Propionispora hippei sp. nov., a novel Gram-negative, spore-forming anaerobe that produces propionic acid

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A Gram-negative, spore-forming anaerobe, KS^T, was isolated from an enrichment culture that was set up for anaerobic degradation of the aliphatic polyester poly(propylene adipate). The strain had the cellular organization of *Sporomusa*, vibrio-shaped cells and terminal round spores, and fermented sugars and sugar alcohols to propionic and acetic acid. Based on the morphological and physiological features as well as on a 16S rRNA gene similarity of 98 %, it was grouped with *Propionispora vibrioides*. A relatively low DNA–DNA hybridization value with the type strain of this species (47 %), and differences in substrate utilization and spore morphology, suggested that the strain should be classified in a separate species, *Propionispora hippei* sp. nov., with KS^T as the type strain (= DSM 15287^T = ATCC BAA-665^T).

Recently, Biebl *et al.* (2000) described a novel genus and species of anaerobes, *Propionispora vibrioides*, which belongs to the *Sporomusa*–*Pectinatus*–*Selenomonas* group, as defined by Strömpl *et al.* (1999). The type species of *Propionispora* resembles *Sporomusa* in cell shape and spore formation (Möller *et al.*, 1984), but its fermentation type is that of propionic-acid bacteria and is not acetogenic. In the course of a screening programme for the anaerobic degradation of natural and synthetic polyesters (Abou-Zeid *et al.*, 2001; Abou-Zeid, 2001), a strain was isolated that proved to be morphologically and physiologically very similar to *P. vibrioides*. However, on the basis of low DNA–DNA hybridization values with *P. vibrioides*, and differences in 16S rRNA gene sequence, substrate utilization and spore morphology, we propose that the isolated strain be classified as a novel species within the genus *Propionispora*.

The isolate was obtained from an enrichment culture set up to examine the biological degradability of the aliphatic copolyester SP 3/6, a condensate of 1,3-propanediol and adipic acid, under anaerobic conditions. A mineral salts medium was used, and was prepared according to the anaerobic cultivation techniques recommended by Hungate

(Abou-Zeid *et al.*, 2001; Holdeman *et al.*, 1977). The heat-labile polymer was prepared as a thin-film disc of 2.5 cm diameter (Witt *et al.*, 1994), sterilized by UV radiation and introduced aseptically into cold, autoclaved medium under sterile nitrogen. Sewage sludge from the municipal treatment plant in Gifhorn (Lower Saxony, Germany) was used as inoculum.

For isolation, 0.1 ml of the culture was plated on three complex agar media (anaerobic TVLS, Brewer's anaerobic and thioglycolate medium; all from Merck). The plates were incubated anaerobically at 35 °C in an anaerobic chamber. A first attempt after 3 months of incubation failed, but, after another 15 months, colonies developed on all three media. Fifteen colonies were checked on mineral salts agar with emulsified SP 3/6 polymer (for preparation see Witt *et al.*, 1994); nine of them formed clear zones indicating degradation of the polymer. They were purified on complex agar medium and rechecked on polymer agar. One strain, KS, attracted attention by its curved cells and conspicuous spores and was characterized further; the others were straight rods and were not investigated further.

Cells of strain KS^T were vibrio-shaped in exponentially growing cultures and formed slightly curved rods during the fermentation phase. Spores were readily formed in peptone-yeast extract-glucose, thioglycolate medium and mineral salts medium containing fructose. The sporangia were distinctly swollen, either dark or refractive, and appeared in terminal position. Vegetative cells could not be

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Propionispora hippei* KS^T is AJ508927.

Details of the time-course of polymer degradation by *P. hippei* are available as supplementary material in IJSEM Online.

Fig. 1. Phase-contrast micrographs of strain KS^T grown on glucose. After 24 h growth (a); after 48 h (b). Bar, 10 μm .

discriminated from *P. vibrioides* (Biebl *et al.*, 2000), while spore-forming cells were somewhat thicker (Fig. 1).

Temperature and pH range and substrate utilization were assessed in 16 ml screw-cap Hungate tubes (Bellco), using the test medium of Holdeman *et al.* (1977). Growth was monitored by measuring the optical density of the entire tube at 600 nm. Strain KS^T behaved as a mesophilic organism, with good growth between 20 and 50 °C, and an optimum at 37 °C. A maximum growth rate of 0.55 h⁻¹ was measured. The pH range for growth was between 5.0 and 8.5, with an optimum at 6.8. Yeast extract was required at a minimum of 0.5 g l⁻¹.

Only a limited number of carbon and energy sources was utilized by strain KS^T , mainly sugars and sugar alcohols, including glycerol (see species description). 1,3-Propanediol and adipate, the depolymerization products of the polyester SP 3/6, did not support growth, nor were the products of other polymers decomposed by the strain (see below): 1,4-butanediol, caproate and terephthalate. The fermentation products were determined by GC (Biebl *et al.*, 2000). It was shown that all utilized substrates were fermented to propionic and acetic acid, CO₂ and H₂. The fermentation balances, as determined in tightly sealed Hungate tubes, are shown in Table 1.

In addition to the polyester SP 3/6, strain KS^T was able to hydrolyse other synthetic polymers, such as SP 4/6, the condensate of 1,4-butanediol and adipic acid, and poly (ϵ -caprolactone). The copolymer of 1,4-butanediol, terephthalic acid and adipic acid (BTA), which exhibits better material properties than the aliphatic polyesters, was weakly degraded, as long as the aromatic component did not exceed 20% of the acidic components. Natural polyesters, such as polyhydroxybutyrate (PHB) and poly(hydroxybutyrate-hydroxyvalerate) (PHBS), were not attacked. The course of depolymerization of these compounds, shown as the increase in the clear zone diameter in agar, can be viewed as Supplementary Fig. A in IJSEM Online. Although the ability to depolymerize synthetic polyesters may be significant for their degradation in nature, it appears to be without significance for the metabolism of the organism, as the products are not further decomposed. The depolymerizing enzyme seems to be a non-specific enzyme, probably a lipase, which affects the ester bonds of the synthetic polymer.

