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Research Article

Paddy husk based formulations of plant growth promoting rhizobacteria for the plant growth

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Abstract

The use of chemical fertilizers in agriculture is a serious problem, which needs to be tackled. The agroindustry by-products viz., peanut shell, corncob, sawdust, paddy husk, press mud etc., are generated in huge amount. These agroindustry by-products are either incinerated or disposed of to landfills. The agroindustry by-products can be converted to value-added products viz; proteins; antibiotics; mushroom production; compost; plant growth promoting substances; etc. The agroindustry by-products can be used as carriers for the plant growth promoting rhizobacteria. The work focuses here on use of paddy husk based formulations of *Bacillus circulans* and *Bacillus subtilis* for the plant growth viz., maize, wheat, jowar and bajra, which was studied by the pot experiment. The paddy husk based formulations of plant growth promoting rhizobacteria showed an increase in the germination and vigour index of maize, wheat, jowar and bajra. There was also a significant increase in the plant growth viz., root length, shoot length and dry weight with paddy husk based formulations of *B. circulans* and *B. subtilis*. The use of paddy husk based formulations of *B. circulans* and *B. subtilis* for plant growth promotion will be eco-friendly and minimize the use of chemical fertilizers.

Keywords: Agroindustry by-product. Carrier. Vigor index. Renewable. Eco-friendly

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Introduction

The use of chemical fertilizers for plant growth in agriculture is a serious problem. There should be some alternative to chemical fertilizers. The agroindustry by-products are generated in a large amount. These are either incinerated or disposed of to landfills. These agroindustry by-products can be converted to different value-added products. The agroindustry by-products can be used as carriers for plant growth, promoting rhizobacteria (PGPR). There are reports where carrier-based formulations of PGPR have enhanced plant growth and yield. Studies have shown where talc-based formulations of *Pseudomonas fluorescens* (Vidhyasekaran *et al.,* 1997; Viswanathan and Samiyappan, 1999), *B. subtilis* and *P. chlororaphis* (PA23) (Kavitha *et al.,* 2003); vermiculite based formulations of *P. fluorescens* (Moenne-Loccoz *et al.,* 1999) and *B. subtilis* (Amer and Utkhede, 2000); chitin based formulations of *B. subtilis* strain GB03 + *B. pumilus* strain INR7 (LS256) and *B. subtilis* strain GB03 + *B. subtilis* strain IN937b (Kokalis-Burelle *et al.,* 2002) have increased the plant growth. Paddy husk is the most significant by-product of rice milling, and one-fifth of the paddy by weight consists of paddy husk. There are few reports on using paddy husk formulations of *B. circulans* and *B. subtilis* for plant growth promotion.

The work here describes paddy husk based formulations of *B. circulans* and *B. subtilis* for the growth of maize, wheat, jowar and bajra plants.

Materials and methodology

Sampling

The root samples, along with adhering soil of maize (*Zea mays*), were collected from a field at Uralikanchan, Pune, India, to isolate PGPR. The samples were stored in sterile plastic bags at low temperature until use.

Enrichment of PGPR

The enrichment of PGPR was done in LGI containing following composition (g/L) (K_2HPO_4 0.2, KH_2PO_4 0.6, $MgSO_47H_2O$ 0.2, $CaCl_22H_2O$ 0.04, sucrose 12, $Na_2MoO_42H_2O$ 0.02, $FeCl_36H_2O$ 0.01, bromothymol blue 5 ml, pH 6.0 and agar 30) and Jenson's Nitrogen free (JNF) media containing following composition were used (g/L) (malic acid 5, K_2HPO_4 0.6, KH_2PO_4 1.8, $MgSO_47H_2O$ 0.2, $CaCl_22H_2O$ 0.2, NaCl 0.1, Fe-EDTA 0.06, KOH 4.5, $FeCl_36H_2O$ 0.01, pH 5.0 and agar 30). The maize roots were disinfected with 70% ethanol (C_2H_5OH) for 30 s and 0.1% mercuric chloride ($HgCl_2$) for 1 min, followed by washing thrice with sterile distilled water (DW). The homogenized root sample (1 g) and rhizosphere soil sample (1 g) were suspended in 9 ml sterile saline. The serial dilutions were made of both the samples and 0.1 mL of each dilution was inoculated separately into 10 mL semisolid LGI and JNF media, incubated at 28 ^oC for three days. The tubes which showed thick yellow pellicles were considered as presumptive PGPR.

Isolation, purification and maintenance of the isolate

The growth from the positive tube was streaked on LGI and JNF solid medium and incubated at 28 ^{o}C for 3 days. Typical colonies were subcultured, and the pure cultures were maintained on Potato Dextrose Agar (PDA) slants at 4 ºC, respectively.

Characterization and identification of PGPR

The morphological, physiological and biochemical characteristics were studied using Bergey's Manual of Determinative Bacteriology (Holt, 1992). The isolates were confirmed by doing 16S rRNA sequencing.

