Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

IN VITRO EVALUATION OF SELECTIVE MAHUA FLOWERS ENDOPHYTES AS A BIOINOCULANT ON GREENGRAM [*Vigna radiata* (L.)]

POOJA PATEL[1], RAMAR KRISHNAMURTHY[1*] AND ASHOK SHAH[2]

*[*1] C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Surat, India*
[2] Prasad Biotech, Valsad, Valsad-395007, India

ABSTRACT

The importance of plant growth promoting microbes in plant growth promotion has been documented. However, reports of effective endophytes from the mahua flowers are limited. In this study, total seven bacteria and two yeast endophytes were isolated. One bacterial isolate (MSB1) and two yeast isolates (MSY1 & MSY2) were found to be most persistent and dominant among all on cultured media. MSB1, MSY1 and MSY2 isolates were further studied for their multiple plant growth promoting traits *viz*; indole acetic acid, siderophore production, phosphorous solubilization and nitrogen fixation. Isolates MSB1, MSY1 and MSY2 were able to produce 18.57, 15.14 and 39.64 µg/ml of indole acetic acid, respectively. Phosphate solubilising activity was found in both yeast; MSY1 (12.24 µg/ml) and MSY2 (10.79 µg/ml). Bacteria (MSB1) produced 47.80% of siderophore unit. Based on microbiological and molecular characteristics, microbes showing PGP traits were identified as *Bacillus amyloliquefaciens* (MSB1), *Rhodotorula* sp. (MSY1) and *Pichia kudriavzevii* (MSY2). *In vitro* study using greengram as an indicator crop revealed that inoculation of individual strain increased shoot length, root length and vigour index of greengram seedling. This study suggests that these isolates have potential to be used as bioinoculants for greengram production.

KEYWORDS: Mahua (*Madhuca longifolia*); *Bacillus amyloliquefaciens*; *Rhodotorula* sp.; *Pichia kudriavzevii*

RAMAR KRISHNAMURTHY*
krishnashanti@gmail.com

C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Surat, India

Received on: 06-07-2017
Revised and Accepted on: 02-08-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b1-7

INTRODUCTION

Madhuca longifolia is commonly known as mahua, belonging to the family of Sapotaceae. Mahua tree is widely distributed among the dry deciduous forest of India. In India, tribal peoples use the leaves, root, bark, fruit, flowers and seeds of mahua tree because of its beneficial and eco-friendly importance in the field of agriculture and medicine. It is reported that the tribal peoples used different parts of mahua tree like fermented waste of flower and seed cake as fertilizer to increase the fertility of soil.[1, 2] In nature, microorganism plays an important role in plant growth promotion (PGP) and as a biocontrol agent.[3] Endophytes are consistently reported in the root, stem, leaf, fruit, and tuber tissues of a wide range of agricultural, horticultural, and forest species.[4] Colonization of bacteria and yeast within plant tissues have been studied intensively for many plants but not for mahua. Being a rich source of sugars, mahua flower can act as outstanding natural habitat for a wide range of microbial diversity.[5] Further, the reports on mahua endophytic bacterial and yeast isolate, and their use in plant growth promotion is scarce. Thus, the present study was conducted to evaluate the PGP activities of endophytes of the mahua flower, on the growth of greengram [*Vigna radiata* (L.)] seedlings.

MATERIAL AND METHODS

Sample collection and surface sterilization

Flowers of the mahua were collected from the Valsad, India (20˚32'N Latitude 73˚18'E Longitudes). Flowers were surface sterilized by washing the samples in running water for two minutes and treated with 70% ethanol for one minute, followed by an immersion in 2.5% of sodium hypochlorite solution for two minutes. Samples were then rinsed three times with sterile distilled water.[6]

Isolation of bacteria and yeast endophytes

Enumeration of endophytic bacterial and yeast was performed using culture method.[6] Initially, surface sterilized flower tissues were ground in 10 ml of normal saline (0.85% NaCl, pH 7.0) under aseptic conditions. Serial dilution technique was performed up to 10^{-5} dilution. An aliquot of this suspension was spread on Nutreint agar (NA) and Malt Extract Glucose Yeast extract Peptone (MGYP) agar (Himedia, India) plates supplemented with 100 mg/L of chloramphenicol,[7] and incubated for 24 h 37°C and 72 h 30°C respectively. After incubation, Colony Forming Units (CFU) per gram was calculated.[8] The strains, which were most dominant among the endophytic bacteria and yeast populations, were selected for further study.

