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Recombination Between Ten Markers in *Phycomyces*

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Summary. Twenty-two sexual crosses between strains of *Phycomyces blakesleeanus* carrying mutations affecting phototropism (*madA, madD, madE*), synthesis of carotenoids (*carA*), auxotrophy (*leu-51, nicA, pur-51*), and resistance to 5-fluorouracil (*fur*) were studied; mating type was also included as a marker. Recombination frequencies were obtained among the ten genes involved. Linkage was found between mating type and *madE*; *leu-51* and *madA; furA401, furB402* and *madD*. All other gene combinations tested are unlinked.

Introduction

Phycomyces blakesleeanus (class *Zygomycetes*) is a low fungus which is currently being used as a model system to study sensory transduction mechanisms. The biosynthesis of carotenoids and its regulation and sexual differentiation are some other main aspects in which research in *Phycomyces* is being carried out.

The sporangiophores of *Phycomyces* show growth responses to blue light, gravity, wind, chemicals, and the presence of objects in the proximity of the growing zone. The mycelium also shows responses to light such as initiation of sporangiophores and induction of β-carotene synthesis. With the use of mutants with abnormal phototropism Bergman et al. (1973) formulated a sensory pathway linking three types of stimulus (light, gravity, and presence of barriers) with the responses (tropism, initiation of sporangiophores, and induction of β-carotene). Analysis by complementation (Ootaki et al. 1974, 1977) revealed the existence, among the mutants tested, of seven complementation groups (*madA* to *madG*) affecting the sensory pathway. Eslava et al. (1976) and Lipson et al. (1980) found no evidence of linkage among any of the *mad* complementation groups. In recent years reviews about genetic and physiological aspects of sensory transduction in *Phycomyces* have been published (Cerdá-Olmedo 1977; Dennison 1979; Russo 1980).

The study of β-carotene biosynthesis and its regulation is facilitated in *Phycomyces* by the existence of mutants which are very easy to isolate. The availability of these mutants and the use of quantitative complementation techniques allowed workers to postulate the existence of an enzyme aggregate carrying out the conversion of phytoene into β-carotene (De la Guardia 1971; Eslava and Cerdá-Olmedo 1974; Aragón et al. 1976). Several other aspects of the regulation of β-carotene biosynthesis have been reviewed recently (Cerdá-Olmedo and Torres Martinez 1979).

Sexual differentiation is mediated by the joint action of mating type specific substances which initiate a complex series of chemical and morphological changes culminating in the production of the zygospores (reviewed by Sutter 1977).

To achieve a deeper understanding in all of these studies it is important to know more about the basic features of this fungus. One of these features is the genetic map which is fundamental to the description in molecular terms of the various mechanisms and processes of interest. In the majority of cases only asexual genetic techniques, mainly complementation and phenotypic characterization, have been used with the mutants already available.

Sexual genetics in *Phycomyces* is difficult to perform because of the long dormancy of the zygospores, the irregularities of the expected genotypes in the progeny, the lack of standard conditions to carry out the crosses and the non isogenicity of the wild-types of different mating types. These difficulties have been in part overcome recently, standard conditions for the germination of the zygospores have been estab-

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Table 1. Strains used

Strains[a]	Genotype[b]	Origin	Comment
C169	*carA5(+)*	UBC21 × C2	
C273	*pur-51(−)*	NRRL1555	By ICR-170 mutagenesis
C264	*nicA101(+)*	C260 × S102	
C244	*leu-51(+)*	UBC21 × H1	
B49	*nicA51, pur-51(+)*	C273 × H4	
A1	*furA401(−)*	NRRL1555	Spontaneous
A2	*furB402(−)*	NRRL1555	Spontaneous
C21	*madA7(−)*	NRRL1555	By NG mutagenesis
C68	*madD59(−)*	NRRL1555	By NG mutagenesis
C149	*madD120(−)*	NRRL1555	By NG mutagenesis
B41	*carA5, pur-51(+)*	C273 × C169	
C110	*madE102(−)*	NRRL1555	By NG mutagenesis
B49	*nicA51, pur-51(+)*	C273 × H4	
B71	*furA401(+)*	A1 × C169	
C47	*madA35(−)*	NRRL1555	By NG mutagenesis
H1	*leu-51(−)*	NRRL1555	By NG mutagenesis
B128	*pur-51, furB402(+)*	A2 × B49	

