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Optimization of Growth Parameters to Enhance the Production of Antifungal Metabolite by Bioactive *Streptomyces pseudogriseolus* VSG-9

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A B S T R A C T

The aim of this study to optimize the growth parameters to significantly enhancing the production of antifungal metabolite by bioactive isolate *Streptomyces pseudogriseolus* VSG-9 isolated from soil sample collected on Gulbarga region Kalaburagi, India. The impact of different factors, including culture media, pH, temperature, agitation, carbon and nitrogen sources varying single parameters at a time. The antifungal activity of the crude extract was evaluated using agar well diffusion method. The *Streptomyces pseudogriseolus* VSG-9 exhibited the highest growth and metabolite production was achieved in Starch casein agar with supplement with % of dextrose at pH 7 and 35^{0}C temperature at stationary condition. A comparative study was performed to evaluate the antifungal activity of amphotericin B, fluconazole, ketoconazole, and nystatin along with extracellular and intracellular crude extract of *S.pseudogriseolus* against *Candida* strains. Ketoconazole and fluconazole showed antifungal activity against tested *Candida* sp., while amphotericin B, and nystatin exhibited resistance. Interestingly, both extract of *S.pseudogriseolus* VSG-9 demonstrated potent and broad spectrum of inhibitory effect against all tested *Candida* sp.

Introduction

Infectious diseases remain a significant health challenge in developing countries, characterized by high rates of morbidity and mortality (Bloom and Cadarette, 2019). The treatment of these diseases is further complicated by the widespread development of multi-drug resistance among pathogenic microorganisms. Although new antimicrobial agents are available, the persistent emergence of drug-resistant pathogens, frequent outbreaks of epidemic disease, and rapid transmission of these pathogens among individuals highlight the critical need for the continuous development of effective antibiotics (Lam, 2006). Actinomycetes hold significant value due to their ability to produce a range of biggest producer of antibiotics, antitumor, immunosuppressive agents, vitamins and enzymes (Ruwandeepika *et al.,* 2022). Among actinomycetes, *Streptomyces* species are particularly notable for their potential as therapeutic antibiotic producers (Mude *et al.,* 2025). About 70% of

known drugs have been derived from actinomycetes, with 75% being utilized in medicine and 60% in agriculture, respectively. The growth of actinomycetes can be optimized by manipulating the cultural conditions such as nutritional, physical and chemical parameters (Reddy *et al.,* 2011; Saurav and Kannabiran, 2010). Establishing optimal conditions for fermentation is essential to enhance the production of antimicrobial metabolites, requiring the standardization of various physical and physiological parameters that influence maximum yield (Osman *et al.,* 2024).

The biological relevance of optimized *Streptomyces* derived extracts is often evaluated against opportunistic fungal pathogens, particularly *Candida* species. These fungi are responsible for a wide spectrum of infections ranging fro superficial mucosal candidiasis to invasive bloodstream infections, with incidence rising due to immunosuppression, prolonged antibiotic use, and invasive medical interventions. Current antifungal therapy mainly relies on polyenes, such as nystatin and amphotericin B, which disrupt fungal cell membranes by binding to ergosterol, and azoles, including ketoconazole and fluconazole, which inhibit ergosterol biosynthesis (Wiederhold, 2021; Pappas *et al.,* 2016). Despite their clinical importance, these drugs face limitations: Amphotericin B is associated with nephrotoxicity, nystatin is largely restricted to topical use, and azole resistance is increasing reported. Resistance mechanisms in *Candida* include mutations in ERG11, overexpression of efflux pumps, and biofilm associated tolerance, with fluconazole resistance particularly common in *C.glabrata* and emerging in *C.auris*. Although amphotericin B and nystatin generally remain effective, reduced susceptibility has also been documented (Kolaczkowska *et al.,* 2016; Ghojoghi *et al.,* 2024).

These challenges highlight the urgent demand for safer and more effective antifungal agents. Secondary metabolites from *Streptomyces* represent a promising alternative due to their structural diversity and potential to overcome current resistance mechanisms. Systematic optimization of fermentation conditions, coupled with comparative evaluation of their antifungal activity against *Candida* species alongside standard drugs, provide a rational framework for identifying novel therapeutic candidates capable of addressing the escalating problem of antifungal resistance. This study focused on Screening of *Streptomyces pseudogriseolus* VSG-9 for its antimicrobial activity and optimizing the conditions of producing antimicrobial compound.

Materials and Methods

Isolation of Streptomyces pseudogriseolus VSG-9

The *Streptomyces pseudogriseolus* VSG-9 was isolated in our previous work from a garden soil of Kalaburgi region, Karnataka, India and screened for its antifungal secondary metabolite production.

The isolate was identified based on cultural, micromorphology, biochemical and molecular identification confirmed its identified as *Streptomyces pseudogriseolus* through the 16S rRNA sequencing and deposited in GeneBank with accession number ON982537 (Fig. 1a, b).

Optimization of growth parameters for antifungal property

Optimization of growth factors for the production of highest secondary metabolites.

