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Structural characterization of the O-polysaccharide antigen of *Edwardsiella tarda* MT 108

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Abstract—*Edwardsiella tarda*, a Gram-negative bacterium, is an important cause of hemorrhagic septicemia in fish and also of gastro- and extraintestinal infections in humans. The lipopolysaccharide produced by the fish pathogenic strain *E. tarda* MT 108 was isolated and the structure of its antigenic O-polysaccharide component determined by the application of chemical analyses, high-resolution 1D and 2D nuclear magnetic resonance spectroscopy, and mass spectrometry. The polysaccharide was found to be a polymer of a repeating pentasaccharide unit composed of 2-acetamido-2-deoxy-D-glucose (D-GlcNAc), 2-acetamido-2-deoxy-D-galactose (D-GalNAc), D-galactose (D-Gal), L-rhamnose (L-Rha), D-galacturonic acid (D-GalA) and (2*S*,3*R*)-threonine (1:1:1:1:1:1) having the structure:

$$[\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow]$$
$$\alpha\text{-D-Gal}p\text{A6Thr-}(1\rightarrow 3)^{]}$$

Keywords: Lipopolysaccharide; *Edwardsiella tarda*, O-antigen

1. Introduction

The genus *Edwardsiella* includes two species of bacteria of the family Enterobacteriaceae that cause disease in at least 20 species of warm water fish and are opportunistic pathogens of humans. *Edwardsiella tarda*[1] infects fish and other animals and *Edwardsiella ictaluri*[2,3] infects fish only. *E. tarda* is common to tropical and subtropical environments and causes a variety of fish diseases collectively referred to as *Edwardsiella* septicemias.[4] Of particular economic interest are the substantial losses through disease, encountered in the commercial pond culture of catfish,[5] caused by both *E. tarda* and *E. ictaluri* infections.[6,7] However, *E. tarda* is also known to cause gastrointestinal infections[8] and extraintestinal infections such as myonecrosis,[9] bacteremia,[10] septic arthritis,[11,12] wound infections[13] in humans and has been associated with septic shock with high mortality.[14] It is reasonable to assume that control of these environmental pathogens would naturally reduce the risk to humans as well as provide a commercial benefit to aquafarms.

In this regard, control of infections would be aided by the development of diagnostic agents and/or effective fish vaccines. In furthering our understanding of the pathogenesis of *Edwardsiella* septicemias, the extraction, purification, and structural characterization of *Edwardsiella* immunodominant antigens is important and of obvious potential benefit to both the aquaculture industry and to human health. In this context, we now report the characterization of the O-polysaccharide antigen of the lipopolysaccharide produced by a clinically isolated typically pathogenic *E. tarda* strain (MT 108).

2. Experimental

2.1. Growth of *E. tarda*

E. tarda (strain #MT 108) was the gift of Dr. Emmett B. Shotts, University of Georgia, and was provided by

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J. Barlow of Microtek Research and Development, Saanichton, BC MT 108 was a typical isolate from a catfish having succumbed to edwardsiellosis and was confirmed biochemically and serologically. The strain was stored at −70 °C in tryptic soy broth (TSB) and streaked onto tryptic soy agar at 25 °C prior to scale up for fermentation. The scale-up procedure was also in TSB at 25 °C and began from a loop of culture to 50 mL then to 500 mL after growth overnight at each stage. The 500 mL inoculum was then used for the final fermentation in a 35 L Chemap fermenter, again at 25 °C in TSB with the initial addition of 0.5% glucose. Once cells had reached late log phase (~8 A_{650}) cells were harvested by ultrafiltration and finally by centrifugation at 10,000*g* and frozen at −70 °C. The completed fermentation was subsequently checked for purity of the production strain, by Gram stain and serologically.

