Reclassification of Clostridium proteoclasticum as Butyrivibrio proteoclasticus comb. nov., a butyrate-producing ruminal bacterium

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It is proposed that Clostridium proteoclasticum be reclassified as Butyrivibrio proteoclasticus comb. nov. on the basis of phylogenetic position, DNA G+C content and physiological traits. Phylogenetic analyses based on 16S rRNA gene sequences from an extensive range of taxa within clostridial rRNA subcluster XIVa grouped C. proteoclasticum together with isolates of the genus Butyrivibrio, though this species was genetically distinct from the extant Butyrivibrio species examined. The DNA G+C content of C. proteoclasticum was originally erroneously reported as 28 mol%. However the genome sequence of the type strain of C. proteoclasticum, strain B316T, and HPLC analysis estimate the DNA G+C content as 40 mol%, which is within the range reported for strains of Butyrivibrio. C. proteoclasticum was distinguishable from other species of the genus Butyrivibrio as the 16S rRNA gene from strain B316T shared less than 97% sequence similarity with sequences from the type strains of Butyrivibrio species. C. proteoclasticum was also able to convert linoleic acid to stearic acid, in contrast to other species of Butyrivibrio. Physiological characteristics, including carbon source utilization, volatile fatty acid production and proteinase activities, were assessed for a panel of representative strains of the genera Butyrivibrio and Pseudobutyrivibrio and C. proteoclasticum. These data, together with the phylogenetic analyses, support the reclassification of Clostridium proteoclasticum as a separate species within the genus Butyrivibrio, Butyrivibrio proteoclasticus comb. nov. (type strain B316T=ATCC 51982T=DSM 14932T).

The original principal characteristics of members of the genus Butyrivibrio (family Lachnospiraceae) (Bryant & Small, 1956; Bryant, 1986) are that they possess curved, strictly anaerobic, non-spore-forming, rod-shaped cells that are generally motile by means of one or more polar to subpolar flagella. They possess an atypical Gram-positive ultrastructure, although stain Gram-negative (Bryant, 1986), and have a fermentative metabolism with butyrate as the main product. Strains of the genus Butyrivibrio are commonly isolated from the rumen and the mammalian gastrointestinal tract and have a DNA G+C content of 36–42 mol% (Bryant, 1986). Phylogenetic analyses have revealed that the genus Butyrivibrio is a diverse polyphyletic assemblage (Willems et al., 1996) for which three species: Butyrivibrio fibrisolvens (the type species), Butyrivibrio crossotus and Butyrivibrio hungateii are currently recognized (Bryant & Small, 1956; Kopečný et al., 2003; Moore et al., 1976). Clostridium proteoclasticum, exemplified by the type strain B316T (= ATCC 51982T = DSM 14932T), was first proposed in 1996 (Attwood et al., 1996) to describe a strictly anaerobic Gram-positive bacterium with straight to slightly curved rod-shaped cells bearing a single subpolar flagellum, isolated from the rumen of a New Zealand cow (Attwood & Reilly, 1995). Accordingly, this highly proteolytic bacterium was originally described as Butyrivibrio-like (Attwood & Reilly, 1995). However, its apparently much lower DNA G+C content (originally

Abbreviation: VFA, volatile fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of Butyrivibrio fibrisolvens C219a and Pseudobutyrivibrio ruminis CF1b are EU346756 and EU346757, respectively.

Supplementary tables detailing the strains used in this study and the 16S rRNA gene sequence similarity for strains of the genera Butyrivibrio and Pseudobutyrivibrio and a supplementary figure showing a multi-sequence alignment of partial 16S rRNA genes from strains of the genera Butyrivibrio, Pseudobutyrivibrio, Clostridium, Ruminococcus and Bacillus generated using CLUSTAL W are available with the online version of this paper.
reported as 28 mol%, which is typical of the Clostridium
type species, Clostridium butyricum), lack of motility and
lack of dextrin utilization (Attwood et al., 1996) distin-
guished strain B316T from B. fibrisolvens. Furthermore,
phylogenetic characterization and phylogenetic analyses of
16S rRNA genes showed that strain B316T was most closely
related to Clostridium aminophilum within clostridial
subcluster XIVa, a strongly supported cluster defined on the
basis of phylogenetic analysis of 16S rRNA genes from an
extensive range of clostridial strains (Collins et al.,
1994). The same analysis demonstrated that the genus
Clostridium is highly diverse, with strains often intermixed
with members of many different genera (Collins et al.,
1994). However, it is generally accepted that the genus
Clostridium should be retained only for organisms belong-
ing to cluster I, which contains the type species (Collins et al.,
1994). It was initially proposed that strain B316T be
assigned to a novel species of the genus Clostridium, C.
proteoclasticus, with a view that classification was likely to
change upon review of clostridial subcluster XIVa
(Attwood et al., 1996).

