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Subgrouping of Lipopolysaccharide O Antigens from *Vibrio anguillarum* Serogroup O2 by Immunoelectrophoretic Analyses

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Abstract. Lipopolysaccharide antigens from 40 *Vibrio anguillarum* slide agglutination serogroup O2 strains, isolated from different species of diseased fish, were studied by means of immunoelectrophoretic techniques. The study divided the examined strains into two O antigenic subgroups, designated O2a and O2b. Subgroup O2a was implicated more frequently than O2b in vibriosis among salmonids as well as other species of fish. Subgroup O2b was primarily associated with disease in nonsalmonids. Bacterial virulence and host preference are considered in relation to O specificity.

Vibrio anguillarum is an important fish pathogenic bacterium, isolated from disease in a wide range of fish species [1, 4]. Previous studies have realized the value of serology for epidemiologic and ecologic studies of this bacterium [6, 11, 14]. Apparently, O antigen determination gives indications of pathogenic potential, including host preference of *V. anguillarum* strains [14], and is, furthermore, considered important with respect to vaccine purposes [6].

Studies of *V. anguillarum* O antigens have been reported from various countries [5, 6, 11]. On the basis of strains from a wide range of different sources, a study defined a new *V. anguillarum* O antigenic scheme and recommended international harmonization of the diagnostic efforts [14]. With this antigenic scheme as basis, the serologic relationships of O antigens from strains contained in same agglutination O serogroup have been analyzed by immunoelectrophoretic procedures. Some of these analyses, concerned with *V. anguillarum* serogroup O3, suggested differences in the O antigenic specificity between strains associated with disease and strains from sea water or sea floor (H.B. Rasmussen, unpublished results). Recently, lipopolysaccharide O antigens from *V. anguillarum* serogroup O2 were also examined by means of immunoelectrophoretic procedures [12]. The present study continues these immunoelectrophoretic analyses of lipopolysaccharide antigens from *V. anguillarum* O2 strains and examines the O antigenic interrelations of strains from different species of fish.

Materials and Methods

Strains. A total of 40 *V. anguillarum* slide agglutination serogroup O2 strains, isolated from diseased fish in Denmark (28 specimens) and Norway (12 specimens) were selected for the study. The strain designed 1173/1 is O2 reference strain, according to the Danish O antigenic scheme [14]. Examined strains were isolated by J.L. Larsen, Royal Veterinary and Agricultural University, Denmark; I. Dalsgaard, Danish Institute for Fisheries and Marine Research, Denmark; T. Håstein, Veterinary Institute, Norway; and E. Egidius, Institute of Marine Research, Norway.

The O antigen determination (slide agglutination) was performed by J.L. Larsen.

Preparation of antigenic extracts. Strains were grown on Marine agar (Difco) containing 5% citrated calf blood. After incubation for 48 h at 20°C, the plates were harvested. The bacterial suspensions were heated to 60°C for 20 min and then centrifuged. Supernatants were collected and boiled for 1 h. Part of the supernatant from one strain was left unboiled for analysis. This procedure for preparation of antigenic extracts was described previously [10].

Antisera. Production of O and OK antisera was carried out by immunization of rabbits [12].

Immunoelectrophoretic procedures. Tris/barbital electrophoresis buffer, pH 8.6, with an ionic strength of 0.02 was applied. Gels were prepared from 1% agarose HSA (Litex, Copenhagen, Denmark) with Gel Bond plastic film (FMC, Marine Colloids Division, Rockland, Maine, USA) as support. Immunoelectrophoretic techniques have been described elsewhere [7–9, 12].

Gels were stained in a 0.5% wt/vol solution of Coomassie brilliant blue R 250.

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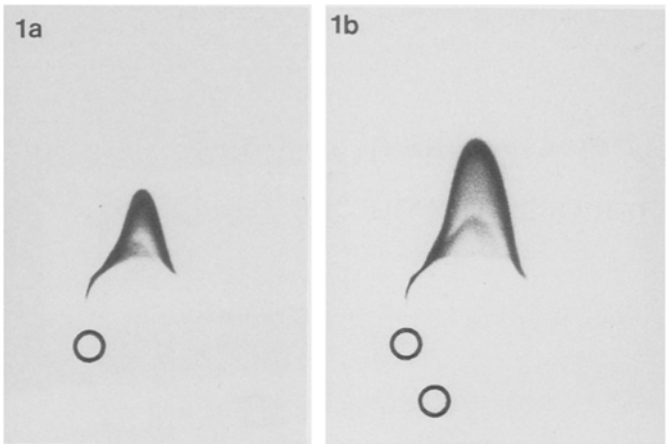

