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Expression Profile of PmiR-31, Novel npcRNA Of *Proteus Mirabilis* Under Different Growth Phases and Stress Conditions

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Abstract: *Proteus mirabilis* (*P. mirabilis*), known for its swarming motility, is a facultatively anaerobic, rod-shaped, Gram-negative bacterium. It forms elongated swarm cells and moves in a distinctive bull's-eye pattern. Proteus mirabilis significantly causes catheter-associated urinary tract infections. Its virulence factors include flagella, fimbriae, hemolysin, urease, and proteases. Flagella-related motility allows P. mirabilis to colonize the urinary system. The flagellum, composed of about 20 proteins, has a basal body that penetrates the cell wall, a curved hook, and a filament extending several micrometers. Previously, we knocked out npcRNA PmiR-137, expected to regulate flhDC, a master transcriptional activator for flagella synthesis, and performed a differential gene expression analysis. We discovered npcRNA PmiR-31, predicted to influence fliZ production via the Target RNA web tool. We analyzed PmiR-31 expression under various stress and growth phases using northern blot. The PmiR-31 was highly expressed in stress conditions but absent in the mutant strain. Since FliZ regulates flagella assembly, npcRNA PmiR-31 may repress fliZ translation, inhibiting flagella synthesis and promoting biofilm formation to evade host immune responses.

Keywords: Proteus mirabilis, non-protein coding RNA, PmiR-31, flagella biosynthesis

1. Introduction

Proteus mirabilis is a Gram-negative bacterium that accounts for more than 90% of Proteus infections. One of the distinguishing traits of Proteus mirabilis is its ability to differentiate from short vegetative swimmer cells to an elongated, highly flagellated swarmer form. It possesses swarming growth and adhesion factors, making it very adhesive and motile. Proteus mirabilis has the ability to resist capture by evading the host's immune system (Chakkour et al., 2024; Scavone et al., 2023). Proteus mirabilis is widely distributed throughout the environment, particularly in water, soil, and the gastrointestinal tracts of humans and animals. It is an opportunistic pathogen that accounts for less than 0.005% of healthy human gut flora (Jamil et al., 2025). Proteus mirabilis is a common cause of complicated urinary tract infection (UTI) in individuals with anatomical or functional urinary tract abnormalities, particularly in those who have long-term indwelling catheters and may develop catheter-associated UTI (CAUTI). It is responsible for around 3% of hospital infections and 44% of CAUTI in the United States.

Proteus mirabilis has a broad combination of virulence factors to achieve effective motility in the face of a stream of urine, nutrient intake, and host defense system protection. The primary virulence factors of *Proteus mirabilis* involve toxins (HpmAB), iron and zinc absorption systems, proteases, fimbriae, flagella, and urease, which hydrolyzes urea into ammonia and carbon dioxide (Filipiak et al., 2020). The bacteria uses flagella for motility, which enable both swimming and swarming (Yang et al., 2024).

Like many bacteria, it uses flagella to move across liquids and towards chemical gradients (Jamil et al., 2025). In a liquid culture, Proteus mirabilis exhibits a small form and a limited number of peritrichous flagella. Proteus mirabilis, however, divides into extraordinarily long (typically 20 to 80 µm, although cells longer than 100 µm have been reported), non-septate polyploid cells with hundreds to thousands of flagella on a nutrient-rich solid medium (Gmiter & Kaca, 2022). The flhDC genes are known to be the primary transcriptional regulators of bacterial flagella synthesis. Similarly, this master regulator is controlled both positively and negatively in Escherichia coli (E. coli) by directly binding to npcRNAs, indicating that npcRNAs have an important role in bacterial virulence (Takada et al., 2023).

