Influence of pH, Temperature and Sodium Chloride Concentration on Growth Rate of *Saprolegnia* sp.

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Water mold samples, 8 isolations, were obtained to study. There were 4, 2 and 2 isolations from Norway, Scotland and Chile respectively. All 8 isolations contained asexual reproductive structures observed under inverted light microscopy and identified as *Saprolegnia* sp. The optimum pH, temperature, and % (w/v) NaCl concentrations were 7-10, 25°C and 0-0.5 respectively. These results are useful for predicting occurrence, epidemiological surveys, and distribution of water molds and aquatic animal health monitoring.

Key words: Maximum growth rate, *Saprolegnia* sp., tolerance and water molds.

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อิทธิพลของ ความเป็นกรด-ด่าง อุณหภูมิและความเข้มข้นของเกลือแกง ต่ออัตราการเจริญของ *Saprolegnia* sp.

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ตัวอย่างราน้ำที่แยกได้จากประเทศนอรเวย์ สกอตแลนด์ และชิลี จำนวน 4, 2 และ 2 ตัวอย่าง ตามลำดับ นำมาศึกษาเพื่อแยกจีนัสของราน้ำ ภายใต้กล้องจุลทรรศน์ชนิดลำแสงธรรมดา พบว่าเป็น *Saprolegnia* sp. ค่า pH อุณหภูมิ และความเข้มข้นของเกลือแกง ที่ทำให้ราน้ำชนิด *Saprolegnia* sp. มีอัตราการเจริญสูงที่สุดคือ 7-10, 25°C และ 0-0.5% (w/v) ตามลำดับ ผลของการศึกษาในครั้งนี้มีประโยชน์ในการสำรวจการแพร่กระจายของราน้ำ การเกิดโรคระบาดในสัตว์น้ำจาก *Saprolegnia* sp. และทำให้เกิดมีการเฝ้าระวังสุขภาพของสัตว์น้ำ

คำสำคัญ อัตราการเจริญสูงสุด *Saprolegnia* sp. ความทนทาน ราน้ำ

INTRODUCTION

Some aquatic fungi are known to be pathogenic to fishes and their eggs. *Saprolegnia parasitica, S. diclina and S. ferax* are known to be pathogens to cold water fishes. Saprolegniasis is a continuing problem for aquatic animal culturists. Any species of fish that is intensively cultured and captured, is at risk of contracting fungal diseases. Fungus has been known to cause serious diseases in fresh water and estuarine aquatic animals in Japan, the Philippines, Australia and throughout South Asia.[1] Genus *Saprolegnia* in the family Saprolegniaceae is ubiquitous in water supplies and often causes losses due to Saprolegniasis in fishes. The occurrence and severity of fungal outbreaks depend on the water sources, water temperature, organic load and length of contact time.[2-5]

To prevent these outbreaks, many attempts have been made to develop both curative and prophylactic fungicides.[6-13] The research has shown that cold water fish (12°C), cool water fish (17°C) and warm water fish (22°C) have varying sensitivities to Saprolegniasis.[14]

This research was intended to study the effect of some water quality characteristics on growth rate of *Saprolegnia* sp. Fungi used in this study were collected from Norway, Scotland and Chile. According to reports, Saprolegniasis is currently occurring in these countries. The study results will be beneficial to many aspects of aquaculture and research.

MATERIALS AND METHODS

Asexual Identification

Experimental isolates were received from Norway (N3, N5, N6 and N33), Scotland (N7 and N12) and Chile (N16 and N18). They were kept on GY agar (1% (w/v) glucose, 0.25% (w/v) yeast extract and 1.5% (w/v) agar) for transportation. Small agar blocks (8 x 8 mm) with hyphae were cut from the edge of each colony and transferred to new GY agar. Water molds were kept in 20°C incubation for 2 days. Small agar blocks were cut from actively growing edges of young fungal colonies and inoculated into disposable plastic petri dishes (90 x 20 mm) which contained 30 ml sterile tap water. All petri dishes were incubated at 20°C for 2 days. Zoosporangial forming and zoospores releasing were observed under inverted microscopy as described by Seymour.[15]

Effect of pH on fungal growth

GY broth (1% (w/v) glucose and 0.25% (w/v) yeast extract) was prepared with pH of 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 as measured by pH meter F-21, Horiba. The 5 ml GY broth was aseptic filtrated with a disposable syringe filter unit (Advantec, DISMI C-25CS) and poured into sterile glass test tubes. An agar block with young mycelia was cut off with a No. 2 cork borer and transferred into the sterile GY broth. All test tubes were incubated at 20°C in a cabinet. Growing hyphae were observed by naked eye every 24 h for 2 weeks and recorded. All experiments were done in 5 replicates. Per cent growth rate was calculated from equation 1.

$$\% \text{ Growth rate} = Hf \times 100 / Hb \quad \ldots (1)$$

Where Hf = height of fungal growth (mm)
Hb = height of GY broth (mm)

