ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 2, pp. 215–223. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © O.I. Guliy, B.D. Zaitsev, I.E. Kuznetsova, A.M. Shikhabudinov, L.Yu. Matora, S.S. Makarihina, O.V. Ignatov, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 2, pp. 218–227.

EXPERIMENTAL ARTICLES

Investigation of Specific Interactions between Microbial Cells and Polyclonal Antibodies Using a Resonator with Lateral Electric Field

O. I. Guliy[a, 1], B. D. Zaitsev[b], I. E. Kuznetsova[b], A. M. Shikhabudinov[b], L. Yu. Matora[a], S. S. Makarihina[a], and O. V. Ignatov[a]

[a] *Institute of Biochemistry and Physiology of Plants and Microorgnaisms, Russian Academy of Sciences, pr. Entuziastov 13, Saratov, 410049 Russia*

[b] *Kotel'nikov Institute of Radio-Engineering and Electronics, Russian Academy of Sciences, Saratov branch, ul. Zelenaya 38, Saratov, 410019 Russia*

Received March 23, 2012

Abstract—The interaction between polyclonal antibodies and *Azospirillum brasilense* Sp7 cells was studied using a resonator with lateral electric field. To this end, specific polyclonal rabbit antibodies against the O-antigen epitopes of the strain *A. brasilense* Sp7 were obtained and the possibility of their application for detection of microbial cells using a piezoelectric resonator with lateral electric field was shown. It was established that frequency dependences of the real and imaginary parts of electrical impedance of such a resonator loaded with the suspension of *A. brasilense* Sp7 cells and antibodies substantially differed from those of the resonator with the control suspension of cells without antibodies. It was shown that the obtained antibodies interacted with azospirilla cells, and the marker was accumulated all over the cell surface. The limit of possible detection of microbial cells during their interaction with antibodies was found to be 10^4 cells/mL. Detection of *A. brasilense* Sp7 cells using antibodies proved to be possible in the presence of foreign bacteria. The presented results demonstrate the possibility of recording the interaction between microbial cells and antibodies and developing a biosensor for quantitative detection of microbial cells.

Keywords: detection, *Azospirillum brasilense* Sp7, antibodies, piezoelectric resonator with lateral electric field

DOI: 10.1134/S0026261713020057

One of the problems of modern microbiology is the study of interactions between antibodies and microbial cell surface. The antigen–antibody interactions are widely used in the sensory systems for detection of various species of microorganisms [1–3]. In recent years, much attention has been given to piezoelectric resonators with lateral electric field for studying the properties of various liquids, including biological fluids. In contrast to conventional resonators with longitudinal field, they are more sensitive to contacting liquid, since they respond to the changes in both its viscosity and conductivity. The possibility of detecting the microbial cells of *Escherichia coli* O157:H7 in suspension by applying the film immobilizing the respective antibodies to the resonator surface has been shown previously [4]. The application of acoustic sensors for *Salmonella typhimurium* detection using bacteriophages has been described as well [5].

The goal of this work was to investigate the possibility of detecting the interaction between microbial cells and antibodies in the case of *A. brasilense* Sp7 using a resonator with lateral electric field.

MATERIALS AND METHODS

Microorganisms. The bacterial cells of *Azospirillum brasilense* Sp7 and *Escherihia coli* BL-Ril were obtained from the Collection of Microorganisms of the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences.

Cultivation of microorganisms. The 24-h culture of *A. brasilense* Sp7 was obtained by inoculating the cells from a petri dish into a flask with the liquid LB medium containing (g/L): NaCl, 10; yeast extract, 5; peptone, 5. The cells were incubated on a circular shaker at 160 rpm and 30°C for 18–20 h. The bacteria were stored on petri dishes with solid agarized medium.

The *E. coli* BL-Ril cells were grown in flasks filled with a liquid LB medium under aerobic conditions on a circular shaker (160 rpm) at a constant temperature (30°C) for 24 h. The bacteria were stored on petri dishes with solid agarized medium.

