Carbohydrate Research, 188 (1989) 105–111
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

LIPOPOLYSACCHARIDES OF *Proteus penneri* SPECIES NOVUM*

KRYSTYNA ZYCH AND ZYGMUNT SIDORCZYK†
Institute of Microbiology, University of Łódź, Banacha 12/16, PL-90-237 Łódź (Poland)
(Received August 16th, 1988; accepted for publication, November 14th, 1988)

ABSTRACT

The lipopolysaccharides (LPS), obtained from twenty strains of *Proteus penneri*, were shown to contain 3-deoxy-D-*manno*-2-octulosonic acid (KDO), L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptoses, glucose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, and galacturonic acid. Galactose was lacking in three LPS and, in some LPS, glucuronic acid, rhamnose, lysine, and two unknown constituents were detected. Chemotypes of the *P. penneri* are discussed.

INTRODUCTION

The current classification of the genus *Proteus* contains three species[1,2], namely, type species for the genus *P. vulgaris*, *P. mirabilis*, and *P. myxofaciens*, isolated from gypsy moth larvae (*Porthetria dispar*) and without clinical importance.

On the basis of fermentation properties, three *P. vulgaris* biogroups were identified[3], namely, (1) indole-, salicin-, and esculin-negative; (2) indole-, salicin-, and esculin-positive; and (3) indole-positive, and salicin- and esculin-negative. DNA hybridization studies[3] showed that the strains in biogroup 1 were highly related to each other, but were more distantly related to the remaining biogroups. Low relatedness as shown by DNA hybridization, phenotypic, and physiological differences have been the main criteria for a recent proposal of a novel species of *P. penneri* for the strains formerly called[3,4] *P. vulgaris* biogroup 1. *P. penneri* strains have been isolated from urine, blood, stools, abdominal wounds, and bronchial exudates[5]. Its natural habitat is unknown and its etiological role in infectious processes has not been established fully. This prompted an examination of isolates of the species in order to determine more precisely their susceptibility to antibiotics[3,5], biochemical reactions[3,5], and role in human infections[7–10].

The above-mentioned genetic and metabolic differences of the novel *Proteus* species could also be reflected in the constitution of its lipopolysaccharides. Hence,

*Presented at the XIVth International Carbohydrate Symposium, Stockholm, Sweden, August 14–19, 1988.
†Author for correspondence.

0008-6215/89/$ 03.50

TABLE I

COMPOSITION OF *P. penneri* LIPOPOLYSACCHARIDES

Strain	*Form*	*Constituents (μmol/mg of LPS)*												
		GlcA	*GalA*	*LD-Hep*	*DD-Hep*	*KDO*	*EtN*	*Glc*	*GlcN*	*GalN*	*Gal*	*Rha*	*X*	*Y*	*Lys*
1	R	–	0.22	0.23	0.14	0.30	0.07	0.18	0.32	0.17	0.18	–	–	–	–
2	S	–	0.09	0.58	0.19	0.27	0.11	0.28	0.25	0.23	0.24	–	–	–	–
3	R	–	0.06	0.25	0.12	0.26	0.07	0.18	0.26	0.18	0.22	–	–	+	–
4	S	–	0.14	0.36	0.12	0.23	0.05	0.57	0.22	0.78	0.54	–	–	–	–
5	S	–	0.61	0.38	0.18	0.23	0.08	0.40	0.42	0.17	0.18	–	–	–	–
6	S	–	0.33	0.28	0.11	0.30	0.08	0.18	0.27	0.31	0.09	0.16	–	–	–
7	S	–	0.27	0.38	0.19	0.33	0.09	0.35	0.37	0.16	–	–	–	–	–
8	S	–	0.27	0.32	0.18	0.27	0.21	0.59	0.30	0.34	–	–	–	–	0.17
9	R	–	0.07	0.30	0.13	0.48	0.13	0.16	0.23	0.22	–	–	–	–	0.02
10	S	0.13	0.12	0.28	0.10	0.30	0.17	0.38	0.35	0.07	0.13	–	+	–	–
11	S	–	0.24	0.43	0.15	0.28	0.12	0.19	0.40	0.45	0.13	0.28	–	–	–
12	R	–	0.06	0.19	0.12	0.42	0.12	0.15	0.25	0.23	0.05	–	–	–	–
13	R	–	0.27	0.13	0.05	0.36	0.10	0.11	0.21	0.17	0.02	–	–	–	–
14	S	–	0.63	0.24	0.14	0.32	0.09	0.28	0.45	0.16	0.17	0.10	–	–	–
15	S	–	0.09	0.43	0.22	0.36	0.13	0.36	0.38	0.19	0.12	–	–	–	–
16	S	0.06	0.11	0.08	0.03	0.30	0.15	0.20	0.28	0.05	0.16	–	+	–	–
17	S	0.11	0.20	0.39	0.12	0.30	0.12	0.21	0.41	0.17	0.49	–	–	–	0.26
18	S	0.08	0.16	0.30	0.08	0.35	0.16	0.42	0.29	0.06	0.12	–	+	–	–
19	S	–	0.18	0.17	0.08	0.34	0.11	0.15	0.35	0.16	0.24	–	–	–	–
20	R	–	0.11	0.27	0.09	0.43	0.13	0.12	0.21	0.18	0.11	–	–	–	–

the chemical composition of the lipopolysaccharides obtained from twenty *P. penneri* strains has been investigated.

