ISSN : 2348-8069 Int.J.Adv. Res.Biol.Sci.2014; 1(6):154-163

International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Different Pre-Treatment methods in Selective Isolation of Actinomycetes from Mangrove Sediments of Ariyankuppam Back water Estuary, Puducherry

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Abstract

Eight different pre-treatment methods and eight different media were used for the isolation of actinomycetes from mangrove sediments of Ariyankuppam, Puducherry, India. The two Sediment soil samples were taken nearer to the root region of *Rhizophora mucronata* mangrove plant from backwater estuary. The dried, sieved soil sample was studied for their physico-chemical nature. The soil sample was pre-treated by $CaCo_3$(0.3%), Phenol (1.5%), dryheat(70^oC), wet heat(50^oC),SDS(0.05g), electromagnetic waves (2460MHz,45sec), Centrifugation(1600xg, 20 min) and by No treatment. The eight different media were supplemented with Flucanazole 80µg/ml and Nalidixlic acid 75µg/ml. The mean actinomycetes population density in sediment sample with eight different media, eight different treatments were recorded. Totally 100 actinomycetes were isolated, out of these 14 were anaerobes. The isolated cultures were subcultured on PDA, SCA, NA,YME for analyzing their better growth, sporulation, duration of sporulation and for soluble pigments. Peptone Yeast Iron Extract Agar for analyzing their melanin pigments.

Keywords: Pre-Treatment, *Rhizophora mucronata*, and selective isolation

Introduction

Actinomycetes are Gram positive bacteria, with a high G+C ratio in their DNA(>55 mol%) which are phylogenetically related from the evidence of 16s ribosomal cataloguing and DNA: rRNA pairing studies (Good fellow and Willians 1983). The name actinomycetes was derived from “atkis”(a ray) and “mykes” (fungus), has features of both bacteria and fungi. Actinomycetes have gained prominence in recent years because of their potential for producing antibiotics (Kumar et al.,2005). 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). Recently marine derived actinomycetes have become recognized as a source of novel antibiotic and anticancerous agent with unusual structure and properties(Jensen et al., 2005).

Actinomycetes are known to produce chemically diverse compounds with a wide range of biological activities (Bredholt et al.,2008). Actinomycetes are potent sources of antibiotics, besides vitamins and enzymes, and such antagonistic actinomycetes of marine origin are being regularly reported (Krasil’nikov, 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). Few reports that soil is a major source of actinomycetes(Sivakumar et al., 2005; Vijayakumar et al.,2007; Dhanasekaran et al., 2008). Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world and especially significant for their role on the recycling of organic matter (Srinivasan et al.,1991). The search of new novel antibiotics and other bio active microbial metabolites is important for the fight against new

emerging pathogens(Good fellow et al.,1989,Berdy.J,2005, Busti et al.,2006). Isolation of actinomycetes from unique natural habitat is of interest to avoid re-isolation of strains that produce known bio active metabolites. Neglected habitats are proving to be a particularly good source of novel actinomycetes that produce bio active compounds. The pre treatment including enrichment, physical and selective media may be used to study the ecology of actinomycetes in natural habitats such as water or soil samples(Jensen et al.,2005). Since the mangrove ecosystem is a very good productive ecosystem enriches with both aerobic and anaerobic actinomycetes, the present study was undertaken to isolate the bio active actinomycets from mangrove sediments of Ariyankuppam back water estuary, Puducherry by using eight different pre treatment methods with eight different culture media.

Materials and Methods

Collection of mangrove sediments

A total of 2 sediment samples from root region of the mangrove plant *Rhizophora mucronata* in Ariyankuppam back water estuary, Puducherry (Lat 11º46´03'' to 11º53´40''North and Longi 79º49´45" to 79º48´00" East) were collected, mixed together in sterile plastic containers and transported immediately to the laboratory. The wet pH of the sediment sample was determined by using glass electrode pH meter described by Reed and Cummmings (1945). The sediment sample was air dried under room temperature for one week. The soil gravels, debris of plants, shells were removed, ground the sediment sample into fine power, sieved to remove big soil particles and stored the soil sample for further studies.

Physico chemical analysis of sediment sample

The air dried ground, sieved fine sediment sample was used for analyzing its available form of macro, micro elements, salinity, dry pH, nature of the soil. The physico-chemical analysis of soil sample was analyzed by the soil testing laboratory, Department of Agriculture, Puducherry.

