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Preliminary genome analysis of psychrotolerant marine bacterium *Pseudoalteromonas* sp. BSw20308 reveals its potential applications

ZENG Yinxin[1, 2*], YU Yong[1], QIAO Zongyun[1,2], LI Huirong[1] & LUO Wei[1]

[1] SOA Key Laboratory for Polar Science, Polar Research Institute of China, Shanghai 200136, China;
[2] College of Biological Engineering, Jimei University, Xiamen 350200, China

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Abstract The genus *Pseudoalteromonas* is ubiquitous in the marine environment and can synthesize a wide range of extracellular compounds. Psychrotolerant *Pseudoalteromonas* sp. BSw20308 was isolated from the Chukchi Sea, a marginal sea of the Arctic Ocean. It produces a number of extracellular enzymes that can degrade polysaccharides and proteins. The BSw20308 genome was sequenced to 38.1-fold coverage, and the sequences were assembled into 146 contigs (≥500 bp). In total, 4 172 open reading frames (ORFs) with an average gene length of 987 bp were detected. At least 86 ORFs were predicted by sequence analysis to encode a variety of catalytic modules involved in the degradation of polysaccharides, proteins, and lipids. In addition, 36 ORFs were predicted to encode catalytic modules involved in the degradation of organic pollutants and halogenated compounds, and in the production of bioactive compounds. The draft genome sequence of BSw20308 provides new information about the ecological function and adaptation of the genus *Pseudoalteromonas* in Arctic marine environments, and also indicates its potential applications in the biotechnology industries (e.g., enzymology, and pollutant degradation).

Keywords ***Pseudoalteromonas*, genome, enzyme, application**

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1 Introduction

Polysaccharides and proteins are the most abundant natural substances in marine systems and serve as nutrient sources for marine microbes[1-2]. Extracellular enzymes play a central role in the microbial degradation of these natural products. The genus *Pseudoalteromonas* comprises a group of marine bacteria that can constitute a significant fraction of the culturable bacteria living in cold marine environments[3-4]. Many species of the genus have been shown to produce a series of extracellular enzymes and bioactive compounds, indicating their ecological significance and their potential for use in biotechnological applications[5-6]. The marine bacterium *Pseudoalteromonas* sp. BSw20308 (previously known as Ar/w/b/75°/10/5) was isolated from the Chukchi Sea of the Arctic Ocean, and confirmed to produce extracellular cold-active enzymes, including agarase, amylase, cellulase, lipase, and protease[7-8]. In this study, we generated a draft genome sequence of *Pseudoalteromonas* sp. BSw20308 and performed sequence analysis to investigate the potential applications of the bacterium as well as to gain insight into the ecological function and adaptation of the genus *Pseudoalteromonas* in cold marine environments.

2 Materials and methods

Bacterial genomic DNA was extracted from strain BSw20308 by standard phenol-chloroform methods and sequenced using the Roche 454 GS FLX system (Roche Diagnostics, Basel, Switzerland). In total, 204 572 whole-genome shotgun reads were produced and assembled into 146 contigs (≥500 bp) using Newbler 2.7 (Roche Diagnostics), providing 38.1-fold coverage of the genome. Putative

* Corresponding author (email: yxzeng@yahoo.com)

protein-coding open reading frames (ORFs) were predicted by combining the results obtained with Glimmer 3.02[9], GeneMark 2.8[10], and Z-Curve 1.02[11], and annotated based on BLASTP searches against the nonredundant GenBank protein sequence database. Putative functions of the translation products were confirmed using the Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases[12-13]. The tRNA genes were predicted directly with tRNAscan-SE 1.23[14]. Genes encoding rRNA were identified using RNAmmer 1.2[15]. The whole-genome shotgun project data has been deposited at DDBJ/EMBL/GenBank under the project accession number AMYA00000000.

3 Results and discussion

3.1 General description of the bacterium

Strain *Pseudoalteromonas* sp. BSw20308 is a slightly halophilic and psychrotrophic bacterium, which produces melanin. It was isolated from ice-covered seawater in the Chukchi Sea in summer[7]. No cell growth was observed at 35℃. Phylogenetic analysis based on 16S rRNA gene indicated that BSw20308 was most closely related to *Pseudoalteromonas haloplanktis* TAC125[16]; however, based on the fragments of four protein-coding housekeeping genes (*atpD*, *gyrB*, *rpoB* and *trpB*), the two strains were found to share 86%–94% sequence similarity suggesting that they actually belong to different species.

