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Some biochemical characteristics of fungi isolated from salmonid eggs

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Fungal isolates from salmonid eggs displayed apparently unique patterns of biochemical characteristics at both the generic and specific levels. Of the five genera examined *Achlya* and *Pythium* were able to assimilate 13–16 out of 19 carbohydrates. *Aphanomyces* was able to assimilate only glucose and starch, which was assimilated by all isolates. Members of *Saprolegnia* displayed identical patterns of carbohydrate assimilation, except for *S. hypogyna*, which was also able to assimilate melibiose, in common with *Achlya*, *Pythium*, and *Leptolegnia*. *Pythium* was the only genus capable of assimilating salicin. Only *Achlya* and *P. monospermum* were able to assimilate rhamnose. In terms of amino acid assimilation, isolates of *Saprolegnia ferax* and *S. diclina* displayed an identical patterns, as did isolates of *S. parasitica* and *S. hypogyna*. Only *Aphanomyces frigidophilus* isolate was capable of assimilating cysteine. All genera except *Pythium* assimilated glutamine, a fundamental amino acid. All isolates exhibited lipase and fatty acid esterase activities but no cellulase activity. The biochemical characteristics discovered in this study offer possibilities for identification and classification of these fungi, which are discussed herein.

Key Words——biochemical characteristic; Oomycetes; salmonid eggs.

In bacteriology, biochemical tests provide the main apparatus for identification and classification. In mycological work, tests are currently being developed for use in the classification of some critical groups, especially among the higher fungi (Ascomycotina, Basidiomycotina and Fungi Imperfecti). Biochemical tests have been used to classify and identify various groups of fungi (Bridge and Hawksworth, 1985; Bridge, 1987; Bridge et al., 1989; Bridge et al., 1993a,b). At present, fungi in the class Oomycetes are mainly identified or classified by their morphological characteristics. A limited number of studies has been conducted in the past five decades on the biochemical characteristics of Oomycetes, specifically their ability to utilize carbohydrates or amino acids as carbon or nitrogen sources (Papavizas and Ayer, 1964; Yuasa, 1995) and their enzymatic activity (Beakes and Ford, 1983; Rand and Munden, 1992), but no study has been reported on any other of their biochemical characteristics. Thus, further basic studies are required of the biochemical characteristics of these fungi, which may provide new data to support their identification or classification. To this end, the present study was undertaken on Oomycetes isolated from salmonid eggs.

Materials and Methods

Fungi and culture conditions Fungal isolates used in this study are detailed in Table 1. Vegetative mycelia of the fungi were obtained by incubating agar blocks (about 5 × 5 mm) with mycelia in 10 ml of GY broth at 20°C for 24–48 h. The mycelia were washed three times in sterilized distilled water before testing. All tests were performed as described by Paterson and Bridge (1994) except for the incubation temperature and observation period, which were modified.

Carbohydrate assimilation test The basal medium consisted of 8.4 mM $NH_4H_2PO_4$, 2.7 mM KCl, and 0.8 mM $MgSO_4 \cdot 7H_2O$, supplemented with 0.035 mM $ZnSO_4 \cdot 7H_2O$ and 0.02 mM $CuSO_4 \cdot 5H_2O$. Brom cresol purple was added as an indicator at a concentration of 50 mg/L, and the medium was autoclaved at 121°C for 10 min. Filter-sterilized carbohydrates were then added to the medium to a final concentration of 1% (w/v), pH was adjusted to 5.4 by adding NaOH or HCl, and 2 ml portions of the medium were dispensed into 10-ml test tubes. The tubes were inoculated with fungal mycelia, and control tubes for each fungus and carbohydrate (+fungus/−carbohydrate and −fungus/+carbohydrate) were also prepared. All tubes were incubated at 20°C for 14 d. A change in the color of the medium to orange or yellow was taken as positive result; a change to pink or purple was considered a negative result.

Amino acid assimilation test The basal medium used in this test contained 7.4 mM KH_2PO_4, 6.7 mM KCl, 0.8 mM $MgSO_4 \cdot 7H_2O$, 0.7 mM $CaCl_2 \cdot 2H_2O$ and 1% (w/v) glucose. Medium preparation and indicator were as described for the carbohydrate assimilation test. Amino acids were added to a final concentration of 0.2% (w/v). Ten-milliliter test tubes containing 2 ml of the medium were inoculated with fungal mycelia, and control

Table 1. Fungi isolated from salmonid eggs used in this study.

