# Strain of *Bacillus circulans* isolated from apple rhizosphere showing plant growth promoting potential

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**Among a sub-sample of 13 isolates, a highly efficient P-solubilizing strain was selected and presumptively identified as *Bacillus circulans* MTCC 8983. The strain solubilized tricalcium phosphate and produced substantial amount of soluble phosphorus (957.3 mg/l) in Pikovskaya's (PVK) broth and exhibited the production of indoleacetic acid (IAA) (15.13 μg/ml), siderophore (57.80%) and growth inhibition against *Dematophora necatrix* (46.57%). Phosphate solubilization of *B. circulans* was inversely correlated with pH ($r = -0.98$) and positively correlated with growth ($r = 0.98$), siderophore production ($r = 0.99$), IAA ($r = 0.78$) and antifungal antibiotic activity against *D. necatrix* ($r = 0.87$). Concurrent production of IAA, siderophore, antifungal antibiotic activity along with phosphorus solubilization revealed its plant growth promotion potential. Thus, the ability of performing multifarious plant growth promoting activities together suggested uniqueness of *B. circulans* MTCC 8983 and its potential use in developing a cost-effective ecofriendly multifunctional biofertilizer for use in apple orchards, the most important cash crop in Himachal Pradesh.**

**Keywords:** *Bacillus circulans*, IAA siderophore production, plant growth promoting rhizobacteria, phosphate solubilizing bacteria.

PLANT growth promoting rhizobacteria (PGPR) are a heterogenous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly or indirectly. Direct promotion by PGPR entails either by providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. Indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic microorganisms. PGPR enhance the adaptive potential of their hosts through a number of mechanisms, such as the fixation of molecular nitrogen, the mobilization of recalcitrant soil nutrients, the control of phytopathogens and the synthesis of phytohormones and vitamins[1,2].

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Phosphate solubilizing microorganisms (PSM) play a significant role in making phosphorus available to plants by bringing about favourable changes in soil reaction in the soil microenvironment leading to solubilization of inorganic phosphate sources. Microbial-mediated solubilization of insoluble phosphates through release of organic acid is often combined with production of other metabolites (siderophores, phytohormones and lytic enzymes), which take part in biological control against soil-borne pathogens[3]. Soil inoculation with PSM improves P-solubilization of fixed soil phosphate and result in higher crop yields[4].

Though much information is available on the activity of soil microorganisms and phosphate solubilization for annual crops, limited information is available in respect of phosphate solubilizing bacteria associated with perennial crops such as apple. Previous research in our laboratory has described beneficial effect of rhizobacteria on growth and nutrient uptake by apple seedlings[5]. However, studies on phosphate solubilizing activity of rhizobacteria associated with apple seedlings have not been conducted. Therefore, the present study deals with an *in vitro* study of phosphate solubilizing ability of apple rhizobacteria along with release of pathogen-suppressing metabolites (indoleacetic acid (IAA) and siderophore production).

Plant growth promoting rhizobacterium, originally isolated from rhizosphere soil of apple (*Malus domestica* Borkh.) seedlings, was maintained on nutrient agar medium at 4°C. The identification of the isolate as *Bacillus circulans* MTCC 8983 was confirmed from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

For P-solubilization, all isolates were first screened on Pikovskaya (PVK)[6] agar plates. Quantitative estimation[7] of phosphorus was done in PVK broth amended with 5.0 g/l tricalcium phosphate (TCP). The logarithmic-phase culture was inoculated and incubated at 37°C for 96 h at 150 rpm. The growth of bacteria was monitored by the spread plate method at definite time intervals.

The CAS assay[8] was used for qualitative detection of siderophore production in PVK broth. To determine the siderophore in liquid culture, 0.5 ml culture supernatant was mixed with 0.5 ml CAS assay solution and the tubes were observed for colour change from dark blue to light blue or orange.

A dual plate method was used for *in vitro* screening of biocontrol PGPR. The inhibitory effect of culture filtrate was determined using dilution technique[9]. Percentage of growth inhibition was calculated using the formula proposed by Vincent[10].

$$I = \frac{c - T}{c} \times 100,$$

where $I$ is the percentage of growth inhibition; $C$ the growth of fungus in control; $T$ the growth of fungus in treatment.

