**Anaerovorax odorimutans gen. nov., sp. nov., a putrescine-fermenting, strictly anaerobic bacterium**

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The strictly anaerobic, Gram-positive, non-spore forming bacterium strain NorPut¹ 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 represents a well-defined monophyletic group. Moderate overall 16S rRNA sequence similarities (<91%) were found for the NorPut¹/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 phylogentic cluster. Therefore, a new genus, *Anaerovorax*, is proposed as harbouring strain NorPut¹ (= DSM 5092T), 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, 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 (= DSM 5092T) 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 5092T. Strain NorPut¹ 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-

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The EMBL accession number for the sequence reported in this paper is AJ251215 (*Anaerovorax odorimutans* strain NorPut¹).
ution (Widdel et al., 1983) and a seven-vitamin solution (Widdel & Pfennig, 1981) under an N₂:CO₂ (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 NorPut1ᵀ (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 NorPut1ᵀ have been documented in detail before (Matthies et al., 1989). Putrescine is fermented in pure culture according to the following reaction equation:

\[
10 \text{putrescine}^{2+} + 26\text{H}_2\text{O} \rightarrow 6 \text{acetate}^- + 7 \text{butyrate}^- + 20\text{NH}_4^+ + 16\text{H}_2 + 13\text{H}^+ 
\]

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 NorPut1ᵀ is a member of the phylogeny 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 NorPut1ᵀ clusters with 'C. aminobutyricum', Eubacterium brachy, Eubacterium infirmum, Eubacterium sphenum 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).

Although strain NorPut1ᵀ 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 two species (strain NorPut1ᵀ/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 reference in the tree shown in Fig. 1 highlights the distance between the type species (Eubacterium limosum) and the remaining included Eubacterium species. Given that the type species of the genus, E. limosum, together with Eubacterium bacteri and Pseudoramibacter alactolyticus keeps a distant phylogenetic position with respect to the above-mentioned NorPut1ᵀ cluster.
fermentation are acetate, butyrate, NH₄NH₄ amino acids and other amines. Products of putrescine substrates tested, e.g. sugars, organic acids, alcohols, substrates utilized. No growth with more than 30 different butyrate and 4-hydroxybutyrate are the only sub-

forming. Chemo-organotrophic, fermentative metab-

membrane but staining Gram-negative. Non-spore-

the cell, Gram-positive cell wall without an outer

motile by 3–5 flagella inserted on the concave side of

MgCl₂ saltwater media containing 2% NaCl and 0

°–

Slightly curved rods, 0–1.2–1.5 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, 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 x 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-amino-

butyrate and 4-hydroxybutyrate are the only sub-

strates 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, NH₄HCO₃ and H₂; 4-

aminobutyrate is fermented to acetate, butyrate and NH₄HCO₃; 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±10 mol%. Habitat: brackish sediment.

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