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EXPERIMENTAL ARTICLES

Comparative Study of the Elemental Composition of Vegetative and Resting Microbial Cells

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Abstract—X-ray microanalysis showed that vegetative cells, viable resting forms, and nonviable forms (micromummies) of the bacteria *Bacillus cereus* and *Micrococcus luteus* and the yeast *Saccharomyces cerevisiae* differ in the content of elements S, P, Ca, and K and Ca/K and P/S ratios. Viable resting forms (cystlike refractive cells and bacillar endospores) had more calcium and less phosphorus and potassium than vegetative cells, the difference being higher for bacilli than for micrococci and yeasts. The distinctive feature of all viable resting microbial forms was their low P/S ratios and high Ca/K ratios. The differences revealed in the cellular content and ratios of elements probably reflect changes in ionic homeostasis accompanying the transition of vegetative microbial cells to the dormant state. Relevant potassium parameters indicate that the membranes of viable resting forms retain their barrier function. At the same time, the nonviable micromummies, even those morphologically intact, of *B. cereus* and *S. cerevisiae* exhibited an anomalously low content of potassium, while those of *M. luteus* had an anomalously high content of this element. This suggests that the cellular membranes of micromummies lose their barrier function, which results in a free diffusion of potassium ions across the membranes. The possibility of using the elemental composition parameters for the quick analysis of the physiological state of microorganisms in natural environments is discussed.

Key words: X-ray microanalysis, elemental composition, cystlike refractive cells, endospores, micromummies.

Improvement of methods for monitoring microorganisms in natural econiches is an important ecological and clinical problem, whose solution may considerably contribute to the control of epidemics and pandemics. It is believed that most microorganisms occur in natural environments in a dormant state, which allows them to survive starvation, extreme temperatures, and other unfavorable conditions [1, 2]. Resting microbial forms differ in morphology and physiological characteristics, such as colony-forming ability, resistance to unfavorable factors, profundity of dormancy, and viability, i.e., the ability to revert to the metabolically active state.

It should be noted that routine culture methods make it possible to reveal only a small fraction of microorganisms detectable by the direct count method [1, 2]. Moreover, the ecological and sanitary value of monitoring is determined not only by the adequacy of bacterial enumeration but also by the possibility of assessing the physiological state of microorganisms in natural environments. Therefore, the development of criteria that would permit the differentiation of microbial cells in natural samples according to their physiological status and their ability to recover to growth is one of the challenging problems of microbial ecology.

The present study focused on the search for such criteria and was performed using three model microorganisms: the spore-forming bacterium *Bacillus cereus*, the non-spore-forming bacterium *Micrococcus luteus* and the yeast *Saccharomyces cerevisiae*. The choice of these microorganisms was dictated by their ability to produce cystlike refractive cells (CRCs), which possess all attributes of dormant cells and are an alternative type of resting forms in spore-forming bacilli and the only type of resting forms in non-spore-forming microorganisms [3–5]. Furthermore, these three microorganisms were found to be able to produce nonviable forms, the so-called micromummies, which are unable to revert to the metabolically active state but retain their morphological integrity [6]. Resting microbial forms, as well as micromummies, are produced in response to the action of anabiosis autoinducers (alkyl-substituted hydroxybenzenes, AHBs) [7, 8], whose mechanism of action lies in the conformational modification of proteins and the structural rearrangement (polycrystallization) of membrane lipids, leading to the alteration of the functional activity of membranes and their permeabil-

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ity to monovalent ions [9, 10]. It seems plausible that metabolically active and inactive microbial cells differ in the content of mono- and divalent ions and main biologically important elements, as was shown for bacillar spores and vegetative cells [11].

The aim of the present work was to study the elemental composition of the vegetative cells, viable resting forms, and nonviable but morphologically intact cells (micromummies) of the bacteria *B. cereus* and *M. luteus* and the yeast *S. cerevisiae* in order to determine the elemental composition parameters that can be used to assess the physiological state of microbial cells without their cultivation.

MATERIALS AND METHODS

Microorganisms. Experiments were carried out with the gram-positive spore-forming bacterium *Bacillus cereus* strain 504, the non-spore-forming bacterium *Micrococcus luteus* NCIMB 13267, and the yeast *Saccharomyces cerevisiae* strain 380. Strains 504 and 380 were obtained from the All-Russia Collection of Microorganisms (VKM).