The draft genome sequence of *Pseudoalteromonas* sp. BSw20308 is 4 757 002 bp long and has a G+C content of 38.9%. It contains 4 172 predicted ORFs (average length = 987 bp), five rRNA operons, and 67 tRNA genes for 18 amino acids. The gene coding sequences cover 86.6% of the genome. Based on BLASTP searches, the proteins encoded by 4 097 of the ORFs were assigned functions, and among them 1 072 were identified as conserved hypothetical proteins. Among the predicted ORFs, 3 109 (74.5%) were assigned to COG families comprising 17 functional categories (Figure 1), and 1 991 (47.7%) were found within KEGG. Based on KEGG pathway classification, 1 274 ORFs (30.5%) were annotated as being involved in metabolism, including amino acid metabolism (345 ORFs), carbohydrate metabolism (296 ORFs), lipid metabolism (85 ORFs), xenobiotic biodegradation and metabolism (66 ORFs), metabolism of terpenoids and polyketides (39 ORFs), glycan biosynthesis and metabolism (37 ORFs), and biosynthesis of other secondary metabolites (26 ORFs).

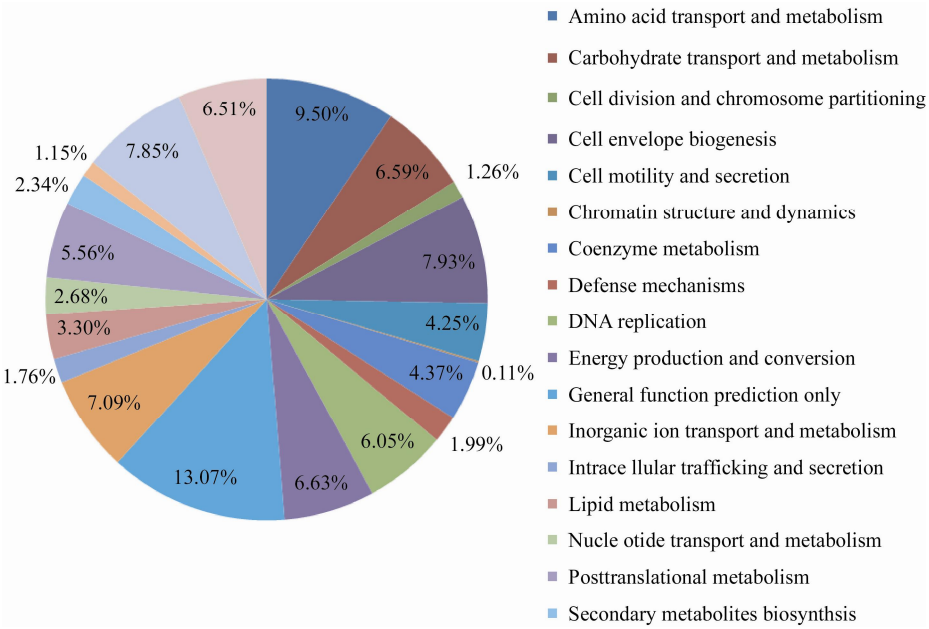

Figure 1 Functional COG categories in the *Pseudoalteromonas* sp. BSw20308 genome.

3.2 Genes involved in polysaccharide metabolism

Degradation of organic matter in oceans is fundamental for the cycling of elements, and is normally undertaken in the pelagic zone by bacteria. Previous studies have shown that strain *Pseudoalteromonas* sp. BSw20308 can produce a series of extracellular enzymes with optimum temperatures between 25℃ and 40℃[7-8], indicating a niche adaptation for the acquisition of substrates for bacterial growth from the extracellular digestion of polymeric substances and the uptake as well as utilization of their respective monomers[17]. Cell wall components of plant cells, particularly the beta-linked polysaccharides cellulose and xylan, are potential substrates for marine algae[17]. Genes encoding polysaccharide-degrading enzymes (e.g., agarase, amylase, cellulase, pectinase, and xylanase) were detected in the BSw20308 genome (Table 1), suggesting that the bacterium

regularly encounters a fairly rich medium as the result of phytoplankton bloom during the Arctic summer. In addition, supported by their demonstrated growth on glucose[7], strain BSw20308 was found to possess a phosphoenolpyruvate-dependent phosphotransferase system (e.g. ORF_2493) for the transport and first metabolic step of carbohydrate degradation. Indeed, the cold-adapted BSw20308 cellulase (Cel308) gene (HQ997897) has been cloned and ligated into plasmid pWD2 to construct the expression vector pWD-cd[18]. Chitin is an important structural element of fungal cell walls and arthropod exoskeletons frequently found in marine environments. The BSw20308 genome contained four genes (i.e., ORF_1342, ORF_1607, ORF_3423, and ORF_2879) involved in chitin hydrolysis, indicating the ability of the strain to degrade chitin.

