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Complete genome sequence of *Pirellula staley* type strain (ATCC 27377^T)

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Pirellula staley Schlesner and Hirsch 1987 is the type species of the genus *Pirellula* of the family *Planctomycetaceae*. Members of this pear- or teardrop-shaped bacterium show a clearly visible pointed attachment pole and can be distinguished from other *Planctomycetes* by a lack of true stalks. Strains closely related to the species have been isolated from fresh and brackish water, as well as from hypersaline lakes. Here we describe the features of this organism, together with the complete genome sequence and annotation. This is the first completed genome sequence of the order *Planctomyces* and only the second sequence from the phylum *Planctomycetes*. The 6,196,199 bp long genome with its 4773 protein-coding and 49 RNA genes is a part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

Introduction

Strain ATCC 27377^T (= DSM 6068 = ATCC 27377) is the type strain of the species *Pirellula staley* and was originally isolated by James T. Staley in the early 1970s [1,2]. Due to superficial similarities with *Pasteuria ramosa* in budding and rosette-formation, strain ATCC 27377^T was for several years considered to belong to the genus *Pasteuria*, the type strain of *P. ramosa* Metchnikoff 1888 [3]. However, Starr *et al.* [4] considered that this strain did not fit the original description of *P. ramosa* published by Metchnikoff in 1888 [3] and formally requested that the Judicial Commission rule that it should not be the type of *P. ramosa* Metchnikoff 1888. An Opinion was published by the Judicial

Commission [5] fixing the type of *P. ramosa* Metchnikoff 1888 as the description of Metchnikoff as emended by Starr *et al.* [3]. At the same time Starr *et al.* [3] also proposed that ATCC 27377^T be used as the type of a new species *Planctomyces staley*. In 1984 Schlesner and Hirsch re-assigned ATCC 27377^T to the new genus *Pirella* [6] as the type strain to the only species *Pirella staley* [6], but realized three years later that this genus name was as later homonym of *Pirella* Bainier 1883 [7], a fungus belonging to the *Mucorales*, and therefore illegitimate according to rule 51b of the *International Code of Nomenclature of Bacteria* [8,9]. In 1987 the strain received its currently validly pub-

lished name *Pirellula staleyi*. *P. staleyi* and close relatives belong to the so called morphotype IV and are of interest because these organisms are usually attached to filamentous algae and cyanobacteria by a holdfast located at the distal end of the fascicle (the multifibrillar major appendage) or at the nonreproductive (nonbudding and nonpiliated) pole of the cell, if a fascicle is not present. *P. staleyi* is of further interest because of its life cycle (see below). It should be noted that members of the genus *Pirellula* (*P. staleyi*, *P. marina*) and other unnamed strains have been variously considered to be rapidly evolving (tachyletic) or ancient lineages. The transfer of *P. marina* to *Blastopirellula marina* and description of *Rhodopirellula baltica* [10] has called this interpretation into question, a theory that the growing number of genomes in the group may also be used to test. Here we present a summary classification and a set of features for *P. staleyi* ATCC 27377^T (Table 1), together with the description of the complete genomic sequencing and annotation.

Classification and features

To date, two strains of the species *P. staleyi* have been described in detail, ATCC 27377^T [6,9] and strain ATCC 35122 [18]. Strain ATCC 27377^T was isolated from the freshwater Lake Lansing, MI,

USA either in 1973 or before [2]. Strain ATCC 35122 was isolated as a “white” subclone of strain ICPB 4232 from a similar habitat, the freshwater Campus Lake, Baton Rouge, LA, USA [18,23]. Both strains are identical in their 16S rRNA gene sequence [18]. Except for an agricultural soil bacterium clone (SC-I-28, AJ252628), and for the isolates ‘Schlesner 516’ and ‘Schlesner 670’ (X81940, X81948) [24], no 16S rRNA gene sequences above 85% sequence similarity were reported in Genbank. Environmental samples from metagenomic surveys also do not surpass 88-90% sequence similarity, indicating that members of the species are not heavily represented in the so far genomically screened habitats (as of August 2009). Interestingly, sequences most closely related to the planktonic, aerobic heterotroph *P. staleyi* have been reported from anoxic sediments of the productive freshwater lake Priest Pot, Cumbria, UK [25]. Also, *Pirellula*-like sequences have been recovered from DNA extracted from marine sediments in Puget Sound [26] and marine snow [27].

