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Ғылыми мақала

Жасөспірімдер депрессиясының ЭЭГ маркерлері

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Аңдатпа. Бүгінде депрессия ең кең таралған психикалық аурулардың бірі болып табылады. Ол көңіл күйдің бұзылуымен, өмірге және күнделікті іс-әрекеттерге деген қызығушылықтың жоғалуымен сипатталады, сондай-ақ басқа да бірнеше симптомдармен қатар жүреді, бұл ақыр соңында жұмысқа қабілеттілікке және жалпы денсаулық жағдайына теріс әсер етеді. Сонымен қатар, депрессия мен суицидтік ойлардың және әрекеттердің жоғары қаупінің арасында дәлелденген байланыс бар. Дүниежүзілік денсаулық сақтау ұйымының мәліметі бойынша, әлемде 280 миллион адам депрессиядан зардап шегеді. Сонымен бірге, соңғы он жылда жастар арасындағы депрессия көрсеткіштерінің өсуі байқалады. Жасөспірімдік кезең елеулі психологиялық өзгерістермен қатар жүреді және олар мидың нейробиологиялық жетілу процестерімен тығыз байланысты. Бұл өзгерістер депрессивті жағдайлардың дамуына осалдықты арттыруы мүмкін, сондықтан осы мәселені терең зерттеу қажеттілігі артып отыр. Ғылыми әдебиеттерді талдау нәтижесінде Қазақстанда жасөспірімдер арасындағы депрессияға қатысты психофизиологиялық зерттеулердің іс жүзінде жоқ екені анықталды, бұл мәселенің өзектілігіне қарамастан. Жасөспірімдердегі депрессияны зерттеудің әртүрлі әдістемелік тәсілдерімен қатар, біз аталған бұзылысы бар жасөспірімдердің тұлғалық ерекшеліктеріне психометриялық бағалау жүргіздік. Эмпирикалық зерттеу барысында эмоционалдық күйлерді түсіну параметрлері мен жағымды және жағымсыз аффект арасындағы байланыстар анықталды. Жасөспірімдердегі депрессия олардың тек жағымсыз эмоцияларды түсінуіне ықпал етеді, сонымен қатар оң эмоционалдық тәжірибелерді реттеу қабілетінің төмендеуіне әсер етеді. Алынған нәтижелер жасөспірімдердегі депрессияны ерте диагностикалау және түзету әдістерін әзірлеуде, сондай-ақ тиімді психологиялық көмек көрсету стратегияларын құруда пайдалы болуы мүмкін.

Түйін сөздер: депрессия, жасөспірімдер, тұлғалық ерекшеліктер, диагностикалық тесттер, биомаркерлер

Кіріспе

Қазіргі уақытта депрессия ең көп таралған психикалық аурулардың бірі болып табылады. Дүниежүзілік денсаулық сақтау ұйымының (ДДСҰ) статистикасына сәйкес,

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әлемде бұл аурумен ауыратын 280 миллион адам бар [1]. Сонымен қатар, жастар арасындағы көрсеткіштер соңғы он жылдықта өсуде. Шоридың және әріптестерінің зерттеуіне сәйкес, 10-19 жас аралығындағы балалардың отыз төрт пайызында клиникалық депрессияның белгілері байқалған [2]. Жасөспірім шағында дер кезінде диагноз қойылмаған жағдайда, ересек өмірде физикалық және психикалық денсаулыққа әсер ету мүмкіндігі жоғары [3,4]. Жоғарыда аталған қауіптерден басқа, депрессия мен суицидтік идеациялар немесе әрекеттер қаупінің жоғарылауы арасында дәлелді байланыс бар. ДДСҰ 15 пен 29 жас аралығындағы адамдар арасында, әсіресе табысы төмен және орташа елдерде суицид өлімнің жетекші себептері арасында төртінші орында тұрғанын хабарлайды [1]. Chen және оның әріптестерінің мәліметінше, когнитивті бұзылулар, әлеуметтік бейімделу қиындықтары және суицидтік мінез-құлық қаупінің жоғарылауы жасөспірім жаста депрессиямен жиі бірге жүретін факторлар болып табылады [5]. Статистикаға сәйкес Қазақстан жасөспірімдер суицидінің ең жоғары көрсеткішіне ие - 100 000 адамға шаққанда 18 адамды құрайды [6]. Аурудың ауырлығына қарамастан, ДДСҰ табысы төмен және орташа елдердегі әлем халқының шамамен 75%-ы уақтылы емделмейтінін хабарлайды. Бұл мәселе біздің еліміз үшін де өзекті болып табылады. Мәселен, Қазақстанда депрессияның таралу пайызы CountryCassete жинаған тәуелсіз статистикаға сәйкес 3,9 пайыз жалпы әлемдік көрсеткішпен салыстырғанда 4,4 пайызды құрайды [7]. Бұл мәселені түзетіп, халықтың өмір сүру сапасын жақсарту үшін депрессияны уақытылы дұрыс диагностикалау қажет. Диагноз қою кейде стигматизация және психикалық денсаулықты субъективті бағалауға байланысты қиындай түседі [8]. Осылайша, денсаулық сақтау және психологиялық көмек беру мамандарына қосымша психофизиологиялық әдістер арқылы депрессияны анықтау оңайырақ болар еді.

Депрессияны зерттеу ежелгі өркениеттерде басталды. Месопотамия, Египет және Грециядан алынған мәтіндер апатия, депрессиялық көңіл-күй және қызығушылықтың жоғалуы сияқты депрессия белгілерін сипаттайды [9]. Ежелгі және орта ғасырларда "қара өттің" шамадан тыс жинақталуы деп аталатын "меланхолия" ұғымы кең таралған және әртүрлі психикалық және эмоциялық бұзылулармен байланысты болды [10]. Ағылшын ғалымы Роберт Бертон (1577-1640) меланхолия туралы алғашқы жан-жақты зерттеулердің бірі болған "меланхолия анатомиясы" трактатын жазды [11]. Бертон меланхолияны физикалық және психологиялық белгілерді қамтитын күрделі ауру деп санады. William Cullen (1710-1790) және Philip Pinel (1745-1826) сияқты басқа ерте зерттеушілер де депрессиялық бұзылуларды түсінуге және осы жағдайларды жіктеуге көмектесті [12].

Депрессия туралы заманауи идеялардың дамуында келесі факторлар рөл атқарды. Біріншіден, Зигмунд Фрейдтің конфликтілер туралы идеялары және олардың қорғаныс және бейсаналық механизмдердегі рөлі бізге психоаналитикалық механизмдерді түсінуге көмектесті. Екіншіден, психикалық бұзылуларды бағалау критерийлері мен әдістері депрессиялық бұзылуларды диагностикалық жүйелерге қосу арқылы стандартталған, мысалы, аурулардың халықаралық жіктелуі (International Classification od Disease, ICD) және психикалық бұзылулардың диагностикалық және статистикалық нұсқаулығы (Diagnostic and Statistical Manual of Mental Disorders, DSM [13, 14].

Депрессияны зерттеу үшін әртүрлі әдістер қолданылады. Клиникалық және эпидемиологиялық зерттеулер аурудың таралуын, қауіп факторларын және емдеу әдістерін зерттеу үшін өте пайдалы [15]. Нәтижесінде депрессияның популяция деңгейіндегі әсерін бағалауға және халықтың осал топтарын анықтауға болады. Нейробиологиялық және генетикалық зерттеулердің мақсаты депрессияға жауап беретін биологиялық

механизмдерді зерттеу болып табылады. Олар мидың құрылымдық және функционалдық өзгерістерін анықтау үшін нейробейнелеу әдістерін қолдануды қамтиды, сондай-ақ депрессияның даму ықтималдығына байланысты генетикалық факторларды зерттеу [16]. Психологиялық және әлеуметтік зерттеулер депрессияның когнитивті, эмоциялық және мінез-құлық элементтерін, сондай-ақ стресс, жарақат және әлеуметтік қолдау сияқты әлеуметтік факторлардың әсерін зерттеуге бағытталған [17]. Зерттеу деректері психологиялық сынақтар, сауалнамалар және эксперименттік әдістер арқылы депрессияның әртүрлі аспектілерін бағалайды.

Эмоция мінез-құлықты, когнитивті функцияларды және тұлғааралық өзара әрекеттесуді реттеуде шешуші рөл атқаратын негізгі психологиялық процесс болып табылады [18]. Эмоция субъективті тәжірибені, физиологиялық реакцияларды, экспрессивті мінез-құлықты және когнитивті бағалауды қамтитын күрделі көп компонентті күй болып табылады [19]. Эмоциялар шешім қабылдауға, мотивацияға, ақпаратты қабылдауға және өңдеуге, сондай-ақ әлеуметтік қызметке айтарлықтай әсер етеді. Күнделікті өмірде эмоцияларды сезінудің қарапайымдылығына қарамастан, олардың нейробиологиялық негізі көптеген ми құрылымдары мен желілерін қамтитын күрделі жүйе болып табылады [20].

Қабылдау, уайымдау, эмоцияны реттеу және эмоцияларды үйрену сияқты эмоциялық процестер мидың әртүрлі аймақтарының, соның ішінде лимбиялық жүйенің, префронтальды қыртыстың, аралық қыртысының, базальды ганглияның және дің құрылымдарының өзара әрекеттесуіне негізделген [21]. Бұл жүйелердегі проблемалар және олардың өзара әрекеттесуі әртүрлі эмоциялық бұзылулардың, соның ішінде депрессияның себебі болуы мүмкін. Көптеген ми құрылымдары мен жүйелері эмоциялық процестерді басқаратын күрделі нейробиологиялық желіні құрайды.

Бадамша денеден, гиппокамптан және белдеу қатпарынан тұратын лимбиялық жүйе маңызды рөл атқарады [21]. Бадамша дене – эмоционалды маңызды тітіркендіргіштерді анықтаудың, эмоционалды реакциялар мен эмоционалды оқытуды қалыптастырудың орталық буыны. Гиппокамп эмоционалды есте сақтау процестеріне және эмоциялардың контекстік модуляциясына қатысады. Белдеу қатпарынан эмоционалды және когнитивті ақпаратты біріктіруге, сондай-ақ эмоционалды реакцияларды реттеуге қатысады. Депрессиялық бұзылыстар кезінде эмоциялық өңдеуге қатысатын негізгі ми құрылымдары мен желілерінде функционалдық және құрылымдық өзгерістер байқалады.

Лимбиялық жүйеде бадамша дене, гиппокамп және белдеулік иірімнің көлемі мен белсенділігінің өзгерістері анықталған [22]. Бадамша дененің теріс стимулдарға гиперактивациясы және оң ақпаратты өңдеу кезінде оның белсенділігінің төмендеуі депрессия кезіндегі жағымсыз бейімділікпен байланысты болуы мүмкін [23]. Префронталды қыртыста эмоцияларды реттеу және когнитивтік бақылауға қатысатын дорсолатералды және вентролатералды аймақтардың белсенділігі мен функционалдық байланыстылығы төмендегені байқалады [24].

Эмоцияларды қабылдау және депрессия

Эмоцияларды тану және интерпретациялау қабілеті эмоцияларды реттеуде, әлеуметтік өзара әрекеттестікте және тұлғааралық қарым-қатынастарда маңызды рөл атқарады. Эмоцияларды қабылдау – бұл бет-әлпет мимикасы, дауыс ырғағы және дене тілі сияқты вербалды емес сигналдарға негізделген басқа адамдардың эмоциялық күйлерін анықтау және тану үдерісі ретінде қарастырылады [25]. Эмоцияларды дәл

қабылдау жеке тұлғаларға айналасындағы эмоциялық сигналдарға сәйкес жауап беруге, өз эмоцияларын реттеуге және эмпатия танытуға мүмкіндік береді, бұл табысты әлеуметтік бейімделу үшін шешуші мәнге ие [26,27]. Керісінше, эмоцияларды қабылдаудағы бұзылыстар әлеуметтік оқшауланға, тұлғааралық қақтығыстарға және өмір сүру сапасының төмендеуіне әкелуі мүмкін [28].

Депрессиялық бұзылыстар жиі эмоциялық саладағы тапшылықтармен қатар жүретіндіктен, эмоцияларды қабылдауды зерттеу ерекше маңызды болып табылады. Бұл депрессияның табиғатын, оның симптомдары мен салдарын жақсырақ түсінуге, сондай-ақ неғұрлым тиімді емдеу әдістерін әзірлеуге ықпал етуі мүмкін [28,29]. Соңғы жылдардағы көптеген зерттеулер депрессиялық бұзылыстар кезінде эмоцияларды қабылдаудағы бұзылыстардың өзіндік ерекшеліктері бар екенін көрсетеді. Негізгі сипаттамалардың бірі – жағымсыз бейімділік, мұнда депрессиямен ауыратын пациенттер теріс эмоциялық стимулдарға гиперсезімталдық танытады және оларға таңдамалы назар аударады [30]. Үлкен депрессиялық ауытқу жасөспірімдер үшін жиі кездесетін аурулардың бірі болып табылады. Зерттеудің мақсаты жасөспірімдердегі депрессия биомаркерлеріне психологиялық зерттеу жүргізу болып табылады.

Зерттеу материалдары мен әдістері

Экспериментке депрессиялы топқа 12 жасөспірім (қатысушылардың орташа жасы 13,58 жас; 6 қыз және 6 ұл), сондай-ақ дені сау бақылау тобына 12 жасөспірім (орташа жасы 13,67 жас; 6 қыз және 6 ұл) қатысты. Қатысушылардың барлығы орыс тілді, оң қолды және олардың көру қабілеті қалыпты немесе түзетілген болды. Олар созылмалы аурулармен ауырмаған.

Үлкен депрессиялық ауытқу (Major Depressive Dissorder, MDD) диагнозын психиатр қысқаша халықаралық нейропсихиатриялық сауалнама (Mini International Neuropsychiatric Interview, MINI) арқылы ICD-10 (F32) классификациясына сәйкес қойды [31]. Бұл құрал екі классификацияға негізделген: психикалық бұзылулардың диагностикалық және статистикалық нұсқаулығы - IV (DSM-IV) және аурулардың халықаралық классификациясы (ICD-10). Бақылау тобындағы қатысушылардың орталық немесе перифериялық жүйке жүйесінде ауытқуы болған жоқ.

Экспериментке дейін қатысушылардың ата-анасы немесе қамқоршылары зерттеуге қатысу үшін әл-Фараби атындағы Қазақ ұлттық университетінің Жергілікті этикалық комитеті мақұлдаған ақпараттандырылған келісімге қол қойды. Сыйақы ретінде қатысушылар жүргізілген кешенді зерттеудің нәтижелерін тегін алды.

Эмоцияны тану тапсырмалары мен Струп тапсырмасының бет-әлпетімен өзгертілген түрі (Face Stroop task) эмоциялық және әлеуметтік функционалды әртүрлі аспектілерін бағалау үшін психодиагностикада кеңінен қолданылады. Бұл тапсырма адамның эмоциялық бейнелерді дәл қабылдау және тану қабілетін бағалауға мүмкіндік береді, бұл әлеуметтік дағдылар мен эмоциялық интеллекттің маңызды бөлігі болып табылады. Мінез-құлық тапсырмасы изоляцияланған, жарықтандыру қуаты 200 Вт жасанды жарық бөлмеде орындалды. Қатысушы экраннан 1 метр қашықтықта отырды. Эмоциялық конфликт тапсырмасы E-Prime 2 бағдарламасында ЭЭГ жазбасымен қатар орындалды. Экранда төрт эмоцияның (қуаныш, қайғы, қорқыныш, ашу) біреуін бейнелейтін ақ-қара түстегі бет-әлпет көрсетілді. Көрнекі стимулмен қатар эмоция атауы айтылып, бұл атау бейнемен сәйкес (конгруэнтті стимул) немесе сәйкес емес (неконгруэнтті стимул) болуы мүмкін. Қатысушының міндеті – визуалды және дыбыстық стимулдардың

сәйкестігін тез және дәл анықтау. Тапсырманы орындау алдында экранда нұсқаулық көрсетіледі. Стимулдар арасындағы үзілісте экранда 3 секундқа «+» белгісі көрсетіліп, назарды тұрақтандыруға көмектеседі. Егер эмоция дұрыс аталған болса, қатысушы «1» батырмасын басуы керек. Егер дыбыстық стимул көрнекі стимулға сәйкес келмесе, «2» батырмасын басуы қажет. Бұл ретте реакция жылдамдығы мен жауаптың дұрыстығы есепке алынады (реакция уақыты жанама түрде бақыланды). Тапсырма мақсаты – стимулдардың сәйкестігін тез әрі дәл бағалау. Эксперименттік тапсырманы орындамас бұрын, қатысушылар тапсырманың нұсқаулығымен танысып, 1 минуттық сынақ кезеңінен өтті. Бұл кезеңнің мақсаты – қатысушыларды мақсатты және мақсатсыз стимулдарды ажыратуға, компьютерлік пернетақтаны пайдаланып мақсатты стимулдың бағытын анықтауға және стимулдар алдында берілген нұсқауларды түсіндіруге үйрету.

Эксперименттік бөлім 4 блоктан тұрды, әр блокта 60 стимул бар, жалпы 240 стимул ұсынылды. Стимулдар кездейсоқ тәртіпте көрсетілді, соңғы блокта тек конгруэнтті емес стимулдар ұсынылды. Эксперименттік бөлімнің жалпы ұзақтығы 20 минут болды.

Электроэнцефалографиялық зерттеу әдісі

ЭЭГ жазбасы әл-Фараби атындағы Қазақ ұлттық университетінің Ми институтының ЭЭГ зертханасында жүргізілді. ЭЭГ-ні тіркеу үшін ANT EEG аппараты мен eegomylab жүйесі қолданылды, ал сынаққа қатысушының басына 64 электродтан тұратын шапканы кигізді. Электродтардың адгезиясын жақсарту және терінің қарсылығын азайту үшін медициналық электрофизиологиялық зерттеулерде қолданылатын арнайы гель пайдаланылды. Бастапқы дискретизация жиілігі (sampling rate) 512 Гц болды. ЭЭГ жазбасы эмоциялық конфликт тапсырмасын орындау кезінде синхронды жүргізілді, бұл тапсырма бірінші реттік (S1) және екінші реттік (S2) стимулдарды көрсетуді қамтыды. S1 стимулдары эмоциялық бет бейнелері болды, ал S2 стимулдары S1-дің эмоциялық валенттілігіне конгруэнтті немесе конгруэнтті емес сөздерден тұрды. S1 және S2 стимулдарын көрсету кезінде ЭЭГ жазбасы визуалды стимулдарды өңдеу мен эмоциялық конфликтіні көрсететін потенциалдарды анықтауға мүмкіндік берді [32]. Талдау үшін келесі ЭЭГ арналары таңдалды: PO4, PO3, CP4, CP3, FC4, FC2, FC3, FCz, O2, O1, POz, P4, Pz, P3, C4, Cz, C3, F4, Fz, F3. Бұл арналар ми қыртысының шүйде, төбе, орталық және маңдай аймақтарын қамтиды. Бұл арналарды таңдау эмоциялық стимулдарды өңдеу кезіндегі туындаған потенциалдардың визуалды компоненттері анықталған алдыңғы зерттеулерге негізделді [33].

Сауалнамалар әдісі

Зерттеу әдістері ретінде сауалнамалар қолданылды. 30 сұрақтан тұратын метакогнитивті көңіл-күй шкаласы (Trait-Meta Mood Scale, TMMS). Бұл сауалнамада үш шкала бар: өз эмоцияларына назар аудару, өзінің эмоциялық күйін түсіну және көңіл-күйді реттеу және жағымсыз эмоциялық тәжірибелерді басқару мүмкіндігі.

- Позитивті және негативті аффект шкаласы (The Positive and Negative Affect Schedule, PANAS). PANAS әртүрлі эмоциялар мен сезімдерді сипаттайтын 20 сын есімнен тұрады. Олардың оны позитивті аффектке (мысалы, "жігерлендірілген", "қызығушылық таныт-қан", "белсенді"), ал қалған оны негативті аффектке (мысалы, "ренжіген", "кінәлі", "ашуланшақ") жатады. Субъектіге эмоциялардың әрқайсысын белгілі бір уақыт аралығында (мысалы, қазір, өткен аптада немесе жалпы) 5 балдық шкала бойынша қанша-лықты бастан өткергенін бағалау ұсынылады. Сауалнаманың нәтижелері позитивті

және негативті аффект деңгейін, сондай-ақ адамның жалпы эмоциялық тепе-теңдігін анықтауға мүмкіндік береді.

– 10 сұрақтан тұратын эмоцияны реттеу сауалнамасы (Emotion Regulation Questionnaire, ERQ). Бұл сауалнама эмоцияларды реттеудің екі стратегиясын көрсететін екі шкаланы өлшейді: 1) жағымсыз эмоцияларды тудыратын жағдайды когнитивті қайта бағалау немесе қайта қарау және 2) эмоцияларыңызды білдіруді басу.

– Спилбергер-Ханин сауалнамасы екі шкалаға бөлінген 40 пікірді қамтиды: ситуациялық (реактивті) мазасыздық шкаласы және тұлғалық мазасыздық шкаласы. Субъектіге 4 балдық шкала арқылы сипатталған күйлерді қаншалықты жиі сезінетінін бағалау ұсынылады.

Нәтижелер

Корреляциялық талдау депрессиясы бар науқастардың жеке қасиеттерінің өзара байланысы туралы болжамды тексеру мақсатында жүргізілді. Корреляцияның статистикалық маңызды нәтижелері 1-3 кестеде келтірілген.

Кесте 1

Психометриялық тесттер арасындағы маңызды корреляциялар. Позитивті және негативті аффект шкаласы (PANAS) және мета көңіл-күйдің ерекшелік шкаласы (TMMS) (r - Пирсон коэффициенті)

Тесттер		Позитивті және негативті аффект шкаласы (PANAS)	
		Позитивті аффект	Негативті аффект
Мета көңіл-күйдің ерекшелік шкаласы (TMMS)	Өз эмоцияларына назар аудару	0,227	0,241
	Өзіңіздің эмоциялық күйлеріңізді түсіну	0,596(*) P=0,041	-0,609(*) P=0,036
	Көңіл-күй мен жағымсыз эмоциялық тәжірибені реттеу қабілеті	0,286	-0,643(*) P=0,024

Кесте 2

Психометриялық тесттер арасындағы маңызды корреляциялар. Позитивті және негативті аффект шкаласы (PANAS) және эмоцияны реттеу сауалнамасы ERQ (r - Пирсон коэффициенті)

Тесттер		Позитивті және негативті аффект шкаласы (PANAS)	
		Позитивті аффект	Негативті аффект
Эмоцияны реттеу сауалнамасы ERQ	Жағымсыз тәжірибе тудыратын жағдайды когнитивті қайта бағалау/қайта қарау эмоцияларыңыздың көрінісін басу	-0,522	-0,272
	Эмоцияларды білдіруді басу	-0,415	0,021

Кесте 3

Психометриялық тесттер арасындағы маңызды корреляциялар. Позитивті және негативті аффект шкаласы (PANAS) және Спилбергер-Ханин сауалнамасы (r - Пирсон коэффициенті)

Тесттер		Позитивті және негативті аффект шкаласы (PANAS)	
		Позитивті аффект	Негативті аффект
Спилбергер-Ханин сауалнамасы	Ситуациялық (реактивті) мазасыздық шкаласы	0,463	0,048
	Тұлғалық мазасыздық шкаласы	-0,318	-0,193

ЭЭГ мен психометриялық тесттер арасындағы маңызды корреляциялар

Алдын ала талдау нәтижелері эксперименттік топтағы тапсырма стимулдарына байланысты ЭЭГ туындаған потенциалдары (ВП) мен сауалнамалар көрсеткіштері арасындағы маңызды корреляцияларды анықтады. Бұл деректер мінез-құлық әдістерімен өлшенген эмоциялық және когнитивті көрсеткіштер мен эксперименттік тапсырмаларды орындау кезінде тіркелген нейрофизиологиялық жауаптар арасындағы байланысты көрсетеді.

Сәйкесінше, 4 кесте деректеріне сүйене отырып, эксперименттік топта «Қайғы (конгруэнтты емес стимул)» және «Бақыт (конгруэнтты емес стимул)» сияқты эмоциялық стимулдар мен негативті аффект арасында маңызды оң корреляциялар анықталды. Бұл осы эмоцияларға қатысты негативті эмоциялық бағалауды көрсетуі мүмкін. Сонымен қатар, диссонансты стимулдың бағалануы экспериментке дейінгі позитивті аффектпен күшейтіледі. Эксперимент басындағы позитивті эмоциялық көңіл-күй диссонансты «Ашу» стимулын тез анықтауға ықпал етуі мүмкін.

Кесте 4

Эксперименттік топтағы тапсырма стимулдарына қатысты сауалнамалардың ЭЭГ-ВП көрсеткіштерімен маңызды корреляциялары

Эмоциялық конфликтке арналған тапсырманың стимулдары	ЭЭГ электроды	Позитивті аффект бойынша PANAS (экспериментке дейін)	Негативті аффект бойынша PANAS (экспериментке дейін)
Ашу конгруэнтті стимул	F3	0,571**	
Қайғы конгруэнтті емес стимул	P3		0,446*
Қуаныш конгруэнтті емес стимул	F3		0,445*

5 кесте деректері бойынша бақылау тобында «Ашу (конгруэнтті стимул)» және «Қайғы (конгруэнтті емес стимул)» сияқты эмоциялық стимулдар арасында маңызды теріс корреляциялар анықталды. Бұл бақылау таңдауының осы эмоциялық стимулдарды анықтауына ситуативті мазасыздықтың әсер етпейтінін көрсетуі мүмкін. Сонымен қатар, негативті аффект «Бақыт» эмоциялық стимулының конгруэнтті және конгруэнтті емес түрлерін ажыратуға әсер етпейді.

Кесте 5

Бақылау тобындағы тапсырма стимулдарына қатысты сауалнамалар мен ЭЭГ-ВП көрсеткіштерінің маңызды корреляциялары

Эмоциялық конфликт тапсырмасының стимулдары	ЭЭГ электроды	Ситуациялық (реактивті) мазасыздық шкаласы	Эксперименттен кейінгі PANAS бойынша негативті аффект
Ашу конгруэнтті стимул	F3	-0,450*	
Қайғы конгруэнтті емес стимул	Cz	-0,675**	
Қуаныш конгруэнтті стимул	Cz		-0,532*
Қуаныш конгруэнтті стимул	FCz		-0,485*

Талқылау

Тиісінше, 4-кестедегі мәліметтерге сүйене отырып, эксперименттік топта «қайғы» (сәйкес келмейтін ынталандыру) және «бақыт» (сәйкес келмейтін ынталандыру) сияқты эмоционалды тітіркендіргіштер мен жағымсыз аффект арасындағы маңызды оң корреляциялар анықталды [34]. Бұл эмоциялардың жағымсыз эмоционалды бағасын көрсетуі мүмкін. Сонымен қатар, диссонанстық ынталандыруды бағалау оң эксперименталды алдындағы аффектпен нығайтылады. Эксперимент басталған кездегі позитивті эмоционалды көңіл-күй диссонанстық ынталандыру – «ашуды» тез анықтауға ықпал етуі мүмкін.

Қатысушылардың эмоционалды күйлерін бағалау үшін жиі позитивті және негативті аффект шкаласы (PANAS) қолданылады. Бұл әдіс позитивті және негативті аффект деңгейлерін сенімді түрде өлшеуге мүмкіндік береді, бұл эмоционалды күйлердің ынталандыруларды қабылдау мен бағалауға әсерін зерттеу үшін маңызды. Негативті аффекттің «қайғы» және «бақыт» сияқты сәйкес келмейтін эмоционалды ынталандыруларды бағалаумен байланысы болуы мүмкін деген байқау бірнеше зерттеулермен расталған. Атап айтқанда, зерттеулер адамның эмоционалды күйі эмоционалды ынталандыруларды қабылдау мен бағалауға әсер ететінін көрсетеді [35]. Бұдан бөлек, Овсянниковаңың зерттеуінде сыналушылардың көңіл-күйі олардың эмоциялық көріністерді тану қабілетіне әсер ететіні атап өтіледі [36].

Сонымен қатар, эксперимент алдындағы позитивті эмоционалды күй диссонанстық стимулдарды тезірек тануға ықпал етуі мүмкін. Бұл позитивті аффекттің зейінді кеңейтіп, ойлаудың икемділігін арттыратынымен байланысты, сондықтан ол стандартты емес немесе күтпеген стимулдарды өңдеуді жеңілдетеді. Осылайша, эксперименттің басындағы позитивті көңіл-күй қатысушылардың «ашу» сияқты диссонанстық стимулдарды жылдам анықтау және оларға жауап беру қабілетін жақсарта алады [37].

5 кестедегі мәліметтерге сәйкес, бақылау тобында «ашу» (конгруэнтті стимул) және «қайғы» (конгруэнтті емес стимул) сияқты эмоционалды стимулдар арасында айтарлықтай теріс корреляциялар анықталды. Бұл жағдайлық мазасыздықтың бақылау таңдауына осы эмоционалды стимулдарды анықтауға әсер етпейтінін көрсетуі мүмкін. Сонымен қатар, негативті аффект «бақыт» эмоционалды стимулының конгруэнтті және конгруэнтті емес формаларын ажыратуға әсер етпейді [38].

Зерттеу көрсеткендей, дені сау адамдарда «ашу» мен «қайғы» эмоциялары бір-бірінен тәуелсіз өңделеді, бұл олардың арасындағы теріс корреляциямен дәлелденеді. Бұл дені сау адамдардың осы эмоционалды күйлерді ажырата алу қабілетін көрсетеді, бұл өз кезегінде қалыпты эмоционалды қызмет етудің белгісі болып табылады [34].

Осылайша, қолданыстағы зерттеулер дені сау адамдарда «ашу» мен «қайғы» арасындағы теріс корреляция байқалатынын, сондай-ақ негативті аффект «бақыт» эмоциясының түрлі формаларын ажыратуға әсер етпейтінін растайды. Бұл дені сау популяциядағы эмоционалды жүйенің қалыпты жұмыс істейтінін көрсетеді.

Қорытынды

Мета көңіл-күй ерекшеліктерінің шкаласы, позитивті және негативті аффект шкаласы, эмоцияларды реттеу сауалнамасы, Спилбергер-Ханин сауалнамасы сияқты әдістер арқылы алынған деректерді талдау әсер көрсеткіштері мен мета көңіл-күй ерекшеліктерінің шкалаларының көрсеткіштері арасындағы статистикалық маңызды байланысты көрсетті. Біз алған мәліметтер қазіргі заманғы сауалнамаларды қолдана отырып, жасөспірімдердің депрессиялық жағдайларын диагностикалау мен түзетуде пайдалы болуы мүмкін. Осылайша, жасөспірімдер депрессиясының психологиялық сипаттамаларын зерттеу деректерін алдын-ала талдау позитивті және негативті аффект шкаласы (PANAS) сияқты сауалнамалардың және мета көңіл-күй ерекшеліктерінің шкалалары көрсеткіштерінің ақпараттылығын көрсетеді.

Авторлардың үлесі

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Қаржыландыру

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Авторлар мүдделер қақтығысы жоқ деп мәлімдейді.

Этикалық нормаларды сақтау

Эксперимент әл-Фараби атындағы Қазақ Ұлттық Университетінің (Алматы, Қазақстан) жергілікті этика комитетінің, 16.02.2023 жылғы № IRB – A267 хаттамасының мақұлдауымен жүргізілді. Адамның қатысуымен жүргізілген зерттеулерде жүргізілген барлық процедуралар зерттеулер жүргізілген мекеменің этикалық нормаларына және Қазақстан Республикасының және халықаралық ұйымдардың бекітілген лига актілеріне сәйкес болды.

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ЭЭГ маркеры подростковой депрессии

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Аннотация. Депрессия на сегодняшний день является одним из самых распространённых психических заболеваний. Она выражается через расстройства настроения, утрату интереса к жизни и привычным занятиям, а также сопровождается рядом других симптомов, что в итоге негативно сказывается на работоспособности и общем состоянии здоровья. Помимо этого, существует доказанная связь между наличием депрессии и повышенным риском суицидальных идеаций и попыток. Согласно данным Всемирной организации здравоохранения, в мире 280 миллионов человек страдают депрессией. Кроме того, за последние десять лет выявлен рост показателей депрессии среди молодежи. Подростковый возраст сопровождается значительными психологическими изменениями, которые тесно связаны с нейробиологическими процессами созревания мозга. Эти изменения могут повышать уязвимость к развитию депрессивных состояний, что подчеркивает необходимость глубокого изучения данного вопроса. Анализ научной литературы также выявил, что в Казахстане практически отсутствуют психофизиологические исследования депрессии среди подростков, несмотря на актуальность данной проблемы. Наряду с различными методическими подходами к изучению подростковой депрессии нами были проведены психометрические измерения личностных особенностей подростков с данным расстройством. В эмпирической части исследования были обнаружены взаимосвязи между параметрами понимания эмоциональных состояний и негативным и позитивным аффектом. Подростковая депрессия способствует осознанию только негативно окрашенных эмоциональных состояний, а также снижает способность к регуляции положительных переживаний. Полученные результаты могут быть полезны при разработке методов ранней диагностики и коррекции подростковой депрессии, а также при создании эффективных стратегий психологической помощи.

Ключевые слова: депрессия, подростки, особенности личности, диагностические тесты, биомаркеры

EEG markers of adolescent depression

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Abstract. Depression is currently one of the most common mental illnesses. It is expressed through mood disorders, loss of interest in life and habitual activities, and is accompanied by a number of

other symptoms, which ultimately negatively affects performance and overall health. In addition, there is a proven link between the presence of depression and an increased risk of suicidal ideation and attempts. According to the World Health Organization, 280 million people worldwide suffer from depression.Moreover, over the past ten years, there has been an increase in rates of depression among young people. Adolescence is accompanied by significant psychological changes that are closely related to the neurobiological processes of brain maturation. These changes may increase vulnerability to the development of depressive states, which highlights the need for in-depth study of this issue. An analysis of the scientific literature also revealed that there are practically no psychophysiological studies of depression among adolescents in Kazakhstan, despite the urgency of this problem. Along with various methodological approaches to the study of adolescent depression, we conducted psychometric measurements of the personality characteristics of adolescents with this disorder. In the empirical part of the study, correlations were found between the parameters of understanding emotional states and negative and positive affect. Adolescent depression promotes awareness of only negatively colored emotional states, and also reduces the ability to regulate positive experiences. The results can help in developing early diagnostic and intervention methods for adolescent depression and enhancing strategies for effective psychological support.

Keywords: depression, adolescents, personality traits, diagnostic tests, biomarkers

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Научная статья

In vitro микроразмножение ирги канадской *(Amelanchier canadensis):* оптимизация цитокининово-ауксиновых комбинаций для индукции побегообразования

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Аннотация. В статье проведено исследование по оптимизации условий микроразмножения трёх сортов ирги канадской *(Amelanchier canadensis)* в условиях *in vitro.* Установлено, что модификация питательной среды Мурасиге и Скуга за счёт введения 6-бензиламинопурина (BAP) в различных концентрациях оказывает дифференцированное влияние на морфогенетическую активность эксплантов. Наиболее эффективной комбинацией для сортов Мартин и Слэйт явилось применение BAP в концентрации 1,0 мг/л и GA_3 – 0,5 мг/л, в то время как для сорта Нортлайн оптимальной оказалась концентрация BAP 0,5 мг/л и GA_3 – 0,5 мг/л. Показано, что увеличение концентрации BAP свыше 1,0 мг/л приводит к снижению как длины, так и количества формирующихся микропобегов. Полученные результаты могут быть использованы для массового размножения и сохранения этих сортов ирги.
Ключевые слова: *Amelanchier Canadensis,* 6-бензиламинопурин, нафтилуксусная кислота, гиббереллиновая кислота, микропобеги, микроразмножение, фотопериод

Введение

Род Amelanchier, также известный как ирга, включает около 20 видов лиственных кустарников и небольших деревьев семейства розоцветных *(Rosaceae)* [1]. Представители этого рода широко распространены в Северной Америке, Европе, а также в Западной и Восточной Азии, при этом видовое разнообразие варьируется в зависимости от региона. Интродукция дикорастущих видов ирги в других странах началась с 1590 года и достигла наиболее интенсивного распространения в XIX веке. Популяризация культуры была обусловлена благодаря ее агрономическим и декоративным качествам. С 1800 года началась целенаправленная селекционная работа по ирге в Канаде, а затем и в США. С 1937 года уже был налажен выпуск коммерческих сортов [2].

Ирга относится к перспективным плодовым культурам благодаря высокой устойчивости к различным абиотическим факторам и множеству хозяйственно ценных признаков [3]. Плоды ирги имеют лечебно-пищевую ценность, что подтверждается их биохимическим составом: 5-12% легкоусвояемых сахаров, 3,7% пектинов, 14% витамина С, в достаточно большом количестве в плодах содержатся также другие витамины

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и много Р-активных соединений и дубильных веществ, нормализующих состояние капилляров и свёртываемости крови. Водорастворимый полисахаридный комплекс из плодов ирги повышает неспецифический иммунитет, улучшает кровообращение, повышает способность тканей к регенерации [4]. Также ирга – очень неприхотливый, холодостойкий кустарник (даже во время цветения переносит понижение температуры до - 50С), устойчивый к задымлению и загазованности городского воздуха, растущий практически на всех типах почв [5].

Канадские сорта ирги выращиваются с 17 века и имеют множество различных названий, таких, как «северный виноград», «коринка», «винная ягода» или «июньская ягода». Благодаря своей способности легко адаптироваться к различным климатическим условиям ирга может расти практически в любом регионе нашей страны.

На рынке Казахстана недавно появились три перспективных сорта ирги канадской, отличающихся высокой урожайностью: сорт ирги Мартин (Martin) выведен в Канаде на основе сорта Тиссен (Thiessen). Изначально широко распространён в Северной Америке, в настоящее время активно используется в садоводстве благодаря устойчивости и высокому декоративному потенциалу.

Растение представляет собой среднерослый, многоствольный кустарник, достигающий высоты до 3 м и ширины до 2 м. Крона плотная, округлая, с выраженным облиственением. Листья матовые, тёмно-зелёные, округлой формы, с заострённой верхушкой и крупнозубчатым краем. Молодые побеги длинные, гибкие, с характерной красновато-коричневой окраской.

Цветение наступает в конце апреля – начале мая. Соцветия крупные, кистевидные, содержат до 20 белых цветков, что обеспечивает высокую декоративность растения в период цветения. Сорт характеризуется выраженной способностью к образованию корневой поросли, что следует учитывать при выборе агротехнических приёмов и способах размножения.

Сорт ирги Слейт получен в Канаде в рамках селекционной программы, направленной на улучшение местных форм ирги ольхолистной. Основными задачами селекционного процесса являлись создание компактной, зимостойкой формы с выраженными декоративными характеристиками, а также выведение растения с крупными, однородными по массе и срокам созревания плодами. Благодаря успешному сочетанию устойчивости к неблагоприятным климатическим условиям, высокой урожайности и декоративной ценности сорт рекомендован как для промышленного садоводства, так и для использования в декоративном озеленении, особенно в условиях северных регионов [6].

Растение представляет собой невысокий кустарник, достигающий около 2 м в высоту. Плоды крупные, округлые, тёмно-синие, с сочной, сладкой мякотью и высоким содержанием витаминов. Семена мелкие, малозаметные при употреблении. Урожай пригоден как для потребления в свежем виде, так и для переработки: используется в производстве варенья, джемов, компотов, а также в виноделии.

Ирга сорта Нортлайн – это высокорослый кустарник (4-5 м) семейства Розоцветных с широкой и плотной кроной, диаметр которой может достигать 6 м. Куст обладает мощной корневой системой с множеством ответвлений, которая залегает на глубину до 2 м. Листья темно-зеленые, с рифлеными краями и слегка заостренной верхушкой. Цветение начинается уже в апреле. Сорт Нортлайн отличается высокой самоплодностью: более 90% цветков превращаются в ягоды [7].

В агрокультуре ирга ценится как декоративный и не требовательный к агротехнике кустарник, поэтому в Европе, Канаде и США активно использовали в декоративных

целях. Данная культура размножается генеративным и вегетативными способами. Из вегетативных способов размножения наиболее простые – это размножение корневой порослью и делением куста, более трудоемкие – зеленое черенкование, прививка [8]. Однако вышеперечисленные методы малоэффективны для коммерческого использования, так как дают недостаточное количество посадочного материала. Одним из современных методов размножения растений является клональное микроразмножение. Данный способ активно практикуется для получения посадочного материала в промышленных масштабах многих плодовых культур. С помощью этого метода можно произвести оздоровление растительного материала, ускоренно размножить ценные отборные формы, а также редкие сорта [9].

Тем не менее при использовании методов клонального микроразмножения ирги выявляется ряд существенных биотехнологических ограничений. Одной из ключевых проблем остаётся низкая эффективность стерилизации первичного растительного материала, что обусловлено высокой контаминированностью тканей и наличием устойчивой эндофитной микрофлоры. Это, в свою очередь, обуславливает высокий уровень инфицирования культур на начальных этапах культивирования in vitro, снижая процент жизнеспособных эксплантов [10].

В связи с этим особое внимание требует решение проблемы отсутствия стандартизированных и воспроизводимых протоколов микроразмножения, которые должны учитывать видовые и сортовые особенности растений. Недостаточная стандартизация этих процессов затрудняет широкое промышленное внедрение технологии и ограничивает её применение в селекционной и питомниководческой практике, что дополнительно осложняет получение качественного посадочного материала.

Для преодоления этих затруднений необходимо проведение комплексной работы, включающей оптимизацию состава питательных сред, подбор эффективных концентраций регуляторов роста и разработку усовершенствованных режимов культивирования и стерилизации, которые будут адаптированы к специфическим биологическим особенностям исследуемой культуры [11].

В результате реализации этих мероприятий возможно создание эффективных протоколов клонального микроразмножения, что обеспечит получение высококачественного, оздоровлённого и генетически однородного посадочного материала в промышленных масштабах, обеспечив успешное применение технологии в агропроизводстве [12].

Целью исследования является установление оптимальных условий для *in vitro* микроклонального размножения 3 сортов ирги канадской *(Amelanchier canadensis)* посредством подбора эффективных концентраций регуляторов роста, обеспечивающих максимальное проявление морфогенетического ответа и регенерационной активности эксплантов.

Материалы и методы исследований

Объектом исследования являлись три сорта канадской ирги *Amelanchier Canadensis:* Мартин (Martin), Слейт (Sleyt), Нортлайн (Northline). В качестве эксплантов для введения в культуру *in vitro* были взяты узловые сегменты стеблевых черенков однолетней ирги.

Стерилизацию проводили на основе двух вариантов обработки, отличающихся по времени экспозиции в каждом растворе стерилизующего агента. Стерилизацию завершали трехкратным промыванием бидистиллированной водой.

Для выявления особенностей развития эксплантов ирги была использована универсальная питательная среда Мурасиге-Скуга [13] с добавлением различных концентраций регуляторов роста NAA, BAP и GA_3 (0,1; 0,3 и 0,5 мг/л). В качестве источника углерода применяли сахарозу в концентрации 30 г/л, что соответствует общепринятому стандарту для большинства древесных культур in vitro и обеспечивает оптимальный энергетический баланс и осмотический потенциал для нормального морфогенеза побегов. Агар добавляли в дозе 6,5 г/л, pH среды доводили до 5,7 перед автоклавированием.

Каждый вариант включал 20 эксплантов, эксперимент проводился в трёх биологических повторностях. Статистическая обработка данных выполнена методом однофакторного дисперсионного анализа (ANOVA). Результаты представлены как средние значения ± стандартное отклонение (SD).

Культивирование растений осуществляли при температуре 22-24 °C, при освещённости 3000 лк и фотопериоде 16/8 (свет/темнота) на стеллажах с использованием люминесцентных ламп в условиях светокультуральной комнаты. Для обработки полученных данных применялись общепринятые методы статистического анализа, осуществленные с использованием программного обеспечения ANOVA [14].

Результаты исследования

Первым этапом микроразмножения являлся отбор эксплантов и введение их в культуру. Узловые сегменты стеблевых черенков обеспечили высокую способность к образованию новых побегов, что делало их подходящим материалом для микроразмножения (Рисунок 1).

Рисунок 1. Экспланты ирги канадской *(Amelanchier canadensis)* для введения в культуру *in vitro*

Процесс стерилизации играет фундаментальную роль в культивировании клеточных культур, так как предотвращает развитие патогенных микроорганизмов и способствует сохранению жизнеспособности эксплантов. Стерилизация эксплантов ирги проводилась в трёх вариантах с различной экспозицией в растворах стерилизующих агентов (Таблица 1).

Таблица 1

Этап стерилизиации эксплантов ирги *Amelanchier Canadensis*

Стерилизующие агенты	Варианты		
	1	2	3
70% C_2H_5OH	1 мин.	2 мин.	3 мин.
3 % NaClO	5 мин.	10 мин.	15 мин.
Twin – 20	10 мин.	15 мин.	20 мин.

В результате проведенных экспериментов установлено, что в 1 варианте стерилизации, где время выдержки эксплантов в 70% этаноле – 1 мин., в 3% растворе гипохлорида натрия – 10 мин., выход стерильных эксплантов составил 17,6%. Во 2-м варианте, где экспозиция в 70% этаноле длилась 2 мин., в 3% растворе гипохлорида натрия – 20 минут, выход стерильных эксплантов был выше и составил – 80,0 %. Из 40 шт. почек – 32 шт. оказались неинфицированными и жизнеспособными. В 3-м варианте все экспланты потеряли жизнеспособность и почернели от продолжительного воздействия стерилизующего агента. На основе полученных данных второй способ стерилизации был далее использован для стерилизации растительных эксплантов ирги (Рисунок 2).

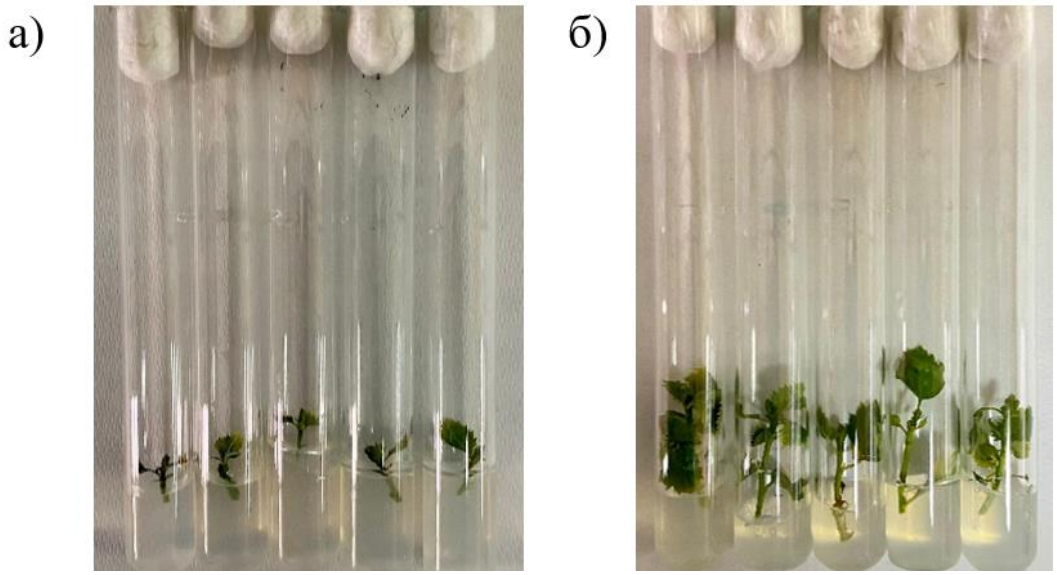

Рисунок 2. Введение в культуру *in vitro* эксплантов ирги: **а)** высаженные экспланты ирги; **б)** экспланты ирги через 14 дней культивирования

Для введения эксплантов ирги в культуру *in vitro* была использована универсальная среда Мурасиге Скуга (МС) с разными концентрациями ВАР:

1. МС – 4,33 г/л, сахароза 30 г/л NAA – 0,1 мг/л, ВАР – 0,1 мг/л, агар – 7 г/л, pH=5,7
2. МС – 4,33 г/л, сахароза 60 г/л, NAA – 0,1 мг/л, ВАР – 0,3 мг/л, агар – 7 г/л, pH=5,7
3. МС – 4,33 г/л, сахароза 60 г/л, NAA –0,1 мг/л, ВАР – 0,5 мг/л, агар – 7 г/л, pH=5,7

Проведённые эксперименты показали, что наибольшая морфогенетическая активность эксплантов ирги наблюдалась на втором варианте питательной среды. При использовании 20 эксплантов на вариант и трёх биологических повторностях однофакторный

дисперсионный анализ (ANOVA) выявил статистически значимые различия между опытными группами ($p < 0,01$). Среднее количество побегов на эксплант во втором варианте составило 5.6 ± 0.1, что достоверно превышало показатели первого (2.7 ± 0.1) и третьего (3.2 ± 0.1) вариантов.

Данные результаты указывают на то, что второй вариант среды обеспечивает оптимальное соотношение экзогенных регуляторов роста, способствующее активации морфогенеза и формированию морфологически стабильных побегов. Варианты 1 и 3, напротив, характеризовались недостаточной либо избыточной регуляторной стимуляцией, что приводило к снижению регенерационного потенциала эксплантов (Рисунок 3).

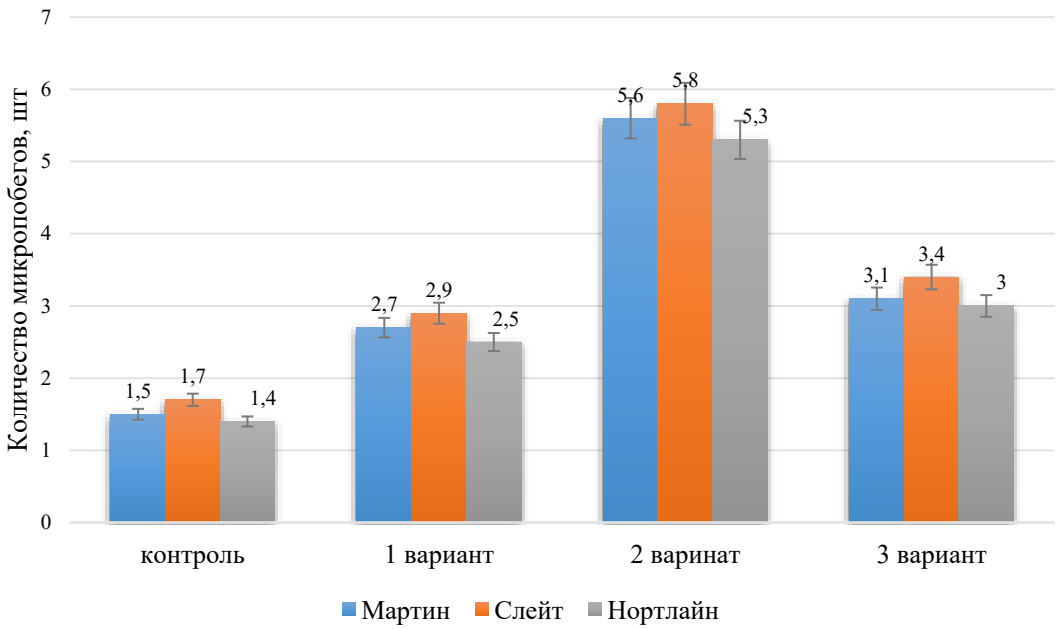

Рисунок 3. Влияние регуляторов роста на количество микропобегов

На этапе микроклонального размножения проводилось тестирование различных комбинаций BAP и GA_3 для сортов ирги. Цитокинин BAP (6-бензиламинопурин) использовался в трёх концентрациях (0,5; 1,0 и 2,0 мг/л) для идентификации оптимального уровня индукции побегообразования. Гиббереллиновая кислота GA_3 была добавлена в фиксированной дозе 0,5 мг/л для поддержания нормального роста микропобегов без риска проявления фитотоксичности.

Оценка эффективности проводилась на протяжении 12 месяцев, с регулярным субкультивированием каждые 4-6 недель. В течение этого периода культура демонстрировала стабильный и высокий морфогенетический потенциал. Оптимальная комбинация, включающая 1,0 мг/л BAP, позволяла получать в среднем 6-8 микропобегов на эксплант.

Культура успешно выдержала до 6 циклов субкультивирования без видимого снижения регенерационной способности и потери морфогенетического потенциала. При более длительном культивировании (после 6-го цикла) отмечалось незначительное снижение скорости пролиферации и появление признаков витрификации у некоторых эксплантов, что указывает на необходимость периодического обновления исходного

материала. Это подтверждает, что данная методика подходит для долгосрочного размножения с периодическим обновлением маточной культуры (Таблица 2).

Проведённый эксперимент подтвердил, что включение фитогормонов в питательную среду оказывает заметное влияние на морфогенез in vitro у различных сортов ирги. Комбинации BAP и GA_3 значительно стимулировали как рост побегов, так и их ветвление по сравнению с контролем, где ростовые процессы оставались на базовом уровне.

Таблица 2

Эффект BAP с GA_3 на морфогенез побегов различных сортов ирги

Сорта	Концентрация гормонов, мг/л	Длина побега, см.	Количество побегов, шт.
Мартин	Контроль (без гормонов)	4,50 ± 0,20	3 ± 0,1
	BAP 0,5 + GA_3 0,5	5,81±0,24	6±0,2
	BAP 1,0 + GA_3 0,5	5,52±0,47	7±0,3
	BAP 2,0 + GA_3 0,5	4,98±0,12	4±0,2
Слэйт	Контроль (без гормонов)	4,40 ± 0,30	3 ± 0,2
	BAP 0,5 + GA_3 0,5	5,76±0,43	5±0,03
	BAP 1,0 + GA_3 0,5	5,78±0,72	6±0,01
	BAP 2,0 + GA_3 0,5	4,90±0,25	5±0,2
Нортлайн	Контроль (без гормонов)	4,60 ± 0,18	4 ± 0,1
	BAP 0,5 + GA_3 0,5	6,32±0,20	8±0,5
	BAP 1,0 + GA_3 0,5	5,75±0,20	6±0,1
	BAP 2,0 + GA_3 0,5	5,02±0,29	4±0,01

У сорта «Мартин» наиболее выраженное удлинение побегов (5,81 ± 0,24 см) было зафиксировано при применении минимальной концентрации BAP (0,5 мг/л) в сочетании с GA_3 (0,5 мг/л). Однако наибольшее количество побегов (7,0 ± 0,3) наблюдалось при средней дозе BAP – 1,0 мг/л.

Сорт «Слейт» продемонстрировал более стабильный отклик: длина побегов и их количество возрастали при увеличении дозы BAP до 1,0 мг/л, при этом разница между 0,5 и 1,0 мг/л была минимальна, а максимальные значения (5,78 ± 0,72 см и 6,0 ± 0,01 шт.) наблюдались именно на средней концентрации. Это подтверждает благоприятное воздействие данных концентраций на рост и развитие ирги этого сорта.

Особый интерес вызвал сорт «Нортлайн», для которого наилучшие результаты были достигнуты уже при самой низкой концентрации BAP (0,5 мг/л): длина побега составила 6,32 ± 0,20 см, а количество – 8,0 ± 0,5 шт., что указывает на высокую морфогенетическую активность даже при умеренном гормональном воздействии.

Во всех вариантах контрольные образцы, культивируемые без гормонов, демонстрировали ограниченное развитие: побеги были короче (4,4-4,6 см), а их количество не превышало 3-4 штук, что подчёркивает необходимость экзогенной стимуляции при микроклональном размножении ирги.

Обсуждение

Первым этапом микроразмножения является отбор эксплантов и введение их в культуру [15]. На данном этапе важную роль играет стерилизация, так как именно она определяет успех дальнейшего культивирования. В проведённом исследовании наилучшие результаты показал второй вариант стерилизации (2 мин в 70% этаноле и 20 мин в 3% NaClO), обеспечивший высокий выход жизнеспособных стерильных эксплантов (80%). Аналогичные данные приводят Hernández-García (2021) и Hunková (2021), отмечавшие, что эффективность стерилизации древесных растений и представителей рода *Amelanchier canadensis* во многом зависит от времени экспозиции в растворах дезинфицирующих агентов [16,17]. Слишком короткая экспозиция (вариант 1) не обеспечивала полной стерильности, тогда как чрезмерная (вариант 3) приводила к некрозу тканей и потере регенерационной способности, что совпадает с общими представлениями о необходимости строгого баланса между дезинфекцией и сохранением жизнеспособности тканей в культуре *in vitro* [18]. Разный подход к подбору концентраций этих регуляторов объясняется спецификой их физиологического действия и функциональной ролью в регенерации. Цитокинины, в частности, BAP, активно используются при микроклональном размножении для стимуляции клеточного деления и индукции побегообразования [19]. Полученные результаты подтверждают ключевую роль экзогенных регуляторов роста в стимулировании морфогенеза у ирги канадской *(Amelanchier canadensis)* при микроклональном размножении. Установлено, что оптимальная концентрация цитокинина BAP варьирует в зависимости от сортовых особенностей, что согласуется с данными Pruski et al. (1990), подчеркивающими необходимость индивидуального подбора фитогормонов для различных генотипов ирги [20].

У сорта Нортлайн высокая эффективность при минимальной концентрации BAP (0,5 мг/л) может быть связана с повышенным уровнем эндогенных цитокининов, что также подтверждалось ранее в опытах Fengli Yang (2017) с сортами *Amelanchier alnifolia* [21]. Это указывает на возможность минимизации гормональной нагрузки при клональном размножении данного сорта, снижая риск фитотоксичности.

В то же время сорта Мартин и Слейт демонстрировали дозозависимое увеличение числа побегов, достигая пика при BAP 1,0 мг/л. Повышение концентрации до 2,0 мг/л, напротив, оказывало ингибирующее действие на рост и ветвление, что вероятно связано с нарушением гормонального баланса и возникновением стресс-реакции у тканей. Подобные наблюдения сделаны Раева-Богословской (2020), которые описывают угнетение побегообразования при избытке цитокининов в культуре Amelanchier Medik. [22]. Сходные сортоспецифические различия в отклике на BAP и GA_3 были ранее зафиксированы у других плодовых культур. Так, у голубики (Vaccinium corymbosum) низкие концентрации BAP обеспечивали максимальное количество побегов, тогда как более высокие дозы вызывали витрификацию [23]. Наличие GA_3 в концентрации 0,5 мг/л обеспечивало улучшение морфологии побегов и их удлинение, не влияя напрямую на количество побегов. Это согласуется с общепринятыми представлениями о роли гиббереллинов в стимуляции клеточного удлинения без индукции меристематической активности [24]. У смородины *(Ribes nigrum)* отмечены различия между сортами по чувствительности к цитокининам, что требовало индивидуальной корректировки протоколов [25]. У жимолости *(Lonicera caerulea)* добавление GA_3 также способствовало удлинению побегов, улучшая морфологию, но не увеличивая их число [26].

Реакция разных сортов ирги на гормоны BAP и GA_3 варьируется из-за их эндогенного гормонального статуса, чувствительности тканей и физиологической адаптации. У каждого сорта свой базовый уровень собственных гормонов и разная плотность рецепторов к ним, что влияет на силу ответа. Это объясняет, почему для сорта «Нортлайн» эффективна низкая доза BAP, а для «Мартина» и «Слейта» требуется более высокая концентрация.

