

Characterization of epitope specificity of *Proteus penneri* 7 lipopolysaccharide core region

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To extend the knowledge on the fragments of *Proteus penneri* lipopolysaccharide core regions, which determine the cross-reactions with specific antibodies, serological studies were performed by use of *P. penneri* 7 core-specific antiserum and *Proteus* sp. lipopolysaccharides. Different reactivity of the tested antiserum with three groups of antigens suggested differences in their core regions' epitope specificity. Comparing the results of the serological investigations with the previously determined structures of the core regions of the tested *P. penneri* lipopolysaccharides allowed distinguishing two potential tri- and tetrasaccharide epitopes and a third fragment which could not be determined precisely.

Keywords: *Proteus penneri*, lipopolysaccharide, epitope, core region

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INTRODUCTION

The lipopolysaccharide (LPS) core region of *Proteus penneri* strains is characterized by higher structural variability than that observed in other representatives of *Enterobacteriaceae*. Thus, twelve different structures of the distal part of *P. penneri* LPS core region and three structural variants of its proximal part have been determined, whereas only five structural types of the whole core region of *Escherichia coli* LPSs and two such types of *Salmonella enterica* LPSs have been identified (Vinogradov *et al.*, 2002b; Holst, 2007). It seemed interesting whether such high structural heterogeneity of *P. penneri* core region also refers to the number of its fragments responsible for the cross-reactions with specific antibodies. Up to date only a few epitopes have been determined in the *P. penneri* LPS core region (Palusiak *et al.*, 2008). Other published data concerning similar epitopes referred to other species of genus *Proteus*: *P. mirabilis* and *P. vulgaris*, and the epitopes were determined by use of antisera containing both O-polysaccharide- and core region-specific antibodies (Sidorczyk *et al.*, 2002b; Kolodziejska *et al.*, 2006). Using in this work an antiserum which contained only the latter type of antibodies enabled a more precise determination of exclusively the core region epitopes.

MATERIALS AND METHODS

Bacterial strains, lipopolysaccharides and antiserum. In all, 41 *Proteus* sp. strains were used. *P. penneri* 7 (O61) was a clinical isolate from the faeces of a patient from Missouri (USA). This strain and 26 others (includ-

ing: *P. penneri* 8 (O67), 13 (R form), 14 (O59), 15 (O52) and 34 (O65)) were kindly provided by Professor D. J. Brenner, Center for Diseases Control and Prevention in Atlanta (GA, USA). Three *P. penneri* strains were isolated from the urine of women with bacteriuria in a hospital in Łódź (Poland). One *P. penneri* and one *P. mirabilis* strain came from the collection of the Department of General Microbiology, University of Łódź (Poland). Five strains, including *P. penneri* 104 (O61), were from Dr. B. Holmes, Central Public Health Laboratory in London (UK) and one strain *P. penneri* 133 (O61) from Dr. G. Giammanco, Institute of Hygiene and Prevention Medicine in Catania (Italy). The remaining three strains were kindly provided by Dr. E. Falsen, Department of Clinical Bacteriology, Göteborg (Sweden) and Dr. B. Senior, Department of Medical Microbiology in Dundee (UK).

The cultivation of bacteria, isolation and saponification of the LPSs, the immunization procedure and obtaining of antiserum were carried out as was described in detail earlier (Westphal & Jann, 1965; Sidorczyk *et al.*, 2002b).

Serological assays. Non-absorbed *P. penneri* 7 antiserum was checked with a set of forty-one *Proteus* sp. LPSs in an enzymatic immunosorbent assay (ELISA). Homologous and cross-reactive LPSs were tested with this antiserum by Western blot technique. In this procedure LPS samples were prepared similarly to the method described elsewhere (Milnerowicz & Bizoń, 2010). The antiserum was absorbed with homologous and respective cross-reactive LPSs and checked with them in the hemagglutination procedure. The first two methods and the absorption procedure were carried out as described in detail elsewhere (Sidorczyk *et al.*, 2002b; Palusiak *et al.*, 2008). Antibody titre determination by ELISA was repeated three times for each system.

Hemagglutination procedure. Alkali-treated LPSs were used for sensitizing sheep red blood cells (SRBC) for 30 min at 37°C (200 µg LPS/0.2 ml SRBC). Such sensitized erythrocytes were washed twice with phosphate-buffered saline (PBS) pH 7.2 (15 mM NaH₂PO₄, 150 mM NaCl) and suspended in the same buffer containing 0.1% bovine serum albumin (BSA) to give 0.1% suspension. To serial twofold dilutions of antiserum, 50 µl PBS buffer with 0.1% BSA and 50 µl suspension of the sensitized SRBC were added to each well of the plate

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Abbreviations: Arap4N, 4-amino-4-deoxy-L-arabinopyranose; ELISA, enzymatic immunosorbent assay; GalA, galacturonic acid; GalN, galactosamine; GalNA, amides of GalA with aliphatic polyamines; Glc, glucose; GlcN, glucosamine; GlcNGly, 2-deoxy-2-glycylamino-D-glucose; Hep, L-glycero-D-manno-heptose; DD-Hep, D-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; LPS, lipopolysaccharide; P_{ET}, 2-aminoethyl phosphate.

