NOTE

Emended descriptions of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and descriptions of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov.

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On the basis of 16S rRNA gene sequencing and DNA–DNA reassociation, industrial solvent-producing clostridia have been assigned to four species. In this study, the phenotypic characteristics of *Clostridium acetobutylicum*, *Clostridium beijerinckii*, '*Clostridium saccharoperbutylacetonicum*', and an unnamed *Clostridium* sp. represented by the strains NCP 262^T and NRRL B643 are compared. In addition, a further 40 strains of solvent-producing clostridia have been classified by biotyping, DNA fingerprinting and 16S rRNA gene sequencing. These included 14 *C. beijerinckii* strains, two strains currently designated as '*Clostridium kaneboi*' and '*Clostridium butanologenum*', and 24 production strains used in the commercial acetone–butanol fermentation. All of the *C. beijerinckii* strains were confirmed to have been classified correctly. The '*C. kaneboi*' and '*C. butanologenum*' strains require reclassification as *C. acetobutylicum* and *C. beijerinckii*, respectively. The commercial production strains were found to belong either to *C. beijerinckii* or to the unnamed *Clostridium* sp. For the comparative phenotypic studies of the four species, representative strains were selected from each of the DNA-fingerprint subgroups within each species. These strains were analysed for their ability to utilize different carbohydrates, hydrolyse gelatin or aesculin, and produce indole, and were tested for the presence of catalase and urease. On the basis of these results, several phenotypic traits were found to be useful for differentiating between the four species. The descriptions of *C. acetobutylicum* and *C. beijerinckii* have been emended. The names *Clostridium saccharoperbutylacetonicum* sp. nov. [type strain = N1-4 (HMT) = ATCC 27021^T] and *Clostridium saccharobutylicum* sp. nov. (type strain = DSM 13864^T = ATCC BAA-117^T) are proposed for the two new species.

Keywords: *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium saccharoperbutylacetonicum* sp. nov., *Clostridium saccharobutylicum* sp. nov.

Solvent-producing clostridia were used extensively from the beginning of the 20th century for the industrial production of acetone and butanol (Jones & Woods, 1986), and have remained a focus of research because of their potential applications in biotechnology. Strains classified as *Clostridium acetobutylicum* were the first industrial cultures to be successfully isolated, patented and used for the large-scale production of solvents from starched-based

substrates. Following the switch (in the mid-1930s) to molasses as the preferred fermentation substrate, numerous new solvent-producing clostridial strains were isolated and patented, and each was given a novel species name (Jones & Keis, 1995). However, none of these saccharolytic industrial strains were recognized as legitimate species, and when the acetone–butanol fermentation process went into decline these names fell into disuse. Subsequently, the majority of these

Table 1. Solvent-producing clostridial strains arranged according to species

Strains of species have been subgrouped on the basis of DNA fingerprints as described by Keis *et al.* (1995). Strains highlighted in bold were chosen for further phenotypic studies. ATCC, American Type Culture Collection, Manassas, VA, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; IAM, Institute of Applied Microbiology, Tokyo, Japan; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCP, National Chemical Products Ltd, Germiston, South Africa; NRRL, Northern Utilization Research and Development Division, Peoria, IL, USA.

Subgroup	Strain	Formerly known as:
<i>C. acetobutylicum</i>		
1	ATCC 824^T (= DSM 792 ^T = NRRL B527 ^T) ATCC 3625 DSM 1733 (= NCIMB 6441) NCIMB 6442 NCIMB 6443 ATCC 43084 ATCC 17792	' <i>C. kaneboi</i> '
2	DSM 1731 (= ATCC 4259 = NCIMB 619 = NRRL B530)	
3	DSM 1737 DSM 1732 (= NCIMB 2951) ATCC 39236	
4	ATCC 8529 (= DSM 1738)	
<i>C. saccharobutylicum</i>		
1	NCP 262^T NCP 249 NCP 265(S) NCP 268 NCP 172(S) NCP 200(S) NCP 202(S) NCP 199 NCP 195 NCP 220 NCP 108 NCP 254(S) BAS/B3 37/3 IMM	<i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i>
2	NRRL B643 NCP 258 NCP 272(S) BAS/B3/SW/336(S) 162/BI	<i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i>
' <i>C. saccharoperbutylacetonicum</i> '		
1	N1-4 (= ATCC 13564) ATCC 27021 ^T	
2	N1-504 (= ATCC 27022)	
<i>C. beijerinckii</i>		
1	NCIMB 9362^T NCIMB 11373	
2	NCIMB 8052 (= DSM 1739 = ATCC 10132 = NRRL B594) NCIMB 8049 NCIMB 6444 NCIMB 6445 NCIMB 8653 NRRL B591 NRRL B597	<i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i> <i>C. acetobutylicum</i>

Table 1 (cont.)

