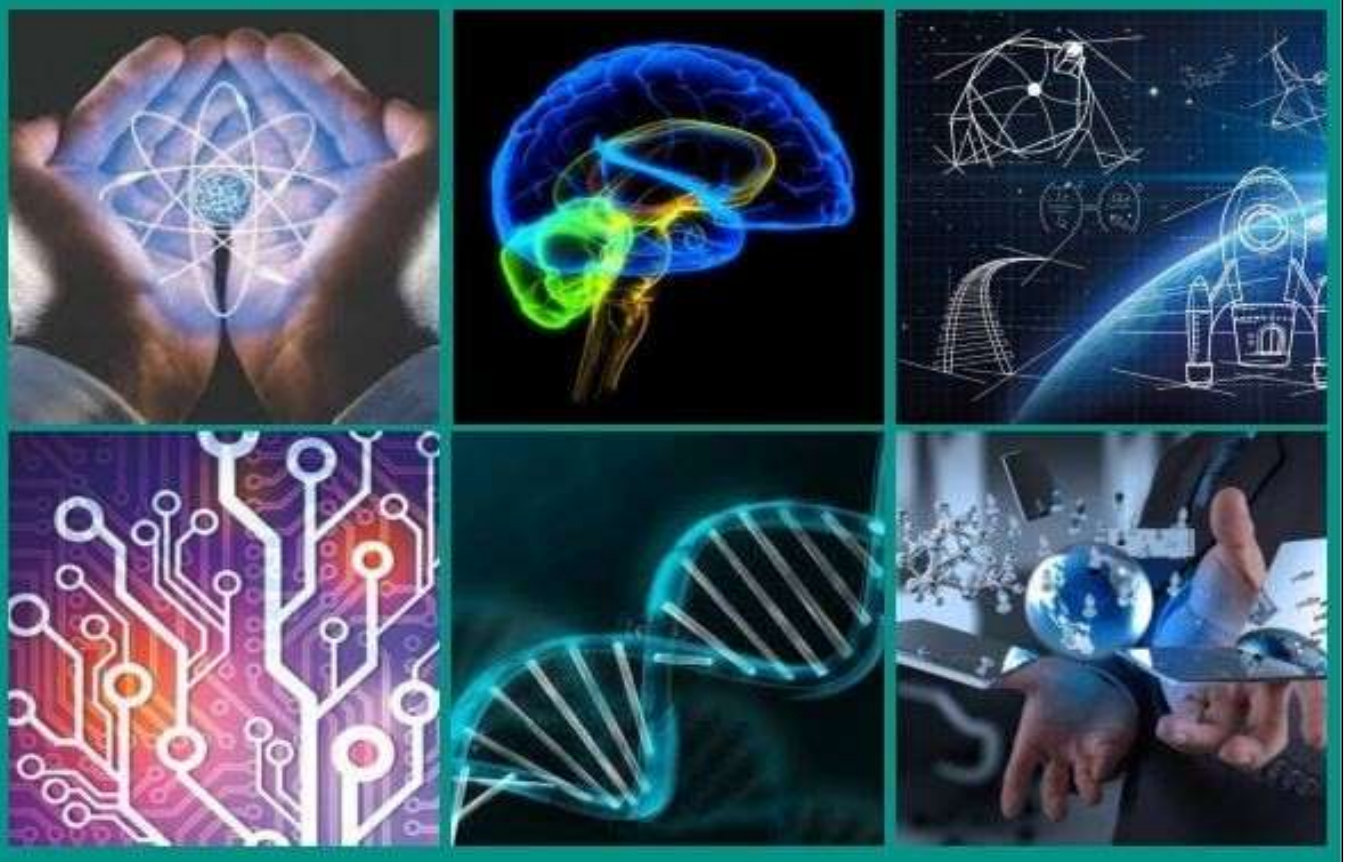
Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

Academia Open



By Universitas Muhammadiyah Sidoarjo

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

Table Of Contents

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

Originality Statement

The author[s] declare that this article is their own work and to the best of their knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the published of any other published materials, except where due acknowledgement is made in the article. Any contribution made to the research by others, with whom author[s] have work, is explicitly acknowledged in the article.

Conflict of Interest Statement

The author[s] declare that this article was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright Statement

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

EDITORIAL TEAM

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

Article information

Check this article update (crossmark)

Check this article impact (*)

Save this article to Mendeley

(*) Time for indexing process is various, depends on indexing database platform

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

Phenotypic and Molecular Profiling of Pseudomonas aeruginosa Under Thermal Stress Conditions: Profil Fenotipik dan Molekuler Pseudomonas aeruginosa Dalam Kondisi Stres Termal

Wameedh Sabah Shukur, wameedalsaady@gmail.com, pad.24.010@alkutcollege.edu.iq (*)
College of pharmacy,University of Al-Kut

(1) Corresponding author

Abstract

General Background: *Pseudomonas aeruginosa* is a versatile opportunistic pathogen frequently associated with hospital-acquired infections and notable for its ability to survive under diverse environmental conditions, including elevated temperatures encountered during fever and clinical disinfection procedures. **Specific Background:** Temperature variation represents a biologically relevant stressor that can alter bacterial physiology, virulence traits, and antimicrobial susceptibility patterns in clinical isolates. **Knowledge Gap:** Despite its clinical importance, the phenotypic and molecular responses of *P. aeruginosa* to thermal stress, particularly the integration of phenotypic assays with transcriptomic analysis, remain insufficiently characterized. **Aims:** This study investigated phenotypic alterations, antibiotic susceptibility modulation, and transcriptomic responses of clinical *P. aeruginosa* isolates exposed to thermal stress conditions. **Results:** Exposure to elevated temperatures produced measurable phenotypic changes, including modified growth rates, colony morphology, motility behavior, and biofilm dynamics, accompanied by shifts in antimicrobial susceptibility profiles and transcriptional reprogramming involving heat-shock proteins, stress-response regulators, virulence factors, and resistance-associated pathways. **Novelty:** The integration of phenotypic characterization with RNA sequencing provides a comprehensive molecular framework for understanding temperature-dependent adaptation in *P. aeruginosa*. **Implications:** These findings highlight temperature variation as a critical environmental signal shaping bacterial survival, virulence potential, and antimicrobial response, providing insights relevant to infection control strategies and clinical management of *P. aeruginosa* infections.

Highlights:

- Thermal exposure modifies bacterial growth patterns, colony structure, and biofilm dynamics.
- Elevated temperature conditions coincide with antimicrobial susceptibility modulation and stress-response activation.
- Integrated phenotypic assays and transcriptomic profiling reveal coordinated adaptive mechanisms.

