Chapter 15
Genomics of Psychrophilic Bacteria and Archaea

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Abstract Genomes are available for a wide range of psychrophilic bacteria and archaea. As of early 2017, approximately 130 cold-adapted species have genome sequences. Several studies complement this data with functional studies. In this review the cold adaptation traits of psychrophilic microorganisms are explored from a genome-centric point of view including surveys of traits across genomes. A broader view of psychrophiles in terms of growth rates amongst life on Earth explaining what a psychrophile represents is presented. Trait surveys, limited to the perspective of gene gain, reveal prevalence of genes demonstratively providing better growth at low temperature including compatible solute uptake and synthesis, antifreeze proteins and polyunsaturated fatty acids and investigate their functional relevance to psychrophily. This includes revealing prevalent antifreeze DUF3494-type proteins that occur in all domains of life but is limited to cold-adapted taxa and is absent in higher-temperature adapted life.

Contents

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15.1 Introduction

Psychrophilic organisms grow best at low temperature and fail to grow or grow slowly at temperatures significantly above room temperature (Morita 1975). This is distinct from organisms termed "psychrotolerant" (or psychroactive; Panikov and Sizova 2006) that typically grow from refrigeration temperatures (4–5 °C) to a temperature equivalent to that of the human body (37 °C). In most taxonomic studies, temperature growth ranges of bacteria and archaea are tested at these temperatures as they are well-known signposts. A few bacteria and even fewer archaea available in culture are bona fide extreme psychrophiles, unable to grow appreciably on agar at 20 °C or higher temperatures. This is not necessarily due to them being hard to grow, but rather environmental conditions do not select from them strongly, and consistently very cold ecosystems will contain many microorganisms that only have mild levels of cold adaptation and are fully capable of growing under standard laboratory temperature environments (22 °C to >30 °C). Given this, psychrophiles have an interesting place in biology and have representatives in all domains of life. Indeed, highly psychrophilic eukaryotic microorganisms are just as likely to be encountered in cold habitats since their inherently more complex structure and biology can be particularly fine-tuned to external temperatures (Mock and Junge 2007; Cvetkovska et al. 2017). Bacteria, on the other hand, have a greater degree of flexibility, and depending on the situation, fast growth rates are not generally selected for in ecosystems with sporadic resource distribution (Roller and Schmidt 2015); thus perfect alignment of growth optima to in situ temperatures of low temperature environments does not occur. For example, most bacteria that dwell in sea ice, which is typically −1 to −10 °C, have optimal growth at 10–25 °C (Bowman et al. 1997). In frozen permafrost, glacier, boreal forest and tundra soils, which can be lower than −10 °C, many bacteria and archaea are found to occur and still show metabolic activity at subzero temperatures (Steven et al. 2008; Drotz et al. 2010; Tuorto et al. 2013), suggesting many could be psychrophilic but usually have reasonably wide growth temperature ranges, for example, *Planococcus halocryophilus* (−15 °C to >30 °C; Mykytczuk et al. 2013) and *Psychrobacter arcticus* (< −5 °C to ~30 °C; Bergholz et al. 2009).

The ability of psychrophiles to grow is also influenced by the presence of other hurdles present in the given ecosystem, for example, pH, water activity and nutrient availability. In the example of sea ice, hurdles include nutrient availability, salinity (water activity), freezing (ice crystal formation and desiccation) and oxidative stress (O_2 supersaturation). Thus, a psychrophile often has a host of adaptations that suit its particular niche inclusive of cold adaptation traits. Typically extreme pH and saline ecosystems have few if any psychrophiles since the organisms would need to successfully develop adaptations to overcome the challenge of multiple hurdles, which have an additive effect on reducing growth rates (McMeekin et al. 2000). Temperature-dependent growth optima as the combination of the rate and biomass generation capability (growth yield) also should be considered in relation to psychrophilic growth. This is because the fastest growth rates are typically close

to the maximum temperatures for growth, and additional stressors reduce the growth rate limits. Cold adaptation traits overlap with traits also required for other forms of stress. For example, compatible solutes (osmolytes), low molecular weight compounds that can be concentrated to high concentrations in the cell cytoplasm (Roberts 2005), are generally beneficial for survival at low temperature as well as salty ecosystems as they stabilise proteins, DNA and cell membranes from temperature-induced entropy in general and help maintain osmotic pressure control. Psychrophilic microorganisms in general have to make trade-offs since the available biological innovation that has evolved on Earth to low temperature appears to have had limits. The innovations are of course coded in and stored in genomes; thus, the study of genomes provides a window into what the diversity and patterns of adaptations of psychrophiles possess within our current state of understanding of biology. This review investigates the state of knowledge of psychrophilic bacteria and archaea in terms of genome-level data. Using the available data, in which approximately 127 genomes are available (compared to only a dozen 10 years ago), the prevalence of cold adaptation traits is examined. The overall consensus of many studies on cold-adapted microorganisms indicates psychrophilic microorganisms achieve efficient growth aided by specific cold adaptation traits and through elimination of unnecessary and deleterious traits. Genomes open the way to study known traits, discover novel proteins and determine the associated biological processes they carry out in greater detail.

15.2 A Broadscale View of Growth Rates and Psychrophily

Crucial to understanding psychrophiles is the concept where they fit in terms of life on Earth. Psychrophiles are determined purely via temperature and growth. In this respect biokinetic temperature/growth rate distributions have notional maximums (T_{max}) and minimums (T_{min}) which are modelled values as they are points in which growth rate is zero (Ratkowsky et al. 1982, 1983). A point in the distribution, T_{opt}, indicates where growth rate is most rapid. The T_{opt} position is usually skewed to the left on the temperature x-axis, and the temperature region between T_{opt} and T_{max} is termed the supraoptimal temperature domain. This represents a range of temperatures and, though growth permissive, causes an organism to experience thermal stress that is compensated for by energy-dependent maintenance processes (Zakhartsev et al. 2015). Since energy for maintenance is finite and entropy from heat grows as the temperature increases, the supraoptimal domain features a rapid decline in growth rate usually within a window of 5–10 °C. At temperatures $>T_{max}$, the temperature is lethal resulting in permanent inactivation (cell death). It has been found where growth is otherwise not possible—due to some other limitation of the system (pH, salt level, lack of nutrients, radiation and more)—that temperature governs the inactivation rate (Ross et al. 2008; Zhang et al. 2010). As a result psychrophiles are constrained by a temperature barrier but are compensated by traits that give them fitness advantages in their particular cold habitats. Some

microorganisms by virtue of a range of stress tolerance traits can achieve high levels of ubiquity, for example, common bacteria of permafrost and Antarctic soils, *Psychrobacter* and *Exiguobacterium*, can be found worldwide in all climatic zones (Rodrigues et al. 2009) partly because the members have wide temperature ranges (<0 °C to ~35–40 °C).

On the opposite side of T_{opt}, growth rate declines as temperature declines. This response essentially follows Arrhenius kinetics (Arrhenius 1889) in that cell reactions are governed by temperature and that activation energies of enzymes within cells collectively operate over defined temperature ranges that set the organisms' temperature growth range. Psychrophiles have enzymes that have low activation energy requirements due to the greater flexibility of the structures (Feller 2013) that aids in formation of enzyme-substrate complexes. Cold temperatures can also cause proteostasis-related problems (Bednarska et al. 2013) for cells due to protein denaturation (Privalov 1990).

It was found quite some time ago that a square-root growth rate model (Ratkowsky et al. 1983) readily interprets temperature growth responses and is able to derive T_{min}, T_{max} and T_{opt} values. The Ratkowsky model forms a reliable albeit empirical interpretation of temperature/growth relationships (Heitzer et al. 1991). The exact same model can be applied to estimate temperature/growth relationships of communities (Pietikäinen et al. 2005). Attempting to bring a level of mechanistic understanding to this relationship is useful and relevant to understanding psychrophily since it provides a better working knowledge of how organisms operate in nature. The main approach is to increase the biological connectivity of models, such as the development of thermodynamic models that try to explain responses to temperature with a biochemical basis. Some recent examples include a soil-oriented model based on activation energy (Schipper et al. 2014); a second utilising the concept of 'exergy', the available energy in a cell for biochemical reactions that takes into account entropy or reduction of available energy (Desmond-Le Quéméner and Bouchez 2014); and a third on protein stability (Corkrey et al. 2012, 2014).

To explore patterns of growth rate temperature, a large number of data sets were compiled from as many sources in the scientific literature as possible to create what is referred to as the biokinetic spectrum of temperature (Corkrey et al. 2016) or the 'BKS'. This triangle-shaped relation peaks at a temperature of about 45 °C, corresponding to the rapid growth rate of *Clostridium perfringens*, and has two distinct descending curves towards increasingly colder and hotter temperatures. The BKS provides an interesting overview of temperature relationships for bacteria and archaea that makes up the bulk of the data used by Corkrey and colleagues. Cold-adapted bacteria occupy the right-hand corner of the BKS (Fig. 15.1). A close-up view shows the overlay of growth rate relationships but also shows that the data has a degree of limitation in that it is especially sparse below 0 °C (Fig. 15.1). Nevertheless, the protein stability thermodynamic model (Corkrey et al. 2014) was found to fit the BKS concept quite well though it only provides so far a partial understanding of the underpinning mechanistics of low- and high-temperature adaptation. The BKS, however, does provide a credible means of assessing the

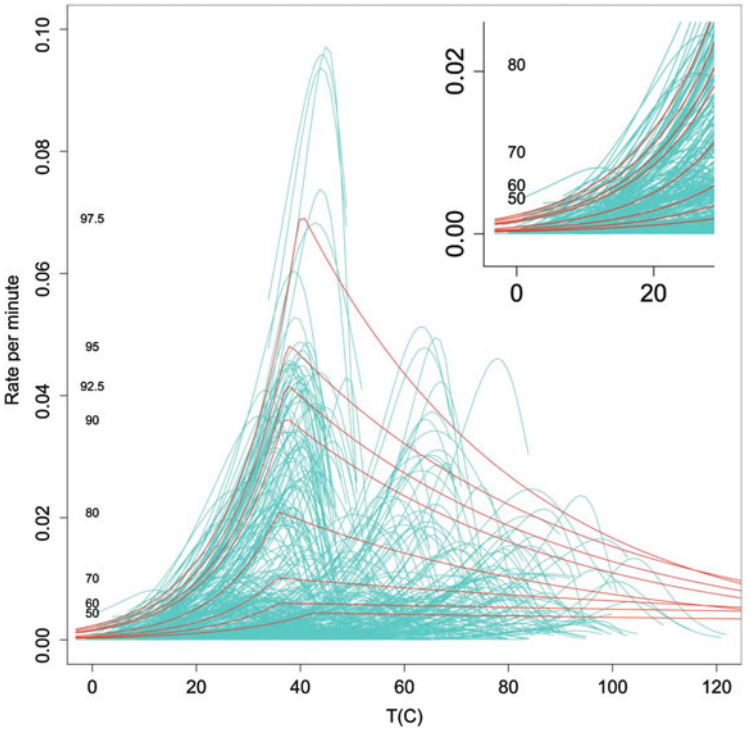

Fig. 15.1 Compilation of temperature versus growth rate *curves* forming the biokinetic spectrum of temperature (Corkrey et al. 2016). The insetted graph magnifying the *bottom right hand corner* of the BKS shows growth *curves* of psychrophilic bacteria and archaea and also shows data sparseness below 0 °C. The graph was adapted from Corkrey et al. (2017)

limits to life on Earth in terms of temperature as well as in terms of growth rate. Consequently, the BKS was used to predict maximum growth rates—essentially the largest possible rates for life on Earth across the entire temperature spectrum. The fastest rates are those performed by mesophiles and moderate thermophiles such as *Vibrio natriegens* and *E. coli* (T_{opt} 35–39 °C, generation time (GT) 10–20 min; Weinstock et al. 2016), *C. perfringens* (T_{opt} 42–46 °C, GT 10 min; Juneja et al. 2010) and *Thermobrachium celere* (T_{opt} 60 °C, GT 10–15 min; Engle et al. 1996). Cold-adapted bacteria obviously grow considerably more slowly, and at the temperature extremes of ≤ -5 °C, growth rates cannot be estimated easily due to freezing problems. Bacteria from permafrost and sea ice show growth down to −10 to −15 °C, including *Colwellia psychrerythraea* (Junge et al. 2003), *Psychrobacter* spp. (Bakemans et al. 2003; Bergholz et al. 2009), *Psychromonas ingrahamii* (Breezee et al. 2004) and *Planococcus halocryophilus* (Mykytczuk et al. 2013) with GT calculated at 10–50 days. *Planococcus halocryophilus*, which doubled roughly every 40–50 days at about −15 °C, is an exemplar of the type of microorganism that would be expected to compete well in frozen and other

cold soils. The maximum growth rate predicted at −20 °C by Corkrey et al. (2017) was estimated at 250 h (95% confidence limits and when pure water is the solute). This is compared to the smallest GT estimated at 6.2–8.5 min at 45–46 °C, a difference of 1800–2400-fold. These data are only inferred from what is currently available in the literature, which as mentioned above tends to be sparse at the extreme ends of the BKS. More data would be valuable in providing more accurate concepts of what is truly the limit to life in the cryosphere and how fast microorganisms grow under subzero conditions taking into account in situ factors of the environment.