Subgroup	Strain	Formerly known as:
3	214	' <i>C. madisonii</i> '
	4J9	' <i>C. madisonii</i> '
	NCP 193	<i>C. acetobutylicum</i>
	NCP 172(B)	<i>C. acetobutylicum</i>
	NCP 259	<i>C. acetobutylicum</i>
	NCP 261	<i>C. acetobutylicum</i>
	NCP 263	<i>C. acetobutylicum</i>
	NCP 264	<i>C. acetobutylicum</i>
	NCP 270	<i>C. acetobutylicum</i>
	NCP 271	<i>C. acetobutylicum</i>
	NCP 200(B)	<i>C. acetobutylicum</i>
	NCP 202(B)	<i>C. acetobutylicum</i>
	NCP 280	<i>C. acetobutylicum</i>
	NCP 272(B)	<i>C. acetobutylicum</i>
	NCP 265(B)	<i>C. acetobutylicum</i>
	NCP 260	<i>C. acetobutylicum</i>
	NCP 254(B)	<i>C. acetobutylicum</i>
	NCP 106	<i>C. acetobutylicum</i>
	BAS/B/SW/136	<i>C. acetobutylicum</i>
	BAS/B3/SW/336(B)	<i>C. acetobutylicum</i>
4	ATCC 39058	<i>C. acetobutylicum</i>
5	NRRL B593	
	ATCC 17791	
6	NRRL B592	
	NRRL B466	<i>C. acetobutylicum</i>
	NRRL B596	<i>C. acetobutylicum</i>
7	NCIMB 9503	
	NCIMB 9504	
8	NCIMB 9579	
	NCIMB 9580	
9	NCIMB 9581	
10	NCIMB 12404	
11	ATCC 17795	
12	IAM 19015	' <i>C. butanologenum</i> '
	ATCC 6014*	
	ATCC 6015*	
	ATCC 14823*	
	ATCC 11914*	

* No DNA fingerprints could be obtained for these strains.

solvent-producing clostridial strains were designated as *C. acetobutylicum* or *Clostridium beijerinckii*. As a result of the renewed interest in the solvent-producing clostridia over the past two decades, it became apparent that a number of the commonly used *C. acetobutylicum* strains differed significantly in their physiological and genetic traits and did not constitute a homologous group (Wilkinson & Young, 1993; Woolley & Morris, 1990). Recent investigations based on biotyping, DNA fingerprinting, pyrolysis mass spectrometry, 16S rRNA gene sequencing and DNA–DNA reassociation established that the various strains of solvent-producing clostridia investigated belong to

four species, namely *C. acetobutylicum*, *C. beijerinckii*, '*Clostridium saccharoperbutylacetonicum*', and an unnamed *Clostridium* sp. represented by the strains NCP 262^T and NRRL B643 (Johnson *et al.*, 1997; Keis *et al.*, 1995; Wilkinson *et al.*, 1995).

The aim of this study was to compare the phenotypic characteristics of representative industrial and culture-collection strains belonging to the four species. Discrepancies were found in the described characteristics of the *C. acetobutylicum* species, and the reclassification of many *C. acetobutylicum* strains as *C. beijerinckii* has required revision of the phenotypic

Fig. 1. *EagI* DNA fingerprints of *Clostridium beijerinckii* strains from the 12 subgroups. The subgroups are indicated above the strain designations. DNA fragments were separated on 1% (w/v) multi-purpose agarose (Roche Molecular Biochemicals) by using the following multi-stage transverse alternating-field electrophoresis program: stage 1, constant current of 250 mA for 5 h with a 4 s pulse; stage 2, constant current of 250 mA for 5 h with an 8 s pulse; stage 3, constant current of 250 mA for 5 h with a 16 s pulse; stage 4, constant current of 300 mA for 5 h with a 24 s pulse; stage 5, constant current for 5 h with a 32 s pulse. Lanes with $\lambda + \lambda/HindIII$ and the λ ladder contained the Low Range PFG Marker and the Lambda Ladder PFG Marker (both New England Biolabs), respectively. All sizes are in kb.