2.2. Extraction of LPS and preparation of O-PS[15]

A thawed wet paste of *E. tarda* cells (~80 g) was extracted with stirred 50% (w/v) aq phenol (800 mL) at 65 °C for 15 min and on cooling (4 °C) and low speed centrifugation (10,000*g*), the top aq and lower phenol phases were separated by aspiration, the separated phases were dialyzed against running water until free from phenol, and the retentates were lyophilized. The two products were each dissolved in 0.02 M sodium acetate (pH 7.0, 80 mL) and treated sequentially for 2 h each at 37 °C with DNase, RNase and proteinase K, and after the removal of precipitated material, were subjected to ultracentrifugation (105,000*g*, 4 °C, 12 h). The precipitated gels were dissolved in distilled water and lyophilized to yield 4.70 g aq phase LPS and 420 mg phenol phase LPS.

The aq phase LPS (1.0 g) was treated with 2% (v/v) AcOH (100 mL) at 100 °C for 2 h and following removal of precipitated lipid A, the concentrated water soluble products were fractionated by Sephadex gel G-50 column chromatography to yield a high molecular mass fraction of O-polysaccharide (K_{av} 0.02, O-PS), a core fraction (K_{av} 0.68), and a fraction (K_{av} 0.97) containing Kdo.

2.3. Chromatography

Gas chromatography (GC) was performed using a HP-1 capillary column (30 m × 0.25 mm) in an Agilent 6850 chromatograph fitted with a flame-ionization detector, and a temperature program 170 °C (4 min delay) to 260 °C at 4 °C/min. GC–MS was made with a Varian Saturn 2000 ion-trap GC–MS instrument using a program 180–260 °C at 3.5 °C/min. Mobilities are quoted relative to hexa-*O*-acetyl-D-glucitol (T_G = 1.00) or 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (T_{GM} = 1.00).

Preparative paper chromatography was made on Whatman 3MM paper using *n*-butanol/pyridine/water (6:3:1 v/v top layer) as the mobile phase and detection was made by 2% *p*-anisidine HCl ethanolic spray reagent.

For the determination of glycose configurations, acetylated 2-(*R*)- and (*S*)-butyl glycosides of isolated monosaccharides were made by the procedure of Gerwig et al.[16] and analyzed by GLC–MS with comparison to authentic reference samples.

Column chromatography was made using Sephadex G-50 (2.5 × 100 cm) and 0.025 M pyridinium acetate (pH 4.5) as the eluant.

2.4. Methylation analysis

Samples (2–3 mg) in Me_2SO (0.5 mL) were methylated by the Ciucanu–Kerek procedure[17] and were hydrolyzed (3 M TFA, 100 °C, 2 h), reduced ($NaBD_4$), converted to their alditol-*1-d* acetate derivatives and analyzed by GC–MS.

2.5. Periodate oxidation

O-PS was oxidized by periodate and subjected to the Smith degradation procedure[18] following previously described experimental details.[19]

2.6. Gel electrophoresis

Deoxycholate-polyacrylamide electrophoresis (DOC-PAGE) was performed on separating gels of 14% acrylamide and 9% sodium deoxycholate and detection was made by silver staining after oxidation with periodate.[19]

2.7. NMR spectroscopy

^1H and ^{13}C spectra were recorded using a Varian 400 MHz spectrometer with samples in D_2O at 50 °C for the polysaccharide and at 25 °C for oligosaccharides and referenced to internal acetone standard (^1H 2.225 ppm, ^{13}C 31.07 ppm). COSY, TOCSY, NOESY, HSQC, and HMBC experiments were used as previously described.[19]

2.8. Amino acid analysis

Separation was performed on a Phenomenex Chirex (D) penicillamine column (250 × 4.60 mm) in 2 mM $CuSO_4$ in 15% aq MeOH at a flow rate of 1 mL/min and detection was made using a UV detector at 254 nm.

2.9. HF solvolysis

O-PS (30 mg) contained in a plastic vial was treated with anhyd HF (2 mL) for 1 h at 25 °C and then dried in a stream of dry N_2. The residue dissolved in water (2 mL) was adjusted to pH 7 with dilute ammonium hydroxide and lyophilized.

