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Synthesis of magneto-sensitive iron-containing nanoparticles by yeasts

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Abstract Industrial production of magneto-sensitive nanoparticles, which can be used in the production of target drug delivery carriers, is a subject of interest for biotechnology and microbiology. Synthesis of these nanoparticles by microorganisms has been described only for bacterial species. At the same time, it is well known that yeasts can form various metal-containing nanoparticles used, for instance, in semiconductors, etc. This paper describes the first results of the biosynthesis of magneto-sensitive nanoparticles by yeasts. The organisms we used—*Saccharomyces cerevisiae* and *Cryptococcus humicola*—represented two different genera. Magneto-sensitive nanoparticles were synthesized at room temperature in bench-scale experiments. The study included transmission electron microscopy of the yeast cells and their energy dispersive spectrum analyses and revealed the presence of iron-containing nanoparticles. Both yeast cultures synthesized nanoparticles at high concentrations of dissolved iron. Electron microscopy showed that nanoparticles were associated mainly with the yeast cell wall. Formation of magneto-sensitive nanoparticles was studied under conditions of applied magnetic fields; a possible stimulating role of magnetic field is suggested. On the whole, the paper reports a novel approach to green biosynthesis of magneto-sensitive nanoparticles.

Keywords Biosynthesis · Biotechnology · Yeasts · Magneto-sensitive nanoparticles

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Introduction

In recent years, various inorganic nanoparticles became subjects of significant interest in view of their possible applications in the areas of chemical, electronic, and biomedical sciences. Medical applications are related mainly to the use of iron-containing magneto-sensitive nanoparticles as target drug delivery systems [23, 29]. According to this approach, drugs are placed on magnetic nanoparticles, injected into a patient's body, and transported to the target organ by means of an applied magnetic field.

Inorganic nanoparticles can be produced both by methods of chemical synthesis and using various biological agents [25, 27, 30]. Microbial synthesis of iron-containing nanoparticles is more advantageous because biogenic particles are more biocompatible than inorganic particles synthesized by chemical methods. Furthermore, biosynthetic processes can be carried out under conditions of ambient temperature and pressure and are thus simpler than some chemical syntheses [13]. Microorganisms as biological agents secrete large amounts of enzymes, which reduce metals and can be responsible for the synthesis of nanoparticles [18, 19, 28].

The most investigated biogenic magnetic nanoparticles are magnetosomes of bacterial origin. They are produced

by a specific group of magnetotactic bacteria; these magnetosomes are formed as intracellular crystals of magnetite or greigite surrounded with a three-layer membrane [20, 34]. Culturing of magnetotactic bacteria on the industrial scale is complicated; nevertheless, it has been shown that some non-magnetotactic bacteria can produce another type of intracellular magneto-sensitive nanoparticles, namely, globular nanoparticles surrounded with a one-layer membrane [31, 32].

Meanwhile, it is well known that some yeasts can form nanoparticles based on metals, for example, Au [1], TiO_2 [14], or Sb_2O_3 [15]. Reductive-enzyme activities in them, coupled with some air oxidative processes, can result in formation of magnetic Fe(II, III) compounds. Thus, it is logical to investigate the possibility of the biosynthesis of iron-containing magneto-sensitive nanoparticles by various yeasts.

In our experiments, yeasts were cultured in media with high concentrations of dissolved iron; the effect of applied magnetic fields on the production of nanoparticles was also investigated. This article presents our experimental results as a possible basis for a new biotechnological approach in producing magneto-sensitive nanoparticles. Our data on the production of magneto-sensitive nanoparticles expand our knowledge about yeasts and show new vista for the development of industrial production of nanoparticles.

Materials and methods

Microorganisms, nutrient media, and culturing

Two yeasts, *Saccharomyces cerevisiae* strain SUF and *Cryptococcus humicola* strain 9-6, representing different species and genera, were used.