Selective Isolation

Pre treatment of sediment soil sample

a. Calcium carbonate treatment

One gram of sediment soil sample was mixed with 0.3g of calcium carbonate. This mixture was wetted with 5ml of double sterilized distilled water and high relative humidity was maintained in the petridish by using sterilized wet filter paper in the upper plate of petridish and incubated it for 10 days. After the incubation period was over, the calcium carbonate treated soil was mixed with 9 ml of dis. Water and further dilutions were made upto10^{-6}. 10^{-6} dilution was taken for inoculation.

b. Phenol Treatment

One ml of soil suspension was taken from one gram of soil sample in 9 ml of sterile 0.85% of NaCl and it was added with 9 ml of 1.5% of phenol solution, it was incubated for 30 min at room temperature(Hayakawa et al.,1991). Serial dilutions 10^{-3} to 10^{-6} were diluted and dilution 10^{-6} was used for inoculation.

c. Wet Heat

One gram of soil in 10 ml of sterilized dis. water was heated in water bath at 50ºC for 6 min and cooled (Duangmal et al., 2005). Serial dilution was done up to 10^{-6}, dilution 10^{-6} was used for inoculation.

d. Centrifugation

One gram of soil in 10 ml sterilized dis. water was centrifuged for 20 min at the centrifugal force 1600xg and it was serially diluted up to 10^{-6}(Rehacek,1959). Final dilution was taken for inoculation.

e. Dry Heat

One gram of soil was dried for 15 min at 70ºC in an oven and diluted up to 10^{-6} dilution(Hayakawa et al.,1991). Final dilution was taken for inoculation.

f. Sodium dodecyl sulfate

10 gram of soil was mixed with 90 ml of sterilized dis. water. From this, 10 ml of soil suspension was taken and added to 90 ml of sterilized dis.water. In this, 6g of Yeast Extract, 0.05g of SDS was added, heated it for 20 min at 60ºC and cooled. From this, 1ml was taken for further dilutions from 10^{-2} to10^{-6}

g. Electromagnetic wave

One gram of soil in 10 ml dis. sterilized water in a test tube was irradiated with the micro wave oven at frequency of 2460MHz and a power setting at 100 watt for 45 sec.T his= pre treatment was adapted from Bulina et al.,(1997).

h. No Treatment

Soil suspension was serially diluted upto10^{-6} without any prior pre treatment.

Preparation of culture media

Eight different media were prepared and used for isolating the selective actinomycetes from the mangrove sediment soil sample. The media used were;

1.Humic acid vitamin agar
2. Water agar
3.Starch casein agar
4.Actinomycetes Isolation agar
5.Kuster's agar
6.Yeast malt extract agar
7. Peptone yeast extract iron agar
8. Arginine glycerol salt agar

Composition of Humic acid vitamin agar(Hayakawa &Nonomura,1987)

Humic acid-1g/l, Na_2H PO_4-0.5g/l, Kcl-1.7g/l, $MgSo_4.7H_2O$-50mg/l, $FeSo_4.7H_2O$-10mg/l , $CaCO_3$ 10mg/l, Agar-18g/l, Sea water-30%/l , PH-7.5. One ml of filter sterilized vitamin stock solution was amended in the medium after its sterilization by autoclaving was over and tempered.

Composition of Water agar(Shirling E.B and Gottlieb.D, 1966)

Yeast extract-100mg/l, Agar-20g/l, Dis.water-700ml, Sea water 300ml, pH-7.5

Composition of Starch casein agar(Kuster and Williams,1964)

Starch-10g/l, KNO_3-2g/l, Nacl-2g/l, K_2H PO_4-2g/l, $MgSo_4.7H_2O$-0.05g/l, $FeSo_4.7H_2O$-0.01g/l $CaCO_3$ - 0.02g/l, Caesin-0.30g/l, Agar-18g/l, Sea water -30%, dis.water-700ml, pH-7.5.

Composition of Actinomycetes Isolation agar(Himedia)

Sodium caseinate-2g/l, L-Asparagine-0.10g/l, Sodium propionate-4g/l, K_2HPO_4-0.5g/l, $MgSo_4.7H_2O$-0.1g/l, $FeSo_4$. $7H_2O$-0.001g/l , Agar-18g/l, Sea water -30%, dis.water-700ml, pH-7.5.

Composition of Kuster's Agar(Balagurunathan and Subramanian,1992) .

Glycerol-10g/l, KNO_3-2g/l, Nacl-2g/l, K_2HPO_4-2g/l, $MgSo_4.7H_2O$-0.02g/l, $FeSo_4.7H_2O$-0.01g/l , $CaCO_3$ - 0.02g/l, Caesin-0.30g/l, Agar-18g/l, Sea water -30%, dis.water-700ml, pH-7.5.

Composition of Yeast Malt Extract Agar(Pridham et al.,1957)

Yeast Extract-4g/l, Malt Extract-10g/l, Soya meal-30g/l, Agar-18g/l, Dis. Water-700ml, Sea water-300ml and pH-7.5.

