Arachidicoccus rhizosphaerae gen. nov., sp. nov., a plant-growth-promoting bacterium in the family Chitinophagaceae isolated from rhizosphere soil

Munusamy Madhaiyan,1,2 Selvaraj Poonguzhali,1 Murugaiyan Senthilkumar,1 Dhandapani Pragatheswari,3 Jung-Sook Lee4 and Keun-Chul Lee4

1Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
2Temasek Lifesciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604
3School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
4Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, 111 Gwahangno, Yusong-gu, Daejeon 305-806, Republic of Korea

Three novel bacterial strains, designated Vu-144T, Vu-7 and Vu-35, were isolated on minimal medium from rhizosphere soil of field-grown cowpea and subjected to a taxonomic study using a polyphasic approach. Cells of the strains were Gram-stain-negative, non-motile, non-spore-forming, coccoid rods, and formed non-pigmented colonies. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Vu-144T was affiliated with an uncultivated lineage of the phylum Bacteroidetes. Its closest phylogenetic neighbour was the recently described species Niastella populi, a member of the family Chitinophagaceae, with just 90.7 % sequence similarity to the type strain. The only isoprenoid quinone detected was menaquinone 7 (MK-7). The fatty acid profiles showed large amounts of iso-C15:0, iso-C17:0 3-OH and iso-C15:0 7c and/or iso-C15:0 2-OH, C16:0 and other fatty acids, allowing the differentiation of the strains from other genera. The G+C content of the genomic DNA of the three strains ranged from 43.1 to 44.3 mol%. In addition to phosphatidylethanolamine, the major polar lipids were three unidentified aminophospholipids (APL1–APL3), two unidentified phospholipids (PL1, PL2) and three unidentified lipids (UL1–UL3). Biochemical test patterns also differed from those of Niastella populi and members of other genera. All three isolates showed plant-growth-promoting properties, e.g. the ability to produce indole-3-acetic acid and NH3 and to solubilize phosphate, utilized 1-aminocyclopropane 1-carboxylate (ACC) as a sole source of nitrogen and possessed the ACC deaminase enzyme. The novel isolates readily colonized roots and stimulated growth of tomato and cowpea under glasshouse conditions. Inoculated plants showed a 45–60 % increase in dry matter weight with respect to uninoculated controls. On the basis of the evidence from our polyphasic study, isolate Vu-144T represents a novel genus and species in the family Chitinophagaceae, for which the name Arachidicoccus rhizosphaerae gen. nov., sp. nov. is proposed. The type strain of Arachidicoccus rhizosphaerae is Vu-144T (=KCTC 22378T=NCIMB 14473T).