S. cerevisiae SUF ("SUF-Moment", Lesaffre, Russia) was incubated in the following medium (g per 200 ml): $Fe(II)SO_4 \times 7H_2O$, 0.05; inoculum was presented with baker's dry yeast enriched with sorbitan monostearate, 11.0. There were no other sources of carbon, phosphorus, and nitrogen, except the large amount of inoculum used to omit long culturing. Two versions of incubation were used: one under conditions of the natural geomagnetic field ("blank") and the other under conditions of an additional magnetic field (static magnetic field, SMF; for description, see the next section).

C. humicola 6-9 [11] was incubated in the medium "Yeast Nitrogen Base Medium" (Difco), 1.7 g/l, and glucose, 20.0 g/l, as described [5]. Before inoculation, the medium was supplemented with a sterile EDTA Fe(III)Na chelate solution to a final concentration of 1.0 g/l. In contrast to the experiment with *S. cerevisiae*, the initial concentration of *C. humicola* cells after inoculation was ~10^4 cells/ml only. As well, two versions of incubation were used: one under conditions of the natural geomagnetic field ("blank") and the other under conditions of an alternating magnetic field (AMF; for description, see the next Section), inside the Helmholtz coils.

Both cultures, *S. cerevisiae* and *C. humicola*, were incubated at laboratory temperature (22–25 °C). Both nutrient media were enriched with the same chemical element, iron, to stimulate the possible formation of iron-containing inorganic nanoparticles. Every 3 days the flasks were thoroughly mixed to create a suspension of the growing cells.

Magnetic fields used

Two types of artificial magnetic fields were used to see if they affect the formation of magneto-sensitive nanoparticles.

A static magnetic field (SMF) was formed by a steel magnet (2) placed on the outer wall of a glass vessel.

The effects of a weak alternating magnetic field (AMF) on some biological systems have already been shown earlier [4, 33]. We used a combined magnetic field containing co-linear oriented static, $\boldsymbol{B}_{DC}$, and alternating, $\boldsymbol{B}_{AC}$, components. The static component is given by the Earth's magnetic field. The alternating (sinusoidal) component was generated by Helmholtz coils (39 cm in diameter) and oriented co-linearly with the vector of the earth's static magnetic field. The Helmholtz coils were fed by a G3-123 signal generator (Astena Ltd, Russia). The control samples were exposed to the earth's magnetic field. The parameters: $\boldsymbol{B}_{DC} = 53.4$ μT, $\boldsymbol{B}_{AC} = 5$, 4 μT, $f = 921.3$ Hz. The values of the Earth's magnetic fields were measured by an SGS-64 fluxgate magnetometer (Geologorazvedka, Russia) ensuring an accuracy of ±0.01 μT. The necessary values of the alternating

Fig. 1 Precipitation of a black sediment under the applied magnet

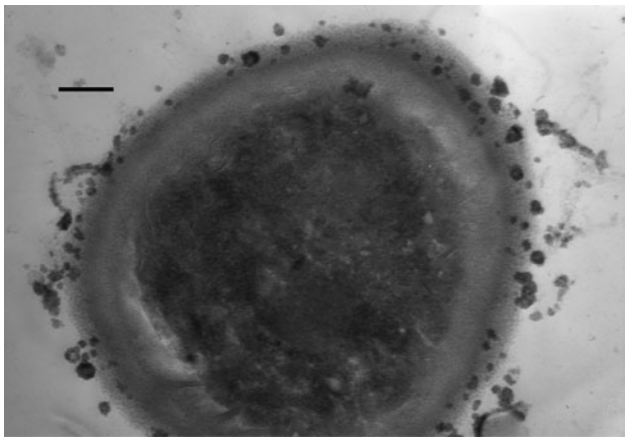

Fig. 2 Thin-section: electron-dense nanoparticles on the envelope of an *S. cerevisiae* cell after its growth in an applied static magnetic field. The cell envelope is surrounded with slime and nanoparticles. Electron microscopy; *scale bar* 100 nm

