Psychrophilic Microorganisms in Alpine Soils

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Rosa Margesin

14.1 Introduction

The Earth is a cold planet. About 85% of the biosphere is exposed to temperatures below 5°C throughout the year. Cold habitats span from the Arctic to the Antarctic, from high mountain range environments to the deep ocean. The major fraction of this low-temperature environment is represented by the deep sea (nearly 75% of the Earth is covered by oceans and 90% of the ocean volume is below 5°C), followed by snow (35% of land surface), permafrost (24% of land surface), sea ice (13% of the Earth's surface) and glaciers (10% of land surface). Psychrophilic microorganisms, including bacteria, archaea, yeasts, filamentous fungi and algae, have successfully colonized these cold environments, because they evolved special mechanisms to overcome the life-endangering influence of low temperature. This chapter describes mechanisms of microbial cold adaptation and aspects of microbial activity and biodiversity in cold alpine soils.

14.2 Mechanisms of Microbial Adaptation to Cold

A change in temperature has an immediate effect on all cellular processes of microorganisms since they are too small to insulate themselves or to use avoidance strategies by moving away from thermal extremes (Russell 2008). To survive and grow successfully in cold environments, psychrophilic microorganisms have evolved a complex range of adaptations of all their cellular constituents, which enable them to compensate for the negative effects of low temperatures on biochemical reactions. These adaptation mechanisms are summarized below. Survival strategies of algae in ice and snow have been described by Remias (see chapter in this book).

14.2.1 Growth Characteristics

14.2.1.1 Arrhenius Law and Growth

When the environmental temperature of a population of microorganisms drops, the growth rate decreases until a point is reached when one or more critical functions proceed so slowly that they are insufficient to support cellular requirements, and cell growth ceases. The effect of temperature on microbial growth is described by the Arrhenius law relating the exponential rise of the reaction rate to the temperature increase:

$$K = A\ e^{-Ea/RT}$$

where A is a constant (relating to steric factors and molecular collision frequency), Ea is the activation energy, R is the gas constant, and T is the absolute temperature.

According to this equation, any decrease in temperature causes an exponential decrease of the reaction rate, the magnitude of which depends on the value of

R. Margesin (✉)
Institute of Microbiology, University of Innsbruck, Innsbruck, Austria
e-mail: rosa.margesin@uibk.ac.at

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the activation energy. The linear range of the Arrhenius plot (the logarithmic value of the growth rate is plotted as the reaction rate constant versus the reciprocal of the absolute temperature) corresponds to a physically "normal" temperature for growth, whereas the plot derives from linearity at temperatures near the upper or lower growth limits. Temperatures outside the linear range are stress-inducing temperatures, as shown by decreased microbial activity (e.g., enzyme production, degradation activities), protein synthesis, membrane permeability, and increased cellular stress (Feller and Gerday 2003; Jaouen et al. 2004; Margesin et al. 2005; D'Amico et al. 2006; Feller 2007). For psychrophiles, Arrhenius plots remain linear down to 0°C, while plots for mesophiles deviate from linearity at about 20°C (Gounot and Russell 1999).

At low temperatures, growth rates of psychrophiles are higher than those of mesophiles. While growth and enzyme production of mesophilic microorganisms is stopped in a refrigerator, psychrophiles actively divide and secrete enzymes under such conditions (see below, Fig. 14.1). Some wild-type psychrophilic bacteria display doubling times at 4°C comparable to that of fast-growing *E. coli* laboratory strains grown at 37°C. The latter fail to grow exponentially below 8°C, whereas psychrophilic bacteria maintain doubling times as low as 2–3 h at 4°C (Margesin and Feller 2010). Both for psychrophiles and mesophiles, the temperature for maximum biomass formation is well below the maximum temperature for growth. Psychrophilic bacteria and yeasts produced the highest amounts of cells per dry mass at 1°C, while cell numbers of mesophiles were highest at 20°C (Margesin 2009).

