Ann Microbiol (2014) 64:1797–1806
DOI 10.1007/s13213-014-0824-0

ORIGINAL ARTICLE

Ochrobactrum sp. Pv2Z2 exhibits multiple traits of plant growth promotion, biodegradation and *N*-acyl-homoserine-lactone quorum sensing

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Received: 21 November 2013 / Accepted: 22 January 2014 / Published online: 19 February 2014

Abstract Rhizosphere bacteria play a vital role in plant growth, pathogen control, biodegradation and rhizosphere signaling. A motile, rod-shaped bacterium, Pv2Z2, isolated from the nodules of the common bean grown in Tanzanian soil was characterized using a polyphasic approach. The traits assessed included the production of indole-3-acetic acid and *N*-acyl homoserine lactone (AHL) molecules, solubilization of insoluble phosphate and zinc compounds and biodegradation of a number of toxic compounds. The 16S rRNA sequence of Pv2Z2 (EU399793) showed 99 % homology to *Ochrobactrum anthropi* isolates (Accession no. AJ867292, AJ867291, AJ867290) from soil samples of wheat root. Phylogenetic analysis showed relatedness to nodulating strain *Ochrobactrum cytisi* rather than to the clinical/pathogenic type strain of *O. anthropi*. Moreover, it showed unique fingerprints in the randomly amplified polymorphic DNA (RAPD) and two primers-RAPD assays which were different from those of the pathogenic type strain of *O. anthropi*. The bacterium produced 6.68 μg/mL^{-1} indoleacetic acid in the presence of tryptophan, released 25.7 μg/mL^{-1} phosphorus from inorganic tri-calcium phosphate in the Pikoviskaya's medium and solubilized zinc sulphate and zinc oxide in the LG1 medium. The production of AHLs (e.g. 3O-C7-HSL, 3OH-C7-HSL) was detected with biosensor strains CV026 using reverse phase thin layer chromatography. The bacterium was able to grow in minimal salt medium supplemented with 100 mg/L each of phenol, 2-bromophenol, 2,4-diamino phenol hydrochloride, 3,4-dimethoxy benzyl alcohol and 4-methoxy benzyl alcohol. Phenol degradation was recorded up to a level of 94 % within 12 days. Inoculation of common bean plants resulted in a significant increase in plant height, fresh/dry weight and nitrogen uptake as compared to non-inoculated plants. The data suggest that the plant growth-promoting and biodegradation potential of this bacterium may be exploited on a large scale. The capacity to produce AHL molecules by members of the *Ochrobactrum* genus has not been previously reported and needs to be explored in detail.

Keywords Legumes · *Ochrobactrum* · Biodegradation · Phosphate solubilization · AHLs

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Introduction

Although nitrogen (N) and phosphorus (P) are vital nutrients for plant growth and development, only 30–50 % of exogenously applied N and 10–45 % of P are taken up by plants (Adesemoye et al. 2009; Garnett et al. 2009). The rest of the fertilizer fraction becomes unavailable either due to leaching or nutrient run-off. In addition, long-term use of synthetic fertilizers negatively affects the overall biogeochemical cycles and ecosystem (Steinshamn et al. 2004) and has become a global environmental concern (Tilman et al. 2002). An alternative to the use of chemical fertilizers is the application of biological fertilizer technology. This technology not only benefits plants in terms of growth and yield but improves soil

health and the environment, reduces reliance on chemical fertilizers and provides economic benefits.

Biological fertilizers composed of plant growth-promoting rhizobacteria (PGPR) are often described as having multiple traits of plant growth promotion. They convert atmospheric N to ammonia, thereby making N available to plants, transform unavailable phosphate or zinc compounds to plant-available fractions, produce different growth hormones and amino acids, produce signal compounds, such as *N*-acyl homoserine lactone (AHL) molecules, to interact with the host and coordinate their activities in the rhizosphere, exhibit 1-aminocyclopropane-1-carboxylate-deaminase activity to mitigate the stress-induced negative impacts and produce different antifungal compounds to combat pathogen attack (Compant et al. 2005).

In addition to the biotic stress caused by pathogen attack, plant growth and yield are seriously affected by abiotic stresses, such as salinity, drought, frost and the presence of toxic compounds in water or soil. Each day huge amounts of organic compounds are released as industrial by-products, pesticides or agrochemicals into the environment, of which phenolic compounds are very hazardous (Afzal et al. 2007). Microbes found in natural waters and soils have an amazing, wide-ranging ability to utilize many of these toxic compounds as carbon (C) and energy sources and have a great potential for use in large-scale bioremediation programs. Biodegradation ability has been reported in many soil bacteria, particularly PGPR [e.g. *Rhizobium* sp. (Amer 2008; Wei et al. 2008), *R. leguminosarum* bv. Trifolli (Parke et al. 1991), *R. meliloti* (Frassinetti et al. 1998), *Ralstonia taiwanensis* (Chen et al. 2004, *Bradyrhizobium japonicum* (Radwan et al. 2007), *Sinorhizobium* (Seo et al. 2007), *Mesorhizobium* (Feng and Lee 2009)].

