OKU Fen Bilimleri Enstitüsü Dergisi
8(4): 1613-1623, 2025

OKU Journal of The Institute of Science and Technology, 8(4): 1613-1623, 2025

Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi

Osmaniye Korkut Ata University Journal of The Institute of Science and Technology

Lack of Pseudouridine Synthase 7 Enzyme Sensitizes Yeast Cells to DNA-Damaging Agents

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Research Article

Article History:
Received: 07.01.2025
Accepted: 27.03.2025
Published online:16.09.2025

Keywords:
Saccharomyces cerevisiae
Pseudouridine synthase
Pus7
Methyl methanesulfonate
Ultraviolet light
DNA damage

ABSTRACT

RNA modifications are essential in reshaping the chemistry of RNA molecules, influencing their structure, function, and stability. Up to now, more than 160 different types of modifications have been discovered among different RNA species. One prominent modification is the enzymatic transformation of uridine (U) into pseudouridine (Ψ), a process facilitated by pseudouridine synthase (Pus) enzymes, which occurs in both coding (mRNA) and non-coding (ncRNA) RNA species. Among the enzymes responsible for this transformation, Pus7 stands out due to its association with certain developmental and physical conditions. Research links Pus7 to intellectual disabilities, delayed speech development, microcephaly, and short stature. Despite its association with these disorders, the exact biological role and mechanism of Pus7 remain poorly understood, leaving room for further investigation. In this study, we aimed to understand the role of Pus7 in *Saccharomyces cerevisiae* cells under DNA damage stress. To achieve this, wild-type and *pus7*Δ cells were subjected to varying concentrations of DNA-damaging agents, including ultraviolet (UV) light and the chemical methyl methanesulfonate (MMS). Survival curves and spot plating assay results demonstrated that *pus7*Δ cells exhibit growth defects when exposed to 2 mM MMS or 15 J/m² UV light. These findings indicate that the absence of Pus7 enzyme renders yeast cells sensitive to DNA-damaging agents. Further research is necessary to investigate the role of Pus7 under DNA damage stress, which contributes to genomic instability - a hallmark of many cancers.

Psödoüridin Sentaz 7 Enzim Eksikliği DNA Hasarına Neden Olan Ajanlara Karşı Maya Hücrelerini Hassas Hale Getirir

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 07.01.2025
Kabul tarihi:27.03.2025
Online Yayınlanma:16.09.2025

Anahtar Kelimeler:
Saccharomyces cerevisiae
Psödoüridin sentaz
Pus7
Metil metansülfonat
Ultraviyole ışık
DNA hasarı

ÖZ

RNA modifikasyonları, RNA moleküllerinin kimyasını değiştirmede önemli bir rol oynayarak onların yapılarını, işlevselliklerini ve kararlılıklarını etkiler. Bugüne kadar yapılan çalışmalarda çeşitli RNA türleri üzerinde 160'tan fazla farklı modifikasyon tipi tanımlanmıştır. Bu modifikasyonlardan biri, üridin (U) molekülünün psödoüridin (Ψ) formuna enzimatik dönüşümüdür ve bu süreç, psödoüridin sentaz (Pus) enzimleri tarafından gerçekleştirilir. Bu modifikasyon, hem kodlama yapan RNA (mRNA) hem de kodlama yapmayan RNA (ncRNA) türlerinde bulunur. Bu dönüşümden sorumlu enzimler arasında, Pus7 belirli gelişimsel ve fiziksel durumlarla ilişkilendirilmesi nedeniyle öne çıkmaktadır. Araştırmalar, Pus7'nin zihinsel yetersizlikler, gecikmiş konuşma gelişimi, mikrosefali ve kısa boy gibi durumlarla bağlantılı olduğunu göstermektedir. Bu rahatsızlıklarla ilişkisine rağmen, Pus7'nin tam biyolojik rolü ve mekanizması yeterince anlaşılmış değildir ve bu durum daha fazla araştırmaya olanak tanımaktadır. Bu çalışmada, *Saccharomyces cerevisiae* hücrelerinde Pus7'nin DNA hasar stresine karşı rolünü anlamayı

