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Isozyme analysis of LE(BIN) Collection Flammulina strains

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Twenty-two dikaryotic *Flammulina* strains from different parts of Europe and the Far East were investigated using isozyme analysis. Clear isozyme polymorphism was detected in three enzymes, which differentiated the majority of *Flammulina* strains from each other, except for two *F. velutipes* strains. In the NJ (Neighbor-Joining) analysis the *Flammulina* strains formed two main groups. Main group I included strains of *F. velutipes* while main group II included the strains of *F. rossica/elastica* along with *F. ononidis* and *F. fennae*. No clear correlation was found between the geographical origin and clustering of strains tested.

Key words: *Flammulina velutipes, Flammulina fennae, Flammulina ononidis, Flammulina rossica*, isozyme analysis

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Introduction

The taxonomy of the genus *Flammulina* P. Karst. is more complicated than has been traditionally thought. For a long time different authors (Metrod 1952, Michael & Hennig 1958, Moser 1978) mostly emphasized variation in spore-size, form, and color in the fruiting bodies of *Flammulina velutipes* (Curtis : Fr.) Singer, but only Bas (1983), gave a more accurate taxonomic treatment and distinguished additional species of *Flammulina* in Western Europe: *F. velutipes*, *F. ononidis* Arnolds and *F. fennae* Bas. In addition, he subdivided *F. velutipes* into two varieties: var. *velutipes* and var. *lactea* (Quel.) Bas and into two formae under var. *velutipes*: f. *velutipes* and f. *longispora* Bas (Bas1983). The existence of three different species was supported by negative infertility tests on monosporic mycelia (Lamoure 1989). Nevertheless, extended studies using numerous specimens have shown that this genus needs further consideration.

Recently several new species were described in the genus *Flammulina*: *F. mexicana* Redhead et al. (Redhead et al. 2000) and a New Zealand species, *F. stratosa* Redhead et al. (Redhead et al. 1998). The systematics, biology and distribution of taxa in *Flammulina* were investigated by Redhead and Petersen (1999). They showed that relationships among the taxa were very complex. After detailed examination of many specimens the authors proposed two new species epithets and one varietal name – *F. populicola* Redhead & R. H. Petersen, *F. rossica* Redhead & R. H. Petersen and *F. velutipes* var. *lupinicola* Redhead & R. H. Petersen, as well as two new combina-

tions, *F. elastica* (Lasch) Redhead & R. H. Petersen and *F. elastica* f. *longispora* (Bas) Redhead & R. H. Petersen. These taxonomic circumscriptions were supported by a mating study in order to confirm or reject the names of taxa (Petersen et al. 1999). Mating experiments could discriminate *F. velutipes* from other taxa, but not between intraspecific taxa of *F. velutipes* (vars. *velutipes, lactea,* and *lupinicola*). Monokaryon isolates of all these taxa were 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. All other taxa (*F. mexicana*, *F. stratosa*, *F. populicola*, *F. fennae*) appeared to be genetically isolated. Low levels of interspecific hybridization were also noted between *F. velutipes* and *F. populicola*, and between *F. velutipes* and *F. rossica/elastica* (Petersen et al. 1999). Furthermore, these conclusions were supported by the parsimony analysis of rDNA (ITS1-5.8S-ITS2 region) sequences, where the main clades were in agreement with defined mating groups (Hughes et al. 1999).

Mycelial cultures of the genus *Flammulina* P. Karst. have been investigated by many authors in connection with their widespread use both in fundamental and applied mycology (Psurtseva 1987, Kinugawa 1993). Cultural studies of *Flammulina* strains have demonstrated the similarity of *F. velutipes* and *F. ononidis* and distinction of *F. fennae* in growth rate and laccase synthesis (Klan & Baudisova 1992). It was shown that *Flammulina* species were variable in laccase activity, and according to authors, cultures of *F. velutipes* and *F. ononidis* produced no laccase, whereas the *F. fennae* strain produced laccase (Klan & Baudisova 1992). The inability or instability of *F. velutipes* to produce laccase was formerly shown by other authors (Nobles 1965, Marr 1979). In our previous studies of *Flammulina* strains from the Komarov Botanical Institute Basidiomycetes Culture Collection [LE(BIN)] laccase activity has been found in all *Flammulina* species, but different isolates showed significant variation (from negative to strong positive) in laccase activity; moreover, it has been found that laccase production varied with growth time and seasonally (Psurtseva & Mnoukhina 1998; Psurtseva, unpublished results).

