ISOGENIC STRAINS OF PHYCOMYCES BLAKESLEEANUS
SUITABLE FOR GENETIC ANALYSIS

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ABSTRACT

The progeny of crosses between wild-type strains of Phycomyces usually do not exhibit all of the expected genotypes from meiosis. By backcrossing, we have isolated a new (+) mating-type strain, A56, which is nearly isogenic with the (-) wild-type NRRL1555 commonly used in Phycomyces research. Tetrad analysis of the backcrosses shows that meiosis becomes more regular as the parental (+) and (-) strains become more isogenic. In our two-factor crosses with unlinked markers, the regularity of meiosis is measured as the percent of reciprocal ditypes plus tetratypes in the progeny. We have shown that this percentage increases from about 15% for crosses between nonisogenic parents to 90% in the eighth backcross. The results indicate that routine, reliable recombination analyses are possible in P. blakesleeanus.

PHYCOMYCES BLAKESLEEANUS is a heterothallic fungus with two mating types, (+) and (-) (BLAKESLEE 1904, 1906), that are morphologically indistinguishable; when they grow near each other, in an appropriate medium, multinucleate zygospores are formed. The zygospore, after a period of dormancy of 2–6 months, germinates, producing a germsporangiophore with a germsporangium containing approximately $10^4$ germspores with an average of three to four haploid nuclei each. The majority of the germspores are homokaryotic; they are formed from protospores containing only one nucleus that undergoes mitotic divisions (BURGEFF 1915; BERGMAN et al. 1969).

On the basis of limited data from sexual crosses involving morphological markers, BURGEFF (1928) postulated that all germspores of a single germsporangium are derived usually from a single meiosis. Recently, BURGEFF's hypothesis has been confirmed, and a general picture of the sexual cycle of Phycomyces can be established (ESLAVA, ALVAREZ and DELBRÜCK 1975; ESLAVA et al. 1975; CERDA-OLMEO 1975). Of the thousands of haploid nuclei of either mating type in the young zygospore, all or some may fuse to form diploid nuclei, and, in general, only one of these will undergo meiosis. The four products of meiosis pass through several postmeiotic mitoses to end up in the germsporangium with thousands of haploid nuclei contained in the germspores. It is not known whether fusion of haploid nuclei to form diploids in the young zygospore is common or rare. Furthermore, the possible mechanism for blocking meiosis in all but one of the diploid nuclei is unknown.

Genetic analysis in Phycomyces seemed possible with the finding by E. W. Goodell (unpublished results) of a (+) strain, UBC21, which in matings with NRRL1555(−), our standard strain, gives a dormancy of approximately 60 days, instead of the usual 4–6 months. With the evidence that a standard meiotic process is operating in the generation of recombinants and with conditions for a reproducible germination of the zygospores (Esalva et al. 1975), it is now possible to carry out genetic analysis in Phycomyces.

Phycomyces is currently being used to study intracellular sensory transduction process, regulation of carotene biosynthesis and sexual differentiation (Cerdá-Olmedo 1977; Cerdá-Olmedo and Torres-Martinez 1979; Sutter 1977). To achieve a deeper understanding of these mechanisms, it is important to know more about the basic features of this fungus. Among these features a fundamental one is the genetic map. Linkage studies involving genes affecting phototropism (Esalva et al. 1976; Lipson, Terasaka and Silvestein 1980), auxotrophy and synthesis of carotenoids (Esalva, Alvarez and Delbrück 1975; Cerdá-Olmedo 1975; Alvarez, Pelaez and Esalva 1980; Roncero and Cerdá-Olmedo 1982) have been reported. In these studies, some inconsistent results were described. For example, Esalva et al. (1976) found that different alleles of the same madC gene, defined by complementation, were at different loci depending on the strain. These results are probably due to differences in the genetic background of the parental strains used, since they come from different geographical areas (Cerdá-Olmedo 1974).

Because the mating of nonisogenic strains may introduce important errors in the genetic analysis of Phycomyces and may also hinder biochemical and physiological studies in this fungus, it was of interest to isolate a (+) mating-type strain isogenic with the wild-type NRRL1555(−). Three criteria have been followed in our work: first, the (+) mating-type strain was made isogenic to the NRRL1555 by backcrossing, since this strain has been used for the last 30 years in Phycomyces research; second, we consciously selected isolates to reduce the dormancy period; third, we have made tetrad analyses of the backcrosses as a measure of the regularity of the meiosis.

