Acta Oceanol. Sin., 2017, Vol. 36, No. 2, P. 88–93
DOI: 10.1007/s13131-017-0979-1
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The complete genome of hydrocarbon-degrading *Pseudoalteromonas* sp. NJ289 and its phylogenetic relationship

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Received 1 November 2015; accepted 18 February 2016

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Abstract

Genus *Pseudoalteromonas* belongs to Family *Pseudoalteromonadaceae* in *Gammaproteobacteria*. A cold-adapted gram-negative bacterium, hydrocarbon-degrading *Pseudoalteromonas* sp. NJ289, was isolated from sea-ice of the Antarctica region, and sequenced the whole genome through the next generation sequencing platform. The assembly yielded three contigs representing two chromosomes and one plasmid with the sizes of 3.2 Mb, 636 kb and 1.8 kb, respectively. The G+C contents of genome were 40.83% and included 3 589 ORFs. Functional annotation indicated some potential roles in enzymatic activity and environmental adaptability. This study may help for understanding the population diverse, evolutionary ecology and the microbial interaction.

Key words: *Pseudoalteromonas*, low temperature, environmental adaptability, sequencing, phylogenetic tree

Citation: Liu Fangming, Wang Yibin, Qu Changfeng, Zheng Zhou, Miao Jinlai, Xu Hua, Xiao Tian. 2017. The complete genome of hydrocarbon-degrading *Pseudoalteromonas* sp. NJ289 and its phylogenetic relationship. Acta Oceanologica Sinica, 36(2): 88–93, doi: 10.1007/s13131-017-0979-1

1 Introduction

Most of the earth is covered by sea, and in some deep sea the temperature remains lower than 15°C. Living beings need evolutionally adapt to the cold environment. Challenges posed by cold to life stem from the slow pace of chemical reactions, from the constraints induced by the stability of hydrogen bonds, and from increased solubility of glasses and stability of radicals (Médigue et al., 2005).

In deep-sea sediment, bacteria and archaea are main population, which play a key role, including organic nitrogen assimilation and remineralization (Kallmeyer et al., 2012; Lloyd et al., 2013). These marine bacteria are mainly divided two branches: Part I. *Proteobacteria*, including *Alteromonas*, *Colwellia*, *Glaciecola*, *Octadecabacter*, *Pseudoaltermonas*, *Shewannella* and *Vibrio*; Part II. Cytophaga-Flexibacter-Bacteroides, including *Cytophaga*, *Flavobacterium*, *Gelidibacter* and *Polaribacter* (Ivanova et al., 2004; Médigue et al., 2005).

Pseudoalteromonas is a gram-negative, psychrophilic bacterium, widely spreading in the sea water and sediment. Recently, the *Pseudoalteromonas* is of interest to biochemists and ecologists due to physiologically cold-adaptive features and pharmacologically active compounds, and organism metabolism (Li et al., 2015). Many psychrophilic *Pseudoalteromonas* species could produce extracellular cold-relative enzymes and exopolysaccharides (Chen et al., 2007; Liu et al., 2013). In *Pseudoalteromonas* sp. CF6-2, a novel metalloprotease named as pseudoalterin, belongs to the M23A subfamily, was found which can hydrolyze elastin through a different mechanism (Zhao et al., 2012). *Pseudoalteromonas* sp. NJ289 was isolated from the Antarctic sea ice, and the whole genome was obtained using Illumina Miseq technology. Finally the complete sequences were assemblized into two chromosomes and one plasmid, with the length of 3.2 Mb, 645 kb and 1.8 kb, respectively. The annotation results and function analyses indicated the presence of genes associated with environmental adaption, biofilm formation, and metabolism cycling. Phylogenetic relationship analysis showed that its closest relative is *Pseudoalteromonas issachenkonii*. Knowledge of the genome will provide aids in understanding the phylogeny and microbial ecology of *Pseudoalteromonas*.

2 Materials and methods

2.1 *Bacterial strain and growth conditions*

The strain NJ289 is reserved at –80°C. The bacteria were grown in 2216E media: 5 g polypepton, 1 g yeast extract, 0.1 g $FePO_4$ and 15 g agar dissolved in 1 L of 75% artificial seawater (ASW pH 7.4) and was incubated at 20°C.

