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Chemotactic and chemokinetic activities of *Saprolegnia parasitica* toward different metabolites and fish tissue extracts

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Abstract The chemotactic and chemokinetic activities in zoospores of *Saprolegnia parasitica* NJM 8604 (= H2) were examined using various amino acids, carbohydrates, fatty acids, and fish tissue extracts to estimate one of the important factors for attachment of zoospores to their host. All the tested six amino acids showed strong chemotactic reactions whereas carbohydrates and fatty acids caused moderate or strong chemotactic reactions. The chemokinetic activities against amino acids and carbohydrates were moderate or weak, whereas almost all fatty acids showed negative chemokinetic responses. Almost all tested fish tissue showed moderate chemotactic response and weak or moderate chemokinetic responses. Generally, chemotactic activity was strong in the amino acids, and the strongest activity was observed in alanine. Based on these facts, we considered that zoospores may react against amino acids of the fish body to attach and establish their colonization.

Key words Chemokinesis · Chemotaxis

Introduction

Occurrence of saprolegniasis at fish hatcheries or farms is one of the serious problems in aquaculture. It can damage the fish life cycle from egg to adult (El-Feki 1991; El-Feki et al. 1995; Kitancharoen and Hatai 1998).

The attraction of *Saprolegnia* zoospores toward fish skin is an important subject in saprolegniasis because it is the essential step of fungal infection against the fish. Fish skin is covered by mucus, which acts as a nutritional and chemotactic substrate for *Saprolegnia* zoospores (Wood et al. 1987). The infection usually occurs at the epidermal layer from which a number of metabolites, free amino acids, fatty acids, and carbohydrates can leak.

Spore motility is important to direct the zoospores to the richest food source (Muehlstein et al. 1988). Obenauf and Smith (1985) defined chemotaxis as migration of the zoospores along an increasing concentration gradient of an attracting substance. In addition, they defined chemokinesis as the speed or frequency of migratory response of the zoospores to a special substance (chemoattractant).

The overall aim of this study was to estimate one of the important factors for attachment of zoospores to their host. For this purpose, chemotactic and chemokinetic activities of *Saprolegnia parasitica* zoospores against different metabolites and different parts of the fish body were examined.

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Methods

Production of zoospores

Zoospores of *Saprolegnia parasitica* NJM 8604 (H2) were prepared after the method described by Kitancharoen and Hatai (1996). The strain was originally isolated from coho salmon, *Oncorhynchus kisutch*, and deposited at ATCC as collection number 90213. The concentration of zoospores was adjusted at 4×10^4 spores/ml.

Tested chemoattractants

Various amino acids, aspartic, alanine, leucine, tyrosine, histidine, and arginine, and carbohydrates, arabinose, sorbose, α-glucose, mannose, sucrose, and trehalose were purchased from Wako (Osaka, Japan). Various fatty acids, myristic, palmitic, aracidic, stearic, oleic, and linolenic were ordered from Sigma (Tokyo, Japan). The concentration of each acid or carbohydrate was adjusted according to its

Table 1. Chemotactic and chemokinetic activities of zoospores of *Saprolegnia parasitica* H2 against different amino acids, carbohydrates, and fatty acids at concentrations corresponding to their natural concentration in salmon flank

Chemoattractant(s)	Concentration[a]	Chemotactic ratio (CR)[b]	Chemokinetic index (CI)[c]
Amino acids:			
Aspartic	0.2329	14.500 ± 2.479 (+++)	7.500 ± 0.680 (++)
Alanine	0.4327	21.563 ± 1.217 (+++)	4.166 ± 1.080 (++)
Leucine	0.2288	10.813 ± 1.504 (+++)	2.500 ± 0.170 (+)
Tyrosine	0.1171	12.000 ± 2.307 (+++)	6.666 ± 0.500 (++)
Histidine	1.1562	12.688 ± 1.201 (+++)	7.833 ± 0.810 (++)
Arginine	0.2970	11.250 ± 0.624 (+++)	4.500 ± 0.400 (++)
Carbohydrates:			
Arabinose	0.090	12.908 ± 3.704 (+++)	3.333 ± 0.199 (++)
Sorbose	0.051	14.940 ± 1.762 (+++)	2.722 ± 0.466 (+)
α-Glucose	0.339	16.846 ± 2.879 (+++)	4.055 ± 0.872 (++)
Mannose	0.009	13.315 ± 0.512 (+++)	1.333 ± 0.233 (+)
Sucrose	3.019	6.907 ± 0.982 (++)	3.000 ± 0.000 (+)
Trehalose	0.055	8.376 ± 0.315 (++)	1.611 ± 0.233 (+)
Fatty acids:			
Myristic	0.636	9.841 ± 1.060 (++)	0.500 ± 0.110 (−)
Palmitic	0.522	11.322 ± 2.173 (+++)	0.533 ± 0.098 (−)
Aracidic	0.659	7.473 ± 1.518 (++)	0.333 ± 0.065 (−)
Stearic	0.455	12.468 ± 2.666 (+++)	1.000 ± 0.996 (+)
Oleic	0.603	7.659 ± 1.401 (++)	0.400 ± 0.101 (−)
Linolenic	0.265	7.399 ± 0.608 (++)	0.333 ± 0.073 (−)

