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SHORT COMMUNICATION

The Cross-Reactivity of *Shewanella fidelis* Lipopolysaccharide with Anti-*Proteus* Antibodies

MAREK KWINKOWSKI[1]*, SEBASTIAN GRABOWSKI[2], IWONA KONIECZNA[1], EVGENY L. NAZARENKO[3] and WIESŁAW KACA[1]

[1] Department of Microbiology, Institute of Biology, Jan Kochanowski University, Kielce, Poland
[2] Department of Immunobiology of Bacteria, Institute of Microbiology and Immunology, University of Łódź, Łódź, Poland
[3] Pacific Institute of Bioorganic Chemistry, Far East Branch of Russian Academy of Sciences, Vladivostok, Russia

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Abstract

The serological cross-reactivity between lipopolysaccharides (LPS) of *S. fidelis* KMM3582^T and rabbit anti-O *P. mirabilis* antibodies was tested. Using ELISA and Western blot cross-reactivity between *S. fidelis* LPS and antisera against *P. mirabilis* O14, O3 LPSs was found. The observed cross-reaction may suggest that anti-*P. mirabilis* S1959 (O3) antibodies may bind to the internal part of *S. fidelis* O-polysaccharides. A weak interaction between *S. fidelis* LPS and antiserum against *P. mirabilis* O13 in Western blot suggests that the absolute configuration of non-sugar "AlaLys" component (N^{ε}-[(S)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine) may influence the affinity of antibodies for *S. fidelis* LPS.

Key words: *Proteus mirabilis*, *Shewanella fidelis*, cross-reactivity, lipopolysaccharides

Proteus mirabilis rods are common inhabitants of human microflora. The bacteria are also regarded as a urinary tract infection pathogen. Polluted water as well as soil are ecological systems inhabited by *Proteus* (Rozalski *et al.*, 1997).

The genus *Shewanella*, a Gram-negative bacterium, is associated with aquatic deep-sea habitats (Ivanova *et al.*, 2003). Several species of *Shewanella* show a capacity for bioremediation of organic pollutants, *i.e.* crude petroleum (Hau and Gralnick, 2007).

The genera *Proteus* and *Shewanella* are in different genetic families – *Enterobacteriaceae* and *Shewanellaceae*, respectively. However these genetically different species have a common, very similar and unique O-specific polysaccharide feature – the presence of amino-acid derivatives, linked to uronic acid by a quasi-amide bond. The common component (named "AlaLys") is N^{ε}-[(S)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine – in *Shewanella fidelis* KMM3582^T (Kilcoyne *et al.*, 2004), or N^{ε}-[(R)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine – in *Proteus mirabilis* O13 (Swierzko *et al.*, 2001). These structures have a different absolute configuration of the alanine carbon α only. Similar structures were found in other *Proteus* species (Sidorczyk *et al.*, 2003) and in *Providencia* spp. (Torzewska *et al.*, 2004). The serological cross-reactivity between lipopolysaccharides (LPS) from *Proteus* and *Shewanella* has not yet been studied and this was the aim of the presented studies. From more than 70 *Proteus* O-antigen structures 5 were chosen for cross-reactivity analysis due to partial structure similarities.

S. fidelis LPS from KMM3582^T strain was from the Pacific Institute of Bioorganic Chemistry, Far East Branch of Russian Academy of Sciences collection. *P. mirabilis* strains were from the Czech National Collection of Type Cultures. Lipopolysaccharide was extracted and all serological methods were performed as described previously (Arabski *et al.*, 2008).

Chemical structures of the LPSs used are presented in Table I. We performed ELISA analysis of *P. mirabilis* O13 and *S. fidelis* lipopolysaccharide with rabbit

* Corresponding author: M. Kwinkowski, Department of Microbiology, Institute of Biology, Jan Kochanowski University, Świętokrzyska 15, 25-406 Kielce, Poland; phone/fax.: (+48) 41 349 63 07; **e-mail: marek.kwinkowski@ujk.edu.pl**

