Bioactive Metabolites from Indigenous Actinomycetes Isolated from Marine Water

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Abstract: Microbial natural products have continued to play an important role in the discovery of novel chemicals for the development of important therapeutic agents. Actinomycetes form a potent reservoir of biologically active secondary metabolites and enzymes. The need for finding novel bioactive compounds for the development of new therapeutic agents is required due to the emergence of antibiotic resistance among pathogenic bacteria. Actinomycetes are considered as one of the best producers of variety of antagonistic compounds that could serve as potential chemotherapeutic agents. The present study was undertaken to find new antagonistic compounds from actinomycetes. Actinomycetes were successfully isolated from marine water samples collected at various locations of Karachi. Initially 39 isolates were collected out of which 23 were found to produce active metabolites against one or more test bacterial cultures. Actinomycetes strains IS26, IS33, and IS39 showed significant potential of having bioactive metabolites. Further, the spectrum of those strains was tested against gram positive and gram negative bacteria and results showed variable potential of actinomycetes to inhibit bacterial growth.

Keywords: Actinomycetes, Bioactive metabolites, Marine water, Antimicrobial, Human Pathogens.

INTRODUCTION

Secondary metabolites are the natural products which are one of the most promising sources in the discovery of drugs [1]. These natural products, possessing unique structures, have principal biological activities and contribute in the development of a number of significant therapeutic [2]. Microorganisms are the chief producers of useful natural products and major portions of the microbial genomes are responsible for the production of these beneficial secondary metabolites. Important therapeutic agents have been isolated from various microbes [3].

The appearance and spreading of antibiotic resistance among pathogens is an alarming issue with reference to the human health [4]. The discovery of new antibiotics is an essential process to tackle the disease causing ability of pathogenic microbes and elimination of infectious diseases among the population of the world. Isolation of these therapeutic compounds involves the screening of microorganisms from natural habitat by using different techniques [2].

Actinomycetes are a rich source of biologically active secondary metabolites having activities such as anticancer, antimicrobial and antiviral [5]. Actinomycetes are the most important members among microbial world for their potential to produce biologically active compounds. It has been reported that about 45% of all bioactive microbial compounds are contributed by actinomycetes [6]. Commonly used commercially important antibiotics such as fosfomycin, lincomycin, neomycin, streptomycin, daptomycin, erythromycin, and tetracycline are produced by the genus *Streptomyces* of actinomycetes [7, 8] and *Streptomyces* species continue to play major role in the production of novel therapeutic compounds [9]. Actinomycetes have a good potential in secondary metabolites production and there is need of continuous exploration of this group of microorganisms for isolation of new compounds [8] and from marine environment as well [10].

MATERIAL AND METHODS

Sampling

Sea water samples were collected from Hawke's Bay beach, located 20km south west of Karachi, Pakistan. Samples were collected in sterile sample collection bottles from the depth of three feet and analysed under sterilized condition.

Chemicals

Chemicals used during analysis included ethanol, NaCl, Na_2HPO_4, KH_2PO_4, H_2SO_4 and $BaCl_2$. Different microbiological media including Nutrient agar, Nutrient broth, Agar technical, Zobell marine agar and Heart Infusion agar (Oxoid) were also used.

Isolation of Actinomycetes from Seawater Sample

For the isolation of actinomycetes, sea water samples were collected during the period March 2014

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to August 2014. 1mL of seawater sample was mixed with 99mL sterile aged seawater and further serially diluted up to 10^{-5} in aged seawater blanks. 100 µL from the final dilution was spread on agar of Zobell medium for isolation [11]. Agar plates were incubated at room temperature for 1 week. Acinomycetes isolates were purified and maintained on half strength Nutrient agar at 4°C [12].

Characterization of Isolates

The isolated actinomycetes strains were characterised on the basis of gram staining and their morphological characteristics [13]. Colonial morphology was observed with respect to colour, pigment, texture, elevation and size on half strength Nutrient agar.

