

# Thermophilic bacteria of the Verhne-Vilyuchinskies and Sredne-Paratunskies hot springs (Kamchatka, Far East)

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**Abstract.** The number of physiological groups, biodiversity, enzymatic activity and some physiological and biochemical properties of microorganisms isolated from thermal waters and microbial mats of Kamchatka were studied. A low content of microorganisms with a predominance of denitrifying (Verhne-Vilyuchinsky) and nitrifying bacteria (Sredne-Paratunsky springs) was shown. Gram-positive spore-forming rods of the genus *Bacillus* predominated among the isolated bacteria. The isolated microorganisms had extracellular enzymatic activity and were capable of growing in wide ranges of temperatures, pH and NaCl concentration. Strains of thermophilic bacteria are of interest for biotechnology purposes.

## 1 Introduction

The Kamchatka Peninsula is one of the largest areas of modern volcanic activity on Earth, which contains many thermal fields with hot springs, geysers, mud volcanoes, fumaroles and other post-volcanic manifestations. Hydrothermal deposits of Kamchatka are currently well studied in both hydrogeological and hydrogeochemical terms [1-5]. Hot springs of Kamchatka are a natural habitat for thermophilic microorganisms. Bacteria in thermal waters play an important role in the geochemical cycle of substances, using a wide range of elements in the process of life, they set in motion biogeochemical cycles that are much faster than inorganic reactions [6,7]. Bacteria are also potential producers of thermostable enzymes [8-10]. The study of thermophilic heterotrophic bacteria, their potential enzymatic activity and physiological and biochemical characteristics is of great practical interest, since bacteria and the enzymes they produce can find wide application in industry, including food, pharmaceutical, textile and other fields. However, the microbiota of Kamchatka thermal springs has not been sufficiently studied to date. The most studied are some springs of the Uzon volcano caldera, as well as the Nachikinsky, Malkinsky and Verkhne-Paratunsky springs [11-16]. The Verkhne-Vilyuchinsky and Sredne-Paratunsky thermal springs have not been studied to date. Only two publications on the Vilyuchinsky thermal waters are noted in the literature [17,18]. In this regard, the aim of the work was to investigate the number of physiological groups, biodiversity, extracellular enzymatic

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activity and some physiological and biochemical properties of isolated bacteria in thermal waters and bacterial mats of the Verkhne-Velyuchinsky and Sredne-Paratunsky springs of Kamchatka.

## 2 Materials and methods

### 2.1 Sampling

Samples of thermal waters and microbial mats were collected in September 2015 in the Verkhne-Velyuchinskiye and Sredne-Paratunskiye hot springs located on the Kamchatka Peninsula. The Verkhne-Vilyuchinskiye thermal springs (N=52°39'15.7"; E=158°13'29.0") are located in the upper part of the Vilyuchi River valley, 1 km upstream from the mouth of the Spokoiny Stream, not far from the foot of the Vilyuchinsky Volcano. The temperature of the springs at the sampling site was 37°C, pH=7.19. The chemical composition of the springs is chloride-hydrocarbonate with a total mineralization of up to 1.2 g/l and a silicic acid content of up to 100 g/l. The springs form a lake. Green microbial mats are formed on the surface of the lake.

The Sredne-Paratunskiye thermal springs are located in the floodplain of the Paratunka River, 6 kilometers southwest of the village of Paratunka, Yelizovsky District, Kamchatka Krai. The springs are represented by many concentrated and scattered outlets of thermal waters over a distance of 1.2 km along the banks and in the bed of a small stream, a tributary of the Paratunka River. Hot water comes out in streams on the left bank of the stream, sometimes it simply oozes through a film of thermophilic green algae covering the bank. Water and mat samples were collected in well GK-6 (N = 52°55'40.1"; E = 158°14'09.4"). The temperature of the springs at the sampling site is 78.3°C, pH = 8.45. The water is sulfate-sodium, siliceous (up to 83 mg/l) with a total mineralization of up to 1.1 g/l. The water contains arsenic 0.16 mg/l. The gas composition is nitrogen. During field work, unstable indicators of chemical composition (pH, temperature) were measured directly on site. Water samples were collected in 1000 ml glass bottles in three replicates, observing sterility conditions, samples of microbial mats were collected in sterile 100 ml glass vials. The collected samples were stored in the refrigerator for no more than 12 hours, then analyzed in the laboratory.

