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Materials for *Pythium* flora of Japan. X. Occurrence, identification and seasonality of *Pythium* spp. in three pond waters and mud soils in Osaka

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Seasonal fluctuations of *Pythium* species in three pond waters and mud soils in Sakai, Osaka, were studied for periods of two years and 14 months, respectively. Thirteen species and four groups were isolated from the pond waters while nine species and three groups were obtained from the mud soils. The highest frequency and total number of species were recorded in the cold and moderately warm seasons and the lowest in summer. Most of the species isolated from the pond waters have been previously recorded from aquatic habitats.

Key Words——mud soil; pond water; *Pythium* spp.; seasonality.

Introduction

Some species of *Pythium* are aquatic, parasitizing algae or living saprophytically on insect cadavers or decaying plant debris. Most, if not all, *Pythium* spp. are capable of parasitizing seeds, roots or aerial parts of a wide range of plants. Usually reported as pathogens of germinating seeds or seedlings, they are also frequently recovered from the diseased roots of plants beyond the seedling stage of growth (Robertson, 1980).

As pointed out by Sparrow (1968), the ecology of fresh-water fungi has not attained the degree of prominence reached by that of soil fungi. Increasing concern for the quality and availability of fresh water as a human resource and the possibility of water-borne inocula emphasize the importance of studies of *Pythium* in watery habitats.

Studies have been made on *Pythium* spp. associated with ponds, lakes and rivers (Ito, 1936, 1942, 1944; Waterhouse, 1942; Beverwijk, 1948; Suzuki, 1960a, b, c, d; 1961a, b, c, d, e; 1962a, b; 1963; 1981; Suzuki and Hatakeyama, 1960, 1961; Suzuki and Nimura, 1961a, b; Suzuki et al., 1961; Suzuki and Takashi, 1966; Howard and Johnson, 1969; Johnson, 1971; Plaats-Niterink, 1975; Manoharachary and Rao, 1978; Park, 1980; Pittis and Colhoun, 1984; El-Hissy and Khallil, 1989; Elnaghy et al., 1991). Reports on the occurrence of aquatic pythia in Japan, however, are scarce. Ito (1944) isolated ten species from pond and lake waters in Kyoto. But some of these isolates have been excluded as doubtful species (Plaats-Niterink, 1981). Suzuki (1960a, b, c, d; 1961a, b, c, d, e; 1962a, b; 1963; 1981), Suzuki and Hatakeyama (1960, 1961), Suzuki and Nimura (1961a, b), Suzuki et al. (1961), and Suzuki and Takashi (1966), have studied the aquatic fungi in ponds, lakes and rivers in different parts of Japan. They did not, however, identify *Pythium* to the species level. Takahashi (1952) isolated some pythia from pond waters but not as a continuous ecological study. No comprehensive work has yet been carried out concerning the *Pythium* flora in the Japanese aquatic ecosystem.

This paper describes the isolation, identification, and seasonality of *Pythium* spp. from three pond waters and from mud soils in the bottom of the connecting ditches. Since these ponds are connected together in the same water system, the relationships between the number of species isolated from each pond and the environmental factors are discussed. The specific habitats of the fungi obtained from the water and the soil are also discussed.

Materials and Methods

Survey ponts Collections were made from three ponds (Nakatsu, Tatsumi and Komoda) for irrigation in Sakai, Osaka, Japan. These ponds are connected together in the same water system, and after flooding water overflows from the highest pond (Nakatsu) to the second (Tatsumi) and then to the lowest (Komoda). Most of the water flowing into Nakatsu pond comes from the even higher Takatsu pond, where rainwater collects. Nakatsu pond is surrounded by grasses and shrubs and has a rubber factory nearby spills some of its by-products into the pond. Tatsumi pond is surrounded by reed grasses (*Phragmites communis* Trin.), paddy fields and some houses. People to go there to fish and water-birds sometimes visit. Komoda pond is surrounded by a residential area and a small plastics factory in town, and there is a forest near one side of the pond. Surface water was collected from a depth of 30 cm at a distance of 1 m from the bank. Mud soil was collected from the bottom of the ditches connecting the ponds and from the inlet above the Nakatsu pond.

Isolation The usual method of setting baits was employed. Water samples were brought into the laboratory in 1 L sterile plastic bottles within 2 h. Water samples of 30 ml were poured into sterilized Petri-dishes and baited with 8 autoclaved *Paspalum thunbergii* Kunth leaf disks (6 mm diam). Autoclaved Satsuma mandarin rind (*Citrus unshiu* Marc.) and filter paper disks (6 mm diam) were also used as baits. Each mud sample (approx. 500 g) was mixed thoroughly and portions (5 g) were placed in several Petri-dishes. Ten ml of sterilized deionized water (Willoughby, 1956, with modifications) was added to enable the baits to float on the surface. After about five days of incubation at 25°C and under net house conditions (1-38°C), the baits were removed, washed thoroughly with sterilized distilled water and blotted dry with sterile filter paper. Four baits were then placed on the edge of a Petri-dish containing VP3 medium (Ali-Shtayeh et al., 1986) for isolating *Pythium* spp. selectively. The baits were incubated at 20°C for 3 days or until the appearance of colonies. Water samples were taken every month for two years (Oct. 1992-Sep. 1994), and soils were collected every month for 14 months (July 1993-Aug. 1994).

pH For water samples, pH was determined in the laboratory by means of a pH meter (model PEC-3C; Sensoni X, Inc., Tokyo). In the case of soils, air-dried samples (10 g) were suspended for 60 min in 25 ml 1.0 N KCl solution and their pH was measured.

Temperature Soil and water temperatures were determined at the sampling points.

Total salinity, transparency and COD for the pond waters These were determined in the laboratory. Total salinity (mg s/ml) was determined with a temperature-pH-conductivity meter (PEC-3C; Sensoni X, Inc., Japan) while transparency was determined by use of a transparency meter cylinder (35 mm diam, 100 cm long and 780 ml water capacity, TO-100, Kagaku Kyoeisha, Inc., Osaka) at 25°C. COD_{Mn} was measured as described by the Japanese Standards Association (1992).

Purification and identification The VP3 selective medium for isolating *Pythium* spp. from the water or the mud soil was found to be effective in inhibiting the development of bacteria whilst not affecting zoospore germination of *Pythium* spp. A new technique (Abdelzaher et al., 1994d) was utilized to obtain *Pythium* free of bacteria. Identification was done with the keys of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990) as well as by reference to the original descriptions (Bary, 1876; Buisman, 1927; Campbell and Hendrix, 1967; Coker and Patterson, 1927; Drechsler, 1930, 1941; Ito, T. 1944; Ito, S and Tokunaga, 1935; Matthews, 1928, 1931; Meurs, 1934; Park, 1977; Petersen, 1909; Vaartaja, 1965; Pringsheim, 1858; Sawada and Chen, 1926; Sparrow, 1960; Trow, 1901). The fungi were maintained as described previously (Ichitani and Kang, 1988). *P. fluminum* Park was maintained in basal salt solution plus filter paper at 17°C and renewed every year (Park, 1975; Abdelzaher, 1994).

