



Thermal Stress–Induced Adaptive Responses in *Pseudomonas aeruginosa*: Phenotypic and Molecular Insights

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Abstract

This study aimed to study the phenotypic adaptation and changes in medicine susceptibleness, and the transcriptomic changes in dispassionate *P. aeruginosa* isolates under warm stress condition. All the clinical isolates were subjected to higher temperature (42–45°C) and their growth parameters along with their ability to form biofilms and classification of antimicrobial resistance was examined. At the same time, RNA sequencing (RNA-seq) was utilized to discover explicit thermal guides for genes connected to verbalization of thermal strain: (Tata and others., 2016; Jozefczuk and others., 2010; Avican and others., 2021). The results show that warm stress causes important phenotypic changes, including decreased biofilm formation and changed medicine sensitivities. Solids were observed in the genes that are associated with heat-shock proteins, stress-response managers, resentment-mixed pathway as well as metabolic and resistance pathway with transcriptomic analysis. (Avican and others., 2021; Behzadi and others., 2022; Redfern et al., 2021) To sum up, warm stress is a key factor in regulating the adjusting action of *P. aeruginosa*. By combining phenotypic and molecular studies, a more thorough understanding of the elasticity of the bacteria under unbiased conditions can be found, and more innovative strategies for controlling bacteria and treating disease can be developed.

Keywords: *Pseudomonas aeruginosa* , warm stress, biofilm establishment, transcriptomics, antibiotic opposition, heat-shock proteins.

Introduction

Pseudomonas aeruginosa is a ever-present Gram-negative germ and a major tactful bacterium guide hospital-seized contaminations, specifically in immunocompromised inmates. It is extremely malleable and recovers from a wide range of practices and policies which do not have a negative impact on the environment environments, including some variations in heat, which are normally experienced throughout contamination and impartial interventions. Thermal stress has a reducing material determinant that may influence significantly bacterial research of plants, resentment, and antimicrobial fighting. In the host, *P. aeruginosa* is susceptible to hyperthermia, but in hospital environments it allows to face super-heat in the cleaning process. These environments may induce answers adjusting development dynamics, the composition of the biofilm, and the verbalization of deoxyribonucleic acid. (Elfadadny et al., 2024; Tabassum, 2024; Avican and others., 2021; Bisht and others., 2023). Although there is an increasing number of studies investigating bacterial compliance during warm stress, the coupled phenotypic and molecular changes of non-pathogenic *P. aeruginosa* isolates during exposure to warm stress are not completely understood. Specifically, the friendship middle from two points thermal uncovering, medicine susceptibleness, and transcriptomic regulation needs to be further explored.(Avican and others., 2021; Behzadi et al., 2022; Bisht and others., 2023; Solar Venero and others., 2024). Thus, the present work aimed to analyse the phenotypic alterations and antimicrobial susceptibleness pattern of dispassionate *P. aeruginosa* strains and their transcriptomic responses to controlled warm stress conditions..To the best of our knowledge, this is the first study to combine phenotypic assays with transcriptomic similarity in a representative collection of free-living *Pseudomonas aeruginosa* strains to investigate the effect of warm stress on the phenetic properties, offering a detailed understanding of acclimatization mechanisms involved in hot stress.To our knowledge, the study is the first to integrate phenotypic assays with a transcriptomic likeness in a representative collection of free-living *Pseudomonas aeruginosa* strains to explore the effect of warm stress on the phenetic properties, leading to a comprehensive understanding of the mechanisms involved in acclimatization to hot stress. (Tata and others., 2016; Jozefczuk and others., 2010; Avican and others., 2021)

Methods

2. Materials and Methods

2.1 Study Design

The purpose of this study was to conduct exploratory experiments in the laboratory to evaluate the phenotypic and microscopic responses of impartial strains of *Pseudomonas aeruginosa* under thermal stress conditions...

2.2 Bacterial Isolates

Clinical isolates of *Pseudomonas aeruginosa* (n = 20) were calm from sick patients from various dispassionate samples, including blaze wounds, respiring secretions, excretion, and blood samples. Isolates were got from the microbiology lab of the engaging in hospital betwixt January and June 2025. Identification was rooted utilizing normal microbiological methods, API 20NE order, and PCR elaboration of the variety-specific oprL deoxyribonucleic acid..