RESULTS AND DISCUSSION

The twenty *P. penneri* strains were tested for "rough" (R) and "smooth" (S) variants (results not included, see Table I for the conclusions). The results of these tests guided the choice of the procedure whereby the individual lipopolysaccharides were extracted. Most LPS were extracted by the hot phenol–water method[11] in yields of 2.5–4.9% dry weight. For strains 1, 3, 9, 12, and 20, the modified light petroleum–chloroform–phenol (PCP) extraction procedure[12] was better and gave yields of 2.2–4.6% dry weight. For strain 3, these extraction procedures gave yields of LPS of 0.17 and 4.24% dry weight; for strain 12, the yields were 0.73 and 3.96%, respectively. The final LPS preparations had no u.v. absorption peaks at 260 or 280 nm, indicating that, at most, only traces of nucleic acid and protein were present.

The *P. penneri* LPS were subjected to polyacrylamide gel electrophoresis in the presence of sodium deoxycholate (DOC-PAGE) (Fig. 1, A and B). The patterns visualized by silver staining revealed at least two types of migration. The first was "ladder"-like, typical for S strains, with high-molecular-weight material at the origin, and was observed for the LPS of strains 5, 6, 8, 11, 14, 16, and 18. The second was characterized by the preponderance of short sugar chains or core chains substituted by only 1–2 repeating units and was detected for the LPS of strains 1, 3, 4, 9, 12, 13, 15, and 20. The migration pattern of the remaining LPS was intermediate. The presence of deoxycholate (DOC) gives[13] a better dissociation of LPS in PAGE than sodium dodecyl sulfate (SDS).

Additional indications of the occurrence of R-type LPS were obtained after separation of the respective LPS-derived polysaccharides on Sephadex G-50 (data

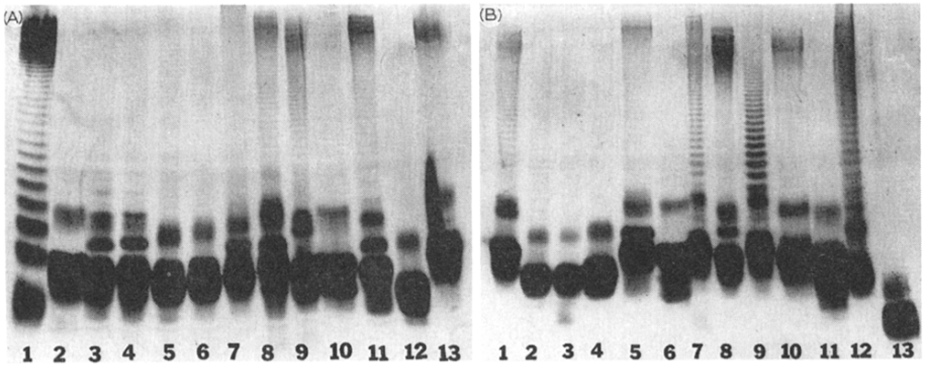

Fig. 1. Silver-stained DOC-PAGE pattern of *P. penneri* LPS; 2 mg of LPS was applied to each well: *A*. 1, *Salmonella abortus equi* LPS (control); 2, *P. penneri* strain 1; 3–4, strain 2 extracted by the phenol–water and PCP methods; 5–6, strain 3 extracted by the phenol–water and PCP methods; 7–13, strains 4–10, respectively; *B*. 1, strain 11; 2–3, strain 12 extracted by the phenol–water and PCP methods; 4–11, strains 13–20, respectively; 12, *P. mirabilis* S 1959; 13, *P. mirabilis* R 45 (control).

TABLE II

CHEMOTYPES[a] OF *P. penneri* LIPOPOLYSACCHARIDES

Chemotype	*Strain*	*Constituents*													*Proteus chemotype*[18,19]
		GlcA	GalA	LD-Hep	DD-Hep	KDO	EtN	Glc	GlcN	GalN	Gal	Rha	X	Y	Lys	
I	7		○	○	○	○	○	○	○	○						–
II	8, 9		○	○	○	○	○	○	○	○					+	VIII
III	1, 2, 4, 5, 12, 13, 15, 19, 20		○	○	○	○	○	○	○	○	+					IX b
IV	6, 11, 14		○	○	○	○	○	○	○	○	+	+				XII
V	3		○	○	○	○	○	○	○	○	+			+		–
VI	10, 16, 18	+	○	○	○	○	○	○	○	○	+		+			–
VII	17	+	○	○	○	○	○	○	○	○	+				+	XV a

[a]○, Common constituent; +, additional constituent.

not included). The degraded polysaccharides were obtained from strains 1, 2, 3, 9, 13, and 20 by mild acid hydrolysis of the LPS. Gel filtration revealed a major fraction which consisted of the core polysaccharide and a minor fraction that contained free KDO. The lack or only traces of the high-molecular-weight fractions showed that the original LPS preparations were R-type.

