WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 256-264. Conference Article ISSN 2277– 7105

STUDY OF GROWTH PROMOTION OF *AVICENNIA MARINA* SEEDLINGS BY PGPB ISOLATED FROM MANGROVES IN NAVI MUMBAI

Joseph Dona[1]*, Jadhav Priyanka[2], Katke Shubham[3], Jaiswar Arpan[4], Thakare D. B.[5]

[1]Associate Professor, Microbiology Department, VES College of Arts, Science and Commerce, Chembur, Mumbai – 400 071.

[2,3,4]Ex- B.Sc Student, Microbiology Department, VES College of Arts, Science and Commerce, Chembur, Mumbai – 400 071.

[5]Retired Associate Professor and Head, Microbiology Department, Karmaveer Bhaurao Patil College, Vashi, Navi Mumbai – 400 703.

Article Received on
03 Jan. 2018,

Revised on 23 Jan. 2018,
Accepted on 13 Feb. 2018

DOI: 10.20959/wjpr20184-10779

***Corresponding Author**
Joseph Dona
Associate Professor,
Microbiology Department,
VES College of Arts,
Science and Commerce,
Chembur, Mumbai – 400
071.

ABSTRACT

Mangroves are essential for coastal cities such as Navi Mumbai, where they act as a buffer between the land and sea. Yet they are destroyed on a large scale for urban development. Mangrove regeneration programs compensate for such losses, by planting mangrove saplings in new areas. The growth of such saplings can be promoted by the use of PGPB (Plant Growth Promoting Bacteria). However, growth promoting bacteria indigenous to mangrove areas in Navi Mumbai have been largely unexplored. This study was aimed at examining bacterial strains isolated from mangroves in Navi Mumbai for their ability to promote the growth of seedlings of *Avicennia marina*, the dominant mangrove species in the area. Seedlings of the plant were grown in mangrove soil inoculated with the test isolates, following which their growth was compared with that of control plants grown in uninoculated mangrove soil. All the inoculated plants showed a significant increase (upto 85%) in their protein content as compared to the control plants. Most of the plants also showed an increase in their chlorophyll-a content (upto 24%), wet weights (upto 28%) and dry weights (upto 57%). These results indicate that the isolates have the ability to promote the growth of *Avicennia marina* plants. Further studies using combinations of the isolates can help in

developing an effective consortium of PGPB for promoting growth of mangrove plants, especially in Navi Mumbai.

KEYWORDS: Mangroves, *Avicennia marina*, PGPB, reforestation, Navi Mumbai.

INTRODUCTION

Mangroves are a valuable ecological and economic resource, being important nursery grounds and breeding sites for birds, fish, crustaceans, shellfish, reptiles and mammals; a renewable source of wood; accumulation sites for sediment, contaminants, carbon and nutrients, offer protection against coastal erosion and diminish the impact of tsunamis. However, they are highly threatened ecosystems and at present are disappearing at a rate of 1 to 2% per year across their range. Habitat destruction through human encroachment has been the primary cause of mangrove loss.[1,2,3]

In Navi Mumbai, about 94 acres of mangroves have to be razed to build Mumbai's second airport. To compensate for this loss, the Environment Ministry has asked CIDCO to come up with a 245-hectare mangrove park, besides regeneration of mangroves at Kamothe (310 hectares) and Moha Creek (60 hectares).[4] Besides, the 'Mumbai Trans Harbour Link' (MTHL) envisages construction of a 6 lane road bridge across the Mumbai Harbour, between Mumbai and Navi Mumbai. 9306 sq. m of mangrove area in Navi Mumbai will be affected by the project. A compensatory mangrove restoration plan, amounting to Rs. 25 crore, is proposed with the help of Forest department.[5]

