Bacteroides xylanisolvens sp. nov., a xylan-degrading bacterium isolated from human faeces

Christophe Chassard,¹ Eve Delmas,¹ Paul A. Lawson²
and Annick Bernalier-Donadille¹

Correspondence

Annick Bernalier-Donadille
bernal@clermont.inra.fr

¹Unité de Microbiologie, INRA, Centre de Recherches de Clermont Ferrand – Theix,
63 122 Saint Genès-Champagnelle, France

²Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA

During the course of a study on the xylan-degrading community from the human gut, six xylanolytic, Gram-negative, anaerobic rods were isolated from faecal samples. 16S rRNA gene sequence analysis showed that the isolates were closely related to each other ($\geq 99\%$ sequence similarity) and that they belonged to the genus *Bacteroides*. On the basis of 16S rRNA gene sequence similarity, representative strain XB1A^T was most closely related to the type strains of *Bacteroides ovatus* (97.5%), *B. finegoldii* (96.5%) and *B. thetaiotaomicron* (95.5%). DNA–DNA hybridization results revealed that strain XB1A^T was distinct from its closest relative, *B. ovatus*. The DNA G+C content of strain XB1A^T (42.8 mol%) and major fatty acid composition (anteiso-C_{15:0}, 33.8%) further supported its affiliation to the genus *Bacteroides*. The novel isolates degraded different types of xylan, and were also able to grow on a variety of carbohydrates. Unlike most other *Bacteroides* species isolated from the human gut, these isolates were not able to degrade starch. Other biochemical tests further demonstrated that strain XB1A^T could be differentiated from the closest related *Bacteroides* species. Xylan and sugars were converted by strain XB1A^T mainly into acetate, propionate and succinate. Based on physiological, phenotypic and phylogenetic data, the six novel strains are considered to represent a novel species of the genus *Bacteroides*, for which the name *Bacteroides xylanisolvens* sp. nov. is proposed. The type strain is XB1A^T (=DSM 18836^T =CCUG 53782^T).

Plant fibres constitute one of the main sources of polysaccharides in the human colon that are degraded extensively by the endogenous microbial community. Plant cell-wall polysaccharides, which mainly include celluloses and hemicelluloses (xylan, arabinoxylan, etc.), represent about 30% of the total dietary fibre ingested by humans daily. Xylan is the most abundant of these cell-wall polysaccharides. Despite the extensive degradation of xylan in the colon (Slavin *et al.*, 1981), the diversity of the predominant bacteria involved in xylan breakdown has not been explored extensively (but see Bayliss & Houston, 1984; Wedekind *et al.*, 1988; Chassard *et al.*, 2007). *Bacteroides* species, which constitute a predominant bacterial group of the human gut microbiota, play key roles in the degradation and fermentation of organic matter present in the colon (Salysers, 1995). Some *Bacteroides* species have been shown to be able to obtain carbon and energy via hydrolysis of soluble or well-hydrated polysaccharides (Salysers, 1995; Robert *et al.*, 2007; Chassard *et al.*, 2007). Significant xylanolytic activity has

been found in some *Bacteroides* species isolated from the human gut, in particular in *Bacteroides ovatus* and *Bacteroides fragilis* subspecies A (Salysers *et al.*, 1977; Hespell & Whitehead, 1990). The xylanase system of *B. ovatus* was subsequently explored in detail by Weaver *et al.* (1992). During an investigation of the xylan-degrading microbial community from the human colon (Chassard *et al.*, 2007), we isolated the xylanolytic strains reported herein.

Six bacterial strains (XB1A^T, XB4A, XB3A, XB2A2, XB41A and XO85C13) were isolated from the faeces of three healthy French adults (strains XB1A^T, XB4A and XO85C13 were from a 31-year-old male, strain XB41A was from a 40-year-old female and strains XB3A and XB2A2 were from a 43-year-old female). All liquid and solid media were prepared, dispensed and inoculated by using strictly anaerobic techniques (Hungate, 1969), with 100% O₂-free CO₂ gas. Freshly voided faeces (1 g) were transferred into 10 ml sterile anoxic mineral solution. Serial 10-fold dilutions down to 10⁻¹¹ of this faecal suspension were then carried out in mineral solution. These faecal dilutions were inoculated (0.3 ml) into liquid basal xylanolytic (BX) medium (10 ml per tube) with commercial (Sigma) oat