Members of the phylum Bacteroidetes have a worldwide distribution in both marine and terrestrial ecosystems. They are abundant in many freshwater and marine systems (Kirchman, 2002). This large group of bacteria was formerly classified under the group ‘Cytophaga–Flavobacterium–Bacteroides’ (Olsen et al., 1994) and has had a long history of nomenclatural modifications, and
large sections are still in need of taxonomic clarification. The order Sphingobacteriales comprises three families, namely Chitinophagaceae, Saprospiraceae and Sphingobacteriaceae. The family Chitinophagaceae was proposed by Kämpfer et al. (2011) to accommodate a group of Gram-stain-negative, non-motile, aerobic or facultatively anaerobic, rod- or coccoid-shaped organisms. This family originally embraced 16 genera (http://www.bacterio.net/chitinophagaceae.html): Balneola (Urios et al., 2008), Chitinophaga (Sangkhobol & Skerman, 1981; Kämpfer et al., 2011), Ferruginibacter (Lim et al., 2009), Filimonas (Shiratori et al., 2009), Flavihuminibacter (Zhang et al., 2010), Flavisolibacter (Yoon & Im, 2007), Flavitalea (Wang et al., 2011), Gracilimonas (Choi et al., 2009), Hydrotalea (Kämpfer et al., 2011), Lacibacter (Qu et al., 2009), Niabella (Kim et al., 2007), Niastella (Weon et al., 2006), Parasegetibacter (Zhang et al., 2009), Sediminibacterium (Qu & Yuan, 2008), Segetibacter (An et al., 2007) and Terrimonas (Xie & Yokota, 2006). Members of the family Chitinophagaceae have been reported to be widely distributed in diverse habitats, such as forest soil, ginseng field soil, Euphrates poplar soil, greenhouse soil, rhizosphere soil of soybean and rice, root endophytes, vermicompost, Mediterranean seawater, sediment of eutrophic lakes and fresh water and air samples (Wang et al., 2009, 2011; Zhang et al., 2009; An et al., 2007; Lee et al., 2007; Kim et al., 2007; Chung et al., 2012; Li et al., 2013; Yasar et al., 2011; Urios et al., 2006; Qu et al., 2009; Lim et al., 2009; Weon et al., 2010). Strains Vu-144T, Vu-7 and Vu-35 were isolated during an investigation of rhizospheric bacterial diversity from different leguminous plant rhizosphere soils. They were found to be members of the family Chitinophagaceae, phylum Bacteroidetes (Garrity & Holt, 2001; Kämpfer et al., 2011), and were subjected to further taxonomic investigation. On the basis of the results obtained in this study, we propose that strains Vu-144T, Vu-7 and Vu-35 represent members of a novel genus.

Strains were isolated from rhizosphere soils of field-grown cowpea and peanut collected from an experimental farm at Tamil Nadu Agricultural University (TNAU), Coimbatore, in April 2001. Soil samples were thoroughly suspended in sterile distilled water and the suspensions were plated on selective ammonium mineral salts (AMS) medium (Whittenbury et al., 1970) and incubated at 30 °C for 7 days. The isolates were routinely cultured on plates of R2A (Difco Laboratories) at 30 °C under aerobic conditions and stored frozen at −80 °C as 50 % (v/v) glycerol stocks. Strains Vu-7, Vu-35 and Vu-144T were deposited in the Korean Collection for Type Cultures as KCTC 22376, KCTC 22377 and KCTC 22378T and in the NCIMB as NCIMB 14477, NCIMB 14474 and NCIMB 14473T, respectively. All physiological and morphological characters were observed with cells cultured on R2A medium under optimal growth conditions, 28–30 °C and pH 6.0–7.0, unless otherwise noted.

Cell morphology was observed under a Nikon light microscope, scanning electron microscopy and transmission electron microscopy, using cells grown for 3 days at 30 °C on R2A medium. Scanning electron microscopic observations were performed on fixed material prepared for routine examinations as described by Bozolla & Russell (1998). Samples were critical-point-dried, mounted on stubs, sputter-coated with gold/palladium and visualized by using a Hitachi S-2500C scanning electron microscope with a Gemini column equipped with a field-emission electron source. For the latter, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air-drying, grids were examined under a transmission electron microscope (model CM-20; Philips). The presence of flexirubin-like pigments was investigated by flooding culture plates with 20 % (w/v) KOH (Fautz & Reichenbach, 1980). Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl p-phenylenediamine, using Difco BBL catalase and oxidase reagent droppers according to the manufacturer instructions (Difco Becton Dickinson). Starch hydrolysis was determined with Lugol’s iodine solution after cultivation on R2A plates containing 0.2 % (w/v) starch. To examine hydrolysis of CM-cellulose, the isolates were cultured on R2A plates supplemented with 0.5 % (w/v) CM-cellulose. After culturing, the plates were stained with 0.2 % aqueous Congo red dye solution and washed with 1 M NaCl to observe the zone of clearing. Lipolytic and proteolytic activities were investigated on tributyrin agar with 1 % (v/v) glycerol tributyrate and 10 % trypticase soy agar (TSA) with 1.5 % skimmed milk, respectively. The plates were incubated for 3–5 days and examined for the presence of a clear zone around the growing colonies. Other tests for the degradation of chitin, gelatin, aesculin and urea were performed and evaluated after 5 days. Enzyme activities of pectinase and cellulase were tested by standard methods (Plazinski & Rolfe, 1985; Andro et al., 1984). Urease activity was tested using urea broth (Difco) according to the manufacturer’s instructions. The carbon source utilization pattern was studied by using Biolog GN2 microplates as described by Madhaiyan et al. (2007). Anaerobic growth was checked on R2A and AMS agar medium by using the GasPak anaerobic system (BBL) at 30 °C for 15 days. Growth at 4, 20, 30, 37 and 45 °C and pH 2.0–10.0 (in increments of 2.0 pH units) was assessed after 5 days of incubation. The Gram-stain reaction was determined using a Gram staining kit according to the manufacturer’s instructions (Difco). Growth on nutrient agar, TSA and MacConkey agar (all from Difco) was also evaluated at 30 °C.