amaçladık. Bu amaçla, yabanıl tip ve *pus7*Δ hücreleri, ultraviyole (UV) ışığı ve metil metansülfonat (MMS) gibi DNA hasarına neden olan ajanların farklı konsantrasyonlarına maruz bırakıldı. Sağ kalım eğri analizleri ve okta ekim sonuçları, *pus7*Δ hücrelerinin 2 mM MMS ve 15 j/m² UV ışığı varlığında büyüme kusurları sergilediğini gösterdi. Bu sonuçlar, Pus7 enziminin yokluğunun maya hücrelerini DNA hasarına neden olan ajanlara duyarlı hale getirdiğini ortaya koymaktadır. Sonuçlar bize, birçok kanser türünün ayırt edici özelliği olan genomik dengesizliğe katkıda bulunan DNA hasarı stresi altında Pus7'nin rolünü araştırmak için acilen kapsamlı araştırmalara ihtiyaç duyulduğunu göstermektedir.

To Cite: Tardu M. Lack of Pseudouridine Synthase 7 Enzyme Sensitizes Yeast Cells to DNA-Damaging Agents. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2025; 8(4): 1613-1623.

1. Introduction

Chemical modifications frequently occur in biological macromolecules, serving to regulate their functional properties. Similar to DNA and proteins, RNA molecules undergo diverse chemical modifications that affect their structure, function, and stability within cells. Up to now, more than 160 distinct RNA modifications have been detected in both coding RNA (mRNA) and non-coding RNA (ncRNA), highlighting the complexity of RNA modification landscape (Cappannini et al., 2023). While these modifications were first discovered more than five decades ago, their importance in cellular functions has only recently garnered significant research attention, giving rise to the field of epitranscriptomics. These RNA modifications are now recognized as crucial contributors to essential biological processes and regulators of gene expression through various mechanisms (Frye et al., 2018; Jin et al., 2022).

Pseudouridine (Ψ), a structural isomer of uridine, is one of the most prevalent RNA modifications. It has been detected both in ncRNAs and as well as in certain mRNAs (Carlile et al., 2014; Lovejoy et al., 2014). Recent studies demonstrate that Ψ modifications in mRNAs influence key processes such as RNA metabolism, pre-mRNA splicing, and the accuracy of mRNA translation across a variety of organisms (Parisien et al., 2012; Nakamoto et al., 2017; Shaheen et al., 2019; Jalan et al., 2023). Transformation of uridine into Ψ is facilitated by pseudouridine synthase (Pus) enzymes, which are highly conserved in both prokaryotes and eukaryotes. Extensive studies in yeast and bacteria have shown that while these enzymes are not essential for survival under standard conditions, their absence can create a competitive growth disadvantage compared to wild-type strains (Gutgsell et al., 2000; Del Campo et al., 2001).

Recent research highlights that mutations in human *PUS* genes, including those encoding *PUS1*, *PUS4*, and *PUS7*, are linked to various disorders. For example, specific mutations in *PUS7* have been associated with intellectual disabilities, speech delays, and physical abnormalities such as microcephaly and short stature (Darvish et al., 2019; Shaheen et al., 2019; Han et al., 2022). Studies have shown that PUS7 predominantly targets RNA sequences with the UGUAR consensus motif. However, not all RNAs containing UGUAR motif undergo pseudouridylation, suggesting that these modification sites are dynamic and may vary depending on the conditions (Purchal et al., 2022).

In mammalian cells, environmental stress is known to trigger changes in pseudouridylation, particularly increasing modification rates in mRNA targets (Carlile et al., 2014; Li et al., 2015). This stress-induced adaptation is thought to be mediated by changes in the cellular localization of Pus enzymes, including Pus7 (Schwartz et al., 2014). In this study, we aimed to explore the role of Pus7 in *Saccharomyces cerevisiae* under conditions of DNA damage stress. To achieve this, wild-type and *pus7Δ* yeast strains were treated with varying concentrations of DNA-damaging agents, such as ultraviolet (UV) light and methyl methanesulfonate (MMS). The growth phenotypes of the wild-type and *pus7*Δ strains were then compared to assess Pus7's involvement in the cellular response to DNA damage. Our findings show that the presence of Pus7 enzyme increases the resistance of yeast cells to UV light and MMS.

