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## Complete genome sequence of the thermophilic sulfur-reducer *Hipaea maritima* type strain (MH<sub>2</sub><sup>T</sup>)

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*Hipaea maritima* (Miroshnichenko *et al.* 1999) is the type species of the genus *Hipaea*, which belongs to the family *Desulfurellaceae* within the class *Deltaproteobacteria*. The anaerobic, moderately thermophilic marine sulfur-reducer was first isolated from shallow-water hot vents in Matipur Harbor, Papua New Guinea. *H. maritima* was of interest for genome sequencing because of its isolated phylogenetic location, as a distant next neighbor of the genus *Desulfurella*. Strain MH<sub>2</sub><sup>T</sup> is the first type strain from the order *Desulfurellales* with a completely sequenced genome. The 1,694,430 bp long linear genome with its 1,723 protein-coding and 57 RNA genes consists of one circular chromosome and is a part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

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### Introduction

Strain MH<sub>2</sub><sup>T</sup> (DSM 10411 = ATCC 700847) is the type strain of the species *Hipaea maritima*, which is the type species of its genus *Hipaea* [1]. The genus currently contains no other validly named species [2], but two other strains belonging to the species were isolated from shallow-water hot vents in New Zealand and Papua New Guinea [1]. The type strain was isolated during a cruise of the Russian scientific vessel *A. Nesmeyanov* through shallow-water hot vents of the south-western Pacific Ocean, environments that are typical for anaerobic, thermophilic,

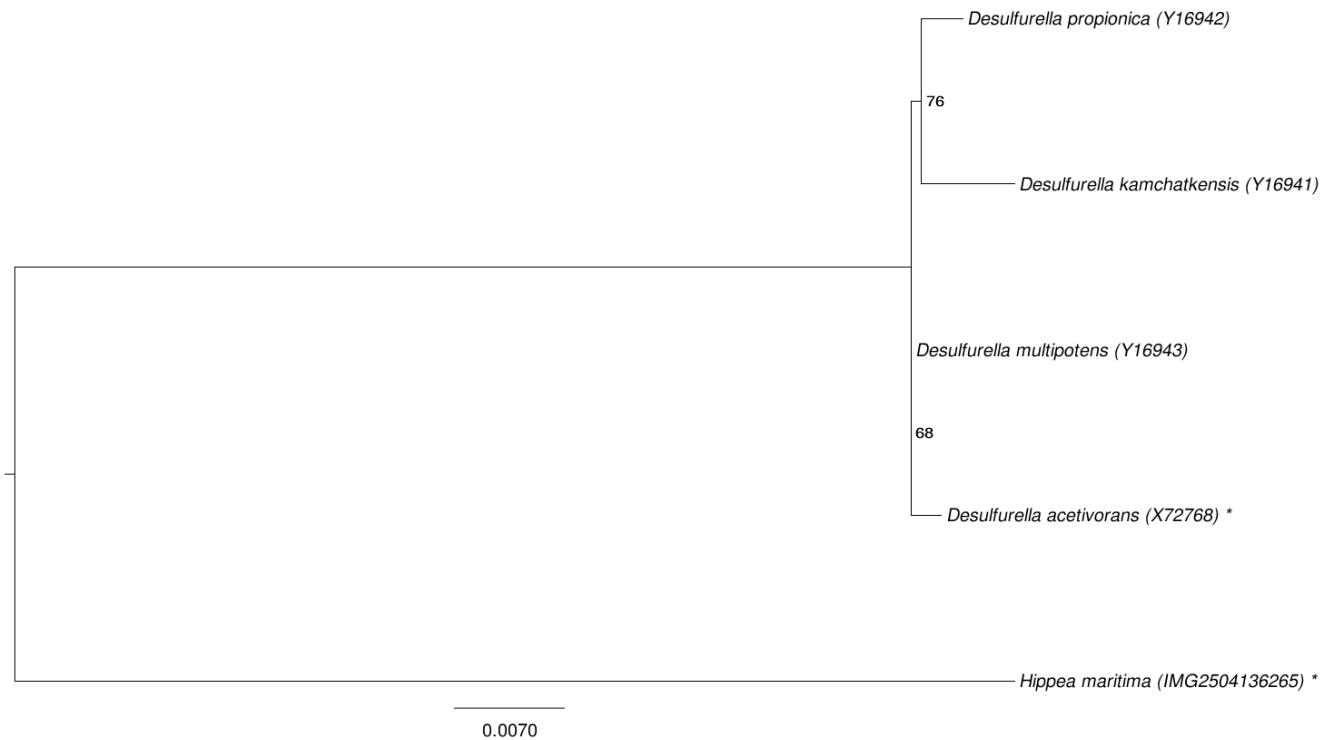
sulfur-reducing bacteria [1]. The genus is named after the German microbiologist Hans Hippe, in recognition of his significant contribution to the characterization of novel, obligately anaerobic prokaryotes and the understanding of their physiology. The species epithet is derived from the Latin word *maritima* (inhabiting marine environments) [2]. Here we present a summary classification and a set of features for *H. maritima* strain MH<sub>2</sub><sup>T</sup>, together with the description of the complete genomic sequencing and annotation.