Test Organisms

The selected human pathogens, which are used to examine the antibacterial activity of the isolates, were obtained from Department of Microbiology, University of Karachi. Bacterial cultures included *Salmonella typhi, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus faecalis, Escherichia coli, Staphylococcus epidermidis* and *Proteus mirabilis.*

Screening of Actinomycetes for Antibacterial Activity

Isolated actinomycetes strains were evaluated for their antibacterial activity by double layer agar method [14]. Cell concentration of test organisms and actinomycetes strains were adjusted at 0.5 McFarland turbidity standards. For determination of antibacterial activity, 8µL of Phosphate buffer saline (PBS) suspension of isolated actinomycetes was inoculated on half strength nutrient agar plates and incubated at room temperature for 2 days. 100µL of PBS suspension of test organisms was inoculated into 5mL 1% Heart infusion soft agar and then poured over the surface of isolates inoculated on half strength nutrient agar plates. Plates were then incubated at 37°C for 24 hours.

Analysis of Bioactive Compound

For determination of nature of bioactive compound, actinomycetes strains were inoculated into 50mL half strength nutrient broth and incubated at room temperature for 2 days at static condition. Then actinomycetes strains inoculated broths were centrifuged at 5000 rpm for 10 min at 4°C. Supernatants obtained were passed through 0.45µm corning membrane filters. The filtered supernatants were divided into two aliquots and one of the aliquots was heated at 100 °C for 1 min. Now agar well diffusion method was followed using *M. luteus* as test organism to determine the activity of both aliquotes. 100µL from both aliquots was inoculated into 10 mm wells on BHI agar plates and incubated at 37° C for 24 hours.

RESULTS AND DISCUSSION

Actinomycetes have been intensively investigated for their tremendous metabolic potential, isolated from various underexplored extreme habitats, niches and environments in the world. The present study was designed to explore the hidden potential of actinomycetes in an unexplored region.

Isolation and Culture Characteristics

Thirty-nine actinomycetes strains were successfully isolated from marine water samples. All the isolates were gram positive and showed different morphological characteristics on half strength Nutrient agar [12]. Many researchers have successfully isolated actinomycetes from various sources like [15], termite gut [16], plant [17], mud [18] and marine [19].

Screening of Actinomycetes Strains for Antimicrobial Activity

In the present study, the antibacterial activity of the isolated actinomycetes strains was determined by measuring the zone of inhibition against *M. luteus*. Among total of thirty-nine actinomycetes strains, twenty-three isolates exhibited antibacterial activity (Table **1**). Isolated strain IS33 showed good inhibitory activity with the highest zone of inhibition against the test organism. *M. luteus* appeared to be the most sensitive organism to antibacterial activity of IS33 (Figure **1**). The same strain was further screened for its activity against gram negative and gram positive bacteria. Gram positive bacteria exhibited more sensitivity towards antibacterial spectrum as compared to gram negative bacteria, *Staphylococcus epidermidis* showed highest sensitivity to antibacterial activity of IS33 (Figures **2** and **3**).

IS39 showed broad range of antibacterial activity against test organisms as it was found to be equally effective against gram positive as well as gram negative clinical isolates (Figures **4** and **5**). IS26 showed good inhibitory activity against test organisms. *Pseudomonas aeruginosa* and *Proteus mirabilis* found

Table 1: Antibacterial Activity of Actinomycetes Strains Against *M. luteus*

Strain No.	Inhibitory activity against *M. luteus*	Strain No.	Inhibitory activity against *M. luteus*
	Diameter of zone (mm)		Diameter of zone (mm)
IS1	14.5	IS21	-
IS2	7.25	IS22	-
IS3	-	IS23	22
IS4	-	IS24	15
IS5	-	IS25	-
IS6	-	IS56	28.5
IS7	25	IS27	-
IS8	43.5	IS28	27
IS9	13.5	IS29	9
IS10	-	IS30	25
IS11	22	IS31	-
IS12	-	IS32	-
IS13	12.5	IS33	63
IS14	13.95	IS34	29
IS15	19.25	IS35	17
IS16	-	IS36	45.5
IS17	-	IS37	8.875
IS18	6	IS38	-
IS19	14.5	IS39	52.5
IS20	-		

