Extremophiles (2017) 21:581–589
DOI 10.1007/s00792-017-0927-1

ORIGINAL PAPER

Comparative genomics analysis of five *Psychrobacter* strains isolated from world-wide habitats reveal high intra-genus variations

Shan Zhang[1,2] · Weizhi Song[1,2] · Min Yu[3] · Xuezheng Lin[4]

Received: 11 September 2016 / Accepted: 6 March 2017 / Published online: 17 March 2017

Abstract *Psychrobacter* has been regarded as an important genus for bacterial cold adaptation studies. However, members of this genus are highly varied in terms of both cold adaptability and genome content. To get an understanding of the diversity of members of this genus, five Psychrobacter strains (G, K5, 273-4, PAMC21119 and PRwf-1), with publicly available complete/draft genome, were selected and comprehensive comparative genomics analyses were performed among them. The closest phylogenetic relationship, highest average nucleotide identity (96.78%) and best sequence synteny were identified between strains G and K5. These findings suggest they belong to the same species, despite the long geographic distance between them (Antarctic and Siberia). 4542 gene clusters in total were identified from the five genomes, and of which 1424 were shared by all of them. The number of genes unique to strains G, K5, 273-4, PAMC21119 and PRwf-1 are 183, 188, 300, 637 and 665, respectively. COG assignment revealed their differences in gene content related to stress response. The extensive sequence rearrangements and the large number of genes unique to strain PAMC21119 and PRwf-1 suggest they may have experienced a high level of gene exchanges in the permafrost soil and the surface of fish skin.

Keywords *Psychrobacter* · Comparative genomics · Intra-genus variation

Abbreviations

ANI	Average nucleotide identity
BLAST	Basic local alignment search tool
BlastN	Nucleotide BLAST
BRIG	BLAST ring image generator
COG	Clusters of orthologous groups
CSP	Cold shock protein
NCBI	National center for biotechnology information
ORF	Open reading frame

Background

The genus *Psychrobacter* comprises a group of bacteria which are gram-negative, spherical to rod-shaped, heterotrophic and cold adapted (Ayala-del-Rio et al. 2010; Bowman 2006; Bozal et al. 2003). Members of this genus have been isolated from various environments, including Antarctic soil and seawater, deep-sea, Siberia permafrost, and the stomach contents of the Antarctic krill (Ayala-del-Rio et al. 2010; Bowman et al. 1997; Denner et al. 2001; Vishnivetskaya et al. 2000; Xuezheng et al. 2010; Yumoto et al. 2003). It is evident that environments with low temperature constitute an ecological niche for members from this genus (Bozal et al. 2003). However, highly varied responses to temperature stresses were observed between the members of this genus. For example, the strain *Psychrobacter* sp. G exhibits an optimal

Communicated by M. da Costa.

✉ Xuezheng Lin
linxz@fio.org.cn

[1] School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW 2052, Australia

[2] Centre for Marine Bio-Innovation, The University of New South Wales, Sydney, NSW 2052, Australia

[3] College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China

[4] The First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China

growth at 25 °C and a maximum growth temperature at 35 °C (Xuezheng et al. 2010); Meanwhile, the *Psychrobacter arcticus* 273-4 is not capable to grow at temperature higher than 28 °C (Bergholz et al. 2009; Vishnivetskaya et al. 2000). Moreover, high variations in genome contents were also found among them. For example, the genome size of the *Psychrobacter* sp. PAMC21119 is 3.35 MB (Kim et al. 2012), whereas the genome size of *Psychrobacter arcticus* 273-4 is only 2.65 MB (Ayala-del-Rio et al. 2010), which is only 79% of the former. These differences suggest that strategies employed by members of this genus to conquer cold stresses maybe highly varied, which limits the universality of conclusions drew based on some particular strains. Despite these obvious intra-genus variations, several members of this genus have been selected as "model strains" for bacterial cold adaptation studies. For example, as stated in Zheng's studies, "*Psychrobacter arcticus* 273-4 was selected as a model to study cold adaptation" (Zheng et al. 2007). In addition to this, *Psychrobacter* sp. PAMC21119 was also "identified as potential model organisms for the study of low-temperature adaptations" (Kim et al. 2012). Given these high intra-genus variations, it is reasonable to question to what extent the results concluded based on one particular strain can be applied to the others or even the whole genus.

The development in sequencing technology and the increasing number of publicly available complete/draft genomes made it possible to get insight into bacterial cold adaptation at a more comprehensive level. However, known studies were mainly focused on the functional comparison among strains which are capable to synthesise certain kinds of biological substances. For example, Fondi et al. (2014) have performed comparative genomics analysis among three *Psychrobacter* strains isolated from Antarctic sponges which can produce antimicrobial compounds. Comparative genomics approach was also used by Moghadam et al. (2016) to identify the differences in laccase-related metabolic profiles between four Arctic *Psychrobacter* strains and other publicly available *Psychrobacter* genomes. In the above studies, the diversity of this genus was not considered, or the analysis was mainly focused on genes/pathways related to the production of certain biological substances.

To address these concerns, five *Psychrobacter* strains, *Psychrobacter* sp. G (Song et al. 2012), *Psychrobacter cryohalolentis* K5 (Bakermans et al. 2006), *Psychrobacter arcticus* 273-4 (Bergholz et al. 2009), *Psychrobacter* sp. PAMC21119 (Kim et al. 2012) and *Psychrobacter* sp. PRwf-1 (Wirth et al. 2012), were selected and comprehensive comparative genomics analyses were performed. Results obtained in current analyses can provide insight into to what extent members of this genus were varied and thus shed light on strain selection for bacterial cold adaptation studies.

