# *Soehngenia saccharolytica* gen. nov., sp. nov. and *Clostridium amygdalinum* sp. nov., two novel anaerobic, benzaldehyde-converting bacteria

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Two anaerobic, benzaldehyde-converting bacteria were isolated from an anaerobic upflow anaerobic sludge bed (UASB)-reactor treating potato starch waste water. Strain BOR-Y$^T$ converted benzaldehyde to benzoate and benzylalcohol in approximately equimolar concentrations. Benzaldehyde conversion did not support growth. Strain BOR-Y$^T$ was Gram-positive and rod-shaped, and its cells were slightly thickened in the middle. The strain was a mesophilic spore-former that grew between 15 and 40 °C, with optimum growth at 30–37 °C. The optimum pH for growth was pH 7·0. Strain BOR-Y$^T$ grew on a wide range of carbohydrates and some other carbon sources including yeast extract, cysteine and serine. The G+C content of its DNA was 42 mol%. According to physiological characteristics and 16S rRNA gene sequence analysis, confirmed by DNA–DNA hybridization with its phylogenetic neighbours, strain BOR-Y$^T$ belongs to a novel genus of cluster XII of the clostridia, namely *Soehngenia*; the name *Soehngenia saccharolytica* is proposed for the type species (type strain BOR-Y$^T$ = DSM 12858$^T$ = ATCC BAA-502$^T$). Strain BR-10$^T$ reduced benzaldehyde to benzylalcohol. This conversion was coupled to growth. In a medium containing yeast extract, the presence of benzaldehyde resulted in the accumulation of more than twofold more cells. Strain BR-10$^T$ was a Gram-positive organism that was characterized by oval- or rod-shaped cells with oval ends, which occurred singly, in pairs or sometimes in chains. The strain was moderately thermophilic and grew between 20 and 60 °C, with optimum growth at 45 °C. The optimum pH for growth was between pH 7·0 and 7·5. Strain BR-10$^T$ grew on a wide range of carbon sources including carbohydrates, yeast extract, casein and some amino acids. The G+C content of its DNA was 32 mol%. As determined by 16S rRNA gene sequence analysis, strain BR-10$^T$ represents a novel species of cluster XIVa of the clostridia; the name *Clostridium amygdalinum* is proposed for this novel species (type strain BR-10$^T$ = DSM 12857$^T$ = ATCC BAA-501$^T$).

## INTRODUCTION

Many anaerobic bacterial strains are able to convert aromatic aldehydes to other compounds (Krumholz & Bryant, 1985; Lux *et al.*, 1990; Sembring & Winter, 1990; Lux & Drake, 1992; Gößner *et al.*, 1994). *Moorella thermoacetica* (formerly *Clostridium thermaceticum*) (Collins *et al.*, 1994) and *Clostridium formicaceticum* were shown to oxidize the aldehyde group of vanillin to the carboxyl level (Lux *et al.*, 1990). *Ruminococcus productus* (formerly *Peptostreptococcus productus*) reduced the aldehyde group of vanillin when co-cultured with CO but was not capable of this reaction when vanillin was the sole substrate (Lux *et al.*, 1990).

*Clostridium aceticum* oxidized aromatic aldehydes (Lux & Drake, 1992). A few *Desulfovibrio* strains oxidized the aldehyde group of vanillin and other aromatic aldehydes with sulfate as an electron acceptor (Zellner *et al.*, 1990). A limited number of the strains were tested for their ability to convert benzaldehyde. *Clostridium acetobutylicum* reduced benzaldehyde, but 10 mM benzaldehyde caused inhibition of growth. This bacterium required the presence

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Soehngenia saccharolytica* BOR-Y$^T$ and *Clostridium amygdalinum* BR-10$^T$ are AY353956 and AY353957, respectively.

Electron micrographs of *Soehngenia saccharolytica* BOR-Y$^T$ (Fig. I) and *Clostridium amygdalinum* BR-10$^T$ (Fig. II) are available in IJSEM Online.

of glucose and butyrate in the medium (Green *et al.*, 1994). *Desulfovibrio desulfuricans* both oxidized and reduced benzaldehyde in the presence of nitrate (Parekh *et al.*, 1996).

In a previous study (Parshina *et al.*, 2000), the isolation of two bacterial strains able to convert benzaldehyde in the absence of inorganic electron acceptors was described. The bacteria were isolated from an anaerobic upflow anaerobic sludge bed (UASB)-reactor treating potato starch waste water. One bacterium (strain BOR-$Y^T$) performs the dismutation of benzaldehyde to benzoate and benzylalcohol. The other bacterium (strain BR-$10^T$) uses benzaldehyde as an electron acceptor resulting in the formation of benzylalcohol and obtains metabolic energy from this reaction. In a medium containing 1 g yeast extract $l^{-1}$, the addition of 10 mM benzaldehyde resulted in a twofold higher number of strain BR-$10^T$ cells. The mechanisms of benzaldehyde conversion by strains BOR-$Y^T$ and BR-$10^T$ have not been described. Therefore, it was expected that the two strains represented novel micro-organisms.

