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Differences in rice rhizosphere bacterial community structure by application of lignocellulolytic plant-probiotic bacteria with rapid composting traits

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ABSTRACT

Plant-probiotic rhizobacteria with abilities of plant growth stimulation and lignocellulosic degradation were isolated from rice rhizosphere, and were used for degradation of rice straw residues to compost. Probiotic bacterial composts formulated with *Bacillus cereus* RSDa2, *Stenotrophomonas maltophilia* RSI6 or *Klebsiella pneumoniae* RSI9 were characterized and applied in rice rhizosphere that significantly ($P \leq 0.05$) enhanced the growth of plants. Variation in rhizosphere bacterial community of rice plants treated with probiotic bacterial compost were studied utilizing the metagenomic analysis of 16S (V3–V4) rRNA gene sequences. Proteobacteria remained the most prominent phyla in control and compost treatments, though relative abundance was lower than control. Also, the relative abundance of Firmicutes and Acidobacteria increased several fold. An unclassified genus from *Xanthomonadaceae* family (26.3%) dominated the rice rhizosphere population in control, while its relative dominance was highly reduced in compost treatments. Several genera including *Geobacter*, *Geothrix*, *Cellulomonas*, *Methylomonas*, *Azospira* were detected in all three compost treatments, which were completely absent in control. The Shannon alpha diversity index was low for control as compared to probiotic bacteria composts treatments. PCoA based on unweighted UniFrac distances placed RS16 and RS19 treatments close to each other, while, unweighted UniFrac distances placed all the treatments at different coordinates.16S (V3–V4) rRNA gene sequence datasets had been submitted to the Sequence Read Archive (SRA) under accession number SRP9811 under Bioproject no. PRJNA411879.

1. Introduction

Rice, (*Oryza sativa* L.) is the principal crop and staple food of nearly half of the world's population. Rice cultivation results in production of rice straw residue (RSR), a by-product and agricultural waste, which remain in the field for long durations if left unattended (Zeigler et al., 2008). Methods for disposal of RSR include burning or mulching in the rice fields that eventually give rise to temporary immobilization of N, and methane (CH_4) emission, that contributes to greenhouse gases (Dobermann et al., 2002). Rice straw comprises mainly lignocellulosic polymers (Shen et al., 1998) that are difficult substrates for microbial decomposition as compared to other grains such as wheat and barley (Parr et al., 1992).

However, the possibility of degrading lignocellulosic by composting process has potential to convert lignocellulosic wastes into value-added biofertilizer (Mishra et al., 2003). Therefore, in present work, a strategy has been applied where plant growth promoting rhizobacteria (PGPR) were isolated and screened for their ability to produce lignocellulolytic enzymes, in addition to plant growth promoting attributes, to produce compost from bio-waste RSR. Plant growth promoting rhizobacteria (PGPR), are known for their ability to enhance growth of host plant by several mechanisms such as synthesis of phyto-hormones, solubilization of inorganic phosphorus, and production of siderophores (Bowen and Rovira, 1999).

Microbial communities within the rhizosphere microbiome tend to impact plant growth and development; however exploitation of their benefits depends on abundances and diversity of bacteria possessing desirable traits. Consequently, these communities are typically less diverse than those in augmented systems. Plant – probiotic bacteria are defined for their ability to improve the growth and nutrition of the plant due to their plant growth promoting attributes (Spence et al., 2012). There are reports where the rhizosphere bacterial community structures had been found to respond to the nutrient rich organic amendments in soil (Bakker et al., 2015; Ling et al., 2014). In fact, Proteobacteria, Acidobacteria and Chloroflexi phyla had been found to dominate bacterial community in response to compost amendments in rice–wheat cropping system (Zhao et al., 2016).

In this study, next generation sequencing approach was used to

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estimate the rhizosphere bacteria community structure, in response to application of RSR–compost formulated with plant probiotic bacteria PGPR, as it imparts beneficial effect to the rice plant. Illumina based 16S rRNA gene amplicon sequencing and QIIME analysis is considered to provide reproducible information (Hong et al., 2016; Johnston-Monje et al., 2016; Cao et al., 2017). Association between microorganisms among bacteria and their environment have been hypothesized to be strategic contributors to microbial community dynamic as a result of complex interactions between microorganisms among themselves and with their environment (Faust et al., 2015). With improved understanding of rhizosphere microbiome, it's pertinent that stability and dynamics of balanced microbiome will be the response for obtaining healthier and more productivity of host plants.

2. Materials and methods

2.1. Isolation

Rhizospheric soils were collected from healthy rice plants (Variety, *Boro*) growing in South Assam, India. Suitable dilutions (10^{-2}–10^{-6}), of soil suspension were plated on Yeast extract mannitol (YEM) agar medium to isolate the rhizospheric bacteria, incubated for 24 h at 30 ± 2 °C. The purified cultures were preserved in agar slants (4 °C) and glycerol stocks (20% v/v, −20 °C).

2.2. Screening of bacterial isolates for in vitro plant growth promotion traits

2.2.1. Indole acetic acid (IAA) production

The indole acetic acid (IAA) production was determined by the method of Devi et al (2017). Strains were allowed to grow on YEM broth containing 2 mg/ml of tryptophan for 5 days and kept incubated in a shaker (150 rpm, 30 °C). 5 ml aliquot was withdrawn periodically from each culture flask at 24 h intervals, centrifuged (11,000 rpm, 15 min) and 1 ml of the supernatant was mixed with 2 ml of Salkowski reagent (Gordon and Weber, 1951). Absorbance was measured at 530 nm and the amount of IAA produced was calculated by comparing with the standard IAA curve.