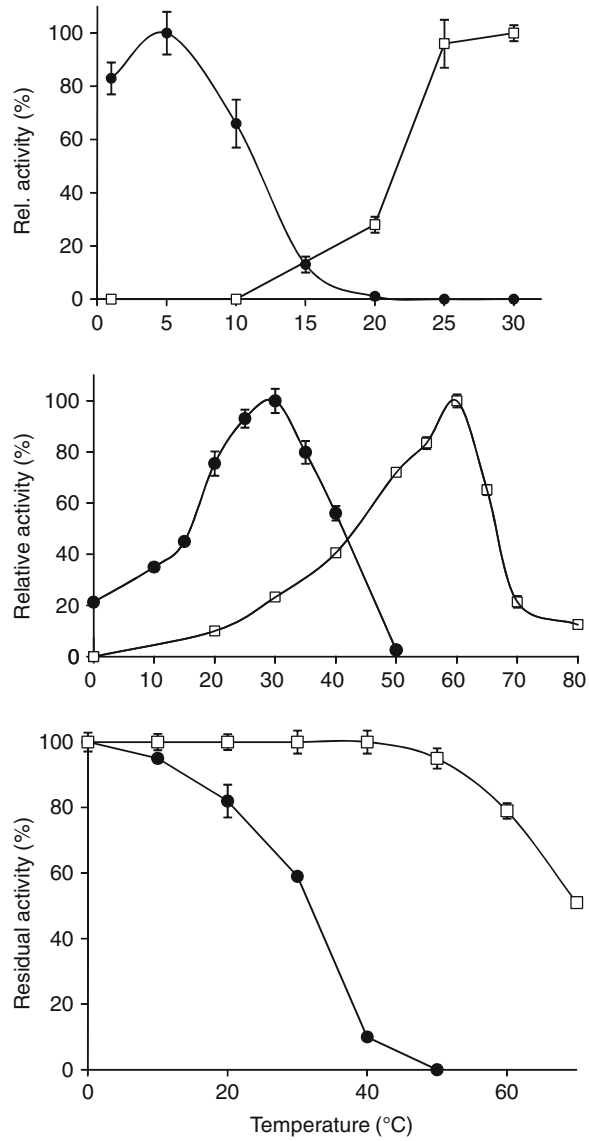

Fig. 14.1 Effect of temperature on enzyme production (*top*), on activity (*middle*) and on stability (*bottom*; residual activity after 15 min of incubation at 25°C) of the cold-active pectate lyase produced by the alpine *Mrakia frigida* strain A15 (●) and its mesophilic counterpart produced by *Bacillus subtilis* (□). Modified from Margesin et al. (2005)

14.2.1.2 Upper and Lower Temperature Limits for Growth

The slope of a microbial growth curve is usually greater at the high temperature compared to the low temperature end of the scale. The reason lies in the different mechanisms that are responsible for setting the upper and lower limits of growth, particularly for psychrophilic microorganisms.

The upper temperature limit for growth results from heat denaturation of cellular proteins. Psychrophiles have lower upper growth temperature limits than mesophiles because of the particular thermolability of one or more of their proteins (e.g. enzymes such as aminoacyl-tRNA synthetases) which are essential for growth/survival of the microorganisms. Other factors that are responsible for the comparatively low upper temperature limit for growth of psychrophiles include the inability to synthesize RNA at superoptimum temperatures, a reduced capacity of ribosomes to bind tRNA, a lower precision of translation, as well as alteration of the cell morphology and inhibition of cell division (Margesin and Schinner 1994).

The lower temperature limit for growth of psychrophiles is usually below 0°C, and its determination in practice is very difficult because of very slow growth rates and the need to include antifreeze in the culture medium which may further reduce the growth rate. The theoretical minimum for psychrophiles may be as low as −26.5°C (Nichols et al. 1997). This estimation assumes that there has been no phase change in the system. However, there are a number of potential phase changes which make such calculated lower temperature limits an overestimate of the true biological value (Margesin et al. 2002). Cold denaturation of proteins generally occurs at a temperature below −15°C (Franks 1995). The lower growth temperature limit is fixed by the physical properties of aqueous solvent systems inside and outside the cell. The major growth-limiting factor at subzero temperatures appears to be the availability of liquid water. Below -10 to -15°C, the cell water begins to freeze and intracellular salt concentrations increase due to the progressive removal of water into ice crystals. The resulting ionic imbalances, lowered water activity, and desiccation have a toxic effect on cells (Ingraham and Stokes 1959; Russell 1990). However, liquid water has been shown to exist at grain contacts as low as -20°C (Jakosky et al. 2003).

Currently the functional low-temperature limits of psychrophiles are -12°C for reproduction and -20°C for metabolism (Bakermans 2008). *Psychromonas ingrahamii* grows exponentially at -12°C with a doubling time of 240 h (Riley et al. 2008). Microbial activity at temperatures ranging from -9°C to -20°C and even below has been convincingly demonstrated by several laboratories and different techniques. Such activities include DNA/protein synthesis at -15°C (Christner 2002) and protein synthesis at -20°C (Junge et al. 2006) by laboratory cultures, as well as CH_4 production at -16.5°C (Rivkina et al. 2002) or glucose oxidation at -20°C (Panikov et al. 2006) in permafrost soil. Permafrost isolates have even been shown to grow and to be active at temperatures as low as -35°C (Panikov et al. 2006; Panikov and Sizova 2007).