In this report, we describe the phenotypic and phylogenetic characteristics of the mesophilic bacterium which converts benzaldehyde to benzoate and benzylalcohol (strain BOR-$Y^T$) and of the moderately thermophilic bacterium able to reduce benzaldehyde to benzylalcohol (strain BR-$10^T$). On the basis of their phenotypic and phylogenetic characteristics, it is proposed that these two bacteria be named *Soehngenia saccharolytica* and *Clostridium amygdalinum*, respectively.

## METHODS

**Organisms and isolation of the strains.** An enrichment culture was obtained from a mesophilic laboratory-scale UASB-reactor treating potato starch waste water. The isolation of strains BOR-$Y^T$ and BR-$10^T$ from this enrichment has been described previously (Parshina *et al.*, 2000). *Clostridium ultunense* $BS^T$ (DSM $10521^T$) was kindly provided by Dr Anna Schnürer (Department of Microbiology, Swedish University of Agricultural Sciences, P.O. Box 7025, SE-750 07 Uppsala, Sweden); *Tissierella creatinini* DSM $9508^T$ was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

**Media and cultivation.** Different media were used for cultivation of the isolates and the culture collection strains. A bicarbonate-phosphate-buffered medium was used. Medium preparation and cultivation of the isolates has been described previously (Parshina *et al.*, 2000). Strain BOR-$Y^T$ was grown at 30 °C and strain BR-$10^T$ was grown at 45 °C. The bacteria were cultivated routinely in 120 ml serum bottles containing 50 ml medium. The amount of inoculum used was 1–2 %. For G+C content and DNA–DNA hybridization analyses, strain BOR-$Y^T$ was grown with 2 g yeast extract $l^{-1}$ plus 10 mM glucose and strain BR-$10^T$ was grown with 2 g yeast extract $l^{-1}$ plus 10 mM crotonate. For the cultivation of *Tissierella creatinini* DSM $9508^T$, *Clostridium* Reinforced Medium (CRM), pH 8·3, supplemented with 0·5 M creatinine was used at 30 °C. CRM supplemented with 20 mmol serine $l^{-1}$, 20 mmol threonine $l^{-1}$ and 5 g yeast extract $l^{-1}$ was used for cultivation of *C. ultunense* $BS^T$ at 37 °C.

**Microscopy.** Cell morphology of the isolates was investigated with a phase-contrast light microscope. Fine structure was studied using a JEM-100 electron microscope as described previously (Svetlichniy *et al.*, 1991). Gram staining was done according to standard procedures (Doetsch, 1981).

**Analytical methods.** Aromatic compounds (benzaldehyde, benzoate and benzylalcohol) and volatile fatty acids were analysed as described previously (Parshina *et al.*, 2000). Organic acids were analysed by HPLC (Merck) with an RI-detector. A Polyspher OAHY column (300 by 6·5 mm) was used. Hydrogen and carbon dioxide were analysed by using a Chrompack gas chromatograph (CP9001) equipped with a TCD-detector. The stainless steel column was filled with Molsieve 13X (60–80 mesh). The TCD-detector run was at 100 °C; argon was used as the carrier gas. Sulfide was analysed by the method Trüper & Schlegel (1964).

**Determination of temperature and pH optima.** For the determination of temperature optima, strain BOR-$Y^T$ was cultivated in a medium containing 2 g yeast extract $l^{-1}$ plus 2 g glucose $l^{-1}$, and strain BR-$10^T$ was cultivated in a medium containing 2 g yeast extract $l^{-1}$ plus 10 mmol crotonate $l^{-1}$. Strain BOR-$Y^T$ was incubated at 5–52 °C and strain BR-$10^T$ was incubated at 10–70 °C. pH optima were determined by incubating strains BOR-$Y^T$ and BR-$10^T$ at 30 and 45 °C, respectively, at initial pH values ranging from pH 5·0 to 9·0. The pH was adjusted by adding 6 M HCl or 6 M NaOH.

