

Bacteriological Study on the Symbiotic Luminous Bacteria
Cultivated from the Luminous Organ of the Apogonid
Fish, *Siphania versicolor* and the Australian Pine
Cone Fish, *Cleidopus gloria-maris*.¹⁾

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(With 1 plate)

ヒカリイシモチ *Siphania versicolor* とオーストラリア
産マツカサウオ *Cleidopus gloria-maris* の
共生発光菌について

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The present paper reports the results of the bacteriological investigation of symbiotic luminous bacteria which were cultivated from the luminous organ of the apogonid fish, *Siphania versicolor* and of the Australian pine cone fish, *Cleidopus gloria-maris*.

The specimen of *Siphania versicolor* were obtained among the long-spined sea urchins, *Diadema setosum* at the sandy bottom of the subtidal zone of coral reefs off Itoman and Minatogawa beach, Okinawa Island, in 1965. The materials were brought to a laboratory and kept alive in a small aerated aquarium 2 or 3 days in order to observe light emission at night and also to cultivate the luminous bacteria of the luminous organ.

The luminous organ of this fish consists of a luminous body, a tube connecting to the intestine and an accessory structure of translucent muscle bundles. The luminous body, a small spherical body, lies below the antero-ventral surface of the liver at the level of the base of the ventral fin.

The specimens of the Australian pine cone fish, *Cleidopus gloria-maris*, were obtained by prawn trawlers in Moreton Bay, Brisbane, Australia, through the courtesy of Mr. G. G. T. HARRISON of the Department of Harbours and Marine, Brisbane. Three dead but fresh specimens were kept in an ice box, and two live specimens were kept in an aquarium for about a week.

The luminous organs are situated on each side of the mandible. In a specimen 82 mm. long, each luminous organ, elliptical in shape, measures 4 mm. in length and

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2 mm. in width. The surface of the organ is reddish-orange in color. Luminescence is continuous and bluish-green in color, but the contents of the luminous organ emit only a bluish light, this color difference being due to the reddish-organe filter at the surface of the lens structure.

Observations on these fish were published in 1965 (Haneda) and 1966 (Haneda).

Haneda has also succeeded in cultivating luminous bacteria from these luminous organ of fish. The isolation of the luminous bacteria was carried out after the following procedures.

The luminous organ was removed with the surrounding muscle from the fish body and washed in water. For the purpose of removing germs from its surface, it was immersed in alcohol several seconds and then washed again in water. The organ was opened with a sterilized knife, and the luminous matter in the organ was taken for the culture media. After 10-15 hours a small, round, transparent and luminous colony appeared. From this start a pure culture which was free from any contaminating bacteria was obtained.

It should be made clear that the same species of fish always possesses the same species of luminous bacteria, 10 different strains of the luminous bacteria of *Siphamia versicolor* as well as 5 different strains of the luminous bacteria of *Cleidopus gloria-maris* showed the same kind of morphological, cultural and physicochemical characteristics.

The characters of these luminous bacteria are as following.

A. Luminous bacteria of *Siphamia versicolor*.

1) Morphology

24 hours cultures in bouillon: bacillary forms dominant, usually occur singly; sizes 1.1 to 2.7 μ .

Agar cultures: bacillary forms dominant; sizes 1.5~2.7 μ .

Spore and capsule absent. Vacuoles are observed; Gram negative.

2) Cultural characters

Agar plate: surface colonies cultivated 24 hours at 20°C; round, convex, amorphous, transparent colonies up to 1 mm. in diameter smooth glistening surface.

Each bacteria from them (are) motile.

Deeper colonies; Globular, all round white.

Agar stab: Surface growth, convex, with irregularly margin, whitish.

Broth: slightly turbid, no ring, sediment.

3) Physiological characters

Gelatin not liquefied. Indol not informed. Milk not coagulated.

Dextrose, Galactose, Laevulose, and Glycerol are fermented with acid, but Arabinose, Rhamnose, Xylose, Lactose, Maltose, Saccharose, Trehalose, Raffinose, Dextrin, Inulin Erythritol, Adonitol, d-Mannitol and Dulcitol not. Optimum temperature 20~25°C.

B. Luminous bacteria of *Cleidopus gloria-maris*

1) Morphology

24 hours cultures in bouillon and on gelatin: Vibrio forms dominant, sizes 1.5 to

2.7 μ long 0.45 to 0.5 breit. Rapid motile, Spore and capsule absent. Gram negative Vacuoles are observed.