Methods

General features prediction

Open reading frame (ORF) prediction was performed with Prokka (Seemann 2014) with default parameters. COG category assignment of predicted genes was performed using BLAST against the NCBI COG database with an E-value cutoff of 1e-5 (NCBI COG database 2016). Average nucleotide identity (ANI) values between each pair of the five genomes were calculated with ANI calculator (Kostas 2016). The completeness of PAMC21119 draft genome was assessed using CheckM v0.9.7 (Parks et al. 2015).

Genome comparison analysis

The evolutionary distances among the five strains were assessed based on 16S rRNA sequences and concatenated amino acid sequences of core genes. Sequences were aligned using Clustal Omega 1.2.3 (Sievers and Higgins 2014) with default parameters. A maximum likelihood phylogenetic tree was constructed by Mega 7.0 (Kumar et al. 2008), the reliability of the tree was analysed using bootstrap probabilities. The pan-genome structure was analysed using Roary (Page et al. 2015) with an identity cutoff of 50%. A Venn diagram of the pan-genome structure was generated with R package VennDiagram (Chen et al. 2011). The circular plot of whole genome identity comparison was generated using BLAST Ring Image Generator (BRIG) (Alikhan et al. 2011) with default parameters. The global alignment of whole chromosomal sequences was performed by M-GCAT software (Treangen and Messeguer 2006).

Results

General overview

A summary of the five strains' habitats is given in Table 1 and Fig. 1. Their habitats range from Siberia (Vishnivetskaya et al. 2000) in the north to Antarctica (Kim et al. 2012; Song et al. 2012) in the south. And their habitat types include seawater (Song et al. 2012), permafrost (Bakermans et al. 2006), soil (Kim et al. 2012) and fish skin (Wirth et al. 2012) (Fig. 1). Their responses to temperature stresses are given in Table 1. The completeness of strain PAMC21119's draft genome is 99.95%. The general genomic information of the five strains is given in Table 2.

Table 1 A summary of published biological properties and habitats of the five strains

	Growth temperature	Habitat	References
Psychrobacter sp. G	The optimal and highest growth temperature of G are 20 and 35 °C	Surface water from south-western King George Island	Song et al. (2012)
Psychrobacter cryohalolentis K5	From −10 to 30 °C	Saline liquid found 11–24 m below the surface of Siberian permafrost	Bakermans et al. (2006)
Psychrobacter arcticus 273-4	From −10 to 28 °C, with an optimal growth temperature of 17 °C	Permafrost in the Kolyma region in Siberia	Bergholz et al. (2009) and Vishnivetskaya et al. (2000)
Psychrobacter sp. PAMC 21119	N/A	Permafrost soil on King George Island, Antarctica	Kim et al. (2012)
Psychrobacter sp. PRwf-1	From 4 to 37 °C	The skin of *Lutjanus vivanus* caught off the coast of Loíza in northeastern Puerto Rico	Wirth et al. (2012)

