Catellibacterium nanjingense sp. nov., a propanil-degrading bacterium isolated from activated sludge, and emended description of the genus *Catellibacterium*

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A novel facultatively anaerobic, non-spore-forming, non-motile, catalase- and oxidase-positive, Gram-negative and rod-shaped bacterial strain, designated Y12^T, was isolated from activated sludge of a wastewater bio-treatment facility. The strain was able to degrade about 90 % of added propanil (100 mg l⁻¹) within 3 days of incubation. Growth occurred in the presence of 0–4.5 % (w/v) NaCl (optimum 0.5 %), at 10–40 °C (optimum 28 °C) and at pH 5.5–10.0 (optimum pH 7.0). Vesicular internal membrane structures and photoheterotrophic growth were not observed. The major respiratory quinone was ubiquinone-10 and the major cellular fatty acid was summed feature 8 (C_{18:1ω6c} and/or C_{18:1ω7c}). The genomic DNA G + C content of strain Y12^T was 63.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparison revealed that strain Y12^T was a member of the genus *Catellibacterium*, as it showed highest sequence similarities to *Catellibacterium caeni* DCA-1^T (99.1 %) and <96.0 % similarities with other species of the genus *Catellibacterium*. Strain Y12^T showed low DNA–DNA relatedness values with *C. caeni* DCA-1^T. Based on phenotypic, genotypic and phylogenetic properties, strain Y12^T represents a novel species of the genus *Catellibacterium*, for which the name *Catellibacterium nanjingense* sp. nov. is proposed. The type strain is Y12^T (=CCTCC AB 2010218^T =KCTC 23298^T). An emended description of the genus *Catellibacterium* is also presented.

The genus *Catellibacterium*, belonging to the ‘*Rhodobacter*’ clade of the family *Rhodobacteraceae* of the class *Alphaproteobacteria*, was proposed by Tanaka *et al.* (2004). At the time of writing, the genus includes four species: *Catellibacterium nectariphilum* (Tanaka *et al.*, 2004), *C. aquatile* (Liu *et al.*, 2010), *C. caeni* (Zheng *et al.*, 2011) and *C. changlense* (Anil Kumar *et al.*, 2007; Zheng *et al.*, 2011). In this study, a propanil-degrading bacterial strain, designated Y12^T, was isolated from activated sludge of a wastewater bio-treatment facility. Based on the results of a polyphasic taxonomic study, this strain is considered to represent a novel species of the genus *Catellibacterium*.

Propanil (3,4-dichloropropionanilide) is an acetanilide post-emergence herbicide, which is widely used against

numerous grasses and broad-leaved weeds in rice, potatoes and wheat. This herbicide is moderately toxic to humans due to its potential to irritate eyes and skin, and is highly toxic to a wide range of aquatic species. One of the major breakdown pathways of propanil in the soil, sediment and water is by microbial degradation (Weed Science Society of America, 1994). To isolate propanil-degrading bacteria, a conventional enrichment procedure was carried out in 10-fold-diluted LB broth supplemented with 100 mg propanil⁻¹ (Wang *et al.*, 2009). The 10-fold-diluted LB broth contained (per litre) 1.0 g tryptone, 0.5 g yeast extract and 1.0 g NaCl. The sludge sample used as the initial inoculant was collected from a wastewater bio-treatment facility in a pesticide manufacturing company in Nanjing city, Jiangsu province, PR China. After five successive steps of subcultivation, the enrichment culture was spread on 10-fold-diluted LB agar supplemented with 100 mg propanil⁻¹, and incubated at 30 °C for 4–5 days. Strain Y12^T was obtained after several streakings and transferred onto fresh plates. *C. caeni* DCA-1^T, *C. aquatile* A1-9^T, *C. nectariphilum* JCM 11959^T and *C. changlense* JCM 14338^T were

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y12^T is EU289803.

Three supplementary figures and a supplementary table are available with the online version of this paper.

employed as controls in phenotypic tests. Unless otherwise mentioned, these strains were grown aerobically on 10-fold-diluted LB agar or in 10-fold-diluted LB broth at 30 °C.