Results

Isolation from pond water Water samples were taken from the ponds for two years and 1775 isolates of *Pythium* were obtained. *Paspalum thunbergii* leaf baits were very useful for isolating different species of *Pythium*. Isolates of *P. fluminum* were, however, obtained only from filter paper disks, while those of *P. marsipium* Drechsler were obtained only from the mandarin baits.

Isolation from mud soil Soil samples were taken from the bottom of the ditches for 14 months and about 800 isolates of *Pythium* were obtained.

Identification The keys of Plaats-Niterink (1981) and Dick (1990) were principally used for identification. Keys and descriptions by Waterhouse (1967, 1968) and Middleton (1943) were also consulted for comparison or confirmation of identifications.

To induce sporangial formation, the colonized grass blades of *Paspalum thunbergii* were put in Petri-dishes (7 cm diam) containing 10 ml of sterilized deionized water and incubated at different temperatures (5, 10, 15, 20, 25 and 30°C). Sexual reproduction is commonly abundant in water cultures. The following description is, however, based on studies of both water cultures and solid agar media such as corn meal agar (CMA), potato carrot agar (PCA) and V8 agar, each supplemented with 500 μg/ml wheat germ oil (Japan Impex Co. Ltd., Tokyo).

Thirty measurements were made of each structure for each isolate whenever possible. Since structures such as antheridia and sporangia may be formed rapidly and then degenerate, cultures were observed about 12 h after inoculation and then periodically until all possible characters had been observed.

Pythium carolinianum Matthews

Colonies on Bacto-CMA with some aerial mycelia. Main hyphae up to 7.0 μm wide. Zoosporangia proliferating internally by forming a new sporangia inside the old one, often 20-35 μm diam, 25 μm diam on average, with short evacuation tubes. Catenulate hyphal swellings present. Zoospores produced at 20°C, encysted zoospores 10-12 μm diam. No sexual reproduction observed. Cardinal temperatures: minimum 5°C, optimum 27°C, maximum 37°C.

Pythium catenulatum Matthews

Colonies on Bacto-CMA at 25°C with rosette pattern, hyphae thin, up to 4.0 μm wide. Sporangia lobulate, irregularly branched, at 20°C forming vesicles with 2-60 or more zoospores. Encysted zoospores 9.0 μm diam. Hyphal swellings often present in chains of 2-8, 10-22 μm diam, sometimes germinate with 1-3 germ tubes. Sexual stage not observed, but oogonia occurred in 12-month-old cultures. Cardinal temperatures: minimum 10°C, optimum 30-35°C, maximum 40°C. Daily growth rate on Bacto-CMA at 25°C 16 mm.

Pythium coloratum Vaartaja

Colonies on Bacto-CMA submerged, without a special pattern. Main hyphae up to 7.0 μm wide. Sporangia filamentous, sometimes slightly inflated, forming zoospores at 15-20°C; encysted zoospores 10-12 μm diam. Oogonia (sub) globose or pyriform, terminal or occasionally intercalary, some with a papilla (7.5 μm long and 2.5-3.0 μm diam at the base). Oogonia (15-)20-28(-30) μm diam, 23 μm diam on average. Vanished oogonia account for about 50% of the total number. Antheridia diclinous and monoclinous, 1-6 per oogonium, clavate, crook-necked; the antheridial stalk sometimes encircling the oogonium. Oospores aplerotic, (14-)16-28, 19 μm diam on average, with yellowish contents and a 2.0-4.0 μm thick, lilac-colored wall. Cardinal temperatures: minimum 1°C, optimum 31°C, maximum 35°C. Daily growth rate on PCA at 25°C 16 mm, on Bacto-CMA at 25°C 18 mm.

Pythium deliense Meurs

Colonies on Bacto-CMA with aerial mycelium. Main hyphae up to 8.0 μm wide. Zoosporangia inflated. Zoospores formed at 20°C; encysted zoospores 8-12 μm diam. Oogonia smooth, terminal, globose, 20-25 μm diam, 22 μm diam on average; oogonial stalks bending towards the antheridium. Antheridia single, with a straight stalk, about 8×8 μm. Oospores aplerotic 16-19 μm diam, 17 μm diam on average, wall up to 2.0 μm thick. Cardinal temperatures: minimum about 10°C, optimum 30°C, maximum over 40°C. Daily growth rate on Bacto-CMA at 25°C 28 mm.

Pythium diclinum Tokunaga

Colonies on Bacto-CMA submerged. Main hyphae up to 5.5 μm wide. Sporangia filamentous non-inflated, at 20°C forming zoospores. Encysted zoospores 6-7 μm diam. Oogonia globose to subglobose, smooth, mostly terminal or subterminal, occasionally intercalary, (18-)19-24 μm diam, 20 μm diam on average. Antheridia typically diclinous, 1-2 per oogonium, about 12×5 μm. Antheridial stalks not branched. Oospores aplerotic, 7-19 (av. 17) μm diam, oospore wall up to 3.0 μm thick. Cardinal temperatures: minimum below 5°C, optimum 30°C, maximum 37°C. Daily growth rate on PCA at 25°C 18 mm.

Pythium dissotocum Drechsler

Colonies on Bacto-CMA submerged, without special pattern. Main hyphae up to 6.5 μm wide. Sporangia filamentous, sometimes slightly inflated, zoospores formed at 20°C after 16 h. Encysted zoospores 8 μm diam. Oogonia terminal or intercalary, subglobose, 20-25 μm diam, 22 μm diam on average. Antheridia 1-3(-5) per oogonium, originating from the oogonial stalk immediately below the oogonium and sessile, monoclinous or diclinous. Oospores aplerotic or nearly so, 18-21 μm diam, 19 μm diam on average, wall 1.0-3.0 μm thick. Cardinal temperatures: minimum 4°C, optimum 25°C, maximum 35°C. Daily growth rate on Bacto-CMA at 25°C 12 mm.

Pythium fluminum Park var. *fluminum*

Descriptions were given elsewhere (Abdelzaher et al., 1994b).

Pythium irregulare Buisman

Colonies on Bacto-CMA with aerial mycelium. Main hyphae up to 8.0 μm wide. Zoosporangia globose, 9-24 μm diam, seldom produced in some isolates while readily produced in others. Hyphal swellings globose or irregular shaped, terminal or intercalary, up to 25 μm diam. Oogonia globose to irregular-shaped, mostly intercalary, sometimes with projections (up to 5), 14-24 μm diam, 18 μm diam on average. Antheridia 1-2(-3) per oogonium, mostly monoclinous, often club-shaped or falcate, making apical contact with the oogonium. Oospores usually aplerotic 12-19 μm diam, 15 μm diam on average; wall up to 1.5 μm thick. Cardinal temperatures: minimum below 5°C, optimum 28°C, maximum 35°C. Daily growth rate on Bacto-CMA at 25°C 27 mm.

Pythium marsipium Drechsler

Descriptions were given elsewhere (Abdelzaher et al., 1994a).

