



ISSN 2320-3862

JMPS 2016; 4(2): 05-08

© 2015 JMPS

Received: 15-01-2016

Accepted: 18-02-2016

T Janaki

Department of Botany, K. M.
Centre for P. G. Studies
(Autonomous), Puducherry-8,
India.

BK Nayak

Department of Botany, K. M.
Centre for P. G. Studies
(Autonomous), Puducherry-8,
India.

T Ganesan

Department of Botany, K. M.
Centre for P. G. Studies
(Autonomous), Puducherry-8,
India.

Correspondence

T Janaki

Department of Botany, K. M.
Centre for P. G. Studies
(Autonomous), Puducherry-8,
India.

Journal of Medicinal Plants Studies

www.PlantsJournal.com

Antifungal activity of soil actinomycetes from the mangrove *Avicennia marina*

T Janaki, BK Nayak, T Ganesan

Abstract

Totally 25 actinomycetes were isolated by dry heat (70 °C) pre-treatment method on Starch casein agar media, from the soil sample nearer to the root region of the mangrove *Avicennia marina* from the back water area, Ariyankuppam, Puducherry (UT). All the 25 actinomycetes were subjected for primary screening against the test fungi by agar plug method. Broad spectrum antifungal activity was confirmed by cross streak method for selected antagonistic actinomycetes. Only 2 actinomycetes were selected from *A. marina* and that were subjected for secondary screening. Finally very active isolate M-20 was selected for further study.

Keywords: Dry heat treatment, Antifungal activity, Agar plug, Cross Streak, Well Diffusion method, *Avicennia marina*, Mangrove Back water area.

1. Introduction

The antibiotic substances elaborated by the actinomycetes display antibacterial, antifungal, anticancer, antiprotozoic and antiviral properties. Of the ten thousand known antibiotics produced by microbes over a decade ago, about 70% are of actinomycete origin: of them, representatives of the genus *Streptomyces* account for two thirds (Miyadoh, 1993) [18]. Actinomycetes are potent source of antibiotics, besides vitamins and enzymes, and such antagonistic actinomycetes of marine origin are being regularly reported (Krasilnikov, 1962; Okami *et al.*, 1976; Pisano *et al.*, 1986; Weyland and Helmke, 1988; Do *et al.*, 1991; Farooq Biabani *et al.*, 1997; Pusecker *et al.*, 1997; Romero *et al.*, 1997; Williams *et al.* 1999) [14, 21, 23, 33, 6, 7, 24, 27, 34]. Few reports that soil is a major source of actinomycetes (Sivakumar *et al.*, 2005; Vijayakumar *et al.*, 2007; Dhanasekaran *et al.*, 2008) [29, 30, 5]. Members of actinomycetes which live in marine environment are poorly understood and only few reports are available pertaining to actinomycetes from mangroves (Sivakumar, 2001; Vikineswari *et al.*, 1997; Rathna kala & Chandrika, 1993; Lakshmanaperumalsamy, 1978) [31, 25, 16]. Mangrove ecosystem is the most productive ecosystem diversified with variety of microbes (Kathiresan and Bingham, 2001) [12]. The search of new and novel antibiotics and other bioactive microbial metabolites is important for the fight against new emerging pathogens (Good fellow *et al.*, 1989; Berdy, 2005, Busti *et al.*, 2006) [8, 2, 3]. Isolation of actinomycetes from unique unexplored natural habitats is of interest to avoid re-isolation of strains that produce known bioactive metabolites. Neglected habitats are proving to be a good source of novel actinomycetes and bio active compounds.

The present investigation aims at finding better antifungal compound for controlling the fungal diseases of plants and man, with the help of mangrove soil actinomycetes of *Avicennia marina* (*Forsk.*). *Vierh - Avicenniaceae*, from the Ariyankuppam back water area, Puducherry, India.

2. Materials and methods

Collection of soil sample

The soil sample near root region of the mangrove plant, *Avicennia marina* (*Forsk.*) *Vierh - Avicenniaceae*) in Ariyankuppam back water estuary, Puducherry (Lat 11 °46'03'' to 11 °53'40'' North and Longi 79°49'45'' to 79°48'00'' East) was collected, packed in sterile plastic containers and transported immediately to the laboratory. The pH of the fresh soil sample was determined (Reed and cummings, 1945). Then the soil sample was air dried for 7-10 days at 40 °C, Crushed and sieved to remove the shells and debris and stored.

Soil analysis

Physio-chemical nature of soil sample was analysed in soil testing laboratory, Department of Agriculture, Puducherry, India.

