6.6 Psychrophiles: Genetics, Genomics, Evolution

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Introduction

From the deepest depths of the ocean to the highest alpine peaks of the mountains, from the darkness of subterranean caves to the intense radiation of the upper atmosphere, and from the Northern to the Southern polar extremes, over two thirds of the Earth's biosphere is dominated by cold habitats. In these cold zones, psychrophilic microorganisms thrive, actively metabolizing at temperatures as low as –20°C, surviving at –45°C (Margesin and Schinner 1999; Feller and Gerday 2003; Cavicchioli 2006) and in the process driving critical global biogeochemical cycles. Yet, despite the fundamental role that these organisms play within the cold biosphere, relatively little is known about their identity, their physiology, how they have evolved, and the biogeochemical processes they perform.

The classic definition of the term, psychrophile, which derives from the Greek words ψυχρος (psukhros, cold) and φιλειν (philein, to love), is for an organism with an optimal growth temperature (T_{opt}) lower than 15°C. However, this definition has important limitations (see, e.g., Feller and Gerday 2003; Cavicchioli 2006). One of the problems with using T_{opt} as a means of defining a psychrophile is that T_{opt} is not known for most microorganisms present in naturally cold environments because, like most environmental microorganisms, the great bulk are not amenable to laboratory cultivation. Moreover, T_{opt} is dependent on growth media and other environmental parameters, making it difficult to extrapolate from laboratory studies back to a natural environmental setting.

While some issues of cultivatablity have been overcome by the use of cultivation-independent approaches (see section Future Prospects in this chapter), and provide information regarding the diversity, relative abundance, and physiology of psychrophilic microbial communities, it is not possible to infer T_{opt} of individual members from this type of data.

The classic definition of a psychrophile also fails to effectively consider the effects of temperature on the rates of enzymatic activity. In effect, cells will continue to grow more rapidly at increasingly higher temperatures until a critical process in the cell becomes sufficiently compromised. As such, many of the autochthonous bacterial and archaeal isolates that dominate permanently cold environments have their T_{opt} and upper temperature limit for growth (T_{max}) well beyond the environmental temperature they would naturally encounter. T_{opt} and $T_{max,}$ therefore, define the unnaturally high temperatures that psychrophiles can tolerate, thereby achieving fastest rates of growth and upper temperature limits tolerated for growth, respectively. Consistent with this view, analyses of molecular markers and physiological responses of psychrophiles have revealed that cells are often stressed when growing at temperatures around T_{opt} (Feller and Gerday 2003; Cavicchioli 2006). In other words, while psychrophiles can grow faster at temperatures above what they typically experience in the natural environment, in many cases they are growing under suboptimal conditions and experiencing heat stress.

An improved way of describing organisms isolated from cold environments is to use the terms "eurypsychrophile" and "stenopsychrophile"(Feller and Gerday 2003; Cavicchioli 2006), where eury and steno are derived from the Greek words ευρυς (broad) and στενος (narrow), respectively, and are terms that are widely used in the field of Ecology. Using these derivations, eurypsychrophiles are organisms that grow in low temperature environments but can tolerate a much wider temperature range extending beyond the 15°C limit imposed in the classical definition. Conversely, stenopsychrophiles have a restricted growth temperature range with a T_{opt} of $\leq$15°C. Good examples of eurypsychrophiles that are numerically abundant in their respective cold environments and thereby demonstrate ecological competitiveness are the

marine bacterium *Sphingopyxis alaskensis* and the Antarctic hypersaline-lake archaeon, *Halorubrum lacusprofundi* (Cavicchioli 2006).

While eury and steno help to classify psychrophiles based on their growth temperature properties, there remains a lack of knowledge defining the molecular properties that enable eurypsychrophiles to tolerate temperatures that stenopsychrophiles cannot (and conversely, the factors that limit the ability of stenopsychrophiles to grow at higher temperatures). In the coming years it will be useful to address these issues within similar classes of microorganisms (e.g., methanogens) with representative eurypsychrophiles (e.g., *Methanococcoides burtonii*) and stenopsychrophiles (e.g., *Methanogenium frigidum*), and between representatives of different ecotypes of eurypsychrophiles and stenopsychrophiles (e.g., sea-ice versus marine planktonic bacteria). Achieving this would expand our understanding of the molecular basis of cold adaptation and the evolutionary paths that have produced these two psychrophilic subclasses.

In addition to naturally cold habitats, some artificial environments support the growth of microorganisms in the cold. Refrigerated appliances and frozen products can harbor potential pathogens such as *Listeria* (Tasara and Stephan 2006). However, microorganisms isolated from artificially cold environments, such as human pathogens, tend to "prefer" hotter environments (e.g., the human body) where they compete effectively and cause disease. As such these types of microorganisms are not psychrophiles, but tolerate cold environments. It will be insightful to assess the molecular and physiological responses of these microorganisms to growth at low temperature, and compare their responses to psychrophiles from naturally cold environments.

This chapter focuses on advances that have been gained through genomic and functional genomic analyses of psychrophilic bacteria and archaea.

Genomes of Cold Adapted Bacteria and Archaea

Currently (in October 2009) the genomes of 28 psychrophilic bacteria have been completed, of which 14 have been published. Most of these isolates are from the Gammaproteobacteria (20 of 28 genomes), with half of all completed bacterial psychrophile genomes belonging to the Alteromonadales (14 genomes) (*Table 6.6.1*).

Sequencing of a further 21 genomes of psychrophilic bacteria is currently underway (*Table 6.6.2*). In addition, genome sequence data for four members of the Archaea is presently available (*Table 6.6.3*). Together, these 53 completed genomes cover six different phyla of the domains Bacteria and Archaea, but even these represent only a small fraction of the phylogenetic diversity existing in low temperature environments. A number of major findings arising from the published psychrophile genomes are detailed below.

Bacterial Genomes

Psychromonas ingrahamii 37 was isolated from Arctic sea ice and is capable of exponential growth at −12°C. The genome contains a single circular chromosome of 4.56 Mb (Riley et al. 2008). The G+C content is 40.1% and there are 3,742 protein coding genes. Ten RNA clusters and 86 tRNA genes are present, along with 81 genes with horizontal gene transfer potential such as transposases and integrases. *P. ingrahamii* has five sigma factors to moderate gene expression by RNA polymerase and 61 regulators of Cgdp, which control motility, adhesion

Table 6.6.1

Completed genome sequences of psychrophilic bacteria

Genome name	Genome publication	Origin of strain	Phylogenetic group	Size (Mb)	Genes
Flavobacterium psychrophilum JIP02/86	Duchaud et al. (2007)	Fish pathogen	Bacteroidetes, Flavobacteria, Flavobacteriales	2.86	2,505
Bacillus cereus cytotoxis NVH 391–98	None yet	Soil	Firmicutes, Bacilli, Bacillales	4.09	4,250
Bacillus weihenstephanensis KBAB4	None yet	Soil	Firmicutes, Bacilli, Bacillales	5.87	5,983
Exiguobacterium sibiricum 255–15	Rodrigues et al. (2008)	Permafrost sediment in Siberia	Firmicutes, Bacilli, Bacillales	3.04	3,151
Leuconostoc citreum KM20	Kim et al. (2008)	The Korean food kimchi	Firmicutes, Bacilli, Lactobacillales	1.90	1,902
Methylocella silvestris BL2	None yet	Acidic forest cambisol	Proteobacteria, Alphaproteobacteria, Rhizobiales	4.31	3,971
Rhodoferax ferrireducens T118	Risso et al. (2009)	Aquifer sediment	Proteobacteria, Betaproteobacteria, Burkholderiales	4.97	4,561
Desulfotalea psychrophila LSv54	Rabus et al. (2004)	Marine sediments off Svalbard	Proteobacteria, Deltaproteobacteria, Desulfobacterales	3.66	3,332
Aeromonas salmonicida salmonicida A449	Reith et al. (2008)	Pathogen of brown trout	Proteobacteria, Gammaproteobacteria, Aeromonadales	5.04	4,609
Colwellia psychrerythraea 34H	Methe et al. (2005)	Arctic marine sediments	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.37	5,066
Idiomarina loihiensis L2TR	Hou et al. (2004)	Marine hydrothermal vent	Proteobacteria, Gammaproteobacteria, Alteromonadales	2.84	2,706
Pseudoalteromonas haloplanktis TAC125	Medigue et al. (2005)	Antarctic coastal seawater	Proteobacteria, Gammaproteobacteria, Alteromonadales	3.85	3,634
Psychromonas ingrahamii 37	Riley et al. (2008)	Sea ice, Northern Alaska	Proteobacteria, Gammaproteobacteria, Alteromonadales	4.56	3,877
Saccharophagus degradans 2–40	Weiner et al. (2010)	Decaying salt marsh cord grass	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.06	4,114

