

STREPTOMYCETES FROM MARINE SEAWEEDS: THEIR ANTIMICROBIAL AND
ANTIBIOTIC POTENTIAL*¹Sridevi K and K Dhevendaran²¹Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom campus,
Thiruvananthapuram, India.²School of Chemical and Biotechnology, SASTRA University, Thanjavur*¹Corresponding author: kanaga_sridevi@yahoo.com; dheven.k@gmail.com

ABSTRACT: Recent research has increased our knowledge and understanding of the antimicrobial effect of marine *Streptomyces*. In a systematic screening effect, *Streptomyces* isolates from different marine seaweeds were investigated for antimicrobial activity against *Vibrio* pathogens (*V. harveyji*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*) and potentially active secondary metabolites. The different solvent extracts (Butanol, Ethyl acetate, Methanol, hexane) of selected isolates were screened for their antivibrio activity by disc method. The Zone of inhibition was measured in all extracts revealed a wide range of antimicrobial activity against pathogenic vibrios. The overall results of the antimicrobial activity indicates the abundance of purine riboside antibiotic in streptomycetes from marine seaweeds evidenced by the presence of precursor molecules such as guanosine, xanthosine in the extractions of *Streptomyces* which can be exploited for the production of purine riboside antibiotics which are in use of pharmaceutical industry.

Key words: Purine riboside antibiotics, Derivatives of antibiotics, Xanthosine, Guanosine, Seaweeds.

INTRODUCTION

Compared to freshwater environments, the marine environment of South India is rich in diversity of plants and microbes. The seaweeds are an untapped resources metabolite and drugs (Gaurav Rajauria and Nissreen Abu-Ghannam, 2013) However, the wealth of the seaweed-associated micro-flora has not been fully investigated till date (Ramesh, 2009). There are approximately 32,500 natural products reported from microbial sources, among 1000 derived from marine microbes (Singh and Pelaz, 2008). Natural compounds are the source of numerous therapeutic agents. The tremendous biochemical diversity of marine microorganisms and their biotechnological potential is becoming more and more recognized, not only by microbiologists, but also by the pharmaceutical industry (Thenmozhi *et al.*, 2010).

Most of the available antibiotics derived from actinomycetes and at present, Streptomycetes occupies the first position in producing a number of antibiotics in the actinomycetae (Waksman and Woodruff 1990). The actinomycetes are noteworthy as antibiotic producers, making three quarters of all known products. They are especially prolific and can produce biologically active secondary metabolites (Hopwood *et al.*, 2000). Beginning with the isolation of actinomycin and streptomycin (Waksman, 1940), they have received a phenomenal amount of attention. Thus actinomycetes are medically significant not so much of their pathogenic effect, which are relatively rare, but for their contributions to the control of other microbial pathogens. Isolation of new strains that have novel secondary metabolites is extremely important in today's industrial world; however, it is becoming very difficult to discover new bioactive compounds produced by these organisms (Piret and Demain, 1988). Members of marine actinomycetes, especially from seaweeds are poorly understood and only a few reports are available (Lakshmanaperumalsamy, 1978; Sivakumar, 2001, Vigneswary *et al.*, 1997; Prasheetha, 2008). The streptomycetes produce numerous chemical and biological active compounds, including antimicrobial, antibiotics, antiviral, enzymes, biofertilizers (Dhevendaran *et al.*, 2004), growth promoters and single cell protein (Dhevendaran *et al.*, 2008), antitumor agents and antibiotics (Be'rdy, 2005), which are widely used in the pharmaceutical industry.

MATERIALS AND METHODS

Sample collection

Muttom located on the south-west coast of India (latitudes 8° 7' 15" N and longitudes 77° 1'E) with luxuriant macroalgal growth. Live and healthy marine algae collected from the intertidal rocky surfaces of Muttom coast at monthly interval by random sampling method, and brought to the laboratory. Herbarium sheets and Photographed were prepared for the identification of seaweeds done by experts.

Isolation of Streptomyces from seaweeds

Seaweeds collected were washed with distilled water, exactly one gram of tissue taken and macerated using sterile mortar and pestle. Aliquots of 1ml serially diluted with sterile distilled water and plated on glycerol asparagine agar media. The plates were incubated at room temperature (28±2°C) for seven days. The number of colonies was expressed as CFU/gm of seaweeds. The isolates were stored in same agar slants at 28±2°C.

