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

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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 (> 99% sequence similarity) and that they belonged to the genus Bacteroides. On the basis of 16S rRNA gene sequence similarity, representative strain XB1A\textsuperscript{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\textsuperscript{T} was distinct from its closest relative, B. ovatus. The DNA G+C content of strain XB1A\textsuperscript{T} (42.8 mol%) and major fatty acid composition (anteiso-C\textsubscript{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\textsuperscript{T} could be differentiated from the closest related Bacteroides species. Xylan and sugars were converted by strain XB1A\textsuperscript{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\textsuperscript{T} (=DSM 18836\textsuperscript{T} = CCUG 53782\textsuperscript{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 (Salyers, 1995). Some Bacteroides species have been shown to be able to obtain carbon and energy via hydrolysis of soluble or well-hydrated polysaccharides (Salyers, 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 (Salyers 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\textsuperscript{T}, XB4A, XB3A, XB2A2, XB41A and XO85C13) were isolated from the faeces of three healthy French adults (strains XB1A\textsuperscript{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% O\textsubscript{2}-free CO\textsubscript{2} gas. Freshly voided faeces (1 g) were transferred into 10 ml sterile anoxic mineral solution. Serial 10-fold dilutions down to 10\textsuperscript{-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\textsuperscript{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 (10⁻⁸⁻¹⁰⁻⁸). 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'-GNTAGCTACGGTAC-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 XB1AT, by using a reduced dataset, is shown in Fig. 1. Analysis showed that this strain clustered with B. ovatus NCTC 11153T (97.5% 16S rRNA sequence similarity), B. finegoldii JCM 13345T (96.6%) and B. thetaiotaomicron ATCC 29148T (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 XB1AT, 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 XB1AT and B. ovatus DSM 1896T 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 x 6 multicell changer and a temperature controller with in situ temperature probe. Strain XB1AT showed a level of DNA–DNA relatedness of 41.9% with its closest neighbour, B. ovatus DSM 1896T. This low level of DNA–DNA relatedness (<70%) with the most closely related Bacteroides species indicated that strain XB1AT represented a novel species. The G + C content of strain XB1AT 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 XB1AT 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 XB1AT 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 XB1AT 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).
Cells of strain XB1AT 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 XB1AT was strictly anaerobic and required an O2-free medium at a redox potential sufficient to decolorize resazurin (E0 5250 mV). Strain XB1AT 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 XB1AT 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 XB1AT and B. ovatus JCM 5824T, B. thetaiotaomicron JCM 5827T and B. finegoldii DSM 17565T are summarized in Table 1. Strain XB1AT could be differentiated from B. ovatus JCM 5824T by its ability to use mannitol, melezitose and sorbitol and to produce acid from glycerol. Furthermore, strain XB1AT expressed glutamyl glutamic acid arylamidase activity, in contrast to the results for B. ovatus, and B. ovatus expressed leucine arylamidase activity, whereas strain XB1AT did not. Several characteristics allowed us to differentiate strain XB1AT from B. thetaiotaomicron DSM 5827T and B. finegoldii DSM 17565T (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 XB1AT and B. finegoldii. Strain XB1AT was able to utilize trehalose, D-mannitol,
glycerol, melezitose and D-sorbitol, whereas \textit{B. finegoldii} is unable to ferment these substrates \cite{Bakir2006}.

The cellular fatty acid composition of strain XB1A\textsuperscript{T} was determined by HPLC at the DSMZ. The major cellular fatty acid was anteiso-C\textsubscript{15:0} (33.8 \%), in agreement with data for the genus \textit{Bacteroides} as described by Miyagawa \textit{et al.} \cite{Miyagawa1979}. Other cellular fatty acids found in strain XB1A\textsuperscript{T} included iso-C\textsubscript{17:0} 3-OH (23.5 \%) and iso-C\textsubscript{15:0} (10.3 \%). \textit{B. ovatus} JCM 5824\textsuperscript{T}, \textit{B. thetaiotaomicron} JCM 5827\textsuperscript{T} and \textit{B. finegoldii} DSM 17565\textsuperscript{T} also contain anteiso-C\textsubscript{15:0} iso-C\textsubscript{15:0} and iso-C\textsubscript{17:0} 3-OH as major cellular fatty acids, but the proportions of iso-C\textsubscript{15:0} and iso-C\textsubscript{17:0} 3-OH were lower in these species than in strain XB1A\textsuperscript{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\textsuperscript{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\textsuperscript{T} was further studied in 4-day cultures. Gases in the headspace of cultures were analysed by gas-phase chromatography \cite{Chassard2005}. Short-chain fatty acids in culture supernatants were quantified by \textsuperscript{1}D\textsuperscript{1}H NMR \cite{Matulova2005}. The xylanase activity of strain XB1A\textsuperscript{T} was determined by enzyme assays in which reducing sugars released from xylan oat spelts were measured colorimetrically \cite{Forano1994}.

