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Unraveling the xylanolytic potential of Acidobacteria bacterium AB60 from Cerrado soils

Gisele Regina Rodrigues[1], Otávio Henrique Bezerra Pinto[1,†], Luís Felipe Schroeder[1], Gabriel da Rocha Fernandes[2], Ohana Yonara Assis Costa[3], Betania Ferraz Quirino[4], Eiko Eurya Kuramae[3,5,‡] and Cristine Chaves Barreto[1,*,§]

[1]Universidade Católica de Brasília, Graduate Program in Genomic Sciences and Biotechnology, SGAN 916, Brasília, DF 70790-160, Brazil, [2]Research Center René Rachou, Oswaldo Cruz Foundation (Fiocruz), Belo Horizonte, MG 30109-009, Brazil, [3]Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708 PB, Wageningen, The Netherlands, [4]Brazilian Agricultural Research Corporation – EMBRAPA/Agroenergy, Brasília, DF 70770-901, Brazil and [5]Ecology and Biodiversity, Institute of Environmental Biology, Utrecht University, 3584 CS, Utrecht, The Netherlands

*Corresponding author: Universidade Católica de Brasília, SGAN 916 Módulo C. Asa Norte. 70709-160 Brasilia DF. Tel: +55-61-3448-9217; E-mail: criscbarreto@gmail.com/cristineb@ucb.br

†Present address: Laboratory of Enzymology, Institute of Sciences Biology, Department of Cell Biology, University of Brasília, Brasília, Brazil.

One sentence summary: Acidobacteria degradation of plant polymers.

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‡Eiko Eurya Kuramae, http://orcid.org/0000-0001-6701-8668
§Cristine Chaves Barreto, http://orcid.org/0000-0001-5067-1329

ABSTRACT

The presence of genes for glycosyl hydrolases in many Acidobacteria genomes indicates an important role in the degradation of plant cell wall material. Acidobacteria bacterium AB60 was obtained from Cerrado oligotrophic soil in Brazil, where this phylum is abundant. The 16S rRNA gene analyses showed that AB60 was closely related to the genera *Occallatibacter* and *Telmatobacter*. However, AB60 grew on xylan as carbon source, which was not observed in *Occallatibacter* species; but growth was not detected on medium containing carboxymethyl cellulose, as observed in *Telmatobacter*. Nevertheless, the genome analysis of AB60 revealed genes for the enzymes involved in cellulose as well as xylan degradation. In addition to enzymes involved in xylan degradation, α-L-rhamnosidase was detected in the cultures of AB60. Functional screening of a small-insert genomic library did not identify any clones capable of carboxymethyl cellulose degradation, but open reading frames coding α-L-arabinofuranosidase and α-L-rhamnosidase were present in clones showing xylan degradation halos. Both enzymes act on the lateral chains of heteropolymers such as pectin and some hemicelluloses. These results indicate that the hydrolysis of α-linked sugars may offer a metabolic niche for slow-growing Acidobacteria, allowing them to co-exist with other plant-degrading microbes that hydrolyze β-linked sugars from cellulose or hemicellulose backbones.

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INTRODUCTION

Acidobacteria are among the most ubiquitous and abundant bacterial phyla in soils (Kielak *et al.* 2016). However, the metabolic underpinnings of this soil prevalence remain unknown, mainly due to the difficulty of culturing Acidobacteria on artificial media. The majority of the data on Acidobacteria occurrence comes from 16S rRNA gene sequencing using metagenomic DNA. These studies revealed that in the Cerrado (Brazilian savanna) soils, Acidobacteria phylum is more abundant than Proteobacteria (Araujo *et al.* 2012; de Castro *et al.* 2016), which is usually the prevalent soil phylum (Kielak *et al.* 2016). The reasons for the abundance of Acidobacteria in the Cerrado are not yet known (Eichorst, Kuske and Schmidt 2011; Kielak *et al.* 2016).

Genomic analyses of Acidobacteria belonging to subgroup 1 from the class Acidobacteriia (Dedysh and Yilmaz 2018) have revealed the presence of an assortment of genes encoding enzymes for degradation of various polysaccharides (Ward *et al.* 2009; Kielak *et al.* 2016). Numerous studies have reported a role of Acidobacteria in the degradation of plant cell wall material (Lee, Ka and Cho 2008; Eichorst, Kuske and Schmidt 2011; Pankratov *et al.* 2012; Navarrete *et al.* 2013). At least 50% of the genera from subgroup 1 are able to use starch, laminarin and xylan as carbon sources (Kielak *et al.* 2016). In addition, most members possess the genes for cellulose hydrolysis (Rawat *et al.* 2012, 2014), although bacterial degradation of cellulose under culture conditions has not been fully demonstrated (Belova *et al.* 2018).

Many members of Acidobacteria were isolated on culture media containing xylan as the main carbon source. Xylan, the most common hemicellulose in hardwood, is a heterogeneous polysaccharide composed of a backbone of β-1,4-linked D-xylose units. A variety of sugars branch out from this linear xylose backbone, such as L-arabinose and D-glucuronic acid, but the chemical composition of these α-linked sugars is unique to each plant species. Xylan hydrolysis is achieved by the activity of several enzymes, such as β-xylosidase and β-1,4-xylanases, which releases xylose residues from the linear xylan backbone. Its full hydrolysis requires additional enzymes, such as α-glucuronidase, α-arabinofuranosidase and esterases, due to the heterogeneity of xylan (Saha and Bothast 1999).

In a previous study, five Acidobacteria were obtained from the Cerrado soil (de Castro *et al.* 2013). Among them, Acidobacteria bacterium AB60 presented the highest growth when cultivated on the chemically defined VL-55 medium containing xylan as the sole carbon source. However, no growth was observed in liquid media using cellulose as the carbon source (de Castro *et al.* 2013). In the present study, we sought to understand AB60's preference for xylan as carbon source over cellulose; we studied the enzymes involved in xylan and cellulose catabolism using biochemical, genomic and functional genomic approaches.