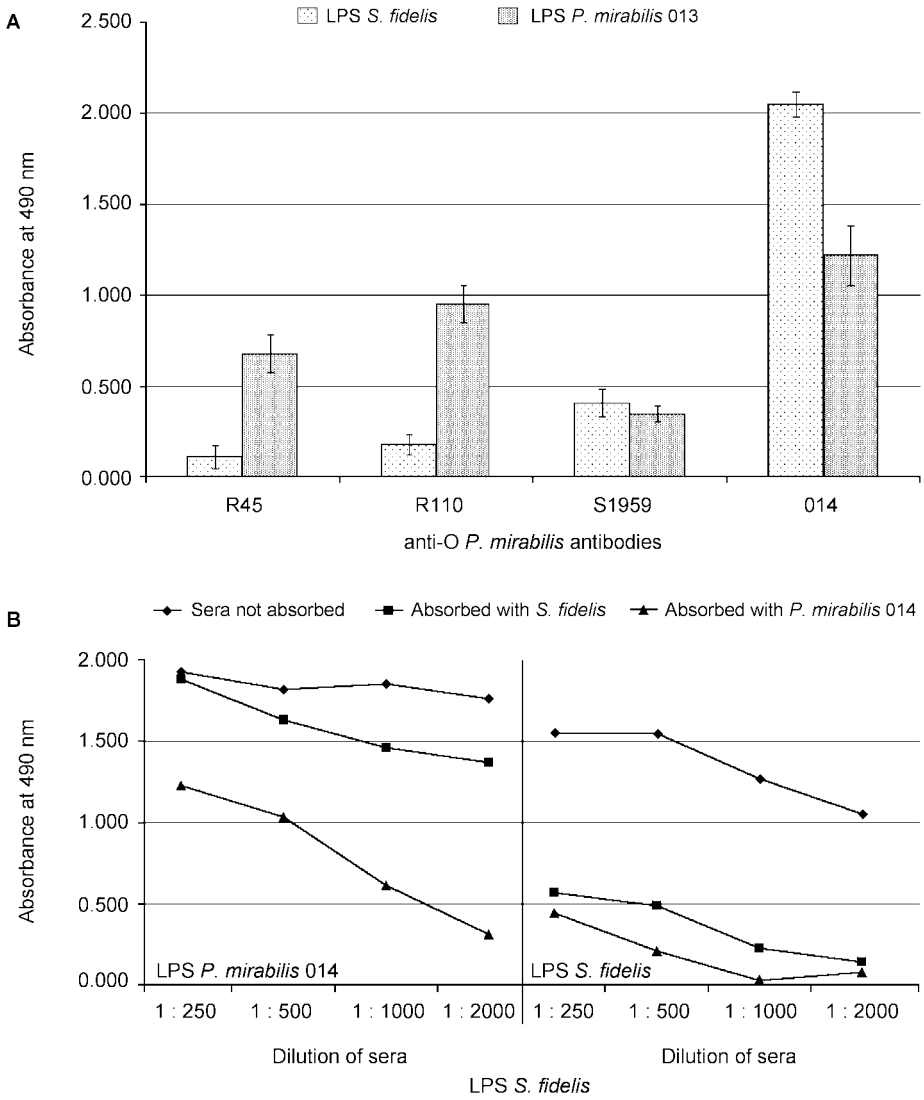

Fig. 1. A: ELISA analysis cross-reaction between anti-O *P. mirabilis* antibodies and LPSs isolated from *P. mirabilis* O13 and *S. fidelis* strains.
B: ELISA absorption test. The anti- *P. mirabilis* O14 serum absorbed with *S. fidelis* and *P. mirabilis* O14 LPSs; left – *P. mirabilis* O14, right panel – *S. fidelis* LPS as antigens.

antisera against *P. mirabilis* strains: R45, R110, S1959 (O3) and O14 (Fig. 1A) and found that *S. fidelis* LPS showed weak cross-reactivity with *P. mirabilis* S1959 and strong with *P. mirabilis* O14 antisera. In a control experiment (with rough mutant: *P. mirabilis* R45 and R110 antibodies) only reactions of antisera with O13 LPS were observed, and this may be caused by interaction with the core part of both LPSs.

To confirm the ELISA results absorption tests with the strongest reacting anti-*P. mirabilis* O14 antibodies were carried out (Fig. 1B). When *P. mirabilis* O14 LPS was used on the plates as antigen – only absorption with homologous O 14 LPS reduced the binding of antibodies (Fig. 1B left panel). However, when the *S. fidelis* LPS was used as an antigen, reduction of binding effect was observed for sera pre-absorbed with both homologous O14 and heterologous *S. fidelis* LPSs (Fig. 1B right panel). This may suggest that *S. fidelis* and *P. mirabilis* O14 LPSs bind different populations of antibodies.