2. Materials and Methods

2.1. Cell Cultivation and DNA Damage Assays

Wild-type (WT) and *pus7*Δ *Saccharomyces cerevisiae* cells were cultured as detailed in Tardu et al., 2019. Briefly, single colonies were picked and cultured overnight in YPD medium (yeast extract and peptone with 2% glucose), with or without selective antibiotics, at 30°C and 250 rpm. The overnight cultures were then diluted to an OD_{600}=0.05 in suitable growth media and grown to an OD_{600}=0.5 for DNA damage assays. For stress experiments, ultraviolet (UV) light and methyl methanesulfonate (MMS) were used as DNA-damaging agents. Stress treatments and survival assays were performed as follows: Mid-log phase WT cells (OD_{600} = 0.5) were plated in triplicate on YPD + 1.5% agar containing varying concentrations of MMS (0–8 mM) using the spreading method and incubated at 30°C for 3 days. For UV stress, WT and knockout cells on YPD + 1.5% agar plates were exposed to UV light (254 nm) at 1.5 J/m² for 10, 25, 45, and 60 seconds. The plates were then wrapped in aluminum foil and incubated in the dark at 30°C for 3 days. After incubation, colony images were captured using the iBright imaging system (Invitrogen, USA), and survival curves were generated from colony counts under each condition.

2.2. Spot Plating Assays

Spot plating assays were conducted using various concentrations of methyl methanesulfonate (MMS) (0, 1 mM, 2 mM) or ultraviolet (UV) light doses (0, 15 J/m², 37.5 J/m²). In summary, single colonies of wild-type (WT) and *PUS7* knockout yeast cells were selected from streak plates and inoculated into culture tubes containing 3 mL of YPD medium.Then, the cultures were incubated overnight at 30°C with shaking. The following day, cultures were diluted 1:10, and the optical density (OD) was measured at 600 nm using a spectrophotometer. Cells were then diluted to OD_{600} = 0.05 and grown until reaching OD_{600} = 0.5. Subsequently, four serial 1:10 dilutions of each sample were prepared. The diluted samples were transferred into 96-well microplates, and 6 µL aliquots were spotted onto the appropriate solid media. Next, plates were incubated at 30°C for 2–3 days. Images of the plates were captured with the iBright Imaging System (Invitrogen, USA). Spot plating assays were performed in two biological replicates for each condition to ensure reproducibility.

2.3. Construction of Saccharomyces cerevisiae pus7Δ deletion mutant

Saccharomyces cerevisiae pus7Δ mutants were generated following the protocol described in Winzeler et al., 1999. This method replaces the *S. cerevisiae PUS7* gene with the kanMX4 selectable marker gene through homologous recombination. The kanMX4 marker provides resistance to the antibiotic Geneticin (G418), facilitating the identification of successful gene knockouts. In summary, the kanMX4 cassette, flanked by 20-nucleotide sequences homologous to the upstream and downstream regions of *PUS7*, was amplified through PCR using the knockout primers specified in Table 1. The PCR products were purified from the gel using the Monarch DNA Gel Extraction Kit (NEB, USA) and then introduced into wild-type *S. cerevisiae* cells via the lithium acetate/single-stranded carrier DNA/PEG transformation method, following the protocol outlined by Gietz et al. (2007). Transformants were plated onto selective media containing 200 mg/L G418. After 3–4 days of incubation at 30°C, colony PCR was performed to screen for mutants using knockout confirmation primers (P3 and P4) detailed in Table 1. Successful integration of the kanMX4 cassette was confirmed through agarose gel electrophoresis of the PCR products.