### 2.2 Sample preparation and analysis

To isolate thermophilic bacteria, various selective nutrient media were used [19]. Heterotrophic bacteria were counted on the YK nutrient medium of the following composition (g/l): CaCO<sub>3</sub> – 1; MgSO<sub>4</sub>×7H<sub>2</sub>O – 1; peptone – 5; yeast extract – 5; glucose – 0.2; K<sub>2</sub>HPO<sub>4</sub> – 0.2 [20]. To identify the maximum diversity of bacteria, due to their very low content in groundwater, the isolation of microorganisms was carried out by membrane filtration, filtering different volumes of water (10-100 ml) under sterile conditions for each dish with a nutrient medium. Bacteria were isolated from microbial mats in a volume of 0.1-1 cm<sup>3</sup> in each dish with a nutrient medium. Bacteria were grown in a thermostat at a temperature of 37 °C and 50 °C for 1-7 days. The morphological properties of the isolated isolates, their sizes, motility, and sporulation were studied by microscopic examination of Gram-stained smears using an AxioStar plus light microscope (Carl Zeiss, Germany) (×1000).

To determine bacteria synthesizing exogenous hydrolases, the strains were plated on plates with YK medium supplemented with olive oil and Tween-80 (lipase), milk (proteinase), starch (amylase), and chicken egg (lecithinase) as substrates. To assess

cellulase activity, carboxymethylcellulose (Sigma-Aldrich, USA) was added to Pfennig's medium. Urease activity was assessed on Sengen's agar medium with the addition of urea by the formation of transparent zones and the release of ammonia [21]. Microorganisms were cultivated in a thermostat at temperatures of 37 (Verkhne-Viluchinsky spring), 50°C (Sredne-Paratunsky) for 24-48 hours. The presence of enzymatic activity was judged by the appearance of substrate hydrolysis zones around the bacterial inoculation. To identify hydrolysis zones on a medium with starch, Lugol's solution was additionally added to the dishes and the appearance of light rings around the inoculations, indicating the presence of amylases, was noted. The ability of the strains to grow at different temperatures, pH, and NaCl concentrations was studied by incubating in a YK medium [20]. For this purpose, the bacteria were grown at different temperatures (35, 50, 60, 65, 70°C) in a thermostat for 2-3 days. Different pH values were adjusted with a 10% hydrochloric acid solution or a 10% sodium hydroxide solution. Various concentrations of NaCl (1.5, 10.15, 20%) were added to the basic YK medium. The result was determined after 2-3 days of incubation in a thermostat. The identification of dominant cultures of heterotrophic microorganisms was conducted using molecular genetic analysis of the 16S RNA gene. For this purpose, biomass obtained from single colonies was concentrated. Genomic DNA was isolated using a method based on hexadecyltrimethylammonium bromide [22]. PCR amplification of 16S rDNA was carried out using primers p27f (5'AGA AGA TGA TCC TGG CAC A) and p1524r (5'AAG CAA CTA ACC ACA CCC CA) [23].