Table 6.6.1 (Continued)

Genome name	Genome publication	Origin of strain	Phylogenetic group	Size (Mb)	Genes
Shewanella baltica OS185	None yet	Baltic Sea	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.31	4,618
Shewanella baltica OS223	None yet	Baltic Sea	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.36	4,622
Shewanella halifaxensis HAW-EB4	None yet	Atlantic Ocean	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.23	4,462
Shewanella loihica PV-4	None yet	Hydrothermal vent	Proteobacteria, Gammaproteobacteria, Alteromonadales	4.60	4,011
Shewanella pealeana ATCC 700345	None yet	Colonizing the squid *Loligo pealei*	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.17	4,434
Shewanella piezotolerans WP3	Wang et al. (2008)	sediment, marine deep sea	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.40	4,944
Shewanella sediminis HAW-EB3	None yet	Marine sediment	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.52	4,666
Shewanella violacea DSS12	None yet*	Deep sea mud	Proteobacteria, Gammaproteobacteria, Alteromonadales	4.90	
Shewanella woodyi ATCC 51908	None yet	Deep marine sediment	Proteobacteria, Gammaproteobacteria, Alteromonadales	5.94	5,085
Psychrobacter arcticus 273–4	None yet	Siberian permafrost, Russia	Proteobacteria, Gammaproteobacteria, Pseudomonadales	2.65	2,215
Psychrobacter cryohalolentis K5	None yet	Siberian permafrost, Russia	Proteobacteria, Gammaproteobacteria, Pseudomonadales	3.10	2,582
Psychrobacter sp. PRwf-1	None yet	From the snapper *Lutjanus vivanus*	Proteobacteria, Gammaproteobacteria, Pseudomonadales	3.00	2,481
Aliivibrio salmonicida LFI1238	Hjerde et al. (2008)	Pathogen of Atlantic cod *Gadus mortua*	Proteobacteria, Gammaproteobacteria, Vibrionales	4.66	4,075
Photobacterium profundum SS9	Vezzi et al. (2005)	2,500m depth from the Sulu Trough	Proteobacteria, Gammaproteobacteria, Vibrionales	6.40	5,754

*Possibly published in Japanese in the journal Idenshi Igaku in 2003.

Table 6.6.2

Current genome sequencing projects for psychrophilic bacteria

Genome name	Sequencing center	Status	Habitat/source	Phylogenetic group	Genes	Size (Mb)
Aequorivita antarctica SW49	DOE JGI, DSMZ	In progress	Marine	Bacteroidetes, Flavobacteria, Flavobacteriales		
Aequorivita sublithincola QSSC9–3	DOE JGI, DSMZ	In progress	Marine	Bacteroidetes, Flavobacteria, Flavobacteriales		
Agreia sp. PHSC20C1	Desert Research Institute, JCVI	Draft available	Surface water off the Western Antarctic Penninsula	Actinobacteria, Actinobacteria, Actinomycetales	2,718	2.77
Colwellia sp. MT41	JCVI	In progress	Fresh water, deep sea	Proteobacteria, Gammaproteobacteria, Alteromonadales		
Gillisia limnaea R-8282	DOE JGI, DSMZ	In progress	Microbial mats in Lake Fryxell, Antarctica	Bacteroidetes, Flavobacteria, Flavobacteriales		
Glaciecola sp. HTCC 2999	JCVI	Draft available	Fresh water	Proteobacteria, Gammaproteobacteria, Alteromonadales	2,290	2.52
Hymenobacter roseosalivarius AA-718	DOE JGI	In progress	Antarctic soil and sandstone	Bacteroidetes, Sphingobacteria, Sphingobacteriales		
Leuconostoc gasicomitatum LMG 18811	University of Helsinki, Finland	In progress	Modified-atmosphere packaged, tomato-marinated broiler meat strips	Firmicutes, Bacilli, Lactobacillales		
Leuconostoc kimchii IMSNU11154	European Consortium	In progress	Kimchi	Firmicutes, Bacilli, Lactobacillales		
Marinobacter sp. ELB17	JCVI, Princeton Univ	Draft available	Permanently ice-covered lake, Antarctica	Proteobacteria, Gammaproteobacteria, Alteromonadales	4,908	4.89

Methylocapsa acidiphila B2T	DOE JGI	In progress	Acidic Sphagnum peat bog	Proteobacteria, Alphaproteobacteria, Rhizobiales		
Mucilaginibacter paludis TPT56	DOE JGI	In progress	Rice paddies	Bacteroidetes, Sphingobacteria, Sphingobacteriales		
Octadecabacter antarcticus 307	ICBM, JCVI	In progress	McMurdo Sound, Antarctica	Proteobacteria, Alphaproteobacteria, Rhodobacterales		
Octadecabacter arcticus 238	JCVI	In progress	350 km offshore off Deadhorse, Alaska	Proteobacteria, Alphaproteobacteria, Rhodobacterales		
Oleispira antarctica RB-8	Max Planck Institute	In progress	Antarctic marine	Proteobacteria, Gammaproteobacteria, Oceanospirillales		
Photobacterium profundum 3TCK	JCVI, Scripps Institute of Oceanography	Draft available	San Diego Bay	Proteobacteria, Gammaproteobacteria, Vibrionales	5,728	6.11
Polaribacter filamentus	Integrated Genomics Inc.	In progress	Fresh water	Bacteroidetes, Flavobacteria, Flavobacteriales		
Polaribacter irgensii 23-P	Desert Research Institute, JCVI	Draft available	Surface waters, Antarctica	Bacteroidetes, Flavobacteria, Flavobacteriales	2,602	2.75
Psychroflexus torquis ATCC 700755	JCVI, Univ of Tasmania	Draft available	Sea-ice algal assemblage, Antarctica	Bacteroidetes, Flavobacteria, Flavobacteriales	6,835	6.01
Psychromonas sp. CNPT3	JCVI, Scripps Institute of Oceanography	Draft available	Central north Pacific Ocean at a depth of 5,800 m	Proteobacteria, Gammaproteobacteria, Alteromonadales	2,829	2.95
Rhodoferax antarcticus Ant.Br	Arizona State Uni	In progress	Microbial mat	Proteobacteria, Betaproteobacteria, Burkholderiales		

Draft genomes are available at http://img.jgi.doe.gov/cgi-bin/pub/main.cgi

Table 6.6.3

Genome sequences for psychrophilic archaea

Genome	Sequencing status	Genome publication	Native environment	Phylogenetic group	Size (Mb)	Genes
Cenarchaeum symbiosum A	Closed	Hallam et al. (2006)	Symbiont of marine sponge off Californian Coast	Crenarchaeota – Ammonia Oxidation Clade	2.05	2,066
Halorubrum lacusprofundi ATCC 49239	Closed	None yet	Deep Lake, Antarctica	Euryarchaeota – Halobacteria	3.69	3,725
Methanococcoides burtonii DSM 6242	Closed	Saunders et al. (2003); Allen et al. (2009)	Ace Lake, Antarctica	Euryarchaeota – Methanomicrobia	2.58	2,506
Methanogenium frigidum Ace-2	Draft	Saunders et al. (2003)	Ace Lake, Antarctica	Euryarchaeota – Methanomicrobia	2–2.5*	

*Estimated genome size based on draft genome.

factors, fimbriae, and biofilm formation. Sixteen glycosyltransferases are present, and it is postulated that extracellular polysaccharide production may help lower the freezing point in the vicinity of the cell and play an important role in cell survival at such low temperatures. Other genes involved in temperature adaptation include a large number encoding cold shock proteins (Csps) (12 genes), heat shock proteins (9 genes), and chaperones (13 genes) along with a polyunsaturase for fatty acid modification. Production and transport of the osmolyte betaine choline is genomically encoded, facilitating osmotic control when the sea ice freezes. Unexpectedly, the proteins of *P. ingrahamii* were more similar to those of *Vibrio cholerae* than to its closer relatives the Shewanellaceae or Collwelliaceae (*Table 6.6.1*).

