ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2007, **55**, 347–352
PL ISSN 0004-069X

DOI 10.1007/s00005-007-0040-8

Serological and structural characterization of the O-antigens of the unclassified *Proteus mirabilis* strains TG 83, TG 319, and CCUG 10700 (OA)

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Received: 2006.12.11, **Accepted:** 2007.05.10, **Published online first:** 2007.10.01

Abstract

Introduction: Lipopolysaccharide (endotoxin, LPS) is an important potential virulence factor of *Proteus* rods. The serological specificity of the bacteria is defined by the structure of the O-polysaccharide chain (O-antigen) of the LPS. Until now, 76 O-serogroups have been differentiated among *Proteus* strains.
Materials and Methods: LPSs were isolated from *Proteus mirabilis* TG 83, TG 319, and CCUG 10700 (OA) strains by phenol/water extraction. Antisera were raised by immunization of rabbits with heat-killed bacteria. Serological investigations were performed using enzyme immunosorbent assay, passive immunohemolysis, inhibition of both assays, absorption of antisera, and Western blot.
Results: The cross-reactive epitope shared by these strains and *P. penneri* O72a,O72b is located on the O-polysaccharide and is most likely associated with an α-D-Glc*p*-(1→6)-β-D-Gal*p*NAc disaccharide fragment. The serological data indicated the occurrence of two core types in the LPSs studied, one characteristic for *P. mirabilis* TG 319 and CCUG 10700 (OA) and the other for *P. mirabilis* TG 83 and O57.
Conclusions: The serological and structural data showed that *P. mirabilis* TG 83, TG 319, CCUG 10700 (OA), and O57 have the same O-antigen structure and could be qualified to the *Proteus* O57 serogroup.

Key words: *Proteus*, lipopolysaccharide, O-serogroup, serological classification.

Abbreviations: EIA – enzyme immunosorbent assay, LPS – lipopolysaccharide, PIH – passive immunohemolysis, CCUG – Culture Collection of the University of Goeteborg.

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INTRODUCTION

Gram-negative bacteria of the genus *Proteus* are widely distributed in the environment, such as soil, water, and sewage, and represent a part of the normal microflora of human and animal intestines. They are opportunistic pathogens which in favorable conditions cause intestine and urinary tract infections that may lead to serious complications, such as acute or chronic pyelonephritis, the formation of bladder and kidney stones, and catheter obstruction [15, 19]. In addition, *Proteus* strains may be a source of wound, burn, skin, nose, and throat infections [10]. *P. mirabilis* also plays an etiopathogenic role in rheumatoid arthritis [21]. The genus *Proteus* is currently divided into five species, including *P. vulgaris*, *P. mirabilis*, *P. penneri*, *P. hauseri*, and *P. myxofaciens*, as well as three unnamed *Proteus* genomospecies 4, 5, and 6 [10, 11].

Lipopolysaccharide (endotoxin, LPS) is an important potential virulence factor of *Proteus* [14]. The serological specificity of the bacteria is defined by the structure of the O-polysaccharide chain (O-antigen) of the LPS. Based on the O-antigens, strains of two species, *P. vulgaris* and *P. mirabilis*, were originally classified into 49 O-serogroups [2]. Our serological studies combined with structural elucidation of the O-polysaccharides resulted in reclassification of some of the existing 49

O-serogroups and the creation of 18 new O-serogroups (O52, O56, O58–O73) for the third medically important species, *P. penneri,* as well as *P. hauseri*, *P. myxofaciens*, and *Proteus* genomospecies [1, 15, 24, 25]. Similar studies were also performed with *P. mirabilis* and *P. vulgaris* strains from the collections of Penner and Hennessy [12] and Larsson et al. [9]. As a result, about half of them (O8, O23, O34, O40, O65, O69) were classified into the existing serogroups and a further six serogroups (O50, O53, O54, O55, O74, O75) were proposed for the others [3–6, 13, 22, 23].

Here we report the serological characterization of the LPSs of three unclassified *P. mirabilis* strains, TG 83, TG 319 [12], and CCUG 10700 (OA) [9], all of which are candidates for the *Proteus* serogroup O57.

