Optimization of growth parameters and pigment production in *Streptomyces. sp.* VITGV38 and analysis of its secondary metabolite properties

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Abstract
Colours are an integral part of daily life, with synthetic dyes widely used in various industries. However, concerns over their toxicity have driven the search for safer, eco-friendly alternatives. Microbial pigments have emerged as a sustainable and biodegradable substitute, with increasing global demand for natural colourants. This study focused on optimizing pigment production and evaluating the antimicrobial activity of secondary metabolites from Streptomyces sp. VITGV38 (MCC 4869). Among different tested media, starch casein agar supported the highest pigment yield whereas Luria-Bertani agar showed the least. Optimal growth and pigment production were achieved with 2% NaCl, pH 8–8.5 and a 21-day incubation period. Nutritional optimization using 2% maltose and potassium nitrate as carbon and nitrogen sources respectively, along with amino acids like tyrosine, threonine and tryptophan, further enhanced bioactive metabolite production.

Ethyl acetate was used to extract the crude pigment. The dark brown extract demonstrated significant antibacterial activity against four pathogens via the agar well diffusion method. The highest inhibition was observed against Staphylococcus aureus (25 mm) followed by Pseudomonas aeruginosa (23 mm), Bacillus subtilis (21 mm) and Escherichia coli (20 mm), highlighting the potential of Streptomyces pigments as promising natural bioactive agents.

Keywords: Actinomycetes, Antimicrobial activity, *Streptomyces*, Secondary metabolites, Pigments.

Introduction

Microorganisms are the most appropriate tools in biotechnology for creating a wide range of substances including pigments, organic acids, enzymes and antibiotics. Recent research has demonstrated that microorganisms have promise as a natural colour source. It has been revealed that pigments are present across the whole microbial kingdom[1]. Microbial hues are extremely fascinating due to the pigment's longevity as well as due to the accessibility and simplicity of cultivation and extraction methods[2]. Actinomycetes are filamentous, Gram-positive bacteria that possess a widespread array. They play an important role in both biological and artificial habitats[3]. A wide range of antibiotics is produced from actinomycetes and these antibiotics also include a variety of pigments[4].

Examples of some naturally coloured antibiotics produced by *Streptomyces* species include tetracycline, a yellow pigment produced by *Streptomyces aureofaciens*; Rifamycin, a reddish-orange antibiotic from *Streptomyces mediterranei*; and Actinorhodin, a blue pigment produced by *Streptomyces coelicolour*. These pigments help to differentiate different antibiotics. *Streptomyces* is the most prevalent genus in Actinobacteria[5]. These bacteria form tacky-coloured colonies with varying colours and physiological traits[6].

Streptomyces species have sparked much interest in biotech applications and this species is well known for creating a broad range of colours including blue, yellow, red, orange, pink, purple, blue-green, brown and black colours[7]. Pigments have various applications in textiles, food, painting, cosmetics and medicines. They play a crucial role in offering the goods an attractive appearance. A food colourant plays a vital role in the food trade as a food additive[8]. The production of natural dyes takes a lot longer and is more complicated and they are also more expensive. Organic hues derived from wildlife and plants may be replaced economically with microbial pigments, which are also more secure than artificially synthesized dyes.

Microbial pigments tend to be safe for human usage and certain kinds even possess antibacterial or anticancer features, in contrast to artificial colours, many of which have been connected to illnesses including cancer, allergies etc.[9] Some common, well-known pigment-producing *Streptomyces* are prodigiosin from *Streptomyces spectabilis*[7], melanin from *Streptomyces lavendulae*[10], actinorohodin from *Streptomyces violaceoruber*[9] and carotenoid from *Streptomyces* strain AQBMM35.

Some of the factors governing these bacteria's capacity to generate pigment include redox level, temperature, pH and medium composition. For instance, low temperatures and aerobic conditions enhance the formation of pigment. Actinomycetes' pigmentation is connected to defence, respiratory and UV protection systems[12]. The pH levels influence the cellular metabolites and secondary metabolite production in *Streptomyces* species[13]. Actinomycetes' ability to produce antibiotics tends to be significantly affected by environmental variables involving temperature, pH and length of incubation, as well as nutritional supplies like carbon, nitrogen and minerals[14]. Carbon and phosphate

sources significantly influence microorganisms' morphological development and the expression of their biosynthetic genes. Additionally, the formation of secondary metabolites is thwarted by high amounts of nitrogen sources like ammonium or amino acids[15]. The present study aimed to optimize the growth conditions of the culture parameters needed for the greatest possible production of secondary metabolites, especially pigments VITGV38 and its antibacterial activity.