Photobacterium profundum SS9 is a Gammaproteobacterium that was isolated at a depth of 2,500 m. The genome is composed of a 4.1 Mb major circular chromosome, a 2.2 Mb minor circular chromosome, and an 80 kb circular plasmid (Vezzi et al. 2005). All but one of the 15 rRNA operons are present on the major chromosome, and the rRNA gene copies exhibit high intragenomic variation (up to 5.13% for the 16S gene) possibly indicating that the various operons operate under distinct physiological conditions. There are a very high number of tRNA genes (164 genes). Transposon sequences were found at higher frequency on the minor chromosome, suggesting the major chromosome is more stable while the minor chromosome may function as more of a "genetic melting pot." *P. profundum* has been adopted as a model piezophile, and much research has focused on its adaptations to life at high pressure rather than at cold temperature; for example, it has been observed that proteins for degradation of polymers such as chitin and cellulose are expressed at depth, while the stress response proteins are upregulated at atmospheric pressure (*Table 6.6.4*).

Shewanella piezotolerans WP3 is an iron-reducing bacterium belonging to the Gammaproteobacteria. It was isolated from sediment at a depth of 1,914 m in the western Pacific Ocean

Table 6.6.4

Proteomic and transcriptomic studies assessing cold adaptation

Study	Organism	Growth temperature (°C)	Transcriptomics	Proteomics
Bakermans et al. (2007)	*P. cryohalolentis*	16, 4, −4		2-DE
Berger et al. (1996)	*A. globiformus*	25, 4		2-DE, immunoblotting
Bergholz et al. (2009)	*P. arcticus*	22, 17, 0, −6	Microarray	
Campanaro et al. (2010)	*M. burtonii*	23, 4	Microarray	
Gao et al. (2006)	*S. oneidensis*	30→15, 30→8	Microarray	
Goodchild et al. (2004a)	*M. burtonii*	23, 4		2-DE, MS/MS
Goodchild et al. (2004b)	*M. burtonii*	4		LC/LC, MS/MS
Goodchild et al. (2005)	*M. burtonii*	23, 4		ICAT, LC/LC, MS/MS
Kawamoto et al. (2007)	*S. livingstonensis*	18, 4	Quantitative RT-PCR	MALDI-TOF-MS
Qiu et al. (2006)	*E. sibiricum*	25, 4		MALDI-TOF-MS
Rodrigues et al. (2008)	*E. sibiricum*	39, 28, 10, −2.5	Microarray	
Saunders et al. (2005)	*M. burtonii*	4		LC/LC, MS/MS
Saunders et al. (2006)	*M. burtonii*	23, 4		LC/LC, MS/MS
Seo et al. (2004)	*B. psychrosaccharolyticus*	30, 30→15, 30→0		2-DE, MS
Ting et al. (2010)	*S. alaskensis*	30, 10		Metabolic labelling, LC/LC, MS/MS
Williams et al. (2010a)	*M. burtonii*	23, 4		iTRAQ, LC/LC, MS/MS
Williams et al. (2010b)	*M. burtonii*	23, 4		iTRAQ, LC/LC, MS/MS
Burg et al. (2010)	*M. burtonii*	23, 4		LC/LC, MS/MS
Zheng et al. (2007)	*P. arcticus*	22, 4		LC/LC, MALDI-MS

and is adapted to growth in the cold and to pressure. The genome is a single 5.4 Mb circular chromosome that encodes 4,944 protein coding genes (Wang et al. 2008). It contains eight rRNA operons and 89 tRNAs. The organism appears well adapted to life in the deep sea, with numerous genes and gene clusters facilitating cold adaptation present in the genome.

Structural RNA modification is an important mechanism for maintaining tRNA flexibility at low temperature, and the *S. piezotolerans* genome contains more pseudouridylate synthase genes than any other genome to date (7 genes). A wide variety of carbon and energy utilization pathways and osmolyte transport and synthesis systems are present in the genome, as are the genes required for synthesis of eicosapentaenoic acid (EPA). EPA and branched chain fatty acid content have been demonstrated to be upregulated at low temperature in this organism (Wang et al. 2009). The genome also contains gene clusters for production of polar and lateral flagella; the lateral flagella are upregulated by exposure to low temperature and are essential for motility at 4°C (Wang et al. 2008). A single filamentous phage is also present in the genome. The phage was demonstrated to be active, and at low temperatures a putative single stranded DNA binding protein and several other key phage genes were significantly upregulated (Wang et al. 2007).

Pseudoalteromonas haloplanktis TAC125 was isolated from Antarctic coastal seawater. It has two circular chromosomes of 3.2 Mb and 0.63 Mb, which encode 3,488 protein coding genes (Medigue et al. 2005). All nine rRNA operons and 106 tRNA genes are present on the larger chromosome. Seventy two percent of the tRNA genes are located on the leading strand and this organization is thought to facilitate the organism's rapid growth at low temperature. Several genes with a role in cold adaptation are clustered together in approximate repeats such as those coding for Csps, *cspA*-paralogs, and putative calcium binding proteins, which may be involved in exopolysaccharide (EPS) production. Nineteen genes coding for RNA binding proteins and RNA chaperones are present. In order to cope with the increased dioxygen solubility at low temperatures *P. haloplanktis* possesses 12 putative dioxygenases along with other proteins capable of scavenging chemical groups that are damaged by reactive oxygen species (ROS).

Although not yet published, the genome of *Psychrobacter arcticus* 273–4 is complete. This aerobic heterotrophic Gammaproteobacterium was isolated from a Siberian permafrost core and is capable of growth at −10°C (Bakermans et al. 2006). The genome is a single circular chromosome 2.6 Mb long, which encodes 2,215 proteins, 4 rRNA operons, and 49 tRNAs.

Desulfotalea psychrophila LSv54 is a sulfate-reducing Deltaproteobacterium. It was isolated from permanently cold Arctic sediments and is capable of growth at −1.8°C. It has one circular chromosome 3.5 Mb long and two plasmids 121 and 14 kb long (Rabus et al. 2004). The genome contains 3,234 protein coding genes, 7 rRNA operons, and 64 tRNA genes. The capacity to produce selenocysteine, the 21st amino acid, is genomically encoded and nine genes which require selenocysteine are present. Five complete IS elements and three partial IS element fragments were identified, along with four phage-related integrases. Nine homologs of known *csps* and a further nine putative *csp* geneswere identified.

All characterized members of the genus *Colwellia* are psychrophilic isolates obtained from stably cold marine environments. *Colwellia psychrerythraea* 34H, the type species, was isolated from Arctic marine sediment and produces maximum cell yield when grown at −1°C. Its single circular chromosome of 5.37 Mb encodes 4,937 protein coding genes, 9 rRNA operons, and 88 tRNAs (Methe et al. 2005). Two phage genomes are integrated into the genome. To retain membrane fluidity at low temperature, several copies of genes involved in fatty acid and phospholipid biosynthesis are present in the genome including a putative operon related to polyketide-like polyunsaturated fatty acid synthases. Transport and production of the compatible solute glycine betaine is genomically encoded, and this compound may perform both osmoprotection and cryoprotection roles in the cell. EPS and extracellular enzyme production is prominent, with many copies of glycosyltransferases (17 proteins) and degradative proteolytic enzymes with a signal peptide (49 proteins) in the genome. Protection from ROS

is provided by a variety of genes encoding antioxidants including three copies of catalase. An unusual Csp appears to localize to the membrane based on the presence of three transmembrane-spanning regions and a further four Csps are found in the cytoplasm.

The genomes of three pathogens from psychrophilic fish are available: *Flavobacterium psychrophilum* JIP02/86, *Aeromonas salmonicida* subsp. *salmonicida* A449, and *Aliivibrio salmonicida* LF11238. *F. psychrophilum* JIP02/86 has one circular chromosome 2.86 Mb long containing 2,432 protein coding genes, 6 rRNA operons, and 49 tRNA genes, and a single plasmid pCP1 (3.4 kb), which encodes four proteins (Duchaud et al. 2007). There are 74 IS elements in the genome. Many genes for the synthesis, export, modification, and polymerization of EPS are found in a 70 kb region of the genome. Genome features that reflect the organism's cold adaptation include lipid desaturases (three proteins), RNA helicases (six proteins, including three which are DEAD/DEAH box RNA helicases), a Csp, three chaperones and numerous enzymes with the capacity to counteract the effects of ROS (30 proteins). *A. salmonicida* subsp. *salmonicida* A449 has one circular chromosome 4.7 Mb long, two large plasmids (pAsa4, 166.7 kb; pAsa5, 155 kb), and three small plasmids (pAsa1, 5.4 kb; pAsa2, 5.2 kb; pAsa3, 5.6 kb) encoding a total of 4,437 protein coding genes (Reith et al. 2008). There are nine rRNA operons and 110 tRNA genes. A total of 170 pseudogenes and 88 insertion sequences are present, along with several large genome inversions when compared with closely related bacteria. *A. salmonicida* lF11238 has two circular chromosomes (3.3 and 1.2 Mb) and four plasmids (pVSAL840, 83.5 kb; pVSAL320, 30.8 kb; pVSAL54, 5.4 kb; pVSAL43, 4.3 kb) (Hjerde et al. 2008). There are a total of 4,286 protein coding genes, 12 rRNA operons, and 107 tRNA. The genome has undergone a high degree of gene rearrangement, deletion, and acquisition, as demonstrated by the presence of 370 pseudo-/partial genes, 521 transposases, and 288 IS elements. However, identifying any role genome plasticity may have in the organism's cold adaptation as distinct from adaptations related to pathogenicity is very difficult.

