Mongolian Journal of Biological Sciences 2003 Vol. 1(2): 37-42

Structure and Functioning of Microbial Community of Mineral Springs in Central Asia

Namsaraev B.B.[1], Barkhutova D.D.[1], Danilova E.V.[1], Dagurova O.P.[1], Namsaraev Z.B.[2], Balzhiin Tsetseg[3], Ayush Oyuntsetseg[4]

[1]*Institute of General and Experimental Biology, Siberian Branch of the Russian Academy of Sciences, Ulan-Ude, Russia e-mail: bairnam@biol.bsc.buryatia.ru*
[2]*Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*
[3]*Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia*
[4]*Faculty of Biology, National University of Mongolia, Ulaanbaatar 210646, Mongolia, e-mail: oyun_a@num.edu.mn*

Abstract

The microbial mats of different types of springs in Central Asia (Zabaikalye and Mongolia) are described. The species diversity of mat-formed phototrophic bacteria was determined. The rates of microbial destruction processes (sulfate reduction and methane formation) were measured. An important role of bacteria and algae in the formation of mineral water composition was shown.

Key words: bacterial processes, Central Asia, microbial mats, mineral springs

Introduction

Functional diversity of microbial communities depends on chemical composition of waters and rocks. The electrons acceptor contents, biogenous elements and other substances influence microbial diversity and microbial activity (Zavarzin, 1984). At the same time microorganisms play an active part in formation of qualitative and quantitative composition of natural waters, as a result of their high biochemical potential and large numbers. Aerobic and anaerobic, autotrophic and heterotrophic bacteria, fungi and algae take part in biogeochemical processes taking place in underground and surface waters. Microorganisms are the main catalysts of multistage processes of production, destruction and transformation of organic material, formation and consumption of gases and synthesis of secondary metabolites.

Geological history, geo-structural peculiarities of territory, chemical composition of rocks, geothermal and climatic conditions cause widespread mineral waters in Central Asia where various types of mineral spring have been determined. They are subdivided into nitrogen, carbonaceous and methane springs as well as hot, warm and cool groups (Tkachuk *et al*, 1962; Pinneker, 1980). Nitric springs form as a result of heat occurrence with infiltrated waters from 2-3 kilometers of the deep kainozoii breaks. The origin of carbonated mineral springs is connected with carbonate and silicate rocks in zones of young volcanic activity.

Hydro-chemical and microbiological research has been conducted in the mineral springs of Zabaikalye and Mongolia (Barkhutova *et al*., 1998; Abidueva *et al*., 1999; Barkhutova *et al*., 2003). The location of springs is shown in Fig. 1. Particular attention in this research has been paid to estimation of geo-chemical activity of microorganisms, to determination of physiological group numbers, and to the study of species diversity of bacteria and algae.

Methods

Samples were taken from 21 springs in Zabaikalye and Mongolia. Samples were taken from the spring basin (water body), from the brook running out from the spring and from giffon formed on the surface of the water. Water samples and silt were taken from 0.1-0.5m; samples of mat were taken from river sides and small lagoons. Water temperature was taken with an electric sensor thermometer "Prima", pH with portative precision pH-meter PRO, redox potential with a redox potential tester ORP and salinity with a portative tester-conduct-meter TDS-4. Concentrations of

