DOI: 10.11134/btp.2.2026.9

UDC 577.15; 577.2 Original Article

FUNCTIONAL ANALYSIS OF AMINO ACID RESIDUES Y61, F72 AND H180 IN URACIL-DNA GLYCOSYLASE FROM *STAPHYLOCOCCUS AUREUS*

Koreneva A.S.[1], Abeldenov S.K.[2], Turgimbayeva A.M.[2]*

[1]NCJSC «S. Seifullin Kazakh Agrotechnical Research University», 010000, Republic of Kazakhstan, Astana, Zhenis avenue, 62
[2]LLP «National center for biotechnology», 010000, Republic of Kazakhstan, Astana, Korgalzhyn highway, 13/5
**Corresponding author: turgimbayeva@biocenter.kz*

ABSTRACT

In this study, bioinformatic and *in silico* structural analysis of uracil-DNA glycosylase from *Staphylococcus aureus* (SaUDG) was performed, leading to identification the amino acid residues Y61, F72 and H180 as potentially important for the catalytic activity of the enzyme. Based on the obtained data, SaUDG Y61S, F72N and H180Q mutant variants were constructed. The results of the enzymatic analysis revealed the absence of detectable catalytic activity in SaUDG F72N and H180Q mutants, whereas the Y61S substitution led to a significant reduction in activity.

These data indicate the key role of the F72 and H180 amino acid residues in the catalytic mechanism of SaUDG and highlight the functional importance of the Y61 residue for maintaining enzymatic activity. Overall, these results provide further insight into the structure and mechanism of action of SaUDG and are consistent with previous data on the conserved organization of the active site in first-family UDG enzymes.

Keywords: *Staphylococcus aureus*, DNA repair, base excision repair, uracil-DNA glycosylase, site-directed mutagenesis, mutant variant.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen that colonizes the anterior nasal passages, skin and mucous membranes in approximately 30% of the human population [1]. Through breaches in host barriers or impaired immune defenses, *S. aureus* can cause a wide range of infectious diseases from mild skin lesions (furuncles) to more severe infections (pneumonia, endocarditis, osteomyelitis, septic arthritis, and bacteremia) [2]. According to epidemiological studies, *S. aureus* bacteremia has a mortality rate of 15-30% and accounts for approximately 300,000 deaths annually worldwide [3]. Treatment of infections caused by the pathogen *S. aureus* is complicated by the spread of antibiotic-resistant strains and their continued evolution. In 2017, the World Health Organization first published a list of bacterial pathogens that pose a serious threat to human health due to their resistance to antimicrobial agents. This list comprises 12 families of bacteria, and *S. aureus* was included in the high-priority category [4].

Infections caused by methicillin-resistant *S. aureus* (MRSA) are of particular concern. Published data indicate that the proportion of MRSA among *S. aureus* isolates is about 25%, reaching 50% or more in certain regions [5]. MRSA is characterized by a high incidence of severe infections and limited therapeutic options. According to the data of the Centers for Disease Control and Prevention (USA), more than 70,000 severe cases of MRSA infections are registered annually, accompanied by more than 9,000 deaths [6]. A similar trend has been observed at the regional level: following the COVID-19 pandemic in Kazakhstan, the proportion of MRSA increased from 11.8% to 35% [7]. Antibiotic resistance in *S. aureus* remains a major global public health concern.

In recent years, DNA repair systems have attracted attention as potential therapeutic targets; inhibition of individual DNA repair enzymes reduces the ability of *S. aureus* to develop resistance and increases its susceptibility to antibiotics [8, 9]. It is well established that base excision repair (BER) plays a key role in the survival of pathogenic bacteria under genotoxic stress induced by drug exposure and immune response factors. DNA damage arising from oxidation, deamination and alkylation of nitrogenous bases is repaired by BER enzymes [10].

BER is initiated by the recognition of the damaged base by DNA glycosylases, which cleave the N-glycosidic bond, forming an apurinic/apyrimidinic (AP) site. As a result of the action of monofunctional DNA glycosylases, AP sites are generated and subsequently processed by AP endonuclease and dRPase to prepare DNA for synthesis. Bifunctional glycosylases additionally cleave the AP site, forming an aldehyde residue that is further processed by AP endonuclease. Next, DNA polymerase inserts the missing nucleotides, and DNA ligase seals the break, restoring the original sequence. In bacteria, BER proceeds via the short-patch pathway (replacement of a single nucleotide) or the long-patch pathway (synthesis of several nucleotides with removal of a fragment of the parental strand) [11, 12].

Among DNA glycosylases, uracil-DNA glycosylases (UDG, UNG) are particularly important enzymes that remove uracil from DNA arising either from spontaneous cytosine deamination or from incorporation of dUTP during replication. In this case, U:A pairs are not premutagenic, whereas U:G pairs, formed after cytosine deamination, can lead to C→T transition mutations in the next replication cycle, posing a significant threat to genome integrity [13]. Experimental studies have demonstrated that UDG is essential for genomic stability and bacterial viability: reduction of UDG activity in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis* is accompanied by a 7-8-fold increase in the mutator phenotype, growth suppression and decreased survival in a macrophage infection model [14, 15].

The body of available data indicates the important role of uracil-DNA glycosylases in maintaining genetic stability and the adaptive potential of microorganisms and points to their possible use as targets for the development of antibacterial

DOI: 10.11134/btp.2.2026.9

agents. At the same time, despite the considerable amount of research on DNA glycosylases, uracil-DNA glycosylase from *S. aureus* (SaUDG) remains insufficiently characterized, which limits the understanding of its structural and functional features. In this regard, the aim of the present study is to analyze the role of individual amino acid residues in the activity of SaUDG.

MATERIALS AND METHODS

2.1. Strains, vectors and media

E. coli DH5α strain was used for plasmid cloning, and *E. coli* Rosetta 2 (DE3) for recombinant protein expression. The plasmid construct pET11a/SaUDG from the laboratory collection served as the template for site-directed mutagenesis.

Low-salt Luria-Bertani broth (1% tryptone, 0.5% NaCl, 0.5% yeast extract) was used for cultivation of *E. coli* strains. SOC medium (2% tryptone, 0.05% NaCl, 0.5% yeast extract, 2.5 mM KCl, 20 mM $MgSO_4$, 20 mM glucose, pH 7.5) was used for incubation of transformed cells. The concentration of ampicillin in the media was 100 μg/mL.

2.2. Oligonucleotides

The following oligonucleotides were used for site-directed mutagenesis of SaUDG:

SaUDG Y61S fw	5’-TTAGGACAAGACCCGTCT-CATGGTCCAAACCAA-3’
SaUDG Y61S rv	5’-TTGGTTTGGACCATGAGAC-GGGTCTTGTCCTAA-3’
SaUDG F72N fw	5’-GCACATGGATTAGCAAAT-TCAGTGCAACCTAAC-3’
SaUDG F72N rv	5’-GTTAGGTTGCACT-GAATTTGCTAATCCATGTGC-3’
SaUDG H180Q fw	5’-ATTATAAAATCAGTGCAG-CCTAGTCCACTGTCT-3’
SaUDG H180Q rv	5’-AGACAGTGGACTAGGCTG-CACTGATTTTATAAT-3’

Codons substituting the original codons in the forward primers and the complementary triplets in the reverse primers are bold.