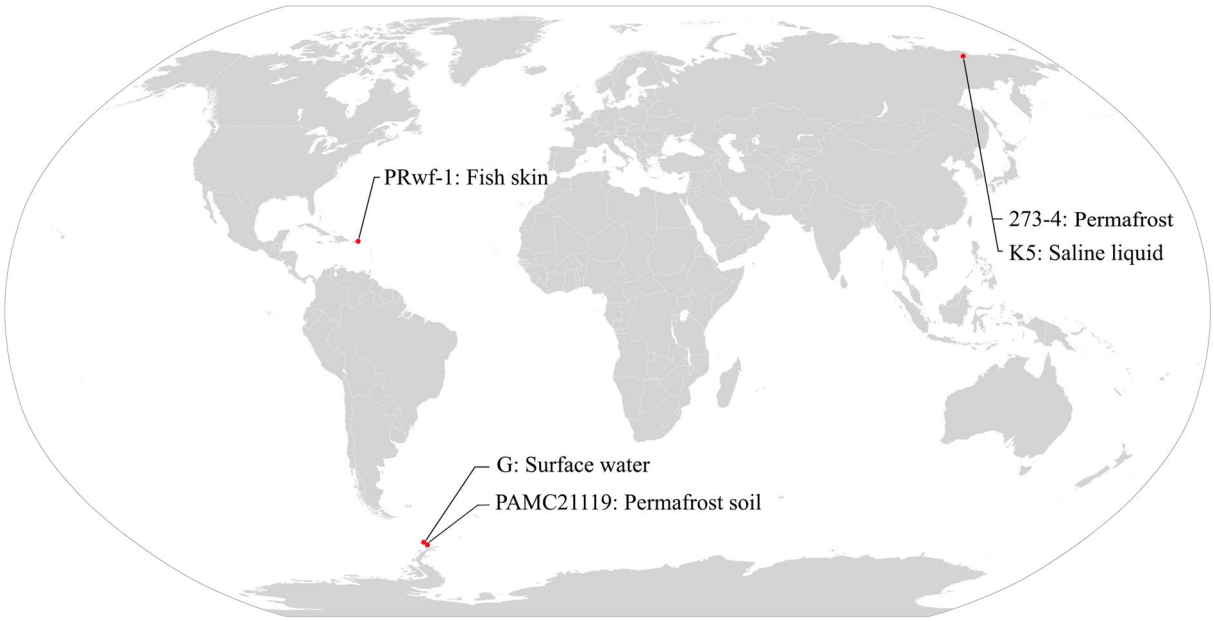

Fig. 1 Habitats location of selected five *Psychrobacter* strains

Phylogenetic analysis

The phylogenetic relationships between the five strains were analysed based on both of 16 S rRNA sequences (Fig. 2a) and concatenated amino acid sequences of core genes (Fig. 2b). More than one 16s rRNA sequence types were identified from the genome of PRwf-1 and PAMC21119. The two phylogenetic trees constructed based on the two methods are well consistent with each other. The closest phylogenetic relationship was identified between strains G and K5 (Fig. 1). While the strain PRwf-1 was most distant from the rest four, its habitat (tropical fish skin) is also distinctively different from the others. The ANI values between each pair of the five genomes are given in Fig. 3. Strains G, K5 and 273-4 share the highest three ANIs among the five strains; while the strain PRwf-1 was most distant from the others. All ANI values between selected genomes are higher than 70%.

Pan-genome structure of selected 5 genomes

The pan-genome structure of the five strains was analysed to get their variations in gene contents (Fig. 4). The results showed that 4542 gene clusters in total were identified from the five genomes, and of which 1424 (31.4%) were identified from all of them (core genes) (Fig. 4). The number of unique gene clusters, which are only detected from one genome, among the five strains is highly varied.

Table 2 A summary of the five strains' general genomic information

	G	K5	273-4	PAMC 21119	PRwf-1
NCBI BioProject accession number	PRJNA231962	PRJNA13920	PRJNA9633	PRJNA76621	PRJNA15759
Genome status	Complete	Complete	Complete	Draft	Complete
Chromosome size (Mbp)	2.94	3.06	2.65	3.51	2.98
Plasmids	3	1	0	N/A	2
Total plasmid size (Mbp)	0.03	0.04	0	N/A	0.02
5S rRNA	4	4	4	3	5
16S rRNA	4	4	4	5	5
23S rRNA	4	4	4	3	5
tRNA	48	48	49	48	58
GC (%)	42.4	42.2	42.8	43.3	44.9
Total gene number	2682	2581	2211	2899	2477
Protein-coding gene number	2614	2511	2120	2841	2385

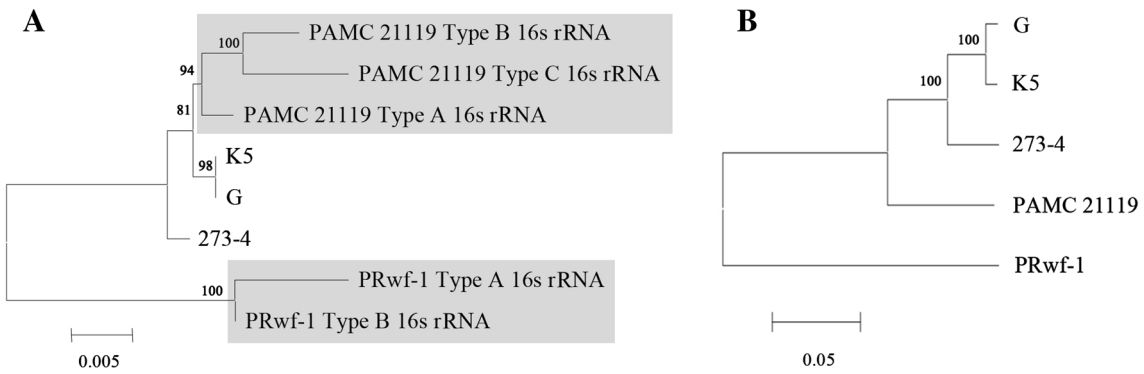

Fig. 2 The phylogenetic tree was constructed based on 16S rRNA sequences (**a**) and the concatenated amino acid sequences of 1424 core genes (**b**). More than one type of 16s rRNA sequences (*grey box*) was identified from strain PAMC21119 and PRwf-1. *Numbers* at each branch indicate the percentage of times a node was supported in 1,000 bootstrap replications by neighbour joining

The numbers of unique gene clusters of stain G and K5 are only 183 and 188, respectively, while that of the numbers of strain PAMC21119 and PRwf-1 are 637 and 665, respectively (Fig. 4). The number of shared gene clusters between strains G and K5 is 2318; meanwhile, that number was decreased to 1661 between strains PAMC21119 and PRwf-1.

COG assignment of each strain's chromosome, plasmid and unique genes as well as the 1424 core genes is given in Fig. 5. The five strains' chromosome genes showed similar COG distribution profile. However, the number of genes related to [L] Replication, recombination and repair in strain PRwf-1's chromosome (194) is much higher than that of the other four strains. On the contrary, its genes related to [K] Transcription (95) is lower than strains G (115), K5 (111) and PAMC21119 (122). Strains PAMC21119 have a large number of genes (243) related to [E] Amino acid transport and metabolism. Of these 1424 core gene clusters, 11.3% of them encode poorly characterised proteins (161). Among the rest 88.7%, the three most enriched categories are [J] translation, ribosomal structure and biogenesis (150), [E] Amino acid transport and metabolism (124) and [C] Energy production and conversion (124). The most enriched COG category for strain G's plasmids is [L] Replication, recombination and repair (8), while most of the genes (15) encoded in strain K5's plasmid were poorly characterised (Fig. 5).

