Microbiology, Vol. 71, No. 6, 2002, pp. 723–734. Translated from Mikrobiologiya, Vol. 71, No. 6, 2002, pp. 836–848.

EXPERIMENTAL ARTICLES

Detection of Microorganisms in the Environment and the Preliminary Appraisal of Their Physiological State by X-ray Microanalysis

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Received March 18, 2002

Abstract—The paper deals with the X-ray microanalysis of the elemental composition of bacteriomorphic particles in 170000-year-old Antarctic permafrost sediments and in indoor dust. A comparative analysis of the phosphorus, sulfur, calcium, and potassium contents and the Ca/K and P/S ratios in these particles and in reference microbial cells occurring in different physiological states showed that the absence of P and/or S peaks in the X-ray spectrum of an object may indicate that it is abiotic. Resting microbial forms can be revealed on the basis of the following characteristic features: an increased content of Ca, a high Ca/K ratio, and a low P/S ratio. Model experiments with nonviable bacterial and yeast micromummies with alterations in the structural and barrier functions of the cytoplasmic membrane showed that micromummies can be recognized by a superhigh content of a marker element (e.g., P, K, or Si), accumulated due to facilitated diffusion along a deliberately created concentration gradient. Such an analysis of the permafrost sediment and dust made it possible to suggest the presence of mummified cells in these objects. The possibility of using X-ray microanalysis for the detection of microbial cells in natural habitats in order to enhance the efficiency of ecological monitoring of the environment is discussed.

Key words: X-ray microanalysis, elemental composition, resting microbial cells, micromummies, ecological monitoring.

In most natural habitats, microorganisms occur in a dormant state, which provides for their long-term survival during nutritional starvation, nonoptimum temperatures, water deficiency, and other detrimental conditions [1, 2]. Resting microbial forms differ in morphology, tolerance to extreme conditions, the degree of inhibition of metabolic processes, and, which is especially important, the ability to revive when environmental conditions become favorable [1].

Standard microbiological culture methods make it possible to reveal only a small fraction of the microorganisms actually present in the environment and yield limited information on the physiological state of microbial cells in situ [1, 2]. It should be emphasized that nondividing cells may represent not only dormant forms, but also nonviable cells that still retain their morphological integrity (the so-called micromummies) [3]. The distinguishing of cells with different physiological statuses is important for ecological monitoring of the environment. One of the related problems is the development of diagnostic criteria that would allow viable and mummified cells to be reliably differentiated. In previous works, we revealed conditions favoring the formation of mummified bacterial and yeast cells and showed that the ultrastructure of micromummies obtained under laboratory conditions [3] is similar to that of the cytoplasm and the cell wall remnants of fossil microorganisms found in 1.8-million-year-old Volyn kerites [4].

However, it is often difficult to distinguish microbial cells and abiotic particles when interpreting the scanning electron microscopic images of natural samples. One of the criteria that may be used to reliably differentiate microbial cells and abiotic particles is their elemental composition. As shown earlier [5], the content of the elements phosphorus, sulfur, calcium, and potassium and the Ca/K and P/S ratios considerably differ in cells with different physiological statuses: vegetative cells, viable resting cystlike refractory cells (CRCs), endospores, and nonviable cells (micromummies). We proposed to use the aforementioned parameters as markers for the direct detection of microbial cells in natural habitats, among which of particular interest are extreme habitats, such as permafrost sediments [6–8]

0026-2617/02/7106-0723$27.00

and dusts, in which microorganisms mainly occur in a resting state. It should be noted that dusts are favorite objects for the ecological, sanitary, and epidemiological monitoring of the environment.

The aim of the present work was to estimate the possibility of using the elemental composition characteristics of cells as obtained by X-ray microanalysis for the direct detection of microbial cells in the environment and for the appraisal of their physiological state.

MATERIALS AND METHODS

Samples. Experiments were performed with two objects. The first object was a sample of the permafrost sediment taken from a depth of 16.4 m in Miers Valley, Antarctica, where the time of the sediment occurrence in a frozen state is about 170000 years. The sediment sample was taken, delivered to the laboratory, and stored at –20°C, with precautions taken to keep the sample in an axenic and frozen state [9]. The second object for study was a sample of dust about 20 mg in weight, which was collected from an indoor laboratory wall area of about 500 cm^2 by scraping with a spatula.

Saccharomyces cerevisiae and *Micrococcus luteus* micromummies were obtained by adding a chemical analogue of the microbial anabiosis autoinducer, hexyl-substituted hydroxybenzene (C_6-AHB), to stationary-phase cultures of these bacteria at a final concentration of 1 mM [3]. After 3–7 days of incubation in the presence of C_6-AHB, mummified micrococci and yeast cells were collected by centrifugation at 4000 *g* and resuspended either in distilled water, or in phosphate buffer (either 50 or 100 mM K_2HPO_4), or in an aqueous solution containing 0.9 g/l SiO_2. To prevent a decrease in the C_6-AHB content of micromummies, all these media were supplemented with 1 mM C_6-AHB. Prior to analysis, the suspensions of micromummies had been kept at room temperature for at least a month.