Fig. 1. Tandem-crossed immunoelectrophoresis. An unboiled extract from strain 820723-2/8 was applied in (a) and the upper well of (b). A preparation, obtained by boiling the above extract for 1 h, was placed in the lower well of (b). The antibody-containing gel was prepared with homologous OK antiserum. The anode is to the right in the first dimension electrophoresis and at the top in the second dimension. The application wells are marked.

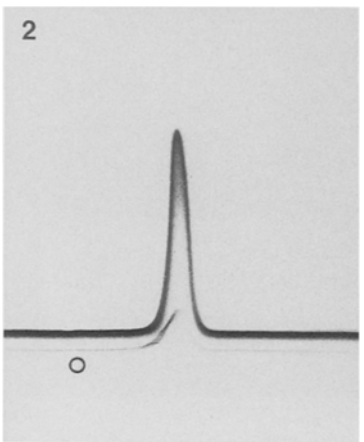

Fig. 2. Crossed-line immunoelectrophoresis. Extract (boiled for 1 h) from strain 820723-2/8 was transferred to the well and also utilized for the antigen-containing gel. The antibody-containing gel was prepared with homologous OK antiserum. The anode is to the right in the first dimension electrophoresis and at the top in the second dimension. The application well is marked.

Results

Antigens from strain 820723-2/8 were analyzed by means of tandem-crossed immunoelectrophoresis. An unboiled extract from this strain produced two peaks, of which one was located below the other (Fig. 1a). Analyses of the unboiled extract as well as the same extract boiled for 1 h (in amounts as applied for the analysis above) also exhibited two peaks (Fig. 1b). The areas of these peaks were at least twice as large as the areas of the corresponding peaks produced by the unboiled extract (Fig. 1a and 1b). With crossed-line immunoelectrophoretic analysis of extract from strain 820723-2/8, two lines elevated by peaks were detected (Fig. 2).

All strains were examined by rocket-line immunoelectrophoreses. Boiled antigenic extracts were applied for these analyses. An analysis with extract from strain 1796 in the antigen-containing gel and OK antiserum against 1173/1 in the antibody-containing gel exhibited two lines completely elevated by extracts from strains 1173/1, 1796, and 1807. In the same analysis, extracts from strains HI 410 and 820723-2/8 produced incomplete line elevations. The reaction between the upper line and strain 820723-2/8 was difficult to distinguish in detail on the photographic reproduction (Fig. 3). Rocket-line immunoelectrophoreses were carried out with extracts from different strains in the antigen-containing gel and in some cases also with a mixture of OK

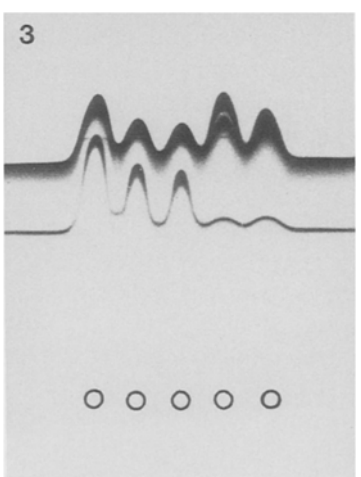

Fig. 3. Rocket-line immunoelectrophoresis. Extracts (boiled for 1 h) from strain 1173/1, 1796, 1807, HI 410, and 820723-2/8 were placed in wells from left to right. Extract (boiled for 1 h) from strain 1796 was utilized for the antigen-containing gel. The antibody-containing gel was prepared with OK antiserum against strain 1173/1. Anode at the top. The application wells are marked.

antiserum against strains 1173/1 and 820723-2/8 in the antibody-containing gel. Using such experimental design, antigens from strains 1173/1, 1796, and 1807 produced incomplete line elevations and

