EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 6 (June-2015) pp. 101-106

www.ijcrbp.com

Original Research Article

Biocontrol Activity of Some Potent Methylotrophs Isolated from Bhitarkanika Mangrove Sediment

Manish Kumar[1], Alok K. Srivastava*[1] and Akhilesh K. Pandey[2]

[1]National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, U.P., India
[2]Department of Biosciences, Rani Durgavati Vishwavidyalaya (RDVV), Jabalpur, M.P., India

**Corresponding author.*

Abstract

Methylotrophs are C1 carbon compound utilizing group of bacteria. These subpopulations of bacteria were isolated from the sediment sample of Bhitarkanika mangrove ecosystem. A total 50 isolates were obtained at the universal media plate specific for methylotrophs, containing methanol as a source of carbon and energy. All the isolates were subjected to screening for the antagonistic activity against fungal pathogen *Macrophomina phaseolina.* The test performed resulted a few of them were designated as potent inhibitor of this rot causing fungal pathogen. The effect of methylotrophic isolate on the fungal mycelia was clearly observed after scanning electron imaging of the interface of fungus and bacteria. Therefore, study indicates the biotechnological potential of methylotrophs isolated from mangrove sediment.

Keywords

Biocontrol
Carbon
Mangrove ecosystem
Methylotrophs

Introduction

Mangrove ecosystem is recognized as a natural resource of importance to mankind by virtue of its efficacy and aesthetic value. The mangrove ecosystem is one of the most fertile ecosystem of tropical and subtropical regions of the world. Such type of dynamic mangrove ecosystem supports numerous diversified soil micro and macro organisms. Soil organisms of mangrove, especially microbial flora, play a very important and crucial role in the degradation of mangrove foliage, which is continuously shed and decomposed in the mangrove base (Kumar, 2000). A total of 4827 km^2 mangrove forests in India is reported which is 0.1% of total geographical area of India and 5% of the world's mangrove vegetation (Kathiresan and Rajendran, 2005). Bhitarkanika mangrove ecosystem, located at the junction of the Brahmani and Baitarni rivers, is the second largest Indian mangrove ecosystem (next to Sundarban, West Bengal). Because of its rich biodiversity, it is regarded as one of the top ranking mangrove forests of the world in terms. High biological species diversity is observed in this mangrove which is evaluated among one of the best in the world (Thatoi et al., 1999).

Bhitarkanika mangrove is deputed as a Ramsar wetland of international importance in year 2002.

Because of richness in carbon and reduced carbon along with other nutrient content, the mangrove ecosystem harbors variant microbial communities which can adapt to the saline condition of this niche. Mangrove forest is environments subject to significant degradation by anthropogenic activities. In coastal area, interfacing the continents and the oceans makes it substantially important in the prediction for biotechnological applications. A number of bacterial groups like phosphate solubilizers, methanogens, methane oxidizers, nitrogen fixers, cellulose decomposers, nitrifiers, archaea, denitrifiers, iron oxidizers, sulphur oxidizers and iron reducers are generally inhabitating this mangrove forest (Holguin et al., 2001).

Macrophomina phaseolina (Tassi) Goid is a well known seed damaging, soil borne pathogen, infecting a number of plant species throughout the world (Kunwar et al., 1986; Mihail and Taylor, 1995, Srivastava and Singh, 1990). Many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot are caused by this fungus under favorable condition in numerous economically important crop plants. The root-rot caused by *Macrophomina* among the fungal diseases, prevails to be a challenging assignment in terms of management. It is prevalent sub-tropical and tropical climate, in arid and particularly in the areas with low rainfall, high temperature and it is distributed worldwide (Raut and Bhombe, 1984). To eradicate pathogenic fungi, numerous disease management methods have been employed to resist. Apart from target organism, several beneficial organisms are also killed by pesticides. In soil and environment their toxic forms persist (Hayes and Laws, 1991).

Due to increment in awareness of humankind for the ecosystem and environment, a remarkable shift from synthetic materials to bio-products was observed. Against several kinds of pests fungi constitute an extensive group of bioagents. Fungi such as *Trichoderma*, *Gliocladium* can minimize, suppress and may inhibit the parasitism of pathogens like *Fusarium* sp., *Rhizoctonia* sp., *Sclerotium* sp. (Rajappan and Ramaraj, 1999; Hadar et al., 1979; Papavizas and Lewis, 1989; Murmanis et al., 1988; Tu, 1991; Kim and Roh, 1987). The present investigation is, however, design in a way to investigate efficacy of some methylotrophic species against pathogenic *M. phaseolina*.