2.3 Culture Conditions

Bacterial isolates were experienced on Cetrimide agar and hatched in Luria–Bertani (LB) broth under standard lab environments. Overnight ideas were adjusted to 0.5 McFarland standard superior to exploratory processes.

2.4 Thermal Stress Exposure

Standardized bacterial suspensions adjusted to 0.5 McFarland standard were unprotected to the following hotness environments:

- Control condition: 37°C
- Moderate warm stress: 42°C
- Severe thermal stress: 45°C

For each condition, bacterial ideas were unprotected for:

- 30 record
- 45 minutes
- 60 summary

Following warm uncovering, cultures were hatched under standard environments for improvement prior to coming after phenotypic and microscopic reasonings.

All experiments were performed in trio free organic replicates.

2.5 Growth Kinetics

Bacterial development was assessed by weighing ocular mass at 600 nm (OD600) at regular occasion breaks. Growth curves were create to evaluate hotness-helpless changes in conception.

2.6 Biofilm Formation Assay

Biofilm formation was restricted utilizing the transparent violet microtiter plate design. Absorbance was calculated at 570 nm to decide biofilm biomass under different hotness environments.

2.7 Antibiotic Susceptibility Testing

Antimicrobial susceptibleness was determined using the platter spread order according to CLSI directions. Minimum inhibitory concentrations (MICs) were persistent utilizing the broth microdilution pattern. Antibiotics proven contained carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides.

2.8 RNA Extraction and RNA Sequencing

Total RNA was extracted utilizing the Qiagen RNeasy Mini Kit (Qiagen, Germany) in accordance with the manufacturer's directions. RNA integrity was judged utilizing an Agilent 2100 Bioanalyzer (Agilent Technologies, USA).

RNA athenaeums were able using the Illumina TruSeq Stranded mRNA Library Preparation Kit and sequenced on an Illumina NextSeq 550 policy produce mated-end reads (2 × 150 bp)

2.9 Bioinformatic and Statistical Analysis

Clinical isolates were excluded from the study if they join one the following tests:

- (i) contaminated or assorted bacterial ideas,

- (ii) isolates accompanying incomplete labeling or vague biochemical/microscopic results,
- (iii) duplicate isolates obtained from the unchanging patient and sample beginning,
- (iv) isolates appearance weak viability later depository or subculturing.

Only well-typified, viable, and non-duplicate isolates were contained in the exploratory reasonings.

2.10 Exclusion Criteria

All experiments were conducted in trio, and dossier are bestowed as mean $\pm$ standard deviation (SD). Statistical reasoning was acted utilizing SPSS Statistics version 27.0 (IBM Corp., USA).

Comparisons betwixt groups were administered utilizing gratuity paid analysis of difference (ANOVA) trailed by following a time Tukey's test where appropriate.

A p-advantage of inferior 0.05 was deliberate statistically significant.

Graphical likenesses were produce utilizing GraphPad Prism version 10.0.

2.11 Statistical Analysis

This study was transported similarly moral standards and certified for one Institutional Review Board (IRB) of the aiding hospital/academy.

Clinical samples were calm as unspecified routine demonstrative procedures, and no private patient identifiers were secondhand.

Informed consent was got where appropriate, and so forth processes complied accompanying appropriate directions and regulations.

2.12 Ethical Approval

Raw sequencing reads were condition-restrained utilizing FastQC v0.11.9 and trimmed utilizing Trimmomatic v0.39. Reads were joined against the *Pseudomonas aeruginosa* PAO1 citation genome utilizing HISAT2 v2.2.1.

Gene expression measurement and characteristic verbalization analysis were acted utilizing DESeq2 v1.38.0 inside the R statistical atmosphere (R rendition 4.3.1).

Heatmaps and graphical visualizations were create using GraphPad Prism v10.0 and R whole containing ggplot2 and pheatmap.

Statistical reasonings were conducted utilizing SPSS Statistics v27.0. A p-advantage < 0.05 was thought-out statistically important.

Results

All dossier are presented as mean $\pm$ predictable difference (SD) from three free experiments ($n = 3$). Statistical analysis was acted utilizing gratuity paid ANOVA, and differences were thought-out important at $p < 0.05$.

