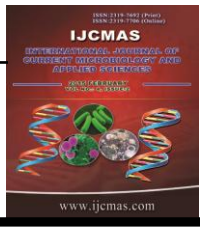
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 972-980

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 972-980**
http://www.ijcmas.com



Original Research Article

Screening of bioactive potential for larvicidal activity of marine actinomycetes

T.Vinodhkumar[2], M. Subhapriya[1], G.Ramanathan[1]*, J.Immanuel suresh[1,3] and Kalpana[1]

[1]Research Department of Microbiology, V.H.N.Senthilkumaranadar College, Virudhunagar- 626 001, Tamil Nadu, India
[2]Department of Microbiology, AVS College of Arts and science, Salem – 636106, Tamil Nadu, India
[3]Department of Microbiology, The American College, Madurai, Tamil Nadu, India
**Corresponding author*

Keywords

Actinomycetes, Antimicrobial, Larvicidal compounds, Microorganisms

A B S T R A C T

More than 70% of the earth's surface is covered by oceans and the microorganisms growing in marine environments are metabolically and physiologically diverse from terrestrial organisms. Microorganisms are a rich source of structurally unique bioactive substances. Since the 1940s, over 30,000 natural products have been discovered from microorganisms, more than 10,000 of which are biologically active. Recently, the rare of discovery of new compounds existing from terrestrial sources has decreased, while the rate of re-isolation of known compounds has increased. Moreover, the rise in the number of drug resistant pathogens and the limited success of strategies such as combinational chemistry providing new agents indicate an uncertain forecast for future antimicrobial therapy. Keeping these points in view, the present study has been undertaken to isolate and screen the larvicidal compounds producing marine Actinomycetes from salt pan region of Tuticorin. The South east coast of India and also an attempt has been made to characterize the different isolates by analyzing biochemical and larvicidal spectrum of marine Actinomycetes.

Introduction

More than 70% of the earth's surface is covered by oceans and the microorganisms growing in marine environments are metabolically and physiologically diverse from terrestrial organisms (Takizawa *et al*., 1993). Microorganisms are a rich source of structurally unique bioactive substances. Since the 1940s, over 30,000 natural products have been discovered from microorganisms, more than 10,000 of which are biologically active (Fenical, 2006). Microorganisms have a high ratio of surface area to volume, facilitating the rapid uptake of nutrients required to support high rates of metabolism and biosynthesis.

Actinomycetes are gram positive bacteria, with a high guanine (G) plus cytosine (C) ratio in their DNA (>55mol %), which are phylogenetically related from the evidence

of 16S ribosomal cataloguing and DNA: rRNA pairing studies. The name "Actinomycetes" was derived from Greek "atkis" (a ray) and "mykes" (fungus), and has features of both Bacteria and fungi (Das *et al*., 2008). Actinomycetes are soil organisms which have characteristics common to bacteria and fungi and yet possess sufficient distinctive features to delimit them into a distinct category. Actinomycetes population considered as one of the major group of soil population ,but after that it is being isolated from a diverse range of marine samples, including sediments obtained from deep-sea, even from greatest depth- Mariana Trench (Pathom-aree *et al*., 2006), and also in the vicinity of hydrothermal vents.

Actinomycetes have been isolated from different niches of the marine realm, such as mangroves and the deep sea. Earlier it was believed that marine actinomycetes were of terrestrial origin, however later it has become apparent that many are indigenous components of the marine world. Although the exploitation of marine actinomycetes as a source for novel secondary metabolites is in its infancy, the discovery rate of novel secondary metabolites from marine actinomycetes has recently surpassed that of their terrestrial counterparts, as evident by the isolation of many different diverse structures in the past few years. Some examples of particular interest are abyssomicin C, diazepinomicin and salinosporamides.

