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Serology of mesophilic *Aeromonas* spp. and *Plesiomonas shigelloides*

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Key words. O-antigen; H-antigen; mesophilic *Aeromonas; Plesiomonas shigelloides;* antigenic relationship.

Mesophilic *Aeromonas* spp. and *Plesiomonas shigelloides* have been noticed as possible etiological agents of diarrheal diseases in recent years. There have been few studies on the serology of these organisms. Ewing et al.[2] distinguished 12 O- and 9 H-antigens of *A. hydrophila,* but their serotyping system is no longer available at the present time. Recently, Leblanc et al.[5] studied O-antigens of *A. hydrophila* isolates from fish and divided them into 12 O-groups with no designation of antigenic symbols. In the serology of *P. shigelloides,* on the other hand, Quincke[6] demonstrated 16 O-groups, but his study has not yielded an applicable serogrouping system for *P. shigelloides* because his reference strains are not available. Based on 307 strains, Sakazaki and Shimada[7] (and unpublished data) recently established an antigenic scheme of mesophilic *Aeromonas* spp. Also, Shimada and Sakazaki[8,9] defined 50 O- and 17 H- antigens of *P. shigelloides* within 194 strains. In this workshop, the serology of *Aeromonas* and *Plesiomonas* is presented on the basis of our studies.

O- and H-antigens

There are two classes of antigens, O- or somatic and H- or flagellar antigens, that are important in the serotyping of *Aeromonas* and *Plesiomonas* strains. Agglutination of many strains of these organisms in O-antisera is inhibited in the living state. The inhibitory effect is inactivated by heating cell suspensions at 100 °C or treating them with 50% ethanol or 1 N HCl. These findings may suggest the presence of some masking antigens. However, those masking antigens have not been taken into consideration in our serotyping system.

O- antigens for antiserum production and agglutination tests are prepared in the same manner as those used for *Salmonella* and *Escherichia coli.* It was recognized, however, that O-antisera contained varying amounts of R antibodies, which cause confusions in O-agglutination even if they are produced with complete S-form cultures. It should be emphasized, therefore, that all O-antisera of *Aeromonas* and *Plesiomonas* should be absorbed before use with the R-form culture of the same species. Slide agglutination is the method of choice.

It was demonstrated that polymeric flagellin extracted with 0.05 N HCl using the method of Fey and Suter[4] was sufficient to produce high-titered H-antisera for *Aeromonas* and *Plesiomonas.* Although *Aeromonas* and *Plesiomonas* are defined as polarly flagellated rods, the majority of strains produce lateral or peritrichous flagella in young agar cultures. It appears, however, that no difference of antigenicity is present between polar and lateral flagella. For the determination of H-antigens of *Aeromonas* and *Plesiomonas* an overnight culture of actively motile organisms in brain heart infusion is prepared, to which one adds an equal volume of PBS containing 0.1% sodium azide. The H-antigen determination is best performed by a quali-

Table 1. O-antigenic relationships between *Plesiomonas shigelloides* and *Shigella* spp.

P. shigelloides	*Shigella* spp.
11	-*S. dysenteriae* 8
17	= *S. sonnei*
22	-*S. dysenteriae* 7
23	-*S. boydii* 13

=, identical relationship; -, a, b-a, c type relationship.

Table 2. Extrageneric relationship of O-antigens of mesophilic *Aeromonas* species

Aeromonas O-antigen	O-antigen of allied species
3	-*V. cholerae* 51
4	-*V. cholerae* 59
11	-*V. cholerae* 19
13	= *P. shigelloides* 5
17	-*V. cholerae* 2
	-*V. cholerae* 9
19	= *P. shigelloides* 15
23	= *V. cholerae* 39
	= *V. fluvialis* 5
28	-*P. shigelloides* 22
29	-*P. shigelloides* 14
38	-*V. cholerae* 62

=, identical relationship; -, a, b-a, c type relationship.

Table 3. Relationships of O-antigen groups of *Plesiomonas shigelloides* between serogrouping systems of Aldova and Shimada-Sakazaki

Aldova	Shimada and Sakazaki	Aldova	Shimada and Sakazaki
1	6	16	39
2	(R)	17	17
3	27	18	12
4	(R)	19	45
5	32	20	47
6	2	21	46
7	33	22	40
8	34	23	41
9	26	24	42
10	25	25	19
11	35	26	43
12	(R)	27	44
13	36	28	7
14	37	29	4
15	38	30	18

tative tube agglutination using a working dilution of H-antisera. The H-agglutination of *Aeromonas* and *Plesiomonas* culture is flocculent in nature but it may only appear after incubation at 50 °C for 4 h or more.

