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ANTIBIOTIC POTENTIAL OF SOIL ACTINOMYCETES UNDER INFLUENCE OF PHYSICAL AND NUTRITIONAL PARAMETERS

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ABSTRACT

A total of 19 soil Actinomycetes were isolated from forest area of Bastar region of Chhattisgarh and screened for antibacterial activity. Among them, the isolate BS 46 exhibited broad spectrum antibacterial activity against gram positive bacterial pathogens, *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacterial pathogens, *Escherichia coli* and *Pseudomonas aeruginosa* obtained from MTCC, IMTECH, Chandigarh, India. It was gram positive, filamentous, producing diffusible pigment on starch casein agar medium. In order to increase its efficiency, the effect of temperature, pH, carbon and nitrogen sources were optimized and its antibacterial activity was examined by agar well diffusion method. The maximum antibacterial activity was observed at 30º C and 7 pH, whereas maltose and malt extract were proved to best carbon and nitrogen sources, respectively. Thus, the results of this study confirmed that the antibacterial substances produced by Actinomycetes isolate BS 46 were found to be more effective after optimization of physical and nutritional parameters.

KEYWORDS: Actinomycetes, Antibacterial Compound, Bacterial Pathogens, Zone of Inhibition

Actinomycetes are proved to be most promising strains for production of various bioactive secondary metabolites, especially the genera *Streptomyces* holds a prominent position of producer of different classes of antibiotics and other pharmaceutically and industrial important compounds (Pereira and Kamat, 2013).There are around one million known natural products and approximately 50000 microbial metabolites including both bioactive and inactive compounds, therefore, there is a need of reinvestigation of microbial products by applying more selective and improved methodologies (Berdy, 2005).Microbial pathogens are increasing their efficiency and resistance to many drugs day by day and becoming more dangerous to the living forms of life, therefore new and potential antibiotics are needed through proper development strategies (Chaudhary *et al.,* 2013).

Actinomycetes are gram positive, spore forming, filametous bacteria, characterized by aerial and substrate mycelium and belonging to order Actinomycetals (Lechevalier and Lechevalier, 1981). The Actinobacteria class holds some resilient species which can grow and survive in variety of habitats including the extreme environments and these adaptations make them capable of synthesizing and producing such important natural compounds which could be useful medical and industrial purposes (Ballav *et al.*, 2012). A total of, 19 actinomycetes were isolated from soil sample collected from forest area of Bastar region of Chhattisgarh and screened for antibacterial activity. The isolate BS 46 had shown broad spectrum antibacterial activity against gram positive and gram negative bacterial pathogens, therefore, in this study various physical and nutritional parameters were optimized for increasing the efficiency of BS 46,So that maximum antibiotic substances could be produced from it and used for extraction, purification and identification of new bioactive metabolite.

MATERIALS AND METHODS

Isolation Actinomycetes from Soil

The Actinomycetes isolate BS 46 was isolated from soil sample collected from forest area of Bastar region of Chhattisgarh. The isolation was done after collection and pre-treatment of soil sample by wet heating method (Hayakawa, 1991). The pre-treated soil sample was serially diluted to 3 folds and spread over Starch casein nitrate agar and incubated at 28 ºC for three weeks. After incubation morphologically distinct colonies were purified and sub cultured on ISP 2 Slants (El- Naggar *et al.* 2006).

Screening of Antibacterial Activity

The isolates were grown in 50 ml Starch casein broth in 250 ml conical flask and incubated for 14 days. After the incubation the culture broth was centrifuged at 15000 rpm for 30 minutes. The resulting supernatant was used for antibacterial activity against the test organisms by agar well diffusion method (Barry & Thornsberry, 1985).

Test Organisms

Antibacterial activity of Actinomycetes were tested against 4 bacterial pathogens, *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 1789), *Escherichia coli* (MTCC 3221) and *Pseudomonas aeruginosa* (MTCC 3163) obtained from Microbial Type Culture Collection & Gene Bank (MTCC), IMTECH, Chandigarh.

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Total 19 isolates had been screened and among them the isolate BS 46 showing maximum antibacterial activity was selected for optimization process.

Characterization of Potential Actinomycetes Isolate

The potent Actinomycetes isolate BS 46 showing antibacterial activity was studied for morphological, physiological and biochemical characteristics by following the methods described in International Streptomyces project (Shirling & Gothlieb 1966).

Optimization of Media for Antibiotic Production

The seed inoculum was prepared by inoculating the isolate BS 46 in 250 ml of Erlenmeyer flask containing 50 ml Glucose soybean broth of (Soybean meal, 10 g/l; Glucose, 10 g/l; NaCl, 10g/l; Calcium carbonate, 1 g/l, and Distilled water 50 ml, pH 7). 1% inoculum is added to basal medium along with the testing parameters and incubated for 7 days.

Effect of Physical Factors

Physical factors used in this study were temperature and pH. The effect of temperature and pH on antibacterial metabolite production was studied by inoculating 4 days old culture in 50 ml Glucose soybean broth. The effect of different ranges of temperature (26-34ºC) and pH (6-10) was examined for production of antibacterial substances by agar well diffusion method.

Effect of Nutritional Factors

Nutritional factors used in this study were carbon and nitrogen sources. The effect of carbon sources on antibacterial metabolite production was studied by adding 1 % carbon sources, such as, Dextrose, Sucrose, Fructose, lactose and maltose in the basal medium. The effect of different nitrogen sources was studied by adding 1 % peptone, Malt extract, Yeast extract, Ammonium sulphate and sodium nitrate into the basal medium. (Narayana and Vijayalaxami, 2008).

