ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (4): 478-480, 2006
ISSN 1682-296X

Isolation and Characterization of *Streptomyces* sp. From Soil Samples for Secondary Metabolite Production

K. Krishna Kumari, P. Ponmurugan and N. Kannan
Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamilnadu, India

Abstract: The present study aims at isolation and characterization of biologically diverse strains of *Streptomyces* from soil samples for the production of bioactive secondary metabolites. There were 10 isolates obtained from different areas in Tamil Nadu and studied for the detection of antimicrobial substances contained it. These antimicrobial compounds were extracted and tested against some pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Serratia marcescens* and *Pseudomonas fluorescence* as well as beneficial microorganisms like Phosphobacteria. The results showed that out of 10 isolates of *Streptomyces* sp. three of the isolates were found to be of potential antagonists against pathogenic organisms and thus providing the production of secondary metabolites which has the potential to control the soil borne pathogens. In contrast, some of the isolates showed no zone of inhibition with Phosphobacteria. This shows that it was not affected their growth.

Key words: Actinomycetes, *Streptomyces*, antimicrobial activity, secondary metabolites

INTRODUCTION

Actinomycetes population has been identified as one of the major groups of the soil population. They are widespread in nature in dry soils which do not grow well in wet soils (Wellington *et al*., 1994; Poornima and Ponmurugan, 2006). Further they can produce an array of secondary metabolites, many of which have antibacterial or antifungal properties. Infact, most antibiotics developed for human pharmaceutical use are actinomycetes metabolites, many being derived from *Streptomyces* sp. (Goodfellow *et al*., 1987). The Streptomycetes are widely used in industry due to their ability to produce numerous chemical compounds including antibiotics, enzymes and anti- tumor agents (Berdy, 1995). Several species from the *Streptomyces* genus produce bioactive molecules, including enzymatic inhibitors with antibiotic activity and many commercially valuable enzymes such as lipases, cellulases and proteases (Ravel *et al*., 2000).

The most promising role for secondary metabolites relies upon deference mechanisms. Inhibiting other competing cells, would leave more nutrients for the survival of the secondary metabolites producing strain. Indeed many secondary metabolites show antibacterial or other inhibitory activities (anti-tumor, anti-fungal) or may function as herbicides (Sanglier *et al*., 1993). The present study aims at isolation and characterization of biologically diverse strains of *Streptomyces* from soil sample for the production of bioactive secondary metabolites. The antimicrobial activity of this genus was studied by performing test against standard pathogenic stains and beneficial organisms.

MATERIALS AND METHODS

The present study was carried out at Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamilnadu, India for a period of two years (2004-2005). Soil samples were collected from fertile lands at different areas in and around Coimbatore district, Tamilnadu, India, from a depth of 6-10 cm in the rhizosphere and non-rhizosphere regions of agricultural crops. The soil samples were allowed to air dry at room temperature and various parameters like soil pH, total organic carbon (Walkley and Black, 1934) and total nitrogen (AOAC, 1990) were determined subsequently.

Isolation and enumeration of actinomycetes present in the soil sample was performed by serial dilution plate technique using starch-casein nitrate agar. Biochemical characterization such as pigment production, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction, indole production, gelatin hydrolysis and hydrogen sulphide production were carried out to identify the name of actinomycetes.

Corresponding Author: P. Ponmurugan, School of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 209, Namakkal District, Tamilnadu, India Tel: 09865857816 Fax: 04288-274745

In order to check the production of antibiotics by actinomycetes, antibiotic production medium was used. The pure culture of actinomycetes was inoculated into 25 mL of seed medium in 250 mL conical flask. The flasks were kept in a rotary shaker at 220 rpm for 25 days. The culture filtrate was centrifuged at 11,000 rpm to get a clear solution.

