Int.J.Curr.Microbiol.App.Sci (2023) *12*(02): 195-202

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 2 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1202.018

Evaluation of *Bacillus strains* for Plant Growth Promoting Properties Isolated from Different Regions of Kashmir Valley

Ulfat Nazir[1*] and M. Y. Zargar[2]

[1]Centre of Research for Development, University of Kashmir, Srinagar - 190006, India
[2]Emeritus Scientist Faculty of Agricultural, Sheri Kashmir University of Agricultural Sciences and Technology Kashmir, Jammu and Kashmir, India
**Corresponding author*

Keywords

Agricultural yields, civilization, industrialization, environmental risks, rhizobacteria

Article Info

Received:
02 January 2023
Accepted:
30 January 2023
Available Online:
10 February 2023

A B S T R A C T

Microbes that are beneficial to plants are used to enhance the crop yield and are alternatives to chemical fertilizers and pesticides. *Bacillus* species are the predominant plant growth-promoting bacteria. This study was aimed to isolate *Bacillus* species from soil of different regions of Kashmir and screen for their ability to promote plant growth directly or indirectly by testing their ability to produce plant growth hormone indole acetic acid, hydrogen cyanide, ammonia and phosphate solubilization. Fifty *Bacillus* strains were isolated from soil samples of different regions of Srinagar and Baramulla districts of Kashmir. These isolates were tested for plant growth promoting traits in vitro. Among the 50 isolates, 35 were indole acetic acid producer, 22 of the isolates showed the ability to solubilize the phosphate, 35 were able to produce ammonia and all the isolates had the ability to produce hydrogen cyanide. The isolated strains showed positive results to maximum PGPR traits and exhibited a potential to be used as alternatives to chemical fertilizers and pesticides and could be used as low-cost bio-based technology to promote plant growth in the agricultural sector.

Introduction

The demand for agricultural productivity has increased dramatically as a result of civilization and industrialization. Chemical fertilizers and pesticides increase agricultural yields, but they can degrade soil fertility and quality, posing environmental risks. As a result, the need for environmentally friendly biological agents, such as plant growth promoting rhizobacteria, has skyrocketed in order to improve soil fertility and agricultural operations while also protecting environmental health (Nagorska *et al.*, 2007). So, substantial research efforts are now focused on finding new alternatives to supplement the use of chemicals in agriculture. The use of beneficial rhizobacteria to increase the productivity and growth of plants could be one of the substitutes to agrochemicals. Plant growth promoting

rhizobacteria (PGPR) are free living,soil borne bacteria which enhance the growth of plant directly or indirectly (Kloepper *et al.,* 1980). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration (Glick and Ibid, 1995; Glick *et al.,* 1999).

The indirect promotion of plant growth occurs when PGPR reduce or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995).

Bacillus species are abundant in the rhizosphere, so they can be one of the major aspects of bio-based products to supplant agrochemicals. *Bacillus*spp are Gram positive common rhizobacteria and widely considered as a major aspect of plant growth promoting rhizobacteria (Fan *et al.,* 2017). The ability to replicate rapidly and resistant to adverse environmental conditions provide a unique feature to *Bacillus* species (Shafi *et al.,* 2017). Their ability to produce hard, resistant endospores and antibiotics that limit wide ranges of phytopathogens make Bacillus spp an attractive option for biocontrol agents (Cavaglieri *et al.,* 2005).

This study was thus aimed to isolate *Bacillus*spp from soil samples of different areas of Kashmir valley and screen the isolates for some direct and indirect plant growth promoting traits. This includes the test for the production of indole acetic acid (IAA), solubilization of phosphate, hydrogen cyanide (HCN) and ammonia production.

Materials and Methods

Sample collection and processing

Soil samples were collected from rhizospheric soils of different district namely Srinagar and baramulla of kashmir. The soil samples were collected from a depth of 5-10 cm in sterile plastic bags and carried to the laboratory, the laboratory work was conducted in the Division of Plant Pathology, Faculty of Horticulture, Shalimar, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir for further processing and analysis.