3. Results and Discussion

To generate *pus7Δ* mutant, kanMX4 selection marker cassette, flanked by 20-nucleotide sequences homologous to the upstream and downstream regions of *PUS7*, is PCR amplified and subsequently used in transformation to achieve targeted replacement of *PUS7* (Figure 1A). Specifically, the kanMX4 cassette from pJET-kanMX4 (conferring resistance to Geneticin antibiotic) is PCR amplified using the appropriate knockout primers (P1 and P2) listed in Table 1. Following transformation, colonies were screened to identify *pus7Δ* mutants using PCR with knockout confirmation primers of P3 and P4 (Table 1). Expected PCR product sizes are 2614 bp for wild-type and 2167 bp for *pus7Δ* mutant yeast cells. Figure 1B confirms the successful deletion of the *PUS7* gene in *Saccharomyces cerevisiae* cells.

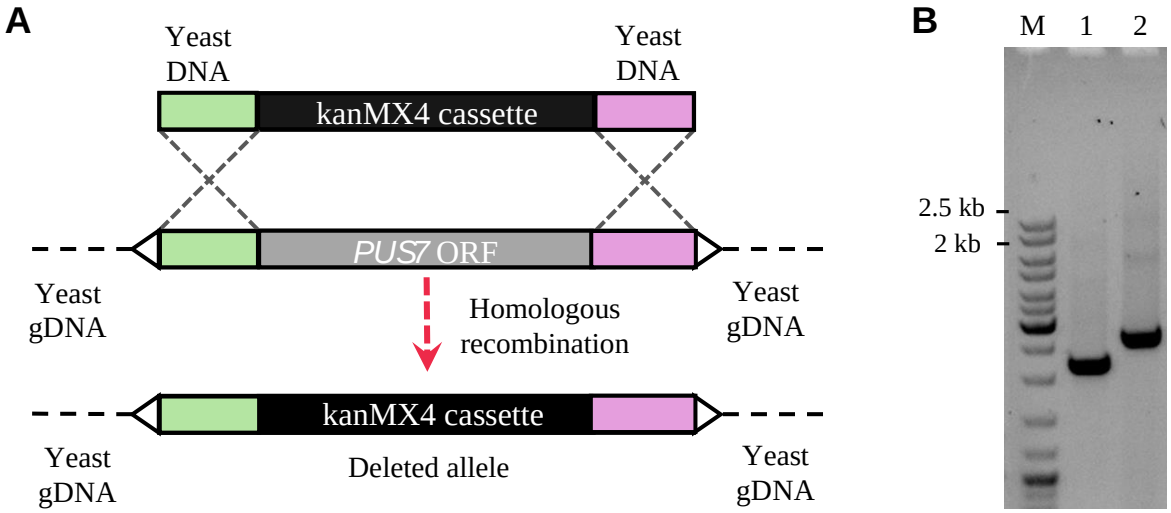

Figure 1. A) Strategy used in this study to generate *PUS7* deleted knockout cells. B) Agarose gel electrophoresis of knockout confirmation PCR. Gel image displaying the PCR products of *pus7Δ* (2167 bp) and wild-type (2614 bp) cells in lanes 1 and 2, respectively. M stands for molecular weight marker.

Table 1. List of oligonucleotides used in this study.

Oligo ID	Oligo Name	Sequence (5'-3')
P1	YOR243C_KO_F	TATCTGTTGCATGCCATGCGTACGCTGCAGGTCGAC
P2	YOR243C_KO_R	GCACCTCGTATAACCTTATCGATGAATTCGAGCTCG
P3	YOR243C_Confirmation_F	ACATCGATGATTTCTTGCAGTATTT
P4	YOR243C_Confirmation_R	CGCGAGAATATATCTGTCTTTTTGT

