Immunocchemical characterization of the O antigens of two Proteus strains, O8-related antigen of Proteus mirabilis 12 B-r and O2-related antigen of Proteus genomospecies 5/6 12 B-k, infecting a hospitalized patient in Poland

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A hospitalized 73-year-old woman was infected with a Proteus mirabilis strain, 12 B-r, isolated from the place of injection of a blood catheter. Another strain, 12 B-k, recognized as Proteus genomospecies 5 or 6, was isolated from the patient's faeces, which was an example of a nosocomial infection rather than an auto-infection. Serological investigation using ELISA and Western blotting showed that strain 12 B-k from faeces belonged to the Proteus O2 serogroup. Strain 12 B-r from the wound displayed cross-reactions with several Proteus O serogroups due to common epitopes on the core or O-specific parts of the lipopolysaccharide. Studies of the isolated 12 B-r O-specific polysaccharide by NMR spectroscopy revealed its close structural similarity to that of Proteus O8. The only difference in 12 B-r was the presence of an additional GlcNAc-linked phosphoethanolamine residue, which creates a putative epitope responsible for the cross-reactivity with Pt. mirabilis O16. The new O-antigen form could appear as a result of adaptation of the bacterium to a changing environment. On the basis of the data obtained, we suggest division of the O8 serogroup into two subgroups: O8a for strains of various Proteus species that have been previously classified into the O8 serogroup, and O8a,b for Pt. mirabilis 12 B-r, where 'a' is a common epitope and 'b' is a phosphoethanolamine-associated epitope. These findings further confirm serological and structural heterogeneity of O antigens of Proteus strains isolated lately from patients in Poland.

INTRODUCTION

Gram-negative bacteria belonging to the genus Proteus are divided into four species: Proteus mirabilis, Pt. vulgaris, Pt. penneri and Pt. hauseri. Additionally, on the basis of molecular investigation, three unnamed genomospecies, 4, 5 and 6, have been separated from Pt. vulgaris (O’Hara et al., 2000a, b). They cannot be fully differentiated by their metabolic features.

The bacteria may play the role of commensals, symbionts or parasites of many domestic and wild animals and are found in faecally polluted water and soil (Drzewiecka, 2016). All Proteus species are recognized as human opportunistic pathogens, most frequently responsible for urinary tract infections. They are also involved in wound infections, especially nosocomially acquired ones. One of the virulence factors of these bacteria is lipopolysaccharide (LPS). Strains producing the full LPS (S – smooth form) with a long-chain polysaccharide called O-polysaccharide or O antigen are usually more virulent than those expressing LPS molecules deprived of the O-polysaccharide (R – rough form). The O antigen facilitates swarming growth of the pathogens, enabling their expansion. It also plays an important role in biofilm formation (Manos & Belas, 2006; Różalski et al., 2012).

A variability of the O-antigen forms accounts for the serospecificity of Proteus spp. rods, giving rise to 80 O serogroups within this genus (Arbatsky et al., 2013; Knirel et al., 2011; Siwińska et al., 2015). However, the number of the O-antigen forms is even larger as several serogroups are divided into subgroups (serotypes), which differ slightly

Abbreviations: EtNP, phosphoethanolamine; HMBC, heteronuclear multiple-bond correlation; LPS, lipopolysaccharide; p, pyranose.
in chemical structure and serospecificity. Some O sero-
groups have an interspecies character, and many cross-
reactions due to common epitopes on the O-polysaccharide
and/or core-oligosaccharide parts of the LPS are observed
(Knirel et al., 2011), complicating the serotyping.

Here we present results of immunochemical studies of two
serologically different Proteus strains, which were isolated
from a hospitalized patient in Poland.

