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Saprolegnia salmonis sp. nov. isolated from sockeye salmon, *Onchrhynchus nerka*

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A saprolegniasis occurred in cultured sockeye salmon, *Onchorhynchus nerka*, raised in Hokkaido, Japan. The lesions were mainly observed in the head, peduncle region and the caudal fin. All strains isolated were morphologically classified in the genus *Saprolegnia*. They were identified as a new species in the genus from the characteristics of the sexual organs, and named *Saprolegnia salmonis*.

Key Words——*Saprolegnia salmonis*; saprolegniasis; sockeye salmon.

Saprolegniasis is a fungal disease of fish and fish eggs caused by a member of the genus *Saprolegnia* (Coker, 1923; Neish and Hughes, 1980) and is very common in adults and eggs of salmonids (Neish and Hughes, 1980). In 1985, however, mass mortality due to saprolegniasis occurred for the first time in 20–60 g freshwater-cultured coho salmon, *Oncorhynchus kisutch* Walbaum, (Hatai and Hoshiai, 1992a; 1992b) in Miyagi Perfecture, Japan. Mortality exceeded 50% and the fungus responsible was identified as *S. parasitica* Coker. This was the first case in Japan in which salmonids of this size had died in such high number. Recently a similar fungal disease occurred in freshwater-cultured sockeye salmon, *O. nerka* Walbaum, in Hokkaido.

This paper describes the morphological and physiological characteristics of a new species isolated from the sockeye salmon, *O. nerka*, with saprolegniasis.

Material and Methods

Specimens Several fish ranging from 20 to 30 g in body weight, dying from saprolegniasis, were received from National Salmon Resources Center, Fisheries Agency, Sapporo, Hokkaido, Japan on 7 May, 1998.

Isolation Isolates were obtained by inoculating samples of infected muscle taken from different parts of the body, approximately 2 mm in diam, from a moribund sockeye salmon onto GY agar consisting of 1% glucose, 0.25% yeast extract, and 1.5% agar (Hatai and Egusa, 1979). To inhibit bacterial growth, traces of ampicillin (Sigma) and streptomycin (Meiji Seika) were added to the medium. Cultured plates were incubated at 15°C for colonial growth, then purified according to the method of Willoughby (1994). The purified fungi were maintained at 15°C on GY agar and transferred to fresh medium monthly.

Identification Nine isolates from sockeye salmon with saprolegniasis were thought to be the same species after a preliminary check of some morphological characteristics made on hemp seed cultures in sterilized tap water (Coker, 1923; Johnson, 1956). Therefore, isolate NJM 9851 was chosen at random and used for all experiments. An agar block (8 × 8 mm) cut from the advancing edge of the fungal colony was inoculated into GY broth and incubated at 15°C for 50–70 h. The growing mycelia were washed in successive baths of sterilized tap water, then transferred to sterilized tap water with halves of hemp seed and kept at 15 and 5°C. The fungus was mainly identified according to Seymour (1970). To observe zoospore germination type, 1 ml of GY broth was placed in a Petri dish with 30 ml sterilized tap water containing encysted zoospores, then incubated at 15°C. Observation of spore germination was conducted 3 h after inoculation (Yuasa et al., 1997).

Effect of temperature on vegetative growth The advancing edges of the fungal colony on GY agar were cut out with a no. 2 cork borer and placed on the center of disposable Petri dishes (90 × 20 mm) containing 20 ml of GY agar, then incubated at 10, 15, 20, 25, and 30°C. The mycelial radial growth from the blocks was measured at intervals of 20, 24, 48, 72, and 96 h and expressed as the mean of two perpendicular radii.

Effect of NaCl on vegetative growth An agar block of the isolate was inoculated on GY agar plates containing various concentrations of NaCl (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5%) and incubated at 15°C for 5 d. The colony radius was measured as mentioned above.