Cultivation conditions for vegetative cells. The bacterium *B. cereus* 504 was grown in a chemically defined medium containing (g/l) glucose, 5.0; $NH_4H_2PO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; KCl, 0.2; $CaCl_2$, 0.2; $FeCl_3 \cdot 6H_2O$, 0.001; $MnSO_4 \cdot H_2O$, 0.017; and yeast extract, 0.2. The pH of the medium after sterilization was 7.0 ± 0.1. The bacterium *M. luteus* NCIMB 13267 was grown in a chemically defined medium containing (g/l) lithium lactate, 5.0; NH_4Cl, 4; KH_2PO_4, 4.0; and $CaCl_2$, 0.2. The medium was supplemented with the following trace elements and growth factors (mg/l): $MgSO_4 \cdot 7H_2O$, 50; $FeSO_4$, 20; $MnCl_2 \cdot 4H_2O$, 20; $ZnSO_4$, 0.4; $B(OH)_3$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.05; $Na_2MoO_4 \cdot 2H_2O$, 0.2; thiamine, 40; and methionine, 20. The pH of the medium after sterilization was 7.2–7.4. The yeast *S. cerevisiae* 380 was grown in a liquid wort (2.3 degrees Balling). Cultivations were conducted at 28°C in 250-ml flasks with 50 ml of the growth medium on a shaker (140–160 rpm). The inoculum was added to give an optical culture density of 0.2 at 650 nm (Specord spectrophotometer; 10-mm-pathlength cuvettes). The cultures were grown to the exponential or stationary growth phases.

Obtaining of resting microbial forms. Bacillar endospores were obtained by cultivating *B. cereus* cells for 7 days in the above medium for this bacterium. Cystlike refractive cells were obtained through minor modifications of the cultivation media: the formation of the CRCs of *B. cereus* [4] and *S. cerevisiae* was induced by increasing the concentration of glucose in the respective cultivation media to 60 g/l and the formation of the *M. luteus* CRCs was induced by decreasing the concentration of phosphates in the medium to 0.4 g/l [4]. Alternatively, the CRCs of *M. luteus* were obtained by incubating thick cell suspensions of this bacterium in 0.1 M KH_2PO_4 (pH 7.25) for 1 month [5] or by adding an ethanol solution of 4-*n*-hexylresorcinol (C_6-AHB), a chemical analogue of anabiosis autoinducers, to the stationary-phase *M. luteus* culture to a final concentration of 0.3 mM. Similarly, the formation of CRCs in the stationary-phase culture of *B. cereus* was induced by adding 1 mM C_6-AHB [3]. In both cases, the ethanol content of the incubation media did not exceed 5 vol %.

Obtaining of nonviable forms. Bacterial and yeast micromummies were obtained by treating *M. luteus* and *S. cerevisiae* cells with C_6-AHB at a concentration of 1 mM [6] and *B. cereus* cells at a concentration of 2 mM. Autolysing yeast cells were obtained through the long-term storage of cultures or thick cell suspensions [5].

Microbiological methods. The viability of vegetative cells, endospores, and CRCs was determined by enumerating colonies grown on solid nutrient media after the plating of appropriate suspension dilutions. The results were expressed in colony-forming units (CFU). The optical density of cultures and cell suspensions was measured using a Specord spectrophotometer (λ = 660 nm; 10-mm-pathlength cuvettes). Microscopic studies were carried out using an Amplival phase-contrast microscope (Germany).

Determination of the elemental composition of cells by X-ray microanalysis. X-ray microanalysis was conducted 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×). Specimens for analysis were prepared as follows: Cells were washed thrice of the incubation medium (centrifugation at 2300–2500 *g*) and resuspended in deionized water. The suspension was mounted on a Formvar-coated copper grid and coated with carbon at an angle of 90°. X-ray spectra were recorded in 3–5 replicates and averaged for 30 cells of each type. The spectra were processed using the ZAP/PB-LINK program. The relative content of each of the elements in cells was defined as the ratio of its peak area (PA) to background (B). The calcium-to-potassium (Ca/K) and phosphorus-to-sulfur (P/S) ratios were calculated as the ratios of the respective peak areas.

RESULTS

To gain insight into the relationship between the metabolic activity of cells and their elemental composition, we investigated various microbial forms, which are ranked, in order of decreasing metabolic activity and viability, as vegetative actively proliferating cells, viable metabolically resting forms (CRCs and endospores), nonviable metabolically inactive forms (micromummies), and dead autolyzed cells.