Antimicrobial activity of *Streptomyces* sp. was tested against pathogenic as well as beneficial organisms. For the present study, a lawn was made using standard strains of pathogenic organism such as *Staphylococcus aureus* (MTCC 740), *Escherichia coli* (MTCC 521), *Bacillus amyloliguefaciens* (MTCC 610) *Serratia marcescens* (MTCC 86) and *Pseudomonas fluorescence* (MTCC 645) and Phosphobacteria onto Mueller Hinton agar plates. Sterilized filter paper discs impregnated with *Streptomyces* broth culture was placed onto the lawn made in Mueller Hinton agar plates. The plates were incubated at 37°C for 24 h and were observed for zone of inhibition, which indicates a positive reaction for antimicrobial activity.

RESULTS AND DISCUSSION

Total number of actinomycetes population present in soil sample collected from different areas in Tamil Nadu indicated that actinomycetes population was found to be more in fertile areas particularly in rhizosphere and less in non-rhizosphere regions of the soil. Total number of actinomycetes colonies was found to be more in red soil and less in black soil which coincided with the soil pH, % of moisture content and total organic carbon content (Table 1). Most of the isolates tend to grow in alkaline soils which is an important characteristic feature of *Streptomyces* sp. (Stackebrandt *et al*., 1991) and with adequate source of carbon and nitrogen present in it that enhance the rate of degradation (Tien *et al*., 1987).

There were ten isolates obtained from these soils. Morphological and physiological characteristics of the isolates were studied and results were presented in Table 2. The results indicated that the purified isolates of actinomycetes belonged to *Streptomyces* sp. as they showed good sporulation with compact, chalk-like dry colonies of different colour variation from pink to white colour. All the isolates were found to be gram-positive organism and showed a branched mycelium in their cell morphology similar to fungal character (Holt, 1989). The result on biochemical characterization indicated that pigment production was very well observed in most of the *Streptomyces* sp. On the other hand, most of the isolates were efficient in hydrolyzing starch and casein (Ravel *et al*., 2000) except *Streptomyces* sp. 9. and *Streptomyces* sp. 7. Indole production was strictly negative but catalase test was positive in all the isolates. Production of hydrogen sulphide, gelatin hydrolysis, casein and starch hydrolysis showed positive result in majority of the isolates (Table 2).

The antimicrobial activity was performed against standard pathogenic organisms as well as beneficial organism like Phosphobacteria. The results showed that inhibition zone was formed around pathogenic stain but there was no inhibition zone formed around beneficial

Table 1: Population and distribution of actinomycetes in soil samples

Sample No.	Type of soil	Population density (cfu × 10^{-3})	Soil pH	Organic carbon (%)	Organic nitrogen (%)
1	Black soil	7	8.7	0.0048	0.00011
2	Black soil	4	8.6	0.0057	0.0002
3	Waste soil	6	8.5	0.0034	0.000084
4	Red soil	9	8.6	0.0036	0.000084
5	Red soil	12	7.7	0.0013	0.00022
6	Red soil	8	8.1	0.0027	0.000056
7	Red soil	7	7.6	0.0016	0.000056
8	Red soil	4	8.2	0.0043	0.00007

Table 2: Biochemical characterization of the *Streptomyces* sp.

	Streptomyces species used									
Biochemical characterization	1	2	3	4	5	6	7	8	9	10
Pigment production	+	+	+	+	-	+	+	+	-	+
Starch hydrolysis	+	+	+	+	+	+	-	+	+	+
Casein hydrolysis	+	+	+	+	-	+	+	+	-	+
Catalase test	+’	+’	+	+’	+	+’	+	+’	+’	+
Nitrate reduction	+	+	+	+	-	+	+	-	+	-
Indole production	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+’	+’	+	+’	+	+’	+	+’	-	-
Hydrogen sulphide production	-	+	+	+	-	+	+	-	-	+

+ Positive reaction for zone of inhibition, - Negative reaction for zone of inhibition, +’ Weakly positive reaction for zone of inhibition

Table 3: Antimicrobial activity of *Streptomyces* sp. against pathogenic and beneficial organisms