Figure 1 shows the phylogenetic neighborhood of *P. staleyi* ATCC 27377^T in a 16S rRNA based tree. The sequence of the sole 16S rRNA gene in the genome is identical to the previously published sequence generated from DSM 6068 (AJ231183).

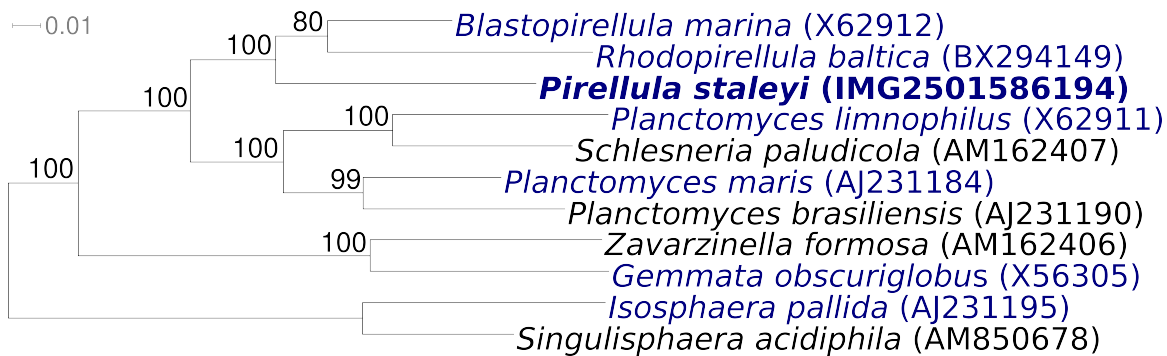


Figure 1. Phylogenetic tree highlighting the position of *P. staleyi* ATCC 27377^T relative to the other type strains within the family Planctomycetaceae. The tree was inferred from 1,316 aligned characters [28,29] of the 16S rRNA gene sequence under the maximum likelihood criterion [30] and rooted in accordance with the current taxonomy. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1,000 bootstrap replicates if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [31] are shown in blue, published genomes in bold.

The cell size of strain ATCC 27377^T is 0.9-1.0 × 1.0-1.5 μm. The mature cell shape is teardrop- to pear-shaped, with the attachment pole slightly pointed (Figure 2). A fibrillar stalk shape and struc-

ture is absent. Crateriform structures are predominantly on the reproductive cell pole only. Occasionally, small crateriform structures may also be observed on the non-reproductive and nonpiliated

pole of the cell opposite the budding site [20]. The position of the monotrichous flagellum is at the reproductive cell pole [6,10]. Strain ATCC 27377^T produces pigmented colonies and motile daughter and sessile mother cells [10].

A unique feature seen in both negatively stained cells and in thin-sectioned cells of strains ATCC 27377^T and ATCC 35122 is the occurrence of 'hump' protrusions including both cell wall and cytoplasm [18]. These protrude 50 ± 111 nm from the cell and are 200 ± 260 nm in diameter measured at the base of the structure (from thin sections and negatively stained cells) [18]. One or two are visible per cell, and when two are visible these are distributed in a characteristic manner opposite to each other in the cell near the narrow pole [18]. They appear to conform to the definition of prosthecae as cellular appendages or extensions of the cell containing cytoplasm [18,32]. However, the prosthecae of strain ATCC 27377 are distributed further from the narrow cell pole than in strain ATCC 35122 [18]. Functions proposed for the prosthecae include increasing surface area, reproduction, and stalk function [18].