14.2.2 Cold Sensing, Lipids and Membrane Fluidity

The ability to adapt to low temperatures depends on the ability to sense changes in temperature. One of the primary cold sensors is the cell membrane that acts as an interface between external and internal environments (Rowbury 2003). At cold temperatures, the membrane becomes more rigid, which activates a membrane-associated sensor. The sensor transduces the signal to a response regulator, which induces up-regulation of genes involved in membrane fluidity modulation, and ultimately results in up-regulation of a number of genes involved in cold adaptation of bacteria, such as genes for fatty acid desaturases, genes that serve as RNA chaperones similar to cold-shock proteins, genes involved in replication, transcription and translation, and genes that encode a number of enzymes (Shivaji and Prakash 2010).

The membranes of microorganisms, like other organisms, contain a lipid bilayer that is essential for many of the major cellular functions, including passive and active permeability, nutrient uptake and electron transport, environmental sensing, photosynthesis and recognition processes. All of these functions demand the maintenance of membrane stability. Lipid fluidity is most influenced by the fatty acyl moieties, whereas lipid phase depends more on the nature of the head-group of the membrane lipids. Both the gel to liquid-crystalline transition as well as the bilayer (lamellar) to non-bilayer phase transition are influenced by growth temperature. However, changes in microbial culture temperature usually lead to greater modifications in the fatty-acyl composition than the head-group composition of membrane lipids, and so the focus of attention has been on fluidity effects (Margesin et al. 2002).

To increase membrane fluidity, microorganisms apply various strategies. When growth temperature is lowered, the most frequently observed change in fatty acid composition is in the extent of unsaturation; increased fatty acid unsaturation has been observed with bacteria, archaea, fungi, and algae. Other bacterial strategies include an increased content in methyl-branched fatty acids, changes in fatty acid isomerization, and an increase in the ratio of anteiso/iso-branched fatty acids. A decrease in the average chain length of fatty acids (only possible in growing cells) as well as in the ratio of sterol/phospholipids has been detected with bacteria, fungi and algae (Robinson 2001; Russell 2008). A further mode of modulation of membrane fluidity includes changes in the composition of carotenoids; polar carotenoids stabilize the membrane to a greater extent

than non-polar ones (Chintalapati et al. 2004; Russell 2008; Shivaji and Prakash 2010). Snow algae also produce large amounts of carotenoids in response to environmental conditions (see chapter by Remias).

Among fatty acid changes in response to temperature, two categories can be distinguished: (1) Alteration of the existing membrane ("modification synthesis"; resulting in fatty acid unsaturation by desaturases) is a more rapid process, especially in response to sudden temperature decrease. (2) Some fatty acid changes ("addition synthesis", such as methyl branching, altered chain length, ratio of sterol/phospholipids) require de novo biosynthesis. In general, after a temperature decrease, modification synthesis takes place to restore membrane fluidity, and later addition synthesis takes over (Russell 2008).

14.2.3 Cold-Active Enzymes

Psychrophiles produce cold-active enzymes. These enzymes can be up to ten times more active at low and moderate temperatures than their mesophilic homologues (D'Amico et al. 2006). Furthermore, psychrophilic enzymes are heat-labile and are frequently inactivated at temperatures that are not detrimental to their mesophilic counterparts (see below, Fig. 14.1).

The conformation and 3D structures of psychrophilic proteins are not markedly different from their mesophilic homologues, and, furthermore, all amino acid side chains that are essential for the catalytic mechanism are strictly identical. It was found, however, that cold-active enzymes maintain the appropriate flexibility and dynamics of the active site at temperatures at which their mesophilic and thermophilic counterparts have severely restricted molecular motions (Feller and Gerday 2003; D'Amico et al. 2006). Thus, cold-active enzymes have a higher structural flexibility in order to compensate for the freezing effect of their cold habitats (Feller 2007). This is achieved by the disappearance of discrete stabilizing interactions either in the whole molecule or at least in structures adjacent to the active site. Amongst these destabilizing factors, the most relevant include a reduced number of proline residues and of electrostatic interactions (ion pairs, H-bonds, aromatic interactions), a weakening of the hydrophobic effect, the strategic location of glycine residues, an improved interaction of surface side chains with the solvent or an improved charge-induced interaction with substrates and cofactors (Siddiqui and Cavicchioli 2006). This adaptive destabilization of psychrophilic enzymes has been demonstrated to be responsible for both cold-activity and low thermal stability (D'Amico et al. 2003; Feller 2007).

