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Comparative evaluation of glucose and glycerol as carbon sources for violacein synthesis in *Janthinobacterium lividum*

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ABSTRACT

Background: Violacein is a purple pigment synthesized by select Gram-negative bacteria, including *Janthinobacterium lividum*, with potential antimicrobial and competitive advantages in environmental niches. Despite extensive research, the optimal conditions for violacein production remain incompletely understood. **Objective:** This study investigates the influence of different carbon sources (glucose and glycerol) on violacein production by *J. lividum* over time and examines the relationship between bacterial viability and pigment synthesis. **Materials and Methods:** *J. lividum* was cultured in LB medium supplemented with either 1% glucose (LB-U) or 1% glycerol (LB-Y). Violacein production was monitored using optical density measurements at 585 nm (OD_{585}) and confirmed by high-performance liquid chromatography (HPLC). Colony-forming unit (CFU) counts were determined at multiple time points over 120 hours. **Results:** Glycerol-supplemented media demonstrated superior violacein production compared to glucose-supplemented media. In LB-Y conditions, violacein production reached 1.0 OD_{585} by 120 hours, while LB-U conditions achieved only 0.1 OD_{585}. HPLC analysis confirmed violacein identity with a characteristic peak at 16.40 minutes retention time. Bacterial viability remained higher in violacein-positive cultures during the death phase. **Conclusion:** These findings demonstrate that carbon source selection critically influences violacein biosynthesis in *J. lividum*, with glycerol promoting enhanced production while glucose suppresses pigment synthesis. The results suggest violacein may contribute to bacterial survival under stress conditions.

Keywords: Violacein, *Janthinobacterium lividum*, Carbon source, Glycerol, Glucose, HPLC, Bacterial pigments.

INTRODUCTION

Violacein is a distinctive purple pigment that has captivated researchers since its initial discovery in *Chromobacterium violaceum* in 1882 [1]. This remarkable bis-indole compound is synthesized by select bacterial groups across diverse ecological niches, and its presence doesn't always denote close phylogenetic relationships among producing organisms. The discovery of violacein-producing bacteria and their striking purple coloration has evolved from an initially intriguing phenomenon to recognition of their vast biological activities and industrial potential *Janthinobacterium lividum* represents one of the most notable violacein producers, first discovered on a wet silk thread, where it caused both the thread and the surrounding environment to adopt a distinctive bluish-purple hue. This psychrotrophic bacterium exhibits prolific production of bluish-purple pigments even on specialized media like Wakimoto medium derived from amino acids, with methanol extraction revealing the presence of both violacein and deoxyviolacein [2]. The geographical distribution of violacein-producing bacteria spans diverse environments worldwide. Various bacterial strains have been extensively researched across different geographical regions and environmental conditions, including Chromobacterium violaceum from tropical forests and rivers [3], *Collimonas* species from Arctic regions [4], *Duganella* species from agricultural soils [5], and marine species such as *Alteromonas* from coastal waters [6] and *Pseudoalteromonas* from deep-sea environments [7,8]. Particularly noteworthy is the psychrotrophic *Janthinobacterium lividum XT1* strain isolated from a glacier in Xinjiang province, demonstrating violacein production under extreme cold conditions [9].

Violacein production is highly dependent on optimal environmental conditions, including temperature, pH, selective carbon sources, and other physiological parameters. Each species exhibits specific requirements influenced by its natural habitat and genetic factors. For instance, Chromobacterium violaceum, typically a saprophytic organism, thrives at temperatures up to 40°C with some isolates from

Brazilian Amazon aquatic environments growing at 44°C, while others show tolerance down to 10°C, albeit without pigment production [10]. Research has demonstrated that factors such as agitation, aeration, inoculum size, and nutrient availability play crucial roles in violacein yields [5,7,11,12].

Beyond its distinctive coloration, violacein exhibits an extraordinary array of biological activities that position it as a promising therapeutic candidate. Its antimicrobial properties demonstrate inhibitory effects against multidrug-resistant bacteria, including *Pseudomonas aeruginosa, Serratia marcescens, and Staphylococcus aureus*, showing four times greater activity than vancomycin against certain S. aureus strains [13,14]. The compound also displays antifungal activities against *Batrachochytrium dendrobatidis* and *Botrytis cinerea* [15], antiviral effects against *Simian rotavirus SA11, HSV-1*, and *Poliovirus type 2* [16], and anti-parasitic activities against *Trypanosoma cruzi* [17] and *Leishmania amazonensis* [18].

