

Evaluation of diverse range microbes for their plant growth promoting abilities and their pesticide compatibility

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) contribute to an increase in crop yield through an environmentally friendly method, therefore eight rhizospheric bacteria, two of each genera *Bacillus*, *Pseudomonas*, *Azotobacter* and *Azospirillum* were examined for their efficacy to solubilize mineral nutrients using atomic absorption spectrophotometry and a flame photometer. Their potency to produce phytohormones, synthesis biocontrol components and their compatibility with pesticides using *in vitro* assays was studied. All of the chosen bacterial isolates proved positive for the above-mentioned Plant Growth Promoting traits. Among the eight bacterial isolates *Pseudomonas* isolate P69 showed the highest phosphorous solubilization efficiency of 190.91 % and another isolate P48 produced a maximum of 27.63 $\mu\text{g mL}^{-1}$ of gibberellic acid, *Bacillus* isolate B120 could solubilize maximum amount of ZnO and ZnCO₃ accounting for 21.3ppm and 25.9ppm, respectively, not merely in terms of solubilization when compared to the other isolates, B120 produced the highest levels of HCN (77.33 ppm TCC) and siderophores (48.87 $\mu\text{g mL}^{-1}$). On day 9 after inoculation, *Azotobacter* isolate AZB17 performed effectively in potassium solubilization of 6.25g mL⁻¹ with a pH drop to 3.83. The *Azospirillum* isolate ASP25 outperformed all other isolates in terms of IAA production (22.64g mL⁻¹) and *Bacillus* isolate B365 was found to be more compatible with eight different pesticides used in the field at varying concentrations. All of these factors point to the possibility of using these bacterial isolates B120, P48, P69, AZB17, and ASP25 as biofertilizers in sustainable agriculture.

Introduction

With the growing global population, food consumption is expected to rise annually and proportionately, resulting in the intensification of agriculture for food security through the continued use of synthetic fertilisers and pesticides to maximise yields (Nirmal *et al.*, 2010). Plant-microbe associations inside the plant root zone are being exploited to produce sustainable crop production systems around the world given by Bhattacharyya and Jha (2012), and one of the most investigated areas is the exploitation of these associations to produce sustainable crop production systems (Naqqash *et al.*, 2016). In plant rhizospheres, there is a complicated interaction

between plant-root-soil bacteria. These complex interactions are thought to occur as a result of root exudations, which serve to attract beneficial soil microorganisms to the plant roots (Zhang *et al.*, 2017; Mhlongo *et al.*, 2018). As a result, plant rhizospheres sustain a large number of bacteria that are thought to promote plant growth rhizobacteria (PGPR) (Bhattacharyya and Jha, 2012). Rapid root colonisation is recognised as a necessity for PGP activities (Kamilova *et al.*, 2015) and is hence one of the most important elements to consider when selecting rhizobacteria for business formation (Shafi *et al.*, 2017). This PGPR are capable of increasing plant growth in a variety of ways, including nitrogen (N₂) fixation, nutrient solubilization and biogenesis of phytohormones (Somers *et al.*, 2004), antibiotics, hydrolytic enzymes, siderophores (Beneduzi *et al.*, 2012), and induced systematic resistance (ISR) in plants to their pathogens (Beneduzi *et al.*, 2012), and are therefore terribly important in redressing soil fertility. For sustainable agricultural production, rhizobacterial-based solutions have been researched as alternatives to synthetic fertilisers. *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, and *Acinetobacter* are some of the most commonly investigated rhizobacteria. Wheat (Govindasamy *et al.*, 2015), beans (Stefan *et al.*, 2013), cucumber (Islam *et al.*, 2016), and a variety of other crops have all demonstrated their promise (Hayat *et al.*, 2010). It is obvious from this that members of the soil microbiome have a lot of potential in terms of encouraging sustainable crop management methods.

As the Plant Growth Promoting Microbes (PGPMs) are shown to benefit plants in many modes, the event of any PGP-based technology can facilitate in reducing the issues arising due to irrational use of inorganic fertilizers or artificial pesticides. However, it is evident that we tend to understand solely a small amount of microbe potential and an awfully flock remains to be done. In this backdrop, for sustainable and eco-friendly agriculture, a study was undertaken to screen the isolates of genus *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp. and *Azospirillum* sp. for their PGP activity at the side of their compatibility with pesticides to use in the future as microbial inoculants that successively correlates to a reduction in environmental pollution

due to the use of harmful fertilizers and pesticides thus ultimately leading to eco-friendly approach for crop yield attributes.