METHODS

Bacterial strains and cultivation. A Proteus sp. strain isolated from
a wound of a hospitalized 73-year-old woman suffering from kidney
failure was kindly provided by the medical laboratory in Barlicki
hospital in Łódź, Poland. The patient’s faeces were screened for the
presence of Proteus spp. bacteria by inoculation on MacConkey and
Nogrady agar media [(g l⁻¹): peptone (10), yeast extract (6), NaCl
(5), Na₂HPO₄ (12), KH₂PO₄ (2.7), natrium deoxycholate (4), lactose
(12.5), bromothymol blue (0.1), neutral red (0.03), urea (2.5), agar
(20), pH 6.5] directly or after 18 h of preliminary selective cultivation
in sodium selenite broth medium. After 24–48 h of cultivation, lac-
tose-negative, colourless colonies (MacConkey) or lactose- and
urease-negative, green colonies (Nogrady) were identified as
Proteus spp. The species of the strains from the wound and faeces were
identified by the investigation of their metabolic properties according
to the methods of Senior (1997) as described previously (Arbatsky
et al., 2013; Drzewiecka et al., 2008).

The Proteus spp. isolates were maintained at −80 °C in stocks made
from Luria Broth cultures in 25 % (v/v) glycerol. Biomass was
obtained from the strains studied by 18 h of cultivation at 37 °C on
nutrient broth medium [(g l⁻¹): beef extract (0.4), peptone (9.4),
yeast extract (1.7), NaCl (3.5), pH 6.8] supplemented with 0.2 %
glucose with aeration, subsequent killing by 1 % (v/v) phenol, cen-
trifugation, washing with distilled water and lyophilization.

Extraction, electrophoresis and degradation of the LPS. The
phenol-water method (Westphal & Jann, 1965) was employed for LPS
extraction from bacterial cells. Proteins and nucleic acids were
removed from the crude extract by precipitation with trichloroacetic
acid (Arbatsky et al., 1997). The yields of LPS preparations were 7.7 %
and 7.5 % from dried bacterial mass of Pt. mirabilis 12 B-r and Proteus
genomospecies 5/6 12 B-k, respectively.

LPS samples were separated in SDS-PAGE followed by silver staining
(Tsai & Frasch, 1982) or by transfer to nitrocellulose membrane.

An LPS sample from Pt. mirabilis 12 B-r (116 mg) was degraded with
2 % acetic acid at 100 °C until precipitation of lipid (1.5 h). The
precipitate was removed by centrifugation (13 000 g, 20 min), and
the supernatant was fractionated by gel-permeation chromatography
on a TSK HW-50 column (90 × 2.5 cm; Toyo Soda) in 0.1 % acetic
acid, monitored with a UV detector (LKB) at 206 nm. An O-polysaccharide sample was obtained in a yield of 35 % of the
LPS mass.

NMR spectroscopy. An O-polysaccharide sample from Pt. mirabilis
12 B-r was deuterium-exchanged by freeze-drying a solution in
99.9 % D₂O and then examined as a solution in 99.95 %
D₂O. NMR spectra were recorded on a Bruker DRX-500 spec-
trometer at 45 °C using internal sodium 3-(trimethylsilyl)pro-
panoate-2,2,3,3-d₄ (δH 0) or acetone (δC 31.45) as references for

Table 1. Biochemical characterization of two Proteus isolates

<table>
<thead>
<tr>
<th>Metabolic feature</th>
<th>Proteus isolate</th>
<th>12 B-r (Pt. mirabilis)</th>
<th>12 B-k (Pt. genomospecies 5/6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine deaminase</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Mannose fermentation</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Urease production</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ornithine decarboxylation</td>
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<td>−</td>
<td></td>
</tr>
<tr>
<td>Salicin fermentation</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>NS</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Rhamnose fermentation</td>
<td>NS</td>
<td>−</td>
<td></td>
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<tr>
<td>Lipase production</td>
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<td>+</td>
<td></td>
</tr>
<tr>
<td>DNase production</td>
<td>NS</td>
<td>+</td>
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</tr>
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</table>

NS, Not studied.

Fig. 1. Silver-stained patterns of electrophoretically separated
LPS molecules from P. mirabilis 12 B-r and Proteus genomospe-
cies 5/6 12 B-k.
calibration. Two-dimensional NMR spectra were obtained using standard Bruker software, and the Bruker TopSpin 2.1 program was used to acquire and process the NMR data. A mixing time of 100 and 150 ms was used in total correlation spectroscopy (TOCSY) and rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY) experiments, respectively.

