

Complete genome sequence of *Paenibacillus* sp. strain JDR-2

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Paenibacillus sp. strain JDR-2, an aggressively xylanolytic bacterium isolated from sweetgum (*Liquidambar styraciflua*) wood, is able to efficiently depolymerize, assimilate and metabolize 4-O-methylglucuronoxylan, the predominant structural component of hardwood hemicelluloses. A basis for this capability was first supported by the identification of genes and characterization of encoded enzymes and has been further defined by the sequencing and annotation of the complete genome, which we describe. In addition to genes implicated in the utilization of β -1,4-xylan, genes have also been identified for the utilization of other hemicellulosic polysaccharides. The genome of *Paenibacillus* sp. JDR-2 contains 7,184,930 bp in a single replicon with 6,288 protein-coding and 122 RNA genes. Uniquely prominent are 874 genes encoding proteins involved in carbohydrate transport and metabolism. The prevalence and organization of these genes support a metabolic potential for bioprocessing of hemicellulose fractions derived from lignocellulosic resources.

Introduction

Paenibacillus sp. strain JDR-2 (Pjdr2) was isolated from wafers cut from live stems of sweet gum (*Liquidambar styraciflua*) placed in soil in an area populated predominantly by this tree species. The ability of this isolate to grow on 4-O-methylglucuronoxylan (MeGX) as the sole carbon source identified a metabolic potential not previously described. MeGX is released along with fermentable xylose during dilute acid pretreatment of lignocellulosic biomass. Since MeGX may represent 5 to 20% of the hemicellulose components from hardwoods and agricultural residues, this ability was of interest for increasing bioconversion yields of fermentable sugars from these resources [1,2].

Growth rates and yields of Pjdr2 with polymeric 4-O-methylglucuronoxylan (MeGXn) as substrate were much greater than with monosaccharides and oligosaccharides derived from MeGXn. These increases are presumably the result of a cell-associated multimodular GH10 endoxylanase that generates xylobiose, xylotriose, and the aldouronate, 4-O-methylglucuronoxylotriose (MeGX3), for direct assimilation and metabolism [2]. A cluster of genes was cloned and sequenced from Pjdr2 genomic DNA which contained two genes encoding transcriptional regulators, three genes encoding ABC transporters, and three sequential structural genes lacking secretion sequences encoding a GH67 α -glucuronidase, a

GH10 endoxylanase catalytic domain and a putative GH43 β -xylosidase. The expression of these genes, as well as a distal gene encoding a secreted cell-associated multimodular GH10 endoxylanase, was coordinately responsive to inducers and repressors, leading to their collective designation as a xylan-utilization regulon [3]. Physiological studies defining the preferential utilization of MeGXn compared to MeGX and MeGX3 support a process in which extracellular depolymerization, assimilation and intracellular metabolism are coupled, allowing the rapid and complete utilization of MeGXn [4].

Pjdr2 was the first member of this genus to have its genome completely sequenced and made available for detailed analysis. The sequences of genomes of 2 strains of *Paenibacillus polymyxa* [5,6], "*Paenibacillus vortex*" [7], and *Paenibacillus* sp. Y412MC10 (NCBI NC_013406.1, unpublished results) have since been completed. The incomplete genome sequence *Paenibacillus larvae* subsp. *larvae*, the causative agent of American Foulbrood disease of honey bees, has also been analyzed [8].

Classification and features

A phylogenetic tree was constructed using the Neighbor-Joining method [9] for complete sequences of genes encoding 16S rRNA derived from sequenced genomes of *Paenibacillus* spp., along with the sequences of some members of the *Bacillus* spp., *Microbacterium* spp. and *Clostridium* spp, is presented in Figure 1. The sequence of the gene encoding 16S rRNA (AF355462) from *Paenibacillus polymyxa* PKB1 is included as representative of the type species of the genus [10].

