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ISOLATION OF NRISW5, NRISS7 STRAINS AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The aim of the present study is to optimize to the medium constituents for maximizing antibiotic production by marine actinomycetes exhibiting promising antibacterial and antifungal activities. Isolation of coloured actinomycetes from the samples with crowded plate technique and confirmed their antibiotic production by giant colony technique. Fifteen coloured strains were isolated and selected the NRISW5, NRISS7 strains to check the antibiotic production with respect to different production medium. The antibiotic activity of crude extract determined by using cup plate method. Both NRISW5 and NRISS showed antibacterial action on Gram-negative bacteria. Both strains have an ability to produce the bioactive compound i.e antibiotic substance.

Key words: actinomycetes, NRISW5, NRISS7 strains

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INTRODUCTION

The name actinomycete was derived from Greek 'aktis'- *a ray* and '*mykas'- fungus.* They were originally considered as intermediate group between Bacteria and Fungi but now are recognized as Prokaryotic organisms. They are generally accepted as bacteria. This is because, they have no nuclear membrane, are sensitive to lysozyme for the most part and to the common antibacterial agents, there is similarity to the type of bacterial flagella when these organelles are present and the type of cell wall resembles those of Bacteria. The hyphal diameters are much smaller then those of fungi and are close to those of bacteria. They are Gram positive thread like filamentous Bacteria having high G+C content in their DNA. The majority of actinomycetes are free living, saprophytic bacteria. Morphologically they share some properties between bacteria and fungi. They form hyphae with true branching as in the case of fungi and exhibit bacterial properties including lack of sterols and cell wall containing mucopeptides. They are unicellular organisms which reproduce either by fission or conidia or by means of special spores. They usually form a mycelium which may be of single kind designated as substrate (vegetative) or of two kinds vegetative and aerial (in part sporogenous). Hyphal diameters of actinomycetes are much smaller than those of fungi. They produce a variety of spore types, which include the endospores long regarded as the typical spore structure of Eubacteriales. They frequently show the presence of lytic viruses (actinophages). Actinomycetes exhibit a morphological differentiation, unique among prokaryotes. The aerial morphological differentiation

coincides with the production of a profusion of "Secondary Metabolites". Actinomycetes are the most economically and biotechnologically priceless prokaryotes. Representative genera of actinomycetes include Streptomyces, *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Micromonospora* and several others. Secondary metabolites produced by actinomycetes possess a wide range of biological activities .The genus Streptomyces alone produces a large number of bioactive molecules .It has an enormous biosynthetic potential that remains unchallenged without a potential competitor among other microbial groups. A large number of Streptomyces spphave1 been isolated and screened from soil in the past several decades. Consequently the chances of isolating a novel Streptomyces strain from terrestrial habitat shave diminished. Above 500 species of Streptomyces account for 70–80% of relevant secondary metabolites with small contributions from other genera, such as *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes* (1-3).

MATERIALS AND METHODS

Screening

A total of 15 isolates were used for the screening of various activities. These isolates were isolated from different samples were collected into the sterile screw cap tubes with a sterile spatula and care was taken to see that the points of collection had as widely varying characteristics as possible with regard to the organic matters, moisture content, particle size, colour of soil. A brief description of marine samples is given below. **Sample No-1**: Sea sediment was collected a depth of 10 mts, Machlipatanum. The sample was muddy, blackish-brown in color; **Sample No-2:** Seawater was collected, at a depth of 10 mts Machlipatanum; **Sample No-3:** Seawater was collected, at a depth of 10 mts Suryalanka, Bapatla; **Sample No-4:** Sea sediment was collected near the shores Suryalanka, Bapatla. The sample was sandy, light brown in colour; **Sample No-5:** Soil was colleted from NRI college ground.

Isolation of actinomycetes from samples (4-6)

About 5 gm of sample was transferred to a sterile Erelenmeyer (E.M) flask containing 50 ml sterile water .The flasks were shaken on rotary shaker for 30 min for the detachment of the spore chains, if any. The flasks were kept aside for 30 min to settle down the particulate matter. The clear supernatant was diluted into sterile water. These dilutions ($10^{-1}$to 10^{-3} for samples) was used as inoculate. One ml of each of this dilution was pieptted out into medium, plated into petridish (8 inch dia) and inoculated at 28^0 C for 2-3 weeks. Sea water sample was also diluted from $10^{-1}$to 10^{-3} for samples. All the media were sterilized by autoclaving .All the glass apparatus were sterilized by dry heat at 160^0.C for 1 hr in hot air oven. Then incubated petriplates were observed from 5 day onwards for 3 weeks. Rifampicin and Flucanazole were used to inhibit the bacterial and fungal growth respectively (Fig-1).

Fig-1 shows the various isolated from marine and soil strains

Study of Antibiotic Activity

The selected two isolates (i.e NRISW5, NRISS7) were sub cultured onto YEME slants and incubated for about 7-10 days. The following production medium was used to test antimicrobial activity.

Screening of antibacterial activity

Cup Plate Method- A 24 hr culture of freshly prepared test organism was seeded into the nutrient agar medium and was placed on to the Petri dishes. The cell free broth (50µl) samples along with the standards (Rifampicin of 1mg/ml conc.) were placed in the cups made at equidistant in the agar plate. Allowed to diffuse for 1 hr and incubated at 37ºC in an used as criterion for selection of isolate and production medium.

