NOTE

Anaerovorax odorimutans gen. nov., sp. nov., a putrescine-fermenting, strictly anaerobic bacteriumCarola Matthies¹, Stefan Evers², Wolfgang Ludwig² and Bernhard Schink³Author for correspondence: Bernhard Schink. Tel.: +49 7531 882140 Fax: +49 7531 882966.
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The strictly anaerobic, Gram-positive, non-spore-forming bacterium strain NorPut1^T ferments putrescine to acetate, butyrate, molecular hydrogen and ammonia. It also utilizes 4-aminobutyrate and 4-hydroxybutyrate as growth substrates. Comparative 16S rDNA sequence analysis confirmed a phylogenetic affiliation of this strain to the phylum of Gram-positive bacteria with low DNA G+C content. Together with its closest relative, 'Clostridium aminobutyricum' (DSM 2634), and several Eubacterium species, strain NorPut1^T represents a well-defined monophyletic group. Moderate overall 16S rRNA sequence similarities (< 91%) were found for the NorPut1^T/Clostridium aminobutyricum pair and several Eubacterium species. The type species, Eubacterium limosum, is not a member of the group and, together with Eubacterium barkeri and Pseudoramibacter alactolyticus, represents a distant phylogenetic cluster. Therefore, a new genus, Anaerovorax, is proposed as harbouring strain NorPut1^T (= DSM 5092^T), which is described as a new species, i.e. Anaerovorax odorimutans.

Keywords: anaerobic degradation, fermentation, biogenic amines, putrescine, Eubacterium spp.

Primary aliphatic amines are formed during oxygen-limited decomposition of organic matter rich in protein. Clostridia, pseudomonads, lactic acid bacteria and some enterobacteria produce biogenic amines, e.g. putrescine or cadaverine, by decarboxylation of the respective amino acids (Andreesen *et al.*, 1989; Geornaras *et al.*, 1995; Madigan *et al.*, 1997). These putrid-smelling and often highly toxic compounds (ptomaines) are also released in food, e.g. in ripening cheese (ten Brink *et al.*, 1990; Stratton *et al.*, 1991).

Aerobic decomposition of amines starts with oxidative deamination or elimination of the amino group by transamination (Prieto-Santos *et al.*, 1986; Lehninger, 1975). In a study on anaerobic degradation of primary amines, a strictly anaerobic, putrescine-degrading, fermenting bacterium that grew only with putrescine, 4-aminobutyrate or 4-hydroxybutyrate as substrates was enriched and isolated (Matthies *et al.*, 1989). This organism initiates putrescine degradation by a transamination reaction forming 4-aminobutyraldehyde

and a subsequent oxidation to 4-aminobutyrate, which is further degraded via 4-hydroxybutyrate to butyrate, acetate and H₂ (Matthies *et al.*, 1989). In the present study, this organism, strain NorPut1^T, is described as the type strain of a new species, *Anaerovorax odorimutans*, on the basis of 16S rDNA sequence comparisons.

A pure culture of strain NorPut1^T (= DSM 5092^T) was taken from our laboratory collection. The strain has been deposited with DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) under accession number DSM 5092^T. Strain NorPut1^T was originally isolated from anoxic brackish water sediments under strictly anoxic conditions in mineral medium with putrescine as sole source of organic carbon and energy (Matthies *et al.*, 1989). The hydrogen gas produced is inhibitory to growth; therefore, sufficient headspace (at least equal to the medium volume) has to be provided in the culture bottles.

The strain was cultivated in a sulfide-reduced, bicarbonate-buffered mineral medium that contained trace-element solution SL10, selenite tungstate sol-

The EMBL accession number for the sequence reported in this paper is AJ251215 (*Anaerovorax odorimutans* strain NorPut1^T).

ution (Widdel *et al.*, 1983) and a seven-vitamin solution (Widdel & Pfennig, 1981) under an $N_2:CO_2$ (90%:10%) atmosphere. Details of cultivation and physiological characterization are given in the original description (Matthies *et al.*, 1989).

