Devosia elaeis sp. nov., isolated from oil palm rhizospheric soil

Muhammad Nuruddin Mohd Nor,¹ ² Vikineswary Sabaratnam¹ and Geok Yuan Annie Tan¹ ³ ⁴

Abstract
A bacterial isolate, designated strain S37ᵀ, was isolated from the rhizosphere of oil palm (Elaeis guineensis). Strain S37ᵀ was found to be Gram-stain-negative, aerobic, motile and rod shaped. Based on 16S rRNA gene sequence analysis, strain S37ᵀ was most closely related to Devosia albogilva IPL15ᵀ (97.3 %), Devosia chinhatensis IPL18ᵀ (96.8 %) and Devosia subaequoris HST3-14ᵀ (96.5 %). The G+C content of the genomic DNA was 63.0 mol%, and dominant cellular fatty acids were summed feature 8 (C₁₈:1ω7c and/or C₁₈:1ω6c), 11-methyl C₁₈:1ω7c and C₁₈:0. The predominant isoprenoid quinone was ubiquinone-10 (Q-10), and the major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, glycolipid and phospholipids. Based on the polyphasic taxonomic data, it is clear that strain S37ᵀ represents a novel species of the genus Devosia within the family Hyphomicrobiaceae, for which we propose the name Devosia elaeis sp. nov., with strain S37ᵀ (=TBRC 5145ᵀ=LMG 29420ᵀ) as the type strain.

The genus Devosia belongs to the class Alphaproteobacteria and family Hyphomicrobiaceae and was created by the reclassification of ‘Pseudomonas riboflava’ [1] as Devosia riboflava [2]. The DNA G+C content of members of the genus Devosia ranges from 59.5 to 66.2 mol% [3]. At the time of writing, the genus Devosia comprises 17 species. Members of the genus Devosia can be found in soil [2-6], glacier [7], dump site [8, 9], nitrifying inoculum [10], marine sediment [11, 12] and even on the surface of a medical leech [13].

In an effort to isolate and characterize plant-growth-promoting bacteria, strain S37ᵀ was isolated from a rhizospheric soil sample collected from an oil palm plantation (03° 00’ 12.1” N 101° 39’ 33.1” E) in Temerloh, Pahang, Malaysia, in January 2015. In brief, 1 g of firm, root-adhering soil [14] was collected aseptically, added to 9 ml quarter-strength Ringer’s solution and shaken at 120 r.p.m. for 30 min at 28 °C. Subsequently, the suspension was serially diluted (10-fold dilutions), inoculated (100 µl) on Luria–Bertani (LB) agar (Sigma) and incubated at 28 °C for 5 days. Bacterial colonies with distinctive morphology were picked, purified and subcultured on LB agar. The colony of strain S37ᵀ was yellowish in colour and slightly convex on LB agar after 5 days of incubation. Strain S37ᵀ was preserved in glycerol (25 %, v/v) at −80 °C and routinely subcultured on LB agar at 28 °C. Devosia albogilva CCM 7427ᵀ, Devosia chinhatensis CCM 7426ᵀ and Devosia subaequoris JCM 14206ᵀ were selected as reference type strains for the phenotypic analysis. All strains were cultivated under the same conditions except for strain D. subaequoris JCM 14206ᵀ, which was grown on LB agar supplemented with NaCl (1 %, w/v).

Crude DNA was extracted and purified using NucleoSpin Tissue kit (Macherey-Nagel) according to manufacturer’s protocol. The 16S rRNA gene was amplified by PCR with universal primers 27F and 1492R [15]. The 16S rRNA gene fragments were sequenced using ABI 3730XL (Applied Biosystems) automated sequencer at the First BASE Laboratories. The 16S rRNA gene sequences of related taxa were obtained from the GenBank database and the EzTaxon-e server (http://www.ezbiocloud.net/.net/) [16]. CLUSTAL W software [17] was used to align the 16S rRNA gene sequence of the novel strain with those of closely related members of the genus Devosia. Genetic distances were calculated using Tamura 3-parameter model [18]. Phylogenetic trees were reconstructed by using the neighbour-joining [19], maximum-likelihood and maximum-parsimony algorithms [20] available within the MEGA 6 software package [21]. The topological structure of each tree was evaluated by bootstrap analysis with 1000 replications [22]. The G+C content was determined using reverse-phase HPLC according to Mesbah et al. [23].

A nearly-complete 16S rRNA gene sequence with a length of 1383 bp was obtained for strain S37ᵀ. Comparison of 16S

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S37ᵀ is KT345712.