Evaluation of plant growth promoting traits

PGP characters *viz*; Indole Acetic Acid (IAA) production, phosphate solubilisation, siderophore production and nitrogen fixation were performed for selected endophytes.

Determination of IAA production

IAA production was estimated using Salkowski reagent.[9] IAA produced by the isolates was estimated by cultured them in yeast extract peptone dextrose (YEPD) medium (Himedia, India) supplemented with 0.1% L-tryptophan.[10] Bacterial and yeast isolates were incubated at 90 rpm for 24 h at 37˚C and 72 h at 30˚C respectively. The supernatant of the culture was obtained by centrifugation for 10 minutes at 10,000 rpm. Salkowski reagent (35% $HClO_4$, 50 ml; 0.5 M $FeCl_3$, 1 ml) and the supernatant were mixed in the ratio of 2:1 and incubated in the dark for 30 minutes. After incubation, the absorbance was recorded by spectrophotometer at 530 nm. The IAA concentration in the culture was estimated based on the IAA standard curve.

Estimation of phosphate solubilisation

Qualitative estimation of phosphate solubilisation was performed using Pikovskaya agar medium (Himedia, India).[11] After 72 h incubation Phosphate Solubilisation Index (PSI) was culturing by dividing phosphate solubilization zone by growth diameter of spot inoculants. For quantitative analysis, the bacterial and yeast isolates were cultured into the Pikovskaya broth at 90 rpm for 24 h at 37˚C and 72 h at 30˚C respectively. Culture supernatant was harvested by centrifugation at 10,000 rpm for 10 minutes and subjected to measure the soluble phosphate by vanadomolybdophosphoric acid.[12] One ml of supernatant was mixed with 2.5 ml of Barton's reagent followed by addition of distilled water to make the final volume up to 50 ml. After 10 minutes, the intensity of yellow color was measured with spectrophotometer at 430 nm. The soluble phosphate concentration was estimated based on the dihydrogen potassium phosphate standard curve.

Estimation of siderophore

Qualitative detection of siderophore was performed using Chrome Azurol S (CAS) agar medium.[13] To detect siderophore production, isolates were inoculated on same medium and incubated at 30˚C for 72 h to observe the orange halo surrounding the colony. Zone was calculated by dividing the color zone on CAS agar by total diameter of colony. For quantification, isolates were inoculated into the iron free M9 broth and incubated for 48 h at 30˚C with constant shaking at 90 rpm. After centrifugation at 10,000 rpm for 5 minutes, 1 ml of culture supernatant was mixed with 1 ml of CAS reagent and incubated for 15 minutes and color change was measured with spectrophotometer at 630 nm. Siderophore content was calculated by using the formula, siderophore unit (%) = $[(Ar - As)/Ar]\ x\ 100$ where, Ar means absorbance of reference CAS solution and As means Absorbance of sample solution.[14]

Detection of nitrogen fixing activity

Qualitative detection of nitrogen fixing activity was performed on the nitrogen free Jensen's agar medium (Himedia, India).[15] Isolates were inoculated on the same medium and incubate for 5 days at 30-37˚C. Growth of isolates on plate indicates qualitative evidence of atmospheric nitrogen fixation.[16]

Effect of endophytes on seed germination and vigour of greengram

Preliminary study on effect of isolates was performed on greengram using standard roll towel method.[17]

Greengram seeds were treated with each of the isolated culture (OD 1.0 at 660 nm) and incubated at 27°C with 80% relative humidity. The root and shoot length of seedlings were measured at sixth day and the percentage of germination and Vigour Index (VI) were calculated using the formula.[18]

Morphological, biochemical and molecular characterization of the isolates

Morphological, biochemical and molecular characterization of the isolates was performed according to the standard procedure.[19-21] For the molecular characterization, cultured cells were subjected to the genomic DNA extraction following supplier's protocol (Saffron Life science, India). 16S universal primers (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-CGGTTACCTTGTTACGACTT-3') and ITS primers (5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATATGC-3') were used to amplify 16S rRNA from bacteria and the fragment of Internal transcribed spacer (ITS) region of yeast, respectively. The resultant sequence from bacterium (MSB1) and yeasts (MSY1 & MSY2) was compared with NCBI-BLASTn homology search tool. Based on the maximum identity score, sequences were selected and aligned using multiple alignment software program Clustal W.[22] A distance matrix was generated and the phylogeneitc tree was constructed using MEGA 7 [23] and the evolutionary history was inferred by using algorithm for Neighbour-Joining.[24]