Material and Methods

In all, 31 strains of *Azotobacter* spp. (Az), 38 strains of *Azospirillum* spp. (As) spp., 82 fluorescent *Pseudomonas* spp. and 19 *Bacillus* spp. were isolated from rhizosphere soils of different cropping systems across 31 different agroclimatic regions from 13 states of India (Figure 1). As shown in Figure 1, it is evident that the collection of strains was done encompassing many states to cover diverse regions spread over different agroclimatic regions. By serial dilution of soil samples and plating on Jensen's agar (Jensen, 1940), N-free bromothymol blue media (Dobereiner *et al.*, 1976), nutrient agar, and King's B (King *et al.*, 1954) media *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* cultures were isolated, respectively. Standard biochemical conventional method tests were used to identify all of the bacterial strains to the genus level (Desai, 2012). Among them, eight bacterial isolates, two from each genus of *Azotobacter* (AZB17, AZB31), *Azospirillum* (ASP25, ASP34), *Bacillus* (B120, B365) and *Pseudomonas* (P48, P69) were randomly procured from ICAR-CRIDA culture bank. The cultures obtained were tested for their purity by plating on their specific media and were screened further for their PGP ability and their pesticide compatibility *in vitro*.

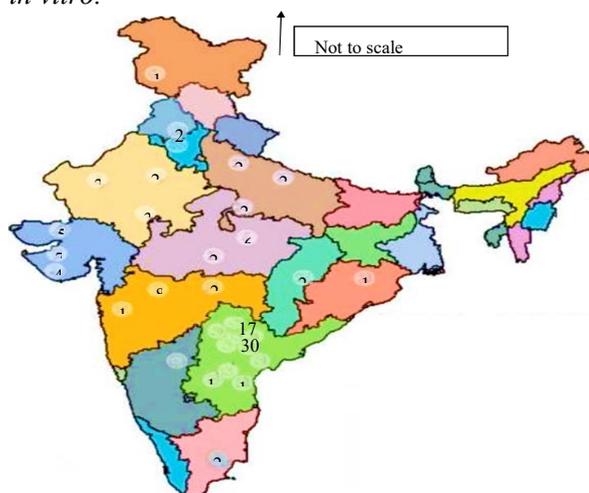


Figure 1: Map showing soils samples from different agro-ecosystems. Figures in parentheses in legends represent number of samples collected.

Qualitative assay for Zinc (Zn), Potassium (K) and Phosphorus (P) solubilization

Two μL bacterial cultures were spot inoculated onto mineral salts agar medium amended with 0.1% of insoluble zinc oxide (ZnO) and zinc carbonate (ZnCO_3). Aleksandrow's medium containing 0.5% potassium aluminium silicate (usually mica) as a source of an insoluble form of potassium and to assess phosphorous solubilization, Pikovskaya's medium amended with tricalcium phosphate (TCP) was used. All the inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ and solubilization zone (in mm) was measured 15, 3 and 14 days after inoculation, respectively.

Solubilization Efficiency (SE) $= \frac{Z}{C} \times 100$;

Solubilizing index (SI) $= C + \frac{Z}{C}$

Where, Z and C stand for clearance zone including bacterial growth and colony diameter, respectively.

Quantitative assay for zinc and potassium solubilization

All strains were tested quantitatively for Zn solubilization in mineral salts broth (100 mL) amended with 0.1 percent insoluble zinc salts (ZnO or ZnCO_3) inoculated with 100 μL of 24 hour old actively developed test bacterial cultures and cultured at 28°C for 10 days with shaking at 140 rpm. On the 3rd, 6th, and 9th days of inoculation, samples were taken and centrifuged at 10000 rpm for 10 minutes to remove cell debris. A pH meter was used to determine the pH of the supernatant, and atomic absorption spectrophotometry was used to quantify the amount of available Zn in the supernatant (AAS-GBC, Australia). Using liquid Aleksandrow's medium supplemented with 0.1 percent potassium aluminium silicate, potassium solubilization was estimated at the 7th and 9th days of incubation, and the amount of 'K' liberated in the broth was estimated. The solubilizing ability of culture media is often related to the degree of acidification of the medium; the pH of the culture supernatant was measured using a pH meter, and the available potash was estimated with a flame photometer.

Ammonium production

After 48 hours of incubation, isolates were tested for ammonia production by inoculating 100 μL of bacterial culture in pre-sterilised peptone water and adding 0.5 mL of Nessler's reagent. Ammonia

production was thought to be aided by a change in the medium's colour from brown to yellow (Kumar *et al.*, 2015)

Indole Acetic Acid (IAA) Production

The active culture of each test isolate was maintained in 5 mL respective specified media broth tubes with 100 mL of L-tryptophan and incubated at $28 \pm 2^\circ\text{C}$ for 4 days to determine indole acetic acid production. Following incubation, these cultures were centrifuged at 3000 rpm for 30 minutes, and 2 mL supernatant was collected, along with two drops of O-phosphoric acid and 4 mL salkowski reagent (0.5 M FeCl_3 in 35 percent perchloric acid) and incubated for 30 minutes to generate pink colour as a positive indicator of IAA generation, and optical density was measured at 530 nm using an UV-Visible spectrophotometer (Elico, India) and the concentrations were plotted against the standard graph (Bric *et al.*, 1991).