Materials and methods

Sampling site and sample collection

Geographically Bhitarkanika is located between 20°4'-20°8'N Latitudes and 86°45'- 87°50' Longitudes. The samples were collected in the month of March from top 4 cm soil profile of sediment where most of the microbial activity takes place, and thus where most of the bacterial population is concentrated. Total five composite samples were made from Gupti (BN1), Habalganda (BN2), Mahisamunda (BN3), Dangmal (BN4) and Kalibhanjadian (BN5). Samples were taken in replicates. Soil samples were collected by using clean, dry and sterile polythene bags along with sterile spatula, marking pen, rubber band and other accessories. 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. Samples were stored in iceboxes and transported to the laboratory where they were kept in refrigerator at -20°C until analysis.

Isolation and enumeration

The samples were taken for the serial dilution up to 10^4 dilution, 0.2 ml of each dilution were inoculated in duplicate plates of the NMS (Nitrate mineral salt) media containing 0.1% methanol as carbon substrate for the isolation of methylotrophs by the spread plate technique (Joshi et al., 2008). After incubation all plates incubated at 30ºC in the incubator for 5-6 days. After incubation the population was recorded for each sample. Cycloheximide was used as antifungal agent in plates. A total 50 pure isolates of methylobacteria have been isolated by streak plate method.

Biocontrol activity against fungal pathogen

The pathogen used in the present study was obtained from DSR (Directorate of seed research), Mau, U.P., India. The fungal pathogen used in present investigation was maintained on PDA (Potato dextrose agar) plates. In vitro antagonistic activity of methylotrophic isolates against *M. phaseolina* was studied in dual culture technique by following the method by (Kucuk and Kivanc, 2003; Dennis and Webster, 1971). Control plate was grown individually on PDA plate after incubated. Three replications were maintained for each treatment. Three times replication

was done for each treatment with incubation of 28±2°C. After 8 days of incubation, percent growth of antagonists, pathogen and zone of inhibition is recorded. Antagonistic activity of methylobacteria was tested in which a 5 mm mycelia agar disc from pathogen plate was placed on the centre of Petriplate containing PDA. Then plates were incubated at 28°C overnight. Methylotrophic cultures were then streaked 3cm away from the disc of *M. phaseolina*. Control containing plates inoculated only with test pathogen. The formula for the calculation of percent growth inhibition (PGI):

$$\% \text{ inhibition} = KR\text{-}R1/KR \times 100$$

Where KR is the distance between point of inoculation to the colony margin on the control plates, and R1 is the distance of fungal growth from the point of inoculation to the colony margin on the treated plates in the direction of the antagonists (Korsten and De Jager, 1995). The percent growth inhibition scale ranges from 0 to 4, where 0= no growth inhibition; 1= 1-25% growth inhibition, 2=26-50% growth inhibition; 3=51-75% growth inhibition; 4=76-100% growth inhibition. After 7 days of incubation, the zone of inhibition (distance between pathogen and are of antagonist) was recorded (Zivkovic et al., 2010).

Scanning electron microscopy

The interaction of the test fungi with antagonistic isolates was studied by scanning electron microscopy (SEM). The hyphae from the interaction zone was transferred on glass coverslip, then fixed with 1.5% gluteraldehyde and dehydrated with graded series of ethanol washes followed by drying in desiccator (Yuan and Crawford 1995). Samples were affixed to SEM stubs using carbon tape followed by thin coating with gold: palladium (60:40) and examined by SEM (JEOL, JSM-62804).

Results

Soil properties and enumeration of methanol oxidizing methylobacteria

Five composite sediment samples were subjected to the estimation of physical properties in which the range of pH was 7.4 to 8.0 while the electrical conductivity ranges from 4.04 dSm^{-1} to 6.67 dSm^{-1} with organic carbon from 0.7 to 1.5 unit.