The following oligonucleotides were used for Sanger sequencing:

T7 fw	5’-TAATACGACTCACTATAGGG-3’
T7 rv	5’-GCTAGTTATTGCTCAGCGG-3’

To determine repair activity, 22-mer oligonucleotides (Eurogentec, Belgium) were used: 5’-TAMRA-d(CACTTCG-GAUTGTGACTGATCC)-3’ with a fluorescent label at the 5’-end and its complementary strand 5’-GGATCAGTCA-CAGTCCGAAGTG-3’, containing guanine complementary to uracil. To generate the double-stranded substrate, oligonucleotides were incubated in a buffer containing 20 mM Tris-HCl, pH 8.0, 50 mM KCl, at 65°C for 5 minutes, followed by cooling to room temperature at a rate of 1°C/min. As a result, the U•G DNA duplex was obtained.

2.3. Transformation by heat-shock

100 ng of plasmid DNA were added to 50 μL of *E. coli* DH5α cell suspension and incubated on ice for 30 minutes. After incubation, the cells were heat-shocked (+42°C, 45 seconds) in a water bath and then cooled on ice for 2 minutes. Subsequently, 200 μL of SOC medium were added, and the culture was incubated in a thermostatic shaker at +37°C with shaking at 180 rpm for 1 hour. The culture was then plated on solid nutrient medium containing the appropriate antibiotic at working concentration and incubated at +37°C for 16 hours.

2.4. Transformation by electroporation

Plasmid DNA (100 ng/μL) was added to 50 μL of electrocompetent *E. coli* Rosetta 2 (DE3) cells and transformed using a MicroPulser electroporator (Bio-Rad, USA) in electroporation cuvettes with a 2 mm gap (Bio-Rad, USA). After electroporation, 950 μL of SOC medium was added to the transformed cells, which were then incubated at +37°C, 180 rpm for 1 hour. The culture was subsequently plated on solid nutrient medium with the appropriate antibiotic and incubated at +37°C for 16 hours.

2.5. Site-directed mutagenesis of SaUDG

The reaction mixture for site-directed mutagenesis: 1× HF buffer, 200 μM of each dNTP, 0.2 μM forward primer, 0.2 μM reverse primer, 40 ng pET11a/SaUDG and 2 U of Phusion DNA polymerase (Thermo Scientific, Lithuania). Amplification program: 98°C – 30 s; 25 cycles: 98°C – 10 s, 70°C – 45 s, 72°C – 5 min; 72°C – 5 min. PCR products were analyzed by electrophoresis in a 1% agarose gel. The remaining volume was treated with 10 U of DpnI endonuclease (New England Biolabs, USA) at +37°C for 1 hour.

Then, the reaction mixture was used to transform chemically competent DH5α cells by heat-shock. Transformed cells were plated on LB agar containing the appropriate antibiotic and incubated at +37°C overnight. Single colonies were inoculated into 5 mL of LB broth with antibiotic and grown for +37°C with shaking at 180 rpm for 16 hours. Plasmid DNA was subsequently isolated from the resulting culture using the Monarch Plasmid Miniprep Kit (New England Biolabs, USA) according to the manufacturer’s instructions.

2.6. Sequencing

The presence of the expected mutations was confirmed by Sanger sequencing. The reaction mixture for : 150 ng of plasmid DNA, 1 μM forward/reverse primer, 1× Sequence Buffer, 1 μl BigDye® Terminator v3.1 ready-to-use reaction mixture. Amplification program: 96°C – 1 min; 25 cycles: 96°C – 10 s, 55°C – 5 s, 60°C – 4 min. After amplification, the products were purified by acetate-alcohol precipitation and analyzed on an ABI 3730xl automated sequencer (Applied Biosystems, USA). Chromatograms were analyzed by alignment to the reference sequence using Vector NTI Advance™ v11.0 software (Thermo Fisher Scientific, USA).

2.7. Expression and chromatographic purification of recombinant proteins

For recombinant protein expression, *E. coli* Rosetta 2 (DE3) cells were transformed with plasmids carrying SaUDG mutant variants obtained by site-directed mutagenesis, as well as wild-type SaUDG. When the optical density reached OD_{600} = 0.6, bacterial cultures were induced with 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) and incubated at +22°C, 90 rpm for 16 hours. Cells were harvested by centrifugation at 6,000×g, +4°C for 7 minutes and resuspended in buffer containing 20 mM Tris-HCl, pH 8.0, 50 mM NaCl.

DOI: 10.11134/btp.2.2026.9

Lysis was performed using lysozyme (3 mg/mL, 20 min, +22 - +25°C) in the presence of 1× protease inhibitor cocktail (Roche, Switzerland) and 0.1 mM phenylmethylsulfonyl fluoride, followed by additional ultrasonic disruption on ice (pulsed mode, 40% amplitude). The lysate was centrifuged at 40,000×g, +4°C for 1 hour to isolate the soluble protein fraction.

Recombinant protein purification was performed in two stages. First, affinity chromatography was carried out on a HiTrap Heparin HP 1 mL column (Cytiva, Sweden) with a NaCl gradient (50-1000 mM) using the ÄKTA Go™ system (Cytiva, Sweden). Protein-containing fractions were pooled and subjected to ion-exchange chromatography on a HiTrap Q HP 1 mL column (Cytiva, Sweden) using a similar NaCl gradient. The purity of the target protein was assessed by electrophoresis in 12% sodium dodecyl sulfate-polyacrylamide gel.

2.8. Determination of DNA repair activity

Repair activity was measured in a 20 µL reaction mixture containing 20 nM U•G DNA duplex, 20 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.5 mM EDTA (pH 8.0), 1 mM DTT, 0.1 mg/mL BSA and the enzyme at the specified concentration (0.25 nM, 0.5 nM, 1 nM for SaUDG WT; 1 nM, 2 nM, 5 nM for SaUDG mutant variants). The reaction mixture was incubated at +37°C for 5 minutes. The reaction was stopped by adding 1 M NaOH followed by incubation at +95°C for 5 minutes, after which 18 mM EDTA (pH 8.0) and 1 M HCl were added. Reaction products were purified on hand-made Sephadex G-25 (Pharmacia, Sweden) columns under denaturing conditions in the presence of 7.5 M urea.

The reaction products were separated on a denaturing 20% polyacrylamide gel containing 7.5 M urea. Reaction products were visualized using the ChemiDoc MP Imaging System (Bio-Rad, USA).

RESULTS AND DISCUSSION

3.1. Selection of amino acid targets for site-directed mutagenesis of SaUDG

The uracil-DNA glycosylase superfamily is classified into six families depending on substrate specificity. Family I (UNG) removes uracil from both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) with high efficiency and is the most extensively studied representative of this superfamily [16]. Family II (MUG/TDG) is specific for U:G and T:G mismatches; the enzymes recognize guanine in the complementary strand and, as a result, are inactive toward ssDNA [17]. Family III (SMUG1) removes uracil in U:G and U:A base pairs, as well as 5-hydroxymethyluracil from both ssDNA and dsDNA [18]. UDG families IV-VI are found in thermophilic and hyperthermophilic bacteria and archaea and are characterized by the presence of a specific Fe-S cluster [19]. Enzymes of these families are predominantly active toward mispaired U:G and T:G. Enzymes of families IV and VI can remove uracil from both ssDNA and dsDNA, whereas representatives of family V generally do not exhibit activity toward single-stranded substrates [20], [21].