3. Results and discussion

Cells of *E. tarda* MT 108 (80 g wet paste) were extracted using a modified hot aqueous phenol method[15] and following sequential digestion of the separated and concentrated dialyzed water and phenol phase extracts with DNase, RNase, and proteinase K, ultracentrifugation gave precipitated aqueous phase LPS (~5.9% yield) and phenol phase LPS (~0.8% yield). DOC-PAGE analysis of both LPS products gave typical S-type banding patterns in which the ladder spacing bands were indicative of an LPS with an O-PS composed of a repeating pentasaccharide unit.[20] Both LPS products were chemically identical, however all subsequently described analyses were made on the aqueous phase LPS preparation. The aqueous phase LPS (1.0 g) on mild hydrolysis (2% AcOH, 100 °C, 2 h) afforded an insoluble lipid A (80 mg) and Sephadex G-50 chromatography of the soluble products afforded O-PS (K_{av} 0.02–0.04, 440 mg), core oligosaccharide (K_{av} 0.68, 130 mg) and a fraction (K_{av} 0.07, 40 mg) containing Kdo. The phenol phase LPS proved to be identical to the aqueous phase LPS and the latter LPS was used in all further studies. The O-PS had $[\alpha]_D$ +66 (*c* 0.2, water).

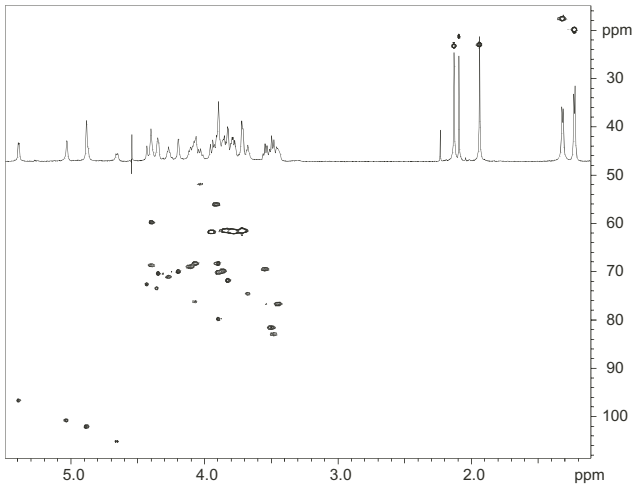
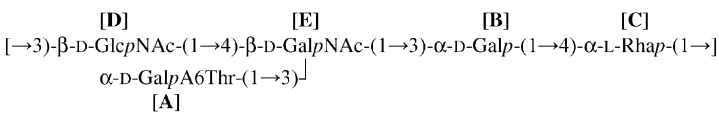


Figure 1. ^{1}H–^{13}C HSQC correlation spectrum of *E. tarda* O-specific polysaccharide.

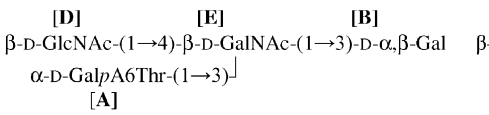
Table 1. NMR data for the *E. tarda* O-PS and derived oligosaccharides **3** and **4**

Unit, compound		1	2	3	4	5	6 (6a)	6b
[A] O-PS, **3**, **4**	^{1}H	5.40	4.07	3.89	4.35	4.43		
	^{13}C	96.7	68.4	70.2	70.4	72.7	171.2	
[B] O-PS	^{1}H	5.04	3.91	3.89	4.20	4.27	3.72	3.72
	^{13}C	100.8	68.4	79.9	70.1	71.1	61.6	
[B] **3**-α	^{1}H	5.21	3.86	3.88	4.16			
	^{13}C	93.7	68.6	80.1	70.4			
[B] **3**-β	^{1}H	4.55	3.53	3.71	4.11			
	^{13}C	97.8	72.2	83.1	69.8			
[C] O-PS	^{1}H	4.89	3.83	3.86	3.50	4.11	1.32	
	^{13}C	102.1	71.8	69.9	81.6	69.1	17.8	
[D] O-PS	^{1}H	4.89	3.91	3.48	3.55	3.45	3.78	3.95
	^{13}C	102.1	56.2	83.1	69.6	76.8	61.8	
[D] **3**, **4**	^{1}H	4.86	3.82	3.41	3.47	3.43	3.80	3.80
	^{13}C	102.8	56.5	75.8	71.0	77.0	61.9	
[E] O-PS, **3**	^{1}H	4.66	4.03	4.08	4.36	3.67	3.79	3.85
	^{13}C	104.3	52.0	76.3	73.6	74.7	61.7	
[E] **4**-α	^{1}H	5.15	4.29	4.17	4.41	4.11		
	^{13}C	92.5	49.7	74.2	74.5	70.8		
[E] **4**-β	^{1}H	4.66	3.99	4.02	4.36	3.69		
	^{13}C	96.6	53.3	76.8	73.5	75.0		
Thr, all	^{1}H		4.40	4.40	1.23			
	^{13}C	176.3	59.9	68.7	20.0			

