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Population structure of the wood-decay fungus *Trichaptum abietinum* (J. Dicks.) Ryvarden in the Carpathian National Nature Park (Ukraine)

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Abstract. This paper provides a list of intracellular isozyme systems of *Trichaptum abietinum* (J. Dicks.) Ryvarden (Basidiomycetes) that can be used for population studies. Population structure of the fungus within the Carpathian National Nature Park (CNNP) was established. Percent of polymorphic loci in general was 83.3%. Groups of rare alleles were assigned Sod^{88}, Sod^{138}, $Sdh1^{91}$, and Est^{111}. Calculated Wright's fixation index allowed establishing privilege of the heterozygotes on locus *Acp* and homozygotes on locus *Sod*. The population of *T. abietinum* in the CNNP was in equilibrium state.

Key words: fungi, Basidiomycetes, population, genetic structure, isozymes, loci, alleles

1. Introduction

Wood for mushrooms is an unlimited source of carbon in their lifecycle. Generally, due to powerful fungal enzyme systems, the type of wood is not highly specific and fungi can develop on different substrates (Boddy *et al.* 2008). Saprotrophic Basidiomycota is able to break down plant litter and wood more rapidly than other fungi (Baldrian 2008; Osono & Takeda 2006). Advanced enzyme systems of mushrooms can be used for biotechnological purposes and in population studies.

Defining the genetic structure of populations is a logical first step in studies of fungal population genetics because the genetic structure of a population reflects its potential to evolve (Hogberg *et al.* 1999). These issues can be solved using DNA technologies or such enzyme systems as genetic markers (Shnyreva *et al.* 2004; Mondini *et al.* 2009; Mishra *et al.* 2010). So, the analysis of molecular variance suggested that native strains of the *Pleurotus* species in Kenya are genetically more diverse than their exotic counterparts cultivated there now (Otieno *et al.* 2015). Genetic analyses of European and Japanese indoor populations showed that *Serpula lacrymans* fungus spreads mainly sexually via basidiospores and significant genetic differentiation was detected among European populations of *Heterobasidion annosum* (Fr.) Bref. (Stenlid *et al.* 1994; Engh *et al.* 2010).

Co-dominant markers are, therefore, ideal for analyzing genotypic data of dikaryotic fungi. Isozymes remain a potent genetic marker in fungi that possess sufficient variation at allozyme loci (Siddiquee *et al.* 2007; Boiko 2015; Eichlerova *et al.* 2015) because it provides a fairly rapid and inexpensive alternative tool for the identification of some fungi species and for taxonomic studies (Bonde *et al.* 1993; Bragaloni *et al.* 1997; Annesi *et al.* 2003; Siddiquee *et al.* 2010).

One of the common species of fungi that colonize coniferous wood is *Trichaptum abietinum* (J. Dicks.) Ryvarden (Gorova 1980; Grand *et al.* 2009). *T. abietinum* is the most frequently observed white rot species, particularly in sapwood. Mycelium of *T. abietinum* colonizes from log surfaces and occupies large volumes of sapwood of *Picea abies* trees (Ovaskainen *et al.* 2013). However, widespread fungus does not indicate a deep knowledge of its biochemistry, genetics and ecology.

Until now, there have only been a few publications of its genetic characteristics and possibilities of application (Jasalavich *et al.* 2000; Kauserud *et al.* 2003a; Zjawiony 2004; Udu-Ibiam *et al.* 2014). There are very few studies aiming at determining the population structure of the *T. abietinum* fungus and they are mostly related to Northern parts of the globe (Kauserud *et al.* 2003a, 2003b; Grand *et al.* 2009; Runnel *et al.* 2015). In this regard, the territory of Ukraine remains unexplored.

The purpose of this article was to analyze the population structure of *T. abietinum* located within boundaries of the Carpathian National Nature Park (CNNP).

2. Material and methods

The research was performed on dikaryotic *T. abietinum* cultures, isolated from basidiocarps growing on fir in the CNNP (Ivano-Frankivsk region of Ukraine). Isolation of pure dikaryotic cultures was performed according to generally accepted methods (Bilay 1982). Briefly, precleaned basidiocarp was cut into pieces 3×3 mm, and transferred by a sterile mycological hook to 8% H_2O_2 solution for 1-2 min. The H_2O_2-treated fragment was placed in a tube with potato agar, and, after the appearance of pure mycelium, re-inoculated on pure culture media. Isolates were cultured on a glucose-peptone liquid medium that was poured into 50 ml Erlenmeyer flasks of 250 ml capacity. The initial pH of the nutrient medium was 5.0. Cultivation occurred from 15-18 days.