Effect of Culture Media

The VSG-9 culture was inoculated on eight different media, namely, inorganic salt starch agar (ISP-4;g/l: soluble starch 10; K_2HPO_4 1; $MgSO_4$ $7H_2O$ 1; NaCl 1; $(NH_4)_2SO_4$ 2; $CaCO_3$ 2; $FeSO_4$ $7H_2O$ 0.1; $MnCl_2$ $4H_2O$ 0.1; $ZnSO_4$ $7H_2O$ 0.1; agar 20), starch casein agar (SCA;g/l: soluble starch 10; casein 0.3; KNO_3 2; NaCl 2; K_2HPO_4 2; $MgSO_4$ $7H_2O$ 0.05; $CaCO_3$ 0.02; $FeSO_4.7H_2O$ 0.01; agar 18), oat meal agar (ISP-3;g/l: oat meal 60;agar 12), tryptone yeast extract agar (ISP-1;g/l: casein enzymic hydrolysate 6; yeast extract powder 3; agar 12), soybean casein digest agar (SBC;g/l: pancreatic digest of casein 15; papaic digest of soybean meal 5; NaCl 5; agar 15), glycerol asparagine agar (ISP-5;g/l: L-Asparagine 1; K_2HPO_4 1; $FeSO_4$ $7H_2O$ 0.001; $MnCl_2$ $4H_2O$ 0.001; $ZnSO_4$ $7H_2O$ 0.001; agar 20), yeast malt agar (ISP-2;g/l: Yeast extract 3; malt extract 3; peptone 5; glucose 10; agar 20), actinomycetes isolation agar (AIA;g/l: sodium caseinate 2; L-asparagine 0.100; sodium propionate 4; K_2HPO_4 0.500; $MgSO_4$ 0.100; $FeSO_4$ 0.001; agar 15) incubated at 37^0C for 7 days. Observations were made on a regular basis and the medium which supported for the growth and sporulation of VSG-9 isolate. The selected media were used for broth culture and the culture filtrate was used for screening the antifungal activity against *Candida* strains (El-Naggar *et al.,* 2016).

Effect of Carbon Source

Different carbon sources namely, dextrose, Fructose, maltose, mannitol and sucrose (10g/l) were used in the basal medium (SC broth with starch being replaced with different carbon sources) and determined their efficacy for the production of secondary metabolites by VSG-9. The actinomycetes isolate was inoculated into flasks containing different carbon sources and the flasks were incubated at 37^0C for 7days. After incubation, the filtrates were extracted using ethyl acetate in separation funnel and the condensed ethyl acetate extracts were used for antifungal activity (Kiranmayi *et al.,* 2011 and Ismail *et al.,* 2017).

Effect of Nitrogen sources

KNO_3, glycine, phenylalanine, Methionine (2g/l) were used in the SC broth medium as a nitrogen source (Casein being replaced with nitrogen sources). The VSG-9 isolate was inoculated in the optimized medium with different nitrogen sources and incubated the flasks at 37^0C for 7days. After incubation, the filtrates were extracted using ethyl acetate in separation funnel. The concentrated ethyl acetate extract were used to assess antifungal activity by agar well method (Al-Ansari *et al.,* 2020).

Effect of pH

Optimum pH was studied by adjusting the pH of growth medium at 5-9 by adding 0.1N NaOH/0.1N HCl. All the flasks were inoculated with VSG-9 culture and incubated at 37^0C for 7days. After incubation the culture filtrates were extracted using ethyl acetate in separation funnel. The concentrated ethyl acetate extracts were analyzed for antifungal activity by agar well-diffusion method against test organisms (Miao *et al.,* 2021).

Effect of Temperature

The influence of temperature on VSG-9 for the production of antifungal metabolite was studied by inoculating the isolate into the optimized medium. The experiment set up was incubated at different temperature viz 15, 20, 30, 35, 40 and 45^0C for 7 days. After incubation, the culture filtrates were extracted using ethyl acetate in separation funnel. The the air dried extracts were tested for antifungal activity (Gebreyohannes, 2015).

Effect of incubation periods

To obtain the high rate of antibiotic production, VSG-9 isolate was inoculated in optimized medium in conical flask and incubated at 37^0C up to 15 days. On 5, 7, 9, 11, 13 and 15^{th} day, the culture filtrates were extracted using ethyl acetate in separation funnel. The solvent layer was separated and air dried. The air dried ethyl acetate extract was used to screen antifungal activity by agar well-diffusion method (Haque *et al.,* 2017).

Effect of Agitation

The VSG-9 isolate was inoculated in optimized medium at various agitation rates of 100, 120, 140, 160 and 180 rotation per minute (rpm) on a rotary shaker. All the optimized flasks were incubated for 7days. After incubation the culture filtrates were extracted using ethyl acetate in separation funnel and the condensed ethyl acetate extracts were analyzed for antifungal activity by agar well-diffusion method (Song *et al.,* 2012).

Extraction of Antimicrobial Compound Production

The broad spectrum of antifungal activity of selected strains was evaluated against *candida* strains. The *S. pseudogriseolus* was cultured in ISP-4 broth and incubated at 37^0 C for 2 weeks. After incubation, the culture medium containing staling substance was filtered through filter paper. The filtrate was aseptically transferred to a conical flask and subjected to liquid-liquid extraction with equal volume of ethyl acetate in a separating funnel. The solvent phase was collected and evaporated to dryness under room temperature to obtain a crude extract and used as extracellular extract (Ex).

The actinomycetes biomass was thoroughly washed to remove any remaining medium components and dried in hot air oven at 60^0 C. The dried biomass was crushed in pistil and mortar and intracellular secondary metabolites were extracted by adding ethyl acetate followed by centrifugation. The resultant supernatant was evaporated to dryness.

The obtained crude was used as intracellular extract (In). The actinomycetes extract and antifungal drugs of ketoconazole, fluconazole, nystatin and amphotericin B were dissolved in DMSO (dimethyl sulfoxide) at a concentration of mg/ml to prepare the test samples.