Our previous work on *F. velutipes* strains from LE(BIN) has resulted in the reidentification of isolates according to Bas' (Bas 1983) classification (Psurtseva & Mnoukhina 1998). Mycelium characteristics, enzymatic activity and spore characteristics from *in vitro* cultured fruiting bodies were used for strain identification (Psurtseva & Mnoukhina 1998). It was doubtful that *Flammulina* species could be determined solely through the use of mycelial features, but cultural characteristics – growth rate, macro- and micromorphology, enzyme activities – have great potential in intrageneric identification of isolates and may be advantageous when used together with other methods of diagnostics, such as morphology of basidiomata, sexual compatibility, molecular studies and isozyme analysis. A recent taxonomic revaluation of the genus *Flammulina* (Redhead & Petersen 1999) has suggested the taxonomy of LE(BIN) strains to be more uncertain than was previously thought. Some LE(BIN) *Flammulina* strains were used in sexual compatibility studies with exemplars of various taxa according to the new classification (Petersen et al. 1999). Some LE(BIN) strains were confirmed to belong to *F. velutipes*, others appeared to be *F. rossica* or *F. elastica*.

To confirm reidentification results, the present study based on isozyme analysis was initiated. Isozyme analysis is rather efficient for determining variation in fungi (Micales et al. 1986, Bonde et al. 1993) and it has been widely used in the identification of species and isolates of a large number of Homobasidiomyceteous genera including *Heterobasidion* (Stenlid 1985), *Pleurotus* (Magae et al. 1990; Zervakis & Labarere 1992), *Lentinus* (Ohmasa & Furukawa 1986), *Armillaria* (Wahlstrom et al. 1991; Agustian et al. 1994) and *Clavicorona* (Wu et al. 1995).

The present study is based on the following investigations: (1) determination of isozyme polymorphism and (2) determination of relationships among strains by phenetic and phylogenetic analyses of zymograms. The aim of our study was to investigate whether *Flammulina* isolates from different parts of Europe and Far East could be sorted by isozyme analysis into groups that correspond to the species, varieties and formae accepted in modern taxonomy (Redhead & Petersen 1999).

Materials and methods

Twenty-two dikaryotic *Flammulina* strains from different parts of Europe and the Far East maintained in the LE(BIN) were studied (Table 1). The strains were identified originally by using mycelium chracteristics, enzyme activities and spore morphology. Strains 803, 904, 905, 389, 535, 386, 388, 671, 817, 936 and N fruited in culture. The latest experiment on fruiting LE(BIN) strains was carried out by N. Psurtseva in My-

Table 1. Collection designation, species and geographic origin of the studied *Flammulina* isolates from the Komarov Botanical Institute LE(BIN) Basidiomycetes culture collection. Strains marked with an asterisk were previously analysed by Psurtseva and Mnoukhina (1998) and strain 0766 is the *F. ononidis* strain I also in the paper of Klan and Baudisova (1992). All strains, except 0386 and 0766, which originated from spores, were isolated from basidiomata.

Species (according to morphology)	LE(BIN) designation	Geographical origin	intersterility group (Petersen et al. 1999) / RFLP pattern (Methven et al. 2000)	Host plant
F. fennae	0882*	Leningrad region, Russia	– –	stump of a deciduous tree
F. ononidis	0766*	Czech Republic	–	*Ononis spinosa*
F. rossica (originally identified as ***F. velutipes var. lactea***)	0803*	Leningrad region, Russia	– –	stump of a deciduous tree
	0904*	Far East, Russia	*F. rossica/elastica*	*Salix sp.*
	0905*	Far East, Russia	*F.velutipes/rossica*	*Chozenia arbutifolia*
F. elastica (originally identified as ***F. velutipes var. velutipes f. longispora)***	0389*	Germany	*F. rossica/elastica*	–
	0535*	Leningrad region, Russia	*F. rossica/elastica*	*Salix caprea*
F. velutipes var. velutipes f.velutipes	0383	Leningrad region, Russia	– /AD	–
	0384	Ukraine	– /AD	–
	0385	Czech Republic	– /AD	*Juglans regia*
	0386	Czech Republic	– /AD	*Salix alba*
	0387	Ukraine	– /AD	–
	0388	Bulgaria	*F. rossica/elastica*	–
	0487	Czech Republic	–	*Crataegus oxycantha*
	0488	Czech Republic	– /AD	*Salix alba*
	0500	Netherlands	– /AD	–
	0515	Estonia	– /AD	–
	0671*	Leningrad region, Russia	*F. velutipes/ononidis*/BD	*Acer platanoides*
	0817*	Belorussia	*F. velutipes* /AD	*Robinia pseudoacaceae*
	0936*	Leningrad region, Russia	– /AD	*Populus alba*
	N	Leningrad	*F. rossica/elastica*	–
	T*	Leningrad region, Russia	*F. velutipes* /AD	stump of *Populus balsamifera*

cology Laboratory of the University of Tennessee, Knoxville, USA. Dried basidiomata of those fruited strains are kept in the UT Mycological Herbarium (TENN).