MATERIALS AND METHODS

Bacterial strains and growth

Proteus mirabilis TG 83 and TG 319 were kindly provided by Prof. J. L. Penner (Department of Medical Genetics, University of Toronto, Canada). *P. mirabilis* OA strain CCUG 10700 as well as *P. myxofaciens* strain CCUG 18769 were obtained from the Culture Collection of the University of Goeteborg, Goeteborg, Sweden. Twenty-four strains of *P. penneri* came from the Collection of the Department of General Microbiology, University of Łódź, Poland. The *P. hauseri* strain was kindly provided by C. M. O'Hara and D. J. Brenner (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). *P. vulgaris* (27) and *P. mirabilis* (39) strains were from the Czech National Collection of Type Cultures (CNCTC, National Institute of Public Health, Prague, Czech Republic). The bacteria were cultivated under aerobic conditions on nutrient broth (BTL, Łódź, Poland). Dry bacterial mass was obtained as described elsewhere [8].

Isolation and degradation of the LPS

LPSs were obtained from bacterial cells by hot phenol/water extraction [20] and purified using a cold aqueous 50% CCl_3CO_2H precipitation procedure as described elsewhere [27].

Rabbit antisera and serological assays

Polyclonal *P. mirabilis* OA, TG 319, and TG 83 O-antisera were obtained by immunization of rabbits with heat-inactivated bacteria according to a published procedure [26]. Passive immunohemolysis test (PIH), enzyme immunosorbent assay (EIA), inhibition of these two tests, as well as absorption experiments were carried out as described elsewhere [17]. SDS/PAGE, electrotransfer of the LPS from gels to nitrocellulose sheets, and immunostaining were performed according to a published procedure [17].

RESULTS AND DISCUSSION

In order to reveal a possible serological relatedness of *P. mirabilis* TG 83, TG 319, and CCUG 10700 (OA) to each other and to other *Proteus* strains, O-antisera against these three strains were tested in PIH and EIA with the LPSs of 94 *Proteus* strains, including 40 *P. mirabilis*, 27 *P. vulgaris*, and 24 *P. penneri* strains as well as one strain each of *P. myxofaciens*, *P. hauseri* and *Proteus* genomospecies 4. Only five LPSs were cross-reactive, namely those of *P. mirabilis* TG 83, TG 319, CCUG 10700, O57 and, in addition, *P. penneri* 4 (O72a,72b). The data of the tests and the amount of each antigen necessary for the inhibition of the reactions in PIH and EIA are given in Table 1.

The strongest cross-reactivity with all O-antisera was observed for the LPSs of *P. mirabilis* TG 83, TG 319, and CCUG 10700 and O57. It was on the same level as the reactivity of the homologous LPS, suggesting serological identity of all four strains. The LPS of *P. penneri* O72a,72b showed a weaker cross-reactivity and a weaker inhibiting activity when tested in the homologous systems with all the O-antisera used.

In Western blot (Fig. 1), all O-antisera clearly recognized slow migrating bands of both homologous and heterologous LPS, and the banding patterns of *P. mirabilis* TG 83, TG 319, CCUG 10700, and O57 were almost identical. These bands correspond to high-molecular-mass LPS species with O-polysaccharides consisting of a large number of repeating units, and hence the cross-reactive epitope(s) is located on the O-polysaccharide. The recognition by heterologous O-antisera of fast migrating bands of *P. mirabilis* TG 83 and O57 LPS, corresponding to the core-lipid A moiety, was different from that of TG 319 and CCUG 10700. No binding was observed for the low-molecular-mass LPS species of *P. penneri* O72a,72b.

For a more detailed epitope characterization, the antigens were tested in PIH with O-antisera after absorption with various LPSs (Table 2). The reactivity of the O-antisera with all the tested antigens was completely abolished when they were absorbed with the homologous LPS. Absorption of O-antisera with LPS from either *P. mirabilis* TG 319 or CCUG 10700 removed all cross-reactive antibodies against the other LPS of the set. In contrast, absorption with *P. mirabilis* TG 83 and O57 LPS of O-antisera against *P. mirabilis* TG 319 or CCUG 10700 left a small fraction of antibodies specific to the homologous LPS (titer 1:800/1600). This finding and the Western blot data (see above) allowed the suggestion that this fraction recognized an epitope(s) in the LPS core region, which seems to be different in TG 83, O57, and two other strains.

Absorption of all O-antisera with the LPS of *P. penneri* O72a,72b decreased the level of antibodies from the titer 1:25,600/51,200 to 1:12,800, thus confirming the presence in all the LPSs studied of an epitope common to the *P. penneri* O72a,72b LPS. As revealed by the Western blot data (see above), this epitope is located in the O-polysaccharide region.