Isolate	Location	Host (egg)	Date
Saprolegnia ferax			
NJM 9580	Shizuoka	rainbow trout	Jul. 95
NJM 9583	Shizuoka	rainbow trout	Jul. 95
NJM 9660	Shizuoka	rainbow trout	Jun. 96
NJM 9676	Gifu	amago salmon	Nov. 96
S. diclina			
NJM 9585	Shizuoka	rainbow trout	Jul. 95
NJM 9587	Shizuoka	rainbow trout	Jul. 95
NJM 9501	Tochigi	sockeye salmon	Dec. 95
NJM 9651	Shizuoka	rainbow trout	Jan. 96
NJM 9654	Tokyo	rainbow trout	Apr. 96
NJM 9661	Shizuoka	rainbow trout	Jun. 96
NJM 9662	Yamanashi	rainbow trout	Jul. 96
NJM 9675	Gifu	amago salmon	Nov. 96
S. parasitica			
NJM 9594	Yamanashi	sockeye salmon	Oct. 95
NJM 9595	Yamanashi	sockeye salmon	Oct. 95
NJM 9672	Nikko, Tochigi	masu salmon	Nov. 96
S. hypogyna			
NJM 9667	Nikko, Tochigi	masu salmon	Nov. 96
NJM 9668	Nikko, Tochigi	masu salmon	Nov. 96
Achlya spp.			
NJM 9586	Shizuoka	rainbow trout	Aug. 95
NJM 9598	Yamanashi	rainbow trout	Dec. 95
NJM 9599	Yamanashi	rainbow trout	Dec. 95
NJM 9656	Tokyo	rainbow trout	Apr. 96
NJM 9674	Gifu	amago salmon	Nov. 96
NJM 9677	Gifu	amago salmon	Nov. 96
Aphanomyces spp.			
NJM 9591	Yamanashi	masu salmon	Oct. 95
NJM 9663	Yamanashi	rainbow trout	Jul. 96
A. frigidophilus			
NJM 9500	Tochigi	Japanese char	Dec. 95
NJM 9665	Yamanashi	masu salmon	Oct. 96
Pythium sp.			
NJM 9592	Yamanashi	masu salmon	Oct. 95
P. monospermum			
NJM 9659	Tokyo	rainbow trout	Apr. 96
Leptolegnia spp.			
NJM 9666	Nikko, Tochigi	masu salmon	Nov. 96
NJM 9669	Nikko, Tochigi	masu salmon	Nov. 96
NJM 9671	Nikko, Tochigi	masu salmon	Nov. 96

tubes were prepared in the same manner as in the carbohydrate assimilation test. All tubes were incubated at 20°C for 14 d. A positive result was determined by a change in the color of the medium to pink or purple, and a change to orange or yellow was considered a negative result.

Other tests

Casein hydrolysis The basal medium was similar to that of the amino acid assimilation test with addition of 0.5% skim milk and 1.2% agar. After autoclaving at 110°C for 30 min, the medium was poured into Petri dishes in 20-ml portions. Fungal mycelia were inoculated onto the center of the plates and incubated at 20°C for 14 d. The appearance of a clear zone around the fungal colony was taken as a positive result.

Lipase activity The medium composed of 0.5% peptone, 0.3% yeast extract, and 1% agar, which was autoclaved at 121°C for 10 min. Filtered and sterilized *n*-tributylin (Sigma) was added to a final concentration of 0.1% (v/v), and the medium was dispensed into sterilized test tubes (10 ml per tube) and chilled rapidly. Fungal mycelia were inoculated onto the surface of the medium and incubated at 20°C for 7 d. The occurrence of clearance in the medium column was taken as a positive result.

Fatty acid esterase activity A medium composed of 1% peptone, 0.7 mM $CaCl_2 \cdot 2H_2O$, 0.08 M NaCl and 1.5% agar with 0.002% brom cresol purple as indicator was prepared and adjusted to pH 5.4. A solution of 10% Tween 80 in distilled water was also prepared, and the medium and Tween solution were autoclaved at 121°C for 10 min. When the medium had cooled to 65–70°C, the Tween solution was added to in a ratio of 1 : 9 by volume the medium. The complete medium was poured into Petri dishes in 10-ml portions. The fungal mycelia were inoculated onto the center of the medium and incubated at 20°C for 14 d. A change in the color of the medium to purple was taken as a positive result.