All three strains were Gram-stain-negative, aerobic, catalase- and oxidase-positive, non-motile and coccoid rod-shaped bacteria (Fig. 1). Colonies were dull white, round and convex after 72 h of growth at 30 °C on R2A agar. The diameter of the colonies on R2A agar plates was 0.3–0.5 mm after 72 h at 30 °C, but growth was not observed at more than 37 °C. The three strains showed growth over a narrow pH range of 6.0–8.0, with an optimum pH between 6.5 and 7.0 and no growth at below
pH 6.0 or above pH 8.0. These strains grew in R2A medium containing 0–2% (w/v) NaCl, but not in media containing ≥3% (w/v) NaCl. The main phenotypic characters that differentiate the strains from other members of the family Chitinophagaceae are shown in Table 1. More detailed results of phenotypic tests and nutritional features of strains Vu-7, Vu-35 and Vu-144T are given in the species description.

Genomic DNA extraction was carried out using the QIAamp DNA mini kit (Qiagen) according to the manufacturer’s instructions. The 16S rRNA gene was amplified using universal primers 27F and 1492R (DeLong, 1992) with the following cycling conditions: 95°C for 10 min, 30 cycles of 95°C for 1.5 min, 55°C for 1.5 min and 72°C for 1.5 min and a final extension for 10 min at 72°C. The full 16S rRNA gene sequence was determined by the fluorescent dye terminator method using a sequencing kit (ABI Prism Big Dye terminator cycle sequencing ready reaction kit version 3.1) and products were run on an ABI3730XL capillary DNA sequencer (ABI Prism 310 Genetic Analyzer). The identification of phylogenetic neighbours was initially carried out by using the BLASTN program (Altschul et al., 1997) against a database containing sequences of type strains of prokaryotic species with validly published names and representatives of uncultured phylotypes (Kim et al., 2012). The sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm (Myers & Miller, 1988), which was implemented at the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012) (Table S1, available in the online Supplementary Material), and aligned using the CLUSTAL W tool in MEGA version 4 (Kumar et al., 2004). Phylogenetic analyses were carried out using three treeing algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods, using MEGA version 5.05 (Tamura et al., 2011) and bootstrap values based on 1000 replications (Felsenstein, 1985). DNA–DNA hybridization was carried out to find the relatedness of the three strains following the filter hybridization method (Seldin & Dubnau, 1985) with some modifications as described previously (Madhaiyan & Poonguzhali, 2014).