2.2.2. Phosphate solubilization

Quantitative estimation of phosphate solubilization was performed according to Singha and Pandey (2017). Strains were inoculated in 100 ml of NBRIP medium (pH 7) and was incubated at 150 rpm, at 30 °C. 5 ml aliquots were withdrawn periodically from each culture flask at 24 h intervals, centrifuged (10,000 rpm, 10 min) and the supernatants were analyzed for P concentration. The amount of phosphate in the culture supernatants were estimated using the vanado-molybdate colorimetric method (Koenig and Johnson, 1942) and expressed as equivalent P (µg/ml). KH_2PO_4 was used as the standard.

2.2.3. Siderophore production

The ability of isolates to produce siderophore was determined by formation of orange halo around bacterial colonies on Chrome Azurol S (CAS) agar plates incubated at 30 °C for 48 h (Schwyn and Neilands, 1987). Quantitative estimation of siderophore production was performed by CAS-shuttle assay (Payne, 1994) on iron deficient broth media.1 ml of 5 d old cultures grown on YEMA were inoculated into 250 ml flasks containing 70 ml different growth media. 5 ml aliquot were withdrawn periodically from the culture flasks at 24 h intervals, centrifuged (10,000 rpm, 10 min) and 1 ml of supernatant was mixed with 1 ml of CAS reagent. Absorbance was measured at 630 nm against a reference consisting of 1 ml of uninoculated broth and 1 ml of CAS reagent. The amount of siderophore produced (percentage siderophore units) was calculated by using the formula [$\mathbf{Ar} - \mathbf{As} \times \mathbf{Ar}^{-1}$] where, **Ar** represent absorbance of reference at 630 nm, and **As** represent absorbance of sample at 630 nm.

2.3. Enzyme assays

2.3.1. Lignolytic enzyme production

The isolates were screened for extracellular enzymes - lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. The isolates were grown in YEM broth amended with lignin (1%), incubated at 120 rpm and at 30 °C, and cells were separated by centrifugation (10,000*g*, 15 min). Supernatant was collected and stored as crude enzyme preparation at 4 °C for enzyme assays (Rahman et al., 2013).

2.3.1.1. Lignin peroxidase. LiP was determined by monitoring the oxidation of dye Azure B (Archibald, 1992). The reaction mixture contained 1 ml of 125 mM sodium tartrate buffer (pH 3.0), 500 ml of 0.160 mM azure B, 500 ml of 2 mM hydrogen peroxide and 500 ml of the culture filtrate. The reaction was started by adding 0.5 ml of H_2O_2 and absorbance was measured at regular intervals at 651 nm. One unit of enzyme activity was defined as 'an absorbance decrease of 0.1 units per minute per ml of the culture filtrate'.

2.3.1.2. Manganese peroxidase. MnP assay was determined by the oxidation of phenol red at 610 nm (Orth et al, 1993). Five millilitre of reaction mixture contained 1.0 ml sodium succinate buffer (50 mM, pH 4.5), 1.0 ml sodium lactate (50 mM, pH 4.5), 0.4 ml manganese sulphate (0.1 mM), 0.7 ml phenol red (0.1 mM), 0.4 ml H_2O_2 (50µ M), gelatin 1 $mg.ml^{-1}$ and 0.5 ml of enzyme extract. The reaction was initiated by adding H_2O_2 and incubated at 30 °C. 40 µl of 5 N NaOH was added on one millilitre of reaction mixture. Absorbance was taken at 610 nm with 1 ml of the reaction mixture up to 4 min. One unit of enzyme activity is equivalent to an absorbance increase of 0.1 units/min/ml.

2.3.1.3. Laccase activity. The reaction mixture was prepared for estimation of laccase activity, which contained 3.8 ml acetate buffer (10 Mm, pH 5.0), 1 ml guaiacol (2 mM) and 0.2 ml of enzyme extract. The solution was incubated at 25 °C for 2 h and absorbance was read at 450 nm. Laccase activity has been expressed in relative term as colorimetric units.$m1^{-1}$ enzyme extract ($CU.ml^{-1}$). (Arora and Sandhu, 1985).

2.3.2. Cellulolytic enzyme production

The isolates were cultured at 37 °C at 150 rpm in enzyme production medium composed of KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, and gelatin 2 g, distilled water 1 L and containing Whatman filter paper No. 1 (1 × 6 cm strip, 0.05 g per 20 ml) (pH 7.0). The liquid culture was centrifuged at 5000 rpm for 15 min at 4 °C after three days of incubation period. The supernatant was collected and stored as crude enzyme preparation at 4 °C for enzyme assays (Gupta et al., 2012).

2.3.2.1. Endoglucanase. Endoglucanase (*β*1-4 endoglucanase) activity was assayed by measuring the amount of reducing sugar from amorphous cellulose. The enzyme activity was determined according to the methods suggested by the International Union of Pure and Applied Chemistry (IUPAC) commission on biotechnology (Ghose et al., 1987). Endoglucanase activity was determined by incubating 0.5 ml of supernatant with 0.5 ml of 2% amorphous cellulose in 0.05 m sodium citrate buffer (pH 4.8) for 30 min. The enzymatic activity of endoglucanase was defined in international units (IU). One unit of enzymatic activity is defined as the amount of enzyme that releases 1 µmol reducing sugars (measured as glucose) per ml per minute.

2.3.2.2. Exoglucanase. Exoglucanase activity was determined by incubating 0.5 ml of supernatant with 1.0 ml of 0.05 M sodium citrate buffer (pH 4.8) containing Whatman no. 1 filter paper strip – 1.0 × 6.0 cm (≈50 mg). After incubation for an hour at 50 °C, the reaction was terminated by adding 3 ml of 3, 5-dinitrosalicylic acid (DNS) reagent to 1 ml of reaction mixture. Reducing sugars were