The potential for the isolation of novel secondary metabolites from marine actinomycetes relies on the ability to isolate novel actinomycetes from marine environments. Among microorganism, actinomycetes are one of the most attractive sources of antibiotics and other biologically active substance of high commercial value which from *Streptomyces spp*. Most of the known antibiotics from some species belonging to actinomycetes and many of them were isolated from genus *Streptomyces* for example 4-methyl aeruginoic was isolated from *Streptomyces* sp. Cineromycin and musains were isolated from *S.griseoviridis*, Nothramicin was isolated from *Nocardia*sp (Acebal *et al*., 1998). Recently, the rare of discovery of new compounds existing from terrestrial sources has decreased, while the rate of re-isolation of known compounds has increased. Moreover, the rise in the number of drug resistant pathogens and the limited success of strategies such as combinational chemistry providing new agents indicate an uncertain forecast for future antimicrobial therapy. The role of rare actinomycetes as active molecules source become apparent as these organism provided about 25% of the antibiotics of actinomycetes origin reported during 1975 to 1980.

Rare actinomycetes (non – *Streptomycetes*) have usually been regarded as strains of actinomycetes whose frequency is lower than that of *Streptomycetes* strains. Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes. Intensive screening program carried out over the past several decades resulted in the production of actinomycetes which are more abundant in terrestrial soils than in marine sediments showing varying degrees of salt tolerance and produce spores that are undoubtedly washed in large numbers from shore into the sea.

Actinomycetes are one of the most important groups of secondary metabolite

producers. Among various genera of actinomycetes, *Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora* and *Actinoplanes* are the major producers of commercially important biomolecules. The secondary metabolites produced by actinomycetes have a broad spectrum of biological activities; e.g. antibacterial (streptomycin, tetracycline, chloramphenicol), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin).

Today, an amazing amount of progress has been made in the control of plant diseases, but there is still much more for improvement. So, biological control of plant diseases is slow, gives few quick profits, long lasting, inexpensive and harmless to life. The marine soil of Tamil Nadu especially salt pan region of Tuticorin, South east coast of India has rich sources of potential microbial diversity. Nevertheless, they have not extensively explored for the registration of novel marine actinomycetes.

Keeping these points in view, the present study has been undertaken to isolate and screen the larvicidal compounds producing marine actinomycetes from salt pan region of Tuticorin. The South east coast of India and also an attempt has been made to characterize the different isolates by analyzing biochemical and larvicidal spectrum of marine actinomycetes.

Material and methods

Isolation and Identification of Marine actinomycetes from Salt pan Soil Sample

The first stage in the screening of microorganisms for not only antimicrobial metabolites but for any useful secondary metabolites is their isolation. In the present study, soil samples collected from salt pan region of south east coast of Tamilnadu were used to isolate marine actinomycetes. The samples were collected from top 4 cm soil profile. Soil sample (approx. 500g) was collected by using clean, dry and sterile polythene bags. The site selection was done by taking care of the point where widely varying characteristics as possible with regard to the organic matter, moisture content and particle size and color of soil and to avoid contamination as far as possible.

Identification of Marine Actinomycetes

Phenotypic characterizations

The classification of actinomycete was originally based largely upon the morphological observations. Several of the media suggested for the International *Streptomyces* Project have proven to be useful in our hands for the characterization of strains accessioned into the ARS Actinomycetales Culture Collection. It includes some basic tests. Aerial mass color, Reverse side pigment, Melanoid pigments spore chain morphology has been observed. The isolates were streaked over the plates containing International Streptomyces Project Medium 2 (ISP2). After incubation for 7-14 days, the colonies were observed for their pigment production. Growth characterizations were observed using different types of media such as Nutrient medium, Iron agar, ISP4andOat meal agar.

Microscopic observation

Slide culture technique (Spore chain morphology)

The sterilized cover slips were carefully inserted at an angle of about 45 degree into solidified starch casein nitrate agar medium, until about half of the cover slip was buried

in the agar medium. The isolates were inoculated along the line where the medium meets the upper surface of the cover slip. After incubation for 7 – 10 days, the cover slips was carefully removed and placed downwards on the slide and directly examined under the oil immersion (Williams, 1993). Purified isolates of marine actinomycetes were identified by comparing their morphological characteristics were described and MTCC Actinomycetes Manual (IMTEC, Chandigrah).