Composition of Peptone Yeast Extract Iron Agar(Himedia)

Peptone-20g/l, Ferric ammonium citrate-0.5 g/l, K_2H PO_4-1g/l, $Na_2S_2O_35H_2O$-0.08g/l, Yeast Extract-1g/l, Agar-18g/l, Dis. Water-700ml, Sea water-300ml and pH-7.5.

Composition of Arginine-Glycerol Salt Agar(EL-Nakeeb, M.A,1961)

Arginine Monohydrochloride-1g/l, Glycerol-12.50g/l, Nacl-1g/l, K_2H PO_4-1g/l, $MgSo_4.7H_2O$-0.5g/l, $FeSo_4.7H_2O$-0.010g/l , $CuSo_4.5H2O$-0.001g/l, $ZnSo_4.7H2O$-0.001g/l, $MnSo_4.H2O$-0.001g/l, Agar-18g/l, Dis. Water-700ml, Sea water-300ml and pH-7.5.

After the eight different media were prepared by autoclaving at 120ºC,15 psi, they were allowed to cool up to 40ºC.The antibacterial antibiotic Nalidixlic acid 75µg/ml and antifungal antibiotic Flucanazole 80µg/ml were added in all the eight different media to minimize the bacterial and fungal contamination. Thirty two plates were plated for each treatment in eight different media, like that for all the 8 treatments in 8 different media, 256 plates were plated with antibiotics.The 64 plates which were plated without any antibiotics used as controls for each treatment in eight different media. Totally 320 plates were plated and plates were inoculated with soil serial dilution 10^{-6} from all the 8 treatments. pour plate method was followed for inoculation. The plates were incubated at 28±2ºC. Actinomycete colonies grown on petriplates were counted at regular intervals. All the morphologically different actinomycete were subcultured on SCA, ISP-2, PDA, NA and ISP6 agar slants for further investigation.

Colour morphology of the actinomycetes

Colour of the aerial mycelium of the subcultured actinomycetes were noted and recorded.

Soluble pigments

The soluble pigments produced by the actinomycetes were observed and the colours were noted down.

Melanoid pigments

Melanins are dark brown to black pigments of macromolecules formed by oxidative polymerization of phenol and/or indolic compounds, which widely exist in actinomycetes also. They showed a broad spectrum of biological roles. Greenish brown, brownish black or distinct brown, pigment modified by other colours on the medium also noted.

Results and discussion

The wet pH of mangrove sediment sample from *Rhizophora murcoanata* was 8.0. The following are the physico-chemical analysis of soil sample, analysed by the soil testing laboratory, Department of Agriculture, Puducherry.

The soil analysis results showed that there were very low available form of Nitrogen and P_2O_5 followed by low amount of Cu. Micro-Nutrients like Zn, Mn and Fe were high in their available form.

Selective Isolation

Eight different pre-treatments were used for selective isolation of actinomycetes. Of which, soil treated by Phenol(1.5%) yielded maximum of 19 colonies from eight different media followed by Dry Heat-17, SDS-17, Electromagnetic induction-17, Calcium carbonate 9, Centrifugation-7,Wet Heat-7 and No treatment-3. Totally 100 actinomycete cultures have been isolated from eight different media by eight different enrichment pre-treatments. Out of 100 isolates, 14 cultures not produce any spores for long time, they remain submerged in the medium, these may be anaerobic actinomycetes.

Influence of media composition on isolating selective actinomycetes

Fast appearance of well spore producing colonies were observed and isolated from Peptone Yeast extract iron agar(ISP_6) followed by Yeast malt extract agar (ISP_2). Some of the cultures were isolated within one week in Humic acid vitamin agar by the pre-treatment-Electromagnetic induction. Late appearance of colonies were observed in Water agar followed by Kuster's agar, Actinomycetes isolation agar and Arginine glycerol salt medium. Moderate duration 30-60 days have been taken by the actinomycetes for their appearance in Starch casein agar also observed and noted. Some cultures from Water agar still appearing in the plate(after one year of inoculation). Non spore producing colonies were observed in Water agar by Electromagnetic induction, SDS, Phenol treatments followed by Kuster's agar, Yeast malt extract aga, Actinomycetes isolation agar by Electromagnetic induction.