Заключение

Таким образом, проведённые исследования позволили оптимизировать протокол микроклонального размножения для трёх сортов *Amelanchier canadensis.* Показано, что эффективность побегообразования носит сортоспецифический характер. Ингибирующее действие на морфогенез при превышении концентрации BAP выше 1,0 мг/л подчёркивает сортоспецифичность и критическую важность точного подбора оптимальной дозы регуляторов роста для каждого генотипа.

Данная методика имеет высокую прикладную ценность, обеспечивая коэффициент размножения 6–8 микропобегов на эксплант за цикл. При этом морфогенетический потенциал культуры сохраняется на высоком уровне в течение шести последовательных циклов субкультивирования, что гарантирует стабильный и прогнозируемый выход посадочного материала.

Предложенный протокол микроклонального размножения обладает высокой прикладной и экономической значимостью для коммерческого садоводства и питомниководства. Технология обеспечивает формирование в среднем 6-8 микропобегов на эксплант за один цикл при сохранении морфогенетического потенциала на протяжении шести последовательных субкультивирований. Это гарантирует стабильный коэффициент размножения и позволяет существенно сократить сроки получения необходимого объёма посадочного материала.

С экономической точки зрения внедрение протокола способствует снижению себестоимости производства за счёт уменьшения потребности в маточных растениях и сокращения трудоёмкости традиционных вегетативных методов. Таким образом, разработанная технология может рассматриваться как эффективное и перспективное решение для широкого применения в практике промышленного размножения сортов ирги.

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Б.Х. и **М.Н. –** осуществили концептуализацию и планирование исследования, провели всесторонний обзор научной литературы, выполнили анализ полученных данных и подготовили первоначальный вариант рукописи. **Т.Б.** и **Х.А.** – предоставили научные консультации по вопросам применения регуляторов роста и оказали методологическую поддержку при разработке экспериментального дизайна. **Б.Х.** и **М.Н.** – выполнили окончательную редакцию и литературное оформление рукописи.

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Соблюдение этических норм

Данная статья не включает описание исследований, проведённых с участием людей или с использованием животных в качестве объектов экспериментальных исследований.

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Канадалық ирганың *(Amelanchier canadensis) in vitro* микрокөбеюі: өркендердің индукциясы үшін цитокинді-акусиндік комбинацияларды оңтайландыру

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Аңдатпа. Мақалада *Amelanchier canadensis* үш сортын *in vitro* жағдайында микрокөбейту шарттарын оңтайландыру бойынша зерттеу жұмыстары жүргізілген. 6-бензиламинопуриннің (БАП) әртүрлі концентрациялары енгізілген Мурасиге және Скуг модификацияланған қоректік ортасының эксплантардың морфогенетикалық белсенділігіне дифференциялды әсер ететіні анықталды. Martin және Slate сорттары үшін 1,0 мг/л ВАР және GA_3 – 0,5 мг/л концентрациясында пайдалану ең тиімді комбинация, ал Northline сорты үшін оңтайлы концентрация 0,5 мг/л ВАР және 0,5 мг/л GA_3 болды. ВАР концентрациясының 1,0 мг/л-ден жоғарылауы түзілген микро өсінділердің ұзындығының да, санының да төмендеуіне әкелетіні көрсетілген. Алынған нәтижелерді ирганың осы сорттарын жаппай көбейту және сақтау үшін пайдалануға болады.

Түйін сөздер: *Amelanchier Canadensis,* 6-бензиламинопурин, нафтилсірке қышқылы, гиббереллин қышқылы, микро өсінділер, микрокөбейту, фотокезең

In vitro micropropagation of Canadian serviceberry *(Amelanchier canadensis)*: optimization of cytokinin-auxin combinations for shoot induction

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Abstract. The article presents a study on optimizing the conditions for micropropagation of three varieties of *Amelanchier canadensis in vitro.* It was found that modification of the Murashige and Skoog nutrient medium by introducing 6-benzylaminopurine (BAP) in various concentrations has a differentiated effect on the morphogenetic activity of explants. The most effective combination for Martin and Slate varieties was the use of BAP at a concentration of 1.0 mg/l and GA_3 – 0.5 mg/l, while for the Northline variety the optimal concentration was 0.5 mg/l BAP and 0.5 mg/l GA_3. Increasing the BAP concentration above 1.0 mg/l led to a decrease in both the length and the number of microshoots. The results obtained can be applied to large-scale propagation and conservation of these varieties of serviceberry.
Keywords: 6-benzylaminopurine, naphthylacetic acid, gibberellic acid, microshoots, micropropagation, photoperiod

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Research article

Optimization of expression and purification of recombinant Apollo *(SNM1B)* protein using affinity chromatography

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Abstract. Apollo, also referred to as SNM1B, is a nuclease involved in telomere stability and DNA repair, and its dysfunction is associated with the development of a number of pathologies, including hereditary kidney cancer. The growing interest to this protein is due to its possible use as a target for antitumor therapy. The present study focuses on the optimization of the expression and isolation of the homogenous recombinant Apollo (SNM1B) WT protein by means of affinity chromatography purification. Expression vector constructs pET28c-hSNM1BcoCut and pETHSUL-hSNM1BcoCut were used to produce the protein after their transformation into *Escherichia coli (E. coli)* Rosetta2 (DE3) and NovaXG strains. Variation in the induction parameters such as concentration of IPTG, incubation temperature, presence of detergent NP-40, glycerol, urea and duration of the post-induction incubations all were subjected to rigorous testing, in order to ascertain the optimal conditions for the obtaining soluble recombinant proteins. The purification process was conducted through the utilization of Ni-NTA affinity chromatography. The purity and solubility of the protein were evaluated by SDS-PAGE. The obtained data allowed to determine effective conditions for producing the soluble and highly homogenous Apollo protein suitable for further studies, such as protein-protein interaction, DNA binding, immunization to obtain specific antibodies, and utilization for screening of inhibitors for cancer therapy.
Keywords: DNA repair, Apollo, SNM1B, renal cell carcinoma, DNA damage, TRF2 protein

Introduction

The stability of the human genome is constantly challenged by damage to cellular DNA by various endogenous and exogenous sources. These include normal by-products of oxidative

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metabolism, UV and ionizing radiation, chemical mutagens, and viral infectious agents. DNA damage such as double-strand breaks, interstrand DNA cross-links, depurination, and deamination can cause genetic mutations and ultimately compromise genome integrity and cellular homeostasis [1-3]. To preserve genetic stability and ensure the accurate transfer of hereditary information, cells employ multiple DNA repair pathways to remove lesions from cellular genome. These encompass excision repair, homologous recombination, non-homologous end joining (NHEJ), and specialized mechanisms responsible for the removal of interstrand DNA cross-links (ICL). Central to these processes are numerous enzymes exhibiting nuclease, helicase, and ligase activities, which cooperate to restore DNA structure and function [4-8].

Among them, Apollo, encoded by the *SNM1B* gene, belongs to the SNM1 family of DNA nucleases and performs a 5'→3' exonuclease function that is essential for proper DNA end resection during replication, telomere protection, double-strand break (DSB) repair, and ICL unhooking [9-12]. Similar to other SNM1 family, Apollo contains a β-CASP domain that forms the enzyme's catalytic center. This structural domain binds divalent metal ions, most commonly Zn^{2+}, which are required for phosphodiester bond cleavage. The β-CASP domain is therefore critical for Apollo's nuclease function, ensuring accurate substrate interaction and efficient catalysis [13-15].

Apollo has an essential role in the protection of telomeric DNA from its misrecognition as a DSB site by the repair system. This is achieved by Apollo interacting with components of the "shelterin" telomere complex, in particular the TRF2 protein. Apollo is involved in the replication of the telomere leader chain. It promotes the formation of the protective telomere structure, the T-loop. Loss of SNM1B function or disruption of its expression can lead to telomere shortening, chromosomal instability and chromosome fragmentation, which may be associated with proliferative and oncological diseases [16-18].

The data collected to date suggest that Apollo *(SNM1B)* may be involved in the pathogenesis of a number of different types of cancer, including kidney cancer, particularly the renal cell carcinoma (RCC). RCC is characterized by high genetic heterogeneity, alterations in cell cycle regulation, apoptosis and DNA damage response and is the most common form of kidney cancer [19]. Although mutations in *SNM1B* are relatively rare in the tumor genome, transcriptomic studies suggest that the expression of *SNM1B* is suppressed or abnormally regulated in certain subtypes of RCC, in particular in tumors with marked telomere dysfunction and high levels of chromosomal instability. These data suggest that functional *SNM1B* deficiency may be involved in tumor progression through accumulation of mutations and genomic rearrangements [20,21].

The additional importance of Apollo (*SNM1B*) in carcinogenesis is underlined by its role in the elimination of ICLs, one of the most cytotoxic types of DNA damage, which occurs particularly under the action of chemotherapeutic agents such as cisplatin and mitomycin C. This function makes Apollo a potential target for anticancer therapy: reducing *SNM1B* activity may increase the sensitivity of tumor cells to drugs, whereas in healthy tissues its function is critical to protect normal cells against genotoxic damage [22-25].

Although the Apollo protein is of great scientific interest, it is difficult to characterize its repair activities *in vitro.* This is due to the problems associated with obtaining the soluble protein in recombinant form. The Apollo protein, like many other eukaryotic enzymes, has a low solubility, a tendency to aggregate, the formation of inclusion bodies in the prokaryotic expression system of *Escherichia coli,* and a susceptibility to proteolysis and denaturation. Another obstacle is the toxicity of the *SNM1B* gene product to the host bacterial cell, since the recombinant protein can cleave bacterial DNA when overexpressed, especially in the absence of controlled regulation of expression and translation [26-30]. Therefore, optimization of the expression of *SNM1B* gene product in the *Escherichia coli* system becomes a high priority, including: selection among available host strains (e.g. BL21(DE3), Rosetta, C41); optimization of induction conditions

(temperature, time, IPTG concentration); construction of the expression vector taking into account rare codons and accessibility of the translation initiation site; addition of soluble fusion tags (MBP, GST, Trx, SUMO); modulation of expression using weak or inducible promoters (e.g. Lac, T7).

Materials and research methods

Molecular cloning, expression, and purification of recombinant proteins

In this study, two commercially available Escherichia coli strains were used for cloning and protein expression. For plasmid construction and propagation, the *E. coli* strain NovaXG purchased from Novagen (Merck Chimie SAS, Fontenay sous Bois, France), was used for plasmid construction because of its recombination-deficient and endonuclease-deficient background, which facilitates high-yield plasmid DNA preparation. The Rosetta 2 (DE3) strain purchased from Millipore was used for recombinant protein expression. This strain is engineered to enhance the expression of eukaryotic proteins by supplying tRNAs for rare codons. It also contains the DE3 lysogen for T7 RNA polymerase expression.

To express the codon-optimized *SNM1B* gene (Apollo) in a prokaryotic expression system, a pETHSUL/hSNM1B plasmid construct containing an *hSNM1B* fragment with an N-terminal His6 tag was used for subsequent protein purification by affinity chromatography. The construct was assembled using standard molecular cloning techniques and verified by DNA sequencing. The hSNM1B gene insertion started with an ATG codon followed by a site encoding a poly-histidine tag: ATGGGTCATCATCACCATCATCATCATCAC (corresponding to the His6 tag). The nucleotide sequence of the insert (from the expression site to the stop codon) was 1983 nucleotides and included a codon optimized version of *hSNM1B* designed with *E. coli* codon preferences and a terminator sequence (lower case). The construct (pET-type vectors) contained EcoRI and BamHI restriction sites flanking the hSNM1B gene. For mutagenesis and subcloning of *hSNM1B* coding DNA sequence (cDNA), several primer sets were designed and used: N246I, Y273H (a point mutation in which tyrosine at position 273 is replaced by histidine). These amino acid substitutions in the Apollo protein sequence will be important for studying the function of the protein and for creating mutant variants. To introduce these mutations, oligonucleotide primers are used Fw-SNM1B-N246I d(CATGCTGCGTTGG**AT**CCAGACCCACCCTACG) and Fw-SNM1B-Y273H d(CCACGTCATCCCT**C**ACTCTGACCATTCCTC), which ensure the introduction of specific changes in the coding sequence of the *SNM1B* gene.

The first step of site-directed mutagenesis is to perform PCR in the presence of the following reagents: 1 x PfuUltra Buffer, 0.25 μM of primers, 200 μM of each dNTP, 1-25 ng matrix DNA, 2.5 U of PfuUltra high-fidelity DNA polymerase, and nuclease-free water. The fully mixed reagents were transferred to a thermocycler. Thermocycling conditions for PCR: denaturation at 98°C - 5 min, 22 cycles of amplification at 98°C - 30 sec, 59°C (depending on primers used) – 10-30 sec, 72°C - 30 sec per kb (depending on the length of cDNA) and final elongation at 72°C for 10 min, followed by a pause at 4°C.

The second step involves DpnI (KLD) treatment. 10μl of PCR product is incubated in a tube containing 1 x reaction buffer and 1U of DpnI. Cleavage of the PCR product by DpnI is an important part of the work. DpnI cuts only a methylated site, cleaving the template plasmid but not the newly synthesized PCR-generated product, which allows selection of transformed cells with a plasmid that has undergone site-directed mutagenesis.

Preparation of competent cells

To prepare competent cells, single colony from fresh plate was inoculated in 30 ml of S.O.C. (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM $MgSO_4$ and 20 mM glucose) and grown overnight at 37°C 200 rpm until OD_{600} ~ 0.4. The following steps

were carried out at 4°C. Cell culture was centrifuged at 4000 rpm for 20 min at 4°C. Then the cell pellet was washed with 0.5 Vol of sterile 10% glycerol. After centrifugation at 4000 rpm, 20 min, 4°C, the cell pellet was washed with 0.5 Vol of sterile 10% glycerol. The next centrifugation step is followed by washing of the pellet with 0.1 Vol 10% glycerol. After the last centrifugation, 0.5-1.0 ml of sterile 10% glycerol was used to resuspend the cell pellet. Cell suspension was aliquoted by 100 µL in pre-cooled 1.5 ml Eppendorf tubes, which were frozen immediately in dry ice and stored at -80°C.

Transformation of E. coli cells

For protein expression, *E. coli* Rosetta 2 (DE3) chemically competent cells (Millipore, Cat. No. 71397-0.2ML) were used for heat shock transformation. Plasmid DNA (pET28c-hSNM1BcoCut, 84 ng/µl; pETHSUL-hSNM1BcoCut, 75 ng/µl) was added to 50 µl of thawed competent cells on ice and gently mixed. After incubation on ice for 30 minutes, cells were heat-shocked at 42°C for 45 seconds, then immediately placed back on ice for 2 minutes. 450 µl of SOC medium was added, and cells were incubated at 37°C with shaking at 200 rpm for 1 hour. Following recovery, 100-200 µl of cell suspension was plated on pre-warmed LB agar plates containing appropriate antibiotics and incubated overnight at 37°C.

Electroporation NovaXG was purchased from Novagen (Merck Chimie SAS, Fontenay sous Bois, France). For plasmid propagation, electroporation was performed using E. coli NovaXG electrocompetent cells. Plasmids (pET28c/hSNM1BcoCut and pETHSUL-hSNM1BcoCut) were added (50-100 ng) to 25 µl of competent cells on ice and transferred into a chilled 1 mm-gap electroporation cuvette. Electroporation was carried out using a Gene Pulser (Bio-Rad) under the following conditions: 1.8 kV, 200 Ω, 25 µF. Immediately after electroporation, 350 µl of SOC medium (37 °C) was added to the cuvette. The cells were gently mixed, transferred to a sterile tube, and incubated at 37 °C with shaking (200 rpm) for 1 hour before plating on LB agar containing antibiotics.

Expression and purification of the hSNM1BcoCut WT protein

E. coli Rosetta2 (DE3) cells (Millipore, 71397-0.2ML) transformed with the expression plasmids pET28c/hSNM1BcoCut WT and pETHSUL/hSNM1BcoCut WT were grown in 2L LB medium supplemented with 50 µg/ml kanamycin or ampicillin at 30°C with vigorous shaking until the optical density at 600 nm (OD_{600}) reached 0.6-0.8. The temperature was then reduced to 14°C, and protein expression was induced by the addition of 0.2 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). The 2L cultures were incubated for an additional 16 hours at 14°C. all subsequent purification steps were carried out at 4°C. Bacterial cells were harvested by centrifugation and lysed using a French press at 18 000 psi in 50 mL lysis buffer containing 20 mM HEPES-NaOH (pH=8.0), 500 mM NaCl, 10 mM Urea, and 0.025% Nonidet P-40, supplemented with a Complete™ protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Cell lysates were clarified by centrifugation at 30 000 × g for 1 hour at 4°C. The 40 mL supernatant was adjusted to 500 mM NaCl and 30 mM imidazole, then loaded onto a HiTrap Chelating HP column (GE Healthcare, Chicago, IL) at a flow rate of 0.5 mL/min. Bound proteins were eluted with a 15 mL 50-800 mM NaCl gradient in Buffer B2 (20 mM HEPES-NaOH, pH 8.0, 1000 mM NaCl, 10% Nonidet P-40) at a flow rate of 0.5 mL/min. The eluted fractions (0.5 mL) were analyzed by SDS-PAGE, and fractions containing pure *hSNM1BcoCut WT* protein were pooled and stored at -80°C in 50% glycerol.

Bradford Assay for Determining Protein Concentration

To quantify the protein concentration of Apollo fractions using bovine serum albumin (BSA) as a standard. BSA stock solution (1 mg/ml) was prepared by diluting 2 µl of 50 mg/ml BSA in 98 µl PBS. This standard was used to prepare a calibration curve by mixing 1-5 µl of 1 µg/µl BSA with 900 µl of PBS and 100 µl of 10x Bradford reagent (Bio-Rad, US), resulting in final concentrations of 1-5 µl of BSA per sample. A blank control was prepared by mixing 900 µl of PBS with 100 µl of 10x Bradford reagent. For experimental samples, 2 or 4 µl of protein was added to 900 µl of PBS, followed by 100 µl of 10x Bradford reagent. All tubes were inverted 10 times to ensure proper mixing and incubated at room temperature for 5 minutes. The solutions were then transferred into 1 ml plastic cuvettes.

The spectrophotometer (Eppendorf BioPhotometer, Germany) was calibrated using the blank control. Then, the absorbance of standard and sample solutions was measured at 595 nm. Based on the absorbance of standard values of BSA standards, an average calibration factor of 0.115 A_{595}/µg was calculated and used to determine protein concentrations in the experiment samples.

Protein analysis by SDS-PAGE

SDS-PAGE was performed using an XCell4 SureLock Midi system (Invitrogen, USA) with a 1 mm thickness on a 10% Bistris Midi Protein Gel (for pure protein fractions) or 4-12% Bistris Midi Protein Gel (for the fractions recovered during chromatography). NuPAGE MES SDS (Invitrogen, USA) was used to premix the running buffer. Four times sample buffer (Invitrogen, USA) and reducing agent, if necessary, were diluted to create the samples, which were then incubated for ten minutes at 75°C. Each well received the equivalent of 20-25 µg of protein for normalized uniform loading. Following incubation in 100 ml of Coomassie solution (0.1% Coomassie R-250 in 40% ethanol, 10% acetic acid), the electrophoresis gel was carefully heated in the oven and then gently shaken on an orbital shaker for 15 minutes at room temperature. The stain was then decanted, and deionized water was used to rinse the gel once. The gel was heated in a destaining solution (10% ethanol and 7.5% acetic acid) to produce the desired background.

Preparation of Cy5-labelled Oligonucleotides

To track enzymatic cleavage, oligonucleotides were tagged with the fluorescent dye Cy5 attached to their 3' ends. Following the labeling processes. The labelled oligonucleotides were purified on a Sephadex G-25 column equilibrated with water. Oligonucleotide duplexes were constructed by annealing the non-radioactively labelled strand in a solution containing 20 mM HEPES-KOH, pH 7.6, and 50mM KCl. The mixture was heated at 80°C for 5 minutes before gradually cooling to 4°C. Cy5-labelled oligonucleotide (10µM) cALi-1403-Cy5 and complementary oligonucleotide. Annealed duplex was kept in small aliquots at -20°C to avoid freeze-thaw cycles.

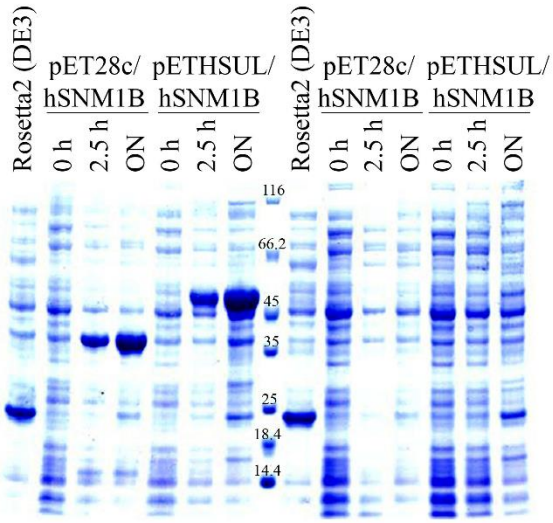
Results

The pET28c-hSNM1B and pETHSUL/hSNM1B expression vectors were transformed into *E. coli* NovaXG to purify recombinant plasmid DNAs. Plasmid DNA was isolated according to the GeneJET plasmid MidiPrep Kit (Thermo Scientific) protocol, and the transformants were verified for the presence of recombinant plasmids by restriction digestion and PCR analysis.

Subsequently, the plasmids were transformed into *E. coli* strain Rosetta2 (DE3), which carries a chromosomal copy of the gene encoding the T7 RNA polymerase under the control of

the lacUV5 promoter. Rosetta2 (DE3) is derived from the *E. coli* BL21 strain and is optimized for enhanced expression of eukaryotic proteins through the additional plasmid pRARE2, which supplies tRNAs for rare codons such as AGG, AGA, AUA, CUA, CCC, and GGA – codons frequently found in eukaryotic genes but uncommon in E. coli.

Induction was performed with 0.2 mM IPTG at +30°C, and after cells were grown to OD600=0.4 at +18°C. Two protein forms were expected: His-hSNM1B (~38.7 kDa) and SUMO/hSNM1B (~48.7kDa). Strong expression of a 38-40 kDa protein was detected in cells carrying pET28c/hSNM1B after 2.5 hours and overnight induction, whereas a strong band of 48-50 kDa was observed in cells containing pETHSUL/hSNM1B under the same conditions. The absence of the target protein before IPTG induction (0 hours) confirmed IPTG-dependent tight regulation of expression (Figure 1).



Note: Total protein samples were collected before induction (0h), after 2.5h, and following overnight induction with 0.2 mM IPTG. Lanes 1-3: E. coli expressing pET28c/hSNM1B; Lanes 4-6: E. coli expressing pETHSUL/hSNM1B. Mw markers: 14.4 kDa-116 kDa. Prominent bands at approximately 38-40 kDa and 48-50 kDa correspond to the expected His-hSNM1Bcut and SUMO-hSNM1Bcut fusion proteins, respectively. Molecular weight (Mw) indicators range from 14.4 to 116 kDa (Thermo Fisher Scientific PageRulerTM).

Figure 1. Expression of recombinant *hSNM1B* in *E. coli* Rosetta 2 (DE3) using pET28c and PETHSUL vectors

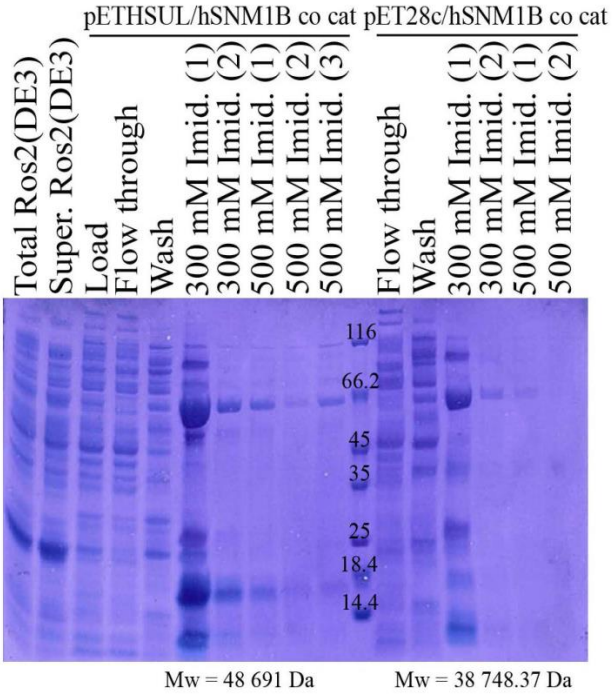
The expression of the recombinant *hSNM1B* protein in *E. coli* Rosetta 2 (DE3) was performed using the *pET28c/hSNM1B* and *pETHSUL/hSNM1B* vectors. Following IPTG induction, cell lysate samples were taken at three time points: before induction (0 hours), after 2.5 hours, and after overnight incubation (ON). SDS-PAGE was used to separate the proteins, and Coomassie staining was applied to visualize the protein bands in the gel. A band with a molecular mass of about 38.7 kDa, which corresponds to the hSNM1B protein with an N-terminal His6-tag, was detected in cell lysates that contained the *pET28c/hSNM1B* construct. A band of around 48.7 kDa, which represents the *hSNM1B* protein fused to the SUMO-tag, was been in bacterial cells containing

the *pETHSUL/hSNM1B* construct. The effectiveness of expression regulation is confirmed by the target band's appearance after and its absence prior to induction. The accumulation of recombinant protein is indicated by the band's increased intensity with extended induction. The presence of extra bands implies the need for additional, more extensive purification since they show concurrent proteins in the cell lysate (Figure 1).

This study compares the expression and purification efficiency of the human *hSNM1B* protein utilizing two distinct expression vectors, pET28c and pETHSUL in E. coli Rosetta2 (DE3) cells (Figure 2). SDS-PAGE analysis demonstrated that both constructs facilitated the successful overexpression of recombinant hSNM1B, exhibiting distinct bands at approximately 38.7 kDa for *pET28c/hSNM1B* and 48.7 kDa for *pETHSUL/hSNM1B*, aligning with the molecular weights of the His6-tagged and SUMO-fused proteins, respectively. Since most of the target protein was eluted at 500 mM imidazole, it was clear that the N-terminal His6-tag was strongly binding to the nickel affinity resin. Nevertheless, copurified contaminating proteins were also present in the elution fractions, indicating that a single affinity purification step is not enough to achieve high purity. According to other research, additional purification steps such as size-exclusion chromatography or a heparin affinity column may be required to obtain homogenous proteins with enzymatic activity towards DNA substrates. Remarkably, the SUMO-fusion construct *(pETHSUL/hSNM1B)* produced a more pronounced and distinct band as compared to that of the pET28c construct, indicating improved expression level and solubility. This conclusion is in line with earlier research that demonstrated that, in bacterial expression systems, SUMO fusion tags frequently enhance protein folding and solubility. The presence of a solubilizing fusion partner like SUMO probably helped to boost the amount of soluble protein because hSNM1B has several hydrophobic patches and predicted secondary structural components that can encourage aggregation (Figure 2).

Furthermore, the use of the Rosetta2 (DE3) strain, which provides tRNAs for rare codons, likely enhanced the production of this eukaryotic protein, consistent with prior findings on expression optimization in similar E. coli strains. Overall, the pETHSUL vector demonstrated superior solubility and expression yield, even though both constructs successfully enabled the recombinant *hSNM1B* protein production in E. coli. Future work will focus on additional purification and tag removal.

Next, we attempted to perform functional assays of the purified protein, such as measuring 5'→3' exonuclease activity toward fluorescently-labelled DNA duplex substrates. In vitro DNA exonuclease degradation assays were performed using Cy5-labelled DNA duplexes (cALi-1404-Cy5/G27 ApA) at 37°C for 30 minutes to assess the exonuclease activity of the recombinant *hSNM1B* protein. The substrates were incubated with varying concentrations of purified *hSNM1B*, and the reaction products were analyzed by denaturing Urea-PAGE followed by fluorescence detection (Figure 3). Lambda exonuclease (1-5U) (purchased from New England BioLabs) served as a positive control and, as shown in Figure 3, efficiently degraded the Cy5-labelled substrates, validating the reliability of assay conditions and the accessibility of DNA substrates to exonucleolytic 5'→3' degradation.



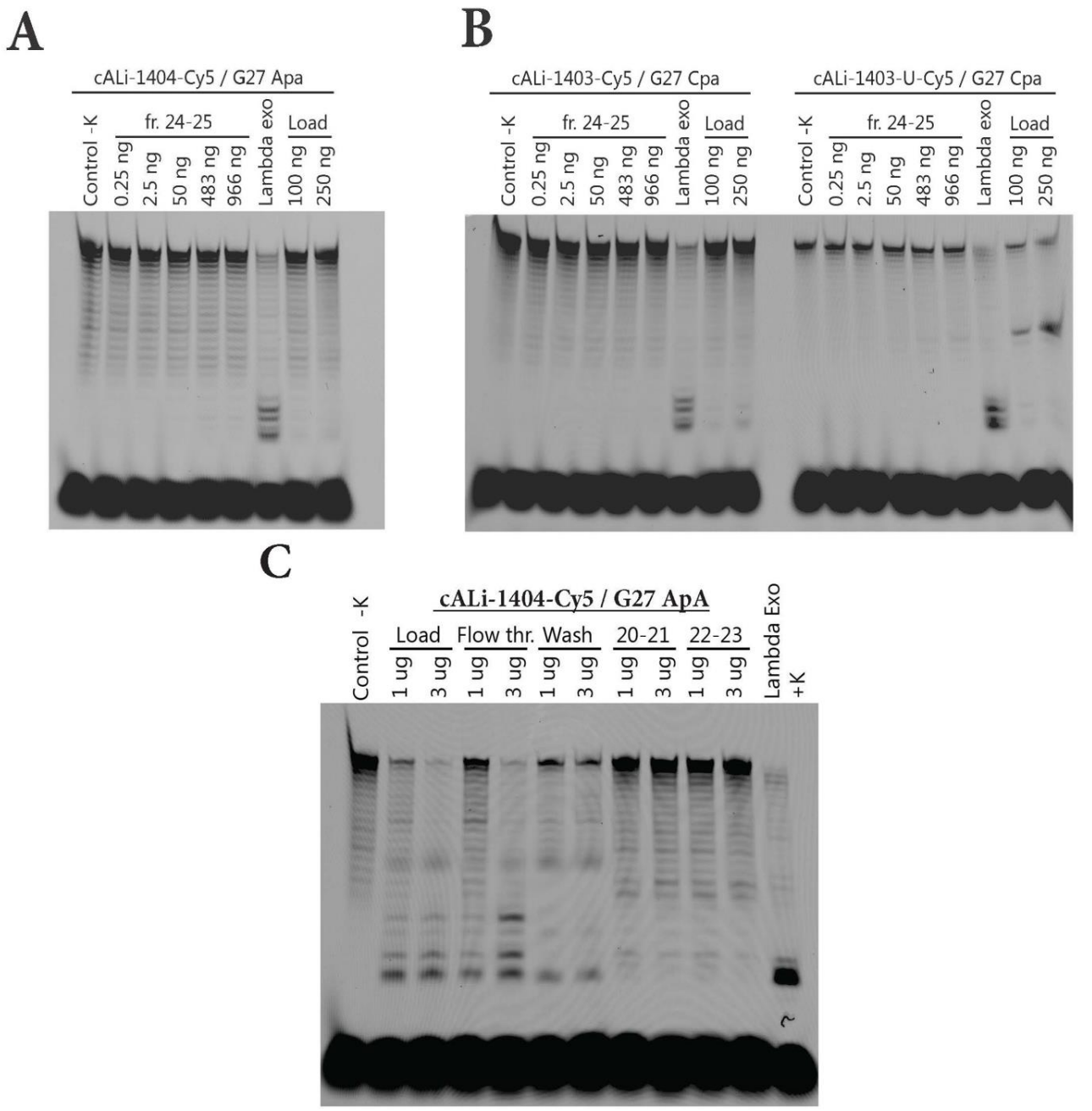
Note: Total lysate, soluble fraction, column load, flow-through, wash, and elution fractions were analyzed by SDS-PAGE. Samples correspond to E. coli Rosetta2 (DE3) cells expressing hSNM1Bcut from pETHSUL and pET28b vectors. Proteins were eluted with imidazole at concentrations of 300-500mM. Distinct bands of ~38.7 kDa (His-hSNM1Bcut) and ~48.7 kDa (SUMO-hSNM1Bcut) were observed in the elution fractions, indicating efficient expression and purification. Mw markers: 14.4-116 kDa (#26610, Thermo Fisher Scientific PageRulerTM).

Figure 2. Purification of recombinant *hSNM1B* proteins from *E. coli* Rosetta2 (DE3) using HisTrap HP columns

In contrast to λ nuclease, the samples incubated with the recombinant *hSNM1B* protein under the standard conditions did not exhibit any degradation of the labelled substrates. No lower-molecular-weight DNA fragments were observed, and the Cy5'-emitted fluorescence signal associated with the full-length substrate remained constant across all tested protein concentrations (2.5 ng to 250 ng). The negative control lanes, which lacked enzyme, confirmed that the substrates were stable and not subject to spontaneous or non-enzymatic degradation.

The presence of intact, full-length fluorescently labelled 27-mer DNA duplex in enzyme-free controls confirmed that any observed degradation would arise solely from enzymatic activity rather than from non-specific breakdown or contamination. As expected, the positive control (λ exonuclease) produced complete digestion of the DNA substrates, confirming the accuracy of the assay and its functionality.

Interestingly, the DNA substrates showed minor variations in degradation susceptibility. The cALi-1404-Cy5/G27 ApA substrate appeared more degraded at lower enzyme concentrations compared with cALi-1403-Cy5/G27 CpA and the modified cALi-1403-U-Cy5/G27 CpA substrate. These differences may reflect DNA sequence or structural variations (e.g., base modifications or secondary structures) that influence *hSNM1B*'s ability to initiate or extend 5′ → 3′ exonucleolytic cleavage. Specifically, the reduced degradation efficiency of the uracil-containing cALi-1403-U-Cy5 construct may indicate impaired recognition or processing efficiency.



Note: Lane labels indicate input load, flow-through, wash fractions, and positive control with λ-exonuclease. Negative controls (–K) confirm substrate stability in the absence of enzyme.

Figure 3. Exonuclease activity assay of the recombinant *hSNM1B* protein using 27-mer Cy5-labeled DNA duplexes. A) Cleavage of cALi-1404-Cy5/G27 ApA, B) cALi-1403-Cy5/G27 CpA, and cALi-1403-U-Cy5/G27 CpA duplexes incubated with increasing concentrations of purified *hSNM1B* (fractions 24–25). C) Cleavage of cALi-1404-Cy5/G27 ApA duplex incubated with increasing concentrations of purified *hSNM1B* (fractions 20–23)

Although the recombinant *hSNM1B* protein did not display measurable exonuclease activity under the current experimental conditions, the data confirm the production of soluble and

structurally intact protein, which represents a key result for further optimization. Future assays should explore alternative metal cofactors (e.g., Zn2+, Mg2+) modified buffer compositions, or DNA substrates that better mimic telomeric or cross-linked DNA structures known to be processed by SNM1B/Apollo.

Our results corroborate the solubility of purified *hSNM1B* proteins and validate fluorescently labeled synthetic DNA duplexes as substrates for 5'→3' DNA processing exonucleases. Also, the substrate-specific variations imply that, consistent with its documented role in DNA repair pathways, *hSNM1B* activity may depend on specific DNA sequence or base modification in the damaged duplex. Our knowledge of the substrate specificity and mechanism of *hSNM1B* will be improved by further biochemical investigations and comparison of the wild-type enzyme with its mutant variants.

Discussion

In this study, we optimized the expression and purification of the recombinant human nuclease Apollo (SNM1B), which plays a key role in telomere maintenance and DNA repair [15]. Our results showed that both the pet28c-*hSNM1B* and *pETHSUL/hSNM1B* constructs enabled robust protein expression in E. coli Rosetta2 (DE3) cells, albeit with significant variations in solubility and yield. Specifically, the SUMO-fusion construct (pETHSUL/hSNM1B) produced a stronger, more defined band (~48.7 kDa) on SDS-PAGE than the His6-tagged construct (~38.7 kDa), indicating improved folding and solubility (Figures 1 and 2). This finding is consistent with previous studies showing that SUMO tags often improve the solubility and stability of eukaryotic proteins in bacterial systems [31,32].

Although both expression vector constructs produce the recombinant protein, which binds efficiently to the Ni-NTA resin, the presence of co-purifying bacterial host proteins indicates that a single affinity step is insufficient to achieve high purity (Figure 2). This limitation has also been reported in studies of other SNM1-family nucleases, where additional purification steps such as size-exclusion chromatography or heparin affinity chromatography were required to obtain homogenous preparations suitable for biochemical analysis [7,9]. Thus, while we successfully established baseline purification conditions, further refinement will be essential for structural and biochemical investigations.

Functional assays using Cy5-labeled DNA duplexes revealed no detectable exonuclease activity of the recombinant *hSNM1B* protein under the conditions used (Figure 3). In contrast, Lambda exonuclease efficiently degraded the same DNA duplex, confirming the validity of the DNA substrate and assay. Several factors may explain this discrepancy. First, Apollo may require post-translational modifications or specific protein - protein interactions, such as those with TRF2 in the "shelterin" complex, to achieve its native activity [15,17]. Co-expression of these partners with Apollo or expression of the human nuclease in eukaryotic systems, such as Sf9 insect cells, HEK293 mammalian cells, or kidney-derived HKC8 epithelial cells, could restore the appropriate folding and post-translational modification states required for enzymatic activity. Second, the divalent cation dependency is a well-established feature of the SNM1 family of nucleases [8,11]. In the current assay, we used Mg2+ as the metal ion cofactor, but varying the type and concentration of metal cofactors (e.g., Mn2+, Co2+, Zn2+) or optimizing buffer conditions (pH, ionic strength, reducing agents) may enhance the catalytic performance of the Apollo enzyme. Third, substrate design could significantly influence the DNA nuclease activity. Although the Cy5-labeled duplexes contained CpA and ApA dinucleotide contexts, they may not fully replicate the physiologically relevant DNA substrates of SNM1B. Testing more complex substrates – such as

G-rich telomeric sequences, gapped or cross-linked DNA, or structures containing abasic sites – could provide better insight into the DNA substrate preference of Apollo.

Notably, DNA substrate-specific variations were observed, with ApA-containing duplexes showing relatively higher susceptibility to degradation compared to CpA or uracil-modified substrates (Figure 3). This observation supports prior reports that SNM1 nucleases exhibit sequence- and damaged base-context dependent activity [6,12].

In summary, this work establishes a strong experimental procedure for the production of the soluble human recombinant *SNM1B* protein in *E. coli,* with the SUMO-fusion strategy providing clear advantages in solubility and yield. Although enzymatic activity was not detected, the results reveal crucial experimental details that must be improved further - namely, expression host, metal cofactor composition, and DNA substrate design. Future work employing the eukaryotic expression system, or co-expression of shelterin components, coupled to systematic divalent cation testing, will be instrumental for reconstituting the active SNM1B protein to advance biochemical and structural studies. These studies will ultimately help to explore the SNM1B-catalyzed activities as potential targets for cancer therapy [6,23].

Conclusion

In summary, although the human recombinant *hSNM1B* protein did not display detectable enzymatic activity under the conditions used, a significant achievement of this study was the successful optimization of its expression and purification as a soluble protein from E. coli. Overcoming the common challenge of producing soluble human proteins in bacterial systems represents an important step toward further biochemical and structural characterization of DNA repair activities of *hSNM1B*.

The lack of observable activity may result from the absence of post-translational modifications, the use of non-specific DNA substrates, or the requirement for specific cofactors or interacting partners. Nevertheless, the established protocol for efficient expression and purification provides a robust foundation for subsequent biochemical studies aimed at refining reaction conditions, exploring eukaryotic expression systems, and testing physiologically relevant DNA substrates. Together, these efforts will facilitate a deeper understanding of the catalytic properties and biological role of *hSNM1B*, as well as its potential role in genome stability and cancer biology.

Author Contributions

A.A.A., A.M.T., U.B.S., K.O.S., M.K.S., and **S.M.T.** – conceptualization; **A.A.A., A.M.T.,** and **U.B.S.** – scientific design development; **A.A.A., A.M.T., U.B.S., K.O.S., M.K.S.,** and **S.M.T.** – implementation of the declared scientific research.

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Conflicts of Interest

Authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Оптимизация экспрессии и очистки рекомбинантного белка Apollo *(SNM1B)* с использованием аффинной хроматографии

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Аннотация. Apollo (также известный как *SNM1B*) – нуклеаза, участвующая в стабилизации теломер и репарации ДНК, и его дисфункция связана с развитием ряда патологий, в том числе почечно-клеточной карциномы. Внимание к этому белку усилилось в связи с его потенциальной функцией в качестве мишени для противоопухолевой терапии. Настоящее исследование посвящено оптимизации экспрессии и очистки рекомбинантного белка Apollo (SNM1B) WT с помощью аффинной хроматографии. Для производства белка были использованы экспрессионные конструкции *pET28c-hSNM1BcoCut* и *pETHSUL-hSNM1BcoCut,* трансформированные в штаммы *Escherichia coli (E. coli)* Rosetta2 (DE3) и NovaXG. Вариации параметров индукции, таких, как концентрация IPTG, температура инкубации, присутствие детергента NP-40, глицерина, мочевины и продолжительность инкубации после индукции, были подвергнуты тщательному тестированию с целью определения оптимальных условий для получения растворимых рекомбинантных белков. Процесс очистки проводился с использованием аффинной хроматографии Ni-NTA. Чистота и растворимость белка оценивались с помощью SDS-PAGE. Полученные данные позволили определить эффективные условия для получения функционального и высокоочищенного белка Apollo, пригодного для дальнейших биохимических исследований, включая скрининг ингибиторов для онкотерапии.

Ключевые слова: репарация ДНК, Apollo, *SNM1B,* почечно-клеточная карцинома, повреждение ДНК, белок TRF2

Apollo *(SNM1B)* рекомбинантты белогының экспрессиясын және тазалығын аффинді хроматография әдісімен оңтайландыру

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Аңдатпа. Apollo *(SNM1B* деп те аталады) – теломерлердің тұрақтылығын қамтамасыз етуде және ДНҚ-ны жөндеуде қатысатын нуклеаза. Бұл белоктың функциясының бұзылуы бірқатар патологиялардың, соның ішінде бүйрек жасушалық карциноманың дамуына байланысты.

Бұл белокқа деген қызығушылық ісікке қарсы терапияның нысанасы ретіндегі потенциалды функциясына байланысты артты. Осы зерттеу рекомбинантты Apollo *(SNM1B)* WT белогының экспрессиясы мен тазалығын аффинді хроматография әдісі арқылы оңтайландыруға бағытталған. Белоктың өндірісі үшін *pET28c-hSNM1BcoCu*t және *pETHSUL-hSNM1BcoCut* экспрессиялық конструкциялары *Escherichia coli (E. coli)* Rosetta2 (DE3) және NovaXG штаммдарына трансформацияланды. IPTG концентрациясы, инкубация температурасы, NP-40 детергентінің, глицерин мен мочевинаның болуы және индукциядан кейінгі инкубация ұзақтығы сияқты параметрлердің өзгерістері еритін рекомбинантты белокты алу үшін оңтайлы жағдайларды анықтау мақсатында жүйелі түрде зерттелді. Тазалау процесі Ni-NTA аффиндік хроматографиясын қолдану арқылы жүргізілді. Белоктың тазалығы мен ерігіштігі SDS-PAGE әдісі арқылы бағаланды. Алынған мәліметтер функционалды және жоғары тазартылған Apollo белогын алу үшін тиімді шарттарды анықтауға мүмкіндік берді, бұл келесі биохимиялық зерттеулерге, соның ішінде онкотерапия үшін ингибиторларды скринингтеуге мүмкіндік береді.

Түйін сөздер: ДНҚ жөндеу, Apollo, *SNM1B*, бүйрек жасушалық карцинома, ДНҚ зақымы, TRF2 белогы

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Advancing livestock waste fermentation via strategic organic substrate selection

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Abstract. Increasing volumes of livestock waste have created a growing demand for efficient biotechnological methods for its management. Properly processed animal waste can not only reduce organic matter accumulation but also serve as an effective substitute for mineral fertilizers, thereby improving soil fertility. This study aimed to investigate the use of various organic substrates in the fermentation of animal waste for the production of biohumus. Thermophilic microorganisms capable of growth at 50°C were isolated from cattle manure samples. All isolates were identified as members of the genus *Bacillus.* Enzymatic activities were evaluated using qualitative assays for cellulase, protease, amylase, and urease, along with biochemical tests for catalase and oxidase. The range of proteolytic index values produced by isolates with protease potential varied from 0.12 to 1.5. Amylase enzyme was detected in isolates I, II, and IV, with the highest activity observed in isolate IV (AI = 1.1). Among them, isolates I and II showed strong enzymatic activity across all investigated substrates, forming distinct hydrolysis zones that indicated efficient degradation of complex organic matter. The most active strains, characterized by high viable cell concentrations, are recommended for incorporation into biopreparations designed to accelerate organic waste fermentation and improve biohumus quality.
Keywords: livestock waste, fermentation, organic substrates, thermophilic microorganisms, microbial activity, biohumus

Introduction

Global agricultural production continues to expand each year in response to population growth, making effective management an increasingly urgent priority [1]. According to the 2023 census, there were more than 8.185 million cattle in Kazakhstan, which is 4.3 percent more than the previous year, according to the National Bureau of Statistics. In January of that

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year, the livestock population increased further, reaching 8.264 million head. The majority of cattle are kept on private subsidiary farms (52.4%), followed by farms (38.2%), and agricultural enterprises (9.4%). Approximately 4.8 million cattle of this, 58.8% are used for dairy farming [2]. Such an increase in the number of farm animals leads to an increase in the volume of organic waste, which in turn creates problems of biological safety.

Animal waste can be broadly divided into two main categories: biodegradable and non-biodegradable. Biodegradable waste consists of solid materials that can be naturally broken down by microorganisms over time, reducing long-term pollution. Examples include animal waste such as paper products, plant residues, carrion, feces, and poultry by-products [3].

Biodegradable waste decomposes relatively quickly, it often produces an unpleasant odor and negatively affects the visual appeal of the environment. In addition, it can contaminate soil and water sources in urban areas, as it is a favorable environment for the growth of pathogenic microorganisms (Figure 1) [4].

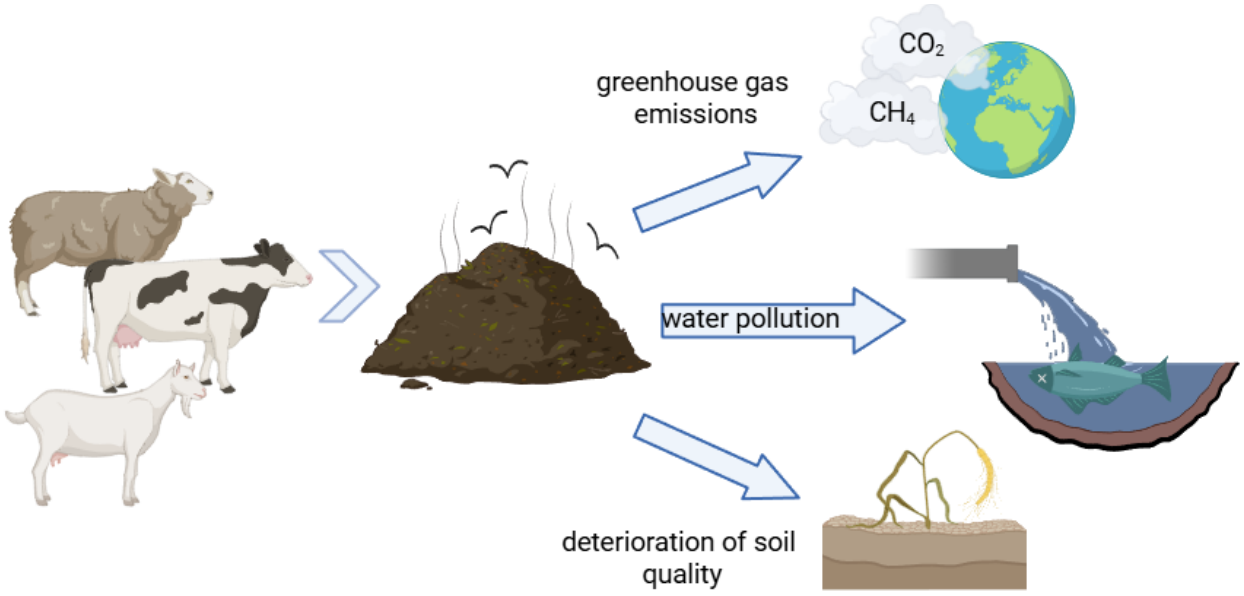

Figure 1. Biosecurity issues arising from the accumulation of animal waste. (This image was created using the BioRender scientific illustration platform)

Furthermore, a substantial amount of ammonia (NH_3), nitrous oxide (N_2O), "knox" (a mixture of NO_x, NO, and NO_2), methane (CH_4), and carbon dioxide (CO_2) are also released into the atmosphere by the agricultural sector. Nonetheless, it is well known that nitrogenous gaseous molecules (NO_x, N_2O, and NH_3) can result in serious biological issues. NO_x helps create ozone in the troposphere, N_2O is a greenhouse gas that contributes to the loss of stratospheric ozone, and ammonia can cause soil acidification and eutrophication of water systems. Methane, with a global warming potential (GWP) of 21, is a potent greenhouse gas that influences the climate directly through interactions with long-wave infrared radiation and indirectly through atmospheric oxidation processes. Methane accounts for around 18% of the greenhouse effect and is the second most important greenhouse gas after CO_2 [5].

However, assuming proper application of animal waste resources can not only decrease the amounts of waste but also effectively replace mineral fertilizers, traditional fodder, and cooking gas [6]. By breaking down organic waste into simpler compounds like carbon dioxide (CO_2), methane (CO_4), hydrogen (H_2), hydrogen sulfide (H_2S), and water (H_2O), microorganisms are

able to receive the energy they require for growth and reproduction. They aid in the breakdown of organic matter, the soil's capacity to clean itself, the development of its fertility, the conversion of humus, and the preservation of the mineral and cellulose balance. Furthermore, it is well known that microorganisms actively participate in the removal of different organic pollutants from the environment [7-8]. Thus, recycling animal waste not only produces energy and organic fertilizers but also reduces the pollution problems associated with such waste. By treating waste as a potential resource rather than a harmful element, air, water, and soil pollution, as well as the spread of toxic substances, can be prevented [9].

In addition, poor soil and erosion can lead to the need to increase its fertility, which in turn opens up the possibility of rational use of livestock waste. The use of agricultural waste based on the bioenzymatic activity of microorganisms that improve soil fertility is an economically rational solution.

The technological parameters of fermentation by microorganisms are aimed at the efficient transformation of the initial mixture into high-quality, environmentally friendly organic fertilizer [10]. Understanding the fermentation processes allows for the controlled fermentation of organic matter, which leads to the production of natural organic fertilizer and the extraction of humic substances from it [11].

The purpose of this study is to investigate the activity of new strains of thermostable microorganisms on various substrates, which can be used to optimize fermentation processes and produce high-quality biohumus.

Materials and research methods

Isolation and selection of thermophilic microorganisms

Isolation of pure bacterial cultures included a number of stages: selection of sources, sampling, inoculation of microorganisms onto solid media by serial dilution, and isolation of pure cultures.

To isolate microorganisms, 1g of cattle manure (Cattle manure samples were collected from farms in the Akmola region, Kazakhstan) was suspended in sterile NaCl (9%) solution, serially diluted, and plated onto meat-peptone agar (MPA) using the 2-4-6 method and incubated at 50°C for 48 hours (Figure 2). After incubation, isolated colonies were selected and isolated onto fresh MPA medium by Gold streaking for further study.

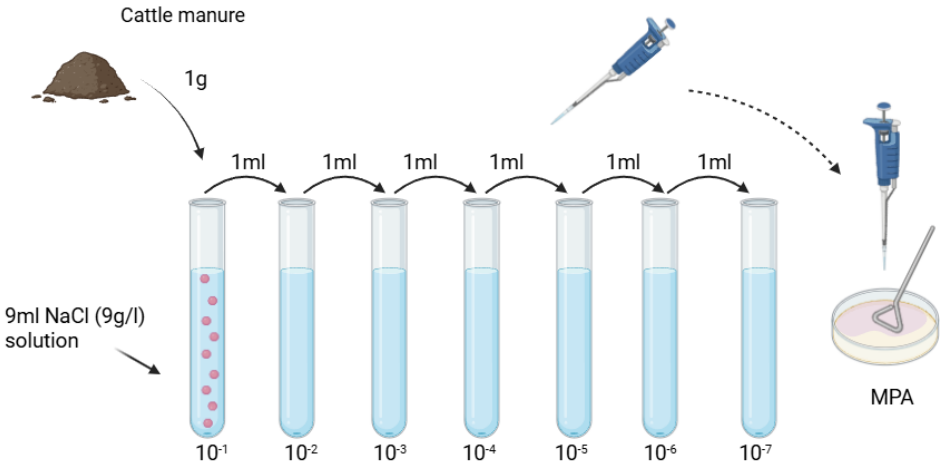

Figure 2. Isolation of microorganisms using the serial dilution method. (This image was created using the BioRender scientific illustration platform)

Cultural and morphological analysis of microbial isolates

For each isolate in Petri dishes, a morphological study was carried out, depending on the shape, color, transparency, and bacterial cell ends. Then, Gram staining was performed for microscopic examination of the morphology of the isolates. First, the suspension of microorganisms was collected from the Petri dish using a bacteriological loop and transferred onto the surface of a glass slide containing a drop of distilled water. The smear was then air-dried or heat-fixed by briefly passing it through a flame. Then the preparation was stained with crystal violet solution for 1 min. Crystal violet was treated with Lugol's solution (1 min), and after stabilization, it was decolorized by dropping ethanol or acetone. The preparation is additionally stained with Safranin solution to give color to Gram-negative bacteria. After each stage, the preparation is rinsed with water.

The stained preparations are examined using immersion microscopy to study the morphology of the bacteria.

Study of the biochemistry of microorganisms

(a) The catalase test was performed using 3% hydrogen peroxide (H_2O_2). A small amount of 24-hour pure culture of bacteria was smeared onto a glass slide using a sterile bacteriological loop. Then, 3% H_2O_2 solution was dripped onto the test (1-2 drops). As a result, the appearance of visual gas bubbles was considered a positive reaction [12]. (b) Commercial oxidase discs (HiMedia, India) were used to conduct the oxidase test. The test was performed according to the manufacturer's instructions: the oxidase disc was treated with the bacterial culture. The results were evaluated by the change in the color of the disc to purple within 30 seconds.

Preparation of various substrates for the evaluation of the enzymatic activity of microorganisms

(a) To test the proteolytic activity of microorganisms, a culture medium based on skim milk was prepared (skim milk agar medium by MicroMaster, prepared according to manufacturer specifications). The culture medium was prepared by dissolving 3.5 g peptone, 3 g yeast extract, and 15 g agar in 700 ml distilled water and sterilized in an autoclave at 1 ATM. 300 ml of skim milk was added to the culture medium after sterilizing in an autoclave at 0.5 ATM and allowed to cool slightly. The culture medium was poured into Petri dishes. Bacterial isolates were inoculated by the spot inoculation method and incubated at 50°C for 48 hours. After incubation, the results were obtained based on the appearance/absence of a clear zone. If the microorganisms are able to degrade casein (a protein in milk), a transparent zone appears around the areas where the bacteria have grown.

(b) The culture medium for determining the amylolytic activity of microorganisms was prepared by dissolving 10 g of peptone, 5 g of KH_2PO_4, 18 g of agar-agar, and 2 g of starch, the main substrate, in 1L of distilled water. The culture medium was sterilized in an autoclave and poured into Petri dishes. A 48-hour culture of bacterial isolates was incubated at 50°C for 48 hours. To visualize the results, the tests were stained with Lugol's solution (2 ml) after incubation [13].

(c) The medium for identification of cellulolytic activity was prepared based on $NaNO_3$-2g, $MgSO_4$-0.5g, KCl-0.5g, K_2HPO_4-1g, peptone-0.2g, agar-agar-17g and the substrate CMC-Na (Sodium Carboxymethyl Cellulose). The medium was sterilized in an autoclave at 1 ATM and poured into Petri dishes. Bacterial isolates were inoculated using the plaque method and incubated at 50°C for 48 hours. To visualize the results, the samples were stained with 5ml of 0.1% Congo red solution for 30 minutes after incubation and washed by treating with 1 M NaCl for 5 minutes [5].

(d) Urea-degrading microorganisms were studied on a medium based on 25 g urea base agar and 20 g urea solution in 1L of distilled water. The medium was sterilized in an autoclave and poured into test tubes in a slanting position. Bacterial isolates were inoculated into test tubes by streaking and incubated at 50°C. Samples were monitored for 6-24 hours, and the results were obtained after 72 hours of incubation. Cultivation of microorganisms in this condition is used to ensure rapid reaction (in the presence of microbial activity, the pH of the medium changes due to the release of nitrogen from urea, turning it pink) and to reduce urea evaporation. The results are visually observed by a change in the intensity of the color of the medium [14].

To quantitatively assess proteolytic, amylolytic, and cellulolytic activity, an additional hydrolysis index (I) was calculated according to the following formula (2) [15]:

$$I = \frac{Dz - Dc}{Dc} \quad (2)$$

Where:
I-activity index
D_z-diameter of the hydrolysis zone
D_c-diameter of the colony

Identification of microorganisms

Pure cultures, which were re-examined microscopically, were grown in the medium for 24 hours at 50°C. After incubation, the samples were sent to a specialized laboratory for identification by MALDI-TOF mass spectrometry, performed according to standard protocols.

Checking the viability of microorganisms

Serial dilutions of the test sample are prepared using the Miles and Misra method. The bacterial culture is serially diluted up to 10^{-10} in physiological saline (0.9% NaCl). Then the agar in a Petri dish is divided into eight equal sectors. Starting from the third dilution (10^{-3}), the resulting test was inoculated in the form of drops on the surface of solid culture medium. The cultures are then incubated to allow the growth of colonies. Colony counting is carried out in the drop zones that show the highest number of discrete colonies that do not coalesce after appropriate incubation. The samples were incubated at 50°C for 24 h. After a specified time, the presence and density of colonies in each sector are assessed, which allows determining the viability of bacteria at different dilution levels.

Statistical analysis

All experiments were performed in quadruplicate, and the results are presented as mean ± standard deviation (SD). Statistical significance of differences among isolates was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test at a significance level of $P < 0.05$. All analyses were performed using Jump (version 8).

Results

Isolation and selection of thermophilic microorganisms

Microorganisms were isolated by the serial dilution method based on samples taken from cattle feces. As a result of incubation on meat-peptone agar (MPA) at a temperature of 50°C for 48 hours, numerous colonies with different morphological features were formed.

After incubation, individual colonies that differed in macroscopic characteristics were selected from the colonies formed. As a result of pure cultures obtained by re-inoculation by

the Gold method, 15 different thermophilic bacterial isolates were obtained. These isolates had different colony morphology and strains were selected from them for further study.

