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Research Article

BIOACTIVE COMPOUNDS PRODUCED BY STREPTOMYCES STRAIN

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ABSTRACT

Objective: To develop a new antibacterial antibiotic from streptomyces strains were isolated from a sample of marine soil in and around Tamil Nadu coastal areas.

Methods: Totally 35 strptomyces species were isolated from marine soil by spread plate method and were screened for their antagonistic activity against five different pathogenic bacterial strains by cross streak method and agar well diffusion method. One of the strain was found to be more active against five different bacterial strains.

Results: The strain was identified as *Streptomyces coelicolor Strain SU6* (JQ828940) by 16S rRNA partial gene sequencing. The antibacterial compound produced by the active strain was identified by GC-MS report as 3-ethyl,3-methyl heptane and Diisodecyl ether.

Conclusion: The observations from this study confirmed that *Streptomyces* isolated from marine environment may be potentially used for extracting novel antibiotics for treating bacterial infections in human.

Keywords: Anti bacterial compound, *Streptomyces,* Marine soil, GC-MS report.

INTRODUCTION

The discovery of antibacterial metabolites for the importance of marine sources with a pharmaceutical potential has been proved in various excellent articles [1]. Isolation of streptomyces from marine sediments and suggested that the isolated streptomyces may be valuable for the production of antibiotics [2]. Most streptomyces species have the ability to synthesis many different biologically active secondary metabolites such as antibiotics, herbicides , pesticides , anti parasitic and enzyme inhibitors. Of these compounds antibiotics are much more important therapeutically , commercially and approximately one third of known antibiotics have been isolated from streptomyces. [3].

In particular, the genus Streptomyces is an important group of actinomycetes because of its ability to produce many types of secondary metabolites.

Streptomyces are the group of Gram positive filamentous bacteria which are ubiquitous various natural environment . [4] . streptomyce are the most economically valuable prokaryotes which are well known to produce chemically diverse metabolites with wide range of biological activity. [5].

The purpose of this study is to evaluate the antagonistic activity of strain *Streptomyces* that active against human pathogenic bacteria by using marine soil sediments.

MATERIALS AND METHODS

Isolation of Streptomyces

Twenty marine soil samples were collected from in and around Tamil Nadu coastal areas in the depth of 2cm. The sediment samples collected were air dried for one week and kept at 45°c for 1 hr to prevent bacterial and fungal contamination. [6]

One gram of each air dried soil sample was processed by serial dilution method. [7] and they were cultured by spread plate technique on starch – casein agar [8] and incubated at 37°C for 2 weeks. Selected colonies were subcultured onto ISP-2 medium. Slants containing pure cultures were stored at 4°C until further examination.

Screening of streptomyces for antibacterial activity

Primary Screening

The isolated and purified streptomycete strains were screened for their antibacterial activity by cross streak method [9] Using modified nutrient agar against five different pathogenic bacterias. The plates were kept for incubation at 37°C for 24hrs . After incubation , results were noted based on the presence and absence of colonies.

Extraction of bioactive compounds

The most potent strain was grown in ISP – 2 medium as a production media for the extraction of crude compound. The active strain was inoculated in ISP-2 (International Streptomyces project – 2)broth and incubated for 7 days in shaker incubator at 28°C. It was centrifuged for 15mins at 8,000 rpm and the supernatant collected was mixed with an equal volume of ethyl acetate. The crude compound were extracted by using ethyl – acetate extraction method [10]. The extracted crude compound were dried to get dry powder by using heating mantle at 40°C.

Secondary screening

The powder form of crude further processed for secondary screening with different pathogenic bacterias by well diffusion method [11] by using modified nutrient agar to confirm the active metabolites. The MIC (minimum inhibitory concentration) of active strain results were noted based on zone of inhibition .

Purification of the bioactive compounds

2grms of crude powder were dissolved in 10ml of ethyl acetate. The solution was passed through a silica gel column in benzene. The active fraction were pooled and subsequently subjected to analysis by Thin layer chromatography. The readymade precoated TLC plates were used. Using the capillary tube a row of spots of the active elutant was applied a line 1.5cm above from the bottom of TLC plates . the spots were left to dry. The TLC plate was placed vertically in a trough containing the solvent (hexane - ethyl acetate) 1:9 ratio. When the solvent moved up to 80% of TLC, the plate was taken out and dried, then sprayed with ninhydrin. [12]. Each bands were eluted out and again checked for antibacterial activity. The active band was identified for the presence of compound.

Identification of bioactive compounds

The compound were identified by using GC-MS technique [13]. The mass spectrum was recorded by using SHIMADZU QP2010. Mass spectrometer under the current (MA) 100 and the temperature at 70*C.

Identification of active strain

The potential strain was identified based on cultural characteristics, microscopic spore morphology, carbon utilization test, growth in

different temperature and pH , biochemical tests and 16S rRNA partial gene sequencing.[14]

RESULTS AND DISSCUSSION

Isolation of Streptomyces

Thirty five isolates were collected based on their different colony morphology and colour variations. The colonies were purified by repeated streak on ISP-2 medium Sub culture plate for active strain (Fig - 1). Of these 5 fast growing ones were further selected and tested for antibacterial activity. The study of [15] low altitude sagebrush found eleven out of 153 isolates tested showed broad spectrum antifungal activity.