Effect of temperature on fungal growth

The advancing edge of each young colony was cut by cork borer No.2 (circular agar with a 5.5 mm diameter). Circular agar was centrally placed on 25 ml GY agar and kept at temperatures of 5, 10, 15, 20, 25, 30, 35, 37, and 40°C in an incubator. Colony radius was measured with venire calipers every 24 h for 2 weeks after inoculation. All experiments were done in 5 replicates. Determination of colony radius was calculated from equation 2.

$$R = F - C \quad \ldots (2)$$

Where R = radius of fungal growth (mm)
F = radius colony of fungal growth (mm)
C = radius of centrally circular agar (mm)

Effect of NaCl concentration on vegetative growth

A circular agar of isolates was cut from the margins of actively growing colonies and inoculated on the center of GY agar containing various concentrations of NaCl (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% (w/v). All petri dishes were kept in a 20°C incubator for 2 weeks. All experiments were done in 5 replicates. Colony radius was measured and calculated as mentioned above.

RESULTS AND DISCUSSION

Growing young mycelia on small agar block were observed everyday under an inverted microscope. The vegetative thallas is tubular, non-septate, multinucleate, variably branched and with transparent hyphae. Active motile Zoospores were discharged one by one in rapid succession through an exit apical pore of Sporangium (Figure 1 A and B). The Zoospore releasing was Saprolegnoid for all isolates.[15-17] Based on the mode of Zoospore release from Zoosporangia, all isolates were identified as aquatic fungi belonging to the genus *Saprolegnia*.

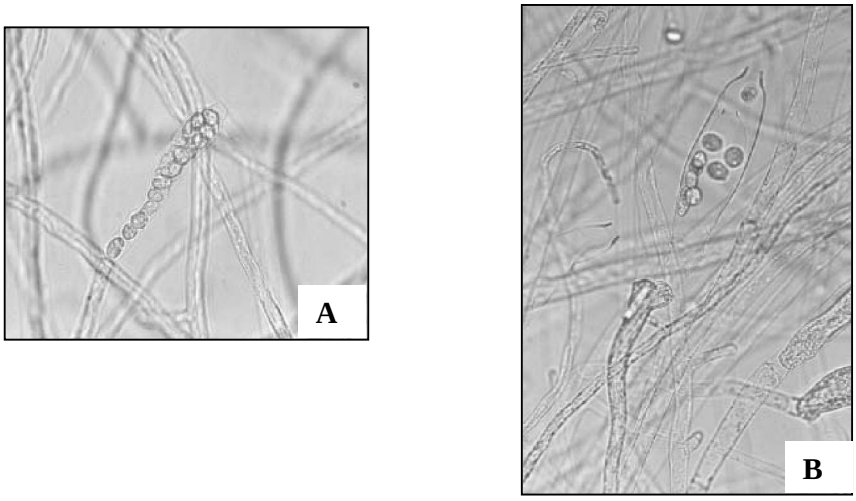

Figure 1. Maturation Zoosporangium (A) and Saprolegnoid type Zoospores releasing from the Zoosprangium (B). Magnification 2.5 x 40.

It is known that water molds can grow well in very dilute nutrients and prefer pH from neutral to weak alkaline conditions.[18] Hence, all isolates in this experiment grew well on pH 7-10. As can be seen in Table 1, water molds from Norway grew well on pH 7-10. The maximum growth rates were: N3-0.75% hyphae growth/h at pH 9, N5-1.03% hyphae growth/h at pH 7, N6-1.11% hyphae growth/h at pH 10 and N33-0.76% hyphae growth/h at pH 10. Aquatic fungi from Scotland had maximum growth when pH was 9-10. The optimum pH for N7 (1.09% hyphae growth/h) was pH 9 and for N12 (1.23% hyphae growth/h) pH 10. For the Chile samples, an optimal pH for N16 (1.09% hyphae growth/h) was pH 7 and for N18 (0.71% hyphae growth/h) was pH 8.

Table 1. Effect of pH on % hyphal growth/h.

Country	Isolate	pH									
		3	4	5	6	7	8	9	10	11	12
Norway	N3	0	0.09	0.55	0.71	0.70	0.73	0.75*	0.71	0.34	0
	N5	0	0.07	0.52	0.71	1.03*	1.02	0.87	1.00	0.95	0
	N6	0	0.08	0.55	0.73	0.80	0.81	0.87	1.11*	0.90	0
	N33	0	0.10	0.62	0.73	0.70	0.70	0.70	0.76*	0.63	0
Scotland	N7	0	0.02	0.44	0.76	0.83	0.94	1.09*	0.71	0.18	0
	N12	0	0.06	0.75	0.76	1.05	1.10	1.03	1.23*	0.87	0
Chile	N16	0	0.13	0.64	0.97	1.09*	1.03	0.83	0.73	0.52	0
	N18	0	0.05	0.60	0.62	0.59	0.71*	0.70	0.66	0.28	0

** Maximum mean of 5 replicates*

Kashiwaki *et al*.,[8] in contrast, addressed the fact that weak alkaline electrolyzed solutions (pH 8.2) had strong fungicidal effects on *S. parasitica* Corker NJM 8604 and were effective antifungal agents. Kitancharoen *et al*.[19] reported that *S. diclina* Hamphrey and *S. parasitica* Corker grew very well in strong acidic condition (pH 3.5). In this study, there was a similar pH range for growth. This information shows that the biological characteristics isolated for study play a major role in fungal growth.

