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TAXONOMIC COMPOSITION AND PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF CULTIVATED MICROORGANISMS ISOLATED FROM KUDURITE ROCKS OF THE PRIMORSKY KRAI AND THE REPUBLIC OF ALTAI (RUSSIA)

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Abstract

The reason for the consumption of rocks (kudurits) by wild animals has not yet been identified. One of the hypotheses of geophagy is a microbiological factor, i.e. rocks can be a source of microorganisms useful for wild animals. In this work, the composition of cultured microorganisms of kudurits of Primorsky Krai and the Altai Republic, as well as some physiological and biochemical properties of the isolated bacteria were studied using molecular genetic analysis of the 16S RNA gene. It has been shown that the studied breeds contain a high amount of physiological groups of bacteria (saprophytic, ammonifying, nitrifying, silicate,

hydrolytic) and yeasts (10^6-10^9 cells/ml) useful for animals. Among the isolated culturable microorganisms in the curls of Primorsky Krai, representatives of the genus *Pseudomonas, Bacillus, Rhodotorula, Paenibacillus, and Stenotrophomonas* predominated. The bacteria of the genus *Bacillus, Chryseobacterium, and Streptomyces* dominated in the edible soils of Altai. Among the isolates, non-pigmented, motile (35-70%) catalase-positive (86-91%) and oxidase-negative (68-76%) rods (67-90%) predominated. All cultures were characterized by a high level of extracellular enzymatic activity, especially in relation to such substrates as casein (57-75%), starch (57-60%), urea (43-45%). The isolated microorganisms are able to develop in a wide range of temperatures (5-50^{0}C), pH (5-12), NaCl concentration (1-18%) and utilize a wide range of carbohydrates and alcohols. It has been established that the microorganisms and biologically active compounds they produce can be a factor that encourages animals to instinctively consume rocks. High antibiotic activity of the isolated cultures has been noted against opportunistic bacteria *Staphylococcus aureus, Micrococcus luteus* and phytopathogenic fungi *Fusarium oxysporum.*

Key words: bacteria, animals, geophagy, yeast, antibiotic, enzymatic activity.

1. Introduction

In many regions of the world, an instinctive form of consumption of rocks (the phenomenon of geophagy) is widespread among herbivorous animals, accompanied by the emergence of kudurs - natural complexes with characteristic traces of animal activity, often existing for many centuries. By geological nature, the consumed rocks (kudurites) are usually represented by clayey varieties with a predominance of smectite or kaolinite, sometimes with zeolites. Kudurs have been described for various regions of the world. In Russia they are known in the Caucasus, in the mountains of Southern Siberia, in Yakutia, Chukotka and Sikhote-Alin (Panichev et al., 2016; Panichev et al., 2021; Panichev et al., 2022a). Their most typical visitors are herbivorous mammals. In the case of Primorsky Krai and Altai, these are mainly red deer *(Cervus elaphus*), elk (*Alces alces*), roe deer *(Capreolus pygargus*) and hares (*Lepus timidus,*

L. brachiurus), sometimes the kudurs are visited by musk deer (*Moschus moschiferus*). In the Altai Mountains, mountain goats (*Capra sibirica*) and livestock are also active visitors to kudurs. Our interest in the microbiological characteristics of mineral substances consumed by animals on kudurs is due to the desire to understand the cause of the phenomenon of geophagy, which still remains largely unknown. Among the causes of geophagy, there are several hypotheses. The most common of them are: the need for sodium and mineral sorbents to normalize the electrolyte balance in the digestive tract (Klaus, Schmid, 1998), the use of bacteriostatic properties of clay minerals in the fight against pathogenic microflora and intestinal parasites; replenishment of symbiont microorganisms, regulation of pH in the digestive tract (Ketch et al, 2001; Wilson, 2003); removal from the body of toxic chemical elements and compounds using mineral sorbents (Houston et al., 2001; Dominy et al., 2004; Ekosse et al., 2020); replenishment of deficient REEs from the group of light lanthanides (Panichev, 2015; Panichev et al., 2021; 2022b).

It is quite possible that animals may be attracted not only by the chemical composition, but also by the specific microbial community characteristic of the mineral substances they consume. Since it is known that various groups of microorganisms participate in the processing of food in the body of animals (Li et al., 2023), it is quite appropriate to assume that the rocks consumed by animals can be sources of beneficial microorganisms that may have various enzymes for digesting plant food, can be a source of protein, participate in the synthesis of vitamins, antibiotics, and are a factor in the nonspecific defense of the animal body. It is also possible that microorganisms can participate in the transformation of mineral forms of trace elements, after which they can be assimilated in the body of mammals. In this regard, the study of the diversity, enzymatic and antibiotic activity of microorganisms in rocks is important for understanding the causes of geophagy, the ecology of animals and their interactions with microorganisms. Moreover, studies of the microbial composition of rocks in the context of

geophagy are still rare. Along with the mentioned works, only (Podchasova, 2020, Lebedeva et al., 2020) can be added.

The purpose of this work is to study the biodiversity of cultivated microorganisms in kudurite rocks of Primorsky Krai and the Altai Republic and to investigate the physiological and biochemical properties, extracellular enzymatic and antibiotic activity of the isolated cultures.

2. Materials and methods

2.1. Subjects and sampling

The object of the microbiological study included 16 rock samples collected in 2020-2021 in Primorsky Krai and the Altai Republic. In Primorsky Krai, rock samples were collected in the territories of the Call of the Tiger and Bikin National Parks located in the southern and central parts of Sikhote-Alin (Olginsky, Terneysky and Pozharsky districts of Primorsky Krai (Fig. 1). In the Altai Republic, samples were collected in the Ondugaisky, Turochaksky and Ulagansky districts (Fig. 1). All kudurite samples are wet or dry mineral soils of various colors with a low proportion of organic matter. The characteristics of the collected rock samples are presented in Table 1. Kudurite samples were collected in sterile disposable containers, observing sterility conditions. Before processing, the samples were stored under anaerobic conditions in a refrigerator at a temperature of 4^0C for no more than 12 hours.

2.2. Chemical methods

The composition of minerals, major elements and microelements in kudurites was studied. Physicochemical methods for studying the kudurites considered in this article are described in detail in already published articles (Panichev et al., 2021, Panichev et al., 2022 a,b). Preparation for analysis and analytical studies of kudurite samples were carried out at the Analytical Center of the Far Eastern Geological Institute, Far Eastern Branch of the Russian Academy of Sciences (AC FEGI). The sum of LOI (loss on ignition) and SiO_2 content were determined by gravimetry. Determination of major and trace elements was carried out by ICP-MS (iCAP 7600 Duo

spectrometer). Preparation of samples for analysis was carried out by open acid decomposition (HNO_3 + $HClO_4$ + HF). To clarify the micromineral composition of kudurite samples, a scanning electron microscope with Tescan Lyra 3 XMH + EDS AZtec X-Max 80 and JSM-6490LV analytical attachments with INCA Energy, X-max EDS and INCA Wave VDS was used at the AC FEGI. The quantitative mineral composition of the samples was determined by X-ray diffraction on an Ultima-IV X-ray diffractometer from Rigaku (Japan) at the Department of Engineering and Environmental Geology of the Geological Faculty of Lomonosov Moscow State University (Moscow).

2.3. Determination of the number of microorganisms and cultivation conditions

The number of microorganisms of a number of physiological groups was determined in rock samples using the Koch method and limiting tenfold dilutions (Lysak et al., 2015). To obtain a microbial suspension, a 10 g sample of rocks was added to 90 ml of sterile physiological solution and mixed on a shaker for 30 minutes at 120 rpm. The aqueous fraction was used to inoculate liquid selective nutrient media. The results were assessed using the most probable number method according to the McCready table. The cultures were repeated three times. Microorganisms were grown in a thermostat at 25^0C for 7-20 days. Anaerobic forms of bacteria were cultivated in an anaerobic jar using BD GasPak EZ gas-generating bags.

2.4. Isolation of pure cultures of microorganisms and study of their physiological and biochemical properties

Pure cultures of heterotrophic microorganisms were isolated by successive subcultures from liquid media to corresponding solid media containing 15 g of agar-agar per liter. The purity of the cultures was controlled microscopically. The morphology, size, and mobility of isolated pure cultures of microorganisms were studied using an AxioStar plus light microscope (Carl Zeiss, Germany) equipped with an Axiovision photo-documentation system and a phase-contrast attachment (x1000). Motility, type of bacterial cell wall, and determination of the production of redox enzymes by cultures were carried out using standard methods (Lysak et al.,

2015). To study the ability of microorganisms to utilize mono-, disaccharides and alcohols, we used ready-made Hiss media “Biocompass-S” (Uglich, Russia), containing the following substrates: glucose, lactose, maltose, mannitol, sucrose, fructose and xylose. Hiss media with carbohydrates were poured into 5 ml test tubes and, using a bacteriological loop, the test microorganism was injected into the medium. Two rows of tubes were used. After sowing, 2 ml of sterile vaseline oil was added to one of them to create anaerobic conditions. They were incubated for 2–4 days at a temperature of 25^{0}C. The results were judged by the change in pH of the medium, as evidenced by a change in the color of the indicator from purple to yellow. The ability of strains to grow at different temperatures, pH, and NaCl concentrations was studied by incubating them in MPA medium (Netrusov et al., 2005). To do this, the tested cultures were streak-sown and grown at various temperatures (5, 10, 25, 35, 42, 50, 60^{0}C) in a thermostat for 4-5 days. Various pH values were adjusted with 10% hydrochloric acid solution or 10% sodium hydroxide solution (5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0). Various concentrations of NaCl were added to the basic MPA medium (1, 3, 6, 8, 10, 15, 18, 20, 22%). The result was determined after 4-5 days of incubation in a thermostat at 25^{0}C.

2.5. Study of extracellular enzymatic and antibiotic activity of isolated cultures

The proteolytic, lipase, lecithinase, pectinase, amylase and cellulase extracellular enzymatic activities of the strains were studied. Testing for proteolytic activity was carried out on milk agar by adding sterile skim milk (15 ml per 100 ml) to sterile starvation agar (1.8%). Lipase activity was determined on a medium of the following composition (g/l): tryptone -10, yeast extract -5, agar - 20, lipid homogenisate - 30 ml. To determine lecithinase activity, chicken egg yolk was used as a substrate, which was carefully suspended and added to YK medium. The prepared medium was poured into sterile dishes. Amylase activity was determined in YK medium without glucose with the addition of 0.2% soluble starch. To determine pectinase activity, a medium of the following composition (g/l) was used: yeast extract - 10, pectin - 10, agar - 15, NaCl - 5. Cellulase activity was determined on Pfennig's medium using Congo red

dye. The cultures were streaked and incubated for 4-5 days at 25^{0}C. Enzymatic activity was recorded by the formation of transparent zones of substrate hydrolysis (milk, starch, Tween 80, egg, pectin, carboxymethylcellulose) (Lysak et al., 2015).

The antibiotic activity of microbial cultures was determined using the agar block method (Zenova et al., 2002). This method is convenient,for,it allows you to select optimal nutrient media for both antimicrobial substance-producing strains and test strains. Museum strains Staphylococcus aureus NCTC 13373, *Enterococcus faecalis* NCTC 12697, *Micrococcus luteus* B-7845, *Saccharomyces cerevisiae* Y-3251, as well as cultures of filamentous phytopathogenic fungi *Phytophthora infestans* and *Fusarium oxysporum* from the collection of the Federal Scientific Center for Agrobiotechnology were used as test cultures. DV im. A.K. Seagulls, kindly provided by N.V. Otsishina, an employee of the laboratory of selection and genetic research. Strains producing antimicrobial substances were sown in a “solid lawn” on solid nutrient media (MPA and Sabouraud) and cultivated in a thermostat at a temperature of 25°C for 24 hours for the diffusion of antimicrobial substances into agar. A day later, the test cultures were also sown in a “solid lawn” on solid nutrient media (MPA - for bacteria; Sabouraud - for *Fusarium* yeasts and fungi; rye-vegetable agar - for *Phytophthora)*. After inoculation, round holes in the medium were immediately prepared using sterile plastic tubes. Next, agar blocks with grown producer cultures were cut out in a similar manner and placed inside the holes on media with test cultures. The finished dishes were incubated in a thermostat at 25°C for 5–7 days. Antibiotic activity was assessed by the presence and size of zones of inhibition or lack of growth of test cultures around agar blocks.