In vitro amplification and sequence analysis of rDNA was performed as described earlier (Springer *et al.*, 1992). The 16S rRNA sequence of strain NorPut1^T (homologous to *Escherichia coli* positions 8–1542) was fitted into an alignment of about 16000 homologous full or partial primary structures available in public databases (Ludwig, 1995), using the respective automated tools of the ARB software package (Ludwig & Strunk, 1997). Distance matrix, maximum-parsimony and maximum-likelihood methods were applied for tree construction, as implemented in the ARB software package. Different datasets varying with respect to the selection of outgroup reference organisms (sequences) as well as alignment positions were analysed according to the criteria and recommendations given by Ludwig *et al.* (1998).

The physiological properties of strain NorPut1^T have been documented in detail before (Matthies *et al.*, 1989). Putrescine is fermented in pure culture according to the following reaction equation:

Further taxonomically relevant features of this strain are summarized in the species description at the end of this section.

As indicated by morphological and physiological characteristics and corroborated by phylogenetic analysis of 16S rDNA sequences, strain NorPut1^T is a member of the phylum of the Gram-positive bacteria with low DNA G+C content. Its closest relative among the bacteria thus far represented in rRNA sequence databases is the not yet validly named species '*Clostridium aminobutyricum*' (Hardman & Stadtman, 1960; Collins *et al.*, 1994). The two organisms share 94.6% overall 16S rRNA sequence similarity. A moderate relationship between this pair and several *Eubacterium* species is indicated by similarity values of 90.5% and lower. On the basis of analysis of the currently available 16S rRNA sequence dataset, these organisms represent a monophyletic group (Fig. 1). The separation into two subclusters was supported by the majority of the various phylogenetic analyses. As shown in Fig. 1, strain NorPut1^T clusters with '*C. aminobutyricum*', *Eubacterium brachy*, *Eubacterium infirmum*, *Eubacterium saphenum* and *Eubacterium timidum* (Cheeseman *et al.*, 1996). A distinct relative order of some of the intracluster branchings could not be defined or was not supported by the results of the different treeing analyses. This is indicated by a multifurcation in the tree. The second subcluster comprises *Eubacterium nodatum*, *Eubacterium tardum*, *Eubacterium minutum* and an unnamed isolate, C2 (Cheeseman *et al.*, 1996; Attwood *et al.*, 1998).

Fig. 1. Maximum-parsimony tree reflecting the phylogenetic position of *Anaerovorax odorimutans* strain NorPut1^T and '*Clostridium aminobutyricum*' as well as the phylogenetic diversity of selected representatives of the genus *Eubacterium*. The tree is based upon the results of an optimized maximum-parsimony analysis of a dataset of about 16000 small-subunit rRNA sequences. The tree topology was evaluated and corrected by performing maximum-parsimony, maximum-likelihood and distance matrix analyses of various datasets, applying the software tools of the ARB program package (Ludwig & Strunk, 1997) according to the criteria and recommendations given by Ludwig *et al.* (1998). The multifurcation indicates that a common significant relative branching order could not be found. Bar, 10% estimated sequence divergence. The following strains were included (sequence accession numbers are given): C2, AF044945; '*C. aminobutyricum*' DSM 2634, X76161; *Eubacterium barkeri* ATCC 25849^T, M23927; *Eubacterium brachy* ATCC 33089^T, Z36272; *Eubacterium infirmum* NCTC 12940^T, AF001766; *Eubacterium limosum* ATCC 8486^T, M59120; *Eubacterium minutum* ATCC 700079^T, AJ005636; *Eubacterium nodatum* ATCC 33099^T; *Eubacterium saphenum* ATCC 49989^T, U65987; *Eubacterium tardum* NTCT 12941^T; *Eubacterium timidum* ATCC 33093^T; *Filifactor villosus* DSM 1645^T, X73452; *Peptostreptococcus anaerobius* ATCC 27337^T, L04168; *Peptostreptococcus magnus* ATCC 15794^T; *Pseudoramibacter alactolyticus* DSM 3980^T, ARB-F8D61E04.