The study was conducted from April to july 2015. One gram of soil sample was dispensed into 99 mL of sterile distilled water and homogenized. One mL of homogenized soil sample was transferred into 9 mL sterile distilled water and serial dilution was carried out up to 10-8 dilution.

Serially diluted bacterial cultures (100 µL) were spread on nutrient agar media and incubated at 37°C for 24 h and examined for the appearance of colonies. Identification of the isolated colonies was done on the basis of colony characteristics, Gram reaction, spore staining and Biochemical test. Screening of isolates was done for plant growth promoting properties – IAA production, phosphate solubilization activity, HCN production and ammonia production.

Indole acetic acid production

IAA production was qualitatively estimated. All the isolates were incubated in Luria Bertani Broth at 28 ºC. The bacterial cells were removed from the culture by centrifugation at 8000 g for 10 min.

A 3ml of supernatant was mixed vigorously with 2 ml of Salkowaski's reagent (2ml0.5MFeCl3+98ml35%HCLO4) and incubated at room temperature in dark for 30 minutes and observed for pink color formation. (Brick *et al.,* 1991).

Phosphorous Solubilization

Phosphate Solubilization was detected by formation of transparent halos surroundings bacterial colonies on the Pikovskaya agar after 72 hour incubation at 28 ºC (Pikovskaya, 1948). Clear zones around the colonies indicated the positive test. The diameter of the halo zone was measured.

Ammonia Production

All the bacterial isolates were tested for the production of ammonia for the detection of ammonia, the method of Cappuccino and Sherman (1992) was followed. The isolates were grown in 10ml peptone water in tubes. The tubes were then incubated at 28±2ºC for 4 days. After 4 days, 1ml of Nessler's reagent was added to each tube. Development of brown to yellow color indicated positive test for ammonia production.

HCN production

The Qualitative estimation of HCN was done by using the method of Baker and Schippers (1987). The isolates were streaked on pre-poured plates of King's B medium amended with 4.4 g/L glycine. The Whatman No. 1 filter paper strips were soaked in 0.5 per cent picric acid in 0.2 per cent sodium carbonate and was placed in between the petriplates. The petriplates were sealed with parafilm and then incubated at 37°C for 1- 4 days. The plates were observed for colour change in filter paper from yellow to orange brown to dark brown.

Results and Discussion

50 isolates of *Bacillus* were obtained. The isolates were Gram positive rods, endospore forming, and positive for catalase, citrate utilization, starch hydrolysis, Voges-proskauer but negative for indole test, oxidase and H_2S production. The colonies on nutrient agar were rough, creamy white, dry and folded, opaque and irregular edged.

Plant growth promoting properties

The plant growth promoting properties of the isolated were evaluated based on the ability to produce IAA, solubilization of phosphate, ability to produce HCN and ammonia.

IAA production

Out of 50 isolates of *Bacillus*, 35isolates showed the ability to produce IAA. Development of pink color after 20 min of addition of 2 drops of Salkowski reagent in 2mL of the cell free supernatant indicated the positive test for IAA (Figure 1)The IAA producing strains were further classified into three groups as +++ (strong), ++ (moderate) and + (weak) based on the intensity of color visible.

The strains showing deep pink, pink and light pink were placed in the group +++, ++ and + respectively. 8 isolates showed deep pink color after 20 min of addition of Salkowski reagent into the cell free supernatant liquid whereas 12 isolates showed pink color and 15 showed light pink color (Table 1).

Phosphate solubilization

The isolates showing a halo zone around the colonies after 48 h of incubation following spot inoculation in Pikovskaya's agar plate were taken as positive tests for Phosphate solubilization (Figure 2). Out of 50 isolates, 22 isolates exhibited the halo zone. The width of the halo zone was also measured (Table 1). The width of the Phosphate solubilization halo zone was as high as 2.80mm (BS15 and BS38) to most of the *Bacillus*spp showing the clear zone of 1mm diameter.

