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ORIGINAL ARTICLE

Composting of rice-residues using lignocellulolytic plant-probiotic *Stenotrophomonas maltophilia*, and its evaluation for growth enhancement of *Oryza sativa* L.

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Abstract

Rice straw residues, a prominent agricultural waste were converted into bioactive compost by application of plant-probiotic bacterium having lignocellulolytic activity. The isolate was also found to have excellent plant growth promoting attributes. *Stenotrophomonas maltophilia* RSD6 was isolated from the rhizospheric soil of *Oryza sativa* L. and was identified on the basis of its phenotypic, physiological features and 16S rRNA sequence analysis. *S. maltophilia* RSD6 had plant growth stimulating attributes including production of siderophore, indole acetic acid and inorganic phosphate solubilization. Eventually, the isolate also has the potential to degrade lignocellulosic agricultural-waste due to production of enzymes including lignases, xylanase and cellulases, and therefore it was used for composting of rice straw residues. There was increase in nitrogen, phosphorus, and potassium content in mature compost, where final content was recorded as 16.26, 0.072, 18.49 (g/kg) after 90 days. The lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase productions for *S. maltophilia* RSD6, were found to be, 1.125 units/min/mL, 0.069 units/min/mL and 0.219 CU mL^{-1} respectively. Endoglucanase, exoglucanase, cellobiase and xylanase activities were also recorded to be 0.445, 0.174, and 0.709 and 0.240 IU/mL, respectively. The treatments of rice plants with RSD6 showed significant enhancement of shoot and root length and biomass as compared to control. *S. maltophilia* RSD6 not only improved the growth of rice plants but also has the potential to degrade lignocellulosic waste because of its rapid compost traits. Therefore, RSD6 was utilized for management of rice straw residues in an eco-friendly manner, resulting in compost that was having plant probiotic characteristics due to presence of RSD6, and it may be used as an alternative to agrochemicals.

Keywords Compost · Lignocellulosic · *Oryza sativa* L · Rhizobacteria · *Stenotrophomonas maltophilia*

Introduction

Rice (*Oryza sativa* L.) is regarded as one of the major source of food worldwide (Ryan et al. 2009). Lignocellulosic agricultural waste products such as rice straw are important sources of lignocellulosic biomass, as millions of tons of rice straw are produced every year globally. In India, due to high production of rice, large quantities of rice residue such as rice straw and stubble are being produced which lie in the field unattended causing arrest of plant nutrients (Abdelhamid et al. 2004); or burned, which results in air pollution. Composting is a strategy by which lignocellulosic biomass may be converted to useful manure for organic agriculture. In the modern day agriculture, excessive use of chemicals has been discouraged, captivating for the search of substitutes enhancing environmental sustainability. One of the alternatives is formulation of organic compost by biodegradation of plant waste material (Golueke 1972). Consequently, augmentation of composted lignocellulosic agricultural waste material enhances soil fertility. Further, if lignocellulosic agricultural waste based compost is unified with plant growth promoting rhizobacteria (PGPR), which directly or indirectly facilitate plant growth (Glick 1995) and act as probiotic for the plants; may result into a value

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added product such as an effective biofertilizer (Bashan et al. 2014). PGPR are known to substantially reduce the use of chemical fertilisers (Lugtenberg and Kamilova 2009). Several bacteria such as *Pseudomonas* (Bais et al. 2006), *Bacillus* (Zakry et al. 2012) and *Stenotrophomonas* (Islam et al. 2015) have been endorsed as PGPR through several direct and indirect mechanism and attributes such as phosphate solubilization, biological nitrogen fixation, production of plant growth regulators and siderophores. Among the PGPRs, *S. maltophilia* member of γ-subclass of the *Proteobacteria* (Palleroni and Bradbury 1993; Moore et al. 1997) is abundant in a wide variety of environments and geographical regions, inhabiting various ecological niches (Denton and Kerr 1998).

The main objective of this study was to select an effective PGPR strain, which has excellent potential to degrade rice straw lignocellulosic waste leading to the formulation of compost, and evaluate the PGPR based bioactive compost in growth enhancement of rice plants. By this strategy, the compost thus formed will have benefits of PGPR amendments in addition to nutrient enrichments via composted rice residues.

Materials and methods

Isolation and screening of PGPR

Rhizospheric soils were collected from the *O. sativa* L. growing at different sites of Cachar, Assam India. Roots tips (3 cm) with sufficient adhered rhizospheric soil were collected using sterile spatula and 1.0 g of soil was taken in the sterilized McCartney bottle. Phosphate buffer saline (9.0 mL) with pH 7.2 was used to suspend the soil sample (Miyazawa et al. 2008) and suitable dilutions (1:10) were inoculated on yeast extract mannitol agar (YEMA) (Kshetri et al. 2018). Plates were incubated at 30 °C for 3 days and colonies were selected and sub-cultured for further analysis. The purified cultures were stored in agar slants (4 °C) and glycerol stocks (20% v/v, − 20 °C) for further use.