The unrooted phylogenetic tree shows Pjdr2 in a branch that includes other *Paenibacillus* spp. in this comparison, supporting a lineage distinct from other Gram positive endospore-forming bacteria. Pjdr2 groups more closely with *Paenibacillus lentimorbus* and other *Paenibacillus* species that are insect pathogens than it does with another group that includes type species *Paenibacillus polymyxa*. From the standpoint of genome size and imputed metabolic potential based on sequence, it is surprising, based on 16S sequence, that it is not more closely related to *Paenibacillus* sp. Y412MC10. Despite a close similarity of *Paenibacillus* JDR-2 to *Microbacterium* species with respect to membrane fatty acids (see discussion below), it is clear that it

is not related to members of the genus *Microbacterium* on the basis of 16S rRNA sequence. When grown on oat spelt xylan agar plates [2], colonies of strain Pjdr2 are white with smooth edges, surrounded by clearing zones resulting from the depolymerization of the xylan. This property was routinely used to monitor the purity of Pjdr2 cultures. As shown in Figure 2, cells of Pjdr2 are rod shaped, with swellings suggestive of sporulation. The properties evaluated for classification allows assignment as an endospore-forming bacterium in the phylum *Firmicutes* and genus *Paenibacillus* as noted in Table 1.

Chemotaxonomy

The fatty acid methyl esters analysis (FAME) of Pjdr2 provided an alternative approach for determination of relatedness to other bacteria. Cultures were grown to exponential phase (24 hrs) on Trypticase soy agars. Bacterial cells were harvested and extracted according to the standard MIDI protocol [26]. FAME analysis was conducted using the Sherlock Microbial Identification System 4.5 [27]. Analyses showed that the predominant fatty acid in Pjdr2 is anteiso-C_{15:0} (46.93%), which in addition to iso-C_{16:0} (23.02%) and C_{16:0} (13.48%), constituted >80% of the fatty acid composition of this strain. Minor fatty acids included iso-C_{14:0} (3.92%), C_{14:0} (2.35%), and iso-C_{15:0} (5.29%).

Strains with a similarity index (SI) value of 0.5 or higher indicate a good library comparison (MIDI 2002). The two strains that most closely match the profile of Pjdr2 are *Microbacterium laevaniformans* (SI = 0.75) and *Cellulobacterium cellulans* (SI = 0.51). We have included these two species in our phylogenetic analysis based upon their 16S rRNA sequences (Figure 1). The FAME analysis provided a rapid assignment of the species by comparing the fatty acid profile(s) with 60 strains (42 species) of *Bacillus*, 2 strains (1 species) of *Cellulobacterium*, 20 strains (19 species) of *Microbacterium* and 20 strains (18 species) of *Paenibacillus*, as well as other aerobic bacteria. Sequence analysis of 16S rRNA provides the acceptable basis for considering phylogenetic relationships. Nevertheless the FAME analysis provides a convenient method with which to confirm the identity of the organism as it is maintained and studied over time.