The production of monokaryotic cultures was performed by the spore prints method. The purity of the spores and their affiliation to monosporous cultures was controlled by microscopy. Total number of dikaryotic and monokaryotic cultures was sixteen and eighty four, respectively.

The mycelium was washed and dried by vacuum filtration, then homogenized in a Tris-citrate buffer system with subsequent filtration. The amount of total protein added into each well ranged from 40 to 60 μg. Electrophoretic separation of intracellular proteins was performed in 7.5% polyacrylamide gel using a Tris-glycine buffer system (pH 8.3) (Hames 1998). Histochemical detection of activity zones was performed for the following enzyme systems: glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), esterase (EST, EC 3.1.1.1), acid phosphatase (ACP, EC 3.1.3.2), sorbitol dehydrogenase (SDH, EC 1.1.1.14), and superoxide dismutase (SOD, EC 1.15.1.1) (Manchenko 2003).

Genetic diversity of populations was characterized by the following indicators: polymorphic loci (P), the average number of alleles per locus (A), effective number of alleles (A_E), the number of alleles per polymorphic loci (Ap), Shannon diversity index (I), observed and expected average genetic heterozygosity per locus (H_o and H_e) and Wright's fixation index (Nei 1978). Data were analyzed using POPGENE 32 computer-based software (Yeh *et al.* 1999).

3. Results

For the calculation of *T. abietinum* population genetic diversity in the CNNP, six isozyme loci were detected in five studied enzyme systems. The analysis of intracellular superoxide dismutase in monokaryotic cultures revealed three loci specific to *T. abietinum* representatives (Fig. 1).

Generally, five enzyme systems were used in the experiment. Twelve allelic variants controlled by six-enzyme gene loci were established. A complete list of enzyme systems, their control and electrophoretic mobility are presented in Table 1.

Data analysis revealed that the number of polymorphic loci amounted to 83.3%. Locus *Sdh2*, which was observed in 100% of studied *T. abietinum* cultures,

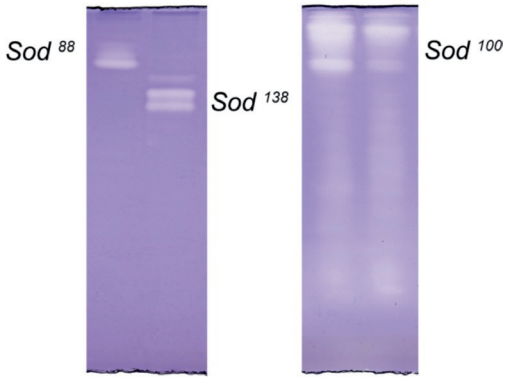

Fig. 1. Electrophoregram of superoxide dismutase allozymes of *Trichaptum abietinum* monokaryotic cultures

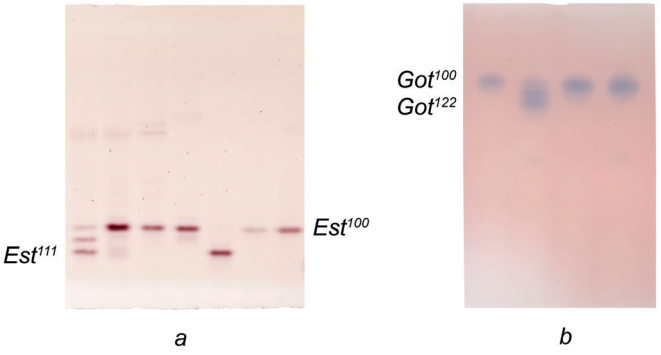

Fig. 2. Electrophoregram of allozymes of *Trichaptum abietinum* dikaryotic cultures
Explanations: a – esterase, b – glutamate oxaloacetate transaminase

Table 1. Enzyme systems, loci and alleles with their electrophoretic mobility of *Trichaptum abietinum*