After successfully confirming the knockout, we performed DNA damage experiments using methyl methanesulfonate (MMS) chemical and ultraviolet (UV) light, both of which are well-known genotoxic agents that generate specific types of DNA damage. To achieve this, survival curves for wild-type *S. cerevisiae* cells were generated under different concentrations of MMS and UV light. As presented in Figure 2A, the percentage of yeast cell survival declined to 67.6% (± 14.8), 58.5% (± 7.7), 51.7% (± 8.5), 42.2% (± 6.0), and 0.25% (± 0.2) following treatment with 0.5 mM, 1 mM, 2 mM, 4 mM, and 8 mM MMS, respectively. MMS acts as a strong alkylating agent, methylating DNA bases mainly at the N3 position of adenine and the N7 position of guanine (Lee et al., 1992). This leads to unstable lesions, like N3-methyladenine, which interfere with DNA replication and transcription (Ma et al., 2008). If not repaired through the base excision repair (BER) pathway, it may cause to strand breaks (Boiteux et al., 2004). Similarly, UV treatment reduced yeast cell survival to 60% (± 15.8), 16% (± 4.1), and 0.4% (± 0.2) after exposure to 15 J/m², 37.5 J/m², and 67.5 J/m² UV, respectively (Figure 2B). UV radiation primarily causes the formation of cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts, which disrupt the structure of the DNA double helix and impede both replication and transcription (Cavga et al., 2017; Kavakli et al, 2017). These bulky lesions are primarily addressed through the nucleotide excision repair (NER) pathway, which eliminates the damaged DNA segments and synthesizes new strands (Krasikova et al., 2021). If left unrepaired, DNA damage caused by MMS and UV radiation can lead to mutations, cell cycle arrest, or apoptosis, emphasizing the critical role of efficient DNA repair systems in maintaining genomic stability (Boiteux et al., 2004; Lo et al., 2005). These complementary models offer valuable insights into cellular responses to genotoxic stress and the mechanisms of DNA repair. In conclusion, the survival analysis of *S. cerevisiae* showed a dose-dependent decrease in cell viability under MMS and UV exposure, confirming successful DNA damage induction (Figure 2). This validates the experimental setup and supports further investigation using spot plating assays on *pus7Δ* cells.

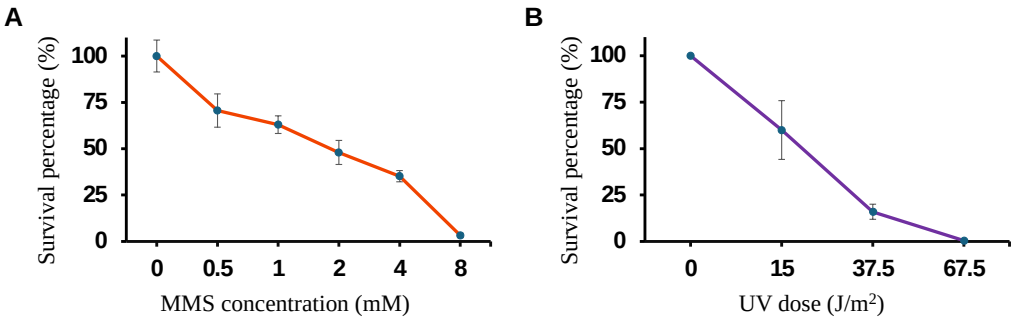

Figure 2. Survival percentages of wild-type *Saccharomyces cerevisiae* cells in the presence of A) methyl methanesulfonate (MMS), and B) Ultraviolet (UV) light.

The spot plating assay is an essential technique in yeast biology, utilized to examine the impact of genetic modifications or environmental conditions on yeast growth. We tested both wild-type and *pus7*Δ cells under MMS and UV DNA damage stress using this assay. Figure 3 shows the spot plating results that compares the growth of both strains under different conditions, including a control (YPD medium) and varying concentrations of MMS (0 mM, 1 mM and 2 mM). Data were recorded over 2 and 3 days with two biological replicates for each strain and condition. Both wild-type and *pus7*Δ strains show comparable growth, indicating no significant growth defect in the *pus7*Δ strain under standard YPD conditions (no-stress). However, while the wild-type strain tolerates 1 mM MMS well, the growth of the *pus7*Δ strain is noticeably impaired, indicating increased sensitivity to MMS (Figure 3). When the MMS concentration is increased to 2 mM, the sensitivity of the *pus7*Δ cells becomes even more obvious, demonstrating a severe vulnerability to higher MMS levels (Figure 3). Previous studies have shown that *PUS7* is involved in modifying RNA to enhance stability and translational fidelity under various stress conditions (Decatur et al., 2002; Shaheen et al., 2019). The absence of Pus7 enzyme could interfere with these processes, leading to increased sensitivity to genotoxic damage induced by MMS. Alternatively, Pus7 might indirectly affect the BER pathway by modifying RNAs that encode repair proteins, potentially influencing the DNA damage response.

