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# Optimization of various physiochemical parameters to enhance production of secondary metabolite from soil actinomycetes against dermatophytes

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## Abstract

**The main objective of the study is to isolates actinomycetes from the Gwalior region and improves the production of secondary metabolite and cell growth of actinomycetes. Total 70 actinomycetes were isolated and screened for maximum secondary metabolites production against the dermatophytes on the bases of agar diffusion method & disc diffusion method. Out of 70 isolate, AP-27 has a good potential to produced secondary metabolite that inhibit the growth of dermatophytes (*Microsporum gypseum, Microsporum fulvum, Tricophyton rubrum, and Tricophyton mentagrophytes*). The optimization of several growth parameters like- growth medium, Incubation time, carbon sources, nitrogen sources, temperature, pH and minerals for the maximum production of secondary metabolites were checked. The optimum growth of AP-27 occurred with starch casein broth containing 2% starch as carbon source and nitrogen source was potassium nitrate nitrate at 2% at pH 7 and temperature 30$^0$C. Soybean meal was also found to be the best nitrogen source after the potassium nitrate which can help in large scale production because In India 74% of soyabean is produced by Madhya Pradesh. $FeSO_4$ (0.01gm/100ml) was selected as optimum mineral source. It was noticed that 6 days old culture was showing the maximum zone of inhibition and cell growth.**

**Keywords: -** ***Actinomycetes, Dermatophytes, optimization, secondary metabolites.***

## Introduction

Over the many past years, many natural compounds were discovered from natural sources as a drug to treat numerous human diseases. Natural products are chemical compounds derived from living organisms e.g. plants, animals and microorganisms. They can be defined as primary or secondary metabolite derived from organisms. The discovery of secondary metabolite exceeded 1 million far by employing different techniques and also by changing physicochemical parameters. 45% of bioactive compound are derived from actinobacteria. Many researchers found that a specific biosynthetic process and specific enzymes are required to produce secondary metabolite Kumar *et al.,* 2012. Cutaneous fungal infection like Dermatophytosis are the common infection in developed and developing country like India, which affect dominantly skin, hair and nails Doddamani *et al.,* 2012. Multiple drug resistance in pathogens intensified the search of new novel antibiotics and bioactive compounds. Actinomycetes found in naturally and manmade environment with divergent physiological and metabolic properties. Production and biosynthesis of secondary metabolite is a remarkable property of actinomycetes which depends on the growth condition. From the minor variation in the nutritional or the environmental condition of fermentation process, quality of product can be changed. Design of the culture media and the culture conditions is an important aspect to influences the growth and metabolism of actinomycetes. Media composition influence the economics of product and process (Mangamuri *et al.,* 2016). Actinomycetes are economically and biotechnologically important and it is also responsible for production of secondary metabolite like antibiotics, anticancer agent and enzymes (Kumari *et al*., 2013). Production of secondary metabolite by fermentation influenced by many factors as fermentation time, ventilation, temperature, initial pH combination of media components, minerals and culture conditions. By the optimization of fermentation process with these factors not only can greatly improve the levels of eventual products, also reduce fermentation costs

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Song Qin *et al.*, 2012. The main objective of this study, optimize fermentation conditions of isolate and determined the optimal combination of the medium composition and culture conditions to improve the active ingredient content, reduce costs, enhance the control effect and lay basis in real production application.

## Materials and Methods

### Isolation of antagonizing actinomycetes

Actinomycetes was isolated from soil of Gwalior region and screened on the basis of agar diffusion and well diffusion methods for their antagonistic activity against dermathophytes which were isolated from soils sample of Gwalior region and identified as *Microsporum gypseum, Microsporum fulvum, Tricophyton ruburum, and Tricophyton mentagrophytes*.