Idiomarina loihiensis is a eurypsychrophilic Gammaproteobacterium isolated from partially oxygenated cold waters at the periphery of a deep-sea vent, Hawaii. Its genome is 2.8 Mb and encodes 2,640 predicted proteins (Hou et al. 2004). There are four rRNA operons and 56 tRNAs. Genomic features that may contribute to cold adaptation in this organism include a cluster of 32 genes related to EPS and capsular polysaccharide synthesis, along with a further cluster for sialic acid biosynthesis and sialyation of surface polysaccharides. In particular, the sialic acid synthetase contains a C-terminal antifreeze domain important for maintenance of enzyme function at low temperatures. Versatile signal transduction machinery allows *I. loihiensis* to sense changes in dissolved oxygen and other environmental parameters in order to regulate EPS production. There are also several diverged copies of fatty acid biosynthesis enzymes, which are important for maintenance of cell membrane fluidity under changing temperature and pressure.

Saccharophagus degradans 2–40 is a eurypsychrophile with a single circular chromosome 5.06 Mb long encoding 4,008 protein coding genes (Weiner et al. 2010). For a Gammaproteobacterium it contains atypically few rRNA operons (two copies) and tRNA genes (41 genes). This is the first single organism (rather than a consortium) that has been demonstrated to degrade cellulosic algae and higher plant material, and the genome organization and composition reflects this unusual capacity. Genes for the degradation of more than 10 complex polysaccharides including cellulose, agar, alginate, and chitin are present, as are 15 megaproteins (>2,000 aa long) each containing domains and motifs reported to bind calcium and mediate protein–protein interactions. The genome is significantly enriched in regulators of

EPS production/degradation and biofilm formation. While all of these features contribute to the organism's success in the cold marine environment, elucidating specific cold adaptations is difficult. Five integron/phage integrases, two integrase psuedogenes, and three IS elements were detected in the genome.

Rhodoferax ferrireducens is a eurypsychrophilic facultative anaerobe belonging to the Betaproteobacteria that possesses the novel ability to convert sugars into electricity. It may play an important role in carbon and metal cycling in sediments and was isolated from subsurface sediment at a depth of 18 feet. The genome is comprised of a circular chromosome 4.7 Mb long (4,451 coding sequences) and a single plasmid 257 kb long (319 coding sequences) (Risso et al. 2009). There are two rRNA operons and 45 tRNA genes. Over 70% of genes on the plasmid are hypothetical. No genes coding for Csps have been identified in the genome even though other Betaproteobacteria such as *Nitrobacter* and *Ralstonia* spp. do contain these proteins. This suggests that *R. ferrireducens* may possess alternative mechanisms for surviving at cold temperatures.

Exiguobacterium sibiricum 255–15 was isolated from 3-million-year-old Siberian permafrost and is capable of growth from −5°C to 39°C. It has one circular chromosome 3 Mb long and two plasmids pEXIG01 (4.9 kb) and pEXIG02 (1.8 kb), which encode a total of 3,054 proteins (Rodrigues et al. 2008). There are nine rRNA operons and 69 tRNA genes. It has the capacity to produce and degrade EPSs, which may be important as cryoprotectants. A number of genes that may be involved in thermal adaptation were identified including translation factors (e.g., chaperones, DEAD-box RNA helicase), DNA replication genes (GyrA, GyrB), genes for maintenance of membrane fluidity (desaturase, beta-ketoacyl carrier protein), and sigma factors.

Leuconostoc citreum KM20 is a member of the Lactobacillales that was isolated from the traditional Korean fermented food kimchi. The genome contains one circular chromosome 1.8 Mb long and four circular plasmids (pLCK1, 38713 bp; pLCK2, 31463 bp; pLCK3, 17971 bp; and pLCK4, 12,183 bp) (Kim et al. 2008). The G+C content is 39.0%, and there are 1,820 protein coding genes in total. Four rRNA operons are present, along with 69 tRNA genes, 5 IS3 insertion sequences, and 5 derivatives of IS30 insertion elements. Research has focused on this organism's ability to suppress the growth of food pathogens such as *Bacillus cereus* and *Listeria monocytogenes* rather than its adaptation to the cold.

Archaeal Genomes

Cenarchaeum symbiosum is the sole archaeal symbiont of the marine sponge *Axinella mexicana* (Preston et al. 1996). Although uncultivated, a full genome sequence of *C. symbiosum* A was obtained through fosmid library construction (Hallam et al. 2006) (*Table 6.6.3*). The single circular chromosome 2.0 Mb long encodes 2,066 predicted ORFs, and contains one rRNA operon, and 45 tRNA genes. Although there is an abundance of *C. symbiosum*-related sequences in marine metagenome data (Hallam et al. 2006), indicating the environmental significance of these members of the Crenarchaeota, little work has focused on low temperature adaptation of *C. symbiosum*.

Methanococcoides burtonii DSM 6242 and *Methanogenium frigidum* Ace−2 are two methanogenic archaea that were isolated from Ace Lake in the Vestfold Hills, Antarctica. Draft genomes of these two organisms were published in 2003 (Saunders et al. 2003). *M. frigidum* has the lowest known T_{opt} of all the methanogens (15°C) (Franzmann et al. 1997; Cavicchioli 2006),

and its draft genome assembly was 1.6 Mb long (estimated total genome size 2–2.5 Mb). A total of 1,815 protein coding regions were identified, including a Csp and five proteins common to *M. frigidum* and *M. burtonii* but not identified in any other species. One of these five had highest structural similarity to a "winged helix" DNA binding domain protein, suggesting that transcriptional regulation may be an important aspect of these organisms' psychrophily. The bulk amino acid composition of proteins from *M. frigidum* and *M. burtonii* was distinct from that of mesophilic and thermophilic archaea, with a roughly linear trend in Gln, Thr, and Leu content over the range of optimal growth temperatures (Saunders et al. 2003).

The subsequent completion of the *M. burtonii* genome in 2009 (Allen et al. 2009) revealed that this organism uses highly skewed amino acid content to facilitate its psychrophilic lifestyle whilst retaining codon usage in common with its close mesophilic relatives. In addition, greater selective pressure was observed on genes that are predicted to be efficiently expressed (Allen et al. 2009). The completed *M. burtonii* genome is a single chromosome 2.57 Mp long encoding 2,494 genes. There are three rRNA operons, each containing two 5S, one 23S and one 16S rRNA gene, and a total of 53 tRNA genes including tRNA-pyl, which codes for pyrrolysine. The capacity for dihydrouridine incorporation into tRNAs is genomically encoded, allowing enhanced tRNA flexibility at cold temperatures. *M. burtonii*'s genome appears to be extremely dynamic with 67 transposons, five transposase fragments, seven transposase-disrupted proteins, and several duplicated cassettes in the genome. A large number of signal transduction proteins (45 genes) are present, providing considerable adaptive potential and allowing *M. burtonii* to sense and respond to its environment. A full pathway for synthesis of unsaturated isoprenoid lipids is present and interestingly is identical to that of *M. jannaschii*, a hyperthermophilic relative. Four operon-like clusters of polysaccharide biosynthesis genes (containing 10, 11, 16, and 39 genes) are present. Based on a comparative analysis of psychrophilic and non-psychrophilic archaeal genomes, the Defense Mechanism COG category of proteins was statistically overrepresented in *M. burtonii* compared to other methanogens or total archaea. These proteins included the restriction-modification systems, which may be required to combat high levels of foreign DNA in Ace Lake, and six putative novel ABC transporters.