Results

Mass mortality in sockeye salmon due to saprolegniasis was first noticed in Hokkaido, Japan in 1992. Since that time epizootics of the disease have occurred annually. The fungal infection first occurs in sockeye salmon fry ranging from 3 g to 5 g in November, but the mortality is not high. However, the infection reccurred in the salmon ranging from 20 g to 30 g in the next April. The water temperature during the course of saprolegniasis occurrence was at 18°C (Nomura, personal communication). Approximately 100,000 sockeye salmon are reared per pond, and 300 to 1,000 died per pond per day. Cotton-like mycelia were present on the head, adipose fin, and caudal fin in the moribund fish (Fig. 1). No bacteria or viruses were detected in the dying fish (Nomura, personal communication). In most cases, no gross signs were observed in the internal organs. Many aseptate hyphae and zoosporangia were observed in various lesions by microscopical examination. Mycelial growth on GY agar reached 82 mm in diam at 15°C within 120 h. Formation of sexual organs was observed at 15°C within 10 d on hemp seed cultures.

Saprolegnia salmonis Hussein et Hatai, sp. nov. Fig. 2

Hyphae, tenues, aseptatae, modice lateraliter ramosae, ca. 22 μm crassae. Zoosporangia, clavata vel cylindrica, recta, interdum curvata, 178–186 × 24–36 μm, orificio dimissionis terminali. Zoosporae primariae saprolegnoides dimissae, pyriformes, biflagellatae, 11 μm diam. Zoosporae secundariae emergentes e zoospora incystata, reniformes. Germinatio oogonii directus vel interdum indirectus. Oogonia abundantia, sphaerica obovata vel pyriformia plerumque 40–90 μm diam ubi antheridio affixo, elongata 90–149 × 50–73 μm ubi antheridio non affixo. Oogonia sphaerica generatim 66.3% et elongata 33.8%. Paries oogonii laevis, foveatus. Stipes oogonii rectus, nonramosus, 20 μm diam. Oosporae sphaericae, 20 μm diam, 4–12 (plerumque 8) inoogoniis sphaericis 16–20 (plerumque 18) inoogoniis elongatis, guttula olei generatim subcentrica interdum centrica includentes. Rami antheridiorum diclini. Cellulae antheridiorum 1-cellulares, tenues, nonramosae, 8 μm diam, lateraliter vel apicaliter adpressae. Gemmae nonobservatae.

Holotypus: NJM 9851, colonia exsiccata e cultura ex musculo, *Onchrhynchi nerka*, Hokkaido in Japonia, 7 Maius 1998, a Hussein isolata et ea collectione culturae in Universitate Veterinarii et Scientificae Animalis Nipponensis (NJM) conservata.

Hyphae delicate, non-septate, slender, moderately branched (laterally), averaging about 22 μm in diam. Zoosporangia clavate or straight, cylindrical, infrequently curved, 178–186 × 24-36 μm. Zoospores discharge saprolegnoid. Primary zoospores biflagellate, pyriform, and 11 μm in diam. Zoospores encysted 30 min to several h after their discharge. The encysted zoospores emerge reniform secondary zoospores. Both direct (dominant 85–90%) and indirect (10–15%) germination types of encysted zoospores observed. Oogonia abundant, spherical, ovate, pyriform, and elongated with or without antheridial attachment. Percentage of spherical and elongated oogonia 66.3 and 33.8%, respectively. Spherical oogonia 40–90(60) μm in diam, elongated oogonia 90–149(132) × 50–73(60) μm in size. Oogonial wall smooth and conspicuously pitted not only under the point of attachement of antheridial cell but also in absence of antheridial attachment. Some spherical oogonia pitted without antheridial attachment. Oogonial stalks straight, slightly bent, not branched, 20 μm in diam. Oospores spherical, 20 μm in diam, 4–12(8) and 16–20(18) in number in spherical and elongated oo-

