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Research Article

ANTIFUNGAL POTENTIAL OF PIGMENTED *STREPTOMYCES* KSRO4 FROM AGUMBE REGION OF WESTERN GHATS

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ABSTRACT
A strain of Streptomyces isolated from agumbe soil of Western Ghats of Karnataka, India was studied with special references to the production of yellow colored pigment and the antibiotic properties of the same. The optimum temperature as 28^{O}C and the most favorable P^{H} was 9. This strain used the glycerol as carbon source and gelatin, as nitrogen source for best pigment production. The methanol and water extract of pigment was examined for the antimicrobial property and it was found to be having strong antimycotic property against many of the skin pathogens like *T.kannei*, *T. mentagrophyta* , *Microsporon. gypsium.* Bacterial pathogens were moderately inhibited while it was ineffective on yeast pathogens.
Key words: Western ghats, Sreptomyces, pigment, antimycotic, peptides

INTRODUCTION

Soil, the natural dwelling habitat and sweet home for millions and billions of micro-organisms has been studied extensively for the presence of many unexplored species of commercially important strains [1.] Actinomycetes, which is one such group, dominantly seen in soil [2]is an extensive and diverse group of gram positive, aerobic mycelial bacteria with high G+C nucleotide content and play an important ecological role in soil cycle [3] .

Hundreds of naturally occurring antibiotics have been discovered in these terrestrial microorganisms, especially from the genus *Streptomyces*. Thus these forms have an important and characteristic biological activity [4].

In the present work interest towards pigments is consequently because of the similar chemical nature of the antibiotics and colouring agents [5]. Also it has been recorded that compounds like anthracyclines and others are red and yellow pigments derived from the strains of actinomycetes are having powerful anti-tumour property [6].

MATERIALS AND METHODS

Soil sampling

The soil samples for selective isolation of suitable actinomycetes were selected from the forest soils of Agumbe,in Shivamogga district. The quadrant method was used to selectively collect the samples, where specific points in a hectare area (one curve in this context) were selected [7, 8] .

The samples are collected in sterilized 'Zip-lock covers', made air tight, brought into the lab. The drying process is done by spreading the collected sample on a blotting sheet.

Media composition

Suitable selective media for the isolation of actinomycetes are used. The two important media which serve the purpose are Kenknight and Munaieer's medium and SCN media. [9].

Isolation of pigmented actinomycetes

The collected soil samples are screened for pigmented actinomycetes by inoculating the serially diluted soil solutions into the selective media- Kenknight and Munaier's media. The plates were incubated at 28^{O}C for 11 days. The typically, pigmented, dry, powdery colonies were observed. These specific colonies were subsequently subcultured and the pure cultures were preserved [10-12].

Identification of the isolates

One promising isolate which showed a unique, stable and interesting property of producing the pigment was selected and characterized [13]. Morphological features of the actinomycete were studied by cover-slip method. The mycelium structure, colour and arrangement of spores on the mycelium were observed under oil immersion microscope (Resolution-2000X). The Bergy's manual of determinative bacteriology (1994) [14] was referred for the confirmation of morphology [15-19].

Bioassay

The well known fact of *streptomyces spp*, as mentioned throughout, is the property of its metabolites exhibiting the antagonistic nature against many pathogens [20].

Among actinomycete isolates found to produce soluble pigments, six isolates were selected to demonstrate antibacterial activity of pigments. The pigments produced in broth cultures of selected isolates were extracted with acetone solvent. Well diffusion technique was employed to assess antifungal activity.Fungi such as *Candida albicans*, *Candida lipolytica, Cryptococcus neoformans* and skin pathogens termed as isolate 1, isolate 2 and isolate 3 are swabbed into respective solid media [21,22]. The 24 hours old cultures of test yeasts and fungi were swabbed on SDA medium in sterile plates and allowed to stand for few minutes. Using sterile cork borer, wells of 9mm diameter were cut in the plates, 300µl of methanol extract, water extract were dispensed into the diffusible agar wells. The plates are then incubated 28^{O}C for fungi and skin pathogens. Methanol alone served as control [12,23-26].

Optimization

Optimization is the technical procedure carried out to find the maximum and minimum pigment production under variable carbon and nitrogen sources and other physical parameters.

The procedure is helpful in determining the quantity of pigment produced

Influence of P^H

70 ml of the SCN media was taken in different flasks with P^H ranges; P^H 4, P^H 6, P^H 9 and P^H 11. This variation is brought about by the addition of 0.1N Hcl and 0.1N NaOH to the media respectively. The standard incubation criterion is followed and the post-incubation results are observed and discussed with respect to the relative pigment intensity [27].

Influence of Temperature

70 ml SCN broth inoculated with the sporulated culture of KSRO4 and grown under various temperature conditions like $4^{\circ}C$, $28^{\circ}C$ and $40^{\circ}C$. The results obtained during the procedure is tabulated and discussed [12].

Influence of carbon source

A series of four 100 ml conical flasks was prepared with 70 ml of SCN broth without starch. [24] The carbon sources (1%) used for the assay were Maltose, Lactose, Sucrose and Glycerol. The broth was inoculated with sporulated culture of KSRO4 and incubated at $30^{\circ}C$ for 9 days. The pigment intensities were measured colorimetrically [28].

Influence of Nitrogen source

Here, the SCN broth is prepared and the nitrogen sources such as Gelatin, Egg albumin, Arginine and Peptone were used. The colorimetric details of the respective pigment are recorded [29].

