Comamonas composti sp. nov., isolated from food waste compost

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A bacterial strain designated YY287\textsuperscript{T}, isolated from food waste compost, was investigated by polyphasic taxonomic approach. The cells were rod-shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analyses using the 16S rRNA gene sequence showed that the strain formed a monophyletic branch towards the periphery of the evolutionary radiation occupied by the genus Comamonas; its closest neighbours were the type strains Comamonas testosteroni DSM 50244\textsuperscript{T} (96.5 %), Comamonas terrigena DSM 7099\textsuperscript{T} (95.4 %), Comamonas odontotermitis Dant 3-8\textsuperscript{T} (95.2 %) and Comamonas koreensis KCTC 12005\textsuperscript{T} (94.6 %). Strain YY287\textsuperscript{T} was clearly distinguished from all of these strains using phylogenetic analysis, DNA–DNA hybridization, fatty acid composition data and a range of physiological and biochemical characteristics. The major fatty acids were 16 : 0 (33 %), 18 : 1\textsuperscript{\textit{v7c}} (13 %) and summed feature 3 (16 : 1\textsuperscript{\textit{v7c}} and/or 15 : 0 iso 2-OH; 41 %). The DNA G + C content of the genomic DNA was 62.8 mol%. It is evident from the genotypic and phenotypic data that strain YY287\textsuperscript{T} represents a novel species in the genus Comamonas, for which the name Comamonas composti sp. nov. is proposed. The type strain is YY287\textsuperscript{T} (=BCRC 17659\textsuperscript{T} =LMG 24008\textsuperscript{T}).

The genus Comamonas proposed by De Vos et al. (1985) belongs to the family Comamonadaceae of the class Betaproteobacteria. At the time of writing the genus Comamonas encompasses nine species with validly published names: Comamonas aquatica, C. badia, C. denitrificans, C. kerstersii, C. koreensis, C. nitrativorans, C. odontotermitis, C. terrigena and C. testosteroni. The aim of the present study was to determine the taxonomic position of a Comamonas-like isolate, YY287\textsuperscript{T}, that was isolated from food waste compost.

During the characterization of micro-organisms from food waste compost collected from Kinmen County, Taiwan, strain YY287\textsuperscript{T} was isolated and maintained on nutrient agar (BD Difco) after incubating at 32 °C for 3 days. Subcultivation was performed on nutrient agar at 25 °C.

A phylogenetic tree based on 16S rRNA gene sequences and an antibiogram of strain YY287\textsuperscript{T} and type strains of other Comamonas species are available with the online version of this paper.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Comamonas composti sp. nov. YY287\textsuperscript{T} is EF015884.

Type strains of C. badia, C. denitrificans, C. koreensis, C. nitrativorans and C. testosteroni were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and the type strains of C. aquatica, C. kerstersii and C. terrigena were obtained from the Laboratorium voor Microbiologie-Bacteriënverzameling (LMG) for comparison. The type strain of C. odontotermitis was from our laboratory (Chou et al., 2007).

Cultural and morphological characteristics were observed on nutrient agar. The morphology of bacterial cells was observed during the lag, exponential and stationary phases of growth under a phase-contrast microscope (Leica DM 2000). Flagellar staining was performed using Spot Test flagella stain (BD Difco). The Gram reaction was performed using a Gram stain set (BD Difco) and the Ryu non-staining KOH method (Powers, 1995). Accumulation of poly-\(\beta\)-hydroxybutyrate granules was observed by light microscopy after staining cells with Sudan black. Colony morphology was examined by using a stereoscopic microscope (Nikon SMZ 800). The optimum growth pH, temperature and tolerance to various NaCl
levels were examined on tryptic soy broth (BD Difco) and nutrient broth (BD Difco) (Chung et al., 1995; Chou et al., 2007). Anaerobic cultivation was performed on nutrient agar using the Oxoid AnaeroGen system.

Cells of strain YY287T were Gram-negative, motile, non-spore-forming rods, 0.5 μm in diameter and 1.0–2.0 μm in length. Strain YY287T formed visible semi-transparent and irregularly edged colonies with umbomate elevation. The colony diameter was approximately 3.0 mm on nutrient agar after 48 h incubation at 25 °C. Strain YY287T grew well at temperatures of 15–35 °C and 0–3 % NaCl with pH ranging from 6 to 9. Optimum growth was observed at 25–35 °C, 0–1 % NaCl and pH 6–8. Strain YY287T was able to grow at 25 °C after 24 h incubation under anaerobic conditions.