MATERIALS AND METHODS

Characterization of the Acidobacteria AB60 strain

Acidobacteria bacterium AB60 was obtained from Cerrado soil (de Castro *et al.* 2013) using VL-55 (Sait, Hugenholtz and Janssen 2002) amended with 0.05% (w/v) oat spelt xylan (Sigma-Aldrich, St Louis, MO, USA). Chemically defined VL-55 medium was prepared as described by Sait *et al.* (2002) by replacing the SL-10 trace elements solution with a modified solution of Balch's trace elements (Balch *et al.* 1979; de Castro *et al.* 2013). The VL-55 medium consisted of the following: 3.9 g L^{-1} of 2-(*N*-morpholino)ethanesulfonic acid (MES), 4 mM $MgSO_4$, 0.6 mM $CaCl_2$, 0.4 mM $(NH_4)_2HPO_4$, 2 mL of a modified solution of Balch's trace elements and 2 mL of Wolfe's vitamin solution (Balch *et al.* 1979; de Castro *et al.* 2013). The pH of the medium was adjusted to 5.5.

After isolation, colonies were maintained in the same VL-55 medium, with the exception that 0.05% (w/v) beechwood xylan (Sigma-Aldrich, St Louis, MO, USA) was used as the carbon source since the production of oat spelt xylan was discontinued by major providers. Solid medium was prepared by the addition of either 1.5% ultrapure agar (Merck Millipore, Germany) or 0.8% gellan gum (CP Kelco, Atlanta, GA, USA) (Sait, Hugenholtz and Janssen 2002) to the liquid medium. Acidobacteria strain AB60 growth was evaluated on solid VL-55 (ultrapure agar or gellan gum) medium using one of the following polysaccharides as the carbon source (0.05%, w/v): beechwood xylan, low-viscosity carboxymethyl cellulose (CMC), crystalline cellulose (Avicel®) or pectin (Sigma-Aldrich, St Louis, MO, USA). Growth at 28°C was measured over a period of four weeks. Acidobacteria bacterium AB60 growth was also followed in MM complex medium (Dedysh *et al.* 2011) to allow comparison with the metabolic profiles of other Acidobacteria. The composition of MM medium was as follows (L^{-1}): 0.1 g KH_2PO_4, 0.1 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.05 g $CaCl_2{\cdot}2H_2O$ and 0.05 g of yeast extract. Glucose was substituted for 0.05% of xylan as carbon source and the pH was adjusted to 5.5.

Phylogenetic analysis using 16S rRNA gene and genomic comparisons

The Acidobacteria bacterium AB60 16S rRNA gene was retrieved from its genome sequence and similarities sequences were obtained using the 16S-based ID tool from the EzBioCloud database (Yoon *et al.* 2017). Sequences were aligned against the SILVA v132 database (Quast *et al.* 2013) and filtered to remove columns with no information using the tools align.seqs and filter.seqs from Mothur software v1.39 (Schloss *et al.* 2009). A phylogenetic tree was created using MEGA X software (Kumar *et al.* 2018) and the evolutionary history was inferred by the maximum likelihood method and application of the Tamura–Nei model (Tamura and Nei 1993).

The average nucleotide identity (ANI) values between the AB60 genome (see below) and other *Acidobacteria* genomes were calculated using the genome-based (TrueBac ID) tool from the EzBioCloud database (Yoon *et al.* 2017).

AB60 genome analysis

Total DNA was obtained from a 200 mL VL-55 culture as described above. Bacterial cells were harvested by centrifugation at 10 000 × *g* for 20 min, and the DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo-Fisher Scientific, Waltham, MA, USA). The DNA was further purified using the DNeasy PowerClean Pro Cleanup Kit (Mo Bio/QIAGEN, Carlsbad, CA, USA), and the DNA concentration was determined using a NanoDrop spectrophotometer (Thermo-Fisher Scientific, Waltham, MA, USA). The genomic DNA library was constructed using a Nextera XT library prep kit (Illumina, Inc., San Diego, CA, USA). Library quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and the DNA library was sequenced (2 × 300 bp) on a MiSeq platform

(Illumina, San Diego, CA) at the Catholic University of Brasília, Brazil. Raw FASTQ sequences were subjected to quality trimming and adaptor removal. The filtering step was performed by Trimmomatic (Bolger, Lohse and Usadel 2014) using the following parameters: sliding window 4:15, quality <5 and min length 40. Genome assembly was performed with SPAdes version 3.7.1 using k-mer sizes of 21, 33, 55, 77, 99 and 127. Genome annotation was performed using the DOE-JGI 'genome annotation pipeline' (Huntemann *et al.* 2016). Genes coding for glycoside hydrolases (GHs) involved in the degradation of cellulose and xylan were identified and classified into families according to the CAZy database (http://www.cazy.org).

The data underlying this article are available in the Genbank Nucleotide databae at he data underlying this article are available at: https://www.ncbi.nlm.nih.gov/nuccore/VANK00000000.1, and can be accessed with accession number: VANK00000000.1.

Enzymatic assays

Xylan degradation was initially tested on solid medium. The Acidobacteria bacterium AB60 isolate was inoculated on VL-55 or MM medium amended with 0.05% beechwood xylan. After an incubation period of four weeks, iodine pellets were added to the culture plates, which were then incubated in a shaker at 120 rpm for 2 h. Xylanase activity was observed as a zone of clearance around the colonies (Gohel *et al.* 2014). Plates containing VL-55 or MM medium with the same carbon source but without bacteria were used as negative controls. Enzymatic analyses using colorimetric substrates were performed from samples of a liquid culture of AB60 grown in 300 mL of VL-55 with 0.05% beechwood xylan or CMC as the sole carbon source. Since no growth was observed on CMC, enzymatic assays were only executed with samples derived from xylan-containing cultures.

After four weeks of growth, the supernatants and cell fractions were separated from 100 mL of the culture by centrifugation at 10 000 × *g* for 15 min. Each fraction (supernatant and cell fraction) was lyophilized and resuspended in 10 mL of 0.5 M MES buffer, pH 6.0. The cell fraction was further sonicated using a tip sonicator (Vibra-Cell, model CV 188 - Sonics Materials, Newton, MA, USA) at 30% amplitude, 30 s pulses with intervals of 20 s for 15 min. Cell debris was removed by centrifugation for 10 min at 5000 × *g*.