Morphological, biochemical and molecular identification of the isolate

The morphological and biochemical characterization of isolates was done according to Bergey's Manual of Systematic Bacteriology (Bergey et al. 1994). Further, QIAamp DNA Mini Kit was used for the extraction of the genomic DNA, and 16S rRNA region was amplified with primers 27F (5′-CAGAGTTTGATCCTGGCT-3′) and 1492R (5′AGGAGG TGATCCAGCCGCA-3′) (Weisburg et al. 1991). The PCR mixture (25.0 mL) consisted of 2 × Master mix (GCC Biotech), 12.5 µL; 27F, 1.0 µL; 1492R, 1.0 µL; DNA, 1.0 µL, and nuclease free water, 9.5 µL. Each cycle consisted of an initial denaturation at 94 °C for 5 min, followed by denaturation of 94 °C for 30 s, annealing 55 °C for 30 s and extension at 72 °C for 1 min 30 s and final extension at 72 °C for 10 min. Sequences were identified using the EzTaxoneserver database (Kim 2012) and NCBI GenBank databases. Phylogenetic analysis was carried out by the software package MEGA 5 (Tamura et al. 2011).

Plant growth promoting attributes

Indole acetic acid production

The indole acetic acid (IAA) production was determined by the method of Bano and Musarrat (2003). Isolates were grown on YEM broth supplemented with different concentrations of L-tryptophan (0, 0.2, 0.4, 0.6, 0.8 or 1%) for 7 days and incubated at shaking conditions (150 rpm, 30 °C). IAA was estimated in supernatants of culture medium collected by centrifugation (10,000 rpm, 15 min), where 1 mL of supernatant was mixed with 2 mL of Salkowski reagent (50 mL 35% perchloric acid with 1 mL 0.5% $FeCl_3$) (Gordon and Weber 1951). Absorbance was measured at 530 nm and the IAA produced was quantified by comparing with known concentration of IAA.

Phosphate solubilization

Tricalcium phosphate (TCP) solubilization was determined by spot inoculation on Pikovskaya's medium containing 0.5% tricalcium phosphate (TCP), bromophenol blue (2.4 mg/mL) and 10 µL of the isolate (Mehta and Nautiyal 2001).The plates were incubated for 48 h at 30 ± 1 °C and observed for the formation of the halo zone around the colony and the diameter of the halo was measured. Quantitative estimation of phosphorous in supernatant was done by vanado-molybdate-yellow colour method in NBRIP broth (Koening and Johnson 1942). For this 100 mL of NBRIP medium (pH 7) was inoculated with respective isolate and incubated in a shaker (150 rpm, 30 °C for 7 days). The total soluble phosphorus (P) was estimated with help of standard curve, which was obtained with known concentrations of potassium phosphate analysed in parallel to test, by vanado-molybdate-yellow colour method as mentioned above. The absorbance was measured at 430 nm using spectrophotometer. The values of soluble phosphate liberated were expressed as µg mL^{-1} over control. The pH of culture supernatants was measured in each case.

Siderophore production

The qualitative screening for siderophore production was done according to You et al. (2005) on Chrome-azurol S

(CAS) medium, where formation of orange to yellow zone around the colonies in medium, after 48 h at 28 °C confirmed siderophore release (Schwyn and Neilands 1987). For quantitative estimation of siderophore, 1 mL of liquid culture of isolates was inoculated into 70 mL of iron-deficient nutrient broth, and analysed by CAS-shuttle assay as described previously (Payne 1994; Sayyed et al. 2005). Briefly, 5 ml aliquot were withdrawn from the culture flasks at every 24 h intervals and centrifuged at 10,000 rpm for 10 min and 1 mL of supernatant was mixed with 1 mL of CAS reagent and allowed the colour to develop before measuring the absorbance at 630 nm. Siderophore content (percentage siderophore units) was estimated as

$$\text{Percentage siderophore units} = \frac{\text{Ar} - \text{As}}{\text{Ar}} \times 100$$

where, **Ar-** $\mathbf{A}_{630}$ of reference, **As**- A_{630} of sample. The reference consisted uninoculated broth + CAS reagent, while culture supernatant + CAS reagent was used to process the sample.

In-vitro assessment of lignocellulolytic activity

Screening of the isolates for the lignin degradation, and Lignin peroxidase (LiP), Manganese peroxidase (MnP), Laccase enzyme activities

Bacterial isolates were screened for their lignin degradation potential. Lignin degrading ability of the isolates was checked on the Minimal salt media (MSM) which contained 0.5% lignin, inoculated and incubated at 28 °C. The MSM-KL medium contained (g/L): Kraft lignin (0.5); K_2HPO_4 (4.55); $CaCl_2$ (0.5); $MgSO_4$ (0.5); NH_4NO_3 (0.5); KH_2PO_4 (0.53); with trace elements $CuSO_4$ (0.002), $MnSO_4$ (0.001), $FeSO_4$ (0.01) and $ZnSO_4$ (0.001) (Rahman et al. 2013). For enzyme assays, 0.8 mg/mL of lignin was added in MSM (Kraft Lignin, Sigma) and inoculated and incubated at 34 ± 1 °C at rpm 120 for 144 h.

The LiP activity was assessed by estimating the oxidation of dye Azure B in the presence of H_2O_2 (Arora and Gill 2001). The reaction mixture comprised of sodium tartrate buffer (50 mM, pH 3.0), Azure B (32 µM), 0.5 mL of enzyme extract and H_2O_2 (100 µM). The reaction was commenced by adding 0.5 mL of H_2O_2. One unit of enzyme activity was defined as decrease in absorbance of 0.1 units per min and reported for one mL of sample.