These plans for regeneration of mangroves involve growing the mangrove seedlings in nurseries and subsequently transplanting them in new areas. The growth of such mangrove plantlets can be promoted by the use of PGPB (Plant Growth Promoting Bacteria), which exhibit activities that benefit the plant either directly or indirectly. The inoculation of plants with PGPB is a common tool in agriculture to enhance crop yields.[6,7] Many PGPB have been identified in influencing the growth and yield of mangrove plants. Though considerable data is available on the use of PGPB to promote the growth of mangroves world-wide, very little work has been carried out in this area in Navi Mumbai. The objective of this study was to evaluate the ability of PGPB isolated from mangroves in Navi Mumbai for their ability to promote the growth of seedlings of *Avicennia marina.* Such PGPB could then be used to develop an effective consortium for promoting growth of mangrove plants, especially in Navi Mumbai.

MATERIALS AND METHODS

Isolation and selection of cultures

Six bacterial isolates exhibiting plant growth promoting activities such as nitrogen fixation, phosphate solubilization, indole acetic acid synthesis, extracellular enzyme production, siderophore synthesis, HCN production and anti-fungal activity were screened from mangrove areas in Vashi, Nerul and Belapur in Navi Mumbai. The isolates were named N5, N11, N12, N13, N14 and N15 and maintained on sterile slants of nutrient agar containing 3% NaCl.

Collection of mangrove seedlings

Seedlings of the grey mangrove *Avicennia marina* were collected from the inter-tidal zone of a creek in Nhava Sheva near the northern part of JNPT port. At low tide, the site is strewn with mangrove seeds and seedlings and is an ideal place for collection of samples. Uniformly coloured, medium sized, healthy seedlings of similar size and weight were selected. Seedlings with evidence of disease, deformities or damage, those that were small, non-uniformly colored, broken or bruised, as well as those showing signs of attack by borer insects were avoided.

Collection of mangrove soil

Mangrove soil was collected from the marshy areas adjoining Palm Beach road in Seawoods, Navi Mumbai. Small roots, branches and stones were removed from the mud to make it uniformly smooth. The mud was collected in plastic buckets and transported to the site of planting.

Fig. 1: Collection of mangrove seedlings and mud.

Planting and inoculation of seedlings

42 seedlings were planted in nursery bags, one per bag. Each bag was filled upto half its capacity with mud and punched to allow water to pass through. The seedlings were divided into 7 sets containing 6 seedlings each. Each set was inoculated with one of the test isolates;

one set was kept uninoculated and maintained as a control. The inoculation of the seedlings was done by the soil inoculation method.[8] A saline suspension of the appropriate test culture was made with density adjusted to an absorbance value of 0.1 at 530 nm. 5 ml of the culture suspension was injected into the soil surrounding the roots, once in 3 weeks. In case of the control plants, sterile saline was injected instead of culture.

Cultivation of the seedlings

The plants were placed in a sunlit area surrounded by a net to prevent attack by birds and insects. They were watered twice a day with non-sterile water collected from the Panvel creek. During the day, the ambient temperature was approximately $30\pm2^{o}C$ with a relative humidity of 75-80% while during the night the ambient temperature was around $26 \pm 2^{o}C$ with a relative humidity of 80-84%. The day length ranged from 10.59 – 12.17 hours over the entire growing period. The intensity of illumination varied from 32,000 - 100,000 lux depending on the weather condition.

Fig. 2: Mangrove seedlings after cultivation for 5 weeks.

Study of growth parameters

The growth of the plants was monitored throughout the growing period of 15 weeks. The stem length and number of leaves were recorded every 3 weeks. At the end of 15 weeks, all the plants were uprooted taking care not to break the roots. The roots were washed carefully to remove the mud sticking to them. The average root length was determined and the number of lateral roots counted. The fresh weight of every seedling was recorded, following which the seedlings were dried in an oven at $60^{o}C$ to constant weight. The dry weight was then determined and recorded.[9] The chlorophyll content of the leaves was extracted using chilled 80% acetone and estimated by measuring the absorbance of the extract at 663.2 and 646.8 nm in a u.v. visible spectrophotometer using 80% acetone as the blank. The Chlorophyll-a and