A comparative antifungal activity of actinomycetes crude extract with standard drugs

Antifungal activity was evaluated using the well-diffusion method. Freshly prepared 24h old *candida* cultures were evenly spread over Sabouraud's dextrose agar medium. Wells of 5 mm diameter were made using sterile cork borer. Each well filled with 100 µl of intracellular (In) and extracellular (Ex) extract, standard antifungal drugs such as ketoconazole, fluconazole, nystatin and amphotericin B and negative control (DMSO). The plates were incubated at 28^0 C for 24 h. After incubation, the diameter of the inhibition zone around the wells was measured. All tests were performed in triplicate and the antifungal activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the secondary metabolite.

Results and Discussion

Optimization of growth parameters for antifungal property

Effect of culture media

The mycelial growth and sporulation were assessed by using different nutrient media. The *S. pseudogriseolus* VSG-9 was grown on eight different media, out of which starch casein agar (SCA) medium was found to be suitable for the luxurious growth of *S. pseudogriseolus* VSG-9 with characteristic soluble pigment as compared to other media. The inorganic salt starch agar medium (ISP-4), Oat meal medium(OA-ISP-3) and actinomycetes isolation agar (AIA) also found suitable for optimum mycelial growth of VSG9 (Figure 2a, b, c, & d).The yeast malt agar (ISP-2) and tryptone yeast extract agar (ISP-1) showed moderate growth, while the soybean casein digest agar (SBC), glycerol aspargine agar medium (ISP-5) showed poor mycelial growth (Fig. 2e, f, g & h). Among the SCA, ISP-4, ISP-3 and AIA culture media, SCA demonstrated the highest ZOI at 15 mm against *C.albicans* 183, followed by ISP-4 with 14 mm ZOI against *C.tropicalis* 230. ISP-3 and AIA extract exhibited 13 mm and 12 mm ZOI against *C.glabrata* 3019 and *C.haemulonii* 2766 (Fig. 3). The maximum antifungal activity of *S. pseudogriseolus* VSG-9 extract was achieved significantly in starch casein agar medium. Hence, the medium was further used to optimize other cultural parameters for better growth and production of antifungal secondary metabolites.

Optimization of carbon sources

The different carbon sources at 10g/l concentration were used for the optimization process. Among the sources used, the maximum of 15±0.66 and 14±0.2 mm ZOI was found against *C.albicans* 183 and *C.glabrata* 3019 in a medium supplemented with dextrose, followed by 14±0.43 and 13±0.2 mm ZOI against *C.albicans* 183 and *C.tropicalis* 230 with fructose, 12±0.5mm ZOI against *C.tropicalis* 230 with mannitol, 11±0.6mm ZOI against *C.albicans* 183 with sucrose and 9±0.3mm ZOI was recorded with maltose against *C.albicans* 183 and *C.glabrata* 3019 (Fig. 4). At 10g/l of dextrose was found to be inhibitory concentration.

Optimization of nitrogen sources

The different nitrogen sources at 2g/l concentration were used for the optimization process. Among the sources used, the maximum of 14±0.66 mm ZOI was found against *C. albicans* 183 in a medium amended with KNO_3, followed by 12±0.83 mm ZOI was found against *C.albicans* 183 and *C.tropicalis* 230 with glycine, 11±0.27 mm ZOI against *C. albicans* 183 with phenylalanine and 9±0.33 mm ZOI was recorded with methionine against *C. albicans* 183 and *C.haemulonii* 2766 (Fig. 5). At 2g/l of KNO_3 was found to be optimum inhibitory concentration.

Optimization of pH

The effect of pH on the growth of *S. pseudogriseolus* VSG-9 and the production of secondary metabolites were investigated at different pH range from 5 to 9. The highest of 16±0.1 mm ZOI against *C. albicans* 183 was recorded at pH 7, followed by 15±0.33 mm ZOI against *C. albicans* 183 at pH 8, 12±0.27 mm ZOI against *C.tropicalis* 230 and *C.haemulonii* 2766 at pH 6. 8±0.1 mm ZOI against *C.glabrata* 3019, 6±0.5 mm ZOI against *C.tropicalis* 230 at pH 5 was recorded (Fig. 6). Therefore, the pH 7 of the culture medium was found effective in the production of antifungal metabolites.

Optimization of temperature

S. pseudogriseolus VSG-9 was grown in the basal medium at different temperature between 15 to 45^0C. Maximum of 16±0.33 mm ZOI was recorded at 35^0C against *C.tropicalis* 230. This was followed by 14±0.3 mm ZOI at 30^0C against *C.tropicalis* 230 and *C.glabrata*

3019, 13±0.2 mm ZOI at 40^{0}C against *C.glabrata* 3019, 10±0.5 mm ZOI at 45^{0}C against *C.glabrata* 3019 and 9±0.27 mm ZOI at 20^{0}C against *C.haemulonii* 2766 and 8±0.66 mm ZOI at 15^{0}C against *C.tropicalis* 230 (Fig.7). The 35^{0}C temperature was found to be optimum for the production of antifungal metabolites and antifungal activity.

Optimization of incubation period

Effect of the incubation period on antifungal activity was investigated and found a maximum of 15±0.43 mm ZOI on 11th day of incubation against *C.glabrata* 3019, followed by 14±0.1 mm ZOI on 9th day of incubation against *C.tropicalis* 230 and *C.haemulonii* 2766, 12±0.66 mm ZOI on the 13th day of incubation against *C.haemulonii* 2766. 11±0.5 mm ZOI on the 15th against *C.glabrata* 3019 and 8±0.33 mm ZOI on the 5th against *C.tropicalis* 230 and *C.haemulonii* 2766. Therefore, the highest antifungal activity was observed on the 11th day of incubation period (Fig.8).