Dikaryotic mycelium was grown on an autoclaved sheet of cellophane (uncoated 325 P, Visella OY, Finland, produced by Courtaulds Films Cellophane, Bridgewater, UK) placed on the surface of beerwort-agar medium (beerwort 4°B, agar 18 g, H_2O 1000 ml, pH 5.8–6.0) in Petri dishes 90 mm in diameter with 20 ml of medium in darkness at 25° C for 7–10 days. Mycelium was thoroughly separated from the membrane and ground in liquid nitrogen in a pre-chilled mortar. The ground mycelium was transferred to an Eppendorf tube kept in ice. Proteins were extracted with STEB-buffer (1 $mlmg^{-1}$ mycelium, pH 8.0, Rosendahl & Sen 1992), after which the tubes were centrifuged at 10,000 g for 4 min (at 4 °C). The supernatant was used for gel electrophoresis and stored at –70 °C. Protein concentrations were estimated according to Bradford (1976).

Enzymes were separated by electrophoresis in vertical 6% polyacrylamide slab gels (7×10 cm) according to Rosendahl and Sen (1992) with an electrode buffer (8.3 mM Tris-barbitone, pH 7.45). Electrophoresis was carried out in a Mini-Protean II Dual Slab cell (Bio-Rad) apparatus at 150 volts at 4°C until the migration front reached the bottom of the gel. About 40 mg of proteins were added to each well of the gels together with bromophenol blue tracking dye.

Four enzyme activities were studied: alchohol dehydrogenase (ADH, EC 1.1.1.1) , malate dehydrogenase (MDH, EC 1.1.1.37), esterase (EST, EC 3.1.1.1) and lactate dehydrogenase (LDH, EC 1.1.1.27). After electrophoresis the gels were immediately stained in enzyme-specific reaction mixture containing dyes and catalysts. The following buffers were used: for ADH – 10 ml 0.5 M Tris/HCl (pH 8.0), 15 mg NAD, 10 mg NBT, 2.5 mg PMS, 0.6 ml ethanol; for MDH – 500 mg maleic acid, 5.0 ml 0.5 M Tris base , adjust to pH 8.0 with 1M KOH, 10 mg NAD, 10 mg NBT, 6 mg PMS; for EST – 2.5 ml 0.5 M Tris/HCl (pH 7.1), 1% α,β -Naphthyl acetate in 50% acetone-water, 25 mg Fast Blue RR salt; for LDH – 2.4 ml 0.5 M Tris/HCl (pH 8.0), 2.4 ml Na^+-lactate, 24 mg NAD, 6 mg MTT, 6 mg PMS. The final volume (50 ml) was obtained by adding H_2O. The positions of the bands were measured directly from the gel. The gels were also photographed.

The data matrix (Table 2), treated as Jaccard's distances, was analysed using NJ (Neighbor-Joining) algorithms of PHYLIP 3.5 package (Felsenstein 1993). The reliability of the phylogenetic NJ tree obtained from the data matrix including only phylogenetically informative characters was explored by using a bootstrapping procedure (SEQBOOT program) of PHYLIP 3.5 in which 3 × 100 different data sets with three different random seed values were analysed. The CONSENSE program of PHYLIP 3.5 was used in order to form consensus tree.

Table 2. Data matrix of isozyme characters in *Flammulina* isolates.

Species	Strain	Enzyme			
		EST	ADH	MDH	LDH
F. flammulina	671	1100100000	0101000	111101000	??????
	817	1100100000	1101000	111101000	010000
	936	1100100000	0101000	111101000	001000
	383	1100100000	1111000	101101000	??????
	N	1100100000	1111000	111101000	000100
	386	1100100000	0111000	111101000	??????
	387	1100100000	1111000	111101000	000010
	487	1100010000	1101000	111101000	001000
	488	1100010000	1110000	111101000	000010
	T	1100010001	1101000	111101000	100100
	500	1100010001	1001000	111101000	001000
	388	1110110001	0011000	111101000	000100
	384	1110010100	0111000	111101000	??????
	385	1110010100	1101000	111101000	100000
	515	1110010100	0111000	100101000	001000
F. rossica	803	1011000000	0111010	111111001	000010
	904	1110000001	0111010	110111010	010000
	905	1111000001	0111010	111111010	001000
F. elastica	389	1010000000	0111100	111101001	000100
	535	1110000011	0111100	110111010	000010
F. fennae	882	1110000000	0101101	110111100	000011
F. ononidis	766	1110001000	0101100	111111010	001000

