Serological and structural features of *Hafnia alvei* lipopolysaccharides containing D-3-hydroxybutyric acid

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**Abstract:** The serological heterogeneity of *Hafnia alvei* lipopolysaccharides from strains ATCC 13337, 1187, 1221, 114/60, 1211 and 1216, that contain D-3-hydroxybutyric acid, was analyzed by rocket immunoelectrophoresis, immunoblotting and passive hemagglutination. The significance of D-3-hydroxybutyric acid component for their cross-reactivity has been discussed. The results obtained allowed us to place four *H. alvei* strains (ATCC 13337, 1187, 1221 and 114/60) in one serotype (A) and to consider two other strains (1211 and 1216) as separate serotypes (B and C, respectively).

**Key words:** *Hafnia alvei* lipopolysaccharide; Serology; D-3-hydroxybutyric acid component

**Introduction**

The enterobacterial genus *Hafnia alvei* contains only one species *Hafnia alvei*, which was divided by Sakazaki [1] into 68 O-serotypes. Strains of *Hafnia alvei* have been isolated from faeces of men and animals, water, soil and dairy products; however many cases of nosocomial infections with *Hafnia* have also been reported [1].

In our previous paper [2] the preliminary chemical characterization of *H. alvei* lipopolysaccharides (LPS) isolated from 33 strains was described. Among these strains a group of six lipopolysaccharides containing D-3-hydroxybutyric acid was identified. Recently the structures of O-antigens from strains ATCC 13337, 1187 and 1211 have been established [3,4]. The O-specific polysaccharides (PS) of 114/60 and ATCC 13337 lipopolysaccharides proved to be identical [5]; on the other hand the O-specific polysaccharide of strain 1221 has the same structure as the de-O-acetylated form of ATCC 13337 polysaccharide (paper in preparation).

The aim of the present work was the immunochemical characterization of *H. alvei* lipopolysaccharides containing D-3-hydroxybutyric acid and...
the elucidation of the role of this component in the serological cross-reactivity.

Materials and Methods

H. alvei standard strain ATCC 13337 and four strains 1187, 1221, 1211 and 1216 derived from the collection of the Pasteur Institute (Paris) and strain 114/60 received from the National Institute of Hygiene (Warsaw) were used in the studies. The origin of the remaining H. alvei strains was described in paper [2]. The growth of bacteria in liquid medium, isolation and purification of the lipopolysaccharides and preparation of the O-specific polysaccharides were carried out as described elsewhere [6].

Determination of D-3-hydroxybutyric acid was performed using D-3-hydroxybutyrate dehydrogenase [2]. Lipopolysaccharide or polysaccharide samples (1 mg) were hydrolyzed with 4 M HCl at 100°C for 2.5 h.

The antisera were prepared by immunization of rabbits with bacteria suspended in phosphate-buffered saline (PBS). The animals were injected first subcutaneously with a dose of 100 μg dry bacteria ml⁻¹ PBS and then intravenously twice a week with increasing amounts of the bacteria (100–6400 μg ml⁻¹ PBS). One week after the last injection the rabbits were bled and the sera collected.

Rocket immunoelectrophoresis was carried out by the method of Weeke [7] with a 1% agarose gel in 0.02 M barbital buffer, pH 8.6, containing 2% polyethylene glycol 6000. The antibody gel contained 5% of the appropriate antiserum. After electrophoresis, gel was washed to rid excess reagent, dried and photographed directly or after staining with 0.5% amido black 10B.

For immunoblotting the separated lipopolysaccharides were transblotted from the gel into nitrocellulose (Schleicher-Schuell pore size 0.45 μm) [8]. Electrophoretic transfer was carried out in 10 mM Tris-150 mM glycine buffer containing 20% methanol, pH 8.3, at 100 mA for 1 h. After transfer, the nitrocellulose was blocked with 3% gelatin in 20 mM Tris, 50 mM NaCl, pH 7.5 (TBS) for 1 h at 36°C. The transblot was incubated overnight at 36°C with appropriate anti-H. alvei serum (primary antibody) diluted 1:200 in

![Fig. 1. Rocket immunoelectrophoresis. Antigens: lipopolysaccharides from H. alvei strains 1, 2, 1M, 17, 23, 31, 32, 37, 38, 39, 481-L, ATCC 13337, 1187, 1188, 1190, 1191, 1192, 1196, 1199, 1200, 1203, 1204, 1205, 1209, 1211, 1213, 1214, 1216, 1220, 1221, 1224 and 114/60 (5 μg/5 μl barbital buffer) were placed in wells from left to right (1-32); antibody: A, anti-ATCC 13337; B, anti-1187 sera.](https://academic.oup.com/femspd/article-abstract/8/1/83/443978/183443978)
1% gelatin. The nitrocellulose was washed twice for 20 min with TBS buffer, prior to the incubation with horseradish peroxidase conjugate with goat anti-rabbit IgG (second antibody) diluted 1:3000 in TBS-1% gelatin for 1 h at 36°C. After five-fold washing with TBS, the nitrocellulose was stained with 4-chloro-1-napthol solution (horseradish peroxidase colour substrate) in the presence of H₂O₂.

Results and Discussion

D-3-Hydroxybutyric acid was found in lipopolysaccharides isolated from 6 strains of

Fig. 2. Immunoblots of *H. alvei* lipopolysaccharides from strains: 1. ATCC 13337, 2. 1187, 3. 1221, 4. 114/60, 5. 1211 with sera: A, anti-ATCC 13337; B, anti-1187; C, anti-114/60; and D, anti-1211.
Table 1

The content of 3-hydroxybutyric acid in *Hafnia alvei* lipopolysaccharides and their O-specific polysaccharides

| Strain   | 3-hydroxybutyric acid | O-specific polysaccharide |
|----------|-----------------------|---------------------------|
| ATCC 13337 | 1.82                  | 6.40                      |
| 1187     | 1.25                  | 8.10                      |
| 1221     | 1.05                  | 7.17                      |
| 114/60   | 1.09                  | 6.30                      |
| 1211     | 1.86                  | 7.40                      |
| 1216     | 2.50                  | 5.64                      |

*Hafnia alvei* [2]. Its content in the LPS preparations and the respective O-specific polysaccharides is given in Table 1.