Table 1. Reactivity of O-antisera against *P. mirabilis* TG 83, TG 319, and CCUG 10700 (OA) strains with the *Proteus* LPS[a, b]

LPS from	Reciprocal titer for the LPS		Minimal inhibitory dose (ng) of the LPS	
	PIH	EIA	PIH	EIA
P. mirabilis TG 83 O-antiserum				
P. mirabilis TG 83	*51 200*	*512 000*	*2*	*2*
P. mirabilis TG 319	51 200	512 000	2	2
P. mirabilis CCUG 10700	51 200	512 000	2	2
P. mirabilis O57	51 200	512 000	2	2
P. penneri O72a,72b	6 400	64 000	250	500
P. mirabilis TG 319 O-antiserum				
P. mirabilis TG 83	25 600	256 000	2	2
P. mirabilis TG 319	*25 600*	*256 000*	*2*	*2*
P. mirabilis CCUG 10700	25 600	256 000	2	2
P. mirabilis O57	25 600	256 000	2	2
P. penneri O72a,72b	3 200	32 000	500	1 000
P. mirabilis CCUG 10700 (OA) O-antiserum				
P. mirabilis TG 83	51 200	512 000	2	2
P. mirabilis TG 319	51 200	512 000	2	2
P. mirabilis CCUG 10700	*51 200*	*512 000*	*2*	*4*
P. mirabilis O57	51 200	512 000	2	2
P. penneri O72a,72b	3 200	8 000	500	500

[a] LPS and alkali-treated LPS were used as antigen in EIA and PIH, respectively.
[b] Data for homologous LPS are italicized.

The serological results were in agreement with the structural analysis of the O-polysaccharides isolated from the LPS of *P. mirabilis* TG 83 and TG 319. Comparison of their ^{13}C-NMR spectra with each other and with that of *P. mirabilis* ATCC 49995 (serogroup O57) studied earlier [18] using the "finger-print" method showed that they are essentially identical, and hence the three O-polysaccharides have the same structure shown in Fig. 2 (for the assignment of the ^{1}H- and ^{13}C-NMR spectra, see published data [18]). Based on the combined serological and structural data, we consider *P. mirabilis* TG 83, TG 319, and CCUG 10700 (OA) as candidates for the *Proteus* O57 serogroup as the next three representatives.

A comparison of the O-polysaccharide structures (Fig. 2) allowed the suggestion that the cross-reactive

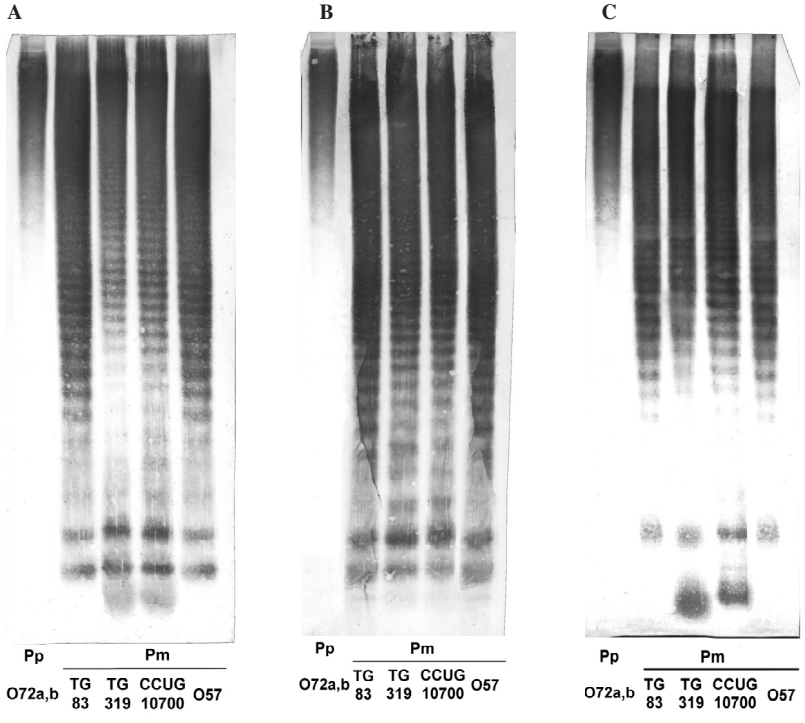


Fig. 1. Western blot of *Proteus* LPS with O--antisera against *P. mirabilis* TG 83 (**A**), TG 319 (**B**) and CCUG 10700 (**C**). Pm and Pp stand for *P. mirabilis* and *P. penneri*, respectively.