2.6. Molecular genetic methods for identifying pure cultures

Identification of the dominant cultures of heterotrophic microorganisms was carried out using molecular genetic analysis of the 16S RNA gene. To do this, the biomass obtained from single colonies was concentrated. Genomic DNA was isolated using a method based on hexadecyltrimethylammonium bromide (Kiselev et al. 2015). PCR amplification of 16S rDNA

was performed using primers 27F 5'AGA GTT TGA TCM TGG CTC AG and 1524R 5'AAG GAG GTG ATC CAR CCG CA (Lane, 1991), and for ITS1 amplification ITS-s 5'AGG AGA AGT CGT AAC was used AAG G and ITS-a 5'TCC TCC GCT TAT TGA TA TGC (White et al. 1990). Amplification was carried out in 30 μl of a reaction mixture containing 20-50 ng of genomic DNA and 2 unitsof Taq DNA polymerase (Evrogen, Moscow, Russia). The final concentration of each dNTP was 200 μM; the forward and reverse primers were 0.5 μM each. The PCR temperature regime included: 1) preliminary denaturation of DNA at 95°C for 2 min; 2) 35 cycles of DNA denaturation at 95°C – 15 sec, primer annealing at 53°C – 10 sec; DNA elongation at 72°C – 90 sec; 3) the last step of DNA elongation at 72°C – 5 min. The reaction results were analyzed by electrophoresis in 1% agarose gel. PCR fragments were purified from an agarose gel using the Cleanup Mini kit (Evrogen, Russia) and sequenced on a 3130xL DNA analyzer (Applied Biosystems, USA) using the primers described above. The search for homologous sequences was carried out on NCBI servers (http://blast.ncbi.nlm.nih.gov). Gene sequence assembly, multiple alignments, and genetic distance calculations were performed using MEGA v.7 (Tamura et al. 2013). Phylogenetic analysis was carried out using the MEGA program version 7.0, Neigh-bor-Joining cluster method Kimura 2-parameters algorithm. The statistical significance of branching was assessed using bootstrap analysis of 100 alternative trees. Sequencing was carried out on the basis of the Federal Research Center for Biodiversity of Terrestrial Biota of East Asia, Far Eastern Branch of the Russian Academy of Sciences.

3. Results

3.1. Mineral and chemical composition of rocks

The detailed mineral and chemical composition (macro- and microelements) of kudurites (except for 4 samples from the Primorsky Krai of Terneysky and Pozharsky districts) is given in the above-mentioned published articles, as well as in the article (Lebedeva et al., 2020). According to X-ray phase analysis, the mineral composition of the collected kudurites in the Primorsky Krai turned out to be of the same type. The rocks are represented either by pure

smectite or smectite-kaolinite clays, or by clay-zeolite mineral mixtures with an admixture of quartz crystals and feldspars of sandy and silty sizes. The amount of quartz crystals and feldspars in kudurites usually ranges from 20 to 50% of the volume, clay minerals from 10 to 80% of the rock volume. The content of zeolite grains (usually heulandite and clinoptilolite of silty size) can vary from 20 to 40%. The mineral composition of Altai kudurites is usually dominated by quartz and feldspars of sand-siltstone fractions (up to 50%), as well as clay minerals, which are dominated by hydromicas and chlorites. In terms of chemical composition, kudurites from the Primorsky Krai correspond to the composition of volcanic tuffs (acidic and intermediate composition), from which, in fact, they are formed. The age interval of deposition of all tuffs, from which kudurites are formed in Primorye, corresponds to the Paleogene. The chemical composition of Altai kudurites is most often inherited from metamorphic primary sedimentary schists of Proterozoic or Early Paleozoic age of greenschist metamorphic facies. Table 2 shows the composition of the main elements in all the studied kudurites from Primorsky Krai and Gorny Altai. According to the silicon oxide content, all kudurites can be classified as rocks of acidic and medium composition with normal alkalinity. According to the content of microelements, such rocks correspond to the average composition of rhyolites and dacites.

3.2. Number of cultivated microorganisms in rocks

The results of microbiological studies of rocks from the Primorsky Territory (12 samples) and Altai (8 samples) showed that the soils contain a fairly high number of bacteria of various physiological groups, reaching values of 5.3×10^6 for some groups (ammonifying, No. Alt 2.2) (Altai)/ 1.2×10^9 cells/g (yeast, No. B-48) (Primorye) (Table 3). In the structure of communities of cultivated microorganisms in rocks of the Primorsky Krai, heterotrophic bacteria significantly predominated. In the soils of two regions of Primorye, aerobic and anaerobic saprophytic microorganisms (0.7×10^2 M14-4.4×10^8 M3-1 cells/g) and hydrolytic bacteria (especially amylolytic and saccharolytic, up to 4.5×10^7 No B- 27 cells/g). Also, the soils of Primorye were characterized by high numbers of silicate (up to 1.2×10^9 No.B-48 cells/g),

nitrogen-fixing (up to 9.2×10^6 M3-1 cells/g), ammonifying (up to 8.4×10^6 M181 cells/g) and carrying out heterotrophic nitrification of bacteria (up to 2.2×10^8 M3-1 cells/g). The minimum quantities of all studied physiological groups of microorganisms were found in a soil sample (M14), externally similar to rocks, but not eaten by animals (Table 3). There was also no yeast in this sample, whereas in other soils their abundance reached high values (Table 3).The Altai rocks were distinguished by lower numbers of saprophytic and silicate bacteria, the absence of yeasts, and the presence of significantly higher numbers of thionic and sulfate-reducing bacteria, as well as autotrophic nitrifying and cellulose-decomposing microorganisms (Table 3). Each rock sample was characterized by its own dominant composition of physiological groups. Soils No. Alt 1.1, Alt 1.2, Alt 2.2 were characterized by a higher average number of bacteria and a predominance in the structure of ammonifying, saprophytic, heterotrophic nitrifying, nitrogen-fixing and saccharolytic microorganisms (Table 3). Rock samples No. Alt 2.3, Alt 2.4, Alt 2.5 contained the largest number of saprophytic, hydrolytic, sulfate-reducing, nitrogen-fixing bacteria. Rocks No. Alt 1.3, Alt 2.1 were distinguished by the absence of hydrolytic bacteria and the predominance in the structure of autotrophic nitrifying, nitrogen-fixing, silicate, saprophytic and sulfate-reducing bacteria (Table 3).

3.3. Diversity of microorganisms in rocks

After initial sowing on selective nutrient media, 101 strains of dominant heterotrophic microorganisms belonging to 31 genera were isolated from rocks (Primorye - 80, Altai - 21). Heterotrophic microorganisms were identified using molecular genetic analysis of the 16S RNA gene. Accession numbers of strains of bacteria and fungi that are isolated from rocks are registered in the international database (NCBI) and are presented in the appendix. The results of the study showed that the isolated bacteria belonged to the phyla *Bacteroidota, Proteobacteria, Firmicutes, Actinobacteria*, yeasts were represented by the *Basidiomycota* department. The main part of cultivated heterotrophic microorganisms in all samples was represented by two dominant phyla *Proteobacteria* (14.3-57.4%) and *Firmicutes* (7.1-57.1%)

(Fig. 2). In the rocks of the Primorsky Krai, a predominance of *Proteobacteria* (43.2%) was noted, while in the rocks of Altai *Firmicutes* (40.9%) significantly dominated. The largest number of taxonomic groups of bacteria from the phylum *Proteobacteria* was identified in rocks M181, the smallest was noted in soils M4-1 (Primorsky Krai) (Fig. 2).Bacteria from the phylum *Bacteroidota* were poorly represented in rocks (3.7-9.1%) and their largest quantities were noted in Altai rocks. In the soils of the Primorsky Krai, bacteria from this phylum were noted only in samples M181, B-42 and were represented by bacteria of the genus *Chryseobacterium, Flavobacterium* (Fig. 3).

Yeasts from the department *Basidiomycota* were found in all rock samples from the Primorsky Krai, except for M14, which was not eaten by animals (Fig. 2). Also, yeasts were not detected in the soils of Altai. The greatest diversity of microbial taxa was noted in rocks M181, B-42, B-47, Alt 2.4, the least was found in samples M3-3, B-48, B-47, Alt 1.1, 1.2. The composition of microorganisms differed in different rocks (Fig. 3). The rocks of the Olga region were characterized by the dominance of bacteria of the genus *Bacillus, Pseudomonas, Acinetobacter*, and *Paenibacillus*. In the rocks of the Terneysky and Pozharsky districts of the Primorsky Krai, the predominance of microorganisms of the genus *Bacillus, Pseudomonas*, and *Stenotrophomonas* was noted. Altai soils were distinguished by the dominance of microorganisms of the genus *Bacillus, Chryseobacterium*, and actinomycetes of the genus *Streptomyces, Rhodococcus*, and *Pseudoarthrobacter* (Fig. 3). Thus, *Bacillus* and *Pseudomonas* bacteria prevailed among bacterial genera, which is generally typical for most types of soils and rocks. It should be noted that bacteria of the genus *Acinetobacter, Chryseobacterium, Novosphingobium, Yersinia, Acidovorax, Micrococcus, Herbiconiux, Variovorax, Serratia* were identified only in rock samples from the Olga region, and representatives of *Brevibacterium, Collimonas, Viridibacillus, Lelliottia, Iodobacter*, Georgian were found only in samples from Pozharsky and Terneysky districts. Only in the Altai rocks

were bacteria of the genus *Alcaligenes, Streptomyces, Brevibacillus, Pseudoarthrobacter, Proteus,* and *Sphingopyxis* noted (Fig. 3).

For all bacterial strains, the structure of the nucleotide sequences of the 16S rRNA gene (about 1300 nucleotide pairs) was determined, which had a high percentage of similarity (98-100%) with the DNA of bacteria from various natural ecosystems. For some strains of bacteria isolated from rocks of the Primorsky Krai and Altai, phylogenetic trees were constructed (Fig. 4, 5). The phylogenetic analysis confirmed that the majority of isolates isolated from the rocks of the Primorsky Krai belong to the genera *Pseudomonas, Bacillus, Paenibacillus*, and *Stenotrophomonas.* The nucleotide sequences of strains No. 8,33, 65, 66, 85, 244, 248, 249, 263 had 99.87-100% similarity to the sequences of the bacteria *Pseudomonas sp.* (CP052860), *Ps. brassicacearum* (KT695827), *Ps. frederiksbergensis* (HQ242750), *Ps. kribbensis* (CP029608), *Ps. anguilliseptica* (AF439803), *Ps. fulva* (MT634251), *Ps. protegens* (MH669312) isolated from various soil types in China and the USA. The sequence of strains no. 5, 26,174, 199 had high similarity (99.86-100%) to *Ps. putida* (AY918067), *Ps. protegens* (MK182884; CP043179), *Ps. fluorescens* (KU173826) isolated from river waters of Africa, China and Germany. The sequences of strains No. 68, 189 had 99.73-100% similarity to *Ps.lurida* (CP023272), *Ps. poae* (KY634431), isolated from a rotting apple and the root of the medicinal plant *Astragalus membranaceus*, China.