Urease activity The same basal medium and indicator were used as those in the carbohydrate assimilation test, with addition of 1% (w/v) glucose and 1.2% (w/v) agar. The medium was autoclaved at 121°C for 10 min, then filter-sterilized urea solution was added to a final concentration of 1% (w/v). The medium was poured into Petri dishes in 10-ml portions. Control plates containing only the basal medium were also prepared. Fungal mycelia were inoculated onto the center of the agar plates, which were then incubated at 20°C for 14 d. A change in the color of the medium to pink or purple was taken as a positive result; a change to orange or yellow was considered a negative result.

Cellulase activity The basal medium used was identical to that used in the carbohydrate assimilation test, with addition of 1% (w/v) microgranular cellulose (Sigma) and 1.2% (w/v) agar. The medium was autoclaved at 121°C for 10 min and dispersed into Petri dishes 10-ml portion. The fungi were inoculated onto the center of the agar plates and incubated at 20°C for 14 d. The appearance of a clear zone around the fungal colony was taken as a positive result.

Nitrite assimilation and resistance A mixture of 5.7 mM $K_2HPO_4 \cdot 3H_2O$, 0.3% (w/v) sucrose and 1.2% (w/v) agar containing 43 mM $NaNO_2$ for the NO_2 resistance test or 5 mM $NaNO_2$ for the NO_2 assimilation test was supplemented with 10 ml of a mineral solution (containing

0.6 M KCl, 0.2 M $MgSO_4 \cdot 7H_2O$, 3.6 mM $FeSO_4 \cdot 7H_2O$). The medium was autoclaved at 121°C for 10 min, then dispensed into plastic Petri dishes in 10-ml portions. The fungi were inoculated onto the center of the plates and incubated at 20°C for 14 d to observe their ability to grow.

Gelatin hydrolysis Medium was prepared by mixing 5% (v/v) solution A (470 mM $NaNO_3$, 135 mM KCl, and 0.8 mM $MgSO_4 \cdot 7H_2O$), 5% (v/v) solution B (115 mM K_2HPO_4), 0.035 mM $ZnSO_4 \cdot 7H_2O$; 0.02 mM $CuSO_4 \cdot 5H_2O$, 1% (w/v) sucrose, and 12% (w/v) gelatin. The medium was dispensed into tubes in 10-ml portions and autoclaved at 121°C for 10 min. Fungal mycelia were inoculated into the tubes and incubated at 20°C for 14 d. Then tubes were chilled at 4°C for 1 h. Liquefaction of the medium after chilling was taken as a positive result.

β-glucosidase activity Medium was prepared by mixing 5% (v/v) solution A, 5% (v/v) solution B, 0.035 mM $ZnSO_4 \cdot 7H_2O$, 0.02 mM $CuSO_4 \cdot 5H_2O$, Fe citrate, 0.5% (w/v) sucrose, 0.3% (w/v) aesculin, and 1.2% (w/v) agar. The medium was autoclaved at 121°C for 10 min and poured into Petri dishes in 10-ml portions. Fungal mycelia were inoculated onto the center of the medium, then incubated at 20°C for 30 d. Blackening of the medium was taken as a positive result.

Results

Carbohydrate assimilation test With the exception of two isolates of *S. diclina* that could not assimilate trehalose, *S. ferax*, *S. diclina* and *S. parasitica* were capable of assimilating 9 carbohydrates, as shown in Table 2. *Saprolegnia hypogyna* was able to assimilate the same 9 carbohydrates and also melibiose. All isolates of *Achlya* could assimilate 14 carbohydrates, including raffinose, sucrose, lactose and rhamnose, which *Saprolegnia* could not. *Aphanomyces* was able to assimilate only glucose and starch. No difference was found between *A. frigidophilus* and the two isolates of *Aphanomyces* spp. in carbohydrate assimilation. *Pythium* was the only genus that could assimilate salicin. *Pythium monospermum* NJM 9659 could be distinguished from *Pythium* sp. NJM 9592 by its ability to assimilate galactose, raffinose and rhamnose. *Leptolegnia* had the same pattern of carbohydrate assimilation as *Saprolegnia*. Glucose and starch were the only 2 carbohydrates assimilated by all fungal isolates. None of the fungal isolates could assimilate arabinose, xylose or sorbose (Table 2).