15.3 Genome Availability Amongst Cold-Adapted Bacteria

Currently, as of early 2017, there are about 91,000 bacterial genomes and 2200 archaeal genomes. A general metadata survey of bacterial and archaeal genera from polar regions, deep sea and marine locations, alpine and glacial environments and other low temperature natural and artificial ecosystems (such as chilled food) was performed. In general the state of metadata for genomes on databases (such as NCBI and PATRIC) is patchy and often of limited value as they usually include scant data and even data that is misleading and inaccurate. As a result literature surveys were necessary to largely cover the available state of knowledge as of the end of 2016 on psychrophilic bacterial and archaeal genomes. Out of 257 bacterial and archaeal genera (1335 species) with sequence data surveyed that had some connection to low-temperature ecosystems, only 59 genera contain genome-sequenced strains that could be considered highly cold adapted. A further 137 genera include a proportion of species that are psychrotolerant, while the remaining genera examined had genome-sequenced species that were exclusively mesophilic. Of the 59 genera with cold-adapted bacteria, almost all also contain psychrotolerant and/or mesophilic sister species; 23 genera contained all three types and so are especially useful for temperature-oriented genome comparisons. A total of 16 genera had only psychrophilic species, but most of these only have a few extant species and are likely incomplete in terms of the coverage of diversity. Time will tell if mesophilic species relatives are found for these genera. These genera are also denoted in Table 15.1. It is notable that at the species level strains can vary in temperature growth range. For example, one strain of *Acetobacterium bakii* grew well at 30 °C, while two other strains stopped growing at 30 °C (Kotsyurbenko et al. 1995). Thus, the distinctions of what is psychrophilic, psychrotolerant and mesophilic are fairly arbitrary. Taken together organisms listed as psychrophilic grow well at low temperatures (~5–10 °C) while mesophiles by comparison will not grow or grow slowly and with reduced biomass levels.

From the data 137 genomes were found that belonged to psychrophilic bacteria ($n = 134$) and archaea ($n = 3$) where corroborating growth data is generally available. The phylogenetic relationship of these bacteria is given in Fig. 15.2. These included strains that grow optimally at 20 °C or less (Table 15.2). Most of the

Table 15.1 List of cold-adapted bacterial and archaeal strains with complete or draft genomes

Species	Strain no. (type strain[T])	Opt. growth temp. (pressure)	Source environment (s)	Accession code	Mbp	G+C mol %	16S rRNA copies, no. of tRNA
Actinobacteria							
Arthrobacter alpinus (3)[a]	R3.8	18	Alpine soil	CP012676-7[b]	4.046	62.2	6, 51
Cryobacterium arcticum	PAMC 27867	16	Permafrost soil	CP016282–4	4.351	68.4	3, 51
Cryobacterium flavum	CGMCC 1.11215	9	Glacier ice core	FNIB00000000	4.040	64.7	–, 45
Cryobacterium levicorallinum	GMCC 1.11211	8	Glacier ice core	FOPW00000000	3.754	64.4	–, 45
Cryobacterium luteum	CGMCC 1.11210	10	Glacier ice core	FOCN00000000	3.834	65.1	–, 47
Cryobacterium psychrotolerans	CGMCC 1.5382	15	Glacial soil	FNFU00000000	3.247	68.3	–, 45
Cryobacterium roopkundense	RuG17	8	Glacial soil	JPXF00000000	4.356	65.3	–, 47
Demequina lutea	NBRC 106155	18	Permafrost soil	BBRC01000000	2.643	65.9	–, 43
Glaciibacter superstes	DSM 21135	15	Permafrost ice wedge	ATWH00000000	4.801	64.4	–, 50
Leifsonia rubra	CMS 76R	15	Polar microbial mat	ATIA00000000	2.671	59.2	–, 45
Tomitella biformata	AHU 1821	17	Permafrost ice wedge	BAVQ00000000	4.688	68.1	–, 50
Firmicutes							
Bacillus cecembensis	DSM 21993	18	Glacial soil	LMBZ00000000	4.782	36.9	–, 49
Bacillus psychrosaccharolyticus	ATCC 23296	18	Polar soil	AJTN00000000	4.590	38.8	–, 85
Brochothrix campestris	FSL F6-1037_c9	18	Chilled meat	AODH00000000	2.372	40.2	–, 73
Lactobacillus algidus	DSM 15638	18	Chilled meat	AZDI00000000	1.590	36.0	–, 46
Paenibacillus antarcticus	CECT 5836	19	Lake sediment	LVJI00000000	5.372	40.5	–, 107

(continued)

Table 15.1 (continued)

Species	Strain no. (type strain^T)	Opt. growth temp. (pressure)	Source environment (s)	Accession code	Mbp	G+C mol %	16S rRNA copies, no. of tRNA
Paenibacillus glacialis	DSM 22343	18	Glacial soil	LVJH00000000	5.709	40.7	–, 108
Paenibacillus macquariensis (2)	DSM 2	18	Polar soils	LVJF00000000	6.274	40.7	–, 100
Planococcus antarcticus	DSM 14505	18	Polar microbial mat	CP016534	3.788	43.2	9, 72
Planococcus faecalis	CECT 8759	18	Polar (ornithogenic) soil	MBMU00000000	3.534	40.8	–, 95
Planococcus halocryophilus	Or1	18	Permafrost soil	CP016537	3.425	40.0	10, 72
Planomicrobium glaciei	CGMCC 1.6846	18	Glacial soil	FNDC00000000	3.917	46.7	–, 55
Carnobacterium alterfunditum	DSM 5972	16	Polar anoxic saline lake	JQLG00000000	2.616	36.0	–, 75
Carnobacterium funditum	DSM 5970	16	Polar anoxic saline lake	JQLL00000000	2.352	34.6	–, 73
Carnobacterium iners	DSM 28070	16	Polar pond sediment	FOAH00000000	2.661	34.0	–,53
Carnobacterium pleistocenium	FTR1	16	Permafrost ice	JQLQ00000000	2.376	35.4	–, 74
Acetobacterium bakii	DSM 8239	18	Freshwater sediment	LGYO00000000	4.135	41.2	–, 47
Bacteroidetes							
Aequorivita sublithincola	DSM 14238	18	Polar sublithic biofilm	CP003280	3.520	36.2	–, 36
Bizionia algoritergicola	APA-3	16	Polar saline lake	LZRO00000000	3.528	34.1	–, 35
Bizionia argentinensis	JUB59	18	Polar seawater	AFXZ00000000	3.279	33.8	–, 36
Bizionia psychrotolerans	PB-M7	18	Marine fauna	JNGS00000000			
Cellulophaga algicola	DSM 14237	18	Sea ice	CP002453	4.888	33.8	5, 44
Chryseobacterium antarcticum	LMG 24720	16	Polar soil and moss	JPEP00000000	3.123	36.1	–, 37

Chryseobacterium frigidisoli	DSM 26000	13	Glacial forefield soil	FOQT00000000	3.021	33.3	–, 35
Crocinitomix catalasitica	ATCC 23190	18	Polar marine sand	JHXV00000000	4.623	34.1	–, 41
Flavobacterium antarcticum	DSM 19726	16	Polar soil	ATTM00000000	3.068	34.9	
Flavobacterium branchiophilum	FL-15	18	Cold freshwater fish	FQ859182–3	3.559	32.9	3, 43
Flavobacterium degerlachei	DSM 15718	18	Polar microbial mat	FNMV00000000	3.856	34.0	–, 43
Flavobacterium frigidarium	DSM 17623	14	Polar marine sediment	AUDO00000000	3.624	34.1	–, 46
Flavobacterium frigoris	DSM 15719	15	Polar microbial mat	FOFZ00000000	4.045	34.2	–, 47
Flavobacterium fryxellicola	DSM 16209	16	Polar microbial mat	LVJE00000000	3.721	34.6	–, 42
Flavobacterium gelidilacus	DSM 15343	16	Polar microbial mat	AUGN00000000	3.428	30.0	–, 37
Flavobacterium gillisiae	DSM 22376	17	Sea ice	FNRD00000000	4.377	34.3	–, 46
Flavobacterium hibernum	DSM 12611	19	Polar freshwater lake	JPRK00000000	5.283	33.2	–, 57
Flavobacterium micromati	DSM 17659	18	Polar microbial mat	FQWF00000000	3.692	33.1	–, 42
Flavobacterium noncentrifugens	CGMCC 1.10076	17	Glacial meltwater	FNEZ00000000	4.032	40.7	–, 41
Flavobacterium omnivorum	CGMCC 1.2747	8	Glacial soil	FNDB00000000	3.808	34.3	–, 43
Flavobacterium rivuli	WB3.3–2	18	Cold mineral spring	ARKJ00000000	4.484	39.6	–, 47
Flavobacterium psychrophilum (29)	DSM 3660	15	Cold freshwater fish	FMVE00000000	2.626	32.3	6, 49
Flavobacterium sinopsychrotolerans	CGMCC 1.8704	16	Glacial soil	FODN00000000	3.520	34.1	–, 44
Flavobacterium sp.	ACAM123	10	Polar saline lagoon	AJXL00000000	3.930	34.8	–, 42

(continued)

Table 15.1 (continued)

Species	Strain no. (type strain[T])	Opt. growth temp. (pressure)	Source environment (s)	Accession code	Mbp	G+C mol %	16S rRNA copies, no. of tRNA
Flavobacterium tegetincola	DSM 22377	17	Polar microbial mat	AUDN00000000	3.759	35.0	–, 39
Flavobacterium xanthum	DSM 3661	17	Polar microbial mat	FRBU00000000	3.755	34.4	–, 44
Flavobacterium xueshanense	CGMCC 1.9227	11	Glacier ice core	FONQ00000000	3.471	34.2	–, 41
Gelidibacter algens	ACAM 536	18	Sea ice, polar saline lakes, sublithic biofilms	LZRN00000000	4.506	37.3	–, 38
Gillisia limnaea	DSM 15749	18	Polar microbial mat	AHKR00000000	3.959	37.6	–, 44
Lacinutrix algicola	AKS293	18	Marine alga	LIQH01000000	3.661	31.4	–, 36
Lacinutrix himadriensis	E4-9a	13	Polar marine sediment	LIQI01000000	4.166	32.6	–, 35
Lacinutrix jangbogonensis	PAMC 27137	10	Polar marine sediment	JSWF00000000	4.017	32.2	–, 37
Lacinutrix mariniflava	AKS432	15	Marine alga	LIQG01000000	3.973	31.8	–, 37
Maribacter antarcticus	DSM 21422	10	Polar marine alga	JHZC00000000	4.853	37.4	–, 38
Polaribacter atrinae	KACC 17473	18	Marine mollusc	LVWE00000000	3.942	30.4	–, 42
Polaribacter franzmannii	ATCC 700399	6	Sea ice	ARJD00000000		32.5	
Polaribacter irgensii	23-P	6	Sea ice	AAOG00000000	2.763	34.5	–, 37
Pricia antarctica	DSM 23421	16	Polar marine sediment	FNAO00000000	4.851	43.8	–, 37
Psychroflexus gondwanensis	ACAM 44	18	Polar hypersaline lake	APLF00000000	3.306	35.8	–, 38
Psychroflexus torquis	ATCC700755	8	Sea ice	AAPR00000000	4.321	34.5	3, 37
Psychroserpens burtonensis	DSM 12212	8	Polar saline lagoon	AUDE00000000	3.961	33.4	–, 35
Psychroserpens jangbogonensis	PAMC 27130	12	Polar marine sediment	JSWG00000000	3.633	32.7	–, 34