## Classification and features

A representative genomic 16S rRNA sequence of strain MH2<sup>T</sup> was compared using NCBI BLAST under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [3] and the relative frequencies, of taxa and keywords (reduced to their stem [4]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Desulfurella* (38.7%), *Desulfovibrio* (15.2%), *Deferribacter* (10.8%), *Thermotoga* (10.8%) and *Hippea* (8.6%) (44 hits in total). Regarding the single hit to sequences from members of the species, the average identity within HSPs was 99.9%, whereas the average coverage by HSPs was 82.7%. Among all other species, the one yielding the highest score was *Desulfurella multipotens*, which corresponded to an identity of 89.6% and an HSP coverage of 82.6%. (Note that the Greengenes database

uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest-scoring environmental sequence was AF232926 ('United Kingdom: Montserrat geothermal springs clone MS10 proteobacterium'), which showed an identity of 88.9% and a HSP coverage of 73.0%. The most frequently occurring keywords within the labels of environmental samples which yielded hits were 'microbi' (5.0%), 'spring' (2.9%), 'sediment' (2.4%), 'soil' (2.3%) and 'industri' (2.2%) (206 hits in total). Environmental samples which yielded hits of a higher score than the highest scoring species were not found.

The 16S rRNA based tree in Figure 1 shows the phylogenetic neighborhood of *H. maritima*. The sequence of the two identical 16S rRNA genes differs by one nucleotide from the previously published 16S rRNA sequence (Y18292).



**Figure 1.** Phylogenetic tree highlighting the position of *H. maritima* relative to the other type strains within the family *Desulfurellaceae*. The tree was inferred from 1,526 aligned characters [5,6] of the 16S rRNA gene sequence under the maximum likelihood criterion [7] and rooted in accordance to the current taxonomy. The branches are scaled in terms of the expected number of substitutions per site. Numbers next to bifurcations are support values from 700 bootstrap replicates [8] if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [9] are shown with an asterisk, those also listed as 'Complete and Published' with two asterisks.

