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Characterization of a New Xylanolytic Bacterium, *Clostridium xylanovorans* sp. nov.

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Summary

A new xylanolytic bacterium designated strain HESP1^T (T = type strain) was isolated from a methanogenic digester. Strain HESP1^T was a motile, rod shaped, spore-forming bacterium, which possessed a Gram-postive type cell wall. Glucose, fructose, lactose, trehalose, maltose, raffinose, sucrose, xylan, mannitol, cellobiose, galactose, mannose, melibiose, ribose were fermented to produce, acetate, butyrate, H_2, CO_2, formate, isobutyrate, and ethanol. Fumarate was fermented to acetate. Glycerol and methanol were also utilized. Sulfate, thiosulfate, nitrate, sulfur and fumarate were not used as electron acceptors. Strain HESP1^T had a G+C content of 40 mol% and grew optimally at 37 °C and pH 7 on a fructose containing medium. Phylogenetically, strain HESP1^T was most related to *Clostridium aminovalericum* (similarity of 94%) than to *C. populeti*, *C. herbivorans* and *Eubacterium xylanophilum* (average similarity of 92%), all members of subcluster XIVa of the low G+C containing Gram-positive branch. However, strain HESP1^T shared little phenotypic and genotypic traits with *C. aminovalericum* and on the basis of this and phylogenetic evidence, we propose to tentatively designate strain HESP1^T as a new species of the genus *Clostridium*, *Clostridium xylanovorans* sp. nov. The type strain is HESP1^T (= DSM 12503).

Key words: *Clostridium xylanovorans* – xylan – methanogenic digester – phylogeny – fermentation

Introduction

Xylan, one of the most important plant cell wall components, is a heteropolymer composed primarily of β-1-4-linked xylose with various amounts of arabinose, glucose, galactose, and other sugars as side group depending on the plant source. Of the numerous diverse microbial species reported, their representation is perhaps the most dominant in the phylum that contains the low G+C Gram-positive clostridial group. *C. aldrichi* (YANG et al., 1990), *C. aerotolerans* (VAN GYLSWYC and VAN DER TOORN, 1987), *C. cellulovorans* (SLEAT et al., 1984), *C. lentocellum* (MURRAY et al., 1986), *C. polysaccharolyticum* (VAN GYLSWYK et al., 1983), *C. populeti* (SLEAT and MAH, 1985), and *C. xylanolyticum* (ROGER and BAECKER, 1991) are such examples of the diversity found in this phylum. In this paper, we provide evidence that a new anaerobic xylanolytic bacterium isolated from an olive mill wastewater treatment digester, and designated HESP1^T, has phenotypic, phylogenetic and genomic characteristics distinct from other xylanolytic bacteria described so far. Based on this evidence we propose that this strain be tentatively placed in the genus *Clostridium* as *Clostridium xylanovorans* sp. nov until the time of revision of the phylum.

Materials and Methods

Source of strains: Strain HESP1^T was enriched from samples collected from an anaerobic olive mill wastewater-fed methanogenic digester (maintained at 37 °C) in Tunisia. Samples were collected and stored under anaerobic conditions in a O_2-free N_2 atmosphere in serum bottles and subsequently transported to the laboratory at ambient temperature.

Enrichment, isolation and cultivation: A basal medium used for enrichment, isolation, and cultivation was prepared by anaerobic techniques described by HUNGATE (1969) and modified for use with syringes (MACY et al., 1972; MILLER and WOLLIN, 1974). The basal medium contained (per liter of deionized water): 1 g of NH_4Cl, 0.3 g of K_2HPO_4, 0.3 g of KH_2PO_4,

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0.6 g of NaCl, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of KCl, 0.5 g of Cysteine-HCl, 1 g of yeast extract (Difco. Laboratories Detroit, Mich.), 1.5 ml of trace element solution (WIDDEL and PFENNIG, 1981), and 1 mg of resazurin. The pH was adjusted to 7 with 10 M KOH solution, the medium boiled under a stream of O_2-free N_2 gas and cooled to room temperature. 5 ml aliquots were dispensed into Hungate tubes, gassed under N_2-CO_2 (80:20%, v/v) and subsequently sterilized by autoclaving at 110 °C for 45 min. Prior to culture inoculation, 0.2 ml of 5% (w/v) $NaHCO_3$ and 0.05 ml of 2.5% (w/v) Na_2S, $9H_2O$ were injected into the pre-sterilized aliquoted medium from sterile stock solutions.