Keywords: Pseudomonas Aeruginosa; Thermal Stress; Phenotypic Adaptation; Antibiotic Susceptibility; RNA Sequencing

Published date: 2026-03-11

Introduction

The opportunistic pathogen Pseudomonas aeruginosa is a highly versatile microorganism that is extensively distributed across a wide array of environments, including but not limited to soil, water bodies, and even artificial settings, demonstrating a remarkable ability to colonize human tissues. This colonization is particularly alarming in people who have compromised immune systems, such as those undergoing chemotherapy, living with HIV/AIDS, or suffering from chronic illnesses, where the likelihood of developing severe infections is significantly heightened. During instances of clinical infections, notable shifts in environmental conditions, particularly temperature changes towards mammalian body temperature, specifically around 37 °C, typically occur in tandem with infection processes. These temperature fluctuations could potentially trigger a variety of adaptive physiological responses within the pathogen, altering its behavior and interaction with the host. Nevertheless, the specific impact of such alterations on microbial populations, both within biofilms that adhere to surfaces and in planktonic states where they exist freely in liquids, is still largely unexplored and warrants extensive further investigation to fully understand the dynamics at play. Moreover, the pathogen's inherent ability to grow and thrive effectively even at lower temperatures, such as 28 °C, provides it with a unique competitive advantage over other microorganisms. This ability allows P. aeruginosa not only to persist outside human hosts within various environmental reservoirs but also raises significant concerns regarding the increased potential for biofilm-associated infections, particularly in climate-controlled environments where specific conditions may inadvertently favor its survival and proliferation. The implications of these characteristics in terms of public health and effective infection control measures need to be thoroughly considered to mitigate the various risks associated with this highly adaptable and resilient pathogen, thereby protecting vulnerable populations from potentially life-threatening infections. [1]

This study undertakes a comprehensive and detailed investigation into the significant phenotypic and molecular changes that arise when a specific strain of Pseudomonas aeruginosa is subjected explicitly to thermal stress conditions. The primary aim of this research is to identify and elucidate the intricate and complex mechanisms that support consistent growth even at elevated temperatures, which could otherwise pose a significant challenge to microbial survival. The research focuses on a particular wild-type strain of Pseudomonas aeruginosa, which consistently exhibits markedly different traits and behaviors compared to other strains when forming biofilms and interacting with various surfaces at lower temperatures, specifically within a precise range of 3 to 30 °C. Furthermore, the study meticulously characterizes the adaptive responses that are activated following a critical and abrupt shift from a baseline temperature to an elevated temperature of 37 °C, carefully observed over the course of several hours. This crucial shift in temperature is essential for understanding how the bacterium adapts to and survives in fluctuating and often unpredictable environmental conditions. This understanding ultimately sheds light on its remarkable resilience and potential for persistence in diverse and challenging ecological niches. By examining both the physiological

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

responses and the underlying molecular adaptations that occur under these pronounced thermal stress conditions, this research contributes significantly to a deeper and more intricate understanding of microbial behavior and the various survival strategies employed in the face of changing temperatures that can impact their environments and ecological fitness. [2]

Research Problem

Clinical P. aeruginosa isolates are frequently exposed to elevated temperatures during infection (e.g., fever) and hospital disinfection processes. The extent to which thermal stress modulates phenotypic behavior, antibiotic susceptibility, and gene expression profiles remains insufficiently characterized, limiting accurate prediction of bacterial responses during infection and treatment

Background on Pseudomonas aeruginosa and Thermal Stress

Pseudomonas aeruginosa, a Gram-negative bacterium classified within the γ-proteobacteria class, is widely recognized as a highly versatile opportunistic pathogen that has the ability to cause infections in both plants and animals alike. This remarkable species has shown a unique capacity to persist in a variety of ecological niches, encompassing environments such as soil, water, and also within living tissues, such as the lungs of cystic fibrosis patients. This highlights the bacterium's dual importance as a valuable model organism for scientific research as well as a significant public health threat that demands attention and management. P. aeruginosa exhibits a notable ability to respond to various environmental stressors, including temperature variations, which may have crucial implications for its physiological behavior and overall survival strategies. This bacterium is capable of thriving at temperatures ranging from a chilly 4 °C to an impressive more than 40 °C, illustrating a remarkably broad thermal tolerance for a mesophilic organism. Specifically, P. aeruginosa can effectively colonize the lungs of cystic fibrosis patients at an optimal temperature of 34 °C; however, it has been observed that its virulence diminishes at lower temperatures, such as 8 °C. This observation underscores the potential influence of temperature on the bacterium’s transition from an environmental reservoir into a mammalian host. Despite the considerable ecological and clinical significance associated with temperature adaptation, there remains a significant gap in our understanding regarding the effects of thermal stress on the bacterium’s transcriptome. Moreover, the phenotypic and molecular profiling of P. aeruginosa during periods of heat-stress exposure has not been systematically characterized, revealing a crucial area for further investigation and research. [2]

This existing knowledge gap has served as a significant motivation for the current investigation focusing on the phenotypic and molecular responses of Pseudomonas aeruginosa to thermal stress. The research specifically targets P. aeruginosa strain PA4, a strain that has been extensively utilized in laboratory settings due to its valuable characteristics. The study carefully considers various temperature treatment ranges, specifically from 30 °C to 40 °C, which represent elevated temperatures that can induce stress responses, as compared to another range from 23 °C to 30 °C, which corresponds to a more typical environmental range. These ranges are reflective of high heat-stress conditions and moderate heat-stress

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

conditions, respectively, allowing for a thorough exploration of the organism's responses. The core objectives of this study are multifaceted: 1) to determine the phenotypic changes that occur when the organism is exposed to elevated temperatures; 2) to profile various adaptations, including transcriptional, metabolic, genomic, and proteomic changes that manifest during this thermal stress; and 3) to elucidate and uncover any relationships that exist between the genotype of the strain, its phenotype, and the specific growth conditions it is subjected to. A more comprehensive understanding of the different phenotypic and molecular responses that arise from high-temperature treatment could potentially aid in the development of effective, target-based therapies in the future, enhancing treatment strategies against this opportunistic pathogen. [1]

