Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [3] 2022: 108-113

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation of KSB, PSB, and NFB and Preparation Of Bio-Formulation

Jasmine Bhullar[1], Dhwani Upadhyay[2], Indrani Bhattacharya[2], Anjali Thakur[2], Prasad Andhare[3]*
[1]Student, M.Sc Microbiology, Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya, Gujarat
[2] Assistant Professor, Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya , Gujarat
[3] Assistant Professor, Biological Sciences, PDPIAS, Charotar University of Science and Technology, Changa, Anand, Gujarat.
*Corresponding Author: Dr. Prasad Andhare;
E-Mail: prasadandhare.as@charusat.ac.in

ABSTRACT
As the world population increases in a geometric manner, it puts great pressure on the agriculture sector to meet the increasing demand for food production. Soil fertility is an important factor when it comes to high crop production not only from an economic aspect but also froman environmental aspect. The increasing dependency on chemical fertilizers to enrich the soil with important nutrients for high crop yields in a long run is not the most sustainable option. The excessive use of these chemical fertilizers has led up to several issues including soil degradation. The detrimental effects of chemical fertilizers possess a great threat not only to the present but also to future generations and the environment. Biofertilizers are a sustainable alternative for good soil and crop management Numerous species of soil bacteria which flourish in the rhizosphere of plants stimulate plant growth by a plethora of mechanisms. The present study was intended to isolate nitrogen-fixing bacteria, potassium-solubilizing bacteria (KSB), and phosphate solubilizing bacteria (PSB) from the rhizosphere soil of Tinospora cordifolia. The isolates were obtained from Arboretum MSU Garden Vadodara. The isolates were screened for their ability to solubilize potassium and phosphate on modified Aleksandrow agar medium and Pikovskaya's Agar respectively. Three isolates from each nitrogen-fixing bacteria, phosphate solubilizing bacteria, and potassium-solubilizing bacteria were selected to test their compatibility. With the three best compatible isolates, a Guar gum-based formulation was prepared.
Keywords *- Sustainable Agriculture, Plant growth-promoting rhizobacteria, biofertilizer, plant and bacteria interaction, formulation.*

Received 02.08.2022 Revised16.09.2022 Accepted 25.10.2022

INTRODUCTION

Soil microbial communities are the world's largest repository of biological variety so far discovered. The rhizosphere, a small zone of soil impacted by root secretions, can include up to 10^{-11} microbial cells per gram root and over 30,000 prokaryotic species. (1) Knowing the dynamic mechanisms that characterize plant-soil connections begins with understanding the interactions between functional groups of the soil micro flora. (2).The rhizosphere, a volume of soil around roots that is impacted chemically, physically, and biologically by the plant root, is an ideal environment for microorganism proliferation, with implications on plant health and soil fertility(3). Rhizobacteria are rhizosphere-competent bacteria that colonies plant roots aggressively. They can be free-living, parasitic, or saprophytic, and their diversity is dynamic, with changes in community structure and species abundance occurring often. (4) It is believed that these microbial communities support plant growth, yield, and quality and are called "plant growth-promoting rhizobacteria (PGPR)" (5). The application of rhizobacteria that promote plant growth can reduce chemical fertilizer use, reduce production costs as well as recognize and implement sustainable soil and crop management practices for more sustainable agriculture and higher soil fertility (6). The major influences that the rhizosphere microorganisms have on plants today become an important tool to guard the health of plants in an eco-friendly manner(7).In recent years, PGPR has received a lot of attention for its potential to replace agrochemicals (fertilizers and pesticides) for plant growth promotion through a variety of mechanisms that include soil structure formation, decomposition of organic matter, recycling of essential elements, solubilization of mineral nutrients, production of numerous plant growth

regulators, degradation of organic pollutants, stimulation of root growth, which is important for soil fertility, and biocontrol of soil and seed-boiling (8).Plants can only use 10–40% of chemical fertilizers in recent years, while the remaining 60–90% of fertilizer is lost due to immobilization, leaching, volatilization, and other processes. (9). The utilization of beneficial microbial strains in the soil is helpful because nutrients are released slowly and continuously. As a result, the demand for chemical fertilizers is reduced while the high goals for integrated nutrient management systems to achieve sustainable agriculture and a healthy environment are maintained (5). Liquid bioinoculants are specific formulations that include not only the necessary microorganisms and their nutrients, but also cell protectants or chemicals that promote a longer shelf life and resistance to unfavorable circumstances[10].Gums, carboxymethyl cellulose, and polyalcohol derivatives are common polysaccharides used to modify the fluid properties of liquid formulations [11].The use of liquid biofertilizer formulations as a solution for extending the shelf life of biofertilizers might be studied. Unlike solid carrier-based biofertilizers, liquid formulation allows for the incorporation of appropriate nutrients, cell protectants, and inducers for cell/spore/cyst formation, resulting in a longer shelf life [12].The use of mixed culture formulations is an area that has not been explored much when compared to single strain inoculants application [10]. The aim of this study is to isolate nitrogen-fixing bacteria, potassium solubilizing bacteria, and phosphate solubilizing bacteria and to test their compatibility for the development of a mixed strain formulation as an alternative to chemical fertilizers.

