Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 02 (Mar–Apr 2024); pp 345–351

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Isolation and Identification of Agricultural Potential Microorganism from Jalgaon, Maharashtra (India)

Kishore Janardhan Patil*[1]

[1] Nilanjan Life Solutions, Jalgaon - 425 001, Maharashtra, India

Received: 14 Dec 2023; Revised accepted: 21 Feb 2024; Published online: 09 Mar 2024

Abstract

Microorganisms are ubiquitous and plays important role for maintaining the nutritive value of soil. Soil microbes are part of soil biological as well as organic matter, responsible for releasing nutrients from organic matter or convert them into more easily accessible form by the plants. The application of chemical fertilizers leads to decrease in microbial flora and fertility of soil. Therefore, it is necessary to explore substitutes to increase the efficiency and quality of crops with minimal environmental impact. The use of plant growth-promoting bacteria can provide solutions to some agri-environmental problems. In this study, Soil samples are collected from different region of Jalgaon area such as "Meharune Pimprala and Paldhi. Two bacterial strains of nitrogen fixing, phosphate solubilizing and potassium solubilizing are isolated and tested for growth promoting ability. Further on basis of biochemical characteristic, the isolated microorganism are identified with the help of ABIS online platform. The isolated organisms are identified as M1D1(*Bacillus megaterium*), M1B1 (*Pseudomonas fluorescens*), M2B2 (*Bacillus bataviensis*), M2C1 (*Bacillus tequilensis*), M3D2 (*Azotobacter chroococcum*) and M3B1 (*Azotobacter beijerinckii*). The results, in vivo, demonstrate that the bacteria increase the length of root and shoot with the number of leaves. The outcome implies that *Bacillus tequilensis* has properties as a plant growth promoter and can be used as a promising inoculant to enhance the growth of seedlings.

***Key words*:** Nitrogen fixing, Phosphate solubilizing, Potassium solubilizing, Growth promoting

Agriculture is an important aspect of the economy in all countries. It involves the production fodder for livestock, of food, medicinal corps and bioenergy. In many developing countries, agriculture is the primary source of income and employment for a significant portion of the population [1]. Microorganisms are ubiquitous in every part of biosphere, including soil, hot springs, inside rocks etc., Microorganisms present in soil play an important role in maintaining the biological balance in the environment. The fertility of soil and the accumulation of organic matter within a short time is dependent on the bacterial amount [2-4]. To enhance the soil fertility, it may contain diverse group of organisms in consortia like nitrogen fixing, phosphate solubilizing, potassium solubilizing and zinc solubilizing, iron oxidizing etc. Nitrogen is not present in soil parent material despite the fact that nitrogen content in the atmosphere is highest among all the atmospheric gases [5-6]. Hence, soil nitrogen input for plant nutrition and crop productivity largely depends on organic matter degradation, synthetic fertilizer applications, and biological nitrogen fixation [7]. Biological nitrogen fixation in plants can be a sustainable source of nitrogen and may divert our current dependence on industrial nitrogen production [8]. After nitrogen (N) and phosphorus (P), potassium (K) is the most important plant nutrient that has a key role in the growth, metabolism and development of plants [9]. Phosphorus (P) is an essential nutrient for plants that is involved in diverse biochemical processes including lipid metabolism and the biosynthesis of nucleic acids and cell membranes [10]. However, P is one of the most limiting nutrients in global agricultural ecosystems [11]. The PSMs, strains from bacterial genera (*Bacillus*, *Pseudomonas*, and *Rhizobium*), fungal genera (*Penicillium* and *Aspergillus*), actinomycetes, and arbuscular mycorrhizal [12]. Organic acids secreted by the microbes will dissolve the insoluble form of elements like phosphates, silicates and convert them into a plant-available soluble form [13]. Overuse of fertilizers can lead to soil degradation and negative environmental impacts, such as water pollution, environmental pollution etc. [14]. A range of microorganisms are known to increase the fertility of soil *A. chroococcum*, *Azospirillum basilensis*, *Bacillus weihenstephanensis*, *Bradyrhizobium sp.*, *Paenibacillus sp.*, *Pseudomonas corrugate*, *Rhizobium sp*., etc. [15]. Therefore, the present study aims to isolate the natural occurring soil organisms and its richness in beneficial microorganisms.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from different region of different zones of Jalgaon area such as "Meharune Pimprala and Paldhi. The sample were collected from the rhizosphere zone from a depth of 10 cm in a sterile container.

Adaptation and enrichment

***Correspondence to:** Kishore Janardhan Patil, E-mail: drkjpatil04@gmail.com; Tel: +91 9096820046

Citation: Patil KJ. 2024. Isolation and identification of agricultural potential microorganism from Jalgaon, Maharashtra (India). *Res. Jr. Agril. Sci.* 15(2): 345-351.