## **3 Results and discussion**

### **3.1 Chemical composition of thermal springs in Kamchatka (according to previously published data)**

The results of the study of the chemical composition of the Verkhne-Vilyuchinsky thermal springs obtained earlier showed that the studied waters have a slightly alkaline reaction, in composition these are sodium hydrocarbonate waters with an  $\text{NH}_4$  content of up to 0.23 mg/l. In the cationic part of the content, sodium and potassium prevail over calcium. The Si content is very stable (54-57 mg/l). The F content exceeds 1 mg/l. Among the microelements, there is an increased content of Mn (0.45-0.54 mg/l), Fe (0.36-0.41 mg/l), Br - (0.4 mg/l), I - about 0.2 mg/l, Co (about 0.2  $\mu\text{g/l}$ ), Ni (up to 5.2  $\mu\text{g/l}$ ), Cu (up to 1.7  $\mu\text{g/l}$ ), Zn (up to 2.8  $\mu\text{g/l}$ ), Ge (7.7- 9.5  $\mu\text{g/l}$ ). A stable presence of Y (0.5-0.6  $\mu\text{g/l}$ ) and La (0.01-0.05  $\mu\text{g/l}$ ) is noted [2]. Thermal waters of the Sredne-Paratunskiy hot springs (well GK-6) belong to the group of nitrogen alkaline thermal springs. Among the anions in the waters, sulfate ion prevails (620 mg/l), and among the cations, sodium (220 mg/l). Among the microelements, an increased content of Si (23 mg/l), F (1.75 mg/l), Li (222.9  $\mu\text{g/l}$ ), Al (29.9  $\mu\text{g/l}$ ), as well as Zn (3.7  $\mu\text{g/l}$ ), Cu (3.1  $\mu\text{g/l}$ ) is noted [2].

### **3.2 The number of different physiological groups of bacteria and their biodiversity**

Studies of the number of physiological groups of bacteria revealed that in the Verkhne-Vilyuchinsky thermal spring, characterized by a temperature of 37° C and a pH of 7.19, a slight development of functional groups of bacteria was observed. At the same time, denitrifying ( $9.5 \times 10^2$  cells/ml), autotrophic iron-oxidizing ( $2.5 \times 10^2$  cells/ml), saprophytic microorganisms ( $1.3 \times 10^2$  CFU/ml) dominated in the springs (Table 1). This indicates the processes of transformation of carbon, nitrogen, iron compounds with the participation of bacteria occurring in thermal waters and microbial mats. Also, only in the water of the

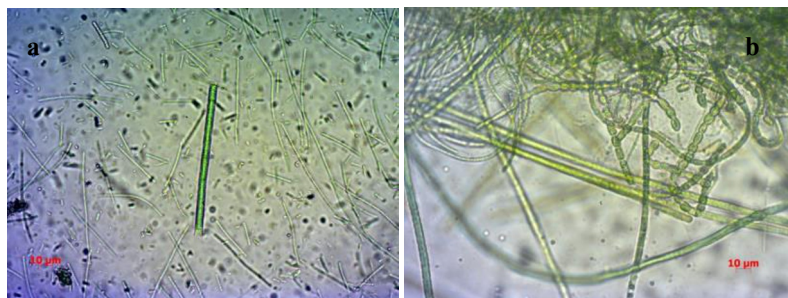
Verkhne-Vilyuchinsky spring were silicate bacteria noted in small quantities, which were capable of destroying silicate minerals (Table 1). In the microbial mats of the Verkhne-Vilyuchinsky springs, the number of bacteria was on average 2 times higher, with a predominance of saprophytic ( $1.2 \times 10^4$  CFU/cm<sup>3</sup>), sulfate-reducing ( $8.6 \times 10^2$  cells/cm<sup>3</sup>), nitrifying ( $6.9 \times 10^2$  cells/cm<sup>3</sup>) and iron and manganese-oxidizing microorganisms ( $1.8-5.4 \times 10^2$  cells/cm<sup>3</sup>) (Table 1). The dominance of heterotrophic bacteria in the microbial mat indicates the presence of organic substances that can be formed with the participation of chemolithotrophic bacteria and cyanobacteria.

**Table 1.** Number of different physiological groups of bacteria in thermal waters and microbial mats of Kamchatka springs.