Optimization of agitation

The *S.pseudogriseolus* VSG-9 was grown at different agitation speeds and recorded a maximum of 17±0.26 mm ZOI against *C.glabrata* 3019 at agitation speed of 140rpm. This was followed by 16±0.83 mm ZOI against *C.haemulonii* 2766 at 120 rpm, and 13±0.2 mm ZOI against *C.glabrata* 3019 at 160rpm, 12±0.43 mm ZOI against *C.tropicalis* 230 at 100rpm and 10±0.33 mm ZOI against *C.glabrata* 3019 at 180 rpm. Significant antifungal activity was recorded at 140rpm. (Fig.9).

A comparative antifungal activity of actinomycetes crude extract with standard drugs

The antifungal activity revealed that all tested *Candida* strains were resistant to nystatin and amphotericin B, while showing varying level of susceptibility to ketoconazole and fluconazole. The extracellular extract showed maximum of 14±0.05 mm ZOI against *C. albicans* 183 as compared to fluconozaole (14±0.33) and Ketaconozole (17±0.57).

The intracellular ethyl acetate crude extract demonstrated a highest of 12±0.57 mm ZOI against *C albicans* 2795 as compared to standard fluconozaole (13±0.26) and Ketaconozole (14±0.57). (Table 1 and Fig. 10). However, *S.pseudogriseolus* VSG-9 extracts showed stronger antifungal activity against *Candida* isolates when used alone compared to combinations with standard drugs, potentially paying the way for the discovery of more powerful and effective antifungal agents. However, further research is needed to validate these findings. Hence this study confirms that *S.pseudogriseolus* VSG-9 is a promising soil actinomycetes for producing antifungal secondary metabolites that are effective against a wide range of *Candida* strains.

Optimization of cultural and physiological parameters plays a crucial role in maximizing the growth of actinomycetes and the production of bioactive secondary metabolites. Factors such as pH, temperature, incubation period, agitation, and nutrient composition significantly influence both biomass accumulation and metabolite biosynthesis. The *S.pseudogriseolus* VSG-9 was grown on different media for the maximum production of antifungal metabolites. Among the media used, starch casein agar (SCA) medium showed excellent growth and highest antifungal activity. Similar studies were reported by Vijayakumar *et al.,* (2012) in *Streptomyces* sp VPTSA18 for grown and produced brown diffusible pigment on SCA medium. Previous studies have shown that the *Streptomyces* sp VPST3-1 grown on starch casein medium exhibits significant antimicrobial activity against *Klebsiella pneumoniae* and *Proteus vulgaris* (Vijayakumar *et al.,* 2012). Al-Ansari *et al.,* (2020) found maximum production of antimicrobial compounds in ISP4, followed by ISP1, ISP6, ISP-3, ISP5, from *Streptomyces* sp. AS11.

According to Rabah *et al.,* (2007) ISP4 medium produces maximum antibiotic in *Streptomyces* sp. RAF-10. Antibiotic synthesis from *Streptomyces* sp. J12 was found improved in oat meal, starch casein and sabouraud media (Al-Zaharani, 2007). Optimizing fermentation conditions is essential for enhancing the production antibiotic by actinomycetes, as nutrient availability directly influences both growth and secondary metabolite biosynthesis (Banga *et al.,* 2008). Dextrose was the most effective substrate for antifungal compound production, followed by fructose, Mannitol, sucrose and Maltose. Maximum of 15±0.66 and 14±0.2 mm ZOI was found against *C.albicans* 183 and *C.glabrata* 3019 in a medium supplemented with dextrose.

Table.1 A comparative antifungal activity of S.pseudogriseolus VSG-9 with different standard antifungal drugs

Candida strains MTCC	N	A	F	K	*S.pseudogriseolus* VSG-9 (In)	*S.pseudogriseolus* VSG-9 (Ex)
***C.albicans* 183**	R	R	14±0.33	17±0.57	12±0.033	14±0.05
***C.albicans* 1637**	R	R	13±0.5	15±0.33	10±0.57	11±0.33
***C.albicans* 3017**	R	R	11±0.43	13±0.26	9±0.83	10±0.43
***C.albicans* 1966**	R	R	12±0.26	12±0.33	11±0.26	10±0.33
***C.albicans* 2795**	R	R	13±0.26	14±0.57	12±0.57	12±0.01
***C.glabrata* 3981**	R	R	12±0.33	14±0.01	11±0.57	11±0.57
***C.tropicalis* 230**	R	R	11±0.33	14±0.05	12±0.5	13±0.01
***C.haemulonii* 8303**	R	R	12±0.83	13±0.26	9±0.33	10±0.026

Note: [N- Nystatin, A- Amphotericin, F-Fluconazole, K-Ketoconazole, In- Intracellular extract, Ex- Extracellular extract]

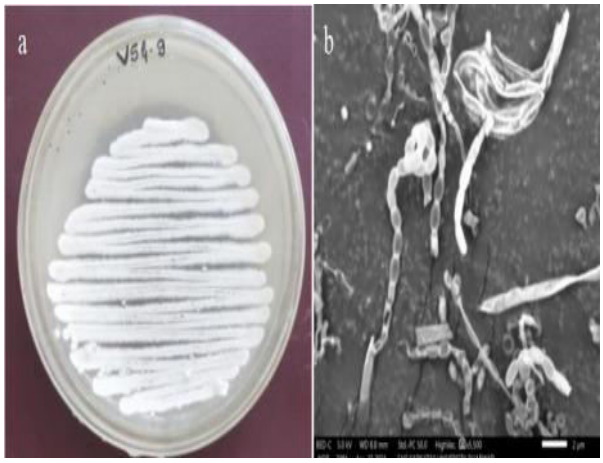

Fig.1 (a) Culture plate of *S.pseudogriseolus* VSG-9 on starch casein agar medium, (b) Scanning electron micrograph showing spore chain morphology.