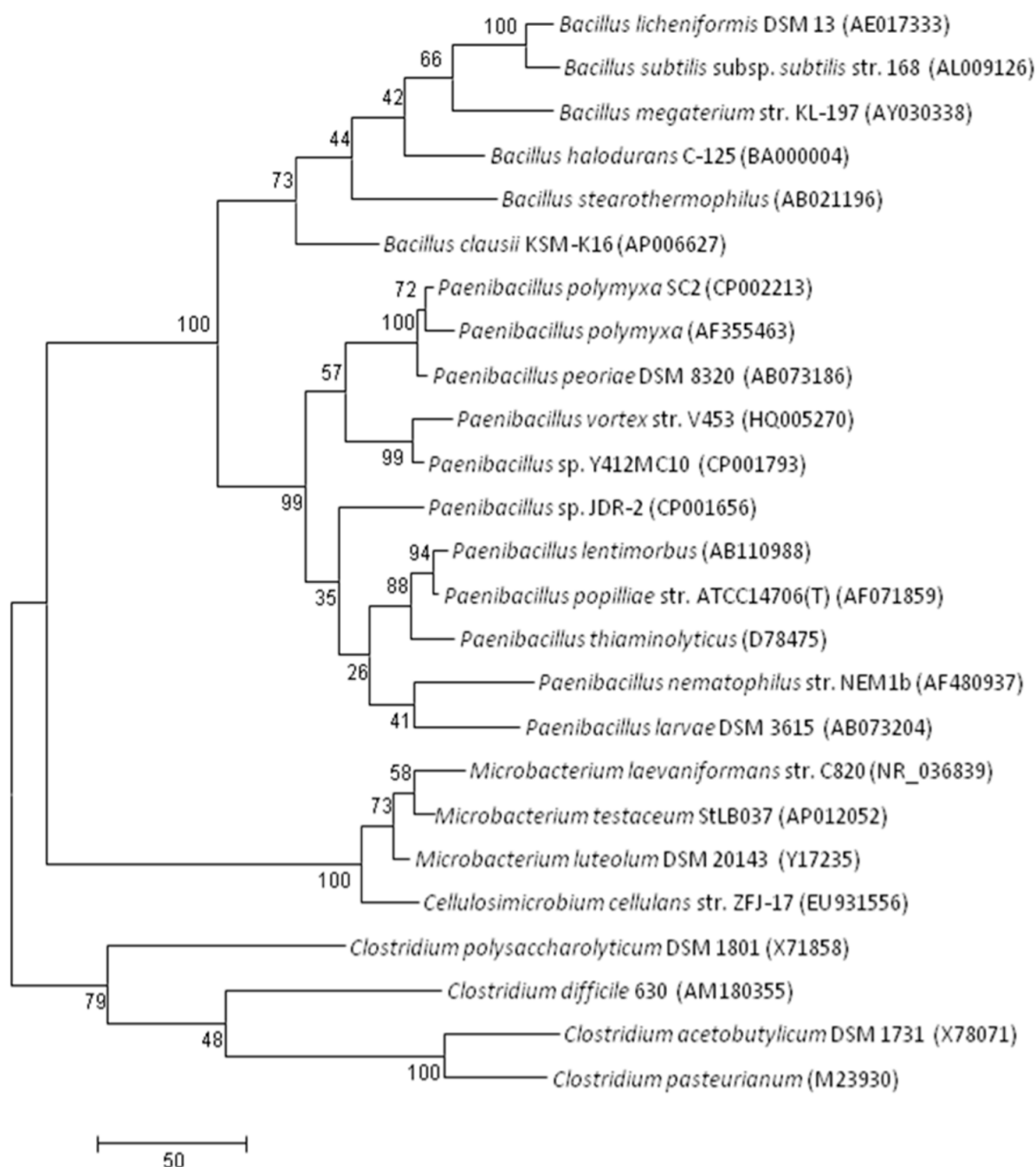


Figure 1. Phylogenetic analysis of *Paenibacillus* sp. JDR-2 was performed using MEGA4 [9] with the Neighbor-Joining method (bootstrap: 2,000 replicates). The species and GenBank accession numbers are: *Paenibacillus larvae* subsp. *pulvifaciens* DSM 3615 (AB073204); *Bacillus halodurans* C-125 (BA000004); *Bacillus subtilis* subsp. *subtilis* str. 168 (AL009126); *Bacillus clausii* KSM-K16 (AP006627); *Bacillus licheniformis* DSM 13 (AE017333); *Bacillus megaterium* str. KL-197 (AY030338); *Bacillus stearothermophilus* (AB021196); *Paenibacillus lentimorbus* (AB110988); *Paenibacillus popilliae* str. ATCC14706(T), (AF071859); *Paenibacillus thiaminolyticus* (D78475); *Paenibacillus nematophilus* str. NEM1b (AF480937); *Paenibacillus polymyxa* (AF355463); *Paenibacillus peoriae* DSM 8320 (AB073186); *Paenibacillus polymyxa* SC2 (CP002213); *Paenibacillus* sp. JDR-2 (CP001656); *Paenibacillus* sp. Y412MC10 (CP001793); “*Paenibacillus vortex*” str. V453 (HQ005270); *Clostridium difficile* 630 (AM180355); *Clostridium polysaccharolyticum* DSM 1801 (X71858); *Clostridium acetobutylicum* DSM 1731 (X78071); *Clostridium pasteurianum* (M23930); *Microbacterium testaceum* StLB037 (AP012052); *Microbacterium laevaniformans* str. C820 (NR_036839); *Microbacterium luteolum* DSM 20143 (Y17235); *Cellulosimicrobium cellulans* str. ZFJ-17 (EU931556).

