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Diversity of DNA and protein contents of spores of the closely related oyster fungi *Pleurotus pulmonarius* and *P. ostreatus* as studied by flow cytometry

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Abstract: For quantitative evaluation of nuclear DNA and protein contents of spores, the flow cytometer (PAS) with staining DAPI SR101 was employed. A total of 22 specimens of *Pleurotus* were studied. Bi-parametric analysis of spore DNA and protein contents revealed that fruitbodies of *P. ostreatus* produce one or two distinct spore populations the DNA and protein contents of bigger of which are comparable to those of *P. pulmonarius* that produces only one distinct spore population. The difference in genome size and chromosome number within *P. ostreatus* appears as heteroploidy (see Fungal Genome Size Database http://www.zbi.ee/fungal-genomesize/). We presume that the divergence that arises from a spore print reflects the fate of a hybrid genome in meiosis. Our results seem to confirm that parental genomes of different sizes segregate in meiosis. Zygotic meiosis can occur even in the case of low density of homology between chromosomes (CLP and aneuploidy) and may ensure distribution of highly different strains.

Kokkuvõte: Lähedaste liikide *Pleurotus pulmonarius* ja *P. ostreatus* eoste DNA ja proteiini sisalduse diversiteet uurituna läbivoolutsütomeetria abil.

Eoste tuuma DNA ja proteiini sisalduse kvantitatiivseks määramiseks kasutati läbivoolutsütomeetriat ja fluorestsentsvärvi DAPI SR101. Uuriti 22 eosproovi, mis olid saadud austerserviku *Pleurotus ostreatus* ja kopsserviku *P. pulmonarius* viljakehadest. Eosete tunnuste kahemõõtmeline analüüs näitas, et austerserviku viljakehad produtseerivad ühesuguseid või kahesuguseid eoseid, kusjuures suurema DNA- ja proteiinisisaldusega eosed on võrreldavad kopsserviku eostega. Kopsserviku eosproovides eospopulatsioonide lahknemist ei esinenud. Austerserviku genoomi suuruse ja kromosoomide arvu erinevus avaldub kui heteroploidsus (vaata Fungal Genome Size Database http://www.zbi.ee/fungal-genomesize/).Võib arvata, et lahknemine eosproovi tuumade DNA- ja proteiinisisalduses peegeldab seene hübriidse genoomi saatust meioosi käigus. Sügootne meioos võib toimuda isegi kromosoomide arvu ja pikkuse poolest erinevate kromosoomikomplektide puhul ja võimaldada väga erinevate tüvede levikut.

INTRODUCTION

The oyster mushroom *Pleurotus ostreatus* is an edible basidiomycete with increasing agricultural and biotechnological importance. A study of the two closely related species, *Pleurotus pulmonarius* and *P. ostreatus,* by Bresinsky et al. (1977, 1987) reported incompatibility between *P. ostreatus* and *P. pulmonarius*. Hilber (1982) showed incompatibility between *P. ostreatus* and all other tested species. Using classical matings and RAPD-PCR analysis, Shnyreva and Shtaer (2006) demonstrated the presence of a reproductive barrier between the above two species and a relatively recent origin of their divergence. Petersen and Ridley (1995) reported collection of *P. pulmonarius* which was weakly compatible with *P. ostreatus*.

Nuclear DNA amount and genome size are important biodiversity characters. The intraspecific variability in the genome sizes of *P. ostreatus* and *P. pulmonarius* reported in the literature data and presented in the Fungal Genome Size Database http://www.zbi.ee/fungal-genomesize/ (Kullman et al., 2005; Gregory et al., 2007), remains within 20.8 Mb to 35.1 Mb. The database also presents the methods used. Microfluorometric measurements of the genome size of *P. pulmonarius* range from 24.2 to 27.5 Mb (14% difference) and of *P. ostreatus* range from 24.0 to 27.5 Mb (15% difference) (data by Wittmann-Meixner, 1989, calculated in Kullman et al., 2005). Electrophoretic karyotyping indicated that genome size in *Pleurotus ostreatus*

varies from 20.8 to 35.1 Mb (relative difference >60%) and chromosome number ranges from 6 to 11 (Sagawa and Nagata, 1992; Peberdy et al., 1993; Ramírez, et al., 2000). Such a difference in chromosome number and genome size appears as heteroploidy. Chromosome length polymorphism (CLP) is rather widespread among fungi and several examples are known where it is accompanied by significant differences in genome size among the strains of one and the same species (Zolan, 1995). Larraya, et al. (1999) found CLP affecting various chromosomes of *P. ostratus*. What happens to the size of a genome of the two closely related species, *P. ostreatus* and *P. pulmonarius,* during meiosis can be established by analysing the DNA content of a spore print of a single fruitbody by flow cytometry. Staining with DAPI SR101 is essential in the study of heteroploidy and especially in the study of the fate of different genomes with CLPs (Kullman, 2000).