Preparation of samples for analysis. The 20-mg dust sample was suspended in 0.6 ml of deionized water, and the permafrost sediment sample was thawed at 20°C for 10 min. Aliquots (0.05 ml) of the dust suspension and the aqueous layer of the thawed sediment were applied to Formvar-coated 200-mesh copper grids for electron microscopic examination. In parallel, the dust suspension and the aqueous layer of the thawed sediment sample were serially diluted for plating on agar media.

Bacterial and yeast micromummies incubated under laboratory conditions were separated from the incubation medium by centrifugation at 4000 *g*, washed twice with deionized water (in some experiments, micromummies were not washed), and resuspended in deionized water or the incubation medium. Aliquots (0.05 ml) of these suspensions were analyzed by electron microscopy as described in the previous paragraph.

Microbiological methods. The number of viable cells in the dust and sediment suspensions was determined by enumerating colonies grown after plating the respective dilutions onto different solid media: nutrient agar, trypticase–soybean agar, starch–ammonia agar, and wort agar. The results were expressed in colony-forming units (CFU). The experiments were performed in triplicate. Each dilution was plated in quintuplicate. The direct count of bacterial cells in suspensions was performed by examining specimens stained with acridine orange (1 : 10000 dilution; pH 6.5–7.0; 15-min incubation without NaCl) under a LYuMAM fluorescence microscope (Russia) with a 90× epiobjective. Fungal cells were enumerated similarly, using specimens stained with calcofluor white (1 : 10000 dilution; 15-min incubation without NaCl).

X-ray microanalysis. Copper grids with applied suspensions of the dust, permafrost sediment, and micromummies were desiccated for 4–6 h, coated with carbon at an angle of 90°, and subjected to electron microscopy and X-ray microanalysis using a JEM-100CXII electron microscope (JEOL, Japan) equipped with an EM-ASID4D scanning device and a Link 860 X-ray microanalyzer with an E5423 detector (Link-System, United Kingdom). The microscope was operated at a voltage of 60 keV (magnification 20000×). X-ray spectra were analyzed for the peak-to-background area ratios (P/B) of elements (P, S, Ca, K, Si, Fe) and the peak-to-peak area ratios (P/P) of the element pairs Ca/K and P/S. The elemental composition parameters P and P/B of bacteriomorphic particles and cells were averaged over the results of three to five replicated measurements. In some experiments, the element content of cells was calculated with a correction made for the peak area of the extracellular element.

RESULTS AND DISCUSSION

The results of the direct microscopic count of bacteriomorphic particles under the fluorescence microscope are presented in Table 1. The presence of viable microorganisms in the sediment and dust samples was confirmed by plating the dust and sediment suspension dilutions on the respective agar media. The number of viable cells estimated in CFU comprised a small fraction (about 0.001%) of the total number of cells detected in the permafrost sediment sample by direct microscopy (Table 1). These data are in agreement with the results of the microbiological investigation of various natural environments [1, 2], including permafrost soils and grounds [7]. The scanning electron microscopy of the dust and permafrost sediment specimens (in total, 20 microscope fields were examined) revealed particles resembling microbial cells in size and morphology (Fig. 1). The total number of such particles in the dust sample was estimated to be about 10^7 per g dust, which is three orders of magnitude more than the number of cells giving rise to colonies (Table 1). Such a great difference between the results of cell counts obtained by the two methods suggested that the samples analyzed contained a large number of abiotic par-

Table 1. Number of cells in the dust and Antarctic permafrost sediment samples

	Permafrost sediment		Dust, CFU/g***
	cells/g*	CFU/g**	
Bacteria	$(1.8 \pm 0.6) \times 10^8$	$(1.3 \pm 0.3) \times 10^3$	$(1.1 \pm 0.1) \times 10^4$
Fungi	$(2.7 \pm 0.7) \times 10^6$	10^2	$(5.8 \pm 0.2) \times 10^3$

* Direct microscopic count of cells with bright green fluorescence.
** Number of colonies grown on trypticase–soybean agar (bacteria) and wort agar (fungi).
*** Number of colonies grown on nutrient agar and starch–ammonia agar (fungi and actinomycetes) and wort agar (fungi).

ticles, as well as microbial cells of different physiological status and viability.

To reveal microbial cells in the dust and sediment samples, we determined the elemental composition of bacteriomorphic particles observed in the scanning electron microscope. Each of the particles was assigned a serial number (nos. 1–14). For comparison, the elemental composition parameters of reference microbial cells occurring in different physiological states were invoked (Table 2). Among them were cells of the spore-forming bacterium *Bacillus cereus*, the non-spore-forming bacterium *M. luteus*, the yeast *S. cerevisiae* [5], and the gram-negative bacteria *Escherichia coli* and *Thioalkalivibrio versutus*. Analysis of the X-ray spectra of bacteriomorphic particles with serial numbers 2, 4, 5, and 7–10 in the permafrost sediment sample (Table 3) and

