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Materials of *Pythium* Flora in Japan (XI): Characterization of *Pythium graminicola* causing seedling blight in rice

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Abstract

Rice-origin *Pythium graminicola* has not been available despite its economical importance. The morphology, sequence of the rDNA internal transcribed spacer (ITS) gene and pathogenicity of isolates of *P. graminicola* causing seedling blight in rice are described.

Key Words: *Pythium graminicola*, rice, seedling blight.

Introduction

Pythium graminicola Subramaniam is pathogenic to many gramineous plants (van der Plaats-Niterink 1981) and is best known as the most common pathogen of rice (*Oryza sativa* L.) seedling blight in Japan (Endo and Ibaraki, 1986; Kato et al., 1985; Ogawa et al., 1983; Ogawa, 1984; Umehara et al., 1983) and Korea (Sung et al., 1983). Despite its economical importance, description of the pathogen has been poorly provided except for Kato et al. (1985) and Kato (1987). In addition, no material of rice-origin *P. graminicola* has been available in public culture collections including National Institute of Technology and Evaluation Biological Resources Center (NBRC), Ministry of Agriculture, Forestry and Fisheries (MAFF) Genebank, Centraalbureau voor Schimmelcultures (CBS) and American Type Culture Collection (ATCC). We describe here its morphology, sequences of the rDNA internal transcribed spacer (ITS) gene and pathogenicity to provide material for the pathogen.

Morphology and growth temperature

Two *Pythium* isolates, OPU480 (=NBRC 33286, MAFF 238432) and OPU481(=NBRC 33287, MAFF 238433), were obtained from rice seedlings (cv. Nipponbare) in a nursery bed at

Shiraoka-cho, Saitama in April 2001 by using *Pythium* selective medium (Ali-Shtayeh et al., 1986) at 25°C. They were maintained on corn-meal agar (CMA) slants at 20°C until use. For morphological analysis, the isolates were grown on grass leaf culture (Martin, 1992) and CMA. Thirty each of morphological criteria listed in the keys of van der Plaats-Niterink (1981) were examined under a light microscope for identification. The two isolates were morphologically the same and identified as *P. graminicola* according to the following features. Main hyphae were up to 5.8 µm wide. Sporangia were terminal or intercalary, lobate and irregular complex (Fig. 1 A and B, Fig. 2 A-C). Zoospores formed at 25°C. Oogonia were terminal or intercalary, globose, smooth-walled, 17-31 µm (mean 24.3 µm) diameter (Fig. 1 C-E, Fig. 2 D-H). Antheridia were terminal, 1-5 (mean 2.5) per oogonium, clavate, 5.6 µm wide, branched, rarely with simple stalks, monoclinal or diclinal. Oospores were simple, plerotic and globose, 14.9-29.9 µm diameter (mean 22.5 µm). The thickness of the oospore wall was up to 2.9 µm. Appressoria were subspherical or irregular in shape. Hyphal swellings were globose, terminal or intercalary, up to 30.8 µm in diameter (Fig. 1 F). Colonies on potato-carrot agar (PCA) and CMA showed a radiate pattern without aerial

mycelium (Fig. 3). The optimal growth of mycelia occurred at 31°C, with the minimum growth temperature at 5°C, and the maximum at 37°C (Fig. 4). The daily growth rate was 37.5 mm at 31°C.

rDNA internal transcribed spacer (ITS) gene sequences

Genomic DNA of isolates OPU480 and 481 was isolated by using Puregene Yeast and Gram-positive Bacteria Kits (Gentra Systems, MN), and sequences of the ITS regions including 5.8S rDNA were determined by the method described elsewhere (Tojo et al., 2001). The sequences of the ITS regions of isolates OPU480 and 481 were the same with each other and identical to those of *P. graminicola* from sugar cane in Kagoshima (Matsumoto et al., 1999). The sequences have been deposited in GenBank as AY099310.