Table 1. X-ray microanalysis of different *B. cereus* cell types

Cell type, medium, and time of cultivation (storage)	Viability, CFU/ml	Peak area (PA), background (B), and PA/B ratio of elements					Peak area ratio	
			S	P	Ca	K	Ca/K	P/S
Five-hour exponential-phase cells grown on 6% glucose	$(3.8 \pm 0.2) \times 10^8$	PA	816 ± 102	2973 ± 130	426 ± 78	998 ± 143	0.427 ± 0.020	3.643 ± 0.339
		PA/B	0.318 ± 0.034	0.846 ± 0.013	0.297 ± 0.054	0.634 ± 0.091		
		B	2566	3514	1434	1574		
Two-day stationary-phase cells grown on 6% glucose	$(6.1 \pm 0.4) \times 10^9$	PA	673 ± 61	2060 ± 123	228 ± 27	779 ± 85	0.293 ± 0.003	3.061 ± 0.104
		PA/B	0.381 ± 0.034	0.844 ± 0.051	0.247 ± 0.029	0.715 ± 0.077		
		B	1766	2440	923	1090		
Endospores from 7-day culture grown on 0.2% glucose	$(3.6 \pm 0.4) \times 10^8$	PA	825 ± 71	1569 ± 140	4578 ± 204	201 ± 30	22.78 ± 2.78	1.902 ± 0.006
		PA/B	0.443 ± 0.038	0.639 ± 0.056	4.150 ± 0.185	0.182 ± 0.027		
		B	1862	2455	1103	1104		
CRCs produced in medium with 6% glucose and stored for 1 month	$(2.3 \pm 0.3) \times 10^9$ (37)*	PA	264 ± 36	342 ± 11	148 ± 20	109 ± 37	1.358 ± 0.412	1.295 ± 0.157
		PA/B	0.143 ± 0.019	0.178 ± 0.005	0.131 ± 0.017	0.085 ± 0.029		
		B	1846	1921	1129	1282		
CRCs produced in medium with 1 mM C_6-AHB and stored for 7 days	$(5.8 \pm 0.4) \times 10^8$ (10)*	PA	579 ± 56	454 ±16	562 ± 66	315 ± 44	1.784 ± 0.041	0.784 ± 0.053
		PA/B	0.444 ± 0.043	0.324 ± 0.011	1.190 ± 0.118	0.169 ± 0.023		
		B	1304	1401	472	1863		
Micromummies produced after treatment with 2 mM C_6-AHB and stored for 1 month	0	PA	88 ± 41	213 ± 42	177 ± 41	15 ± 32	–	2.420 ± 1.227
		PA/B	0.236 ± 0.112	0.408 ± 0.084	0.850 ± 0.209	–0.063 ± 0.138		
		B	372	522	208	238		

* Parenthesized is the percentage of viable cells with respect to the beginning of storage.

Table 2. X-ray microanalysis of different *M. luteus* cell types

Cell type, medium, and time of cultivation (storage)	Viability, CFU/ml	Peak area (PA), background (B), and PA/B ratio of elements					Peak area ratio	
			S	P	Ca	K	Ca/K	P/S
One-day stationary-phase cells	$(3.5 \pm 0.3) \times 10^9$	PA	1673 ± 151	5839 ± 185	630 ± 115	1321 ± 135	0.477 ± 0.034	3.490 ± 0.177
		PA/B	0.277 ± 0.025	0.842 ± 0.031	0.197 ± 0.036	0.358 ± 0.037		
		B	6040	6935	3198	3690		
CRCs produced in phosphorus-deficient medium and stored for 2 months	$(3.8 \pm 0.2) \times 10^8$ (11)*	PA	2756 ± 172	7859 ± 205	2072 ± 138	1487 ± 138	1.393 ± 0.040	2.852 ± 0.110
		PA/B	0.390 ± 0.025	0.992 ± 0.032	0.531 ± 0.037	0.329 ± 0.031		
		B	7066	7922	3902	4520		
CRCs from 10-fold thickened cell suspension stored for 1 month	$(5.9 \pm 0.8) \times 10^9$ (23)*	PA	1028 ± 144	2392 ± 143	1854 ± 151	979 ± 141	1.894 ± 0.138	2.327 ± 0.246
		PA/B	0.230 ± 0.032	0.494 ± 0.031	0.619 ± 0.052	0.312 ± 0.045		
		B	4470	4842	2995	3138		
CRCs produced in medium with 0.3 mM C_6-AHB and stored for 1 month	$(1.3 \pm 0.1) \times 10^9$ (72)*	PA	797 ± 109	2922 ± 109	1710 ± 111	5047 ± 168	0.339 ± 0.011	3.667 ± 0.365
		PA/B	0.238 ± 0.033	0.780 ± 0.039	0.788 ± 0.056	2.469 ± 0.104		
		B	3349	3746	2170	2044		
Micromummies produced after treatment with 1 mM C_6-AHB and stored for 2 months	0	PA	441 ± 71	1044 ± 80	524 ± 62	1500 ± 93	0.350 ± 0.022	2.367 ± 0.236
		PA/B	0.350 ± 0.058	0.720 ± 0.062	1.064 ± 0.139	2.536 ± 0.202		
		B	1260	1450	492	591		

* Parenthesized is the percentage of viable cells with respect to the beginning of storage.