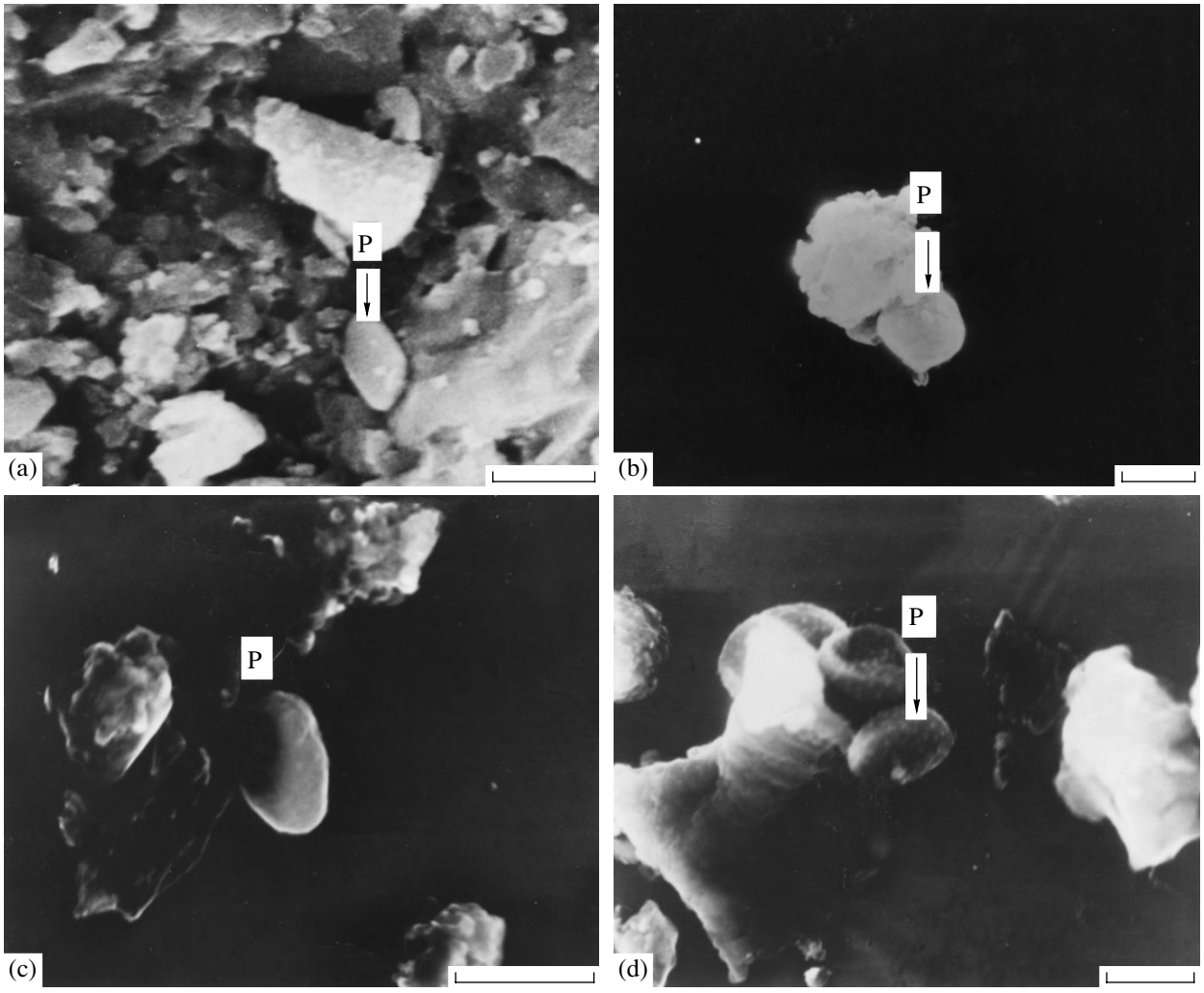

Fig. 1. Micrographs of bacteriomorphic particles (P) in (a, b) the Antarctic permafrost sediment sample and (c, d) the dust sample. The scale bar represents 1 μm.

Table 2. Tentative diagnostic key for the detection of microbial cells in natural samples based on X-ray microanalysis data

Element	P/B interval for cells*	Objects resembling cells in elemental composition		Objects with elemental composition atypical of cells	
		in permafrost sediment	in dust	in permafrost sediment	in dust
S	0.143–1.600	1, 3, 4, 5, 6, 8, 10	1, 2 (?), 3, 5, 8–11, 13, 14 (?)	2, 7, 9	4, 7, 12
P	0.173–1.250	1, 3, 6	8, 9, 11, 13, 14 (?)	2, 4, 5, 7–10	1–7, 10, 12
Ca	0.131–4.150	1–6, 8–10	1–4, 6, 8–14	7	5, 7 (?)
K	0.065–0.920	1, 2, 3, 4, 5, 6 (?), 7, 9, 10	1, 4, 6, 7(?), 8–13	8	2, 3, 14
Si	0.000–0.120	3 (?)	8, 9(?), 11, 13 (?)	1, 2, 4, 5, 6–10	3, 4, 6, 7, 10, 12, 14
Fe	0.079–1.332	3	8, 9, 11, 13 (?)	1, 4, 5, 6–10	1, 4, 7, 13
In the content of these six elements		Objects 1 and 3 are probable cells; object 6 is an anomalous cell	Objects 8, 9, 11, and 13 are probable cells; object 14 is an anomalous cell	Objects 2, 4, 5, and 7–10 are not cells	Objects 1–7, 10, and 12 are not cells

Tentative diagnosis of the physiological state of probable cells

Differentiating parameter*	Vegetative cells*	Objects resembling vegetative cells		Resting cells*	Objects resembling resting cells	
		in permafrost sediment	in dust		in permafrost sediment	in dust
Ca/K ratio	0.342–0.592	–	–	1.285–25.58	1, 3	8, 9, 11, 13
P/S ratio	2.342–3.433	–	–	0.697–2.148	1, 3	8, 9, 11, 13