Family I is the most extensively studied in the UDG superfamily, found in a variety of organisms – from prokaryotes to higher eukaryotes, as well as in several DNA-containing viruses [22]. The high degree of evolutionary conservation of UDG is reflected in the preservation of their spatial organization: the core of the protein structure consists of a four-stranded parallel β-sheet flanked by two pairs of α-helices [23]. Specific recognition and catalytic removal of uracil from DNA are provided by five key structural and functional motifs of *E. coli* UNG (EcUNG): the catalytic water-activating loop (62-GQDPYH-67 EcUNG), the proline-rich loop (84-AIPPS-88 EcUNG), the uracil-binding motif (120-LLLN-123 EcUNG), the glycine-serine loop (165-GS-166 EcUNG) and the leucine intercalation loop (187-HPSPLS-192 EcUNG). Individual amino acid residues within these motifs form the uracil-binding pocket, the dimensions of which restrict access of larger purine bases, thereby ensuring the high specificity of the enzyme – Q63, D64, Y66, F77, S88, N123, H187 in EcUNG [24].

Comparative analysis of the amino acid sequences of uracil-DNA glycosylase from *S. aureus* (GenBank: KII22023.1), *E. coli* (GenBank: CAD6006455.1), human (GenBank: CAA70211.1), and herpes simplex virus type 1 (GenBank: CAA32338.1) suggests that SaUDG contains regions corresponding to known structural motifs: the catalytic water-activating loop (57-GQDPYH-62 SaUDG), the pro-rich loop (79-KFPPS-83 SaUDG), the uracil-binding motif (113-LLLN-116 SaUDG), the Gly-Ser loop (158-GK-159 SaUDG) and the leucine intercalation loop (180-HPSPLS-185 SaUDG) (Fig. 1A). Based on the alignment, it is proposed that the uracil-binding pocket in SaUDG is formed by residues Q58, D59, Y61, F72, S83, N116 and H180 (Fig. 2B).

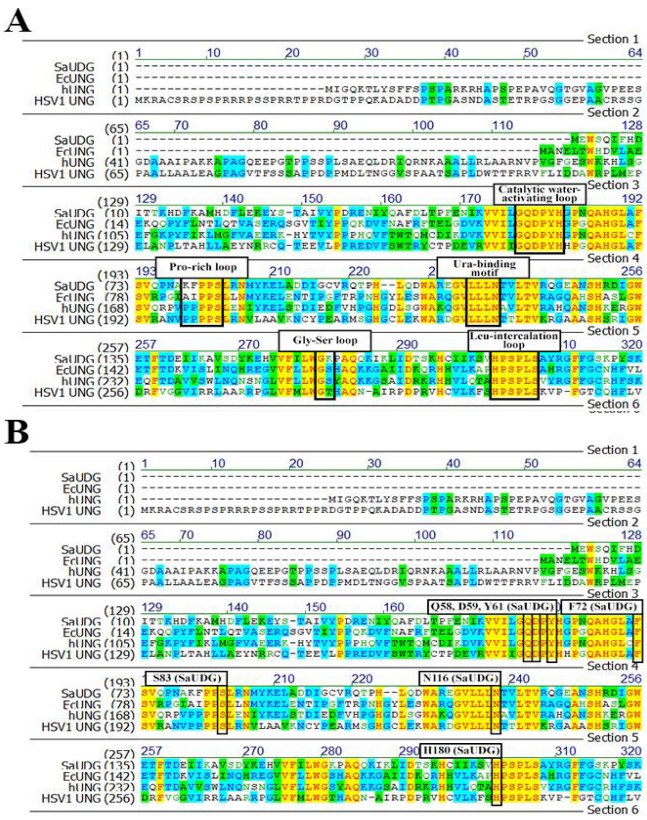

Figure 1. Amino acid alignment of selected representatives of family I uracil-DNA glycosylases: conserved structural motifs(A) and amino acids residues forming the uracil-binding pocket (B)

DOI: 10.11134/btp.2.2026.9

It has been previously shown that the Y66 residue in EcUNG engages in van der Waals interactions with the C5 atom of the uracil ring, with its aromatic plane oriented orthogonally to the uracil plane. This orientation creates steric hindrance for the binding of thymine with its 5-methyl group, as well as purines with bulky ring structures, ensuring the specificity for uracil excision. Substitution of Y66 with Ser or Cys results in approximately a 1000-fold decrease in catalytic activity compared to the wild-type enzyme [25].

The F77 residue in EcUNG is located in the active site and interacts with the uracil ring, stabilizing it in the active center, facilitating base flipping from DNA and promoting glycosidic bond cleavage; replacement of F77 with Asn leads to an approximately 1200-fold reduction in kcat/KM and over 1000-fold decrease in single-turnover excision rate [26].

The H187 residue in EcUNG forms a hydrogen bond with O2 of uracil and participates in stabilizing base after flipping into the active site, ensuring positioning of the base and facilitating glycosidic bond cleavage; substitution with Gln significantly reduces reaction rate and disrupts transition-state stabilization, as evidenced by a more than 1000-fold decrease in single-turnover excision rate [27]. Alignment of SaUDG amino acid sequences with homologs from various organisms revealed that these target residues are conserved in structurally significant positions of family I enzymes (Fig. 2).

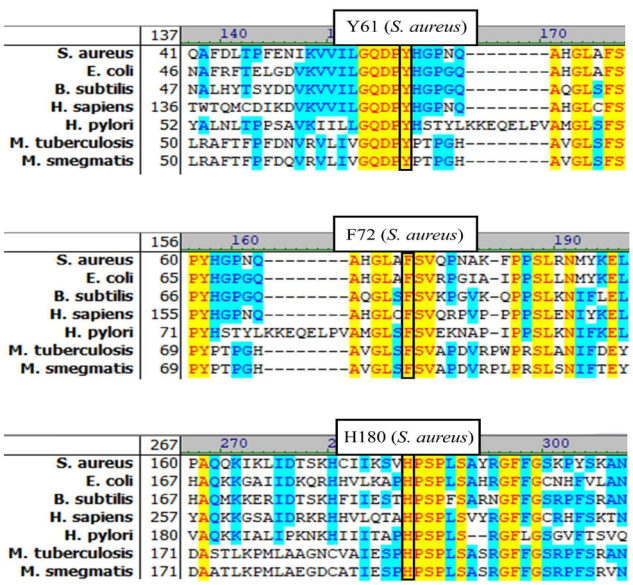

Figure 2. Amino acid alignment of family I uracil-DNA glycosylases from various organisms

Structural alignment of SaUDG and EcUNG, based on models from the AlphaFold Protein Structure Database, confirms the structural and functional similarity of the enzymes: the superposition of 1080 out of 1477 atoms with an RMSD of 0.41 Å indicates preservation of the active site organization (Fig. 3A-B).

Based on an analysis of literature data, as well as a comparison of the amino acid sequence and three-dimensional structure the amino acid residues Y61, F72 and H180 were selected as targets for site-directed mutagenesis to assess their role in the catalytic activity of SaUDG (substitutions Y61S, F72N and H180Q, respectively).

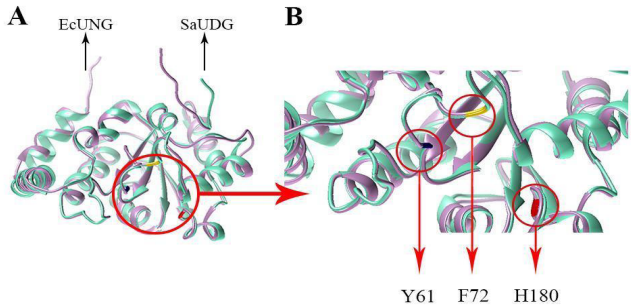

Figure 3. Structural alignment of EcUNG and SaUDG (UCSF Chimera) [28]:

overall protein alignment (A) and active site with target residues (Y61, F72, H180 SaUDG) (B)

Generation of SaUDG mutant variants by site-directed mutagenesis

Overlapping primer pairs were designed to introduce the point mutations Y61S, F72N and H180Q: SaUDG Y61S fw – SaUDG Y61S rv, SaUDG F72N fw – SaUDG F72N rv, SaUDG H180Q fw – SaUDG H180Q rv, respectively. The plasmid construct pET11a/SaUDG was used as the template DNA. Figure 4 shows the PCR product of the site-directed mutagenesis.

