

## Serological Studies of *Proteus penneri* Strains Determining Qualification to Appropriate O-serogroup

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### Abstract

Our Department of General Microbiology created a wide collection of *P. penneri* isolates and classified most of them into 19 different O-serogroups. This work describes the classification of 12 remaining *P. penneri* strains. The lipopolysaccharides extracted from *P. penneri* strains were tested in an enzyme-linked immunosorbent assay (ELISA) with selected O-antisera against *Proteus* sp. strains. Homologous and cross-reacting systems were checked in: passive immunohemolysis (PIH), inhibition of ELISA and PIH and Western blot procedure. These studies led to the qualification of tested *P. penneri* strains to five *Proteus* sp. O-serogroups, thus completing the serological classification of the whole collection.

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

*Proteus* bacteria are Gram-negative, peritrichously flagellated rods belonging to the *Enterobacteriaceae* family and capable of swarming growth on solid media. These bacteria are human opportunistic pathogens that in favourable conditions may cause numerous infections, among which the urinary tract infection is the most common (Penner, 1992). Currently, the genus *Proteus* consists of five named species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, *P. myxofaciens* and three species without a specific name *P. genomospecies* 4, 5 and 6 (O'Hara *et al.*, 2000). The fifth named species of the genus, *P. myxofaciens*, has been recently proposed to be classified into a new genus, *Cosenzaea* gen. nov. (Giammanco *et al.*, 2011).

*Proteus* sp. strains have been divided into O-serogroups mainly on the grounds of the serological specificity of the O-polysaccharide part of their lipopolysaccharides. This classification scheme founded by Kauffmann (1966) and Perch included 49 O-serogroups consisting of *P. mirabilis* and *P. vulgaris* species only. More O-serogroups were added to this scheme by Penner and Hennessy (1980) (11 O-serogroups) and Larsson *et al.* (1978) (6 O-serogroups) and they also contained only *P. vulgaris* and/or *P. mirabilis* species. The above-mentioned scheme was completed also with the third *Proteus* species, *P. penneri* – first described and named by Hickman in 1982 (Hickman *et al.*, 1982). A wide and

unique collection of these strains has been created in the Department of General Microbiology (University of Łódź, Poland) and comprises 133 *P. penneri* strains isolated from diverse clinical sources from different sites in the world. The serological and chemical studies of *P. penneri* LPSs have led to classifying 19 *P. penneri* strains to the existing *Proteus* O-serogroups and the other 97 *P. penneri* strains to the new selected O-serogroups consisting of the strains of this species only (Drzewiecka *et al.*, 2004; Knirel *et al.*, 2000; Knirel *et al.*, 2011; Kondakova *et al.*, 2003a, 2003b; Sidorczyk *et al.*, 2002a, 2002b; Shashkov *et al.*, 2000; Zych *et al.*, 2000a, 2000b, 2005a, 2005b). Four of 133 *P. penneri* strains appeared to be rough forms without the O-polysaccharide part in their LPSs (Sidorczyk and Zych, 1993; Zych and Sidorczyk, 1989). Only one strain from this collection, *P. penneri* 97, remained without a defined O-serogroup. Other authors proposed to classify this strain to a new separate *Proteus* O-serogroup since the antiserum against this strain did not react strongly with any LPSs from strains representing all known *Proteus* O-serogroups (Drzewiecka *et al.*, 2004). Unfortunately, the antiserum against this strain appeared to contain core-specific antibodies mainly (*P. penneri* 97 LPS has very short O-polysaccharide chains) and there was no similar LPS to compare with.

To complete the classification of 128 *P. penneri* strains the next 12 *P. penneri* strains were selected for this work.

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Table I  
The strains tested

The strain name	The isolation sources (place)	Provided by:
<i>P. penneri</i> 3	urine (Cansas)	Professor D.J. Brenner, Center for Diseases Control and Prevention in Atlanta (USA)
<i>P. penneri</i> 5	feces (Texas)	
<i>P. penneri</i> 6	urine (Louisiana)	
<i>P. penneri</i> 7*	feces (Missouri)	
<i>P. penneri</i> 9	urine (Georgia)	
<i>P. penneri</i> 12*	urine (Alaska)	
<i>P. penneri</i> 14*	bronchial secretion (Georgia)	
<i>P. penneri</i> 17	urine (New Jersey)	
<i>P. penneri</i> 23	unknown (Zurich)	
<i>P. penneri</i> 40*	unknown (Toronto)	
<i>P. penneri</i> 47*	feces (Stockholm)	Dr. E. Falsen, Department of Clinical Bacteriology, Göteborg (Sweden)
<i>P. penneri</i> 135	unknown (Paris)	Dr. G. Giammanco, Institute of Hygiene and Prevention Medicine in Catania (Italy)