Among the isolates obtained, five strains demonstrating the highest enzymatic activity were selected for further analysis.

Colony description

The colonies of the selected strains grown on nutrient media had different morphological characteristics (Figure 3):

Colonies of isolate I were shiny, yellowish, with a dry shell, had a complex shape, and were distinguished by a convex structure, teardrop-like elevations, and crater-like formations.

Isolate II formed yellowish colored colonies with a dry surface; their edges were rhizoid, and their structure was flat.

Colonies of isolate III were flat, shiny, and round, with irregular, jagged ends.

Isolate IV produced dark, flat colonies with a yellow-orange hue and a structure resembling concentric rings.

Isolate V developed flat, dark-yellowish colonies with a round shape and clearly irregular ends.

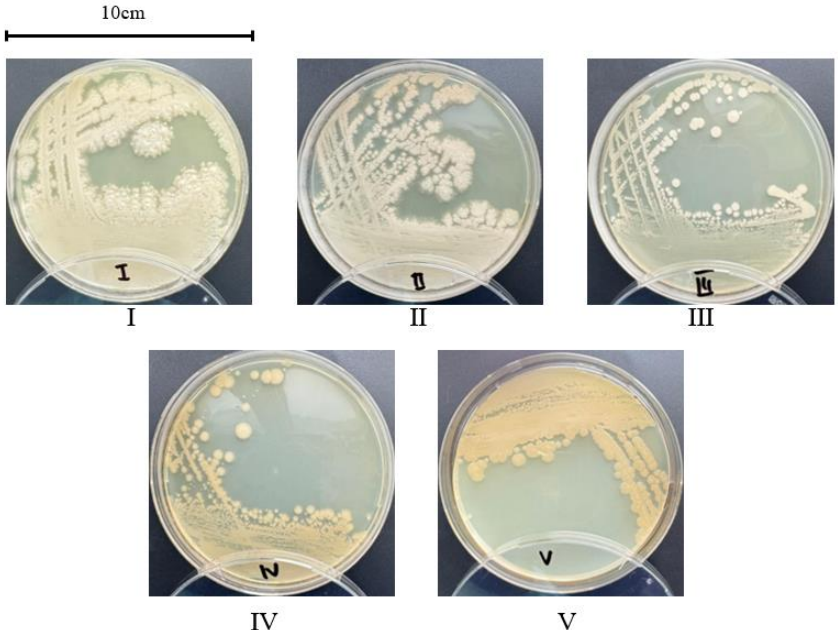

Figure 3. Colonies of microorganisms. I-V-correspond to isolates numbers; Scale bar = 10cm

Microscopic description

When examining the microscopic appearance of microorganisms, it was found that all the isolates studied were gram-positive, round rods with pointed ends (Figure 4). Spores were observed in all isolates. They are often located on the outside, but also inside the bacteria. The morphology of bacteria differs from each other in shape and length of the rods.

Gram +, short rods with rounded ends, spores on the outside and in the middle.

Gram +, short rods with rounded ends, rare spores on the outside and in the middle of the rods

Gram +, short rods with rounded ends, many spores, located on the outside and inside (in the middle)

Gram +, short rods, "microphone"-shaped rods are often found, spores are located separately.
Gram +, short, thin rods with rounded ends, few spores, "microphone"-shaped rods are found.

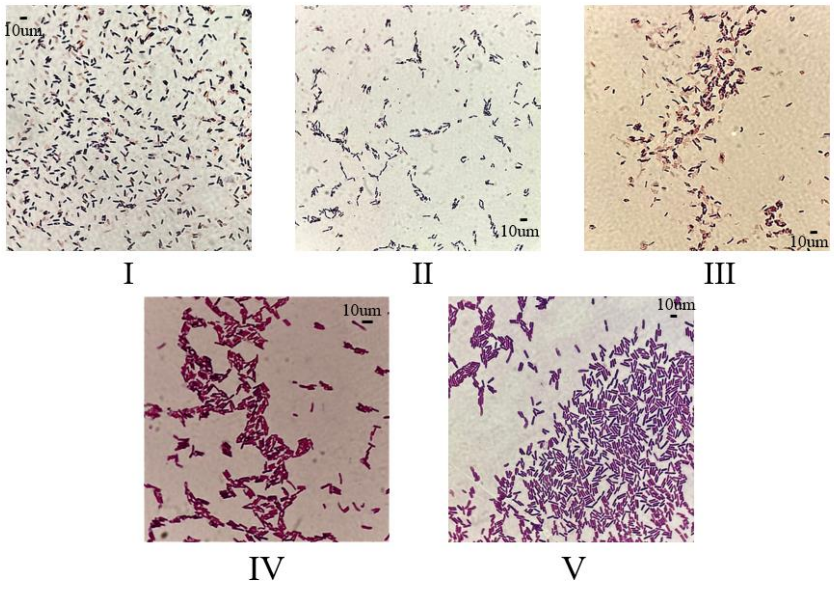

Figure 4. Microscopic morphology of bacterial isolates. **I-V** corresponds to isolates numbers; Scale bar = 10 μm.

Biochemical assay results

Assays performed to characterize the biochemical properties of the bacterial isolates, all tested isolates exhibited positive activity for both enzymes. However, the intensity of the oxidase test showed lower indicators.

As a result of the catalase reaction (1), the decomposition of hydrogen peroxide on the surface of the glass slide by the catalase enzyme led to the formation of O2 gas bubbles (Figure 5a):

$$2H_2O_2 \rightarrow 2H_2O+O_2\uparrow \quad (1)$$

A positive result of the oxidase test confirms that the bacteria possess the *cytochrome C oxidase* enzyme, which uses oxygen as an electron donor in the final stage of the respiratory chain. The result of the reaction can be observed by the color change of the *cytochrome-C* oxidase enzyme when it interacts with a chemical indicator on the oxidase disk (Figure 5b).

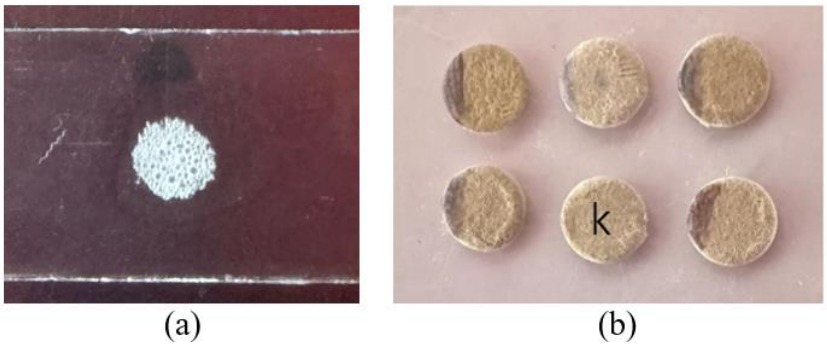

Figure 5. Biochemical **(a)** catalase and **(b)** oxidase tests. k-indicates control

Evaluation of the enzymatic activity of microorganisms based on various substrates

The qualitative assessment of four enzymatic activities: proteolytic, amylolytic, cellulolytic, and urease, was performed on the isolated microorganisms, representing a key component of the experimental work. The results were observed due to the appearance of transparent zones around the colony or a change in the color of the culture medium. The clear zones formed in the activity test qualitatively indicate the ability of bacterial isolates to produce hydrolysis enzymes. It is believed that the larger the formed transparent zone, the more positively it correlates with the ability of these isolates to produce the corresponding enzymes.

Calculating the activity index allowed us to compare the efficiency of enzymatic activity between different isolates, since it took into account not only the presence of the zone, but also the ratio of the hydrolysis zone diameter to the diameter of the bacterial colony itself. A higher ratio indicates a greater enzymatic activity exhibited by the microorganism.

Proteolytic activity was determined on a medium containing casein proteins. In this study, the appearance of transparent zones around the colonies indicates the area of protein degradation. Hydrolysis by the protease enzyme was shown by isolates I, II, IV, and V (Figure 6). The proteolytic activity index values are shown in Table 1 and Figure 7. The range of proteolytic index values produced by isolates with protease potential varied from 0.12 to 1.5. Isolate No. V showed the most pronounced proteolytic activity in terms of the volume of the hydrolysis zone and the calculated index.

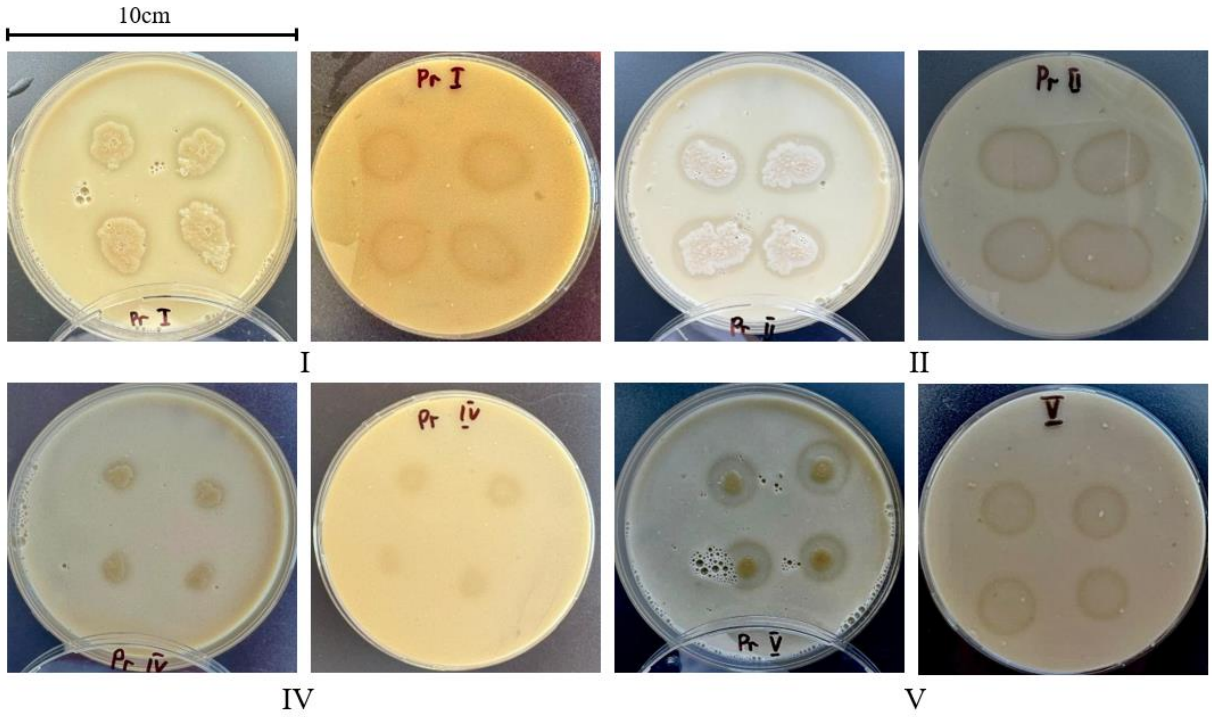

Figure 6. Proteolytic activity. **I, II, IV, V** correspond to isolates numbers; Scale bar = 10cm

Table 1

Average proteolytic index (PI) of thermophilic bacteria isolated from cattle manure (n-4)

№	Isolate	Diameter of the colony (mm)	Diameter of the hydrolysis zone (mm)	Proteolytic Index (PI)
1	I	13	17	0.30

1	I	15 22 17	18 25 20	0.2 0.14 0.18
2	II	17 18 17 24	21 23 19 27	0.23 0.28 0.12 0.125
3	IV	8 7 7 7	10 9 9 8	0.25 0.28 0.28 0.143
4	V	8 6 7 7	16 15 14 14	1 1.5 1 1

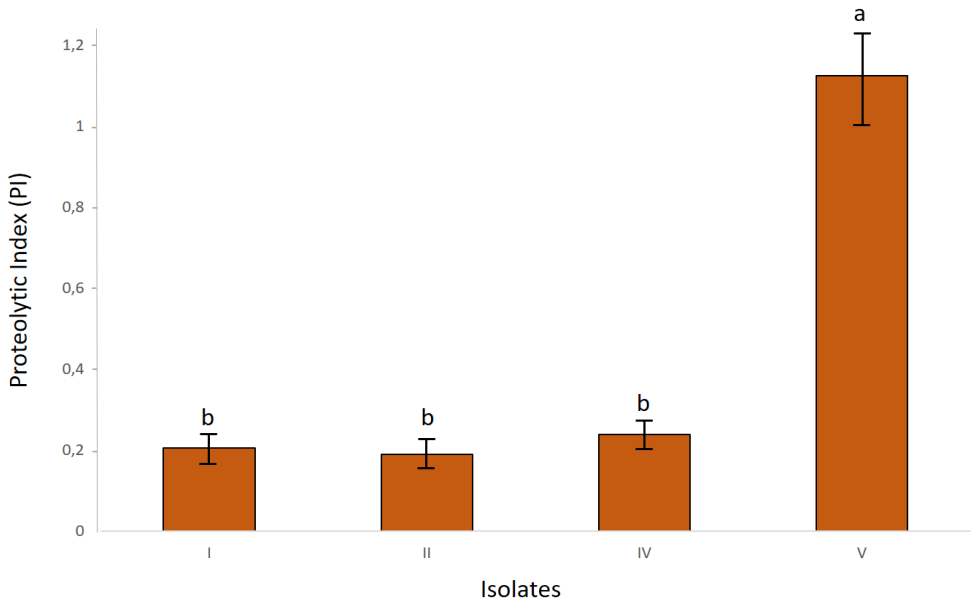

Figure 7. Comparison of proteolytic activity (PI) among thermophilic isolates. Bars represent mean ± SD (n = 4). Different letters indicate significant differences between isolates (Tukey's HSD, $P < 0.05$)

Amylolytic bacteria break down starch and glycogen using amylases, including α- and β-amylases. The resulting hydrolysis products, such as glucose, serve as a source of carbon and energy for microorganisms and a source of nutrients for plants [16].

Therefore, amylolytic activity was assessed on a starch-based medium. The results were visualized, and the area of hydrolysis was stained with iodine solution, an indicator of starch. Clear zones around the colonies indicate starch degradation. Amylase enzyme was detected in isolates I, II, and IV, with very high activity observed in isolate IV (AI=1.1). The results are shown in Figure 8, and the amylolytic index is shown in Table 2 and Figure 9.

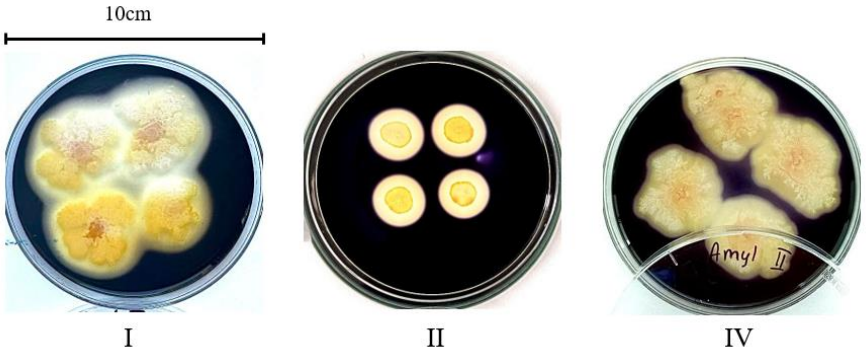

Figure 8. Amylolytic activity. **I, II, IV** correspond to isolates numbers; Scale bar = 10cm

Table 2

Average amylolytic index (AI) of thermophilic bacteria isolated from cattle manure (n-4)

№	Isolate	Diameter of the colony (mm)	Diameter of the hydrolysis zone (mm)	Proteolytic Index (PI)
1	I	24 20 23 27	30 32 28 34	0.25 0.6 0.30 0.26
2	II	27 33 28 28	35 35 30 30	0.29 0.06 0.07 0.07
3	IV	11 10 9 10	21 20 19 20	0.9 1 1.1 1

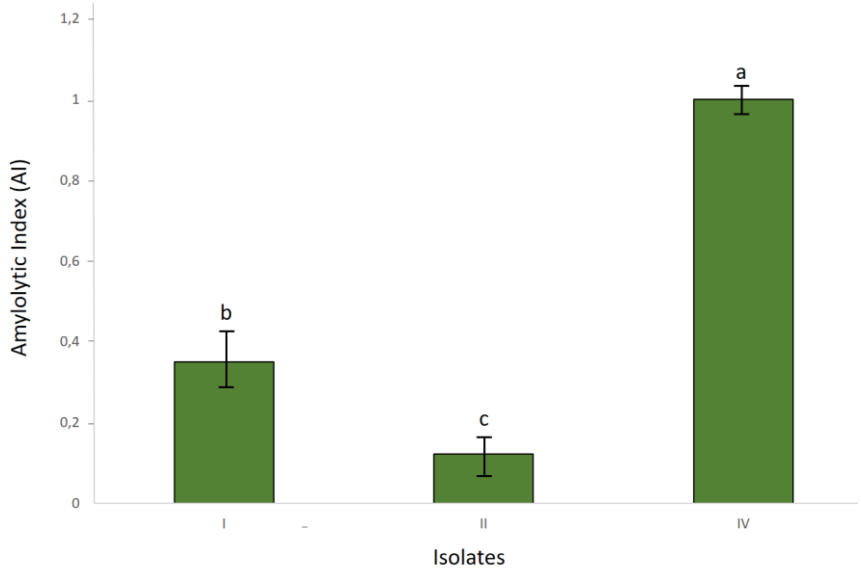

Figure 9. Comparison of amylolytic activity (AI) among thermophilic isolates. Bars represent mean ± SD (n = 4). Different letters indicate significant differences between isolates (Tukey's HSD, $P < 0.05$)

Cellulolytic activity was studied in a medium containing sodium carboxymethylcellulose (CMC-Na). Degradation was visualized as decolorized zones surrounding the colonies following staining of the medium with Congo red solution. Cellulolytic activity was detected in isolates I, II, III, and V (Figure 10). However, the hydrolytic activity exhibited by isolates III and V was markedly low (Table 3, Figure 11).

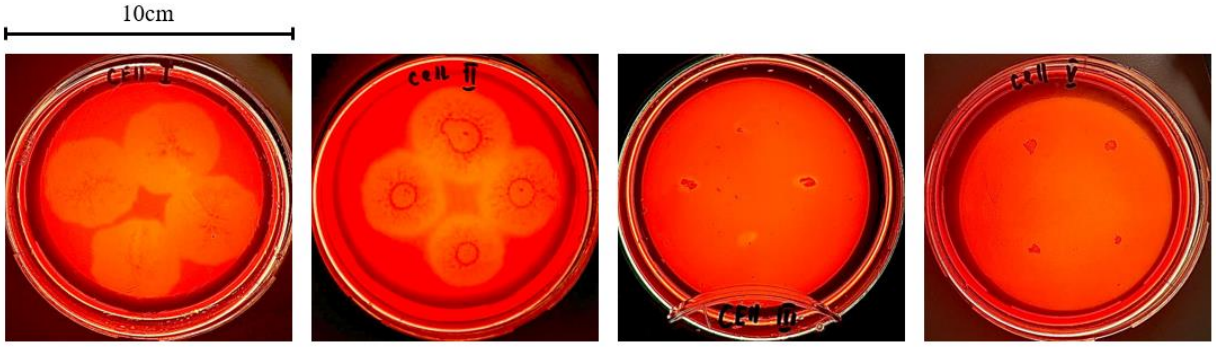

Figure 10. Cellulolytic activity. **I, II, III, V** correspond to isolates numbers; Scale bar = 10cm

Table 3

Average cellulolytic index (CI) of thermophilic bacteria isolated from cattle manure (n-4)

№	Isolate	Diameter of the colony (mm)	Diameter of the hydrolysis zone (mm)	Proteolytic Index (PI)
1	I	18 16 17 17	30 28 29 30	0.66 0.75 0.70 0.76
2	II	18 20 19 20	25 33 27 33	0.39 0.65 0.63 0.65
3	IV	5 5 6 4	7 7 8 5	0.4 0.4 0.33 0.25
4	V	4 4 3 4	5 5 4 5.5	0.25 0.25 0.33 0.375

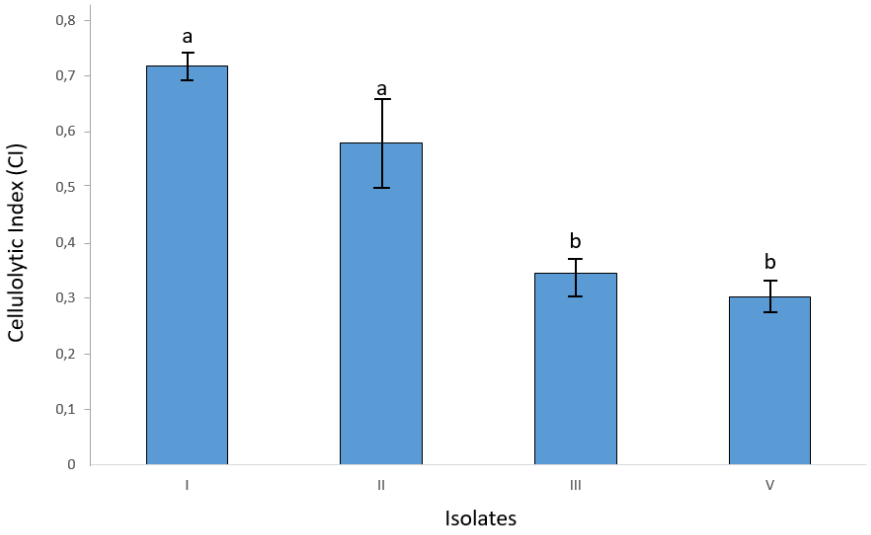

Figure 11. Comparison of cellulolytic activity (CI) among thermophilic isolates. Bars represent mean ± SD (n = 4). Different letters indicate significant differences between isolates (Tukey's HSD, $P < 0.05$)

Urease enzyme activity was assessed by the color change of a medium containing urea and red phenol as a pH indicator. The results were interpreted in terms of the intensity of the pink color change of the medium, which occurred due to the formation of ammonia during the decomposition of urea and the increase in the pH of the medium. Isolates I, II, III, and IV, shown in Figure 12, showed very high urease activity.

A culture of *Klebsiella pneumoniae,* known for its high urease activity, was used as a positive control, which caused the medium to turn a deep pink color. As a negative control, E. Coli, which does not show urease activity, was inoculated, resulting in a yellow coloration. Also, as a control, the pure medium without inoculation retained its original orange color.

Note: Klebsiella-positive control, Empty-clean medium without microorganisms, *E. Coli*-negative control

Figure 12. Urease Activity Assay. **I, II, III, IV** correspond to isolates numbers

Currently, microbial enzymes have almost completely replaced synthetic analogues in a number of industrial sectors [17]. Thus, the obtained results showed that most of the isolates studied had several types of enzymatic activity. Isolates I and II exhibited positive results across

all four tested enzymatic activities, demonstrating notable activity potential. Based on their high enzymatic performance, these isolates were selected for further investigation.

Identification of microorganisms

To determine the taxonomic affiliation of the isolated microorganisms, identification was carried out using MALDI-TOF mass spectrometry. The results of the analysis of protein profiles showed that all the isolates studied belong to the genus Bacillus.

As a result of the identification, it was determined that the isolates studied belong to the species *Bacillus licheniformis, Aeribacillus pallidus, Geobacillus thermodenitrificans,* and *Lysinibacillus sphaericus* (Table 4). All of these species are microorganisms with high enzymatic activity, the ability to decompose complex organic compounds, and have valuable properties from a biotechnological point of view.

Table 4

Taxonomic affiliation of the studied isolates

№	Isolate	Genus	Species
1	I	*Bacillus*	*B. licheniformis*
2	II	*Bacillus*	*B. licheniformis*
3	III	*Aeribacillus*	*A. pallidus*
4	IV	*Geobacillus*	*G. thermodenitrificans*
5	V	*Lysinibacillus*	*L. sphaericus*

Assessment of microbial viability

To assess the viability of microorganisms, a tenfold dilution series of the test samples was performed up to the 10^{-10} level. 10 µl of the sample from each dilution was evenly dropped onto the surface of the solid medium in separate sectors of a Petri dish. After incubation under optimal conditions, the number of colonies grown was counted.

Since the droplet volume used was 10 µl (i.e., 1/100 of 1 ml), the following formula (3) was used to convert the obtained data to a standard unit of measurement (CFU/ml) [18]:

$$CFU/ml = Number\ of\ colonies \times 100 \times Dilution\ level \quad (3)$$

Where:

Number of colonies – the number of colonies grown in 10 µl of suspension,

100 – the conversion factor for 1 ml of solution,

Dilution level – takes into account the dilution of the original sample.

Thus, each cultured colony showed the presence of one viable cell per drop of the test dilution, and the final re-counting allowed us to determine the total number of viable microorganisms in the original sample (Table 5).

Table 5

Determination of the viability of isolates

№	Isolates	Dilution degree	Number of colonies	CFU/ml
1	I	10^{-7}	no separate colony	> 10^{10} CFU/ml

2	II	10^{-6}	2	2×10^{8} CFU/ml
3	III	10^{-6}	20	2×10^{9} CFU/ml
4	IV	10^{-6}	9	9×10^{8} CFU/ml
5	V	10^{-6}	5	5×10^{8} CFU/ml

The obtained data show that the viability of microorganisms was maintained in all studied samples to the order of 10^{-6} and 10^{-7} (Figure 13), and the concentration of viable cells varied from 5×10^{8} to 10_{10} CFU/ml. In the first sample, the concentration of viable cells was so high that it was not possible to separate colonies even at a dilution of 10^{-7}, which indicates that the permissible limit of standard counting was exceeded. The viability of samples II, III, IV, and V ranged from 5×10^{8} to 2×10^{9} CFU/ml. These values confirm the presence of a large number of viable microorganisms in the studied samples.

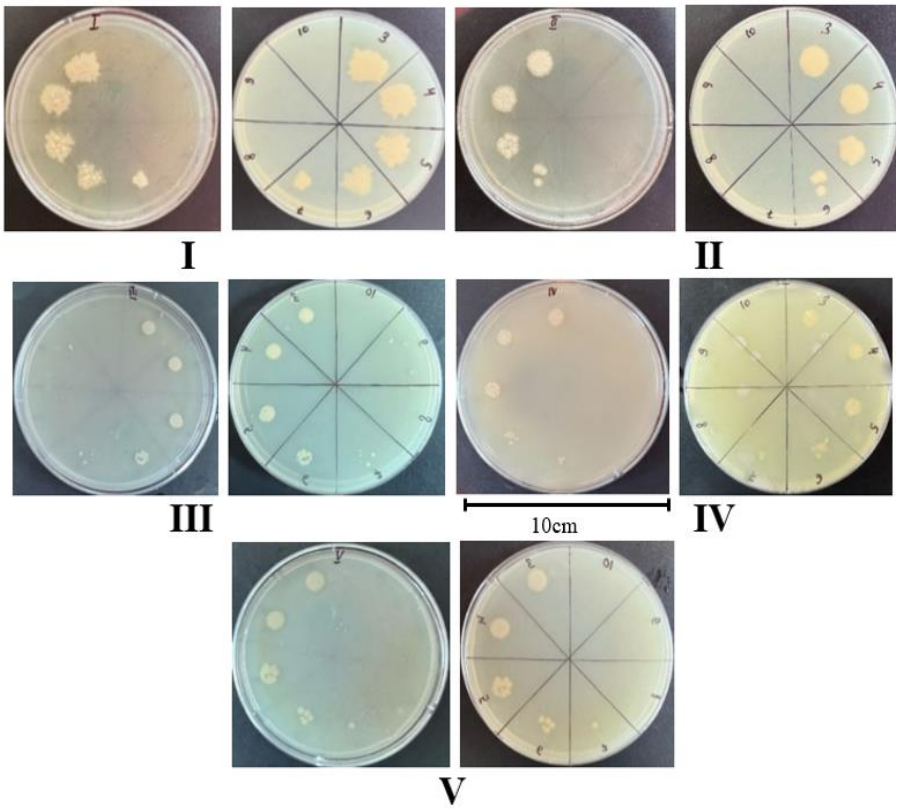

Figure 13. Bacterial viability. **I-V** correspond to isolates numbers; Scale bar = 10cm

Discussion

Cattle feces are a rich source of microbial diversity, as evidenced by the isolation of thermophilic microorganisms from them. The obtained isolates show potential for biotechnological applications, particularly in the context of fermenting organic waste.

These strains were distinguished by their ability to accelerate the decomposition of substrates and were recognized as promising for future use as the main components of a bioconsortium in the production of biopreparations.

Recent research highlighting the significance of thermophilic bacterial strains in high-temperature composting and waste bioconversion is consistent with the isolation and characterization of these organisms from cattle manure. Temperatures between 50 and 80°C promote the growth of thermophilic microorganisms during the thermogenic phase of composting, especially spore-forming Gram-positive bacteria like those in the Bacillus genus, which predominate microbial communities and play a major role in the breakdown of organic matter [19].

The tests showed that the isolates exhibit various enzymatic activities, indicating their role in organic matter decomposition. Studies by T. A. Ogunnusi and O. Olorunfemi have shown that cattle manure is a rich substrate for the growth and activity of proteolytic microorganisms due to the high content of organic compounds, including proteins. The protein components of manure serve as a source of amino acids and peptides formed as a result of enzymatic decomposition [20]. Protein hydrolysis in this substrate is carried out due to the activity of bacteria that synthesize proteases capable of breaking down casein and other complex protein structures.

The observed amylase activity, especially pronounced in isolate IV, indicates that these microorganisms are capable of efficient starch degradation. Such enzymatic potential not only reflects their adaptability but also points to their possible role in enhancing nutrient cycling and soil fertility. Amylolytic activity has also been reported in other Bacillus strains isolated from cattle manure. For example, Bacillus subtilis CM3 produces thermostable α-amylase with an optimum at 50-70°C and pH 5-9, making it highly relevant for composting and feed bioconversion applications [21].

Cellulose can be degraded by cellulases produced by thermophilic bacteria, mainly Bacillus spp., effectively hydrolyzing cellulose even at temperatures up to 70°C and under variable pH conditions [22].

Cellulose is the most abundant renewable organic resource in nature, comprising approximately 45% of the dry weight of wood. The microbial degradation of cellulose has been extensively studied for decades. The function of cellulase is to hydrolyze cellulose by breaking the α-1,4-glycosidic bond in the main structural polysaccharide of plants. The mechanisms of cellulose degradation are constrained by many factors, including the complexity of the substrate and the number of enzymes involved [23].

Recent data show that thermotolerant Bacillus inoculation increased cellulose degradation rates up to 57% in composting systems, supporting the reliability of our observed enzymatic potential [24].

Several isolates demonstrated cellulolytic potential, consistent with previous findings that thermophilic Bacillus strains are key degraders of cellulose in compost environments. Nevertheless, the hydrolytic index of isolates III and V is significantly lower than other indicators. Notably, the third isolate demonstrates weak cellulase activity and have no detectable proteolytic, amylolytic or catalase functions. However, it showed strong urease activity, indicating that, despite its low enzymatic activity, this isolate could still contribute to nitrogen cycling during organic matter decomposition.

The main source of nitrogen in animal waste is urea, which is converted to ammonia by the enzyme urease [25]. Urease is highly specific and catalyzes the breakdown of urea to unstable ammonium carbonate, which then decomposes to form ammonia and carbon dioxide [26]. Supporting our findings on urease activity and nitrogen-cycle involvement of isolates, Cao et al. (2024) reported significant enhancement of nitrification gene abundances and nitrogen retention in manure composts inoculated with Bacillus strains [27].

The results of the oxidase and catalase tests, in addition to serving as indicators of the biochemical characteristics of the studied isolates, provide essential preliminary data for their further identification. These enzymatic assays offer insight into the physiological properties of the bacterial strains and contribute to their classification within specific taxonomic groups.

Catalases are one of the ROS defense enzymes, which can decompose harmful oxygen compounds such as hydrogen peroxide (H_2O_2) [28]. Therefore, this property proves that they are adapted to live in aerobic or facultative anaerobic environments.

Oxidative activity indicates that the bacteria use oxygen for energy metabolism, which confirms that they belong to aerobic or facultative aerobic organisms. However, the weak reaction may indicate low enzymatic activity in the studied bacteria.

The above-mentioned species belonging to Bacillus genus are considered promising agents for the creation of bioconsortiums and their use in various application areas, such as environmental biotechnology, agriculture, and organic waste processing. Choudhary & Johri (2009) and Ubani et al. (2016) found that 87% of randomly selected colonies in the thermophilic phase of compost belonged to the genus Bacillus [29, 30]. Then, Nayara Noreen et al. (2019) reported similar results. The abundance of thermotolerant and thermophilic Bacillus species in compost samples is due to the thick spore walls of these microorganisms, which are characterized by their high adaptability to extreme conditions [31].

These isolates were recovered from cattle manure in the Akmola region, which to our knowledge has not been previously reported for thermophilic Bacillus strains applied in biohumus production, thereby representing a locally-adapted candidate set for industrial waste-fermentation applications. In this context, the genus Bacillus includes a wide range of species with important biotechnological properties, such as enzymatic activity, antagonism to pathogens and the ability to biodegrade complex compounds. Therefore, the combined enzymatic potential (proteolytic, amylolytic, cellulolytic, and ureolytic activity), together with oxidative stress resistance and high adaptability, suggests that Bacillus isolates from cattle manure are excellent candidates for industrial bioprocesses, compost enhancement, and sustainable waste valorization strategies. What makes them useful for the formation of bioconsortiums and further biotechnological applications.

Conclusion

The development of microbial technologies that allow the reuse of organic waste as fertilizer ensures the efficient use of valuable biological resources [32]. Thermophilic bacteria are particularly promising in the management of livestock waste, since their activity at high temperatures allows for significantly reducing the fermentation time and obtaining a product with high agrotechnical properties. Therefore, in the context of the present study, thermophilic bacterial cultures belonging to the Bacillus genus were isolated from cattle manure, which are distinguished by high viability and enzymatic activity. The study of their activity on four different organic substrates showed that a number of isolated microorganisms exhibited stable activity on all tested substrates. This indicates their ability to effectively decompose complex organic compounds into simpler forms, which significantly improves the availability of nutrients to plants. The results obtained confirm the prospects for further study of the isolated strains. Since this study is part of a larger scientific project aimed at creating a biopreparation based on thermophilic bacteria, it is planned to further study the antagonistic activity and biocompatibility of the isolated strains in order to create an effective bioconsortium for obtaining an industrial biopreparation in the future. Thus, the practical significance of the conducted studies, in turn,

opens prospects for environmentally safe processing of livestock waste and contributes to solving the problems of increasing soil fertility in conditions of sustainable agriculture.

Authors contributions

M.E., N.Zh., and **T.A. –** conceptualization; **T.Zh., K.K.** – data curation; **T.A., E.D., A.A.** – formal analysis; **M.E.** and **N.Zh., T.A.** – writing – original draft; **M.E., N. Zh., T.A.,** and **T.Zh.** – writing – review & editing; **N.Zh.,** and **T.A.**– supervision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest. Compliance with ethical standards. This article does not contain a description of studies performed by the authors involving people or using animals as objects.

Compliance with ethical standards

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Органикалық субстратты стратегиялық таңдау арқылы мал шаруашылығы қалдықтарының ферментациясын оңтайландыру

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Аңдатпа. Жануарлар қалдықтарының өсіп келе жатқан көлемі оны өңдеудің тиімді биотехнологиялық әдістеріне сұраныстың артуына әкеліп соғады. Мұндай қалдықтар тез ыдырайды, жиі жағымсыз иістерді шығарады және қоршаған ортаның эстетикалық сапасын нашарлатады. Сонымен қатар, топырақ пен судың ластануы патогендік микроорганизмдердің көбеюіне қолайлы жағдай болып табылады. Мал шаруашылығы қалдықтарын дұрыс өңдеу органикалықзаттардыңжинақталуыназайтыпқанақоймай,минералдытыңайтқыштардытиімді алмастырып, топырақ құнарлылығын арттырады. Бұл зерттеудің мақсаты биогумус өндіру үшін жануарлар қалдықтарын ашытуда әртүрлі органикалық субстраттарды пайдалануды зерттеу болып табылады. Ірі қара мал көңі үлгілерінен 50°C температурада өсуге қабілетті термофильді микроорганизмдер бөлініп алынды. Барлық изоляттар *Bacillus* туысының өкілдері ретінде анықталды. Ферменттік белсенділік целлюлаза, протеаза, амилаза және уреаза үшін сапалы талдаулар және каталаза мен оксидаза биохимиялық сынақтары арқылы бағаланды. Өміршең

жасушаларының концентрациясы жоғары, ең белсенді штаммдар органикалық қалдықтардың ферментациясын жеделдетуге және биогумус сапасын арттыруға арналған биопрепараттардың құрамына енгізу үшін ұсынылады.
Түйін сөздер: мал шаруашылығы қалдықтары, ферментация, органикалық субстраттар, термофильді микроорганизмдер, микробтық белсенділік, биогумус

Оптимизация ферментации отходов животноводства посредством стратегического выбора органического субстрата

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Аннотация. Растущие объемы отходов животноводства создали растущий спрос на эффективные биотехнологические методы их переработки. Такие отходы быстро разлагаются, часто выделяя неприятные запахи и ухудшая эстетическое качество окружающей среды. Более того, загрязнение почвы и воды создает благоприятные условия для размножения патогенных микроорганизмов. Правильно переработанные отходы животноводства могут не только снизить накопление органического вещества, но и служить эффективной заменой минеральных удобрений, тем самым повышая плодородие почвы. Целью данного исследования было изучение использования различных органических субстратов при ферментации отходов животноводства для производства биогумуса. Из образцов навоза крупного рогатого скота были выделены термофильные микроорганизмы, способные расти при температуре 50 °C. Все изоляты были идентифицированы как представители рода *Bacillus.* Ферментативная активность оценивалась с помощью качественных анализов на целлюлазу, протеазу, амилазу и уреазу, а также биохимических тестов на каталазу и оксидазу. Наиболее активные штаммы, характеризующиеся высокой концентрацией жизнеспособных клеток, рекомендуются для включения в состав биопрепаратов, предназначенных для ускорения ферментации органических отходов и улучшения качества биогумуса.
Ключевые слова: отходы животноводства, ферментация, органические субстраты, термофильные микроорганизмы, микробная активность, биогумус

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Iron homeostasis disruption suppresses viral infection via ferroptosis-like cell death and RNA interference in plants

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Abstract. Ferroptosis-like cell death in plants is a recently described mechanism triggered by iron accumulation and lipid peroxidation; however, its role in viral infection remains unclear. In this study, we investigated the effect of iron (Fe-EDTA) treatment on *Nicotiana benthamiana* plants infected with wild-type tomato bushy stunt virus (wtTBSV). Morphological and biochemical analyses under combined stress revealed pronounced symptoms, including growth inhibition, necrosis, leaf yellowing, chlorosis, and leaf deformation, while the expression of the viral suppressor protein P19 was reduced. Biochemical assays showed that low Fe-EDTA concentrations maintained the photosynthetic apparatus and increased chlorophyll content, whereas high concentrations induced lipid peroxidation and ferroptosis-like cell death. Our results suggest that excess iron activates RNA interference and ferroptosis-like death, thereby suppressing viral infection, while low iron concentrations preserve photosynthetic activity and alleviate symptoms. In addition, combined stress led to reduced levels of HSP70 and HSP90, indicating that iron homeostasis may interfere with cellular chaperones essential for viral replication. These findings highlight the key role of ferroptosis-like cell death in plant-virus interactions.

Keywords: ferroptosis, plant virus, combined stress, ROS, TBSV

Introduction

Abiotic stress refers to a set of unfavorable non-living factors whose impact disrupts the physiological processes of plants, alters their metabolism, and suppresses growth and development. The major abiotic factors include extreme temperatures, water deficit or excess, soil salinization, as well as disturbances in ion balance caused by mineral deficiency or overaccumulation. Metals act as significant abiotic stressors: although plants require them in small amounts for normal growth, excessive accumulation can poison cells [1]. Metals of this type include iron (Fe). Within plant cells, it can exist in two oxidation states, Fe^{2+} and Fe^{3+}, and serves as a cofactor in respiration, DNA synthesis, photosynthesis, and chlorophyll formation

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[2]. However, under neutral and especially alkaline soil conditions, most iron exists in the form of poorly soluble compounds, which limits its availability to the root system. To overcome this problem, chelated forms of iron are widely used in crop production. In particular, the Fe-EDTA complex stabilizes iron ions in a soluble form, prevents their precipitation, and facilitates their transport to plant cells. Chelates provide a convenient and effective tool for fertigation, alleviating iron deficiency [3]. Despite its clear advantages, an excessive influx of iron into the cell is associated with certain risks. One of the most serious consequences is ferroptosis, a form of cell death induced by iron overload [4].

Ferroptosis is an iron-dependent form of regulated necrotic cell death, triggered by excessive lipid peroxidation and leading to the destruction of cellular membranes [5]. Ferroptosis is characterized by a decrease in the level of reduced glutathione (GSH) and inactivation of glutathione peroxidase 4 (GPX4). Under normal conditions, GPX4 reduces lipid hydroperoxides to non-toxic alcohols using GSH, thereby preventing the accumulation of lipid peroxidation products. When ferroptosis begins, cells suppress GPX4 activity or degrade the enzyme, which rapidly accumulates lipid peroxides and triggers cell death. Stress induces other antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which temporarily limit ROS accumulation; in plants, in particular, ethylene enhances the activity of these enzymes, while transcription factors of the ERF family increase the expression of antioxidant defense genes. Nevertheless, it is the inactivation of GPX4 that defines the specificity of ferroptosis, since the activation of other antioxidants reflects only a general stress response and does not prevent cell death [6,7]. At the same time, the molecular mechanisms underlying changes in the expression and activity of antioxidant enzymes in plants under ferroptosis conditions remain largely unexplored, highlighting the relevance of further research.

Heat shock proteins (HSPs) represent an evolutionarily conserved family of molecular chaperones that play a key role in maintaining protein homeostasis. Initially described as proteins induced by elevated temperatures, they are now known to be upregulated under a wide range of abiotic and biotic stresses [8,9]. During ferroptosis in animal cells, the HSP70 family exhibits a protective role: HSP70 has been shown to enhance GPX4 expression and antioxidant activity, thereby increasing resistance to ferroptosis. In contrast, HSP90 may promote ferroptosis by binding to timosaponin AIII, thereby inducing ubiquitination and degradation of GPX4, which enhances lipid peroxidation [7,10]. In plants, the direct roles of HSP70 and HSP90 in ferroptosis remain poorly understood and require further investigation.

Viral infections trigger ROS generation and cell death by employing virus-encoded suppressor proteins that inhibit RNA interference, antioxidant systems, and hormone-mediated defense pathways in plants. One such suppressor protein is p19, encoded by the tomato bushy stunt virus (TBSV). This protein binds double-stranded siRNAs, thereby suppressing post-transcriptional gene silencing and facilitating the spread of the viral genome within the plant. Previous studies have shown that p19 forms dimers and interacts with the host RNA-binding protein Hin19. The resulting complex plays a key role in the effective suppression of RNA interference and promotes viral progression in plant cells [11]. As viruses spread through plant cells, they cause significant changes in the activity of antioxidant enzymes. For example, cucumber mosaic virus (CMV) disrupts the plant's antioxidant defense by binding its 2b protein to catalase CAT3, initiating its degradation via the ubiquitin–proteasome pathway. This process leads to the accumulation of H_2O_2 and the development of necrosis [12].

Simultaneous exposure to multiple stressors usually worsens plant damage as signaling pathways interact in complex ways. The relationship between viral infections and ferroptosis has been studied in detail in animals: viruses can both activate and suppress iron-dependent cell

death by altering Fe metabolism, enhancing lipid peroxidation, and influencing ROS-dependent mechanisms of cell death, thereby promoting either viral replication or evasion of the immune response [13].

In plants, these processes have been studied to a limited extent. It is known that they involve mechanisms similar to those in animals but also exhibit specific features, such as the presence of multiple GPX isoforms and the involvement of chloroplasts and peroxisomes. Suppression of GPX4 in *Nicotiana benthamiana* has been shown to enhance ferroptosis-like cell death during infection with a mutant *tobacco mosaic virus;* however, systematic data remain scarce [6]. Studying such interactions is essential for understanding multistress responses, plant immunity, and adaptation, as well as for breeding resistant cultivars. This is particularly important in the context of combined viral infections and toxic metals, which are critical factors for predicting crop yield and ensuring food security. Identifying common signaling nodes of multistress responses may provide a basis for discovering genetic markers of resistance [14].

Materials and research methods

Plant preparation

In this study, *Nicotiana benthamiana* plants were used. Soil and pots were sterilized by autoclaving, after which the soil was mixed with vermiculite at a ratio of 3:1 and watered every other day for 30 days. We grew plants under controlled conditions with a 16-hour light/8-hour dark photoperiod at 25°C during the day and 23°C at night.

Plant treatment

On day 30, plants were mechanically inoculated with tomato bushy stunt virus (TBSV) in 10 mM PBS buffer (1:3). One day post-inoculation, plants were irrigated with iron chelate (Fe-EDTA) solutions at various concentrations: 0.05 mM, 0.1 mM, 0.5 mM, 0.8 mM, and 1 mM. Morphometric measurements were performed on day 7 post-inoculation (dpi). Plants were harvested, their root systems rinsed with water, and then fixed for measurement of shoot and root length.

Chlorophyll content determination

Chlorophyll content in plant leaves was determined according to Arnon's method [15]. We measured chlorophyll using a Multiskan SkyHigh spectrophotometer (Thermo Fisher Scientific) with SkanIt Software at wavelengths of 664 nm and 647 nm. The contents of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the equations proposed by Lichtenthaler and Wellburn (1985) [16].

SDS-PAGE

For protein analysis, 0.1 g of plant material was collected and homogenized in 200 µL of extraction buffer (1×TE: 10 mM Tris, pH 7.4–7.6; 1 mM EDTA, pH 8.0). SDS-PAGE was performed according to the protocol developed by Laemmli (1970) using a polyacrylamide gradient gel with a concentration range of 5–20% [17]. The gel was prepared from a separating gel (5 mL 30% acrylamide/bisacrylamide solution [29:1], 2.5 mL 1.5 M Tris, pH 8.8, 2.3 mL dH_2O, 100 µL 10% SDS, 100 µL 10% APS, 5 µL TEMED) and a stacking gel (1.35 mL 30% acrylamide/bisacrylamide solution [29:1], 1 mL 1 M Tris, pH 6.8, 7.2 mL dH_2O, 100 µL 10% SDS, 100 µL 10% APS, 5 µL TEMED). Samples were mixed with β-mercaptoethanol (1:3). Electrophoresis was carried out at 120 V, 110 mA, and 50 W using 1×TGS buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS) for 2–3 hours.

Protein transfer and immunodetection

Proteins were transferred onto nitrocellulose membranes using a Mini Trans-Blot® Cell (Bio-Rad). Transfer efficiency was verified by staining the membranes with Ponceau S. Non-specific binding sites were blocked with 7.5% non-fat dry milk in TBS-Tween-20 (50 mM Tris, 200 mM NaCl, 0.25% Tween-20). Membranes were then washed three times for 7 min each in 1× TBS-Tween-20.

Immunodetection was performed using monoclonal primary antibodies against HSP70 (Agrisera, #AS08371) and HSP90 (Agrisera, #AS08346), as well as polyclonal primary antibodies against P19. Secondary anti-rabbit antibodies (Rockland Immunochemicals, 611-1502) were applied. Protein bands were visualized by incubating the membranes with NBT/BCIP substrate in the dark for 5 min.

Native-PAGE

A 7% polyacrylamide gel was prepared under native conditions to determine enzyme activity. The separating gel contained 3.75 mL N3 buffer (pH 8.5; 1 M Tris base, 2 M Tris-HCl), 2.82 mL N5 buffer (40% acrylamide/bisacrylamide solution, 19:1), 8.44 mL dH_2O, 100 µL APS, and 5 µL TEMED. The stacking gel contained 5 mL N6 buffer (6.24% acrylamide/bisacrylamide solution, 4:1), 2.5 mL N4 buffer (pH 6.9; 18 mM Tris base, 2 mM Tris-HCl), 2.5 mL dH_2O, 100 µL APS, and 5 µL TEMED.

Electrophoresis was carried out at 150 V, 120 mA, and 50 W in the UPPER buffer (pH 8.88; 50 mM Tris, 7 mM Tris-HCl, 60 mM glycine) for 2–3 h. To determine AO activity, gels were incubated in a solution containing 4 mM Tris-HCl, 3 mM indole-3-carboxyaldehyde, 0.6 mM MTT, and 0.7 mM PMS at 37 °C for 40 min [18].

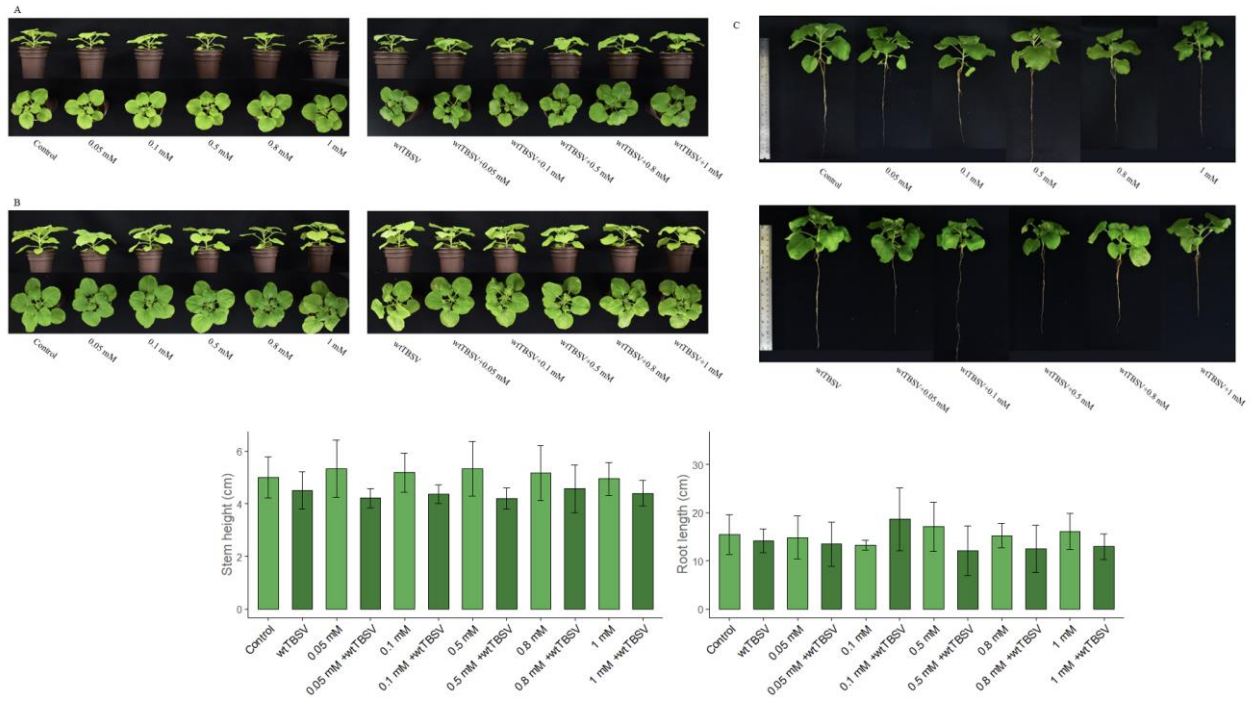
Catalase activity was determined by incubating samples with 0.03% hydrogen peroxide solution (500 µL H_2O_2 in 49.5 mL distilled water) for 10 min. Subsequently, 0.6 g $K_3[Fe(CN)_6]$ dissolved in 26.4 mL distilled water and 0.6 g $FeCl_3$ dissolved in 26.4 mL distilled water were added, and the reaction was incubated in the dark for 3 min [19].

Results

Nicotiana benthamiana plants were inoculated with wtTBSV, followed by irrigation with Fe-EDTA solution starting at 2 days post-inoculation (dpi) (Figure 1A). At 7 dpi, plants exhibited symptoms such as chlorosis and leaf wilting. Under combined stress, however, symptoms of viral infection were alleviated compared with control plants inoculated only with wtTBSV. The most pronounced improvement was observed at 0.8 mM and 1 mM Fe-EDTA.

Plants inoculated with wtTBSV displayed typical symptoms of TBSV infection, including leaf curling, chlorosis, growth suppression, necrotic spots, and wilting (Figure 1B). Morphometric analysis showed that the average shoot height of control plants was 5.0 ± 0.7 cm. Irrigation with Fe-EDTA at concentrations ranging from 0.05 to 1 mM did not significantly affect shoot growth ($p > 0.05$) (Figure 1C). In wtTBSV-infected plants, shoot height decreased to 4.5 ± 0.6 cm, and under combined stress, the reduction was more pronounced.

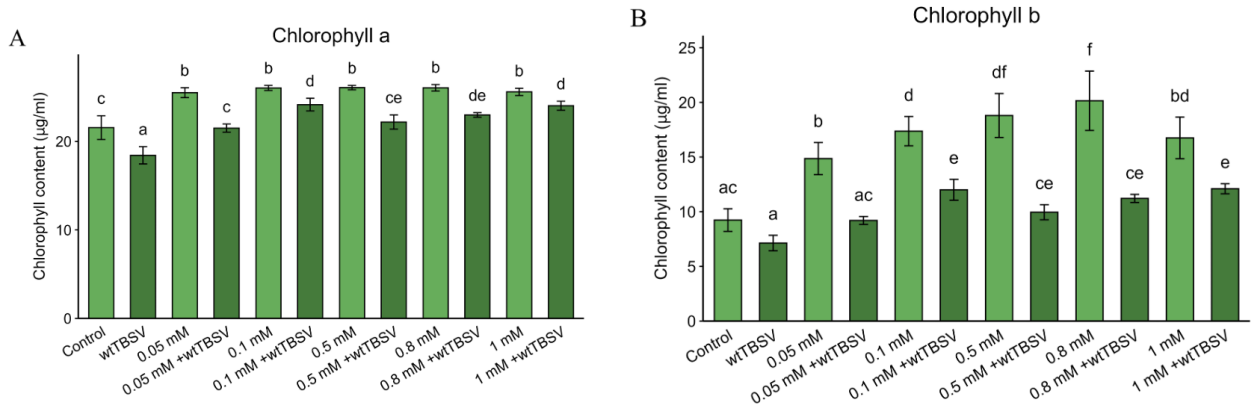
Analysis of root length revealed an average of 15.8 ± 4.2 cm. Fe-EDTA irrigation at concentrations from 0.05 to 1 mM had no significant effect compared with the control ($p > 0.05$), although an increase was observed at 0.5 mM (17.1 ± 4.8 cm) and a decrease at 0.1 mM (13.2 ± 0.9 cm). In wtTBSV-infected plants, root length moderately decreased to 14.2 ± 2.3 cm. Under combined stress, root length increased to 18.9 ± 6.8 cm at wtTBSV+0.1 mM, whereas at wtTBSV+0.8 mM it decreased to 10.0 ± 5.2 cm. However, these changes did not reach statistical significance ($p > 0.05$).

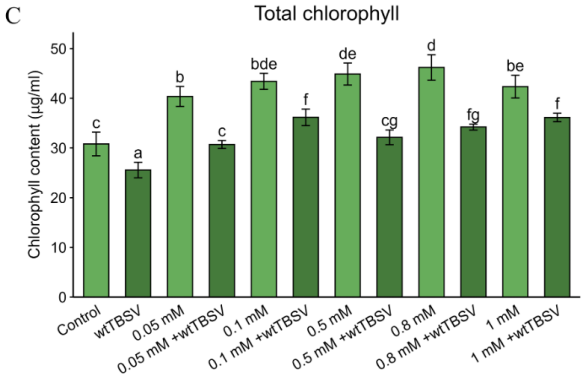


Note: Differences between groups were considered statistically significant at $p < 0.001$. Data were analyzed using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

Figure 1. Morphometric parameters of *Nicotiana benthamiana*. **(A)** Day 1 after inoculation with wtTBSV and Fe-EDTA irrigation; **(B)** Day 7 after inoculation with wtTBSV and Fe-EDTA irrigation; **(C)** Morphometric measurements of plant shoots and roots

To assess the activity of the photosynthetic apparatus, the contents of chlorophyll a, chlorophyll b, and total chlorophyll were analyzed in *N. benthamiana*. It was found that infection with wtTBSV significantly reduced chlorophyll levels compared to control plants. Treatment with Fe-EDTA markedly increased chlorophyll levels, with the highest values observed at 0.1 mM and 0.5 mM Fe-EDTA. Under combined stress, chlorophyll content was restored to control levels or even exceeded them (Figure 2).





Note: Differences between groups were considered statistically significant at $p < 0.001$. We performed the analysis using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

Figure 2. Analysis of chlorophyll content in the leaves of *N. benthamiana* plants. **(A)** Chlorophyll a content; **(B)** Chlorophyll b content; **(C)** Total chlorophyll content

Differences between groups were considered statistically significant at $p < 0.001$. We performed the analysis using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

To determine the levels of host proteins HSP70, HSP90, and the viral RNA silencing suppressor P19, western blot analysis was performed. The detection of the viral protein P19 was carried out using polyclonal antibodies against P19. Under combined stress conditions (Fe-EDTA + wtTBSV), the level of the viral protein P19 was significantly reduced compared to the wtTBSV control (Figure 3A). In addition, the levels of host proviral heat shock proteins (HSPs) were examined. The level of HSP70 increased at Fe-EDTA concentrations of 0.05 mM, 0.1 mM, and 1 mM, but decreased at 0.5 mM and 0.8 mM. Under combined stress, the lowest levels were observed at 0.05 mM, 0.5 mM, and 0.8 mM Fe-EDTA, whereas a significant increase was recorded at 1 mM (Figure 3B). Analysis of HSP90 revealed maximum accumulation in control plants and in response to 0.05 mM Fe-EDTA, while the lowest accumulation was observed at 1 mM Fe-EDTA. Under combined stress conditions, HSP90 levels remained moderate compared to wtTBSV but also decreased at 1 mM Fe-EDTA, similar to the results observed with Fe-EDTA treatment alone (Figure 3C).

