

Is Proteorhodopsin a General Light-driven Stress Adaptation System for Survival in Cold Environments?

By

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Shi Feng (60%) designed and conducted the experiment, analysed the data and wrote the manuscript. Shane Powell (10%), and John Bowman (25%) contributed to experimental design, analysed the data, and edited the manuscript. Richard Wilson (5%) offered 1D LC/MS proteomic service and interpretation of proteomic raw data.

Paper 2: Light stimulated growth of proteorhodopsin bearing sea-ice psychrophile *Psychroflexus torquis* is salinity-dependent

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Paper 3: Life in sea ice – proteomic insights into a proteorhodopsin-containing sea-ice dwelling flavobacteria

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List of Abbreviations

CCCP	Carbonylcyanide-3-chlorophenylhydrazine
CHASE	Cyclase/histidine kinase associated sensory extracellular
CRISPR	Clustered regularly interspaced short palindromic repeats
CRT	CRISPR recognition tool
EPS	Exopolysaccharides
EPA	Eicosapentaenoic acid
GAF	cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA domain
GIs	Genomic islands
HGT	Horizontal gene transfer
IVYWREL	Ile, Val, Tyr, Trp, Arg, Glu, Leu
MS	Mass spectra, mass spectrometer or mass spectrometry
NCBI	National Center for Biotechnology Information
ORFs	Open reading frames
<i>oriC</i>	Origin of replication
PAS	Per-Arnt-Sim domain
PEA	2-phenylethylamine
PR	Proteorhodopsin
PUFA	Polyunsaturated fatty acid
SIMCO	Sea-ice microbial communities

Abstract

This study aimed to achieve a better understanding of microbial adaptations in sea ice focusing on the physiological role of the light harvesting proton pump proteorhodopsin. To carry out these aims the research mainly focused on exploring the genome biology, physiology and life strategy of the model sea-ice bacterial species *Psychroflexus torquis*, an extremely psychrophilic member of the family *Flavobacteriaceae* (phylum *Bacteroidetes*). *P. torquis* has a bipolar distribution and is only known to occur in polar sea-ice and associated polar waters. It possesses proteorhodopsin and is believed to have a predominantly epiphytic lifestyle, mainly dwelling on sea-ice diatoms in sea-ice basal assemblages. This study extensively used gel-free label-free based proteomic approach to explore *P. torquis*' genome biology and unravel the role of proteorhodopsin in aiding the species adaptation to the extreme sea ice environment.

Sea ice has been estimated to have only become a stable feature on Earth in the last few million years ago thus it has been hypothesized that bacteria adapted to sea-ice acquired or exchange survival traits via horizontal gene transfer (HGT) between other sea ice dwelling microorganisms relatively recently. To examine the question whether sea-ice bacteria, such as *P. torquis* are endemic and display sea ice-ecosystem specialism a comparison of *P. torquis*' genome to its very closely related (99% 16S rRNA gene sequence similarity) sister species, *P. gondwanensis* ACAM 44^T, which is only known to dwell in Antarctic hypersaline lakes, was performed. This comparison

allowed for the determination of the level of HGT, what traits show evidence of HGT, what traits are relevant to the sea-ice ecosystem, and whether these genes are highly expressed, which would be indicative of their biological importance to *P. torquis*. The results show that in *P. torquis* ATCC 700755^T (genome size 4.3 Mbp) HGT has occurred much more extensively compared to *P. gondwanensis* (genome size 3.3 Mbp) and genetic features that can be linked as a sea ice specific adaptation are mainly concentrated on numerous genomic islands absent from *P. gondwanensis*. Genes encoding sea-ice ecosystem relevant traits, such as secreted exopolysaccharide, poly-unsaturated fatty acids, and ice binding proteins, form gene clusters on a number of these genomic islands. Proteomic analysis revealed that the encoded proteins for many sea-ice relevant traits are highly abundant under standard laboratory growth conditions. The genomic islands feature comparatively low gene density, a high concentration of pseudogenes, repetitive genetic elements, and addiction modules, indicative of large scale HGT either via phage or conjugation driven insertions. The overall results suggest the extensive level and nature of gene acquisition in *P. torquis* indicates its potential evolution to sea-ice ecosystem specialism. In that respect *P. torquis* seems to be an excellent model to study sea-ice functional biology. The initial screening of the *P. torquis* ATCC 700755^T genome revealed the presence of a proteorhodopsin gene. Previous studies have demonstrated proteorhodopsin-based phototrophy can enhance bacterial growth and survival during nutrient-stress conditions. But proteorhodopsins are widespread in natural environments and these environments may have other stress conditions for which proteorhodopsin can be

advantageous. So it can be hypothesized that proteorhodopsin may provide growth/survival advantage under stress conditions that are associated with a specific econiche. Growth studies on proteorhodopsin-containing *P. torquis* have demonstrated for the first time that light-stimulated growth can be linked to salinity stress rather than nutrient limitation. In addition, proteorhodopsin abundance and associated proton-pumping ability is also salinity dependent. The results extend the existing hypothesis that light can provide energy for marine prokaryotes through proteorhodopsin under stress conditions other than nutrient stress.

To gain a deeper insight into the physiological role of proteorhodopsin and the life strategy of *P. torquis*, a gel-free label-free quantitative proteomic approach was used. Proteome analysis revealed how *P. torquis* responded to different salinity and illumination levels by regulating its energy generation, nutrient uptake transporters, adhesion ability and gliding motility. The protein expression patterns of *P. torquis* indicates that it can use light to gain an advantage in colonizing phytoplanktonic surfaces, taking up more nutrients, and optimizing energy production. This study provided a comprehensive understanding of life style in sea ice and also partly revealed the physiological role of proteorhodopsin and its complex interrelationships.

Summary of Major Findings

Through this study, we examined the genome and the protein expression level of model sea-ice bacterial species *P. torquis* bacteria and revealed a high level of HGT seems to have driven the evolution process by which it became adapted to the adapt

dynamic sea-ice environment. We also examined the physiological role of PR within *P. torquis*, and extended the current hypothesis that PR provides light derived energy for marine prokaryotes under specific stress condition within econiches rather than only being linked to nutrient limitation. Finally, we assessed the proteome of *P. torquis* under different levels of salinity and illumination and achieved a better understanding in the proteorhodopsin-phototrophy, life strategies and physiological processes of this microorganism. Overall, we conclude that PR can be a general light-driven stress adaptation system for survival in cold environments. However, further studies are required on additional proteorhodopsin-containing strains to provide more evidence regarding this question.