RESULTS

Isolation of endophytes from the flower of mahua

Total seven bacteria and two yeast endophytic strains were isolated on NA and MGYP agar medium respectively, from the flower of mahua. Among all, MSB1 (3.04 ± 0.13 CFU/g), MSY1 (2.99 ± 0.09 CFU/g) and MSY2 (2.95 ± 0.16 CFU/g) were found to be persistent and recurrent (Table I) that indicates its dominancy in the mahua flower. Thus, bacteria MSB1 and yeast MSY1 and MSY2 were selected for further study.

Plant growth promoting traits of endophytes

Bacteria (MSB1) and yeast (MSY1 and MSY2) were screened for their plant growth promoting abilities, *i.e.* IAA production, siderophore production, phosphate solubilisation, and nitrogen fixation. IAA production among the isolates ranged from 15.14 to 39.64 µg/ml (Table II), where highest production of IAA was recorded in MSY2 (39.64 ± 1.78 µg/ml). Qualitative assay for phosphate solubilisation shows that yeast endophytic strains MSY1 and MSY2 were able to produce clear zone on Pikovskaya agar medium. The highest phosphate solubilisation index was recorded 1.6 ± 0.1 mm for MSY1 followed by MSY2 (1.5 ± 0.2 mm). Further, quantitative assay indicates that yeast strain MSY1 and MSY2 shows 12.24 ± 0.38 µg/ml and 10.79 ± 0.28 µg/ml phosphate solubilisation, respectively but, MSB1 did not show phosphate solubilisation. Results of CAS agar assay indicated that only bacterial isolate (MSB1) gave positive response to siderophore production by producing orange color halo (0.9 ± 0.1 mm). Qualitative assay using CAS solution shows that bacteria MSB1 produce 47.80 ± 1.93 percentages of siderophore units. While both the yeasts showed negative results for siderophore production. None of the test isolates were able to grow on nitrogen free medium.

***In-vitro* evaluation of isolates on seed germination and vigour**

Effect on seed germination and vigour index of greengram seeds was varied with different isolates treatment. All treatments had a significant effect on root length, shoot length and vigour index, compare to control. Isolates were found to increase the vigour index by 16.56 to 59.61%, but rate of enhancement was varied with different treatments (Table III). Highest vigour index was found with yeast MSY2 (1605.40) followed by MSY1 (1290.40) and bacteria MSB1 (1172.40).

Identification of bacteria and yeast isolates

Identification of all three endophytic isolates was carried out using morphological, biochemical and molecular approaches. Microbiological observation showed isolate MSB1, as aerobic, motile, gram-positive rods and spore forming. Colony was found intermediate, irregular, undulate, opaque and dull white on NA. Biochemical tests such as catalase, nitrate reduction, starch hydrolysis, Voges-Proskauer, gelatin liquefaction, casein hydrolysis and fermentation of glucose, lactose, maltose, sucrose were positive. Test for urease, H_2S production, methyl red, and indole production were reported negative. MSY1 isolate produced intermediate, round, regular, convex, moist, smooth, orange colony while in contrast to it, MSY2 isolate produced intermediate, round, regular, convex, moist, smooth, opaque, white colony. Both the yeast showed, bud tied to the mother cell but ascospore was observed only in MSY2. Isolate MSY1 was found positive for glucose, maltose, sucrose, glycerol, and nitrate assimilation while MSY2 was positive only for glucose and glycerol. Test for urea hydrolysis was found positive only for MSY2. All above data confirmed that both yeast isolates (MSY1 & MSY2) were from different division under eukaryotic classification.The identity of isolates was confirmed by sequencing of amplified fragments of rDNA and ITS regions of the isolates and NCBI-BLAST (Basic Local Alignment Search Tool), online homology search program, where MSB1, MSY1 and MSY2 significantly aligned with *Bacillus amyloliquefaciens* (Figure. I), *Rhodotorula* sp. and *Pichia kudriavzevii*, respectively (Figure. II). Based on nucleotide homology, the tree is drawn with the bootstrap (5000 replicates) and the evolutionary distances were computed using the p-distance method. The 16S rDNA gene sequences of *B. amyloliquefaciens* and ITS gene sequences of *Rhodotorula* sp. and *P. kudriavzevii* determined in this study were deposited in GenBank database under accession number KY680541, KY680542 and KY680543 respectively.