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Messenger RNAs (mRNAs) and non-protein coding RNAs (npcRNAs) are two types of RNA molecules (Wang & Farhana, 2025). Protein translation is carried out by messenger RNAs. N contrast, non- protein coding RNA transcripts are RNA that do not encode proteins yet and are generated by a large number of genome sequences. Despite 90% of bacterial genomes being transcribed, mRNAs account for just a small fraction of RNAs (1-2%), while npcRNAs account for a larger proportion of total RNAs (Kishanraj et al., 2021a). Non-protein coding RNAs are categorized as either regulatory or housekeeping RNAs depending on their functions. Bacterial npcRNAs are a type of small regulatory RNA (sRNA) with less than 500 bp in length that performs important physiological functions in various biological processes by binding to the mRNA or proteins (Ratti et al., 2020). The regulatory actions of npcRNAs affect both bacterial pathogenesis and therapeutic treatment (Kishanraj et al., 2021b).

We previously identified and evaluated Hfq-bound npcRNAs in P. mirabilis, reported 182 npcRNAs, 13 of which bind to the Hfq protein, and validated their expression using Northern blot analysis (unpublished data). According to the target mRNA prediction tool, these 13 npcRNAs regulate the mRNAs associated with P. mirabilis virulence factors, such as flagella protein, which is essential for the bacteria's motility. The goal of this study is to delete the npcRNA PmiR-137 gene from the P. mirabilis genome since it has been shown to play an important role in P. mirabilis virulence. The experiment was then extended to investigate the expression profile of selected novel npcRNA PmiR-31 under various stress conditions, including oxidative stress, heat shock, acidic stress, osmolarity stress, stationary phase, and in wild-type and mutant strains of P. mirabilis. This npcRNA is believed to regulate the mRNA of flagella biosynthesis protein FliZ (fliZ), a protein required for the formation of flagella in P. mirabilis. While several studies have examined the regulatory mechanisms of flagella biosynthesis, the role of npcRNAs in this process remains underexplored. This study investigates the expression of PmiR-31 under different growth and stress conditions to clarify its regulatory function. The findings offer insight into the role of npcRNAs in gene regulation of P. mirabilis.

2. Method

2.1 Bacterial Gene Knock-Out in Single-Step

The npcRNA PmiR-137 was identified in P. mirabilis and selected for the gene knockout experiments because it is more likely to affect and modify the production of bacterial virulence factors. The npcRNA PmiR-137 was knocked out from the P. mirabilis genome using a one-step gene knockout method as described by prior work (Sanniraj et al., 2022).

2.2 Total RNA Extraction under Various Stress Conditions and Growth Phases

The total RNA was extracted from cell pellets of the wild-type strain at the exponential and stationary phases. Several stress conditions were applied to the wild-type during the exponential stage, including oxidative stress, osmotic stress, acidic stress, and heat shock stress. Meanwhile, the total RNA was extracted from the mutant strain during the exponential phase of bacterial growth without exposing it to any stress conditions.

In 10 ml of Luria Bertani (LB) broth, an overnight inoculum of P. mirabilis wild-type and mutant strains was introduced and incubated at 37°C with agitation at 180 rpm. The following day, 250 µl of the overnight culture was added to 250 ml of LB broth under various growth and stress conditions. The cultures were incubated at 37°C at 180 rpm. Total RNA was obtained from both wild-type and mutant cells at the mid-exponential phase (OD600 0.5–0.6).

Oxidative stress was induced in the wild-type cells by adding 5.6 µl of hydrogen peroxide (H_2O_2) during mid-exponential phase and incubating the culture for 30 minutes at 37°C and 180 rpm. To induce heat shock stress, the cell culture at the exponential phase was incubated at 42°C and 180 rpm for 30 minutes. For the osmolarity and acidic stress tests, cultures at OD_{600} 0.5–0.6 were treated with 3 g of sodium chloride and a few drops of hydrochloric acid, respectively, then incubated at 37°C at 180 rpm for 30 minutes. Bacterial cells were harvested at the stationary phase when OD600 reached 0.9–1.0. Total RNA was isolated from bacterial cells under various growth and stress conditions using the Trizol method as directed by the manufacturer. The extracted total RNA's quality and quantity were assessed using the NanoDrop Spectrophotometer and the Qubit 4 Fluorometer, respectively.