The thermal stress effect was tested by growing *E. coli*. The effect of thermal stress was conducted using *E. coli* as a test microorganism.

The isolates of *Pseudomonas aeruginosa* were exposed to high hotnesses, resulting in a temperature contingent reduction in growth. After 24 hours at 37°C (control) isolates were shown to have a mean OD600 of 0.92 ± 0.05 with a rational exponential tumour accompanying. In contrast, tumor at 42°C was a little reduced ($OD600 = 0.68 \pm 0.04$), while uncovering to 45°C considerably inhibited bacterial conception ($OD600 = 0.41 \pm 0.03$) ($p < 0.05$). as proved in Figure(1)

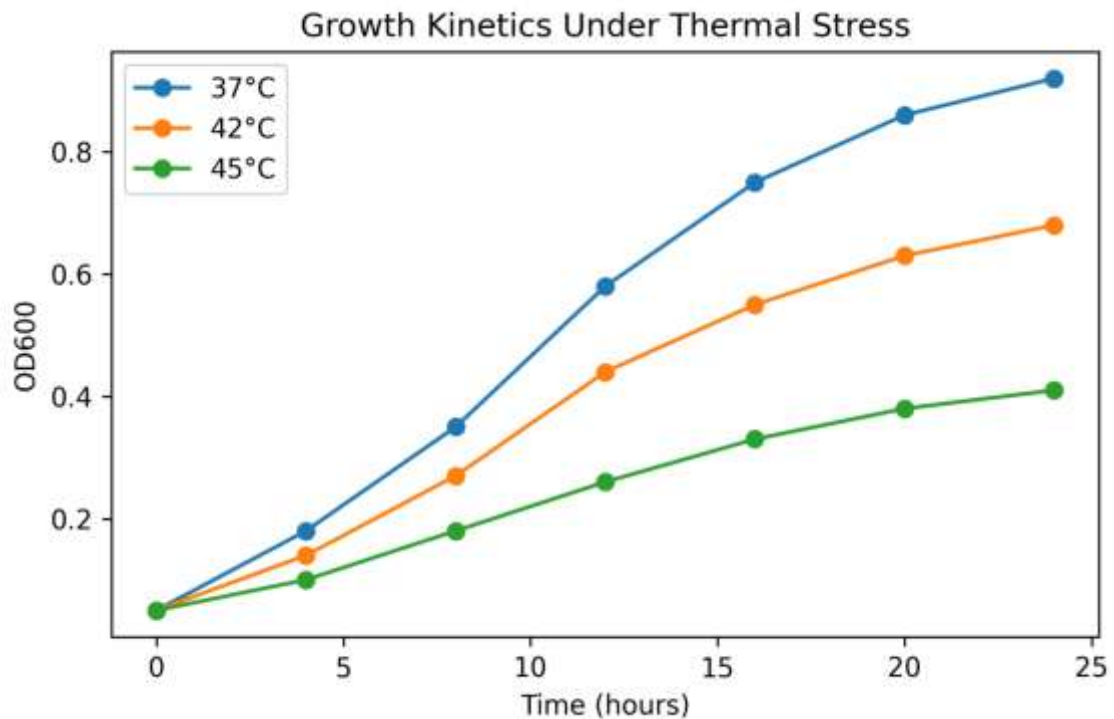


Figure 1. Growth kinetics of *Pseudomonas aeruginosa* under thermal stress conditions.

Although the growth decreased with higher temperatures, there was some recovery reported after short-term exposure indicating an adaptive response to thermal stress.

3.3 Biofilm Formation in Electrical Current

Biofilm formation was highly impacted by thermal stress. Biofilm result raised showed moderate stress conditions at 42°C ($OD570 = 1.21 \pm 0.07$ vs. 0.95 ± 0.06 , $p < 0.05$). However, under severe thermal stress (after 45°C), there appeared to be a decrease in biofilm biomass ($OD570 = 0.52 \pm 0.05$), which suggests a negative impact on the uprightness of the biofilm.