Serological studies. A set of Proteus O1–O80-specific rabbit polyclonal antisera and O1–O80 LPS samples came from the collections of the Department of General Microbiology and the Department of Immunobiology of Bacteria, University of Łódź, Poland. Polyclonal Pt. mirabilis 12 B-r specific serum was obtained by immunization of rabbit with the heat-killed whole-cell vaccine as described previously (Sidorczyk et al., 2002). Specific antibodies were removed from the antisera by adsorption with whole bacterial cells (wet biomass) as described previously (Drzewiecka et al., 2008). ELISA and Western blotting were performed according to the protocols of Sidorczyk et al. (2002) using LPS samples as the antigens and rabbit polyclonal antisera as a source of specific antibodies.

RESULTS

Bacterial strains, isolation and characterization of lipopolysaccharides

A hospitalized 73-year-old woman suffering from a kidney failure was found to be infected with Proteus strain 12 B-r. The strain infected a wound in the place of the injection of a dialysis catheter and was isolated from its end. It was classified as Pt. mirabilis by biochemical tests (Drzewiecka et al., 2008; Senior, 1997) (Table 1).

Another strain, 12 B-k, was found to inhabit the intestines of the patient. It was recognized as Proteus genomospecies 5 or 6 (Table 1) on the basis of its metabolic abilities checked as described by Arbatsky et al. (2013).

The strains were cultivated, and LPS preparations were extracted from bacterial cells using the phenol-water method (Westphal & Jann, 1965). The pattern of electrophoretically separated and silver-stained LPS samples from both strains showed intense bands for R-form LPS and weak bands for S-form LPS (Fig. 1).

Table 2. Cross-reactivity in ELISA (reciprocal titres) of Proteus antisera (native and adsorbed with 12 B-k cells) with the respective LPSs

<table>
<thead>
<tr>
<th>Serum against:</th>
<th>LPS</th>
<th>Strain 12 B-k</th>
<th>Homologous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt. vulgaris O2</td>
<td>Native</td>
<td>1 024 000</td>
<td>1 024 000</td>
</tr>
<tr>
<td></td>
<td>Adsorbed</td>
<td>&lt;1000</td>
<td>2000</td>
</tr>
<tr>
<td>Pt. penneri O68</td>
<td>Native</td>
<td>256 000</td>
<td>512 000</td>
</tr>
<tr>
<td></td>
<td>Adsorbed</td>
<td>&lt;1000</td>
<td>256 000</td>
</tr>
<tr>
<td>Pt. mirabilis O77</td>
<td>Native</td>
<td>32 000</td>
<td>1 024 000</td>
</tr>
<tr>
<td></td>
<td>Adsorbed</td>
<td>&lt;1000</td>
<td>32 000</td>
</tr>
<tr>
<td>Pt. penneri O65</td>
<td>Native</td>
<td>64 000</td>
<td>256 000</td>
</tr>
<tr>
<td></td>
<td>Adsorbed</td>
<td>&lt;1000</td>
<td>32 000</td>
</tr>
<tr>
<td>Pt. vulgaris O22</td>
<td>Native</td>
<td>64 000</td>
<td>1 024 000</td>
</tr>
<tr>
<td></td>
<td>Adsorbed</td>
<td>&lt;1000</td>
<td>128 000</td>
</tr>
</tbody>
</table>

Serological studies of the O antigen of Proteus genomospecies 5/6 12 B-k

When examined with the full set of Proteus O1–O80 antisera, LPS from strain 12 B-k exhibited a marked cross-reactivity in ELISA with five antisera (Table 2). The strongest reaction, similar to that of the homologous LPS, was observed with Pt. vulgaris O2 antisera, and the reaction was almost completely abolished by the adsorption of O2 antiserum with 12 B-k cells. In Western blotting, antibodies present in O2 antiserum recognized electrophoretically separated LPS molecules from both O2 and 12 B-k strains (Fig. 2a).