MATERIAL AND METHODS

Sample collection

Rhizosphere soil samples were collected from Arboretum MSU Garden in November 2021. At a depth of 10–15 cm underneath the surface of soil, a sample was taken and collected into a sterile vial sieved through a 4-mm mesh sieve and stored at 48°C at field moisture content [13]. Rhizosphere soil samples were collected from a healthy field-grown plant of *Tinospora cordifolia*.

Azotobacter isolation and characterization

The collected soil sample was serially diluted from 10^{-1} to 10^{-10} and was spread plated on Azotobacter Agar (Mannitol) media and incubated for 48h at 30°C [14].Bacterial culture was repeated three times to obtain the purity of the cultured isolate of bacteria. . The strains were inoculated in nutrient broth and incubated for 48 hours at 100 rpm at 30°C.

Isolation of Phosphate Solubilizing Bacteria

To isolate PSRB rhizosphere soil samples were serially diluted from 10^{-1} to 10^{-10}were spread plated and onto Pikovskaya's agar medium and incubated at 30°C [15]. Using the quadrant streak method, single, well-separated colonies with clear zones were streaked onto new Pikovskaya's solid medium. This process was continued until a pure culture was produced. The strains with clear zones were inoculated into nutritional broth and incubated for 48 hours at 100 rpm at 30°C. [16].

Isolation Potassium Solubilizing Bacteria

To isolate KSB from the rhizosphere soil sample was serially diluted from 10^{-1} to 10^{-10} were spread plated on Aleksandrow agar medium. The isolates were then incubated at 30°C for 4 days [17]. Detection of potassium solubilization was based upon the ability of solubilization zone formation. This technique is based on using an acid-base indicator dye (bromothymol blue, BTB) to modify the Aleksandrow medium to facilitate observation of halo zone formation surrounding colonies on agar plates [18]. The bacterium which showed a zone of clearance on repeated subculture onto Aleksandrow agar medium was selected were inoculated into nutritional broth and incubated for 48 hours at 100 rpm at 30°C, for further studies.

Morphological and microscopic observation

On selective mediums, the bacterial species form distinct colonies. Gram staining was used to examine the isolates' morphology. Under a compound microscope, the stained cells were examined. The Gram reaction as well as the shape of the cells was recorded.

Compatibility test

Bacterial cultures were streaked on an agar plate with one culture streaked in the center of the agar plate while the other two cultures were streaked as radiating streaks from the Centre [6]. The plates were incubated at 30°C for 24 hours and the zone of inhibition was observed.

Liquid bio-Formulation preparation

After 48 h of incubation, the nutrient broth culture of the three isolates containing 9×10^{8} cfu/ml was used for the preparation of the liquid bio-formulation. In 15 ml of bacterial suspension, 5g of the Guar gum and 5 mL of glycerol were mixed under sterile conditions and viability of bacteria in the formulation was noted [19].

RESULTS

Sample collection

The sampling approach used in this study was established after considering the various factors that influence the occurrence of *Azotobacte*r in soil. Because *Azotobacter* distribution in the rhizosphere is not dependent on plant type [13], soil samples from the rhizosphere of *Tinospora cordifolia* were obtained.

Isolation of Azotobacter, PSB AND KSB-

The figure 1 shows the three bacterial species isolated using the three selective media (Azotobacter Mannitol agar, Pikovskaya agar and Aleksandrow agar).Among them the isolate JB1 is Azotobacter while isolate JB2 is phosphate solubilizing bacteria - *Pseudomonas* and isolate JB3 is potassium solubilizing bacteria.

Colony morphology and microscopic observation -

All of the isolated bacteria showed typical bacterial colonisation characteristics morphologically. Two of the bacterial isolates (JB1, JB2) developed white colonies, whereas the isolate JB3developed a creamy white colony. In 6-7 days of incubation, the isolates had produced well-developed colonies. Bacterial species were further examined for their Gram's reaction and shape. Isolates JB1, JB2 were Gram negative whereas Isolate JB3 was Gram positive. The results are listed in Table 1.

