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Efficient expression and purification of recombinant MUTYH protein in Escherichia coli

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Abstract. MUTYH is a DNA glycosylase involved in the excisional base repair (BER) process responsible for the recognition and removal of adenine improperly bonded to 8-oxoguanine. Pathogenic variants or impaired MUTYH function are associated with MUTYH-associated polyposis and an increased risk of colorectal cancer. Recombinant MUTYH production is necessary for biochemical and structural studies. In this study, we developed and optimized a protocol for the expression and purification of recombinant human MUTYH protein fused with the 6×His N-terminal label. Wild-type constructs were cloned into the pET-28c expression vector and expressed in Escherichia coli Rosetta (DE3), ArcticExpress(DE3), and SHuffle® T7 Express strains. Low-temperature induction and optimized IPTG concentrations improved protein solubility. Recombinant MUTYH was purified using $Ni2^{+}$-affinity chromatography followed by affinity chromatography with heparin. SDS-PAGE and Western blot analyses confirmed a high level of expression and effective isolation of the recombinant protein, with a significant portion isolated in a soluble form. The optimized workflow provides a reproducible method for obtaining purified MUTYH, suitable for subsequent biochemical and structural studies. In the future, we plan to evaluate its enzymatic activity.

Keywords: DNA repair, MUTYH, Base excision repair, oxidative DNA damage, recombinant protein expression, affinity chromatography, colorectal cancer.

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Introduction

Numerous endogenous and exogenous DNA-damaging agents, such as reactive oxygen species (ROS) produced during regular cellular metabolism, ultraviolet and ionizing radiation, chemical mutagens, and viral infections, are constantly present in the genome [1-4]. Numerous DNA lesions, including oxidative base modifications, point mutations, depurination, and single- and double-strand breaks, can be caused by these factors and endanger cellular homeostasis and genomic integrity [5]. Cells use highly conserved DNA repair pathways, such as base excision repair (BER), nucleotide excision repair, homologous recombination, and non-homologous end joining (NHEJ), to combat these insults [6].

Among these mechanisms, the BER pathway plays a central role in repairing oxidative DNA damage. The DNA glycosylase family is a crucial class of BER enzymes that starts repair by identifying and eliminating damaged or mispaired bases [7, 8]. The MUTYH gene encodes MUTYH (MutY Homolog), one of this family's essential glycosylases [9]. MUTYH specifically recognizes adenines misincorporated opposite 8-oxoguanine (8-oxoG), a highly mutagenic lesion arising from oxidative stress. MUTYH plays a crucial role in maintaining genomic stability by preventing G:C→T:A transversion mutations by eliminating the mispaired adenine [10, 11].

Structurally, MUTYH contains a conserved helix–hairpin–helix (HhH)-GTP motif, a hallmark of many DNA glycosylases, which mediates damaged base recognition and catalytic excision [5]. This domain forms a defined binding pocket that accommodates the aberrant base, ensuring precise cleavage while minimizing collateral DNA damage [12]. Furthermore, proliferating cell nuclear antigen (PCNA), replication protein A (RPA), and apurinic/apyrimidinic endonuclease 1 (APE1) are among the other parts of the BER machinery and the DNA replication complex with which the C-terminal region of MUTYH mediates interactions. These interactions enable coordination of repair processes, particularly at replication forks [7].

MUTYH also functions in close cooperation with other DNA glycosylases, such as OGG1, which removes 8-oxoG from G: C base pairs, and members of the NEIL (NEIL1-3) family, which recognize distinct classes of oxidized bases [13-16]. These enzymes work together to create an integrated BER network that guarantees precise lesion identification, effective transfer of repair intermediates, and prompt DNA repair completion [6, 7].

Particularly significant is the functional interaction between MUTYH and APE1. An abasic (AP) site is created after adenine excision by MUTYH and needs to be processed right away to prevent strand breaks or damage related to replication. APE1 efficiently cleaves these AP sites, allowing downstream repair steps to proceed and ensuring restoration of DNA integrity. This coordinated action enhances BER efficiency, reduces mutational burden, and preserves genome stability [15-19].

MUTYH-associated polyposis (MAP), an autosomal recessive cancer predisposition syndrome marked by multiple colorectal adenomas and a markedly increased risk of colorectal cancer, is directly associated with loss of MUTYH function or the presence of pathogenic variants. Aside from MAP, sporadic colorectal cancer, gastric cancer, and other cancers linked to oxidative stress and impaired BER have also been linked to altered MUTYH expression or activity [7, 18, 19].

MUTYH is highly relevant to biomedicine because of its crucial function in genome maintenance. The accumulation of mutations is caused by reduced glycosylase activity in cancer-associated variants of MUTYH, whereas functional MUTYH inhibits mutagenesis and tumor development. Therefore, to clarify its molecular mechanisms and develop treatment approaches, recombinant production and thorough biochemical characterization of MUTYH and its disease-associated variants are crucial [1, 3, 6].