**MATERIALS AND METHODS**

*Strains:* The parental strains used and the pedigree of A56, the (+) strain largely isogenic to the wild-type NRRL1555 (−), are shown in Figure 1. The wild-type strains UBC21 and NRRL1555 and the mutant H1 have been described elsewhere (Esalva et al. 1975).

*Media:* Solid minimal medium (SIV; Sutter 1975), included glucose, asparagine and trace elements. SIV medium was supplemented with leucine at 200 μg/ml, nicotinic acid at 10 μg/ml and 5-fluorouracil at 100 μg/ml, as needed.

For crosses a complete medium (PDA) included 4% potato-dextrose agar (Difco) plus 5 μg/ml

**Figure 1.**—Pedigree of A56, a (+) strain isogenic with NRRL1555(−). UBC21 and NRRL1555 are wild types. S102 is a mutant isolated after nitrosoguanidine treatment; A1 is a spontaneous mutant; both are derived from NRRL1555. The genotype is shown below each strain. S, G, B and A refer to strain collection at University of Sevilla, California Institute of Technology, Max Plank Institute for Molecular Genetics in Berlin and University of Salamanca, respectively. nic designates requirement for nicotinic acid and fur resistance to 5-fluorouracil. D indicates the shortest dormancy, in days, of the respective cross.
UBC21 x S102
(+) \(\text{nica}101(-)

C247 x NRRL1555
\(\text{nica}101(+)\) \(-\)

C260 x S102
(+) \(\text{nica}101(-)

C264 x NRRL1555
\(\text{nica}101(+)\) \(-\)

C268 x S102
(+) \(\text{nica}101(-)

B36 x A1
\(\text{nica}101(+)\) \(\text{furA}401(-)

B101 x NRRL1555
\(\text{furA}401,\text{nica}101(+)\) \(-\)

B146 x NRRL1555
(+) \(-\)

A11 x S102
(+) \(\text{nica}101(-)

A16 x NRRL1555
(+) \(-\)

A32 x NRRL1555
(+) \(-\)

A56
(+)

D = 52
D = 59
D = 52
D = 55
D = 70
D = 51
D = 68
D = 85
D = 74
D = 88
D = 78
### TABLE 1
Crosses analyzed

<table>
<thead>
<tr>
<th>Type*</th>
<th>Cross</th>
<th>Genotype</th>
<th>% Zygospores germinated</th>
<th>Shortest dormancy (days)</th>
<th>% Germspores fertile*</th>
<th>No. of germospores analyzed*</th>
<th>Average germospores/germosporangium*</th>
<th>Viability of pooled germospores on SIVYCA (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>UBC21 × H1</td>
<td>(+) × <em>leuA51</em> (-)</td>
<td>72</td>
<td>57</td>
<td>37 (40)</td>
<td>14</td>
<td>6500 (122)</td>
<td>19</td>
</tr>
<tr>
<td>P</td>
<td>UBC21 × S102</td>
<td>(+) × <em>nicA101</em> (-)</td>
<td>79</td>
<td>52</td>
<td>35 (42)</td>
<td>39</td>
<td>7800 (122)</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>C247 × NRRL1555</td>
<td><em>nicA101</em> (+) × (-)</td>
<td>75</td>
<td>59</td>
<td>74 (83)</td>
<td>51</td>
<td>9500 (60)</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>C260 × S102</td>
<td>(+) × <em>nicA101</em> (-)</td>
<td>70</td>
<td>52</td>
<td>60 (63)</td>
<td>53</td>
<td>9000 (80)</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>C264 × NRRL1555</td>
<td><em>nicA101</em> (+) × (-)</td>
<td>60</td>
<td>55</td>
<td>73 (66)</td>
<td>64</td>
<td>16000 (110)</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>C268 × S102</td>
<td>(+) × <em>nicA101</em> (-)</td>
<td>68</td>
<td>70</td>
<td>60 (61)</td>
<td>30</td>
<td>20000 (61)</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>C268 × H1</td>
<td>(+) × <em>leuA51</em> (-)</td>
<td>70</td>
<td>70</td>
<td>65 (68)</td>
<td>58</td>
<td>14000 (100)</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>A11 × S102</td>
<td>(+) × <em>nicA101</em> (-)</td>
<td>72</td>
<td>74</td>
<td>96 (103)</td>
<td>76</td>
<td>15000 (112)</td>
<td>35</td>
</tr>
</tbody>
</table>

* P represents parental crosses and the numbers correspond to backcrosses.