Table 1. Classification and general features of *Paenibacillus* sp. JDR-2 according to the MIGS recommendations [11].

MIGS ID	Property	Term	Evidence code
		Domain <i>Bacteria</i>	TAS [12]
		Phylum <i>Firmicutes</i>	TAS [13,14]
		Class <i>Bacilli</i>	TAS [15,16]
	Current classification	Order <i>Bacillales</i>	TAS [17,18]
		Family <i>Paenibacillaceae</i>	TAS [16,19]
		Genus <i>Paenibacillus</i>	TAS [20-24]
		Species <i>Paenibacillus</i> sp. Strain JDR-2	TAS [2]
	Gram stain	Positive	NAS
	Cell shape	Rod-shaped	NAS
	Sporulation	Spore-forming	NAS
	Temperature range	Mesophile,	TAS [2]
	Optimum temperature	30°C	TAS [2]
	Salinity		
MIGS-22	Oxygen requirement	Aerobic	IDA
	Carbon source	Glucose, xylose, β 1,4-xylan, β 1,4-1,3-glucan, 4-O-methyl-glucuronoxyllose	TAS [2]
	Energy source	chemoorganotrophic	
MIGS-6	Habitat	Sweet Gum stem wood	TAS [2]
MIGS-15	Biotic relationship	Free living	TAS [2]
MIGS-14	Pathogenicity	Non pathogenic	NAS
	Biosafety level	1	NAS
	Isolation	Sweet Gum stem wood in soil	TAS [2]
MIGS-4	Geographic location	Florida	TAS [2]
MIGS-5	Sample collection time	2000	TAS [2]
MIGS-4.1	Latitude	29.4°	TAS
MIGS-4.2	Longitude	82.3°	TAS
MIGS-4.3	Depth	1 inch	TAS [2]
MIGS-4.4	Altitude	180 feet above msl	NAS

Evidence codes – IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [25].