Methodological Framework

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium that serves as an opportunistic pathogen primarily associated with endemic infections among individuals who are immunocompromised and with outbreaks occurring in patients who are mechanically ventilated. This organism demonstrates a remarkable ability to thrive in a diverse range of environments, including naturally contaminated water and soil, irrigated crops, sinks, and disinfected hospital supplies. It possesses an extensive and broad profile of metabolic versatility, being capable of utilizing over one hundred different carbon sources. This ability significantly sets it apart from other nonfermenters such as Acinetobacter baumannii, Burkholderia cepacia, and Stenotrophomonas maltophilia. In clinical settings, Pseudomonas aeruginosa commonly exhibits pseudo- and mucoid colony morphologies. The former morphology predominates during the early stages of infections, while a switch to the latter often occurs as infections progress, which is frequently associated with biofilm formation. This shift is indicative of the bacterium's demand for amino acids that become critical in restricting overall growth. Additionally, the ambient temperature in a given environment influences not only the growth rate of this bacterium but also its metabolism and production of various virulence factors. These factors, in turn, directly or indirectly affect the expression of numerous regulatory genes, including those that are controlled by AraC, LysR, and LacI-type transcriptional regulators. Understanding these dynamics is essential for developing effective strategies for treatment and prevention in clinical settings where such infections can pose severe threats to vulnerable patients. [1] [3] [4]

Pathogens possess the remarkable ability to sense and respond to a variety of environmental changes, including significant temperature fluctuations, which are often associated with fever and play a crucial role in enabling the transition to a parasitic lifestyle. Such thermal stresses create a favorable environment for the human pathogen Staphylococcus aureus, leading to enhanced pathogenicity and an increased capacity to cause disease. Conversely, temperature shifts encountered within non-human hosts can have the opposite effect, potentially diminishing virulence and the ability of these pathogens to thrive. The phenomenon of temperature mismatches between the ambient environment and the host body is frequently observed, especially after pathogens are transmitted from their environmental reservoirs. This mismatch prompts a

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

carefully coordinated response orchestrated by CbrAB—the principal regulatory system for carbon sources—as well as the alternative sigma factor σE, which collectively manage temperature adaptation when conditions are at 30 °C and below. This temperature range is especially relevant for understanding the ambient environment of the opportunistic pathogen Pseudomonas aeruginosa. Consequently, the genomic, transcriptomic, and phenotypic responses to thermal stress are thoroughly investigated across an extensive range of temperatures. This range encompasses ambient, physiological, and post-physiological set points, providing a comprehensive understanding of how these pathogens adjust and adapt to varying thermal conditions to ensure survival and maintain pathogenic potential.

A. Experimental Design

Pseudomonas aeruginosa has increasingly become recognized as a significant opportunistic pathogen in the realm of human health, contributing to noteworthy morbidity and mortality rates in hospitalized patients. This bacterium has also been identified prominently as an increasingly adaptive microorganism that responds to climate change in various soil ecosystems. The environmental microbe possesses the remarkable ability to reproduce across a broad temperature spectrum, ranging from a low of 4 degrees Celsius to a high of 42 degrees Celsius. However, comprehensive molecular profiling that examines the intricacies of temperature adaptation remains limited. The primary aim of this study is to delve into the temperature-aware phenomic and molecular profiles during growth under conditions that are non-optimal for P. aeruginosa. To achieve this goal, various factors such as colony morphology, motility, the capacity for biofilm formation, and the production rate of a distinctive blue-green pigment were meticulously characterized. Furthermore, the transcriptomic and multi-omics data were thoroughly analyzed at multiple temperatures, specifically at 20, 25, 28, 33, 37, 42, and an elevated threshold of 44 degrees Celsius. These innovative approaches provide valuable insights into the temperature-dependent phenotypic alterations and the multi-omics changes exhibited by P. aeruginosa over the complete spectrum of growth under liquid-nutrient-requirement conditions. [2] [5]

B. Phenotypic Assessments

Thermal stressors, particularly those represented by elevated temperatures, exert significant influence on the growth patterns, behavioral traits, and morphological characteristics of Pseudomonas aeruginosa, a bacterial species widely recognized for its opportunistic pathogenicity in human hosts. In order to elucidate the intricate and multifaceted temperature-mediated responses exhibited by this organism, an exhaustive and comprehensive evaluation of its phenotypic adaptations was meticulously planned and conducted. To achieve this, a broad series of specialized assays were employed systematically to monitor and assess temperature-dependent alterations across a multitude of biological parameters, which included growth kinetics, variations in colony morphology, different motility patterns, dynamics associated with biofilm formation, levels of pyocyanin production, as well as tolerance assessments to both oxidative and osmotic stresses; all while additionally evaluating heat inactivation responses when subjected to varying

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

thermal conditions. Growth kinetics were measured rigorously across a wide range of temperatures that were set methodically at 22, 30, 37, and 40˚C under a variety of media conditions, specifically designed to gauge the organism's adaptability and responsiveness to thermal stress. Notably, expanded colony sizes were prominently observed at the elevated temperature of 40˚C for cultures that were grown in minimal medium specifically supplemented with 0.2% malate as a designated carbon source, which provided useful insights into the organism's metabolic responses. In terms of motility, P. aeruginosa PA4 exhibited a clear and marked ability to migrate well beyond the original inoculation site on motility agar at both 30 and 37˚C, thereby revealing a clear facilitation of movement and enhanced locomotion under these warmer conditions. However, motility was markedly absent when cultures were held at either 22 or 40˚C, indicating a complex relationship between temperature fluctuations and locomotion capabilities of this bacterium. Comprehensive biofilm assessments conducted under both closed and open well formats revealed an intriguing and distinct inverse relationship between biofilm production and growth temperature, highlighting how different thermal conditions can profoundly influence microbial community structures and their functional dynamics. Furthermore, under suboptimal growth conditions induced by thermal exposure, heat-exposed populations of P. aeruginosa displayed the formation of characteristic micro-colonies along with a noticeable increase in pigment production, which may suggest specific stress responses that are adapted to addressing unfavorable or challenging environmental conditions. Assays specifically targeting pyoverdine and hydrogen cyanide production yielded negative results, indicating that under certain specific stressors, the regulatory mechanisms in place governing these processes may become altered or entirely curtailed. Remarkably, P. aeruginosa PA4 successfully maintained its viability post-exposure to an elevated temperature of 45˚C for a duration of 30 minutes; this resilience was further substantiated by the detection of enriched mutations and high-frequency variants emerging following selective growth phases, which highlights the organism's remarkable capacity to adapt and thrive in extremely adverse thermal conditions. [1]