Bacterial activities (either beneficial or pathogenic) are mainly controlled by different signal molecules which are produced by bacteria to regulate the expression of specific genes and to interact with the environment. The AHLs are signal compounds that are produced specifically by Gram-negative bacteria and are involved in signaling processes such as antibiotic production and conjugal plasmid transfer. This kind of signaling has been identified in *Pseudomonas*, *Agrobacterium* and *Rhizobium* (An et al. 2006; Abbas et al. 2007; Antunes and Ferreira 2009). The members of family Brucellaceae are known to produce signal molecules (Taminiau et al. 2002), but to date this activity has not been reported for the genus *Ochrobactrum*. Quorum-quenching activity, however, has been reported for one member of this genus (Jafra et al. 2006; Czajkowski et al. 2011).

The aim of this study was to isolate the nodulating bacteria that can be used for inoculum production for common bean, cowpeas and soybean in Zanzibar, Tanzania. For that purpose, soil was collected from different regions of Zanzibar, Tanzania, and soil inoculum was applied to common bean, cowpea and soybean to trap the indigenous rhizobia specific to these hosts. After screening a number of isolates, we identified one nodule isolate having the ability to produce AHL compounds and multiple traits of plant growth promotion and biodegradation. The isolate, when characterized in detail, showed great promise for use in the detoxification of hazardous chemicals and as a biofertilizer. The AHL quorum-sensing system might be involved in regulating its function in the rhizosphere and environment.

Materials and methods

Strain isolation

The strain was isolated from soil collected from the Maruhubi region of Tanzania, which was a sandy-loam soil having a pH of 5.69, electrical conductivity of 10.2 dS/m^{-1}, 11 % C content, 2.2 % organic matter content, 0.08 % total N content, 10 mg/kg P, 0.72 mg/100 g calcium, 0.48/100 g magnesium, 0.50 mg/100 g potassium and a C/N ratio of 13. The last crop grown in the soil was common bean. Serial eightfold dilutions were prepared from 1 g of soil (Somasegaran and Hoben 1994), and 1 mL of each dilution was applied to the base of the root tip of 3-day-old seedlings of common bean (var. Lyamungu 90). The plants were irrigated with N-free Hoagland solution and observed for nodule formation. Nodules were detached from the root, flame sterilized, crushed on a sterile glass surface and streaked onto YEM-Congo red plates as described by Shah et al. (1995) to isolate the bacteria. The plates were incubated at 28±2 °C for 3–4 days until the appearance of separate colonies.

Biochemical characterization

Colony morphology, size, color, shape, gum production and growth pattern were recorded after 24 h of growth on Yeast Extract Mannitol (YEM) and Luria–Bertani (LB) agar plates at 28±2 °C as described by Somasegaran and Hoben (1994). Cell size and motility was observed by light microscopy. Acid/alkali production was tested on LB-agar plates containing 0.025 % (w/v) bromothymol blue as pH indicator. The Gram reaction was performed as described by Vincent and Humphrey (1970). Amino-peptidase and cytochrome oxidase tests were performed by using commercially available strips (Merck, Darmstadt, Germany). Catalase production was checked by placing a drop of H_2O_2 onto the bacterial colony on a glass slide. Resistance to the antibiotics chloromphenicol (30 μg), erythromycin (15 μg), aztreonam (30 μg), carbenicillin (100 μg), ampicillin (10 μg), gentamycin (10 μg), amikacin (30 μg), streptomycin (10 μg), doxicycline (30 μg), neomycin (30 μg) and trimethoprim/sulfamethoxazole (25 μg) was determined on antibiotic sulphonamide sensitivity-test agar (Merck) plates

using commercially available discs (Bioanalyse®, Ankara, Turkey). The utilization of different carbon sources and enzymatic reactions were performed using the QTS-24 kit (DESTO, Karachi, Pakistan) following the manufacturer's protocol. The capacity to utilize mannitol, maltose, fructose, sucrose, D-glucose, levulose, sorbitol, myo-inositol, glycerol and yeast extract as carbon sources was determined as described by Somasegaran and Hoben (1994).

Molecular characterization

Total genomic DNA of the bacterium was isolated by the alkaline lysis method (Maniatis et al. 1982) and used to amplify the 16S rRNA gene with primers P1/P6 (Tan et al. 1997). The PCR assay was carried out in a thermal cycler (Eppendorf, Hamburg, Germany) as described by Imran et al. (2010). The amplified PCR product was purified using the QIA Quick PCR Purification kit (Qiagen, Hilden, Germany), ligated in TA cloning vector pTZ57R/T (Fermentas, Thermo Fisher Scientific, Waltham, MA) and cloned in *Escherichia coli* strain DH5α as described by Maniatis et al. (1982). Cloned PCR products were sequenced commercially from Macrogen (Seoul, Korea). The gene sequence was compared with others in the GenBank database using the NCBI BLASTn. Multiple sequence alignments were performed by ClustalX, and phylogeny was determined by neighbor-joining method. Random amplified polymorphic DNA (RAPD) analysis was performed as described by Williams et al. (1990) using primers OPC-13 and OPC-15 (Operon Technologies, Alameda, CA). Two primers (TP)-RAPD was performed as described by Imran et al. (2010). The DNA of the *O. anthropi* type strain LMG3331^T and other type strains of the genus obtained from DSMZ (German Collection of Microorganisms and Cell Cultures) were used as reference.