14.2.4 Cold-Shock Proteins and Cold-Acclimation Proteins

As a response to sudden temperature changes, representatives of all thermal classes of bacteria (psychro-, meso- and thermophilic) display cold-shock responses. Mesophilic bacteria react with a transient overexpression of cold-shock proteins (CSPs) that are involved in a number of cellular processes, e.g., transcription, translation, protein folding, regulation of membrane fluidity, general metabolism, and chemotaxis (Phadtare 2004; Phadtare and Inoue 2008). The basic principles of cold-shock response are similar in psychrophiles and mesophiles. However, the cold-shock response in psychrophiles differs from that in mesophiles or thermophiles bacteria in two major aspects: cold shock does not repress the synthesis of housekeeping proteins, and the number of CSPs is higher and increases with the severity of the cold shock. In addition, psychrophiles permanently produce one set of proteins (cold-acclimation proteins, CAPs) during growth at low temperature and increase the steady-state level of CAPs when the temperature is lowered. These CAPs are mostly constitutively (rather than transiently) expressed at low temperatures and may be fundamental to life in the cold and ensure improved protein synthesis at low temperature (Gounot and Russell 1999; Margesin et al. 2002; Phadtare and Inoue 2008).

14.2.5 Cryoprotectants and Ice-Binding Proteins

In frozen environments, bacteria are exposed to conditions that require the partial removal of water from the intracellular space to maintain the structure and function of the cell. Since water is essential for the functioning of macromolecular structures, any significant deviation in the accessibility of water, such as the physical state (alteration from the aqueous phase to an

ice crystal), poses a severe threat to the survival of organisms (Beall 1983). Psychrophilic microorganisms produce various compounds to protect themselves or the extracellular environment against intracellular freezing or to minimize the deleterious effects of ice crystal formation (Kawahara 2002).

14.2.5.1 Low-Molecular Mass Cryoprotectants

Freezing results in an osmotic shock. Osmoprotection of bacterial and fungal cells is achieved by the accumulation of compatible solutes (low molecular mass compounds) after cold shock in bacteria and fungi (Gounot and Russell 1999; Robinson 2001; Kawahara 2008; Shivaji and Prakash 2010). These compounds include polyamines, sugars (e.g., glucose, fructose, sucrose, trehalose, ribose), polyols (a class of alcohols derived from sugar; e.g., glycerol, sorbitol, mannitol), and amino acids (e.g., alanine, proline).

Ribose-1-phosphate acts as cryoprotectant of enzymes (observed with *Pantoea agglomerans*), while the accumulation of glucose results in the depression of freezing points (observed with *Pantoea ananatis*) (Kawahara 2008). Trehalose accumulation in bacteria plays a role in preventing protein denaturation and aggregation (Phadtare 2004). This sugar is also accumulated in alpine mycorrhizal roots and in fungal hyphae in response to low temperatures (Niederer et al. 1992; Weinstein et al. 2000). For example, trehalose accumulation in *Mortierella elongata* at 5°C increased by 75% compared to the accumulation at 15°C. Polyols (e.g. glycerol, mannitol) act as cryoprotectants in fungi (Robinson 2001).

Glycine betaine aids to maintain optimum membrane fluidity at low temperatures by preventing cold-induced aggregation of proteins; this compound has been shown to enhance growth of *Listeria monocytogenes* at low temperatures (Chattopadhyay 2002). *Colwellia psychrerythraea* (Methé et al. 2005) and *Psychromonas ingrahmii* (Riley et al. 2008) have genes for the production of compatible solutes, such as glycine betaine and betaine cholin, which may balance the osmotic pressure under freezing conditions.

14.2.5.2 Ice-Nucleation Proteins

Some bacteria (at least six Gram-negative and epiphytic species of the genera *Pseudomonas*, *Pantoea* and *Xanthomonas*) and fungi (e.g., *Fusarium* and related genera) produce proteins that can induce ice-nucleation at temperatures higher than -3°C. Ice-nucleating agents serve as templates for ice crystallization and provide resistance to desiccation. The induction of frost damage in plants by bacteria that produce ice-nucleating agents can be an adaptive advantage to get access to nutrients from plants (Lundheim 2002).

According to the sequences of genes conferring ice-nucleating activity in six bacterial strains, all strains encode ice-nucleating proteins with a molecular mass of 120–150 kDa and similar primary structures. The ice-nucleating proteins contain three domains: the N-domain is responsible for the binding of lipids, polysaccharides and ice-nucleating proteins; the R-domain acts as a template for ice formation, and their length (ca. 800–1,300 amino acids) is correlated with the amplitude of ice nucleation activity; the C-terminal domain is required for ice-nucleation activity as demonstrated with mutants (Kawahara 2008).