Colony size

Well developed colonies were observed in Peptone yeast extract iron agar followed by Yeast malt extract agar and Starch casein agar. Small and very small colonies were observed in Humic acid vitamin agar followed by Water agar. Since the delayed appearance of colonies in Arginine glycerol salt medium, Kuster's agar, most of the plates were contaminated by fungal and bacterial pathogens. From the No treatment method, only 3 isolates were isolated and the remaining plates were contaminated. The soil sample without any pre treatment allowed the bacterial and fungal pathogens to grow in the plates and did not allow the actinomycetes to grow in the plates. The present study reports that the different pre- treatments of soil and the different enrichment media allowed the isolation of maximum number of morphologically different actinomycetes and no treatment in soil sample allowed the isolation of very less number of actinomycetes with high level of contamination. Previously, this type of pre-treatment methods for isolation of actinomycetes has also been suggested by several researchers (Hayakawa and Nonomura, 1987; Hayakawa *et al*., 1991; Jensen *et al.*, 1991; Kim *et al.*, 1994; Seong *et al*., 2001 ; Bulina etal.,1997.,). Supplementation of antibiotics like Nalidixlic acid (75µg/ml) and Flucanazole(80µg/ml) also influenced the suppression of bacterial and fungal pathogens. In our study, totally 100 isolates were isolated from root region(nearer) of the mangrove *R.mucronata.* Maximum actinomycetes colonies were found on Starch casein agar(19CFU/ 10^{-6}), Peptone

Table-1. Physico-Chemical analysis of mangrove sediment sample from root region (nearer) of *Rhizophora mucronata* from Ariyankuppam backwater estuary

Parameter	Soil status
pH	7.9
E.C status	0.3
Lime status	Normal
Soil Texture	Clay-loamy
Macro-Nutrients	
Nitrogen	45.00(VL)
P_2O_5	0.56(VL)
K_2O	69(M)
Micro-Nutrients	
Cu	0.448(L)
Zn	2.7088(H)
Mn	8.702(H)
Fe	>8.1(H)

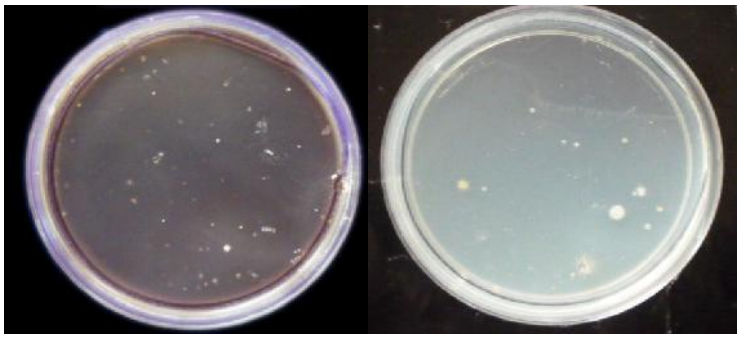
VL-Very low, L- Low, M-Medium, H- High

Table-2: Different Pre-Treatment methods in selective isolation of actinomycetes from mangrove *R. mucronata* sediment soil sample(nearer to root region)

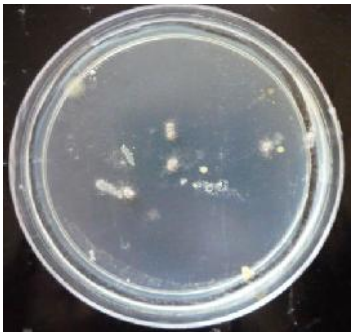
S.No	TREATMENT	MEDIA USED FOR ISOLATION								
		HVA	WA	SCA	AIA	KA	YME	PIYE	AGS	TOTAL
1	CALCIUM CARBONATE (0.3:1)g	-	1	3	1NS	1-NS	2	1	-	9
2	PHENOL(1.5%)	5	1	4	1-NS	1	4	2	1	19
3.	WET HEAT(50°C-6")	-	1-NS	1	-	1	1	2	1-NS	7
4.	CENTRIFUGATION 1600Xg-20"	1	1	1	-	2	3	3	-	11
5	DRY HEAT 70°C-15"	2	3	4	1	2	1-NS	3	1-NS	17
6.	SDS (0.05g)	1	4	3	1	1	2	4	1	17
7.	ELECTROMAGNETIC(2460,100W,45')	3	3+1 NS	2	1-NS	3-NS	1-NS	2	1-NS	17
8	NO TREATMENT	-	1	1	-	-	1	-	-	3
	TOTAL	12	16	19	5	11	15	17	5	100

HVA- Humic acid vitamin agar, WA-Water agar, SCA-Starch casein agar, AIA-Actinomycetes isolation agar, KA-Kuster's agar, YME-Yeast malt extract agar, PIYEA-Peptone iron yeast extract agar, AGS-Arginine glycerol salt medium and NS-Non sporulated