MnP activity was estimated by the phenol red oxidation method of Orth et al. (1993). Reaction mixture (5 mL) contained 1 mL sodium succinate buffer (50 mM, pH 4.5), 0.4 mL manganese sulphate (0.1 mM), 1 mL sodium lactate (50 mM, pH 5), 0.7 mL phenol red (0.1 mM), gelatin (1 mg mL^{-1}) 0.4 ml, H_2O_2 (50 µM) and 0.5 ml of enzyme extract.

H_2O_2 was added in mixture at 30 °C to start the reaction. Later, 40 µl of 5 N NaOH was added to 1 mL of reaction mixture, and absorbance was measured at 610 nm. The activity was measured at different time intervals starting from one min, till four minutes for each sample. One unit of enzyme activity is equivalent to an absorbance increase of 0.1 units min^{-1} mL^{-1}.

For the estimation of Laccase activity, reaction mixture was prepared having 3.8 mL of acetate buffer (10 mM, pH 5), 1 mL of guaiacol (2 mM) and 0.2 mL of enzyme extract, which was incubated for 2 h at 25 °C (Arora and Sandhu 1985). The activity was calculated by measuring the absorbance at 450 nm and expressed as colorimetric units mL^{-1} (CU mL^{-1}).

Screening of the isolates for the cellulose degradation and production of endoglucanase, exoglucanase and cellobiase

Cellulolytic activity was checked using the CMC agar plates, which were inoculated with test isolate and colonies were flooded with 1% congo red, later on allowed to stand for 15 min at room temperature. Plates were counterstained with NaCl (1 M). Clear zones around growing bacterial colonies were considered as positive results for cellulose hydrolysis (Andro et al. 1984). The isolates were cultured 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) and at pH 6.8–7.2 (37 °C, 150 rpm). The broth cultures were subjected to centrifugation at 5000 rpm for 15 min at 4 °C after 3 days of incubation and supernatant was collected for estimation of activity (Gupta et al. 2012). Amount of reducing sugar released from amorphous cellulose was used to determine endoglucanase activity (Ghose 1987). Exoglucanase activity was determined by measuring amount of reducing sugar released from cellulose filter paper; by DNS method (Miller 1959). Cellobiase activity was determined according to the method described by Eriksson (1997). One unit of enzymatic activity is defined as the amount of enzyme that releases 1 µmol glucose per mL.

Screening of the isolates for hemicellulose activity, and production of xylanase

The ability of isolates to grow on xylan agar medium (g/L): yeast extract (2.0); $CaCl_2$ (0.15); peptone (5.0); NaCl (0.5); $MgSO_4$(0.5); agar(20.0); birch wood xylan (20.0) and pH 7, was checked. The colonies were flooded with 1% aqueous Congo red dye for 1 h followed by de-staining with 1 M NaCl (Teather and Wood 1982). Formation of clear zone around the colonies was taken as positive for release of xylanase. The selected isolates were cultured at 37 °C, pH 7.0

at 100 rpm in medium containing 0.5% birchwood xylan in 100 mL of minimal medium (0.7% KH_2PO_4, 0.2% K_2HPO_4, 0.05% $MgSO_47H_2O$, 0.1% $(NH_4)_2SO_4$ supplemented with 0.06% yeast extract. After 2 days of incubation, broth cultures were subjected to centrifugation at 10,000 rpm for 20 min at 4 °C (Medeiros et al. 2003). Xylanase activity was estimated by the method of Bisaria and Ghose (1987) using 1% birchwood xylan. One unit of xylanase activity (IU) was defined as the amount of enzyme that released 1 µM of D-xylose.

Composting

In sterile water, rice straw residues were soaked for 24 h and sterilized by autoclaving. These residues were packed tightly in compost bins (0.5 m × 0.4 m × 0.45 m) of 3 kg capacity (Nevita et al. 2018). The rice straw biomass was augmented with the isolate *S. maltophilia* RSD6 with ≈ 10^9 cfu g^{-1} inoculum ($O.D._{600} = 1.0$), and mixed in sterile conditions. The compost was incubated and matured till 90 days in indoor conditions at ambient temperature. Samples after 0, 30, 60 and 90 days were collected and throughout the composting process physico-chemical properties were analysed. Carbon (Navarro et al. 1993) and N contents (Jackson 1973) were estimated by standard procedures while total P was determined by ammonium molybdate method (Murphy and Riley 1962) whereas K content was measured by flame photometric method (Jankowski and Freiser 1961).

Seed germination index

Isolates were grown on YEM broth for 5 days and harvested by centrifugation (10,000 rpm, 10 min). The pellets were washed three times with sterile distilled water (SDW). Rice (variety: Boro) seeds were surface sterilized as described previously (Nevita et al. 2018). Seeds were soaked in cell suspension, of late log phase culture. Further, seeds were also suspended in aqueous extract of RSD6-compost (1 g in 10 mL water), and checked separately. The seeds were shifted to sterile plates with wet filter papers (10 seeds per plate). Rice seeds soaked in SDW were used as control. Plates were incubated at 28 °C. Germination of seeds, and other seedling growth parameters were recorded and compared with control. Four replications of each treatment were experimented in parallel, while each experiment was repeated twice. Vigor index was calculated as per Baki and Anderson (1973), formula as given below:

Vigor index = Percent germination × Seedling length *

*seedling length was reached by adding shoot and root lengths.

