

Anaerobic psychrophiles from Lake Zub and Lake Untersee, Antarctica

Alisa Townsend, Elena V. Pikuta*, Melissa Guisler, Sarah Stahl and Richard B. Hoover
Astrobiology Laboratory, National Space Science and Technology Center/University of Alabama in
Huntsville, 320 Sparkman Dr., Huntsville, AL, USA, 35805

* Pikuta@UAH.edu ; phone: +1 (256) 961-7868; fax: +1 (256) 961-7524.

ABSTRACT

The study of samples from Antarctica 2008 and 2009 expeditions organized and successfully conducted by Richard Hoover led to the isolation of diverse anaerobic strains with psychrotolerant and psychrophilic physiology. Due to the fact that Lake Untersee has never been subject to microbiological study, this work with the samples has significant and pioneering impact to the knowledge about the biology of this unique ecosystem. Also, the astrobiological significance for the study of these ecosystems is based on new findings of ice covered water systems on other bodies of our solar system.

Anaerobic psychrotolerant strain LZ-22 was isolated from a frozen sample of green moss with soils around the rhizosphere collected near Lake Zub in Antarctica. Morphology of strain LZ-22 was observed to be motile, rod shaped and spore-forming cells with sizes 1 x 5-10 μm . This new isolate is a mesophile with the maximum temperature of growth at 40°C. Strain LZ-22 is able to live on media without NaCl and in media with up to 7 % (w/v) NaCl. It is catalase negative and grows only on sugars with the best growth rate being on lactose. The strain is a neutrophile and grows between pH 5 and 9.0 with the optimum at 7.8.

Another two strains UL7-96mG and LU-96m7P were isolated from deep water samples of Lake Untersee. Proteolytic strain LU-96m7P had a truly psychrophilic nature and refused to grow at room temperature. Sugarlytic strain UL7-96mG was found to be psychrotolerant, but its rate of growth at 3°C was very high compared with other mesophiles.

Two homoacetogenic psychrophilic strains A7AC-96m and AC-DS7 were isolated and purified from samples of Lake Untersee; both of them are able to grow chemolithotrophically on H_2+CO_2 . In the presence of lactate, these strains are able to grow only at 0-18 °C, and growth at 22 °C was observed only with yeast extract stimulation.

In this paper, physiological and morphological characteristics of novel psychrophilic and psychrotolerant isolates from Antarctica 2008 and 2009 expeditions will be discussed.

Keywords: Antarctica, psychrophiles, proteolytic anaerobe, fermentation, Lake Zub, Untersee Lake

1. INTRODUCTION

During the last two decades extensive studies of microorganisms inhabiting the Antarctica continent showed the presence of a wide variety of phylogenetically distant groups of prokaryotes. Historically, the first marine microorganisms growing at low temperature regimes were diatoms.¹ These algae were collected near the Antarctica coast. Much later there were reports of isolated aerobic, fermentative, and sulfate-reducing anaerobic bacteria.²⁻⁷ Currently, the scientific effort of microbiologists from different countries has reached its highest level, and as a result many updates were received for these unique and unstudied Antarctica ecosystems.

Very exciting research of microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys in Antarctica was initiated by a group of scientists from California, Florida and Colorado universities.⁸ This group surveyed the microbial diversity of selected cryptoendolithic communities by analyzing clone libraries of rRNA genes amplified from environmental DNA. As a result, a giant piece of new information about genomic composition within these unique ecosystems was obtained. Over 1,100 individual clones from two different types of cryptoendolithic communities were analyzed with one community dominated by cyanobacteria and another community dominated by lichens. There were 51 phylotypes with 46 of which were bacterial and 5 eukaryal were determined. No representatives of *Archaea* were detected. It was shown that the lichen-dominated community contained 25 unique phylotypes.

Representatives of fungi (29 % of clones), some chloroplasts (22 % of clones), and green algae (22 % of clones) were determined within this community. The study of the cyanobacterial community showed the presence of truly psychrophilic cyanobacterial cultures that previously had not been described. The dominant cyanobacterial sequence type identified in this study was phylogenetically most closely related to the filamentous cyanobacterial *Phormidium* spp. and *Plectonema* spp.

Recent productive investigations of methanogenic bacteria and sulfate-reducing bacteria in Lake Fryxell in Antarctica were conducted at Southern Illinois University. The study of diversity and distribution of sulfate-reducing bacteria in permanently frozen Lake Fryxell had showed that at least four of the six major phylogenetic groups of sulfate-reducing bacteria (*Desulfovibrio*, *Desulfosarcina*, *Desulfotomaculum*, *Desulfobulbus*, *Desulfobacter*, and *Desulfobacterium*) were present in the lake.⁹ All of these cultures should be active at 4 °C which means psychrophilic or psychrotolerant physiology. The study of biodiversity of methanogenic and other *Archaea* in the permanently frozen Lake Fryxell has also been successfully started by the same research group.¹⁰ The phylogenetic analysis of the 16S rRNA gene sequences obtained in this work showed that at least four clusters of *Archaea* inhabited Lake Fryxell - three clusters of *Euryarchaeota* and one cluster of *Crenarchaeota*. Within *Euryarchaeota*, at least two clusters of methanogens were detected. Some phylotypes were closely related to 16S rRNA gene sequences from the *Methanosarcina* species. The presence of *Methanosarcina*-like methanogens in Lake Fryxell sediments has been confirmed by the isolation of strain FRX-1, a methylotrophic *Methanosarcina* species growing at 5 °C.¹¹ The other phylotypes of methanogens in Lake Fryxell sediments clustered with species of *Methanoculleus*. Unlike *Methanosarcina*, no cultures of *Methanoculleus* species have been obtained from Lake Fryxell; however, a *Methanoculleus*-like 16S rRNA gene was previously detected in a molecular diversity study of the cyanobacterial mats that develop in the peripheral melt waters of this lake.^{12,13}

Our research will focus on the microorganisms of Lake Untersee (71°20'S 13°27'E); and other lakes of the Schirmacher Oasis (70°46'S, 11°44'E).