Results

Esterase (EST), alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH) activity was studied in all 22 strains, while lactate dehydrogenase (LDH) activity was only studied in 18 strains representing the main types of EST, ADH and MDH activities. Each studied strain produced four or more EST isozymes (Fig.1A). The total number of EST isozymes was ten, of which three (E1, E2, E9) were major. No differences were detected in esterase profiles between strains 671, 817, 936, 383, N, 386, 387. Variability was considerable among the other strains. Practically all patterns were strain-specific, whereas Ea pattern was common to seven strains, patterns Eb and Ef were each common to two strains. Using the differences in the enzyme profiles the strains were divided into three main EST group: EST group I (pattern Ea) – presence of isozyme E5 and absence of E6 – strains 671, 817, 936, 383, N, 386 and 387; EST group II (patterns Eb to Eg) – presence of isozyme E6 – strains 487, 488, T, 500, 388, 384, 385, 515; EST group III (patterns Eh to En) – presence of isozyme E3 and absence of isozyme E5 and E6 – strains 803, 904, 905, 389, 535, 882 and 766. Strain 766 (pattern En) produced isozyme E7 not presented in other strains.

Eleven distinct ADH patterns (Aa to Ak) consisting of two to four isozymes were found in the *Flammulina* strains tested (Fig.1B). The A2 and A4 isozymes were present in nearly all the strains, except in strains 500, 388 and 488. Five patterns (Ad, Ae, Ag, Aj and Ak) were strain-specific, while Aa, Ab, Ac, Af and Ah were specific to two, three or four strains. According to ADH profiles, strains tested could be divided for two groups. Within the ADH group I (absence of isozymes A5, A6, A7) seven distinct patterns (Aa–Ag) consisting of two to four isozymes were differentiated for majority of strains. ADH group II (presence of isozymes A5, A6, A7) included the same strains as represented by the Est group III – 803, 904, 905, 389, 535, 882 and 766. These strains were characterized by specific isozymes A5, A6 and A7. Strain 882 produced isozyme A7 not present in other strains, while strains 803, 904, 905 produced isozyme A6 not present in other strains.

Eleven zymogram patterns for nine isozymes were produced for MDH activity (Fig. 1C). For this enzyme it was also possible to define two groups which were the same as for ADH. Within main group I all strains had the same pattern (Ma) composed of M1, M2, M3, M4 and M6 isozymes, except for strains 383, 515 and 388. The latter strains were characterized by slightly different patterns produced by absence of M2, M3 or M6 isozymes respectively. Other strains (803, 904, 905, 389, 535, 882 and 766), – group II, – had unique patterns caused by the presence of M5, M7, M8 or M9 isozymes (patterns Me to Mk). Again strain 0882 produced isozyme M7 (pattern Mj) not present in other strains, while strains 803, 904, 905 together with strains 535, 882 and 766 produced isozyme M5 not present in other strains.

Seven zymogram patterns formed by six LDH isozymes were studied in 18 strains (patterns not shown). As for ADH and MDH, strain 882 produced a LDH isozyme, which was not present in other strains (Table 2).

A phenogram representing isozyme relationships based on Jaccard's genetic distances using 17 (excluding LDH data) phylogenetically informative characters (present in at least two strains) of Table 2 is shown in the NJ tree (Fig. 2). In this tree all *F. velutipes* strains along with strains 388 and N of *F. rossica/elastica* were separated from other strains with bootstrap value 64 %. Only four other branches within the *F. velutipes* cluster had bootstrap support more than 50% (Fig.2), including the subgroup of strains 671 and 936 of *F. velutipes* from Leningrad region, which had identical isozyme patterns. Any other correlation between geographical origin and clustering of strains was not found.

Discussion

A total of 32 isozymes was detected for the four activities examined. Enzyme polymorphism and interstrain variability was found in all enzymes tested, but esterase was the most polymorphic and most variable enzyme within the strains examined. These results confirm a high degree of esterase polymorphism for some basidiomycetes, which previously also found in *Agaricus* and *Pleurotus* (Iracabal & Labarere 1993).