Despite extensive research into violacein's biological functions and therapeutic potential, the environmental and nutritional factors that regulate its biosynthesis remain incompletely understood [9]. Carbon source availability has been identified as a critical factor influencing secondary metabolite production in bacteria, with previous studies indicating that different carbon sources can dramatically affect violacein yields. Research has shown that variables such as nutrient availability, including specific carbon sources, play crucial roles in violacein production optimization [7,12].

This study compares the effects of glucose and glycerol on violacein production by *Janthinobacterium lividum*. It examines temporal production patterns, bacterial viability, and pigment identity, highlighting how carbon source selection influences secondary metabolite biosynthesis. The findings provide insights for optimizing culture conditions and advancing biotechnological applications.

MATERIAL AND METHODS

Chemicals, Glassware, and Apparatus

All experimental procedures were conducted using analytical-grade and high-purity chemicals. For bacterial culture media preparation, tryptone, yeast extract, nutrient broth, and nutrient agar were obtained from Loba Chemie, India, while potassium dichromate and 1,5-diphenylcarbazide were purchased from Merck Chemicals, India. The stock solution of hexavalent chromium [Cr(VI)] was prepared following the standard protocol described in EPA Method 7196A [19]. For the preparation of LB medium (25 mL), 0.25 g tryptone, 0.125 g yeast extract, 0.25 g NaCl, and 0.375 g nutrient agar were dissolved in distilled water.

All glassware items required for the study, including conical flasks, graduated cylinders, beakers, Petri plates, and test tubes, were of Borosil make and sourced from M/S India Scientific, Patna, India.

Bacterial Strain and Culture Conditions

The *Janthinobacterium lividum* strain *MTCC 2655T* was preserved at -80°C in Brain–Heart Infusion (BHI) broth (HiMedia, Mumbai, India) supplemented with 25% glycerol. Prior to experimental use, the culture's purity was verified by streaking onto Trypticase Soy Agar (TSA; Soybean-Casein Digest Agar, Merck Millipore, India) as described by [20]. For experimental studies, Luria-Bertani (LB) broth served as the basal growth medium, following the formulation reported earlier [21]. Bacterial viability was assessed using the colony-forming unit (CFU) method. Samples were withdrawn from broth cultures, serially diluted, and spread onto TSA plates. The inoculated plates were incubated at 25°C for 24 hours. After incubation, colonies were enumerated, and the CFU count was used as a quantitative measure of viable bacterial cells. Colony-forming unit (CFU) counts were determined by serial dilution plating on LB agar at 48, 72, 96, and 120 hours of incubation. Viable counts were expressed as log CFU ml^{-1}.

Carbon Source Supplementation

Two experimental media were prepared:

1. LB-U medium: LB medium supplemented with 1% (w/v) glucose
2. LB-Y medium: LB medium supplemented with 1% (w/v) glycerol

Control cultures were maintained in standard LB medium without additional carbon sources.

Violacein Production Monitoring

Janthinobacterium lividum was cultured in 2 ml LB broth in 24-well plates. Cells were harvested by centrifugation (16,000 g, 20 min) and lysed with 10% SDS (5 min, RT). Violacein was extracted using water-saturated butanol (1:2 v/v), vortexed, and centrifuged (16,000 g, 10 min). The violacein-rich upper phase was collected, and absorbance was measured at 585 nm. Quantification was based on the extinction coefficient of 0.05601 ml μg^{-1} cm^{-1} [12,20].

High-Performance Liquid Chromatography (HPLC) Analysis

Violacein identity was confirmed using HPLC analysis. Culture supernatants were analyzed using a reverse-phase column with detection at multiple wavelengths (220, 270, 290, 310, and 340 nm). Retention times and spectral characteristics were compared to violacein standards.

Statistical Analysis

All experiments were conducted in triplicate with three independent biological replicates. Standard deviations were calculated and represented as error bars in graphical presentations. Statistical significance was assessed where appropriate.