Enzymatic assays for β-glucosidase, cellobiohydrolase, β-galactosidase, α-rhamnosidase, β-xylosidase and α-glucosidase were performed using 10 μL of each resuspended fraction. The enzymatic reaction consisted of 50 mM substrate in a final volume of 200 μL of MES buffer (pH 6.0) and was incubated for 20 min at 50°C. The reaction was stopped by the addition of 100 μL of 1 M sodium carbonate. Assay controls were prepared by adding substrate without the cell fraction sample and by adding the cell fraction sample without substrate. Liberation of pNP was detected at 405 nm with a SpectraMax M3 spectrophotometer (Molecular Devices, San Jose, CA, USA) . All results were reported in μmol of pNP mL^{-1} h^{-1}. Enzyme activity was evaluated using the following *p*-nitrophenolate (pNP) substrates: *p*-nitrophenyl-β-D-glucopyranoside—pNPG (for β-glucosidase); *p*-nitrophenyl-β-D-cellobioside—pNPC (for cellobiohydrolase); *p*-nitrophenyl-β-D-galactopyranoside—pNPGal (for β-galactosidase); *p*-nitrophenyl-α-L-rhamnopyranoside—pNPR (for α-rhamnosidase); *p*-nitrophenyl-β-D-xylopyranoside—pNPX (for β-xylosidase); and *p*-nitrophenyl-α-D-glucopyranoside—pNPαG (for α-glucosidase). The protein concentration was determined using the Bradford reagent (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's recommendations.

Functional genomic assays—BAC library screening

Acidobacteria bacterium AB60 genomic DNA was treated with the restriction enzyme *Eco*RI, and fragments of 4–8 kb were extracted from low-melting-point agarose gels (Invitrogen, Carlsbad, CA, USA). A small-insert genomic library was obtained by cloning the DNA fragments into the pCC1BAC vector and then transforming the clones into *Escherichia coli* EPI300, according to the manufacturer's instructions for the Copy Control BAC Cloning Kit (Epicentre, Madison, WI, USA). Clones were screened by plating the genomic library on 0.5× Luria–Bertani medium (0.5% peptone, 0.5% NaCl, 0.25% yeast extract, 1.5% agar) supplemented with 12.5 μg mL^{-1} chloramphenicol. Screenings were executed on plates amended with 0.1% (w/v) beechwood xylan or 0.1% CMC (Sigma-Aldrich, St Louis, MO, USA). After 12 days of incubation, colonies were removed with a sterile toothpick, and the plate was overlaid with 0.1% (w/v) Congo red dye (Sigma-Aldrich, St Louis, MO, USA) for 15 min. The culture plate was then washed with 0.1 M NaCl for 90 min. After removing the NaCl solution, 1 M HCl was added, reducing the pH and the degradation halo to be visualized on the blue culture medium (Teather and Wood 1982).

A total of 1100 clones were screened, and clones presenting the most visible blue halos were selected. Plasmids were extracted from these clones and retransformed into *E. coli* EPI 300. The resulting clones were then assayed as described above to confirm their phenotype. *Escherichia coli* EPI 300 containing the pCC1BAC plasmid without any DNA insert did not produce a blue halo. Clones obtained on CMC-containing medium did not present clear blue halos after retransformation. Clones showing the strongest visible halos on the xylan-containing medium were sequenced. The nucleotide sequences of clones D10-2, E8-9, F10-5, G4-5 and E4-7 were determined using Illumina technology, as described above for the genome sequencing. Due to the smaller size of the fragments compared with the genomic data, contig assembly and open reading frame (ORF) identification were performed within Geneious 10.0 (Kearse *et al.* 2012). Each ORF was then analyzed by BLASTp using UniProtKB/Swiss-Prot database and the Conserved Domain Database. Peptide signal was detected using SignalP 5.0 (Almagro Armenteros *et al.* 2019).

RESULTS

Taxonomic characterization of Acidobacteria bacterium AB60

Phylogenetic analysis of the 16S rRNA gene revealed that strain AB60 is a member of the phylum Acidobacteria, class Acidobacteria, presenting 97.47% similarity to *Occallatibacter riparius* 277; 96.91% to *O. savannae* A2-1c; and 96.90% to *Telmatobacter bradus* TPB6017 (Pankratov *et al.* 2012; Foesel *et al.* 2016). All other Acidobacteria type species were more distantly related to AB60, showing similarity values below 96%. The '16S-based ID' tool from the EzBioCloud database classifies Acidobacteria bacterium AB60 as a new species within the genus *Occallatibacter*. However, the phylogenetic tree shows that the 16S rRNA gene sequence of Acidobacteria AB60 clusters with *T. bradus*, which is a genus closely related to *Occallatibacter*, but the bootstrap value for this clustering is below 80% (Fig. 1).