Gibberellic acid (GA_3) production

Borrow and his coworkers (1955) developed a method for determining the gibberellic acid-producing capacity of bacterial strains (Loper, 1986). 100 mL sterile TSB was added to 1 mL culture broth in flasks and incubated at 37°C for seven days. After incubation, 15 ml of cell-free supernatant was pipetted out of the broth culture after centrifugation at 8000 rpm for 10 minutes, and two ml of zinc acetate solution (21.9 g zinc acetate dissolved in 80 ml distilled water and one ml glacial acetic acid, and the volume was raised up to 100 ml with distilled water) was added and left for two minutes. Five millilitres of supernatant was collected, five millilitres of 30% hydrochloric acid was added, and the mixture was incubated for 75 minutes at 27°C . At 430 nm, absorbance was measured using a UV-VIS spectrophotometer. Each bacterial isolate's amount of GA_3 liberated was determined and expressed in g mL^{-1} broth.

Siderophore Production

The potential of bacterial isolates to produce siderophores was assessed qualitatively and quantitatively using the universal Chrome Azurol Sulphonate (CAS) test (Schwyn and Neilands, 1987). CAS agar plates were made by combining 100 mL CAS reagent with 900 mL sterilised Tryptone soy agar for the qualitative analysis (TSA). After eight bacterial isolates were spot

injected on a single plate and incubated at 28°C for 5–7 days, the pigmented zone formed around the bacterial colonies.

For the quantitative experiment, 24-hour-old bacterial cultures were inoculated into sterile Tryptone soy broth (TSB) and incubated at 30°C for 24 hours at 120 rpm with continual shaking. Following incubation, the fermented broth was centrifuged at 10000 rpm for 15 minutes, and 100 µl of cell-free supernatant was used to estimate siderophores by placing 100 µl of CAS reagent in separate wells of a microplate (CLS3474 Sigma). Following incubation, the optical density of each sample was measured using a microplate reader at 630 nm against a reference consisting of 100 µL of uninoculated broth and 100 µl of CAS reagent. Isolates' siderophores were measured in percent siderophore unit (psu), which was determined using the formula below (Payne, 1993).

Siderophore production (psu) = $(Ar - As) \times 100 \div Ar$
Where Ar is the reference absorbance (CAS solution and uninoculated broth) and As is the sample absorbance (CAS solution and cell-free supernatant of sample).

Hydrogen Cyanide (HCN) Production

Castric & Castric's technique for determining HCN production was used (1983). Under aseptic conditions, a strip of Whatmann filter paper No.1 (10 x 0.5 cm²) was impregnated with an alkaline picric acid solution (0.5 percent picric acid (w/v) in 1 percent sodium carbonate) and inserted in the broth tubes containing individual bacterial cultures modified with glycine (4.4g/L). The tubes were incubated for 48-72 hours at 28°C. HCN production was determined by a change in filter paper colour from yellow to light brown or strong reddish-brown. The shift in colour is due to sodium picrate being reduced to a reddish compound proportional to the amount of HCN released. The colour was eluted by inserting the filter paper in a test tube with 10 ml of distilled water and measuring the absorbance at 510 nm.

Total cyanides contents (ppm) = $396 \times A_{510}$

Where, A_{510} = absorbance at 510nm

Pesticide compatibility assay

The bacterial isolates were replicated in their respective broths and incubated for 48 hours at 28°C with continuous agitation at 150 rpm on a rotary shaker. The bacterial cultures were centrifuged at 3750 rpm for 20 minutes at 10°C to

remove the supernatant and harvest the bacterial cells the bacterial cultures were centrifuged at 3750 rpm for 20 minutes at 10°C to remove the supernatant and harvest the bacterial cells.. To obtain a bacterial concentration of 10^8 cfu mL⁻¹, the supernatant was suspended in sterile phosphate buffer. Compatibility of test isolates was done with commonly used pesticides (Supplementary Table S1) following the Disc diffusion method. Dimethoate, imidacloprid, quinolphos, mancozeb, acephate and bavistin were used as test pesticides at eight different concentrations 0.01%, 0.05%, 0.1%, 0.2%, 0.5%, 1.0%, 1.5% and 2% by maintaining four replications. 100 µl bacterial suspension was spread on TSA media. Subsequently, sterile Whatmann paper plugs of 5 mm diameter were moistened with 50 µl pesticide, at the mentioned concentrations, and placed on the surface of bacteria inoculated plates. All plates were incubated at 28°C for 72 hours before being examined for bacterial growth suppression (Mukherjee *et al.*, 2017). Bacterial strain sensitivity to pesticides was measured based on the inhibition zone (mm) around the disc.

Statistical Analysis

According to Panse and Sukhatme (1954), the data from various experiments were statistically evaluated using Completely Randomized Design (CRD).

Results and Discussion

These eight bacterial isolates were chosen for their PGP characteristics, such as solubilization of phosphorus, zinc and potassium; production of siderophore, ammonia, IAA, GA₃, HCN and compatibility with commonly used pesticides in crops (*in vitro*). The results are presented below.