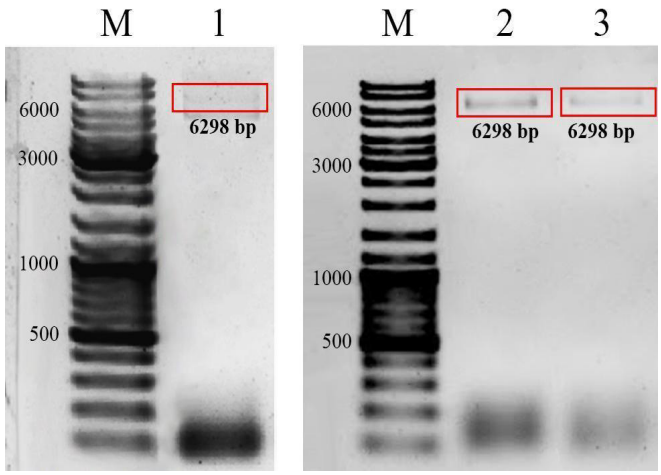

Figure 4. Electrophoretic analysis of Quick Change PCR.

M – DNA marker SM0333 (Thermo Fisher Scientific, USA); 1 – pET11a/SaUDG Y61S; 2 – pET11a/SaUDG F72N; 3 – pET11a/SaUDG H180Q

As a result of Quick Change PCR, 6298 bp products corresponding to the original pET11a/SaUDG plasmid were obtained. To remove the parental non-mutated plasmid, the reaction mixture was digested with the DpnI endonuclease, after which the resulting mixture was used to transform *E. coli* DH5α cells. The transformed colonies obtained on selective media were used for plasmid DNA isolation and subsequent sequencing.

Alignment of the mutant sequences against the reference wild-type gene from the NCBI GenBank database (NC_007795.1: region 571090-571746) confirmed the presence of the intended amino acid substitutions and the absence of additional mutations (Fig. 5).

3.2. Expression and purification of recombinant wild-type and mutant SaUDG proteins

E. coli Rosetta 2 (DE3) cells were transformed with plas-

DOI: 10.11134/btp.2.2026.9

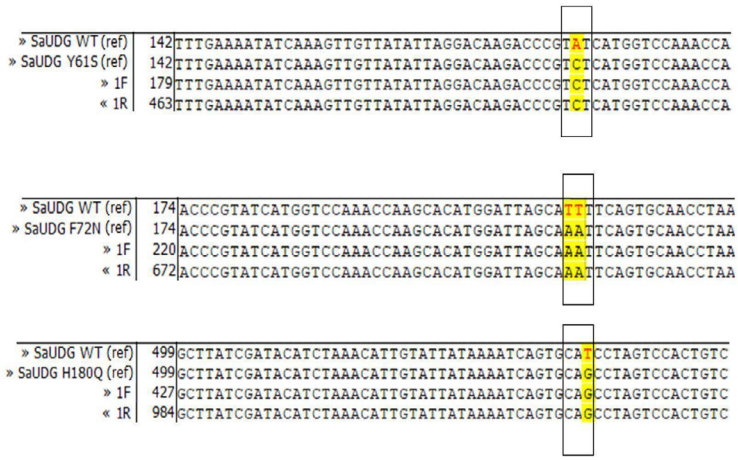

Figure 5. Sequencing alignment of SaUDG mutant variants (Y61S, F72N, H180Q) with wild type

Thus, mutant variants of the *udg* gene from *S. aureus* encoding proteins with the amino acid substitutions Y61S, F72N and H180Q were successfully generated and confirmed by sequencing.

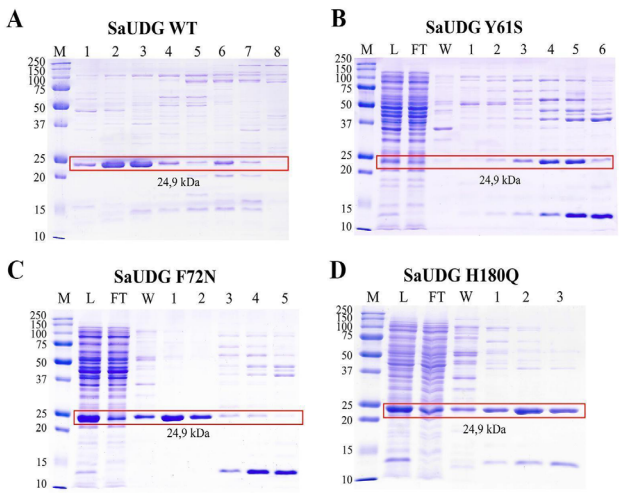

Figure 6. Electropherograms of SaUDG WT (A), SaUDG Y61S (B), SaUDG F72N (C), SaUDG H180Q (D) after chromatographic purification on HiTrap Heparin HP.

M – protein marker #1610373 (Bio-Rad, USA); L – load; FT – flow-through; W – wash; 1-8 – fractions of the corresponding recombinant protein

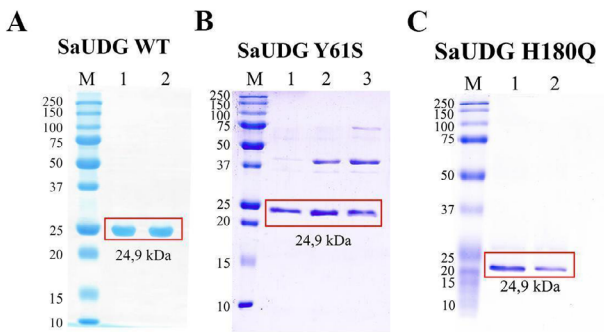

Figure 7. Electropherograms of SaUDG WT (A), SaUDG Y61S (B), SaUDG H180Q (C) proteins after chromatographic purification on HiTrap Q HP column.

M – protein marker #1610373 (Bio-Rad, USA); 1-3 – fractions of the corresponding recombinant protein

mid vectors pET11a/SaUDG WT, pET11a/SaUDG Y61S, pET11a/SaUDG F72N and pET11a/SaUDG H180Q for recombinant protein expression.

The first stage of chromatographic purification was performed by affinity chromatography on a HiTrap Heparin HP 1 mL column with protein elution using a linear gradient of 50 mM-1 M NaCl. The obtained fractions of the recombinant protein were analyzed by electrophoresis in a 12% sodium dodecyl sulfate-polyacrylamide gel (Fig. 6A-D).

According to the electropherograms in Figure 6, the highest content of recombinant proteins after chromatographic purification was observed in the following fractions: SaUDG WT fractions 2-3; SaUDG Y61S fractions 4-5; SaUDG F72N fractions 1-2; SaUDG H180Q fractions 1-3. Fraction 2 of SaUDG F72N was used for subsequent experiments. The above-mentioned fractions of SaUDG WT, SaUDG Y61S, and SaUDG H180Q contained protein impurities. Therefore, an additional purification step using anion-exchange chromatography on a HiTrap Q HP 1 mL column with a similar NaCl gradient was performed (Fig. 7A-B).