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XB1A^T is AM230650.

spelts or birchwood xylan as the sole energy source (Chassard *et al.*, 2007). After 15 days incubation at 37 °C, the presence of xylanolytic organisms in faecal dilution cultures was estimated by measuring xylanase activity. Detection of xylanase activity was performed by using an agar plate method as described by Forano *et al.* (1994) and Chassard *et al.* (2007). Xylanolytic bacteria were then isolated from the highest-dilution faecal cultures showing xylanase activity (10^{-8} – 10^{-9}). The medium used for isolation of xylanolytic strains was solid BX medium containing oat-spelt xylan as energy source. All strains were isolated by using the roll-tube technique. After three to five successive subcultures on roll-tubes and BX medium broth, isolates were examined for purity by phase-contrast microscopy, from cultures grown with oat-spelt xylan and xylose (both at 2 g l⁻¹). The medium used for further routine cultivation of the xylanolytic isolates was BX medium with oat-spelt xylan as substrate.

DNA extraction, PCR and sequencing of the 16S rRNA gene of the novel strains were performed as described by Robert *et al.* (2007). Xylanolytic strains were grown for 24 h in 50 ml semi-synthetic BC medium (Robert & Bernalier-Donadille, 2007) with glucose (2 g l⁻¹) as carbon source. Cells were harvested by centrifugation for 15 min at 9000 g at 4 °C. The bacterial pellet was then subjected to DNA extraction (Easy DNA kit genomic DNA isolation; Invitrogen). 16S rRNA genes of the strains investigated were then amplified by using the universal primers F8 (5'-AGAGTTTGATCMTGGCTC-3') and 1492R (5'-GNTA-CCTTGTTACGACTT-3'). PCR products were purified by using the Strataprep PCR purification kit (OZYME) and were sequenced by use of a Big Dye Terminator Cycle Sequencing kit and ABI PRISM 310 automated sequencer (Applied Biosystems) according to the manufacturer's specifications, yielding continual stretches of >1400 bases. The closest known relatives of the new isolates were determined by performing database searches with the program FASTA (Pearson & Lipman, 1985). These reference sequences and those of other related strains were retrieved from GenBank and aligned with the newly determined sequences by using the program SEQtools (Rasmussen, 2002). The resulting multiple-sequence alignment was corrected manually by using the program GeneDoc (Nicholas *et al.*, 1997) and a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) with the programs SEQtools and TreeView (Page, 1996). The G + C content of the genomic DNA was determined by HPLC by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany).

The six new isolates were found to be genetically highly related to each other, displaying levels of 16S rRNA gene sequence similarity of between 99 and 100 %. Treeing analysis revealed that the new isolates were members of the *Bacteroides*–*Prevotella*–*Porphyromonas* rRNA supercluster of organisms (data not shown), and demonstrated a specific affinity with members of the genus *Bacteroides*

(Chassard *et al.*, 2007). The phylogenetic position of strain XB1A^T, by using a reduced dataset, is shown in Fig. 1. Analysis showed that this strain clustered with *B. ovatus* NCTC 11153^T (97.5 % 16S rRNA sequence similarity), *B. finegoldii* JCM 13345^T (96.6 %) and *B. thetaiotaomicron* ATCC 29148^T (95.6 %). There is no precise correlation between the level of 16S rRNA gene sequence divergence and species delineation, but it is generally recognized that similarity values of ≤97 % are significant (Stackebrandt & Goebel, 1994). However, Stackebrandt & Ebers (2006) have made the recommendation that this value can be increased to 98.7–99 % without sacrificing the quality and precision of a 'species' description, and as an aid to taxonomists. To determine the genotypic relatedness of strain XB1A^T, DNA–DNA hybridization experiments between this xylanolytic isolate and the type strain of its closest relative, *B. ovatus*, was performed by the DSMZ Identification Service. DNA from strain XB1A^T and *B. ovatus* DSM 1896^T was isolated by using a French pressure cell from cells harvested from BHI broth medium and was further purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA hybridization experiments were carried out as described by De Ley *et al.* (1970) as modified by Huß *et al.* (1983) by using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe. Strain XB1A^T showed a level of DNA–DNA relatedness of 41.9 % with its closest neighbour, *B. ovatus* DSM 1896^T. This low level of DNA–DNA relatedness (<70 %) with the most closely related *Bacteroides* species indicated that strain XB1A^T represented a novel species. The G + C content of strain XB1A^T was 42.8 mol%, compared with values of 40–42, 42–43 and 42.4–43.0 mol% for *B. ovatus*, *B. thetaiotaomicron* and *B. finegoldii*, respectively (Table 1). The DNA G + C content of strain XB1A^T thus supported its affiliation with members of the genus *Bacteroides*, which have a DNA G + C content of 40–48 mol% (Shah, 1992).