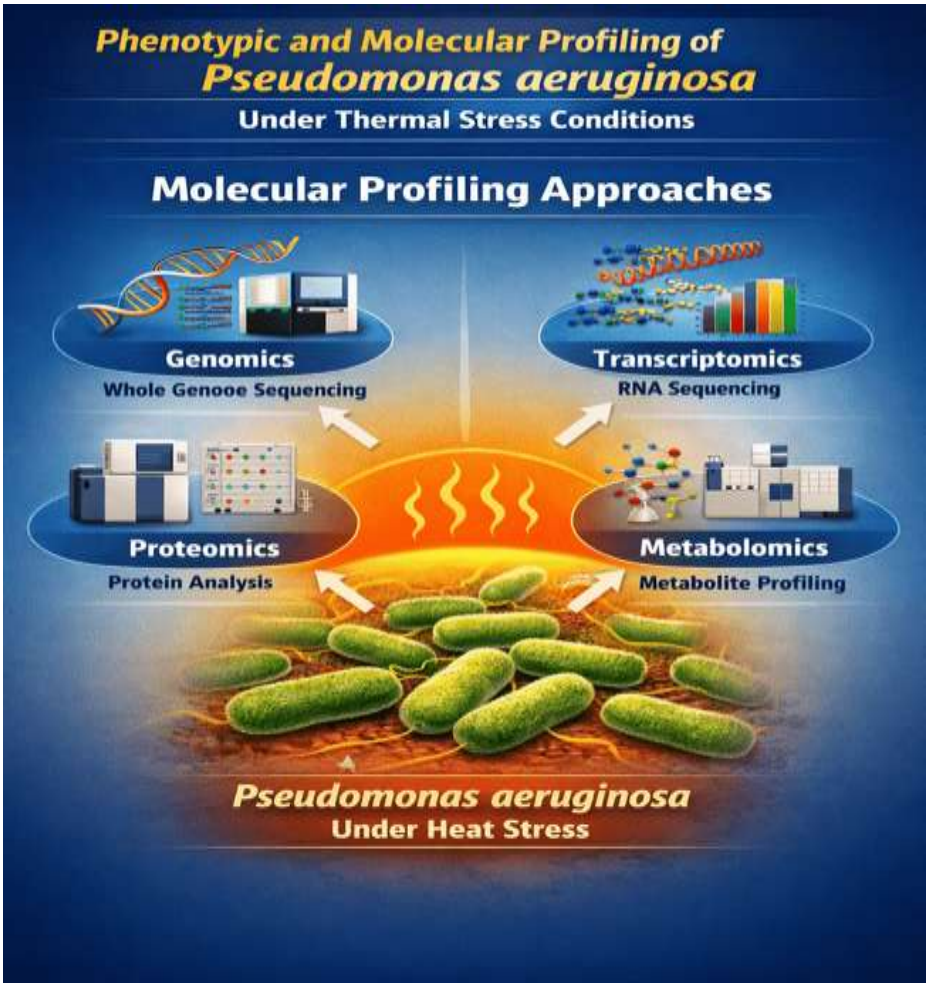
The dynamic interplay between temperature and various stressors was further examined through tolerance tests. Resistance to hydrogen peroxide, sodium chloride, sodium carbonate, and sodium acetate was retained at 37˚C, whereas 40˚C exposure resulted in enhanced susceptibility. Examination with fluorescent probes for membrane integrity, metabolic activity, and superoxide production indicated altered resilience—exposure to 37˚C and subsequently to higher temperatures was more detrimental than vice-versa. Notably, a precise 40˚C step-hold for precisely 30 minutes allowed survival, emphasizing the significance of timely recovery periods in expediting population re-establishment. [6]

C. Molecular Profiling Approaches

Pseudomonas aeruginosa is widely recognized as a common opportunistic pathogen that frequently causes infections in individuals with cystic fibrosis as well as in those who are immunocompromised. It also poses a significant threat in cases related to burn wounds, endocarditis, and ventilator-associated pneumonia. This bacterium is categorized under the Group of Environmental Pathogens, which are

specifically adapted to thrive in a wide range of temperatures, particularly between 4 and 30 °C. Due to its resilience and adaptability, P. aeruginosa can be found in various natural environments, including but not limited to soil, water bodies, and a variety of vegetation types. Experimental studies have provided insights that demonstrate that growth at elevated temperatures significantly influences its virulence factors, which are essential for establishing infections in hosts. When cultured at the human body temperature of 37 °C, P. aeruginosa exhibits a growth rate that is approximately 25% higher, an increase in pigment production that is six times greater, and biofilm formation that rises by more than 70% when compared with the standard culture temperature of 28 °C. These impactful temperature-induced phenotypes highlight why this pathogen serves as a prominent model for researchers aiming to investigate temperature-induced phenotypic changes and molecular adaptations in bacteria as shown in Figure (1) . [2] [7] [8]

Figure 1. *Molecular Profiling Approaches*



Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13865

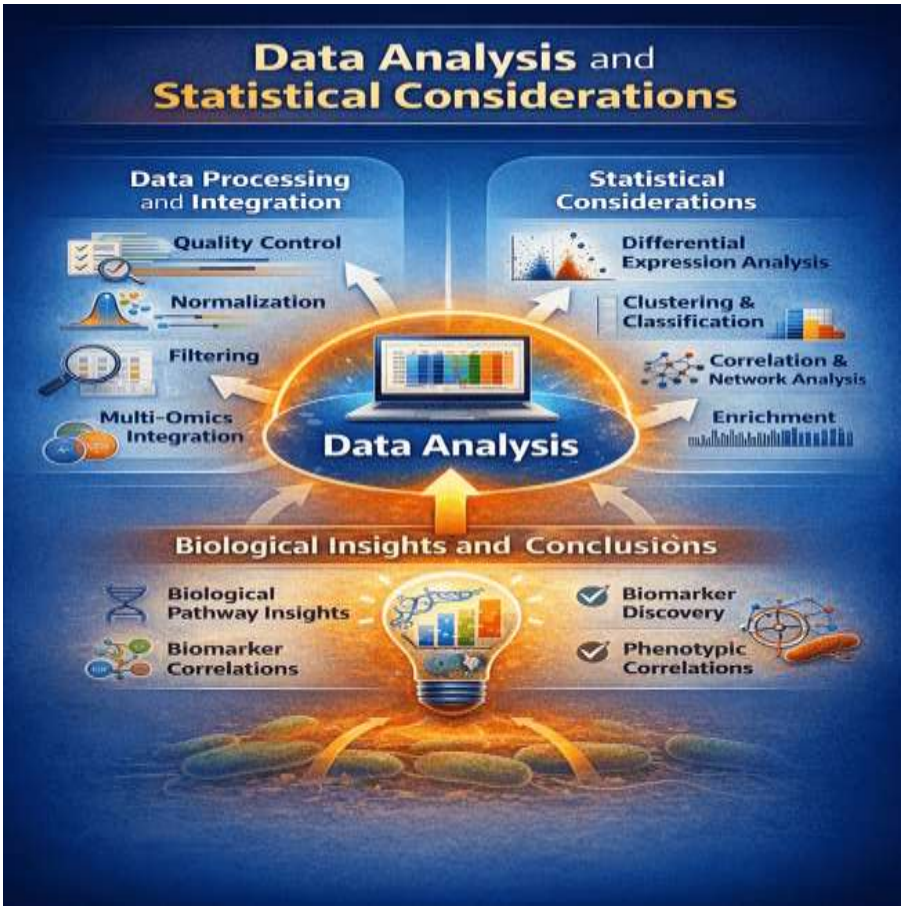
D. Data Analysis and Statistical Considerations