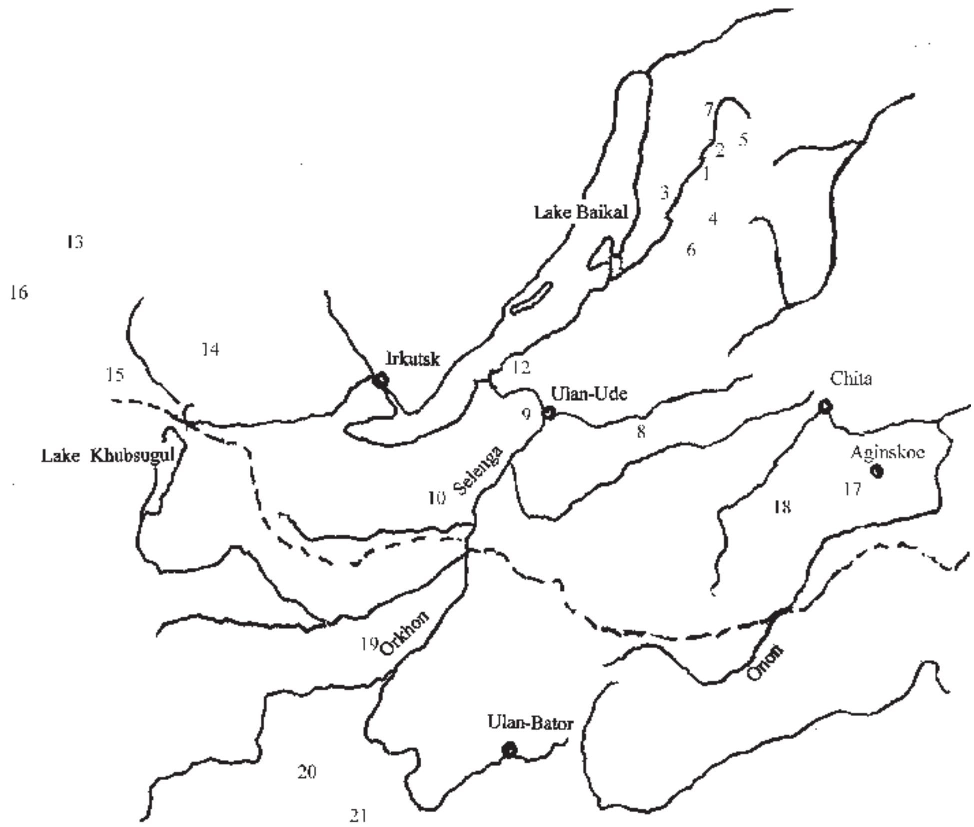

Fig. 1. Location scheme of studied mineral springs (Zabaikalye and Mongolia regions)
1 – Alla, 2 – Kuchiger, 3 - Bolshaya Rechka, 4 – Garga, 5 – Seiya, 6 – Uro, 7 – Umkhei, 8 – Bakhlaita, 9 – Khalyuta, 10 – Gegetui, 11 – Zmeyinaya, 12 - Sukhaya Zagza, 13 - Khoito Gol, 14 – Shumak, 15 - Gadan Gembe, 16 – Zhoigon, 17 – Suduntui, 18 – Alkhanai, 19 – Erdenet, 20 – Khuzhirta, 21 - Devsen Bulag

carbonates, sulfides and sulfates were measured by colorimeter (Kuznetsov *et al*., 1989). Microbial process rate was determined by radioisotope method (Wainshtein *et al.*, 1988).

Results

Water temperature in the springs under study was 1.2-76.2^{0}C (Table 1). Maximum water temperature was in Alla spring and minimum in Gegetui spring. Salinity of water in the springs was 0.1-1.55 g/l. Maximum salinity was measured in Zhoigan spring. The pH values varied from 2.8 (Erdenet spring) to 9.6 (Alla spring). Hydrogen sulfide concentration was 0.46-45.5 mg/l. The highest values were observed in water from Alla and Zmeinaya springs.

In spring outlets and brooks microbial mats have been noticed (Tables 2-3). Cyanobacterial, green and purple mats grow at temperatures below 60^{o}C. Sulfur mats have been noticed in hot and cold springs with a high concentration of sulfide, such as Alla, Umkhei, Zmeyinaya, Kuchiger, Devsen Bulag and Bakhlaita. High methane concentration of Sukhaya Zagza spring is favorable for activity of methanotrophic bacteria. Perennial thick mats with a thickness of 2-7 cm are found in hot brooks of Seiya, Garga, Alla and Khuzhirta springs. At the outlet of Alla spring, situated on the left side at 58^{o}C microbial mat is not formed, on the bottom there is a thin brown film covering black sand and silt, which provides evidence of active sulfate-reducing bacteria.

At 49^{o}C cyanobacterial mats grow and the color gradually changes from bright green to dark yellow. When plating from the mat, *Heliobacter* sp., *Chromatium* sp., *Thiocapsa* sp. and *Desulfobacter* sp. were isolated. At 35^{o}C *Thiothrix* sp. and Cyanobacteria (*Phormidium* sp.) develop, attaching to stones.

At 65-79^{0}C in the spring, situated on the right-bank, there are no visible bacterial communities.

It can be explained by the combined influence of 3 environmental factors: high temperature, pH and sulfide existence. However, when plating sand samples from this mat culture *Chloroflexus aurantiacus* was isolated. 70 cm downstream from the spring outlet a dark-brown mat is developing, formed by *Chloroflexus aurantiacus* and *Termus ruber*. At 59^0C a thick cyanobacterial mat grows, *Chloroflexus* dominating. Thickness of this mat reaches 3-4 cm. Under microscope *Chloroflexus aurantiacus* dominates in the upper layer of the mat; cyanobacteria have not been recorded. In deeper layers *Synechococcus* sp. appears. When temperature decreases cyanobacteria begin to dominate. At 40^oC layers of purple bacteria appear. At 35^oC the color of the mat changes. The surface becomes covered with a white layer of sulfur bacteria. Under this layer cyanobacteria and beneath this purple bacteria grow. At lower temperatures microbial mats are not found. For 20 m downstream colorless sulfuric bacteria *Thiothrix* develops (Barkhutova *et al*., 1998).

