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RESEARCH ARTICLE

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Anti-infective potential of halophilic actinomycetes isolated from saltpan

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ABSTRACT

Microorganisms are well known for their beneficial characters to human out of their infectious disease causing ability. Out of their vast diversity few class of extremophiles are well known for their ability to produce to novel bioactive compounds which act against deadly diseases and disorders. Halophiles are known for their ability to produce some rare secondary metabolites, enzymes and peptides among the group of extremophiles. In the process of screening bioactive compound from halophilic microorganisms two potential actinomycetes were isolated from salt pan near suburban of Mumbai (Maharashtra, India). Anti-infective screening of fermented crude extract against drug resistant human pathogenic shown promising activity in the present study.

***Keywords*:** *Actinomycetes, anti-infective, halophiles, human pathogens.*

1. INTRODUCTION

Natural products are generally either of prebiotic origin or originate from microbes, plants, or animal sources [1]. As chemicals, natural products include such classes of compounds as terpenoids, polypetides, carotenoids, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and so forth. Natural products are products of convenience of nature. Interestingly natural sources provide treatments for pain, palliatives, or curatives for deadly disease like cancer, HIV and tuberculosis etc. [2]. Microorganisms have proven to be an excellent source of novel natural products including polypeptide and peptide antibiotics and other classes of biologically active compounds [3]. So far microbial metabolites are used as antineoplastic agents (e.g. mitomycin), immunosuppressive agents (rapamycin), hypocholestrolemic agents (pravastatin) enzyme inhibitors (desferal), antimigraine agents (ergot alkaloids), herbicides (bialaphos), antiparasitic agents (salinomycin), bioinsecticides (tetranactin), and ruminant growth promoters (monensin) [4].

The potency of the *Streptomyces* species should not be underestimated. Their capacity to produce promising new compounds will certainly be unsurpassed and for a long time and they still have been producing the majority of the chemotherapeutically applied antibiotics. It is estimated that the total number of antimicrobial compounds from this genus is capable of producing to be of the order of a 100,000 [5]. Almost about 120 glycopeptide antibiotics are produced exclusively by these rare actinomycetes species. The macrolides, polyene antibiotics, amino glycosides, anthracyclines (each groups covering about 400~500 compounds), are all produced exclusively from actinomycetales species. Other smaller groups, such as polyether antibiotics

(~250 compounds); the novobiocin related glycosidic antibiotics, streptothricins, actinomycin and echinomycin-like quinoxalin peptides (each covers 80~120 compounds); elfamycins, glutarimides, orthosomycin are also exclusive actinomyces products. The predominant part of the large, 18 to 60 membered macrocyclic lactone derivatives (including over 1000 compounds), ansa lactones (~150), benzanthraquinon -e derivatives (~200), thiostrepton-like thiazolyl peptides (140), cyclopolylactones (~40), benzadiaze-epine antibiotics (~60), tetracyclines (~40), macrodi- and -tetrolides (30~40 compounds) are derived also from various actinomycetes species [6].

Halophiles are salt-loving organisms that inhabit hypersaline environments. They mainly include prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. Among halophilic microorganisms are a variety of heterotrophic and methanogenic archaea; photosynthetic, lithotrophic, and heterotrophic bacteria; and photosynthetic and heterotrophic eukaryotes. Examples of well-adapted and widely distributed extremely halophilic microorganisms include archaeal *Halobacterium species*, *cyanobacteria* such as *Aphanothece halophytica*, and the green algae *Dunaliella salina*. Actinomycetes can be isolated from soil relatively easily. For this purpose a selective agar medium is prepared which contains the usual assortment of inorganic salts to which starch, asparagine or calcium malate is added as a source of carbon and undigested casein or potassium nitrate as source of nitrogen unexplored producers [7]. In general, the sea and to a lesser extent the rainforests are almost inexhaustible, untapped reservoirs for novel compounds. Besides the marine microorganisms, the soft-bodied marine animals and the endophytic fungi living together with the green plants, represents important sources for new compounds [8].

2. MATERIALS AND METHODS

2.1. Sample collection

The soil sediments were collected from Mira road, Mumbai-Maharashtra, using a clean polyvinyl cover of 10 cm, which is previously disinfected and dried with alcohol, into the sediments at each ecological unit. The central portion of the top 2 cm sediment sample was taken out with the help of a sterile spatula. This sample was transferred to a sterile polythene bag, labeled and transported immediately to the laboratory and stored at 4°C till processing.

2.2. Isolation of actinomycetes

International *Streptomyces* project II media and Starch Casein gar amended with increased salt concentration was used for the isolation. To avoid growth of accompanying flora higher concentration of NaCl of 10% and Amphotericin B (50µg/ml) concentration was used to suppress the fungal growth. Plates were incubated at 25°C for 5-40 days and observed periodically using stereo microscope. Typical actinomycetes, especially *Streptomycetes* colonies observed under stereo microscope are picked and streaked on a fresh media in a slant to obtain pure isolate.

2.3. Fermentation and preparation of crude extract

Loop full of culture from the axenic culture slant were inoculated into seed medium ISP2 broth for 72 hrs. (29-30^{o} C at 200 rpm) and further 5% (v/v) inoculum from seed culture were aseptically inoculated in the batch culture of 100ml and incubated for 72 to 96 hours (29-30^{o} C at 200 rpm). The batch was harvested with 10% methanol in ethyl acetate. Upper clear solvent layer was collected without disturbing the spent (cell debris). Whole broth extract (upper layer) was subjected to layer separation using separating funnel. The elutes collected were concentrated to remove the solvent and used as crude for primary screening against various anti-infective test models.

