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## Short Communication

# Some biochemical characteristics of the genera *Saprolegnia*, *Achlya* and *Aphanomyces* isolated from fishes with fungal infection

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**Carbohydrate and urease tests were performed on the genera *Saprolegnia*, *Achlya* and *Aphanomyces*. These tests may be used as additional criteria for the identification of fungi belonging to these genera.**

Key Words——*Achlya*; *Aphanomyces*; carbohydrate test; *Saprolegnia*; urease test.

The identification of three genera, *Saprolegnia*, *Achlya* and *Aphanomyces*, in Saprolegniaceae has traditionally been based on the features of their asexual and sexual reproduction (Sparrow, 1960). However, some species need long periods to produce oogonia or lack them, and this makes identification difficult. Recently, the distinction between parasitic *Saprolegnia parasitica* Coker and saprophytic *S. diclina* Humphrey has relied on the characteristics of the ornaments on the spore and germination type of the spores (Pickerling et al., 1979; Beakes, 1983; Willoughby, 1985). Furthermore, characteristically catenulated gemmae in *S. parasitica*, which are observed in the isolates showing strong pathogenicity to rainbow trout by artificial infection, is also an important feature (Yuasa and Hatai, 1995). On the other hand, there are a few reports about biochemical characteristics of fungi in Saprolegniaceae. Wolf (1937) discovered that *Achlya bisexualis* Coker et Couch has a strong ability to hydrolyze peptone compared to *S. ferax* (Gruith.) Thuret. Gleason et al. (1970) having tested carbohydrates for the culture of lower fungi, reported that *A. ambisexualis* Raper showed a more rapid growth on the medium with sucrose than the genera *Saprolegnia*, *Leptolegnia* and *Dictyuchus*. The urea utilization test was conducted to identify some species of Fungi Imperfecti, but there has been no such report on lower fungi. In this study, carbohydrate and urease tests were performed on the genera *Saprolegnia*, *Achlya* and *Aphanomyces*. The possibility of using these characteristics for identification is also discussed.

The isolates used are shown in Table 1. *Saprolegnia parasitica* is divided into three groups according to the pathogenicity to rainbow trout fingerling and gemmae production (Yuasa and Hatai, 1995). Groups 1, 2 and 3 represented highly pathogenic, moderately pathogenic and non-pathogenic strains, respectively. For the carbohydrate utilization test, the medium used for culture the fungal isolates was Yeast Nitrogen Base agar (Difco). GY agar (Hatai and Egusa, 1979) was used for the urease test. To prepare the fungal isolates for the experiment, the isolates of the genus *Saprolegnia* were cultured at 20°C for 3 d, whereas the genera *Achlya* and *Aphanomyces* were cultured at 20°C for 7 d. For carbohydrate utilization test, Yeast Nitrogen Base broth (Difco) with 1% carbohydrate was prepared, adjusted to pH 7.2 and autoclaved at 110°C for 10 min. For urease test, GY broth with 1% urea was prepared, adjusted to pH 6.8 and filtered through a 0.45 $\mu$m Millipore filter (Whatman). Brom thymol blue (BTB) and phenol red (PR) were used as indicators, added into Yeast Nitrogen Base broth and GY broth, respectively. Each test was performed in test tubes (1.5 mm × 20 mm) containing 5 ml of the medium. Inocula were taken from the edge of a colony with a No. 2 cork borer (5.5 mm diam) and inoculated into each tube. The tubes were incubated at 20°C for 7 d, then the color change of the indicator was observed. Positive reaction was determined by the change of BTB to yellow in the carbohydrate utilization test, and of PR to pink in the urease test (Table 2).