RESULTS

The isolate BS 46 had shown maximum antibacterial activity among all 19 actinomycetes isolates. On studying it's cultural and morphological characters it was found that it has cream colour aerial mycelium and purple colour substrate mycelium, producing diffusible pigment in starch caesium agar medium. The microscopic examination at 1000 X had shown that, it was gram positive, filamentous, branched, bearing spiral spore chains. It has shown positive results for biochemical test such as, citrate utilization, nitrate reduction, H_2S production, urease test, catalase test. It was capable of hydrolysis of starch, gelatine, tween 20, and Caesin.

Upon optimization of Physical parameters for production of antibacterial substance it was found that, the isolate has shown maximum zone of inhibition at temperature 30º C and minimum 34 ºC and intermediate at 28 ºC (Table. 1). The strain has reached maximum level of antibacterial production at pH 7, intermediate at pH 6 and 8. No activity was observed at pH 10 (Table. 2).

The effect of nutritional parameters, carbon sources and nitrogen sources were presented in table 3 and 4 respectively. It was found that the strain has shown maximum activity when carbon source was maltose and minimum in the presence of sucrose, whereas malt extract proved to be the best nitrogen sources and least activity was observed in medium containing yeast extract.

Table 1: Effect of Temperature on Antibacterial activity of BS 46

Temperature in ºC	Zone of Inhibition in mm			
	SA	BS	EC	PA
26	22	20	18	20
28	25	22	20	21
30	30	28	25	24
32	24	23	20	22
34	18	17	15	16

SA- *Staphylococcus auerues*, BS- *Bacillus subtilis* , EC- *Escherichia coli,* PA-*Pseudomonas aeroginosa*

Table 2: Effect of pH on Antibacterial activity of BS 46

pH	Zone of Inhibition in mm			
	SA	BS	EC	PA
6	30	27	24	21
7	32	28	25	26
8	26	26	22	23
9	24	23	19	21
10	12	11	9	10

SA- *Staphylococcus aureus*, BS- *Bacillus subtilis* , EC- *Escherichia coli,* PA-*Pseudomonas aeruginosa*

Table 3: Effect of Carbon Sources on Antibacterial activity of BS 46

Carbon Source	Zone of Inhibition in mm			
	SA	BS	EC	PA
Dextrose	26	25	20	21
Sucrose	27	28	25	26
Maltose	30	28	24	26
Lactose	24	23	19	21
Fructose	22	18	16	17

SA- *Staphylococcus aureus*, BS- *Bacillus subtilis* , EC- *Escherichia coli,* PA-*Pseudomonas aeruginosa*

Table 4: Effect of Nitrogen Sources on Antibacterial activity of BS 46

Nitrogen Sources	Zone of Inhibition in mm			
	SA	BS	EC	PA
Peptone	24	20	18	17
Yeast Extract	25	23	18	20
Malt Extarct	28	24	20	21
Ammonium Sulphate	20	17	14	15
Sodium Nirtae	16	13	11	13

SA- *Staphylococcus aureus*, BS- *Bacillus subtilis* , EC- *Escherichia coli,* PA-*Pseudomonas aeruginosa*

DISCUSSION

Actinomycetes had produced major bioactive compounds of health benefits and high commercial value and are in continue involved in screening programmes for production of bioactive secondary metabolites (Shahidi *et al.,* 2004). On the basis of morphological and biochemical characteristics, the isolate BS 46 was found to be belonging to genus *Streptomyces* (Shirling and Gottlib, 1996). *Streptomyces* is the largest antibiotic producer in the world but the discovery of new antibiotic producing *Streptomyces* has decreased in the past decade (Watve et al. 2001). The isolate BS 46 had shown maximum antibacterial activity against *Staphylococcus aureus* and minimum against *Escherichia coli* which is in similarity with findings of Selvameenal 2009, who had shown the antimicrobial activity of pigment produced by *Streptomyces hygroscopicus sp*. against the bacterial pathogens such as, S*taphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp in which maximum zone of inhibition was observed against *Staphylococcus aureus.* Antibiotic production by *Streptomyces* can be influenced by modulating the type and concentration of nutrients and conditions of the culture medium (Slavica *et al.*, 2010). Thakur *et al.*, 2009, had shown that temperature 30ºC and pH 7.5 were optimum for antibiotic production by *Streptomyces* sp.201. In the present study maltose containing medium has shown the maximum active among all carbon sources used in this study which is in similarity with findings of Himabindu and Jetty (2006) and Narayana *et al.,* 2008. Malt extract was found to be best nitrogen source which is in accordance with the study of Sharon et al. 2014 who had also found malt extract to be the good nitrogen source in the basal medium for antibiotic production against *Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus faecalis*.

CONCLUSION

From the present study it can be concluded that the Actinomycetes isolate BS 46 belongs to genera *Streptomyces* and possessing broad spectrum

antibacterial activity. It has shown good production of antibiotic in the culture medium containing maltose and malt extract and pH 7 at 30ºC.Thus, the efficiency of the *Streptomyces* isolate has increased after the optimization of physical and nutritional parameters. Therefore, in order to increase the chances of discovery of new and effective antibiotic against pathogenic bacteria it is necessary to isolate and screen new species from unexplored regions. Improved methodologies and cultural conditions also plays crucial role in increasing their efficiency. Thus, this isolate could be used for further processes like extraction, purification and identification of new bioactive metabolites.

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