Pathogenicity

Pathogenicity of isolates OPU480 and 481 to rice seedlings (cv. Koshihikari) was examined by the following procedure. The fungal isolates

were inoculated on 5 cm³ of autoclaved bent-grass seeds (Tojo et al., 1993) in a 300-ml Erlenmeyer flask and incubated at 25°C for 6 days. The mycelial mat with the medium seeds was homogenized in 200 ml of 0.08% water agar for a few seconds. Germinated rice seeds (3.2 g) were sown in a plastic pot (7 cm diameter, 9.5 cm depth) containing a nursery medium for rice (Kumiai Ube Ryujo-baido 2, Zen-noh, Tokyo). Six ml of mycelial suspension were spread over the surface of rice seeds in the pot. The seeds were irrigated with tap water and covered by a thin layer of nursery medium. Six pots were used for each isolate. Six pots inoculated with the autoclaved inoculum acted as control. The pots were incubated in a growth chamber at 28 °C with continuous light (52 μmol m⁻² s⁻¹). Eight days after inoculation, the level of virulence of the isolates was expressed as disease severity based on the percentage of seedlings wilted or with root discoloration. Length of roots and leaves of surviving plants were measured at the same time. Values of the disease severity were analyzed by analysis of variance (Kruskal-Wallis test) and significant differences detected by

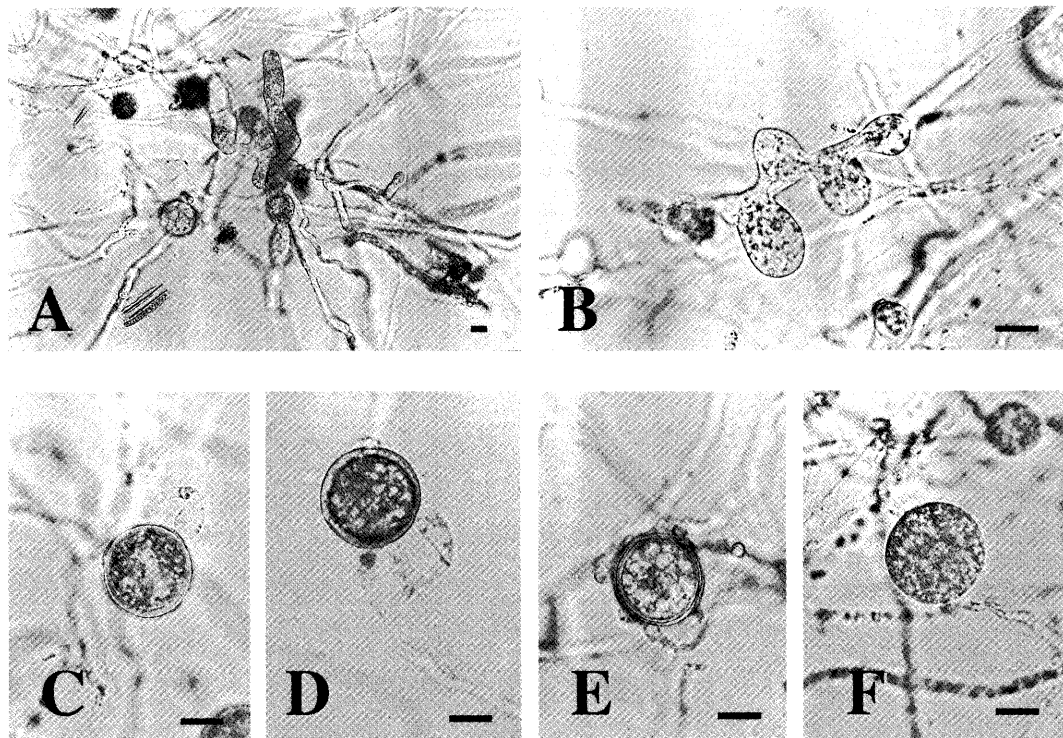


Fig. 1. Sexual and asexual organs of isolate OPU480 (=NBRC 33286, MAFF 238432) of *P. graminicola* from rice seedlings. Lobate sporangia (A and B). Terminal oogonium and pleurotic oospores with monoclinal antheridia (C and D). Oogonium and pleurotic oospore with antheridia (E). Terminal and globose hyphal swelling (F). Bar, 10 μm.