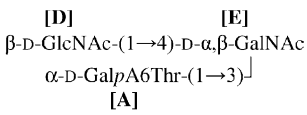
O-Polysaccharide



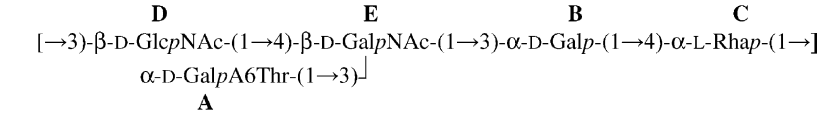
Oligosaccharide **3**



Oligosaccharide **4**



The set of 2D NMR spectra of the O-PS (COSY, TOCSY, NOESY, HSQC, and gHMBC were recorded and completely interpreted (Table 1, Fig. 1). For the NMR analysis, the glycose lycoses were arbitrarily labeled (**A**–**E**) in the order of decreasing anomeric proton chemical shifts. 2D NMR spectra identified the glycose residue followed from a HMBC correlation between Thr H-2 and GalA C-6. From the NMR data the O-PS was concluded to be a repeating unit having the structure as shown below, the D-configuration of the GalA and GalNAc residues being defined from the results of subsequent analyses:



units to be in the pyranose form, and the presence of signals from a threonine residue, and two *N*-acetyl substituents were observed. Anomeric configurations were deduced from the $J_{1,2}$ coupling constants and the chemical shifts of the H-1, C-1, and C-5 signals. The glycose residues α-Gal*p* ($J_{1,2}$ 3.5 Hz), α-Rha*p* ($J_{1,2} \sim 2$ Hz), β-Glc*p*NAc ($J_{1,2}$ 8.0 Hz), α-GalA ($J_{1,2}$ 3.5 Hz), and α-Gal*p*NAc $J_{1,2}$ 3.4 Hz) were identified on the basis of their vicinal coupling constants and ^{13}C NMR chemical shifts.[21,22]

Acid hydrolysis of the O-PS (3 M TFA, 100 °C, 3 h) and GLC analysis of the reduced ($NaBH_4$) and acetylated products identified the acetylated derivatives of rhamnitol, galactitol, and 2-amino-2-deoxyglucitol in a 1:1:1 ratio. Preparative paper chromatography of the hydrolyzate afforded L-rhamnose (R_G 3.25), and D-galactose (R_G 1.02). The L-Rha had $[\alpha]_D$ +8 (*c* 0.1, water) and on methanolysis gave methyl-α-L-rhamnopyranoside having $[\alpha]_D$ −59 (*c* 0.1, MeOH) whose ^1H NMR spectrum was identical with that of an authentic reference sample. The D-Gal had $[\alpha]_D$ +78 (*c* 0.1, water) and on reduction ($NaBH_4$) and acetylation afforded hexa-*O*-acetyl-galactitol having mp and mixed mp 168 °C and $[\alpha]_D$ 0 (CH_2Cl_2). 2-Amino-2-deoxy-D-glucose isolated from the above hydrolyzate by adsorption on Rexyn 101 (H^+) ion-exchange resin and subsequent elution with 0.1 M HCl, had $[\alpha]_D$ +68 (*c* 0.12, water). The D-configuration of galactose and 2-amino-2-deoxy-D-glucose were confirmed by the GLC analysis of the 2-(*R*)-butyl glycosides comparing with those of reference derivatives. The amino acid component released from the O-PS by HCl hydrolysis was identified as (2*S*,3*R*)-threonine (L-threonine) by separation made using a Phenomenex Chirex (D) penicillamine column and reference samples. The characterization of the above glycose components as 1:1:1 L-Rha, D-Gal, and D-GlcN, at this point leaves two unidentified O-PS glycose residues, which were however determined from 2D NMR analysis of O-PS.