HCN production

All 50 isolates of *Bacillus* produced HCN as evidenced by the change in color of the Whatmann filter paper from yellow to brown. In the presence of glycine, the brown color of filter paper was observed giving a clear indication of HCN production by *Bacillus* strains (Figure 3).

However, different strains produced the different intensity of brown color as light brown, orange brown to reddish brown. Based on the distinction in the color of filter paper the strains are grouped into +++ (strong), ++ (moderate) and + (weak) for those producing reddish brown, orange brown and light brown respectively (Table 1). Ten strains changed the color of filter paper to reddish brown, 13 changed the color to orange brown while 27 strains changed the color to only light brown.

Table.1 Properties of 50 isolates tested for different plant growth promoting traits.

Bacillus strains	IAA production	P-Solubilization Width of halo zone(mm)	HCN production	Ammonia production
BS01	+	1.50	+	+++
BSO2	++	1.30	+	+
BSO3	+++	-	++	++
BSO4	-	2.00	+	+
BSO5	++	-	+++	+++
BSO6	+++	1.00	++	+
BSO7	-	-	+	+
BSO8	+	2.30	+++	++
BS09	+++	-	+	+++
BS10	+	1.95	+++	+
BS11	-	-	+	+
BS12	+	-	++	+
BS13	-	1.20	+++	++
BS14	+++	-	+	+
BS15	++	2.80	++	+++
BS16	-	1.00	+	++
BS17	-	-	++	+
BS18	+	-	+	++
BS19	+++	1.00	+++	+
BS20	+	-	+	+
BS21	-	1.65	++	+++
BS22	++	1.00	+	+
BS23	+	1.20	+	++
BS24	++	1.00	+	+
BS25	+	-	+++	++
BS26	+	1.60	+	-
BS27	-	-	++	+
BS28	-	-	+	++
BS29	-	1.00	++	+++
BS30	++	-	+	-
BS31	+	-	++	+
BS32	+++	1.90	+	-
BS33	-	-	+++	++
BS34	+	-	++	-
BS35	++	1.95	+	+
BS36	-	-	+	+
BS37	++	-	+	++
BS38	+++	2.80	++	+++
BS39	++	-	+	+
BS40	-	-	+	-
BS41	+++	2.30	+++	++
BS42	-	-	++	+

BS43	++	-	+	-
BS44	+	-	+++	+
BS45	+	1.50	+++	+++
BS46	-	-	+	++
BS47	++	-	++	+
BS48	+	1.20	+	-
BS49	++	-	+	+++
BS50	+	-	+	-

Fig.1 Appearance of pink color after the addition of Salkowski reagent in IAA test.