Despite its significance, active recombinant MUTYH production is still technically difficult. Like many eukaryotic DNA repair enzymes, Escherichia coli-expressed MUTYH frequently exhibits poor solubility, forms inclusion bodies, or degrades proteolytically. These restrictions can result from cytotoxic effects linked to high-level expression, incorrect folding, or the need for particular redox conditions [3, 20]. The selection of appropriate host strains (such as Rosetta (DE3), Arctic Express (DE3), and SHuffle® T7 Express), modification of induction parameters (temperature, IPTG concentration, induction time), codon optimization, or the application of solubility-enhancing techniques are therefore essential steps in the optimization of recombinant expression [3].

To isolate a functionally active protein suitable for biochemical and structural analyses, the current study aimed to develop and refine an effective protocol for the recombinant expression and purification of human MUTYH using affinity chromatography.

Materials and research methods

Molecular cloning and plasmid construction

Gene-specific primers intended to introduce NdeI and BamHI restriction sites for directional cloning into the pET28c(+) expression vector were used to amplify the full-length human MUTYH coding sequence by PCR.

The primers that were utilized were Rv-MUTYH (32-mer, reverse): 5′-TATAGGGATCCTCGAAACTCGATCTCGAAGAG-3′ and Fw-MUTYH (30-mer forward): 5′-TATACATATGATGACACCGCTCGTCTCCCG-3′.

PCR was carried out in a total volume of 50 μL, containing 10× high-fidelity reaction buffer, between 5 and 50 ng of plasmid DNA, 125 ng of each primer, dNTP mix, and PfuUltra HF DNA polymerase. Thermal cycling is a way to change the DNA from a regular structure to a different structure, and then back again. First, the DNA is heated to 95°C for 30 seconds, then it is cooled down to 68°C for 1 minute. This is then repeated 25 to 30 times.

The product from the PCR was made pure with a PCR purification kit, cut with NdeI and BamHI, and joined to the cut pET28c(+) vector using T4 DNA ligase. The ligation mixture was then added to some bacteria called Escherichia coli DH5α cells. The transformed cells were then collected in a special liquid called SOC medium at 37 °C for 1 hour. After this, they were put onto a plate of LB agar containing 50 μg/mL kanamycin.

Individual colonies were selected and plasmid DNA was isolated using the GeneJET Plasmid Midi Prep Kit (New England Biolabs). We can be sure that the correct version of the MUTYH gene was put in and that no mistakes were made by checking it using restriction digestion and Sanger sequencing.

The transformed WT MUTYH plasmids, which had been checked using a process called ‘sequence verification’, were then introduced into E. coli Rosetta (DE3), Shuffle T7 Express and Arctic Express (DE3) strains. This was done to make a recombinant protein.

Preliminary protein expression and optimization

We tried out several prokaryotic expression systems to make a working version of the human MUTYH gene. The pET28c-MUTYH construct was introduced into E. coli strains Rosetta(DE3), Arctic Express(DE3), and Shuffle® T7 Express. In Rosetta (DE3) cells, we made the proteins using 0.5 mM IPTG, and then took samples at 2, 4, 6, and 16 hours after we had added the IPTG. The protein fractions were separated into soluble and insoluble parts, and their levels were assessed using a method called SDS-PAGE.

Arctic Express (DE3) cells, which co-produce cold-adapted chaperones, were put through their paces together to make proteins more soluble. We made the cells produce a certain substance by adding a chemical called IPTG, and then we put the cultures in a fridge at 15°C. We collected samples at the same times. Another way of doing this was to add 0.2% glucose and 3% ethanol to the liquid mixture. This is because these substances can stop the body from breaking down muscle and can increase protein production.

For the Shuffle® T7 Express, we tested IPTG concentrations of 0.1, 0.2, 0.3, and 0.5 mM. We also tested post-induction incubation times of 2, 4, 6, and 16 hours at 15°C. They then collected and analyzed the soluble and insoluble parts of the sample. This was done by SDS-PAGE to find the best conditions for identifying the soluble WT MUTYH protein. These experiments showed the best ways to grow and purify large quantities of protein.

Purification of recombinant WT proteins

We produced the soluble MUTYH in E. coli Shuffle® T7 Express cells, then harvested it after adding 0.2 mM IPTG at 15°C for four hours. Bacterial cells were harvested using a centrifuge and then put back into a liquid by adding a chemical called 'lysis buffer' which contains a number of things including 30 mM of Tris-HCl (pH 7.5), 1 M NaCl, 30 mM of β-mercaptoethanol, 5% (volume for volume) glycerol and a complete EDTA-free protease inhibitor (Roche). The cells were disrupted using a French press at 18,000 psi, and the lysates were clarified by spinning at 30,000×g for 1 h at 4 °C, to yield clear liquids containing soluble MUTYH.

