Anaerostipes caccae gen. nov., sp. nov., a New Saccharolytic, Acetate-utilising, Butyrate-producing Bacterium from Human Faeces

ANDREAS SCHWIERTZ1, GEORGINA L. HOLD2, SYLVIA H. DUNCAN2, BARBEL GRUHL1, MATTHEW D. COLLINS3, PAUL A. LAWSON3, HARRY J. FLINT2 and MICHAEL BLAUT1

1Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Bergholz-Rehbrücke, Germany
2Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK
3School of Food Biosciences, University of Reading, Reading, UK

Received January 28, 2002

Summary

Two strains of a previously undescribed Eubacterium-like bacterium were isolated from human faeces. The strains are Gram-variable, obligately anaerobic, catalase negative, asporogenous rod-shaped cells which produced acetate, butyrate and lactate as the end products of glucose metabolism. The two isolates displayed 99.9% 16S rRNA gene sequence similarity to each other and treeing analysis demonstrated the faecal isolates are far removed from Eubacterium sensu stricto and that they represent a new sub-line within the Clostridium coccoides group of organisms. Based on phenotypic and phylogenetic criteria, it is proposed that the two strains from faeces be classified as a new genus and species, Anaerostipes caccae. The type strain of Anaerostipes caccae is NCIMB 13811T (= DSM 14662T).

Key words: Anaerostipes caccae – 16S rRNA – taxonomy – phylogeny – human faeces

Introduction

The human intestinal tract harbours an immense diversity of bacteria and the total number of resident bacteria has been estimated to reach 10^{14} cells (SAVAGE, 1977; SUAU et al. 2001). Although the human gut flora has been studied intensively over several decades using traditional culture-based approaches and phenotypic methods of identification, it is now universally recognised that knowledge of the diversity of species present is far from complete. Culture-independent rRNA-based studies (such as PCR rRNA cloning/sequencing and rDNA TGGE) have revealed that the majority of the dominant species within the gut have so far eluded taxonomic description (WILSON and BLITCHINGTON 1996; ZOETENDAL et al. 1998; SUAU et al. 1999). In a recent investigation of butyrate-producing bacteria isolated from human faeces, using 16S rRNA as a tool to aid identification, numerous novel isolates were recovered within the Clostridium coccoides rRNA lineage which did not correspond to recognised species (BARCENILLA et al. 2000). In a subsequent and independent investigation of the human faecal flora, a second Eubacterium-like strain designated P2 was isolated which displayed a high degree of relatedness (16S rRNA) to the butyrate-producing strain L1-92 described by BARCENILLA et al. (2000). In this article we report the phenotypic characteristics of these two isolates and the results of a phylogenetic analysis. Based on the findings presented, we propose the novel bacterium from human faeces be classified as a new genus and species, Anaerostipes caccae.

Materials and Methods

Cultures and cultivation

Strains L1-92T and P2 were isolated from faecal samples from two different healthy volunteers that had not undergone antibiotic therapy in the previous six months. Strains L1-92T was isolated as described by BARCENILLA et al. (2000) and deposited in the National Collection of Industrial and Marine Bacteria (UK) under accession number NCIMB 13811T. For the isolation of strain P2, fresh faecal samples were transferred into an anaerobic workstation (MK3; DW Scientific, Shipley, UK) and diluted serially ten-fold up to 10^{10} in Sorensen buffer (25 mM KH2PO4, 33 mM Na2HPO4·12 H2O, 0.04% (v/v) thioglycolic acid, 0.06% (w/v), cysteine, pH 6.8). Aliquots (1 ml) of the dilutions were plated onto starch plates containing (l–l): 4 g NaHCO3, 33 mM Na2HPO4 × 12 H2O, 0.04% (v/v) thioglycolic acid, 0.06% (w/v), cysteine, pH 6.8).
0.5 g MgCO₃ × 7 H₂O, 0.07 g CaCl₂ × 2H₂O, 5 mg FeSO₄ × 7H₂O, 0.15 mg NaSeO₃, 2 g tryptically digested peptone from casein, 2 g yeast extract, 2 g soluble starch, 15 g agar, 1 mg resazurin, 3 ml trace element solution SL 10 (Wöelle et al., 1983) and 0.5 mg cysteine × HCl, at pH 7.0. Following autoclaving, 1 l medium was supplemented with 20 ml of a filter-sterilised vitamin solution as described by Wöllner et al. (1964). Single colonies were isolated and re-streaked until pure cultures were obtained.

