Malaysian Journal of Microbiology, Vol 3(2) 2007, pp. 15-18
http://dx.doi.org/10.21161/mjm.00907

Useful extracellular activity of bacteria isolated from Bhitarkanika mangrove ecosystem of Orissa coast

Gupta, N., * Das, S. and Basak, U.C.

Microbiology Laboratory, Regional Plant Resource Centre,
Bhubaneswar – 751 015 (Orissa).
E-mail: nguc2003@ yahoo.co.in

ABSTRACT

This paper describes the isolation of bacteria from Bhitarkanika mangrove ecosystem of Orissa and screening for their antifungal properties against five pathogenic fungi, extracellular enzyme activity like amylase, protease and lipase, and phosphate solubilization capacity. From 567 bacterial isolates obtained, 26 bacterial isolates have exhibited wide spectrum antifungal activity against all five fungi tested. Most of the bacterial isolates were found to be amylase and protease producers. In present study, 41 lipase producers and 33 phosphate solubilisers were also found. Bacterial isolates from plant origin exhibited all the four extracellular enzyme activity except lipase. The maximum % occurrence of phosphate solubilisers was found in soil and plant system of mangrove. This screening study opens an avenue to work with some of the potent strains for useful product formation at large scale.

Keywords: Mangrove, Phyllosphere, Bacteria, lipase, phosphate solubilization, antifungal, Extracellular enzymes

INTRODUCTION

The microbial metabolism at intracellular and extracellular level is gaining much importance to provide various useful products of industrial importance viz. enzymes, sugars, antibiotics and organic acids etc. Such diversified microbial systems are also reported from mangrove ecosystem (Christophersen, 1999; Kathirasen and Selvam, 2006). Mangroves, over millions of years, have evolved both morphologically and physiologically to adapt to swampy and saline environments. Similar adaptive characteristics in the form and function may occur with the associated microflora in such environments. Some reports are available on the occurrence of free living and symbiotic microorganisms in such saline habitats. However, information on the microbial flora of Bhitarkanika mangrove ecosystem is scanty. Keeping this in view, the present study was carried out for evaluation of extracellular activities of the microbial diversity in the Bhitarkanika mangrove ecosystem of Orissa coast.

MATERIALS AND METHODS

Study sites

The study was carried out in the Bhitarkanika mangrove forests of Orissa coast which is located on the East Coast of India (20° 4' 20° 8' N latitude and 86° 45' 87° 5' E Longitude) and extends up to the northeastern part of the Mahanadi delta in the Kendrapara district of Orissa. Three distinct seasons can be recognized while the temperature has an average minimum and maximum of 18° C and 33° C respectively. The maximum temperature recorded was 43°C. The average number of rainy days was between 70 to 96 with most rains occurring in the monsoon seasons between July and September. The area is prone to severe cyclones. Tides are semi-diurnal in nature with an altitude of 2-3 m in upstream areas and 3.5-6.0 m near the river mouth. Due to regular inundations through tidal action the soil is highly slushy. The surface soil is composed of silt and clayey loam and is about 3-4 m in depth. The soil though well-aerated, is saline. In the elevated areas away from the creeks and channels, the soil is more sandy and comparatively less moist and saline. The soil pH varies from 6.3 - 7.1

Collection of samples

Location
Soil samples were collected from different locations including outer estuaries, inner estuaries, creeks at lower elevation, creeks at higher elevation and transitional zones

Seasons
Samples were collected thrice in a year for three consecutive years to record data on the seasonal variations.

Source material
Various types of soil and roots of mangrove plant species growing in Bhitarkanika mangrove ecosystem served as source material.

Isolation of Bacteria

Bacteria from different sources were isolated by dilution plate technique by using media of different pH and composition.

***Corresponding author**

Source of Test fungi

The five test pathogenic fungi namely Black mold (*Chalaroprirthielavioides* sp.), Common canker (*Coninothyrium fuckelli*), Dieback (Nectria *cinnabarina*), Verticillium wilt (*Verticillium albo-atrum*) and *Fusarium* sp. used in this study were obtained from culture collection of Microbiology Laboratory of Regional Plant Resource Centre. These fungi coded as BM, CC, VW and DB, *Fusarium*, respectively in the results and discussion.

