

## CURRENT MICROBIOLOGICAL ASPECTS IN HIGH MOUNTAIN LAKE RESEARCH

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### ABSTRACT

Remote and normally unpolluted high mountain lakes provide habitats with no or very limited anthropogenic influences and, therefore, their hydrodynamics are mostly regulated by the natural conditions. Researches in high mountain lakes deal with measuring and modeling the response of the habitats to environmental changes especially correlated to acid deposition, pollutants influx and climatic variability. The microbial world has also become a focus in many studies of these extreme ecosystems. Despite the pressure of harsh and extreme conditions, microbial communities living in these habitats developed flexible strategies and show quick adaptation to climate oscillation. New aspects in microbiological studies in recent high mountain lake research are presented in this paper.

**Key words** : High mountain lake, extreme environment, microbial communities, microbial diversity, psychrotolerant microbe, molecular microbe

### INTRODUCTION

In recent years, interests in the presence and survival of microorganisms which inhabit extreme environments have been increasing. Attempts to discover novel microorganisms possessing special metabolic characters have encouraged microbiologists to intensive researches on microorganisms which are able to survive and sustain in extreme habitats. Extremophiles dwelling in extreme environments have attracted the interest of microbial ecologists because of the uniqueness of peculiar physiological properties.

Life strategies of microbes evolved in low temperatures and nutrient limited environments have also attracted the attention of those who are studying conditions for life on other planets, such as Mars (Monastersky 1997). Naturally cold ecosystems, such as Antarctic and Arctic regions, cryoconite holes, ice-covered lakes, the deep sea, high altitude mountain environments and glaciers (Morgan-Kiss *et al.* 2006) may represent analogs on earth to the cold extra terrestrial habitats.

High mountain ecosystems are also of ecological interest because of their richness in natural resources, their biodiversity, the clean water, healthy climate, original soil, and diverse geology which are relevant to issues of environmental protection. On the other hand, the utilization of the mountain areas e.g. for the tourist industry is an issue of socio-economic importance.

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Microbiologists are mainly interested in the following questions:

- What microbial diversity is present in high mountain lake habitats? Are there unknown microorganisms which are characteristic for the extreme conditions?
- Are psychrotolerant organisms phylogenetically closely related to each other?
- Do microbes present in oligotrophic cryo-habitats possess special life strategies?
- Do the communities possess physiological and ecological flexibilities with which they can respond to the fluctuating physico-chemical conditions?
- What are the ecological parameters determining the community structures and how rapidly do they respond to environmental changes?

In tropical regions, the extreme cold environment can be found for instance in areas at high elevations of ice-covered mountains, i.e. Mount Jayawijaya in Papua Island, Indonesia, Mount Kilimanjaro in Tanzania, and the Tropical Andes in South America. Despite of the cold temperature and oligotrophic characteristics, these ecosystems are different to those found in temperate and polar regions. They exist only at altitudes of 4500 m a.s.l. and higher, whereas in temperate and polar regions they normally begin at lower altitudes (e.g. > 1 400 m a.s.l.) depending on the season.

Strong UV radiation, because of the continuous sun ray exposure throughout the year and thinner ozone layer at higher altitudes, might influence the physico-chemical characteristics of tropical mountain habitats. In addition, atmospheric deposition of pollutants (acidity and toxic air pollutants) that strongly influences the ecology of temperate high mountain lakes (Mosello *et al.* 2002), may occur less in tropical mountain habitats, because of the higher elevation. So far, very limited information is available on (sub) tropical high mountain lake habitats. Therefore, this article presenting various studies on temperate high mountain areas may encourage for new research and exploration of our tropical high mountain lake habitats.

### **High mountain lake habitats as indicators of environmental changes**

#### *Global warming indicators*

For decades mountain areas have been used as sites to evaluate possible effects of global warming and its consequences. They are sensitive indicators for the early detection of the impacts of rapid climatic changes on the hydrological cycle, water chemistry and ice melting. Even a small temperature change can strongly influence their hydrological cycle (Schindler *et al.* 1990). The climatic warming observed in the last 25-30 years seems to be most pronounced in the mountain regions all over the world (Beniston *et al.* 1997; Rogora *et al.* 2003). The temperatures measured in these areas have increased by 1.5 - 2.0°C on the average since 1980, whereas an increase of approximately 0.5°C was observed on a global scale (Beniston *et al.* 1997). The climatic changes and environmental fluctuations particularly affect open waters of high mountain lakes (Psenner and Schmidt 1992; Sommaruga-Wograth *et al.* 1997; Koinig *et al.* 1998). In addition, other factors such as the atmospheric dust

deposition (Psenner 1999) and long periods of anthropogenic impacts (Kamenik *et al.* 2000) also shape the ecology of these areas.