Rhodonellum psychrophilum	GCM71	12	Fjord tufa columns	AWXR00000000	5.120	41.8	–, 37
Winogradskyella psychrotolerans	RS-3	18	Polar marine sediment	ATMR00000000	4.279	33.5	–, 40
Arcticibacter eurypsychrophilus	MJ9–5	15	Glacier ice core	MDFN00000000	4.867	38.5	–, 37
Arcticibacter svalbardensis	MN12–7	16	Polar soil	AQPN00000000	4.681	38.2	–, 39
Pedobacter arcticus	A12	15	Polar soil	AKZJ00000000	4.221	36.9	–, 39
Pedobacter hartonius	DSM 19033	18	Cold mineral spring	FNRA00000000	5.188	43.0	–, 51
Hymenobacter psychrophilus	CGMCC 1.8975	15	Alpine soil	FNOV00000000	4.758	60.8	–, 44
Alphaproteobacteria							
Devosia psychrophila	CGMCC 1.10210	13	Glacier cryoconite	FOMB00000000	4.328	61.2	–, 46
Erythrobacter sp.	QSSC1–22B	15	Polar sublithic biofilm	LZRP00000000	3.344	63.5	1, 46
Phaeobacter arcticus	DSM 23566	15	Polar marine sediment	AXBF00000000	5.049	59.3	–, 58
Octadecabacter antarcticus	307	10	Sea ice	CP003740–1	4.875	54.6	2, 42
Octadecabacter arcticus	238	10	Sea ice	CP003742–44	5.478	55.2	2, 42
Octadecabacter temperatus	SB1	17	Seawater	CP012160–1	3.264	54.7	1, 38
Robiginitomaculum antarcticum	DSM 21748	17	Polar seawater	AQOY00000000	2.768	52.5	–, 36
Deinococcus-Thermus							
Deinococcus frigens	DSM 12807	15	Polar endolith rocks	JNIW00000000	4.031	65.4	–, 52
Deinococcus marmoris	DSM 12784	15	Polar endolith rocks	JNIV00000000	4.800	64.4	–, 54

(continued)

Table 15.1 (continued)

Species	Strain no. (type strain[T])	Opt. growth temp. (pressure)	Source environment (s)	Accession code	Mbp	G+C mol %	16S rRNA copies, no. of tRNA
Betaproteobacteria							
Polaromonas glacialis	R3–9	18	Glacier cryoconite	JMDZ00000000	5.284	62.3	–, 53
Polaromonas naphthalenivorans	CJ2	18	Cold freshwater stream	CP000529–37	5.366	61.7	2, 51
Simplicispira psychrophila	DSM 11588	17	Polar moss	JHYS00000000	3.600	61.5	–, 50
Gammaproteobacteria							
Aliivibrio logei	ATCC 35077	18	Cold-water marine fish	ASAH00000000	5.288	38.9	–, 70
Aliivibrio salmonicida	LFI1238	10	Cold-water marine fish	FM178379–84	4.531	39.0	12, 91
Aliivibrio wodanis	AWOD1	15	Cold-water marine fish	LN554846–51	4.518	38.2	7, 87
Colwellia chukchiensis	CGMCC 1.9127	18	Polar seawater	FOBI00000000	4.023	41.9	–, 55
Colwellia hornerae	PAMC 20917	12	Sea ice	CP014944	4.683	37.9	6, 73
Colwellia piezophila	ATCC BAA-637	5 (60 MPa)	Deep sea fauna	ARKQ00000000	5.475	38.9	–, 67
Colwellia psychrerythraea (3)	34H	8	Marine fauna, sea ice	CP000083	5.373	38.0	9, 88
Dasania marina	DSM 21967	18	Polar marine sediment	ARDZ00000000	4.101	47.4	–, 45
Glaciecola pallidula	ACAM 615	13	Sea ice	BAEQ00000000	4.355	41.2	–, 46
Glaciecola punicea (2)	ACAM 611	14	Sea ice	BAET00000000	3.072	43.1	–, 45
Idiomarina abyssalis	KMM 227	18	Deep seawater	LGOW01000000	2.684	47.2	–, 55
Idiomarina zobellii	KMM 231	18	Deep seawater	LHSG01000000	2.618	47.1	–, 52
Marinobacter psychrophilus	20,041	15	Sea ice	CP011494	3.998	53.8	3, 49
Marinomonas ushuaiensis	DSM 15871	18	Subpolar seawater	JAMB00000000	3.332	41.1	–, 58
Moritella dasanensis	ArB 0140	10	Polar seawater	AKXQ00000000	4.882	40.8	–, 90
Moritella marina	ATCC 15381	12	Marine sediment	ALOE00000000	4.624	40.5	–, 48
Moritella viscosa		16	Cold-water fish	LN554852–4	5.093	39.4	11, 131

Neptunomonas antarctica	S3–22	15	Polar marine sediment	LJSI01000000	4.568	45.7	–, 60
Neptunomonas japonica	DSM 18939	17	Whale carcass	AUGR00000000	4.389	43.7	–, 54
Oleispira antarctica	RB-8	15	Polar seawater	FO203512	4.406	42.2	5, 50
Paraglaciecola arctica	BSs20135	16	Polar marine sediment	BAEO00000000	5.961	40.1	–, 50
Paraglaciecola chathamensis	S18 K6	18	Marine sediment	BAEM00000000	5.255	44.1	–, 49
Paraglaciecola polaris	LMG 21857	18	Sea ice, polar seawater	BAER00000000	5.225	44.1	–, 48
Paraglaciecola psychrophila	170	10	Sea ice	CP003837	5.374	40.4	6, 59
Photobacterium profundum	SS9	10 (20 MPa)	Deep sea samples	CR354531	6.403	41.7	16,169
Pseudoalteromonas arctica	A 37–1-2	16	Sea ice, polar seawater	AHBY00000000	4.621	39.0	–, 94
Pseudoalteromonas denitrificans	DSM 6059	12		FOLO00000000	6.094	34.7	–, 72
Psychrobacter arcticus	273–4	16	Permafrost	CP000082	2.650	42.8	4, 49
Psychrobacter cryohalolentis		18	Permafrost	CP000323	3.101	42.2	4, 48
Psychrobacter glacincola	BNF20	10	Sea ice	LIQB01000000	3.490	42.8	–, 47
Psychrobacter urativorans	R310.10B	16	Polar soil	CP012678	2.931	42.1	6, 52
Psychromonas arctica	DSM 14288	17	Sea ice, polar seawater	AXWP00000000	4.719	37.8	–, 65
Psychromonas hadalis	ATCC BAA-638	6 (60 MPa)	Deep-sea sediment	ATUO00000000	3.977	39.1	–, 42
Psychromonas ingrahamii	37	8	Sea ice	CP000510	4.559	40.1	10, 86
Psychromonas ossibalaenae	ATCC BAA-1528	18	Whale carcass	ARML00000000	5.200	41.9	–, 57
Shewanella benthica	KT99	7 (60 MPa)	Deep-sea samples	ABIC00000000	4.320	46.0	13, 86
Shewanella frigidimarina	NCBM4000	16	Sea ice, seawater	CP000447	4.845	41.6	9, 96
Shewanella halifaxensis	HAW-EB4	16	Deep-sea sediment	CP000931	5.226	44.6	10, 125

(continued)

Table 15.1 (continued)

Species	Strain no. (type strain[T])	Opt. growth temp. (pressure)	Source environment (s)	Accession code	Mbp	G+C mol %	16S rRNA copies, no. of tRNA
Shewanella piezotolerans	WP3	16 (20 MPa)	Deep-sea sediment	CP000472	5.396	43.3	8, 89
Shewanella sediminis	HAW-EB3	16	Deep-sea sediment	CP000821	5.517	46.1	12, 125
Shewanella violacea	DSS12	7 (60 MPa)	Deep-sea samples	AP011177	4.962	44.7	14, 126
Shewanella woodyi	ATCC 51908	16	Seawater	CP000961	5.935	43.7	10, 126
Thiomicrospira arctica	DSM 13458	11	Polar marine sediment	ARLF00000000	2.551	41.9	–, 45
Deltaproteobacteria							
Desulfotalea psychrophila	LsV54	7	Polar marine sediment	CR522870–2	3.523	46.6	7, 65
Desulfovibrio ferrireducens	DSM 16995	18	Polar marine sediment	FNGA00000000	3.872	42.8	–, 57
Desulfovibrio frigidus	DSM 17176	18	Polar marine sediment	JONL00000000	4.185	42.7	–, 56
Epsilonproteobacteria							
Sulfurimonas gotlandica	GD1	12	Hypoxic seawater	AFRZ00000000	2.952	33.6	–, 47
Archaea (Methanomicrobia)							
Methanococcoides burtonii	DSM 6242	19	Polar saline lake	CP000300.1	2.575	40.8	3, 49
Methanogenium frigidum		10	Polar saline lake		~2.2		
Methanolobus psychrophilus	R15	15	Alpine wetland sediment	CP003083.1	3.072	44.6	–, 52

[a]Number of strains sequenced
[b]Genome includes multiple elements (multiple chromosomes and/or plasmids)

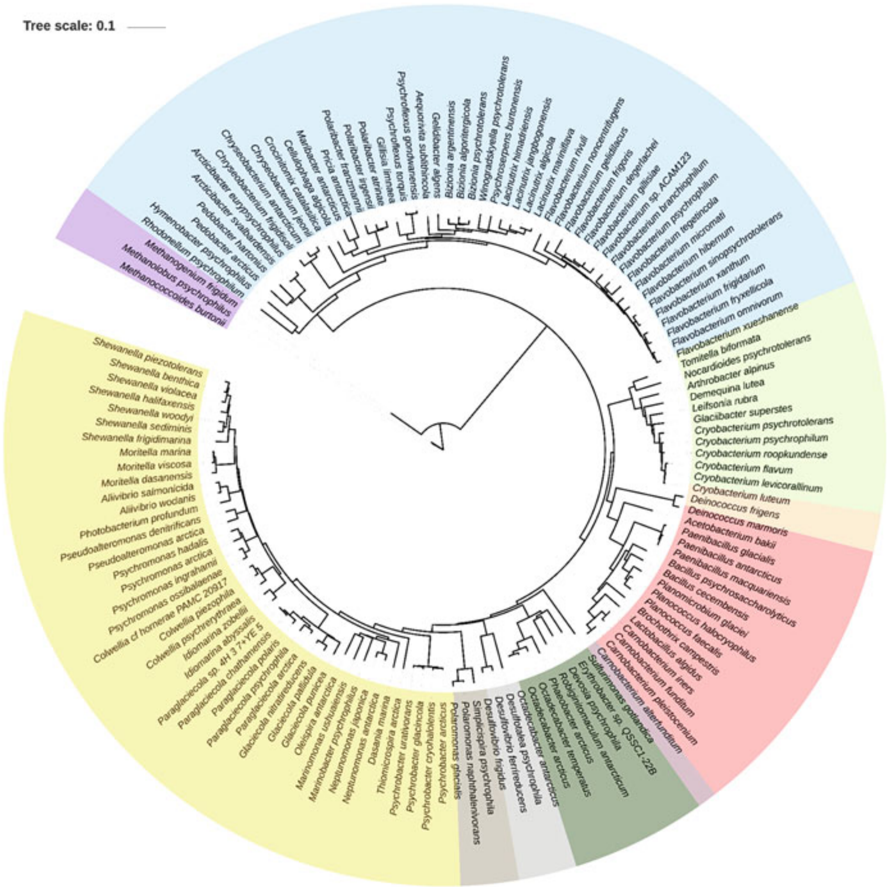

Fig. 15.2 A phylogenetic tree showing 16S rRNA gene sequences of genome-sequenced psychrophilic species. The *coloured* sections correspond to the major taxonomic groups to which the species belong (see Table 15.1). The tree was created using the Interactive Tree of Life online application (itol.embl.de/) using maximum likelihood and neighbour joining algorithms

strains are type strains. All speciated strains are taxonomically valid except for *Methanolobus psychrophilus* (Zhang et al. 2008). A temperature of 25–30 °C represents supraoptimal to lethal temperatures for these strains. Many strains that are not speciated, and potentially represent undescribed species, are also available with genomes, but a few have any available growth data and so were not considered further here except in a few cases where data was available. Genomes extracted from environmental metagenomes are also not considered for the same reason since little data is available that provides credible growth rate versus temperature data for uncultured microorganisms, let alone microorganisms of low-temperature systems. The use of amino acid distributions could be used to assess their potential for psychrophily (see next section); however, this was not done here. Overall a much

