Thauera butanivorans sp. nov., a C_2–C_9 alkane-oxidizing bacterium previously referred to as '*Pseudomonas butanovora*'

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The placement of '*Pseudomonas butanovora*' in the genus *Thauera* was proposed previously, based on 16S rRNA gene sequence analysis, upon further studies of taxonomical characteristics. In this study, physiological characteristics and DNA–DNA reassociation data are presented and the transfer of '*P. butanovora*' to the genus *Thauera* is proposed. The original description of the strain (strain Bu-B1211) indicated that it was capable of denitrification but not anaerobic growth. '*P. butanovora*' is capable of anaerobic respiration and growth, utilizing nitrate as a terminal electron acceptor during the oxidation of organic acids and alcohols, but not aromatic hydrocarbons or open-chain terpenoids. The total fatty acid composition supported the assignment of strain Bu-B1211 to the *Betaproteobacteria* and resembled that of members of the genus *Thauera*. The combination of 16S rRNA gene phylogenetic evidence, physiological and taxonomical characteristics and DNA–DNA reassociation data supported the placement of '*Pseudomonas butanovora*' Bu-B1211 in the genus *Thauera* as representing a novel species, for which the name *Thauera butanivorans* sp. nov. is proposed. The type strain is Bu-B1211^T (=IAM 12574^T=ATCC 43655^T=DSM 2080^T).

The genus *Thauera* was described originally by Macy *et al.* (1993) and comprises eight species with validly published names: *Thauera selenatis* (Macy *et al.*, 1993), *Thauera aromatica* (Anders *et al.*, 1995), *Thauera chlorobenzoica* (Song *et al.*, 2001), *Thauera linaloolentis* (Foss & Harder, 1998), *Thauera mechernichensis* (Scholten *et al.*, 1999), *Thauera terpenica* (Foss & Harder, 1998), *Thauera aminoaromatica* (Mechichi *et al.*, 2002) and *Thauera phenylacetica* (Mechichi *et al.*, 2002). '*Pseudomonas butanovora*' strain Bu-B1211 (=IAM 12574=ATCC 43655=DSM 2080) was isolated originally from activated sludge sampled from an oil-refining plant in Japan by enrichment with n-butane as the sole source of carbon and energy (Takahashi *et al.*, 1980). The initial characterization of the isolate placed the bacterium in the genus *Pseudomonas*, based solely on morphology and phenotypic characteristics (Takahashi *et al.*, 1980). A comprehensive study of the phylogenetic affiliation of the pseudomonads using 16S rRNA gene analysis showed that, phylogenetically, '*P. butanovora*' was a member of the *Rhodocyclus* group, with the closest relatives belonging to the genus *Thauera* (Anzai *et al.*, 2000). The level of 16S rRNA gene sequence similarity between '*P. butanovora*' and recognized *Thauera* species ranged between 96.2 and 98.7 %, prompting the authors to propose the transfer of '*P. butanovora*' to the genus *Thauera* based upon additional taxonomic studies.

Growth of '*P. butanovora*' (ATCC 43655), *T. aromatica* S100 (DSM 14793) and *T. linaloolentis* 47Lol^T (DSM 12138^T) was carried out using the method and conditions described in Sluis *et al.* (2002), with the exception that, for aerobic growth, and when alkane was supplied as the sole source of carbon energy, the medium was amended with 100 μM Fe^{3+}-EDTA. Anaerobic conditions were established in 50 ml growth medium in serum vials (160 ml) by sparging with O_2-free nitrogen, and crimp sealing with butyl-rubber septa followed by sparging of the headspace. Organic acid stock solutions (1 M) were neutralized (pH 7.0) and made anaerobic as described above.

The fatty acid profile of '*P. butanovora*' (grown in LB medium) was determined in this study by Microbial ID (Newark, DE, USA). The profile was composed of summed feature 3 ($C_{16:1}\omega6c/C_{16:1}\omega7c$; 43.6 %), $C_{16:0}$ (27.6 %), $C_{18:1}$ (14.8 %), $C_{10:0}$ 3-OH (4.7 %) and $C_{12:0}$ (4.6 %). The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of '*Pseudomonas butanovora*' IAM 12574 is AB021377.

main components of the profile were in the range of those of recognized *Thauera* species (Song *et al.*, 2001) and agreed with the 16S rRNA gene phylogenetic placement in the class *Betaproteobacteria*. The DNA G+C content (67.3 mol%) determined by Takahashi *et al.* (1980) was also similar to those of members of the genus *Thauera* (Song *et al.*, 2001).

In the original paper describing the isolation of '*P. butanovora*', the bacterium was reported to be capable of NO_3^- reduction and denitrification, but not anaerobic growth (Takahashi *et al.*, 1980). Growth medium for assessing terminal electron acceptors for anaerobic respiration was supplemented with 10 mM NH_4Cl as a supplementary nitrogen source. '*P. butanovora*' was capable of utilizing KNO_3 (10 mM), $NaNO_2$ (10 mM) and N_2O as terminal electron acceptors for growth when butyrate was supplied as the electron donor. Utilization of N_2O as the terminal electron acceptor required complete reduction of the growth medium (5 mM ascorbate, which is not utilized as a source of carbon under anaerobic conditions). Selenate did not serve as an electron acceptor for anaerobic growth of '*P. butanovora*'.