The duration and extent of the snow cover in a lake catchment area are dominant factors governing the release of weathering products from rocks and soils into the water (Wright and Schindler 1995). A recent study shows that the effect of climate warming has influenced the weathering rate in the mountains (Rogora *et al.* 2003). Through the chemical and physical degradation of rocks and soil minerals, solute contents increased. Warmer temperatures are also enhancing biological processes by increasing the primary productivity.

### **Biochemical characteristics and dynamics of high mountain lake habitats**

#### *Trophic condition of high mountain lake habitats*

High altitude lakes are normally situated above the timberline and have catchment areas with little or no vegetation. Consequently, they are often oligotrophic as defined by the concentration of nitrogen and phosphorus. Examples include the lakes which became subject of the European project MOLAR (Mountain Lake Research) such as in the High Tatra Mountains (Slovakia, 2 655 m a.s.l.) (Kopacek *et al.* 1995), and the Sumava Mountains (Czech, 1456 m. a.s.l.); Schwarzsee (Austria, 2799 m a.s.l.), Lake Paione Superiore (Italy, 2269 m a.s.l.) (Mosello *et al.* 2002), Yellow Belly Lake (U.S.A, 2157 m a.s.l.) (Pilati and Wurtsbaugh 2003), Gossenkollesee (Austria, 2417 m a.s.l.) (Kamenik *et al.* 2000), Jori lakes III and VII (Switzerland, 2557 m a.s.l.) (Hinder *et al.* 1999a). Lake Jori XIII does not meet the oligotrophy criteria especially in summer season. Low N:P ratios were measured at the end of the ice cover period, whereas the highest ratios were measured during the ice free period (Iqbal-Nava 2003).

The physico-chemical conditions strongly depend on the duration of the ice and snow cover. The nutrient conditions in Lake Jori XIII initiate high biomass and remarkable microbial activities and population dynamics.

#### *Atmospheric deposition influences mountain lakes' hydrochemistry*

Atmospheric depositions have been demonstrated to be one of the key factors to influence long-term changes in the chemical characteristics of high mountain lakes (Kamenik *et al.* 2000). Airborne desert dust depositions contain major basic cations, carbonates, sulphates and other anions of nutrient value for residents of remote lakes (Psenner 1999). The high calcium contents of the Saharan aerosols, which are also found in aerosols from other deserts, might significantly contribute to the biogeochemical cycles involving acid neutralization (De Angelis and Gaudichet 1991). Airborne dusts in snow and rain have also contributed to the high buffering capacity of some mountain lakes and to the elevated seasonal nutrient levels (De Angelis and Gaudichet 1991; Psenner 1999).

*Environmental changes demonstrated by microbial community succession*

Physicochemical conditions in high mountain ecosystems are exposed to strong environmental fluctuations, seasonally as well as diurnally. These habitats (Figure 1) are good models for studies of microbial dynamics of cold environments with a simple and short food web. During the long ice-cover period, they are exposed to cold temperatures, nutrient limitation, darkness below the thick ice cover, and anoxic conditions at larger water depths. Whereas in the short summer season, they are exposed to high sun radiation, oxic conditions and wind-driven water masses upwelling. Despite low nutrient input and commonly limited concentrations, some lakes also showed self-trophication capability, which is particularly observable during the summer season (Iqbal-Nava 2003). These environmental pressures provide heterogeneous ecological niches in the habitats. Therefore, the microbial community adapts itself by continuously selecting for those members which are best adapted to the particular conditions in the ecosystem.

A recent study observes dynamic changes in planktonic bacterial community composition, seasonally as well as spatially (Yuhana 2005). These changes are a consequence of strongly fluctuating environmental conditions in which the communities show a quick response to fulfill the requirements for various



Photo by Munii Yuhani

ecological niches. Their dynamics were not only demonstrated by the seasonal changes in the genera diversities, but also their abundances. The quick adaptation was also observed in the planktonic microalgal populations. The autotrophic and heterotrophic flagellate as well as ciliate communities showed dynamics and high activities as indicated by high [ $^3\text{H}$ ]thymidine and [ $^3\text{H}$ ]leucine or [ $^{14}\text{C}$ ]leucine uptake rates during the short ice-free period (Felip *et al.* 1995; Hinder *et al.* 1999a). Their population was supported with the availability of organic matters as nutrients which originate from allochthonous and autochthonous sources. Two different external

sources can contribute to nutrient enrichment inside a lake: atmospheric input or input from the catchment. The input can consist of dissolved organic matter, organisms, particulate matter, vegetation debris including pollen grains, and insects (*Fclipetal.* 1995).