The other cross-reactions revealed by ELISA were weaker and had a different nature. Western blot indicated that Pt. mirabilis O77 and Pt. penneri O68 antisera recognized an O-polysaccharide(s)-linked epitope on 12 B-k LPS (Fig. 2b, c). In contrast, Pt. penneri O65 antibodies bound fast-migrating LPS molecules devoid of the O-polysaccharide part (Fig. 2d) and hence are specific to the core part of the LPS. Pt. penneri O65 antibodies also recognized a core-linked epitope(s) on Pt. vulgaris O22 LPS (Fig. 2d). A similarity of the LPS core regions of Pt. penneri O65, Pt. vulgaris O22 and Pt. penneri O67 has been demonstrated previously using O67 antiserum (Knirel et al., 2000). These findings indicated a relatedness of the LPS core
regions of Proteus genomospecies 5/6 12 B-k, Pt. penneri O65 and Pt. vulgaris O22.

The O2, O68 and O77 antigens share the β-D-GlcNAc-(1→2)-β-D-Glc disaccharide fragment (where p represents pyranose; Fig. 3), which has been demonstrated to play a role in their cross-reactivity (Drzewiecka et al., 2008). O68 and O77 antisera were evidently deprived of antibodies against the common disaccharide fragment by the adsorption with 12 B-k cells as their reactivity in ELISA with the homologous LPS decreased (Table 2). The data obtained indicate that the Proteus genomospecies 12 B-k isolate from the patient’s faeces belongs to Proteus serogroup O2.

**Immunochromosomal studies of the O antigen of Pt. mirabilis 12 B-r**

LPS of Pt. mirabilis 12 B-r was recognized in ELISA by antisera against Pt. vulgaris O8 and Pt. mirabilis O16 (reaction to the dilution 1 : 64 000 and 1 : 16 000, respectively) (Table 3), whereas reactions with the remaining antisera from the full set of antisera against Proteus O serotypes were weaker (to the dilutions < 1 : 1000–1 : 8000). O8 antiserum adsorbed with 12 B-r biomass lost its reactivity with the homologous O8 LPS. Adsorption of O16 antiserum with 12 B-r cells decreased its reactivity in the homologous system (Table 3). As no cross-reactivity was observed between the O8 and O16 antigens (Table 3) and they do not possess any common fragment (Fig. 4), it was suggested that the 12 B-r O-polysaccharide shares different epitopes with the O8 and O16 polysaccharides. Accordingly, in Western blotting, O8 and O16 antisera recognized epitopes on slowly migrating S-form molecules of both homologous LPS and LPS of Pt. mirabilis 12 B-r but no cross-reaction was observed between O8 and O16 (Fig. 5a, b).

In ELISA, antiserum against Pt. mirabilis 12 B-r cross-reacted with the LPS of Pt. vulgaris O8 and Pt. mirabilis O16 only (Table 3). In Western blotting (Fig. 5c), it bound O-polysaccharide-containing LPS molecules of 12 B-r, O8 and O16, the reaction with the O16 LPS being weak. The adsorption of 12 B-r antiserum with O8 or

**Table 3. Cross-reactivity in ELISA (reciprocal titres) of Pt. vulgaris O8, Pt. mirabilis O16 and Pt. mirabilis 12 B-r antisera (native and adsorbed) with the respective LPSs**

<table>
<thead>
<tr>
<th>Serum against:</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pt. mirabilis 12 B-r</td>
</tr>
<tr>
<td>Pt. vulgaris O8</td>
<td>Native</td>
</tr>
<tr>
<td></td>
<td>12 B-k adsorbed</td>
</tr>
<tr>
<td>Pt. mirabilis O16</td>
<td>Native</td>
</tr>
<tr>
<td></td>
<td>12 B-k adsorbed</td>
</tr>
<tr>
<td>Pt. mirabilis 12 B-r</td>
<td>Native</td>
</tr>
<tr>
<td></td>
<td>O8 adsorbed</td>
</tr>
<tr>
<td></td>
<td>O16 adsorbed</td>
</tr>
<tr>
<td></td>
<td>O8 and O16 adsorbed</td>
</tr>
</tbody>
</table>

NS, Not studied.
O16 biomass considerably decreased the level of antibodies reacting in the homologous system, whereas the simultaneous adsorption by both strains totally abolished the reactivity and hence removed all anti-LPS antibodies (Table 3). These findings further confirmed that the 12 B-r O antigen shares different epitopes with the O8 and O16 antigens.