Pot experiment

The experimental soil (Hill soils) was collected from 0 to 15 cm soil depth from Cachar, south Assam, India (24.6850°N, 92.7512°E). Air dried, ground, sieved (2 mm mesh) soils weighing exactly 10 kg was sterilized and packed in pots of 60 cm diameter and 50 cm height. The experiment was carried out in a randomised complete block design, with harvests at 120 days after planting with four blocks. The treatments enacted were: (1) autoclaved uninoculated culture medium (control) (2) *S. maltophilia* RSD6 inoculum (3) Compost formulated with *S. maltophilia* RSD6 inoculum. Each pot were sowed with ten rice seeds of similar size and shape, which were later on thinned to five per pot. The bacterial culture was grown in YEM medium for use as inoculum, where cell suspension was adjusted to $OD_{600} \approx 10^9$ cfu mL^{-1} ($O.D._{600} = 1.4$). Each pot received 20 mL of respective inoculum. Throughout the experiment, the pots were watered daily with sterilized water.

Statistical analysis

Statistical analysis were done by using Analysis of Variance (ANOVA) statistical package for social sciences (SPSS) software, version 21 where control were compared with the multiple treatment, using Tukey's test and the poshocat $P \leq 0.05$.

Result

Isolation and identification of isolates from the rhizospheric soil

A total of 60 bacteria were isolated from different regions of Cachar, Assam (India), from the rhizospheric region of *O. sativa* L (supplementary data, Table 1). Among them, RSD6 was selected for further study because of its unique assortment of PGPR attributes in addition to production of lignocellulolytic enzymes to degrade lignocellulosic waste (Fig. 1). Isolate RSD6 was found to be Gram–negative small rods, catalase and oxidase positive, facultative anaerobe, forming irregular whitish, circular and smooth colonies on YEMA. It was able to ferment glucose but unable to ferment fructose, mannitol, sucrose, cellulose and maltose. It was methyl red, indole and Voges-proskauer negative. BLAST search result of the 16SrRNA gene sequence indicated RSD6 isolate to be a close relative of *S. maltophilia* strains. Based on the phylogenetic tree constructed with the 16SrDNA sequence analysis, it was identified as *S. maltophilia*. RSD6 showed maximum similarity with strain JCM 5962T (Fig. 2). The 16SrDNA sequence of RSD6 has been submitted in NCBI with accession number KX832346.

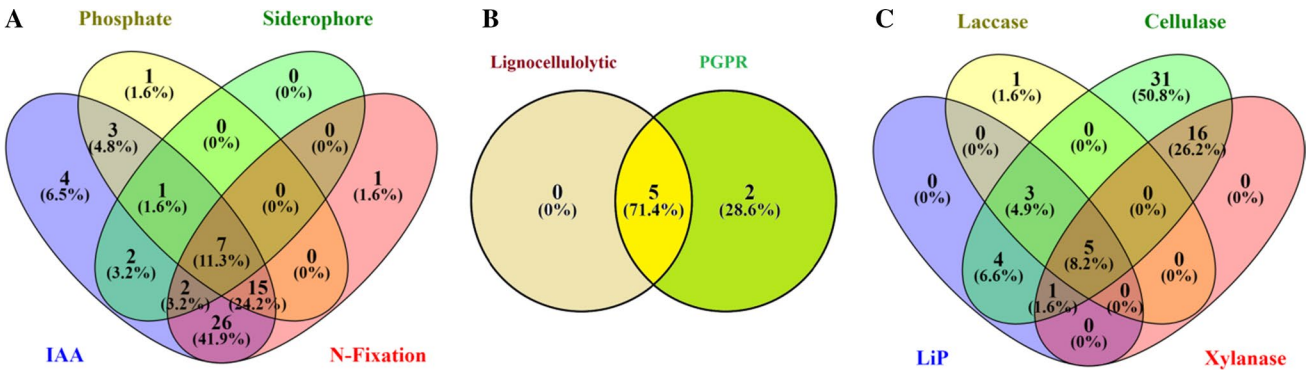

Fig. 1 Venn diagram representing the sixty number of isolates with plant growth promoting attributes (**a**), and lignocellulotic abilities (**c**). Out of these two categories, seven isolates were picked (given in core of **a**, **b**), having all the respective attributes and among these, five were found to have lignocellulolytic activity in addition to plant growth promoting attributes (**b**). The isolate RSD6 was selected for this study as elaborated in the text. *IAA* indole-3-acetic acid, *LiP* lignin peroxidase, *PS* phosphate solubilization