One supplementary table and four supplementary figures are available with the online Supplementary Material.
rRNA gene sequences revealed that strain S37\textsuperscript{T} belonged to the genus *Devosia* and shared closest similarities with *D. albogilva* ILP15\textsuperscript{T} (97.3 % similarity), *D. chinhatensis* ILP18\textsuperscript{T} (96.8 %) and *D. subaequoris* HST3-14\textsuperscript{T} (96.5 %). Kim et al. [24] have shown that a 16S rRNA gene sequence similarity of 98.65 % can be used as the threshold for differentiating two species, thus DNA–DNA hybridization studies of strain S37\textsuperscript{T} with its close relatives were not performed. Strain S37\textsuperscript{T} formed a reliable and monophyletic cluster with *D. albogilva* ILP15\textsuperscript{T} in the neighbour-joining tree (Fig. 1). Similar tree topologies were obtained when phylogenetic trees were reconstructed using maximum-likelihood (Fig. S1, available in the online Supplementary Material) and maximum-parsimony algorithms using MEGA version 6.0 software [21]. The DNA G+C content of strain S37\textsuperscript{T} was 63.0 mol%, which was within the range described for the genus *Devosia* [3].

Cell morphology was observed with a transmission electron microscope (LIBRA120, Carl Zeiss AG) after negative staining with 0.2 % (w/v) uranyl acetate using cells grown for 1 day at 28 °C on tryptic soy agar (BD). Gram staining was performed by using a Gram-staining kit (bioMérieux) according to the manufacturer’s instructions. Growth on tryptic soy agar, nutrient agar (BD) and LB agar was also assessed. Growth at various temperatures (5, 10, 15, 20, 25, 28, 30, 35, 40 and 45 °C) was observed. Growth at varying pH (pH 4.0 – 10.0, at intervals of 0.5 pH units) was monitored in tryptic soy agar after incubation at 28 °C. Salt tolerance was determined in tryptic soy agar supplemented with 0 – 7 % (w/v) NaCl (at intervals of 1.0 %). After cells were grown for 1 day at 28 °C on tryptic soy agar, catalase activity was determined with 3 % (v/v) H\textsubscript{2}O\textsubscript{2}, and oxidase activity was determined by using 1 % (w/v) \(N,N',N',N''\)-tetramethyl-1,4-phenylenediamine reagent. Test for hydrolysis of DNA was tested using DNase media (Difco); DNase activity was revealed by flooding the plates with 1 M HCl. Assimilation of different carbohydrates was detected in basal media as described by Gordon et al. [25]. Production of indole was

![Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence analysis showing phylogenetic relationships of strain S37\textsuperscript{T} and members of the genus *Devosia*. Only bootstrap values >50 %, based on 1000 replications, are shown. *Rhizobium leguminosarum* USDA 2370\textsuperscript{T} (U29368) was used as the outgroup. Solid circles indicate that the same branch was recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

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done as described by Miller and Wright [26]. Hydrolysis of Tween 80, casein, gelatin, starch and aesculin was carried out as described by Smibert and Krieg [27]. Citrate utilization was tested on Simmons’ citrate agar (HiMedia). All tests described above were evaluated after incubation for 1 day at 28°C, unless otherwise indicated. Susceptibility to antibiotics was tested on tryptic soy agar plates, using antibiotic discs containing the following amounts (in micrograms unless otherwise indicated): streptomycin (10), penicillin G (10 units), cefixime (5), compound sulfonamides (300), ceftriaxone (30), sulfamethoxazole/trimethoprim (25), erythromycin (15), novobiocin (5), bacitracin (10 units), ampicillin (10), chloramphenicol (30), vancomycin (30), ciprofloxacin (5), tetracycline (30), polymyxin B (300), kanamycin (30), amikacin (30), chloramphenicol (30) and rifampicin (5). Additional physiological characteristics and enzyme activities were tested by using API ZYM and API 20 NE kits at 28°C as recommended by the manufacturer (bioMérieux). The API ZYM was read after 4.5 h, and the API 20 NE strips were read after 24 and 48 h. Motility was tested on sulfide indole motility medium (SIM; Difco).

Strain S37T is Gram-stain-negative, aerobic, motile and rod shaped with a single polar flagellum (Fig. S3). The isolate grew on tryptic soy agar, nutrient agar and LB agar. Growth was observed on tryptic soy agar at 10–45°C, and the

Table 1. Differential biochemical and physiological characteristics of strain S37T and type strains of closely related species of the genus Devosia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Short rods</td>
</tr>
<tr>
<td>Colony colour*</td>
<td>Deep yellowish brown</td>
<td>Yellowish</td>
<td>Cream</td>
<td>Light yellow</td>
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<tr>
<td>Urease activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Utilization of sucrose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Utilization of inositol</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Growth at/in</td>
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<td>5°C</td>
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<td>10°C</td>
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<td>45°C</td>
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<td>pH 5.0</td>
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<td>pH 11.0</td>
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<td>6% NaCl</td>
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<td>Enzyme activities (API ZYM):</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
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<td>+</td>
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<td>Leucine arylamidase</td>
<td>w</td>
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<td>Valine arylamidase</td>
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<td>Cystine arylamidase</td>
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<td>Trypsin</td>
<td>w</td>
<td>+</td>
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<td>w</td>
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<tr>
<td>Acid phosphatase</td>
<td>w</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>β-Galactosidase</td>
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<td>α-Mannosidase</td>
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<td>API 20NE tests:</td>
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<tr>
<td>Tryptophan deaminase</td>
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<td>D-Glucose utilization</td>
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<td>L-Arabinose utilization</td>
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<td>D-Mannose utilization</td>
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<tr>
<td>D-Mannitol utilization</td>
<td>+</td>
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<tr>
<td>N-Acetylglucosamine utilization</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Maltose utilization</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>w</td>
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*When grown on TSA in this study.
optimum growth temperature was at 28–37 °C and pH 6.0–11.0, optimally at pH 7.0–9.0. Strain S37T is able to tolerate up to 6.0 % NaCl. Positive for oxidative and catalase. Strain S37T did not hydrolyse DNA, starch, casein, gelatine and urea. Strain S37T is susceptible to the following (in micrograms unless otherwise indicated): streptomycin (10), penicillin G (10 units), ceftriaxone (30), sulfamethoxazole/trimethoprim (25), erythromycin (15), novobiocin (5), bacitracin (10 units), ampicillin (10), chloramphenicol (30), vancomycin (30), ciprofloxacin (5), tetracycline (30), polymyxin B (300), kanamycin (30), amikacin (30), chloramphenicol (30) and rifampicin (5). Physiological characteristics of strain S37T are summarized in the species description, and the differences of biochemical and physiological characteristics between strain S37T and three reference strains are shown in Table 1.