The O-polysaccharide was isolated from the 12 B-r LPS and studied by one- and two-dimensional $^1$H, $^{13}$C and $^{31}$P NMR spectroscopy (for assigned $^1$H and $^{13}$C chemical shifts see Table 4). The $^1$H and $^{13}$C NMR spectra of the 12 B-r O-polysaccharide (Fig. 6) were similar to those of *Pt. vulgaris* O8 (Perepelov et al., 1999) but showed additional signals for a phosphoethanolamine group (EtNP): CH$_2$Na at $\delta$H 3.30 and $\delta$C 41.6, CH$_2$Oa at $\delta$H 4.14 and $\delta$C 63.3, phosphate at $\delta$P 1.0. Analysis of the two-dimensional NMR spectra as described by Perepelov et al. (1999) indicated that the 12 B-r and O8 O-polysaccharides have the same carbohydrate structure. The location of EtNP at position 6 of the GlcNAc residue was established by a two-dimensional $^1$H–$^{31}$P heteronuclear multiple-bond correlation (HMBC) experiment (Fig. 7), which showed a cross-peak between H-6a and H-6b of GlcNAc and phosphorus of the phosphate group at $\delta$ 4.07/1.0 and 4.22/1.0. This finding was confirmed by downfield displacements, due to deshielding effects, of the signals for H-6 and C-6 of GlcNAc from $\delta$H 3.85 and $\delta$C 62.4 in the spectra of the O8-polysaccharide and phosphorus of the phosphate group at $\delta$P 1.0. Therefore, the O-polysaccharide of *Pt. mirabilis* 12 B-r has the structure shown in Fig. 4.

**DISCUSSION**

Two strains, 12 B-r and 12 B-k, were isolated from a wound and faeces of the same hospitalized patient in Łódź and classified to *Proteus* serogroups O8 and O2, respectively. Strains of these O serogroups have not been reported as the most prevalent clinical isolates in different countries (Larsson, 1984), including central Poland (Drzewiecka, 2016; D. Drzewiecka, unpublished data).

In addition to urine, wounds are frequent sources of *Proteus* spp. isolates, causing 4% of wound infections (Różalski et al., 2007). Strain 12 B-r that infected the patient’s wound belonged to *Pt. mirabilis*, the species responsible for the majority of *Proteus* infections and

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**Fig. 4.** Structures of the O antigens of *Pt. mirabilis* 12 B-r, O8 and O16. The proposed epitope shared by 12 B-r and O16 is shown in bold type. EtNP, Phosphoethanolamine; P, phosphate; Rib-ol, ribitol.

**Fig. 5.** Western blot after SDS-PAGE of *Pt. mirabilis* 12 B-r, O8 and O16 LPSs with O8 (a), O16 (b) and 12 B-r (c) antisera.
more pathogenic than the other species. Particularly, *Pt. mirabilis* is an important cause of nosocomial wound infections. Recently, it has been found to be the third most common species after *Staphylococcus aureus* and *Pseudomonas aeruginosa*, recognized in 10% of wound swabs from hospitalized patients, including polymicrobial infections (Bessa et al., 2015).

Patients’ faeces may be an original source of facultative pathogens from the genus *Proteus* leading to auto- and cross-infections. The bacteria in the conditions of an impaired immunity system can easily reach niches other than the intestines and the same clone may be detected in different materials from an infected patient (Drzewiecka et al., 2008, 2010; Gibbs & Greenberg, 2011). However, *Pt. mirabilis* 12 B-r was not detected in the faeces of the woman, and therefore its occurrence in the wound might result from a nosocomial infection rather than an auto-infection. Instead, a representative of *Proteus* genomospecies 5/6, 12 B-k, was a constituent of the intestinal microflora of the hospitalized patient, and this work is among few reports on the habitats and properties of *Proteus* genomospecies separated from *Pt. vulgaris* (Arbatsky et al., 2013; Janda et al., 2001; O’Hara et al., 2000b).