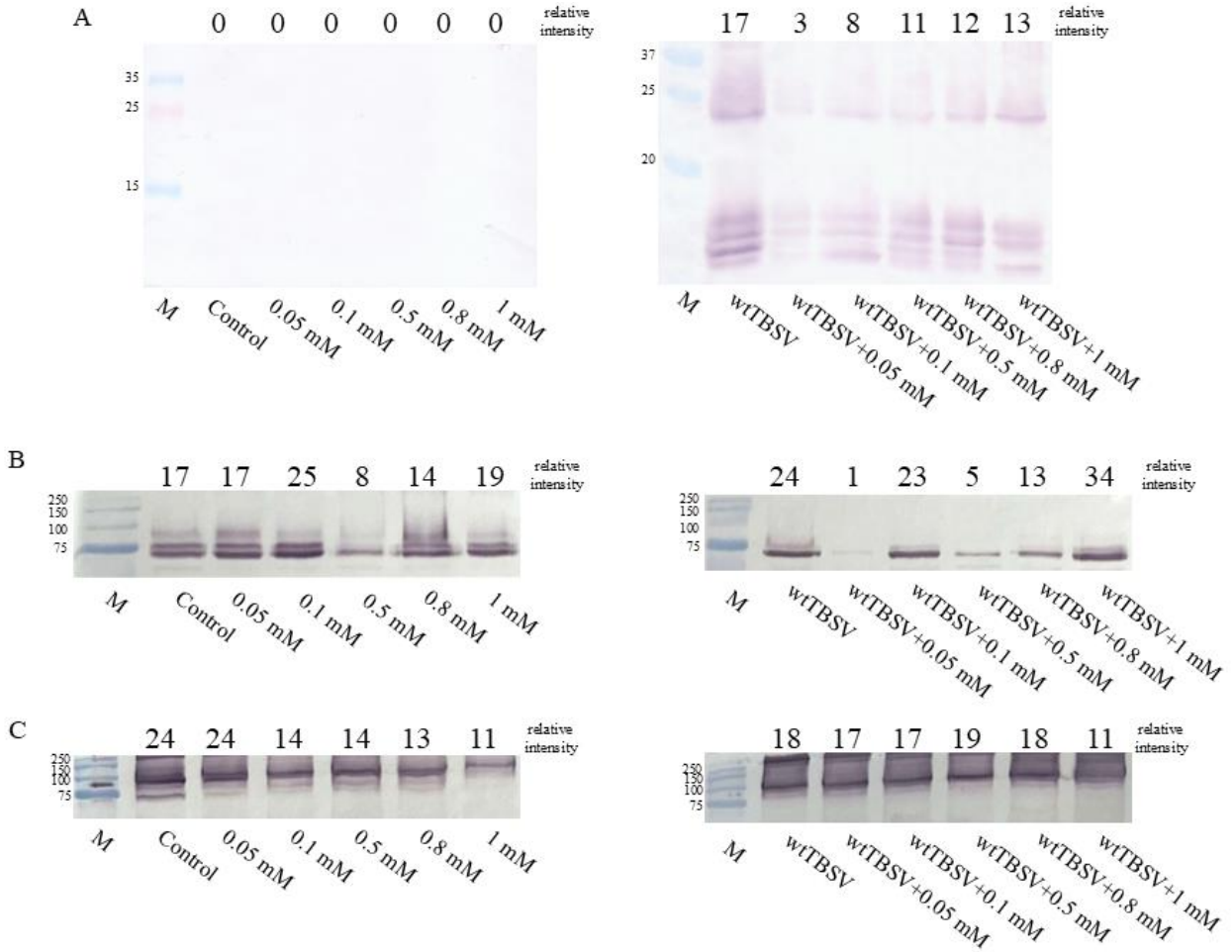


Figure 3. Western blot analysis of P19, HSP70, and HSP90 in *N. benthamiana* leaves. **(A)** Immunoblot detection of the viral protein P19. **(B)** Immunoblot detection of HSP70. **(C)** Immunoblot detection of HSP90. Data were analyzed using Image J. All experiments included at least three biological replicates

Discussion

The term ferroptosis was first described in animals as a novel form of cell death triggered by erastin in tumor cells [20]. Later, ferroptosis was also identified in plants [21], where it is activated in response to various abiotic and biotic stresses [22], including Fe^{2+} overload, accumulation of reactive oxygen species (ROS), and deficiencies in antioxidant defenses, particularly glutathione-dependent antioxidants such as GPX4 [23,24]. Lipid peroxidation in the membranes of mitochondria, chloroplasts, and peroxisomes, where ROS and iron-dependent radicals accumulate, has been shown to act as the key trigger of ferroptosis initiation [25,26]. Damage to membrane lipids disrupts membrane integrity and suppresses respiration and photosynthesis, thereby initiating a cascade of ferroptosis-like cell death.

Ferroptosis is considered a relatively recently described type of programmed cell death, and therefore, information about its regulation in plants under abiotic and biotic stresses remains fragmented. Consequently, the influence of viruses on ferroptosis is still unclear.

Our study demonstrated that Fe-EDTA treatment significantly influenced plant development and the symptoms of wtTBSV infection in *N. benthamiana*, as well as the levels of host heat shock proteins (HSPs) and the viral suppressor P19.

To analyze ferroptosis-like cell death, *N. benthamiana* plants were treated with Fe-EDTA solutions at varying concentrations to determine the optimal range. Fe-EDTA was selected as the iron source because its chelated form ensures high bioavailability and promotes the accumulation of Fe^{2+} ions in plant cells. The buildup of free iron ions triggers the Fenton reaction, which generates hydroxyl radicals (·OH):

$$Fe^{2+}+H_2O_2 \longrightarrow Fe^{3+}+OH^-+\cdot OH$$

Subsequently, the hydroxyl radical (·OH) reacts with polyunsaturated fatty acids (PUFAs), generating lipid radicals. This initiates a chain reaction in which the lipid radicals propagate by reacting with other lipids, leading to the accumulation of reactive oxygen species (ROS) [23]. Ultimately, this process results in damage to organelle membranes, proteins, and DNA [24].

In the assessment of physiological and morphometric parameters, we found that the effects of Fe-EDTA treatment and viral infection were accompanied by symptoms such as growth inhibition, leaf mottling, curling, necrosis, and chlorosis (Figure 1A, B). Iron (Fe) is essential for many cellular functions in plants, including chlorophyll biosynthesis, photosynthesis, and respiration [27]. It is well established that viral infection reduces photosynthesis and causes severe alterations in the ultrastructure of chloroplasts [28]. Previous studies have shown that treatment with iron oxide nanoparticles (Fe_3O_4) at high concentrations under high light intensity (300 μM $m^{-2}\cdot s^{-1}$) significantly increased plant biomass, as well as the contents of chlorophyll a, chlorophyll b, and carotenoids in leaves. At the same time, the nanoparticles did not have a noticeable effect on primary photochemical processes or stomatal conductance. Treatment with Fe_3O_4 nanoparticles led to a reduction in malondialdehyde (MDA) levels in roots and leaves, indicating the absence of oxidative stress. This was supported by increased activity of antioxidant enzymes, such as ascorbate peroxidase (APX) and superoxide dismutase (SOD). The authors suggest that elevated iron content in leaves promotes higher chlorophyll levels and enhances the activity of enzymes such as RuBisCO, ultimately increasing the rate of CO_2 assimilation [29]. An increase in chlorophyll content was also observed following foliar application of $Fe_2(SO_4)_3$ and EDTA-Fe·Na fertilizers to potato (Solanum tuberosum L.) tubers [30]. Iron deficiency in pea (Pisum sativum L.) plants led to a reduction in chlorophyll a and b, accompanied by an increase in dry biomass per unit of fresh shoot weight. This resulted in a significant decrease in photosynthetic rate per leaf area, as well as increased stomatal resistance and reduced transpiration rate. However, under partial iron deficiency, the photosynthetic rate per leaf area was not reduced, even though chlorophyll content decreased [31]. This may be because the reduction in chlorophyll likely did not result from the destruction of all components of the photosynthetic apparatus, but rather from an adaptive response to stress conditions. In our study, we also observed an increase in chlorophyll content following both Fe-EDTA treatment and combined stress from wtTBSV infection and Fe-EDTA, compared to control plants (Figure 2). This effect is likely due to the crucial role of iron as a component of various photosynthetic electron carriers, such as cytochrome (Cyt) b_6f and Cyt c_6, and as an integral part of both photosystem I (PSI) and photosystem II (PSII) [32–34]. Iron deficiency induces significant changes in the structure of the thylakoid membrane and in the core processes involved in photochemical energy conversion. The first transcriptomic studies conducted on

P. tricornutum cells under iron-deficient conditions identified novel regulators of the iron deficiency response, such as iron starvation-induced proteins (ISIPs) [35].

Tomato bushy stunt virus (TBSV) is a typical member of the genus Tombusvirus within the family Tombusviridae. The P19 protein encoded by TBSV acts as a potent suppressor of RNA interference (RNAi) and blocks a key post-transcriptional defense mechanism in plants. RNAi is a conserved eukaryotic mechanism that degrades RNA with high sequence specificity [36–38]. A DICER-containing complex recognizes and cleaves double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA) with hairpin structures, producing short interfering RNAs (siRNAs) of approximately 20–25 nucleotides. The RNA-induced silencing complex (RISC) incorporates these siRNAs and directs the endonucleolytic cleavage of complementary viral RNAs, thereby limiting viral replication. P19, however, binds viral siRNAs (vsiRNAs) with high affinity, preventing their incorporation into RISC. By doing so, P19 inhibits the RNAi pathway, allowing the virus to evade degradation [39–41].

In our study, combined stress from wtTBSV infection and Fe-EDTA treatment led to a reduction in the level of the viral suppressor P19 compared to virus-infected control plants. The decrease in P19 protein levels indicates an effective RNAi response, resulting in a reduced wtTBSV viral titer in N. benthamiana (Figure 3A). Interestingly, at higher Fe-EDTA concentrations, P19 protein levels increased compared to 0.1 mM Fe-EDTA, highlighting the dose-dependent role of iron in regulating cellular metabolism. Similar studies in Cucumis sativus L. under combined stress with Glomus mosseae showed significant improvements in key physiological parameters, including chlorophyll content, photosynthetic rate, stomatal conductance, and accumulation of phenolic compounds. These results also demonstrated that combined stress increased the activity of antioxidant enzymes such as SOD, POD, CAT, and APX, mitigating oxidative stress and promoting plant health [42]. Previous studies have shown that foliar application of Fe-EDTA at a concentration of 3.36 mg·L^{-1} in N. benthamiana plants infected with potato virus Y (PVY) also reduced symptom severity and suppressed the accumulation of viral RNA and proteins, particularly during the early stages of infection [43]. These findings support our results showing a reduction in viral titer in plants undergoing ferroptosis-like cell death. As noted earlier, viral infection suppresses photosynthesis. Iron treatment may assist the plant by regulating genes associated with cytochromes, chlorophyll, and photosystems (PSI and PSII). Similar observations were reported by Bwalya, Alazem, and Kim (2021), where photosynthesis-related genes (PSaC and ATPsyn-α) conferred resistance to soybean mosaic virus (SMV) in N. benthamiana through RNAi [44]. Additionally, Xu et al. (2023) employed virus-induced gene silencing (VIGS) and found that suppressing the expression of the NbHSP90 gene in N. benthamiana significantly increased PVY accumulation [43].

Heat shock proteins (HSPs), particularly HSP70, play a key role in the formation of the viral replication complex. HSP70 interacts with the viral protein p33 and activates RNA-dependent RNA polymerase (RdRp), ensuring efficient viral genome replication. HSP90 also contributes by stabilizing and activating the viral RdRp. Together with the cofactor CDC37, HSP90 forms a complex with the viral RdRp p92, which is essential for initiating replication [45–48]. The role of HSP proteins in host cells is critical, as they participate in protein folding, prevent the aggregation of denatured proteins, and maintain cellular homeostasis under stress conditions [9]. Analysis of HSP70 protein expression showed a significant decrease under combined stress compared to control plants, except for wtTBSV + 1 mM Fe-EDTA (Figure 3B). These results suggest possible degradation of host proteins, in which HSPs normally play a protective role, as also indicated by HSP70 expression under single stress with 1 mM Fe-EDTA. Interestingly, treatment with 0.5 mM Fe-EDTA caused a marked reduction in HSP70 expression compared to

control plants. Analysis of HSP90 expression revealed that under combined stress (wtTBSV + 1 mM Fe-EDTA), its level significantly decreased (Figure 3C).

Based on all our experiments, we propose that the combined stress of wtTBSV infection and 1 mM Fe-EDTA causes more severe damage to plant cells. We suggest that high iron concentrations induce an enhanced form of programmed cell death, ferroptosis. Accumulation of Fe^{2+} and the activation of lipid peroxidation may trigger localized necrosis by disrupting cellular membranes, which in turn reduces viral titer in the plants. In contrast, lower Fe concentrations help maintain the photosynthetic apparatus in N. benthamiana.

Conclusion

Our study provided new insights into the role of ferroptosis-like cell death in plant-virus interactions with TBSV. We showed that Fe-EDTA treatment alters the accumulation of wtTBSV in *N. benthamiana*. High concentrations of Fe-EDTA induced oxidative stress and ferroptosis-like cell death, accompanied by membrane damage, necrosis, and growth inhibition, while simultaneously leading to a decrease in the level of the suppressor protein P19. At the same time, low concentrations of Fe-EDTA maintained photosynthetic activity and alleviated symptoms. In addition, a decrease in HSP70 and HSP90 was observed under combined stress, suggesting a possible interaction of iron with host chaperones required for viral replication.

Thus, viral infection accumulation is regulated by iron availability in the cell: limited iron supply supports photosynthesis and RNA interference activation, whereas its excess may trigger ferroptosis-like cell death pathways. These findings highlight the importance of iron homeostasis in studying plant defense mechanisms and open perspectives for the practical application of iron-containing treatments to control viral diseases. However, further studies are needed to clarify the precise mechanisms of interaction between viral infection and ferroptosis-like cell death in plants.

Author Contributions

N.I. and **Z.M. –** conceptualization; **N.I.** and **D.A. –** methodology; **N.I., D.A., T.E., S.B., Z.B.,** and **Z.T. –** validation; **N.I.** and **Z.M. –** investigation; **N.I., D.A.,** and **Z.B. –** data curation; **N.I., D.A., T.E., S.B.,** and **Z.B. –** writing-original draft preparation; **N.I.** and **D.A. –** writing-review and editing; **D.A., T.E., S.B.,** and **Z.T. –** visualization; **N.I. –** supervision; funding acquisition **– N.I.** All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain any description of studies performed by the authors involving human subjects or using animals as objects.

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Темір гомеостазының бұзылуы, ферроптоз тәрізді жасушалық өлімі және өсімдіктердегі РНҚ интерференциясы арқылы вирустық инфекцияны басуы

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Аңдатпа. Ферроптоз-тәрізді жасушалық өлім - бұл өсімдік жасушаларында темірдің жиналуы мен липидтердің асқын тотығуынан қоздырылатын жаңадан сипатталған механизм. Алайда оның вирустық инфекциядағы рөлі әлі толық анықталмаған. Осы зерттеуде *Nicotiana benthamiana* өсімдіктеріне темірмен (Fe-EDTA) өңдеудің, wild type tomato bushy stunt virus (wtTBSV) инфекциясы жағдайындағы ықпалы талданды. Кешенді стресс жағдайында жүргізілген морфологиялық және биохимиялық талдау өсімдіктер өсуінің тежелуі, некроз, жапырақтардың сарғаюы, хлороз және жапырақтардың жиырылуы сияқты айқын симптомдардың байқалғанын көрсетті, ал супрессорлық P19 ақуызының экспрессиясы төмендеді. Биохимиялық талдау жағынан Fe-EDTA төмен концентрациялары өсімдіктердің фотосинтетикалық аппараттың тұрақтылығын қамтамасыз етіп, хлорофилл құрамын арттыратыны анықтады, ал жоғары концентрациялар липидтердің асқын тотығуын және ферроптоз тәрізді жасуша өлімін тудырды. Алынған нәтижелер темірдің артық мөлшерінде РНҚ-интерференциясының белсенуі мен ферроптоз тәрізді жасушалық өлімнің іске қосылатынын, сондай-ақ осы жағдайда вирустық инфекцияның тежелетінін дәлелдейді. Ал темірдің төмен концентрацияларында фотосинтетикалық

белсенділік сақталып, симптомдардың айқындылығы төмендеді. Сонымен қатар, кешенді стресс жағдайында HSP70 және HSP90 жылулық шок ақуыздарының деңгейі төмендегені анықталды, бұл микроэлементтік гомеостаздың вирус репликациясына қажетті жасушалық шаперондардың жұмысына араласуын көрсетуі мүмкін. Бұл зерттеу ферроптозға ұқсас жасушалық өлімнің вирус пен өсімдік арасындағы өзара әрекеттесудегі негізгі рөлін көрсетеді.
Түйін сөздер: ферроптоз, өсімдік вирустары, құрама стресс, белсенді оттек түрлері (БОТ), TBSV

Нарушение гомеостаза железа подавляет вирусную инфекцию посредством клеточной смерти, подобной ферроптозу, и РНК-интерференции в растениях

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Аннотация. Ферроптоз-подобная клеточная гибель у растений [1] это недавно описанный механизм, запускаемый накоплением железа и перекисным окислением липидов, однако его роль в вирусной инфекции остаётся неясной. В данном исследовании было проанализировано влияние обработки железом (Fe-EDTA) на растения *Nicotiana benthamian*а, инфицированные wild type tomato bushy stunt virus (wtTBSV). Морфологические и биохимические анализы растений под воздействием комбинированного стресса показали более выраженные симптомы, такие, как угнетение роста растений, некроз, пожелтение листьев, хлороз и сморщивание листьев, тогда как экспрессия супрессорного белка P19 снижалась. Биохимический анализ выявил, что низкие концентрации Fe-EDTA поддерживали фотосинтетический аппарат растений и повышали содержание хлорофиллов, тогда как высокие концентрации индуцировали перекисное окисление липидов и ферроптоз-подобную гибель клеток. Результаты указывают на активацию РНК-интерференции и ферроптоз-подобной клеточной гибели в условиях избытка железа, при котором вирусная инфекция подавлялась. В то время как при низких концентрациях железа сохранялась фотосинтетическая активность и снижалась выраженность симптомов. Кроме того, было выявлено снижение уровня белков теплового шока HSP70 и HSP90 при комбинированном стрессе, что может отражать вмешательство микроэлементного гомеостаза в работу клеточных шаперонов, необходимых для репликации вируса. Это указывает на ключевую роль ферроптоз-подобной клеточной гибели во взаимодействии вирус-растение.
Ключевые слова: ферроптоз, вирус растений, комбинированный стресс, активные формы кислорода (АФК), TBSV

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HLA system nomenclature and discovery of novel allelic variants in the Kazakh population

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Abstract. The human leukocyte antigen (HLA) system is among the most genetically diverse in humans, encompassing over 220 genes that encode immune proteins essential for transplant compatibility, immune regulation, and disease susceptibility. This review outlines the fundamentals of HLA nomenclature, standardized by the World Health Organization (WHO) and curated in the IPD-IMGT/HLA database. We describe the gradual improvements in HLA typing methods, ranging from serological assays to molecular-based techniques, including Sanger sequencing and next-generation sequencing (NGS), as well as interpretation software, and evaluate their strengths and limitations in allele discovery. We discuss allelic variants of HLA genes, methods for sequencing HLA alleles, and their variants. Additionally, we report the identification of four novel HLA alleles in the Kazakh population: *DQB1*03:82*, *C*06:256*, *B*13:150*, and *A*32:95*. All four alleles feature non-synonymous substitutions within peptide-binding domains, suggesting potential immunological relevance. Comparative analysis reveals that NGS enhances allele detection efficiency by 2.8-fold compared to Sanger sequencing (one novel allele per 635 typings vs. 1,773). These findings demonstrate the significant HLA diversity present in Central Asian populations, which remain underrepresented in global databases. The identification of population-specific alleles reveals critical gaps in international donor databases, underscoring the urgent need to expand HLA profiling in ethnically diverse regions to improve transplant outcomes and advance personalized immunotherapy.
Keywords. Allelic variants, HLA typing, IPD-IMGT/HLA database, Kazakhstan, Next-generation sequencing

Introduction

The genes encoding human leukocyte antigens (HLA) are known for their high level of polymorphism, making the systematic classification of HLA genes essential. Located on the short arm of chromosome 6 at position 6p21.3, the HLA complex includes over 220 genes, many of which play crucial roles in immune function. Oversight of HLA allele naming and quality

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control is managed by the WHO Nomenclature Committee for Factors of the HLA System [1]. Comprising both classical (HLA-A, -B, -C, -DRB1, -DQB1, -DPB1) and non-classical loci, the HLA complex displays remarkable genetic diversity that has evolved under pathogen-driven selective pressures. This diversity enables populations to respond to various infectious challenges, although it also makes clinical applications such as transplantation and disease association studies more complex.

Understanding HLA diversity across global populations is essential for several reasons. First, successful hematopoietic stem cell transplants depend on close HLA matching between donors and recipients, yet European and North American populations are overrepresented in donor registries. Second, certain HLA alleles are associated with either increased risk or protection against autoimmune diseases, infectious diseases, and adverse drug reactions, making population-specific HLA analysis important for medical purposes. Third, the extensive polymorphism of HLA genes provides a valuable model for studying balancing selection and host-pathogen coevolution.

Established in 1968, the committee set the initial criteria for nomenclature and has since met regularly, publishing 19 key reports that have progressed from identifying HLA antigens to documenting genes and alleles. Standardization efforts were traditionally supported through the exchange of reagents and reference cells via the International Histocompatibility Workshops. The systematic naming of HLA allele sequences began in 1989, marking a significant milestone in immunogenetics. Since then, the development and maintenance of a centralised sequence database have become essential. The assignment and distribution of allele names are particularly important in clinical settings. Thanks to the efforts of the HLA biinformatics team, in collaboration with the European Bioinformatics Institute (EBI), this information is publicly accessible through both the EBI website (http://www.ebi.ac.uk/ipd/imgt/hla) and http://hla.alleles.org [2-4].

The IPD-IMGT/HLA database compiles newly identified and validated HLA sequences, which undergo expert curation and approval by the WHO Nomenclature Committee. Only sequences meeting strict quality standards are incorporated into the database and associated resources available on the official platforms. Continuous updates ensure that researchers and clinicians worldwide have access to the latest HLA allele data [5].

Molecular genetic diagnostics has become one of the fastest-growing fields in the detection and management of various diseases. The introduction of the polymerase chain reaction (PCR) technology, a method of specific DNA amplification developed over the past two decades, marked a significant turning point in the clinical application of genetic testing [6]. Today, next-generation sequencing (NGS) is widely employed for the diagnosis and prediction of graft rejection in organ and hematopoietic stem cell transplantation. This technology is also used to detect leukocyte antigens associated with specific diseases. In these applications, the patient's polymorphic HLA variants are compared with those of potential donors. A donor with a fully or closely matched HLA genotype is selected to reduce the risk of transplant rejection and improve the overall success of transplantation procedures [8].

History

The role of the major histocompatibility complex (MHC) in allograft rejection was first suggested by Bover [9], who noted that skin grafts between identical twins were not rejected in the same manner as those from genetically dissimilar individuals. The MHC genes implicated in the allograft rejection process were initially characterized in mice by Gorer [10].

Following this, Snell [11] utilized mouse cell lines to further delineate a locus known as H for histocompatibility. Gorer [10] referred to the gene products of locus H as antigens II, leading to the combined designation H-2 for the mouse MHC. The human leukocyte antigen (HLA) system was subsequently identified in the 1950s. Multiple researchers independently found that sera from individuals who had received previous blood transfusions and from multiparous women contained antibodies that agglutinated leukocytes [12]. This finding led to the development of serological typing methods that identified a single locus, which was later divided into two loci: HLA-A and HLA-B [13-16].

Initially, various techniques were employed for serological typing; however, the microlymphocytotoxicity assay became the most common method. It was later observed that when lymphocytes from unrelated individuals matched at the HLA-A and HLA-B loci and were cultured together in a "mixed lymphocyte culture" (MLC) [17,18], they exhibited a strong proliferative response. This led to the discovery of an additional locus initially called HLA-D [19-21]; it was later shown that mismatches at HLA-DR and HLA-DQ contributed to the lymphocyte activation seen in the MLC. Extensive serological studies soon revealed the existence of HLA-C [22]. Later, HLA-DP, originally known as the "secondary B cell" (SB) antigen, was discovered through a secondary stimulation assay called the "primed lymphocyte test" (PLT), which indicated recognition of another HLA molecule distinct from those identified in the primary mixed lymphocyte culture [23,24].

The IPD-IMGT/HLA database and nomenclature committee

Since its launch in 1998, the IPD-IMGT/HLA database has served as a central repository for information on immune system gene polymorphisms. The initial version included 964 HLA allelic variants, and the database has since expanded considerably, becoming a vital reference resource for HLA researchers and clinicians [5]. In addition to storing allele sequences, the database provides comprehensive metadata about the biological source of each sequence and the methods used for sequence validation. Today, it is standard practice for researchers to submit newly identified sequences directly to the IPD-IMGT/HLA database for expert review and official naming before publication.

This approach helps prevent confusion caused by the renaming of already published sequences or the use of multiple identifiers for the same allele [3]. The growing importance of timely reporting of novel HLA allele sequences has led to the regular convening of the WHO Nomenclature Committee for HLA Factors, with annual meetings dedicated to maintaining the consistency and accuracy of HLA nomenclature. To ensure rapid access to newly approved sequences, monthly nomenclature updates are also published online and in scientific journals.

In collaboration with the Imperial Cancer Research Fund (ICRF; now part of Cancer Research UK) and the European Bioinformatics Institute (EBI), a sophisticated Oracle-based database was developed. This system enables users to perform complex queries and access detailed data on sequence features, references, contact information, and official allele designations via a user-friendly graphical web interface. The initial creation of this database was supported by European Union BIOMED1 (BIOMED1-930038) and BIOTECH2 (BIOTECH-960037) grants awarded to the ICRF as part of the International Immunogenetics (IMGT) initiative.

Development and curation of the HLA database were carried out in collaboration with Julia Bodmer (ICRF), James Robinson (formerly at ICRF, now with the HLA Informatics Group), and Peter Parham (Stanford University). The WHO Nomenclature Committee is currently chaired by Professor Steven G.E. Marsh, and its headquarters are located at the Anthony Nolan Research

Institute in London, UK [25]. Since its establishment, the committee has held 18 international meetings, covering a wide range of topics, from classical serological HLA typing methods to advanced molecular genetic techniques, including mixed lymphocyte reactions, PCR-based DNA typing, and next-generation sequencing (NGS). A summary of these international workshops is provided in Table 1, detailing the seminars organized under the auspices of the WHO Nomenclature Committee for HLA.

Table 1

International seminars of the WHO nomenclature committee for HLA

Seminar	Year	Committee Chair	Location	Seminar topic
1	1964	DB Amos	Durham, USA	Determination of the specificity of antigens Hu-1, LA, and Four
2	1965	JJ van Rood	Leiden, The Netherlands	Testing of mixed lymphocyte culture.
3	1967	R Ceppellini	Torino, Italy	Family studies; HLA in kidney transplantation
4	1970	PI Terasaki	Los Angeles, USA	Determination of the specificity of 27 HLA-A, HLA-B, and HLA-C
5	1972	J Dausset	Evian, France	Typing of 49 populations worldwide
6	1975	F Kissmeyer-Nielsen	Aarhus, Denmark	Description of the characteristics of Dw.
7	1977	WF Bodmer	Oxford, UK	Determination of the characteristics of DR1-7; HTC testing
8	1980	PI Terasaki	Los Angeles, USA	Determination of MB (DQ) and MT (DR52/53); HLA in transplantation and diseases.
9	1984	EA Albert/W Mayr	Munich, Germany Vienna, Austria	New features of class I and II; HLA class II in kidney transplantation
10	1987	B Dupont	Princeton, USA	Creation of RFLP; T-cell clones; GTK methods; Biochemistry of 1D IEF, 2D gels; Creation of a panel of homozygous cell lines.
11	1991	T Sasazuki/K Tsuji/M Aizawa	Yokohama, Japan	DNA PCR typing of HLA class II; Anthropology.
12	1996	D Charron	St Malo/Paris, France	DNA PCR typing of HLA class I; Anthropology.
13	2002	J Hansen	Victoria, Canada Seattle, USA	Virtual DNA analysis; Identification of SNP markers; Anthropology; Disease association; GWAS (Genome-Wide Association Studies)
14	2005	J McCluskey	Melbourne, Australia	MHC and anthropology; Disease; Infectious disease; GWAS (Genome-Wide Association Studies); Cancer; KIR (Killer Immunoglobulin-like Receptors); Cytokine genes.

15	2008	M Gerbase de Lima/ME Moraes	Buzios/Rio de Janerio, Brazil	Anthropology; GWAS (Genome-Wide Association Studies); Informatics.
16	2012	SGE Marsh/D Middleton	Liverpool, UK	NGS (Next-Generation Sequencing), GWAS (Genome-Wide Association Studies)
17	2012	M Fernandez-Viña	Asilomar, USA	NGS
18	2021	S Heidt/E Spierings	Amsterdam, The Netherlands	Topic under clarification

Note: *(website source https://hla.alleles.org/nomenclature/workshops.html)

The International ImMunoGeneTics Information System (IMGT) is a comprehensive set of databases and bioinformatics tools dedicated to immunogenetics and immunoinformatics. It focuses on the sequences of V (variable), D (diversity), J (joining), and C (constant) genes, which are crucial components of the adaptive immune system. IMGT was established in June 1989 by Marie-Paule Lefranc, an immunologist at the University of Montpellier. The initiative was formally introduced at the 10th Human Genome Mapping Workshop, where the V, D, J, and C regions were officially recognized as genes. The first IMGT database, IMGT/LIGM-DB, was created to compile nucleotide sequences of human immunoglobulins and T-cell receptors, later expanding to include sequences from various vertebrate species. The project was launched at the Laboratoire d'Immunogénétique Moléculaire at the University of Montpellier and supported by the French National Centre for Scientific Research (CNRS) [26].

Given that T-cell receptors and immunoglobulins are generated through somatic recombination of nucleotide segments, the genomic annotation of these gene regions poses unique challenges. To address this, IMGT-NC, the nomenclature committee, was formed in 1992 to provide standardized terminology. This subcommittee is officially recognized by the International Union of Immunological Societies [1]. In addition to its databases, IMGT offers several key tools:

• IMGT/Collier-de-Perles: provides a two-dimensional graphical representation of receptor amino acid sequences.

• IMGT/mAb-DB: a curated database of monoclonal antibodies.

• IPD-IMGT/HLA database: a critical resource for HLA allele data maintained by the HLA Informatics Group, was developed in part through IMGT's efforts and remains integrated within its broader infrastructure [2,25].

Polymorphisms

The HLA system is the most polymorphic genetic system in the human genome. HLA polymorphisms were first identified phenotypically by observing acceptance or rejection of tissue and/or through reactions with specific alloantibodies (serological typing methods). Later, molecular typing methods revealed HLA polymorphisms that range from a single nucleotide change to the loss or gain of an entire genetic region. Initially, HLA polymorphisms were identified using serological and cell proliferation assays [14-16, 21]. These methods were primarily used to characterise the HLA system; however, despite their widespread use, they have notable limitations in accuracy and reproducibility [26]. Moreover, alloimmune antisera are often limited in supply, and both serological and cellular HLA typing require live cells. Overall, a key limitation of serological HLA typing is its inability to detect minor polymorphic differences that can activate CD4+ or CD8+ T lymphocytes.

A general characteristic of the HLA genes is that the distal membrane domains are highly polymorphic. In contrast, the proximal membrane domains, the transmembrane, and cytoplasmic domains have very low or no polymorphisms. The heavy chain of the HLA class I molecule is composed of 3 extracellular domains, and both the α and β chains of the HLA class II molecule contain 2 extracellular domains (Figure 3). Of note, the HLA genes, like all eukaryotic genes, contain both coding (exons) and non-coding (introns) regions. The HLA class I genes contain 8 exons, while the HLA class II genes contain 6 or 7 exons [27,28]. The widespread application of molecular typing techniques has enabled the characterization of thousands of HLA alleles [29]. The current number of HLA alleles is shown in Table 4 and can also be found on the IPD-IMGT/HLA Database [30,31].

Analysis of the nucleotide sequences of the HLA genes indicates that most of the polymorphisms are found in exons 2 and 3 of the HLA class I genes and in exon 2 of the HLA class II genes. These exons encode for the distal membrane domains called the "peptide-binding region" [2, 29-31]. It has been observed that most nucleotide polymorphisms within the "peptide-binding regions" involve changes that induce a change in the corresponding amino acid sequence (non-synonymous substitutions) and have a high level of correlation with phenotype differences detected by serological and cellular methods [32]. However, serological equivalents are not available for all described alleles [2, 30, 33], and it is difficult to predict the serological specificities of selected alleles with polymorphisms corresponding to more than one antigenic group [34,35] (Table 2).

It has been observed that most of the polymorphisms are restricted to certain segments of the gene, known as variable regions. Allele pairs associated with the same serotype (e.g., A*02:01, A*02:02) differ only by a few nucleotides. At the same time, distinguishing sequences are observed in alleles of other serotypes, indicating the patchwork nature of HLA polymorphisms. The significant HLA polymorphism probably evolved from the existence of a few allelic lineages, followed by short segmental exchanges, to increase the number of alleles at a given location. It appears that most of the HLA polymorphisms were generated by this mechanism. Then, selected natural selection events must have been necessary for new alleles to reach a significant frequency in the population. Nevertheless, it is worth noting that many alleles arise from single-point mutations.

With an understanding of the significant level of HLA polymorphism and the improvement of molecular techniques, several molecular HLA typing methods have been developed [44-46]. These methods have focused on detecting polymorphisms in exons 2 and 3 of HLA class I genes and in exon 2 of HLA class II genes. The application of these molecular techniques has led to the development of accurate and reproducible HLA typing methods suitable for clinical use [30-32, 36]. The wide application of these methods has led to the identification of many new alleles that were previously undetectable with the serological and cellular methods. The molecular HLA typing methods are widely used and take advantage of the efficiency of DNA amplification by polymerase chain reaction (PCR). The more widely used molecular HLA typing methods currently used in histocompatibility laboratories are: (1) amplification with sequence-specific primers (SSP), (2) hybridization with sequence-specific oligonucleotide probe hybridization (SSOPH), and (3) direct analysis of the DNA sequence (sequence-based typing, SBT) by means of Sanger sequencing or next-generation sequencing (NGS).

Table 2

HLA specificities 1 identified by serological and molecular methods (as of September 2022)

Gene	Serology	Proteins	Alleles	Null alleles
HLA-A	28	4450	7644	397
HLA-B	60	5471	9097	318
HLA-C	10	4218	7609	330
HLA-DRA1	0	5	43	0
HLA-DRB1	21	2203	3389	115
HLA-DRB3	1	334	446	22
HLA-DRB4	1	144	223	25
HLA-DRB5	1	142	187	23
HLA-DQA1	0	244	508	13
HLA-DQB1	9	1455	2330	102
HLA-DPA1	0	233	491	21
HLA-DPB1	6	1325	2221	113

Note:[1] Obtained from the IPD-IMTG/HLA Database [30, 31, 36].

Naming of HLA alleles

G-codes are used to identify ambiguous HLA allele types that share identical nucleotide sequences in the peptide-binding domains. Specifically, they refer to exons 2 and 3 for class I HLA genes and exon 2 for class II genes. These alleles are grouped under a "G" designation, which includes three fields and must contain at least six digits, such as A*01:01:01G [39,40]. Groups may include alleles with unsequenced genomic regions, as well as those obtained through whole-gene sequencing that differ only in silent (synonymous) nucleotide substitutions. When full sequencing is not available, alternative alleles within the same group may be used to infer the missing parts. A detailed list of such alleles, including information about unsequenced regions and substituted sequences, can be found in the ambiguous typing files available in the IMGT/HLA database. If a sequence is revised or removed and only one allele remains in a G group, the G-code designation is kept and can be expanded later if new alleles with identical nucleotide sequences in the peptide-binding domains are identified.

P-codes, on the other hand, are used to report alleles that encode identical antigen-binding domains at the protein level. For class I HLA alleles, this includes proteins encoded by exons 2 and 3, while for class II, it refers to exon 2. The P-code follows the two-field nomenclature of the allele with the lowest number in the group and must include at least four digits, for example, A*01:01P. A full list of alleles grouped by P-code can be found in the downloadable file HLA_nom_p.txt at https://hla.alleles.org [39,40]. Because exon boundaries often intersect codons, meaning one base of a codon may be in one exon and the remaining two in another, such partial codons are excluded from P group comparisons. For example, in the analysis of HLA-A, codons 1 and 183 are excluded since they straddle the boundaries between exons 1/2 and 3/4, respectively.

The allotype of an allele is represented by the numbers preceding the first column, which typically correspond to serological antigen types. The second field identifies subtypes, assigned sequentially as DNA sequences are discovered. In cases where two alleles differ in the first or second field, this implies they differ by at least one nonsynonymous substitution, a change

in the nucleotide sequence that alters the resulting amino acid. The third field distinguishes alleles that differ only by synonymous (silent) mutations within coding regions. In contrast, a fourth field is used when alleles differ only in non-coding regions, such as introns or the 5′/3′ untranslated regions (UTRs) adjacent to exons and introns (Figure 1).

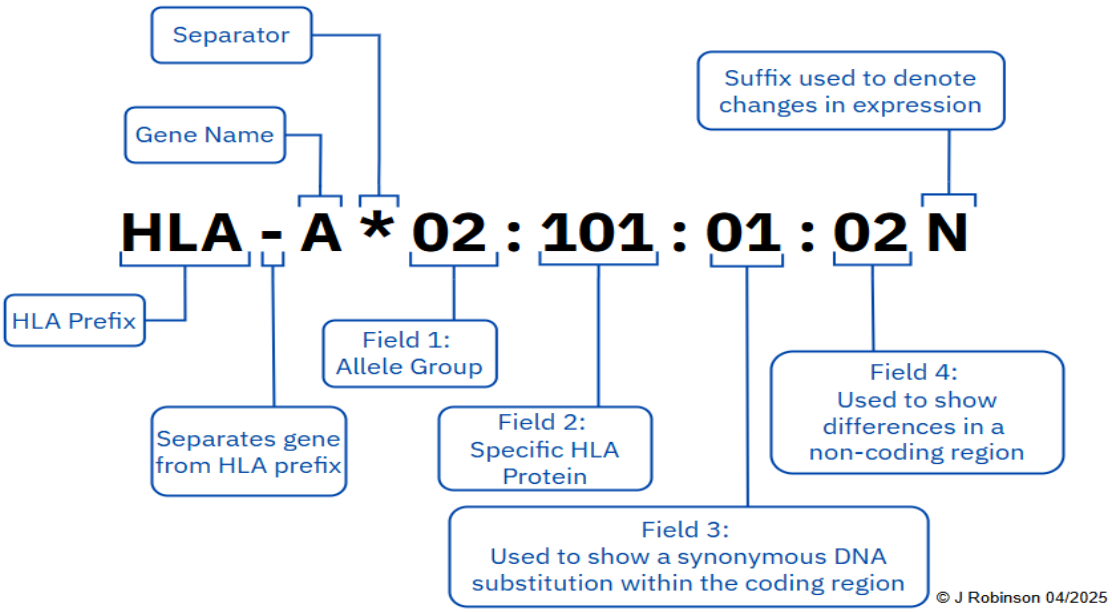

Figure 1. Nomenclature of the HLA alleles

In addition to the unique allele number, there are additional optional suffixes that can be added to the allele to indicate its expression status. Alleles that are not expressed, i.e. 'Null' alleles, are assigned the suffix 'N'. Alleles that are alternatively expressed may have suffixes L, S, C, A, or Q (Table 3).

Table 3

Designation of alleles by additional suffixes *

Suffix	Interpretation
N	An allele that is not expressed on the cell surface.
L	An allele that has been shown to have "low" cell surface expression compared to normal levels.
S	An allele that encodes a protein expressed as a soluble, "secreted" molecule, but not present on the cell surface.
C	Assigned to alleles that produce proteins present in the "cytoplasm," rather than on the cell surface.
A	Indicates "deviant" expression, when it is doubtful that the protein is actually expressed.
Q	Used when the expression of the allele is "questionable," considering that a mutation observed in the allele has been shown to affect normal expression levels in other alleles.

Note: * Taken from www.hla.alleles.org, Anthony Nolan Research Institute, London, UK.

According to the WHO Nomenclature Committee for Histocompatibility and the *IMGT/HLA* database, there are three principal levels of resolution used in *HLA* typing [4]:

• Low-resolution typing: molecular HLA typing results defined by the first field of the allele nomenclature *(e.g., A*31, B*07)*, which often correspond to the serological equivalents of *HLA* antigens [5,36].

• High-resolution typing: typing results that include both the first and second fields of the nomenclature *(A*31:01, B*07:02, etc.)*, and distinguishes alleles encoding identical peptide-binding domains, excluding those that are not expressed as surface proteins. The antigen-binding domain includes domains 1 and 2 of the α polypeptide chain (class I), and domain 1 of the α and β chains (class II).

• Intermediate-resolution typing: based on results of the first field of the nomenclature, irrespective of the specific position between low- and high-resolution distinctions. This includes allele groups such as *DRB1*11:01/11:09/11:28,* which may be represented using *NMDP* (National Marrow Donor Program) codes, such as 11:BYCC [6,33].

The HLA system consists of a highly diverse group of genes and their associated molecules, which are crucial for immune regulation, transplant compatibility, and transfusion success. These antigens are encoded by genes found within the *MHC* on the short arm of chromosome 6. HLA molecules are essential for differentiating self from non-self, triggering immune responses to antigenic stimuli, and coordinating both cell-mediated and humoral immunity (Figure 2).

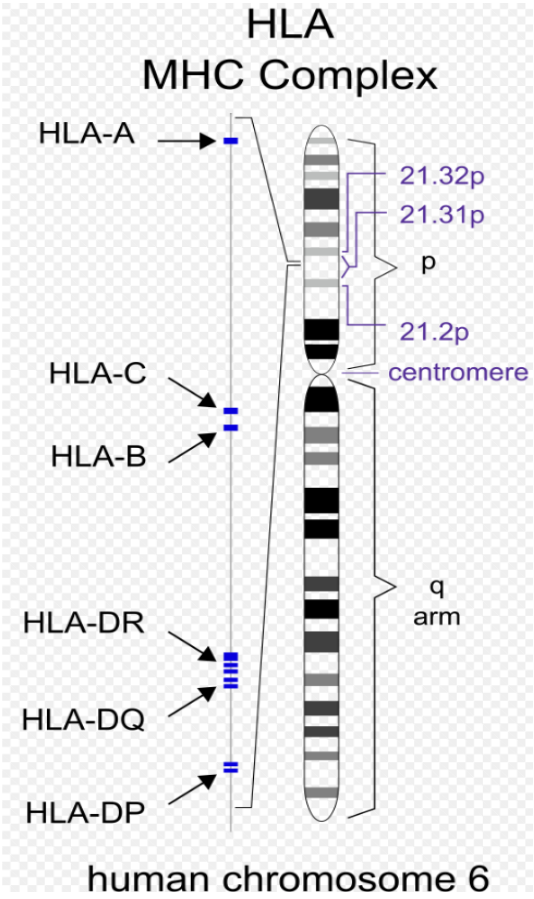

Figure 2. The HLA complex on chromosome 6 comprises the 3-6 kb class I region and the 4-11 kb HLA class II region. The HLA class III is not part of the polymorphic HLA system

The products of HLA genes are crucial in triggering various immune responses by presenting both self and non-self peptide antigens to T lymphocytes. Most polymorphisms in class I HLA genes are found in exons that encode the peptide-binding groove and regions involved in interacting with the T cell receptor. This high level of polymorphism is seen as an evolutionary adaptation, boosting immune defence by increasing the diversity of peptides that can be presented to T cells. Additionally, the codominant expression of HLA genes further enhances this diversity, as both maternal and paternal alleles are expressed, broadening the range of peptides that can be recognized and presented [7,8,35].

Class I MHC molecules are heterodimeric molecules, comprising a single heavy α-chain (~45 kDa) that is non-covalently associated with the invariant 12 kDa β2-microglobulin, and are expressed on the surface of all nucleated cells and platelets (Figure 4). The heavy α-chain is organized into three extracellular domains (α1, α2, α3), a transmembrane segment, and a cytoplasmic tail. Of these, the α1 and α2 domains are or particular importance, as they form an elongated groove through their α-helical structures, which functions as the binding site for processed antigens. This antigen-binding complex is essential for immunogenic recognition by T cells. The α1 and α2 domains are encoded by exons 2 and 3 of the HLA-A, HLA-B, and HLA-C genes, as illustrated in Figure 3 [8,34].

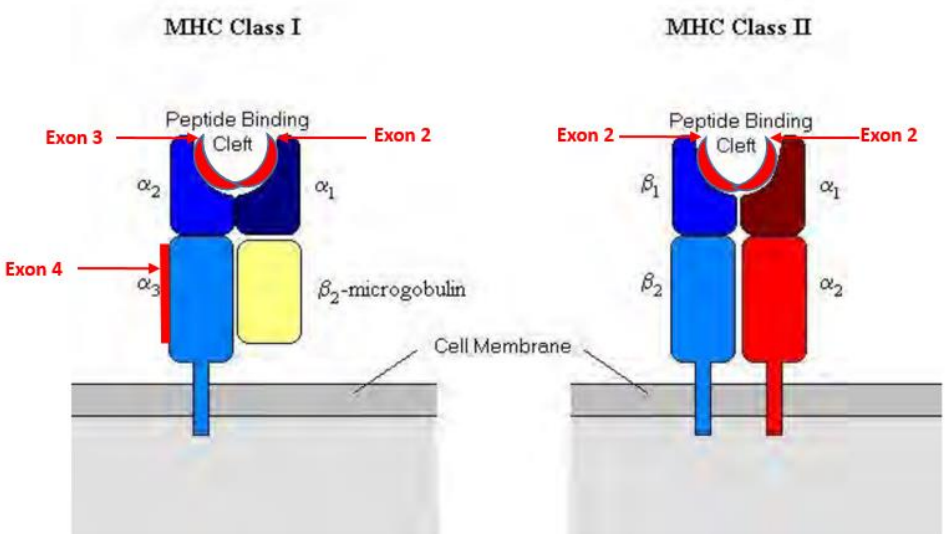

Figure 3. Structure of HLA class I and class II molecules. HLA class I molecules consist of one polymorphic heavy chain (α) associated with a non-polymorphic β2-microglobulin (β2m). The HLA class II molecules consist of two disulphide-linked polymorphic α and β chains

Conversely, class II molecules are mainly expressed on the surface of B lymphocytes, activated T cells, monocytes, macrophages, and dendritic cells, where they are vital in directing the interactions between T lymphocytes and antigen-presenting cells during immune responses. Structurally, class II molecules are heterodimers composed of non-covalently linked α- and β-chains [9,34]. Each chain features an extracellular region folded into two domains, connected to the transmembrane domain by a short linker peptide. Unlike class I MHC molecules, the peptide-binding site of class II MHC consists of domains from both chains, especially the α1 and β1 domains. Therefore, exon 2 of both the α and β genes is central to forming this binding groove.

The structure of classical HLA genes, schematically shown in Figure 3, illustrates the exons responsible for encoding the peptide-binding region of the antigen-binding site (highlighted in red). Other exons encoding the transmembrane domain and cytoplasmic tail do not contribute to antigen presentation and are therefore considered of clinical relevance only when non-expressed (null) alleles are present. Such alleles may result from premature stop codons within exons or from mutations at splice sites that disrupt normal mRNA processing [41,42].

The current number of known HLA alleles can be found on the official HLA nomenclature committee website and in the IPD-IMGT/HLA database [25, 32, 43]. As noted previously, most polymorphisms in class I HLA genes are located in exons 2 and 3, while in class II genes, they are mainly found in exon 2. These regions encode the membrane-distal domains that form the peptide-binding site [30]. Importantly, most nucleotide polymorphisms within these regions lead to nonsynonymous amino acid substitutions, which strongly correlate with phenotypic variation observed through serological and cellular typing methods [30, 31]. However, serological equivalents have not been established for all HLA alleles, and in some cases, allelic polymorphisms may correspond to multiple antigenic groups, complicating the prediction of serological specificity [41].

Methods for obtaining HLA allele sequences

Sanger sequencing, also known as Sequence-Based Typing (SBT), is widely recognized as one of the most precise techniques for HLA typing, as it allows for the direct determination of nucleotide sequences. Until the advent of newer technologies, the most commonly employed method for sequence-based HLA typing was the chain termination technique, developed by Dr. Frederick Sanger in the 1970s [44]. This approach relies on the random incorporation of four dideoxyribonucleotide triphosphates (ddNTPs), ddATP, ddCTP, ddGTP, and ddTTP, each tagged with a unique fluorescent dye. During the second phase of the process, the DNA fragments terminated by ddNTPs are separated by size using gel electrophoresis.

To determine the nucleotide sequence, the gel is read from bottom to top, as the smallest fragments (which migrated the farthest) represent the nucleotides closest to the 5' end of the DNA strand. DNA synthesis by DNA polymerase proceeds in the 5' to 3' direction, starting from a specific primer. Therefore, each fragment ends with a ddNTP that corresponds to a particular position in the original sequence. A computerized detection system was introduced to analyze the gel, with a laser exciting the fluorescent dyes at the ends of the fragments. The final output is a chromatogram, which presents a series of fluorescent peaks, each representing one nucleotide in the 5’ to 3’ direction of the DNA strand (Figure 4).

Sanger sequencing is commonly employed to obtain clinically relevant HLA exon sequences, particularly exons 2 and 3 for class I genes and exon 2 for class II genes, which are critical for transplantation. Depending on the design of primers in commercial reagent kits and the specific sequencing requirements, additional exons may also be sequenced. For example, the “Protrans S4” kit (Protrans medizinische diagnostische Produkte GmbH) enables the sequencing of exons 1 through 4 for class I HLA genes, full exon 2 for HLA-DRB1*, and exons 2 and 3 for HLA-DQB1* of class II.

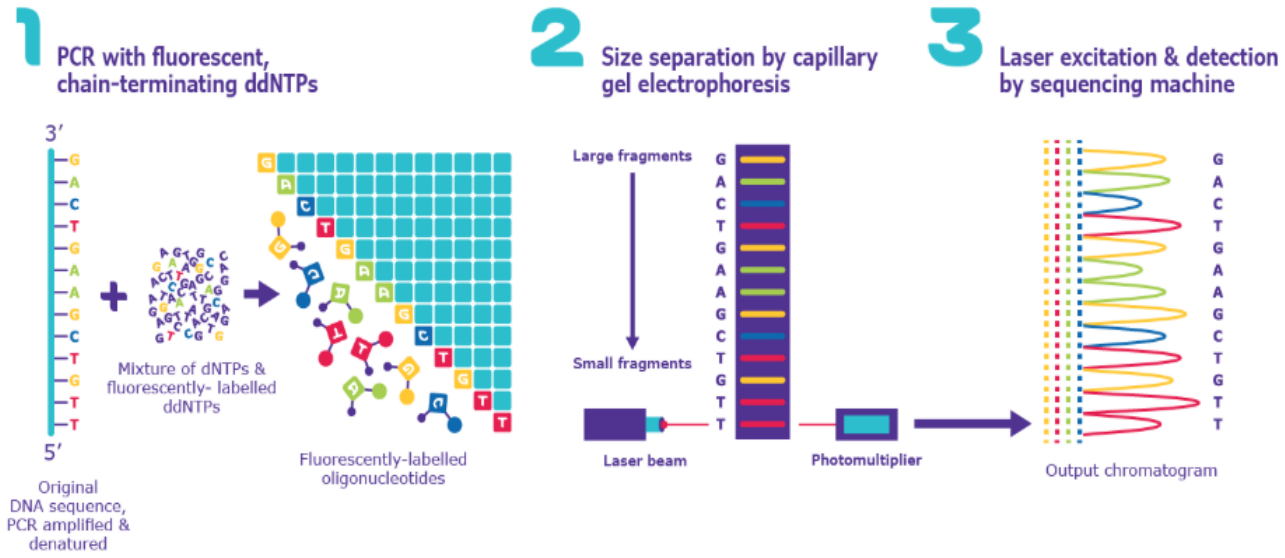

Figure 4. Three basic steps of automated sanger sequencing

Despite its high accuracy, Sanger-based HLA typing has several limitations, including low throughput and high operational costs. A notable drawback is its inability to phase heterozygous nucleotide positions, which often results in ambiguous typing that requires additional, labor-intensive testing to resolve. These limitations are increasingly being addressed by next-generation sequencing (NGS) technologies, specifically short-read NGS (second-generation sequencing). These methods rely on the high-throughput parallel sequencing of clonally amplified short DNA fragments (typically 250–800 base pairs in length). Several commercial NGS-based HLA typing kits are now available, offering comparable accuracy to Sanger sequencing, with the added advantages of efficient multiplexing and the ability to sequence both class I and class II HLA genes relevant to clinical practice. A standard NGS workflow typically involves DNA extraction, library preparation, and sequencing (Figure 5) [45].

Library preparation involves a series of additional steps, such as DNA fragmentation, end-repair, adapter ligation, and size selection. Fragmentation is crucial for producing DNA fragments within the optimal size range for a specific NGS platform. Methods used include sonication, transposase-mediated “tagmentation”, or thermal fragmentation using divalent metal ions. Shorter DNA fragments generally provide higher sequencing accuracy, while longer fragments offer valuable phasing information over greater genomic distances. After fragmentation, DNA ends are repaired to facilitate the ligation of NGS-compatible adapters. These adapters contain both platform recognition sequences and barcodes, enabling sample multiplexing, the simultaneous sequencing of multiple samples in a single run.

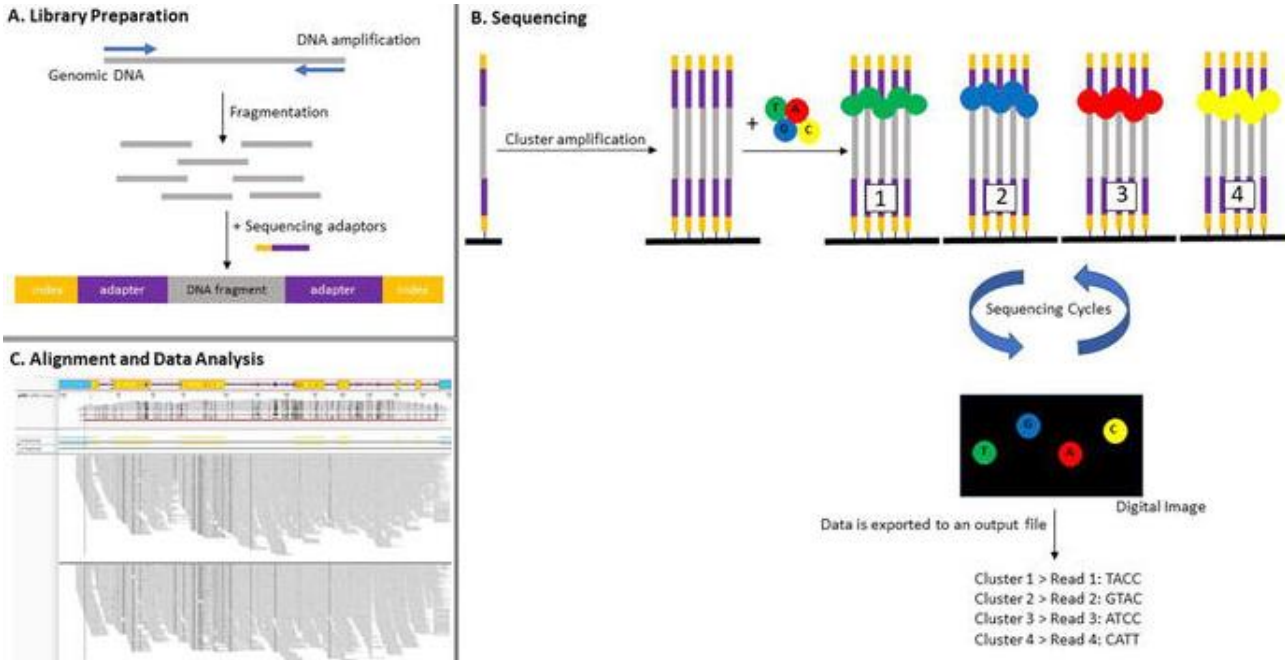

Figure 5. Steps of next-generation sequencing (NGS)-based HLA typing. **(A)** A sequencing library is generated by fragmenting amplified genomic DNA and attaching platform-specific adapters, which include index sequences (barcodes), to both ends of each DNA fragment. **(B)** The prepared library is then loaded into a flow cell, where DNA fragments bind to the surface and are amplified to form clonal clusters. Sequencing begins with the addition of fluorescently labelled nucleotides and other reagents. Each cycle incorporates one nucleotide per cluster, and the emitted fluorescence is recorded. The wavelength and intensity of the light emitted by each cluster identify the incorporated base. This sequencing-by-synthesis cycle is repeated over multiple rounds. **(C)** The resulting sequencing reads are then aligned to reference sequences from the IPD-IMGT/HLA database [4,5], allowing for the identification of nucleotide variations between the reference genome and the newly sequenced DNA

Size selection, performed after adapter ligation, enriches DNA fragments of a desired size and removes residual contaminants, enhancing sequencing efficiency. This can be achieved via bead-based or electrophoretic methods. Bead-based methods enable concurrent DNA concentration, whereas electrophoresis offers higher precision. Alternatively, on-bead tagmentation protocols streamline the process by combining fragmentation, adapter ligation, and normalization into a single step. A PCR amplification step follows, adding platform-specific adapters and sample-identifying barcodes. This streamlined workflow enables library generation in under 90 minutes, with less than 15 minutes of hands-on time, making it both efficient and scalable for clinical and research applications [46].

Given the large volume of data generated by NGS, efficient bioinformatics analysis and data management are essential for its successful application in HLA laboratories. The initial step in sequencing data analysis is carried out by the instrument itself, which performs base-calling for each clonally amplified DNA fragment. During this process, quality control procedures such as read filtering and trimming are also applied. The sequencing data, along with associated quality metrics, is stored in a FASTQ file format. For HLA genotyping, specialized commercial software programs are available for the final analysis phase.

Due to the high polymorphism of HLA genes, aligning sequences to the human reference genome is often inadequate for precisely identifying the specific HLA alleles in a patient's

sample. Instead, alignment is conducted against the IPD-IMGT/HLA Database, which contains sequences of all currently known HLA alleles [30,31]. Coverage is another crucial quality metric at this stage, comprising both depth of coverage (the number of times a base is sequenced) and breadth of coverage (the percentage of the reference genome covered). It is important to note that coverage may not be uniform across the amplicon, and insufficient coverage in critical regions such as exons can affect the accuracy of HLA typing results. Commercial HLA typing software generally includes filters to ensure the minimum coverage required for reliable genotyping. However, in some cases, lower thresholds may be acceptable, such as when polymorphisms between alleles at a locus are phased or when low-coverage regions, like introns and untranslated genomic areas, do not impact typing accuracy. A major concern in HLA data analysis is ensuring balanced allele representation to avoid allele dropout, which can result from preferential amplification due to technical factors or the patient's disease state, where one allele may be lost (loss of heterozygosity).

Recently, third-generation sequencing technologies have been developed, enabling the direct sequencing of DNA fragments over 10 kb from the genome. Although earlier versions of these methods had limitations, recent improvements have significantly enhanced their accuracy. NGS technologies enable the resolution of polymorphisms at various stages, eliminating ambiguities and providing high-resolution HLA typing without the need for re-testing, thereby offering a potential solution to previous challenges in HLA typing. Many HLA typing kits, such as Holotype HLA (Combion Biocomputing Ltd., Hungary), focus on amplifying long PCR fragments of HLA genes and perform sequencing on platforms like Illumina MiSeq. These kits amplify genes such as A, B, C, DQA1, and DQB1 across their entire coding region, including parts of the untranslated 5' and 3' regions. In contrast, DRB1 is amplified from intron 1 to intron 4, and DPB1 is amplified from intron 1 to intron 3 (Figure 6).