Analysis of the nucleotide sequences of the 16S rRNA gene fragment of strains No. 31, 46, 90,121a, 152, 158, 160, 200 A, 222, 277 showed their belonging to the class *Bacilli,* family *Bacillaceae* and 98.93-100% similarity with *Bacillus amyloliquefaciens* (KT588663), *B. nakamurai* (NR_151897), *Bacillus sp* (JN874755; KU598979; MN855347), *B. thuringiensis* (CP050183), *B. cereus* (MT611946), *B. mycoides* (MN710448), isolated from soils of China, Africa, Argentina, India , Canada, Pakistan. The sequences of strains No. 56, 262a, 262b were highly similar (100%) to *B. proteolyticus* (MT184819) and *B. nakamurai* (MH057385), isolated in India from the seeds of *Triticum aestrivum* and the plant *Dioscorea bulbosa*. The nucleotide

sequence of strain No. 121 is 99.92% similar to *Bacillus foraminis* (KJ575004) (renamed Mesobacillus), isolated from deep-sea sediments of India (Bezplodny Island). The closest homologues of isolates no. 44, 60, 62b, 83, 193 were strains of *Paenibacillus sp.* (JQ345703; DQ243814; GU733398*), P. glucanolyticus* (CP028366), *P. taichungensis* (KT933220), (more than 99% homology), isolated from groundwater, an aqueous solution of lignin residues and hemicellulose, sedimentary rocks of Brazil and soils in Sweden. Strains No. 15,165,168 were homologous (100%) in the 16 pRNA gene to the bacteria *Stenotrophomonas rhizophila* (MK371075) *and S. maltophilia* (AB680524) and were isolated from permafrost soils of central Evenkia, Russia and soils of Japan. 100% homologues of strains No. 210 and 225 were *S. maltophilia* (DQ113454; MG905309), which showed resistance to β-lactam antibiotics and organic solvents and were isolated from nematode tissue and water.

On the phylogenetic tree (Fig. 4), sequences of actinomycete strains No. 54, 77, 96, 120, 159, 245, 257 cluster with bacteria isolated from soil ecosystems *Rhodococcus erythropolis (*CP050124), *Arthrobacter sp.* (DQ157998), *Brevibacterium aureum* (KY910099), from the rhizosphere of plants *Herbiconiux ginseng* (NR_043879), *Arthrobacter methylotrophus* (NR_025083), as well as from a medieval wall painting of *Georgenia muralis* (NR_112820). The nucleotide sequences of *Arthrobacter methylotrophus* and *Georgiania muralis* strains showed the least similarity (98.5-98.9%) to their closest relatives from the International Data Bank, which indicates possible new species of bacteria and requires further research.

The structure of nucleotide sequences of 16S rRNA gene fragments was also studied in pure cultures of bacteria isolated from Altai rocks. Of the 21 strains studied, 5 were represented by bacteria of the genus *Bacillus*. Sequences No. 46, 60, 62 Alt 2.3, 64 Alt 2.4, 91 Alt 2.5 belong to the same morphological group, and, as shown on the phylogenetic tree (Fig. 4), they form three separate subgroups: one clustered with subgroup *B. subtilis* (strains 60, 62 Alt 2.3, 64 Alt 2.4), the second - with the *B. nakamurai* subgroup (strain 46 Alt 2.3), the third - with the *B. tequilensis* subgroup (strain 91 Alt 2.5). On the phylogenetic tree, the strain sequences form a

cluster with sequences from fresh river water *B. subtilis* (MH010185), cow rumen *B. subtilis* (MK367788), Dioscorea bulbosa plant *B. nakamurai* (MH057385) and Chinese soil *B. tequilensis* (MK615608) (Fig. 5). The nucleotide sequences of strains 45 Alt 2.2, 62 Alt 1.2, 50 Alt 2.5, 68 Alt 2.4, 111 Alt 1.1 cluster with representatives of the phylum *Actinobacteria*, the family *Streptomycetaceae, Nocardiaceae, Micrococcales.* The sequences of strains 45 Alt 2.2, 62 Alt 1.2 had 98.87-100% similarity to homologues of the bacteria *Streptomyces pratensis* (MH473002), *S. sclerogranulatus* (MK367601), isolated from the soils of Algeria, Korea and the rice rhizosphere, India. The sequences of strains 50 Alt 2.5, 68 Alt 2.4 had high similarity (99.79-100%) with *Rhodococcus opacus* (CP051855) and *R. jostii* (KF976882) isolated from water and sediments in caves in Turkey. Sequences of representatives of different *Rhodococcus* species form a common cluster on the phylogenetic tree, and therefore it was not possible to determine the exact species affiliation of the strains (Fig. 5). The resulting sequence of strain 111 Alt 1.2 was most similar (100%) to an uncultured bacterial clone (JQ684260) isolated from permafrost, Kunlun Mountain Pass (China), and was also 99.8% similar to *Pseudarthrobacter sulfonivorans* (CPO13747), isolated from soils in China.

3.4. Physiological and biochemical properties of isolated cultures of heterotrophic microorganisms

3.4.1. Morpho-physiological properties

As a result of research, dominant heterotrophic microorganisms were isolated from rock samples taken in the Primorsky Krai and the Altai Republic and a collection consisting of 101 strains was formed. Among the isolated cultures, pigmented forms of colonies of yellow-orange, red-pink and greenish shades were often distinguished; sometimes colonies were found that synthesized black and purple pigments. The study of the morphology, motility and type of cell wall of the isolated cultures showed that among the heterotrophic microflora isolated from rocks, non-pigmented, mobile (35-70%) rods (67-90%) dominated (Fig. 6). A study of the production of redox enzymes by microbial strains isolated from rocks showed that catalase-

positive (86-91%) and oxidase-negative (68-76%) bacteria predominated among the isolated isolates, 21-37% of strains possessed both redox enzymes, another 9-12% of isolates did not exhibit any of the activities. For further study of physiological and biochemical characteristics, 34 dominant strains were selected (20 crops - Primorsky Krai, 14-Altai), characterized by stable growth and normal biomass.

3.4.2. Study of exogenous hydrolytic activity of isolated cultures of heterotrophic microorganisms

As a result of the studies, it was shown that pure cultures of 34 strains isolated from rocks of Primorsky Krai (20 cultures) and Altai (14 cultures) had multiple enzymatic activities. 75% of the strains isolated from the rocks of Primorye had proteolytic activity, 60-65% had amylolytic and pectinolytic activity, 40% had lipolytic and cellulase activity, and only 5% had lecithinase activity. 45% of cultures had urease activity. Altai isolates exhibited lecithinase (71%), proteolytic, amylase (57%), cellulase (43%) and pectinase (14%) hydrolytic activity. 43% of strains showed urease activity. 43% of strains showed urease activity. Strains of the genus Bacillus (No. 31, 62 Alt 2.3) were distinguished by multiple enzymatic activities, which formed the largest clearing zones and were capable of hydrolyzing 5 out of 7 substrates. Yeast strains (No. 37, No. 83.1) (*Saitozyma podzolica, Rhodotorula mucilaginosa*), as well as bacteria of the genus *Pseudomonas* (No. 5), *Rhodococcus* (No. 54,50 Alt 2.3, 68 Alt 2.4), *Delftia* (No. 48) showed the lowest enzymatic activity .

3.4.3. Antibiotic activity of isolated microorganisms to test cultures

To study the antibiotic activity of microorganisms in relation to test strains of some opportunistic bacteria (*Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis*), yeast (*Saccharomyces cerevisiae*) and phytopathogenic fungi (*Phytophthora infestans, Fusarium oxysporum*), 16 strains of heterotrophic bacteria isolated from rocks of the Primorsky Krai and 22 bacterial cultures isolated from soils of the Altai Republic. The research results showed that all studied strains of microorganisms exhibited a wide range of antagonistic activity

against opportunistic bacteria and fungi (Table 4). The greatest inhibitory effect on the growth of test cultures was observed for isolates from Altai. The strains were characterized by a fairly high level of bactericidal activity, especially against the bacteria *Staphylococcus aureus* and *Micrococcus luteus* (59% of cultures inhibited growth). 32% of bacterial cultures were able to inhibit the growth of *Enterococcus faecalis.* The greatest suppressive effect on S. aureus bacteria was exerted by strains of the genus *Staphylococcus* (31 A-1), *Bacillus* (60 Alt 2.3, 64 Alt 2.4, 91 Alt 2.5), *Pseudarthrobacter* (111 Alt 1.2) and *Sphingopyxis* (70 Alt 2.3), while the width of the growth inhibition zone was 8-17 mm (Table 2). The growth of *M. luteus* was inhibited to the greatest extent by strains of the genus *Bacillus* (46, 60 Alt 2.3), *Brevibacillus* (73 Alt 2.4), *Chryseobacterium* (14 Alt 2.1), and the diameter of the growth inhibition zone ranged from 10 to 16 mm. The most intense effects on *E. faecalis* were demonstrated by the strains *B. tequilensis* (91 Alt 2.5) and *B. subtilis* (60 Alt 2.3), as evidenced by the maximum diameter of the inhibition zone halos (6 mm). 27-45% of crops showed fungicidal activity against phytopathogenic fungi and yeasts. 45% of isolates from Altai were able to inhibit the growth of *F. oxysporum* and *S. cerevisiae*. Actinomycetes of the genus *Streptomyces* (45 Alt 2.2) and *Rhodococcus* (50 Alt 2.3) had the greatest antagonistic activity towards the fungi *F. oxysporum*, with the fungal growth inhibition zone being 16 and 10 mm, respectively. He width of the growth inhibition zone of the yeast *S. cerevisiae* was 1-9 mm, with bacteria of the genus *Pseudarthrobacter* (111 Alt 1.2) and *Bacillus* (60 Alt 2.3) having the greatest suppressive effect (Table 4). The development of *P. infestans* was inhibited by 27% of the studied strains, while the width of the zone of growth inhibition was 1-7 mm. Bacteria of the genus *Sphingopyxis* (70 Alt 2.4) and *Streptomyces* (62 Alt 1.2) showed the greatest antibiotic effect on *P. infestans.*

Strains isolated from rocks of Primorsky Krai were distinguished by lower antibiotic activity against test microorganisms (Table 4). The growth of opportunistic bacteria was suppressed by 6-31% of cultures. Strain No. 31 (*Bacillus amylolyticus*), active against *M. luteus* (15 mm) and *S. aureus* (7 mm), proved to be especially effective. 13-56% of isolates were able

to inhibit the growth of phytopathogenic fungi and yeasts. The largest number of strains exhibited antagonistic activity against *F. oxysporum* (Table 4). Bacteria of the genus *Georgenia* (No. 159, 13 mm) and *Paenibacillus* (No. 42, 11 mm) were especially active in inhibiting the growth of *F. oxysporum*. Strains No. 83 (*Paenibacillus chibensis*), 31 (*Bacillus amyloliquefaciens*), No. 15 (*Stenotrophomonas rhizophila*), 210 (*Stenotrophomonas maltophilia*) had antibiotic activity against the yeast *S. cerevisiae*. Strain No. 31 (*Bacillus amyloliquefaciens*) suppressed the growth of the yeast *S. cerevisiae* to the greatest extent, with a growth inhibition zone of 10 mm. Species of the genus *Bacillus* had multiple antibiotic activities: *B. amyloliquefaciens* (No. 31), *B. tequilensis* (91 Alt 2.5), *B. subtilis* (60 Alt 2.3, 64 Alt 2.4), while they showed both bactericidal and fungicidal activity against all test cultures (Table 2). Strain No. 31 (*B. amyloliquefaciens*) did not exhibit antagonistic activity only to *E. faecalis*. Strains No. 83.1 (*Rhodotorula mucilaginosa*), 88 (*Micrococcus yunnanensis*), 49 Alt 2.3 (B. subtilis), 18 Alt 2.1 (*Acinetobacter calcoaceticus*), 68 Alt 2.4 (*Rhodococcus jostii*) did not exhibit antibiotic activity against test cultures.