Almost-complete 16S rRNA gene sequences for strains Vu-144T, Vu-7 and Vu-35 were determined and subjected to comparative analysis. These strains showed the highest levels of pairwise 16S rRNA gene sequence similarity (89.0–90.7%) with respect to the type strains of species of the genus Niastella, followed by the type strains of species of Sediminibacterium (88.6%), Chitinophaga (87.5–89.7%), Lacibacter (89.7%), Flavisolibacter (89.2–89.4%), with a unique phylogenetic position (Table S1). A phylogenetic analysis based on 16S rRNA gene sequences indicated that the three isolates were clustered with several uncultured bacterial clones and with established genera in the family Chitinophagaceae (Kämpfer et al., 2011), but were clearly separated from these genera (Fig. 2). The neighbour-joining method, as well as the other two treeing algorithms, maximum-likelihood and maximum-parsimony, clearly indicated a distinct line of descent for strains Vu-144T, Vu-7 and Vu-35 (Fig. S1a, b); in view of their phylogenetically deep branching point within the order Sphingobacterales, the strains could be considered as representing a novel genus. Strains Vu-7 and Vu-35 exhibited DNA–DNA relatedness values of more than 70% with strain Vu-144T. From this result, and the other characteristics described above, strains Vu-7 and Vu-35 were identified as members of the same novel species. The whole-genome sequence of strain Vu-144T has been accepted through the Genomic Encyclopedia of Bacteria and Archaea (GEBA) III project of the Community Science Program (CSP) of the DOE Joint Genomes Institute (data not shown).

The three strains were screened for indole-3-acetic acid (IAA) production by growing in 50 ml R2A medium supplemented with 100 µg L-tryptophan ml−1 for 96 h. After incubation, 2 ml of the cell suspension was transferred to a tube and then mixed vigorously with 100 µl 10 mM orthophosphoric acid and 4 ml Salkowski’s reagent (1 ml 0.5 M FeCl3 in 50 ml 35 % HClO4) and allowed to stand at room temperature for 30 min for the

---

**Fig. 1.** (a, b) Scanning electron micrographs of cells of strain Vu144T grown on R2A agar supplemented with 0.5% (v/v) methanol. Bars, 1 µm. (c) Transmission electron micrograph of cells of strain Vu144T, showing the general morphology of negatively stained cells from an exponentially growing culture. Bar, 500 nm.
Table 1. Characteristics that serve to differentiate strains Vu-144T, Vu-7 and Vu-35 from members of genera classified in the family Chitinophagaceae

Strains: 1, Vu-144T; 2, Vu-7; 3, Vu-35; 4, Niastella koreensis GR20–10T; 5, Flavisolibacter ginsengisolii Gsoil 643T; 6, Sediminibacterium salmoneum NJ-44T; 7, Chitinophaga pinensis ACM 2034T; 8, Lacibacter cauensis NJ-8T; 9, Hydrotalea flava CCUG 51397T; 10, Niabella aurantiaca R2A15–11T. Unless indicated, data for reference strains were taken from Kim et al. (2007), Weon et al. (2006), Kämpfer et al. (2006), Qu et al., (2009), Qu & Yuan (2008) and Kämpfer et al. (2011). +, Positive; −, negative; ND, no data available. All strains have an ML-7 quinone system.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Rhizosphere soil</td>
<td>Rhizosphere soil</td>
<td>Rhizosphere soil</td>
<td>Soil</td>
<td>Sediment</td>
<td>Soil</td>
<td>Sediment</td>
<td>Sediment</td>
<td>Water</td>
<td>Greenhouse soil</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Milky white</td>
<td>Milky white</td>
<td>Milky white</td>
<td>Light yellow</td>
<td>Yellow</td>
<td>Salmon pink</td>
<td>Yellow</td>
<td>Orange</td>
<td>Orange</td>
<td>Orange</td>
</tr>
<tr>
<td>Cellular morphology</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Filamentous</td>
<td>Rods</td>
<td>Filamentous</td>
<td>Rods</td>
<td>Filamentous</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>0.5–0.6</td>
<td>0.5–0.6</td>
<td>0.4–0.5</td>
<td>10–50</td>
<td>0.3–6.0</td>
<td>1.0–1.2</td>
<td>0.8–40</td>
<td>1.0–2.0</td>
<td>1.5–2.0</td>
<td>1.0–1.5</td>
</tr>
<tr>
<td>Gliding motility</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Flexirubin reaction</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polar lipid(s)*</td>
<td>PE, APL, PL, UL</td>
<td>PE, APL, PL, UL</td>
<td>PE, APL, PL, UL</td>
<td>ND</td>
<td>PE†</td>
<td>PE, APL, AL, PL, UL</td>
<td>PE, APL, AL, PL, UL†</td>
<td>ND</td>
<td>PE, APL, AL, PL, UL</td>
<td>PE, PS, APL, AL, UL†</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;15% ‡)</td>
<td>i-C_{15.0}, i-C_{17.0}</td>
<td>i-C_{15.0}</td>
<td>i-C_{15.0}</td>
<td>i-C_{15.0}, i-C_{17.0}</td>
<td>3-OH</td>
<td>i-C_{15.0}, i-C_{17.0}</td>
<td>3-OH</td>
<td>i-C_{15.0}, i-C_{17.0}</td>
<td>G</td>
<td>i-C_{15.0}, i-C_{17.0}, G, i-C_{17.0}</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>43.1</td>
<td>44.8</td>
<td>44.3</td>
<td>45.8</td>
<td>42.7</td>
<td>38.6</td>
<td>45.2</td>
<td>46.6</td>
<td>42.0</td>
<td>45.0</td>
</tr>
</tbody>
</table>