[a] Prefixes C, B, A and H refer to strain collection at California Institute of Technology, The Max Planck Institute for Molecular Genetics in Berlin, University of León, and Cold Spring Harbor Laboratory, respectively

[b] *mad* indicates a mutant with abnormal phototropism. *car* indicates a mutant with abnormal carotene production. *leu, nic* and *pur* indicate mutants requiring leucine, nicotinic acid and purine, respectively. *fur* indicates a mutant resistant to 5-fluourouracil. (+) and (−) indicate the two mating types. The *fur* genes (A and B) are inferred from the present work

lished and clear evidence found that a standard meiotic process was operating in the generation of recombinants, establishing *Phycomyces* as an organism amenable to genetic analysis (Eslava et al. 1975a, b; Cerdá-Olmedo 1975).

Few genetic markers are available in *Phycomyces* and few linkage relationships have been established among them (Eslava et al. 1975b, 1976). In this paper we report for the first time a systematic genetic analysis of a total of ten different markers in all pair-wise combinations to determine the linkage among them as a first step toward the construction of a genetic map in *Phycomyces*.

Materials and Methods

Strains. The strains used in this work are listed in Table 1. All cultures were incubated at room temperature (22° C).

Media. Minimal medium (SIV), Sutter (1975), included glucose, asparagine, and trace elements. A complete medium (SIVYC) consisted of minimal medium supplemented with 0.1% Yeast Extract (Difco) and 0.1% Bacto-Casitone (Difco). For colonial growth the medium was acidified to pH = 3.2 by adding 1 N HCl after autoclaving (SIVYCA). Potato Dextrose Agar medium (PDA) was used for sexing plates and for testing phototropism. Minimal medium was supplemented with leucine at 200 μg/ml, nicotinic acid at 10 μg/ml, hypoxanthine at 100 μg/ml, and 5-fluorouracil at 100 μg/ml, depending on the cases. The media were solidified with 1.5% Agar (Difco). These media have been described in detail elsewhere (Eslava et al. 1975a).

Mutagenesis. All mutations used in this work, except one, (see Table 1) were induced by N-methyl-N′-nitro-N-nitrosoguanidine (NG). The mutagenesis procedure and isolation of these mutants have been described elsewhere (Bergman et al. 1973; Eslava et al. 1975a).

ICR-170 (Terochem. Lab. Ltd, Edmonton, Canada) was used for the isolation of the purine requiring mutant C273. A suspension of spores at a concentration of 10^6 per ml. was shaken in the dark for 60 min at 22° C with 40 μg/ml of ICR-170 in 0.1 M phosphate buffer pH 7. Treatment was stopped by adding five times as much of 1% NaCl solution. The spores were washed three times by centrifugation and resuspension in water. About 50 viable spores per plate were plated on SIVYCA medium, the colonies that grew were divided and tested for differential growth on minimal and complete medium. One purine requiring mutant, C273, was found among 500 colonies tested.

Crosses. Sexual crosses were performed as described previously (Eslava et al. 1976). In brief, mycelia of two strains of opposite mating type were inoculated at opposite margins of PDA plates and incubated for 42 days in the dark at 22° C. At that time 200 or more zygospores for each cross were picked up individually and transferred to filter paper kept moist at 22° C in daylight conditions. An average of 100 germsporangia were pooled per cross in 2 ml of water. The shortest dormancy, defined as the time elapsed from the day of the inoculum to the germination of the first zygospore, was recorded for each cross.

Analysis of the Progeny. Samples from the pool of germspores were plated on SIVYCA medium. After 2–3 days the colonies were analyzed for the specific markers in each cross. In general, mating type and auxotrophy were tested as described by Eslava et al. (1975a). Phototropism was tested according to Bergman et al. (1973) and Eslava et al. (1976).

Results

Linkage Tests

Table 2 shows the characteristics of the 22 sexual crosses analyzed. The average shortest dormancy was around 60 days (range 49 to 99 days). The average number of spores per germsporangium ranged from 6000 to 30000 with an average of 13000. The viability of the germspores on SIVYCA medium was 10%–30%, average 18%.