Table I
Endophyte isolates from the flower of mahua

Serial number.	Total Endophytic population (Colony Forming Unit/g)	Endophytic isolates	Colonization level (Colony Forming Unit/g)
1	Bacteria 3.43 ± 0.13	MSB1	3.04 ± 0.13
2		MSB2	2.46 ± 0.15
3		MSB3	2.39 ± 0.36
4		MSB4	2.56 ± 0.24
5		MSB5	1.59 ± 1.38
6		MSB6	2.33 ± 0.35
7		MSB7	1.79 ± 1.56
8	Yeast 3.28 ± 0.07	MSY1	2.99 ± 0.09
9		MSY2	2.95 ± 0.16

The figures in the column are the mean value of 3 replicates ± Standard Error (SE).

Table II
Plant growth promoting properties of isolates

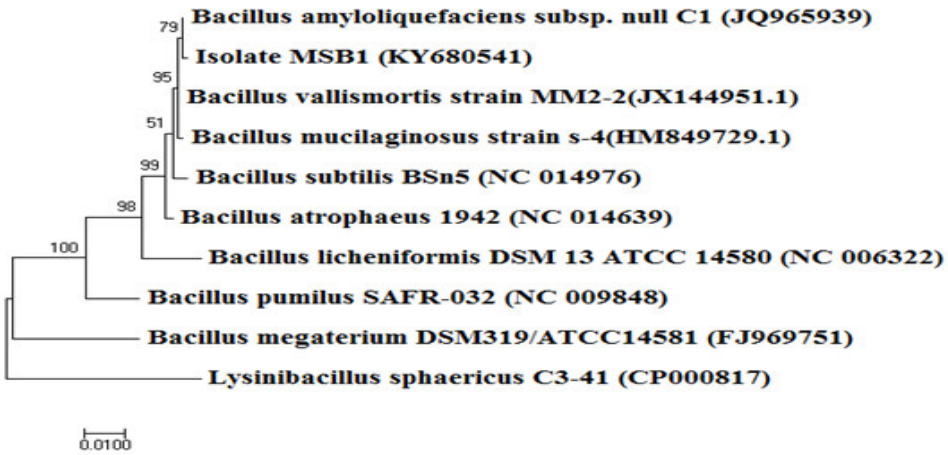
Isolates	Plant growth promoting properties					
	IAA	Phosphate solubilisation		Siderophore		Nitrogen fixation
	Quantitative (µg/ml)	Qualitative (mm)	Quantitative (µg/ml)	Qualitative (mm)	Quantitative (%)	Qualitative
MSB1	18.57 ± 1.04	-	-	0.9 ± 0.1	47.80 ± 1.93	-
MSY1	15.14 ± 0.80	1.6 ± 0.1	12.24 ± 0.38	-	-	-
MSY2	39.64 ± 1.78	1.5 ± 0.2	10.79 ± 0.28	-	-	-

The figures in the column are the mean value of 3 replicates ± Standard Error (SE). (−) indicate negative test

Table III
Effect of isolates on seed germination and seedling vigour of greengram (cv. Pusa Vishal)