*PE, Phosphatidylethanolamine; PS, phosphatidylserine; AL, unidentified aminolipid(s); APL, unidentified aminophospholipid(s); PL, unidentified phospholipid(s); UL, unidentified lipid(s).
†Data from Baik et al. (2014), Proença et al. (2014) and Glaeser et al. (2013).
‡i, iso-branched.
cell suspension to turn pink in colour. The presence of indoles in the culture supernatant was determined spectro-photometrically (UV-1601; Shimadzu) at 530 nm and the assay was calibrated by generating a standard curve for samples containing IAA (Sigma). Uninoculated medium served as a control. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity of the three strains was checked by the ability of the strains to utilize ACC as a nitrogen source by assessing their growth in DF minimal salts medium (Dworkin & Foster, 1958) supplemented with 3 mM ACC. ACC deaminase activity of the isolates was measured by spectrophotometry at 540 nm as described previously by Shah et al. (1998), and a modified procedure described by Honma & Shimomura (1978). The α-ketobutyrate produced by the strains was estimated by comparing with a standard curve of α-ketobutyrate (Sigma-Aldrich). A stock solution of α-ketobutyrate was prepared in 0.1 M Tris/HCl (pH 8.5) and stored at 4 °C.

To measure the specific activity of the cultures, protein concentration was estimated according to the Lowry method. Bacterial strains were tested by plate assay using Pikovskaya’s agar medium (Pikovskaya, 1948) with 0.5 % tricalcium phosphate as the inorganic phosphate source. The plates were incubated at 28 °C for 72 h; solubilization of mineral phosphate was characterized by a clear halo around the bacterial colonies, indicating the capacity for phosphate solubilization. Siderophore secretion by the strains was detected by the universal method of Schwyn & Neilands (1987) using blue agar plates impregnated with chrome azurol S dye (Sigma-Aldrich). Orange haloes around the colonies on blue agar were indicative of siderophore production. Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48 h at 30 °C. Nessler’s reagent (0.5 ml) was added to each tube. Development of a brown to yellow colour reflected a positive test for ammonia production (Cappuccino & Sherman, 1992).