Table 2. Passive immunohemolysis data of alkali-treated LPS with absorbed O-antisera against *P. mirabilis* TG 83, TG 319, and CCUG 10700 (OA) strains[a, b]

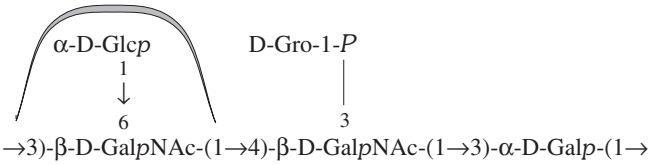
O-antisera absorbed with the alkali-treated LPS from	Reciprocal titer of absorbed O-antisera with the alkali-treated LPS from				
	P. mirabilis				*P. penneri*
	TG 83	TG 319	CCUG 10700	O57	O72a,72b
P. mirabilis TG 83 O-antiserum					
Control	51 200	51 200	51 200	51 200	6 400
P. mirabilis TG 83	*<100*	*<100*	*<100*	*<100*	*<100*
P. mirabilis TG 319	800	<100	<100	800	<100
P. mirabilis CCUG 10700	800	<100	<100	800	<100
P. mirabilis O57	<100	<100	<100	<100	<100
P. penneri O72a,72b	12 800	12 800	12 800	12 800	<100
P. mirabilis TG 319 O-antiserum					
Control	25 600	25 600	25 600	25 600	3 200
P. mirabilis TG 83	<100	800	800	<100	<100
P. mirabilis TG 319	*<100*	*<100*	*<100*	*<100*	*<100*
P. mirabilis CCUG 10700	<100	<100	<100	<100	<100
P. mirabilis O57	<100	<100	<100	<100	<100
P. penneri O72a,72b	12 800	12 800	12 800	12 800	<100
P. mirabilis CCUG 10700 O-antiserum					
Control	51 200	51 200	51 200	51 200	3 200
P. mirabilis TG 83	<100	1 600	1 600	<100	<100
P. mirabilis TG 319	<100	<100	<100	<100	<100
P. mirabilis CCUG 10700	*<100*	*<100*	*<100*	*<100*	*<100*
P. mirabilis O57	<100	<100	<100	<100	<100
P. penneri O72a,72b	12 800	12 800	12 800	12 800	<100

[a] Sheep red blood cells were used as a control.
[b] Data for homologous LPS are italicized.

epitope of *P. penneri* O72a,72b [7, 16] is associated with an α-D-Glc*p*-(1→6)-β-D-Gal*p*NAc disaccharide fragment shared by all the strains studied.

In conclusion, 1) the serological and structural data showed that *P. mirabilis* TG 83, TG 319, CCUG 10700 (OA), and O57 have the same O-antigen structure and may be considered candidates for the *Proteus* O57 serogroup; 2) the cross-reactive epitope shared by these strains and *P. penneri* O72a,72b is located on the O-polysaccharide and is most likely associated with an α-D-Glc*p*-(1→6)-β-D-Gal*p*NAc disaccharide fragment; 3) the serological data pointed to the occurrence of two

Proteus mirabilis O57 [18, this work]



Proteus penneri O72a, 72b (*P. penneri* 1, 4) [16, 24]

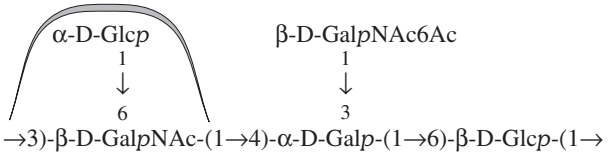


Fig. 2. Chemical structures of the O-specific polysaccharides of the investigated *P. mirabilis* and *P. penneri* O72a,72b LPS with signed epitope responsible for cross-reactions.

core types in the LPSs studied, one characteristic for *P. mirabilis* TG 319 and CCUG 10700 (OA) and the other for *P. mirabilis* TG 83 and O57.

Acknowledgment: This work was supported by the State Committee for Scientific Research (KBN, Poland, grant No. 2 PO5A 086 26) and the Council on Grants of the President of the Russian Federation for the Support of Young Russian Scientists (project No. MK-2204.2006.4). The authors thank Janusz Włodarczyk for excellent technical assistance in the preparation of the manuscript.

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