Qualitative Assay for phosphorus, potassium and zinc solubilization by Plate Assay

Because phosphate solubilizing bacteria may convert insoluble phosphate to soluble orthophosphate ions that may be taken up and used by plants, the B120, B365, P69, AZB17, and ASP25 bacterial isolates demonstrated a zone of solubilization after 14 days of incubation by differing in their ability to solubilize TCP. The highest 'P' solubilization was noted for P69 with an index of 2.91 and 190 % efficiency and Lowest was observed by B365 with a solubilization index (SI) of 2.36 and solubilization efficiency (SE) of

136.36%. The solubilization results are shown in Figures 2a, 2b (Supplementary Table S2, Figure S1). Isolates may solubilize 'P' due to phosphatase synthesis or release of low molecular weight organic acids and production of inorganic acids such as sulphuric acid, nitric acid, and carbonic acid (Vyas and Gulati, 2009), generation of protons and ligands (Hinsinger *et al.*, 2011), mobilise phytate (organic P) possibly by phytase synthesis (Jorquera *et al.*, 2008). *Pseudomonas* isolate P33, *Azospirillum* (As-22) showed TCP solubilization (Desai, 2012).

Similarly, among all the eight isolates tested for 'K' solubilization, only AZB17 could solubilize potassium aluminium silicate supplemented in Aleksandrow's medium, with a SI of 2.8 and 180% of SE as shown in Figure-2a, 2b (Supplementary Table S3, Figure S2). The release of potassium caused by the creation of a clearing zone surrounding the colonies could be related to exopolysaccharide synthesis.

All the selected bacterial isolates were able to solubilize ZnO and ZnCO₃ except ASP34 which could solubilize only ZnCO₃ when supplemented as an insoluble source and has shown the highest solubilization with an index of 5.33 and efficiency of 433.00%. In ZnO amended media as insoluble mineral form, SI and SE were highest for B120 and least for B365 (8.75 and 775%; 2.50 and 150%, respectively) (Supplementary Table S4, Figure S3, S4). Hence, it is clear that Zn metal is known to be immobilised by bacteria by precipitation and adsorption. All the isolates differed significantly in their solubilization ability are shown in Figure 2a, 2b. In line with the results obtained, Zn solubilization efficiency of bacterial isolates collected from legume crop rhizosphere in ZnO modified media ranged from 117.2 percent to 466 percent, according to the Kumar *et al.*, 2014 research group.

Quantitative estimation of potassium solubilization efficiency

The amount of potassium released from potassium aluminium silicate by all isolates rose with incubation time and peaked at 9 days after inoculation (DAI), according to the findings as shown in Figure-3. The 'K' released from the broth by the isolate at 9 DAI measured using a flame photometer, ranged from 2.93 µg mL⁻¹ to 6.25 µg

mL⁻¹. With an increase in incubation time, a fall in pH was detected, resulting in media acidification (Supplementary Table S5). The increase in potassium solubilization and reduction in pH in culture broth over time could be due to the production of large amounts of organic acids that can form bidentate complexes with metal ions and chelate silicon ions, which are more effective in increasing mineral dissolution. Our findings are similar to those of Verma and colleagues' (2017) research, five bacterial strains, including four *Pseudomonas* sp. and one *Azotobacter* sp., were shown to be capable of releasing 'K' from muscovite and biotite.

Quantitative assay of Zn by ZnO and ZnCO₃ as supplement sources

The results showed significant heterogeneity in the isolates' ability to solubilize the same or different source of insoluble Zn compounds, which could be attributable to the generation of organic acids in the culture broth, such as gluconic acids (particularly 2-keto-gluconic acids). Maximum solubilization of 'Zn' with 0.1% ZnO and ZnCO₃ amended media was observed on 9 DAI by all the isolates. The available 'Zn' concentration in ZnO and ZnCO₃ amended broth was in the range of 14.8 to 21.3 ppm and 20.3 to 25.9 ppm, respectively on 9 DAI, when measured in AAS. Among all the isolates, B120 showed the highest solubilization with ZnO and also ZnCO₃ with the lowest pH as shown in Figure 4a, 4b. On the other hand, AZB31 showed the lowest solubilization with ZnO and B365 with ZnCO₃ as an insoluble mineral source, as shown in Figure 4a, 4b. The drop in pH of the culture medium was usually accompanied by an increase in incubation duration (Supplementary Table S6.1, S6.2). Microorganisms that solubilize zinc do so through a variety of methods, one of which is acidification through the release of organic acids (Alexander, 1997), chelated ligands and oxidoreductive systems on cell membranes (Chang *et al.*, 2005). On inoculation of *Bacillus* sp., *Azospirillum*, and *Pseudomonas aeruginosa* to plants, they showed increased growth and zinc content (Fasim *et al.*, 2002). From the results obtained we can conclude that the bacterial isolates of *Bacillus*, *Pseudomonas*, *Azotobacter* and *Azospirillum* were effective in solubilizing the inorganic insoluble forms of zinc,