Isolation of mangrove actinomycetes

The soil sample was subjected to dry heat (70 °C for 15 min) (Hayakawa *et al.*, 1991) [9] pretreatment to enhance the chances of isolating rare and novel actinomycetes. After pretreatment, one gram soil was mixed and serially diluted in sterile water blanks. 0.1 ml of last two dilutions (10⁻⁵ and 10⁻⁶) was inoculated by pour plate method (Zheng *et al.*, 2000) [35] using Starch casein agar (Kuster and Williams, 1964) [15] supplemented with Fluconazole 80 µg/ml and Nalidixic acid 75 µg/ml. Plates were incubated at 30 ± °C for up to 30 days. Plates were periodically examined for actinomycetes colonies. Selected colonies were transferred to Yeast Malt extract agar slants and maintained in the same medium.

Test organisms used in this study

The fungi used were *Colletotrichum capsici* (MTCC-3414), *Aspergillus fumigatus* (MTCC-3377), *Aspergillus niger* (MTCC-872), *Fusarium oxysporum* (MTCC-1755) and *Rhizoctonia solani* (MTCC-1236), procured from MTCC, Chandigarh. The following fungi- *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus-1, 2*, *Aspergillus terreus*, *Curvularia lunata* and *Colletotrichum gloeosporioides* were obtained from the laboratory collection.

Preparation of test fungi

Test fungi were maintained in potato dextrose broth and in PDA slants, pH-7. These were stored in refrigerator at 4°C for future use. 3-5 days old fungal liquid cultures and plate cultures were used for antifungal study

In vitro screening for antibacterial activity

Primary screening by agar plug method was studied by following Mohan raj *et al.*, 2011, Secondary screening by agar well diffusion method was done by using Murrey *et al.*, 1995 procedure and Cross streak method was studied by Lemos *et al.*, 1985.

Screening of selected actinomycetes for antifungal activity

Finally 12 most potent cultures were selected for antifungal activity screening by dual culture assay. From these, 4 were

selected for secondary screening in antifungal activity. Only one isolate M20 was selected for further characterization studies based on its antifungal activity.

Antimicrobial spectrum of active isolate M20

Antimicrobial activity of isolate M20 was tested with 12 multicellular fungi. Inhibition zones were noted down in millimeter.

3. Results and Discussion

The wet pH of mangrove sediment sample collected from *Avicennia marina* was 7.7. The following are the physico-chemical analysis of soil sample. The soil analysis results showed that there were very low available Nitrogen, P₂O₅ and Cu. Micro-Nutrients like Zn and Fe were high in their available form, Mn was medium.

Isolation and maintenance of actinomycetes

Totally 25 actinomycetes were isolated from soil sample of collected near *Avicennia marina* by after dry heat (70 °C for 15 min) pretreatment method. Dry heat method yielded bioactive actinomycetes for antimicrobial activity (Janaki *et al.*, 2015, Baskaran *et al.*, 2011) [1]. The isolated actinomycetes were subcultured in yeast malt extract agar-ISP2. Rare actinomycetes are considered as the strains whose isolation frequency by conventional methods is much lower than that of commonly occurring actinomycete strains. Subsequently, employing pretreatments of soil i.e. Drying and heating enhanced the isolation of rare actinomycetes. The great majority of antibiotics that have been isolated in numerous screening programs concerned with the search for new therapeutic agents have been tested primarily for their activity against different pathogenic microbes (Waksman *et al.*, 1952) [32]. Accordingly, some pathogenic fungi were procured from MTCC, Chandigarh for antifungal study.

The antifungal potential of isolate M20 was also observed in confirmatory test by direct streak method, dual culture plate method. The fungal test species in the order of decreasing sensitivity were *Alternaria alternata* (22 mm) > *Aspergillus sp-1* (20 mm) > *Colletotrichum gloeosporioides* (16 mm) > *Curvularia lunata* (12 mm) = *Rhizoctonia solani* (12 mm) = *Colletotrichum capsici* (12 mm) > *Fusarium oxysporum* (10 mm) = *Aspergillus sp-2* (10 mm) > *Aspergillus niger* (6 mm) = *Aspergillus flavus* (6 mm) = *Aspergillus fumigatus* (6 mm) = *Aspergillus terreus* (6 mm)