Enumeration of culturable, aerobic, methylotrophic bacteria was carried out by standard spread plate count dilutions using specific media. The population of methylotrophs ranged from 5 -300 x 10^4 g^{-1} soil at different sampling sites. The bacterial diversity found in the isolations from the five different locations revealed to be statistically different (p<0.05). At location BN1, the number of bacteria had a log value of 4.69, location BN3 and BN5 had a value of 5.69 while at location BN2 and BN4 the value was 6.47, maximum among different sites (Table 1).

Table 1. Enumeration of methylobacteria population at different sampling sites.

Sample	Sample type	Sampling site	CFU	Log CFU
BN1	Sediment	Gupti	5×10^4	4.69 ± 0.33
BN2	Sediment	Habalganda	300×10^4	6.47 ± 0.37
BN3	Sediment	Mahisamunda	50×10^4	5.69 ± 0.46
BN4	Sediment	Dangmal	300×10^4	6.47 ± 0.48
BN5	Sediment	Kalibhanjadian	50×10^4	5.69 ± 0.49

However, no significant differences were observed between samples collected from different locations. From these soil samples, a total of 50 isolates were randomly selected for their antagonistic activity against pathogenic fungus. The methylotrophic population was higher in the sediment sample from Dangamal and Habalganda district. Methylotrophs with different morphology and pigmentation were observed from this ecosystem. All of the isolates were aerobic, catalase, urease positive and weakly oxidase positive. The halotolerant ability of the isolate was observed and NaCl tolerance ranges from 100 mM to 550 mM in most of the methylobacteria.

Biocontrol activity against pathogenic fungus *M. phaseolina* and SEM

In this screening, some isolates were found potential to antagonize the pathogen at considerable level ranging from 25.45- 54.65% inhibition. Antagonistic potential of some selected methylobacteria was concluded and

validated by restriction and inhibition of pathogen growth by showing zone of inhibition towards the antagonists as shown in photo-plates of dual culture plate assay (Fig. 1). The isolates NBMS37 and NBMS48 showed maximum antagonism (54.65% and 51.23% respectively) followed by isolates NBMS19, NBMS5, NBMS33, NBMS11 and NBMS9. SEM images of the interface of pathogenic fungus and methylobacteria reveals that fungal growth is inhibited by methylobacteria (Fig. 2). The growth of fungus is relatively checked by inhibiting the dispersal of hyphae in the plate. The observation revealed the disruption of the fungal hyphae by the activity of methylobacteria.

Fig.1: Biocontrol activity of the methylotrophic isolates against *M. phaseolina*.

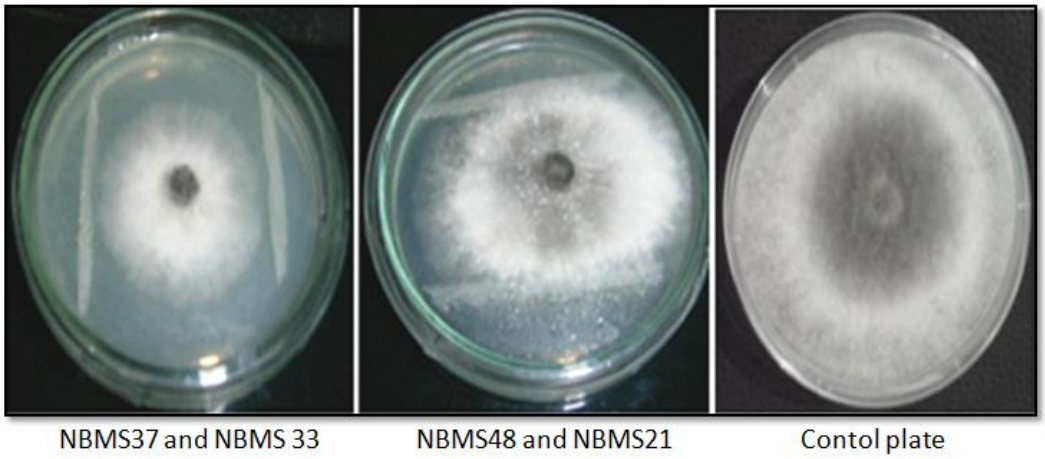
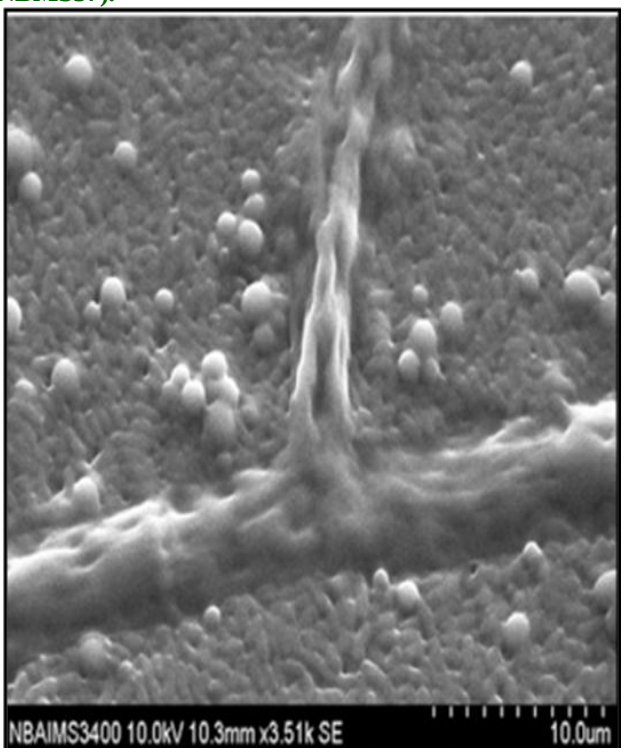