As a result of the two-step chromatographic purification, the recombinant proteins reached a purity suitable for further experiments. The yields of the purified proteins were as follows: SaUDG WT – 6.31 mg from 300 mL of culture; Y61S, F72N and H180Q mutant variants – 0.15 mg, 0.27 mg and 0.17 mg from 200 mL of culture, respectively. The following fractions were selected after the second purification step for subsequent analysis of repair activity: SaUDG WT – fraction 1, SaUDG Y61S – fraction 1, SaUDG H180Q – fraction 1.

3.3. Analysis of the repair activity of SaUDG mutant variants

UDG enzymes of the first family exhibit high specificity for uracil: their mechanism of action, thoroughly studied using the EcUNG model, is based on recognition of uracil itself rather than its complementary base [29]. Consequently, UDGs can remove uracil from various types of DNA, including ssDNA and dsDNA, as well as from different complementary base pairs [30]. U:G mismatches are of particular biological significance because they can lead to C→T transition mutations, making them an appropriate substrate for assessing enzyme activity [31].

DOI: 10.11134/btp.2.2026.9

Thus, the repair activity of wild-type SaUDG and its mutant variants was evaluated by monitoring their ability to excise uracil from a dsDNA substrate in which uracil was paired with guanine. Mutant proteins were tested at higher enzyme concentrations to compensate for the anticipated reduction in catalytic activity. Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis (20%) in the presence of urea. The results of these reactions are presented in Figure 8A-C.

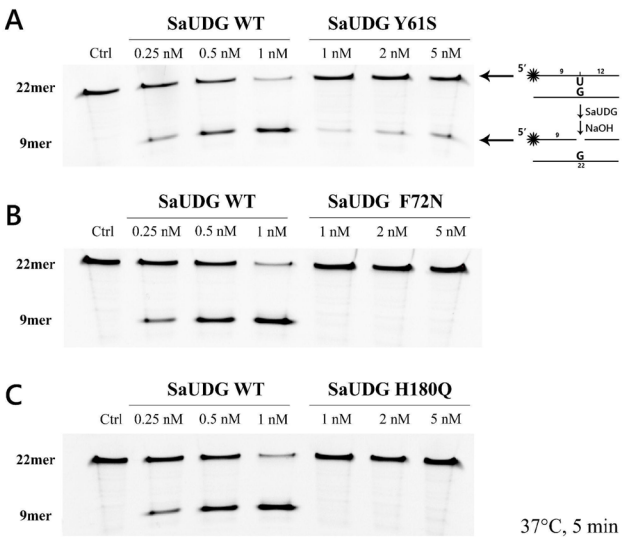

Figure 8. Comparative analysis of the repair activity of wild-type SaUDG and its mutant variants: SaUDG Y61S (A), SaUDG F72 (B), SaUDG H180Q (C)

The results show that the F72N and H180Q mutants lacked detectable catalytic activity, whereas the Y61S substitution caused a pronounced reduction in activity. These data highlight the functional importance of the selected amino acid residues in the catalytic mechanism of SaUDG and are consistent with previously reported effects of analogous substitutions in other uracil-DNA glycosylases.

CONCLUSION

Previous studies on the EcUNG have shown that the Y66 residue contributes to excision specificity via Van der Waals interactions with the uracil base [25], and its substitution causes a pronounced decrease in catalytic activity. The F77 residue stabilizes uracil within the active site [26], while H187 ensures proper positioning of the base, facilitating efficient glycosidic bond cleavage [27]. Mutations of these residues in EcUNG lead to a significant reduction in enzyme activity.

In the present study, based on *in silico* bioinformatic and structural analyses of SaUDG, the residues Y61, F72 and H180 were identified as potentially critical for catalytic activity. Experimental assessment of the corresponding mutant variants of SaUDG (Y61S, F72N and H180Q) revealed a complete loss of detectable activity for F72N and H180Q and a pronounced reduction in activity for Y61S.

These results confirm the critical role of residues F72 and H180 in the repair activity of SaUDG and indicate the functional significance of Y61 in the enzyme's activity. The observed data correspondence with previously reported effects of analogous substitutions in EcUNG underscores the high degree of conservation in the structural and functional organization of the active site among family I UDG enzymes. Overall, this study provides further insight into the molecular mechanisms underlying SaUDG function and the general principles of uracil-DNA glycosylase activity.

ATHOUR CONTRIBUTIONS

Conceptualization - A.S.K. and A.T.M.; methodology - A.S.K. and A.T.M.; software – K.A.S. and A.T.M.; validation - K.A.S. and A.T.M.; formal analysis - A.T.M.; investigation - K.A.S.; resources - A.S.K.; data curation - A.T.M.; writing—original draft preparation - K.A.S. and A.T.M.; writing—review and editing - A.S.K. and A.T.M.; visualization - K.A.S. and A.T.M.; supervision - A.T.M.; project administration - A.T.M.; funding acquisition - A.T.M. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (grant AP23488467 «Functional characteristics of DNA glycosylase from *Staphylococcus aureus*», 2024-2026).

REFERENCES

1. Laux C., Peschel A., Krismer B. *Staphylococcus aureus* Colonization of the Human Nose and Interaction with Other Microbiome Members // *Microbiology Spectrum*. – 2019. – Vol. 7, № 2, P. 10.1128/microbiolspec.gpp3-0029-2018. doi: 10.1128/microbiolspec.GPP3-0029-2018. PMID: 31004422

2. Touaitia R., Mairi A., Ibrahim N.A., Basher N.S., Idres T., Touati A. *Staphylococcus aureus*: A Review of the Pathogenesis and Virulence Mechanisms // *Antibiotics*. – 2025. – Vol. 14, № 5, P. 470. doi: 10.3390/antibiotics14050470. PMID: 40426537

3. Tong S.Y.C., Fowler V.G. Jr, Skalla L., Holland T.L. Management of *Staphylococcus aureus* Bacteremia: A Review // *JAMA*. – 2025 – Vol. 334; № 9, P. 798-808. doi: 10.1001/jama.2025.4288. PMID: 40193249

4. World Health Organization. *WHO publishes list of bacteria for which new antibiotics are urgently needed* – 2022 – URL: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

5. Garoy E.Y., Gebreab Y.B., Achila O.O., Tekeste D.G., Kesete R., Ghirmay R., Kiflay R., Tesfu T. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea // *Canadian Journal of Infectious Diseases and Medical Microbiology.* – 2019. – P. 8321834. doi: 10.1155/2019/8321834. PMID: 30881532

6. Centers for Disease Control and Prevention. *Infection Control Guidance: Preventing Methicillin-resistant Staphylococcus aureus (MRSA) in Healthcare Facilities* – 2025. – URL: https://www.cdc.gov/mrsa/hcp/infection-control/

DOI: 10.11134/btp.2.2026.9

7. Turgunova L., Klodzinskiy A., Lavrinenko A., Kolesnichenko S., Seidalin N., Zinchenko A. Growth of antibiotic resistance in the hematology department of central Kazakhstan during the covid-19 pandemic // *HemaSphere*. – 2021. – Vol. 5, SUPPL 2, P. 387-388

8. Bradbury J.D., Hodgkinson T., Thomas A.M., Tanwar O., La Monica G., Rogga V.V., Mackay L.J., Taylor E.K., Gilbert K., Zhu Y., Sefton A.Y., Edwards A.M., Gray-Hammerton C.J., Smith G.R., Roberts P.M., Walsh T.R., Lanyon-Hogg T. Development of an inhibitor of the mutagenic SOS response that suppresses the evolution of quinolone antibiotic resistance // *Chemical Science*. – 2024. – Vol. 15, № 25, P. 9620-9629. doi: 10.1039/d4sc01234k

