A TAXONOMIC STUDY ON GENUS AEROMONAS

Numerical classification and bacteriophage typing of motile Aeromonas

isolated from river mud, river water and fresh-water fishes

Masafumi FUKUYAMA

Bacterial species being classified into genus Aeromonas

are known to have been having properties quite resembled with

those of the ones in genus Vibrio, and extensively inhabited

in natural environment, such as in water and/or mud of river,

pond, lake or marsh.

Among the species, A.hydrophila has been known as a

pathogene for fresh-water-fishes, reptiles and amphibians, since

the first recognition of the organisms as a causal agent of

red leg disease of frogs. The evidence has been accumulated on

the bacterium, that it is also capable of producing hemorrhagic

septicemia or red pest in fresh-water eel(s) and perforation

disease in artificially breeded fresh-water fishes, and often

giving much economic losses to the fish breeders.

On the other hand, a weak pathogenisity of the bacterium

against humanbeings is also reported. Namely, a frequent isolation

of the bacterium from the clinical specimens, obtained from per-

itonitis, meningitis, pneumonia, otitis interna, arose thesuspicion,

and now regarded as a causal agent of oppotunistic infection or

autogenous infection.

In addition to the above mentioned information, a frequent isolation of <u>A.hydrophila</u> and <u>A.sobria</u>, from the feces of diarrheal disease patients, has also been reported. It is suggesting that the presence of some pathogenic factor(s) in these two organism s affecting on the occurrance of human diarrhea.   At present, however, little is known about <u>Aeromonas</u> which mightbe involve in human diarrheal diseases.

Thus, in the first part of this study, a survey was conducted on the incidence of <u>Aeromonas</u> in natural environment, and the taxonomic investigation of the isolates was made by the method described by Popoff et al. (1984).

In the second part, Adansonian (or numerical) classification was performed on the 73 items of biochemical characteristics of the isolates, and results obtained were compared with those of the ones obtained by the conventional Popoff's classification.

In the last part, isolation of 105 bacteriophages from river water and river mud and establishment of a new phage set, consisted of 48 phage types, were reported. Followed by the results on the phage typing made on the isolates and its advantageous point were discussed.

1 . Incidence of Aeromonas in natural environment

During the period from October 1982 through July 1984, a total of 1,157 specimens, consisted of 132 river- and lake-waters, 514 river- and lake-muds, and 511 fresh-water fishes caught at both River-Tama and River-Sagami were examined for the presence of the organisms.

Of them, 132 (100%) river- and lake-waters, 304 (59.1%) river- and lake-muds, and 462 (90.4%) intestinal contents of fresh -water fishes were found to had been harboring a mean concentration of $1.3 \times 10^3/l$, $1.6 \times 10^6/g$, and $1.1 \times 10^6/g$ of Aeromonas respectively. However, no seasonal variation was observed on the incidence of the organisms throughout the investigation period.

When attempts were made to classify the isolates by the method described Popoff et al., 17 (14.2%) of 120 water-isolates were typed as A.hydrophila, 33 (27.5%) as A.sobria and 35 (29.2%) as A.caviae respectively. And rest of the 35 (29.2%) were remained as untypable.

As for the 176 mud-isolates, 38 (21.6%) were typed as A.hydrophila 23 (13.1%) as A.sobria and 43 (24.4%) as A.caviae respectively. And rest of the 74 (42.0%) were remained untypable.

Same efforts were made on the 1,056 strains obtained from fresh-water fishes, and 182 (17.2%) were typed as A.hydrophila, 332 (31.4%) as A.sobria and 206 (19.5%) as A.caviae respectively. And rest of the 336 (31.8%) were remained as untypable.

2. Adansonian classification of the isolates

Since many strains remained as untypable by the conventional Popoff's criteria, further studies were conducted by themethod of Adansonian classification (or numerical classification), on the 595 representative strains, consisted of 72 water-isolates,82 mud-isolates and 441 fish-isolates, on the 73 items of their biochemical characteristics.

Through the analysis made on the results obtained, water- and mud-isolates were classified into groups I to X, and fish -isolates into groups I to IX by the similarity value of greater than 70% respectively.

Among the above mentioned 595 strains, 103 (17.3%) were classified into A.hydrophila, 292 (49.1%) into A.sobria and 148 (24.8%) into A.caviae respectively. And rest of the 52 (8.7%) were remained as untypable. The reduction of untypable strains from 42.2% to 8.7% by the adoption of Adansonian classification reveals that some inaccuracy points of the present classification criteria of Popoff et al.

