Marine Genomics 41 (2018) 54–56

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

Complete genome sequence of *Colwellia hornerae* PAMC 20917, a cold-active enzyme-producing bacterium isolated from the Arctic Ocean sediment

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ARTICLE INFO

Keywords:
Colwellia hornerae
Cold-active enzyme
Thermolabile industrial enzymes

ABSTRACT

Psychrophilic bacteria are considered a source of cold-active enzymes that can be used in industrial applications. The Arctic bacterium *Colwellia hornerae* PAMC 20917 strain has been isolated from the offshore sediment near Ny-Ålesund, Svalbard. The optimal growth temperature of the strain was 10 °C on marine agar. The cell lysate showed alkaline phosphatase activities. Analysis of the enzymatic properties showed that the alkaline phosphatase was cold-active and thermolabile. To explore useful cold-active industrial enzymes further, the entire genome of the PAMC 20917 strain was sequenced. The genome of the strain contained 4,684,314 nucleotides, with 37.87% G + C content. Genome mining analysis revealed that, in the complete genome sequence, three proteins were annotated as alkaline phosphatases. The genome of PAMC 20917 encodes cold shock proteins and an ice-binding protein that inhibits the growth of ice, allowing the bacterium to adapt to cold environments. This genome information may be useful for understanding mechanisms of adaptation to cold stress.

1. Introduction

The genus *Colwellia*, within the γ-proteobacteria, consists of psychrophilic, gram-negative bacteria living in cold environments (below 20 °C), which have been mainly isolated from cold marine environments, including deep sea, and Arctic and Antarctic sea ice (Bowman et al., 1998; Methé et al., 2005; Nogi et al., 2004; Zhang et al., 2008). These bacteria are good sources of cold-active and cold-adapted enzymes that have low temperature optima in activity and marked thermolability (Methé et al., 2005). These biochemical properties of cold-active enzymes may be useful in various industrial applications, such as detergent additives, within the food industry, in chemical synthesis, and bioremediation at low temperatures (Gerday et al., 2000). In molecular biology, heat-labile enzymes are advantageous for obtaining irreversible enzyme inactivation by mild heat treatment without interference from subsequent reactions. The most valuable cold-active DNA-modifying enzyme is alkaline phosphatase, which dephosphorylates DNA (De Prada et al., 1996; Kobori et al., 1984). Alkaline phosphatase has also been used as a reporter enzyme for secreted proteins and as an indicator enzyme in diagnostic kits. An alkaline-phosphatase-productin bacterium, PAMC 20917, was isolated from the offshore sediment near Ny-Ålesund, Svalbard (N78.55000, E11.53000). The bacterial strain was assigned to *Colwellia hornerae* based on its ≥99% 16S rRNA sequence match. The temperature range for PAMC 20917 was 4–15 °C, and the optimal growth temperature was 10 °C (Table 1). The strain could not be cultivated at 20 °C. This growth pattern is considered to be a characteristic of strictly psychrophilic bacteria. *Colwellia* sp. PAMC 20917 may be a good source of the useful cold-active and heat-labile alkaline phosphatase (De Prada et al., 1996).

2. Data description

The PAMC 20917 strain showed alkaline phosphatase activities in the cell lysate. The activity was determined using *p*-nitrophenyl phosphate as a substrate. In order to examine the enzymatic properties of the alkaline phosphatase, we prepared a crude enzyme extract by sonicating the cell suspension with buffer A (pH 8.0, 20 mM Tris-HCl, 200 mM NaCl, 10 mM PMSF). Only alkaline phosphatase activity, and not acid phosphatase activity, was observed in the extract. The optimum temperature of activity was 50 °C, but only approximately 60% of the maximal activity was observed at 20 °C (Fig. 1B), indicating that the enzyme is well-adapted to low temperatures. In the thermostability experiment, enzyme activity was lost when the enzyme was placed at 60 °C for 1 h (Fig. 1C). These results suggest that alkaline phosphatase from the PAMC 20917 strain is cold-active and thermolabile. To obtain genomic information about the alkaline phosphatase for its future application, the genomic DNA of *Colwellia* sp PAMC 20917 was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison,

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https://doi.org/10.1016/j.margen.2018.03.004
Received 27 October 2017; Received in revised form 16 March 2018; Accepted 18 March 2018
Available online 26 March 2018
1874-7787/

Table 1
General features of *Colwellia hornerae* PAMC 20917 and MIGS mandatory information.