The life cycle of *P. staleyi* has been described in great detail elsewhere [23]. Briefly, the mature bud develops a sheathed flagellum attached near the piliated pole (opposite the fascicle origin) and becomes a swarmer; the swarmer loses its flagellum and becomes a sessile mother cell (with a distal holdfast and eventually a fascicle at the pole

opposite the piliated and budding pole); the mother cell develops a bud [20,23].

Strain ATCC 27377^T hydrolyses casein, aesculin, gelatin and starch, but not DNA [10]. It produces H₂S from thiosulfate and is negative for lipase (pH 7) and phosphatidyl choline [10]. It utilizes fucose as carbon source, but not glycerol, glutamic acid, or chondroitin sulfate [10]. Contrary to the original description [2], the cells are Gram-negative and do not utilize lyxose, D-ribose, fucose, L-rhamnose, fructose, or inulin as a carbon source. Additional characteristics include the following. Pectin, lactose, maltose, melibiose, raffinose, sucrose, and trehalose are utilized as carbon sources. The maximum salt tolerance is 50% artificial seawater (Lyman & Fleming, 1940), with 100% ASW corresponding to 3.5% salinity [4]. The cells are weakly inhibited by artificial light (2,400 lx). The following carbon sources are not utilized: adipate, citrate, L-alanine, L-glutamate, gluconate, and urea [4,7]. Strain ATCC 27377^T is resistant to ampicillin and penicillin (1000 $\mu\text{g ml}^{-1}$), cephalothin (100 $\mu\text{g ml}^{-1}$), streptomycin (500 $\mu\text{g ml}^{-1}$) and cycloserine (100 $\mu\text{g ml}^{-1}$), but not to tetracycline (10 $\mu\text{g ml}^{-1}$ is lethal) [10]. The primary sequence and secondary structure of the ribonuclease P RNA of strain *P. staleyi* ATCC 27377^T and other planctomycetes has been described in detail and has been evaluated for their suitability as a taxonomic marker [20].

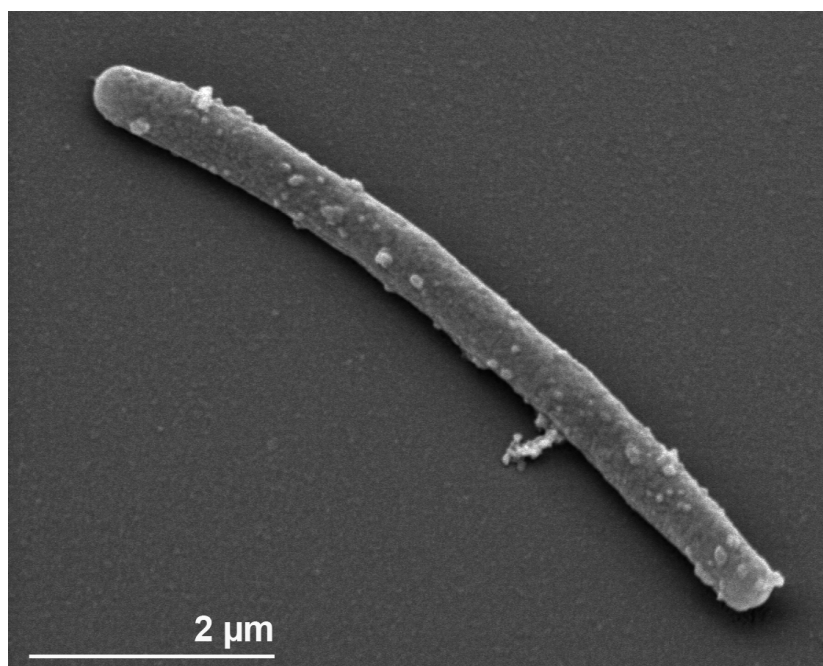


Figure 2. Scanning electron micrograph of *P. staleyi* ATCC 27377^T

Chemotaxonomy

The cell envelope of strain *P. staleyi* ATCC 27377^T contains no peptidoglycan but consists almost entirely of protein. The cell wall amino acids (molar ratio) are threonine (3.0), glutamate (9.0), cysteine (3.6) and valine (1.7) [22]. Further details on the amino acids, NH₃, hexosamine and neutral sugar contents of the cell envelope of strain ATCC 27377^T are published elsewhere [10]. The major fatty acids (relative %) are C_{16:0} (33.8), C_{18:1Δ9} (26.6), C_{20:1Δ11} (15.7), C_{17:1Δ9} (14.4), C_{17:0} (5.3), C_{16:1Δ9} (3.5), C_{18:0} (3.3), and C_{18:1Δ11} (2.0) [10]. The