1.1 Lake Untersee

The perennially ice covered Lake Untersee is in a deep glacier carved basin 563 M above sea level. It is the largest freshwater lake in the interior of East Antarctica with a surface area of 11.4 km² (Fig. 1a). Lake Untersee is a perennially ice-covered, ultra-oligotrophic lake. Wand with co-researchers have reported the detection of sharp vertical gradients of temperature, pH, dissolved oxygen, and electrical conductivity with an anoxic zone below 80 meters in the southwestern area of the lake.^{14,15} They also found that Lake Untersee has the highest observed maximum methane concentration of any known natural aquatic ecosystem. Although they concluded that this resulted from methanogenic archaea, the microorganisms of this environment have not previously been investigated. Their bathymetry cross-sectional profile of Lake Untersee showing the deep anoxic trough is presented in Figure 1b.

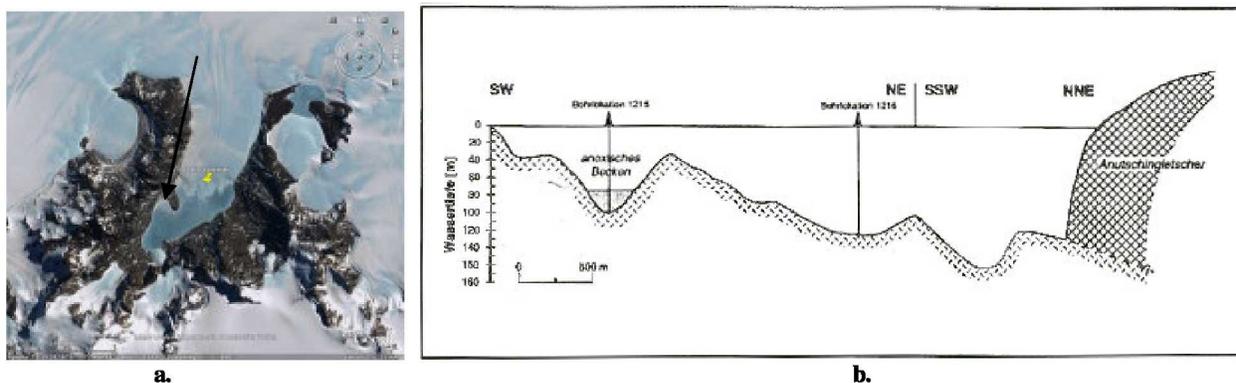


Fig. 1a,b: a) The location of the Lake Untersee in the Droning Maud Land, East Antarctica; b) The cross-sectional profile of Lake Untersee with the Anuschin Glacier (on right) and the deep anoxic trough. From Wand *et al.*¹⁴

Lake Untersee is surrounded on three sides by the steep cliffs of the Otto-von-Gruber-Gebirge (Gruber Mountains) of the central Droning Maud Land. The other side is sealed off by the Anuschin Glacier, and there is no outlet for the water due to the glacier ice sheet cover. The permanent ice cover eliminates wind-generated currents

allowing chemical gradients to persist, reducing light penetration, and limiting gas exchange with the atmosphere. Therefore, Lake Untersee and other perennially ice-covered lakes are physically driven systems with their environmental properties being determined primarily by the physical effects of the ice cover. Lake Untersee has remained a completely closed and isolated ecosystem for many millennia.

Wand and colleagues reported that the pH of the water in Lake Untersee was unusually high (meaning 11.34 ± 0.12 from the ice-water interface to a depth of about 75 m, the location of the chemocline).¹⁵ They attributed the high pH to the addition of OH⁻ into the lake water, which is likely poorly buffered in the rocky basin. This was slightly higher than the pH that was measured in November 2008 during our expedition. The Hach Hydrolab was used to measure temperature, dissolved oxygen, Eh, pH, and conductivity at each collection site.¹⁶

The data on water temperature, pH, and dissolved oxygen as a function of depth in the water column at the deep anoxic trough sampling site (S 71° 21' 21.5"; E 013° 25' 37.8") was obtained on the Tawani International Expedition on November 22, 2008, and it is shown in Figure 2. These results were in relatively good agreement with the Wand's measurements^{14,15}; although, the maximal pH (10.2) was notably lower than their measurement of pH maximum 12.1. Also, the data from the 2008 Expedition indicated that the oxycline was located at 70 m (rather than 80 m), while the temperature was found to be in the same range as their data (0.5 - 5°C).

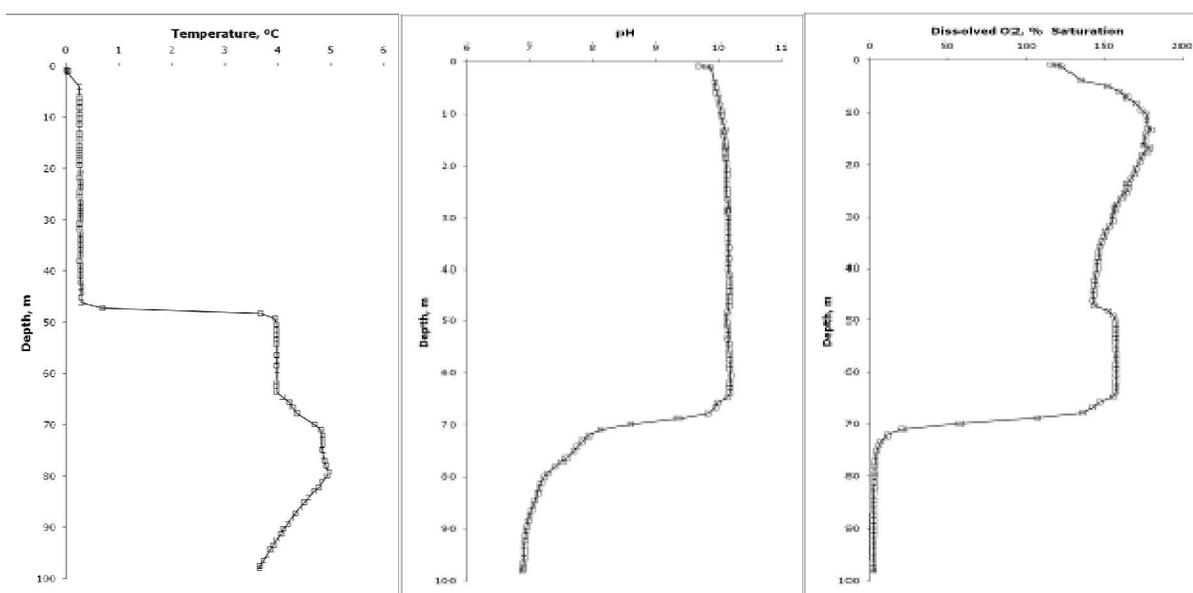


Fig. 2. The measured temperature, pH, and dissolved oxygen profiles at Lake Untersee deep anoxic trough.

