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Phytophthora gallica sp. nov., a new species from rhizosphere soil of declining oak and reed stands in France and Germany

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ABSTRACT

A non-papillate, slow-growing *Phytophthora* species, which could not be assigned to any existing taxon, was isolated from rhizosphere soil of a declining oak in Northeast France, and from the rhizosphere of *Phragmites australis* at Lake Constance in south-west Germany in 1998 and 2004, respectively. We describe this species, previously informally designated *Phytophthora* taxon 'G', as *Phytophthora gallica* sp. nov. Morphology, growth rates, and pathogenicity against cuttings of riparian tree species and leaves of reed are described and compared with those of morphologically and phylogenetically similar *Phytophthora* species. *P. gallica* produces colonies with limited aerial mycelium and variable growth patterns. Gametangia are not formed in single or mixed cultures with tester strains of known mating types. *P. gallica* produces globose and elongated irregular chlamydospores, of which a high proportion is abortive. In water culture irregular hyphal swellings and non-papillate persistent sporangia are formed abundantly. *P. gallica* is moderately aggressive to *Alnus glutinosa* and *Fagus sylvatica*, weakly aggressive to *Quercus robur* and *Salix alba* and non-pathogenic to *Fraxinus excelsior* and *Phragmites australis*. According to ITS and mtDNA sequence data *P. gallica* belongs to a distinct *Phytophthora* clade, with *P. boehmeriae* and *P. kernoviae* being the closest relatives. The origin of *P. gallica* and its ecological role in wet ecosystems remain unclear.

Introduction

Phytophthora is a major genus of plant pathogens within the *Oomycota*, kingdom *Straminipila*. *Phytophthora* species are responsible for some of the most devastating diseases of tree species (Erwin & Ribeiro 1996). Since the early 1990s several projects in Europe were funded by the European Commission and national governments and research councils, investigating the involvement of *Phytophthora* species in the declines and diebacks of oaks, beech, chestnut, and alders (Brasier & Jung 2003, 2006; Jung *et al.* 2000, 2005; Gibbs *et al.* 2003; Vettraino *et al.* 2005; Jung & Blaschke 2004). Systematic searches for *Phytophthora* species were performed in more than a thousand stands in natural and semi-natural ecosystems, and 18 unknown *Phytophthora* taxa were detected of which 12 have been formally described as new species (Brasier & Jung 2003, 2006; Brasier *et al.* 2003a,b, 2004, 2005; Jung *et al.* 1999, 2002, 2003).

In 1998, and 2004, respectively, two isolates of a yet undescribed taxon that was previously informally named

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Phytophthora taxon G (Brasier & Jung 2003, 2006) were isolated from a declining oak stand in France, and from a reed (*Phragmites australis*) stand in south-western Germany that has been reported to be in decline for about five decades (Nechwatal *et al.* 2005). Based on its unique combination of cultural and morphological characters, temperature–growth relationships, and ITS DNA and mtDNA sequence data, we formally describe this taxon as *P. gallica* sp. nov.

Materials and methods

Isolation methods

Soil samples (approximately 1 l) from the rhizosphere of mature declining oak trees in the forest stand, Forêt d'Illwald, in Northeast France (48°13′36″ N, 7°27′22″ E) and from common reed growing in the littoral of Lake Constance in south-western Germany (47°41′48″ N, 9°11′20″ E) were removed to the laboratory, carefully mixed, and isolation tests for *Phytophthora* spp. carried out according to Jung *et al.* (1999, 2000) using *Quercus robur* leaflets as baits.

Voucher material of the isolates of *Phytophthora gallica* sp. nov. has been permanently preserved at the living strain culture collection of the Centraalbureau voor Schimmelcultures (CBS) Utrecht, under accession numbers CBS 111474 and CBS 111475. Additional strains are held at the culture collection (PRC) of the Phytopathology Department of the University of Konstanz, Germany.

Morphology and physiology

Isolates were grown at 20 °C in the dark on carrot agar (CA; 16 g agar, 3 g $CaCO_3$, 100 ml carrot juice, 900 ml distilled water), V8-agar (V8A; 16 g agar, 3 g $CaCO_3$, 100 ml V8 juice, 900 ml distilled water), Sigma, Sigma-Aldrich GmbH, Deisenhofen, Germany, malt-extract agar (MEA) and Sigma corn meal agar (CMA) in 90 mm Petri dishes, and colony morphology recorded after 10 d.