Fig. 1: Subculture plates for active strain on ISP – 2 medium

Primary screening of antibacterial activity

The cross streak method of five fast growing isolates were tested against pathogenic bacteria . Out of five strains , fourth strain more active against five pathogenic bacteria.

Secondary screening of antibacterial compound

The fourth strain selected for extraction of crude compound by ethyl acetate extraction method. Dry powder form extract was obtained by this method. The MIC of antibacterial activity was carried out by agar well diffusion method . The MIC of fourth strain showed most active against *Psedomonas aeruginosa.* MIC of active strain showed in Table – 1.

Table 1: MIC of active strain

Organism	Zone of inhibition in (mm)			
	25(µl)	50 (µl)	75 (µl)	100 (µl)
Bacillus subtilis	13	14	15	20
Staphlococcus aureus	10	12	16	23
E.coli	12	15	21	26
Klebsiella pneumonia	11	16	21	28
Psedomonas aeruginosa	14	18	20	26

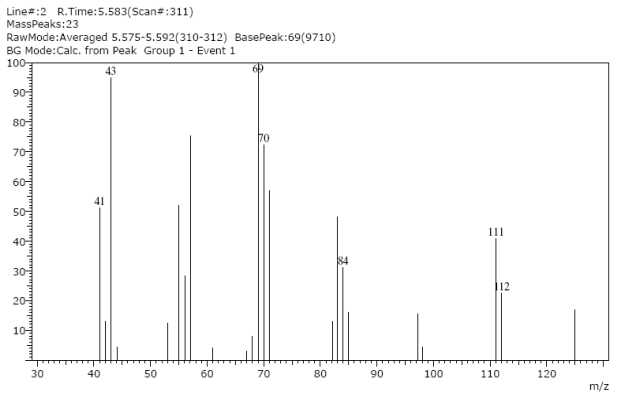

Fig. 2: Mass spectrum of Diisodecyl ether.

Identification of compound

The extracted compound was purified and separated by column and thin layer chromatography. Single separated band was observed in Thin layer chromatography. The Rf value of active compound was 0.45cm in a thin layer chromatography. The name of the compound identified by mass spectrum was 3-ethyl,3-methylheptane and Diisodecyl ether. Bioactive actinomycetes have been reported from Lake Baikal in Russia [16], Nile river in Egypt [17], Krishna river in Andhra Pradesh, India [18]. Mass spectrum and structure of diisodecyl ether showed in Fig 2 and Fig 3.

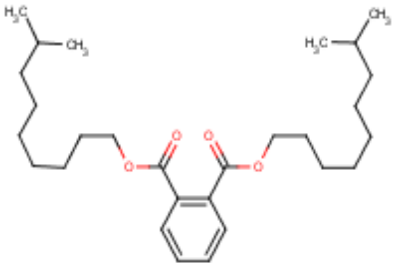

Fig. 3: Structure of Diisodecyl ether

Identification of active strain

The active strain was identified by morphological characteristics, carbon utilization test, biochemical test , growth in different temperature and pH and 16S rRNA partial gene sequencing. Results were showed in (Table 2 , Table 3 and Table 4)

Table 2: Identification of strains by different methods

Morphological Characteristics	Active strain
Colour of aerial mycelium	Grey
Reverse side colour	Pale yellow
Spore chain	Flexibilis
Spore surface	Smooth
Growth	Good
Test for utilization of carbon sources	
Carbon sources	**Active strain**
Glucose	+
Sucrose	+/-
Lactose	+
Fructose	+
Maltose	+
Galactose	-
Mannitol	+/-
Starch	+

+ : presence of growth , - : no growth , +/- : partial growth

Table 3: Biochemical Tests

Chemicals	Active strain
Citrate	-
Indole	-
MR	-
VP	-
TSI	A/A, gas -, H2S Absent
Urease	+

+: positive , - : Negative

Table 4: Growth in different temperature and pH of SU2

Temperature	Growth	pH	Growth
10°C	-	3	-
20°C	-	5	+
30°C	+	7	+
40°C	+	9	+
50°C	-	11	-

16S rRNA partial gene sequencing

The active strain identified as ***Streptomyces coelicolor Strain SU6*** **(JQ828940)** by 16S rRNA partial gene sequencing.

CONCLUSION

For this study, samples were collected from the Tamil Nadu coastal areas. A total of 35 isolates were collected , Of these 5 fast growing ones were further selected and tested for antibacterial activity. The five strains showed broad-spectrum activity against all the six pathogenic bacteria . The active strain showed more resistant to *Psedomonas aeruginos*a. The pure compounds 3-ethyl,3-methylheptane and Diisodecyl ether.more active against pathogenic bacteria . The most effective strain was identified as *Streptomyces coelicolor Strain SU6* (JQ828940) based on morphological , biochemical features and 16S rRNA sequencing. The observations from this study suggest that *Streptomyces* isolated from marine environment may be potentially used for extracting novel antibiotics for treating bacterial infections in human.

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