**Physiological tests.** The following substrates were tested as carbon and energy sources (20 mmol $l^{-1}$ each, unless indicated): yeast extract (2 g $l^{-1}$), formate, acetate, propionate, isobutyrate, glucose, fructose, sucrose, xylose, arabinose, rhamnose, mannose, ribose, maltose, cellobiose, galactose, melibiose, lactose, cellulose, xylan, mannitol, casitone, inositol, methanol, ethanol, ethylene glycol, ethylamine, lactate, succinate, fumarate, crotonate, pyruvate, malate, starch (2 g $l^{-1}$), glycerol, cysteine, serine, arginine, leucine, glycine, alanine, glutamate, methionine, casein (2 g $l^{-1}$), casein hydrolysate (2 g $l^{-1}$), peptone (2 g $l^{-1}$), gelatin, betaine, $H_2/CO_2$ [80 % : 20 % (v/v) in the gas phase], creatine and creatinine. To investigate the utilization of electron acceptors, strains were cultivated in a medium containing 2 g yeast extract $l^{-1}$. The following electron acceptors were tested: $Na_2SO_4$ (10 mM), $Na_2SO_3$ (2 mM), $Na_2S_2O_3$ (10 mM), $Na_2S_2O_4$ (10 mM), $Na_2S_2O_5$ (10 mM), $S^0$ (2 g $l^{-1}$) and $NaNO_3$ (2 g $l^{-1}$). To test the ability of molecular nitrogen fixation, the medium described by Skinner (1971) was used. Standard medium supplemented with 2 g yeast extract $l^{-1}$ plus 10 mM pyruvate flushed with $N_2$ served as a control. The aerotolerance of the strains was determined as described for *Clostridium aerotolerans* (van Gylswyk & van der Toorn, 1987) in the medium supplied with 20 mM glucose. The air was injected into the closed bottles through a membrane filter. The volume of the air was 0·5–100 % (v/v).

**Biochemical tests.** Several physiological and biochemical characteristics of the cultures were analysed using API 20 E biochemical kits (Identification system for *Enterobacteriaceae* and other Gram-negative rods; bioMérieux). To test for the presence of catalase, cell material was exposed to 10 % $H_2O_2$.

**Isolation of genomic DNA.** Wet biomass was washed with a solution containing 0·15 M NaCl and 0·1 M EDTA, pH 8·0, and transferred to a medium containing 0·6 M sucrose, 0·015 M Tris/HCl, pH 7·5, 0·015 M NaCl and 0·01 M EDTA. The bacterial cell wall was digested by the addition of 50 mg egg white lysozyme $ml^{-1}$ (Fluka) to the suspension and incubation at 37 °C for 1 h and subsequent addition of 1 % SDS. Further purification was carried out according to previously described methods (Marmur, 1961; Marmur & Doty, 1961).

**DNA G+C content determination.** This was done by thermal denaturation using a Pye Unicum SP 1800 spectrophotometer. The G+C content (mol%) was calculated as described by Owen *et al.* (1969).

**DNA–DNA hybridization.** DNA homology was determined using the reassociation method described by De Ley *et al.* (1970).

**16S rRNA gene sequence analysis.** This was done at the DSMZ. Approximately 95 % of the 16S rRNA gene sequence of the strains was determined by direct sequencing of PCR-amplified 16S rDNA. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of the PCR products was carried out as described by Rainey *et al.* (1996). Purified PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) as described in the manufacturer's protocol. Sequence reactions were determined with an Applied Biosystems 373A DNA Sequencer.

The resulting sequence data from the isolates were put into the alignment editor AE2 (Maidak *et al.*, 1996), aligned manually and compared with representative 16S rRNA gene sequences of organisms belonging to the clostridia. For comparison, 16S rRNA gene sequences were obtained from the EMBL database or RDP (Maidak *et al.*, 1996). The 16S rRNA gene sequence similarity values were calculated by pairwise comparison of the sequences within the alignment. For construction of the phylogenetic dendrogram, the PHYLIP package (Felsenstein, 1993) was used: pairwise evolutionary distances were computed from percentage similarities by the correction method of Jukes & Cantor (1969). Based on the evolutionary distance values, phylogenetic trees for strains BOR-Y$^T$ and BR-10$^T$ (Figs 1 and 2, respectively) were constructed by the neighbor-joining method (Saitou & Nei, 1987).

## RESULTS AND DISCUSSION

### Isolation of strains BOR-Y$^T$ and BR-10$^T$

The enrichment and isolation of strains BOR-Y$^T$ and BR-10$^T$ have been described previously (Parshina *et al.*, 2000).

### Morphological characterization of strains BOR-Y$^T$ and BR-10$^T$

**Strain BOR-Y$^T$.** Some morphological characteristics of strain BOR-Y$^T$ can be found in the genus and species descriptions for *Soehngenia* and *Soehngenia saccharolytica*, respectively (see below). Strain BOR-Y$^T$ was rod-shaped; its cells were slightly thickened in the middle and occurred singly, in pairs or in chains [Fig. Ia, supplementary data (http://ijs.sgmjournals.org)]. In the early-exponential phase of growth, cells were slightly motile by means of peritrichous flagella, but older cells lost their motility (Fig. Ib, supplementary data). Electron micrographs of thin sections revealed a cell-wall architecture typical for Gram-positive bacteria (Fig. Ic, supplementary data). Spores were formed in the medium supplemented with pyruvate and occasionally in the nitrogen-free medium. Colonies of strain BOR-Y$^T$ on agar were rhizoid, resembled a snowflake, were dark cream in colour and reached 1·0–1·2 mm in diameter.