### Optimization of physicochemical Parameters

Several physical and chemical parameters were optimized like media, temperature, pH, carbon, nitrogen sources, and minerals for maximum production of antimicrobial metabolites by the test potent strain AP-27. In order to check the effect of medium various media were used like Yeast extract-Malt extract broth, Oatmeal broth, Inorganic Starch-Salt broth, Glycerol asparagines broth, Starch casein broth, Starch broth and Peptone yeast extract iron broth. The influence of carbon sources glucose, galactose, sucrose, starch, xylose, raffinose and rhamnose, nitrogen sources ammonium sulphate, glutamic acid, L-arginine, peptone, soyabean meal, potassium nitrate, L-phenylalanine and their concentration 0.1, 0.25, 0.5, 1.0, 2.0, 2.5 and 3.0 g/ 100ml. The effect of different pH 3,4,5,6,7,8 and 9 and temperature 25,28,30,35,40,45 and 50°C. Different minerals such as $MgCl_2$ $CuSO_4$, $MnSO_4$, $FeSO_4$, $CoCl_2$, $ZnCl_2$ and $KNO_3$ and their concentration (0.001-0.25 mg/ml) and the incubation time were also optimized from 1 to 15 days (Majumdar *et al.*, 1965; Jyotsnamayee and Gupta, 2010; Kumar *et al.*, 2012; Kumari *et al.*, 2013; Shukla *et al.*, 2014; Parmar *et al.*, 2010). The young broth culture of selected isolates were inculcated into the broth medium and incubated in the orbital shaker at 130 rpm, 30º C for 7 days. The culture broth was centrifuged at 5000 rpm for 10 min at 30ºC ± 2˚C. The pellet of the isolate was washed twice by suspension in sterile distilled water and resuspended in 10 ml of sterile distilled water. This suspension was used as inoculums (2% v/v) (Bundale *et al.*, 2015). The maximum secondary metabolite production was checked by measuring the zone of inhibition. The triplicate flasks were used for each parameter. Mean ± SD values were recorded.

## Results and Discussion

### Isolation and screening identification of actinomycetes

Total 70 actinomyctes were isolated from collected soil samples. Out of them, only AP-27 showed the good potential to produced secondary metabolize that inhibit the growth of dermathopytes and identified as *Stereptomycetes griseus* AP-27.

### Isolation and identification of fungal dermathopytes

The fungal dermathophyte were isolated from from soils sample of Gwalior region and identified as *Microsporum gypseum, Microsporum fulvum, Tricophyton rubrum, and Tricophyton mentagrophytes*.

### Optimization of physio-chemical Parameters

### Growth medium

Growth medium is important for secondary metabolite production. The results showed that the maximum growth of potential actinomycets was found with starch casein broth (Figure 1).

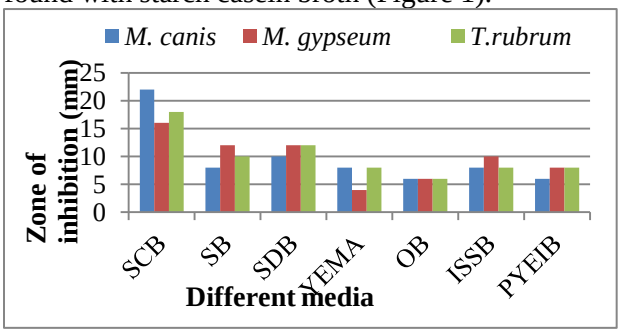


**Figure 1 Optimization of different media for production of secondary metabolite by *S. griseus AP-27.***

### Carbon Sources and their concentration

Different carbon sources were used in the production medium to determine the maximum yield of secondary metabolite production and cell growth. The results revealed that polysaccharides (starch) were more effective carbon sources for

growth and secondary metabolite production with the concentration of 2%. (Figure 3,4,5,6).