Bioassays for plant growth-promoting activities

Production of indole-3-acetic acid

The production of indole-3-acetic acid (IAA) was determined using the colorimetric method of Gordon and Weber (1951). Quantitative estimation was done by growing Pv2Z2 in LB-broth supplemented with 100 mg/L tryptophan as IAA precursor for 5 days. IAA was extracted with an equal volume of ethyl acetate as described by Tien et al. (1979) and finally collected in ethanol. A 100-μL sample of the extract was analysed on high-performance liquid chromatograph equipped with Turbochrom software (Perkin Elmer Life Science, Boston, MA) and on a C-18 column at a flow rate of 0.5 mL/min.

Solubilization of tri-calcium phosphate, zinc oxide and zinc sulphate

Aliquots (10 μL) of Pv2Z2, after overnight growth in LB medium, were spot inoculated onto Pikovskaia's agar (Pikovskaia 1948) containing tri-calcium phosphate as the insoluble P source and onto LGI medium (Cavalcante and Döbereiner 1988) supplemented with 0.1 % zinc oxide and zinc sulphate. The plates were incubated at 28±2 °C for 10–14 days and examined for the formation of a clear zone around the bacterial growth spot daily. The appearance of a clear zone was considered to be a positive indication for phosphate and zinc solubilization activities. P solubilization was quantified using the phosphomolybdate blue colour method on a spectrophotometer (λ=882 nm) as described by Murphy and Riley (1962)

Antifungal activity

Antifungal activity was tested using a dual-culture assay as described by Sakthivel and Gnanamanickam (1986). A drop of the exponentially grown bacterial culture (approx. 20 μL) was spotted onto potato dextrose agar (PDA) plates close to the walls of the petri dish on both sides and the plate left in laminar air flow cabinet until dry. A 6-mm agar disk of each of three fungal species, namely, *Fusarium solani*, *F. oxysporum* and *F. moniliforme* (obtained from the lab collection), were separately placed at the centre of PDA plates. The plates were incubated at 28±2 °C for 5 days to measure the inhibition of radial fungal growth between fungal and bacterial colonies. Percentage inhibition was determined by the reduction in fungal growth compared to control (un-inoculated bacterial strain).

The AHL assay and detection by reverse-phase thin-layer chromatography

The Pv2Z2 isolate was streaked onto the center of a Tryptone Yeast extract (TY) (Beringer 1974) agar plate and grown overnight at 28 °C to achieve appreciable growth. The indicator strain *Chromobacterium violaceum* CV026 (obtained from John Innes Centre, Norwich, UK) was grown separately in LB broth at 30 °C with constant shaking overnight up to an OD of 10^6 CFU/mL. AHLs were detected by the plate overlay assay as described by McLean et al. (2004). The reference strain A34 (obtained from the John Innes Centre) was used as the positive control. A34 is a *Rhizobium leguminosarum* 8401 containing pRLIJ1. Plates were incubated at 30±2 °C overnight, and purple pigmentation produced by the indicator strain on the plates was considered to indicate AHL production.

AHLs were extracted from the culture supernatant with an equal volume of ethyl acetate (acidified with 0.1 % v/v glacial acetic acid).Water residues were removed by treating the extract with anhydrous Na_2SO_4. The extract was filtered, concentrated in a rotary evaporator

(Rota vapor R-210; Buchi Corp, New Castle, DE) and re-confirmed by reverse plate assay as described by McLean et al. (2004). The AHL extract (10 μL) was separated on glass-backed C18 reverse-phase thin-layer chromatography (RP-TLC) plates (Merck) in methanol/water (60:40 v/v) along with the AHL extract of A34 as reference. The TLC plate was developed with the overlay of an exponentially grown culture of indicator strain CV026, and AHLs were identified as purple spots on the off-white background.

Plant inoculation assay

The Pv2Z2 strain, which had been grown in yeast-extract-mannitol (YEM) medium to a concentration of up to 10^9 cells/

Table 1 Morphological and biochemical characters of *Ochrobactrum* sp. Pv2Z2

Cultural/Morphological characters			
Growth on		**Colony morphology on LB agar**	Circular,
Nutrient agar	+		Low convex, entire
LB agar	+		Smooth, shining
YEM agar	+		
Optimum growth temperature on LB, NA, YEM agar	28°C	**Colony color on LB agar**	Light-Yellow
		Colony color on Nutrient agar	Off-white to creamy
Pigment production on LB, NA, YEM & TY agar	No	**Cell shape in Nutrient agar**	Small rods
Gum production on LB, NA, TY & YEM agar	+	**Cell motility in LB and Nutrient broth**	+
	Biochemical characters		
Reaction/test	**PV_2Z_2**	**Reaction/test**	**PV_2Z_2**
Cytochrome oxidase	+	Catalase	+
Aminopeptidase	+	Nitrate reduction	+
β-galactosidase production	+	Production of acid from Glucose	+
Sodium citrate utilization	UI	Production of acid from Maltose	+
Sodium malonate fermentation	-	Production of acid from Sucrose	-
Lysine decarboxylase	-	Production of acid from Mannitol	+
Arginindihydrolase	-	Production of acid from Arabinose	+
Ornithine decarboxylase	-	Production of acid from Rhamnose	+
H_2S production	-	Production of acid from Sorbitol	+
Urea hydrolysis	-	Production of acid from Inositol	+
Tryptophanedeaminase	+	Production of acid from Adonitol	-
Indole production	-	Production of acid from Melibiose	+
Acetion formation (Voges-Proskauer test)	+	Production of acid from Raffinose	-
		Gelatin hydrolysis	-
Utilization of carbon source			
Mannitol	+++	Yeast extract	++
Maltose	++	Myo-inositol	+++
Sucrose	+++	Sorbitol	+++
Fructose	+	Levulose	+++
D-Glucose	-	Glycerol	+++
Resistant to antibiotic			
Erythromycin (15 μg)	+	Kanamycin (10 μg)	+
Ampicillin (100 μg)	+	Spectinomycin(50 μg)	+
Aztreonam (30 μg)	+	Chloramphenicol (30 μg)	+
Carbenicillin (100 μg)	+	Gentamycin (10 μg)	-
Streptomycin (10 μg)	-	Neomycin (10 μg)	-