2.4. Test Organisms

The test bacteria used for primary screening were *Staphylococcus aureus* 209P, *Staphylococcus aureus* E710 (MRSA), *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218. Anti-fungal activity of actinomycetes was determined using *Candida albicans*, *Candida glabrata* HO5 Flu^{R} ,*Candida krusei* GO3 Flu^{R}, *Aspergillus fumigatus* HMR, *Aspergillus fumigatus* ATCC 16424.

2.5. Anti-infective screening

The antiinfective screeing of the crude extract were carried out by modified Kirby Bauer agar well diffusion assay using nutrient agar medium (Himedia) and Potato dextrose agar (Himedia) for fungai. Each plates were prepared by and pre inoculated with test organisms. Well size of 10mm diameter was made using sterile wellpuncture and loaded with 100 µL per well. 50µg/ml concentration of Gentamicin (Himedia) and Amphotericin B (Himedia) were used as the standard. Plates were inc--ubated for 37°C for 24-48 hours and results obtained were tabulated.

Table 1. Anti-infective potential of actinomycetes against test pathogens

S.No	Sample Code	*E.coli* 35218	*E.coli* 25922	*S.aereus* 209P	MRSA E710	*C. glabrata* HO5 Flu^R	*A. fumigatus* HMR	*A.fumigatus* HMR + Ergosterol	*A.fumigatus* ATCC 16424	*C.albicans*	*C.albicans* + Ergosterol	*C.albicans* GO3 $Fluc^R$
1	SP-A1	10	-	15	17	20	11	9	15	21	19	10
2	SP-A1	11	10	19	24	23	10	11	20	24	24	13
3	SP-A1	-	-	21	25	18	9	-	12	19	20	12
4	SP-A1	-	-	18	20	18	9	-	12	20	20	12
5	SP-A2	12	10	18	19	23	13	12	22	24	23	14
6	SP-A2	9	-	14	20	23	13	10	20	22	22	15
7	SP-A2	-	-	19	21	23	10	9	15	21	20	13
8	SP-A2	9	9	22	25	20	11	10	17	20	21	-

(Zone of inhibition in mm)

3. RESULTS

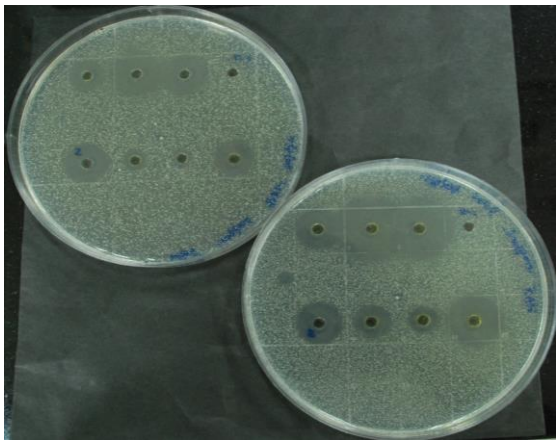
3.1. Anti-infective activity

In this present study the salt pan soil sediments were collected from different locations of Mira road, Mumbai, Maharashtra. The actinomycetes were isolated and identified based on its morphological, cultural characters and microscopic analysis culture techniques. Total of 45 actinomycetes screened for anti-infective potential, two isolates SP-A1 and SP-A2 produced a clear zone of 24-25 mm for MRSA and 19-22 mm for *S.aureus* respectively as shown in Figure.1. The inhibition zone produced was compared with that of the standard bacterial agent (Table 1) Gentamicin sulphate (50µg/ml per well). Potential isolates SP-A1 and SP-A2 also produced activity against other fungal test models *viz Candida glabrata* HO5 Flu^R, *Candida krusei* GO3 FluR, *Aspergillus fumigatus* HMR, *Aspergillus fumigatus* ATCC 16424. Then the isolates were designated as *Streptomyces sp*. SP-A1 and SP-A2.

4. DISCUSSION

Development of drug resistance against available standard dugs becomes the challenges for effective and curative treatment for the infectious diseases. Actinomycetes consider to be a largest group of the bacterium which plays vital role in natural cycle [9].

Figure 1. Antibiotic well diffusion method



Antibiotics from actinomycetes will be a possible way in identifying new drug of its diversified ecological niches [10]. Actinomycetes are consider to be the higher producers of metabolic compounds which as commercial importance in the industries as antibiotics and other novel drugs [11]. Observation of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities of antibiotics extracted from actinomycetes against test organisms. Gurung *et al*., 2009 [10] reported 0-18 mm inhibition zone of crude extracts against test organisms were consider as the potential. From the present study, a range of recorded inhibition zone of crude extract from isolates against test organisms were 10-25 mm which were higher

than the result reported by previous study [10]. In the present study, the randomly selected soil samples, sediments were taken from of the saltpan residue and sediment for isolation of actinomycetes. The successful isolation of actinomycetes from environmental samples requires an understanding of the potential soil sample areas and environmental factors affecting their growth. Previous studies shown that selection of different potential areas such as rhizosphere soil samples were an important activity for isolation of different types of potent antibiotic producing [12]. Actinomycetes isolated in the present study shown resistant to the higher salt concentration of 10-15% of Nacl and potential anti-infective activity against various drug resistant human bacterial and fungal pathogens. Crude extracts will show good inhibition zones better than the standard used. Therefore, further purification process should be done to get pure antibiotic substance for the application of treatment and industrial process.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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