Pythium middletonii Sparrow

Colonies on Bacto-CMA submerged without special pattern. Main hyphae up to 8.0 μm wide. Zoosporangia globose, ovoid or limoniform, proliferating internally (22-)24-38(-40)×12-32 μm diam, 29×22 μm diam on average. Zoospores formed at 20°C; encysted zoospores 10-14 μm diam. Evacuation tubes usually short, sometimes 18.0 μm long and 8.0 μm wide. Oogonia mostly intercalary, occasionally terminal (18-)20-26(-29) μm diam, 23 μm diam on average. Antheridia (1-3) monoclinous, stalked or often sessile, both diclinous and hypogynous. Oospores aplerotic (15-)18-23(-26) μm diam, 20 μm diam on average, wall 1.5-2.0 μm thick. Cardinal temperatures: minimum 5°C, optimum 30°C, maximum 37°C. Daily growth rate on PCA at 25°C 15 mm.

Pythium monospermum Pringsh.

Colonies on Bacto-CMA showing a radiate pattern. Main hyphae up to 6.0 μm wide. Sporangia strictly filamentous, non-inflated. Zoospores formed at 20°C after 18 h. Encysted zoospores 8-10 μm diam. Oogonia terminal or intercalary, globose, (12-)14-16(-18) μm diam, 15 μm diam on average. Antheridia 1-2(-4) per oogonium, monoclinous and diclinous, stalks originating at various distances from the oogonium; antheridial cells making rather broad apical contact with the oogonium. Oospores plerotic, wall 1.0-2.0 μm thick. Cardinal temperatures: minimum below 5°C, optimum 25°C, maximum 35°C. Daily growth rate on PCA at 25°C 5 mm.

Pythium myriotylum Drechsler

Colonies on Bacto-CMA with little aerial mycelium. Main hyphae up to 10.0 μm wide; appressoria clavate, sickle-shaped, or banana-shaped, often up to 65× 12 μm, usually formed in clusters. Sporangia filamentous with inflated lobulate or digitate elements of variable length and mostly 7-20 μm wide. Zoospores formed at 20°C after 20 h. Discharge tubes up to 140 μm long, 2-4 μm wide. Encysted zoospores 10-12 μm diam. Oogonia (sub) globose, terminal or intercalary 25-32 μm diam, 30 μm diam on average. Antheridia 3-6(-10) per oogonium, stalks branched sometimes enveloping the oogonium, diclinous occasionally monoclinous. Antheridial cells crook-necked, making apical contact with the oogonium. Oospores aplerotic 20-28 μm diam, 25 μm diam on average; wall up to 2.0 μm thick. Cardinal temperatures: minimum 5°C, optimum 35°C, maximum 40°C. Daily growth rate on PCA at 25°C 25 mm.

Pythium papillatum Matthews

Colonies on Bacto-CMA without special pattern. Main hyphae up to 10 μm wide. Sporangia filamentous, non-inflated. Zoospores formed at 20°C after 18 h. Encysted zoospores 10-12 μm diam. Oogonia terminal or intercalary, often catenulate, globose to oval, smooth or with 1 or 3 papillae, 16-27 μm diam. Antheridia lacking. Oospores plerotic. Daily growth rate on PCA at 25°C 18 mm.

Pythium pleroticum T. Ito

Colonies on Bacto-CMA submerged without special pattern. Main hyphae up to 5.0 μm wide. Zoosporangia and zoospores not formed. Hyphal swellings abundant, globose to pyriform, 18-42 μm diam, germinate by one or two germ tubes. Oogonia terminal, sometimes intercalary, 14-19 μm diam. Antheridia monoclinous or diclinous 1-4. Oospores plerotic, wall 1.0-1.5 μm thick. Cardinal temperatures: minimum 5°C, optimum 25-30°C, maximum 35°C. Daily growth rate on PCA at 25°C 13 mm.

Pythium spinosum Sawada

Colonies on Bacto-CMA with cottony aerial mycelium. Main hyphae up to 7.0 μm wide, septate when old. Zoospores not formed. Hyphal swellings usually limoniform, rarely globose, up to 28 μm diam, sometimes with projections. Oogonia globose, rarely fusiform, usually intercalary, 23-29 μm diam, 25 μm diam on average, with a varying number of blunt, digitate projections, 2.0-13 μm long and 2.0-4.5 μm wide at the base. Antheridia 1(-2) per oogonium, monoclinous, sometimes diclinous. Oospores plerotic, occasionally aplerotic, 20-26 μm diam, 22 μm diam on average; wall up to 2.0 μm thick. Cardinal temperatures: minimum below 5°C, optimum 30°C, maximum 35°C. Daily growth rate on Bacto-CMA at 25°C 28 mm.

Pythium sylvaticum Campbell et Hendrix

Colonies on Bacto-CMA with cottony aerial mycelium. Main hyphae up to 11.0 μm wide, septate when old. Zoospores not formed. Hyphal swellings frequently, globose or limoniform, intercalary or terminal, up to 35 μm diam. Oogonia sometimes produced in single cultures (homothallic isolates), but mostly heterothallic and produced in the line of contact between two compatible isolates. Oogonia smooth, terminal or intercalary, 16-24 μm diam, 20 μm diam on average. Antheridia 2-5 per oogonium, diclinous; antheridial stalks branched, often encircle the oogonium, soon vanishing after fertilization; antheridial cells inflated. Oospores aplerotic, 14-19 μm diam, 17 μm on average; wall 1.0-2.0 μm thick. Cardinal temperatures: minimum below 5°C, optimum 28°C, maximum 37°C. Daily growth rate on Bacto-CMA at 25°C 27 mm.

Pythium torulosum Coker et Patterson

Colonies on Bacto-CMA submerged. Zoosporangia toruloid. Zoospores formed at 20°C; encysted zoospores 8.0 μm diam. Oogonia terminal or intercalary, smooth, 12-22 μm diam, 15 μm diam on averagre. Antheridia 1-2(-3) per oogonium, monoclinous, sometimes diclinous. Oospores plerotic, 11-21 μm diam, 14 μm diam on average; wall 1.5 μm thick. Cardinal temperatures: minimum below 5°C, optimum 28°C, maximum 35°C. Daily growth rate on PCA at 25°C 14 mm.

Pythium ultimum Trow var. *ultimum*

Colonies on Bacto-CMA with aerial mycelium. Main hyphae up to 11.0 μm wide, septate when old. Appressoria sickle-shaped. Zoospores not formed even at 5°C. Hyphal swellings globose, limoniform, terminal, up to 30 μm diam. Oogonia usually terminal, sometimes intercalary, globose, smooth, occasionally with 1 projection, 21-28 μm diam, 25 μm diam on average. Antheridia 1(-2) per oogonium, sac-like, sessile, mostly monoclinous, sometimes hypogynous. Oospores aplerotic, globose, 18-24 μm diam, 21 μm diam on average; wall up to 2.0 μm thick. Cardinal temperatures: minimum 5°C, optimum 28°C, maximum 34°C. Daily growth rate on Bacto-CMA at 25°C 25 mm.

Pythium undulatum H. E. Petersen

Colonies on Bacto-CMA submerged with a radiate pattern. Main hyphae up to 7.0 μm wide. Sporangia proliferating internally by one or more sporangiophores, often long 40-80×20-40 μm diam, 60×30 μm on average, mostly forming short evacuation tubes. Zoospores produced at 20°C; encysted zoospores 10-14 μm diam. No sexual reproduction observed. Cardinal temperatures: minimum 5°C, optimum 25°C, maximum 37°C. Daily growth rate on PCA at 25°C 18 mm.

