Streptacidiphilus oryzae sp. nov., an actinomycete isolated from rice-field soil in Thailand

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The taxonomic position of ten acidophilic actinomycetes isolated from an acidic rice-field soil was established using a polyphasic approach. 16S rRNA gene sequences determined for the isolates were aligned with corresponding sequences of representatives of the genera Kitasatospora, Streptacidiphilus and Streptomyces and phylogenetic trees were inferred using four tree-making algorithms. The isolates had identical sequences and formed a distinct branch at the periphery of the Streptacidiphilus 16S rRNA gene tree. The chemotaxonomic and morphological properties of representative isolates were consistent with their assignment to the genus Streptacidiphilus. The isolates shared nearly identical phenotypic profiles that readily distinguished them from representatives of the established species of Streptacidiphilus. It is evident from the genotypic and phenotypic data that the isolates form a homogeneous group that corresponds to a novel species in the genus Streptacidiphilus. The name proposed for this new taxon is Streptacidiphilus oryzae sp. nov.; the type strain is strain TH49T (=CGMCC 4.2012T = JCM 13271T).

Acidophilic actinomycetes with streptomycete-like properties are common in terrestrial habitats with low pH values, notably in acid forest soils (Williams et al., 1971; Goodfellow & Dawson, 1978; Seong et al., 1993). Acidophilic strains grow at pH values between about 3·5 and 6·5, with an optimal pH around 4·5; their neutrotolerant counterparts grow at pH values between 4·5 and 7·5, with optimal growth between pH 5·0 and 5·5 (Williams et al., 1971; Kim et al., 2004; Xu et al., 2006). It is important to clarify the taxonomic position of acidophilic sporactinomycetes, as they are involved in the turnover of fungal biomass in acid litters and soils (Williams & Robinson, 1981) and are a source of antifungal antibiotics (Williams & Khan, 1974) and acid-stable enzymes (Williams & Robinson, 1981).

Kim et al. (2003) proposed the genus Streptacidiphilus for a group of strictly acidophilic strains. They recognized three species, Streptacidiphilus albus, the type species, Streptacidiphilus carbonis and Streptacidiphilus neutrinimicus. A further species, Streptacidiphilus jiangxensis Huang et al., 2004, has been added to the genus. Representatives of these taxa form a distinct 16S rRNA gene clade within the evolutionary radiation encompassed by the family Streptomycetaceae Waksman and Henrici 1943 emend. Kim et al., 2003, a taxon that also contains the genera Kitasatospora and Streptomyces. Representatives of these genera have many properties in common but can be distinguished from one another using a range of genotypic and phenotypic properties (Groth et al., 2003, 2004; Kim et al., 2003). In a continuation of studies on the biodiversity of acidophilic sporactinomycetes, a group of strains were isolated which grew well at pH 3·5. These organisms were the subject of a polyphasic study that showed that they formed a novel species within the genus Streptacidiphilus.

Strains TH31, TH35, TH37, TH49T, TH51, TH54, TH63, TH69, TH74 and TH78 were isolated on starch-casein-nitrate agar (Küster & Williams, 1964) adjusted to pH 4·5 using a citric acid/disodium hydrogen phosphate buffer (McIlvaine, 1921) and supplemented with the antifungal antibiotics cycloheximide and nystatin (each at 50 μg ml⁻¹). The isolation plates had been inoculated with a soil suspension prepared using a modification of the dispersion and differential centrifugation procedure (Hopkins et al., 1991), as described by Sembiring et al. (2000), and incubated at 28 °C for 14 days. The soil sample had been collected from a rice field in Nontaburi Province, Thailand. The isolates were maintained on modified Bennett’s agar.
plates (Jones, 1949), adjusted to pH 5.0, for short-term storage, and as suspensions of mycelial fragments and spores in 20 % (v/v) glycerol at −20 °C for long-term maintenance.

The phylogenetic relationships of the isolates were determined by 16S rRNA gene sequence analysis using biomass grown in shake flasks of modified Bennett’s broth (Jones, 1949), adjusted to pH 5.0, and incubated for 5 days at 28 °C. The biomass preparations were washed twice in Tris-EDTA buffer (0.03 M Tris, 0.1 M EDTA; pH 8.0) and stored at −20 °C. Genomic DNA preparations were prepared and used as templates for PCR amplification and sequencing according to the procedure of Kim et al. (1998). The resultant almost complete 16S rRNA gene sequences were aligned manually, using the pairwise alignment option and 16S rRNA secondary structural information held in the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/), against corresponding sequences of representatives of the genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* retrieved from the GenBank/EMBL/DDBJ databases.

Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated after Jukes & Cantor (1969). The unrooted tree topologies were evaluated by using a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset based on 1000 resamplings using the TREECON program (Van de Peer & De Wachter, 1994).