[1] Corresponding author; e-mail: guliy_olga@mail.ru

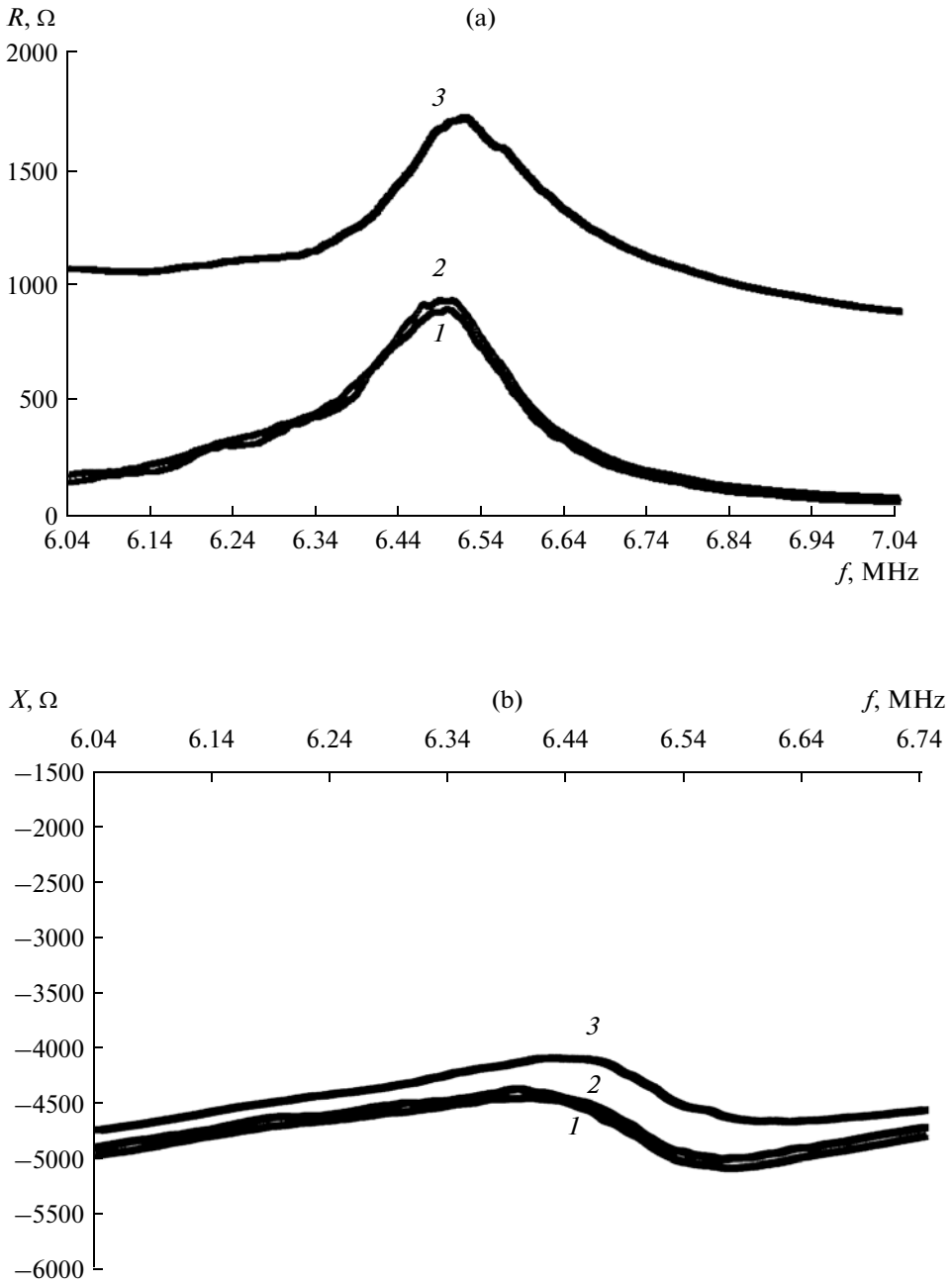

Fig. 1. Dependence of the real (a) and imaginary (b) parts of electrical impedance on the frequency (10^2 cells/mL in the analytic cell) during the interaction between *A. brasilense* Sp7 cells and specific antibodies. Distilled water (*1*), the suspension of *A. brasilense* Sp7 cells without antibodies (*2*); the suspension of *A. brasilense* Sp7 cells with antibodies (*3*).

The antibodies against the somatic antigen of *A. brasilense* Sp7 were obtained as described previously [6].

Preparation of the cells for electroacoustic analysis. Prior to the analysis, the cells were washed in distilled water by 3-fold centrifugation at 2800 *g* for 5 min and then resuspended in a small volume of water (electrical conductivity 1.8 μS/cm). Optical density of the bacterial suspension was adjusted to $OD_{660} = 0.4–0.42$.

Electroacoustic sensor analysis. Investigation of the changes in the mechanical and electrical properties of cell suspensions during biospecific interactions between microorganisms and antibodies was carried out using a specially made sensor based on a piezoelectric resonator with lateral electric field within the frequency range of 6–7 MHz. This resonator was made of X-cut 0.5-mm thick lithium niobate plate. Two rectangular electrodes (5 × 10 mm) with a 3-mm

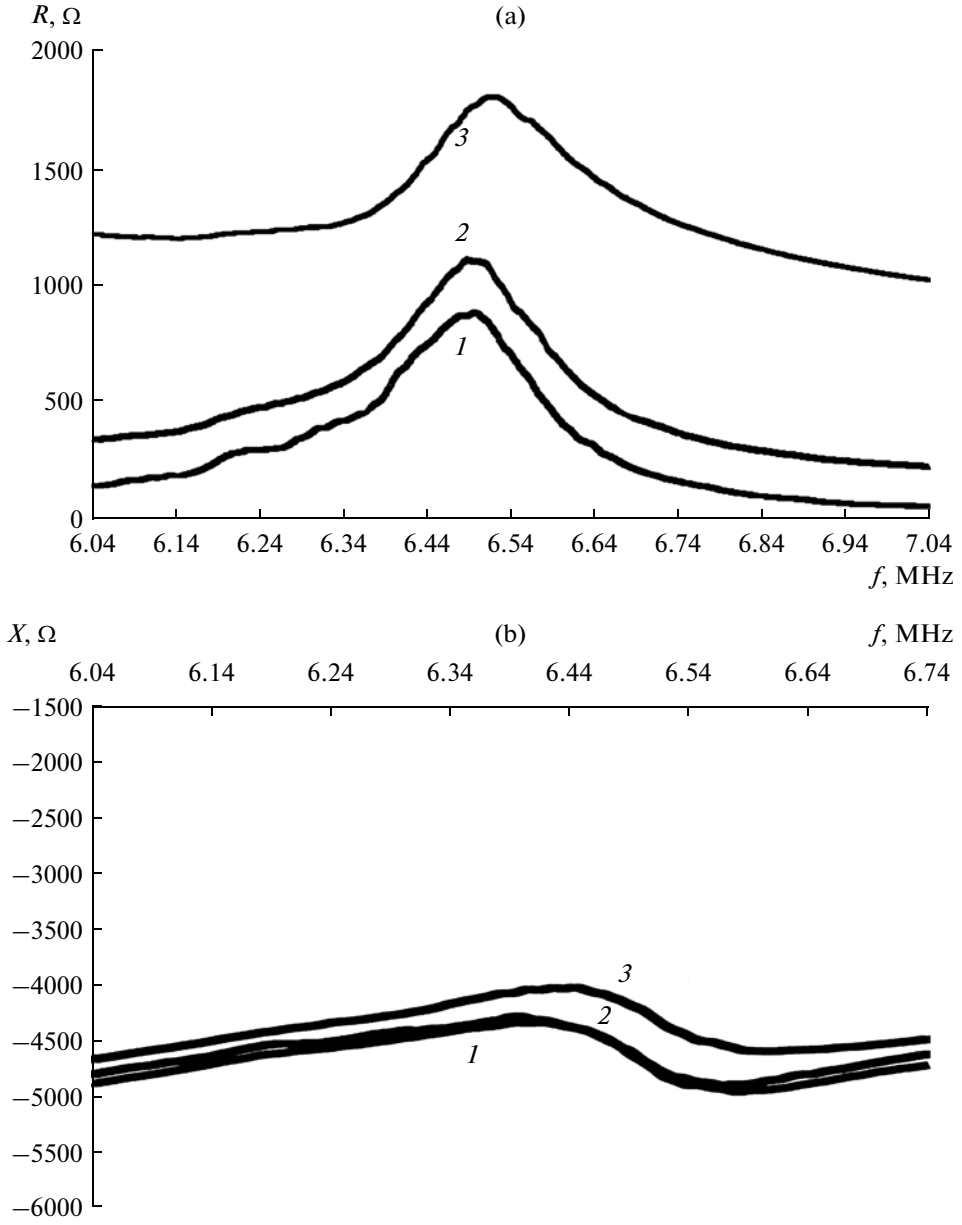

Fig. 2. Dependence of the real (a) and imaginary (b) parts of electrical impedance on the frequency (10^4 cells/mL in the analytic cell) during the interaction between *A. brasilense* Sp7 cells and antibodies. Designations are as in Fig. 1.

gap between them were placed onto the lower side of the plate. The area around the electrodes and some part of the electrodes were coated with a special lacquer to damp extraneous Lamb waves [7] and to provide a rather high Q factor (~630). A liquid cell (~1 mL) was glued on the upper side of the plate.

The prepared microbial cells with or without specific antibodies were placed into the above liquid cell, and the real and imaginary parts of electrical impedance of the sensor were measured with an Agilent 4285A precision LCR meter.

Electron microscopy. For electron microscopic identification of the interaction between the *A. brasilense* Sp7 cells and the antibodies, 0.5 mL preparations of the bacterial suspension (10^6 cells/mL) were incubated in phosphate buffered saline (PBS)

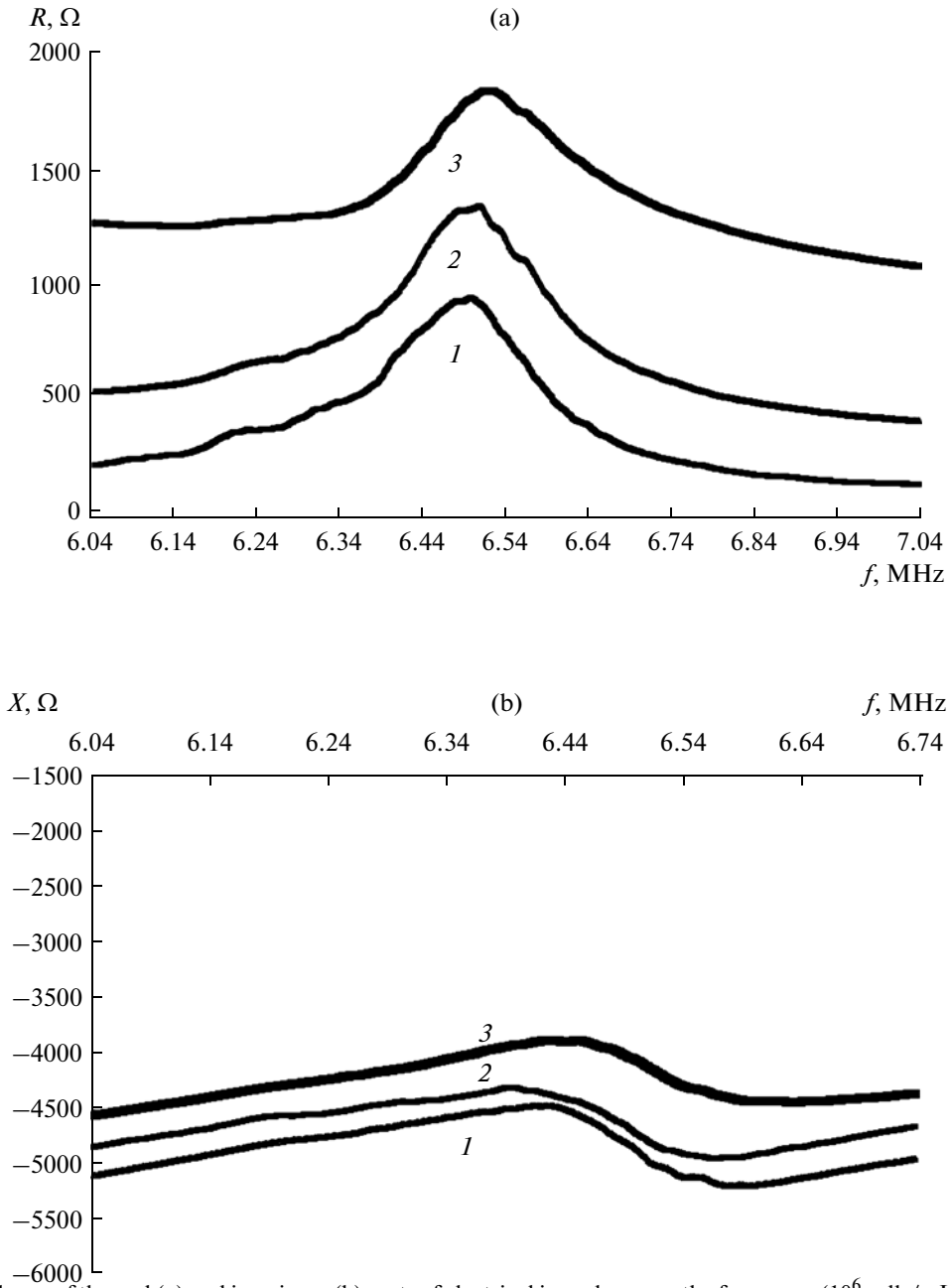

Fig. 3. Dependence of the real (a) and imaginary (b) parts of electrical impedance on the frequency (10^6 cells/mL in the analytic cell) during the interaction between *A. brasilense* Sp7 cells and antibodies. Designations are as in Fig. 1.

with 10 μL of the solution of homologous antibodies (100 μg/mL) for 1 h on a shaker. The suspension was then centrifuged at 12000 *g* for 5 min and the cell pellet was resuspended in 0.5 mL of PBS. The resulting suspension was incubated with 100 μL of the protein A–colloidal gold marker solution (OD_{520} = 0.5) for 1 h, and the precipitation and resuspension procedures were repeated. About 20 μL of the suspension was applied onto the film (Parafilm, United States), and a nickel wire grid (200 mesh) with a carbon-reinforced nitrocellulose membrane was placed over the drop for 20 min. The grid was held close to an incandescent lamp for 2 min for thermal attachment. Excessive liquid was removed by touching a filter paper strip. The grid was washed in a drop of deionized water, dried, and placed into a container. The analysis was performed with a Libra 120 electron microscope (Germany).

RESULTS AND DISCUSSION

Lipopolysaccharide, or O-antigen, is of particular interest among the major antigens of bacterial surface of azospirilla (typical gram-negative microorganisms).

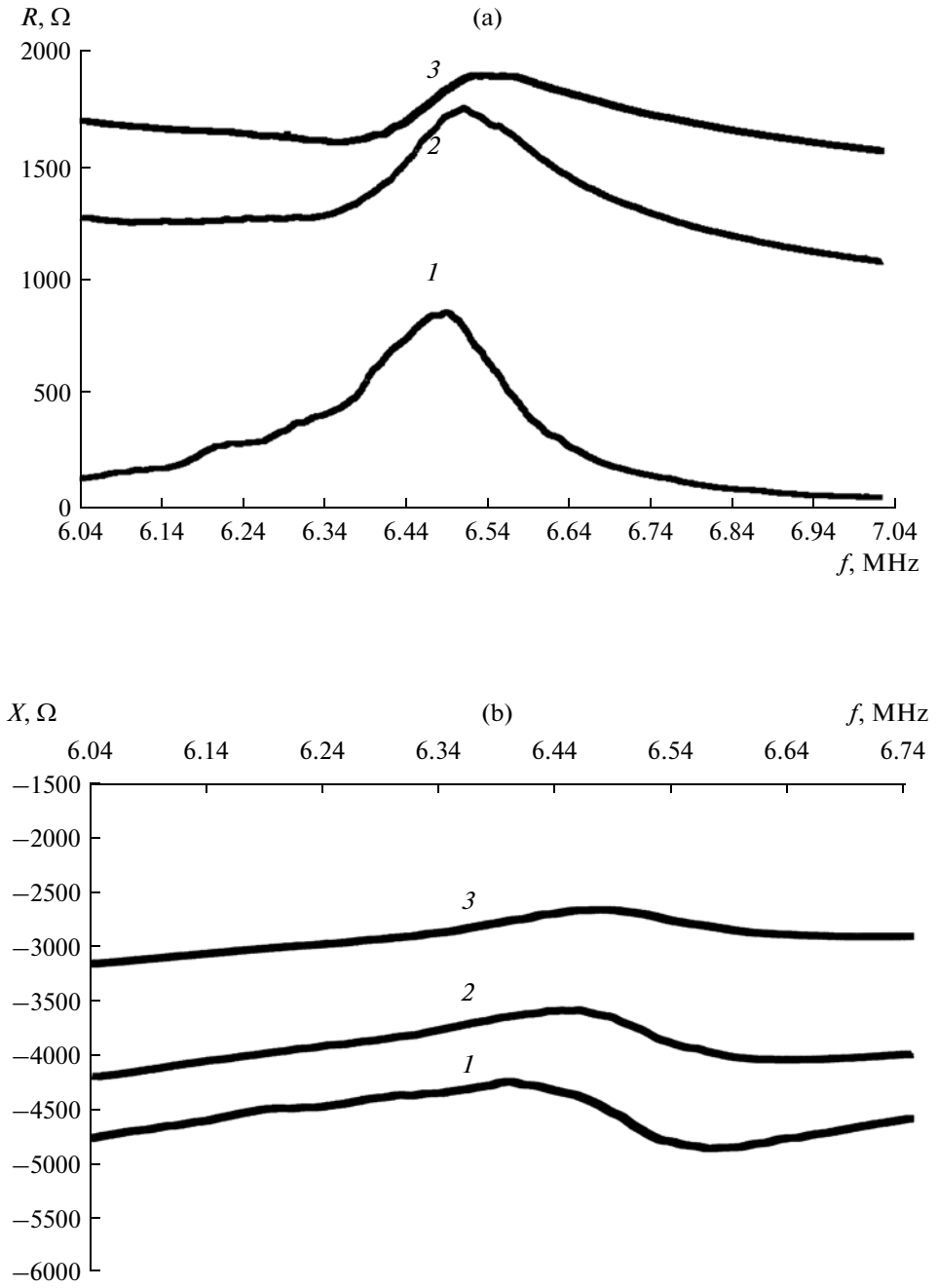

Fig. 4. Dependence of the real (a) and imaginary (b) parts of electrical impedance on the frequency (10^8 cells/mL in the analytic cell) during the interaction between *A. brasilense* Sp7 cells and antibodies. Designations are as in Fig. 1.

The structure of its O-specific polysaccharide determines the immunochemical specificity of microorganisms, providing the basis for their intraspecies classification.

The changes in the physical parameters of *A. brasilense* Sp7 cell suspension during the interaction with O-specific antibodies were investigated using an electroacoustic sensor at different cell concentrations in the suspension. To this end, frequency dependences of the real and imaginary parts of electrical impedance were measured in a liquid cell containing *A. brasilense* Sp7 microbial cells with and without specific antibodies. In our experiments, the suspensions were studied at cell numbers of 10^2, 10^4, 10^6, and 10^8 cells/mL. The antibody concentration was 3.2 μg/mL. This concentration was chosen because our previous studies of variations in the electro-optical parameters of *A. brasilense* Sp7 cell suspensions

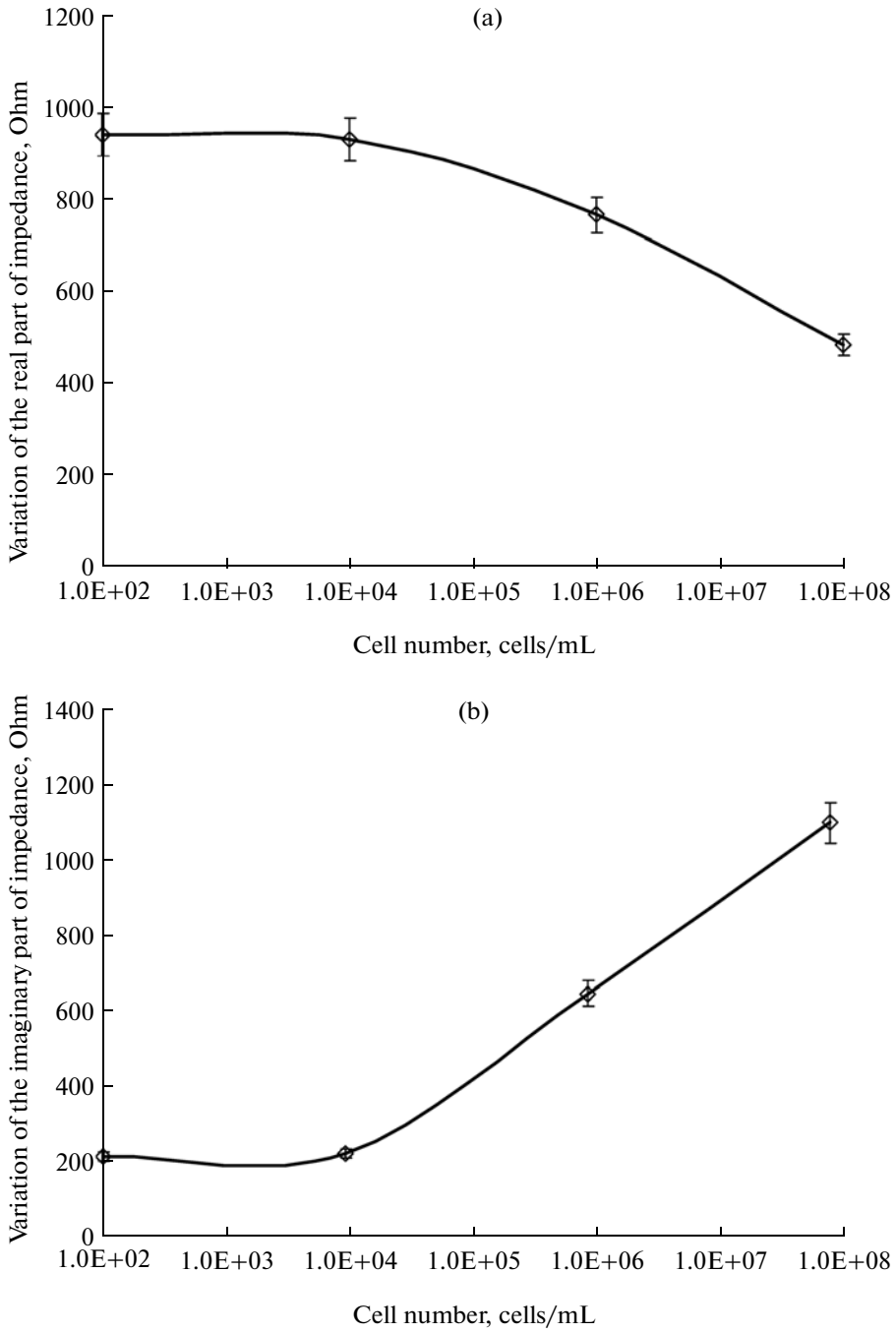

Fig. 5. Dependences of variation in the values of the real (a) and imaginary (b) part of electrical impedance of the sensor for the suspension of *A. brasilense* Sp7 cells with the addition of antibodies on cell concentration for the frequency of 6.74 MHz.

treated with specific antibodies had shown that the maximum changes in their values were recorded upon addition of antibodies to a final concentration of 3.2 μg/mL [8].

Figures 1–4 show the measured frequency dependences of the real (a) and imaginary (b) parts of electrical impedance of the sensor for pure cell suspensions (10^2, 10^4, 10^6, and 10^8 cells/mL), respectively, and after the interaction of these suspensions with the antibodies. The relevant dependences for distilled water are given here for comparison. These dependences lead to the following conclusions.

First, one can see that the sensor distinctly discriminates the situations when bacterial cells interact with specific antibodies from the control experiments when such interaction is absent. Second, the above dependences show the possibility of using the value of the real or imaginary part of electrical impedance at a

fixed frequency as an analytic signal. The figures show that the fixed frequency value may be arbitrarily selected in a certain vicinity of the resonance frequency. Figure 5 shows the dependences of the changes in the real (a) and imaginary (b) parts of impedance caused by the interaction between cells and antibodies on cell concentration for the frequency of 6.74 MHz. The analytic signal does not depend on the concentration in the range of 10^2–10^4 cells/mL, while a one-to-one correspondence exists between the analytic signal and concentration within the range of 10^4–10^8 cells/mL. Thus, the analysis shows the possibility of cell detection using specific antibodies, with the lower detection limit of 10^4 cells/mL.

Moreover, our findings lead to some conclusions concerning the variation in the physical properties of cell suspensions upon addition of antibodies. First, the increase in the real part of impedance implies the decrease in the electrical conductivity of the suspension. At that the decrement in conductivity decreases at increasing cell concentration. Second, the decrease in the absolute value of the imaginary part of impedance, which is capacitive component, is evidence of the INCREASE in the dielectric PERMITTIVITY of the suspension. With increasing cell concentration the degree of the permittivity variation increases.

Simultaneously, the specificity of antibody interaction was controlled by electron microscopic identification of the interaction between *A. brasilense* Sp7 cells and colloidal gold-labeled antibodies. The electron microphotograph shows that the obtained antibodies interact with azospirilla cells, with the marker accumulating over the entire cell surface (Fig. 6).

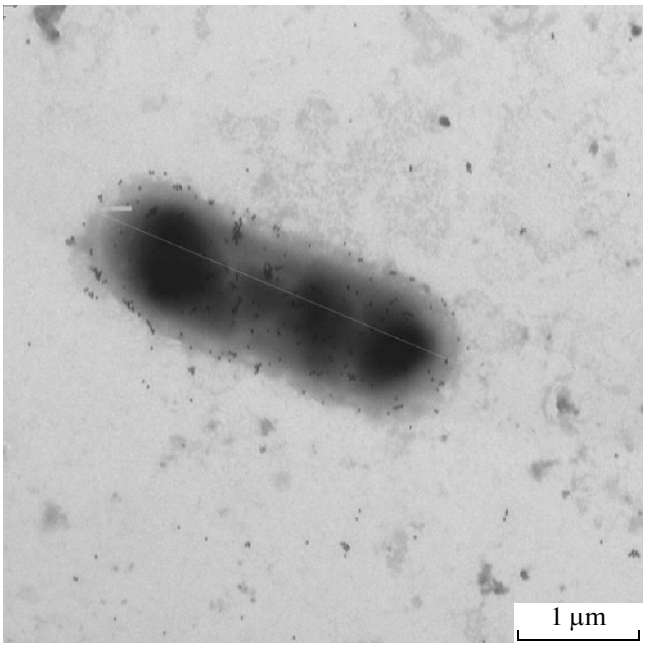

Fig. 6. Electron microscopic image of the cell of *A. brasilense* Sp7 labeled with the protein A–colloidal gold conjugate after interaction between the cells and the antibodies (magnification of 10000).

One of significant factors in the development of electroacoustic analysis is analytic signal generation in the presence of foreign microflora. Therefore, at the next stage of the work we measured the frequency dependences of the real and imaginary parts of impedance for the *A. brasilense* Sp7 cell suspension during its interaction with the antibodies in the presence of *E. coli* BL-Ril cells. This bacterium was selected because of its different taxonomic position and the cell size similar to that of azospirilla cells. To this end, the antibodies were added up to a final concentration of 3.2 μg/mL to the mixed suspension of *A. brasilense* Sp7 and *E. coli* BL-Ril cells (10^4 cells/mL in the measuring cell) taken in equal proportions (1 : 1). The mixed suspension of *A. brasilense* Sp7 and *E. coli* BL-Ril cells without the antibodies was used as the control. The resulting dependences (Fig. 7) show that the interaction between *A. brasilense* Sp7 cells and the antibodies in the presence of foreign microflora also leads to variations in the recorded dependences mentioned above. Simultaneously, the control experiments were performed to study the nonspecific interaction between the antibodies and *E. coli* BL-Ril cells. It was discovered that the parameters changed only in the cells of *A. brasilense* Sp7 during their interaction with specific antibodies, while the suspension of *E. coli* BL-Ril cells showed no changes in the values of the real and imaginary parts of impedance under the influence of antibodies to *A. brasilense* Sp7.

Thus, the above studies revealed that frequency dependences of the real and imaginary parts of impedance of the resonator on introduction of specific antibodies into the cell suspension were substantially different from the parameters of the control cell suspension without the antibodies. The above dependences show the possibility of using the value of the real or imaginary part of electrical impedance at fixed frequency as an analytic signal. The lower limit of potential detection of microbial cells was determined to be ~10^4 cells/mL during the interaction with the antibodies. The detection of *A. brasilense* Sp7 cells in mixed suspension using specific antibodies was also possible in the presence of the foreign bacterium *E. coli* BL-Ril.

Since the results of electroacoustic analysis are in good agreement with the results of electron microscopy, we believe that our findings demonstrate the possibility of developing an acoustic biosensor for the quantitative analysis of microbial cells with the lower detection limit of ~10^4 cells/mL. The piezoelectric resonator with lateral electric field for detection of microbial cells during their interaction with specific antibodies is fundamentally different from the described methods of microbial cell detection by the