	Streptomyces species used									
Culture used	1	2	3	4	5	6	7	8	9	10
Staphylococcus aureus	-	-	+	-	-	+	-	-	-	-
Escherichia coli	-	+	-	-	-	-	-	-	-	-
Bacillus amyloliguefaciens	-	-	-	-	-	-	-	-	-	-
Serratia marcescens	-	-	-	-	-	+'	-	-	-	-
Pseudomonas fluorescence	-	-	-	-	-	-	-	-	-	-
Phosphobacteria	-	-	-	-	-	-	-	-	-	-

+ Positive reaction for zone of inhibition, - Negative reaction for zone of inhibition, +' Weakly positive reaction for zone of inhibition

organism (Table 3). Similar result was observed by Zahner *et al*. (1979). The formation of inhibition zone around the pathogenic strain is due to the production of secondary metabolites by *Streptomyces* sp. (Demain, 1983; Sanglier *et al*., 1993).

In this study, out of 10 isolates of *Streptomyces* sp. used three of the isolates were found to be of potential antagonists against pathogenic organisms and thus proving the production of secondary metabolites that has the potential to control variety of pathogens. Some of the isolates of *Streptomyces* sp. showed no zone of inhibition with phosphate solubilizing microorganisms. This shows that it was not affected the growth. Based on these result it can be inferred that the *Streptomyces* sp. can be used as a soil inoculants to prevent bacterial pathogens and at the sometime, this may have synergistic affect with beneficial organisms.

REFERENCES

AOAC., 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. (Ed), Helrich, K., 15th Edn., Vol. 1 and 2. AOAC Inc. USA.

Berdy, J., 1995. Are actinomycetes exhausted as a source of secondary metabolites? Biotechnologia, 7-8: 13-34.

Demain, A.L., 1983. New applications of microbial products. Science, 219: 709-714.

Good fellow, M., J. Lacey and C. Todd, 1987. Numerical classification of thermophilic *Streptomyces*. J. Gen. Microbiol., 133: 3135-3149.

Holt, J.G., 1989. Bergey's Manual of Systemic Bacteriology. Vol. 4. Williams, S.T. and M.E. Sharpe, (Eds.), Baltimore, Cambridge University Press. UK.

Poornima, R. and P. Ponmurugan, 2006. Studies on actinomycetes diversity in Namakkal and Erode districts of Tamilnadu, India. National seminar on Emerging trends in Industrial Biotechnology. Bioxplore' 06. Dept. of Industrial Biotechnology, Vivekanandha college of Engineering for woman. 17-18th February 2006.

Ravel, J., M.H. Wellington and R.T. Hill, 2000. Interspecific transfer of *Steptomyces* inear plasmids in sterile amended soil microorganisms. Applied Environ. Microbiol., 66: 529-534.

Sanglier, J.J., H. Haag, T.A. Huck and T. Fehr, 1993. Novel bioactive compounds from actinomycetes. A short review (1988-1992). Res. Microbiol., 144: 633-642.

Stackebrandt, E., D. Witt, C. Kemmerling, M. Kroppenstedt and W. Liesack, 1991. Designation of *Streptomycete* 16S and 23S rRNA based target regions of oligonucleotide probes. Applied Environ. Microbiol., 57: 1468-1477.

Tien, M., P.J. Kersten and T.K. Kirk, 1987. Selection and improvement of lignin-degrading microorganisms: Potential strategy based on lignin model-amino acid adducts. Applied Environ. Microbiol., 53: 242-245.

Walkley, A. and C.A. Black, 1934. An examination of the Degtjareff method for determining soil organic matter and proposed modification of chromic valid titration method. Soil Sci., 37: 29-38.

Wellington, E.M.H., E. Stackebrandt, D. Sanders, J. Wolstrup and N.O.G. Jorgensen, 1994. Taxonomic status of Kitasatosporia and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and enrici 1943, 339AL. Intl. J. Syst. Bacteriol., 42: 156-160.

Zahner, H., W. Weber, J. Siebers, K. Schroder and A. Zeeck, 1979. *Streptomyces*. Arch. Microbiol., 124: 111-116.