Samples of the pooled germspores from each cross were plated on SIVYCA medium with about 20 viable spores per plate. Of the colonies formed after 2 or 3 days a sample ranging from 200 to 500 was picked

Table 2. Crosses analyzed

Cross	Genotypes	Shortest dormancy	Number of pooled germ.	Average spor./ germsporangium	Viability of germspores on SIVYCA (%)
C169 × C273	*carA5(+) × pur-51(−)*	50	270	10000	10
C264 × C273	*nicA101(+) × pur-51(−)*	68	94	20000	18
C244 × C273	*leu-51(+) × pur-51(−)*	84	103	17000	11
B49 × A1	*nicA51, pur-51(+) × furA401(−)*	59	87	11000	25
B49 × A2	*nicA51, pur-51(+) × furB402(−)*	59	97	9000	22
C169 × A1	*carA5(+) × furA401(−)*	49	63	16000	13
C169 × C21	*carA5(+) × madA7(−)*	52	133	11000	15
C169 × C68	*carA5(+) × madD59(−)*	52	129	12000	12
C169 × C149	*carA5(+) × madD120(−)*	52	51	12000	10
C244 × C68	*leu-51(+) × madD59(−)*	71	62	13000	30
B41 × C68	*carA5, pur-51(+) × madD59(−)*	55	51	11000	13
C169 × C110	*carA5(+) × madE102(−)*	52	130	10000	10
C244 × C110	*leu-51(+) × madE102(−)*	63	73	19000	20
B49 × C21	*nicA51, pur-51(+) × madA7(−)*	71	88	30000	15
B49 × C149	*nicA51, pur-51(+) × madD120(−)*	70	103	12000	20
B71 × C21	*furA401(+) × madA7(−)*	66	52	8000	10
B71 × C149	*furA401(+) × madD120(−)*	56	52	11000	20
C244 × C21	*leu-51(+) × madA7(−)*	88	124	12000	26
B71 × H1	*furA401(+) × leu-51(−)*	52	91	6000	25
B71 × C149	*furA401(+) × madD120(−)*	53	174	14000	30
B71 × A2	*furA401(+) × furB402(−)*	56	120	16000	25
B128- × A1	*pur-51, furB402(+) × furA401(−)*	99	50	6000	23

Table 3. Occurrence of parental alleles in the genotypes of the progeny

Cross	*carA*	*carA*$^+$	*nic*	*nic*$^+$	*leu*	*leu*$^+$	(+)	(−)	*furA401*	*furA401*$^+$	*furB402*	*furB402*$^+$	*pur*	*pur*$^+$	*madA*	*madA*$^+$	*madD*	*madD*$^+$	*madE*	*madE*$^+$
C169 × C273	300	276											295	281						
C264 × C273			172	167			180	159					163	176						
C244 × C273					173	171							186	158						
B49 × A1			200	230					213	217			205	225						
B49 × A2			197	237							218	216	204	230						
C169 × A1	99	94					103	90	101	92										
C169 × C21	77	80					74	83							84	73				
C169 × C68	112	100					102	110									111	101		
C169 × C149	129	120					130	119									126	123		
C244 × C68					134	150											132	152		
B41 × C68													162	173			169	166		
C169 × C110	161	175					155	181											178	158
C244 × C110					112	108	114	106											107	113
B49 × C21			160	150									158	152	148	162				
B49 × C149			148	146													156	138		
B71 × C21									105	129					108	126				
B71 × C149									96	87							86	97		
B71 × C149							185	215	186	214							213	187		
C244 × C21					96	93	87	102							103	86				
B71 × H1					94	106			96	104										

at random and tested for the different markers depending on the cross.

Table 3 lists the distribution of the parental alleles in the progeny of each cross. It demonstrates the absence of allele specific selection.