14.2.5.3 Antifreeze Proteins

Antifreeze proteins (AFPs) are ice-binding proteins that have the ability to modify the ice crystal structure and inhibit the growth of ice in two ways. (1) Prior to freezing, they lower the freezing point of water without altering the melting point (thermal hysteresis activity). (2) In the frozen state, AFPs show ice recrystallization inhibition activity, whereby the proteins inhibit the growth of large crystals at the expense of small crystals at subzero temperatures (Gilbert et al. 2004).

AFPs have been detected in bacteria, fungi, plants and animals (Margesin et al. 2007). Bacterial and plant AFPs generally show substantially lower thermal hysteresis compared to AFPs from animals. Insects and fish have up to 2°C and 5°C of thermal hysteresis, respectively, while bacterial and fungal representatives show values of $\leq$0.1°C (Gilbert et al. 2005; Hoshino et al. 2009). Bacteria that produce AFPs with a low thermal hysteresis activity, however, use the recrystallization inhibition activity of the AFPs (Xu et al. 1998; Yamashita et al. 2002; Gilbert et al. 2005). As an exception, the AFP produced by the Antarctic lake-ice bacterium *Marimonas primoryensis* has a thermal hysteresis activity (lowers the freezing point of water) of more than 2°C, which is higher than the maximum activity of most fish AFPs. The protein is Ca^{2+}-dependent and located in the periplasmic

space, while bacterial and fungal AFPs are generally secreted extracellularly (Gilbert et al. 2004; Gilbert et al. 2005).

Thus, bacteria may apply different strategies: freeze tolerance can be obtained with low levels of thermal hysteresis activity but high recrystallization inhibition activity, a strategy similar to the one employed by some plants (rye grass, carrot, winter rye) (Griffith et al. 1992; Worrall et al. 1998; Sidebottom et al. 2000). On the other hand, freeze avoidance by high thermal hysteresis activity can inhibit the growth of ice crystals before they propagate into the bacterium (Gilbert et al. 2005).

Among AFP-producing bacteria from Antarctic lakes, members of the *Gammaproteobacteria* dominated (Gilbert et al. 2004). The AFP produced by an Arctic plant growth promoting rhizobacterium (*Pseudomonas putida*) is an extracellular glycolipoprotein that also has ice-nucleating activity (Xu et al. 1998).

In fungi, extracellular AFPs are assumed not only to prevent hyphae from freezing, but also to ensure substrate availability by preventing nutrients from freezing at subzero temperatures (Robinson 2001). AFPs have been detected in psychrophilic phythopathogenic fungi causing snow molds. These fungi belong to various taxa (*Oomycetes, Ascomycetes* and *Basidiomycetes*), grow at temperatures as low as $< -7°C$, and can grow and attack dormant plants (crops, winter cereals and conifer seedlings) at low temperatures under snow cover (Hoshino et al. 2009). Basidiomycetous snow molds produce extracellular AFPs to keep the extracellular environment unfrozen, which, however, does not support mycelial growth. In contrast, the ascomycete *Sclerotia borealis* does not produce extracellular AFPs but grows at subzero temperatures due to osmotic stress tolerance; its mycelial growth is even higher under frozen conditions compared to unfrozen conditions (Hoshino et al. 2009).

14.2.5.4 Exopolymers

Exopolymeric substances (EPS) are complex organic materials composed primarily of high-molecular mass exopolysaccharides. Exopolysaccharides contain major amounts of hexose and pentose. Contrary to intracellular adjustments to cold stress, EPS are secreted as mucous slime by many aquatic microorganisms. Key functions of EPS include the mediation of adhesion to wet surfaces and the formation of the biofilm matrix, which traps nutrients, protects the cell against unfavorable environmental conditions and mediates biochemical interaction (Mancuso Nichols et al. 2005). EPS production is high in bacteria living in aquatic environments; high EPS abundance has been found in Antarctic and Arctic sea ice (Krembs et al. 2002; Mancuso Nichols et al. 2005).

14.2.6 Antioxidant Defense

Protection against reactive oxygen species (ROS) is important for survival at low temperatures where the solubility of gases is increased. ROS can result in significant damage to cell structures. Bacterial strategies for the detoxification of ROS include the production of high amounts of antioxidant enzymes (catalase, superoxide dismutases, dioxygen-consuming lipid desaturases) or the absence of ROS-producing pathways. *Pseudoalteromonas haloplanktis* employs both strategies; it entirely lacks the ROS-producing molybdopterin metabolism. In addition, the bacterium produces dioxygen-consuming lipid desaturases in order to obtain protection against oxygen and to maintain membrane fluidity at the same time. By contrast, *Colwellia psychrerythraea* achieves an enhanced antioxidant capacity through the presence of catalase and superoxide dismutases (Medigue et al. 2005; Methé et al. 2005).