Enrichments were performed by inoculating 0.5 ml of sample into 5 ml of basal medium containing 1% xylan followed by incubation at 37 °C. The enrichments that developed were sub-cultured several times under the same growth conditions. Pure cultures were obtained by serially diluting the enrichment ten-fold, and inoculating roll tubes (HUNGATE, 1969). The roll tubes contained basal medium supplemented with 1% xylan and 1.6% agar. Single colonies that developed in the last dilution series were picked, serially diluted and the roll tube procedure repeated. Several pure cultures were obtained and a culture picked at random, and designated strain HESP1^T, was characterized further.

Characterization: 0.05 ml of substrates were injected into 5 ml basal media from ×100 concentrated filter-sterilized stock solutions. All substrates were tested at a final concentration of 20 mM with the exception of peptides which were tested at 0.2%. The substrates included carbohyrates (glucose, fructose, xylose, sorbose, sorbitol, sucrose, melibiose, raffinose, galactose, myo-inositol, sucrose, lactose, cellobiose, mannitol, mannose, arabinose, arabitol, raffinose and ribose), organic acids (formate, acetate, propionate, butyrate, valerate, fumarate, crotonate, malonate, malate, lactate, citrate and succinate), alcohols (methanol, ethanol, propanol, isopropanol, butanol, isobutanol, and glycerol) and peptides (biotrypcase, yeast extract, casamino acids, gelatin and peptone). Xylan and cellulose were weighed directly into Hungate tubes before the media was dispensed to give final concentration of 1%.

For determining pH growth range, the pH of the pre-reduced anaerobic basal medium was adjusted by injecting 5% $NaHCO_3$, 5% Na_2CO_3 or 0.1 M HCl to give a pH range between 5 to 9.5. Different amounts of NaCl were weighed directly into Hungate tubes prior to dispensing of 5 ml of basal medium to give the desired concentration range (0 to 4%). The temperature range for growth was determined between 20 °C to 45 °C.

Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur and fumarate were tested as electron acceptors at a final concentration of 10 mM.

Analytical methods: Light and electron microscopy were performed as previously described (FARDEAU et al., 1997). Growth was measured at 580 nm by inserting anaerobic Hungate tubes directly into the cuvette holder of a Shimadzu Model UV 160A spectrophotometer. Acetate, formate isobutyrate, ethanol and butyrate were measured by HPLC (Spectra Series 100 model; Thermo Separation Products, les Ulis France) equipped with an Aminex HPX-87X 300 mm by 7.8 mm [inside diameter] column (Bio-Rad Laboratories 200 Alfred Nobel Dr., Hercule, CA 94547) connected to a differential refractometer (RID-6A Shimadzu). Analysis was performed using a CR-6A Shimadzu integrator. The mobile phase was 0.005N H_2SO_4 at a flow rate of 0.6 ml min^{-1}, and the column temperature was 35 °C. The volume of the injection loop was 20 µl.

H_2 and CO_2 were measured on a Chromosorb WAW 80/100 mesh sp100 column (Alltech France) using N_2 as a carrier gas and a Girdel gas chromatograph (Girdel, Suresnes, France) equipped with a flame ionization detector, and a C-R6A Chromatopac integrator (Shimadzu Co., Kyoto, Japan).

DNA base composition: The G+C content of DNA was determined at DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The DNA was isolated and purified by chromatography on hydroxyapatite, and the G+C content was determined by using high-performance liquid chromatography (HPLC) as described by MESBAH et al., (1989). Nonmethylated lambda DNA (Sigma) was used as the standard.