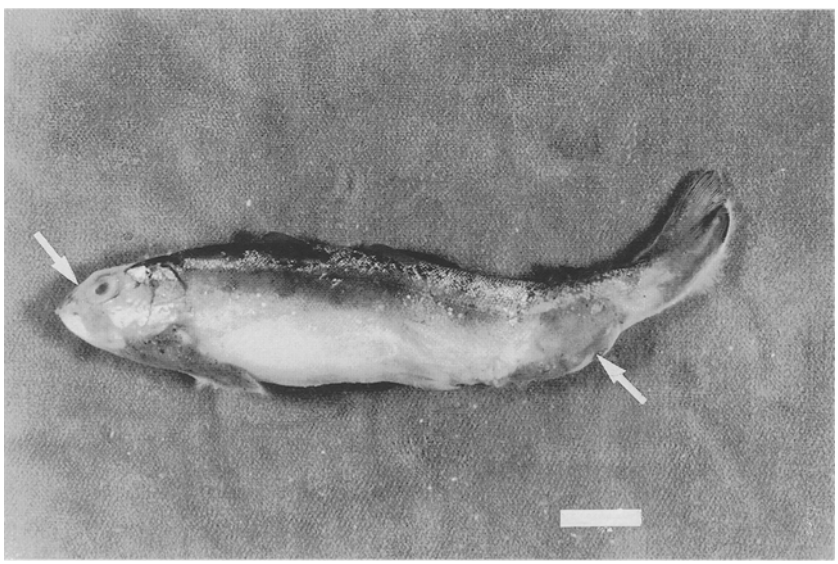

Fig. 1. Gross appearance of sockeye salmon fingerling with saprolegniasis. The fungal growth appears adjacent to head and peduncle region (arrows). Scale bar = 1 cm.

