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Serological classification and epitope specificity of *Proteus penneri* S29 lipopolysaccharide

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: Gram-negative bacteria of genus *Proteus* are common human intestinal and urinary tract pathogens. In the genus *Proteus* there are four clinically important named species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, and *P. hauseri*, and three unnamed *Proteus* genomospecies: 4, 5, and 6. The clinical significance of *P. penneri*, described in 1982 as a new species, is poorly documented. The aim of this work is serological characterization and classification of a ceftriaxone-susceptible *P. penneri* S29 strain isolated from a 34-year-old patient with postneurosurgical meningitis. In this characterization we will also include a ceftriaxon-resistant strain, *P. penneri* R15, isolated from the same patient after 12 days' treatment with ceftriaxon and other antibiotics.

Materials and Methods: Rabbit polyclonal O-antisera were obtained against these two strains and purified lipopolysaccharides (LPS) were extracted from the bacterial mass of the *P. penneri* S29 and R15 strains. In the serological investigations the following tests were used: enzyme immunosorbent assay (EIA), passive immunohemolysis (PIH), inhibition of these tests, absorption of rabbit O-antisera with the respective LPS, and repeated PIH, SDS/PAGE, and Western blot techniques.

Result The serological studies of the LPS extracted from both *P. penneri* strains showed the identity of both preparations of O-polysaccharides from LPS. In *P. penneri* S29 O-antiserum, four different types of antibodies were described and characterized.

Conclusions Both investigated *P. penneri* S29 and R15 strains were classified to the *Proteus* O31ab serogroup.

Key words: ***Proteus penneri*** • **lipopolysaccharide** • **O-serogroup** • **epitope** • **serological classification**

Abbreviations: **EIA** – enzyme immunosorbent assay, **LPS** – lipopolysaccharides, **PAGE** – polyacrylamide gel electrophoresis, **PIH** – passive immunohemolysis.

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INTRODUCTION

Bacteria of the genus *Proteus* are opportunistic pathogens which, under favorable conditions, cause urinary tract infections leading to severe complications, such as acute and chronic pyelonephritis, formation of bladder and kidney stones, and catheter obstruction[15, 16]. The genus *Proteus* includes 5 named species, i.e. *P. mirabilis*, *P. vulgaris*, *P. myxofaciens*, *P. penneri*, *P. hauseri*, and the three unnamed *Proteus* genomspecies 4, 5, and 6[12, 13]. *P. penneri* was described in 1982 as a new species[3], previously known as *P. vulgaris* indol-negative. These bacteria are isolated mainly from the urine of patients with urinary tract abnormalities and from abdominal wounds after bowel resections[3, 8]. *P. penneri* strains also cause urosepsis[10], subcutaneous[2], and nosocomial infections[1]. More recently, *Proteus* infections caused by some species resistant to a variety of antibiotics have been described[11, 20]. The serological specificity of *Proteus* strains is defined by the chemical structure of the O-specific polysaccharide chain (O-antigen) of the lipopolysaccharide (LPS, endotoxin). Based on the O-antigens, strains of two species, *P. mirabilis* and *P. vulgaris*, were classified first into 49 *Proteus* O-serogroups[4, 9] and later into 12 additional O-serogroups[14, 17]. Fifteen further O-serogroups have been proposed for the strains of *P. penneri*[5, 18, 24]. In this study we report the serological characterization of two *P. penneri* S29 and R15 isolates[11] from a patient with postneurosurgical meningitis treated with antibiotics. Classification of these strains into the *Proteus* O31ab serogroup and the LPS epitopes responsible for cross-reactions are described.

MATERIALS AND METHODS

Bacterial strains and growth

A *P. penneri* S29 isolate, susceptible to ceftriaxon, from a cerebral abscess and *P. penneri* R15, resistant to ceftriaxon, from the cerebrospinal fluid from the same 34-year-old patient after 12 days' treatment with various antibiotics[11] were kindly provided by N. Liassine (Central Laboratory of Bacteriology, University Hospital, Geneva, Switzerland). Thirty-nine *P. mirabilis* and 27 *P. vulgaris* strains were from the Czech National Collection of Type Cultures (CNCTC, National Institute of Public Health, Prague, Czech Republic). Twenty-four *P. penneri,* one *Proteus* genomospecies, and one *P. hauseri* strains were kindly provided by D. J. Brenner and C. M. O'Hara (Center for Disease Control and Prevention, Atlanta, Georgia, USA). A *P. myxofaciens* strain (CCUG 18769) was received from E. Falsen [Cultures Collection University of Goeteborg (CCUG), Goeteborg, Sweden]. Dry bacterial mass was obtained from aerated culture as described previously[7].