LB=Luria-Bertani; YEM=Yeast Extract Mannitol, TY=Trypton Yeast medium.

- shows the reaction/test is negative

+ shows that reaction is positive

+++/++ shows that growth is sufficiently good on agar plates containing respective carbon source

UI reaction/test cannot be categorized as positive or negative

mL, was inoculated (1 mL inoculum) onto the base of the root tips of common bean (var. Lyamungu 90) in growth pouches (Asad et al. 1991). The plants were harvested after 30 days, and the effectiveness of nodules was determined by the acetylene reduction assay as described by Hafeez et al. (2005). Total N content of the plants was determined by the micro-Kjeldahal method (Yoshida et al. 1976) on a Kjeldahal system (Rapid Kjeldahl System; Labconco Corp., Kansas City, MO), and the results were statistically analysed using the MSTAT Version C statistical package. Significance at the 5 % level was tested by New Duncan's Multiple Range.

Biodegradation potential

A 1 mL sample of bacterial culture, after overnight growth in LB medium, was inoculated into 100 mL of minimal salts medium (MSM) supplemented with 100 mg L^{-1} each of the phenol, 2-bromo-phenol, 4-nitrophenol, 2, 4-diamino phenol HCL, 3, 4-dimethoxy benzyl alcohol, 1, 3-dimethoxy benzene and 2(5-bromo-2-pyridylazo)-5-diethylamino phenol. Cultures were grown with constant shaking for 12 days at 28 °C, and growth was monitored by measuring the optical density of the culture at λ_{610} with a CamSpec M350 double-beam UV-visible spectrophotometer (Spectronic Camspec, Leeds, UK) using non-inoculated MSM medium as blank. Bacterial growth was quantified by counting the colony-forming units (CFU) as described (Somasegaran and Hoben 1994). The cells after growth in phenol-supplemented MSM were centrifuged, and the residual phenol concentration in the cell-free supernatant was estimated at λ_{510} as described (Greenburg et al. 1985) based upon the reaction between phenol and 4-amino antipyrine in the presence of ferricyanide under alkaline conditions. Percentage degradation was calculated by the formula

$$\%\,\text{phenol degradation} = [(C_f - C_0)/C_0] \times 100$$

where C_f is the final concentration of phenol in medium, and C_0 is the initial concentration of phenol in the medium.

Results

Physicochemical characters and identification of Pv2Z2

The detailed physicochemical characters of the bacterial strain Pv2Z2 are given in Table 1. Based on the test results, cells of the strain Pv2Z2 are Gram negative (aminopeptidase positive) and aerobic (catalase and oxidase positive), and they appear as highly motile, short rods commonly observed as single cells under the light microscope. Growth occurs on LB, YEM, nutrient agar (NA) and TY media within 10–12 h at 28 °C, resulting in light-yellow colonies in TY and LB medium. Colonies are generally convex and shiny, with smooth margins, and produce high amounts of gum. It is an acid-producing strain and showed complete resistance to chloromphenicol (30 μg), erythromycin (15 μg), aztreonam (30 μg) and carbenicillin (100 μg), but was highly sensitive to ampicillin (10 μg), gentamycin (10 μg), amikacin (30 μg), streptomycin (10 μg), doxicycline (30 μg), neomycin (30 μg) and trimethoprim/ sulfamethoxazole (25 μg).

The almost full-length 16S rRNA (1,461 bp) fragment of PV2Z2 showed its maximum homology (99 %) to *Ochrobactrum anthropi* strain CLM6 (AJ867292). The nucleotide accession number for strain PV2Z2 is EU399793. Full-length 16S rRNA gene sequences were obtained from the NCBI (www.ncbi.nlm.nih.gov) site and were aligned with PV2Z2 to generate a phylogenetic tree. This phylogenetic tree (Fig. 1) showed that the strain falls in the same cluster with the type strains of *O. anthropi* LMG3331^T, *O. lupini* LUP21^T and *O. cytisi* ESC1^T and that it is more related to *O. cytisi* from an evolutionary point of view than to *O.*