For the analysis of fatty acids, strain S37T and three reference strains, *D. albogilva* CCMM 7427T, *D. chinhatensis* CCMM 7426T and *D. subaequoris* JCM 14206T, were cultured on tryptic soy agar under the same condition, except for *D. subaequoris* JCM 14206T that was cultured on tryptic soy agar supplemented with 1 % (w/v) NaCl. The cellular fatty acids were analysed by GC (Agilent 7890A) using the Microbial Identification software package with the Sherlock system Version 6.1. Analyses of respiratory quinones and polar lipids were carried out by the Identification Service, Leibniz-Institut DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

The major fatty acids (>10 %) of strain S37T were summed feature 8 (C18:1ω7c and/or C18:1ω6c) (52.9 %), 11-methyl C18:1ω7c (15.6 %) and C16:0 (13.9 %) (Table S1). The major isoprenoid quinone of strain S37T was ubiquinone-10 (Q-10), which is consistent with the genus *Devosia*. The polar lipid profile of strain S37T was composed of phosphatidylglycerol, diphosphatidylglycerol, glycolipid and phospholipids (Fig. S4).

Phenotypic examination revealed that strain S37T shared most of the common characteristics of members of the genus *Devosia*. In addition, strain S37T cannot produce alkaline phosphatase and assimilate N-acetylglucosamine; the colony colour of strain S37T is deep yellowish brown on tryptic soy agar, and 16S rRNA gene sequence analysis confirmed the placement of strain S37T within the genus *Devosia*. This phylogenetic inference is supported by the unique combination of chemotaxonomic and biochemical characteristics of the novel strain. Hence, it can be concluded that strain S37T represents a novel species of the genus *Devosia*, for which the name *Devosia elaeis* sp. nov. is proposed.

**DESCRIPTION OF DEVOSIA ELAEIS SP. NOV.**

*Devosia elaeis* (e.lae/ is, N.L. gen. n. elaeis of the oil palm genus *Elaeis*).

Cells are Gram-stain-negative, catalase and oxidase positive, aerobic, motile and rod shaped (0.889 × 0.348μm) with a single polar flagellum. Colonies are circular, deep yellowish brown and 0.5–2.0 mm in diameter after incubation for 5 days on tryptic soy agar. Cells grow on tryptic soy agar, LB agar and nutrient agar. Growth occurs at 10–45 °C (optimum, 37 °C), at pH 6.0–11.0 (optimum, pH 9.0) and in the presence of 0–6 % (w/v) NaCl. Starch, casein, Tween 80 and DNA are not hydrolysed. Esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, N-glucosidase and N-acetyl-N-glucosaminidase activities are present. Leucine arylamidase, trypsin, acid phosphatase, β-galactosidase, α-glucosidase and α-mannosidase activities are weakly present. Alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, β-chymotrypsin, α-galactosidase, β-glucuronidase and α-fucosidase are absent. Nitrate is not reduced to nitrite, but asacul hydrolis is positive. Glucose fermentation, arginine dihydrolase, urease and protease (gelatin hydrolysis) activities are absent. L-Arabinose, D-glucose, D-mannitol, D-mannose, maltose, N-acetylglucosamine, inositol and sucrose were utilized as carbon sources but not potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylactic acid. Ubiquinone-10 (Q-10) is the sole isoprenoid quinone. The major cellular fatty acids are summed feature 8 (C18:1ω7c and/or C18:1ω6c), 11-methyl C18:1ω7c and C16:0. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, glycolipid and phospholipids.

The type strain is S37T (=TBRC 5145T=LMG 29420T) and was isolated from a rhizospheric soil of an oil palm (*Elaeis guineensis*) from a plantation in Temerloh, Pahang, Malaysia. The DNA G+C content of the type strain is 63.0 mol%.

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**Conflicts of interest**

The author(s) declare that there are no conflicts of interest.

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