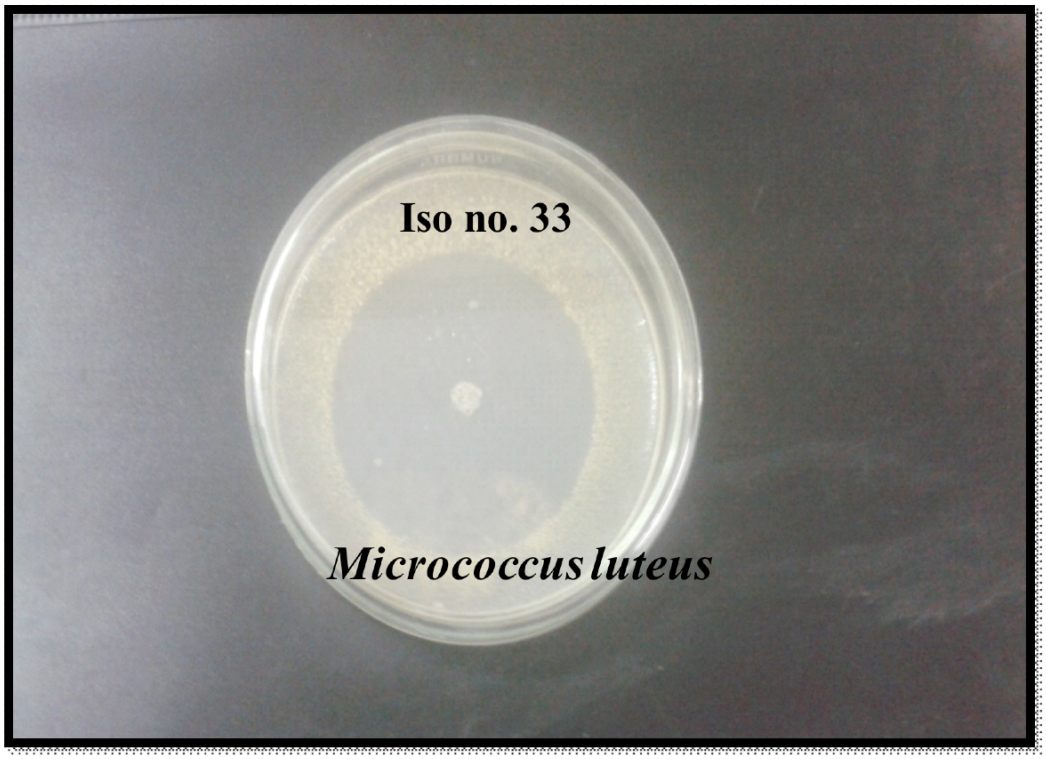

Figure 1: Determination of antibacterial activity of the isolate IS33 against *M. luteus.*

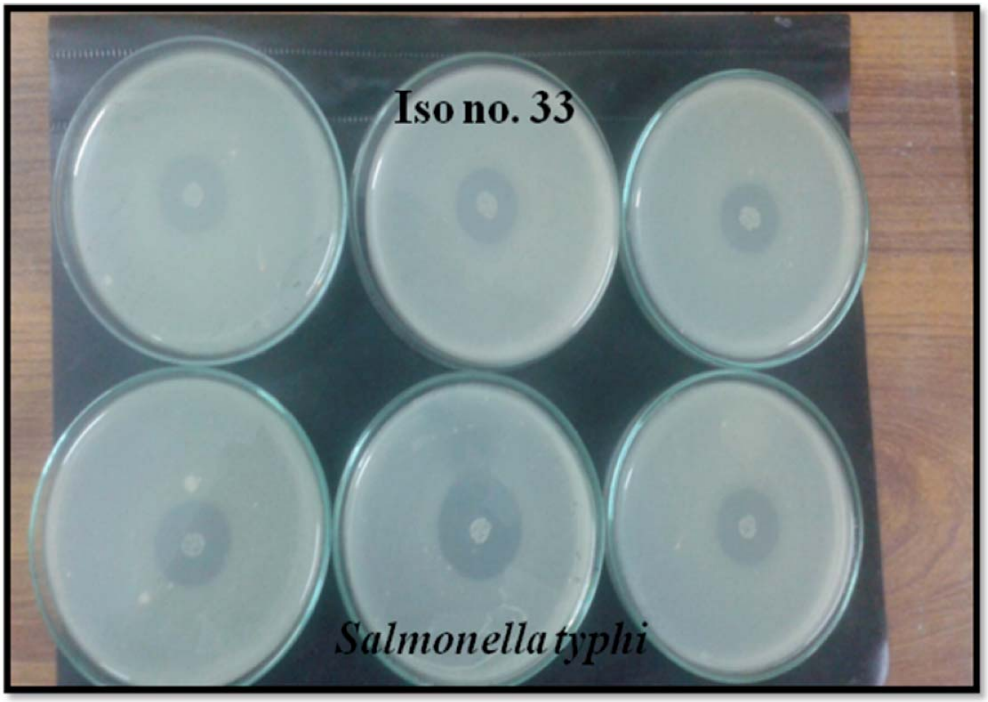

Figure 2: Determination of spectrum of antibacterial activity of the isolate IS33 against *S. typhi.*