In Table 4 linkage tests for each pair of markers are shown. In those cases in which linkage between a pair of markers was detected a second cross with different strains containing at least the same pair of linked markers was set up and analyzed. The cross

Table 4. Linkage tests for the markers used

Cross	Markers	Parentals	Recombinants	% recombination
C169 × C273	*carA5 and pur-51*	319	257	45
C264 × C273	*nicA101 and pur-51*	185	154	45
	mt and pur-51	183	156	46
	mt and nicA101	187	152	45
C244 × C273	*leu-51 and pur-51*	157	187	54
B49 × A1	*furA401 and pur-51*	187	253	58
	nicA51 and pur-51	213	227	52
	furA401 and nicA51	228	212	48
B49 × A2	*furB402 and pur-51*	242	192	44
	nicA51 and pur-51	253	181	42
	furB402 and nicA51	231	203	47
C169 × A1	*furA401 and carA5*	52	141	73
	furA401 and mt	54	139	72
	carA5 and mt	117	76	39
C169 × C21	*carA5 and madA7*	80	77	49
	mt and madA7	71	86	55
	carA5 and mt	90	67	43
C169 × C68	*carA5 and madD59*	127	75	37
	mt and madD59	89	113	56
	carA5 and mt	82	120	59
C169 × C149	*carA5 and madD120*	107	142	57
C244 × C68	*leu-51 and madD59*	143	141	50
B41 × C68	*pur-51 and madD59*	149	186	56
C169 × C110	*carA5 and mt*	162	174	52
	carA5 and madE102	159	177	53
	mt and madE102	315	21	6
C244 × C110	*leu-51 and mt*	92	128	58
	leu-51 and madE102	101	119	54
	mt and madE102	195	25	11
B49 × C21	*nicA51 and madA7*	172	138	44.5
	pur-51 and madA7	162	148	48
	nicA51 and pur-51	156	154	50
B49 × C149	*nicA51 and madD120*	136	158	54
B71 × C21	*furA401 and madA7*	99	135	58
B71 × C149	*furA401 and madD120*	169	14	7.6
B71 × C149	*furA401 and madD120*	387	13	3.2
	furA401 and mt	195	205	51
	mt and madD120	198	202	50
C244 × C21	*leu-51 and madA7*	163	26	14
	leu-51 and mt	96	93	49
	mt and madA7	112	77	41
B71 × H1	*furA401 and leu-51*	88	112	56

C169 × C110 showed that mating type and *madE* are linked with a recombination frequncy of 6%. The other pair combinations of markers in the same cross were unlinked. In the cross C244 × C110 mating type and *madE* showed a recombination frequency of 11% and the other markers showed a recombination frequency around 50%.

In those cases in which different strains carrying the linked markers were not available the same cross was repeated. The first time the cross B71 × C149 was analyzed, the pair *furA401 – madD120* showed 7.2% recombination. When the cross was repeated recombination between the same pair of markers was 3.2%. The other pairs of markers in the same cross were unlinked.

In the cross C244 × C21 recombination between *leu-51* and *madA7* was 14%. The other pairs of markers in the same cross were unlinked. When the same cross was repeated the recombination frequency between *leu-51* and *madA7* was 17% (results not shown).

All the other pairs of markers analyzed in the crosses were unlinked (see Table 4) with the exception of the pair *furA401 – furB402*, the analysis of which is shown below.

Analysis of Crosses Involving Linked Markers

Complete data from crosses involving linked markers are shown in Table 5. All are three factor crosses with two linked markers and the third unlinked. When crossing over occurs between the linked markers the two types of recombinants appear with about the same frequency. Because the third marker is unlinked, four different genotypes with about the same frequency are found among the recombinants involving the linked markers.

Linkage between the two genes *furA401* and *furB402* was observed in the cross B71 × A2. A total of 120 germsporangia were pooled and a sample of 501 germspores was tested for resistance to 5-fluorouracil. The resistant colonies were 487 and 14 were sensitive to the drug. Among the sensitives, four were of mating type (−) and 10 were (+), ruling out the possibility of contamination with a wild type. The recombination frequency is $2 \times 14/501 \times 100 = 5.6\%$.

A second cross was analyzed between the strains B128 (*pur-51, furB402(+)*) and A1 (*furA401 (−)*). A total of 50 germsporangia were pooled and a sample of 500 germspores was tested. The number of colonies resistant to the drug was 487 and 13 were sensitive: of these, two carried the genotype *pur-51 (+)*, three were *pur-51 (−)*, two were *(−)* and six *(+)*. These results show that the colonies sensitive to 5-fluorouracil were not derived from contamination. In this last cross the recombination frequency was 5.2%.