2.2 *Genome sequencing*

When the OD_{600} reached 0.6, the culture was collected to extract the genomic DNA using commercial kit (OMEGA, bacterial DNA extraction kit, USA). The DNA was suspended in distilled water and stored at –80°C until use. The complete genome sequence was determined by using the whole shotgun method.

Foundation item: The National Natural Science Foundation of China under contract Nos 31200097, 41576187, U1406402-5 and 31202024; the Basic Scientific Fund for National Public Research Institutes of China under contract Nos 2013G33 and 2015G10.
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Two libraries were constructed, named 400 bp-insert paired-end fragment library and 5 kb-insert Mate-paired fragment library following the manufacture's protocol.

2.3 *Annotation and bioinformatics analysis*

After sequencing, base quality was evaluated and poor bases were filtered using using Cutadapt (Martin, 2011). Then assembly was performed using three kinds of software, including ALLPATH-LG software (v52488), Spades (v3.6) and DISCOVAR-denovo with default parameters, respectively (Ribeiro et al., 2012; Bankevich et al., 2012). Finishing was completed by PCR amplification of gaps.

Gene was predicted by using Glimmer (v3.02) trained with minimum length of 110 bp, and verified by manually analysis (Delcher et al., 1999). And putative ORFs were submitted to functional annotation. Blastx searches against the NR database (from NCBI database) were performed to determined significant homology. By the Inter Proscan software, the protein motifs and domains were identified. TMHMM was used to identify the transmembrane domains, and Signal P was used to predict signal peptides (Petersen et al., 2011). tRNA scan-SE was used to identify tRNAs and RNAmmer to identify rRNAs (Lowe and Eddy, 1997; Lagesen et al., 2007). Other non-coding RNAs were predicted in Rfam database. Finally, CRISPR was analyzed by using CRISPR recognition tool. The resulting genes were determined the classification of GO term and COG term, and were further analyzed using KEGG database. The circular genome maps were drawn using CGVIEW software (Stothard and Wishart, 2005).

2.4 *Phylogenetic analysis based on 16S rRNA gene*

Thirty-one presentative sequences of 16S rRNA gene were downloaded from NCBI, including *Pseudoalteromonas* sp., *Pseudoalteromonas flavipulchra*, *Pseudoalteromonas lipolytica*, *Pseudoalteromonas translucida*, and so on. *Pseudomonas aeruginosa* were used as outgroups.

2.5 *Bayesian inference (BI)*

Substitution models for 16S rRNA were selected in Model Generator based on Akaike Information Criterion (Keane et al., 2006). A GTR+I+Γ substitution model was used. Bayesian analysis was run for 10 000 000 generations in MrBayers (v3.2). Trees were sampled every 1 000 times and the first 25% resulting trees were excluded. Then the remaining trees were merged into a consensus tree with posterior probabilities (PP) indicating the support information for each clade.

3 Results and discussion

3.1 *De novo assembly and genome organization*

Three kinds of assembly software were used to perform the analysis. And the outputs were compared to confirm the best assembly through QUAST. The resulting N_{50} of contigs showed that the software ALLPATHLG performed better (data not shown). Table 1 summarizes the genome assembly statistics.

As in many *γ-proteobacteria*, *Pseudoalteromonas* is made of two chromosomes. For *Pseudoalteromonas* sp. NJ289, there are two circular chromosomes, in addition one plasmid. Chromosome I is 3 245 449 bp in size and Chromosome II is 636 246 bp, while the plasmid is 1 806 bp in length. The atlas of the whole genome was showed in Fig. 1.

Following gene annotation, a total of 3 589 protein-coding genes, accounting 86.83% of the whole genome (Altschul et al., 1990). Three rRNA operons and 78 tRNA loci were detected in the genome. Interestingly, for this strain, the number of tRNA genes was very high (78 copies), which was in common with that in *Pseudoalteromonas haloplanktis* TAC125, in *vibrios* and in *Photobacterium profundum* (Médigue et al., 2005). In *Pseudoalteromonas haloplanktis* TAC125, the number of tRNA was 106. The genes were organized in long runs of repeated. This maybe suggested the situations of bacterial rapid growth. The replication origin of Chromosome I mapped near dnaA, which is highly conserved in *γ-proteobacteria* (Lobry and Louarn, 2003). The plasmid was aligned to NCBI databases and showed 68% similarity to *pVT*2-4 from *Vibrio* sp. VT2.