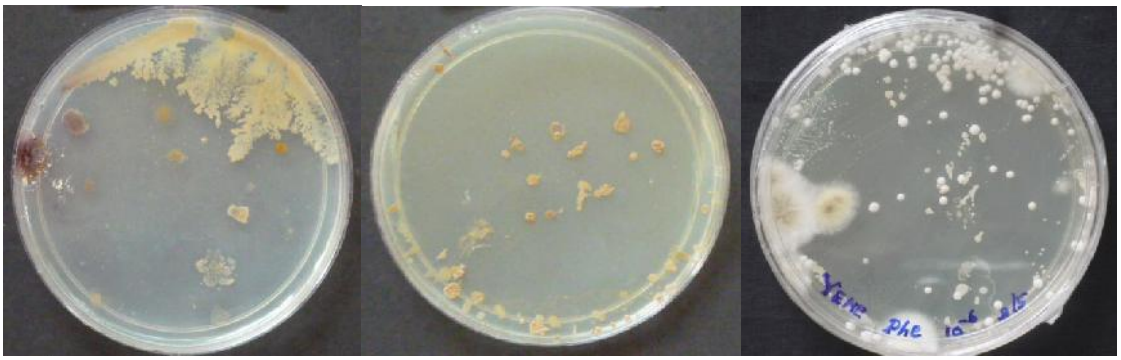
Figure-1. Plates show the different types of actinomycete by Phenol treatment on different media used for isolation.



a. HV-AGAR



b&c . WATER AGAR



d.STARCH CASEIN AGAR

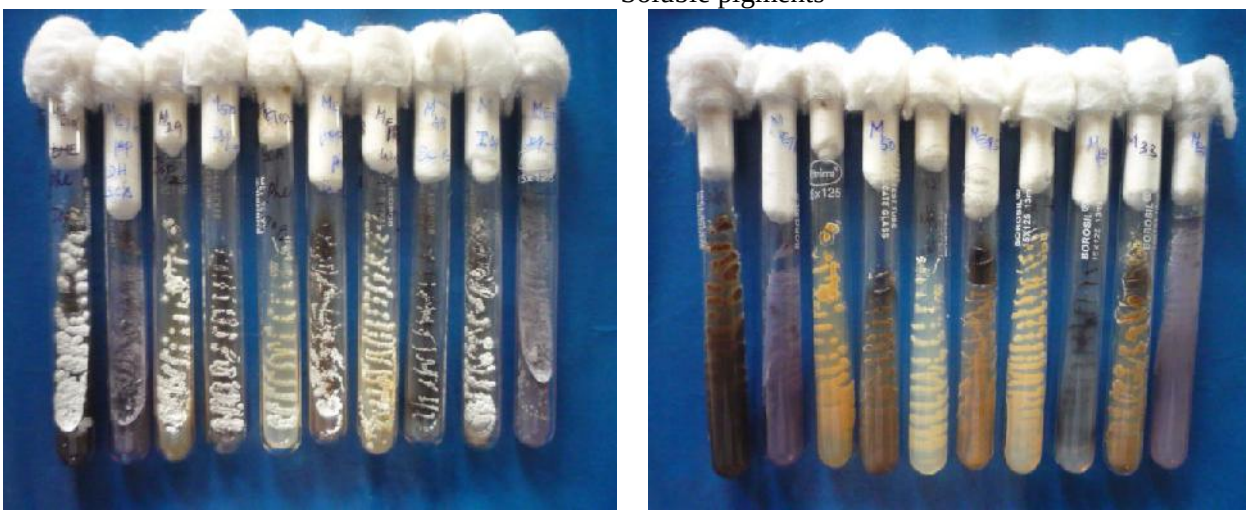
e&f. YEAST MALT EXTRACT AGAR

g&h. PEPTONE YEAST EXTRACT IRON AGAR

i.YEAST MALT EXTRACT AGAR

Figure-2. Soluble and Melanoid pigments

Soluble pigments



Melanoid pigments.