Amino acid assimilation test As shown in Table 3, 6 of the 12 amino acids tested, namely, methionine, lysine, ornithine, phenylalanine, leucine, and glycine, could not be assimilated by the fungi. *Saprolegnia ferax* and *S. diclina* were identical in their capability to assimilate 5 amino acids: asparagine, glutamine, arginine, alanine and histidine. *Saprolegnia parasitica* and *S. hypogyna* were identical in their capability to assimilate 3 amino acids, asparagine, glutamine, and alanine, and incapable of assimilating arginine and histidine. All isolates of the genus *Achlya* were able to assimilate the same 4 amino acids: asparagine, glutamine, arginine and alanine. *Achlya frigidophilus* could assimilate glutamine, alanine and cysteine; but the two isolates of *Aphanomyces* spp. could assimilate only glutamine. Of all isolates tested, only *A. frigidophilus* was capable of assimilating cysteine. *Pythium* sp. NJM 9592 was not capable of assimilating any of the amino acids tested, while *P. monospermum* was able to assimilate one amino acid, histidine. *Leptolegnia* was able to assimilate 2 of the amino acids tested, asparagine and glutamine.

Other tests As shown in Table 4, all fungal isolates revealed lipase and fatty acid esterase activity by hydrolyzing tributylin and Tween 80, respectively, but none showed cellulase activity by hydrolyzing cellulose. Casein hydrolysis tests showed that only members of the genera *Achlya* and *Pythium* could hydrolyze casein, whereas the other three genera could not. The ability to hydrolyze urea was found in all isolates of *S. ferax*, *S. parasitica*, *S. hypogyna*, *Pythium*, *Leptolegnia* and some isolates of *S. diclina*. Most of the isolates could assimilate NO_2 (5 mM), but only *Saprolegnia* and *Leptolegnia* could tolerate high concentrations of NO_2 (43 mM). All genera, except for *Pythium* could hydrolyze gelatin. β-Glucosidase activity shown by hydrolysis of aesculin was found only in the genus *Saprolegnia*, although not in *S. diclina*.

Discussion

The morphological characteristics of fungi in the class Oomycetes are highly dependent upon culture conditions, and characteristics in some of the morphological parameters tested. This has led, in the past, to its well-known doubtful classification as *S. diclina-parasitica* complex (Willoughby, 1978; Beakes and Ford, 1983; Woods et al., 1987). Therefore, supporting criteria, such as physiological or biochemical characteristics, should be introduced to aid in the classification of these fungi. The results of carbohydrate assimilation tests in this study are quite different from those obtained by Yuasa (1995) for the isolates the genera *Aphanomyces*, *Achlya* and *Saprolegnia*, except for *S. ferax*. This might be due to the difference in approach between the two studies, the carbohydrates examined or the fungal isolate itself. *Achlya euteiches*, as reported by Papavizas and Ayer (1964), was able to use more kinds of carbohydrate than the *Aphanomyces* examined in this study. It is of particular interest that arabinose, xylose and sorbose were not utilizable as sole carbon sources by any of the isolates tested. These carbohydrates may actually be toxic to the fungi, because little or no growth was evident after their addition.

The results of amino acid assimilation tests contribute to taxonomic values among the fungi examined. Schreurs et al. (1989) reported that the amino acids phenylalanine, methionine, arginine and glutamine induced hyphal growth and branching in *Achlya bisexualis*. But none of the fungal isolates in the present study could use methionine or phenylalanine. Among the genera studied, hydrolysis of casein was found only in *Achlya*

Table 2. Carbohydrate assimilation by the fungi isolated from salmonid eggs.

Isolate	Fru	Glu	Ara	Man	Gal	Xyl	Sor	Raf	Suc	Mal	Lac	Mel	Cel	Tre	Sta	Dex	Rha	Gly	Sal
Saprolegnia ferax																			
NJM 9580	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9583	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9660	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9676	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
S. diclina																			
NJM 9585	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9587	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9501	+	+	−	+	*	*	*	−	−	+	*	−	+	−	+	+	−	+	−
NJM 9651	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9654	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9661	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9662	+	+	−	+	*	*	*	−	−	+	*	−	+	−	+	+	−	+	−
NJM 9675	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
S. parasitica																			
NJM 9594	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9595	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
NJM 9672	+	+	−	+	*	*	*	−	−	+	*	−	+	+	+	+	−	+	−
S. hypogyna																			
NJM 9667	+	+	−	+	−	−	*	−	−	+	−	+	+	+	+	+	−	+	−
NJM 9668	+	+	−	+	−	−	*	−	−	+	−	+	+	+	+	+	−	+	−
Achlya spp.																			
NJM 9586	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
NJM 9598	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
NJM 9599	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
NJM 9656	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
NJM 9674	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
NJM 9677	+	+	−	+	*	*	*	+	+	+	+	+	+	+	+	+	+	+	−
Aphanomyces spp.																			
NJM 9591	−	+	−	−	*	*	*	−	−	−	*	−	−	−	+	−	−	−	−
NJM 9663	−	+	−	−	*	*	*	−	−	*	*	−	−	−	+	−	−	−	−
A. frigidophilus																			
NJM 9500	−	+	−	−	*	*	*	−	−	*	*	−	−	−	+	*	−	−	−
NJM 9665	−	+	−	−	*	*	*	−	−	*	*	*	−	+	−	−	−		
Pythium sp.																			
NJM 9592	+	+	−	+	−	*	*	−	+	+	+	+	+	+	+	+	−	+	+
P. monospermum																			
NJM 9659	+	+	−	+	+	*	*	+	+	+	+	+	+	+	+	+	+	+	+
Leptolegnia spp.																			
NJM 9666	+	+	*	+	−	*	*	−	−	+	−	+	+	+	+	+	−	+	−
NJM 9669	+	+	*	+	−	*	*	−	−	+	−	+	+	+	+	+	−	+	−
NJM 9671	+	+	*	+	−	*	*	−	−	+	−	+	+	+	+	+	−	+	−