16S rDNA studies: DNA was extracted from the isolate as described previously (REDBURN and PATEL, 1993; ANDREWS and PATEL, 1996). The universal primers Fd1 and Rd1 were used to obtain a PCR product of approximately 1.5 kb corresponding to base positions 14 to 1539 based on *E. coli* numbering of the 16S rDNA (WINKER and WOESE, 1991). A 50-µl reaction contained 1–20 ng of genomic DNA, 1 µM of each primer, 5 µl of 10 × buffer, 200 µM of dNTP, 3.5 mM of $MgCl_2$, and 2.5 U of *Taq* polymerase (Promega). PCR was carried out by an initial denaturation at 94 °C for 7 min, then 29 cycles of annealing at 55 °C for 2 min, extension at 72 °C for 4 min, denaturation at 94 °C for 1 min, and finally an extension cycle of 55 °C for 2 min and 72 °C for 20 min.

PCR products were purified using a QIAquick Kit (Qiagen). DNA concentration of purified PCR product was estimated by comparison with the Low Mass Ladder (Gibco BRL) on an agarose gel containing ethidium bromide. QIAquick purified PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit containing AmpliTaq FS DNA polymerase and ABI 373A sequencer. A 10-µl reaction contained 35 ng of PCR product, 4 µl of cycle sequencing reaction mix, 3.2 pmol of primer, and 2.5 µg of BSA. Thermal cycling was carried out using a RapidCycler (Idaho Technology) at a temperature transition slope of 2, an initial denaturation of 94 °C for 15 sec, then 25 cycles of denaturation at 94 °C for 0 sec, annealing at 50 °C for 10 sec, extension at 60 °C for 3 min.

The new sequence data that was generated was aligned, an almost full length consensus 16S rRNA gene sequence assembled and checked for accuracy manually using the alignment editor, ae2 (MAIDAK et al., 1997). These were compared with other sequences in the GenBank database (BENSON et al., 1993) using BLAST (ALTSCHUL et al., 1997), and in the Ribosomal Database Project, version 7.0 using SIMILARITY_RANK and SUGGEST_TREE (MAIDAK et al., 1997). If necessary, reference sequences most related to our newly generated sequence were extracted from these databases and aligned. Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 1280 unambigious nucleotides were computed using dnadist (JUKES and CANTOR) and neighbor-joining programs that form part of the PHYLIP suite of programs (FELSENSTEIN, 1993). TREECON was used extensively for bootstrap analysis (VAN DE PEER and DE WACHTER, 1993).

Results

Morphology

Cells of strain HESP1^T were rod-shaped (0.4–0.8 µm × 7–14 µm) and usually occurred singly. The strain stained Gram positive and thin section of cells examined under an electron microscope revealed a single thick tri-layered cell wall structure typical of Gram-positive type walls (Fig. 1). The presence of terminal spherical spores distended the cells. Cells were motile and negatively stained cell preparations showed the presence of subpolar to laterally inserted flagella.

FICHE DESCRIPTIVE

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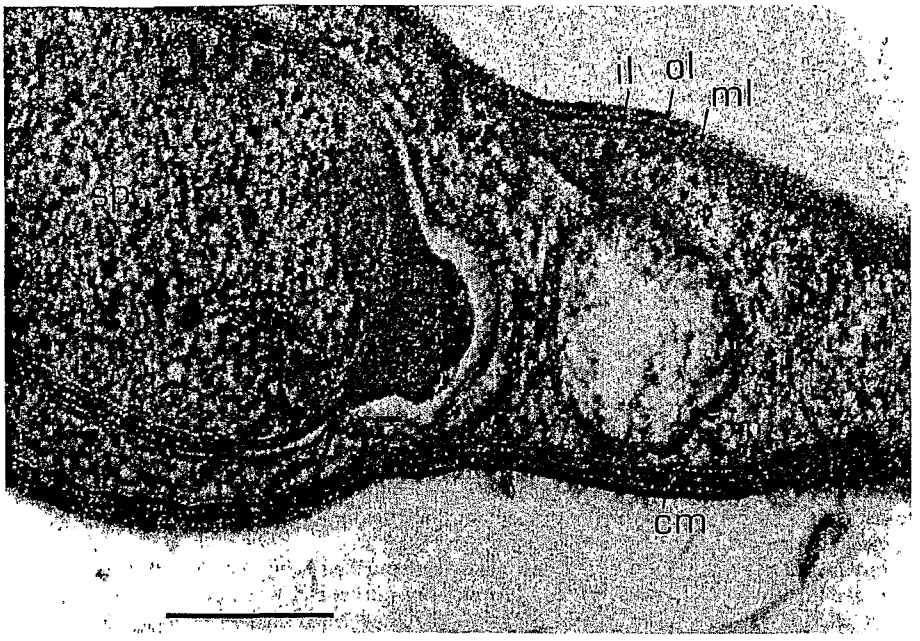