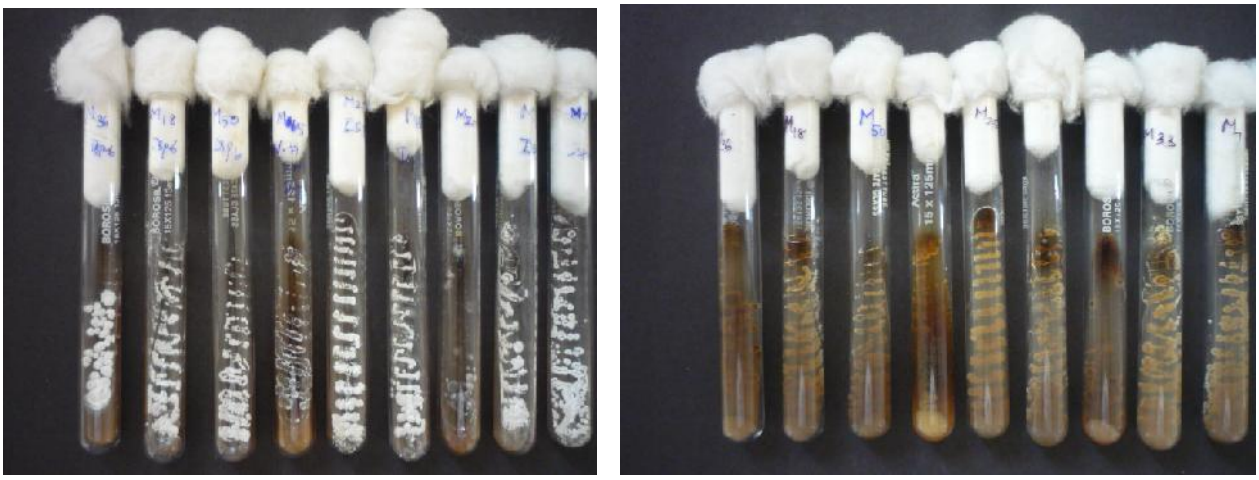
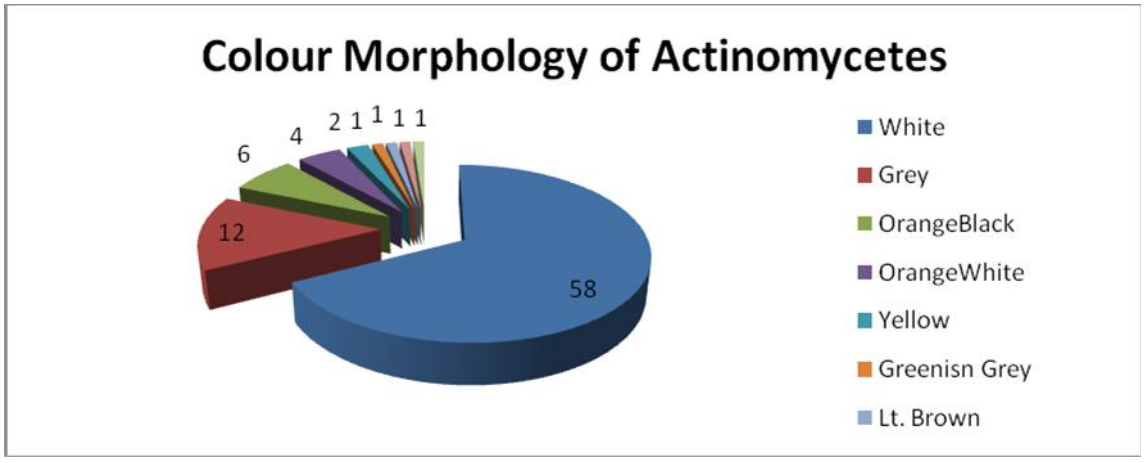


Figure-3. Colour Morphology of Actinomycetes



Yeast extract iron agar(17CFU/10^{-6}), Water agar(16CFU/10^{-6}), Yeast malt extract agar(15 CFU/10^{-6}), Humid acid vitamin agar(12CFU/10^{-6}), Kuster's agar(11CFU/10^{-6}), Actinomycetes isolation agar(5CFU/10^{-6}) and the Arginine Glycerol salt medium(5CFU/10-6). The actinomycetes which require less nutrients, emerged fastly in water agar, those that require high amount of nutrients appeared fastly in Starch casein, Peptone yeast extract iron agar fol lowed by Yeast Malt extract agar and Humic acid vitamin agar. Starch casein agar medium and the phenol treatment yielded the maximum number of actinomycetes.

SDS, Dry heat, Electromagnetic induction pre-treatments helped to isolate more or less same number of isolates but they are morphologically different. The actinomycetes isolated by Electromagnetic induction method were late spore producers above that they were sensitive to heat. Colouless transparent bulged actinomycete colonies were isolated from calcium carbonate treatment. Centrifugation method helped to isolate white coloured actinomycetes.

In the Wet heat treatment, the sample was heated only for 6 minutes so that the inoculated plates were contaminated by the bacteria, only 7 isolates were isolated from the Wet heat treatment. No treatment yielded only 3 isolates. From these it is clear that pre-treated soil with selective enriched media can yield maximum number of selective and rare actinomycetes.

Subculture of actinomycetes

4 different media-Starch casein agar, Yeast malt extract agar, Potato dextrose agar, Peptone yeast extract iron agar were used for subculturing of isolated isolates. Cultures on SCA, YME and PDA used for observing the soluble pigments and colour morphology. PYEIA was used for observing their melanoid pigment production. It was noted that only 10% of cultures produced soluble pigments and 18% of cultures produced melanoid pigments.

