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ORIGINAL ARTICLE

Phage antibodies for the immunochemical characterization of *Herbaspirillum seropedicae* Z78 glycopolymers

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Abstract

Purpose Microbial carbohydrate antigens are targets of the immune systems of hosts. In this context, it is of interest to obtain data that will permit judgment of the degree of heterogeneity, chemical makeup, and localization of the antigenic determinants of the *Herbaspirillum* surface glycopolymers.

Methods A sheep single-chain antibody-fragment phage library (Griffin.1, UK) was used to obtain miniantibodies to the exopolysaccharides (EPS-I and EPS-II), capsular polysaccharides (CPS-I and CPS-II) and lipopolysaccharide (LPS) of *Herbaspirillum seropedicae* Z78. To infer about the presence or absence of common antigenic determinants in the cell-surface polysaccharides of *H. seropedicae* Z78, we ran a comparative immunoassay using rabbit polyclonal and phage recombinant antibodies to the surface glycopolymers of *H. seropedicae* Z78.

Results We isolated and purified the exopolysaccharides (EPS-I and EPS-II), capsular polysaccharides (CPS-I and CPS-II), and lipopolysaccharide (LPS) of *Herbaspirillum seropedicae* Z78. Using rabbit polyclonal antibodies, we found that these cell-surface polysaccharides were of a complex nature. EPS-I, EPS-II, CPS-I, CPS-II, and LPS contained common antigenic determinants. CPS-I, CPS-II, and LPS also contained individual antigenic determinants composed of rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine—sugars responsible for cross-reactions with miniantibodies.

Conclusions The anti-LPS miniantibodies were more specific for the core region of the LPS, in which rhamnose was the most abundant sugar, than they were specific for its O portion. The miniantibodies we isolated can be useful reagents not only in basic biochemical research but also in clinical diagnostic and therapeutic applications.

Keywords *Herbaspirillum seropedicae* · Polysaccharides · Lipopolysaccharide · Antibodies · Immunodetection

Introduction

Herbaspirillum, a member of the *Betaproteobacteria*, enjoys intense current interest (Bajerski et al. 2013; Lin et al. 2013; Chemaly et al. 2015; Batista et al. 2018; Correa-Galeote et al. 2018). Except several phytopathogenic strains (Valdameri et al. 2017), herbaspirilla can promote plant growth and development (Pedrosa et al. 2001). *Herbaspirillum* also colonizes human organs and tissues (Baldani et al. 1996; Michael and Oehler 2005; Tan and Oehler 2005) and has been identified in clinical isolates and human secretions (Coenye et al. 2002; Spilker et al. 2008; Ziga et al. 2010; Chen et al. 2011). Although the ability of these bacteria to colonize their hosts has been proven, the data on the mechanisms of such associations are fragmentary.

The mechanisms of host-bacterium associations involve the glycopolymers of the bacterial surface. Characterization of the structure of glycopolymers is necessary for understanding their properties and functions, including those operating in the interaction of bacteria with other organisms and with the surroundings. The principal macromolecules implicated in the recognition of symbiotic partners are exopolysaccharide (EPS), capsular polysaccharide (CPS), and lipopolysaccharide (LPS), which determine the antigenic specificity of gram-negative bacteria (Kato et al. 1980; Konnova et al. 1994; Skvortsov and Ignatov 1998; Whitfield and Roberts 1999; Newman et al. 2000; Yirmiya et al. 2000; Smol'kina et al. 2010). Much information about the structure of these glycopolymers can be acquired not only by the destructive chemical methods but also by immunochemistry, which enables the study of antigenic determinants *in vitro*, *in vivo*, and *in planta*.

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Serological and immunochemical investigations have traditionally used polyclonal or monoclonal antibodies raised by animal immunization. If, however, the antigen used is poorly immunogenic or highly toxic, immunization may be difficult to carry out. The problem is solved with antibodies based on variable antigen-binding fragments that are obtained from antibody fragment libraries by phage display (MacCafferty et al. 1990). The most commonly used are Fab and scFv fragment libraries, in which the antigen-binding fragment is present at the surface of bacteriophage M13 as part of its pIII protein (miniantibodies (miniAbs) in phage format) (Asadi-Ghalehni et al. 2015; Petrenko 2018). Phage display technology, proposed by Smith (Smith 1985; MacCafferty et al. 1990; Smith and Petrenko 1997), replaces all work stages with simple manipulations with DNA and bacteria, yielding stable antibody-producing clones within weeks rather than months and decreasing the associated costs.

Here, we use a sheep single-chain antibody-fragment library (Griffin.1, UK) to raise miniAbs against the main surface antigens of *Herbaspirillum seropedicae* Z78, and we report a comparative immunochemical characterization of these antigens.

Materials and methods

Strain and growth conditions

Herbaspirillum seropedicae Z78 (IBPPM 217) was from the IBPPM RAS Collection of Rhizosphere Microorganisms (http://collection.ibppm.ru). Cells were grown in a vitamin-supplemented liquid synthetic nutrient medium (Smol'kina et al. 2012) at 30 ± 1 °C for 24 h (until the end of the exponential growth phase).

Isolation and purification of bacterial polysaccharides

Cells were sedimented by centrifugation at 3000×*g* for 40 min. Capsular polysaccharides were removed from the cell surface by resuspending the cells three times in 0.15 M NaCl, agitating the cells on a magnetic stirrer, and resedimenting the cells. The cells were then degreased with petroleum ether, dried with acetone, and finely dispersed. CPS-I, CPS-II, EPS-I and EPS-II were isolated as described by Smol'kina et al. (Smol'kina et al. 2012). All carbohydrate-containing fractions that did not give absorption between 240 and 260 nm were pooled, concentrated, and lyophilized. LPS was extracted from the acetone-treated cells (10 g) with hot 45% aqueous phenol by a modified Westphal procedure (Velichko et al. 2018), purified by ultracentrifugation two times (each at 105000×*g* for 4 h), and lyophilized with a Benchtop 2K apparatus (VirTis, USA).