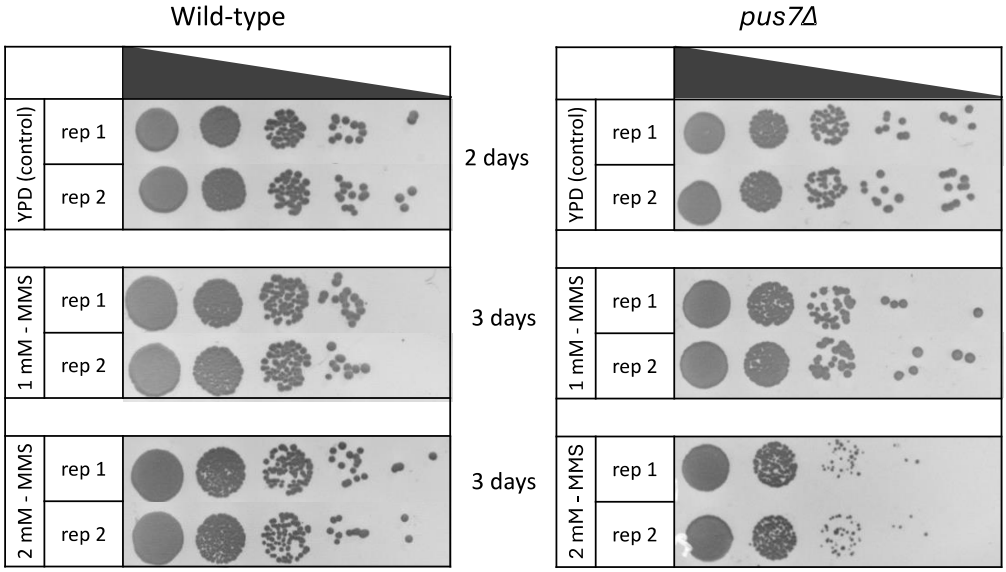

Figure 3. Spot plating assay of wild-type and *pus7*Δ *Saccharomyces cerevisiae* cells in the presence 0 mM (YPD (control)), 1 mM, and 2 mM methyl methanesulfonate (MMS). Plates are incubated at 30 ^{0}C for 2-3 days with two biological replicates (rep 1 and rep 2).

To further investigate the role of *PUS7* under DNA-damage stress, we evaluated its impact under UV stress conditions. At 15 J/m² UV exposure, the wild-type strain displays robust growth across replicates, whereas the *pus7*Δ strain exhibits a marked decrease in growth, suggesting sensitivity to DNA damage at this level (Figure 4). This effect is even more evident at 37.5 J/m², where wild-type growth is reduced but still visible, while the *pus7*Δ strain shows almost no growth, indicating extreme sensitivity to high

UV doses (Figure 4). Similar to MMS, UV exposure revealed a consistent pattern of increased sensitivity in the *pus7*Δ strain, suggesting a broader role for *PUS7* in mitigating diverse genotoxic conditions. These results emphasize the critical role of Pus7 in cellular tolerance to MMS- and UV-induced DNA damage and suggest its involvement in stress-response pathways, potentially through its pseudouridine synthase activity and influence on RNA stability or translation fidelity.