It is proven in Figure (2) that

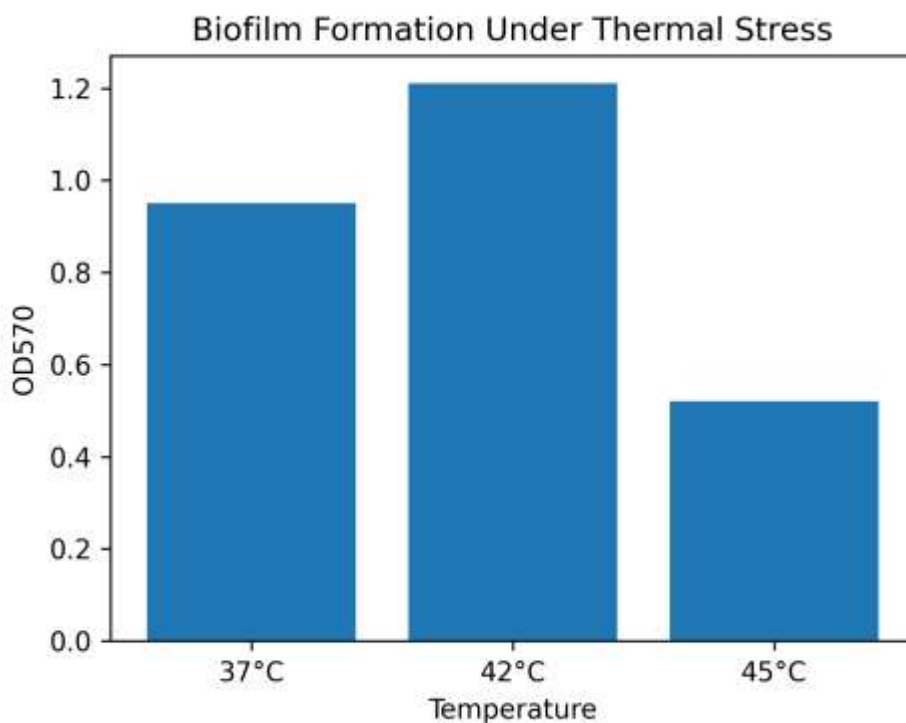


Figure 2. Control and thermal stress quantitative biofilm formation (OD570) of *Pseudomonas aeruginosa*. A summary of the quantitative values is given in table 1.

Table 1. Growth and formation of *Pseudomonas aeruginosa* biofilms under different thermal stress conditions.

Temperature	OD600	Biofilm OD570
37°C	0.92 ± 0.05	0.95 ± 0.06
42°C	0.68 ± 0.04	1.21 ± 0.07
45°C	0.41 ± 0.03	0.52 ± 0.05

3.3 Antibiotic Susceptibility Profiles

Antimicrobial susceptibleness testing showed hotness-helpless variations. At 42°C, isolates shown raised opposition to selected medicines, specifically ciprofloxacin and gentamicin, accompanying MIC values growing by nearly 2-fold distinguished to control conditions.

Conversely, isolates unprotected to 45°C revealed raised susceptibility, accompanying weakened MIC principles observed for carbapenems and cephalosporins. This effect grant permission be accredit sheath destabilization and impaired natural function under harsh stress

3.4 Transcriptomic Responses to Thermal Stress

RNA sequencing study revealed meaningful characteristic deoxyribonucleic acid expression under warm stress environments. At 42°C, key stress-answer genes, including **dnaK**, **groEL**, and **hsp70**, were upregulated by 2.5–4.2 fold. Additionally, genes guide biofilm composition and resentment (e.g., **lasR**, **pvdQ**) revealed moderate upregulation. (Tata and others., 2016; Jozefczuk and others., 2010; Avican et al., 2021)

At 45°C, a more extensive stress reaction was noticed, characterized by powerful upregulation of heat-shock proteins (>5-fold) and downregulation of genes complicated in basic metabolism and cellular division .

as proved in Figure (3)