Plant growth promoting traits of *B. circulans* and *B. subtilis*

The initial studies of the plant growth promoting traits viz., phosphate solubilization (El-Azouni, 2008), ammonia (NH_3) (El-Azouni, 2008), Indole Acetic Acid (IAA) (Egamberdiyeva *et al.,* 2008), Gibberellins (Holbrook *et al.,* 1961), exopolysaccharides (EPS) production of *B. circulans* and *B. subtilis* have been carried out.

Carrier-based formulations for the plant growth

Preparation of the inoculum

B. circulans and *B. subtilis* were grown in 100 ml potato dextrose broth on a shaker at 28 ^{o}C at 165 rpm for three days. The initial density was 2.0 x 10^5 cfu/g, respectively.

Selection and disinfection of the seeds

The seeds selected were viz., maize, wheat, jowar and bajra. The disinfection of the seeds was done as per the method of Sachdev *et al.,* 2009.

Source of agroindustry by-product, i.e., paddy husk

Paddy husk was purchased from Surve rice mill located at Karad, Maharashtra, India.

Processing of the paddy husk

The paddy husk was processed by making fine powder using the mixer. One kg of powdered paddy husk was passed through a sieve of the size of 72 µm, packed in polythene bags and used for further study.

Preparation of the formulations

Powdered paddy husk (50 g) was sterilized in 250 ml conical flask in an autoclave at 121 ^{o}C, 15 min to which was added 250 mL sterile DW, mixed and inoculated with 10 ml of each of *B. circulans* and *B. subtilis* cultures separately.

Preparation of slurry and bacterization of seeds

The slurry was prepared by mixing paddy husk based formulations (10 g) with 0.25 g calcium carbonate ($CaCO_3$) and 0.50 g carboxymethyl cellulose (CMC). $CaCO_3$ was used to neutralize the pH and CMC as adhesive. Ten disinfected seeds each of maize, wheat, jowar and bajra were pelleted for 5 min by mixing in the prepared slurry.

Pot experiment

The pot experiment was carried for the evaluation of carrier-based formulations using the randomized block design (RBD). The sterilized garden soil was used. The seeds without treatment were kept as control. The seeds were sown at an equal distance (depth 1 cm) in the pots. The seedlings were watered daily. After 25 days, the plants were carefully uprooted for the study of plant growth parameters viz., seed germination, vigour index, root length, shoot length and total dry weight.

Statistical analysis

The statistical analysis of the data was done using Microsoft Excel software to see any significant difference between the treatment and control.

Results and discussion

Characterization and identification of the PGPR

The isolates were named A8 and H8. The isolates were Gram +ve motile rods, and optimum salt concentration was 2.50%, pH 5.0-7.0 and temperature 37 ^{o}C for the growth of the isolates. The isolates did not show hydrogen sulphide (H_2S) and indole production. Both the isolates were fermentative and showed citrate utilization. The isolate H8 was positive for Voges-Proskauer (VP) test and also showed urea degradation. The isolates were oxidase and catalase-positive and could liquefy gelatin. The isolate A8 was protease positive. The isolates also showed starch hydrolysis.

The 16S rRNA sequencing confirmed the isolate A8 as *Bacillus circulans* (Accession Number: AY043084.1) and H8 as *Bacillus subtilis* (Accession Number: FJ413049.1).

Plant growth promoting traits

B. circulans and B. subtilis showed plant growth promoting properties. Both isolates were found to solubilize phosphorus. The phosphorus solubilization by *B. circulans* and *B. subtilis* was 3.16 $\pm$ 0.00 and 2.40 $\pm$ 0.00 µg/mL respectively. The isolates also showed NH_3 production. The IAA production by *B. circulans* and *B. subtilis* was 8.50 $\pm$ 0.02 and 12.50 $\pm$ 0.00 µg/mL and gibberellins 8.00 $\pm$ 0.36 and 6.40 $\pm$ 0.02 µg/mL respectively. The EPS production by *B. circulans* was found to be 0.63 $\pm$ 0.01 g/mL.

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Evaluation of paddy husk formulations of PGPR on the growth of plants

Effect of paddy husk formulations of PGPR on the maize seed and growth

Increase in the vigor index of maize plant was found by paddy husk formulations of *B. circulans* and *B. subtilis* (Table 1).

Table 1 Effect of paddy husk based formulation of PGPR on maize seed and growth

Formulation of	Avg Root length (cm)	Avg Shoot length (cm)	Dry weight (mg/plant)	Germination (%)	Vigor index
Control	3.25 ± 2.50	3.75 ± 1.03	3.93 ± 0.82	90 ± 1.41	630.00
B. circulans	3.43 ± 2.14 (0.45)^{c}	2.36 ± 1.79 (0.09)^{ab*}	3.53 ± 2.46 (0.38)^{c*}	80 ± 0.00	463.20
B. subtilis	4.33 ± 2.50 (0.26)^{c}	4.67 ± 2.50 (0.25)^{c}	4.24 ± 2.88 (0.42)^{c}	90 ± 0.00	810.00

Effect of paddy husk formulations of PGPR on the wheat seed and growth

A significant increase in the root length of the wheat plant was found by paddy husk formulations of *B. circulans* and *B. subtilis* (Table 2). There was a significant increase in the shoot length and dry weight by paddy husk formulation of *B. subtilis*. The seed germination was also increased by paddy husk formulations of PGPR (Table 2). The vigour index of the wheat plant was found to increase by paddy husk formulation of *B. subtilis*.