RESULTS AND DISCUSSION

Time-course of violacein production under different carbon sources

The temporal dynamics of violacein production by *Janthinobacterium lividum* were evaluated over 72 hours in LB medium supplemented with either 1% glucose or 1% glycerol. During the initial 24-hour period, violacein production remained negligible in both conditions ($OD_{585} \approx 0$). Between 24-48 hours, glucose-supplemented cultures initiated violacein synthesis earlier, reaching 0.5 ± 0.05 OD_{585}, while glycerol cultures showed delayed production at 0.2 ± 0.02 OD_{585}. However, during the late phase (48-72 hours), production patterns reversed dramatically. Glucose cultures exhibited only a modest increase to 0.6 ± 0.05 OD_{585}, whereas glycerol cultures demonstrated substantial acceleration, achieving the highest production level of 0.9 ± 0.1 OD_{585}. Overall, glycerol supplementation supported a 1.5-fold higher final pigment yield compared to glucose (Figure 1).

These findings align with previous studies on *Chromobacterium violaceum*, where violacein synthesis was shown to be repressed in the presence of glucose but enhanced in glycerol-supplemented media [22]. Similar carbon source-dependent effects on violacein yield have been reported in other bacterial systems, where glucose imposes catabolite repression on secondary metabolite biosynthesis [23,24]. The contrasting dynamics observed here suggest that glucose may facilitate early activation of metabolic pathways linked to pigment initiation but restrict downstream biosynthetic steps, thereby limiting final yields. Glycerol, in contrast, avoids strong catabolite repression and appears to channel carbon flux more efficiently into violacein precursors, supporting sustained pigment biosynthesis during later stages of growth [25,20].

Furthermore, the delayed yet enhanced production pattern in glycerol-grown cultures may also contribute to improved stress tolerance during the stationary phase, consistent with the proposed ecological role of violacein in protecting cells from predation and microbial competition [18,26,27]. Collectively, these results reinforce the notion that violacein production is strongly modulated by carbon source availability, with glycerol providing a metabolic context that favors high-yield pigment synthesis and survival benefits.

Long-term growth dynamics and violacein accumulation

Violacein biosynthesis was closely associated with the stationary and death phases of bacterial growth, with pigment production initiating in the stationary phase and reaching its peak during the early death phase. Interestingly, cultures producing violacein retained higher numbers of viable cells during the death phase compared to violacein-negative counterparts, indicating a protective role of the pigment under stress. This protective effect can be attributed to the bioactive properties of violacein, which are known to inhibit protozoal predation and suppress microbial competition [16,18,20,26,28].

Extended cultivation (48-120 hours) under different carbon sources further highlighted the interplay between bacterial viability and violacein production (Figure 2). In LB–glycerol cultures, CFU counts gradually declined from 8.5 to 7.0 log CFU ml^{-1}, while violacein accumulation increased steadily from 0.8 to 1.0 OD_{585}. In contrast, LB-glucose cultures showed a sharper loss in viability, with CFU counts dropping from 8.8 to 5.9 log CFU ml^{-1} (a 10-fold reduction), coupled with negligible pigment accumulation (0.06–0.1 OD_{585}). Notably, violacein yield in glycerol-supplemented cultures was nearly 10-fold higher than in glucose cultures, indicating strong catabolite repression by glucose. The observed correlation between sustained pigment production and improved viability in glycerol-grown cultures further supports the hypothesis that violacein plays a survival-enhancing role during nutrient-limited or stress conditions

HPLC confirmation of violacein production

High-performance liquid chromatography analysis provided definitive chemical confirmation of violacein biosynthesis by *J. lividum*. The chromatographic profile revealed a distinct and reproducible peak at retention time 16.40 minutes, consistently detected across multiple wavelengths (A_{220}, A_{270}, A_{290}, A_{310}, and A_{340} nm). This multi-wavelength detection pattern confirms the identity of the compound as violacein, based on its characteristic conjugated aromatic structure and absorption properties. The consistent peak appearance across all tested wavelengths validates the analytical method's specificity and eliminates potential interference from other cellular metabolites. Peak area quantification at the 16-minute mark provides a reliable measure for violacein concentration, establishing a robust analytical framework for monitoring production efficiency under various cultivation conditions.

The analytical approach is consistent with previous reports using HPLC-DAD for violacein detection and quantification [25,29]. Importantly, violacein-producing cultures maintained higher cell viability during the stationary and death phases, suggesting that pigment accumulation enhances stress tolerance and competitive fitness [20,26]. Furthermore, quantification by HPLC enables direct correlation of pigment production with metabolic regulation-for instance, the delayed but higher yields observed in glycerol-supplemented cultures, which reflect reduced catabolite repression compared to glucose [22,30]. Together, these findings highlight both the robustness of HPLC as a monitoring tool and the ecological and biotechnological significance of violacein.