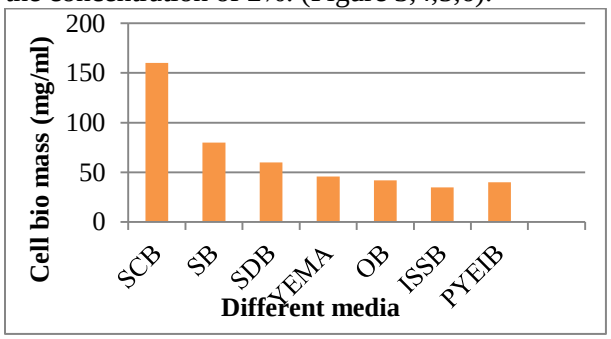


**Figure 2. Optimization of different media for production cell growth by *S. griseus AP-27.***

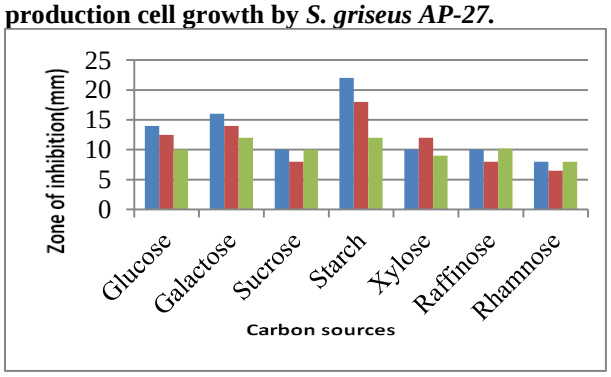


**Figure 3. Optimization of carbon source of carbon source on secondary metabolite production by *S. griseus AP-27.***

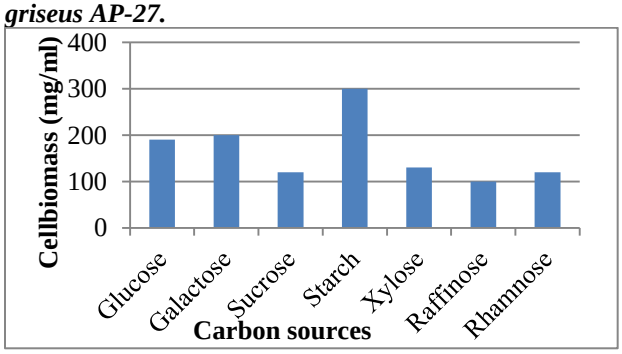


**Figure 4. Optimization of carbon source of carbon source on cell growth production by S. *griseus AP-27.***

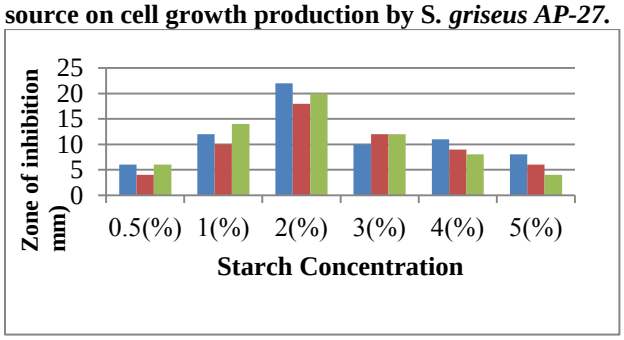


**Figure 5. Optimization of carbons source concentration for maximum secondary metabolite production by *S. griseus AP-27.***

