Microbiology, Vol. 73, No. 2, 2004, pp. 159–162. Translated from Mikrobiologiya, Vol. 73, No. 2, 2004, pp. 199–203.

EXPERIMENTAL ARTICLES

Cobalt- and Chromium-Containing Inclusions in Bacterial Cells

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Received July 21, 2003; in final form, September 1, 2003

Abstract—Bacteria belonging to different taxonomic and physiological groups (members of the genera *Pseudomonas, Brevibacterium, Rhodopseudomonas*, and *Lactococcus*) are able to form intracellular cobalt- and chromium-containing magnetic inclusions. The paper deals with the structure and the intracellular localization of these inclusions and their similarity to the known noncrystalline iron-containing magnetic inclusions. The possible biological role of the magnetic inclusions is discussed.

Key words: bacteria, magnetic inclusions, cobalt, chromium.

It is well known that bacteria are able to take up metal cations from the environment and to hold them on the cell wall and in protein S layers, capsules, and intracellular polyphosphate granules. These processes are widespread and relatively nonspecific [1–3]. The formation of intracellular structured metal depositions (such as magnetosomes in magnetotactic bacteria [4], noncrystalline magnetic inclusions [5], and iron oxide granules in the dissimilatory iron-reducing bacterium *Shewanella putrefaciens* [6]) is a more rare phenomenon. The first two types of metal inclusions make bacterial cells capable of motion in a magnetic field and are responsible for magnetotaxis and passive attraction to a magnet, respectively [7]. Earlier, we reported on the ability of bacterial cells to accumulate a new type of noniron ferromagnetic inclusions [8].

This paper describes the structure and the localization of cobalt- and chromium-containing magnetic inclusions in certain bacterial cells.

MATERIALS AND METHODS

Microorganisms. Experiments were carried out with *Pseudomonas fluorescens* VKM B-2170, *Lactococcus lactis* subsp. *lactis* VKM B-978, *Rhodopseudomonas palustris* VKM B-1620, *Rhodospirillum rubrum* VKM B-1621, *Brevibacterium linens* VKM Ac-2112 and Ac-2119, and *Brevibacterium* sp. VKM Ac-2118, which were obtained from the All-Russia Collection of Microorganisms.

Media and cultivation conditions. *P. fluorescens* VKM B-2170 was grown on nutrient agar (medium 5 in the VKM catalog [9]) supplemented with 1 g/l $NaNO_3$; *L. lactis* subsp. *lactis* VKM B-978 was grown in tryptose–soybean broth (Difco, no. 0370-01-1); *Brevibacterium* strains were grown in glucose–peptone–yeast extract medium; and phototrophic bacteria were grown in a medium containing (g/l) yeast extract, 0.5; sodium succinate, 1.0; sodium acetate, 2.0; NH_4Cl, 0.5; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.4; and sea salt, 0.5. The final pH of these media was 6.5–7.2. The microorganisms were grown in Hungate tubes under microaerobic conditions at optimal growth temperatures for 7–14 days.

Conditions promoting the formation of Co and Cr inclusions. The ability of the bacteria to form cobalt- and chromium-containing magnetic inclusions was studied by supplementing the respective growth medium with one of the following saline solutions: (1) 40 mg of $K_2Cr_2O_7$ and 50 mg of EDTA in 10 ml of distilled water; (2) 40 mg of $K_2Cr_2O_7$ in 10 ml of distilled water; (3) 60 mg of $CoCl_2 \cdot 6H_2O$ and 50 mg of EDTA in 10 ml of distilled water; and (4) 60 mg of $CoCl_2 \cdot 6H_2O$ in 10 ml of distilled water. The final concentrations of metal ions in the media were 0.25, 0.5, 0.7, 1, 2, 4, and 9 mM. The control variants of the growth media were not supplemented with the heavy metals. All the media were sterilized at 110°C for 30 min. It should be noted that the control experiments on the formation of iron-containing magnetic inclusions, described earlier [5, 10], were performed by using the Na,Fe(III)–EDTA chelate complex.

The observation of bacterial motion to a magnet. Bacterial cells were collected by centrifugation and washed twice with physiological saline solution (0.8% NaCl), which was acidified to pH 5.0 with HCl to remove metal ions adsorbed on the cell surface. The washed cells were resuspended in physiological saline solution with pH 6.5–7.0. After spontaneous cell precipitation, the test tube was brought in contact with a magnet. In the case of positive magnetotaxis, the precipitated bacterial cells moved as a whole toward the

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magnet. If the magnet was shifted to the positive wall of the test tube, all the cells moved toward the new location of the magnet.