Table 1. Summary statistics of *Pseudoalteromonas* sp. sequencing assembly

Description	Data
PE400 raw data/bp	5 363 432
MP5K raw data/bp	2 880 854
Average length raw/bp	251
PE400 clean data/bp	5 363 432
MP5K clean data/bp	2 880 854
Average length trim/bp	190
G+C content/%	40.83
Total bases/bp	8 244 286
Contigs number	9
Scaffold number	3

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are direct repeats found in many bacteria and archaea. These repeats range in size from 23 to 47 base pairs. CRISPR-Cas systems provide adaptive immunity against foreign elements (Marraffini, 2015). For NJ289, there were no CRISPR elements to be predicted from the whole genome (Bland et al., 2007).

3.2 *Gene functional classification*

3.2.1 *GO term*

Gene Ontology (GO) is used to establish to provide a common language to describe aspects of a gene product's biology, involved in biological process (BP), molecular function (MF), and cellular component (CC) of the gene products. These terms are to be used as attributes of gene products by relative organism databases, facilitating uniform queries across them. As shown in Fig. 2 by Level 2, 2 679 of 3 589 predicted gene products were classified into 31 functional subcategories, and each gene product was assigned to more than one GO term and together 5 240 genes were assigned to GO term. In BP category, the dominant subcategories were "metabolic process" (1 583, 30.2%), followed by "cellular process" (1 320, 25.2%). In MF category, the dominant subcategories were "catalytic activity" (1 295, 24.7%) and "binding" (1 030, 19.7%). And for CC category, the main subcategories were "cell" (890, 17.0%) and "membrane" (485, 9.3%).

3.2.2 *KEGG pathways*

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were surveyed to functionally the gene product profiles. A large number of pathways were enriched, including those associated with amino acid metabolism, carbohydrate and energy metabolism, oxidative phosphorylation et al. as shown in Fig. 3. Using KAAS, a total 2 342 of 3 589 gene products were involved into 18 main categories.