To find out what part of *S. fidelis* LPS is responsible for the observed cross-reaction a Western blot was done. No reaction was observed with anti-*P. mirabilis* O14 antibodies (data not shown). A positive and strong reaction of *S. fidelis* O-polysaccharide was observed with anti-*P. mirabilis* S1959 (O3) serum (Fig. 2A), and a weak reaction with anti-O13 serum (Fig, 2 B). In both cases only the fraction of LPSs with long O-polysaccharide part bound rabbit anti-O *Proteus* antibodies.

In conclusion – the observed cross-reaction of anti-*Proteus* antibodies with *S. fidelis* LPS may suggest that in case of anti-*P. mirabilis* S1959 (O3) antibodies bind to the internal part of O-polysaccharides

Table I
Structures of O-specific polysaccharide of LPSs used in this study

Strain	Structure of O-specific polysaccharide	Reference
S. fidelis KMM3582^T	→3)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→2)-β-D-Gal*p*A-(1→ 6 | *2S*,*8S*-AlaLys	Kilcoyne *et al*., 2004
P. mirabilis O13	→3)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→2)-α-D-Gal*p*A-(1→ 6 | *2R*,*8S*-AlaLys	Swierzko *et al*., 2001
P. mirabilis O14	D-AlaEtn*P*¬ 6 →3)-α-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→	Perepelov *et al.*, 1999
P. mirabilis S1959 (serotype O3)	α-D-Gal*p*A6(L-Lys)-(1¬ α-D-Glc*p*-(1¬ 4 2 →6)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→	Kaca *et al*., 1987
P. mirabilis R110	Rough mutant (Ra) of *P. mirabilis* S1959 (only core oligosaccharide, without O-specific polysaccharides)	Vinogradov *et al.*, 2000
P. mirabilis R45	Deep rough mutant (Re) of *P. mirabilis* S1959 (only two Kdo, without O-specific polysaccharides and major part of core oligosaccharide)	Sidorczyk *et al.*, 1987

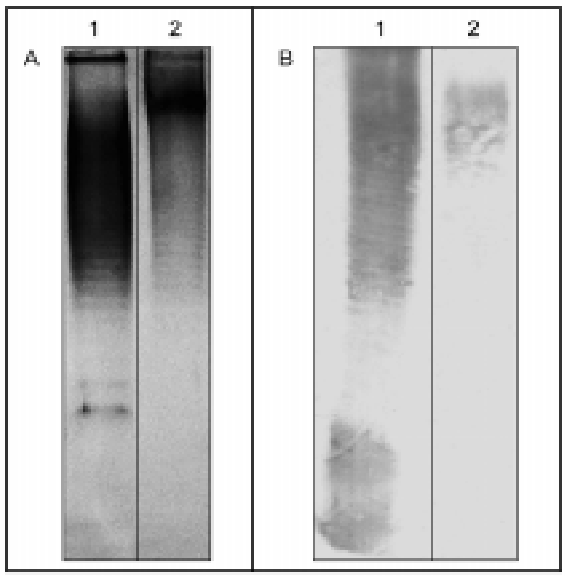

Fig. 2. Western blot serological reactions.
A: Anti-*P. mirabilis* S1959 (O3) antibodies reacting with: lane 1 – *P. mirabilis* S1959, lane 2 – *S. fidelis* LPSs,
B: Anti-*P. mirabilis* O13 antibodies reacting with: lane 1 – *P. mirabilis* O13, lane 2 – *S. fidelis* LPSs.

of *S. fidelis* LPS (-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc- (Table I). The weak interaction between *S. fidelis* LPS and antiserum against *P. mirabilis* O13 in Western blotting suggests that the inverted configuration in "AlaLys" may influence the affinity of the antibodies for *S. fidelis* LPS. Heterologous reactions of rabbit polyclonal antibodies with *Shewanella* LPS may also depend on the type test used – ELISA or Western blotting.

The genetic determination of N^{ε}-[(R)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine in *P. mirabilis* O13 LPS is currently under investigation.

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