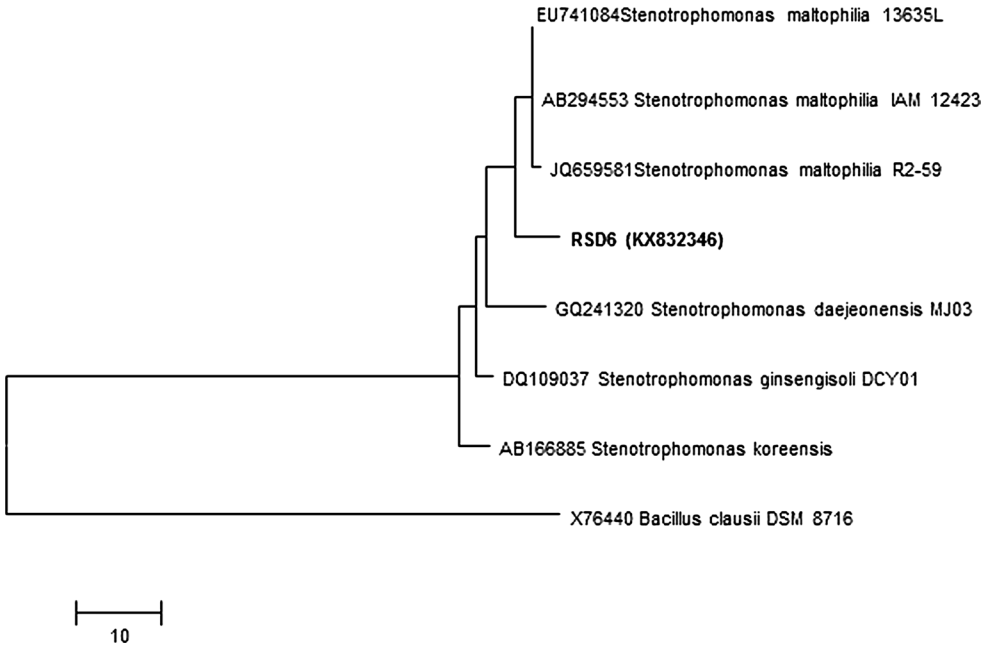

Fig. 2 Phylogenetic analysis of 16S rRNA sequence of *S. maltophilia* RSD6 isolated from *Oryza sativa* L. The analysis was conducted with MEGA5 using neighbor-joining method

PGPR attributes

IAA production assay

IAA production was recorded for RSD6, with different concentrations of L-tryptophan, in the range of 0.102 to 80.934 µg/mL. The optimum concentration of L-tryptophan was found to be 0.8% for IAA production by RSD6. IAA production decreased at higher concentrations of tryptophan. Except for 0.6% L-tryptophan concentration, the amount of IAA production was maximum after 96 h of incubation for all other concentrations of tryptophan (Fig. 3).

Phosphate solubilization

The isolate RSD6 solubilized tri-calcium phosphate by forming clear halo around the colony on Pikovskaya's agar. The P-solubilizing ability of isolate RSD6 varied from 96 to 221 µg/mL in TCP amended NBRIP medium, as a source of insoluble P in the broth. There was an increase in the content of phosphate to 221 µg/mL at 96 h and 3.9 pH. It was observed that there was an increase in the phosphate solubilization with decrease in pH (Fig. 4).

Siderophore production

In the CAS medium inoculated with RSD6, formation of orange halo zones was detected after 24 h. Increase in zone

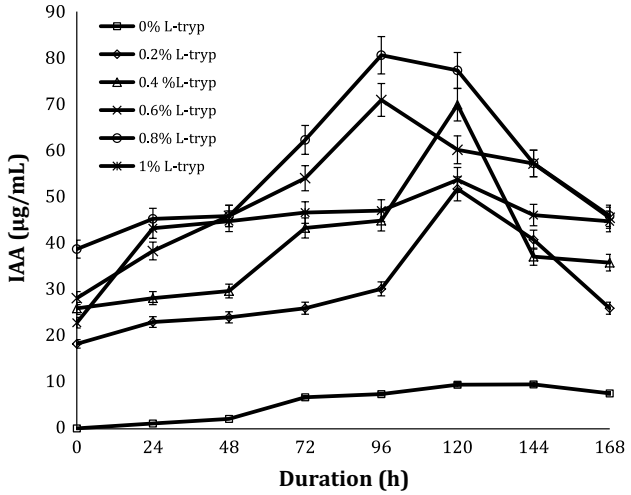

Fig. 3 Quantitative estimation of indole acetic acid (IAA) produced by *S. maltophilia* RSD6 at different L-tryptophan (L-tryp) concentrations. Error bars represent standard deviation

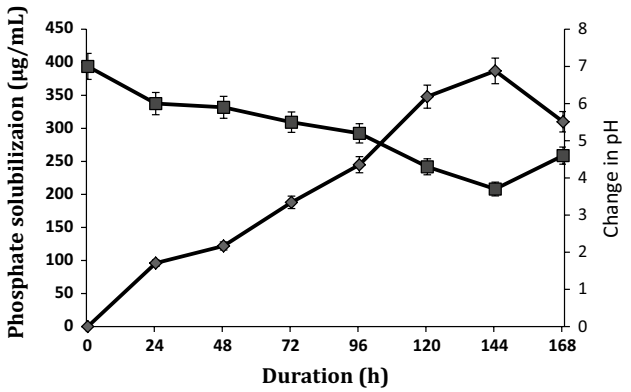

Fig. 4 Phosphate solubilization by *S. maltophilia* RSD6 at different time intervals. Soluble free-phosphate (PO_4) concentration is given against primary Y-axis (unfilled triangle), whereas variation of pH (unfilled square) in the culture medium is given at secondary Y-axis. Standard deviation is showed as bars

size and decolourization of CAS reagent from blue to orange was observed with increase in incubation period. A total of 81.71% unit of siderophore was recorded for RSD6 in succinate broth after 96 h of incubation whereas siderophore production was low in first 24 h of incubation. It was obvious that at late log phase there was a high siderophore production and the amount of siderophore is proportional with the growth profile of the isolates (Fig. 5).