Soluble pigments

Colour of aerial mycelium of the actinomycetes were observed and noted. Out of 100 isolates, 14 were non spore producers with yellow, orange , pink, white morphology. They may come under Nocardias. 86 actinomycetes produced aerial spores with different colour morphology. Out of 86 spore producers, 58 were White(67.4%), 12 were Grey(13.9%),6 were Orange to black(6.9%), 4 were orange to white(4.7%), Yellow 2(2.3%), Greenish grey1(1.2%), light Brown 1(1.2%), Lavender1(1.2%) and purple pink 1(1.2%).

The isolates with different colours indicate that high degree of diversity in the backwater mangrove soil near the root region of the *Rhizophora mucronata* plant in Ariyankuppam. Colour grouping provides a valuable index of taxonomic diversity of actinomycetes in natural habitats(Antony babu et al.,2008)

Conclusion

It is an antibiotic resistant era by the human and plant disease causing pathogens. Microbiologists are searching for novel antibiotics to cure the human and plant diseases. Actinomycetes are most potent source for producing novel secondary metabolites like antibiotics, vitamins, enzymes, organic acids, antioxidants and anti-tumor substances. Considering this, there is a search for isolating novel actinomycetes from the neglected areas like mangrove back water estuary in Ariyankuppam is selected. Different coloured selective actinomycetes are isolated by using eight different pretreatment with eight different media. Phenol treatment followed by Dry heat, SDS and electromagnetic induction yielded more numbers of rare actinomycetes than other methods used here.

References

Antony-Babu S. and Goodfellow M., 2008. Biosystematics of alkaliphilic streptomycetes isolated from several locations across a beach and dune sand system, *Anton van Leeuwenhoek*. 94: 581-591.

Balagurunathan, R.1992. Antagonistic actinomycetes from Indian shallow sea sediments with reference to α, β - unsaturated γ - lactone type of antibiotic from *Streptomyces griseobrunneus*. Ph. D. Thesis, Annamalai University, India, 88 pp.

Berdy J., 2005. Bioactive microbial metabolites, *J. Antibiot.,* 58: 1-26.

Bredholt, H., Fjaervik, E., Jhonsen, G. and Zotechev,S. B. 2008. Actinomycetes from sediments in theTrondhein Fjrod, Norway: Diversity and biologicalactivity. *Journal of Marine Drugs* 6, 12-24.

Bulina T.I., Alferova I.V. and Terekhova L.P.,1997. A novel approach to isolation of actinomycetes involving irradiation of soil samples with microwaves, Microbiol., 66: 278-282.

Busti E., Monciardini P., Cavaletti L., Bamonte R., Lazzarini A., Sosio M. and Donadio S., 2006, Antibiotic producing ability by representatives of a newly discovered lineage of actinomycetes, *Microbiol*., 2006; 152: 675-683.

Dhanasekaran, D., Panneerselvam, A. and ThajuddinN. 2008. An antifungal compound: 4' phenyl-1-napthyl–phenyl acetamide from *Streptomyces* spp.DPTB16. *Facta Universitatis Series: Medicine and Biology* 15, 7-12**.**

Do,H.K., Kogure,K., Imada,C.,Noguchi, T., Ohwada,K., Simidu,U.,1991. Tetrodotoxin production of actinomycetes isolated from marine sediments. *J. Appl. Bacteril*. 70,464-468.

Duangmal K., Ward A.C. and Good fellow M.,2005. Selective isolation of members of the Streptomyces violaceoruber clade from soil, *FEMS Microbiol. Lett.*, 245,321-327.

EL-Nakeeb, M.A.1961. Selective isolation of aerobic actinomycetes. M.S. Thesis, Rutgers, The state University, New Brunswick,N.J.

Farooq Biabani, M.A., Laatsch, D., Helmke,E., Weyland,H., 1997. Δ-Indomycinone: a new memberof pluramycin class of antibiotics isolated from marine *streptomyces sp. J.Antibiot.*50,874-877.

Goodfellow, M. and Williams, E. 1983. Ecology of Actinomycetes. *Annual Review of Microbiology* 37,189–216.

Goodfellow M. and O'Donnel A.G.,1989. 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, 343-383.

Hayakawa M. and Nonomura H., 1987. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes, *J. Ferment. Technol.*, 66: 501-509.

Hayakawa, M., Sadaka, T., Kayiura, T. and Nonomura,H. 1991. New methods for the highly selective isolation *Micromonospora* and *Microbispora*. *Journal of Fermentation and Bioengineering* 72, 320-326.

Jensen, P. R., Dwight, R. and Finical, W. 1991. Distribution of Actinomycetes in near shore tropical marine sediments. *Journal of Applied Environmental Microbiology* 57, 1102-1108.

