Mycoscience 35: 315–318, 1994

Effect of temperature, hydrogen ion concentration and osmotic potential on oospore germination of five *Pythium* spp. isolated from pond water

Hani M. A. Abdelzaher[1], Takio Ichitani[1] and Mohamed A. Elnaghy[2]

[1] College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 593, Japan
[2] Department of Botany, Minia University, Minia, Egypt

Accepted for publication 15 August 1994

Oospore germination occurred over a temperature ranging of 15–35°C for *Pythium coloratum*, 10–35°C for *P. diclinum*, 15–30°C for *P. dissotocum*, 7–30°C for *P. monospermum*, and 10–30°C for *P. pleroticum*. Optimum temperature was 25°C for all species tested. In case of pH, oospore germination occurred over a range of 4.76–8.55 with an optimum of 6.40–7.40. The least germination occurred at pH 4.76 for *P. coloratum, P. diclinum, P. monospermum* and *P. pleroticum*, while *P. dissotocum* germinated from pH 5.02. Oospores of the all tested pythia were able to germinate at −0.13 to −1.65 MPa and could not germinate at −3.40 MPa, with the highest germination rate at −0.27 to −0.47 MPa. The effect of temperature, pH and osmotic potential on oospore germination was discussed in relation to pollution of pond water.

Key Words——oospore germination; osmotic potential; pH; pond water; *Pythium* spp.; temperature.

Oospores are considered to be resistant structures which allow fungi to survive adverse conditions. Most investigators have studied the dynamics of oospores in soil, the biology of oospores and factors affecting their germination in the genus *Pythium* (Peter, 1971; Stanghellini and Burr, 1973; Stanghellini and Russel, 1973; Ayers, 1975; Ge and Ichitani, 1992). No work has, however, been done on oospore germination of *Pythium* spp. in an aquatic ecosystem.

The present study examined oospore germination of five aquatic *Pythium* spp. from pond water in Osaka, Japan, under various cultural conditions in order to clarify the relationship between their behavior under polluted conditions and their life cyclic patterns. Three main factors of temperature, hydrogen ion concentration and osmotic potential were selected. Part of the work has been reported elsewhere (Abdelzaher et al., 1993).

Materials and Methods

Fungi and isolates *Pythium coloratum* Vaartaja (isolate No. 187), *P. diclinum* Tokunaga (114), *P. dissotocum* Drechsler (250), *P. monospermum* Pringsh. (237) and *P. pleroticum* T. Ito (125) were isolated from pond water in Sakai, Osaka, Japan, during an ecological survey of aquatic pythia (Abdelzaher et al., 1993). Table 1 represents some ecological information about the species tested.

Oospore production and germination The fungi were cultured to produce oospores at 20°C for 1–4 weeks in 100-ml Erlenmeyer flasks containing 10 ml of V-8 juice. Oospore suspensions were then obtained by mincing mycelial mats in a blender for 3 minutes. The resulting suspension was filtered through a sieve, the size of which was chosen in relation to the oospore diameter in order to produce a suspension of oospores reasonably free of hyphal fragments. An attempt to separate viable hyphae from oospores by freezing (Kusunoki and Ichitani, 1982) resulted in negative effect on some species (*P. coloratum, P. dissotocum* and *P. monospermum*) and a positive effect on others (*P. diclinum* and *P. pleroticum*), and therefore this method was not selected for this experiment. This suspension was used directly after counting and adjusting the number of mature oospores and incubated on the following media for 24 hours at fixed temperature in the dark. Oospore germination was examined microscopically and 200 oospores were chosen at random for calculation of germination rate. All the results are the mean of five replicate tests, each repeated twice.

Media for germination tests Bacto-CMA medium (CMA) was employed for the investigation of temperature response of oospore germination. V-8 juice (10%) liquid medium supplemented with 500 μg/ml of wheat germ oil (Japan Impex, Tokyo) was used for producing oospores.

