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Characterization and serological classification of O-specific polysaccharide of *Proteus mirabilis* TG 276-90 from *Proteus* serogroup O34

Katarzyna Kołodziejska[1], Małgorzata Siwińska[1], Krystyna Zych[1], Antoni Różalski[2] and Zygmunt Sidorczyk[1]

[1] Department of General Microbiology, Institute of Microbiology and Immunology, University of Łódź, Poland
[2] Department of Immunobiology of Bacteria, Institute of Microbiology and Immunology, University of Łódź, Poland

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Abstract

Introduction: Gram-negative bacteria of the genus *Proteus* from the family *Enterobacteriaceae* are currently divided into the five species *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, and *P. myxofaciens* and three unnamed *Proteus* genomospecies 4, 5, and 6. They are important facultative human and animal pathogens which, under favorable conditions, cause mainly intestinal and urinary tract infections, sometimes leading to serious complications such as acute or chronic pyelonephritis and the formation of bladder and kidney stones. In this study we report on the serological properties of the lipopolysaccharide (LPS) of *P. mirabilis* TG 276-90, whose O-polysaccharide chemical structure was described earlier.
Materials and Methods: LPS and alkali-treated LPS of a few serologically related *Proteus* strains and O-antisera against *P. mirabilis* TG 276-90 and CCUG 4669 (O34) were used. Serological characterization of *P. mirabilis* TG 276-90 O-specific polysaccharide was done using enzyme immunosorbent assay, passive immunohemolysis test (PIH), inhibition of these tests, SDS/PAGE and Western blot techniques, absorption of rabbit polyclonal O-antisera, and repeated PIH test.
Results: Structural and serological investigations showed that the O-polysaccharides of *P. mirabilis* TG 276-90 and *P. vulgaris* O34 are identical and that their LPSs differ only in epitopes in the core part. Therefore these two strains could be classified into the same *Proteus* O34 serogroup.
Conclusions: The serological data showed that the β-D-Gal*p*NAc-(1→4)-α-D-Gal*p*NAc disaccharide is an important epitope of the *P. mirabilis* TG 276-90 and *P. vulgaris* O34 LPSs, shared by the *P. mirabilis* O16 and *P. vulgaris* TG 251 LPSs. It is responsible for cross-reactions with *P. mirabilis* TG 276-90 and *P. vulgaris* O34 O-antisera.

Key words: *Proteus mirabilis*, *Proteus vulgaris*, lipopolysaccharide, O-serogroup, O-antigen.

Abbreviations: EIA – enzyme immunosorbent assay, LPS – lipopolysaccharide, O-PS – O-specific polysaccharide, PIH – passive immunohemolysis test.

Corresponding author: Prof. Zygmunt Sidorczyk, Ph.D., Department of General Microbiology, Institute of Microbiology and Immunology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland. tel.: +48 42 635-44-67, fax: +48 42 665-58-18, e-mail: zsidor@biol.uni.lodz.pl

INTRODUCTION

The genus *Proteus* includes the five named species *P. mirabilis*, *P. vulgaris*, *P. myxofaciens*, *P. penneri*, and *P. hauseri* and three unnamed *Proteus* genomospecies 4, 5, and 6 [7, 8]. The medically important bacteria of genus *Proteus* are usually found in soil, water, and sewage and are also part of normal fecal microflora. They are important human opportunistic pathogens that cause a variety of community-acquired and nosocomial diseases, including urinary tract infections, septicemia, and wound infections [4, 13]. Several morphological and biochemical features of *Proteus* species and a number of virulence factors, such as swarming growth, fimbriae, flagella, enzymes (urease, IgA proteases), siderophores, hemolysins, and endotoxic lipopolysaccharide (LPS), have been described [1, 5, 6, 12]. LPS and capsular polysaccharides of *Proteus* are considered to play an important role in the infections and in the formation of kidney and bladder stones [2, 11].