Strain XB1A\textsuperscript{T} was able to degrade xylan from different botanical origins (oat spelts and birchwood), as also described for \textit{B. ovatus} \cite{Cooper1985}. Whereas most of the \textit{Bacteroides} species, including \textit{B. ovatus} and \textit{B. thetaiotaomicron}, isolated from the human gut are able to ferment starch \cite{Salyers1977}, one of the main components of dietary fibre, strain XB1A\textsuperscript{T} did not exhibit the ability to degrade this substrate (Table 1). Strain XB1A\textsuperscript{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\textsuperscript{T}, high xylanase activities [14.1 ± 1.6 μg xylene (mg protein\textsuperscript{-1})\textsuperscript{1}] were measured that are similar to those reported for \textit{B. ovatus} \cite{Weaver1992}, and further demonstrate the great xylanolytic potential of the new isolate. The end-products of xylan or xylene fermentation by strain XB1A\textsuperscript{T} were mainly acetate, propionate and succinate; small amounts of H\textsubscript{2} were also produced. Similarly, the majority of \textit{Bacteroides} species from the human gut reportedly produce acetate, propionate and succinate from sugar fermentation as main end-products \cite{Moore1974,Salyers1981}.

On the basis of these phenotypic, genotypic and phylogenetic differences from recognized species, we propose that strain XB1A\textsuperscript{T} represents a novel species of the genus \textit{Bacteroides}. Strain XB1A\textsuperscript{T} showed properties typical for the genus \textit{Bacteroides}: Gram-negative, non-spore-forming, non-motile rods; obligately anaerobic growth; DNA G+C content in the range 40–48 mol\% \cite{Shah1992}; saccharolytic, producing acetate and succinate as the major metabolic end-products \cite{Holdeman1974}; and anteiso-C\textsubscript{15:0} as the major cellular fatty acid \cite{Miyagawa1979}. The closest recognized relative of strain XB1A\textsuperscript{T} was \textit{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\textsuperscript{T} and \textit{B. ovatus} DSM 1896\textsuperscript{T}). Strain XB1A\textsuperscript{T} and \textit{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 \textit{Bacteroides xylanisolvens} sp. nov. is proposed to accommodate the novel strains described herein.

### Description of \textit{Bacteroides xylanisolvens} sp. nov.

\textit{Bacteroides xylanisolvens} (xy.lan.i.sol\textsuperscript{’}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.

### Table 1. Differential characteristics between strain XB1A\textsuperscript{T} and related species of the genus \textit{Bacteroides}

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch utilization</td>
<td>−</td>
<td>+*</td>
<td>+*</td>
<td>ND</td>
<td></td>
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<tr>
<td>Acid produced from:</td>
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<tr>
<td>Salcin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Melezitose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Enzyme activities</td>
<td></td>
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<tr>
<td>Arginine arylamidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Phenylalanine arylamidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Leucine arylamidase</td>
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<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Tyrosine arylamidase</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Glycine arylamidase</td>
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<td>+</td>
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<td>−</td>
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<tr>
<td>Histidine arylamidase</td>
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<td>Glutamyl glutamic acid arylamidase</td>
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<td>+</td>
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<tr>
<td>Serine arylamidase</td>
<td>−</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>42.8</td>
<td>42</td>
<td>43</td>
<td>42.4</td>
<td></td>
</tr>
</tbody>
</table>

*Data from Salyers \textit{et al.} (1977).
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 arabinobiose, 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, mannohexosyl, 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, N-acetyl-β-glucosaminidase, 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-aminohexanoic acid arylamidase, ornithine arylamidase, tyrosine arylamidase, phenylalanine arylamidase, threonine arylamidase, serine arylamidase. The major fatty acids are anteiso-C15:0 (33.8%), iso-C17:0 3-0H (23.5%) and iso-C15:0 (10.3%). The DNA G+C content of the type strain is 42.8 mol%.

The type strain, XB1A® (=DSM 18836® =CCUG 53782®), was isolated from human faeces of a non-methane-excreting individual. Strains XB4A, XB3A, XB2A, XB41A and XO85C13, isolated from similar sources, are also included in the species.

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References