The results of the present study are in agreement with the previous morphological, growth and enzymatic studies (Bas 1983, Klan & Baudisova 1992, Psurtseva 1987, Psurtseva & Mnoukhina 1998) according to which LE(BIN) strains la-

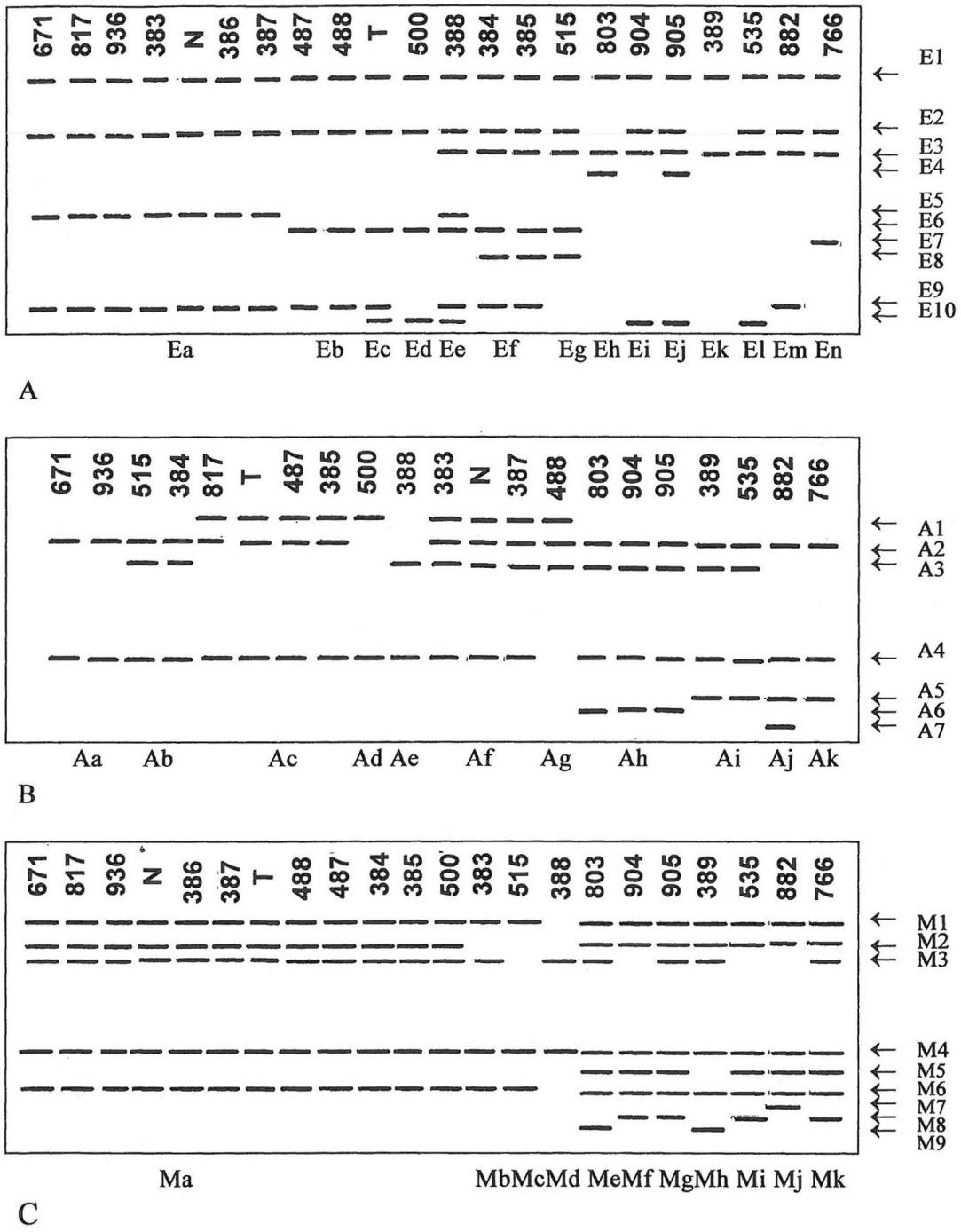

Fig.1. Diagrammatic representation of electrophoretic patterns of enzyme activities in *Flammulina* strains. Each isozyme is named by a code composed of the letter for the activity and a number corresponding to the migration order from the cathode to the anode. **A**. Esterase activities. **B**. Alcohol dehydrogenase activities. **C**. Malate dehydrogenase activities.