In the pH experiment, CMA was adjusted to pH values between 4.50 and 8.50 at 0.50 pH unit intervals with 50 mM MES buffer (Inoue and Ichitani, 1990). Double strength of the buffer salt was dissolved in a fixed volume of distilled water and adjusted to the desired pH with 1 N HCl or 1 N NaOH. Double strengths of MES buffer and CMA were separately autoclaved at 121°C for

Table 1. Ecological data on species tested.

Fungi (Isolate no.)	Date of isolation	Pond	Temp. (°C)	pH value	Transparency (ml)	Conductivity (ms/ml)
P. coloratum (187)	Jan., '93	Nakatsu	5.5	8.71	24.5	0.35
P. diclinum (114)	Dec., '92	Nakatsu	10.0	8.08	32.0	0.35
P. dissotocum (250)	Mar., '93	Nakatsu	9.0	7.88	17.5	0.34
P. monospermum (237)	Mar., '93	Nakatsu	7.0	7.90	26.5	0.36
P. pleroticum (125)	Dec., '92	Tatsumi soil[1)]	10.0	6.36	N.D.[2)]	N.D.

[1)] Bottom soil of the pond while there was no water.
[2)] Not determined.

15 minutes, and the buffer was then added to an equal volume of the medium to give a final buffer concentration of 50 mM. The initial pH was measured and the medium was dispensed into 90-mm diam Petri dishes (10 ml/dish). Oospore geremination tests were carried out at 25°C.

In the osmotic potential experiment, mannitol, which was found not to be utilized by *Pythium* as a nutrient (Thill et al, 1979), was added to CMA. The osmotic potential of CMA was adjusted to −0.13, −0.27, −0.47, −1.00, −1.65 and −3.40 MPa by adding mannitol in a ratio of 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28 mol/kg, respectively, according to Robinson and Stokes's formula (Robinson and Stokes, 1949). The osmotic potential was determined with a Dew Point Microvoltmeter (HR-33T; Wescor, Logan, USA). Incubation temperature for the test was 25°C.

Results

Influence of temperature on oospore germination As shown in Fig. 1, oospores of *P. monospermum* germinated between 7°C and 30°C, but not at 4°C or 35°C; those of *P. diclinum* and *P. pleroticum* germinated from 10°C, and those of *P. coloratum* and *P. dissotocum* germinated from 15°C. Oospores of *P. coloratum* and *P. diclinum* germinated at 35°C, while those of *P. dissotocum*, *P. monospermum* and *P. pleroticum* did not. Maximum germination rate of all the pythia tested was at 25°C.

Influence of hydrogen ion concentration on oospore germination As shown in Fig. 2, all the pythia tested germinated over the whole experimental pH range at 25°C, except for *P. dissotocum*, which germinated from pH 5.02. The optimum pH value for oospore germination ranged from 6.40 to 7.40 for all species tested.

Influence of osmotic potential on oospore germination The effect of osmotic potential on oospore germination at 25°C is indicated in Fig. 3. The pythia tested showed similar responses. At −3.40 MPa, none could germinate. They germinated at the other osmotic potentials tested, with good germination between −0.13 and −0.27 MPa and the optimum at −0.47 MPa.

Discussion

During our ecological study of aquatic pythia (Abdelzaher et al., 1993), water temperature at the survey points was