Antigens and serological assays

LPS were obtained by the extraction of bacterial mass with hot phenol/water[21] and purified with aqueous 50% trichloroacetic acid at 4°C followed by dialysis of the supernatant[26]. Alkali-treated LPS were prepared by saponification of LPS with 0.25 M NaOH (56°C, 2 h) followed by precipitation with ethanol. Purified LPS were used as antigens in the enzyme immunosorbent assay (EIA), SDS/polyacrylamide gel electrophoresis (SDS/PAGE), and Western blotting and alkali-treated LPS in the passive immunohemolysis (PIH) test, inhibition of PIH, and absorption of antisera. All tests were carried out according to procedures described in detail elsewhere[19].

Rabbit antisera

Polyclonal O-antisera were obtained by immunization of rabbits with heat-inactivated bacteria of *P. penneri* S29 and 28, as described previously[25].

RESULTS AND DISCUSSION

Serological classification

LPS of a number of *Proteus* strains with known O-polysaccharide structure were tested with polyclonal rabbit O-antiserum against *P. penneri* S29 by EIA to reveal possible serological relatedness of these bacteria. Of the 93 tested *Proteus* LPS, including those of 39 *P. mirabilis*, 27 *P. vulgaris*, 24 *P. penneri*, one *P. myxofaciens*, one *P. hauseri*, and one *Proteus* genomospecies, only 6 were cross-reactive, namely those of *P. penneri* 19, 26, 28, 31, and 62 and of *P. vulgaris* OX2 and OX19. The strongest cross-reactivity of *P. penneri* S29 O-antiserum with *P. penneri* 28 LPS was on the same level as with the homologous *P. penneri* S29 LPS, which suggested a possible antigenic identity of both strains. Therefore, *P. penneri* 28 O--antiserum was also included in the subsequent detailed serological investigation, which involved the PIH test, EIA, inhibition of these tests, absorption of the O-antisera with those LPS, and repeated PIH as well as Western blotting (Table 1, Fig. 1). Data obtained for the LPS of the two *P. penneri* strains S29 and 28 were essentially identical in all the tests used. These results were supported by structural investigations of *P. penneri* S29 and R15 LPS, which revealed identical repeating units of O-specific polysaccharides of LPS of these strains to those of *P. penneri* 28 LPS described in literature[6]. Therefore, we propose

Table 1. Reactivity of O-antisera against *P. penneri* S29 and 28 with both these LPS

LPS from *Proteus* strain	Reciprocal titer for LPS in		Minimal inhibitory dose (ng) of the LPS in		Reciprocal titer (in PIH) of antisera absorbed with the alkali-treated LPS from *P. penneri*	
	PIH	EIA	PIH	EIA	S29	28
P. penneri S29 O-antiserum						
P. penneri S29	51 200	102 400	1	4	<100	<100
P. penneri 28	25 600	256 000	4	32	<100	<100
P. penneri 28 O-antiserum						
P. penneri S29	51 200	102 400	2	4	<100	<100
P. penneri 28	51 200	512 000	8	16	<100	<100

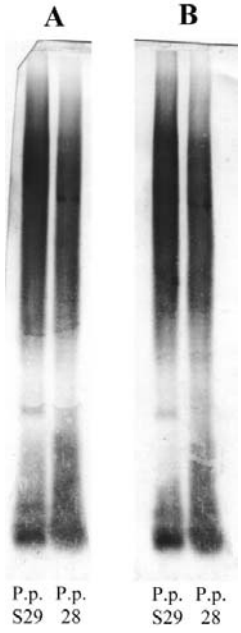


Figure 1. Reactivity of O-antisera against *P. penneri* S29 (**A**) and 28 (**B**) with LPS of the investigated *Proteus* strains in Western blot.

classifying the *P. penneri* S29 and R15 strains to the *Proteus* O31ab serogroup, in which, until now, the *P. penneri* 28 strain has been the only representative.