Soil samples collected from different regions of Jalgaon kept at room temperature for one week. 10 g of soil sample taken in 250 mL Erlenmeyer flask, 90 mL of 0.85% NaCl solution and kept on an orbital shaker at 120 rpm for 30 min. Serial dilution of the rhizosphere soil was carried till 10^{-7} and 0.1 mL of the aliquot was inoculated into Jeansons agar, Pikovskayas agar and Aleksandrow agar and incubated at 30°C for 72 to 96 hrs. Bacterial colonies were isolated, sub-cultured, and stored in Luria Bertani agar slants at 4°C [16].

Isolation of potassium solubilizing bacteria

The enriched soil samples were serial diluted up to 10^{-7} and inoculated on Aleksandrov agar medium which constitute glucose 5-0 g, Magnesium sulphate 5.0 g, Ferric chloride 0.006 g, calcium carbonate 0.6 g, Calcium phosphate 2 g, Mica 3.0 g, and agar 30 g/l, pH was adjusted to 6.5 and phenol red 0.1g/l dye as indicator, plates incubated at 37 °C for 4 days. Potassium solubilizers selected on the basis of zone formed around the colonies [17-18]. Potassium solubilization was determined on the basis of zone formed around the colonies D/d ratio according to Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance / Diameter of growth

Isolation of nitrogen solubilizing bacteria

The enriched soil samples were serial diluted up to 10^{-7} and inoculated on Jeansons agar composed of Sucrose 20.000, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Sodium chloride 0.5 g, Ferrous, sulphate 0.1 g, Sodium molybdate 0.005 g, Calcium carbonate 2.0 g, Agar 15.0 g/l, plates incubated at 37 °C for 4 days [19].

Phosphorus solubilizing bacteria

The enriched soil samples were serial diluted up to 10^{-7} and inoculated on Pikovskayas Agar composed of Yeast extract 0.5 g, Dextrose 10 g, Calcium phosphate 5 g, Ammonium sulphate 0.5 g, Potassium chloride 0.2 g, Magnesium sulphate 0.1 g, Manganese sulphate 0.0001 g, Ferrous sulphate 0.0001g, Agar 15 g/l, plates incubated at 37 °C for 4 days. Phosphorous solubilizing isolates selected on the basis of zone formed around the colonies [20]. phosphate solubilization efficiency was calculated on the basis of value of the diameter of the colony (C), diameter of halo zone (H), diameter of colony+ halo zone (Z) and the ratio Z/C of different isolates obtained on PKV agar plates. The use of the ratio Z/C helps to evaluate the activity of a given microorganism [21].

Study of cultural morphological and biochemical nature of isolates

In order to study the cultural morphology and biochemical characteristic of isolates modified medium such as Aleksandrove's agar media, Jeanson's agar medium and Pikovskays agar medium was used to grow isolates. Colony characteristics such as size, shape, texture, color, opacity and consistency were examined. Gram staining, endospore and capsule staining carried out for the purified isolates. Indole test, Methyl red test, Starch hydrolysis test, Simmons Citrate test, Triple Sugar Iron test, Voges Proskauer test, Casein hydrolysis test, H_2S production catalase test, motality test, oxidase test, urease test, arginine test, lysine decarboxilase test, sugar production test (lactose, fructose, sucrose, mannitol, and glucose) determined. The experiment performed by the method reported in Aneja [22], Dubey and Maheshvari [23] and Harley and Prescott [24].

Identification of bacterial isolates

Identification of bacterial isolates carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining, Motility test etc. The bacteria are identified by using ABIS software on basis of morphological and biochemical characters.

The estimation of phyto-toxicity and phyto-stimulation activity by Cucumber cotyledon bioassay

Cucumber cotyledon bioassay performed by the method described by Patil *et al*. [25]. The cucumber seeds were purchased from local market. Seeds were germinated on tissue paper saturated with autoclaved distilled water and test suspension in the Petri plates. For germination, seeds were incubated at room temperature in dark for 7 days. Further, the number of sprouted seeds was calculated, then the length of root and stem in cucumber seeds was measured to determine phyto-stimulation activity of suspension; and lastly the ability of isolates to promote growth was calculated (as % of the control response). Cotyledons were excised from cucumber seedlings (7 day old) that were grown in the dark condition. Cotyledons are pulverized in mortal and pistel in chloroform. The chloroform extract was used for determination of total chlorophyll content. A negative control with sterile distilled water alone and a synthetic cytokine 6-Benzylaminopurine (BAP) at 25 ppm is used as positive control. After the incubation, the cotyledons were collected and grounded with 80% acetone with mortar and pestle. The chlorophyll extract was collected and then centrifuged at 4000 rpm for 10 minutes. The resultant supernatant was analyzed for total amount of chlorophyll estimation using spectrometer [26].

