ENZYMATIC SCREENING AND ANTIBACTERIAL POTENTIAL OF ACTINOMYCETES FROM FOREST SOIL OF BASTAR, INDIA

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Abstract - Forest soils of unexplored regions are important source of potential, industrially important and novel microorganisms. Actinomycetes are prominent decomposers of various organic matters by producing wide variety of degrading enzymes and also produces various antimicrobial secondary metabolites. In the present study, 20 actinomycetes were isolated from forest soil samples of Bastar region of Chhattisgarh, India,. The isolates were screened for their ability to produce amylase, pectinase, and lipases by qualitative test on agar plates using specific substrate and also screened for antibacterial activity. The results revealed that 45% of isolates had shown pectinase activity, 65% had shown lipases activity while 90% had shown amylase activity. Screening for antibacterial activity revealed that 25% of total isolates inhibited gram positive bacteria and 15% inhibited gram negative bacteria. While only 1% exhibited broad spectrum antibacterial activity. The excellent enzymatic and antibacterial activities of these actinomycetes isolates might prove beneficial for industrial and pharmaceutical applications, respectively.

Keywords - Forest soil, Actinomycetes, Enzymes, Antibacterial activity.

I. INTRODUCTION

Actinomycetes have exceptional and unusual ability for surviving in diverse habitats and yet are leading producers of various industrial and pharmaceutical compounds. They are important component of soil microbial population and plays significant role in degradation and digestion of various complex naturally occurring organic compounds [1]. Actinomycetes are Gram positive filamentous bacteria; they perform saprophytic activities in soil and contribute in turnover of complex biopolymers, such as, lignocelluloses, hemicellulose, pectin, keratin and Chitin [2].They are most widely distributed group of microorganisms in nature and due to their diversity and ability for the production of most of the discovered bioactive secondary metabolites, mainly antibiotics [3], [4], Enzymes [5], Enzyme inhibitors [6], Immunosuppressive agents [7], holds a prominent position as targets in screening programs. Over 22, 00 bioactive microbial compounds (including antibiotics) were published on the scientific and patent literature and about a half of them were produced by Actinomycetes [8]. About 150 antibiotics are in direct practical use in human therapy and most of them were derived from Actinomycetes species. And from these common Actinomycetes the rate of discovery of new compounds has declined, therefore improved methodologies for isolating the uncommon and rare actinomycetes from the diverse habitats is required to avoid the re isolation of same strains that produce known bioactive compounds and to improve the quality of natural products screened [9]. Bastar region of Chhattisgarh, India, also known as "Island of Sal", is rich in natural resources like medical plants and not much explored yet for screening of potential micro organisms, therefore present study is designed to isolate soil actinomycetes and screen them for their ability to produce degrading enzymes like pectinases, amylase and lipases and determine their antibacterial potential against gram positive and gram negative bacteria.

II. MATERIALS AND METHODS

A. Soil sample collection and pretreatment

Soil samples were collected randomly from forest area of Bastar region of Chhatisgarh, India, from 10 - 15 cm depth, transported to laboratory in sterile polythene bags, where the soil samples were meshed, sieved and air dried at room temperature for 7 days and treated by wet heating or dry heating method [10].

B. Actinomycetes isolation

Pretreated soil samples were serially diluted in distilled water and spread on Humic acid vitamin agar [11], Starch Caesin agar and Actinomycetes isolation and incubated at 28˚ C for minimum three weeks or till the appearance of actinomycetes colonies. Morphologically distinct colonies were subcultured and pure isolates were preserved on ISP 2 medium.

C. Enzymatic Screening

Amylase*:* Pure actinomycetes isolates were streaked over starch agar plates containing peptic digest of animal tissue, 5.0 g; yeast extracts, 1.5 g; beef extract, 1.5g; starch soluble, 2.0 g; NaCl, 5.0 g; agar, 20.0 g; distilled water, 1000 ml and incubated at 28˚ C. After proper growth, iodine solution is added to the medium which will turn the starch into blue color, while the starch hydrolysis will be observed by clear zone around the isolates.

Pectinase*:* Actinomycetes isolates were streaked on hankin medium containing Yeast extract, 1.0g; pectin, 5.0g; agar, 15.0g; distilled water 500ml, (pH

7).Mineral salt solution: (NH4)2SO4,2.0g; KH2PO4, 4.0g; Na2PO4, 6.0g; FeSO4.7H2O,0.2g; CaCl2, 1.0mg; H3BO3, 10mg; MnSO4, 10mg; ZnSO4, 70mg; MoO3 10mg, distilled water 500ml. After incubateion plates were flooded with 1% aquous solution of hexadecyl-trimethyl-ammonium bromide. Presence of clear zone around the colonies in plates was the indicative of degradation of pectin [12].

Lipase: Lipid hydrolysis was observed by inoculating the colonies on basal medium (Peptone, 10.0g; NaCl, 5.0g; CaCl2.2H20, 0.1g; agar, 15.0g; distilled water, 1000 ml.) containing tween 20 (Sorbitol monolaurate). When lipase enzyme utilizes the Tween 20, then visible precipitate (calcium salt of the lauric acid) will be formed on surrounding of the culture.

D. Antibacterial screening

Isolates were screened against reference strains of common bacterial pathogens, gram-positive bacteria such as, *Staphylococcus aureus*, *Bacillus cereus and* gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumonia* by cross streak technique on starch casein agar [13], [14].

III. RESULTS AND DISCUSSION

Isolation* of *actinomycetes

A total 20 morphological distinct actinomycetes were isolated from forest soil sample collected from Bastar region of Chhattisgarh, India, by using two different pretreatment methods. Humic acid vitamin agar medium rich in soil humic acid, is a good medium for the growth of large number of actinomycetes genera such as, *Streptomyces, Micromonospora, Microbispora, Streptosporangium, Nocardia, Dactylosporangium, Microtetraspora* and *Thermomonospora* [11].