### **The role and the physiological adaptation of microorganisms in high altitude habitats**

#### *The role of microorganisms in high altitude habitats*

In aquatic ecosystems, the bacterioplankton community is an integral part of the food web, which is essential for protists and metazoans (Cho and Azam 1988). Temporal and spatial alterations of the microbial community in pelagic food webs of high mountain lake habitats have been studied (Felip *et al.* 1995). Recent studies focus on more specific subjects such as the effect of the UV radiation on the bacterivory (Sommaruga *et al.* 1996), microbial diversity and activity (Alfreider *et al.* 1996) and palaeolimnology (Koinig *et al.* 1998). Compared to eutrophic ecosystems, the pelagic food webs in these oligotrophic ecosystems appeared to be less complex and the microbial loops might play an essential role in recycling nutrients for the higher trophic levels (Hinder *et al.* 1999b). The significance of the pelagic microbial assemblage increases with the level of oligotrophy of the lake water (Hahn *et al.* 1999). This is true for pelagic food webs in circumcontrol as well as in acidified high mountain lakes (Wille *et al.* 1999).

Microorganisms in high mountain habitats play a key role in biogeochemical cycles such as subglacial rock weathering and nutrient cycling. Microorganisms living in phosphate-limited mountain lakes are able to mobilize particulate iron phosphates from sedimentary deposits and biofilm-associated microbes can develop on iron-phosphate-oxyhydroxide coated surfaces (Amberg-Brunner 2002). Microbially mediated pyrite oxidation occurs at low temperature and glacier bed microbial communities contribute to sulfate release to the environment (Sharp *et al.* 1999). From these biochemical processes, furthermore the microbial population derives energy from the oxidation of reduced mineral or organic carbon within the sediments.

#### *Physiological adaptation to low temperature*

Organisms that live and actively grow at near freezing temperatures and limited-nutrient conditions face a number of growth constraints. Under the cold conditions the enzyme reaction rates are generally lower, uptake and transport systems function more slowly, membranes become less fluid and nucleic acid structures become more stable (Feller *et al.* 1996; Graumann and Marahiel 1996). However, microorganisms have evolved various strategies to adapt to these hindrances. They range from molecular level to cell and ecosystem levels (Gerday *et al.* 1997). The evolution of cold shock and antifreeze proteins, the modulation of the kinetic key enzymes, and the development of more fluid biological membranes

through the accumulation of polyunsaturated fatty acyl chains are among the means of adaptation to low temperature (Morgan-Kiss *et al.* 2006).

#### *Adaptation to high-level UV exposure*

Attenuation coefficients (K<sub>d</sub>) of the photosynthetically active radiation (PAR) and UV were used for indirect determination of the chlorophyll concentrations. K<sub>d</sub> values, which are based on the upper and lower UV radiation intensities, are used to illustrate the strength of UV intensity at different wave lengths i.e. 305, 320, 340, and 380 nm. Results from Iqbal-Nava's study (2003) showed that the diffuse attenuation coefficients (K<sub>d</sub>) measured in Swiss Alps Lake Jori XIII (ca. 2640 m a.s.l.) were ranging from 0.68 to 1.54. Whereas K<sub>j</sub> values of the Austrian Lake Gossenkolle at 2417 m a.s.l were ranging from 0.14 to 0.32 (Sommaruga and Psenner 1997), and the mean K<sub>d</sub> values of 13 oligotrophic lakes in the Bariloche region in Argentina (ca. 2000 m a.s.l.) were between 0.3 and 0.8 (Morris *et al.* 1995).

Microorganisms living at high altitudes are challenged by intense UV radiation. UV B radiation (280-320 nm) is potentially the most damaging for living cells (Morris *et al.* 1995). The UV B level in the winter cover of high mountain lakes can reach up to 50% higher than at sea level (Psenner and Sattler 1998). The strong UV exposure can lead to growth inhibition of benthic diatoms (Bothwell *et al.* 1994); causes damage in heterotrophic flagellates (Sommaruga *et al.* 1996), and inhibits the rate of nutrient uptake by bacterioplankton in the water column (Sommaruga *et al.* 1997). Some adaptive strategies against strong UV radiation, that were found in permanently low temperature high mountain habitats are, for instance, the capability to screen UV and the photosynthetically active radiation (PAR) by *Chlamydomonas* sp. In addition, *Chloromonas* sp. possesses UV-screening pigments called mycosporine-like amino acids (MAA), whereas some Cyanobacteria like *Nostoc*, *Phormidium*, and *Anabaena* spp. segregate a mucopolysaccharide matrix (Morgan-Kiss *et al.* 2006).