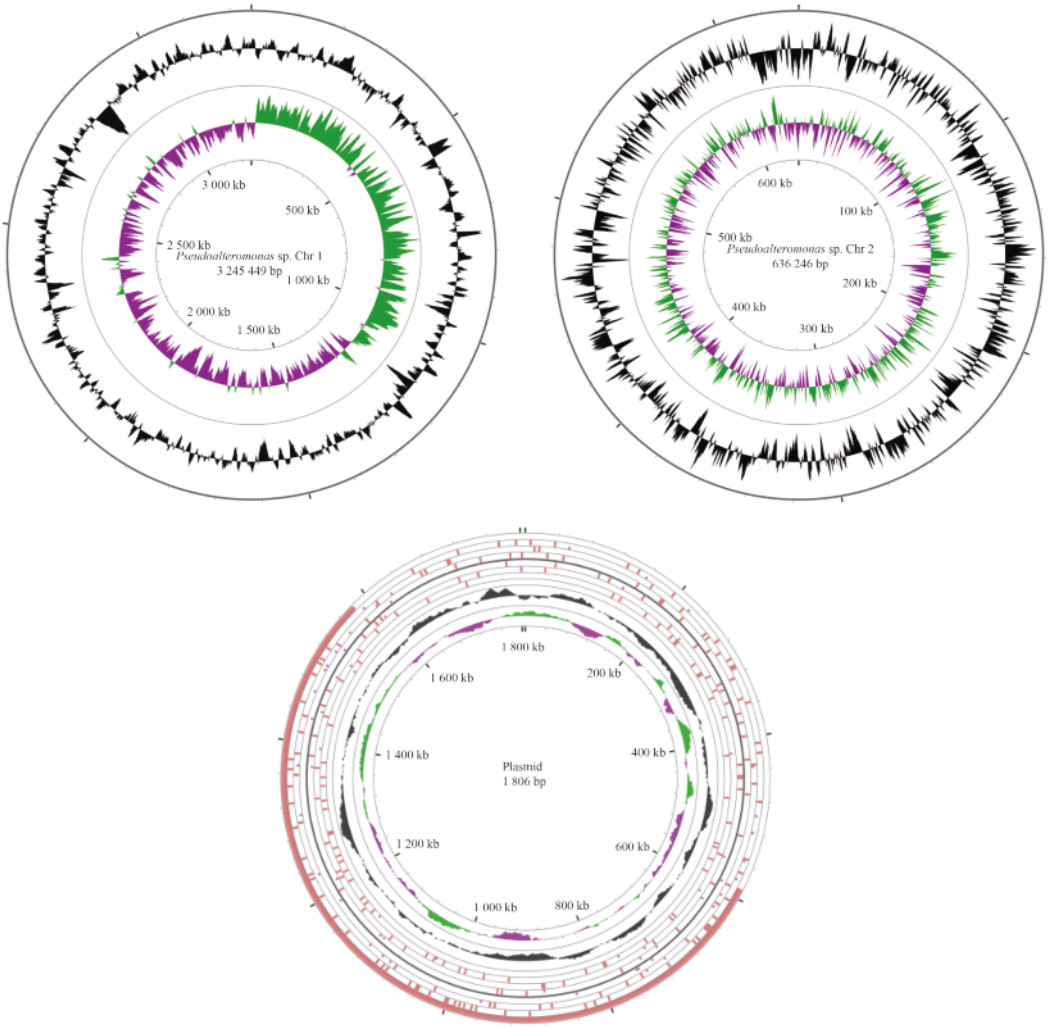

Fig. 1. Atlas of the whole genome of *Pseudoalteromonas* sp. NJ289.

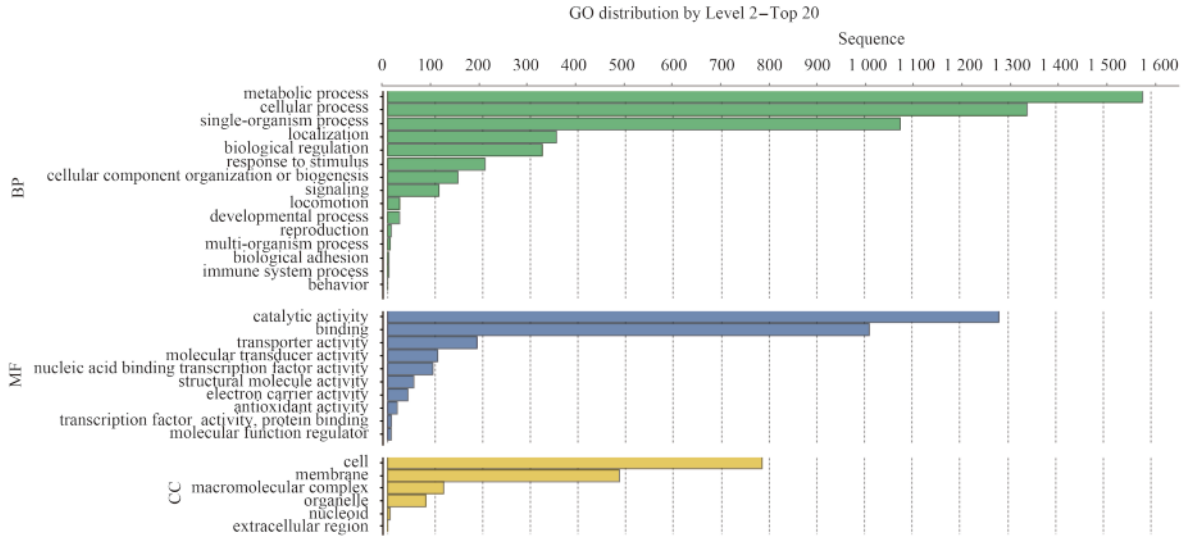

Fig. 2. GO distribution of genes in *Pseudoalteromonas* sp. NJ289.

3.3 *NJ289 phylogenetic comparison*

In order to determine the phylogenetic relationship of NJ289 to the other species *Pseudoalteromanos*, a phylogenetic analysis was performed from 30 *Psedudomonas* 16S ribosomal RNA including NJ289 and one *Pseudomonas* aeruginosa as outgroup. The phylogenetic tree was constructed based on Mrbays method (Fig. 4). In this study, NJ289 was clustered in Group I and closed to *Pseudoalteromonas citrea*. The resulting genomic comparison