Screening for the Lignolytic, Cellulolytic and Xylanolytic enzyme activity

The isolate *S. maltophilia* RSD6 inoculated on the plates amended with lignin, CMC and birchwood xylan fortified

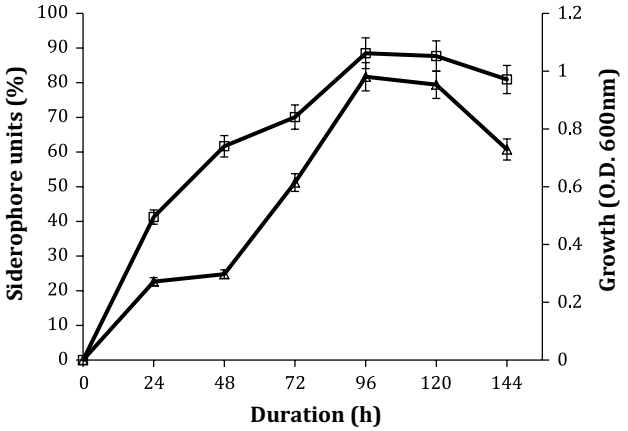

Fig. 5 Quantitative estimation of siderophore (unfilled square) released by *S. maltophilia* RSD6 given against primary Y-axis, and growth of bacteria is given as absorbance (Optical density at 600 nm) represented on secondary Y-axis (unfilled triangle). Standard deviation is showed as bars

media showed clear zone, suggesting its ability to release lignase, cellulase and xylanase. Further, maximum enzyme activity for MnP, LiP and laccase production by *S. maltophilia* RSD6, were found to be 0.069 units/min/mL, 1.125 units/min/mL and 0.219 CU mL^{-1} respectively. Also, the maximum amount of xylanase production was 0.240 IU/mL by the *S. maltophilia* RSD6. Endoglucanase, exoglucanase and cellobiase activities of *S. maltophilia* RSD6 were recorded to be 0.445, 0.174 and 0.709 IU/mL respectively (Table 1).

Composting

RSD6 degraded the rice straw residues rapidly, as the physical and chemical properties of mature compost suggested high nutritive index. The initial organic carbon in treatment was 34% in 30 days which gradually decreased to 26 in 90 days. As decomposition advanced there was decrease in the organic carbon. Eventually, the NPK value were found to be 7.72, 0.045, 13.67 mg/kg, respectively, on 30 days from the inoculation time which increased to 16.26, 0.072, and 18.49 mg/kg respectively, with the maturation of the compost i.e. 90 days (Table 2).

Germination index

Vigor index of RSD6, and compost (formulated and amended with RSD6) were found to be 706 and 930 respectively. Compost formulated with RSD6 showed higher germination percentage, vigor indices and significant increase ($P \leq 0.05$) in root and shoot length over the control and RSD6. Consequently, RSD6 treated rice seedlings also exhibited similar germination percentage as that of

Table 1 Maximum Ligninases, Cellulase and Xylanase activities produced by S. *maltophilia* RSD6

Ligninase			Cellulase			Xylanase
Lignin peroxidase (units/min/mL)	Manganese peroxidase (units/min/mL)	Laccase (CU mL^{-1})	Endoglucanase (IU/mL)	Exoglucanase (IU/mL)	Cellolbiase (IU/mL)	Exoglucanase (IU/mL)
1.125	0.069	0.219	0.445	0.174	0.709	0.240

the compost treatment, though vigor indices and increase ($P \leq 0.05$) in the root and shoot length were recorded better over the control, yet it was slightly lesser than compost treatment. Compost formulated with RSD6 showed better results than the *S. maltophilia* RSD6 alone (Table 3).

Pot trials

The treatment of rhizobacteria RSD6 and probiotic-compost (formulated with RSD6 and rice straw) enhanced the growth of *O. sativa* L. Increase in growth was recorded to be 1.43, 70.58, 19.04, 95.5 and 150% for shoot length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight respectively, due to bacterial treatment (RSD6) which was significant ($P \leq 0.05$) as compared to control. However, application of compost formulated with *S. maltophilia* RSD6 also resulted in the increase in all growth parameters of *O. sativa* L. Compost with *S. maltophilia* significantly increased shoot length by 7.4%, root length by 5.09%, fresh shoot weight by 48.23%, dry shoot weight by 16.6% ($P \leq 0.05$) (Table 4, Fig. 6).