Since the original description of C. proteoclasticus, a body
of evidence to support its reclassification to the genus
Butyrivibrio has arisen. It has been found that the DNA
G + C content originally reported for strain B316T,
28 mol% (Attwood et al., 1996), was erroneous and recent
evaluations have found the value in the range of that
characteristic for species of the genus Butyrivibrio.
Furthermore, phylogenetic analyses involving 16S rRNA
gen sequences from a wider range of taxa that fit into
clostridial subcluster XIVa, including those from the genera
Butyrivibrio and Pseudobutyrivibrio, have been performed
(Forster et al., 1996; Kopečný et al., 2001, 2003; Wallace et al.,
2006; Willems et al., 1996). Such analyses show that C.
proteoclasticus sequences cluster closely with sequences of
the genus Butyrivibrio, where it is most closely related to B.
hungatei (Kopečný et al., 2003; Wallace et al., 2006). In
addition, PCR primers designed specifically to amplify 16S
rRNA gene sequences from species of the genus
Butyrivibrio (Mrázek & Kopečný, 2001) were able to
amplify sequences from C. proteoclasticus isolates
(Kopečný et al., 2003). Despite its close relationship with
B. hungatei, C. proteoclasticus can be distinguished from
this species by its ability to form stearic acid from linoleic
acid (Paillard et al., 2007; Wallace et al., 2006), a feature
that has not been observed in other strains of the genera
Butyrivibrio or Pseudobutyrivibrio (Paillard et al., 2007).

To support the reclassification of C. proteoclasticus to B.
proteoclasticus comb. nov., we present phylogenetic anal-
yses and a phenotypic characterization of strains of the
genera Butyrivibrio and Pseudobutyrivibrio (listed in
Supplementary Table S1 in IJSEM Online), including the
type strains B. crosstus DSM 2876T, B. fibrisolvens ATCC
19171T, B. hungatei JK 615T, Pseudobutyrivibrio ruminis
DSM 9787T and Pseudobutyrivibrio xylanivorans Mz 5T.
Hereafter, we refer to C. proteoclasticus as B. proteocлас-
ticus.

The evolutionary positions of strains of B. proteoclasticus
[B316T, UC142, H17c(SA), DSM 10301 and DSM 10304]
relative to other strains of the genera Butyrivibrio,
Pseudobutyrivibrio, Clostridium and Ruminococcus from
clostridial cluster I and subcluster XIVa (Collins et al.,
1994) were inferred by 16S rRNA gene sequence-based
phylogenetic analyses. Near full-length 16S rRNA gene
sequences (>1400 bp) were obtained by PCR amplification
and automated sequencing or were retrieved from
GenBank and the Ribosomal Database II databases (Cole
et al., 2007). Sequence alignment and phylogenetic analyses
were implemented in MEGA4 (Tamura et al., 2007). A
multi-sequence alignment was generated using CLUSTAL W
corrected manually for alignment ambiguities (see
Supplementary Fig. S1 in IJSEM Online). A phylogenetic
tree was constructed using the neighbour-joining method
(Saitou & Nei, 1987) with the Kimura two-parameter
model (transition to transversion ratio=2.0) for distance
estimation (Kimura, 1980). Bootstrap values (Felsenstein,
1985) were determined from 10000 replicates. The
resulting phylogenetic tree (Fig. 1) clearly shows that B.
proteoclasticus sequences are located in clostridial subclu-
ster XIVa (rather than cluster I), and group together in a
well-supported clade (88 % bootstrap support), which is
closely related to clades containing B. hungatei strains and
Butyrivibrio strains NCDO 2222 and NCDO 2434. Despite
this close relationship, the 16S rRNA gene sequence
similarity between the type strains of B. proteoclasticus
and B. hungatei is 95.7 % (see Supplementary Table S2 in
IJSEM Online), which is below the cut off value (97 %) that
is generally accepted for two strains to be considered
members of the same species (Stackebrandt & Goebel,
1994; Staley, 2006). Interestingly, strains NCDO 2222 and
NCDO 2434 were also considered to be isolates of C.
proteoclasticus on the basis of their ability to convert
linoleic acid to stearic acid (Paillard et al., 2007), and
NCDO 2222 was earlier considered to be a B. hungatei
strain (Kopečný et al., 2003). However, the distinct
position of these two strains within the 16S rRNA gene
phylogeny (Fig. 1 and Wallace et al., 2006) and <97 % 16S
rRNA gene sequence similarity to other type strains of
the genus Butyrivibrio (see Supplementary Table S2) suggests
that strains NCDO 2222 and NCDO 2434 are neither
representatives of B. proteoclasticus nor B. hungatei, and
possibly represent a novel species of the genus Butyrivibrio
(and thus are referred to only as Butyrivibrio strains in this
study).