**Phenotypic characterisation**

All incubations were performed using strictly anaerobic conditions. For morphological and physiological studies both strains were grown on Columbia blood agar (bioMérieux), in ST medium (Schwertz et al. 2000) or in a medium used for culturing acetogenic bacteria (Kamlage et al. 1997) but modified by adding 0.5 g Proteose-Peptone No.2 (Difco) per litre (HA medium). The morphology of the isolates was examined by phase contrast microscopy. Growth was monitored by changes in pH and optical density, measured at 600 nm. Cells were tested for catalase and oxidase as described by Simbert and Krieg (1994). Other biochemical features were determined with the API rapid ID32A, API ZYM and API50 CHL systems (bioMérieux) according to the manufacturer’s instructions. Experiments with resting cells were performed as described by Kamlage et al. (1997). Acetate, butyrate, propionate, valerate, iso-valerate and hydrogen were determined by gas chromatography (Hartmann et al. 2000; Schneider et al. 1999). Succinate, ethanol, formate, acetate, D-lactate, L-lactate and glucose were determined enzymatically (Bergmeyer and Grassl, 1984). Acetate utilisation was tested on M2GSC medium (Miyazaki et al. 1997) and the acetate concentrations determined by capillary GC as described in Richardson et al. (1989).

**DNA base composition**

The mol% G + C content of DNA was determined by HPLC according to Misbah et al. (1989) except that the methanol content of the chromatographic buffer was decreased to 8% and the temperature was increased to 37 °C.

**16S rRNA gene sequencing and phylogenetic analysis**

The 16S rRNA genes of the isolates were amplified by PCR and directly sequenced using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, USA) and an automatic DNA sequencer (model 377; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. These sequences and those of the known related strains were retrieved from the GenBank or the Ribosomal Database Project (RDP) databases and aligned with the newly determined sequences using the program DNATOOLS (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually using the program GeneDoc (Nicholas et al. 1997) and a distance matrix was calculated using the program DNADIST (using Kimura’s two-parameter correction) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR and the stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE Felsenstein, 1989).

**Results and Discussion**

The two faecal isolates (L1-92T and P2) were non-sporing, non-motile, strictly anaerobic, rod-shaped organisms. Cells were 0.5-0.6 µm wide × 2.0-4.3 µm long and occurred sometimes in short chains of approximately 2 to 4 cells. In the exponential growth phase cells stained Gram-positive, while stationary phase cells stained Gram-negative. Cultures in ST medium had aropy sediment with little or no turbidity. Both strains formed white-opaque, non-haemolytic colonies on Columbia blood agar and Wilkens Chalgren agar; the colonies were approximately 1 to 3 mm in diameter, circular, convex, smooth and shiny. Using traditional testing the two strains produced acid from D-fructose, fructooligosaccharides (FOS), D-glucose, D-galactose, inositol, maltose, D-mannose, ribose (weak), soluble starch, sucrose, L-sorbosate and sorbitol. Strain L1-92T produced acid (weak) from salicin whereas strain P2 did not. Aesculin was hydrolysed only by strain L1-92T. Both organisms failed to produce acid from L-arabinose, cellobiose, glycerol, inulin, lactose, lactulose, melibiose, melezitose, L-rhamnose, D-trehalose or D-xylene. Using the commercially available API 50 system both strains displayed very similar carbohydrate reactions: acid was produced from adonitol, D-arabitol, D-arabinose, dulcitol, erythritol, galactose, D-glucose, D-fructose, inositol, D-lyxose, maltose, D-mannose, mannitol, melibiose, α-methyl-D-glucoside, N-acetyl-glucosamine, D-raffinose (weak), ribose (weak), L-sorbate, sorbitol, sucrose, D-tagatose, D-turanose and xylose but not from amygdalin, L-arabinose, cellobiose, D-fucose, β-gentiobiose, gluconate, glycerol, glycogen, inulin, lactose, 5-keto-glucuronate, melezitose, α-methyl-D-mannoside, β-methyl-D-xylloside, rhamnose, trehalose, D-xylene or L-xylene. Different results were obtained for arbutin, salicin and 2-keto-glucunonate: strain L1-92T produced acid from arbutin and salicin but not from 2-keto-glucunonate, whereas strain P2 failed to produce acid from the former two substrates but produced acid albeit weakly from 2-keto-glucuronate. In addition strain L1-92T hydrolysed aesculin whereas strain P2 showed a weak reaction.