Regarding plant-growth-promoting traits, the isolates presented diverse activities. The strains possessed ACC deaminase activity and produced IAA when examined through qualitative and quantitative assays. The highest ACC deaminase activity was detected in a cell-free extract of strain Vu-144T [14.12 nmol α-ketobutyrate (mg protein)⁻¹ h⁻¹], followed by Vu-7 [9.31 nmol α-ketobutyrate (mg protein)⁻¹ h⁻¹] and Vu-35 [6.21 nmol α-ketobutyrate (mg protein)⁻¹ h⁻¹]. All three strains were able to solubilize tricalcium phosphate, and the solubilization zone ranged from 1.3 to 1.8 cm in diameter, as confirmed by plate assay. This characteristic trait allows the strains to

Fig. 2. Phylogenetic dendrogram, based on 16S rRNA gene sequences and reconstructed from evolutionary distances (Kimura, 1980), showing the position of strains Vu-144T, Vu-7 and Vu-35 within the radiation of members of the family Chitinophagaceae (Kämpfer et al., 2011). Filled circles at nodes indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Numbers at branching points refer to bootstrap percentages (based on 1000 resamplings); only values above 50 % are shown. GenBank accession numbers for the sequences are in parentheses. Bar, 0.01 substitutions per nucleotide position.
increase the available phosphate in soil which, in turn, promotes plant growth. All three isolates did not show acetylene reduction activity on the nitrogen-free media tested, indicating the absence of the nitrogenase enzyme that is responsible for \( \text{N}_2 \)-fixation. Strains were positive for \( \text{NH}_3 \) production and negative for siderophore production.

A gnotobiotic root elongation assay was performed with canola to study the effect of the treatment of seeds with ACC-deaminase-producing bacterial isolates. The culture conditions and the procedure for the gnotobiotic growth pouch assay were described previously (Penrose & Glick, 2003; Madhaiyan et al., 2006). The root and shoot lengths of the canola seedlings were measured following 7 days of growth at \( 20^\circ \text{C} \), beginning with a cycle of 12 h of dark followed by 12 h of light (18 \( \mu \text{mol m}^{-2} \text{s}^{-2} \)). For pot experiments, tomato and peanut seeds were surface-sterilized with 70 % ethanol for 1 min followed by 5 % \( \text{NaOCl} \) with 0.6 % Tween 20 for 20 min. The bacterial cultures that proliferated in R2A medium (\( \text{OD}_{600}=1.0 \)) were pelleted (8000 \( \text{g}, \text{10 min at} \ 4^\circ \text{C} \)), washed and resuspended in sterile 0.01 M \( \text{MgSO}_4 \) (10^7 \( \text{c.f.u. ml}^{-1} \)). Seeds were added to the bacterial suspension and incubated for 2 h. Untreated control seeds were treated with 0.01 M \( \text{MgSO}_4 \). After inoculation, seeds were allowed to germinate on germination trays filled with sand. Plastic covers were put on the trays until the cotyledons were visible. Ten- to twelve-day-old seedlings of similar size were transferred to single pots (inner diameter 15 cm) containing garden soil. Each treatment had 20 replicates for both non-inoculated and bacteria-inoculated plants. Plants were harvested after 5–7 weeks and plant growth parameters were analysed. Total carbon and nitrogen content were determined by the combustion method in an elemental analyser (Vario EL Elemental Analyser; Elementar). The concentration of phosphorus was measured according to Jackson (1973) after digesting the plant samples with concentrated sulfuric acid, perchloric acid and ammonium metavanadate reagent. Standards were prepared with potassium dihydrogen phosphate (Sigma).