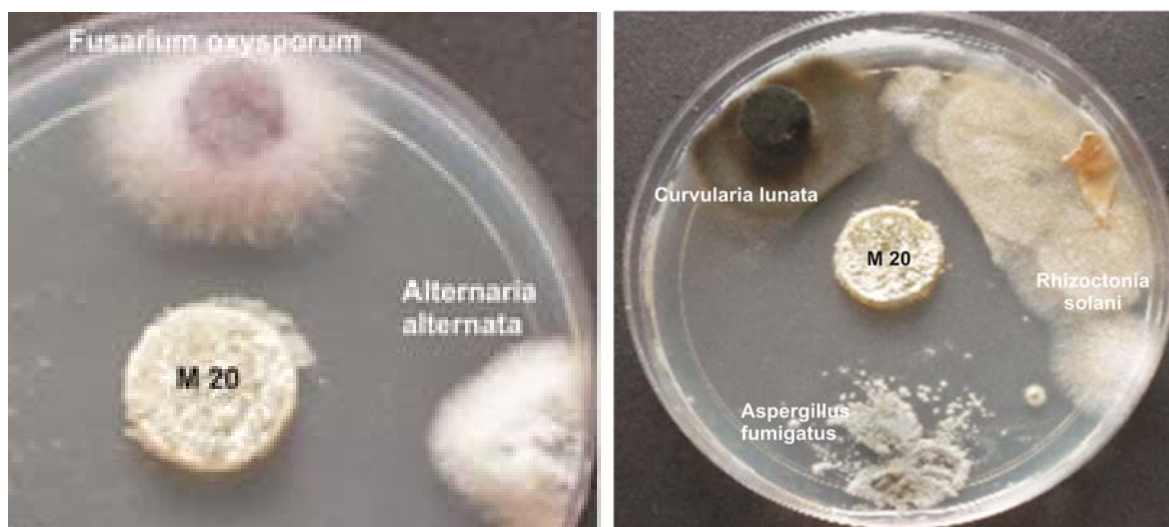


Plate 1: Antifungal activity of isolate M20 by agar plug method

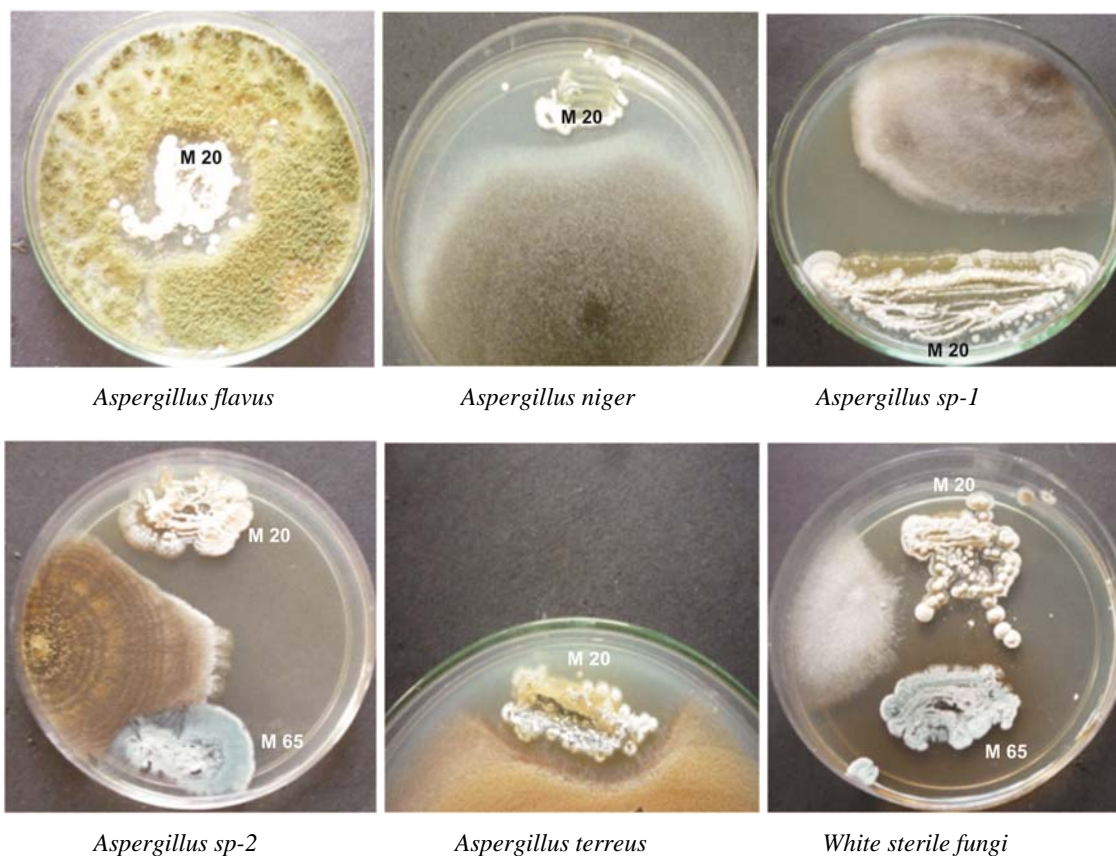


Plate 2: Antifungal activity of isolate M20 by direct streak method

Table 1: Antifungal spectrum of isolate M20

S.no	Test organisms used for antifungal activity	Inhibition in mm
Multicellular human fungi		
1	Curvularia lunata	12
2	Alternaria alternata	22
3	Aspergillus fumigatus	4
4	Aspergillus niger	6
5	Aspergillus flavus	6
6	Aspergillus sp-1	20
7	Aspergillus sp-2	10
8	Aspergillus terreus	6
Multicellular plant fungi		
9	Rhizoctonia solani	12
10	Colletotrichum capsici	12
11	Colletotrichum gloeosporioides	16
12	Fusarium oxysporum	10

The inhibition of multicellular fungi by isolate M20 was noted in the Table 1. The broad spectrum activity of the isolate M20 against the tested fungi (multicellular) was unique and better. Actinomycete-fungus antagonism has been demonstrated for a wide variety of plant pathogens such as *Alternaria sp.* (Chattopadhyay and Nandi, 1982) [4], *Rhizoctonia sp.* (Merriman *et al.*, 1974; Rothrock and Gottlieb, 1984) [28], *Macrophomina sp.* (Hussain *et al.*, 1990) [10], *H. oryzae* (Chattopadhyay and Nandi, 1982); *Pythium sp.* (Knauss 1976) [13], *Colletotrichum sp.* (Kathiresan *et al.*, 2005) [29] and *Curvularia sp.* (Paul and Banerjee, 1986) [22].

4. Conclusion

Mangrove actinomycetes are very potential in producing valuable secondary metabolites like antibiotics. Even though