While there are interesting biological effects on the environment of the Antarctica lakes, they are minimal in comparison to the biological effects influencing temperate lake ecosystems, therefore leading to the limited species diversity and the community structure expected.¹⁷ The physicochemical characteristics including a sharp gradient of dissolved oxygen, temperature, pH, and sulfur compounds, along with its ultraoligotrophic nature, makes Lake Untersee unique for microbiological study in comparison to all other lakes that have been explored on the Antarctica continent.

1.2 The Schirmacher Oasis

Other studied ecosystems of Antarctica in this research are samples of the lakes in the Schirmacher Oasis. The Schirmacher Oasis is 3 km wide, 20 km long and consists of ~180 lakes (<http://www.antarctic-company.com/antarctic-weather.htm>) (Fig. 3). Three categories of lakes (land-locked lakes, proglacial lakes, and epishelf lakes) are found in this region. Several of the epishelf lakes are perennially ice-covered, while most all of the others melt out by mid-austral summer. The land-locked lakes are primarily fresh water from snow, glacial melts, and melting of the permafrost under the soil during summer months. The epishelf lakes are the ocean tidal water mixed with the fresh water from the snow and glacier melts. The proglacial lakes are the melting glacier or the melt-water trapped against an ice sheet. The distributions of these three categories of lakes are elaborated in Figure 3. Unlike Lake Untersee, the lakes at

the Schirmacher Oasis are open systems and are expected to be cross-mixing of microorganisms either through the melting of the snow during summer months or by the almost continuous high wind (15-100 km h⁻¹). The open water system is exposed to seasonal and often diurnal freezing and thawing cycles: high levels of solar radiation during summer and completely frozen and covered with snow during the winter months. A complex physico-chemical, biotic, and extreme environmental conditions play crucial roles in the occurrence, distribution, and biodiversity of the microorganisms in this segment of the Droning Maud Land.

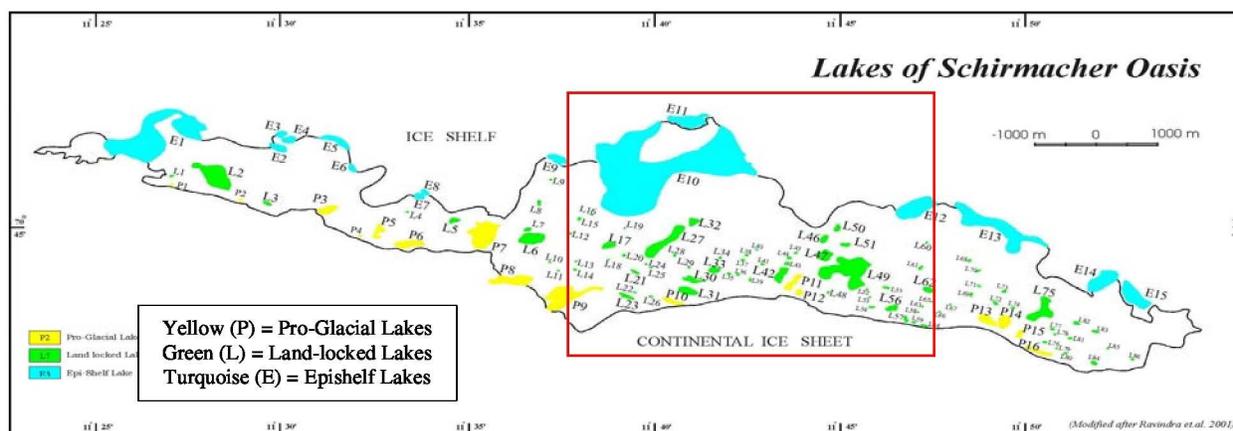


Fig. 3. Map of the Schirmacher Oasis showing the three types of lakes: Green - land-locked; Turquoise - epishelf; and Yellow - proglacial. The lakes within the boxed area of the Schirmacher Oasis were explored and samples collected for analysis during the 2008 Tawani International Antarctic expedition.

2. MATERIALS AND METHODS

During the 2008 Tawani International Antarctica Expedition, water and ice samples from Lake Untersee and the Schirmacher Oasis were collected to study the taxonomically important and diverse group of extremophilic microorganisms expressing unique physiological and metabolic characteristics necessary to cope with and/or adapt to the Antarctica cold and subzero temperature environments. This part of the project is focused on the anaerobic/facultative anaerobic microorganisms, and accordingly the collections of samples from Lake Untersee and Schirmacher Oasis were performed by anaerobic technique.^{18,19} Sterile gloves, Whirl-Pak bags, and sample bottles; Sipre ice augers, core samplers, and Kemmerer bottles were used for collecting samples of ice, anoxic bottom sediments, and water from selected depths within the oxic and anoxic zones of the water column. The physico-chemical parameters of local ecosystems (pH, Eh, salinity, conductivity, and temperature) were measured *in situ* and documented at the moment of sampling. Water samples were collected from surface and near bottom areas for determination of mineral composition and redox potential. For obtaining enrichment cultures, the inoculations of sample materials were performed in the field into sterile media prepared in advance. These inoculated tubes were then maintained and transported to the NSSTC Astrobiology Laboratory and UAB in liquid state at 4 °C. Other samples were stored in frozen state in coolers with Blue Ice while in Antarctica; they were kept frozen during transport to South Africa. One half of the samples were hand-carried in the Blue Ice coolers. The other portion was shipped in styrofoam coolers with Dry Ice and subsequently have been stored in freezers at -80 °C.