Redetermination of objects 6 and 14, tentatively determined as cells with an anomalous content of some elements

Peculiar elemental composition of micromummies	Anomalously low or high P/B for K	Elevated content of Si and Fe	Lower background (B)	Probable status
is typical of object 6 (sediment)?	No, although the peak area of K is small	Yes	Yes	Micromummy
is typical of object 14 (dust)?	Yes	Yes	Yes	Micromummy

* Data obtained by Mulyukin et al. [5] for laboratory cultures and unpublished data on the content and proportion between elements in *E. coli* and *T. versutus* cells.

with serial numbers 1–7, 10, and 12 in the dust sample (Table 4) showed that none of them exhibited P and/or S peaks. This fact allows these objects to be considered abiotic, since all of the reference microbial cells, irrespective of their taxonomic affiliation [10] and physiological status (vegetative cells, resting forms, nonviable micromummies, and partially autolyzed cells [5]) always exhibit the presence of the elements P and S, albeit in different amounts. At the same time, some bacteriomorphic particles (object nos. 1, 3, and 6 in Table 3 and object nos. 8, 9, 11, and 13 in Table 4) were characterized by P/B values of the elements S, P, Ca, and K within ranges typical of viable cells (Table 2). The difference in the elemental compositions of abiotic particles and bacteriomorphs is easily seen from their X-ray spectra presented in Figs. 2 and 3. The comparison of the P/B values of P and S in the X-ray spectra of objects with the respective values of viable cells (Table 2) makes it possible to differentiate abiotic particles and microbial cells.

Objects nos. 1 and 3 in Table 3 and 8, 9, 11, and 13 in Table 4, ascribed to microorganisms (the category "bioobjects"), were characterized by elemental composition parameters typical of resting microbial forms [5] (an increased Ca/K ratio and a decreased P/S ratio) (Table 2). In spite of the fact that such values of these ratios are also typical of slow-growing stress-resistant oligotrophic bacteria [10], we may suggest that the dust and permafrost sediment samples contain microorganisms occurring in a resting state. This suggestion does not contradict data on the ultrastructural organization of objects isolated from permafrost sediments [6–8], which resemble resting cystlike microbial cells [11, 12]. Some bacteriomorphic particles found in the permafrost sediment sample (objects nos. 1 and 6 in Table 3) exhibited an increased content of Si and Fe as compared with cells grown under laboratory conditions and with the bacteriomorphs found in the dust sample (objects nos. 8, 9, 11, and 13 in Table 4). This fact can be explained by the in situ adsorption of mineral particles on the cell surface, a process that was observed

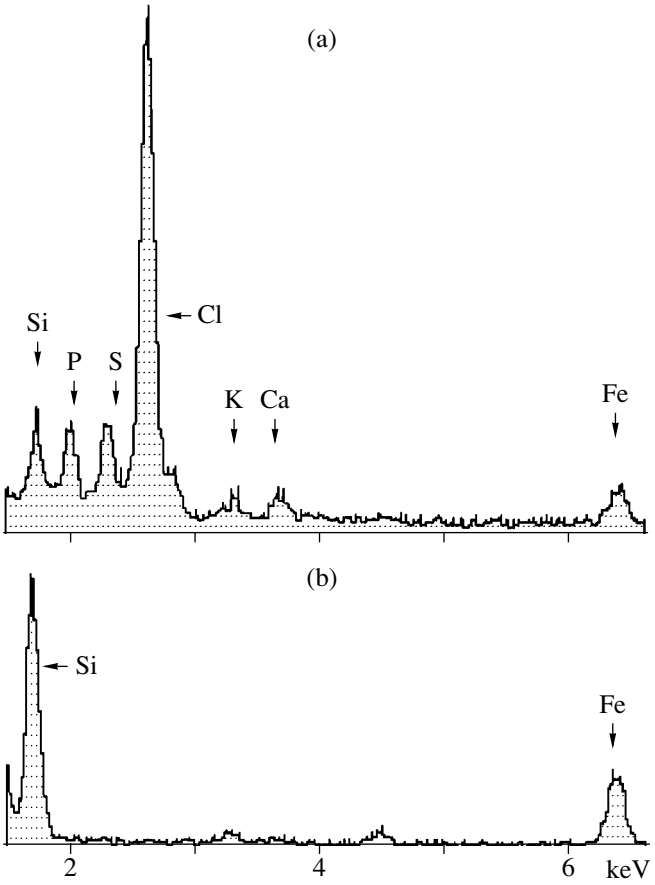

Fig. 2. The X-ray spectra of (a) object 3, resembling a microbial cell, and (b) abiotic object 2 in the Antarctic permafrost sediment sample.