Table 15.2 Distribution of DUF3494 type AFPs in cold-adapted bacteria[a]

Taxonomic group	Species or strain
Bacteroidetes	
Aequorivita	*A. sublithincola*
Arcticibacter	*A. eurypsychrophilus*, *A. barbardensis*
Chryseobacterium	*C. antarcticum*, *C. gregarium*, *C. jeonii*, C. *luteum*
Flavobacterium	*F. degerlachei*, *F. frigoris*, *F. fryxellicola*, *F. geldilacus*, *F. gillisiae*, *F. micromati*, *F. noncentrifugens*, *F. omnivorum*, *F. urumqiense*, *F. xanthum*, *F. xinjiangense*, *F. xueshanense*
Gelidibacter	*G. algens*
Gillisia	*G. limnaea*, *Gillisa* sp. CAL575, *Gillia* sp. JM1
Hymenobacter	*Hymenobacter* sp. DG5B
Lacinutrix	*Lacinutrix* sp. Hel_I_90
Lutibacter	*Lutibacter* sp. BRH_c52
Mucilaginibacter	*M. paludis*
Pedobacter	*P. arcticus*, *P. oryzae*, *P. ruber*
Polaribacter	*P. irgensii*
Psychroflexus	*P. sediminis*, *P. torquis*
Psychroserpens	*P. burtonensis*
Rhodonellum	*R. antarcticum*
Spirosoma	*S. spitsbergense*
Firmicutes	
Paenibacillus	*P. wynnii*
Paenisporosarcina	*Paenisporosarcina* sp. TG-14
Actinobacteria	
Demequina	*D. aestuarii*, *D. lutea*
Cryobacterium	*C. flavum*, *C. luteum, C. roopkundense, C. psychrotolerans, Cryobacterium* sp. MLB-32
Gammaproteobacteria	
Colwellia	*Colwellia hornerae* PAMC 20917, *Colwellia* sp. SLW05
Dasania	*D. marina*
Idiomarina	*I. salinarum*, *Idiomarina* sp. A28L
Marinobacter	*Marinobacter* sp. ELB17, *M. subterrani*
Pseudomonas	*P. xinjiangensis*, *P. pelagia*
Psychromonas	*P. ingrahamii*
Saccharospirillum	*S. impatiens*
Shewanella	*S. frigidimarina*, *S. denitrificans*
Thiomicrospira	*T. arctica*
Deltaproteobacteria	
Desulfovibrio	*Desulfovibrio* sp. DV
Geospychrobacter	*G. electrodiphilus*

[a]Also detected in the following species and strains: *Cytophaga aurantiaca*, *Cytophaga hutchinsonii*, *Sulfuricaulis limicola*, *Roseivirga echinicomitans*, *Nafulsella turpanensis*, *Marivirga tractuosa*, *Pontibacter akesuensis*, *Pelosinus* sp. UW01, *Streptomyces* spp. and some other actinomycetes, *Rhodoferax ferrireducens*, *Janthinobacterium* sp. CG3, *Candidatus* 'Gallionella acididurans', *Sphaerochaeta* spp. and some other spirochaetes. Also observed in several uncultured taxa from metagenomes (e.g. *Candidatus* 'Ignavibacteria'). Archaea that contain orthologs include *Methanoregula boonei* from a cold acidic bog, *Haloterrigena* sp., *Halohasta litchfieldiae* (from Deep lake, Antarctica) and *Halovivax asiaticus* from a lake from Inner Mongolia. Eukaryotes possessing similar proteins include fungi (*Piloderma croceum*, *Fibulorhizoctonia* sp., *Typhula ishakariensis* and others) and sea-ice diatom (*Fragilariopsis cylindrus*)

improved pool of data is available for comparative analysis as compared to a decade ago. Amongst psychrophilic strain genomes, the specific statistics (Table 15.1) do not show any unusual features, genome lengths are typical and G+C mol% content is consistent with the genus where they belong and includes high and low values depending on the source phyla. The number of rRNA operons (were available) and tRNAs also highly varies between taxa but is generally consistent with higher-temperature relatives.

15.4 Protein Amino Acid Content as Markers for Psychrophily as Discerned from Genomes

Cold-active enzymes and low-temperature adjusted cell structures, such as tRNA and ribosomes, make life possible in freezing conditions since they can process biochemical reactions and mechanically function at low temperature successfully. For example, tRNA nucleotide content in *Archaea* seems to increase with increasing T_{opt} and thus alters in flexibility based on genome data (Saunders et al. 2003); however, psychrophiles cannot be readily distinguished from mesophiles on this basis. The G+C content of genomes has not been found to be related to T_{opt} when relatively large genome sets are compared (Zeldovich et al. 2007). Similarly for proteins the differences in amino acid content between psychroactive and mesophilic counterparts are subtle (Georlette et al. 2004; D'Amico et al. 2006; Siddiqui and Cavicchioli 2006; De Vendittis et al. 2008). Only specific changes are needed to affect significantly the temperature-based catalytic properties of an enzyme. Folding also effects discrete changes in protein structure that affect catalytic efficiency at different temperatures (Feller 2013). It was found that the Hsp70 (GroEL/GroES) complex from *Oleispira antarctica* (Table 15.1) when expressed in *E. coli* resulted in *E. coli* being able to grow at low temperature where before it could not (Ferrer et al. 2003). Based on their data, the growth rate of the *E. coli* transgenic train was improved 141-fold at 8 °C (close to the wild-type strain notional T_{min} value) and was threefold faster at 15 °C. Growth occurred at 4 °C, and the notional T_{min} was estimated to be −13.7 °C after using the Ratkowsky square-root model. The result importantly shows that the capability of psychrotolerant bacteria to grow at low temperature is strongly controlled by rate-limiting steps in protein folding, a process that requires a substantial investment of cell energy (Rothman and Schekman 2011). Other traits possessed by *E. coli* seem to provide fitness for low-temperature growth, in the absence of other challenges of cold ecosystems (such as freezing).

Cold-active enzymes are generally comparatively thermolabile due to a different distribution of non-polar and charged amino acid residues. This allows substantial activity at ecosystem relevant temperatures, such as ≤0 °C in sea ice (Huston et al. 2000). Several studies have examined genome data to determine trends in amino acid substitutions affecting low-temperature catalytic rates and/or stability. Some

groups of microorganisms such as the haloarchaea seem to have an inherent limit to the flexibility of their enzymes. For example, *Halorubrum lacusprofundi*, one of the dominant life forms in Deep Lake, Antarctica, and one of the most extreme still liquid environments on Earth (annual temperature 5 °C to −21 °C, ~30% salinity), has subtle changes to proteins that help with both high-salinity and low-temperature adaptation (DasSarma et al. 2013). This is of interest when considering the limits of life on Earth. Examination of a substantial number of proteomes of psychrophilic versus mesophilic bacteria and archaea have provided further insights into amino acid preferences and the locations of amino acid changes that influence protein structure (Metpally and Reddy 2009; Ayala-del-Río et al. 2010). A number of criteria have been defined for cold-active proteins that can be useful in predicting psychrophily of overall proteomes when compared to mesophilic relatives:

1. Relative levels of arginine (R) versus arginine plus lysine (E). Arginine creates more salt bridges than lysine, promoting stability (Siddiqui et al. 2006). It should be noted higher levels of lysine also stabilise thermophilic proteins through entropic processes (Berezovsky et al. 2005).
2. Relative amount of acidic residues—glutamate (Q) and aspartate (D). Collectively reduced levels of Q and D reduce the number of salt bridge forming with R and E.
3. Amount of proline (P). Less proline enhances chain flexibility of protein secondary structure (Sakaguchi et al. 2003). Less proline also helps avoid the limitations of the rate-limiting step of proline isomerisation during protein folding, which is slowed further at low temperature (Feller 2013). Trigger factor, which acts as a prolyl *cis-trans* isomerase, is more abundant at low temperature (Piette et al. 2010), and cold-active versions have efficient prolyl isomerase activity (Godin-Roulling et al. 2015).
4. Lower levels of hydropathicity, scored as the grand average of hydropathicity (GRAVY) (Kyte and Doolittle 1982). Typically cold-adapted bacteria have less buried hydrophobic residues that would affect folding processes (Sælensminde et al. 2009; Feller 2013).
5. A fifth criterion is the measure of the aliphatic index, which provides an estimate of the protein packing volume (Ikai 1980). However, this measure appears less sensitive unless buried amino acids are specifically analysed since the link with cold-active enzymes seems to mainly relate to buried amino acid residues and overall measurements lose information (Ayala-del-Río et al. 2010).

Though there is little universality of these amino acid substitutions, they can provide valuable insight into modifications that can be applied to improve enzyme function for practical applications (Metpally and Reddy 2009). In the big picture sense, seven amino acids (I, V, Y, W, R, E, L) were found to be collectively the most influential, correlating broadly to overall bacterial and archaeal T_{opt} (Zeldovich et al. 2007). For psychrophilic bacteria the ratios of these amino acids are typically 38–39% of total amino acid residues versus 40–41% for most mesophiles. The relationship of IVYWREL content to T_{opt} is not strong enough for it to be able to accurately predict the T_{opt} of psychrophiles. In the study of the

Planococcus halocryophilus strain Or1, which has a large temperature growth range (Mykytczuk et al. 2013), only three of the five above criteria fitted. The wide temperature growth range of this organism suggests it has not undergone selection for enhanced protein flexibility and thus allows it to maintain fitness under a range of conditions. It is also recognised that low temperature effects the permeability of the lipid bilayer and could denature membrane proteins that carry out vital processes such as transport, adhesion and motility. Data suggests that exposed parts of membrane proteins seem more likely to have amino acid differences that provide cold adaptation benefits (Kahlke and Thorvaldsen 2012). This in turn suggests the lipid portion of the membrane is also crucial for cold adaptation.

15.5 Comparative and Functional Studies of Genomes of Psychrophilic Bacteria and Archaea

Comparative genomic studies provide a perspective on traits acquired and lost in microorganisms and are especially useful when empirical phenotypic, biochemical and genetic data is available to support interpretations. Furthermore, annotated genomes can provide predictions on unrealised functionality as well as provide a detailed view of evolutionary and taxonomic relationships. For psychrophilic archaea and bacteria, comparative genomics places emphasis on the basis of cold adaptation. This includes questions related to what are the cold adaptation-linked traits present; how they might contribute to temperature preferences, and how these traits collectively relate to the ecosystem the psychrophile inhabits.