For the cultivation of the anaerobic microorganisms, the mineral medium was applied (1 L): 10 g NaCl, 0.3 g KCl, 0.3 g KH₂PO₄, 0.1 g MgSO₄•7H₂O, 1 g NH₄Cl, 0.0125 g CaSO₄•7H₂O, 0.4 g NaHCO₃, 0.4 g Na₂S•9H₂O, 1 ml resazurin, 0.1 g yeast extract, 2 ml vitamin solution²⁰, and 1 ml trace elements²¹. The final pH was adjusted to 7.8. To obtain a pure culture, the serial dilution and the roll tube methods were used. Also, the roll tube method allowed the determination of the colony appearance. The pH that the strain grew optimally on was determined by using the above media and adjusting the pH by 0.5 increments. The pH was adjusted using 6N H₂SO₄ or 6N NaOH. Next, to determine the dependence from sodium ions, the above media was used with the sodium ions being replaced with potassium ions in equal-molar amounts. Since the medium was made without any sodium ions, various amounts of NaCl were added to the medium before autoclaving. The determination of the temperature optimum and range was done by inoculating hungate tubes containing 10 ml of the media with substrate and culture. The hungate tubes were incubated at

temperatures ranging from -5° to 50°C . The substrates that the strain could successfully grow on were determined by injecting culture and the substrates into a hungate tube containing 10 ml of medium.

3. RESULTS

3.1 Strain LZ-22 from Lake Zub

The strain LZ-22 was isolated from a sample containing living green moss and its rhizosphere. The colonies of this strain that grew on 3% agar medium (prepared by roll-tube method) had light cream color, circular and concave shape, smooth and shiny surface, and a transparent circle area surrounding the colony itself. The size of the colonies varied within 0.5 – 4.0 mm in diameter. The older colonies had a more dense consistency in the center compared to the perimeter of the colony. The cells had a diameter of $1\ \mu\text{m}$ and length of 10-15 μm (Fig. 4). Also, the cells were rounded at the ends, showed gliding motility, and formed round/oval terminally located endo-spores. The strain LZ-22 was capable of growing on media with a pH range of 5 – 9 with an optimum at 7.8 (Fig. 6). The optimum salinity was determined to be 0.25 % (w/v) NaCl with the range of 0 - 7 % (w/v) NaCl (Fig. 7). Also, this strain could grow at a temperature of 3° to 40°C , and the optimum temperature was 30°C (Fig. 5). It was capable of surviving at -5°C and 0°C . New isolate LZ-22 was able to grow on the following substrates: yeast extract, chitin, pectin, D-glucose, D-fructose, maltose, sucrose, D-trehalose, D-ribose, D-cellobiose, lactose, and minor growth on starch. Also, this strain was catalase negative and gram positive.

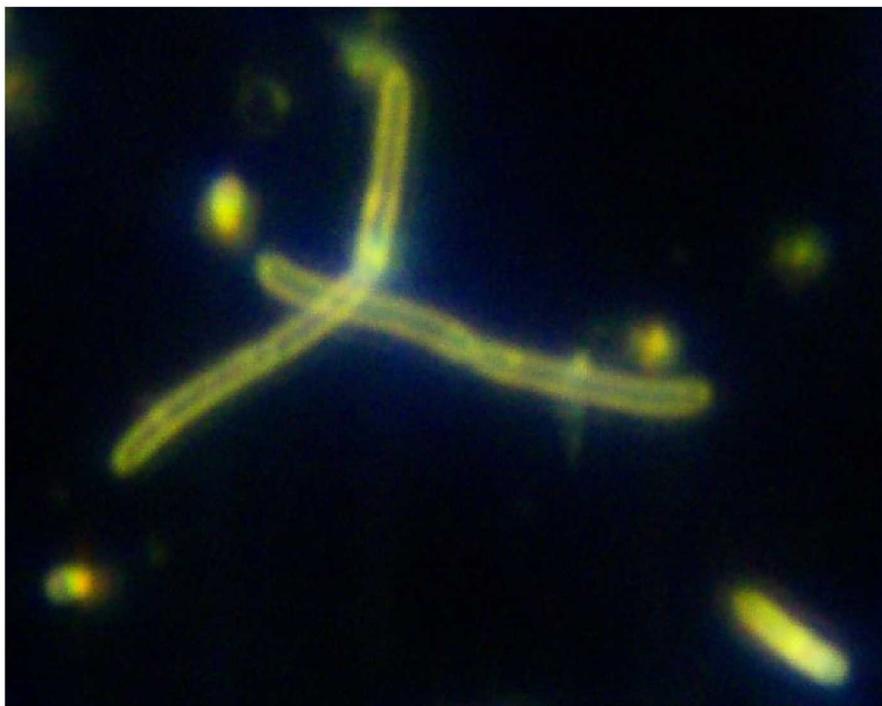


Fig. 4. The cell morphology of strain LZ-22 growing on *D*-glucose at 3°C . Cells are motile by peritrichous flagella and are spore-forming (oval spores are located sub-terminally).

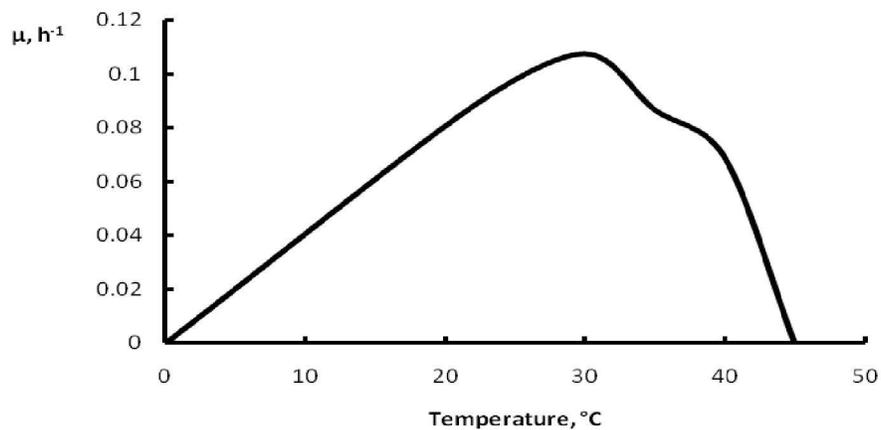


Fig. 5. Growth of strain LZ-22 in dependence upon the temperature.