2) Cultural characters

Agar colonies: Surface colonies; Delicate, with spreading, circular with irregular margin, yellowish white untransparent.

Deeper colonies; Globular or spindle shape.

Agar stab: Surface growth, convex, with irregularly waved margin, yellowish white.

Broth: slightly turbid, ring and pellicle on the surface abundant sediment.

3) Physiological characters

Gelatine not liquefied, Indol not formed. Milk not coagulated. aerobic; Limits of pH for growth: 6.8 to 9.2 in pure culture. Optimum temperature 20~25°C Dextrose, Galactose, Laevulose, Glycogen, Maltose Dextrin, Inulin, Glycerol and d-Mannitol are fermented with acid, but Arabinose, Rhamnose, Xylose, Lactose, Saccharose, Trehalose, Raffinose, Erythritol, Adonitol and Dulcitol not.

The description "milk coagbated", in the former report (HANEDA 1966) is corrected as above. From the view point of cultural characters, these bacteria are different from other symbiotic luminous bacteria in that the bacteria of *Cleidopus gloria-maris* are for weaker than other bacteria in culture media.

In regard to other symbiotic luminous bacteria for example, the bacteric obtained from the luminous organ of fish belonging to the families Macrouridae and Acropomidae live for two to three months at 5°C; however, almost luminous bacteria of *Cleidopus gloria-maris* die within one month. It is especially susceptible to low temperatures.

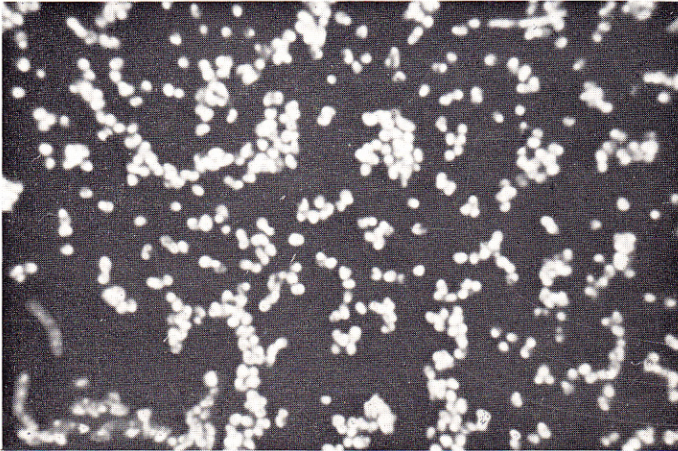
A more distinct difference is shown in fermentation. As it is these two species of bacteria might be recognized as new species. However, since the symbiotic luminous bacteria of fishes belonging to the families Macrouridae, Acropomidae, Gadidae, Leiognathidae, Monocentridae and *Paratrachycthis* have not been sufficiently investigated, the nomination of new species is reserved for the future.

References

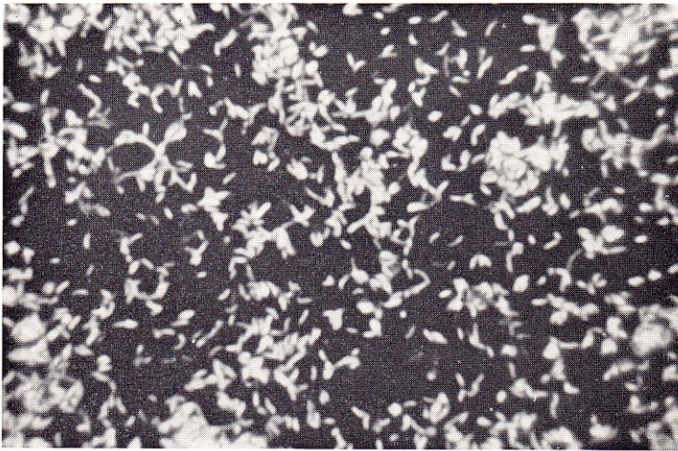
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抄 録

ヒカリイシモチ *Siphamia versicolor* とオーストラリアのマツカサウオ *Cleidopus gloria-maris* の発光器より培養した共生発光細菌について細菌学的研究を行なった。マツカサウオの共生発光菌は他の共生発光菌に比して、種々の点で異なっているが、特に低温に抵抗力がない。多くの発光菌が低温に抵抗力が強いのと大きな相違である。



Symbiotic luminous bacteria of *Siphania versicolor*
24 hours culture Phot by fluorescent microscope



Symbiotic luminous bacteria of *Cleidopus gloria-maris*
24 hours culture, Phot by fluorescent microscope