Analysis of antifungal activity

Co-plating method was used for inoculation and evaluation of antifungal activity of all bacterial isolates against test fungi (Sood, 1991)

Analysis of extracellular enzymes

The three enzymes i. e. amylase, protease and lipase were analyzed through plate test and qualitative method by growing these individual bacterial isolates in Starch casein (amylase), Gelatin agar (Protease) and peptone agar media (lipase). After seven days incubation at 30 ºC and/or 37 ºC, culture plates were tested for enzyme activity by adding iodine solution in amylase plates, HgCl2 (10%) in protease plates. The clear zone formation around the growing colony was considered as positive. The lipase activity of bacterial isolates was determined on lipase test medium and the formation of opaque whitish zone around the growing colony was considered as positive (Booth, 1978).

Analysis of phosphate solubilization

The phosphate solubilization capacity was determined qualitatively by growing bacterial isolates on Pikovaskaya medium of 7.2 (Chung *et al*., 2005) and the halo zone formation around the growing colony was treated as positive.

RESULTS AND DISCUSSION

Antifungal activity

Total of 567 bacterial isolates were isolated from different sources of mangrove plants area. Like air (110 isolates), water (162 isolates), soil (53 isolates) and Plants (242 isolates). Evaluation test for their activity against fungi exhibited wide spectrum performance where 26 bacterial isolates showed the activity against all four pathogens tested. It is interesting to note that more or less similar number of bacterial isolates ranged 110-129 were found to be active against four fungi CC, BM, DB and VW where 144 no. of bacterial isolates were exhibited antifungal activity against *Fusarium*. It was quite obvious that air bacteria also showed antifungal activity. The differences in antifungal performance among the different groups of bacteria that isolated as per the source materials indicated the presence of biological competition in mangrove ecosystem. The more number of bacteria in population revealed the more competitive metabolism and physiological versatility.

The antifungal activity of 26 selected bacterial isolates against individual pathogenic fungi presented in Figure 1 A-E. B5 and B6 exhibited highest zone of inhibition i. e. 7 and 8 mm against black mold fungi (Fig. A). The dieback pathogen fungi was also found to be inhibited by 14 bacterial isolates (Fig. B). Similarly, B8 exhibited highest activity against VW whereas B42 and 43 shoed maximum zone of inhibition against CC fungi i. e. 6 mm (Fig. C and D) Four bacteria namely, 11, 18, 23 and 26 have shown 5 mm inhibition zone against fusarium sp. (Fig. E). It is very surprising to observe that all 26 bacteria were active against all 5 pathogen fungi. In spite of similarity in their activity, differences were recorded for their potentials like B7 and 8 that sowed higher zone of inhibition could not inhibit other fungi in similar way.

We found that several bacteria among the 26 bacterial isolates showed the wide spectrum of antifungal activity but their performance was quite poor. The occurrence of antifungal activity against all the two pathogen by those bacteria may be due to the production of similar kind of antifungal compound against one pathogen and /or all pathogens. Different observations in formation of different inhibition zone may be due to the potency of different and/or same antifungal metabolite. However, with this preliminary study we found that 26 bacterial isolates were active against all 5 pathogens tested and showed wide spectrum of antifungal activity. That highest % occurrence showed by plant bacteria against 41-32 against *Fusarium* followed by air bacteria active against dieback pathogen (Figure 2) Very low % of occurrence of bacteria was found with antifungal activity in water samples of mangrove area. Where as a good number of bacteria was observed as antifungal from the air samples.

Very rare reports are available on the mangrove lipases, phosphate solubiliser. In our study, maximum bacterial isolates obtained from plant system and air was found to be extracellular enzyme producers especially amylase and protease (Figure 3). The phosphate solubilizing capacity was observed more in soil bacteria and plants than those other sources. The maximum % of bacteria was found to be lipase producer that were obtained from air, water, soil, except plant. Similarly, phosphate solubilization bacteria were found to be more in number from soil and plant system. The important phosphate solubiliser is self explanatory which can be used as bioinoculant and potential tool for and mangrove reforestation (Bashan *et al*., 2000; Bashan and Holguin, 2003; Kaitheresan and Salvam, 2006).