Genes related to stress responses were also identified from the five genomes and are given in Fig. 6. These stresses include temperature, osmotic, oxidative and starvation. The most abundant osmotic regulation factor in the five genomes is the choline–glycine betaine transporter. ABC-type proline/glycine betaine transport systems were absent in strain 273-4's genome. Several oxidative stress response-related protein families were identified from the five genomes, including three peroxiredoxin families, two superoxide dismutases and one catalase family. The catalase subfamily peroxidase I was

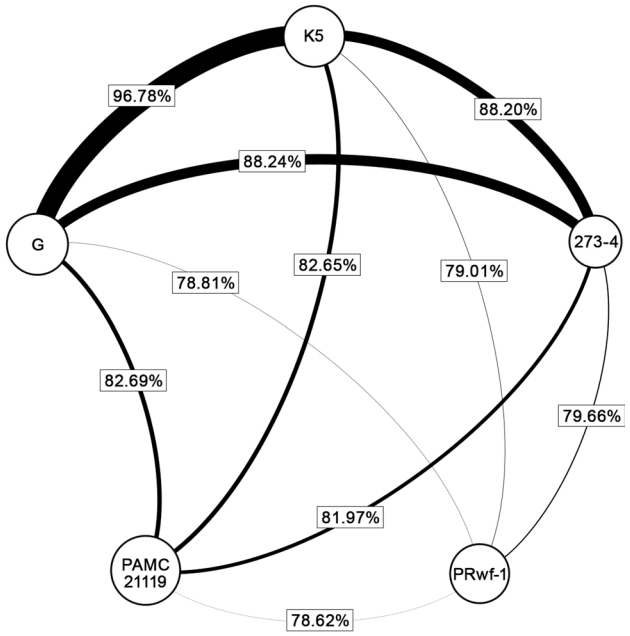

Fig. 3 ANI Comparison between each pair of the five genomes. The area of a *circle* is proportional to the genome size it represented. The width of an edge connecting two *circles* indicates the degree of identity between the two genomes

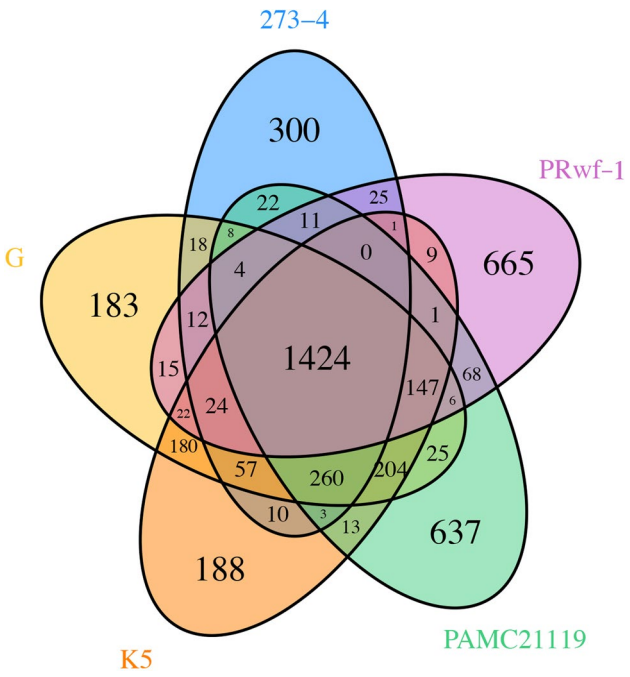

Fig. 4 Pan-genome structure of selected five *Psychrobacter* strains

only identified in strain PAMC21119's genome. Three starvation-related protein families were identified from the each of the five genomes (Fig. 6).

Multi-genome alignment of the five *Psychrobacter* strains

Global genome alignment was performed by BRIG and M-GCAT to illustrate the conservation and variation between the five genomes (Figs. 7, 8). High sequence similarity and extensive sequence synteny were observed between strains G and K5 (Fig. 7). Despite their high similarity, several sequences insertion/deletion were identified from their genomes (Fig. 8). A large inverted DNA fragment (about 660 kb) was identified in the genome of strain 273-4. Sequences except for the large inverted part in the genome of 273-4 showed high similarity and good synteny with stain G and K5 (Fig. 8). Extensive sequence rearrangements were found in the genome of strain PAMC21119 and PRwf-1 (Fig. 8). Sequence similarity of PAMC21119 and PRwf-1 to the reference genome was also much lower than that of strain K5 and 273-4 (Fig. 7).

Discussion

Among the five selected strains, K5 and 273-4 have been classified to different species within the *Psychrobacter* genus, while the rest three were only classified at the genus level. The closest phylogenetic relationship (Fig. 2) and highest sequence similarity (Fig. 7) were identified between strains G and K5. According to Konstantinidis' statements, two genomes that show higher than 95% ANI are belonging to the same species (Konstantinidis et al. 2006), while genomes sharing 70–95% ANI are regarded as belonging to distantly or closely related species of the same genus (Konstantinidis and Tiedje 2007). The high ANI (96.78%), close phylogenetic relationship and high sequence similarity between strains G and K5 suggest that they belong to the same species. This is quite interesting considering the long geographic distance between their habitats (from Antarctic to Siberia) (Fig. 1).

