RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF ANTIBIOTIC PRODUCING *ACTINOMYCETES* AGAINST CERTAIN PATHOGENS

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Abstract: Aim of the present study was isolation and identification of *Actinomycetes* against certain pathogens. *Actinomycetes* strains were isolated from cultivated field of Sudhowala, Dehardun and *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* were also isolated from sewage at Dehradun. Both *Actinomycetes* and pathogens were characterized on the basis of microscopy and various biochemical tests. Further, we evaluated antimicrobial activity of *Actinomycetes* strains against isolated pathogens. Microscopic examination and biochemical tests confirmed that isolated strains were *Actinomycetes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. *Actinomycetes* did not show inhibition zone against *Staphylococcus aureus*. But crude extract of *Actinomycetes* showed 191.66, 181.81 % more inhibition zone as compare to 25% extract concentration against pathogenic *E. coli* and *Salmonella typhi* repectively. It confired that Actinimycetes effectively control growth of *E. coli* and *Salmonella typhi*.

Keywords: *Actinomycetes*, Antibacterial, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*

INTRODUCTION

Antibiotic is one of the important secondary metabolites or chemical compounds which are produce by few micro-organisms. These antibiotics are metabolic products of one organism which directly inhibit or kill growth of other organisms. The various types of microorganisms are present in soil. *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* are responsible for various type of disease in human. *Staphylococcus aureus* is a major cause of bacteremia, and it is associated with higher morbidity and mortality (Naber, 2009, Van Hal *et al*., 2012). Food and water contamination by *Escherichia coli* has been a serious public health problem and a cause of huge economic losses worldwide. Foodborne pathogenic *Escherichia coli* contamination, such as that with *E. coli* O157 and O104, is very common, even in developed countries (Yang *et al*., 2017) and these *E. coli* strains cause diarrheal illness (Ramanathan *et al*., 2010). Salmonella represents the major cause of bacterial foodborne infection in the United States and is considered the major cause of human salmonellosis outbreaks in worldwide (Tarabees *et al*., 2017).

Antibiotics have been used since being discovered and used as remedy for infections, inflammations and diseases (Bhuyan *et al*., 2017). Total world production of antibiotics is more than one million tons per annum. Over 5,000 antibiotics have been identified from the cultures of Gram-positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used for treatment of diseases (Thomson and Bonomo, 2006). Fungal strains and streptomyces members are extensively used in industrial antibiotic production. The *Streptomycetes* are responsible for production of major antibiotics among antibiotics producing microorganisms (Singh *et al*., 2012). *Actinomycetes* from the genera *Actinoplane*, *Streptomyces*, and *Actinopolyspora* have been reported to produce over 300 broad-spectrum antibiotic substances (Kieser *et al*., 2000, Wynands and van Pee, 2004). So aim of present study is isolation and characterization of antibiotic producing *Actinomycetes* against certain pathogens.

MATERIALS AND METHODS

Isolation of pathogens: 0.5 ml sewage water sample was spreaded on Mannitol salt agar (MSA), Eosin Methylene Blue (EMB) agar medium, BSA agar medium for isolation of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* respectively. These plates were incubated at 37^{o}C for 48 hours.

Isolation and characterization of antibiotic producing actinomycetes: Actinomycetes was isolated from soil of cultivated field at Sudhowala, Dehradun (U.K).

(i) Sample preparation: Soil samples were dried separately at 65^{O}C for 1 hr, in a hot air oven and stored at room temperature for further work.

(ii) Isolation of Actinomycetes: Soil sample was diluted and spreaded on Actinomycetes Isolation Agar (AIA) medium plates and starch-casein agar medium plates aseptically in a laminar-air flow cabinet. The plates were incubated at 27 ± 2 °C for 5 days. The plates were observed intermittently during incubation. After 72 h, whitish pin-point colonies, characteristic of actinomycetes.

(iii) Characterization of isolated *Actinomycetes* strains and Pathogens: Isolated strains were characterized according to Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

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(iv) **Preparation and inoculation of Antibiotic production medium:** The isolated Actinomycetes strains were transferred in sterilized production medium ISP-1 (Starch-10g, Yeast extract- 4g, Peptone-2g, Potassium bromide-5g, Iron sulphate tetrahydrate-4.76g per liter) and incubated at 27 ^{0}C for 10 days.

(v) **Extraction of crude antibiotic metabolites from the production medium:** Extract was separated by the centrifugation at 3000 rpm for 20 minutes. The pellets were discarded and supernatant was mixed with double volume of chilled acetone and left overnight. The mixture was centrifuged at 5000 rpm for 20 minutes. The crude extract of antibiotic was washed with tris-phosphate buffer.

(vi) **Determination of the antibiotic activity of partially purified extract of Actinomycetes:** Twenty milliliters of sterilized molten Mueller Hinton agar (MHA) was seeded with 50 µl of each test organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* aseptically poured into three different sterilized Petri dishes and allowed to solidify. Sterile cork borer (6 mm diameter) was used to bore wells in the plate, and 100 µl of the extract was then carefully dispensed into the bored holes. The extract was allowed to diffuse for about 1 h before incubating aerobically at 37°C for 24 h. The presence of a zone of inhibition around each well was indicative of antibacterial activity.