Epitope characterization of P. penneri S29 LPS

The reactivity of *P. penneri* S29 O-antiserum with the 6 cross-reacting *Proteus* LPS listed at the beginning was checked using the same serological tests (Table 2). The strongest reaction with the investigated O-antiserum and the highest inhibitory activity was shown by the homologous *P. penneri* S29 LPS. Of the heterologous LPS, *P. penneri* 62 and *P. vulgaris* OX2 and OX19 showed the strongest reactions as well as inhibition of the homologous system.

Table 2. Reactivity of *P. penneri* S29 O-antiserum with investigated *Proteus* LPS[a]

LPS from *Proteus* strain	Reciprocal titer for LPS in		Minimal inhibitory dose (ng) of the LPS in	
	PIH	EIA	PIH	EIA
P. penneri S29	*51 200*	*1 024 000*	*1*	*0.5*
P. penneri 19	1 600	16 000	>1 000	>1 000
P. penneri 26	3 200	16 000	>1 000	>1 000
P. penneri 31	800	8 000	>1 000	>1 000
P. penneri 62	12 800	64 000	62.5	125
P. vulgaris OX2	1 600	32 000	1 000	>1 000
P. vulgaris OX19	1 600	32 000	1 000	>1 000

[a] Data for the homologous LPS are italicized.

In Western blot, *P. penneri* S29 O-antiserum showed strong reactivity with the slowly migrating bands of homologous *P. penneri* S29 LPS and heterologous *P.*

Table 3. Passive immunohemolysis of the alkali-treated *Proteus* LPS with absorbed O-antiserum against *P. penneri* S29[ab]

O-antiserum absorbed with the alkali-treated LPS from *Proteus* strain	Reciprocal titer of absorbed O-antiserum for the alkali-treated LPS from *Proteus* strain						
	P. penneri					*P. vulgaris*	
	S29	19	26	31	62	OX2	OX19
SRBC (control)	51 200	1 600	3 200	800	12 800	1 600	1 600
P. penneri S29	*<100*	*<100*	*<100*	*<100*	*<100*	*<100*	*<100*
P. penneri 19	25 600	<100	3 200	800	3 200	1 600	1 600
P. penneri 26	25 600	1 600	<100	800	12 800	<100	<100
P. penneri 31	51 200	1 600	3 200	<100	12 800	1 600	1 600
P. penneri 62	25 600	<100	3 200	800	<100	1 600	1 600
P. vulgaris OX2	25 600	1 600	800	800	12 800	<100	<100
P. vulgaris OX19	25 600	1 600	800	800	12 800	<100	<100

[a] Non-absorbed O-antiserum was used as a control. [b] Data for the homologous LPS are italicized.

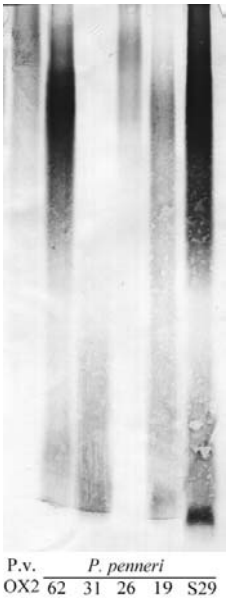
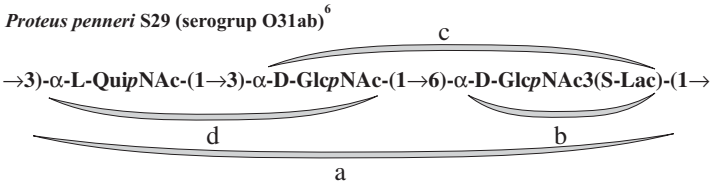


Figure 2. Western blot of the investigated *Proteus* LPS with *P. penneri* S29 O-antiserum.