For affinity purification, the mixture was adjusted to a final mix of 20 mM HEPES (pH 8.0), 500 mM NaCl, 30 mM imidazole, and 5% glycerol, and then loaded onto a HisTrap Chelating Ni^{2+}-Affinity column (GE Healthcare). The protein stuck tightly to the resin and was separated into clear lines when a specific imidazole solution was added (between 30 and 500 mM). Imidazole was removed straight away after elution to keep the protein stable.

To make the product even purer, the eluate was then passed through a heparin affinity chromatographic column. Before loading, the protein sample was made weaker by mixing it with low-salt buffer (30 mM HEPES, pH 7.6; 5% glycerol; 1 mM DTT; 1 mM EDTA) to reduce the NaCl concentration to approximately 100 mM. Proteins were extracted using a gradually increasing amount of sodium chloride in the same buffer system.

We then looked at the samples using something called SDS-PAGE. The ones that contained the most purified MUTYH were put together. The purified protein was stored at -80°C in 50% glycerol and remained stable under these conditions for later biochemical and functional analyses.

Protein analysis using SDS-PAGE

Protein samples were mixed with a 5-fold SDS-PAGE loading buffer (125 mM Tris-HCl, pH 6.8; 10% β-mercaptoethanol; 4% SDS; 0.02% bromophenol blue; 20% glycerol) and denatured at 80°C for 10 minutes. The samples were loaded onto a 10% polyacrylamide gel for electrophoresis. Electrophoretic analysis was performed in two stages: first, the proteins were concentrated in a gel and run at 100 V for 20 minutes, and then separated in a soluble gel at 200 V for 40-45 minutes. Tris-Glycine-SDS buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH ~8.3) was used as the working buffer. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (0.1% Coomassie R-250, 40% ethanol, 10% acetic acid) for 30 minutes. Destaining was performed either with running water or with a solution containing 40% ethanol and 10% acetic acid, repeated 4-5 times for 20 minutes each, until the protein bands became clearly visible.

Results

Construction of a Recombinant DNA Expression Vector for MUTYH.

In this study, we created a special DNA design that allowed us to express the human MUTYH gene in bacteria. We cloned a synthetic cDNA sequence corresponding to the full-length MUTYH gene into the multiple cloning site of the pET-28c(+) expression vector (see Figure 1). This vector was chosen because it has a strong T7 RNA polymerase-dependent promoter and an N-terminal 6×His tag. These things allow the vector to be expressed at a high level in Escherichia coli. They also make it easy to purify the recombinant protein using an affinity chromatography process.

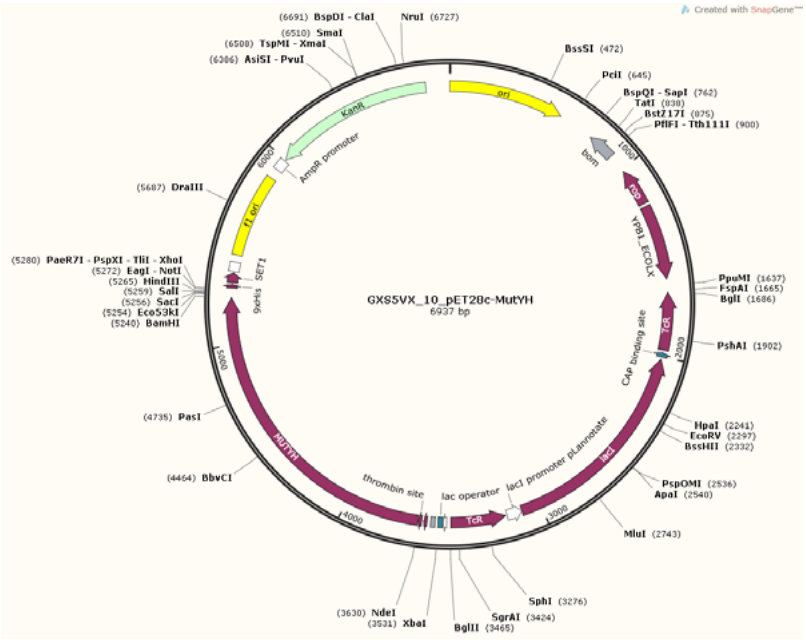

Figure 1. Map of the recombinant pET-28c/MUTYH expression vector.

First, the MUTYH coding fragment and the pET-28c(+) vector were cut into smaller pieces with the restriction endonucleases NdeI and BamHI. Next, the fragments were joined together using T4 DNA ligase. The resulting construct contained the MUTYH insert in the correct position, downstream of the T7 promoter and fused to an N-terminal His tag.