RESULTS AND DISCUSSION

Isolation and screening of agriculturally important microbes

The purified bacteria grown on selective media, showed clear zone around colonies were selected, only those colonies were selected which showed distinct morphology. Out of 22 isolates only 06 bacterial isolates which showed distinct morphology were selected, having any two amongst PSB, KSB or nitrogen fixing ability. The isolates are abbreviated (Table 1) as M1D1, M2B1, M2B2, M2C1, M3D2 and M3B1. Where, 'B' represents soil sample from Meharun, 'C' Pimprala and 'D' Paldhi. On basis of mineral solubilization of fixing M1 stands for phosphate solubilizing, M2 potassium solubilizing and M3 nitrogen fixing. The ratio of potassium solubilization zone was measured according to Khandeparkar's selection ratio as listed in (Table 2).

Table 1 Abbreviation and characteristic of isolated microorganisms and site of collection

Abbreviation	Characteristic	Site of collection
M1D1	Phosphate solubilizing	Paldhi
M1B1	Phosphate solubilizing	Meharun
M2B2	Potassium solubilizing	Pimprala
M2C1	Potassium solubilizing	Pimprala
M3D2	Nitrogen fixing	Paldhi
M3B1	Nitrogen fixing	Meharun

Potassium solubilizing activity of isolates

The selected isolates are grown on both modified Alkendrove media containing phenol red dye and without dye showed clear zone around colonies. The (Table 2) indicated the value of the diameter of the colony (d), diameter of halo zone (D), and the ratio D/d of different isolates obtained on

alkendrove agar plates. The use of the ratio D/d helps to evaluate the activity of given microorganism. The potassium solubilization activity of these selected isolates on alkendrove agar plates ranged from 1 to 2.5 (Table 2). This suggests that the isolates have varying abilities to solubilize potassium, which could be an important characteristic depending on the context of the experiment or application.

Phosphate solubilizing activity of bacterial isolates

The results showed that the section of efficient phosphate solubilizing bacterial isolated on qualitative basis. The table 3 indicated the value of the diameter of the colony (C), diameter of halo zone (H), diameter of colony+ halo zone (Z) and the ratio Z/C of different isolates obtained on PKV agar plates. The use of the ratio Z/C helps to evaluate the activity of given microorganism. The phosphate solubilization activity of these selected isolates on PVK agar plates ranged from 1.67 to 6.5 (Table 3).

Table 2 Potassium solubilization of bacterial isolates (Alkendrove media without dye) by Khandeparkar's selection ratio

Bacterial isolates	Zone of clearance (D) in mm	Colony diameter (d) mm	D/d (ratio)
M1D1	6	3	2
M1B1	5	2	2.5
M2B2	6	4	1.5
M2C1	12	5	2.4
M3D2	3	3	1
M3B1	4	4	1

Table 3 Phosphorus solubilization efficiency of bacterial isolates Z/c ratio

Bacterial isolates	Diameter of halo zone (H) in mm	Colony diameter (C) mm	Diameter of Colony and halo zone (Z)	Solubilization activity (Z/C)
M1D1	12	6	18	3
M1B1	11	2	13	6.5
M2B2	6	5	11	2.2
M2C1	10	6	16	2.67
M3D2	2	3	5	1.67
M3B1	12	6	18	3

Table 4 Colony and morphological characteristic of agricultural importance isolates

	M1D1	M1B1	M2B2	M2C1	M3D2	M3B1
Gram character	Positive	Negative	Positive	Positive	Negative	Negative
Shape	Irregular	Circular	Irregular	Irregular	Circular	Circular
Surface	Rough	Smooth	Rough	Rough	Mucoid	Mucoid
Elevation	Raised	Convex	Raised	Raised	Convex	Convex
Margin	Undulate	Entire	Undulate	Undulate	Entire	Entire
Color	White color	Yellow color	White color	White color	Cream color watery colony	Cream color watery colony
Motility	Motile	Motile	Motile	Motile	Motile	Non-motile
Spore	Positive	Negative	Positive	Positive	Positive	Positive
Capsule formation	Absent	Negative	Positive	Positive	Positive	Positive
Pigment production	Negative	Positive	Negative	Negative	Negative	Negative

Table 5 Biochemical characteristic of agricultural importance isolates

	M1D1	M1B1	M2B2	M2C1	M3D2	M3B1
Casein hydrolysis	+	+	-	+	+	+
Gelatin hydrolysis	+	+	-	+	-	-
Starch hydrolysis	+	-	+	+	-	-
Catalase	+	+	-	+	+	+
Oxidase	+	+	+	+	+	+
Urease	-	-	-	-	+	+
Arginine Dihydrolase Test	-	+	-	+	-	-
Lysine Decarboxylase Test	-	-	-	+	-	-
Indole production	-	-	-	+	-	-
Citrate utilization	+	+	-	+	-	-
Fructose fermentation	+	+	+	+	+	+
Glucose fermentation	+	+	+	+	+	+
Lactose fermentation	+	-	+	+	-	-
D-Mannitol fermentation	+	-	+	+	+	+
Maltose fermentation	+	-	+	+	+	+
Sucrose fermentation	+	-	+	+	+	+
Starch fermentation	+	-	+	+	+	+
D-Xylose fermentation	+	-	-	+	-	-
Phosphatase test	+	-	+	+	-	-
Acid production from glucose	+	-	+	+	-	-

