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Cold-adapted Microorganisms in Alpine Environments

Rosa Margesin

Institute of Microbiology, Leopold Franzens University, A-6020 Innsbruck, Austria

Microorganisms have successfully colonized cold environments because they have evolved special mechanisms to thrive successfully at low temperatures. A wide diversity of microorganisms has been isolated from various cold environments. Compared to the Arctic and Antarctica, there is little knowledge of microbial life in alpine ecosystems. The term "alpine" is generally accepted as a term for a high-altitude vegetation belt above continuous forests on mountains, ranging from ca. 1800 to 2500 m above sea level in the European Alps. Alpine environments are subjected to large temperature fluctuations, high precipitation and frequent freeze-thaw events.

A largely unexplored habitat is glacier cryoconite. The term "cryoconite" (ice dust) is a description for microcavernes (melt holes) on the ice surface, which contain dark-colored organic and inorganic material and are saturated with cold meltwater. The characterization of culturable viable heterotrophic microorganisms from **alpine glacier cryoconite** showed the predominance of bacteria over yeasts and filamentous fungi. The proportion of microorganisms able to grow at 2°C but unable to grow above 20°C was higher among yeasts than among bacteria. A high percentage (55-83%) of heterotrophs utilized various organic compounds s the sole source of carbon (cellulose, lignin) or nitrogen (casein) at 2°C, which points to the ecological significane of cold-adapted microorganisms in this habitat. 33% of the isolates utilized diesel oil, showing the ubiquity of hydrocarbon degraders. However, only 1-3% utilized xenobiotic compounds (polyaromatic hydrocarbons) for growth.

The identification of selected alpine isolates resulted in the detection of novel bacterial (*Pedobacter cryoconitis*, *Arthrobacter psychrophenolicus*) and yeast species (*Rhodotorula glacialis, Rh. psychrophila, Rh. psychrophenolica*).

Further studies were focused on **cold-active enzymes** from alpine microorgansims. As examples, pectate lyase and protease production will be demonstrated. Such enzymes could be useful for a wide range of applications, e.g. low-temperature pretreatment of wastewater containing pectic substances or useful new tools in molecular biology. The psychrophilic yeast *Mrakia frigida* A15 was isolated from glacier cryoconite and showed good growth at 1-15°C; production of alkaline pectate lyase was highest

at 5°C. *Pedobacter cryoconitis* A37^T, also isolated from alpine glacier cryoconite, produced highest amounts of an extracellular metalloprotease at 15°C. Both enzymes were cold-active; they displayed apparent optimal activity at 30°C (pectate lyase) or 40°C (protease) and were thermolabile, but resistant to repeated freezing and thawing.

To investigate **low-temperature biodegradation**, the potential of alpine cold-adapted bacteria and yeasts to degrade **phenol** was examined. Phenol and phenolic compounds are widely distributed in nature and as environmental pollutants. In cold climatic regions, wastewater temperature can decrease to 10°C and below, which requires the activity of cold-adapted degraders. Yeast strains were characterized by a lower apparaent optimum temperature for growth and phenol biodegradation compared to bacterial strains. Representatives of the genera *Rhodococcus*, *Arthrobacter* and *Pseudomonas* utilized up to 12.5 mM phenol as the sole carbon source. All yeast strains investigated were basidiomycota (*Cryptococcus, Rhodosporidium*, *Rhodotorula*, *Mastigobasidium*, *Sporobolomyces, Trichosporon*) and degraded up to 15 mM phenol at 10°C. Investigations on the biodegradability and toxicity of phenol and phenol-related monoaromatic compounds showed that *Rhodotorula creatinivora* strains were characterized by a higher metabolic versatility and higher IC_{50} values than other yeast species.

Studies on the feasibility of **low-temperature bioremediation of alpine soils contaminated with petroleum hydrocarbons** demonstrated the significance of indigenous soil microorganisms. Enrichments of oil degraders occurred soon after contamination. Biostimulation of the indigenous soil population with nutrients resulted in a significant increase of hydrocarbon biodegradation and was more efficient than bioaugmentation with cold-adapted hydrocarbon-degrading inocula. The feasibility of bioremediation as a treatment option for a diesel-oil-polluted soil in a Tyrolean glacier ski resort (3000 m above sea level) was determined during a 3-year field study. To examine the efficiency of natural attenuation and biostimulation, soil in mesocosms remained untreated or was amended with an inorganic slow-release N-P-K fertilizer. Most of the hydrocarbon loss occurred during the first summer season (42% loss) with fertilization and during the second summer season (41% loss) without fertilization. At the end of the third summer season, the initial contamination was reduced by 70% and 50% in the fertilized and unfertilized soil, respectively, resulting in a still high residual contamination. Nonetheless, the initial fertilization treatment was appropriate in termes of accelerated biodegradation.

The molecular characterization of 12 alpine soils contaminated with petroleum hydrocarbons (0.4-30 g TPH/kg soil) and corresponding pristine soils demonstrated a different distribution pattern of bacterial classes depending on the contamination. Among Proteobacteria, the relative amount of microorganisms belonging to the Alphaproteobacteria was larger in pristine (46%) than in contaminated (24%) soils, whereas Beta- and Gammaproteobacteria were only detected in the contaminated soils. Actinobacteria were present to a similar extent in contaminated (20%) and pristine (18%) soils. This was confirmed

in a study on the prevalence of genotypes involved in the degradation of representative fractions of petroleum hydrocarbons. Hydrocarbon-degrading genotypes, derived from Gram-negative representatives (*Pseudomonas* and *Acinetobacter*) of the Gammaproteobacteria, were detected to a significantly higher percentage in contaminated (50-75%) than in pristine (0-12.5%) soils, indicating that these bacteria had been enriched following contamination. In contrast, Gram-positive members of the Actinobacteria phylum, represented by *Rhodococcus* and *Mycobacterium*, were already found in soils before contamination events occur. These results demonstrated a significant shift in the microbial community structure in alpine soils following contamination.