Phenotypic characterization of isolated strains

Bacterial strains, streptomycetes used in the study were characterized according to aerial and substrate mycelial production on different media, morphological characteristics, soluble and melanoid pigmentation and carbon utilization characteristics as described in Shrilling and Gottlieb (1966). Species level identification done by comparing the phenotypic properties with the representative species found in the key of Nonomura (1974) and Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974).

Preparation of sample of Antimicrobial activity:

The selected strains were grown in 250ml flask containing 50ml of FM medium. The flasks were inoculated with active culture and incubated at 28±2°C for 120hr. After sufficient growth, the contents of the each flask were extracted twice with n-Butanol and ethyl acetate (1-2.5v/v). The Butanol and ethyl acetate extracts containing bioactive components used for testing antimicrobial activity. Then the Butanol extracts were concentrated in water bath at 80°C. The concentrated residue was dissolved in methanol and also used as a sample for antimicrobial activity.

Antivibriostatic activity by disc diffusion method:

Antivibriostatic activity was carried out against four different Vibrio species by disc method. The assay system was prepared with Vibrio agar plates. The test culture was aseptically inoculated on the surface of the Vibrio agar plates and left to dry to be perfect. The crude culture extract of 1mg was dissolved in 1mL of the solvent from which 10µL (10µg) concentrations were taken and loaded on the disc using micropipette. The disc was aseptically transferred into petridish which was pre-seeded with Vibrio species. The plates were incubated 24hrs at 37°C.

Preparation of sample for GCMS analysis

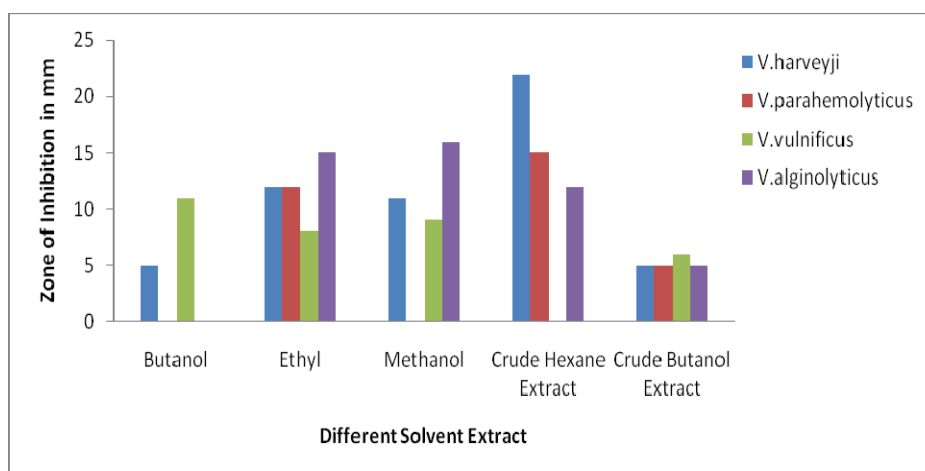
The selected strains were grown in 500ml Glycerol asparagine broth medium, incubated for 7days at 28±2°C. The cell mass was harvested by filtering. The harvested cell mass were homogenized with hexane. The filtrate was concentrated through the rotary evaporator at 80°C for 8-10hrs. 1mg of crude powder was dissolved in 1ml of hexane. The extract was filtered through a micro - filter with 0.4mm in diameter. The extract was stored at 4°C in airtight plastic vials for further studies.