The ten isolates had identical 16S rRNA gene sequences. Phylogenetic analyses showed that they formed a distinct lineage at the periphery of the evolutionary radiation encompassed by representatives of the genus *Streptacidiphilus* (Fig. 1). This association was supported by the results from the four tree-making algorithms and by a bootstrap value of 71 % in the neighbour-joining analysis. The isolates shared 16S rRNA gene similarities of between 95.6 and 96.7 % with representatives of *Streptacidiphilus* species with validly published names, values that corresponded to between 46 and 62 nucleotide differences at 1407 locations.

Chemo-taxonomic studies were carried out on four representative strains, namely isolates T31, TH49<sup>T</sup>, TH54 and TH74, to determine whether they had properties consistent with their assignment to the genus *Streptacidiphilus*. Biomass needed to determine the chemical properties of the strains was grown in shake flasks of modified Bennett’s broth, as described above except that cells for quantitative fatty acid analyses were prepared on tryptic soy agar plates as described by Zhang et al. (2003). Harvested biomass was

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**Fig. 1.** Neighbour-joining tree (Saitou & Nei, 1987), based on nearly complete 16S rRNA gene sequences, showing the position of *Streptacidiphilus oryzae* sp. nov. TH49<sup>T</sup> in the phylogenetic tree of the *Streptomycetaceae*. Asterisks indicate branches that were recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. Bar, 0-01 nucleotide substitutions per nucleotide position.
washed twice with distilled water and freeze-dried. Standard procedures were used for the extraction and analysis of fatty acids (MIDI System; http://www.midi-inc.com), diagnostic isomers of diaminopimelic acids (Lechevalier & Lechevalier, 1980; Hasegawa et al., 1983), isoprenoid quinones (Collins, 1985; Wu et al., 1989), polar lipids (Minnikin et al., 1984) and sugars (Lechevalier & Lechevalier, 1980).

The diamino acid of the peptidoglycan was lL-diaminopimelic acid, although traces of the meso-isomer were detected. The representative strains contained hexahydrogenated and octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologues, contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids (phospholipid type II sensu Lechevalier et al., 1977) and were shown to be rich in saturated straight-chain and iso- and anteiso-branched fatty acids (fatty acid type 2c sensu Kroppenstedt, 1985). All of these attributes are typical of members of the genus *Streptacidiphilus* (Kim et al., 2003; Huang et al., 2004). However, whole-organism hydrolysates of the strains contained galactose, glucose, mannose and ribose, not the combination of galactose and rhamnose reported for representatives of the *Streptacidiphilus* species with validly published names (Kim et al., 2003; Huang et al., 2004).

The ten strains were grown on modified Bennett’s agar (Jones, 1949), inorganic salts-starch agar (ISP 4 medium; Shirling & Gottlieb, 1966) and oatmeal agar (ISP 3 medium; Küster, 1959) plates, adjusted to pH 5.0, at 28 °C for 14 days. The incubated plates were examined by eye to determine aerial spore-mass colour, substrate mycelial pigmentation and the colour of any diffusible pigments; colours were recorded using the National Bureau of Standards Color Name Charts (Kelly, 1958; National Bureau of Standards, 1964). The undisturbed arrangement of hyphae and spore-chain morphology were observed on oatmeal agar plates using the cover-slip technique of Kawato & Shinobu (1959). Spore-chain arrangement and spore-surface ornamentation were examined on dehydrated, gold-coated specimens of strains TH35, TH49T, TH54 and TH63 by using a FEI Table 1. Phenotypic properties that separate the new isolates from representatives of established *Streptacidiphilus* species


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td><strong>Growth on acidified oatmeal agar</strong></td>
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<td></td>
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<tr>
<td>Aerial spore mass colour</td>
<td>Greyish white</td>
<td>Brown</td>
<td>White</td>
<td>White to greyish white</td>
<td>White</td>
</tr>
<tr>
<td>Substrate mycelium colour</td>
<td>Golden brown</td>
<td>Cream</td>
<td>None</td>
<td>Cream</td>
<td>None</td>
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<td>Colour of diffusible pigment</td>
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<td><strong>Degradation of:</strong></td>
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<tr>
<td>Tweens 40 and 60</td>
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<td>+</td>
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<tr>
<td>Xanthine</td>
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<td>+</td>
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<td><strong>Growth on sole carbon sources at 1 % (w/v)</strong></td>
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<tr>
<td>D-Gluconic acid</td>
<td>+</td>
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<td>D-Glucosamine hydrochloride</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Inulin</td>
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<td>+</td>
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<tr>
<td>D-Melibiose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>D-Sorbitol</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Growth on sole carbon sources at 0·1 % (w/v)</strong></td>
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<tr>
<td>L-Arginine</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>L-Aspartic acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Sodium oxalate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td><strong>Growth on sole carbon and nitrogen source at 0·1 % (w/v)</strong></td>
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<tr>
<td>L-Isoleucine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td><strong>Growth at:</strong></td>
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<tr>
<td>30 °C</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>35 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>pH 6·0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>
Quanta scanning electron microscope. The strains tested formed extensively branched substrate mycelia carrying abundant aerial hyphae that differentiated into long straight to flexuous chains of smooth-surfaced spores, these properties being consistent with their assignment to the genus *Streptacidiphilus*.