Table 1 Genome annotation and potential applications of genes in *Pseudoalteromonas* sp. BSw20308

Enzyme description	ORF	COG	Potential application
Polysaccharide metabolism			
Glycoside hydrolase, superfamily 16	ORF_0001, ORF_2168	COG2273	Food, health products, detergents, textiles, fuel
Glycoside hydrolase, superfamily 8	ORF_0024	COG3405	
Glycoside hydrolase, family 77	ORF_2210	COG5434	
Xylan 1,4-beta-xylosidase	ORF_0027, ORF_0051, ORF_2205	COG3507	
Xylose isomerase	ORF_0030	COG2115	
Endo-1,4-beta-xylanase A	ORF_0035, ORF_0042	COG3693	
Family GH10 xylanase	ORF_0041	COG3693	
Cellulase	ORF_1261	COG2730	
Endoglucanase	ORF_1855	COG3405	
Alpha-glucosidase	ORF_1359		
Probabl alpha-glucosidase	ORF_0254	COG0366	
Beta-glucosidase	ORF_0245, ORF_0333, ORF_0364	COG1472	
Beta-glucosidase	ORF_0336, ORF_0365	COG2723	
Alpha amylase (neopullulanase)	ORF_0250, ORF_0251, ORF_1418	COG0366	
Gamma amylase (glucoamylase)	ORF_0248	COG3387	
1,4-alpha-glucan branching enzyme	ORF_1279	COG0296	
Alpha,alpha-trehalase	ORF_3431	COG1626	
Licheninase (Endo-beta-1,3-1,4 glucanase)	ORF_0246, ORF_1573	COG2273	
Pectate lyase	ORF_0193, ORF_1055, ORF_1757	COG3866	
Candidate pectate lyase, polysaccharide lyase family 10	ORF_2194	COG4677	
Pullulanase	ORF_1376	COG1523	
Glycogen operon protein GlgX	ORF_1278	COG1523	
Beta-agarase (Glycoside hydrolase, superfamily 16)	ORF_0583, ORF_1582, ORF_3063, ORF_3068		
Beta-agarase B	ORF_1564, ORF_1565, ORF_3053		
Chitin deacetylase 1	ORF_3423	COG0726	
Carbohydrate esterase family 4/polysaccharide deacetylase	ORF_1342	COG0726	
Chitin-binding, domain 3 protein	ORF_2879		
Beta-N-acetylhexosaminidase (exochitinase)	ORF_1607	COG1472	
Protein metabolism			
Putative collagenase	ORF_0572	COG0826	Food, health products, detergents, cosmetics
Putative collagenase	ORF_2861		
Peptidase U32	ORF_2417	COG0826	
Secreted cyanophycinase	ORF_3342	COG4242	
Zinc protease	ORF_3610	COG0612	
Peptidase M16-like protein	ORF_2811	COG0612	

