A new species of Pythium isolated from a vineyard in France

Bernard Paul
Laboratoire des Sciences de la Vigne, Institut Jules Guyot, Université de Bourgogne, Dijon, France

Abstract

Pythium apiculatum sp. nov. is a new oomycete characterized by the presence of both ornamented and smooth-walled oogonia. The ornamentations are blunt, and at times, bent. The oomycete was isolated from soil samples taken in a vineyard in the Burgundian region of France. Morphologically, it resembles some species having ornamented oogonia like Pythium radiosum, Pythium echinulatum, and resembles, also the species having smooth-walled oogonia like Pythium hypogynum and Pythium acrogynum. However, the oomycete has its own distinguishing characteristics which, when combined with molecular features, enables us to describe it as a new species. The taxonomic description of this new oomycete, its comparison with related species, and the sequence of the ITS region of its rRNA gene, are described in this article.

Introduction

The members of the genus Pythium are distributed throughout the world (Middleton, 1943). Quite a number of the members of the genus Pythium are known for their ornamented oogonia (female gametangia), and of these members the most commonly found is Pythium echinulatum. The oogonial ornamentations not only make these members 'spectacular', but are also of great taxonomic value. Plaats-Niterink (1981) recognized 21 species of Pythium having ornamented oogonia. Since then, the present author has added four more: Pythium ornamentatum, Pythium radiosum, Pythium ornacarpum and Pythium spiculum (Paul, 1987, 1992, 1999; Paul et al., 2006). This is the fifth to be added to the group. The name ‘apiculatum’ refers to the ‘pricking’ nature of the oogonial spines. The species has been erected on the basis of the differences of morphological characters, and the sequence of the ITS region of its rRNA.

Pythium apiculatum was isolated from soil samples collected in two different vineyards in Marsannay in the Burgundian region of France. The isolate F-1326 is the type species as it produces both sexual and asexual structures. The new species is being deposited at the CBS culture collection in BAARN (Holland). The following description of the oomycete will explain why the new species is erected within the genus Pythium.

Materials and methods

Oomycete isolates

Soil samples, together with plant root debris, were collected in sterile, capped bottles and were brought to the laboratory. Oomycetes were isolated from these samples by the usual baiting techniques described elsewhere (Middleton, 1943; Paul et al., 1998). These were purified by repeated culturing in sterile distilled water and ultimately grown and maintained on solid media like PCA (potato carrot agar) and PDA (potato dextrose agar). The temperature–growth relationship of the oomycete was taken when it was grown on PCA incubated at 25°C. All isolates are maintained at ‘Institut Jules Guyot’, in Dijon, France. Pythium apiculatum was identified with the help of keys provided by Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981), and also by its ITS sequences using the BLAST search. The type specimen has been deposited at the CBS culture collection (BAARN, the Netherlands).

DNA extraction and PCR amplification

All three isolates of P. apiculatum were grown in PDB (potato dextrose broth). The culture conditions and procedures used for DNA isolation and the PCR of the internal
transcribed spacer (ITS) region of the ribosomal nuclear DNA were those described earlier (Paul et al., 1999; Paul, 2001). ITS amplifications of *Pythium* samples were carried out using previously described universal primers ITS1 and ITS4 that target conserved regions in the 18S and 28S rRNA genes (White *et al*., 1990). These primers [(ITS1: TCC GTA GGT GAA CCT GCG G) and ITS4: (TCC TCC GCT TAT TGA TAT GC)] were synthesized by Genome Express (Paris). ITS1 is at the 3′ end of the 18S rRNA gene and ITS4 is at the 5′ end of the 28S rRNA gene. The sequencing of the amplified PCR product was performed by the Laboratoire de Génétique Appliquée, Ecole d’Ingénieurs de Lullier, Jussy in Switzerland. The sequences obtained were compared with the ITS1 sequences of related species of *Pythium*: *P. radiosum* (Genbank accession AY598695), *P. echinulatum* (AY598639), *Pythium erinaceum* (AY598694), *Pythium hypogynum* (AY598693), *Pythium acrogynum* (AY598638), *Pythium* sp. strain F-1216 (AY455697) and *P. irregulare* (AF271226). The sequence of the ITS region of the nuclear ribosomal DNA of *P. apiculatum* (F-1326) has been deposited in Genbank (accession DQ211530).