The spore suspensions used to inoculate a broad range of phenotypic tests were prepared from 14-day-old acidified oatmeal agar plates by following the procedure described by Hopwood et al. (1985). The strains were examined for their ability to grow on oatmeal agar adjusted to pH 3.0, 3.5, 4.5, 5.5, 6.5 and 7.5 using the citric acid/disodium hydrogen phosphate buffer system (McIlvaine, 1921); the tests were carried out in Replidishes (Sterilin) 14 days after the inoculation of compartments with the appropriate spore suspension (5 μl). The remaining phenotypic tests were carried out using established procedures (Williams et al., 1983; Seong et al., 1993), albeit with acidified media. All of the isolates grew well at pH values from 3.5 to 6.5; only strains TH31, TH74 and TH78 failed to grow at pH 3.0. The isolates also grew at 28 and 37 °C, but not at 10 or 45 °C. The isolates shared many properties, as shown in Table 1 and in the species description. It is evident from Table 1 that the isolates can be separated from representatives of *Streptacidiphilus* with validly published names by means of a combination of phenotypic tests.

It is apparent from the genotypic and phenotypic data that the isolates belong to a distinct homogeneous taxon within the genus *Streptacidiphilus*. It is proposed that these organisms be assigned to a novel *Streptacidiphilus* species. The name proposed for this taxon is *Streptacidiphilus oryzae* sp. nov.

**Description of *Streptacidiphilus oryzae* sp. nov.**

*Streptacidiphilus oryzae* (o.ry.za’e. L. gen. n. oryzae of rice, denoting the isolation of the strains from a rice field).

Aerobic, Gram-positive, non-acid–non-alcohol-fast actinomy-cetes that form a brown substrate mycelium and abundant greyish-white aerial hyphae on acidified modified Bennett’s, inorganic salts-starch, oatmeal and yeast extract-malt extract agar. Aerial hyphae differentiate into long flexuous chains of spores (0.7 × 1.0 μm) with smooth surfaces. Golden brown diffusible pigments are formed on acidified modified Bennett’s, oatmeal and yeast extract-malt extract agar, but not on inorganic salts-starch-agar. Growth occurs at pH 3.0–6.5 and at 28–37 °C. Degradates adenine, casein, starch and uric acid, but not elastin, guanine, hypoxanthine, Tween 80, L-tyrosine, xanthine or xylan. Nitrate is reduced. Aesculin, allantoin and urea are not hydrolysed. L-Arabino, D-arabitol, D-cellulose, D-fructose, D-galactose, D-glucose, glycogen, D-lactose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-salicin (weak), sucrose (weak), D-trehalose, D-xylitol (each at 1 %, w/v) and L-alanine, DL-x-amino- butyric acid, 2-aminoethanol, L-histidine (weak), L-isoleucine (weak), L-phenylalanine, sodium fumarate (weak), sodium pyruvate (weak), L-threonine and L-valine (each at 0.1 %, w/v) are used as sole carbon sources for energy and growth, but adonitol, dextran, methyl D-glucoside, ethanol, glycine (each at 1 %, w/v), adipic acid, L-arginine, L-aspartic acid, L-cysteine, potassium nitrate and sodium oxalate (each at 0.1 %, w/v) are not. L-Alanine, 2-aminoethanol, L-aspartic acid, L-isoleucine (weak) and L-phenylalanine (each at 0.1 %, w/v) are used as sole carbon and nitrogen sources. Growth occurs in the presence (μg ml⁻¹) of amoxicillin (16), fusidic acid (16), gentamicin sulphate (16, weak), lincomycin hydrochloride (16) and penicillin G (8, weak) but not in the presence of amikacin (32), amoxicillin (32), ampicillin (16, 32), cephalaxin (16, 32), cephaloridine hydrochloride (32, 64), clindamycin hydrochloride (4), doxycycline hydrochloride (16), gentamicin sulphate (16), neomycin sulphate (16, 32), novobiocin (8), penicillin G (16), streptomycin sulphate (8, 16), tetracycline hydro- chloride (16, 32) or lead acetate (100). Weak growth occurs in the presence of 5 % (w/v) NaCl.

The type strain, TH49T (= CGMCC 4.2012T = JCM 13271T), was isolated from a rice-field soil sample collected in Nontaburi Province, Thailand.

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**References**


Streptacidiphilus oryzae sp. nov.