The serological specificity of *Proteus* strains is defined by the chemical structure of the O-specific polysaccharide chain (O-PS, O-antigen) of the LPS. Based on the O-antigens, the strains of two species, *P. vulgaris* and *P. mirabilis*, were classified into 49 O-serogroups [3]

and later into 11 additional serogroups [9]. Fifteen further O-serogroups have been proposed for the third medically important species, *P. penneri* [5, 14, 18].

In an attempt to correlate surface antigen structures with the immunospecificity of bacteria, we investigated the structural and immunochemical characteristics of the O-PS chains (O-antigens) of the LPSs of the three medically important *Proteus* species *P. mirabilis*, *P. vulgaris*, and *P. penneri* [5, 18]. Here we report on the serological properties of *Proteus mirabilis* TG 276-90 LPS, whose chemical structure of the O-polysaccharide repeating unit was described earlier [10]. Serological investigations support the conclusions drawn from the structural studies to classify the *P. mirabilis* TG 276-90 strain into the *Proteus* O34 serogroup.

MATERIALS AND METHODS

Bacterial strains

P. mirabilis TG 276-90 and *P. vulgaris* TG 251 were kindly donated by Prof. Dr. John L. Penner, Department of Medical Genetics, University of Ontario, Canada. *P. vulgaris* CCUG 4669 (O34) and *P. myxofaciens* CCUG 18769 (O60) came from the Culture Collection of the University of Goeteborg (Sweden). *P. mirabilis* O16 (PrK 31/57) as well as another 38 *P. mirabilis* and 27 *P. vulgaris* strains were purchased from the Czech National Collection of Type Cultures (CNCTC, National Institute of Public Health, Prague, Czech Republic). Twenty-four *P. penneri,* one *P. genomospecies*, and one *P. hauseri* strain were kindly provided by D. J. Brenner and C. M. O'Hara (Center for Disease Control and Prevention, Atlanta, Georgia, USA).

Cultivation of bacteria, isolation of the LPS, and serological assays

The bacteria were cultivated under aerobic conditions in nutrient broth (BTL, Łódź, Poland). The bacterial masses were harvested at the end of the logarithmic growth phase, centrifuged, washed with water, and lyophilized. The LPS was isolated from dried bacterial cells by hot phenol/water extraction [17] and purified by treatment with cold 50% aqueous trichloroacetic acid, followed by dialysis of the supernatant [20]. Alkali-treated LPS was prepared by saponification with 0.25 M NaOH (56°C, 2 h) and precipitation with ethanol. Purified LPS was used as antigen in the enzyme immunosorbent assay (EIA), polyacrylamide gel electrophoresis (SDS/PAGE), and Western blot techniques, and alkali-treated LPS in the passive immunohemolysis (PIH) test, inhibition of PIH, and absorption of antisera. All the tests were performed according to the procedures described in detail elsewhere [15].

Rabbit antisera

The polyclonal O-antisera were obtained by immunization of New Zealand white rabbits with heat-inactivated bacteria (100°C, 2.5 h) as described previously [19].

RESULTS AND DISCUSSION

In this study we the present the serological and immunochemical characterization of a LPS from the *P. mirabilis* strain described in the J. L. Penner collection as TG 276-90 [9]. Structural analysis of the O-polysaccharide from this strain showed the same structure of the repeating unit as that of *P. vulgaris* CCUG 4669 (O34) [10]. Therefore, in the serological investigations performed in this study, the second O-antiserum, that against *P. vulgaris* O34, was also included.

LPSs from 93 strains representing all the *Proteus* O-serogroups (39 *P. mirabilis*, 27 *P. vulgaris*, 24 *P. penneri*, 1 *P. myxofaciens*, 1 *Proteus* genomospecies 4, 1 *P. hauseri*) were tested in PIH and EIA with polyclonal O-antisera against *P. mirabilis* TG 276-90 and *P. vulgaris* O34. The cross-reactivities of both O-antisera were observed for four LPSs, i.e. of *P. mirabilis* TG 276-90 and O16 and of *P. vulgaris* O34 and TG 251 (Table 1).