Extraction of genomic DNA and PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Chen et al. (2001). Sequence reaction fragments were separated by using a DNA sequencer (ABI PRISM 310 instrument; Applied Biosystems). DNA sequences were assembled by using the Fragment Assembly System program from the Wisconsin Package 9.1 (GCG, 1995). The resulting assembled sequence was compared with available 16S rRNA gene sequences from the Ribosomal Database Project II and the EMBL database (accession numbers in parentheses) constructed after multiple alignments of data showing the position of Comamonas compositi sp. nov. (YY287T) in the genus Comamonas. Distances were calculated and clustering with the neighbour-joining method was performed by using the software package MEGA version 3.1. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. Bar, 2 % sequence dissimilarity.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences from the EMBO database (accession numbers in parentheses) constructed after multiple alignments of data showing the position of Comamonas compositi sp. nov. (YY287T) in the genus Comamonas. Distances were calculated and clustering with the neighbour-joining method was performed by using the software package MEGA version 3.1. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. Bar, 2 % sequence dissimilarity.
For G+C content calculations, the DNA sample was prepared in duplicate and degraded enzymically into nucleosides as described by Mesbah et al. (1989). The obtained nucleoside mixture was then separated with a HPLC system. The G+C content of strain YY287T was 62.8±1.0 mol%, which was within the range of DNA G+C contents previously reported for Comamonas species (60.8–66.3 mol%; Table 2).

Biomass for fatty acid studies was grown in nutrient medium for 2 days at 28 °C, as described by Chang et al. (2002), Tago & Yokota (2004) and Chou et al. (2007). Fatty acid methyl esters were prepared, separated and identified according to the instructions of Microbial Identification System (MIDI; Microbial ID) (Sasser, 1990). Predominant fatty acids of strain YY287T were 16:0 (33.3 %), 18:1ω7c (12.9%) and summed feature 3 (16:1ω7c and/or 15:0 ω2OH; 40.8%). The fatty acid pattern of the strain YY287T is shown in Table 1 in comparison with other representative Comamonas species. The fatty acid profile of strain YY287T was in good agreement with data obtained for other members of the genus Comamonas (Chang et al., 2002; Wauters et al., 2003; Tago & Yokota, 2004; Chou et al., 2007) (Table 1).

Strain YY287T was examined for a broad range of phenotypic properties. Additional biochemical tests were performed to assess the carbon source utilization pattern with the Biolog GN2 (Biolog), API ZYM and API 20NE (bioMérieux) microtest systems, according to the methods outlined by the manufacturers. Sensitivity of strain YY287T to various antibiotics was examined by disc diffusion assay (Bauer et al., 1966). The antibiotic discs contained ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 U), streptomycin (10 µg), tetracycline (30 µg) and sulfamethoxazole (23.25 µg) plus trimethoprim (1.25 µg). The effect of antibiotics on cell growth was assessed after 3 days of incubation.

Detailed results of biochemical characterization and antibiotic sensitivity are provided in Table 2, Supplementary Table S1 and in the species description. It is clear from Table 2 that there are several phenotypic characters that readily distinguish strain YY287T from other phylogenetically related species. Based on the 16S rRNA gene sequence data, DNA–DNA hybridization and chemotaxonomic analyses, it is evident that strain YY287T should be classified as the type strain of a novel species in the genus Comamonas, for which the name Comamonas composti sp. nov. is proposed.

**Description of Comamonas composti sp. nov.**

Comamonas composti (com.pos‘ti.N.L. gen. n. composti of compost).

Aerobic, Gram-negative, non-spore-forming, motile and rod-shaped. After 24 h of growth on nutrient agar at 25 °C, the mean cell size is 0.5 µm in width and 1.0–2.0 µm in length. Optimum growth occurs at 25–35 °C, 0–1 % NaCl.