Isolation of O polysaccharide and core oligosaccharides

Lyophilized LPS was heated in 1% acetic acid at 100 °C for 4 h (Müller-Seitz et al. 1968). Lipid A was sedimented by centrifuging the reaction mixture at 12000×*g* for 20 min. The supernatant liquid was dialyzed against distilled H_2O and fractionated by gel filtration. The high molecular weight O polysaccharide (OPS) fraction was separated from the oligosaccharide fraction on a Sephadex G-50 column (Pharmacia, Sweden). The OPS and core oligosaccharide solutions were concentrated, lyophilized, and analyzed.

Preparation of rabbit antibodies

Antibodies to whole cells of *H. seropedicae* Z78 were kindly provided by the IBPPM RAS Immunochemistry Laboratory. Rabbits were immunized with whole *H. seropedicae* Z78 cells treated with 2% glutaraldehyde. Rabbits were also immunized with a mixture of LPS and Freund's complete adjuvant into popliteal lymph nodes, three times (0.5, 1.0, and 1.5 mg) at 2-week intervals. The antigen concentration was 1 mg ml^{-1} in all immunizations. The animals were bled 6 days after the last immunization. Antibody titers were determined by agglutination tests. IgG fractions were isolated from antisera by ammonium sulfate precipitation.

Antibody selection from phage-displayed library

For selection of phage-carrying antibodies to the EPS-I, EPS-II, CPS-I, CPS-II, and LPS of *H. seropedicae* Z78, an enzyme immunoassay plate was used as a solid support for antigen immobilization. The selection procedure was described in detail elsewhere (Dykman et al. 2012). The concentration of the sheep phage recombinant library (Charlton et al. 2000) was 10^{12} phagemids ml^{-1}. The phage specificity was determined by dot and enzyme-linked immunosorbent assays (ELISA). The serum titer was measured by conventional ELISA (Beatty et al. 1987). The titer of the resultant phage antibodies was 1:4000.

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} (Smith & Scot, 1993).

Enzyme-linked immunosorbent assay (ELISA)

For ELISA, 96-well polystyrene plates were used. EPS-I, EPS-II, CPS-I, CPS-II, and LPS with two-fold dilution were immobilized on a plate through simple adsorption. The enzymatic label was horseradish peroxidase conjugated to goat antibodies. 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).

Competitive immunoassay

The inhibition of the immunochemical reactions was assessed by ELISA as described by Kabat and Mayer (1961). Inhibitory monosaccharides were added to miniAbs to 10^{-4} M, a concentration purposely chosen in excess of the miniAb concentration. The miniAb concentration was 1.2×10^{13} virions ml^{-1}, and the LPS concentration was 0.015 mg ml^{-1}. Solutions of the miniAbs, LPS, OPS, and monosaccharides were made in a buffer of 0.1 M NaCl and 0.01 Tris-HCl (pH 7.2). The miniAbs were mixed with each inhibitor, and the mixture was incubated at 4 °C for 24 h.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed with Excel 2010 software and with standard methods of statistical data processing. Correlation coefficients and unpaired *t*-tests were used when appropriate. All confidence intervals are for 95% confidence. Differences between means at a confidence level of 5% ($P < 0.05$) were considered statistically significant. Data are presented as the mean ± the standard deviation (SD).

Results

Preparation of rabbit polyclonal antibodies

Purified preparations of CPS-I, CPS-II, EPS-I, EPS-II and LPS were isolated as described by Smol'kina et al. (2012) and Velichko et. al. (2018).

All preparations were tested by ELISA for their ability to interact with rabbit polyclonal antibodies to glutaraldehyde-treated *H. seropedicae* cells (Fig. 1). LPS, EPS-II, and CPS-II interacted with the antibodies, with the highest interaction being observed for LPS. Conversely, EPS-I and CPS-I did not interact with the antibodies at all. Effors to prepare rabbit polyclonal antibodies against purified LPS were unsuccessful.

Antibody selection from phage library

Because it was difficult to interpret the results of the experiment in Fig. 1, we selected phage recombinant anti-LPS, anti-EPS-I, anti-EPS-II, anti-CPS-I, and anti-CPS-II antibodies ($miniAbs_{LPS}$, $miniAbs_{EPS\text{-}I}$, $miniAbs_{EPS\text{-}II}$, $miniAbs_{CPS\text{-}I}$, and $miniAbs_{CPS\text{-}II}$). There were four rounds of selection of phage antibodies. As suggested by Griep et al. (1998), the antigen concentration in each round was reduced twofold to increase miniAb specificity. The starting antigen concentration was 1 mg ml^{-1}. Measuring the UV absorbance allowed calculation of the numbers of phage in the final dialysates. These were 1.2×10^{13}, 0.6×10^{13}, 0.5×10^{13}, and 2.6×10^{13} particles ml^{-1} for LPS, EPS-I, EPS-II, CPS-I, and CPS-II, respectively.