All the plasmids that were made were designed and looked at using software called Snap-Gene. During vector design, compatibility, reading frame integrity, and restriction site positioning were carefully checked.

The resulting pET-28c/MUTYH plasmid was then added to E. coli DH5α cells, which had been treated with heat, to make them ready for the next step in the process. The transformants were selected on LB agar plates with added kanamycin (50 μg/mL). The transformation was a success, as shown by the colonies growing well in the right conditions.

The DNA was taken from certain groups of bacteria and made into a copy (a «copy», or «template», of the original DNA) using something called a «PCR reaction» (a special process that makes lots more copies of the DNA). We used agarose gel electrophoresis of the PCR products to reveal the DNA fragment (Figure 2). This revealed that the expected DNA size for the MUTYH cDNA was 1,621 bp.

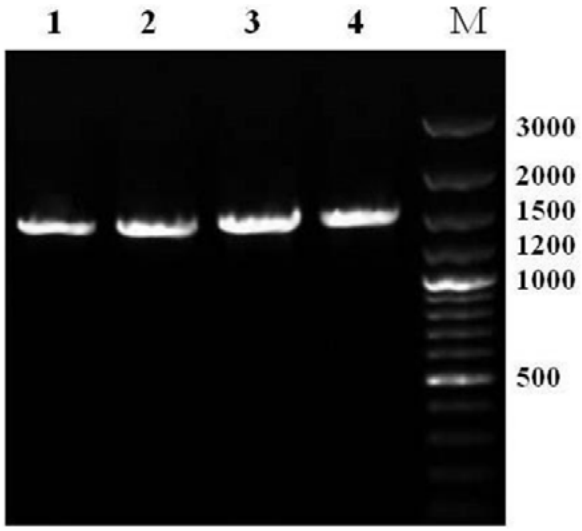

Figure 2. Agarose gel electrophoresis (1%) of PCR products confirming the presence of the MUTYH insert in recombinant pET-28c/MUTYH plasmids. M – 1 kb DNA ladder; lanes 1-4, PCR products (~1621 bp) amplified from selected clones.

Additional verification was performed by restriction digestion with NdeI and BamHI, yielding two fragments of approximately 5,367 bp (vector backbone) and 1,621 bp (MUTYH insert). This further confirmed the integrity of the recombinant construct.

Preliminary expression analysis.

To achieve functional expression of the human MUTYH protein, several prokaryotic expression systems were comparatively evaluated. At the initial stage, the recombinant pET-28c/MUTYH construct was transformed into Escherichia coli Rosetta(DE3) (Novagen) and Arctic-Express(DE3) (Agilent Technologies) strains, and protein expression was induced with IPTG (Isopropyl β-D-1-thiogalactopyranoside, Thermo Fisher Scientific).

In E. coli Rosetta(DE3), protein expression was induced with 0.5 mM IPTG, and cells were incubated for 2, 4, 6, and 16 hours. SDS-PAGE analysis demonstrated that, under all tested conditions, the recombinant MUTYH protein accumulated exclusively in the insoluble fraction as inclusion bodies (Figure 3). This result indicates improper protein folding and suggests limited biological activity of the expressed protein in this host strain.