**Strain BR-10$^T$.** Some morphological characteristics of strain BR-10$^T$ can be found in the species description for *Clostridium amygdalinum* (see below). The strain was Gram-positive (Fig. II, supplementary data), and motile in the early-exponential phase of growth. Cell chains, as well as swelling cells, were sometimes formed in the late-stationary phase of growth. Round, free spores were observed only in the nitrogen-free medium. Colonies of strain BR-10$^T$ on agar were circular, about 1 mm in diameter, creamy with a yellowish elevated centre and had a slightly undulating margin.

### Physiological characterization and metabolism of strains BOR-Y$^T$ and BR-10$^T$

**Strain BOR-Y$^T$.** Strain BOR-Y$^T$ grew at initial pH values of between pH 6·5 and 7·5; the optimum pH for growth was around 7·0. The temperature range for growth was 15–40 °C; the optimum temperature for growth was 30–37 °C. Strain BOR-Y$^T$ was anaerobic. A remarkable property of the strain was its aerotolerance. Strain BOR-Y$^T$ grew until 50 % air in the gas phase at standing conditions in the medium supplemented with 20 mM glucose without reductant; however, it did not grow in non-reduced medium under air. The strain was capable of molecular nitrogen fixation. After 2 weeks cultivation, good growth was observed in a medium without $NH_4Cl$, flushed with $N_2$, supplemented with 10 mM of pyruvate and 8 mg yeast extract $l^{-1}$. The strain was able to convert benzaldehyde. In our previous study (Parshina *et al.*, 2000), the conversion of benzaldehyde by strain BOR-Y$^T$ was described in more detail. Strain BOR-Y$^T$ required yeast extract in the medium (0·2 g $l^{-1}$), and higher concentrations (1–2 g $l^{-1}$) stimulated growth. In the medium supplemented with carbohydrates, fast cell lysis was observed at the stationary phase of growth. Strain BOR-Y$^T$ used a wide range of carbon and energy sources, but could not utilize formate, acetate, propionate, isobutyrate, butyrate, casitone, inositol, glycerol, methanol, ethanol, *p*-cresol, casein, peptone, gelatin, lactate, succinate, fumarate, $H_2$/$CO_2$, creatine, creatinine, ethylene glycol, betaine, ethylamine, crotonate, arginine, leucine, glycine, alanine, glutamate, methionine or casein hydrolysate. The main products formed from the conversion of yeast extract were acetate, $H_2$ and $CO_2$. Products formed after fermentation of glucose were formate, $H_2$, $CO_2$, acetate and ethanol. Using the API 20 E system, strain BOR-Y$^T$ showed $\beta$-galactosidase activity but no activity for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan desaminase, gelatinase or cytochrome oxidase. It also had positive reactions for $H_2S$ formation, indole and acetoin production, sorbitol and amygdalin fermentation and a negative reaction for citrate utilization.

**Strain BR-10$^T$.** Strain BR-10$^T$ grew between pH 6·5 and 8·0; the optimum pH for growth was 7·0–7·5. The temperature range for growth was 20–60 °C; the optimum temperature for growth was 45 °C. The strain was aerotolerant. Growth occurred till 50 % of air in the gas phase at standing conditions. The strain did not fix molecular nitrogen. Strain BR-10$^T$ required yeast extract (1–2 g $l^{-1}$) in the medium. Benzaldehyde, sulfite and thiosulfate were used as electron acceptors. Hydrogen in the gas phase

instead of $N_2$ served as an additional electron donor and this resulted in the stimulation of benzaldehyde conversion. Strain BR-10$^T$ used a wide range of carbon and energy sources, but could not utilize formate, acetate, propionate, isobutyrate, butyrate, methanol, benzoate, fumarate, malate, succinate, $H_2/CO_2$, galactose, rhamnose, lactose, mannose, cellulose, casein, casein hydrolysate, gelatin, aspartate, creatine, creatinine, ethylene glycol, ethylamine, arginine, leucine, lysine, alanine, glycine, glutamate or methionine. The main products formed from the conversion of yeast extract were $H_2$, $CO_2$ and acetate. Products from glucose fermentation were ethanol, acetate, $H_2$ and $CO_2$. Using the API 20 E system, strain BR-10$^T$showed no $\beta$-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan desaminase, gelatinase or cytochrome oxidase activity. It had positive reactions for $H_2S$, indole and acetoin production, and sorbitol and amygdalin fermentation, and a weak-positive reaction for citrate utilization.