Electron microscopy. To prepare a whole specimen, a drop of a suspension of bacterial cells (either washed or not) was placed onto a Formvar-coated 300-mesh copper grid for 30–40 s. The stage of contrasting was omitted. Thin sections were prepared as described by Vainshtein *et al.* [10]. The specimens were examined with a JEM-100B electron microscope (JEOL, Japan), which was operated at 80 kV.

X-ray microanalysis. The chemical composition of magnetic inclusions was studied using a JEM-100CXII electron microscope (JEOL) 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 60 keV, which provided a magnification of ×20000. Specimens for analysis were either thin sections of bacterial cells with magnetic inclusions or the magnetic particles themselves. In the latter case, a suspension of magnetic particles isolated from bacterial cells was placed onto a Formvar-carbon-coated grid and subjected to the vacuum deposition of carbon at an angle of 90°. The spectra obtained were processed with the aid of the LAF/PB computer program.

RESULTS

Bacterial cells grown in the media that contained either free Cr(VI) or Co(II) ions or their chelate complexes were found to contain specific cytoplasmic inclusions, which made the cells capable of passive motion in a magnetic field. The inclusions were spheric or globular in shape and had a diameter ranging from 20 to 250 nm. Electron microscopy showed that the structure of these inclusions was similar in all the bacteria under study and resembled the structure of the iron-containing magnetic inclusions of purple phototrophic bacteria [10, 11]. The inclusions had electron-transparent central parts and an electron-opaque homogeneous matrix enriched in the respective metal. Such a specific structure of the inclusions made their identification possible without additional assays.

We were unable to reveal a statistically significant dependence of the number, size, and localization of magnetic inclusions in bacterial cells on the type of metal ions and their concentration in the incubation medium, although we observed a direct correlation between the concentrations of cobalt and chromium in the medium and the relative number of respective magnetic inclusions in the cells.

Unlike iron magnetic inclusions, which could be formed only in the media containing Fe(III)–EDTA chelate complexes, cobalt and chromium magnetic inclusions were found to be formed in media containing either free Co or Cr ions or their chelate complexes. The bacteria under study were able to grow and form magnetic inclusions in the presence of Co, Co–EDTA, Cr, and Cr–EDTA at concentrations of up to 2, 9, 1, and 0.5 mM, respectively. As can be seen from these data, Cr ions were most toxic in the chelate form, whereas Co ions were most toxic in the free form. The low toxicity of the Co–EDTA chelate complex was likely to be due to its high stability.

The distribution pattern of Co- and Cr-containing magnetic inclusions in certain bacterial cells was found to be different from that of iron-containing inclusions. For instance, the *P. fluorescens* VKM B-2170 cells cultivated in the medium with the iron–EDTA chelate complex produced numerous small (below 25 nm in diameter) inclusions, which occurred in the central part of the cells (Fig. 1). The same cells incubated in the media with cobalt and chromium ions formed no more than 10–15 relatively large (80 to 170 nm in diameter) magnetic inclusions, which were distributed nonuniformly throughout the cytoplasm [11]. At the same time, the size and the distribution pattern of cobalt- and chromium-containing magnetic inclusions in the cells of *Brevibacterium* sp. and *L. lactis* VKM B-978 did not differ from the size and the distribution pattern of iron-containing inclusions (numerous inclusions from 30 to 50 nm in diameter were localized in the cytoplasm and near the cytoplasmic membrane). In phototrophic bacteria, large cobalt and chromium inclusions were predominantly localized near the cell poles (Fig. 2).

Like iron-containing magnetic inclusions [5, 8, 10], cobalt and chromium inclusions did not contain phosphorus and sulfur, as is evident from the X-ray analysis of the thin sections of bacterial cells (Figs. 3a, 3b). The X-ray analysis also showed that cobalt and chromium concentrated only in the peripheral electron-opaque regions of the magnetic inclusions and were absent in the central parts of the inclusions and in the cytoplasm.

DISCUSSION

It is well known that certain bacteria are able to form magnetic inclusions containing iron, which is a ubiquitous chemical element in natural bacterial habitats. The specific biological role of these inclusions may lie in providing for the motion of anaerobic bacteria to the nearest natural magnets, such as magnetic greigite depositions in bodies of water [5]. Due to magnetotactic motion, bacterial cells can come to zones with favorable redox conditions and can utilize reduced compounds occurring in these zones.