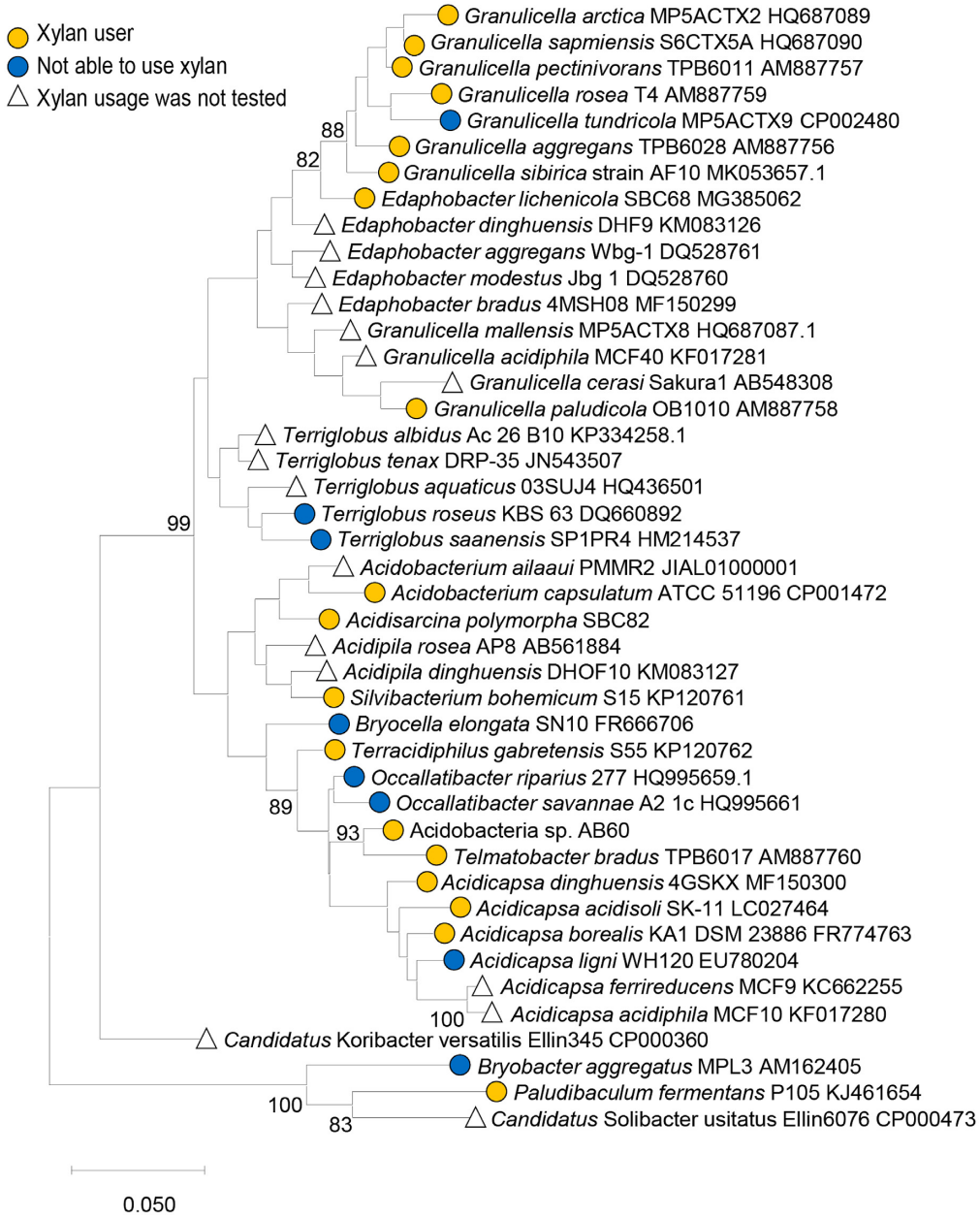

Figure 1. Phylogenetic relationships of Acidobacteria AB60 with Acidobacteria type species belonging to the class Acidobacteriia and their usage of xylan as carbon source determined in culture conditions. The evolutionary history was inferred from the 16S rRNA gene nearly full sequences, using the maximum likelihood method and Tamura–Nei model. Acidobacteria AB60 16S rRNA gene was obtained directly from the genome sequence. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) above 80% are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 43 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1178 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

The ANI values could not be obtained for *O. riparius*, *O. savannae* and *T. bradus* due to the lack of available genomes for these species. Acidobacteria AB60 showed the highest ANI values with the genomes of *Silvibacterium bohemicum* S15 (83.90%); followed by *Terracidiphilus grabetensis* S55 (83.87%), and *Acidobacterium capsulatum* ATCC 51196. Acidobacteria AB60's ability to metabolize xylan but not cellulose differs from its closest relatives. Growth on xylan was observed for Acidobacteria bacterium AB60 and *T. bradus*, but not for *O. riparius* and *O. savannae* (Table 1 and Fig. 1). In addition, only *T. bradus* could grow on crystalline cellulose, which was not observed for isolate AB60 under our culture conditions (Table 1).

Acidobacteria AB60 genome analysis

Genome assembly resulted in a draft genome size of 6.6 Mbp with 20 contigs, an N50 of 466 258 bp and G + C content of 61.86% (Table S1, Supporting Information). Several genes encoding hemicellulose degradation enzymes were identified, including genes involved in the xylan backbone degradation: endo-1,4-β-xylanase, xylan 1,4-β-xylosidase, α-D-xyloside xylohydrolase, α-L-fucosidase, feruloyl esterase and α-D-galactosidase, as well as genes encoding α-L-rhamnosidase. Almost all of these genes presented signal peptide sequence (Table 2). In addition, genes involved in the degradation of cellulose such as endoglucanases

Table 1. Growth on different polymers by Acidobacteria bacterium sp. AB60 and its closest relatives.

Growth observed	*Occallatibacter* sp.	*Occallatibacter riparius*	*Occallatibacter savannae*	*Telmatobacter bradus*
	AB60[a]	277[b]	A2-1c[b]	TPB6017[c]
Xylan	+	−	−	+
Chitin	+	−	−	−
CMC	−	ND[d]	ND[d]	−
Crystalline cellulose (Avicel)	−	ND[d]	ND[d]	+
Pectin	+/−	−	+	+
Yeast extract	+	+	+	+

Growth evaluated on: a) chemically defined medium VL-55, b) complex medium SSE amended with 50 mg yeast extract L^{-1} and c) complex medium MM.
[d]Growth was not observed using cellulose, but it is not clear whether CMC or crystalline cellulose was used.