+: positive, −: negative, *: no growth.

Abbreviations. Fru: Fructose, Glu: Glucose, Ara: Arabinose, Man: Mannose, Gal: Galactose, Xyl: Xylose, Sor: Sorbose, Raf: Raffinose, Suc: Sucrose, Mal: Maltose, Lac: Lactose, Mel: Melibiose, Cel: Cellobiose, Tre: Trehalose, Sta: Starch, Dex: Dextrin, Rha: Rhamnose, Gly: Glycerol, Sal: Salicin.

and *Pythium*. Casein hydrolysate was proved to affect the chemotropism of germlings of *A. bisexualis*. Fungi in the class Oomycetes, especially the family Saprolegniaceae, have been widely reported as plant pathogens, and this suggests that they produce cellulase, which is important for invasion of plant tissues. Hill and Mullins (1980) found cellulase in the hyphal tip of *Achlya ambisexualis*. None of the isolates tested in the present

Table 3. Amino acid assimilation by the fungi isolated from salmonid eggs.

Isolate	Met	Asp	Lys	Glu	Orn	Phe	Arg	Leu	Ala	His	Cys	Gly
Saprolegnia ferax												
NJM 9580	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9583	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9660	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9676	−	+	−	+	−	−	+	−	+	+	−	−
S. diclina												
NJM 9585	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9587	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9501	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9651	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9654	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9661	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9662	−	+	−	+	−	−	+	−	+	+	−	−
NJM 9675	−	+	−	+	−	−	+	−	+	+	−	−
S. parasitica												
NJM 9594	−	+	−	+	−	−	−	−	+	−	−	−
NJM 9595	−	+	−	+	−	−	−	−	+	−	−	−
NJM 9672	−	+	−	+	−	−	−	−	+	−	−	−
S. hypogyna												
NJM 9667	−	+	−	+	−	−	−	−	+	−	−	−
NJM 9668	−	+	−	+	−	−	−	−	+	−	−	−
Achlya spp.												
NJM 9586	−	+	−	+	−	*	+	−	+	−	−	−
NJM 9598	−	+	−	+	−	*	+	−	+	−	−	−
NJM 9599	−	+	−	+	−	*	+	−	+	−	−	−
NJM 9656	−	+	−	+	−	*	+	−	+	−	*	−
NJM 9674	−	+	−	+	−	*	+	−	+	−	−	−
NJM 9677	−	+	−	+	−	*	+	−	+	−	−	−
Aphanomyces spp.												
NJM 9591	−	−	−	+	−	*	−	−	−	−	−	−
NJM 9663	*	−	−	+	−	*	−	−	−	−	*	−
A. frigidophilus												
NJM 9500	−	−	−	+	−	*	−	−	+	−	+	−
NJM 9665	*	−	−	+	*	*	−	−	+	−	+	−
Pythium sp.												
NJM 9592	−	−	−	−	−	−	−	−	−	−	−	−
P. monospermum												
NJM 9659	−	−	−	−	*	*	−	−	−	+	*	−
Leptolegnia spp.												
NJM 9666	−	+	−	+	−	−	−	−	−	−	−	−
NJM 9669	−	+	−	+	−	−	−	−	−	−	−	−
NJM 9671	−	+	−	+	−	−	−	−	−	−	−	−

+: positive, −: negative, *: no growth.