2.3 Northern Blot Analysis

The total RNA was isolated from various growth and stress conditions and separated on 8% polyacrylamide denaturing gels containing 7 M urea, then transferred to positively charged nylon membranes (Ambion Ltd, Cambridgeshire, UK) using a semi-dry blotting transfer method (Bio-Rad, USA). Northern blot hybridization was performed using radioactively labeled oligonucleotide probes (Table 1) that were complementary to the npcRNA PmiR-31 as described by Chinni et al. 2010 (Chinni et al., 2010; Kishanraj et al., 2021b).

Table 1. List of DNA oligonucleotide probes designed for Northern blot hybridization

npcRNA	Oligonucleotide sequences (5' to 3')
PmiR-31	GTCGATTGCAAGGTTCTCTT
5S rRNA	TGGCAGTTCCCTACTCTCACAT

3. Results and discussion

3.1 Differential expression of the npcRNA PmiR-31 in wild-type and mutant strains of P. mirabilis under varied environmental conditions.

PmiR-31, a novel npcRNA found in P. mirabilis, was selected to study the expression profile of this npcRNA under different environmental conditions along with the wild-type and mutant strain. In the P. mirabilis genome, the npcRNA PmiR-31 is located between the succinate dehydrogenase iron-sulfur protein (sdhB) and the 2-oxoglutarate dehydrogenase E1 component (sucA) with

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5' and 3' coordinates of 613505-613788 (Figure 1). In our previous study, the npcRNA showed low expression in the log phase but was highly expressed during the stationary phase. However, the expression in the exponential phase was low. Based on Figure 2, the npcRNA PmiR-31 was shown to be expressed during stress conditions.

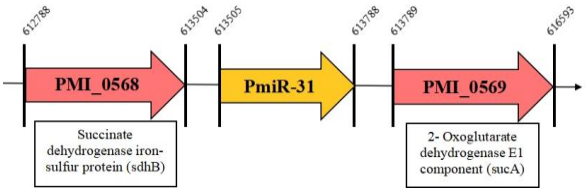

Figure 1. The genomic location of npcRNA PmiR-31 in *P. mirabilis*. Boxes indicate the gene's transcriptional orientation. The orange arrows represent gene locus markers.

According to the published genome of P. mirabilis H14320, the numbers indicate the genomic position of the npcRNA and its flanking genes. The npcRNA gene is represented by the yellow box.

The TargetRNA2 webtool predicts that the PmiR-31 target mRNA is the flagella biosynthesis protein (fliZ) (Table 2). The interaction between npcRNA PmiR-31 and fliZ mRNA occurs with a minimum free energy of -14.43 kcal/mol and a p-value of 0.001. The npcRNA interacts with fliZ from -7 to 9 at the 5'UTR region.

Flagella are complex surface structures that serve as the principal mode of motility for many bacterial species and allow numerous bacterial pathogens to attach, infiltrate, and produce virulence factors (Akahoshi & Bevins, 2022). More than 50 genes are involved in the biosynthesis and function of an E. coli or Salmonella enterica serovar Typhimurium flagellum. FlhDC is a class 1 operon whose products, FlhD4C2 heterohexamers, are essential for the expression of all other flagellar genes. The E. coli FlhD4C2 complex activates class 2 operons, including structural genes for flagella hook-basal-body components (type III secretion system) and the alternative sigma factor fliA (Avelino-Flores et al., 2022; Sassi et al., 2020). In E. coli and Salmonella enterica, the product of the fliA gene, σ28, regulates the transcription of class 3 genes producing filament proteins, hook-associated proteins, motor proteins, and various chemotaxis proteins (Kurniyati et al., 2023). FliZ, encoded in the fliAZY operon, is a FlhD4C2-dependent activator of class 2 gene expression. It promotes the expression of class 2 flagellar genes in Salmonella enterica (Das et al., 2018). Additionally, E. coli FliZ can block RpoS post-translationally, which prevents the development of curli fimbria. The FliZ protein contains a region similar to the core DNA binding domain of phage integrases while exhibiting evident posttranslational effects in Salmonella and E. coli (Ponath et al., 2022; Ravishankar et al., 2024). In Xenorhabdus nematophila, FliZ binds directly to the flhDC promoter to initiate transcription of class 2 flagellar genes (Bientz et al., 2024; Trouillon et al., 2023).