#### **Molecular approaches applied to study the microbial succession in high mountain aquatic ecosystems**

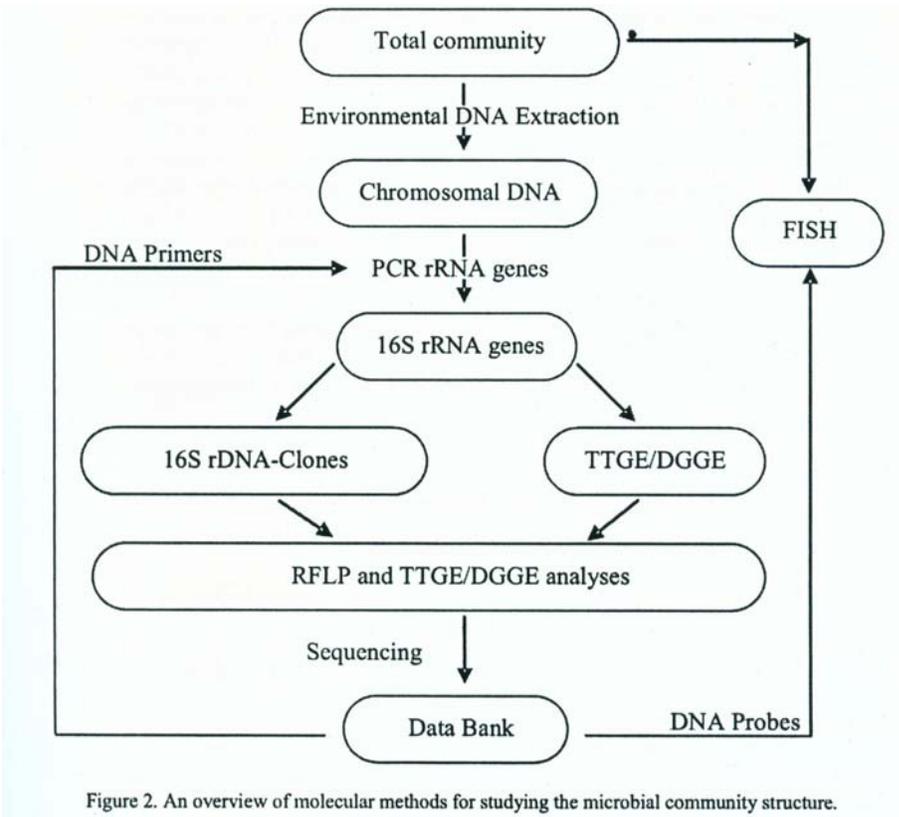
PCR-based methods provide information on the nucleic acid composition of microorganisms isolated from different habitats which mostly are still not (yet) cultivable. When Carl Woese introduced the use of 16S rRNA sequences for molecular phylogeny (Woese 1987), only 12 microbial phyla could be phylogenetically compared. Currently, 26 phyla of approximately 52 identifiable major phyla within the bacterial domain have cultivated representatives (Rappe and Giovannoni 2003). The number of environmentally retrieved bacterial 16S rRNA genes has been increasing rapidly and now is exceeding 30 000 (Rappe and Giovannoni 2003; Wagner 2004). Carl Woese's approach has also uncovered a new domain of life, the *Archaea*. Formerly, *Archaea* were thought to exclusively consist

of thermophiles, halophiles, and strictly anaerobic methanogenic microorganism inhabiting extreme environments. Today, *Archaea* are recognized as ubiquitous microorganisms, also present in high mountain lake habitats (Yuhana 2005).

In recent years, advanced techniques have been developed to study the composition of microbial communities (Figure 2). PCR-based community fingerprinting techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) or Temporal Temperature Gradient Gel Electrophoresis (TTGE) allow us to monitor the microbial structure based on community banding patterns. These techniques are based on the separation of the PCR products of genes isolated from mixed populations possessing different nucleotide sequences (Muyzer *et al.* 1993). Nucleic acid is extracted from the natural samples and DNA amplification is performed by PCR with primers targeting for instance the small subunit ribosomal RNA genes. The PCR products are subsequently analyzed by loading them onto a polyacrylamide gel containing a linearly increasing gradient of denaturants (in DGGE) or a linearly increasing temperature gradient (in TTGE).