3.4.4. Studying the ability of isolated crops to utilize some carbohydrates and alcohols

The research results showed that the majority of strains isolated from rocks of Primorsky Krai and Altai were capable of oxidizing and fermenting a wide range of carbohydrates and alcohols (Table 5). Microorganisms isolated from the soils of Primorye were distinguished by a greater ability to ferment carbohydrates (5-50%), while cultures from Altai rocks could oxidize them more (14-79%). The largest number of cultures utilized glucose (79-80%), maltose (50-57%), and sucrose (71%) (Altai). Glucose was oxidized by 30-79% of the studied microorganisms, 50% (Primorye) were only capable of fermenting it (Table 5). Strains No. 77 *Arthrobacter oryzae*, 24 *Chryseobacterium scophthalmum*, 31 *Bacillus amyloliquefaciens*, 88 *Micrococcus yunnanensis* (Primorye) and No. 17 Alt 2.1 *Chryseobacterium indoltheticum*, 50 Alt 2.3 *Rhodococcus opacus*, 68 Alt 2.4 *Rhodococcus jostii* (Altai) did not have the ability to use glucose. The strains of the genus *Paenibacillus* (No. 44, 35 Alt 1.1) had the greatest

saccharolytic activity; they could both oxidize and ferment all types of carbohydrates and alcohols used in the study. Cultures No. 211 (*Staphylococcus arlettae*) No. 73 Alt 2.4 (*Brevibacillus agri*) and No. 277, 62, 46 Alt 2.3, which belonged to the genus *Bacillus*, also showed a high ability to utilize carbohydrates. Yeast (No. 37; 83.1) showed activity towards glucose and sucrose, strain 37 (*Saitozyma podzolica*) was also able to oxidize maltose, and strain 83.1 (*Rhodotorula mucilaginosa*) - fructose. The least studied strains were able to oxidize and ferment lactose (14-20%), mannitol (5-21%) and xylose (5-43%).

3.4.5. The ability of isolated microorganisms to grow at different temperatures, pH, and NaCl concentrations.

The study was carried out on pure cultures of 34 strains isolated from rocks of Primorsky Krai (20 cultures) and Altai (14 cultures). The results of the study showed that the tested crops grew over a wide range of temperatures, pH and NaCl concentrations (Table 6). The largest number of microorganisms, 85-100%, showed growth at 25-35^0 C; at this temperature, all cultures had the most active growth, which characterizes them as mesophiles. 14-70% of the studied strains were capable of growth at 5^0 C, while strains isolated from Altai rocks were more sensitive to low temperatures. Strains from Altai (62 Alt 1.2 *Streptomyces,* 18 Alt 2.1 *Acinetobacter)* showed the ability to grow at a temperature of 5^0 C. Some microorganisms isolated from rocks of Primorsky Krai (yeast No. 37 *Saitozyma*, No. 24 *Chryseobacterium,* No. 189 *Pseudomonas*) were characterized by a narrow growth zone (5-25^0 C) and stopped development at 35^{0}C. The maximum temperature value for 35-50% of isolates was 42^0 C; at higher temperatures they stopped growing. The strains No. 31, 210 (*B. amyloliquefaciens* and *S. maltophilia*) (Primorye), 62 Alt 2.3 (*B. subtilis*), 35 Alt 1.1 (*P. terrae*) (Altai) were the most tolerant to high temperatures; they showed growth at 50^0 C (Table 6). The growth of all cultures was completely absent at a temperature of 60^0 C.

The studied strains were highly resistant to different acidity levels. The overwhelming majority of strains (95%) were able to grow at pH 5.0-12.0. At the same time, many of them

retained growth activity on media with all acidity values. In strains No. 20 *Acidovorax*, 85 Alt 2.5 *Proteus*, 18 Alt 2.1 *Acinetobacter*, the narrowest optimum zone was observed (pH 6.0-7.0).

In relation to NaCl concentration, microorganisms showed a wide growth range (1-20% NaCl). The largest number (80-100%) of strains showed growth at a NaCl concentration in the medium of 1-6%. The most sensitive were the strains isolated from the rocks of Primorsky Krai. Thus, cultures No. 24 and 20 of *Chryseobacterium* and *Acidovorax* lost their ability to grow even at a NaCl concentration of 3%. 45-86% of microorganisms grew at a concentration of 8% NaCl, 7-20% of strains developed in the presence of 15% NaCl in the medium, and 5-7% of isolates grew at 18% NaCl. At a concentration of 20% NaCl, 5% of the cultures isolated from the rocks of Primorye developed. The greatest ability to grow at high concentrations of NaCl (10-15%) was characterized by strains of the genus *Bacillus* (No. 49 Alt 2.3, 62 Alt 2.3, 31), *Brevibacillus* (73 Alt 2.4), *Brevibacterium* (No. 245) and yeast of the genus *Rhodotorula* (No. 83.1) (Table 6). Cultures of the genus *Rhodococcus* (50 Alt 2.3) and *Staphylococcus* (No. 211) were distinguished by particularly high osmotolerance; they stopped growing only at a NaCl concentration of 20-22%.

4. Discussion

In terms of mineral composition, the rocks are typical clays with an admixture of quartz and feldspar. Among the main components, silicon, aluminum, iron and potassium predominate (Table 2). The rocks of Altai are distinguished by the predominance of quartz, feldspars and clay minerals in the form of hydromicas and chlorites. They also have the highest content of iron, magnesium, calcium, sodium and manganese. Zeolites were found only in the rocks of Primorsky Krai.

Rocks can be a source of beneficial microorganisms for animals. To test this hypothesis, studies were conducted on the number of physiological groups and the taxonomic composition of microorganisms, exogenous hydrolytic, antibiotic, saccharolytic activities and the ability of cultures to grow at different temperatures, pH, and NaCl concentrations.

The composition of the dominant physiological groups of microorganisms in the rocks of Altai and Primorsky Krai differed significantly (Table 3). In the soils of Primorsky Krai, heterotrophic bacteria (saprophytic, amylolytic, saccharolytic, nitrifying, iron-oxidizing, silicate) prevailed in terms of the number of physiological groups, which indicates the presence of organic matter in the rocks. The presence of these bacteria in the rocks indicates the ongoing processes of decomposition of nitrogen-containing organic substances, hydrolysis of polysaccharides and fermentation of sugars, oxidation of iron and destruction of silicate minerals. Whereas in the rocks of Altai, autotrophic nitrifying, thione, cellulose-decomposing, sulfate-reducing and manganese-oxidizing bacteria dominated. This indicates the processes of oxidation of nitrogen, sulfur, manganese compounds, and decomposition of cellulose with the participation of bacteria taking place in the rocks. The presence of sulfate-reducing bacteria in a fairly high number (up to 8.3×10^5 cells/g) indicates the ongoing processes of sulfate reduction to sulfides. The difference in the composition of physiological groups of bacteria in rocks from different regions is probably due to the peculiarities of the chemical composition of soils and tree roots that permeate the rocks. Only in the rocks of Primorye (except M-14) were yeasts found in high quantities (up to 10^9 CFU/g). This coincided with the presence of smectite in all rock samples (Table 1). The number of yeasts in soils rarely exceeds 10^3 CFU/g (Botha, 2022). The main factor determining the development of yeast in the soil is the concentration of readily available organic matter. It is also known that iron ions, which are part of the rocks, can stimulate the growth of *Rhodotorula mucilaginosa* yeast (Cudodski, Pietryczuk, 2019). Probably, smectite in combination with plant residues could somehow stimulate the development of yeast in the soils. The absence of yeast in the Altai rocks is probably due to the different composition of the rocks, which does not allow these microorganisms to develop.

As a result of the studies, pure cultures of dominant microorganisms were isolated and identified. In general, the rocks were characterized by a low diversity of heterotrophic bacteria, which is associated with a low concentration of organic matter. The main part of the cultivated

heterotrophic microorganisms in all samples was represented by two dominant phyla Proteobacteria (14.3-57.4%) and Firmicutes (7.1-57.1%), which is consistent with the literature data (Lopez-Fernandez et al., 2015). Bacteria of the genus *Pseudomonas, Bacillus, Paenibacillus, Stenotrophomonas* prevailed in the rocks of Primorsky Krai (Fig. 3). Numerous literature data show that bacteria of the genera *Bacillus, Pseudomonas, Stenotrophomonas, Micrococcus, Arthrobacter, Acinetobacter* are often found in soils and various clay rocks, which is generally consistent with our data (Lopez-Fernandez et al., 2014, Okereafor et al., 2018, Mitzscherling et al., 2023).

It is known that bacteria of the genus *Pseudomonas* are typical representatives of the plant rhizosphere, have high chitinolytic and nitrogenase activity and are capable of exhibiting antagonistic activity against a wide range of fungi. Therefore, it is not surprising that these bacteria were found in rocks that are penetrated by the roots of spruce and Siberian larch. The largest numbers of pseudomonads were noted in rock samples No. 47, M3-1, M181 (Fig. 3). The isolated bacteria of the genus *Bacillus*, according to the literature, have a wide range of enzymes that break down various organic compounds, and are also capable of producing antibiotics and antifungal substances and degrading a number of toxins (Danilova, Sharipova, 2020; Alqahtani et al., 2023).The largest numbers of bacteria of the genus *Bacillus* were observed in samples No. M14, M4-1, B-47. Bacteria of the genus *Paenibacillus* are widely distributed in various environments, especially in soil, where they play a role in biological nitrogen fixation, phosphate solubilization, production of the phytohormone indole-3-acetic acid, and release of siderophores. Various species of these bacteria also produce glucans, chitinases, cellulases, and proteases involved in the degradation of eukaryotic cell walls. Paenibacillus is also capable of producing antimicrobial agents such as bacteriocins and antimicrobial peptides that can inhibit phytopathogenic microorganisms (Li et al., 2023). They are recommended as a feed additive for farm animals.

Bacteria of the genus *Stenotrophomonas* are often found in association with plants and are isolated from soils, rhizosphere, or internal plant tissues. They are capable of metabolizing a wide range of organic compounds, including phenolic compounds, polycyclic hydrocarbons, benzene and promote plant growth through nitrogen fixation and elemental sulfur oxidation. They are resistant to various heavy metals. *S. maltophilia* strains have extremely high hydrolytic potential; they produce a variety of proteases, chitinases, DNases, lipases, and antifungal compounds. They are able to absorb iron because they produce siderophores (Pan et al., 2022). Despite its wide biotechnological applications, *S. maltophilia* is an opportunistic, multidrug-resistant opportunistic bacteria. *S. maltophilia* is capable of causing a variety of infections, including bacteremia, endocarditis, pneumonia, meningitis, eye infections, urinary tract infections, enteritis, and skin/soft tissue infections (Kalidasan et al., 2018).

