Diversity in the spore print of the hybrid of

*Lentinula* and *Pleurotus* on the basis of nuclear DNA content

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Abstract. Four spore prints of the strains of *Lentinula edodes* L18, *L. edodes* L19, *Pleurotus ostreatus* × *P. ostreatus* P403×P404 and *L. edodes* × *P. ostreatus* L19-1×P404 were studied with flow cytometry. Diversity was found in the spore print of the interspecies hybrid fruitbody L19-1×P404 and in the spore print of the fruitbodies of the parental strains P403×P404 and L19. One fruitbody can produce two or less equally represented subpopulations of spores with different nuclear DNA and protein contents. There exist subpopulations with similar DNA content among the spore prints produced by parental and hybrid fruitbodies. The dikaryon stage in the fungal life cycle allows for the genomes of different species to coexist and to even produce a fruitbody. Diversity of the nuclear DNA content of a spore print reflects the fate of the hybrid genome during meiosis. After karyogamy, this fruitbody distributes viable spores. It means that meiosis can even occur in the case of low density of homology between the chromosomes (CLP and aneuploidy) ensuring distribution of highly different genome sizes of strains.

Keywords: DNA content, meiosis, heteroploidy, flow cytometry, DAPI-SR101, fungi

Introduction

Nuclear DNA content and genome size are important biodiversity characters the study of which has both practical and theoretical uses in biology (Bennett and Leitch, 1998, 2005). The total DNA content of the unreplicated haploid nuclear genome is characteristic of each species and is known as its C-value (for terminology see Greilhuber et al., 2005). Genome DNA amount serves as a criterion for genome mutation and speciation.

To measure the size of plant or animal genome, the flow cytometry (FCM) has met ever wider application. FCM is a convenient and rapid tool for estimation of nuclear DNA and cellular protein content. Staining with DAPI-SR101 is essential in the study of aneuploidy and especially in genetic characterisation of heteroploids (Kullman, 2000, 2002b).

It is argued that if one basidioma can produce two different kind of spores with different DNA contents, then the hybrid dikaryons of these basidioma my also differ in genome size. In the case of a commercial oyster mushroom, the difference in spore DNA content of two subpopulations was 1.5 - fold, and in the case of a wild oyster mushroom, 1.2 – fold (Kullman, 2002b). This indicates differences in many chromosomes. It was suggested that during meiosis two hybrid genomes can diverge back regarding their size.

The aim of this study was to compare the nuclear DNA content of spore prints of an interspecies hybrid of Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) and of oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm.), as well as their parental strains, in order to clarify the stability of their genome size in the course of meiosis.

Material and Method

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 Institute of Radiobiology (Westfälische Wilhelms-Universität, Münster, Germany). DAPI-SR101 allows bi-
parametric analysis of nuclear DNA and protein content. The number of nuclei in cells 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 (Kullman, 2000).

The spore print of the interspecies hybrid of Shiitake mushroom and oyster mushroom (produced by protoplast fusion of neohaplonts for commercial cultivation) as well as their parental strains were obtained from Mexico (Department of Food Science and Biotechnology, Ciudad Universitaria, Col. Copilco). Four spore prints of the strains of *Lentinula edodes* L18, *L. edodes* L19, *Pleurotus ostreatus* × *P. ostreatus* P403×P404 and *L. edodes* × *P. ostreatus* L19-1×P404 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 the 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 intermittently two times and then left overnight in the refrigerator. Before use, the spores were filtered through 20 µm mesh nylon.

**Results**

Measurement of the studied material demonstrated that one fruitbody can produce two more or less equally represented subpopulations of spores with different genome sizes. Diversity was found in the spore print of an interspecies hybrid fruitbody (L19-1×P404) and in the spore print of the fruitbodies of the parental strains (P403×P404 and L19). Diversity was not found in the spore print of the fruitbody of the strain L18 (Table 1. Fig. 1).

**Table 1. Nuclear DNA and protein contents of the studied strains.**

<table>
<thead>
<tr>
<th>Spore print</th>
<th>Mean DNA content in Mb ±CV%</th>
<th>Mean protein content in a.u. ±CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subpopulation 1</td>
<td>Subpopulation 2</td>
</tr>
<tr>
<td>L19</td>
<td>14.6±19%</td>
<td>18.9±14%</td>
</tr>
<tr>
<td>L19</td>
<td>14.6±18%</td>
<td>19.1±14%</td>
</tr>
<tr>
<td>L19</td>
<td>14.6±19%</td>
<td>18.9±14%</td>
</tr>
<tr>
<td>L19×P404</td>
<td>13.7±20%</td>
<td>25.6±13%</td>
</tr>
<tr>
<td>L19×P404</td>
<td>13.7±18%</td>
<td>25.0±12%</td>
</tr>
<tr>
<td>P403×P404</td>
<td>13.7±21%</td>
<td>22.4±14%</td>
</tr>
<tr>
<td>P403×P404</td>
<td>13.2±18%</td>
<td>22.4±14%</td>
</tr>
<tr>
<td>L18</td>
<td>14.6±19%</td>
<td>-</td>
</tr>
<tr>
<td>L18</td>
<td>14.6±19%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Discussion**

For the quantitative evaluation of nuclear DNA and protein of spores DAPI-SR101 staining was employed and samples were analysed by flow cytometry. The spore print of interspecies hybrid of Shiitake mushroom and of oyster mushroom (produced by protoplast fusion of neohaplonts for commercial cultivation (Ramirez and Leal Lara, 2000)), as well as their parental strains were studied.

