

Modern Taxonomy and Medicinal Value of the *Flammulina* Mushrooms

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Flammulina mushrooms have an important place among Basidiomycetes as well as being useful in the production of medicines. In China, Japan, and other oriental countries these mushrooms (trivial name: enoki-take) have been cultivated traditionally since ancient times because of their nutritional and therapeutic value. Nowadays, they are among the six most actively cultivated mushroom species in the world. During the last decade of the last century, world production of *Flammulina* constantly increased. By

the end of the 20th century, its production was over 300,000 tons per year.

In nature *Flammulina* is a cosmopolite inhabiting deciduous forests during cold seasons. It can survive even light freezing temperatures and, therefore, it has been named “winter mushroom.” These mushrooms can be easily cultured on various media. Good growth and fruiting in culture as well as the capacity for biosynthesis of different biologically active metabolites make *Flammulina* cultures very popular for study.

Before the 1980s this genus was represented by only one species—*Flammulina velutipes* (W.Curt.: Fr.) Singer—although many authors pointed out varieties and forms of the species. In 1983, Bas made the first taxonomical record of *Flammulina* for Western Europe (Bas, 1983). He distinguished three species: *F. velutipes*, including two varieties—var. *velutipes* with f. *velutipes* and f. *longispora* Bas, and var. *lacteal* (Quél.) Bas; *F. ononidis* Arnolds; and *F. fennae* Bas. The validity of the species was later supported by the mating study of monokaryon isolates (Lamoure, 1989).

Nevertheless, study of numerous *Flammulina* specimens from different geographical regions showed that this genus needed more detailed investigation. In the late 1990s, a Mexican species, *F. mexicana* Redhead et al. (Redhead et al., 2000), and a New Zealand species, *F. stratosa* Redhead et al. (Redhead et al., 1998), were described. The taxonomy, biology, and distribution of taxa in *Flammulina* were investigated by Redhead and Petersen (1999). They showed the complexity of relationships among the taxa. After detailed examination of many specimens, the authors proposed two new species epithets and one varietal name—*F. populicola* Redhead et Petersen, *F. rossica* Redhead et Petersen, and *F. velutipes* var. *lupinicola* Redhead et Petersen—as well as two new combinations, *F. elastica* (Lasch) Redhead et Petersen and *F. elastica* f. *longispora* (Bas) Redhead et Petersen.

Genetic and molecular studies were carried out to support the described taxa. The mating study showed that *F. mexicana*, *F. stratosa*, *F. populicola*, and *F. fennae* were genetically isolated taxa. *F. velutipes* could be separated from the other species, but its intraspecific taxa (vars. *velutipes*, *lactea*, and *lupinicola*) could not be distinguished. They were also partially compatible with those of *F. ononidis*. Similarly, monokaryon isolates of *F. rossica* and *F. elastica* were partially compatible with one another but incompatible with those of other taxa. Low levels of interspecific hybridization were noted between *F. velutipes* and *F. populicola* and between *F. velutipes* and *F. rossica/elastica* (Petersen et al., 1999). These data were supported by the parsimony analysis of rDNA (ITS1-5.8S-ITS2 region) sequences, wherein the main clades were in agreement with defined mating groups and showed the phylogenetic

relationships between the species (Hughes et al., 1999). Isozyme analysis also could help to separate *F. velutipes* cultures from *F. rossica/elastica*, *F. ononidis*, and *F. fennae* (Alekhina et al., 2001).

Using these means, the modern molecular and genetic methods allow us to distinguish not only *Flammulina* specimens, but also cultures maintained in culture collections. This is most important considering the possibility of using *Flammulina* cultures in medicine. Much physiological and biochemical research has been carried out on *Flammulina* cultures all over the world since 1950s. Before this, only one species, *F. velutipes*, was found in the literature.

During recent years field trips, several new cultures of *F. velutipes* and *F. fennae* were isolated. Mating study and DNA analysis confirmed species names. The new strains were included in the LE (BIN) Culture Collection.

Much physiological and biochemical research has been carried out all over the world since 1950. Research had mainly been done on one species: *F. velutipes*. Literary data showed that *F. velutipes* obtained various biologically active compounds. Antibiotic properties and different enzymes such as proteinase, ribonuclease, phosphodiesterase, chitinase, acyl-KoA-synthetase, laccase, hydrolase complexes, hypocholesterol, and blood sugar had decreasing effects. Polysaccharides with antitumor activity were studied using *Flammulina* cultures. In Japan these mushrooms were used in making healthful beverage preparations (Psurtseva, 1987).

Study of *Flammulina* cultures from the Komarov Botanical Institute Basidiomycetes Culture Collection showed that the biosynthesis of proteolytic enzymes with thrombolytic and fibrinolytic activity was one of the specific characteristics. Strain producers of proteinase were selected. Methods of surface and submerged fermentations for exoenzymes were developed (Psurtseva and Mnoukhina, 1996 a,b).

Cultural study using morphological, physiological, and biochemical methods helped to verify *Flammulina* cultures from the LE (BIN) Culture Collection as *F. velutipes* var. *velutipes* f. *velutipes* and f. *longispora*, *F. velutipes* var. *lactea*, *F. ononidis* and *F. fennae* (Psurtseva and Mnoukhina, 1998). For biological species determination, the cultures were cultivated for fruit bodies and monokaryons were obtained. The

mating study allowed the distinguishing of *F. velutipes* strains from *F. rossica/elastica* complex (Petersen et al., 1999; Psurtseva and Petersen, 2000). Taxonomic verification following up-to-date nomenclature was done on *Flammulina* strains maintained in the LE (BIN) Culture Collection. These strains could be of considerable interest for medicinal investigation.

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