Compatibility test

The plate contained all of the test bacteria in culture compatibility testing, with one bacterial strain in the centre and other strains streaks radiating from it. The experiment's results are shown in Figure 3.The plates were observed and no zone of inhibition was seen present between the radiating streaks and the streak present in the centre. This helped in concluding that the three bacterial isolates were compatible with each other and can be together grown in the same nutrient media to develop a mixed culture liquid Bioformulation in figure 4 [6].

Viability of bacteria in bio-formulation

Absorbance spectrophotometry, which estimates the optical density at a certain wavelength and correlates it to the number of bacterial cells present in a sample, has also been used to track cell populations shown in figure 5.

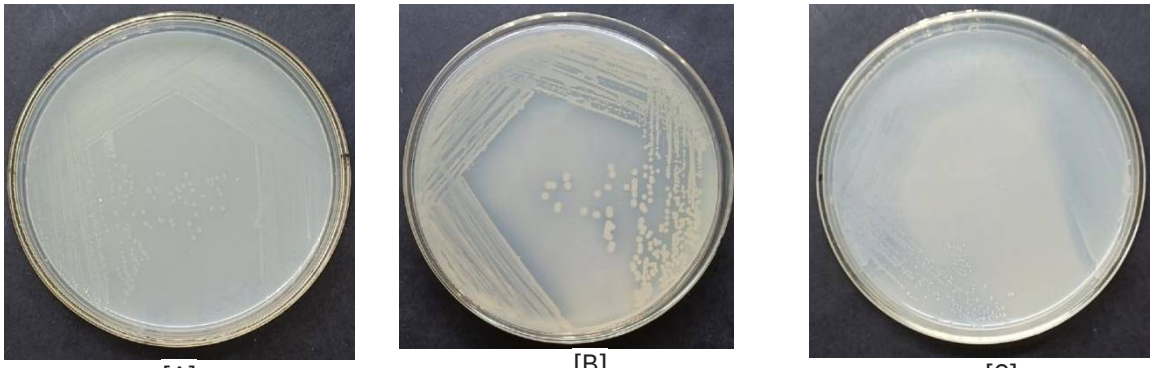
DISCUSSION

Several strains of bacteria were obtained during the isolation and characterization of bacteria from plant roots and rhizosphere soil by using dilution series on selective media including Azotobacter agar, Pikovskaya's agar, and Aleksandrow agar. The sampling technique employed in the process for isolation was implanted after considering various factors that can affect the occurrence of rhizobacteria in the soil.

As described by Kole and Altosaar**,** *Azotobacter* distribution in rhizosphere is independent of the plant type [20]. Because richness in organic matter and phosphates, which are particularly crucial for *Azotobacter* metabolism, encourages *Azotobacteria* diffusion in soil, the samples were taken from cultivated soils just like PSB that are found abundantly in soils with rich organic matter. The aim of this study was to discover compatible strains of rhizobacteria that can be used to create microbial consortia for bio-formulation preparation. The microbial strains are considered compatabile when they have no growth suppressive effect on each other .The compatibility test showed no apparent zone of inhibition between the bacteria strain streaked in centre and the streaks of strain radiating from it, indicating that these three isolates were compatible with each other. The discovery of Plant Growth Promoting Rhizobacteria (PGPR) gave rise to the idea that combining beneficial microbial isolates might improve the efficacy of single isolates [21]**.** The mixed culture technique is essentially an attempt to apply these ideas to natural systems like agricultural soils to alter the microbial balance in favor of enhanced plant growth, productivity, and protection. (18).Gaur gum is a natural polysaccharide that is used as a carrier for bio-formulation preparation. The presence of viable cells in the formulation is important to ensure its effectiveness when applied to the plants. The steady growth curve of bacteria in the formulation that was later maintained a stationary growth. This led us to conclude that this formulation could be applied to the plants as a sustainable alternative to chemical fertilizers.