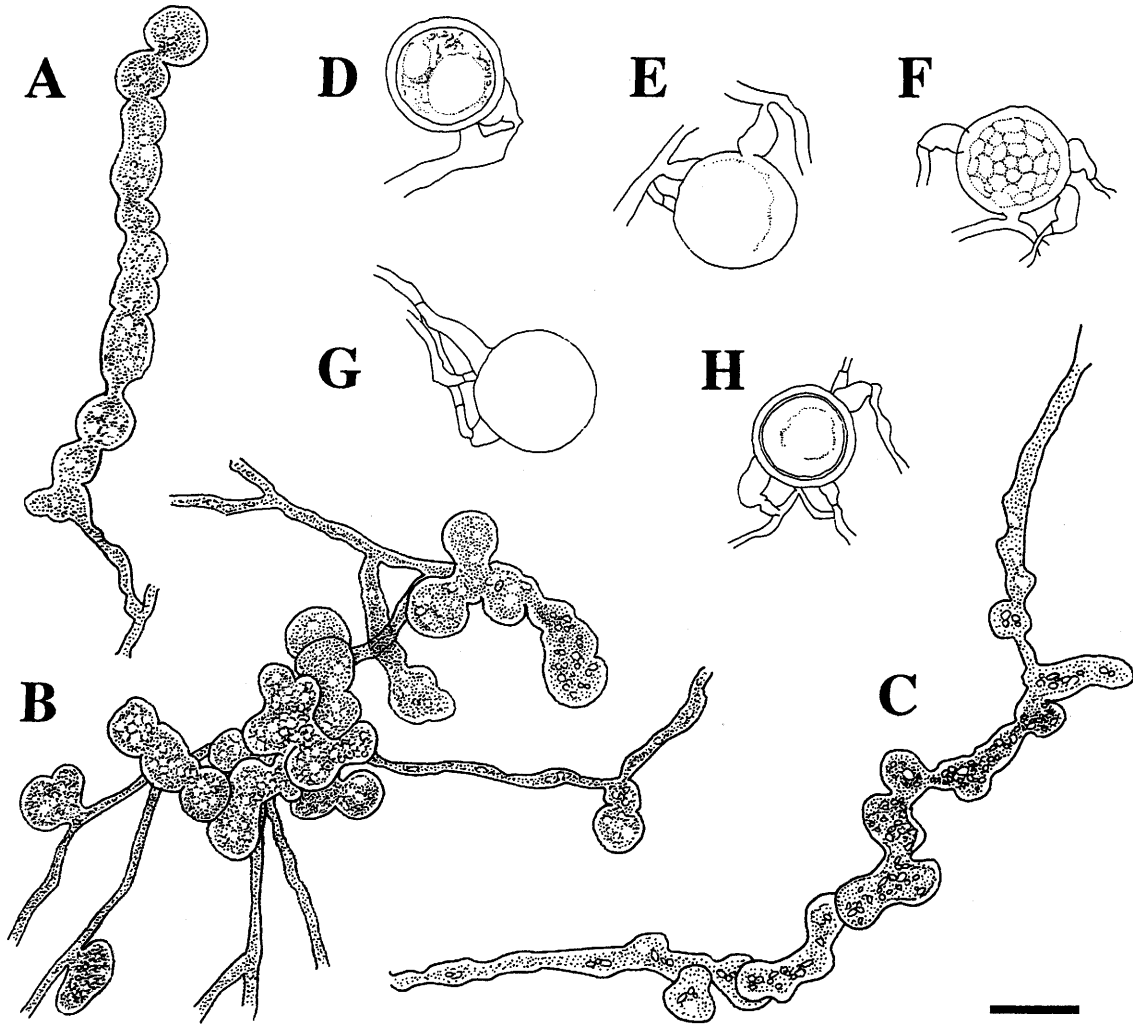


Fig. 2. Sporangia and sexual organs of isolate OPU480 (=NBRC 33286, MAFF 238432) of *P. graminicola* from rice seedlings. A, terminal complex; B, irregular complex; C, intercalary sporangia; D, terminal oogonium and pleurotic oospores with monoclinous antheridium, E and F, terminal oogonium with diclinous antheridia; G, terminal oogonium with diclinous and monoclinous antheridia; H, terminal oogonium and pleurotic oospore with diclinous and monoclinous antheridia. Bar, 20 μ m.

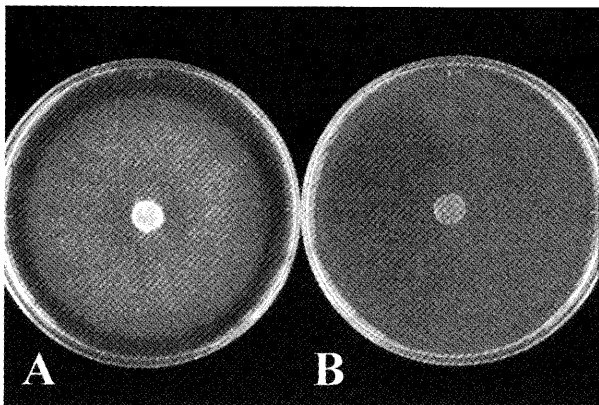


Fig. 3. Colony morphology of isolate OPU480 of *P. graminicola* 1 day after inoculation on Bacto-CMA (A) and PCA (B) media at 31°C.