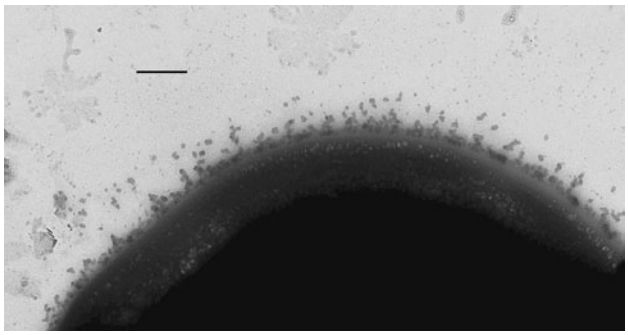

Fig. 3 Thin-section: nanoparticles on the envelope of an *S. cerevisiae* cell after its growth in the natural geomagnetic field ("blank"). The cell envelope is surrounded with slime and nanoparticles. Electron microscopy; *scale bar* 300 nm

magnetic field were established taking into account the transmission coefficient for a Helmholtz coil. The frequency was provided and monitored with a Ch3-36 counter (Russia) to an accuracy of up to ±0.01.

Magnetic sensitivity analysis

Magnetic sensitivity of the formed nanoparticles was checked by their direct attraction to the 2-T steel static magnet. There was no cell motion in the cell suspension as described for bacteria [31], most likely because the concentration of magnetic nanoparticles bound by a single yeast cell is too low for their magnetic forces to move the cell. Still, we did observe attraction of free nanoparticles in the media: (1) in the case of *S. cerevisiae*, it was the accumulation of nanoparticles near the magnet: they joined into clusters, which was followed by a precipitation visible as a black-colored sediment under the magnet (Fig. 1), and (2) in the case of *C. humicola*, it was an oriented movement of the foam to the magnet immediately after the magnet was applied to the glass wall of the flask pre-incubated inside the Helmholtz coils.

Transmission electron microscopy

To prepare ultrathin sections, the cells were centrifuged, washed, and fixed with 1.5 % glutaraldehyde in cacodylate buffer (0.05 M, pH 7.2; 4 °C; 1 h) and with 1 % OsO_4 (4 °C; 12 h) in the same buffer. The specimens were dehydrated in an ethanol series and embedded in Epon-812 epoxy resin. Thin-sections were cut on an LKB Ultratome III and stained with 2 % uranyl acetate. The prepared specimens were placed on a 300-mesh copper grid, and the supporting Formvar film was coated with evaporated carbon. No additional