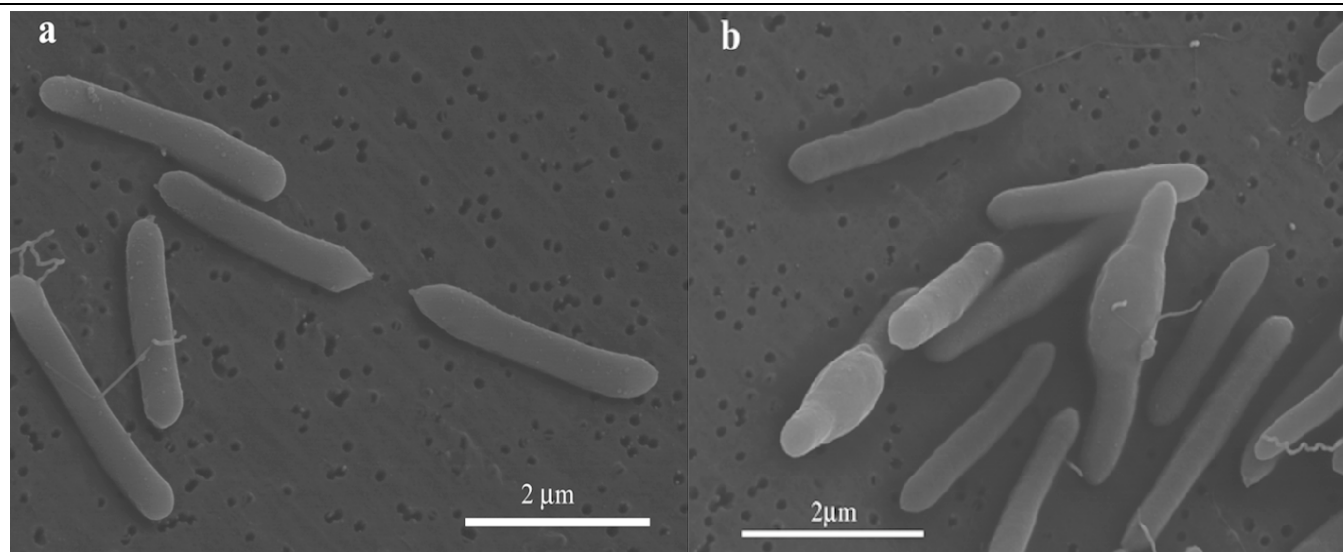


Figure 2. Scanning electron micrographs of *Paenibacillus* sp. JDR-2. Panel (a) is representative of the bacilli harvested in the vegetative state and panel (b) indicates individuals with expanded midsections which are entering the sporulation phase. Pjdr2 cells were grown in Luria Broth and harvested by centrifugation at the exponential growth phase (a) and post exponential phase (b), the pellets washed with water 3 times and prepared for scanning electron microscopy by the Electron Microscopy and Bio-Imaging laboratory, ICBR of the University of Florida.

Growth conditions and DNA isolation

For the preparation of genomic DNA, one of several colonies surrounded by a clear zone was picked from an agar plate (0.1% oat spelt xylan/ 0.1% yeast extract/ Zucker-Hankin medium [2], and grown in Zucker-Hankin/1% yeast extract at 30°C with shaking at 240 rpm. A culture (8 ml) at 0.6 OD_{600nm} was inoculated into 48 ml of culture media (Zucker-Hankin, 1% yeast extract). The latter was grown to 0.6 OD_{600nm} and cells were collected by centrifugation. High molecular weight DNA was prepared from these cells as per the protocol provided by JGI. Cells were suspended in TE buffer (10 mM Tris-HCl, 1.0 mM EDTA), pH 8.0 and treated with lysozyme to lyse the cell wall. SDS and Proteinase K were added to denature and degrade proteins. NaCl and CTAB were added to facilitate subsequent precipitation. Cell lysates were extracted with phenol and chloroform and the DNA was precipitated by addition of isopropanol. The nucleic acid pellet was washed with 70% ethanol, dissolved in water and then treated with RNase A.

Genome sequencing and assembly

The genome of Pjdr2 was sequenced at the JGI using a combination of 8 kb and 40 kb (fosmid) DNA libraries. In addition to Sanger sequencing, 454 pyrosequencing [28] was performed to a

depth of 20× coverage. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website [29]. Draft assemblies were based on 39,689 total reads. All three libraries provided 5.1× coverage of the genome. The Phred/Phrap/Consed software package [30] was used for sequence assembly and quality assessment [31-33]. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with Dupfinisher [34] or transposon bombing of bridging clones (Epicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, custom primer walk, or PCR amplification (Roche Applied Science, Indianapolis, IN). A total of 1,028 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. The completed sequence analysis of Pjdr2 contained 45,057 reads, achieving an average of 5.5-fold sequence coverage per base, with an error rate less than 1 in 100,000. The complete nucleotide sequence of *Paenibacillus* sp. strain JDR-2 and its annotation can be found online at the IMG (Integrated Microbial Genome) portal of JGI [35], as well as at the genome resource site of NCBI [36].

Genome annotation

Genes were identified using Prodigal [37] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by manual curation using the JGI program GenePRIMP [38]. The predicted CDSs were translated and searched with the following databases to assign a product description for each predicted protein: the National

Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [39], RNAMmer [38], Rfam [40], TMHMM [41], and SignalP [42]. Genome statistics are provided in Table 2, and a full circular map in Figure 3 below.