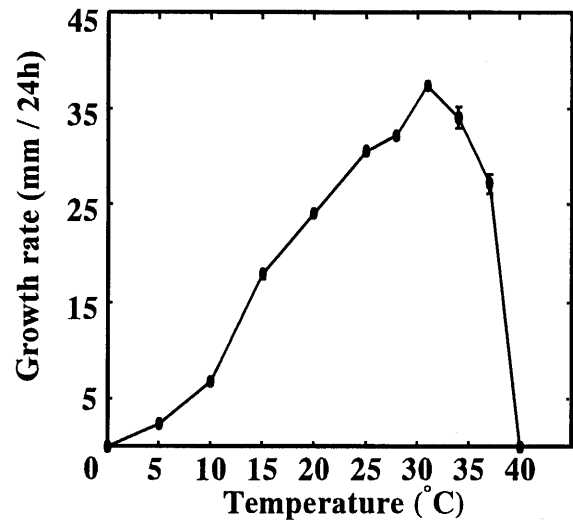


Fig. 4. Growth-temperature relationships of isolate OPU480 of *P. graminicola* on PCA medium. Bar represents standard deviation of 4 replications.

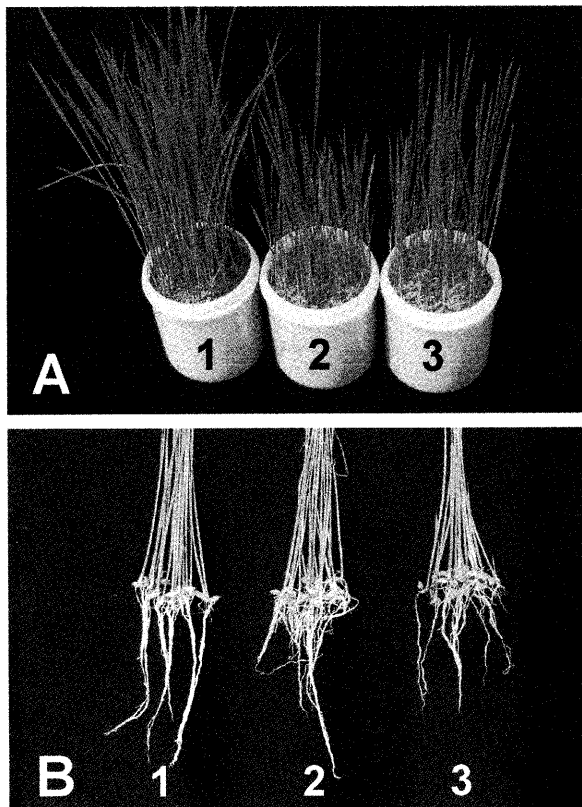


Fig. 5. Growth of leaves (A) and roots (B) of rice seedlings 8 days after inoculation with *P. graminicola*. Uninoculated control plants (1), and plants inoculated with isolates OPU480 (2) and OPU481 (3).

multiple range test (Fisher's PLSD-test). Values of the length of root and leaves were analyzed by the Bartlett test and statistically analyzed by analysis of variance (ANOVA). The two isolates of *P. graminicola* caused wilt and root discoloration (Fig. 5, Table 1). Roots and leaves were significantly shorter in the inoculated plants than in control plants ($P \leq 0.05$). Infection of the pathogen to damaged plants was confirmed by

reisolation of the fungus on the *Pythium* selective medium (Ali-Shtayeh et al., 1986).

Discussion

Morphological description of rice-origin *P. graminicola* has been limited, although the descriptions of the species from other gramineous plants have been provided (Ichitani and Kinoshita, 1991; Subramaniam, 1928; van der Plaats-Niterink, 1981). Morphological characteristics of *P. graminicola* are overlapping to its related species such as *P. arrhenomanes* Drechsler and *P. aristosporum* Vanterpool (van der Plaats-Niterink, 1981; Kajihara et al., 2002). Therefore morphological observation combined with molecular analysis should be provided for more reliable description of this species. In this report, we described isolates of the rice-origin *P. graminicola* by morphological observation along with rDNA analysis. Morphology of the isolates corresponded to the former descriptions of *P. graminicola* (Kato et al., 1985; Ichitani and Kinoshita, 1991; Subramaniam, 1928; van der Plaats-Niterink, 1981), and their sequences of rDNA ITS region were identical to those of sugar cane isolates (Matsumoto et al., 1999). These results show that the present isolates can be used as materials for taxonomic study of rice-origin *P. graminicola*. The isolates readily cause seedling blight on rice seedlings by artificial inoculation. An accurately identified material of rice-pathogenic *P. graminicola* has been required for development of new chemicals and biocontrol agents to control the disease. The present isolates will also be useful for testing these control agents.