characteristics for these two species. As a consequence, emended descriptions for these two species are provided. It is also recommended that the name '*C. saccharoperbutylacetonicum*' sp. nov. be accepted for the third species of solvent-producing clostridia, with ATCC 27021^T as the type strain (as this name is in widespread use in the scientific literature). The name *Clostridium saccharobutylicum* sp. nov. is proposed for the fourth species. Since NCP 262^T is the most extensively characterized strain belonging to this new species, this strain has been designated as the type strain. Cultures of NCP 262^T have been lodged with the American Type Culture Collection (ATCC) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under accession numbers ATCC BAA-117^T and DSM 13864^T, respectively.

The solvent-producing clostridial strains used in this study are shown in Table 1. These included the 55 strains investigated in a previous study (Keis *et al.*, 1995), as well as an additional 40 strains consisting of 14 *C. beijerinckii* strains held in international culture collections, two strains currently designated as

'*Clostridium kaneboi*' and '*Clostridium butanologenum*', and 24 commercial production strains. The industrial strains originated from the strain collection previously maintained by National Chemical Products (NCP) Ltd, Germiston, South Africa, and are now held in the Department of Microbiology at the University of Otago. The taxonomic relationships of these additional 40 strains was determined as described previously (Keis *et al.*, 1995), using biotyping (viz., rifampicin-susceptibility testing, bacteriocin typing, and bacteriophage typing), DNA-fingerprint analysis and 16S rRNA gene sequencing.

To characterize the four species of solvent-producing clostridia phenotypically, representative strains were chosen for each species and tested for their sugar-fermentation abilities by using two different methods. The tests using the API 20A system (bioMérieux) were carried out essentially according to the manufacturer's instructions. However, instead of harvesting cells from agar plates, cells were harvested by centrifuging a culture broth grown in *Clostridium* basal medium (CBM; O'Brien & Morris, 1971) to an OD₆₀₀ of

0.3–0.4, and the pH of the API 20A medium was adjusted. The pH for the starch-utilizing *C. acetobutylicum* strains was adjusted to 6.2, whereas the pH for the remaining saccharolytic strains was adjusted to pH 6.8. Because of the limited range of carbohydrates included in the API 20A system, and the ambiguity of some of the results obtained, a second assay method was used. This involved growing the strains in supplemented tryptone–yeast–glucose medium (Keis *et al.*, 1995) in which the standard glucose had been replaced with one of the various carbohydrates (20 g l⁻¹) being tested. Each strain was first grown in CBM, and a small inoculum of actively growing vegetative cells was then transferred into the test media. The results were considered positive when the OD₆₀₀ of the culture was greater than 1.0 within 24 h. The strain growth was described as weak when the OD₆₀₀ was > 0.4 but never reached > 1.0, even after 72 h. The results were considered to be negative when the strain never exceeded an OD₆₀₀ of 0.15. Each of the sugar-fermentation tests was carried out at least three times. The API 20A system was also used to determine the indole, urea and catalase reactions for each strain. To determine whether the strains were capable of gelatin liquefaction, bacterial strains were inoculated onto CBM gelatin agar [CBM supplemented with 1.5% (w/v) agar and 0.4% (w/v) gelatin] and incubated anaerobically at 34 °C for 2–3 d. The presence of clear zones around bacterial colonies indicated that the bacteria had hydrolysed the gelatin in the medium.