Table 3. X-ray microanalysis of different *S. cerevisiae* cell types

Cell type, medium, and time of cultivation (storage)	Viability, CFU/ml	Peak area (PA), background (B), and PA/B ratio of elements					Peak area ratio	
			S	P	Ca	K	Ca/K	P/S
Two-day stationary-phase cells	$(1.0 \pm 0.1) \times 10^9$	PA	1560 ± 151	5900 ± 183	3561 ± 159	1577 ± 144	2.258 ± 0.116	3.782 ± 0.275
		PA/B	0.265 ± 0.026	0.910 ± 0.033	0.924 ± 0.046	0.382 ± 0.035		
		B	5887	6484	3853	4128		
CRCs produced in medium with 6% glucose and stored for 18 months	$(9.2 \pm 0.5) \times 10^7$	PA	1012 ± 133	1267 ± 113	2016 ± 120	338 ± 32	5.964 ± 0.191	1.252 ± 0.046
	(9.2)*	PA/B	0.199 ± 0.026	0.234 ± 0.021	0.804 ± 0.052	0.118 ± 0.032		
		B	5058	5415	2507	2864		
Autolyzing cell suspension stored for 1 month	$(2.0 \pm 0.2) \times 10^6$	PA	318 ± 77	595 ± 76	90 ± 29	33 ± 9	2.727 ± 0.28	1.871 ± 0.140
	(0.18)*	PA/B	0.174 ± 0.013	0.311 ± 0.041	0.101 ± 0.067	0.031 ± 0.024		
		B	1828	1913	891	1064		
Micromummies produced after treatment with 1 mM C_6-AHB and stored for 1 month	0	PA	708 ± 104	1350 ± 105	260 ± 78	115 ± 38	2.261 ± 0.181	1.907 ± 0.154
		PA/B	0.219 ± 0.033	0.387 ± 0.031	0.180 ± 0.054	0.061 ± 0.021		
		B	3233	3488	1444	1885		

* Parenthesized is the percentage of viable cells with respect to the beginning of storage.