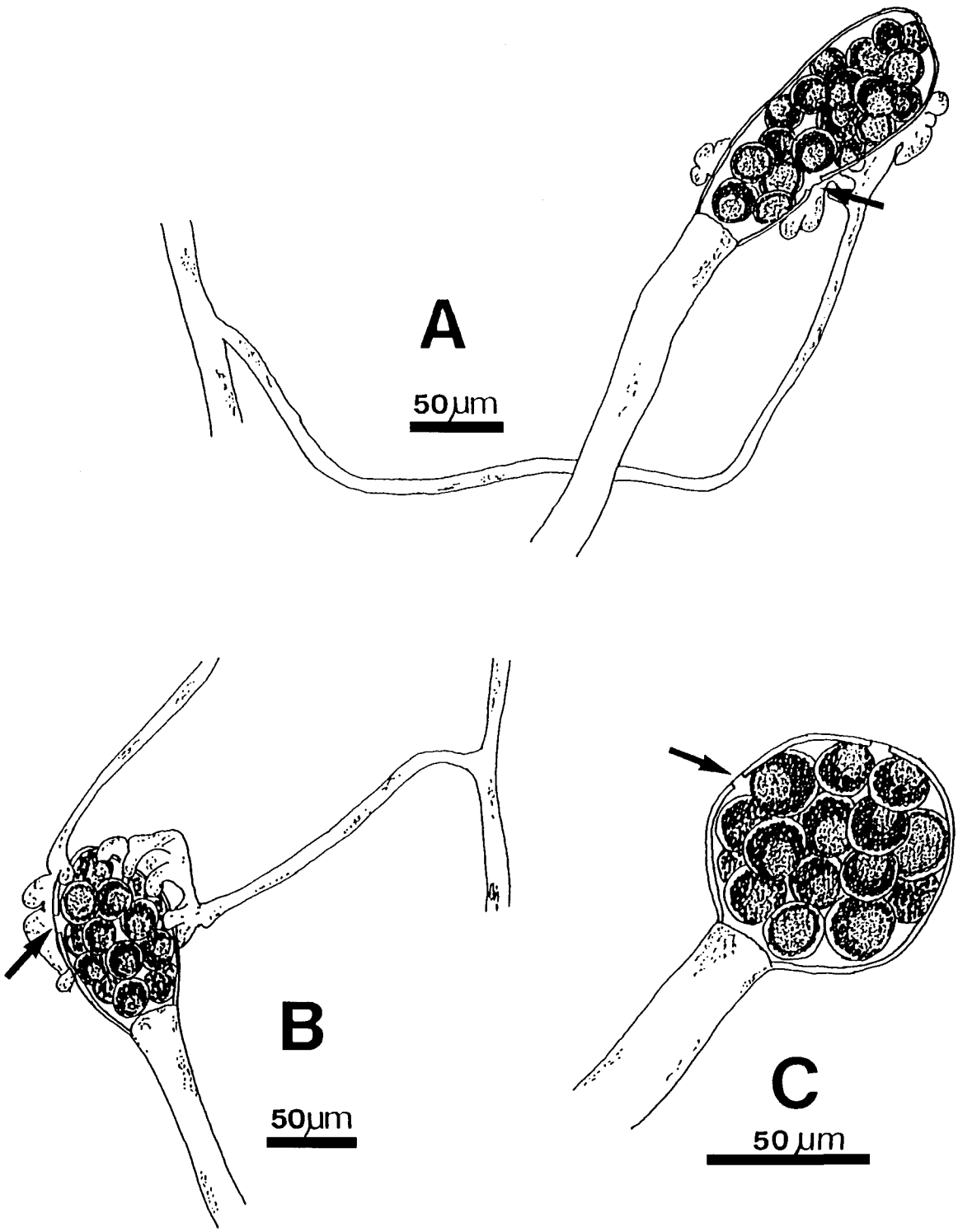

Fig. 2. Morphological characteristics of sexual organs of *Saprolegnia salmonis* NJM 9851 isolated from sockeye salmon. A. Elongated oogonium pitted under the point of diclinous antheridial attachment (arrow); B. Pyriform oogonium pitted under the point of diclinous antheridial attachment (arrow). C. Spherical oogonium pitted without antheridial attachment (arrow). Scale bar=50 μm.

gonia, respectively. Internal structure of oospores not only centric but also subcentric. Subcentric prevalent. Antheridial branches diclinous. Antheridial cells simple, tubular, delicate, not branched, 8 μm in diam, and laterally or apically apressed (sometimes attachment by foot-like projections). Gemmae not observed.

Effect temperature on vegetative growth The effect of temperature on vegetative growth is summarized in Fig. 3. The optimum temperature for vegetative growth was between 15 and 20°C. Growth at 30°C was abnormal and stopped at 96 h.

Effect of NaCl on the vegetative growth As shown in Fig. 4, the isolate was able to tolerate up to 3.0% of NaCl. No growth was observed on the GY agar containing 3.5% NaCl.

Discussion

Water molds of the genus *Saprolegnia* are known as external parasites of freshwater fishes, amphibians, and reptiles, which notably cause saprolegniasis (Scott and Warren, 1964; Willoughby, 1970, 1978, 1994; Hatai and Hoshiai, 1992a, 1992b; Bly et al., 1992; Blausteine et al., 1994; Kitancharoen et al., 1995; Dieguez-Uribeondo, 1995)

Seymour (1970) considered that the difference between *S. parasitica* and the closely related *S. diclina* lies in the internal stucture of the oospore, the former being subcentric type and the latter centric type. Willoughby (1978) proposed that *S. diclina* could be classified into three types, I, II, and III, based on the length (L) / breadth (B) ratio of the oogonia. The isolate studied here showed abundant oogonial formation with two different shapes, spherical (66.3%), 40–90(60) μm in diam, and elongated (33.8%), 90–149(132) × 50–73(60) μm with L/B ratio ≥2. These correspond to Willoughby's type III and type I, respectively. Our fungus is distinguished from *S. diclina* type III by the presence of several pitted oogonia without antheridia as well as the dominance of subcentric oospores separate our fungus from *S. diclina* Type III. In addition, gemma formation, which is one of the characters of *S. parasitica* (*S. diclina* type I), was not observed in our fungus.

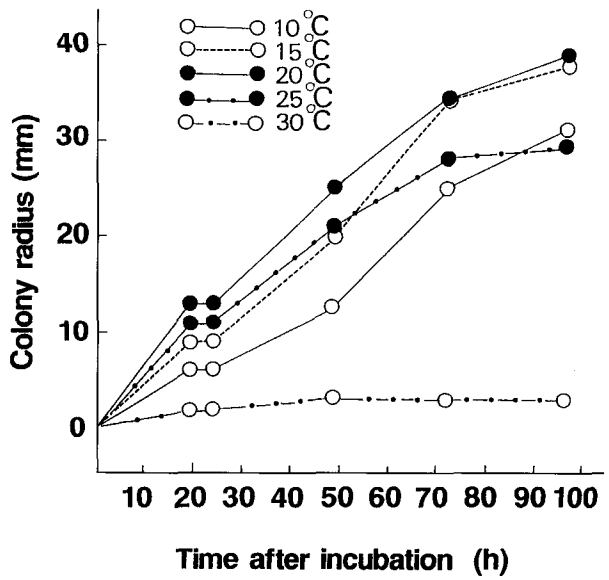

Fig. 3. Effect of temperature on mycelial growth of *Saprolegnia salmonis* NJM 9851.