Data were preprocessed using the fastq-sanger quality format. For transcriptomic data, sequencing reads were mapped onto the P. aeruginosa PAO1 reference genome (NCBI accession number NC_002516) using the Spliced Transcripts Alignment to a Reference (STAR) aligner, adopting two-pass mode 2. Quality control was performed to assess overall read quality and compare pre- and postprocessing per sample using FastQC v0.11.3

(https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Genomic and proteomic data were processed using Galaxy (https://usegalaxy.org/; a web-based platform allowing management of bioinformatic data) to assess contamination.

Transcriptomic and metabolomic data were normalized to account for systematic biases and for the detection of genuine biological differences using a combination of approaches 7. The modelling framework was adjusted to enable integration of multiple data types and the generalized linear model. Differential analysis was performed applying a negative-binomial distribution model to consider overdispersion and fitted using the R package DESeq2 , as shown in Figure (2)

Figure 2. *Data Analysis and Statistical Considerations*



(http://bioconductor.org/packages/deSeq2/) for both transcriptomic and proteomic data (v1.4.5). Proteomic and genomic analyses were conducted independently to avoid assumptions that the observed responses would affect the underlying mutations. After mapping sequencing reads onto the reference genome, variant calling for genomic analysis (SNVs and InDels) was conducted with the free bayes and varscan2 software (https://github.com/vcflib/vcflib). Multi-omics integration across transcriptomic and metabolomic data was performed with the R package mixOmics.

Statistical analyses were thoroughly performed utilizing the R package stats v3.4.3, which is an essential tool for conducting various statistical evaluations. For the comprehensive phenotypic assessment of motility, biofilm formation, and thermal tolerance among the subjects under study, statistical significance was meticulously determined using the Kruskal-Wallis test, which is particularly effective for comparing three or more independent groups. Following this, Dunn's post-hoc test was employed to delve deeper and identify specifically which groups differed from each other significantly. Additionally, to compare pigment production in different environments, specifically on solid versus liquid medium, a one-way ANOVA was adopted, allowing for a clearer understanding of the variance across the different conditions tested. Prior to conducting these tests, the Shapiro-Wilks normality test was first applied rigorously to identify any deviations from a Gaussian distribution within the data sets; in instances where normality was not present, the non-parametric Kruskal-Wallis test was appropriately employed as it is more suitable for non-normally distributed data. Moreover, to ensure the reliability of the findings, multi-test adjustments across the genome were conducted utilizing the Benjamini–Hochberg procedure. This method is crucial in controlling the false discovery rate, ensuring that the results maintain a level of statistical rigor. The threshold for log2 fold-change was set at 1.5, which helped in determining the biological significance of the results obtained from the statistical evaluations. [9]

Materials and Methods

A. Study Design

An experimental laboratory-based study using clinical isolates of P. aeruginosa, combining phenotypic assays, antimicrobial susceptibility testing, and RNA sequencing.

B. Clinical Isolates

Clinical isolates will be collected from hospital specimens (burn wounds, respiratory samples, urine, blood) following ethical approval. Identification will be confirmed using standard microbiological methods and PCR targeting species-specific genes (e.g., oprL).

C. Phenotypic Identification

1. Gram staining and microscopy
2. Culture on Cetrimide agar and Blood agar
3. Oxidase test and biochemical profiling (API 20NE)

D. Thermal Stress Exposure

Standardized bacterial suspensions (0.5 McFarland) will be incubated at: - Control temperature: 37°C

- Thermal stress conditions: 42°C and 45°C for defined exposure periods

E. Growth and Phenotypic Assays

1. Growth kinetics measured by OD600
2. Colony morphology assessment
3. Biofilm formation assay using crystal violet microtiter plate method

F. Antibiotic Susceptibility Testing

1. Disk diffusion method (CLSI guidelines)
2. MIC determination using broth microdilution
3. Antibiotics tested: carbapenems, cephalosporins, fluoroquinolones, aminoglycosides

G. RNA Extraction and RNA-Seq Analysis

1. Total RNA extraction using commercial bacterial RNA kits
2. RNA quality assessment (Bioanalyzer)
3. Library preparation and Illumina sequencing
4. Bioinformatic analysis: quality control, read alignment, differential expression analysis

H. Statistical Analysis

1. Descriptive statistics (mean ± SD)
2. Paired t-test or ANOVA for phenotypic and AST comparisons
3. Differential gene expression analysis with false discovery rate correction
4. Correlation analysis between phenotypic traits and transcriptomic data

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

DOI: 10.21070/acopen.11.2026.13865

Expected Results

- Reduced growth rates and altered biofilm formation under thermal stress
- Modulation of antibiotic susceptibility profiles
- Upregulation of heat-shock proteins, stress-response regulators, and resistance-associated genes

I. Phenotypic Responses to Thermal Stress

Pseudomonas aeruginosa, a remarkably versatile pathogen, is capable of causing various infections in both humans and animals alike. These infections frequently arise in individuals with compromised immune defenses, such as those suffering from chronic illnesses or those undergoing medical treatments that weaken their immune systems. High temperatures, which often accompany fever during infection, serve to inactivate many bacterial pathogens that threaten human health. However, it is noteworthy that some pathogens, including Pseudomonas aeruginosa, have evolved mechanisms that allow them to survive and even replicate at approximately 37 °C, which corresponds to the normal human body temperature. Furthermore, while bacteria can replicate at temperatures reaching 43 °C, the fitness of these organisms typically decreases rapidly as they move outside of the normal physiological range. After exposure to increased heat, metabolic activity can resume in bacterial populations within a span of minutes or hours, even in instances where those populations cannot proliferate at the elevated temperatures. This phenomenon allows for adjuvant treatments to be conducted at higher temperatures, which permits researchers to carry out metabolic measurements without requiring growth, thereby aiding in the identification of mutations that may affect bacterial fitness specifically under heat stress conditions. As a result of these factors, P. aeruginosa strains are most commonly cultivated on saturated plates at the standard temperature of 37 °C. Interestingly, there have been considerably fewer studies conducted at 28 °C, and incubation of these organisms at 43 °C remains a relatively rare practice. [10] [2]