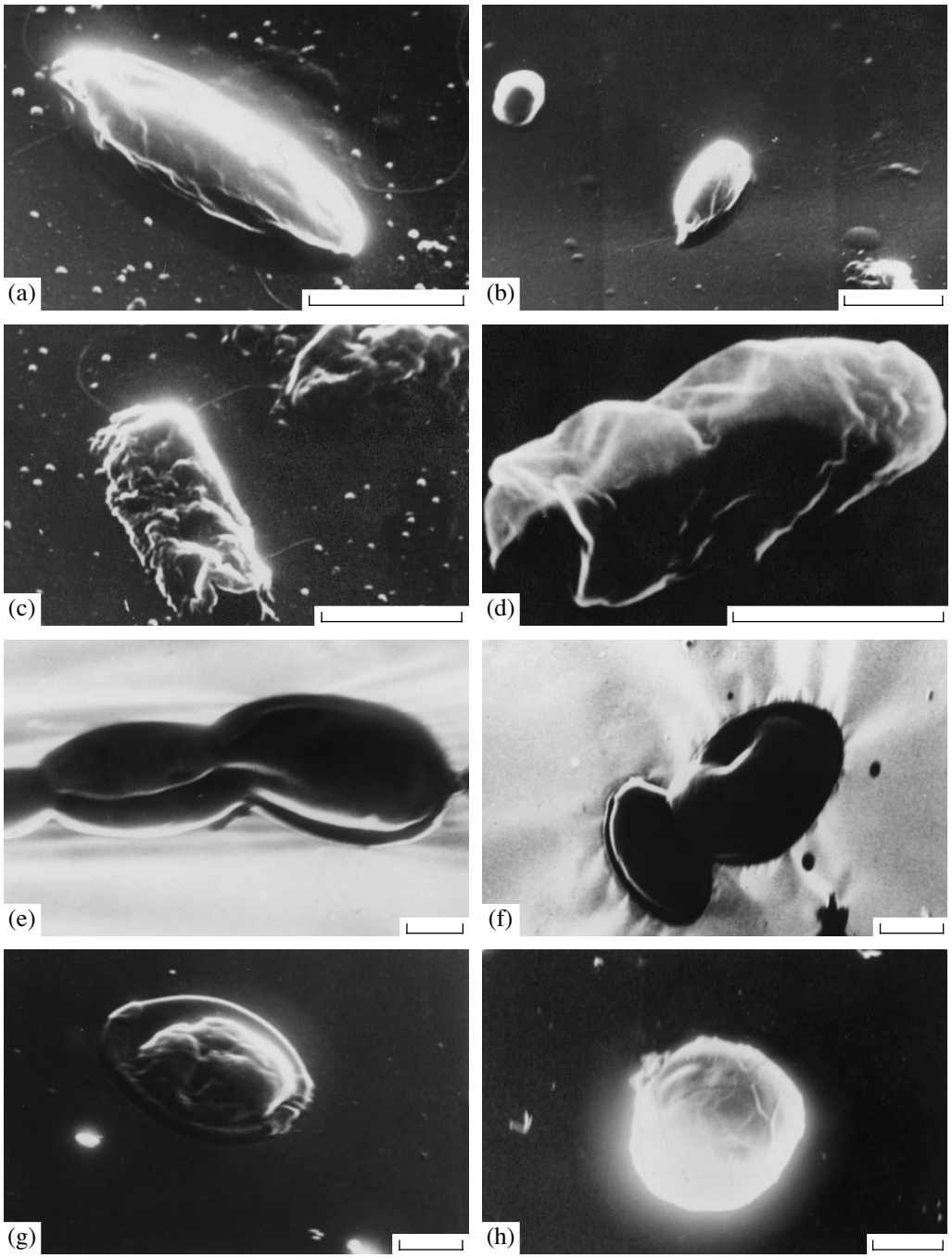

Fig. 1. Micrographs of (a) an exponential-phase vegetative *B. cereus* cell; (b) *B. cereus* spores; (c) *B. cereus* CRCs formed in the presence of C_6-AHB; (d) an *B. cereus* CRC formed in the presence of 6% glucose; (e) an exponential-phase vegetative *S. cerevisiae* cell; (f) a stationary-phase *S. cerevisiae* cell; and (g, h) *S. cerevisiae* CRCs formed in the presence of 6% glucose and stored for 18 months. Bars represent 1 μm.

The CRCs obtained from the sporogenic bacterium *B. cereus* (under conditions inhibitory to sporogenesis), the asporogenic bacterium *M. luteus*, and the yeast *S. cerevisiae* using the methods that we developed earlier [3–6] retained their viability after long-term storage at levels from 10 to 72% of the initial value (Tables 1–3). After the storage of autolyzed bacterial and yeast suspensions for 1–6 months, the percentage of viable CRCs varied from 0.004 to 23% of the initial value. The treatment of *M. luteus* and *S. cerevisiae* cells with high concentrations of C_6-AHB resulted in the formation of morphologically intact and microscopically

refractive cells, which never produced colonies when plated on nutrient agar (the so-called micromummies [6]).

The elemental composition of various microbial forms was studied by X-ray microanalysis. The results are presented in Tables 1–3. Particular attention was paid to calcium (as a secondary cellular effector and the stabilizer of biomacromolecules and membranes), potassium (as an element involved in the formation of transmembrane potential and in the maintenance of osmotic cell pressure), sulfur (as a constituent of proteins), and phosphorus (as a constituent of nucleotide phosphates, nucleic acids, and phospholipids). According to the working hypothesis, the Ca/K and P/S ratios must well reflect the specificities of metabolically active cells, resting forms, and nonviable micromummies.

Bacillus cereus

Scanning electron microscopy showed that the resting forms and vegetative cells of bacilli differ in the structure of the cell surface. Unlike the stationary-phase cells of *B. cereus* (Fig. 1a), the CRCs of this bacterium obtained under the conditions of glucose excess (60 g/l medium) were characterized by an uneven cell surface and the absence of flagella (Fig. 1d). The CRCs of this bacterium obtained by treating it with 1 mM C_6-AHB also had a wrinkled surface, but flagella were present (Fig. 1c). The alterations observed in the surface structure of CRCs may be due to a structural modification of the cell-wall constituents [10] and/or the dehydration of bacterial cells caused by an impairement of the permeability of cell membranes to monovalent ions under the action of anabiosis autoinducers [9]. Bacillar endospores distinctly differed from vegetative cells and CRCs in that they were oval and had a smaller size and smooth surface (Fig. 1b). The micromummies of *B. cereus* were morphologically similar to the CRCs of this bacterium formed under the action of C_6-AHB, but did not have flagella.

X-ray microanalysis showed that vegetative and resting bacillar cells considerably differ in the PA/B values of Ca, K, P, and S and Ca/K and P/S ratios (Table 1 and Fig. 2).

Vegetative cells of bacilli. The exponential-phase cells of *B. cereus* exhibited a larger PA/B value for Ca and smaller PA/B values for K and S than the stationary-phase cells of this bacterium (Table 1). However, the difference between these two types of vegetative cells was less pronounced than the difference between them and the endospores and CRCs (Table 1 and Fig. 2). For this reason, further studies were carried out using the stationary-phase vegetative cells.