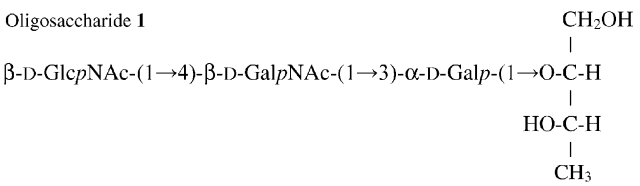
Connections between the glycose residues were determined on the basis of observed interresidue NOE and HMBC correlations (H-1of **D** to C-4 of **E**, H-1 of **E** to C-3 of **B**, H-1 of **B** to C-4 of **C**, H-1 of **A** to C-3 of **E**, and H-1 of **C** to C-3 of **D**). The linkage between the carbonyl C-6 of the D-Gal*p*A residue (**A**) to Nα of the Thr

Methylation analysis of the native O-PS followed by hydrolysis, reduction ($NaBD_4$), acetylation, and GC–MS analysis identified the products as 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-rhamnitol-*1-d* (T_G 0.89), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-galactitol-*1-d* (1.39) and 1,5,3-tri-*O*-acetyl-2-deoxy-2-(*N*-methylacetamido)-4,6-di-*O*-methyl-glucitol-*1-d* (2.60) (ca. 1:1:1). The analysis is consistent with the NMR evidence and confirms the presence in the O-PS chain of the respective units →4)-L-Rha*p*-(1→, →3)-D-Gal*p*-(1→ and →3)-D-Glc*p*NAc-(1→.

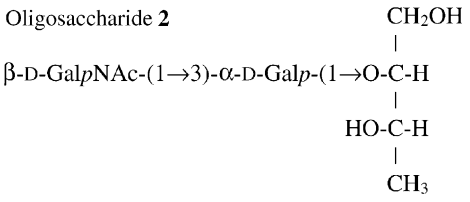
The failure to detect GalN [**E**] and GalA [**A**] in the initial O-PS hydrolysis experiment is no doubt due to the acid stability of the glycoside linkage of the Gal*p*A residue. The identity of these residues and their linkage positions were confirmed from the results of subsequent methylation and periodate oxidation studies made on the O-PS.

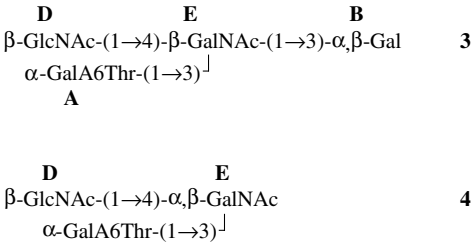
Periodate oxidation of the O-PS followed by reduction ($NaBH_4$) and mild hydrolysis afforded an oligosaccharide (**1**), which on hydrolysis, reduction ($NaBD_4$), acetylation, and GC–MS analysis, showed three major peaks identified as hexa-*O*-acetyl-galactitol-*1-d* (T_G 1.05), 1,3,4,5,6-penta-*O*-acetyl-2-acetamido-2-deoxy-glucitol-*1-d* (T_G 1.28), and 1,3,4,5,6-penta-*O*-acetyl-2-acetamido-2-deoxy-galactitol-*1-d* (T_G 1.32) (ca. 1:1:1). The identification of the residual O-PS glycoses Gal, GlcN, and GalN is consistent with the proposed O-PS structure in which the (1→4)-linked Rha*p* [**C**] residues and Gal*p*A [**A**] residues would have been oxidized by periodate. The removal of the GalA residue facilitated the release of the GalN residue whose identity was confirmed from the GLC identification of its acetylated alditol derivative.