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Zn-dependent protease with chaperone function PA4632	ORF_0728	COG4783	
Peptidase M48, Ste24p	ORF_3089	COG4783	
Hemagglutinin/protease	ORF_1029	COG3227	
Metalloprotease	ORF_1212	COG3227	
Pseudolysin	ORF_2858, ORF_2864	COG3227	
Metalloprotease MEP2	ORF_1604		
Probable zinc metalloprotease Lema_P086240	ORF_0589	COG2234	
Protease IV	ORF_3045	COG0616	
SohB protein, peptidase U7 family	ORF_0260	COG0616	
Protease BcsE	ORF_1860		
Zn-dependent protease	ORF_1099	COG1994	
Probable zinc metalloprotease Lema_P086240	ORF_0589	COG2234	
Putative peptidase precursor	ORF_0661	COG2234	
Serine protease	ORF_1211	COG2234	
Peptidase M28	ORF_2322, ORF_3239	COG2234	
Serine protease	ORF_2273, ORF_3632	COG1404	
Subtilisin	ORF_2719	COG1404	
Deseasin MCP-01	ORF_3424	COG1404	
Aminopeptidase	ORF_0613, ORF_1630, ORF_1923	COG0308	
Peptidase, M13 family	ORF_2236	COG3590	
Peptidase	ORF_0418, ORF_2725	COG1025	
Oligopeptidase B	ORF_2274, ORF_2878	COG1770	
Lipid metabolism			
Lipase-like protein	ORF_0261	COG1073	Food, health products, detergents, asymmetric synthesis
Lipase class 3	ORF_1775		
Predicted lipase	ORF_2034, ORF_2733		
Esterase/lipase/thioesterase family protein	ORF_0467	COG4757	
Esterase/lipase/thioesterase	ORF_2204	COG0657	
Organic pollutant degradation			
2-hydroxychromene-2-carboxylate isomerase/ DsbA-like thioredoxin domain	ORF_0450	COG2761	Environmental protection
Glutathione S-transferase	ORF_0084, ORF_0430, ORF_0488, ORF_0571, ORF_1830, ORF_2389, ORF_2737, ORF_3887	COG0625	
Glutathione S-transferase, omega	ORF_1948	COG0435	
Nitrilotriacetate monooxygenase component B	ORF_0448, ORF_1819	COG1853	
Dienelactone hydrolase	ORF_0709	COG0412	
Nitroreductase	ORF_0718, ORF_1382, ORF_3048	COG0778	
Homogentisate 1,2-dioxygenase	ORF_0429	COG3508	
Haloacid dehalogenase	ORF_0156, ORF_2301	COG1011	
Secondary metabolites biosynthesis and catabolism			
Nonribosomal peptide synthetase	ORF_1196, ORF_1617, ORF_1618, ORF_1620, ORF_4028	COG1020	Pharmaceutical industry
$FADH_2$ O_2-dependent halogenase I	ORF_0047, ORF_0252		
$FADH_2$ O_2-dependent halogenase I	ORF_0363	COG0644	
Putative Tryptophan halogenase	ORF_1164	COG0644	
Tryptophan halogenase	ORF_0362	COG0654	

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Tryptophan halogenase	ORF_3073		
Isochorismate synthase/isochorismate-pyruvate lyase mbtI	ORF_1621	COG0147	
Cytochrome P450	ORF_3057	COG2124	
dTDP-glucose 4,6-dehydratase	ORF_1679	COG1088	
Penicillin amidase	ORF_2288	COG2366	
Isopenicillin N synthase	ORF_2978	COG3491	
Phenazine biosynthesis like protein	ORF_2374	COG0384	

3.3 Genes involved in protein and lipid metabolism

In accordance with its ability to degrade casein and gelatin, at least 35 genes encoding proteases and peptidases (Table 1) were found in the BSw20308 genome, indicating the potential of BSw20308 for proteolytic degradation of high-molecular-weight organic matter in marine environments. Three putative peptidases encoded in the BSw20308 genome, namely ORF_0572, ORF_2417, and ORF_2861, match collagenases found in *Pseudoalteromonas* sp. Collagens are an important component of marine invertebrates (including chordates) and sponges[19-20]. In addition, two genes encoding putative pseudolysin, which cleaves elastin and collagen, were identified in the BSw20308 genome, namely, ORF_2858 and ORF_2864. The putative serine protease (ORF_3424) matched deseasin MCP-01, a bacterial collagenolytic serine protease found in deep-sea *Pseudoalteromonas* sp. SM9913[21]. Another proteolytic enzyme (ORF_3342) was annotated as a putative cyanophycinase, which can degrade the amino-acid polymer cyanophycin, an important intracellular nitrogen-storage polymer predominantly found in cyanobacteria[17,22]. The BSw20308 genome possessed at least six genes that encode lipases; two of them (ORF_0467 and ORF_2204) encode putative esterases/lipases found in *Pseudoalteromonas* sp. and ORF_0261 encodes an extracellular lipase (COG1073) that matches hypothetical proteins in *Pseudoalteromonas* sp. Growth experiments confirmed the ability of BSw20308 to utilize Tween 80 and hydrolyze olive oil[8]. A large number of ORFs in the BSw20308 genome were predicted to encode putative hydrolytic enzymes, indicating that the bacterium is an efficient degrader of complex organic matter in marine environments and thus may provide novel enzymes for use in the biotechnology industry.