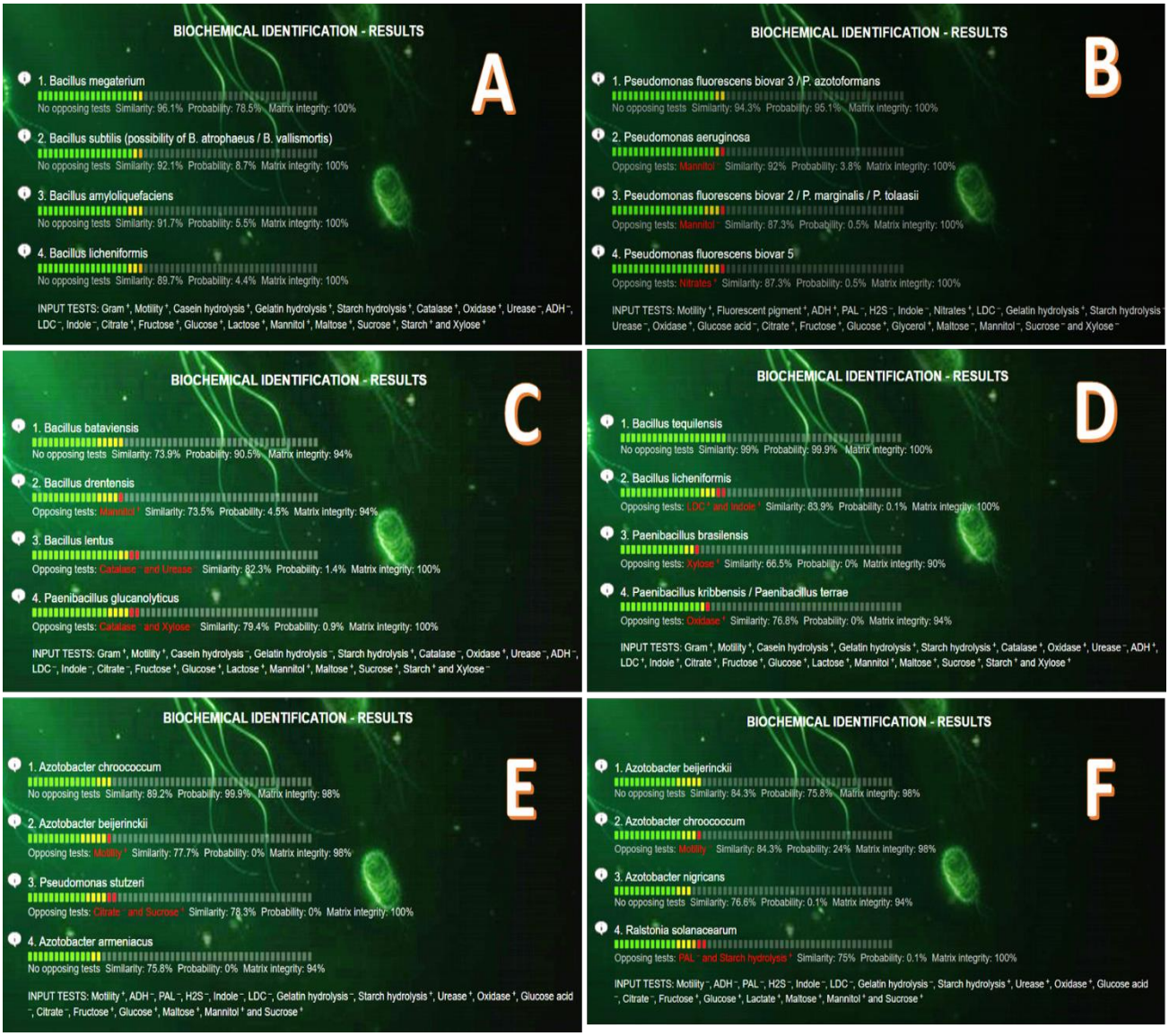

Fig 1 ABIS online tool report of isolated microorganisms (Fig A): M1D1(*Bacillus megaterium*), B: M1B1 (*Pseudomonas fluorescens*), C: M2B2 (*Bacillus bataviensis*), D: M2C1 (*Bacillus tequilensis*), E: M3D2 (*Azotobacter chroococcum*) and F:M3B1 (*Azotobacter beijerinckii*)