	Phosphorous	Potassium	Zinc oxide	Zinc carbonate
B 120	2.64	0	8.75	4.09
B 365	2.36	0	2.5	2.56
P 48	0	0	3.17	4.5
P 69	2.91	0	3.33	4.86
AZB 17	2.9	2.8	3	4.75
AZB 31	0	0	2.82	4.2
ASP 25	2.5	0	3	4.8
ASP 34	0	0	0	5.33

Figure 2a: Solubilization index (SI) of bacterial isolates to solubilize different mineral sources (in vitro)

	Phosphorous	Potassium	Zinc oxide	Zinc carbonate
B 120	163.64	0	775	309.09
B 365	136.36	0	150	155.56
P 48	0	0	216.67	350
P 69	190.91	0	233.33	385.71
AZB 17	190	180	200	375
AZB 31	0	0	181.82	320
ASP 25	150	0	200	380
ASP 34	0	0	0	433.33

Figure 2b: Solubilization efficiency (SE %) of bacterial isolates to solubilize different mineral sources (in vitro)

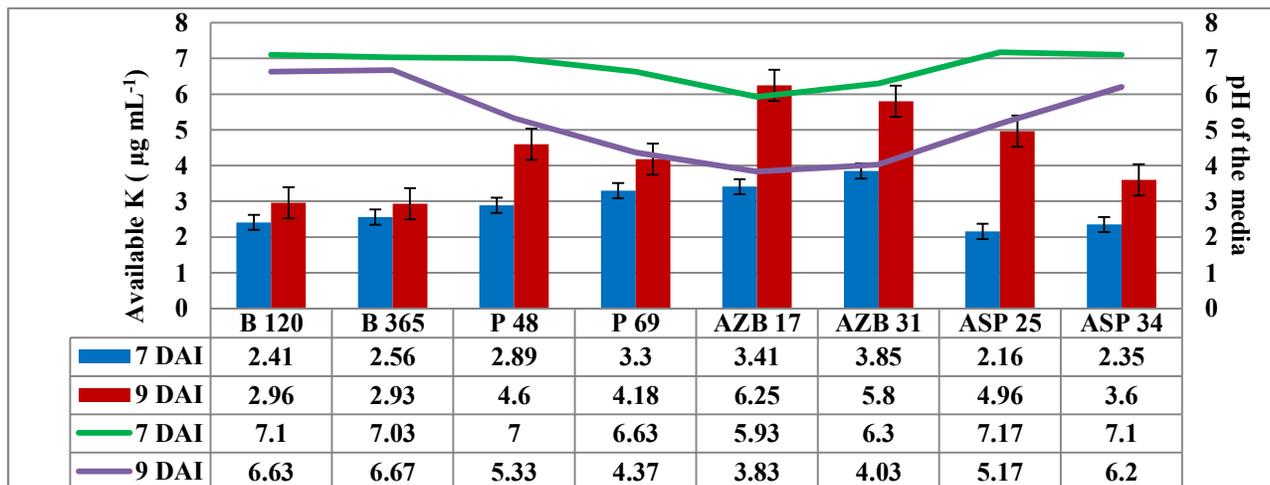


Figure 3: Evaluation of selected isolates for their ability to solubilize potassium present in the media over a while by decreasing the pH of the broth (in vitro)

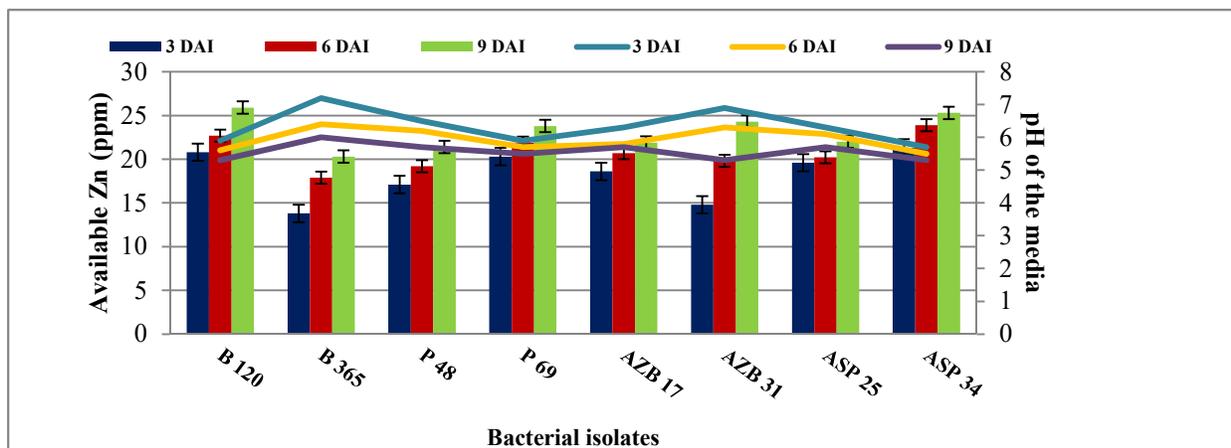


Figure 4a: Evaluation of selected isolates for their ability to solubilize Zn present in ZnO, over a period of time by decreasing the pH of the broth (in vitro) Where, DAI is Days after inoculation, ppm is Parts per million