In the brook of the hydrogen sulfide spring Devsen Bulag the layer of elemental sulfide is seen as a yellowish film on stones and a white film is noticed on the water surface. Sulfur mats *Thiothrix* have been found in the brook. Cyanobacterial communities appear at 13.5^oC and pH 8.2 and decrease of hydrogen sulfide concentration to 5 mg/l. The total productivity of mat in thermal springs increases when temperature decreases. So, in Alla spring the total productivity increases from 360 mg C/ (m^2 day) in the point BG-8 (temperature 65^oC) to 840 mg C/(m^2 day) (temperature 45^oC). In Garga spring the rate of oxygenous photosynthesis is 203 mg C/(m^2 day), while the rate of anoxygenous photosynthesis is 50 mg C/(m^2 day). On terminal stages of organic matter destruction participates sulfate-reducing acetogens, methanogens and methanotrophs (Table 4). Their activities primarily depend on sulfate concentration in the spring water. In Alla, Zmeyinaya and Garga springs, sulfate concentration is not high and its maximum was noticed in Zmeyinaya spring. Sulfate reduction rate in the studied probes was 0.01-10.4 mg S/(kg day). Maximum rate was determined in the mats of Zhoigon spring. High values for this process have been measured in mat samples from Khoito Gol, Garga and Zmeyinaya spring. In the spring of Barguzin valley and Eastern Sayans part of the organic matter is used for sulfate restoration. A low rate of sulfate reduction has been measured in the

Table 1. Physical and chemical characteristics of mineral springs in Central Asia

Spring	Situation	T^0C	pH	Salinity g/l	Type
Alla	Barguzin range, Buryatia	76.2	9.6	0.24	Nitric
Kuchiger	-“-	43.0	9.8	0.6	Nitric
Bolshaya Rechka	-“-	74.5	10.2	0.39	Nitric
Garga	Ikatskii range	72.7	7.9	1.0	Nitric
Seiya	-“-	50.3	9.6	0.32	Nitric
Uro	-“-	67.2	8.7	0.25	Nitric
Umkhei	The upper Barguzin river	47.6	9.1	0.45	Nitric
Bakhlaita	Valley of Kizhinga river	5.0	8.2	0.5	Sulfide-hydrogen
Khalyuta	Range Khamar Daban	4.2	6.8	0.15	Carbonated
Gegetui	-“-	1.2	7.3	0.24	Carbonated
Zmeinaya	Chivirkuiski Bay, Lake Baikal shore	52.3	8.9	0.43	Nitric
Sukhaya Zagza	Lake Baikal shore	48.0	8.4	0.51	Methane
Khoito Gol	The upper Sentsi river, Eastern Sayans	34.0	8.3	0.62	Carbonated
Shumak	The upper Shumak river, Eastern Sayans	34.0	7.2	0.56	Carbonated
Gadan Gembe	Mountain Tengesiin Dabaan, Eastern Sayans	20.8	6.5	0.56	Carbonated
Zhoigon	Valley of Arzham-Khem river, Tiva	38.5	8.2	1.55	Carbonated
Suduntui	The upper Suduntui river, Aginski region	3.7	6.0	0.25	Carbonated
Alkhanai	Spurs of the mountain Alkhanai, Aginski region	3.0	6.7	0.34	Carbonated
Erdenet	Range Burengiin nuruu, Orkhon aimak, Mongolia	11.6	2.8	0.7	Acid without gas
Khuzhirta	The upper Orkhon river, Uburkhangai district, Mongolia	49.1	9.2	0.24	Nitric
Devsen Bulag	Erden Sant somon, Tov aimak, Mongolia	4.2	7.1	0.4	Sulfide-hydrogen