The increase in miniAb specificity was evaluated by ELISA. Figure 2 shows the results obtained after the first and fourth rounds of selection of $miniAbs_{CPS\text{-}II}$. After round 1, the $miniAbs_{CPS\text{-}II}$ interacted with all preparations included in the figure, and after round 4, they interacted equally intensely with CPS-I and CPS-II but did not interact with EPS-I. The $miniAbs_{LPS}$ interacted with LPS, CPS-I, and CPS-II. The activity toward CPS-II was the highest, which suggests that the specific antigenic determinants of CPS-II were better surface-exposed. By contrast, the interaction of the $miniAbs_{LPS}$ with EPS-II and EPS-I was very weak, almost absent. Like the $miniAbs_{LPS}$, the $miniAbs_{CPS\text{-}II}$ barely interacted with EPS-I and EPS-II, but the reaction with LPS was weaker and that with CPS-I was stronger than was the reaction with CPS-II (Table 1). The $miniAbs_{EPS\text{-}I}$ and $miniAbs_{EPS\text{-}II}$ interacted with all antigens used. The reactions with EPS-I and EPS-II were weaker than the reaction with CPS-II, the absorbance for which was maximal. The interaction of the $miniAbs_{EPS\text{-}I}$ and $miniAbs_{EPS\text{-}II}$ with LPS was stronger than it was with CPS-I (Table 1).

The $miniAbs_{CPS\text{-}I}$ interacted with all antigens of *H. seropedicae* Z78, but the interaction peaked for LPS, EPS-II, and CPS-II to an equal degree. The reaction with CPS-II was somewhat stronger than it was with CPS-I.

Analysis of the data indicates that the polysaccharide-containing antigens of the *H. seropedicae* Z78 surface have a complex nature. Clearly, LPS, CPS-II, and CPS-I have individual antigenic determinants which EPS-I and EPS-II lack and which are recognized by $miniAbs_{LPS}$ and $miniAbs_{CPS\text{-}II}$. In LPS, CPS-II, and CPS-I, the $miniAbs_{EPS\text{-}II}$ detect antigenic determinants that were present in EPS-II and EPS-I.

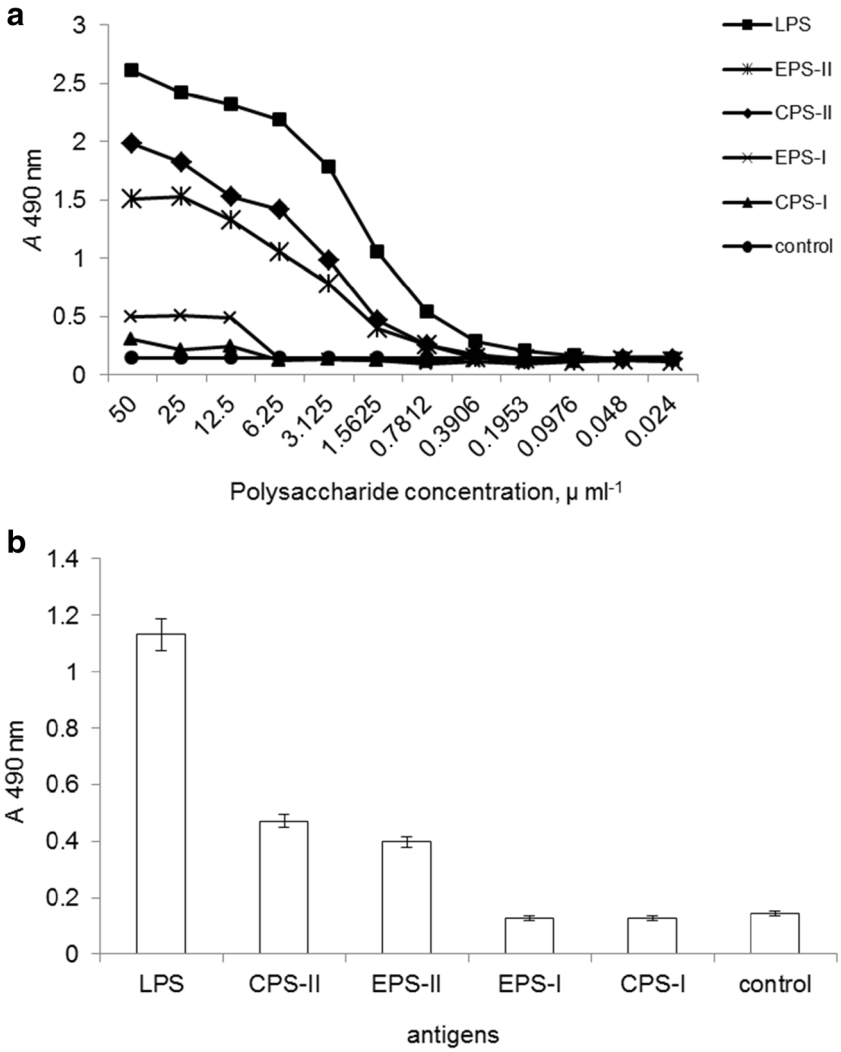

Fig. 1 a - ELISA of LPS, CPS-II, EPS-II, EPS-I, and CPS-I by using rabbit antibodies to glutaraldehyde-treated *H. seropedicae* cells. The wells in a polystyrene plate were coated with two-fold dilution of EPS-I, EPS-II, CPS-I, CPS-II, and LPS through simple adsorption; b - ELISA of LPS, CPS-II, EPS-II, EPS-I, and CPS-I by using rabbit antibodies to glutaraldehyde-treated cells with polysaccharide concentration 1.56 μ $ml^{-1.}$

Inhibition of the glycopolymer–miniAb interaction

We examined the inhibition of miniAbs by commercial rhamnose, galactose, glucose, and *N*-acetyl-D-glucosamine (all sugars part of LPS), as well as by the OPS and core oligosaccharide of *H. seropedicae* Z78 (Velichko et al. 2018). The negative control was commercial altrose. The monosaccharide concentration (10^{-4} M) was purposely chosen in excess of the concentration of the miniAbs. The concentration of $miniAbs_{LPS}$ was 1.2×10^{13} virions ml^{-1}, and that of LPS was 0.015 mg ml^{-1}.