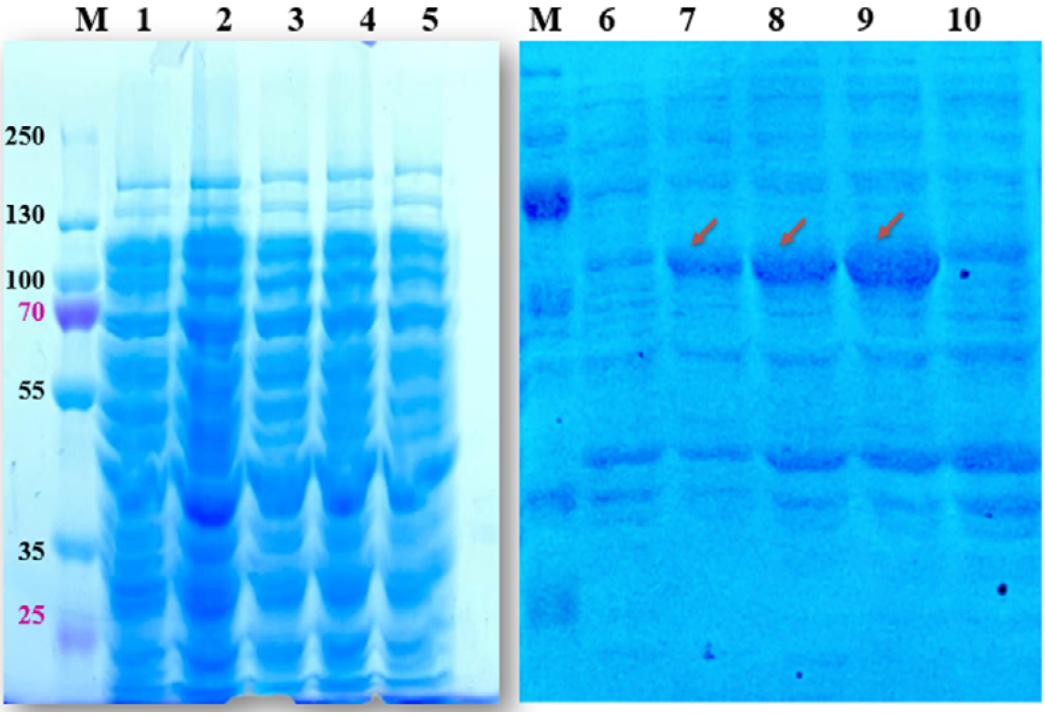

Figure 3. SDS-PAGE analysis of recombinant MUTYH expression in E.coli Rosetta (DE3) following IPTG induction for 2, 4, 6, and 16 hours. M – protein marker, lanes 1-5 soluble fractions; lanes 6-10 insoluble fractions corresponding to the indicated induction times.

Given the tendency of ArcticExpress(DE3) to improve the solubility of recombinant proteins at low temperatures, this strain was evaluated as an alternative host. Protein expression was induced at 15°C using 0.5 mM IPTG, with incubation times of 2, 4, 6, and 16 hours. In addition, an auto-induction strategy was tested by supplementing the growth medium with 0.2% glucose (Sigma-Aldrich) and 3% ethanol (Merck) to alleviate catabolite repression and enhance membrane permeability. However, SDS-PAGE analysis revealed that, similar to Rosetta(DE3), the majority of MUTYH protein in ArcticExpress(DE3) accumulated in the insoluble fraction, and no substantial increase in soluble protein was observed (Figure 4).

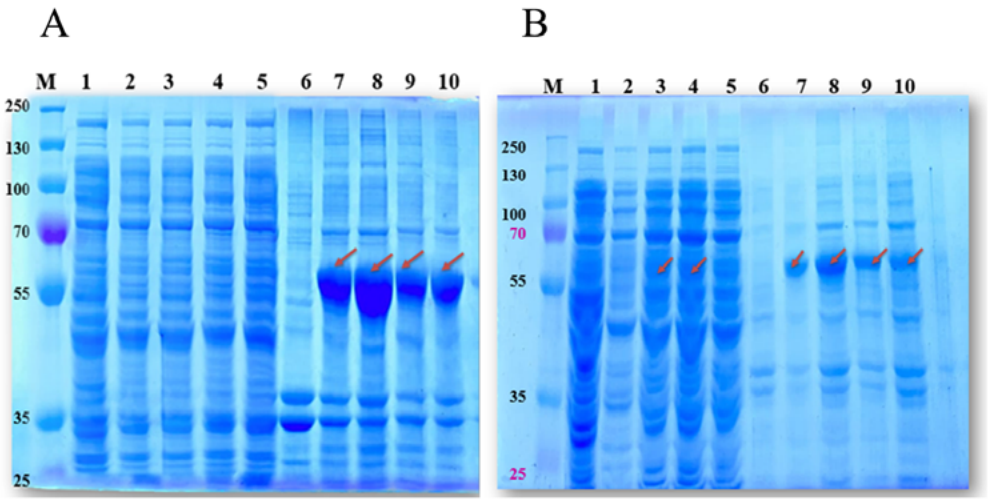

Figure 4. SDS-PAGE analysis of recombinant MUTYH expression in E.coli ArticExpress (DE3). (a) Induction with 0.5 mM IPTG at 15°C for 2, 4, 6, and 16 hours. (b) Auto-induction conditions with supplementation of glucose and ethanol.

Overall, these results demonstrate that, in both Rosetta(DE3) and ArcticExpress(DE3) strains, recombinant MUTYH predominantly formed insoluble inclusion bodies, indicating inefficient folding and limited functional expression.

To overcome this limitation, E. coli SHuffle® T7 Express was employed. This strain carries deletions in the trxB and gor genes, enabling the formation of disulfide bonds within the cytoplasm. Additionally, SHuffle cells overexpress the disulfide bond isomerase DsbC, which facilitates correction of mispaired disulfide bonds and promotes proper protein folding. Since the MUTYH protein contains two cysteine residues, the formation of correct disulfide bridges is critical for its functional conformation.