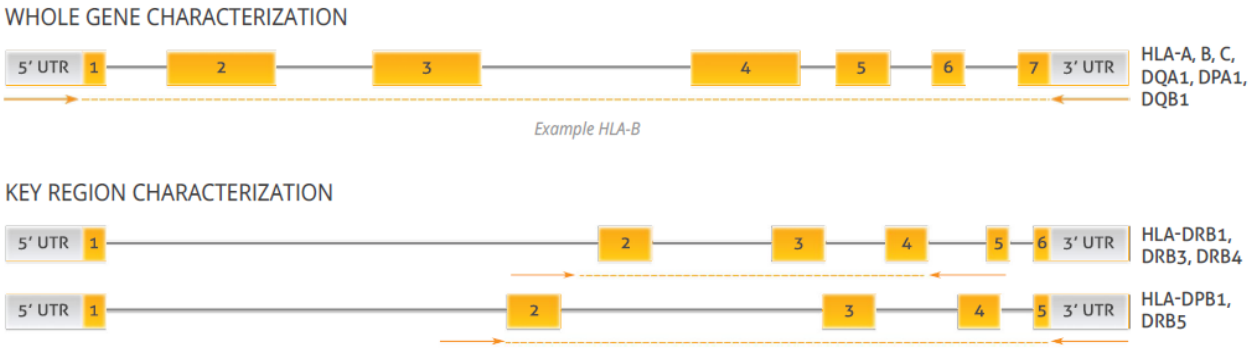

Figure 6. Gene coverage by NGS sequencing method

The WHO Nomenclature Committee for Factors of the HLA System releases an HLA antigen database every three months at the request of the Working Committee on Information Technology of the World Marrow Donor Association (WMDA) for the interpretation of sequencing in various computer programs. These files comprise the IMGT/HLA database, which documents the official HLA nomenclature, the relationships between serologically defined antigens, and the relationships between HLA allele sequences [30, 31, 36]. The database comprises five files, each with a short header containing four lines of information: the file name, the date of file creation (YYYY-MM-DD), the file format (URL), and the author. The files contain information about all

current and retired HLA antigens and alleles, sorted by locus and antigen/allele number. For HLA antigen names assigned before November 1987, dates are assigned approximately.

Conditions for accepting new allelic sequences

To accept DNA sequences as a new gene variant and assign an official name, the following conditions must be met:

• Material used for sequencing: These must be clearly specified.

• Sequencing direction: to be conducted in both directions, using forward and reverse primers.

• PCR amplification requirements: If sequencing is done using PCR-amplified material in the forward direction, the products must come from two separate PCR reactions.

• Heterozygous individuals: If one allele is new, it must be sequenced separately from the other allele. Sequencing both alleles together (as in SBT typing) is insufficient to assign an official name to a new allele.

• Primer-derived sequences: these should not be included in the submitted data.

• Confirmation through other methods: If the new sequence contains a novel mutation or an uncharacterized nucleotide combination, it must be confirmed by DNA typing methods (PCR-SSOP, PCR-SSP). This might require new mutation-specific probes or primers which should be documented.

• Database registration: A registration number must be obtained for the new sequence. Sequences can be submitted to online databases such as:

o EMBL: www.ebi.ac.uk/Submissions/index.html

o GenBank: www.ncbi.nlm.nih.gov/Genbank/submit.html

o DDBJ: www.ddbj.nig.ac.jp/sub-e.html

• Sequence length requirements: though preferred, full-length sequences are not required. Minimum sequence requirements are exons 2 and 3 for class I genes and exon 2 for class II genes.

• Intron or non-coding differences: The entire gene sequence, including coding and non-coding regions, must be sequenced if the new sequence differs only in introns or non-coding regions. In the absence of a full-length sequence of the closest related allele, it may also need to be sequenced and presented before an official name can be assigned.

• Manuscript submission: A manuscript describing the new sequence is recommended to be submitted for publication. Draft copies can be sent to the database via email or fax.

• Tumor-derived sequences: Sequences obtained solely from tumor material are generally not accepted by the nomenclature committee.

• Complete HLA type: A complete HLA type for HLA-A, -B, and -DRB1 genes must be provided for any material where a new allele is identified. The sample must also have a second allele at the locus of interest in a heterozygous individual.

• Repository submission: DNA or other materials, preferably cell lines, should be submitted to a public repository or remain in the originating laboratory. The WHO Nomenclature Committee will maintain documentation of this material.

• Online submission: Sequences should be submitted to the WHO Nomenclature Committee using their online submission tool at www.ebi.ac.uk/imgt/hla/subs/submit.html. Researchers must complete a questionnaire comparing their new sequence to related known alleles [47,48].

New alleles identified in the Kazakh population

From 2011 to 2022, four new HLA alleles at the *HLA-A, -B, -C,* and *-DQB1* loci were identified in individuals of Kazakh nationality in Kazakhstan. These were *DQB1*03:82*, *C*06:256*, *B*13:150,* and *A*32:95 [49-52],* identified using capillary sequencing (SBT method). Initial typing of blood samples was performed using SBT sequencing for the *HLA-A, -B, -C, -DRB1,* and *-DQB1* loci with Protrans S4 technology (Protrans, Hockenheim, Germany). During typing at the PCR product stage, haplotypes were separated, resulting in a heterozygous sequence. The nucleotide sequence was obtained using BigDye v1.1 Terminator Reagent (Applied Biosystems, Foster City, CA) and analyzed on a 3730XL Genetic Analyzer (Applied Biosystems). Sequencing was conducted in both forward and reverse directions for exons 2, 3, and 4 of the HLA-A, B, and C loci; for exon 2 of DRB1; and for exons 2 and 3 of DQB1. The results were analyzed using SeqPilot software (JSI Medical Systems, Germany, version 3.35.0):

1. DQB1*03:82: Identified in a patient with acute myeloid leukemia, this allele differs from DQB1*03:01 at exon 2 at position 223, where adenine (A) replaces guanine (G), resulting in an amino acid substitution from Cysteine to Tyrosine. Inheritance was confirmed through family analysis. The allele is registered in the EMBL and IMGT/HLA databases (HWS10018423) and has been named HLA-DQB1*03:82 [49].
2. C*06:256: Discovered while typing a potential hematopoietic stem cell donor, this allele differs from C*06:02:01:01 by a C to A substitution at exon 3, leading to an amino acid change from Glutamine to Lysine (Q→K). Identification was confirmed by NGS. The new allele is registered under the number HWS10027913 and has been named HLA-C*06:256 [50].
3. B*13:150: Found in a child with acute leukemia, this allele differs from B*13:02:01 by an A to C substitution at exon 2 that causes an amino acid change from Methionine to Leucine (M→L). Family analysis confirmed inheritance of the variant from the mother and brother. It is registered in the database under number HWS10060741 and has been named HLA-B*13:150 [51].
4. A*32:95: Identified in a potential stem cell donor, this allele differs from A*32:01:01 by a C to A substitution at exon 2 at position 28 that leads to an amino acid change from Lysine to Threonine (K→T). The allele is registered in the database under number HWS10027001 and has been named HLA-A*32:95 [52].

Following the implementation of the NGS sequencing technology, our laboratory identified an additional ten new sequences with changes in clinically significant exons (exons 2 and 3), as well as four sequences in clinically insignificant exons, such as exons 1, 4, 5, and 8 [53, 54]. Despite the short time of using NGS technology, the identification of new sequences has increased. The frequency of detecting new allelic variants using capillary sequencing is one new allele per 1,773 typings; whereas with NGS technology, detection of new alleles occurs every 635 typings.

The 2.8-fold increase in detection efficiency after implementing NGS technology results from NGS's superior ability to phase heterozygous positions across larger genomic areas and to sequence intronic regions where additional polymorphisms may be found. Additionally, NGS workflows enable simultaneous typing of multiple loci with high coverage depth, reducing ambiguities that often affect Sanger-based approaches. All four identified alleles feature non-synonymous substitutions within exons encoding peptide-binding domains (exons 2 and 3 for class I; exon 2 for class II). These amino acid changes: Cys→Tyr (DQB1*03:82), Gln→Lys (C*06:256), Met→Leu (B*13:150), and Lys→Thr (A*32:95), occur at positions that may influence peptide repertoire and T cell receptor recognition. Nevertheless, functional validation

through peptide-binding assays and T cell activation studies remains essential to determine the immunological impact of these substitutions.

Discussion

During this study, previously unreported HLA class I and II allelic varFor example, the "Protrans S4" kit (Protrans medizinische diagnostische Produkte GmbH) enables the sequencing of exons 1 through 4 for class I HLA genes, full exon 2 for HLA-DRB1*, and exons 2 and 3 for HLA-DQB1* ofnary pressures related to pathogen antigen recognition [55]. According to the IMGT/HLA Database (version 3.57.0, January 2025), there are currently over 37,000 different HLA gene alleles registered, including 8,123 alleles for HLA-A, 9,276 for HLA-B, and 6,475 for HLA-DRB1, with this number continually increasing [2].

In our analysis, new variants were identified, including B*51:01:XX and DRB1*13:XX, which contain single-nucleotide substitutions in coding exons that could potentially alter the peptide-binding domain structure. Such mutations can significantly affect antigen presentation and are associated with an increased risk of developing autoimmune and infectious diseases [56].

While African and South Asian populations have historically been considered the primary reservoirs of HLA diversity due to longer evolutionary timescales and larger effective population sizes [57], our findings indicate that substantial unique ancient migration waves and prolonged isolation of certain sub-ethnic groups persist in Central Asian populations. The Kazakh population occupies a geographic crossroads that has historically been traversed by migration waves connecting Europe, East Asia, and Central Asia. This positioning likely facilitated admixture between diverse ancestral groups, potentially generating novel allelic combinations through recombination events. Furthermore, subsequent isolation of certain sub-ethnic groups within Kazakhstan's vast territory may have preserved rare variants through genetic drift. The four novel alleles identified in this study show no exact matches in the Allele Frequency Net Database (AFND), which aggregates HLA data from over 1,500 worldwide [58].

This absence could reflect either recent mutational origin, preservation of ancient variants in isolated populations, or simply the historical undersampling of Central Asian groups in global HLA studies. Distinguishing among these scenarios requires expanded sampling with dense geographic coverage and phylogenetic analysis of allelic relationships. Comparing the obtained alleles with global panels revealed that several commonly occurring alleles in our study sample are absent from most standard diagnostic panels used in bone marrow donor typing. This may complicate donor matching for Kazakh recipients. According to the World Marrow Donor Association (WMDA), as of 2024, there are over 41 million potential donors registered in international registries; however, a significant portion is represented by European and North American populations. This creates a disparity and reduces the effectiveness of finding HLA-compatible donors for recipients from Central Asia [59].

From a practical standpoint, identifying new alleles highlights the need to adapt HLA typing panels to local ethnic characteristics. This is particularly important in the context of developing biomedical products, such as personalized vaccines, immunotherapeutic agents, and transplant matching algorithms. Moreover, accounting for rare and unique alleles can enhance the accuracy of population genetic and epidemiological models while minimizing the risks of transplant rejection and immunological complications [60]. Thus, our findings expand existing knowledge about HLA gene diversity and underscore the importance of deep local analysis within global biobanks and registries.

Several limitations are noteworthy. The sample sizes for individual novel alleles remain small (n = 1-4 carriers), which hampers the accurate estimation of allele frequencies and restricts understanding of linkage disequilibrium patterns within the Kazakh population [61-63]. The functional attributes of the identified amino acid substitutions were not experimentally validated, for example, through peptide-binding assays, thermal stability measurements, or T cell activation studies. Additionally, our sequencing strategy focused on clinically relevant exons (exons 2-3 for class I; exon 2 for class II), potentially missing variants in promoter regions or intronic enhancers that could influence expression levels. Furthermore, the comparison of detection rates between Sanger and NGS technologies may be confounded by temporal differences in sample composition, typing indications (e.g., patient versus donor typing), and operator experience [64]. Future plans include increasing the sample size and conducting functional tests to assess the impact of identified mutations on antigen binding, which could help advance personalized medicine in the region.

Conclusions

Our study identified the novel HLA alleles that contain non-synonymous substitutions within peptide-binding domains: *HLA-DQB1*03:82, HLA-C*06:256, HLA-B*13:150*, and *HLA-A*32:95* in the Kazakh population. This addition to the IPD-IMGT/HLA database enhances our understanding of HLA diversity in Central Asia, a region that has historically been underrepresented in global immunogenetic studies. The transition from Sanger sequencing to NGS-based typing in our laboratory increased the efficiency of novel allele detection by 2.8-fold, further demonstrating the superiority of NGS for HLA characterization, which stems from its ability to resolve phase ambiguities and sequence extended genomic regions with high throughput. Several aspects must be considered when identifying a new allele based on DNA sequence. It is essential to have knowledge of the structure of a similar allele, and the location (exon, intron) of the nucleotide substitution and its relation to the codon. It is necessary to describe the protein sequence and determine whether this substitution will lead to an amino acid change. These and related factors are needed when submitting a new gene to the nomenclature committee and for its registration.

Kazakhstan's multi-ethnic composition (comprising over 124 ethnic groups) and its location along migration routes support ongoing efforts to identify additional unique HLA variants. These population-specific data are crucial for improving transplant donor matching for Kazakh recipients, who are currently underserved by registries dominated by European and North American data, for developing personalized immunotherapeutic strategies that consider local HLA diversity, and for studying disease associations in Central Asian populations. Future research will focus on expanding sample sizes, validating the immunological effects of identified substitutions through peptide-binding and T-cell assays, and establishing collaborations to enhance Central Asian representation in international HLA registries and biobanks. These initiatives will significantly enhance our understanding of HLA genetic diversity in Central Asia and promote the clinical use of HLA typing in personalized medicine.

Author Contributions

A.T. – conceptualization and writing the draft of the manuscript; **S.A. –** discussion of main findings and project administration; **W.Y.A. –** supervision and editing of the article.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Compliance with ethical standards

Not applicable, as it is a review article.

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Номенклатура HLA системы и открытие новых аллельных вариантов в казахской популяции

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Аннотация. Система человеческих лейкоцитарных антигенов (HLA) является одной из наиболее генетически полимерфных в организме человека, которая включает более 220 генов, кодирующих иммунные белки, играющие ключевую роль в трансплантационной совместимости, регуляции иммунного ответа и предрасположенности к заболеваниям. В данном обзоре представлены основы номенклатуры HLA, стандартизированной Всемирной организацией здравоохранения (ВОЗ) и поддерживаемой в базе данных IPD-IMGT/HLA. Описана эволюция методов типирования HLA - от серологических тестов до молекулярных технологий, включая секвенирование по Сэнгеру и секвенирование нового поколения (NGS), а также использование специализированного программного обеспечения для интерпретации данных. Проведена оценка их преимуществ и ограничений при выявлении новых аллелей. В работе рассмотрены аллельные варианты генов HLA, методы их секвенирования и анализа. Кроме того, сообщается

об идентификации четырёх новых аллелей HLA в казахской популяции: *DQB1*03:82, C*06:256, B*13:150 и A*32:95.* Все четыре аллеля содержат несинонимичные замены в пептидсвязывающих доменах, что указывает на их возможную иммунологическую значимость. Сравнительный анализ показал, что технология NGS повышает эффективность обнаружения новых аллелей в 2,8 раза по сравнению с секвенированием по Сэнгеру (один новый аллель на 635 типирований против 1773). Полученные результаты демонстрируют значительное генетическое разнообразие HLA среди населения Центральной Азии, которое остается недостаточно представленным в международных базах данных. Выявление специфичных для популяции аллелей подчеркивает необходимость расширения HLA-профилирования в этнически разнообразных регионах для улучшения исходов трансплантации и развития персонализированной иммунотерапии.
Ключевые слова: аллельные варианты, HLA-типирование, база данных IPD-IMGT/HLA, Казахстан, секвенирование нового поколения (NGS)

HLA жүйесі номенклатурасы және қазақ популяциясында жаңа аллельдік түрлерінің анықталуы

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Аңдатпа. Адам лейкоцитарлық антигендер жүйесі (HLA) - адам ағзасындағы ең генетикалық тұрғыда әртүрлі жүйелердің бірі. Ол 220-дан астам генді қамтиды және трансплантациялық сәйкестікке, иммундық жауаптың реттелуіне және әртүрлі ауруларға бейімділікке жауапты иммундық ақуыздарды кодтайды. Бұл шолуда Дүниежүзілік денсаулық сақтау ұйымы (ДДҰ) бекіткен және IPD-IMGT/HLA деректер базасында сақталатын HLA номенклатурасының негіздері баяндалады. Сондай-ақ, HLA типтеу әдістерінің дамуы — серологиялық талдаулардан бастап Сэнгер секвенирлеуі мен жаңа буын секвенирлеуіне (NGS) дейінгі технологиялық жетілдірулер қарастырылған. Деректерді интерпретациялауға арналған бағдарламалық құралдардың мүмкіндіктері мен олардың артықшылықтары мен шектеулері талданады. Зерттеу барысында HLA гендерінің аллельдік вариантты, олардың секвенирлеу әдістері мен талдау тәсілдері сипатталады. Сонымен қатар, қазақ популяциясында төрт жаңа HLA аллелі анықталды: *DQB1*03:82, C*06:256, B*13:150* және *A*32:95.* Бұл аллельдердің барлығында пептид байланыстыру домендерінде мағынасын өзгертетін ауысулар барын және бұл олардың иммунологиялық маңыздылығын көрсетуі мүмкін. Салыстырмалы талдау нәтижелері көрсеткендей, NGS технологиясын қолдану жаңа аллельдерді анықтау тиімділігін 2,8 есеге арттырады (әрбір 635 типтеуге бір жаңа аллель, ал Сэнгер әдісінде – 1773 типтеуге бір жаңа аллель). Бұл нәтижелер Орталық Азия халықтарындағы HLA жүйесінің жоғары генетикалық әртүрлілігін айғақтайды. Аталған аймақтар халықаралық деректер базасында жеткіліксіз қамтылғандықтан, этникалық тұрғыдан алуан түрлі популяцияларда HLA-профильдеуді кеңейту қажеттілігі туындайды. Бұл өз кезегінде трансплантация нәтижелерін жақсартуға және жекелендірілген иммунотерапияны дамытуға ықпал етеді.
Түйін сөздер: Аллельдік түрлер; HLA типтеу; IPD-IMGT/HLA деректер базасы; Қазақстан; жаңа буынды секвенирлеу (NGS)

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Morphological characteristics of the spotted thicklip loach *Triplophysa strauchii (Nemacheilidae)* from the Uba River (upper Irtysh basin)

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Abstract. The spotted thicklip loach is a Central Asian fish species native to many water bodies of Kazakhstan. This species belongs to a complex taxonomic group of fish, the genus *Triplophysa.* Three subspecies of the spotted thicklip loach are known for water bodies of Kazakhstan: *T. s. strauchii, T. s. ruzskyi,* and *T. s. zaisanicus,* but their taxonomic status and distribution require clarification. The modern distribution of the spotted thicklip loach is very wide; it inhabits the basins of Balkhash-Alakol, Tarim, the Chu and Syr Darya rivers, and is also known for the basin of Lake Zaysan. Currently, this species expands its range far to the north, where it has successfully acclimatized in the endorheic rivers and lakes of Central and Northern Kazakhstan. In 2016, the spotted thicklip loach was discovered in the Uba River of the upper Irtysh basin. Here we present a description of the external morphology, including coloration, plastic and meristic features, body proportions, fin shape, lip structure, features of the intestine, and swim bladder. Features of sexual dimorphism were also investigated. Characteristic features of *Triplophysa strauchii* have been revealed. In order to clarify the range of this species, further research is needed in the Irtysh basin.

Key words: spotted thicklip loach, *Nemacheilidae, Triplophysa strauchii,* morphology, upper Irtysh basin, Uba River

Introduction

The loaches of the genus *Triplophysa* are widely distributed in Central and East Asia. It is one of the largest genera of the family *Nemacheilidae*, currently comprising approximately 160 species [1]. Moreover, the number of described species of this genus is increasing every year [1-6]. The taxonomy and phylogeny of the genus *Triplophysa*a are undergoing a period of revisions and descriptions of new species [7–12]. In this regard, the identified subspecies and morphs of loaches from the Kazakhstan water bodies also require more detailed research using modern approaches.

One of the most widespread species of the genus is the spotted thicklip loach *T. strauchii,* which inhabits both flowing and stagnant water bodies in Central Asia. This species is considered ecologically flexible, capable of inhabiting a variety of habitats, where it creates morphologically distinct forms [13]. The modern range of the spotted thicklip loach includes the basins of lakes

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Balkhash, Alakol, Tarim, the Chu and Irtysh rivers (upper reaches), and the Syr Darya river [13, 14]. Moreover, along with seed from southern fish hatcheries, this species has spread beyond its natural range far to the north, and today is quite common in the endorheic rivers of Central Kazakhstan. There is some data about his presence in the lakes of the North Kazakhstan region – Shalkar, Katarkol, and Bolshoye Chebachye [15].

The following subspecies of *T. strauchii* are known:

– *T. s. strauchii*, inhabiting the basins of Lake Balkhash and Lake Alakol, the Chu River, and Lake Biylikol;

– *T. s. ruzskyi,* inhabiting the basin of the Alakol-Sasykkol lake system;

– *T. s. zaisanicus),* described for Lake Zaisan and the rivers of the northern slopes of the Tarbagatai Mountains.

Data on the last two subspecies are very scarce, and the systematic status of these forms requires verification.

Previous data on the distribution of *T. strauchii* in the upper Irtysh basin are known for the rivers of the northern slopes of the Tarbagatai Mountains. In 2016, a sample of the spotted thicklip loach was collected from a more northern location – the tributary of the Uba River [16], where it had not been previously mentioned.

The aim of the study was to investigate the external morphology of the spotted thicklip loach *T. strauchii* from the Uba River in the upper Irtysh basin. Here we confirm the new location for this species and provide new data on its morphology.

Material and methods

Fish were caught with net traps on July 20, 2016, in the tributary of the Uba River (the Poperechka River, near Shemonaikha, East Kazakhstan, 50.3751°/ 81.5228°). The fish were fixed in a 4% formaldehyde solution in horizontally placed plastic bottles, so the fish had straightened bodies and fins.

Analysis of morphological traits was conducted according to Prokofiev (2010) [17]. Plastic features were measured on 10 specimens (TL – 121–133 mm – 6 males and 4 females) using a digital caliper with an accuracy of 0.1 mm. All measurements were made in a straight line directly between two points. To increase the accuracy of the analysis of meristic characters, fish were stained in potassium hydroxide solution (0.3%) with the addition of Alizarin Red. The last fin rays in the dorsal and anal fins, located on a single pterygiophore, were counted as 11/2. The intestine and gas bladder were also investigated in all the specimens.

Results and discussion

Meristic features

Number of lateral line pores – 70–94.

Dorsal fin with 4 unbranched and 7½–8 ½ branched rays; anal fin with 3 unbranched and 51/2 branched rays; caudal fin with 16 branched rays (C I+8+8+I); pectoral fin with 1 unbranched and 13–14 branched rays; ventral fin with 1 unbranched and 7½–8½ branched rays. Scales absent.

Coloration

The body is light brown with clear brown spots of variable shape and size on the lateral and dorsal sides of the body (Figure 1). On the back, the spots are often small and unclear. The

lateral line is usually distinguished by a white stripe. The abdomen is whitish. On the lateral sides of the head, clusters of melanophores might form a marble pattern. In some specimens, the top of the head is uniformly brown, while in others it is spotted.

Figure 1. Examples of coloration of the spotted thicklip loach *(Triplophysa strauchii)* from the Uba River (upper Irtysh basin) – specimens fixed in formaldehyde. Female (**A**, *TL* – 121 mm) and male (**B,** *TL* – 128 mm)

Some specimens have vertically elongated spots of irregular shape on the dorsal fin. The caudal fin is mottled with rows of spots. The anal and pelvic fins are uniformly whitish or have a few small spots. The pectoral fins have small spots.

Plastic features

Results of measurements are presented in Table 1.

Table 1

Plastic features of males and females of *T. strauchii* from the Uba River (upper Irtysh basin)

Feature	Uba River, males, n=6		Uba River, females, n=4	
	Range	Mean ± m	Range	Mean ± m
Total length (mm)	121–133	128±0,17	121–139	129±0,47
Standard length (mm)	102–113	108±0,15	100–116	107±0,39
Percentage of standard length				
Predorsal length	53,1–56,86	54,44±0,05	54,46–56,76	55,53±0,22
Postdorsal length	34,8–37,27	36,51±0,09	35,14–38	36,89±0,15
Pre-anal length	69,44–70,91	70,12±0,11	68,97–72	70,25±0,24
Prepelvic length	51,87–54,72	53,34±0,08	52,48–54	53,11±0,19

Prepectoral length	22,43–23,64	23,06±0,05	22,77–24,32	23,56±0,13
Pectoral-pelvic distance	29,09–32,35	30,68±0,03	30,17–33	31,28±0,09
Pelvic-anal distance	16,67–18,69	17,5±0,04	16,22–17,33	16,82±0,05
Pelvic-anus distance	10,19–12,15	11,1±0,04	9,9–11	10,52±0,05
Anus-anal distance	2,39–2,84	2,68±0,01	3,02–3,6	3,35±0,01
Body depth maximum	17,29–23,53	19,85±0,07	19,8–20	19,88±0,08
Body depth at dorsal-fin origin	16,36–17,94	17,24±0,02	17,33–18,1	17,73±0,09
Body depth minimum	6,02–6,94	6,49±0,01	6,03–6,44	6,26±0,02
Body width maximum	14,49–17,94	16,17±0,04	17,33–18,62	18,06±0,1
Body width at dorsal-fin origin	11,68–12,75	12,08±0,01	14–15,32	14,5±0,08
Caudal peduncle length	22,12–24,07	22,84±0,04	21–23,76	22,41±0,11
Caudal peduncle depth	8,14–9,26	8,66±0,02	7,84–9,01	8,47±0,03
Caudal peduncle width	7,01–8,33	7,59±0,02	7,76–9,01	8,24±0,05
Pectoral-fin length	15,93–18,18	17,23±0,04	15,95–17,33	16,46±0,05
Pelvic-fin length	14,16–16,67	15,41±0,04	15–17,12	16,17±0,09
Dorsal-fin depth	15,93–19,81	17,83±0,06	18,5–18,97	18,78±0,08
Dorsal-fin base length	11,95–12,75	12,31±0,01	11–12,87	11,93±0,06
Anal-fin depth	13,27–14,81	14,01±0,03	14–15,32	14,73±0,08
Anal-fin base length	6,64–7,73	7,04±0,02	6,63–7,66	7,03±0,05
Caudal-fin length	17,70–20,37	19,2±0,06	19,8–21,62	20,45±0,1
Caudal-fin longest branched ray length	18,14–20,94	19,45±0,06	19,8–22,07	20,6±0,11
Caudal-fin shortest branched ray length	14,95–16,7	15,85±0,05	16,81–17,57	17,1±0,07
Lateral head length	22,12–23,64	22,84±0,03	22,77–24,32	23,56±0,13
Percentage of lateral head length				
Head depth at nape	55,77–62,5	59,04±0,02	55,56–58,7	57,03±0,07
Head depth at the middle of eye	44–47,5	45,99±0,01	46,3–48,21	47,45±0,06
Interorbital width	32,5–36	33,77±0,02	29,63–33,48	31,65±0,03
Head width	65,38–71,43	68,16±0,02	66,67–69,57	68,02±0,08
Head width at the preopercle	57,69–63,27	60,73±0,02	61,11–65,22	62,77±0,07
Snout length	41,67–46,8	44,21±0,02	44,44–43,59	43,59±0,06
Postorbital head length	40–45,83	42,05±0,02	43,48–42,53	42,53±0,04
Eye diameter	18,75–20,8	19,73±0,01	21,3–19,91	19,91±0,01
Inner rostral barbel length	23,75–30,61	26,37±0,03	25,93–23,08	23,08±0,04
Outer rostral barbel length	25–40,82	34,49±0,05	35,56–33,35	33,35±0,04
Maxillary barbel length	28,85–44,90	34,01±0,06	30,43–29,71	29,71±0,04
Mouth length	22,92–29,17	26,18±0,02	26,09–25,77	25,77±0,04
Mouth width	36,8–40,82	38,67±0,01	39,57–37,95	37,95±0,04

Body shape and proportions

The body is elongated and only slightly laterally compressed. The origin of the dorsal fin starts closer to the end of the snout than to the end of the caudal fin. The dorsal profile of the back is nearly arch-like. The deepest body point is between the occiput and the dorsal fin. The maximum body depth is greater than its width at this point and smaller than the caudal peduncle length. The caudal peduncle is short, not compressed laterally; its length is much greater than its depth and width. The width of the caudal peduncle is equal to, or slightly smaller than, its depth. The depth of the caudal peduncle is 2.5-2.9 times its length. The minimum body depth is smaller than the caudal peduncle depth.

The dorsal fin is longer than the anal fin. The pectoral fins in males are slightly longer than the pelvic fins, and in some females, they are equal in length. The pectoral fins extend beyond half the distance between the bases of the pectoral and pelvic fins. The pelvic fins extend beyond the anus and do not reach the anal fin. The anus is closer to the bases of the anal fin than to the bases of the pelvic fins. The distal margin of the caudal fin is slightly concave.

The head is not compressed laterally and dorsally resembles a truncated cone with a rounded top (Figure 2).

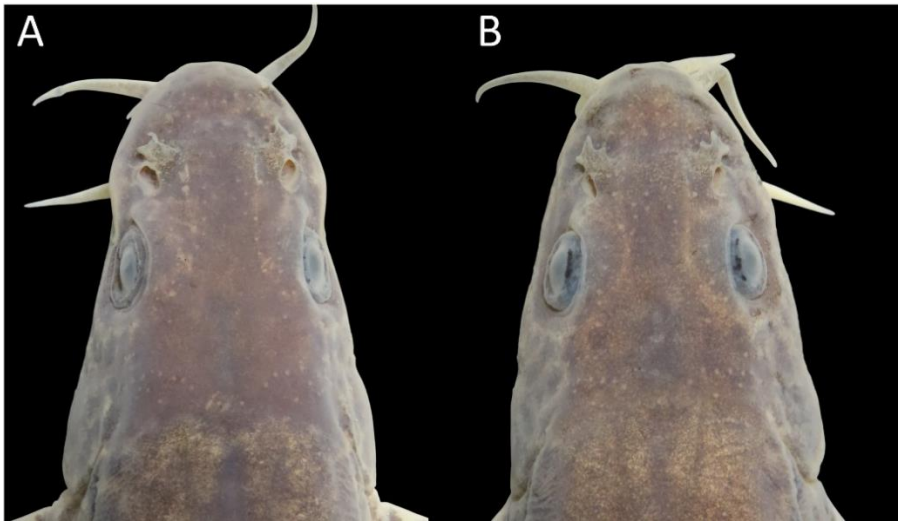

Figure 2. Head of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin), dorsal view. Male (**A,** *TL* – 126 mm) and female (**B,** *TL* – 121 mm)

The head length is more than 22% of SL, exceeds the greatest body depth, and is approximately equal to the caudal peduncle length. The head depth at the occiput is greater than half its length and smaller than the head width here. The interorbital space is flat and moderately wide; the eye diameter is less than 1.8 times its width. The dorsal profile of the head is straight to the nostrils, where it is slightly or noticeably convex (Figure 3). The snout is rounded and moderately elongated; it is usually shorter than the postorbital distance. In males, the anterolateral edges of the snout form small ridges - thus, dorsally, their snout is more rounded than in females. In addition, a well-formed swelling extends from the eye to the upper jaw in males (Figure 3A).

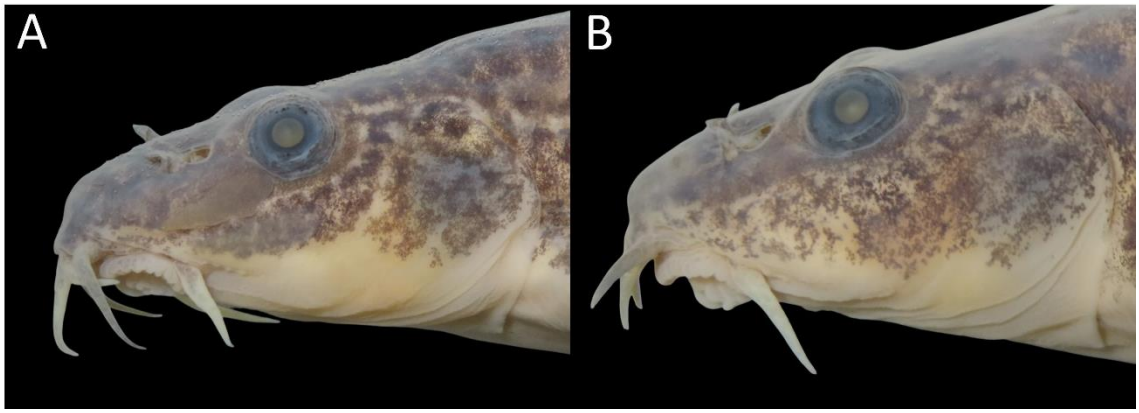

Figure 3. Head of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin). Male (**A,** *TL* – 131 mm) and female (**B**, *TL* – 139 mm)

The eyes are situated rather laterally and, when viewed from the side, are located above the forehead line. The horizontal diameter of the eye is 2.0-2.4 times the length of the snout. The barbels' length is greater than the eye diameter. The longest is the outer rostral one. The maxillary barbels extend beyond the middle of the eye, or further beyond the posterior edge of the eye.

Shape of the mouth and nostrils

The mouth is short, inferior, and surrounded by three pairs of barbels (Figure 4). The length of the oral opening is less than its width, and the oral slit is crescent-shaped. The lower lip covers the edge of the lower jaw; the edge is visible in the middle of the lip. The upper lip is without a medial notch; in some individuals, it covers the edge of the upper jaw, but in most fish, the edge is visible. The processus dentiformis is absent. The lower lip is interrupted in the middle and divided into three or four paired and more or less isolated lobes. The mental lobes are well-formed and disc-shaped. The lateral lobes are not formed. The lips with short thickened papillae. The upper lip is not interrupted, the folds on the lower lip are well expressed. The anterior and posterior nostrils adjacent, the posterior nostril is triangular in shape, the internasal septum is wide.

Figure 4. Lips of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin), ventral view. Female (**A**, *TL* – 135 mm) and male (**B**, *TL* – 128 mm)

Fins shape

The distal end of the dorsal fin is rounded, the posterior margin is straight, the longest ray is the second branched one. The distal end of the anal fin is rounded, its posterior margin is rather convex, the longest ray is the second branched one (Figure 5).

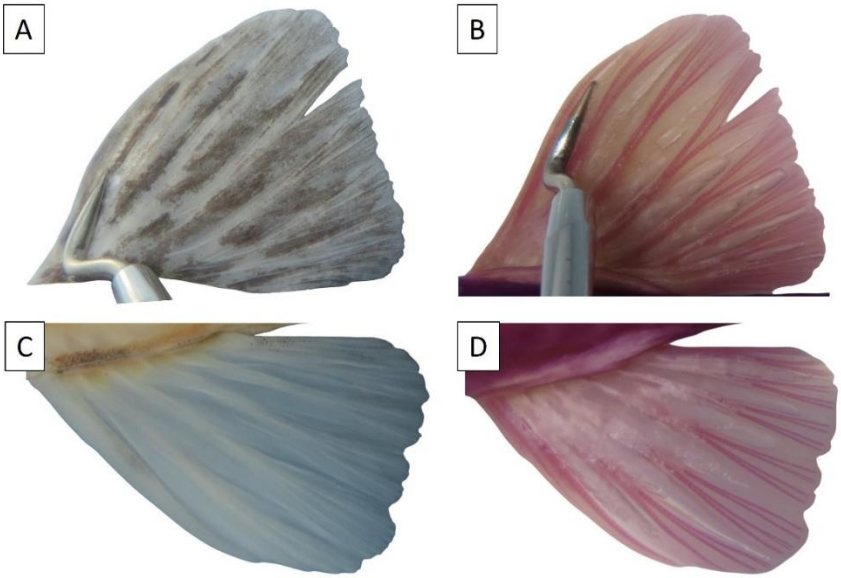

Figure 5. Dorsal (**A-B**) and anal (**C-D**) fins of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin). On the right are fins with stained rays

The lobes of the caudal fin are rounded, the upper lobe is slightly longer than the lower one, the fin is slightly notched, the longest upper lobe rays the second and third branched ones, the longest lower lobe rays are the third or the third and fourth branched ones (Figure 6).

Figure 6. Caudal fin of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin). On the right is fin with stained rays

The distal end of the pectoral fin is rounded, the posterior margin is convex, and the longest rays are usually the third and fourth branched ones. The distal end of the pelvic fin is rounded, the posterior margin is convex, the longest rays are the second branched, or the second and third branched ones (Figure 7).

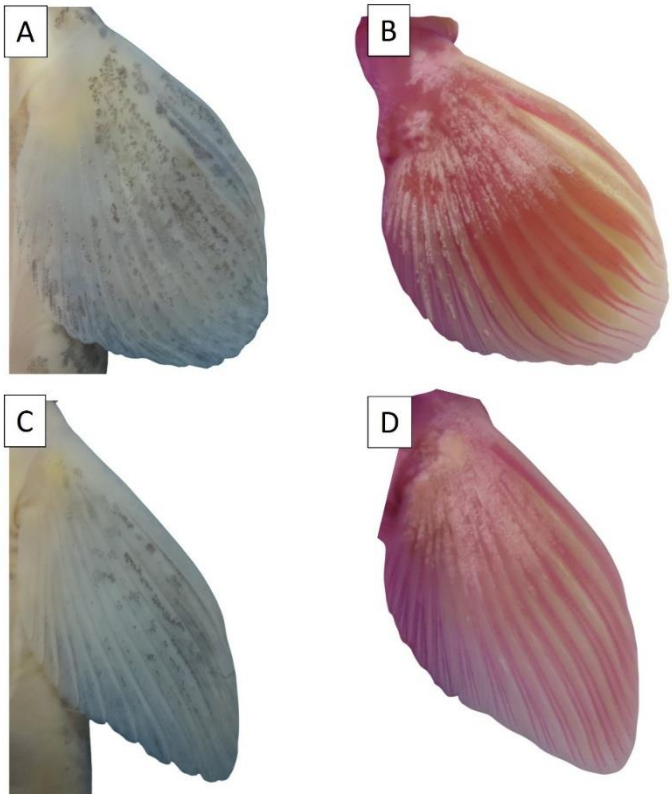

Figure 7. Left pectoral fin of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin). Male (**A-B)**, *TL* – 128 mm) and female (**C-D,** *TL* – 121 mm). On the right are fins with stained rays

Internal organs

The posterior part of the swim bladder is well developed. The swim bladder is widened in the back part of the abdominal cavity and has a round or oval shape. It is connected to the bone capsule by a thin canal. The intestine is of medium length, usually forming two main loops – ascending and descending (Figure 8). Sometimes there are one to three additional loops.

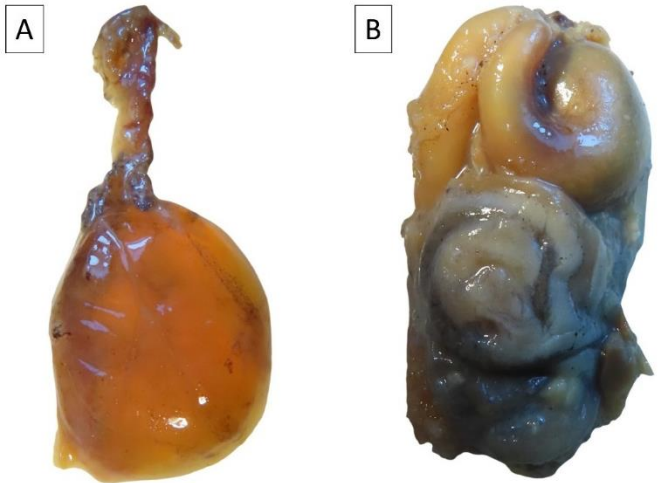

Figure 8. Swim bladder (**A**) and intestine (**B**) of the spotted thicklip loach (*T. strauchii*) from the Uba River (upper Irtysh basin)

Sexual dimorphism

Males are distinguished by the following characteristics: the first four-five branched pectoral fin rays are thickened in males, the anterolateral edges of the snout with ridges, a swelling extends from the eye to the snout, some males have the brush-like agglomerations of spawning tubercles on sides of the head, characteristic for T. *strauchii* [17] (Figure 9).

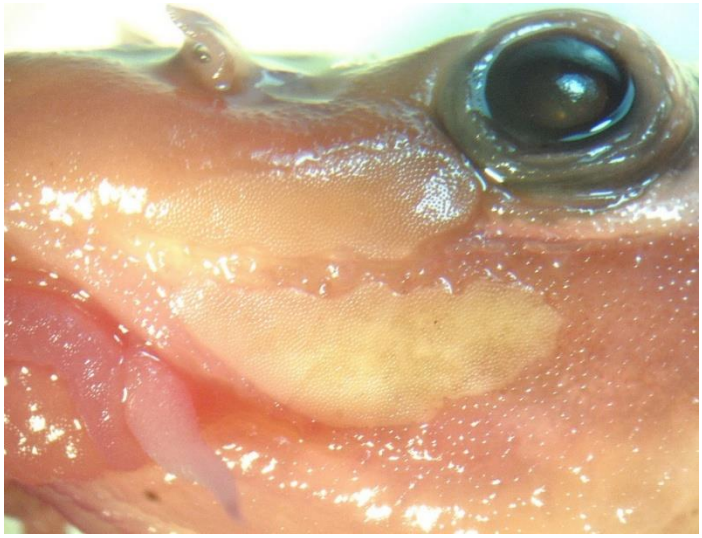

Figure 9. The brush-like agglomerations of spawning tubercles on sides of the head of the spotted thicklip loach (T. *strauchii*) from the Uba River (upper Irtysh basin). Male, TL – 131 mm)

The results of the morphological analysis showed that the studied fish from the Uba River have features characteristic of the spotted thicklip loach [13,17,18]: rounded black spots on the body, a caudal peduncle not compressed at the base, caudal peduncle width exceeds the minimum body depth, the minimum body depth more than 3 times in the caudal peduncle, the dorsal fin depth does not exceed the maximum body depth, the pectoral fins are longer than the pelvic fins, the anterior and posterior nostrils adjacent, the upper lobe of the caudal fin is slightly longer than the lower one. The structure of the swim bladder and intestine, the structure of the mouth and lips, as well as the location of the brush-like agglomerations of spawning tubercles on the head [17] are also characteristic of T. strauchii.

The spotted thicklip loach is subject to increased morphological variability, and individual populations differ significantly in many plastic features [13]. According to Prokofiev [17], most plastic features are of secondary importance in diagnosing the loach species, therefore, when describing new forms, it is necessary to study in more detail the qualitative features related to body shape, mouth structure, nuptial elements on the body of males, internal organ structure and, if possible, osteological features.

Conclusion

The spotted thicklip loach *(T. strauchii)* is a widespread Central Asian species, for which many populations have been poorly studied in the water bodies of Kazakhstan, and the distribution requires clarification. The range of this species is moving further north. In the upper Irtysh basin, T. strauchii was mentioned only for the rivers of the northern slopes of the Tarbagatai

Mountains. This study confirms its presence in the Uba River. The plastic and meristic features, body proportions, qualitative features related to color, shape of fins, head, lips, as well as the structure of the intestine and swim bladder were studied. Characteristic features of males were revealed. Most of the analyzed features are characteristic of Triplophysa strauchii. To clarify the range of this species, further studies are needed, including in the basin of the middle and lower Irtysh, and possible places for its penetration.

Authors' contributions

T.D.A. – idea and structure of the study, implementation of the experimental part of the study, writing and editing the text of the article; **S.M.B. –** implementation of the experimental part of the study, writing the text.

Conflicts of Interest

Authors declare no conflicts of interest.

Compliance with ethical standards

All animal procedures complied with the ethical standards of the institution where the studies were conducted and the approved legal acts of the Republic of Kazakhstan and international organizations. (No. 25/2).

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Үбі өзені (Жоғарғы Ертіс бассейні) теңбіл талма балығының *Triplophysa strauchii (Nemacheilidae)* морфологиялық сипаттамасы

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Аңдатпа. Теңбіл талма балығы – Қазақстанның көптеген су қоймаларында мекендейтін Орталық Азиялық балықтардың аборигенді түрі. Бұл түр балықтардың күрделі таксономиялық тобына жатады – *Triplophysa* туысы. Қазақстанның су қоймаларындағы теңбіл талма балығының үш түршесі белгілі: теңбіл талма балығы *(T. s. strauchii),* көл талма балығы *(T. s. ruzskyi)* және Зайсан талма балығы *(T. s. zaisanicus)*, бірақ олардың таксономиялық орны мен таралуы нақтылауды қажет етеді. Теңбіл талма балығының қазіргі таралу аумағы өте кең, ол Балқаш-Алакөл, Тарим, Шу және Сырдария өзендерінің бассейндерін мекендейді, сонымен қатар Зайсан көлінің бассейнінде де кездеседі. Қазіргі уақытта бұл түр өзінің таралу аймағын солтүстікке қарай кеңейтуде, ол Орталық және Солтүстік Қазақстанның ағынсыз өзендері мен көлдеріне сәтті бейімделген. 2016 жылы Жоғарғы Ертіс бассейнінің Үбі өзенінен теңбіл талма балығы табылды. Сыртқы морфологиялық сипаттамасы, оның ішінде түсі, пластикалық және меристикалық белгілері, дене пропорциялары, жүзбеқанаттарының пішіні, еріннің құрылымы, ішек пен жүзу торсылдағының

ерекшеліктері берілген. Жыныстық диморфизмге қатысты белгілер зерттелді. *Triplophysa strauchii* балығына тән белгілер анықталды. Осы түрдің ареалын нақтылау мақсатында Ертіс бассейнінде одан әрі зерттеу жүргізуді қажет етеді.

Түйін сөздер: теңбіл талма балығы, *Nemacheilidae, Triplophysa strauchii,* морфология, Жоғарғы Ертіс бассейні, Үбі өзені

Морфологическая характеристика пятнистого губача *Triplophysa strauchii (Nemacheilidae)* из реки Уба (верхне-иртышский бассейн)

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Аннотация. Пятнистый губач – аборигенный центральноазиатский вид рыб, обитающий во многих водоемах Казахстана. Этот вид относится к сложной таксономической группе рыб – роду *Triplophysa.* Для водоемов Казахстана известно три подвида пятнистого губача: пятнистый губач *(T. s. strauchii),* озерный губач *(T. s. ruzskyi)* и зайсанский губач *(T. s. zaisanicus),* однако их таксономический статус и распространение требуют уточнения. Современное распространение пятнистого губача очень широкое, он обитает в бассейнах Балхаш-Алаколя, Тарима, рек Чу и Сырдарья, также известен для бассейна озера Зайсан. В настоящее врем этот вид расширяет свой ареал далеко на север, где успешно акклиматизировался в бессточных реках и озерах Центрального и Северного Казахстана. В 2016 году пятнистый губач был обнаружен в реке Уба бассейна верхнего Иртыша. Приведено описание внешней морфологии, включающее окраску, пластические и меристические признаки, пропорции тела, форму плавников, строение губ, особенности кишечника и плавательного пузыря. Изучены признаки, касающиеся полового диморфизма. Выявлены характерные черты Triplophysa strauchii. С целью уточнения ареала данного вида, необходимы дальнейшие исследования в бассейне Иртыша.

Ключевые слова: пятнистый губач, *Nemacheilidae, Triplophysa strauchii,* морфология, верхне-иртышский бассейн, река Уба

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CRISPR/Cas12a-RPA integrated assay for potential rapid detection of *Fusarium* spp.

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Abstract. Timely detection and accurate identification of crop pathogens are considered fundamental components of sustainable agricultural systems alarming prompt and effective crop protection management. The cutting-edge CRISPR/Cas12a technology is emerging as a nucleic-acid-based platform with high potential for rapid, sensitive, and specific phytopathogen detection. In this study, we aimed to develop a CRISPR/Cas12a-based method to detect *Fusarium* spp., targeting the acl1 gene. A plasmid carrying the target gene fragment was successfully amplified using Recombinase Polymerase Amplification (RPA), confirming the feasibility of integrating this isothermal amplification with the CRISPR/Cas12a detection method. Optimization of the CRISPR-Cas12a reaction revealed optimal conditions at 100 nM MbCas12a, 100 nM crRNA, and 5 μM ssDNA reporter. Sensitivity assays demonstrated reliable detection of the *acl1* gene up to 1:1000 dilution. Specificity evaluation involving different plant-pathogenic fungal species confirmed the high specificity of the developed method. Altogether, this study highlights the potential of the CRISPR/Cas12a-based system as a promising diagnostic approach for agricultural applications to detect *Fusarium* spp.
Keywords: CRISPR, Cas12a, *Fusarium,* Recombinase Polymerase Amplification, nucleic acid detection

Introduction

Food Security is one of the 17 Sustainable Development Goals (SDGs) that require substantial efforts to address it. Sustainable agricultural production is challenged by different factors, including plant diseases caused by diverse pathogens [1,2]. The global economic loss attributed to plant diseases is estimated at approximately 40 billion USD annually, emphasizing the urgent need for efficient and sustainable crop protection strategies [2]. According to the Bureau of National Statistics of Kazakhstan (https://stat.gov.kz/), cereals, particularly wheat, occupy the largest share of cultivated land, accounting for approximately 68-72% of total sown areas in

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2022–2023. However, combating factors that limit crop productivity remains a critical challenge in agriculture, with biotic stresses such as pathogenic diseases posing significant threats to yield.

Fusarium spp. are considered one of the most destructive plant pathogens, affecting a wide range of agricultural crops worldwide [3]. Species within this genus infect wheat, barley, rice, tomato, and potato, causing severe yield reductions and producing toxic secondary metabolites known as mycotoxins. Diseases like *Fusarium* head blight and root rot cause billions of dollars in crop losses per annum. For instance, wheat rust and Fusarium infections alone are responsible for 5 and 3 billion USD in annual losses, respectively [4].

Rapid and precise detection of plant pathogens is essential for sustainable agriculture and effective crop protection [5]. Conventional detection methods include morphology- or PCR-based approaches [6]. Such methods are often time-consuming and require well-equipped laboratories, limiting their applications for rapid in situ diagnostics. Consequently, precise, nucleic-acid-based, portable detection methods attract considerable interest as alternative methods for validating plant infections under field conditions [5,6].

In recent years, molecular biology tools have advanced significantly [7], and CRISPR/Cas systems have emerged as powerful tools not only for genome editing but also for sensitive and specific pathogen detection [8,9]. Among CRISPR-associated nucleases, Cas12a represents remarkable potential for diagnostics due to its ability to recognize specific DNA sequences, process its own guide RNA (crRNA), and exhibit trans-cleavage activity toward single-stranded DNA once activated by the target sequence [10–12]. Such characteristics are crucial for developing portable, rapid, and sensitive detection platforms for agricultural pathogens. Moreover, isothermal amplification techniques, like Recombinase Polymerase amplification (RPA) [13], were developed to enable amplification of target genetic loci at relatively low temperatures without the need for advanced laboratory equipment. Such isothermal amplification methods integrated with CRISPR/Cas12a remarkably expand their application potential as highly sensitive and field-deployable assays [14]. Additionally, potential coupling with lateral flow devices or portable fluorescence readers for real-time visualization underscores their potential for monitoring disease outbreaks in remote agricultural fields as well as for implementing early intervention strategies and applying appropriate management strategies [15,16].

Recent studies already demonstrated the potential of CRISPR/Cas12a for detecting various agricultural pathogens [9]. This approach was successfully applied for the detection of *Alternaria solani* [17], *Puccinia striiformis* f. sp. tritici [18], and *Phytophthora ramorum* [19]. *Regarding Fusarium,* the CRISPR/Cas12a method was reported for identifying *Fusarium circinatum* [20], *Fusarium asiaticum* [21], and *Fusarium graminearum* [22] in different crops. Nevertheless, further research is required to extend this approach to other agriculturally significant Fusarium species. Therefore, the objective of the present study was to develop a CRISPR/Cas12a-based method for the detection of Fusarium spp.

Materials and research methods

Selection of genetic locus, design of RPA primers, and crRNA

The sequence of the *acl1* gene from the *Fusarium tricinctum* strain KAS:1036 (accession number JX397819.1) was selected as the target locus. RPA primers were designed with a melting temperature of 40–60 °C, GC content of 40–60%, and length of 30–35 bp. crRNA was designed based on a TTTG PAM site. All designs were performed using VectorNTI software. crRNA was transcribed *in vitro* using the HiScribe T7 Quick High Yield RNA Synthesis Kit and purified with the Monarch RNA Cleanup Kit (New England Biolabs) according to the manufacturer's instructions.

Identification of fungal isolates

Preliminary morphology-based identification, microscopy observation, DNA extraction, and ITS-based identification were as reported in our previous study [23].

Construction of the positive control carrying the acl1 gene and RPA validation

Positive control plasmids were obtained using the NEB PCR cloning kit according to the manufacturer's instructions. Successfully cloned plasmids were purified with the GeneJET Plasmid Miniprep Kit. RPA was performed following TwistAmp Basic kit official protocols (TwistDX, Cambridge, United Kingdom). Negative control was H2O throughout all relevant studies.

Optimization of CRISPR/Cas-12a-based reaction

Various concentrations of MbCas12a (10 nM, 50 nM, 100 nM, 250 nM, and 500 nM), crRNA (10 nM, 50 nM, and 100 nM), and ssDNA reporter (1.0, 2.0, 3.0, 4.0, and 5.0 μM) were tested to determine the optimal conditions. Recombinant MbCas12a protein obtained from *Moraxella bovis* in our laboratory, as described previously [24], was verified by reverse-phase C18 liquid chromatography-tandem mass spectrometry (LG-MS/MS) and employed in all corresponding studies.

Cis-activity of MbCas12a toward the acl1 gene

Cis-activity of MbCas12a was assessed using purified PCR amplicons (QIAquick PCR Purification kit, QIAQEN GmbH, Hilden, Germany) of the acl1 target sequence, amplified with RPA-designed primers (described above). MbCas12a was incubated with crRNA to form a ribonucleoprotein complex, followed by the addition of the PCR product and further incubation at 37°C. Cleavage of the target amplicon was evaluated via horizontal agarose gel electrophoresis and visualized using a GelDoc system.

Sensitivity validation and specificity verification

Positive control plasmid was serially diluted (1:1, 1:10, 1:100, and 1:1000) and amplified via RPA. Amplicons were analyzed by gel electrophoresis and fluorescence assays.

Various fungal plant pathogens commonly hosted wheat, such as *Nigrospora oryzae, Bipolaris sorokiniana,* and 2 strains of *Alternaria spp.*, were included to evaluate specificity. All samples were amplified with RPA and analyzed using agarose gel and naked-eye fluorescence readout.

Results

Selection of genetic locus, design of RPA primers, and crRNA

Based on the sequence of the acl1 gene available in the NCBI database (accession number JX397819.1), one set of RPA primers was designed according to the parameters described above. The forward (RPA-Phyto-Fus-acl1) and reverse primers (RPA-Phyto-Fus-acl1-RV) were GTGTCTTGAGACAATTCCTCGTTGAGCCTT and TAATGAAGTCGACAAGGACGTTGTGGACAC, respectively. The crRNA (Figure 1) was designed according to the TTTG PAM site in the *acl1* sequence (MbCas12a-crRNA-Fus-*acl1*: ggactctcgtctactggcgaggaaATCTACAAACAGTAGAAATTCC CTATAGTGAGTCGTATTAGAATT).

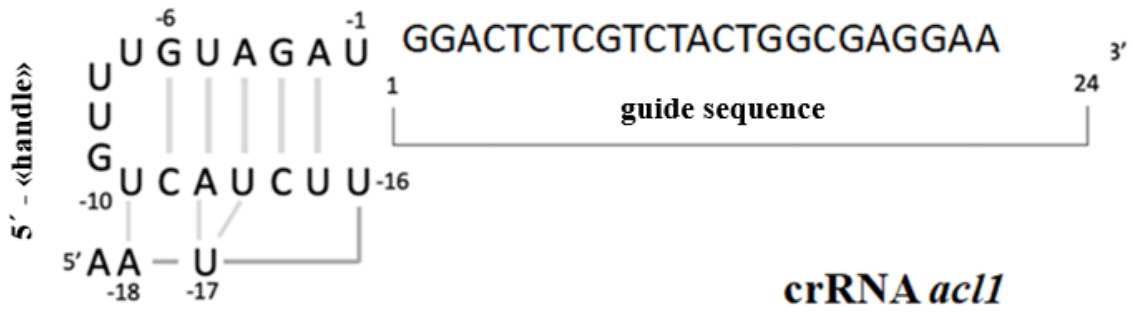

Figure 1. Design of the guide RNA targeting the acl1 gene

Identification of fungal isolates

A total of 12 fungal strains were isolated from cereal seeds samples [23]. BLAST analysis of ITS sequences revealed that eight strains (4/1, 7/2, 8/1, 8/5, 8/7, 11/1, 41/1, and 42/1) belong to *Alternaria* spp. and their sequences were deposited in NCBI database under accession numbers PQ056909-PQ056916, respectively. The remaining four strains – 22/1, 465, 1/3, and 25/1 - deposited as PQ056917-PQ056920 were identified as *Nigrospora oryzae, Bipolaris sorokiniana, Fusarium equiseti,* and *Fusarium acuminatum,* respectively. The *Fusarium* strain 25/1 was selected to validate the specificity of the designed RPA primers.

Construction of a positive control carrying the acl1 gene and RPA validation

Amplification of the Fusarium acuminatum 25/1 strain confirmed the functionality of the designed RPA primers (Figure 2a). The obtained PCR product was cloned into the pJET cloning kit (Thermo Scientific), yielding four positive clones out of five tested (Figure 2b)

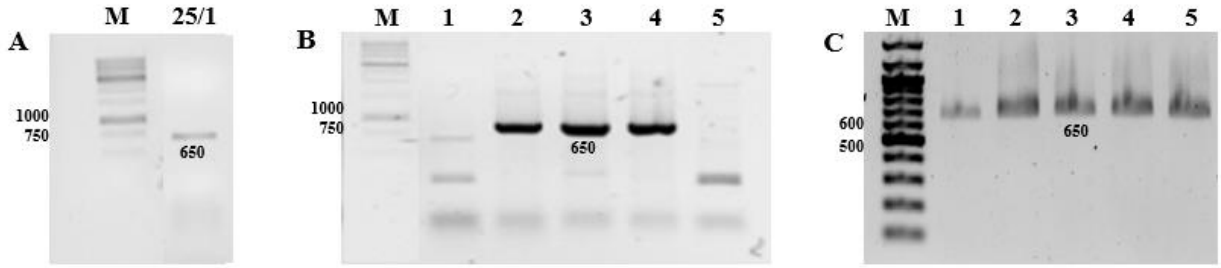

Figure 2. Construction of the plasmid carrying the *acl1* gene: (**a)** PCR amplification of the *acl1* gene from Fusarium strain 25/1 using RPA primers; (**b**) number of E. coli clones carrying plasmids with the cloned *acl1* gene; (**c**) RPA-based amplification of the *acl1* gene in the positive control (five technical copies)

The plasmid containing the cloned acl1 fragment was purified and subsequently used as a positive control in all subsequent assays.