\* LPSs of these strains were also used as antigens in the work concerning the *P. penneri* LPS core region (Palusiak *et al.*, 2008). The authors mentioned the O-serogroup numbers of these strains (in the materials and methods section) on the basis of the unpublished partial results of preliminary studies to justify the choice of antigens.

All tested strains (Table I) were collected in the Department of General Microbiology (University of Łódź). *P. penneri* 12 strain (American Type Culture Collection (ATCC) 33519) is a type strain of *P. penneri* species (Hickman *et al.*, 1982).

All polyclonal rabbit *Proteus* sp. O-antisera came from the Department of General Microbiology and they were obtained as described in another article (Sidorczyk *et al.*, 2002c).

The bacteria were grown under aerobic conditions in liquid nutrient broth containing 0.1% glucose (BTL, Łódź, Poland) at 37°C. The bacterial masses were harvested after 18 h of incubation, killed with phenol, centrifuged, washed twice with water and lyophilized in order to obtain dry bacterial cells.

Isolation of the LPSs from lyophilized bacterial cells of smooth strains was performed according to the procedures described in detail elsewhere (Westphal and Jann, 1965).

Alkali-treated LPSs used for PIH and antisera absorption procedures were prepared by saponification of LPSs with 0.25 M NaOH (2 h, 56°C) and followed by precipitation with ethanol.

All serological assays used in this work including: ELISA, Western blot procedure after sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of LPSs, passive immunohemolysis (PIH), inhibition of ELISA and PIH and absorption of antisera procedure

were described in detail elsewhere (Palusiak *et al.*, 2008; Sidorczyk *et al.*, 2002c). In PIH, sheep red blood cells were coated with a maximum concentration of alkali-treated LPSs (200 µg/0.2 ml of sheep red blood cells).

The serological characterization of the *Proteus penneri* 3, 5, 6, 7, 9, 12, 14, 17, 23, 40, 47 and 135 strains is presented. In the preliminary studies the reactivity of all tested LPSs was checked in ELISA with the selective polyclonal rabbit antisera specific to the *Proteus* sp. strains representing all defined O-serogroups of the genus. Only strong reactions (reciprocal titres ranging from 1 : 256.000 to 1 : 1.024.000) were taken into account. They were observed in four heterologous sets: *P. penneri* 6 and 12 LPSs with the antiserum against *P. penneri* 11 strain (O58); *P. penneri* 7 and 40 LPSs with *P. penneri* 52 (O61) and *P. penneri* 62 (O64 a,b,d) antisera, respectively. LPS of *P. penneri* 17 reacted with the antiserum against *P. vulgaris* 17/57 (O8) to a lower reciprocal titre (1 : 32.000) (Table II). Due to the lack of the sera reactions with the remaining tested LPSs, *P. penneri* 3, 5, 9, 14, 23, 47 and 135, it was necessary to examine the possibility of cross-reactions within this group. Thus, an additional serum against *P. penneri* 14 was obtained and checked with these antigens using ELISA. This strain was chosen since among seven LPSs from this group, only that one has a known chemical structure of LPS O-polysaccharide and may serve as a future standard to which these LPSs can be compared (Vinogradov *et al.*, 1991). The strong cross-reactivity of these systems suggests high degree serological similarities of *P. penneri* 3, 5, 9, 23, 47 and 135 LPSs to *P. penneri* 14 LPS (Table II). In order to confirm the obtained results an additional antiserum against one of the tested LPSs cross-reacting with *P. penneri* 14 antiserum (*P. penneri* 3 LPS) was selected randomly. This antiserum reacted with *P. penneri* 3, 5, 9, 14, 23, 47 and 135 LPSs in ELISA in a way identical to *P. penneri* 14 antiserum (data not shown).