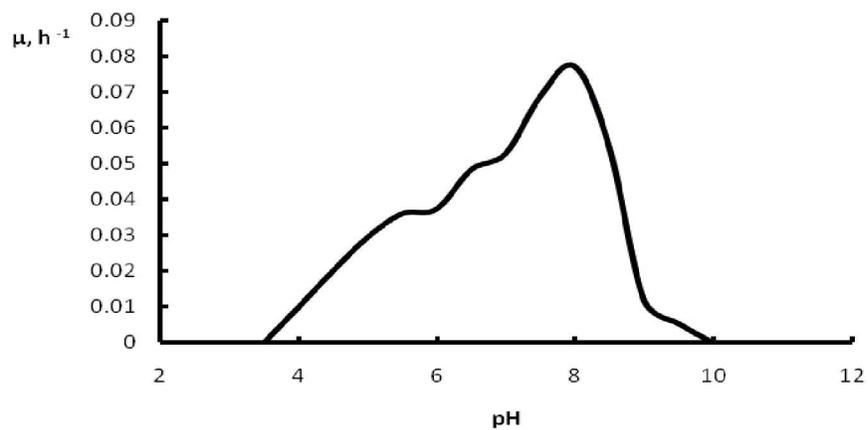


Fig. 6. Growth of strain LZ-22 in dependence upon pH.

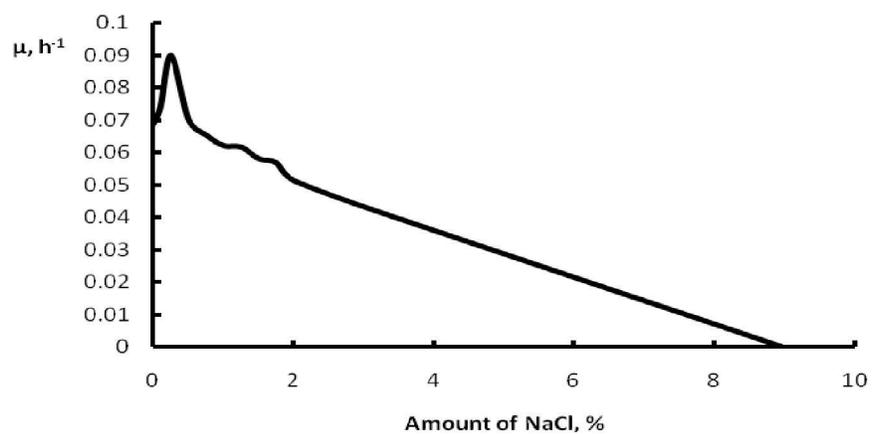


Fig. 7. The growth of strain LZ-22 in dependence upon NaCl.

3.2 Strain UDS7-G and UL7-96mG

The strain UDS7-G (Fig. 8a) was isolated from an anaerobic, deep sediment sample. The strain UDS7-G is a sugarlytic bacterium which is able to grow on anaerobic medium at pH 7, salinity 0.5 % NaCl, and *D*-glucose as the substrate. The morphology of the cells showed straight swallowed rods with pointed ends. The sizes of the cells were 0.7 x 2 - 3µm. The new isolate UDS7-G is motile, spore-forming (central location with the swallowed sporangium), and capable of growing at a temperature range of 3 - 18°C.

The sugarlytic strain UL7-96m (Fig. 8b) was isolated from a water sample of Lake Untersee collected at a depth of 96 m. It has similar morphology to strain UDS7-G except for the growth rate. The strain UL7-96mG grew much faster at 3°C than strain UDS7-G.

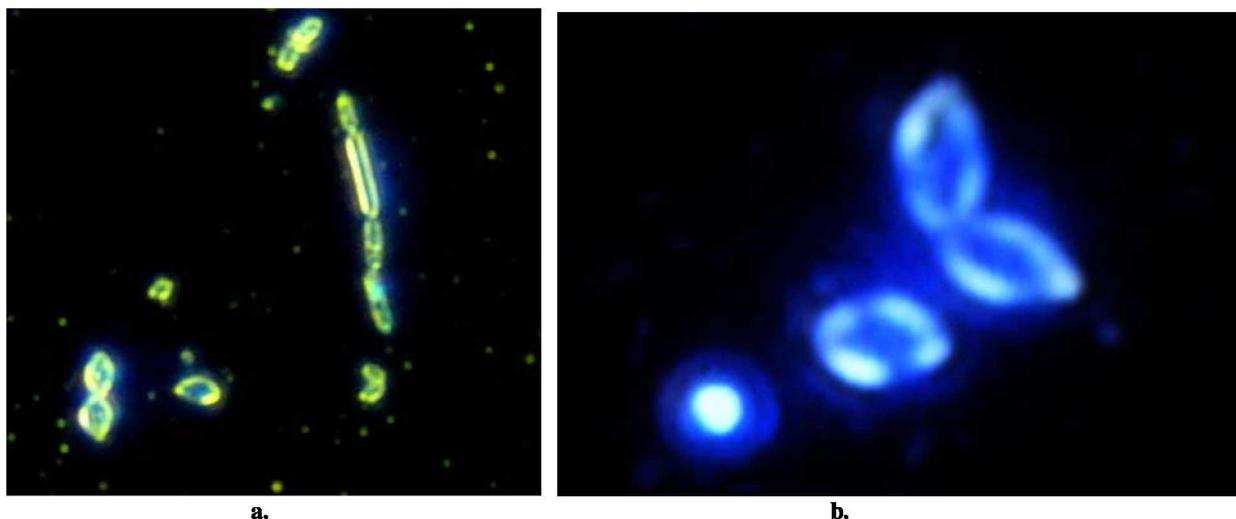


Fig. 8. Two psychrophilic, sugarlytic, and anaerobic strains from samples of Lake Untersee: a. strain UDS7-G isolated from the deep anoxic sediment sample; b. Strain UL7-96mG isolated from 96 m deep anoxic trough.