### Table 1. Comparison of the fatty acid compositions of strain YY287T and other Comamonas species

<table>
<thead>
<tr>
<th>Fatty acid</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>
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<tbody>
<tr>
<td>10:0 3-OH</td>
<td>5.6</td>
<td>3.8</td>
<td>3.5</td>
<td>4.8</td>
<td>5.3</td>
<td>4.2</td>
<td>5.0</td>
<td>5.0</td>
<td>4.5</td>
<td>2.4</td>
</tr>
<tr>
<td>12:0</td>
<td>3.2</td>
<td>2.7</td>
<td>2.3</td>
<td>2.4</td>
<td>2.8</td>
<td>3.0</td>
<td>2.9</td>
<td>3.0</td>
<td>2.6</td>
<td>2.9</td>
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<td>14:0</td>
<td>1.4</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>3.3</td>
<td>3.2</td>
<td>3.4</td>
<td>3.9</td>
<td>2.9</td>
<td>1.5</td>
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<td>9.4</td>
<td>1.0</td>
<td>3.7</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15:0 2-OH</td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>16:0</td>
<td>33.3</td>
<td>33.6</td>
<td>29.9</td>
<td>30.4</td>
<td>27.5</td>
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<td></td>
<td></td>
<td>2.5</td>
<td>2.2</td>
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<td>16:1 2-OH</td>
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<tr>
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<td>2.6</td>
<td>0.8</td>
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<td></td>
<td></td>
<td>0.6</td>
<td></td>
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<tr>
<td>17:0 cyclo</td>
<td>1.4</td>
<td>5.9</td>
<td>12.3</td>
<td>3.8</td>
<td>2.4</td>
<td></td>
<td></td>
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<td></td>
<td>0.7</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>18:1/18:1ω7c</td>
<td>12.9</td>
<td>16.2</td>
<td>9.6</td>
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<td>14.9</td>
<td>22.4</td>
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<tr>
<td>20:0 iso</td>
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<td></td>
<td>1.1</td>
<td></td>
<td></td>
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<tr>
<td>Summed feature 3*</td>
<td>40.8</td>
<td>33.9</td>
<td>26.1</td>
<td>33.1</td>
<td>38.4</td>
<td>48.6</td>
<td>42.9</td>
<td>42.4</td>
<td>28.2</td>
<td>41.9</td>
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</table>

*Summed feature 3 comprises 16:1ω7c nad/or 15:0 iso 2-OH. The summed fatty acids cannot be separated by the GLC of MIDI system.
and pH 6–8. In API 20NE tests, strain YY287<sup>T</sup> shows positive results for oxidase (weak), catalase, nitrate reduction and assimilation of gluconate, adipate and malate reactions, and negative results for indole production, hydrolysis of aesculin and gelatin, glucose fermentation, arginine dihydrolase, urease, β-galactosidase and assimilation of glucose, arabinose, mannose, maltose, N-acetylglucosamine, caprate, citrate and phenyl acetate. In API ZYM tests, positive results are recorded for alkaline phosphatase, C4 esterase, C8 lipase, C14 lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, while negative results

Table 2. Genotypic, phenotypic and nutritional characteristics that distinguish strain YY287<sup>T</sup> from other Comamonas species