The binding of npcRNA PmiR-31 with the fliZ mRNA suggests that the expression of PmiR-31 suppresses the translation of fliZ mRNA as it might block the ribosome binding site of the mRNA.

The expression of PmiR-31 is high under all various stress conditions as well as in different growth phases of P. mirabilis compared to the mutant strain. The knockout of npcRNA PmiR-137 from the P. mirabilis genome could play a role in the lower expression of npcRNA PmiR-31. Since the absence of PmiR-137 may influence flagella synthesis, the bacteria could experience pressure for survival; thus, it may adhere to the host epithelial cell and start producing biofilm to survive and protect themselves from the host immune system (Sanniraj et al., 2022). This could explain the lower expression of npcRNA PmiR-31 in the mutant strain of P. mirabilis.

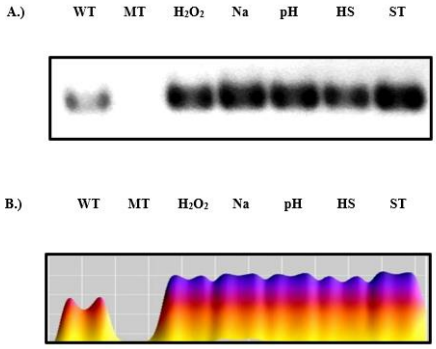

Figure 2. *Proteus mirabilis* npcRNA PmiR-31 expression profile. A.) Northern blot analysis of PmiR-31 expression in *P. mirabilis* under various environmental conditions (WT: Wild-type, MT: Mutant type, H_2O_2: Oxidative stress, Na: Osmolarity stress, pH: acidic stress, HS: heat shock and ST: Stationary phase). B.) ImageJ software was used to construct an interactive 3D surface graphic of the band intensities of the npcRNA northern blot.

Under stress conditions, the expression of PmiR-31 is higher compared to the wild-type strain, which could downregulate the expression of fliZ mRNA. The fliZ showed poor hybridization on the chip and no discernible change in transcription in the presence or absence of chlorine or H2O2 in E. coli (Roth et al., 2022). Our results predict that when the cells are exposed to oxidative stress under severe circumstances, the bacteria protect the cells through reduced permeability and biofilm formation (Shahryari et al., 2021). The expression of PmiR-31 is high during oxidative stress, osmotic stress, acidic stress, and heat shock stress, suggesting a decrease in FliZ protein production. Thus, the cells may proceed to form biofilm for their adaptation and survival.

These results show that npcRNA PmiR-31 expression is slightly higher during the stationary phase compared to the wild-type strain of P. mirabilis. According to Zhuang et al. (2019), the percentage of flagellated bacteria (PFB) further decreased when the culture approached the stationary phase, and the cells actively lost their flagella, as evidenced by the drastic reduction in the number of flagellated cells (Zhuang et al., 2019). The other stages of the E. coli development cycle and the accompanying regulatory circuits are closely linked with the stationary-phase response. At least five RNAP sigma subunits (σ70, σFliA, σS, σE, σ54), as well as the flagellar master regulator FlhDC and the

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second messengers cAMP, (p)ppGpp, and c-di-GMP, are key players in these circuits. For metabolic adaptation to available resources, and regulation of motility, cell shape, stress resistance, and biofilm functions during the exponential, post-exponential, and stationary phases of the growth cycle, the interaction of all these components is essential (Hengge, 2020; Hengge et al., 2023; Kędzierska-Mieszkowska, 2023). Interestingly, Salmonella's fliZ gene may also be directly related to biofilm development (Eran et al., 2020). As mentioned earlier, high expression of PmiR-31 could reduce the translation of the FliZ protein, which is directly associated with FlhDC in flagella formation. As a result, the bacteria may protect themselves to adapt against unfavorable conditions. Although TargetRNA predicts PmiR-31 interaction with fliZ mRNA, further validation using electrophoretic mobility shift assays (EMSA) or ribonucleoprotein immunoprecipitation (RIP) is required. Given that non-coding RNAs in Gram-negative bacteria often regulate multiple virulence pathways, future studies should investigate whether PmiR-31 affects urease expression, fimbriae production, or biofilm formation.