Fig. 1. Transmission electron micrograph of the cell wall ultrastructure of strain HESP1^T showing the presence of cytoplasmic membrane (cm) and a single thick tri-layered electron dense cell wall composed of the inner layer (il) adjacent to the cytoplasmic membrane, the middle layer (ml) and the outer layer (ol). Part of the spore (sp) of the strain can also be seen. The cell wall of the spore has the same structure as the bacterial cell wall, and is typical for endospores. Bar = 0.2 µm.

Physiology and G+C content

Strain HESP1^T was a strictly anaerobic chemoorganotrophic bacterium. The temperature range for growth was between 25 and 42 °C, and the optimum 37 °C. The pH range for growth was between 6-8 and the optimum pH 7. Strain HESP1^T was inhibited in the presence of 1.5% NaCl but could grow in the presence of 0 to 1% NaCl. Strain HESP1^T grew in the presence of cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, ribose, sucrose, trehalose, xylan, glycerol, methanol and fumarate but not on xy-

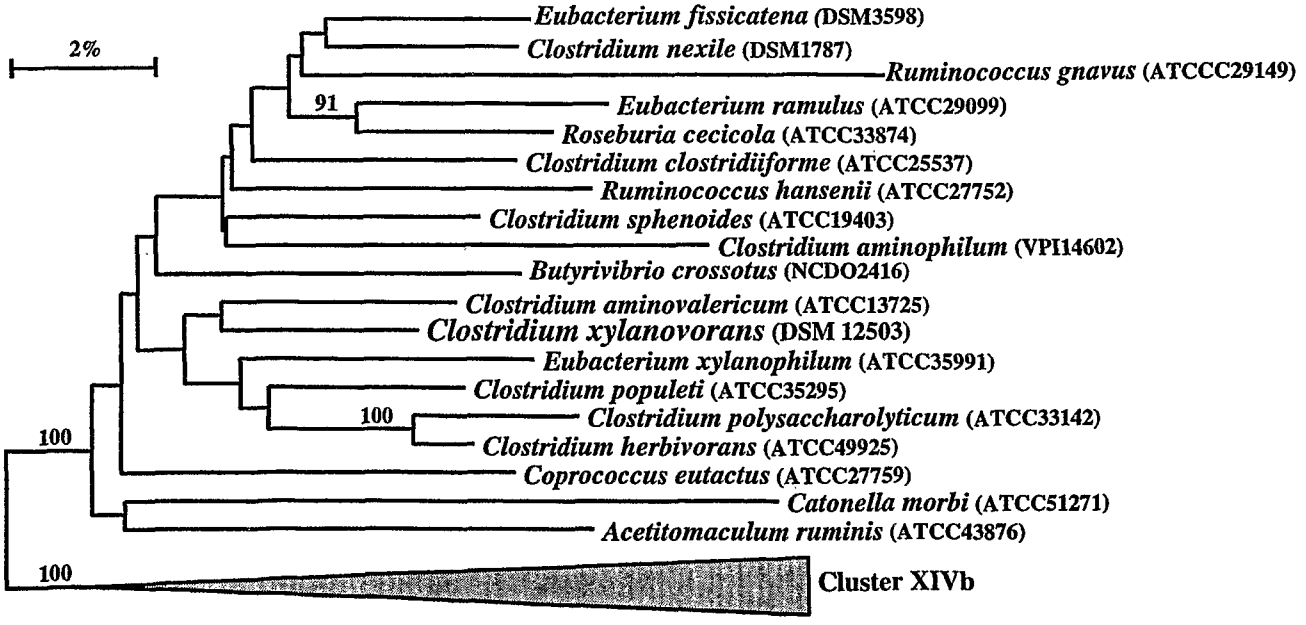

Fig. 2. Unrooted dendrogram based on 16S rRNA sequence data indicating the phylogenetic position of *Clostridium xylanovorans* (strain HESP1^T = DSM 12503^T) within the radiation of representatives of subcluster XIVa of the low G+C containing gram positive bacteria. Sequences represented in subcluster XIVb (indicated as a triangle) were used as outgroup and include *Clostridium lentocellum* and *Clostridium propionicum*. The clusters are defined as suggested by COLLINS et al., 1994. All the sequences used in the analysis were obtained from the RDP (Ribosomal Database Project), version 7.0 (MAIDAK et al., 1997). Bootstrap values, expressed as a percentage of 100 replications are shown at the branching points. Only values above 90% were considered significant and therefore reported. Scale bar indicates 2 nucleotide substitutions per 100 nucleotides.