14.2.7 Genomic and Proteomic Insights into Microbial Cold Adaptation

Microbial adaptation to low temperatures requires a vast array of metabolic and structural adjustments at nearly all organization levels of the cell, which are gradually being understood thanks to the availability of genome sequences and proteomic studies of a number of psychrophilic bacteria. A survey of these data shows that the main up-regulated functions for growth at low temperatures are protein synthesis (transcription, translation), RNA and protein folding (adaptation of the molecular structure of proteins to ensure increased flexibility at low temperatures), maintenance of membrane fluidity, production and uptake of compounds for cryoprotection (extracellular

polysaccharides, compatible solutes), antioxidant activities and regulation of specific metabolic pathways. However, only few features are commonly shared by all psychrophilic genomes and proteomes, which suggests that cold adaptation superimposes on pre-existing cellular organization and, accordingly, the strategies to cope with cold environments may differ among psychrophiles (Medigue et al. 2005; Methé et al. 2005; Kurihara and Esaki 2008; Riley et al. 2008; Bakermans et al. 2009; Qiu et al. 2009).

14.3 Microbial Activity and Biodiversity in Alpine Soils

Compared to the Arctic, the European Alpine region is characterized by higher maximum temperatures, lower minimum temperatures, large and frequent (diurnal) temperature fluctuations and freeze-thaw events, higher precipitation (up to 2,000–3,000 mm per year) and air humidity, lower atmospheric pressure, and higher intensity of solar radiation.

Alpine microorganisms are equally well-adapted to low temperatures as polar microorganisms. The comparison of cold-active enzymes (pectate lyase) from alpine and Siberian psychrophilic yeasts (*Mrakia frigida*) clearly showed that the enzymes produced by these strains had an almost identical activity and stability pattern (Fig. 14.1). Both enzymes were thermolabile, but resistant to repeated freezing and thawing (Margesin et al. 2005). The two strains had almost identical growth characteristics (high cell densities at 1–15°C, no growth above 20°C), yet their enzyme production patterns were completely different. The Siberian strain produced pectate lyase over the entire growth temperature range, with a maximum at 1°C, whereas enzyme production by the alpine strain was highest at 5°C, very low at 15°C and absent at 20°C. Enzyme production patterns may be related to the natural environmental conditions of the strains.

14.3.1 Soil Microbial Activity at Low Temperatures

Soil microorganisms play an essential role in soil organic matter turnover and biogeochemical cycling. Soil microbial activity and community composition are influenced by a number of biotic and abiotic factors, such as vegetation type, soil type, and a range of environmental conditions including temperature. Low temperature is not a limiting factor for microbial activity in cold soils. There is evidence of a wide range of metabolic activities in all cold ecosystems; microbial activity in soil has been reported to occur at subzero temperatures down to -20°C (Lipson and Schmidt 2004; Panikov and Sizova 2007) and substantial carbon mineralization has been described to occur in cold soils during winter months (Clein and Schimel 1995).

A change in temperature affects soil microbial communities and nutrient cycling (Uchida et al. 2000; Hart 2006). Microbial activities in cold soils respond quickly to seasonal changes (Lipson 2007; Edwards and Jefferies 2010). In seasonally frozen soils from some alpine and arctic sites, microorganisms metabolize slowly at subzero temperatures, presumably in contact with unfrozen water. However, microbial biomass declines in late winter (at the winter-spring transition), before the soil temperature rises above 0°C. This decline in biomass has been attributed to low levels of available nutrients, rupture of cell membranes due to repeated freeze-thaw cycles, and the loss of compatible solutes from viable cells due to an abrupt change in osmotic potential (Jefferies et al. 2010).

Like polar microorganisms, psychrophilic alpine microorganisms, able to grow and to be active at low temperatures, play a key ecological role in their natural habitats. Measurement of microbial activities in the Austrian Central Alps at altitudes of 2,300–2,500 m a.s.l. included litter decomposition, CO_2-release and enzyme activities (phosphatase, urease, xylanase, cellulase). Soil activities were generally lower on wind-exposed sites and were low in poorly drained soils of the snowbed, which was explained by a deficiency of substrates and frequent drought stress during the vegetation period. Irrespectively of the site, soil microbial activities increased immediately after the frozen topsoils thawed, when bacterial and fungal populations increased (Schinner 1982a, 1983). Another factor influencing soil microbial respiration and enzyme activities is soil depth; activities were considerably higher in surface layers of alpine soils and sharply decreased with depth. Soil microbial activities are further influenced by vegetation. For example, activities in soils with

alpine dwarf shrubs (*Loiseleuria procumbens*) were higher by a factor of five compared to activities in *Carex curvula* grassland soils (Schinner 1982a).