It is known that bacteria of the genus *Pseudomonas* that bear cobalt and nickel resistance plasmids can accumulate cobalt in the form of electron-opaque granules on the cell surface [12]. It is also known that the bacterium *Staphylococcus cohnii* can accumulate chromium, although the cellular localization of this metal remains unknown [13]. Earlier, we showed that certain bacteria are able to form intracellular inclusions that contain cobalt and chromium [8, 14].

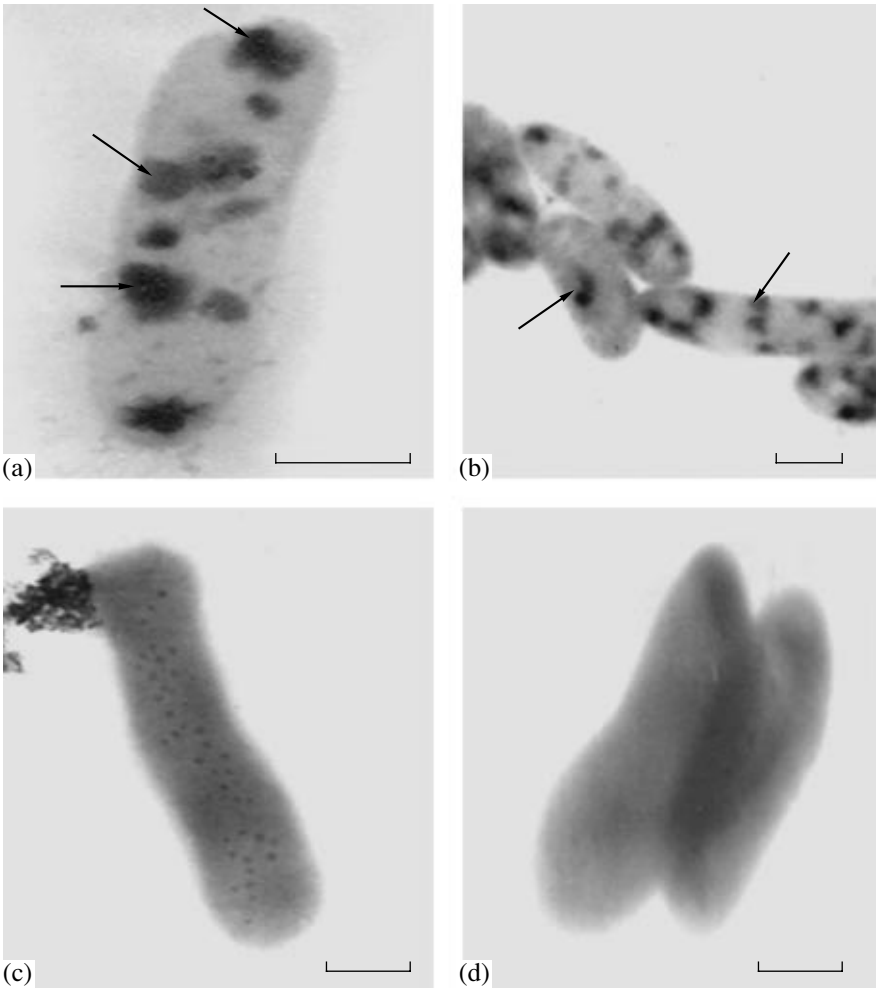

Fig. 1. The electron microscopy of whole specimens of *P. fluorescens* VKM B-2170 cells with magnetic inclusions that contain (a) cobalt, (b) chromium, and (c) iron. (d) Control cells cultivated in the medium without the metals. The cells were taken from the zone located close to the test tube wall that was in contact with a magnet. The arrows point to magnetic particles. The scale bars represent 0.5 nm.