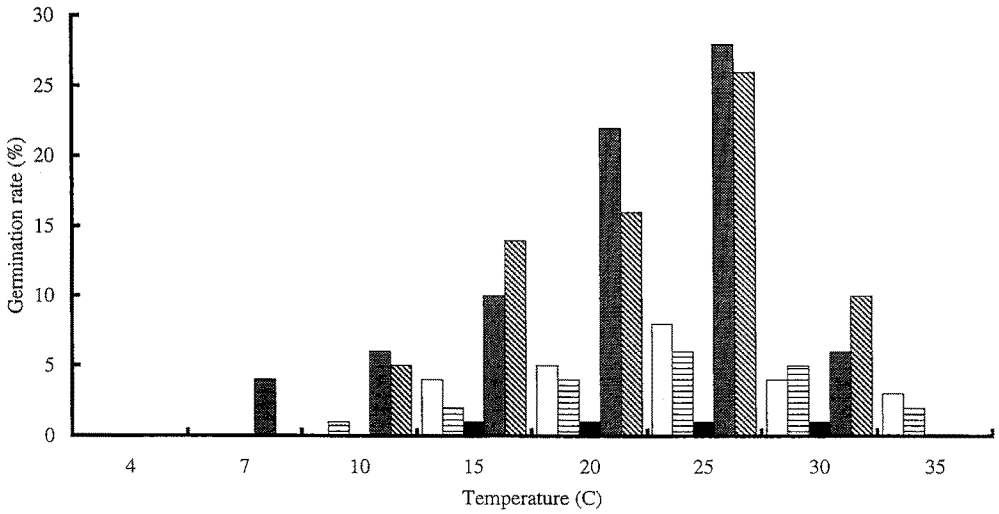

Fig. 1. Effect of temperature on oospore germination of five aquatic *Pythium* spp. (*P. coloratum* □, *P. diclinum* ⊟, *P. dissotocum* ■, *P. monospermum* ▩, *P. pleroticum* ▧).

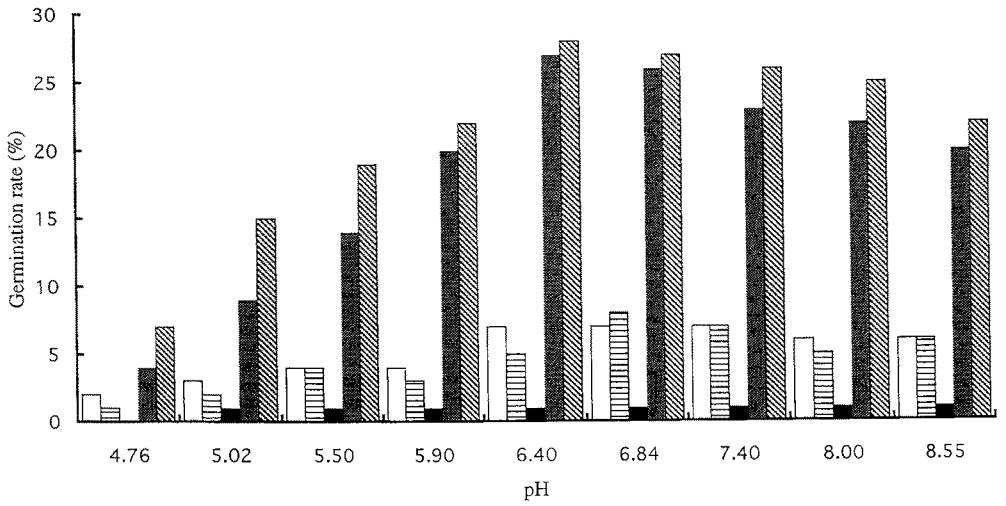

Fig. 2. Effect of hydrogen ion concentration on oospore germination of five aquatic *Pythium* spp. (*P. coloratum* □, *P. diclinum* ▤, *P. dissotocum* ■, *P. monospermum* ▩, *P. pleroticum* ▧).

ranged from 2 to 30°C. The very cold water in winter was unsuitable for oospore germination of the pythia tested. Temperatures of 7°C or 10°C, however, support some oospore germination. For this reason, oospore germination at different temperatures in the laboratory suggests that the germination in water or in plant debris in water may be stimulated by temperatures in the range of 7-30°C.

Oospore germination occurred over a broad pH range with an optimum around neutral. *Pythium* appears to be intolerant of acid soils (Webster, 1991). We can assume, therefore, that acidic ponds (pH below 4.5) are unsuitable for establishment of *Pythium* spp. *Pythium* species have been absent from heavily polluted water and seldom found in partially polluted places (Harvey, 1952). High polluted pond water with osmotic potential above −1.65 MPa, located downstream of a pollution source such as factories, represents an inhibitive medium toward oospore germination of aquatic pythia. Further studies on pollution elements and their effect on oospore germination in the aquatic habitat should be done.

Literature cited

Abdelzaher, H. M. A., Ichitani, T. and Elnaghy, M. A. 1993. Effect of temperature, hydrogen-ion concentration and osmotic potential on oospore germination of five aquatic *Pythium* spp. Trans. Nishi-Nippon Div. (Mycol. Soc. Japan). p. 3.