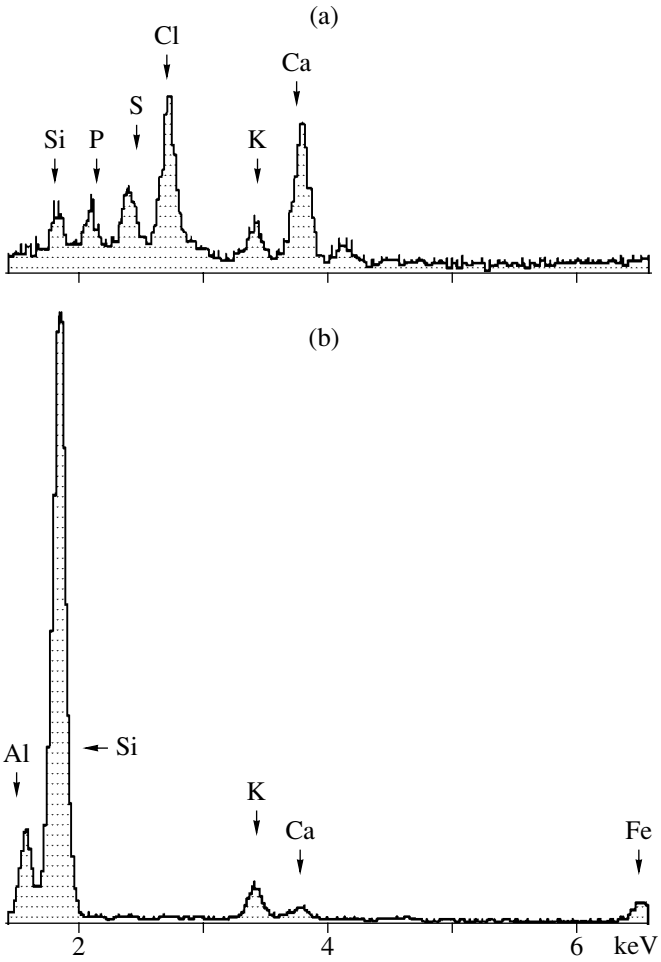

Fig. 3. The X-ray spectra of (a) object 13, resembling a microbial cell, and (b) abiotic object 12 in the dust sample.

during the ultrastructural study of native microbial populations isolated from ancient permafrost sediments [6, 8]. The possibility also cannot be excluded that mineral elements are incorporated into cellular structures.

It is obvious that the elemental composition of microbial cells in natural samples must differ from that of cells grown under laboratory conditions, since these types of cells occur in very different environments. Furthermore, the ability of microbial cells to adsorb [13] or absorb [14] mineral elements depends on the viability and physiological state (or age) of these cells. In nature, nonviable cells can be fossilized due to their impregnation with solutions of silicon oxide, carbonates, iron oxides, phosphorites, pyrite, etc. [15]. For instance, the X-ray spectra of cyanobacteria subject to postmortem fossilization and sheath mineralization with hydroxy- and fluoroapatite were found to be dominated by Ca and P peaks [16]. Lysed or heat-inactivated *Candida utilis* cells exhibited an increased ability to accumulate Ag^+ ions [17]. In our experiments, mummified bacterial and yeast cells contained an anomalously high amount of potassium ions [5], presumably due to a failure of the barrier function of cell membranes in micromummies [3]. For this reason, micromummies must undergo the facilitated diffusion of mineral elements along the concentration gradient, resulting in the fossilization of the micromummies due to the substitution of organic substances by inorganic components. Bearing this in mind, in the next set of model experiments, we studied the elemental composition of mummified microbial cells as a function of the salt composition of the incubation medium.

In these experiments, the *S. cerevisiae* and *M. luteus* micromummies obtained under laboratory conditions were suspended in distilled water, or in phosphate buffer (either 50 or 100 mM K_2HPO_4), or in an aqueous solution containing 0.9 g/l of water-soluble SiO_2, as recommended in [15], and the suspensions of micromummies were kept at room temperature for a month. When suspended in distilled water, the yeast micromummies that had been washed twice with deionized water did not contain potassium, whereas the unwashed yeast micromummies suspended in distilled water exhibited a low content of potassium, which was close to the level of detectability of this element (Table 5). These data suggest that these micromummies undergo

Table 3. X-ray microanalysis of bacteriomorphic particles in the Antarctic permafrost sediment sample

Object no.	The peak areas (P) and the P/B ratios of elements						Element ratios (P/P)		The most probable diagnosis
		S	P	Ca	K	P/B for other elements	Ca/K	P/S	
1	P	399 ± 64	813 ± 73	2038 ± 91	302 ± 56	Fe: 2.342 ± 0.389	6.748 ± 1.1667	2.038 ± 0.171	Resting cell
	P/B	0.594 ± 0.104	1.135 ± 0.135	4.286 ± 0.455	0.548 ± 0.109	Cl: 1.286 ± 0.149			
						Si: 1.412 ± 0.106			
						Al: 0.365 ± 0.059			
						Mg: 0.092 ± 0.043			
2	P	~0	~0	143 ± 47	63 ± 47	Fe: 0.338 ± 0.146	2.270 ± 1.370	–	Not cell
	P/B	~0	~0	0.214 ± 0.071	0.091 ± 0.068	Cl: 0.192 ± 0.058			
						Si: 9.127 ± 1.135			
						Al: 0.372 ± 0.129			
						Mg: 0.110 ± 0.065			
3	P	1236 ± 107	1008 ± 92	386 ± 65	225 ± 63	Fe: 1.382 ± 0.193	1.716 ± 0.266	0.816 ± 0.077	Resting cell
	P/B	0.695 ± 0.069	0.470 ± 0.047	0.392 ± 0.068	0.219 ± 0.062	Cl: 5.284 ± 0.458			
						Si: 0.287 ± 0.026			
						Al: 0.063 ± 0.020			
						Mg: 0.053 ± 0.020			
4	P	738 ± 77	~0	134 ± 54	134 ± 52	Fe: 21.094 ± 3.313	1.000 ± 0.011	0	Not cell
	P/B	0.516 ± 0.058	~0	0.210 ± 0.086	0.179 ± 0.069	Cl: 0.101 ± 0.054			
						Si: 1.898 ± 0.110			
						Al: 0.875 ± 0.095			
						Mg: 1.125 ± 0.097			
5	P	98 ± 34	~0	33 ± 26	193 ± 34	Fe: 0.985 ± 2.661	0.337 ± 0.293	0	Not cell
	P/B	0.358 ± 0.132	~0	0.405 ± 0.317	1.624 ± 0.332	Cl: ~0			
						Si: 6.548 ± 0.890			
						Al: 3.171 ± 0.628			
						Mg: 1.262 ± 0.258			