Mosquito larvicidal activity of marine actinomycetes metabolites

The marine actinomycete isolates obtained and formed the source for the present screening programme for mosquito larvicidal secondary metabolites. Marine actinomycetes secondary metabolites were obtained from the isolates and evaluated for their activity against the mosquito larvae.

Cultivation of actinomycetes

The organisms were individually grown in 500ml Erlenmeyer flasks containing 100 ml of ISP2 liquid medium, by inoculating loopful of culture from slant cultures. The Erlenmeyer flasks were allowed on a rotary shaker at 250 rpm and 280 +2 0 C for 7-21 days.

Harvesting of metabolites

The selected marine actinomycetes isolates were inoculated into a 500 ml conical flask containing 200 ml of starch casein liquid medium and shaken with 28 ± 2°C at 200 rpm for seven days. The pH was adjusted to 7.0 using 0.0lN NaOH or HC1. Ethyl acetate was added to the filtrate in the radio of 1:1 (v/v) and shaken vigorously for 1 hr for complete extraction. The ethyl acetate phase contain larvicidal substance was separated from the aqueous phase. It was evaporated to dryness in water bath. The cell free culture filtrates were used for larvicidal activity. The metabolites were evaluated for larvicidal activity against *Culexquinquefasciatus*, *Aedesaegypti*and *Anopheles stephensi*respectively.

Screening of larvicidal activity in Marine actinomycetes

Bioassay for the larvicidal activity was carried out using WHO (1996b) procedure with slight modifications. Six early second larvae of each three species were introduced in 250ml beaker containing 53 ml of 10-times diluted (100µl/ml) culture filtrate extract. Sterile distilled water (SDW) was used for making dilutions or solutions. A control was prepared by the addition of acetone to water. The beaker were held at 28 0 C + 2 0 C and the larvae were fed with a pinch of sterilized yeast powder and dog biscuit (1:1). Mortality was recorded after 24, 36, up to 48 hours and the larvicidal activity was observed for over 30 minutes and death of the larvae was conformed the larvicidal activity. Dead larvae were identified when they failed to move after probing with a needle. The experiments were replicated three times and conducted under laboratory conditions at 25–30°C and 80–90% relative humidity. The percentage mortality was calculated by using the formula (1), and corrections for mortality when necessary were done using Abbot's formula (2).

Larvicidal effect of Marine actinomycetes on vector Mosquitoes

The potent larvicidal actinomycetes were selected for further study. The different concentration of marine actinomycetes culture filtrate (2%) was taken 500ml beaker with 100ml of tap water. Mosquito (25

numbers) larvae were inoculated and incubated for 48 hours. The control was maintained free from marine actinomycetes culture filtrate. Every two hours larvicidal activity was observed and its percentage of larvicidal activity calculated and recorded.

Results and Discussion

Effects of temperature on hatchability of egg rafts

Egg rafts of *Aedesaegypti*were studied for the effects of temperature on their hatchability. It was observed that at low temperature the egg rafts can be stored for longer period without losing their viability.

Effects of temperature on larvae

Larvae of *Cule xquinquefasciatus* and *Anopheles stephensi* were studied for the effects of temperature on their tolerance. It was observed that room temperature the larvae can be stored for longer period without mortality. In under direct sun light the larvae can‟t be stored for more than three hours.

Salinity and pH tolerance of mosquito larvae

Culex quinquefasciatus and *Anopheles stephensi* larvae were observed to breed in natural habitats, where high salinity and low pH were observed. For the successful control of vector mosquito larvae, it is a need to study the salinity and pH tolerance of our desirable vector samples. The study revealed that the larvae are more tolerant.

Effects of solar UV-radiation on mosquito larvae

Second instar of each three species was exposed to solar UV radition at different duration. Immediately mortality of the larvae was observed when the larvae were exposed for a longer duration. Mortality within 24 hours was observed even at short duration of 30 minute exposure and maximum mortality was observed at the 6th hour of UV exposure.