Material and Methods

***Streptomyces* vitgv38:** *Streptomyces* VITGV38 was purchased from MCC, Pune and its accession no. was ID: MCC4869.

Pigment Production on Different Agar Media: Nutrient agar, Luria-Bertani agar, starch casein agar, actinomycetes isolation agar, international *Streptomyces* project 2 agar (ISP2) and yeast malt agar were the six agar media that were used to assess the pigment synthesis of the *Streptomyces* strain. Each plate was streaked with a standardised *Streptomyces* VITGV38. The plates were incubated at 30°C for 14 days and pigment production was checked daily with a focus on colour, intensity and diffusion.

Effect of Different pH: The effect of initial pH on pigment production by *Streptomyces* VITGV38 was investigated using eight different pH levels in 250 mL Erlenmeyer flasks, each containing 200 mL of nutrient broth. The pH of the medium was adjusted to eight different values (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5) using 0.1 M NaOH before sterilization through autoclaving. After sterilization, the flasks were inoculated with *Streptomyces* VITGV38 and incubated at room temperature (30°C) for 21 days on a rotary shaker (150 rpm). Growth and pigment production were monitored throughout the incubation period. Following incubation, the cultures were harvested and the pigments were extracted.

Effect of Different Incubation Periods: Three 250 mL conical flasks containing 200 mL of sterile nutrient broth were inoculated with 1 loopful of *Streptomyces* VITGV38 culture. The inoculated flasks were incubated at 30°C for 7, 14 and 21 days to study the impact of incubation time on growth and pigment production. The cultures were observed periodically for changes in growth characteristics including turbidity and colour, indicating microbial activity.

Effect of Different NaCl Concentrations: To determine the optimal NaCl concentration for growth and pigment, five 250 mL conical flasks containing 200 mL of nutrient broth were supplemented with NaCl at concentrations of 2%, 4%, 6%, 8% and 10%. The media were sterilized, inoculated with a loopful of *Streptomyces* VITGV38 culture and incubated at 30°C for 21 days.

Effect of Different Carbon Sources: To evaluate the impact of various carbon sources on growth and pigment production by *Streptomyces* VITGV38, four 250 mL Erlenmeyer flasks, each containing 200 mL of nutrient broth, were prepared. Before sterilization, each flask was supplemented with 1% (w/v) of one of the following carbon sources: glucose, sucrose, maltose and lactose. The pH of the medium was 7.4, which is the nutrient broth's optimal pH for pigment synthesis. The mixture was then sterilized by autoclaving. After sterilization, 1 loopful of *Streptomyces* VITGV38 sporulated culture was inoculated into each flask. The flasks were incubated at room temperature (30°C) for 21 days on a rotary shaker (150 rpm) to ensure proper aeration and agitation. After the incubation period, the cultures were visually examined for the extent of pigment production.

Effect of Different Nitrogen Sources: To evaluate the effect of different nitrogen sources on growth and pigment production, 250 mL Erlenmeyer flasks were prepared with 200 mL of nutrient medium. Various nitrogen sources including peptone, tryptone, potassium nitrate, ammonium chloride, phenylalanine and histidine, were added individually at a concentration of 1% (w/v) before sterilization. The prepared medium was sterilized by autoclaving and subsequently inoculated with *Streptomyces* VITGV38. The inoculated flasks were incubated at room temperature (30°C) for 21 days on a rotary shaker (150 rpm) to ensure uniform aeration and mixing. After the incubation period, pigment intensity in the cultures was visually assessed.

Effect of different Amino Acids: Five 250 ml of Erlenmeyer flasks were loaded with 200 ml of nutrient broth and the pH was 7.4. The amino acids to be tested were tyrosine, threonine, tryptophan, glycine and arginine at a concentration of 1% w/v. The medium was inoculated and incubated at room temperature for 21 days on a rotary shaker (150 rpm). Growth and pigment were recorded.