J. Transcriptomic and Genomic Adaptations

Pseudomonas aeruginosa adapts transcriptionally and genomically to thermal stress, as measured through RNA deep sequencing, mutation spectra, and allele frequencies. Exposure to elevated temperatures results in substantial shifts in expression of genes such as the primary sigma factor rpoD, numerous virulence factors, and genes involved in the synthesis of pigments, exopolysaccharides, and biofilm formation in the cystic fibrosis pathogen P. aeruginosa strain PA14. These observations mirror similar adaptation to the mammalian–host-related temperature of 37 °C. Biofilm-type modulation of rpoD expression contributes to greater colony-size variation on solid media and underpins extensive transcriptional remodelling during early biofilm establishment. Accompanying temperature-mediated shifts in P. aeruginosa physiology include

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

increased production of the pyrogenic virulence factor 1-phenazine–1-carboxylic acid and altered biofilm dynamics. Bioinformatic analyses of P. aeruginosa genomes from diverse environments reveal that heat-exposed cultures accumulate more mutations than those under optimal growth conditions, with pathogenicity islands being preferentially targeted. Genomic profilers indicate that the most prevalent mutations occur in a modulatable ribosomal RNA methyltransferase associated with increased thermal–stress resilience; supplementation with an aminoglycoside that inhibits this target restores temperature–sensitivity [11] [2].

K. Proteomic and Metabolomic Alterations

In response to thermal stress, Pseudomonas aeruginosa experiences substantial proteomic and metabolomic changes, providing insight into adaptive strategies at a systems level. Temperature significantly influences protein abundance, leading to differential expression of over 500 proteins, and commonly drives shifts in post-translational modification status. Adaptation is accompanied by alterations in the production of key metabolites and downstream proteins, revealing tight regulatory coupling between primary and secondary metabolism even under minimal nutritional variation. Perturbations in metabolic fluxes are evident for primary pathways (glycolysis, TCA cycle, central nitrogen, and phosphorous metabolism) and several secondary routes (tryptophan, phenylalanine, and polyhydroxybutyrate biosynthesis), with transcriptional, proteomic, and metabolomic analyses indicating complex layered regulation. Cross-species comparisons reveal conservation of temperature-dependent proteomic and transcriptomic signatures with largely parallel absolute abundance alterations, whereas an unexpected divergence in metabolomes under common heat shock treatments highlights the scale of environmental perturbations yet greater diversity in regulatory networks across taxa [12] .

Integration with transcriptomic data significantly furthers our understanding of the coordinated responses in biological systems, pinpointing over 160 co-regulated genes and their corresponding flux changes that operate downstream of the transcriptional signature along conserved metabolic pathways. Such multi-layered adaptation strategies are critical, with discrete metabolic regulators emerging as central hubs in the network of responses. This underscores the intricate physiological mechanisms that guide P. aeruginosa through various transitional environments, highlighting how these processes adapt to changes and challenges in their surroundings. Through careful analysis and integration of these data, we can better grasp the complexities of how this organism navigates its ecological niche.[13]

L. Regulatory Networks and Stress Response Pathways

As a model organism, Pseudomonas aeruginosa has become a paradigm for studying adaptive responses against multiple environmental stresses. Due to its intrinsic resistance mechanisms, P. aeruginosa is readily isolated from chronically infected cystic fibrosis patients and is known to switch between different mutational patterns and phenotypic states, acquiring biofilm-enhancing traits. Between 75 % and 85 % of

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

regulated genes co-localize in at least one transcriptional regulon of 83 different carbon-source transitions even when strains are exchanged. The bacterium responds to chronic environmental changes and antibiotics through a complex regulatory network, with more than 36 transcription factors of 9 distinct families. Extracellular features such as biofilm formation and pyocyanin production are extensively characterized under various stresses. The bacterium possesses a high number of two-component regulatory systems, which increase sensitivity to growth-limiting environments, thus providing evolutionary advantages during stress adaptation. Such regulatory networks that coordinate a large number of signals are important during thermal stress to ensure cell growth, motility, and biofilm formation. Characterization of regulons and signalling pathways under thermal stress conditions is fundamental to understanding stress response at both transcriptional and post-transcriptional levels.[14]

To characterize the stress response at the regulatory level, P. aeruginosa PAO1 was cultivated at different temperatures in synthetic minimal media. Analysis of the stress response connections revealed that (i) a large number of genes were regulated in a temperature-dependent manner, (ii) different dynamic patterns were observed at higher temperatures, (iii) the number of induced genes at higher temperatures progressively increased until cell lysis, (iv) both transcriptomic and proteomic analyses indicated that temperature stress without other external signals resembles ammonia-shock adaptation, and (v) a temperature-specific signalling network that controls an intricate variety of stress responses was revealed. Under thermal stress, the sigma factors σ28 (rpoF) and σS (rpoS) are up-regulated, providing a unique modular network with high flexibility in adaptation [12] [15] [16].

M. Comparative Analyses Across Strains and Conditions

Temperature serves as a crucial environmental factor that significantly influences the growth, survival, and overall development of microbes in various natural ecosystems. A comprehensive overview of the temperature ranges of interest allows for the comparison of the intensity of both phenotypic and molecular responses to thermal stress across different microbial strains in distinct experimental setups and conditions. As climate change scenarios continue to unfold, projections suggest that temperatures will experience notable increases compared to the current norm, posing challenges for many microbial communities. The temperature commonly utilized in body temperature experiments, specifically set at 37 °C, establishes a critical threshold for P. aeruginosa species, directly impacting their fitness levels and potential selection pressures that may arise as a result. [17]

Numerous species of Pseudomonas are found in specific ecological niches, which enable them to function as opportunistic pathogens in various environments. The temperature conditions to which these organisms are subjected in their natural settings serve as a significant and influential factor that shapes their thermal stress response. This unique thermal constraint influences how these bacteria adapt and thrive under fluctuating temperature conditions, ultimately affecting their pathogenic potential and survival strategies.[5] [18]

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

N. Implications for Virulence, Biofilm Formation, and Antibiotic Susceptibility

As the ambient temperature rises towards febrile thermal states (37–40 °C), the clinical relevance of P. aeruginosa is amplified, and the pathogenicity-enhancing effects of heat become a topic of concern. Previous studies have reported that the biofilm-forming ability increases significantly in biofilm-associated strains at 37 °C as compared with 22 °C 1. The 37 °C biofilm formed by several of the same isolates shows conspicuous differences in colony morphology and rate of expansion 19. In addition, certain virulence-associated genes and/or regulatory elements are upregulated at 37 °C in biofilm-forming clonal populations, such as lasR, pvdQ, pviB, aesY, and Hfq, which may serve as potential pathogenicity markers when exposed to therapeutic thermal stresses (and/or other clues to correlate temperature dependence). Furthermore, as biofilm dispersal is critical to biofilm establishment and in vivo transmission yet displays an inverse association with thermally elevated growth rates, the rate of biofilm dispersal remains to be characterized using such biofilm-associated strains. Specific disruption of motility may also contribute to the establishment of initially rapid, thermotolerant P. aeruginosa biofilms and/or delayed dispersal, complementing investigation of control at the physiological level through the Multi-Omics framework. Temperature modulation effects on pathogenicity-related traits (biofilm ability and gene repertoire) warrant further exploration to provide insight into pathogen proliferation coupled with febrile thermal response.