Table 2 Effect of paddy husk based formulation of PGPR on wheat seed and growth

Formulation of	Avg Root length (cm)	Avg Shoot length (cm)	Dry weight (mg/plant)	Germination (%)	Vigor index
Control	1.60 ± 0.89	2.60 ± 1.14	2.00 ± 0.93	80 ± 0.00	336.00
B. circulans	2.76 ± 0.75 (0.02)[a]	4.79 ± 3.02 (0.08)[ab]	2.66 ± 1.63 (0.22)[c]	85 ± 0.70	336.00
B. subtilis	5.00 + 2.44 (0.01)[a]	4.00 ± 0.81 (0.03)[a]	3.87 ± 1.74 (0.42)[a]	100 ± 0.00	900.00

Effect of paddy husk formulations of PGPR on the jowar seed and growth

The root length of jowar plant was significantly increased by paddy husk formulation of *B. circulans* (Table 3). The seed germination and vigour index were also increased by paddy husk formulations of PGPR.

Table 3 Effect of paddy husk based formulation of PGPR on jowar seed and growth

Formulation of	Avg Root length (cm)	Avg Shoot length (cm)	Dry weight (mg/plant)	Germination (%)	Vigor index
Control	2.40 ±1.14	2.40 ± 1.14	2.96 ± 2.25	70 ± 0.00	336.00
B. circulans	4.94 ± 2.22 (0.02)[a]	3.63 ± 2.65 (0.18)[c]	3.12 ± 2.23 (0.45)[c]	80 ± 0.00	685.60
B. subtilis	3.22 ± 1.12 (0.14)[c]	3.38 ± 2.30 (0.20)[c]	2.90 ± 2.24 (0.48)[c*]	100 ± 0.00	660.00

Effect of paddy husk formulations of PGPR on the bajra seed and growth

The seed germination was increased by paddy husk formulation of *B. circulans*. The vigour index of bajra plant was increased by paddy husk formulations of *B. circulans* and *B. subtilis* (Table 4).

Table 4 Effect of paddy husk based formulation of PGPR on bajra seed and growth

Formulation of	Avg Root length (cm)	Avg Shoot length (cm)	Dry weight (mg/plant)	Germination (%)	Vigor index
Control	2.50 ±0.70	2.50 ± 0.93	2.68 ± 1.24	80 ± 0.00	400.00
B. circulans	1.44 ± 0.50 (0.02)[a*]	4.19 ± 3.23 (0.09)[ab]	2.57 ± 1.25 (0.45)[c*]	90 ± 0.00	506.70
B. subtilis	2.48 ± 0.97 (0.48)[c*]	3.50 ± 2.37 (0.11)[c]	3.00 ± 0.81 (0.34)[c]	80 ± 0.00	478.40

*Vigor index = (mean root length + mean shoot length) x % germination (Abdul-Baki and Anderson, 1973)

All values are average of two readings. Figures in parenthesis, *P* value of T test

[a] Significant increase, [ab] weakly significant increase, [c] no significant increase, [a*] Significant decrease; [ab*] weakly significant decrease, [c*] no significant decrease

Effects were significant if $P \leq 0.05$, weakly significant if $0.05 < P \leq 0.1$ and not significant if $P > 0.1$

There is a report where composted paddy husk improved the growth of sunflower plants (Badar and Qureshi, 2014). Also, a report where paddy husk composted with 2% lignocellulolytic fungus *Aspergillus* sp. can be alternate organic manure to enhance the yield and quality of black gram crop (Thiyageshwari *et al.,* 2018). Fly-ash based formulation of *Azotobacter chroococcum* has been reported to increase seed germination, plant height and biomass of wheat plant (Kumar and Gupta, 2010). A report showed an increase in the root length, dry matter, and phosphorus uptake of the maize plant by the carrier material prepared using lignite enriched with 1% soybean powder (SP) and 2% Mussoorie Rock Phosphate (Menaka and Alagawadi, 2007). The enhanced growth and dry matter content of different crop plants due to the amendment of carrier materials has been reported (Mudenoor, 2002).

Conclusion

Paddy husk carrier-based formulations of *B. circulans* and *B. subtilis* improved the growth of maize, wheat, jowar and bajra plants. The use of carrier-based formulations in agriculture for plant growth will be eco-friendly, reduce the use of chemical fertilizers and ultimately benefit the farmers.

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The social relevance of the study

The social impact of the study is that the biofertilizer product developed will be beneficial to the farmers and will be economically feasible.

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