Jensen, P. R., Gontang, E., Mafnas, C., Mincer, T. J. and Fenical, W. 2005. Culturable marine Actinomycetes diversity from tropical Pacific Ocean sediments. *Applied and Environmental Microbiology* 7, 1039-1048.

Kim, C. M., Lec, K. H., Kwon, O. S., Shimazu, A. and Yoo I. D. 1994. Selective isolation of Actinomycetes by physical pre-treatment of soil sample. *Journal of Applied Environmental Microbial Biotechnology* 22, 222-225.

Krasil'nikov, N.A.,1962. Antibiotic properties of micro organisms isolated from various depths of world's oceans. *Microbiology* 30,545-550.

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

Kuster E. and Williams S.T.,1964. Selection of media for isolation of streptomycetes, *Nature*, 202: 928-929.

Lakshmanaperumalsamy, P. 1978. Studies on actinomycetes with special reference to antagonistic *streptomycetes* from sediments of Porto Novo coastal zone. Ph.D. thesis, Annamalai University, India, 192pp.

Okami,Y., Okazaki, T., Kitahera, T., Umezawa, H., 1976. A new antibiotic aplasmomycin produced by a streptomycete isolated from shallow sea mud. *J. Antibiot.* 29, 1019-1025.

Pisano, M.A., Sommer, M.J., Lopez, M.M., 1986. Applications of pretreatments for the isolation of bioactive actinomycetes from marine sediments. *Appl. Microbiol. Biotechnol*. 25, 285-288.

Pridham,T.G., Anderson, P., Foley, E., Lindenfelser.L.A., Hesseltine. E.W and Benedict, R.G,1957 . A selection of media for maintenance and taxonomic study of streptomyces. *Antibiot.*, 947-953.

Pusecker, K., Laatsch, H., Helmke,E., Weyland, H., 1997. Dihydropencomycin methyl ester, a new phenazine derivative from a marine streptomycete. *J. Antibiot.* 50, 47-483.

Rathna Kala, R. and Chandrika, V. 1993. Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. *Indian J. mar. sci*., **22**: 297 - 299.

Reed, J., Cummings, F.S., 1945. Soil reaction-glass electrode and colorimetric methods for determining PH values of soil, *Soil Sci.*, 59: 97-104.

Rehacek, z., 1959. Isolation of actinomycetes and determination of the number of their spores in soil. Microbiology USSR(English Transl.) 28;220-225.

Romero, F., Espliego, F., Baz, J.P., De Quesada, T.G., Gravalos, D., De La Calle, F., Fernandez-Puentes, J.L., 1997. Thiocoraline, a new depsipeptide with anti-tumour activity produced by a marine micromonospora. *J. Antibiot*. 50, 734-737.

Seong, C. H., Choi, J. H. and Baik, K. S. 2001. An improved selective isolation of rare actinomycetes from forest soil. *Journal of Microbiology* 17, 23-39.

Shirling, E. B. and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology* 16, 312-340.

Siva Kumar, K. (2001). Actinomycetes of an Indian Mangrove (Pichavaram) environment: An Inventory. Ph.D. thesis, Annamalai University, India, 91 pp.

Sivakumar, K., Sahu, M. and Kathiresan, K. 2005. Isolation and characterization of streptomycetes producing antibiotic from mangrove environment. *Asian Journal of Microbial Biotechnology and Environmental Science* 7, 457-764.

Srinivasan, M. C., Laxman R. S. and Deshpande M. V. (1991). Physiology and nutrition aspects of actinomycetes – An overview. *World Journal of Microbiol and Biotechnology* 7, 171-184.

Vijayakumar, R., Muthukumar, C., Thajuddin, N., Pannerselvam A. and Saravanamuthu R. 2007. Studies on the diversity of Actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica* 21, 59-65.

Vikineswary, S., Nadaraj, P., Wong, W.H. and Balabaskaran, S. 1997. Actinomycetes from a tropical mangrove ecosystem - Antifungal activity of selected strains. *Asia Pacific J. Mol. Biol. Biotec*., 5(2) : 81-86.

Weyland, H. and Helmke, E. 1988. Actinomycetes in the marine environment. *In:* The Biology of Actinomycetes. Okami, Y., Beppu, T. and Nagamura H. (eds.). Japan Scientific Society Press, Tokyo. pp.294.

Williams, D.E., Bernan,V.S., Ritacco, F.V., Maiese, W.M., 1999. Holyrines A and B, possible intermediates in staurosporine biosynthesis produced in culture by a marinr actinomycete obtained from the North Atlantic ocean. *Tetrahedron Lett.* 40, 7171-7174.

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