All homologous and cross-reacting systems were checked in detailed serological studies. In PIH *P. penneri* 11, 14, 52 and 62 antisera reacted identically with (or similarly to) respective homologous and cross-reacting antigens (Table II). The complete abolition of the observed reaction after antisera absorption with cross-reacting antigens confirmed the identity of LPSs within the appropriate tested system. Only in one case the absorption of antiserum was not complete. After antibodies specific to *P. penneri* 3 LPS were removed from *P. penneri* 14 O-antiserum, immunoglobulins that remained in this antiserum slightly reacted with *P. penneri* 47 LPS (reciprocal titre – 1 : 400) (data not shown). This reaction probably concerned the core region of *P. penneri* 47 LPS, which is serologically similar to but not identical with the core oligosaccharide of *P. penneri* 14 LPS as has been shown in another article (Palusiak and Sidorczyk, 2009).

Table II  
Serological cross-reactivity of tested *P. penneri* LPSs with the respective antisera\*.

Serial number	Number of <i>Proteus</i> O-serogroup/subgroup	Reciprocal titre	Antiserum directed against strain	Tested LPSs	Previous contest of O-serogroup		Minimal inhibitory dose (ng)		Reciprocal titre for absorbed antiserum in PIH
					ELISA	PIH	ELISA	PIH	
1	O58	<i>P. p.</i> 115, 125	<i>P. p.</i> 11	<i>P. p.</i> 11, 6, 12	512.000–1.024.000 (512.000)	25.600–51.200 (25.600)	0.5–2 (0.5)	0.5–1 (0.5)	<100
2	O59	–	<i>P. p.</i> 14	<i>P. p.</i> 14, 3, 5, 9, 23, 47, 135	128.000–256.000 (128.000)	12.800	1–4 (1)	1–4 (2)	<100
3	O61	<i>P. p.</i> 21, 33, 43, 50–55, 57, 58, 66–69, 72, 92, 104, 109, 116, 118, 127, 133, 136	<i>P. p.</i> 52	<i>P. p.</i> 52, 7	256.000	51.200	2 16	4 16	<100
4	O64a,b,d	<i>P. p.</i> 29, 39	<i>P. p.</i> 62	<i>P. p.</i> 62, 40	256.000	25.600 12.800	2 62.5	2 62.5	<100
5	O8	<i>P. p.</i> 106, 107, 112, <i>P. m.</i> TG326, <i>P. genomospecies</i> 5	<i>P. v.</i> 17/57	<i>P. v.</i> 17/57, <i>P. p.</i> 17	1.024.000 32.000	51.200 6.400	8 2.500	2 2.500	<100

\* Respective data for homologous strains, *P. p.* 11 and 14, are in parenthesis

The results of PIH and ELISA inhibition were consistent for homologous and cross-reacting systems with only one exception: *P. penneri* 62 LPS inhibited the reaction with the homologous antiserum in a definitely stronger way than *P. penneri* 40 LPS did (Table II). The Western blot procedure also revealed some differences in both antigens specificity: *P. penneri* 62 antiserum strongly recognized only the high-molecular-mass species from the homologous strain and weakly reacted with high-molecular-mass LPS of *P. penneri* 40 strain and low-molecular-mass species of *P. penneri* 62 LPS (Fig. 1a). In order to find the explanation for the antigenic differences between these two LPSs, another antiserum, directed against *P. penneri* 40 strain, was added and checked with both LPS preparations in the Western blot technique (Fig. 1b). This time the antiserum reaction with *P. penneri* 40 LPS referred only to low-molecular-mass species (strong immunoreactivity pattern) whereas the reaction with the other antigen concerned both high- and low-molecular-mass species (weak immunoreactivity pattern). The reasons for differences in serological specificity of both LPSs could be a different length of their O-polysaccharide chains (shorter O-polysaccharides (O-PS) of *P. penneri* 40 LPS) and a different composition of antibodies in both antisera (a higher level of core-specific antibodies in *P. penneri* 40 antiserum). Taking into account the above explanation and the outcomes of PIH with the absorbed *P. penneri* 62 antiserum (Table II), both LPSs: *P. penneri* 40 and 62 can be regarded as identical and classified into *Proteus* serogroup O64, subgroup O64 a,b,d.