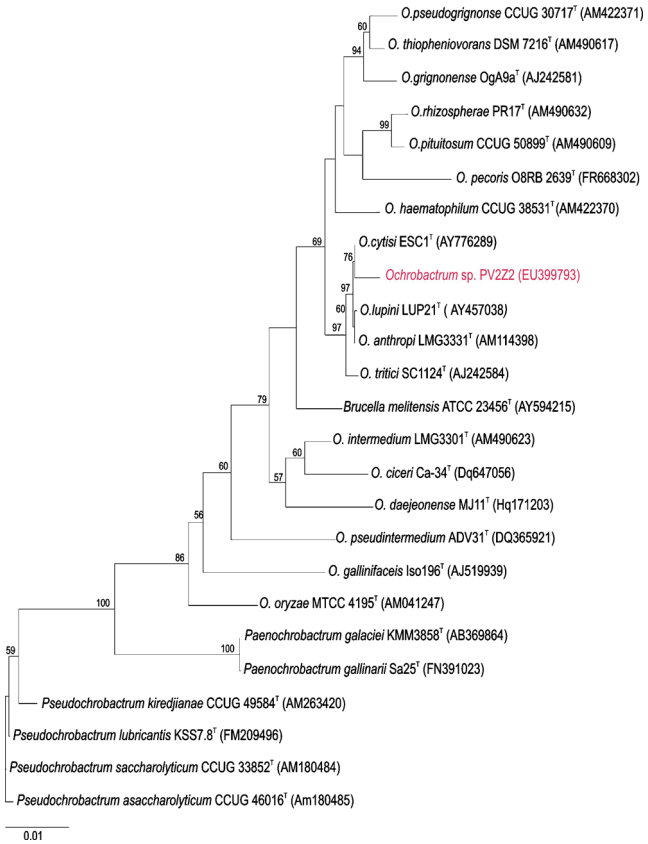

Fig. 1 Neighbour-joining tree, based on 16S rRNA gene sequences showing the phylogenetic position of strain Pv2Z2 among recognized members of the genus *Ochrobactrum* and other related species of the family Brucellaceae. Bootstrap values of >50 %, based on 1,000 replications, are shown at branch points. The tree was rooted with *Pseudochrobactrum asaccharolyticum* CCUG 46016 ^{T} (accession no. AM180485). *Bar* 0.01 substitutions per nucleotide position

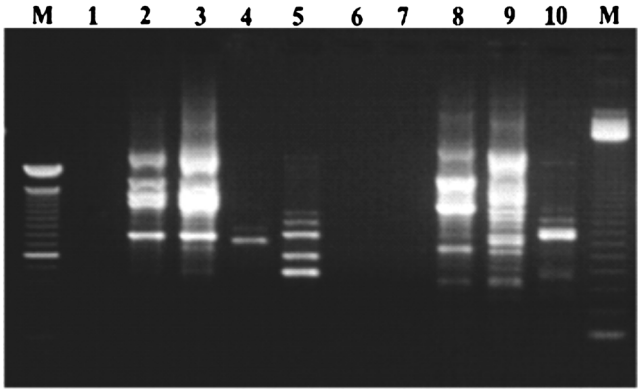

Fig. 2 Representative random amplified polymorphic DNA profile of strain Pv2Z2 and some type strains of genus *Ochrobactrum* generated with primer OPC-15. *Lanes*: *M* DNA marker (1 kb/100 bp), *1 O. ciceri*, *2 O. tritici* LAIII106, *3 O. tritici* ScII24^T, *4 O. lupini* LUP-21^T, *5 O. oryzae* MTCC4195^T, *6*, *7 O. gallinifaecis* ISO196^T, *8 O. intermedium* DSM 17986^T, *9 O. anthropi* DSM 6882^T, *10 Ochrobactrum* sp. PV_2Z_2

anthropi. The RAPD results (Fig. 2) further validated the genetic differences between strains PV2Z2 and *O. anthropi* LMG3331^T.

Bioassay for beneficial plant traits

After 7 days of incubation a clear halo zone around the bacterial growth spot was clearly observed, showing the solubilization of zinc oxide (Fig. 3a) and inorganic phosphate (Fig. 3b). The quantification data of these and other beneficial plant traits are given in Table 2. No antagonistic activity was observed against *Fusarium solani*, *F. oxysporum* and *F. moniliforme*.

AHL assay and RP-TLC

The production of a violet colour in the TY/LB media detected with the biosensor strain after overnight incubation demonstrated the production of AHL molecules in the medium (Fig. 3c). These AHLs after extraction with ethyl acetate were analysed by RP-TLC, and the chromatogram (Fig. 4) showed that strain Pv2Z2 produces the AHLs 3O-C7-HSL and 3OH-C7-HSL.

Plant inoculation assay

The inoculation of common bean with Pv2Z2 resulted in increased plant weight, plant height and N uptake as compared to the non-inoculated plants (Fig. 5).