Bacillar endospores were characterized by a very high content of Ca and a low content of K (Table 1 and Fig. 3). The level of P in the endospores was lower than in vegetative cells (Table 1 and Fig. 2a). The ratios Ca/K (22.8) and P/S (1.9) of endospores considerably

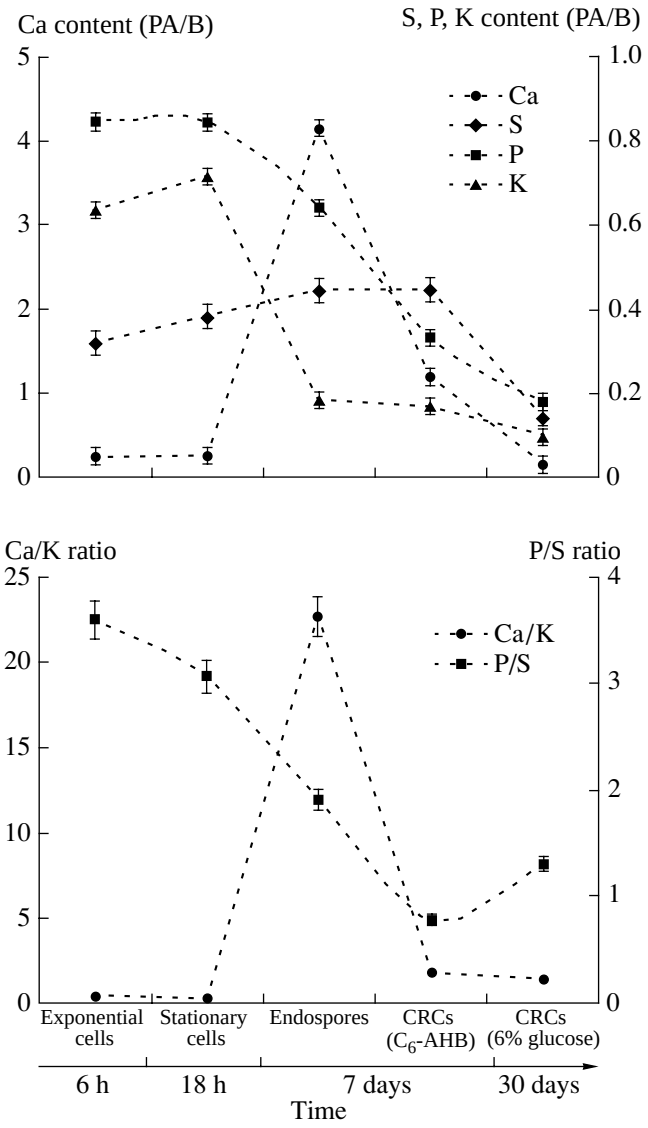

Fig. 2. (a) Levels of sulfur, phosphorus, calcium, potassium, and (b) Ca/K and P/S ratios for different forms of *B. cereus*.

differed from the respective parameters of vegetative cells (Table 1 and Fig. 2b). It should be noted that the Ca/K and P/S ratios (13.2 and 1.2) that we calculated for the endospores of *B. cereus* strain T from the data presented in the publication [12] were roughly close to our estimations of these ratios for *B. cereus* 504. Likewise, our estimations showing a decrease in P and K levels and a rise in the Ca level are in agreement with the data obtained by other methods [11] and indicate the validity of X-ray microanalysis for the comparative study of the elemental composition of vegetative cells and resting forms.

Cystlike refractive cells. The content of Ca in the CRCs induced by treating with C_6-AHB and stored for 7 days was significantly higher and that of K and P was much lower than in vegetative cells (Table 1 and Fig. 2a).