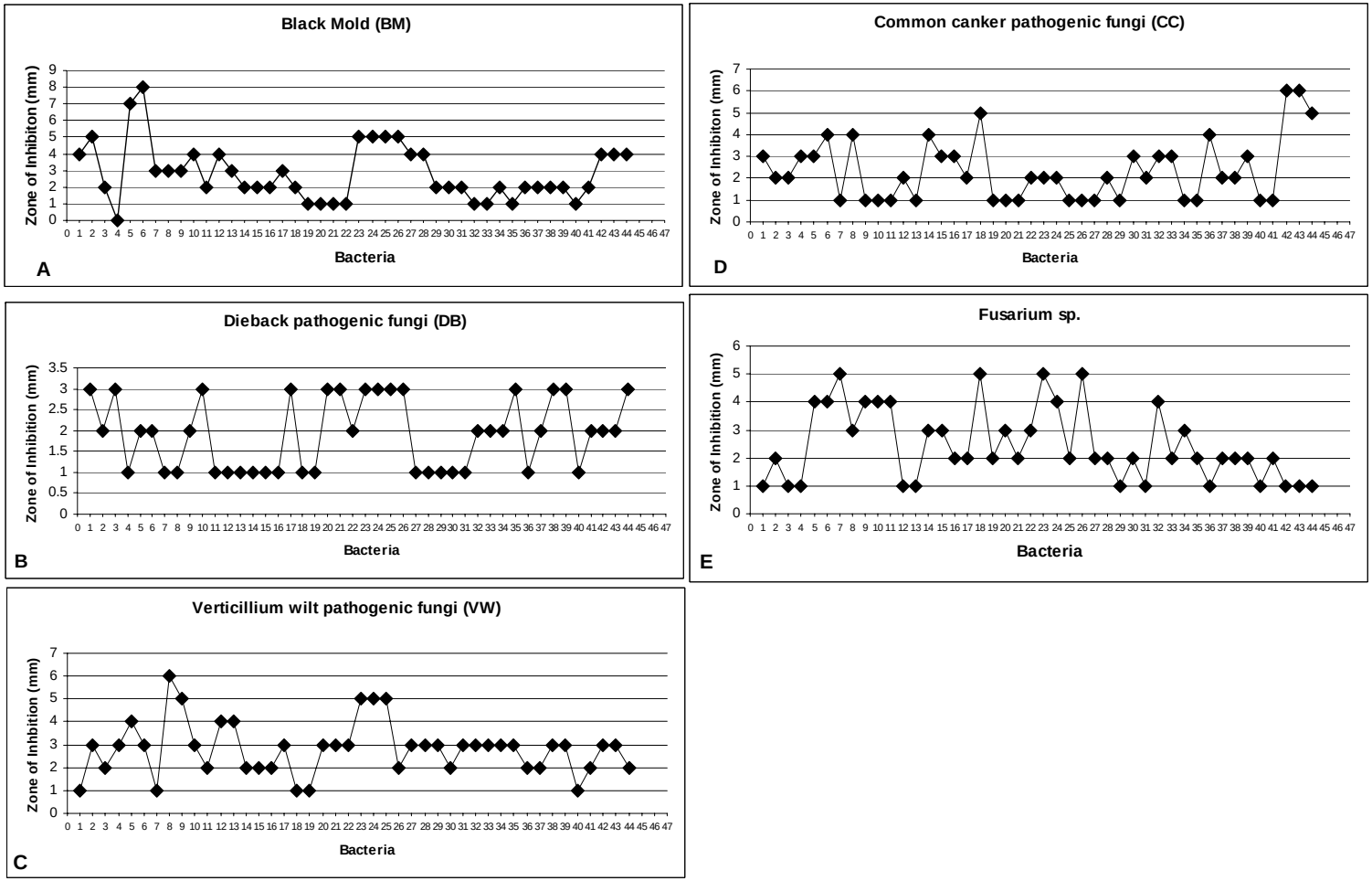
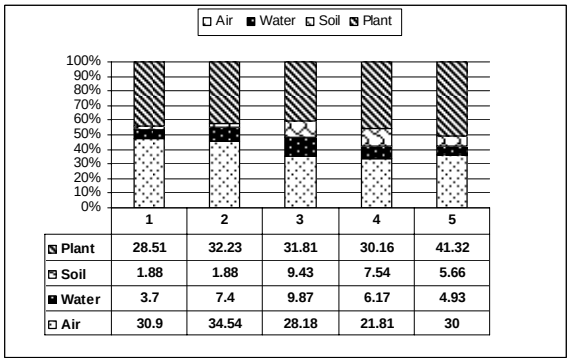
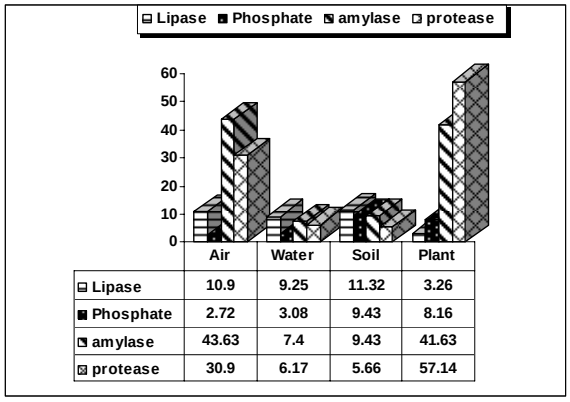