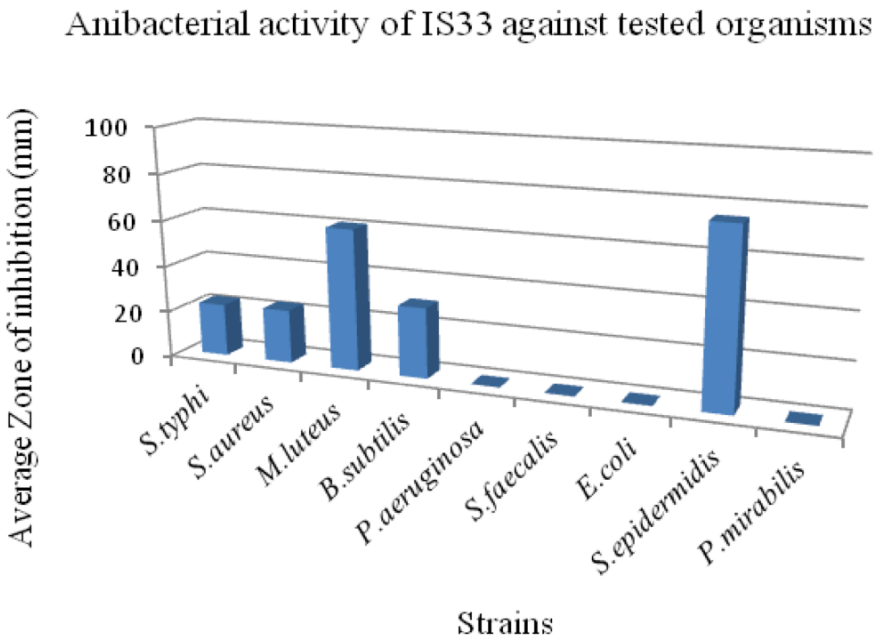

Figure 3: Antibacterial activity of IS33 against tested gram positive and gram negative bacteria.

to be resistant to antibacterial activity of IS26 while *Staphylococcus epidermidis* showed high sensitivity with greater zone of inhibition (Figures **6** and **7**). Our finding is in agreement with published reports that actinomycetes also exhibit antibacterial activity against many human pathogens [20]. Actinomycetes isolated from soil and marine environment are extensively studied for their antimicrobial potential [21, 22]. Dissemination of antibacterial resistance among pathogens had forced researchers to search new and more effective therapeutic agents from various possible sources such as biologically active secondary metabolites produced by actinomycetes [15].

CONCLUSION

By virtue of the present study we can conclude that the marine environment is a rich source of actinomycetes which produce metabolites that exerts

