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Influence of parental strains on the germination of *Phycomyces blakesleeanus* zygospores

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Summary

Phycomyces blakesleeanus wild-type NRRL1555(−), the standard strain, when crossed with UBC21(+), another wild type, gives zygospores that germinate in 50–60 days. By backcrossing to UBC21 and selecting for shorter dormancy we have isolated a (−) strain, A803, and a (+) strain A804, which when crossed give zygospores that germinate in 32 days, the shortest dormancy period found in *Phycomyces*. The same result was obtained when A803 was crossed with UBC21. Zygospore dormancy decreased as the parental strains became more isogenic with UBC21, but the number of zygospores giving germsporangia with viable germspores also decreased to zero in the third backcross. The existence of germspore-killer alleles in the strain UBC21 is postulated. The strains of shortest dormancy can be used as helper strains (Orejas *et al.* 1985) in sexual crosses. Tetrad analysis of the cross NRRL1554 × S102, a two-factor cross, showed 90% of reciprocal ditypes plus tetratypes in the progeny, indicating that the (+) wild-type strain NRRL1554, when crossed with the standard strain, gives regular meiosis and, contrary to current beliefs, may be used in *Phycomyces* genetic analysis.

1. Introduction

The sporangiophores of the zygomycete fungus *Phycomyces blakesleeanus* show growth responses to light, gravity, wind, chemicals and the presence of objects in the proximity of the growing zone (avoidance response). The mycelium also shows responses to light such as the initiation of sporangiophores and induction of β-carotene synthesis. All those properties have made *Phycomyces* a model system for the study of intracellular sensory transduction processes (Bergman *et al.* 1969, 1973; Cerdá-Olmedo, 1977; Galland & Lipson, 1984, 1987). The regulation of carotene biosynthesis (Cerdá-Olmedo, 1985) and sexual differentiation (Sutter, 1977; Sutter & Whitaker, 1981) are other active areas of *Phycomyces* research.

Phycomyces is a heterothallic fungus with two morphologically indistinguishable mating types (+) and (−) (Blakeslee, 1904, 1906). Multinucleate Zygospores are formed when opposite mating types meet. The zygospore germinates after a dormancy of 2–6 months, producing a germsporangiophore with a germsporangium containing multinucleate germspores. 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). Of the thousands of hapliod nuclei of either mating type that enter the zygospore, all or only some may fuse to form diploid nuclei. In general, only one of these nuclei undergoes meiosis, yielding four meiotic products which, after several mitotic divisions, become the nuclei of the germspores (Cerdá-Olmedo, 1975; Eslava *et al.* 1975*a*, *b*).

The study of the sexual genetics of *Phycomyces* is difficult owing to the long dormancy of the zygospores, the irregularities of the expected genotypes in the progeny, the non-isogenicity of the different wild types and the limited number of markers available. In spite of these problems a tentative genetic map of *Phycomyces blakesleeanus* has been constructed recently (Orejas *et al.* 1987). A more complete genetic map would be very useful for better understanding of the different mechanisms of interest in this fungus, particularly the sensory transduction processes.

In most of the crosses performed, the parental strains have been NRRL1555(−), the wild-type strain commonly used in *Phycomyces* research, and UBC21(+), another wild-type strain, or strains derived from these. UBC21, when mated with NRRL-1555, gives zygospores with a dormancy of about

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50–60 days, the shortest dormancy found in *Phycomyces*. However, some irregularities have been described in the above crosses, probably due to the non-isogenicity of the parental strains used. Regular meiotic segregation of two unlinked markers (e.g. *sex* and *nicA*) leads to either tetrads with two reciprocal genotypes (2Trec) or tetrads with four different genotypes (4T). Apart from rare gene conversion events, other segregation patterns can arise only from irregularities in meiosis or spore inviability. These irregular segregations can lead to tetrads containing a single spore genotype (1T), two non-reciprocal types (2Tmix) or three different genotypes (3T). An estimate of the regularity of meiosis in *Phycomyces* is the percentage of 2Trec+4T germsporangia present. In normal meiosis this percentage should be 100.

A different (+) wild-type strain, NRRL1554, was used in earlier crosses with NRRL1555, the dormancy of the zygospores being about 6 months (Cerdá-Olmedo 1975; Eslava *et al.* 1975*b*). At that time no standard conditions for sexual crosses and zygospore germination were established. The isolation, by backcrossing, of a (+) strain A56, which is largely isogenic with the (−) wild-type NRRL1555 (Alvarez & Eslava 1983), improved the viability of the germspores and the regularity of meiosis when the (+) and (−) isogenic strains were mated. However, when this is done, the dormancy period of the zygospores rises from 50–60 days when UBC21(+) is used to about 80–100 days and sometimes more.