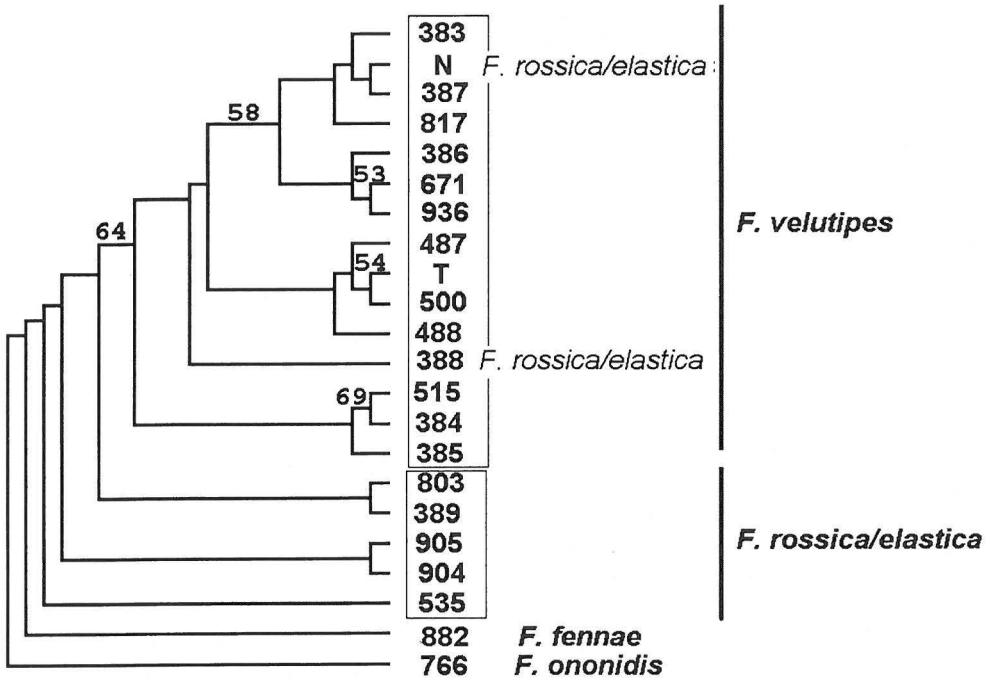

Fig. 2. Phylogenetic analysis (NJ) based on Jaccard's distances for the isozyme characters of Table 1 for *Flammulina* species.

beled as *Flammulina velutipes* can be divided into several species and/or varieties and formae. The isozyme data appear to be rather efficient in precision of correct grouping and in distinguishing different strains within species, which is in accordance with our previous study of *Fusarium* fungi (Yli-Mattila et al. 1996).

According to the results of the present work all *F. velutipes* isolates from different parts of Europe fall into one group which is separated from *F. fennae* and *F. ononidis* and most of *F. rossica/elastica* isolates where most differences in isozyme profiles were found. These *F. velutipes* strains were identified as *F. velutipes* var. *velutipes* f. *velutipes* by culture morphology in our previous studies (Psurtseva & Mnoukhina 1998) and taxonomic identity of some isolates was supported by sexual compatibility tests (Petersen et al. 1999). The bootstrap value supporting the separation is, however, only above 60%, probably, because of unsufficient number of characters. It should be noted that according to sexual compatibility studies of Petersen et al. (1999) two of the isolates (388 and N), were compatible with *F. rossica/elastica* but not with *F. velutipes*. In our study both isolates were, however, nested in *F. velutipes* group but not with *F. rossica/elastica*. It was interesting that one ADH isozyme (A6) was specific for all *F. rossica* strains. No characters supporting *F. elastica* group was found.

Flammulina rossica (originally labeled as *F. velutipes* var. *lactea*) and *F. elastica* (previously labeled *F. velutipes* var. *velutipes* f. *longispora*) isolates of the present work did not form a monophyletic group, but all of them but strains N and 388 were separated from *F. velutipes* as well as from *F. ononidis* and *F. fennae* strains. The group also included strain 905, which according to the sexual compatibility studies data showed a rather high level of compatibility with *F. velutipes* and to a lesser extent with *F. rossica* but was not compatible with *F. elastica* (Petersen et al. 1999).

More strains and isozyme characters are probably, required before the relationships between *F. velutipes*, and *F. rossica/ elastica* isolates can be

confirmed. Our results are, however, in most cases in agreement with conclusions of Redhead and Petersen (1999) who suggested new species and combinations in the genus *Flammulina*. Also it is well in accordance with the sequencing data of the ribosomal ITS1-5.8S-ITS2 area (Hughes et al. 1999) where *F. velutipes* formed its own cluster clearly separated from *F. fennae*, *F. ononidis*, *F. rossica/elastica* groups. The branches of the ITS tree of Hughes et al. (1999) are supported by higher bootstrap values than in the present isozyme tree, which is due to the much higher number of characters obtained by ITS sequencing. Unfortunately, in mentioned ITS analysis only one strain of *F. ononidis* and two strains of *F. rossica* and *F. elastica* were tested and none of the strains were the same as in our study.