Table 6 Identification of isolated organism by using ABIS online tool

Abbreviation	Similarity (%)	Probability (%)	Matric integrity (%)	Organism
M1D1	96.1	78.5	100	*Bacillus megaterium*
M1B1	94.3	98.1	100	*Pseudomonas fluorescens*
M2B2	73.9	90.5	94	*Bacillus bataviensis*
M2C1	99	99.9	100	*Bacillus tequilensis*
M3D2	89.2	99.9	98	*Azotobacter chroococcum*
M3B1	84.3	75.8	98	*Azotobacter beijerinckii*

Cultural, microscopical and biochemical characteristics

The selective medias are used to study cultural behaviour of purified bacterial isolates. From the 22 isolates only six bacterial isolates were picked to identify and study their morphological (Table 5) and biochemical characteristic (table 6), by using ABIS online tool the probable identification of organisms given in (Table 6).

The estimation of phyto-toxicity and phyto-stimulation activity by Cucumber cotyledon bioassay

The data arrived from the studying of phyto-toxicity and phyto-stimulation activity shows high growth stimulating activity. Interestingly, the isolates are free of toxicity for cucumber seeds and results are almost comparable to that of control. All isolates displayed Phyto stimulation activity (Table 7). The highest Phyto stimulation activity was exhibited by M2C1 (*B. tequilensis*) i.e, 238.64% and 207.69% in terms of root and shoot respectively. The highest chlorophyll content was observed in M2C1 treated seeds, 41.72, while lowest was found in M2B2 25.90. whereas, the lowest activity is shown by M2B2 (*B. bataviensis*). On the basis of results of Cucumber cotyledon bioassay, the consortia of M1B1, M2C1 and M3B1 is prepared and tested for growth promotion activity on maize seeds. The results are enthralling and proved that the organisms in consortia can be used as biofertilizer (Figure 2 and Table 8). As compared to BAP in consortia, the growth promoting activity was exhibited by consortia is, 100 % and 108.82% in terms of root and shoot hight respectively. Such beneficial effect of this synergistic bacterial mixture has previously been reported in maize [27-28].

Table 7 Plant growth promoting activity of isolated microorganisms

Isolate abbreviation	Mean of No. of seeds sporulated	Root		Stem		% of control and response		total chlorophyll content
		Mean	SD	Mean	SD	Root	Stem	
Distilled water	4	0.88	0.54	3.9	1.4	100.00	100.00	29.71
M1D1	5	1.4	0.41	4.6	1.2	159.09	117.95	36.86
M1B1	4	1.1	0.67	5.2	1.9	125.00	133.33	31.47
M2B2	3	0.9	0.76	3.8	0.8	102.27	97.44	25.9
M2C1	5	2.1	0.34	8.1	1.45	238.64	207.69	41.72
M3D2	4	1.5	0.43	6.3	1.87	170.45	161.54	35.41
M3B1	4	1.44	0.47	6.9	1.94	163.64	176.92	34.76
BAP (25 PPM)	5	1.9	0.37	7.3	1.56	215.91	187.18	39.46

Table 8 growth promoting activity of Consortia of potential isolates

Test organisms	Mean of No. of seeds sporulated	Root		Stem		% of control and response	
		Mean	SD	Mean	SD	Root	Stem
Control	4	19	2.3	18	1.98	100.00	100.00
BAP	5	24	2.45	34	3.1	126.32	188.89
Consortia	5	24	3.21	37	2.9	126.32	205.56

