Cultural Characteristics of the Bacterium Causing Jaundice of Yellowtail, *Seriola quinqueradiata*

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The present investigation was conducted to determine optimal cultural conditions of the causative bacterium of jaundice in yellowtail.

This microorganism grew in L-15 medium, Eagle MEM, 0.85% NaCl solution and 1/3 sea water, each supplemented with 10% of fetal bovine serum (FBS). FBS was essential for growth of the bacterium. The bacterium did not grow in any other media usually used for bacterial isolation or culture. The optimal growth conditions for culture of the agent causing jaundice determined from this study are at temperature of 23–26°C using L-15 medium at pH 7.0–7.5 supplemented with more than 10% of FBS and 1.6–2.0% of NaCl.

An epizootic disease called “jaundice” has been known in cultured yellowtail, *Seriola quinqueradiata*, in Japan since 1980. The cause of this epizootics had not been known until recently. However, Sorimachi *et al.* (1993) succeeded in isolating a bacterium from yellowtail suffering from the jaundice using either L-15 medium with 10% fetal bovine serum (FBS) or nutrient broth, and proved that this bacterium is the causative agent of this disease. From its characteristic morphology and the distinctive motility, they thought that this microorganism was a new species. However, the taxonomic position of this bacterium has not been determined, because of its difficulty in stable subculture. In the present study we described the cultural characteristics of this bacterium.

Materials and Methods

Bacteria

Five isolates from yellowtail suffering from the jaundice were used in this study (Table 1). Two or three strains were tested for each experiment, though JBKA-6 was used in all experiments. Bacteria were subcultured in L-15 medium (Sigma, USA) supplemented with 10% fetal bovine serum (FBS; ICN

Biochemicals, Australia).

Cultural media

Following media were tested for culturing the agent, i.e. L-15 medium (Sigma, USA), Eagle MEM (Nissui, Japan), Marine Broth 2216 (Difco, USA), Brain Heart Infusion Broth (Nissui, Japan), Heart Infusion Broth (Nissui, Japan), Nutrient Broth (Difco, USA), MR-VP Broth (Nissui, Japan), Cytophaga medium (Anacker and Ordal, 1959) in 70% sea water, 0.85% NaCl solution, 1/3 sea water and 1/3 sea water with 0.5% (w/v) peptone (Difco, USA), tryptone (Difco, USA), yeast extract (Difco, USA) or Lab-Lemco Powder (Oxoid, England). All media were supplemented with 10% FBS. Five ml of media were inoculated with 0.1 ml of subculture of the bacteria grown in L-15 medium with 10% FBS, and incubated at 25°C with occasional agitation. Growth of bacteria was judged by the turbidity of media.

Effects of cultural conditions on growth of the isolates

The effects of FBS concentration, temperature, NaCl concentration and pH on growth of the isolates were studied. For the effects of FBS or NaCl concentration, L-15 medium or distilled water supplemented with 10% FBS were used as the basal medium, respectively. For the effects of the others,

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Table 1. Bacterial strains used in this study

Strain	Isolation	
	Date	Location
JBKA-6	17 Oct., 1991	Kagawa Pref.
JBE-4	11 Dec., 1991	Ehime Pref.
JBEU-1	14 Jul., 1992	Ehime Pref.
JBMKA-4	17 Jul., 1992	Mie Pref.
JNMO-1	24 Jul., 1992	Mie Pref.

L-15 medium with 10% FBS was used as the basal medium. After 4 days shaken culture, the absorbance of the incubated media was measured at 660 nm in a spectrophotometer (U-2000, Hitachi, Japan) with a supernatant of the same media as a blank. Inoculation and incubation methods of the bacterium were described above.

Survival in water

The strain JBKA-6 was suspended in distilled water, 0.85% NaCl solution, 1/3 sea water, 1/2 sea water and sea water. An aliquot of the suspension was transferred to L-15 medium with 10% FBS at appropriate intervals. Survival of the strain in each water was determined by growth in the medium.

Results

Cultural media

The isolates grew in L-15 medium, Eagle MEM, 0.85% NaCl solution and 1/3 sea water, and the best growth was obtained in L-15 medium. The bacteria grown in 0.85% NaCl solution or 1/3 sea water with 10% FBS able to be subcultured in L-15 medium with 10% FBS, but not in the 0.85% NaCl solution or 1/3 sea water with 10% FBS.