Functional groups of bacteria: (cells/cm <sup>3</sup> )	Verkhne-Vilyuchinskie		Sredne-Paratunskie	
	Water	Mat	Water	Mat
Carbon cycle:				
Saprophytic bacteria	$1.3 \times 10^2$	$1.2 \times 10^4$	$1.5 \times 10^2$	$8.2 \times 10^3$
Nitrogen cycle:				
Nitrogen-fixing	0	$1.5 \times 10^2$	0	0
Ammonifiers	0	0	0	$7.9 \times 10^2$
Autotrophic nitrifiers	0	0	$6.5 \times 10^2$	0
Heterotrophic nitrifiers	$1.2 \times 10^2$	$6.9 \times 10^2$	$0.4 \times 10^2$	$3.4 \times 10^2$
Denitrifying	$9.5 \times 10^2$	0	0	0
Sulfur cycle:				
Thionaceous	0	0	0	$5.0 \times 10^2$
Sulfate-reducing	0	$8.6 \times 10^2$	0	0
Iron, manganese cycle:				
Iron-oxidizing heterotrophs	$0.5 \times 10^2$	$5.4 \times 10^2$	0	0
Iron-oxidizing autotrophs	$2.5 \times 10^2$	0	0	0
Iron-reducing	0	0	0	0
Manganese-oxidizing heterotrophs	$0.2 \times 10^2$	$1.8 \times 10^2$	$0.01 \times 10^1$	0
Manganese-reducing	$1.0 \times 10^2$	0	$0.3 \times 10^2$	0
Silicon cycle:				
Silicate bacteria	$0.8 \times 10^1$	0	0	0

In the Sredne-Paratunskie thermal springs, which have a higher temperature and pH (78°C, 8.45), the composition and quantity of physiological groups were smaller (Table 1). Nitrifying ( $0.4-6.5 \times 10^2$  cells/ml), saprophytic ( $1.5 \times 10^2$  CFU/ml) and manganese-reducing bacteria ( $0.3 \times 10^2$  CFU/ml) predominantly developed in the water. Saprophytic ( $8.2 \times 10^3$  CFU/cm<sup>3</sup>), ammonifying, nitrifying ( $3.4-7.9 \times 10^2$  cells/ml) and thiobacteria ( $5.0 \times 10^2$  cells/ml) prevailed in the mats. High amounts of sulfates (620 mg/l) noted in the Sredne-Paratunskie thermal waters should have stimulated the growth of bacteria of the sulfur geochemical cycle, but their growth was not noted. This may be due to the low numbers of these bacteria and the high temperature of the thermal waters, which could inhibit bacterial growth. It is known that microbial mats are a kind of filter that accumulates deep microorganisms from thermal waters. Thiobacteria were found in medium numbers in microbial mats, indicating their presence in groundwater. Thiobacteria are known to obtain energy from the oxidation of mineral sulfur compounds such as hydrogen sulfide, sulfides, sulfite, thiosulfate, etc., as well as molecular sulfur. And their presence can contribute to the formation of sulfates in thermal waters.

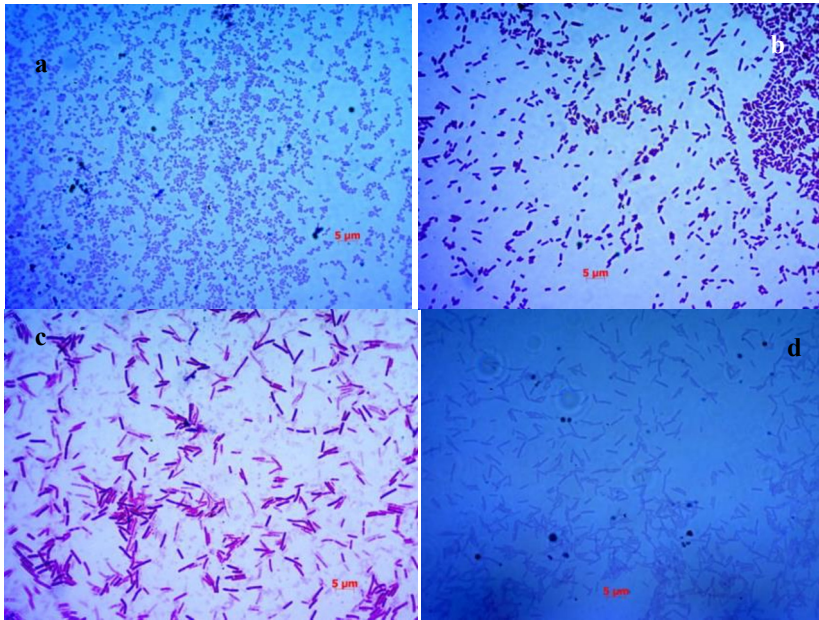
Cyanobacteria were also noted in the microbial mats of Kamchatka hydrotherms, the composition of which varied (Fig. 1). Larger forms of cyanobacterial cells were found in the hot Sredne-Paratunsky springs. Whereas in the composition of microbial mats of the warm Verkhne-Velyuchinsky thermal springs we have previously shown the prevalence of cyanobacteria of the species *Chroococcus turgidus*, *Homoeothrix varians*, *Lyngbya aestuarii*, *Phormidium autumnale*, *Planktolyngbya circumcreta*, *Planktolyngbya cf. Limnetica* [16].