Table 2. Characteristics of microbial mats of mineral springs

Spring	Sample	Probe type	T°C	pH	Dominant species
Seya	Water body	Grey fine sand	49.7	9.6	-
		Low mat	-	-	*Phormidium,Oscillatoria*
		Floating mat			*Oscillatoria,Phormidium*
	Brook	Thin green mat	47.2	9.6	*Phormidium, Chloroflexus* *Phormidium,Oscillatoria*
	Brook	Thick green mat	46.3	9.7	*Chloroflexus*
Uro	Brook	Emerald-green mat	46.5	8.8	*Phormidium laminosum, Synechococcus elongatus S. elongatus,Ph. laminosum Oscillatoria limosa*
	Brook	Orange Cyanobacterial mat	47.6	9.1	*O.limosa, O. chalibea*
	Brook	Black Cyanobacterial mat	38.5	9.7	*Ph. laminosum, Ph. fragile, G.minor*
	Brook	Yellow-green Cyanobacterial mat	33-40	9.2	*Anabaena*
	Giffon	Mat with sand	64	8.7	*Chloroflexus,* Purple bacteria
	Brook	Sulphur mat	63-61	8.8	*Thermothrix*
Alla	Brook	Green mat	56-45	9.0	*Chloroflexus*
	Brook	Green cyanobacterial mat	30	7.0	*Oscillatoria, Oscillochloris*
	Brook	Green mat	55	-	*Chloroflexus*
	Brook	Sulphur mat	35	-	*Thiothrix, Oscillatoria*
	Brook	Cyanobacterial green mat	40	9.6	*Synechococcus, Chloroflexus*
Garga	Giffon		72.7	7.9	-
	Brook	Dying mat	70	8.0	-
	Brook	Cyanobacterial mat	44	8.5	*Phormidium, Oscillatoria*
	Brook	Green mat	54-49	8.5	*Cloroflexus*
	Brook	Orange-olive-green mat	36.6	9.0	*Oscillatoria Phormidium, Anabaena,Cloroflexus*

mats on the left bank of Alla spring. Sulfate reduction in bottom sediments of Devsen Bulag spring occurred at a rate of 0.81mg S/ (kg day). In the brook in this spring the rate of sulfate reduction increased to 8.8 mg S/ (kg day). Methane formation rate in this spring varied from 32 to 5720.6 µl CH_4/ (kg day). High rates of methane oxidation (up to 13708.2 µl CH_4/ (kg day)) have been observed in sediments of Sukhaya Zagza spring. In this spring most of the organic matter (up to 973.8 mg C/ (kg day) is used for methane formation.

Discussion

Microbiological research carried out in various types of mineral springs shows an abundance of bacteria (about 300,000 cells per ml in spring waters), in sediments (960 to 7,500 cells per ml), in microbial mats (1,200 to 8,000 cells per ml) (Barkhutova *et al*, 1998). Among them aerobic, anaerobic, psychrophilic and thermophilic, autotrophic and heterotrophic, acidophilic and alkaliphilic bacteria, all potential producers of

Table 3. Dominant species of microbial mats of mineral springs

Mats	T^{0}C	pH	Spring	Dominant
Sulfur	4.2-71	8.7-9.3	Bakhlaita, Uro, Umkhei, Devsen Bulag, Zmeyinaya	*Thiothrix, Thiobacillus, Thermothrix*
Purple	5-53	8.8-9.3	Alla, Garga, Uro, Kuchiger, Umkhei	*Thiocapsa roseopersicina, Chromatium venosum*
Green	46-50	8.0-9.3	Garga, Kuchiger, Alla, Umkhei	*Chloroflexus aurantiacus*
Green	1.2-5	2.8-7.3	Alkhanai, Gegetui, Khalyuta, Erdenet, Suduntui	Green filamentous algae
Cyanobacterial	8-54	8.9-9.8	Alla, Garga, Uro, Khuzhirta, Sukhaya Zagza, Khoito Gol, Devsen Bulag, Gadan Gembe, Zhoigon, Bolchaia Rechka	*Oscillatoria, Synechocystis, Phormidium, Synechococcus, Anabaena*

Table 4. Consumption of organic matter in microbial processes in mineral springs

Spring	Sample type	Sulfate reduction, mg C/ (kg day)	Methane formation, mg C/ (kg day)
Alla	Cyanobacterial mat	0.21-0.27	0.16-0.18
	Black silt	0.08-0.22	0.01-0.09
Zmeyinaya	Cyanobacterial mat	3.6	1.1
Kuchiger	Dark-grey silt	0.3	0.03
	Sulphur mat	0.09	0.008
	Cyanobacterial mat	0.4	0.07
Umkhei	Silted sand	0.37-0.52	0.04-0.7
	Cyanobacterial mat	0.45	0.08
Bolshaya rechka	Cyanobacterial mat	4.15	0.62
Bakhlaita	Silted sand	0.63	0.08
	Sulphur mat	0.5	0.16
	Cyanobacterial mat	0.85	0.22
Khoito Gol	Cyanobacterial mat	0.9-6.8	0.001-0.003
Zhoigon	Cyanobacterial mat	0.8-7.5	0.001-0.04
Sukhaya Zagza	Black silt	0.2-3.5	35.0-973.8
Devsen Bulag	Black silt	0.14-1.73	0.08-0.47
	Cyanobacterial mat	0.04-6.6	0.06-12.3