Table 2. Predicted target mRNA for PmiR-31 using TargetRNA2 tool

Target mRNA	Energy (kcal/mol)	P- Value
Flagella biosynthesis protein (fliZ)	-14.43	0.001

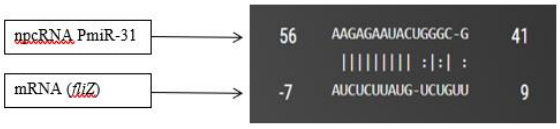

Figure 3. Sequence alignment information of npcRNA PmiR-31 with predicted target mRNA.

4. Conclusion

We have successfully obtained a P. mirabilis strain lacking npcRNA PmiR-137. Northern blot analysis was conducted to examine the differential expression of npcRNA PmiR-31 in both wild-type and mutant strains as well as under various stress conditions. Using the TargetRNA web tool, the potential role of this npcRNA was predicted to regulate the mRNA of the FliZ flagellar biosynthesis protein. Compared to the wild-type of P. mirabilis and under other stress conditions, our results showed that the expression of npcRNA PmiR-31 is significantly increased in stress conditions, as well as during the stationary phase. However, npcRNA PmiR-31 is absent in the mutant strain of P. mirabilis. Instead of prioritizing flagella synthesis in a stressful environment, the bacteria may adopt biofilm formation as an adaptive strategy.

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6. References

Akahoshi, D. T., & Bevins, C. L. (2022). Flagella at the Host-Microbe Interface: Key Functions Intersect With Redundant Responses. *Frontiers in Immunology*, *13*, 828758. https://doi.org/10.3389/fimmu.2022.828758

Avelino-Flores, F., Soria-Bustos, J., Saldaña-Ahuactzi, Z., Martínez-Laguna, Y., Yañez-Santos, J. A., Cedillo-Ramírez, M. L., & Girón, J. A. (2022). The Transcription of Flagella of Enteropathogenic Escherichia coli O127:H6 Is Activated in Response to Environmental and Nutritional Signals. *Microorganisms*, *10*(4), Article 4. https://doi.org/10.3390/microorganisms10040792

Bientz, V., Lanois, A., Ginibre, N., Pagès, S., Ogier, J.-C., George, S., Rialle, S., & Brillard, J. (2024). OxyR is required for oxidative stress resistance of the entomopathogenic bacterium Xenorhabdus nematophila and has a minor role during the bacterial interaction with its hosts. *Microbiology*, *170*(7), 001481. https://doi.org/10.1099/mic.0.001481

Chakkour, M., Hammoud, Z., Farhat, S., El Roz, A., Ezzeddine, Z., & Ghssein, G. (2024). Overview of Proteus mirabilis pathogenicity and virulence. Insights into the role of metals. *Frontiers in Microbiology*, *15*, 1383618. https://doi.org/10.3389/fmicb.2024.1383618

Chinni, S. V., Raabe, C. A., Zakaria, R., Randau, G., Hoe, C. H., Zemann, A., Brosius, J., Tang, T.-H., & Rozhdestvensky, T. S. (2010). Experimental identification and characterization of 97 novel npcRNA candidates in Salmonella enterica serovar Typhi. *Nucleic Acids Research*, *38*(17), 5893–5908. https://doi.org/10.1093/nar/gkq281

Das, C., Mokashi, C., Mande, S. S., & Saini, S. (2018). Dynamics and Control of Flagella Assembly in Salmonella typhimurium. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00036