In SHuffle® T7 Express cells, recombinant MUTYH was predominantly detected in the soluble fraction, indicating successful folding and functional expression. To optimise expression conditions, IPTG concentrations of 0.1, 0.2, 0.3, and 0.5 mM were systematically evaluated. SDS-PAGE analysis revealed that induction with 0.2 mM IPTG resulted in the highest yield of soluble recombinant MUTYH protein.

Subsequently, the effect of induction time was examined using 0.2 mM IPTG, with incubation periods of 2, 4, 6, and 14-16 hours. Optimal expression was observed after 4 hours of induction, at which point the majority of the recombinant MUTYH protein was present in the soluble fraction (Figure 5). A distinct protein band corresponding to approximately 60 kDa was clearly visible, consistent with the expected molecular weight of His-tagged MUTYH.

The presence and correct synthesis of the recombinant protein were further confirmed by Western blot analysis. Proteins were transferred onto a nitrocellulose membrane and probed with anti-His monoclonal antibodies. A specific immunoreactive band at approximately 60 kDa was detected exclusively in induced samples, confirming the expression of 6×His-tagged recombinant MUTYH protein.

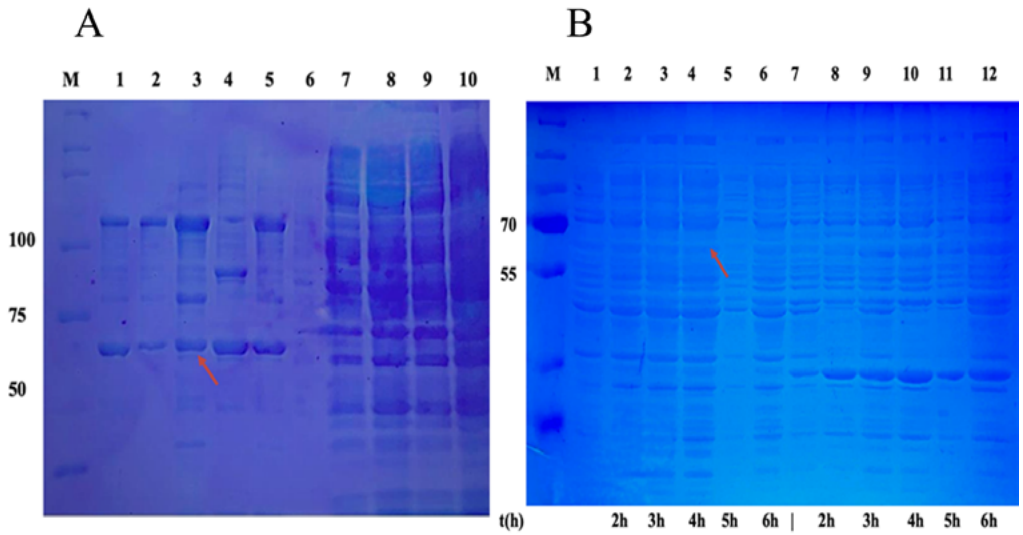

Figure 5. SDS-PAGE analysis of recombinant MUTYH expression in E. coli SHuffle® T7 Express. (a) Effect of IPTG concentration (0.1-0.5 mM) on recombinant MUTYH expression, after 15–16 hours of induction. (b) Time-course analysis of recombinant MUTYH expression following induction with 0.2 mM IPTG.

Purification of recombinant MUTYH protein

Following optimisation of expression conditions, soluble WT MUTYH was successfully purified from E. coli SHuffle® T7 Express cells using a two-step chromatographic strategy. After cell lysis and clarification, the soluble fraction containing recombinant MUTYH was subjected to Ni^{2+}-affinity chromatography. MUTYH bound efficiently to the HisTrap column and eluted as a well-defined, symmetrical peak upon application of an imidazole gradient, indicating homogeneous binding behaviour and minimal nonspecific interactions.

Analysis of the eluted fractions by SDS-PAGE revealed a dominant protein band with an apparent molecular mass of approximately 60 kDa, consistent with the predicted size of His-tagged WT MUTYH (Figure 6A). Minor contaminating proteins were largely removed during this initial affinity purification step.

To further improve protein purity, the pooled Ni^{2+}-eluted fractions were subjected to heparin affinity chromatography. MUTYH bound strongly to the heparin matrix and eluted at elevated salt concentrations during the NaCl gradient, consistent with its DNA-binding properties. SDS-PAGE analysis of the heparin-eluted fractions demonstrated a substantial increase in purity, with WT MUTYH appearing as a single, predominant band and only trace levels of residual contaminants (Figure 6 B).