Soil bacteria play important role in maintaining nutritive value of soil. The bacteria may include different groups phosphate, potassium, zinc, iron solubilizing bacteria and nitrogen fixing bacteria are able to enhance the availability of different nutrients by utilizing different mechanisms. Plant growth promoting bacteria are able to enhance the availability of different nutrients including N, P and micronutrients e.g. *Rhizobium sp*., in symbiosis with their legume host plant, and *Azospirillum* in non-symbiotic association with their host plant, can fix atmospheric N_2 [29]. The present work showed the role of a specific PGPB strain on leaf and root of tested plants. The M1D1 isolate (*Bacillus megaterium*) shows both phosphate and potassium solubilizing activity. Similar results are reported by Amalraj *et al*. [30], *Bacillus megaterium* var. phosphaticum improved significantly the uptake of nitrogen (7.97mg/100g dry mass), phosphate (3.41mg/100g dry mass), potash (38.12mg/100g dry mass), zinc (184mg/100g dry mass), iron (743mg/100g dry mass) and manganese (138mg/100g dry mass). The findings of Amalraj *et al*. [30] suggest reduction of 25% recommended dose of chemical fertilizers in combination with *Bacillus megaterium* as seed dresser and soil application. The role of *Bacillus megaterium* for enhancing mineral phosphorus (P) solubilization is well documented [31]. Several mechanisms have been proposed to explain the P solubilization as they are associated with the release of organic and inorganic acids and the excretion of protons that accompanies to the ammonia assimilation [32]. Whereas, Antonia and Ricardo [33] proved the *Bacillus megaterium* strain improve uptake of plant potassium nutrition without affecting K^+ availability in the soil. The results demonstrate the potential of this bacteria for using as a biofertilizer to reduce the amount of potassium fertilizers to be applied in the field. The isolate M2BC1 (*Pseudomonas fluorescens*) also shows both the activity viz., phosphate and potassium solubilization. The Amri *et al*. [34] proved that the *Pseudomonas fluorescencs* shows the highest capability of phosphate solubilization of 618.57 µg mL^{-1} in NBRIP phosphate-enriched liquid culture medium. Previously, results obtained by Gupta *et al*. [35], Ahmad *et al*. [36], showing that *Pseudomonas sp.* is an effective phosphate solubilizer. Whereas, Ashrafi *et al*. [37] showed that pH had a significant impact on K release by *Pseudomonas fluorescens* using response surface methodology from minerals. The isolate M2B2 (*Bacillus bataviensis* or *Neobacillus bataviensis*) shows negligible potassium solubilization activity. No supporting evidences are observed on potassium solubilizing activity of *Bacillus bataviensis*. Although, it is isolated from the roots/rhizosphere of maize (*Zea mays*) [38]. The isolate M2C1 (*Bacillus tequilensis*) is found to be the most efficient in terms of overall growth promoting organisms. Since is shows all Phosphate solubilizing bacteria (PSB), KSB and nitrogen fixing ability. This finding is supported by the results of Mukherjee and Dutta [39] CP6 (*Bacillus tequilensis*) was found to be the best-performing strain among the nine isolates. CP6 has both phosphate solubilizing and nitrogen fixing activity. Similarly, the studies of Shultana *et al.* [40], the isolated strain 'UPMRE6' (*Bacillus tequilensis*) showed better performance towards phosphorous and potassium solubilization. Since it is proved form the evidence that the *Bacillus tequilensis* has PSB, KSB and nitrogen fixing ability. While, the isolate M3D2 and M3B1 are nitrogen fixing isolates and shows matches with *Azotobacter chroococcum* and *Azotobacter beijerinckii* respectively. These two strains of azotobacter are already known for their nitrogen fixing ability by plenty of researchers. The most well-known species of the genus are *Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter chroococcum, Azotobacter armeniacus, A. paspali, Azotobacter salinestris*, and *Azotobacter nigricains* [41-46].

CONCLUSION

Sustainable agriculture requires the use of strategies to increase fertility of soil and food production while reducing damage to the environment. The use of microbial plant growth promoters is an alternative to chemical fertilizer and conventional agricultural technologies. Plant growth-promoting microbe can affect plant growth directly. The PRPG produce certain chemicals, help to dissolve insoluble minerals from rhizosphere and make it available to plants. The need of today's country is full fill demands of the increasing population in terms of food, biomass and resources. The high output of agriculture yield and enhanced production of the crop as well as fertility of soil to get in an ecofriendly manner. Hence, the research has to be focused on isolation and application of this microorganisms in field. The application of multi strain bacterial consortium over single inoculation could be an effective approach for reducing the harmful impact of stress on plant growth.