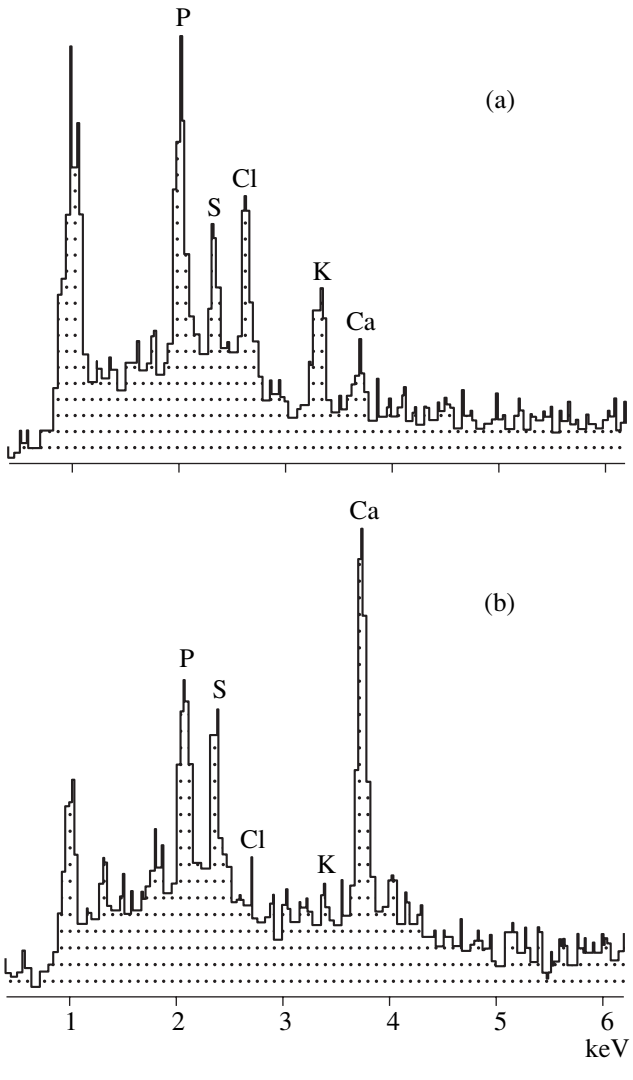

Fig. 3. X-ray spectra of (a) vegetative cells and (b) endospores.

It should be noted that the CRCs obtained in the modified medium were characterized by lesser PA/B values of all elements then CRCs formed under the influence of C_6-AHB (Table 1). Generally, the Ca/K and P/S ratios for CRCs were, respectively, 4–5 times higher and 2.5–4 times lower than for vegetative bacillar cells (Table 1), correlating with the dynamics of these elements in endospores.

Bacillar micromummies obtained by treating vegetative cells with a high concentration (2 mM) of C_6-AHB and stored for 2 months virtually did not contain K. The content of Ca increased and that of P declined as compared to vegetative cells, showing a dynamics similar to that described above for endospores and CRCs. The peak areas and backgrounds (Table 1) of all elements in *B. cereus* micromummies were considerably smaller than in the case of bacillar vegetative cells and viable resting forms.

Micrococcus luteus

Scanning electron and phase-contrast microscopy showed that the CRC of micrococci differ from vegetative cells in size and a higher index of refraction.

Vegetative cells of micrococci. The content of elements and their ratios in the vegetative cells of *M. luteus* (Table 2) were similar to those in the vegetative cells of bacilli (Table 1).

Cystlike refractive cells of micrococci obtained through the alteration of cultivation conditions (P deficiency) and stored for 2 months were characterized by elevated levels of Ca and P and a lowered level of K as compared to the stationary-phase vegetative micrococci (Table 2). The increase in the content of phosphorus can be explained by the accumulation of this element in resting cells in response to its deficiency in the medium. As in the case of bacilli, the Ca/K and P/S ratios of the CRCs of micrococci were, respectively, higher and lower than those of vegetative cells. The CRCs of micrococci present in their autolyzing thick suspensions after 1 month of storage showed a higher content of Ca and lower contents of K and P (Table 2). These data, however, cannot be considered completely correct because of the presence in such suspensions not only of CRCs but also of autolyzed cells.

The CRCs of micrococci obtained by treating them with 0.3 mM C_6-AHB retained their viability at a level of 72% during storage for 1 month. In this case, the content of P somewhat decreased, whereas the content of Ca and especially K drastically increased (Table 2). The high content of K can be explained by the impairement of the permeability of cellular membranes due to the polycrystallization of their lipid stroma under the action of C_6-AHB [9]. Since the incubation medium contained a great amount of K^+ ions (0.1 M), it can be suggested that the diffusional flow of potassium across the cell membrane was directed into the cell along its concentration gradient. In view of this, it is not surprising that, despite the high intracellular content of Ca, the Ca/K ratio of the CRCs of micrococci turned out to be low, which is not typical of endospores and the CRCs of other microorganisms.

Micromummies of *M. luteus*. Taking into account our earlier data that the membranes of bacterial and yeast micromummies lose their barrier function [6], we suggested that, during cell mummification, Ca and K ions must diffuse along their concentration gradients. If so, mummification in media with a high concentration of either of these elements must lead to a high content of this element in micromummies. Experiments showed that, indeed, the *M. luteus* micromummies that were produced in a medium with 0.1 M K^+ under the action of 1 mM C_6-AHB [6] contained larger amounts of Ca and especially K than the vegetative cells of micrococci (Table 2). As in the case of bacillar micromummies, the backgrounds of these elements (the B values) in the *M. luteus* micromummies were low. This phenomenon can be considered a distinguishing feature

of micromummies. At the same time, the viable CRCs and micromummies of micrococci showed no significant differences in the contents (PA/B values) of P, S, Ca, and K.

Saccharomyces cerevisiae

Stationary-phase vegetative yeast cells contained a greater amount of Ca (Table 3) than vegetative bacterial cells, whereas the intracellular contents of P, K, and S of bacterial and yeast cells were close. The high content of Ca in yeast cells may be due to the fact that these cells, unlike bacterial cells, possess a depot of intracellular Ca associated with the endoplasmic reticulum.