Recently (Orejas *et al.* 1985), the advantages of isogenicity and short dormancies have been combined using heterokaryons carrying nuclei of UBC21 (the helper strain) and A56 as the (+) parental strain and the standard wild-type NRRL1555 as (−) parental strain. Under these conditions, the zygospores show early germination (2 months) and, with appropriate markers, the germsporangia whose germspores originate from meiosis of the diploid nuclei corresponding to the isogenic strains can be analysed. In fungi, dormancy involves two phenomena (Sussman, 1969, 1979): exogenous dormancy, caused by adverse environmental conditions, and constitutive dormancy, which is due to an innate property of the spores. In *Phycomyces* vegetative spores, these aspects have been extensively studied (reviewed by Van Laere, 1986). However, little is known about the factors that are involved in the blocking of zygospore germination and the mechanisms of release from dormancy. Considerable progress has been made in the establishment of standard conditions for zygospore formation and germination (Cerdá-Olmedo, 1975; Eslava *et al.* 1975*a*, *b*), but the nature of the above mechanisms remains unknown. The aims of the present work were (1) to shorten the dormancy period of the zygospores by making a (−) strain partially isogenic to UBC21(+) by backcrossing and (2) to measure the regularity of meiosis when another (+) wild-type strain, NRRL1554, is used in crosses with the (−) wild-type strain NRRL1555 under standard environmental conditions.

2. Material and methods

(i) *Strains*

NRRL1555 is the standard wild type of (−) mating type and NRRL1554 is a wild-type of (+) mating type; both come from Northern Regional Research Laboratories, USDA, Peoria, I11., USA. UBC21 is a wild type of (+) mating type from R. J. Bandoni, Botany Department, University of British Columbia, Vancouver, BC, Canada. A56 is a (+) strain which is nearly isogenic with NRRL1555. S102 is a nicA101(−) mutant isolated from NRRL1555 by nitrosoguanidine mutagenesis. These strains have been described elsewhere (Eslava *et al.* 1975*a*; Cerdá-Olmedo, 1975; Alvarez & Eslava, 1983). A800, A801, A803 and A804 were isolated by backcrossing with UBC21, and A812 and 813 were isolated from the cross NRRL1554 × NRRL1555. The pedigree and genotypes of these strains are shown in Fig. 1. A and S refer to strain collections at the University of Salamanca and University of Sevilla, respectively; nic designates requirement for nicotinic acid.

(ii) *Media*

Minimal medium (SIV) (Sutter, 1975) included glucose, asparagine and trace elements. Rich medium (SIVYC) included SIV plus 0·1% yeast extract (Difco) and 0·1% Bactocasitone (Difco). The rich medium was acidified to pH 3·2 with 1 N-HCl when colonial growth was required. For crosses Potato Dextrose Agar medium (PDA from Difco) was used. The media were solidified with 1·5% Agar (Difco). The minimal medium (SIV) was supplemented with nicotinic acid at 10 μg/ml as needed. These media have been described in detail elsewhere (Eslava *et al.* 1975*a*).

(iii) *Crosses*

Crosses were carried out essentially as described previously (Eslava *et al.* 1975*b*). Pieces of mycelium from the strains to be mated were inoculated at opposite sides of plates containing PDA medium. The plates were incubated for 25 days in the dark at 22 °C. At that time, the zygospores were picked individually and transferred to moist filter paper at 22 °C under continuous illumination. Individual germsporangia were collected by picking them up in a drop of sterile water between the tips of tweezers, crushing them and dropping the germspore suspension into a tube containing 0·5 ml of sterile water. After heat-shocking (15 min at 48 °C), the activated germspore suspensions were plated on rich acid medium (SIVYCA). If after three days no colonies were seen the corresponding germsporangium was classified as sterile. In the cases

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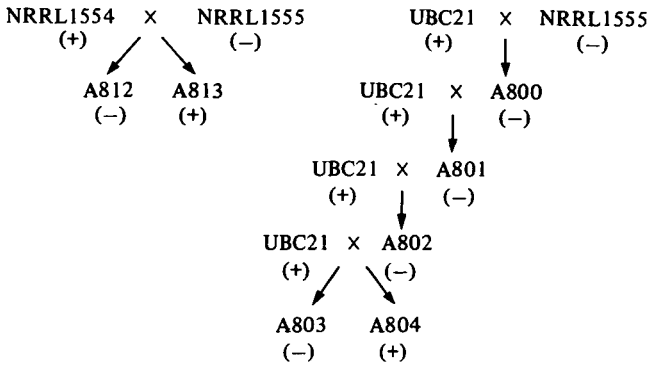

Fig. 1. Pedigree of the strains used. UCB21, NRRL1554 and NRRL1555 are the wild types. The genotype is shown below each strain.

in which the regularity of meioses was measured the method of unordered and amplified tetrad analysis was followed. This method has been described in detail for *Phycomyces* elsewhere (Eslava *et al.* 1975*a*, *b*; Alvarez & Eslava, 1983).