Fig. 2: Scanning electron micrographs of bicontrol activity of isolate NBMS48 against pathogenic fungus *M. phaseolina*. Picture indicates the ruptured fungal mycelia surrounded by methylotrophic bacterial cells (NBMS37).



Discussion

Biological control is considered as an environmental friendly strategy to minimize and eradicate the crop damage caused by plant pathogens. Pesticide use by the general public and governmental agencies are increasing concerns now a day, severely restricting the availability and use of several important pesticides. In vitro antagonistic activity of methylotrophic isolates against pathogen was estimated and observed in dual culture technique by following the method of Kucuk and Kivanc (2003).

Differential biocontrol ability among the antagonists was noticed against *M. phaseolina*. Seven days of incubation represent various degrees of mycelial growth inhibition of *Macrophomina*. The intermingled contact zone of methylotrophs and pathogenic fungus was cut and was observed under the electron microscopy. The electron micrograph of the intermingled region shows the collapse, shrinkage and disruption of cytoplasm of the fungal mycelia with the healthy methylotrophic cells around mycelia. Baker and Cook (1979) have reported that the mycelial walls and septal walls may be digested by the enzymes produced or antibiotic formation that inhibit growth or cause endolysis. The isolates NBMS37 and NBMS48

were found to be most potent in reducing and inhibiting the growth of pathogen. The hypothesis of antibiosis, i.e. the secretion of extracellular toxic metabolites is supported by the results obtained, led to the fact that the main mode of action by which methylotrophs exhibit its biocontrol potential (Hajlaoui and Belanger, 1993).

In many disease control programs, fungicides as standards have been used, been increasingly regulated. Research efforts with pathogens have indicated that most of the biocontrol organisms either did not control the pathogen or they did not perform as well as selected fungicides. Reduced carbon utilizing microbes, Methylotrophs are a physiologically interesting group of bacteria that preferentially utilize methanol and other reduced one carbon compounds such as formate and formaldehyde as sole source of carbon and energy give a good biocontrol activity against phytopathogens like *M. phaseolina*, *Fusarium oxysporum* and *Sclerotium rolfsii* (Poorniammal et al., 2009). In this study the bacterial isolates with methylotrophic potential were subjected to check their activity against phytopathogen like *M. phaseolina*. Few isolates like NBMS5, NBMS37 and NBMS48 were giving very considerable antagonistic activity against pathogenic fungi while others were not showing antagonism. The scanning electron micrograph clears the image of interface of bacteria and fungi in which the fungal mycelia were disrupted and growth was inhibited by the healthy bacterial community present.

Acknowledgement

Authors are thankful to ICAR (Indian council of agricultural research) for financial assistance and laboratory facilities. Authors are also thankful to the Director, NBAIM and HOD, Department of Biosciences, RDVV, Jabalpur.

References

Baker, F.K., Cook, R.J., 1979. Biological control of plant pathogens. S. Chand and Company Limited, New Delhi, India. 433p.

Dennis, C.J., Webster, J., 1971. Antagonistic properties of species - group of *Trichoderma* III. Hyphal interaction. Trans. British Mycol. Soc. 57, 363-369.