The serological relationships between the lipopolysaccharides were studied by several methods, like rocket immunoelectrophoresis, immunoblotting and passive hemagglutination.

Rocket immunoelectrophoresis of *H. alvei* lipopolysaccharides isolated from 32 strains with anti-ATCC 13337 and anti-1187 sera is shown in Fig. 1A,B. Only four lipopolysaccharides (ATCC 13337, 1187, 1221, and 114/60) gave precipitin lines. No reaction occurred between LPS preparations of strains 1211 and 1216 and anti-ATCC 13337 or anti-1187 sera.

Immunoblotting experiments confirmed the results obtained in the immunoelectrophoresis. A ladder-like pattern of transblotted lipopolysaccharides of ATCC 13337, 1187, 1221 and 114/60 strains after their reaction with anti-ATCC 13337, anti-1187 and anti-114/60 sera (Fig. 2A,B,C) show evidence that they have a common epitope which is located in their O-specific polysaccharide chain.

Lipopolysaccharide of 1211 strain reacted with the homological serum only (Fig. 2D).

For quantitative evaluation of the cross-reactivity of *H. alvei* lipopolysaccharides a passive hemagglutination test was employed. It is clearly visible from the results presented in Table 2 that the lipopolysaccharides of four strains (ATCC 13337, 1187, 1221 and 114/60) cross-reacted distinctly, whereas no reactivity was shown with lipopolysaccharides of strains 1211 and 1216.

As the serological results showed, the antisera used are directed mainly to the O-specific region of the lipopolysaccharides. In some of the sera a minute portion of anti-core antibodies is also present which could be observed in immunoblotting (Fig. 2B,C,D), but not in rocket immunoelectrophoresis (Fig. 1).

The cross-reactions of the lipopolysaccharides (ATCC 13337, 1187, 1221, 114/60) with the homologous and heterologous sera differed in the intensity in immunoblotting and passive hemagglutination (Fig. 2 and Table 2).

For a better understanding of the serological relations within the group of LPS preparations containing 3-hydroxybutyric acid, the structures of their repeating units are shown in Fig. 3. As can be seen, the base chains of the O-specific polysaccharides of strains ATCC 13337, 1187, 1221 and 114/60 are identical. Additional α-glucosyl and O-acetyl side chains occur in ATCC 13337 and 114/60 strains but only α-glucosyl side chains in the 1221 strain. This is in good agreement with the serological evidence on a common

Table 2

Passive hemagglutination of the lipopolysaccharides isolated from *H. alvei* strains with the homologous and heterologous antisera

| Anti-*Hafnia alvei* serum | Lipopolysaccharides | ATCC 13337 | 1187 | 1221 | 114/60 | 1211 | 1216 |
|--------------------------|---------------------|------------|------|------|-------|------|------|
|                          | Passive hemaglutination reciprocal titre |
| anti-ATCC 13337          | 10240               | 20480      | 81900| 1280 | 320   | 10   |
| anti-1187                | 10240               | 20480      | 81900| 640  | 160   | 0    |
| anti-1221                | 5120                | 5120       | 5120 | 5120 | 0     | 0    |
| anti-114/60              | 5120                | 1280       | 5120 | 2560 | 20    | 80   |
| anti-1211                | 160                 | 0          | 0    | 0    | 5120  | 0    |
| anti-1216                | 0                   | 0          | 0    | 0    | 81920 | 0    |
Fig. 3. The structure of O-specific polysaccharides isolated from *H. alvei* strains ATCC 13337, 1187 [3], 114/60 [5], 1211 [4], 1216 (submitted manuscript) and 1221 (paper in preparation). Acyl stands for 3-O-hydroxybutyryl, D-Fuc3NAcyl for 3-amino-N-(3’-O-hydroxybutyryl)-3,6-dideoxy-D-Galp, DQui3NAcyl for 3-amino-N-(3’-O-hydroxybutyryl)-3,6-dideoxy-D-Glcp.

epitope present in the four lipopolysaccharides. The α-glucosyl side chains are not an essential element of the epitope. O-Acetyl groups, however, are a part of the epitope of ATCC 13337 LPS, but they hinder reaction of this LPS with anti-1187 antibodies. D-3-Hydroxybutyryl groups present in the lipopolysaccharides are important for their serological activity [3], but they do not have sufficient power to cause cross-reactivity, e.g. LPS preparations of ATCC 13337 and 1187
strains are not serologically related to 1211 LPS or 1216 LPS although all of them contain D-3-hydroxybutyric acid.

From the results presented above it may be suggested that *H. alvei* strains containing D-3-hydroxybutyric acid in their O-antigens can be divided into 3 serotypes. Four strains (ATCC 13337, 1187, 114/60 and 1221) belong to one serotype (A) and their O-antigens have the common basic structure containing D-3-hydroxybutyric acid. Their antigenic structures are not uniform, however. The differences in the length and density of O-specific chains in the LPS molecules correspond to the serological microheterogeneity within this serotype.

*H. alvei* strains 1211 and 1216 differ serologically and represent two separate serotypes (B and C, respectively). The structures of *H. alvei* 1211 and 1216 O-specific polysaccharides are unique and very much different from those of the four polysaccharides (serotype A) mentioned above as well as amongst themselves.

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