Optimized Culture Medium: To optimize pigment production, prepare a medium containing maltose as the carbon source, potassium nitrate as the nitrogen source, tyrosine and tryptophan as amino acids and 2% NaCl. Inoculate the medium with a seed culture of *Streptomyces sp.* VITGV38 and adjust the pH to 8.5. Incubate the culture under natural conditions at a temperature of 30°C for 21 days. During incubation, pigment production is monitored and the pigment is extracted.

Extraction: After 21 days of incubation, the media were filtered and centrifuged at 10,000 rpm for 10 minutes using a cooling centrifuge to separate the biomass. The supernatant was filtered using Whatmann filter paper to remove any remaining solid particles. To extract the pigments, 200 mL of the filtrate was mixed with ethyl acetate in 1:2 ratio and shaken vigorously for 20 minutes. The mixture was then transferred to a separating funnel and the organic phase was separated from the aqueous phase. The combined organic phases were collected and evaporated under reduced pressure at 40°C using a rotary evaporator. This process yielded a dark brown extract, which was further analyzed.

Antimicrobial activity: The antibacterial activity of crude extracts was evaluated using the agar-well diffusion method. Four different human pathogens, namely *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa* were uniformly spread onto Mueller-Hinton agar plates. In each plate, three different crude samples were tested against the respective pathogen for comparative analysis**.** Wells of 6 mm diameter were created using a sterile cork borer filled with 100 µl of the crude metabolite extract. The plates were incubated at 37°C for 12-16 hours, allowing bacterial growth and diffusion of the extracts into the medium. After incubation, the inhibition zones surrounding each well were measured to determine the antibacterial potential of the crude extracts. The presence of clear zones around the wells indicated microbial inhibition.

Results

Pigment Production on Different Agar Media: The results of *Streptomyces* VITGV38 pigment production on various agar media revealed significant differences in both growth and pigment intensity (Figure 1). The highest pigment production was observed on starch casein agar where the strain exhibited vibrant, diffusible pigments, suggesting that the medium provided optimal conditions for pigment biosynthesis. In contrast, ISP2 and nutrient agar supported moderate growth and resulted in less intense pigment formation. Both yeast malt and actinomycetes isolation agar promoted good growth, but pigment production was lower compared to starch casein agar, indicating that these media were less effective in inducing pigment synthesis. Finally, LB agar showed the least pigment production, highlighting that it may not be the most suitable medium for enhancing pigment biosynthesis in this strain (Table 1).

Effect of Different pH: The effect of initial pH on pigment production and growth of *Streptomyces* VITGV38 was evaluated at pH levels ranging from 6.0 to 9.5. After 21 days of incubation, both growth and pigment production (Fig. 2A) were significantly influenced by pH. Minimal pigment synthesis and low growth (0.24 absorbance at 570 nm) were observed at pH 6.0. Growth and pigment production improved at pH 7.0 and 7.5, with optimal levels achieved at pH 8.0 and 8.5 where absorbance peaked at 0.87. Beyond pH 8.5, pigment production and growth declined, with absorbance dropping to 0.23 at pH 9.5. These findings indicate that a slightly alkaline pH (7.5-8.5) is ideal for maximum growth and pigment production in *Streptomyces* VITGV38.

Effect of Different Incubation Periods: The bar chart (Fig. 2B) illustrates the absorbance values measured at different incubation periods (Day 7, Day 14 and Day 21) to evaluate the time-dependent changes in the process under study. Absorbance showed a progressive increase from day 7 (0.18) to day 14 (0.35), reaching its highest value at day 21 (0.38). These results emphasise that the incubation period significantly influences the observed absorbance, with day 21 being the optimal time point for peak performance in the analysed process.

Effect of Different NaCl Concentrations: The *Streptomyces sp*. VITGV38 strain was cultivated under optimal conditions to evaluate growth and pigment synthesis (Fig. 2C) at different NaCl concentrations. At 2% NaCl (absorbance: 2.697), which also demonstrated significant biomass, the highest growth and pigment production were observed.

Table 1
Comparative Analysis of Streptomyces VITGV38 growth, colony size, colour, texture and pigment production on different agar media.