By using DGGE or TTGE, the diversity and microbial community composition can be described without enriching them or performing the cloning techniques (Muyzer and Smalla 1998; Muyzer 1999). The number of bands on a gel may not accurately reflect the number of different species in their habitat, but the DNA of the most abundant representatives of the communities normally gets amplified and should be represented in the band pattern (Muyzer *et al.* 1993). These techniques can also be applied for the screening of clone libraries (e.g. Bosshard *et al.* 2000). Both techniques provide an alternative way for microbial community fingerprinting without application of cloning strategies which is more time consuming. DGGE and TTGE combine a direct visualization of community diversity and the opportunity for subsequent identification of microbial population members by sequence analysis or hybridization experiments using taxon specific probes (Muyzer 1999).

This community fingerprinting technique has been successfully applied in high mountain lake ecosystems. The population dynamics and the community succession can be followed seasonally as well as spatially which has been done for example in the alpine shallow lake Jori XIII (10.4 m, maximum depth). While this lake was temperature-stratified, it showed distinct TTGE banding patterns, whereas the patterns were identical at all depths during summer upwelling events (Yuhana 2005). A different study was carried out in the meromictic lake Cadagno (Bosshard *et al.* 2000). This lake shows a permanent stratification, chemically as well as physically, represented by an oxic mixolimnion, a chemocline water column and an anoxic, monimolimnion of high salinity. The spatial community structure, as revealed by TTGE banding patterns, of the chemocline and the monimolimnion were highly similar whereas the mixolimnion showed a distinctly different pattern. For the temporal community shift, the authors reported that community structure of these three zones varied with time. In the mixolimnion and the chemocline, the community composition changed greatly during the sampling period, whereas in the monimolimnion, the microbial community shift was less distinctive. The distribution of microbial populations was mainly correlated to the spatial or temporal fluctuation



in their micro-environmental conditions, such as organic matter concentration (Crump *et al.* 2003) or nutrient availability (Yuhana 2005). It has also been demonstrated that the spatial distribution of the phototrophic community present in a dense layer changed diurnally by active vertical movement in response to the light conditions and the chemical gradient (Egli *et al.* 2004).

A direct and rapid detection technique to taxonomically identify the members of microbial communities is Fluorescent *In Situ* Hybridization (FISH). The use of specific rRNA-targeted oligonucleotide probes allows one to visualize the morphotype and size of hybridized cells; as well as to determine quantitatively the species, sub groups, or domains among the DAPI-stained cells (Amann *et al.* 1990; Amann *et al.* 1995). The *in situ* assessment for the abundances and the microbial composition has been widely applied to various habitats. Different studies were carried out to investigate the community compositions of habitats from high mountain lakes (Alfreider *et al.* 1996; Pernthaler *et al.* 1998). Alfreider *et al.* (1996)

studied the microbial composition in the different snow, slush, and pelagic layer of Lake Gossenkölle in the Tyrolean Alps (2417 m a.s.l.). They reported that the application of probes specific for the alpha, beta, and gamma subclasses of Proteobacteria and the Cytophaga-Flavobacterium group showed a very distinct bacterial community composition within different habitats (snow, slush, and lake water). The community, in most cases, was dominated by members of the beta subclass (6.5 to 11.6% of the bacteria detectable with the probe HUB).

The PCR-based (culture-independent) analyses, however, also have their limitations. Without having appropriate culture representatives, it is quite difficult to predict phenotypic properties of the detected but uncultured microorganisms. It is still a challenge for microbial ecologists to assign functions and activities to populations within those communities in complex ecosystems (Wagner 2004; Paerl and Steppe 2003). The microbial communities, which consist of mixed groups of microbial species having different functions and metabolic activities, are responsible for maintaining the ecosystem fitness (Paerl and Steppe 2003), i.e. microbial-mediated chemical transformations and habitat alterations (Boetius *et al.* 2000). In spite of extreme conditions, highly active and diverse microbial communities occupying the ice and snow cover of high mountain lakes have been demonstrated (Felipe *et al.* 1995).

## CONCLUSIONS

Although mountain environments have been exploited for a number of purposes, the giant microbial gene pool present in these extreme habitats has been neglected so far. Their aquatic areas are environments often characterized by simple food chains, low in species diversity and species richness. Appreciation of microbial diversity, their genomic richness and metabolic capabilities is very much supported by advances in molecular techniques. These techniques allow us to phenotypically and genotypically characterize microbes, as well as to assign their ecological roles, without the need for cultivation processes. Hence, the remote mountain habitats which typically contain low nutrient concentrations can no longer be underestimated as genetic pools. This untouched reservoir of genomes might be exploited for biotechnological purposes, one day, e.g. for cold active enzymes.

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