3.3 Proteolytic strains LU-96m7P and strain AP7-90

Two proteolytic, truly psychrophilic isolates LU-96m7P and AP7-90 were purified in pure cultures from different samples of Lake Untersee. The strain LU-96m7P is a proteolytic stain that was isolated from a Lake Untersee water sample at a depth of 96 m. It can grow on medium with pH 7, salinity of 0.5 % (w/v) NaCl, and peptone as a substrate. The morphology of strain LU-96m7P is presented by rod-shaped cells which are motile and spore-forming. The sizes of the cells are 0.6 x 3.0-5.0 µm (Fig. 9). The strain LU-96m7P is a true psychrophile; it grows rapidly at 3 - 5°C and does not grow at room temperature (22°C). Another proteolytic strain AP7-90 was grown on anaerobic medium of pH 7 and peptone as the substrate. The cell morphology is straight thin rods with the size of the cells being 0.3 - 0.4 x 10 - 15µm with laterally located large spores (diameter 1.5-2 mm).

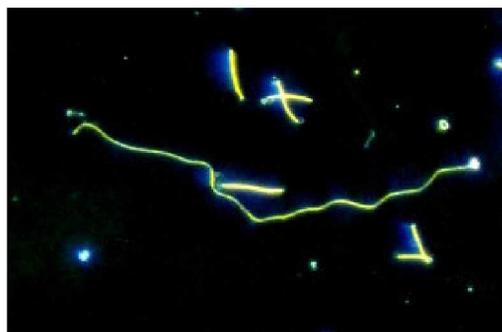


Fig. 9. Psychrophilic, proteolytic strain AP7-90 growing at 3 °C with peptone

3.4 Strain A4P-85m

Proteolytic strain A4P-85m was isolated on the low-mineralized medium with pH 4 from the 85 m anoxic trough of Lake Untersee. The morphology of this new isolate is represented by straight rods dividing under rectangular, perpendicular to the original cell direction (Fig. 10). These cells are spore-forming (round, located terminally) and motile in the beginning of the cell cycle; older cells are non-motile and covered by excreted shine, reflecting light material, which makes the cell diameter almost twice thicker. This strain grows on peptone and triethylamine as substrates. Growth of this isolate was never observed at 22 °C. The best growth rate was documented at 5-8 °C.

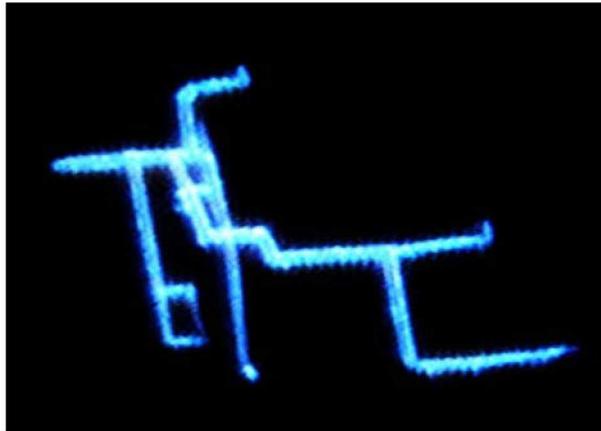


Fig. 10. Strain A4P-85m was isolated on anaerobic medium with pH 3.5 and peptone.

3.5 Homoacetogenic strains A7AC-96m and AC-DS7

These strains were obtained from a 96 m deep water sample from Lake Untersee and a sediment sample from a deep (96 m) anoxic trough. Both of these strains grow on lactate. These strains are vibrios that are motile and capable of growing at 5°C and pH 7. The size of the cells for both strains are 0.6 – 0.7 x 2 – 4 µm (Fig 11). The maximum temperature to produce growth was 14°C which showed slow growth during a three week period. Due to the growth at this temperature, these strains are truly psychrophilic. Both new isolates were able to grow lito-autotrophically during two days after inoculation on the medium with H₂ + CO₂ as the only sources of energy and carbon (three consequent transfers).

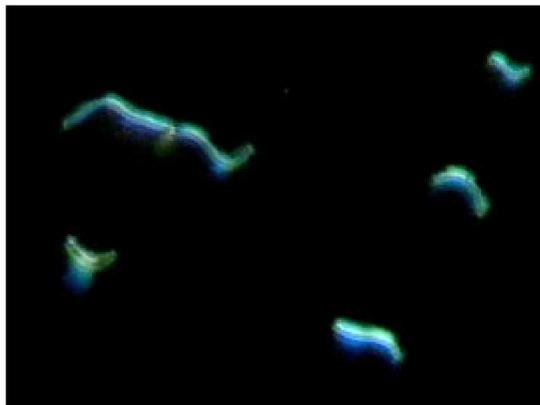


Fig. 11. Psychrophilic homoacetogenic anaerobe from Lake Untersee, strain A7AC-96m growing on H₂ + CO₂ at 5 °C.

The preliminary study of strain AC-DS7 showed that it grew well on the following substrates: H₂+CO₂, lactate, cellobiose, chitin, peptone, casamino acids, and yeast extract. No growth was observed on the following substrates:

formate, acetate, methanol, ethanol, acetone, *D*-mannitol, *D*-fructose, *D*-glucose, fumarate, betaine, trimethylamine, and glycerine. The optimum temperature for this strain was 15 °C, and no growth was observed at 22 °C on the lactate medium without yeast extract being added. Also, it was able to grow at 0 °C. On the medium with yeast extract, the strain grew within a temperature range of 0-30 °C (Fig. 12).