lose, sorbose, sorbitol, myo-inositol, arabinose, arabitol, cellulose, formate, acetate, propionate, butyrate, valerate, crotonate, malonate, malate, lactate, citrate, succinate, ethanol, propanol, isopropanol, butanol, isobutanol, biotrypcase, yeast extract, casamino acids, gelatin and peptone.

The major fermentation end-products of glucose, fructose, lactose, trehalose, maltose, raffinose, sucrose, xylan, mannitol, cellobiose, galactose, mannose, melibiose and ribose were acetate and butyrate and minor products were H_2, CO_2, formate, isobutyrate, and ethanol.

Fumarate was fermented to acetate. None of the electron acceptors tested were utilized.

The DNA G+C base composition of strain HESP1^T was 40 mol% as determined by HPLC method.

Phylogeny

Using 12 primers, we determined an almost complete 16S rRNA gene sequence of strain HESP1^T consisting of 1533 bases corresponding to. *E. coli* positions 14 to 1539 (nomenclature of WINKER and WOESE, 1991). Phylogenetic analysis revealed that strain HESP1^T was a member of low G + C containing Gram-positive branch as defined by COLLINS et al (1994) and grouped with members of the subcluster XIVa. The closest relative was *Clostridium aminovalericum* (similarity of 94%) with *E. xylanophilum*, *C. populeti* and *C. polysaccharolyticum* slightly distantly related (average similarity of 92%).

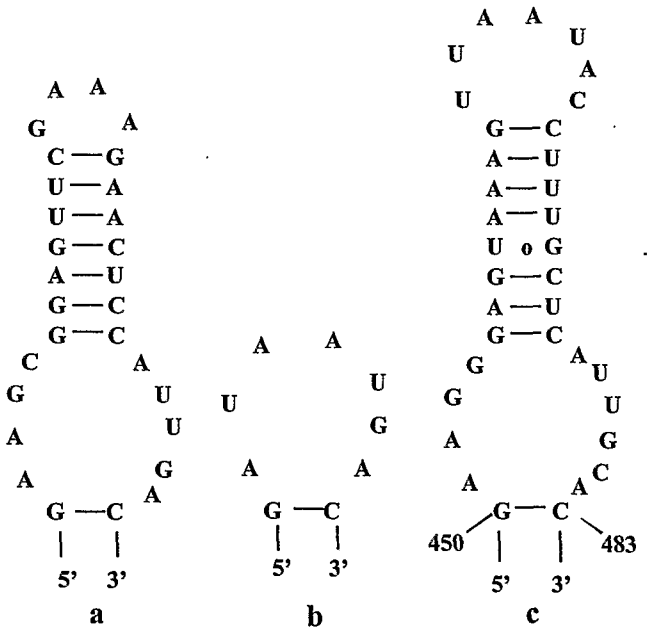

Fig. 3. Secondary structure of a part of the helix of 16S rRNA from base positions 450 to 483 (*E. coli* numbering, WINKER and WOESE, 1991). A 29 nucleotide version is found in strain HESP1^T (a) a shortened 9 nucleotide version in *C. aminovalericum* and most members of the subcluster XIVa (b), and a 35 nucleotide version in *E. coli* (c).

Fig. 2 represents a dendrogram generated by the neighbor-joining method (FELSENSTEIN, 1993) from the JUKES and CANTOR evolutionary distance matrix (JUKES and CANTOR, 1969) depicting the relationship of strain HESP1^T with representative members of the subcluster XIVa.