Gas Chromatography – Mass Spectrometry (GC-MS) details

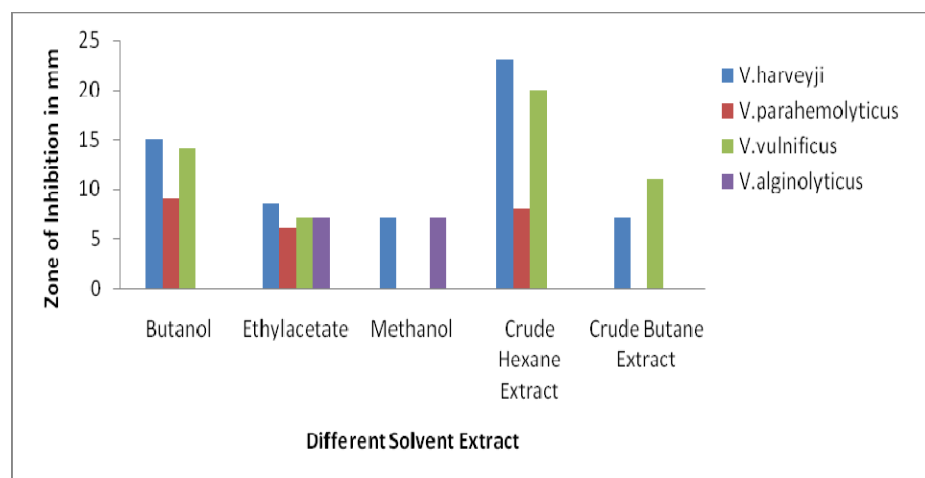
Description	Details
Instrument	Shimadzu GC-17A 2P5000
Carrier gas	Helium
Column	Diphenyl-dimethyl silofane
Sample injected	0.1ul
Flow rate	0.4ml/min
Oven temperature	70°C
Injested temperature	25°C
Deductor temperature	300°C

RESULTS

The isolation of *Streptomyces* was made by using different media by the different scientist. The present study, isolation was made by using medium consists of L-asparagine and it is considered as the potential source for isolating novel antibiotic producing strains. The selected strain AQB.SKKU 10 and AQB.SKKU37 shows rectiflexus morphology. The phenotypic characteristic of selected strains closely related to the *Streptomyces autotrophicus* (AQB.SKKU 10) and *Streptomyces nov.spp.* (AQB.SKKU37). Strains were subjected to an extraction process with different solvents like butanol, ethyl acetate, methanol, and hexane. The crude hexane extracts (10µg/ml conc.) showed remarkable antimicrobial activity with a zone of inhibition of 22mm against *V. harveyji* and 15mm against *V. parahemolyticus*, followed by ethyl acetate extract of *Streptomyces* active against *V. alginolyticus* (15mm), crude Butanol and Butanol extract showed moderate activity in the *Streptomyces* AQB.AKKU 10 (Graph 1). Comparison between four solvent extracts of *Streptomyces* AQB.AKKU 37 at 10µg/ml conc. of butanol, ethyl acetate, methanol and hexane extracts. In these four extracts, crude hexane extract showed more activity *V. harveyji* (23mm) and *V. vulnificus* (20mm). The methanol extract showed moderate activity against *V. harveyji* and *V. alginolyticus* (Graph 2). Statistical analysis revealed that there was no significant difference found. Gas chromatography Mass spectrum analysis was used for the chemical characterization of solvent extracted potential isolates (AQB.SKKU 10 & AQB.SKKU 37). The GC-MS results of active extracts revealed that the active principals were xanthosine in the 5th peak (Figure 1) and guanosine at peak 4 (Figure 3). The retention times of isolated compounds were 12.064 and 13.874 respectively (Table 1). The chemical structures of identified compounds were given in Figure 4 and Figure 6 respectively.



Graph: 1. Antimicrobial activity of Strain AQB.SKKU 10 against four *Vibrio* spp.



Graph: 2. Antimicrobial activity of Strain AQB.SKKU 37 against four *Vibrio* sp

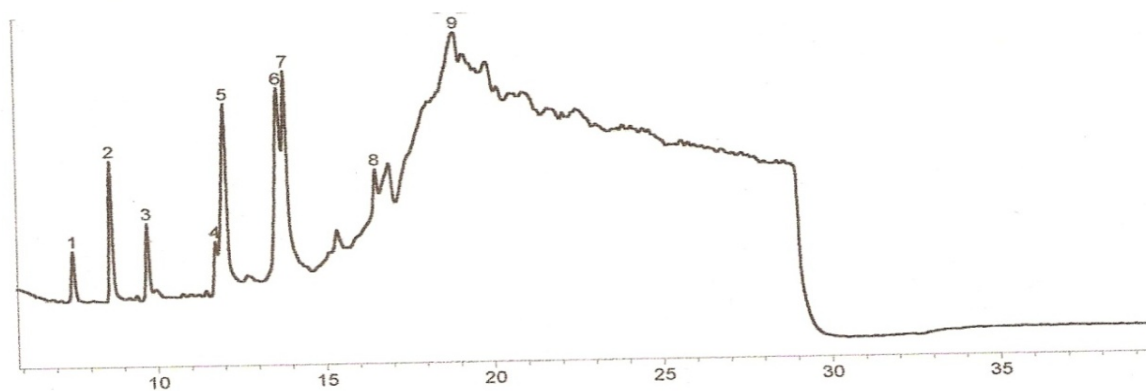


Figure 1. Represents the different peaks obtained from GCMS analysis of hexane extract of *Streptomyces* isolate AQB.SKKU10, peak 5 showing the representative compound