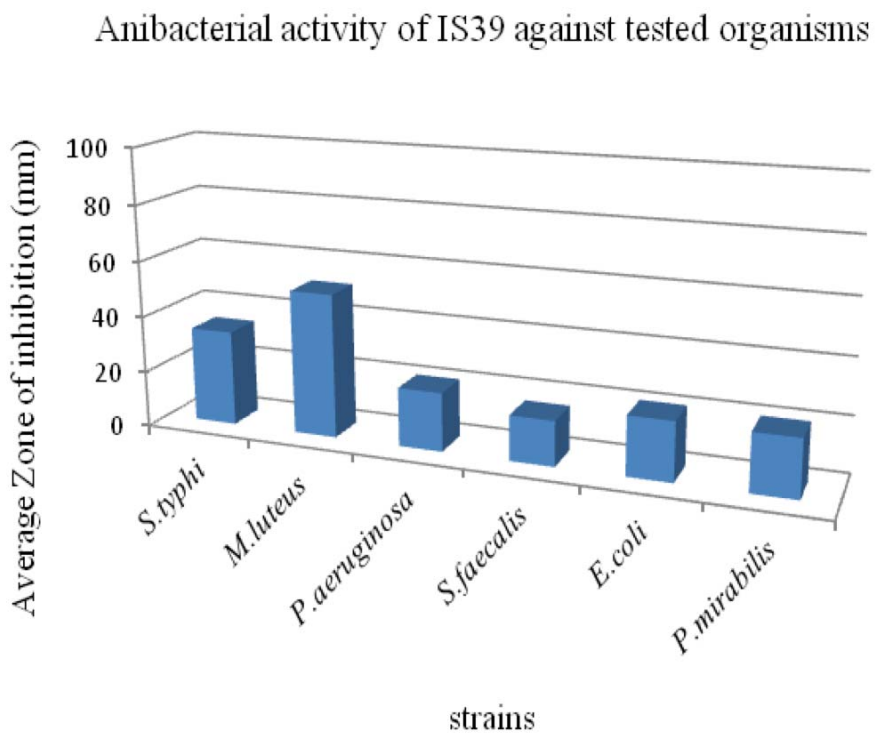

Figure 4: Antibacterial activity of IS39 against tested organisms.

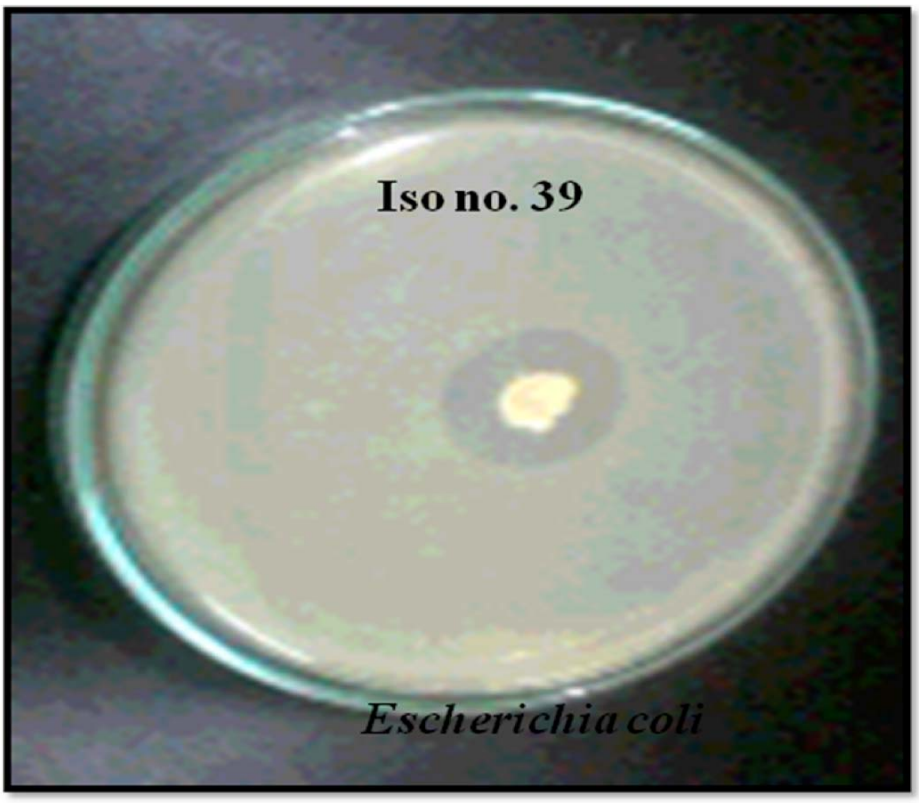

Figure 5: Determination of antibacterial activity of the isolate IS39 against *E. coli.*

inhibitory effects on both gram positive and gram negative bacteria. It was found that 58.9% of the isolates were active against the test bacterial cultures such as *E. coli, S. typhi, P. aeruginosa, S. aureus, S. epidermidis, B. subtilis,* and *M. luteus*. The great potential of marine actinomycetes and their antimicrobial metabolites can be studied upto molecular level in future studies.