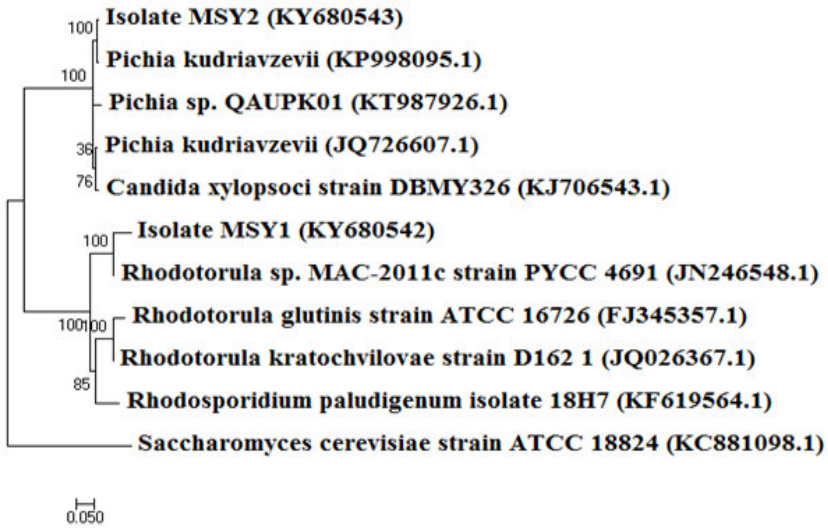
Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
Control	100	7.75[d]	2.31[d]	1005.80[d]
MSB1	100	8.53[c]	3.20[c]	1172.40[c]
MSY1	100	9.56[b]	3.35[b]	1290.40[b]
MSY2	100	12.43[a]	3.62[a]	1605.40[a]
SEM	0	0.09	0.05	9.44
C.D at 0.05	NS	0.26	0.14	28.28
C.V %	0	2.04	3.35	1.66

Data are mean of four replicates; data followed by same letter in a column are not significantly different (p = 0.05)



The evolutionary history was inferred using the Neighbour-Joining method with 5000 replicates and branch length 0.15688505

Figure I
Phylogeneitc tree for evolutionary relationships of bacterial isolate MSB1.



The evolutionary history was inferred using the Neighbour-Joining method with 5000 replicates and branch length 1.13515784

Figure II
Phylogenetic tree for evolutionary relationships of yeast isolates MSY1 & MSY2

DISCUSSION

India being an agricultural country, it is a great challenge for the nation to increase the food production for the escalating population which is growing at immersion line rate against food. Usage of chemical fertilizers is greater than never before, and the heavy dependence on costly chemical fertilizers is strongly linked with health hazards and environmental pollution. Therefore, eco-friendly techniques are needed for agriculture sustainability. There has been a large body of literature describing potential uses of plant associated microbes as agents stimulating plant growth and managing soil and plant health.[25] Therefore, isolation and identification of novel endophytes with plant growth promoting microbes is need of the nation. Traditional knowledge of tribal farmers to use mahua flower, seed cake and fermented waste as organic manures for crop improvement provided a prospect to this study. Importantly, reports on PGP endophytes of mahua flower is limited thus; enumeration of dominant endophytic bacterial and yeast population having PGP properties was isolated and identified from flower of mahua. Interestingly, the population densities of cultivable bacteria found in the present study was similar to the previous study in grapevine flowers, berries, and seeds. [6] The dominance of endogenous yeast flora within mahua flowers was expected. [1] Role of these mahua flower endophytic microorganisms was unknown. Therefore, role of cultivable endophytes for PGP traits such as production of IAA and siderophore, nitrogen fixation and phosphate solubilization were evaluated. IAA is the most common growth regulator produced by PGP microbes and is responsible for plant growth. Production of IAA found commonly in the strains belonging to *Bacillus* sp., which have an effect on plant growth attributes. In the current study, we found that isolate *B. amyloliquefaciens* MSB1 produced 18.57 µg/ml of IAA in presence of L-tryptophan, which was much higher than the previous report where strain of *B. amyloliquefaciens* FZB42 produced 0.051 µg/ml IAA this also confirms that our strain is different from the previously reported *B. amyloliquefaciens* strain.[10] In the current study IAA producing yeast *Rhodotorula* sp. MSY1 and *Pichia kudriavzevii* MSY2 were also isolated, and found to produce 15.14 µg/ml and 39.64 µg/ml, respectively. This particular aspect agrees with the results obtained in similar researches on different plant species where three endophytic *Rhodotorula* yeast species and *P. kudriavzevii* LM128 were isolated, identified and evaluated for their capability to produce IAA and among them *P. kudriavzevii* LM128 were able to produce 27.1 µg/ml IAA.[26] Phosphorus (P) is one of the major essential macronutrients for plants growth and most of the cultivated soils are deficient in available forms of P.[27] Microorganisms are situated at the central position of the soil P cycle and play a significant role in mediating the transfer of P between different inorganic and organic soil P fractions, subsequently releasing available P for plant acquisition. [28, 29] Bacteria and yeast isolates were screened for phosphate solubilization. Unlike, *B. amyloliquefaciens* (CM-2 & T-5) phosphate solubilisation was not observed in MSB1, this further proves that MSB1 is novel strain of *B. amyloliquefaciens*.[30] Captivatingly, both yeast strains showed positive results for phosphate solubilisation. The production of soluble phosphate from $Ca_3(PO_4)_2$ was higher 12.24 µg/ml in MSY1 strain compared to MSY2 strain (10.79 µg/ml). It is in agreement with the previous work on *Rhodotorula* sp. PS4.[31] Role of siderophore in plant growth promotion is well documented for bacteria as well as for yeasts.[32, 33] In the present study, only bacterial isolate *B. amyloliquefaciens* MSB1 but no isolated endophytic yeast strains produced siderophore. Thus, it confirms that the ecological roles played by endophytic microbes could be diverse and varied depending on the source and site of isolation. Those strain which have high potential for plant growth could be used as biofertilizers through the production of IAA, production of siderophore and N_2 fixation, as well as by converting insoluble phosphorous to soluble one, making it accessible to plant absorption. Therefore, research that