Detailed analysis of the 16S rRNA gene from position 450 to 483 (*E. coli* nomenclature of WINKER and WOESE, 1991) revealed a unique stretch of 29 nucleotides in strain HESP1^T (Fig. 3a) but a reduced 9 nucleotide stretch in all other members of the subcluster XIVa (Fig. 3b) and 35 nucleotides in *E. coli* (Fig. 3c). In general, a reduced length is present in a vast majority of the members of the clusters of the low G+C containing Gram positive bacteria, with the exception of members of cluster IX.

Nucleotide sequence accession number

The 16S rRNA gene sequence of strain HESP1^T has been deposited in the GenBank database under accession number AF116920.

Discussion

The ability to degrade xylan in mesophilic environments is widely distributed amongst anaerobic bacteria and includes some members of the genera *Clostridium* (VAREL et al., 1995), *Eubacterium, Fibrobacter, Butyrivibrio, Selenomonas* (COTTA et al. 1995) and *Prevotella* (AVGUSTIN et al., 1995). These bacteria have been isolated from a variety of different ecosystems such as animal intestinal tract, anaerobic digester, sewage sludge and soil. The isolation of strain HESP1^T from a methanogenic digester fed with olive oil mill wastewater extends the known habitat of xylan degrading anaerobic bacteria. This type of environment is rich in plant xylans, celluloses and aromatic compounds. Whether it is a dominating member of the digester can be undertaken by developing probes targeted against the unique region of the 16S rRNA of strain HESP1^T (Fig. 3b) identified during the course of this investigation.

Strain HESP1^T is an anaerobic, mesophilic, spore-forming, motile, rod-shaped bacterium which stains Gram-positive and has a Gram-positive type cell wall ultra-structure. It does not reduce sulfate and is a carbohydrate fermenter. Phylogenic analysis indicated that strain HESP1^T was a member of the subcluster XIVa (COLLINS et al. 1994) in the low-G+C containing Gram-positive bacteria. On this basis, its taxonomic relationship to other xylanolytic *Clostridium* species distributed in cluster I (*C. cellulovorans*, cluster III (*C. aldrichi*) and subcluster XIVb (*C. lentocellum*)) can be ruled out. Subcluster XIVa currently comprises of several cellulolytic and / or xylanolytic clostridial species including *C. aerotolerans, C. celerecresens, C. sphenoides, C. xylanolyticum, C. populeti* and *C. polysaccharolyticum*. The closest relative of strain HESP1^T in subcluster XIVa was *C. aminovalericum* (similarity of 94%). Phenotyically, both strain HESP1^T and *C. aminovalericum* (HARDMAN and

STADTMAN, 1960) are rod-shaped, motile, sporulates and grow at mesophilic temperatures. However C. *aminovalericum* ferments a limited number of carbohydrates, has a G+C content of 33 mol% and utilizes aminovalerate and peptides but strain HESP1^T has a wider carbohydrate utilization spectrum, has a G+C content of 40% and is unable to utilize aminovalerate and peptides (Table 1).

Based on the evidence presented and based on the current taxonomic guidelines, we propose that strain HESP1^T be designated a new member of the genus *Clostridium* in the subcluster XIVa. However, a major taxonomic revision for the members of the low G+C containing Gram-positive bacteria has been proposed. Under this proposal, cluster I is to be reserved for members of the redefined genus *Clostridium* (COLLINS et al., 1994). Strain HESP1^T is not a member of cluster I and hence this will mean a change in the genus name for strain HESP1^T at a later date. An alternative is to propose that a new genus be created in subcluster XIVa to accommodate strain HESP1^T and C. *aminovalericum*. However, we believe that it would be premature to attempt this in this communication. Any revision should wait until formal discussions on taxonomic changes are completed. Accordingly, we propose that strain HESP1^T be considered a temporary member of the genus *Clostridium*, *Clostridium xylanovorans* sp. nov., in the subcluster XIVa until such time when the revision of cluster I has been completed.

Table 1. Characteristics of strain HESP1^T and *Clostridium aminovalericum*.