Comparative and functional studies have been performed utilising sequenced complete and draft genomes of single psychrophilic strains and, where available, multiple genomes from closely related species. In several cases these studies have been coupled with transcriptomic and proteomic analysis. Comparative studies have been performed on groups of species including cold-adapted archaeal species *Methanococcoides burtonii* and *Methanogenium frigidum* (Saunders et al. 2003) and species of the bacterial genera *Shewanella* (Zhao et al. 2010), *Octadecabacter* (Vollmers et al. 2013), *Glaciecola* (Qin et al. 2014), *Psychroflexus* (Feng et al. 2014, 2015), *Paenibacillus* (Moreno Switt et al. 2014), Arctic seawater-derived *Psychrobacter* strains (Moghadam et al. 2016), cold-water fish pathogens (Touchon et al. 2011; Castillo et al. 2016; Vincent et al. 2016) and *Pseudoalteromonas* (Bosi et al. 2017). Examples of intensive genome studies on specific psychrophilic and psychrotolerant strains include *Colwellia psychrerythraea* strain 34H, the first psychrophile to be genome sequenced (Methé et al. 2005; Nunn et al. 2015); *Methanococcoides burtonii* (Allen et al. 2009; Burg et al. 2010; Campanaro et al. 2011; Williams et al. 2010a, b, 2011); *Methanolobus psychrophilus* R15 (Chen et al. 2012, 2015); the deep sea species *Luteimonas abyssi* (Zhang et al. 2015); Arctic *Mesorhizobium* strain N33 (Ghobakhlou et al. 2015); *Shewanella livingstonensis* Ac10 (Kawamoto et al. 2007; Park et al. 2012); *Psychromonas*

ingrahamii strain 37 (Riley et al. 2008); *Planococcus halocryophilus* Or1 (Mykytczuk et al. 2013); *Pseudoalteromonas haloplanktis* TAC125 (Fondi et al. 2015); the type strain of *Pseudomonas extremaustralis* (Tribelli et al. 2015); and Antarctic desert-soil strain *Nesterenkonia* sp. AN1 (Aliyu et al. 2016). The genomes of these species have been investigated in terms of ecology, lifestyle, metabolic models, environmental processes, and almost universally cold adaptation strategies.

Through research and bioinformatics investigations, certain cellular traits can be discerned that are closely connected to psychrophily and cold adaptation though it is rare to find any that are exclusively connected to only cold adaptation since most traits have broad functionality and thus have a degree of temperature independence. The main traits discussed further in this review have been distilled from genomic comparisons and functional studies as listed above. In some cases relevant traits are firmly linked to cold adaptation either because they are induced at low temperature or when disabled result in poor or no growth at low temperature, for example, osmolyte uptake (Angelidis et al. 2002) and fatty acid biosynthesis (Zhu et al. 2005). Psychrophily also can represent the loss of traits that allow growth at higher temperatures. These are much more difficult to discern since the phenotype of psychrophily needs to be evaluated in the context of temperature by empirical experiments. Thus, this review focuses only on traits gained rather than lost. Furthermore, the traits considered are generally stable within genomes since they would theoretically contribute to positive and purifying selection (Bosi et al. 2017) in a given cold ecosystem.

More specifically comparisons of genomes coupled with functional studies provide insights that in general suggest that psychrophiles in their diversity have arrived at their psychrophily in a myriad of different ways though they use a limited pool of traits that are just part of a fundamental conserved framework found in psychrophilic to at least mesophilic species. For example, gene expression and proteome studies on the saline Antarctic lake-derived methanogen *Methanococcoides burtonii* have demonstrated that cell envelope, RNA and protein structure are compromised at low temperatures if not appropriately maintained and managed (Williams et al. 2010a, b, 2011; Campanaro et al. 2011). This lead to the discovery of TRAM domain proteins that seem to act as RNA chaperones analogous to cold-shock proteins (CspA family proteins) in bacteria. These proteins are found in most if not all archaea (Taha et al. 2016). The studies also demonstrated that growth rates are directly linked with metabolic and energy-generation pathway activity as inferred from transcript levels and via proteomics. The overall activity declines with temperature as would be expected with a declining growth rate with lowering temperature. In *M. burtonii* transporters that import glycine betaine, molybdate, iron and phosphate were more abundant indicating the greater need for the resources for maintaining fitness under cold conditions.

A second example is the species *Psychroflexus torquis* ATCC 700755, a strictly aerobic flavobacterial epiphyte which occurs in sea ice algal assemblages. Genome comparative analysis with the closely related Antarctic hypersaline lake species *P. gondwanensis* was performed (Feng et al. 2014) (Fig. 15.3). Genome data

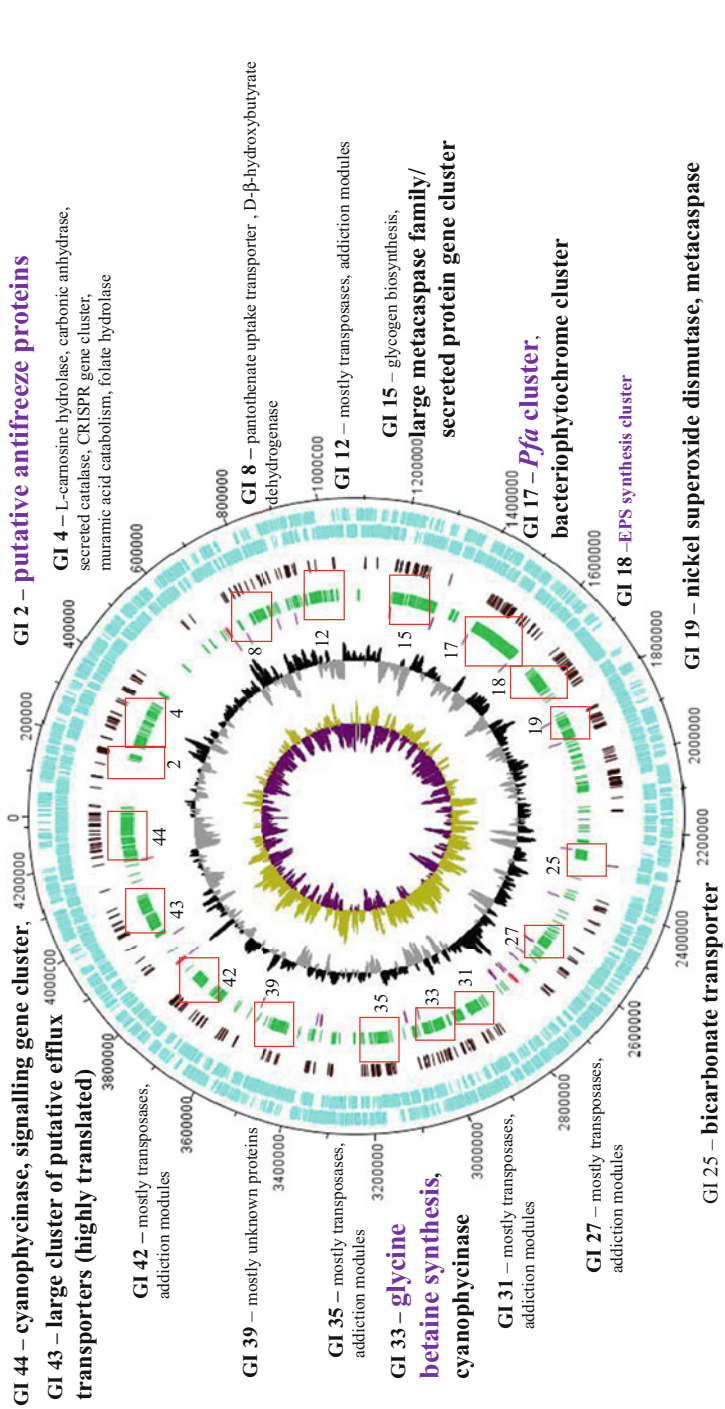

Fig. 15.3 Standout genes located on the genome of *Psychroflexus torquis* ATCC 700755 compared to close relative *Psychroflexus gondwanensis* ACAM 44 (99% 16S rRNA gene sequence similarity). Genomic islands containing genes of interest are defined from the *green ring* that denotes genes present in *Psychroflexus torquis* ATCC 700755 but absent in *Psychroflexus gondwanensis* ACAM 44. Genes that are relevant to a psychrophilic lifestyle are shown in *purple* while other genes potentially relevant to the unique lifestyle of the species (in sea ice and as an algal epiphyte) are in bold. Other features of the genome can be obtained from Feng et al. (2014)

revealed *P. torquis* has a consistently larger genome than all of its five genome-sequenced sister species (4.3 Mb vs. 2.7–3.3 Mb) (Table 15.1). This extra genetic material forms 'genomic islands' (Langille et al. 2010) that are completely absent in the other genomes and are likely acquired through horizontal gene transfer (HGT) processes based on their divergent G+C values and the presence of HGT-related genes (transposases, integrons) that might be selfish (addiction modules, group II retrons) (Zimmerly and Wu 2015) and thus could affect fitness. Likewise, the other species have their own unique genomic islands but on a smaller scale and which are not linked to cold adaptation. There seems extensive evidence of both gene gain and loss through HGT processes in *P. torquis* (Feng et al. 2014). This has been observed in other cold-adapted bacteria such as *Octadecabacter arcticus* and *O. antarcticus* (Vollmers et al. 2013) and in fish pathogen *Aeromonas salmonicida* (Vincent et al. 2016). In the case of *P. torquis* ATCC 700755, these islands contain genes that provide psychrophilic traits including the ability to synthesise polyunsaturated fatty acids (see following section), compatible solute uptake, putative antifreeze proteins and exopolysaccharides (EPS) (Feng et al. 2014).

Similar cold traits have been frequently identified in the genomes of other psychrophiles. For example, in the analysis of the *Psychromonas ingrahamii* strain 37 genome there was suggestion of:

1. Hypothetical proteins that seem cold adapted based on their amino acid composition.
2. An EPS gene cluster that formed an EPS that seems to protect cells from freezing by acting as a cryoprotectant (Breezee et al. 2004); a similar phenotype was found for *Colwellia psychrerythraea* 34H (Methé et al. 2005; Marx et al. 2009; Nunn et al. 2015).
3. The main osmolyte accumulated seemed to be glycine betaine.
4. A large number of TRAP family transporters could aid nutrient acquisition (Riley et al. 2008). Similar types of adaptations though never exactly the same are noted in other studies.

The study of the *M. burtonii* genome suggested it had high plasticity and seemed to have acquired traits that allowed it to fit into a cold niche (Allen et al. 2009). A similar observation was observed in the genomes of *O. arcticus* and *O. antarcticus* isolated from sea ice, which have very high levels of infiltration of HGT elements that have brought along features to the cell that could aid in survival, including xanthorhodopsin, a means to energise membrane electron transport via light harvesting, and also cyanophycinase, a means to obtain nitrogen from poly-L-aspartic acid (cyanophycin), a nitrogen storage polymer common in algae (Vollmers et al. 2013). The ability to combat oxidative stress was also noted as a significant trait, for example, aiding the cold adaptation and survival of *Nesterenkonia* sp. AN1, in soils of the Antarctic Ross Desert, one of the driest and coldest environments on Earth that can sustain some life. This strain relative to its warm temperature-adapted relatives (such as *Nesterenkonia alba*) had a greater proportion of cold-shock protein, DNA repair, fatty acid biosynthesis, osmoprotective and

oxidative management stress genes in terms of genome gene proportion (Aliyu et al. 2016).

In the case of *Colwellia psychrerythraea* 34H, a number of cold adaptation traits are identified including those already mentioned including EPS, compatible solutes, omega-3 polyunsaturated fatty acids, oxidative stress management and enzyme flexibility (Methé et al. 2005). Proteomics performed on cells of 34H grown at −1 °C and at −10 °C in ice confirmed these observations especially in regards to EPS and osmolyte management (Nunn et al. 2015). At −10 °C more effort was emphasised in cells in terms of DNA repair, chemotaxis and sensing processes. Modulation also occurred in cell envelope, metabolism, iron and nitrogen uptake (Nunn et al. 2015). Overall, the differences were still rather subtle and tend to suggest that the strain uses a similar array of traits and works at a slowed pace at subzero temperatures and that energy is directed to cell maintenance and stress responses, which are largely compensatory in nature ensuring cell survival. This is not unlike other forms of stress where when growth permissiveness is challenged, the overall processes become increasingly temperature dependent since disorder (entropy) in the system rapidly increases, especially when moving beyond growth limits (Ross et al. 2008; Zhang et al. 2010; Corkrey et al. 2014).