biologically active substances, have been identified. Synthesized by phototrophic and chemolithotrophic microorganisms, autochthonous and allochthonous organic matter is subjected to aerobic and anaerobic breakdown. As a result different substances and metabolites with medicinal effects are formed such as hydrogen sulfide, sulfides, vitamins, volatile organic acids and polysaccharides. Close trophic relationships between the different groups of microorganisms enable them to participate effectively in the transformation of organic and inorganic matters of underground waters. Functional activity of the microbial community depends on physical and chemical conditions in the springs. High temperature and presence of microbial substrates favor bacterial development of different physiological groups. In all the thermal springs of Barguzin valley, Eastern Sayans and Mongolia, development of microbial mats has been noticed. Microbial mats are found in hydrogen sulfide and methane, cold and thermal springs of Buryatia and Mongolia. Quantitative estimation of microorganism activity of thermal and cold mineral springs shows that bacteria and algae play an important role in formation processes of quantitative and qualitative composition of mineral waters and their medicinal factor in mineral springs of Central Asia.

Acknowledgements

The study was supported by Russian Fund for Basic Research (grant ^{1} 03-04-48047, 01-05-97256), Siberian Branch of the Russian Academy of Sciences (grant ^{1} 170) and the Ministry of Education of the Russian Federation (grant ^{1} 02-6.0-294). Thanks also due to the anonymous referees and editors for their useful comments and suggestions.

References

Abidueva E.Yu., Danilova E.V., Namsaraev Z.B. & Namsaraev B.B. 1999. Microbial diversity of mineral springs of Buryatia. *Biodiversity of Baikal, Siberia*. Novosibirsk: Nauka. Siberian FERM, RAS, p. 49-60.

Barkhutova D.D., Danilova E.V., Namsaraev B.B., Gorlenko V.M. & Namsaraev Z.B. 2000. Microbial diversity of thermal springs of Pribaikalye. In: *Biodiversity and Dynamics of Ecosystems in North Eurasia.* Novosibirsk: IC&G., Vol. 5, p. 7-9.

Barkhutova D.D., Danilova E.V. & Namsaraev B.B. 1998. Microbial characteristic of water ecosystems of Zabaikalye. J. Scientific works. Chemistry of biologically active substances. Ulan-Ude: ESSÒU, T.4, 75-83.

Barkhutova D.D., Dagurova O.P., Namsaraev B.B., Marzhangul N. & Oyuntsetseg A. 2003. Sulfate reduction process in the sulfide mineral spring Devsen Bulag (Mongolia). In: *Biodiversity and Function of Microbial Communities of Water and Ground Systems of Central Asia.* Ulan-Ude, BSAA. p. 17-20.

Kuznetsov S.I. & Dubinina G.A. 1989. Methods of research of water microorganisms. Moscow, Nauka, 288 pp.
Pinneker E.V. (ed.). 1980. Geo-chemistry of water of the Mongolian People's Republic. Novosibirsk: Nauka, 80 pp.
Tkachuk V.G., Tolstikhina N.I. (ed.). 1962. Mineral springs of the Southern part Eastern Siberia. Moscow, AS SU, 200 pp.
Wainshtein M.B. & Laurinavichus K.S. 1988. Consideration and cultivation of anaerobic bacteria. Pushino: DNTI NCBI AS SU, 64 pp.
Zavarzin G.A. 1984. Bacteria and atmosphere composition. Moscow, Nauka, 199 pp.

Received: September 2003
Accepted: December 2003

Хураангуй

Рашааны найрлага хэлбэршин тогтоход бактери болон замаг ихээхэн үүрэгтэй болохыг харуулж, ялангуяа бичил биетний тархалт рашааны зарим нэг физик-химийн шинж төлөв, температураас хэрхэн хамаарахыг судлав. Төв Азийн (Монгол ба Байгалийн чандах зарим нутгийн) янз бүрийн хэв шинжийн рашаан дахь микробын бүрдлийг судалсны үндсэнд фототроф бактерийг тодорхойлсны зэрэгцээ тэдгээр рашаан дахь микробын задралын үйл явцын хэм хэмжээг мөн тогтоов.