Items	Description
General feature	
Current classification	Domain *Bacteria*
	Phylum *Proteobacteria*
	Class *Gammaproteobacteria*
	Order *Alteromonadales*
	Family *Colwelliaceae*
	Genus *Colwellia*
	Species *hornerae*
	Strain PAMC 20917
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Temperature range	4–15 °C, and optimally at 10 °C
MIGS data	
Geographic location	Ny-Ålesund, Svalbard (78°55′N, 11°53′E)
Isolation source	Sediment
Collection date	2015–11-04
Habitat	Seawater
Oxygen requirement	Facultative anaerobe
Pathogenicity	None
Biotic relationship	Free-living
Sequencing platform	PacBio RS II with P6-C4 chemistry
Fold coverage	269.35 x
Assembler	Canu (Ver. 1.1)

WI, USA), according to the manufacturer's specification. We sequenced its entire genome using Single molecule Real Time (SMRT) technology (Eid et al., 2009). A 20-kb insert library was constructed and sequenced using the PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) at MACROGEN (Seoul, Korea); 1,261,699,172 base pairs were obtained from 96,006 subreads. Assembly was performed using Canu (Ver. 1.1) (Berlin et al., 2015). One complete circular chromosome was generated. Genome annotation was performed using the Rapid Annotation, using Subsystems Technology server (Aziz et al., 2008). Functional annotation was done using the classic RAST scheme and RAST gene caller. Release70 was selected for FIGfam version. The database of Clusters of Orthologous Groups of proteins (COGs) was also used for functional annotation (Tatusov et al., 2000).

The complete genome contains 4,684,314 nucleotides, with 37.87% G + C content (Table 2). A total of 4071 protein-coding sequences were predicted, and 73 tRNA genes and 19 rRNA genes were identified in the genome, from which 7 copies were of 5S rRNA genes, 6 copies of 16S rRNA genes, and 6 copies of 23S rRNA genes (Table 2). Among the 4071 genes, we assigned 2771 to a subsystem using the SEED method (Overbeek et al., 2014), and the function of 2887 genes was categorized by comparison with the Clusters of Orthologous Groups (COGs) (Tatusov et al., 2000). The graphic circular map of the genome was generated using DNAPlotter (Ver. 10.2) (Carver et al., 2009) (Fig. 2).

Table 2
Genomic features of *Colwellia hornerae* PAMC 20917.

Attributes	Chromosome
Genome size (bps)	4,684,314
GC content (%)	37.87
CDSs	4071
rRNA operons	6
tRNA genes	73

Genome mining analysis revealed that three proteins were annotated as alkaline phosphatases in the complete genome sequence of *C. hornerae* PAMC 20917. These proteins showed a 58–64% match with databases of non-redundant protein sequences, and were not highly conserved with each other. Therefore, this bacterial species may provide a new candidate for a heat-labile alkaline phosphatase. Together with alkaline phosphatases, the genome of *C. hornerae* PAMC 20917 encoded various degradative enzymes. Alpha-amylase, alpha-glucosidase, 2 beta-glucosidases, 8 proteases, extracellular protease, 4 beta-glucanases, and 3 pullulanases were identified.

Additionally, the genome of PAMC 20917 encodes an ice-binding protein (IBP) that inhibits the growth of ice. The ice-binding protein showed a 98% match only with the ice-binding protein of the Antarctic bacterium *Colwellia* sp. SLW05, and no similar proteins were found in other *Colwellia* sp. The recombinant ice-binding protein of strain SLW05 exhibited thermal hysteresis activity of approximately 4 °C (Hanada et al., 2014; Raymond et al., 2007). Four cold-shock proteins (CSPs) were identified, and showed a 93–99% match with other *Colwellia* CSPs. Their expression levels were increased under cold shock, and CSPs can melt secondary structures in nucleic acids and affect both transcription and translation (Bisht et al., 2014; Phadtare, 2004). Therefore, the presence of an ice-binding protein and cold-shock proteins allowed *C.* sp PAMC 20917 to adapt to cold environments.

The complete genome of PAMC 20917 may be a good source of cold enzymes and provide basic information for the wider exploitation of cold-active and thermolabile industrial enzymes.

3. Nucleotide sequence accession number

The complete genome sequence of *C. hornerae* PAMC 20917 has been deposited at DDBJ/EMBL/GenBank under the accession number CP014944. This strain is available from the Polar and Alpine Microbial Collection (PAMC) of Korea Polar Research Institute with the accession number PAMC 20917.