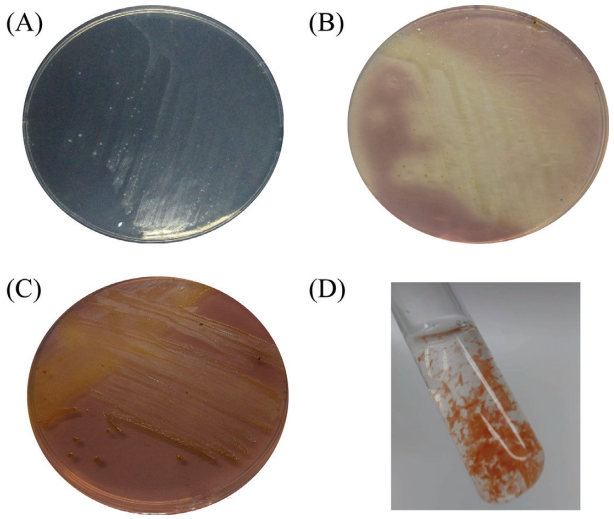

Figure 2. Growth of Acidobacteria AB60 on xylan-containing media. **(A)** Growth on VL-55 medium solidified with agar and containing xylan as sole carbon source; **(B)** iodine staining showing xylan degradation halo; **(C)** MM medium solidified with agar amended with xylan and stained with iodine; and **(D)** growth on liquid medium showing bacterial cell clumps. VL-55 is a chemically defined medium and xylan in the sole carbon source. MM medium is a complex medium containing yeast extract and xylan as carbon sources.

and β-glucosidases were identified, but no gene for a cellobiohydrolase and any other exocellulase was observed. These results indicate that Acidobacteria bacterium AB60 should be able to grow on either xylan or cellulose as carbon source.

Acidobacteria AB60 enzymatic activity

Since AB60 grew on media containing xylan but not CMC or crystalline cellulose as the sole carbon source, only samples from xylan-containing media were evaluated. Xylan degradation was initially observed on a solid medium amended with xylan. Although AB60 was able to grow on MM medium, a degradation halo was more clearly observed on VL-55 solidified with agar (Fig. 2). In complex media, the presence of a degradation halo should be interpreted with caution, since some dyes may react with degraded medium components other than xylan (Meddeb-Mouelhi, Moisan and Beauregard 2014).

The enzymatic profile from liquid cultures in VL-55 amended with xylan as the sole carbon source revealed that activities were higher in the cell fraction of the culture, with low enzymatic activity in the supernatant samples (Fig. 3). In fact, cells of AB60 form clumps in liquid media amended with xylan (Fig. 2D). Among all enzymes tested, β-galactosidase, β-xylosidase and α-L-rhamnosidase presented the highest activity. Cellobiohydrolase activity, which is involved in cellulose degradation, had the lowest value observed among all enzymes tested. The concentration of total proteins in both fractions was very similar; the supernatant fraction presented a concentration of 191.6 mg/mL and the pellet fraction, 217.2 mg/mL.

Acidobacteria AB60 functional genomic assays

The cloning and heterologous expression of genes from AB60 was used in an attempt to avoid intrinsic gene regulation due to growth on xylan as the sole carbon source. It was expected that some genes for cellulose degradation, for example, would be expressed using the promoter from the BAC vector. In addition, this application of functional genomics could allow us to detect new genes involved in a phenotype, such as xylan or cellulose degradation (Tuffin *et al.* 2009; Ngara and Zhang 2018). However, clones on CMC-containing medium did not present clear halos at any stage of the experiment. Five randomly chosen clones exhibiting putative halos were sequenced, but did not reveal the presence of any glycosyl hydrolase or sugar transport gene (data not shown). These clones were considered to be false positives and were not investigated further. Clones obtained on xylan-containing media, however, presented clear halos, and even after retransformation, five clones continued to exhibit degradation halos on xylan. Alignment of the sequences from these five clones showed that these DNA fragments originated from distinct regions of the genome (Table S2, Supporting Information; Fig. 4). Clones E4-7 and D10-2 contained glycosyl-hydrolase coding regions. Clone G4-5 contained a region coding for a domain found in pectate lyases. Clones E8-9 and F10-5 did not present any ORFs for glycosyl hydrolases, but they did contain coding regions for hypothetical proteins and one 'Domain of Unknown Function', DUF 305 (Fig. 4; Table S3, Supporting Information). Therefore, for clones E8-9 and F10-5, it is unclear whether any of these ORFs were responsible for the halos observed or whether they were false positives.

Clones E4-7 and D10-2 contained the same genomic region. The 5′ region of clone E4-7 contained 5631 bp with 100% nucleotide similarity to clone D10-2, whereas the 3′ region of clone E4-7 comprised 5269 bp originating from a distinct region of the genome (Fig. 4). Consequently, clone E4-7 must be a chimera resulting from the ligation of two genomic fragments at the *Eco*RI site used for library preparation. A gene for α-L-arabinofuranosidase was identified in the 3′ region of clone E4-7; this enzyme is directly involved in the degradation of the lateral chains of xylan (Yang *et al.* 2015). Clone D10-2 and the 5′ region of clone E4-7 presented three ORFs: a partial sequence for a β-glucosidase, BglX (GH3); a PAP2-like gene that likely

Table 2. Genes for enzymes involved in cellulose and hemicellulose degradation found in the genome of *Acidobacteria* AB60.