In PIH and EIA, both O-antisera reacted equally strongly with homologous LPSs. A weaker reaction was

Table 1. Reactivity of O-antisera against *P. mirabilis* TG 276-90 and *P. vulgaris* O34 with *Proteus* LPSs

LPS from *Proteus* strain	Reciprocal titer for LPS in		Minimal inhibitory dose (ng) of the LPS in	
	PIH	EIA	PIH	EIA
P. mirabilis TG 276-90 O-antiserum				
P. mirabilis TG 276-90	*51200*	*1024000*	*1*	*2*
P. vulgaris O34	51200	512000	2	4
P. mirabilis O16	6400	64000	62.5	125
P. vulgaris TG 251	6400	32000	125	250
P. vulgaris O34 O-antiserum				
P. mirabilis TG 276-90	51200	1024000	2	2
P. vulgaris O34	*51200*	*512000*	*2*	*2*
P. mirabilis O16	6400	64000	125	250
P. vulgaris TG 251	3200	64000	500	500

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

observed with the heterologous *P. mirabilis* O16 and *P. vulgaris* TG 251 LPSs. Accordingly, the homologous LPSs were also the strongest inhibitors of the reaction in both PIH and EIA, whereas the heterologous LPSs (*P. mirabilis* O16 and *P. vulgaris* TG 251) showed weaker inhibitory activity.

In Western blot, both *P. mirabilis* TG 276-90 and *P. vulgaris* O34 O-antisera clearly recognized both high-molecular-mass O-polysaccharide-containing LPS species and low-molecular-mass LPS species restricted to the core-lipid A moiety from homologous strains (Fig. 1A, B). The heterologous LPSs (*P. mirabilis* O16 and *P. vulgaris* TG 251) reacted significantly more weakly, and this referred only to high-molecular-mass LPS species.

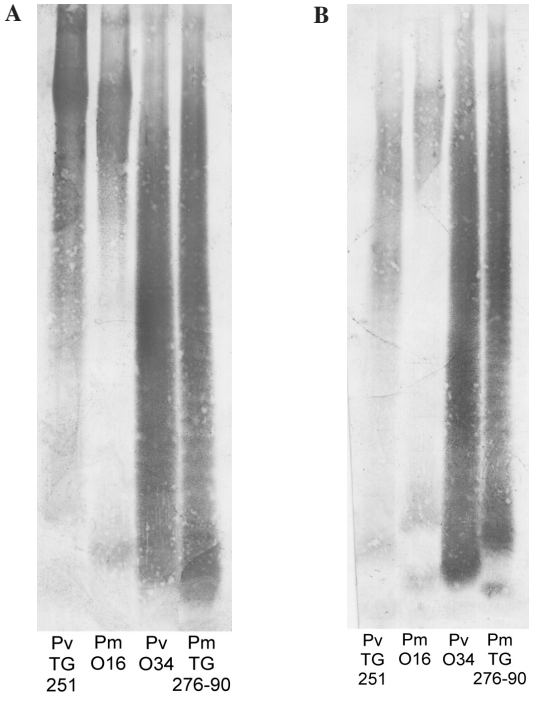

Fig. 1. Western blot of LPSs from *Proteus* strains with O-antisera against *P. mirabilis* TG 276-90 (**A**) and *P. vulgaris* O34 (**B**). Pm and Pv stand for *P. mirabilis* and *P. vulgaris*, respectively.

Absorption of *P. mirabilis* TG 276-90 and *P. vulgaris* O34 O-antisera by homologous LPSs completely abolished their reactivity with all the tested antigens (Table 2). Absorption of these O-antisera by the same antigens (having identical structure of the repeating unit of O-PS [10]) but in heterological systems left small fractions of antibodies, recognizing most probably different epitopes localized in the core part of their LPS.

Neither of the O-antisera absorbed by the heterologous *P. mirabilis* O16 and *P. vulgaris* TG 251 LPSs reacted with both antigens, which suggests the presence of the same fragments in their LPSs reacting as an epitope with the O-antisera used. Such data indicate the presence of two different kinds of epitopes in the O-PS of the investigated LPSs. The serological data are consistent with the chemical data described earlier [10] which demonstrated the structural identity of O-PS from the two investigated *P. mirabilis* TG 276-90 and *P. vulgaris* O34 strains and allowed their classification into the same *Proteus* O34 serogroup.