major polyamine is sym-homospermidine [50.2 μmol (g dry weight)⁻¹] [19]. The major respiratory lipoquinone present is MK-6. One of the major phospholipid present that has been identified is phosphatidylglycerol [10]. Other lipids have not been identified based on R_f values and staining behavior, indicating that novel lipids are an important constituent of the cell membrane. The production of spermidine distinguishes *P. staleyi* from the closely related *R. baltica* DSM 10527 and *B. marina* DSM 3645.

Table 1. Classification and general features of *P. staleyi* ATCC 27377^T according to the MIGS recommendations [11]

MIGS ID	Property	Term	Evidence code
		Domain <i>Bacteria</i>	TAS [12]
		Phylum <i>Planctomycetes</i>	TAS [13]
		Class " <i>Planctomycetacia</i> "	TAS [13]
	Current classification	Order <i>Planctomycetales</i>	TAS [14-17]
		Family <i>Planctomycetaceae</i>	TAS [14,15]
		Genus <i>Pirellula</i>	TAS [9,10]
		Species <i>Pirellula staleyi</i>	TAS [10,15]
		Type strain ICPB 4128	TAS [6]
	Gram stain	negative	TAS [6]
	Cell shape	pear or teardrop shaped	TAS [6]
	Motility	with flagella	TAS [6]
	Sporulation	sporulation has not been reported	TAS [6,10]
	Temperature range	mesophile, range has not been determined	TAS [10,18]
	Optimum temperature	20-25°C	TAS [10,19]
	Salinity	50% artificial seawater (100% ASW = 34.5 ‰ salinity)	TAS [6,10]
MIGS-22	Oxygen requirement	aerobic	NAS [6,10]
	Carbon source	fucose, pectin, lactose, maltose, melibiose, raffinose, sucrose, and trehalose	TAS [6,10]
	Energy source	carbohydrates	TAS [6,10]
MIGS-6	Habitat	aquatic	TAS [20]
MIGS-15	Biotic relationship	free-living, but also attached to filamentous algae and cyanobacteria	TAS [20]
MIGS-14	Pathogenicity	non pathogenic	NAS
	Biosafety level	1	TAS [21]
	Isolation	fresh and brackish water	TAS [6,20]
MIGS-4	Geographic location	Lake Lansing, Michigan, USA	TAS [20]
MIGS-5	Sample collection time	early 1970s	TAS [20]
MIGS-4.1	Latitude, Longitude	42.759, -84.399	NAS
MIGS-4.2			
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

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). These evidence codes are from the Gene Ontology project [22]. If the evidence code is IDA, then the property was directly observed for a living isolate by one of the authors or an expert mentioned in the acknowledgements.

Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position, and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project. The genome project is deposited in the Genome OnLine Database [31] and the complete

genome sequence is deposited in Genbank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	One 8kb pMCL200 genomic library One 454 pyrosequencing standard library and one Illumina library
MIGS-29	Sequencing platforms	ABI3730, 454 GS FLX, Illumina GA
MIGS-31.2	Sequencing coverage	10.6× Sanger; 20.4× pyrosequencing
MIGS-30	Assemblers	Newbler version 1.1.03.24, PGA
MIGS-32	Gene calling method	Prodigal, GenePRIMP
	INSDC ID	CP001848
	Genbank Date of Release	December 30, 2009
	GOLD ID	Gi02538
	NCBI project ID	29845
	Database: IMG-GEBA	2501533211
MIGS-13	Source material identifier	DSM 6068
	Project relevance	Tree of Life, GEBA

Growth conditions and DNA isolation

P. staley ATCC 27377^T, DSM 6068, was grown in DSMZ medium 595 (Caulobacter Medium) [33], at 26°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram-positive DNA Purification Kit (Epicentre MGP04100) with doubled volume (2 µl) lysozyme and incubated for one hour at 37°C according to Wu *et al.* [34].