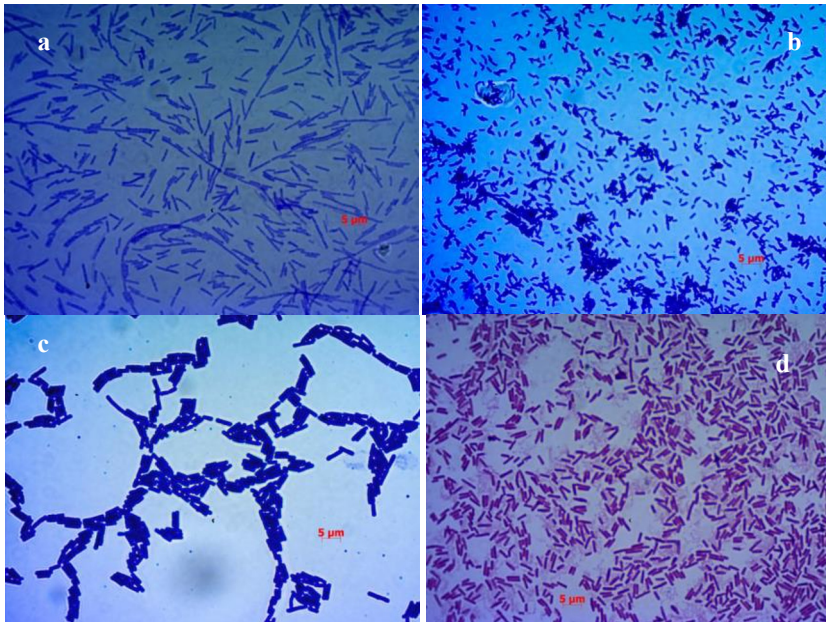
**Fig. 1.** Dominant cyanobacteria in microbial mats of the Verkhne-Velyuchinsky (a) and Sredne-Paratunsky (b) thermal springs of Kamchatka.

From the thermal waters and microbial mats of the Kamchatka hydrotherms, 150 cultures were isolated on various selective media, of which 26 strains of dominant heterotrophic bacteria were purified and used in the work (Verkhne-Vilyuchinskies – 13 cultures, Sredne-Paratunskie – 13 cultures). The isolated strains formed colonies on selective media that were mainly light gray, transparent, beige, brown, yellow, orange and pink. Among the isolated strains, rod-shaped forms significantly prevailed (Fig. 2, 3). At the same time, in the Sredne-Paratunsky spring, both in the water and in the mats, larger rod-shaped bacterial cells were found (Fig. 3).

