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Note

Physiological characteristics of the trunk sap rot pathogen *Fomitiporia* sp. on the "Sanbu-sugi" cultivar of *Cryptomeria japonica*

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ABSTRACT

An unidentified *Fomitiporia* sp. initially identified as *Fomitiporia punctata*, causes severe trunk sap rot on *Cryptomeria japonica* cultivar "Sanbu-sugi". We investigated the physiological characteristics of the mycelia of the causal fungus (F2, F6, and F43), in comparison with *F. punctata* (Fp) as a reference, in eight different experiments. The three unidentified isolates showed similar tendencies in change in mycelial growth during incubation, optimal growth temperatures (25 °C), optimal pH range (pH 5–6), glucose to yeast extract ratio (45), utilizable carbon sources (amylose, CM-cellulose, and pectin), utilizable nitrogen sources (yeast extract and polypepton), and water potential (−1.7 Mpa).

An unidentified *Fomitiporia* sp. causes severe sap rot with elongated cankers on the trunks of the economically important cultivar "Sanbu-sugi" (Okizumi 1993; Yoshimaru et al. 1995; Kondo and Watanabe 2005) of Japanese cedar, *Cryptomeria japonica* D. Don (Fig. 1A, B; Imazeki 1960; Aoshima et al. 1964). Aoshima et al. (1964) named this causal fungus from Chiba Prefecture, Japan, "Cha-ana-take-modoki" in Japanese, trunk sap rot, and identified it as *Fomitiporia punctata* (Pilát) Murrill. This fungus is a known pathogen of grapevine (Cortesi et al. 2000) and other angiosperm trees (Dai et al. 2007). Hattori et al. (2009, 2010) morphologically and phylogenetically analyzed the preserved fruit bodies and mycelial collections of this fungus in the Forestry and Forest Product Research Institute, Tsukuba, Japan, and found it to be distinct from *F. punctata* (Wagner and Fischer 2002; Cony et al. 2007; Dai et al. 2008), but a sister species within the genus. The fungus was observed to cause white rot on Sawara cypress, *Chamaecyparis pisifera* (Siebold & Zucc.) Endl., Hinoki cypress, *Ch. obtusa* Siebold & Zucc., and Japanese pear, *Pyrus pyrifolia* var. *culta* (Makino) Nakai (Kaneko et al. 2011). Ota et al. (2009, 2010) reported that the fungi isolated from *Cr. japonica*, from trunk rot of *Ch. pisifera*, and from *P. pyrifolia* var. *culta* were phylogenetically conspecific and distinct from *F. punctata*.

In previous research, trunks of living "Sanbu-sugi" trees were artificially inoculated and cankers were formed (Hayashi et al. 1980; Abe and Hattori 1991). Studies of the physiological characteristics of the fungus are important for understanding invasion strategy and prevention. Physiological studies, however, have not been reported. Previously there is no physiological characteristic study on the polypore. The aim of this study was to clarify the physiological characteristics of

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Fig. 1 – (A) Sap rot with elongate canker on the trunk of the "Sanbu-sugi" cultivar of *Cryptomeria japonica*, caused by *Fomitiporia* sp., Sammu, Chiba. (B) Fruit body of *Fomitiporia* sp. formed on trunk of a "Sanbu-sugi" cultivar. (C) Mycelia of isolates F2 (*Fomitiporia* sp. – left) and Fp (*Fomitiporia punctata* – right).

the mycelia of the causal fungus in preparation for ecological studies of invasion strategies and prevention.