The enzyme profiles of the two strains were identical using the API ZYM and API rapid ID32A systems. The API ZYM kit, both strains gave positive reactions only for acid phosphatase and phosphoamidase, with all other tests being negative. Using the API rapid ID32A system both isolates displayed activity for arginine dihydrolase and α-galactosidase and were weakly nitrate reductase positive. To ascertain further information about the catabolic potentials of the two isolates, resting cells in 50 mM anoxic potassium phosphate buffer (pH 7.0, 1 mg of resazurin per litre, 5 mM dithioerythritol) were incubated with glucose under N₂/CO₂ (80/20) and the degradation products analysed. The fermentation balance was as follows (CO₂ content was calculated from redox balance): 1 glucose → 0.2 acetate + 0.9 lactate + 0.4 butyrate + 1.2 H₂ + 1 CO₂. The carbon recovery in this experiment was 95%. Growth of L1-92T and P2 on anaerobic M2GSC revealed that both strains were net acetate utilisers in this medium (utilising 6.29 and 6.41 mM respectively). The DNA base composition values for the two faecal isolates L1-92T and P2 were 46.0 and 45.4 mol% G + C, respectively. The genotypic relatedness of the isolates was further investigated by comparative 16S rRNA gene sequence analysis. Almost complete sequences of L1-92T...
(1455 bp) and P2 (1506bp) were determined. Pair-wise analysis showed that the two strains had almost identical 16S rRNA gene sequences (99.9% sequence similarity) and searches of GenBank and RDP databases revealed that the isolates were closely related to members of the Clostridium coccoides group of organisms (Clostridium rRNA cluster XIVa, [COLLINS et al., 1994]). The novel bacterium (as exemplified by isolate L1-92T) formed a distinct sub-line within this cluster, but did not display a particularly close nor statistically significant association (as shown by bootstrap re-sampling) with any recognised species of the Clostridium coccoides group (Fig. 1).

Fig. 1. Unrooted phylogenetic tree showing the phylogenetic relationships of Anaerostipes caccae gen. nov., sp. nov. within the Clostridium coccoides rRNA group or organisms. The tree constructed using the neighbor-joining method was based on a comparison of approximately 1300 nucleotides. Bootstrap values expressed as a percentage of 500 replications are given at the branching points.
It is evident from the results of the taxonomic investigation that the two faecal isolates belong to a hitherto undescribed species. As a strictly anaerobic non spore-forming rod, the unidentified bacterium somewhat resembles the genus *Eubacterium*. It is now recognised however that the genus *Eubacterium* is phenotypically and phylogenetically diverse and should be restricted to *Eubacterium limosum*, the type species of the genus, and its close relatives (viz: *E. barkeri*, *E. callanderi* and *E. aggregans*; Willems and Collins, 1996). The human isolates described here are phylogenetically only remotely related to *E. limosum* and close relatives (i.e. *Eubacterium sensu stricto*, Willems and Collins, 1996), with the latter species forming a different cluster (rRNA cluster XV, Collins et al. 1994) and displaying approximately 20% sequence divergence with the unidentified rod-shaped bacterium. Hence the new faecal bacterium cannot be assigned to the genus *Eubacterium sensu stricto*. Phylogenetically the novel rod-shaped bacterium is only distantly related to recognised species and genera within the *Clostridium coccoides* group (displaying divergence values of greater than 10%). The unidentified bacterium is also phenotypically incompatible with all currently named genera within this rRNA cluster. For example the new rod-shaped bacterium can be distinguished from *Clostridium* spp. and *Sporobacterium* in not producing endospores, from *Coprococcus*, *Lachnospira* and *Ruminococcus* by cellular morphology and end-products of glucose metabolism, and from *Butyrivibrio* and *Roseburia* in being non-motile and end-products of glucose metabolism. The human faecal bacterium can also be biochemically readily distinguished from miss-classified *Eubacterium* species (such as *E. hallii*) within the *C. coccoides* cluster (see Table 1). These miss-classified eubacterial species invariably display 10% or more sequence divergence with the unidentified bacterium and therefore cannot be considered members of the same genus. Thus it is clear that the novel faecal bacterium reported here, is both phylogenetically and phenotypically incompatible with *Eubacterium sensu stricto* and all recognised genera within the *C. coccoides* rRNA cluster, and merits classification in a separate genus. Therefore based on the presented findings we propose that the unknown rod-shaped organism be classified as a new genus and species, *Anaerostipes caccae*.