Table 2 shows the effect of temperature on *Saprolegnia* sp. All water molds in this experiment had maximum growth at a temperature of 25°C. Growth of samples from Norway; N3, N5, N6 and N33 were 0.50, 0.32, 0.45, and 0.81 mm/h respectively. Samples N7 and N12 from Scotland grew at 0.52 and 0.49 mm/h respectively. The maximum growth rate of N16 and N18 from Chile were 0.70 and 0.59 mm/h respectively. The colony radius growth recording stopped if the petri dish was full. Samples N5, N6, N7, and N12 remained viable on the available agar surface for 15 days but did not grow well. No growth of aquatic fungi in this experiment was noted at temperatures over 30°C.[20]

In Saprolegniaceae, differences in growth rate and other physiological characteristics not only might be correlated to host specificity, but may also serve to identify different subgroups within the species[21] and indicate strain differences.[22] Wood *et al*.[23] indicated that colony morphology and growth at 25°C could distinguish *S. diclina* from *S. parasitica* from salmonids. Hatai and Hoshiai[24] noted that growth rate at 30°C could be used for rapid identification of *Saprolegnia* sp. Hussein and Hatai[25] classified *Saprolegnia* sp. based on growth at temperatures ranging from 3-33°C. These studies show that aquatic fungi can survive in many environments.

Table 2. Effect of temperature on vegetative mycelia radial growth (mm/h).

Country	Isolate	Temperature (°C)								
		5	10	15	20	25	30	35	37	40
Norway	N3	0.22	0.26	0.31	0.49	0.50*	0.10	0	0	0
	N5	0.10	0.12	0.13	0.29	0.32*	0.07	0	0	0
	N6	0.20	0.26	0.28	0.41	0.45*	0.13	0	0	0
	N33	0.32	0.38	0.45	0.68	0.81*	0.52	0	0	0
Scotland	N7	0.20	0.22	0.26	0.45	0.52*	0.13	0	0	0
	N12	0.21	0.25	0.30	0.48	0.49*	0.14	0	0	0
Chile	N16	0.21	0.27	0.40	0.57	0.70*	0.20	0	0	0
	N18	0.25	0.31	0.35	0.52	0.59*	0.33	0	0	0

** Maximum mean of 5 replicates*

Table 3. Effect of NaCl concentration on colony radius (mm/h).

Country	Isolate	% NaCl								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Norway	N3	0.49	0.60*	0.45	0.34	0.20	0.08	0	0	0
	N5	0.33	0.35*	0.24	0.18	0.08	0.02	0	0	0
	N6	0.39	0.44*	0.34	0.17	0.14	0.08	0	0	0
	N33	0.82*	0.77	0.46	0.33	0.24	0.15	0.01	0	0
Scotland	N7	0.45	0.48*	0.33	0.25	0.15	0.05	0	0	0
	N12	0.45	0.46*	0.38	0.26	0.14	0.08	0	0	0
Chile	N16	0.62*	0.53	0.35	0.23	0.12	0.06	0	0	0
	N18	0.58	0.59*	0.55	0.4	0.22	0.09	0	0	0

** Maximum mean of 5 replicates*

According to Table 3, NaCl concentration affected vegetative growth of aquatic fungi. In the samples from Norway the maximum growth rate for N3 was 0.60 mm/h at 0.5% NaCl, for N5 growth was 0.35 mm/h at 0.5% NaCl, for N6 it was 0.44 mm/h at 0.5% NaCl and for N33 it was 0.82 mm/h at 0% NaCl. Samples N7 and N12 from Scotland were 0.48 and 0.46 mm/h at an optimal saline amount of 0.5%. The maximal growth observed in N16 and N18 from Chile was 0.62 and 0.59 mm/h at 0 and 0.5% NaCl respectively. No water molds in this experiment, excluding N33 from Norway, grew in salinity over 2.5%. N33 grew very slowly (0.01 mm/h) in 3% NaCl but did not grow if the salinity was 3.5% or more.

Mycotic infections associated with Saprolegniacea are widely reported in fresh water fish.[26] They are rarely found in brackish water.[27] However, *Saprolegnia* spores can take advantage of nutrient availability and germinate in 1.5% artificial sea water[28] under laboratory conditions.[29] It is interesting that Singhal *et al*.[13] dipped fish in 3% NaCl for 2 min and reduced *S. parasitica* infection by 96%. Also, Marking[17] and Schreier *et al*[12] recommended the use of 3% NaCl to improve fry hatching. This study showed that 3% NaCl inhibited the growth rate of fungi in 7 out of 8 isolates.

Our findings indicate that research needs to be directed toward understanding the environmental factors that allow aquatic fungi to proliferate and cause disease. Although it may be necessary to use chemicals to control disease, it is also important to find alternative control methods. Reducing chemical usage can reduce the subsequent concern for the environmental damage and can also lower the cost of raising fish.

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