The utilization of organic substrates as carbon sources for growth of '*P. butanovora*' was assessed under aerobic and anaerobic conditions. The results are summarized in Table 1. Takahashi *et al.* (1980) reported previously that aerobic growth occurred with n-alkanes (C_2–C_9), primary alcohols and carboxylic acids (C_2–C_4). The pathway for butane assimilation was determined to occur via the terminal alcohol (1-butanol), with subsequent oxidation to butyraldehyde and butyrate. Presumably, butyrate was metabolized further to acetyl-CoA via the β-oxidation pathway (Arp, 1999). One major characteristic of recognized *Thauera* species is the utilization of aromatic or terpenoid hydrocarbons under denitrifying conditions (Anders *et al.*, 1995; Foss & Harder, 1998; Mechichi *et al.*, 2002; Scholten *et al.*, 1999; Song *et al.*, 2001). '*P. butanovora*' was not capable of growth on aromatic or terpenoid hydrocarbons when NO_3^- was supplied as the terminal electron acceptor. Interestingly, aerobic growth with the branched hydrocarbons isobutane and isopentane was possible due to a soluble butane monooxygenase (Dubbels *et al.*, 2007; Sluis *et al.*, 2002). Cell-free extracts, prepared from aerobic, acetate-grown '*P. butanovora*' and assayed according to the protocol of Alber *et al.* (2006), showed isocitrate lyase activity indicating a functional glyoxylate cycle and the ability to synthesize precursors of amino acids and sugars from the carboxylic acid end products of alkane oxidation.

DNA–DNA reassociation was performed as described by Simbahan *et al.* (2004) and Urbance *et al.* (2001) with DNA from two species of *Thauera* (*T. aromatica* S100 and *T. linaloolentis* 47Lol^T) that exhibit high 16S rRNA gene sequence similarity to '*P. butanovora*' (97 and 98.7 %, respectively). The results showed DNA–DNA reassociation values of about 20 % between '*P. butanovora*' and either *Thauera* species examined. Based on the data above and the established classification that two strains within the same genus represent distinct species when the level of DNA–DNA reassociation is below 70 % (Stackebrandt & Liesack, 1993; Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987), we propose a novel species with the name *Thauera butanivorans* sp. nov.

Table 1. Characteristics that differentiate strain Bu-B1211^T and two species of the genus *Thauera*

Taxa: 1, strain Bu-B1211^T (*T. butanivorans* sp. nov.); 2, *T. aromatica*; 3, *T. linaloolentis* 47Lol^T. All taxa grew under aerobic conditions with butyrate. Strain Bu-B1211^T and *T. aromatica* grew under aerobic conditions with acetate, propionate and succinate/fumarate; no data are available for *T. linaloolentis* 47Lol^T. All taxa grew under denitrifying (NO_3^-) conditions with acetate, propionate, butyrate, succinate/fumarate and ethanol (1–5 mM; Takahashi *et al.*, 1980 and this study). ND, No data available; +, positive; −, negative; d, different reactions from strains of the same species.

Characteristic	1	2	3
Aerobic growth			
Ethanol	+	d*	ND
Benzoate	−	+*	ND
Butane	+†	−	−
Isobutane	+	−	−
Isopentane	+	−	−
Leucine	−	+*	ND
Valine	−‡	−*	ND
Denitrifying growth			
Benzoate	−	+*	−*
Phenol	−	d*	ND
Toluene	−	+*	−*
Geraniol	−	ND	+*
Linalool	−	ND	+*

*Data from Heider & Fuchs (2005).
†Data from Takahashi *et al.* (1980).
‡Takahashi *et al.* (1980) reported growth on valine. Growth was not observed under the conditions employed in this study.

Description of *Thauera butanivorans* sp. nov.

Thauera butanivorans (bu.tan.i.vo′rans. N.L. n. *butanum* butane; L. part. adj. *vorans* devouring; N.L. part. adj. *butanivorans* butane-devouring).

Morphology and general characteristics are as described for the genus. Cells are Gram-negative, rod-shaped (0.6–0.8 μm wide and 1.1–2.4 μm long), and motile by means of a polar flagellum. Fatty acid profile comprises summed feature 3 ($C_{16:1}\omega6c$/$C_{16:1}\omega7c$), $C_{16:0}$, $C_{18:1}$, $C_{10:0}$ 3-OH and $C_{12:0}$. Shows facultatively anaerobic chemo-organotrophic metabolism. O_2, NO_3^-, NO_2^- and N_2O (requires reduction of medium) are utilized as terminal electron acceptors. Capable of utilizing C_2–C_9 alkanes for carbon and energy, aerobically. Negative for anaerobic catabolism

of aromatic hydrocarbons (benzoate, toluene or phenol) or open-chain terpenoids (linalool or geraniol). The DNA G+C content of the type strain is 67.3 mol%.

The type strain, Bu-B1211^T (=IAM 12574^T=ATCC 43655^T=DSM 2080^T), was isolated from activated sludge sampled from an oil-refining plant in Japan. Originally isolated and identified as '*Pseudomonas butanovora*' by Takahashi *et al.* (1980).

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