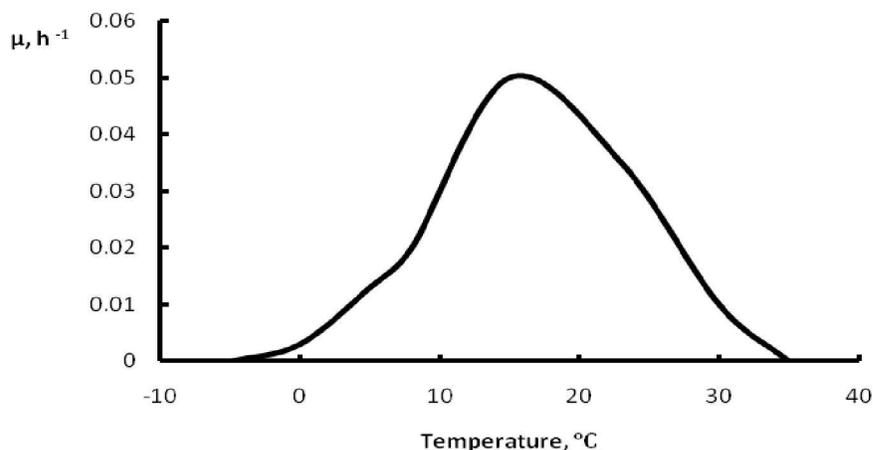


Fig. 12. The growth of strain AC-DS7 on the medium with yeast extract in dependence on temperature.

4. DISCUSSIONS

Since the most interesting results were received for truly psychrophilic homoacetogenic strains, a brief chronological review of these bacteria is considered below.

4.1 Acetogens

The first report of a psychrophilic, homoacetogenic bacterium was *Acetobacterium carbinolicum* strain HP4, which was isolated by Bak in 1988.²² It was shown later that the strain HP4 was phylogenetically identified as *Acetobacterium carbinolicum*, the species that was previously described by Eichler & Schink.²³ This strain had a growth range of 1- 25 °C. Chronologically, the next three homoacetogenic isolates were described as the new psychrophilic species *A. bakii*, *A. paludosum* and *A. fimetarium*.²⁴ Later, another psychrotolerant homoacetogen *A. tundrae* was isolated from the tundra wetland soil sample collected in Polar Ural.²⁵ However, all of these species were able to grow at 30 or 35 °C with optimal growth at 20-30 °C, indicating they are psychrotolerant microorganisms rather than true psychrophiles. New subspecies *kysingense* of the species *A. carbinolicum* strain SyrA5 was isolated from anaerobic sediments of brackish fjord.²⁶ This isolate was also psychrotolerant and able to grow lithotrophically on H₂+CO₂ and on CO.

Recently, two new strains of acetogenic bacteria LS1 and LS2 were isolated from surficial sediments of the permanently ice-covered, meromictic Lake Fryxell in McMurdo Dry Valleys, Antarctica.²⁷ It was shown that these isolates represent the first acetogens able to grow at subzero temperatures, but their range of growth, -2.5 °C to 25 °C, exceeds the upper limit (20 °C) for psychrophiles in accordance with the definition provided by Morita.²⁸

The homoacetogenic bacteria are competing with methanogens and sulfate-reducing bacteria for hydrogen and other inorganic and organic substrates within oligotrophic polar environments. It was shown that at low temperatures homoacetogenic bacteria had significantly predominant activity compared to methanogens and sulfate-reducing bacteria.^{25,29,30} Gaidos and colleagues studied the microbial diversity of one of Iceland's volcanic subglacial lakes, and they made an unsuccessful attempt to isolate a new psychrophilic acetogenic strain found there, the phylotype of which belongs to the genus *Acetobacterium*.³¹

In our study of samples from Lake Untersee, we have isolated two strains (str. A7AC-96m and str. A7AC-DS7) of truly psychrophilic, homoacetogenic bacteria. Both strains were isolated from water and sediment samples correspondently. These samples were collected from the 96 m region of the deep anoxic trough of Lake Untersee in Antarctica. Both of these strains have maximum temperature for growth at 15 °C and were able to grow on H₂ + CO₂. Interestingly, the effect of yeast extract added to the lactate-containing medium for both homoacetogenic strains led to growth at 22-30 °C; this effect needs to be studied in more detail.

5. CONCLUSIONS

The preliminary study of the samples delivered by Tawani Antarctica 2008-2009 Expeditions showed the presence of different anaerobic bacterial cultures. Several of them have been purified, and their physiology has been described. The following phylogenetic description will provide precise information about an evolution position for each new organism.

This research will provide new data concerning the biodiversity of studied ecosystems, diversity of metabolisms, and physiology of the anaerobic prokaryotes that inhabit these unique ecosystems on Earth. This research may result in the discovery of several new taxa with novel biochemical structures, functionally critical biomolecules, enzymes, proteins, and cytological characteristics. The study of these microorganisms may significantly impact many branches of fundamental and applied science as well as data about the evolutionary development of “closed” and “open” ecosystems of Antarctica. The psychrophilic bacteria investigated during this study may have many important biotechnological, biomedical, and pharmaceutical applications which may yield great benefits for mankind.