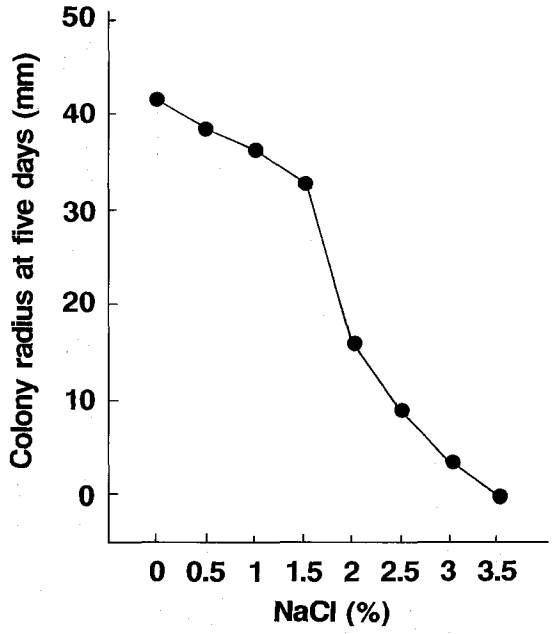

Fig. 4. Effect of NaCl on mycelial growth of *Saprolegnia salmonis* NJM 9851.

Hatai et al. (1977) identified a strain isolated from rainbow trout fingerling with saprolegniasis as *S. australis* Elliott. Oospores in *S. australis* are of subcentric type I or II in internal stucture 22–24 μm in diam, with an average number of 6–12 per oogonium (Seymour, 1970). The oospores of the present isolate were spherical, 20 μm in diam, with an average number of 8 oospores per spherical oogonium and 18 oospores per elongated oogonium, and their internal structure was not only centric but also subcentric. Thus, although the present isolate is morphologically similar to *S. australis*, it differs in 1) type, number, and size of oospores, 2) absence of elongated oogonia, 3) presence of pitted oogonia without antheridial branches.

The present isolate also closely resembles *S. ferax* (Seymour, 1970; Beakes, 1980; Willoughby, 1994) in the presence of spherical pitted oogonia without an antheridial attachment, but differs in having antheridial attachments not only on spherical oogonia but also on elongated ones.

The present isolate thus closely resembled *S. parasitica* Coker (Coker, 1923; Hatai and Hoshiai, 1992a, 1992b), *S. diclina* (Hatai and Egusa, 1977; Willoughby, 1977; Hatai et al., 1990; Kitancharoen et al., 1995), *S. ferax* (Seymour, 1970; Beakes, 1980; Willoughby, 1994), and *S. australis* (Seymour, 1970; Hatai et al., 1977). In comparison with the descriptions of these

species, however, our isolate is characterized as follows: 1) oogonia are predominantly spherical (66.3%); 2) the oogonial wall is pitted, especially under the antheridial attachment; 3) pitted oogonia without antheridia are present; 4) the origin of antheridial branches is exclusively of the diclinous type; 5) oospores are predominantly subcentric; 6) zoospore germination was predominantly direct; and 7) gemmae were not observed. From these characteristics, isolate NJM 9851 was identified as a new species in the genus *Saprolegnia*.

Hatai et al. (1990) studied the effects of temperature on hyphal growth of eight isolates of *S. diclina* and found that only three isolates (H10, H13, H18) grew at 30°C, while all strains showed similar mycelial growth at 10°C. The present isolate showed an optimum temperature for growth of between 15 and 20°C, while at 30°C it exhibited scant abnormal growth which stopped at 96 h, then died. The water temperature and the climate of Hokkaido is usually around 15 to 20°C, and does not exceed 25°C. This means that this fungus is adapted to the environment of Hokkaido. Willoughby and Copland (1984) classified pathogenic *Saprolegnia* of fish into three groups (a, b and c) based on the temperature-growth relationships. All groups are investigated at a wide range of temperature ranging from 3–33°C. They found that group a shows fast-growing at high and low temperature; group b shows better growth at high temperature and less competent at low temperature; and group c is able to grow at low temperature but less competent at high temperature. Our isolate can be included in the Willoughby's group c. The optimum temperature for the growth of *Saprolegnia* sp. reported by Post (1987) coincided with our results.

Although our isolate can grow on 3% NaCl GY agar, the fungi in the genus *Saprolegnia* generally only grow in freshwater (Post, 1987). It is thought that our fungus developed tolerance to NaCl through being exposed to estuarine water, because saprolegniasis occurs in salmonide even in an estuarine environment.

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