Enzymatic Screening

Screening of enzymatic potential of actinomycetes isolates is indicated in table no. 1. Amylase is an exoenzyme produed by various microorganisms specially fungi that hydrolyses starch into maltose and glucose. Out of 20 isolates, 18 had shown clear zone around actinomycetes colonies in Starch agar, exhibiting good amylase activity. Pectinolytic activity of the actinomycetes isolates was determined, in which only 9 isolates had shown pectinase activity which is due to secretion of extracellular polygalactouranase and pectate lyase in test culture [Fig 1]. Lipid hydrolysis by actinomycetes lipases was done by 13 isolates by showing visible precipitate (calcium salt of the lauric acid) on surrounding area of culture [Fig 1]. Among all actinomycetes isolates, 50% had produced all degrading enzymes tested in this study.

Antibacterial Screening:

Actinomycetes are among the world's largest producer of various commercially available antibiotics and among them *Streptomyces* genera holds a major share. In the present study, out of 20 isolates, only 5 isolates exhibited antibacterial activity against gram positive bacteria and only 3 isolates exhibited activity against gram negative bacteria while 2 isolates proved to be most potential among all isolates and exhibited broad spectrum antibacterial activity against all test organism used in this study were shown in table No. 2. Meklat [15] reported diverse population of 52 halophillic actinomycetes, among which 33% of *Saccharomonospora* and *Streptomonospora*, 27% of *Actinoplanes* and all *Nocardiopsis* strains showed broad-spectrum antimicrobial activity. Bioactive compounds are produced by both *Streptomyces* and non-*Streptomyces* actinomycetes but the non-S*treptomyces* actinomycetes were less screened due to traditional and difficult methods of isolation, some actinomycetes such as, *Actinomadura, Micromonospora, Microbispora* also produces antimicrobial compounds against both gram-positive and gram-negative pathogens [16.]

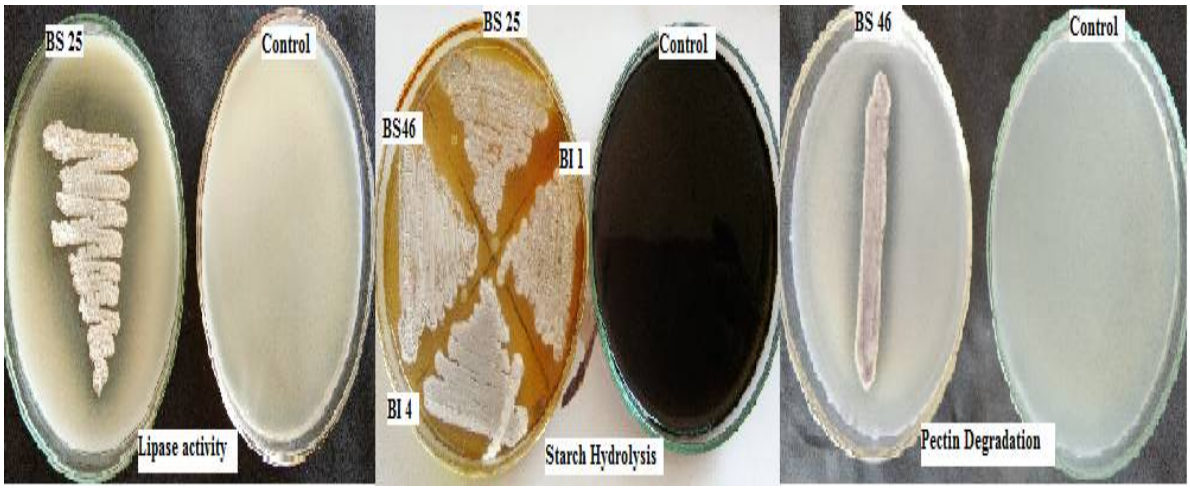

Fig: 1 Enzyamatic Screening of actinomycetes isolates

Isolate.No.	Amylase	Pectinase	Lipase
BSA1	+	+	+
BSA2	+	+	+
BSA 4	+	-	-
BS25	+	+	+
BS27	+	-	-
BS46	+	-	+
BI 1	+	-	+
BT 2	+	-	+
BT 3	+	+	-
BI4	+	+	+
STA 1	+	+	+
ST A3	+	+	-
SC 1	+	-	-
AC1	+	-	+
AC2	-	-	-
A10	+	-	+
A11	+	+	+
SS16	-	-	+
SS45	+	+	+

Table No. 1 Enzyamatic Screening of actinomycetes isolates

Isolate.No.	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumonia*
BSA1	+	+	-	-
BSA2	-	-	-	-
BSA 4	-	+	-	-
BS25	+	-	+	+
BS27	-	-	-	-
BS46	-	-	+	+
BI 1	+	+	-	-
BT 2	-	-	-	-
BT 3	-	-	-	-
BI4	-	-	-	-
STA 1	+	+	-	+
ST A3	-	-	-	-
SC 1	-	-	-	-
AC1	-	-	-	-
AC2	-	-	-	-
A10	-	-	-	-
A11	+	+	-	-
SS16	-	-	-	-
SS45	-	-	-	-

Table No. 2. Antibacterial Screening of actinomyctes isolates against bacterial pathogens

CONCLUSION

From the above study it can be concluded that the forest soil of bastar area has prove to be good source of potential antinomyctes isolates. Maximum number of isolates were found to be having good enzymatic activity which could be further used for production and purification of important and useful enzymes used in this study at large scale. While two actinomycetes isolates, BS 25 & BS 46 which exhibited broad spectrum antibacterial activity could be used for antibiotic production and for further steps in drug discovery.

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