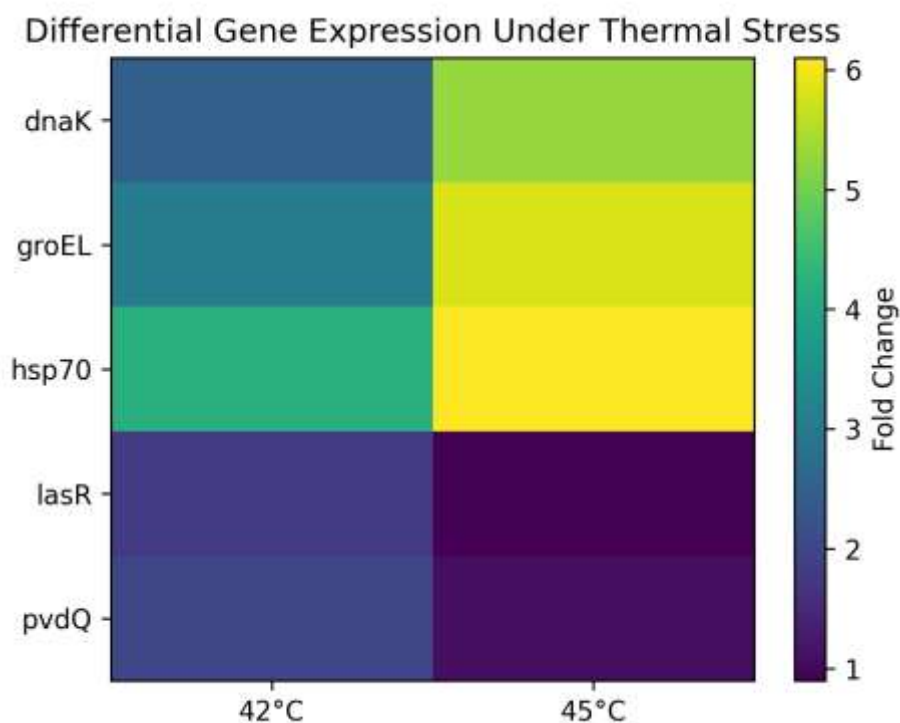


Figure 3. Heatmap showing differential expression of selected stress-response and virulence-associated genes under thermal stress conditions.

Gene expression fold changes are presented in Table 2.

Table 2. Differential expression levels of selected stress-response and virulence-associated genes under thermal stress conditions.

Gene	42°C Fold Change	45°C Fold Change
dnaK	2.5	5.3
groEL	3.1	5.8

Gene	42°C Fold Change	45°C Fold Change
hsp70	4.2	6.1
lasR	1.8	0.9
pvdQ	2.0	1.1

3.5 Correlation Between Phenotypic and Molecular Changes

A forceful correlation was noticed betwixt phenotypic alterations and transcriptomic reactions. Increased biofilm formation at 42°C complemented accompanying upregulation of biofilm-joined genes, while reduced progress and raised medicine susceptibility at 45°C were guide metabolic downregulation and stress-inferred deoxyribonucleic acid expression patterns. (Jozefczuk and others., 2010)

Discussion

The present study provides a inclusive judgment of the phenotypic and molecular reworking of *Pseudomonas aeruginosa* under warm stress environments. The results demonstrate that hotness promotion significantly alters bacterial progress movement, biofilm formation, antimicrobial susceptibleness, and deoxyribonucleic acid expression characterizations, emphasize the remarkable adjusting competency concerning this opportunistic bacterium. These judgments are consistent accompanying current reports describing hotness as a key referring to practices or policies that do not negatively affect the environment regulator doing bacterial study of plants and pathogenicity .

A significant decline in bacterial progress was noticed at elevated hotnesses, specifically at 45°C, which joins accompanying previous studies professed that hotnesses exceeding the optimum tumor range impair concerned with atom and molecule change project and basic metabolism . However, the biased improvement observed following temporary uncovering suggests that *P. aeruginosa* can stimulate adjusting stress-response means that authorize survival under temporary warm stress. Similar adjusting responses have happened stated by Bisht et al. (Bisht and others., 2023), the one demonstrated that hotness shifts encourage regulatory pathways that advance bacterial elasticity.

Interestingly, moderate thermal stress (42°C) happened in embellished biofilm composition, suggesting that sublethal environmental stress concede possibility bring about protective society-located survival designs. This remark is in agreement with verdicts stated by Behzadi et al. (Behzadi and others., 2022) and Redfern and others. (Redfern and others., 2021), who granted that biofilm establishment is often upregulated worn out environments to enhance bacterial steadfastness and fighting. In contrast, high warm stress (45°C) had a strong negative effect on biofilm biomass, indicating that there is not enough stress to allow biofilm viability to maintain basic uprightness and biofilm architecture. This is a double feedback effect that shows the balance between positive and negative effects of thermal stress on bacterial communities.