Biodegradation assays

The strain Pv2Z2 was able to grow on MSM containing 100 mg/L of each of the phenols 2-bromophenol, 2,4-diamino phenol hydrochloride, 3,4-dimethoxy benzyl alcohol and 4-methoxy benzyl alcohol, demonstrating that it was able to utilize these substrates as carbon sources, forming populations of $>10^7$ cells per millilitre (Table 2). Maximum and minimum growth was observed in phenol (8.88 CFU) and 3,4-dimethoxy benzyl alcohol (7.53 CFU), respectively. Strain Pv2Z2 was unable to grow in MSM containing 4-nitrophenol and 2 (5-bromo-2-pyridylazo)-5-diethyl amino phenol. Within 12 days, it degraded 94 % of 100 mg/L phenol (Fig. 6).

Discussion

The beneficial effects of the rhizosphere microbiome have been known about for some time, but the wide-spread use of microbes as a crop supplement and bioremediation has increased in recent years due to the deleterious effects of chemical fertilizers/pesticides on the environment and non-target organisms, the increasing costs of chemical fertilizers and the massive increase in environmental pollution and contaminated water due to rapid industrialization (Agrawal et al. 2010). Beneficial bacteria generally include members of the genera *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Serratia*, *Enterobacter*, *Arthrobacter*, *Burkholderia* and *Ochrobactrum* (Lugtenberg and Kamilova 2009; Bhattacharyya and Jha 2012). These bacteria have been reported to act as plant

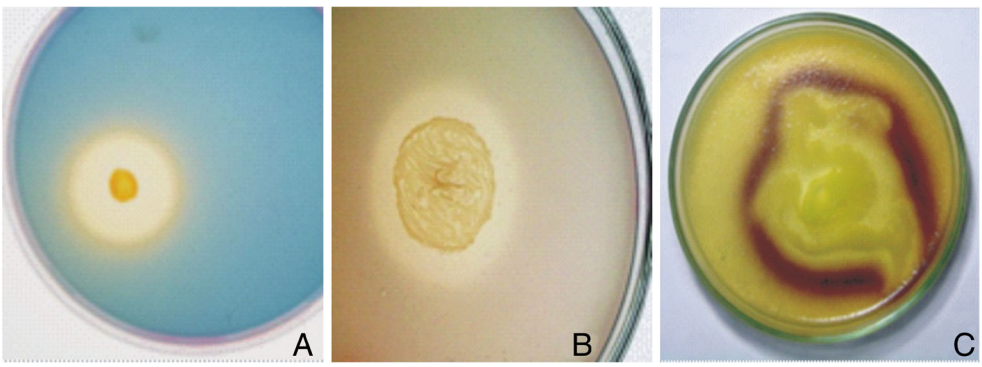

Fig. 3 Solubilization of zinc oxide (**a**), tri-calcium phosphate (**b**) and the production of *N*-acyl homoserine lactone (AHL) molecules (**c**) by *Ochrobactrum* sp. Pv2Z2 in vitro

Table 2 Plant growth promoting and biodegradation potential of strain PV2Z2

Beneficial plant traits		Growth in the MSM-containing substrate	
Trait/character	Values	Substrate/compound	Colony-forming units
Indole-3-acetic acid production	6.68 μg mL^{-1}	Phenol	8.88
Solubilization of inorganic phosphate	25.7 μg mL	2-Bromophenol	8.17
Production of AHL signal molecule	+	2,4-diaminophenol dihydrochloride	8.25
Solubilization of zinc oxide	+	4-Nitrophenol	0
Solubilization of zinc sulphate	+	2 (5-Bromo-2-pyridylazo)-5-diethyl amino phenol	0
Antagonistic activity	-	3,4-Dimethoxybenzyl alcohol	7.53
		4-Methoxybenzyl alcohol	7.82

MSM, Minimal salts medium; AHL, *N*-acyl homoserine lactone

-, The reaction/test is negative; +, the reaction/test is positive

growth-promoting factors (Hafeez et al. 2006; Kantar et al. 2007; Mirza et al. 2007; Bhattacharyya and Jha 2012) and in biodegradation or bioremediation (Smejkal et al. 2003; Afzal et al. 2007; Anwar et al. 2009).

In the study reported here we describe the taxonomic affiliation of a common bean nodule isolate Pv2Z2 and demonstrate its beneficial plant traits and biodegradation traits as well as its likely contribution in promoting plant growth. The strain was found to be metabolically diverse, to utilize a number of carbon sources and substrates, to be resistant to different antibiotics and to show the presence of different enzymes. These characteristics show the ecological fitness of the strain in a range of environments. Strain Pv2Z2 was isolated from sterilized nodules, indicating its endophytic nature. Its colony morphology was similar to that of other rhizobia, and nodulation assays were performed to confirm its nodulation ability. The *Ochrobactrum* strain Pv2Z2 induced nodulation in plant inoculation assay on common bean and showed ARA activity (3.34 μmol of ethylene g^{-1} h-1). However we were unable to confirm the identity of the endophyte to our inoculated strain using in situ hybridization or other molecular techniques. The improved N contents in common