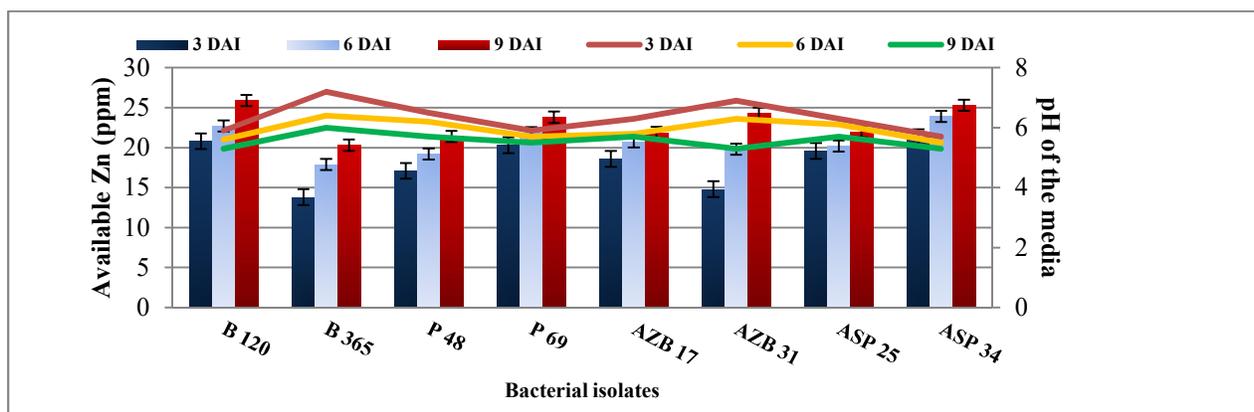


Figure 4b: Evaluation of selected isolates for their ability to solubilize Zn present in ZnCO₃ over a period of time by decreasing the pH of the broth (in vitro)

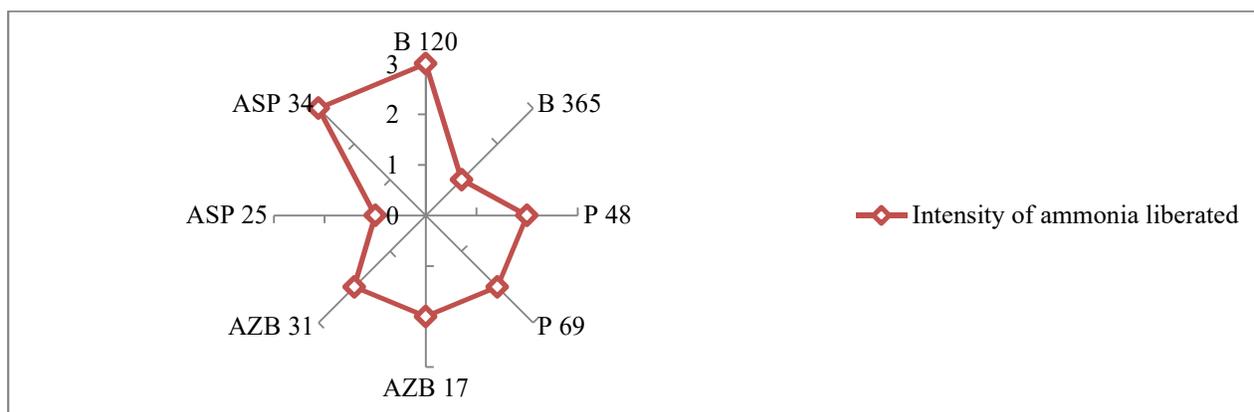


Figure 5: Indication of Ammonia production by the bacterial isolates based on colour intensity (in vitro). The highest value showing isolates are strong ammonia producers (B120, ASP34), a medium value indicating moderate ammonia producers (P48, P69, AZB17, AZB31) and the lowest value showing isolates represents weak producer of ammonia (B365, ASP25)

potassium, and phosphorous found in the soil and making them available for plant uptake, enhancing plant growth and improving crop production.

Production of Ammonia (Qualitative assay)

Ammonia production is an essential PGP feature of bacteria that indicates nitrogenase activity (Kundu and Gaur, 1984). Ammonia generation was detected in all of the PGPR isolates tested. When Nessler's reagent was added to the isolates cultured in peptone water, the broth colour changed brown to yellow, suggesting a positive test for ammonia generation. The isolates were divided into three categories based on colour intensity: strong, moderate, and weak. As shown in Figure-5, B120 and ASP34 were substantially positive; P48, P69, AZB17, AZB31 were moderately positive; and B365 and ASP25 were faintly positive (Supplementary Table S7, Figure S5). All of the ammonia-producing bacterial isolates give evidence for the ammonification process, which is a key part of the nitrogen cycle (Suja *et al.*, 2010). Reeves *et al.*, (1983) discovered that increasing the iron concentration boosted *Azospirillum* nitrogenase activity. Soil microorganisms that produce phytohormones can drive plant development and increase plant tolerance to a variety of biotic and abiotic challenges (Cho *et al.*, 2015; Mustafa *et al.*, 2020). IAA may be produced as a result of the conversion of tryptophan to indole-3-acetic aldehyde *via* a different mechanism than tryptamine formation. In *Azotobacter*, *Bacillus*, *Azospirillum*, and *Pseudomonas*, this pathway could provide a reason to operate.