All *Saprolegnia* isolates were found to utilize dextrose, maltose and soluble starch. Only *S. parasitica* group 1 could utilize cellobiose among *S. parasitica*. *Saprolegnia parasitica* group 1, except for NJM 9115, and *S. parasitica* group 2 was capable of utilizing trehalose, but *S. parasitica* group 3 was not. *Saprolegnia ferax* was able to utilize mannose and glycerol, but the other isolates of *Saprolegnia* were not. From these results, it is clear that *S. ferax* is distinguished from the

Table 1. Details of isolates used in this study.

| Species Isolate | Host/Habitat | Sites | Host location[a] | Year of isolation |
|---|---|---|---|---|
| *Saprolegnia* | | | | |
| *S. parasitica* group 1 | | | | |
| NJM 8604 (H2) | coho salmon | E[b] | Miyagi | 1986 |
| NJM 8761 (H15) | coho salmon | E | Miyagi | 1987 |
| NJM 8629 | coho salmon | E | Miyagi | 1986 |
| NJM 9106 | ayu | E | Wakayama | 1991 |
| NJM 9115 | ayu | E | Tochigi | 1991 |
| NJM 9305 | ayu | E | Tochigi | 1993 |
| *S. parasitica* group 2 | | | | |
| NJM 8602 (H7) | pejerry | E | Kanagawa | 1986 |
| NJM 8737 (H14) | rainbow trout | E | Gumma | 1987 |
| NJM 9111 | coho salmon | E | Tochigi | 1991 |
| NJM 9302 | pejerrey | E | Tochigi | 1993 |
| *S. parasitica* group 3 | | | | |
| NJM 8535 (H5) | pond water | — | Kanagawa | 1985 |
| NJM 9101 | ayu | I[c] | Tochigi | 1991 |
| NJM 9102 | ayu | I | Tochigi | 1991 |
| NJM 9115 | ayu | E | Shizuoka | 1991 |
| NJM 9225 | ayu | I | Tochigi | 1992 |
| *S. diclina* | | | | |
| NJM 0005 (H3) | coho salmon | E | Miyagi | 1986 |
| NJM 0009 (H4) | ayu | E | Kanagawa | 1985 |
| NJM 1010 (H10) | yamame salmon | I | Tokyo | 1986 |
| NJM 0010 (H13) | yamame salmon | I | Tokyo | 1986 |
| NJM 1014 (H17) | yamame salmon | I | Tokyo | 1986 |
| NJM 8536 (H18) | pond water | — | kanagawa | 1985 |
| NJM 9105 | ayu | I | Tochigi | 1991 |
| NJM 9218 | pond water | — | Tokyo | 1992 |
| NJM 9222 | ayu | — | Tochigi | 1992 |
| *Achlya* | | | | |
| *A. prolifera* | | | | |
| NJM 8505 | pond water | — | Gifu | 1985 |
| NJM 9117 | corydoras catfish | E | Tokyo[d] | 1991 |
| NJM 9227 | dwarf gourami | E | Tokyo[d] | 1992 |
| *A. bisexualis* | | | | |
| NJM 9205 | sturgeon catfish | E | Tokyo[d] | 1992 |
| *Achlya* spp. | | | | |
| NJM 9116 | dwarf gourami | E | Tokyo[d] | 1991 |
| *Aphanomyces* | | | | |
| *A. piscicida* | | | | |
| NJM 8997 | ayu | I | Tochigi | 1989 |
| NJM 9030 | ayu | I | Shizuoka | 1990 |
| *Aphanomyces* spp. | | | | |
| NJM 9114 | ayu | I | Tochigi | 1991 |
| NJM 9226 | pond water | — | Tokyo | 1992 |

a) Coho salmon, *Oncorhynchus kisutch* (Walbaum); ayu, *Plecglossus altivelis* Temminck et Schlegel; pejerrey, *Odontesthes bonariensis* Cuvier et Valenciennes; yamame salmon, *Onchrhynchus masou* (Brevoort); rainbow trout, *Oncorphynchus mykiss* Walbaum; dwarf gourami, *Colisa lalia* (Hamilton et Buchanan); sturgeon catfish, *Platystomatichthys sturio*; corydoras catfish, *Corydoras* sp.

b) Isolated from fish with external saprolegniasis.

c) Isolated from fish with internal saprolegniasis.

d) These fishes were imported from Singapore.