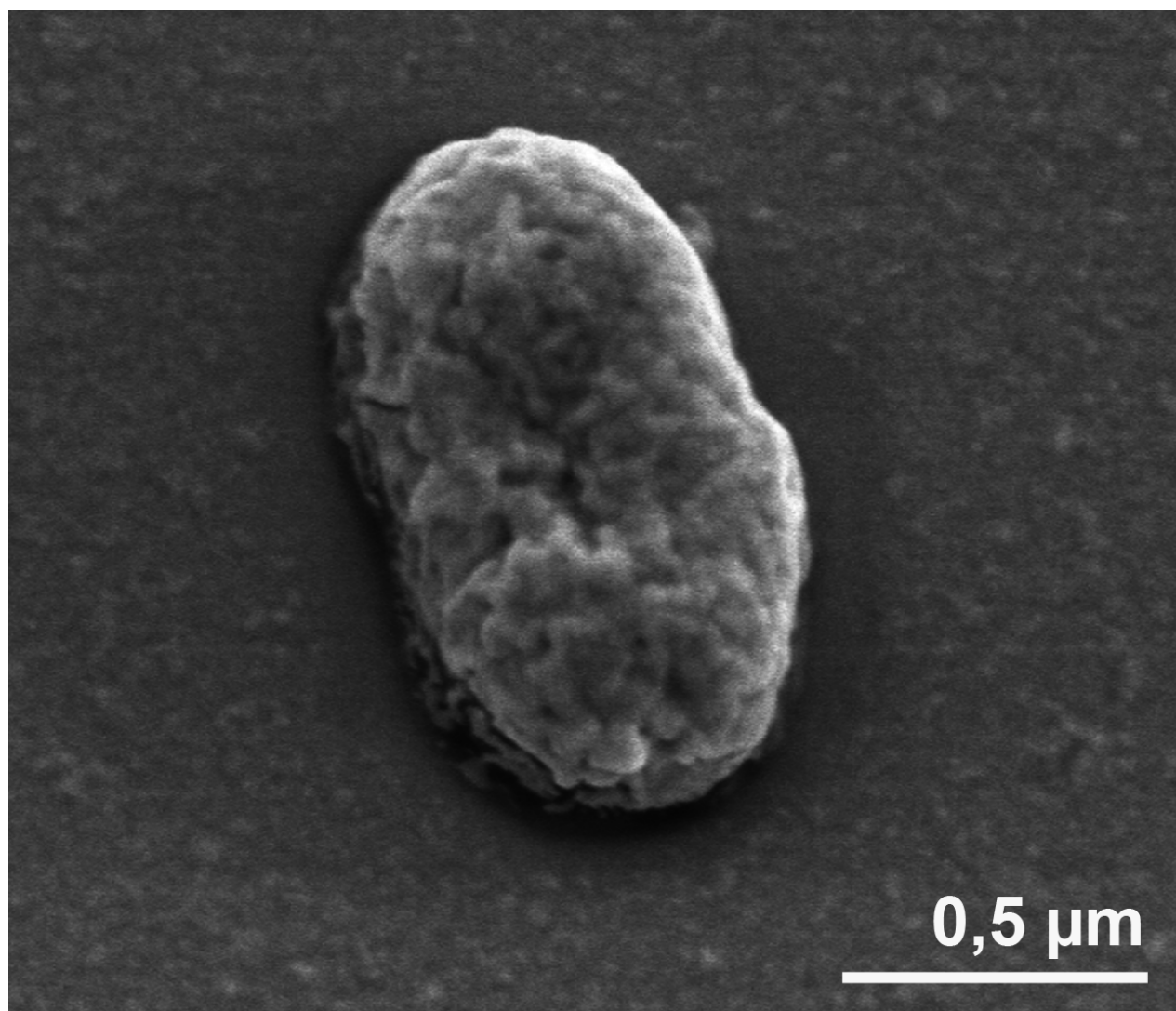
The cells of *H. maritima* are short rods ranging from 1-3 x 0.4–0.8  $\mu\text{m}$  (Figure 2 and Table 1) that occur singly or in pairs [1]. *H. maritima* is motile by one polar flagellum [1] (not visible in Figure 2). Colonies are whitish-gray with diameters up to 0.5 mm [1]. *H. maritima* cultures require 2.5-3% NaCl and 0.02% (w/v) yeast extract for growth [1]. The temperature range for growth is between 40°C and 65°C, with an optimum at 52–54°C [1]. Growth was observed over a pH range of 5.7 to 6.5 with an optimum around 6.0 [1].

All *H. maritima* strains can grow on molecular hydrogen, acetate, and saturated fatty acids and require elemental sulfur as the only known electron acceptor [1]. Strain MH<sub>3</sub>, isolated from Matupi

Harbor, was the only *H. maritima* strain growing on ethanol in the presence of elemental sulfur [1]. Fumarate supported only weak growth for all three known strains [1], whereas formate, propionate, butyrate, pyruvate, lactate, succinate, glucose, starch, peptone, methanol did not support growth [1]. CO<sub>2</sub> and H<sub>2</sub>S were the only detected end products [1].

### Chemotaxonomy

No chemotaxonomical data were reported in the initial description of the organism [1] nor elsewhere, subsequently.



**Figure 2.** Scanning electron micrograph of *H. maritima* MH<sub>2</sub><sup>T</sup>

**Table 1.** Classification and general features of *H. maritima* MH<sub>2</sub><sup>T</sup> according to the MIGS recommendations [10].