[a] μg/mg salmon muscles /ml

[b] $\mathrm{CR} = \dfrac{\text{Mean spore numbers of reacted}}{\text{Mean spore number of control}} \pm \mathrm{SD}$; (−), negative; (+), weak; (++), moderate; (+++), strong

[c] $\mathrm{CI} = \dfrac{\text{Mean spore numbers of reacted}}{\text{Mean spore number of control}} \pm \mathrm{SD}$; (−), negative; (+), weak; (++), moderate; (+++), strong

natural concentration in salmon flank, as indicated in Table 1 (Ogata and Arai 1985).

Fish tissue extracts

Two healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), of average weight 37.0 ± 2 g and average length 15.0 ± 1 cm, were collected from Oshino Trout Hatchery Experimental Station, Yamanashi Prefectural Fisheries Technology Center, Oshino, Yamanashi, Japan.

The fish were anesthetized with AF 100 (Tanabe, Tokyo, Japan), and samples of mucus, blood, skin, peduncle muscle, chin, knuckle, adipose fin, and gills were aseptically collected, immersed in 4 ml saline solution (0.65% NaCl), homogenized, and centrifuged at 1000 rpm for 5 min, respectively. Then, the pellet was discarded and the supernatant used for chemotaxis and chemokinesis assays.

Chemotaxis assay

The modified capillary root model (Halsall 1976), based on the technique of Royle and Hickman (1964), was adapted for assay. The assay chamber was prepared by bending a capillary tube (1.45–1.65 mm external diameter) into a uniform angular U-shape; this was placed on a microscopic slide and covered with a coverslip. Capillary tubes were filled with each tested attractant, and the zoospore suspension was introduced into the chamber. Ten minutes later, the number of zoospores at the tip of the capillary tube was counted. Controls were conducted at the same manner using saline (0.65% NaCl) instead of the attractant solution. The chemotactic ratio was determined by the following formula:

$$\text{Chemotactic ratio}\,(\mathrm{CR}) = \frac{\text{Mean spore numbers of reacted}}{\text{Mean spore numbers of control}}$$

CR values were then compared with the control using Student's t test, and $P < 0.15$ was taken to indicate a positive chemotactic response (Muehlstein et al. 1988). CR values were further graded into positive, weak (+) (CR = 1.1–3.0), moderate (++) (CR = 3.1–10.0), strong (+++) (CR ≥ 10.1), and negative (−) (CR = 0.0–1.0).

As shown in Table 1, all tested amino acids and carbohydrates showed strong chemotactic activities, except sucrose and trehalose (moderate), but all tested fatty acids showed moderate responses with the exception of palmitic acid and stearic acid (strong). In amino acids, the strongest chemotactic reaction was observed in alanine whose value of CR was almost twice those of others.

On the other hand, all the fish tissue extracts showed moderate chemotactic activities except the gills (weak) (Table 2). The most attractive site of the fish was skin, followed by chin, mucus, and adipose fin, then by knuckle and blood, and last the peduncle and gills.

Chemokinesis assay

The assay was carried out according to Gearing and Rimmer (1985) and El-Feki (1994). A modified Boyden chamber was prepared by using two microtitration plates

Table 2. Chemotactic and chemokinetic activities of zoospores of *Saprolegnia parasitica* H2 against different tissue extracts of rainbow trout, *Oncorhynchus mykiss* Walbaum

Tissue sample	Chemotactic ratio (CR)[a]	Chemokinetic index (CI)[b]
Skin	8.466 ± 0.654 (++)	1.500 ± 0.408 (+)
Blood	4.643 ± 0.339 (++)	1.500 ± 0.141 (+)
Mucus	5.000 ± 0.254 (++)	3.100 ± 0.697 (++)
Peduncle	3.443 ± 0.193 (++)	1.200 ± 0.286 (+)
Chin	6.243 ± 0.339 (++)	1.175 ± 0.418 (+)
Knuckle	4.821 ± 0.278 (++)	3.100 ± 0.015 (++)
Adipose fin	5.133 ± 0.298 (++)	5.750 ± 0.816 (++)
Gills	2.421 ± 0.232 (+)	1.200 ± 0.286 (+)