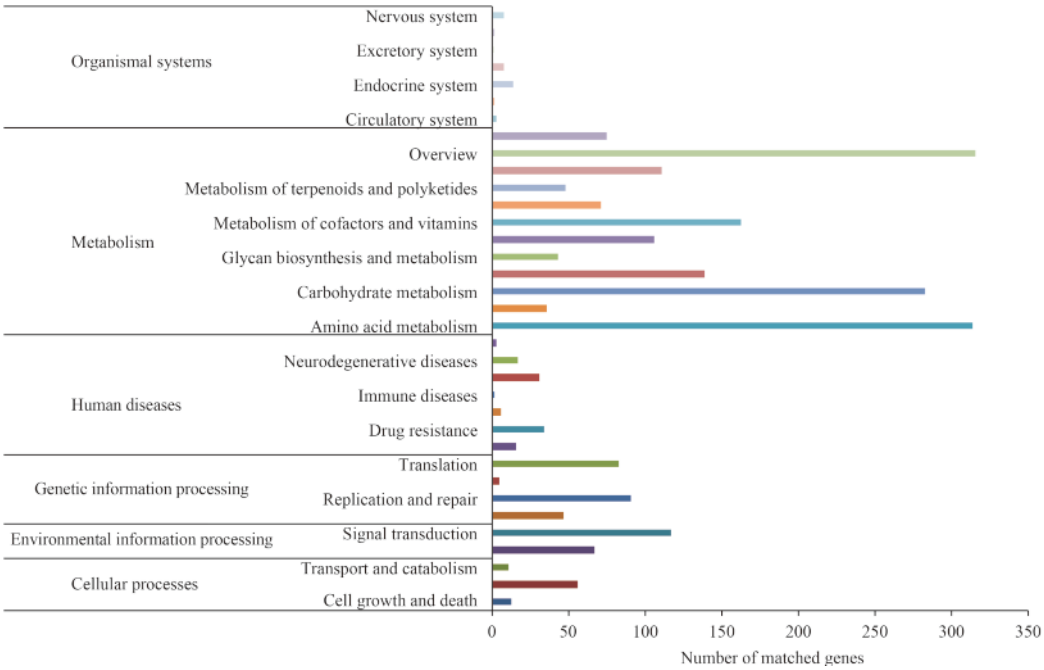

Fig. 3. Statistics of KEGG pathways in *Pseudoalteromonas* sp. NJ289.