<table>
<thead>
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<th>Characteristic</th>
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<tbody>
<tr>
<td>Source</td>
<td>Food waste</td>
<td>Termite gut</td>
<td>Wetland</td>
<td>Soil</td>
<td>Hay-infusion filtrate</td>
<td>Activated sludge</td>
<td>Sludge</td>
<td>Freshwater</td>
<td>Human</td>
<td>Activated sludge</td>
</tr>
<tr>
<td>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>Nitrite reduction to N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>C8 lipase</td>
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<td>+</td>
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<tr>
<td>C14 lipase</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
| Assimilation of (API 20NE):
  D-Gluconate | + | + | + | + | + | − | − | + | + | + |
  Caprate | − | − | − | − | − | − | − | − | − | − |
  Adipate | + | + | + | + | + | − | − | + | + | + |
  Malate | + | + | + | + | + | − | − | + | + | + |
  Citrate | − | + | + | + | + | − | − | − | − | − |
  Phenylacetate | − | + | − | − | − | − | − | − | − | − |
| Oxidation of (Biolog GN2):
  Acetate | + | + | + | + | + | − | − | + | + | + |
  cis-Aconitate | + | − | − | − | − | + | + | + | + | + |
  Propionate | + | + | + | + | + | − | − | + | + | + |
  γ-Hydroxybutyrate | + | + | + | − | + | − | − | + | + | + |
  Itaconate | + | − | − | − | − | − | − | − | − | − |
  d-Serine | − | + | + | + | + | − | − | − | − | − |
  l-Serine | − | − | − | − | − | − | − | − | − | − |
  L-Threonine | − | + | + | + | + | − | − | + | + | + |
  L-Phenylalanine | − | + | + | + | + | − | − | + | + | + |
  Hydroxy-L-proline | + | + | + | + | + | − | − | + | + | + |
  l-Ornithine | − | − | − | − | − | − | − | − | − | − |
  Glycyl-L-aspartate | − | + | − | − | − | − | − | − | − | − |
  Glycyl-L-glutamate | − | + | − | − | − | − | − | − | − | − |
  N-Acetyl-d-galactosamine | − | − | − | − | − | − | − | − | − | − |
  Tween 40 | + | + | + | + | + | − | − | + | + | + |
  Tween 80 | + | + | + | + | + | − | − | + | + | + |
| Susceptibility to:
  Ampicillin | S | R | R | R | S | S | S | S | S | R |
  Gentamicin | R | S | S | S | S | S | S | S | S | S |
  Penicillin G | S | R | R | R | S | S | S | S | S | S |
  Rifampicin | S | R | S | S | S | S | S | S | S | R |
  Streptomycin | R | S | S | R | S | S | S | S | S | R |
| G + C content (mol%) | 62.8 | 61.6 | 66 | 62.5–64.5 | 64 | 60.8 | ND | 64 | 61 | 66.3 |
are recorded for cystine arylamidase, trypsin, z-chymotrypsin, z-galactosidase, b-glucuronidase, z-glucosidase, z-mannosidase, N-acetyl-b-glucosaminidase and z-fucosidase reactions. The following compounds are oxidized in the Biolog GN2 microtitre test system: i-erythritol, acetic acid, bromosuccinic acid, urocanic acid, cis-aconitic acid, itaconic acid, succinic acid, hydroxy-L-proline, citric acid, z-ketobutyric acid, L-leucine, glycogen, formic acid, z-ketoglutaric acid, Tween 40, Tween 80, DL-lactic acid, L-prolylglutamic acid, propionic acid, L-glutamic acid, z-hydroxybutyric acid, pyruvic acid methyl ester, b-hydroxybutyric acid, sebacic acid, succinic acid monomethyl ester, y-hydroxybutyric acid, succinic acid, z-ketovaleric acid and L-asparagine. It cannot oxidize melibiose, p-hydroxyphenylacetic acid, L-histidine, z-cyclodextrin, D-fructose, methyl b-D-glucose, inosine, dextrin, L-fucose, D-psicose, glucuronamide, uridine, D-galactose, raffinose, L-alaninamide, L-ornithine, thymidine, gentiobiose, L-rhamnose, D-galactonic acid lactone, D-alanine, L-phenylalanine, phenethylamine, z-D-glucose, D-sorbitol, D-galacturonic acid, L-alanine, L-proline, putrescine, N-acetyl-D-galactosamine, myo-inositol, sucrose, d-gluconic acid, malonic acid, L-alanylglycine, 2-aminoethanol, N-acetyl-D-glucosamine, z-D-lactose, trehalose, D-glucosaminic acid, D-serine, 2,3-butanediol, adonitol, lactulose, turanose, D-glucuronic acid, quinic acid, L-aspartic acid, L-serine, glycerol, L-arabinose, maltose, xylitol, D-saccharic acid, L-threonine, DL-2-glycerol phosphate, D-arabitol, D-mannitol, glycol L-aspartic acid, DL-carnitine, z-D-glucose 1-phosphate, D-cellulose, D-mannose, glycol L-glutamic acid, D-glucose 6-phosphate and y-aminobutyric acid. Strain YY287T is resistant to streptomycin and gentamicin but sensitive to ampicillin, chloramphenicol, rifampicin, penicillin G, sulfa methoxazole plus trimethoprim, kanamycin, nalidixic acid, novobiocin and tetracycline. The major fatty acids are 16:0 (33.3 %), 18:1ω7c (12.9 %) and summed feature 3 (16:1ω7c and/or 15:0 iso 2-OH; 40.8 %). The DNA G+C content is 62.8 mol%. The type strain YY287T (=BCRC 17659T=LMG 24008T) was isolated from food waste compost, Kinmen County, Taiwan.

Acknowledgements

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References