* Among the germinated zygospores, percent of germosporangia with viable germospores. The rest yielded no viable germospores. The total number is in parentheses.

* Tetrad analysis of these germosporangia is shown in Table 2.

* Ripe germosporangia are picked and put into 1 ml of sterile water. The number of pooled germosporangia is in parentheses.

* Samples from the germospores suspension are plated on SIVYCA to calculate the viability.
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of thiamine HCl and 0.1 g/liter of hypoxanthine. Rich medium (SIVYC) included SIV plus 0.1% yeast extract (Difco) and 0.1% Bacto-casitone (Difco). The rich medium was acidified to pH 3.2 with 1 N HCl (SIVYCA) if colonial growth was desired.

Crosses: The several crosses and the analysis of genotypes in the progeny were performed as described previously (Eslava et al. 1975). Samples of germspores of germsporangia of the first four germinated zygospores were analyzed, and a desired genotype was selected and used as parent in the next backcross.

The method of unordered and amplified tetrad analysis was followed in those backcrosses analyzed. This method has been described in detail for Phycomyces elsewhere (Eslava et al. 1975).

RESULTS

Figure 1 shows the pedigree of the isogenic strain A56(+) . The (−) recurrent parental strain was either the wild-type NRRL1555 or strains derived from it such as S102, a nicotinic acid-requiring mutant, and A1, a 5-fluorouracil-resistant spontaneous mutant. In all crosses the (+) strain used to start the next generation was derived from the first germinated zygospore of the set, that is, the one with the shortest dormancy. It may be seen that the shortest dormancy, defined (Eslava et al. 1975) as the time elapsed from the day at which mating plates were inoculated to the germination of the first zygospore, increases slightly through the backcrosses.

Table 1 shows the characteristics of the eight crosses analyzed. The first two crosses were between parental strains; the (+) strain was the wild-type UBC21 and the (−) were H1 and S102, both isolated from the wild-type NRRL1555 after one-step nitrosoguanidine mutagenesis. Crosses C268 × S102 and C268 × H1 both correspond to the fourth backcross in which we used both (−) strains again for comparison with the parental crosses. The last cross shown in Table 1 corresponds to the eighth backcross, the last analyzed in detail. It may be seen that as the (+) strain becomes more isogenic with respect to the (−) strain, the percent of germsporangia with at least one viable germspore increased from 35 and 37% in the parental crosses to 60 and 65% in the fourth backcross and to 96% in the eighth backcross. The average number of germspores per germsporangium increased from approximately 7000 to 15,000, and the viability of germspores increased from about 17 to 35%.

The products of a regular meiosis with two unlinked markers (our case) may be either tetrads with two types of reciprocal genotypes (2T_{rec}) or tetrads with four different types of genotypes (4T), as long as there is a crossing over between at least one of the markers and its centromere. In no case may the products of a meiosis be of a single genotype or three different genotypes, unless there are some irregularities such as inviability due to the lack of isogenicity among the strains. The measurement of the normality of meiosis in Phycomyces, in the case of two unlinked markers, may be considered as the percentage of the number of 2T_{rec} + 4T germsporangia present. In normal meiosis this percentage should be equal to 100. Table 2 shows the results of the crosses from which samples of germspores from individual germsporangia were analyzed. In crosses between parental wild types, the proportion of 2T_{rec} + 4T was approximately 15%. This percentage increased in the successive backcrosses until it reached about 90% in the eighth backcross, which was the last one we analyzed.
TABLE 2

