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An easy way of obtaining *Aspergillus nidulans* haploids in the parasexual cycle using *N*-glycosyl polifungin

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SUMMARY

A considerable improvement in the selection of haploids in *Aspergillus nidulans* is described. Haploidization is used to assign genes to linkage groups (chromosomes) in fungi. The differential effect of a polyene antibiotic, *N*-glycosyl-polifungin, in respect to haploids and diploids, was utilized. In minimal medium only diploids (constructed from a 'Master Strain' and the investigated strain), which are fully heterozygous and thus have no growth requirements, germinate and are killed by the antibiotic. However, auxotrophic haploids, either induced or spontaneously formed, survive. These can then be used for linkage studies.

1. INTRODUCTION

In fungi mitotic segregation is commonly used in early stages of gene location. In *A. nidulans* this process occurs during haploidization (Pontecorvo, Tarr-Gloor & Forbes, 1954), the transition from diploid to haploid that is believed to result from mitotic non-disjunction (Käfer, 1961). The fact that only one of a pair of chromosomes of a diploid will be transmitted to the haploid is of great value in mapping genes. The crucial point is that during mitotic segregation, in contrast to meiotic processes, recombination is very rare (Pontecorvo *et al.* 1954). A diploid constructed from a strain with an unlocated gene plus a 'Master Strain' bearing a marker in every chromosome will give haploid segregants in which the unknown gene will fail to recombine with the marker on the homologous chromosome from the Master strain (McCully & Forbes, 1965).

Initially spontaneous haploid segregants were isolated (Forbes, 1959). This process is rare, therefore D,L-*p*-fluorophenylalanine was introduced by Lhoas (1961) as an agent inducing haploid formation. The way in which this is brought about is not understood, though D,L-*p*-fluorophenylalanine is known to cause chromosome losses in mitosis (Lhoas, 1968).

Even though D,L-*p*-fluorophenylalanine increases the frequency of haploid formation it is still difficult and time-consuming to obtain haploids free from contamination with diploids. Often, after tedious work, the number of haploids found is insufficient to allow complete linkage studies to be made.

The discovery by Ditchburn & MacDonald (1971) that polyene antibiotics have a differential effect on the growth of auxotrophic and prototrophic strains of *A. nidulans* could be of value in obtaining haploid segregants free from contamination with diploids.

The aim of this work was to improve the technique of haploidization by increasing the yield of haploids and removing contaminating diploids. *N*-Glycosylpolifungin (NGP) was used because, as was shown previously (Bal, Balbin & Pieniążek), this derivative gave very good results in the selection of *A. nidulans* auxotrophs.

2. MATERIALS AND METHODS

All procedures using NGP have been described previously (Bal *et al.* 1974).

(*a*) *Strains.* All of the strains used in the present work originate from the collection of the Department of Genetics, University of Warsaw. The genotype of MSE is *yA2, wA3, galA1, pyroA4, facA303, sB3, nicB8, riboB2.* MSP II is the same as MSE with the exception of the first chromosome which is *proA6 pabaA9 biA1.* All the diploids were constructed using the standard technique of McCully & Forbes (1965).

(*b*) *Media.* The media described by Cove (1966) modified after Pontecorvo *et al.* (1953) were used. All incubations were carried out at 37 °C in a thermostatic incubator or in the case of liquid media in an orbital shaker at 34 °C.

3. RESULTS

(i) *Determination of optimal conditions for NGP*

Conidia of the diploid strain MSP II/*yA2, argB2, methH2* were suspended in liquid minimal medium and incubated for 7 h. Every hour a small sample of conidial suspension was withdrawn and the percentage of conidia germinated was estimated. All the viable conidia germinated between 3 and 6 h after the beginning of the incubation. Therefore the antibiotic in all subsequent experiments was added after 3 h and 3 further hours of incubation with the antibiotic were then applied. The final NGP concentration was 500 units/ml.

(ii) *Selection of haploids by NGP*

Conidia of a diploid strain were plated on solid complete medium containing D,L-*p*-fluorophenylalanine at the concentrations established by McCully & Forbes (1965). There were approximately 1 million conidia per plate. After 10–14 days conidia were harvested and subjected to NGP treatment on liquid minimal medium. After the end of the incubation conidia were separated from the antibiotic by centrifugation and plated on solid complete medium in order to obtain single colonies. Next, the colonies obtained in this way were tested on selective media.

(iii) *Comparison of various methods of obtaining haploids*

The classical and polifungin method of obtaining haploids were compared (Table 1). As was expected, the polifungin treatment greatly increased the number of haploid segregants. Although the clonal effect could not be avoided, the ease of obtaining haploid segregants by this method makes this inconvenience negligible.

(iv) *Analysis of segregation of the* argB2 *marker in haploid segregants obtained by means of NGP treatment*

Haploidization of the diploid strain MSE/*argB2*, *biA1* with NGP selection was used. The percentages of recombination between *argB2* and markers of MSE are presented in Table 2. Only 5·9 % of non-parental phenotypes between *argB2* and *galA1* were found among 117 analysed segregants, which fully agrees with the data cited by Roberts (1963) as both *galA* and *argB* belong to linkage group III.

Table 1. *Comparison of various methods of obtaining haploids*

Diploid strain	Method of isolation	No. of colonies tested	Haploids (%)
MSP II/*yA2*, *argB2*, *methH2*	Polifungin	264	96·8
	Classical	343	10·6
MSE/*argB2*, *biA1*	Polifungin	260	45·0
		372	87·7
	Classical	260	1·5
		476	8·4

When two results are given, the experiments were performed in duplicate.

Table 2. *Recombination of the* argB2 *marker with markers of MSE in 117 mitotic segregants obtained with the aid of NGP*

Marker tested	Linkage group of MSE marker	Non-parental phenotypes (%)
yA2	I	36·8
wA3	II	58·9
galA1	III	5·9
pyroA4	IV	43·6
facA303	V	50·4
sB3	VI	64·9
nicB8	VII	62·4
riboB2	VIII	65·8

4. DISCUSSION

The polyene antibiotic *N*-glycosyl polifungin proved to be very effective in selection of mitotic segregants for linkage studies. The value of 5·9 % non-parental phenotypes between *argB2* and *galA1* is higher than values obtained by McCully &

Forbes (1965). The reason for this is not known, and there are no data on the effects of polyene antibiotics on the genome (Hamilton-Miller, 1973). Two points of caution in the method presented should be kept in mind:

(*a*) Plating of about 1 million diploid conidia per plate containing complete medium with D,L-*p*-fluorophenylalanine and then harvesting a mixture of various diploid and haploid conidia found on these plates saves time and space but can result in repeated isolation of the same genotypes. The percentage of certain genotypes in this conidial mixture may depend on their ability to conidiate. In most cases this has no effect on the assigning of genes to a linkage group.

(*b*) The use of the polyene antibiotic for killing prototrophic diploids and preserving auxotrophic haploids may eliminate leaky phenotypes. In haploidization this does not matter unless more than one marker of the 'Master Strain' is lost in this way. Such phenotypes as *yA2*, *wA3* and *sB3* could also be partly eliminated. However, this did not appear to affect the results obtained. Moreover, this potential difficulty may be overcome by the construction of appropriate 'Master Strains'.

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