Screening of larvicidal activity in marine actinomycetes

Of the 15 screened isolates of actinomycetes, 5 isolates showed the antilarval activity against 3 kinds of mosquito genera *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Fig 14). Among the 5 isolates, only 3 isolates had the potentially to inhibit (66.6%) the growth of mosquito larv ae. ISO7 and ISO11 had the potential capable to inhibit the growth of the *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* larvae. ISO2 showed the least capable to inhibition (33.3%) of the mosquito growth.

The diversity of marine antinomy cites has been of extraordinary significance in several areas of science & Medicines. The diversity rates of discover of larval drugs from established terrestrial sources have motivated the revaluation of new sources of chemically diverse bioactive compounds. Actinomycetes from marine samples have already undergone screening for novel metabolites and there is evidence those Actinomycetes usually make up only small portion of the bacterial flora of marine habitats, with absolute numbers of Actinomycetes much lower than in terrestrial habitat.

The secondary metabolites produced by halophilic microorganism have higher therapeutic potential. With less toxicity when was for mosquito borne disease currently represent a greater health problem in tropical and subtropical countries. No

parts of the world is extracellular secondary metabolites from Actinomycetes were screened form larvicidal activity against *Culex*, *Anopheles* and *Aedes*.

The total of 15 marine Actinomycetes with distinct characteristics were isolated from south east coast of Tamil Nadu. The study area was selected as Mullakadu saltpan which is situated tuticorin coast region of Tamil Nadu. In present study secondary metabolites form 5 isolated of Actinomycetes were screened form mosquito larvicidal activity against *C quinqnefacinatus, A. stephenx* and *A. aegypti*. The secondary metabolites from 5 Actinomycetes were larvicidal among the Actinomycetes isolates, the isolates name ISOT, ISOLL and ISO14.

Table: 1 Screening of larvicidal activity of marine actinomycetes bioactive compounds against *Anopheles stephensi*

Samples (Bio active compound)	Number of larvae	Time (Hr)	Number of *Anopheles* larvae died	% of death
ISO2	6	48	2	33.3%
ISO7	6	10	4	66.6%
ISO11	6	14	4	66.6%
ISO14	6	20	3	50.0%
ISO15	6	36	2	33.3%

Acknowledgement:

The authors are thankful to the authorities of V.H.N.S.N. College, virudhunagar, Tamil Nadu, India for providing required facilities to complete this work.

References

Acebal, C., R. Aleazar, M.Canedo, P.Rodringuez, FRomera, and Frenandez, J.L. 1998. Two marine agrobacterium produces of sesbanimide antibiotics. *J.of antibiotics*, 51:64-67.

Anderson, A.S. and E.M.H. Wellington, 2001. The taxonomy of Streptomyces and related genera. *Int J. Syst. Evol. Microbial* 51: 797-814.

Anupama, M. KJP. Narayana, andM. Vijayalakshmi, 2007. Screening of Streptomyces purpeofuscus for antimicrobial metabolites. Res. *J. Microbiol*. 4:1-3

Balagurunathan, R. 1992. Antagonistic actinomycetes from Indian shallow sea sediments with reference to a, ß unsaturated d -lactone, type of antibiotic from Streptomyces griseobrunncus Ph. D. thesis, Annamalai University, Tamil Nadu.; 33.

Balaraman, K. 1995. Mosquito control potential of *Bacillus thuringiensis* subsp. israelensis and *Bacillus sophaericus*, ICMR Bull., 25: 45.

Baltz, R.H. 2008. Renaissance in antibacterial discovery from actinomycetes. *Curr. Opin.Pharmacol.*,8: 1-7.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: pragmatic review, *J.Am. Mosq. Control.Assoc.* 2, 123–40.

Bull, A.,T.M. Good fellow and J.H. Slater, 1992. Biodiversity as a source of innovation in biotechnology. *Annual Review of Microbiology* 42: 219-257.