Cell morphology and dimensions were determined by transmission electron microscopy (H-7650; Hitachi). In preparation for electron microscopy, bacterial cells were suspended in 0.85 % NaCl. Subsequently, the cells were dried on a nickel-coated mesh, negatively stained with phosphotungstic acid and examined. The motility of cells was tested by the hanging drop method (Suzuki *et al.*, 2001). The internal membrane structures were viewed with a transmission electron microscope after the cells had been processed as described by Hanada *et al.* (2002). Gram staining and the KOH lysis test were carried out according to Gregersen (1978) and Buck (1982), respectively. Growth was tested at different temperatures (4, 10, 15, 25, 28, 30, 35, 37, 40 and 45 °C), pH (5.0–10.0, in increments of 0.5 pH units), and NaCl concentrations [final concentrations of 0–7.0 % (w/v) at increments of 0.5 %] in 10-fold-diluted LB broth. Anaerobic growth was assessed in serum bottles containing 10-fold-diluted LB broth and sodium thioglycolate (1 g l⁻¹) with the upper air layer replaced with nitrogen gas. The catalase test was carried out as described previously (Ohta & Hattori, 1983). Growth on TSA (Difco) was evaluated at 30 °C for 72 h. Hydrolysis of starch, Tweens 20 and 80, and tyrosine was determined as described by Cowan & Steel (1965). Other physiological and biochemical tests were assayed by using API ZYM and 20NE strips (bioMérieux) according to the manufacturer's instructions. Phototrophic growth was assessed in the minimal medium of Pfennig & Trüper (1974) in completely filled screw-cap bottles under incandescent illumination (2000 lx); sodium succinate, sodium malate, sodium pyruvate, sodium citrate and glucose (4.0 g l⁻¹) were supplemented as electron donors. Ability to degrade propanil was determined according to the methods described by Guo *et al.* (2009); the propanil was added at a final concentration of 40 mg l⁻¹.

Strain Y12^T was facultatively anaerobic and the cells were Gram-stain-negative, non-motile, non-sporulating rods covered with a thick slime layer (Fig. 1). Colonies were white to beige, convex and circular with entire margins. The strain grew in the presence of 0–4.5 % (w/v) NaCl (optimum 0.5 %), at 10–40 °C (optimum 28 °C) and at pH 5.5–10.0 (optimum pH 7.0). Strain Y12^T was able to degrade about 90 % of added propanil (100 mg l⁻¹) within 3 days of incubation. A vesicular internal membrane structure was not detected and photoheterotrophic growth was not observed. Detailed morphological, physiological and biochemical characteristics of strain Y12^T are summarized in the species description below and in Table 1. The HPLC data of propanil degradation by strain Y12^T is shown in Supplementary Fig. S1 (available in IJSEM Online).

Genomic DNA was extracted according to standard procedures (Sambrook & Russell, 2001). The G + C content

Fig. 1. Transmission electron micrograph of a negatively stained cell of strain Y12^T, characterized by short, non-spore-forming rods, no flagella and a thick slime layer. Bar, 0.5 µm.

of the genomic DNA was determined by thermal denaturation (Mandel & Marmur, 1968). The 16S rRNA gene was amplified by PCR and sequenced as described by Wang *et al.* (2011). Calculations of pairwise sequence similarity were carried out using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic analysis was performed using MEGA version 4.1 (Tamura *et al.*, 2007). Distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis of 1200 resamplings was used to evaluate tree topology (Felsenstein, 1985).

An almost complete 16S rRNA gene sequence (1334 nt) was obtained for strain Y12^T. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain Y12^T was a member of the genus *Catellibacterium* and was related most closely to *C. caeni* DCA-1^T (99.1 % sequence similarity); it showed less than 96.0 % 16S rRNA gene sequence similarity to *C. aquatile* A1-9^T (95.9 %), *C. changlense* JCM 14338^T (95.3 %), *C. nectarophilum* JCM 11959 (93.6 %). In the neighbour-joining phylogenetic tree, strain Y12^T formed a clade within the genus *Catellibacterium* (Fig. 2; Supplementary Fig. S2).