Cell morphology and motility were studied in 18 h xylose-grown cultures by phase-contrast microscopy and by electron microscopy after negative staining of whole cells with 2 % uranyl acetate. The Gram reaction was determined by using the conventional Gram stain. Cell morphology and cell-wall structure were further observed on ultrathin sections by transmission electron microscopy (Philips 400) after staining with uranyl acetate and lead citrate (Bernalier *et al.*, 1996). The presence of catalase and cytochrome oxidase was examined by standard methods. The optimal growth temperature of strain XB1A^T was determined in semi-synthetic BC medium (Robert & Bernalier-Donadille, 2007) containing xylose (2 g l⁻¹), at pH 6.8, over the temperature range 25–45 °C (at 1 °C intervals). The influence of pH on growth of strain XB1A^T was evaluated in semi-synthetic BC medium containing xylose (2 g l⁻¹) at 37 °C. The pH range studied was 5.5–7.5 (at 0.1 pH unit intervals, adjusted with varying concentrations of NaHCO₃ in the medium).

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain XB1A^T and some related members of the genus *Bacteroides*. The tree was based on a comparison of approximately 1350 nt. Bootstrap values, expressed as percentages of 500 replications, are given at branch points (only values $\geq 90\%$ are shown). Bar, 1% sequence divergence.

Cells of strain XB1A^T were rods with rounded ends, with a mean length of 1.8–2.5 μm and mean width of 0.2–0.3 μm . Cells occurred singly or in pairs. Cells stained Gram-negative and thin sections examined by transmission electron microscopy showed a Gram-negative cell-wall structure. Negatively stained cells revealed the absence of flagella. Viable cells could not be recovered from cultures held at 100 °C for 10 min and no spores were observed in xylan- or in xylose-grown cultures incubated at 37 °C for almost 1 month. Strain XB1A^T was strictly anaerobic and required an O₂-free medium at a redox potential sufficient to decolorize resazurin ($E'_0 = -50\text{ mV}$). Strain XB1A^T was catalase- and cytochrome oxidase-negative, unlike *B. ovatus* and *B. thetaiotaomicron*, which are catalase-positive (Moore & Holdeman, 1974). With xylose as substrate, the optimal growth temperature was 38 °C, with growth occurring from 25 to 42 °C. Strain XB1A^T grew from an initial pH of 6.0 up to 7.2, with optimal growth at pH 6.8. These optimal growth conditions are in line with those found in the human colon. Rumen fluid was not required for growth.

The results of further phenotypic analyses are given in the species description. Other physiological, biochemical and enzyme activity tests were performed by using API 20A and API Rapid ID 32A kits (bioMérieux), according to the manufacturer's instructions, with incubation at 37 °C in an anaerobic chamber. Results of chemotaxonomic analyses of strain XB1A^T and *B. ovatus* JCM 5824^T, *B. thetaiotaomicron* JCM 5827^T and *B. finegoldii* DSM 17565^T are summarized in Table 1. Strain XB1A^T could be differentiated from *B. ovatus* JCM 5824^T by its ability to use mannitol, melezitose and sorbitol and to produce acid from glycerol. Furthermore, strain XB1A^T expressed glutamyl glutamic acid arylamidase activity, in contrast to the results for *B. ovatus*, and *B. ovatus* expressed leucine arylamidase activity, whereas strain XB1A^T did not. Several characteristics allowed us to differentiate strain XB1A^T from *B. thetaiotaomicron* DSM 5827^T and *B. finegoldii* DSM 17565^T (Table 1). In particular, *B. thetaiotaomicron* showed a large number of positive results in tests for enzyme activities, in contrast to the results for strain XB1A^T and *B. finegoldii*. Strain XB1A^T was able to utilize trehalose, D-mannitol,