S.N.	Agar Medium	Growth	Size of the colony	Colour of the colony	Texture of the colony	Pigment Production	Observations
1	Starch Casein Agar	High	1-3 mm	Dark brown to black	Smooth	Excellent pigment formation	Optimal conditions for secondary metabolite biosynthesis supporting robust pigment formation.
2	ISP2 Agar	Moderate	2-4 mm	Dark reddish brown	Dry	Moderate pigment formation	Suitable for moderate pigment production, though not as intense as on starch casein agar.
3	Nutrient Agar	Moderate	2 mm	reddish-brown	Smooth	Less intense pigment formation	It supports moderate growth but less synthesis-friendly pigment intensity.
4	YM Agar	Good	2-5 mm	Dark brown	Brittle	Lower pigment production	Good growth, but pigment production is still lower than starch casein agar.
5	AI Agar	Good	1-7 mm	Dark red	Dry	Moderate pigment production	Promotes good growth but pigment formation is not 6as significant as on starch casein agar.
6	LB Agar	Low	1-6 mm	Light beige to brown	Smooth	Least pigment production	Minimal pigment production, indicating it is less effective for pigment biosynthesis in this strain.

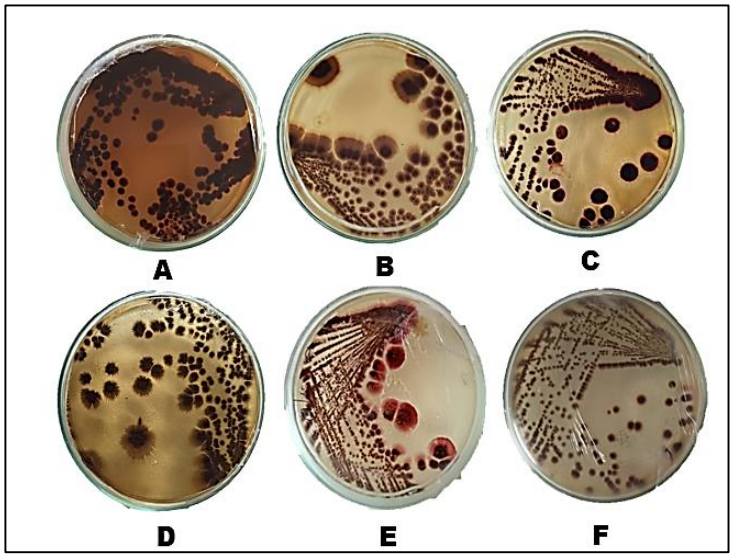

Figure 1: Petri plates showing the dark colour pigments in *Streptomyces* VITGV38. 1A. Starch casein agar, 1 B. ISP2 Agar, 1C. Nutrient agar, 1D. Yeast Malt Agar, 1E. Actinomycetes Isolation Agar and 1F. Luria Britania Agar

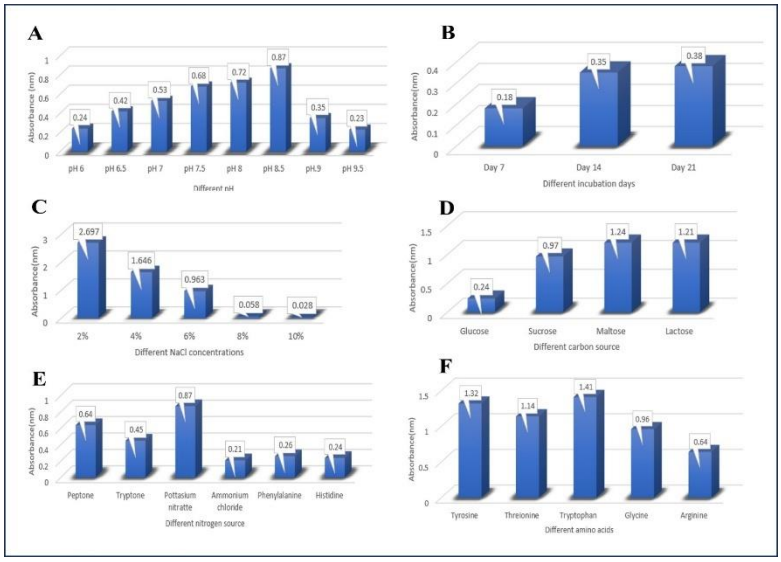

Figure 2 (A-F): Shows the effect of various nutritional parameters on the growth and pigment production by *Streptomyces* sp. VITGV38. (A) Effect of different pH on pigment production, (B) Effect of different incubation period, (C) Effect of different NaCl (%) concentrations, (D) Effect of different carbon sources, (E) Effect of different nitrogen sources, (F) Effect of various amino acids