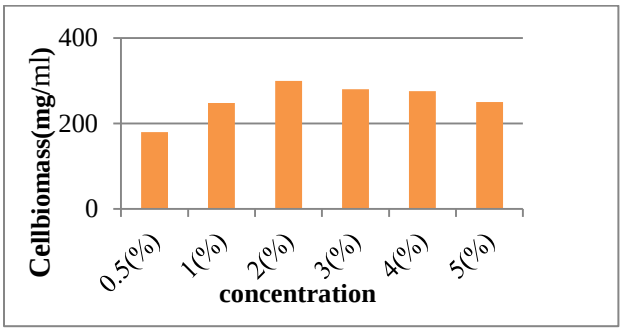


**Figure 6. Optimization of carbons source concentration for maximum cell growth by *S. griseus AP-27.***

## Nitrogen Sources and its concentration

Different nitrogen sources ammonium sulphate, glutamic acid, L-arginine, peptone, soyabean meal, potassium nitrate, L-phenylalanine and their concentration 0.1, 0.25, 0.5, 1.0, 2.0, 2.5and 3.0 g/ 100ml were used to determined the optimum production of secondary metabiolits and cell growth. The suitable nitrogen sources was potassium nitrate with concentration of 2 % (Figure 7,8,9,10).

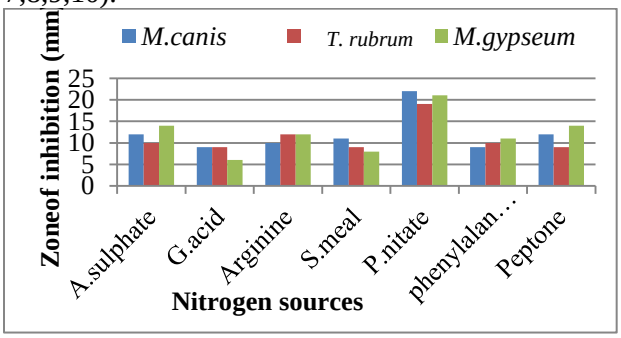


**Figure 7. Optimization of nitrogen sources on secondary metabolite production by *S. griseus AP-27.***

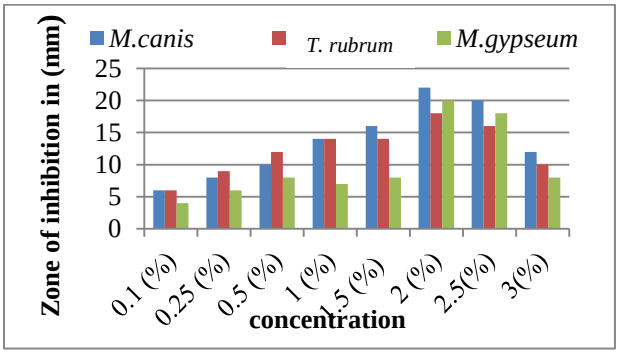


**Figure 8 Optimization of Potassium nitrate concentration for maximum Secondary metabolite production by *S. griseus AP-27.***