Enzyme systems	Loci	Alleles	Rf (mobility relative to dye marker)
Glutamate oxaloacetate transaminase	*Got*	1 – 100	0.23
		2 – 122	0.28
Superoxide dismutase	*Sod*	1 – 88	0.14
		2 – 100	0.06
			0.16
		3 – 138	0.22
			0.26
Sorbitol dehydrogenase	*Sdh1*	1 – 91	0.60
		2 – 100	0.66
	Sdh2	1 – 100	0.31
			0.35
Esterase	*Est*	1 – 100	0.63
		2 – 111	0.70
Acid phosphatase	*Acp*	1 – 100	0.19
			0.33
		2 – 137	0.26
			0.33

Table 2. Genetic diversity of *Trichaptum abietinum* in the Ivano-Frankivsk population Carpathian National Nature Park

Locus	A	A_E	I	H_O	H_E
Got	2	1.3243	0.4101	0.2857	0.2637
Est	2	1.1529	0.2573	0.1429	0.1429
Acp	2	1.6897	0.5983	0.5714	0.4396
Sdh1	2	1.1529	0.2573	0.1429	0.1429
Sdh2	1	1.0000	0	0	0
Sod	3	1.3425	0.5091	0.1429	0.2747
Average	2	1.2771	0.3387	0.2143	0.2106

Explanations: A – the number of alleles per locus, A_E – the effective number of alleles per locus, I – Shannon diversity index, H_O – observed heterozygosity per locus, H_E – expected heterozygosity per locus

was monomorphic. Frequencies of allele isozymes are presented in Table 3.

The group of the most common alleles included: Got^{100}(one-band variant with Rf 0.23), Sod^{100}(two-band variants with Rf 0.06; 0.16), $Sdh1^{100}$(one-band variant with Rf 0.66), and Est^{100}(one-band variant with Rf 0.63); the group of rare alleles comprised: Sod^{88}(one-band variant with Rf 0.14), Sod^{138}(two-band variants with Rf 0.22; 0.26), $Sdh1^{91}$(one-band variant with Rf 0.60), and Est^{111}(one-band variant with Rf 0.70) (Table 1; Fig. 2).

Genetic diversity indicators of the *T. abietinum* population in the Carpathian NNP are presented in Table 2. In general, loci of the studied enzyme systems were not distinguished by the number of alleles and had an average of two alleles per locus (2.2 per polymorphic loci).

Table 3. The allele frequency of *Trichaptum abietinum* from Ivano-Frankivsk population in the Carpathian National Nature Park

Allele	Loci					
	Got	*Sod*	*Sdh1*	*Sdh2*	*Est*	*Acp*
1	0.8571	0.0714	0.0714	1.0000	0.9286	0.2857
2	0.1429	0.8571	0.9286	-	0.0714	0.7143
3	-	0.0714	-	-	-	-

Table 4. Wright's fixation index (Fis) according to the data from the Ivano-Frankivsk population *Trichaptum abietinum* in the Carpathian National Nature Park

Allele	Locus					
	Got	*Est*	*Acp*	*Sdh1*	*Sdh2*	*Sod*
1	-0.1667	-0.0769	-0.4000	-0.0769	-	-0.0769
2	-0.1667	-0.0769	-0.4000	-0.0769	-	1.0000
3	-	-	-	-	-	-0.0769
Total	-0.1667	-0.0769	-0.4000	-0.0769	-	0.4400

4. Discussion

Experiments failed to establish a high level of polymorphism for the *Schizophyllum commune* fungus: five alleles per locus, on average (James *et al.* 1999), while for the *Trichoderma harzianum* fungus, this value did not exceed 1.57 (Siddiquee *et al.* 2010). The polymorphism value depends strongly on the used set of isozymes. Monomorphic loci greatly reduce this index, but on the other hand, can serve as species molecular markers (Annesi *et al.* 2003; Boiko 2015). More informative measure is the mean number of alleles per polymorphic locus because Ap is independent of the proportion of polymorphic loci (Berg *et al.* 1997). The number of alleles per polymorphic loci was 2.2 for our set of isozymes (Table 2). It is well known that the effective number of alleles maintained in a population is defined as the inverse of the homozygosity. As it can be seen, the effective number of alleles, generally, is below the average and is inherent to many ecosystems (Huang *et al.* 1998; Shnyreva *et al.* 2004; Xin Qian *et al.* 2013). On average, within population the effective number of alleles per locus is 1.27.