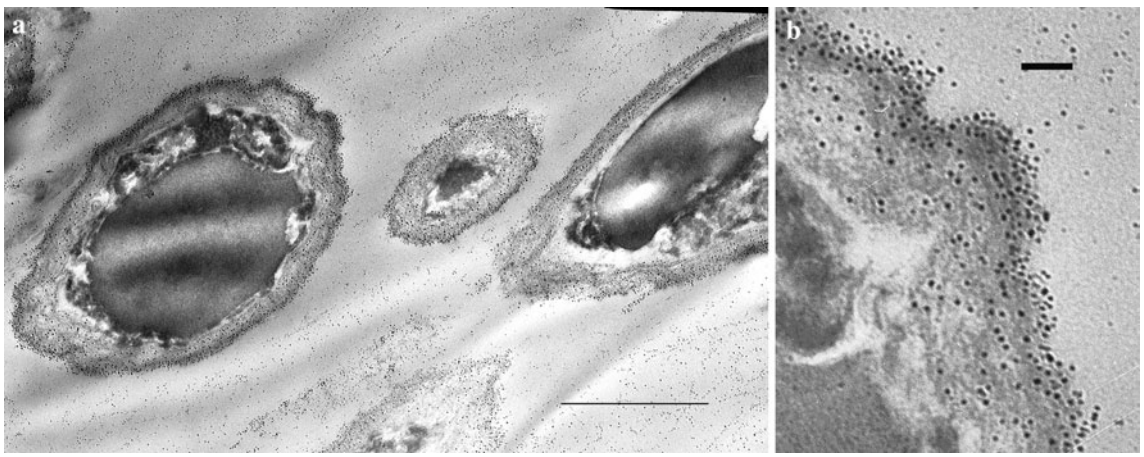

Fig. 4 Thin-section: electron-dense nanoparticles on the envelope of a *C. humicola* cell and in the surrounding medium after the growth in an alternating magnetic field. The cell envelope is surrounded with slime and nanoparticles. Electron microscopy; **a** *scale bar* 1 μm, **b** a scaled up fragment of the same image; the *scale bar* 100 nm

contrast staining was used. All preparations were examined by a JEM-100B transmission electron microscope.

X-ray analyses

The X-ray microanalyses were made to estimate the energy-dispersion spectra of the elemental composition. They were carried out in a JEM-100C XII electron microscope with the EM-ASID 4D scanning unit equipped with a Link 860 X-ray analyzer with the E54233 detector. The areas of the scanned fields and the time intervals of the scanning procedure were the same for all analyses.

Results

Yeasts form nanoparticles in media enriched with dissolved iron compounds with/without magnetic fields applied

Our preliminary experiments with the bacterial strain *P. fluorescens* [2] found that a magnetic field stimulated the bacterial production of intracellular iron-containing nanoparticles as compared with the absence of any magnetic field. Based on this experience, we studied the formation of nanoparticles by the yeasts both under conditions of applied additional magnetic fields and, as a blank, in the natural geomagnetic field.

Two yeast cultures were exposed in the media enriched with the dissolved iron compounds: *S. cerevisiae* was placed in the medium with Fe(II) and *C. humicola* was grown in the medium with Fe(III). The presented photographs of *S. cerevisiae* (Figs. 2, 3, with and without the applied magnetic field, respectively) and *C. humicola* (Figs. 4, 5, with and without the applied magnetic field, respectively) demonstrate that both yeast cultures could form electron-dense nanoparticles. The yeast species studied belong to different genera, the used media drastically differ by the supplemented iron compounds, the experiments also differ by the kinds of the applied magnetic fields (static magnetic field, SMF, and alternating magnetic field, AMF) but in both cases nanoparticles were formed mainly in the cell envelope.

The experiments with *S. cerevisiae* used a high concentration of Fe(II). In this case, a mixture of small spherical and larger nanoparticles emerged in the applied SMF (Fig. 2). On the whole, the described experiments resulted in the formation of relatively large particles of irregular morphology and some particle aggregation.

In contrast, the experiments with *C. humicola*, which used a high concentration of chelated Fe(III), resulted not only in the formation of regular nanoparticles in the applied AMF but also showed their translocation through the cell envelope and their presence outside the cells, too.