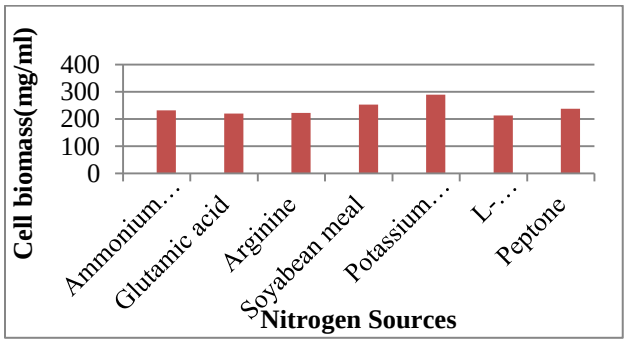


**Figure 9. Optimization of nitrogen sources for cell growth of *S. griseus AP-27.***

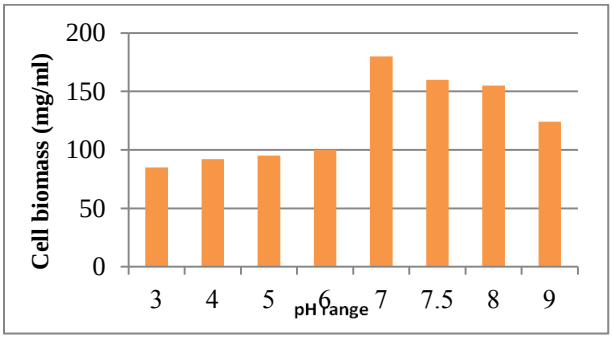


**Figure12. Optimization of medium pH for cell growth of *S. griseus AP-27.***

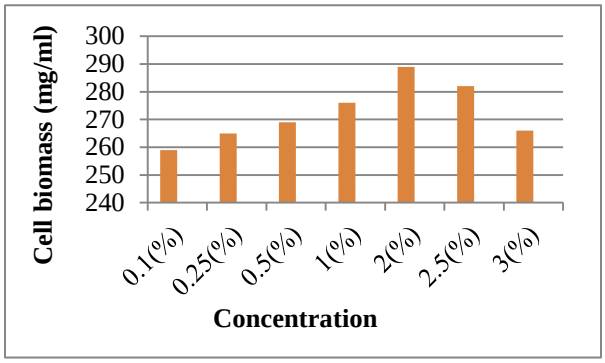


**Figure 10. Optimization of Potassium nitrate concentration for cell growth of *S. griseus AP-27.***

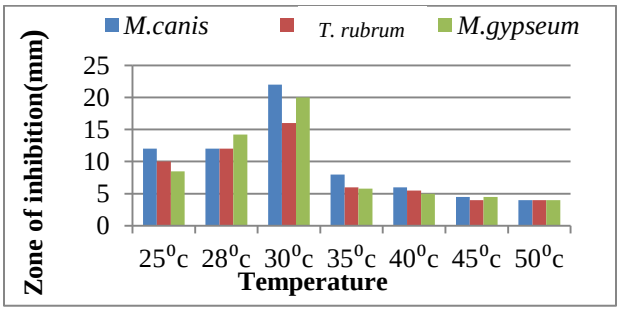


**Figure 13. Optimization of temperature for secondary metabolite production by *S. griseus*. *AP-27.***

## pH of medium

It was found that the cellular regulation process and biosynthesis of active metabolite production get affected by different pH level. The results of study showed that cell growth and metabolite production was maximum at pH 7.0 and 7.5 (Figure 10, 11).

## Growth Temperature

The growth of selected actinomycetes was studied at different temperature range. The maximum production of secondary metabolite and cell growth was found at 30°C (Fig 13, 14).

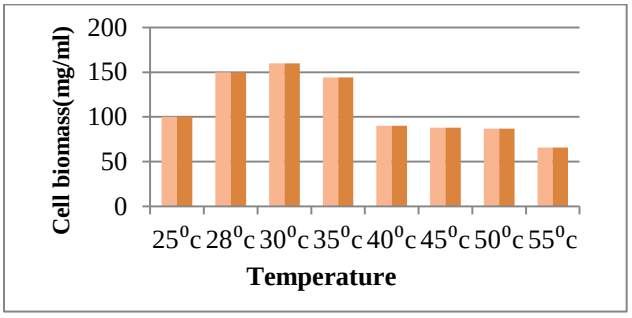


**Figure 14. Optimization of temperature for cell growth of *S. griseus AP-27.***

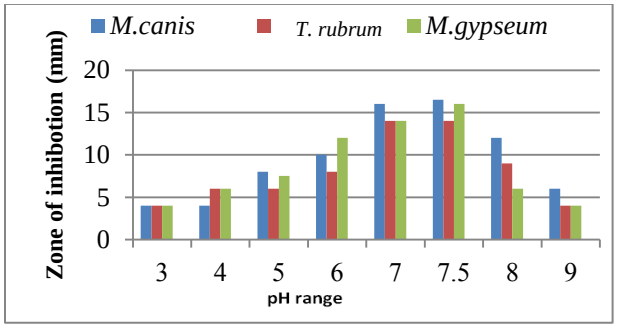


**Figure11. Optimization of medium pH for secondary metabolite production by *S. griseus AP-27.***

## Optimization of minerals

It was found that $FeSo_4$ showed maximum cell growth and metabolite production. Different concentration of $FeSo_4$ was used. It was found that concentration of 0.01gm/100ml shown maximum growth and secondary metabolite production. $CoCl_2$ and $ZnCl_2$ did not seem to have much effect on growth of isolate whereas $MnSO_4$ and $CuSO_4$ significantly enhanced the growth and secondary metabolite production. (Figure 15,16, 17, 18).