RESULTS AND DISCUSSION

Various samples are collected from the different sources from the sample isolated the 15 strains, from these strains we are selected i.e. NRISW5, NRISS7.These two strains are selected because they shows the anti microbial properties in crowded plate technique and gaint colony technique. These strains inoculated into different production media and checked the anti microbial activity to know the nutrient effect on antibiotic activity of these micro organisms. The antibiotic activity of these strains was determined by cup plate method.

To screen the anti microbial activities, we are selecting the two organisms they are gram positive *Bacillus subtilis* and gram negative *E.coli.* The cup plate method shows no inhibition property of these strains are gram positive bacteria with respective production media PM_1,PM_2,PM_3 but we are observe inhibition zones of gram negative bacteria(*E.coli)* with PM_2 of NRISW5 and PM_1 of NRISS7(Table-1). The inhibition zones are compare with reference standard Rifampicin(1mg/ml).And observe NRISW5 shows large inhibition zones when compared reference standard (Fig-2 and 3).

This indicates these two strains are produced anti bacterial substance with respective nutrient compiosition of the production media.

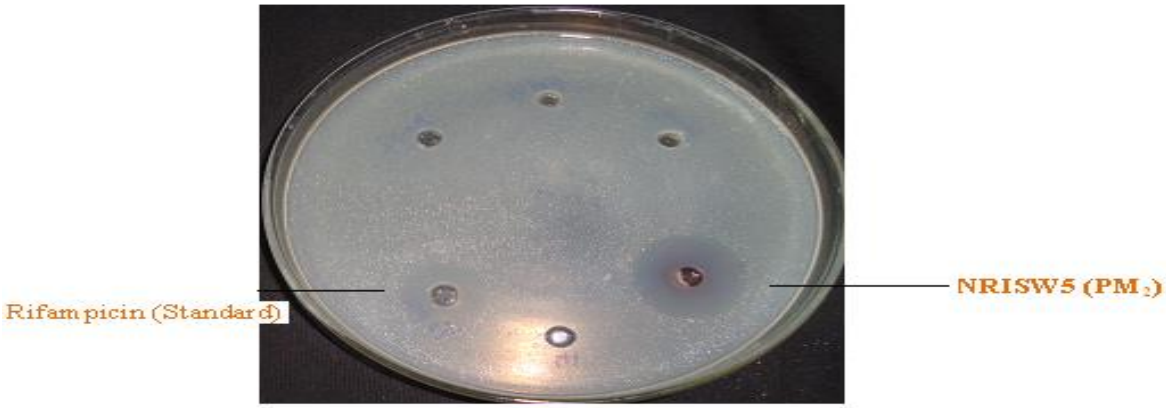

Fig-2 Crude extract (PM_2) of NRISW5 shows the inhibition zones of Gram-Negative strain *E.Coli*

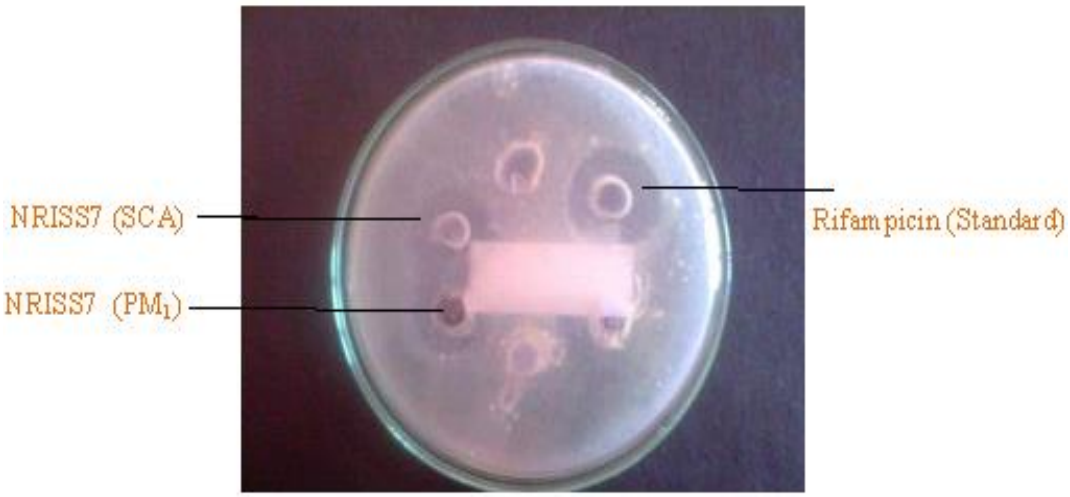

Fig-3 Crude extract (PM_1) and SCA of NRISW5 shows the inhibition zones of Gram-Negative strain *E.Coli*

Table- 1 Shows the inhibition zones of crude extracts and compared with reference standard

S.NO	STRAIN.NO	INHIBITION ZONE DIAMETER(mm)							
		GRAM +VE BACTERIA (BS)			STANDARD	GRAM –VE BACTERIA(EC)			STANDARD
		PM_1	PM_2	PM_3		PM_1	PM_2	PM_3	
1	NRISW5	NIL	NIL	NIL	20	NIL	28	NIL	19
2	NRISS7	NIL	NIL	NIL	21	18	NIL	NIL	20

BS: *Bacillus subtlis;* EC: *Escherichia coli;* Standard: Rifampicin 1mg/ml;
PM_1, PM_2 PM_3: Producation medium

CONCLUSION

It was concluded that both NRISW5 and NRISS7 showed anti bacterial activity only on gram negative bacteria with respective nutrient composition of production media.

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