Table 1. Differential characteristics between strain XB1A^T and related species of the genus *Bacteroides*

Strains: 1, XB1A^T; 2, *B. ovatus* JCM 5824^T; 3, *B. thetaiotaomicron* JCM 5827^T; 4, *B. finegoldii* JCM 13345^T. Data for reference strains are from Bakir *et al.* (2006) except where indicated. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Starch utilization	–	+	+	ND
Acid produced from:				
Salicin	+	+	–	+
Trehalose	+	+	+	–
D-Mannitol	+	–	–	–
Glycerol	+	–	–	–
Melezitose	+	–	–	–
D-Sorbitol	+	–	–	–
Enzyme activities				
Arginine arylamidase	–	–	+	–
Phenylalanine arylamidase	–	–	+	–
Leucine arylamidase	–	+	+	–
Tyrosine arylamidase	–	–	+	–
Glycine arylamidase	–	–	+	–
Histidine arylamidase	–	–	+	–
Glutamyl glutamic acid arylamidase	+	–	+	–
Serine arylamidase	–	–	+	–
DNA G + C content (mol%)	42.8	42	43	42.4

*Data from Salyers *et al.* (1977).

glycerol, melezitose and D-sorbitol, whereas *B. finegoldii* is unable to ferment these substrates (Bakir *et al.*, 2006).

The cellular fatty acid composition of strain XB1A^T was determined by HPLC at the DSMZ. The major cellular fatty acid was anteiso-C_{15:0} (33.8 %), in agreement with data for the genus *Bacteroides* as described by Miyagawa *et al.* (1979). Other cellular fatty acids found in strain XB1A^T included iso-C_{17:0} 3-OH (23.5 %) and iso-C_{15:0} (10.3 %). *B. ovatus* JCM 5824^T, *B. thetaiotaomicron* JCM 5827^T and *B. finegoldii* DSM 17565^T also contain anteiso-C_{15:0}, iso-C_{15:0} and iso-C_{17:0} 3-OH as major cellular fatty acids, but the proportions of iso-C_{15:0} and iso-C_{17:0} 3-OH were lower in these species than in strain XB1A^T (7–9 and 12–16 %, respectively).

Degradation and fermentation of different polysaccharides, such as xylan, starch or cellulose found in dietary fibres, by strain XB1A^T were studied in BX broth medium (10 ml per tube) containing 100 mg polysaccharide as sole energy source. Cultures were incubated at 37 °C for 2–4 days. After incubation, substrate utilization was estimated by measuring bacterial growth [optical density of the cultures at 600 nm or determination of bacterial protein content by the method of Bradford (1976)]. Xylan fermentation by strain XB1A^T was further studied in 4-day cultures. Gases in the headspace of cultures were analysed by gas-phase chromatography (Chassard *et al.*, 2005). Short-chain fatty

acids in culture supernatants were quantified by ¹D¹H NMR (Matulova *et al.*, 2005). The xylanase activity of strain XB1A^T was determined by enzyme assays in which reducing sugars released from xylan oat spelts were measured colorimetrically (Forano *et al.*, 1994).

Strain XB1A^T was able to degrade xylan from different botanical origins (oat spelts and birchwood), as also described for *B. ovatus* (Cooper *et al.*, 1985). Whereas most of the *Bacteroides* species, including *B. ovatus* and *B. thetaiotaomicron*, isolated from the human gut are able to ferment starch (Salyers *et al.*, 1977), one of the main components of dietary fibre, strain XB1A^T did not exhibit the ability to degrade this substrate (Table 1). Strain XB1A^T was also unable to use cellulose as an energy source and did not show cellulase activity. In 2-day-old xylan-grown cultures of strain XB1A^T, high xylanase activities [14.1 ± 1.6 µg xylose (mg protein^{–1})] were measured that are similar to those reported for *B. ovatus* (Weaver *et al.*, 1992), and further demonstrate the great xylanolytic potential of the new isolate. The end-products of xylan or xylose fermentation by strain XB1A^T were mainly acetate, propionate and succinate; small amounts of H₂ were also produced. Similarly, the majority of *Bacteroides* species from the human gut reportedly produce acetate, propionate and succinate from sugar fermentation as main end-products (Moore & Holdeman, 1974; Salyers *et al.*, 1981).