The soils of Altai were characterized by the dominance of bacteria of the genus *Bacillus, Chryseobacterium* and actinomycetes of the genus *Streptomyces*. It is known that bacteria of the genus *Chryseobacterium* are chemoorganotrophic, have the pigment flexirubin, which colors the colonies orange. These bacteria have the ability to dissolve organophosphorus compounds and obtain iron from the environment using siderophores and the production of indoleacetic acid. Moreover, they have antibiotic resistance genes (Zhang et al., 2023), which probably allows them to survive together with actinomycetes. They exist in fresh water, soils, and were also isolated from deep-sea sediments of the Tibetan Plateau (Xu et al., 2015). Actinomycetes are widespread in soils under unfavorable conditions (dry, salty, alkaline, with high and low temperatures). The largest number of actinomycete taxa was noted in the Altai Territory, characterized by the lowest water content in the samples (0.17-1.04%) (Table 3). Perhaps, this factor could contribute to the dominance of actinomycetes in rocks. Metabolic versatility allows them to survive in a wide range of soil environments. Thus, actinomycetes of the genus *Streptomyces* were found in the dominant in granite rocks of Egypt and stone ruins of Tunisia (Abdulla, 2009, Saadouli et al., 2022). The same authors showed the ability of

Streptomyces to actively extract trace elements from minerals. It is known that representatives of the families *Streptomycetaceae, Nocardiaceae, Micrococcales* are able to synthesize many hydrolytic enzymes (especially cellulase, chitinase, amylase), are producers of fatty acids, amino acids, antitumor substances. These bacteria are the most important producers of antibiotics and a number of anticancer, antihelminthic, antifungal and immunosuppressant substances (Farda et al., 2022). Thus, eating rocks with actively developing actinomycetes by animals can not only saturate them with essential microelements, but also be a way to prevent and treat gastrointestinal disorders. Animals can detect the presence of actinomycetes in rocks by the smell of geosmin, a volatile organic substance of terpene nature. For animals, especially in arid biocenoses, the smell of geosmin can serve as an indicator of the presence of moisture (Podchasova et al., 2020).

Only in the rocks of Primorsky Krai the presence of yeast species *Rhodotorula mucilaginosa* (7.2-16.7%) and *Saitozyma podzolica* (0-10%) (only M3-1) was established. It is known that yeasts are typical inhabitants of the rhizosphere and are widespread in soils (Bota, 2011). *Rhodotorula mucilaginosa* yeast is widely found in the environment and was also isolated from clay deposits in Spain and cosmetic clays in Africa (Mpuchane et al., 2010, Lopez-Fernandez et al., 2014, Jach et al., 2022). *Saitozyma podzolica* yeast was the dominant species in Brazilian soils and positively correlated with the presence of aluminum in them (Moreira, Vale, 2018). *Saitozyma podzolica* were first described in soils with the presence of organic matter, iron and aluminum oxides (Fell, Statzell-Tallman, 1998). Thus, the presence of yeasts of the genus *Rhodotorula* and *Saitozyma* in the rocks of Primorsky Krai may be associated with the presence of organic matter, iron and aluminum.

Yeasts of the genus *Rhodotorula* and *Saitozyma* are capable of synthesizing many useful nutrients, such as proteins, folic acid, carotenoids, lipids containing polyunsaturated fatty acids (oleic, linoleic, palmitic, stearic, etc.), which make up to 70% of the dry weight of these cells (Gorte, Kugel, 2020; Hu et al., 2022). Yeast protein is digested in the body of animals by 95%

and thus can enrich the body with essential amino acids, vitamins, and microelements (Patterson et al., 2023). The presence of beneficial microorganisms in soils can probably instinctively attract animals to consume rocks due to the formation of volatile metabolites with a specific odor. When entering the animal's body, the identified microorganisms are able to participate in the processing of plant food, decomposition of organic matter, destruction of silicates, enrich the animal's body with protein, valuable vitamins and amino acids. In addition, it is known that yeasts of the genus *Rhodotorula* and other microorganisms are able to dissolve phosphate minerals and weathered granites (Xiao et al., 2012, Voutsinos et al., 2024), which can affect the extraction of many trace elements, including REE in rocks. It is known that an important factor in maintaining animal health is the balance of trace elements. Currently, animals are experiencing an acute shortage of available trace elements. Trace elements play an important role in biological systems that are involved in gene regulation, nucleic acid metabolism, anti-inflammatory and antioxidant functions (Sun et al., 2022). Inorganic forms of trace elements are toxic and poorly absorbed by humans and animals. REE are an important group of trace elements, as they play a key role in the functional and structural molecules in biological systems. In China, REE have been used as feed additives in livestock farming for more than 40 years. It has been shown that REE has a significant effect on improving the health and productivity of animals. Moreover, the introduction of REE had anti-inflammatory, antioxidant, and immunostimulating effects on pigs, cattle, sheep, and chickens (Abdelnour et al., 2019). Thus, microorganisms as intermediaries can participate in the transformation of inorganic forms of trace elements (including REE) from rocks into biologically active organic forms, making them available for absorption by animals. Therefore, the consumption of rocks by animals can significantly improve digestion, stimulate the immune system of animals, and protect them from various bacterial and fungal diseases.

Studies of extracellular hydrolytic and saccharolytic activity of microbial cultures showed that the strains had multiple enzymatic activities and were able to utilize a wide range of

carbohydrates and alcohols. It should be noted that all strains showed enzymatic activity, but none of them had the ability to produce enzymes of the entire studied spectrum. It was found that microorganisms isolated from the rocks of Primorsky Krai actively produced proteases, amylases, pectinases and, to a lesser extent, lecithinases. Altai isolates were characterized by the highest production of lecithinases, proteinases and amylases and the lowest production of lipases. It is known that lecithinase is a pathogenic enzyme and is characteristic of opportunistic bacteria (Lysak et al., 2015). Since the highest number of opportunistic bacteria (*Pseudomonas, Bacillus, Acinetobacter, Stenotrophomonas, Staphylococcus, Alcaligenes*) is found in the rocks of Altai, this can explain the higher lecithinase activity of the strains. The difference in the production of exogenous enzymes by microorganisms is probably due to differences in the chemical composition of rocks and the individual characteristics of bacterial strains. There are also studies demonstrating that calcium ions can affect the virulence of soil bacteria, which can increase their enzymatic activity (Keskina, Kahramanb, 2022). The calcium content in rocks is 0.09-7.47%, and can affect the production of enzymes by strains. The highest production of amylases and proteases is characteristic of cultures of the genus Bacillus and Pseudomonas, which is confirmed by literary data (Afrin et al., 2024). The highest percentage of protease and amylase activity of strains from Primorsky Krai can be explained by the dominance of bacteria of the genus Bacillus and Pseudomonas in the rocks of Primorye.

A fairly high percentage of cultures isolated from rocks were able to exhibit cellulase enzymatic activity (40-43%). The highest cellulase activity was shown by strains of the genus *Bacillus* and *Paenibacillus*. According to literature data, representatives of the genus *Bacillus* and *Paenibacillus* are characterized by high cellulolytic ability, which is associated with the presence of a wide range of glycoside hydrolase enzymes in their genomes (Li et al., 2023). Cellulose is the main resource in feed for ruminants. Some animals have a limited ability to decompose cellulose. Bacteria of the genus *Bacillus* and *Paenibacillus* have the necessary enzymes to destroy plant cell walls, thereby releasing nutrients and improving the rate of

absorption of feed nutrients. Therefore, consumption of rocks with microorganisms, especially *Bacillus* and *Paenibacillus*, can have a positive effect on the health of animals. Tree roots and plant remains may contain pectins. Pectins are polysaccharides formed by residues mainly of galacuronic acid. These substances are present in all higher plants (Netrusov et al., 2005). The pectolytic activity of strains isolated from different areas of rocks differed. Strains isolated from rocks of Primorye showed higher pectinase activity (65%), while cultures from Altai were characterized by lower activity (14%). This may be due to the high content of pectin in plant residues of rocks of Primorsky Krai and the greater adaptability of bacteria to metabolize pectins.

The isolated cultures were also characterized by high saccharolytic activity (Table 5). The greatest number of strains isolated from rocks utilized glucose, maltose, and sucrose to the greatest extent, which is typical for many bacteria. Glucose, maltose, and sucrose are the most common sugars in the environment, contained in the tissues of higher plants, in grain crops, and are also part of the pollen and nectar of a number of plants (Lysak et al., 2015). A limited number of cultures were able to utilize mannitol, lactose, and xylose (Table 5). Xylose is the main component of lignocellulose and the second most common sugar in nature. It is known that only a limited number of bacterial cultures are capable of processing xylose (Li et al., 2023). Only 10% of cultures from the rocks of Primorsky Krai were able to ferment xylose. The strains isolated from the Altai rocks were characterized by the highest ability to oxidize xylose (43%). According to the literature, it is known that the wood and roots of coniferous trees, especially larch, contain lignocellulose, and xylose is part of it (Fuchtner et al., 2020). Siberian larch is widespread in the Altai region (Klyuchnikov et al., 2008), while it is much less common in Primorsky Krai. Therefore, the higher ability to oxidize xylose by the Altai strains is probably due to their high adaptability to this substrate. Bacteria of the genus *Bacillus, Paenibacillus, Arthrobacter, Pseudomonas* were characterized by the greatest ability to utilize xylose. Strains capable of effectively utilizing xylose are of industrial interest and require

further research. According to the literature, many yeasts are capable of using xylose to produce ethanol (Zha et al., 2021). However, the yeast isolated from rocks did not have this ability. This difference is due to the individual characteristics of the microorganism strains. Therefore, rocks contain a complex of microorganisms with different hydrolytic enzymes and the ability to utilize various sugars. These properties of microorganisms can contribute to better digestion of plant feed eaten by animals.

The isolated microorganisms were capable of exhibiting antibiotic activity against bacteria and fungi. The strains isolated from the Altai rocks were characterized by the highest antibiotic activity, especially against *S. aureus, M. luteus* cultures. The widest clearing zones were formed by strains of the genus Staphylococcus (31 Alt 1.1), Bacillus (64 Alt 2.4, 91 Alt 2.5) (Table 3). Literature data show that *Staphylococcus epidermidis, Bacillus* strains are capable of suppressing the growth of S. aureus due to the production of antimicrobial peptides, polysaccharides specific to these bacteria, as well as due to the rapid and efficient absorption of nutrients (Hardy et al., 2020, Sabino et al., 2023). The highest antagonistic activity against the test culture of *Micrococcus luteus* was demonstrated by the strains of *Chryseobacterium* (14 Alt 2.1), *Bacillus* (60 Alt 2.3), *Brevibacillus* (73 Alt 2.4). It is known from the literature that cultures of the genus *Bacillus* and *Brevibacillus* are rich sources of antimicrobial compounds and are able to suppress the growth of opportunistic bacteria (Jahne et al., 2023). Actinomycetes isolated from rocks of the Primorsky and Altai Territories were characterized by the highest activity against the fungi *F. oxysporum* and *Ph. infestans*. It is known that *F. oxysporum* and *Ph. infestans* are phytopathogenic fungi that produce many toxins and are the causative agents of fusarium and late blight, especially in potatoes and tomatoes (Lastochkina et al., 2020). The highest antagonistic activity against *F. oxysporum* was demonstrated by actinomycetes of the genus *Streptomyces* (45 Alt 2.2), *Georgenia* (159), *Rhodococcus* (50 Alt 2.3). It is known from the literature (Polyak, Sukharevich, 2021) that actinomycetes are producers of a wide range of antibiotics and other biologically active substances and are capable of exhibiting biocidal

activity against fungi, which is also confirmed by our data. Actinomycetes producing many different antimicrobial compounds were isolated from mangrove sediments in India (Naligama et al., 2022). In addition to antimicrobial compounds, actinomycetes are capable of producing various bioactive compounds, such as antitumor and antiviral agents, antioxidants, enzymes and antifibrotic agents. This in turn can have a beneficial effect on the animal body.

It is known that *Ph. infestans* are resistant to the effects of bacteria. Our data show that the greatest inhibitory effect on *Ph. infestans* is exerted by strains of the genus *Sphingopyxis* (70 Alt 2.4), *Bacillus amyloliquefaciens* (31), *Bacillus subtilis* (60 Alt 2.3), which form clearing zones with a diameter of 3-4 mm. Literature data show that *Bacillus amyloliquefaciens, Bacillus subtilis* cultures are able to suppress mycelial growth, cyst germination and zoospore motility in *Ph. infestans* (Lin et al., 2023), which is confirmed by our results. It is also reported that bacteria of the genus *Pseudomonas* are able to suppress the growth of *Ph. Infestans* due to the production of antimicrobial phenazine compounds and volatile organic compounds (Lin et al., 2023). However, in our case, the strains of the genus *Pseudomonas* isolated from rocks of the Primorsky and Altai regions did not have an inhibitory effect on the *Ph. infestans* fungi. This may be due to the characteristics of the strains.