**Table 1.** Tricalcium phosphate solubilization by *Bacillus circulans* MTCC 8983 and production of IAA in Pikovskaya's medium under optimum conditions

| Incubation period (h) | P-solubilization (mg/l) | IAA (μg/ml) | Viable count (log CFU/ml) | Final pH of supernatant |
|---|---|---|---|---|
| 0 | 0.00 | 2.53 | 6.18 | 6.92 |
| 24 | 577.0 | 3.60 | 6.56 | 5.89 |
| 48 | 906.0 | 7.27 | 6.81 | 4.87 |
| 72 | 957.3 | 15.13 | 6.83 | 4.69 |
| 96 | 910.0 | 14.13 | 6.73 | 4.70 |
| $CD_{0.05}$ | 3.351 | 0.342 | 0.022 | 0.021 |

**Table 2.** Tricalcium phosphate solubilization by *B. circulans* MTCC 8983 and production of siderophore and antifungal antibiotic activity in Pikovskaya's medium under optimum conditions

| Incubation period (h) | P-solubilization (mg/l) | Viable count (log CFU/ml) | Siderophore unit (%) | Growth inhibition of *Dematophora necatrix** (%) |
|---|---|---|---|---|
| 0 | 0.00 | 6.18 | 0.00 (0.00) | 17.67 (24.86) |
| 24 | 577.0 | 6.56 | 35.57 (36.61) | 24.63 (29.76) |
| 48 | 906.0 | 6.81 | 53.53 (47.03) | 37.63 (37.84) |
| 72 | 957.3 | 6.83 | 57.80 (49.49) | 46.57 (43.03) |
| 96 | 910.0 | 6.73 | 55.60 (48.22) | 31.67 (34.25) |
| $CD_{0.05}$ | 3.351 | 0.022 | 0.192 | 0.253 |

Figures in parentheses are arc-sin transformed value. *10% of culture filtrate.

*B. circulans* MTCC 8983 was inoculated to PVK medium not supplemented with 500 μg/ml L-tryptophan and incubated at 37°C for 96 h. The IAA concentration in the culture supernatant was determined by colourimetric method[11].

All the experiments were conducted in triplicates along with equal number of appropriate controls. Data were statistically analysed by analysis of variance technique (one way classification) following Gomez[12].

A total of 13 bacterial strains were isolated and all of them exhibited phosphate solubilization, siderophore production and antifungal activity against *Dematophora necatrix*. However, none of the isolates was found positive for HCN production. One of them showed maximum effectiveness of multifarious plant growth promoting activities.

On the basis of cultural, morphological and biochemical characteristics, the isolate was presumptively identified as *B. circulans* MTCC 8983. *B. circulans* MTCC 8983 had the ability to grow over a wide range of temperature (20–42°C), pH (5.0–9.0) and NaCl concentration (2.5–8.5%).

It was observed that maximum P-solubilization (957.3 mg/l) occurred up to 72 h of incubation. P-solubilization was accompanied by a decrease in pH of the culture filtrate from 6.92 initially to 4.69 (Table 1). The decrease in pH indicates the production of organic acids considered responsible for P-solubilization[13]. Maximum growth (6.83 log cfu/ml) coincides with the maximum amount of P-solubilization and is in agreement with the earlier reports on P-solubilization[14]. The extent of solubilization with the release and accumulation of 957.3 mg/l (95.7%; repeated at least three times with reproducible results) of phosphate from TCP by *B. circulans* MTCC 8983 is high in comparison to earlier report on P-solubilization, i.e. 502.4 mg/l by *Bacillus megaterium*[15] and 785 mg/l by an induced mutant of *Aspergillus tubingensis*[16].

Substantial production of IAA (2.53–15.13 μg/ml) was observed by *B. circulans* MTCC 8983 when it was grown in PVK broth without tryptophan (Table 1). The production of IAA increased up to 72 h. IAA production by microbes promotes the root growth by directly stimulating plant cell elongation or cell division[17]. Increase in IAA production in medium supplemented with tryptophan is not surprising because tryptophan is a known precursor for synthesis of IAA[18].

Another important trait of PGPR, that may indirectly influence the plant growth, is the production of siderophores. They bind to the available form of iron ($Fe^{3+}$) in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant health. In the present study, *B. circulans* MTCC 8983 showed maximum antagonism against *D. necatrix* (46.57%) and siderophore production (57.80%) at 72 h of incubation (Table 2). The production of maximum antifungal antibiotic activity at the end of exponential phase is in agreement with the earlier studies on the production of peptide antibiotic by *B. megaterium*[19] that usually begins at late log phase of growth and continues with the stationary