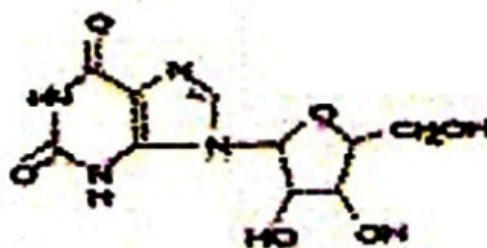


Figure 2. The chemical structure of the Xanthosine compound identified from *Streptomyces* AQB.SKKU 10

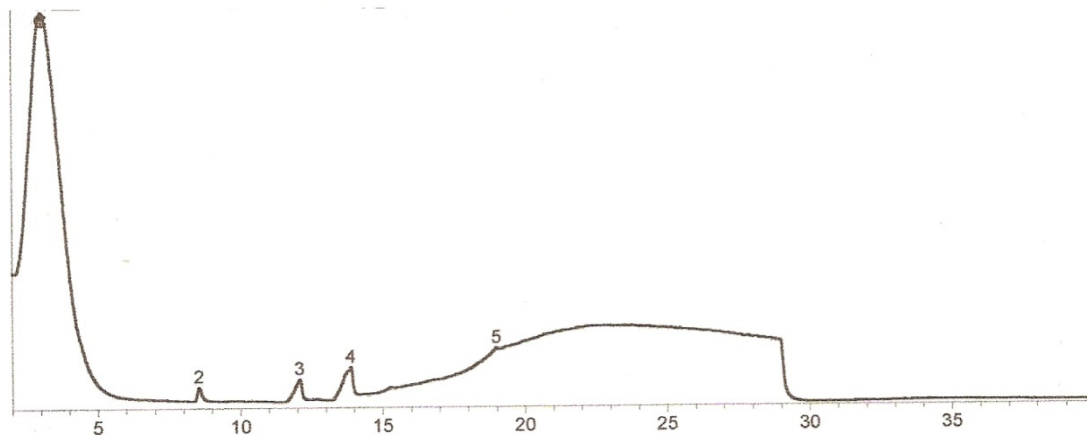


Figure 3. GCMS peaks showing different compounds separated from the hexane extract of *Streptomyces* isolate AQB.SKKU37

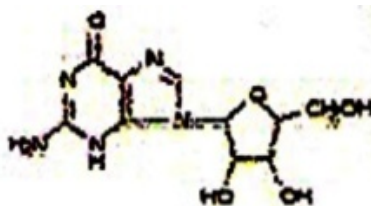


Figure 4. Chemical Structure of the identified compound Guanosine from Strain AQB.SKKU 37

Table 1. Details of Compound reported in *Streptomyces* strains

Strain No.	Retention time	Initiation time	Finishing Time	Area	Height AH(Sec)	AH (Sec)	MK %
AQB.SKKU10	12.064	11.833	12.367	25873749	2181774	11.859	23.61
AQB.SKKU37	13.874	13.217	14.158	87478245	3645583	23.996	3.09

DISCUSSION

The pharmaceutical industries gradually advance towards the chemical aspects of drug discovery for various diseases of humans as well as of fishes which includes both qualitative and quantitative analyses of the constituents. In the present study, Xanthosine, Guanosine derivative of flavor enhancing nucleotides were recovered. This finding was also an agreement with Schwartz and Margalith (2008), who found Xanathosine 5'-monophosphate, Inosine-5'-monophosphate and guanosine-5'-monophosphate from streptomycetes. Muhsin *et al.* (1996), investigated purine riboside antibiotic from *Streptomyces yokosukanensis* ATCC 25520, and he identified 14 purine bases, ribosides and ribotides. Inosine is a precursor of purine riboside antibiotic. They were purine xanthine, guanine, adenine, hypoxanthine, nebularine, xanthosine, guanosine, adenosine, and inosine, AMP, IMP, GMP and GDP. Therefore, the compounds identified in the present study are derivatives of purine riboside antibiotics.