Acknowledgements

This work was supported by grants from the National Research

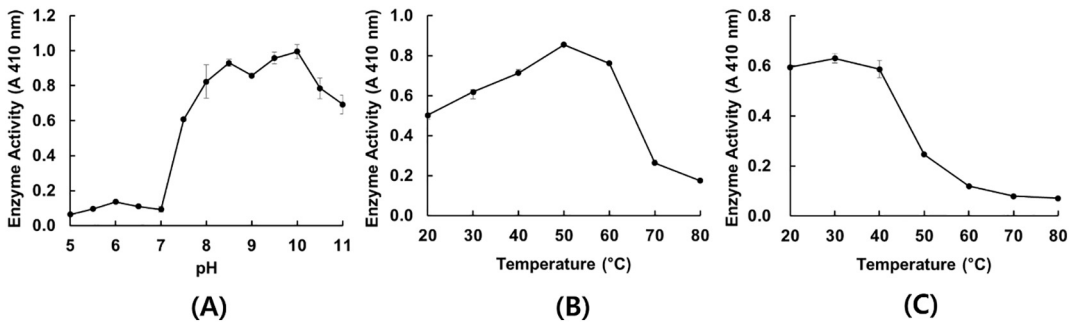

Fig. 1. Enzymatic properties of alkaline phosphatase from the PAMC 20917 strain. (A) pH dependence of enzyme activity. Enzyme activity was determined at 30 °C in 50 mM Na-acetate (pH 5.0–5.5), 50 mM Na-phosphate (pH 6.0–7.0), 50 mM Tris-HCl (pH 7.5–8.5), or 50 mM Gly-NaOH (pH 9.0–11.0). (B) Temperature dependence of enzyme activity. The reaction mixture containing 50 mM Tris-HCl buffer (pH 8.0) was incubated at each temperature for 10 min. (C) Thermostability of the activity of the cell lysate. The crude enzyme extract was preincubated at each temperature for 1 h. Residual activity was determined at 30 °C for 10 min.