3.4 Genes involved in pollutant degradation

Genes encoding putative enzymes for pollutant degradation were detected in the BSw20308 genome. Two ORFs (ORF_0448 and ORF_1819) were predicted to encode putative nitrilotriacetate monooxygenase component B, which can degrade nitrilotriacetate, an important industrial chelating agent that has been widely used for various radionuclide processing and decontamination procedures. Polycyclic aromatic hydrocarbons are well-known ubiquitous environmental contaminants. The ORF_0450 gene encodes the putative 2-hydroxychromene-2-carboxylate isomerase/DsbA-like thioredoxin domain. 2-Hydroxychromene-2-carboxylate isomerase is a glutathione-dependent enzyme involved in the naphthalene catabolic pathway, and it is unique to bacteria that degrade polycyclic aromatic compounds. In addition, at least nine glutathione S-transferase-encoding genes, which are a potential probe for bacteria capable of degrading polycyclic aromatic hydrocarbons[23], were identified in the BSw20308 genome. ORF_0429 encodes a putative homogentisate 1,2-dioxygenase (HGD) found in *P. haloplanktis* ANT/505. HGD is involved in the catabolism of aromatic rings, and the HGD gene in the fungus Exophiala lecanii-corni was found to be up-regulated in the presence of ethylbenzene and may be responsible for the ring cleavage step in the degradation pathway[24]. Another putative dienelactone hydrolase encoded by ORF_0709 also matches the enzyme found in *P. haloplanktis* ANT/505. Dienelactone hydrolase may be involved in the degradation of protoanemonin, a toxic metabolite formed during the degradation of polychlorinated biphenyls[25]. At least two predicted hydrolases encoded by ORF_0156 and ORF_2301 match haloacid dehalogenases found in Gammaproteobacteria, including the genera of *Alcanivorax*, *Glaciecola*, and *Pseudoalteromonas*. Halogenated organic compounds are one of the largest groups of environmental pollutants, and dehalogenases that catalyze the cleavage of the carbon-halogen bond of these compounds have attracted a great deal of attention because of their potential applications in bioremediation. In addition, haloacid dehalogenases are useful for the production of optically active 2-hydroxyalkanoic acids and 2-haloalkanoic acids, indicating their potential applications in the pharmaceutical and fine chemical industry[26]. At least three genes in the BSw20308 genome encode nitroreductases (COG0778), which play a central role in the activation of a variety of nitro-compounds (e.g., nitrofurans, nitrobenzenes, nitrophenols, nitrotoluenes, and nitroimidazoles) and have received much attention for their environmental importance[27]. In addition, as agents used to activate prodrugs in directed anticancer therapies and with susceptibility to antibiotics, nitroreductases have also received considerable interest from the medical community[27].

3.5 Genes involved in secondary metabolites biosynthesis and catabolism

The genus *Pseudoalteromonas* is well known for its ability

to produce a series of bioactive compounds[5]. The polyketide and non-ribosomal peptide synthases constitute a class of multifunctional proteins that may create a multitude of secondary metabolites, and many of them have become important drugs[28]. At least five genes in the BSw20308 genome were predicted to encode non-ribosomal peptide synthase modules and related proteins (COG1020) (Table 1); however, no genes involved in polyketide synthases were detected. At least six genes encoding FADH2 O2-dependent halogenases and tryptophan halogenases were detected, which may play an important role in the enzymatic incorporation of halogens during natural product assembly and enable coordinate regulation to activate the secondary metabolite pathways[29]. ORF_1621 encodes putative isochorismate synthase/isochorismate-pyruvate lyase mbtI, which is involved in the biosynthesis of salicylic acid in bacteria. ORF_3057 encodes a putative cytochrome P450 and, because of their catalytic diversity and broad substrate range, cytochrome P450 enzymes are attractive biocatalyst candidates for the production of pharmaceuticals, including biosynthesis of the glycopeptide antibiotics vancomycin and balhimycin[30]. ORF_1679 encodes a putative dTDP-glucose 4,6-dehydratase, which is known to participate in metabolic pathways involved in the biosynthesis of vancomycin group antibiotics and streptomycin[31-32]. In addition, at least three of the genes (ORF_2288, ORF_2978 and ORF_2374) in the BSw20308 genome encode putative penicillin amidase, isopenicillin N synthase, and phenazine biosynthesis like protein, which play major roles in the synthesis of clinically useful antibiotics, such as penicillin, cephalosporin, and phenazine respectively. The genome sequence of *Pseudoalteromonas* sp. BSw20308 indicates the potential to use this bacterium for the production of bioactive compounds in the pharmaceutical industry.

Preliminary analysis of the *Pseudoalteromonas* sp. BSw20308 genome indicated that this bacterium possesses various enzyme-encoding genes that may have significant potential applications in various industries, including the chemical, detergent, food, fuel, textile, environmental protection, and pharmaceutical industries. Some of the enzymes have been confirmed to exhibit hydrolytic activity. Further research is required to investigate the enzyme-encoding genes for application in biotechnology, as well as their ecological function and adaptation in Arctic cold marine environments.

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