Extrageneric relationships of mesophilic Aeromonas and Plesiomonas

Since *P. shigelloides* was first noted by Ferguson and Henderson[3] because of O-antigenic identity of their isolates with *Shigella sonnei*, additional antigenic relationships have been recognized between *Plesiomonas* and certain serovars of *S. dysenteriae* and *S. boydii*, as shown in table 1. Furthermore, many close O-antigenic relationships are also recognized among *A. hydrophila*, *P. shigelloides*, *Vibrio cholerae* and *Vibrio fluvialis*. Those are summarized in table 2. Whang et al.[10] reported the presence of the enterobacterial common antigen in strains of *P. shigelloides*.

Further problems with serotyping systems

Mesophilic *Aeromonas* includes three species: *A. hydrophila*, *A. sobria* and *A. caviae*. Since O- and H-antigens of the latter 2 species can be determined with antisera prepared against *A. hydrophila*, serovars of these species are included in a single scheme. Recently, Aldova[1] also published her own antigenic scheme of *Plesiomonas*, independently of Shimada and Sakazaki[2, 3], and includes 30 O-groups. Table 3 shows the relationships betwen the two schemes. It is desirable that one species have a single antigenic scheme which is used internationally. That aim will be achieved in collaboration between Aldova and Shimada and Sakazaki in the near future.

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II. Non-gastrointestinal diseases

Fish-pathogenic aeromonads, with emphasis on the ecology of *Aeromonas salmonicida*

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Key words. Aeromonas salmonicida; Aeromonas hydrophila; fish pathogens; aeromonads; uptake into fish.

Aeromonads, particularly *Aeromonas salmonicida* and to a lesser extent *A. hydrophila*, are recognised to be a scourge of fresh water fish farming worldwide[3]. *A. salmonicida*, which is the causal agent of furunculosis in salmonids, was initially recognised in the last century[6, 15] and has emerged as the best studied of all the bacterial fish pathogens. The significance of this pathogen has overshadowed *A. hydrophila*, which has been described as causing haemorrhagic septicaemia in a diverse array of fish species, including ayu, bass, carp, catfish, perch and salmonids[3].

Aeromonas salmonicida. Although Emmerich and Weibel[6] have been credited with the first description of furunculosis and the classification of the aetiological agent as *'Bacterium salmonicida'*, recognition should also go to Zimmermann[15], who named *'Bacillus devorans'*, a synonym of *'Bacterium salmonicida'*. An identical organism was described independently by Marsh[12] and classified as *'Bacterium truttae'*. Against this confusion, the pathogen was re-classified again, as *Aeromonas salmonicida*[7].

Ecology of Aeromonas salmonicida. By definition, *A. salmonicida* occurs only in fish but not in surface waters. Indeed, microbiological surveys of fresh water, even during the height of furunculosis epidemics, would seem to support this contention[1, 5]. However, such negative data do not explain the reasons for the spread of furunculosis between segregated fish populations or indicate the precise route of infection. Certainly, carrier or subclinically diseased fish may be partially responsible for transmitting the pathogen over small distances[13]. Yet, it seems unwise to neglect exploring fully the possibility of water-borne transmission. Consequently, against this background, work has been carried out to examine aspects of the survival of *A. salmonicida* in fresh water.

A detailed survey of two freshwater sites in England, using selective and non-selective media for aeromonads, failed to reveal the presence of *A. salmonicida* even from the water and sediments around populations of fish which were succumbing to furunculosis[1]. Nevertheless, the use of fluorescent antibody techniques (FAT) incorporating polyclonal antibodies to *A. salmonicida*, revealed the presence of the pathogen in aquatic samples during the height of furunculosis epidemics in July and August. Thus during these periods, micro-colonies of 5–10 coccoid cells, each of 1 μm in length/diameter, were observed on the surface of some water-borne particulates[2]. This suggested a total population of approximately 10^3 *A. salmonicida* cells/ml of fresh water. The viability of the fluorescing cells, which were attached to particulates, was confirmed using nalidixic acid and brain heart infusion broth (BHIB), in a procedure described by Kogure et al.[9]. Here, such coccoid cells enlarged to 2–3 μm in length within 6 h at 18 °C. Unfortunately, it was difficult to discern the exact nature of these particulates, but it was considered possible that they represented pieces of organic material, such as faecal matter or leaf debris. From September to June, there was a marked reduction in the number of *A. salmonicida* cells observed in the water column by FAT. At most, an occasional fluorescing coccoid bacterial-like cell was observed attached to particulates. Such data indicated a population density of ≤ 1 cell/ml. It is relevant to note that from laboratory-based experiments, Sakai[14] correlated the long term survival of virulent cells of *A. salmonicida* with their attachment to particulates, notably sand grains. However, it is impossible to relate such data to seasonal population trends in the natural environment.

From laboratory-based experiments, it was demonstrated that *A. salmonicida* could indeed survive in fresh water, albeit in an