For temperature–growth relationships, four replicate CA plates of two isolates of each *Phytophthora gallica* and *P.* taxon 'PgChlamydo', and one isolate of each *P. gonapodyides* and *P.* taxon 'salixsoil' were incubated for 24 h at 20 °C to stimulate onset of growth, and then transferred to 5, 10, 15, 20, 25, 30, 33, and 35 °C. The growth rate was recorded 5–7 d after the onset of linear growth along two lines intersecting the centre of the inoculum at right angles (Jung *et al.* 1999).

Characterisation and measurements of the morphological structures of the two isolates of *P. gallica* and comparisons with known species (Table 1) were made under the light microscope on CA at ×320 according to Jung *et al.* (2002, 2003). For each isolate dimensions and characteristic features of 50 fully-mature sporangia, the diameters of 50 globose chlamydospores and length and breadth of 50 elongated chlamydospores, and diameters of 25 primary hyphae, chosen at random, were measured.

Sexual compatibility type was tested using 'direct' pairing tests on 9 cm CA plates. Both isolates of *P. gallica* were paired with each other and with A1 and A2 tester isolates (Table 1).

Table 1 – Species and isolates of *Phytophthora* spp. examined

Phytophthora spp.	ITS clade[a]	PRC no.[b]	Other references[b]	Geographical location, year	Isolated from	Source[b]
P. gallica	10	GAL 1	CBS 111474	NE France, 1998	*Quercus robur*[c]	PRC
	10	GAL 2	CBS 111475	Konstanz, Germany, 2004	*Phragmites australis*[c]	UKN
P. gonapodyides	6	GON 3		Freising, Germany, 1994	*Q. robur*[d]	PRC
	6	GON 18		Bad Aibling, Germany, 1998	*Alnus glutinosa*[d]	PRC
P. taxon 'PgChlamydo'[e]	6	CHLA 5		Nursery, Germany, 2001	*A. glutinosa*[c]	PRC
	6	CHLA 7		Nursery, Germany, 2001	*A. glutinosa*[c]	PRC
P. taxon 'salixsoil'[e]	6	SAL 1[f]	UKN 1	Konstanz, Germany, 2003	*P. australis*[c]	UKN
P. cambivora	7	CAM 109		Freising, Germany, 2004	*Fagus sylvatica*[d]	PRC
P. citricola	2	CIT 55[f]	BU 137	Garmisch, Germany, 1997	*F. sylvatica*[c]	UKN
	2	CIT 135		Nursery, Germany, 2002	*F. sylvatica*[d]	PRC
P. alni ssp. *alni*	7	ALN 377		Bamberg, Germany, 2002	*A. glutinosa*[d]	PRC
P. cambivora (A1)	7	CAM A1	CBS 356.78	Belgium, 1978	*Chamaecyparis* sp.	Kamoen O.
P. cambivora (A2)	7	CAM 1[f]		Freising, Germany, 1995	*F. sylvatica*[d]	PRC
P. cinnamomi (A1)	7	CIN A1	CBS 341.72	California, 1972	*Camelia japonica*	Zentmyer G.A.
P. cinnamomi (A2)	7	CIN 8		Dominican Republic, 2002	*Pinus occidentalis*[c]	PRC
P. drechsleri (A2)	8	DRE 3460	TUM 3460	Germany	NK	Zinker-nagel V.
P. cryptogea (A2)	8	CRY 1		Nursery, Germany, 1999	*Q. robur*[c]	PRC

NK, not known.
a ITS clades according to Cooke *et al.* (2000) and http://www.phytophthoradb.org.
b PRC, Phytophthora Research and Consultancy, Thomas Jung, Germany; TUM, Technische Universität München, Germany; UKN, University of Konstanz, Jan Nechwatal, Germany; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.
c Soil.
d Bark.
e Taxon not yet formally described in the literature; according to ITS sequence analysis related to *P. gonapodyides* (Brasier *et al.* 2003b).
f Isolate also used in the underbark inoculation test.