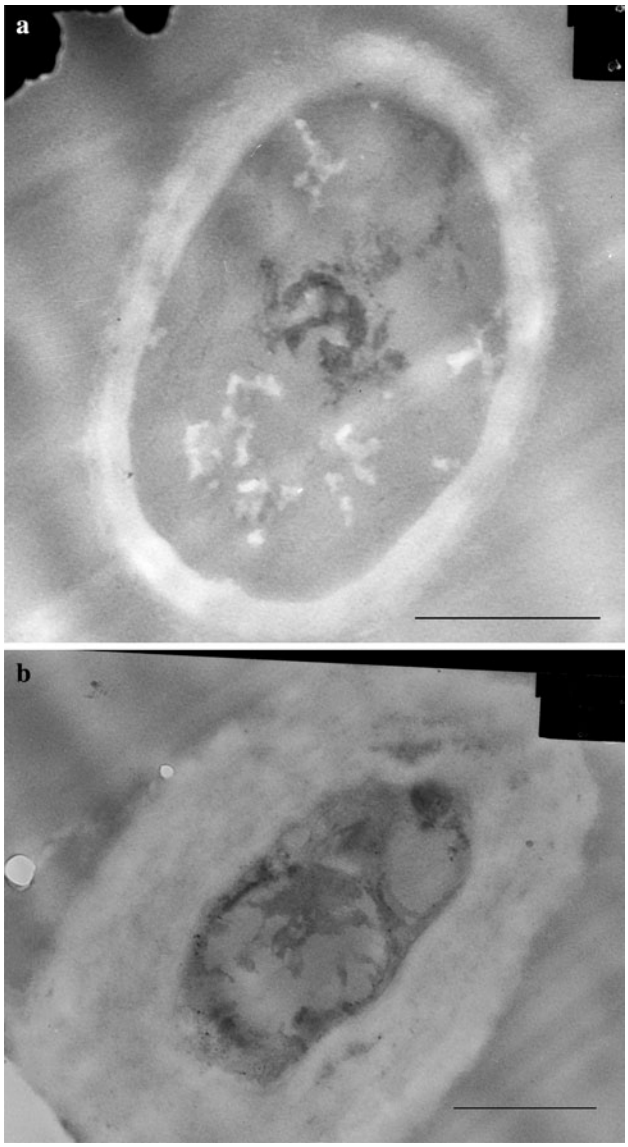

Fig. 5 Thin-section of a *C. humicola* cell after its growth in the natural geomagnetic field ("blank"). Electron microscopy; *scale bar* 1 μm; **a** no nanoparticles; **b** just a few electron-dense nanoparticles

All particles were similar in size and shape (on average, 8–9 nm only) (Fig. 4b). Association of the produced nanoparticles with the cell wall may suggest that some cell wall enzymes (reductases?) are very active in iron reduction linked with the formation of nanoparticles.

Unclear role of magnetic fields in the formation of inorganic nanoparticles

The possible effects of magnetic fields on yeasts are of interest for researchers and biotechnologists. Examples are some data presented in Table 1 as the published results of experiments with *S. cerevisiae* since 2001.

Table 1 Examples of the effects of magnetic fields on *S. cerevisiae* according to some published data [3, 7, 8, 12, 22, 24, 26]

Nos.	Type of magnetic field (static, SMF; alternating, AMF)	Characteristics of the field	Main suggested effect	References
1	SMF	0.220 T	An increment in cell proliferation (1.84 %) and an increased CO_2 production (36.1 %)	[24]
2	SMF	14 T	A decreased rate of yeast proliferation under magnetic fields after 16 h of incubation	[12]
3	SMF	10 T	Cell cycle not affected	[22]
4	SMF	0.006 T	Induced modifications of cell shape, cell surface, and cytoskeleton. Apoptosis affected in a cell type-dependent manner; induction of apoptosis likely to be due to Ca^{2+} increment during the exposure; cell proliferation affected only slightly	[7]
5	AMF	50 Hz/0.010 T	Magnetic field decreases the number of yeasts [estimated as CFU] and slows down their growth	[26]
6	SMF	16 T; pulsed 55 T and 4 ×20 T	Genome-scale gene expression, proteome profile, cell viability, morphology, and growth, metabolic and fermentation activity after magnetic field exposure not impaired	[3]
7	SMF	2.93 T	The budding angle of the yeast cell clearly affected by the direction of homogeneous and inhomogeneous magnetic fields	[8]

These data show that the authors [3, 7, 8, 12, 22, 24, 26] used different strains and different stages of their growth, different incubation/exposure times, and different conditions of their experiments. Thus, the presented results do not coincide and do not permit one to describe a common mechanism of the possible effects. Returning to our experiments on the formation of inorganic nanoparticles, we need to mention just one interesting result: application of a weak static magnetic field (0.006 T) led to an increment of Ca^{2+} during the exposure [7].