## Phylogenetic, G+C content and DNA–DNA hybridization analyses

**Strain BOR-Y$^T$.** Comparative sequence analysis of the 16S rRNA gene sequence of strain BOR-Y$^T$ showed that it is a member of cluster XII of the clostridia (Collins *et al.*, 1994) (Fig. 1). This cluster is very heterogeneous. Among the bacteria most closely related to strain BOR-Y$^T$ in the phylogenetic tree are members of the genus of spore-forming bacteria *Clostridium* and non-spore-forming *Eubacterium* and *Tissierella*. Strain BOR-Y$^T$ showed highest sequence similarity with *Tissierella creatinini* DSM 9508$^T$ (93·6 %), *Tissierella praeacuta* NCTC 11158$^T$ (93·1 %), *Tissierella creatinophila* DSM 6911$^T$ (93·1 %), *Clostridium hastiforme* DSM 5675$^T$ (93·2 %) and *C. ultunense* DSM 10521$^T$ (92·2 %). Sequence similarity values of less than 97 % (Stackebrandt & Goebel, 1994) justify the creation of a novel species for strain BOR-Y$^T$.

The G+C content of the DNA of strain BOR-Y$^T$ was 43 mol%.

DNA–DNA hybridization of strain BOR-Y$^T$ with *C. ultunense* BS$^T$ and *Tissierella creatinini* DSM 9508$^T$ revealed reassociation values of 9 and 13 %, respectively. A DNA similarity value below 20 % is an indicator for a novel genus (Johnson, 1984).

**Strain BR-10$^T$.** According to the results of a phylogenetic analysis, strain BR-10$^T$ falls into cluster XIVa of the clostridia (Collins *et al.*, 1994), with highest sequence similarity (97–98 %) with a few representatives of the genus *Clostridium* (Fig. 2). Strain BR-10$^T$ also showed 98 % sequence similarity with the sulfate-reducing bacterium *Desulfotomaculum guttoideum* (Gogotova & Vainstein, 1983), but it does not use sulfate and thus does not belong to the genus *Desulfotomaculum*. All other phylogenetic neighbours belong to the genus *Clostridium*.

The G+C content of the DNA of strain BR-10$^T$ was 32 mol%.

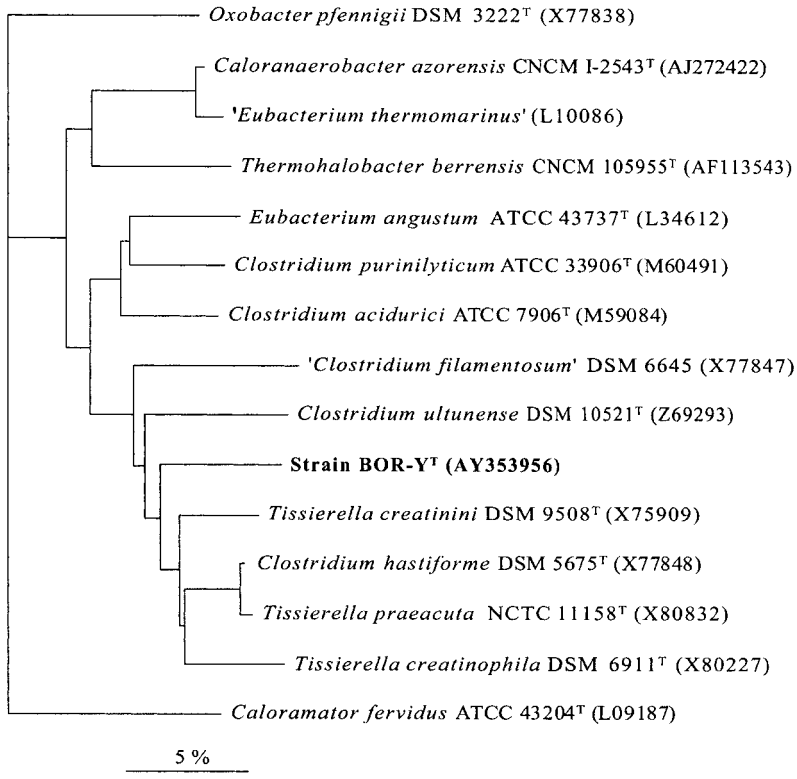


**Fig. 1.** Phylogenetic tree showing the position of strain BOR-Y$^T$ among representatives of cluster XII of the clostridia (Collins *et al.*, 1994). The tree is based on a distance matrix of 16S rRNA gene sequences and was constructed using the neighbor-joining method (Saitou & Nei, 1987), corrected by the method of Felsenstein (1993). The 16S rRNA gene sequence of *Caloramator fervidis* was used to root the tree. Bar, sequence divergence.