| MIGS ID  | Property               | Term  | Evidence code |         |
|----------|------------------------|---|---------------|---------|
|          |                        | Domain <i>Bacteria</i>  | TAS [11]      |         |
|          |                        | Phylum <i>Proteobacteria</i>  | TAS [12]      |         |
|          |                        | Class <i>Deltaproteobacteria</i>  | TAS [13,14]   |         |
|          | Current classification | Order <i>Desulfurellales</i>  | TAS [13,14]   |         |
|          |                        | Family <i>Desulfurellaceae</i>  | TAS [14,15]   |         |
|          |                        | Genus <i>Hippea</i>   | TAS [1]       |         |
|          |                        | Species <i>Hippea maritima</i>  | TAS [1]       |         |
|          |                        | Type strain MH <sub>2</sub>   | TAS [1]       |         |
|          |                        | Gram stain  | negative      | TAS [1] |
|          |                        | Cell shape  | short rods    | TAS [1] |
|          | Motility               | motile, one polar flagellum   | TAS [1]       |         |
|          | Sporulation            | never observed  | TAS [1]       |         |
|          | Temperature range      | 40-56°C   | TAS [1]       |         |
|          | Optimum temperature    | 52-54°C   | TAS [1]       |         |
|          | Salinity               | 2.5-3% NaCl   | TAS [1]       |         |
| MIGS-22  | Oxygen requirement     | anaerobic   | TAS [1]       |         |
|          | Carbon source          | saturated fatty acids (stearate, palmitate)   | TAS [1]       |         |
|          | Energy metabolism      | acetate, long-chain saturated fatty acids; lithotrophic growth with H <sub>2</sub> and S <sub>0</sub> | TAS [1]       |         |
| MIGS-6   | Habitat                | submarine hot vents   | TAS [1]       |         |
| MIGS-15  | Biotic relationship    | free-living   | NAS           |         |
| MIGS-14  | Pathogenicity          | none  | NAS           |         |
|          | Biosafety level        | 1   | TAS [16]      |         |
|          | Isolation              | hot vents in tidal zone   | TAS [1]       |         |
| MIGS-4   | Geographic location    | Matupi Harbour, Papua New Guinea  | TAS [1]       |         |
| MIGS-5   | Sample collection time | 1999  | TAS [1,17]    |         |
| MIGS-4.1 | Latitude               | -4.23   | NAS           |         |
| MIGS-4.2 | Longitude              | 152.2   | NAS           |         |
| MIGS-4.3 | Depth                  | not reported  |               |         |
| MIGS-4.4 | Altitude               | approximately sea level   | 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). These evidence codes are from of the Gene Ontology project [18]. If the evidence code is IDA, the property was directly observed 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 [19], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [20]. The genome project is deposited in the Genomes On Line Database [9] 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.

### Growth conditions and DNA isolation

*H. maritima* MH<sub>2</sub><sup>T</sup>, DSM 10411, was grown anaerobically in medium 554 (HIPPEA medium) [21] at 55°C. DNA was isolated from 0.5-1 g of cell paste using Jetflex Genomic DNA Purification Kit (GENOMED 600100) following the standard protocol as recommended by the manufacturer with the following modification to improve cell lysis: additional 20µl lysozyme (100mg/µl) and 10µl mutalysin were used for 30 min incubation at 37°C, followed by three hours incubation at 58°C with 20µl proteinase K. DNA is available through the DNA Bank Network [22].

**Table 2.** Genome sequencing project information

| MIGS ID   | Property                   | Term  |
|-----------|----------------------------|---|
| MIGS-31   | Finishing quality          | Finished  |
| MIGS-28   | Libraries used             | Three genomic libraries: one 454 pyrosequence standard library, one 454 PE library (7.3 kb insert size), one Illumina library |
| MIGS-29   | Sequencing platforms       | Illumina GAii, 454 GS FLX Titanium  |
| MIGS-31.2 | Sequencing coverage        | 1,213 × Illumina; 29.6 × pyrosequence   |
| MIGS-30   | Assemblers                 | Newbler version 2.3, Velvet version 0.7.63, phrap version SPS-4.24  |
| MIGS-32   | Gene calling method        | Prodigal 1.4, GenePRIMP   |
|           | INSDC ID                   | CP002606  |
|           | Genbank Date of Release    | March 29, 2011  |
|           | GOLD ID                    | Gc01705   |
|           | NCBI project ID            | 48195   |
|           | Database: IMG-GEBA         | 2504136000  |
| MIGS-13   | Source material identifier | DSM 10411   |
|           | Project relevance          | Tree of Life, GEBA  |

## Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [23]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly, consisting of 70 contigs in one scaffold, was converted into a phrap [24] assembly by making fake reads from the consensus to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (4,403.8 Mb) was assembled with Velvet [25] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 66.2 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are `-consed -a 50 -l 350 -g -m -ml 20`. The Phred/Phrap/Consed software package [24] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [23], Dupfinisher [26], or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Epicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 357 additional reactions and one shatter library were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [27].

The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 1,241.6 × coverage of the genome. The final assembly contained 112,403 pyrosequence and 57,283,044 Illumina reads.