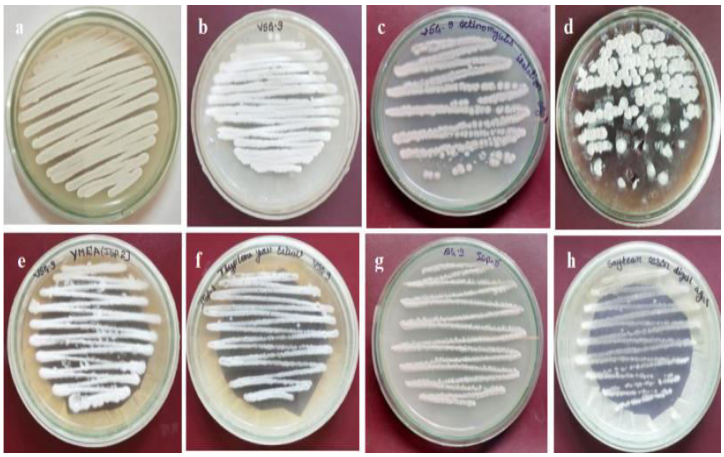

Fig.2 Mycelial growth and sporulation of *S.pseudogriseolus* VSG-9 on different medium after 7 days of incubation period was observed on (a) Starch casein agar, (b) Inorganic soluble starch agar (ISP-4), (c) Actinomycetes isolation agar, (d) Oat meal agar (ISP-3), (e) Yeast malt agar (ISP-2), (f) Tryptone yeast extract agar (ISP-1), (g) Glycerol aspargine agar (ISP-5), (h) Soybean casein digest agar.

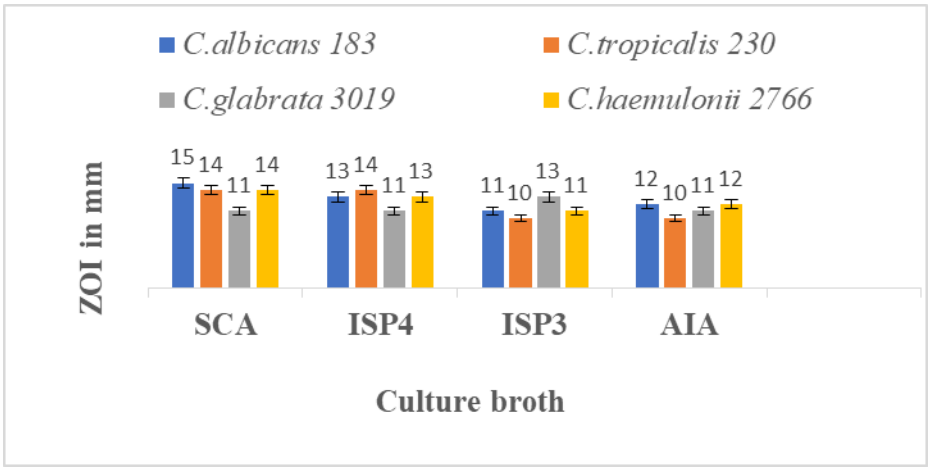

Fig.3 Effect of different culture broth growth and antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

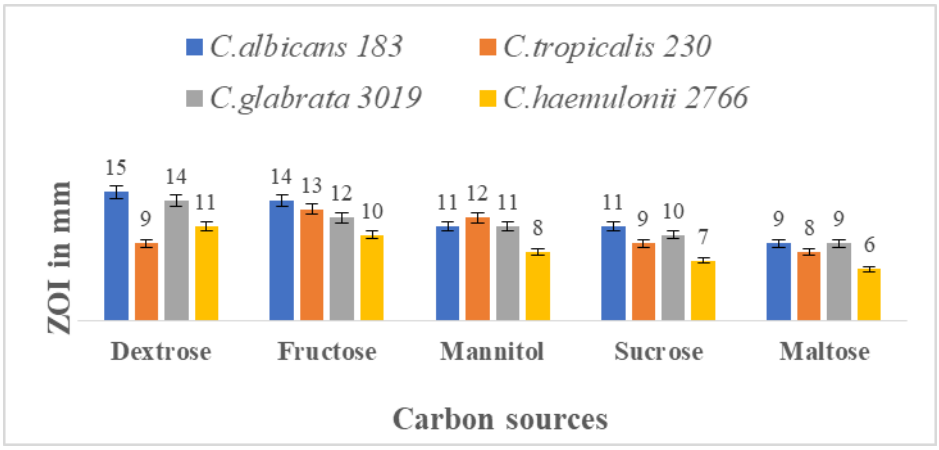

Fig.4 Effect of carbon sources on antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