Figure 1(A-E): Antifungal activity of bacteria against pathogenic fungi



	1	2	3	4	5
Plant	28.51	32.23	31.81	30.16	41.32
Soil	1.88	1.88	9.43	7.54	5.66
Water	3.7	7.4	9.87	6.17	4.93
Air	30.9	34.54	28.18	21.81	30

Figure 2: % Occurrence of bacteria active against pathogenic fungi 1=Common canker, 2=Dieback, 3=BM= Black mold, 4= Verticillium wilt, 5= *Fusarium*



	Air	Water	Soil	Plant
Lipase	10.9	9.25	11.32	3.26
Phosphate	2.72	3.08	9.43	8.16
amylase	43.63	7.4	9.43	41.63
protease	30.9	6.17	5.66	57.14

Figure 3: Distribution of bacteria with reference to their extracellular activity

ACKNOWLEDGEMENTS

Authors are thankful to Ministry of Environment and Forests, Govt. of India for financial assistance though project no. 22/7/2003 –CS/BR dt. 29/3/2004.

REFERENCES

Bashan, Y. and Hologuin G. (2002). Plant growth promoting bacteria a potential tool for arid mangrove reforestation. *Trees structure and Function* **16(2): 159 – 166.**

Bashan, Y., Moreno, M. and Troyo E. (2000). Growth promotion of the seawater irrigated oilseed halophyte *Salicornia bigelovii* inoculated with magrove rhizosphere bacteria and halotolerant *Azospirrilum* sp. *Biology and fertility of soil* **32(4): 265 – 272.**

Booth, C. (1978). Introduction to general methods. In Methods in Microbiology. Eds. C. Booth, Acad. Press NY. vol. 4 **pp: 57– 91.**

Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H. and Sa, T. (2005). Isolation and characterization of phosphate solubilising bacteria from the rhizosphere of crop plants of Korea. *Soil Biology and Biochemistry* **37(10): 1970 – 1974.**

Cristophersan, C., Crescente O., Frisvad J. C., Gram L., Nielsen J., Nielsen P.H. and Rahbaek L. (1999) . Antibacterial activity of marine derived fungi, *Mycopathologia* 143**(3): 135 – 138.**

Kathiresan , K. and Salvam M. M (2006). Evaluation of beneficial bacteria from mangrove soil. *Botanica Marina* **49(1): 86 – 88.**

Sood, M. (1990). Studies on *Aspergillus umbrosus* (Bainier and Sartory) as related with the physiology of growth and antibiotic production. Ph. D. Thesis, Department of Bioscience, Ravisankar University, Raipur (Chhattisgarh), India.