LITERATURE CITED

1. Jayaraj J, Shibila S, Mathan KS, Mariselvam R, Tahani AA, Sulaiman AA, Ponnani K M, Sakthiganesh K, Ananthi S. 2023. Isolation and Identification of bacteria from the agricultural soil samples to tolerate pesticides dimethoate, thiamethoxam and Imidacloprid. *Environmental Research Communications* 5(7): 075011, DOI 10.1088/2515-7620/ace68b.
2. Kummerer K. 2004. Resistance in the environment. *Journal of Antimicrobial Chemotherapy* 45: 311-320.
3. Martínez CB, Juan FG, Arantza ES, Manuel TA, Genaro MS. 2022. Isolation and characterization of plant growth-promoting compost bacteria that improved physiological characteristics in tomato and lettuce seedlings. *Agriculture* 12(1): 3. https://doi.org/10.3390/agriculture12010003.
4. Girmay K. 2019. Phosphate solubilizing microorganisms: Promising approach as biofertilizers. *International Journal of Agronomy*. https://doi.org/10.1155/2019/4917256.
5. Paun CV, Radu CM, Vladimir MT, Dan CD. 2019. The impact of financial sector development and sophistication on sustainable economic growth. *Sustainability* 11(6): 1713. https://doi.org/10.3390/su11061713
6. Hedin LO, Brookshire EJ, Menge DN, Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. *Annu. Rev. Ecol. Evol. System* 40: 613-635. doi: 10.1146/annurev.ecolsys.37.091305.110246.
7. Vitousek PM, Menge DNL, Reed SC, Cleveland CC. 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philos. Trans. R. Soc. B: Boil. Science* 368: 20130119. doi: 10.1098/rstb.2013.0119.
8. Mahmud K, Makaju S, Ibrahim R, Missaoui A. 2020. Current progress in nitrogen fixing plants and microbiome research. *Plants (Basel)* 9(1): 97 doi: 10.3390/plants9010097.
9. Etesami H, Emami S, Alikhani HA. 2017. Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects - A review. *Journal of Soil Science and Plant Nutrition* 17(4): http://dx.doi.org/10.4067/S0718-95162017000400005.
10. Ha S, Tran LS, 2014. Understanding plant responses to phosphorus starvation for improvement of plant tolerance to phosphorus deficiency by biotechnological approaches. *Crit. Rev. Biotechnology* 34: 16-30. doi: 10.3109/07388551.2013.783549
11. Lin S, Litaker RW, Sunda WG. 2016. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. *Journal of Phycology* 52: 10-36. doi:10.1111/jpy.12365
12. Girmay K. 2019. Phosphate solubilizing microorganisms: Promising approach as biofertilizers. *Hindawi International Journal of Agronomy.* Article ID 4917256, 7 pages https://doi.org/10.1155/2019/4917256
13. Zhao F, Qiu G, Huang Z, He L, Sheng X. 2013. Characterization of Rhizobium sp. Q32 isolated from weathered rocks and its role in silicate mineral weathering. *Geomicrobiol. Journal* 30: 616-22.
14. Bisht N, Singh CP. 2021. Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. *Soil Contamination - Threats and Sustainable Solutions* (https://doi.org/10.5772/intechopen.94593).
15. Bhattacharyya1 N, Goswami MP, Bhattacharyya LH. 2016. Perspective of beneficial microbes in agriculture under changing climatic scenario: A review. *Journal of Phytology* 8: 26-41. doi: 10.19071/jp.2016.v8.3022
16. Sukumar K, Swarnabala G, Gangatharan M. 2023. Experimental studies on isolation and characterization of silicate solubilizing *Bacillus tequilensis* SKSSB09. *Journal of Applied Biology and Biotechnology* 1(4): 135-140. DOI: 10.7324/JABB.2023.119115.
17. Verma A, Patidar Y, Vaishampayan A. 2016. Isolation and purification of potassium solubilizing bacteria from different regions of India and its effect on crop's yield. *Indian Jr. Microbiol. Research* 3(4): 483-488. DOI: 10.18231/2394-5478 .2016.0029.
18. Khanghahi MY, Pirdashti H, Rahimian H, Nematzadeh G, Sepanlou MG. 2018. Potassium solubilizing bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. *Symbiosis* 76: 13-23.
19. Vishwakarma D, Thakur JK, Gupta SC. 2017. A study on nitrogen fixing ability of soil isolates and quantitative estimation of K solubilization by soil and plant isolates. *International Journal of Current Microbiology and Applied Sciences* 6(12): 2641-2647. https://doi.org/10.20546/ijcmas.2017.612.305.
20. Sanjotha G, Manawadi S. 2016. Isolation, screening and characterization of phosphate solubilizing bacteria from Karwar Costal Region. *International Journal of Research Studies in Microbiology and Biotechnology* 2(2): 1-6. http://dx.doi.org/10.20431/2454-9428.0202001.
21. Xess N, Sao S. 2020. Isolation, identification and characterization of phosphate solubilizing bacteria in soils of coal mines landfills of Chhattisgarh. *Annals of Plant and Soil Research* 22(4): 437-441. https://doi.org/10.47815/apsr.2020.10018
22. Aneja KR. 2007. *Experiments in Microbiology, Plant Pathology and Biotechnology*. (4th Edition). New Age International.
23. Dubey RC, Maheshwari DK. 2010. *A Textbook of Microbiology*. S. Chand Publication.
24. Harley JP, Prescott LM. 2002. *Laboratory Exercises in Microbiology*. 5th Edition, The McGraw-Hill Companies.
25. Patil KJ, Harshada N, Mahajan RT, Banerjee C, Shukla P, Bandopadhyay R. 2015. Plant growth stimulating activity of fresh water micro algae. *International Journal of Advanced Research* 3(5): 284-288.
26. Sadasivam S, Manickam A. 2010. *Biochemical Methods*. New Age International Publishers (P) Ltd., New Delhi.
27. Pereira SIA, Castro PML. 2014. Phosphate-solubilizing rhizobacteria enhance *Zea mays* growth in agricultural P-deficient soils. *Ecol. Eng.* 73: 526-535. https://doi.org/10.1016/j.ecoleng.2014.09.060
28. Jesus A. Ibarra-Galeana, Claudia Castro-Martínez, Rosario A. Fierro-Coronado, Adolfo D. Armenta-Bojórquez, Ignacio E. Maldonado-Mendoza. 2017. Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (*Zea mays* L.) in calcareous soils of Sinaloa, *Mexico. Ann Microbiology* 67: 801-811. https://doi.org/10.1007/s13213-017-1308-9.
29. Yadav AN, Verma P, Singh B, Chauahan VS, Suman A, Saxena AK. 2017. Plant growth promoting bacteria: Biodiversity and multifunctional attributes for sustainable agriculture. *Adv Biotech and Micro.* 5(5): 102-116.
30. Amalraj E, Leo D, Maiyappan S, Peter JA. 2012. In vivo and In vitro studies of *Bacillus megaterium* var. phosphaticum on nutrient mobilization, antagonism and plant growth promoting traits. *Journal of Ecobiotechnology* 4(1): 35-42.
31. Vazquez PG, Holguin ME, Puente A. Lopez-Cortes, Bashan Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* 30: 460-468.