The other tested antisera, *P. penneri* 11, 14 and 52 antisera, reacted in the Western blot procedure identically with high-molecular-mass species concerning the O-polysaccharide part of respective homologous and cross-reacting LPSs (Fig. 1c-e). Some differences were observed only for *P. penneri* 14 antiserum at the level of low-molecular-mass LPS species (Fig. 1d). The immunoreactivity patterns observed for the *P. penneri* 3 and 14 LPSs were the weakest and the difference between the reactivity of *P. penneri* 47 LPS and the other antigens was the most significant. The classification of *Proteus* sp. strains into appropriate O-serogroups is based on the serological specificity of the LPSs O-PS mainly, so the small differences in the LPSs core region do not influence this classification.

The last tested antiserum, against *P. vulgaris* 17/57 (O8), exhibited significantly lower reactions with *P. penneri* 17 LPS as compared with the homologous system in all serological methods (Table II, Fig. 1f). Only the triple absorption of the tested antiserum with *P. penneri* 17 LPS completely removed all specific antibodies. The final explanation for the serologically different specificity of both antigens (*P. penneri* 17 and *P. vulgaris* 17/57 LPSs) was provided by the results of the Western blot procedure. *P. vulgaris* 17/57 antiserum clearly recognized both low- and high-molecular-mass LPS species from the homologous strain only and slightly bound to low- and high-molecular-mass LPS species of *P. penneri* 17 strain (Fig. 1f). To make the serological classification more precise *P. penneri* 17 antiserum was additionally selected. This antiserum strongly reacted only with