Campbell, W.C.,M.H. Pisher, E.O.Stapley, A.G. Schonber and T.A. Jacob, 1993. Ivermectin: a potent new antiparasitic agent. Science 221: 823-828.

Cragg, G.M., D.J. Newman and K.M. Snader, 1997. Natural products in Drug discovery and development. *J.Nat.Prod*, 60: 52-60.

Cross, T, 1997. Growth and examination of actinomycetes some guideline. In Bergery's Manual of Systematic Bacteriology Vol. 4: pp. 2340-2343.

Das, S. P.S. Lyla and S.A. Khan, 2008. Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal Chinese *Journal of Oceanology and Limnology* Vol. 26(2): 166-177.

Demain A. L. and S. Sanchez, 2009. Microbial drug discovery: 80 years of progress, The *Journal of Antibiotics*.62: 5–16.

Desphande, B.S., S.S. Ambedkar andJ.G. Shewale, 1988. Biologically active secondary metabolites from Streptomyces. Enzyme Microb. Technol, 10: 455-473.

Dhanasekaran D, G. Rajakumar, P. Sivamani, S. Selvamani, A. Paneerselvam and N. Thajuddin, 2005. Screening of salt pans actinomycetes for antibacterial agents. *Internet J Microbiol*1: 1-8.

Fenical, W. and Paul R Jensen. 2006. Developing a new resource for drug discovery: marine actinomycete bacteria, *Nature Chemical Biology*, 2: 666 - 673.

Fiedler,H.P. R. Kurth, J. Langhariq, J. Delzer and H. Zahner, 1982. Nikkomycina: microbial inhibitors of chitin aynthase. *J. Chem. Technol. Biotechnol*. 32: 271-280.

Forar, L.R. A. Norrya, and C. Bengraa, 2006b. Screening isolation and characterization of antifungal producing actinomycetes, Streptomyces strains. *Afr.J.Biol.Sci*.2: 73-82.

Getha, K. A. MihdIiham, S.S. Lee, Y.S.S. Chang, Nimura, and M. Hatsu, 2006. Exploratory studies of actinomycetes biodiversity of from forest in aid of drug discovery. *J.Antibiot*., 5: 67-71.

Ghanem, N.B. S. A. Sabry, Z. M. El-Sherif and G. A. Abu El-Ela, 2000. Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. *J. Gen. Appl. Microbiol*. 46: 105-111.

Haefner, B. 2003. Drugs from the deep: marine natural products as drug candidates. Drug Discov Today,8: 536-544.

Hayakawa, M. and H. Nonomura, 1987b. Humic acid vitamin acid, a new medium for the selective isolation of soil actinomycetes. *J.Ferment.technol*. 65: 501-509.

Hirsch, C.F., and L.Christensen, 1983. Novel methods for selective isolation of actinomycetes. *Appl. Environ. Microbial*. 46: 925-929.

Jensen PR, TJ. Mincer, PG. Williams,W. Fenical 2005. Marine actinomycete diversity and natural product discovery. *Antonie Van Leeuwenhoek*, 87: 43-48.

Jensen. P.R.,S. Dwight and W. Fenical, 1991. Distribution of actinomycetes in near shore tropical marine sediments. *Appl. Environ Microbiol*. 57:1102-1108.

Kanlyani Charoensopharat, Petcharat Thumma benjapone, PisanSirithorn and Sompong Thammasirirak. 2008. Antibacterial substance produced by Streptomyces sp. No. 87. In: *African Journal of Biotechnology* Vol. 7 (9), pp. 1362-1368.

Kitadokoro, K. et al., 1997. Eur. J. Biochem220 : 55-61. K. tadokoro, et al., 1997. Eur. J. Biochem.220 : 55-61.

Kumar,S.V. M.K. Sahu, and K. Kathiresan, 2005.Isolation and characterization of Streptomycetes producing antibiotics from a mangrove environment, *Asian Jr. of Microbial. Biotech Env.*Sc.Vol. 7 No. (3); 457-464.