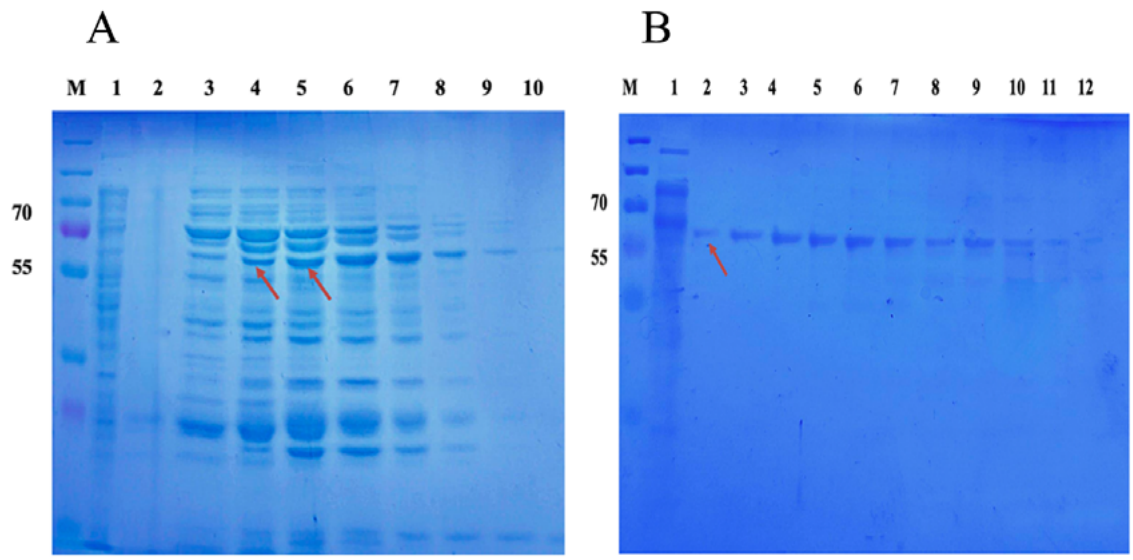

Figure 6: Purification of recombinant WT MUTYH expressed in E. coli SHuffle® T7 Express.(a) SDS-PAGE analysis of Ni^{2+}-affinity (HisTrap) chromatography. (b) SDS-PAGE analysis of heparin affinity chromatography. M – protein molecular weight marker; lane 1 – non-induced cell lysate; lanes 2-10 – eluted fractions containing recombinant WT MUTYH.

The final purification yielded WT MUTYH with high homogeneity and good stability. The purified protein remained soluble and structurally intact upon storage at -80 °C in the presence of glycerol, enabling its use in subsequent biochemical, enzymatic, and functional assays.

Discussion

In this study, we established an optimized workflow for the expression and purification of recombinant human MUTYH in Escherichia coli. The choice of host strain proved to be critical for obtaining soluble protein. Both Rosetta(DE3) and ArcticExpress(DE3) strains produced predominantly insoluble MUTYH, likely due to misfolding and inclusion body formation, even when using low-temperature induction and autoinduction strategies. These results highlight the challenges associated with expressing eukaryotic DNA repair proteins in standard prokaryotic hosts.

SHuffle® T7 Express cells proved to be the most effective host for producing soluble MUTYH. The ability of this strain to form disulfide bonds in the cytoplasm, combined with overexpression of the disulfide isomerase DsbC, likely contributed to the proper folding of MUTYH, which contains cysteine residues important for structural stability. Optimization of IPTG concentration and induction time further increased soluble protein yield, with 0.2 mM IPTG and 4 hours of induction resulting in the highest soluble protein.

A two-step purification strategy combining Ni^{2+}-affinity and heparin chromatography yielded highly pure, homogeneous, and structurally intact MUTYH. Ni^{2+}-affinity chromatography effectively captured the His-tagged protein, while heparin chromatography removed residual impurities and exploited the DNA-binding properties of MUTYH to increase purity. The resulting protein remained soluble and structurally stable during storage, providing a reliable preparation for subsequent applications.

Recombinant MUTYH is suitable for biochemical and structural studies. The enzymatic activity of recombinant MUTYH has not yet been tested; future studies will focus on evaluating its DNA glycosylase activity using classical substrates containing A:8-oxoG, including fluorescently labeled 24AACy5/24ToG duplexes.

This work provides a reproducible and efficient method for producing soluble, structurally intact MUTYH, addressing common challenges in bacterial expression of eukaryotic DNA repair proteins. Given the key role of MUTYH in base excision repair and the prevention of mutagenic G:C→T:A transversions, this protocol provides a valuable platform for studying MUTYH function, elucidating mechanisms of genome maintenance, and investigating disease-associated variants relevant to colorectal cancer and other malignancies.

Conclusion

A special plan was made for making and cleaning up a special protein called MUTYH in a type of bacteria called Escherichia coli. SHuffle® T7 Express cells were the best for making soluble proteins. The most was made when they were triggered with 0.2 mM IPTG at 15°C for 4 hours. A two-step purification strategy combining Ni2+-affinity and heparin chromatography yielded highly pure, homogeneous, and structurally intact MUTYH.