involves those endophytes which have more than one feature for promoting plant growth is important for a better understanding of their interaction with the plant. Thus, *in vitro* study with greengram seed treated with bacteria (MSB1) as well as yeast (MSY1 and MSY2) was carried out. Treated seed significantly increase the green gram root length, shoot length and vigour index of seedling over control. Similar results were reported in previous study with *B. amyloliquefaciens*[34] as well as for yeast species belong to *Rhodotorula* genus that shows beneficial effect on plant growth in field condition.[35] To best of our knowledge presence of yeast belongs to *Rhodotorula* genera within mahua flower, was not reported previously. Thus, our investigation extends the spectrum of endophytic organism of mahua with PGP traits.

CONCLUSION

In present investigation one bacterial (*B. amyloliquefaciens* MSB1) and two yeast (*Rhodotorula* sp. MSY1 and *Pichia kudriavzevii* MSY2) endophytic strains with PGP traits and positive effect on plants were isolated and *in vitro* characterized.

ACKNOWLEDGEMENT

Authors are grateful to Mr. Jitendra Patel and Komal Chandarana from the Department of Asia Green Bio Crops Science for their support and encouragement. We would also like to thank Dr. Himanshu Desai, Dr. Jayesh Pastagia and Dr. Priti Parmar from Navsari Agricultural University for their invaluable guidance.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Gupta A, Chaudhary R, Sharma S. Potential applications of Mahua (*Madhuca indica*) biomass. Waste Biomass Valorization. 2012 Jun ;3(2):175-89.
2. Jha S, Vaibhav V, Suneetha V. A Culinary Mahua (*Madhuca indica*) flower from Bihar, India? A potential in Production of Jam, Alcohol for Pharmacological benefits with Fertilizer value. Int j drug dev res.2013 Apr-Jun;5(2):362-7.
3. Higa T, Parr JF. Beneficial and effective microorganisms for a sustainable agriculture and environment. Atami, Japan: International Nature Farming Research Center ; 1994 Dec.
4. Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, et al. Endophytic bacteria and their potential applications. Crit Rev Plant Sci. 2002;21(6):583-606.
5. Gavankar R, Chemburkar M. Isolation and Characterization of Native Yeast from Mahua Flowers. Int J Curr Microbiol App Sci. 2016;5(11):305-14.
6. Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol. 2011 July 1;;62(1):188-97.
7. Manwar J, Mahadik K, Paradkar A, Sathiyanarayanan L, Vohra M, Patil S. Isolation, biochemical and genetic characterizations of alcohol-producing yeasts from the flowers of Woodfordia fruticosa. J Young Pharm. 2013 Dec 31 ;5(4):191-4.
8. Sutton S. Accuracy of plate counts. Journal of validation technology. 2011july 1;17(3):42-6.
9. Glickmann E, Dessaux Y. A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol. 1995 Feb;61(2):793-6.
10. Idris EE, Iglesias DJ, Talon M, Borriss R. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol Plant-Microbe Interact. 2007 Jun;20(6):619-26.
11. Nosrati R, Owlia P, Saderi H, Rasooli I, Malboobi MA. Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iran J Microbiol. 2014 Aug;6(4):285-95.
12. Subba Rao N. Phosphate solubilization by soil microorganisms. Advances in agricultural microbiology. Oxford & IBH publishing Co. 1982:1-149.
13. Schwyn B, Neilands J. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 1987 Jan;160(1):47-56.
14. Payne SM. Detection, isolation, and characterization of siderophores. Methods Enzymol. 1994; p. 235:329.
15. Reddy CN, Arunasri K, Kumar YD, Krishna KV, Mohan SV. Qualitative in vitro evaluation of plant growth promoting activity of electrogenic bacteria from biohydrogen producing microbial electrolysis cell towards biofertilizer application. J Energy Environ Sustainability. 2015 Nov;1:47-51.
16. Döbereiner J, Alef K, Nannipieri P. Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. Methods in Applied Soil Microbiology and Biochemistry. 1995:134-41.
17. Association IST, editor International rules for seed testing: edition 2010: International Seed Testing Association.
18. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Sci. 1973 Nov;13(6):630-3.
19. Murray R, Costilow RN, Nester E, Wood W, Krieg N, Phillips G. Manual of methods for general bacteriology. American society for microbiology, Washington, DC. 1981:31.
20. Pelczar MJ BR, Burnett GW, Conn HJ, Demoss RD, Euans, EE WF, Jennison MW, Meckee AP, Riker AJ, Warren J,, OB W. Manual of