(incubation for 1 h at 4°C and for 16 h at 37°C). The titre was established as the highest dilution of antiserum resulting in 50% hemagglutination (Schmidt *et al.*, 1970).

RESULTS

This work presents serological characterization of the *P. penneri* 7 LPS core region. First, rabbit polyclonal antiserum against heat-killed *P. penneri* 7 was absorbed with alkali-treated LPS from *P. penneri* strain 104, whose LPS has an O-polysaccharide chain serologically identical to that of *P. penneri* 7 LPS and a different core region (unpublished). That procedure gave an antiserum containing antibodies against the core region only. The elimination of the O-polysaccharide-specific antibodies from the antiserum was confirmed by Western blotting after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) of LPS preparations from the strains tested (Fig. 1). As expected, the tested antiserum did not react with the high-molecular mass O-polysaccharide containing LPS species of *P. penneri* 104 and 7 strains, which confirmed the efficiency of the absorption procedure. This serum will be further referred to as core-specific antiserum.

The reactivity of the tested antiserum was checked by ELISA with a set of forty-one *Protens* sp. LPSs selected on the basis of the results of previous serological studies conducted in the Department of General Microbiology (Zych *et al.*, 2000; 2005; Sidorczyk *et al.*, 2002a; Kondakova *et al.*, 2003; Drzewiecka *et al.*, 2004). The core-antiserum reacted with seven LPSs. The strongest and identical reactions were observed with *P. penneri* 7, 14 and 15 LPSs (reciprocal titre: 256 000), and a less strong one with *P. penneri* 13 (reciprocal titre: 16 000). LPSs of *P. penneri* 8, 34 and 133 bound the antibodies with the lowest but identical strength (reciprocal titre: 8 000).

In the Western blot, whose main aim was to show which region of LPSs was involved in the observed reactions, the tested antiserum clearly recognized the low-molecular-mass LPS species restricted to the core-lipid A moiety from the homologous and six cross-reactive *P. penneri* strains (Fig. 1). Similarly to the results obtained in ELISA, the strongest reactions were observed for *P. penneri* 7, 14 and 15 LPSs.

In the next step of the investigation, the core-antiserum was first absorbed with the applied seven LPSs and then tested with these LPS preparations in the hemagglutination procedure. Each time the antiserum was absorbed with the LPS of one *P. penneri* strain. The absorption of the tested antiserum with any of the *P. pen-*

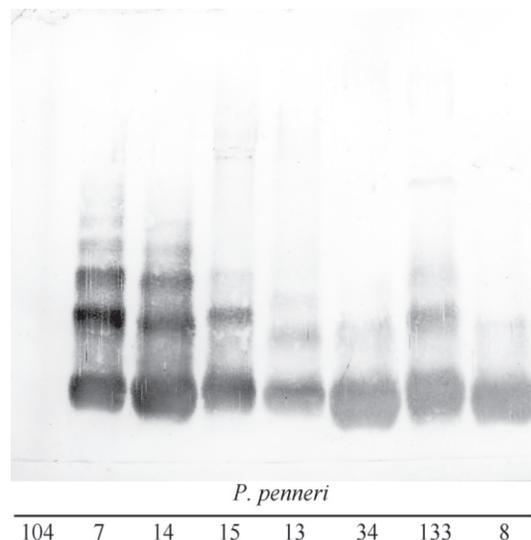


Figure 1. Western blot of *P. penneri* LPSs with core-specific antiserum against *P. penneri* 7.

The antiserum was diluted 1:100 in dot-blot-10% skimmed milk buffer. The reactions were developed with alkaline phosphate-conjugated goat anti-rabbit antibodies diluted 1:2000. Alkaline Phosphatase Conjugate Substrate Kit (Bio-Rad) was used.

neri 7, 14 or 15 LPSs completely abolished its reactivity with all the antigens tested, which suggests that the core regions of these three LPSs were serologically identical (Table 1). The absorption of antiserum with the second group of identically reactive antigens, *P. penneri* 8, 34 and 133 LPSs, caused a complete elimination of antibodies to these antigens and to *P. penneri* 13 LPS, and decreased the antiserum titres with *P. penneri* 7, 14 and 15 LPSs. This result indicates some differences between epitopes located in LPS core regions of *P. penneri* 7, 14, 15, 13 and those of the second group: *P. penneri* 8, 34 and 133.