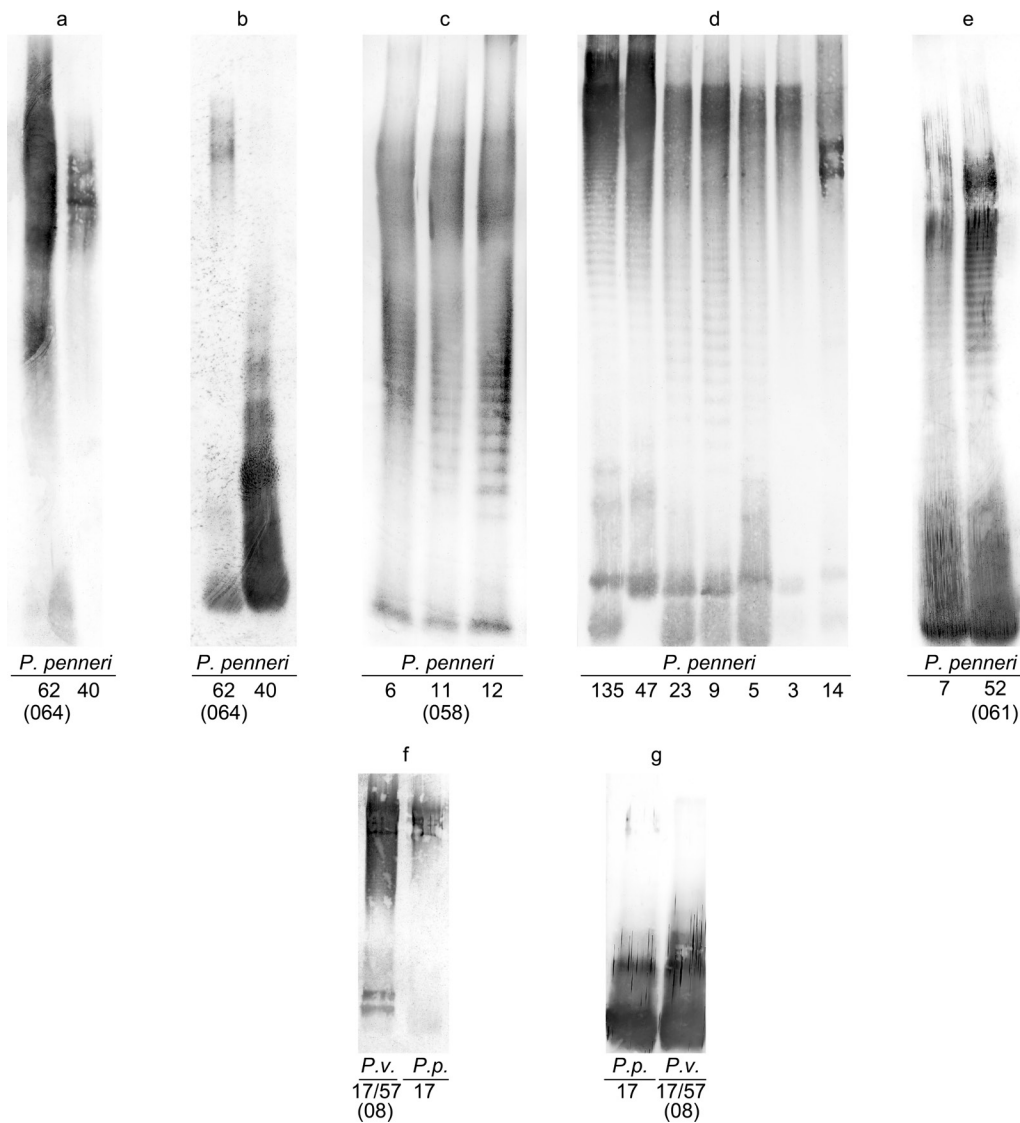


Fig. 1. Western blot of *Proteus* sp. LPSs with antisera directed against: a *P. penneri* 62 (O64 a,b,d) strain, b *P. penneri* 40, c *P. penneri* 11 (O58) strain, d *P. penneri* 14 strain, e *P. penneri* 52 (O61) strain, f *P. vulgaris* 17/57 (O8), g *P. penneri* 17

low-molecular-mass LPS species restricted to the core-lipid A moiety of both tested strains (Fig. 1g). It is another example of two antisera of a different antibodies composition: one containing a higher level of core-specific antibodies (*P. penneri* 17 antiserum) and the other one containing a higher level of O-polysaccharide-specific antibodies (*P. vulgaris* 17/57 antiserum). Taking into account this observation and the PIH outcomes obtained for the *P. vulgaris* 17/57 antiserum after its absorption with *P. penneri* 17 LPS, both strains (*P. penneri* 17 and *P. vulgaris* 17/57) can be classified into one common *Proteus* O-serogroup – O8.

It is worth mentioning that all doubts concerning the results obtained for the tested systems have been resolved after using the absorption procedure, a fundamental method in serological classification.

In conclusion, the results of all serological assays performed have led to a classification of the 12 tested

*P. penneri* strains into five different *Proteus* O-serogroups: *P. penneri* 17 – into serogroup O8, *P. penneri* 6 and 12 – O58, *P. penneri* 3, 5, 9, 14, 23, 47 and 135 – O59, *P. penneri* 7 – O61 and *P. penneri* 40 – serogroup O64 (subgroup O64 a,b,d) and allowed completing the serological classification of a unique collection of 128 smooth *P. penneri* strains collected in the Department of General Microbiology. Seven tested *P. penneri* strains 3, 5, 9, 14, 23, 47 and 135 formed a new O59 *Proteus* serogroup and the remaining ones were incorporated into *Proteus* sp. O-serogroups, which already contained other *Proteus* strains as shown in table II (Drzewiecka *et al.*, 2004; Kauffmann, 1966; Perepelov *et al.*, 1999; Zych *et al.*, 2000b, 2005c). Only serogroup O8 consists of four different *Proteus* species and the other four O-serogroups contain *P. penneri* strains only.

Completing the classification of the collection of 128 smooth *P. penneri* strains is of high importance for



the diagnostics of new isolated *P. penneri* strains since it may serve as a reference scheme according to which new isolates may be serologically classified.

*Proteus* classification scheme including 78 O-serogroups is not closed and new *Proteus* sp. strains isolated from different materials from patients e.g. in Central Poland are the subject of serological investigation (Drzewiecka *et al.*, 2010). The growing number of multi-drug-resistant strains occurring among *P. mirabilis* clinical isolates e.g. in Łódź area (unpublished data) necessitates the development of alternative methods of combating or preventing *Proteus* sp. infections, often persistent and complicated. Using the classification scheme might be a crucial part of *Proteus* sp. diagnostics. It will also provide information which O-serogroup is prevalent within a certain area. Such data could be also helpful in choosing proper vaccine antigens (LPS) shared by a large number of strains and in obtaining a protective antiserum.