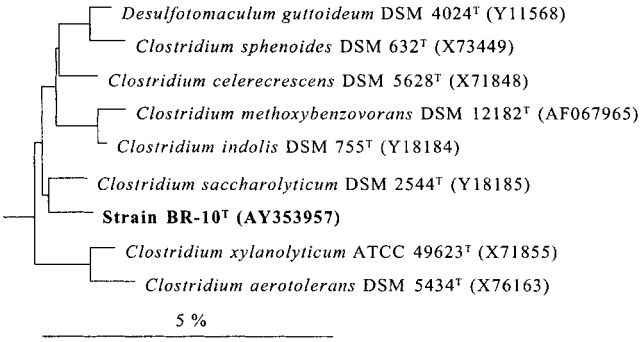


**Fig. 2.** Phylogenetic tree showing the position of strain BR-10$^T$ among representatives of cluster XIVa of the clostridia (Collins *et al.*, 1994). The tree is based on a distance matrix of 16S rRNA gene sequences and was constructed using the neighbor-joining method (Saitou & Nei, 1987), corrected by the method of Felsenstein (1993). Bar, sequence divergence.

## Comparison of strains BOR-Y$^T$ and BR-10$^T$ with related strains

**Strain BOR-Y$^T$.** Table 1 gives characteristics useful for distinguishing strain BOR-Y$^T$ from related species.

*C. aerotolerans* can grow in media without added reducing agent but exposed to air (van Gylswyk & van der Toorn, 1987). The acetogens *Sporomusa silvacetica*, *M. thermoacetica*, *Clostridium magnum*, *Acetobacterium woodii* and *Thermoanaerobacter kivui* grow in semi-solid and liquid cultivation media containing $O_2$, consume small amounts of $O_2$ and do not lose the ability to synthesize acetate via the acetyl CoA pathway (Karnholz *et al.*, 2002). Strain BOR-Y$^T$ is aerotolerant. It can grow till 50 % of air in the gas phase. Aerotolerance is not an unusual property of anaerobic bacteria. Nevertheless, no one strain from cluster XII of the clostridia has been reported as being aerotolerant until now. In addition to the traits shown in Table 1, *C. ultunense* (Schnürer *et al.*, 1996) differs from strain BOR-Y$^T$ by its inability to grow on fructose and its production of acetate, formate, $H_2$ and $CO_2$ from glucose fermentation. *C. hastiforme* (Holdeman *et al.*, 1977; Cato *et al.*, 1982; Suen *et al.*, 1988) is proteolytic, but not saccharolytic, unlike strain BOR-Y$^T$. In addition to the traits shown in Table 1, *Tissierella creatinini* DSM 9508$^T$ differs from strain BOR-Y$^T$ in that it grows on *N*-methylhydantoin, and does not use carbohydrates or various other substrates including threonine (Gauglitz, 1988; Hermann *et al.*, 1992), while *Tissierella creatinophila* (Harms *et al.*, 1998) utilizes creatinine via creatine and does not grow on carbohydrates. *Tissierella praeacuta* (Tissier, 1908; Cato *et al.*, 1979; Collins & Shah, 1986; Farrow *et al.*, 1995) is Gram-negative, and is weakly or non-fermentative.

On the basis of its phylogenetic, genetic and physiological

**Table 1.** Characteristics useful for distinguishing strain BOR-Y$^T$ from related species

Strain/species: 1, strain BOR-Y$^T$ (this study); 2, *Tissierella creatinini* DSM 9508$^T$ (Gauglitz, 1988; Hermann *et al.*, 1992); 3, *Tissierella praeacuta* (Tissier, 1908; Cato *et al.*, 1979; Collins & Shah, 1986; Farrow *et al.*, 1995); 4, *Tissierella creatinophila* (Harms *et al.*, 1998); 5, *C. hastiforme* (Holdeman *et al.*, 1977; Cato *et al.*, 1982; Suen *et al.*, 1988); 6, *C. ultunense* (Schnürer *et al.*, 1996). −, Negative; +, positive; NR, not reported; W, weak.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Utilization of: | | | | | | |
| Formate | − | − | − | + (with creatinine) | NR | + |
| Pyruvate | + | − | NR | − | − | + |
| Glucose | + | − | − | − | − | + |
| Sucrose | + | − | − | − | − | − |
| Betaine | − | − | NR | − | NR | + |
| Starch | + | NR | − | NR | − | NR |
| Ethylene glycol | − | NR | NR | NR | NR | + |
| Cysteine | W | NR | NR | NR | NR | + |
| Arginine | − | − | NR | − | + | NR |
| Serine | + | − | NR | − | + | − |
| Creatine | − | − | NR | + | − | NR |
| Creatinine | − | + | NR | + | − | NR |
| Temp. opt. (°C) | 30–37 | 37 | 37 | 30–34 | 37 | 37 |
| pH opt. | 7·0 | 8·3 | 7·5 | 7·4 | 7·5 | 7·0 |
| G+C content (mol%) | 43 | 32 | 28 | 30 | 28 | 32 |
| Colonies | Rhizoid, resemble snow-flakes, dark cream | NR | Pinpoint, circular, translucent, colourless | Circular, flat with rough surface, greyish and milky | Pinpoint, circular, entire, convex to peaked, opaque, greyish-white | Disk-shaped, whitish |