Eran, Z., Akçelik, M., Yazıcı, B. C., Özcengiz, G., & Akçelik, N. (2020). Regulation of biofilm formation by marT in Salmonella Typhimurium. *Molecular Biology Reports*, *47*(7), 5041–5050. https://doi.org/10.1007/s11033-020-05573-6

Filipiak, A., Chrapek, M., Literacka, E., Wawszczak, M., Głuszek, S., Majchrzak, M., Wróbel, G., Gniadkowski, M., & Adamus-Białek, W. (2020). *Correlation of pathogenic factors with antimicrobial resistance of clinical Proteus mirabilis strains* (p. 2020.02.24.962514). bioRxiv. https://doi.org/10.1101/2020.02.24.962514

Gmiter, D., & Kaca, W. (2022). Into the understanding the multicellular lifestyle of Proteus mirabilis on solid surfaces. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.864305

Hengge, R. (2020). Linking bacterial growth, survival, and multicellularity – small signaling molecules as triggers and drivers. *Current Opinion in Microbiology*, *55*, 57–66. https://doi.org/10.1016/j.mib.2020.02.007

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Malaysian Journal of Science 44 (Special Issue 1): 25-29 (December 2025)

Hengge, R., Pruteanu, M., Stülke, J., Tschowri, N., & Turgay, K. (2023). Recent advances and perspectives in nucleotide second messenger signaling in bacteria. *MicroLife*, *4*, uqad015. https://doi.org/10.1093/femsml/uqad015

Jamil, R. T., Foris, L. A., & Snowden, J. (2025). Proteus mirabilis Infections. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK442017/

Kędzierska-Mieszkowska, S. (2023). Sigma factors of RNA polymerase in the pathogenic spirochaete Leptospira interrogans, the causative agent of leptospirosis. *The FASEB Journal*, *37*(10), e23163. https://doi.org/10.1096/fj.202300252RRR

Kim, S. W., Li, Z., Moore, P. S., Monaghan, A. P., Chang, Y., Nichols, M., & John, B. (2010). A sensitive non-radioactive northern blot method to detect small RNAs. *Nucleic Acids Research*, *38*(7), e98. https://doi.org/10.1093/nar/gkp1235

Kishanraj, S., Sumitha, S., Tang, T.-H., Citartan, M., & Chinni, S. V. (2021a). Comparative genomic identification and characterization of npcRNA homologs in Proteus vulgaris. *Journal of Biosciences*, *46*, 108.

Kishanraj, S., Sumitha, S., Tang, T.-H., Citartan, M., & Chinni, S. V. (2021b). Comparative genomic identification and characterization of npcRNA homologs in Proteus vulgaris. *Journal of Biosciences*, *46*, 108.

Kurniyati, K., Chang, Y., Guo, W., Liu, J., Malkowski, M. G., & Li, C. (2023). Anti-σ28 Factor FlgM Regulates Flagellin Gene Expression and Flagellar Polarity of Treponema denticola. *Journal of Bacteriology*, *205*(2), e00463-22. https://doi.org/10.1128/jb.00463-22

Ponath, F., Hör, J., & Vogel, J. (2022). An overview of gene regulation in bacteria by small RNAs derived from mRNA 3' ends. *FEMS Microbiology Reviews*, *46*(5), fuac017. https://doi.org/10.1093/femsre/fuac017

Raabe, C. A., Sanchez, C. P., Randau, G., Robeck, T., Skryabin, B. V., Chinni, S. V., Kube, M., Reinhardt, R., Ng, G. H., Manickam, R., Kuryshev, V. Y., Lanzer, M., Brosius, J., Tang, T. H., & Rozhdestvensky, T. S. (2010). A global view of the nonprotein-coding transcriptome in Plasmodium falciparum. *Nucleic Acids Research*, *38*(2), 608–617. https://doi.org/10.1093/nar/gkp895

Ratti, M., Lampis, A., Ghidini, M., Salati, M., Mirchev, M. B., Valeri, N., & Hahne, J. C. (2020). MicroRNAs (miRNAs) and Long Non-Coding RNAs (lncRNAs) as New Tools for Cancer Therapy: First Steps from Bench to Bedside. *Targeted Oncology*, *15*(3), 261–278. https://doi.org/10.1007/s11523-020-00717-x