Abbreviations. Met: Methionine, Asp: Asparagine, Lys: Lysine, Glu: Glutamine, Orn: Ornithine, Phe: Phenylalanine, Arg: Arginine, Leu: Leucine, Ala: Alanine, His: Histidine, Cys: Cysteine, Gly: Glycine.

study, however, showed cellulase activity. This may be explained by differences in the host, which affect essential enzyme production. All of the fungal isolates in the present study were obtained from fish eggs, and most of them were able to hydrolyze gelatin, the protein found in animals. All of the fungal groups tested could reduce NO_2, but *Aphanomyces* sp. NJM 9663 was very sensitive to NO_2 and incapable of growth even at 5 mM NO_2. NO_2 is known to be toxic to living cells (Pateman and Kinghorn, 1976). However, at the high concentration

Table 4. Some biochemical characteristics of the fungi isolated from salmonid egg.

Isolate	Biochemical characteristic								
	Casein	Lipase	FA esterase	Urease	Cellulase	NO_2 ass.	NO_2 Res.	Gelatin	β-gluco
Saprolegnia ferax									
NJM 9580	−	+	+	+	−	+	+	+	+
NJM 9583	−	+	+	+	−	+	+	+	+
NJM 9660	−	+	+	+	−	+	+	+	+
NJM 9676	−	+	+	+	−	+	+	+	+
S. diclina									
NJM 9585	−	+	+	−	−	+	+	+	−
NJM 9587	−	+	+	−	−	+	+	+	−
NJM 9501	−	+	+	+	−	+	+	+	−
NJM 9651	−	+	+	−	−	+	+	+	−
NJM 9654	−	+	+	+	−	+	+	+	−
NJM 9661	−	+	+	+	−	+	+	+	−
NJM 9662	−	+	+	+	−	+	+	+	−
NJM 9675	−	+	+	+	−	+	+	+	−
S. parasitica									
NJM 9594	−	+	+	+	−	+	+	+	+
NJM 9595	−	+	+	+	−	+	+	+	+
NJM 9672	−	+	+	+	−	+	+	+	+
S. hypogyna									
NJM 9667	−	+	+	+	−	+	+	+	+
NJM 9668	−	+	+	+	−	+	+	+	+
Achlya spp.									
NJM 9586	+	+	+	−	−	+	−	+	−
NJM 9598	+	+	+	−	−	+	−	+	−
NJM 9599	+	+	+	−	−	+	−	+	−
NJM 9656	+	+	+	−	−	+	−	+	−
NJM 9674	+	+	+	−	−	+	−	+	−
NJM 9677	+	+	+	−	−	+	−	+	−
Aphanomyces spp.									
NJM 9591	−	+	+	−	−	+	−	+	−
NJM 9663	−	+	+	−	−	−	−	+	−
A. frigidophilus									
NJM 9500	−	+	+	−	−	+	−	+	−
NJM 9665	−	+	+	−	−	+	−	+	−
Pythium sp.									
NJM 9592	+	+	+	+	−	+	−	−	−
P. monospermum									
NJM 9659	+	+	+	+	−	+	−	−	−
Leptolegnia spp.									
NJM 9666	−	+	+	+	−	+	+	+	−
NJM 9669	−	+	+	+	−	+	+	+	−
NJM 9671	−	+	+	+	−	+	+	+	−

Abbreviations. Casein: Casein hydrolysis, Lipase: Lipase activity, FA esterase: Fatty acid esterase activity, Urease: Urease activity, Cellulase: Cellulase activity, NO_2 ass.: NO_2 assimilation, NO_2 Res.: NO_2 resistance, Gelatin: Gelatin hydrolysis, β-gluco: β-glucosidase activity.

(43 mM) that some fungal groups could tolerate, this may be of value in taxonomic studies (Frisvad, 1981). Aesculin used for presumptive β-glucosidase activity, which has been used to distinguish the race of *Fusarium oxysporum* (Bridge et al., 1993a), was also suggested to be a taxonomic criterion in this study. In particular, it supported the segregation of *S. diclina* and *S. parasitica*, the doubtful species in genus *Saprolegnia*.

The results in Tables 2–4, support the proposition that biochemical characteristics should be considered as an additional approach in identification and classification of fungi in the class Oomycetes. Although only a limited number of fungal isolates were examined in this study, the results are of considerable taxonomic value. Each genus and certain species seemed to have a unique pattern of biochemical characteristics. Therefore, it is recommended that further investigation be undertaken into this approach to the taxonomy of the Oomycetes.

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