Since isothermal amplification is the crucial element to perform the CRISPR/Cas12a methods without thermal equipment, positive control was amplified using RPA to validate this amplification method for obtaining amplicons required by the subsequent assays. RPA

amplification of the positive control in five technical replicates produced consistent amplicon yields (Figure 2c), validating the suitability of RPA for generating sufficient template DNA for CRISPR/Cas12a detection.

Optimization of CRISPR/Cas-12a-based reaction

The crucial elements of the CRISPR/Cas12a reaction include MbCas12a, crRNA, and ssDNA reporter. Various concentrations of MbCas12a (10-500 nM), crRNA (10 nM-100 nM), and ssDNA reporter (1.0-5.0 μM) were evaluated based on end-point fluorescence after 30 minutes of incubation (Figure 3).

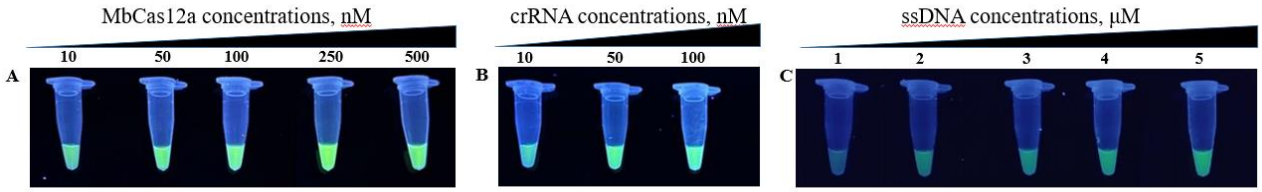

Figure 3. Optimization of CRISPR/Cas12-based reaction: (**a**) effect of MbCas12a concentration; (**b**) effect of crRNA concentration; **(c)** effect of ssDNA reporter concentration

Optimal signal intensity for the naked eye was achieved using 100 nM MbCas12a, 100 nM crRNA, and 5.0 μM ssDNA reporter.

Cis-activity of MbCas12a toward the acl1 gene

The cis-activity of MbCas12a, i.e., its ability to cleave the target site guided by the designed crRNA, was studied using the purified *acl1* PCR product (~650 bp). Partial cleavage was observed across all reaction durations ranging from 5 to 60 min (Figure 4).

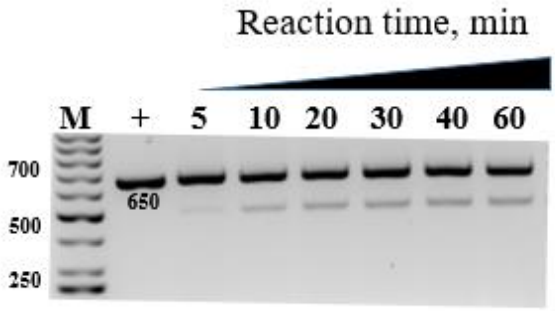

Figure 4. Evaluation of MbCas12a cis-activity using 650 bp acl1 PCR amplicon. The + lane represents the uncleaved control (PCR amplicon without MbCas12a reaction), whereas lanes 5–60 min correspond to reaction time intervals

Sensitivity validation and specificity verification

The analytical sensitivity and specificity of the developed method were evaluated using serial dilutions of the plasmid template and different fungal DNA samples, respectively. Both conventional gel electrophoresis and fluorescence signal with the naked eye assays demonstrated a sensitivity level up to a 1:1000 dilution of the origin plasmid (Figure 5a, b).

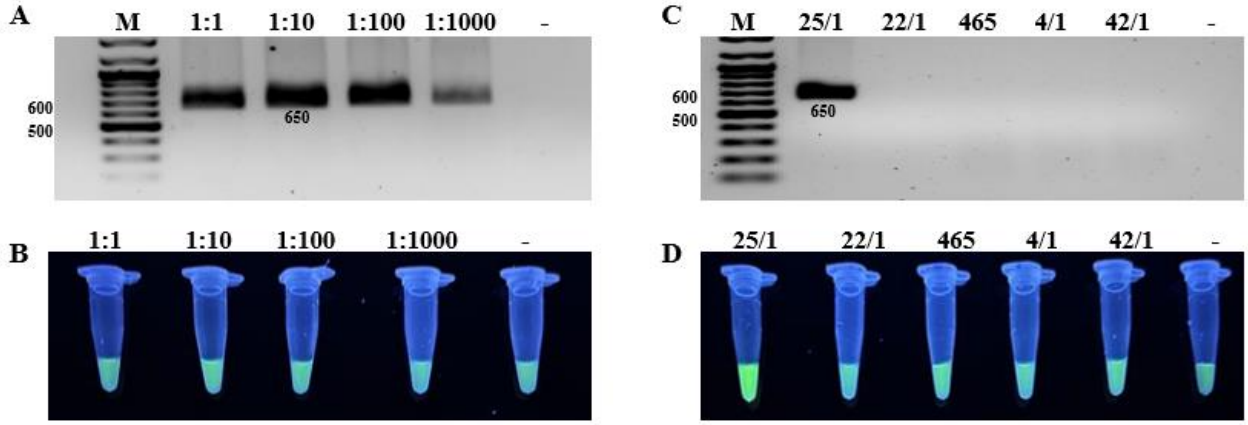

Figure 5. Evaluation of assay sensitivity and specificity. **(a**) sensitivity: gel electrophoresis results; serial dilutions of the target DNA fragment (1:10 to 1:1000); the minus sign indicates the negative control (H2O). **(b**) sensitivity: fluorescence assay results (legend as in panel a). **(c)** specificity: lanes 25/1, 22/1, 465, 4/1, and 42/1 correspond to Fusarium spp. positive controls: N. oryzae, B. sorokiniana, and Alternaria spp. were tested for cross-reactivity; the minus sign indicates the negative control (H2O). (**d**) Specificity: fluorescence assay results (legend as in panel c)

Specificity examination confirmed high selectivity of the assay toward the acl1 gene: none of the non-Fusarium fungal isolates was amplified during RPA reaction (Figure 5c). In the fluorescence assay, only the positive control exhibited visible fluorescence, further validating assay specificity (Figure 5d).

Discussion

The CRISPR/Cas system, originally discovered in bacteria and archaea as a form of adaptive immunity, has rapidly developed into a powerful platform for molecular diagnostics [25]. Since the first observation of clustered repeats in E. coli by Ishino et al. in 1987 [26], successive studies have revealed the system's biological function and its application potential, including diagnostics of various pathogenic agents [8,9,25].

Among the Cas nucleases, Cas12a is particularly attractive for diagnostic purposes due to its RNA-guided DNase activity and unique collateral (trans) cleavage property, which allows for the detection of target DNA sequences through a simple fluorescence readout [10,27,28]. Cas12a recognizes specific DNA targets guided by crRNA, and upon activation, it non-specifically cleaves single-stranded DNA molecules such as fluorescent reporters [29,30]. This mechanism forms the basis for highly sensitive detection systems that can operate under isothermal conditions. When combined with recombinase polymerase amplification (RPA), which efficiently amplifies DNA at constant temperatures, the Cas12a system can detect even minimal amounts of pathogen DNA without requiring thermal cyclers or complex laboratory equipment [15]. Such features make it a promising platform for rapid and field-adapted diagnostics.

Several recent studies have demonstrated the potential of Cas12a-based diagnostics for plant pathogens. For example, RPA–CRISPR/Cas12a systems have been developed for Diaporthe aspalathi and D. caulivora causing soybean stem canker [31], Verticillium dahliae associated with wilt disease [32], and Leptosphaeria maculans responsible for phoma stem canker in oilseed rape [30]. These methods achieved detection within 30–60 minutes and with

sensitivities as low as a few DNA copies, showing clear applicability in agricultural pathogen monitoring. Furthermore, CRISPR/Cas12a has been successfully used to identify several plant viruses, including tomato mosaic virus, tomato brown rugose fruit virus, and beet necrotic yellow vein virus, demonstrating its versatility across taxonomic groups [33,34].

In the present study, we applied this concept to develop a CRISPR/Cas12a-based diagnostic assay targeting the acl1 gene to detect *Fusarium*spp. To the best of our knowledge, this is the first report describing a CRISPR/Cas12a-based detection system for *Fusarium* spp. developed in Kazakhstan. The designed RPA primers and crRNA enabled specific recognition of the target fragment, with optimal reaction parameters determined for Cas12a, crRNA, and ssDNA reporter concentrations. The assay showed both high specificity and remarkable sensitivity, detecting target DNA dilutions up to 1:1000 of the original plasmid. Importantly, the system distinguished Fusarium species from other common cereal-associated fungi such as *Alternaria spp., Bipolaris sorokiniana,* and *Nigrospora oryzae.*

Altogether, our results support the potential of Cas12a-based systems for accurate, portable, and cost-effective detection of fungal pathogens. By enabling on-site diagnostics, such approaches could facilitate early detection and management of *Fusarium*-related diseases, helping to reduce crop losses and improve yield stability. Continued optimization of reaction conditions and integration into simple field-deployable formats, such as lateral flow strips or smartphone-based fluorescence readers, will further expand the practical use of this method in agricultural monitoring and plant health management.

Conclusion

The CRISPR/Cas12a-based method targeting the *acl1* genetic locus was developed to detect Fusarium spp. with high specificity and sensitivity. Integrated with the isothermal Recombinase Polymerase Amplification and a fluorescence assay, this method demonstrates strong potential for rapid and reliable detection of *Fusarium* pathogens under field conditions. The proposed system can contribute to improved plant disease diagnostics and sustainable crop protection practices.

Author Contributions

All authors contributed to the article and approved the submitted version. **A.Sa., Zh.Zh., A.Z. –** formal analysis, investigation, writing – original draft preparation; **A.Sh., M.A., S.A. –** methodology, conceptualization, discussion, writing – review and editing; **S.A., A.Z.** – funding acquisition, project administration, data curation, supervision.

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Conflicts of Interest

The authors declare the absence of any conflict of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Fusarium spp. жылдам анықтауға арналған CRISPR/Cas12a-RPA біріктірілген талдау әдісінің потенциалы

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Аңдатпа. Егін ауруларын дер кезінде анықтау мен дәл идентификациялау – ауыл шаруашылығындағы тұрақты жүйелердің негізгі құрамдас бөлігі болып табылады және өсімдіктерді жедел әрі тиімді қорғауды қамтамасыз етеді. Алдыңғы қатарда саналатын CRISPR/Cas12a жүйесі фитопатогендерді жоғары дәлдікпен, сезімталдықпен және жедел анықтауға мүмкіндік беретін нуклеин қышқылдарына негізделген заманауи платформа ретінде қарқынды дамып келеді. Осы зерттеуде біз Fusarium туысының саңырауқұлақтарын анықтауға арналған acl1 генін нысана еткен CRISPR/Cas12a негізіндегі әдісті әзірледік. Нысана ген фрагментін қамтитын плазмида Рекомбиназалық полимеразалық амплификация (RPA) әдісімен сәтті амплификацияланды, бұл изотермиялық амплификацияны CRISPR/Cas12a детекция әдісімен біріктірудің орындылығын растады. CRISPR-Cas12a реакциясын оңтайландыру нәтижесінде ең тиімді шарттар 100 нМ MbCas12a, 100 нМ crRNA, және 5 μM ssDNA репортері ретінде анықталды. Сезімталдықты анықтау сынақтары acl1 генін 1:1000 дейінгі сұйылтуда сенімді анықтауға болатынын көрсетті. Түрлі өсімдік патогенді саңырауқұлақтар түрлерін қамтыған ерекшелік сынақтары әзірленген әдістің жоғары ерекшелігін дәлелдеді. Осылайша, бұл зерттеу Fusarium туысының өкілдерін анықтау үшін CRISPR/Cas12a негізіндегі жүйенің ауыл шаруашылығында қолдануға арналған перспективалы диагностикалық құрал екенін көрсетеді.
Түйін сөздер: CRISPR, Cas12a, *Fusarium,* Рекомбиназалық полимеразалық амплификация, нуклеин қышқылдарын анықтау

CRISPR/Cas12a-RPA интегрированный метод для потенциала быстрой детекции *Fusarium* spp.

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Аннотация. Своевременное обнаружение и точная идентификация возбудителей болезней сельскохозяйственных культур являются ключевыми элементами устойчивых агросистем, обеспечивающими оперативное и эффективное управление защитой растений. Современная технология CRISPR/Cas12a активно развивается как платформа на основе нуклеиновых кислот с высоким потенциалом для быстрого, чувствительного и специфичного выявления фитопатогенов. В данной работе разработан метод на основе CRISPR/Cas12a для детекции грибов рода Fusarium, нацеленный на ген acl1. Плазмида, содержащая фрагмент целевого гена, была успешно амплифицирована с использованием метода Рекомбиназной полимеразной амплификации (RPA), что подтвердило возможность интеграции этой изотермической

амплификации с методом детекции CRISPR/Cas12a. Оптимизация реакции CRISPR-Cas12a показала, что наилучшие условия достигаются при концентрациях 100 нМ MbCas12a, 100 нМ crRNA и 5 μM ssDNA-репортера. Тесты чувствительности показали надёжное выявление гена acl1 при разведении до 1:1000. Оценка специфичности с участием различных фитопатогенных грибов подтвердила высокую селективность разработанного метода. В целом результаты исследования демонстрируют потенциал системы на основе CRISPR/Cas12a как перспективного диагностического инструмента для выявления грибов рода Fusarium в сельскохозяйственных применениях.

Ключевые слова: CRISPR, Cas12a, *Fusarium,* Рекомбиназная полимеразная амплификация, детек-ция нуклеиновых кислот

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Dynamics of *Acinetobacter baumannii* isolation and antibiotic resistance in a multidisciplinary clinic for 2021-2025

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Abstract. *Acinetobacter baumannii* is one of the most influential pathogens causing nosocomial infections characterized by high resistance to antibacterial drugs. Thus, this study aimed to evaluate the dynamics of *A. baumannii* isolation and the level of antibiotic resistance in a multidisciplinary hospital for the period 2021-2025. For this reason, a comparative retrospective analysis was conducted on 13 221 clinical isolates obtained from various departments. The analysis was conducted using phenotypic identification and susceptibility testing methods. Susceptibility testing (antibiograma) was performed using the Vitek 2 automated system and the disk diffusion method for nine antimicrobial drugs. These antibiotics included carbapenems (imipenem, meropenem), aminoglycosides (gentamicin, amikacin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin), colistin, and a trimethoprim/sulfamethoxazole combination. The results showed a decrease in the frequency of *A. baumannii* isolation over the observation period ($\chi^2 = 19.29$, $p = 0.0007$), with the majority of isolates retaining multiple-drug resistance (MDR). The highest resistance was detected to fluoroquinolones and carbapenems, while susceptibility to colistin remained high throughout all years. The lack of molecular analysis limits the interpretation of the results; however, the resulting resistance profile suggested genes that could act as pathogenicity factors. These comparative retrospective analyses of resistance highlight the need for ongoing local monitoring of A. baumannii antibiotic resistance and the rational use of antimicrobials in hospital settings.

Keywords: *Acinetobacter baumannii,* antibiotic resistance, multidrug resistance (MDR), carbapenems, fluoroquinolones, colistin, clinical isolates

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Introduction

Antibiotic resistance (AR) remains one of the most significant public health threats in the 21st century [1,2]. The widespread spread of resistant pathogens and the declining effectiveness of essential antimicrobials lead to increased mortality, prolonged hospitalization, and increased healthcare costs [1,2]. Among hospital-acquired pathogens, *Acinetobacter baumannii* poses a particular danger [3–6]. It is a non-fermenting Gram-negative rod characterized by its ability to survive in the hospital environment [3–6]. Its rapidly accumulating resistant determinants cause severe nosocomial infections (including ventilator-associated pneumonia and bacteremia) with high mortality in intensive care units [3–6].

Current systematic reviews and meta-analyses demonstrate wide geographic variability in *A. baumannii* resistance levels [3,4]. At the same time, many regions exhibit persistently high rates of resistance to carbapenems, fluoroquinolones, and aminoglycosides. Carbapenem-resistant *A. baumannii* (CRAB) strains are listed by the World Health Organization (WHO) as priority bacterial pathogens requiring the development of new therapeutic agents and enhanced surveillance [7,8]. Furthermore, there are alarming reports of an increase in cases resistant even to reserve drugs (colistin, tigecycline) [9–14]. Molecular epidemiological analyses highlight the role of clinical clones and plasmid-mediated transmission of resistance genes in the formation of local and interregional outbreaks [11,15,16].

Reliable epidemiological surveillance of AR requires continuous monitoring through the analysis of obtained data, which allows us to separate random annual fluctuations from stable trends [17–20]. Timely detection of outbreaks of clonal spread and assessment of the impact of interventions (antibiotic stewardship, infection control measures) are important for sustainable control [17–20]. Thus, local and regional studies assessing the annual dynamics of S/R for individual antibiotics and the distribution of non-MDR (non-multi-drug-resistant)/ MDR (multi-drug-resistant)/PDR (pan-drug-resistant) categories are a valuable source of clinical and epidemiological information [1,8,12]. They inform empirical treatment regimens, antimicrobial stewardship policies, and infection control measures in hospitals [1,8,12]. With limited therapeutic options and the slow development of new antibiotics, timely monitoring and transparent reporting remain crucial elements in responding to the A. baumannii threat.

Materials and research methods

Object and research basis

The study involved clinical isolates of *Acinetobacter baumannii* isolated from various biological specimens of patients hospitalized at the National Research Medical Center (NRMC) between 2021 and 2025. The study included specimens from the anesthesiology and intensive care departments (ICU), urology, surgery, and therapy patients. In some cases, specimens were received from the microbiology department (MOB-2). Over five years, a total of 13 221 clinical specimens were analyzed, from which a varying number of A. baumannii isolates were identified by year (Table 1).

Table 1

Annual distribution of the number of tested samples and positive isolates of *A. baumannii* at the NRMC (2021-2025)

Year	Number of samples tested (n)	*A. baumannii* isolates (n)	Main departments where the pathogen was isolated
2021	2 939	12	ICU, urology, outpatient department, day hospital

2022	2 746	28	ICU, urology, surgery, and the outpatient department
2023	2 505	16	Urology, internal medicine, outpatient clinic, surgery
2024	2 739	12	Urology, surgery, outpatient clinic, ICU
2025	2 292	6	ICU, Urology, CCD, Urology, MOB-2

Note: the calculation was based on data from the microbiological registry of the NRMC (2021-2025). ICU – intensive care departments, MOB-2 – microbiology department.

Selection of biological materials

Between 2021 and 2025, the study included clinical specimens collected from patients in various departments of the NRMC (Table 1). The predominant samples examined were from the urinary tract (urine), the respiratory tract (bronchial washings, sputum, throat swabs, pleural fluid, and contents of intubation and suction catheters), as well as wound, drainage, wound and pressure ulcer swabs, and contents of central venous catheters. All specimens were collected using an aseptic technique and transported to the microbiology laboratory within two hours after collection [21].

Microbiological analysis and identification of isolates

Clinical specimens were cultured on standard nutrient media, including blood agar, Endo agar, egg yolk-salt agar, Kalina agar, chocolate agar, and chromogenic media. Incubation was performed at 37°C for 18-24 hours. Preliminary identification of microorganisms was performed by morphological characteristics, Gram staining, and biochemical tests [21]. Pure cultures were also identified using the automated Vitek 2 Compact system [22,23].

Antimicrobial susceptibility testing (antibiograma) of *A. baumannii* isolates was performed using two methods. The first method was a disk diffusion assay on a nutrient medium using commercial antibiotic disks [22]. Inhibition zones were recorded after 18-24 hours of incubation at 37°C and interpreted according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [24]. The second method was automated using the Vitek 2 Compact system [23].

The comparative retrospective analyses of resistance included drugs from various pharmacological groups: imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and colistin. Results were interpreted according to EUCAST criteria, classifying isolates as intermediate (S), intermediate (I), or resistant (R) [24]. Intermediate (I) and resistant (R) isolates were combined during the analysis to compare intermediate and resistant isolates.

Statistical data analysis

Statistical data processing was performed using GraphPad Prism 8.0.1. For each year, the proportion of positive A. baumannii isolates among the total number of tested samples was calculated, expressed as a percentage. Based on these data, the mean proportion (%) and standard error of the mean (SE) were determined and used to plot error bars (values are presented as mean ± SE). Comparisons of proportions between groups were performed using the χ^2 (chi-square) test or Fisher's exact test, depending on the sample size [21]. Differences were considered statistically significant at $p < 0.05$. All graphical representations included legends indicating the analysis method used and confidence levels.

Results

The proportion of A. baumannii among clinical isolates in 2021-2025

The analysis revealed a significant downward trend in the frequency of *Acinetobacter baumannii* isolation from patient clinical specimens over the period 2021-2025. The proportion of positive isolates was 1.09% in 2021, 1.02% in 2022, 0.64% in 2023, 0.44% in 2024, and 0.26% in 2025, representing a total of 4.49% across the five years. The highest number of *A. baumannii* cases was recorded in 2021-2022. The decrease in the proportion of the pathogen in subsequent years likely reflects the effectiveness of infection control measures and the rationalization of antibacterial therapy in the hospital. The statistical analysis performed using the χ^2 method confirmed the reliability of differences between the years of observation (χ^2 = 19.29; df = 4; p = 0.0007). This indicates a statistically significant decrease in the proportion of *A. baumannii* isolates over the five-year observation period.

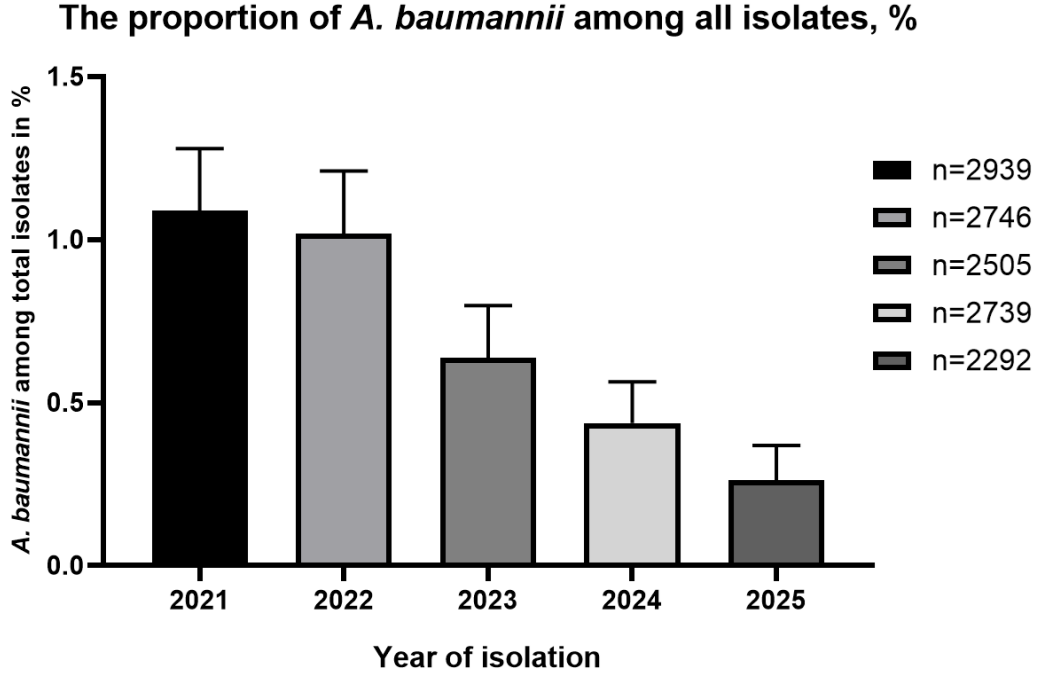


Figure 1. Yearly changes in the proportion of *Acinetobacter baumannii* among all isolates (2021-2025). The total number of isolates (n) for each year is indicated in the diagram. The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test comparing positive and negative *A. baumannii* cases across the years 2021-2025 (χ^2 = 19.29; df = 4; p = 0.0007)

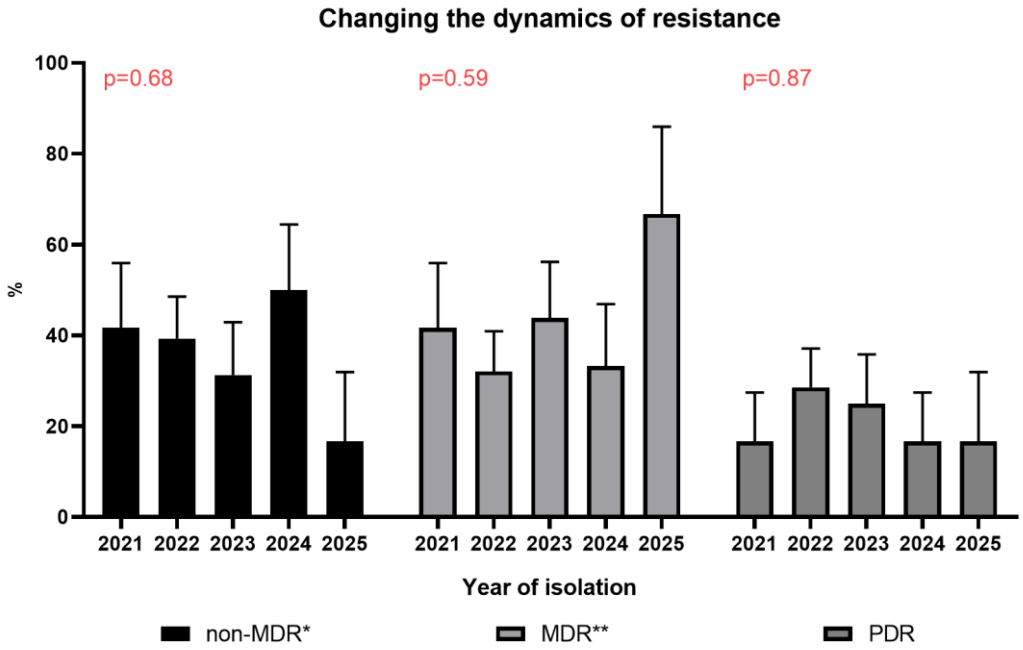
Changes in the resistance structure of A. baumannii isolates

An analysis of the distribution of *A. baumannii* isolates by resistance categories (non-MDR*, MDR**, and PDR) showed that multidrug-resistant strains predominated in all observation years (Figure 2). In 2021, the proportion of MDR isolates was approximately 42%, non-MDR 25%, and PDR 8%. In subsequent years, slight fluctuations between categories were observed, but without significant dynamics (p > 0.05). Overall, the resistance pattern remained stable throughout the study period. At the same time, the proportion of MDR isolates remained high, indicating the persistence of resistant clones of the pathogen in hospital settings. The recorded

periodic variability in the ratios of non-MDR and PDR strains likely reflects the influence of factors associated with changes in antibiotic therapy tactics, drug rotation, and hospital environmental characteristics. Despite the absence of an increase in PDR forms, the persistence of a significant proportion of MDR isolates poses a serious problem for clinical practice.

Antibiotic susceptibility of A. baumannii isolates

The results of antibiotic susceptibility testing of *A. baumannii* isolates collected between 2021 and 2025 indicate widespread drug resistance and a strong tendency toward the persistence of a multidrug-resistant phenotype. The overall picture (Figure 3) shows that *A. baumannii* exhibited low susceptibility to most antimicrobial agents. The exception was colistin, whose activity remained high over the last three years of observation. However, the data were not presented graphically due to the small sample size.



Note: *Because there were only 3 cases of *A. baumannii* that were completely susceptible to antibiotics during the entire observation period, it was combined into a group as non-MDR, which includes those resistant to 1 and 2 antibiotics. **The MDR group included resistance to equal or more than 3 antibiotics. The PDR group includes isolates that were resistant to all antibiotics tested.

Figure 2. Yearly changes in the resistance dynamics of *Acinetobacter baumannii* among all isolates (2021, n=12; 2022, n=28; 2023, n=16; 2024, n=12; 2025, n=6). The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test comparing response type with other *A. baumannii* cases across the years 2021-2025

Aminoglycosides

Susceptibility of isolates to amikacin was 58% in 2021, decreased to 50% in 2022, and reached a minimum of 33% by 2025. A similar trend was observed for gentamicin. In 2021, the proportion of susceptible strains was 58%, in 2023 it was 50%, and by 2025 it had decreased to 20%. Similar fluctuations were observed for tobramycin, from 70% of isolates susceptible in 2021 to 30% in 2025. Although the differences between years did not reach statistical significance ($p > 0.05$), the overall trend indicates a gradual decline in aminoglycoside activity.

This indicates the emergence of resistance in the hospital strain population. The high level of cross-resistance between amikacin, gentamicin, and tobramycin indicates the accumulation of similar genetic determinants (aacC, aphA, and aadB) typical for *A. baumannii* [25,26].

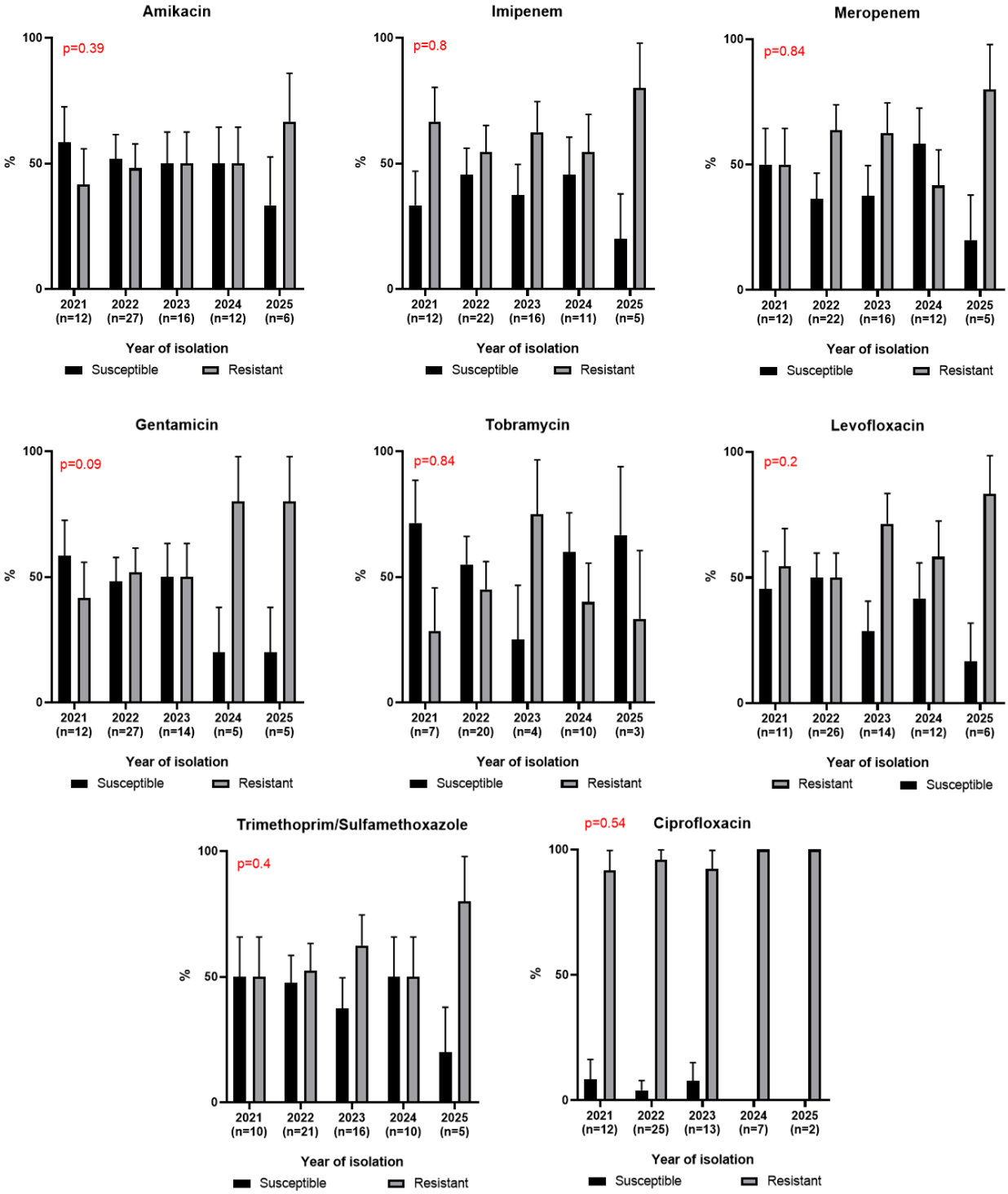


Figure 3. Changes in the sensitivity of *A. baumannii* isolates to antibiotics from various pharmacological groups from 2021 to 2025. The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test to compare susceptible and resistant *A. baumannii* cases across the years 2021-2025

Carbapenems

The most alarming situation is observed for imipenem and meropenem. In 2021, susceptibility to imipenem was 33%, by 2024 it was 45%, and by 2025 it had decreased to 20%. Similar trends were observed for meropenem (50%, 58%, and 20%, respectively). These data confirm the presence of high levels of carbapenem resistance, likely associated with the spread of OXA-type D carbapenemases (primarily OXA-23-like, OXA-24/40-like и OXA-58-like), consistent with global trends [7,27–29]. Carbapenem-resistant strains typically exhibit multiple resistance and limited therapeutic options, as evidenced by the high correlation with the MDR and PDR categories in this study.

Fluoroquinolones

Fluoroquinolone activity remained low throughout the study period. While susceptibility to levofloxacin was 45% in 2021, 30% in 2023, and decreased to 17% by 2025, susceptibility to ciprofloxacin did not exceed 10% in all observation years. These values indicate the practical ineffectiveness of fluoroquinolones against clinical isolates of *A. baumannii*. This is likely due to mutations in the gyrA and parC genes, as well as activation of the efflux systems [30,31].

Sulfonamides

For trimethoprim/sulfamethoxazole, moderate fluctuations in susceptibility were observed, ranging from 50% (2021) to 20% (2025). Although there were no statistically significant differences ($p > 0.05$), a gradual decrease in susceptibility persisted. Given the limited use of this drug in hospital settings, the decrease in activity may be due to cross-resistance via plasmid determinants such as dfrA1, dfrA5, sul1, and sul2 [26,29].

Polymyxins

Of particular importance is the high activity of colistin, to which over 60% of isolates remained susceptible throughout the observation period (2023-2025). In 2024-2025, susceptibility increased to 80% and 100%, respectively. This makes colistin a "last resort" drug for the treatment of infections caused by MDR/XDR *A. baumannii* strains. However, the development of resistance to polymyxins remains extremely rare. This is likely due to the limited use of the drug and the high fitness cost of mutations in the lpxA/D, lpxACD, and pmrB genes [32,33].

Discussion

The results of this study demonstrate that, over the period 2021-2025, a clear downward trend in the frequency of *A. baumannii* isolation among all clinical isolates was observed in a large multidisciplinary hospital. The significant decrease in the proportion of positive isolates ($\chi^2 = 19.29$; $p = 0.0007$) may reflect the effectiveness of the implemented infection control measures and optimization of antibacterial therapy in various departments. Similar trends have been described in a number of publications where the implementation of antimicrobial stewardship programs led to a reduction in the prevalence of hospital isolates of *A. baumannii* [3,5,12].

Despite the positive trend in the reduction of pathogen isolation frequency, the structure of drug resistance in *A. baumannii* remained tense. MDR strains predominated in all study years, which is consistent with the results of similar observations conducted in other countries [2,6,10]. The high proportion of MDR strains indicates stable circulation of hospital clones possessing a combination of defense mechanisms [4,11]. This is possibly associated with the rapid production of broad-spectrum β-lactamases, the activity of efflux systems, and changes in the permeability of the outer membrane [4,11].

The most pronounced resistance was observed to carbapenems (imipenem, meropenem) and fluoroquinolones, which is consistent with international observations, in which the spread of carbapenem-resistant clones of A. baumannii (CRAB) is considered one of the main challenges of clinical microbiology [7,14,32]. Given the lack of molecular typing in our study, it is assumed that the obtained phenotypic manifestations are associated with the spread of OXA-type β-lactamases [7,27–29]. We also assume that this is associated with possible mutations in the gyrA and parC genes and the activation of adeABC-type efflux systems, which has been confirmed by other authors [34–36].

An interesting feature is the persistence of high susceptibility of isolates to colistin, which is consistent with the results of studies conducted in Europe and the Middle East [6,37,38]. The lack of growth of colistin-resistant strains in our study is likely due to the limited use of this drug in clinical practice. Mutations in the pmrA/pmrB and lpxACD systems responsible for resistance to polymyxins are known to be accompanied by a significant fitness cost, which reduces the likelihood of their persistence in the population [32,33].

It should be emphasized that the study was phenotypic in nature and did not include molecular analysis of resistance genes. This limits the ability to accurately identify resistance mechanisms and genetically characterize circulating clones. However, the results obtained allow us to formulate hypotheses about the likely mechanisms of multiple resistance. This, in turn, allows us to formulate a direction for further research, including genome sequencing and the identification of pathogenicity factors (OXA, NDM, sul, aad, adeABC, etc.). From a practical standpoint, the data confirm the need for continuous local monitoring of *A. baumannii* susceptibility. Notably, phenotypic shifts can serve as an early indicator of the emergence of new resistant clones. Going forward, it seems advisable to combine epidemiological observations with molecular genetic methods (WGS, MLST) to clarify clonal affiliation and sources of nosocomial spread. In the future, the results of this study can be used to adjust empirical treatment regimens, optimize antimicrobial control programs, and develop regional protocols for the rational use of antibiotics.

Conclusion

The study assessed the isolation dynamics and antibiotic resistance levels of *A. baumannii* in hospitals from 2021 to 2025. The comparative retrospective analyses of resistance data obtained indicate a decrease in the rate of pathogen isolation. This may reflect the effectiveness of implemented infection control measures and the rationalization of antibacterial therapy. However, despite this positive trend, a high level of multidrug resistance (MDR) persists, including to β-lactam antibiotics and carbapenems, confirming the persistent circulation of hospital clones. Of particular note is the high susceptibility of isolates to colistin, which persisted throughout the observation period. This is likely due to the limited use of the drug and the high fitness cost of mutations conferring resistance to polymyxins. Meanwhile, moderate susceptibility rates to trimethoprim/sulfamethoxazole may be due to cross-resistance associated with plasmid determinants of the sul1/sul2 type. It should be noted that this study was phenotypic in nature and did not include molecular verification of resistance mechanisms. This limits the ability to accurately identify resistance determinants and assess the clonal structure of the population. Nevertheless, the identified patterns allow us to hypothesize possible mechanisms of multiple resistance and outline areas for further research, including molecular genetic methods to detect pathogenicity factors. The results of this study can potentially be used to improve surveillance systems for hospital-acquired infections, optimize empirical antibiotic therapy, and develop

regional antimicrobial control protocols. Continued studies using molecular technologies will provide a deeper understanding of the spread of resistance and develop more effective strategies for its containment.

Author Contributions

N.S.S., N.B., and **M.D. –** conceptualization and design of the study; **N.S.S.** and **N.B.** –methodology and visualization; **N.S.S., N.B., M.D.,** and **A.B.** – writing – original draft; **N.S.S., N.B., M.A., S.A., A.B.,** and **G.D. –** writing – review & editing; **N.S.S., G.D., M.A., A.S.** – investigation, data curation, and formal analysis.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

All procedures performed in studies involving people comply with the ethical standards of the National Committee on Research Ethics and the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards.

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Көпбейінді ауруханадағы 2021-2025 жылдар аралығында *Acinetobacter baumannii* оқшаулану жиілігі және антибиотикке төзімділігінің динамикасы

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Аңдатпа. *Acinetobacter baumannii* – ауруханаішілік инфекцияларды тудыратын ең қауіпті патогендердің бірі, ол бактерияға қарсы препараттарға жоғары деңгейдегі төзімділігімен сипатталады. Сондықтан, бұл зерттеудің мақсаты 2021-2025 жылдар аралығындағы көп салалы ауруханада *A. baumannii* оқшаулану динамикасын және антибиотикке төзімділік деңгейін бағалау болды. Осы мақсатта әртүрлі бөлімдерден алынған 13 221 клиникалық изоляттың салыстырмалы ретроспективті талдауы жүргізілді. Талдау фенотиптік идентификация және антибиотиктерге сезімталдықты тексеру арқылы жүргізілді. Сезімталдықты тексеру (антибиограммалар) автоматтандырылған Vitek 2 жүйесі және тоғыз микробқа қарсы агент үшін диск диффузиясы әдісін қолдану арқылы жүргізілді. Бұл антибиотиктерге карбапенемдер (имипенем, меропенем), аминогликозидтер (гентамицин, амикацин, тобрамицин), фторхинолондар (ципрофлоксацин, левофлоксацин), колистин және триметоприм/сульфаметоксазол комбинациясы жатады. Нәтижелер саралау кезеңінде *A. baumannii* оқшаулану жиілігінің төмендегенін көрсетті ($\chi^2 = 19.29$, $p = 0.0007$), көптеген изоляттар көп дәрілік төзімділікті (КДР) сақтап қалды. Фторхинолондар мен карбапенемдерге ең жоғары төзімділік анықталды, ал колистинге сезімталдық барлық жылдар бойы жоғары болып қалды. Молекулалық талдаудың болмауы нәтижелерді түсіндіруді шектейді. Дегенмен, алынған төзімділік профилі патогенділік факторлары ретінде әрекет етуі мүмкін гендердің бар екенін көрсетеді. Салыстырмалы ретроспективті төзімділік талдауының нәтижелері *A. baumannii* антибиотиктерге төзімділігін үздіксіз жергілікті бақылау және аурухана жағдайында микробқа қарсы препараттарды ұтымды пайдалану қажеттілігін көрсетеді.

Түйін сөздер: *Acinetobacter baumannii,* антибиотиктерге төзімділік, көп дәрілік төзімділік (КДР), карбапенемдер, фторхинолондар, колистин, клиникалық изоляттар

Динамика выделения *Acinetobacter baumannii* и антибиотикорезистентности в многопрофильном стационаре за 2021-2025

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Аннотация. *Acinetobacter baumannii* является одним из наиболее опасных возбудителей нозокомиальных инфекций, характеризующихся высокой устойчивостью к антибактериальным препаратам. В связи с этим целью данного исследования была оценка динамики выделения *A. baumannii* и уровня антибиотикорезистентности в многопрофильном стационаре за период 2021-2025 гг. Для это был проведен сравнительный ретроспективный анализ 13 221 клинических изолятов, полученных из различных отделений. Анализ проводился с использованием методов фенотипической идентификации и определения чувствительности к антибиотикам. Определение чувствительности (антибиограммы) проводилось с помощью автоматизированной системы Vitek 2 и диско-диффузионного метода для девяти антимикробных препаратов. К этим антибиотикам относятся карбапенемы (имипенем, меропенем), аминогликозиды (гентамицин, амикацин, тобрамицин), фторхинолоны (ципрофлоксацин, левофлоксацин), колистин и комбинация триметоприма/сульфаметоксазола. Результаты показали снижение частоты выделения A. baumannii за период наблюдения (χ^2 = 19.29, p = 0.0007), при этом большинство изолятов сохраняли множественную лекарственную устойчивость (МЛУ). Наибольшая резистентность была выявлена к фторхинолонам и карбапенемам, в то время как чувствительность к колистину оставалась высокой на протяжении всех лет. Отсутствие молекулярного анализа ограничивает интерпретацию результатов, однако полученный профиль резистентности позволяет предположить наличие генов, которые могут выступать в качестве факторов патогенности. Результаты сравнительного ретроспективного анализа резистентности подчеркивают необходимость постоянного локального мониторинга резистентности *A. baumannii* к антибиотикам и рационального использования антимикробных препаратов в условиях стационара.

Ключевые слова: *Acinetobacter baumannii,* устойчивость к антибиотикам, множественная лекарственная устойчивость (МЛУ), карбапенемы, фторхинолоны, колистин, клинические изоляты

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Biological features of ixodid tick dispersal in biotopes of the West Kazakhstan region

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Abstract. A biological and faunal analysis of the species distribution of Ixodid ticks in the West Kazakhstan region was carried out. The ecological features of the distribution of Ixodid mites in the biogeocenosis of the studied region, as well as their species association with certain biotopes, are studied. The highest species diversity of ticks is distinguished by the elevated steppe zone. The ixodid fauna in this zone is represented by 5 species belonging to 4 genera: *D. marginatus, D. reticulatus, H. detritum, Rh. rossicus,* and *I. persulcatus.* Mites of the genus Dermacentor prefer toinhabit areas with sufficiently moist soil, with mixed grass and meadow vegetation. *I. persulcatus* and *Rh. rossicus species* are found in forested floodplains, *Hyalomma* species are pastures. In the collected specimens of Ixodid ticks, a significant predominance of females compared to males was noted. Ticks of the genus *Dermacentor* are distributed mainly in flat steppe landscapes. Forest and forest-steppe zones are the most suitable for the ixodid family. For *Ripicephalus*, they are forest-steppe and semi-desert. Ticks of the genus *Hyalomma* are found only in areas of cattle breeding and adjacent territories.
Keywords: ixodid ticks, biogeocenosis, dominant species, biological analysis, faunal analysis

Introduction

Ixodid mites belong to the ecological group of temporary ectoparasites with long-term feeding [1-4], so the life cycle of ixodids includes four stages: egg, larva, nymph, and imago. The nature of the effects of tick bites on the skin of animals is determined by their type, the duration of suction, and the immune status of the host. Ixodid ticks are temporary ectoparasites that feed for a long time, spending a significant part of their life cycle in the external environment.

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In addition, much attention was paid to species of epizootological and epidemiological significance. As a result of numerous studies and observations, an extensive factual material was accumulated, so there was a need to systematize and generalize it, which was implemented by a number of authors in works of a monographic nature [5-10]. A number of authors have found that ixodids contain tularemia, plague, tick-borne encephalitis, ixodic tick-borne borelliosis, and animal piroplasmidosis. For many natural focal diseases, ixodid ticks are special carriers. The range of pathogens transmitted by ixodid ticks is constantly expanding.

Currently, diseases associated with tick bites dangerous for animals and humans, are everywhere, from tropical, subtropical, and temperate zones to rural areas and large cities. The constant movement of animals and surrounding people contributes to the spread of ticks. The study of the ecological features of the distribution of ixodid ticks in the biome of West Kazakhstan, as well as the exact location of a particular biome, allows you to determine in time the places of accumulation of the most dangerous ixodid tick species. The direction of activity in determining the environmental conditions on the icon of the dependence of the problem is also important. In the Ural region of Kazakhstan, this issue deserves special attention. In this area, the different natural-geographical conditions and various autobiographies, which are characterized by the Icon of the distribution of diversity, lead to the population of social welfare, and the area of the protection of the environment is affected. The living conditions of the landscape, and ticks of the biological distribution, as well as the ticks of the species composition and specific biological communities of the relationship, for the details of the study are necessary. Based on such studies, it is possible to assess the risk of diseases of natural vectors to identify the main points of accumulation of ixodides [11-17].

In 2011, during the spring and summer period, 138 residents of the West Kazakhstan region suffered from ixodic tick bites and sought medical help. A total of 100 ticks taken from humans were tested in the laboratory for tick-borne infections, with a negative result. An annual stable circulation of Congo-Crimean hemorrhagic fever (CCGF) virus was detected in the studied region. There is a high concentration of *Hyalomma marginatum* in cattle in this area. This type of tick is the main vector of the CCGF virus in neighboring regions of Russia [3,18-20]. Interestingly, they can be attracted to human breath and its components, such as acetone, nitric oxide, and carbon dioxide, which can also be produced by plants [15, 17-23].

The study of spontaneous CCGF virus infection in various animals is carried out by enzyme immunoassay (ELISA) using the Vectorkrim-CCGF-antigen test system manufactured by Vector-Best CJSC. The detected CCGF virus should be additionally examined by real-time PCR. In the territories of Russia adjacent to the West Kazakhstan region, Congo-Crimean hemorrhagic fever virus RNA was detected in 10 samples of the *H. marginatum* tick. The 661 *H. asiaticum* mites were examined in the Zhangali region, and 8 of the 187 samples tested positive for Congo-Crimean hemorrhagic fever virus antigen (4.2%). As a result of comprehensive studies in the west of the West Kazakhstan region, the virus was detected in *H. asiaticum*. Thus, a new natural outbreak of Congo-Crimean hemorrhagic fever has also been identified in Kazakhstan.

Based on the above, the aim of this study is to conduct a comparative analysis of changes in faunal complexes of Ixodid mites in various biogeocenoses of the territory of Western Kazakhstan.

Materials and research methods

This work is based on materials obtained after field and laboratory studies in 3 administrative districts of the West Kazakhstan region (Bokeyorda, Zhanibek, and Syrym districts), including

1,825 biomass and 2,712 ticks according to a single methodology using standard flags and fibers (Figure 1). When monitoring large mammalian infections, direct collection of ticks from vertebrate hosts in the study area is the main method [21-24].

The determination of the systematic affiliation of ticks was carried out using determinants together with specialists from the Uralsk sanitary and Epidemiological laboratory, the Ural Anti-plague station, and the district sanitary and Epidemiological station.

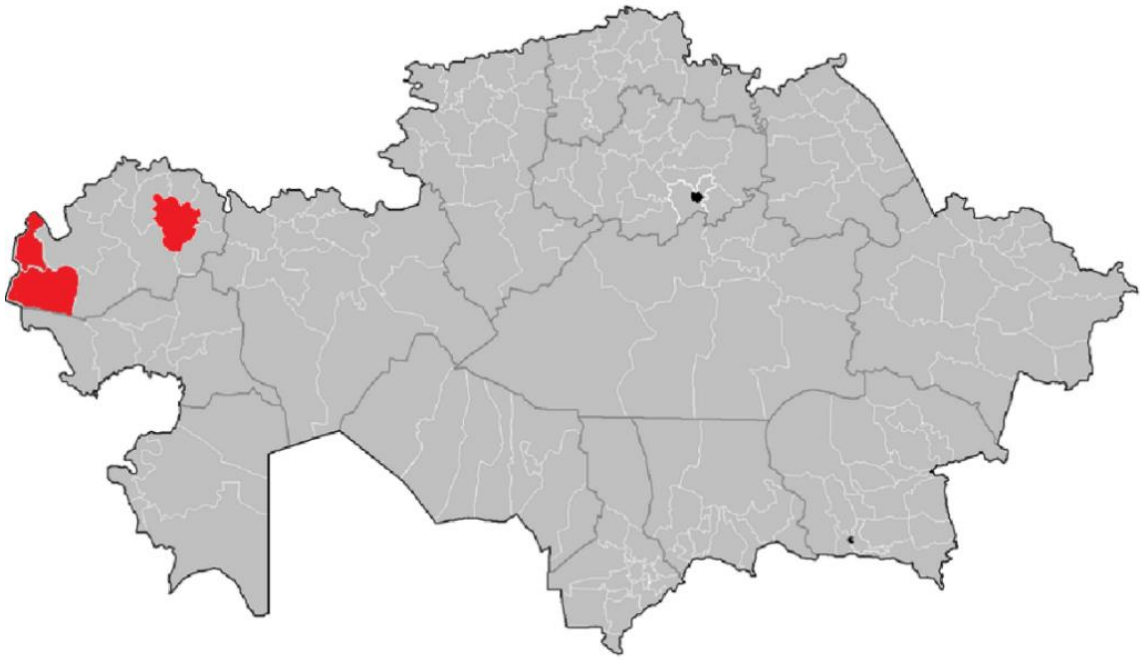

Figure 1. Research areas

From vertebrates, ticks were collected on farms and pastures. When collecting ticks, attention was paid to the places where they were concentrated in the feeder: neck, underbelly, ears, eyelids, axillary and groin areas, nipples, bottom and tail tip. Fixed ixodid ticks were removed manually with medical gloves, grabbing the base of the beak. In some cases, tweezers were used. Tear off the ticks by any means, with caution it is necessary to release movements or rotate the tick around the longitudinal axis of the body, so as not to tear the burrow. Gloves and tools are disinfected after work, and hands are treated with 70% alcohol [25-28].

The ticks collected in various biotopes were fixed with 70% alcohol. Before the study, the ticks were removed from a test tube, placed on filter paper, removed with tweezers, and examined under a magnifying glass under side lighting. The table "tick identifier of the Ixodidae family" was used to determine the genus and species of ticks.

The data obtained were processed using statistical methods. The work used standard environmental indicators (dominance index (id), indices of species diversity and wealth). Basic statistical calculations were performed using Microsoft Excel 2007 and Statgraphics 5.0.

Results

Today, the ixodofauna in western Kazakhstan biotope is represented by five species of four genera: *Dermacentor marginatus, Dermacentor reticulatus, Hyalomma detritum, Ixodes persulcatus,* and *Rhipicephalus rossicus.*

The fauna of tick species of the western region of Kazakhstan, by quantitative distribution, is very uneven. *Dermacentor* ticks (*D. marginatus* and *D. reticulatus)* occupy a dominant place in terms of abundance and appearance. *D. marginatus* species are found in all three regional settlements (49.60% in collections). Divisions of the ixodid fauna of the Kazakh Urals are formed on *D. reticulatus* (21.12% in collections). *H. detritum* species -at least than 50%.

The share of all listed types of ixodid ticks in the region is 87.90% of the total tick fauna of the studied biogeocenoses. The *Ixodes persulcatus* and *Rhipicephalus rossicus* can be considered the ixodid mite species for the fauna of the biotopes of the three studied regions, of the region 0.95% and 6.30% in the collections, respectively, the least.

The distribution of ixodid mites in different biogeocenoses is uneven and mosaic. Quantitative characteristics of the Ixodid tick fauna also depend on natural and geographical conditions. The natural and climatic conditions of the West Kazakhstan region are characterized by a high degree of mosaic landscapes, soils, vegetation cover, and economic development of the territory. The tick fauna of the southern upland Uvalisto plain forest-steppe part of the right bank of the Chagan River is represented by 3 species belonging to 2 genera. The dominant species in this zone was *D. marginatus* (ID was 84. 5%). The percentage of the 2 other species was distributed as follows: *D. reticulatus* – 14.0%, I. persulcatus – 1.4%.

Analysis of the faunal data of ixodid ticks in the West Kazakhstan region showed that the highest species diversity of ticks is found in the high steppe belt on the right bank of the Derkul River. As a result of the conducted studies, it was determined that the ixoid fauna in this zone is represented by 5 species belonging to 4 families: *D. marginatus, D. reticulatus, H. detritum, Rh. rossicus,* and *I. persulcatus.* A total of 425 tick specimens were collected. The dominant species in the study area was *D. marginatus* (ID was 42.3%). The percentage of other species had the following distribution: *D. reticulatus* – 21.7%, *H. detritum* – 12.3%, *Rh. rossicus* – 9.6%, I. persulcatus - 2.3%.

On the territory of the Bokeyorda district, in the biogeocenoses of low-mountain steppes, 4 species of ixodids were also recorded: *D.marginatus, D. reticulatus, H.detritum, Rh. rossicus. D.marginatus dominates* (ID 49,6%), D.5.3 is subdominant (ID 2%). The percentage of other species was distributed as follows: *H. detritum* – 16,4%, *Rh. rossicus* – 1,6%. Three types of ticks were found in the high-level steppe biogeocenoses of the Syrym district: *D. marginatus, D. reticulatus,* and *H.detritum.* The following species dominated: *D. marginatus* (ID 58.4%) and *D. reticulatus* (ID 40.1%). In the elevated mountainous biogeocenoses near the village of Mashtakov, only 2 species are found: *D. reticulatus* and *D. marginatus.* The territory is dominated by ticks of the *D. reticulatus* species by a large margin (ID 82.0%). The *D. marginatus* species was less common in this zone (8.4%). The biotopic distribution of ixodid ticks in the West Kazakhstan region is as follows. Forest areas and floodplains of the Chagan and Derkul Rivers were characterized by the greatest species diversity and richness. The Macintosh diversity index was 0.487 ±0.042 and 0.395 ± 0.040, respectively; the Shannon index was 1.973 ± 0.132 in forest areas and 1.841 ± 0.067 in floodplains, respectively. The species composition in forests is 4 species, in floodplains, 5 species.

The isotopic ixodic ticks of the territory are distributed in the West Kazakhstan region as follows. The Chagan River's largest forest areas of were distinguished by their diversity. The Macintosh index is 0.487 ±0.042 with composition diversity, respectively, not 0.395 ± 0.040; in the forest area, the Shannon index is 1.973± 0.132, in the floodplain, 1.841 ± 0.067, respectively. c. the species composition of the forest is 4 forces, floodplain – 5 species. According to the results of the survey, small animals had a lower occlusion rate than large animals, and their occurrence index was recorded, respectively, 91.0% and 92.3% (Table 1).

Table 1

Damage to various animal species in western Kazakhstan

Type of tested animal	Number of examined Animals	Ticks collected	Maximum number of ticks per 1 animal	Abundance index, pcs	Index of occurrence, %
Small animals	996	2960	19	2.97	91.0
Large animals	13	98	24	7.5	92.3

In addition, there are three most common species in the region: ticks – *I. persulcatus, D. reticulatus, and D. marginatus*. In addition, based on the fact of predominance of these types, we focused on the study of these three types of ixodids, which we found and are practically widespread in western Kazakhstan. The results of collecting ixodid ticks in the context of natural and climatic zones and their types are presented below in Table 2. Our observations and studies were carried out in the forest steppe zone and the steppe zone of western Kazakhstan.

Analysis of the data in Tables 1 and 2 shows that animals in forest-steppe territory are infected with three species of ixodids: *D. reticulatus* – 49.5%, *D. marginatus* – 8.6%, *I. persulcatus* – 41.9%. The maximum number of ticks found on one cattle was 24, and the maximum number of Ixodes ticks on one cattle was 19. On average, 7.5 Ixodes ticks were found per examined cattle, and 2.97 Ixodes ticks per cattle.

Table 2

Types of ixodids in different natural and climatic zones of western Kazakhstan

Natural-Climate belt	Collected ticks, pieces	Among them					
		D. reticulatus		*D. marginatus*		*I. persulcatus*	
		pieces	%, M+m	pieces	%, M+m	pieces	%, M+m
Forest-steppe	3100	1535	49,5+2,8	266	8,6±1,1	1299	41,9+2,6
Steppe	2835	1252	44,2+2,5	412	14,5±1,7	1171	41,3±2,7
Total	5935	2787	47,4±2,6	678	10,6±1,4	2470	41,6±2,7

According to Table 3, the dominant place in terms of numbers in cattle is occupied by ticks of the genus Dermacenter – *D. marginatus.* This type of tick is found in 65 percent of the cattle population. The subdominant form includes *H. detritum* (30%). Significantly fewer species were found in cattle: *D. reticulatus* (3.0%) and *Rh. rossicus* (2.0%). Ticks of the *I. persulcatus* species have not been detected on cattle.