Table 1. Virulence of *Pythium graminicola* isolates from rice seedlings*.

Isolates	Diseased plants (%)	Length of roots (cm)	Length of leaves (cm)
OPU480	38.33 ^b	8.10 ^b	22.11 ^b
OPU481	18.33 ^c	8.83 ^b	23.28 ^b
Control	0 ^a	10.31 ^a	26.98 ^a

*, Disease severity was recorded as percentage of seedlings showing wilt or with root discoloration. Measurements were made 8-days after inoculation. Values of disease severity followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's PLSD-test ($n=6$). Values of the length of root and leaves followed by different letters are significantly different ($P \leq 0.05$) according to analysis of variance (ANOVA).

References

- Ali-Shtayeh, M. S., Len, L.-H. C., and Dick, M. W. 1986. An improved method and medium for quantitative estimates of populations of *Pythium* species from soil. *Trans. Br. Mycol. Soc.* **86**, 39-47.
- Endo, Y. and Ibaraki, T. 1986. Association of some fungal species to damping-off of aged seedlings of rice in nursery containers. *Ann. Rept. Pl. Prot. North. Japan* **37**, 189. (in Japanese)
- Ichitani, T. and Kinoshita, T. 1991. Materials for *Pythium* Flora of Japan (IV) *Pythium graminicola* from rhizosphere soil of zoysia green. *Bull. Univ. Osaka Pref. Ser.* **B43**, 1-9.
- Kajihara, Y., Nakashima, A., Suga, H., and Kageyama, K. 2002. Classification of *Pythium graminicola* and related species based on polymorphisms of rDNA-ITS region and cytochrome oxidase II gene and morphology. *Ann. Phytopath. Soc. Japan* **69**, 63. (in Japanese)
- Kato, S. 1987. Studies on *Pythium* damping-off of rice seedlings in nursery flats. Ph. D. Thesis of Kyoto University, 178pp. (in Japanese)
- Kato, S., Nakanishi, T., Takahi, Y., Nakagami, K., and Ogawa, M. 1985. Studies on *Pythium* damping-off of rice seedlings (2) *Pythium* species associated with damping-off at the middle and latter growth stages of rice seedlings in nursery flats. *Ann. Phytopath. Soc. Japan* **51**, 168-175. (in Japanese with English summary)
- Martin, F. N. 1992. *Pythium*. In "Methods for research on soilborne phytopathogenic fungi" (eds. Singleton, L. L., Mihail, J. D., & Rush, C. M.) APS Press, St. Paul, 39-49.
- Matsumoto, C., Kageyama, K., Suga, H., and Hyakumachi, M. 1999. Phylogenetic relationships of *Pythium* species based on ITS and 5.8S sequences of the ribosomal DNA. *Mycoscience* **40**, 321-331.
- Ogawa, K. 1984. Diseases of rice seedlings in nursery flat caused by *Pythium* sp. *Shokubutu boeki (Plant Protection)* **38**, 218-221. (in Japanese)
- Ogawa, K., Kageyama, K., and Suwa, M. 1983. Occurrence of seedling blight of rice caused by *Pythium graminicolum* in Japan. *Ann. Phytopath. Soc. Japan* **49**, 389. (in Japanese)
- Subramaniam, L. S. 1928. Root rot and sclerotial diseases of wheat. *Bull. Agric. Res. Inst. Pusa* **177**, 1-17.
- Sung, J. M., Jin, K. S., Lee, S. C., and Park, J. S. 1983. Identification and pathogenicity of *Pythium* spp. associated with seedling damping off of rice. *Kor. J. Mycol.* **11**, 27-32.
- Tojo, M., Fujita, Y., Awad, H. M., and Ichitani, T. 1993. Preparation of *Pythium* inocula using bentgrass seeds for glasshouse studies. *Proc. Kansai Pl. Prot.* **35**, 1-5. (in Japanese with English summary)
- Tojo, M., Hoshino, T., Herrero, M. L., Klemsdal, S. S., and Tronsmo, A. M. 2001. Occurrence of *Pythium ultimum* var. *ultimum* in a greenhouse on Spitsbergen Island, Svalbard. *Eur. J. Pl. Path.* **107**, 761-765.
- Umehara, Y., Sakui, H., and Nakagawa, T. 1983. Environmental effects on the development of seedling wilt of rice caused by *Pythium* spp. *Ann. Phytopath. Soc. Japan* **49**, 389. (in Japanese)
- Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. *Stud. Mycol.* **21**, 1-242.

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