The chemical compositions of the LPS from the 20 strains of *P. penneri* are presented in Table I. Constituents common to all LPS were KDO, ethanolamine, L-*glycero*-D-*manno*-heptose, D-*glycero*-D-*manno*-heptose, glucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and galacturonic acid. Galactose was lacking in three lipopolysaccharides. The presence of glucuronic acid was observed in four lipopolysaccharides and, in some, rhamnose, lysine, and two unknown constituents (X and Y) were detected also. The structures of X and Y are being investigated. Lysine was the only amino acid detected and its role in the serological specificity of some *Proteus* LPS has been shown[14,15].

On the basis of chemical composition, the LPS of the *P. penneri* strains were divided into seven chemotypes (Table II). The LPS of chemotype I contained the eight sugars common to all the preparations and occurred in one strain only. LPS of four other chemotypes contained 1–3 additional components, namely, lysine (II), galactose (III), rhamnose (IV), or X and Y (V), and were represented by two (II), nine (III), three (IV), and one (V) strain. LPS of the remaining two chemotypes were characterized by the presence of glucuronic acid, and the other constituents were galactose and X (VI) or lysine (VII). Chemotype III was observed most frequently (eight strains). As compared to *P. vulgaris* and *P. mirabilis* chemotypes[16–19], two chemotypes IXb and XVa are present in all three *Proteus* species and the next two (VIII and XII) in *P. vulgaris* and *P. penneri* only. Chemotypes I, V, and VI were found exclusively in *P. penneri* strains. Chemotype IXb, which was most frequently observed in *P. mirabilis* (nine) and *P. vulgaris* (four) serogroups[19], also occurred most often in *P. penneri* strains.

Thus, the R-type *P. penneri* LPS do not contain high-molecular-weight material. The *P. penneri* LPS, besides constituents typical for the genus *Proteus* such as uronic acids, hexoses, heptoses, KDO, and EtN[19], also contained occasionally rhamnose, lysine, and two unknown sugars. From seven chemotypes of *P. penneri* LPS, four were already described in genus *Proteus* and three were novel. Similarities in the chemical composition of some *P. penneri* LPS shown in this work were also supported by the results of serological tests (data not included).

EXPERIMENTAL

Strains. —The 20 *P. penneri* strains were provided by Professor D. J. Brenner (Center for Diseases Control, Atlanta, U.S.A.). Dry bacteria were obtained from aerated liquid cultures, as described[20].

Isolation and purification of LPS. — Crude LPS preparations obtained after extraction of the bacteria with hot phenol–water mixtures[11] were purified by ultra-

centrifugation and then digestion with nucleases[21]. LPS from some strains were extracted by the phenol–chloroform–light petroleum method[12]. The purity of the LPS preparations was ascertained by absorbance scans from 300–200 nm.

The DOC-PAGE procedure. — The deoxycholate–polyacrylamide gel electrophoresis of LPS preparations was carried out with a modified buffer system[22] where the separating gels were formed with 13% of polyacrylamide (PA) and 0.5% of sodium deoxycholate (DOC) instead of SDS[13], and the stacking gel with 5% of PA[23] and 0.5% of DOC. Gels were oxidized with periodate and stained with silver nitrate[23].

Analytical methods. — Neutral sugars, which were liberated by hydrolysis in 0.1M HCl for 48 h at 100°, were converted into their alditol acetate derivatives and determined by g.l.c.[25] using a Varian 3700 gas chromatograph equipped with a fused-silica capillary column (SE-54, 25 m × 0.22 mm). Hexosamines, amino acids, and ethanolamine were determined after hydrolysis of LPS (4M HCl, 10 h, 100°) in a Jeol, JLC-6AH amino acid analyzer. Uronic acids were identified after hydrolysis of LPS (0.5M H_2SO_4, 4 h, 100°) by high-voltage paper electrophoresis (pH 2.8) with staining with naphthoresorcinol and alkaline silver[18]. Uronic acids were determined by the Scott[26] and orcinol methods[27]. Qualitative identification of KDO was performed after hydrolysis (M HCl, 2 h, 100°) of LPS by high-voltage paper electrophoresis (pH 2.8) and staining with alkaline silver[18] and thiobarbituric acid[28]. Quantitative determination of KDO was carried out by the thiobarbituric acid assay[29].

ACKNOWLEDGMENTS

We thank Professor D. J. Brenner (CDC Atlanta, U.S.A.) for supplying the *P. penneri* strains, Dr. U. Zähringer (Forschungsinstitut Borstel, F.R.G.) for access to g.l.c. equipment, and Dr. A. Rózalski for DOC-PAGE analysis of LPS. This work was partly supported by grant 06.01 of the Polish Academy of Sciences.

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