Characteristics	Strain HESP1^T[1])	C. *aminovalericum*[2])
Cell size	0.8–1×4–10 µm	0.3–0.5×1.5–5.2 µm
pH optima	7	nr
Temperature range	20–45 °C	25–45 °C
G+C content	40%	33%
Utilization of:		
glucose	+	+
fructose	+	–
cellobiose	+	–
maltose	+	–
sucrose	+	–
trehalose	+	–
xylose	–	–
Proteins[3])	–	+
Aminovalerate	–	+
Yeast extract (0.2%)	–	nr

Both, strain HESP1^T and C. *aminovalericum*, are motile, sporulating rods and have a temperature optimum of 37 °C.
[1]) This study. Culturing was performed in basal medium (pH 7) supplemented with the indicated substrates at 20 mM, and the results recorded after two weeks of incubation at 37 °C
[2]) According to the data of HARDMAN and STADTMAN (1960).
[3]) Proteins tested were peptone, meat extract.
nr – not reported; + positive growth; – negative growth

Description of strain *Clostridium xylanovorans* sp. nov.

Clostridium xylanovorans (xy.la.no.vo'rans. Gr. n. xylanosum, xylan; L. v. vorare devour to eat, xylan-eating bacterium). Cells stain Gram positive, are rod-shaped and form terminal spherical spore which swell the cell. The cells are motile by means of subpolar to laterally inserted flagella.

Growth is strictly anaerobic and chemoorganotrophic. Cells grow in the temperature range between 25 and 42 °C, with an optimum at 37 °C. The pH range for growth is between 6–8 with an optimum of pH 7. NaCl concentrations in the range of 0 to 1% do not inhibit growth.

The isolate grows on cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, ribose, sucrose, trehalose, xylose, glycerol, methanol and fumarate but not on xylose, sorbose, sorbitol, myo-inositol, arabinose, arabitol, cellulose, formate, acetate, propionate, butyrate, valerate, crotonate, malonate, malate, lactate, citrate, succinate, ethanol, propanol, isopropanol, butanol, isobutanol, biotrypcase, yeast extract, casamino acids, gelatin and peptone.

The major end products from fructose, glucose, lactose, trehalose, maltose, raffinose, sucrose xylan, mannitol. cellobiose, galactose, mannose, melibiose and ribose fermentation are acetate and butyrate with H_2, CO_2, formate, and ethanol also produced. Fumarate is reduced to acetate.

The DNA base composition of strain HESP1^T is 40 mol% as determined by HPLC. The closest phylogenetic relative is C. *aminovalericum*. The type strain is HESP1^T (= DSM 12503).

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References

ALTSCHUL, S. F., MADDEN, T. L., SCHÄFFER, A. A., ZHANG, J., ZHANG, Z., MILLER, W., LIPMAN, D. J.: "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25, 3389–3402 (1997).

ANDREWS, K. T., PATEL, B. K. C.: *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. Int. J. Syst. Bacteriol. 46, 265–269 (1996).

AVGUSTIN, G., WALLACE, R. J., FLINT, H. J.: Phenotypic diversity among ruminal isolates of *Prevotella ruminicola*: proposal of *Prevotella brevis* sp. nov., *Prevotella bryantii* sp. nov., and *Prevotella albensis* sp. nov. and redefinition of *Prevotella ruminicola*. Int. J. Syst. Bacteriol. 47, 284–288 (1997).

BENSON, D., LIPMAN, D. J., OLSTELL, J.: GenBank. Nucleic Acids Res. 21, 2963–2965 (1993).

COLLINS, M. D., LAWSON, P. A., WILLEMS, A., CORDOBA, J. J., FERNANDEZ-GARAYZABAL, J., GARCIA, P., CAI, J., HIPPE, H., FARROW, J. A. E.: The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol 44, 812–826 (1994).

COTTA, M. A., ZELTWANGER, R. L.: degradation and utilization of xylan by the ruminal bacteria *Butyrivibrio fibrosolvans* and *Selenomonas ruminantium*. Appl. Environ. Microbiol. 61, 4396–4402 (1995).

FARDEAU, M.-L., OLLIVIER, B., PATEL, B. K. C., MAGOT, M., THOMAS, P., RIMBAULT, A., ROCCHICCIOLI F., GARCIA, J.-L.: *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. Int. J. Syst. Bacteriol. 47, 1013–1019 (1997).