O. Limitations and Considerations

Numerous characteristics of the study must be meticulously taken into account when interpreting results. The efficiency of the experiment is significantly influenced by the growth stage of the bacteria at the precise time of impartation. Before being exposed to heat, the bacterial cultures were carefully diluted to achieve a concentration of approximately 06 cells mL-1. Effective transmission at this specific density has been previously shown to be nearly optimal. The selection of strain PA14, along with the deliberate choice of media, aims to accurately pinpoint adaptations that are particularly relevant to various environmental niches, which include non-human transmission, potential clinical reservoirs found in cystic fibrosis patients, and so forth. Nevertheless, it is important to highlight that these characteristics might still exert various effects that extend beyond the limits of the detection window. Some of these alternative factors appear sufficiently unlikely to warrant deeper consideration; however, it is crucial to note that their potential impact cannot be entirely disregarded or overlooked. In summary, a comprehensive understanding of these dynamics is essential for accurate interpretation. [1] [20]

P. Future Directions

Directly following extensive and comprehensive broad phenotypic and molecular profiling studies that reveal an intricate and extensive degree of flexibility in Pseudomonas aeruginosa’s response to varying temperature conditions, several additional experimental approaches and advanced analytical strategies could further enhance our understanding of this remarkable environmental adaptation and its significant

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

implications for virulence in this opportunistic pathogen. Given that the genome of Pseudomonas aeruginosa is known to be notably resilient and robust to mutation under a diverse range of stress conditions, including both temperature fluctuations and nutritional limitations, genome sequencing and variant analysis provide a valuable complementary perspective on the adapted phenotypes and the selective pressures they experience. Furthermore, since temperature exerts a strong and profound influence on growth rates and overall physiology, tracing the temperature-dependent variation in transcriptomic, proteomic, or metabolic responses across the broad spectrum of conditions applied during influential biofilm or infection studies would greatly illuminate the more subtle effects that occur and their interactions with broader ecological and evolutionary shifts. This holistic approach, integrating various levels of biological organization and adaptation, will certainly deepen our insights into Pseudomonas aeruginosa's dynamic capabilities and its pivotal role in environmental and clinical contexts. [12]

Integrating considerage of alternative substrates or environmental parameters could further enrich the existing data set: although temperature must rise substantially for substantial metabolic alteration, broad haploinsufficiency and haplo-advantage studies demonstrate interactions between temperature and diverse nutrient incorporation. Linking osmolarity, pH, and other hanitory traits modifies phenotypic analysis of larger-scale population behavior in community studies of surface colonization; and analogously, temperature is known to modulate interfaces in joint pathways such as c-di-gmp and PA-1. Given the extensive impacts of temperature on Pseudomonas aeruginosa strain PA14 behavior, reinforcing existing techniques with these follow-up options promises deeper mechanistic understanding both within clustered profiles and across broader networks.

Thermal stress has significant implications for all biological systems, yet our understanding of its effects continues to be limited and requires further investigation. This study specifically implicated temperature as both a crucial signal and a potent stressor for the bacterium Pseudomonas aeruginosa. In addition to this, complete temperature-related growth rates along with differential gene expression were thoroughly analysed to provide deeper insights. P. aeruginosa demonstrated distinct and notable phenotypic changes at varying temperatures, which were further supported by comprehensive data regarding protein abundance and metabolite levels. Through this research, key connections between the organism's genotype and observable phenotype started to emerge clearly. The growth behaviour of six distinct P. aeruginosa strains at temperatures of both 27°C and 37°C was meticulously recorded; interestingly, five of these privileged strains displayed the same consistent 27°C–37°C switching pattern when subjected to standard batch culture conditions. This observation suggests a potentially important adaptability mechanism within P. aeruginosa in response to thermal fluctuations.[21]

Even thermophilic microorganisms experience a loss of temperature homeostasis when the temperature exceeds 80°C. This phenomenon is significant as it highlights the limits of thermal tolerance in these organisms. For instance, Pseudomonas aeruginosa showcases notable changes on multiple levels,

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

including phenotypic, molecular, and phylogenetic switches, when subjected to temperatures within the range of 25°C to 37°C. This range is considerably lower than the typical threshold associated with heat shock, which generally denotes a temperature differential (ΔT) that organisms can withstand temporarily. However, when temperatures reach 80°C, this threshold is surpassed, leading to effects that go beyond mere transitory or inherent heat stress conditions. The genus Pseudomonas has expanded its ecological reach far beyond mere laboratory cultures, emerging from varied ecosystems such as temperate, coliforming, aerobic, and even polar, brackish, and estuarine environments. Significantly, Pseudomonas thrives in a diverse range of habitats, and its secondary niches appear to commence at temperatures above 27°C. This expansion is notable as it may have led to evolutionary adaptations that optimize survival strategies under diverse environmental stresses. Additionally, the preference for terrestrial environments has encouraged the development of non-culturable forms that exhibit remarkable resilience, with survival periods becoming increasingly prolonged in conditions where temperatures soar above 73°C. Such adaptations underline the intricate relationship between temperature and microbial life, demonstrating how heat affects ecological niches and environmental dynamics. [1] [5] [22]

Conclusions

This study thus enhances our understanding of the role of temperature as a key environmental factor for the phenotypic and molecular adaptation of Pseudomonas aeruginosa to thermal stress as well as their associated mechanisms driving changes in bacterial survival and virulence and antimicrobial resistance. Our results show that high temperature causes significant phenotypic changes including altered growth rates, colony morphology, motility and biofilm formation, and activates a substantial transcriptional and molecular response to heat-shock genes, stress-response regulators and virulence-associated pathways. By coupling sensitive phenotypic assays with high-resolution RNA sequencing we provided novel insights into the transcriptional changes and metabolic reprogramming that thermal stress induces and how they function to promote bacterial acclimation and survival. These findings highlight that temperature variation, both as manifested during fever and during clinical disinfection procedures, may modulate antibiotic susceptibility profiles and play a role in environmental selection of stress-adapted bacteria. Therefore, greater understanding of the temperature-dependent adaptive mechanisms of P. aeruginosa, a major opportunistic pathogen, may inform therapeutic drug intervention and other clinical responses. Future studies should be directed to address the following issues: multi-omics studies in clinical strains of P. aeruginosa, the role of other environmental conditions such as pH and starvation, alone or in combination with thermal stress, and the elucidation of the role of specific regulatory networks in the forward and reverse flux of stress-induced virulence and resistance mechanisms across the P. aeruginosa genus.