Discussion

In this work, 60 different bacteria were isolated from different regions of Silchar-Assam, India (details not given). One of these isolates, RSD6 was selected on the basis of its capability to degrade ligninase, cellulase and xylanase, along with its plant growth promoting attributes. The potential bacterial isolate, RSD6, was identified as *S. maltophilia* on the basis of 16S rDNA sequence homology (accession number KX832346). In fact, among the *Stenotrophomonas* species, *S. maltophilia* is branded as an important plant growth promoting species in agriculture (Kumar and Audipudi 2015). *S. maltophilia* is known to be usually associated and reported from rhizosphere of diverse plants. (Lottmann et al. 1999). The isolate *S. maltophilia* RSD6 had excellent attributes desired in a PGPR. IAA producers are known to enhance root growth (Kampert and Strzelczyk 1975), which was indicated in our results, where IAA producing *S. maltophilia* was found to effect the growth of rice plants and induced better development of root tissues in pot trials. The isolate *S. maltophilia* RSD6 was found to produce significant amount of IAA (80.934. mg/mL), in L-tryptophan amended medium, illustrating the tryptophan dependant IAA release mechanism. Sachdev et al. (2009) reported that maximum IAA was released after 96 h of incubation which is in accordance with the results obtained in this study. In one of the previous report, *S. maltophilia* was found to produce 26.78 µg/mL of IAA, which was an isolate from rhizosphere of cucumber (Islam et al. 2015). Beside IAA, ability of P solubilization is also a desired attribute in rhizobacteria, where the PGPR solubilize inorganic phosphate by releasing inorganic phosphate (Khan et al. 2007). The isolate *S. maltophilia* RSD6 solubilized tri-calcium on the Pikovskaya's agar medium amended with TCP. There was a decline in the pH with subsequent enhancement in the amount of soluble phosphate depicting the maximum P-solubilization at pH 3.9 after 144 h of incubation (221 mg/mL), which is in agreement to the findings of Xiao et al. (2009). Maximum solubilization of TCP was found on the 5th day, where the concentration of soluble P reached 180.5 mg/L after 120 h of incubation, and then decreased gradually. This may be attributed to decrease in the production of organic acid and bacterial activity, due to the reduction of the substrate concentration, and also re-immobilization of a portion of the soluble P as suggested previously (Kim et al. 2005). There are reports on the wide range of bacteria belonging to diverse genera possessing the P-solubilizing potential (Sharma et al. 2005). Another vital trait of PGPR, that may ultimately effect the plant growth, is the production of siderophores which is produced by bacteria as a direct mechanism of plant growth promotion by rhizobacteria, which in turn contribute in enhancing the growth of plants (Meyer and Abdallah 1978). In the rhizosphere, the available form of iron Fe^{3+} becomes bound to the siderophore thus making it inaccessible to the phytopathogens and thus it protects the plant. *S. maltophilia* RSD6 was observed to release significant amount of siderophore. It produced 81.71 unit of siderophore in succinate broth after 96 h of incubation. Siderophore release was in correlation with growth of bacteria, where the result is in accordance with reports of *Azospirillum* by Saxena et al. (1986), where after 20 h of growth maximum siderophore production was recorded. Further, in addition to the PGPR attributes, *S. maltophilia* RSD6 had superior lignocellulolytic activity which was very unique. It showed positive result on the preliminary test for lignases, cellulase and xylanase production.

Table 2 Carbon, Nitrogen, Phosphorous and Potassium content of rice straw compost after 30, 60 and 90 days of bioconversion (Results are mean of five replicates ± standard deviation)

Isolates↓	Organic C (%)			Total N (g/kg)			Total P(g/kg)			Total K(g/kg)		
Days	30	60	90	30	60	90	30	60	90	30	60	90
RSD6	34 ± 0.2	28 ± 0.4	26 ± 0.4	7.72 ± 0.34	8.78 ± 0.70	16.26 ± 0.61	0.045 ± 0.01	0.065 ± 0.01	0.072 ± 0.01	13.67 ± 0.72	20.55 ± 0.053	18.49 ± 0.44

In fact, Samira et al. (2011) reported cellulase activity in *S. maltophilia* on the CMC agar plate, which resulted in clear zone, though without any mention of PGP attributes. The unique lignocellulolytic ability of RSD6 was utilized for the degradation of lignocellulosic waste viz rice straw, making use of synergistic action of enzymes. The result is in accordance with Ping et al. (2008) where *S. maltophilia* was found to produce all the three cellulolytic enzymes-endoglucanase, exoglucanase and cellobiase. Tian et al. (2016) had recently reported isolation of lignin degrading bacteria, though in present study, lignin degradation was explained in a plant probiotic bacteria, which was further utilized for degradation of lignocellulosic biomass viz rice straw. Similarly, in some previous reports, Galai et al. (2009) stated the production of laccase, while Raj et al. (2013) reported the production of highly thermostable xylanase from *S. maltophilia*, which is in accordance with our result. Production of xylanase by *S. maltophilia* on agroindustrial residues like wheat bran has been reported. During the process of composting, the initial organic carbon varied from 34 to 33% and reduced as the decomposition progressed. The total nitrogen (N) content of the compost varied from 7.72 to 16.26 g/kg as it increased with decomposition (Table 2). There was an increase in both P and K content with the progress in the composting. Hence, rice straw, which is more resistant to degradation due to the higher content of lignin was degraded by the inoculation of *S. maltophilia* RSD6. There are reports on the composting of paddy straw using microbial consortium (Pan et al. 2012; Sharma et al. 2014). Though, in present work, a single strain of bacteria was successfully utilized, and this isolate also had attribute for plant growth promotion. It is known that organic carbon is converted into energy and CO_2 as metabolic end product during the biodegradation process, which results in reduction in the carbon content of the rice straw as the degradation continues. In the present study, the maximum carbon mineralization rate was recorded during the 30–60 days of incubation owing to presence of high content of easily degradable organic carbon in the substrate enhancing the progress of microbial population during biodegradation. Huang et al. (2006) also reported that during the composting of straw dust and cattle manure, there is continuous decline in the total organic carbon. However there is an increase in the N content of the lignocellulosic waste during the biodegradation process which might be due to the formation of new cell structure, enzymes and hormones, as well as nitrification by the microorganisms. Although, there are factors like formation of new cell structure, enzymes and hormones, as well as nitrification (Zhu 2007) which may be accounted for increase in the N content of the lignocellulosic waste during the degradation process by microorganisms. Similarly we observed that the total N content was increased during the process of biodegradation, which is similar to the findings of Veeken et al. (2001).