On the basis of these phenotypic, genotypic and phylogenetic differences from recognized species, we propose that strain XB1A^T represents a novel species of the genus *Bacteroides*. Strain XB1A^T showed properties typical for the genus *Bacteroides*: Gram-negative, non-spore-forming, non-motile rods; obligately anaerobic growth; DNA G + C content in the range 40–48 mol% (Shah, 1992); saccharolytic, producing acetate and succinate as the major metabolic end-products (Holdeman & Moore, 1974); and anteiso-C_{15:0} as the major cellular fatty acid (Miyagawa *et al.*, 1979). The closest recognized relative of strain XB1A^T was *B. ovatus*, which was isolated from the human colon, but these two taxa showed a low level of DNA–DNA relatedness (<70 % between strain XB1A^T and *B. ovatus* DSM 1896^T). Strain XB1A^T and *B. ovatus* are able to degrade xylan but they showed important differences in their phenotypic characteristics, such as the expression of catalase, ability to degrade starch and ability to ferment glycerol, melezitose and D-sorbitol. The name *Bacteroides xylanisolvens* sp. nov. is proposed to accommodate the novel strains described herein.

Description of *Bacteroides xylanisolvens* sp. nov.

Bacteroides xylanisolvens (xy.lan.i.sol'vens. N.L. n. *xylanum* xylan, a vegetal polymer; L. part. adj. *solvens* dissolving; N.L. part. adj. *xylanisolvens* xylan-dissolving, referring to the ability of the micro-organism to degrade xylan).

Cells are non-motile rods, 1.8–2.5 µm long and 0.2–0.3 µm wide. Gram-negative by staining and cell-wall ultrastructure.

No heat-resistant endospores are formed. Strictly anaerobic. Cytochrome oxidase- and catalase-negative. Metabolizes xylan to acetate, propionate and succinate. Poor growth is exhibited with arabinoxylan, pectin and galacturonic acid. No growth occurs with starch or cellulose. Growth occurs between 25 and 42 °C and at pH 6.0–7.2. Optimal growth conditions are 38 °C and pH 6.8. Rumen fluid is not required for growth. Indole-negative but able to hydrolyse aesculin. Nitrate is not reduced. No activity is detected for urease, and gelatin is not hydrolysed. Acid is produced from glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose. Also able to ferment fructose, ribose, galactose and melibiose. Positive reactions are obtained (API Rapid ID 32A) for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -arabinosidase, *N*-acetyl- β -glucosamidase, glutamic acid decarboxylase, α -fucosidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Negative reactions are obtained for arginine dihydrolase, 6-phospho- β -galactosidase, β -glucuronidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. The major fatty acids are anteiso-C_{15:0} (33.8%), iso-C_{17:0} 3-OH (23.5%) and iso-C_{15:0} (10.3%). The DNA G+C content of the type strain is 42.8 mol%.

The type strain, XB1A^T (=DSM 18836^T =CCUG 53782^T), was isolated from human faeces of a non-methane-excreting individual. Strains XB4A, XB3A, XB2A2, XB41A and XO85C13, isolated from similar sources, are also included in the species.

Acknowledgements

C. C. was supported by a fellowship from the French Ministère de la Recherche et de l'Enseignement Supérieur. We express our thanks to V. Goumy and A. Ameilbone for technical assistance.