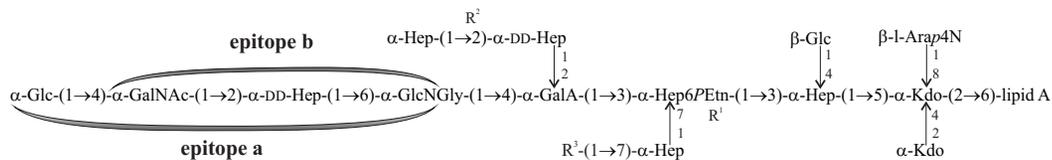
After the absorption of the core-antiserum with *P. penneri* 13 LPS, the reactivity with *P. penneri* 7, 14 or 15 was markedly decreased and only slightly decreased in the case of the other three antigens *P. penneri* 8, 34 and 133. The antibodies removed by this absorption from the antiserum probably bound to another common fragment located in the core region of all the mentioned LPSs. Accordingly, immunoglobulins that remained in the antiserum absorbed in this way recognized core region fragments which differ *P. penneri* 13 LPS and all the remaining LPSs tested.

Table 1. Hemagglutination of alkali-treated *P. penneri* LPSs with absorbed core-specific antiserum against *P. penneri* 7^{a,b}

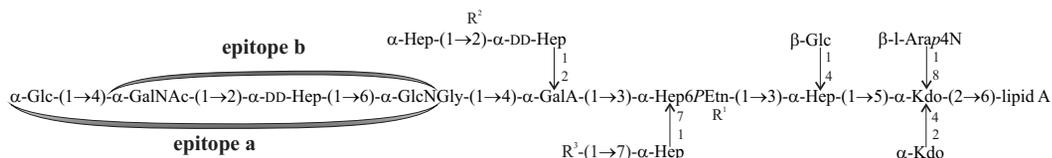
^aNon-absorbed core-specific antiserum was used as control; ^bData for homologous LPS are italicized.

Alkali-treated LPS used for absorption	Reciprocal titer of absorbed core-specific antiserum for alkali-treated LPS from <i>P. penneri</i> strains						
	7	14	15	8	34	133	13
Control	51 200	51 200	51 200	3 200	3 200	3 200	1 600
<i>P. penneri</i> 7	<100	<100	<100	<100	<100	<100	<100
<i>P. penneri</i> 14	<100	<100	<100	<100	<100	<100	<100
<i>P. penneri</i> 15	<100	<100	<100	<100	<100	<100	<100
<i>P. penneri</i> 8	12 800	12 800	12 800	<100	<100	<100	<100
<i>P. penneri</i> 34	12 800	12 800	12 800	<100	<100	<100	<100
<i>P. penneri</i> 133	12 800	12 800	12 800	<100	<100	<100	<100
<i>P. penneri</i> 13	1 600	1 600	1 600	800	800	800	<100

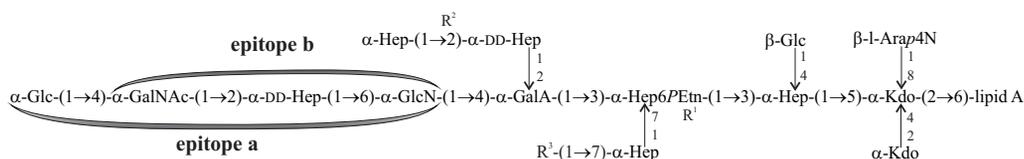
a. *P. penneri* 7 ($R^3=H$) (Vinogradov et al., 2002a)



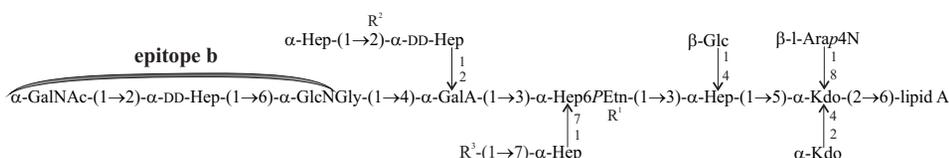
b. *P. penneri* 14 ($R^1=PEtn$ or H ; $R^3=H$ or β -GalNA) (Vinogradov et al., 2002a)



c. *P. penneri* 15 ($R^3=H$) (Vinogradov et al., 2002a)



d. *P. penneri* 8 ($R^3=H$) (Vinogradov et al., 2002a)



e. *P. penneri* 13 ($R^1=PEtn$ or H ; $R^3=H$ or β -GalAN) (Vinogradov & Sidorczyk, 2002)