3. Results

(i) *UBC21 decreases the zygospore dormancy period.*

Fig. 1 shows the pedigree of the strains A800, A801, A802, A803, and A804 which were isolated after backcrossing the (+) recurrent wild-type strain UBC21, to NRRL1555, the (−) strain commonly used in *Phycomyces* research. 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.

Table 1 shows the dormancy period and the percentage of zygospores with viable spores of the crosses analysed. A decrease can be seen in the dormancy period of the zygospores as the strains become more isogenic with UBC21. The shortest dormancy observed (32 days), in the crosses UBC21 × A803 and A804 × A803 was nearly half that shown by the parental cross UBC21 × NRRL1555 and in fact this is the shortest dormancy period reported in the literature on *Phycomyces*. The strain UBC21 must be responsible for this shortening of the dormancy because in those crosses in which at least one of the parental strains was UBC21, or a strain derived from it by backcrossing, dormancy was considerably shorter than in the rest. The percentage of zygospores with viable germspores (fertile germsporangia) decreased in the successive backcrosses until it reached zero at the fourth backcross (UBC21 × A803). Those strains isolated from the backcrosses which showed the shortest dormancy of the zygospores when mated, were crossed with strains not related to UBC21. When the wild-type NRRL1555(−) was crossed with A804, a (+) strain from the progeny of the third backcross, the percentage of zygospores with viable germspores was 27. However, when A804 was crossed with A803, viability was zero. The (−) strain A801 from the progeny of the first backcross (see Fig. 1), when crossed with the (+) wild-type strains UBC21, NRRL1554, and A56 (a strain isogenic with NRRL155) showed a percentage of zygospores with viable germspores of 3, 27–30, and 32, respectively.

Table 1. *Crosses analysed*

Cross	Dormancy (days)	Germsporangia fertile (%)
UBC21 × NRRL1555	55–60	20–25
UCB21 × A800	34	3
UCB21 × A801	36	3
UCB21 × A802	34	3
UCB21 × A803	32	0
A804 × A803	32	0
A804 × NRRL1555	43	20–35
A56 × A801	41–45	27–30
A56 × NRRL1555	113	90
NRRL1554 × A801	41	32
NRRL1554 × NRRL1555	69–75	95
NRRL1554 × A812	80	98
A813 × NRRL1555	136	88

In conclusion, it may be said that as the strains become more isogenic with the wild-type UBC21, the dormancy and the viability of the zygospores decrease. By contrast, when the strains become more isogenic with the wild-type NRRL1555 the dormancy and the viability of the zygospores increase.

(ii) *Crosses with the wild-type strain NRRL1554*(+)

At the beginning of *Phycomyces* genetics, when the (+) wild-type strain NRRL1554 was crossed with the (−) wild-type standard strain NRRL1555, the zygospores remained dormant for more than 5 months (Bergman *et al.* 1969; Cerdá-Olmedo, 1975). Due to this long dormancy period the strain NRRL1554 has not been used in recent *Phycomyces* crosses. The results in the section above (see Table 1) reveal a dormancy period of 69–75 days in zygospores from the cross NRRL1554 × NRRL1555, the percentage of zygospores with viable germspores being about 95. This unexpectedly short dormancy, in comparison with early results, may be due to the use of standard environmental conditions which were lacking in the past for reproducible zygospore germination. To test the regularity of meiosis in those crosses in which NRRL1554 was used as a parental strain, the crosses shown in Table 2 were set up and analysed.

Table 2 shows the results of the crosses from which samples of germspores from individual germsporangia were analysed: the rationale is presented in the Introduction. In the cross UBC21 × S102 the proportion of 2Trec+4T was about 15% (Alvarez & Eslava, 1983); in the cross A56 × S102 this percentage reached 80%, and in the cross NRRL1554 ×

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Table 2. *Tetrad analysis*

Crosses	Class of germsporangium[a]					
	1T	2Trec	2Tmix	3T	4T2	Trec+4T
UCB21 × S102[b]	24 (61·5)	5 (13)	5 (13)	4 (10)	1 (2·5)	6 (15·5)
A56 × S102	0 (0)	16 (32)	9 (18)	1 (2)	24 (48)	40 (80)
NRRL1554 × S102	1 (2)	14 (28)	4 (8)	0 (0)	31 (62)	45 (90)

[a] The germosporangia 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 analysed. 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.

[b] The data of the cross UBC21 × S102 were taken from Alvarez & Eslava, 1983.

NRRL1555 the percentage increased to 90%. Accordingly, the wild-type strain NRRL1554(+) may be used in further genetic analysis of *Phycomyces* because when crossed with our standard wild-type strain NRRL1555(−) the meiosis is regular and the dormancy period of the zygospores lasts about 70 days.