Although strain NorPut1^T and '*Clostridium aminobutyricum*' clearly cluster with *Eubacterium* species, we wish to propose a new genus. This should be done for two major reasons. First, only a moderate relationship between the pair (strain NorPut1^T/*C. aminobutyricum*) and the *Eubacterium* species is indicated by the results of the 16S rRNA based analyses (Fig. 1). Second, it is well known that the current genus *Eubacterium* combines a phylogenetically diverse collection of species and requires taxonomic revision. The inclusion of *Peptostreptococcus* and *Filifactor* (Paster *et al.*, 1992; Ezaki *et al.*, 1994; Collins *et al.*, 1994) as outgroup references in the tree shown in Fig. 1 highlights the distance between the type species (*Eubacterium limosum*) cluster and the remaining included *Eubacterium* species. Given that the type species of the genus, *E. limosum*, together with *Eubacterium barkeri* and *Pseudoramibacter alactolyticus* keeps a distant phylogenetic position with respect to the above-mentioned NorPut1^T cluster

(Weisburg *et al.*, 1989; Weizenegger *et al.*, 1992; Willems & Collins, 1996), the genus *Eubacterium* should be confined to a phylogenetic group defined by *E. limosum*, *E. barkeri* and closely related organisms. Consequently, the species groups within the above defined cluster should be assigned to new genera. A new genus, *Anaerovorax*, is proposed comprising a new species, *Anaerovorax odorimutans*, as the type species. '*C. aminobutyricum*' could perhaps be added to this genus later, as a renamed species, *Anaerovorax aminobutyricus*. Its sequence similarity to *A. odorimutans* is just on the borderline to allow assignment to the same genus (around 95% sequence similarity, as suggested by Ludwig *et al.*, 1998). The other *Eubacterium* spp. mentioned in Fig. 1 are more distinct and have to be grouped as a separate genus.

Description of *Anaerovorax* gen. nov.

Anaerovorax (An.ae.ro.vo'rax.. Gr. pref. *an-* non-; Gr. n. *aer* air; L. adj. *vorax* voracious; M.L. masc. n. *Anaerovorax* an anaerobic, voracious bacterium).

Strictly anaerobic, non-spore-forming bacteria of fermentative metabolism, often metabolizing amino acids. They have a low DNA G+C content (approx. 30 mol%).

Description of *Anaerovorax odorimutans* sp. nov.

Anaerovorax odorimutans (o.do.ri.mu'tans. L. masc. n. *odor* smell; L. v. *mutare* to change, *mutans* changing; *odorimutans* changing the smell, referring to the degradation of the odorous compound putrescine to form another odorous one, i.e. butyric acid).

Slightly curved rods, 0.7–0.8 × 1.9–2.7 µm in size, motile by 3–5 flagella inserted on the concave side of the cell, Gram-positive cell wall without an outer membrane but staining Gram-negative. Non-spore-forming. Chemo-organotrophic, fermentative metabolism; external electron acceptors are not used. Contains no cytochromes. Putrescine, 4-aminobutyrate and 4-hydroxybutyrate are the only substrates utilized. No growth with more than 30 different substrates tested, e.g. sugars, organic acids, alcohols, amino acids and other amines. Products of putrescine fermentation are acetate, butyrate, NH₄⁺ and H₂; 4-aminobutyrate is fermented to acetate, butyrate and NH₄⁺; 4-hydroxybutyrate is fermented to acetate and butyrate. Growth rate (µ) with putrescine at 37 °C is 0.044 h⁻¹. Grows at pH 5.1–8.0, with the optimum at pH 7.2–7.6. Temperature optimum, 37 °C; temperature limits, 12 and 50 °C. Grows in freshwater and saltwater media containing 2% NaCl and 0.3% MgCl₂·6H₂O (w/v). The DNA G+C content is 29.6 ± 1.0 mol%. Habitat: brackish sediment.

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