**Table 3.** Antifungal spectrum of antifungal metabolites produced by *B. circulans* MTCC 8983 in Pikovskaya's medium containing tricalcium phosphate under optimum condition in Pikovskaya's medium under optimum conditions

| Sample | Sample details | Antifungal activity (% growth inhibition) against different fungal pathogen | | | |
|---|---|---|---|---|---|
| | | *Dematophora necatrix* | *Rhizoctonia solani* | *Sclerotinia sclerotiorum* | *Fusarium oxysporum* |
| Control | MEA | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| Test | MEA + CF 5% | 28.73 (32.42) | 30.33 (33.42) | 23.67 (29.11) | 26.23 (30.81) |
| | MEA + CF 10% | 46.17 (42.80) | 44.20 (41.67) | 39.67 (39.04) | 44.60 (41.90) |
| | MEA + CF 20% | 48.17 (43.95) | 40.31 (37.53) | 46.83 (43.19) | 48.60 (44.20) |
| | MEA + CF 40% | 58.47 (49.88) | 50.83 (45.48) | 55.73 (48.29) | 59.37 (50.40) |
| $CD_{0.05}$ | | 0.432 | 1.617 | 0.423 | 0.312 |

MEA: Malt extract agar; CF: Culture filtrate. Figures in parentheses are arc-sin transformed values.

phase. The antifungal activity exhibited a close relationship with production of siderophore.

*B. circulans* MTCC 8983 also showed antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum* (Table 3) in addition to *D. necatrix*. These results are in agreement with earlier report that *Bacillus* sp. produced antifungal metabolites with activity against a number of mycelial fungi[20,21]. We propose that this effect may be caused by the different antifungal metabolites including siderophores, organic acids, IAA and antifungal antibiotics in the culture filtrate. Other workers[22,23] have reported that the inhibition of growth of phytopathogens was due to the production of some specific siderophores, antibiotics, secondary metabolites or hydrolytic enzymes. Therefore, phosphate solubilizing bacteria (PSB) with antifungal activity could be further exploited both as a biofertilizer and an effective biocontrol agent.

It has now been well established that a single PGPR has several modes of action. We have recently published the work on characterization of a novel carbendazim tolerant *B. subtilis* strain isolated from tomato rhizosphere that exhibits multiple plant growth promoting activities[24]. To the best of our knowledge, this is the first report of a phosphate solubilizing *B. circulans* MTCC 8983 isolated from apple seedlings simultaneously producing siderophore, IAA and antagonistic activity that continue during the logarithmic phase parallel with growth.

To know the overall impact of P-solubilization on pH ($X_1$), viable count ($X_2$), IAA ($X_3$), percentage of siderophore unit ($X_4$) and percentage of growth inhibition against *D. necatrix* ($X_5$) and regression analysis were carried out. The fitted regression lines along with standard error and adjusted $r^2$ have been given below. Their graphical representations have also been presented in Figure 1.

(i) $Y = 7.0134 - 0.0024X_1$ (0.000011) adj $r^2 = 0.97$.

(ii) $Y = 6.1766 + 0.007X_2$ (0.00003) adj $r^2 = 0.98$

(iii) $Y = 0.9239 + 0.0114X_3$ (0.0024) adj $r^2 = 0.62$

(iv) $Y = 0.2359 + 0.0601X_4$ (0.00054) adj $r^2 = 0.99$

(v) $Y = 15.15 + 0.0241X_5$ (0.0038) adj $r^2 = 0.75$

These fitted regression lines can be successfully employed to work out the value of P-solubilization for a given value of viable count, final pH, IAA, percentage of siderophore unit and percentage of growth inhibition against *D. necatrix* and hence these regression lines can be regarded as estimation equation.

P-solubilization of inorganic phosphate has been attributed to the production and release of organic acids[25,26], but others suggested some additional mechanisms as is confirmed by weak or even lack of linear correlation between pH and the amount of P-solubilized[27]. Strong negative correlation between pH and P-solubilized ($r = -0.97$) in the present study (Figure 1 *a*) is similar to that reported earlier[28–30]. A positive correlation observed between soluble P and growth ($r = 0.98$, Figure 1 *b*) is in agreement with earlier studies where the increase of P-solubilization was more closely related to the pattern of increasing population[31]. It was observed that there was no clear relationship between P-solubilization and growth of *A. tubingensis*[16].

Although simultaneous solubilization of phosphorus and production of IAA has recently been demonstrated[19], highly significant positive correlation between P-solubilization and concomitant production of IAA ($r = 0.62$; Figure 1 *c*), siderophore ($r = 0.99$; Figure 1 *d*) and antagonistic activity against *D. necatrix* ($r = 0.75$; Figure 1 *e*) has been recorded for *B. circulans* MTCC 8983 in the present study.