A gnotobiotic assay using growth pouches was performed with canola to study the root elongation activity induced by ACC deaminase, since canola plants show high sensitivity to ethylene (Li et al., 2000; Penrose & Glick 2003; Ghosh et al., 2003; Madhaiyan et al., 2006). The root length of the canola seedlings was measured after 7 days of growth at \( 20^\circ \text{C} \), beginning with a cycle of 12 h of dark followed by 12 h of light (18 \( \mu \text{mol m}^{-2} \text{s}^{-2} \)). Inoculation of the novel strains significantly increased the seedling vigour index, with the increase over uninoculated controls ranging from 25.3 to 77.7 % (Fig. S2a). The maximum biomass (228.4 mg per plant) was observed in canola seeds treated with strain Vu-144\text{T} when compared with the other strains and uninoculated controls (Fig. S2b). Fig. S3 shows the results of tomato and peanut plant growth promotion on day 45 post-infection with strain Vu-144\text{T}. The inoculated tomato and peanut plants had significant increases of 126.3 and 141.8 % in biomass, respectively, with respect to the uninoculated control (Fig. S3b, g). The content of plant nutrients (N, P and C) was increased significantly in tomato and peanut plants inoculated with strain Vu-144\text{T}, compared with the uninoculated control (Fig. S3c–e, h–j). These results indicated that the novel strains were good plant-growth-promoting bacteria, not only because of their ability to solubilize phosphates and to synthesize phytohormones, but also through diverse plant-growth-promoting mechanisms that require further study.

For fatty acid methyl ester analysis, strains Vu-144\text{T}, Vu-7 and Vu-35 and two reference strains, \textit{Niastella koreensis} \textit{KACC} 11465\text{T} and \textit{Flavisolibacter ginsengisoli} \textit{KACC} 12722\text{T}, were grown on R2A agar at 30 \( ^\circ \text{C} \) for 72 h. All strains included in the fatty acid analyses exhibited similar growth, and sufficient cells of comparable physiological age could be harvested from the third streak quadrant of the R2A agar plates after cultivation under the same conditions. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI) (Sasser, 1990), using a gas chromatograph (model 6890; Hewlett Packard), and were identified using the TSBA 50 database (version 5.00). Isoprenoid quinones were analysed by using reversed-phase HPLC as described by Komagata & Suzuki (1987). Polar lipids were analysed according to Minnikin et al. (1984). DNA G+C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah et al. (1989), using a reversed-phase column (Supelcosil LC-185; Supelco). Strains Vu-7 and Vu-35 contained phosphatidylethanolamine, two unidentified phospholipids (PL1, PL2), three unidentified aminophospholipids (APL1–APL3) and three unidentified lipids (UL1–UL3); strain Vu-144\text{T} did not contain PL2 (Fig. S4a–c). The polar lipid profiles were largely consistent with previous reports (Lee et al., 2013; Kang et al., 2014; Kim et al., 2013; Proença et al., 2014; Wang et al., 2014; Lin et al., 2014; Jin et al., 2013). All the strains identified had MK-7 has the major ubiquinone. The fatty acid profiles of the three strains consisted mainly of iso-C\textsubscript{15:0} (47.8–59.8 %) and iso-C\textsubscript{17:0} 3-OH (11.2–15.4 %). Previous work has shown that the predominance of fatty acids iso-C\textsubscript{15:0}, iso-C\textsubscript{17:0} 3-OH and iso-C\textsubscript{15:1} is a distinct feature of the vast majority of members of the family \textit{Chitinophagaceae} (Kämpfer et al., 2011) (Table S2). The presence of iso-C\textsubscript{15:1} G (9.2–11.5 %), C\textsubscript{16:0} (4.8–6.4 %), iso-C\textsubscript{15:0} 3-OH (1.6–2.0 %) and summed feature 3 (C\textsubscript{16:1} \( \omega 7c \) and/or iso-C\textsubscript{15:0} 2-OH; 3.6–8.3 %), and some quantitative differences in fatty acid composition, served to distinguish strains Vu-144\text{T}, Vu-7 and Vu-35 from members of phylogenetically related genera.

On the basis of the phylogenetic, chemotaxonomic and physiologochemical data, strains Vu-144\text{T}, Vu-7 and Vu-35 differ from related genera (Table 1). For this reason, a new genus is proposed with the name \textit{Arachidicoccus} gen. nov., with the novel species \textit{Arachidicoccus rhizosphaerae} sp. nov.
Description of Arachidicoccus gen. nov.