32. Whitelaw MA. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agronomy* 69: 99-151.
33. Antonia RM, Ricardo A. 2023. A non-K+-solubilizing PGPB (*Bacillus megaterium*) increased K^+ deprivation tolerance in *Oryza sativa* seedlings by up-regulating root K^+ transporters. *Plant Physiology and Biochemistry* 196: 774-782. https://doi.org/10.1016/j.plaphy.2023.02.027.
34. Amri M, Rjeibi MR, Gatrouni M, Mateus DMR, Asses N, Pinho HJO, Abbes C. 2023. Isolation, identification, and characterization of phosphate-solubilizing bacteria from Tunisian soils. *Microorganisms* 11(3): 783. doi: 10.3390/microorganisms11030783. PMID: 36985356; PMCID: PMC10052640.
35. Ahmad F, Ahmad I, Khan MS, 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Research* 163: 173-181. doi: 10.1016/j.micres.2006.04.001.
36. Gupta A, Meyer JM, Goel R. 2002. Development of heavy growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiol. Research* 158: 163-168.
37. Ashrafi-Saeidlou AS, Samadi MH, Rasouli-Sadaghiani M, Barin E. Sepehr. 2019. Optimization of potassium dissolution by *Pseudomonas fluorescens* using placket-Burman design and response surface methodology. *Journal of Water and Soil* 33(4): 647-658. 10.22067/JSW.V0I0.80017.
38. Peter Kampfer, Stefanie P. Glaeser, John A. McInroy, Dominique Clermont, Andre Lipski, Alexis Criscuolo. 2022. *Neobacillus rhizosphaerae* sp. nov., isolated from the rhizosphere, and reclassification of *Bacillus dielmonensis* as *Neobacillus dielmonensis* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* 72(11): https://doi.org/10.1099/ijsem.0.005636.
39. Mukherjee B, Dutta S. 2019. Isolation of a phosphate solubilizing bacterial strain bacillus Tequilensis mcc 3872 from the rice field of Burdwan district and characterization of its plant growth promoting traits. *The Pharma Innovation Journal* 8(4): 956-962.
40. Shultana R, Kee Zuan AT, Yusop MR, Saud HM, El-Shehawi AM. 2021. *Bacillus tequilensis* strain 'UPMRB9' improves biochemical attributes and nutrient accumulation in different rice varieties under salinity stress. *Plos One* 16(12): e0260869. https://doi.org/10.1371/journal.pone.0260869
41. Patel R, Patel A. 2023. Nitrogen fixation in nonlegume plants and genomics of nitrogen-fixing nonrhizobium symbioses. *The Chemical Dialogue Between Plants and Beneficial Microorganisms* 10: 145-152. https://doi.org/10.1016/B978-0-323-91734-6.00017-X.
42. Plunkett MH, Knutson CM, Barney BM. 2020. Key factors affecting ammonium production by an *Azotobacter vinelandii* strain deregulated for biological nitrogen fixation. *Microb. Cell Fact.* 19: 107. https://doi.org/10.1186/s12934-020-01362-9
43. Chen SL, Tsai MK, Huang YM. 2018. Diversity and characterization of azotobacter isolates obtained from rice rhizosphere soils in Taiwan. *Ann. Microbiology* 68: 17-26. https://doi.org/10.1007/s13213-017-1312-0
44. Gothandapani S, Sekar S, Padaria JC. 2017. *Azotobacter chroococcum*: Utilization and potential use for agricultural crop production: An overview. *Int. Jr. Adv. Res. Biol. Science* 4(3): 35-42. http://dx.doi.org/10.22192/ijarbs.2017.04.03.004.
45. Jain D, Kaur G, Bhojiya AA. 2021. Phenetic characterization of nitrogen fixing azotobacter from rhizospheric soil of Southern Rajasthan. *Jr. Pure Applied Microbiology* 15(1): 428-436. doi:10.22207/JPAM.15.1.40
46. Wani SA, Chand S, Ali T. 2013. Potential use of *Azotobacter chroococcum* in crop production: An overview. *Curr. Agri. Research* 1(1): 35-38. doi : http://dx.doi.org/10.12944/CARJ.1.1.04.