From the literature it is known that spore-forming bacteria of the genus *Bacillus* are producers of a wide range of biologically active substances, including lipopeptide and other antibiotics, which determines their high bactericidal and fungicidal activity (Lelyak, 2014). A number of authors have shown a high antagonistic effect of microorganisms of the genus *Bacillus* on a number of bacteria (*S. aureus, B. licheniformis, P. aeruginosa*, *Klebsiella, Citrobacter, Salmonella*) and phytopathogenic fungi (*Alternaria, F. oxysporum, F. solani, P. infestans*) (Wang et al., 2023, Lelyak, 2014, Khan et al., 2019). The wide spectrum of antibiotic action of bacteria of the genus *Bacillus* on test organisms suggests that the cultures form not one, but several compounds with antagonistic properties. *B. subtilis* strains (No. 62, 49, 60 Alt 2.3), isolated from Altai rocks, were characterized by different levels of antibiotic activity

against opportunistic bacteria and phytopathogenic fungi, while isolate 49 Alt 2.3 (*B. subtilis*) did not suppressed the growth of test cultures. This can be explained by the fact that, due to their genetic and metabolic diversity, different strains belonging to the same *Bacillus* species may have markedly different inhibitory efficacies against test cultures (Wang et al., 2023). Differences in antagonistic activity by strains of the same species can be explained by the variable composition of the peptidoglycan of the bacterial cell, which is a regulator of relationships in the prokaryotic-prokaryotic system, as well as a regulator of intrageneric and intergeneric antagonism in conditions of intermicrobial interactions (Lelyak, 2014).

Most of the isolated strains were able to grow at temperatures of 25-42^0 C, pH = 5-12, NaCl concentration of 1-6%. As the author notes (Zvereva, 2014), cocci have the greatest resistance to environmental factors, so this can explain the higher ability of strains of the genus *Staphylococcus, Rhodococcus* to grow at high NaCl concentrations. It is known that in the rumen of ruminants, the pH is neutral, and the temperature varies from 37-39^0 C (DelCurto-Wyffels et al., 2021). Therefore, such a wide range of culture growth makes it possible for microorganisms to survive when they enter the body of animals along with rocks.

5. Conclusions

Consequently, the rocks of Primorsky Krai and the Altai Republic contain a high number of potentially beneficial physiological groups of microorganisms for animals (cellulose-degrading, amylolytic, saccharolytic, saprophytic, yeast, silicate, etc.), as well as various bacteria (*Bacillus, Pseudomonas, Paenibacillus, Stenotrophomonas, Rhodococcus, Streptomyces*) and yeast (*Rhodotorula, Saitozyma*), which have high extracellular enzymatic activity and antagonistic activity against opportunistic bacteria and phytopathogenic fungi and yeast. These microorganisms are able to grow in a wide range of temperatures, pH, NaCl concentrations and utilize a wide range of carbohydrates and alcohols. These microorganisms are also capable of participating in the transformation of inorganic forms of microelements (including REE) from rocks into biologically active organic forms, making them available for

assimilation by animals. Thus, microorganisms of rocks and the biological active compounds they produce can be one of the factors that encourage animals to instinctively consume rocks to improve the processes of digestion of plant feed, supply the animal body with proteins, amino acids, vitamins, microelements, stimulate the immune system and protect animals from various diseases.

Conflict of interest

Authors have no conflict of interests.

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Table 1.

Characteristics of rock samples

Sample:	**Sampling area:**	**Sampling date:**	**Mineral composition:**
M181, M 4-1, M3-1	Olginsky district, Primorsky Krai	July, September 2020	Smectite-zeolite mineral mixtures
M14 (not eaten by animals), M 3-3			Smectite
B-27, B-42, B-47, B-48	Terneysky and Pozharsky districts of Primorsky Krai	September 2020	Smectite with zeolites (clinoptilolite-heulandite) with quartz and feldspar crystals
Alt 1.1-Alt 2.5	Altai Republic	April-August 2021	Finely dispersed quartz, hydromicas and chlorites

Table 2.
Composition of the main elements in the rocks of Primorsky Krai and Gorny Altai (weight %)

Samples:	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	H_2O-	LOI	Σ
M3-1	69,93	0,19	10,60	2,09	0,06	0,29	0,85	1,99	2,23	0,01	6,14	5,24	99,62
M3-3	65,96	0,26	13,39	3,79	0,04	0,63	0,80	1,87	1,65	0,02	6,75	4,56	99,72
M4-1	66,89	0,21	12,08	2,16	0,02	0,36	0,88	1,28	1,24	0,00	6,43	7,96	99,53
M14	61,89	0,33	15,41	5,08	0,04	0,60	0,92	0,69	2,93	0,02	6,66	5,03	99,61
M181	70,67	0,43	13,40	3,20	0,04	0,47	0,40	1,70	2,35	0,07	2,08	4,79	99,60
B-27	76,65	0,24	11,17	1,04	0,01	0,28	0,43	0,32	6,66	0,03	1,24	1,55	99,63
B-42	66,59	0,34	12,66	2,25	0,06	0,76	2,86	0,76	1,28	0,02	5,28	6,93	99,79
B-47	76,53	0,08	11,82	1,73	0,01	0,10	0,09	3,20	4,23	0,01	0,63	1,17	99,60
B-48	54,72	1,36	16,73	9,29	0,15	3,16	7,47	3,32	1,68	0,29	1,13	0,56	99,85
Alt 1.1	53,60	0,58	15,47	8,23	0,18	3,70	5,43	2,13	2,25	0,19	0,38	7,54	99,67
Alt 1.2	62,57	0,86	14,35	6,01	0,11	3,14	4,09	3,04	1,77	0,20	0,17	3,36	99,68
Alt 1.3	56,19	0,76	15,45	8,68	0,15	4,20	3,61	2,91	2,33	0,16	1,04	4,05	99,52
Alt 2.1	61,70	0,83	14,64	7,56	0,11	3,54	4,79	3,30	1,48	0,18	0,39	1,21	99,73
Alt 2.2	66,32	0,54	12,45	4,77	0,08	2,57	4,33	2,67	1,57	0,17	0,54	3,61	99,61
Alt 2.3	65,72	0,63	13,64	5,91	0,17	3,09	3,58	2,48	1,84	0,15	0,28	2,32	99,82
Alt 2.4	64,50	0,61	11,64	4,11	0,07	2,35	7,08	2,43	1,26	0,14	0,17	5,27	99,63
Alt 2.5	62,18	0,75	14,04	6,96	0,12	3,48	3,78	2,95	1,94	0,16	0,55	2,89	99,79

Table 3.

Range of numbers of dominant physiological groups of microorganisms in rock samples from Primorsky Krai and the Altai Republic

Functional groups of microorganisms, cells/g:	**Primorsky Krai**			**Altai**
	Olginsky area		**Terneysky, Pozharsky districts**	**Ongudaisky and Turochaksky districts**
	M14*	**M181, M4-1, M3-1, M3-3**	**B-27, B-42, B-47, B-48**	**Alt 1.1-Alt 2.5**
Saprophytes, aerobes	2.2×10^{2}	6.2×10^{6} - $4,4\times10^{8}$	5.3×10^{6} – 1.1×10^{8}	8.8×10^{4}-3.5×10^{6}
Saprophytes, anaerobes	0.7×10^{2}	4.8×10^{5} - $3,9\times10^{6}$	5.0×10^{5} – 1.0×10^{8}	3.6×10^{4}-2.0×10^{6}
Yeast	0	1.4×10^{3}- $4,5\times10^{6}$	4.0×10^{8} – 1.2×10^{9}	0
Amylolytic	0	2.5×10^{4} – 4.8×10^{6}	5.0×10^{3} – 8.8×10^{5}	0-1.9×10^{5}
Saccharolytic	0.4×10^{2}	9.5×10^{2} – 2.5×10^{6}	9.5×10^{3} – 4.5×10^{7}	0-1.6×10^{6}
Cellulose decomposing	0	0 – 0.9×10^{2}	0 – 4.5×10^{2}	0 – 2.4×10^{4}
Nitrogen-fixing	2.4×10^{3}	5.0×10^{3} – 9.2×10^{6}	1.5×10^{4} – 3.0×10^{5}	7.2×10^{3} – 1.6×10^{6}
Ammonifying	0	0 – 8.4×10^{6}	0 – 1.2×10^{6}	1.5×10^{3} – 5.3×10^{6}
Nitrifying autotrophic	0	0	0	0-7.3×10^{5}
Nitrifying heterotrophs	0	5.1×10^{6} – 2.2×10^{8}	0 – 1.4×10^{5}	5.0×10^{3} – 1.8×10^{6}
Denitrifying	2.5×10^{2}	0 – 9.5×10^{3}	0 – 2.5×10^{2}	0 – 2.5×10^{3}
Thionic	0	0	0 – 9.5×10^{2}	0 – 1.6×10^{4}
Sulfate-reducing	0	0 – 4.5×10^{3}	0 – 9.5×10^{3}	0 – 8.3×10^{5}
Iron oxidizing	0	0 – 1.7×10^{3}	0 – 1.2×10^{4}	0
Manganese oxidizing	0	0 – 3.3×10^{2}	0 – 5.2×10^{3}	0 – 6.7×10^{4}
Silicate	1.2×10^{3}	1.1×10^{4} - $6,4\times10^{6}$	8.6×10^{3} – 1.2×10^{9}	8.0×10^{3} – 6.2×10^{5}

M14* - Soils not eaten by animals

Table 4.

Antibiotic activity of some strains of heterotrophic microorganisms isolated from rocks of Primorsky Krai and Altai

Strains, no.:	*Phytophthora infestans*	*Fusarium oxysporum*	*Saccharomyces cerevisiae* Y-5	*Staphylococcus aureus*	*Micrococcus luteus* B-7845	*Enterococcus faecalis*
	Diameter of growth inhibition zone, mm					
4 (*Acinetobacter guillouiae*)	-	-	-	-	3±0.7	1±0.5
31 (*Bacillus amyloliquefaciens*)	3±0.4	10±2	4±2	7±2	15±2	-
159 (*Georgenia muralis*)	-	13±3	-	-	-	-
210 (*Stenotrophomonas maltophilia*)	-	4±1	2±1	-	-	-
5 (*Pseudomonas putida*)	-	-	-	2±0.8	-	-
42 (*Paenibacillus glucanolyticus*)	-	11±1	-	-	3±0.5	-
45 Alt 2.2 (*Streptomyces pratensis*)	-	16±4	-	9±1	3±1	-
50 Alt 2.3 (*Rhodococcus opacus*)	-	10±2	-	-	-	-
111 Alt 1.2 (*Pseudarthrobacter sulfonivorans)*	-	-	9±1	10±2	-	-
31 Alt 1.1 (*Staphylococcus epidermidis)*	-	2±0,5	8±2	15±3	4±0.8	-
73 Alt 2.4 (*Brevibacillus agri*)	-	1±0.3	-	3±0.3	10±3	2±0.3
62 Alt 2.3 (*Bacillus subtilis*)	2±1	6±1	1±0.5	-	-	-
91 Alt 2.5 (*Bacillus tequilensis)*	1±0.5	6±1	7 ±1	15±1	7±2	6±1
60 Alt 2.3 (*Bacillus subtilis)*	3±0.7	8±2	9±3	10±3	10±1	6±0.8
64 Alt 2.4 (*Bacillus subtilis)*	2±0.8	3±0.5	7±2	17±4	2±0.4	3±1
74 Alt 2.4 (*Pseudomonas* sp.)	-	-	-	1±0.6	1±0.3	3±0.8
70 Alt 2.4 (*Sphingopyxis bauzanensis)*	4±1	-	7±0.9	8±0.5	8±0.5	-
14 Alt 2.1 (*Chryseobacterium haifense*)	-	-	-	-	16±0	-

- no activity.