Microbiological Methods by the Society of American Bacteriologist. Mc Graw Hill, New York; 1957.
21. Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and identification: Cambridge University Press; 1983.
22. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994 Nov;22(22):4673-80.
23. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016 Mar; 33(7): 1870-4.
24. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987 Jul;4(4):406-25.
25. Pindi PK, Satyanarayana S. Liquid microbial consortium-a potential tool for sustainable soil health. J Biofertil Biopestic2013 Aug;4:1-9.
26. Limtong S, Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. World J Microbiol Biotechnol.2012 Dec;28(12):3323-35.
27. Goldstein AH. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Alternative Agric. 1986 Apr;1(02):51-7.
28. McLaughlin MJ, Alston A, Martin J. Phosphorus cycling in wheat pasture rotations. II. The role of the microbial biomass in phosphorus cycling. Aust J Soil Res. 1988;26(2):333-42.
29. Oberson A, Friesen DK, Rao IM, Bühler S, Frossard E. Phosphorus transformations in an Oxisol under contrasting land-use systems: the role of the soil microbial biomass. Plant Soil. 2001 Dec;237(2):197-210.
30. Tan S, Jiang Y, Song S, Huang J, Ling N, Xu Y, et al. Two *Bacillus amyloliquefaciens* strains isolated using the competitive tomato root enrichment method and their effects on suppressing Ralstonia solanacearum and promoting tomato plant growth. Crop Protect. 2013 Jan;43:134-40.
31. Mundra S, Arora R, Stobdan T. Solubilization of insoluble inorganic phosphates by a novel temperature-, pH-, and salt-tolerant yeast, *Rhodotorula* sp. PS4, isolated from seabuckthorn rhizosphere, growing in cold desert of Ladakh, India. World J Microbiol Biotechnol. 2011 Oct;27(10):2387-96.
32. Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, et al. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microb. Cell Fact. 2009 Nov;8(63):1-12.
33. Ferramola MS, Benuzzi D, Calvente V, Calvo J, Sansone G, Cerutti S, et al. The use of siderophores for improving the control of postharvest diseases in stored fruits and vegetables. Microbial Pathogens and Strategies for Combating Them: Science: Technology and Education. Formatex Research Center Spain; 2013. p. 1385-94.
34. Chowdhury SP, Dietel K, Rändler M, Schmid M, Junge H, Borriss R, et al. Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. Plos one. 2013 Jul;8(7):1-10.
35. El-Kholy M, El-Ashry S, Gomaa A. Biofertilization of maize crop and its impact on yield and grains nutrient content under low rates of mineral fertilizers. J App Sci Res.2005;1(2):117-21.

Reviewers of this article

Dr. (Mrs.) FARIDA P. MINOCHEHERHOMJI

Associate Professor,
B.P. Baria Science Institute, Near Fuwara,
NAVSARI-396445, India

Sanjay Jha, Ph.D

Associate Professor, Aspee Shakilam Biotech Institute, Navsari Agricultural Univ, Surat.

Asst. Prof. Dr. Deepansh Sharma, M.Sc, M.Phil, Ph.D.

Assistant Professor, School of Biotechnology and Bioscience, Lovely Professional University, Phagwara, Punjab, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript