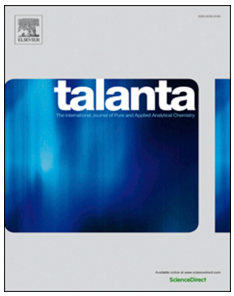
Accepted Manuscript



Use of an electro-optical sensor and phage antibodies for immunodetection of *Herbaspirillum*

O.I. Guliy, N.S. Velichko, Y.P. Fedonenko, V.D. Bunin

PII: S0039-9140(19)30484-9

DOI: https://doi.org/10.1016/j.talanta.2019.04.086

Reference: TAL 19885

To appear in: *Talanta*

Received Date: 28 February 2019

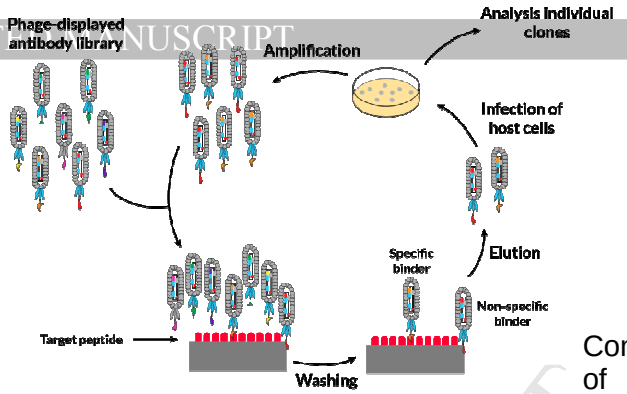
Revised Date: 25 April 2019

Accepted Date: 30 April 2019

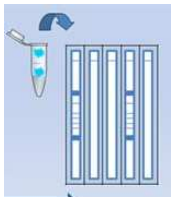
Please cite this article as: O.I. Guliy, N.S. Velichko, Y.P. Fedonenko, V.D. Bunin, Use of an electro-optical sensor and phage antibodies for immunodetection of *Herbaspirillum*, *Talanta* (2019), doi: https://doi.org/10.1016/j.talanta.2019.04.086.

Preliminary preparation of microbial cells

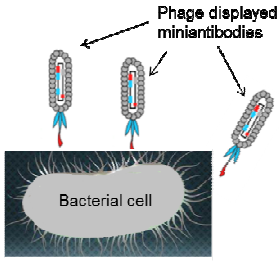
Preparation of phage antibodies and their control



Control specifity of antibodies by dot-immunoassay



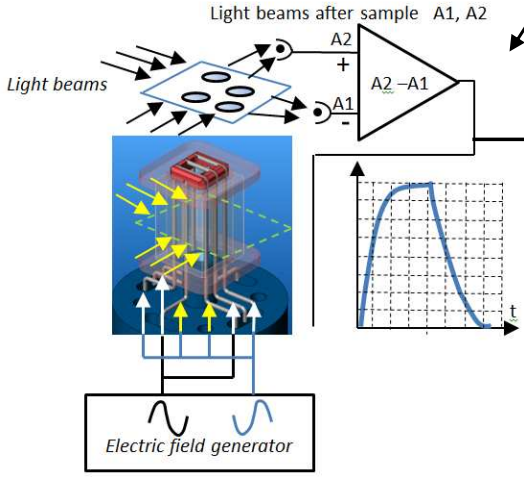
antibodies interact with the microbial cells



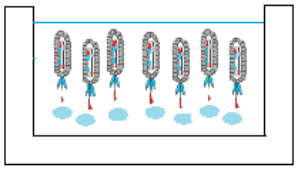
The use of phage antibodies and microbial cells for analysis

Control interaction with electro microscopy

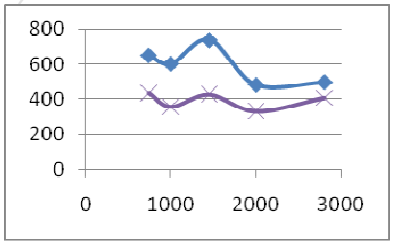
Biological optical sensor



Signal registration

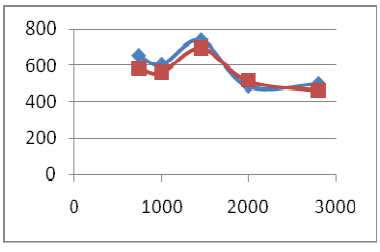


Result analysis



phage-antibodies interaction with microbial cells

microbial cells are present in the sample



phage-antibodies not interaction with microbial cells

microbial cells are absent in the sample

Use of an electro-optical sensor and phage antibodies for immunodetection of *Herbaspirillum*

O.I.Guliy[a,*1], N.S.Velichko [a], Y.P. Fedonenko [a], V.D. Bunin [b]

[a] Institute of Biochemistry and Physiology of Plants and Microorganisms,

Russian Academy of Sciences, Saratov 410049, Russia

[b] EloSystemGbR, Berlin 13407

[1]These authors contributed equally to this work.
*Corresponding author at: Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov 410049, Russia.
E-mail address: guliy_olga@mail.ru: (O.I. Guliy).

ABSTRACT

A sheep single-chain antibody-fragment library (Griffin.1, UK) was used to obtain miniantibodies to the lipopolysaccharide of *Herbaspirillum seropedicae* Z78. Using electro-optical analysis and electron microscopy, we recorded a biospecific interaction of antigenic determinants on the cell surface with phage antibodies against the LPS of *H. seropedicae* Z78 (mini-Abs_{LPS}). Control experiments were run to rule out nonspecific binding of the mini-Abs_{LPS} to cells of *Azospirillum brasilense* Sp245. Use of the highly specific mini-Abs_{LPS} enabled the lipopolysaccharide of *H. seropedicae* Z78 to be detected in a mixture of bacterial cells by electro-optical means (analysis time, ~5 min). This report is the first to show the possibility of rapid detection of *Herbaspirillum* on the basis of electro-optical analysis coupled with the use of mini-Abs_{LPS}. The results are promising for the development of biosensor-based methods to detect potentially human-harmful prokaryotes whose structures either have not been studied or are absent from commercial databases.

Keywords:

Herbaspirillum

lipopolysaccharide

phage antibodies

electro-optical sensor

detection

1. Introduction

Detection of microorganisms in different environmental constituents is a topical problem in current microbiology and biotechnology. For detection purposes, immunosensors have been used widely. Such sensors are highly selective and sensitive owing to the ability of antigens and antibodies to form immune complexes. The complexes are formed mostly with the complementary antigen, despite many other components being present. The recording of microbial cell interactions with specific antibodies is a prerequisite to the successful use of such antibodies in cell detection. In addition to polyclonal and monoclonal antibodies, use has been made of antibodies made by phage display technology [1–4]. An effective instrument for the recording of binding of phage antibodies to the cell-surface antigenic determinants is electro-optical (EO) analysis. The analysis is based on measuring the optical properties of a bacterial suspension in an alternating electric field [5–7]. The recording of changes in the EO characteristics of a suspension of cells interacting with specific antibodies makes it possible to make preliminary conclusions about the presence or absence of cells in that suspension. Previously, we have prepared cell-specific miniantibodies (mini-Abs) and have shown their usability in the electrophysical detection of bacteria [8, 9]. This technology is also applicable to members of different taxonomic groups. In this context, of special interest is *Herbaspirillum*, first described in 1984 [10]. Herbaspirilla belong to β-Proteobacteria and have been found in tissues of agriculturally important plants such as maize, rice, sorghum, wheat, and sugar beet [11,

12]. Except several phytopathogenic strains [13, 14], herbaspirilla promote plant growth and development [15, 16] and are considered nonpathogenic to humans [17, 18].

Some *Herbaspirillum* species have biological activity toward humans as well as plants, which shows that they potentially can provoke diseases in immunodeficient patients [17–22]. Such activity is due to the similar mechanisms that govern rhizosphere colonization, antagonistic activity against phytopathogens, and the early stages of human pathogenesis.

Herbaspirilla are normally identified by polymerase chain reaction or by study of their morphological, tinctorial, cultural, enzymatic, and antigenic properties. The methods used are time-consuming and require special conditions. A further complication is that herbaspirilla are absent from the databases of commercial identification systems. The rapid detection of *Herbaspirillum* spp. in water, tissues, or biological fluids is, therefore, a topical problem.

We used phage display technology to prepare mini-Abs to the lipopolysaccharide (LPS) of *H. seropedicae* Z78, and we examined whether it was possible to use these mini-Abs_{LPS} for the EO detection of *H. seropedicae* Z78.

2. Materials and methods

2.1 Bacteria and culture conditions

Herbaspirillum seropedicae Z78 (IBPPM 217), *Azospirillum brasilense* Sp245 (IBPPM 219), and *Escherichia coli* XL-1 (IBPPM 632) were from the IBPPM RAS Collection of Rhizosphere Microorganisms (http://collection.ibppm.ru). *H. seropedicae* Z78 was grown in a vitamin-supplemented liquid synthetic medium [23] at 30±1 °C for 22–24 h (until the end of the exponential growth phase). *A. brasilense* Sp245 was grown in Luria–Bertani's liquid medium on a rotary shaker (160 rpm) at 30±1 °C for 18–20 h, as described [8]. *E. coli* XL-1 was grown in Luria–Bertani's liquid medium, as described [8].

2.2 LPS isolation and purification

Cells were sedimented by centrifugation at 3000*g* for 40 min, resuspended three times in 0.15 M NaCl, agitated on a magnetic stirrer, and resedimented. LPS was extracted from an acetone-dried bacterial mass with 45 % aqueous phenol by a modified Westphal procedure [24]. LPS was purified by ultracentrifugation two times, each at 105000 *g* for 4 h, and was lyophilized in a BenchTop 2K freeze dryer (VirTis, NY, USA).

2.3 Antibody selection from phage-displayed library

For selection of miniantibodies against the LPS of *H. seropedicae* Z78 (mini-Abs_{LPS}), a Western S membrane was chosen as the optimal carrier for antigen immobilization. The selection procedure was described in detail elsewhere [8]. A 200-µl portion of LPS was placed in a well, and the plate was incubated overnight at 4 °C. The LPS-free space on the plate walls was then blocked with 2% BSA for 1 h. After a 200-µl portion of a sheep single-chain antibody-fragment library (Griffin.1, UK; 10^{12} phagemids ml^{-1}) [25] was added, the plate was incubated overnight at 4 °C. After incubation, the plate was washed three times with 0.1 M Tris–HCl buffer containing 0.05% Tween 20. Phage particles were eluted with triethylamine, and the eluate served to infect *E. coli* XL-1.

The infected cells were grown overnight in 2TY medium (trypton, 10 g l^{-1}; yeast extract, 5 g l^{-1}; NaCl, 5 g l^{-1}) containing 100 mg ml^{-1} of ampicillin and 1% glucose. A 1/100 portion (100 µl) of the resultant culture was inoculated into 10 ml of 2TY medium, and the culture was grown in a thermostatted shaker at 37 °C for 6 h until an absorbance (A_{600}) of 0.3 (~10^{12} cells/ml) was achieved. This was followed by the addition of helper phage M13K07 and by incubation at 37 °C for 1 h. After incubation, the cells were sedimented by centrifugation at 2000 *g* for 10 min. The cell sediment was resuspended in 50 ml of 2TY medium containing 100 µg ml^{-1} of ampicillin, 50 µg ml^{-1} of kanamycin, and 100 µg ml^{-1} of isopropyl-β-D-thiogalactoside and was grown

overnight at 37 °C in a thermostatted shaker. The overnight cell culture was again centrifuged at 3000 *g* for 40 min. To the supernatant liquid containing phage particles was added a 1/5 volume of 20% PEG 6000/2.5 M NaCl, and the mixture was incubated on ice for 1.5 h. Phage particles were sedimented by centrifugation at 8000 *g* for 10 min, and the sediment was resuspended in 5 ml of 10 mM TE buffer (1/10 the original culture volume; pH 7.5). The resultant preparation was clarified by centrifugation under the same conditions, and the phage particles were again precipitated by adding a 1/5 volume of PEG 6000/NaCl (1 ml) and were centrifuged. The sediment was suspended in 1 ml of TE buffer.

Increasing the sensitivity of mini-Abs requires no less than three selection rounds. There were four rounds of selection of mini-Abs_{LPS}. As suggested by Griep *et al.* [27], the LPS concentration in each round was reduced twofold to increase the specificity of the mini-Abs_{LPS}. The starting LPS concentration was 1 mg ml^{-1}.

The phage particle concentration was calculated spectrophotometrically by using a Specord BS-250 UV–vis instrument (Analytic Jena, Germany). The spectrophotometry was done at the Simbioz Center for the Collective Use of Research Equipment in the Field of Physical–Chemical Biology and Nanobiotechnology, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov. The calculations were based on the relation $(A_{269}–A_{320}) \times 5 \times 10^{14} / 15$, where A_{320} is the absorbance of the suspension at 320 nm and A_{269} is the absorbance of the suspension at 269 nm. The virion concentration can be estimated if we know that $A_{269}–A_{320}$ = 30 absorbance units, which correspond to 2×10^{14} virions ml^{-1} [26]. The phage particle concentration was 1.2×10^{13} particles ml^{-1}. The serum titer was measured by conventional enzyme-linked immunosorbent assay (ELISA) [28]. The titer of the resultant phage antibodies was 1:4000.

For ELISA, 96-well polystyrene plates were used. LPS was immobilized on a plate through simple adsorption. The enzymatic label was horseradish peroxidase conjugated to goat antirabbit antibodies. Rabbit antiphage antibodies were used as the first antibodies, with a

dilution of 1:2 [28]. The substrate reagent was *o*-phenylenediamine in the presence of hydrogen peroxide. The absorbance of the samples was measured at 490 nm on a Multiskan Ascent reader (ThermoLabsystems, Finland).

Fig.1 shows the results obtained after the first to fourth rounds of selection of mini-Abs_{LPS}. It can be seen that after round 1, the mini-Abs_{LPS} interacted with *A. brasilense* Sp245 LPS, as well as with *H. seropedicae* LPS. After round 4, the interaction of the mini-Abs_{LPS} with *A. brasilense* LPS was very weak, almost absent.

The specificity of the mini-Abs_{LPS} was checked by dot assay. This was done with a Western S membrane (Sigma–Aldrich), which had been disinfected in a methanol solution for 30 s. A suspension of *H. seropedicae* Z78 (1.06×10^{11} cells ml^{-1}) was prepared by reference to a turbidity standard and was applied onto the Western S membrane. The membrane was blocked for 1 h with 2% BSA diluted in a pH 7.0 10 mM phosphate buffer and was then dipped into a solution of specific phagemids diluted to 1×10^{13} particles in 1 ml of 10 mM phosphate buffer. After overnight incubation at 4 °C, the membrane was washed free of nonspecifically bound mini-Abs_{LPS} in a phosphate buffer and was incubated overnight with colloidal gold conjugated to rabbit antiphage antibodies (A_{520}=0.5) at 25 °C.

2.4 Electron microscopy

Samples were prepared as described [29] and were viewed with a Libra 120 transmission electron microscope (Carl Zeiss, Germany) at an accelerating voltage of 120 kV. The microscopy was done at the Simbioz Center of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences.

2.5 Preparation of cells for EO analysis

Before analysis, the cells were washed three times with distilled water by centrifugation at 2800 *g* for 5 min and were then resuspended in a little water (conductivity, 1.8 μ cm^{-1}). Cellular aggregates were removed by re-centrifuging the cell suspension at 110 *g* for 1 min, and further work was done with the suspension that had remained in the supernatant liquid. The A_{660} of the suspension prepared in this way was adjusted to 0.40–0.42.

2.6 EO analysis

The orientational spectra of the cells were measured with the ELUS EO analyzer, developed by the State Research Center for Applied Microbiology, Obolensk, Russia [30]. The analysis conditions were: electric field strength, 93.1 V cm^{-1}; light wavelength, 670 nm (relative to vacuum); field application time, 3 s; measuring-cell volume, 1 ml; cell concentration (as absorbance, *A*) A_{670}=0.40–0.42. The number of cells was calculated conventionally by using direct light microscopy. The orienting-field frequencies were 740, 1450, 200, and 2800 kHz.

The EO analysis of bacterial suspensions is based on the recording of changes in the optical properties of a suspension that are induced by an alternating field. The field-induced changes are measured at the cell surface. The interaction of the surface charges with an electric field changes the orientation of bacteria in the surrounding milieu and, ultimately, the optical properties of the suspension. An integral variable covering all these cell-structure characteristics is the frequency dispersion of cell polarizability (the dependence of the magnitude of the induced charges on the electric-field frequency). In EO measurements, this variable is determined directly by the change in the absorbance of the suspension exposed to an electric field and is called the “EO signal” or “cellular EO characteristics” [30].

Orientational spectra were presented as the frequency dependence of the difference (δA) between the absorbance values of cell suspensions. This difference was measured during the

preparation of a beam of unpolarized light along and across the orienting-field direction and was normalized to the absorbance value measured for randomly oriented cells [31].

2.7 Statistics

Each set of experiments had no less than five measurements. Data were analyzed with Excel 2010 software and with standard methods of statistical data processing.

3. Results and discussion

Two directions exist in the current immunodetection of microorganisms: (i) design of complex automated systems and (ii) development of rapid detection approaches [32, 33]. All immunochemical methods depend primarily on the quality of the specific antibodies used to make diagnostic systems. The simplest and most common method is to generate antibodies by animal immunization with either whole bacterial cells or isolated antigen preparations. A more complex yet more reliable method is hybridoma technology [34]. However, like most other animal cell cultures, hybridomas grow relatively slowly, do not reach high densities, and require complex, expensive media; as a result, their use in clinical practice is restricted. Recent research in molecular biology has used genetic engineering technologies for the cloning of recognizing fragments—hypervariable immunoglobulin regions (mini-Abs). Phage display technology, proposed by Smith [1, 35, 36], is based on simple manipulations with DNA and bacteria and enables the preparation of stable clones within a short period and at decreased cost [37, 38].

We have previously obtained mini-Abs to the surface antigens of *A. brasilense* Sp245 and have used them to detect LPS and flagellin in a dot assay and an EO study [8, 9, 30]. The resultant data permitted the use of phage display technology to select mini-Abs_{LPS}.

LPSs localized on the outer membrane of gram-negative bacteria, largely determine the antigen and surface properties of microorganisms [39–42]. They are implicated in adhesion, recognition of foreign objects, and induction of defense reactions in the host organism. The LPS structure data form a basis for intraspecies classifications of microorganisms.

Because the capsular polysaccharide of *H. seropedicae* Z78 is an extracellular form of LPS [23], the removal of a capsule from the cell surface was absolutely necessary to prevent the LPS from being contaminated. The use of modified phenolic extraction afforded a purified LPS. The isolation method used and the structure of *H. seropedicae* Z78 LPS were described elsewhere [24].

The selected mini-Abs_{LPS} were used in the EO detection of *H. seropedicae* Z78. We had previously optimized the measuring conditions, including the strength, frequency, and application time of an electric field. The orientation of *H. seropedicae* Z78 in an alternating field was measured with the ELUS EO analyzer [30] under the following conditions: field strength, 17 V cm^{-1}; application time, 16 s; frequencies, 740, 100, 1450, 2000, and 2800 kHz.

We examined changes in the magnitude of the EO signal from a suspension of Z78 cells interacting with mini-Abs_{LPS} (Fig. 2). After the microbial cells were prepared for EO measurements and the mini-Abs_{LPS} were added to the cell suspension at 1, 2, and 3 μl ml^{-1} (11, 22, and 33 phage $cell^{-1}$, respectively), the EO signal was measured. The mini-Abs_{LPS} interacted strongly with the cells (Fig. 2). For convenience of presentation, Fig. 2 (B) reports the results for the signal change at 1000 kHz. In subsequent experiments, the interaction was recorded with 3 μl ml^{-1} of mini-Abs_{LPS}.

We next investigated the time course of the changes in the magnitude of the EO signal. Bacteria were incubated with the mini-Abs_{LPS} for 1, 5, 10, 20, 30, and 40 min, after which signal changes were recorded. The effect was maximal as early as 1 min after the addition of the mini-Abs_{LPS} and changed insignificantly when the incubation time was extended. The changes in the signal magnitude after 5, 10, 20, 30, and 40 min of incubation were not greater than 5%. For

convenience of presentation, Fig. 3 (B) reports the signal value at 1000 kHz. We conclude that the optimal time for recording the presence of Z78 cells in the sample is, on average, 1–5 min.

The antigen–antibody reaction has two stages [43]. The first, specific stage involves the coupling of the antigenic determinant to the antigen's active center or to its active Fab fragment. The coupling is attended by a change in the spatial structure of the antigen molecule. This stage is rapid and has no visible effects even at low temperatures. The EO signal changes recorded within the first minutes after the addition of specific mini-Abs to a bacterial cell suspension may be due to the specific stage of antigen interaction with the mini-Abs and to the conformational restructuring of the mini-Ab and antigen molecules.

Therefore, the changes in the EO signal from a cell suspension are caused by the changes in the electrophysical variables of the cell surface structures. These latter changes result from the formation of the miniAb–cell complex when the mini-Abs bind to the antigenic determinants on the cell surface. The dielectric properties of the suspension change the most, which is what is recorded with an EO sensor.

The second, nonspecific stage involves further changes in the above characteristics after exposure of bacteria to specific mini-Abs. In this work, these changes were due to the precipitation of the antigen–antibody complex and to the formation of aggregates. Because of this, it was not worthwhile to extend the incubation time beyond 5 min, because the method put forward here was devised to record specific antigen–antibody interactions within a short time.

The interaction specificity was checked by electron microscopy by using colloidal-gold-labeled mini-Abs_{LPS}. Figure 4 (B) shows that the mini-Abs_{LPS} interacted with *H. seropedicae*, with the label accumulating throughout the cell surface. A no-antibody photo of *H. seropedicae* cells is provided in Fig. 4 (A).

In cell detection, it is important to rule out nonspecific interactions of bacterial cells with phage antibodies, which are active toward other cells. The control in this part of the study was mini-Abs specific for the LPS of *A. brasilense* Sp245 (for characterization of these mini-Abs, see

[8]). The experimental conditions were the same as described above. Fig. 5 (A) shows that *H. seropedicae* Z78 did not interact with those mini-Abs. We then examined the analytical signal in the presence of a hindering factor, *A. brasilense* Sp245 (a contaminant culture). This bacterium was chosen because both *Azospirillum* and *Herbaspirillum* are soil rhizospheric microorganisms that can promote plant growth and development. Cells of *A. brasilense* Sp 245 were added 1:1 to a suspension of cells of *H. seropedicae* Z78, after which mini-Abs_{LPS} were added. The control was a mixed suspension of *H, seropedicae* Z78 and *A. brasilense* Sp245 without the mini-Abs_{LPS}. Fig. 5 (B) shows that the addition of the mini-Abs_{LPS} brought a change in the signal magnitude.

In addition, we ran experiments to rule out nonspecific interaction of *A. brasilense* Sp245 with mini-Abs_{LPS}. No changes in the signal magnitude were recorded (Fig. 5 (C)), a finding in agreement with the data of Schmid et al. [44], who used RNA–RNA hybridization to show the absence of a close relationship between *Herbaspirillum* and *Azospirillum* or *Aquaspirillum*. The structures of the LPSs of Sp245 [45] and Z78 [24] are different; consequently, the mini-Abs_{LPS} that we used recognized only those antigenic determinants located on the surface of *H. seropedicae* Z78 and did not interact with the antigenic determinants of *Azospirillum*.

In summary, we have used a sheep phage library to conduct four rounds of selection of mini-Abs to *H. seropedicae* Z78 LPS. The mini-Abs_{LPS} were used for the first time to detect LPS in an EO analysis. The results show that when *H. seropedicae* Z78 interacted with the specific mini-Abs_{LPS}, the magnitude of the EO signal changed appreciably. Our data can be used in the design of rapid assays for bacterial cell detection and in the assessment of the exposure of antigenic determinants on the bacterial cell surface.

The structure and immunospecificity of bacterial polysaccharides are conserved phenotypic characteristics of microorganisms that are of great taxonomic importance. With *H. seropedicae* Z78 as an example, we have shown that the use of highly specific mini-Abs_{LPS} makes it possible to detect identical antigenic determinants of bacterial cells in an EO analysis.

The use of such mini-Abs is promising for the development of rapid methods of the detection of potentially human-harmful prokaryotes whose structures either have not been studied or are absent from commercial databases. Although there are no data on the role of *Herbaspirillum* in human pathologies, the detection of this bacterium is important for obtaining exhaustive information about the bacterial flora of the sample being examined.

4. Conclusion

The use of phage display technology for the obtainment of mini-Abs has additional advantages in our case, because efforts to develop polyclonal antibodies to a purified LPS preparation were unsuccessful [24], possibly owing to the structural peculiarities of *H. seropedicae* Z78 LPS [24]. The repeating unit of its O polysaccharide is formed from glycerol-1-phosphate substituted by *N*-acetyl-D-glucosamine residues. Such structures are characteristic of the teichoic acids of gram-positive bacteria [46] and are rare in gram-negative bacteria [47–49].

Immunological and PCR methods used for bacterial cell detection are highly sensitive but require long analysis times. The approach proposed here differs fundamentally from the use of mini-Abs in immunotherapy [50], proteomics [51], and biosensor construction [52] in that it is rapid (analysis time, about 5 min) and includes no additional immobilization of the components. It forms a basis for the future development of rapid tests for *Herbaspirillum* immunodetection using mini-Abs and an EO sensor in a liquid medium.

Declaration of interest

The authors declare that they have no conflicts of interest.

References

[1] J. McCafferty, A.D. Griffiths, G. Winter, D.J. Chiswell, Phage antibodies: filamentous phage displaying antibody variable domains, Nature 348 (1990) 552–554. https://doi.org/10.1038/348552a0.

[2] V. Nanduri, I.B. Sorokulova, A.M. Samoylov, A.L. Simonian, V.A. Petrenko, V. Vodyanoy, Phage as a molecular recognition element in biosensors immobilized by physical adsorption, Biosens. Bioelectron. 22 (2007) 986–992. https://doi.org/10.1016/j.bios.2006.03.025.

[3] G.C. Paoli, C.Y. Chen, J.D. Brewster, Single-chain Fv antibody with specificity for *Listeria monocytogenes*, Immunol. Methods. 289 (2004) 147–155. https://doi.org/10.1016/j.jim.2004.04.001.

[4] D.D. Williams, O. Benedek, C.L. Turnbough Jr., Species-specific peptide ligands for the detection of *Bacillus anthracis* spores Appl. Environ. Microbiol. 69 (2003) 6288–6293. https://doi.org/10.1128/AEM.69.10.6288-6293.2003.

[5] V.D. Bunin, A.G. Voloshin, Determination of cell structures, electrophysical parameters and cell population heterogeneity, J. Colloid. Interface Sci. 180 (1996) 122–126. https://doi.org/10.1006/jcis.1996.0281.

[6] A. Miroshnikov, V.M. Fomchenkov, A.Yu. Ivanov, Electrophysical Analysis and Cell Separation, Nauka Publishers, Moscow, 1986 p. 486, [in Russian].

[7] O.V. Ignatov, O.I. Guliy, V.D. Bunin, Electro-optical analysis as a tool for determination of microbial cells with the help of specific bacteriophages, in: L.M. Lechuga, F.P. Milanovich, P. Skladal, O.V. Ignatov, T. Austin (Eds.), Commercial and pre-commercial cell detection technologies for Defence against Bioterror – Technology, Market and Society, IOS Press, The Netherlands, 2008, pp. 45–53.

[8] L.A. Dykman, S.A. Staroverov, O.I. Guliy, O.V. Ignatov, A.S. Fomin, I.V. Vidyasheva, O.A. Karavaeva, V.D. Bunin, G.L. Burygin, Preparation of mini-antibodies to *Azospirillum brasilense* Sp245 surface antigens and their use for bacterial detection, J. Immunoassay and Immunochem. 33 (2012) 115–127. https://doi.org/10.1080/15321819.2011.603775.

[9] O.I. Guliy, B.D. Zaitsev, I.E. Kuznetsova, A.M. Shikhabudinov, O.A. Karavaeva, L.A. Dykman, S.A. Staroverov, O.V. Ignatov, Phage mini-antibodies and their use for detection of microbial cells by using electro-acoustic sensor, Biophysics. 57 (2012) 336–342. https://doi.org/10.1134/S0006350912030086.

[10] J.I. Baldani, V.L.D. Baldani, M.J. Sampaio, and J. Dobereiner, A found *Azospirillum* species from cereal roots, An. Acad. Bras. Cienc. 56 (1984) P. 365–368.

[11] J.I. Baldani, V.L.D. Baldani, L. Seldin, and J. Dobereiner, Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium, Int. J. Syst. Bacteriol. 36 (1986) 86–93. https://doi.org/10.1099/00207713-36-1-86.

[12] E.C. Falk, J.L. Johnson, V.L.D Baldani, J. Dobereiner, and N.R. Krieg, Deoxyribonucleic and ribonucleic acid homology studies of the genera *Azospirillum* and *Conglomeromona*, Int. J. Syst. Bacteriol. 36 (1986) 80–85. https://doi.org/10.1099/00207713-36-1-80.

[13] J.I. Baldani, V.L.D. Baldani, History of biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience, Ann. Brasil Acad. Sci. 77 (2005) 547–579. http://dx.doi.org/10.1590/S0001-37652005000300014.

[14] G. Valdameri, D. Alberton, V.R. Moure, T.B. Kokot, C. Kukolj, L.C.C., Brusamarello-Santos, R.A. Monteiro, F.O. Pedrosa, E.M. Souza, *Herbaspirillum rubrisubalbicans*, a mild pathogen impairs growth of rice by augmenting ethylene levels, Plant Mol Biol. 94 (2017) 625–640. https://doi.org/10.1007/s11103-017-0629-1.

[15] F.O. Pedrosa, E.M. Benelli, M.G. Yates, R. Wassem, R.A. Monteiro, G. Klassen, M.B. Steffens, E.M. Souza, L.S. Chubatsu, L.U. Rigo, Recent developments in the structural organization and regulation of nitrogen fixation genes in *Herbaspirillum seropedicae*, J Biotechnol. 91 (2001) 189–195. https://doi.org/10.1016/S0168-1656(01)00343-1.

[16] S. Ishii, M. Yamamoto, M. Kikuchi, K. Oshima, M. Hattori, S. Otsuka, and K. Senoo, Microbial populations responsive to denitrification-inducing conditions in rice paddy soil, as revealed by comparative 16S rRNA gene analysis, Appl. Environ. Microbiol. 75 (2009) 7070–7078. https://doi.org/10.1128/AEM.01481-09.

[17] T. Spilker, A.Z. Uluer, F.M. Marty, W.W. Yeh, J.H. Levison, P. Vandamme, J.J. Lipuma, Recovery of *Herbaspirillum* species from persons with cystic fibrosis, J Clin Microbiol. 46 (2008) 2774–2777. https://doi.org/10.1128/JCM.00460-08.

[18] E.D. Ziga, T. Druley, C.A. Burnham, *Herbaspirillum* species bacteremia in a pediatric oncology patient, J. Clin. Microbiol. 48 (2010) 4320–4321. https://doi.org/10.1128/JCM.01479-10.

[19] J.I. Baldani, B. Pot, G. Kirchhof, E. Falsen, V.L. Baldani, F.L. Olivares, B. Hoste, K. Kersters, A. Hartmann, M. Gillis, J. Döbereiner, Emended description of *Herbaspirillum*: inclusion of [*Pseudomonas*] *rubrisubalbicans*, a milk plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3, Int. J. Syst. Bacteriol. 46 (1996) 802–810.https://doi.org/10.1099/00207713-46-3-802

[20] J. Michael, T.R. Oehler, Lower Extremity Cellulitis and Bacteremia With *Herbaspirillum seropedicae* Associated With Aquatic Exposure in a Patient With Cirrhosis, Infectious Disease in Clinical Practice 13 (2005) 277–279. https://doi.org/10.1097/01.idc.0000170026.41994.8d.

[21] T. Coenye, J. Goris, T. Spilker, P. Vandamme, J.J. LiPuma, Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinuslimosus* gen. nov., sp. nov., J. Clin. Microbiol. 40 (2002) 2062–2069. https://doi.org/10.1128/JCM.40.6.2062-2069.2002.

[22] J. Chen, Z. Su, Y. Liu, S. Sandoghchian, D. Zheng, S. Wang, H. Xu, *Herbaspirillum* species: a potential pathogenic bacteria isolated from acute lymphoblastic leukemia patient, Curr. Microbiol. 62 (2011) 331–333. https://doi.org/10.1007/s00284-010-9703-5.

[23] O.N. Smol'kina, N.S. Shishonkova (Velichko), N.A. Yurasov, V.V. Ignatov, Capsular and extracellular polysaccharides of the diazotrophic rhizobacterium *Herbaspirillum seropedicae* Z78, Microbiology 81 (2012) 317–323. https://doi.org/10.1134/S0026261712030113

[24] N.S. Velichko, A.K. Surkina, Y.P. Fedonenko, E.L. Zdorovenko, S.A. Konnova, Structural Peculiarities and Biological Properties of the Lipopolysaccharide from *Herbaspirillum seropedicae* Z78, Microbiology 87 (2018) 635–641. https://doi.org/10.1134/S002626171805017X.

[25] K.A. Charlton, S. Moyle, A.J Porter, W.J Harris, Analysis of the diversity of a sheep antibody repertoire as revealed from a bacteriophage display library, The Journal of Immunology 164 (2000) 6221–6229. https://doi.org/10.1016/S0956-5663(01)00192-0

[26] G.P. Smith, J.K. Scott, Libraries of peptides and proteins displayed on filamentous phage, Methods in enzymology 217 (1993) 228–257. https://doi.org/10.1016/0076-6879(93)17065-D

[27] R.A. Griep, van C. Twisk, van J.R. Beckhoven, van der J.M. Wolf, A. Schots, Development of specific recombinant monoclonal antibodies against the lipopolysaccharide of *Ralstoniasolanacearum* race 3, Phytopathology 88 (1998) 795–803. https://doi.org/10.1094/PHYTO.1998.88.8.795.

[28] J.D. Beatty, B.G. Beatty and W.G. Vlahos, Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay, Journal of Immunological Methods 100 (1987) 173–179. https://doi.org/10.1016/0022-1759(87)90187-6.

[29] O.I. Guliy, B.D. Zaitsev, I.A.,Borodina, A.M. Shikhabudinov, A.A Teplykh., S.A. Staroverov, A.S. Fomin, The biological acoustic sensor to record the interactions of the microbial cells with phage antibodies in conducting suspensions, Talanta 178 (2018) 569–576. https://doi.org/10.1016/j.talanta.2017.09.076.

[30] O.I. Guliy, V.D. Bunin, V.I. Korzhenevich, O.V. Ignatov, Electro-optical assays for immunoindication of microbial cells, Curr. Immun. Rev. 13 (2017) 153–162. https://doi.org/10.2174/1573395513666171010142039.

[31] V.D. Bunin, A.G. Voloshin, Determination of cell structures, electrophysical parameters and cell population heterogeneity, J. Colloid. Interface Sci. 180 (1996) 122–126. https://doi.org/10.1006/jcis.1996.0281.

[32] P. Von Lode, Point-of-care immunotesting: Approaching the analytical performance of central laboratory methods, Clinical Biochemistry 38 (2005) 591–606. https://doi.org/10.1016/j.clinbiochem.2005.03.008.

[33] J. Hu, S. Wang, L Wang., F. Li, B. Pingguan-Murphy, T.J. Lu, F. Xu, Advances in paperbased point-of-care diagnostics, Biosensors and Bioelectronics 54 (2014) 585–597. https://doi.org/10.1016/j.bios.2013.10.075.

[34] G. Kohler, C. Milstein, Continuous cultures of fused cells secreting antibody of predetermined specificity, Nature 256 (1975) 495–497.

[35] G.P. Smith, Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface, Science 228 (1985) 1315–1317. https://doi.org/10.1126/science.4001944.

[36] G.P. Smith, V.A. Petrenko, Phage display, Chem. Rev. 97 (1997) 391–410. https://doi.org/10.1021/cr960065d.

[37] S. Chassagne, E. Laffly, E. Drouet, F. Hérodin, M.P. Lefranc, P. Thullier, A high-affinity macaque antibody Fab with human-like framework regions obtained from a small phage display immune library, Mol. Immunol. 41 (2004) 539–446. https://doi.org/10.1016/j.molimm.2004.03.040.

[38] K. Jacobsson, A. Rosander, J. Bjerketorp, L. Frykberg, Shotgun Phage Display – Selection for Bacterial Receptins or other Exported Proteins, Biol. Proced. Online 5 (2003) 123–135. http://doi.org/:10.1251/bpo54.

[39] O. Hols, A.J. Ulme, H. Brade, H-D. Fla, E.T. Rietsche, Biochemistry and cell biology of bacterial endotoxins, FEMS Immunol. Med. Microbiol. 16 (1996) 83–104. https://doi.org/10.1111/j.1574-695X.1996.tb00126.x.

[40] L. Mora, and W.E. Newton, Isolation, identification and localisation of diazotrophic bacteria from C4-plant *Miscanthu*, in B. Eckert (Eds.), New Horizons in Nitrogen fixation, Kluwer academic publishers, Dordrecht, 2008. pp. 705.

[41] N.J. Saunder, J.F. Pede, D.W. Hoo, E.R. Moxon, Simple sequence repeats in the *Helicobacter pylori* genome, Mol. Microbiol. 27 (1998) 1091–1098. https://doi.org/10.1046/j.1365-2958.1998.00768.x.

[42] E.L. Kannenberg, R.W. Carlson, Lipid A and O-chain modifications cause *Rhizobium* lipopolysaccharides to become hydrophobic during bacteroid development, Mol. Microbial. 39 (2001) 379–391. https://doi.org/10.1046/j.1365-2958.2001.02225.x.

[43] W.E. Paul, Fundamental Immunology, Raven Press, New York, 1984.

[44] M. Schmid, J.I. Baldani, A. Hartmann, The Genus *Herbaspirillum*, The Prokaryotes, third ed., Springer, New York, 2006. https://doi.org/10.1007/0-387-30745-1_7.

[45] Y.P.Fedonenko, E.L. Zdorovenko, V.V. Ignatov, Structure of the O-specific polysaccharide of the lipopolysaccharide of *Azospirillum brasilense* Sp245, Carbohydrate Research 337 (2002) 869–872. https://doi.org/10.1016/S0008-6215(02)00061-7.

[46] I.B. Naumova, A.S. Shashkov, E.M. Tul'skaya, G.M. Streshinskaya, Y.I Kozlova, N.V. Potekhina, L.I. Evtushenko, E. Stackebrandt, Cell wall teichoic acids: structural diversity, species specificity in the genus *Nocardiopsis*, and chemotaxonomic perspective, FEMS Microbiol. Rev. 25 (2001) 269–284. https://doi.org/10.1111/j.1574-6976.2001.tb00578.x.

[47] A.N. Kondakova, R. Fudala, S.N. Senchenkova, A.S. Shashkov, Yu.A. Knirel, W. Kaca, Structure of a lactic acid ether-containing and glycerol phosphate-containing O-polysaccharide from *Proteus mirabilis* O40, Carbohydr. Res. 340 (2005) 1612–1617. https://doi.org/10.1016/j.carres.2005.04.002.

[48] A.S. Shashkov, M. Wang, E.M. Turdymuratov, Sh. Hu, N.P. Arbatsky, X. Guo, L. Wang, Yu.A. Knirel, Structural and genetic relationships of closely related O-antigens of *Cronobacter*spp. and *Escherichia coli*: *C. sakazakii* G2594 (serotype O4)/*E. coli* O103 and *C. malonaticus* G3864 (serotype O1)/*E. coli* O29, Carbohydr. Res. 404 (2015) 124–131. https://doi.org/10.1016/j.carres.2014.11.014.

[49] E.L. Zdorovenko, L.D. Varbanets, O.S. Brovarskaya, O.A. Valueva, A.S. Shashkov, Yu.A. Knirel, Lipopolysaccharide of *Budvicia aquatica* 97U124: Immunochemical properties and structure, Microbiology 80 (2011) 372–377. https://doi.org/10.1134/S0026261711020196.

[50] W.J. Harris, C. Cunniugham, Therapeutic Antibodies, Springer-Verlag and Heidelberg GmbH &Co.K, Berlin, 1995.

[51] B. Liu, J.D. Marks, Applying phage antibodies to proteomics: Selecting single chain Fv antibodies to antigens blotted on nitrocellulose, Anal. Biochem. 286 (2000) 119–128. https://doi.org/10.1006/abio.2000.4788.

[52] N. Stich, A. Gandhum, V. Matyushin et al., Phage display antibody-based proteomic device using resonance-enhanced detection, J. of Nanoscience and Nanotechnology 2 (2002) 375–381. https://doi.org/10.1166/jnn.2002.111.

Captions for figures

Fig.1 ELISA of *H. seropedicae* Z78 LPS and *A. brasilense* Sp245 LPS by using mini-Abs_{LPS} obtained in selection round 1 to 4. (A) round 1; (B) round 2; (C) round 3; (D) round 4

Fig. 2 (A) Change in the magnitude of the EO signal from a suspension of *H. seropedicae* Z78 cells versus the amount of mini-Abs_{LPS}. 1, control (no mini-Abs); 2, 3 μl of mini-Abs; 3, 6 μl of mini-Abs; 4, 9 μl of mini-Abs. (B) Change in the signal magnitude at 100kHz.

Fig. 3 Time course of changes in the signal magnitude, as recorded during the interaction of *H. seropedicae* Z78 with mini-Abs_{LPS} at different orienting-field frequencies (A) and at 1000 kHz (B). 1, control (no mini-Abs); 2, 1-min incubation; 3, 5-min incubation; 4, 10-min incubation; 5, 20-min incubation; 6, 30-min incubation; 7, 40-min incubation.

Fig. 4 Electron microscopy of (A) cells of *H. seropedicae* Z78 without mini-Abs_{LPS} added (×10000) and (B) cells of *H. seropedicae* Z78 after addition of colloid-gold-labeled specific mini-Abs_{LPS}.

Fig. 5 (A) Change in the magnitude of the EO signal from *H. seropedicae* Z78 cells interacting with mini-Abs to *A. brasilense* Sp245 LPS. (B) Change in the magnitude of the EO signal from mixed *H. seropedicae* and *A. brasilense* Sp245 cells interacting with mini-Abs_{LPS}. (C) Change in the magnitude of the EO signal from *A. brasilense* Sp245 cells interacting with mini-Abs_{LPS}.
1, control (no mini-Abs); *2*, cells after exposure to mini-Abs.

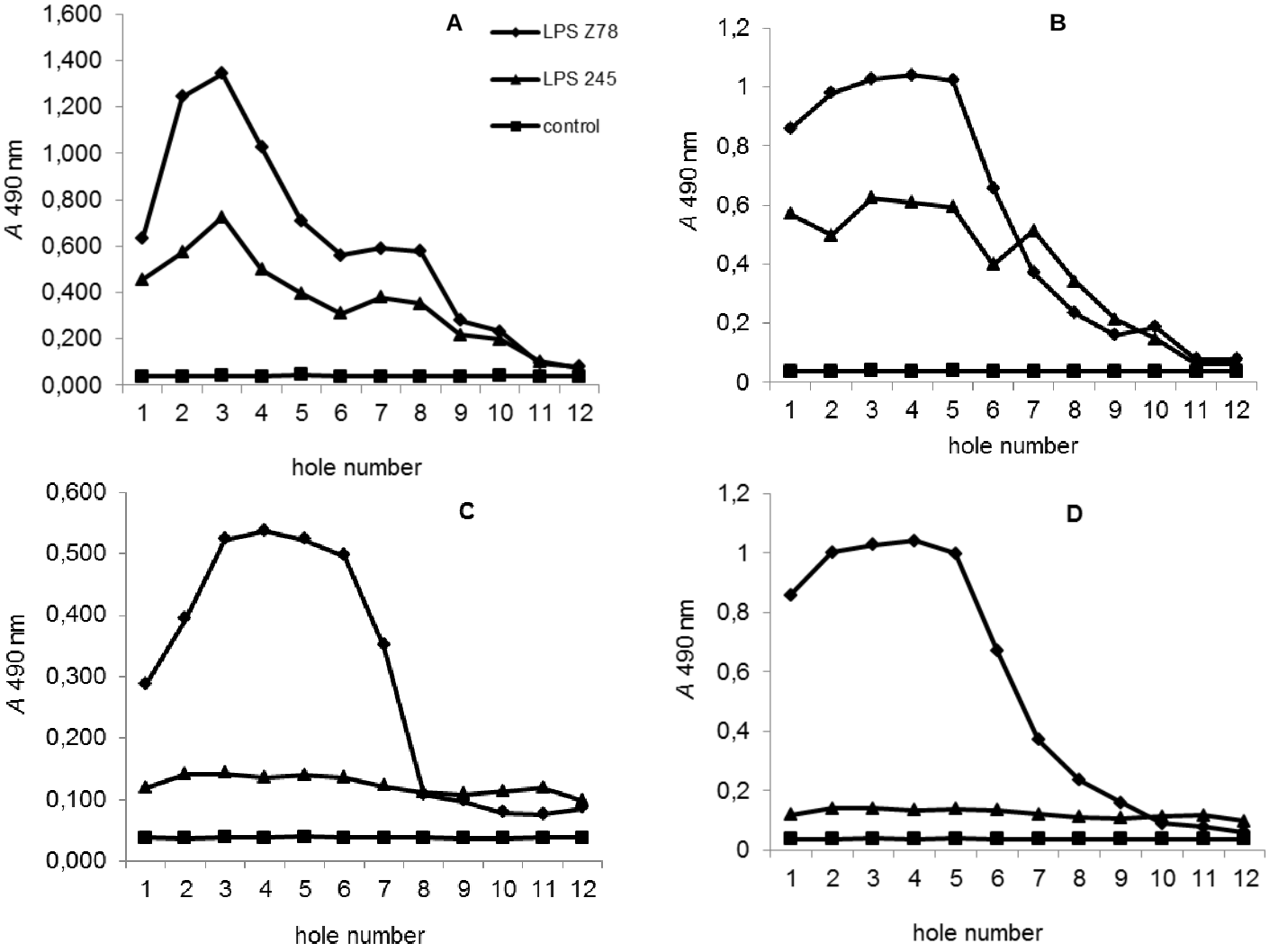


Fig.1 ELISA of *H. seropedicae* Z78 LPS and *A. brasilense* Sp245 LPS by using mini-Abs_{LPS} obtained in selection round 1 to 4. (A) round 1; (B) round 2; (C) round 3; (D) round 4

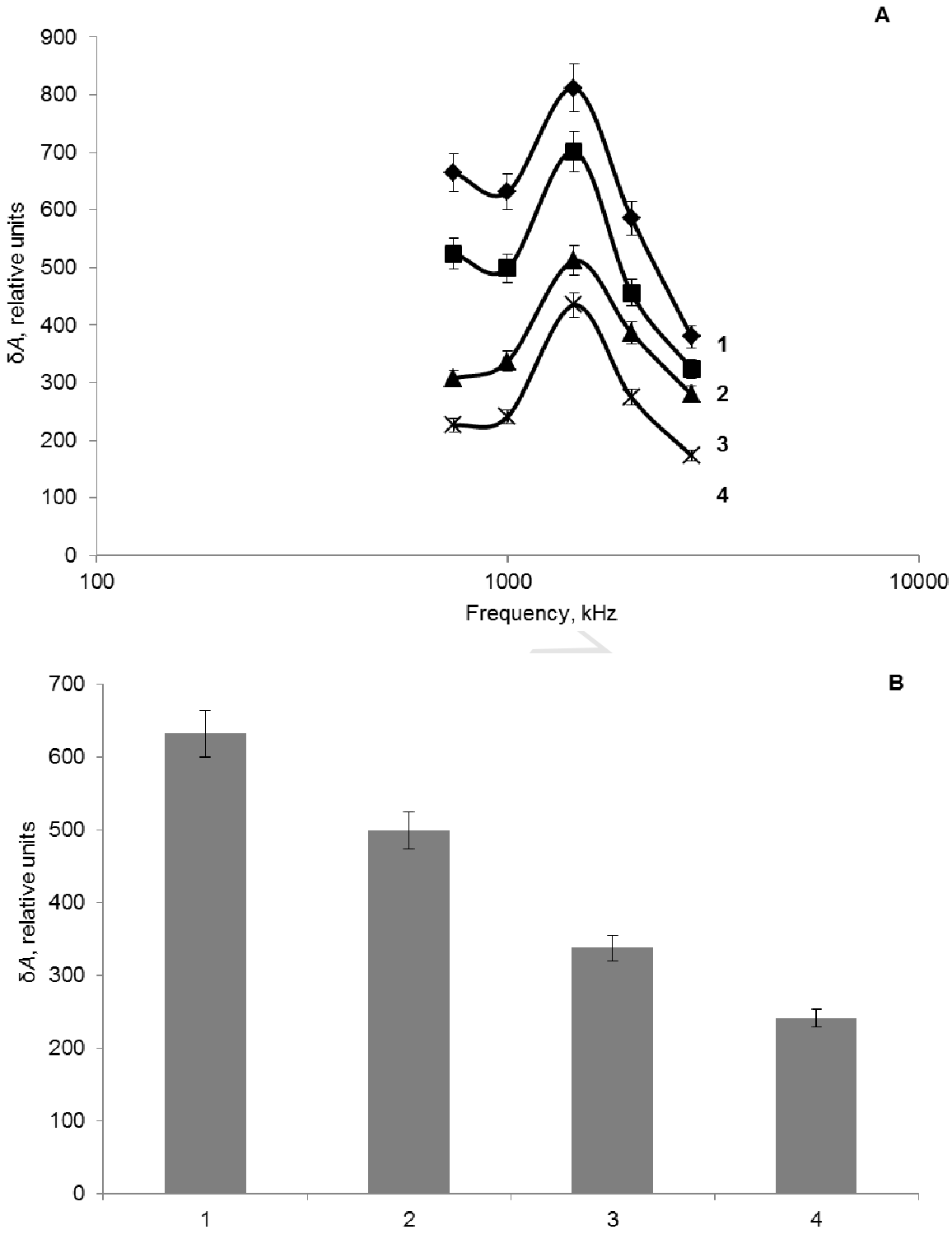


Fig. 2 (A) Change in the magnitude of the EO signal from a suspension of *H. seropedicae* Z78 cells versus the amount of mini-Abs$_{LPS}$. 1, control (no mini-Abs); 2, 3 µl of mini-Abs; 3, 6 µl of mini-Abs; 4, 9 µl of mini-Abs. (B) Change in the signal magnitude at 100kHz.

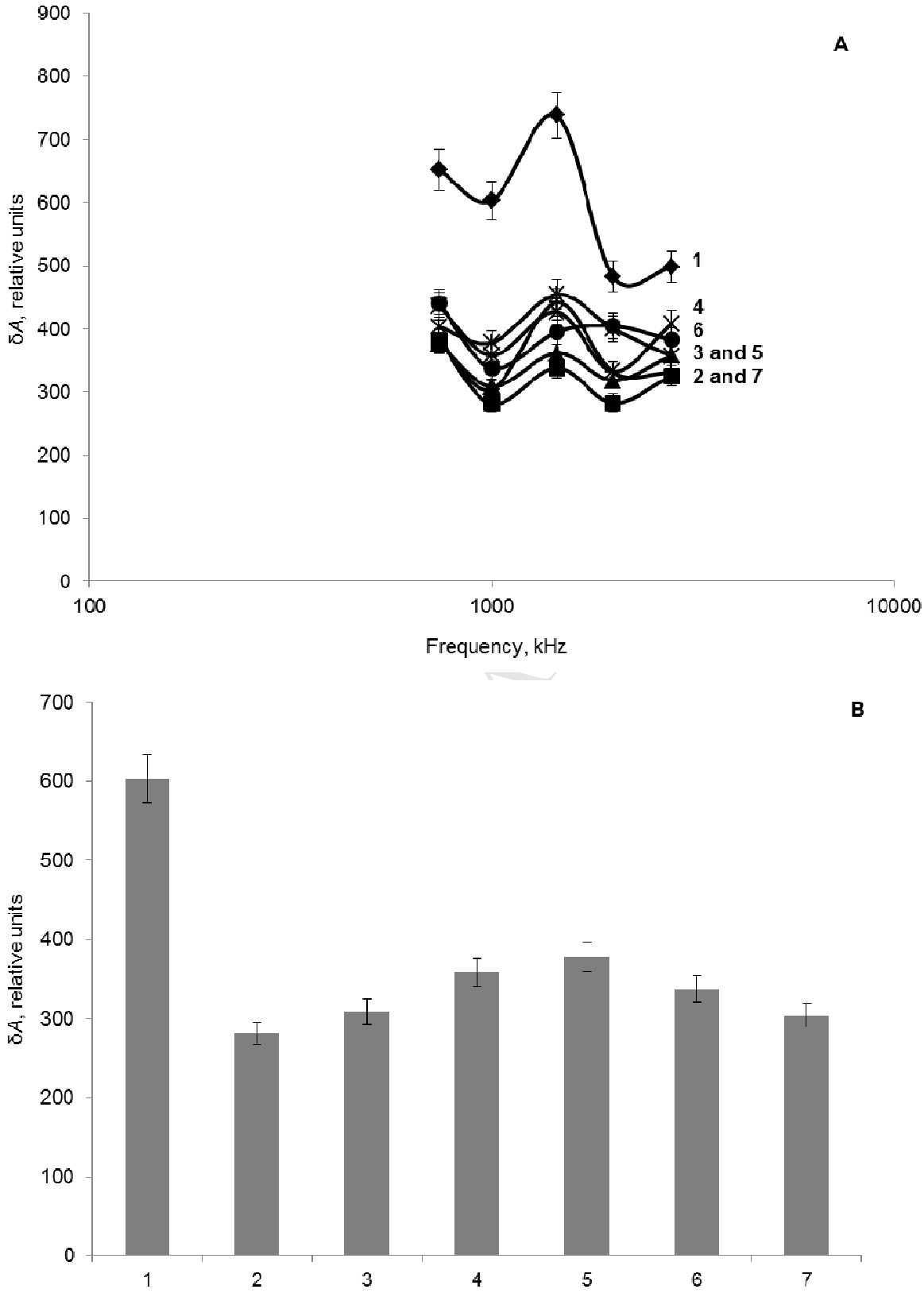


Fig. 3 Time course of changes in the signal magnitude, as recorded during the interaction of *H. seropedicae* Z78 with mini-Abs_{LPS} at different orienting-field frequencies (A) and at 1000 kHz (B). 1, control (no mini-Abs); 2, 1-min incubation; 3, 5-min incubation; 4, 10-min incubation; 5, 20-min incubation; 6, 30-min incubation; 7, 40-min incubation.