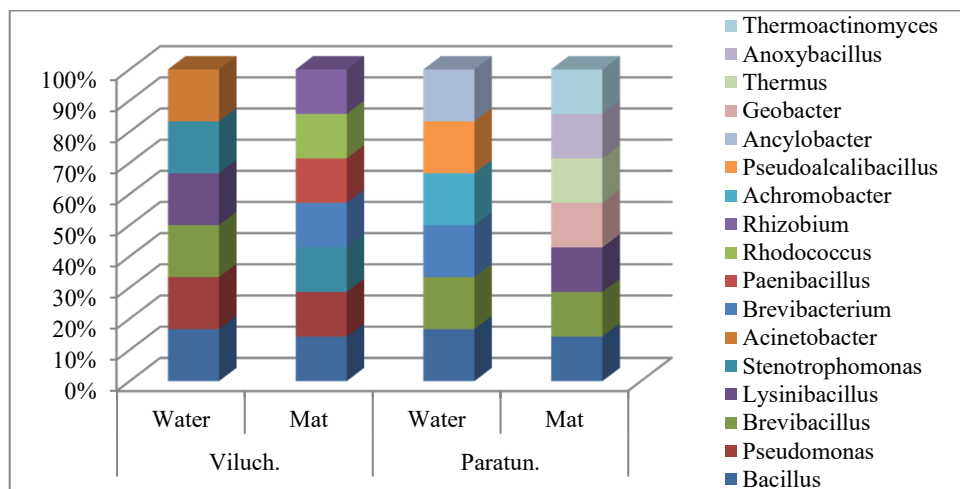
Using molecular genetic analysis of the 16S rRNA gene, the isolated cultures were identified. The results of the study showed that the composition of bacteria in different thermal springs differed (Fig. 4). Bacteria of the genus *Bacillus*, *Pseudomonas*, *Brevibacillus*, *Stenotrophomonas*, and *Acinetobacter* were found in the Verkhne-Vilyuchinskies warm waters. Microorganisms of the genus *Bacillus*, *Brevibacterium*, *Pseudomonas*, *Stenotrophomonas*, *Rhodococcus*, *Rhizobium*, and *Paenibacillus* were detected in the microbial mats of the same warm springs. Bacteria of the genus *Bacillus*, *Brevibacterium*, *Achromobacter*, *Pseudoalcalibacillus*, *Brevibacillus*, and *Ancylobacter* predominated in the Sredne-Paratunskie hot springs. Thermophilic bacteria of the genus *Bacillus*, *Lysinibacillus*, *Geobacter*, *Thermus*, *Anoxybacillus*, *Thermoactinomyces*, *Brevibacillus* were noted in the microbial mats of the same springs. As can be seen, bacteria of the genus *Bacillus* were identified in all the studied springs and in the microbial mats (Fig. 4). It is known that representatives of the genus *Bacillus* are widespread and are often isolated from thermal springs around the world [24-27]. The wide distribution of microorganisms of the genus *Bacillus* in the environment is due to the fact that they are spore-forming and have simple nutritional needs, and therefore are able to colonize oligotrophic niches, such as thermal springs.



**Fig. 2.** Morphological forms of heterotrophic bacteria isolated from thermal waters and microbial mats of the Verkhne-Vilyuchinsky spring (a – strain No. 9; b – No. 20; c – No. 80; d – No. 101).



**Fig. 3.** Morphological forms of heterotrophic bacteria isolated from thermal waters and microbial mats of the Sredne-Paratunsky spring (a – strain No. 50; b – No. 67; c – No. 78; d – No. 146).



**Fig. 4.** Identification of isolated bacterial cultures from thermal waters and microbial mats of the Verkhne-Vilucinsky and Sredne-Paratunsky springs of Kamchatka.

### 3.3 Extracellular enzymatic activity and ecological-physiological properties of isolated bacteria

Among the isolated cultures, spore-forming (75%), catalase-positive (96%) and oxidase-positive (68%), motile (57%) rods sized 0.1-1.5/0.50-8.0  $\mu\text{m}$  in length significantly prevailed. It is known that thermophilic bacteria are capable of forming exoenzymes belonging to the group of hydrolases capable of breaking down large macromolecules into more accessible compounds that penetrate the membrane of the bacterial cell. In this regard, the isolated thermophilic heterotrophic bacteria were tested for the presence of exogenous hydrolytic activity (proteinase, amylase, lipase, lecithinase, cellulase, urease). The results showed that the cultures isolated from microbial mats had the highest enzymatic activity (Table 2). In the Verkhne-Vilyuchinsky spring, the greatest number of strains had amylase (64-95%) and proteinase (34-56%) activity, the smallest number of cultures showed lipase and lecithinase hydrolytic activity (0-21%) (Table 2). Cellulase activity was possessed by 12-23% of the studied strains. Among the isolated strains with enzymatic activity, 67% were represented by gram-positive rods. Bacteria isolated from the Sredne-Paratunsky hot springs showed multiple enzymatic activity (Table 2). In this case, the greatest number of cultures were capable of showing amylase (84-91%), lecithinase (45-64%) and least of all showed lipase (5-10%) and proteinase (15-21%) enzymatic activity. Bacterial strains No. 31 (*Lysinibacillus*), 42 (*Paenibacillus*), 50 (*Brevibacterium*), 56 (*Bacillus*), 101 (*Stenotrophomonas*) had multiple enzymatic exogenous activity. They had the ability to produce 5 of the 6 studied enzymes. Thus, the isolated thermophilic heterotrophic bacteria were mostly able to synthesize the enzymes amylase, proteinase, lecithinase and use polysaccharides as a source of carbon and energy.