DNA–DNA hybridization is necessary to clarify the taxonomic relationship of strains when these strains share more than 97 % 16S rRNA gene sequence identity (Tindall *et al.*, 2010). To further clarify the taxonomic relationship of strain Y12^T with its closest phylogenetic neighbour, *C. caeni* DCA-1^T, DNA–DNA hybridization was performed in triplicate with photobiotin-labelled probes as described by Ezaki *et al.* (1989). Additionally, reciprocal analysis was carried out to avoid any inconsistency during DNA binding. DNA–DNA hybridization

Table 1. Phenotypic characteristics that differentiate strain Y12^T from type strains of the genus *Catellibacterium*

Taxa: 1, Y12^T; 2, *C. caeni* DCA-1^T; 3, *C. aquatile* A1-9^T; 4, *C. changlense* JCM 14338^T; 5, *C. nectarophilum* JCM 11959^T. +, Positive reaction; –, negative reaction. Data on the requirement for diffusible compounds from other bacterial cultures for vigorous growth from Tanaka *et al.* (2004); other data from this study.

Characteristic	1	2	3	4	5
Colony pigmentation	White to beige	Pale yellow	White	Yellowish brown	White to beige
Motility	–	–	+	–	–
Requirement for diffusible compounds from other bacterial cultures for vigorous growth	–	–	+	–	–
Production of slime layer	+	–	–	+	–
Facultatively anaerobic growth	+	–	–	–	–
Photoheterotrophic growth	–	–	–	+	–
Conditions for growth					
Temperature range (°C)	10–40	15–35	10–40	5–37	20–35
pH range	5.5–10.0	5.5–9.0	5.5–9.0	6.5–9.0	6.0–8.0
NaCl range (% w/v)	0–4.5	0–6.5	0–1.5	0–4.0	0–2.5
Hydrolysis of:					
Starch	–	–	–	–	+
Tween 20	–	–	+	–	–
Tween 80	–	–	+	–	+
Tyrosine	–	–	+	–	–
API ZYM results					
Valine arylamidase	+	–	–	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	–	–	–
β-Glucosidase	+	–	+	–	–
Lipase (C14)	+	–	–	–	–
API 20NE results					
Hydrolysis of aesculin	+	–	+	+	–
Hydrolysis of urea	–	–	–	+	–
Assimilation of D-glucose	+	–	–	+	–
Assimilation of D-mannose	+	–	–	+	–
Assimilation of N-acetylglucosamine	+	–	–	+	–
Assimilation of maltose	+	–	–	–	–
Assimilation of malate	+	–	–	+	–
Assimilation of mannitol	+	–	–	+	+

results indicated low levels of relatedness between strain Y12^T and *C. caeni* DCA-1^T (18.9 %; reciprocal, 14.3 %). These values are well below the threshold of 70 % that is

suggested for species delineation (Wayne *et al.*, 1987), indicating that strain Y12^T represents a separate genomic species.

Fig. 2. Phylogenetic tree reconstructed by the neighbour-joining method based on 16S rRNA gene sequences of strain Y12^T and type strains of the genus *Catellibacterium*. *Filomicrobium fusiforme* DSM 5304^T was used as an outgroup. Bootstrap values, generated from 1200 resamplings, at or above 70 % are indicated at branch points. Bar, 0.01 nt substitutions per nucleotide position.

The cell mass of strains used for chemotaxonomic analyses was produced on 10-fold-diluted LB agar incubated at 30 °C for 3 days. Isoprenoid quinones were extracted and purified according to the method of Collins *et al.* (1977) and analysed by HPLC as described by Kroppenstedt (1982). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System following the manufacturer's instructions. Fatty acid methyl esters were obtained from cells by saponification, methylation and extraction as described by Kämpfer & Kroppenstedt (1996) and were separated in a gas chromatograph (Agilent 6890N). Peaks were automatically integrated and fatty acid names and percentages were determined using the MIDI Sherlock MIS system (library: TSBA6; version, 6.0B) (Sasser, 1990). Polar lipid analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ, Braunschweig, Germany.