RESULTS

The soil samples were collected from the Agumbe Ghats, which is one of the rich bio-diversity hot spot belonging to the Western Ghats. The collected samples earned us one of the best streptomycetes strain KSRO4 known for its better growth in the moist soils and dark yellow pigmentation (Figure 1).

Effect of P^H

Dark colored pigmentation was observed in the alkaline pH ranges of 9 and11.The colour ranges vary from dark yellow to reddish brown showing that alkaline P^H was best for production

Effect of Temperature

The suitable temperatures are found to be $28^{\circ}C$ and $50^{\circ}C$. Even though the isolate grew and got sporulated at $4^{\circ}C$, but it failed to produce the pigment. The colour of the pigment in $28^{\circ}C$ and $50^{\circ}C$ is as similar to that as observed for the P^H. And hence these two temperatures can be considered as the optimum one for the production of metabolite.

Effect of Nitrogen sources

Among the nitrogen and carbon sources tested for optimization, both exhibited some interesting features. With respect to the intensity of the pigment measured, the best suitable nitrogen source was gelatin. Arginine and peptone supported to lesser extent but egg albumin was among the least where the pigment was not at all produced.

Effect of Carbon sources:

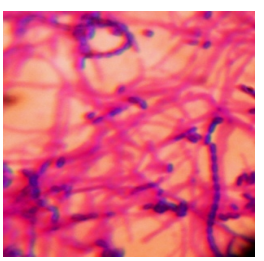
Glycerol, lactose, maltose and sucrose as carbon source were utilized by the strain ,regarding the influence of carbon source, the colour intensity was maximum in media with glycerol. Lactose and maltose also showed the positive results but with lesser colour intensity. Sucrose supported much less than all other carbon sources (Fig 2).

Bioassay

Skin pathogens, were inhibited to the maximum extent by the pigment extracts. The test organisms such as *T.kannei, M.gypsisum* and *T.mentagrophyta* got inhibited by the activity of *streptomyces* metabolite having the zone of inhibition up to 12 and 13 mm. Among the three test organism, the inhibition zone is more in *T.mentagrophyta* and hence by using increased concentrated extract of the metabolite, the pathogen can be restricted during infection.

Table 1: Antifungal activity of KSRO4 pigment against skin pathogens

Organisms		Zone of inhibition (in mm)
T.kannei	Water extract	12 mm
	Solvent extract	8 mm
	Methanol	3 mm
M.gypsisum	Water extract	5 mm
	Solvent extract	2 mm
	Methanol	—
T.mentagrophyta	Water extract	12 mm
	Solvent extract	13 mm
	Methanol	



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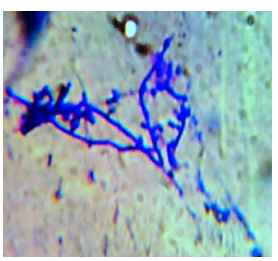

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Figure 1: *Streptomyces KSRO4*

Table 2: Influence of temperature

Temperature range	Pigmentation
4°C	—
28°C	++
50°C	+

— → No pigmentation
+ → Pigmentation

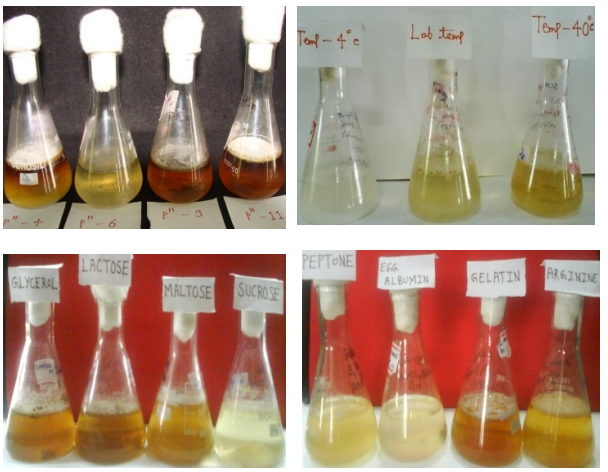

Figure 2: optimization of temperature, P^H, nitrogen and carbon sources

DISCUSSION

The *streptomyces* species has been studied more predominantly with respect to its pigment production and antimicrobial activity [30]**.** The comparative analysis of the pigments shows that, the nature and chemistry of the metabolites are purely dependent on the kind of carbon and nitrogen sources that has been provided in the media. We have got some variations in this regard where there is least production of pigments when the nitrogen source is egg albumin. The intensity of the colour also varies with the sources. The utilization of glycerol by streptomyces strains is also evident from the similar work done by Dastager *et al*., (2006)[27]**.**

Maltose and Soya meal has been found to be the best carbon and nitrogen sources as recorded in the work done by Narayana and M.Vijayalakshmi (2007)) [28]**.** Maltose seems to be the best carbon source for that species, but for our isolate it stands in third spot and arginine was best nitrogen source. Similarly Soya meal is nitrogen source and we have opted some other different nitrogen sources for the pigment production.

Carbohydrate such as glycerol, maltose, mannose, sucrose and xylose are reportedly known to interfere in the production of metabolites. With regard to carbon sources, species specific variation occurs in *Streptomyces* species for cell growth and production of secondary metabolite. Even the temperature and P^H will play a significant role. As tabulated in table 1 and table 2, the pigment production and the growth of isolate is nil at 4°C temperature which indicates that the particular temperature is not suitable in this regard.

The antimicrobial activity of *streptomyces* is well known and hence we used the pigments to verify the same. Collectively the metabolites showed the positive result up to a maximum extent against the skin pathogens. On the other hand a fungal pigment against the same test pathogens exhibited similar and variable results [31]**.**

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