Chlorophyll-b contents were then quantified by using the formula: Ch-a=12.25 * A663.2 – 2.79 A646.8; Ch-b=21.5 A646.8 – 5.1 A663.[10] The leaf protein content was extracted by resuspending the pellet obtained after chlorophyll extraction in 5 mL 1:1 ethanol: ether (v:v) to remove lipids followed by centrifugation at 10,000 rpm for 15min at 4^{o}C. The pellet was re-suspended in 20 ml of 0.1N NaOH and allowed to dissolve for 15 minutes. Dilutions were made using 0.1N NaOH and the protein content was determined by Lowry's method.[11]

RESULTS AND DISCUSSION

Stem length, number of leaves and average root length

There was no significant difference between the stem lengths of the test and control plants. Plants inoculated with two of the test cultures N13 and N14 showed an increase in the number of leaves as compared to the controls. All the test plants, except the ones inoculated with N12, showed an increase in average root length as compared to the control set. However, none of the above was found to be significant when analysed by the Student's t-test and Anova. Thus the PGPB inoculation had no effect on the stem length, number of leaves and root length of the plants.

Wet and dry weight of the plants

The wet weight of the inoculated plants was positively affected by the culture inoculation. All the test plants showed an increase in wet weight as compared to the control. The plants inoculated with N14 showed a 26% increase and this was found to be statistically significant (p=0.03). The dry weight of all the test plants was significantly higher than that of the control plants. The set inoculated with N5 showed the highest percentage increase in dry weight (57%), followed by those inoculated with N13, N12 and N14 (41%, 34% and 21% respectively). However only the increase induced by N14 was found to be statistically significant (p=0.01).

Chlorophyll content

Most of the test plants showed higher chlorophyll-a content as compared to the controls. The increase was found to be statistically significant in case of N5 (p = 0.0008), N12 (p = 0.003), N14 (p = 0.002) and N15 (p = 0.0007). The control plants showed a higher chlorophyll-b content as compared to the test plant. The total chlorophyll content of the plants inoculated with the culture N15 was significantly higher than that of the controls.

Leaf protein content

There was a statistically significant increase in leaf protein content of all the test plants as compared to the controls. The highest protein content was shown by the plants inoculated with N5 (p= 0.015), N14 (p= 0.011) and N15 (p= 0.022).

Table 1 summarizes the results obtained for the different sets of plants considering all the growth parameters while Fig. 3 indicates the percentage increase/decrease in values of various growth parameters of the test plants as compared to the control plants. The results indicate significant differences between the control and test plants in terms of leaf protein content, chlorophyll a content, wet and dry weight, indicating that the test cultures enhanced the growth of the plants.

Table 1: Summary of effect of PGPB inoculation on mangrove seedlings.

Sample	Stem length (cm)	No. of leaves	Average length of the primary root (cm)	Wet weight (gm)	Dry weight (gm)	Ptn content (%)	Chlorophyll a content (mcg/ml)	Chlorophyll b content (mcg/ml)
Control	16.67 ±1.48	8.33 ±0.61	12.52 ±1.68	15.11 ±1.12	4.41 ±0.29	5.12 ±0.56	2.29 ±0.06	2.16 ±0.51
N5	15.25 ±1.9	8.67 ±0.42	16.8 ±3.39	19.33 ±4.74	6.94 ±1.84	9.55 ±0.98	2.65 ±0.03	1.4 ±0.03
N11	14.58 ±1.17	7.67 ±0.61	17.5 ±3.61	14.86 ±1.55	4.48 ±0.60	7.09 ±0.47	2.38 ±0.11	1.52 ±0.01
N12	15.08 ±1.50	7.67 ±0.95	10.3 ±1.58	16.39 ±3.02	5.92 ±1.03	7.08 ±0.19	2.5 ±0.02	1.49 ±0.04
N13	14.33 ±1.22	8.33 ±0.61	13.35 ±2.64	18.09 ±2.27	6.22 ±1.2	7.21 ±0.33	2.3 ±0.01	1.35 ±0.02
N14	15.17 ±1.38	8.67 ±0.42	14.83 ±1.91	19.00 ±1.07	5.36 ±0.37	8.07 ±0.37	2.73 ±0.01	1.35 ±0.01
N15	13.67 ±2.11	7.33 ±0.42	13.58 ±2.24	17.93 ±3.5	5.45 ±0.94	7.77 ±0.09	2.98 ±0.01	1.35 ±0.01