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REFERENCES

- [1]. Castracane, A. F. A., "Report on the Diatomaceae collected by the *H. M. S. Challenger* during the years 1873-76," *Reports of the Scientific Voyage of H. M. S. Challenger-Botany* 2, 1-178 (1886).
- [2]. McClean, A. L., "Bacteria of the Ice and Snow in Antarctica," *Nature* 102, 35-39 (1918).
- [3]. Darling, C. A. and Siple, P. A., "Bacteria of Antarctica," *J. Bacteriol.* 42, 83-98, (1941).
- [4]. Straka, R. P. and Stokes, J. L., "Psychrophilic Bacteria from Antarctica," *J. Bacteriol.* 80, 622-625 (1960).
- [5]. Iizuka, H., Okazaki, H. and Naosuke, S., "A new sulfate-reducing Bacterium Isolated from Antarctica," *J. Gen. Appl. Microbiol.* 15, 11-18 (1969).
- [6]. Reddy, G. S. N., Prakash, J. S. S., Srinivas, R., Matsumoto, G. I. and Shivaji, S., "*Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica," *Int. J. Syst. Evol. Microbiol.* 53, 977-984 (2003).
- [7]. Mountfort, D. O., Rainey, F. A., Burghardt, J., Kasper, H. F. and Stackebrandt, E., "*Clostridium vincentii* sp. nov., a new obligately anaerobic, saccharolytic, psychrophilic bacterium isolated from low-salinity pond sediment of the McMurdo Ice Shelf, Antarctica," *Arch. Microbiol.* 167, 54-60 (1997)
- [8]. De la Torre, J. R., Goebel, B. M., Friedmann E. I. and Pace, N. R., "Microbial Diversity of Cryptoendolithic Communities from the McMurdo Dry Valleys, Antarctica," *Applied and Environmental Microbiology* 69, 3858-3867 (2003).
- [9]. Karr, E. A., Sattley, W. M., Rice, M. R., Jung, D. O., Madigan, M. T. and Achenbach, L., "Diversity and distribution of sulphate-reducing bacteria in permanently frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica," *Appl. Environ. Microbiol.* 71, 6353-6359 (2005).
- [10]. Karr, E. A., Ng, J. M., Belchik, S. M., Sattley, W. M., Madigan, M. T. and Achenbach, L., "Biodiversity of methanogenic and other *Archaea* in the permanently frozen lake Fryxell, Antarctica," *App. Environ. Microbiol.* 72, 1663-1666 (2006).
- [11]. Singh, N., Kendall, M. M., Liu, Y. and Boone, D. R., "Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska, description of *Methanococcoides alaskense* sp. nov., and emendation of *Methanosarcina baltica*," *Intl. J. Syst. Evol. Microbiol.* 55, 2531-2538 (2005).
- [12]. Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B. J. and Stackebrandt, E., "16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica," *Extremophiles* 5: 22-33 (2001).
- [13]. Taton, A., Grubisic, S., Brambilla, E., De Wit, R. and A. Wilmotte, A., "Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach," *Appl. Environ. Microbiol.* 69, 5157-5159 (2003).
- [14]. Wand, U., Schwarz, G., Brüggemann, E. and Bräuer, K., "Evidence for physical and chemical stratification in Lake Untersee (central Dronning Maud Land, East Antarctica)," *Antarctic Sci.* 9, 43-45 (1997).
- [15]. Wand, U., Samarkin, V. A., Nitzsche, H-M. and Hubberten H. W., "Biogeochemistry of methane in the permanently ice-covered Lake Untersee, central Dronning Maud Land, East Antarctica," *Limn Oceanogr* 51, 1180-1194 (2006).

- [16]. Hoover, R.B., Pikuta, E.V., Townsend, A., Anthony, J., Guisler, M., McDaniel, J., Bej, A. and Storrie-Lombardi, M., "Microbial extremophiles from the 2008 Schirmacher Oasis expedition: Preliminary results," *Proc. SPIE 7097*: 70970L-70970L-9 (2008).
- [17]. Vincent, W.F., "Microbial Ecosystems in Antarctica," Cambridge University Press, Cambridge, UK (1988)
- [18]. Hungate, R. E., "A roll tube method for cultivation of strict anaerobes," In *Methods in Microbiology* (Eds. J.R. Norris and D.W. Ribbons), Academic Press, New York, 3B, 117-132 (1969).
- [19]. Miller, T.L. and Wolin, M. J., "A serum bottle modification of the Hungate technique for cultivating obligate anaerobes," *Appl. Microbiol.* 27, 985-987 (1974).
- [20]. Wolin, E.A., Wolin, M. J. and Wolfe, R. S., "Formation of methane by bacterial extracts," *J. Biol. Chem.* 238, 2882-2886 (1963).
- [21]. Whitman, W.B., Ankwarda, E. and Wolfe, R. S., "Nutrition and carbon metabolism of *Methanococcus voltae*," *J. Bacteriol.* 149, 852-863 (1982).
- [22]. Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., Mayer, H. P. and Schulz H., "Hydrogen turnover by psychrophilic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment," *FEMS Microbiol Ecol* 62: 285-294 (1989).
- [23]. Eichler, B. and Schink, B., "Oxydation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe," *Arch Microbiol* 140: 147-152 (1984).
- [24]. Kotsyurbenko, O. R., Simankova, M. V., Nozhevnikova, A. N., Zhilina, T. N., Bolotina, N. P., Lysenko, A. M. and Osipov, G. A., "New species of psychrophilic acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov.," *Arch. Microbiol.* 163, 29-34 (1995).
- [25]. Simankova, M. V., Kotsyurbenko, O. R., Stackebrandt, E., Kostrikina, N. A., Lysenko, A. M., Osipov, G. A. and Nozhevnikova, A. N., "*Acetobacterium tundrae* sp. nov., a new psychrophilic acetogenic bacterium from tundra soil," *Arch Microbiol* 174, 440-447 (2000).
- [26]. Paarup, M., Friedrich, M. W., Tindall, B. J. and Finster, K., "Characterization of the psychrotolerant acetogen strain SyrA5 and the emended description of the species *Acetobacterium carbinolicum*," *Antonie van Leeuwenhoek* 89, 55-69 (2005).
- [27]. Sattley, W. M. and Madigan, M. T., "Cold-active acetogenic bacteria from surficial sediments of perennially ice-covered Lake Fryxell, Antarctica," *FEMS Microbiol. Lett.* 272, 48-54 (2007).
- [28]. Morita, R.Y., "Psychrophilic Bacteria," *Bacteriol Rev* 39, 144-167 (1975).
- [29]. Kotsyurbenko, O. R., Glagolev, M. V., Nozhevnikova, A. N. and Conrad, R., "Competition between homoacetogenic and methanogenic archaea for hydrogen at low temperature," *FEMS Microbiol. Ecol.* 38, 153-159 (2001).
- [30]. Nozhevnikova, A. N., Simankova, M. V., Parshina, S. N. and Kotsyurbenko, O. R., "Temperature characteristics of methanogenic archaea and acetogenic bacteria isolated from cold environments," *Water Science & Technology* 44, 41-48 (2001).
- [31]. Gaidos, E., Marteinsson, V., Thorsteinsson, T., Johanneson, T., Runarsson, A. R., Stefanson, A., Glazer, B., Lanoil, B., Skidmore, M., Han, S., Miller, M., Rusch, A. and Foo, W., "An oligarchic microbial assemblage in the anoxic bottom waters of a volcanic subglacial lake," *The ISME Journal* 1-12 (2008). www.nature.com/ismej