Oligosaccharide **1** subject to further periodate oxidation, reduction ($NaBH_4$), and mild hydrolysis yielded a second oligosaccharide (**2**) that on hydrolysis afforded



Oligosaccharide **2**





Gal and GalN (1:1). The aminoglycose was identified as D-GalN by GC–MS analysis of its acetylated 2-(*S*)-butanolysis products with reference to a standard.[6]

Methylation analysis of the product **1** identified the acetylated methyl ethers of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-galactitol-*1-d* (T_{GM} 1.41), 1,5-di-*O*-acetyl-2-(*N*-methylacetamido)-2-deoxy-3,4,6-tri-*O*-methyl-glucitol-*1-d* (T_{GM} 2.15), 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-(*N*-methylacetamido)-2-deoxy-galactitol-*1-d* (T_{GM} 2.55) (1:1:0.8), and a minor amount (~0.2 mol) of 1,3,4,5-tetra-*O*-acetyl-2-(*N*-methylacetamido)-2-deoxy-6-*O*-methyl-galactitol-*1-d* (resulting from incomplete removal of *O*-3 linked residual oxidation product), thus identifying the linkage positions and the presence of the respective units →3)-D-Gal*p*-(1→ and →4)-D-Gal*p*NAc-(1→, in the O-PS.

Confirmation of a (1→4) linkage to the D-Gal*p*NAc [**E**] residue in the backbone chain and its (1→3) linkage substitution by an α-D-Gal*p*A residue was obtained from methylation analysis of the periodate oxidized and reduced ($NaBH_4$) O-PS product (without subsequent mild hydrolysis) in which the oxidation fragment of the D-GalA was attached at its O-3 position. The GLC–MS characterization of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-galactitol-*1-d* (T_{GM} 1.41), 1,3,5-tri-*O*-acetyl-2-(*N*-acetamido)-2-deoxy-4,6-di-*O*-methyl-glucitol-*1-d* (T_{GM} 2.15), and 1,3,4,5-tetra-*O*-acetyl-2-(*N*-methylacetamido)-2-deoxy-6-*O*-methyl-galactitol-*1-d* (T_{GM} 3.22) (1:1:1) as the major products provides confirmatory evidence that the D-GalA [**A**] residue is linked to the O-3 position of the D-GalNAc residue [**E**] in the O-PS repeating pentasaccharide repeating unit.

The D-configuration of the GalA O-PS component was established by GLC–MS analysis of the 2-(*R*)-butyl glycoside derivatives of the 2-(*R*)-butyl ester of the galacturonic acid made by butanolysis of the HPLC isolated corresponding methyl glycoside methyl ester of D-GalA (**A**) present in the methanolysis product of the native O-PS.

Further confirmation of the proposed O-PS structure was obtained by its depolymerization with anhydrous hydrogen fluoride that led to the formation of numerous products, among them two oligosaccharides **3** and **4**, which were isolated by reverse phase chromatography and analyzed by NMR spectroscopy (Table 1). The results show that, as expected, the glycoside bonds of the Rha and Gal residues were the most unstable in HF and were completely cleaved; some minor cleavage also occurred however at the glycoside linkage of GalNAc [**E**].

The combined NMR and chemical analysis results have given an unequivocal characterization of the antigenic O-chain of the LPS produced by *E. tarda*. To the best of our knowledge, this paper records the first structural characterization of an *E. tarda* LPS O-antigen. Lipopolysaccharide structural elucidation of another important fish pathogen, *Flavobacterium psychrophilum*[23] has recently been shown to facilitate the development of a rapid diagnostic test[24] for this pathogen. Early serological typing systems for *E. tarda*[25,26] recognized multiple serotypes for both O-antigens and flagella-based H-antigens. There is little information however regarding possible relationships between O-antigen serotypes, their predominance, contribution to virulence, or for the geographic distribution of pathogenic strains of the bacterium.[27] Further investigation of the chemical nature of the O-antigens of *E. tarda* isolates would be useful in establishing a facile, structurally based typing system, in indicating the feasibility of O-antigen based vaccine development, and establishing the role of LPS in pathogenesis. While the present characterization of the O-antigen of a single serotype LPS of a fish pathogenic strain of *E. tarda* provides little assistance to the overall development of a structurally based antigen serological typing system, the currently obtained chemical information can be used for quantitative experimental studies in pathogenesis and in polysaccharide-based vaccine development related to experimental fish infections with the studied strain of *E. tarda* the results of which could forseeably be used to diminish the environmental persistence of *E. tarda* and be reflected in a reduction of both fish and human infections.