**Optimization of Incubation Period on Growth and Bioactive Metabolite**

It has been observed that isolate AP-27 shown a progressive increase of biomass during the first 4-8 days of incubation. Secondary metabolite production started from third and fourth day. Maximum antimicrobial activity was found after six days of incubation and remains constant up to the eight days. From the ninth day of incubation, secondary metabolite production and cell biomass production get decreases (Figure 19,20).

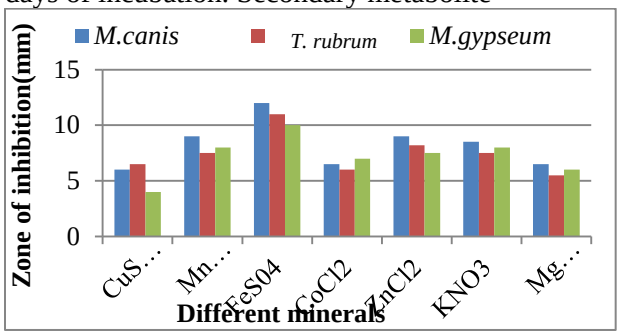


**Figure 15. Optimization of minerals on secondary metabolite production by *S. griseus AP-27***

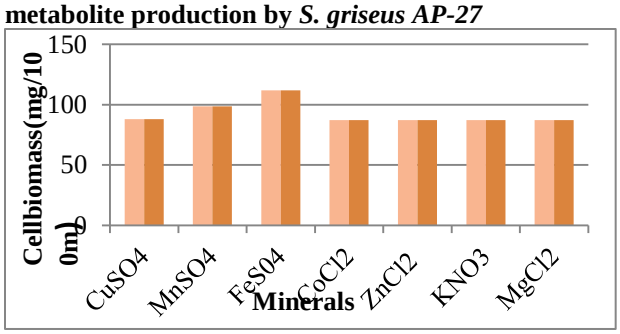


**Figure 16. Optimization of minerals for cell growth of *S. griseus AP-27***

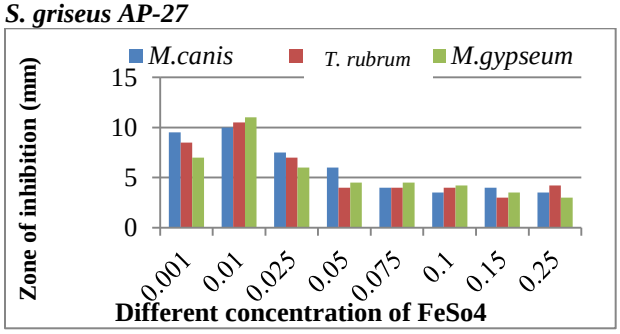


**Figure 17. Optimization of mineral concentration on secondary metabolite production by *S. griseus AP-27***

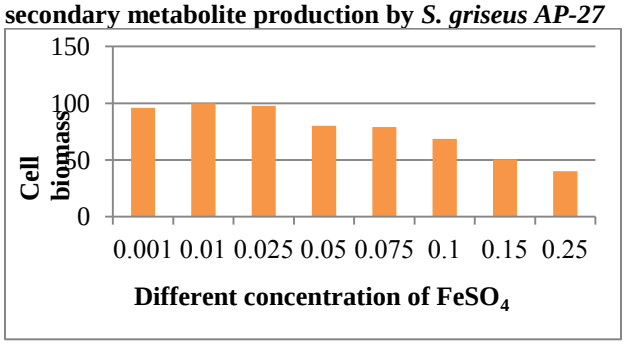


**Figure 18. Optimization of mineral concentration for cell growth of *S. griseus AP-27***

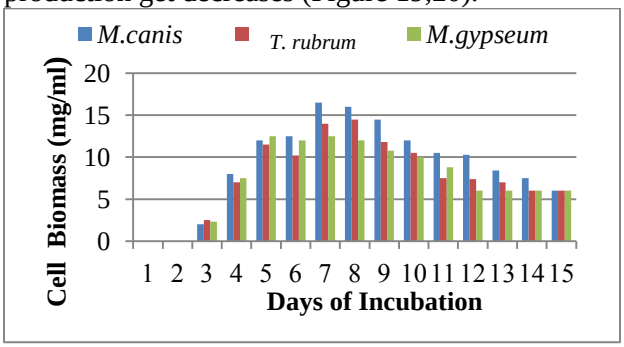


**Figure 19. Optimization of Incubation periods on secondary metabolite production by *S. griseus AP-27***

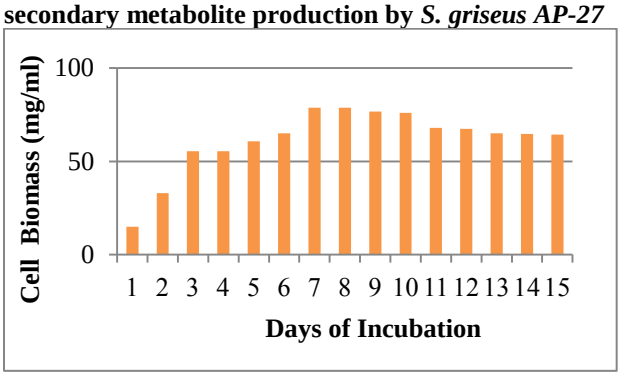


**Figure 20. Optimization of Incubation periods on cell growth of *S. griseus AP-27.***

The resistance of various pathogenic fungal species to antifungal necessitate search for new antifungal agents. Secondary metabolites produced by actinomycetes still interested, due to their promising effects. Actinomycetes are a rich source of secondary metabolites. It has been reported by the several researchers Dahiya *et al*. (2006) and Devi *et al*. (2008), that the nutritional requirements of actinomycetes play an important role during the secondary metabolite synthesis process. Amongst various nutritional requirements, antifungal production has been known to be influenced by media components and cultural conditions, such