In summary, the completed psychrophilic genomes reveal a number of common traits involved in cold adaptation and survival. These include:

- Genome arrangement and content, including genome recombination through the presence of transposons, insertion sequences, and phage
- Capacity to modulate and maintain membrane fluidity via lipid unsaturation
- EPS production
- Altered thermodynamics of proteins and enzymes through amino acid skew or addition of antifreeze domains
- Presence of Csps, chaperones, RNA helicases
- tRNA modifications (e.g., pseudouridine, dihydrouridine)
- Osmolyte production and transport systems
- Mechanisms to cope with ROS
- Sensitive signal transduction systems

While many of these features are common to several or more organisms, no two species share the exact same set of adaptations suggesting there is a wide variety of ways to reach the same goal of growth at low temperature. Undoubtedly, as more psychrophilic genomes are completed further cold-adaptation strategies will be uncovered. It will then be possible to begin

to link specific genomic traits (and hence mechanisms of adaptation) to ecologically relevant selection pressures (e.g., subsurface versus sea-ice versus planktonic habitat; aerobic versus anaerobic; salinity; nutrient flux; temperature stability).

Functional Genomics of Psychrophilic Bacteria and Archaea

Transcriptomics and proteomics provide global views of RNA and protein levels in the cell, respectively (*Table 6.6.4*). By measuring RNA and protein abundance, the functional genomic methods provide a combined measure of both gene expression or protein synthesis, and mRNA or protein stability/turnover. By comparing RNA or protein abundances from the same organism under at least two test conditions, changes in abundance can be measured and related to the specific test parameters. For example, by comparing the growth of a psychrophile at a relatively low versus high temperature, inferences can be made about mechanisms of growth temperature adaptation from the quantitative changes in gene product abundance. In addition to global gene expression studies, studies of cellular composition (e.g., intracellular solutes, membrane lipid composition) and targeted studies of specific genes and proteins, have provided important functional information about cold adaptation. These functional approaches have often been led by inferences made from genomic analyses.

Membrane and Cell Wall

Low temperatures reduce membrane fluidity and permeability, and microorganisms respond by producing less saturated fatty acids to improve membrane fluidity (Russell 2008). While many microorganisms respond by modifying the types and proportions of membrane lipids, this can occur in different ways. Fatty acid desaturases reduce saturation of preexisting fatty acids and a desaturase is upregulated at low temperature in *E. sibiricum* (Rodrigues et al. 2008). In contrast, the Antarctic methanogen *M. burtonii* does not encode a desaturase but alters its expression of several lipid biosynthesis genes resulting in less saturated isoprenoid lipid precursors (Nichols et al. 2004). A similar observation has been made for the archaeon *H. lacusprofundi* (Gibson et al. 2005). *P. arcticus* appears to upregulate a fatty acid desaturase, a fatty acid synthase and a phosphatidylethanolamine synthase, thereby modifying existing lipids and regulating de novo synthesis pathways (Zheng et al. 2007).

Other lipid modifications can also improve membrane fluidity. *S. oneidensis* upregulates two lipid biosynthesis proteins, so2088 and so3179, which synthesize Lipid A modified by palmitoleate acylation (Gao et al. 2006). It is also likely that isoleucine and valine degradation at low temperature produces intermediates, which increase the proportion of anteiso-methyl-branched fatty acids in the membrane of *S. oneidensis* (Gao et al. 2006). Membranes with a higher proportion of anteiso-methyl-branched fatty acids are more fluid than those with a higher proportion of iso-methyl-branched fatty acids (Russell 2008).

P. arcticus achieves changes to its cell wall composition at low temperature by downregulating transcription of peptidoglycan biosynthesis genes including murein disaccharides, transglycosylases, and peptidoglycan transpeptidases while upregulating genes for peptidoglycan breakdown. Transcription of one peptidoglycan crosslinking DD-peptidase isozyme (*dac2*) was upregulated at low temperature while another (*dac1*) was downregulated, and it was

speculated that the suppression of peptidoglycan crosslinking may function to maintain cell wall elasticity at low temperatures (Bergholz et al. 2009). *E. sibiricum* similarly strengthens its cell wall at −2 to 10°C, upregulating the *murADEI* peptidoglycan biosynthesis and *dupABD* lysine biosynthesis operons (Rodrigues et al. 2008). In *M. burtonii*, numerous surface layer proteins were found to be more abundant at 4°C, indicating an extensive remodeling of the cell envelope in response to low temperature (Williams et al. 2010a). These include a large number of putative S-layer proteins containing domains that point to roles in protein–protein or protein–carbohydrate interactions. It was speculated that deployment of cell surface proteins that promote intercellular interactions may facilitate nutrient exchange under challenging environmental conditions, and/or improve the stability of the cell membrane (Williams et al. 2010a).

Membrane transporters and other membrane-bound proteins are upregulated at low temperatures in several bacterial species and in *M. burtonii* (Table 6.6.5). This is likely to reflect, at least in part, a compensation for lower transporter efficiency at low temperature; in the cold, membrane transport and diffusion are impeded by reduced membrane permeability and lower thermodynamic efficiency (Kurihara and Esaki 2008; Russell 2008). However, in some species the upregulation of a transporter may be a way of increasing the import or export of a specific compound (e.g., cryoprotectant or accommodating a shift in metabolism).

Table 6.6.5

Membrane-bound proteins upregulated at low temperature

Species	Gene/protein
B. psychrosaccharolyticus	ATP-binding ABC transporter, ABC transporter-associated protein (Seo et al. 2004)
E. sibiricum	BetT (choline-glycine-betaine transporter), carnitine transporter (Rodrigues et al. 2008)
M. burtonii	Mbur_0060 (YVTN/NHL protein), Mbur_0314 (cadherin), Mbur_2003 (Ig-like domain protein), Mbur_0714 (MxaI/MoxI-like protein), Mbur_0728, and Mbur_0729 (proteins with dockerin and cohesin domains), other putative S-layer proteins (DUF1608, etc.), glycine betaine ABC transporter, GspE-3 (type II secretion system protein), probable TolB related transporter, probable potassium, sodium and cation transporters, SufB ABC transporter (Goodchild et al. 2004a; Goodchild et al. 2005; Burg et al. 2010; Williams et al. 2010a, b)
P. arcticus	Psyc_1070 (periplasmic subunit for ABC sulfate transporter), Psyc_2041 (sulfate uptake transporter), Psyc_2033 to Psyc_2035 (ABC zinc transporter) (Bergholz et al. 2009)
P. cryohalolentis	AfuA and FecA (ferric iron transporters), LolD (lipoprotein transporter), TolC (efflux protein), DctP (TRAP-T dicarboxylate transporter), Uup (ABC transporter) (Kurihara and Esaki 2008; Bakermans et al. 2007)
S. livingstonensis	OmpA, OmpC (probable outer membrane porins) (Kawamoto et al. 2007)
S. oneidensis	LolE (lipoprotein releasing system transmembrane protein), LolA (outer membrane lipoprotein carrier protein), LolD (lipoprotein trasporter), LolB (unspecified outer membrane lipoprotein) (Gao et al. 2006)

In *P. cryohalolentis*, the upregulation of a putative acetate transporter and acetate kinase at low temperature may increase acetate transport and processing to accommodate energy and carbon demands (Bakermans et al. 2007). Upregulated ferric iron transporters may also relieve oxidative stress and allow increased production of iron-dependent enzymes to counter decreased rates of enzyme activity (Bakermans et al. 2007). The upregulation of an ABC zinc transporter and downregulation of Fe^{2+} uptake transporters in *P. arcticus* also appears to be an adaptative strategy for oxidative stress (Bergholz et al. 2009).

In *E. sibiricum*, the upregulation of choline, glycine, betaine, and carnitine transporters at −2–10°C compared to 28°C is a possible osmoregulatory response triggered by water flow from the cell at low temperatures (Rodrigues et al. 2008). A similar type of response has been observed for a glycine betaine ABC transporter at 4°C in *M. burtonii* (Williams et al. 2009a).

DNA Modulating and Translational Proteins

At low temperatures, many microbial species upregulate DNA-modulating and translational proteins to compensate for reduced efficiency of transcription, translation, and DNA replication (*Tables 6.6.6* and *6.6.7*). The most prominent class of upregulated proteins from this category are ribosomal proteins, which is likely to reflect a need to compensate for reduced translational efficiency at low temperatures. RNA helicases also appear to be generally important for cold adaptation and are likely to facilitate the unwinding of secondary structures in nucleic acids. For example, a DEAD-box RNA helicase, *csdA* (Psyc_1082), is upregulated in *P. arcticus* and is important for low temperature growth and possesses a highly disordered C-terminal extension (Bergholz et al. 2009), similar to that observed in *M. burtonii* (Lim et al. 2000).