Pythium vexans de Bary

Colonies on Bacto-CMA with cottony aerial mycelium. Main hyphae up to 6.0 μm wide. Sporangia globose, ovoid or pyriform, occasionally proliferating, intercalary or terminal, 18-25 μm long, 22 μm long on average and 14-23 μm broad, 17 μm broad on average. Oogonia mostly terminal or short side branches, sometimes intercalary or terminal, globose, 15-24 μm diam, 21 μm diam on average. Antheridia 1(-2) per oogonium, monoclinous, rarely diclinous, arising at some distance below the oogonium or from the parent hyphae, antheridial cells large, typically bell-shaped. Oospores aplerotic, 13-20 μm diam, 17 μm diam on average; wall up to 1.5 μm thick. Cardinal temperatures: minimum 5-7°C, optimum 31°C, maximum 37°C. Daily growth rate on Bacto-CMA at 25°C 17 mm.

Pythium 'group F'

This group of *Pythium* is heterogeneous and contains members differing in their cultural characteristics, but all of them have the following characters: zoosporangia filamentous non-inflated, evacuation tubes up to 160 μm long. Zoospores produced at 4-35°C with optimum at 20°C.

Pythium 'group HS'

Colonies on Bacto-CMA with aerial mycelium. Main hyphae up to 10.0 μm wide. No zoospore formation. Hyphal swellings abundant with 19-27 μm diam, 22 μm diam on average. Oospores are not formed, but some spherical structures enmassed by numerous swollen

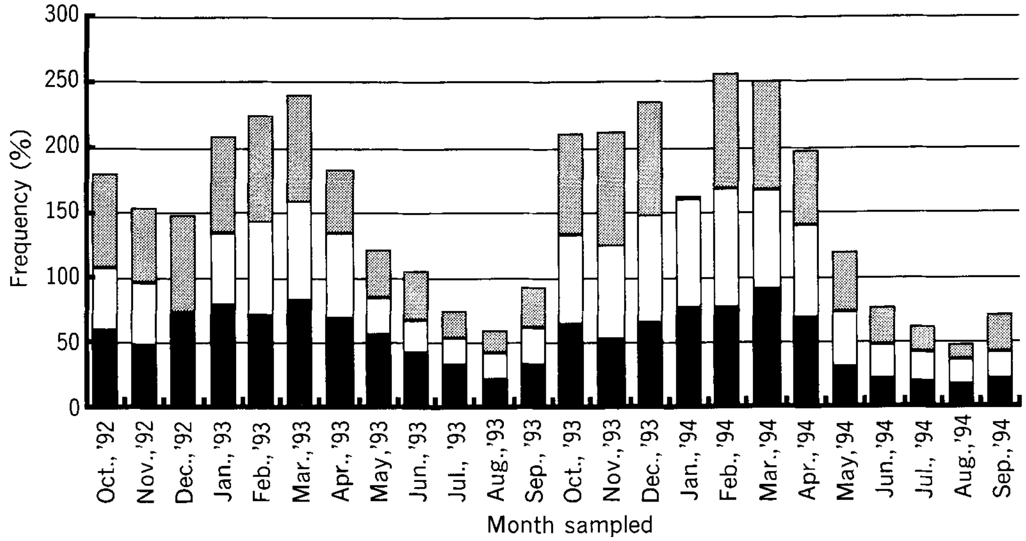

Fig. 1. Frequency percentage of pythia isolated from three pond waters (Nakatsu ■, Tatsumi □ and Komoda ▒) during two years of study. Frequency %=(Number of samples of occurrence on VP3 selective medium/Total number of baits used)×100. Sampling from Tatsumi pond in Dec. 1992 was not possible because of no water, while Komoda pond in Jan. 94 contained dirty water derived from the adjacent residential area.

hyphae which appeared to be morphologically similar to antheridia. None of these structures appear to give rise to mature oospores. Cardinal temperatures: minimum 5°C, optimum 30°C, maximum 37°C. Daily growth rate on Bacto-CMA at 25°C 27 mm.

Pythium 'group P'

Colonies on Bacto-CMA with aerial mycelia. Main hyphae up to 7.0 μm wide. Zoosporangia proliferating internally by forming a new sporangium inside the old one, often 20-35 μm diam, 27 μm diam on average, mostly forming short evacuation tubes, sometimes germinate by one or two germ tubes. Zoospores produced at 20°C, after 18 h; encysted zoospores 10-14 μm diam. No sexual reproduction observed. Cardinal temperatures: mini-

Table 1. Seasonal variation in the occurrence of different *Pythium* spp. in Nakatsu pond water (Oct. 1992-Sep. 1993).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	0	0	0	0	0	0	0	0	0	0	0	0
P. catenulatum	0	0	2	0	0	0	0	0	0	0	0	0
P. coloratum	0	0	4	6	5	0	0	0	0	0	0	0
P. diclinum	0	0	7	8	7	1	0	0	0	0	0	0
P. dissotocum	0	0	0	0	0	4	2	0	0	0	0	0
P. fluminum	0	0	0	0	0	0	0	0	0	0	0	0
P. marsipium	0	0	0	0	0	0	0	0	0	0	0	0
P. middletonii	0	0	0	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	0	0	1	3	3	5	2	0	0	0
P. myriotylum	0	0	0	0	0	0	0	0	0	0	0	0
P. papillatum	0	0	0	0	0	0	0	0	0	0	0	0
P. pleroticum	0	0	0	0	0	0	0	0	0	0	0	0
P. undulatum	0	0	0	0	0	3	2	0	0	0	0	0
Pythium 'group F'	20	23	22	23	21	24	24	20	18	16	10	16
Pythium 'group HS'	0	0	0	1	0	1	0	0	0	0	0	0
Pythium 'group P'	9	0	0	0	0	7	5	2	0	0	0	0
Pythium 'group T'	0	0	0	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	2	1	4	4	4	7	5	3	2	1	1	1

[a] Each entry represents the total number of colonies obtained on VP3 selective medium, using 48 baits for each sample.

Table 2. Seasonal variation in the occurrence of different *Pythium* spp. in Nakatsu pond water (Oct. 1993–Sep. 1994).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	0	0	0	0	0	0	0	0	0	0	0	0
P. catenulatum	0	1	1	0	0	0	0	0	0	0	0	0
P. coloratum	0	3	5	9	8	0	0	0	0	0	0	0
P. diclinum	0	0	5	8	9	2	0	0	0	0	0	0
P. dissotocum	0	0	0	0	2	4	2	0	0	0	0	0
P. fluminum	0	0	0	0	0	0	0	0	0	0	0	0
P. marsipium	0	0	0	0	0	0	0	0	0	0	0	0
P. middletonii	0	0	0	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	0	0	1	5	3	1	2	0	0	0
P. myriotylum	0	0	0	0	0	0	0	0	0	0	0	0
P. papillatum	0	0	0	0	0	0	0	0	0	0	0	0
P. pleroticum	0	0	0	0	0	0	0	0	0	0	0	0
P. undulatum	0	0	0	0	1	6	3	0	0	0	0	0
Pythium 'group F'	24	21	20	20	16	22	19	11	8	9	8	10
Pythium 'group HS'	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group P'	6	0	0	0	0	4	6	3	0	0	0	0
Pythium 'group T'	0	0	0	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	2	3	4	3	6	6	5	3	2	1	1	1

[a] As in Table 1.