Description (Pfam domain)	EC	Name (JGI annotation)	Signal peptide prediction	NCBI accession number*
Xylan degradation				
Glycosyl hydrolase—GH10	3.2.1.8	endo-1,4-beta-xylanase	TAT(Tat/SPI)	WP_150164758.1
Glycosyl hydrolase—GH10	3.2.1.8	endo-1,4-beta-xylanase	SP(Sec/SPI)	WP_150167716.1
Glycosyl hydrolase—GH10	3.2.1.8	endo-1,4-beta-xylanase	SP(Sec/SPI)	WP_150169159.1
Glycosyl hydrolase—GH39	3.2.1.37	xylan 1,4-beta-xylosidase	SP(Sec/SPI)	WP_150164468.1
Glycosyl hydrolase—GH39	3.2.1.37	xylan 1,4-beta-xylosidase	LIPO(Sec/SPII)	WP_150169980.1
Glycosyl hydrolase—GH39	3.2.1.37	xylan 1,4-beta-xylosidase	SP(Sec/SPI)	WP_150173848.1
Glycosyl hydrolase—GH39	3.2.1.37	xylan 1,4-beta-xylosidase	TAT(Tat/SPI)	WP_150166526.1
Glycosyl hydrolase—GH67	3.2.1.139	alpha-glucuronidase	Other	WP_150173286.1
Alpha-L-arabinofuranosidase—GH51	3.2.1.55	alpha-N-arabinofuranosidase	SP(Sec/SPI)	WP_150168673.1
Alpha-L-arabinofuranosidase—GH51	3.2.1.55	alpha-N-arabinofuranosidase	TAT(Tat/SPI)	WP_150168679.1
Alpha-L-arabinofuranosidase—GH51	3.2.1.55	alpha-L-arabinofuranosidase	SP(Sec/SPI)	WP_150169763.1
Alpha-L-arabinofuranosidase—GH51	3.2.1.55	alpha-N-arabinofuranosidase	SP(Sec/SPI)	WP_150171870.1
Alpha-L-arabinofuranosidase—GH51	3.2.1.55	alpha-N-arabinofuranosidase	Other	WP_150173674.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	SP(Sec/SPI)	WP_150164232.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	TAT(Tat/SPI)	WP_150164497.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	SP(Sec/SPI)	WP_150165177.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	SP(Sec/SPI)	WP_150167217.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	TAT(Tat/SPI)	WP_150168111.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	TAT(Tat/SPI)	WP_150168912.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	SP(Sec/SPI)	WP_150168936.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	SP(Sec/SPI)	WP_150173220.1
Alpha-L-fucosidase—GH29	3.2.1.51	alpha-L-fucosidase	TAT(Tat/SPI)	WP_150173668.1
Glycosyl hydrolase—GH65	3.2.1.51	alpha-L-fucosidase 2	SP(Sec/SPI)	WP_150167113.1
Glycosyl hydrolases—GH31	3.2.1.177	alpha-D-xyloside xylohydrolase	SP(Sec/SPI)	WP_150165230.1
Glycosyl hydrolases—GH31	3.2.1.177	alpha-D-xyloside xylohydrolase	Other	WP_150168313.1
Glycosyl hydrolases—GH31	3.2.1.177	alpha-D-xyloside xylohydrolase	SP(Sec/SPI)	WP_150173664.1
Alpha galactosidase A—GH27	3.2.1.22	alpha galactosidase A	LIPO(Sec/SPII)	WP_150168005.1
Alpha galactosidase A—GH27	3.2.1.22	alpha galactosidase A	SP(Sec/SPI)	WP_150171701.1
Alpha galactosidase A—GH36	3.2.1.22	alpha-galactosidase	SP(Sec/SPI)	WP_150166173.1
Alpha galactosidase A—GH36	3.2.1.22	alpha-galactosidase	Other	WP_150167494.1
Alpha galactosidase A—GH36	3.2.1.22	alpha-galactosidase	TAT(Tat/SPI)	WP_150173666.1
Alpha galactosidase A—GH36	3.2.1.22	alpha-galactosidase	SP(Sec/SPI)	WP_150174740.1
Alpha-L-rhamnosidase—GH78	3.2.1.40	alpha-L-rhamnosidase	SP(Sec/SPI)	WP_150165416.1
Alpha-L-rhamnosidase—GH106	3.2.1.40	alpha-L-rhamnosidase-like protein	SP(Sec/SPI)	WP_150168187.1
Alpha-L-rhamnosidase—GH78	3.2.1.40	alpha-L-rhamnosidase-like protein	Other	WP_150168189.1
Tannase and feruloyl esterase	3.1.1.73	feruloyl esterase	SP(Sec/SPI)	WP_150166293.1
Tannase and feruloyl esterase	3.1.1.73	feruloyl esterase	SP(Sec/SPI)	WP_150169984.1
Tannase and feruloyl esterase	3.1.1.73	feruloyl esterase	Other	WP_150173850.1
Cellulose degradation				
Glycosyl hydrolase -GH9[a]	EC 3.2.1	cellulase-like Ig domain-containing protein	SP(Sec/SPI)	WP_150172866.1
Glycosyl hydrolase—GH5[b]	EC 3.2.1	cellulase (glycosyl hydrolase family 5)	SP(Sec/SPI)	WP_150174270.1
Glycosyl hydrolase—GH8[c]	EC 3.2.1	endoglucanase	LIPO(Sec/SPII)	WP_150165560.1
Glycosyl hydrolase—GH5	EC 3.2.1	endoglucanase	Other	WP_150167270.1
Glycosyl hydrolase—GH5	EC 3.2.1	aryl-phospho-beta-D-glucosidase BglC	SP(Sec/SPI)	WP_150169849.1
Beta-glucosidase—GH1	EC:3.2.1.21	broad-specificity cellobiase	TAT(Tat/SPI)	WP_150169554.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	TAT(Tat/SPI)	WP_150167489.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	Other	WP_150166310.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150168315.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150168569.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	LIPO(Sec/SPII)	WP_150168571.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150168669.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150170787.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150170706.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150171270.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150173744.1
Beta-glucosidase—GH1	EC:3.2.1.21	beta-glucosidase	SP(Sec/SPI)	WP_150174667.1
Cellobiohydrolase was not found in the genome of AB60				

[a]Cellulases from family 9 are exo- or endoglucanases. [b]Cellulases from family 5 present endoglucanase and xylanase activity among others. [c]This enzyme is part of a putative cellulose biosynthetic gene cluster.