9. Lim C.S.Q., Ha K.P., Clarke R.S., Gavin L.A., Cook D.T., Hutton J.A., Sutherell C.L., Edwards A.M., Evans L.E., Tate E.W., Lanyon-Hogg T. Identification of a potent small-molecule inhibitor of bacterial DNA repair that potentiates quinolone antibiotic activity in methicillin-resistant *Staphylococcus aureus* // *Bioorganic & Medicinal Chemistry*. – 2019. – Vol. 27, № 20, P. 114962. doi: 10.1016/j.bmc.2019.114962. PMID: 31307763

10. Krwawicz J., Arczewska K.D., Speina E., Maciejewska A., Grzesiuk E. Bacterial DNA repair genes and their eukaryotic homologues. 1. Mutations in genes involved in base excision repair (BER) and DNA-end processors and their implication in mutagenesis and human disease // Acta. Biochim. Pol. – 2007. – Vol. 54, P. 413-434. doi: 10.18388/abp.2007_3219. PMID: 17893748

11. Kurthkoti K., Kumar P., Sang P.B., Varshney U. Base excision repair pathways of bacteria: new promise for an old problem // *Future Medicinal Chemistry*. – 2020. – Vol. 12, № 4, P. 339-355. doi: 10.4155/fmc-2019-0267. PMID: 32031026

12. Ha K.P., Edwards A.M. DNA Repair in *Staphylococcus aureus* // *Microbiology and Molecular Biology Reviews*. – 2021. – Vol. 85, № 4, P. e0009121. doi: 10.1128/MMBR.00091-21. PMID: 34523959

13. Krokan H.E., Drabløs F., Slupphaug G. Uracil in DNA--occurrence, consequences and repair // *Oncogene*. – 2002. – Vol. 21, № 58, P. 8935-8948. doi: 10.1038/sj.onc.1205996. PMID: 12483510

14. Venkatesh J., Kumar P., Krishna P.S., Manjunath R., Varshney U. Importance of uracil DNA glycosylase in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*, G+C-rich bacteria, in mutation prevention, tolerance to acidified nitrite, and endurance in mouse macrophages // *Journal of Biological Chemistry*. – 2003. – Vol. 278, № 27, P. 24350-24358. PMID: 12679366

15. Malshetty V.S., Jain R., Srinath T., Kurthkoti K., Varshney U. Synergistic effects of UdgB and Ung in mutation prevention and protection against commonly encountered DNA damaging agents in *Mycobacterium smegmatis* // *Microbiology (Reading)*. – 2010. – Vol. 156, Pt 3, P. 940-949. PMID: 19942658

16. Liu P., Burdzy A., Sowers L.C. Substrate recognition by a family of uracil-DNA glycosylases: UNG, MUG, and TDG // *Chemical Research in Toxicology*. – 2002. – Vol. 15, № 8, P. 1001-1009. doi: 10.1021/tx020030a. PMID: 12184783

17. Barrett T.E., Savva R., Panayotou G., Barlow T., Brown T., Jiricny J., Pearl L.H. Crystal structure of a G:T/U mismatch-specific DNA glycosylase: mismatch recognition by complementary-strand interactions // *Cell*. – 1998. – Vol. 92, № 1, P. 117-129. PMID: 9489705

18. Wibley J.E., Waters T.R., Haushalter K., Verdine G.L., Pearl L.H. Structure and specificity of the vertebrate anti-mutator uracil-DNA glycosylase SMUG1 // *Molecular Cell*. – 2003. – Vol. 11, № 6, P. 1647-1659. doi: 10.1016/s1097-2765(03)00235-1. PMID: 12820976

19. Knævelsrud I., Moen M.N., Grøsvik K., Haugland G.T., Birkeland N.K., Klungland A., Leiros I., Bjelland S. The hyperthermophilic euryarchaeon *Archaeoglobus fulgidus* repairs uracil by single-nucleotide replacement // *Journal of Bacteriology*. – 2010. – Vol. 192, № 21, P. 5755-5766. doi: 10.1128/JB.00135-10. PMID: 20453094

20. Kosaka H., Hoseki J., Nakagawa N., Kuramitsu S., Masui R. Crystal structure of family 5 uracil-DNA glycosylase bound to DNA // *Journal of Molecular Biology*. – 2007. – Vol. 373, № 4, P. 839-850. doi: 10.1016/j.jmb.2007.08.022. PMID: 17870091

21. Sartori A.A., Fitz-Gibbon S., Yang H., Miller J.H., Jiricny J. A novel uracil-DNA glycosylase with broad substrate specificity and an unusual active site // *EMBO Journal*. – 2002. – Vol. 21, № 13, P. 3182-3191. PMID: 12065430

22. Kwon E., Pathak D., Chang H.W., Kim D.Y. Crystal structure of mimivirus uracil-DNA glycosylase // *PLoS One*. – 2017. – Vol. 12, № 8, P. e0182382. doi: 10.1371/journal.pone.0182382. PMID: 28763516

23. Aravind L., Koonin E.V. The alpha/beta fold uracil DNA glycosylases: a common origin with diverse fates // *Genome Biology*. – 2000. – Vol. 1, № 4, P. RESEARCH0007. doi: 10.1186/gb-2000-1-4-research0007. PMID: 11178247

24. Schormann N., Ricciardi R., Chattopadhyay D. Uracil-DNA glycosylases–structural and functional perspectives on an essential family of DNA repair enzymes // *Protein Science*. – 2014. – Vol. 23, № 12, P. 1667-1685. doi: 10.1002/pro.2554. PMID: 25252105

25. Handa P., Acharya N., Varshney U. Effects of mutations at tyrosine 66 and asparagine 123 in the active site pocket of *Escherichia coli* uracil DNA glycosylase on uracil excision from synthetic DNA oligomers: evidence for the occurrence of long-range interactions between the enzyme and substrate // *Nucleic Acids Research*. – 2002. – Vol. 30, № 14, P. 3086-3095. doi: 10.1093/nar/gkf425. PMID: 12136091

26. Shaw R.W., Feller J.A., Bloom L.B. Contribution of a conserved phenylalanine residue to the activity of *Escherichia coli* uracil DNA glycosylase // *DNA Repair*. – 2004. – Vol. 3, № 10, P. 1273-1283. doi: 10.1016/j.dnarep.2004.05.003. PMID: 15336623

27. Drohat A.C., Jagadeesh J., Ferguson E., Stivers J.T. Role of electrophilic and general base catalysis in the mechanism of *Escherichia coli* uracil DNA glycosylase // *Biochemistry*. – 1999. – Vol. 38, № 37, P. 11866-11875. doi: 10.1021/bi9910878. PMID: 10508389

28. Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C., Ferrin T.E. UCSF Chimera–a visualization system for exploratory research and analysis // *Journal of Computational Chemistry*. – 2004. – Vol. 25, № 13, P. 1605-1612. PMID: 15264254

DOI: 10.11134/btp.2.2026.9

29. Lindahl T., Ljungquist S., Siegert W., Nyberg B., Sperens B. DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli* // *Journal of Biological Chemistry*. – 1977. – Vol. 252, № 10, P. 3286-3294. PMID: 324994

30. Parker J.B., Stivers J.T. Uracil DNA glycosylase: revisiting substrate-assisted catalysis by DNA phosphate anions // *Biochemistry*. – 2008. – Vol. 47, № 33, P. 8614-8622. doi: 10.1021/bi800854g. PMID: 18652484