3. Establishment of a new phage set and its apprication

In order to establish a new phage set for the first time in Japan, a total of 285 specimens, consisted of 195 river waters and 90 river muds, was examined for the presence of bacteriophages against Aeromonas, and obtained 105 strains of bacteriophages. Then, established types A1 through A26 for A.hy-

drophila, A.sobria and A.caviae, and types AM1 through AM22 for

the rest of Aeromonas spp.

When newly established phage set was applied on the

71 water-, 82 mud- and 441 fish-isolates, 11 (15.5%), 29 (35.4%)

and 89 (20.2%) were typed into some of the 48 types respectively.

Namely, when a total of 543 strains which had been

tagges as A.hydrophila, A.sobria and A.caviae by the conventional

bio-typing methods (including Adansonian classification) were tested

by A-type phages, and it was found that 7 strains were types

as type A4, 6 strains as type A12, 3 as type A18, 2 as type

A1, 2 as type A22, 1 as type A8, 1 as type A13, and rest

of 64 strains as mixed A-types respectively. As for the rest of

the 51 strains, those being failed to tag by the conventional

bio-typing methods, 3 strains were types as type AM7,2 strains

as type AM9, 2 as type AM16, 1 each as types AM2,AM3, AM4,

AM8, AM10, AM12, AM14, AM15, AM17, AM22, and rest of 25 strains

as mixed AM-types respectivly.

4. Distribution of genus Aeromonas and classification from river

     mud, river water and fresh-water fishes.

1)Distribution of genus Aeromonas and classification of its

species by the method of Popoff et al.

A total of 662 specimens , consisted of 48 river- and

lake-waters , 200 river- and lake-muds, and 414 fresh-water fishes

caught at bith River-Tama and River-Sagami were examined for the

presence of the organisms.

When attempts were made to classify the isolates by the method described Popoff et al., 15(31.3%) of 48 water-isolates were typed as A.hydrophila, 21(43.8%) as A.sobria and 22(45.8%) as A.caviae respectively. And rest of the 21(43.8%) were remained as untypable.

As for the 200 mud-isolates, 33(16.5%) were typed as A.hydrophila, 21(10.5%) as A.sobria and 39(19.5%) as A.caviae respectively. And rest of the 60(30.0%) were remained as untypable.

Same efforts were made on the 414 obtained from fresh -water fishes, and 149(36.0%) were typed as A.hydrophila , 204 (49.3%) as A.sobria and 159(38.4%) as A.caviae respectively. And rest of the 210(50.7%) were remained as untypable.

2) Adansonian classification (Numerical classification)

A total of 285 specimens, consisted of 24 river- and lake-water, 100 river- and lake-muds, and 161 fresh-water fishes caught at both River-Tama and River-Sagami were examined for the presence of the organisms. When attempts were made to classify the isolates by the method of Adansonian classification., 3(12.5%) of 24 water-isolates were typed as A.hydrophila, 18(75.0%) as A.sobria and A.caviae respectively. And rest of the 4(16.7%) were remained as untypable.

As for the 100 mud-isolates, 15(15.0%) were typed as A.hydrophila, 25(25.0%) as A.sobria and 20(20.0%) as A.caviae respectively. And rest of the 13(13.0%) were remained as untypable.

Same efforts were made on the 161 obtained from fresh
-water fishes, and 59(36.6%) were typed as A.hydrophila, 100(62.1%)
as A.sobria and 71(44.4%) as A.caviae respectively. And rest of
the 34(21.1%) were remained as untypable.

3) Bacteriophage typing of Aeromonas

When newly established phage set was applied on the 24
water-, 100 mud- and 161 fish-isolates, 9(37.5%), 30(30.0%) and
78(48.4%) were typed into some of the 48 types respectively.

On the basis of the above mentioned results, it can
be concluded that: 1) Extreamely high incidence of Aeromonas in
river waters, river muds and fresh-water fishes, suggest us that
some of the organisms in the genus may be constituting a normal
intestinal flora of fresh-water fishes; 2) The present conventional
bio-typing method for the organisms, described by Popoff et al.,
is likely to have some defective points, since there were many
strains failed to tagges at the begining, and later some of
these could be confirmed as A.hydrophila, A.sobria or A.caviae
by the Adansonian classification; and 3) Application of the newly
developed phagetyping method will be a good tool for the
clarification of the ecology of Aeromonas in natural environment,
and may contribute in future for the epidemiologicalsurveys of
unknown human diarrheal diseases.