At increasing NaCl concentrations, growth significantly decreased with minimal absorbance at 8% (0.058) and 10% (0.028). According to these results, *Streptomyces* VITGV38 grows best at low salt concentrations, but greater concentrations inhibit its growth.

Effect of Carbon Source: All carbon sources that were evaluated, allowed the organism to develop; however, the maximum biomass, pigment intensity and growth (Fig. 2D) were found with lactose and maltose. The highest growth (absorbance: 1.24) was enhanced by maltose which was closely followed by lactose (1.21). Glucose produced low growth (0.24), but sucrose produced significant growth (0.97). These findings suggest that the best carbon sources for promoting Streptomyces sp. VITGV38 growth and pigment production are maltose and lactose.

Effect of Nitrogen Sources: The most effective nitrogen sources for Streptomyces VITGV38 were potassium nitrate and peptone, which promoted the maximum growth and pigment synthesis (Fig. 2E). The absorbance of potassium nitrate was the greatest (0.87), followed by that of peptone (0.64). Ammonium chloride and tryptone promoted considerable development, but histidine and phenylalanine made only minor contributions. These findings demonstrate that the best nitrogen source for enhancing growth and pigment production is potassium nitrate.

Effect of Amino Acids: Amino acid supplementation has a major impact on Streptomyces growth and pigment synthesis (Fig. 2F). Tyrosine and tryptophan produced the highest pigment development and intensity (absorbance 1.32 and 1.41 respectively), making them the most effective.

Threonine (1.14) and glycine (0.96) exhibited moderate pigment synthesis and growth, but arginine (0.64) had the least amount of pigment intensity and growth. According to these findings, tyrosine and tryptophan promote the development and synthesis of pigments, possibly serving as regulators or precursors in the biosynthesis process.

Optimised Culture Medium: The optimised medium, containing maltose as the carbon source, potassium nitrate as the nitrogen source, tyrosine and tryptophan as amino acids and 2% NaCl, led to significant growth and pigment production in *Streptomyces sp.* VITGV38 after 10 days of incubation at 30°C under natural conditions. The pH of 8.5 was maintained throughout the experiment and the highest pigment yield was observed on day 10. The extracted pigment showed a strong colour intensity, indicating effective secondary metabolite synthesis. The production of pigment increased steadily during the incubation period, with the peak concentration of pigment achieved by day 10.

Comparative analysis with control media revealed that the optimized medium supported higher growth and pigment biosynthesis, the optical density (OD) absorbance was 2.36 at 570 nanometers, confirming that the selected carbon and nitrogen sources, along with the addition of pH, NaCl and amino acids, are key factors in promoting enhanced pigment production. This result suggests that the conditions used in this experiment are suitable for optimizing pigment production in *Streptomyces* species and could be applied in the industrial-scale production of microbial pigments.

Extraction: After incubation, the culture was centrifuged at 10,000 rpm for 10 minutes, which separated the biomass from the clear liquid. The liquid was then filtered to remove any remaining solids. 200 mL of the filtered liquid was mixed with ethyl acetate in 1:2 ratio and shaken for 20 minutes, causing the ethyl acetate layer to turn yellow, indicating that pigments were extracted. The mixture was placed in a separating funnel where the two layers separated clearly. The organic layer was collected and the solvent was removed by evaporation at 40°C, leaving a dark brown pigment. This crude pigment was further analyzed.

Different pH: Figure 3 (A) illustrates the zone of inhibition for four different bacterial species *E coli, B.subtilis, S.aureus and P.aeruginosa* under different pH levels (6.0 to 9.5).