CONCLUSION

The present study concluded that continuous isolation, screening of rare actinomycetes from unexploited source is the simple way to discover the novel drugs or bioactive metabolites. The hexane extract of *Streptomyces* showed better antimicrobial activity against pathogenic vibrios and also the producer of purine riboside antibiotic derivatives such as xanthosine, guanosine and are off-flavor producing compounds. So the present study also concludes that *Streptomyces* strain AQB.SKKU10 and AQB.SKKU37 isolated from seaweeds will be a potent source of purine riboside antibiotic. Further research should be made to molecular characterization of strains and identify the biosynthetic pathway of purine riboside antibiotic for pharmaceutical research.

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REFERENCES

- Berdy, J., (2005). "Bioactive microbial metabolites", *J. Antibiotics*, 58(1), pp.1-26.
- Buchanan, R.E and Gibbons, N.E., eds.(1974).Bergey's Manual of Determinative Bacteriology.8th ed. Williams & Wilkins Co., Baltimore, Md.21202.xxvi+1246pp.
- Dhevendaran, K., Shanmugam, R., Jasmine, A., Prasheetha, P.K and Anithakumari, Y.K. (2004). Studies on Streptomycetes associated with seaweeds with Kovalam coast. Kerala. Seaweed Res. Utilin: 26(1&2): 253-259.
- Dhevendaran, K., Sukumaran, M and Georgekutty, M.I. (2004). Growth hormone (IAA) producing bacteria and Streptomycetes and their biofertilizer effect on mangrove seedling, *Avicennia officinalis*, L., Marine Biotechnology, 6: 209-213.
- Gaurav Rajauria and Nissreen Abu-Ghannam (2013). Isolation and Partial Characterization of Bioactive Fucoxanthin from *Himan*. International Journal of Analytical Chemistry, Article ID 802573, 6 pages.
- Hopwood, D.A, Buttner M.J., Bibb M.J., Kiesser, T and Chater, K.F.(2000).Antibiotic production by *Streptomyces*. Pract. *Streptomyces* Genetics, 1:1-42.
- Lakshmanaperumalsamy P (1978). Studies on actinomycetes with special reference to antagonistic *streptomycetes* from sediments of Porto Novo coastal zone. Dissertation, Annamalai University, Chidambaram, India.
- Mushin KONUK, (1996).Inosine Metabolism by *Streptomyces yokosukanensis* ATCC 25520. Turkish Journal of Biology, 20: 235-239.
- Nonomura, H. (1974). Key for classification and identification of 458 species of the *Streptomycetes* included in ISP. Journal of Fermentation Technology 52(2): 78-92.
- Piret JM, Demain AL (1988). Actinomycetes in biotechnology In: Actinomycetes in Biotechnology. Academic Press, London, pp.461-482.a

- Ramesh, S. (2009). Marine actinomycetes diversity in Bay of Bengal, India: isolation and characterization of bioactive compounds from *Streptomyces fungicidicus* MML 1614. India. Dissertation, University of Madras, Chennai, India.
- Schwartz, J. Margalith, P. (2008). Production of flavor-enhancing Materials by *Streptomyces*: Screening for 5'-Nucleotides. *Journal of Applied Microbiology*, 34(2):347-359.
- Shirling EB, Gottlieb D (1966). Co-operative descriptions of type cultures of *Streptomyces*. The International *Streptomyces* Project. *International Journal of Systemic Bacteriology*, 17: 315-322.
- Singh SB, Pelaez F (2008). Biodiversity, chemical and drug discovery. *Progr Drug Res*, 65:143-74.
- Sivakumar K (2001). Actinomycetes of an Indian mangrove (Pichavaram) environment: An inventory. Dissertation, Annamalai University, Chidambaram, India.
- Thenmozhi M, Sindhura S, Kannabiran K (2010). Characterization of Antioxidant activity of *Streptomyces* species VITTK3 isolated from Puducherry Coast, India. *J Adv Sci Res.*, 1:46-52.
- Vikineswary S, Nadaraj P, Wong WH (1997). Balabaskaran S. Actinomycetes from a tropical mangrove ecosystem - Antifungal activity of selected strains. *Asia Pacific J Mol Biol Biotechnol*, 5:81-6.
- Waksman SA, Woodruff HB. (1990). Bacteriostatic and bactericidal substances produced by soil actinomycetes, *Biomedical Health Publication*, 45:609-614.
- Waksman SA (1940). On the classification of Actinomycetes. *Journal of Bacteriology*, 39: 549-558.