The chemotaxonomic data obtained for strain Y12^T were consistent with the assignment of the strain to the genus *Catellibacterium* (Tanaka *et al.*, 2004; Liu *et al.*, 2010; Zheng *et al.*, 2011). The DNA G + C content of strain Y12^T was 63.7 mol%. The strain contained ubiquinone-10 as the predominant respiratory quinone. The major cellular fatty acids of strain Y12^T were summed feature 8 (C_{18:1}ω6c and/or C_{18:1}ω7c; 70.9%), C_{16:0} (5.1%), C_{18:0} (6.6%), C_{10:0} 3-OH (4.1%) and 11-methyl C_{18:1}ω7c (8.8%). The minor components C_{14:0} (1.9%), C_{18:0} 3-OH (1.3%) and summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c; 2.7%) were also present. The fatty acid profile of strain Y12^T was similar to those of the type strains of species of the genus *Catellibacterium*, although the proportions of some major fatty acids were different (Supplementary Table S1).

The polar lipids of strain Y12^T consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phospholipid, an unidentified lipid and an unidentified aminolipid (Supplementary Fig. S3). The polar lipid profile of strain Y12^T was not identical to those of *C. caeni* DCA-1^T and *C. nectariphilum* JCM 11959^T. Phospholipid (PL1) was absent from the polar lipid profiles of *C. caeni* DCA-1^T and *C. nectariphilum* JCM 11959^T but present in strain Y12^T, glycolipid (GL) was absent in strain Y12^T and *C. caeni* DCA-1^T but present in *C. nectariphilum* JCM 11959^T.

On the basis of the results of the phylogenetic analysis, morphological and chemotaxonomic investigations, and DNA–DNA hybridization, strain Y12^T represents a novel species of the genus *Catellibacterium*, for which the name *Catellibacterium nanjingense* sp. nov. is proposed.

Emended description of the genus

***Catellibacterium* Tanaka *et al.* 2004 emend. Liu *et al.* 2010 emend. Zheng *et al.* 2011**

The formal descriptions given by Tanaka *et al.* (2004), Liu *et al.* (2010) and Zheng *et al.* (2011) remain correct except that some species are facultatively anaerobic.

Description of *Catellibacterium nanjingense* sp. nov.

Catellibacterium nanjingense (nan.jing.en'se. N.L. neut. adj. *nanjingense* of or pertaining to Nanjing, Jiangsu Province, PR China, the geographical origin of the type strain).

Cells are Gram-stain-negative, catalase- and oxidase-positive, facultatively anaerobic, non-spore-forming, non-motile and short rods, 0.5–0.6 µm in length and 1.0–1.2 µm in diameter. After 3 days of incubation on 10-fold-diluted LB agar, colonies are 1.0–2.0 mm in diameter, white to beige, convex and circular with entire edges. Growth occurs in 0–4.5% (w/v) NaCl (optimum 0.5%), at 10–40 °C (optimum 28 °C) and at pH 5.5–10.0 (optimum pH 7.0). Vesicular internal membrane structure was not detected and photoheterotrophic growth was not observed. Negative for hydrolysis of starch, Tween 20, Tween 80 and tyrosine. In the API 20NE test, positive for hydrolysis of aesculin, assimilation of D-glucose, D-mannose, maltose, mannitol, malate and N-acetylglucosamine, and negative for urease, arginine dihydrolase, gelatinase, fermentation of glucose, nitrate reduction, indole production, and assimilation of arabinose, gluconate, caprate, adipic acid, citrate and phenylacetic acid. In the API ZYM test, positive for alkaline phosphatase, esterase C4, esterase lipase C8, lipase C14, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and β-galactosidase; negative for cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, N-acetyl-β-glucosaminidase, α-fucosidase, β-glucuronidase and α-mannosidase. Major fatty acid is C_{18:1}ω7c/C_{18:1}ω6c. The major respiratory quinone is ubiquinone-10. The DNA G + C content is 63.7 mol%.

The type strain Y12^T (=CCTCC AB 2010218^T =KCTC 23298^T) was isolated from activated sludge in a wastewater bio-treatment facility in a pesticide manufacturing company in Nanjing city, Jiangsu province, PR China.

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