Studies on the antibacterial activity of the actinomycetes isolated from the fish cultivating reservoirs of Tamil Nadu

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ABSTRACT

Discovery of new drug is the need of the hour due to the identification of Multi Drug Resistant (MDR) pathogens in the environment. Apart from the existence of MDR strains, the uprising awareness on the side effects of the chemical drugs has put forth the want of discovery from biological sources. Hence, the present study was designed to isolate and identify potential actinomycetes strains from fish cultivating reservoir habitat soil and to find out antibacterial efficiency against the common human pathogens. A total of 38 actinomycetes strains isolated from reservoirs of Tamil Nadu, were subjected to primary screening by perpendicular streak method against Gram-positive (*Enterococcus* sp., *Staphylococus epidermis*, *Staphylococus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella dysentriae* and *Klebsiella* sp.) pathogenic bacteria. It was observed that four isolates were active against both Gram-positive and Gram-negative bacteria. Four isolates showed antibacterial activity in secondary screening by well diffusion assay to further confirm the activity of primarily screened organisms. The result showed that three of the isolates BR-12, BR-13, and BR-14 were highly active producing inhibition zone of 20mm and broad spectrum antibacterial activity against dreadful pathogenic bacteria. These Strains were identified by using morphological, physiological and biochemical characteristics and found to be *Streptomyces* sp.

Keywords: actinomycetes, antibacterial metabolites, antibacterial activity, well diffusion assay, *Streptomyces*

INTRODUCTION

Antibiotics - the wonder drug of 20th century is losing their clout. Bacteria naturally develop resistance to antimicrobial drugs. In recent years, however over use of antibiotics has caused resistance to certain infections. The dramatic rise of antibiotic resistance in human pathogenic microorganisms became the foundation for continuous development of novel antibiotics. Microbial diversity is a vast frontier and potential goldmine for the biotechnology industry because it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules [1]. The actinomycetes are Gram positive bacteria having high G+C (>55%) content in their DNA. The name 'Actinomycetes' was derived from Greek 'aktis' (a ray) and 'mykes' (fungus) and given to these organisms form initial observation of their morphology. Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms.

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The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomycetes population has been identified as one of the major group of soil population [2], which may vary with the soil type. The actinomycetes are noteworthy as antibiotic producers, making three quarters of all known products; Streptomyces are still rich sources of bioactive natural products with a great functional diversity [3]. They are extensively used as pharmaceuticals and agrochemicals [4-6]. They cover around 80% of total antibiotic product, with other genera trailing numerically; *Micromonospora* is the runner up with less than one-tenth as many as Streptomyces. Due to unique sample collection site and variable atmosphere it is quite likely to expect that the distribution of antibiotic producing actinomycetes is also variable. This study is carried out to screen the antibiotic producing actinomycetes from fish cultivating reservoir soil from Manchester of south India.

MATERIALS AND METHODS

Collection of soil samples and sample processing

Soil samples were collected from different sites of fish cultivating reservoirs in Tamil Nadu. Preferably wet samples (4-5g each) were collected from the depth of 4 to 6 cm and taken in a clean polythene bag. Collected samples were dried for about 2 to 3 weeks until the moisture contents gets removed completely. Dried samples were grinded finely using a motar and pestle. 1g of finely powdered soil was subjected to heat treatment by heating at 60°C for about 10 to 15 minutes. Treated soil was added to 100ml of distilled water and kept for 30 minutes incubation in an orbital shaker.

Isolation of actinomycetes

Actinomycetes were isolated from the processed sample by serial dilution technique. The samples were diluted (ten-fold) to give final concentrations of 10^{-3}, 10^{-4} and 10^{-5}. The best dilution was considered for counting. Isolation of Actinomycetes was carried out in accordance with standard method using Starch Casein Nitrate Agar medium (SCA). Colonies were isolated and purified by streak plate technique.

Isolation of antibacterial metabolites

Actinomycetes isolates were inoculated in 100 ml SCA medium in a 250 ml capacity Erlenmeyer flask. Flasks were lodged on the orbital shaker at a speed of 150 rpm at room temperature for 4 days. After fermentation, the medium was harvested and centrifuged to remove cells and debris. Filtrate is collected in a sterilized screw cap bottle. Antibacterial compound was recovered from the filtrate by solvent extraction method. The filtrate was mixed with Ethyl acetate, Chloroform, Ethanol and Butanol in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour in a solvent extraction funnel/separatory funnel. The solvent phase that contains antibacterial compound was separated from the aqueous phase. Solvent extract was used to determine antimicrobial activity.

Determination of the antibacterial activity

Antibacterial activity of the partially purified extract was determined by the agar well diffusion method using test pathogens *Enterococcus* sp., *Staphylococus epidermis*, *Staphylococus aureus*, *Bacillus cereus* (Gram positive), *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella dysentriae* and *Klebsiella* sp. (Gram negative).

Characterization of actinomycetes

The potent Actinomycetes isolates selected from primary screening were characterized by morphological, biochemical and physiological methods. The morphological method consists of macroscopic and microscopic characterization. Macroscopically the Actinomycetes isolates were differentiated by their colony characters, e.g. size, shape, color, consistency, etc. To microscopically characterize, the isolates were grown by cover slip culture method [7]. They were then observed for their mycelial structure, and conidiospore and arthrospore arrangements on the mycelia under microscope (1000X). The observed morphology of the isolates was compared with the Actinomycetes morphology provided in Bergey's Manual [8] for the presumptive identification of the isolates. Various biochemical tests performed were catalase, oxidase, citrate utilization, nitrate reduction, starch hydrolysis, tween-20 hydrolysis, urea hydrolysis, gelatin hydrolysis, esculin hydrolysis, acid production from sugar, and the physiological test included motility, NaCl resistance, and temperature tolerance.