4. Discussion

The duration of zygospore dormancy in *Phycomyces* varies between different strains and can be influenced by extraneous substances. The shortening of dormancy has been attempted by several workers since the beginning of this century. Blakeslee (1906) assayed several physical and chemical treatments with negative results. Schwartz (1926) observed that the shortest dormancies were achieved when the zygospores were grown at 22 °C. Hocking (1967) combined physical treatments (light and temperature) with chemical substances (nutrients, indoleacetic acid, gibberellic acid), also with negative results. Eslava *et al.* (1975*a*) found that zygospore dormancy is determined polygenically.

A search for a pair of strains giving zygospores that would have shorter dormancy (E. W. Goodell, unpublished) yielded a (+) strain, UBC21, which in matings with NRRL1555(−) – our standard strain in physiological research – gives a dormancy of about 60 days instead of the usual 3–6 months (Bergman *et al.* 1969).

We have described a series of backcrosses with the strain UBC21 to find a (−) strain with the shortest dormancy possible when mated. The results show that the dormancy period decreases as the parental strains become more isogenic with UBC21, and reaches a minimum of 32 days, the shortest dormancy found for *Phycomyces* zygospores in the literature. Little is known about the mechanisms of release from dormancy in *Phycomyces*. Our results indicate the existence of some kind of factor(s) produced by UBC21 and its derivatives which, when present, cause a shortening of the zygospore dormancy period.

An unexpected result in the backcrosses with UBC21 (Table 1) is the lack of viability of the germspores from the germsporangia when the two parental strains are relatively isogenic with UBC21. This loss of viability might be explained as a consequence of the short dormancy period preventing the maturation of the zygospores to form viable germspores. However, this explanation can be ruled out because zygospores from the third backcross (UBC21 × A803) maintained on the mating-plates for 42 days showed the same lack of viability as the zygospores kept for 25 days on the mating-plates as controls. The zygospores maintained on the mating-plates do not germinate (Bergman *et al.* 1969). It is not known whether the factor(s) responsible for the shortening of dormancy are related to those responsible for the lack of viability.

A kind of incompatibility which does not prevent fertilization but causes the death of meiotic products has been described in *Neurospora* and has been explained in terms of the existence of spore-killer alleles in some *Neurospora* strains (reviewed by Fincham *et al.* 1979). In view of the results reported in this work, the existence of germspore-killer alleles may also be postulated in certain *Phycomyces* strains. When UBC21 is crossed with partially isogenic strains (crosses UBC21 × A803 and A804 × A803, Table 1) the number of viable germspores decreased to nearly zero, which is in agreement with the existence of germspore-killer alleles in UBC21. The wild types NRRL1554 and NRRL1555, which come from different geographical areas from UBC21 (Cerdá-Olmedo, 1974), are probably free of these germspore-killer alleles because the crosses A56 × NRRL1555 and NRRL1554 × NRRL1555 (Table 1) showed a high proportion of fertile germsporangia.

Crosses of UBC21 with NRRL1555 showed around 25% of fertile germsporangia. This result may be explained by assuming the presence of killer alleles in UBC21 and partial resistance to the spore-killer effect of the NRRL1555 strain. At present, the effect of these allelic differences is unexplained at the genetic and biochemical levels.

In all previous work on *Phycomyces* sexual crosses

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(reviewed by Eslava, 1987) the lack of viability of the germspores in crosses using UBC21 as a parental was mainly explained as a consequence of the non-isogenicity of the parental strains used, although the presumed germspore-killer alleles, carried by UBC21 and its derivatives, can also contribute to the sterility of the germspores.

The strains obtained showing the shortest dormancy, A803 and A804, can be used in sexual crosses of *Phycomyces* as helper strains by taking as the (+) parental strain the heterokaryon A56 * A804 and NRRL1555 as the (−) parental strain. When using A803, the (+) parental strain should be A56 and the (−) parental strain the heterokaryon NRRL1555 * A803. Only the germsporangia whose germspores originate from meiosis of the diploid nuclei corresponding to the isogenic strains (A56/NRL1555) or their derivatives should be analysed. This analysis can be carried out by using appropriate markers (Orejas *et al.* 1985). In this way, the advantages of viability and shortest dormancies can be combined.

The results of tetrad analysis of the cross NRRL-1554 × S102 (Table 2) show that, contrary to current beliefs, the (+) wild-type strain NRRL1554 may be used in the genetic analysis of *Phycomyces* sexual crosses because, when crossed with NRRL1555 or its derivatives, dormancy is not too long (65–75 days) and meiosis is regular as measured by the number of 2Trec + 4T germsporangia present, i.e. around 90%.

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