Measurements of the studied material proved heteroploidy in the spore print (Fig. 1). One fruitbody can produce two more or less equally represented subpopulations of spores with different genome sizes.
genome sizes. Diversity was found in the spore print of the interspecies hybrid fruitbody L19-1×P404 as well as in the spore print of the fruitbodies of the parental strains P403×P404 and L19. It is evident that there exist subpopulations with similar genome sizes among the spore prints produced by parental and hybrid fruitbodies (Table 1, Fig. 1).

Heteroploidy was also found in the spore prints of *P. ostreatus* produced by wild fungi (Kullman, 2000, 2002b) and in a commercial strain (Kullam, 2002b). Aneuploidy seems to be a common phenomenon for *P. ostreatus*. Different chromosome numbers and genome sizes, reported for this species by several authors, may also refer to the existence of true aneuploidy and indicate the plasticity of this fungal genome (Table 2).

The dikaryon stage in the fungal life cycle allows the genomes of species *L. edodes* and *P. ostreatus* to coexist and even to produce a fruitbody. Moreover, after karyogamy, this fruitbody distributes viable spores (not shown in this paper). It means that meiosis can even occur in the case of low density of homology between the chromosomes (chromosome-length polymorphism - CLP and aneuploidy) ensuring distribution of highly different genome sizes of strains. 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).

![Fig. 1. Diversity of nuclear DNA and protein contents in the spore prints of *L. edodes* L19, *P. ostreatus* P403×P404 and their hybrid L19-1×P404 on the basis of nuclear DNA and protein content. The spore print of *L. edodes* L18 is not diverged. Different signs denote spore prints from different fruitbodies (Mb - DNA content in megabase pairs of nucleotides, a.u. – protein content in arbitrary units).](image)

Table 2. Total genome sizes and chromosome numbers and sizes of *Pleurotus ostreatus* (Mb - DNA content in megabase pairs of nucleotides).

<table>
<thead>
<tr>
<th>Total genome size (Mb)</th>
<th>Chromosome number</th>
<th>Chromosome sizes (Mb)</th>
<th>Research technique</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.4</td>
<td></td>
<td></td>
<td>Calculated</td>
<td>Wittmann-Meixner, 1989 from data of Horgen <em>et al.</em>, 1984</td>
</tr>
<tr>
<td>20.8</td>
<td>6</td>
<td>2.1 to 5.2</td>
<td>PFGE</td>
<td>Sagawa and Nagata, 1992</td>
</tr>
<tr>
<td>31.3</td>
<td>9</td>
<td>1.1 to 5.7</td>
<td>PFGE</td>
<td>Peberdy <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>35.0</td>
<td>11</td>
<td>1.4 to 4.8</td>
<td>PFGE</td>
<td>Larraya <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>24.0</td>
<td></td>
<td></td>
<td>FCM</td>
<td>Kullman, 2000</td>
</tr>
</tbody>
</table>
Diversity of nuclear DNA content of a spore print reflects the fate of the hybrid genome during meiosis. When in the zygote (in the basid) two nuclei are only weakly conjugated, then their mitotic haploidisation may result in dimorphism of the spore nuclear DNA content (Kullman, 2002b). It can be suggested that the hybrid genome is diverged back regarding its size, indicating the primitive nature of zygotic meiosis. All studied strains have first subpopulations with a more or less similar DNA content, 13.2-14.6 Mbp, where L19 and L18 have exactly the same DNA content (Table 2). Regrettably, as we could not study P403, exact comparison of the DNA content of the hybrids and their ancestral strains was not possible.

Another explanation for the above described divergence in DNA content can be that spore DNA replication is arrested differently by different strains and subpopulations. If this is the case, then DNA replication of spores in L18 is arrested at 14.6 Mbp. The strain L19 has two subpopulations, in which the first is arrested in the same stage as L18, while the second is arrested later, at 19 Mbp. The second subpopulation can be more viable with twice as large protein content as the first. Both hybrid strains produce one part of spores with particularly large nuclear protein content, 131-137 a.u., which can indicate their heterosis, or 'hybrid vigour'. This protein content is twice as large as the protein content of the first subpopulations of the both strains (63-68 a.u.). Interestingly, L19 has a second subpopulation whose protein content is equal to that of the first subpopulation of the hybrids.

It should be emphasized that such ambiguity cannot be resolved simply using flow cytometry data. We conclude that the diversity revealed by measurement of the DNA and protein contents of the studied spore prints requires further research.

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References