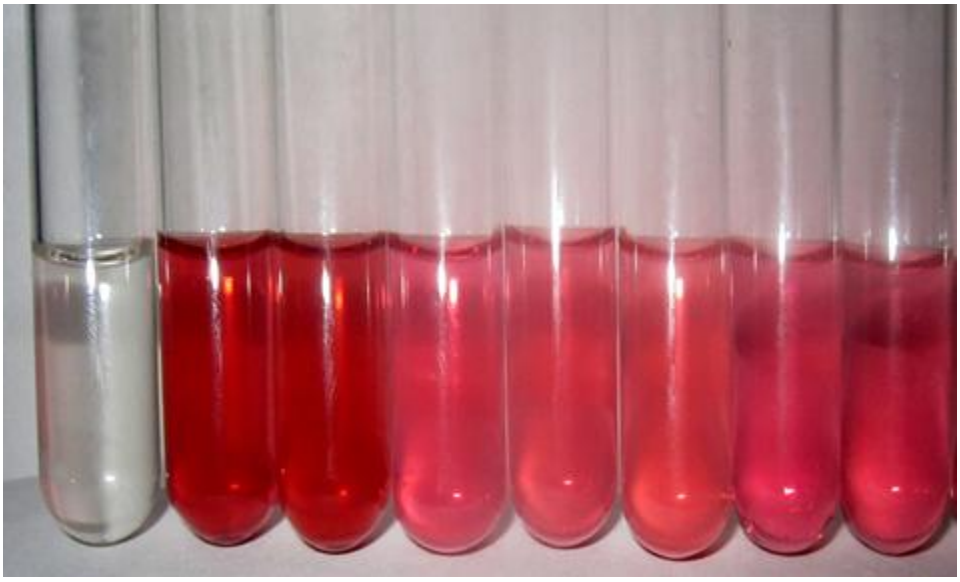

Fig.2 Appearance of halo zone around colony in phosphate solubilization test

Fig.3 Change in color of filter paper in HCN Production test

Fig.4 Change in color of peptone water in ammonia test

Ammonia production

For ammonia production, the development of brown to yellow color after the addition of 0.5 mL of Nessler's reagent was observed as a positive test (Figure 4). Among 50 isolates, 35 isolates developed the color of the medium to brown or yellow following the addition of Nessler's reagent. However, different strains produced different intensity of color as yellow, light brown and deep brown. Based on the distinction in the color of media the strains are grouped into +++ (strong), ++ (moderate) and + (weak) for those producing deep brown, light brown and yellow respectively (Table 1). Nine strains changed the color of media to deep brown, 12 changed the color to light brown while 21 strains changed the color to yellow.

Though there is a high demand for food production in the current scenario, a focus on sustainable agricultural practices without harming the environment. PGPR, frontbenchers of the next-

generation green revolution, are good alternatives to chemical fertilizers. *Bacillus* is one of the common PGPR to be used efficiently for optimization of plant growth and yield. Therefore, in this study, isolates of *Bacillus* spp. obtained from soil of different regions of Kashmir valley were primarily tested for the plant growth promoting traits in vitro.

The capability to increase plant growth parameters is highly related to the IAA level, which was produced by *Bacillus*spp isolates. IAA, the major auxin in plants, plays a major role in both the shoot and root development (Prusty *et al.*, 2004). Among the 50 isolates, 35 isolates were able to produce IAA. Despite phosphorus being abundant in soil it is insoluble and cannot contribute to the plant growth (Glick, 2012). So, the solubilization and mineralization of insoluble phosphate in soil by rhizobacteria makes an important property of plant growth promoting bacteria. Rodriguez and Fraga studied that *Bacillus* and other phosphate solubilizing bacteria (PSB) like *Pseudomonas* and *Rhizobium* were capable of converting insoluble phosphate available in the soil into soluble form (Rodriguez *et al.*, 1999). Phosphate solubilizing ability of isolated strains was tested using Pikovskaya's agar medium. 22 out of the 50 isolates produced halo zone on Pikovskaya's agar medium after incubation following the spot inoculation on the plates as a result of phosphate solubilization.

Production of HCN by rhizobacteria is believed to promote plant growth by indirect mechanism. Hydrogen cyanide is supposed to act synergistically with bacterially encoded antibiotics (Glick, 2012). Rijavec and Lapanje in their study concluded that HCN increases the availability of phosphate for rhizobacteria and plant hosts, especially in oligotrophic alpine environments and thus indirectly contributing to plant growth (Rijavec *et al.*, 2016).

Picric acid present in the filter paper reacts with free cyanide produced by the bacteria to produce colored iso purpuric acid, thus the change in color of filter paper is visible.

The color developed is directly proportional to free cyanide. Plants can only utilize the reduced forms of the nitrogen; hence, nitrogen first must be fixed and converted to a combined form (either ammonia/nitrate) and then trapped by the plants (Smil, 2011). 35 isolates were able to produce ammonia.