Serological and structural analyses showed the identity of the O antigens of strain 12 B-k and *Pt. vulgaris* O2, whereas the O antigens of *Pt. mirabilis* 12 B-r and *Proteus* spp. O8 were found to be closely related but not identical. They share the branched carbohydrate backbone composed of tetrasaccharide repeating units (Fig. 4), which defines a common epitope called ‘a’ responsible for the strong cross-reactivity of these bacteria. 12 B-r has an additional epitope called ‘b’, which is evidently associated with EtNP linked to position 6 of α-2-3 linked GalpNAc component as *Pt. mirabilis* O16, which possesses the same EtNP-6-α-2-3-GalpNAc O-antigen component as *Pt. mirabilis* 12 B-r (Toukach et al., 2001). The common fragments of the 12 B-r, O8 and O16 O-polysaccharide that function as putative cross-reactive epitopes are depicted in Fig. 4.

Although the α-2-GlcNAc-linked EtNP group is also present in the O-polysaccharide of *Pt. mirabilis* O17 (Toukach et al., 2002), no significant cross-reactivity was observed between this O serogroup and either *Pt. mirabilis* O16 (Torzewska et al., 2006) or *Pt. mirabilis* 12 B-r [LPS and antisera of the latter reacted in ELISA very weakly with antisera and LPS of *Pt. mirabilis* O17 (reciprocal titres 4000 and 1000, respectively)]. This phenomenon could be accounted for by a different environment of the EtNP-associated epitope resulting in its different accessibility for interactions with recognizing proteins.

Contrary to the closely related genus *Providencia* (Ovchinikova et al., 2013) or, e.g. *Citrobacter* spp. (Knirel, 2011),

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**Table 4.** $^1$H and $^{13}$C NMR chemical shifts (δ, p.p.m)

<table>
<thead>
<tr>
<th>Sugar residue</th>
<th>Nucleus</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td><em>Pt. mirabilis</em> 12 B-r (this work)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→3)-α-2-GlcNAc(1→a</td>
<td>$^1$H</td>
<td>5.12</td>
<td>4.12</td>
<td>3.90</td>
<td>3.73</td>
<td>4.18</td>
<td>4.07, 4.22</td>
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<td></td>
<td>$^{13}$C</td>
<td>99.7</td>
<td>55.1</td>
<td>77.5</td>
<td>69.1</td>
<td>72.2</td>
<td>65.5</td>
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<tr>
<td>→3)-β-2-GlcNAc(1→ →3)-β-2-GlcNAc(1→</td>
<td>$^1$H</td>
<td>4.67</td>
<td>3.59</td>
<td>3.59</td>
<td>3.83</td>
<td>3.88</td>
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<td>$^{13}$C</td>
<td>105.1</td>
<td>73.6</td>
<td>83.6</td>
<td>73.2</td>
<td>77.0</td>
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<td>4.05</td>
<td>4.12</td>
<td>4.48</td>
<td>1.31</td>
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<td></td>
<td>$^{13}$C</td>
<td>99.1</td>
<td>50.3</td>
<td>74.8</td>
<td>80.8</td>
<td>69.1</td>
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<td>α-2-GalpNAc(1→</td>
<td>$^1$H</td>
<td>5.05</td>
<td>3.69</td>
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<td>3.85</td>
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<td>$^{13}$C</td>
<td>102.1</td>
<td>69.9</td>
<td>70.8</td>
<td>70.9</td>
<td>72.8</td>
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<tr>
<td>EtNP</td>
<td>$^1$H</td>
<td>4.14</td>
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<tr>
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<td>$^{13}$C</td>
<td>63.3</td>
<td>41.6</td>
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<td><em>Pt. vulgaris</em> O8 (Perepelov et al. 1999)</td>
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<td>→3)-α-2-GlcNAc(1→</td>
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<td>5.16</td>
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<td>→3)-β-2-GlcNAc(1→</td>
<td>$^1$H</td>
<td>4.65</td>
<td>3.58</td>
<td>3.63</td>
<td>3.83</td>
<td>3.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{13}$C</td>
<td>105.3</td>
<td>74.2</td>
<td>84.2</td>
<td>73.6</td>
<td>77.9</td>
<td>173.7</td>
</tr>
<tr>
<td>→4)-α-2-FucNAc(1→</td>
<td>$^1$H</td>
<td>5.04</td>
<td>4.44</td>
<td>4.03</td>
<td>4.12</td>
<td>4.46</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C</td>
<td>99.6</td>
<td>50.6</td>
<td>75.3</td>
<td>81.1</td>
<td>69.7</td>
<td>16.7</td>
</tr>
<tr>
<td>α-2-GalpNAc(1→</td>
<td>$^1$H</td>
<td>5.05</td>
<td>3.71</td>
<td>3.78</td>
<td>3.91</td>
<td>4.10</td>
<td>3.69</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C</td>
<td>102.5</td>
<td>70.4</td>
<td>71.4</td>
<td>71.5</td>
<td>73.2</td>
<td>63.4</td>
</tr>
</tbody>
</table>