Csps are a family of nucleic acid binding proteins, which share a Cold Shock Domain (CSD), and are frequently associated with cold shock responses in bacteria (Gao et al. 2006). CspA is thought to moderate RNA secondary structure by chaperoning unwound RNA (Jiang et al. 1997), and may also act as a transcriptional inducer and antiterminator (Kurihara and Esaki 2008). Csps are upregulated in a broad range of bacteria including *P. cryohalolentis*, *P. arcticus*, *S. oneidensis*, *S. livingstonensis* and *Arthrobacter globiformus* following cold shock

Table 6.6.6

Ribosomal proteins upregulated at low temperature

Species	Ribosomal protein
B. psychrosaccharolyticus	S30P, L7/L12, L10, S6 (Seo et al. 2004)
E. sibiricum	L25, L7AE (Rodrigues et al. 2008)
M. burtonii	L37E, L24E, L22P, L24P, S4E, L5P, L21E, L3P, L23P, L19E, L30P, L15P, S13P, S4P, S11P, S19E, L15E, L7Ae, S7P, S12P, L1P, L10E, L12P, S8E, L18E, L13P, S9P (Goodchild et al. 2005; Burg et al. 2010; Williams et al. 2010a)
P. arcticus	S3, S4, S6, S15, L2, L7/L12, L15, L28 (Zheng et al. 2007)
P. cryohalolentis	S2, L25 (Bakermans et al. 2007)

Table 6.6.7

Non-ribosomal transcription and translation proteins upregulated at low temperature

Species	Gene/protein
A. globiformus	CspA, CspB-like protein (Berger et al. 1996)
B. psychrosaccharolyticus	HU-like DNA binding protein, putative elongation factor (Seo et al. 2004)
E. sibiricum	GyrAB (gyrase A/B), NusA (transcription terminator/antiterminator), RpoN (RNA polymerase sigma 70), numerous RNA helicases, IF-1 and IF-2 (transalation initiation factors), RbfA (ribosome-binding factor A), EF-Ts (elongation factor), CspABCD homologs (Qiu et al. 2006; Rodrigues et al. 2008)
M. burtonii	CheY-like DNA binding protein, TATA-box binding protein (TBP), three TRAM-domain proteins (Mbur_0304, Mbur_0604, Mbur_1445), DEAD-box RNA helicases (Mbur_1950, Mbur_0245), RNase J-like protein (Mbur_2398), aRadC (RecA family recombinase; Mbur_2095), RadA (DNA repair and recombinase) (Goodchild et al. 2004a; Burg et al. 2010; Williams et al. 2010a)
P. arcticus	gene 14 (tRNA synthetase), gene 1195 (EF-Tu elongation factor), DNA-directed RNA polymerase subunit, CspA homolog, rbfA (ribosomal binding factor), NusA and NusB (transcription terminator/antiterminators), IF-2 (translation initiation factor) (Zheng et al. 2007; Bergholz et al. 2009)
P. cryohalolentis	CspA, EF-Ts and EF-Tu (elongation factors), NusA (transcription terminator/antiterminator) (Bakermans et al. 2007)
S. livingstonensis	CspA, RpoA (RNA polymerase subunit), GreA (transcriptional regulator/elongation factor), TufB (elongation factor), Efp (translation elongation/initiation factor), lysU (tRNA synthase) (Kawamoto et al. 2007)
S. oneidensis	so1648 (CspA-like protein), TopB (topoisomerase), Rbn (ribonuclease), three HU-like DNA binding proteins, EF-Tu (elongation factor), yfiA-2 (ribosomal subunit interface protein), NusA (transcription terminator/antiterminator), IF-1 and IF-2 (translation initiation factors), TufB (elongation factor) (Gao et al. 2006)

and/or during low-temperature growth (Berger et al. 1996; Gao et al. 2006; Kawamoto et al. 2007; Bakermans et al. 2007; Bergholz et al. 2009). Enhanced Csp synthesis is not exclusively associated with cold shock/growth, and Csps appear to play diverse cellular roles. In *S. oneidensis*, only one of three Csps appears to play a particular role in low temperature growth (Gao et al. 2006). Only few archaea possess *csp* genes, and the function of a Csp protein from *M. frigidum* and a protein with a CSD-fold (but little sequence identity to Csp proteins) from *M. burtonii*, have been examined (Giaquinto et al. 2007). In addition, small proteins each composed of a single TRAM domain (unique to archaea) were found to be upregulated at low temperature in *M. burtonii*, and proposed to serve as RNA chaperones in an analogous manner to Csp proteins (Williams et al. 2010a).

Modification of nucleosides (e.g., methylation) can stabilize tRNA, and as a result, the extent of tRNA modification in archaea and bacteria tends to be much higher in

hyperthermophiles (Dalluge et al. 1997; Noon et al. 2003). On the other hand, dihydrouridine is a specific modified nucleoside that can enhance tRNA flexibility. Consistent with this, relative to hyperthermophilic archaea, tRNA in *M. burtonii* is characterized by an overall low extent of modification, but a high proportion of dihydrouridine per tRNA molecule (Noon et al. 2003).

In *S. oneidensis*, a topoisomerase (TopB), a ribonuclease (Rbn), and three DNA binding proteins (with identity to *Escherichia coli* HU family DNA binding proteins) are upregulated by the cold, and may function to minimize DNA and RNA secondary structure (Gao et al. 2006). A HU family DNA-binding protein is also upregulated at 4°C in *B. psychrosaccharolyticus* (Seo et al. 2004). In *M. burtonii* numerous proteins with relatively clear functional annotations and others with nucleic acid binding domains but with less confident predictions of cellular functions have been found to be upregulated at low temperature (Saunders et al. 2005; Williams et al. 2010a).

Global regulation studies have illustrated the complexity of molecular responses for some cellular processes, particularly those involving numerous individual gene products, such as the transcription and translation machinery. In *P. arcticus*, while proteins which promote tRNA-ribosome binding and translational accuracy (tRNA synthetase and EF-Tu) are upregulated at 4°C, a number of ribosomal proteins (S3, S4, S6, S15, L2, L7/L12, L15, and L28) are simultaneously downregulated (Zheng et al. 2007; Kurihara and Esaki 2008). This has been suggested to reflect a strategy of conserving energy by minimizing ribosomal count while simultaneously ensuring extant ribosomes are fully utilized. However, in a different *Psychrobacter* species, *P. cryohalolentis*, ribosomal proteins S2 and L25 and two elongation factors, EF-Ts and the EF-Tu-like TypA, were found to be upregulated at −4°C (Bakermans et al. 2007). The differences in response between the two *Psychrobacter* species may in part reflect the 8°C temperature difference used in the two studies.

In *M. burtonii*, proteins involved in translation initiation rather than elongation are upregulated at low temperature, and it has been speculated that this may be to ensure that the translation machinery is ready to process mRNA before inhibitory secondary structures form that would otherwise stall polypeptide synthesis (Williams et al. 2010a). At the transcriptional level, the basal transcription machinery is less influenced by temperature than a host of bacterial-like regulatory proteins, indicating that transcriptional regulation is mainly facilitated by these types of transcriptional regulators (Williams et al. 2010a). Consistent with this, a specific cold responsive mechanism of gene regulation that involves a long 5′-untranslated region has been identified as a feature of an RNA helicase gene in *M. burtonii* and a number of genes from several bacteria (Lim et al. 2000).

In *E. sibiricum*, GyrAB gyrase is upregulated at −2°C and may counteract increased DNA supercoiling that could occur at low temperature (Rodrigues et al. 2008). In *M. burtonii*, an archaeal RecA family recombinase (aRadC) was reported to be upregulated at 4°C and may function to rescue collapsed DNA replication forks as a consequence of increased duplex DNA stability at low temperature (Williams et al. 2010a). In contrast, PCNA (DNA sliding clamp protein) and XPB (catalyze ATP-dependent local DNA strand opening) are upregulated at 23°C (high temperature) and have been proposed to effect nucleotide excision repair in response to heat and oxidative stress that may occur at this elevated growth temperature (Williams et al. 2010a). Interestingly, transposases are not only highly represented in the genomes of some psychrophiles (e.g., *M. burtonii*; Allen et al. 2009), but appear to be expressed (Goodchild et al. 2004b), indicating that they are active and genome rearrangement may be occuring.

