

FULL PAPER

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Genetic relationship of *Tricholoma matsutake* and *T. nauseosum* from the Northern Hemisphere based on analyses of ribosomal DNA spacer regions

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Abstract The genetic relationship among *Tricholoma matsutake* and *T. nauseosum* strains collected from various parts of the Northern Hemisphere was investigated using sequence analysis of the rDNA ITS region and PCR-RFLP analysis of the rDNA IGS-1 region. ITS sequence similarity between *T. matsutake* and *T. nauseosum* ranged between 98.1% and 100%. The strains of *T. matsutake* from coniferous forests and those from broad-leaved forests showed more than 99.8% similarity in their ITS sequences. Three distinct RFLP types were detected when IGS-1 regions were digested with *Cfr*13I. RFLP patterns showed no variability among the strains of *T. nauseosum* and those of *T. matsutake* from broad-leaved forests. This pattern corresponded to the dominant RFLP type in the Japanese population of *T. matsutake*. Thus, strains belonging to this RFLP type are widely distributed throughout East Asia and Europe and associated with many tree species of Pinaceae and Fagaceae. The result suggests that *T. matsutake* in coniferous and broad-leaved forests and *T. nauseosum* should be treated as the same species genetically.

Key words Ectomycorrhizal fungi · Intergenic spacer (IGS) · Internal transcribed spacer (ITS) · *Tricholoma matsutake* · *Tricholoma nauseosum*

Introduction

Tricholoma matsutake (S. Ito & Imai) Sing. is one of the most economically important ectomycorrhizal mushrooms in the world. In the early 1940s, about 12000 tons of *T. matsutake* were harvested each year in Japan, but since then production has dramatically decreased to less than 100 tons per year. In recent years, more than 2000 tons of *T. matsutake* or closely related species are imported annually, mainly from Bhutan, Canada, China, Korea, Mexico, Morocco, Turkey, and the United States (Japan Tariff Association 1994–2003; Suzuki 2005).

Among closely related species, *T. bakamatsutake* Hongo, *T. caligatum* (Viv.) Ricken, and *T. magnivelare* (Peck) Redhead are clearly distinguished from *T. matsutake* by morphological features (Hosford et al. 1997; Wang et al. 1997). Ito and Imai (1925) reported that *T. matsutake* (syn. *Armillaria matsutake*) and *T. caligatum* (syn. *A. caligata*) are quite different in the form of stipe base, i.e., the stipe of *T. matsutake* is enlarged and that of *T. caligatum* is tapered downward. However, the characteristics of stipe base could not be differentiated between *T. matsutake* and *T. nauseosum* because the stipe of *T. nauseosum* varies from tapered downward to enlarged (Kytövuori 1988). According to morphological studies with 139 specimens from Europe, North Africa, and Japan, Kytövuori (1988) proposed that *T. matsutake* was identical to *T. nauseosum*. Because the sequences of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region were 98%–99% matched between *T. nauseosum* and *T. matsutake*, they were considered to be the same species (Bergius and Danell 2000). However, only four strains of *T. nauseosum* from Sweden and one strain of *T. matsutake* from Japan were examined for sequencing. It seems to be too small a number to examine among the many strains throughout Japan.

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Table 1. Nucleotide sequence length of the internal transcribed spacer (ITS) region and restriction fragment sizes of the amplified IGS-1 region by *Cfr13I* of *Tricholoma matsutake* and *T. nauseosum* strains analyzed