Shannon index of genetic diversity was the highest for *Acp* and *Sod* loci, and these values were 0.5983 and 0.5091, respectively. The high Shannon index was also indirectly confirmed by high values of the effective allele number. The average heterozygosity indicating, which part of the population consisted of individuals that were heterozygous by studied markers had maximum values for the *Acp* locus. This demonstrated the important role of this locus in *T. abietinum* genetic diversity.

The investigated quantitative status of heterozygotes in the *T. abietinum* population made it possible to answer the question whether the population was in equilibrium state or not, according to the Hardy-Weinberg law. The impact of environmental factors can lead to significant changes in species genotype and, in extreme cases, can cause elimination of certain genes. According to the data, the average observed heterozygotes (0.2143) was almost equal to the expected heterozygote value (0.2106), and this is evidence that the Hardy-Weinberg equation for the studied population was performed. Thus, the population of *T. abietinum* in the Carpathian NNP was in equilibrium state. Performance of panmictic conditions in this species was observed due to high outcrossing and abundance of *T. abietinum* in this region. Fennoscandian geographic populations of *T. abietinum* also satisfied Hardy-Weinberg conditions (Kauserud & Schumacher 2003b).

Negative Wright's fixation index indicated a small excess of heterozygous genotypes for *Got*, *Est*, and *Sdh1* loci, and domination of *Acp* (Table 4). This indicated an important role of the sexual process in the development of the *T. abietinum* population.

It is worth emphasizing a significant advantage of the homozygous state of the *Sod* locus. It is difficult to explain such "contrasting" indicators. Increasing the number of natural materials and expanding research area will probably answer this question in future.

5. Conclusions

The obtained data made it possible to determine that *T. abietinum* population in the Carpathian National Nature Park was in equilibrium state. The proposed enzyme systems are indicative and informative features to be used in population studies of the fungus. Polymorphic loci, as a whole, reached 83.3%, and Sod^{88}, Sod^{138}, $Sdh1^{91}$, and Est^{111}were included in the group of rare alleles. The obtained Wright's fixation index allowed establishing prepotency of heterozygotes in the *Acp* locus and homozygotes – in the *Sod* locus of *T. abietinum* populations in the Carpathian NNP. An important role of the sexual process in the development of the *T. abietinum* population was established.

References

Annesi T., Coppola R. & Motta E. 2003. Isozyme analysis on some wood decay fungi. Journal of Plant Pathology 85: 87-90.

Baldrian P. 2008. Enzymes of saprotrophic basidiomycetes. In: L. Boddy, J. C. Frankland & P. van West (eds.). Ecology of Saprotrophic Basidiomycetes, pp. 19-41. Academic Press, Amsterdam.

Berg E. E. & Hamrick J. L. 1997. Quantification of genetic diversity at allozyme loci. Can. J. For. Res. 27: 415-424.

Bilay V. I. 1982. Metodyi eksperimentalnoy mikologii. Naukova dumka, Kyiv.

Boddy L., Frankland J. & West P. 2008. Ecology of saprotrophic Basidiomycetes. 372 pp. Published by Elsevier Ltd.

Boiko S. M. 2015. Allozyme polymorphism in mono- and dikaryotic cultures of fungus *Schizophyllum commune* Fr. (*Basidiomycetes*). Cytology and Genetics 49: 27-31.

Bonde M. R., Micales J. A. & Peterson G. L. 1993. The use of isozyme analysis for identification of plant-pathogenic fungi. Plant Disease 77: 961-968.

Bragaloni M., Anselmi N. & Cellerino G. P. 1997. Identification of European *Armillaria* species by analysis of isozyme profiles. European Journal of Forest Pathology 27: 147-157.

Eichlerova I., Homolka L., Zifcakova L., Lisa L., Dobiasova P. & Baldrian P. 2015. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. Fungal ecology 13: 10-22.

Engh I. B., Carlsen T., Kauserud H., Hogberg N., Saetre G.-P. & Doi S. 2010. Two invasive populations of the dry rot fungus *Serpula lacrymans* show divergent population genetic structures. Mol. Ecol. 19: 706-715.

Gorova T. L. 1980. Macromycetes derivatives spruce Ukrainian Carpathians. Ukr. Bot. J. 37: 44-50 (in Ukraine with English summary).

Grand L. F., Vernia C. S. & Munster M. J. 2009. Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Trametes* and *Trichaptum*. Mycotaxon 106: 243-246.

Hames B. D. 1998. Gel Electrophoresis of Proteins: A Practical Approach 3rd ed. 352 pp. Oxford University Press, New York.