On the basis of serological studies, two kinds of antibodies were detected in *P. mirabilis* TG 276-90 and *P. vulgaris* O34 O-antisera. The first kind reacted with epitopes localized in the core part of *P. mirabilis* TG 276-90 and *P. vulgaris* O34 LPS, while the other recognized an epitope localized in the O-PS part of the LPSs of all the investigated strains.

Comparison of the known chemical structures of the O-polysaccharides investigated in this study with the obtained serological results suggests the existence of an epitope responsible for the serological reaction (Fig. 2). This epitope consists of the β-D-Gal*p*NAc-(1→4)-α-D-Gal*p*NAc disaccharide and is present in all four investigated LPSs.

The presence in both O-antisera, after their absorption by heterologous antigens, of significant fractions of

Table 2. PIH of alkali-treated *Proteus* LPSs with absorbed O-antisera against *P. mirabilis* TG 276-90 and *P. vulgaris* O34

O-antisera absorbed by the alkali-treated LPS from *Proteus* strain	Reciprocal titer of absorbed O-antiserum for the alkali-treated LPS from *Proteus* strain			
	P. mirabilis TG 276-90	*P. vulgaris* O34	*P. mirabilis* O16	*P. vulgaris* TG 251
P. mirabilis TG 276-90 O-antiserum				
SRBC/Control	51200	51200	6400	6400
P. mirabilis TG 276-90	<100	<100	<100	<100
P. vulgaris O34	1600	<100	<100	<100
P. mirabilis O16	25600	25600	<100	<100
P. vulgaris TG 251	25600	25600	<100	<100
P. vulgaris O34 O-antiserum				
SRBC/Control	51200	51200	6400	3200
P. mirabilis TG 276-90	6400	<100	<100	<100
P. vulgaris O34	<100	<100	<100	<100
P. mirabilis O16	25600	25600	<100	<100
P. vulgaris TG 251	25600	25600	<100	<100

Sheep red blood cells were used as a control.

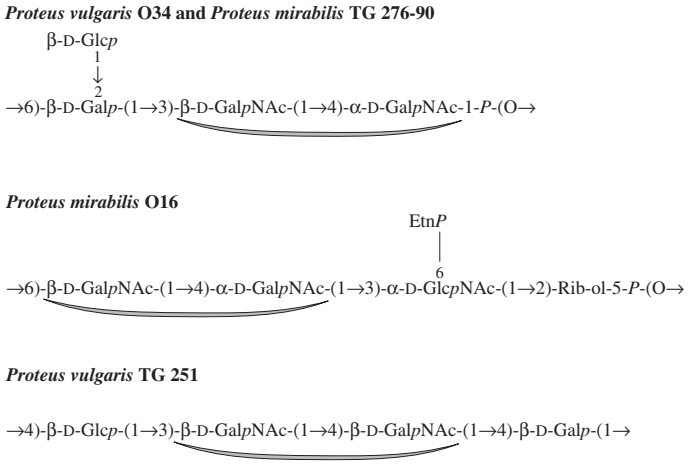

Fig. 2. Structure of the O-SPs of cross-reactive *P. vulgaris* O34 [10], *P. mirabilis* TG 276-90 (this work), *P. mirabilis* O16 [16], and *P. vulgaris* TG 251 (unpublished data) LPSs with a putative common epitope are shown by an arc.

antibodies reacting with homologous *P. mirabilis* TG 276-90 and *P. vulgaris* O34 LPSs suggests that the distinct part of the O-polysaccharide structures, i.e. a lateral Glc residue and 1-phosphate, also seems to play the role of a particularly specific minor epitope. The remaining antibodies in *P. mirabilis* TG 276-90 and *P. vulgaris* O34 O-antisera, after their absorption by the same LPSs but in heterological systems (Table 2), recognized epitopes localized in the core part of their LPS, which, additionally, are most probably different in both the investigated LPSs (Fig. 1).

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