31. Zharkov D.O., Mechetin G.V., Nevinsky G.A. Uracil-DNA glycosylase: Structural, thermodynamic and kinetic aspects of lesion search and recognition // *Mutation Research*. – 2010. – Vol. 685, № 1-2, P. 11-20. doi: 10.1016/j.mrfmmm.2009.10.017. PMID: 19909758

LITERATURE

1. Laux C., Peschel A., Krismer B. *Staphylococcus aureus* Colonization of the Human Nose and Interaction with Other Microbiome Members // *Microbiology Spectrum*. – 2019. – Vol. 7, № 2, P. 10.1128/microbiolspec.gpp3-0029-2018. doi: 10.1128/microbiolspec.GPP3-0029-2018. PMID: 31004422

2. Touaitia R., Mairi A., Ibrahim N.A., Basher N.S., Idres T., Touati A. *Staphylococcus aureus*: A Review of the Pathogenesis and Virulence Mechanisms // *Antibiotics*. – 2025. – Vol. 14, № 5, P. 470. doi: 10.3390/antibiotics14050470. PMID: 40426537

3. Tong S.Y.C., Fowler V.G. Jr, Skalla L., Holland T.L. Management of *Staphylococcus aureus* Bacteremia: A Review // *JAMA*. – 2025 – Vol. 334; № 9, P. 798-808. doi: 10.1001/jama.2025.4288. PMID: 40193249

4. World Health Organization. *WHO publishes list of bacteria for which new antibiotics are urgently needed* – 2022 – URL: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

5. Garoy E.Y., Gebreab Y.B., Achila O.O., Tekeste D.G., Kesete R., Ghirmay R., Kiflay R., Tesfu T. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea // *Canadian Journal of Infectious Diseases and Medical Microbiology.* – 2019. – P. 8321834. doi: 10.1155/2019/8321834. PMID: 30881532

6. Centers for Disease Control and Prevention. *Infection Control Guidance: Preventing Methicillin-resistant Staphylococcus aureus (MRSA) in Healthcare Facilities* – 2025. – URL: https://www.cdc.gov/mrsa/hcp/infection-control/

7. Turgunova L., Klodzinskiy A., Lavrinenko A., Kolesnichenko S., Seidalin N., Zinchenko A. Growth of antibiotic resistance in the hematology department of central Kazakhstan during the covid-19 pandemic // *HemaSphere.* – 2021. – Vol. 5, SUPPL 2, P. 387-388

8. Bradbury J.D., Hodgkinson T., Thomas A.M., Tanwar O., La Monica G., Rogga V.V., Mackay L.J., Taylor E.K., Gilbert K., Zhu Y., Sefton A.Y., Edwards A.M., Gray-Hammerton C.J., Smith G.R., Roberts P.M., Walsh T.R., Lanyon-Hogg T. Development of an inhibitor of the mutagenic SOS response that suppresses the evolution of quinolone antibiotic resistance // *Chemical Science*. – 2024. – Vol. 15, № 25, P. 9620-9629. doi: 10.1039/d4sc01234k

9. Lim C.S.Q., Ha K.P., Clarke R.S., Gavin L.A., Cook D.T., Hutton J.A., Sutherell C.L., Edwards A.M., Evans L.E., Tate E.W., Lanyon-Hogg T. Identification of a potent small-molecule inhibitor of bacterial DNA repair that potentiates quinolone antibiotic activity in methicillin-resistant *Staphylococcus aureus* // *Bioorganic & Medicinal Chemistry*. – 2019. – Vol. 27, № 20, P. 114962. doi: 10.1016/j.bmc.2019.114962. PMID: 31307763

10. Krwawicz J., Arczewska K.D., Speina E., Maciejewska A., Grzesiuk E. Bacterial DNA repair genes and their eukaryotic homologues. 1. Mutations in genes involved in base excision repair (BER) and DNA-end processors and their implication in mutagenesis and human disease // Acta. Biochim. Pol. – 2007. – Vol. 54, P. 413-434. doi: 10.18388/abp.2007_3219. PMID: 17893748

11. Kurthkoti K., Kumar P., Sang P.B., Varshney U. Base excision repair pathways of bacteria: new promise for an old problem // *Future Medicinal Chemistry*. – 2020. – Vol. 12, № 4, P. 339-355. doi: 10.4155/fmc-2019-0267. PMID: 32031026

12. Ha K.P., Edwards A.M. DNA Repair in *Staphylococcus aureus* // *Microbiology and Molecular Biology Reviews*. – 2021. – Vol. 85, № 4, P. e0009121. doi: 10.1128/MMBR.00091-21. PMID: 34523959

13. Krokan H.E., Drabløs F., Slupphaug G. Uracil in DNA--occurrence, consequences and repair // *Oncogene*. – 2002. – Vol. 21, № 58, P. 8935-8948. doi: 10.1038/sj.onc.1205996. PMID: 12483510

14. Venkatesh J., Kumar P., Krishna P.S., Manjunath R., Varshney U. Importance of uracil DNA glycosylase in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*, G+C-rich bacteria, in mutation prevention, tolerance to acidified nitrite, and endurance in mouse macrophages // *Journal of Biological Chemistry*. – 2003. – Vol. 278, № 27, P. 24350-24358. PMID: 12679366

15. Malshetty V.S., Jain R., Srinath T., Kurthkoti K., Varshney U. Synergistic effects of UdgB and Ung in mutation prevention and protection against commonly encountered DNA damaging agents in *Mycobacterium smegmatis* // *Microbiology (Reading)*. – 2010. – Vol. 156, Pt 3, P. 940-949. PMID: 19942658

16. Liu P., Burdzy A., Sowers L.C. Substrate recognition by a family of uracil-DNA glycosylases: UNG, MUG, and TDG // *Chemical Research in Toxicology*. – 2002. – Vol. 15, № 8, P. 1001-1009. doi: 10.1021/tx020030a. PMID: 12184783

17. Barrett T.E., Savva R., Panayotou G., Barlow T., Brown T., Jiricny J., Pearl L.H. Crystal structure of a G:T/U mismatch-specific DNA glycosylase: mismatch recognition by complementary-strand interactions // *Cell*. – 1998. – Vol. 92, № 1, P. 117-129. PMID: 9489705

18. Wibley J.E., Waters T.R., Haushalter K., Verdine G.L., Pearl L.H. Structure and specificity of the vertebrate anti-mutator uracil-DNA glycosylase SMUG1 // *Molecular Cell*. – 2003. – Vol. 11, № 6, P. 1647-1659. doi: 10.1016/s1097-2765(03)00235-1. PMID: 12820976

19. Knævelsrud I., Moen M.N., Grøsvik K., Haugland

DOI: 10.11134/btp.2.2026.9

G.T., Birkeland N.K., Klungland A., Leiros I., Bjelland S. The hyperthermophilic euryarchaeon *Archaeoglobus fulgidus* repairs uracil by single-nucleotide replacement // *Journal of Bacteriology*. – 2010. – Vol. 192, № 21, P. 5755-5766. doi: 10.1128/JB.00135-10. PMID: 20453094

20. Kosaka H., Hoseki J., Nakagawa N., Kuramitsu S., Masui R. Crystal structure of family 5 uracil-DNA glycosylase bound to DNA // *Journal of Molecular Biology*. – 2007. – Vol. 373, № 4, P. 839-850. doi: 10.1016/j.jmb.2007.08.022. PMID: 17870091

21. Sartori A.A., Fitz-Gibbon S., Yang H., Miller J.H., Jiricny J. A novel uracil-DNA glycosylase with broad substrate specificity and an unusual active site // *EMBO Journal*. – 2002. – Vol. 21, № 13, P. 3182-3191. PMID: 12065430