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

References

[1] K. Bisht, A. R. Luecke, and C. A. Wakeman, “Temperature-specific adaptations and genetic requirements in a biofilm formed by Pseudomonas aeruginosa,” 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov

[2] O. Wurtzel, D. R. Yoder-Himes, K. Han, A. A. Dandekar, et al., “The single-nucleotide resolution transcriptome of Pseudomonas aeruginosa grown at body temperature,” 2012. [Online]. Available: https://www.ncbi.nlm.nih.gov

[3] M. Letizia, S. P. Diggle, and M. Whiteley, “Pseudomonas aeruginosa: Ecology, evolution, pathogenesis and antimicrobial susceptibility,” Nature Reviews Microbiology, 2025.

[4] T. Krell and M. A. Matilla, “Pseudomonas aeruginosa,” Trends in Microbiology, 2024.

[5] P. M. Tribelli and N. I. López, “Insights into the temperature responses of Pseudomonas species in beneficial and pathogenic host interactions,” Applied Microbiology and Biotechnology, 2022.

[6] Z. Li, N. Tan, J. Huang, J. Wang, Y. Xiao, J. Xu, Q. Wang, B. Wu, Z. Luo, and Y. Xu, “H_2O_2-mediated cell wall remodeling and pectin demethylesterification are involved in maintaining postharvest texture of table grape by sulfur dioxide,” Food Chemistry, vol. 464, p. 141838, Feb. 2025.

[7] R. D. Waite, A. Paccanaro, A. Papakonstantinopoulou, and J. M. Hurst, “Clustering of Pseudomonas aeruginosa transcriptomes from planktonic cultures, developing and mature biofilms reveals distinct expression profiles,” 2006. [Online]. Available: https://www.ncbi.nlm.nih.gov

[8] P. Behzadi, M. Gajdács, P. Pallós, B. Ónodi, A. Stájer, D. Matusovits, K. Kárpáti, K. Burián, B. Battah, M. Ferrari, and C. Doria, “Relationship between biofilm formation, phenotypic virulence factors and antibiotic resistance in environmental Pseudomonas aeruginosa,” Pathogens, vol. 11, no. 9, p. 1015, Sep. 2022.

[9] J. Ling, X. Xie, Y. Wang, W. Huang, J. Luo, J. Su, H. Fan, S. Wu, and L. Liu, “Differential expression profiles of miRNA in granulomatous lobular mastitis and identification of possible biomarkers,” Experimental and Therapeutic Medicine, vol. 24, no. 2, p. 500, Jun. 2022.

[10] S. M. Hug and B. S. Gaut, “The phenotypic signature of adaptation to thermal stress in Escherichia coli,” 2015. [Online]. Available: https://www.ncbi.nlm.nih.gov

[11] M. Tata, M. T. Wolfinger, F. Amman, N. Roschanski, et al., “RNA-seq based transcriptional profiling of Pseudomonas aeruginosa PA14 after short- and long-term anoxic cultivation in synthetic cystic fibrosis sputum medium,” 2016. [Online]. Available: https://www.ncbi.nlm.nih.gov

[12] S. Jozefczuk, S. Klie, G. Catchpole, J. Szymanski, et al., “Metabolomic and transcriptomic stress response of Escherichia coli,” 2010. [Online]. Available: https://www.ncbi.nlm.nih.gov

[13] A. Elfadadny, R. F. Ragab, M. AlHarbi, F. Badshah, E. Ibáñez-Arancibia, A. Farag, A. O. Hendawy, P. R. De los Ríos-Escalante, M. Aboubakr, S. A. Zakai, and W. M. Nageeb, “Antimicrobial resistance of Pseudomonas aeruginosa: Navigating clinical impacts, current resistance trends, and innovations in breaking therapies,” Frontiers in Microbiology, vol. 15, p. 1374466, Apr. 2024.

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

[14] T. Zhou, J. Huang, Z. Liu, Q. Lin, Z. Xu, and L. H. Zhang, "The two-component system FleS/FleR represses H1-T6SS via cyclic di-GMP signaling in Pseudomonas aeruginosa," Applied and Environmental Microbiology, vol. 88, no. 2, p. e01655-21, Jan. 2022.

[15] K. Avican, J. Aldahdooh, M. Togninalli, A. K. M. F. Mahmud, et al., "RNA atlas of human bacterial pathogens uncovers stress dynamics linked to infection," 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov

[16] A. Dötsch, M. Schniederjans, A. Khaledi, K. Hornischer, et al., "The Pseudomonas aeruginosa transcriptional landscape is shaped by environmental heterogeneity and genetic variation," 2015.

[17] J. Arizti-Sanz, A. D. Bradley, Y. B. Zhang, C. K. Boehm, C. A. Freije, M. E. Grunberg, T. S. Kosoko-Thoroddsen, N. L. Welch, P. P. Pillai, S. Mantena, and G. Kim, "Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants," Nature Biomedical Engineering, vol. 6, no. 8, pp. 932–943, Aug. 2022.

[18] K. Bisht, A. R. Luecke, and C. A. Wakeman, "Temperature-specific adaptations and genetic requirements in a biofilm formed by Pseudomonas aeruginosa," Frontiers in Microbiology, 2023.

[19] J. Redfern, J. Wallace, A. van Belkum, M. Jaillard, et al., "Biofilm-associated genotypes of multiple antibiotic-resistant Pseudomonas aeruginosa," 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov

[20] P. Shunmugam, N. Ahmed, and K. K. B. Singh, "ipaH-targeted electrochemical genosensor: A fast and reliable diagnostic approach for simultaneous detection of Shigella species and enteroinvasive Escherichia coli," Microchemical Journal, 2025.

[21] E. C. Solar Venero, J. Wallace, A. van Belkum, M. Jaillard, et al., "Fever-like temperature impacts on Staphylococcus aureus and Pseudomonas aeruginosa interaction, physiology, and virulence both in vitro and in vivo," BMC Biology, vol. 22, no. 1, p. 27, Feb. 2024.

[22] A. Tabassum, "Effects of temperature, pH, and salinity on Pseudomonas species from household water supplies and their multidrug resistance profiles," Journal of Bacteriology and Virology, 2024.