Genomic-DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products was carried out as described by Rainey *et al.* (1996). Purified PCR products were sequenced directly using the *Taq*

Table 1. Fermentation balances of strain KS^T from various carbon substrates

Substrate	Product recovery (mol per 100 mol substrate)				Carbon recovery (%)	Electron recovery (%)
	Propionic acid	Acetic acid	CO ₂	H ₂		
Glucose	119	60	75	2	92	90
Fructose	104	55	67	5	82	80
Mannitol	119	55	55	21	87	83
Xylitol	90	40	40	15	78	75
Erythritol	98	29	35	16	96	91
Glycerol	79	24	37	23	108	97

DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems), according to the manufacturer's instructions. An Applied Biosystems 373A DNA genetic analyser was used for electrophoresis of the sequence reaction products. Although nearly complete sequence determination was achieved by this method, part of the 5' region of the gene, corresponding to helix 6 (De Rijk *et al.*, 1992), could not be resolved. In accordance with the characterization of *P. vibrioides* (Biebl *et al.*, 2000), the 16S rRNA gene PCR product had to be cloned to resolve the sequence in this region. Insertions of about 119 nt were found in several individual clones (data not shown). For alignment and subsequent analysis, sequences from clones carrying no insertion were used.

Using the ae2 editor (Maidak *et al.*, 1999), the almost complete 16S rRNA gene sequence of one clone of strain KS^T was aligned manually with those of all currently available nucleotide sequences of the *Sporomusa*–*Selenomonas*–*Pectinatus* group retrieved from GenBank and EMBL. The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were reconstructed according to the method of De Soete (1983) and the neighbour-joining and maximum-likelihood methods contained in the PHYLIP package (Felsenstein, 1993). The highest binary similarity value was found to the sequence of *P. vibrioides* (97.9%). Lower values, ranging between 92.2 and 89.8%, were found for the type strains of *Sporomusa* species. Different treeing algorithms (De Soete, 1983; Felsenstein, 1993) placed the novel strain on a separate line of descent together with *P. vibrioides* (Fig. 2).

The DNA–DNA hybridization between *P. vibrioides* strain FKBS1^T (=DSM 13305^T) and strain KS^T was 46.7%. The G+C content was 42.3 mol% for strain KS^T and 48.7 mol% for strain FKBS1^T.

With the isolation of strain KS^T, a second representative of the genus *Propionispora* (Biebl *et al.*, 2000) was found. This genus possesses properties that also occur in other related genera belonging to the *Selenomonas*–*Sporomusa*–*Pectinatus* group (Strömpl *et al.*, 1999), but in an unusual combination. It shares the typical cellular organization, i.e. vibrio-shaped cells with flagella inserted at the concave side, with *Selenomonas* and *Sporomusa*, and the ability to form spores with *Sporomusa* and *Dendrosporobacter*. The strain ferments propionic acid, in common with many other members of the group, including *Selenomonas*, but not *Sporomusa*, which is acetogenic (Möller *et al.*, 1984).

Strain KS^T matches the type strain (DSM 13304^T) of *P. vibrioides* in essential criteria. Cells are vibrio-shaped and form terminal, round spores. They produce propionic and acetic acid from a limited number of sugars and sugar alcohols. However, there are differences in the utilization of glucose and glycerol, which are not attacked by the *P. vibrioides* strain. Also, spore-forming KS^T cells are distinctly thicker than those of *P. vibrioides*. The 16S rRNA gene sequence similarity between KS and *P. vibrioides* of

Fig. 2. 16S rRNA gene sequence dendrogram showing the phylogenetic position of strain KS^T. Bar, 5 nt substitutions per 100 nt.

97.9% corresponds to values found among *Sporomusa* species (mean, 97%). The DNA–DNA hybridization was far below 70%, indicating an evolutionary distance at the species level (Johnson, 1984). We therefore propose to classify strain KS^T as a separate species within the genus *Propionispora* as *Propionispora hippei* sp. nov.

Description of *Propionispora hippei* sp. nov.

Propionispora hippei (hip'.pe.i. N.L. gen. n. *hippei* named after Dr Hans-H. Hippe, DSMZ, for his numerous contributions to the cultivation and taxonomy of anaerobes).

Cells appear as vibrios to slightly curved rods in phase-contrast microscopy. Spores are round and in terminal position; spore-forming cells are distinctly swollen. Growth and fermentation substrates are glucose, fructose, mannitol, xylitol, erythritol and glycerol. Propionic and acetic acids are the fermentation products. Lactose, lactate, pyruvate, 3-hydroxybutyrate, 2,3-butanediol, ethanol, methanol and H₂/CO₂ are not used. Yeast extract is required. The species differs from *P. vibrioides* in its ability to ferment glucose and glycerol. It depolymerizes the aliphatic polyesters of 1,3-propanediol or 1,4-butanediol and adipic acid, as well as poly(ε-caprolactone), without degrading the monomers.

The type strain is strain KS^T (=DSM 15287^T=ATCC BAA-665^T), isolated from an enrichment culture for decomposition of the aliphatic copolyester of 1,3-propanediol and adipic acid.

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