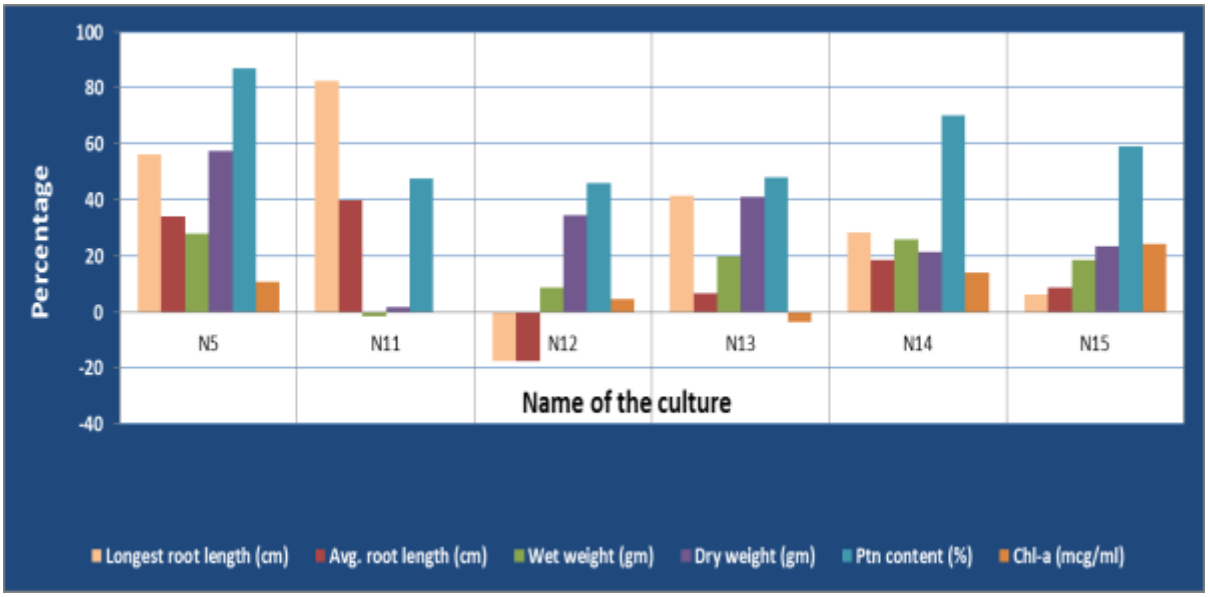

Fig. 3: Percentage difference in values of growth parameters (test v/s control).

Growth promotion of mangroves and marine plants after inoculation with PGPB has benn demonstrated in earlier studies. Mangrove seedlings grow better after inoculation with the diazotrophic filamentous cyanobacteria *Microcoleus chthonoplastes*.[12,13]

Inoculation of a marine plant *S. bigelovii* with eight species of halotolerant PGPBs resulted in significant plant growth promotion. Statistical analyses demonstrated that inoculation with terrestrial halotolerant *Azospirillum halopraeferens*, a mixture of two halotolerant *A. brasilense* strains, a mixture of marine *Vibrio aestuarianus* and *V. proteolyticus*, or a mixture of marine *Bacillus licheniformis* and *Phyllobacterium* sp. significantly increased plant height and dry weight at the end of the season.[14]

In the current study, considering all the parameters tested, the cultures N5, N13 and N14 showed the greatest enhancement of plant growth (Fig.3). Hence these cultures can be studied further to gain a better understanding of their potential to enhance mangrove growth.