The degradation of xylan, the major polysaccharide in plant cell walls, occurs mainly by microbial xylanases. Recently it has been shown that xylanase activities in cold alpine tundra soil are very diverse and widely distributed among soil bacteria; they could be clustered into six groups and were related to xylanases from *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Firmicutes* and *Acidobacteria* (Wang et al. 2010).

Soil microbial respiration is a critical component of the global carbon cycle. In subalpine coniferous forest soil, microbial communities isolated from under-snow soil were characterized by high biomass-specific respiration rates, i.e. higher growth rates and lower growth yields. Bacteria may contribute to soil heterotrophic respiration to a greater extent, as demonstrated by higher bacterial growth rates and lower growth yields compared to those from fungi. In winter, psychrophilic bacteria of the genus *Janthinobacterium* dominated (Lipson et al. 2009).

Litter decomposition is an important factor in nutrient cycling. Microorganisms decomposing plant litter belong to phylogenetically diverse taxa. In cold Arctic and Antarctic ecosystems, wood decomposition appears to proceed via "soft rot" by anamorphous ascomycetes, rather than by "white rot" or "brown rot" basidiomycetes (Ludley and Robinson 2008).

Cold periods during the growing season can significantly limit the symbiotic association of legumes with rhizobia. Cold-adapted rhizobia, isolated from alpine or arctic legumes, are useful to improve the symbiosis under cold stress. Arctic rhizobia increased the production of legumes by 30% through improved nitrogen fixation (Prevost et al. 2003).

14.3.2 Microbial Activity and Biodiversity Related to Altitude

The change of temperature and other environmental conditions with altitude in mid-latitude mountains has often been compared to their change with latitude: a 1,000 m higher altitude in the Alps may roughly be equivalent to a 1,000 km move northward (Kuhn 2008). Thus, temperature gradients in mountains can be similar to those relating to latitude; the altitude-controlled vegetation belts on mountain slopes represent an analogue to the different latitudinally controlled climatic zones. The annual average temperature decreases with increasing latitude; in mountain areas the temperature decreases with increasing altitude. While climate changes (e.g. temperature decrease) are spread over thousands of kilometres along latitude gradients, they occur on a comparatively small scale along altitude gradients, which makes mountain regions useful for climate change studies (Diaz et al. 2003).

Altitudinally defined climatic conditions, soil properties, and vegetation regulate microbial community structures and metabolic rates in mountain soils (Whittaker 1975; Schinner and Gstraunthaler 1981). An increase in altitude, and thus in environmental harshness (lower annual temperature, lower soil nutrient contents), generally results in a decrease in microbial abundance and activity (respiration rate, microbial biomass, litter degradation, enzyme activities), as well as in shifts in microbial (bacterial and fungal) community composition (Schinner and Gstraunthaler 1981; Schinner 1982b; Väre et al. 1997; Ma et al. 2004; Giri et al. 2007; Lipson 2007; Niklinska and Klimek 2007). With increasing altitude, and thus colder climate conditions (lower air and soil temperatures, more ice and frost days, higher precipitation) over a gradient ranging from 1,500 to 2,530 m in the Austrian Central Alps, a number of significant changes were observed: an increase in altitude resulted in a significant decrease of bacterial and fungal biomass, on one hand, and in a significant increase in the relative amounts of psychrophilic heterotrophic bacteria and fungal populations, on the other hand. Gram-negative bacteria detected by FISH (fluorescence in situ hybridization) increased with altitude. Since FISH is based on the detection of rRNA, and the rRNA content is associated with the metabolic state of microbial cells, FISH-detected cells represent the active, ecologically relevant part of the microbial community (Wagner et al. 2003). *Proteobacteria* dominated at high altitudes, while the amount of members of the *Cytophaga-Flavobacterium-Bacteroides* group decreased with altitude (Margesin et al. 2009).

With increasing altitude, and thus colder climate conditions, microorganisms are better adapted to the cold. Microbial activity (soil dehydrogenase) decreased with altitude, yet relative activities at low temperatures were significantly higher in alpine than