Table 3 *In vitro* rice seed germination (Vigor index) by *S. maltophilia* RSD6

Treatment	Germination percent	Root length[a] (cm)	Shoot length[a] (cm)	Vigor index
Control	99	4.06 ± 0.32a	1.5 ± 0.58a	550.44
RSD6	100	5.26 ± 0.48c	1.8 ± 0.69a	706
RSD6 compost amended	100	6.5 ± 0.54b	2.8 ± 0.45b	930

[a]Values with the same letter within a column are not significant at $P \leq 0.05$

Table 4 Different growth parameters of rice plants treated with *S. maltophilia* RSD6 in comparison with the untreated soil

Parameters	Control	RSD6	RSD6 based compost
Shoot length (cm)	29.9 ± 2.45a	30.33 ± 1.52b	32.3 ± 3.48c
Root length (cm)	14.33 ± 2.08a	15.66 ± 2.51b	15.06 ± 4.74c
Fresh shoot weight (g)	1.7 ± 0.05a	2.9 ± 0.58b	2.52 ± 0.264c
Dry shoot weight (g)	0.42 ± 0.02a	0.5 ± 0.02b	0.49 ± 0.04c
Fresh root weight(g)	0.9 ± 0.05a	1.76 ± 0.15b	1.2 ± 0.26c
Dry root weight(g)	0.14 ± 0.03a	0.35 ± 0.03b	0.48 ± 0.03c

Each value is the mean of five plants. Values with the same letter within a row are not significant at $P \leq 0.05$

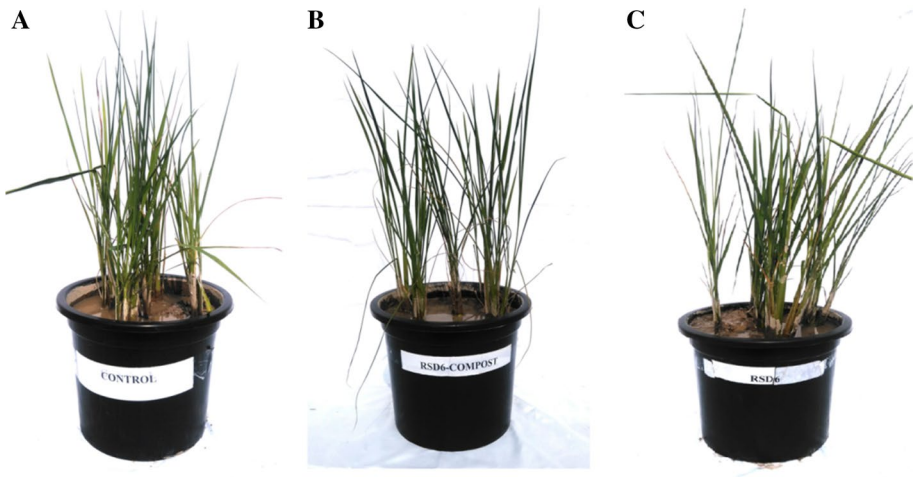

Fig. 6 Pot trials of rice plant (Boro) (**a**) untreated soil (**b**) *S. maltophilia* RSD6 bacterial compost treated (**c**) *S. maltophilia* RSD6 treated

Subsequently, the findings of this work confirmed that soil quality can be enhanced by the addition of rice straw which was being decomposed by a PGPR thereby transforming it into a value added biofertilizer that enhances the soil quality. Moreover, the high N content of the compost adding to the slow-release character of P and potassium components in its residue represent a good bio-fertilizer for plant growth. Potassium, which usually leaches out during composting process was found to be increasing with maturation of compost. Retention of potassium in the compost may be explained by the usage of rice straw as a medium that can absorb moisture, maintaining its structural integrity and porosity (Iyengar and Bhave 2005). Further, the phosphate content of the composting mixture was observed to increase with the duration of composting. This result is in agreement with Singh et al. (1983) and Singh (1985) for the composting of pearl millet, boobla and paddy straw.

Further, the selected bacterial isolate *S. maltophilia* RSD6 and the compost formulated with *S. maltophilia* RSD6 showed higher vigor index over the control and also stimulated growth of *O. sativa*. L. Earlier *S. maltophilia* has been reported as an excellent plant growth promoter (Alavi et al. 2013). However, growth promotion and augmentation of the compost formulated with *S. maltophilia* has not been reported earlier.

Conclusion

In conclusion, *S. maltophilia* RSD6, had excellent plant growth promoting attributes, including siderophore, IAA, inorganic phosphate solubilization, and therefore promoted the growth of rice plants. Further, it also proved to be a potential lignocellulosic degrader which induces rapid composting of rice straw residues. The compost formed by application of RSD6 was effective in promoting growth of rice plants. Hence, the unique combination of traits of isolate RSD6 highlights its application to be exploited as biofertlizer by composting of agricultural waste, which may be an eco-friendly alternative to agrochemicals leading to sustainable agriculture.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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