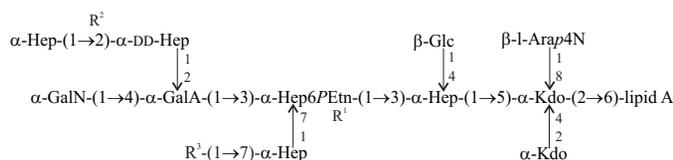


Figure 2. Structures of core-lipid A region of *P. penneri* LPSs

Common epitopes are indicated by arcs. Arap4N, 4-amino-4-deoxy-L-arabinopyranose; GalA, galacturonic acid; GalN, galactosamine; GalNA, amides of GalA with aliphatic polyamines; Glc, glucose; GlcN, glucosamine; GlcNGly, 2-deoxy-2-glycylamino-D-glucose; Hep, L-glycero-D-manno-heptose; DD-Hep, D-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; PEtn, 2-aminoethyl phosphate.

DISCUSSION

The outcome of the serological studies reported here can be referred to the previously determined chemical structures of the core regions of *P. penneri* 7, 14, 15, 8 and 13 LPSs (Fig. 2a, b, c, d, e) (Vinogradov & Sidorczyk, 2002; Vinogradov *et al.*, 2002a). Our analyses, especially the results of hemagglutination with antisera after their absorption with an appropriate LPS sample (Table 1), allowed suggesting that the cross-reactions of the tested antiserum were determined by three common fragments located in the core regions of homologous and respective cross-reactive LPSs. The first epitope, marked in Fig. 2 as epitope a, seems to be associated with the following tetrasaccharide fragment: α -Glc-

(1 \rightarrow 4)- α -GalNAc-(1 \rightarrow 2)- α -DDHep-(1 \rightarrow 6)- α -GlcN found in the distal part of *P. penneri* 7, 14 and 15 LPS core regions (Fig. 2a, b, c). Additionally, *P. penneri* 7 and 14 LPS core regions have a glycine residue attached to α -GlcN of epitope a. The lack of reaction of core-antiserum absorbed with *P. penneri* 15 LPS with LPSs which possess this amino acid in their core regions (*P. penneri* 7 and 14) suggests that the glycine residue does not play a role in the epitope specificity.

The second fragment referred to as epitope b, α -GalNAc-(1 \rightarrow 2)- α -DDHep-(1 \rightarrow 6)- α -GlcN, occupying distal part of *P. penneri* 7, 14, 15 and 8 LPS core regions might account for the respective cross-reactions observed in this work (Fig. 2a, b, c, d). This epitope might also be located in the LPS core regions of *P. penneri* 34 and 133,

whose core oligosaccharide structures remain unknown. This suggestion could be made only on the grounds of the identical serological reactivity of the tested antiserum with *P. penneri* 34, 133 and 8 LPSs observed in this work. It is worth mentioning that these data are also confirmed by the results of immunochemical studies obtained with *P. penneri* 8 anti-conjugate and anti-core sera, which also indicated the presence of epitope b in the *P. penneri* 8, 34 and 133 LPS core regions (Palusiak *et al.*, 2008). This fragment differs from epitope a by the lack of the terminal glucose residue. The higher antiserum titres with LPSs having the terminal glucose residue in their core regions (*P. penneri* 7, 14 and 15) compared with those obtained with LPSs which do not have it (*P. penneri* 8, and probably *P. penneri* 34 and 133) showed that this component may largely contribute to the epitope specificity of the homologous and *P. penneri* 14 and 15 antigens. The reaction of the tested antiserum after its absorption with *P. penneri* 13 LPS indicated the presence of a third epitope located in the core region of *P. penneri* 7 and the remaining LPSs. A comparison of the core region structures of *P. penneri* 7 and 13 LPSs showed that they share most components in the inner part of the core oligosaccharide and their distal parts of the core region are completely different (Fig. 2a, e). This fact indicates that this third epitope is associated with a fragment of the inner part of the core regions of all the investigated LPSs. However, the great similarity of the mentioned part of all LPSs core regions makes it impossible to identify the fragment acting as the third epitope. The most likely option is that α -Hep-(1 \rightarrow 2)- α -DD-Hep could play a role of such an epitope. That residue occupied the most distal fragment of the inner part of the core oligosaccharide of all LPSs studied (Fig. 2a, b, c, d, e) and that is why it may have been quite easily recognized by the specific antibodies. Apart from these assumptions, the third epitope located in the core regions of *P. penneri* 7 and other investigated LPSs remains unknown.

Gathering the information on the presence of common epitopes in *P. penneri* LPS core regions should be helpful in finding R antigens of LPS, necessary for preparing a vaccine protecting not only against one *Proteus* sp. strain but also effective with other strains for which it will be possible to obtain protective immunoglobulins of broad specificity.

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