The genome of *Planococcus halocryophilus* strain Or1 featured several cold adaptation traits, such as a large number of compatible transporters (Mykytczuk et al. 2013). Expression studies revealed cell envelope modification including EPS synthesis, energy metabolism (increased ATP synthesis), ion transport, transcription and translation modulation that were important for survival at ≤−10 °C. The studies of Nunn et al. (2015) and those on *M. burtonii* mentioned above parallel these findings. The overall interpretation is that psychrophily relies at least to some extent on adaptive plasticity to acquire more cold-adaptation-relevant traits. These seem to be coupled with efficient use of resources allowing generation of residual energy above and beyond what is needed to counter deleterious effects of extremely low temperature including protein denaturation, slow enzymatic reactions and associated metabolic bottlenecks and membrane permeability limitations. The combined use of genomics, gene expression and proteomic analysis has the power to develop a more systems-based understanding of psychrophily in model organisms, for example, as done with *Pseudoalteromonas haloplanktis* TAC125 in terms of metabolic models (Fondi et al. 2015). Studying strains spread over a number of phyla and from contrasting ecosystems would be illuminating for investigating the limits to life.

Metagenomic studies also clearly have their place in this area. Many psychrophiles are likely being uncovered by the ever growing number of population genomes and metagenomes created in recent times from samples collected from cold ecosystems (Simon et al. 2009; Berlemont et al. 2011; Han et al. 2012; DeMaere et al. 2013; Lay et al. 2013; Ugalde et al. 2013; Wright et al. 2013; Bowman et al. 2014; Choudhari et al. 2014; Glass et al. 2014; Klippel et al. 2014; Tveit et al. 2014; Anderson et al. 2015; Bowman and Ducklow 2015; Lee et al. 2015; Chrismas et al. 2016; Colangelo-Lillis et al. 2016; Le et al. 2016; Lopatina et al. 2016; Raymond 2016; Tschitschko et al. 2016; Goordial et al. 2017).

However, proving they are psychrophilic may require use of predictive tools such as using developing inferences from amino acid content and the presence of genes found in cultured psychrophilic and psychrotolerant species. This has yet to be done in any systematic way and could be useful, especially aligned with larger scale concepts such as the BKS.

An exemplary example of a metagenomic analysis of cold ecosystems was the study of Ace-C, a dominant bacterial taxon within meromictic, marine saline Ace Lake located in the Vestfold Hills of Eastern Antarctica (Ng et al. 2010). Ace-C, a phototrophic member of the phylum *Chlorobia* (green sulphur bacteria), is the only reported cold-adapted bacteria within this phylum to date with substantial data, including genomic, transcriptomic and proteomic information. The study by Ng et al. (2010) revealed the traits these organisms use to survive without needing to culture, though this was aided by the microbe completely dominating a particular depth of Ace Lake, at about the oxycline. Primary adaptations used by Ace-C include (1) specific bacteriochlorophyll and pigment production that likely maximises photon capture; (2) tight regulation of its sulphur metabolism in addition to syntrophic relations developed with sulphate-reducing bacteria in the anoxic layer of Ace Lake for sulphur supply; and (3) oxidative stress management that was found to be important for its survival since its biomass concentrated near the oxycline where it could be periodically exposed to cold O_2-rich waters in the upper layer of the lake.

Genomics has been useful in revealing features of cold adaptation including cold-inducible proteins that provide a perspective on how bacterial and archaeal cells manage low temperature at a fundamental level. For example, cold shocking of *Shewanella livingstonensis* Ac10 (Kawamoto et al. 2007) revealed via proteomics that it increased the abundance of proteins associated with RNA synthesis (RpoA, GreA, CspA), protein folding (including Tuf, Efp, LysU, Tig), membrane transport (OmpA and OmpC) and flagella proteins (FlgE and FlgL). Experiments can also tend to be more practical and provide underpinning knowledge in man-made systems. An example of such a study investigated enzymes produced by pasteurisation of cold-adapted *Paenibacillus* spp. at 6–7 °C (Moreno Switt et al. 2014). This is useful since most wastage of food via spoilage in modern systems is due to a 'food microbiome' consisting largely of psychrotolerant and psychrophilic bacteria including *Pseudomonas*, *Shewanella*, *Psychrobacter*, *Photobacterium*, *Carnobacterium* and other cold-adapted lactobacilli, *Brochothrix*, *Clostridium* and other spore-forming bacilli for which much genome data is available now. Genomic approaches may also pave the way to counter cold-water fish infectious diseases caused by the pathogens *Flavobacterium psychrophilum* (Castillo et al. 2016), *Aeromonas salmonicida* (Vincent et al. 2016) and *Aliivibrio salmonicida* (Kashulin et al. 2017).

It was found that by examining the evolutionary connections of cold-adapted traits between *Pseudoalteromonas* spp. (Bosi et al. 2017) and *Glaciecola* spp. (Qin et al. 2014), there is support of the concept that they are highly beneficial and would support increased fitness in appropriate cold ecosystems. Traits that have been highlighted tended to focus on central processes or comprise a particular pool of

traits. The distribution of major cold adaptation traits amongst psychrophilic microorganisms as determined by examining genome data is covered in the next sections.

15.6 Membrane Phospholipid Content in Psychrophiles and Associated Gained Genes and Proteins

Membrane fluidity homeostasis is critical for growth at low temperature. Disabling the ability to synthesise certain enzymes in model bacterial species leads to mutants unable to grow at low temperature. For example, disabling branched chain fatty acid synthesis in the psychrotolerant human pathogen *Listeria monocytogenes* leads to a mutant with very poor growth at 10 °C (Zhu et al. 2005). The proportionally increased levels of fatty acids that relatively promote increased fluidity are a major tactic by bacteria to enable growth over a wide temperature range. The shift by some microorganisms to the colder end of the BKS may suggest that the fluidisation of membranes becomes 'fixed' and that the ability to apply thermotolerance to membranes becomes lost. Fatty acid profiles tend to be relatively conserved at the genus level with quantitative rather than qualitative differences predominating. Changes in the ratios of acyl chain length, proportion of unsaturated and branched chain fatty acid types, comprise a homeostatic mechanism that involves enzymes in at least some bacteria activated transiently by a thermosensory two-component histidine kinase/response regulator system. These systems are likely widespread in bacteria and archaea (Sengupta and Garrity 2013; Inda et al. 2014; Porrini et al. 2014). Thus, psychrotolerant versus mesophilic bacteria could differ in that psychrotolerant strains can better fluidise their membranes via either desaturation of lipids or changing branched chain fatty acid (BCFA) levels, for example, by increasing anteiso C15:0 levels as found in *L. monocytogenes*. Various mesophiles like *E. coli* already have some flexibility in this sense, while other bacteria, such as *Campylobacter jejuni*, seem inflexible and may reflect their econiche preferences and degree of host adaptation (Hughes et al. 2009). Various psychrophiles as well as psychrophilic piezophiles have overtly adapted to low temperature by gaining genes that allow synthesis of omega-3 polyunsaturated fatty acids (PUFA) including eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3c). Some PUFA-producing bacteria also can produce arachidonic acid (AA, 20:4ω6c). AA, EPA and DHA all increase membrane fluidity while shorter chain, less unsaturated lipids such as oleic acid (18:1), linoleic acid (18:2), and α-linolenic acid (18:3) have substantially weaker effects (Yang et al. 2011).

The synthesis of PUFA has come through the apparent HGT of a polyketide synthase (PKS) gene cluster that includes five genes (*pfaABCDE*). Three classes of bacterial PUFA synthesis PKS are known (Shulse and Allen 2011a, b) (Fig. 15.4). One class termed group A by Shulse and Allen (2011b) creates EPA, group B creates DHA, and class D creates AA and EPA depending on the species. PUFA *pfa*

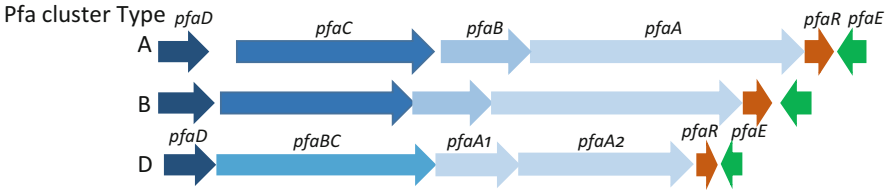

Fig. 15.4 Polyketide synthase type *pfaABCDE* gene clusters that allow synthesis of PUFA as defined by Shulse and Allen (2011a, b). Group A, B and D *pfa* gene cluster synthesise EPA, DHA and EPA and/or AA, respectively. The gene pfaR is a putative transcriptional regulator.

gene clusters are only found in marine bacteria (Shulse and Allen 2011a), so far restricted to *Gammaproteobacteria* and *Bacteroidetes*, primarily in psychrophiles. Based on surveys of the list of psychrophilic and other cold-adapted bacterial and archaeal genomes mentioned previously (Table 15.1), group A *pfa* genes are mainly possessed by members of the genus *Shewanella* as well as in the deep sea moderate piezophile *Photobacterium profundum* and include all psychrophilic species with genomes available, as well as some psychrotolerant species (such as *S. putrefaciens*). Group A *pfa* cluster genes are also found in some psychrotolerant *Vibrio* spp. such as *V. splendidus* and *V. tasmaniensis*. Group B *pfa* genes are found in most if not all *Colwellia*, *Psychromonas* and *Moritella* species, as well as in the species *Pseudoalteromonas denitrificans*, the most cold-adapted species of that genus. Group D *pfa* genes are found in members of the phylum *Bacteroidetes* and is rare, spread across several genera but typically just in specific psychrophilic species or strains including *Psychroflexus torquis*, *Maribacter antarcticus*, *Psychroserpens jangbogonensis*, *Aureispira* spp. and unclassified strains within the genera *Ulvibacter* and *Dokdonia*. *Aureispira* species, which predate other bacteria (via ixotrophy), produce large amounts of AA, and this has been shown to be made by the class D pfa/PKS system (Ujihara et al. 2014). Ujihara and colleagues also showed PUFA synthesis requires the PfaE protein, a 4-′-phosphopantetheinyl (4′-PP) transferase that post-translationally modifies apo-acyl carrier protein to holo-ACP by adding a 4′-PP moiety to an invariant serine residue. Holo-ACP is activated to allow thioesterification of the bound fatty acid acyl chain from the 4′PP distal end. In *P. torquis* the *pfa* cluster is located on a genomic island (see Fig. 15.3) and unlike *Aureispira* spp. instead makes mainly EPA and only some AA. Regulatory systems involved in controlling expression of PUFA proteins are unstudied, but a putative regulator gene is located between *pfaA* and *pfaE* in all PUFA-producing strains suggesting it plays a role in controlling expression of some or all *pfa* genes. There is possibly a link to thermosensing since in *P. torquis* the EPA/AA ratio increases as temperature drops (Nichols et al. 1997) suggesting expression is temperature dependent. At 2 °C proteomics revealed the *pfa* cluster in *P. torquis* was strongly translated (Feng et al. 2014); however, the specific thermosensory regulation if any remains to be elucidated.

15.7 Antifreeze Proteins with the DUF3494 Domain Are Prevalent Amongst Psychrophilic Bacteria

Freezing of water is potentially detrimental to microorganisms with cryodamage worsened when the freezing process is relatively slow. This is due to formation of larger ice crystals than what is found in flash freezing. Ice crystals that form in the cytoplasm act as a solute, and large amounts lead to the intake of water resulting in the increase of osmotic pressure and subsequent membrane rupture. Alternatively, external ice formation causes water loss from cells as it reduces water availability. Bacteria combat freezing in two ways: first by concentrating compatible solutes in the cytoplasm and second by utilising ice-active or antifreeze proteins (AFPs) that hinder or control the recrystallisation of water in the immediate region around the cell or within the cytoplasm itself (Lorv et al. 2014; Bar Dolev et al. 2016a, b; Cheung et al. 2017). Compatible solutes are discussed in the next section.

AFPs are a diverse set of proteins belonging to a number of different protein superfamilies, several with no classification. In general AFPs are not subgrouped owing to their diverse nature both structurally and functionally. Until more data is available, AFPs are generally only associated with the groups of organisms they are found (Lorv et al. 2014; Bar Dolev et al. 2016b; Cheung et al. 2017). AFPs have been studied extensively in bacteria and eukaryotes.