Table 1.Morphological characters of bacterial Isolates on selective media

Characteristic	Colony shape	Cell shape	Colony colour	Transparency	Margin of colony	Surface of colony	Gram stain
Isolate A	Circular	Rod	White	Opaque	Entire	Smooth, flat	Negative
Isolate B	Circular	Rod	White	Opaque	Entire	Smooth elevated	Negative
Isolate C	Circular	Rod	Creamy white	Translucent	Entire	Smooth, elevated	Positive



[A] [B] [C]

Figure 1; [A]isolate JB1 on azotabacter agar , [B]Isolate JB2 on Pikovaskaya agar media , [C]Isolate JB3 on Aleksandrow agar Aleksandrow agar

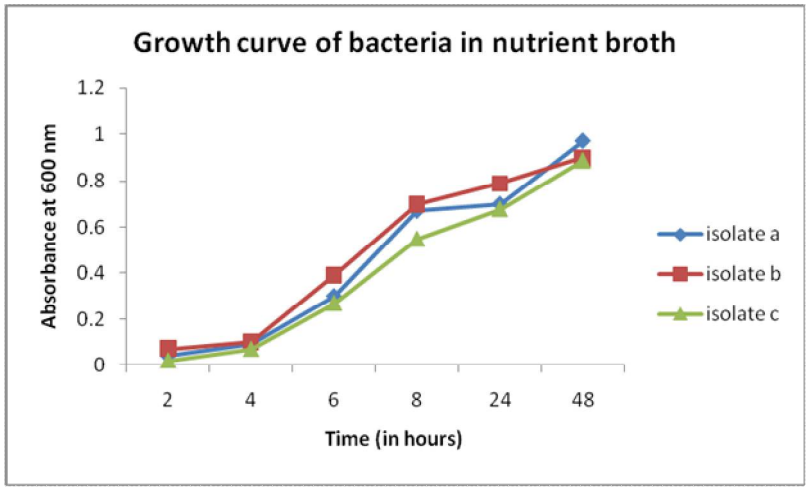


Figure 2.Growth curve of bacteria in nutrient media
Isolate a—JB1,isolate b-JB2,isolate c-JB3

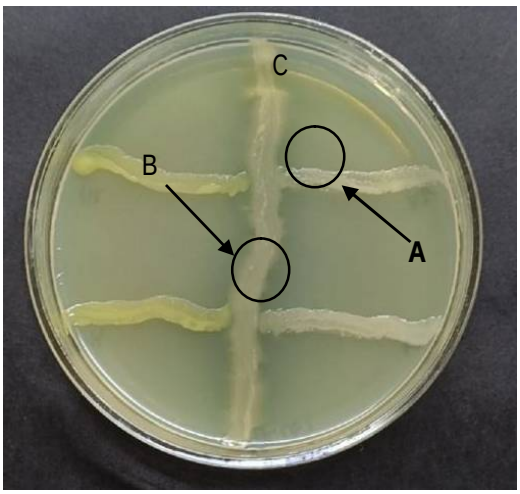


Figure 3.Compatibility test where no zone of inhibition was observed
A-JB1,B-JB2,C-JB3

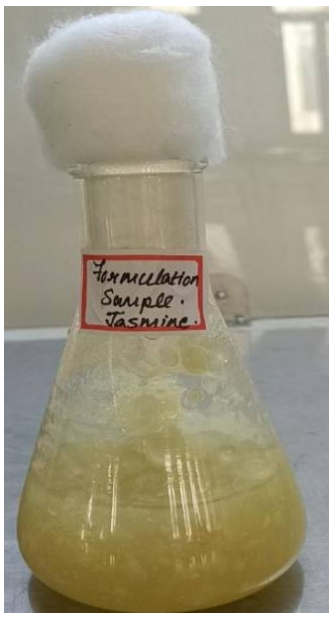


Figure 4. Bio-formulation

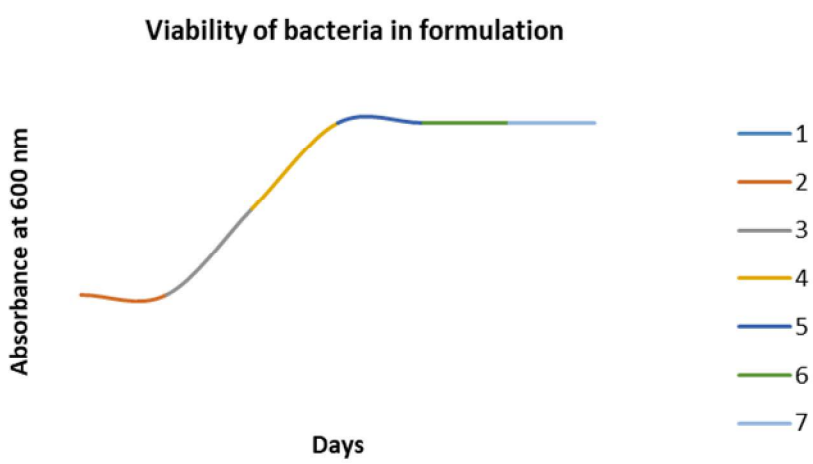


Figure 5.Spectrophotometric measurements of the optical density for the bacterial cell count.