Tetrad analysis of the backcrosses

<table>
<thead>
<tr>
<th>Crosses</th>
<th>(1T)</th>
<th>(2T_{rec})</th>
<th>(2T_{res})</th>
<th>(3T)</th>
<th>(4T)</th>
<th>(2T_{rec} + 4T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC21 (\times) H1</td>
<td>7 (50)</td>
<td>0 (0)</td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>2 (14.3)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>UBC21 (\times) S102</td>
<td>24 (61.5)</td>
<td>5 (13)</td>
<td>5 (13)</td>
<td>4 (10)</td>
<td>1 (2.5)</td>
<td>6 (15.5)</td>
</tr>
<tr>
<td>C247 (\times) NRRL1555</td>
<td>14 (27.5)</td>
<td>11 (21.5)</td>
<td>7 (13.7)</td>
<td>11 (21.5)</td>
<td>8 (15.7)</td>
<td>19 (37.2)</td>
</tr>
<tr>
<td>C260 (\times) S102</td>
<td>18 (34)</td>
<td>10 (19)</td>
<td>10 (19)</td>
<td>8 (15)</td>
<td>7 (13)</td>
<td>17 (32)</td>
</tr>
<tr>
<td>C264 (\times) NRRL1555</td>
<td>9 (14)</td>
<td>18 (28)</td>
<td>4 (6)</td>
<td>12 (19)</td>
<td>21 (33)</td>
<td>39 (61)</td>
</tr>
<tr>
<td>C268 (\times) S102</td>
<td>5 (17)</td>
<td>13 (43)</td>
<td>3 (10)</td>
<td>1 (3)</td>
<td>8 (27)</td>
<td>21 (70)</td>
</tr>
<tr>
<td>C268 (\times) H1</td>
<td>6 (10)</td>
<td>18 (31)</td>
<td>8 (14)</td>
<td>10 (17)</td>
<td>16 (28)</td>
<td>34 (59)</td>
</tr>
<tr>
<td>A11 (\times) S102</td>
<td>4 (5.2)</td>
<td>26 (34.2)</td>
<td>2 (2.6)</td>
<td>2 (2.6)</td>
<td>42 (55.3)</td>
<td>68 (89.5)</td>
</tr>
</tbody>
</table>

*The germsporangia are classified as monotype (1T), ditype (2T), tritype (3T) and tetratype (4T) depending on whether one, two, three or four different genotypes, respectively, are found in the sample of germspores analyzed. Among the ditype, the two genotypes may be reciprocal pairs, parental or recombinant (2Trec) or they may not (2Tmixed). The percentage of the total is shown in parentheses.

In conclusion it may be said that genetic analysis shows that meiosis becomes increasingly more regular as isogenicity increases.

DISCUSSION

The sexual genetics in Phycomyces has been difficult because of the long dormancy and erratic germination of zygospores and also because of irregularities in meiotic segregation of genotypes (CERDA-OLMEDO 1974, 1975; ESLAVA et al. 1975). The wild-type UBC21, of (+) mating type, crossed with the (−) wild-type NRRL1555 gives zygospores that take less than 2 months to germinate. This is the shortest dormancy period found in Phycomyces. UBC21 and NRRL1555 are two independent wild-type isolates; they probably have a different arrangement of their genetic material. Due to this, irregularities may arise resulting in lack of viability and unexpected ratios of postmeiotic segregants. Furthermore, strains isogenic for all but the mating-type gene(s) are desirable for genetic mapping and for analysis of the physiology and biochemistry of Phycomyces.

The results obtained show that the dormancy period increased slightly as the strains became more isogenic; some of the crosses with A56 (M. I. ALVAREZ and A. P. ESLAVA, unpublished) have shown a dormancy of 70 days, whereas others take 3–4 months. Factors such as temperature, light, humidity and the culture medium (ESLAVA et al. 1975) affect the germination of the zygospores, and it should be noted that the most suitable conditions for early germination of the zygospores are still unknown.

By backcrossing through ten generations a wild-type (+) strain to the (−) strain commonly used in studies of P. blakesleeanus, we have derived isogenic (+) and (−) strains which, when mated, show increased viability of germspores and regular patterns of meiotic segregation. With these features it is now possible to carry out a detailed genetic analysis of this fungus.
We are greatly indebted to the late Max Delbrück in whose lab we started this project several years ago. We would further like to thank María José Jiménez and Fernando Diez for their technical assistance. This work was supported in part by the Alexander von Humboldt Foundation and Comisión Asesora de Investigación Científica y Técnica of Spain.

LITERATURE CITED


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