One famous group of AFPs are called ice-nucleation proteins (INPs). INPs are mainly restricted to plant-associated bacteria including *Pseudomonas* spp., *Xanthomonas* spp. and *Pantoea* spp. and can trigger ice crystal nucleation events at high-subzero temperatures (Bar Dolev et al. 2016a, b). The ice crystal formation is used as a means to disrupt plant cells to gain access to nutrients. INP-forming bacteria, such as the plant pathogen *Pseudomonas syringae*, are psychrotolerant but not psychrophilic. Screening for INPs has failed to find them in freezing ecosystems such as sea ice or polar lakes where one theory was that they could act as nucleators of ice formation. However, they are ubiquitous in precipitation worldwide (Christner et al. 2008), and thus wind- and rain-transported bacteria with INPs could act as nuclei for ice formation. INPs are large proteins of around 130 kDa with numerous repeat domains. No INP-type orthologs are found in the strain genomes listed in Table 15.1 and seem entirely restricted to pseudomonads and some other members of class *Gammaproteobacteria*.

Several diverse proteins have been found to have antifreeze properties, which allow bacteria and eukaryotes living in freezing-prevalent environments to control the size and shape of ice crystals in supercooled liquids. These proteins do this by thermal hysteresis and by controlling ice recrystallisation. Based on the literature, AFPs use both of these processes though the level of hysteresis can be quite minimal. Some AFPs can strongly depress the equilibrium freeze-temperature point (by up to 6 °C), a process called hyperactive thermal hysteresis, and simultaneously raise the melting temperature of ice (hysteresis melting), essentially heating the ice by up to around 0.1–0.2 °C. Ice crystals neither grow nor melt in the hysteresis-controlled-temperature range; however, when the temperature drops

below the equilibrium point, crystal formation occurs, often explosively, in a burst pattern. The temperature range of hysteresis varies widely, and in general it was thought bacterial thermal hysteresis ranges of bacterial AFPs tend to be smaller (0.1–0.3 °C) than those of fish, plants and insects (Middleton et al. 2012; Lorv et al. 2014). Several recently studied recombinant bacterial hyperactive AFPs (see below) have hysteresis temperatures equivalent to insect and fish AFPs (2–5 °C) (Kawahara et al. 2007; Hanada et al. 2014) when at sufficient concentrations and in the presence of ligands and solutes. Bacterial extracts alone give weak results likely because the protein is not highly abundant and possibly because it requires some form of activation.

Reducing ice recrystallisation by AFPs seems a prevalent activity and prevents ice crystal size increasing in size. This is because the amount of protein required can be very low, indeed nanomolar levels (Mangiagalli et al. 2017). This is important since as subzero temperatures drop ice crystals tend to be bigger due to small ice crystals combining together. This impediment of crystal size reduces the probability of cryodamage and applies to either the cytoplasm if the AFP is cytosolic or the external environment if the AFP is secreted. AFPs have been noted in a range of bacteria, but relatively few examples have been studied in depth with functionality characterised and crystal structures of proteins also obtained. An AFP from *Pseudomonas putida* strain GR12–2 (Sun et al. 1995; Muryoi et al. 2004) was characterised that had modest thermal hysteresis activity but promoted survival of the bacterium in freezing conditions. The protein precursor of this AFP is 50 kDa in size and is unusual in that is highly glycosylated and lipidated (increasing molecular mass to 164 kDa). Based on genome comparisons performed here, this AFP is restricted to only strain Gr12–2 so far based on conserved domain structure and homology. Another AFP was isolated from an Antarctic psychrophile classified oddly as *Moraxella catarrhalis* (Yamashita et al. 2002); most likely this is a member of the genus *Psychrobacter*; however, no sequence is available to confirm this nor is there a protein sequence for the AFP. This is the case for many other reports of AFP activity in bacteria but still suggests AFP activity is widespread (Wilson and Walker 2010; Wilson et al. 2012; Wu et al. 2012).

An isolate of the species *Marinomonas primoryensis* (not the type strain) detected in a large screen for bacterial AFP activity from Antarctic lakes in the Vestfold Hills region produces an unusual large (1.5 MDa) adhesin-like AFP that has a large thermal hysteresis (2 °C) and seems to be used by the bacterium to survive freezing (Gilbert et al. 2005). This AFP is complex in having five subunits and requires Ca^{2+} for stabilisation of the complex that forms (Garnham et al. 2011). Only subunit IV interacts with ice clathrates and is about 30 kDa in size. It was shown that this definitively is able to bind to ice and that the ice interaction is not just a chance feature (Bar Dolev et al. 2016a). Most of the size of this adhesin is due to 120 tandem repeats of subunit II that forms a kind of cell extension to provide better access to surfaces. Comparison with available genomes indicates this AFP/adhesin is unique to the *M. primoryensis* strain it is from. The type strain of the species has not been sequenced yet, and so no data is available if it is prevalent

in the species *M. primoryensis* or relatively unique being acquired by horizontal gene transfer.

Proteins that have been classified with functionally unknown DUF3494 domains have been shown to be AFPs. These AFPs were initially characterised from a number of polar environment-sourced bacteria including *Flavobacterium frigoris* PS1 (Raymond et al. 2007), *Flavobacterium xanthum* (Kawahara et al. 2007) and *Colwellia* sp. SWL05 (Hanada et al. 2014) (Fig. 15.5). Similar proteins have been found in metagenome surveys of Antarctic moss bacteria (Raymond 2016), and orthologs are found in yeast and other ascomycetous fungi, algae and ciliates. Crystal structures have been solved for the versions from *F. frigoris* PS1 (Do et al. 2014), *Colwellia* sp. SWL05 (Hanada et al. 2014) and the snow mould *Typhula ishikariensis* (Kondo et al. 2012) (Fig. 15.5). These AFPs have large thermal hysteresis values, lack repeat domains and can control recrystallisation and formation of ice crystals. Surveying psychrophile genomes here reveals orthologs of this AFP family are prevalent in psychrophilic bacterial species and are also found in some archaea from cold ecosystems (Table 15.2). Most importantly they rarely if at all appear in bacteria that do not have some level of cold adaptation, either proven or inferred (from their ecosystem source). Orthologs detected in archaea include a methanogen *Methanoregula boonei* (Bräuer et al. 2011) and haloarchaea, including *Halohasta litchfieldiae* from Deep Lake, Antarctica (Mou et al. 2012) (Table 15.2). Some interesting variations of DUF3494 AFPs occur including 35 kDa orthologs in the Antarctic species *Aequorivita sublithincola* that have C-terminal T9SS secretion domains. The type IX secretion system (T9SS) terminal domains represent characteristic signal peptide domains used by members of the *Bacteroidetes* for the export of many proteins (McBride and Nakane 2015), including those involved in gliding motility. Another version occurs in *Psychroflexus torquis* in which the DUF3934 domain is located at the N-terminal ends of putatively T9SS secreted proteins of 100–110 kDa. The DUF3494 domains

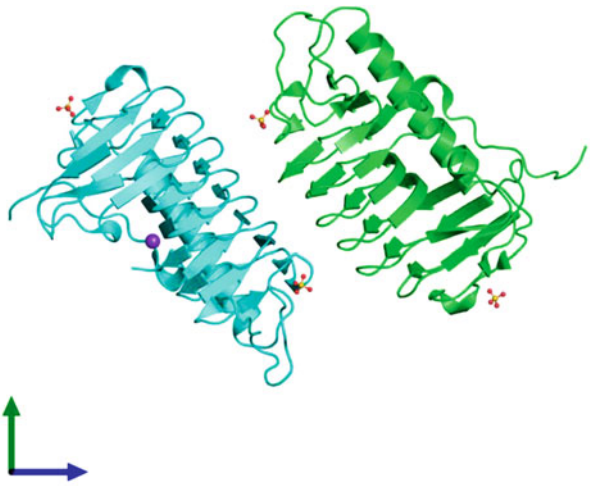

Fig. 15.5 Diagrammatic representation of the structure of the DUF3494-type antifreeze protein from *Flavobacterium frigoris* PS1 as determined by Do et al. (2014). The two chains forming the protein are separately coloured and viewed from the front. The associated single Na^+ (*purple sphere*) and four sulphate ligands are also shown. Image obtained from the EMBL-EBI Protein Data Bank

in this case are quite divergent. The aforementioned AFP from *F. xanthum* has several repeat regions accompanying the DUF3494 domain (Hanada et al. 2014). Orthologs of this protein are prevalent in *Flavobacterium* and some other members of the phylum *Bacteroidetes*. Other proteins with DUF3494 domains occur with additional domains in other taxa. Functionally it remains to be seen if these proteins also have antifreeze activity. The survey suggests AFPs are commonly distributed in bacteria and other life forms from cold regions though they require further study to verify functionality and cellular roles.

15.8 Compatible Solute Uptake Transporters and Synthesis Pathways Revealed by Genomics in Psychrophiles

Compatible solutes are low molecular mass compounds that can be accumulated to high levels in the cytoplasm without affecting protein and other cell functions. Their main role is to act as osmoprotectants and stabilise proteins and membranes; doing so provides defence against a range of stress including mainly low water activity but also low temperature, freezing, heat, starvation, desiccation and solvent stresses (Wood et al. 2001; Roberts 2005; Hoffmann and Bremer 2011, 2017; Vyrides and Stuckey 2017).

Bacteria and archaea can accumulate compatible solutes from the external environment via a range of transporters (Hoffmann and Bremer 2011, 2017). Compatible solutes can also be synthesised de novo (Roberts 2005). Most bacteria and archaea have a capacity to either accumulate or create compatible solutes, and so distinctions in terms of capability are usually not relevant when comparing psychrophiles to mesophiles. This is also complicated further by the fact that osmoprotection by accumulated solutes is also highly important for halophilic and halotolerant species. The most simple solute that is accumulated and usually held at constant levels in the cytoplasm is K^+, commonly used by haloarchaea and other archaea as their means of osmotic control (Grant 2004). Transporters for K^+ come in several forms and occur virtually universally. Thus, it is assumed most psychrophiles accumulate K^+ as part of normal cytoplasmic homeostasis (Binepal et al. 2016; Checchetto et al. 2016). The main organic compatible solutes include amino acids such as L-proline; amino acid derivatives such as glycine betaine, carnitine and ectoine; and disaccharides and polyols, in particular trehalose, sucrose and inositol. A range of other compounds are formed as compatible solutes in thermophilic archaea and bacteria (Roberts 2005; Empadinhas et al. 2007; Lamosa et al. 2013; Borges et al. 2014).

Most psychrophilic taxa contained betaine-choline-carnitine transporter (BCCT)-type uptake systems that take up a combination of glycine betaine, choline, carnitine or (hydroxy)ectoine. By comparing sequences of BCCT transporters to the genomes listed in Table 15.1, they appear to be quite prevalent in psychrophiles

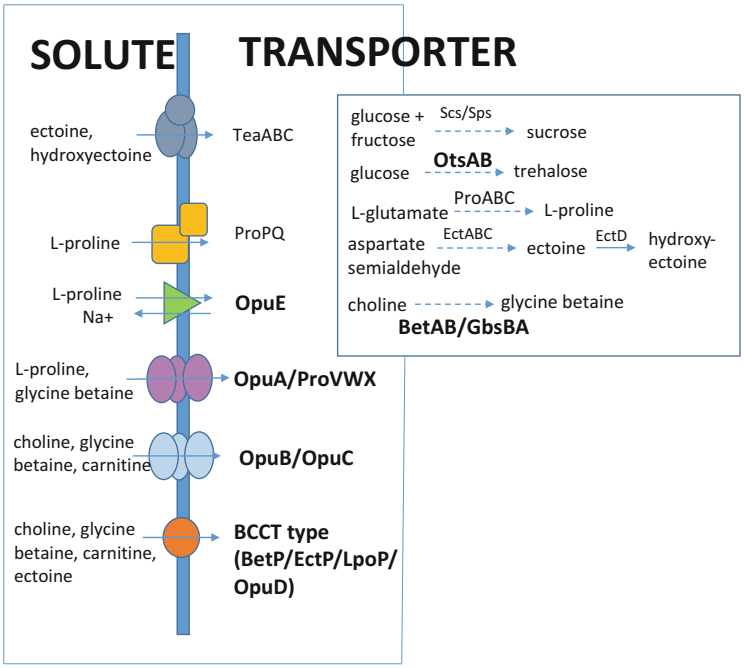

Fig. 15.6 The main types of compatible solute uptake systems and synthesis pathways present in bacterial and archaeal psychrophiles. Proteins shown in bold are the most extensively distributed

(Fig. 15.6). Not all species possess them, and the pattern seems random, not linked to halophily or halotolerance. Many species have multiple paralogs present in their genomes, for example, *Planococcus halocryophilus* contains four paralogs likely working together to allow growth at both low temperatures and high-salt levels (Mykytczuk et al. 2013). The distribution of transporters is widespread in terms of phylogenetic groups.