The inhibition of the immunochemical reactions decreased in the order core oligosaccharide > rhamnose > OPS > glucose > galactose > *N*-acetyl-D-glucosamine (Fig. 3). Altrose did not affect the completeness of the LPS–miniAb reaction and did not inhibit the miniAbs. The extent of interaction of altrose-treated miniAbs with LPS was the same as in the nontreated control. The core oligosaccharide inhibited the $miniAbs_{LPS}$ completely; OPS inhibited them to a lesser extent; and glucose, galactose, and *N*-acetyl-D-glucosamine had equally intense inhibitory effects, which were greater than the effect of OPS.

Discussion

Knowledge concerning surface polymers is conducive to a better understanding of bacterial interactions with macrosymbionts. Studies of biopolymer structure are important from both basic and applied perspectives. The major, highly conserved structures, which are important for the immunochemical behavior of microorganisms, are LPS, CPS, and EPS (Holst et al. 1996; Ovodov 2006; Weidenmaier et al. Weidenmaier and Peschel 2008). Besides being implicated in the mechanical attachment of bacteria to the root surface, CPS and EPS ensure the error-free recognition of the plant host. The unique chemical structure of LPS, formed from three structurally different portions (lipid A, core oligosaccharide, and O polysaccharide), determines its broad

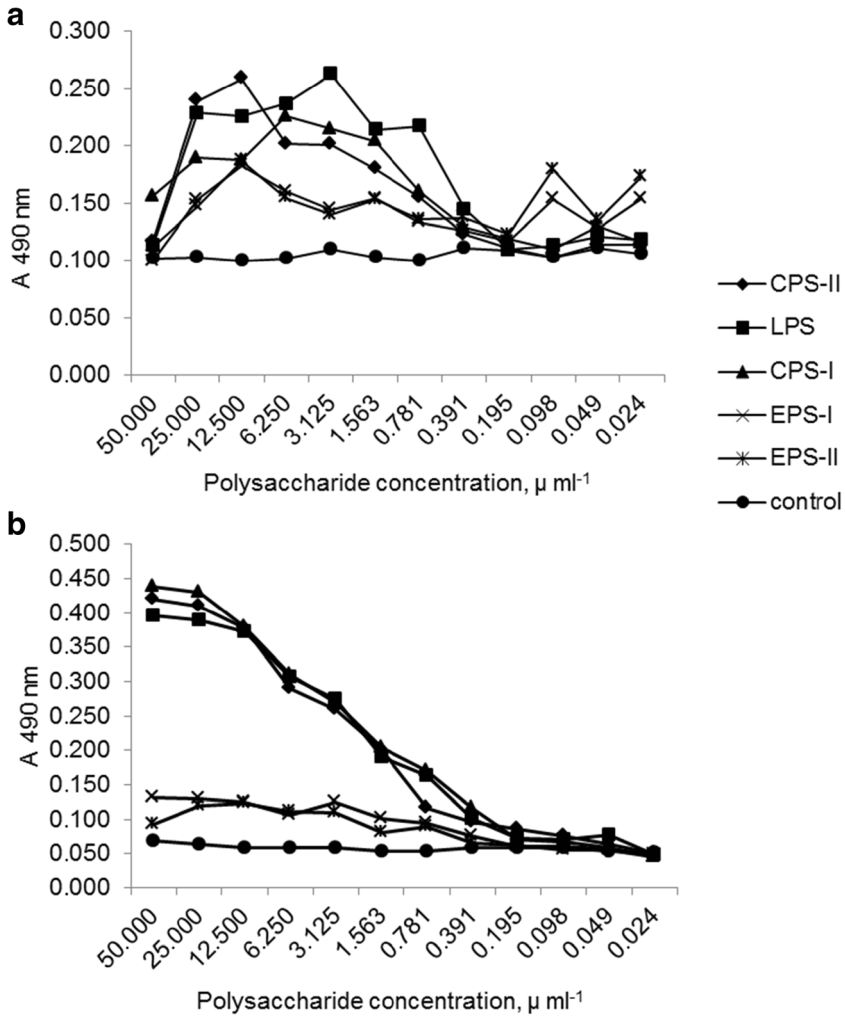

Fig. 2 ELISA of CPS-I, CPS-II, EPS-I, EPS-II, and LPS by using miniAbs$_{CPS-II}$ prepared after the first (a) and fourth (b) selection rounds

biological activity (Holst et al. 1996). Under certain conditions, some bacteria can produce LPS extracellularly. The capsular glycans of some bacteria are represented by LPS (Konnova et al. 1994; Whitfield and Roberts 1999; Smol'kina et al. 2010).

The fine mechanisms of *Herbaspirillum* interactions with host organisms have been understudied. Silva-Froufe et al. (2009) used polyclonal antibodies raised against whole bacterial cells to detect bacteria in the plant tissue interior. Antibodies to component II (nifH or Fe protein) of the nitrogenase complex from *Rhodospirillum rubrum* were used to evaluate the nitrogenase activity of endophytic herbaspirilla (Reinhold et al. 1987; James et al. 1997; Olivares et al. 1997; James et al. 2002).