All of the 14 *C. beijerinckii* strains examined were found to be correctly classified and exhibited 100% 16S rRNA gene-sequence homology to one another, as well as to the other *C. beijerinckii* strains examined previously (data not shown). Furthermore, the '*C. butanologenum*' strain was also found to be 100% identical to *C. beijerinckii* (according to 16S rRNA gene-sequence analysis) and requires reclassification. On the basis of DNA fingerprints, however, the various *C. beijerinckii* strains can be grouped into at least 12 subgroups (Fig. 1, Table 1), indicating that this species consists of a genetically diverse collection of strains. This is supported by the broad range of DNA–DNA reassociation values exhibited by the *C. beijerinckii* strains investigated by Johnson *et al.* (1997). The '*C. kaneboi*' strain was found to be identical to the *C. acetobutylicum* type strain (ATCC 824^T) on the basis of the 16S rRNA gene sequence and the DNA-fingerprint pattern (data not shown). Hence, this strain also requires reclassification. The 24 NCP industrial strains, designated as *C. acetobutylicum*, have been reclassified as either *C. beijerinckii* (subgroup 3) or *C. saccharobutylicum*, on the basis of their 16S rRNA gene sequences and DNA fingerprints (Table 1). The latter strains could be further divided into two subgroups (subgroup 1 or subgroup 2) on the basis of differences in their DNA-fingerprint patterns (Table 1).

Comparative phenotypic studies were carried out on representative strains from the four species of solvent-

producing clostridia, selected on the basis of the subgroups identified by biotyping and genomic DNA fingerprints. These strains are highlighted in bold in Table 1. The phenotypic characteristics tested included carbohydrate utilization, aesculin hydrolysis, gelatin hydrolysis, indole production, catalase activity and urease activity.

The range of carbohydrates used in this study is given in Table 2 and included common pentose sugars, monosaccharide and disaccharide hexose sugars, sugar alcohols, modified sugars and complex polysaccharides. All of the strains from the four species grew on the pentose sugars arabinose and xylose; with ribose, however, the growth of the strains belonging to *C. acetobutylicum*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum* was either negative or weak (Table 2). In the case of *C. beijerinckii*, nine of the 16 strains tested were able to utilize this pentose sugar. All four species were able to utilize all of the hexose sugars tested, whereas the utilization of the alcohol sugars was variable between the species, glycerol being utilized only weakly or not at all by all four species (Table 2). The modified sugars methyl-glucopyranoside, raffinose, salicin and amygdalin supported the growth of all four species, whereas the rhamnose-containing media supported the growth of the strains from the four species only weakly or not at all (Table 2). The various complex polysaccharides tested all proved to be good growth substrates for the majority of the strains. Pectin was the only complex polysaccharide not utilized by *C. saccharobutylicum*; consequently, this substrate is useful for discriminating this species from the other three species (Table 2).

None of the species was found to produce indole or to display catalase or urease activity, but all four species were able to hydrolyse aesculin (Table 2). Furthermore, *C. acetobutylicum*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum* were all able to hydrolyse gelatin, whereas all of the strains belonging to *C. beijerinckii* were unable to hydrolyse gelatin, except for the NRRL B593 strain, which tested positive for gelatin liquefaction (Table 2).

On the basis of the results of these phenotypic tests, several traits can be identified that are useful for differentiating the four species of solvent-producing clostridia (Table 2). Particularly useful in combination are rifampicin sensitivity, gelatin hydrolysis, pectin utilization and riboflavin production from milk. For example, *C. acetobutylicum* can be differentiated from *C. beijerinckii* by its sensitivity to rifampicin, by its ability to produce riboflavin and by gelatin hydrolysis. *C. beijerinckii* is unable to produce riboflavin, only NRRL B593 is able to hydrolyse gelatin, and only the strains NCIMB 9579 and NCIMB 9580 are sensitive to rifampicin. *C. acetobutylicum* can also be differentiated from *C. saccharobutylicum* by its ability to utilize pectin and produce riboflavin; *C. saccharobutylicum*, however, cannot utilize this complex sugar or produce riboflavin. *C. saccharoperbutylacetonicum* can be

Table 2. Differences in carbohydrate utilization and other phenotypic traits of the four species of solvent-producing clostridia

Species: 1, *C. acetobutylicum*; 2, *C. beijerinckii*; 3, *C. saccharoperbutylacetonicum*; 4, *C. saccharobutylicum*. Characteristics are scored as follows: s, sensitive to 10 ng per disc and 100 ng per disc of rifampicin; r, resistant to 10 ng per disc and 100 ng per disc of rifampicin; (r), most strains are resistant; +, positive; –, negative; w, weak; (+), most strains are positive; (–), most strains are negative; (w), most of the strains are weak; d, 40–60% of strains are positive; +/–, positive most of the time, but sometimes negative; –/+, negative most of the time, but sometimes positive. All strains were negative for indole production, urease and catalase activity. All strains hydrolysed aesculin and utilized arabinose, xylose, glucose, mannose, cellobiose, lactose, maltose, saccharose, methyl-glucopyranoside, raffinose, salicin, amygdalin, starch and dextrin.