Proline can also be transported by the MHS-type transporter called ProP. This transporter has an osmosensor regulatory domain and is activated by the effector protein ProQ. ProP and ProQ were only detected in *Pseudomonas* spp. and *Devosia* spp. amongst the cold-adapted taxa. Virtually all taxa could in any case synthesise proline from glutamate (via proABC), but whether proline is used as a compatible solute requires direct chemical investigation. Data from studies on *B. subtilis* suggests L-proline accumulation does not provide cold tolerance (Hoffmann and Bremer 2011), which may explain the limited distribution of ProPQ. Various halophilic and halotolerant species that include many marine psychrophiles, for example, species of *Colwellia*, *Glaciecola*, *Oleispira*, *Paraglaciecola*, *Psychrobacter*, *Psychromonas*, *Shewanella*, *Planococcus*, *Psychroflexus* and *Psychroserpens*, can transport proline via Na^+-based symporter OpuE (Fig. 15.6). Whether L-proline only provides osmotolerance protection requires further analysis in marine psychrophiles.

Compatible solutes can also be transported via ABC-type transporters, notably the OpuA/ProVWX and OpuB/OpuC systems (Hoffmann and Bremer 2017). The OpuA/ProVWX system mainly transports glycine betaine and proline while OpuB/OpuC transports choline, carnitine and glycine betaine (Fig. 15.6). These transporters occur primarily in the more cold-adapted species, but this was not a strict relationship. It is likely that Opu-type transporters contribute equally to osmotic and cold tolerance as found in other bacteria such as *L. monocytogenes* (Angelidis et al. 2002) and *B. subtilis* (Hoffmann and Bremer 2011).

Ectoine and hydroxyectoine have been found to be transported by TRAP-type proteins called TeaABC (Grammann et al. 2002). TeaABC proteins are only found amongst *Halomonas* and its relatives including *Marinobacter* spp. and the Antarctic hypersaline lake psychrophile *Saccharospirillum impatiens* (Labrenz et al. 2003). Ectoine and hydroxyectoine are synthesised from aspartate semialdehyde by a pathway including three enzymes (EctABC) for ectoine and an additional hydroxylase EctD for hydroxyectoine (Cánovas et al. 1997; Roberts 2005). *Devosia*, *Marinobacter*, *Marinomonas*, some *Paenibacillus* spp., *Photobacterium halotolerans*, *Pseudomonas*, *Dasania marina* and the permafrost ice species *Tomitella biformata* (Katayama et al. 2010) possessed either EctABC or EctABCD pathways (Fig. 15.6). The results suggest only a small proportion of psychrophiles accumulates these compounds and most that do have both psychrotolerant and halotolerant capabilities. Ectoine, like proline, was found not to confer cold tolerance to *B. subtilis* (Hoffmann and Bremer 2011) and is likely more used for survival at low water activity.

De novo synthesis of compatible solutes is very prevalent but not universal amongst cold-adapted bacteria and occurs as frequently as in their more warm-temperature adapted relatives. The pathway for glycine betaine synthesis (BetA/GpsB and BetB/GpsB) was, however, very commonly detected in taxa listed in Table 15.1 across all major taxonomic groups. The ability to synthesise trehalose (via OtsAB) was also relatively common, especially in the more salt-tolerant taxa but was more species specific in terms of distribution. Sucrose synthase or sucrose phosphate synthase, which can potentially allow accumulation of sucrose as a compatible solute, often observed in cyanobacteria and proteobacteria with low halotolerance (Roberts 2005), was found in a few taxa including several *Flavobacterium* species and the glacial soil species *Planomicrobium glaciei* (Zhang et al. 2009).

Overall, psychrophiles have a broad capability to take up and synthesise compatible solutes of conventional sorts. The BCCT and OpuA/OpuB/OpuC transporters are the most prevalent accumulating choline, glycine betaine and carnitine. Many can make glycine betaine from choline or synthesise and accumulate trehalose. Whether there are any other forms of solutes present that are more novel in nature or known compounds not covered here requires direct chemical and biochemical analysis to be conducted.

15.9 Other Traits Relevant to but Not Directly Connected to Psychrophily

Certain stress protective traits seem universal at least in many well-known mesophiles often used as genetic models, including *E. coli*, *B. subtilis*, *P. aeruginosa* and *Streptomyces coelicolor*. All these species have wide growth temperature ranges (typically 40 °C) and are well endowed with protective systems. These species seem to have equal or more capability than many psychrophiles in some aspects of stress defence such as oxidative stress management and possession of multiple cold-shock proteins (CspA family proteins), which act as RNA chaperones, and other enzymes linked to cold temperature survival. These additional enzymes include, for example, as tRNA-dihydrouridine synthetases (Dus proteins) and ATP-dependent RNA DEAD-box helicases (CshA, CshB, RhlB, SrmB) that provide flexibility to tRNA and stabilise ribosomes against cold denaturation (Saunders et al. 2003). Chill stress and low-temperature shocks often lead to these proteins becoming more abundant as mentioned previously.

Low temperature encourages greater solubility of O_2, which can become supersaturated especially where there is active primary production (Marks 2008); thus, cold environment-associated bacteria likely need some form of oxidative stress protection. But this equally applies to any aerobic environment where high dissolved O_2 levels can accumulate or where production of reactive oxygen is used as a chemical defence. The above model species have 17–22 proteins associated with dealing with toxic oxidative reaction products including catalases (KatE, KatG, KatN), thiol peroxidases (peroxiredoxin Tpx and Bcp types), glutathione peroxidase (BtuE), cytochrome *c* and heme-type peroxidases, dye-reducing peroxidase (EfeB), vanadium-dependent haloperoxidases and superoxide dismutases (SodN, SodA/SodB, SodC). They also have multiple cold-shock proteins, Dus and RNA helicase proteins and compatible solute uptake systems and often some flexibility in terms of fatty acid synthesis. It is possible psychrophiles may possess unknown means for oxidative stress protection synthesising unusual or novel antioxidants; however, data suggests at present psychrophiles do not generally possess innovative or unusual distributions of stress protection mechanisms.

Light-harvesting proton and Na^+ pump proteins proteorhodopsin and xanthorhodopsin (Pinhassi et al. 2016) seem concentrated in psychrophilic species. These membrane proteins have a β-carotene-derived cofactor; thus, most strains produce carotenoid-pigmented colonies. *Glacieola* spp. are exceptions, and their pigmentation (pale to bright pink) is due to the accumulation of proteorhodopsin in cell membranes. Psychrophiles possessing these proteins are listed in Table 15.3. Most are marine where rhodopsins abound in marine bacteria to such an extent genes have been observed in viruses through which they are believed to have been spread via HGT (Philosof and Béjà 2013). Proteorhodopsin likely accumulates because large marine systems are cold, and in all probability most marine bacteria are thus cold-adapted to some extent. It is possible rhodopsins provide energetic advantages

Table 15.3 Psychrophilic and psychrotolerant bacterial species and strains possessing rhodopsin family proteins

Genus	Species or strain
Bacteroidetes	
Bizionia	*Bizionia* sp. PZ-13
Cellulophaga	*Cellulophaga* sp. He1_I_12
Chryseobacterium	*C. frigidisoli*, *C. humi*
Erythrobacter	*Erythrobacter* sp. QSSC1–22B[a]
Flavobacterium	*F. antarcticum*, *F. aquatile*, *F. fontis*, *F. frigidarium*, *F. frigoris*, *F. fryxellicola*, *F. gelidilacus*, *F. gillisiae*, *F. micromati*[b], *F. omnivorum*, *F. segetis*, *F. sinopsychrotolerans*, *F. urumqiense*, *F. xanthum*, *F. xinjiangense F. xueshanense*, *F. cucumis*, *Flavobacterium* sp. ACAM 123
Gelidibacter	*G. algens*
Gillisia	*G. limnaea*[b], *Gillisia* sp. JM1
Lacinutrix	*L. jangbogonensis*, *Lacinutrix* sp. He_I_90
Maribacter	*M. antarcticus*, *M. orientalis*
Pedobacter	*P. glucosidilyticus*
Polaribacter	*P. irgensii*, *P. dokdonensis*
Pricia	*P. antarctica*
Psychroflexus	*P. torquis*, *P. gondwanensis*, *P. salarius*, *P. sediminis*[b], *P. tropicus*
Psychroserpens	*P. burtonensis*
Rhodonellum	*R. psychrophilum*
Spirosoma	*S. linguale*, *S. spitsbergense*
Alphaproteobacteria	
Devosia	*D. psychrophila*
Octadecabacter	*O. antarcticus*, *O. arcticus*
Gammaproteobacteria	
Glaciecola	*G. punicea, G. pallidula, G. nitratireducens*
Marinobacter	*M. psychrophilus*[a]
Paraglaciecola	*P. psychrophila*
Photobacterium	*P. angustum*
Pseudomonas	*P. psychrotolerans*, *P. graminis*, *P. coleopterorum* (very diverged orthologs)
Actinobacteria	
Cryobacterium	*C. arcticum*, *C. flavum, C. levicorallinum, C. luteum, C. roopkundense*
Leifsonia	*L. rubra*
Firmicutes	
Carnobacterium	*C. iners*, *C. funditum* (diverged orthologs)
Exiguobacterium	*E. profundum*, *E. acetylicum*, *E. antarcticum*, *E. aurantiacum, E. enclense*, *E. indicum*, *E. sibiricum*, *E. undae*
Deinococcus-Thermus	
Deinococcus	*D. pimensis*, *D. marmoris*, *D. aquatilis*, *D. puniceus*

[a]Xanthorhodopsin present
[b]Also has a Na^+ pump rhodopsin

in marine systems such as sea ice (Koh et al. 2010; Feng et al. 2013, 2014, 2015) where several species with proteorhodopsin were isolated.

In summary, a rate-limiting step, such as protein folding (Ferrer et al. 2003), likely stops mesophiles from growing at low temperature. Otherwise many environmental mesophiles, not closely linked to host systems (such as gut microbiota), are not any different to psychrophiles in the sense of stress protection and thus have the ability to survive chill stress. Furthermore, gene content does not necessarily translate to a heightened tolerance; such phenotypes need to be empirically tested.

15.10 Conclusions and Future Prospects

Beneficial traits that seem more species (or strain) specific and potentially more concentrated in psychrophiles include traits mentioned above. They provide fitness in the context of particular econiches where psychrophiles abound. Since cold ecosystems are diverse—ranging from high mountains to the deepest oceans—the adaptations though having some general similarity have substantial variation in terms of specific details. The sum of these differences likely defines the ecophysiological profile of the psychrophile, including the temperature range for growth and their inherent growth rate. Genomes provide sources of knowledge and a start point to further explore evolved cold adaptation traits and other phenotypes, some potentially innovative and unrealised. From genome surveys and functional discovery, some traits could indeed develop greater significance, for example, proteins that provide unrealised cold stress protection but have unknown functionality. One recent example was a sequence extracted from an Antarctic desert soil metagenome that coded a protein with a WHy (water stress and hypersensitivity response) domain (Singh et al. 2005) that when expressed in *E. coli* provided chill and desiccation stress protection (Anderson et al. 2015). When WHy proteins are compared to psychrophile genomes, they can only be found in some psychrotolerant *Pseudomonas* species. The protein type seems associated with species with limited halotolerance and could act to protect bacteria against desiccation stress possibly by acting as an effector protein. There could be other proteins with analogous functions that could be found in psychrophilic bacteria that tangibly provides benefits for survival. Further research into the functional aspects of psychrophilic bacteria and archaea is now well served with the abundance of genome data currently available.

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