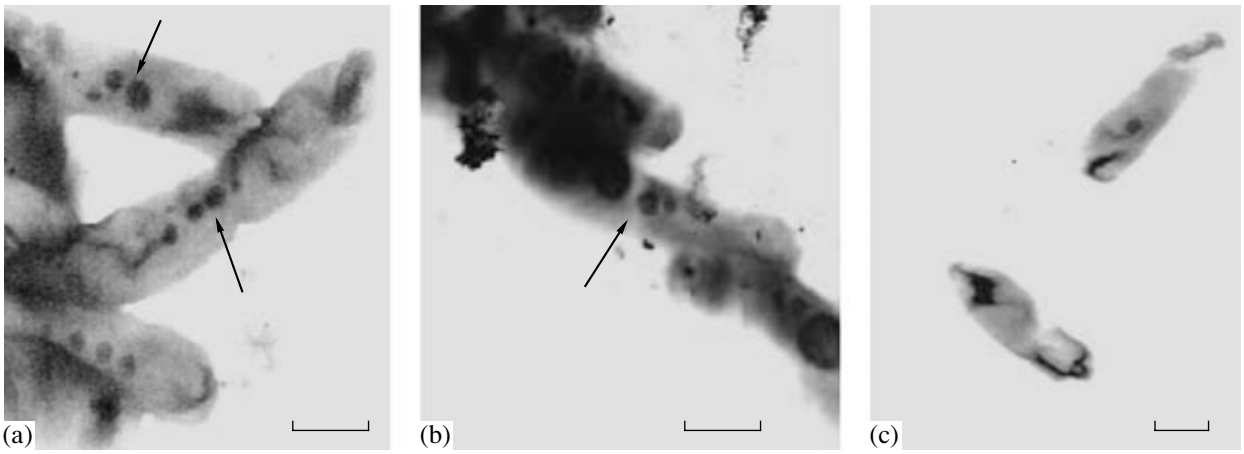

Fig. 2. The electron microscopy of whole specimens of *R. palustris* VKM B-1620 cells with magnetic inclusions that contain (a) cobalt and (b) chromium. (c) Control cells cultivated in the medium without the metals. The cells were taken from the zone located close to the test tube wall that was in contact with a magnet. The arrows point to magnetic particles. The scale bars represent 0.5 nm.

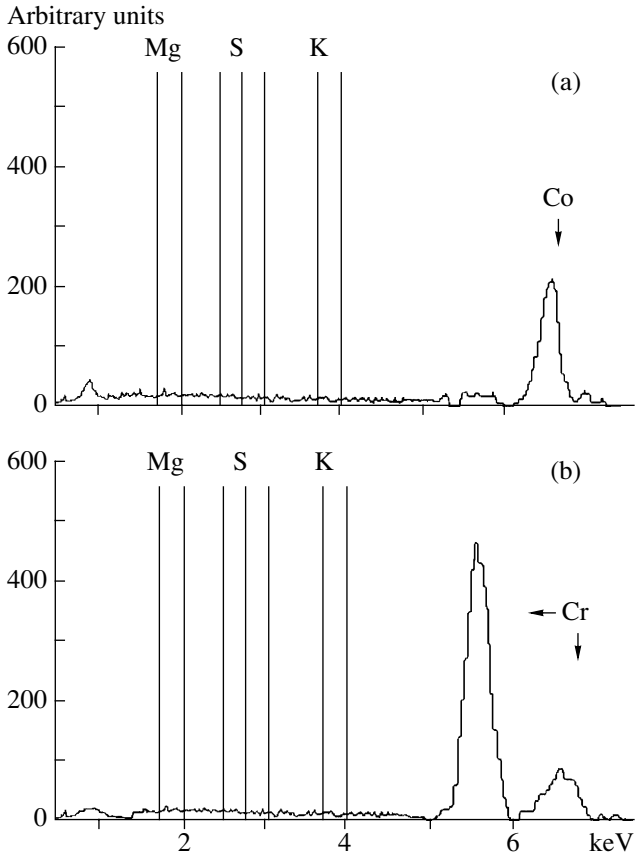

Fig. 3. The X-ray spectra of magnetic inclusions in intact *R. palustris* VKM B-1620 cells that were cultivated in the medium with (a) cobalt and (b) chromium. The vertical lines show the spectral position of certain chemical elements.

In natural bacterial habitats, cobalt and chromium are not so widespread as iron. Correspondingly, the magnetic properties of intracellular Co- and Cr-containing inclusions can be considered as a side effect. On the other hand, the formation of magnetic inclusions that contain ferrimagnetic metals may contribute to bacterial defense against the toxic action of high concentrations of dissolved heavy metals. Magnetic inclusions may contain metal ions occurring in different redox states, such as the Fe^{2+} and Fe^{3+} ions of magnetite [15]. This implies that the metal ions of intracellular inclusions can protect bacterial cells from the toxic action of oxygen by serving as a specific redox buffer.

ACKNOWLEDGMENTS

We are grateful to M.B. Vainshtein of the Institute of Biochemistry and Physiology of Microorganisms for his help with the experiments and discussion of the paper and to V.V. Sorokin from the Institute of Microbiology for his assistance in analyzing the chemical composition of intracellular inclusions.

This work was supported by grant nos. 02-04-49202 and 02-05-65406 from the Russian Foundation for Basic Research.

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