Table 3

Collected ticks from different animals

Types of ticks	Number of ticks											
	From cattle		From horses		From sheep		From dogs		From humans		From roe	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
D. marginatus	138	65	71	60,4	221	88,6	15	42,6	43	55,0	2	7,0
D. reticulatus	6	3,0	47	39,6	29	11.4	12	32,4	19	25,0	3	8,0
H. detritum	64	30	0	0	0	0	0	0	0	0	0	0
I. persulcatus	0	0	0	0	0	0	0	0	12	15,0	0	0
Rh.rossicus	6	2,0	0	0	0	0	9	25,0	4	5,0	23	85,0
Total	214	100	118	100	250	100	36	100	78	100	28	100

D.reticulatus (32.4%) and *Rh. rossicus species* (25.2%) is more common. The species *H. detritum* and *I. persulcatus* have not been found. For horses, *D. marginatus* is the dominant species (60.4%), and *D. marginatus* (42.4%) is a common species of ixodic ticks for dogs.

Subdominant *D.reticulatus* (39.6%). *Rh. rossicus, H. detritus,* and *I. persulcatus* could not be identified. Only two types of ticks have been removed from sheep: *D.marginatus* and *D.reticulatus. D. marginatus* predominates (88.6% in collections). *D. reticulatus* was significantly fewer (11.4% in collections) (Figure 2).

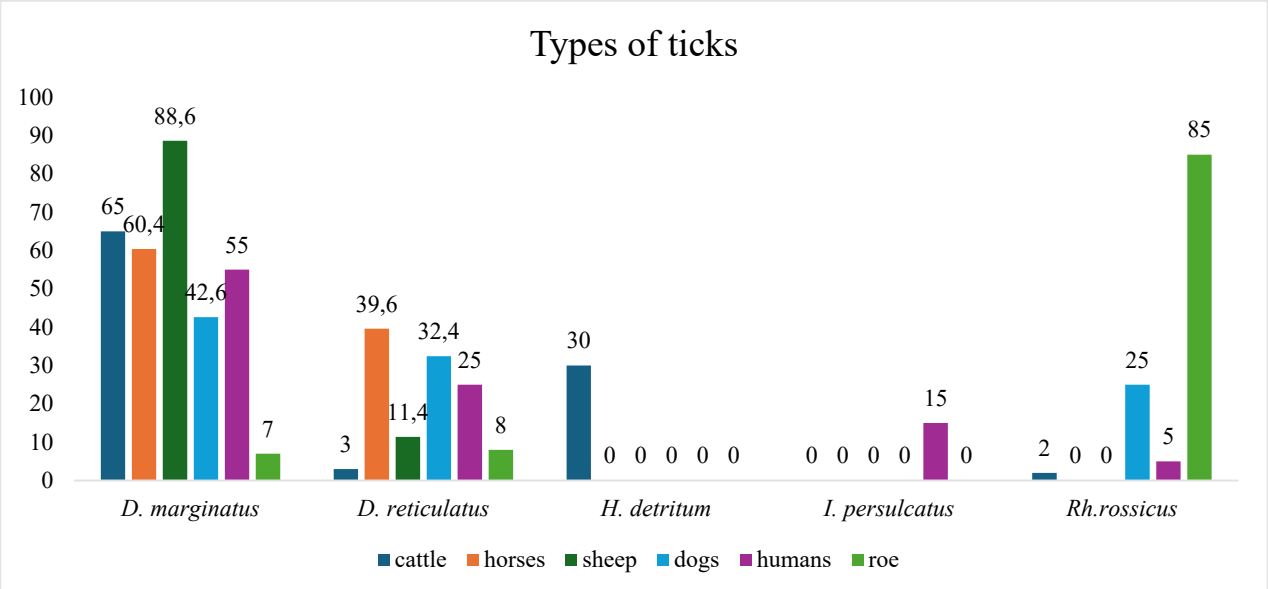

Figure 2. Specific occurrence of ticks in different hosts

Rh. rossicus is the dominant species of roe deer (85.0%). *D. marginatus (*7.0%) and the species *D. reticulatus* (8.0%) is much less common in roe deer. Humans are often the breadwinners of ixodic ticks. The species, *I. persulcatus,* was only found in human collections. Dominant type *D. marginatus* (55,0%), *D. reticulatus* – 25,0%. Ticks of the type I. persulcatus – 15.0% and *Rh. rossicus* – 5.0%.

As a result of mass parasitization of ticks of the genus Dermacentor, the amount of hemoglobin in the blood of animals decreases by 12-15%, red blood cells by 1.5 - 2 million, and leukocytosis develops with significant eosinophilia, 7-13%, which leads to exhaustion and death of animals.

When 60-100 pairs of ticks are parasitized on sheep, severe intoxication, nervous system disorders, and sometimes death are observed. Milk yields of cows can decrease by 18-20% (with mild tick damage) and 40-50% (with severe tick damage, more than 3 thousand ticks per animal). Many researchers have found that the time of falling off drunk ixodids falls on certain periods of the day. For example, female ticks Ixodes ricinus and I. persulcatus [25]. They usually finish the last stage of feeding on the night before falling off and leave the owner in the morning, when the cattle are grazing on pastures. In arid climates, the larvae, nymphs, and females of Hyalomma anatolicum fell off cattle during the summer months, and in Tajikistan at night, when the animals were resting in primitive pens. In the cracks of the mud walls of these rooms, the eggs of *H. anatolicum* molt or develop, all stages of which feed on farm animals [26].

Data on the species belonging to ixodid ticks collected from different vertebrate species in the Bokeyorda and Zhanibek districts are presented in Table 4.

Table 4

Species belonging of ixodids collected from animals

Study area	Total ticks collected, ex.	Among them					
		D. reticulatus		*D. marginatus*		*Ixodes persulcatus.*	
		Ex.	%	Ex.	%	Ex.	%
Bokeyorda	531	244	45.9	89	16.8	198	37.2
Zhanibek	2181	947	43.4	314	14.4	920	42.2
Total:	2712	1191	43,9±2,5	403	14.8±1,6	1118	41.2±2,2

As the analysis of the tabular data shows, during the study of cattle and small animals on farms, 2712 tick samples were removed, we identified them as *D. reticulatus* – 1191 samples (43.9%), *D. marginatus* – 403 samples (14.8%), and Ixodes persulcatus – 1118 samples (41.2%) (Figure 3).

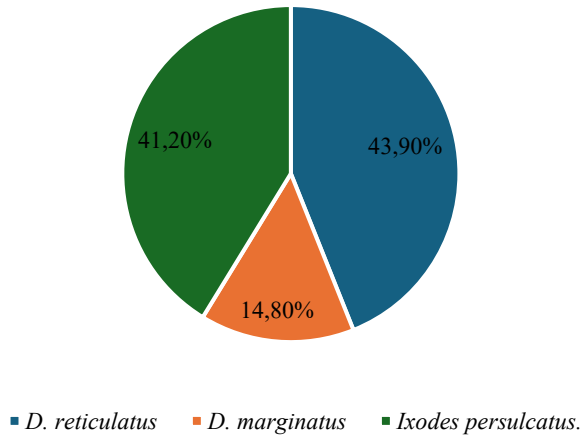

Figure 3. Species ratio of epidemiologically significant ticks

In addition, in MNT – the maximum number of ticks was 11 individuals, and in cattle the maximum was slightly higher – 14 ixodids. The tick abundance index in these animal species differed significantly from each other. Thus, the average abundance index in cattle was 5.7 individuals, while in MNT there were only 3.1 ixodids per animal studied.

After wintering, the emergence of ticks occurs gradually, so their number in pastures and, accordingly, in animals increases until the last decade of May, then reaches a certain maximum and begins to decrease. In the last days of the season, active ticks are practically not detected, and ticks of the genus Dermacentor reappear in the second half of August.

In the West Kazakhstan region, the first cases of activity of *D. reticulatus* ticks were recorded in mid-April, when adults of the parasite were caught from plants. The first peak of maximum abundance of *D. reticulatus* was recorded from April 21 to June 4, when 30 individuals were recorded per hour of flight, and the abundance index (IO) was 13.4 individuals. The second peak of activity of Dermacentor ticks was recorded later, from August 15 to October 3.

In addition, the first cases of detection of a species such as Ixodes persulcatus in nature were recorded on April 13, and the last cases of activity were recorded in the third decade of June. The highest number of ixodids in animals was recorded from May 7 to June 3, and during this period their incubation reached 77.2-100% of the population, depending on the region, with an abundance index (AI) of 14.2 individuals per animal. The maximum number of ixodids per animal reached 29 individuals. The last cases of attacks on cattle by Ixodid ticks of the Dermacentor genus were recorded on September 21, and the last ticks were removed from plants on October 7. During the observation, it was also noted that ticks attack animals only when they go out to pasture. With the increase in the number of ticks in nature, the number of their warm-blooded hosts increased.

Although some studies were conducted later, when the parasitic season of adult ticks began to decrease, we observed a high degree of damage to farm animals. Thus, the ixodid tick occurrence index in MNT was 88.6%, and ixodid tick infestation in cattle was 65.0%.

The sexual structure of the population of ixodids collected on the surface of vertebrate bodies and biogeocenoses in the West Kazakhstan is presented in Table 5.

Table 5

The ratio of males and females in the biogeocenoses of the West Kazakhstan region

Species	Total number of	Males		Females	
	1833	Ex.	%	Ex.	%
D. marginatus	670	269	40,2	401	59,8
D. reticulatus	524	169	32,4	355	67,6
I. persulcatus	228	47	20,4	181	79,6
H. detritum	215	103	48,0	112	52,0
Rh. rossicus	196	109	55,4	87	44,6

According to the table, there are significant predominance of females than males. The ratio of males and females is obviously biologically important for preserving the diversity of species in the region. This pattern is due to the desire to increase the number of offspring. A special feature is the Rh. rossicus: the females are more numerous than in the collections. Over the years of research, there has been a tendency for Ixodes ticks to change their body shape. During the dry years, various species of Ixodes mites were suppressed.

Discussion

The scientific and practical significance of the work lies in the fact that scientific research was conducted in Kazakhstan, mainly of an epizootological nature, which determined the species composition of ticks. The leading measure in the system of combating pathogens of vector-borne diseases is the destruction of their vectors, Ixodes ticks. The development of new methods for trapping ticks will help prevent economic and social damage and is aimed at improving the safety of the environment and humans. The proposed technology will use compositions of environmentally friendly products to prolong the action of drugs in order to reduce the number of treatments with acaricidal drugs and reduce the burden on the animal body and the environment.

Epidemiologically, the importance of arthropods as carriers of pathogens of infectious diseases of humans and animals is incomparably greater than their importance as parasites, and in obligate-transmissible diseases, vector ticks play a leading role. They actively participate in the mechanism of transmission of pathogens of infectious diseases, especially from protozoan, rickettsial, viral, and bacterial.

Ticks carry hemorrhagic fevers, tularemia, pyroplasmosis, and tick-borne encephalitis. The Ixodid tick order (*Ixodidae*) occupies a leading position among blood-sucking arthropods in terms of the number of transmitted diseases. Among them, there are such dangerous infections as tick-borne typhus, encephalitis, tularemia, pyroplasmosis, and hemosporidial diseases of domestic animals and humans. These ticks are under the close attention of medical and veterinary acarology and parasitology in general. Ixodes ticks are the most common carriers of dangerous encephalitis [29, 30]. The harmful effects of parasites as carriers of infectious diseases are many times greater than their actual parasitic harm. Diseases transmitted to humans and animals by tick bite are called vector-borne diseases. In this case, ticks are a kind of reservoir of infectious agents; they retain them throughout their lives and are often transmitted from generation to generation, laying infected eggs, from which infected larvae are born. Encephalitis mite is one of the most common and well-known. It is important to note that the encephalitic tick is not a separate breed (species) of arthropod insects. Any type of tick can become infected with encephalitis, so it is impossible to identify signs that determine the degree of danger. But it should be remembered that such an infection can lead to the death of a person [6,10].

The Ixodid tick order (*Ixodidae*) occupies a leading position among blood-sucking arthropods in terms of the number of transmitted diseases. Among them, there are such dangerous infections as tick-borne typhus, encephalitis, tularemia, pyroplasmosis, and hemosporidial diseases of domestic animals and humans. These ticks are under the close attention of medical and veterinary acarology and parasitology in general. Our results have shown that using this feature of tick behavior is very effective in collecting and trapping ticks and other blood-sucking insects.

Conclusion

Field trips were conducted, and new knowledge was gained about the patterns of formation of tick foci in the West Kazakhstan region, and their role in the spread of diseases carried by ectoparasites.

In Western Kazakhstan the analysis of the changes in the ixodofauna of the biogeocenoses of in the period over the past 10 years has been carried out. Changes in the species composition, dominant type, and abundance of ixodid ticks have been identified.

In the Urals, among the studied landscape zones, the mountain steppe zone was characterized by the largest number of species, diversity, and richness. The composition of the species is 5 species, which is a consequence of the significant diversity of landscape and zoological conditions in this region. However, ticks of the genus Dermacentor sharply dominated in number. Ecological and faunal analysis showed the features of the spread of Ixod ticks on the territory of Kazakhstan. In the coastal forest-steppe zones, the diversity of species is higher, and the abundance ratio of individual ixodid species was more uniform.

A relationship has been established between the species composition of Ixodes mites and the landscape conditions of the West Kazakhstan region. Ticks of the genus Dermacentor are distributed mainly in flat steppe landscapes. Forest and forest-steppe zones are the most suitable for the ixodid family. For Ripicephalus, they are forest-steppe and semi-desert. Ticks of the genus Hyalomma are found only in areas of cattle breeding and adjacent territories.

The greatest diversity of ticks was recorded in dogs (3 species); the Macintosh variety index was 0.336 ± 0.021, and the Shannon index was 1.452 ± 0.072; for cattle (4 species), the Macintosh variety index was 0.342 ± 0.04, and the Shannon index was 1.068 ± 0.032.

From an epidemiological point of view, the importance of ixodids as carriers of infectious diseases of humans and animals is incomparably higher than their parasitic significance. The harmful effects of parasites as carriers of infectious diseases are many times greater than their real parasitic harm. Based on the data obtained, it is possible to develop regional measures for the treatment and prevention of ectoparasites in animals.

Author Contributions

B.E. – concept and supervision of the work; **R.T.–** conducting the experiments; **A.N.** – discussion of the research results; **R.T.** and **B.B. –** writing the text; **K.A.–** editing the text of the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Батыс Қазақстан облысының биотоптарында иксодидті кенелердің таралуының биологиялық ерекшеліктері

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Аңдатпа. Батыс Қазақстан облысында иксодидті кенелердің түрлерінің таралуына биологиялық және фауналық талдау жүргізілді. Зерттелетін аймақтың биогеоценоздарында иксодидті кенелердің таралуының экологиялық ерекшеліктері, сондай-ақ олардың белгілі бір биотоптармен түрлік байланысы зерттелген. Кенелердің ең жоғары түрлерінің әртүрлілігі биік дала аймағымен ерекшеленеді. Бұл аймақтағы иксодид фаунасы 4 туысқа жататын 5 түрден тұрады: *D. marginatus, D. reticulatus, H. detritum, Rh. rossicus* және *I. persulcatus.* Dermacentor тұқымдасының кенелері жеткілікті ылғалды топырақты, аралас шөпті және шалғынды өсімдіктері бар мекендеу орындарын жақсы көреді. *I. persulcatus* және *Rh. rossicus* түрлері жайылмалардағы ормандар, *Hyalomma* түрлері жайылымдарды мекендейді. Иксодидті кенелердің жиналған үлгілерінде аналықтардың еркектерге қарағанда едәуір басым екендігі байқалды. *Dermacentor* тұқымдасының кенелері негізінен жазық дала ландшафттарында таралған. Ixodidae тұқымдасы үшін орманды және орманды дала аймақтары ең қолайлы. Ripicephalus үшін олар орманды дала және шөлейт жерлер. Hyalomma тұқымдасының кенелері тек мал өсіретін аудандарда және оған іргелес аумақтарда кездеседі.

Түйін сөздер: иксодидті кенелер, биогеоценоздар, басым түрлер, биологиялық талдау, фауналық талдау

Биологические особенности распространения иксодового клеща в биотопах Западно-Казахстанской области

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Аннотация. Проведен биолого-фаунистический анализ распространения видов иксодовых клещей в Западно-Казахстанской области. Изучены экологические особенности распространения иксодовых клещей в биогеоценозах исследуемого региона, а также их видовая связь с определенными биотопами. Наибольшим видовым разнообразием клещей отличается возвышенная степная зона. Фауна иксодовых клещей в этой зоне представлена 5 видами, относящимися к 4 родам: *D. marginatus, D. reticulatus, H. detritum, Rh. rossicus* и *I. persulcatus.* Клещи рода *Dermacentor* предпочитают заселять участки с достаточно влажной почвой, со смешанной травянистой и луговой растительностью. Виды *I. persulcatus* и *Rh. rossicus* обитают в поймах рек, виды *Hyalomma* – на пастбищах. В собранных экземплярах иксодовых клещей отмечено значительное преобладание самок по сравнению с самцами. Клещи рода *Dermacentor* распространены преимущественно в равнинных степных ландшафтах. Лесная и лесостепная зоны являются наиболее подходящими для семейства иксодовых. Для *Ripicephalus* это лесостепь и полупустыня. Клещи рода *Hyalomma* встречаются только в местах скотоводства и на прилегающих территориях.

Ключевые слова: иксодовые клещи, биогеоценозы, доминирующие виды, биологический анализ, фаунистический анализ

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Ғылыми мақала

Солтүстік-батыс Қазақстандағы масалар популяциясының қазіргі жағдайы

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Аңдатпа. Мақалада Солтүстік - батыс Қазақстанда масалардың фаунасын зерттеу, бірқатар эпидемиологиялық қауіптілік белгілері бойынша масалардың неғұрлым қауіпті түрлерін есепке алу, оларға қарсы күрес шараларын негіздеуге арналған. Солтүстік-батыс Қазақстанда, атап айтқанда Ақмола, Атырау, Батыс-Қазақстан, Павлодар, Қостанай, Солтүстік батыс Қазақстан облыстарында 2020 және 2024 жылдардың жазғы маусымдарында жүргізілген бұл жұмыстың мазмұны осы аймақтар таралған әр түрлі экологиялық топтардағы жекелеген түрлердің түрлік құрамы мен экологиясын, оның ішінде масалардың аймақтық және тәуліктік белсенділігін анықтау, популяциялардың жас құрамы мен өмір сүру ұзақтығын анықтау, жұмыртқалау орындарын, қансорғыштардың ең көп шоғырланған жерлерін, масалардың ұшып шығу мерзімдерін анықтаудан құралды. Масалар – қан сорғыш буынаяқтылардың арасында практикалық тұрғыдан маңызы зор топтарының бірі: бұл жәндіктер адамға жабылатын қансорғыш қосқанатты жәндіктердің ішінде бірқатар ландшафттарда негізгі массасын құрап қана қоймай, адам мен жануарларға ортақ көптеген трансмиссивті ауру қоздырғыштарын тасымалдайды. Алынған ғылыми нәтижелер мен тұжырымдамалар Солтүстік -батыс Қазақстанда масалардың популяцияларының қазіргі жағдайын бағалауға мүмкіндік береді, өңірдің масаларының түр құрамы мәліметтерін жаңа ақпараттармен толықтырады.

Түйін сөздер: масалар, түр құрамы, тіршілігінің биологиялық ерекшеліктері, ұшудың тәуліктік және маусымдық динамикасы

Кіріспе

Масалардың *Culicidae* үлкен тұқымдасының 34 туысқа жататын 3000-дай түрі белгілі [1,2]. Қазіргі мәліметтер бойынша, ТМД территориясында 8 туыстың 98 түрі кездеседі

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**Хат-хабар авторы*

[3,4]. Олардың ішінде маңыздылары Anopheles («безгек» масалары), *Culex* және *Aedes* туыстары өкілдері; кейбір ландшафттарда *Mansonia* және *Culiseta* туысы масалары маңызды роль атқарады [5-7].

Қазақстанда қос қанатты қан сорғыштарды зерттеу көлемді және түбегейлі жүргізілген деуге болады. Бұл зиянды жәндіктердің барлық компоненттері толық зерттелген. Қазақстан масаларының фаунасы жеті туысқа *(Anopheles, Aedes, Culiseta, Culex, Ochlerotatus, Uranotaenia* және *Mansonia)* жататын 53 түрден тұрады. Географиялық біртектілігі, таралу типі және экологиялық ұқсастығы бойынша олар орман, орманды дала, дала және шөл деп жіктелетін төрт экологиялық-фауналық кешенге жатқызылады [8-10].

Солтүстік батыс Қазақстанның қан сорғыш масаларының фаунасын зерттеуге арналған ғылыми деректер зерттеу пункттеріндегі масалардың жекелеген түрлерінің түр құрамы мен саны туралы кейбір деректерді, көбінесе безгек масаларының көбею орындары, күнделіктері және жаппай жаз уақыты туралы азды-көпті мәліметтерді береді [11-16]. Осы мәліметтерге сәйкес, Солтүстік батыс Қазақстан аумағында бес туысқа *(Anopheles, Theobaldia, Mansonia, Aedes, Culex)* жататын қан сорғыш масалардың 25 түрі тіркелген.

Әдеби деректер бойынша Маңғыстау, Атырау, Ақтөбе және Батыс Қазақстан облыстарының шегінде Каспийдің осы бөлігіндегі масалардың фаунасы 25 түрден тұрады. Осы аймақтардың ландшафты-климаттық аймақтарында арбовирустардың ықтимал тасымалдаушылары төрт туыс масалары болуы мүмкін: *Anopheles (An. maculipennis, An. hyrcanus), Aedes (Ae. vexans) Ochlerotatus (Och. caspiis, Och. dorsalis, Och. cantans, Och. detritus, Och. subdiversus) және Culex (Cx. modestus, Cx. pusillus, Cx. pipiens)* [17-19].

Қазақстанда қосқанатты қансорғыш масалардың зерттелу жағдайы осындай. Республиканың солтүстік батысындағы қосқанатты қансорғыш масаларды биологтар және паразитолог ғалымдар көптеген жылдар бойы зерттеп келеді. Бұл жоғарырақ көрсетілген болатын.

Дегенмен де Солтүстік - батыс Қазақстан масаларының түрлері туралы мәліметтер әлі де аз және шашыраңқы. Біздің зерттеуіміздің мақсаттары мен міндеттері осы аймақпен байланысты болғандықтан, аталған аймақтың қосқанатты қансорғыш масаларының зерттелу жағдайына талдау жасау және зерттелген облыстардағы өлкелік паразитология мәселелерінің олқы жерлерін толтыру қажет деп есептейміз.

Сонымен қатар климаттың өзгеруінен көптеген түрлердің, соның ішінде масалардың да үлкен қоныс аударуы байқалады – олар планетаның жаңа аймақтарында қолайлы өмір сүру жағдайларын табуда. Жаһандық жылыну қауіпті тропикалық ауруларды тарататын азиялық жолбарыс масаларының *(Aedes albopictus)* және сары безгекті масалардың *(Aedes aegypti)* көші-қонына ықпал етеді [20-29]. Осыған байланысты масалар туғызатын қауіпті ауралардың таралуының алдын алу үшін Солтүстік-батыс Қазақстан аумағында мекендейтін масалардың популяциясы мен климаттық өзгерістерге сай түрлердің географиялық таралуын жаңадан зерттеу қажеттілігі туындады.

Зерттеу материалдары мен әдістері

Жұмыстың негізін 2020 жылдан 2024 жылға дейін жүргізген жинақтар, бақылаулар және эксперименттік зерттеулер құрады. Стационарлық зертханалық зерттеулер, жиындар мен бақылаулар негізінен Солтүстік батыс Қазақстанның 30-дан астам елді мекендерінде, алты әкімшілік облыс шегінде (Ақмола, Атырау, Батыс-Қазақстан, Павлодар, Қостанай,

Солтүстік батыс Қазақстан) және Астана қаласының жекелеген аудандарында (Алматы, Есіл, Нұра, Сарыарқа және Байқоңыр) жүзеге асырылды.

Көп жылғы маусымдық бақылаулар әртүрлі табиғи-климаттық жағдайларда (жазық даладан ұсақ шоқылы төбелер мен орманды шалғындарға дейін) орналасқан елді-мекендерде жүргізілді.

Стационарлық зерттеу пункттері: Павлодар облысында 03.06.2020-11.06.2020; 23.05.2022 аралығында Павлодар қаласы (Ертіс жайылмасы, ГРЭС, Ленинск); Ақсу қаласы, 23.05.2022-02.06.2022 аралығы Павлодар ауданы, Чернорецк, Ямышево, Мичурин, ауылдары; Железнин ауданы, Пятирыжск, Ертіс ауылдары; Аққулы ауданы, Аққу елді мекені; Атырау қаласында 16.06.2020-19.06.2020; 17.05.2022- 19.05.2022 аралығында (Лесхоз, Еркін қала, Құрсай, Өркен-1, Жұмыскер ш/а); Маңғыстау облысы, Каспий теңізі, 15.04.2021; Батыс Қазақстан облысында 22.06.2020- 12.08.2020; 18.06.2021 - 25.08.2021; 20.05.2022 аралығында Орал қаласы, Шаған өзені; Тасқала ауданы, Тасқала ауылы; Сырым ауданының Сырым ауылы; Бәйтерек ауданы, Новенький, Күшім ауылдары; Бурлы ауданы, Қанай, Аралтал, Жарауат ауылдары; Шыңғырлау ауданы, Шыңғырлау ауылы; Ақжайық ауданы, Чапаев ауылы; Меловые ауылы; 30.05.2021-15.07.2021 Ақмола облысы, Көкшетау қаласы; 28.06.2022-28.08.2025 Целиноград ауданы, Жартыкөл және Қоянды ауылдары; Аршалы ауданы, Күйгенжар және Жібек жолы ауылдары; 06.06.2022-08.06.2022 Қостанай облысы, Әулие көл ауданы, Черниговка ауылы (Сарай); Ища өзені; 08.06.2022; Ақтөбе қаласы, Сазанка өзені; 14.06.2022- 16.06.2022 Хромтау ауданы, Абай ауылдық округі; 24.06.2022-25.06.2022 Солтүстік батыс Қазақстан облысы, Қызылжар ауданы, Новопавловская ауылы; Кожазавод ауданы; 15.05.2023-15.08.2025 Астана қаласы, Нұра ауданы, Қараөткел, Үркер елді мекендері, қалаішілік Алматы, Есіл, Нұра, Сарыарқа, Сарайшық және Байқоңыр аудандарында Астана қаласы әкімдігінің тапсырысымен «Столичная дезинфекция» ЖШС бірлесіп энтомологиялық зерттеулер жүргізілді.

Қан соратын масалардың түрлік құрамы, биологиясы мен экологиясын зерттеу жалпы қабылданған әдістермен жүргізілді (Павловский,1935; Стакельберг,1937; Беклемишев, 1944; Мончадский, 1951,1962; Гуцевич, Глухова, 1970; Лутта, 1970; Скуфьин, 1973 және т.б.) [8,9,12].

Нәтижелер

Қазақстанның солтүстік-батысында біз қан сорғыш масалардың 28 түрін тіркедік. Олардың 25-і бұрын белгілі болған: *Anopheles maculipennis messeae, Mansonia richiardii, Aedes caspius, Ae. dorsalis, Ae. maculatus, Ae. cyprius, Ae. flavescens, Ae. excrucians, Ae. subdiversus, Ae. lepidonotus, Ae. cataphylla, Ae. intrudens, Ae. vexans, Ae. cinereus, Ae, rossicus, Culex modestus, C. pipiens. Theobaldia longiareolata, Th. alaskaensis, Th. annulata, Th. ochroptera, Ae. behningi, Ae. beklemishevi, Ae leucomelas, Ac. sticticus.*

Бұл зерттелген аумақта алғаш рет табылғандары: *Ae. euedes, Ae. communis, Ae. Punctor* (кесте 1).

Кесте 1

Солтүстік батыс Қазақстандағы қос қанатты қан сорғыш масалардың түр құрамы және ұшу кезеңі

№	Түрлер	Ара қатынасы		Ұшу кезеңі			ұшу ұзақтығы, күн
		сандық	үлесі,%	басталуы	жаппай ұшуы	аяқталуы	
1	*Anopheles maculipennis messeae*	232	4,19	16.VI	29.VI-2.VII	13.VII	57
2	*Aedes cataphylla*	768	13,8	3.VI	14.VI-5.VII	12.VII	39
3	*Ae. intrudens*	355	6,42	15.V	13.VI-3.VII 24VII-4.VIII	3.IX	78
4	*Ae. vexans*	137	2,47	6.VI	19.VI-28.VI	26.VII	51
5	*Ae. cinereus*	783	14,1	29.V	14.VI-23.VI	5.VII-21.VII 26.VIII	57
6	*Ae. rossicus*	391	7,07	13.VI	28.VI-19.VII	24.VIII	71
7	*Culex modestus*	52	0,93	22.VI	5. VII-9.VII	19.VII	27
8	*C. pipiens.,*	79	1,42	24.V	4.VI-19.VI	16.VII	52
9	*Ae. behningi,*	348	6,28	27.V	6.VI-16.VI	24.VII	57
10	*Ae. beklemishevi*	146	2,64	29.V	14.VI-18.VI	29.VI	31
11	*Ae leucomelas*	1071	19,3	12.VI	28.VI-23.VII	11.VIII	59
12	*Ae. sticticus.*	501	9,06	2.V	9.V-19.V	12.VI	41
13	*Theobaldia longiareolata*	3	0,05	15.V	13.VI-3.VII 24VII-4.VIII	3.IX	78
14	*Th. alaskaensis*	2	0,03	6.VI	19.VI-28.VI	26.VII	51
15	*Th. annulata*	1	0,01	29.V	14.VI-23.VI 5.VII-21.VII	26.VIII	57
16	*Th. ochroptera,*	1	0,01	13.VI	28.VI-19.VII	24.VIII	71
17	*Mansonia richiardii*	5	0,9	22.VI	5. VII-9.VII	19.VII	27
18	*Ae. caspius*	17	0,3	24.V	4.VI-19.VI	16.VII	52
19	*Ae. lepidonotus*	49	0,88	27.V	6.VI-16.VI	24.VII	57
20	*Ae. subdiversus*	54	0,97	29.V	14.VI-18.VI	29.VI	31
21	*Ae. excrucians*	26	0,46	12.VI	28.VI-23.VII	11.VIII	59
22	*Ae. flavescens*	127	2,29	2.V	9.V-19.V	12.VI	41

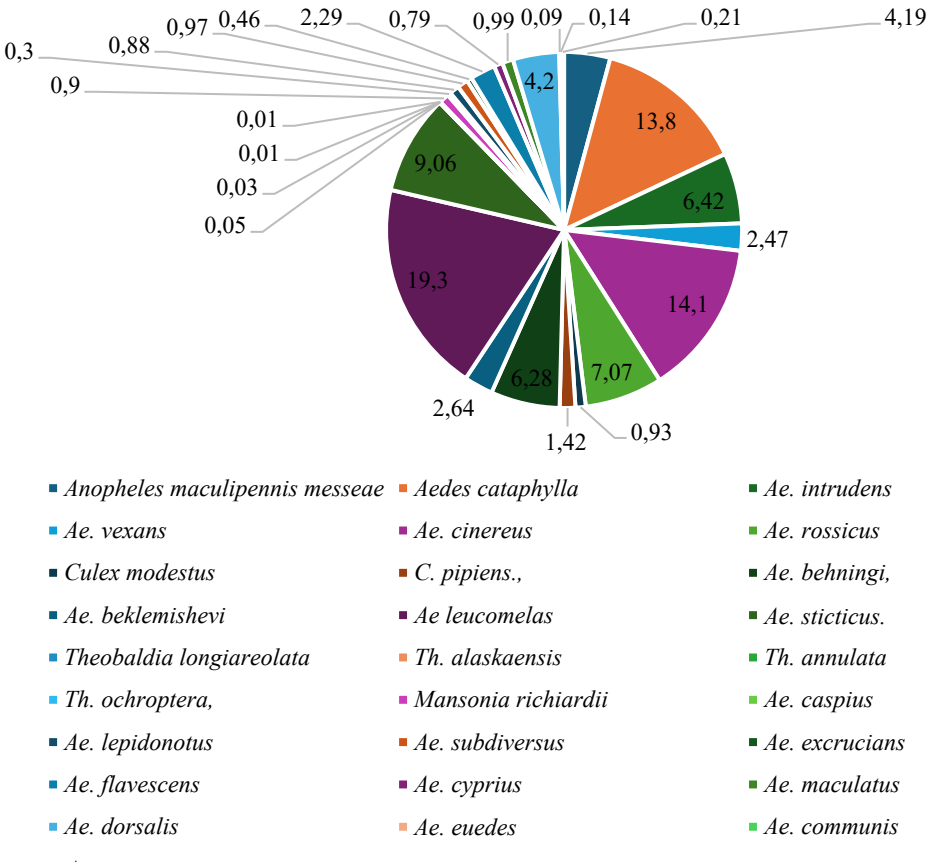
23	*Ae. cyprius*	44	0,79	15.V	13.VI-3.VII 24VII-4.VIII	3.IX	78
24	*Ae. maculatus*	55	0,99	6.VI	19.VI-28.VI	26.VII	51
25	*Ae. dorsalis*	257	4,2	29.V	14.VI-23.VI 5.VII-21.VII	26.VIII	57
26	*Ae. euedes*	5	0,09	13.VI	28.VI-19.VII	24.VIII	71
27	*Ae. communis*	8	0,14	22.VI	5. VII-9.VII	19.VII	27
28	*Ae. punctor*	12	0,21	24.V	4.VI-19.VI	16.VII	52
Барлығы		5529	100,0	2.V	9.V– 4.VIII	3.IX	52

Ескертулер:
1 басым – 8% және одан көп;
2 суб. басым – 2%-дан 8%-ға дейін;
3 аздаған – 0,5%-дан 2%-ға дейін;
4 сирек-жалпы санының 0,5%-нан кем;
5 БК – басымдылық көрсеткіші,%.

Қос қанатты қансорғыш масалардың өсіп-өну ортасын тұрақты және уақытша деп бөлуге болады. Тұрақты су қоймалары ұзағырақ сақталуымен, бентос пен планктонның әркелкілігімен, құрамында органикалық тұздар мен минералды тұздар болуымен, ортаның реакциясы әлсіз қышқылдыдан сілтіліге дейін болуымен сипатталады. Жалпы көлеміне, тереңдігіне және шөп-шалам басу деңгейіне байланысты ондағы су температурасы біршама (8-25°С) ауытқуы мүмкін. Көктемде мұндай су қоймаларында судың температурасы қоршаған орта температурасынан төмен болады.

Тұрақты су қоймаларына көлшіктер, өзен жайылмалары мен арналары, қара сулар, өзен-көл жағалауларындағы кең ауқымды батпақты саздар, тұрақты өзен және бұлақ аңғарлары жатады. Барлық қысқа мерзімді су қоймалары уақытша су қоймалары болып табылады. Оларға: әртүрлі шалшықтар (қар, жаңбыр суынан түзілген), жер бедерінің анда-санда сумен толатын ойпат жерлері – орлар мен жыралар, сондай-ақ адамның шаруашылықтық әрекетімен байланысты болатын жасанды уақытша су қоймалары жатады. Таяз, күн жақсы қыздыратын, шөбі аз су қоймалары масалардың өсіп-өнуі үшін қолайлы орта болып табылады.

Маса тіршілігіне ең қолайлы экологиялық жағдай орманды – шалғынды белдік. Бұл белдікте қалың маса *Ae leucomelas*, шалғынды белдікте оның басымдық индексі 19,3%. Осындай қасиетті *Ae. cinereus* (БК-14,1%) сан өзгерісінен де байқауға болады (Сурет 1).



Сурет 1. Солтүстік батыс Қазақстанда таралған масалардың түр арақатынасы, %

Орманды–шалғынды белдеуде қос қанатты қансорғыш масалардың дернәсілдері төбералық сайларда, өзендердің алқаптарында, батпақты, төмен орналасқан жерлерде, жер бедерінің тегіс емес, су жиналған жерлерінде өсіп-өнеді. Стенобионтты, моноциклді, орманды көптеген түрлер теректі-қайыңды орман шетінде және қалың бұта арасында орналасқан шағын су қоймаларында өсіп-өнеді. Олар су температурасы 6-18 ºС аралығында болғанда дамиды. Онда дернәсілдер тығыздығы шамалы, 1 м^2 су ауданында 2-26 дернәсіл кездеседі.

Зерттелген аймаққа жататын Ақмола облысында ұсақ су жайылмалары көп. Олар жаз бойы бастаулар мен бұлақтар ағынымен толықтырылып отырады, климаттық ерекшеліктерге байланысты бұл аймақтың сулары құрғамайды, яғни қос қанатты қан сорғыш масалардың жаз бойы өсіп-өнуіне қолайлы жағдай жасайды (Сурет 2).

Сурет 2. Масалардың дернәсілдері дамитын типті су қоймасы

Солтүстік батыс Қазақстанда орманды-далалық және орманды белдеулерінде масалар дернәсілдері мамырдың ортасынан маусымның ортасына дейінгі аралықта пайда болады.

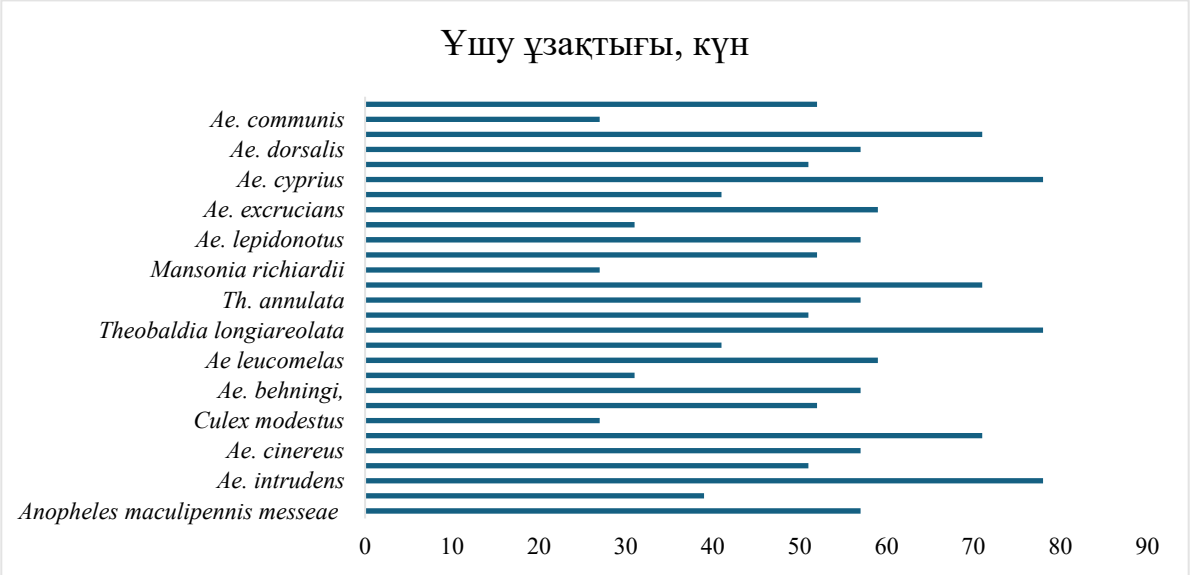
Мамырдың бас кезінде полициклді түрлер мен кейбір эврибионтты моно-циклді масалардың дернәсілдері еріген қар суын және арнасынан асып, тасыған өзендерден түзілген су жайылмаларын жайлайды. Дернәсіл тығыздығы 1 м^2 су ауданында 30-дан *(Ae. leucomelas)* 90-ға дейін *(Ae. c dorsalis)* болады.

Масалар әртүрлі көлемдегі, түбі батпақты шалшық суларда өсіп-өнеді. Көпшілік су қоймаларының суы мөлдір, әлсіз сілтілі реакциялы, құрамында сульфатты тұздары көп болды. Бұл су қоймаларында орташа температура 12-24^{o}С болғанда, 1м^2 ауданға 100 ден 5 мыңға дейін дернәсілдер келді. Үш жыл бойы біздің бақылауымызда болған масалардың әртүрлі өсіп-өну орталарын-дағы түр құрамы аса өзгерген жоқ. Ауылдар маңындағы ұзындығы 5 м ені 3,5 м, тереңдігі 1,5 м болатын суы жартылай мөлдір тұрақты су қоймасында *Ae. intrudens, Ae. c dorsalis, Ae. flavescens* көп мөлшерде өсіп-өнеді.

Маса түрлерінің басым көпшілігі осындай жартылай ашық және ашық су қоймаларында өсіп-өнеді. Солтүстік батыс Қазақстанда таралған маса түрлерінің 70-75% осындай су қоймаларында дамиды. Орман арасындағы көлеңкелі, көктемгі шалшық сулар мен өзен жағалауындағы тал-бұталар арасындағы жартылай көлеңкелі суларда тайгалы-орманды түрлер *Ae. impiger, Ae. nigripes, Ae. sticticus, Ae. pullatus* өсіп-өнеді. Орман шетіндегі ұзындығы 3,2 м, ені 1,5 м тереңдігі 0,2 м болатын, түбі құмдауыт, шетін сарғалдақтар, қияқ көмкерген суы мөлдір тұрақсыз су қоймасында *Ae. punctor* және *Ae. leucomelas* дернәсілдері ауланды.

Моноциклді *Ae. intrudens, Ae. pullatus, Ae. punctor, Ae. leucomelas, Ae. cataphylla* жартылай ашық су қоймаларында 7-19 ºС температурада 23-32 күн аралығында дамиды. Аталған түрлерден сәл кешірек, көктемнің соңында моноциклді түрлер дами бастайды. Бұлардың пайда бола бастауы су айдынын-дағы жылылық тұрақтануымен байланысты. *Ae. flavescens*-тің көктемгі популяциясының дамуы 11-18 ºС жылылықта жүреді. Бұл түрлердің ұшып шығуы негізінен алғанда, ерте көктемдік түрлерден кейін, кейде олармен бірге жүреді. Олардың дамуының жалпы ұзақтығы 28-30 күнді құрайды. Кейбір моноциклді түрлер (*Ae. flavescens, Ae. punctor*) жазда қайталама ұрпақ беруі мүмкін. Бұл

түрлердің ұшуы едәуір ұзаққа созылады. *Ae. flavescens*-тің ересегі мамырдан қыркүйекке дейін кездеседі, *Ae. punctor* соңғы рет тамыздың ортасында ауланды (Сурет 3).



Сурет 3. Зерттелген аймақ масаларының жаз маусымындағы ұшу ұзақтығы

Полициклді *Ae. c dorsalis, Ae. v. vexans* ашық және жартылай ашық шалшықтар мен көлшіктерде, басқа да тұрақты су қоймаларында үзіліссіз дамиды. Зерттеу аймағындағы метеорологиялық жағдай мен гидро тәртіпке байланысты олар екі -үш, тіпті одан да көп ұрпақ бере алады.

Қан соратын масалардың таралуын анықтайтын негізгі фактор-олардың дамуы байланысты интразональды биотоптардың болуы.

Солтүстік батыс Қазақстанда тұрақты және уақытша типтегі келесі су айдындары бар, онда біздің бақылауларымыз бойынша масалардың өсіп-өнуі жүреді:

1. Ірі өзендердің батпақты жерлері, ағыстан таяз қайраңдармен бөлінген жағалау учаскелері.

2. Жайылма өзен-көлдер (Тобыл, Есіл, Ертіс өзендерінің жайылмаларында).

3. Үлкен және кіші тұщы көлдер.

4. Қамыс өскен батпақты ойпаттар (үлкен көлдер мен батпақтардың бір бөлігі солтүстік ойпатты аудандарда шоғырланған).

5. "Қарасу" деп аталатындар-кеуіп жатқан шағын өзендер, жаз мезгілінде батпаққа және бөлшектелген сортаңға айналатын су қоймалары.

Олар негізінен Ақмола мен Целиноград ауданының оңтүстігінде шоғырланған.

6. Жасанды тоғандар, қараусыз қалған суару каналдары, шұңқырлар және басқа да тұрақты су қоймалары, шаруашылық қызметтің өнімі болып табылатын сулар

7. Ашық ландшафттардың уақытша су қоймалары.

8. Солтүстік батыстағы уақытша орман тоғандары.

Аталған тұрақты су объектілері атмосфералық жауын-шашынның жиналуынан пайда болады, күнмен жақсы жылынады, қамысты өсімдіктерімен қоршалған және *Anopheles, Theobaldia, Mansonia* және *Culex* туысы масаларының көбею ошағы ретінде қызмет етеді. Біз олардың әрқайсысында *An. mac. messeae* және *C. modestus* масаларын таптық.

Солтүстік батыс Қазақстанның бүкіл аумағында осындай су айдындарының болуы оның осы түрлердің кең таралуын шарттайды.

Ашық ландшафттардың уақытша су айдындары Солтүстік батыс Қазақстанда да кездеседі, сондықтан оның аумағын *Ae. caspius, Ae. dorsalis, Ae. cyprius, Ae. flavescens, Ae. excrucians, Ae. subdiversus, Ae. cataphylla, Ae. vexans, Ae. cinereus, Ae. rossicus, Ae. intrudens* кеңінен мекендейді.

Бұл түрлер Солтүстік батыс Қазақстан аумағының көп бөлігін алып жатқан дала аймағында да, орманды дала мен шөлейт аймақтардың іргелес шетінде де кең таралған. Ae. cataphylla солтүстік аудандарында көбірек таралады, Ae. caspius батыстың оңтүстік және құрғақ аймақтарына тән.

Солтүстік батыс Қазақстан шегінде *Th.. annulata, Th. longiareolata* және *A lepidonotus* сирек кездесуі диапазонның негізгі бөлігінен үлкен қашықтыққа байланысты болуы мүмкін. Солтүстік батыс Қазақстандағы *Th. longiareolata* (Ақмола облысының солтүстігі) солтүстікке таралудың ең шеткі нүктелерінің бірі болып табылады (Мончадский, 1951).

Солтүстік батыс Қазақстанның басым бөлігінде *Ae. sticticus* кездейсоқ пайда болуы оның дамуы үшін қажетті жағдайлардың болмауымен түсіндіріледі. Бұл масалар уақытша орман тоғандарымен байланысты және тек осы су қоймалары бар Ақмола облысының солтүстігінде байқалады.

Жекелеген аудандарда немесе тіпті пункттерде Mansonia richiardii (Ақмола облысының солтүстігі және Көкшетау облысының батыс бөлігі), *Ae. beklemishevi, Ae. leucomelas* (Ақмола облысының солтүстігі), *Ae. behningi* (Батыс Қазақстан облысының оңтүстігіндегі шөлейт ландшафттар) тіркелді. Солтүстік батыс Қазақстанның басқа аудандарында олардың болуы туралы нұсқаулардың болмауы мәліметтердің жеткіліксіздігімен түсіндіріледі.

Солтүстік батыс Қазақстан жағдайында масалар ұшуы сәуір айының соңында басталып, қыркүйектің соңына дейін созылады. Ең көп саны маусым, шілде және тамыз айларында байқалады.

Қан сорғыш масалар санының маусымдық барысын талдау олардың ішінен жаз мезгілімен және ең жоғары белсенділік кезеңімен сипатталатын үш топты бөлуге мүмкіндік береді: көктем, жаз-күз және маусым бойы ұшатын түрлер.

Бірінші топқа мамырдың басынан маусымның аяғына дейін ұшатын барлық ерте көктемгі Aedes моноциклді түрлері кіреді. Санының шыңы мамырдың аяғында-маусымның басында байқалады. Бұлар *Ae. cyprius, Ae. subdiversus, Ae. lepidonotus, Ae. cataphylla, Ae. leucomelas, Ae. maculatus, Ae. intrudens, Ae. sticticus* масалары.

Жазғы-күзгі түрлер тобына біз шілде және тамыз айларында ұшатын M. richiardii, Ae. Vexans жатқызамыз, олар үш ай бойы: шілде, тамыз және қыркүйекте кездеседі. Тек шіліңгір ыстық жазда (шілденің ортасынан тамыздың аяғына дейін) Ae. rossicus ауланды.

Маусым бойы полициклді түрлер: *An. mac. messeae, S. modestus, Ae. caspius, Ae. dorsalis, Ae. cinereus*, кейбір жылдары *Ae. flavescens* және *Ae. echsrucians* ұшады (2024 жылы бұл масалардың ұшуы мамырдың аяғынан қыркүйектің ортасына дейін байқалды). Біздің коллекцияларымызда сирек кездесетін *Th. longiareolata, Th. alaskaensis, Th. annulata, Th. ochroptera* масалары осы топқа жатады. *C. pipiens* масаларын біз тек күзде ұстадық.

Маусымның әртүрлі кезеңдерінде түрлер құрамының осындай өзгеруіне байланысты масалардың әртүрлі түрлері басым болады. Ақмола облысында көктемде *Ae. cataphylla* және *Ae. leucomelas* жаппай болып табылады. Ae. subdiversus айтарлықтай орын алады. Павлодар Ертіс жайылмасында – *Ae. intrudens, Ae. cyprius* және *Ae. cataphylla* тіркелді. Маусымның екінші жартысынан бастап ерте көктемгі доминанттардың орнына *Ae. flavescens және Ae. excrucians* келеді, олар: адам жақсы игерген аудандарда кең тараған,

An. mac. messeae бұларға кейінірек қосылады. 2024 жылы шілде-тамыз айларының ортасында осы түрлермен бірге *M. richiardii* және көптеген *Ae. dorsalis,* пайда болды, олардың саны күзге қарай өсті. Соңғысы *Ae. vexans* мен бірге тамыз және қыркүйек айларында басым болды. 2024 жылы уақытша су айдындарын жайылған масалардың саны аз болды, ал күзгі кезеңде шабуылдаушылар арасында *An. mac. messeae* және *C. modestus* ерекше белсенді болды.

Осылайша, Солтүстік батыс Қазақстанда масалардың саны жаз маусымының барлық жылы кезеңінде жоғары болып келеді.

Талқылау

Біздің бақылауларымыз климаттық жағдайлары бойынша күрт ерекшеленетін жылдары жүргізілді. 2022 жылдың ұзақ және суық көктемі, суық ауа райының жиі оралуы ерте көктемнің өте жылдам өтуіне және *Aedes* полициклді түрлерінің алғашқы генерациясының шығуына себеп болды. Жауын-шашынның аздығы нәтижесінде уақытша су объектілері бір рет толтырылды. Маусымда екі *Ae. dorsalis* ұрпағы және бір Ae. vexans ұрпағы дамыды. Күзгі масалардың саны аз болды.

2024 жылдың жылы және жылы көктемі, жауын-шашынның көп мөлшері, 2022 жылмен салыстырғанда ауаның ылғалдылығы көктемде де, жазғы-күзгі кезеңдерде де *Aedes* туысы масаларының көбеюіне себеп болды. *Ae. dorsalis* үш ұрпағы, *Ae. cinereus* және *Ae. vexans.* екі ұрпағы дамыды. Әр түрлі масалардың популяциясының тіршілік ұзақтығы 2022 жылмен салыстырғанда 20-50 күнге ұзағырақ болды. *Ae. flavescens, Ae. excrucians, Ac. sticticus*-да ішінара екінші ұрпақтың дамуы байқалды.

Аз дәрежеде ауа температурасы мен жауын-шашын мөлшері тұрақты су объектілерінің жағдайына және *Anopheles, Mansonia* және *Culex* туысы масаларының дамуындағы байланысты мөлшерге әсер етеді. 2022 жылы олардың жалпы саны 2024 жылмен салыстырғанда аз болғанына қарамастан, қансорғыштардың жалпы санында олардың 2022 жылғы үлес салмағы 2024 жылмен салыстырғанда (16,4%) айтарлықтай жоғары болды (30,2%).

Қан соратын масалардың көпшілігі *(Ae. caspius, Ae. dorsalis, Ae. flavescens, Ae. excrucians, Ae. subdiversus, Ae. cataphylla, Ae. intrudens, Ae. vexans, Ae. cinereus, Ae. leucomelas, C. modestus)* ең көп белсенділікті таңертең ерте, мамыр мен маусымда 6-7-ден 8-9-ға дейін, шілдеде сағат таңғы бестен бастап және кешкі және ымырт сағаттарында көрсетеді. Күндізгі уақытта біз барлық ерте көктем түрлерінде айтарлықтай белсенділікті байқадық. Масалар олжаны іздеу және қудалау үшін өсіп-өнетін және ең көп шоғырланған жерлерінен айтарлықтай арақашықтықты игереді және жолдарда, төбелерде, ашық дала учаскелерінде адамдарға және жануарлар табындарына шабуыл жасайды.

M. richiardii және *An. mas. messeae* белсенділігі толық қараңғылықтың басталуымен күрт көтеріледі. Қараңғыда 20 минут ішінде, масалар әрең ерекшеленетін кезде, 20 минут бұрын өндірілген жинау уақытына қарағанда екі есе көп *An. mas. messeae* (27 шілде 2024 жылы 112 масалар 20 сағат 50 минуттан 21 сағат 10 минутқа дейін және 229 маса 21 сағат 10 минуттан 21 сағат 30 минутқа дейінгі аралықта) ұсталды.

Масалардың күндіз шоғырланған жерлерінде (орман шоқтары, су қоймаларына жақын шөптер мен бұталар) сирек кездесетін масалар, соның ішінде *M. richiardii,* едәуір мөлшерде жалқаулықпен шабуыл жасайды, олар екі-үш метрден кейін олжа іздемейді. *An. mac. messeae* түрінің ерекшілігі, біз күндізгі уақытта шабуылдарды олар күндіз көп шоғырланатын орманды жерлерде де, қараңғы жерлерде де байқамадық.

Қорытынды

Зерттеу нәтижелеріне сүйенсек, Солтүстік батыс Қазақстанда эпидемиологиялық тұрғыдан ең қауіпті әрі жаппай және көп кездесетін масалар *An. mac. messeae, Ae. dorsalis, Ae. flavescens, Ae. excrucians, Ae. vexans* бір маусымда бірнеше ұрпақ береді және гонотрофиялық циклдардың ең көп санын жасай алады. Сондықтан, негізгі шаралар халыққа және мал шаруашылығына барынша зиян келтіретін, Солтүстік батыс Қазақстанда кең таралған *Aedes* туысы масаларын жоюға бағытталуы тиіс. Біз уақытша су қоймаларын суспензияланған деларвациялық препараттармен өңдеу арқылы масалардың преимагинальды фазаларын жоюды ең ұтымды шара деп санаймыз. Ерте көктемгі моноциклді және полициклді *Aedes* түрлерінің алғашқы ұрпақтарының алғашқы генерациясы сәуір айының ортасынан аяғына дейін қар жамылғысы ерігеннен кейін көп ұзамай пайда болады. Осы сәттен бастап 2-3 километр радиуста елді мекендер маңын қоршап тұрған су объектілерін бастапқы өңдеуді бастау керек. Полициклді *Aedes* түрлерінің екінші және үшінші ұрпақтарын жою үшін уақытша су объектілерін кейінгі өңдеу жазда, олар жауын-шашынмен толғаннан кейін қайталануы керек. Деларвацияны бірінші жастағы дернәсілдер пайда болған кезде жасау керек.

Анофелогенді су объектілерінің деларвациясы мамырдың екінші немесе үшінші онкүндігінде алғашқы дернәсілдер пайда болған кезде басталуы керек. *An. mac. messeae* – мен бірге тіршілік ететін және дамитын *Culex* туысы масаларының дернәсілдерін жою мақсатында: безгек масаларының дернәсілдерін жою үшін қолданылатын эмульсиялар мен газдарды суспензиялармен біріктіру керек. Май тәрізді ларвоцидтер *An. mac. messeae* үшін де, басқалар үшін де жойқын әсер көрсетеді. Әсер болмаған жағдайда, тоғандарды өңдеу бірінші ұрпақтың масалары жаппай ұшып шыққаннан кейін бір аптадан кейін қайталануы керек.

Авторлардың үлесі

В.Д., С.У., Р.О. – жұмыстың тұжырымдамасы және оны басқару; **А.О., М.С.–** эксперименттер жүргізу; **К.А.** – зерттеу нәтижелерін талқылау; **А.О. –** мәтінін жазу; **А.О.** және **М.С. –** мақала мәтінін өңдеу.

Мүдделер қақтығысы

Бұл материал бұрын жарияланбаған және басқа баспаларда қаралмағанын мәлімдейміз, сол себепті мүдделер қақтығысы жоқ.

Этикалық нормаларды сақтау

Авторлар орындаған бұл мақалада адамдарды немесе жануарларды объектілер ретінде пайдаланған зерттеулер жоқ.

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Современное состояние популяции комаров в северо-западном Казахстане

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Аннотация. Статья посвящена изучению фауны комаров в северо-западном Казахстане, учету наиболее опасных видов комаров по ряду эпидемиологических признаков опасности, обоснованию мер борьбы с ними. Содержание данной работы, проведенной в летнем сезоне 2020 и 2024 годов в северо-западном Казахстане, в частности, в Акмолинской, Атырауской, Западно-Казахстанской, Павлодарской, Костанайской областях, включает определение видового состава и экологии отдельных видов в различных экологических группах, в которых распространены эти регионы, в том числе региональной и суточной активности комаров, определение возрастного состава популяций и определение продолжительности жизни состояло из определения мест откладывания яиц, мест наибольшей концентрации кровососов, сроков вылета комаров. Комары - одна из наиболее важных с практической точки зрения групп среди кровососущих членистоногих: эти насекомые не только составляют основную массу в ряде ландшафтов среди кровососущих насекомых, но и переносят множество трансмиссивных болезнетворных микроорганизмов, общих для человека и животных. Полученные научные результаты и концепции позволят оценить современное состояние популяций комаров в северо-западном Казахстане, дополнят данные видового состава комаров региона новой информацией.

Ключевые слова: комары, видовой состав, биологические особенности жизни, суточная и сезонная динамика лета

The current state of the mosquito population in North-West Kazakhstan

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Abstract. The article is devoted to the study of mosquito fauna in North-West Kazakhstan, accounting for the most dangerous types of mosquitoes for a number of signs of epidemiological danger, and justification of measures to combat them. The content of this work, which was carried out in the summer seasons of 2020 and 2024 in North – West Kazakhstan, in particular in Akmola, Atyrau, West Kazakhstan, Pavlodar, Kostanay, North-West Kazakhstan regions, consisted in determining the species composition and ecology of certain species in various ecological groups in which these regions are distributed, including regional and daily activity of mosquitoes, determining the age composition and life expectancy of populations, determining the places of laying eggs, places of the largest concentration of bloodsuckers, mosquito flight dates. Mosquitoes are one of the most important groups among blood-sucking arthropods from a practical point of view: these insects have added a blood-sucking that is close to man, not only forming the main mass in a number of landscapes within the blood-sucking insects, but also carrying many transmissible pathogens common to humans and animals. The obtained scientific results and concepts make it possible to assess the current state of mosquito populations in North-West Kazakhstan, supplement the data on the species composition of mosquitoes of the region with new information.