FELSENSTEIN, J.: PHYLIP (Phylogenetic Inference Package) version 3.51c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA (1993).

HARDMAN, J.K., STADTMAN, T. C.: Metabolism of ω-amino acids. II Fermentation of Δ-aminovaleric acid by *Clostridium aminovalericum*. J. Bacteriol. 79, 549–552 (1960).

HUNGATE, R. E.: A roll-tube method for the cultivation of strict anaerobes. Methods Microbiol. 136, 194–198 (1969).

JUKES, T. H., CANTOR, C. R.: Evolution of protein molecules. In Mammalian protein metabolism, pp. 211–232. Edited by H. N. MUNRO. New York: Academic Press (1969).

MACY, J. M., SNELLEN, J. E., HUNGATE, R. E.: Use of syringe methods for anerobiosis. Am. J. Clin. Nutr. 25, 1318–1323 (1972).

MAIDAK, B. L., OLSEN, G. J., LARSEN, N., OVERBEEK, R., MCCAUGHEY, M. J., WOESE, C. R.: The RDP (Ribosomal Database Project). Nucleic Acids Res. 25,109–111 (1997).

MESBAH, M., PREMCHANDRAN, U., WHITMAN, W. B.: Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. Int. J. Syst. Bacteriol. 39, 159–167 (1989).

MILLER, T. L., WOLLIN, M. J.: A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. 27, 985–987 (1974).

MURRAY, W. D., HOFMANN, L., CAMPBELL, N. L., MADDEN, R. H.: *Clostridium lentocellum* sp. nov., a cellulolytic species from river sediment containing paper-mill waste. Syst. Appl. Microbiol. 8, 181–184 (1986).

REDBURN, A. C., PATEL, B. K. C.: Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. FEMS Microbiol. Lett. 113, 81–86 (1993).

ROGERS, G. M., BAECKER, A. A. W.: *Clostridium xylanolyticum* sp. nov., an anaerobic xylanolytic bacterium from decayed *Pinus patula* Wood Ships. Int. J. Syst. Bacteriol. 41, 140–143 (1991).

SLEAT, R., MAH, R. A.: *Clostridium populeti* sp. nov., a cellulolytic species from a woody-biomass digestor. Int. J. Syst. Bacteriol. 35, 160–163 (1985).

SLEAT, R., MAH, R. A., ROBINSON, R.: Isolation and characterization of an anaerobic, cellulolytic bacterium, *Clostridium cellulovorans* sp. nov. Appl. Environ. Microbiol. 48, 88–93 (1984).

VAN DE PEER, Y., DE WACHTER, R.: TREECON: a software package for the construction and drawing of evolutioary trees. Comput. Applic. Biosc. 9, 177–182 (1993).

VAN GYLSWYK, N. O., VAN DER TOORN, J. J. T. K.: *Clostridium aerotolerans* sp. nov., a xylanolytic bacterium from corn stover and from the rumina of sheep fed corn stover. Int. J. Syst. Bacteriol. 37, 102–105 (1987).

VAN GYLSWYK, N. O., MORRIS, E. J., ELS, H. J.: Sporulation and cell wall structure of *Clostridium polysaccharolyticum* comb. nov. (formerly *Fusobacterium polysaccharolyticum*). J. Gen. Microbiol. 121, 491–493 (1983).

VAREL, V. H., TANNER, R., WOESE, C. R.: *Clostridium herbivorans* sp.nov., a cellulolytic anaerobe from the pig intestine Int. J. Syst. Bacteriol. 45, 490–494 (1995).

WIDDEL, F., PFENNIG, N.: Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. Isolation of new sulfate reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov. sp. nov. Arch Microbiol 129, 395–400 (1981).

WINKER, S., WOESE, C. R.: Definition of the domain *Archaea*, *Bacteria* and *Eucarya* in terms of small ribosomal RNA characteristics. Syst. Appl. Microbiol. 13, 161–165 (1991).

YANG, J. C., CHYNOWETH, D. P., WILLIAMS, D. S., LI, A.: *Clostridium aldrichii* sp. nov., a cellulolytic n.. ophile inhabiting a wood-fermenting anaerobic digester. Int. J. Syst. Bacteriol. 40, 268–272 (1990).

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