References

- Bakir, M. A., Kitahara, M., Sakamoto, M., Matsumoto, M. & Benno, Y. (2006). *Bacteroides finegoldii* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **56**, 931–935.
- Bayliss, C. E. & Houston, A. P. (1984). Characterization of plant polysaccharide- and mucin-fermenting anaerobic bacteria from human feces. *Appl Environ Microbiol* **48**, 626–632.
- Bernalier, A., Willems, A., Leclerc, M., Rochet, V. & Collins, M. D. (1996). *Ruminococcus hydrogenotrophicus* sp. nov., a new H₂/CO₂-utilizing acetogenic bacterium isolated from human feces. *Arch Microbiol* **166**, 176–183.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chassard, C., Gaillard-Martinie, B. & Bernalier-Donadille, A. (2005). Interaction between H₂-producing and non-H₂-producing cellulolytic bacteria from the human colon. *FEMS Microbiol Lett* **242**, 339–344.
- Chassard, C., Goumy, V., Leclerc, M., Del'Homme, C. & Bernalier-Donadille, A. (2007). Characterization of the xylan-degrading microbial community from human faeces. *FEMS Microbiol Ecol* **61**, 121–131.
- Cooper, S. W., Pfeiffer, D. G. & Tally, F. P. (1985). Evaluation of xylan fermentation for the identification of *Bacteroides ovatus* and *Bacteroides thetaiotaomicron*. *J Clin Microbiol* **22**, 125–126.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Forano, E., Brousolle, V., Gaudet, G. & Bryant, J. A. (1994). Molecular cloning, expression and characterization of a new endoglucanase gene from *Fibrobacter succinogenes* S85. *Curr Microbiol* **28**, 7–14.
- Hespell, R. B. & Whitehead, T. R. (1990). Physiology and genetics of xylan degradation by gastrointestinal tract bacteria. *J Dairy Sci* **73**, 3013–3022.
- Holdeman, L. V. & Moore, W. E. C. (1974). Gram-negative anaerobic bacteria. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 384–385. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- Hungate, R. E. (1969). A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Matulova, M., Nouaille, R., Capek, P., Péan, M., Forano, E. & Delort, A. M. (2005). Degradation of wheat straw by *Fibrobacter succinogenes* S85: a liquid and solid NMR study. *Appl Environ Microbiol* **71**, 1247–1253.
- Miyagawa, E., Azuma, R. & Suto, T. (1979). Cellular fatty acid composition in Gram-negative obligately anaerobic rods. *J Gen Appl Microbiol* **25**, 41–51.
- Moore, W. E. C. & Holdeman, L. V. (1974). Gram-negative anaerobic bacteria. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 384–385. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- Nicholas, K. B., Nicholas, H. B., Jr & Deerfield, D. W., II (1997). GeneDoc: analysis and visualization of genetic variation. *EMBnet News* **4**, 2, 1–4. http://www.embnet.org/download/embnetnews/embnet_news_4_2.pdf
- Page, R. D. M. (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Pearson, W. R. & Lipman, D. J. (1985). Rapid and sensitive protein similarity searches. *Science* **227**, 1435–1441.
- Rasmussen, S. W. (2002). SEQtools, a software package for analysis of nucleotide and protein sequences. <http://www.seqtools.dk>
- Robert, C. & Bernalier-Donadille, A. (2003). The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. *FEMS Microbiol Ecol* **46**, 81–89.
- Robert, C., Chassard, C., Lawson, P. A. & Bernalier-Donadille, A. (2007). *Bacteroides cellulosilyticus* sp. nov., a cellulolytic bacterium from the human colon. *Int J Syst Evol Microbiol* **57**, 1516–1520.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Salysers, A. A. (1995). Fermentation of polysaccharides by human colonic anaerobes. In *Dietary Fibre*, pp. 29–35. Edited by

C. Cherbut, J. L. Barry, D. Lairon & M. Durand. Paris: John Libbey Eurotext.

Salyers, A. A., Vercellotti, J. R., West, S. & Wilkins, T. D. (1977). Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl Environ Microbiol* **33**, 319–322.

Salyers, A. A., Gherardini, F. & O'Brien, M. (1981). Utilization of xylan by two species of human colonic *Bacteroides*. *Appl Environ Microbiol* **41**, 1065–1068.

Shah, H. N. (1992). The genus *Bacteroides* and related taxa. In *The Prokaryotes*, 2nd edn, pp. 3593–3607. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. Schleifer. New York: Springer.

Slavin, J. L., Brauer, P. M. & Marlett, J. A. (1981). Neutral detergent fiber, hemicellulose and cellulose digestibility in human subjects. *J Nutr* **111**, 287–297.

Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Weaver, J., Whitehead, T. R., Cotta, M. A., Valentine, P. C. & Salyers, A. A. (1992). Genetic analysis of a locus on the *Bacteroides ovatus* chromosome which contains xylan utilization genes. *Appl Environ Microbiol* **58**, 2764–2770.

Wedekind, K. J., Mansfield, H. R. & Montgomery, Y. L. (1988). Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. *Appl Environ Microbiol* **54**, 1530–1535.