**Table 2.** Characteristics useful for distinguishing strain BR-10$^T$ from related species

Strain/species: 1, Strain BR-10$^T$ (this study); 2, *C. saccharolyticum* (Murray *et al.*, 1982); 3, *Clostridium xylanolyticum* (Rogers & Baecker, 1991); 4, *C. aerotolerans* (van Gylswyk & van der Toorn, 1987; Chamkha *et al.*, 2001); 5, *Clostridium sphenoides* (Walther *et al.*, 1977); 6, *Clostridium celerecrescens* (Palop *et al.*, 1989; Chamkha *et al.*, 2001); 7, *C. indolis* (McClung & McCoy, 1957; Corry, 1978); 8, *C. methoxybenzovorans* (Mechichi *et al.*, 1999). +, Positive; NR, not reported; −, negative; W, weak.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Utilization of: | | | | | | | | |
| Pyruvate | + | + | NR | NR | + | − | + | − |
| Glucose | + | + | + | + | + | + | + | + |
| Arabinose | + | + | − | + | + | + | NR | − |
| Xylose | + | + | + | + | + | + | NR | − |
| Galactose | − | + | NR | + | NR | + | NR | + |
| Rhamnose | − | + | + | + | + | + | NR | NR |
| Lactose | − | + | NR | + | + | W | NR | + |
| Mannose | − | + | + | + | + | + | NR | NR |
| Xylan | + | NR | + | + | NR | NR | NR | − |
| Mannitol | + | + | − | − | + | + | NR | NR |
| Gelatin | − | NR | − | − | − | + | NR | − |
| Cellulose | − | − | − | − | − | + | NR | − |
| Starch | + | − | NR | NR | − | − | NR | NR |
| Methanol | − | NR | NR | NR | NR | NR | NR | + |
| Crotonate | + | NR | NR | NR | NR | NR | NR | − |
| Betaine | + | NR | NR | NR | NR | NR | NR | + |
| $H_2/CO_2$ | − | NR | NR | NR | NR | NR | NR | + |
| Temp. opt. (°C) | 45 | 37 | 35 | 22–38 | 30–37 | 35 | 37 | 37 |
| pH opt. | 7·4 | 7·4 | 7·0–7·2 | 7·0 | NR | 7·0 | NR | 7·4 |
| G+C content (mol%) | 32 | 28 | 40 | 40 | 41 | 38 | 44 | 44 |

properties, we propose to create a novel genus, *Soehngenia*, and a novel species, *Soehngenia saccharolytica*, to accommodate strain BOR-Y$^T$.

**Strain BR-10$^T$.** Table 2 gives characteristics useful for distinguishing strain BR-10$^T$ from related species.

Species of cluster XIVa of the clostridia are saccharolytic, but the types of sugars used by the different species vary (Table 2). In addition to the traits shown in Table 2, *Clostridium saccharolyticum* differs from strain BR-10$^T$ in that it is Gram-negative, its cells are shorter (0·6–3·0 μm), it is non-motile and it has no flagella. Strain BR-10$^T$ is moderately thermophilic (optimum temperature is 45 °C): all species closely related to it are mesophilic. *C. aerotolerans* can grow in a non-reduced medium, exposed to air. Strain BR-10$^T$ is aerotolerant, but can grow only till 50 % of air in the gas phase. As mentioned above, the aerotolerance of the anaerobic acetogenic bacteria *S. silvacetica*, *M. thermoacetica*, *C. magnum*, *A. woodii* and *Thermoanaerobacter kivui* has been determined. In these bacteria, as in strains BOR-Y$^T$ and BR-10$^T$, catalase activity was not detected (Karnholz *et al.*, 2002). For optimal growth, strain BR-10$^T$ requires 1–2 g yeast extract per litre of medium: all referred strains of cluster XIVa need 1–5 g yeast extract $l^{-1}$. Strain BR-10$^T$ uses benzaldehyde as an electron acceptor and forms benzylalcohol during growth on yeast extract or other electron donors. *Clostridium methoxybenzovorans* oxidizes methoxylated aromatic compounds, with an aldehyde group to their corresponding carboxylic derivatives (Mechichi *et al.*, 1999). Strain BR-10$^T$ can use sulfite and thiosulfate as electron acceptors: this property has not been reported for any of the species closely related to it. *Clostridium indolis* is poorly described, but known properties are distinct from those of strain BR-10$^T$ (Table 2).

On the basis of its phylogenetic, genetic and physiological properties, we believe that strain BR-10$^T$ is clearly different from related species and can be considered as a novel species of *Clostridium*, namely, *Clostridium amygdalinum*.

## Description of *Soehngenia* gen. nov.

*Soehngenia* [Soehn.ge′ni.a. N.L. fem. n. *Soehngenia* named in honour of Nicolas L. Soehngen, the founder and first head (1911–1937) of the Laboratory of Microbiology of Wageningen University, The Netherlands, where this strain was isolated and described].