ACKNOWLEDGMENTS

It is our privilege and honor to express our sincerest gratitude to the Parul University, Vadodara, Gujarat for providing me with all the necessary support and facilities including state-of-the-art infrastructural facilities with advanced technological scientific laboratories and everything else that was required to carry out this work.

REFERENCES

1. Berendsen RL, Pieterse CM, Bakker PA. The rhizosphere microbiome and plant health. Trends in plant science. 2012 Aug 1;17(8):478-86.]
2. Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and soil. 1997 May;192(1):71-9
3. Kaymak HC. Potential of PGPR in agricultural innovations. Plant growth and health promoting bacteria. 2010:45-79.
4. Kloepper JW. Plant growth-promoting rhizobacteria on radishes. InProc. of the 4th Internet. Conf. on Plant Pathogenic Bacter, Station de PathologieVegetale et Phytobacteriologie, INRA, Angers, France, 1978 1978 (Vol. 2, pp. 879-882).

5. Singh TB, Sahai V, Goyal D, Prasad M, Yadav A, Shrivastav P, Ali A, Dantu PK. Identification, characterization and evaluation of multifaceted traits of plant growth promoting rhizobacteria from soil for sustainable approach to agriculture. Current Microbiology. 2020 Nov;77(11):3633-42.
6. Prasad M, Srinivasan R, Chaudhary M, Choudhary M, Jat LK. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. InPGPR amelioration in sustainable agriculture 2019 Jan 1 (pp. 129-157). Woodhead publishing.
7. Akhtar N, Qureshi MA, Iqbal A, Ahmad MJ, Khan KH. Influence of Azotobacter and IAA on symbiotic performance of Rhizobium and yield parameters of lentil. J Agric Res. 2012 Jul 1;50:361-72.
8. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J MicrobBiochem Technol. 2015 Mar;7(2):096-102.
9. Adesemoye AO, Kloepper JW. Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied microbiology and biotechnology. 2009 Nov;85(1):1-2.
10. Vora MS, Shelat HN, Vyas RV. Liquid biofertilizers: a new vistas. Handbook of biofertilizers and microbial pesticides. Satish serial publishing house, New Delhi. 2008:87-90.
11. Paau AS. Formulation of beneficial organisms applied to soil. InFormulation of microbial biopesticides 1998 (pp. 235-254). Springer, Dordrecht.
12. Brar SK, Sarma SJ, Chaabouni E. Shelf-life of biofertilizers: an accord between formulations and genetics. J Biofertil Biopestici. 2012;3(5).
13. Aquilanti L, Favilli F, Clementi F. Comparison of different strategies for isolation and preliminary identification of Azotobacter from soil samples. Soil Biology and biochemistry. 2004 Sep 1;36(9):1475-83.
14. Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N. Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma. 2014 May;251(3):511-23.
15. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus. 2013 Dec;2(1):1-4.
16. Ranjan A, Mahalakshmi MR, Sridevi M. Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2013 Jan 1;3(1):29.
17. Prajapati KB, Modi HA. Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. CIBTech J Microbiol. 2012;1(2-3):8-14.
18. Etesami H, Emami S, Alikhani HA. Potassium solubilizing bacteria (KSB):: Mechanisms, promotion of plant growth, and future prospects A review. Journal of soil science and plant nutrition. 2017 Dec;17(4):897-911.
19. Santhosh GP. Formulation and shelf life of liquid biofertilizer inoculants using cell protectants. IJRBA T, II. 2015(7):243-7.
20. Kole MM, Page WJ, Altosaar I. Distribution of Azotobacter in Eastern Canadian soils and in association with plant rhizospheres. Canadian journal of microbiology. 1988 Jun 1;34(6):815-7.
21. Kloepper JW, Scher FM, Laliberte M, Tipping B. Emergence-promoting rhizobacteria: description and implications for agriculture. InIron, siderophores, and plant diseases 1986 (pp. 155-164). Springer, Boston, MA.

CITATION OF THIS ARTICLE

J Bhullar, D Upadhyay, I Bhattacharya, A Thakur, P Andhare. Isolation of KSB, PSB, and NFB and Preparation of Bio-Formulation. Bull. Env.Pharmacol. Life Sci., Vol Spl Issue [3] 2022: 108-113