Table 5. Crosses involving linkaged markers

Strains Geno-types	C169 × C110 *carA5(+) madE102(−)*		C244 × C110 *leu-51(+) madE102(−)*		B71 × C149 *furA401(+) madD120(−)*		C244 × C21 *leu-51(+) madA7(−)*	
	Genotypes of the progeny	Total number	Genotypes of the progeny	Total number	Genotypes of the progeny	Total number	Genotypes of the progeny	Total number
	carA5(+)	65	*leu-51(+)*	44	*furA401(+)*	81	*leu-51(+)*	38
	madE102(−)	85	*madE102(−)*	40	*madD120(−)*	109	*madA7(−)*	54
	(+)	81	*(+)*	57	*(+)*	4	*(+)*	3
	carA5, madE102(−)	84	*leu-51, madE102(−)*	54	*furA401, madD120(−)*	4	*leu-51, madA7(−)*	2
	madE102(+)	3	*madE102(+)*	8	*madD120(+)*	98	*madA7(+)*	31
	carA5(−)	6	*leu-51(−)*	9	*furA401(−)*	99	*leu-51(−)*	42
	(−)	6	*(−)*	3	*(−)*	3	*(−)*	5
	carA5, madE102(+)	6	*leu-51, madE102(+)*	5	*furA401, madD120(+)*	2	*leu-51, madA7(+)*	15
		336		220		400		190

Discussion

We have described a total of 22 sexual crosses between strains of *Phycomyces* involving 10 markers. Our results show that mating type and *madE* are linked with a distance between them of around 8 map units; *leu-51* and *madA* are linked at a distance of about 15 map units. *furA401, furB402* and *madD* belong to the same linkage group. The distances between *furA401* and *madD* and between *furA401* and *furB402* are both about 5 map units. We do not yet know the relative position of these three genes because the cross involving *furB402* and *madD* was not done. This is the first time that linkage between several markers have been reported in *Phycomyces*. The other genes studied, *nicA*, *carA*, and *pur-51* are all unlinked.

With the data obtained a tentative enumeration of the linkage groups found so far in *Phycomyces* is shown in Fig. 1. This scheme is necessarily incomplete due to the small number of markers used.

Mating type, *leu-51, nicA,* and *carA* are on separate chromosomes. Previously it hat been inferred by tetrad analysis that each was located about 15 map units from its centromere (Eslava et al. 1975b). It is reasonable to assume that the three markers *furA401, furB402,* and *madD* are located in a different linkage group, the same type of argument can be applied to the gene *pur-51*.

We do not know the chromosome number in *Phycomyces*. Cytological studies have been inconclusive and the sexual genetics is only just begun. The facts that the DNA complement per genome in *Phycomyces* is about seven times that of *Escherichia coli* (Dusenbery 1975) and that no linkage was found in previous

Linkage groups	Markers
I	Mating type, madE
II	leu-51, madA
III	nicA
IV	carA
V	pur-51
VI	furA401, furB402, madD

Fig. 1. Tentative linkage groups found in *Phycomyces* among the ten markers used

crosses suggested that in *Phycomyces* the genome consists of a relatively large number of small chromosomes (Eslava et al. 1976). This view may now have to be modified because from a total of ten markers we have found only six linkage groups.

We have routinely used a large sample of germspores from a pool of germsporangia (random spores analysis). This method gives information only about the linkage of the markers but it is very convenient in this early stage of *Phycomyces* genetics. The germspores from individual germsporangia are usually derived from mitotic amplification of the four products of a single meiosis (Eslava et al. 1975a, b). The analysis of samples of germspores from individual germsporangia formally corresponds to the analysis of unordered tetrads. This kind of analysis can be very useful in calculating the distances of the markers *madE, madA, pur-51, furA401, furB402* and *madD* from their respective centromeres.

There are several sources of error in all crosses with *Phycomyces*. We have been taking a sample from the pool of germspores and this may have led to sampling errors. In addition, the viability of the germspores on rich media (see Table 2) is only around

20%. This poor germination may be due to genotypic differences between the segregants because of non-isogenicity of the original pair. This problem has been solved with the isolation, by backcrossing, of a (+) wild-type, A56, which can be shown, by genetic evidence, to be largely isogenic with the (−) wild type commonly used in *Phycomyces* research (Alvarez and Eslava, in preparation). We have almost completed the transfer of all alleles found so far in *Phycomyces* to the (+) isogenic strain in order to avoid the gross errors which could be introduced with the use of non-isogenic strains not only in genetic but also in physiological experiments.