Chaperonins and Proteolysis

Protein misfolding is a major cellular challenge at both high and low temperature, and proteins involved in chaperoning, refolding and turnover of nascent, and mature proteins are involved in microbial adaptive responses (Table 6.6.8). Following cold shock, chaperonin *groES, groEL, dnaK, dnaJ, htpGd*, and *hslU* and protease *lon, aprE, so3942*, and *so4162* genes are upregulated in *S. oneidensis*. With the exception of *aprE*, all these genes are also upregulated by heat shock (Gao et al. 2006). Similarly, chaperonins Hsp10 and Hsp60 were upregulated at 4°C in *P. arcticus* (Zheng et al. 2007), and Hsp10 is upregulated in *B. psychrosaccharolyticus* at the same temperature (Seo et al. 2004). Hsp70 is upregulated at 4°C in *E. sibiricum* (Qiu et al. 2006). It is noteworthy that cold shock or heat shock conditions confer an inherently greater challenge to cells than steady-state growth, that is, stress caused by a sudden change often invokes a more pronounced and transient response until the cell adapts to growth (if possible) at the new temperature. As a result, molecular responses to these types of conditions should not be equated to an adaptive response to growth at relatively low or high growth temperatures.

Peptidyl-prolyl *cis-trans* isomerases (PPIases) have been reported to be upregulated at low temperature in *S. oneidensis, S. livingstonensis*, and *Shewanella* sp. SIB1 (Kurihara and Esaki 2008; Suzuki et al. 2004), *E. sibiricum* (Qiu et al. 2006), and *M. burtonii* (Goodchild et al. 2004a; Goodchild et al. 2005; Williams et al. 2010a). This observation in bacteria and archaea highlights the importance of the functions that the PPIases perform to optimize protein folding at low temperature, which includes their ability to isomerize proline imide bonds, and possibly refold proteins.

In *M. burtonii*, several chaperones are upregulated at 23°C (compared to 4°C) indicating that they are likely to play a more important role in rescuing protein function under conditions of high temperature stress (Goodchild et al. 2004b, 2005; Williams et al. 2010a; Burg et al. 2010). In contrast, an atypical J-domain (type III) protein, which may bind specific protein

Table 6.6.8

Chaperonins and proteolysis proteins upregulated at low temperature

Species	Gene/protein
B. psychrosaccharolyticus	HSP10 (heat shock chaperonin) (Seo et al. 2004)
E. sibiricum	Pnp (polyribonucleotide nucleotidyltransferase/RNase) (Rodrigues et al. 2008), HSP70, peptidyl-prolyl *cis-trans* isomerase (Qiu et al. 2006)
M. burtonii	Peptidyl-prolyl *cis-trans* isomerase (cyclophilin-type and FKBP-type), Mbur_1212 (possible DnaK recruiter) (Goodchild et al. 2004a; Goodchild et al. 2005; Burg et al. 2010; Williams et al. 2010a)
P. arcticus	HSP10, HSP60 (heat shock chaperonins) (Zheng et al. 2007), 5 RNases, 12 peptidases, ClpB homolog (chaperone) (Bergholz et al. 2009)
S. livingstonensis	Peptidyl-prolyl *cis-trans* isomerase (Kawamoto et al. 2007)
S. oneidensis	GroES, GroEL, DnaK, DnaJ, HtpGd and HslU (chaperones), Ion, AprE, so3942 and so4162 (proteases), tatA, tatB and tatC (Sec-independent translocases), peptidyl-prolyl *cis-trans* isomerase (Suzuki et al. 2004; Gao et al. 2006)

substrates and recruit these to DnaK was upregulated at 4°C and may therefore play a specific role as part of a low temperature chaperone system (Burg et al. 2010). In *Shewanella* sp. Ac10, DnaK has been reported to be upregulated at low temperature (Yoshimune et al. 2005).

In *P. arcticus*, 12 peptidases and 5 RNases were upregulated at low temperature, although two RNases were downregulated (Bergholz et al. 2009). RNases probably serve to maintain the turnover of biosynthesis precursors at low temperature, with some proteolysis enzymes possibly serving the same role. In *M. burtonii* the α-subunit of the proteasome and a number of secreted proteins that were predicted to have proteolytic function were upregulated at 4°C, indicating that degradation and subsequent recycling of proteins, and/or post-translational processing of secreted proteins may be important for cold adaptation of this archaeon (Williams et al. 2010a).

In *S. oneidensis*, Sec-encoding genes were either downregulated or unaffected following cold shock, while genes encoding Sec-independent translocases *tatA*, *tatB*, and *tatC* were upregulated more than 3.4-fold, indicating a shift from Sec-mediated to Sec-independent protein translocation (Gao et al. 2006). Conversely, in the archaeon *M. burtonii* a Tat system is not present and secretion appears to be primarily mediated by the Sec pathway (Saunders et al. 2006). The abundance of numerous secreted proteins in *M. burtonii* were found to be upregulated at 4°C, being either released on the external side of the membrane or anchored via a C-terminal membrane anchor (Williams et al. 2010a). The proteins are likely to form part of the protective glycoprotein and protein S-layer.

Metabolic Proteins

Metabolic responses to low temperature are dependent on the specific physiology of individual psychrophiles (*Table 6.6.9*). *M. burtonii* is a methylotrophic methanogen capable of growth with trimethylamine (TMA) and methanol as sole sources of carbon. The abundance of methanogenic and biosynthetic proteins has been found to be greatly affected by both substrate (TMA versus methanol) and growth temperature (4 versus 23°C) (Williams et al. 2010b). The strong influence of substrate on abundance of substrate-specific methanogenesis enzymes, and higher protein abundance at 23°C consistent with higher growth rate, indicates that these core metabolic enzymes do not play a central role in cold adaptation per se.

However, despite this overall metabolic response in *M. burtonii*, it has been noted that while the membrane-bound proton pump subunit $F_{420}H_2$ dehydrogenase is upregulated at 4°C, ATP synthesis and several oxidative methylotrophic genes are downregulated (Goodchild et al. 2004b, 2005; Kurihara and Esaki 2008). This has been interpreted as a simultaneous downshift in biosynthesis and a switch from a sodium to proton motive force, which is thermodynamically economical at low temperature. A similar downshift and switch, also characterized by decreased ATP synthesis, was proposed for *E. sibiricum*, to exploit higher oxygen solubility at low temperature and switch from substrate level to oxidative phosphorylation and a proton motive force (Rodrigues et al. 2008).

In *S. oneidensis*, a large number of energy metabolism genes are downregulated during cold shock to 8°C (Gao et al. 2006). However, a large pyruvate synthesis operon (so2486–so2489) and genes from pathways for other fermentative end products (formate, acetyl-CoA, lactate, and aceto-lactate) are upregulated. This has been interpreted as *S. oneidensis* preferentially utilizing fermentative end products upon cold shock, although it is not clear why this may be advantageous (Gao et al. 2006).

Table 6.6.9

Metabolic proteins differentially regulated at low temperature

Species	Upregulated	Downregulated
B. psychrosaccharolyticus	Five glycolytic and four other metabolic proteins (Seo et al. 2004)	
E. sibiricum	glpA (glycerol 3-phosphate dehydrogenase), glpKF (glycerol degradation), D-galactose catabolism genes, PTS glucose transport genes, Exig_1739 (alpha-amylase), PflD (pyruvate formate lyase), histidine, serine, aerginine, and lysine synthesis genes (Rodrigues et al. 2008)	Exig_2537 (alpha-amylase), numerous exopolysaccharide synthesis genes, ATPase synthase, cyoCBAE (cytochrome synthesis), PorA (pyruvate ferredoxin oxidoreductase alpha subunit) (Rodrigues et al. 2008)
M. burtonii	ketol-acid reductoisomerase, Mbur_0686 (possible RimK-like amino acid ligase), L-threonine O-3-phosphate decarboxylase, Mbur_1269 (6-pyrovoyl tetrahydrobiopterin synthase) (Burg et al. 2010; Williams et al. 2010a, b)	Methanogenesis (methyltransferases; oxidative and reductive methylotrophy proteins) and ATP synthesis proteins, pyrophosphate proton pump (HppA), acetyl-CoA decarbonylase/synthase complex and pyruvate synthase complex subunits, ThiC and Thi4 thiamine biosynthesis proteins, dihydrodipicolinate synthase, Mbur_2001 (2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate synthase) (Goodchild et al. 2004a; Goodchild et al. 2005; Burg et al. 2010; Williams et al. 2010a, b)
P. arcticus	TrpG, TrpD (tryptophan synthesis), Psyc_2024–2027 and Psyc_2028–2031 (ATP synthesis), relA (amino acid biosynthesis regulator), acs (acetyl co-A sythetase), Psyc_0728 (choline dehydrogenase) (Bergholz et al. 2009)	Branched-chain amino acid, arginine, and lysine biosynthesis genes, Atk (acetate kinase), Pta (phosphotransacetylase), Psyc_1301 (betatine-carnitine-choline type transporter), Psych_0729 (betaine aldehyde dehydrogenase), numerous TCA cycle and glycoxylate shunt genes, numerous amino acid synthesis genes excluding tryptophan synthesis, GcvH and GcvP (glycine cleavage), Psyc_0826 (betatine-carnitine-choline type transporter) (Bergholz et al. 2009)
S. oneidensis	Pyruvate synthesis operon so2486-so2489, pathways for other fermentative end products (formate, acetyl co-A, lactate and aceto-lactate) (Gao et al. 2006)	Several energy metabolism genes (Gao et al. 2006)