MATERIAL AND METHODS

For the quantitative evaluation of the nuclear DNA and protein contents of spores, the flow cytometer Particle Analysing System (PAS) with staining with DAPI-SR101 was employed at the Institute of Radiobiology (Westfälische Wilhelms-Universität, Münster, Germany). DAPI-SR101 allows bi-parametric analysis of nuclear DNA and protein contents. The number of nuclei in spores was determined using the fluorescence microscope Olympus. The spore print of the oyster mushroom *P. ostreatus* (TAA 142824) was applied as the standard (24 Mb). The spore nuclei of *P. ostreatus* are mononuclear and unreplicated and hence their DNA content corresponds to genome size (Kullman, 2000).

The spore print of 22 specimens of *P. ostreatus,* and *P. pulmonarius* from TAA(M) (Table 1) were studied.

Staining Protocol: The DNA stain DAPI in combination with the protein fluorochrome SR 101 was used for bivariate DNA and protein analysis. For preparation and staining of the fungal material, a slightly modified method (Ulrich and Ulrich, 1991) was employed at the laboratory at Münster University. 1 ml 0.5% Pepsin pH 1.8 was added to the spore print by briefly vortexing and incubated for 3 min at room temperature. Then a 4- fold volume of DAPI-SR101 (Partec GmbH, Münster, Federal Republic of Germany) was added, and the sample was incubated for 20 min by vortexing intermittenly two times and then left overnight in the refrigerator. Before use, the spores were filtered through 20µm nylon mesh.

RESULTS

Measurement of the studied material demonstrated that some fruitbodies of *P. ostreatus* could produce two more or less equally represented subpopulations of spores with different genome sizes and protein content. Diversity was found in the spore print in half of the studied specimens of *P. ostreatus*. Diversity was not detected in the spore print of all specimens of *P. pulmonarius* and in half of the specimens of *P. ostreatus* (Table 1 and Fig. 1).

DISCUSSION

Our previous studies involving flow cytometry have revealed that spore prints of *Pleurotus* and *Lentinula* species may represent spore populations with two different DNA contens (Kullman 2000, 2002, Kullman at al., 2005, 2006). Divergence was even more evident in a spore print of an industrially cultivated strain of *P. ostreatus* (Kullman 2002).

The intraspecific variability in the genome sizes of *P. ostreatus* found in our study remains largely within the limits reported in the literature data and presented in the Fungal Genome Size Database http://www.zbi.ee/fungal-genomesize/ (Kullman et al., 2005; Gregory et al., 2007). Measurements of our material proved presence of heteroploidy in *P. ostreatus* Using flow cytometry we found that genome size in the same species appears to range from 18.7 Mb (mean genome size of two equally represented spore subpopulations in the spore print of a specimen, 14.0–23.3 Mb) to 28.7 Mb (53 % difference between the fruitbodies, 105% difference between the spore subpopulations). We studied the spore prints of fruit bodies of *P. ostreatus* as well as *P. pulmonarius* (Table 1, Fig 1). The intraspecific variability in the genome sizes of *P. pulmonarius,* 23.3 to 32.9 (44.1) Mb (41.2% (89.3%) difference) is higher than reported earlier, 24.2 to 27.5 Mb (14% difference)