lot of antifungal antibiotics available in the market, still the problem in controlling the fungal diseases not gets an end, this is because of drug resistance by pathogens. So, in order to overcome these problems, there is a need for searching new and newer compounds from the new habitats like mangroves.

5. References

- Baskaran R, Vijaya kumar R, Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from Mangrove sediments of Andaman Islands, India, Malaysian. J Microbiol. 2011; 7:26-32.
- Berdy J. Bioactive microbial metabolites, J Antibiot. 2005; 58(1):1-26.
- Busti E, Monciardini P, Cavaletti L, Bamonte R, Lazzarini A, Sosio M *et al.* Antibiotic producing ability by representatives of a newly discovered lineage of actinomycetes. *Microbiol* 2006; 152:675-683.
- Chattopadhyay SK, Nandi. Inhibition of *Helminthosporium oryzae* and *Alternaria* by *Streptomyces longisporus* (Krassilnikov) Waksman. *Plant Soil* 1982; 69:171-175.
- Dhanasekaran D, Panneerselvam A, Thajuddin N. An antifungal compound: 4' phenyl-1-naphthyl-phenyl acetamide from *Streptomyces* spp.DPTB16. *Facta Universitatis Series: Medicine and Biology* 2008; 15:7-12.
- Do HK, Kogure K, Imada C, Noguchi T, Ohwada K, Simidu U. Tetrodotoxin production of actinomycetes isolated from marine sediments. *J Appl. Bacteriol.* 1991; 70:464-468.
- Farooq Biabani MA, Laatsch D, Helmke E, Weyland H. Δ -Indomycinone: a new member of pluramycin class of antibiotics isolated from marine streptomyces sp. *J Antibiot.* 1997; 50:874-877.
- Goodfellow MO, Donnel AG. Search and Discovery of