Table 3. Seasonal variation in the occurrence of different *Pythium* spp. in Tatsumi pond water (Oct. 1992–Sep. 1993).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	4	2	N.A.[b]	0	0	0	0	0	0	0	0	0
P. catenulatum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. coloratum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. diclinum	0	0	N.A.	10	6	0	0	0	0	0	0	0
P. dissotocum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. fluminum[c]	N.D.[d]	N.D.	N.A.	N.D.	16	13	8	2	0	0	0	2
P. marsipium	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. middletonii	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. myriotylum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. papillatum	0	0	N.A.	0	0	0	0	0	0	0	0	0
P. pleroticum	4	7	N.A.	0	0	0	0	0	0	0	0	0
P. undulatum	0	0	N.A.	0	0	3	4	0	0	0	0	0
Pythium 'group F'	15	14	N.A.	17	13	21	16	12	9	10	10	12
Pythium 'group HS'	0	0	N.A.	0	0	0	0	0	0	0	0	0
Pythium 'group P'	0	0	N.A.	0	0	0	3	0	3	0	0	0
Pythium 'group T'	0	0	N.A.	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	3	3	N.A.	2	3	3	4	2	2	1	1	2

[a] As in Table 1.
[b] N.A. = not assayed because of no water.
[c] *P. fluminum* was only isolated by use of filter paper disks as baits.
[d] N.D. = not determined.

Table 4. Seasonal variation in the occurrence of different *Pythium* spp. in Tatsumi pond water (Oct. 1993–Sep. 1994).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	0	0	0	0	0	0	0	0	0	0	0	0
P. catenulatum	0	1	1	0	0	0	0	0	0	0	0	0
P. coloratum	0	0	4	4	5	0	0	0	0	0	0	0
P. diclinum	0	2	6	5	6	3	0	0	0	0	0	0
P. dissotocum	0	0	0	0	2	1	2	0	0	0	0	0
P. fluminum[b]	9	12	14	16	16	13	10	0	0	0	0	0
P. marsipium	0	0	0	0	0	0	0	0	0	0	0	0
P. middletonii	0	0	0	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	0	0	1	1	3	1	2	0	0	0
P. myriotylum	0	0	0	0	0	0	0	0	0	0	0	0
P. papillatum	0	0	0	0	0	0	0	0	0	0	0	0
P. pleroticum	3	3	2	2	1	0	0	0	0	0	0	0
P. undulatum	0	0	0	0	0	2	0	1	0	0	0	0
Pythium 'group F'	18	17	13	14	15	17	18	16	11	10	9	10
Pythium 'group HS'	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group P'	3	0	0	0	0	0	1	2	0	1	0	0
Pythium 'group T'	0	0	0	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	4	5	6	5	7	6	5	4	2	2	1	1

[a] As in Table 1.
[b] As in c) of Table 3.

Table 5. Seasonal variation in the occurrence of different *Pythium* spp. in Komoda pond water (Oct. 1992–Sep. 1993).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	0	0	0	0	0	0	0	0	0	0	0	0
P. catenulatum	0	4	3	2	2	3	3	2	1	1	1	0
P. coloratum	0	0	6	7	5	0	0	0	0	0	0	0
P. diclinum	0	0	7	6	9	0	0	0	0	0	0	0
P. dissotocum	0	0	0	0	0	3	0	0	0	0	0	0
P. fluminum[b]	N.D.[c]	N.D.	N.D.	N.D.	8	8	3	0	0	0	0	0
P. marsipium	3	0	0	0	0	0	0	0	0	0	0	0
P. middletonii	6	0	0	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	0	0	2	3	2	1	1	0	0	0
P. myriotylum	0	0	0	0	0	0	0	0	0	0	0	6
P. papillatum	0	2	0	0	0	0	0	0	0	0	0	0
P. pleroticum	0	0	0	0	0	0	0	0	0	0	0	0
P. undulatum	0	0	0	0	0	2	1	2	1	0	0	0
Pythium 'group F'	21	20	19	20	16	21	15	14	12	9	8	15
Pythium 'group HS'	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group P'	3	0	0	0	0	0	0	0	3	2	1	0
Pythium 'group T'	0	2	1	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	4	4	5	4	6	6	5	4	5	3	3	2

[a] As in Table 1.
[b] As in c) of Table 3.
[c] N.D. = not determined.

Table 6. Seasonal variation in the occurrence of different *Pythium* spp. in Komoda pond water (Oct. 1993-Sep. 1994).

Pythium species	Month sampled and total number of colonies obtained[a]											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
P. carolinianum	0	0	0	0	0	0	0	0	0	0	0	0
P. catenulatum	2	5	4	0	2	4	2	3	1	1	0	0
P. coloratum	0	0	2	0	2	0	0	0	0	0	0	0
P. diclinum	0	1	4	0	5	1	0	0	0	0	0	0
P. dissotocum	0	0	0	0	2	2	0	0	0	0	0	0
P. fluminum[c]	3	6	8	0	10	6	3	0	0	0	0	0
P. marsipium	0	0	0	0	0	0	0	0	0	0	0	0
P. middletonii	3	0	0	0	0	0	0	0	0	0	0	0
P. monospermum	0	0	0	0	1	2	2	2	1	0	0	0
P. myriotylum	3	0	0	0	0	0	0	0	0	0	0	0
P. papillatum	0	0	0	0	0	0	0	0	0	0	0	0
P. pleroticum	3	3	2	0	1	0	0	0	0	0	0	0
P. undulatum	0	0	0	0	0	1	0	0	0	0	0	0
Pythium 'group F'	22	23	22	1	19	24	20	15	12	8	6	13
Pythium 'group HS'	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group P'	2	1	0	0	0	0	1	2	0	0	0	1
Pythium 'group T'	0	3	0	0	0	0	0	0	0	0	0	0
Number of species or groups isolated	7	7	6	1	8	7	5	4	3	2	1	1

a) As in Table 1.
b) The water was cut during this month because of construction. There was only dirty water derived from the adjacent residential area.
c) As in c) of Table 3.