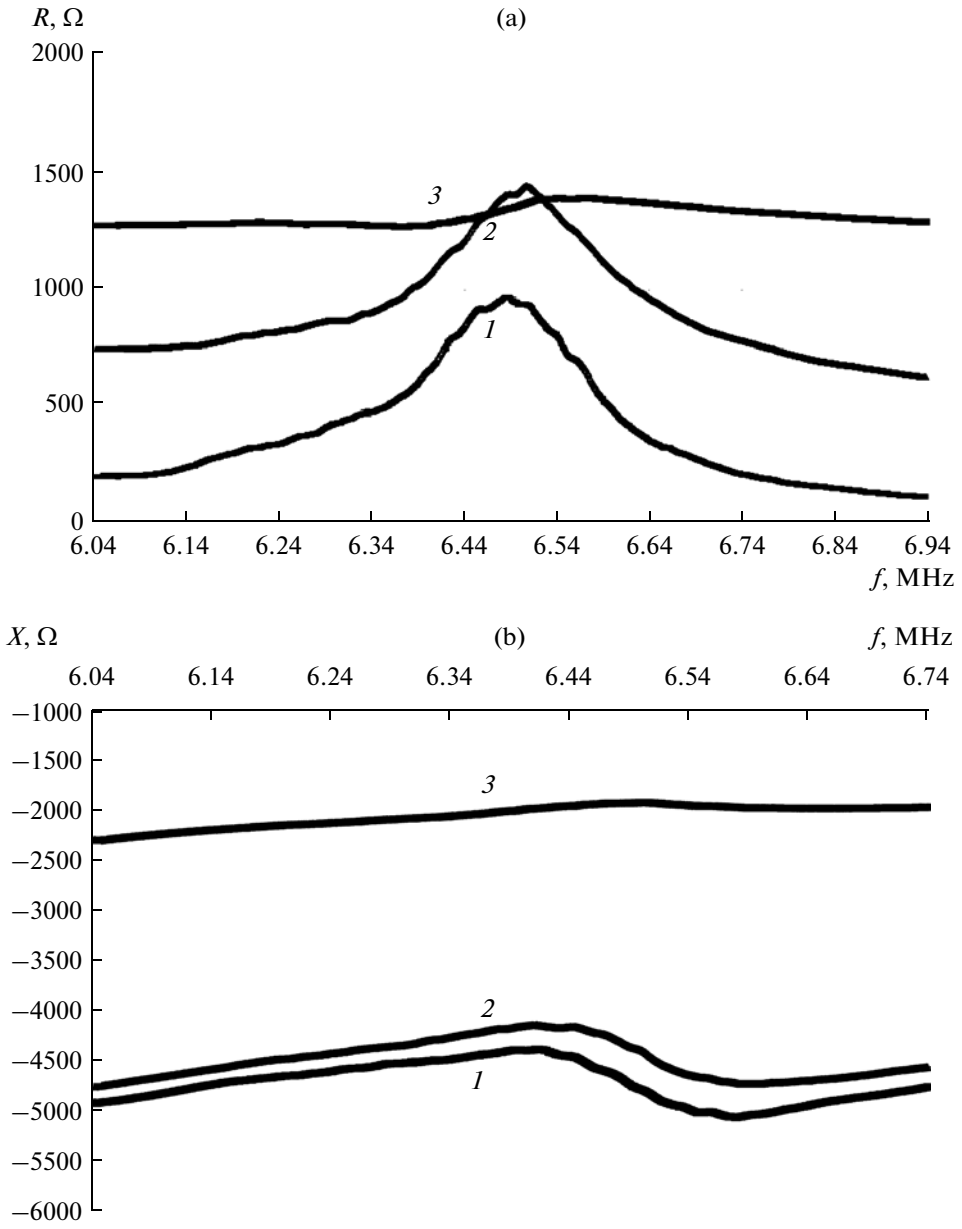

Fig. 7. Dependence of the real (a) and imaginary (b) parts of electrical impedance on frequency (the mixed suspension of *A. brasilense* Sp7 and *E. coli* BL-Ril cells) during the interaction with antibodies. Distilled water (*1*), the mixed suspension of *A. brasilense* Sp7 and *E. coli* BL-Ril cells without antibodies (*2*); the mixed suspension of *A. brasilense* Sp7 and *E. coli* BL-Ril cells with antibodies (*3*).

biosensor systems with immobilized antibodies [4] and bacteriophages [5] in simplicity of the analytical procedure, sufficient sensitivity and promptness.

ACKNOWLEDGMENTS

The work was supported by the Minisrty of Education and Science of Russian Federation, project no. 8852 and the Russian Foundation for Basic Researches (grants 10-02-01313a and 12-02-01057a).

REFERENCES

1. Bunin, V.D., Ignatov, O.V., Guliy, O.I., Voloshin, A.G., Dykman, L.A., O'Neil, D., and Ivnitskii, D., Study of the Electrophysical Properties of *Listeria monocytogenes* Cells during Interaction with Monoclonal Antibodies, *Biophysics,* 2005, vol. 50, no. 2, pp. 299–302.

2. Guliy, O.I., Matora, L.Yu., Burygin, G.L., Dykman, L.A., Ostudin, N.A., Bunin, V.D., Ignatov, V.V., and Ignatov, O.V., Electrophysical Characteristics of *Azospirillum brasilense* Sp245 during Interaction with

Antibodies to Various Cell-Surface Epitopes, *Anal. Biochem.,* 2007, vol. 370, pp. 201–205.

3. Jyoung, J.Y., Hong, S., Lee, W., and Choi, J.W., Immunosensor for the Detection of *Vibrio cholerae* O1 Using Surface Plasmon Resonance, *Biosens. Bioelectron.*, 2006, vol. 21, no. 12, pp. 2315–2319.
4. Vetelino, J.F., A Lateral Field Excited Acoustic Wave Sensor Platform, *Proc. IEEE Ultrason. Symp.*, San-Diego, 2010, pp. 2269–2272.
5. Handa, H., Gurczynski, S., Jackson, M.P., Auner, G., and Mao, G., Recognition of *Salmonella typhimurium* by Immobilized Phage P22 Monolayers, *Surf. Sci.*, 2008, vol. 602, pp. 1392–1400.
6. Matora, L.Yu., Shvartsburd, B.I., and Shchegolev, S.Yu., Immunochemical Analysis of O-Specific Polysaccharides from the Soil Nitrogen-Fixing Bacterium *Azospirillum brasilense, Microbiology,* 1998, vol. 67, no. 6, pp. 677–681.
7. Zaitsev, B.D., Kuznetsova, I.E., Shikhabudinov, A.M., and Vasil'ev, A.A., New Method of Parasitic Mode Supression in Lateral-Field-Excited Piezoelectric Resonator, *Tech. Phys. Lett.*, 2011, vol. 37, no. 6, pp. 473–476.
8. Gulii, O.I., Bunin, V.D., Neil, D., Ivnitskii, D., Matora, L.Yu., Ignatov, V.V., and Ignatov, O.V., Investigation of Electrophysical Properties of the Cells of *Azospirillum brasilense* SP7 during Interaction with Polyclonal Antibodies, *Zhurn. Mikrobiol. Epidemiol. I Immunol*, 2007, no. 5, pp. 80–82.