Mycelia of three isolates of the causal *Fomitiporia* sp., F2, F6, and F43, and those of one isolate of *F. punctata* (designated as Fp hereafter), as a reference, were used (Fig. 1C, Table 1). The mycelia of F2 isolated from basidiospores of a fruit body developed on a "Sanbu-sugi" trunk in Matsuo, Sammu area, Chiba, Japan, were obtained from the Ministry of Agriculture, Forestry and Fisheries (MAFF). Mycelia of F6 were isolated from decomposed sapwood just under the bark on which fruit bodies of the fungus had developed on "Sanbu-sugi" in Sammu, Chiba, Japan, and mycelia of F43 were isolated from decomposed wood just under the bark on which *Fomitiporia* sp. fruit bodies had developed on a *P. pyrifolia* var. *culta* tree in Ichihara, Chiba. The wood was placed in potato dextrose agar (PDA, Tanabe, Tokushima, Japan) medium using sterilized cork borers (8 mm in diameter). These three isolates showed the same phylogenetic clade (Hattori et al. 2009; Ota et al. 2009). The isolate Fp originated from Fujisan, Yamanashi, and was obtained from MAFF; it was known to belong to a different phylogenetic clade from the above tree isolates (Hattori et al. 2009, 2010). Mycelia were grown on PDA medium in 90 mm Petri dishes, and 4 mm agar disks of mycelia were taken from the peripheries of the dense colonies and used as inocula.

Eight kinds of experiments were undertaken to analyze weight change during incubation, optimal temperature, optimal pH, glucose to yeast extract ratio (GY ratio), carbon sources, nitrogen sources, amino acid assistance, and water potential, for growth under the conditions shown in Table 2.

For the weight change experiment, mycelia were incubated from 0 to 70 days, and weighed at 7 days intervals. Media after incubation were filtered with membranes (0.45 μm in pore diameter) and glucose concentrations were determined by enzymatic method using an F-Kit for D(+)-glucose (Roche Diagnostics, Tokyo, Japan), measuring light absorbance at 340 nm using a double beam spectrophotometer (U-2900, Hitachi High Technologies Co., Tokyo, Japan). For the different temperature experiment, incubation temperatures (5–45 °C) were set at 5 °C intervals. For the different pH experiment, a pH range (ca. 3–9) was created by adjustment with sterilized 1 N-KOH or 1 N-HCl solution, and actual initial pH values after sterilization and final pH values after incubation were recorded. For the GY ratio, the media were adjusted to a ratio from 0 to100 with glucose (0–100 g/l) and yeast extract (1 g/l, Difco, Tokyo, Japan). For the carbon sources, each of 17 different carbon sources (30 g/l) was combined with yeast extract (1 g/l). The insoluble carbon sources were put into 100 ml Erlenmeyer flasks with distilled and deionized water (water hereafter) prior to sterilization. For the nitrogen sources, each of 10 different nitrogen sources (1 g/l) was combined with glucose (30 g/l). In the amino acid assistance experiment only the mycelia of F6 were used in comparison with the reference fungus. Each of 18 amino acids (0.4 g/l) was added to glucose (30 g/l) and of yeast extract (0.6 g/l). For the insoluble amino acids, 1 N-HCl was added and dissolved. Media without carbon, nitrogen, or amino acids were used as controls for the carbon and nitrogen source experiments and the added amino acid experiment. For the water potential experiment, to the media were added different concentrations of polyethylene glycol 6000 (0, 1, 2, 3, 4, 5, 10, 20, 30, 40, and 50% (w/v)); the water potential of the media measured using a vapor-pressure osmometer (Aqualab CX-2, Decagon devices, Inc., Washington, USA) at 25 °C was −0.69, −0.76, −0.83, −0.87, −0.92, −0.96, −1.10, −1.66, −2.49, −4.32, and −8.34, respectively.

Grown mycelia were collected using a nylon cloth (125 mesh), rinsed with water, dried at 95 °C for more than 24 h to constant weight, and weighed. The data were statistically

Table 1 – List of fungal mycelia used.

Isolate no.	Scientific name	Original name/number	Location	Host	Isolated section
F2	*Fomitiporia* sp.	MAFF420111, Pa46f	Matsuo, Chiba	*Cryptomeria Japonica*	Basidiospores
F6	*Fomitiporia* sp.		Sammu, Chiba	*C. japonica*	Decomposed sapwood
F43	*Fomitiporia* sp.		Ichihara, Chiba	*Pyrus pyrifolia* var. *culta*	Decomposed wood
Fp	*Fomitiporia punctata*	MAFF420211, WD-568	Fujisan, Yamanashi	Unknown	Fungal tissue of *F. punctata* fruit body