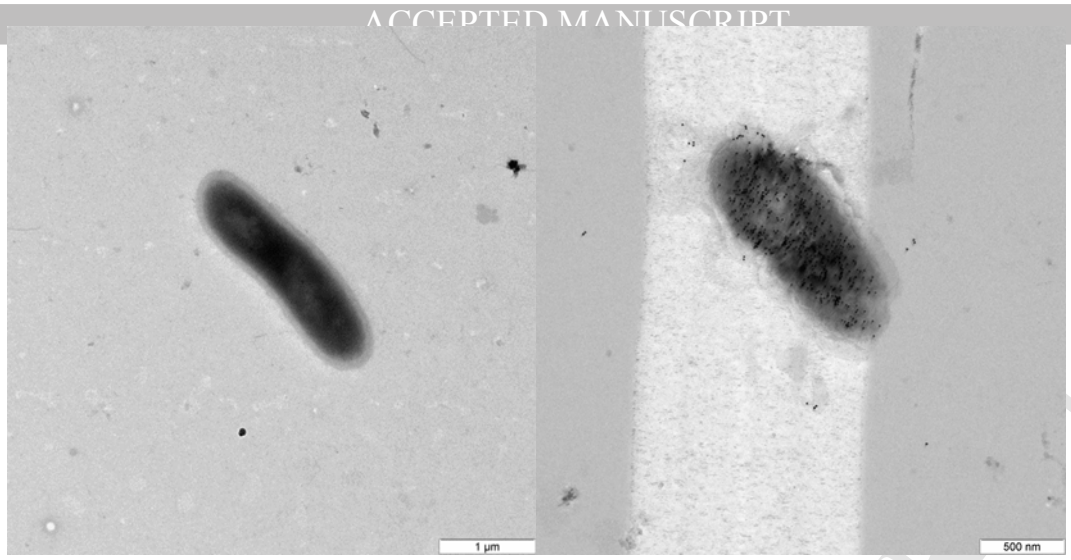


Fig. 4 Electron microscopy of (A) cells of *H. seropedicae* Z78 without mini-Abs_{LPS} added (×10000) and (B) cells of *H. seropedicae* Z78 after addition of colloid-gold-labeled specific mini-Abs_{LPS}.

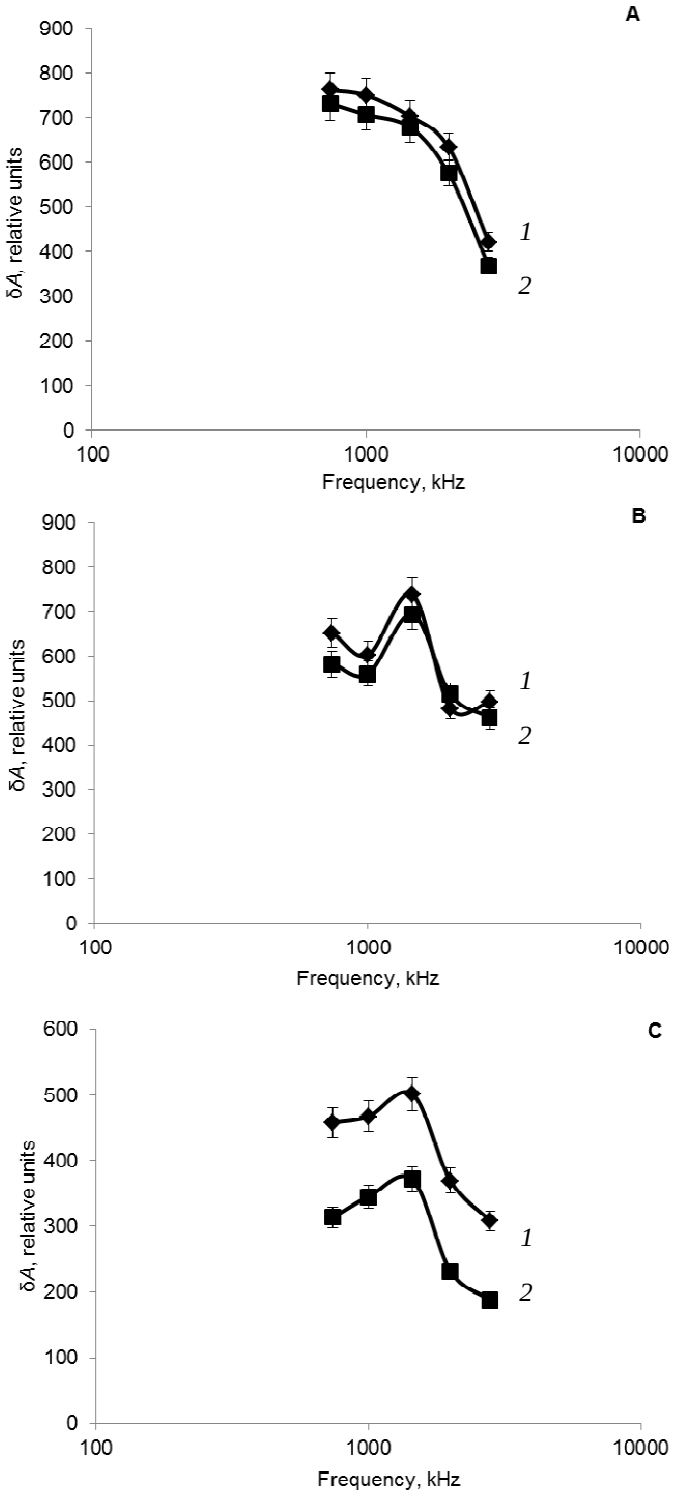


Fig. 5 (**A**) Change in the magnitude of the EO signal from *H. seropedicae* Z78 cells interacting with mini-Abs to *A. brasilense* Sp245 LPS. (**B**) Change in the magnitude of the EO signal from mixed *H. seropedicae* and *A. brasilense* Sp245 cells interacting with mini-Abs_{LPS}. (**C**) Change in the magnitude of the EO signal from *A. brasilense* Sp245 cells interacting with mini-Abs_{LPS}. *1*, control (no mini-Abs); *2*, cells after exposure to mini-Abs.

Highlights

1. A sheep single-chain antibody-fragment library (Griffin.1, UK) was used to obtain miniantibodies to the lipopolysaccharide of *Herbaspirillum seropedicae* Z78.

2. By using electro-optical analysis and electron microscopy, a biospecific interaction of the lipopolysaccharide with the miniantibodies was recorded.

3. Bacterial cell detection is based on the recording of changes in the EO signal before and after cell interaction with specific phage antibodies even in the presence of other bacterial cells.

4. The sensor successfully recorded specific interactions of *Herbaspirillum seropedicae* Z78 with phage antibodies in a mixture of bacterial cells with a detection limit of 10^4 cells/ml, with an ~5 min recording time.