Table 3. (Contd.)

Object no.	The peak areas (P) and the P/B ratios of elements							Element ratios (P/P)		The most probable diagnosis
		S	P	Ca	K	P/B for other elements		Ca/K	P/S	
6	P	351 ± 52	2.87 ± 55	155 ± 38	47 ± 36	Fe:	4.643 ± 0.677	3.298 ± 5.973	0.818 ± 0.033	Probable micromummy
	P/B	0.73 ± 0.135	0.537 ± 0.122	0.588 ± 0.148	0.159 ± 0.122	Cl:	0.112 ± 0.103			
						Si:	2.946 ± 0.256			
						Al:	1.517 ± 0.168			
						Mg:	~0			
7	P	~0	95 ± 76	8053 ± 177	81 ± 62	Fe:	4.271 ± 0.400	99.420	–	Not cell
	P/B	~0	0.046 ± 0.036	14.848 ± 1.47	0.088 ± 0.068	Cl:	~0			
						Si:	5.656 ± 0.270			
						Al:	1.150 ± 0.101			
						Mg:	0.788 ± 0.056			
8	P	246 ± 62	~0	180 ± 58	1051 ± 87	Fe:	9.140 ± 0.941	0.171 ± 0.038	0	Not cell
	P/B	0.171 ± 0.044	~0	0.173 ± 0.05	1.004 ± 0.089	Cl:	0.170 ± 0.051			
						Si:	3.376 ± 0.191			
						Al:	1.583 ± 0.136			
						Mg:	0.538 ± 0.057			
9	P	~0	~0	71 ± 35	105 ± 39	Fe:	10.026 ± 1.583	0.676 ± 0.131	–	Not cell
	P/B	~0	~0	0.216 ± 0.10	0.295 ± 0.109	Cl:	~0			
						Si:	3.628 ± 0.276			
						Al:	1.769 ± 0.191			
						Mg:	0.392 ± 0.076			
10	P	47 ± 30	~0	77 ± 29	45 ± 28	Fe:	17.080 ± 5.461	1.711 ± 0.375	0	Not cell
	P/B	0.144 ± 0.092	~0	.524 ± 0.203	0.234 ± 0.148	Cl:	~0			
						Si:	2.590 ± 0.214			
						Al:	0.158 ± 0.099			
						Mg:	0.940 ± 0.126			

Note: In this and the other tables, ~0 indicates that the content of the particular element is below the detectable level.

Table 4. X-ray microanalysis of bacteriomorphic particles in the dust sample

Object no.	The peak areas (P) and the P/B ratios of elements						Element ratios (P/P)		The most probable diagnosis
		S	P	Ca	K	P/B for other elements	Ca/K	P/S	
1	P	266 ± 48	46 ± 35	472 ± 49	64 ± 33	Fe: ~0	7.375 ± 2.003	0.204 ± 0.142	Not cell
	P/B	0.492 ± 0.111	0.086 ± 0.066	1.985 ± 0.285	0.279 ± 0.145	Cl: 2.689 ± 0.348			
						Si: 0.073 ± 0.048			
2	P	55 ± 30	~0	157 ± 33	~0	Fe: 0.814 ± 0.637	–	0	Not cell
	P/B	0.221 ± 0.122	~0	0.683 ± 0.152	~0	Cl: 0.982 ± 0.210			
						Si: 0.133 ± 0.058			
3	P	461 ± 70	68 ± 61	1495 ± 87	1065 ± 86	Fe: 1.485 ± 0.292	1.409 ± 0.029	0.147 ± 0.129	Not cell
	P/B	0.377 ± 0.061	0.048 ± 0.042	1.951 ± 0.152	1.469 ± 0.140	Cl: 1.032 ± 0.210			
						Si: 1.160 ± 0.051			
4	P	143 ± 61	145 ± 67	702 ± 68	751 ± 56	Fe: 5.780 ± 0.632	0.935 ± 0.023	1.014 ± 0.074	Not cell
	P/B	0.096 ± 0.042	0.082 ± 0.039	0.999 ± 0.109	0.887 ± 0.098	Cl: 0.394 ± 0.061			
						Si: 1.445 ± 0.041			
5	P	167 ± 55	73 ± 51	11547 ± 201	~0	Fe: 0.712 ± 0.263		0.437 ± 0.241	Not cell
	P/B	0.145 ± 0.048	0.059 ± 0.041	20.648 ± 3.23	~0	Cl: ~0			
						Si: 0.180 ± 0.032			
6	P	1570 ± 97	84 ± 62	2304 ± 103	437 ± 68	Fe: 1.134 ± 0.201	5.272 ± 0.693	0.053 ± 0.038	Not cell
	P/B	0.096 ± 0.042	0.056 ± 0.041	2.673 ± 0.211	0.523 ± 0.086	Cl: 1.031 ± 0.110			
						Si: 0.595 ± 0.032			
7	P	~0	~0	61 ± 46	71 ± 46	Fe: 4.028 ± 0.527	0.859 ± 0.259		Not cell
	P/B	~0	~0	0.135 ± 0.102	0.144 ± 0.094	Cl: 0.233 ± 0.071			
						Si: 1.129 ± 0.052			