RESULTS AND DISCUSSION

The isolates share their morphological similarities to the genus *Streptomyces*

The actinomycetes bacteria isolated from the fish cultivating reservoir exhibited different morphologies on the third day of incubation, the colonies appeared as chalky white spots, and the aerial mycelium arose from the surface of the agar plate in the form of single hyphae that subsequently branched heterogeneously. After five days they showed distinct differences in their aerial mycelia color, and some of the grey and white colonies showed fine droplets of extracellular exudates on their surface. 38 different isolates of actinomycetes with white, pale pink, brown and grey colonies were isolated from the fish cultivating reservoir soil. The aerial and substrate mycelia of all these colonies were observed by light microscopy and they were well branched and non-fragmentary.

The morphological characteristics like musty odor, spore formulation, dimorphic mycelia forms such as aerial and substrate mycelium, and the gram positive non-motile nature of the colonies indicated that they belong to the genus *Streptomyces* of the bacterial community. All the isolates could be grown on starch casein agar medium supplemented with 0.1% NaCl and they display proliphic mycelium formation and spore production within two days of incubation. The organisms was also able to grow well on the other growth medium tryptone-yeast extract medium (ISP medium 1), yeast extract-malt extract agar (ISP medium 2) and inorganic starch agar (ISP medium 4). They utilized a wide range of carbon sources glucose, fructose, sucrose, lactose, starch, mannitol, arabinose, raffinose and all xylose. The utilization of starch, tributryn and casein showed that these isolates produce extracellular enzymes amylase, lipase and protease to metabolize the polymeric components of the nutrient mixture. Positive reaction for the catalase enzyme revealed that the isolates could withstand the stress conditions generated by reactive oxygen spp. The test on triple sugar iron agar revealed that these organisms would not produced gas and acid when incubated in carbon sources such as glucose, sucrose and lactose (Table 1).

Antimicrobial effects of isolates from fish cultivating reservoir

The antimicrobial bioassay revealed that most of the isolated strains showed significant antimicrobial effects on the pathogenic bacteria, and four Actinomycetes isolates (BR-11, BR-12, BR-13, BR-14) exhibited strong inhibitory effects on (zone of inhibition $\geq$ 20mm) pathogenic bacteria microorganism (Figure 1).

Table 1. Biochemical characterization of actinomycetes isolates.

Biochemical tests	Actinomycete isolates			
	BR-11	BR-12	BR-13	BR-14
Hydrolysis of casein	-	-	-	-
Hydrolysis of Starch	-	-	-	-
Hyrdrolysis of xanthine	+	+	+	+
Nitrate Reduction	-	+	+	-
Indole	-	-	-	-
MR	-	-	-	-
VP	-	-	-	+
Citrate	+	+	+	+
TSI	+	+	+	+

Note: + (Positive), – (Negative).

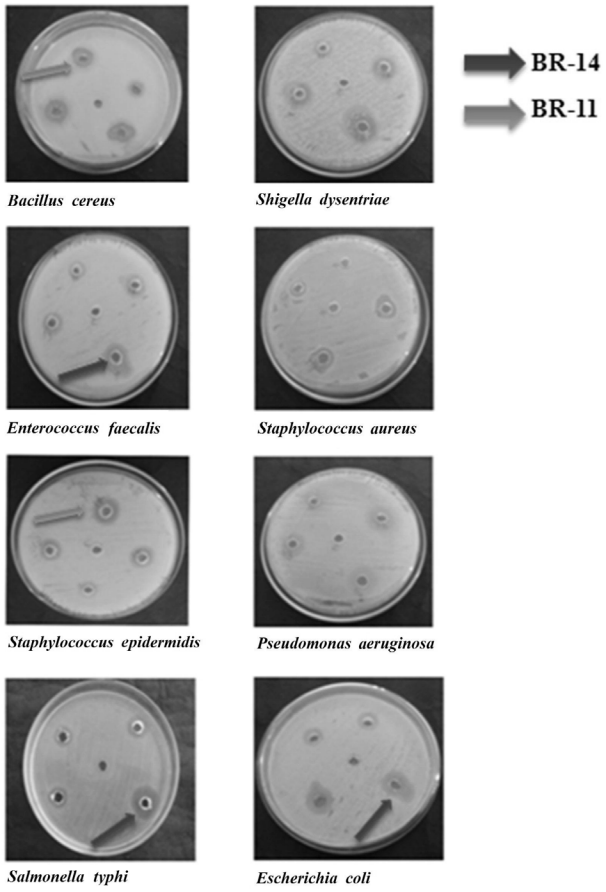

Figure 1. Plates showing zone of inhibition against clinical pathogens.

Actinomycetes isolates of the clusters were also characterized by diverse antimicrobial spectra BR-11 exhibited antimicrobial potential against *Staphylococus epidermis*, *Bacillus cereus* where as BR-15 showed no antibacterial or antifungal activity and the lineage BR-14 exerted antibacterial

activity against *Enterococcus faecalis*, *Escherichia coli* and *Salmonella typhi*. The genus of the strains with antagonistic activity against the pathogens was identified using morphological, biochemical and physiological studies based on Bergey's manual determinative biology (Table 1) [7]. In the previous study reported *Streptomyces* species isolated from forest soil [9] shows similar characteristic features of BR-11, BR-14 from fish cultivating reservoir soil which is the first evidence of *Streptomyces* from reservoirs.

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