RESULTS AND DISCUSSION

All pathogenic strains were isolated from sewage water. Selective media was used for isolation of pathogens. *Staphylococcus aureus* showed bright yellow coloured colonies on MSA agar medium which is selective medium. *E. coli* strains showed distinctive metallic green sheet colonies on EMB agar medium which confirmed that isolated strains were *E. coli*. *Salmonella typhi* strains were showed distinctive black coloured colonies on BSA agar medium which confirmed that isolated strains were *Salmonella typhi.* All pathogenic strains were characterized on the basis of various biochemical tests. *Staphylococcus aureus* showed negative result in Indole test, VP test, Urease test, starch hydrolysis and sugar fermentation but showed positive reaction in MR test, Citrate test, Gelatin utilization. *E. coli* and *Salmonella typhi* showed similar biochemical tests except citrate test i.e. *Salmonella typhi* strains were citrate positive (Table 1). All biochemical tests confirmed that isolated strains were *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*. Similar observations have been mentioned in Bergey's manual of determinative bacteriology (Holt *et al*., 1994) and Cowan and Steel's Manual for the identification of medical bacteria (Barrow and Feltham, 1993). Pure Actinomycetes strains were selected for biochemical identification. Actinomycetes strains showed positive tests such as Indole test, VP (Voges-Proskauer) test, Urease test, Gelatin utilization test, Starch hydrolysis test, Sucrose fermentation test, Lactose fermentation test, Glucose fermentation test but Methyl red test and citrate test negative (Table 2). All the biochemical tests are confirmed that isolated strains belong to genus *Actinomycetes*. Similarly, Dhananjeyan *et al*. (2010) reported that *Actinomycetes* strains showed positive results in methyl red, indole test, starch hydrolysis, vogus-proskauer test, catalase test and triple sugar iron test and shown negative result in fermentation of citrate. Previously, Abbas (2006) isolated actinomycetes from Kuwait saline soil and characterized *Actinomycetes* on the basis of biochemical tests. Similar observation has been observed by (Varghese *et al*., 2012, Chaudhary *et al*., 2013). Crude extract of *Actinomycetes* did not show any antibacterial activity against *Staphylococcus aeurus* but significantly inhibited the growth of *E. coli* and *Salmonella typhi*. Our result suggested that as we reduced concentration of crude extract of the *Actinomycetes* than there is redcution of antibacterial activity agianst *E. coli* and *Salmonella typhi*. 100% extract showed 191.66, 181.81 % more inhibition zone as compare to 25% extract concentration against *E. coli* and *Salmonella typhi* repectively (Table 3). Previously, Chaudhary *et al*. (2013) observed that Actinomycete Isolates AS14, AS27, and AS28 were highly active, while AS1 showed less activity against the pathogenic microorganisms i.e. Isolate AS7 exhibited the highest antagonistic activity against *Bacillus cereus* (24 mm) and AS16 showed the highest activity against *Enterococcus faecalis* (21 mm). further, Chaudhary *et al*. (2013) reported that MIC of actinomycete isolates was found to be 2.5 mg/ml against *Shigella dysenteriae*, Vancomycin-resistant enterococci, and *Klebsiella pneumoniae*, and was 1.25 mg/ml for *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus*, *Bacillus*.

Table 1. Biochemical test for isolated pathogen or test organisms

Biochemical test	***Staphylococcus aureus***	***Escherichia . coli***	***Salmonella typhi***
Indole test	Negative	Positive	Positive
MR test	Positive	Positive	Positive
VP test	Negative	Negative	Negative
Citrate test	Positive	Negative	Positive
Urease test	Negative	Negative	Negative
Gelatin utilization	Positive	Negative	Negative

Starch hydrolysis	Negative	Negative	Negative
Sucrose fermentation	Negative	Positive	Positive
Lactose fermentation	Negative	Positive	Positive
Glucose fermentation	Negative	Positive	Positive

Table 2. Biochemical tests results for Actinimycetes strain

Biochemical test	**Result**
Indole test, VP (Voges-Proskauer) test, Urease test, Gelatin utilization test, Starch hydrolysis test, Sucrose fermentation test, Lactose fermentation test, Glucose fermentation test	Positive
MR (Methyl red) test, Citrate test	Negative

Table 3. Antibacterial activity of crude extract of *Actinomycetes*

S. No.	**Concentration of extract (%)**	Inhibition zone (mm)		
		Staphylococcus aeurus	*Escherichia coli*	*Salmonella typhi*
1	25	0	12±0.5	11±0.5
2	50	0	16±0.5	15±0.0
3	75	0	20±0.5	17±0.5
4	100	0	23±0.0	20±0.5

Values are mean of three replicates

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