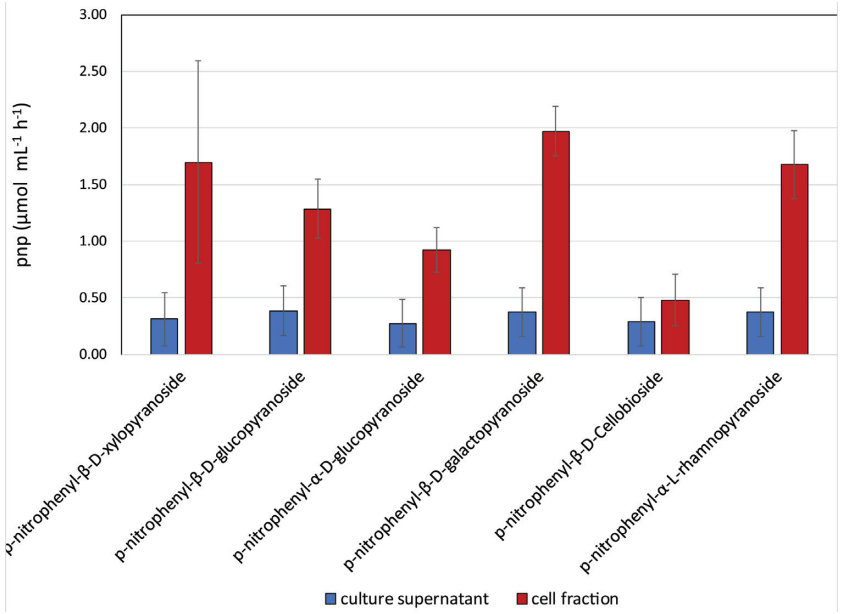

Figure 3. Enzymatic activity in the cell fraction of Acidobacteria AB60 for each substrate. Activity was expressed as µmol mL^{-1} h^{-1} of pNP released from the reaction. *p*-nitrophenyl-*β*-D-glucopyranoside—pNPG (for *β*-glucosidase); *p*-nitrophenyl-*β*-D-cellobioside—pNPC (for cellobiohydrolase); *p*-nitrophenyl-*β*-D-galactopyranoside—pNPGal (for *β*-galactosidase); *p*-nitrophenyl-*α*-L-rhamnopyranoside—pNPR (for *α*-rhamnosidase); *p*-nitrophenyl-*β*-D-xylopyranoside—pNPX (for *β*-xylosidase); and *p*-nitrophenyl-*α*-D-glucopyranoside—pNP*α*G (for *α*-glucosidase).

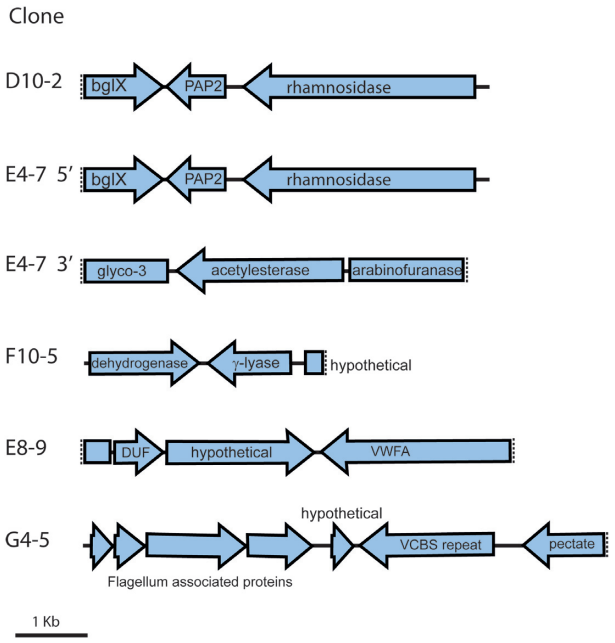

Figure 4. Identification of ORFs from the nucleotide sequences of genomic library clones with activity on xylan. bglX: beta-glucosidase [EC:3.2.1.21]; PAP2: PAP2 superfamily protein; *α*-L-rhamnosidase [EC:3.2.1.40]; glyco-3: glycosyl hydrolase GH3; acetylesterase: sialate O-acetylesterase; arabinofuranosidase: *α*-L-arabinofuranosidase [EC:3.2.1.55]; DUF: uncharacterized protein (DUF305 family); hypothetical: hypothetical protein; dehydrogenase: phenylacetaldehyde dehydrogenase [EC:1.2.1.39]; gamma lyase: cystathionine gamma-lyase [EC:4.4.1.1]; flagellum proteins: FlgM family anti-sigma-28 factor, flagellar hook-associated protein 1, FlgK flagellar hook-associated protein 3 FlgL; pectate: right-handed beta helix region, pectate lyase-3 family domain. Dashed areas represent incomplete gene sequence in the cloned region.

encodes either an acid phosphatase or glucose-6-phosphatase (EC:3.1.3.9); and the full sequence for an *α*-L-rhamnosidase (EC:3.2.1.40). Of these three ORFs, the rhamnosidase is most likely to be involved in xylan degradation.

DISCUSSION

Metagenomic studies using the 16S rRNA gene revealed that members of the phylum Acidobacteria are abundant in Cerrado soils (Araujo *et al.* 2012; de Castro *et al.* 2013). However, Acidobacteria members are well known for their recalcitrance to lab culturing. The use of techniques for culturing slow-growing bacteria allowed for the isolation of Acidobacteria bacterium AB60 from Cerrado soils (de Castro *et al.* 2013). Similar to other members of this phylum, strain AB60 was able to use xylan as its carbon source; however, no growth was observed on cellulose. Acidobacteria bacterium AB60 is closely related to *Telmatobacter bradus* and *Occallatibacter* species (Pankratov *et al.* 2012; Foesel *et al.* 2016), but 16S rRNA gene similarity analysis did not conclusively reveal whether it is a new species within the genus *Occallatibacter* or a new closely related genus. The genomes of *T. bradus* and *Occallatibacter* species are not currently available in the databases, and overall genome similarities could not be used to correctly assign AB60 to a new taxon, as currently recommended by the International Committee on Systematics of Prokaryotes (Chun *et al.* 2018).

The most striking metabolic differences between AB60 and its close relatives are the use of cellulose and hemicellulose. *Occallatibacter* species do not grow on xylan (Foesel *et al.* 2016), whereas strain AB60 clearly degrades this polysaccharide. Growth was observed on xylan from oat spelts (de Castro *et al.* 2013) and beechwood. On the other hand, *T. bradus* was reported to degrade several polysaccharides, including xylan and crystalline cellulose (Avicel®) (Pankratov *et al.* 2012), while AB60 was not able to grow on crystalline cellulose or CMC.