Table 4. (Contd.)

Object no.	The peak areas (P) and the P/B ratios of elements							Element ratios (P/P)		The most probable diagnosis
		S	P	Ca	K	P/B for other elements		Ca/K	P/S	
8	P	286 ± 66	300 ± 62	897 ± 70	182 ± 55	Fe:	0.027 ± 0.003	4.929 ± 1.617	1.049 ± 0.033	Resting cell
	P/B	0.257 ± 0.060	0.232 ± 0.048	1.765 ± 0.187	0.314 ± 0.096	Cl:	1.518 ± 0.128			
						Si:	0.041 ± 0.031			
9	P	506 ± 74	254 ± 58	1152 ± 70	285 ± 56	Fe:	0.333 ± 0.213	4.042 ± 0.648	0.502 ± 0.048	Resting cell
	P/B	0.516 ± 0.081	0.224 ± 0.053	2.301 ± 0.233	0.538 ± 0.112	Cl:	2.986 ± 0.269			
						Si:	0.133 ± 0.034			
10	P	832 ± 92	~0	1135 ± 89	1448 ± 100	Fe:	0.712 ± 0.117	0.784 ± 0.008	0	Not cell
	P/B	0.328 ± 0.038	~0	0.765 ± 0.064	0.523 ± 0.086	Cl:	0.436 ± 0.050			
						Si:	0.959 ± 0.025			
11	P	646 ± 77	704 ± 67	1395 ± 84	319 ± 56	Fe:	~0	4.373 ± 0.611	1.090 ± 0.030	Resting cell
	P/B	0.765 ± 0.105	0.741 ± 0.086	2.508 ± 0.236	0.640 ± 0.121	Cl:	3.937 ± 0.399			
						Si:	0.098 ± 0.035			
12	P	~0	~0	1541 ± 134	3034 ± 163	Fe:	1.094 ± 0.068	0.508 ± 0.018	–	Not cell
	P/B	~0	~0	0.293 ± 0.026	0.596 ± 0.033	Cl:	0.040 ± 0.019			
						Si:	2.966 ± 0.051			
13	P	927 ± 92	583 ± 76	2116 ± 102	413 ± 70	Fe:	0.162 ± 0.124	5.123 ± 0.720	0.629 ± 0.022	Resting cell
	P/B	0.584 ± 0.063	0.352 ± 0.048	2.239 ± 0.166	0.386 ± 0.068	Cl:	1.665 ± 0.117			
						Si:	0.243 ± 0.033			
14	P	91 ± 36	60 ± 29	415 ± 46	117 ± 32	Fe:	1.530 ± 1.377	3.547 ± 0.794	0.659 ± 0.095	Probable micromummy
	P/B	0.595 ± 0.277	0.357 ± 0.181	4.790 ± 1.011	1.488 ± 0.507	Cl:	6.901 ± 2.067			
						Si:	0.572 ± 0.110			

Table 5. The effect of the incubation medium composition on the content of elements in *S. cerevisiae* and *M. luteus* cells according to X-ray microanalysis data

Medium	The peak areas (P) and the P/B ratios of elements					
		S	P	Ca	K	Si
Micromummies of *Saccharomyces cerevisiae*						
Distilled water, unwashed cells	P	88.2 ± 112	1451 ± 112	250 ± 82	546 ± 94	232 ± 80
	P/B	0.359 ± 0.046	0.484 ± 0.040	0.382 ± 0.126	0.662 ± 0.116	0.060 ± 0.021
Distilled water, washed cells	P	1592 ± 136	3549 ± 152	274 ± 96	~0	~0
	P/B	0.331 ± 0.029	0.648 ± 0.031	0.127 ± 0.045	~0	~0
100 mM KH_2PO_4, washed cells	P	~0	2386 ± 108*	96 ± 66	1931 ± 108*	~0
	P/B	~0	1.306 ± 0.075	0.085 ± 0.058	1.908 ± 0.124	~0
0.9 g/l SiO_2, unwashed cells	P	1439 ± 108	1005 ± 93	480 ± 74	684 ± 82	1104 ± 85*
	P/B	0.886 ± 0.073	0.540 ± 0.055	0.468 ± 0.075	0.627 ± 0.078	0.495 ± 0.040
0.9 g/l SiO_2, washed cells	P	1263 ± 87	425 ± 65	181 ± 49	267 ± 57	569 ± 59*
	P/B	1.434 ± 0.125	0.443 ± 0.074	0.407 ± 0.113	0.512 ± 0.112	0.465 ± 0.054
Micromummies of *Micrococcus luteus*						
Distilled water, washed cells	P/B	1498 ± 127	1044 ± 80	1031 ± 95	747 ± 95	~0
	P	0.430 ± 0.038	0.720 ± 0.062	0.575 ± 0.056	0.385 ± 0.050	~0
50 mM KH_2PO_4, washed cells	P/B	458 ± 78	2420 ± 108	298 ± 68	3148 ± 129	180 ± 62
	P	0.323 ± 0.056	1.468 ± 0.093	0.364 ± 0.084	3.653 ± 0.198	0.079 ± 0.027
100 mM KH_2PO_4, washed cells	P/B	436 ± 99	5648 ± 156	612 ± 94	8842 ± 206	~0
	P	0.157 ± 0.036	1.747 ± 0.075	0.443 ± 0.069	5.591 ± 0.196	~0
0.9 g/l SiO_2, washed cells	P/B	723 ± 102	1315 ± 107	1448 ± 99	576 ± 86	1810 ± 80*
	P	0.338 ± 0.049	0.553 ± 0.050	2.247 ± 0.217	0.617 ± 0.096	0.556 ± 0.024