Characteristic	1	2	3	4
Number of strains	7	16	2	4
Susceptibility to rifampicin	s	(r)	r	s
Riboflavin (yellow pigment) produced in milk*	+	–	–	–
Gelatin liquefaction	+	(–)	+	+
Utilization of:				
Ribose	–	d	–	w
Glycerol	w	w	–	–
D-Arabitol	–	(+)	+	–/+
L-Arabitol	–	(+)	+	–/+
Dulcitol	–	(+)	d	–
Inositol	–	+	d	+
Mannitol	+	+	+	–/+
Sorbitol	d	+	d	–
Melezitose	(–)	+	+	–
Melibiose	(–)	(+)	+	+
Rhamnose	–	(w)	w	–
Trehalose	(–)	+	+	+
Turanose	(w)	+	+	+
Glycogen	+	(+)	+	+
Inulin	d	+	+	+/–
Pectin	+	+	+	–

* Result from Johnson *et al.* (1997).

differentiated from *C. beijerinckii* by its ability to hydrolyse gelatin, and, according to Johnson *et al.* (1997), by its inability to cause curd formation in milk during the first 48 h (*C. beijerinckii* caused curd formation within 24 h).

On the basis of their fermentation abilities and the results of the catalase-, urease-, indole- and gelatin-hydrolysis tests, emended and proposed descriptions of the four species of solvent-producing clostridia are presented below.

Emended description of *Clostridium acetobutylicum* McCoy *et al.* 1926

The description of *Clostridium acetobutylicum* in *Bergey's Manual of Systematic Bacteriology* (Cato *et al.*, 1986) is based on the description by Smith & Hobbs (1974) and Holdeman *et al.* (1977). Smith & Hobbs (1974) group this species within Group II of the genus *Clostridium*, on the basis of its ability to hydrolyse gelatin. However, Holdeman *et al.* (1977) describe this species as being unable to hydrolyse gelatin, as in *Bergey's Manual*. We found that all seven

representative strains of *C. acetobutylicum* tested positive for gelatin liquefaction. This is in agreement not only with the formal description of *C. acetobutylicum* by McCoy *et al.* (1926), but also with the description given in the patent, issued to Weizmann (1919), describing the original acetone–butanol fermentation process. The description of *C. acetobutylicum* should therefore be emended accordingly. Furthermore, we found that amygdalin and raffinose were fermented by *C. acetobutylicum*. This is contrary to the description given in *Bergey's Manual* but is in agreement with the formal description given by McCoy *et al.* (1926). In addition, we found that *C. acetobutylicum* was able to ferment saccharose and pectin but was able to ferment turanose only weakly.

Emended description of *Clostridium beijerinckii* Donker 1926

The phenotypic description of *Clostridium beijerinckii* is essentially the same as that given in *Bergey's Manual of Systematic Bacteriology* (Cato *et al.*, 1986), based on those of Smith & Hobbs (1974) and Holdeman *et al.*

(1977). In some instances in this study, we found the percentage of strains able to ferment certain carbohydrates to be higher than that given in *Bergey's Manual*, perhaps because we analysed fewer strains (16, as opposed to 60). As in *Bergey's Manual*, we found that the *C. beijerinckii* strains were unable to hydrolyse gelatin (with the exception of strain NRRL B593). Characteristics additional to the description of *C. beijerinckii* include the following: the ability to ferment saccharose, and the ability of the majority of the strains to utilize the alcohol sugars D- and L-arabitol, dulcitol and inositol, but glycerol only weakly. All of the strains of *C. beijerinckii* were also able to ferment methyl-glucopyranoside, turanose, dextrin and pectin.

Description of *Clostridium saccharoperbutylacetonicum* sp. nov.