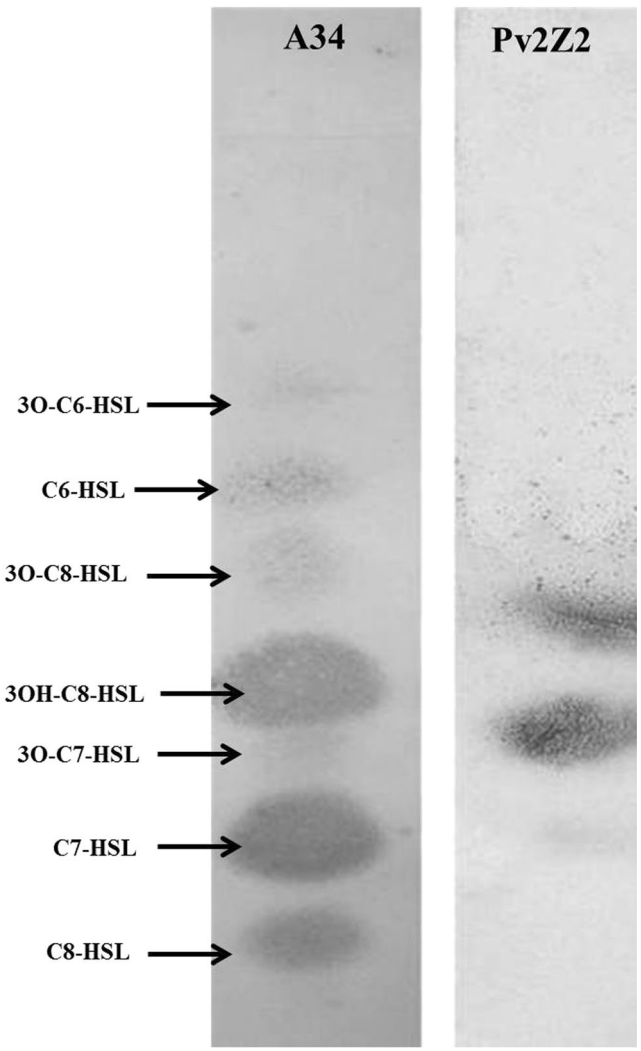

Fig. 4 Thin-layer chromatogram of AHL extracts of *Ochrobactrum* sp. Pv2Z2 compared with reference strain *Rhizobium leguminosarum* (A34). *HSL* Homoserine lactone

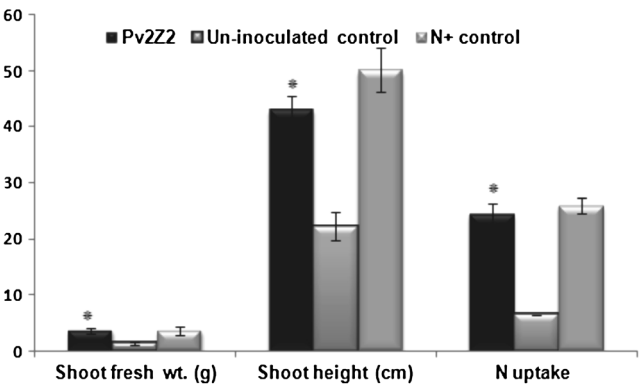

Fig. 5 Effect of inoculation with *Ochrobactrum* sp. Pv2Z2 on common bean in growth pouches. Nitrogen uptake is given in units of mg g^{-1} shoot dry weight. The results are the average of three replicates. Un-inoculated control contained sterile water. The concentration of the N+control was 1 M potassium nitrate

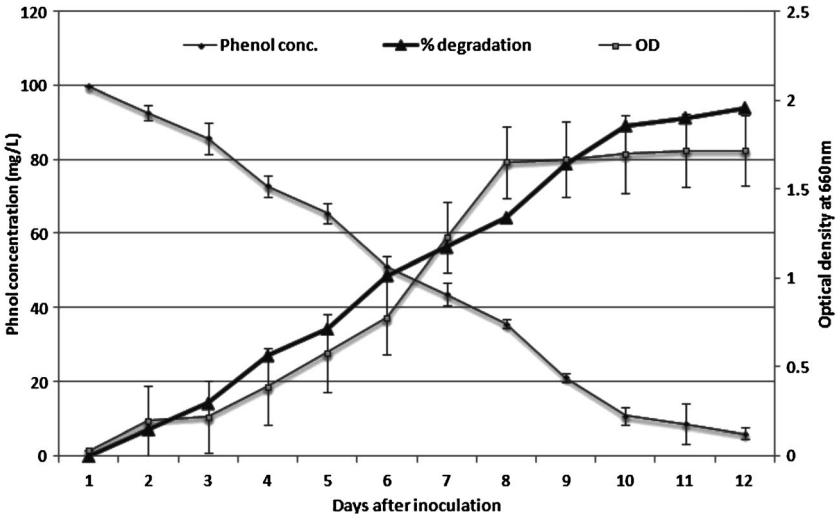

Fig. 6 Degradation of phenol in comparison with the growth [optical density (*OD*)] of *Ochrobactrum* sp. Pv2Z2

bean plants inoculated with the Pv2Z2 may be attributed to its N-fixing ability.

