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Vibrio anguillarum isolated from the European Eel (*Anguilla anguilla*) cultured in Japan

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(Tables 1 ~ 2)

Vibrio anguillarum has been isolated as a causative agent of epizootics in ayu (*Plecoglossus altivelis*), rainbow trout (*Salmo gairdneri*) and some other fishes cultured in Japan (MUROGA 1975)[1]. This bacterium was originally described as a pathogen of the eel (*Anguilla anguilla*) living in salt and brackish waters in Europe. According to SINDERMANN[2], of all the known bacterial diseases of marine fishes none has a longer or more fascinating history than the "red disease" of eels caused by *V. anguillarum*. But in Japan, the vibriosis of the eel (*A. japonica*) has never been observed except in one district, that is, the northern coastal area of Tokushima prefecture in Shikoku Island (JŌ and MUROGA 1972)[3]. Perhaps, it may be due to the fact that the eels are usually cultured in freshwater ponds in Japan and that the susceptibility of *A. japonica* to *V. anguillarum* is not so high as that of ayu.

Since 1969, to meet a shortage of elvers of *A. japonica*, not a little amount of elvers of *A. anguilla* were imported mainly from France, and cultured in various districts of Japan. But vibriosis in *A. anguilla* has not yet occurred in Japan.

In the late spring of 1975, we examined diseased European eels cultured in an eel-farm in Tokushima prefecture, and isolated *V. anguillarum* from the European eel for the first time in our country.

Materials and Methods

In the spring of 1974, red spot disease (Sekiten-byō, *Pseudomonas anguilliseptica* infection of eels, WAKABAYASHI and EGUSA 1972)[4] occurred in the European eels cultured

in a private eel-farm named Shikoku Yōshoku (situated in Anan City in Tokushima prefecture), and the disease remained chronically in the farm (Jō et al. 1975)[5]. At the end of April 1975, we examined parasitologically and bacteriologically eleven diseased eels (*A. anguilla*) cultured in the same farm. These eels had been introduced as seed fish from France about 2 years before, and cultured in concrete tanks supplied with running water which contained some amounts of saline (chlorinity 5.7 ~ 6.2‰). The water temperature of the pond was 18°C at the time of the investigation.

These specimens seemed inactive and showed discoloration or petechia in the skin. In the parasitological examination, the number of parasites on the gills was counted for each species under a binocular dissecting microscope. Especially, the dactylogyrids were examined morphologically in detail using both live and stained specimens.

In the bacteriological examination, streak cultures were made from the liver, spleen and the kidney using nutrient agar plates, and they were incubated at 20°C. After a 2 day-incubation, two types grew purely or dominantly, then these two isolates were submitted to characterization tests.

Results

From the characterization tests the two isolates were identified as *Pseudomonas anguilliseptica* and *Vibrio anguillarum*, respectively. The results of both parasitological and bacteriological examinations of the eleven specimens are presented in Table 1. As seen in the table, *P. anguilliseptica*, the causative bacterium of red spot disease, was isolated from 8 fish and *V. anguillarum* from 3 fish. The latter pathogen was isolated together with the former, so the 3 fish were infected with both pathogens.

In the parasitological examination, *Pseudodactylogyrus* sp. (accords with *Pseudodactylogyrus anguillae* described by GUSSEV[6] only except the length or width ratio of ovary to testis) and *Trichodina* sp. were found on the gills of every fish. It was noticeable that more than one hundred individuals of the monogenean parasite attached to the gills of every fish.

The characteristics of *P. anguilliseptica* and *V. anguillarum* isolated from these specimens are listed in Table 2. The characteristics of *V. anguillarum* isolated are coincident with those of strains isolated from ayu and some other fishes (MUROGA 1975).

Discussion

As mentioned above, considerable amounts of elvers of the European eel were introduced and cultured in Japan. It seems, however, that the results are not satisfactory in many farms because of their relatively inferior growth and high susceptibility to some ectoparasites. From the taxonomical point of view, the European eel is closely related

Table 1. Results of the examinations of the European eels (*Anguilla anguilla*) cultured in a pond

Fish examined			Incidence of bacteria and gill parasites				
No.	Body weight (g)	Body length (cm)	*Pseudomonas anguilliseptica*	*Vibrio anguillarum*	*Pseudodactylogyrus* sp.	*Trichodina* sp.	*Mxydium* sp.
1	43.7	35.0	+	–	+++*	++*	+*
2	62.5	32.3	+	+	+++	+++	–
3	70.5	32.3	+	+	+++	+	–
4	54.7	32.0	+	+	+++	++	–
5	89.5	35.5	–	–	+++	++	–
6	47.7	29.6	+	–	+++	+	++
7	63.8	32.5	+	–	+++	++	–
8	71.0	35.2	–	–	+++	+	–
9	85.7	34.4	–	–	+++	+	–
10	48.7	30.5	+	–	+++	++	–
11	76.6	35.0	+	–	+++	+	–
Mean & Incidence	64.9	33.1	8/11	3/11	11/11	11/11	2/11