Nine isolates BS01, BS06, BS08, BS15, BS19, BS24, BS38, BS41 and BS45 showed positive results for PGPR traits assessed in the study. Among these, BS38 showed maximum IAA production, phosphate solubilization, HCN production and ammonia production.

It is evident from the present studies that the *Bacillus* species tested were able to demonstrate multiple plant growth promoting traits. The results concluded that *Bacillus* strains have a huge potential to be used as alternatives to chemical fertilizers and pesticides for the promotion in growth and production of plants. The study on *Bacillus* strains can be further detailed so that these strains can commercially be developed as low-cost biobased products to promote plant growth in the agricultural sector.

Acknowledgement

The authors are thankful to staff of the Department of plant pathology, SKUAST K Shalimar for providing laboratory facilities during the period of study. The authors have no conflict of interest to declare.

References

Baker, P. D. and Schippers 1987. Microbial cyanide production in the rhizophere in relation to potato yield production and Pseudomonas spp mediated plant growth stimulation. *Soil Biology and Biochemistry* 9: 451-457.

Brick, J. M., Bostock, R. M. and Silverstone, S. E. 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl. Environ.*

Microbiol 57: 535-538. https://doi.org/10.1128/aem.57.2.535-538.1991

Cappuccino, J. G. and Sherman, N. 1992. Microbiology: A laboratory manual. *The Benjamin/Comings Publishing Company, Inc. California* 46: 334-336.

Cavaglieri, L., Orlando, J. and Etcheverry, M., 2005. In vitro influence of bacterial mixtures on *Fusarium verticillioides* growth and fumonisin B1 production: effect of seeds treatment on maize root colonization. *Letters in Applied Microbiology*, 41(5), pp.390-396. https://doi.org/10.1111/j.1472-765X.2005.01785.x

Fan, B., Blom, J., Klenk, H. P. and Borriss, R., 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. *Frontiers in microbiology*, 8, p.22. https://doi.org/10.3389/fmicb.2017.00022

Glick, B. R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012. https://doi.org/10.6064/2012/963401

Glick, B. and Ibid, R., 1995. Genotyping of antifungal compounds producing PGPR *Pseudomonas. Canadian Journal of Microbiology*, 41, pp.107-109.

Glick, B. R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*, 41(2), pp.109-117. https://doi.org/10.1139/m95-015

Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N., 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286(5776), pp.885-886. https://doi.org/10.1038/286885a0

Nagórska, K., Bikowski, M. and Obuchowski, M., 2007. Multicellular behaviour and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *ActaBiochimicaPolonica*, 54(3), pp.495-508.

Pikovskaya, R. I. 1948. Mobilization of phosphorus in soil connection with vital activity of some microbial species. *Microbiology* 17: 362-370.

Prusty, R., Grisafi, P. and Fink, G. R., 2004. The plant hormone indoleacetic acid induces invasive growth in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, 101(12), pp.4153-4157. https://doi.org/10.1073/pnas.0400659101

Rijavec, T. and Lapanje, A., 2016. Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. *Frontiers in microbiology*, 7, p.1785. https://doi.org/10.3389/fmicb.2016.01785

Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 1999;17(4–5):319–39. https://doi.org/10.1016/s0734-9750(99)00014-2

Shafi, J., Tian, H. and Ji, M., 2017. Bacillus species as versatile weapons for plant pathogens: a review. *Biotechnology &* Biotechnological *Equipment*, 31(3), pp.446-459. https://doi.org/10.1080/13102818.2017.1286950

Smil, V., 2011. Nitrogen cycle and world food production. *World Agriculture*, 2(1), pp.9-13.

How to cite this article:

Ulfat Nazir and Zargar, M. Y. 2023. Evaluation of *Bacillus strains* for Plant Growth Promoting Properties Isolated from Different Regions of Kashmir Valley. *Int.J.Curr.Microbiol.App.Sci.* 12(02): 195-202.
doi: https://doi.org/10.20546/ijcmas.2023.1202.018