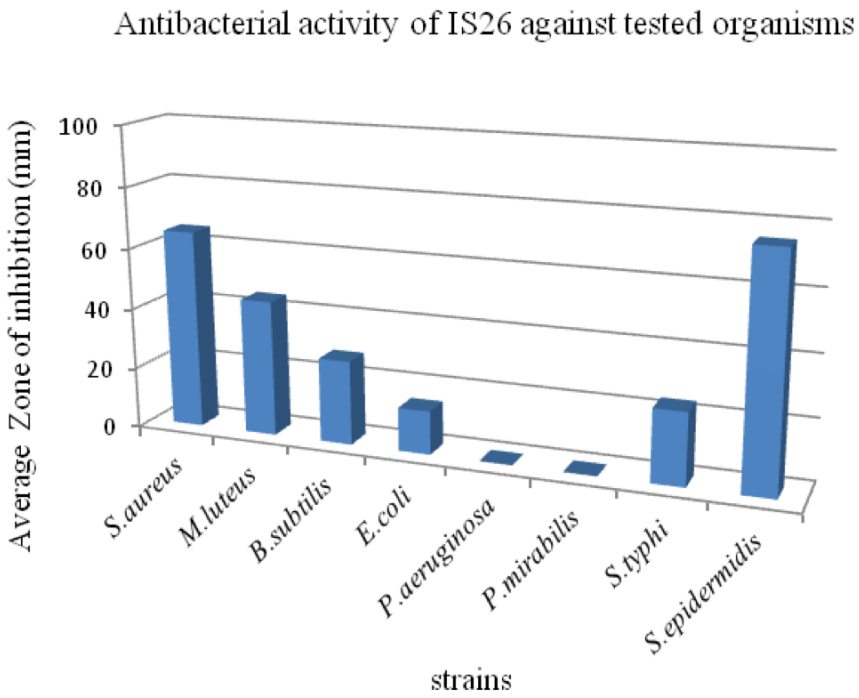

Figure 6: Antibacterial activity of IS26 against tested organisms.

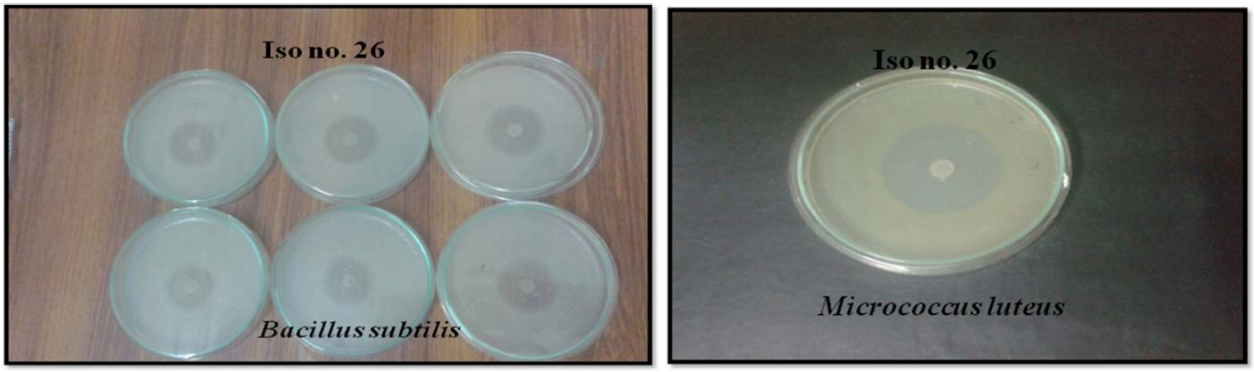

Figure 7: Antibacterial activity of IS26 against a. *B. subtilus* b. *M. luteus.*

REFERENCES

[1] David B, Wolfender JL, Dias DA. The pharmaceutical industry and natural products: historical status and new trends. Phytochem Rev 2014; 1-17.

[2] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012; 75(3): 311-335. http://dx.doi.org/10.1021/np200906s

[3] Demain AL. Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 2014; 41(2): 185-201. http://dx.doi.org/10.1007/s10295-013-1325-z

[4] Martinez JL, Baquero F. Emergence and spread of antibiotic resistance: setting a parameter space. Upsala J Med Sci 2014; 119(2): 68-77. http://dx.doi.org/10.3109/03009734.2014.901444

[5] Singh LS, Sharma H, Talukdar NC. Production of potent antimicrobial agent by actinomycete, Streptomyces sannanensis strain SU118 isolated from phoomdi in Loktak Lake of Manipur, India. BMC Microbiol 2014; 14(1): 278. http://dx.doi.org/10.1186/s12866-014-0278-3

[6] Berdy J. Bioactive microbial metabolites. J Antibiot 2005; 58(1): 1-26. http://dx.doi.org/10.1038/ja.2005.1

[7] Mahajan GB, Balachandran L. Antibacterial agents from actinomycetes-a review. Frontiers in bioscience (Elite edition) 2011; 4: 240-253.