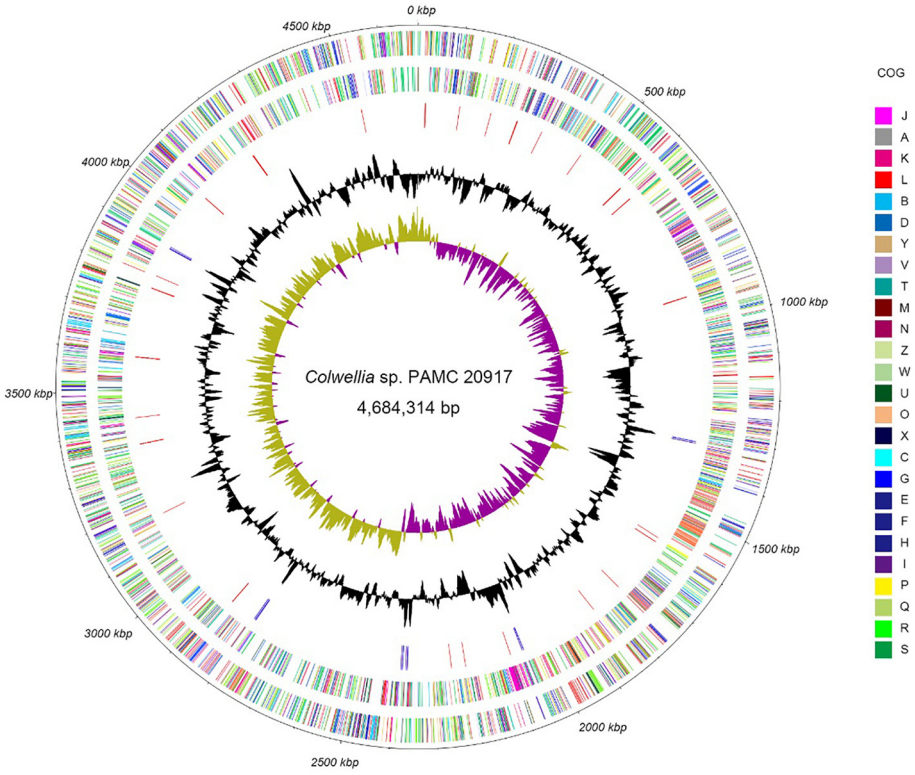

Fig. 2. Circular map of the *Colwellia hornerae* PAMC 20917 genome. Labeling from outside to the center: genes on forward strand, genes on reverse strand, RNA genes (tRNAs red, rRNAs blue), GC content (black), and GC skew (olive/purple). Individual genes are colored by COG categories: J (translation, ribosomal structure, and biogenesis), A (RNA processing and modification), K (transcription), L (replication, recombination, and repair), B (chromatin structure and dynamics), D (cell cycle control, cell division, and chromosome partitioning), Y (nuclear structure), V (defense mechanisms), T (signal transduction mechanisms), M (cell wall/membrane/envelop biogenesis), N (cell motility), Z (cytoskeleton), W (extracellular structures), U (intracellular trafficking, secretion, and vesicular transport), O (post-translational modification, protein turnover, and chaperones), X (mobilome: prophages and transposons), C (energy production and conversion), G (carbohydrate transport and metabolism), E (amino acid transport and metabolism), F (nucleotide transport and metabolism), H (coenzyme transport and metabolism), I (lipid transport and metabolism), P (inorganic ion transport and metabolism), Q (secondary metabolites biosynthesis, transport, and catabolism), R (general functional prediction only), and S (function unknown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Foundation of Korea (NRF) which were funded by the Ministry of Science and ICT (MSIT) (Grant no. NRF-2017M1A5A1013568) (Title: Application study on the Arctic cold-active enzyme degrading organic carbon compounds) and KOPRI's basic research project (Grant no. PN17083 and PN18082).

References

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., et al., 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9, 75.

Berlin, K., Koren, S., Chin, C.S., Drake, J.P., Landolin, J.M., et al., 2015. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. Nat. Biotechnol. 33, 623–630.

Bisht, S.C., Joshi, G.K., Mishra, P.K., 2014. CspA encodes a major cold shock protein in Himalayan psychrotolerant pseudomonas strains. Interdisc. Sci. Comput. Life Sci. 6, 140–148.

Bowman, J.P., Gosink, J.J., McCAMMON, S.A., Lewis, T.E., Nichols, D.S., et al., 1998. Colwellia demingiae sp. nov., Colwellia hornerae sp. nov., Colwellia rossensis sp. nov. and Colwellia psychrotropica sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22: ω63). Int. J. Syst. Evol. Microbiol. 48, 1171–1180.

Carver, T., Thomson, N., Bleasby, A., Berriman, M., Parkhill, J., 2009. DNAPlotter: circular and linear interactive genome visualization. Bioinformatics 25, 119–120.

De Prada, P., Loveland-Curtze, J., Brenchley, J.E., 1996. Production of two extracellular alkaline phosphatases by a psychrophilic arthrobacter strain. Appl. Environ. Microbiol. 62, 3732–3738.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., et al., 2009. Real-time DNA sequencing from single polymerase molecules. Science 323, 133–138.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J.-P., Claverie, P., et al., 2000. Cold-adapted enzymes: from fundamentals to biotechnology. Trends Biotechnol. 18, 103–107.

Hanada, Y., Nishimiya, Y., Miura, A., Tsuda, S., Kondo, H., 2014. Hyperactive antifreeze protein from an Antarctic sea ice bacterium Colwellia sp. has a compound ice-binding site without repetitive sequences. FEBS J. 281, 3576–3590.

Kobori, H., Sullivan, C.W., Shizuya, H., 1984. Heat-labile alkaline phosphatase from Antarctic bacteria: rapid 5′ end-labeling of nucleic acids. Proc. Natl. Acad. Sci. 81, 6691–6695.

Methé, B.A., Nelson, K.E., Deming, J.W., Momen, B., Melamud, E., et al., 2005. The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses. Proc. Natl. Acad. Sci. U. S. A. 102, 10913–10918.

Nogi, Y., Hosoya, S., Kato, C., Horikoshi, K., 2004. Colwellia piezophila sp. nov., a novel piezophilic species from deep-sea sediments of the Japan Trench. Int. J. Syst. Evol. Microbiol. 54, 1627–1631.

Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., et al., 2014. The SEED and the rapid annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 42, D206–214.

Phadtare, S., 2004. Recent developments in bacterial cold-shock response. Curr. Issues Mol. Biol. 6, 125–136.

Raymond, J.A., Fritsen, C., Shen, K., 2007. An ice-binding protein from an Antarctic sea ice bacterium. FEMS Microbiol. Ecol. 61, 214–221.

Tatusov, R.L., Galperin, M.Y., Natale, D.A., Koonin, E.V., 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. 28, 33–36.

Zhang, D.-C., Yu, Y., Xin, Y.-H., Liu, H.-C., Zhou, P.-J., et al., 2008. Colwellia polaris sp. nov., a psychrotolerant bacterium isolated from Arctic sea ice. Int. J. Syst. Evol. Microbiol. 58, 1931–1934.