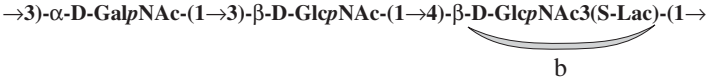
penneri 62 LPS, and weaker reactivity with *P. penneri* 19 and 26 and *P. vulgaris* OX2 and OX19 LPS (Fig. 2). These bands correspond to high-molecular-mass LPS species, consisting of the core lipid A moiety with O-chain polysaccharide attached. Weaker reactions of this O-antiserum with low-molecular-mass bands of *P. penneri* S29, 19, 31, and 62, corresponding to an LPS moiety without the O-chain polysaccharide, were also observed. This O-antiserum recognized the low-molecular-mass bands only of *P. penneri* 31 LPS.

In order to define epitope specificity, the antigens were tested by PIH with *P. penneri* S29 O-antiserum (Table 3). The reactivity of the antiserum with all the tested antigens was completely abolished when it was absorbed with the homologous LPS. Further experiments with heterologous LPS revealed two sets of similar antigens, the first being *P. penneri* 19 and 62 LPS, and the second *P. penneri* 26 and *P. vulgaris* OX2 and OX19 LPS. The absorption of O-antiserum with one strain of the each set (*P. penneri* 26 and 62) completely removed all cross-reactive antibodies against the other LPS of the set. The opposite, absorption with *P. penneri* 19, *P. vulgaris* OX2 and OX19 LPS only decreased the reactivity level with the remaining *P. penneri* 26 and 62 LPS. In both cases, absorption did not change the reactivity of O-antiserum with the other LPS used. The absorption of the O-antiserum with *P. penneri* 31 LPS did not influence the reactivity with other antigens except the homologous one, which indicated the antigenic distinction of this LPS and supported the result obtained in Western blot analysis (Fig. 2).

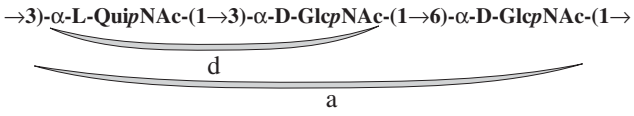
***Proteus penneri* S29 (serogrup O31ab)**[6]



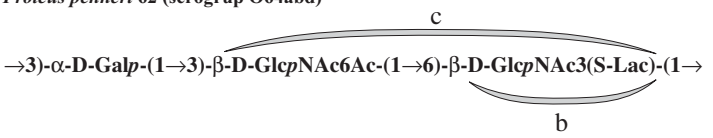
***Proteus penneri* 19 (serogrup O64abc)**[23]



***Proteus penneri* 26 (serogrup O31a)**[6]



***Proteus penneri* 62 (serogrup O64abd)**[23]



***Proteus vulgaris* OX2 (serogrup O2)**[23]

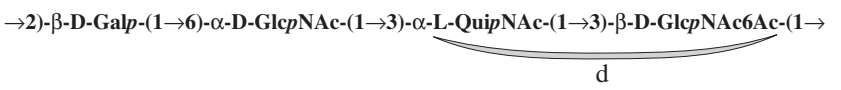


Figure 3. Chemical structures of O--specific polysaccharides of the investigated *Proteus* LPS with the signed epitopes responsible for serological reactions.

a **α-L-Qui*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→6)-α-D-Glc*p*NAc** – in *P. penneri* S29 and 26 LPS

b **D-Glc*p*NAc-(S-Lac)** – in *P. penneri* S29, 19 and 62 LPS

c **D-Glc*p*NAc-(1→6)-α-D-Glc*p*NAc3(S-Lac)** – in *P. penneri* S29 and 62 LPS

d **α-L-Qui*p*NAc-(1→3)-α-D-Glc*p*NAc** – in *P. penneri* S29, 26 and *P.vulgaris* OX2 and OX19 LPS

Figure 4. Common epitopes in the investigated *Proteus* LPS.

A comparison of these serological investigations with the chemical structures of the repeating units of O--specific polysaccharides of cross-reacting LPS described in the literature suggests the presence of four types of antibodies in *P. penneri* S29 O-antiserum which recognize the LPS epitopes (Fig. 3). Common epitopes in O-specific polysaccharides of the investigated *Proteus* LPS responsible for their serological reactions with *P. penneri* S29 are listed in Fig. 4. Furthermore, in the investigated O-antiserum a fifth independent fraction of antibodies recognizing an epitope present in the core part of *P. penneri* 31 LPS was found. Its nature, as well as the structure of the whole core part of that LPS, remains unknown.

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