Strain ^a	Geographical origin		Sample ^b	Dominant host tree ^c	ITS ^d		IGS-1		
	Locality	Country			Accession no.	Length (bp)	Reference ^e	RFLP type ^f	Fragment size (bp)
<i>T. matsutake</i>									
TmK1	Yangyang	Korea	f	<i>Pinus densiflora</i>	AB 188533	634	-	C	348, 215, 133, 63, 45
TmC1	Jiaohu, Jilin	China	f	<i>Pinus densiflora</i>	AB 188534	634	-	B	348, 63, 45
TmC2	Fuyu, Jilin	China	f	<i>Pinus densiflora</i>	AB 188535	634	-	A	215, 133, 63, 45
TmC3	Antu, Jilin	China	f	<i>Pinus densiflora</i>	AB 188536	634	-	C	348, 215, 133, 63, 45
TmC4	Wangqing, Jilin	China	f	<i>Pinus densiflora</i>	-	-	-	A	215, 133, 63, 45
TmY1	Lufeng, Yunnan	China	m	<i>Castanopsis orthacantha</i>	AB 188537	634	-	A	215, 133, 63, 45
TmY2	Lufeng, Yunnan	China	m	<i>Castanopsis orthacantha</i>	AB 188538	634	-	A	215, 133, 63, 45
TmY3	Lijiang, Yunnan	China	m	<i>Quercus</i> spp.	AB 188539	634	-	A	215, 133, 63, 45
TmY4	Lijiang, Yunnan	China	m	<i>Quercus</i> spp.	AB 188540	634	-	A	215, 133, 63, 45
TmY5	Lijiang, Yunnan	China	m	<i>Quercus</i> spp.	AB 188541	634	-	A	215, 133, 63, 45
TmY6	Lijiang, Yunnan	China	m	<i>Pinus yunnanensis</i>	AB 188542	634	-	A	215, 133, 63, 45
TmY7	Lijiang, Yunnan	China	m	<i>Pinus yunnanensis</i>	AB 188543	634	-	A	215, 133, 63, 45
TmY8	Lijiang, Yunnan	China	m	<i>Pinus yunnanensis</i>	AB 188544	634	-	A	215, 133, 63, 45
TmY9	Lijiang, Yunnan	China	m	<i>Pinus yunnanensis</i>	AB 188545	634	-	A	215, 133, 63, 45
TmY10	Zhongdian, Yunnan	China	m	<i>Quercus pannosa</i>	AB 188546	634	-	A	215, 133, 63, 45
TmY11	Zhongdian, Yunnan	China	m	<i>Quercus pannosa</i>	AB 188547	634	-	A	215, 133, 63, 45
TmY12	Zhongdian, Yunnan	China	m	<i>Quercus pannosa</i>	AB 188548	634	-	A	215, 133, 63, 45
Tm1	Ina, Nagano	Japan	-	<i>Pinus densiflora</i>	AF 204868	634	K	-	-
Tm4	Iwaizumi, Iwate	Japan	-	<i>Pinus densiflora</i>	-	634	K	-	-
Tm33	Minakuchi, Shiga	Japan	-	<i>Pinus densiflora</i>	AF 204806	634	K	-	-
Tm0945	Miyazu, Kyoto	Japan	-	<i>Pinus densiflora</i>	-	634	K	-	-
TmA-5	Kake, Hiroshima	Japan	-	<i>Pinus densiflora</i>	AF 202772	634	K	-	-
<i>T. nauseosum</i>									
Tn1	Valtellina	Italy	f	<i>Picea abies</i>	AB 188549	634	-	A	215, 133, 63, 45
Tn2	Lario	Italy	f	<i>Picea abies</i>	AB 188550	634	-	A	215, 133, 63, 45
Tn3	Varese	Italy	f	<i>Picea abies</i>	AB 188551	634	-	A	215, 133, 63, 45
Tn4	Grognardo	Italy	f	<i>Castanea setiva</i>	AB 188552	634	-	A	215, 133, 63, 45
Tn5	Val Gerola	Italy	f	<i>Picea abies</i>	AB 188553	634	-	A	215, 133, 63, 45
Tn6	Ticino	Switzerland	f	<i>Picea abies</i>	AB 188554	634	-	A	215, 133, 63, 45
Tn7	unknown	Sweden	f	<i>Pinus sylvestris</i>	AB 188555	634	-	A	215, 133, 63, 45
Tn8	unknown	Sweden	f	<i>Pinus sylvestris</i>	AB 188556	634	-	A	215, 133, 63, 45
Tn9	unknown	Sweden	f	<i>Pinus sylvestris</i>	AB 188557	634	-	A	215, 133, 63, 45
F-013392	Uppsala	Sweden	-	<i>Pinus sylvestris</i>	AF 145447	637	B	-	-
F-013393	Arjeplog	Sweden	-	<i>Pinus sylvestris</i>	AF 145448	636	B	-	-
F-013394	Vagnhärad	Sweden	-	<i>Pinus sylvestris</i>	AF 145446	635	B	-	-
F-013395	Kiruna	Sweden	-	<i>Pinus sylvestris</i>	AF 145449	639	B	-	-

^aSpecies names were according to collectors

^bDNA was extracted from hyphae sampled directly from the fruiting body (f) or from pure cultured mycelium (m)

^cDominant tree(s) at the collection site

^dTmC4 was not used for ITS sequence analysis

^eB, published by Bergius and Daneil (2000); K, published by Kikuchi et al. (2000); -, determined in this study

^fPublished by Guerrin-Laguette et al. (2002)

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