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Pasteurellosis in Cultured Black Seabream (*Mylio macrocephalus*)

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(Tables 1–2)

Fish pasteurellosis was first described by SNIESZKO *et al.* (1964)[1] among white perch (*Roccus americanus*) in Chesapeake Bay. The etiological agent isolated by S. F. SNIESZKO was further characterized and identified as *Pasteurella piscicida* by JANSSEN and SURGALLA (1968) [2].

In Japan, a bacterial tuberculoidosis or pseudotuberculosis has been prevailing in cultured populations of yellowtail (*Seriola quinqueradiata*) since 1969 (KUBOTA *et al.* 1970)[3]. The causative agent was characterized by KIMURA *et al.* (1971) [4], and identified as *P. piscicida* by KUSUDA and YAMAOKA (1972) [5].

The case described in this paper is assumed to be the first report of *P. piscicida* causing mortality in black seabream (*Mylio macrocephalus*) reared in sea floating net cages.

Materials and Methods

In the summer of 1976, an epizootic occurred in a young population of black seabream cultured in floating net cages. These fish were cultured under a series of experiments on artificial spawning and larval rearing conducted by Fisheries Experimental Station of Okayama Prefecture. They had hatched out at the beginning of May in that year and had been reared in a seawater tank up to the end of July, and then they were transferred into net cages floating inshore. The epizootic occurred among these fish at about the middle of August when the water temperature was about 27°C, and lasted about 2 weeks killing about 8,000 fish out of 9,000 (mortality 90%).

The affected fish lost their appetite and became inactive and somewhat dark-coloured. But other remarkable symptoms such as haemorrhage of the skin or white spots on the internal organs, of which the latter is the most prominent symptom of pseudotuberculosis in yellowtail, were not recognized. An abundance of bacilli were observed microscopically in the blood smear from the diseased fish.

From the beginning to the end of the epizootic, a total of 25 diseased fish were submitted to bacterial isolations by using nutrient agar (NaCl 3%). Some of these specimens were dead, others still alive. They measured 3.2 – 7.5 cm in body length and 1.1 – 12.0g in body weight. After incubation at 25°C, a pure or predominantly pure growth

of a non-motile bacterium was obtained from liver and kidney materials of all the specimens with one exception. Then, 3 isolates were taken up for characterization tests.

In this study, heart infusion agar (NaCl 3%, Eiken) was used to maintain these isolates because of their poor growth on nutrient agar. Characterization test media contained 3 percent of NaCl, and they were incubated at 28°C.

As a test of the pathogenicity, the three isolates were inoculated intramuscularly into healthy black seabream kept in a tank at about 22°C. The injection dose was 1 mg of wet bacteria per 100g of fish body weight. The average size of the material fish was 6 cm in body length and 6g in body weight.

Results

The characteristics of the three isolates examined completely coincide with each other.

Table 1. Morpho-physiological and biochemical characteristics of the isolates and *Pasteurella piscicida*

Test	Present isolates 3 strains (black seabream)	*Pasteurella piscicida* (KUSUDA & YAMAOKA 1972) 4 strains (yellowtail)	*Pasteurella piscicida* (KUSUDA & YAMAOKA 1972) 1 strain (white perch)	*Pasteurella piscicida* (SIMIDU & EGUSA 1972) 5 strains (yellowtail & white perch)
Motility	−	−	−	−
Bipolar staining	+w	+	+	+w
Gram staining	−	−	−	Intermediate
Growth on nutrient agar	+w	+	+	+w
in nutrient broth	+w	+w	+w	+w
in peptone water	+w	+w	+w	
on heart infusion agar	+			
on brain heart infusion agar	+	+	+	+
on SS agar	−	−	−	
on MacConkey agar	−	−	−	
on BTB agar	−	−	−	
on Endo agar	+w	−	−	
Growth at 10°C	−	−	−	−
20°C	+			+
25°C	+	+	+	+
30°C	+			+
35°C	+w	−	−	−
37°C	−			
pH range	6–9	5.5–8	6–7.5	6.6–8.8
NaCl range	0.5–4%	0.5–4	0.5–3	0.5–5
Oxidase	+	+	+	+
Catalase	+	+	+	+
Hugh-Leifson test	Ferment.	F.	F.	F.
Gas from glucose	−	−	−	−
Gelatin liquefaction	−	−	−	−
Casein digestion	−	−	−	−
Litmus milk	+w	+	+	−
Indole production	−	−	−	−
Starch hydrolysis	+	−	−	−
Hydrogen-sulphide product.	−	−	−	−
Nitrate reduction	−	−	−	−

Test	Present isolates	*Pasteurella piscicida* (KUSUDA & YAMAOKA) 4 strains	*Pasteurella piscicida* (KUSUDA & YAMAOKA) 1 strain	*Pasteurella piscicida* (SIMIDU & EGUSA) 5 strains
Ammonia production	−	−	−	
Urease production	−	−	−	−
Voges-Proskauer test	+w	+w	+w	+w
Methyl red test	+	+	+	+
Cholera red test	−	−	−	
Methylene blue reduct.	−	−	−	
Tween 80	+w			
Phenylalanine deamin.	−			−
Chitin decomposion	−			±
Gluconate oxidation	−	−	−	
2,3-butanediol dehydro.	+	+	+	
Arginine hydrolysis	+	+	+	
Arginine decarboxylation	+	+	+	+
Lysine	−	−	−	±
Ornithine	−	−	−	−
β-galactosidase	−			
Citrate utilization	−	−	−	−
Tartrate	−	−	−	−
Malonate	−	−	−	−
Mucate	−	−	−	
Propionate	−	−	−	
Pyruvate	−	−	−	
Succinate	−	−	−	±
Butyrate	−			−
Sensitivity to				
pteridine (0/129)	+	+	+	
penicillin	+	+	+	+
novobiocin	+	+	+	+
chloramphenicol	+			+
tetracycline	+			+
nalidixic acid	+			

+w : weakly positive ± : some strains positive, some negative

The results of the tests are shown in Tables 1 and 2, compared with the characteristics of *P. piscicida* tested by KUSUDA and YAMAOKA (1972)[5] and SIMIDU and EGUSA (1972).[6] Table 1 shows the morpho-physiological and biochemical characteristics of those strains, and Table 2 shows the carbohydrates utilization. As shown in these two tables, the characteristics of the present isolates well agree with those of *P. piscicida* examined by the other researchers except only for the result of starch hydrolysis.