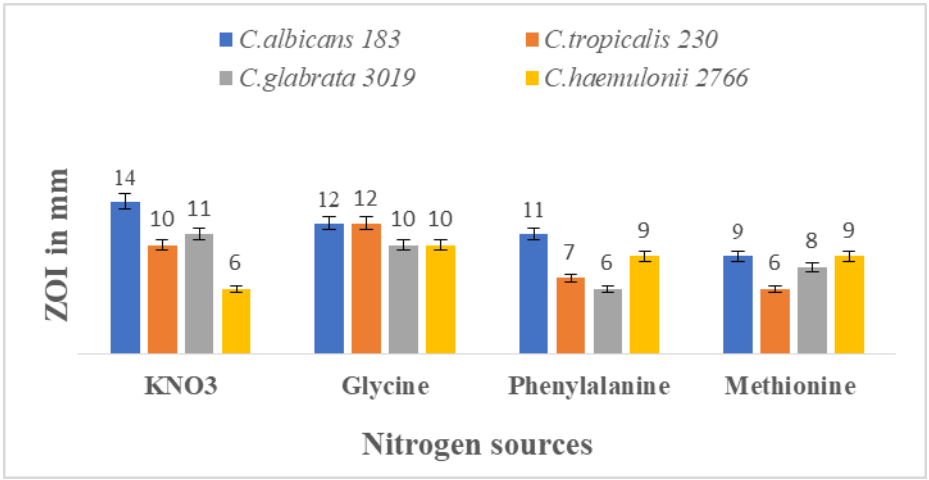

Fig.5 Effect of various nitrogen sources on antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

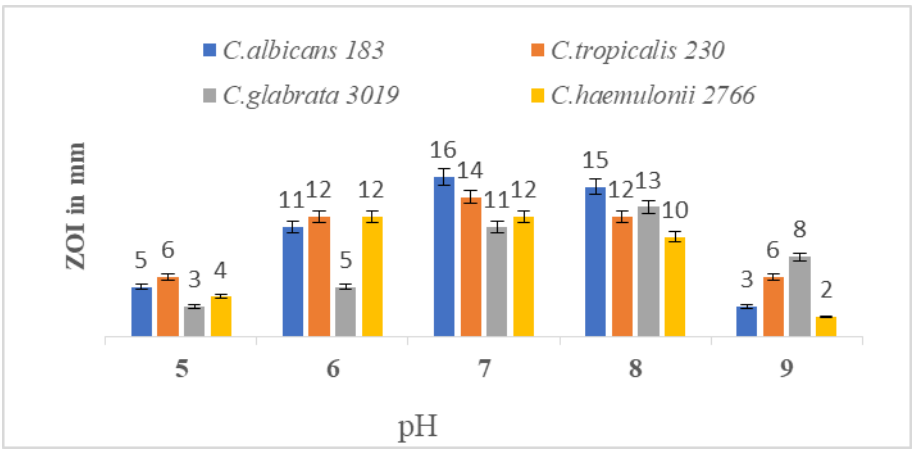

Fig.6 Effect of different pH for antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

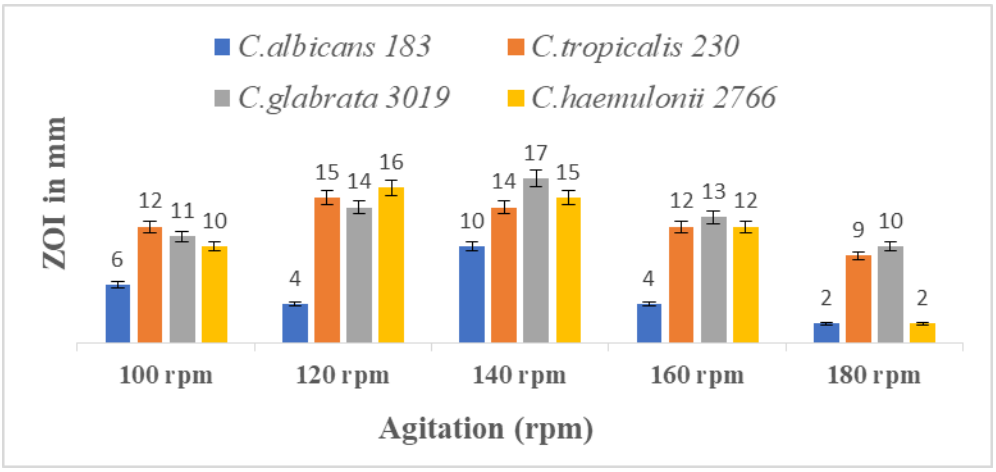

Fig.7 Effect of temperature for antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

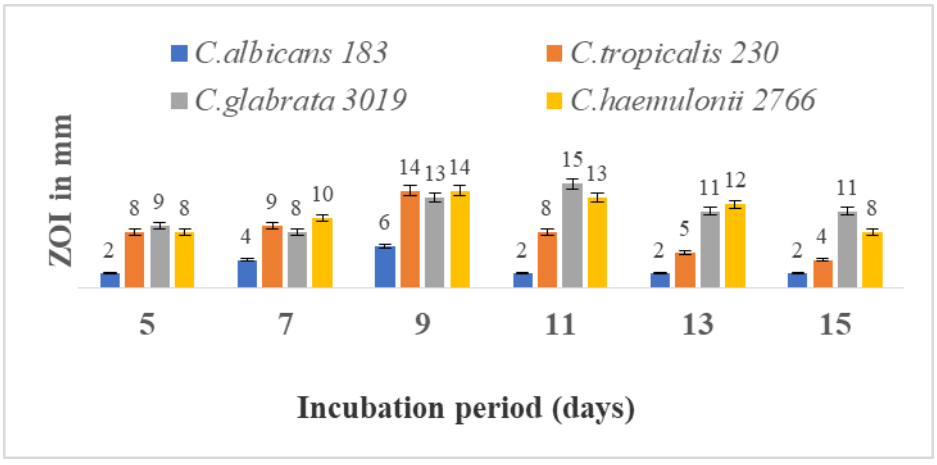

Fig.8 Effect of incubation period on antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