Genome sequencing and assembly

The genome was sequenced using a combination of Sanger, 454 and Illumina sequencing platforms. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website (<http://www.jgi.doe.gov/>). 454 Pyrosequencing reads were assembled using the Newbler assembler version 1.1.03.24 (Roche). Large Newbler contigs were broken into 6,869 overlapping fragments of 1,000 bp and entered into assembly as pseudo-reads. The sequences were assigned quality scores based on Newbler consensus q-scores with modifications to account for overlap redundancy and adjust inflated q-scores. A hybrid 454/Sanger assembly was made

using the PGA (Paracel Genome Assembler) assembler. Possible mis-assemblies were corrected and gaps between contigs were closed by custom primer walks from sub-clones or PCR products. Illumina reads were used to improve the final consensus quality using an in-house developed tool (the Polisher). The error rate of the completed genome sequence is less than 1 in 100,000. The final assembly consists of 70,045 Sanger and 450,004 pyrosequence reads. Together all sequence types provided 31.0× coverage of the genome.

Genome annotation

Genes were identified using Prodigal [35] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline (<http://geneprimp.jgi-psf.org/>) [36]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and

functional annotation was performed within the Integrated Microbial Genomes - Expert Review (<http://img.jgi.doe.gov/er>) platform [37].

Genome properties

The genome is 6,196,199 bp long and comprises one main circular chromosome with a 57.5% GC content (Figure 3 and Table 3). Of the 4,822 genes

predicted, 4,773 were protein coding genes, and 49 RNAs. In addition, 56 pseudogenes were also identified. The majority of the protein-coding genes (54.5%) were assigned with a putative function while those remaining were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