Because we had only one isolate each of *F. fennae* and *F. ononidis*, it was not possible to compare them to *F. velutipes* strains in details in the phenetic analyses. Both strains were, however, clearly different from *F. velutipes* strains and produced strain-specific bands. It should be noted here that in the present study *F. fennae* strain of Russian origin was used. This strain is different from the well-known CBS strain (419.83), which seems to be misidentified (see discussion by Petersen et al. 1999; see also review of Petersen et al. <http://fp.bio.utk.edu/mycology/Flammulina/default.html>). Initially we differentiated *F. fennae* strain (882) from the other LE(BIN) *Flammulina* strains mainly by spore size according to Bas (1983). In pure culture our strain had the same morphological and growth characteristics as *F. velutipes* (Psurtseva & Mnukhina 1998), while in the CBS strain the cultural characteristics were different from *F. velutipes* (Klan & Baudisova 1992).

It was also possible to separate *Flammulina* strains of different species by RFLP patterns of ITS region (Methven et al. 2000). *F. velutipes* strains were divided in this work into three types, of which one was mainly American, the other one European and the third one Asian. According to the RFLP data 11 of our *F. velutipes* strains belong to the European RFLP type AD, while one (671) belongs to an American RFLP type BD (Table 1).

In future, more strains per species, varieties and formae should be compared to LE(BIN) *Flammulina* strains with identified reference strains, especially with *F. fennae*, *F. velutipes* and *F. rossica/elastica* and all isolates should be analyzed together before a final conclusion is drawn. In addition, further molecular studies, such as RAPD-PCR (Williams et al. 1990), the UP-PCR hybridization approach (Bulat et al. 1992, Yli-Mattila et al. 1997) and sequencing of more different DNA regions may be useful to confirm the status of different *Flammulina* isolates.

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References

Appel, D. J. & Gordon, T. R. 1996: Relationships among pathogenic and nonpathogenic isolates of Fusarium oxysporum based on the partial sequence of the intergenic spacer region of the ribosomal DNA. – Mol. Plant-Micr. Interact. 9: 125–138.

Agustian, A., Mohammed, J., Guillaumin, J. & Botton, B. 1994: Discrimination of some African Armillaria species by isozyme electrophoretic analysis. – New Phytologist 128: 135–143.

Bas, C. 1983: Flammulina in Western Europe. – Persoonia 12: 51–66.

Bonde, M. R., Micales, J. A. & Peterson, G. L. 1993: The use of isozyme analysis for identification of plant-pathogenic fungi. – New Phytologist 128: 135–143.

Bradford, M.M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. 72: 248–254.

Bulat, S.A., Mironenko, N.V., Zharova, V.P., Nagornaya, S.S. 1992: Species identification of fungi by polymerase chain reaction method with universal oligonucleotides. Genotyping of taxonomic species Issatchenkia orientalis Kudriavzev and Candida krusei (Cast.) Berkhout. – Cytologia i genetica (Kiev, Ukraine) 26: 25–31. [In Russian.]

Edel, V., Steinberg, C., Avelange, I., Laguerre, G. & Alabouvette, C. 1995: Comparison of three molecular methods for the characterrization of Fusarium oxysporum strains. – Phytopathology 85: 579–585.

Felsenstein, J. 1993: PHYLIP (Phylogeny Inference Package) version 3.5. University of Washington, Seattle, USA.

Hughes, K.W., McGhee, L.L., Methven, A.S., Johnson, J.E., & Petersen, R.H. 1999: Patterns of geographic speciation in the genus Flammulina based on sequences of the ribosomal ITS1-5.8S-ITS2 area. – Mycologia 91: 978–986.

Hughes, K.W. & Petersen, R.H. 2000: Apparent recombination or gene conversion in the ribosomal ITS region of a Flammulina (Fungi, Agaricales) hybrid. – Mol. Biol. Evol. 18: 94–96.

Iracabal, B. & Labarere J. 1993: Comparison of polymorphism and phenetic variability as determined by the study of hydrolases and oxidoreductases in two cultivated mushrooms, Agaricus bisporus and Pleurotus cornucopiae. – Exp. Mycology 17: 90–102.

Klan, J. & Baudisova, D. 1992: Cultural, enzyme and genetic studies in the genus Flammulina Karst. – Mycotaxon 43: 341–350.

Lamoure, D. 1989: Species concept in the Flammulina velutipes group. – Opera Bot. 100: 163–167.