In our crosses the two alleles of each marker gene appear in the progeny tested in a ratio not significantly different from 1:1 showing the absence of allele specific selection (see Table 3).

Our results are internally consistent for all crosses as can be seen in Table 4. In those cases in which linkage was detected a second cross was made to confirm the results. The recombination frequencies should be taken as indicators only. Final linkage groups and genetic distances must wait until we have enough markers in *Phycomyces*.

The results from the crosses involving the two 5-fluorouracil resistance mutations, *furA401* and *furB402*, show that they are in different, but linked, genes. These two spontaneous mutations were isolated independently. It is not possible to perform complementation tests with them because preliminary results show that the allele *furA401* is dominant and the allele *furB402* is partially dominant. Therefore they are defined as two different genes (see Fig. 1) solely on the basis of their genetic distance, of 5 map units.

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References

Aragon CMG, Murillo FJ, De la Guardia MD, Cerdá-Olmedo E (1976) An enzyme complex for the dehydrogenation of phytoene in *Phycomyces*. Eur J Biochem 63:71–75

Bergman K, Eslava AP, Cerdá-Olmedo E (1973) Mutants of *Phycomyces* with abnormal phototropism. Mol Gen Genet 123:1–16

Cerdá-Olmedo, E (1975) The genetics of *Phycomyces blakesleeanus.*: Genet Res 25:285–296

Cerdá-Olmedo E (1977) Behavioral genetics of *Phycomyces.*: Annu Rev Microbiol 31:535–547

Cerdá-Olmedo E, Torres-Martínez S (1979) Genetics and regulation of carotene biosynthesis.: Pure and Appl Chem 51:631–637

De la Guardia MD, Aragón CMG, Murillo FJ, Cerdá-Olmedo E (1971) A carotenogenic enzyme aggregate in *Phycomyces*: Evidence from quantitative complementation. Proc Natl Acad Sci USA 68:2012–2015

Dennison DS (1979) Phototropism. In: Pirson A, Zimmerman ME, Haupt W, Feinleib ME (eds) Encyclopedia of plant physiology, vol 7. Springer, Berlin Heidelberg New York, p 506

Dusenbery RL (1975) Characterization of the genome of *Phycomyces blakesleeanus*. Biochim Biophys Acta 378:363–377

Eslava AP, Cerdá-Olmedo E (1974) Genetic control of phytoene dehydrogenation in *Phycomyces*. Plant Science Letters 2:9–14

Eslava AP, Alvarez MI, Burke PV, Delbrück M (1975a) Genetic recombination in sexual crosses of *Phycomyces*. Genetics 80, 445–462

Eslava AP, Alvarez MI, Delbrück M (1975b) Meiosis in *Phycomyces*. Proc Natl Acad Sci USA 72:4076–4080

Eslava AP, Alvarez MI, Lipson ED, Presti D, Kong K (1976) Recombination between mutants of *Phycomyces* with abnormal phototropism. Mol Gen Genet 147:235–241

Lipson ED, Terasaka DJ, Silverstein PS (1980) Double mutants of *Phycomyces* with abnormal phototropism. Molec Gen Genet (in press)

Ootaki T, Fischer EP, Lockhart P (1974) Complementation between mutants of *Phycomyces* with abnormal phototropism. Molec Gen Genet 131:233–246

Ootaki T, Kinno T, Yoshida K, Eslava AP (1977) Complementation between *Phycomyces* mutants of mating type (+) with abnormal phototropism. Mol Gen Genet 152:245–251

Russo VEA (1980) Sensory transduction in phototropism: Genetic and physiological analysis in *Phycomyces*. In: Leuci F (ed) Photoreception and sensory transduction in aneural organisms. (NATO Advanced Study Institute, Versilia, Italy). Plenum New York London (in press)

Sutter RP (1975) Mutations affecting sexual development in *Phycomyces blakesleeanus*. Proc Natl Acad Sci USA 72:127–130

Sutter RP Regulation of the first stage of sexual development in *Phycomyces blakesleeanus* and in other mucoraceous fungi. In: O'Day DH, Hogen PA (eds) Eukariotic microbes as model development systems. Dekker, New York, p 251

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