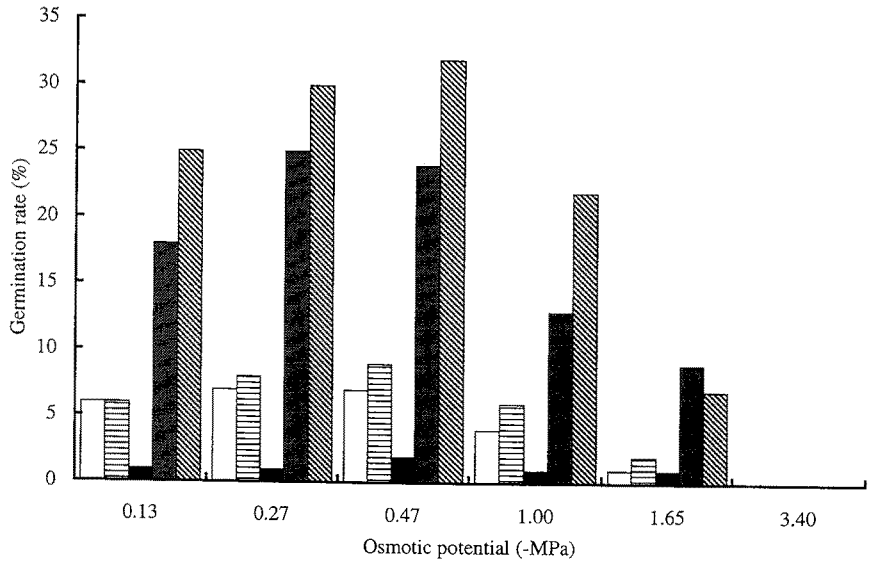

Fig. 3. Effect of osmotic potential on oospore germination of five aquatic *Pythium* spp. (*P. coloratum* □, *P. diclinum* ▤, *P. dissotocum* ■, *P. monospermum* ▩, *P. pleroticum* ▧).

Ayers, W. A. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology **65**: 1094-1100.

Harvey, J. V. 1952. Relationship of aquatic fungi to water pollution. Sewage and Industrial Wastes **24**: 1159-1164.

Inoue, M. and Ichitani, T. 1990. Optimum pH range for mycelial growth of *Pythium* spp. estimated by MES [2-(N-morpholino) ethanesulfonic acid] buffer. Trans. Mycol. Soc. Japan **31**: 191-196.

Kusunoki, M. and Ichitani, T. 1982. Preparation of mycelium-free oospores of *Pythium butleri* by a freezing method. Ann. Phytopath. Soc. Japan **48**: 695-698.

Ge, L. P. and Ichitani, T. 1992. Influence of temperature, hydrogen-ion concentration and osmotic potential on oospore germination of *Pythium paddicum* and *P. iwayamai*. Bull. Univ. Osaka Pref. Ser. B **44**: 7-11.

Peter, B. A. 1971. *Pythium aphanidermatum* oospore germination as affected by time, temperature, and pH. Phytopathology **61**: 1149-1150.

Robinson, R. A. and Stokes, R. H. 1949. Tables of osmotic and activity coefficients of electrolytes in aqueous solution at 25°C. Trans. Faraday Soc. **45**: 612-624.

Stanghellini, M. E. and Burr, T. J. 1973. Effect of soil water potential on disease incidence and oospore germination of *Pythium aphanidermatum*. Phytopathology **63**: 1496-1498.

Stanghellini, M. E. and Russel, J. D. 1973. Germination in vitro of *Pythium aphanidermatum* oospores. Phytopathology **63**: 133-137.

Thill, D. C., Schirman, R. D. and Appleby, A. P. 1979. Osmotic stability of mannitol and polyethylene glycol 20,000 solutions used as seed germination media. Agronomy Journal **71**: 105-108.

Webster, J. 1991. "Introduction to Fungi, 2nd ed.," Cambridge University Press, Cambridge. 169p.