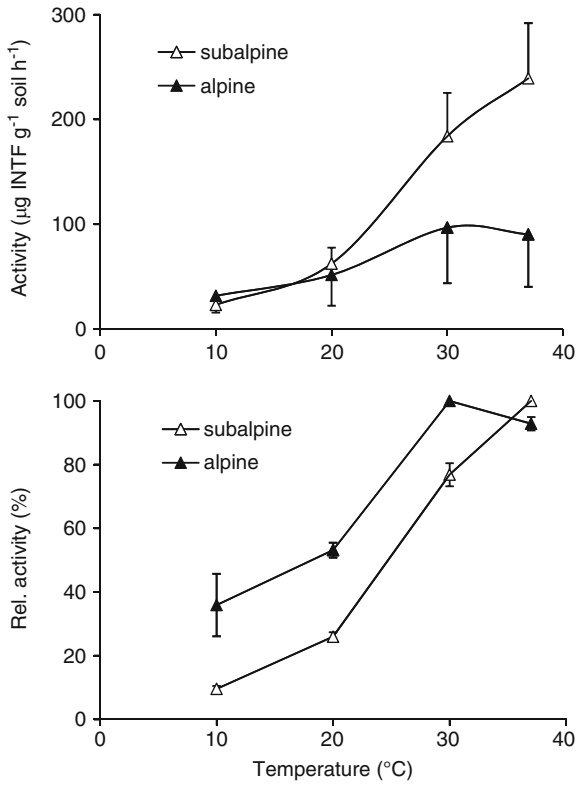

Fig. 14.2 Effect of temperature on soil dehydrogenase activity (*top*) and on the relative enzyme activity (*bottom*; maximum activity as determined in the figure on *top* = 100%) in subalpine (1,500–1,900 m) and alpine (2,100–2,530 m, above the forest line) soils (Margesin et al. 2009). INTF = iodonitrotetrazoium formazan

in subalpine soils (Fig. 14.2), which means that enzymes from soils from higher altitudes are better adapted to the prevailing cold climate conditions. This can be attributed partly to the influence of altitude on physicochemical properties; e.g., lower contents of clay, humus and nitrogen due to unfavorable conditions for soil formation with increasing altitude; slower nutrient cycling at high altitudes due to cold temperatures could possibly affect organic matter structure and quality (Margesin et al. 2009).

Similarly to European alpine soils, the diversity of the psychrophilic bacterial community in high altitude cold soils of the Himalayan mountains decreased with increasing altitude. The culture-independent approach revealed a dominance of *Preoteobacteria*. However, viable bacteria consisted of almost equal amounts of Gram-negative bacteria (with a dominance of *Gammaproteobacteria* and a low amount of *Bacteroidetes*) and Gram-positive bacteria (with a dominance of *Firmicutes*). Isolates produced a number of hydrolytic enzymes; the most frequently observed enzyme was lipase (Gangwar et al. 2009). The abundance of ammonia-oxidizing bacteria and archaea in high-altitude soils (4,000–6,500 m) of Mt. Everest was also influenced by altitude. Archaeal ammonia oxidizers were more abundant than bacterial ones at altitudes below 5,400 m, while the situation was reversed at higher altitudes (Zhang et al. 2009).

Soils at high altitudes (3,000–5,400 m) in Annapurna Mountains, Nepal, are characterized by low water activity due to dry climate, and consequently these soils contained psychrophilic fungi with xerophilic characteristics; the most extreme xerophiles belonged to the ascomycetous genera *Eurotium* and *Aspergillus* (Petrovic et al. 2000). *Chytridiomycota* dominated fungal diversity in periglacial soils at high altitudes in the Himalayans and Rockies, which can be attributed to the high abundance of carbon sources that support chytrid growth (eolian deposited pollen and microbial phototrophs) as well to the saturation of soils with water under snow (Freeman et al. 2009).

Conclusions

A change in temperature has an immediate effect on all cellular processes of microorganisms, since they are too small to insulate themselves from the cold or to use avoidance strategies such as moving away from thermal extremes. Therefore, they alter their cellular composition. To survive and grow successfully in cold environments, psychrophilic microorganisms have therefore evolved a complex range of adaptations of all their cellular constituents, which enable them to compensate for the negative effects of low temperatures on biochemical reactions. The main up-regulated functions for growth at low temperatures are protein synthesis (transcription, translation), RNA and protein folding, maintenance of membrane fluidity, production and uptake of compounds for cryoprotection (extracellular polysaccharides, compatible solutes), antioxidant activities and regulation of specific metabolic pathways. The emerging fields of genome and proteome analyses will give further new insights into the psychrophilic lifestyle.

Microorganisms in cold soils play an essential role in organic matter turnover and biogeochemical cycling. Like polar microorganisms, psychrophilic alpine microorganisms, able to grow and to be active at low temperatures, play a key ecological role in their natural habitats. An increase in environmental harshness (e.g. lower air and soil temperatures, more frost and ice days, higher precipitation at higher altitudes) generally results in a decrease in microbial abundance and activity, as well as in shifts in microbial community composition. On the other hand, microorganisms living in colder climate conditions are better adapted to the cold, as shown by higher relative amounts of psychrophilic bacterial and fungal populations and higher relative enzyme activities.

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