* Data corrected for the extracellular content of the particular element.

the diffusion of potassium from cells. At the same time, the micromummies kept in the K-phosphate buffers showed the reverse process (the diffusion of extracellular elements into cells). For instance, the yeast micromummies that were kept in 100 mM K_2HO_4 were characterized by anomalously high values of the P/B ratio for phosphorus and potassium, even if they were preliminarily washed with deionized water (Table 5). These results were similar to those obtained for cyanobacteria grown at an elevated concentration of phosphate (in this case, cyanobacteria accumulated polyphosphates) and at a high phosphate concentration (in this case, cyanobacteria died, the death being accompanied by the formation of hydroxyapatite sheaths around dead cells) [16]. The X-ray microanalysis of the *S. cerevisiae* micromummies that were kept in the aqueous SiO_2 solution revealed an elevated content of Si in these micromummies (Table 5). Similarly, *M. luteus* micromummies, they exhibited elevated contents of P and K when incubated in the K-phosphate buffer and of Si when incubated in the SiO_2 solution (Table 5). These data are important for the proper appraisal of the viability and the physiological state of laboratory cultures and native microbial populations isolated from natural samples.

Based on the X-ray microanalysis data showing (1) the presence of P and S (even in small amounts), (2) an anomalously low or high content of K, and (3) an elevated content of Si and other mineral elements, it can be suggested that objects no. 6 (Table 3) and no. 14 (Table 4) represent micromummies. The low background value of some elements may provide further evidence for this suggestion [5].

Thus, the qualitative (the presence of corresponding peaks) and quantitative (the P/B ratio values) results of the X-ray microanalysis of the elements S, P, Ca, and K in the bacteriomorphic particles found in the dust and the Antarctic permafrost sediment samples, performed in accordance with the diagnostic key presented in Table 2, make it possible to differentiate microbial cells from abiotic bacteriomorphic particles. Namely, the absence of S and/or P in a particle may be considered as convincing evidence that this particle is abiotic. Anomalously low or high concentrations of mineral elements (K, Fe, Si, and others) in particles ascribed to microbial cells may indicate their complete nonviability. The possibility of using X-ray microanalysis to assess the physiological state of microorganisms (vegetative or resting) in natural samples is of great interest for the ecological monitoring of the environment,

which calls for further investigation in this respect of a wider range of microorganisms belonging to different taxonomic groups.

In particular, studies should be performed to elucidate whether the reliable differentiation of microorganisms and bacteriomorphic particles necessitates analysis of light elements (C, O, and N) in addition to S, P, Ca, and K. It should be noted in this regard that the comparative analysis of the cellular content of C, O, P, N, and S made it possible to differentiate exponential- and stationary-phase *E. coli* cells in laboratory cultures [18]. However, natural samples usually contain a great amount of organic residues, which hinders the identification of microbial cells in these samples from the content of the elements C, O, and N. On the other hand, Kajander and Ciftcioglu reported that the X-ray spectrum of nannobacteria from human blood serum was similar to that of inorganic hydroxyapatite, except that the latter spectrum did not contain the potassium peak present in the spectrum of nannobacteria [19]. Furthermore, Cisar *et al.* demonstrated that the abiotic particles formed from phospholipid and hydroxyapatite were morphologically similar to nannobacteria and virtually did not differ from the nannobacteria and hydroxyapatite in the X-ray spectrum [20]. Consequently, living cells and abiotic particles cannot be reliably differentiated in natural samples based only on the qualitative X-ray spectral characteristics (the presence or absence of certain elements). Therefore, we consider it important to take into account the ratios between these elements. The proposed method of differentiation of microbial cells and abiotic particles certainly needs further improvements at the stage of sample preparation for X-ray microanalysis.

ACKNOWLEDGMENTS

We are grateful to D.A. Gilichinskii, head of the Laboratory of Soil Cryology at the Institute of Physicochemical and Biological Problems of Soil Science, Pushchino, Russia, for providing the permafrost sediment sample.

This work was supported by grant no. 02-04-49150 from the Russian Foundation for Basic Research and by contract no. 101-10(00)-P-D01 within the scope of the Russian Federal Scientific and Technical Program "Research and Development in Priority Directions of Science and Civil Engineering."

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