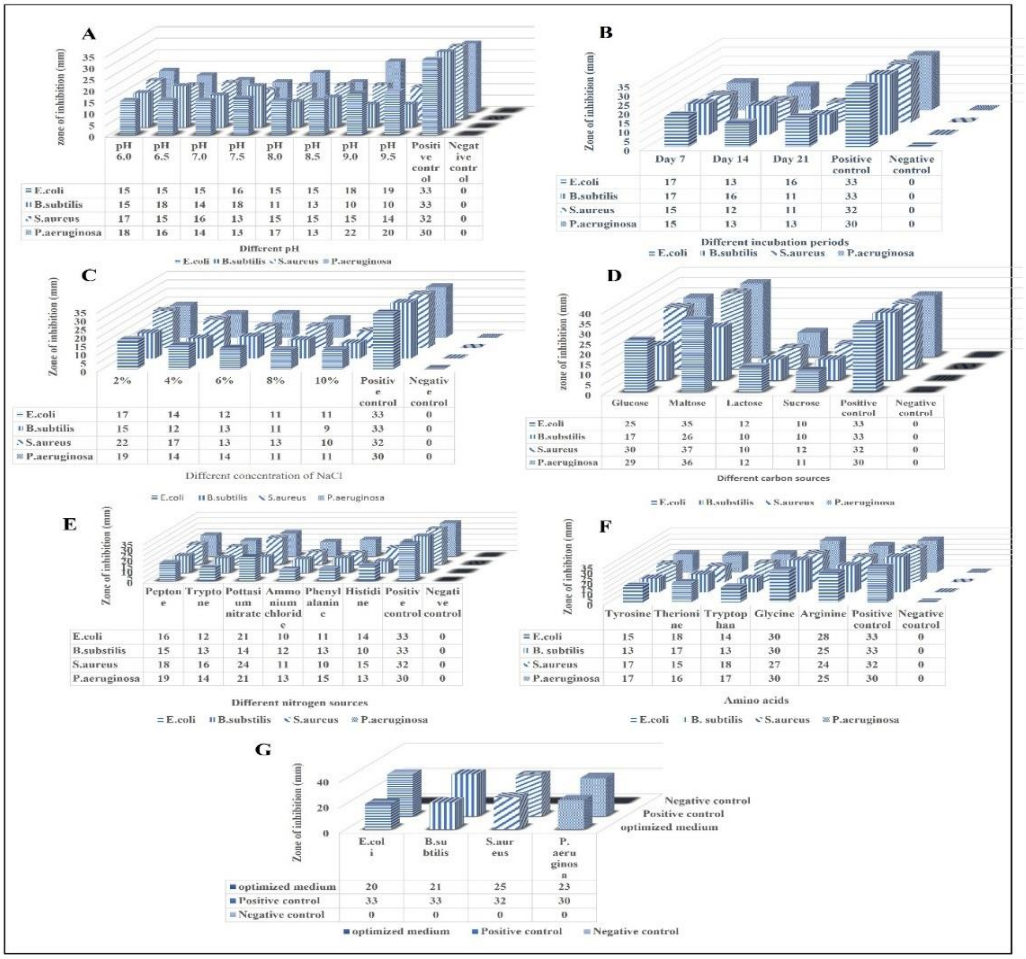

Figure 3 (A-G): Shows the antimicrobial activity of *Streptomyces* sp. VITGV38 against pathogenic bacteria under different conditions: (A) Effect of different pH on pigment production, (B) Effect of different incubation period, (C) Effect of different NaCl (%) concentrations, (D) Effect of different carbon sources, (E) Effect of different nitrogen sources, (F) Effect of various amino acids and (G) optimised medium

At pH 9.0, *P.aeruginosa* (22 mm) showed the highest inhibition zone followed by *E. coli* (18 mm), while *B.subtilis* and *S.aureus* were less inhibited (10 mm and 15 mm, respectively). Similarly, at pH 9.5, *P.aeruginosa* (20 mm) exhibited significant inhibition, whereas other bacteria showed much lower values. At neutral to slightly acidic pH (6.0-7.5), Bacillus displayed higher inhibition (18 mm) at pH 6.5 compared to other bacteria, whereas *S. aureus* maintained moderate inhibition (13-17 mm) across most pH values. In contrast, *E. coli* and *P. aeruginosa* showed relatively stable inhibition zones across pH 6.0-8.5, ranging between 13-18 mm. Overall, *P. aeruginosa* exhibited the highest inhibition at alkaline pH (9.0-9.5), while *B.subtilis* performed better under slightly acidic conditions (pH 6.5), suggesting pH-dependent variations in antimicrobial effectiveness.