In this context, it is of interest to obtain data that will permit judgment of the degree of heterogeneity, chemical makeup, and localization of the antigenic determinants of the *Herbaspirillum* surface glycopolymers. In addition, results from comparisons of the immunochemical specificities of LPS, CPS, and EPS may serve the needs of microbiology

Table 1 Comparison of the ELISA results for the polysaccharide-containing antigens of *H. seropedicae* Z78

Antigen	Mini*Abs$_{LPS}$	Mini*Abs$_{CPS-I}$	Mini*Abs$_{EPS-I}$	Mini*Abs$_{CPS-II}$	Mini*Abs$_{EPS-II}$
LPS	++++	+++	+++	+++	+++
CPS-I	++	++	++	++++	++
EPS-I	–	++	++	–	++
CPS-II	+++	+++	++++	+++	++++
EPS-II	–	+++	+++	–	+++

++++, maximal interaction; +++, strong interaction; ++, medium-strong interaction; –, no interaction

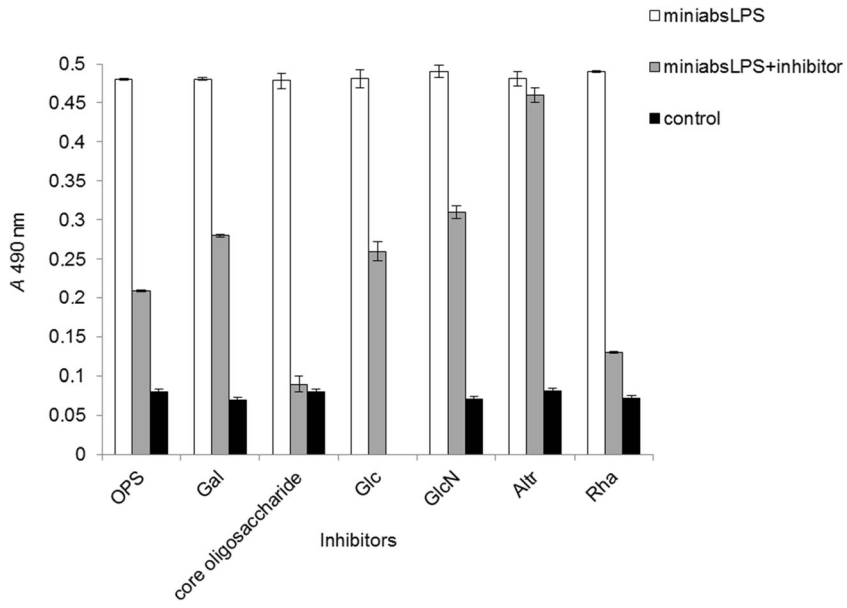

Fig. 3 Inhibitor effects on miniAbs$_{LPS}$, as evaluated by ELISA

and immunology. Because the CPS of *H. seropedicae* Z78 is an extracellular form of LPS [Smol´kina et al. 2012], the removal of the capsules from the cell surface was absolutely necessary to prevent contamination of the LPS preparations with the surface glycopolymers. The monosaccharide composition of *H. seropedicae* Z78 CPS-I, CPS-II, EPS-I, and EPS-II was described by us earlier [Smol´kina et al. 2012], as were the monosaccharide composition and the structure of *H. seropedicae* Z78 LPS [Velichko et al. 2018]. In this study, we used the following preparations: EPS-I, EPS-II, CPS-I, CPS-II, and LPS.

To infer about the presence or absence of common antigenic determinants in the EPS-I, EPS-II, CPS-I, CPS-II, and LPS of *H. seropedicae* Z78, we ran a comparative immunoassay with rabbit polyclonal and phagerecombinant antibodies to the surface glycopolymers of *H. seropedicae* Z78. Experiments with rabbit polyclonal antibodies to glutaraldehyde-treated whole cells of strain Z78 showed that the CPS-I, CPS-II, EPS-I, EPS-II, and LPS, had some antigenic differences. Glutaraldehyde modifies protein epitopes and makes impossible an immune response to native membrane proteins, enabling the preparation of antibodies against bacterial LPS. The antibodies so prepared specifically interact with the carbohydrate components of the bacterial surface glycopolymers. These results indicate that although the polysaccharide components of EPS-I and CPS-I containe the same sugars [Smol´kina et al. 2012], they lack common antigenic determinants. Similar findings have been reported elsewhere for structurally similar glycoconjugates of other bacteria in which serological differences were found. For instance, the K and O antigens of *Proteus mirabilis O*40 are structured similarly but differ serologically [Kenne & Lindberg, 1983]. Rabbit anti-LPS antibodies did not cross-react with any of the antigens used, including LPS.

Efforts to raise antibodies against purified LPS were unsuccessful, possibly owing to the structural peculiarities of *H. seropedicae* Z78 LPS. Previous work by us (Velichko et al. 2018) has found that the OPSrepeating unit in *H. seropedicae* Z78 consists of glycerol-1-phosphate substituted by residues of *N*-acetyl-D-glucosamine. Structures of this kind are typical of the teichoic acids of gram-positive bacteria (Naumova et al. 2001) and are rare in gram-negative bacteria (Kondakova et al. 2005; Zdorovenko et al. 2011; Shashkov et al. 2015). Many studies of teichoic acids in a range of microorganisms, including staphylococci, bacilli, pneumococci, lactobacilli, and listeria, have shown that these acids have antigenic properties and can induce immune responses (Baddiley and Davison 1961; Clark et al. 2000; Wicken and Knox 2016). An important condition for an immune response is the natural surroundings of teichoic acids in an intact cell or cell wall, because purified teichoic acids are nonimmunogenic. Antigenic properties may also be determined by the nature of the polyol and glycosyl substituents in a biopolymer (Naumova et al. 2001).

