

Can we regulate the growth of shiro?

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'Shiro' refers to the dense mat of fungal filaments that *Tricholoma Matsutake* forms in soil in association with pine roots and soil particles. It is considered that the amount of mycelia in *T. matsutake* shiros is closely related to the amount of emerged *T. matsutake* fruiting body. However, under natural condition it is difficult to form shiro due to the poor growth of *T. matsutake* mycelia.

Therefore, focusing on the extremely slow growth of *T. matsutake* mycelia as one of the factors that hinder artificial cultivation of *T. matsutake*, several studies involving culture substrates, saprophytic ability of *T. matsutake*, which including total 12 isolates originate from Japan, North and South of China, by measuring the quantity of ergosterol, the activity of related enzymes and observing under the transmission electron microscope have been demonstrated. The results have been reported on the following subjects: (1) Cellobiose, pine (*Pinus densiflora*) bark and beech (*Fagus crenata*) sawdust could be carbon source for *T. matsutake* mycelia *in vitro* culture system, respectively; (2) The activity of *p*-nitrophenyl- β -D-lactopyranosidase (*p*-NPL), a cellulolytic enzyme, was significantly higher in *T. matsutake* cultures which incubated on sawdust-containing medium than on glucose-containing medium; (3) The transmission electron microscope observations indicated that hyphae of *T. matsutake* were able to degrade the xylem cell walls of sawdust in a sawdust-containing glucose-free liquid nutrient medium; (4) Growth of *T. matsutake* mycelia could be accelerated 15 times by incubating them on culture substrates that containing surfactants such as Tween 40 and Tween 80, which control the cell membrane permeability of the mycelia, or natural vegetable oils such as olive oil that increase the hydrophilicity of the mycelia. Such growth stimulation was associated with a sharp increase in protein and β -glucosidase excretion by hyphae in culture filtrates; (5) Typical ectomycorrhizal symbiosis was also confirmed between 5-month-old seedling of *P. densiflora* and *T. matsutake* on soil substrate containing 2% olive oil under controlled condition and semi controlled condition, respectively.

The path toward the artificial induction of *T. matsutake* shiros, which is the first step toward the artificial cultivation of *T. matsutake*, has been paved by the rapid cultivation of large amounts of *T. matsutake* mycelia. Further, the function of artificial shiros on forest ecosystems need to be investigated *in situ*, and also the dynamics of nutrient metabolism of the shiro need to be reexamined *in situ* by taking into consideration the new concept of ectomycorrhizal symbiosis in the rhizosphere.