Earlier, we have already shown [2] that a magnetic field affected both the bacterial activity in *P. fluorescens* as the specific rate of carbonate assimilation and the penetration of dissolved iron into bacterial cells. Those experiments compared the geomagnetic field or some artificial "magnetic disturbance" versus "magnetic vacuum" (the compensated geomagnetic field). Concerning the formation of inorganic nanoparticles in *P. fluorescens*, we need to mention that an increased magnetic field resulted in the translocation of iron into the cells.

In our present experiments, the comparative analyses of the ultrathin sections of the yeast cells, with or without a magnetic field applied, showed a drastic difference between these two variants. Large irregular electron-dense nanoparticles were formed by *S. cerevisiae* in a static magnetic field (Fig. 2) while in the natural geomagnetic field some nanoparticles around the cells were smaller and not dense (Fig. 3).

Numerous inorganic nanoparticles were formed by *C. humicola* in a flask exposed in an AMF (Fig. 4a, b). In contrast, there were no inorganic nanoparticles (Fig. 5a) or just a few of them were formed under conditions of the natural geomagnetic field (Fig. 5b). We suggest that a weak alternating magnetic field leads to a significant increment of iron penetration into the cells, which, in turn, could stimulate the formation of nanoparticles.

Nanoparticles formed are iron-bearing and magneto-sensitive

The composition of the media initially suggested that any inorganic particles produced by the yeasts have to be iron-containing. Nevertheless, we verified their composition with the X-ray microanalyses, which confirmed that the nanoparticles in the cell envelopes had a high concentration of iron (Fig. 6a, b). The predominance of iron over other chemical elements, which was detected with X-ray analyses, means that the nanoparticles are mainly iron oxides.

It is well known that bacterial cells containing magnetosomes or globular magnetic inclusions can be made to move by applying a magnetic field. In our experiments, the cells had no taxis and were too heavy to be moved by small nanoparticles attached to them. Nevertheless, we observed the accumulation of black flakes precipitated directly under the applied magnet in our experiments with *S. cerevisiae* (Fig. 1) and the movement of the foam when the flask with *C. humicola* was taken out of the Helmholtz coils and the magnet was applied to the flask. There was no analogous movement of foam in the blank flask with the *C. humicola* culture grown without an AMF. Thus, both the accumulated black sediment under the magnet and the foam motion have to be caused by magneto-sensitive particles from disrupted cells. The released nanoparticles outside the cells are clearly visible in the intercellular space in Fig. 4a.