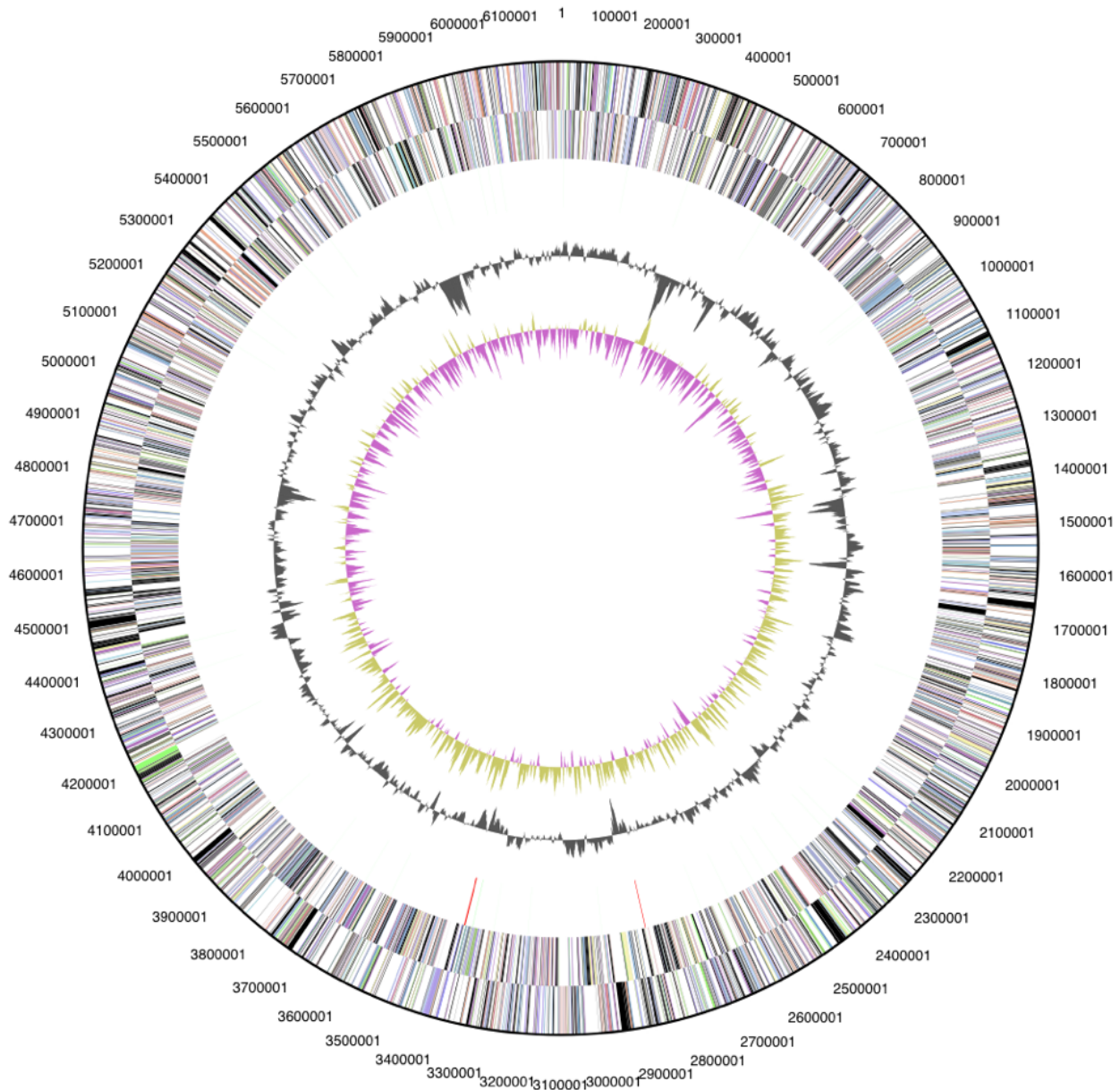


Figure 3. Graphical circular map of the genome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

Table 3. Genome Statistics

Attribute	Value	% of Total
Genome size (bp)	6,196,199	100.00%
DNA coding region (bp)	5,362,662	86.55%
DNA G+C content (bp)	3,560,627	57.46%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	4,822	100.00%
RNA genes	49	1.02%
rRNA operons	1	
Protein-coding genes	4,773	98.98%
Pseudo genes	56	1.16%
Genes with function prediction	2,629	54.52%
Genes in paralog clusters	471	9.77%
Genes assigned to COGs	2,755	57.13%
Genes assigned Pfam domains	2,895	60.04%
Genes with signal peptides	1,414	29.32%
Genes with transmembrane helices	1,309	27.15%
CRISPR repeats	2	

Table 4. Number of genes associated with the general COG functional categories

Code	value	%age	Description
J	149	3.1	Translation, ribosomal structure and biogenesis
A	2	0.0	RNA processing and modification
K	198	4.1	Transcription
L	153	3.2	Replication, recombination and repair
B	1	0.0	Chromatin structure and dynamics
D	21	0.4	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	70	1.5	Defense mechanisms
T	197	4.1	Signal transduction mechanisms
M	164	3.4	Cell wall/membrane biogenesis
N	159	3.3	Cell motility
Z	1	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	191	4.0	Intracellular trafficking and secretion
O	138	2.9	Posttranslational modification, protein turnover, chaperones
C	164	3.4	Energy production and conversion
G	169	3.5	Carbohydrate transport and metabolism
E	213	4.5	Amino acid transport and metabolism
F	64	1.3	Nucleotide transport and metabolism
H	135	2.8	Coenzyme transport and metabolism
I	96	2.0	Lipid transport and metabolism
P	150	3.1	Inorganic ion transport and metabolism
Q	60	1.3	Secondary metabolites biosynthesis, transport and catabolism
R	432	9.1	General function prediction only
S	285	6.0	Function unknown
-	2018	42.3	Not in COGs

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