Indole acetic acid (IAA) & Gibberellic acid (GA₃) Production (Qualitative and Quantitative assay)

IAA is a signal molecule for plant development, stimulates root growth, and modifies root architecture, increases water absorption and water holding capacity. The data presented in Table-1 revealed the IAA produced by different isolates varied from 0.25 to 22.63 $\mu\text{g mL}^{-1}$ after 72 h of incubation. The IAA production by ASP25 isolate was maximum and was statistically superior over all other isolates and the least was showed by P69 isolate. Similar findings were discovered by Islam and colleagues (2016), who tested 66 bacterial isolates from the cucumber rhizosphere and discovered that 10 isolates produced high quantities of IAA (26.78–51.28 g mL^{-1}). However, bacterial

isolates produced variable amounts of IAA, which could be attributable to their involvement in biosynthetic pathways, gene placement, regulatory sequences, and the presence of enzymes that convert active free IAA into conjugated forms.

Another growth regulator, gibberellin, impacts seed germination, boosts plant development, and slows ageing (Gou *et al.*, 2011), hence microorganisms were tested for their ability to produce GA₃. All of the isolates were able to generate GA₃ in a variation of quantities, 5.9 and 31.8 $\mu\text{g mL}^{-1}$. All the isolates differed significantly as shown in Table 1 (Figure S6, S7). Similarly, the generation of GA and ABA by rhizosphere microorganisms has been widely reported in prior research (Janzen *et al.*, 1992, Gutierrez *et al.*, 2001). The studies of Kapoor *et al.*, 2016 also indicated the generation of GA₃ by *Pyrus* and *Malus* rhizospheric bacteria. In the genera *Azotobacter* spp., *Bacillus* spp., and *Azospirillum* spp., PGPB synthesis of GAs has been found (Deka *et al.*, 2015; Dodd *et al.*, 2010).

Siderophore & HCN Production (Qualitative and Quantitative assay)

Another secondary metabolite produced during the stationary growth phase is cyanide (Knowles and Bunch, 1986). All eight bacterial isolates tested positive for HCN production capacity, which is thought to be linked to one of the microbes' biocontrol mechanisms. The ability of HCN synthesis, measured as Total Cyanide Content (TCC) in ppm was maximum by B120 (77.2 ppm) and the lowest by AZB17 (27.3 ppm) presented in Table 2 (Supplementary Figure S8, S9). HCN production varies between isolates, possibly because the oxidative decarboxylation route, which uses glycine as a precursor, produces HCN at higher rates (Trolldenier *et al.*, 1986). Bacterial genera including *Pseudomonas* and *Bacillus* have been discovered to release HCN (Das *et al.*, 2017; Zachow *et al.*, 2017). The action of HCN has been linked to the suppression of *Meloidogyne javanica*-caused tomato root knot disease (Siddiqui *et al.*, 2006).

Siderophore production is an indirect mode of biocontrol ability of microorganisms to chelate the iron and starve the pathogens for iron. All of the isolates tested positive for siderophore synthesis in this investigation. Table 2 shows that B120 exhibited maximum percent of siderophore units (48.00%) followed by P69 which was 43.00% and

Table 1: Screening of selected isolates for their ability to produce indole Acetic acid (IAA) and gibberellic acid (GA₃)

Isolates	IAA µg/mL	GA ₃ µg/mL
B 120	4.86 ± 0.24	5.96 ± 0.30
B 365	5.71 ± 0.17	22.31 ± 0.08
P 48	5.23 ± 0.18	27.63 ± 0.96
P 69	0.25 ± 0.05	31.89 ± 0.32
AZB 17	5.98 ± 0.10	22.39 ± 0.39
AZB 31	9.97 ± 0.23	8.32 ± 0.19
ASP 25	22.64 ± 1.13	26.27 ± 1.31
ASP 34	9.97 ± 0.35	22.32 ± 0.77
S.Em (±)	0.025	0.026
C.D. (p=0.05)	0.077	0.078
CV %	0.545	0.213

Table 2: Production of HCN and Siderophore by selected bacterial isolates

Isolates	TCC (ppm)		psu (%)
B120	77.22 ± 3.86	+++	48.87 ± 2.44
B365	40.78 ± 0.43	++	36.23 ± 0.73
P48	63.36 ± 2.19	+++	22.25 ± 0.77
P69	72.86 ± 0.99	+++	43.66 ± 1.57
AZB17	27.32 ± 0.47	+	12.27 ± 0.21
AZB31	72.86 ± 1.68	+++	19.74 ± 0.46
ASP25	35.24 ± 1.76	++	6.51 ± 0.33
ASP34	32.87 ± 1.14	++	37.65 ± 1.30
S.Em (±)	0.023	-	0.007
C.D. (p=0.05)	0.071	-	0.022
CV %	0.079	-	0.044