Estimation of DNA G + C content was determined by
enzymic hydrolysis of total genomic DNA, followed by
HPLC analyses as previously described (Kopečný et al.,
2003). For each strain, the mean G + C content value was
determined from two replicate experiments. Values for B.
proteoclasticus strains (B316T, 40.04 mol%; DSM 10301,
40.09 mol%; DSM 10304, 39.72 mol%; DSM 10305,
42.67 mol%; JK 669, 39.79 mol%, and UC 142,
41.06 mol%) were within the general range specified for
species of the genus Butyrivibrio, in contrast to the original
reported G+C value for strain B316 T of 28 mol% (Attwood et al., 1996). These values were further supported by the recent availability of the complete genome sequence of strain B316 T, where the G+C content of the main chromosome is 40.21 mol% and an estimated value for the whole genome (considering one copy of each extrachromosomal element) is 40.01 mol% (W. J. Kelly, E. Altermann, S. C. Leahy, C. J. Yeoman, C. D. Moon and G. T. Attwood, unpublished information).

A panel of fifteen strains of the genera Butyrivibrio and Pseudobutyrivibrio was assessed for carbon source utilization, proteinase activity and volatile fatty acid (VFA) production. The ability to utilize 30 different carbon sources (modified by omission of all carbohydrates and containing four times the specified amount of yeast extract and doubling the specified volume of mineral solution), with addition of each specific carbon source at 0.5% final concentration, was tested in triplicate using API 20A test kits (bioMe`rieux; Table 1). In contrast to the original description, strain B316 T grew on dextrin and trehalose, but growth on mannose and rhamnose was not observed as originally reported (Fig. 1). Cultures were inoculated in duplicate and grown under anaerobic conditions overnight at 39 °C, with results shown in Table 1. Additionally, the substrate utilization abilities of the B. proteoclasticus strains DSM 10301, DSM 10304, DSM 10305, JK 669, JK 722, UC 142 and X2D62 were tested in triplicate using API 20A test kits (bioMe`rieux; Table 1). In comparison to its closest relative, B. hungatei and Butyrivibrio sp. NCDO 2222, B. proteoclasticus differed in its ability to utilize amygdalin, glycogen, mannan, melibiose, rhamnose and trehalose (Table 1). In contrast to the original description, strain B316 T grew on dextrin and trehalose, but growth on mannose and rhamnose was not observed as originally reported (Fig. 1).

FIG. 1. 16S rRNA gene phylogeny using near-full-length sequences from clostridial cluster I and subcluster XIVa strains. The tree was constructed by the neighbor-joining method, and the alignment gap and missing data were eliminated only in pairwise sequence comparisons. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The optimal tree had a sum of branch lengths = 0.9572951, and the tree with the sum of branch lengths = 0.9572951 is shown. Percentage bootstrap values are shown at nodes if > 50%. GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per site.
Table 1. Carbon source utilization of strains of the genera Butyrivibrio and Pseudobutyrivibrio

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With all strains of Butyrivibrio proteoclasticus, B. hungatei JK 615T, B. fibrisolvens DSM 10295, and P. xylanivorans DSM 14809T.

Phylogenetic, genetic, morphological and physiological data all support the reclassification of C. proteoclasticum to the genus Butyrivibrio where it is genetically and physiologically distinct from extant species of the genus. Hence it is proposed that Clostridium proteoclasticum be reclassified as Butyrivibrio proteoclasticus comb. nov.

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Butyrivibrio fibrisolvens (1996). Butyrate-producing anaerobic bacteria from the rumen of white-tailed deer.


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