**Table 1. Characteristics which are useful in differentiating *Anaerostipes caccae* from some miss-classified *Eubacterium* species of the *Clostridium coccoides* rRNA group.**

<table>
<thead>
<tr>
<th>Test/Species</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid end products</td>
<td>A&lt;sup&gt;1&lt;/sup&gt;, B&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, E&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, E&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, E&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>A, B&lt;sup&gt;1&lt;/sup&gt;, F&lt;sup&gt;1&lt;/sup&gt;, L&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Motility</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>+</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>v&lt;sup&gt;1&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> – *Anaerostipes caccae*; 2 – *E. cellulosolvens*; 3 – *E. contortum*; 4 – *E. eligens*; 5 – *E. fiscicatena*; 6 – *E. formicigenerans*; 7 – *E. hallii*; 8 – *E. ramulus*; 9 – *E. rectale*; 10 – *E. uniforme*; 11 – *E. ventriosum*; 12 – *E. xylanophilum*; 13 – *E. ruminantium*.

<sup>1</sup> A – Acetic acid; B – butyric acid; But – butanol; E – ethanol; F – formic acid; L – lactic acid.

<sup>1</sup> v – variable; nd – not determined.
Description of Anaerostipes

_Anaerostipes_ (an. ae. ro. sti'pes) Gr. pref. an not; Gr. n. aer air; anaero not living in air; L. masc. n. stipes club of stick; _Anaerostipes_, a stick not living in air) consists of non-motile, non spore-forming rod-shaped cells. Cells are Gram-positive, but in older cultures may stain Gram-negative. Strictly anaerobic and catalase and oxidase negative. Non-haemolytic. Glucose and some other sugars may be fermented. Butyrate, acetate and lactate are the major products of glucose metabolism. Acetate is utilised. Arginine dihydrolase, phosphoamidase, and α-galactosidase are produced. Gelatin and urease are not hydrolysed. Indole is not produced. Nitrate is reduced. The DNA G + C content is 45.5–46.0 mol%. The type species is _Anaerostipes cacaee_.

Description of _Anaerostipes cacaee_ sp. nov.

_Annaerostipes cacaee_ (cacaee, pronounced kak’ka Gr. n. kakke faeces: N.L. gen. n. cacaee of faeces) cells consist of non-motile, non spore-forming rods. Individual cells are 0.5–0.6 μm in width × 2.0–4.0 μm in length and occur in chains of up to 4 cells. Cells stain Gram-positive but older cultures (>16 h) may stain Gram-negative. On Columbia blood agar, white-opaque colonies are formed that are 1–3 mm in diameter, circular, convex, smooth, shiny and sticky, which are non haemolytic. The strains are strictly anaerobic and catalase and oxidase negative. Butyrate, acetate and lactate are the major products of glucose metabolism. Acetate is utilised. Using conventional tests acid is produced from D-fructose, FOS, D-glucose, D-galactose, inositol, maltose, D-mannose, ribose (weak), soluble starch, sucrose, L-sorbose and sorbitol. Acid may or may not be produced from salicin. Acid is not produced from L-arabinose, cellobiose, glycerol, inulin, lactose, lactulose, melibiose, melizitose, L-rhamnose, D-trehalose or D-xylene. Using the miniaturised API 50 system acid is produced from adonitol, D-arabitol, L-arabinose, dextrose, erythritol, galactose, D-glucose, glucuronate, D-fructose, inositol, D-lyxose, maltose, D-mannose, mannitol, melibiose, α-methyl-D-glucoside, N-acetyl-glucosamine, D-arabinose (weak), D-sorbitose, sorbitol, sucrose, D-tagatose, D-turanose and xylitol but not from amygdalin, L-arabinose, cellobiose, D-fucose, β-gentiobiose, glycerol, glycogen, inulin, lactose, 5-keto-gluronate, melezitose, α-methyl-D-mannose, β-methyl-D-xylloside, rhamnose, trehalose, D-xylene or L-xylene. Acid may or may not be produced from arbutin, salicin and 2-keto-gluronate. Aesculin hydrolysis is variable. Using API ZYM and API rapid ID32A systems activity is detected for acid phosphatase and phosphoamidase, and arginine dihydrolase, α-galactosidase and nitrate reductase (weak reaction) respectively. No activity is detected for alanine arylamidase, alkaline phosphatase, arginine arylamidase, α-arabinosidase, chymotrypsin, cystine arylamidase, esterase C4, ester lipase C8, α-fucosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, β-glucuronidase, glycine arylamidase, glutamylglutamic acid arylami-


Corresponding author:: Andreas Schwiertz, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Arthur-Scheunert-Allee 114–116, D - 14558 Bergholz-Rehbrücke, Germany. Tel.: ++49-3320088440; Fax: ++49-3320088407; e-mail: andy@www.dife.de