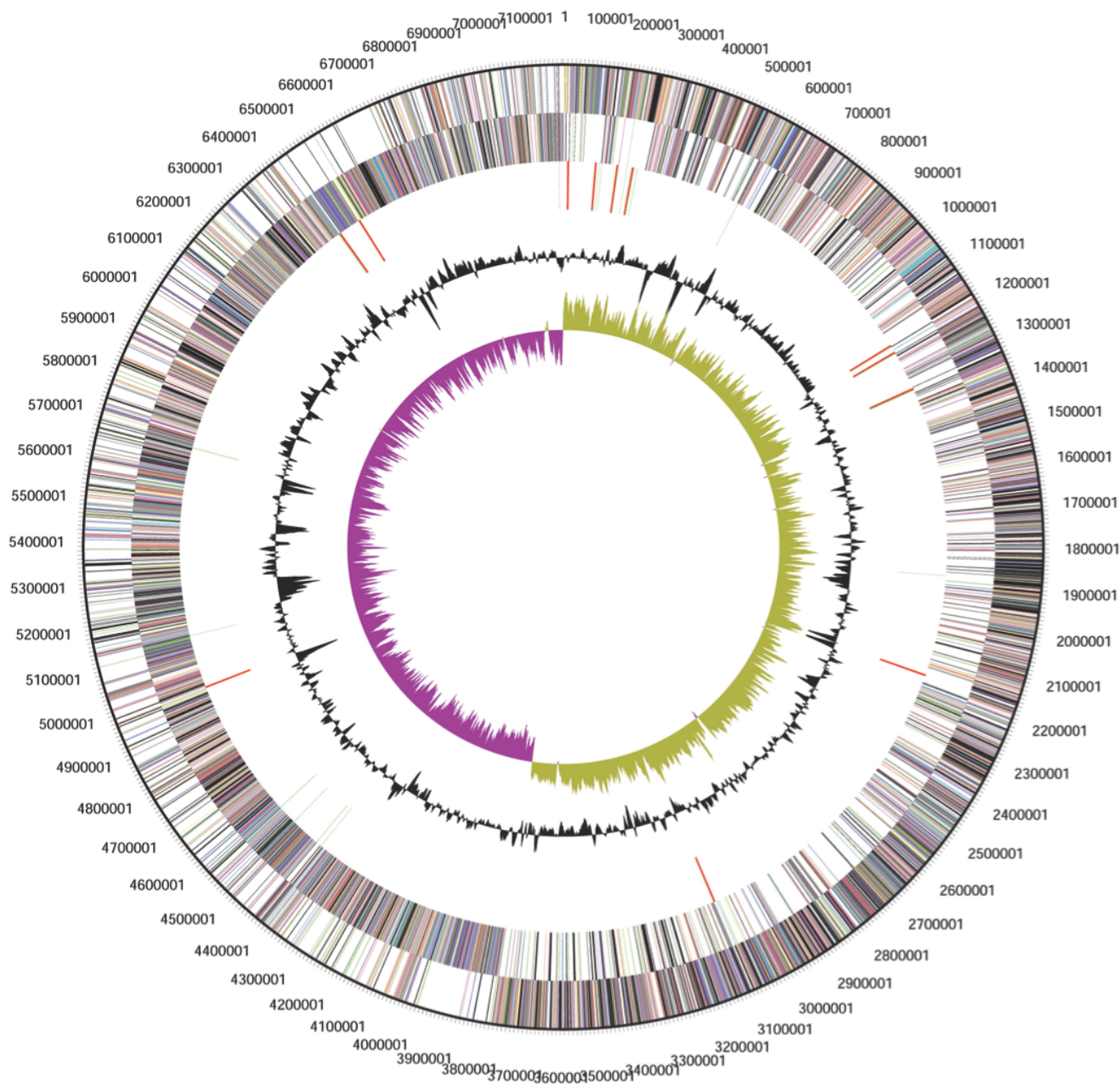


Figure 3. Circular map of the genome of *Paenibacillus* sp JDR-2. Labeling from the outside circle towards the inside circles: circle 1. Nucleotide numbering system; circle 2 and 3. Predicted coding sequences on the forward strand and on the reverse strand with each gene colored by its assigned COG category; circle 4. RNA genes (tRNAs in green, rRNAs in red, other RNAs in black); circle 5. GC content; circle 6. GC skew.