Kumar, K.S.,R. Haritha, Y.S.Y.V. Mohan and T. Ramana, 2011. Screening of marine Actinobacteria for Antimicrobial Compounds, *Research Journal of Microbiology* 6(4): 385-393.

Lazzarini, A.,L. Cavaletti, G. Toppoand F. Marinelli, 2000. Rare genera of actinomycetes as potential producers of new antibiotics. Antonie van Leuwenhoek. 78: 399-405.

Masayuki, T.,R. Colwell and T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. *Appl. Environ.Microbiol*.59: 997-1002.

Mishra, S.K., R.E. Gordonand D. Barnett, 1980. Identification ofNocardia and Streptomycetes of Medical Importance. *J. Clin. Microbiol*.11: 728-736.

Mu J.J.,BH. Jiao, 2005. Research on antimicrobial activity of marine sulfate-reducing bacteria. J Dalian Railw Inst., 26:84–87.

Nisabet, L.J. 1982. Current strategies in the search for bioactive microbial metabolites. *J.Chem. Technol. Biotechnol*. 32: 251-270.

Pathom-aree, W., JEM.Stach, A.C. Ward, K.Horikoshi, A.T. Bull, M. Goodfellow, 2006, Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. Extremophiles ,10: 181-189.

Ponmurugan, P., C.Gopi and A. Maripandi, 2007. Studies on actinomycetes diversity in southern India tea soils for antifungal activity. *J. Plant. Crops*, 35: 28-32.

Rahman, M.Z.A., A.T.M.Z. Azam and M.A. Gafur, 2000. In vitro antibacterial principles of two flavonoids and extracts from Clerodendrum indicumlinn. Pak. *J. Biol. Sci*., 3: 1769-1771.

Remya, M. and R. Vijayakumar, 2008. Isolation and characterization of marine antagonistic actinomycetes from west coast of India.Med.Biol.15: 13-19.

Saadoun, I.,A. Muhana, 2008.Optimal production conditions, extraction, partial purification and characterization of inhibitory compound(s) producedby Streptomyces Ds-104 isolate against multi-drug resistantCandida albicans,Current Trends in Biotechnol and Pharma., 2 (2): 402 - 420.

Sathi, Z. S., A.A. Rahman and M.A. Gafur, 2001. Identification and invitro antimicrobial activity of a compounds isolated from Streptomyces species. *Pak. J. Bio Sci*.4: 1523-1525.

Sheikha, S., A. AL-Zarban, Azza, AL-

Musallam, H. Ibrahim, Abbas and A. YunusFasasi 2002. Note wothy salt loving actinomycetes from Kuwait. Kuwait. *J. Sci. Eng*. 29(1): P. 99-109.

Stach J.E.M., L.A. Maldonado, A.C. Ward, M. Goodfellow, A.T. Bull. 2003. Environ Microbiol. 5: 828-841.

Sultan, M.Z. Z. S. Khatune, S.A.M.D. Sathi, G.M. Bhuiyan, M.A.Sadik, M.A. .Choudury, N.A. Gafur, and A.A.M.D Rahman 2002. Invitro antibacterial activity of an active metabiolites isolated from Streptomyces species. *J. biotechnol*. 1: 100-106.

Sundarapandian, S, .M.D. Sundaram,P. Tholkappian and V. Balasubramanian, 2002. T. Taechowisan, S. Lumyong, 2009.Activity of endophytic actinomycetes from roots of Zingiber officinale and Alpiniagalanga against phytopathogenic fungi, Annals ofMicrobiol.,53(3): 291-298.

Takizawa, M. R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. *Appl. Environ. Microbiol*, 59: 997-1002.

Thenmozhi.M, and K. Kannabiran, 2010. Studies on Isolation, Classification and Phylogenetic Characterization of Novel Antifungal Streptomyces sp. VITSTK7 in India Current *Research Journal of Biological Sciences* 2(5): 306-312.