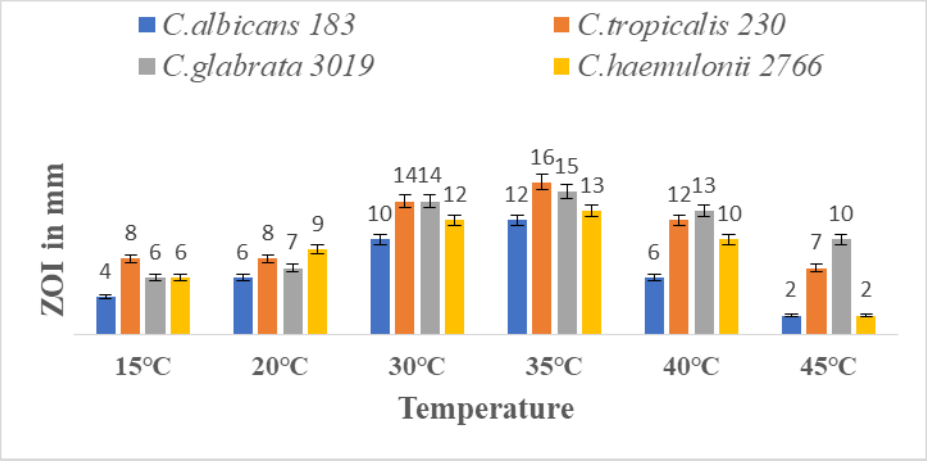

Fig.9 Effect of temperature on antifungal activity against *candida* strains [Data expressed as means ± SD (n=3)]

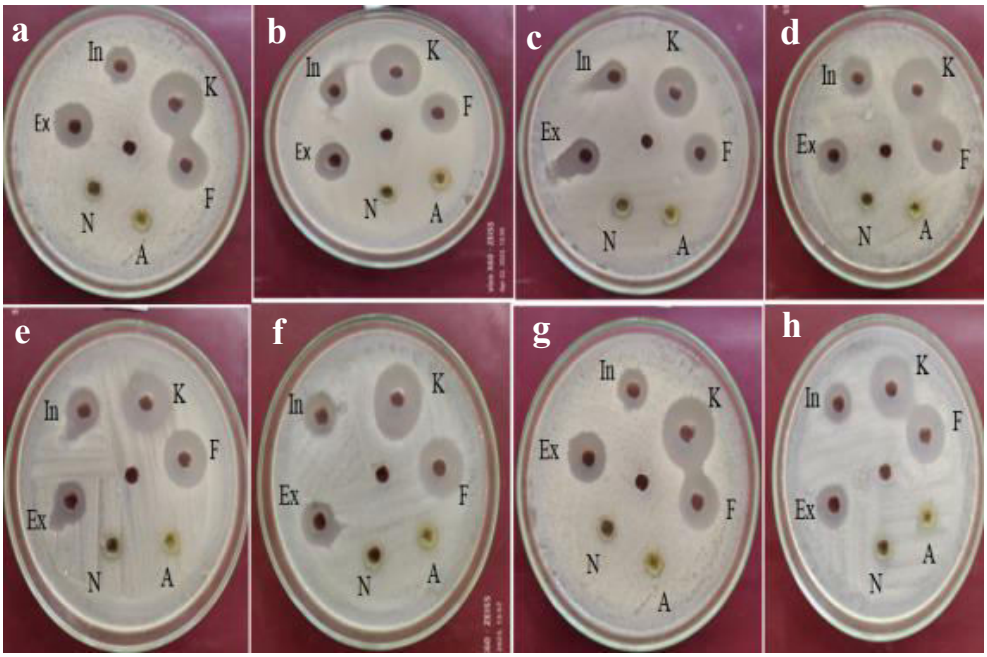

Fig.10 Antifungal activity of *S,pseudogriseolus* VSG-9 [In- Intracellular extract, Ex- Extracellular extract, N- Nystatin, A- Amphotericin, F-Fluconazole, K-Ketoconazole]showing ZOI against *Candida* sp., (a) *C.albicans* 183, (b) *C.albicans* 1637, (c) *C.albicans* 3017, (d) *C.albicans* 1966, (e) *C.albicans* 2795, (f) *C.glabrata* 3981, (g) *C.tropicalis* 230, (h)

Similar studies were reported by Rakesh *et al.,* (2014) in *Streptomyces* species SRDP-TK-07 isolated from rhizosphere soil with highest antimicrobial effect against *S.aureus* in the medium supplemented with Starch and lowest antimicrobial activity against *S.aureus* and *P.aeruginosa* in the medium supplemented with glucose. Microorganisms are capable of metabolizing diverse carbon sources, which not only sustain their growth but also influence the composition of structural and energy molecules, serving as key precursors in antibiotic biosynthesis (Bhosale *et al.,* 2018). Nitrogen sources and amino acids are vital for antibiotic synthesis. In this study *S. pseudogriseolus* VSG-9 showed maximum antifungal activity in medium containing KNO_3, while