Acknowledgements

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References

1. Ewing, W. H.; McWhorter, A. C. *Int. B. Bact. Nomencl. T.* **1965**, *15*, 33–38.

2. Hawke, J. P. *J. Fish. Res. Board Can.* **1979**, *36*, 1508–1512.
3. Hawke, J. P.; McWhorter, A. C.; Steigerwalt, A. G.; Brenner, D. J. *Int. J. Syst. Bacteriol.* **1981**, *13*, 396–400.
4. Plumb, J. A. In *Fish Diseases and Disorders*; Woo, P. T. K., Bruno, D. W., Eds.; CABI, 1999; Vol. 3, pp 479–521.
5. Meyer, F. P.; Bullock, G. L. *Appl. Microbiol.* **1973**, *25*, 155–156.
6. Thune, R. L.; Stanley, L. A.; Cooper, R. K. *Annu. Rev. Fish. Dis.* **1993**, *3*, 37–68.
7. Plumb, J. A. *Edwardsiella* septicaemia. In *Bacterial Diseases of Fish*; Inglis, V., Roberts, R. J., Broomage, N. R., Eds.; Cambridge University Press: Cambridge, United Kingdom, 1993; pp 61–79.
8. Janda, J. M.; Abbott, S. L. *Clin. Infect. Dis.* **1993**, *17*, 742–748.
9. Slaven, E. M.; Lopez, F. A.; Hart, S. M.; Sanders, C. V. *Clin. Infect. Dis.* **2001**, *32*, 1430–1433.
10. Yang, C. H.; Wang, C. K. *J. Infect.* **1999**, *38*, 124–126.
11. Ashford, R. U.; Sargeant, P. D.; Lum, G. D. *Med. J. Aust.* **1998**, *168*, 443–444.
12. Osiri, M.; Tantawichien, T.; Deesomchok, U. *Southeast Asian J. Trop. Med. Public Health* **1997**, *28*, 669–672.
13. Banks, A. S. *J. Am. Pediatr. Med. Assoc.* **1992**, *82*, 529–531.
14. Wilson, J. P.; Waterer, R. R.; Wofford, J. D.; Chapman, S. W. *Arch. Int. Med.* **1989**, *149*, 208–210.
15. Johnson, K. G.; Perry, M. B. *Can. J. Microbiol.* **1976**, *22*, 29–34.
16. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, F. G. *Carbohydr. Res.* **1978**, *62*, 349–357.
17. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
18. Goldstein, I. J.; Hay, G. W.; Lewis, B. A.; Smith, F. *Methods Carbohydr. Chem.* **1970**, *5*, 361–370.
19. MacLean, L. L.; Perry, M. B. *Eur. J. Biochem.* **2001**, *268*, 40–57.
20. Perry, M. B.; Babiuk, L. A. *Can. J. Biochem. Cell Biol.* **1984**, *62*, 108–114.
21. Jansson, P.-E.; Kenne, L.; Widmalm, W. *Carbohydr. Res.* **1989**, *188*, 169–191.
22. Lipkind, G. M.; Shashkov, A. S.; Knirel, Y. A.; Kochetkov, N. K. *Carbohydr. Res.* **1988**, *175*, 59–75.
23. MacLean, L. L.; Vinogradov, E.; Crump, E. M.; Perry, M. B.; Kay, W. W. *Eur. J. Biochem.* **2001**, *268*, 1–8.
24. Crump, E. M.; Perry, M. B.; Gale, S.; Crawford, E.; Kay, W. W. *J. Mol. Microbiol. Biotechnol.* **2004**, *6*, 182–190.
25. Tamura, K.; Sakazaki, R.; McWhorter, A. C.; Kosako, Y. *J. Clin. Microbiol.* **1988**, *26*, 2343–2346.
26. Sakazaki, R. In *Methods in Microbiology*; Bergen, T., Ed.; Academic: London, 1957; Vol. 15, pp 213–225.
27. Rao, P. S. S.; Lim, T. M.; Leung, K. Y. *Infect. Immun.* **2003**, *71*(3), 1343–1351.