In the inoculation experiment, the isolates killed almost all the black seabream (8/9) within a week.

Table 2. Carbohydrates utilization of the isolates and *Pasteurella piscicida*

Species & Strain / Carbohydrate	Present isolates	*Pasteurella piscicida* (Kusada & Yamaoka) yellowtail	white perch	(Simidu & Egusa) yellowtail & white perch
Acid from				
Glucose	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Mannose	+	+	+	+
Glycerol	–	–	–	±
Xylose	–	–	–	–
Arabinose	–	–	–	–
Rhamonse	–	–	–	–
Sucrose	–	–	–	–
Maltose	–	–	–	–
Lactose	–	–	–	–
Trehalose	–	–	–	–

Species & Strain / Carbohydrate	Present isolates	*Pasteurella piscicida* (Kusuda & Yamaoka) yellowtail	white perch	(Simidu & Egusa) yellowtail & white perch
Raffinose	–	–	–	–
Cellobiose	–	–	–	–
Starch	–	–	–	
Dextrin	–	–	–	–
Inulin	–	–	–	–
Glycogen	–	–	–	
Adonitol	–	–	–	–
Mannitol	–	–	–	–
Sorbitol	–	–	–	
Inositol	–	–	–	
Dulcitol	–	–	–	–
Salicin	–	–	–	

±: some strains positive, some negative.

Discussion

The results of morpho-physiological and biochemical examinations show that the present isolates are identical with *P. piscicida* examined by Kusuda and Yamaoka[5] or Simidu and Egusa[6]. Simidu and Egusa[6] made a re-examination of *P. piscicida*, and indicated that genus *Arthrobacter* would be the most suitable genus to which the species should be classified in. But referring to Bergey's Manual of Determinative Bacteriology, 8th ed. (1974),[7] which was published 2 years after their report, it seems improper to include *P. piscicida* in the genus *Arthrobacter* because of its cell morphology, fermentative metabolism and glucose utilization in peptone medium. On the other hand, many of the characteristics of the species support its placement in the genus *Pasteurella*. Thus, the present isolates are identified as *P. piscicida* as they were in the past.

In Japan, the pasteurellosis (pseudotuberculosis) has been particularly devastating for populations of yellowtail, though red seabream (*Chrysophrys major*) and some other fishes cultured unintentionally with yellowtail in the same net cages were found to be infected occasionally by *P. piscicida* (Kusuda 1974).[8] And still, another pasteurellosis was reported among ayu (*Plecoglossus altivelis*) cultured in freshwater ponds, the causative agent was named *P. plecoglosacida* by Kusuda and Miura (1972)[9]. But, black seabream has never been reported as a victim of pasteurellosis before, and the case described here is thought to be the first record of pasteurellosis in this fish.

In addition, the positive cross reactions in serological tests were found between the present isolates and a strain (Ehime-70) of *P. piscicida* isolated from yellowtail (personal information from Dr. T. AOKI of Miyazaki University, 1977). From the results of characterization tests and serological tests, the present isolates prove identical with the strains from yellowtail. Therefore, some relations were suspected between the present epizootic and pasteurellosis in yellowtail, but in fact, no yellowtail had been cultured in that area for several years. Food did not seem to be the source of the infection, either. Because these black seabream were administered dry pellet, at least after the transfer into net cages.

The source of the infection could not be made clear, but anyway, hereafter some attention should be paid to the occurrence of pasteurellosis in black seabream culture. We note in parentheses that oral administration of chloramphenicol appeared to be effective to some extent for checking the disease, yet this was applied but in the last stage of the epizootic.

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養殖クロダイ（*Mylio macrocephalus*）のパストレラ症

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1976年8月，岡山県水産試験場において種苗生産に関する一連の実験のために，沖出し後海面小割中で飼育されていたクロダイ（*Mylio macrocephalus*）幼魚に細菌性疾病と思われる流行病が発生し，約2週間の間に飼育されていた9,000尾のうち約8,000尾が死亡した。

それらの病魚には遊泳及び摂餌活動の低下ならびに体色の黒化が認められたが，そのほかの目立った病徴は認められなかった。検査したほとんどの病魚の内臓諸器官から一種の非運動性細菌がほぼ純粋に分離された。

分離菌の形態学的・生理学的及び生化学的性状を調べた結果，本菌は *Pasteurella piscicida* に同定された。また分離菌をクロダイに接種したところ，比較的短時間で死亡せしめることも確認された。

なお，これらのクロダイが飼育されていた水域ではここ数年ハマチ養殖は行なわれておらず，また少なくとも沖出し後はこれらの魚にペレットのみを餌として与えており，今回の流行の感染源を明らかにすることはできなかった。

P. piscicida は従来我が国ではハマチの類結節症の原因菌としてよく知られてきたが，今後はクロダイにおいても本菌感染症の発生に対する注意が必要と考えられた。