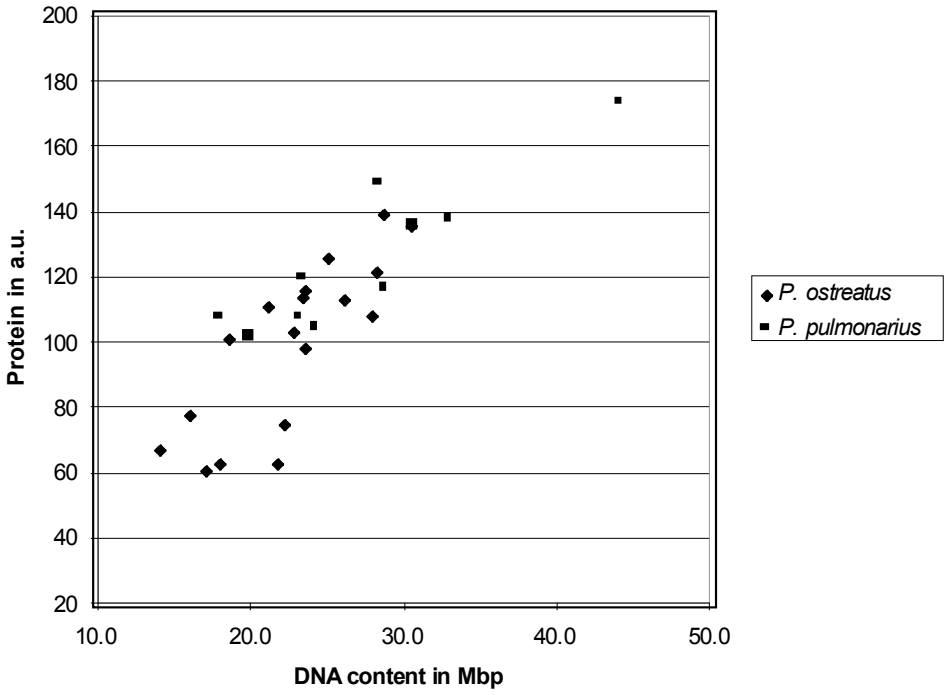

Fig. 1. Diversity of nuclear DNA and protein contents in spore prints from different specimens. Half of the specimens of *P. ostreatus* have diverged on the basis of their nuclear DNA and protein contents. All specimens of *P. pulmonarius* and half of the specimens of *P. ostreatus* have not diverged on the basis of their nuclear DNA and protein contents (see Table 1). (Mb - DNA content in megabase pairs of nucleotides, a.u. – protein content in arbitrary units).

(data by Wittmann-Meixner, 1989 calculated in Kullman et al., 2005). The specimen of *P. pulmonarius* TAA 179857 with genome size 44.1 Mb can be diploid.

Bi-parametric analysis of spore DNA and protein contents revealed that fruitbodies of *P. ostreatus* may produce two distinct spore populations the DNA and protein contents of bigger of which are comparable to those of *P. pulmonarius* that produces only one distinct spore population. This can indicate polyploidisation in this complex, and/or the hybrid origin of *P. osteratus,* which evidently supports the opinion about ongoing speciation in this case. Obviously, a dikaryon may originate from the parents with different genome sizes. Our results seem to confirm that parental genomes of different sizes can segregate in meiosis. We presume that the divergence arising from a spore print reflects the fate of a hybrid genome in meiosis (Kullman 2000). Primitive, zygotic meiosis can occur even in the case of low density of homology between chromosomes (aneuploidy and CLP) and may ensure distribution of highly different strains (Kullman, 2002; Kullman et al., 2005).

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Table 1. Nuclear DNA and protein contents of the studied specimens

		Mean DNA content in Mbp ±CV%		Mean protein content in a.u. ±CV%	
Spore print		Subpopulation 1	Subpopulation 2	Subpopulation 1	Subpopulation 2
Pl. ostreatus	TAA77603	14.0±19%	23.3±16%	67±18%	114±16%
Pl. ostreatus	TAA125925	16.0±17%	25.0±15%	78±17%	126±17%
Pl. ostreatus	TAA126992	17.0±12%	26.1±11%	61±13%	113±08%
Pl. ostreatus	TAA179856	17.9±25%	30.4±14%	63±25%	136±15%
Pl. ostreatus	TAA151033	18.5±14%		101±10%	
Pl. ostreatus	TAA150933	21.2±11%		111±08%	
Pl. ostreatus	TAA142824	21.7±19%	27.8±12%	63±10%	108±08%
Pl. ostreatus	TAA175016	22.2±16%	28.3±14%	75±17%	122±15%
Pl. ostreatus	TAA009135	22.7±10%		103±09%	
Pl. ostreatus	TAA150066	23.5±17%		98±08%	
Pl. ostreatus	TAA 151089	23.5±22%		116±18%	
Pl. ostreatus	TAA107777	28.7±16%		139±17%	
Pl. pulmonarius	TAA106162	17.9±13%		108±16%	
Pl. sp.	TAA107552	19.9±13%		102±12%	
Pl. sp.	TAA107549	23.1±14%		108±11%	
Pl. pulmonarius	TAA 107504	23.3±09%		120±07%	
Pl. pulmonarius	TAA 157788	24.2±14%		105±17%	
Pl. pulmonarius	TAA 093854	28.3±09%		149±09%	
Pl. pulmonarius	TAA 179250	28.7±15%		117±18%	
Pl. pulmonarius	TAA 179798	30.5±10%		136±08%	
Pl. pulmonarius	TAA 179231	32.9±11%		138±13%	
Pl. pulmonarius	TAA 179857	44.1±11%		174±14%	

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