Ravishankar, S., Baldelli, V., Angeletti, C., Raffaelli, N., Landini, P., & Rossi, E. (2024). Fluoropyrimidines affect *de novo* pyrimidine synthesis impairing biofilm formation in *Escherichia coli*. *Biofilm*, *7*, 100180. https://doi.org/10.1016/j.bioflm.2024.100180

Roth, M., Jaquet, V., Lemeille, S., Bonetti, E.-J., Cambet, Y., François, P., & Krause, K.-H. (2022). Transcriptomic Analysis of E. coli after Exposure to a Sublethal Concentration of Hydrogen Peroxide Revealed a Coordinated Up-Regulation of the Cysteine Biosynthesis Pathway. *Antioxidants*, *11*(4), Article 4. https://doi.org/10.3390/antiox11040655

Sanniraj, A., Chinni, S. V., Samuggam, S., Ong, E. B. B., Samrot, A. V., & Yin, L. S. (2022). Morphological And Physiological Responses To Npcrna Pmir-137 Knockout In Proteus Mirabilis. *Journal of Pharmaceutical Negative Results*, 723–729. https://doi.org/10.47750/pnr.2022.13.S09.82

Sassi, A. S., Garcia-Alcala, M., Kim, M. J., Cluzel, P., & Tu, Y. (2020). Filtering input fluctuations in intensity and in time underlies stochastic transcriptional pulses without feedback. *Proceedings of the National Academy of Sciences*, *117*(43), 26608–26615. https://doi.org/10.1073/pnas.2010849117

Scavone, P., Iribarnegaray, V., González, M. J., Navarro, N., Caneles-Huerta, N., Jara-Wilde, J., Härtel, S., & Zunino, P. (2023). Role of Proteus mirabilis flagella in biofilm formation. *Revista Argentina De Microbiologia*, *55*(3), 226–234. https://doi.org/10.1016/j.ram.2023.01.005

Shahryari, S., Talaee, M., Haghbeen, K., Adrian, L., Vali, H., Shahbani Zahiri, H., & Noghabi, K. A. (2021). New Provisional Function of OmpA from Acinetobacter sp. Strain SA01 Based on Environmental Challenges. *MSystems*, *6*(1), e01175-20. https://doi.org/10.1128/mSystems.01175-20

Takada, H., Kijima, K., Ishiguro, A., Ishihama, A., & Shimada, T. (2023). Genomic SELEX Reveals Pervasive Role of the Flagella Master Regulator FlhDC in Carbon Metabolism. *International Journal of Molecular Sciences*, *24*(4), Article 4. https://doi.org/10.3390/ijms24043696

Trouillon, J., Attrée, I., & Elsen, S. (2023). The regulation of bacterial two-partner secretion systems. *Molecular Microbiology*, *120*(2), 159–177. https://doi.org/10.1111/mmi.15112

Vogel, J., & Sharma, C. M. (2005). How to find small non-coding RNAs in bacteria. *Biological Chemistry*, *386*(12), 1219–1238. https://doi.org/10.1515/BC.2005.140

Wang, D., & Farhana, A. (2025). Biochemistry, RNA Structure. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK558999/

Yang, A., Tian, Y., & Li, X. (2024). Unveiling the hidden arsenal: New insights into Proteus mirabilis virulence in UTIs. *Frontiers in Cellular and Infection Microbiology*, *14*, 1465460. https://doi.org/10.3389/fcimb.2024.1465460

Zhuang, X.-Y., Guo, S., Li, Z., Zhao, Z., Kojima, S., Homma, M., Wang, P., Lo, C.-J., & Bai, F. (2019). *Dynamic production and loss of flagellar filaments during the bacterial life cycle* (p. 767319). bioRxiv. https://doi.org/10.1101/767319

DOI: https://doi.org/ 10.22452/mjs.vol44sp1.4
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