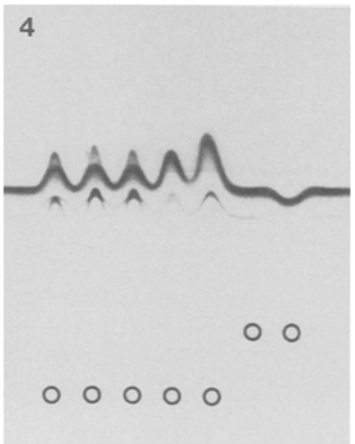

Fig. 4. Rocket-line immunoelectrophoresis. Extracts (boiled for 1 h) from strain 1173/1, 1796, 1807, HI 410, and 820723-2/8 were placed in the wells from left to right on the cathodic side of the antigen-containing gel. In the wells on the anodic side of the antigen-containing gel, PBS with pH 7.2 (to the left) and O antiserum against 1173/1 (to the right) were applied. Extract (boiled for 1 h) from strain 820723-2/8 was utilized for the antigen-containing gel. The antibody-containing gel was prepared with a mixture of OK antisera against strain 1173/1 and 820723-2/8. Anode at the top. The application wells are marked.

caused precipitations below lines. The peaks, exhibited by strain HI 410 and 820723-2/8, fused completely with the lines. Application of O antiserum against 1173/1 in a well on the anodic side of the antigen-containing gel gave rise to cathodic deflections of the lines (Fig. 4). On the basis of rocket-line immunoelectrophoretic patterns, the strains were divided into two antigenic groups (Table 1).

Table 1. *Vibrio anguillarum*, distribution of O2 antigenic subgroups

	Strains	
Origin	Subgroup O2a	Subgroup O2b
Salmo gairdneri and *Salmo salar*[a]	6087 D/2, 9020-1/2, 840613-2/1, 840614-1/1 A, 840614-1/1 B, 840817-2/4 B, 840817-2/6, 840829-1/3 B, 840830-1/3 B, 1782, 1783, 1792, 1796, 1800, 1801, 1807, 1809, 1820, 1821,	820723-2/8
Gadus morhua	1037/1, 1045/2, 1049/2, 1088/1, 1089/1, 1173/1, 1222/1, 1466/1, 1896 S/1	1474/1[c]
Anguilla anguilla	1570/1, 1701/2, 860226-1/7, 860226-1/9	840814-1/5, 840814-1/10
Scophthalmus maximus	None	6828 C[c], 6828 H
Pollachius virens[b]	None	HI 410, 1790
	Number of strains	
Salmonids[d]	19 (20)	1 (20)
Other species[d]	13 (20)	7 (20)

[a] The latter ten strains derived from diseased salmonids in Norway.
[b] Strains isolated from diseased saithe in Norway.
[c] In two strains only one lipopolysaccharide entity was detected.
[d] Numbers in parentheses, total number of strains from salmonids and other species of fish, respectively.

Discussion

Host-specific pathogenicity has been reported in *Vibrio anguillarum* strains isolated from diseased saithe and rainbow trout [3]. The extent of this feature is not clear [4]. In feral fish, vibriosis is most frequently associated with *V. anguillarum* serogroup O2; this serogroup is also commonly involved in vibriosis among saltwater-reared salmonids [14]. A recent examination has suggested that *V. anguillarum* serogroup O2 strains, derived from different species of diseased fish, exhibit serologically closely related lipopolysaccharide antigens. Furthermore, two lipopolysaccharide entities, differing in immunoelectrophoretic characteristics, were demonstrated in extracts from the examined strains [12]. The present study, dealing with a more comprehensive number of O group 2 strains, provided evidence for the existence of two O antigenic subgroups (designated O2a and O2b) and observed two lipopolysaccharide entities. Analyses suggested that these lipopolysaccharide entities were present in unboiled as well as boiled extracts.

The subgroup O2a was detected more frequently than O2b. This finding can reflect difference in virulence. The study, furthermore, indicates that *V. anguillarum* subgroup O2a is commonly involved in vibriosis in nonsalmonids as well as salmonid species, whereas the subgroup O2b strains were isolated primarily from nonsalmonids. Strain HI 410, derived from diseased saithe, was recorded as O2b. In challenge test, this strain produced disease in saithe but not in rainbow trout [3]. The quality as well as amount of O antigen is important with

respect to bacterial virulence, at least in some Gram-negative infections [2, 13, 15, 16], and different host species probably vary in their response toward the same O specificity [15]. Additional studies should clarify possible effects of O specificity on virulence and host preference in *V. anguillarum*. Such studies can be of interest from an epidemiologic point of view and for vaccine purposes.

ACKNOWLEDGMENT

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