**Results**

**Morphological characteristics**

*Pythium apiculatum* Paul sp. nov. (Figs 1–5)

Sporangio et zoosporis non observata, Corporibus hypharum globosa, cylindrosa, intercalaria, catenaria, vel terminalia, 10–30 µm diam., zoosporae non observata. Oogonia terminalia, vel intercalaria, globosa, cylindrosa 9–25 µm diam., ornata vel non-ornata, spiculis 2–5 µm longis. Antheridia, monoclinata, raro diclinata, 1–3 in singulo oogonia Oosporae, pleroticae vel apleroticae, globosae, vel cylindrosae 8–19 µm diameter, paries 1–3 µm crassus. Incrementum radiale quotidianum 8–10 mm 25°C in agaro Solani tuberosi et Dauci carotae (PCA). Holotypus in herbario Universitatis Bourgogne conservatus (F-1326).

The oomycete grows well both on solid media and on hemp-seed halves in water. Its mycelium in water is hyaline, well-branched with the main hyphae measuring up to 5–7 µm in width. Colonies on PCA are submerged and show a broad chrysanthemal pattern. Average radial growth of the fungus at 25°C on PCA is 8–10 mm per day.

**Fig. 1.** Asexual and sexual reproductive bodies of *Pythium apiculatum*: (a) terminal hyphal body; (b) Intercalary hyphal body; (c and d) Elongated hyphal body bearing some spines; d–f, Catenulate hyphal bodies. All figures bar = 20 µm.
Sporangia or hyphal bodies are produced plentifully (Fig. 1a–f). These are terminal (Fig. 1a), intercalary (Fig. 1b) and also catenulate (Fig. 1c–f). These asexual structures are mostly spherical, but at times cylindrical to irregular and occasionally bear one to five spines (Fig. 1c and d). The spherical hyphal bodies measure 10–30 μm in diameter (av. 20.5 μm), the elongated and irregular ones (Fig. 1c–f) can measure up to 60 μm in length. Zoospores were not observed.

Fig. 2. Sexual reproduction of Pythium apiculatum: smooth-walled oogonia. (a) Oogonia with U-shaped monoclinous antheridia; (b) oogonia with both mono and diclinous antheridia; (c) oogonia supplied with diclinous antheridia; (d) oogonia bearing one spine and supplied with monoclinous antheridia. Bar = 10 μm.

Fig. 3. Sexual reproduction of Pythium apiculatum: Ornamented oogonia. (a and b) terminal oogonia; (c) oogonia supplied with a monoclinous antheridia; (d) intercalary oogonia containing an oospore, (e and f) Terminal oogonia with monoclinous antheridia. Bar = 10 μm.
The female gametangia (oogonia) are smooth-walled (Fig. 2) and, ornamented (Figs 3–4). Occasionally, the smooth-walled oogonia may bear one or two papilla-like structures (Fig. 2d). The ornamented ones have blunt spines measuring 2–5 μm in length and generally 3 μm at the base, rarely one or two spines on the oogonia can measure up to 10 μm. The oogonial spines are at times bent. Oogonia are terminal (Figs 2d and 3a–c) or intercalary (Fig. 3d), spherical, ovoidal or cylindrical measuring 9–25 μm in diameter (av. 18 μm). The elongated oogonia can measure up to 50 μm in length and 25 μm in breadth (Fig. 3d). These are filled with dense, coarsely granulated protoplasm.

The male gametangia (antheridia) are mostly monoclinous (Figs 2a, 3c and e–f) and stalked, rarely diclinous (Fig. 2c), one to three per oogonium (Fig. 2b and c). The antheridial cells make a broad apical contact with the oogonia and at times these are U-shaped (Fig. 2a). They are usually persistent and remain attached to the oogonia, even after fertilization (Fig. 2d).

The zygotes (oospores) are rarely formed; when present, they are mostly plerotic and globose in spherical oogonia (Fig. 4a and b), whereas aplerotic and dumb-bell shaped or peanut-shaped in intercalary oogonia (Fig. 4c and d). The oospore walls are generally thick, varying between 1 and 3 μm (Fig. 4a–d). Oospores measure between 8 and 19 μm in diameter, and these can attain the length of 35 μm in the elongated oogonia.

The ITS region of the rRNA gene sequence of *P. apiculatum* (ITS1, 5.8 S and ITS2) is comprised of 870 bases and is subdivided as follows: Bases 1–236 = internal transcribed spacer 1, complete sequence; Bases 237–395 = 5.8 S gene, complete sequence; Bases 396–870 = internal transcribed spacer 2, complete sequence.