EtNP, Phosphoethanolamine.

*Chemical shifts for the N-acetyl groups are: a, δ$_H$ 1.96, δ$_C$ 23.3 (CH$_3$) and 175.2 (CO); b, δ$_H$ 2.01, δ$_C$ 23.7 (CH$_3$) and 175.8 (CO).
the genus *Proteus* comprises many complex O serogroups that are subdivided into different subgroups (O serotypes), and the number of serotypes is constantly growing. O8 is the thirteenth O serogroup in the genus *Proteus* subdivided into subgroups (Knirel *et al.*, 2011). It has an interspecies character as it includes strains belonging to *Pt. mirabilis*, *Pt. vulgari*, *Pt. penneri* and *Proteus* genospecies 5 (Drzewiecka *et al.*, 2004; Knirel *et al.*, 2011; Perepelov *et al.*, 1999; Zych *et al.*, 2005). One can suggest that this O-antigen form might spread by a horizontal gene transfer in the course of the convergent adaptation of strains. Inter- or intra-species transfers of O-antigen biosynthesis genes or clusters by insertion sequence elements, plasmids or phages have been reported for other genera of Gram-negative bacteria (Wang *et al.*, 2010).

On the other hand, it may be speculated that *Pt. mirabilis* 12 B-r evolved from a *Pt. mirabilis* O8a strain by acquisition of an O-antigen-specific EtNP-transferase gene, which could be an example of the divergent adaptation. O-antigens that contain this constituent are rather common among *Proteus* spp. having been found so far in 11 *Proteus* O serotypes. Therefore, its occurrence in the O-polysaccharide may be advantageous for the bacteria, helping their adaptation to changing environmental conditions or the evasion of the host defence mechanisms. EtNP is always linked to position 6 in different sugars (glucose, galactose, GlcNAc or GalNAc) (Knirel *et al.*, 2011), giving rise to a component that is important for the serospecificity of the bacteria (this work; Drzewiecka *et al.*, 2002).

**Fig. 6.** $^1$H NMR (top) and $^{13}$C NMR (bottom) spectra of the O-polysaccharide of *Pt. mirabilis* 12 B-r. Numbers refer to protons or carbons in phosphoethanolamine (EtNP) and sugar residues denoted as follows: F, FucNAc; G, Gal; GA, GlcA; GN, GlcNAc.
The O8a,b serotype is the fifth Proteus O serotype that has been described recently in Poland, further confirming serological and structural heterogeneity of the O antigens of Proteus spp. isolates from Polish patients (Arbatsky et al., 2013; Drzewiecka et al., 2008, 2010; Siwińska et al., 2015). On the other hand, multiple similarities are observed among Proteus spp. O antigens, which are manifested in frequent cross-reactivity between various O serotypes (Knirel et al., 2011) and in some cases, make serotyping of Proteus spp. difficult. These similarities highlight the constituents and epitopes, which are important for the survival of bacteria in various conditions and could be used for design of polyvalent vaccines against these opportunistic pathogens.

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