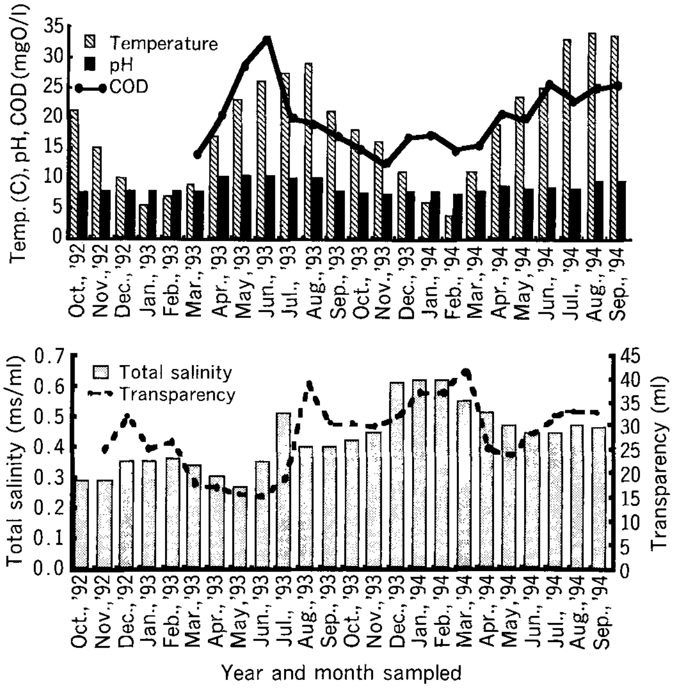

Fig. 2. Monthly changes of water characteristics of Nakatsu pond during 24 months of study.

mum 5°C, optimum 27°C, maximum 37°C.

Pythium 'group T'

Colonies on Bacto-CMA with a radiate pattern without aerial mycelia. Main hyphae up to 6.0 μm wide. Zoosporangia filamentous, inflated. Zoospores formed at 20°C; encysted zoospores 8-10 μm diam. No sexual reproduction observed. Cardinal temperatures: minimum below 5°C, optimum 25°C, maximum 35°C. Daily growth rate on PCA at 25°C 8 mm.

***Pythium* spp. in pond waters** The percentage of occur-

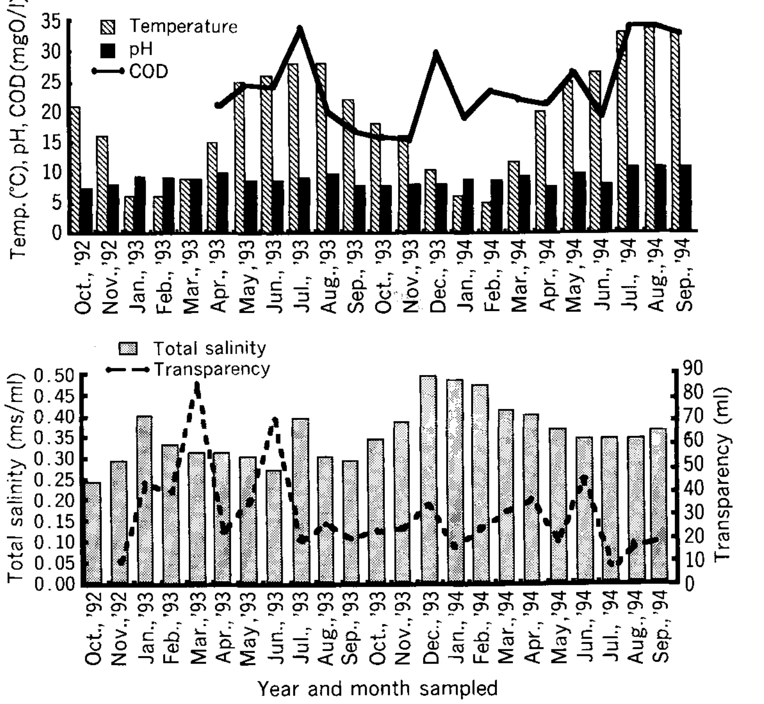

Fig. 3. Monthly changes of water characteristics of Tatsumi pond during 24 months of study. There was no sampling in Dec. 1992 because of drying of the pond.

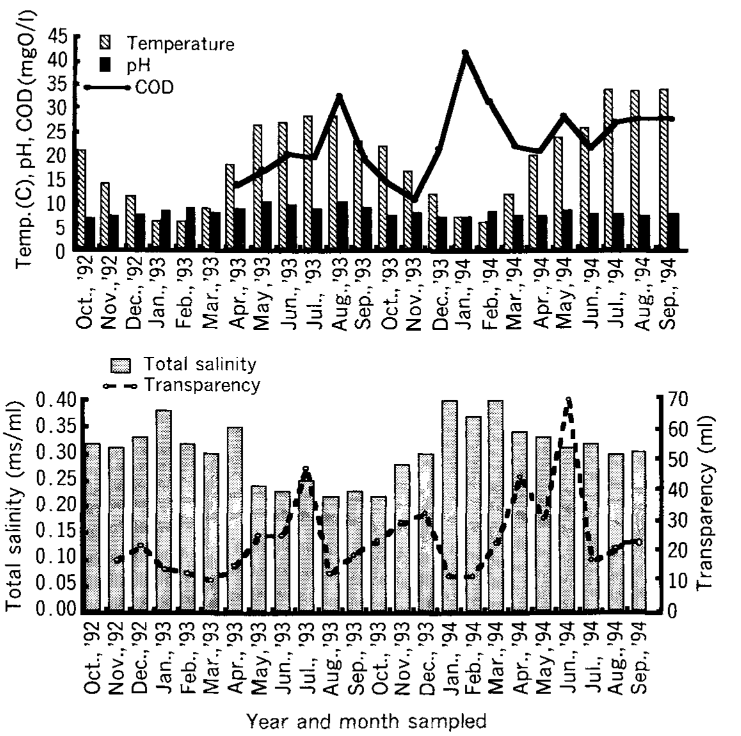

Fig. 4. Monthly changes of water characteristics of Komoda pond during 24 months of study.

rence of the pythia was highest from winter to early spring (February-March), decreased from late spring to the summer months (April to September), and was at the minimum in August (Fig. 1). The occurrence of 13 species and 4 groups of *Pythium* in 72 collections and their seasonality are given in Tables 1-6. The most commonly occurring *Pythium* was *Pythium* 'group F'. Some species such as *P. coloratum* and *P. diclinum* were isolated only in winter. *P. fluminum* was isolated from autumn to the next spring, while *P. marsipium* and *P. middletonii*

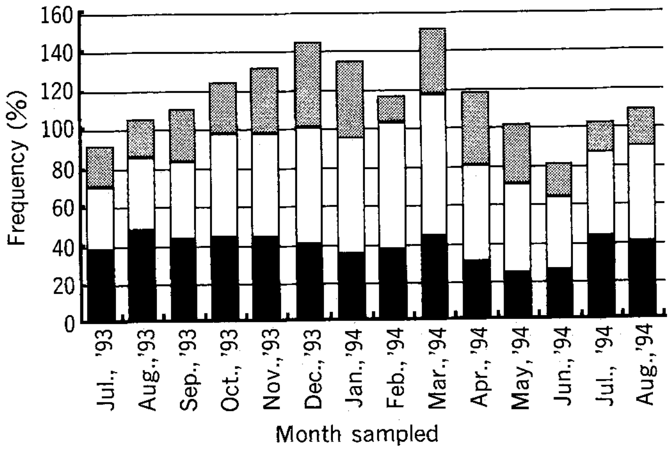

Fig. 5. Frequency percentage of pythia isolated from mud soils beside three ponds (Nakatsu ■, Tatsumi □ and Komoda ▒) during 14 months of study. Frequency % = (Number of samples of occurrence on VP3 selective medium/Total number of baits used) × 100.

were obtained only in the early autumn. *Pythium* 'group P' was isolated in spring and summer. *Pythium* 'group HS' appeared only two times during the collection. Some species (*P. catenulatum, P. coloratum, P. diclinum, P. dissotocum, P. monospermum, P. undulatum, Pythium* 'group F' and *Pythium* 'group P') were common to the three ponds, but others (*P. marsipium, P. middletonii* and *P. myriotylum*) were recorded only from Komoda pond. *P. fluminum* was isolated from both Tatsumi and Komoda ponds.