A study of the eco-physiology of cultures isolated from thermal springs showed that the strains were capable of developing in a wide range of temperatures (35-70°C), pH (5.0-12.0) and NaCl concentrations (1-20%), exhibiting properties of halo-, alkali- and thermotolerance. Most of the tested strains showed growth at a temperature of 35-60°C, 5 strains out of 26 were capable of growing in the range of 50-60°C, and one strain (No. 68) showed growth at 65°C. In relation to the acidity of the medium, most strains were able to develop at pH 5-9, growth in more alkaline conditions (pH 11-12) was typical of gram-positive cultures No. 3, 42, 56, 78, 86, 148. Most of the studied strains showed growth at a

NaCl concentration in the medium within 1-8%, 14 of 26 isolates were able to develop at a high NaCl concentration of 10-12% (Table 2). A salt concentration of 15% was critical for strain growth. The noted wide range of growth of the studied cultures indicates a high adaptability of the strains to growth at different temperatures, pH and salinity. High enzymatic activity of bacteria and their ability to grow in a wide range of temperatures, pH, NaCl concentration indicate the prospects for using the isolated cultures in the field of biotechnology.

**Table 2.** Extracellular hydrolytic activity of bacterial strains isolated from hot springs of Kamchatka.

Thermal springs:	t° C	Place of allocation	% of crops exhibiting hydrolytic activity					
			Proteinase	Amylase	Lipase	Lecithinase	Cellulase	Urease
Verkhne-Vilucinskic	37	Water	34	64	0	10	12	40
		Mat	56	95	0	21	23	35
Sredne-Paratunskic	78.3	Water	15	84	5	45	43	14
		Mat	21	91	10	64	31	37

## 4 Conclusions

Thus, the thermal springs of Kamchatka have a low number of physiological groups of bacteria and a small diversity of taxonomic groups. At the same time, bacteria of the geochemical cycles of nitrogen, carbon and iron prevailed in the Verkhne-Vilyuchinskic springs. Bacteria of the geochemical cycles of nitrogen, carbon and sulfur prevailed in the Sredne-Paratunskic thermal springs. The conducted studies made it possible to isolate thermophilic bacteria from the thermal springs of Kamchatka and study some of their cultural, morphological and physiological-biochemical properties. It was shown that most thermophilic bacteria are represented by gram-positive spore-forming rods. Identification of the strains showed a significant predominance of bacteria of the genus *Bacillus* in the water and in the microbial mats of the studied springs. The isolated isolates had the highest amylase extracellular hydrolytic activity and were able to grow in a wide range of temperatures, pH and NaCl concentrations, which indicates their adaptability to high temperatures, mineralization and alkaline conditions of the environment. The diversity of growth conditions of the strains suggests that their hydrolytic enzymes have a set of useful characteristics, including thermal stability and resistance in a wide range of pH and mineralization values. The data obtained suggest that the water and microbial mats of the Verkhne-Vilyuchinsky and Sredne-Paratunsky thermal springs of Kamchatka are populated by a unique community of thermophilic microorganisms. There is no doubt that this microbial community plays a special role in the functioning of the hydrothermal ecosystem, including in biogeochemical transformations, precisely due to its high potential metabolic activity.