CONCLUSION

This study was carried out to test the effect of PGPB isolated from mangroves in Navi Mumbai on the growth of *Avicennia marina* seedlings. All the isolates increased the protein content of the plant and four of the isolates increased the chlorophyll-a content. One culture N14 increased the wet and dry weight of the plant. Three of the isolates (N5, N13 and N14) showed the best overall enhancement of plant growth. These isolates can be tested in various combinations to determine synergistic relationships between them and create consortia that

would show optimum plant growth promotion when applied on the field. Since the cultures were isolated from local mangrove environments, they are already adapted to the environmental stresses found in these environments. Further, since the soil and water used for growing the plants were natural and non-sterile, we can predict that the added cultures will promote the growth of the plants even in the presence of the indigenous microflora and nutrients that occur in natural mangroves. Further experiments can be carried out to determine whether the isolates can enhance the growth of mangroves in nutrient deficient, non-mangrove soils, which are likely to be found in areas allocated for mangrove reforestation programs. If the isolates are successful in such conditions, their significance as PGPB in mangrove reforestation programs will increase manifold.

ACKNOWLEDGEMENTS

We thank the management of KBP and VES College for providing us with the facilities to carry out this work. Special thanks to the staff of the Microbiology department of VES College for their unstinting support and encouragement.

REFERENCES

1. Alongi DM. Present state and future of the world's mangrove forests. Environmental Conservation, 2002; 29(3): 331–349.
2. Gomes Newton C. M. et al. Taking Root: Enduring Effect of Rhizosphere Bacterial Colonization in Mangroves. PLoS One Biodiversity, 2010; 5(11): e14065.
3. Kathiresan K, Bingham L. Biology of Mangroves and Mangrove Ecosystems. Advances in Marine Biology, 2001; 40: 81-251.
4. Hiranandani A., Levitt T. Mangroves v/s airport: Mumbai's development battle. The Ecologist, 2010, http://www.theecologist.org/News/news_analysis/492153/mangroves_vs_airport_mumbais_development_battle.html
5. Government of India, Ministry of Environment, Forest and Climate Change, F.No.11-65/2012-IA.111, https://mmrda.maharashtra.gov.in/documents/10180/5928836/MTHL+-+CRZ+Clearance+25-01-2016.pdf/dacec6dc-93fb-4d6d-805e-70c558f55732?version=1.0
6. Bashan Yoav, Holguin Gina. Plant growth-promoting bacteria: a potential tool for arid mangrove reforestation. Trees, 2002; 16: 159-166.
7. Glick BR, Bashan Yoav. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnology Advances, 1997; 15(2): 353-378.

8. Shukla JP. Technologies for sustainable rural development having potential of socio-economic upliftment. Allied Publishers Pvt. Ltd., 2014; 140.
9. Ravikumar S, Shanthy S, Kalaiarasi A, Sumaya S. Bioinoculation of halophilic phosphobacteria for vigorous seedlings of *Rhizophora mucronata*. Applied Science Research, 2010; 2(6): 261-266.
10. Nayek Sumanta, Choudhury Imranul Haque, Jaishee Nishika3 and Roy Suprakash. Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. Research Journal of Chemical Sciences, 2014; 4(9): 63-69.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem, 1951; 193(1): 265-75.
12. Bashan Y, Puente ME, Myrold, DD and Toledo G. In vitro transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. FEMS Microbiol. Ecol, 1998; 26: 165-170.
13. Bashan Y, Moreno M, Puente ME, Holguin G, Vazquez P, Troyo E and Glick B R. Inoculation of marine plants with marine plant growth- promoting bacteria and halotolerant Azospirillum - a novel approach of employing PGPBs for environmental use and seawater agriculture, http://www.bashanfoundation.org/gmaweb/pdfs/inoculation.pdf
14. Toledo G, Bashan Y, and Soeldner A. In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Can. J. Microbiol, 1995; 41: 1012-1020.