Considering the high variations in genome size between the five strains (Table 2), the pan-genome structure was analysed to show their differences in gene contents. The core genome of selected five genomes in current study consists of 1424 genes. In Fondi's study, 2073 genes were shared by three *Psychrobacter* strains (Fondi et al. 2014), which is much higher than the number obtained in the current study. However, it is reasonable to conclude that with the increased number of genomes, the size of core genome will be inevitably decreased. The number of genes unique to strain PRwf-1 (665) and PAMC21119 (637)is much higher than that unique to strains G (183) and K5 (188), which is consistent with the fact that the number of shared gene clusters between strains G and K5 (2318) is much higher than the number between strains PAMC21119 and

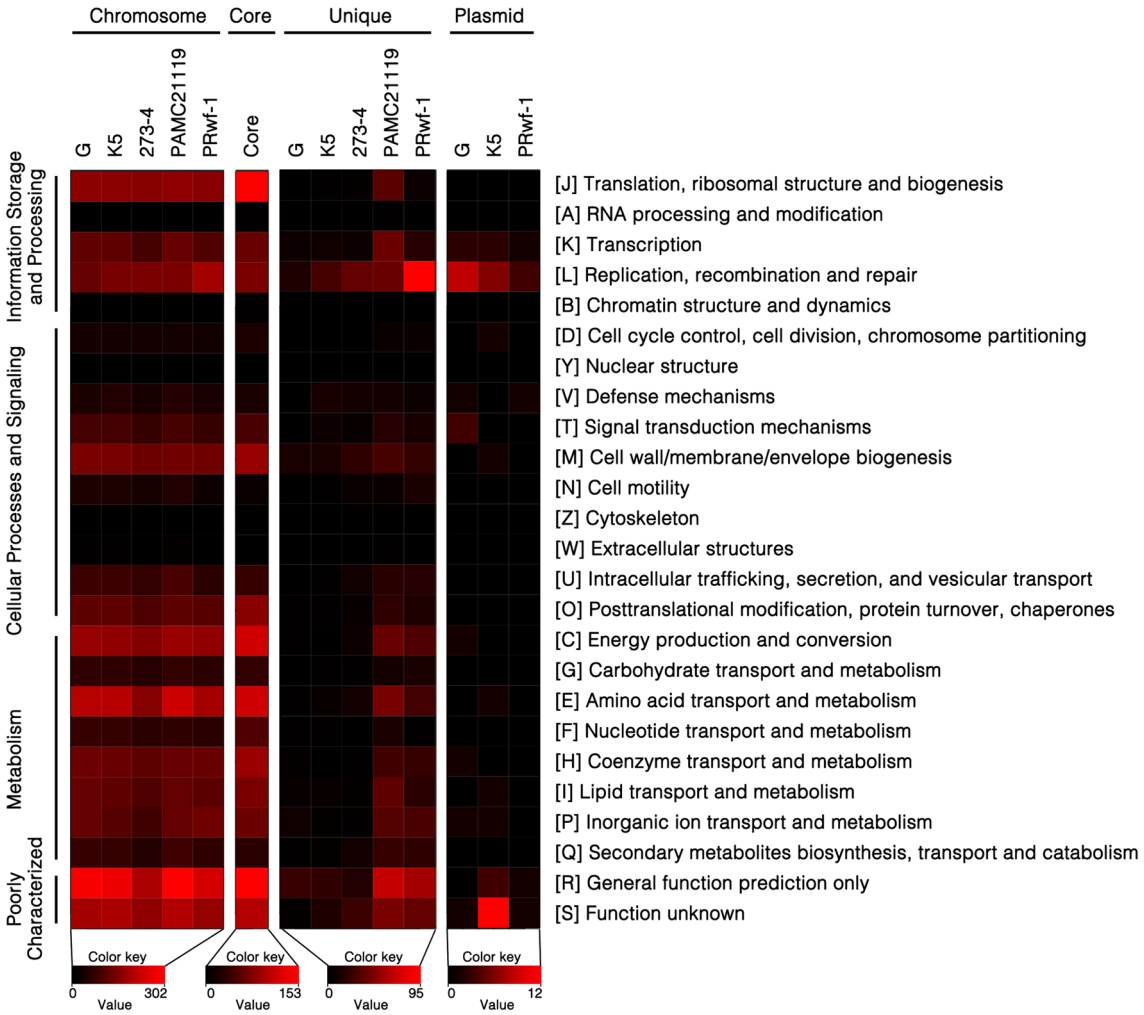

Fig. 5 COG assignment of chromosome, core, unique and plasmid genes for the five genomes

PRwf-1(1661) (Fig. 4). There are 665 genes unique to strain PRwf-1. Considering the distinct geographic location (tropical region) and habitat type (fish skin) of strain PRwf-1, these genes may play important roles in its adaptation to this niche.

COG assignment of the core genome as well as each strain's chromosome, plasmid, and unique genes was performed to provide insight into their differences in gene functions. More than 22% (95) of strain PRwf-1's unique genes are related to [L] Replication, recombination and repair (Fig. 5), which is much higher than the other four strains. The abundant genes related to replication, recombination and repair as well as the extensive sequence rearrangements (Fig. 8) detected from its genome indicate that strain PRwf-1 may undergo frequent gene gain and loss for adaptation to its distinct niche. The identification of stresses related genes revealed that at least three CSPs were identified from each of the five genomes. Cold shock protein (CSP) has been reported to be involved in the cold response of strains G (Song et al. 2012) and 273-4 (Ayala-del-Rio et al. 2010). The universal existence of CSPs may help *Psychrobacter* strains adapt to the frequently encountered cold stresses. Temperature changes in polar regions are often accompanied with salinity fluctuations, as salts in seawater are pushed out during ice formation (Thomas and Dieckmann 2002). Five intracellular osmotic regulation factors, including osmosensitive K^+ channel histidine kinase, ABC-type proline/glycine betaine transporter, Na^+/H^+ antiporter, choline-glycine betaine transporter and trehalose-6-phosphatase, were identified from the five genomes. The different distributions of these intracellular osmotic regulation factors in the five genomes indicate that multiple strategies may be employed by the five strains to cope with osmotic stresses. The interlinked stress response to low temperature and oxidation has been found in Antarctic bacterium *Pseudomonas fluorescens* MTCC 667 (Chattopadhyay et al. 2011). Several oxidative stress response-related protein families were identified from the five genomes, including