[a] $\mathrm{CR} = \dfrac{\text{Mean spore numbers of reacted}}{\text{Mean spore number of control}} \pm \mathrm{SD}$; (−), negative; (+), weak; (++), moderate; (+++), strong

[b] $\mathrm{CI} = \dfrac{\text{Mean spore numbers of reacted}}{\text{Mean spore number of control}} \pm \mathrm{SD}$; (−), negative; (+), weak; (++), moderate; (+++), strong

with 20-μm nylon Millipore filters (NY20). Then, 0.2 ml zoospores (4×10^4/ml) was introduced into each upper well and the lower test wells were filled with 0.2 ml chemoattractant. Controls were sited in the same manner using saline (0.65% NaCl) instead of the attractant solution. The nylon Millipore filters (triples for both tested and control zoospores) were stained with Giemsa after 10, 20, and 30 min, and the mean numbers of both reacted and control zoospores were calculated. The chemokinetic effect was expressed by a chemokinetic index calculated as follows:

$$\text{Chemokinetic index (CI)} = \frac{\text{Mean spore numbers of reacted}}{\text{Mean spore numbers of control}}$$

The values of CI were also graded into four categories as indicated in CR.

The chemokinetic activities of all used amino acids showed moderate reactions except leucine (weak). The strongest chemokinetic reaction was recognized in histidine and aspartic. All tested carbohydrates exhibited weak to moderate responses, with the exception of arabinose and α-glucose (moderate), but the values of CI were low. All tested fatty acids showed negative or weak responses (see Table 1).

All fish tissue extracts (Table 2) showed weak to moderate chemokinetic responses. Generally, the strongest chemotactic and chemokinetic activities were recognized in amino acids. In fish tissue extracts, the adipose fin was the most attractive tissue.

Saprolegniaceous fungi are known to utilize a wide range of natural organic compounds. The leakage of these compounds from the body of the fish could act as a good chemoattractant to the zoospores of pathogenic *Saprolegnia* species and supply the species with essential nutrients for their growth (Rand and Munden 1993). This observation may explain why injured fish are more susceptible to the zoospores of *Saprolegnia* species than those not injured.

Discussion

The present study revealed that all the selected amino acids had strong chemotactic and chemokinetic activities to the zoospores of *S. parasitica* H2. These results are comparable with those reported by Lehnen and Powell (1989) and Alexopoulos (1962). On the other hand, Kitancharoen and Hatai (1998) reported that the vegetative (mycelial) stages in the three isolates of *S. parasitica* could not assimilate arginine, leucine, and histidine.

The present study revealed that among the six sugars selected, namely arabinose, sorbose, α-glucose, mannose, sucrose, and trehalose, the four former ones had strong chemotactic activities whereas the two latter ones showed moderate responses. In addition, arabinose and α-glucose had strong chemokinetic activity and the rest of the four sugars had weak chemokinetic responses. It can be explained that α-glucose, mannose, and trehalose are good attractants for *S. parasitica* zoospores, because they can be also assimilated by the vegetative stage of *S. parasitica* (Yuasa and Hatai 1996). In contrast, although arabinose, sorbose, and sucrose are also good attractants for *S. parasitica* zoospores, they cannot be assimilated by the vegetative stage of *S. parasitica.* No growth occurred after the addition of these sugars to the growth media (Kitancharoen and Hatai 1998). These results suggest that some constituents (nutrients) are shared in necessity for the different life stages of *Saprolegnia* (e.g., zoosporic or vegetative stages) but some are not.

The tested fatty acids showed the lowest chemotactic and chemokinetic responses in comparison with the tested amino acids and carbohydrates. It seems that fatty acids are not in serious demand by the zoosporic and vegetative stages of *Saprolegnia*. As a result, El-Feki (1987) and Smith et al. (1994) demonstrated that S. *diclina* and *S. ferax* also had relatively slow growth rates on the media with different concentrations of fatty acids.

Some authors reported that fish tissues are very rich in amino acids. For example, fish skin is covered by mucus, whose main constituent is glycoprotein (Pickering 1974; Pickering and Macy 1977). These amino acids in mucus act as good chemoattractants for the secondary zoospores that can germinate later on fish skin (Smith et al. 1994; Willoughby and Pickering 1977). Fish muscle is considered to be a preferable substrate for fungal growth as *Saprolegnia* can grow on mullet muscle extract agar (Fraser et al. 1992) and snakehead muscle extract agar (Lilley and Roberts 1997). In addition, Ogata and Arai (1985) reported that the blood plasma and RBCs of carp, catfish, coho salmon, and rainbow trout were rich in free amino acids (FAA) and intercellular FAA. These FAA are the main nutritional sources for *Saprolegnia.* However, in this investigation the fish tissue extracts showed moderate chemotactic and moderate to weak chemokinetic activities, which were lower than expected.

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