Table 2. Carbohydrate and urea utilization test of three genera, *Saprolegnia*, *Achlya* and *Aphanomyces*.

| Fungus | Carbohydrate utilization test | | | | | | | | | | | | | | | | | | | | | | | | Urea utilization test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dextrose | Maltose | Dextrin | Starch | Fructose | Trehalose | Cellobiose | Mannose | Glycerol | Sucrose | Arabinose | Xylose | Sorbitol | Sorbose | Salicin | Melezitol | Glucoside | Raffinose | Adonitol | Galactose | Dulcitol | Inositol | Lactose | Mannitol | Rhamnose | |
| *Saprolegnia* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *S. parasitica* group 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| NJM9115 | + | + | + | + | + | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Others | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *S. parasitica* group 2 | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *S. parasitica* group 3 | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *S. diclina* | | | | | | | | | | | | | | | | | | | | | | | | | |
| NJM0009 | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| NJM0005, NJM1014, NJM8540 | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| NJM1010, NJM0010 | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + |
| others | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + |
| *S. ferax* | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + |
| *Achlya* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *A. prolifera* | | | | | | | | | | | | | | | | | | | | | | | | | |
| NJM8505, NJM9227 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | + |
| NJM9117 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | + |
| *A. bisexualis* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | + |
| *Achlya* sp. | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | + |
| *Aphanomyces* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *A. piscicida* | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *Aphanomyces* spp. | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |

other species in *Saprolegnia*. The isolates of *Achlya* could utilize 18–20 of the 25 carbohydrates tested, of which sucrose gave the strongest reaction. This result indicates that sucrose is useful for distinction of the genus *Achlya* from the other two genera. The isolates of the genus *Aphanomyces* could not use any of the carbohydrates tested. All isolates of *S. parasitica* showed negative reaction in the urea utilization test, except for *S. ferax*. Among *S. diclina*, isolates NJM 0005, NJM 0009, NJM 1014 and NJM 8536, which could utilize trehalose, showed a negative reaction in the urea utilization test, but the other trehalose-negative isolates of *S. diclina* showed a positive reaction in this test. *Aphanomyces* isolates studied here could not utilize urea. However, it is known that *Aphanomyces* spp. isolated from turtle, *Clemmys japonica* (Temminck et Schlegel, 1835), and soft-shelled turtle, *Pelodiscus sinensis* (Wiegmann), could utilize urea (unpublished data). Further studies on urea utilization are considered to be necessary for the genus *Aphanomyces*. As the above results, carbohydrate and urea utilization tests may be used as additional criteria for the identification of fungi belonging to the genera *Saprolegnia*, *Achlya* and *Aphanomyces*.

## Literature cited


Beakes, G. W. 1983. A comparative account of cyst coat ontogeny in saprophytic and fish-lesion isolates (pathogen) of the *Saprolegnia diclina-parasitica* complex. Can. J. Bot. **61**: 603–625.

Gleason, F. H., Rudolph, C. R. and Price, J. S. 1970. Growth of certain aquatic Oomycetes on amino acids 1. *Saprolegnia*, *Achlya*, *Leptolegnia* and *Dictyuchus*. Physiol. Plant. **23**: 513–516.

Hatai, K. and Egusa S. 1979. Studies on the pathogenic fungus of mycotic granulomatosis Ⅲ. Development of the medium for MG-fungus. Fish Pathol. **13**: 147–152.

Pickerling, A. D., Willoughby, L. G. and Mc Grory, C. B. 1979. Fine structure of secondary zoospore cyst cases of *Saprolegnia* isolates of infected fish. Trans. Br. Mycol. Soc. **72**: 427–436.

Sparrow, F. K. 1960. Aquatic Phycomycetes, 2nd ed., Univ. Michigan Press, Ann Arbor.

Willoughby, L. G. 1985. Rapid preliminary screening of *Saprolegnia* on fish. J. Fish Dis. **8**: 473–476.

Wolf, F. T. 1937. A nutritional study of *Achlya bisexualis* and *Saprolegnia ferax*. Am. J. Bot. **24**: 119–123.

Yuasa, K. and Hatai, K. 1995. Relationship between pathogenicity of *Saprolegnia* spp. isolated to rainbow trout and their biological characteristics. Fish Pathol. **30**: 101–106.