- Industrially Significant Actinomycetes; in Baumberg S, Hunter I.S., and Rhodes P.M., eds., *Microbial Products: New Approaches*, Cambridge University Press, Cambridge, 1989, 343-383.
9. Hayakawa M, Sadaka T, Kayiura T, Nonomura H. New methods for the highly selective isolation Micromonospora and Microbispora. *Journal of Fermentation and Bioengineering*. 1991; 72:320-326.
 10. Hussain N, Ghaffar A, Aslam M. Biological control of Macrophomina phaseolina charcoal rot of sunflower and mung bean. *J Phytopathol*. 1990; 130:157-160.
 11. Janaki T, Nayak BK, Ganesan T. Different Pre-treatment methods in Selective Isolation of Actinomycetes from Mangrove sediments of Ariyankuppam, Back water Estuary, Puducherry. *Int. J Adv. Res. Biol. Sci*. 2014; 1 (6):154-163.
 12. Kathiresan NK, Bingham BL. *Biology of Mangroves and Mangrove Ecosystems*. *Advances in Marine Biology* 2001; 40:81-251.
 13. Knauss JS. In-vitro antagonistic activity of several Streptomyces spp. against species of Pythium and Phytophthora. *Pl. Dis. Repr*. 1976; 60:846-850.
 14. Krasil'nikov NA. Antibiotic properties of microorganisms isolated from various depths of world's oceans. *Microbiology* 1962; 30:545-550.
 15. Kuster E, Williams ST. Selection of media for isolation of streptomycetes, *Nature* 1964; 202:928-929.
 16. Lakshmanaperumalsamy P. Studies on actinomycetes with special reference to antagonistic *streptomycetes* from sediments of Porto Novo coastal zone. Ph.D. thesis, Annamalai University, India, 1978, 192.
 17. Lemos ML, Toranzo AE, Barja JL. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microbial Ecol* 1985; 11:149-163.
 18. Miyadoh S. Research on antibiotic screening in Japan over the last decade: a producing microorganism approach. *Actinomycetologica* 1993; 9:100-106.
 19. Mohanraj D, Bharathi S, Radhakrishnan M, Balagurunathan R. Bioprospecting of actinobacteria from Yelagiri hills with special reference to antibacterial activity. *J Chem. Pharm. Res*. 2011; 3(3):439- 446.
 20. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover HR. *Manual of Clinical Microbiology*, 6th Edition. ASM Press, Washington, DC, 1995, 15-18.
 21. Okami Y, Okazaki T, Kitahara T, Umezawa H. A new antibiotic aplasmomycin produced by a streptomycete isolated from shallow sea mud. *J Antibiot*. 1976; 29:1019-1025.
 22. Paul AK, Banerjee AK. In vitro effect of an antifungal antibiotic produced by *Streptomyces galbus* SME-13. *Hind. Antibiot. Bullet* 1986; 28:15-19.
 23. Pisano MA, Sommer MJ, Lopez MM. Applications of pretreatments for the isolation of bioactive actinomycetes from marine sediments. *Appl. Microbiol. Biotechnol* 1986; 25:285-288.
 24. Pusecker K, Laatsch H, Helmke E, Weyland H. Dihydropencomycin methyl ester, a new phenazine derivative from a marine streptomycete. *J Antibiot*. 1997; 50:47-483.
 25. Rathna Kala R, Chandrika V. Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. *Indian J mar. sci*. 1993; 22:297-299.
 26. Reed J, Cummings, F.S., Soil reaction-glass electrode and colorimetric methods for determining PH values of soil, *Soil Sci* 1945; 59:97-104.
 27. Romero F, Espieigo F, Baz JP, De Quesada TG, Gravalos D, De La Calle F *et al*. A new depsipeptide with anti-tumour activity produced by a marine micromonospora. *J Antibiot*. 1997; 50:734-737.
 28. Rothrock CS, Gottlieb D. Role of antibiosis in antagonism of *Streptomyces hygroscopicus* var *geldanus* to *Rhizoctonia solani* in soil. *Can. J Microbiol*. 1984; 30:1140-1147.
 29. Sivakumar K, Sahu M, Kathiresan K. Isolation and characterization of streptomycetes producing antibiotic from mangrove environment. *Asian Journal of Microbial Biotechnology and Environmental Science*. 2005; 7:457-764.
 30. Vijayakumar R, Muthukumar C, Thajuddin N, Pannerselvam A, Saravanamuthu R. Studies on the diversity of Actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica* 2007; 21:59-65.
 31. Vikineswary S, Nadaraj P, Wong WH, Balabaskaran S. Actinomycetes from a tropical mangrove ecosystem - Antifungal activity of selected strains. *Asia Pacific J Mol. Biol. Biotec*. 1997; 5(2):81-86.
 32. Waksman SA, Lechevalier HA, Romano AH, Raubitschek F. Antifungal antibiotics. *Bull.org. mond. sante. Bull. wld. hth. Org* 1952; 6:163-172.
 33. Weyland H, Helmke E. Actinomycetes in the marine environment. In: *The Biology of Actinomycetes*. Okami, Y., Beppu, T. and Nagamura H. (Eds.). Japan Scientific Society Press, Tokyo, 1988, 294.
 34. Williams DE, Berman VS, Ritacco FV, Maiese WM, Holyrines A, possible B. intermediates in staurosporine biosynthesis produced in culture by a marine actinomycete obtained from the North Atlantic ocean. *Tetrahedron Lett* 1999; 40:7171-7174.
 35. Zheng Z, Zeng W, Huang Y, Yang Z, Li J, Cai H *et al*. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. *FEMS Microbiol. Lett*. 2000; 188:87-91.