Magae, Y., Haga, K., Taniguchi, H. & Sasaki, T. 1990: Enzymes of strains of Pleurotus species (Basidiomycetes) compared by electrophoresis. – J. Gen. Appl. Microbiol. 36: 69–80.

Marr, C.D. 1979: Laccase and peroxidase oxidation of spot test reagents. – Mycotaxon 9: 244–276.

Methven, A.S., Hughes, K.W., & Petersen, R.H. 2000: Flammulina RFLP patterns identify species and show biogeographical patterns within species. – Mycologia 92: 1064–1070.

Metrod, G. 1952: Les collybies. – Rev. Mycol. 17: 60–93.

Micales, J.A., Bonde, M.R. & Peterson, G.L. 1986: The use of isozyme analysis in fungal taxonomy and genetics. – Mycotaxon. 27: 405–449.

Michael, E. & Hennig, B. 1958: Handbuch fur Pilzfreunde. Erster Band, Die wichtigsten und häufigsten Pilze. Jena.

Moser, M. 1978: Die Röhrlinge und Blätterpilze (Polyporales, Boletales, Agaricales, Russulales). (Kleine Kryptogamenflora IIb/2, 4. Aufl).

Nobles, M.J. 1965: Identification of cultures of wood-inhabiting Hymenomycetes. – Can. J. Bot. 26: 281–413.

Ohmasa, M. & Furukawa, H. 1986: Analysis of esterase and malate dehydrogenase isozymes of Lentinus edodes by isoelectric focusing for the identification and discrimination of stocks. – Trans. Mycol. Soc. Jpn. 27: 79–90.

Paavanen-Huhtala, S., Hyvönen, J., Bulat, S.A. & Yli-Mattila, T. 1999: RAPD-PCR, isozyme, rDNA RFLP and rDNA sequence analyses in identification of Finnish Fusarium oxysporum isolates. – Mycol. Res. 103: 625–634.

Petersen R.H., Hughes K.W., Redhead S.A., Psurtseva N., Methven A.S. 1999: Mating systems in the Xerulaceae (Agaricales, Basidiomycotina): Flammulina. – Mycoscience 40: 409–424.

Psurtseva N.V.1987: Culture of Flammulina velutipes (Fr.) P.Karst. (biology and economic importance). – Mikol. i Fitopatol. 21: 477–486. [In Russian.]

Psurtseva N.V. & Mnoukhina A.Y. 1996: Cultural characteristic and exoproteinase activity of Flammulina species (Basidiomycetes). I. Surface cultivation. – Mikol. i Fitopatol. 30: 44–50. [In Russian.]

Psurtseva N.V. & Mnoukhina A.Y. 1998: Morphological, physiological and enzyme variability of Flammulina P. Karst. cultures. – Mikol. i Fitopatol. 32: 49–54.

Redhead S.A., Petersen R.H., Methven A.S. 1998: Flammulina (Agaricales): F. stratosa, a new New Zealand species distantly related to the cultivated Enoki mushroom. – Can. J. Bot. 76: 1589–1595.

Redhead S.A. & Petersen R.H. 1999: New species, varieties and combinations in the genus Flammulina. – Mycotaxon 71: 285–294.

Redhead S.A., Estrada-Torres A., Petersen R.H. 2000: Flammulina mexicana, a new Mexican species. – Mycologia 92:1009–1018.

Rosendahl, S. & Sen, R. 1992: Isozyme analysis of mycorrhizal fungi and their mycorrhiza. – Methods in Microbiology 24: 169–194.

Stenlid, J. 1985: Population structure of Heterobasidion annosum as determined by somatic incompatibility, sexual incompatibility, and isozyme patterns. – Can. J. Bot. 63: 2268–2273.

Wahlstrom, K., Karlson, J.O., Holdenrieder, O. & Stenlid, J. 1991: Pectinolytic activity and isozymes in European Armillaria species. – Can. J. Bot. 69: 2732–2739.

Yli-Mattila, T., Paavanen, S., Hannukkala, A., Parikka, P., Tahvonen, R. & Karjalainen, R. 1996: Isozyme and RAPD-PCR analyses of Fusarium avenaceum strains from Finland. – Plant Pathol. 45: 126–134.

Yli-Mattila, T., Mironenko, N.V., Alekhina, I.A. & Bulat, S.A. 1997: Universally primed polymerase chain reaction analysis of Fusarium avenaceum isolated from wheat and barley in Finland. – Agric. Food. Sci. Finland 6: 25–36.

Zervakis, G. & Labarere, J. 1992: Taxonomic relationships within the fungal genus Pleurotus as determined by isoelectric focusing analysis of enzyme patterns. – J. Gen. Microbiol. 138: 635–645.