## Genome annotation

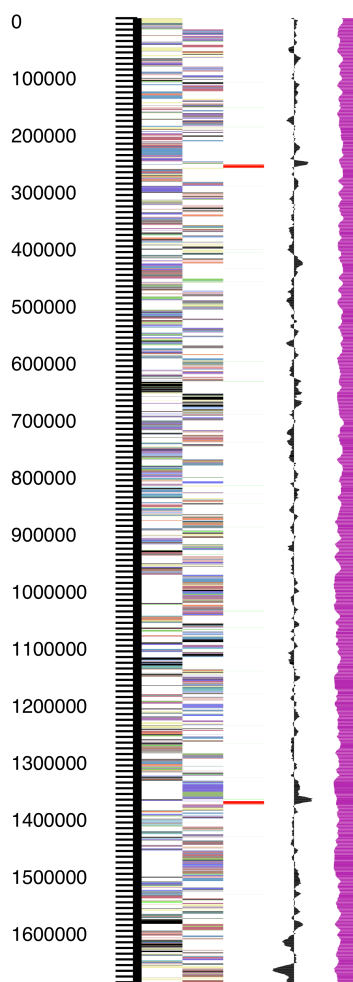
Genes were identified using Prodigal [28] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [29]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation were performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [30].

## Genome properties

The genome consists of a 1,694,430 bp long linear chromosome with a G+C content of 37.5% (Table 3 and Figure 3). Of the 1,780 genes predicted, 1,723 were protein-coding genes, and 57 RNAs; 46 pseudogenes were also identified. The majority of the protein-coding genes (76.4%) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

**Table 3.** Genome Statistics

| Attribute                        | Value     | % of Total |
|----------------------------------|-----------|------------|
| Genome size (bp)                 | 1,694,430 | 100.00%    |
| DNA coding region (bp)           | 1,580,424 | 93.27%     |
| DNA G+C content (bp)             | 634,975   | 37.47%     |
| Number of replicons              | 1         |            |
| Extrachromosomal elements        | 0         |            |
| Total genes                      | 1,780     | 100.00%    |
| RNA genes                        | 57        | 3.20%      |
| rRNA operons                     | 2         |            |
| Protein-coding genes             | 1,723     | 96.80%     |
| Pseudo genes                     | 46        | 2.58%      |
| Genes with function prediction   | 1,360     | 76.40%     |
| Genes in paralog clusters        | 182       | 10.22%     |
| Genes assigned to COGs           | 1,414     | 79.44%     |
| Genes assigned Pfam domains      | 1,485     | 83.43%     |
| Genes with signal peptides       | 261       | 14.66%     |
| Genes with transmembrane helices | 423       | 23.76%     |
| CRISPR repeats                   | 0         |            |



**Figure 3.** Graphical map of the linear chromosome. From left to right: 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 4.** Number of genes associated with the general COG functional categories

| Code | value | % age | Description   |
|------|-------|-------|---|
| J    | 133   | 8.5   | Translation, ribosomal structure and biogenesis               |
| A    | 0     | 0.0   | RNA processing and modification                               |
| K    | 45    | 2.9   | Transcription   |
| L    | 119   | 7.6   | Replication, recombination and repair                         |
| B    | 0     | 0.0   | Chromatin structure and dynamics                              |
| D    | 19    | 1.2   | Cell cycle control, cell division, chromosome partitioning    |
| Y    | 0     | 0.0   | Nuclear structure   |
| V    | 11    | 0.7   | Defense mechanisms  |
| T    | 78    | 5.0   | Signal transduction mechanisms                                |
| M    | 110   | 7.1   | Cell wall/membrane/envelope biogenesis                        |
| N    | 69    | 4.4   | Cell motility   |
| Z    | 0     | 0.0   | Cytoskeleton  |
| W    | 0     | 0.0   | Extracellular structures                                      |
| U    | 59    | 3.8   | Intracellular trafficking, secretion, and vesicular transport |
| O    | 68    | 4.4   | Posttranslational modification, protein turnover, chaperones  |
| C    | 107   | 6.9   | Energy production and conversion                              |
| G    | 62    | 4.0   | Carbohydrate transport and metabolism                         |
| E    | 147   | 9.4   | Amino acid transport and metabolism                           |
| F    | 46    | 3.0   | Nucleotide transport and metabolism                           |
| H    | 108   | 6.9   | Coenzyme transport and metabolism                             |
| I    | 52    | 3.3   | Lipid transport and metabolism                                |
| P    | 66    | 4.2   | Inorganic ion transport and metabolism                        |
| Q    | 22    | 1.4   | Secondary metabolites biosynthesis, transport and catabolism  |
| R    | 146   | 9.4   | General function prediction only                              |
| S    | 93    | 6.0   | Function unknown  |
| -    | 366   | 20.6  | Not in COGs   |

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