Different Incubation Periods: The data shows (Fig. 3B) the zone of inhibition for four different bacterial species *E coli, B.subtilis, S.aureus and P.aeruginosa* over the time. On the day 7, *E. coli* and *B.subtilis* had the highest zones (17 mm), while *S.aureus* and *P.aeruginosa* showed 15 mm. By day 14, inhibition decreased slightly, with *B.subtilis* (16 mm) showing the largest zone. On the day 21, *E. coli* (16 mm) increased, while others ranged from 11-13 mm. By the day 28, inhibition peaked, with *S.aureus* (30 mm) and *P.aeruginosa* (28 mm) showing the highest values, followed by *B.aureus* (21 mm) and *E. coli* (19 mm). Overall, inhibition fluctuated, with day 28 showing maximum zones, particularly for *S.aureus* and *P.aeruginosa.*

Different NaCl Concentration: The data shows (Fig. 3C) the zone of inhibition for four different bacterial *E.coli, B.subtilis, S.aureus* and *P.aeruginosa* at different NaCl concentrations. At 2% NaCl, inhibition was highest, with *S.aureus* (22 mm) leading, followed by *P.aeruginosa* (19 mm), *E. coli* (17 mm) and *B.subtilis* (15 mm). As NaCl increased to 4%, inhibition decreased with *S.aureus* still highest at 17 mm, while others dropped to 12-14 mm. At 6% NaCl, zones further declined to 12-13 mm. By 8% and 10% NaCl, inhibition was minimal, with *S. aureus* (10-13 mm) slightly higher than others (9-11 mm). Overall, inhibition decreased with rising NaCl, with *S.aureus* most susceptible at lower concentrations.

Different Carbon Sources: The data shows (Fig. 3D) the zone of inhibition for four different bacterial species such as *E.coli, B.subtilis, S.aureus and P.aeruginosa* using different carbon sources. Maltose produced the highest inhibition, with *S. aureus* (37 mm) and Pseudo (36 mm) showing the largest zones. Glucose also had significant inhibition, particularly for *S. aureus* (30 mm) and *P.aeruginosa* (29 mm).

In contrast, lactose and sucrose caused minimal inhibition, with *E. coli* and *P. aeruginosa* showing the largest zones at 12 mm for lactose and *S. aureus* at 12 mm for sucrose. Maltose was the most effective, while lactose and sucrose had the least impact.

Different Nitrogen Sources: The data shows (Fig. 3E) the zone of inhibition for four different bacterial *E coli, B.subtilis, S.aureus and P.aeruginosa* under six nitrogen sources. Peptone had the highest inhibition with *P.aeruginosa* (19 mm) and *S.aureus* (18 mm) showing the largest zones. Potassium nitrate was also effective with *S.aureus* (24 mm) and *P.aeruginosa* (21 mm) showing significant inhibition. Tryptone showed moderate inhibition, while ammonium chloride and phenylalanine had lower effects, with zones ranging from 11-13 mm. Histidine showed the least inhibition overall, with *E.coli* (14 mm) and *P.aeruginosa* (13 mm) performing slightly better. Overall, peptone and potassium nitrate were the most effective, particularly for *S. aureus* and *P.aeruginosa*, while histidine and ammonium chloride were the least effective.

Different Amino Acids: The data (Fig. 3F) compares the zone of inhibition for four different bacterial species *E coli, B.subtilis, S.aureus and P.aeruginosa* under different amino acids. Glycine showed the highest inhibition for *E.coli, B.subtilis* and *P.aeruginosa* (30 mm), while *S.aureus* had 27 mm. Arginine was also effective with *E.coli* (28 mm) leading, followed by *P.aeruginosa*, *B.subtilis* (25 mm) and *S.aureus* (24 mm). Tyrosine had moderate effects with *S.aureus* (17 mm) having the largest zone and others between 13-15 mm. Threonine showed moderate inhibition, with *E.coli* (18 mm) leading. Tryptophan had the lowest but consistent effects, with *S.aureus* (18 mm) showing the highest zone. Overall, glycine and arginine were the most effective, particularly for *E.coli, B.subtilis and P.aeruginosa*, while tyrosine and tryptophan had the least impact.