Very few reports have used scFv antibodies for immunochemical studies of bacterial glycopolymers. However, those reports show that such antibodies prove more sensitive that traditional monoclonal antibodies. Thus, Griep et al. (1998), using recombinant antibodies against the LPS of *Ralstonia solanacerum* (biovar 2, race 3), recorded 5×10^3 microbial cells in potato tuber extracts. For *Herbaspirillum*, this is the first time that miniAbs to the exopolysaccharides (EPS-I and EPS-II), capsular polysaccharides (CPS-I and CPS-II) and lipopolysaccharide (LPS) have been obtained.

The procedure that we used to increase miniAb specificity proved highly effective. From round to round, there were increases in the number of phage carrying specific variable domains to the corresponding antigens (Fig. 2). To look into the structure of the antigenic determinants, we inhibited the formation of antigen–antibody complexes with various competitive components of known chemical composition (Kabat and Mayer 1961). The results for the inhibition of LPS–miniAb precipitation with mono- and disaccharides suggest that these carbohydrates are part of the immunodominant sites of the O antigens.

The results obtained correlate well with the data on the monosaccharide composition of the antigens examined (Smol'kina et al. 2012; Velichko et al. 2018). LPS, CPS-II, and CPS-I contain rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine, which may form part of their antigenic determinants. This explains why the $miniAbs_{LPS}$, $miniAbs_{CPS-I}$, and $miniAbs_{CPS-II}$ not only interacted but also cross-reacted with LPS, CPS-II, and CPS-I. We speculate that EPS-II and EPS-I did not react with $miniAbs_{LPS}$ and $miniAbs_{CPS-II}$ because they contained no rhamnose and only trace amounts of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine.

The interaction of $miniAbs_{CPS-I}$ and $miniAbs_{CPS-II}$ with LPS, CPS-II, and CPS-I could be explained by the presence in them of *N*-acetyl-D-glucosamine and galactose. On the basis of the foregoing, we speculate that $miniAbs_{LPS}$ and $miniAbs_{CPS-II}$ should be more specific for the determinants containing rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine; $miniAbs_{CPS-I}$ and $miniAbs_{CPS-II}$ should be more specific for the determinants containing *N*-acetyl-D-glucosamine and galactose; and $miniAbs_{CPS-I}$ should be specific for the determinants including all the above sugars.

The $miniAbs_{LPS}$ were more specific for the core region of the LPS, in which rhamnose was the most abundant sugar, than they were specific for its O portion. These results are in harmony with our speculation that $miniAbs_{LPS}$ have the highest specificity for rhamnose-carrying determinants. The inhibition of $miniAbs_{LPS}$ by a wide range of sugars possibly indicates that the core oligosaccharide is highly branched and heterogeneous.

The use of highly specific miniAbs makes it possible to detect identical antigenic determinants in samples whose structures have not yet been examined, to compare those samples with polysaccharides of known structure, and to detect microorganisms and bioactive molecules.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This study was approved by the Committee of Experts of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS; record no. 1049). Animals were cared for and handled in accordance with the Guide for the Care and Use of Laboratory Animals, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the legislation of the Russian Federation. Informed consent was obtained from all human participants.

Informed consent Not applicable.

References

Asadi-Ghalehni M, Ghaemmaghami M, Klimka A, Javanmardi M, Navari M, Rasaee MJ (2015) Cancer immunotherapy by a recombinant phage vaccine displaying EGFR mimotope: an in vivo study. Immunopharmacol Immunotoxicol 37:274–279. https://doi.org/10.3109/08923973.2015.1027917

Baddiley J, Davison AL (1961) The occurrence and location of teichoic acid in lactobacillus. J Gen Microbiol 24:295–299. https://doi.org/10.1099/00221287-24-2-295

Bajerski F, Ganzert L, Mangelsdorf K, Lipski A, Busse H-J, Padur L, Wagner D (2013) *Herbaspirillum psychrotolerans* sp. nov., a member of the family *Oxalobacteraceae* from a glacier forefield. Int J Syst Evol Microbiol 63:3197–3203. https://doi.org/10.1099/ijs.0.046920-0

Baldani JI, Pot B, Kirchhof G, Falsen E, Baldani VL, Olivares FL, Hoste B, Kersters K, Hartmann A, Gillis M, Döbereiner J (1996) 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 46:802–810. https://doi.org/https://doi.org/10.1099/00207713-46-3-802

Batista MB, Teixeira CS, Sfeir MZT, Alves LPS, Valdameri G, Pedrosa FO, Sassaki GL, Steffens MBR, de Souza EM, Dixon R, Müller-Santos M (2018) PHB biosynthesis counteracts redox stress in *Herbaspirillum seropedicae*. Front Microbiol 9(472). https://doi.org/10.3389/fmicb.2018.00472

Beatty JD, Beatty BG, Vlahos WG (1987) Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay. J Immunol Methods 100:173–179. https://doi.org/10.1016/0022-1759(87)90187-6

Charlton KA, Moyle S, Porter AJ, Harris WJ (2000) Analysis of the diversity of a sheep antibody repertoire as revealed from a bacteriophage display library. J Immunol 164:6221–6229. https://doi.org/10.1016/S0956-5663(01)00192-0

Chemaly RF, Dantes R, Shah DP, Shah PK, Pascoe N, Ariza-Heredia E, Perego C, Nguyen DB, Nguyen K, Modarai F, Moulton-Meissner H, Noble-Wang J, Tarrand JJ, LiPuma JJ, Guh AY, MacCannell T, Raad I, Mulanovich V (2015) Cluster and sporadic cases of *Herbaspirillum* species infections in patients with cancer. Clin Infect Dis 60:48–54. https://doi.org/10.1093/cid/ciu712