Table 5

The ability to utilize some carbohydrates and alcohols by strains of heterotrophic microorganisms isolated from rocks of the Primorsky Territory and the Altai Republic

Substrate:	Strains from rocks of Primorsky Krai,%			Strains from rocks of Altay, %		
	OF	F	NU	OF	F	NU
Glucose	30	50	20	79	0	21
Lactose	20	0	80	14	0	86
Sucrose	20	10	70	64	7	29
Maltose	45	5	50	50	7	43
Mannitol	10	5	85	21	7	72
Fructose	25	15	60	50	7	43
Xylose	10	5	85	43	21	36

OF - oxidize, ferment, F - only ferment, NU - do not utilize

Table 6.

Growth range of some strains of heterotrophic microorganisms isolated from rocks of Primorsky Krai and Altai

Strain:	Growth range								
	Temperature (^{0}C)			pH values			NaCl concentration,%		
	min	opt	max	min	opt	max	min	opt	max
83.1 *Rhodotorula mucilaginosa*	5	25	35	5	6-10	12	1	1-8	15
31 *Bacillus amyloliquefaciens*	25	25-35	50	5	6-10	12	1	1-6	15
210 *Stenotrophomonas maltophilia*	5	25-35	50	5	6-11	12	1	1-3	10
245 *Brevibacterium aureum*	5	25	35	5	6-11	12	1	1-8	15
211 *Staphylococcus arlettae*	10	25-35	50	5	6-11	12	1	1-6	20
62 Alt 1.2 *Streptomyces sclerogranulatus*	5	25-35	42	5	6-9	11	1	1-3	8
62 Alt 2.3 *Bacillus subtilis*	10	25-35	50	5	6-9	11	1	1-6	10
49 Alt 2.3 *Bacillus subtilis*	25	25	35	5	6-10	12	1	1-6	10
35 Alt 1.1 *Paenibacillus terrae*	10	25-35	50	5	6-10	12	1	1-3	8
50 Alt 2.3 *Rhodococcus opacus*	10	25-35	42	5	6-10	12	1	1-10	18
74 Alt 2.4 *Brevibacillus agri*	10	25-35	42	5	6-8	9	1	1-8	10

min – minimum value, opt – optimal, max – maximum value of the factor under study.

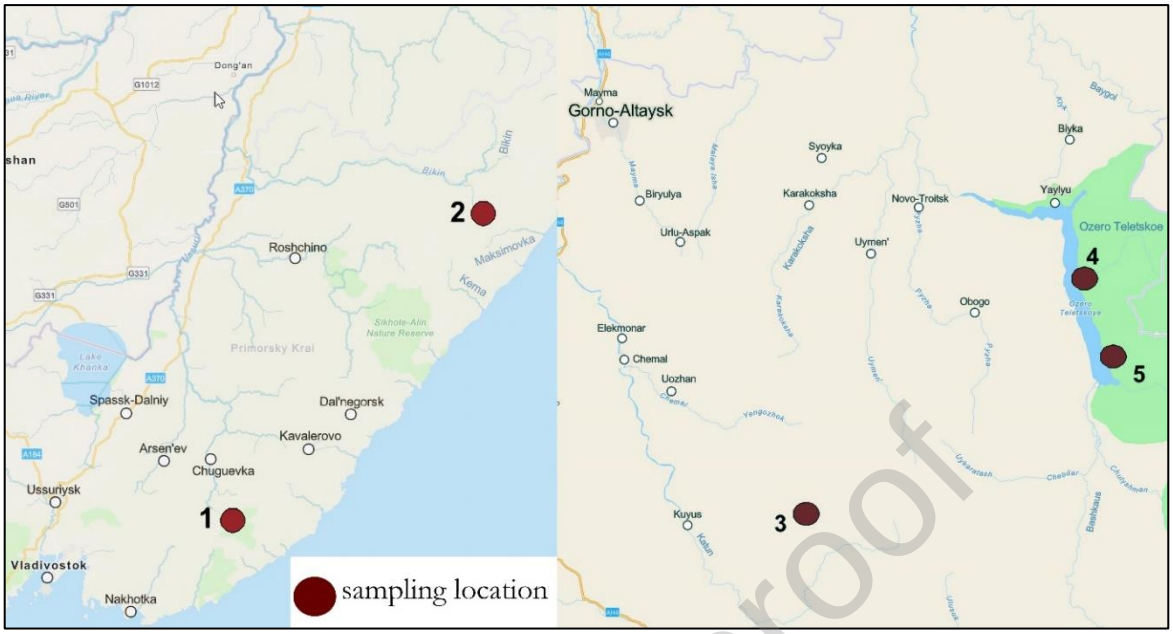

Fig. 1. Map-scheme of rock sampling sites (kudurites) in the Olginsky (1), Terneysky and Pozharsky districts (2) in the Primorsky Territory, as well as in the territory of the Altai Republic along the river Malaya Sumulta (3) in the Ongudai district, along the river Kokshi (4) in the Turochaksky district and in the area of the Bele cordon (5) in the Ulagansky district.

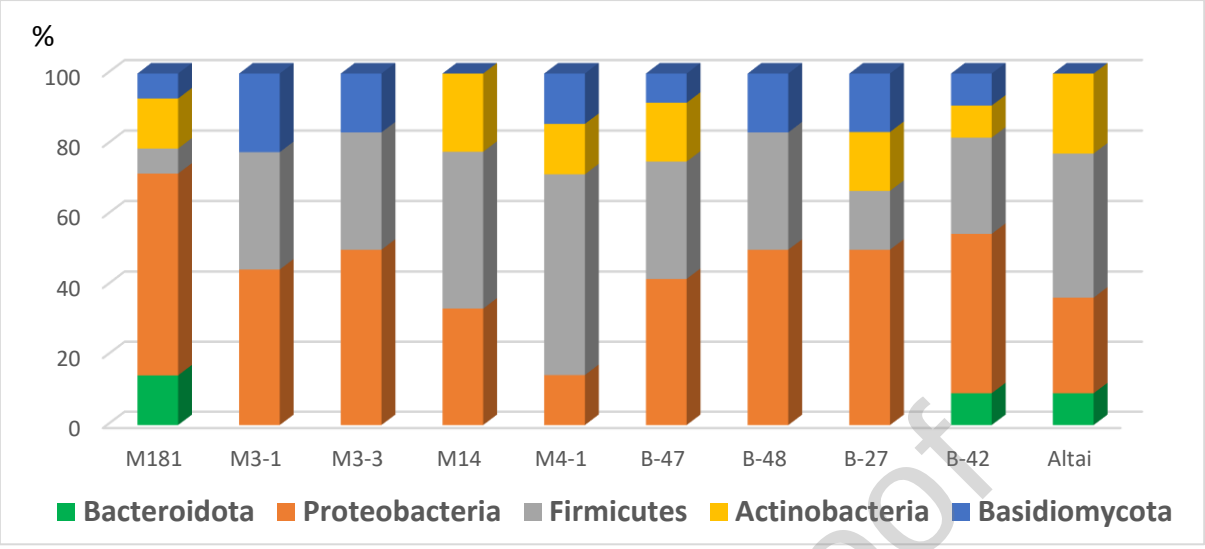

Fig. 2. Taxonomic diversity of cultivated heterotrophic microorganisms at the phylum level in rocks of the Primorsky Territory and the Altai Republic, %

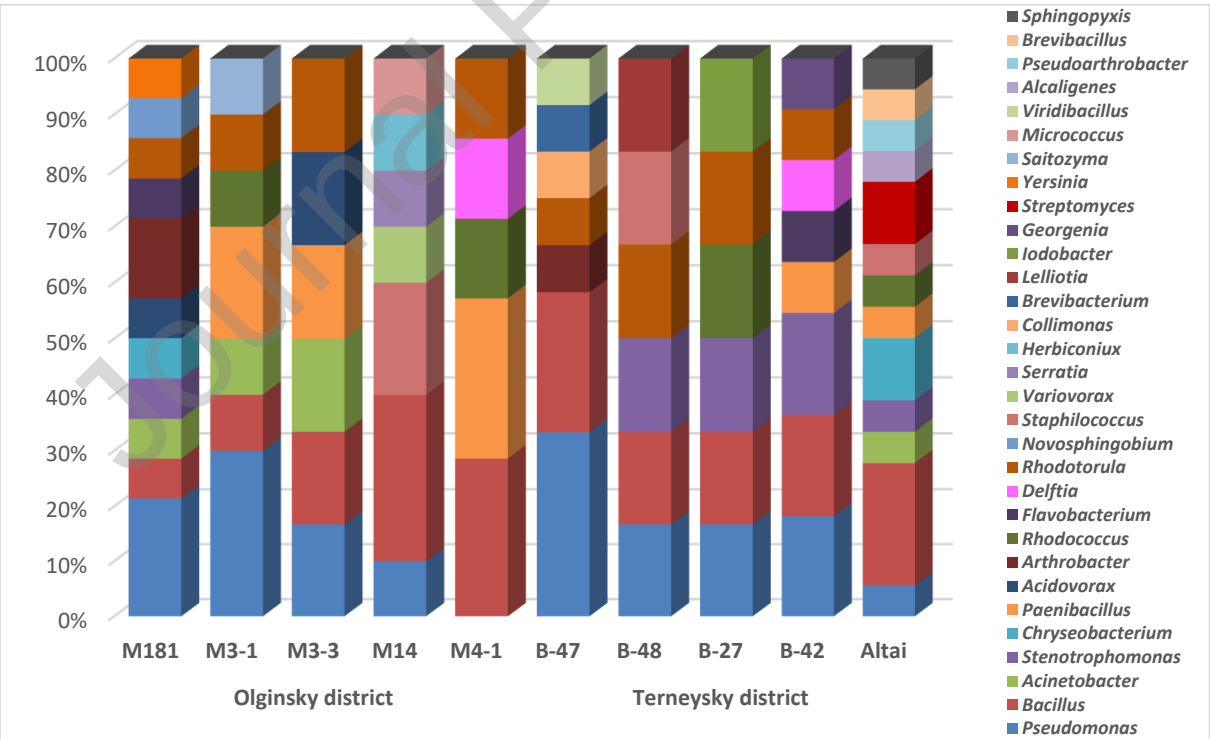

Fig. 3. Taxonomic diversity of cultivated heterotrophic microorganisms at the genus level in rocks of the Primorsky Territory and the Altai Republic