Table 2. Genomic Statistics

Attribute	Value	% of Total
Genome size (bp)	7,184,930	100.00%
DNA coding region (bp)	6,384,736	88.86%
DNA G+C content (bp)	3,612,449	50.28%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	6,410	100.00%
RNA genes	122	1.90%
rRNA genes	35	0.55%
Protein coding genes	6,288	98.10%
Pseudo Genes	75	1.17%
Genes with function prediction	4,737	73.90%
Protein coding genes with COGs	4,667	72.81%
Protein coding genes with Pfam	5,128	80.00%
Genes in paralog clusters	1,614	25.18%
Protein coding genes coding signal peptides	1,629	25.41%
Genes connected to transporter classification	1,090	17.00%

Insights from genome sequencing

Utilization of lignocellulosics

The nucleotide sequence of a cluster of genes which included the α -glucuronidase gene served as a marker for the sequenced genome. The sequence of this cluster was previously determined in a cosmid clone of the genomic DNA of Pjdr2. The presence of this unique contiguous sequence in a single copy without orthologs or paralogs supported the final genomic sequence as representative of a single genome from a pure culture. This aldouronate-utilization gene cluster, in conjunction with the distal gene encoding a multimodular cell-associated GH10 endoxylanase, constitutes a xylan-utilization regulon as previously defined [3]. The coordinate expression of the genes in this regulon supports a process in which assimilation of the aldouronate, 4-O-methylglucuronoxylotriose, generated by a cell-associated GH10 endoxylanase, is coupled to extracellular depolymerization, facilitating

depolymerization, assimilation and metabolism as previously described [4]. The sequencing of the genome of *Paenibacillus* sp. strain JDR-2 has allowed further analysis of its xylan-utilization regulon and the identification of similar regulons involved in the depolymerization and utilization of soluble β -glucans.

A noteworthy feature of the genome of Pjdr2 is the large number (874) of genes involved in carbohydrate metabolism and transport constituting 17% of the genome (Table 3). This characteristic contrasted with 9% and 291 genes in *Bacillus subtilis* 168 and 11% and 481 genes in *Paenibacillus polymyxa* E861. The recently completed genome *Paenibacillus* sp. Y412MC10, however, is quite similar to Pjdr2 and contains 16% and 828 genes in this category.

Table 3. Number of genes associated with the general COG functional categories

Code	value	%age	Description
J	199	3.89	Translation, ribosomal structure and biogenesis
A	-	-	RNA processing and modification
K	580	11.34	Transcription
L	149	2.91	Replication, recombination and repair
B	1	0.02	Chromatin structure and dynamics
D	36	0.70	Cell cycle control, cell division, chromosome partitioning
Y	-	-	Nuclear structure
V	104	2.03	Defense mechanisms
T	426	8.33	Signal transduction mechanisms
M	255	4.98	Cell wall/membrane/envelope biogenesis
N	70	1.37	Cell motility

Table 3 (cont.) Number of genes associated with the general COG functional categories

Code	value	%age	Description
Z	1	0.02	Cytoskeleton
W	-	-	Extracellular structures
U	57	1.11	Intracellular trafficking, secretion, and vesicular transport
O	116	2.27	Posttranslational modification, protein turnover, chaperones
C	180	3.52	Energy production and conversion
G	874	17.08	Carbohydrate transport and metabolism
E	316	6.18	Amino acid transport and metabolism
F	115	2.25	Nucleotide transport and metabolism
H	151	2.95	Coenzyme transport and metabolism
I	120	2.35	Lipid transport and metabolism
P	273	5.34	Inorganic ion transport and metabolism
Q	99	1.94	Secondary metabolites biosynthesis, transport and catabolism
R	613	11.98	General function prediction only
S	381	7.45	Function unknown
-	1,743	27.19	Not in COGs

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