Genomic analysis revealed that AB60 possessed genes involved in xylan and cellulose degradation, but no growth was observed on cellulose. Genes for the enzymes involved in the degradation of the xylan backbone, such as endo-1,4-β-xylanase and xylan 1,4-β-xylosidase, are present in its genome, as well as genes that encode enzymes involved in the lateral chain degradation of xylan from different plant sources.

The enzymatic profile of AB60 showed activities of enzymes known to be involved in xylan degradation, α- and β-glycosyl hydrolases, but these enzymes seemed to be mostly associated with the cell fraction of the culture, indicating substrate binding to the bacterial cell. This result is in agreement with the proposed presence of a xylanase in the periplasm of *Acidobacterium capsulatum* cells (Inagaki *et al.* 1998). Furthermore, a β-glucosidase from *A. capsulatum* (Kishimoto *et al.* 1991) was previously purified and characterized. Similar to the xylanase described above, it was postulated that this enzyme is located in the cell periplasm, since its activity was highest at pH 3.0 rather than intracellular pH 7.0. Periplasmatic glycosyl hydrolases such as β-glycosidases (Bai *et al.* 2017), endoglucanases (Zhu *et al.* 2016) and the xylanase XylC (Fontes *et al.* 2000) were reported in aerobic and anaerobic Gram-negative bacteria.

Among the enzymatic activities exhibited by AB60 grown on xylan, the activity on *p*-nitrophenyl-β-D-cellobioside was lowest. This result matches the enzymatic assays previously reported for Acidobacteria from forest soil (Lladó *et al.* 2016). The observed breakdown of *p*-nitrophenyl-β-D-cellobioside, which is normally a result of the activity of exoglucanases, could be the result of an endoxylanase activity. Enzymes belonging to the GH10 family were reported to exhibit some activity on low-mass cellulose substrates, such as aryl-cellobiosides (Collins, Gerday and Feller 2005). This is consistent with a previous study showing that an *A. capsulatum* endo-β-1,4-xylanase was active on both xylan and *p*-nitrophenyl-β-D-cellobioside, but had low activity on CMC and no activity on filter paper (Inagaki *et al.* 1998). The low activity observed on *p*-nitrophenyl-β-D-cellobioside agrees with the lack of detectable growth on cellulose, as well as the absence of cellobiohydrolase genes in the AB60 genome.

Genes for cellobiohydrolases (GH families 6, 7 and 48) are not detected in most genomes of *Acidobacteria* (Eichorst *et al.* 2018). However, we detected genes for endoglucanases in the genome of Acidobacteria AB60. As mentioned before, strong physiological evidence for cellulose degradation is still absent in most *Acidobacteria* from subgroup 1 (Belova *et al.* 2018). One possible explanation is that synthetic cellulose substrates are not suitable for AB60, or for any other *Acidobacteria* from subgroup 1. Alternatively, it is possible that xylan downregulates the expression of some endoglucanase genes.

Regulation of the expression of glycoside hydrolases by sugars has been widely described, including a negative effect on cellulose utilization in bacteria grown on xylan (Han *et al.* 2003). To test this hypothesis, a small-insert clone library was constructed in order to provide the expression of DNA fragments controlled by a strong promoter. Our attempts to detect genes involved in CMC degradation by functional screening yielded no clones expressing clear halos; however, an increase in the number of clones analyzed might produce positive results. On the other hand, the heterologous expression of the endoglucanases present in the genome may provide further information on their substrate specificities.

The screening on xylan-containing medium yielded clones that consistently presented degradation halos. The ORFs encoded by these clones revealed that clone E4-7 had an ORF coding for an α-L-arabinofuranosidase (EC 3.2.1.55), which catalyzes the hydrolysis of α-1,2-, α-1,3- or α-1,5-L-arabinofuranoside linkages. This enzyme acts synergistically with hemicellulases or pectic enzymes for the complete degradation of hemicellulose, pectin and other arabinose-containing polysaccharides (Yang *et al.* 2015). In addition, two independent clones (E4-7 and D10-2) had identical regions containing an ORF for an α-L-rhamnosidase (EC 3.2.1.40), which is not associated with xylan degradation in most studies. Genes for α-L-rhamnosidases are found in many genomes of the class Acidobacteria, and although some copies are closely related, there is evidence for a lateral transfer origin (Naumoff and Dedysh 2012). The physiological role of α-L-rhamnosidase is not well understood but is probably linked to the broad distribution of L-rhamnose as a component of bacterial and plant cell walls, glycosides, biofilms and glycolipids (Avila *et al.* 2009; Yadav *et al.* 2010).

Rhamnose may not be a major component of most xylans, but the reducing ends of xylan from dicots form a tetrasaccharide containing rhamnose (Rennie and Scheller 2014). The beechwood xylan used in this study is mostly composed of xylose and glucuronic acid, but may contain other sugars (Megazyme, CAS 9014-63-5), such as rhamnose, arabinose, galactose and glucose (Daus *et al.* 2011).The halos observed in the functional genomics analysis were a result of the degradation of xylan lateral chains, indicating that α-L-rhamnosidases and α-L-arabinofuranosidases may be involved in hemicellulose degradation by Acidobacteria bacterium AB60 in nature.

Research on the use of plant-derived polymers by subgroup 1 of the class Acidobacteria has usually focused on the degradation of cellulose, a β-1,4-D-glucose homopolymer, or the backbone of xylan, which is composed of β-1,3-D-xylose units. The results presented here show the importance of enzymes that hydrolyze α-linked carbohydrates, which are typically found in the lateral chains of heteropolymers such as xylan and pectin. The presence of many genes for glycosyl hydrolases that break lateral chains of heteropolymers indicates that Acidobacteria can degrade hemicellulose from various plant sources, which may explain their ubiquity in soils. Finally, this metabolic characteristic may provide a niche for the slow-growing Acidobacteria in soils, allowing its coexistence with other well-known plant-degrading microbes such as fungi and many soil bacteria. Cellulolytic microbes secrete hydrolytic enzymes, including cellulases and hemicellulases that break β-linked sugar bonds, and Acidobacteria may benefit from a partial degradation of these plant polymers.

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SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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