Environmental factors concerning the pond waters Environmental factors studied here are illustrated in Figs. 2–4. Temperature and pH values showed seasonal variations. They reached the maximum in summer and the minimum in winter. Although COD, transparency and total salinity did not show a clear seasonality, total salinity decreased while COD increased in summer. The dirty water collected from Komoda pond in January 1994 supported only one isolate of *Pythium* 'group F' (See Table 6).

***Pythium* spp. in mud soils** The collections near the three ponds showed a maximum fungal frequency in winter and spring with the minimum in summer, except for a low frequency in February near Nakatsu pond (Fig. 5). The occurrence of 9 species and 3 groups of *Pythium* in 42 collections and their seasonality are given in Tables 7–9. The most commonly occurring fungi were *Pythium* 'group F', *Pythium* 'group HS' and *Pythium* 'group P'. *P. deliense* was isolated only in summer, while *P. vexans* and *Pythium* 'group P' were isolated in summer and au-

Table 7. Seasonal variation in the occurrence of different pythia in the mud soil beside Nakatsu pond.

Pythium species	Month sampled and total number of colonies obtained[a]													
	Jul.'93	Aug.'93	Sep.'93	Oct.'93	Nov.'93	Dec.'93	Jan.'94	Feb.'94	Mar.'94	Apr.'94	May '94	Jun.'94	Jul.'94	Aug.'94
P. coloratum	0	0	0	0	0	2	3	3	0	0	0	0	0	0
P. deliense	3	4	2	0	0	0	0	0	0	0	0	0	3	5
P. dissotocum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. irregulare	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. spinosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. sylvaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. torulosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. ultimum var. *ultimum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. vexans	6	5	5	4	4	0	0	0	0	0	0	0	4	5
Pythium 'group F'	3	6	6	5	8	7	4	3	8	8	6	5	5	2
Pythium 'group HS'	2	3	5	8	9	10	10	12	13	7	5	4	4	3
Pythium 'group P'	4	5	3	3	0	0	0	0	0	0	1	4	5	4
Number of species or groups isolated.	5	5	5	4	3	3	3	3	2	2	3	3	5	5

[a] As in Table 1.

Table 8. Seasonal variation in the occurrence of different pythia in the mud soil beside Tatsumi pond.

Pythium species	Month sampled and total number of colonies obtained[a]													
	Jul.'93	Aug.'93	Sep.'93	Oct.'93	Nov.'93	Dec.'93	Jan.'94	Feb.'94	Mar.'94	Apr.'94	May '94	Jun.'94	Jul.'94	Aug.'94
P. coloratum	0	0	0	0	0	0	0	3	0	0	0	0	0	0
P. deliense	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. dissotocum	0	2	0	0	0	0	0	0	0	0	0	0	0	0
P. irregulare	0	0	0	0	0	0	0	0	3	4	2	1	0	0
P. spinosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. sylvaticum	4	4	3	4	3	4	4	3	4	0	0	0	4	5
P. torulosum	0	0	2	4	0	0	0	0	0	0	0	0	0	0
P. ultimum var. *ultimum*	3	3	4	7	9	9	8	8	7	4	4	4	3	3
P. vexans	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group F'	4	4	4	5	6	8	7	6	8	6	5	4	3	3
Pythium 'group HS'	3	3	4	4	8	8	10	11	13	8	8	4	3	4
Pythium 'group P'	2	2	2	1	0	0	0	0	0	2	3	5	8	9
Number of species or groups isolated	5	6	6	6	4	4	4	5	5	5	5	5	5	5

[a] As in Table 1.

Table 9. Seasonal variation in the occurrence of different pythia in the mud soil beside Komoda pond.

Pythium species	Month sampled and total number of colonies obtained[a]													
	Jul.'93	Aug.'93	Sep.'93	Oct.'93	Nov.'93	Dec.'93	Jan.'94	Feb.'94	Mar.'94	Apr.'94	May '94	Jun.'94	Jul.'94	Aug.'94
P. coloratum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. deliense	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. dissotocum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. irregulare	0	0	0	0	0	0	0	0	2	2	1	0	0	0
P. spinosum	4	4	5	5	4	5	6	4	7	4	4	2	3	2
P. sylvaticum	3	3	4	3	2	3	2	0	2	3	4	2	2	3
P. torulosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. ultimum var. *ultimum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. vexans	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pythium 'group F'	3	2	2	3	7	8	6	2	6	6	4	3	2	3
Pythium 'group HS'	0	0	2	2	4	5	5	0	0	3	2	1	0	1
Pythium 'group P'	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of species or groups isolated.	3	3	4	4	4	4	4	2	4	5	5	4	3	4

[a] As in Table 1.

Table 10. Monthly values of pH and temperature of mud soil samples during 14 months (Jul. 1993–Aug. 1994).

Pond	Environmental factor	Month sampled													
		Jul.'93	Aug.'93	Sep.'93	Oct.'93	Nov.'93	Dec.'93	Jan.'94	Feb.'94	Mar.'94	Apr.'94	May '94	Jun.'94	Jul.'94	Aug.'94
Nakatsu	pH	7.1	7.8	6.9	7.2	7.4	7.5	7.5	7.7	7.7	6.6	7.3	7.7	7.7	7.9
	Soil temp. (°C)	26.5	31.0	27.0	19.0	17.0	9.0	5.0	2.5	10.0	19.0	25.0	26.0	35.0	34.0
Tatsumi	pH	6.8	7.4	6.2	7.1	7.3	6.9	6.7	7.5	7.2	6.4	7.1	7.5	7.5	7.5
	Soil temp. (°C)	28.0	30.5	27.5	21.0	18.0	10.0	5.0	4.0	11.0	21.0	26.0	28.0	36.0	34.0
Komoda	pH	4.9	5.2	5.1	5.0	5.6	5.5	5.4	5.1	5.5	5.7	5.6	5.6	5.4	5.5
	Soil temp. (°C)	30.0	31.5	27.5	19.0	18.0	10.0	6.0	5.0	14.0	19.5	25.0	27.0	35.0	34.0

tumn. *P. coloratum* was obtained only in winter. Some *Pythium* species such as *Pythium* 'group F' and *Pythium* 'group HS' were common to the three ponds, but others (*P. coloratum, P. deliense* and *P. vexans*) were restricted to Nakatsu pond. *P. spinosum*, and *P. torulosum* and *P. ultimum* were restricted to Komada pond and Tatsumi pond, respectively. *P. sylvaticum* was widely found in Tatsumi and Komoda ponds.

Environmental factors concerning the mud soils Temperature and pH values measured are depicted in Table 10. Soil temperature showed seasonal variations, while soil pH did not.