Optimized Medium: Fig. 3G shows the zone of inhibition (mm) for four different bacterial species in an optimized medium. *S. aureus* had the largest inhibition zone at 25 mm, followed by *P.aeruginosa* at 23 mm, *B.subtilis* at 21 mm and *E.coli* at 20 mm. This highlights the optimized medium's effectiveness, with *S.aureus* showing the highest susceptibility.

Discussion

Actinomycetes, a diverse group of Gram-positive bacteria, play a crucial role in natural ecosystems and industrial applications. Among them, *Streptomyces* is the most dominant genus, widely recognized for its ability to produce a vast array of bioactive pigments. These pigments have extensive applications in industries such as textiles, food, painting, cosmetics and medicine, making *Streptomyces* a valuable resource in biotechnology. The optimization of pigment production in *Streptomyces* species involves fine-tuning various physical and nutritional parameters to enhance yield, stability and bioactivity, thereby increasing their industrial and therapeutic potential. pH levels influence the production of secondary metabolites and cellular metabolisms in *Streptomyces* species.

The pH was determined to be appropriate for the highest metabolite synthesis in this investigation when it was close

to neutral[13]. According to Ripa et al[18], high pH conditions adversely affect the production of secondary metabolites, inhibiting their growth. As a previous report, the pH 8 to 8.5 was the most suitable range for growth and pigment synthesis in *Streptomyces* VITGV38. Glycerol, lactose, maltose and a few other carbohydrates are known to interfere with the synthesis of secondary metabolites[17].

Our results align with studies on *S. hygroscopicus* isolates AK-111-81 and CH-7 where lactose as a carbon source led to significant antimicrobial metabolite production. Similarly, we observed enhanced antimicrobial compound synthesis using lactose. This supports lactose's role in stimulating the production of bioactive metabolites in *S. hygroscopicus* [18]. The subsequent results were obtained from experiments that were carried out to optimize different culture factors. Maltose produced the greatest amount of biomass, although lactose and maltose aided in forming pigment. Rajendran et al[16] for *Kocuria sp*. K70, reported that 2% NaCl had remarkable pigment biomass. In our study, peptone significantly enhanced pigment production in *Streptomyces sp.* VITGV38, consistent with previous reports on *Streptomyces sp.* PM4,[20] where peptone supplementation increased pigment yield with *Streptomyces lavendulocolour* VHB-9. Peptone was identified as the most suitable nitrogen source for pigment production[18,21]. The greatest biomass output was obtained from the production of potassium nitrate. Tyrosine and tryptophan, which have the best growth and pigment richness, were recorded.

Consequently, a considerable increase in growth and pigmentation was achieved at a concentration of 2g (mg/ml). For VITGV38, tryptophan produced robust dark colouration and great development[22]. Secondary metabolites were widely extracted using ethyl acetate[13]. More research is needed to develop a cost-effective fermentation medium for large-scale *Streptomyces* pigment synthesis utilizing readily available resources. Therefore, it is justified to seek a commercial manufacturing procedure for its manufacture.

Conclusion

This study identifies *Streptomyces spp.* VITGV38 is a highly promising natural pigment producer distinguished by its antimicrobial properties. This species is well-suited for industrial applications in the pharmaceutical and food industries. Cultivating the organism in a starch casein agar medium resulted in the highest pigment production, fostering optimal growth and achieving the most intense pigment synthesis with notable antimicrobial activity. While optimising growth conditions may influence secondary metabolite synthesis, the organism's ability to generate high-pigment yields under controlled conditions presents a sustainable alternative to synthetic pigments.

This offers great potential for environmentally friendly solutions in various industries. Future research should aim to refine the separation of pigments and bioactive compounds and explore genetic engineering strategies to scale up production, making it cost-effective for large-scale applications. Additionally, further investigation into its antimicrobial properties could expand its utility in developing novel therapeutics.

Acknowledgement

The authors of the study acknowledge the Management of Vellore Institute of Technology, Vellore, for providing all the facilities.

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(Received 16th June 2025, accepted 20th August 2025)