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#### Literature

- Drzewiecka D., N.P. Arbatsky and P. Stączek. 2010 Structural and serological studies of the O-polysaccharide of strains from a newly created *Proteus* O78 serogroup prevalent in Polish patients. *FEMS Immunol. Med. Microbiol.* 58: 269–276.
- Drzewiecka D., K. Zych and Z. Sidorczyk. 2004. Characterization and serological classification of a collection of *Proteus penneri* clinical strains. *Arch. Immunol. Ther. Exp.* 52: 121–128.
- Giammanco G.M., P.A.D. Grimont, F. Grimont, M. Lefevre, G. Giammanco and S. Pignato. 2011. Phylogenetic analysis of the genera *Proteus*, *Morganella* and *Providencia* by comparison of rpoB gene sequences of type and clinical strains suggests the reclassification of *Proteus myxofaciens* in a new genus, *Cosenzaea* gen. nov., as *Cosenzaea myxofaciens* comb. nov. *Int. J. Syst. Evol. Microbiol.* 61: 1638–1644.
- Hickman F.W., A.G. Steigerwalt, J.J. Farmer III and D.J. Brenner. 1982. Identification of *Proteus penneri* sp. nov., formerly known as *Proteus vulgaris* indole negative or as *Proteus vulgaris* biogroup 1. *J. Clin. Microbiol.* 15: 1097–1102.
- Kauffmann F. 1966. *The bacteriology of Enterobacteriaceae*. 3<sup>rd</sup> ed. The Williams & Wilkins Co., Baltimore.
- Knirel Y.A., A.V. Perepelov, A.N. Kondakova, S.N. Senchenkova, Z. Sidorczyk, A. Rozalski and W. Kaca. 2011. Structure and serology of O-antigens as the basis for classification of *Proteus* strains. *Innate Immunity* 17: 70–96.
- Knirel Y.A., K. Zych, E.V. Vinogradov, A.S. Shashkov and Z. Sidorczyk. 2000. Structure of a 2-aminoethyl phosphate-containing O-specific polysaccharide of *Proteus penneri* 8 from a new serogroup O67. *Eur. J. Biochem.* 267: 815–820.
- Kondakova A.N., K. Zych, S.N. Senchenkova, B. Bartodziejska, A.S. Shashkov, Y.A. Knirel, A. Różalski and Z. Sidorczyk. 2003a. Structure of the O-polysaccharide of *Proteus penneri* 28 and *Proteus vulgaris* O31 and classification of *P. penneri* 26 and in *Proteus* serogroup O31. *FEMS Immunol. Med. Microbiol.* 39: 87–93.
- Kondakova A.N., K. Zych, S.N. Senchenkova, A. Zablotni, A.S. Shashkov, Y.A. Knirel and Z. Sidorczyk. 2003b. Structure of the O-polysaccharide leads to classification of *Proteus penneri* 31 in *Proteus* serogroup O19. *FEMS Immunol. Med. Microbiol.* 39: 73–79.
- Larsson P., H.E. Andersson and B. Norlen. 1978. Serotyping in epidemiological tracing of nosocomially acquired *Proteus mirabilis* in a geriatric ward. *Infection* 6: 105–110.
- O'Hara C.M., F.W. Brenner and J.M. Miller. 2000. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin. Microbiol. Rev.* 13: 534–546.
- Palusiak A., M. Dzieciatkowska and Z. Sidorczyk. 2008. Application of two different kinds of sera against *Proteus penneri* lipopolysaccharide core region in search of epitopes determining cross-reactions with antibodies. *Arch. Immunol. Ther. Exp.* 56: 135–140.
- Palusiak A. and Z. Sidorczyk. 2009. Serological characterization of the core regions of lipopolysaccharides of rough *Proteus* sp. strains. *Arch. Immunol. Ther. Exp.* 57: 303–310.
- Penner J.L. 1992. The genera *Proteus*, *Providencia* and *Morganella*, pp. 2849–2853. In: Balows A., H.G. Trüper, W. Harder and K.H. Schleifer (eds). *The Prokaryotes*. Springer-Verlag, Berlin, Germany.
- Penner J.L. and J.N. Hennessy. 1980. Separate O-grouping schemes for serotyping clinical isolates of *Proteus mirabilis* and *Proteus vulgaris*. *J. Clin. Microbiol.* 12: 304–309.
- Perepelov A.V., D. Babicka, A.S. Shashkov, N.P. Arbatsky, S.N. Senchenkova, A. Rozalski and Y.A. Knirel. 1999. Structure and cross-reactivity of the O-antigen of *Proteus vulgaris* O8. *Carbohydr. Res.* 318: 186–192.
- Shashkov A.S., A.N. Kondakova, S.N. Senchenkova, K. Zych, E.V. Toukach, Y.A. Knirel and Z. Sidorczyk. 2000. Structure of 2-aminoethyl phosphate-containing O-specific polysaccharide of *Proteus penneri* 63 from a new serogroup O68. *Eur. J. Biochem.* 267: 601–605.
- Sidorczyk Z., F.V. Toukach, K. Zych, N.P. Arbatsky, D. Drzewiecka, A. Ziółkowski, S. Shashkov and Y.A. Knirel. 2002a. Structural and serological characterization of the lipopolysaccharide from *Proteus penneri* 20 and classification of the cross-reacting *Proteus penneri* strains 10, 16, 18, 20, 32 and 45 in *Proteus* serogroup O17. *Arch. Immun. Ther. Exp.* 50: 345–350.
- Sidorczyk Z., F.V. Toukach, K. Zych, D. Drzewiecka, N.P. Arbatsky, A.S. Shashkov and Y.A. Knirel. 2002b. Structural and serological relatedness of the O-antigens of *Proteus penneri* 1 and 4 from a novel *Proteus* serogroup O72. *Eur. J. Biochem.* 269: 358–363.
- Sidorczyk Z. and K. Zych. 1993. Wybrane właściwości szczepów nowego gatunku *Proteus penneri* z drugiej kolekcji amerykańskiej. *Med. Dośw. Mikrobiol.* 45: 69–73.
- Sidorczyk Z., K. Zych, F.V. Toukach, N.P. Arbatsky, A. Zablotni, A.S. Shashkov and Y.A. Knirel. 2002c. Structure of the O-polysaccharide and classification of *Proteus mirabilis* strain G1 in *Proteus* serogroup O3. *Eur. J. Biochem.* 269: 1406–1412.
- Vinogradov E.V., A.S. Shashkov, Y.A. Knirel and N.K. Kochetkov. 1991. The structure of *Proteus penneri* 14 O-specific polysaccharide containing D- and L-alanine. *Carbohydr. Res.* 219: 1–3.
- Westphal O. and K. Jann. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. *Methods Carbohydr. Chem.* 5: 83–91.
- Zych K., N.A. Kocharova, M. Kowalczyk, F.V. Toukach, D. Kaminśka, A.S. Shashkov, Y.A. Knirel and Z. Sidorczyk. 2000a. Structure of the O-specific polysaccharide of *Proteus penneri* 71 and classification of cross-reactive *P. penneri* strains to a new proposed serogroup O64. *Eur. J. Biochem.* 267: 808–814.
- Zych K., M. Kowalczyk, Y.A. Knirel and Z. Sidorczyk. 2000b. New serogroups of the genus *Proteus* consisting of *Proteus penneri* strains only, pp 339–344. In: Hacker J., G. Blum-Ochtev, T. Pal and