Discussion

Spring was the most favorable season for growth, and a drastic seasonal variation was shown in the great majority of Saprolegniaceae (Coker, 1923). Winter is the most suitable season for growth of aquatic fungi (Dayal and Tandon 1963; Khulbe and Bhargava 1977; Manoharachary and Ramarao, 1981; Misra, 1982; Gupta and Mehrotra, 1989), while two maxima were found, one in early spring and the other in late autumn (Perrot, 1960; Dick and Newby, 1961). Roberts (1963) recorded low numbers of aquatic fungi during the warm season, while fungal species began to build up in autumn and reached a maximum in the spring. Recently, Elnaghy et al. (1991) demonstrated that the occurrence of aquatic zoosporic fungi was inversely correlated with temperature. Hunter (1975), Roberts (1963) and Manoharachary and Ramarao (1981) have designated the aquatic mycoflora as winter, summer, monsoon and constant species based on their monthly frequency of isolation. The fungi isolated here can be classified as low temperature-favoring species (4-25°C), high temperature-favoring species (>25°C) and constant species.

Our findings provide clear evidence that the occurrence of *Pythium* species in the pond water studied was affected by the water temperature and had a close relation to the pond type. The number of species isolated from each pond fluctuated seasonally. The maximum was observed in winter, autumn and spring, and the minimum in summer. As shown in Figs. 2-4, the highest temperatures and pH values were recorded in June, July and August. Consequently, the frequency of the pythia obtained is inversely proportional to temperature. *P. catenulatum, P. monospermum, P. myriotylum, P. undulatum, Pythium* 'group F' and *Pythium* 'group P' were the only species isolated in summer. *P. marsipium* and *P. middletonii* were isolated only from Komoda pond in autumn (water temperature, 21°C), and were not obtained from the other ponds during the study. *P. coloratum, P. diclinum, P. dissotocum, P. fluminum, P. papillatum, P. pleroticum* and *Pythium* 'group T' were isolated in winter or early spring. Therefore, isolation of *Pythium* species from pond water was strongly influenced by the water temperature. Because of the stagnation of water in the pond, the temperature increases in summer, which induces algal multiplication. The algae absorb carbon dioxide from the water, which increases its pH value. The high pH values in summer together with the high temperature retard zoospore formation by some *Pythium* species (Abdelzaher et al., 1994c).

The origin of *Pythium* is a matter of discussion (Butler, 1907; Park, 1980). Most of the species isolated from pond waters in the present study have been recorded from aquatic habitats. *Pythium* 'group F' with filamentous non-inflated zoosporangia, which are very common in open water, have been recorded several times from water (Plaats-Niternik, 1975, 1981; Pittis and Colhoun, 1984). *P. marsipium* was first isolated in the USA from a water plant in water (Drechsler, 1941). T. Ito (1942) isolated it from water of a nursery bed of rice and from a pond in Kyoto. This species has also been reported from water of a sewage purification plant in Ukraine (Meshcheryakova, 1970; Meshcheryakova and Logvinenko, 1970). Recently, this fungus has been isolated from Komoda pond in Osaka (see Abdelzaher et al., 1994a). *P. fluminum* var. *fluminum* was first obtained from a river in Ireland in 1972 (Park, 1975). About 21 years later, Abdelzaher et al. (1994b) have isolated this taxon from Tatsumi pond in Osaka.

Some species of *Pythium* have been recorded only from water habitats, others from both land and water. A specific group of pythia such as *P. oligandrum* Drechsler is strictly terrestrial forms and has only been obtained from land (Plaats-Niterink, 1981; Dick, 1990). The water environment seems to be the origin of *Pythium* species (Butler, 1907). Therefore, studying *Pythium* flora in their natural origin should reveal more about its occurrence and distribution. Many species, however, have migrated toward the land and adapted themselves to behave as terrestrial fungi. They then lost the ability to produce zoospores and the asexual propagules become able to germinate directly by germ tubes. Botha (1993) has stimulated zoospore production of a land inhabitant, *P. spinosum*, and this might explain the aquatic origin of *Pythium* spp. *P. spinosum* has been obtained from irrigation ponds in Georgia (Shokes and McCarter, 1976, 1977). On the other hand, some species of *Pythium* cannot tolerate at shortage of water and cannot survive on the land, and this may explain the occurrence of such species only in the water ecosystem (Plaats-Niterink, 1981).

Generally, species with filamentous zoosporangia represent the most primitive forms of the Pythiaceae and they are very common in the water habitats (Butler, 1907). Species with proliferating zoosporangia are dominant in the aquatic habitats and the proliferation phase corresponds to the aquatic atmosphere. In general, the aquatic forms are characterized by a great spore production in comparison to the size of the thallus (Butler, 1907). Since *Pythium* 'group HS', which was detected twice in only one pond water, does not produced zoospores, it might be a temporary inhabitant of the aquatic habitat. On the other hand, *P. coloratum*, the only species not previously recorded from water, has filamentous zoosporangia indicating its aquatic origin.

The most recent key of the genus *Pythium* by Dick (1990) includes filamentous zoosporangial species such as *P. adhaerens* Sparrow, *P. angustatum* Sparrow, and *P.*

apleroticum Tokunaga. All of them have been obtained from water, except *P. capillosum* Paul, *P. capillosum* Paul var. *helicoides* Paul, *P. coloratum, P. destruens* Shipton, *P. perniciosum* Serbinov and *P. sulcatum* Pratt & Mitchell (Plaats-Niterink, 1981; Dick, 1990). This indicates that the *Pythium* species with filamentous zoosporangia are very common in water and represent the most primitive forms of *Pythium*.

Pythium spinosum, P. sylvaticum, P. ultimum, P. deliense, P. torulosum, P. irregulare and *P. vexans* have only been isolated from the mud soils, never from the pond waters. On the other hand, *P. coloratum, P. dissotocum, Pythium* 'group F', *Pythium* 'group HS' and *Pythium* 'group P' have been isolated from both waters and mud soils. We can, therefore, assume that some species of *Pythium* are strictly aquatic and others are strictly terrestrial, while the rest can survive both in water and on land. The species which can survive both in water and on land might be attributable to substrata brought into water by wash-in from terrestrial habitats and so made available for colonization there, while the species recorded only from the watery ecosystems could be attributed to the inability of those species to survive in the absence of adequate water. Most of the terrestrial species have lost the ability to produce zoospores and adapted themselves to multiply on land. While the findings reported here on the specific habitats of *Pythium* species in pond water and mud soil suggest possible explanations in terms of the ecology of the species, conclusive statements are not yet possible. More information is needed on the nature of water-borne pythia, and also on the natural substrata occupied by these fungi during their multiplication.

Dick (1971) distinguished the aquatic fungi as 'inhabitants', 'aliens' and 'active aliens' in lentic environments, while Park (1972) classified them as 'indwellers', 'versatiles' or 'transients' and 'immigrants'. It can be concluded from the present study and on the basis of Park's classification that species of *Pythium* such as *P. fluminum, Pythium* 'group F' and others are indwellers as they are typically aquatic pythia recorded several times from the watery ecosystems, while species like *Pythium* 'group HS' might be transients in the water but indwellers in the mud soil. Species such as *P. coloratum, P. diclinum, P. dissotocum* and *Pythium* 'group P' might be versatiles between aquatic and terrestrial ecosystems.

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