PSM microorganisms benefit plant growth and development not only by the increase in uptake of phosphate but is often combined with the production of other metabolites, which take part in biological control against

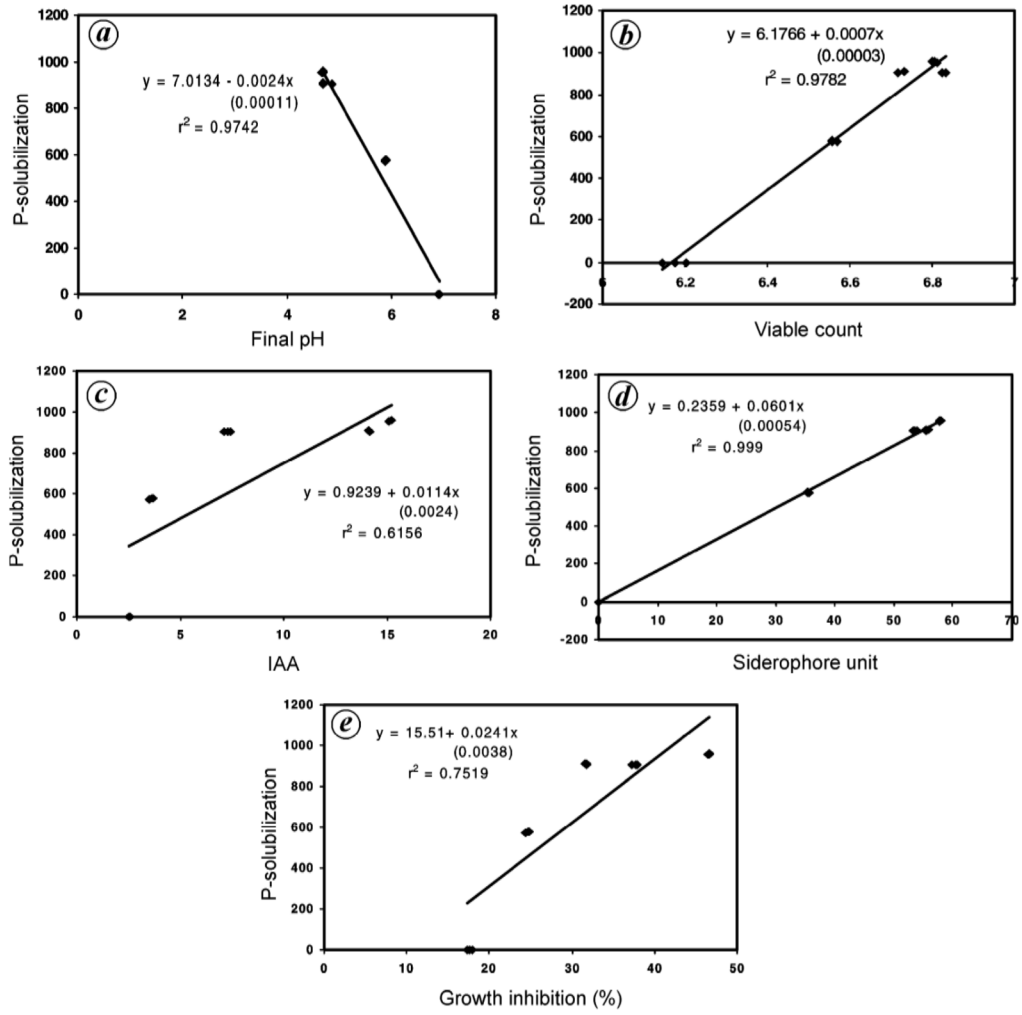


**Figure 1.** ***a***, Linear correlation between phosphate solubilization and final pH; ***b***, Growth, i.e. viable count; ***c***, IAA production; ***d***, Siderophore, and ***e***, Percentage of growth inhibition of *Dematophora necatrix*.

soil-borne plant pathogens. Our result showed the potential of P-solubilizing *B. circulans* MTCC 8983 for the simultaneous synthesis of IAA and release of pathogens suppressing metabolites, mainly siderophores.

This study elucidates the multifarious role of *B. circulans* MTCC 8983 with plant growth promoting potentials. This is the first example of a P-solubilizing bacterial agent isolated from apple plant rhizosphere to exhibit multiple plant growth promoting activities together. The choice of such bacteria can further augment their utility as bio-inoculations in sustainable organic farming.

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# Using fallout $^{210}$Pb measurements to estimate sedimentation rate in Lam Phra Phloeng dam, Thailand

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**The Lam Phra Phloeng dam was constructed in 1963 and is located in the Nakhon Ratchasima province. The dam has severely reduced water level caused by deforestation and agriculture at the upper land. Sediment cores were collected using a gravity corer. The $^{210}$Pb activities were measured using alpha and gamma spectrometry and sedimentation rates were determined. The sedimentation rates decreased gradually from the upstream to the crest of the dam. The high sedimentation rate may be due to the inflow from the tributary as well as eroded materials that come from the upland area to the dam.**

**Keywords:** Lam Phra Phloeng dam, $^{210}$Pb, sedimentation rate.

SEDIMENTATION and siltation in water supply dams are widespread problems affecting the viability of the water supply systems. The siltation results from settlement of sediments carried by rivers and causes a number of pro-

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