Keywords: mosquitoes, species composition, biological features of life, daily and seasonal dynamics of flight

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Environmental controls on sedimentation, habitat development, and implications for palaeobiological reconstruction in the Middle Siwalik Group, Southern Indus Basin

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Abstract. The Middle Siwalik Group, located near Sehwan Town in the Northern Laki Range of the Southern Indus Basin, Sindh, Pakistan, extends for a stratigraphic thickness of approximately 1055 meters at the Kari Buthi Section (KBS). This study investigates the environmental controls on sedimentation and their role in the development of habitats within the region. The lithology of the area is composed of a range of sedimentary rocks, including Sandstone, Conglomerate, Conglomeratic Sandstone, Shale, Clay, and Mudstone. The facies analysis revealed six primary depositional facies: Conglomerate and Conglomeratic Sandstone (GT), Fine to Coarse-Grained Trough Cross-Bedded Sandstone (St), Fine to Coarse-Grained Flat-Bedded Sandstone (Sh), Shale (Fm), Mudstone (Mf), and Clay (Cf), each of which reflects distinct environmental and biological conditions during sediment deposition. Grain size distribution analysis, based on sieve data from seven representative loose sandstone samples, shows a mixture of fine to medium grains, with occasional very coarse grains. The sub-angular to sub-rounded grain shapes suggest a low-energy environment of deposition, characteristic of a braided fluvial system. Petrographic analysis conducted using a LEICA 2500p Transmitted Light Polarizing Microscope identified quartz (50-60%), feldspar (15-16%), and rock fragments (5%) as the primary constituents, with minor muscovite and biotite. This mineral composition, along with the sedimentary characteristics, indicates proximity to the sediment source, providing further evidence for the presence of a braided fluvial system. From a biological perspective, the sedimentary environment likely facilitated the development of various habitats for early biota, especially within the fine-grained deposits of mudstones and shales. These depositional settings would have provided potential substrates for microbial life and early forms of aquatic organisms, contributing to the overall habitat development within this ancient fluvial system. This study emphasizes the significant role of environmental factors-

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sediment supply, water energy, and biological influences in shaping both the sedimentary architecture and the habitat conditions of the Middle Siwalik Group. Understanding these interactions enhances our ability to reconstruct past ecosystems and the biological processes that governed sedimentation and habitat formation in the Southern Indus Basin.

Keywords: Petrography, Grain Size Distribution, Facies Categorization, Depositional Environment, Middle Sawilik Group of Northern Laki Range, Southern Indus Basin

Introduction

The Siwalik Group, a major Neogene-Quaternary stratigraphic unit of the Himalayan foreland basin, represents one of the most complete continental sedimentary archives of fluvial, climatic, and biological evolution in South Asia [1]. Extending in an east–west orientation along the southern Himalayan foothills, it provides an exceptional record of the interplay between tectonics, sedimentation, and biotic adaptation associated with the uplift of the Himalayas [2]. The Middle Siwalik Group, in particular, chronicles the phase of intensified Himalayan exhumation and foreland sedimentation, marking a key period of paleoenvironmental transformation and ecosystem diversification within the Indus Basin. These deposits capture the coupling between mountain-building processes, sediment dispersal systems, and biological responses to changing habitats and hydrological regimes [3].

The Siwalik Group extends over a 6-90 km wide belt across Bhutan, Nepal, India, and Pakistan, forming a regionally continuous fluvial succession that spans from the Middle Miocene to the Upper Pleistocene [4]. This broad temporal and spatial coverage provides an unparalleled opportunity to reconstruct the Neogene terrestrial paleoecology and sedimentary dynamics of the Himalayan foreland. Sedimentary structures typical of the Middle Siwaliks, such as trough cross-bedding, planar lamination, and ripple stratification, reflect high-energy braided and meandering fluvial processes that shaped both the geomorphic and ecological landscape [5]. Within these deposits, distinct lithofacies associations, including conglomerate and conglomeratic sandstone (Gt), trough cross-bedded sandstone (St), flat-bedded sandstone (Sh), shale (Fm), mudstone (Mf), and clay facies (Cf), record a spectrum of depositional environments from channel bars to floodplains [6].

In the present study, the Kari Buthi Section, located in the northern part of the Laki Range near Sehwan Town, District Jamshoro, Sindh, Pakistan, provides an excellent natural laboratory for investigating these fluvial and biological processes [7]. The section lies at coordinates 26°20'12"N and 67°50'42"E (Topographic Sheet No. 35N/15) and exposes one of the most complete Middle Siwalik successions in the southern Indus Basin (Figure 1), reaching a thickness of approximately 1055 feet near Manchar Lake [8]. Stratigraphically, the succession forms part of a sequence of five major formations: Laki, Kirthar, Nari, Middle Siwalik, and Dada Conglomerate, representing progressive sedimentary and tectonic evolution from marine to continental conditions in the Sindh region (Table 1)

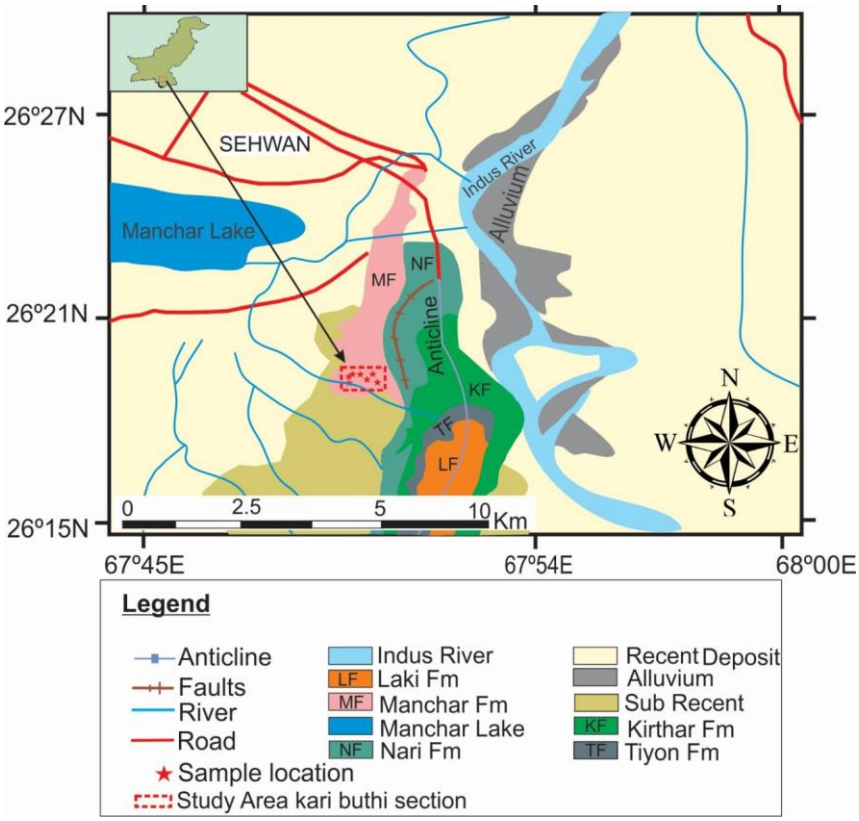

Figure 1. Geological setting of the Middle Siwaliks at southern Indus Basin

Lithologically, the Kari Buthi Section is composed primarily of interbedded sandstone, shale, and subordinate conglomerate layers [9]. The lower part of the succession is dominated by soft, yellowish-brown shale interbedded with fine-grained sandstone and siltstone (Figure 2), while the upper part transitions into grey to greenish-grey, gritty to pebbly sandstone with pronounced cross-bedding [10,11]. Conglomeratic beds are typically poorly cemented and contain sandstone pebbles along with fragments of arenaceous and fossiliferous limestone, suggesting periodic high-energy depositional episodes related to river channel migration and debris flow events sourced from the Himalayan hinterland [12]. These coarse-grained intervals record the proximal fluvial input of clastic detritus, whereas the fine-grained shale and mudstone represent low-energy overbank and floodplain deposits conducive to vegetation establishment and soil development.

Table 1

Five formations are arranged from oldest to youngest: Laki, Kirthar, Nari, Middle Siwalik, and Dada Conglomerate Formations

Age	Formation	Lithology
Pleistocene	Dada Conglomerate	Conglomerate and Boulders and Pebbles
		Conglomerate and Sandstone

Miocene to Pliocene	Middle Siwalik Group	Shale and Conglomeratic Sandstone
		Mudstone
	Unconformity	
Oligocene	Nari Formation	Sandstone, Limestone and Shale
	Unconformity	
Eocene	Kirthar Formation	Limestone
	Laki Formation	Limestone, Shale, and Sandstone

The lithological composition of the Kari Buthi Section shows strong correlation with the Nari, Gaj, and Kirthar formations, all of which exhibit mixed siliciclastic and carbonate facies, reflecting the gradual transition from marine to continental sedimentation during the late Neogene [11]. The sedimentological features, such as trough cross-bedding, planar lamination, and gradational facies contacts, indicate an active fluvial regime dominated by alternating braided-channel and floodplain environments [13]. These dynamic depositional conditions were likely modulated by monsoonal climatic variations, leading to cyclic patterns of flooding, erosion, and soil formation.

Beyond its sedimentological importance, the Middle Siwalik Group is of profound biological and palaeoecological significance, preserving evidence of fluvial ecosystem evolution during the Neogene. Fossil plant remains, root traces, and paleosol horizons document the establishment of riparian vegetation and terrestrial habitats within this dynamic floodplain system. These vegetated surfaces played a critical role in sediment stabilization and nutrient cycling, representing early instances of biogeomorphic feedback between vegetation and sedimentation [14]. The alternation of high-energy channel fills with low-energy over bank deposits further suggests fluctuating ecological niches that supported diverse terrestrial and aquatic communities.

The present study, therefore, aims to evaluate the environmental controls on sedimentation, habitat development, and palaeobiological reconstruction of the Middle Siwalik Group in the Southern Indus Basin. By integrating lithofacies analysis, grain-size data, and petrographic observations with palaeoecological interpretations, this research seeks to elucidate how hydrodynamic variability, sediment supply, and biological adaptation collectively shaped the fluvial landscapes of the Middle Siwaliks. This multidisciplinary approach provides new insights into the linkages between sedimentary processes and ecosystem resilience, contributing to a more comprehensive understanding of Neogene paleoenvironmental evolution in the Himalayan foreland domain.

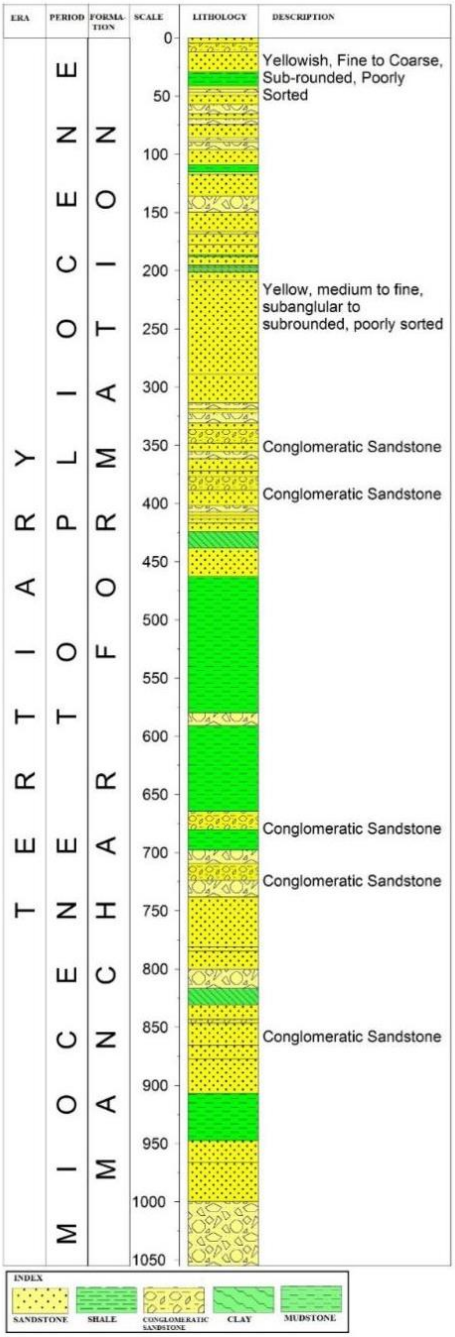

Figure 2. Columnar section of the Middle Siwaliks at southern Indus Basin

Materials and research methods

For the current study on the Middle Siwalik group, two primary methodologies were employed: fieldwork and laboratory analysis. A total of twenty sandstone samples, both loose and compact, were collected from the field. Seven of these samples underwent thin-section analysis at the laboratory of the Geological Survey of Pakistan. Field-based data collection involved the systematic sampling of outcrops, with a comprehensive stratigraphic section of the

Middle Siwalik at Kari Buthi being measured. Rock samples were collected for detailed analysis and further investigation.

Section measurement

The Kari Buthi Section was selected for this investigation, and the geological section was surveyed using the compass and tape method; the layers were measured directly where applicable. During the section measurement, 14 samples were selected from each lithofacies. The conformable contacts of the Middle Siwalik with the Nari Formation were chosen as the measurement's starting point (Figure 3), and it was measured continually with recent to sub-recent deposits up to its unconformity at the top of the section.

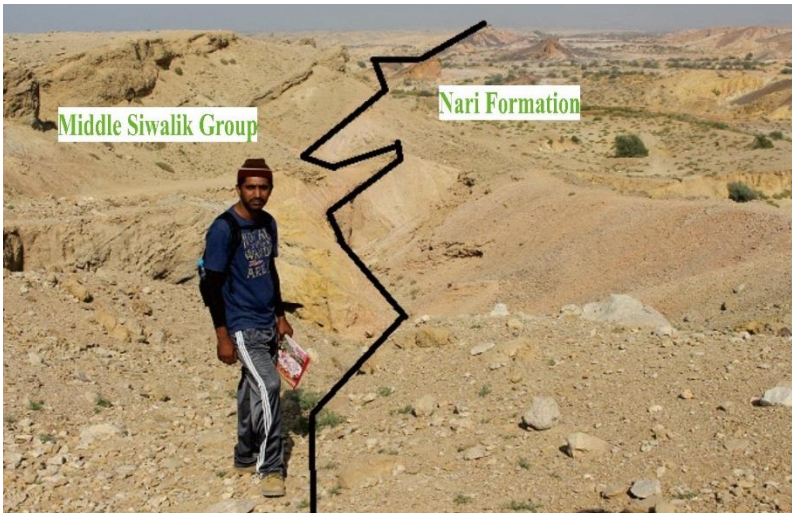

Figure 3. Lower contact of the Middle Siwalik group with the Nari Formation

At the Kari Buthi Section, the entire thickness of the Middle Siwalik group is 1055 meters (Figure 1). Sample (a) is provided by the Middle Siwalik Group on the Kari Buthi trend with a thickness of 2,719 m, (b) Samples were taken from a bed 4,436 m impenetrable. Calcareous cement was reacted with HCL. (c) It has a sub-angular to sub-rounded shape, is well-sorted, and measures 16.472 meters in unit thickness. (d) It has minimal sorting, a 24.629-meter bed thickness, and calcareous cement that can be identified by its reaction with HCl. (e) It is made up of a 5.56-meter-thick layer with calcareous cement visible, and the sandstone is grey with medium to fine grain sizes, angular to sub-rounded shapes, and is fairly sorted. (f) Its shape is subangular to subrounded, and the grain size is moderately sorted. Calcareous cement is present in the sandstone, and the bed thickness is 13.515 meters. (g) The sample has a sub-angular to sub-rounded shape, a poorly sorted texture, calcareous cement, and a bed thickness of more than 3 meters in the study area.

Petrographic analysis

For the petrographic study, standard thin sections were prepared from seven hard, consolidated to semi-consolidated materials. The selected samples for this section preparation (Figure 4) were dispatched to Geosciences Advance Research Laboratories, Islamabad. Thin sections were examined under the Polarizing Microscope at the Centre for Pure and Applied Geology, University of Sindh, Jamshoro, to assess the mineral composition and textural characteristics. The Sawliks group (Miocene to Pliocene) is exposed at Manchar Lake in Sehwan,

Sindh, Pakistan; its lithofacies were studied to establish the environment and mechanism that caused its deposition [15]. Our current study has identified four lithofacies types in the Middle Siwaliks at the southern Indus Basin: (Gt, St, Sh, Fm), which occur in a particular sequence and are characterized by a coarsening upward trend in grain size. The Middle Siwalik group in the studied area is predominantly clastic, with lithology ranging from conglomerate to conglomeratic sandstone, sandstone, shale, clay, and mudstone [16]. Boring and burrows, wood fossils, and roots can be observed in conglomerate, conglomeratic sandstone, and sandstone with trough cross-bedding [17].

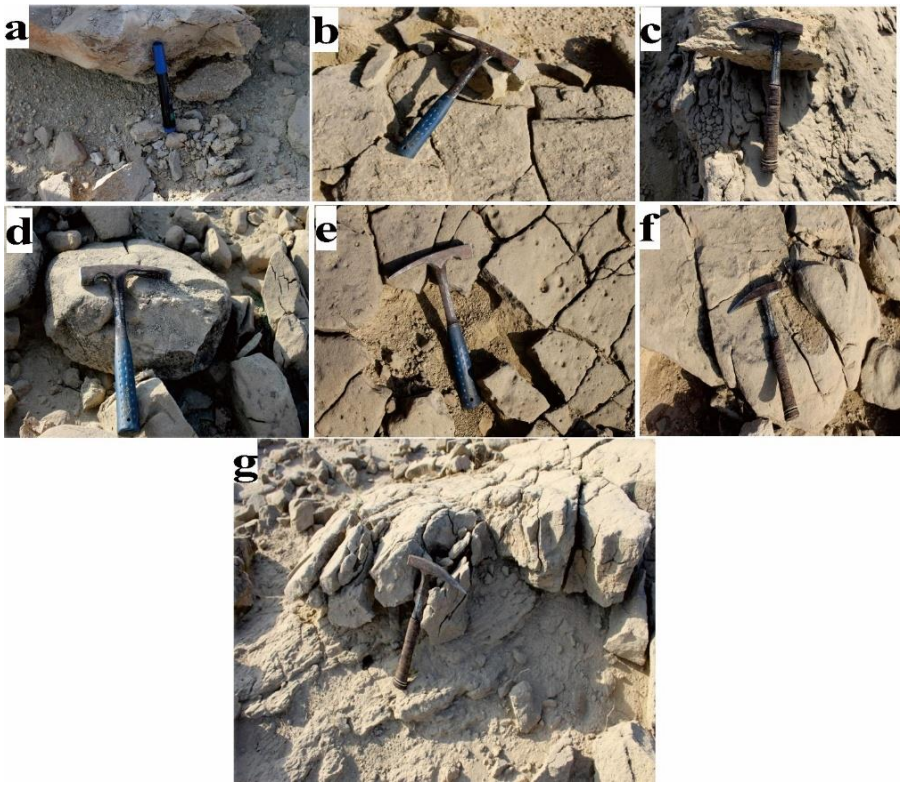

Figure 4. (a) The sample is sandstone, with fine to extremely fine grains and some coarse-grain particles. It is greenish-white. **(b)** Sandstone is semi-consolidated, fine to medium-grained in size, and yellow. The calcareous cement was identified when it was treated and reacted with HCL**. (c)** Sandstone reveals that it is grey and has a medium to fine grain size. **(d)** Sandstone is grey, with medium to small grain-size particles that are angular to surround it. **(e)** The sample of sandstone is grey in hue, with medium to fine grain size, angular to sub-round in shape, and fairly sorted. **(f)** Sandstone is grey in hue, with fine to very fine grain size**. (g)** Sandstone is grey, with medium to coarse grain sizes, it contains subangular to subrounded form, and a poorly sorted texture

Grain size distribution

Grain size analysis is one of the most important techniques in sedimentology for extracting geological information from loose sand and sandstone [18]. Defined that the size of grains and their statistical parameters are helpful in the interpretation of the depositional environment of friable sandstones and for facies characterization [19]. Tucker (1991) suggested that the transportation and deposition of pre-existing rock particles are well understood through grain analysis, and it also helps to know the lithologies of the different environments [20]. The grain size analysis helps to interpret the sedimentological process involved and the type of prevalent

environment of deposition [21]. During fieldwork, four principle lithofacies were identified and seven (7), loose sandstone samples from those lithofacies were selected and prepared for sieving by using "The Octagon Digital sieve shaker" available at the sedimentological laboratory of the Centre for Pure and Applied Geology. The selected (-1 φ, 0φ,1φ,2φ,3φ,4φ, and Pan) mesh sizes were stacked on the sieving machine and operated for the standard time of ten minutes. After the finishing of the standard time of 10 minutes shaker sieve screens were removed, and every screen was weighed on the digital electronic balance and noted on the data sheet. Sieved data (raw data) was calculated by following the formula for the use of statistical work (cumulative frequency curves).

Results

Facies analysis

The Conglomeratic Sandstone Facies suggests stream flow deposits but may also result from rapid, decelerating, high-magnitude, gravel-dominated stream flow under flashy discharge conditions. Coarse channel-floor deposits form from lag gravel in deeper channel parts after finer material is winnowed. The subangular fragments and poor sorting indicate minimal stream reworking, likely due to the collapse of cohesive bank sediments into nearby channels, caused by rotational slumping that brecciates material below the thalweg depth. Some channel conglomerate bodies fine upward into sandstone, indicating flow diminution from channel diversion or lateral migration (Figure 5a). The Sandstone Facies (St) consists of laterally persistent sandstone sheets, dominated by large trough cross-stratification with subordinate small-scale planar and trough cross-stratification, interpreted as distal, sand-dominant braided fluvial deposits. Individual channel-fill sequences stack without vertical accretion deposits.

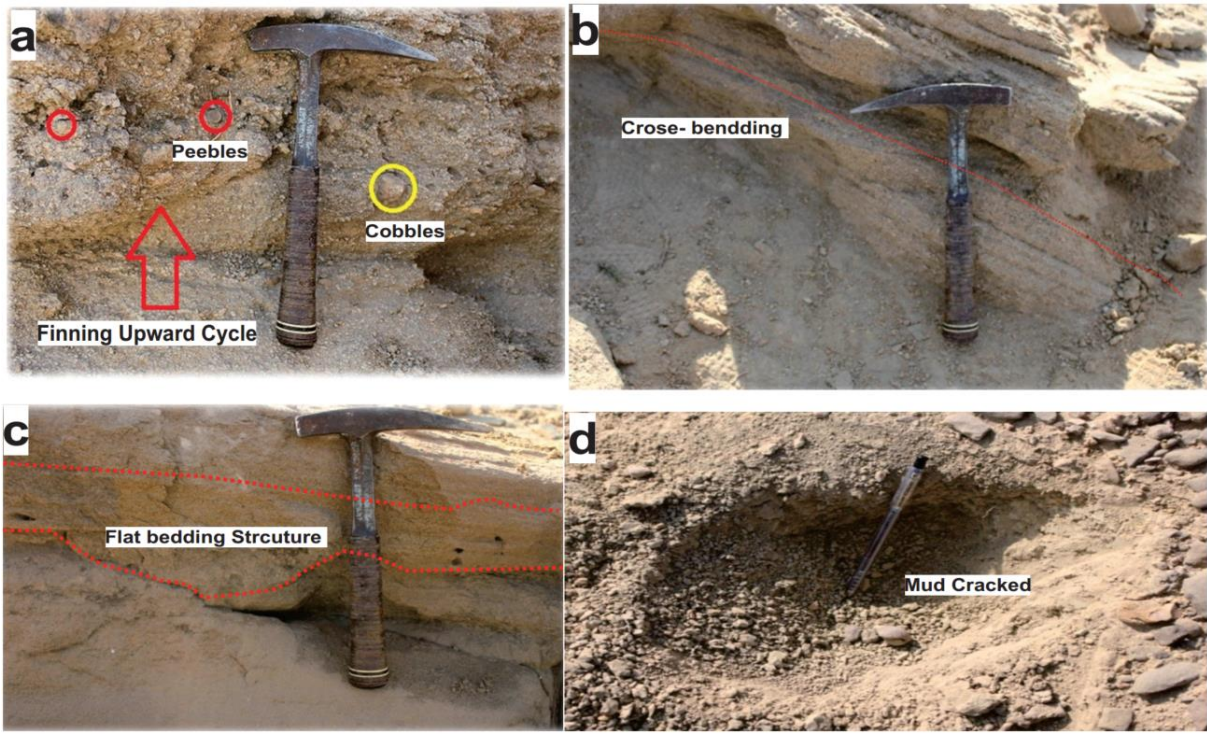

Figure 5. (a) Conglomeratic Sandstone Facies indicates stream flow deposits, (**b**) Sandstone Facies (St) with crose bending, **(c)** plane bedding sandstone (SH), **(d)** Shale facies (FM), sandy silt and mud-cracked

Large-scale inclined strata represent channel bar deposits from lateral migration or superposition of different bars or channel belts. Intraformational conglomerates along erosion surfaces represent cut-bank material eroded during lateral channel migration. Multistory sandstone bodies reflect channel bar superposition within aggrading belts before abandonment. Trough and planar cross-stratification suggest sinuous- and straight-crested dunes, with the latter forming under higher velocities. The absence of mud cracks and root traces indicates perennial river flow, while the lack of vertical accretion deposits points to the erosive capability of shifting channels (Figure 5b). The Fine to Coarse-grained Flat-bedding Sandstone (Sh) is a crevasse channel-fill deposit, with the lack of fining upward trends implying low sinuosity paleo-streams. Mud clasts, derived from levee and floodplain sediments, are intraformational. Rapid sedimentation from mixed-load streams is reflected in textural immaturity, while the upward increase in shaly lenses, burrows, and root traces suggests crevasse channel abandonment and waning current energy. Features like current ripple cross-lamination, trough cross-stratification, and planar stratification reflect deposition from migrating ripples, dunes, and upper-stage plane beds. Trace fossils and pedogenic features suggest overbank sand deposits were sites of insect burrowing, plant growth, and weak soil development (Figure 5c). The Shale Facies (Fm), consisting of sandy silt and mud-clay units, likely represents levee and distal splay deposits as indicated by burrows and calcareous concretions, with burrows and rootlets facilitating calcareous solution movement. The Middle Siwalik group at KBS typifies a braided river system, with variability in grain size reflecting differences in provenance and/or water stage fluctuations, while fine-grained lithological characteristics suggest significant overbank deposition (Figure 5d).

Grain size distribution analysis (GSD)

This report focuses on seven sandstone samples (Figure 6, KBS-3 to KBS-9), characterized through their respective cumulative frequency curves. These curves graphically represent the relationship between grain size, expressed in phi (φ) units, and the cumulative weight percentage of sediment finer than that size. The shape and position of these curves provide a visual representation of the sediment's sorting, a key indicator of the depositional environment. Samples KBS-3 and KBS-4 Exhibit steep cumulative frequency curves, signifying well-sorted sediments with a narrow range of grain sizes. This characteristic suggests deposition under relatively consistent hydrodynamic conditions, where prolonged or repetitive processes effectively sorted the grains based on size. Such well-sorted sediments are often associated with environments characterized by continuous winnowing and reworking, such as beach settings or aeolian dunes. In these environments, the constant action of waves or wind selectively transports and deposits grains of similar sizes. Conversely, samples KBS-5C and KBS-6B display more gradual curves, indicative of poorly sorted sediments with a wide range of grain sizes.

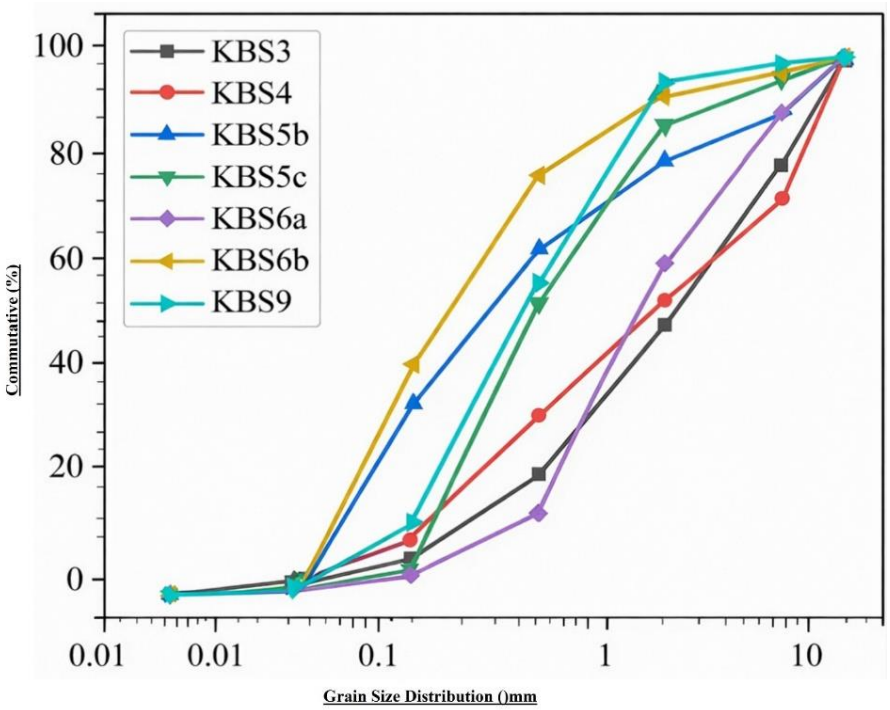

Figure 6. The grain size distribution of seven sandstone samples (KBS-3 to KBS-9) is represented as cumulative frequency curves. The x-axis depicts grain size using the phi (φ) scale, while the y-axis represents the cumulative weight percentage of sediment finer than the corresponding grain size

This pattern suggests deposition under fluctuating energy conditions, where rapid deposition or limited reworking prevented effective grain size sorting. Such poorly sorted sediments are commonly associated with environments characterized by rapid depositional events, such as alluvial fans, debris flows, or glacial outwash plains. In these settings, the sudden influx of sediment from various sources, coupled with limited reworking, results in a heterogeneous mixture of grain sizes. The inflection point of each curve offers further insights into the dominant grain size fraction within the sample. For instance, the rightward inflection of KBS-9 suggests a predominance of fine-grained material, potentially indicative of a low-energy depositional environment or the influence of suspension settling. This pattern could point towards deposition in a lacustrine or distal marine setting, where fine-grained sediments settle out of suspension in relatively calm waters. On the other hand, the more symmetrical curve of KBS-6A suggests a more balanced distribution of grain sizes, potentially reflecting a depositional environment with moderate energy levels and a mixed sediment supply (Figure 6).

Petrography result

There exists a relationship between the detrital components of clastic sedimentary rocks and the tectonic context of their source location. It should be noted, however, that detrital composition is influenced by several other critical factors, including travel history. In addition to tectonic origin, the depositional environment and paleoclimate cannot be overlooked, nor can diagenetic alteration of sand composition. Climate and tectonics are intertwined. Statistical analyses of the relationship between composition and depositional facies revealed that the association remains weak and that the control imposed by changes in source-area geology

was significantly overwhelmed by the relationship. With the use of petrography, sedimentary rocks may be easily identified and categorized by considering the megascopic and microscopic features of the constituent minerals. Standard thin slices are regularly made and examined under a polarized microscope for any petrographic inquiry. The consolidated to semi-consolidated samples of the Middle Siwalik group from the Kari Buthi Section were chosen for the thin section investigation in this regard. The constituent minerals, textural characteristics, and alteration products of all created thin sections were extensively examined. The outcomes are presented in the following sections (Figure 7a). Both types of feldspar (Plagioclase and Orthoclase) are present in the quartz (55 to 60%), which is sub-angular to sub-rounded, very fine to fine-grained, and occasionally coarse-grained. The 10% sedimentary lithic pieces are bonded to the grains with calcareous cement (Figure 7b).

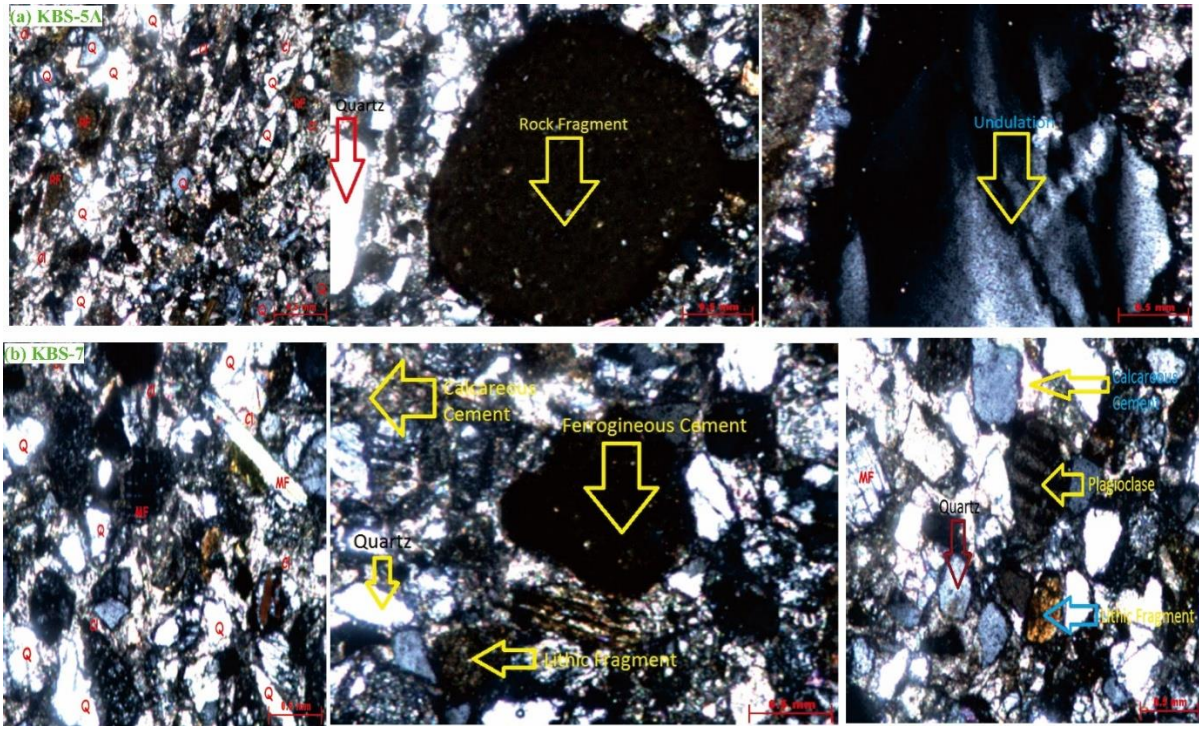

Figure 7. The appearance of sandstone thin section and photomicrograph of the middle sialic group at Kari Buthi section **(a)** quartz grain to fine grain and plagioclase, orthoclase, and calcareous. **(b)** Muscovite, biotite lithic pieces, Quartz ingredient

The main component, Quartz, is angular to sub-angular, fine to extremely fine-grained, and occasionally very coarse-grained (40 to 45 percent). The grains exhibit point-to-point contact. Both orthoclase and plagioclase feldspar are present, and a small percentage of straight muscovite and biotite flakes were also noted. It contains some sedimentary lithic fragments, and the grains are joined by calcareous cement. (Figure 8a). The fine to extremely fine quartz grains are encased in calcareous cement. The loose, angular to sub-angular shape and point contact of the grains indicate loose compaction. This sample's main components are quartz (between 55 and 60 percent), calcareous cement (between 35 and 37 percent), and lithic fragments (3 to 4 percent). Significant amounts of Plagioclase and K-feldspar are also present, along with 2% Muscovite and less than 1% Biotite (Figure 8b).

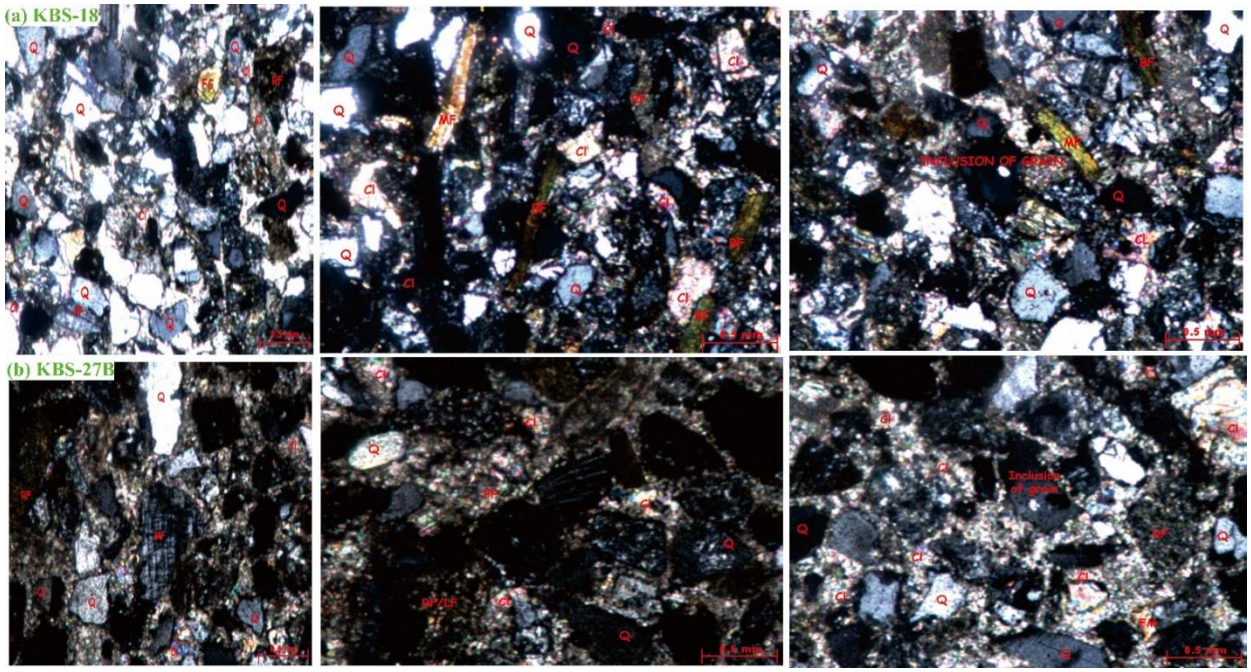

Figure 8. Show The appearance of sandstone thin section and photomicrograph **(a)** Lithic Fragments, Plagioclase Feldspar, K-feldspar, Quartz, and Biotite. **(b)** Plagioclase contains cross-aged rock fragments of Quartz and Biotite

Quartz has parts with small grain size and parts with medium to coarse grain size in the thin section. From point-to-point grain contact, it is angular to sub-angular in shape and loosely compacted. The main component of the sample is quartz (55 to 60%). Both feldspar types (Plagioclase and Orthoclase) have been identified, but only Plagioclase exhibits cross-aged twinning and contains significant amounts of calcareous cement (30 to 33%), rock fragments, and other minerals like Biotite (1%). (Figure 9a) In the thin section, quartz accounts for (50 to 55%). Microcline and K-plagioclase are examples of feldspar that are found. While ferruginous cement is only found in a small amount, it contains 30-35 percent calcareous cement. There are fewer than 1% sedimentary lithic fragments, Biotite, and Muscovite found. (Figure 9b) Quartz appears to be the most abundant mineral, accounting for 55 to 58% of all minerals. Plagioclase and Orthoclase are the two types of feldspars discovered in the thin section. Calcareous cement (30-32%), sedimentary lithic fragments, and needle inclusions in quartz are abundant in the thin sections. (Figure 8c). It is composed of medium to coarse-grained elements that range in shape from sub-angular to sub-rounded. It has poor compaction due to point-to-point contact. Needle inclusions can be seen in quartz. Quartz is the most common mineral found in this sample (55 to 56%), along with both types of feldspar (Orthoclase and Plagioclase), as well as calcareous cement (30%) and lithic fragments (4%). Biotite (less than 1%) and Muscovite (less than 1%).

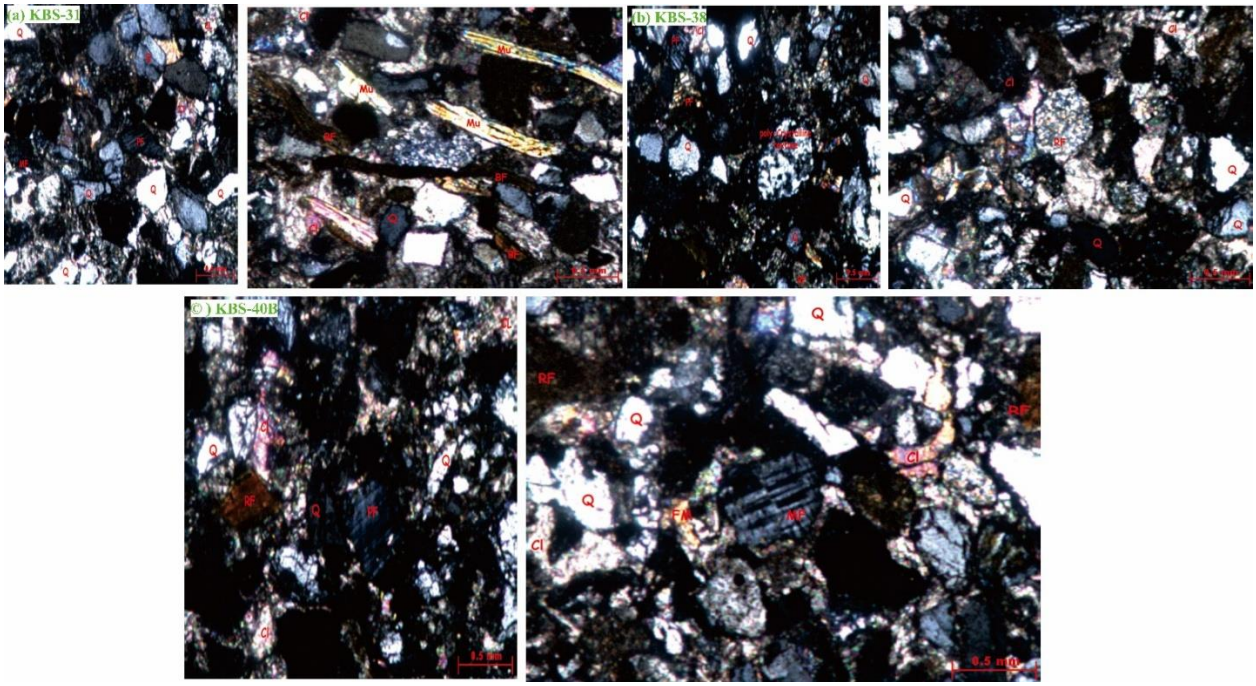

Figure 9. Show the appearance of sandstone thin section and photomicrograph of the middle sialic group at Kari buthi section **(a)** Microcline and k-plagioclase, Quartz and other sedimentary lithic fragments, Biotite and Muscovite. **(b)** Plagioclase, orthoclase, and sedimentary lithic fragments such as quartz. **(c)** Quartz is common in Orthoclase, Plagioclase, Biotite, and Muscovite

The QFR (Quartz-Feldspar-Rock Fragments) triangular classification diagram categorizes sandstones based on the Dot classification. The apexes represent quartz (Q), feldspar (F), and rock fragments (RF). The findings show over 90% quartz as Quartzarenite, located at the top. Subarkose and Sublitharenite occupy intermediate fields between Quartz arenite and the other two components, with 75–90% quartz, indicating moderate amounts of feldspar and rock fragments. Arkose, on the left, signifies feldspar-dominant sandstones (over 25%), while Lithic Arenite, on the right, marks sandstones with a greater proportion of rock fragments. The red dots represent samples primarily composed of quartz with moderate amounts of feldspar and rock fragments, placing them near the boundaries between Subarkose and Sublitharenite. The matrix percentage (0-15%) at the bottom corresponds to the proportion of finer material in the sandstone (Figure 10).

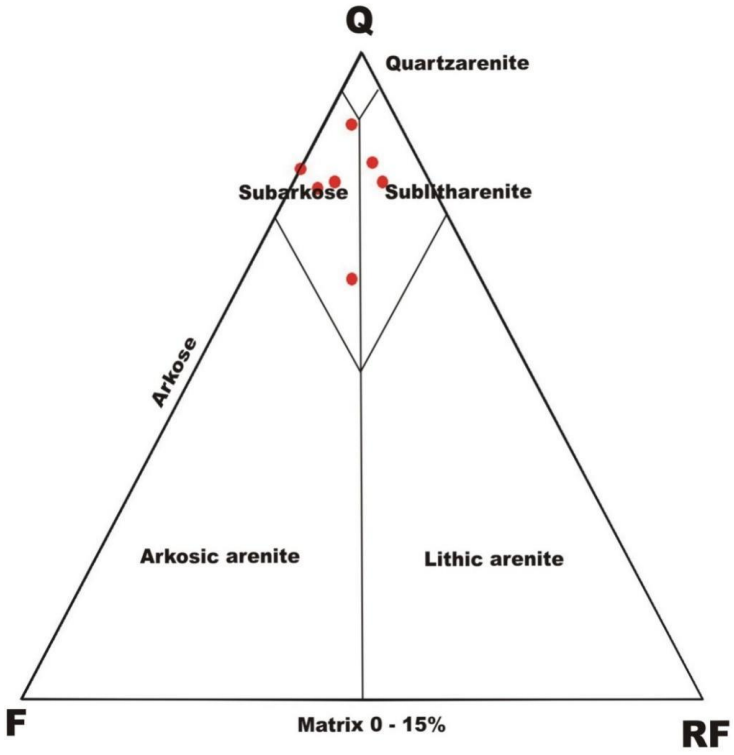

Figure 10. Show the Dot Classification of Middle Siwaliks at southern Indus Basin

Discussion

Sedimentary architecture and environmental controls

The Middle Siwalik Group exposed near Manchar Lake, Sehwan Town, District Jamshoro, Sindh, Pakistan, represents a dynamic fluvial succession shaped by variable hydrological regimes and climatic oscillations. Lithofacies, textural, and petrographic analyses collectively suggest deposition within a braided fluvial system characterized by alternating high- and low-energy depositional episodes. The dominance of conglomeratic sandstone (Gt), trough cross-bedded sandstone (St), and fine-grained flat-bedded sandstone (Sh) indicates a complex interaction between channel migration, sediment supply, and flow variability [22–24].

The Gt facies, with a total thickness of 263.334 m (24.952% of the succession), represents proximal channel deposits formed under rapid sediment aggradation and turbulent flow. These coarse-grained, matrix-supported conglomerates reflect episodic floods that transported sediments from the Himalayan hinterland through high-gradient channels [24]. The St facies (168.308 m; 15.948%) developed from the migration of sinuous-crested dunes in moderate flow conditions, representing point-bar and mid-channel accretion surfaces [25–28]. The Sh facies (395.692 m; 37.494%) corresponds to lower-energy bar-top and levee deposits, where finer sediments accumulated from waning flows [27]. The Fm facies (20.862 m; 1.976%) marks suspension fallout in floodplain and overbank settings during low-energy episodes. Collectively, these facies record lateral and vertical facies transitions typical of multi-channel braided systems dominated by episodic floods and sediment pulses [23].

Hydrological variability, likely governed by monsoonal climate forcing, exerted primary control on sedimentation. Intense rainfall during peak monsoons led to flash floods, high sediment discharge, and channel avulsion, while dry phases promoted pedogenic stabilization

and vegetation colonization. These alternating processes established a sedimentary architecture in which energy regimes directly influenced habitat structure, setting the foundation for subsequent biotic colonization and paleoecological development.

Textural and petrographic signatures of environmental energy

Grain size and textural parameters provide crucial evidence of depositional processes and paleoenvironmental energy. Based on Folk's (1951) classification [29], the sandstones of the Middle Siwalik Group range from immature to sub-mature, characterized by moderate to poor sorting and subangular to subrounded grains. The presence of >5% clay matrix in some horizons indicates limited sedimentary reworking and rapid deposition, consistent with proximal alluvial and braided river settings. These immature textures, coupled with high feldspar (10-12%) and lithic fragment content (6–7%), point to a short transport distance from the Himalayan arc and minimal compositional maturity.

Petrographically, the Manchar Lake sandstones are composed predominantly of quartz (50-55%), cement (30-32%), and minor feldspar and lithic fragments, corresponding to lithic arenite to sublitharenite composition. The high cement proportion suggests early diagenetic processes under fluctuating groundwater saturation conditions, reflecting alternating wet-dry cycles typical of monsoon-dominated floodplains [24]. These mineralogical and textural features underscore the rapid sedimentation and dynamic hydrological regime that governed the environmental evolution of the Middle Siwalik landscape.

Fluvial habitat zonation and ecosystem structuring

The sedimentary framework of the Middle Siwalik Group provides a basis for reconstructing fluvial habitat zonation and associated biological gradients. High-energy channels (Gt and St) formed unstable habitats dominated by sediment mobility and scouring, restricting permanent vegetation but allowing colonization by pioneer flora during hydrological recessions. Root traces and rhizoliths preserved within bar-top and levee deposits (Sh facies) record phases of riparian vegetation establishment following flood events [30].

These vegetation patches, likely comprising flood-tolerant angiosperms, grasses, and early C_4 taxa, acted as biogeomorphic agents, reinforcing channel banks through root stabilization and influencing sediment entrainment dynamics [31-33]. The development of root mats within fine-grained intervals indicates repeated colonization and erosion cycles, evidencing close feedback between biological stabilization and sedimentary reorganization. Over time, these processes facilitated the emergence of riparian microhabitats characterized by high nutrient turnover, moisture retention, and bioturbation activity.

In contrast, low-energy floodplain and overbank environments represented by Fm facies hosted stable, waterlogged substrates suitable for aquatic and semi-aquatic communities. The fine-grained, anoxic shales and mudstones likely supported benthic invertebrates, ostracods, mollusks, and early fish larvae. Trace fossils and burrow structures preserved in these facies indicate a thriving benthic ecosystem with microbial mat colonization as the basal trophic component [34,35]. Photosynthetic algae and cyanobacterial biofilms contributed to organic matter cycling and sediment biostabilization, forming micro-ecosystems resilient to hydrological fluctuations [34,35].

Bio-geomorphic feedback and environmental evolution

The interaction between biological colonization and sedimentary dynamics established biogeomorphic feedback mechanisms that shaped habitat persistence and sedimentation

patterns. Vegetation colonization on bar surfaces reduced flow velocity, encouraged fine-sediment deposition, and led to localized overbank accretion. Conversely, vegetation removal during floods accelerated erosion, resetting ecological succession. These alternating processes created a patchy mosaic of habitats, ranging from bare sandbars to vegetated levees and organic-rich floodplains, that collectively enhanced biodiversity and ecological resilience.

Sedimentological evidence, such as interbedded root traces, mud drapes, and pedogenic horizons, attests to short-lived stability phases between successive floods [30,31]. Such cycles of disturbance and recovery likely drove evolutionary pressures favoring species with rapid life cycles, dispersal capabilities, and physiological tolerance to moisture stress. The recurring colonization events reflect an ecosystem functioning under disturbance-mediated equilibrium, where resilience and opportunism determined biotic survival and proliferation [36, 37].

Conclusion

This study provides a detailed sedimentological and paleoecological assessment of the Middle Siwalik Group at the Kari Buthi Section, Southern Indus Basin. The findings present the first integrated analysis of facies architecture, depositional environment, and biological interactions within this Neogene fluvial system. The succession is characterized by repetitive fining-upward cycles composed of four major lithofacies: conglomeratic sandstone (Gt), trough cross-bedded sandstone (St), flat-bedded sandstone (Sh), and shale (Fm). These facies reflect deposition in a braided fluvial environment where alternating high- and low-energy regimes controlled sedimentation. The Gt facies, consisting of coarse-grained, poorly sorted conglomerates, represents high-energy channel deposits formed by rapid aggradation during flood events. The St and Sh facies correspond to moderate-energy bar and levee deposits formed under fluctuating discharge conditions, whereas the Fm facies marks fine-grained overbank and floodplain accumulation during flow quiescence.

Grain-size and textural analyses indicate that the sandstones are immature to sub-mature, with poorly sorted, subangular to subrounded grains. High feldspar (10–12%) and lithic fragment contents (6–7%) suggest a proximal Himalayan source and short transport distance. Petrographic observations reveal quartz dominance (50–55%) with calcareous cement (20–30%), supporting rapid burial and limited diagenetic alteration. According to the DOT classification, these sandstones range from sub-arkosic to sublitharenite, consistent with active fluvial channel deposition in a tectonically dynamic foreland basin.

From a biological perspective, the Siwalik fluvial system hosted dynamic riparian ecosystems adapted to frequent disturbance. Root traces and rhizoliths within the Sh facies record colonization by flood-tolerant vegetation that stabilized sediments and modified local hydrology. Fine-grained Fm deposits, representing low-energy settings, supported aquatic and benthic communities, including invertebrates, ostracods, and microbial mats. These organisms contributed to organic matter cycling and substrate bio stabilization, forming an early example of biogeomorphic feedback within a foreland river system.

Overall, the lithological, petrographic, and biological evidence collectively indicate that the Middle Siwalik deposits at Kari Buthi formed in a braided river system influenced by monsoonal climate and Himalayan tectonism. Environmental energy regimes, sediment supply, and vegetation interactions jointly controlled sediment dispersal, habitat development, and fossil preservation, providing new insights into Neogene fluvial ecosystem evolution within the southern Indus Basin.

Author Contributions

S.S.J. – Writing–review and editing, writing original draft, visualization, validation, software, methodology, formal analysis, data curation, and conceptualization; **A.A.K. –** Review and editing, visualization; **K.J. –** Review and editing, visualization; **M.A.N. –** Validation, formal analysis, data curation; **N.A. –** Review and editing.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could possibly influence the results of the work presented in this article.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Оңтүстік Инд бассейнінің Орта Сивалик тобында шөгінділердің түзілуіне, тіршілік ету ортасының дамуына қоршаған орта факторларының әсері және олардың палеобиологиялық қайта құрудағы маңызы

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Аңдатпа. Пәкістанның Синд қаласындағы Оңтүстік Инд бассейнінің Солтүстік Лаки жотасындағы Сехван қаласының маңында орналасқан Орта Сивалик тобы Кари Бути учаскесінде (KBS) шамамен 1055 метр стратиграфиялық қалыңдыққа созылып жатыр. Бұл зерттеу шөгінділердің түзілуіне және олардың аймақтағы тіршілік ету ортасының дамуындағы рөліне қоршаған ортаны бақылау шараларын зерттейді. Ауданның литологиясы құмтас, конгломерат, конгломерат

құмтасы, тақтатас, саз және балшық тас сияқты шөгінді жыныстардың кең ауқымынан тұрады. Фацияларды талдау алты негізгі шөгінді фациясын анықтады: конгломерат және конгломерат құмтасы (GT), ұсақтан ірі түйіршікті ойпатқа дейінгі көлденең қабатты құмтасы (St), ұсақтан ірі түйіршікті жалпақ қабатты құмтасы (Sh), тақтатас (Fm), балшық тас (Mf) және саз (Cf), олардың әрқайсысы шөгінділердің шөгіндісі кезіндегі әртүрлі экологиялық және биологиялық жағдайларды көрсетеді. Жеті типтік борпылдақ құмтас үлгілерінен алынған елек деректеріне негізделген түйіршік өлшемінің таралуын талдау ұсақтан орташа түйіршіктердің қоспасын, кейде өте ірі түйіршіктерді көрсетеді. Бұрыштан дөңгелектенген түйіршік пішіндері өрілген өзен жүйесіне тән төмен энергиялы шөгінді ортасын көрсетеді. LEICA 2500p жарық поляризациялық микроскопын пайдаланып жүргізілген петрографиялық талдау кварцты (50-60%), дала шпаты (15-16%) және тау жыныстарының сынықтарын (5%) негізгі құрамдас бөліктер ретінде, аз мөлшерде мусковит пен биотитпен анықтады. Бұл минералды құрам, шөгінді сипаттамаларымен қатар, шөгінді көзіне жақындығын көрсетеді, бұл өзен жүйесінің өрілген жүйесінің болуының қосымша дәлелдерін береді. Биологиялық тұрғыдан алғанда, шөгінді орта ерте биота үшін әртүрлі тіршілік ету орталарының дамуына ықпал еткен болуы мүмкін, әсіресе балшық тастар мен тақтатастардың ұсақ түйіршікті шөгінділерінде. Бұл шөгінді орталары микробтық тіршілік пен су организмдерінің ерте формалары үшін әлеуетті субстраттарды қамтамасыз етіп, осы ежелгі өзен жүйесіндегі тіршілік ету ортасының жалпы дамуына ықпал еткен болар еді. Бұл зерттеу қоршаған орта факторларының - шөгінділермен қамтамасыз етудің, су энергиясының және биологиялық әсерлердің Орта Сивалик тобының шөгінді архитектурасын да, тіршілік ету ортасының жағдайларын да қалыптастырудағы маңызды рөлін атап көрсетеді. Бұл өзара әрекеттесулерді түсіну өткен экожүйелерді және Оңтүстік Инд бассейнінде шөгінділер мен тіршілік ету ортасының қалыптасуын басқаратын биологиялық процестерді қалпына келтіру мүмкіндігімізді арттырады.

Түйін сөздер: Петрография, түйіршіктердің мөлшерінің таралуы, фациялардың жіктелуі, шөгінді ортасы, Солтүстік Лаки жотасының ортаңғы Савилик тобы, Оңтүстік Инд бассейні

Влияние факторов окружающей среды на осадконакопление, развитие местообитаний и их значение для палеобиологической реконструкции в Средней группе Сивалик, Южный бассейн Инда

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Аннотация. Средняя группа Сивалик, расположенная недалеко от города Сехван в северной части хребта Лаки Южного бассейна Инда, Синд, Пакистан, простирается на стратиграфическую мощность приблизительно 1055 метров в разрезе Кари Бути (KBS). В данном исследовании изучаются факторы окружающей среды, влияющие на осадконакопление, и их роль в развитии местообитаний в этом регионе. Литологический состав района включает в себя различные осадочные породы, в том числе песчаник, конгломерат, конгломератный песчаник, сланец, глину и аргиллит. Фациальный анализ выявил шесть основных фаций осадконакопления: конгломерат и конгломератный песчаник (GT), мелко- и крупнозернистый косослоистый песчаник (St), мелко- и крупнозернистый плоскослоистый песчаник (Sh), сланец (Fm), аргиллит (Mf) и глина (Cf), каждая из которых отражает различные экологические и биологические

условия во время осадконакопления. Анализ гранулометрического состава, основанный на данных ситового анализа семи репрезентативных образцов рыхлого песчаника, показывает смесь мелких и средних зерен с редкими очень крупными зернами. Субоугловая и субокруглая форма зерен указывает на низкоэнергетическую среду осадконакопления, характерную для разветвленной речной системы. Петрографический анализ, проведенный с использованием поляризационного микроскопа LEICA 2500p, выявил кварц (50-60%), полевой шпат (15-16%) и фрагменты горных пород (5%) в качестве основных компонентов, а также незначительное количество мусковита и биотита. Этот минеральный состав, наряду с характеристиками осадконакопления, указывает на близость к источнику осадочного материала, что является дополнительным доказательством наличия разветвленной речной системы. С биологической точки зрения, осадочная среда, вероятно, способствовала развитию различных местообитаний для ранней биоты, особенно в мелкозернистых отложениях аргиллитов и сланцев. Эти условия осадконакопления могли обеспечить потенциальные субстраты для микробной жизни и ранних форм водных организмов, способствуя общему развитию местообитаний в этой древней речной системе. Данное исследование подчеркивает значительную роль факторов окружающей среды - поступления осадочного материала, энергии воды и биологических факторов - в формировании как осадочной архитектуры, так и условий обитания Средней Сиваликской группы. Понимание этих взаимодействий расширяет наши возможности по реконструкции прошлых экосистем и биологических процессов, которые определяли осадконакопление и формирование местообитаний в южной части бассейна Инда.

Ключевые слова: Петрография, гранулометрический состав, категоризация фаций, условия осадконакопления, средняя группа Савилик северного хребта Лаки, южная часть бассейна Инда

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