Hogberg N, Holdenreider O. & Stenlid J. 1999. Population structure of the wood decay fungus *Fomitopsis pinicola*. Heredity 83: 354-360.

Huang H., Dane F. & Kubisiak T. 1998. Allozyme and RAPD analysis of the genetic diversity and geographic variation in wild populations of the American chestnut (Fagaceae). Am. J. Bot. 85: 1013-1021.

James T. Y., Porter D., Hamrick J. L. & Vilgalys R. 1999. Evidence for limited intercontinental gene flow in the cosmopolitan mushroom *Schizophyllum commune*. Evolution 53: 1665-1677.

Jasalavich C. A., Ostrofsky A. & Jellison J. 2000. Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Applied and environmental microbiology 66: 4725-4734.

Kauserud H. & Schumacher T. 2003a. Ribosomal DNA variation, recombination and inheritance in the basidiomycete *Trichaptum abietinum*: implications for reticulate evolution. Heredity 91: 163-172.

Kauserud H. & Schumacher T. 2003b. Regional and local population structure of the pioneer wood-decay fungus *Trichaptum abietinum*. Mycologia 95: 416-425.

Manchenko G. P. 2003. Handbook of detection of enzymes on electrophoretic gels. 553 pp. CRC Press.

Mishra A. K., Sharma K. & Misra R. S. 2010. Isozyme and PCR-based genotyping of epidemic *Phytophthora colocasiae* associated with taro leaf blight. Archives of Phytopathology and Plant Protection 43: 1367-1380.

Mondini L., Noorani A. & Pagnotta M. 2009. Assessing plant genetic diversity by molecular tools. Diversity 1: 19-35.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Osono T. & Takeda H. 2006. Fungal decomposition of *Abies* needle and *Betula* leaf litter. Mycologia 98: 172-179.

Otieno O. D., Onyango C., Onguso J. M., Matasyoh L. G., Wanjala B. W., Wamalwa M. & Harvey J. J. 2015. Genetic diversity of Kenyan native oyster mushroom (*Pleurotus*). Mycologia 107: 32-38.

Ovaskainen O., Schigel D., Ali-Kovero H., Auvinen P., Paulin L., Norden B. & Norden J. 2013. Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi. ISME Journal 7: 1696-1709.

Runnel K., Tamm H. & Lohmus A. 2015. Surveying wood-inhabiting fungi: Most molecularly detected polypore species form fruit-bodies within short distances. Fungal Ecology 18: 93-99.

Shnyreva A. V. Belokon Yu. S., Belokon M. M. & Altukhov Yu. P. 2004. Interspecific genetic variability of the Oyster mushroom *Pleurotus ostreatus* as revealed by allozyme gene analysis. Russian Journal of Genetics 40: 871-881.

Siddiquee S., Abdullah F., Guan T. S. & Rohaza E. 2007. Level in allozyme variations of Malaysian isolates of *Trichoderma harzianum* and its taxonomic implications. Research journal of microbiology 10: 717-726.

Siddiquee S., Guan T. S. & Kalsom Y. 2010. Isozyme analysis and relationships among three species in Malaysian *Trichoderma* isolates. J. Microbiol. Biotechnol 20: 1266-1275.

Stenlid J., Karlsson J. O & Hogberg N. 1994. Intraspecific genetic variation in *Heterobasidion annosum* revealed by amplification of minisatellite DNA. Mycol. Res. 98: 57-63.

Udu-Ibiam O. E., Ogbu O., Ibiam U. A., Nnachi A. U., Agah M. V., Ukaegbu C. O., Chukwu O.S., Agumah N. B. & Ogbu K. I. 2014. Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria. IOSR Journal of Pharmacy and Biological Sciences 9: 86-91.

Xin Qian, Cai-xia Wang & Min Tian. 2013. Genetic diversity and population differentiation of *Calanthe tsoongiana*, a rare and endemic orchid in China. Int. J. Mol. Sci. 14: 20399-20413.

Yeh F. C., Yang R. & Boyle T. 1999. POPGENE Version 1.32. Microsoft window-based freeware for population genetic analysis. Univ. Alberta. Center Intern. Forestry Res.

Zjawiony J. K. 2004. Biologically active compounds from Aphyllophorales (Polypore) fungi. J. Nat. Prod. 67: 300-310.