22. Kwon E., Pathak D., Chang H.W., Kim D.Y. Crystal structure of mimivirus uracil-DNA glycosylase // *PLoS One*. – 2017. – Vol. 12, № 8, P. e0182382. doi: 10.1371/journal.pone.0182382. PMID: 28763516

23. Aravind L., Koonin E.V. The alpha/beta fold uracil DNA glycosylases: a common origin with diverse fates // *Genome Biology*. – 2000. – Vol. 1, № 4, P. RESEARCH0007. doi: 10.1186/gb-2000-1-4-research0007. PMID: 11178247

24. Schormann N., Ricciardi R., Chattopadhyay D. Uracil-DNA glycosylases–structural and functional perspectives on an essential family of DNA repair enzymes // *Protein Science*. – 2014. – Vol. 23, № 12, P. 1667-1685. doi: 10.1002/pro.2554. PMID: 25252105

25. Handa P., Acharya N., Varshney U. Effects of mutations at tyrosine 66 and asparagine 123 in the active site pocket of *Escherichia coli* uracil DNA glycosylase on uracil excision from synthetic DNA oligomers: evidence for the occurrence of long-range interactions between the enzyme and substrate // *Nucleic Acids Research*. – 2002. – Vol. 30, № 14, P. 3086-3095. doi: 10.1093/nar/gkf425. PMID: 12136091

26. Shaw R.W., Feller J.A., Bloom L.B. Contribution of a conserved phenylalanine residue to the activity of *Escherichia coli* uracil DNA glycosylase // *DNA Repair*. – 2004. – Vol. 3, № 10, P. 1273-1283. doi: 10.1016/j.dnarep.2004.05.003. PMID: 15336623

27. Drohat A.C., Jagadeesh J., Ferguson E., Stivers J.T. Role of electrophilic and general base catalysis in the mechanism of *Escherichia coli* uracil DNA glycosylase // *Biochemistry*. – 1999. – Vol. 38, № 37, P. 11866-11875. doi: 10.1021/bi9910878. PMID: 10508389

28. Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C., Ferrin T.E. UCSF Chimera–a visualization system for exploratory research and analysis // *Journal of Computational Chemistry*. – 2004. – Vol. 25, № 13, P. 1605-1612. PMID: 15264254

29. Lindahl T., Ljungquist S., Siegert W., Nyberg B., Sperens B. DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli* // *Journal of Biological Chemistry*. – 1977. – Vol. 252, № 10, P. 3286-3294. PMID: 324994

30. Parker J.B., Stivers J.T. Uracil DNA glycosylase: revisiting substrate-assisted catalysis by DNA phosphate anions // *Biochemistry*. – 2008. – Vol. 47, № 33, P. 8614-8622. doi: 10.1021/bi800854g. PMID: 18652484

31. Zharkov D.O., Mechetin G.V., Nevinsky G.A. Uracil-DNA glycosylase: Structural, thermodynamic and kinetic aspects of lesion search and recognition // *Mutation Research*. – 2010. – Vol. 685, № 1-2, P. 11-20. doi: 10.1016/j.mrfmmm.2009.10.017. PMID: 19909758

DOI: 10.11134/btp.2.2026.9

УДК 577.15; 577.2

ФУНКЦИОНАЛЬНЫЙ АНАЛИЗ АМИНОКИСЛОТНЫХ ОСТАТКОВ Y61, F72 И H180 В УРАЦИЛ-ДНК-ГЛИКОЗИЛАЗЕ *STAPHYLOCOCCUS AUREUS*

Коренева А.С.[1], Абельденов С.К. [2], Тургимбаева А.М.[2*]

[1]НАО «Казахский агротехнический исследовательский университет имени С. Сейфуллина», 010000, Республика Казахстан, г. Астана, проспект Женис, 62
[2]ТОО «Национальный центр биотехнологии», 010000, Республика Казахстан, г. Астана, шоссе Коргалжын, 13/5
**Автор для корреспонденции: turgimbayeva@biocenter.kz*

АННОТАЦИЯ

В рамках настоящего исследования проведен биоинформатический и структурный анализ *in silico* урацил-ДНК-гликозилазы *Staphylococcus aureus* (SaUDG), позволивший идентифицировать аминокислотные остатки Y61, F72 и H180 как потенциально значимые для каталитической активности фермента. На основе полученных данных были сконструированы мутантные варианты SaUDG Y61S, F72N и H180Q. Результаты ферментативного анализа показали отсутствие детектируемой каталитической активности у мутантов SaUDG F72N и H180Q, тогда как замена Y61S сопровождалась её существенным снижением.

Полученные данные свидетельствуют о ключевой роли аминокислотных остатков F72 и H180 в обеспечении каталитического механизма SaUDG и указывают на функциональную значимость остатка Y61 для поддержания ферментативной активности. В целом, результаты исследования углубляют представления о структуре и механизме действия SaUDG и согласуются с данными о консервативной организации активного центра ферментов первого семейства UDG.

Ключевые слова: *Staphylococcus aureus*, репарация ДНК, эксцизионная репарация оснований, урацил-ДНК-гликозилаза, сайт-направленный мутагенез, мутантный вариант.

ӘОЖ 577.15; 577.2

STAPHYLOCOCCUS AUREUS УРАЦИЛ-ДНҚ-ГЛИКОЗИЛАЗАСЫНДАҒЫ Y61, F72 ЖӘНЕ H180 АМИНҚЫШҚЫЛ ҚАЛДЫҚТАРЫНЫҢ ФУНКЦИОНАЛДЫҚ ТАЛДАУЫ

Коренева А.С.[1], Абельденов С.К.[2], Тургимбаева А.М.[2*]

[1]КеАҚ «С.Сейфуллин атындағы Қазақ агротехникалық зерттеу университеті», 010000, Қазақстан Республикасы, Астана қ., Жеңіс даңғылы, 62
[2]ЖШС «Ұлттық биотехнология орталығы», 010000, Қазақстан Республикасы, Астана қ., Қорғалжын тас жолы, 13/5
**Корреспондент автор: turgimbayeva@biocenter.kz*

АҢДАТПА

Зерттеу аясы *Staphylococcus aureus* урацил-ДНҚ-гликозилазасына (SaUDG) *in silico* биоинформатикалық және құрылымдық талдау жүргізілді, бұл ферменттің каталитикалық белсенділігі үшін Y61, F72 және H180 аминқышқыл қалдықтары әлеуетті маңызды рөл атқаратыны анықталды. Алынған деректер негізінде SaUDG Y61S, F72N және H180Q мутантты варианттары құрастырылды. Ферментативтік талдау нәтижелері SaUDG F72N және H180Q мутанттарында анықталатын каталитикалық белсенділіктің жоқтығын көрсетті, ал Y61S алмастыруы оның едәуір төмендеуін көрсетті.

Алынған деректер SaUDG каталитикалық механизмін қамтамасыз етуде F72 және H180 аминқышқыл қалдықтарының шешуші рөлін дәлелдейді және ферментативтік белсенділікті сақтауда Y61 қалдығының функционалдық маңыздылығын көрсетеді. Жалпы алғанда, зерттеу нәтижелері SaUDG құрылымы және механизмі туралы түсініктерді тереңдетеді әрі, UDG бірінші отбасы ферменттерінің белсенді орталығының консервативті ұйымдасуы туралы мағлұматтармен сәйкес келеді.

Кілт сөздер: *Staphylococcus aureus*, ДНҚ репарациясы, негіздердің эксцизиялық репарациясы, урацил-ДНҚ-гликозилаза, сайт-бағытталған мутагенез, мутантты вариант.