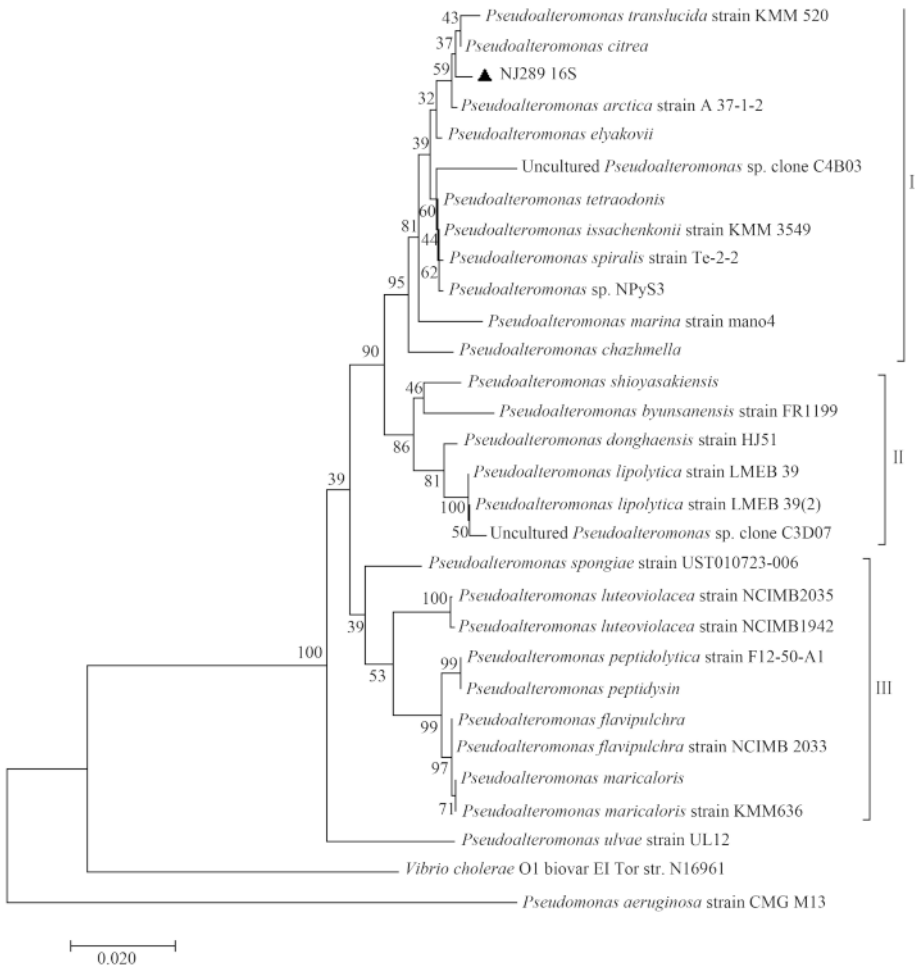

Fig. 4. Phylogenetic relationships for the 16S rRNA gene sequences among *Pseudomonas* species. Numbers at the each node indicated bootstrap values calculated using Mrbayes probabilities. *Pseudomonas aeruginosa* strain CMG M13 served as the outgroup.

showed that the similarity between NJ289 and *Pseudoalteromonas haloplanktis* is only 86%, indicating NJ289 possible a new species.

3.4 *Cold-relative enzymes and environment adaption*

After annotation, there were a few cold-relative enzymes including cold-active aminopeptidase (neutral zinc metallopeptidase, M1 family) (Fig. 5, indicted in red) and cold-shock protein cluster (Fig. 6, indicted in red and in green). The near gene arrangement of aminopeptidase of NJ289 was similar to *Pseudoalteromonas tunicata* D2. The amino acid sequence of cold-active aminopeptidase was with 100% similarity to *Pseudoalteromonas haloplanktis*, and with molecular weight of 68.98 kDa. In marine psychrophile *Colwellia psychrerythraea* strain 34H, the purified extracellular proteinase with molecular weight of 71 kDa displayed a low optimum temperature (19°C) and showed the activity in the cold (Huston et al., 2004).

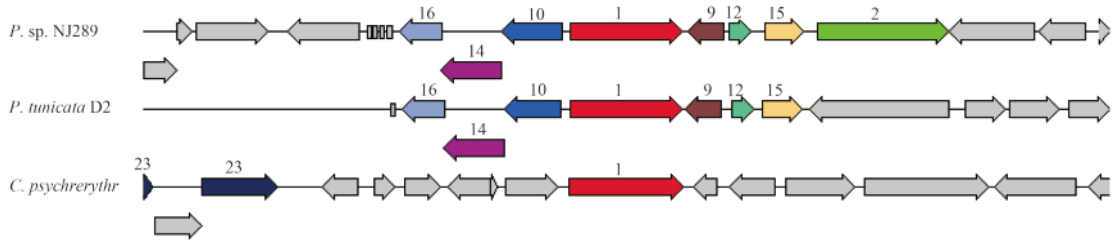

Fig. 5. Comparison atlas of cold-active aminopeptidase.

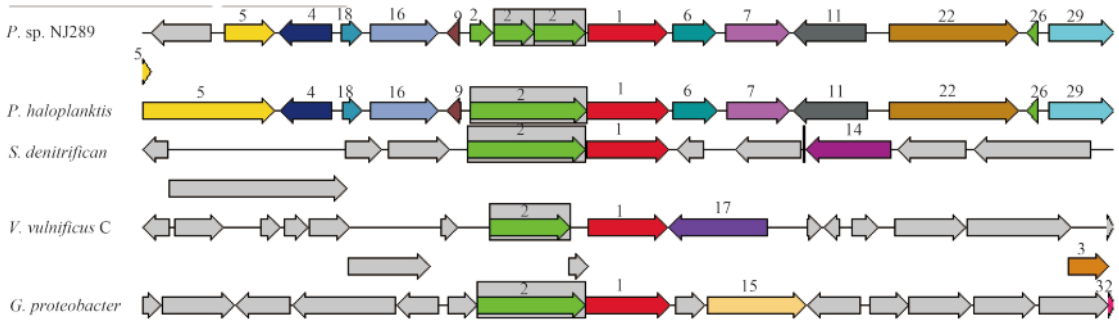

Fig. 6. Graphic map of cold-shock protein cluster of NJ289.

4 Conclusions

This study analyzed a hydrocarbon-degrading bacterial genome from *Pseudoalteromonas* sp. NJ289 using high throughout sequencing. Associated gene function and phylogenetic relationship was analyzed. Some important metabolic enzymes and key factors coping with cold were found from predicted ORFs. This maybe makes NJ289 a model for the research of adaptation to cold marine conditions and for further investigations for applicable enzymes.

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