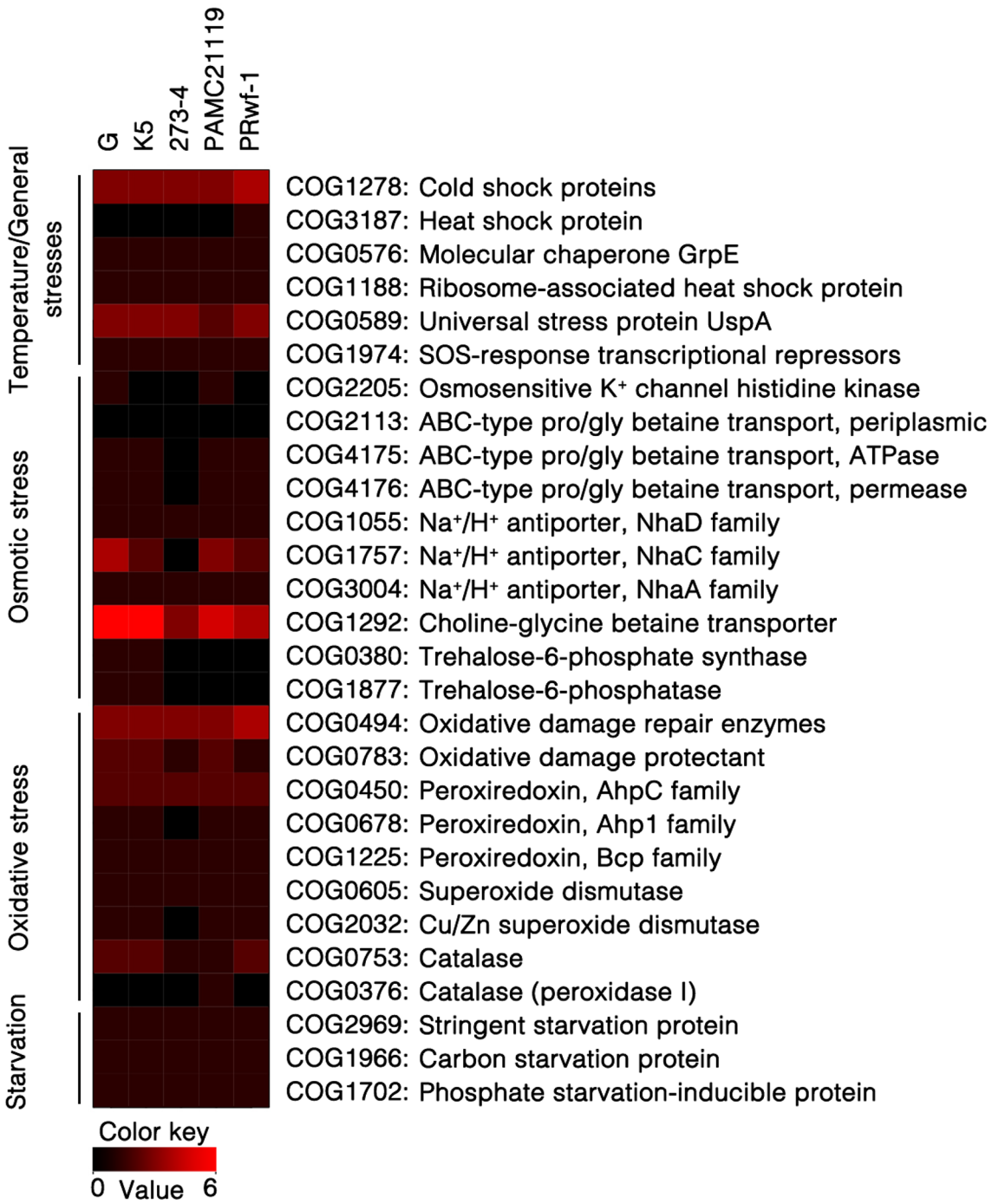

Fig. 6 Abundance of stress response-related genes from the five genomes

three peroxiredoxin families, two superoxide dismutases and one catalase family.

Multi-genome alignment of the five genomes revealed high identity (Fig. 7) and extensive sequence synteny (Fig. 8) between strains G and K5 indicate their close phylogenetic relationship. This is consistent with the results obtained based on phylogenetic trees and ANI values, the closer the phylogenetic relationships between genomes, the higher the synteny level detected, on the contrary, higher gene rearrangements were detected between them (Fig. 8). Bacteria community in soil, sediment, and host surfaces are much more complex and dense than surrounding water column in the marine system (Wiese et al. 2009). Previous studies have found that gene exchange rate is typically higher in high cell density communities compared with those in planktonic states (Davey and O'Toole 2000;