Chen J, Su Z, Liu Y, Sandoghchian S, Zheng D, Wang S, Xu H (2011) *Herbaspirillum* species: a potential pathogenic bacteria isolated from acute lymphoblastic leukemia patient. Curr Microbiol 62:331–334. https://doi.org/10.1007/s00284-010-9703-5

Clark EE, Wesley I, Fiedler F, Promadej N, Kathariou S (2000) Absence of serotype-specific surface antigen and altered teichoic acid glycosylation among epidemic-associated strains of *Listeria monocytogenes*. J Clin Microbiol 38:856–859

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

Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize endophytic bacterial diversity as affected by soil cultivation history. Front Microbiol 9(484). https://doi.org/10.3389/fmicb.2018.00484

Dykman LA, Staroverov SA, Guliy OI, Ignatov OV, Fomin AS, Vidyasheva IV, Karavaeva OA, Bunin VD, Burygin GL (2012) Preparation of mini-antibodies to *Azospirillum brasilense* Sp245 surface antigens and their use for bacterial detection. J Immunoass Immunochem 33:115–127. https://doi.org/10.1080/15321819.2011.603775

Griep RA, van Twisk C, van Beckhoven JR, van der Wolf JM, Schots A (1998) Development of specific recombinant monoclonal antibodies against the lipopolysaccharide of *Ralstonia solanacearum* race 3. Phytopathology 88:795–803 https://doi.org/10.1094/PHYTO.1998.88.8.795

Holst O, Ulmer A, Brade H, Flad HD, Rietschel ET (1996) Biochemistry and cell biology of bacterial endotoxins. EMS Immunol Med Microbiol 16:83–104. https://doi.org/10.1111/j.1574-695X.1996.tb00126.x

James EK, Olivares FL, Baldani JI, Dobereiner J, Moench L (1997) *Herbaspirillum*, an endophytic diazotroph colonizing vascular tissue in leaves of *Sorghum bicolor*. J Exp Bot 48:785–789. https://doi.org/10.1093/jxb/48.3.785

James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PP, Olivares FL, Ladha JK (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant-Microbe Interact 15:894–906. https://doi.org/10.1094/MPMI.2002.15.9.894

Kabat E and Mayer M (1961) Experimental immunochemistry in: Kabat EA, Thomas CC (ed) 2nd edn. Springfield, USA, Blackwell Scientific Publications, pp 905

Kato G, Maruyama Y, Nakamura M (1980) Role of bacterial polysaccharides in the adsorption process of the *Rhizobium*-pea symbiosis. Ag Biol Chem 44:2843–2855. https://doi.org/10.1080/00021369.1980.10864422

Kenne L, Lindberg B (1983) Bacterial polysaccharides. In: Aspinall GO (ed) The polysaccharides. Academic Press, New York, pp 287–363

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

Konnova SA, Makarov OE, Skvortsov IM, Ignatov VV (1994) Isolation, fractionation and some properties of polysaccharides produced in a bound form by *Azospirillum brasilense* and their possible involvement in *Azospirillum* – wheat root interaction. FEMS Microbiol Lett 118:93–99. https://doi.org/10.1111/j.1574-6968.1994.tb06809.x

Lin SY, Hameed A, Arun AB, Liu YC, Hsu YH, Lai WA, Rekha PD, Young CC (2013) Description of *Noviherbaspirillum malth*ae gen. nov., sp. nov., isolated from an oil-contaminated soil, and proposal to reclassify *Herbaspirillum* soli, *Herbaspirillum aurantiacum*, *Herbaspirillum canariense* and *Herbaspirillum psychrotolerans* as *Noviherbaspirillum* soli comb. nov., *Noviherbaspirillum aurantiacum* comb. nov., *Noviherbaspirillum canariense* comb. nov. and *Noviherbaspirillum psychrotolerans* comb. nov. based on polyphasic analysis. Int J Syst Evol Microbiol 63:4100–4107. https://doi.org/10.1099/ijs.0.048231-0

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

Michael J, Oehler TR (2005) Lower extremity cellulitis and bacteremia with *Herbaspirillum seropedicae* associated with aquatic exposure in a patient with cirrhosis. Infect Dis Clin Pract 13:277–279

Müller-Seitz E, Jann B, Jann K (1968) Degradation studies on the lipopolysaccharide from *E. coli* O71:K1:H12. Separation and investigation of O-specific and core polysaccharides. FEBS Lett 1:311–314. https://doi.org/10.1016/0014-5793(68)80141-3C

Naumova IB, Shashkov AS, Tul'skaya EM, Streshinskaya GM, Kozlova YI, Potekhina NV, Evtushenko LI, Stackebrandt E (2001) Cell wall teichoic acids: structural diversity, species specificity in the genus *Nocardiopsis*, and chemotaxonomic perspective. FEMS Microbiol Rev 25:269–284. https://doi.org/10.1111/j.1574-6976.2001.tb00578.x

Newman MA, von Roepenack E, Daniels M, Dow M (2000) Lipopolysaccharides and plant responses to phytopathogenic bacteria. Mol Plant Pathol 1:25–31. https://doi.org/10.1046/j.1364-3703.2000.00004.x

Olivares FL, James EK, Baldani JI, Dobereiner J (1997) Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph *Herbaspirillum*. New Phytol 135:723–737. https://doi.org/10.1046/j.1469-8137.1997.00684.x

Ovodov YS (2006) Capsular antigens of bacteria. Capsular antigens as the basis of vaccines against pathogenic bacteria. Biochemistry (Mosc) 71:1175–1182. https://doi.org/10.1134/S0006297906090021