The resulting protein is stable, soluble, and suitable for further biochemical and structural studies. This preparation can be used in future studies to evaluate how well enzymes work and how they interact with DNA, including the study of disease-related variants. This workflow provides a strong foundation for advancing studies into how MUTYH works and understanding the molecular mechanisms of MUTYH in genome maintenance and disease.

Author Contributions

U.B.S., A.A.K., A.A.A., U.B.S., K.O.S., M.K.S., and S.M.T. – conceptualization; U.B.S., A.A.K. and A.A.A. – scientific design development; U.B.S., A.A.A., 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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Эффективная экспрессия и очистка рекомбинантного белка MUTYH в Escherichia coli

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Аннотация. MUTYH – это ДНК-гликозилаза, участвующая в процессе эксцизионной репарации оснований (BER), ответственная за распознавание и удаление аденина, неправильно соединенного с 8-оксогуанином. Патогенные варианты или нарушение функции MUTYH связаны с полипозом, ассоциированным с MUTYH, и повышенным риском развития колоректального рака. Рекомбинантная продукция MUTYH необходима для биохимических и структурных исследований.В этом исследовании мы разработали и оптимизировали протокол экспрессии и очистки рекомбинантного белка MUTYH человека, слитого с N-концевой меткой 6×His. Конструкции дикого типа были клонированы в экспрессирующий вектор pET-28c и экспрессированы в штаммах Escherichia coli Rosetta (DE3), ArcticExpress(DE3) и SHuffle® T7 Express. Низкотемпературная индукция и оптимизированные концентрации IPTG улучшили растворимость белка. Рекомбинантный MUTYH очищали с помощью $Ni2^{+}$ - аффинной хроматографии с последующей аффинной хроматографией с гепарином. Анализы SDS-PAGE и Вестерн-блоттинга подтвердили высокий уровень экспрессии и эффективное выделение рекомбинантного белка, при этом значительная часть была выделена в растворимой форме. Оптимизированный рабочий процесс обеспечивает воспроизводимый метод получения очищенного MUTYH, пригодного для последующих биохимических и структурных исследований. В будущем мы планируем оценить его ферментативную активность.

Ключевые слова: репарация ДНК, MUTYH, эксцизионная репарация оснований (BER), окислительное повреждение ДНК, рекомбинантная экспрессия белков, аффинная хроматография, колоректальный рак.

Escherichia coli жүйесінде рекомбинантты MUTYH белогының тиімді экспрессиясы мен тазартылуы

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Аңдатпа. MUTYH – негіздердің эксцизиялық репарациясы (BER) жолына қатысатын ДНҚ гликозилаза болып табылады және 8 оксогуанинмен қате жұптасқан аденинді тану мен алып тастауға жауап береді. MUTYH функциясының бұзылуы немесе патогенді нұсқаларының болуы MUTYH-пен ассоциацияланған полипозбен және колоректалды қатерлі ісіктің даму қаупінің жоғарылауымен байланысты. MUTYH ақуызының рекомбинантты түрде алынуы оны биохимиялық және құрылымдық тұрғыдан зерттеу үшін аса маңызды. Бұл зерттеуде N-соңында 6xHis таңбасы бар адамның рекомбинантты MUTYH белогының экспрессиясы мен тазартылуына арналған протокол әзірленіп, оңтайландырылды. Жаабйы типті MUTYH конструкциялары pET28c экспрессиялық векторына клондалып, Escherichia coli Rosetta (DE3), ArticExpress(DE3) және SHuffle® T7 Express штамдарында экспрессияланды. Төмен температура жағдайында индукция және IPTG концентрацияларын оңтайландыру белок ерігіштігін арттыруға мүмкіндік берді. Рекомбинантты MUTYH $Ni2^{+}$ - аффинді хроматография арқылы тазартылды. SDS-PAGE және Вестерн-блот талдаулары рекомбинантт белоктың жоғары деңгейде экспрессияланғанын және тиімді тазартылғанын, сондай-ақ оның едәуір бөлігі ерігіш күйде алынғанын көрсетті. Оңтайландырылған жұмыс протоколы биохимиялық және құрылымдық зерттеулерге жарамды тазартылған MUTYH белогын алудың қайталынымды әдісін қамтамасыз етеді. Болашақта оның ферментативтік белсенділігін бағалау жоспарлануда.

Түйін сөздер: ДНҚ репарациясы, MUTYH, негіздердің эксцизиялық репарациясы (BER), ДНҚ-ның тотығу арқылы зақымдалуы, рекомбинантты белок экспрессиясы, аффинді хроматография, колоректалды қатерлі ісік.

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