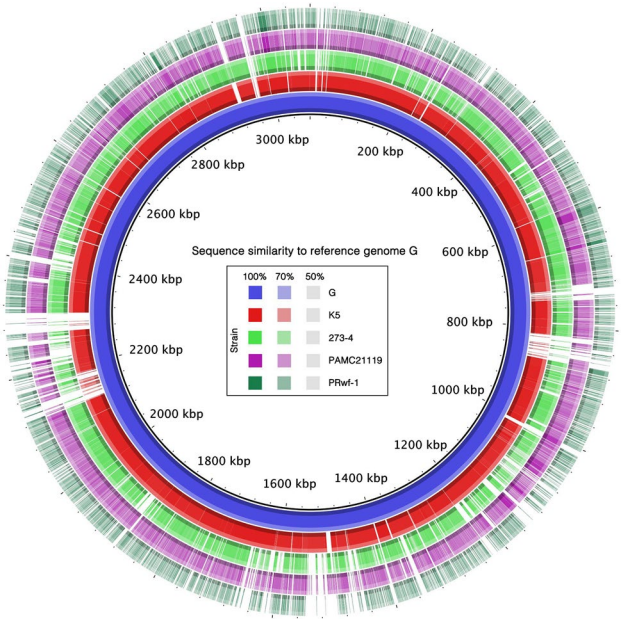

Fig. 7 Sequence similarity of genome K5, 273-4, PAMC21119 and PRwf-1 to reference genome G. Sequence similarity was calculated with BlastN

Kurokawa et al. 2007). Strains PAMC21119 and PRwf-1 were isolated from permafrost soil and fish skin, respectively. The extensive sequence rearrangements and the large number of unique genes suggest they may have experienced a high level of gene exchanges in such habitats.

Conclusions

Comparative genomics analyses were performed among five *Psychrobacter* strains isolated from world-wide habitats, which to our knowledge are the first comprehensive analyses among the highly varied members within this genus. Our results showed that the closest phylogenetic relationship and highest genome similarity were identified between two strains (G and K5) with distantly separated habitats (Antarctica and Siberia). Both of the two strains were isolated from aquatic habitats. On the contrary, another two strains with highest sequence rearrangements were isolated from soil or fish skin. These may indicate a lower genome variation progress in aquatic habitats. Although strains G and K5 share the highest genome similarity, their responses to temperature stresses are quite different. Strain G was capable of reproducing at 35 °C, while the highest growth temperature of K5 was only 30 °C. The most differentiated COG categories between their unique genes belong to the poorly characterised group and the [L] Replication, recombination and repair group. It should be interesting to get a more detailed understanding of these genes in the future studies. High differences in genes related to stress response were identified between the five genomes, which suggest that stress response mechanisms employed by members of this genus may be highly diversified. The profiles of pan and core genome as well as the extensive sequence rearrangements found in the genome of strain PAMC21119 and PRwf-1 suggest they may have experienced high genome diversification rate in permafrost soil and the surface of fish skin.

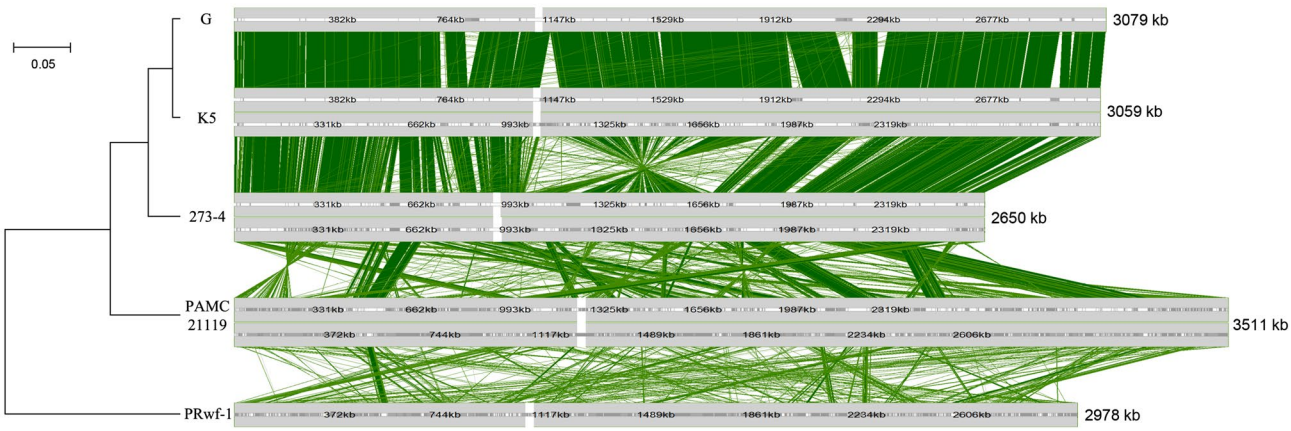

Fig. 8 Global alignment was performed by M-GCAT to illustrate the conservation and divergence between the five genomes. The phylogenetic tree on the *left* is constructed based on the concatenated amino acid sequences of 1424 core genes (Fig. 2b). The M-GCAT genome comparison between neighbouring genomes is given on the *right*. The five genomes are shown in full length and drawn to scale. The *green lines* link homology sequences between neighbouring genomes

Acknowledgements This research was funded by the National Natural Science Foundation of China (41176174), awarded to Xuezheng Lin. Weizhi Song was funded by the China Scholarship Council (201508200019).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genom 12:402

Ayala-del-Río HL, Chain PS, Grzymski JJ, Ponder MA, Ivanova N, Bergholz PW, Di Bartolo G, Hauser L, Land M, Bakermans C, Rodrigues D (2010) The genome sequence of *Psychrobacter arcticus* 273–4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. Appl Environ Microbiol 76:2304–2312. doi:10.1128/AEM.02101-09