Pedrosa FO, Benelli EM, Yates MG, Wassem R, Monteiro RA, Klassen G, Steffens MB, Souza EM, Chubatsu LS, Rigo LU (2001) Recent developments in the structural organization and regulation of nitrogen fixation genes in *Herbaspirillum seropedicae*. J Biotechnol 91:189–195. https://doi.org/10.1016/S0168-1656(01)00343-1

Petrenko VA (2018) Landscape phage: evolution from phage display to nanobiotechnology. Viruses 10:311–318. https://doi.org/10.3390/v10060311

Reinhold B, Hurek T, Fendrik I, Pot B, Gillis M, Kesters K, Thielenmans S, De Ley J (1987) *Azospirillum halopraeferens* sp. nov., a nitrogen-fixing organism associated with the roots of *Kallar* grass (*Leptochloa fucsa* (L) Kunth). Int J Syst Bacteriol 37:43–51

Shashkov AS, Wang M, Turdymuratov EM, Hu S, Arbatsky NP, Guo X, Wang L, Knirel YA (2015) 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 404:124–131. https://doi.org/10.1016/j.carres.2014.11.014

Silva-Froufe LG, Boddey RM, Reis VM (2009) Quantification of natural populations of *Gluconacetobacter diazotrophicus* and *Herbaspirillum* spp. in sugar cane (Saccharum spp.) using different polyclonal antibodies. Braz J Microbiol 40:866–878. https://doi.org/10.1590/S1517-83822009000400018

Skvortsov M, Ignatov VV (1998) Extracellular polysaccharides and polysaccharide-containing biopolymers from *Azospirillum* species: properties and the possible role in interaction with plant roots. FEMS Microbiol Lett 165:223–229. https://doi.org/10.1111/j.1574-6968.1998.tb13150.x

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

Smith GP, Petrenko VA (1997) Phage display. Chem Rev 97:391–410. https://doi.org/10.1021/cr960065d

Smith GP, Scot JK (1993) Libraries of peptides and proteins displayed on filamentous phage. Methods Enzymol 217:228–257. https://doi.org/10.1016/0076-6879(93)17065-D

Smol'kina ON, Kachala VV, Fedonenko YP, Burygin GL, Zdorovenko EL, Matora LY, Konnova SA, Ignatov VV (2010) Capsular polysaccharide of the bacterium *Azospirillum lipoferum* Sp59b: structure and antigenic specificity. Biochemistry (Mosc) 75:606–613. https://doi.org/10.1134/S000629791005010X

Smol'kina ON, Shishonkova Velichko NS, Yurasov NA, Ignatov VV (2012) Capsular and extracellular polysaccharides of the diazotrophic rhizobacterium *Herbaspirillum seropedicae* Z78. Microbiology 81:317–323. https://doi.org/10.1134/S0026261712030113

Spilker T, Uluer AZ, Marty FM, Yeh WW, Levison JH, Vandamme P, Lipuma JJ (2008) Recovery of *Herbaspirillum* species from persons

with cystic fibrosis. J Clin Microbiol 46:2774–2777. https://doi.org/10.1128/JCM.00460-08

Tan MJ, Oehler RL (2005) Extremity cellulitis and bacteremia with *Herbaspirillum seropedicae* associated with aquatic exposure in a patient with cirrhosis. Infect Dis Clin Pract 13:277–279. https://doi.org/10.1097/01.idc.0000170026.41994.8d

Valdameri G, Alberton D, Moure VR, Kokot TB, Kukolj C, Brusamarello-Santos LCC, Monteiro RA, Pedrosa FO, de Souza EM (2017) *Herbaspirillum rubrisubalbicans*, a mild pathogen impairs growth of rice by augmenting ethylene levels. Plant Mol Biol 94:625–640. https://doi.org/10.1007/s11103-017-0629-1

Velichko NS, Surkina AK, Fedonenko YP, Zdorovenko EL, Konnova SA (2018) Structural peculiarities and biological properties of the lipopolysaccharide from *Herbaspirillum seropedicae* Z78. Microbiology 87:635–641. https://doi.org/10.1134/S002626171805017X

Weidenmaier C, Peschel A (2008) Teichoic acids and related cell-wall glycopolymers in Grampositive physiology and host interactions. Nat Rev Microbiol 6:276–287. https://doi.org/10.1038/nrmicro1861

Whitfield C, Roberts IS (1999) Structure, assembly and regulation of expression of capsules in *Escherichia coli*. Mol Microbiol 31:1307–1319. https://doi.org/10.1046/j.1365-2958.1999.01276.x

Wicken AJ, Knox KW (2016) Amphipathic antigens of oral microorganisms - immunogenicity and other biological properties. In: Cohen IR, Lajtha A, Lambris JD, Paoletti R, Rezaei N (eds) Advances in experimental medicine and biology. Plenum Press, New York, pp 1161–1167

Yirmiya R, Pollak Y, Morag M, Reichenberg A, Barak O, Avitsur R, Shavit Y, Ovadia H, Weidenfeld J, Morag A, Newman ME, Pollmächer T (2000) Illness, cytokines, and depression. Ann N Y Acad Sci 917:478–487. https://doi.org/10.1111/j.1749-6632.2000.tb05412.x

Zdorovenko EL, Varbanets LD, Brovarskaya OS, Valueva OA, Shashkov AS, Knirel YA (2011) Lipopolysaccharide of *Budvicia aquatica* 97U124: immunochemical properties and structure. Microbiology 80:372–377. https://doi.org/10.1134/S0026261711020196

Ziga ED, Druley T, Burnham CA (2010) *Herbaspirillum* species bacteremia in a pediatric oncology patient. J Clin Microbiol 48:4320–4321. https://doi.org/10.1128/JCM.01479-10

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