CONCLUSION

This study highlights that *Janthinobacterium lividum* produces violacein more efficiently in glycerol-supplemented media than in glucose-supplemented conditions, even when bacterial growth is comparable or slightly reduced. While glycerol promotes higher but more variable pigment yields, glucose supports consistent yet moderate production. These insights are significant for optimizing culture conditions aimed at maximizing violacein yield for biotechnological and pharmaceutical applications. Moreover, the findings indicate a potential protective function of violacein in enhancing bacterial survival under stress, suggesting new avenues for exploiting this pigment in the development of antimicrobial strategies.

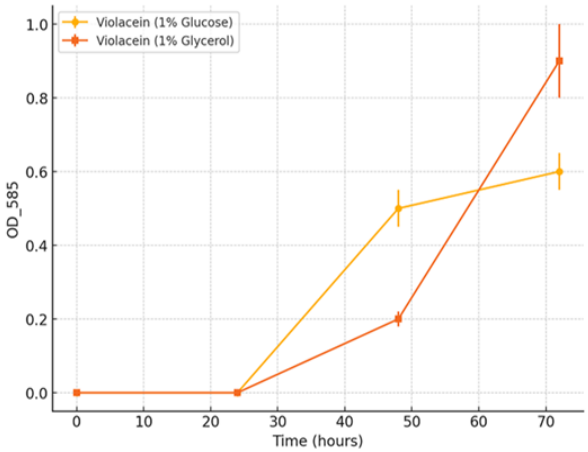

Figure 1: Graph showing the time course of violacein production by *Janthinobacterium lividum* in LB media containing either 1% glucose or 1% glycerol. The graph includes error bars representing the standard deviation of the measurements, providing insights into the variability of the data

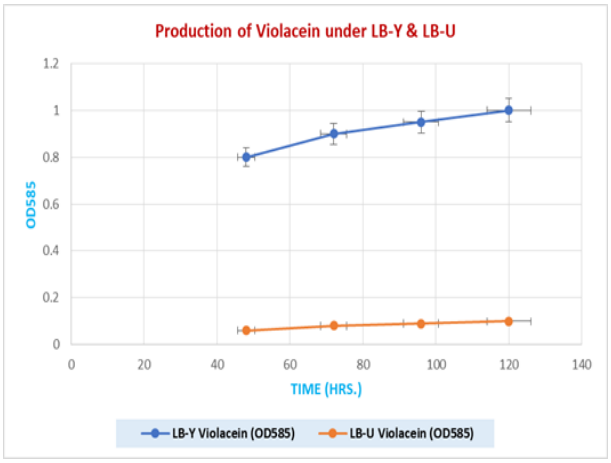

Figure 2: Violacein Production in *Janthinobacterium lividum* Grown in LB Medium with 1% Glucose (LB-U) or 1% Glycerol (LB-Y)

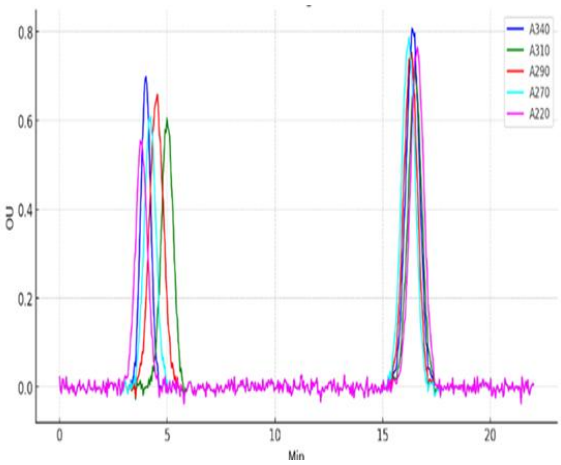

Figure 3: Chromatogram of Violacein Production by *Janthinobacterium lividum* (red peak indicates the production of Violacein)

Author Contributions

AK conceptualized the research idea, conducted the experiments, collected and analysed the data, and drafted the manuscript. SD critically reviewed and revised the manuscript for important intellectual content. Both authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declared no conflict of interest.

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