* +++ : more than 100 parasites, ++ : from 20 to 100 parasites, + : less than 20 parasites per one fish

Table 2. Characteristics of *Vibrio anguillarum* and *Pseudomonas anguilliseptica* isolated from the European eels cultured in a pond

Character	*Vibrio anguillarum* (ET-507)	*Pseudomonas anguilliseptica* (ET-508)	Character	*Vibrio anguillarum* (ET-507)	*Pseudomonas anguilliseptica* (ET-508)
Form	short rod	short rod	Acid from		
Length	0.5–1.5 μ	2.0–3.0 μ	Fructose	+	–
Single polar fragellum	+	+	Galactose	+	–
Motility	+	+	Glucose	+	–
Gram stain	–	–	Mannose	+	–
Swarming	–	–	Maltose	+	–
O–F test	ferment.	–	Trehalose	+	–
Gas from glucose	–	–	Dextrin	+	–
Cytochrome oxidase	+	+	Mannitol	+	–
Sensitivity to 0/129	+	–	Starch	+	–
Novobiocin	+	+	Sucrose	+	–
Penicillin	–	–	Glycogen	+	–
Catalase	+	+	Glycerin	–	–
Litmus milk peptoniz.	+	–	Cellobiose	–	–
Nitrate reduction	+	–	Arabinose	+	–
Gelatin liquefaction	+	+	Sorbitol	–	–
Indole	+w	–	Lactose	–	–
Voges-Proskauer test	+	–	Inulin	–	–
2, 3-butanediol product.	+	–	Rhamnose	–	–
Methyl red test	–	–	Xylose	–	–
Hydrogen sulphide	–	–	Raffinose	–	–
Arginine decarboxyl.	+		Adonitol	–	–
Lysine	–		Salicin	–	–
Ornithine	–		NaCl 0 %	–	+
Phenylalanine deamin.	–		0.5	+	+
Urease	–	–	1	+	+
Cholera red	–	–	3	+	+
Citrate (Simmons)	+	–	5	+	+
Tartrate (Jordan)	+	–	7	–	+
Malonate	+	–	10	–	–
Starch hydrolysis	+	–	Pathogenicity for eel	+	

+w : weakly positive

to the Japanese eel, but some physiological differences of the two kinds of eels have been observed in culture ponds. For instance, as compared with the Japanese eel, the European eel is susceptible to some chemical compounds and also several parasites such as *Ichthyophthirius multifiliis* and *Pseudodactylogyrus* spp. MUROGA et al. (1975)[7] demonstrated not only by field investigations but also by injection experiments that the European eel is less susceptible to *Pseudomonas anguilliseptica* than the Japanese eel. On the other hand, MUROGA (1975) pointed out from experimental results that the susceptibilities of both species to *V. anguillarum* are almost the same, but that their susceptibilities are lower than those of ayu and rainbow trout.

In this study, *V. anguillarum* was isolated for the first time from the European eels cultured in Japan. But the pathogen was isolated from a small number of the eels examined and it has not produced a significant mortality. Though the lower temperature (18°C) and clean running water might support the fish, the low mortality must be mainly due to the resistance of the European eel to *V. anguillarum*. Therefore, it was demonstrated that not only the Japanese eel but also the European eel has a relatively low susceptibility to *V. anguillarum*, as MUROGA (1975) pointed out before.

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養殖ヨーロッパウナギ (*Anguilla anguilla*) から分離された *Vibrio anguillarum*

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Vibrio anguillarum はヨーロッパにおいて海水中および汽水中のウナギに鰭赤病を起こすものとして古くから知られている。1969年以来，ニホンウナギ (*Anguilla japonica*) の種苗不足を補うためヨーロッパウナギ (*A. anguilla*) のシラスが大量に輸入され，日本各地で養殖されているが，我が国においてはヨーロッパウナギでの *V. anguillarum* 感染症の発生は現在まで報告されていない。

1975年4月末，徳島県下のやや塩分を含む養殖池において，前年から赤点病（*Pseudomonas anguilliseptica* 感染症）が発生していた魚群について検査したところ，*P. anguilliseptica* とともに *V. anguillarum* が分離され，ヨーロッパウナギにおける本菌感染症が我が国でも確認された。

しかしながら，その感染率は比較的低く，また本病による大量斃死も認められなかった。これはヨーロッパウナギがニホンウナギと同様，*V. anguillarum* に対してある程度抵抗性を有しているためと考えられた。