Gram-positive. In the early-exponential phase of growth, cells are slightly motile by means of peritrichous flagella; older cells lose their motility. Rare terminal or subterminal spore formation. Mesophilic. Anaerobic, but aerotolerant. Fixes molecular nitrogen. Saccharolytic and weakly proteolytic. Major end products from yeast extract conversion are

$H_2$, $CO_2$ and acetate. Products of glucose fermentation are formate, $H_2$, $CO_2$, acetate and ethanol.

Type species is *Soehngenia saccharolytica*.

### Description of *Soehngenia saccharolytica* gen. nov., sp. nov.

*Soehngenia saccharolytica* (sac.cha.ro.ly′ti.ca. Gr. n. *sakkharos* sugar; Gr. adj. *lytikos* loosening, dissolving; N.L. fem. adj. *saccharolytica* sugar dissolving).

Has the features of the genus. Straight or slightly thickened at the middle rod-shaped cells (0·5–0·7 μm by 2–11 μm) that occur singly, in pairs or in chains. Cell chains are formed in the stationary phase of growth. Terminal or subterminal spores are formed occasionally on pyruvate medium or in nitrogen-free medium. Spores are round to oval in shape. Colonies on agar are rhizoid, resemble a snow-flake, are dark cream in colour and reach 1·0–1·2 mm in diameter. Fermentative metabolism. Able to grow till 50 % of air in the gas phase. Sulfite and thiosulfate are weakly used as electron acceptors (reduced to $H_2S$). Sulfate, dithionite, disulfite, sulfur and nitrate are not used as electron receptors. Benzaldehyde is dismutated to benzoate and benzylalcohol. Catalase-negative. Produces indole. Does not liquefy gelatin. Substrates used as carbon and energy sources include yeast extract, glucose, fructose, sucrose, xylose, arabinose, rhamnose, mannose, ribose, maltose, cellobiose, galactose, melibiose, lactose, cellulose, xylan, mannitol, pyruvate, malate, starch, cysteine and serine. Minor product from yeast extract is $NH_4^+$. Moderate growth occurs in mineral medium supplemented with 0·2 g yeast extract $l^{-1}$. Abundant growth occurs in mineral medium with 2 g yeast extract $l^{-1}$or plus 10 mol glucose $l^{-1}$ or some other carbohydrates. In medium supplemented with carbohydrates, cells lyse rapidly in the stationary phase of growth. Growth temperature range is 15–40 °C; optimum growth at 30–37 °C. pH range for growth is pH 6·0–7·5; optimum growth around pH 7·0.

The type strain is BOR-Y$^T$ (=DSM 12858$^T$=ATCC BAA-502$^T$). Isolated from an anaerobic-digester sludge. G+C content of its DNA is 43 mol%.

### Description of *Clostridium amygdalinum* sp. nov.

*Clostridium amygdalinum* (a.myg.da.li′num. L. neut. adj. *amygdalinum* made from almonds, referring to the smell of benzaldehyde, which is reduced by the type strain).

Oval or straight rod-shaped cells (0·5–1·0 μm by 0·5–10 μm) that occur singly, in pairs or in chains. In the early-exponential phase of growth, cells are motile by means of one terminal flagellum; older cells lose their motility. Cell chains, as well as swelling cells, are formed in the late-stationary phase of growth. Round, free spores are formed only in nitrogen-free medium. Colonies on agar are circular, about 1 mm in diameter, cream in colour with a yellowish elevated centre and have a slightly undulated margin. Gram-positive. Anaerobic, but aerotolerant. Growth occurs till 50 % of air in the gas phase. Does not fix molecular nitrogen. Obligate requirement for yeast extract (1–2 g $l^{-1}$) in the growth medium. Substrates used as carbon and energy sources include yeast extract, glucose, sucrose, fructose, ribose, arabinose, xylose, melibiose, maltose, cellobiose, crotonate, casitone, pyruvate, lactate, ethanol, inositol, glycerol, mannitol, xylan, betaine, starch, casein, cysteine, serine and threonine. Catalase-negative. Produces indole. Does not liquefy gelatin. Major products formed from yeast extract are $H_2$, $CO_2$ and acetate; minor amounts of propionate, butyrate and valerate are formed. Products from glucose fermentation are ethanol, acetate, $H_2$ and $CO_2$. Benzaldehyde, sulfite and thiosulfite are used as electron acceptors. Sulfate, dithionite, disulfite, sulfur and nitrate are not used as electron acceptors. Moderately thermophilic. Temperature range for growth is 20–60 °C; optimum growth at 45 °C. pH range for growth is pH 6·5–8·0; optimum growth at pH 7·0–7·5.

The type strain is BR-10$^T$ (=DSM 12857$^T$=ATCC BAA-501$^T$). Isolated from an anaerobic-digester sludge. G+C content of its DNA is 32 mol%.

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