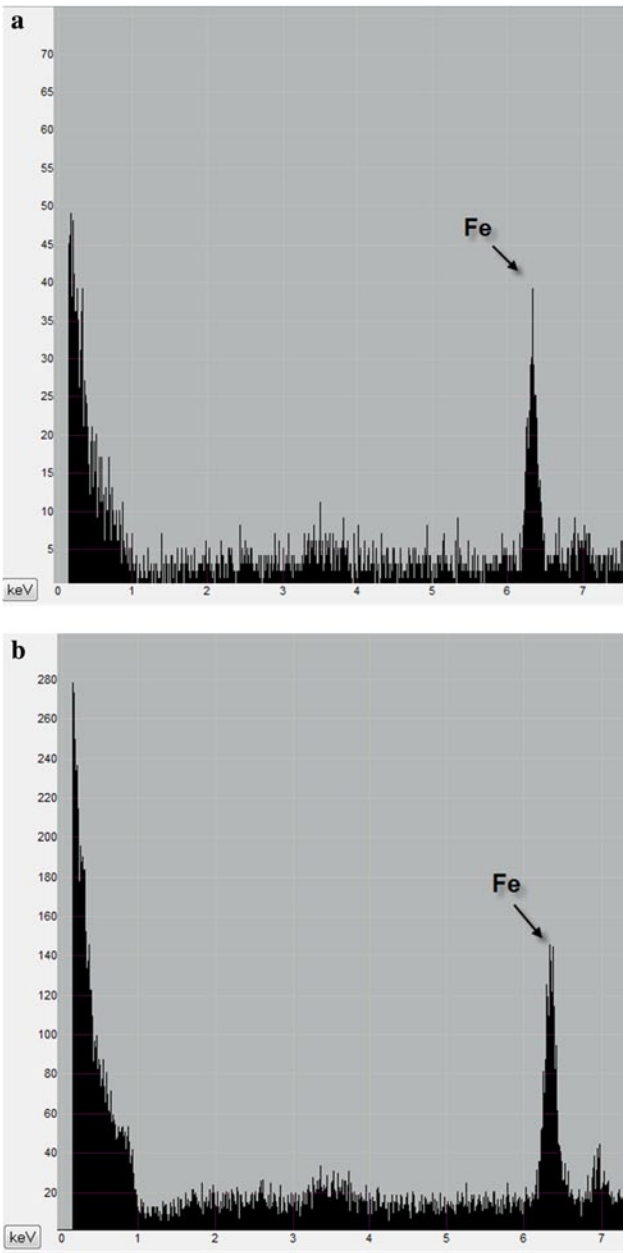

Fig. 6 Data of the X-ray microanalyses on the elemental composition: **a** *S. cerevisiae*, a scanned yeast cell with nanoparticles; **b** *C. humicola*, a scanned yeast cell with nanoparticles

Discussion

Possible industrial production of magneto-sensitive nanoparticles is of interest for biotechnology. Until now, bacteria have been the main known source and tool to produce biogenic magneto-sensitive nanoparticles at the semi-model or bench-scale level [21]. Production of nanoparticles by various yeast cultures has been exploited mainly for the synthesis of semiconductors. For example, *Candida glabrata* [6] and *Schizosaccharomyces pombe* [16] produced intracellular CdS crystallites used as industrial semiconductors. Similar synthesis of PbS semiconductors by the yeasts was also described [17]. *Yarrowia lipolytica* [1] and *S. cerevisiae* can form gold-bearing nanoparticles in the cell wall. Our electron microscopy analyses showed that the iron-bearing nanoparticles in our experiments were associated mainly with the yeast cell wall, too. In contrast, *Pichia jadinii* (*Candida utilis*) formed intracellular gold nanoparticles [9, 10]. The mechanisms of the biosynthesis of metal nanoparticles by yeasts are not yet clear. Following other investigators [14], we suggest the participation of oxidoreductases in these processes, at least in our experiments, where *C. humicola* transformed Fe(III) into a magneto-sensitive form, i.e. partly reduced it to Fe(II).

In our experiments, the investigated organisms were representatives of two different genera: *S. cerevisiae* and *C. humicola*. Both yeast cultures synthesized nanoparticles at high concentrations of dissolved iron. Formation of magneto-sensitive nanoparticles was studied under conditions of applied magnetic fields, and the possible stimulating role of magnetic field is suggested. There are different theories describing the possible mechanisms of the effects of magnetic fields on cells; our choice of the AMF parameters was based on Lednev's theory [4].

Conclusions

Our experiments confirmed the known ability of the yeasts to form metal-bearing nanoparticles and showed production of iron-containing magneto-sensitive nanoparticles. Magneto-sensitive iron-containing nanoparticles are significant for the use as target drug delivery carriers, but their synthesis by microorganisms was described only for bacterial species. Yeast cultures could be more helpful in developing a new biotechnology for production of magnetic nanoparticles.

We also showed that applied magnetic fields stimulated the formation of nanoparticles. The mechanism of the bioreduction of metal ions by yeasts is still an open question. Also, the mechanisms of the effects of magnetic fields are not clear yet. The paper reports a novel approach for the green biosynthesis of magneto-sensitive nanoparticles.

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