The Pv2Z2 strain was isolated from the soil of Zanzibar, Tanzania, where crop productivity is usually low due to soil acidity and moisture stress. Cell and colony morphology features, biochemical reactions and the antibiotic resistance pattern showed a maximum relatedness with genus *Ochrobactrum*. The 16S *rRNA* sequence analysis also supported the genus affiliation. The strain exhibited maximum similarities (99 %) with *Ochrobactrum anthropi* strains CLM6 (AJ867292), CLM5 (AJ867291) and SAIII104 (AJ867290). All of these strains showing maximum similarities to *Ochrobactrum* strain Pv2Z2 are isolates from the wheat rhizosphere (Lebuhn et al. 2000). Although phylogenetic analysis grouped Pv2Z2 in the same phylogenetic lineage in which the type strain of *O. anthropi* was present, the nearest phylogenetic relative was the nodulating strain *O. cytisi* rather than the clinical isolate or any pathogenic strain (Fig. 1), suggesting the non-pathogenic nature of the strain Pv2Z2 which was further validated by the RAPD and TP-RAPD assays (Fig. 2). Moreover, the plant inoculation data obtained in this and other studies (Faisal and Hasnain 2004; Chakraborty et al. 2009; Riaz et al. 2010) show that *O. anthropi* or *O. intermedium* can be safely used as PGPR.

The production of IAA, solubilization of inorganic phosphate/zinc and the promotion of growth of common bean plants in the pot experiment as well as the significant increase in N uptake by plants suggest that the strain can be a potential PGPR candidate for field application. The production of AHL was found to be a novel trait that had not earlier been reported for the members of this genus. N2 fixation, phosphate solubilization and biocontrol activities of bacteria are known to be controlled by the AHL signal molecules. The varied characters of this strain suggest that its quorum sensing signals might be involved in all of these processes as well as in many other physiological phenomena. Moreover, the ability to produce AHL will help in rhizosphere colonization and might give this strain a competitive advantage under field conditions. To date, no member of genus *Ochrobactrum* has been reported to contain AHL production activity, although it does have the activity to produce quorum quenching lactonase enzymes. This *Ochrobactrum* sp. was isolated from potato rhizosphere and produces lactonase enzyme which is able to disrupt the signals of potato pathogen *Erwinia carotovora* (Jafra et al. 2006). Following separation by RP-TLC, the crude AHL extract showed the presence of 3O-C7-HSL and 3OH-C7-HSL, but the exact mass and nature of these compounds will remain questionable unless confirmed through detailed mass spectrometry analysis.

The biodegradation study is the most disparate of the investigations since it does not directly address plant–microbe interaction but was undertaken because the root exudates contain a number of phenolic compounds and bacteria contain the ability to utilize these compounds as an energy source. This ability might give the bacterium an ecological advantage in the rhizosphere, which (due to its nutrient richness) works as "microbial hot-spot" for diverse plant–plant, plant–microbe and microbe–microbe interactions. The strain proved itself a candidate of choice for the biodegradation of phenol-contaminated soils. The degradation of phenol by *Ochrobactrum* species (Lechner et al. 1995; El-Sayed et al. 2003) has been reported to be fourfold higher than that of other reported phenol-degrading bacteria. The known phenol-degrading *Ochrobactrum* species include both those isolated from activated sludge and contaminated industrial environments or the plant-associated *O. tritici* NBRC 102585, *O. grignonense* NBRC 102586, and *O. lupini* NBRC 102587 (Yamada et al. 2008). Phenol degradation by nodule bacteria, such as *Rhizobium leguminosarum* bv trifolli, has been reported (Parke et al. 1991), as has the degradation of

brominated phenols by plant-associated *Ochrobactrum* sp. TB01, *O. tritici* NBRC102585, *O. grignonense* NBRC 102586 and *O. lupini* NBRC 102587 (Yamada et al. 2008). These studies show that this phenol-degradation phenomenon is wide-spread in the genus. The Pv2Z2 strain was, however, not able to grow in the medium containing 4-nitrophenol and 2-(5-bromo-2-pyridylazo)-5-diethyl amino phenol. One possible explanation for the failure of PV2Z2 to grown on nitrophenol-supplemented medium might be the unfavourable conditions, as *p*-nitrophenol degradation has been shown to be accelerated at alkaline pH (8–10) and following the addition of organic N (i.e. yeast extract and peptone; Qiu et al. 2007). The ability of plant-associated bacteria to degrade aromatic compounds allows these microorganisms to utilize multiple energy sources, thereby improving their ecological success in the rhizosphere.

Ochrobactrum species are widespread and frequent inhabitants of the rhizosphere and other environmental habitats. The results presented here have implications for using *Ochrobactrum* strain Pv2Z2 as biofertilizer and in biodegradation. The strain Pv2Z2 was isolated from the root nodule of common bean; it is genetically different from clinical/pathogenic *O. anthropi/intermedium* isolates and has positive effect on plant growth. Therefore, its use as plant and/or soil inoculum is not expected to cause any human pathogenic consequences. In this study, we report for the first time a new strain of *Ochrobactrum* sp. Pv2Z2 isolated from the common bean nodules with the ability to produce AHL molecules.

Acknowledgements The authors are grateful to Dr. Allan J. Dowine (JIC. Norwich, UK) for providing reference and indicator strains for the AHL assay and to Dr. Erko Stackebrandt (DSMZ, Germany) for providing the DNA of *O. anthropi* and other type strains of the genus. The research work was partially supported by IDB-BIRCEN Project on "Use of Biofertilizers for Increasing Sustainable Crop Production in Developing Countries: Establishment of a Biofertilizer Resource Center (BIRCEN) at NIBGE" (2000–2005).

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