Multiple studies have revealed a strong link between PUS7 enzyme and various cancers (Cui et al., 2021; Du et al., 2022). For example, research has shown that *PUS7* is significantly upregulated in glioblastoma, a highly aggressive form of brain cancer (Cui et al., 2021). Similarly, in colorectal cancer, *PUS7* overexpression has been associated with increased metastasis, highlighting its role in cancer progression (Du et al., 2022) Moreover, elevated *PUS7* expression in non-small cell lung cancer (NSCLC) tissues has been observed, with patients exhibiting higher *PUS7* levels experiencing poorer prognoses (Zhang et al., 2023). In another study, it was shown that *PUS7*-dependent pseudouridylation of ALKBH3 mRNA inhibits gastric cancer cell proliferation and tumor growth. This suggests that *PUS7*'s catalytic activity plays a role in suppressing gastric cancer progression (Chang et al., 2024). These findings indicate that *PUS7* may play a significant role in modulating cancer cell responses to genotoxic stress by enhancing RNA pseudouridylation, a process that may facilitate adaptive stress responses and drive tumor progression. PUS7's involvement in multiple cancer types emphasizes its potential as a promising therapeutic target, particularly in malignancies characterized by elevated cellular stress levels.

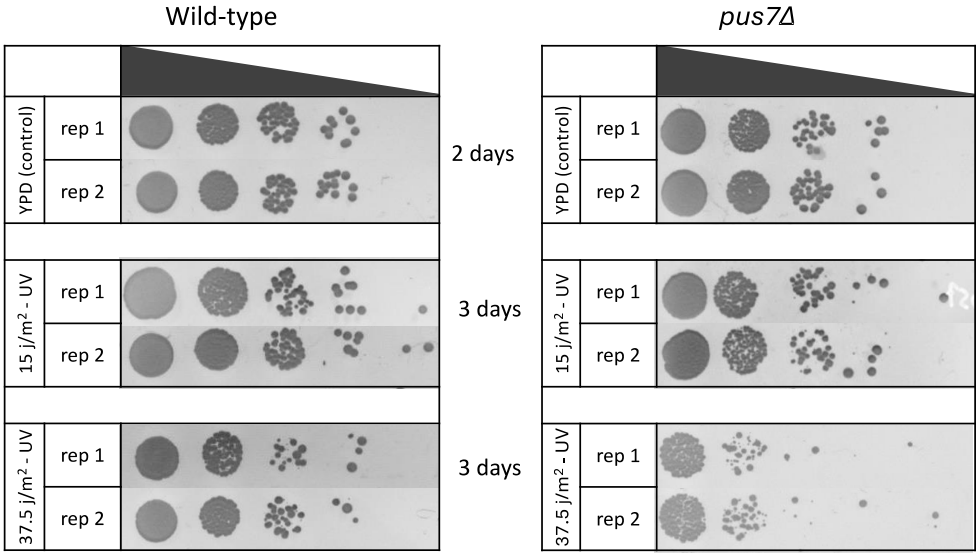

Figure 4. Spot plating assay of wild-type and *pus7*Δ *Saccharomyces cerevisiae* cells in the presence 0 j/m^2 (YPD (control)), 15 j/m^2, and 37.5 j/m^2 ultraviolet (UV) light. Plates are incubated at 30 ^{0}C for 2-3 days with two biological replicates (rep 1 and rep 2).

4. Conclusion

This study demonstrates that Pus7 is critical for yeast cell survival under MMS- and UV-induced DNA damage, particularly during elevated genotoxic stress. However, the molecular mechanisms underlying its protective function remain unclear. Exploring how Pus7 enhances resistance to DNA-damaging agents is essential for understanding its role in maintaining genome stability.

Beyond its role in DNA damage protection, Pus7 may also regulate DNA repair genes at the post-transcriptional level. Pseudouridylation enhances RNA stability and translation efficiency, suggesting that Pus7-mediated modifications may influence the expression of DNA repair factors. Investigating how Pus7 modulates gene expression under genotoxic stress could offer valuable insights into broader cellular stress response networks. To clarify its contribution to MMS and UV resistance, further studies should utilize transcriptomic analysis and complementation assays. These approaches will be crucial in uncovering the role of Pus7-mediated RNA modifications in genome stability and cellular adaptation to DNA damage.

Acknowledgements

This work was supported by research infrastructure provided through the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project No. 121C066) and the Scientific Research Projects Coordination Unit of Istanbul University (IU-BAP, Project No. FHZ-2024-40796).

Statement of Conflict of Interest

The author declares that he has no conflict of interest related to this work.

Author's Contributions

The author declares that he contributed 100% to this work.

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