In *P. cryohalolentis*, two glyoxylate cycle enzymes (malate dehydrogenase *mdh* and isocitrate lyase *aceA*) and acetate kinase *ackA* were upregulated at −4°C (Bakermans et al. 2007). As the cells were grown with acetate as the sole carbon source, and an acetate membrane transport protein DctP was also upregulated (see *Table 6.6.5*), it was suggested the changes reflect an increase in cellular energy production rather than compensation for lower enzymatic activity at low temperature (Bakermans et al. 2007). It was also postulated that the glyoxylate cycle may also be upregulated to produce intermediate products needed in other stress response pathways (Bakermans et al. 2007). In *P. arcticus*, two genes from one acetate activation pathway (acetate kinase *atk* and phosphotransacetylase *pta*) were downregulated at low temperature while acetyl coenzyme A synthetase *acs* from an alternate pathway is upregulated. This implies a switch to the alternate pathway as a response to cold temperature (Bergholz et al. 2009). In *P. arcticus*, a large number of energy and metabolism genes including NADH dehydrogenase, ATP synthase, sodium-translocating NADH-ubiquinone oxidoreductase, tricarboxylic acid cycle, and glyoxylate shunt genes were also downregulated at low temperature, perhaps reflective of a low temperature regulated stringent response (Bergholz et al. 2009) (*Tables 6.6.9* and *6.6.10*).

In *E. sibiricum* the expression of two α-amylase genes was found to be inversely regulated by growth temperature, and it was proposed that this may reflect the thermal properties of the individual enzymes (Rodrigues et al. 2008).

Future Prospects

Functional genomic studies of a few psychrophiles indicate that growth temperature causes relatively few quantitative changes in gene expression; for example, based on transcriptome analysis, *E. sibiricum* gene expression is largely unchanged within the growth temperature range 4°C–28°C, with differential gene expression mostly occurring at growth temperature extremes

Table 6.6.10

Miscellaneous proteins upregulated at low temperature

Species	Gene/protein
E. sibiricum	Proline dehydrogenase, PspA (Qiu et al. 2006; Rodrigues et al. 2008)
M. burtonii	ParA partitioning protein, Mbur_2028 (exopolysaccharide synthesis), Mbur_0356 (chemotaxic protein), CheW (chemotaxic protein), Mbur_0104 and Mbur_0346–0347 (flagellins) (Goodchild et al. 2004a; Goodchild et al. 2005; Burg et al. 2010; Williams et al. 2010a, b)
P. arcticus	ahpC, hsp33, isc operon encoded by Psyc_1477–1482, Psyc_1043 and Psyc_1950 (peptide methionine sulfate reductases), peroxide-resistant aconitase A (Bergholz et al. 2009)
P. cryohalolentis	OsmC (organic hydorperoxide detoxifier), cheA (chemotaxic protein histidine kinase) (Bakermans et al. 2007)
S. livingstonensis	FlgE and FlgL (hook-related flagellum proteins), FtsZ (septum formation protein) (Kawamoto et al. 2007)
S. oneidensis	so0584, so1056, so3282 and so4053 (methyl-accepting chemotaxic proteins), so2125, so2318, so3202 (chemotaxic proteins) (Gao et al. 2006)

(i.e., at −2.5°C and 39°C) (Rodrigues et al. 2008). A similarly small number of changes (in this case in the proteome) were observed for *Desulfobacterium autotrophicum*; a marine sulfate-reducing bacterium described as being psychrotolerant (Rabus et al. 2002). In contrast, extensive changes in differential abundance have been observed for global levels of proteins and mRNA in a range of other psychrophiles (see section Functional Genomics of Psychrophilic Bacteria and Archaea above). To better understand the molecular mechanisms of adaptation in psychrophiles it will be valuable to extend functional genomic studies to a range of other psychrophiles that represent broader phylogenetic diversity and ecotypes. Performing studies using a range of relevant growth substrates (and varying growth temperature) will also help to identify genes that are core to a growth temperature response of an individual organism (e.g., Williams et al. 2010a, b). Similarly, determining quantitative changes in different subcellular fractions (e.g., cytosolic versus membrane versus secreted) and adopting robust statistical methods for evaluating quantitative changes in gene product abundance will help to clarify the role that specific proteins play in the cell (e.g., Williams et al. 2010a, b; Burg et al. 2010; Ting et al. 2009). Moreover, it will be useful to assess changes that occur in gene product abundance across a range of temperatures that span the growth temperature range of an individual psychrophile, rather than limiting quantitative assessments to a binary comparison of two growth temperatures. These types of studies will provide important insight into the capacity of psychrophiles to regulate their response to growth temperature and help to clarify core psychrophilic versus organism specific mechanisms of cold adaptation.

It has been well established that cultivation approaches typically recover only a small, skewed fraction of the total cells present in many environmental samples. Moreover, molecular ecological surveys of PCR amplified rRNA genes do not allow inferences about cell physiology or biological capacity. Random shotgun sequencing of DNA extracted from entire environmental samples (metagenomics) provides information about which types of microorganisms are present and what their functional capacities are likely to be. More than 30 microbial communities from diverse polar and permanently cold environments are currently at various stages of sequencing. Metagenome sequencing efforts include Antarctic lakes, polar ocean waters, permafrost, and glacial ice. These data sets are likely to reveal an entirely new level of understanding about psychrophilic microbial communities and the microbial processes the microorganisms are driving (Cavicchioli 2007; Murray and Grzymski 2007). The metagenome data will also greatly enhance metafunctional studies, for example, providing the DNA sequence baseline for protein identifications from mass spectrometry data when performing metaproteomics. To date, no metatranscriptomic or metaproteomic studies have been published for cold adapted microbial communities. However, this will change in the near future with studies, such as those from an Antarctic lake, which incorporate comprehensive genome coverage and a high level of proteomic coverage for unique psychrophilic microorganisms (R. Cavicchioli et al. unpublished data).

Integrating meta/genomics and meta/functional genomics with meteorological, geological, chemical, and physical data will produce a considerably more comprehensive understanding of how psychrophiles have evolved and how they have transformed and presently interact with permanently cold environments. In this regard, as an environmental parameter, low temperature has been shaping the genomes of psychrophilic microorganisms since life first emerged on Earth. In fact, based on the stability of macromolecules and a range of other pertinent factors, there has been speculation that life may have evolved in low temperature environments (Bada and Lazcano 2002; Price 2009). Therefore, understanding the adaptations that allow

psychrophilic microorganisms to successfully compete in their environment is not just an exercise in comparative genomics, physiology, and biochemistry but a quest for understanding the fundamentals of life.

Cross-References

- 6.1 Ecology of Psychrophiles: Subglacial and Permafrost Environments
- 6.2 Taxonomy of Psychrophiles
- 6.3 Diversity of Psychrophilic Bacteria from Sea Ice - and Glacial Ice Communities
- 6.4 Adaptation Mechanisms of Psychrotolerant Bacterial Pathogens
- 6.5 Ecological Distribution of Microorganisms in Terrestrial, Psychrophilic Habitats
- 6.7 Psychrophilic Enzymes: Cool Responses to Chilly Problems
- 9.1 Sub-seafloor Sediments: An Extreme but Globally Significant Prokaryotic Habitat (Taxonomy, Diversity, Ecology)
- 9.2 Physiology
- 9.3 Biochemistry

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