L. Emödy (eds). *Genes and proteins underlying microbial urinary tract virulence. Basic aspects and applications*. Kluwer Academic/Plenum Publishers, New York.

**Zych K., A.V. Perepelov, A. Baranowska, A. Zabłotni, Y.A. Knirel and Z. Sidorczyk.** 2005a. Structure and serological studies of the O-polysaccharide of *Proteus penneri* 75. Epitopes and subgroups of *Proteus* serogroup O73. *FEMS Immunol. Med. Microbiol.* 43: 141–148.

**Zych K., A.V. Perepelov, A. Baranowska, A. Zabłotni, A.S. Shashkov, Y.A. Knirel and Z. Sidorczyk.** 2005b. Structure of the

O-polysaccharide and serological studies of the lipopolysaccharide of *Proteus penneri* 60 classified into a new *Proteus* serogroup O70. *FEMS Immunol. Med. Microbiol.* 43: 351–356.

**Zych K., A.V. Perepelov, M. Siwińska, Y.A. Knirel and Z. Sidorczyk.** 2005c. Structures of the O-polysaccharides and classification of *Proteus* genomospecies 4, 5 and 6 into respective *Proteus* serogroups. *FEBS Journal* 272: 5536–5543.

**Zych K. and Z. Sidorczyk.** 1989. Lipopolysaccharides of *Proteus penneri* species novum. *Carbohydr. Res.* 188: 105–111.