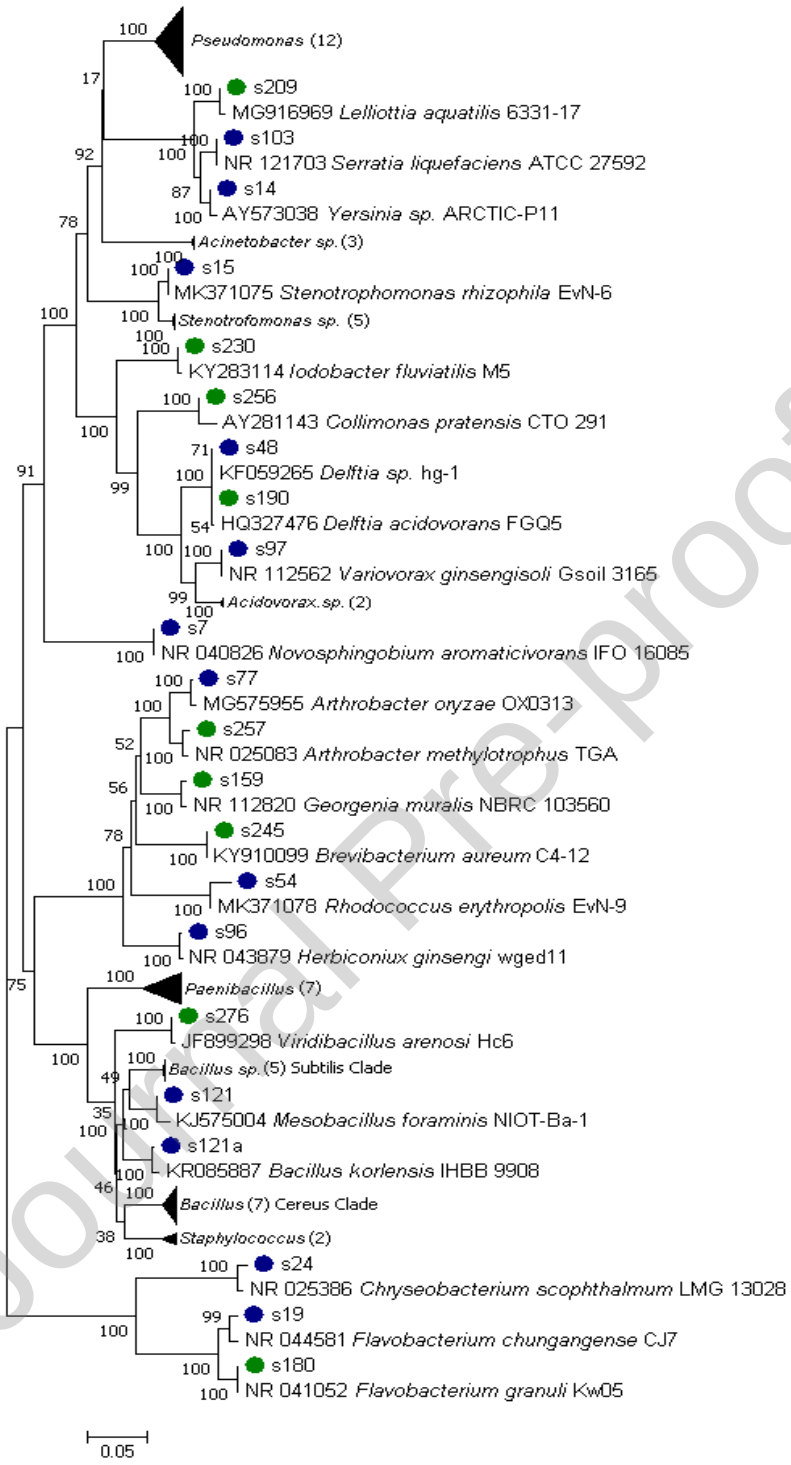

Fig.4. Phylogenetic tree constructed based on the analysis of sequences of 16S RNA gene fragments of bacteria isolated from rocks of the Primorsky Krai. The dendrogram was constructed based on the nearest neighbor-joining algorithm using the MEGA 7.0 software package. ● Olginsky district, ● Terneysky and Pozharsky districts.

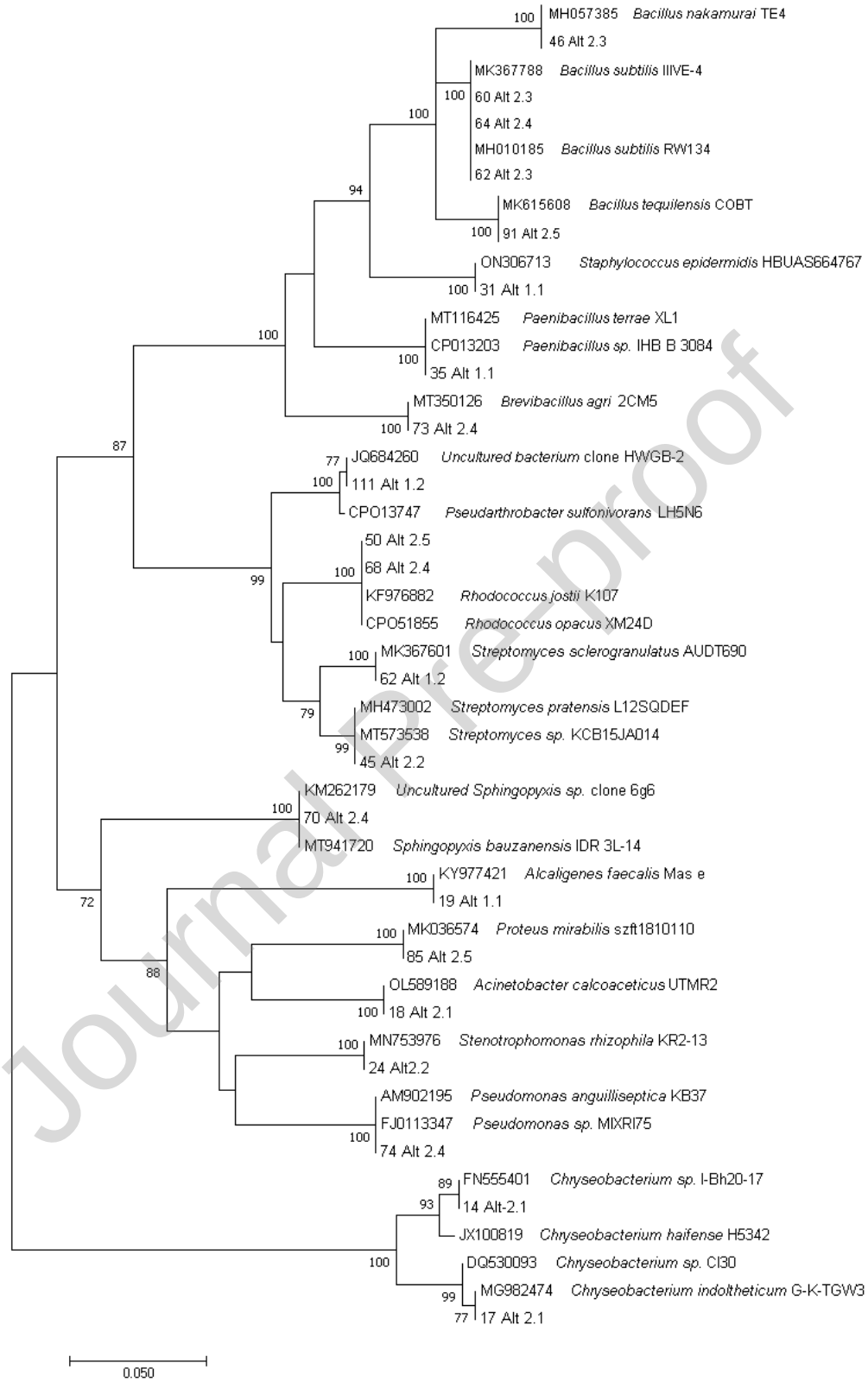

Fig. 5. Phylogenetic tree constructed based on analysis of sequences of 16S RNA gene fragments of bacteria isolated from rocks of the Altai Republic. The dendrogram was constructed based on the nearest neighbor-joining algorithm using the MEGA 7.0 software package.

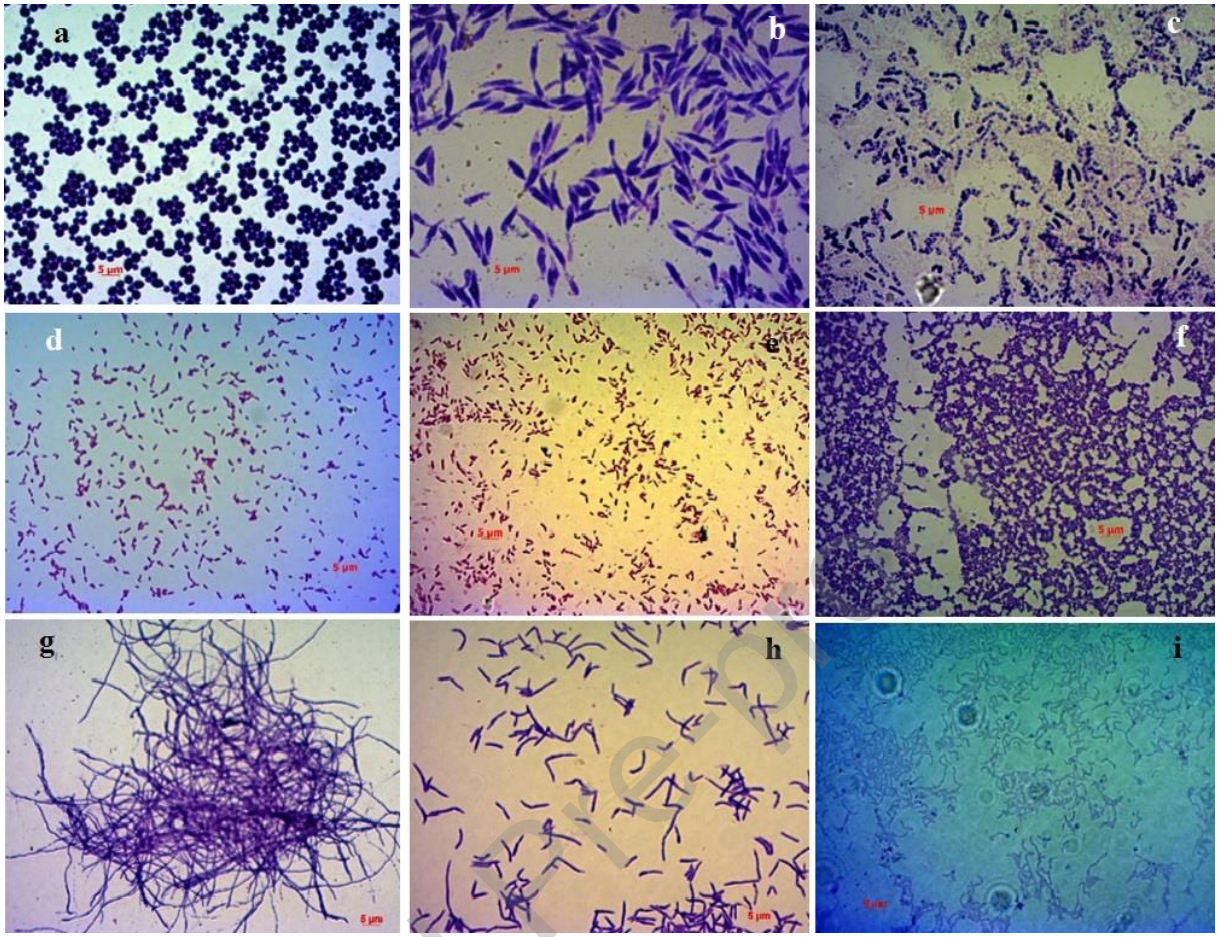

Fig. 6. Morphological forms of microorganisms isolated from rocks of the Primorsky Territory (a-f) and the Altai Republic (g-i) (a – strain No. 239 yeast of the genus *Rhodotorula*, b – strain No. 37 yeast of the genus *Saitozyma*, c – strain No. 200 a genus *Bacillus*, d – strain No. 65 *Pseudomonas*, e – strain No. 225 genus *Stenotrophomonas*, f – strain No. 257 genus *Arthrobacter*, g – strain No. 45 Alt 2.2 genus *Streptomyces*, h – strain No. 50 Alt 2.5 genus *Rhodococcus*, i – strain No. 111 Alt 1.2 *Pseudarthrobacter*).

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Elena Lebedeva reports administrative support and equipment, drugs, or supplies were provided by Russian Science Foundation, grant. Panichev A.M. reports a relationship with Pacific Institute of Geography FEB RAS that includes: employment, funding grants, and non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights:

1. This study aims to understand the reasons why wild animals consume rocks.

2. One of the hypotheses of geophagy is the microbiological factor.

3. A wide range of microorganisms were identified that had high biological activity.

4. The important role of microorganisms in the geophagy of rocks has been established.