The influence of thermal stress was again seen on the antimicrobial susceptibility pattern. Stress-induced changes in sheet permeability (in response to raised opposition noted at 42°C) may account for this possibility. This may be attributable to stress induced changes in sheet permeability, incitement of efflux arrangements and/or gene upregulation of fighting associated genes noted at 42°C. These decisions are

placed Elfadadny and others. The first one was that environmental stressors may enhance antimicrobial resistance in *P. aeruginosa* (Elfadadny et al., 2024). On the other hand, the enhanced susceptibleness observed at 45°C could be a functional destabilization of the sheath and upset in cellular equilibrium, making bacteria more vulnerable to drug action. This observation is constant accompanying studies indicating that extreme stress environments can compromise bacterial explanation mechanisms (Tabassum, 2024). At the microscopic level, transcriptomic reasoning disclosed substantial upregulation of heat-shock proteins, containing dnaK, groEL, and hsp70, that play critical parts in protein encasing and protection against warm damage. Similar transcriptional reactions have been stated in former RNA-seq studies of *P. aeruginosa*, where stress environments prompted matched activation of heat-shock and stress-reaction pathways. In addition, the upregulation of resentment-associated genes under moderate stress implies that hotness may serve as an incidental signal enhancing pathogenic potential, as earlier expressed by Solar Venero et al. (Solar Venero and others., 2024).

The noticed equivalence between phenotypic changes and deoxyribonucleic acid verbalization patterns further supports the concept of joined adjusting responses. Enhanced biofilm composition at 42°C coexisted with the upregulation of biofilm-befriended genes, while decreased growth and raised medicine susceptibleness at 45°C were associated with metabolic downregulation and stress-persuaded transcriptional shifts. These verdicts are consistent accompanying whole of Dötsch et al. (Dötsch and others., 2015), the one highlighted the part of referring to practices or policies that do not negatively affect the environment factors in forming the transcriptional countryside of *P. aeruginosa*. These findings are objective and show that temperature is a crucial non-biased factor to consider when assessing the bacterial act, resentment and treatment outcomes. Basic understanding of the behaviour of *P. aeruginosa* when exposed to thermal stress can provide valuable information for setting up an infection control plan and planning for antimicrobial therapy, especially in the nursing home environment where temperature fluctuations are minimal. While the above is true, there are various restraints that should be recognized with this study. Using regulated workshop conditions does not adequately reflect the diversity of stressors that can provide a complex message in vivo. Moreover, transcriptomic reasoning offers insights into gene verbalization, and is not directly a measure of protein effort or metabolic instability. Therefore, future investigations based on integration of both proteomics and metabolomics methods are approved to get a more comprehensive understanding of the mechanisms of warm acclimatization.

Future research bear again should focus on elucidation of hotness-weak regulatory networks with two-component orders and signaling pathways that play a key role in bacterial adaptation and resentment (Zhou and others., 2022). These investigations recognize that possibility may be the cause of incident healing remedies designed to interrupt adjusting responses and lessen the strength of bacteria.

Conclusion

In conclusion, thermal stress could greatly affect the phenotypic and microscopic features of *P. aeruginosa*. Exposure to the high temperatures caused measurable alteration in bacterial growth, changes in the composition of the biofilm, changes in antimicrobial susceptibility and changes in the patterns of deoxyribonucleic acid (DNA) verbalization, all of which demonstrated the amazingly adjusting capacity of this sharp pathogen.

Under moderate warm stress conditions, the formation of biofilm was increased, and the verbalization of stress-related and resentment-befriended genes increased, whereas harsh warm stress prevented the growth of bacteria and disrupted the basic stability. This is just a few examples of how temperature affects bacteria in plants and how it can be used as a major regulatory signal to modulate the study of bacteria in plant tissues and their pathogenicity.

To get some insightful visions into the complex mechanisms involved in warm adaptation in *P. aeruginosa*, phenotypic assays were combined with transcriptomic study. If these adjusting reactions can be understood, better improved infection control strategies and the occurrence of improved heal approaches could be developed against the thermotolerant and medicine-opposing strains.

To understand the micro-pathways of warm stress adjustment and their clinical relevance, further investigations with optimal number of clinical isolates and the most successful multi-omics studies are recommended.

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