the other nitrogen sources showed moderate activity. Maximum of 14±0.66 mm ZOI was found against *C. albicans* 183 in a medium amended with KNO_3. *Streptomyces rimosus* 93060 has been reported to produces oxytetracycline in soybean meal and peptone as nitrogen source (Omura *et al.,* 1973). The pH of the medium favors the enzyme stability, nutrient solubility, and metabolic activity. The *S. pseudogriseolus* VSG-9 was cultured on a SCA medium with a varying pH range from 5 to 9. Maximum antifungal activity was recorded against *C. albicans* 183 at pH 7. Al-Ghazali and Omran (2017) reported maximum antimicrobial activity by *Streptomyces* sp. LHR 9 at pH 7 against *E.coli*, *P.aeruginosa* and *S. aureus*. Neutral pH was essential for supporting the growth of *Streptomyces* sp. JRG-04 and in enhancing its potential for bioactive compound production (Ganesan *et al.,* 2017). *Streptomyces* sp. Strain MFB27 showed optimum antibiotic production at pH 7.5, (Zineb *et al.,* 2025). Similar results were also reported by Oskay (2009) by *Streptomyces* sp. KHH23 at pH 7.5. The *S. pseudogriseolus* VSG-9 was grown in the basal medium at different temperature between 15 to 45^{0}C. Highest (16±0.33mm) antifungal activity was recorded at 35^{0}C against *C.tropicalis* 230. Temperature and growth rate have a significant impact on the amount of secondary metabolite synthesized by *Streptomyces* (James *et al.,* 1991). Al-Ghazali and Omran (2017) reported that optimum temperature for maximum antibiotic production in *Streptomyces* sp LHR 9 was 35^{0}C, which was evidenced by the increased inhibition zones against *E.coli*, *P.aeruginosa*, *S.aureus* and *S.agalactiae*. Saha *et al.,* (2010) reported 35^{0}C as the optimum fermentation temperature for antimicrobial metabolite production by *Streptomyces* sp MNK7. De *et al.,* (1992) reported the culture filtrate of *Streptomyces* sp., with notable antifungal activity against *Helminthosporium oryzae* and *F.solani* at 30^{0}C. Thus, temperature greatly influences both on the morphological and physiological traits of the microorganism. The incubation period requires for growth and metabolite production varied depending on the type of actinomycetes strain to be used. Secondary metabolite like antibiotic is produced by the organism at the particular growth stage. Therefore, the highest antifungal activity was recorded on 11th day of the incubation period against *Candida* strains. Similar studies were reported by Kathiresan *et al.,* (2005), optimal antifungal activity against *Fusarium solani,* with the highest inhibition was observed at 120 h. Sujatha *et al.,* (2005) reported good antimicrobial activity on 4th day of incubation by *Streptomyces psammoticus*. Similar, studies were reported by Kavitha and Vijayalakshmi (2009) with *Nocardia levis* MK_VL113. Agitation enhances aeration to the cells and ensures better nutrient accessibility to the cultures. The *S.pseudogriseolus* VSG-9 was grown at different agitation speeds exhibited maximum growth and antifungal activity at 140rpm, with a 17±0.26 mm ZOI against *C.glabrata* 3019. Wadetwar and Patil, (2013) reported *Streptomyces violaceorubidus* with highest inhibitory effect against *B.cereus, E.coli* and least inhibition against *Candida albicans* at 150 rpm. Yang *et al.,* (2024) reported that 200rpm was optimal to *S.yanglinesis* for the production of antifungal substances. The antifungal potential of both extracellular and intracellular crude extracts of *S.pseudogriseolus* VSG-9 was found to be significant, surpassing the efficacy of commonly used antifungal drugs such as fluconazole, ketoconazole, amphotericin B and nystatin against *candida* strains. *Streptomyces* are renowned for producing clinically important antifungals, including amphotericin B, nystatin, and natamycin (Ali Mude *et al.,* 2025; Omelchuk *et al.,* 2018). Several studies have reported strong anticandidal activity of crude actinomycete extracts, with ZOIs ranging from 12-20mm against *C.albicans* (Flora *et al.,* 2015; Sarika *et al.,* 2021). Our results consistent with these reports, suggesting that crude extracts may contain bioactive compounds capable of inhibiting *candida* strains. The resistance of *C.albicans* to amphotericin B and nystatin observed in this study is unusual but aligns with reports of emerging polyene resistance due to alterations in ergosterol biosynthesis (Kanafani & Perfect, 2008). Similarly, reduced susceptibility to azoles is often associated with efflux pump overexpression and ERG11 mutations (Pristov & Ghannoum 2019). This increasing resistance underscores the need for novel antifungal alternatives. The present study revealed *Streptomyces pseudogriseolus* VSG-9 extract with significant inhibition against *Candida* strains. The genus *Streptomyces* is well known for the production of effective antibacterial and antifungal secondary metabolites (Alam *et al.,* 2022) and it has been further confirmed that the *Streptomyces pseudogriseolus* is a potential for the production of antifungal secondary metabolites against the broad spectrum of *Candida* sp.

In this study, *Streptomyces pseudogriseolus* VSG-9 produced a diverse range of antifungal metabolites through optimization of growth parameters. The crude extract showed significant antifungal activity against drug resistant *Candida* sp., inhibiting the growth of *C.*

albicans, *C. tropicalis*, *C. glabrata*, and *C. haemulonii*, which are resistant to amphotericin B and nystatin. These finding suggest the potential bioactive compounds that could lead to the development of new antifungal agents, subject to further chemical analysis and clinical validation.

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Conflict of interest

The authors declare that they have no conflict of interest

Declaration of competing interest

The authors declare that they have no potential competing financial interests

Ethical approval

Not applicable

Author Contributions

Shweta Mallikarjun: Conceptualization, Methodology, Wrote the manuscript, Writing original draft. Vidyasagar Gunagambhire M: Conceptualization, Investigation, Supervision, Writing-review and Editing.

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