_Arachidicoccus_ (A.ra.chi.di.coc’cus. N.L. fem. n. _Arachis_ botanical generic name for peanut; N.L. masc. n. _coc’cus_ a coccos from Gr. masc. n. _kokkos_ a grain or berry; N.L. masc. n. _Arachidicoccus_ coccos associated with peanuts).

Cells are Gram-stain-negative, aerobic, non-motile, coccoïd rods (0.40–0.55 × 0.52–0.64 μm). Nitrate is reduced under aerobic conditions. Major fatty acids are iso-C_{15:0} G and iso-C_{17:0} 3-OH. In addition, C_{16:0} iso-C_{15:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), which are produced in moderate amounts, are unique characteristic features of this genus. The major respiratory quinone is MK-7. The major polar lipids are phosphatidylethanolamine, three unidentified aminophospholipids (APL1–APL3), two unidentified phospholipids (PL1, PL2) and three unidentified lipids (UL1–UL3). The G+C content of the genomic DNA of known strains ranges from 43.1 to 44.3 mol%. The type and only species is _Arachidicoccus rhizosphaerae._

Description of Arachidicoccus rhizosphaerae sp. nov.

_Arachidicoccus rhizosphaerae_ (rhi.zo.sphae’rae. N.L. gen. n. _rhizosphaerae_ of the rhizosphere).

The description is the same as for the genus, with the following additions. Colonies are creamy white and 2–4 mm in diameter after 96 h at 28 °C on AMS and R2A agar. Good growth on Colby and Zatman medium and nutrient agar, but unable to grow on TSA and MacConkey agar. Growth occurs at 20–37 °C (optimal temperature 30 °C) and at pH 6.0–8.0 (optimal pH 6.5–7.0); does not grow in the presence of 3.0 % NaCl or higher. Tests for catalase, oxidase and pectinase are positive, but urease activity is absent. Nitrate reduction is positive, but hydrogen sulfide production is negative. Aesculin hydrolysis is positive (Fig. S5). Gelatin, starch, chitin, casein and urea are not hydrolysed. Lipolytic activity (glycerol tributyrate) and proteolytic activity are positive. Methanol, succinate, α-cyclodextrin, dextrin, glycogen, N-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannose, melibiose, turanose, pyruvic acid methyl ester, D-glucic acid, α-ketobutyric acid, DL-lactic acid, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-ornithine, L-proline, L-threonine, uridine, α-D-glucose 1-phosphate and D-glucose 6-phosphate are utilized as sole carbon and energy sources, but Tweens 40 and 80, N-acetyl-D-galactosamine, adonitol, D-arabitol, L-erythritol, L-fucose, myo-inositol, D-mannitol, C_{2}-methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, xyitol, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinic anhydride, L-alaninamide, D- and L-alanine, L-asparagine, glycyl L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L- pyroglutamic acid, D- and L-serine, DL-carnitine, γ-amino butyric acid, urocanic acid, inosine, thymidine, phenylethylamine, putescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL-α-glucol phosphate are not.

The type strain, Vu-144T (=KCTC 22378T =NCIMB 14473T), was isolated from rhizosphere soil of field-grown cowpea [Vigna unguiculata (L.) Walp.] and peanut (_Arachis hypogaea_ L.) at TNAU, Coimbatore, Tamil Nadu, India. The DNA G+C content of the type strain is 43.1 mol%.

Acknowledgements

This work was supported by a grant from the KRIBB Research Initiative Program, Daejeon, Republic of Korea. This work was partially supported by the Indian Council of Agricultural Research, New Delhi, India. We wish to thank Samantha Law and Peter Green, National Collection of Industrial, Food and Marine Bacteria (NCIMB), Buckburn, Aberdeen, Scotland, for their valuable advice. We wish to thank Professor Jean P. Euzéby for his valuable advice on nomenclature.

References


sp. nov.