## References

1. Yu.F. Manukhin, L.A. Vorozheikina, *Hydrothermal Systems and Thermal Fields of Kamchatka* (Vladivostok, 1976)
2. O.V. Chudaev, V.A. Chudaeva, G.A. Karpov, M. Edmunds, P. Shand, *Geochemistry of waters in the main geothermal areas of Kamchatka* (Dalnauka Publishing House, Vladivostok, 2000)

3. O.V. Chudaev, G.A. Chelnokov, I.V. Bragin et al., *Russ. J. Pacific Geol.* **35(6)**, 458–475 (2016)
4. I.V. Bragin, N.A. Kharitonova, G.A. Chelnokov et al., *Environm. Earth Sci.* **77**, 376–389 (2018)
5. G.A. Chelnokov, N.A. Kharitonova, I.V. Bragin et al., *Moscow Univ. Geol. Bull.* **75**, 196–204 (2020)
6. E.V. Lazareva, A.V. Bryanskaya, S.M. Zhmodik et al, *Reports of the Academy of Sciences* **430(5)**, 675–680 (2010)
7. R.C. Beaver, J.D. Neufeld, *The ISME Journal* **18(1)**, 091 (2024)
8. E.G. Lebedeva, N.A. Kharitonova, *Samara Scientific Bulletin* **9**, 79-85 (2020)
9. E.G. Lebedeva, N.A. Kharitonova, I.V. Bragin, T.V. Kuzmina, *Bull. of Tomsk Polytech. Univer. Geores. Engin.* **334 (1)**, 116-125 (2023)
10. M. Guta, G. Abebe, K. Bacha, P. Cools, *Microbiol. Spectr* **12(3)**, e03710-23 (2024)
11. V.M. Gumerov, A.V. Mardanov, A.V. Beletsky, E.A. Bonch-Osmolovskaya, N.V. Ravin, *Microbiology* **2**, 258-265 (2011)
12. N.A. Chernyh, A.V. Mardanov, V.M. Gumerov et al, *Extremophiles* **19**, 1157-1171 (2015)
13. A.S. Rozanov, A.V. Bryanskaya, T.K. Malup et al, *BMC Genomics* **15**, 12 (2014)
14. A.Y. Merkel, N.V. Pimenov, I.I. Rusanov et al, *Extremophiles* **21**, 307–317 (2017)
15. T. V. Kochetkova, O. A. Podosokorskaya, A. G. Elcheninov, I. V. Kublanov, *Microbiology* **91**, 1–27 (2022)
16. T.V. Nikulina, E.G. Kalitina, N.A. Kharitonova et al, *Diatoms: Fundamentals & Applications* (Wiley-Scrivener, Beverly, MA, USA, 2019)
17. N. Belkova, J.R. Zakharova, K. Tazaki, V.M. Okrugin, V. V. Parfenova, *International Microbiology* **7(3)**, 193-198 (2004)
18. N.L. Belkova, V.M. Okrugin, K. Tazaki, *Microbiology* **5**, 713-716 (2006)
19. S.I. Kuznetsov, G.A. Dubinina, *Methods for studying aquatic microorganisms* (Nauka Publishing House, Moscow, 1989)
20. M. Youchimizu, T. Kimura, *Fish Pathology* **10(2)**, 243-245 (1976)
21. V.V. Lysak, R.A. Zheldakova, O.V. Fomina, *Microbiology workshop* (BSU, Minsk, 2015)
22. K.V. Kiselev, A.S. Dubrovina, A.P. Tyunin, *J. Plant. Physiol.* **175**, 59-67 (2015)
23. D.J. Lane, *Nucleic acid techniques in bacterial systematic* (John Wiley and Sons, Chichester, 1991)
24. R. Safitri, D.P. Kusumawardhani, A. Annisa, R. Partasasmita, S. Asharina, A.M. Maskoen, *Biodiversitas* **21**, 3444-345 (2020)
25. O. Ulucay, A. Gormez, C. Ozic, *Antonie van Leeuwenhoek* **115**, 253-270 (2022)
26. S.P. Bernardo, A.R. Rosana, A.N.de Souza, S. Chiorean, M.L. Martins, J.C. Vederas, *Microbiol. Resource Announc* **17**, 106-120 (2020)
27. A. Saghatelyan, A. Margaryan, H. Panosyan, N. Birkeland, *Microorganisms* **9**, 1473 (2021)