[8] Ullah I, Arshad M, Chuadhry MJI, Noureen U, Jadoon WA, Jadoon MA. *Actinomycetes* screening for bioactive potential isolated from the moist forest soils of Pakistan. Rec Zool Surv Pakistan 2012; 21: 10-13.

[9] Hwang KS, Kim HU, Charusanti P, Palsson BO, Lee SY. Systems biology and biotechnology of *Streptomyces* species for the production of secondary metabolites. Biotechnol Adv 2014; 32(2): 255-268. http://dx.doi.org/10.1016/j.biotechadv.2013.10.008

[10] Duncan KR, Haltli B, Gill KA, Correa H, Berrue F, Kerr RG. Exploring the diversity and metabolic potential of actinomycetes from temperate marine sediments from Newfoundland, Canada. J Ind Microbiol Biotechnol 2014; 1-16.

[11] Park SH, Kwon KK, Lee DS, Lee HK. Morphological diversity of marine microorganisms on different isolation media. J Microbiol 2002; 40(2): 161-165.

[12] Taha MPM, Drew GH, Vestlund AT, Aldred D, Longhurst PJ, Pollard SJ. Enumerating actinomycetes in compost bioaerosols at source—use of soil compost agar to address plate 'masking'. Atmos Environ 2007; 41(22): 4759-4765. http://dx.doi.org/10.1016/j.atmosenv.2007.03.057

[13] Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). J Adv Pharm Tech Res 2013; 4(2): 118. http://dx.doi.org/10.4103/2231-4040.111528

[14] Ramazani A, Moradi S, Sorouri R, Javani S, Garshasbi M. Screening for antibacterial activity of streptomyces species isolated from zanjan province, Iran. Int J Pharm Chem Biol Sci 2013; 3(2): 342-349.

[15] Patel JD, Parmar M, Patel P, Rohit P, Taviyad R, Ansari P, Singh PK. Dynamism of Antimicrobial Activity of Actinomycetes—A Case Study from Undisturbed Microbial Niche. Adv Microbiol 2014; 4(06): 324. http://dx.doi.org/10.4236/aim.2014.46039

[16] Watanabe Y, Shinzato N, Fukatsu T. Isolation of Actinomycetes from Termites' Guts. Biosci Biotechnol Biochem 2003; 67: 1797-1801. http://dx.doi.org/10.1271/bbb.67.1797

[17] Gangwar M, Dogra S, Gupta UP, Kharwar RN. Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. Afr J Microbiol Res 2014; 8(2): 184-191. http://dx.doi.org/10.5897/AJMR2012.2452

[18] Kumar V, Naik B, Gusain O, Bisht GS. An actinomycete isolate from solitary wasp mud nest having strong antibacterial activity and kills the Candida cells due to the shrinkage and the cytosolic loss. Front Microbiol 2014; 5: 446. http://dx.doi.org/10.3389/fmicb.2014.00446

[19] Poosarla A, Krishna RM. Isolation of potent antibiotic producing Actinomycetes from marine sediments of Andaman and Nicobar Marine Islands. J Microbiol Antimicrob 2013; 5(1): 6-12. http://dx.doi.org/10.5897/JMA11.075

[20] Francis PM, Manickam R, Venkataesan BP. Crude protein extract of Actinobacteria exhibits antibacterial activity against *Salmonella typhi*. Int J Curr Microbiol App Sci 2014; 3(7): 319-326.

[21] Sarker AK, Haque MA, Rishikesh Ghosh DR, Islam MAU. Isolation, Characterization and Screening of Actinomycetes for Antibacterial Activity from the Soil of Gazipur, Bangladesh. WJPPS 2014.

[22] Arasu MV, Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activities of polyketide metabolite from marine Streptomyces sp. AP-123 and its cytotoxic effect. Chemosphere 2013; 90(2): 479-487. http://dx.doi.org/10.1016/j.chemosphere.2012.08.006

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