Effects of cultural conditions on growth of the isolates

The isolates did not grow in L-15 medium without FBS. Within the FBS concentrations from 0 to 20% (v/v), higher FBS concentrations yielded better growth of the isolate (Fig. 1). The strains grew only in a narrow range of temperature (20–26°C), and optimal temperature for growth was from 23 to 26°C (Fig. 2). A NaCl concentration needed for growth of the isolates ranged from 0.8 to 3.2% (w/v). The isolate JBKA-6 had the optimal NaCl concentration at 1.6% (Fig. 3), while the strain JBE-4 had the optimum at 2.0%. A prolonged incubation made the isolates grow in a NaCl concentration of 4.0%. No

Concentration of fetal bovine serum

Fig. 1. Effect of concentration of fetal bovine serum on the growth of strain JBKA-6.

Temperature

Fig. 2. Effect of temperature on the growth of strain JBKA-6.

growth of the isolates was obtained at pH 6.0 and 8.5. The optimal pH for culturing the isolates was from 7.0 to 7.5 (Fig. 4).

Survival in water

The strain JBKA-6 was inactivated in distilled water within 3 h. Survival of the bacterium in 1/3 sea water was longer than in 0.85% NaCl solution.

Fig. 3. Effect of NaCl concentration on the growth of strain JBKA-6.

There was no difference of survival period of JBKA-6 among in 1/3, 1/2 and 1/1 sea water. In sea water, the bacterium did not detected at day 5.

Discussion

From the shape of the causative agent of yellowtail jaundice which is the long rod of 4–6 μm in length, the bacterium was thought to belong to the genus *Campylobacter*, *Leptospira* or *Spirochaeta*. Therefore, Sorimachi *et al.* (1993) used Modified Butzler Agar (Nissui, Japan), Fletcher medium with 10% FBS or 10% yellowtail serum, and Korthof medium with 10% FBS or 10% yellowtail serum for the isolation of the agent from diseased fish, but no bacterium grew in these media. Electron microscopy showed that the microorganism did not take a helical form and not have a flagellum. Sorimachi *et al.* (1993) judged by those observations that the isolate was not *Campylobacter*, *Leptospira* or *Spirochaeta* and was probably a new species; however, the taxonomic position is not determined at all until now. The cultural characteristics of the bacterium will be useful for the bacteriological examinations.

The strains isolated at different dates and locations showed almost the same cultural characteristics in this study. In conclusion, it seems at present that the

Fig. 4. Effect of pH on the growth of strain JBKA-6.

causative agent of yellowtail jaundice can be most efficiently cultured at temperatures of 23–26°C using L-15 medium at pH 7.0–7.5 supplemented with 10% or more of FBS and 1.6–2.0% of NaCl. And shaken cultures are superior in growth of the bacterium to stationary cultures (data not shown).

In this study, in addition to L-15 medium, the isolate was able to be cultured in Eagle MEM, 0.85% NaCl solution and 1/3 sea water, but not in nutrient broth supplemented with FBS. Nutrient broth was available to culture the bacterium at the primary isolation, but L-15 medium yielded far better growth than nutrient broth (Sorimachi *et al.*, 1993). Yellowtail whole blood was inoculated into media for isolating the agent. It is thought that the blood made possible to isolate the bacterium by using nutrient broth. Nutrient broth seems to be not good to isolate the agent by nature.

Sea water with 10% FBS allowed the proliferation of the isolate. However addition of nutrients such as peptone, tryptone, yeast extract or Lab-Lemco Powder to sea water with 10% FBS inhibited the growth of the isolate. Addition of agar of 0.125% (w/v) also caused inhibition of the bacterial growth (data not shown). Thus, up to this time, we can steadily culture the bacterium only in L-15 medium with FBS. In this best medium for cultivation of the

agent, bacterial density did not exceed 10^7 cells/ml. This density is too low to study the biochemical characteristics. Screening essential substances for the cultivation of the bacterium is needed for determining the taxonomic position of this bacterium. Analysis of the 16S rRNA sequence of this bacterium may also provide the useful data, since the 16S rRNA is informative to determine the phylogenetic position (Kita-Kukamoto *et al.*, 1993).

Jaundice in yellowtail usually occurs throughout the year. The damage from this disease increases with the increase of the environmental water temperature, and decreases with the decrease of the environmental water temperature. This phenomenon is considered to be related to the bacterial characteristic, since the isolate can not grow under 20°C *in vitro*.

The relatively longer survival of this agent in sea water suggests that the bacterium is adapted to sea water. Sorimachi *et al.* (1993) succeeded in developing jaundice by bath infection. From these results, this disease seems to spread horizontally over culture fields. However, if the diagnosis of the infection at the early stage is possible, the spread of this disease will be prevented, because chemotherapy is thought to be effective to this disease (Sorimachi and Maeno, 1993).

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