Clostridium saccharoperbutylacetonicum (sac.cha.ro.per.bu.tyl.a.ce.to'ni.cum. Gr. n. *saccharon* sugar juice; Gr. pron. *per* throughout; N.L. n. *butylum* butanol; N.L. adj. *acetonicus* acetic; N.L. adj. *saccharoperbutylacetonicum* denoting the production of a large amount of butanol and acetone from sugar).

C. saccharoperbutylacetonicum was first named and described by Hongo (1960) in US patent no. 2945786. The species status of *C. saccharoperbutylacetonicum* has now been firmly established by 16S rRNA gene-sequence analysis (Keis *et al.*, 1995) and DNA-DNA reassociation (Johnson *et al.*, 1997), and, since this name is in widespread use in the scientific literature, we recommend that it be validated. The original description by Hongo (1960) has been incorporated in the description provided below. Strain N1-4 was deposited by Hongo & Murata in the American Type Culture Collection as strain ATCC 13564, along with its derivatives N1-4 (HMT) (ATCC 27021^T) and N1-504 (ATCC 27022).

Cells in most broth cultures consist of straight, short and long rods with rounded ends, measuring 0.4–0.8 µm × 3.1–6.2 µm. Rods occur singly, or occasionally in pairs, and are motile by means of peritrichous flagella. They are initially phase-dark and Gram-positive, becoming Gram-negative in older cultures. Towards the end of exponential growth, the rod-shaped cells usually begin to accumulate granules. These morphological changes are typically associated with the metabolic shift from acid production to solvent production. Endospores are oval, measuring 0.8–1.5 µm × 1.6–2.2 µm. Existing strains sporulate poorly on most common laboratory culture media. Colonies on CBM agar are 2–3 mm in diameter, domed, white and have smooth surfaces and entire/undulated margins. The species is mesophilic, optimum growth for solvent production occurring between 25 and 35 °C; the optimum pH range is between pH 5.6 and pH 6.7. Catalase, oxidase and indole are not produced. The strains are resistant to rifampicin (100 ng). Aesculin and gelatin are hydro-

lysed. Nitrate is not reduced to nitrite. Ammonia is produced from nitrite. The results for the production of hydrogen sulphide are as follows: negative from peptone-containing medium; negative or weakly positive from the reduction of sulphites; and positive from the reduction of thiosulphates. Digestion of coagulated albumin is very weak or negative. Strains ferment arabinose, xylose, glucose, mannose, cellobiose, lactose, maltose, saccharose, D-arabitol, L-arabitol, manitol, melibiose, methyl-glucopyranoside, raffinose, salicin, trehalose, turanose, amygdalin, starch, glycogen, dextrin, pectin, melezitose and inulin. The utilization of the latter two sugars is in contrast to the original description given in the patent by Hongo (1960). The sugars ribose and glycerol were not utilized and weak fermentation was observed with rhamnose. Strain N1-4 was able to ferment sorbitol, dulcitol and inositol, but strain N1-504 was unable to utilize these alcohol sugars. No curd formation of milk occurs during the first 48 h and no riboflavin is produced (Johnson *et al.*, 1997). The G + C content of the DNA (T_m) is 31 mol% (Hongo *et al.*, 1966). Fermentation products include acetic and butyric acids, acetone, butanol, ethanol, CO₂ and H₂. These industrial strains were used in the applied fermentation process for the production of the solvents acetone, butanol and ethanol from a variety of sugar- and starch-based substrates. Commercial production of solvents commonly used various types of molasses mash supplemented with nitrogen in the form of ammonium salts and organic nitrogen. Typically, industrial fermentations utilized molasses containing 4–6% fermentable sugars at temperatures of 25–35 °C for 48–72 h, giving a final pH of 5.5–8.0, solvent yields of 27–34% and solvent concentrations of 15–17 g l⁻¹ (maximum 19 g l⁻¹); the proportion of butanol ranged from 73 to 85%. Corn mash typically required longer fermentation times and produced significantly lower yields and concentrations of solvents. More detailed information on solvent ratios, concentrations and yields for both sugar-based and starch-based substrates with two strains belonging to this species is available in a recent publication by Shaheen *et al.* (2000). Since the ATCC 13564 strain has been deaccessioned, it is recommended that the type strain for this species should be ATCC 27021^T.