Cystlike refractive yeast cells that were formed in the suspensions of *S. cerevisiae* cells grown in media with 60 g/l glucose and stored for 18 months (Fig. 1g) differed from vegetative yeast cells (Figs. 1e and 1f) in that they had a specific surface structure and a more condensed cytoplasm. The population of cells in senescent yeast cultures was heterogeneous and contained oval cells of a high electron density (Fig. 1h). Yeast CRCs differed from the stationary-phase vegetative cells in that they contained lower amounts of K, P, and Ca (Table 3). Yeast CRCs and stationary-phase cells also differed in the Ca/K and P/S ratios.

Yeast micromummies produced in the presence of 1 mM C_6-AHB were nonviable but microscopically refractive and morphologically intact. The peak areas and PA/B ratios of all elements, especially Ca and K, were very low (Table 3). According to the experimental data presented in our previous paper [6], the decrease in the phosphorus content of yeast micromummies is due to the degradation of membrane phospholipids. As in the micromummies of bacilli and micrococci, the background of all elements in yeast micromummies was low.

Autolyzing nonviable yeast cells that were formed in thick cell suspensions exhibited considerably lower levels of Ca and especially K than stationary-phase and cystlike cells, although the decrease in the contents of P and S was not so drastic (Table 3). These data are in agreement with those obtained by the X-ray microanalysis of another yeast, *Candida utilis* [13].

It should be noted that we failed to reveal any difference in the levels of other elements, such as silicon, iron, and chlorine, in different types of microbial cells. The content of silicon (as judged from the PA/B value) was low (from 0 to 0.056). In several cases, we revealed a low content of chlorine, whose role in bacterial and yeast cells remains poorly studied.

DISCUSSION

The investigation of the elemental composition of the vegetative cells, resting forms (CRCs and bacillar endospores), and nonviable mummified cells of bacteria and yeast showed that viable resting forms differ from vegetative cells in that they have low P/S and high Ca/K ratios. In resting forms, the contents of P and K were typically low and the content of Ca was high. The parameters that characterize the absolute and relative amounts of Ca and K in endospores and cystlike cells probably reflect changes in their homeostasis due to transition to the dormant state. The low levels of the P and P/S ratio common to resting forms are probably due to a decline in the cellular pool of ATP and nucleic acids.

The nonviable micromummies of bacteria and yeast differed from their vegetative cells and viable resting forms in that the content of K was very low in the *B. cereus* and *S. cerevisiae* micromummies and very high in the *M. luteus* micromummies. This phenomenon can be explained by the fact that the cellular membranes of mummified bacteria and yeasts lose their barrier function, as follows from the total or partial degradation of membranes easily seen in thin sections and freeze-fracture replicas [6]. Autolyzing yeast cells are characterized by low contents of Ca and K. As for nonviable mummified bacterial and yeast cells, they are distinguished by the low values of the background of all elements in their X-ray spectra.

In general, the data obtained in this study confirm the hypothesis that microbial forms differing in physiological state and viability can be distinguished through the levels of Ca, K, P, S and Ca/K and P/S ratios. The relevant data for *B. cereus, M. luteus,* and *S. cerevisiae* are in agreement with those obtained for other organisms. For instance, the phosphorus content of the stationary-phase *Escherichia coli* cells was found to be lower than that of the actively growing exponential-phase cells of this bacterium [14]. A part of phytoplankton cells taken from deep lake waters exhibited lower levels of K than those taken from the surface water layer, which was related to a senescence of phytoplankton in the deep waters [15]. Senescent human erythrocytes were found to contain less K and more Ca (therefore, the Ca/K ratio was high) than young human erythrocytes, whereas the P levels were comparable [16].

The high levels of the Ca and Ca/K ratio typical of endospores and resting forms can be accounted for by the stabilizing effect of calcium on membranes and proteins and its involvement in other processes related to cell response to extreme factors. This also explains why the relatively radioresistant bacteria *Methylobacterium organophilum*, *"Arcocella aquatica"*, and *Flectobacillus major* showed higher levels of Ca and Ca/K ratio than the relatively radiosensitive bacterium *E. coli* [17, 18]. The fact that the vegetative cells of the facultatively oligotrophic bacterium *Hyphomicrobium vulgare* and the oligotrophic bacteria *Caulobacter bacteroides* and *F. major* exhibit a low value of the P/S ratio [18], which seems to contradict our hypothesis, may actually imply that oligotrophs have reduced metabolic activity (this is obvious from the typically low growth rates of oligotrophs).

In this work, the taxonomic range of the subjects studied was confined to three representatives of pro-

and eukaryotic microorganisms. In our opinion, in further studies this range should be extended.

Further information on the use of the elemental composition parameters for the analysis of the physiological state of microorganisms in natural samples without their cultivation can be found in our next paper.

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