Hadar, Y., Chet, I., Heins, Y., 1979. Biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum*. Phytopathol. 69, 64-68.

Hajlaoui, M.R., Belanger, R.R., 1993. Antagonism of the yeastlike phylloplane fungus *Sporothrix focculosa* against *Erysiphe graminis* var. *tritici*. Biocontrol Sci. Technol. 3, 427-434.

Hayes, W.J., Laws, E.R., 1991. Handbook of Pesticide Toxicology. Vol. 1, Academic Press Inc., New Delhi.

Holguin, G., Vazquez, P., Bashan, Y., 2001. The role of sediment microorganisms in the productivity, conservation and rehabitation of mangrove ecosystems: An overview. Biol. Fertil. Soils 33, 265-278.

Joshi, A. A., Kanekar, P. P., Kelkar, A. S., Shouche, Y. S., Vani, A. A., Borgave, S. B., Sarnaik, S. S., 2008. Cultivable bacterial diversity of alkaline Lonar lake, India. Microb. Ecol. 55, 163–172.

Kathiresan, K., Rajendran, N., 2005. Mangrove ecosystem of Indian Ocean region. Indian J. Mar. Sci. 34, 104-113.

Kim, H.K., Roh, M.J., 1987. Isolation, identification and evaluation of biocontrol potential of rhizosphere antagonists to *Rhizoctonia solani*. Korean J. Plant Protec. 26, 26-87.

Korsten, L., De Jager, E.S., 1995. Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. S. Afr. Avocado Growers Assoc. 18, 124-130.

Kucuk, C., Kivanc, M., 2003. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turk. J. Biol. 27, 247-253.

Kumar, R.S., 2000. A review of biodiversity studies of soil dwelling organisms in Indian mangroves. Zoo's Print J. 15(3), 221-227.

Kunwar, I.K., Singh, T., Machado, C.C., Sinclair, J.B., 1986. Histopathology of soybean seed and seedling infection by *Macrophomina phaseolina*. Phytopathol. 76, 532–535.

Mihail, J.D., Taylor, S.J., 1995. Interpreting variability among isolates for *Macrophomina phaseolina* in pathogenicity, pycnidium production and chlorate utilization. Canadian J. Bot. 10, 1596–1603.

Murmanis, L.L., Highley, T.L., Richard, J., 1988. Hyphal interaction of *Trichoderma harzianum* and *T. polysporum* with wood decay fungi. Material Organis. 23(4), 271-279.

Papavizas, G.C., Lewis, J.A., 1989. Effect of *Gliocladium* and *Trichoderma* on damping off and blight of snapbean caused by *Sclerotium rolfsii* in

green house. Plant Pathol. 38, 277-286.

Poorniammal, R., Sundaram, S.P., Kumutha, K., 2009. *In vitro* biocontrol activity of *Methylobacterium extorquens* against fungal pathogens. Int. J. Plant Prot. 2(1), 59-62.

Rajappan, K., Ramaraj, B., 1999. Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing wilt of cauliflower, Ann. Plant Prot. Soc. 7(2), 205-207.

Raut, J.G., Bhombe, B.B., 1984. Longevity of *M. phaseolina* in sunflower seeds. Indian Phytopathol. 37(2), 333-334.

Srivastava, A.K., Singh, R.B., 1990. Effect of organic amendment on interaction of *Macrophomina phaseolina* and *Melpidogyne incognita* on fresh bean (*Phaseolus vulgaris*). New Agriculturist 1, 99–100.

Thatoi, H.N., Ouseph, A., Mishra, P.K., Mohanty, J.R., Acharjyo, L.N., Daniels, R.J., 1999. Mangrove restoration in Odisha, South India: An experiment. Tiger Paper 26, 23-26.

Tu, J.C., 1991. Comparison of the efficacy of *Gliocladium virens* and *Bacillus subtilis* in the control of seed rot and root - rot of navy beans. Mededelingen Van de Faculteit Landbourn Weteschappen Rijks Universiteit Gent. 56, 229-234.

Yuan, W. M., Crawford, D. L., 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. Appl. Environ. Microbiol. 61, 3119-3128.

Zivkovi, S., Stojanovi, S., Ivanovi, Z., Gavrilovi, V., Popovi, T., Balaž, J., 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Arch. Biol. Sci. Belgrade 62, 611-623.