Description of *Clostridium saccharobutylicum* sp. nov.

Clostridium saccharobutylicum (sac.cha.ro.bu.ty'li.cum. Gr. n. *saccharon* sugar juice; N.L. n. *butylum* butanol; N.L. neut. adj. *saccharobutylicum* denoting the production of butanol from sugar).

The industrial strains belonging to this species were originally isolated and patented by the Commercial Solvents Corporation under the name '*Clostridium saccharo-butyl-aceticum-liquefaciens*' (Jones & Keis, 1995). The US patents describing this saccharolytic,

solvent-producing *Clostridium* species were filed by Arzberger (1938) and Carnarius & McCutchan (1938). These industrial strains subsequently became designated as *C. acetobutylicum*. As the original name never gained widespread usage, we propose the less cumbersome name *Clostridium saccharobutylicum* for this species.

Cells in most broth cultures consist of straight, short and long rods with rounded ends, with a mean size of $1.4\ \mu\text{m} \times 6.3\ \mu\text{m}$ and lengths ranging from $3.8\ \mu\text{m}$ to $10\ \mu\text{m}$ (Long, 1984). Rods may occur singly or in pairs and short chains, and are motile by means of peritrichous flagella; they are initially phase-dark and Gram-positive, becoming Gram-negative in older cultures (Jones *et al.*, 1982). Towards the end of exponential growth, the rod-shaped cells typically begin to accumulate granules, assume a swollen cigar-shaped clostridial form, and produce extracellular slime or capsules (Jones *et al.*, 1982). These morphological changes are typically associated with the metabolic shift from acid production to solvent production. Endospores are oval, measuring $1.1\text{--}1.8\ \mu\text{m} \times 1.7\text{--}3.9\ \mu\text{m}$, terminal or subterminal with up to 15% being bipolar (Long, 1984). Colonies on CBM agar are 2–3 mm in diameter, domed, creamy yellow, have smooth surfaces and have circular/irregular margins. The species is mesophilic: the optimum growth for solvent production is between $30\ ^\circ\text{C}$ and $34\ ^\circ\text{C}$ and the optimum pH ranges between 6.2 and 7.0. All of the strains tested are catalase-negative and susceptible to rifampicin (10 ng). Urease and indole are not produced. Aesculin and gelatin are hydrolysed. All strains ferment arabinose, xylose, glucose, mannose, cellobiose, lactose, maltose, saccharose, inositol, melibiose, methyl-glucopyranoside, raffinose, salicin, trehalose, turanose, amygdalin, starch, glycogen and dextrin. Weak fermentation was observed with ribose, and no fermentation was seen with glycerol, dulcitol, sorbitol, melezitose, rhamnose or pectin. Variable results were obtained with the sugars D- and L-arabitol, mannitol and inulin. Curd formation in milk occurs within the first 24 h, but no riboflavin is produced (Johnson *et al.*, 1997). The G+C content of the DNA has not been determined but DNA sequencing data indicate that it is likely to fall in the range 28–32 mol%. Fermentation products include acetic and butyric acids, acetone, butanol, ethanol, CO_2 and H_2 . These industrial strains were used in the applied fermentation process for the production of the solvents acetone, butanol and ethanol from a variety of sugar- and starch-based substrates. Commercial production of solvents commonly used various types of molasses mash supplemented with nitrogen in the form of ammonium salts and organic nitrogen. Typically, industrial fermentations utilized molasses containing 6–7.5% fermentable sugars at temperatures of $29\text{--}33\ ^\circ\text{C}$, for 29–33 h, giving a final pH of 5.2–6.4, solvent yields of 27–33% and solvent concentrations of $17\text{--}20\ \text{g l}^{-1}$, (maximum $21\ \text{g l}^{-1}$); the proportion of butanol ranged from 55% to 74%. Corn mash

typically required longer fermentation times and produced significantly lower yields and concentrations of solvents. More detailed information on solvent ratios, concentrations and yields for both sugar-based and starch-based substrates with various strains belonging to this species is available in a recent publication by Shaheen *et al.* (2000). The type strain is NCP 262^T, which has been lodged with the DSMZ and the American Type Culture Collection under accession numbers DSM 13864^T and ATCC BAA-117^T, respectively.

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