+++; High; ++: Medium; +: Low

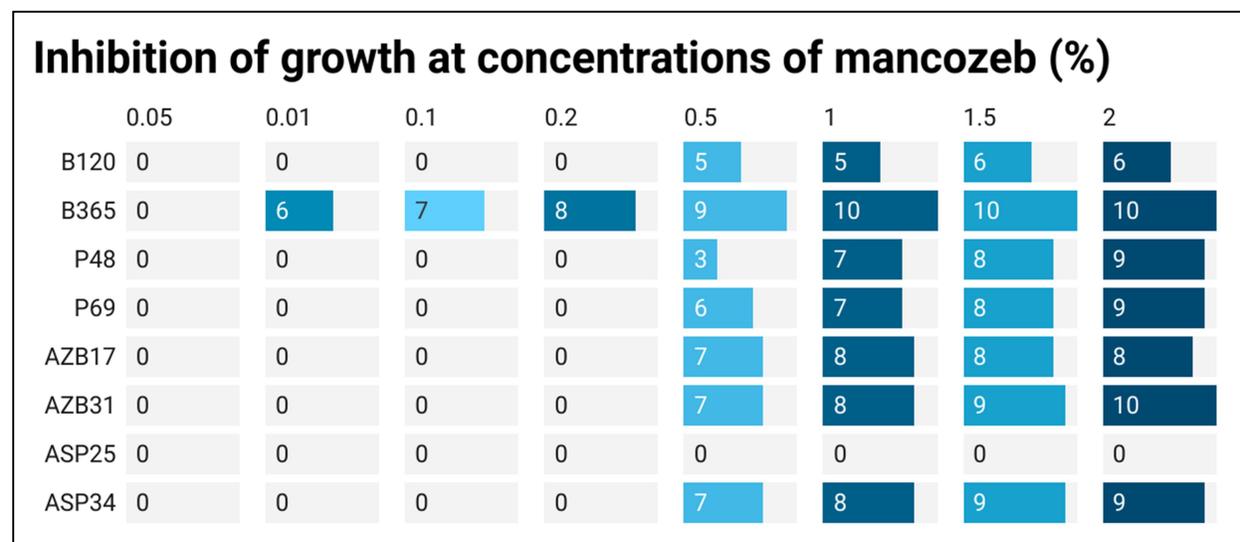


Figure 6: Zone of Inhibition (mm) of selected bacterial isolates with mancozeb at different concentrations (Plate assay)

ASP34 (37.00%) (Supplementary Figure S10). Similar findings were made by Ahmad *et al.* (2008) showed that 12.77 percent of *Azotobacter* isolates were able to manufacture siderophores after screening 72 isolates belonging to *Azotobacter*, *Pseudomonas*, *Mesorhizobium*, and *Bacillus*. *Bacillus halotolerans* produced the most siderophores (73 percent), followed by *Bacillus subtilis* (69 percent), and *Bacillus safensis* (68 percent) in the quantitative estimation of siderophores (Sarwar *et al.*, 2020).

Compatibility of selected isolates with pesticides

If the biological control approach has to be successful, the biocontrol agents should be compatible with the commonly used pesticides. All the eight potential isolates selected were compatible with carbendazim, acephate, imidacloprid, dimethoate and quinalphos at all concentrations and mancozeb at lower concentrations. Except for ASP25, remaining all bacterial isolates showed a zone of inhibition by the use of mancozeb. Zone of inhibition is a measure of the effectiveness of the pesticide (Figure 6). B365 was more sensitive to mancozeb at all concentrations except at 0.01%. Hence only 0.01% concentration of mancozeb was a safe tolerance limit for B365. The isolates B120, B365, P48, P96, AZB17, AZB31 and ASP34 showed inhibition zone ranging from 3 mm to 10 mm on treating with mancozeb fungicide at 0.5%, 1%, 1.5% and 2% concentrations.

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Conclusion

There is a pressing need to develop efficient and cost-effective microorganisms that may be utilised singularly or in groups to ensure constant performance in the farmer's field. More research is needed to determine the quality of this PGPM in cropping systems. The microbiome has enormous potential for agricultural profit in terms of global food security, crop production sustainability, and establishing agricultural systems that are resilient to climate change. It's fascinating to go into the genomes of specific microorganisms to see if they're physiologically hardy enough to be useful in the field. By boosting soil fertility, plant tolerance, crop yield, and maintaining a balanced nutrient cycle, the use of modern technologies and techniques for enhancing PGPR with multidisciplinary analysis will play a significant role in sustainable agriculture, say the researchers. The study was undertaken to develop organic fertilizers and meet the requirements of eco-friendly crop farming packages. The research found that eight selected bacteria exhibit the features that can contribute to plant development and biocontrol activities.

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