The sequence of the entire ITS region is on the Genbank (accession DQ211530). The comparison of the ITS1 sequences of *P. apiculatum* and related species is given in Fig. 5.

**Discussion**

The isolates of *P. apiculatum* were collected from Burgundian vineyards. Lack of zoosporangia, zoospores, presence of intercalary catenulate hyphal bodies, ornamented oogonia bearing blunt spines mixed with nonornamented oogonia, and thick-walled oospores are the characteristics of *P. apiculatum*. The morphology and temperature–growth relationship of the oomycete brings it close to other species of the genus bearing ornamented oogonia like *P. radiosum*, *P. erinaceum* and *P. echinulatum*. However, it is also close to the species that bears smooth-walled oogonia. This is supported by molecular analyses, and a BLAST search with the sequence of the ITS region of the rRNA genes of *P. apiculatum* gives close resemblance with species of *Pythium* having both ornamented and smooth-walled oogonia. The closest relative is *P. radiosum* (Genbank accession, AY598695), a new species described by the author from soil samples taken in Switzerland (Paul, 1992) with a 96.3% resemblance. However, the ITS region of this oomycete is bigger than *P. apiculatum* (873 bases instead of 870). Morphologically, *P. radiosum* is a different oomycete with only one type of oogonia (ornamented), and unlike *P. apiculatum* all the ornamentations are conical to
mammiform. The comparison of these two species is given in Table 1. Other species coming close to the new species are *P. echinulatum* (AY598639) with the ITS size of 871 bases and similarity with *P. apiculatum* of 96.3%; *P. erinaceum* (AY598694) with the ITS size of 871 bases and a homology with the new species of 95.9%. Other close relatives of *P. apiculatum* are those having smooth-walled oogonia like *P. hypogynum* (AY598693), 871 bases and 95.5% homology; an undescribed *Pythium* sp. F-1216 (AY455697), 872 bases and 95% homology; *P. acrogynum* (AY598638) 873 bases, and

**Fig. 5. CLUSTAL W (1.81) Multiple sequence alignment of the ITS1 sequences of *Pythium apiculatum* (DQ211530) with those of *Pythium radiosum* (Genbank accession AY598695), *Pythium erinaceum* (AY598694), *Pythium echinulatum* (AY598639), *Pythium hypogynum* (AY598693), *Pythium* sp. F-1216 (AY455697), *Pythium acrogynum* (AY598638) and *Pythium irregularare* (AF 271226).**

**Table 1. Comparison of morphological characters of *Pythium apiculatum* and *Pythium radiosum***

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>P. radiosum</em></th>
<th><em>P. apiculatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS region</td>
<td>873 bases</td>
<td>870 bases</td>
</tr>
<tr>
<td>Oogonia</td>
<td>Mostly spherical, all are provided with conical to mammiform spines of 2–15 μm in length</td>
<td>Both ornamented and smooth-walled, spines blunt 2–5 μm in length</td>
</tr>
<tr>
<td>Antheridia</td>
<td>Usually absent, when present, hypogynous</td>
<td>Always present, monoclinous and stalked, rarely diclinous, one to three per oogonium</td>
</tr>
<tr>
<td>Oospores</td>
<td>Globose, plerotic in smaller oogonia and aplerotic in bigger ones. Wall 0.75–2 μm in diameter</td>
<td>Globose, cylindrical to peanut shaped, Plerotic and aplerotic, thick-walled, Oospore wall 1–3 μm in diameter</td>
</tr>
</tbody>
</table>

&copy; 2006 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved
94.9% homology. All these species have either an ornamented type of oogonia or are of the smooth-walled type. None of these have both types like that found in the case of *P. apiculatum*. Within the genus *Pythium*, it is rare to have two types of oogonia. One such case is that of *P. irregularare* (AF 271226) which is an entirely different oomycete with only 69.2% similarity as far as the ITS region is concerned. A clustal multiple alignment of the ITS1 regions of the rRNA sequences of all these species is given in the Fig. 5. Morphological details of *P. apiculatum* and its molecular characteristics enables us to describe it as a new taxon. It is a ‘link’ species between the ornamented and nonornamented members of the genus *Pythium*, and it easily fits into the ‘clade E’ erected by Lévesque & de Cock (2004).

**Acknowledgements**

The author wishes to thank the team of Professor F. Lefort of the ‘Ecole d’Ingénieur de Lullier’ in Jussy, Switzerland, for co-operation in sequencing of the ITS region of the rRNA of the new species.

**References**