as aeration, agitation, pH, temperature, and carbon source, which vary from organism to organism. Gebreel *et al*. (2008), studied the antibacterial activity of isolated actinomycetes and also observed the effect of different carbon and nitrogen sources on the production of bioactive compound against

several human & plant pathogenic fungi. It was found during the optimization of isolate AP-27 that among various carbon and nitrogen sources starch and potassium nitrate selected as best carbon and nitrogen sources for secondary metabolite production and cell growth. Kumari *et al.* (2013), optimized the nutritional condition by using 8 different media for antibiotic production by *Streptomyces* sp. and starch casein broth was selected as best medium. They also estimated the dry weight of mycelium to check the growth of secondary metabolite producing actinomycetes. It was suggested in this study that starch casein broth supported the maximum metabolite production and cell growth of isolate AP-27 as compare to other medium used. Mangamuri *et al*. (2014), conducted a study to optimize the various parameters for maximum production of bioactive metabolites by *Streptomyces tritolerans* DAS 165. They concluded that at the $9^{th}$ day of incubation period at $35^{0}$C was ideal for that strain and chemical parameters like pH 7.5 and manitol as carbon source, yeast extract as nitrogen source was ideal for metabolite production.

## Conclusion

The present study showed that the selected actinomycetes possessed the good potentiality to produce secondary metabolites that inhibit the growth of dermatophytes, *Microsporum gypseum, Microsporum canis and Tricophyton rubrum.* This study also represents that various growth parameters like media, pH, temperature, incubation time, carbon, nitrogen source and minerals directly influenced the production of bioactive metabolites and cell growth.

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