Bakermans C, Ayala-del-Río HL, Ponder MA, Vishnivetskaya T, Gilichinsky D, Thomashow MF, Tiedje JM (2006) *Psychrobacter cryohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. Int J Syst Evol Microbiol 56:1285–1291

Bergholz PW, Bakermans C, Tiedje JM (2009) *Psychrobacter arcticus* 273-4 uses resource efficiency and molecular motion adaptations for subzero temperature growth. J Bacteriol 191:2340–2352

Bowman JP (2006) The genus *Psychrobacter*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 920–930

Bowman JP, Nichols DS, McMeekin TA (1997) *Psychrobacter glacincola* sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic sea ice. Syst Appl Microbiol 20:209–215. doi:10.1016/s0723-2020(97)80067-7

Bozal N, Montes MJ, Tudela E, Guinea J (2003) Characterization of several *Psychrobacter* strains isolated from Antarctic environments and description of *Psychrobacter* luti sp. nov. and *Psychrobacter* fozii sp. nov. Int J Syst Evol Microbiol 53:1093–1100

Chattopadhyay MK, Raghu G, Sharma YVRK, Biju AR, Rajasekharan MV, Shivaji S (2011) Increase in oxidative stress at low temperature in an antarctic bacterium. Curr Microbiol 62:544–546

Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 12:35

Davey ME, Otoole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

Denner EB, Mark B, Busse HJ, Turkiewicz M, Lubitz W (2001) *Psychrobacter proteolyticus* sp. nov., a psychrotrophic, halotolerant bacterium isolated from the Antarctic krill Euphausia superba Dana, excreting a cold-adapted metalloprotease. Syst Appl Microbiol 24:44–53

Fondi M, Orlandini V, Perrin E, Maida I, Bosi E, Papaleo MC, Michaud L, Giudice AL, de Pascale D, Tutino ML, Liò P (2014) Draft genomes of three Antarctic *Psychrobacter* strains producing antimicrobial compounds against *Burkholderia cepacia* complex, opportunistic human pathogens. Mar Genom 13:37–38

Kim SJ, Shin SC, Hong SG, Lee YM, Choi IG, Park H (2012) Genome sequence of a novel member of the genus *Psychrobacter* isolated from Antarctic soil. J Bacteriol 194:2403. doi:10.1128/JB.00234-12

Konstantinidis KT, Tiedje JM (2007) Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead. Curr Opin Microbiol 10:504–509

Konstantinidis KT, Ramette A, Tiedje JM (2006) The bacterial species definition in the genomic era. Philos Trans R Soc Lond Ser B Biol Sci 361:1929–1940

Kostas lab-ANI calculator (2016) http://enve-omics.ce.gatech.edu/ani/. Accessed 5 Jan 2016

Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9:299–306

Kurokawa K, Itoh T, Kuwahara T, Oshima K, Toh H, Toyoda A, Hattori M (2007) Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. DNA Res Int J Rapid Publ Rep Genes Genom 14:169–181

Moghadam MS, Albersmeier A, Winkler A, Cimmino L, Rise K, Hohmann-Marriott MF, Kalinowski J, Rückert C, Wentzel A, Lale R (2016) Isolation and genome sequencing of four Arctic marine *Psychrobacter* strains exhibiting multicopper oxidase activity. BMC Genom 117:17

NCBI COG database (2016) http://www.ncbi.nlm.nih.gov/COG/. Accessed 20 Apr 2016

Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J (2015) Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31:3691–3693

Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069

Sievers F, Higgins DG (2014) Clustal Omega, accurate alignment of very large numbers of sequences. Mult Seq Alig Methods 1079:105–116

Song W, Lin X, Huang X (2012) Characterization and expression analysis of three cold shock protein (CSP) genes under different stress conditions in the Antarctic bacterium *Psychrobacter* sp. G. Polar Biol 35:1515–1524. doi:10.1007/s00300-012-1191-6

Thomas DN, Dieckmann GS (2002) Antarctic Sea ice—a habitat for extremophiles. Science 295:641–644

Treangen TJ, Messeguer X (2006) M-GCAT: interactively and efficiently constructing large-scale multiple genome comparison frameworks in closely related species. BMC Bioinform 7:433. doi:10.1186/1471-2105-7-433

Vishnivetskaya T, Kathariou S, McGrath J, Gilichinsky D, Tiedje JM (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4:165–173

Wiese J, Thiel V, Nagel K, Staufenberger T, Imhoff JF (2009) Diversity of antibiotic-active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. Mar Biotechnol 11:287–300

Wirth SE, Ayala-del-Río HL, Cole JA, Kohlerschmidt DJ, Musser KA, Sepúlveda-Torres LD, Thompson LM, Wolfgang WJ (2012) Psychrobacter sanguinis sp. nov. recovered from four clinical specimens over a 4-year period. Int J Syst Evol Microbiol 62:49–54. doi:10.1099/ijs.0.029058-0

Xuezheng L, Shuoshuo C, Guoying X, Shuai W, Ning D, Jihong S (2010) Cloning and heterologous expression of two cold-active lipases from the Antarctic bacterium *Psychrobacter* sp. G. Polar Res 29:421–429

Yumoto I, Hirota K, Sogabe Y, Nodasaka Y, Yokota Y, Hoshino T (2003) *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. Int J Syst Evol Microbiol 53:1985–1989

Zheng S, Ponder MA, Shih JYJ, Tiedje JM, Thomashow MF, Lubman DM (2007) A proteomic analysis of *Psychrobacter articus* 273-4 adaptation to low temperature and salinity using a 2-D liquid mapping approach. Electrophoresis 28:467–488