

Use of 3-aminotriazole to isolate oleate/acetate non-utilizing mutants of *Aspergillus nidulans*

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Oleate non-utilizing (*olu*) mutants of *Aspergillus nidulans* were isolated on selective medium containing the catalase inhibitor, 3-aminotriazole (3-AT); in an attempt to obtain strains that would enable analysis of peroxisome biogenesis and function in filamentous fungi. Out of 816 putative mutants recovered from the selective medium, 40 were *olu* mutants. All of the *olu* mutants were unable to utilize acetate for growth, and so resembled the *acu* mutants obtained by previous workers. The 40 *olu* mutants were placed in 5 different complementation groups.

Several methods have been developed to isolate peroxisome-deficient mutants of yeasts. These methods are based on the inability of cells to grow on carbon sources (e.g. oleic acid) for which functional peroxisomes are required. In this communication, we describe how a positive method of selection used to isolate peroxisomal assembly mutants of *Saccharomyces cerevisiae* (Van der Leij *et al.* 1992 J. Cell Biol. **119**:153-162) was modified to isolate oleate non-utilizing (*olu*) mutants of *Aspergillus nidulans*.

The selection procedure is based on the lethality of H₂O₂ formed during peroxisomal -oxidation of fatty acids in the presence of the catalase inhibitor 3-aminotriazole (3-AT). Mutant cells that do not accumulate H₂O₂ (due, for example, to a nonfunctional -oxidation system or ill-assembled peroxisomes) will be able to grow.

The selection medium should contain: at least, two different carbon sources: a fatty acid, to induce peroxisomal fatty acid oxidation, and a second carbon source (other than a fatty acid) to allow growth of mutants disturbed in peroxisomal fatty acid oxidation. In addition, this medium should allow normal growth of the wild type in the absence of 3-AT, but prevent growth in the presence of the catalase inhibitor.

In preliminary experiments, the nutritionally-supplemented *Aspergillus* minimal medium (MM) (Pontecorvo *et al.* 1953 Adv. Genet. **5**:141-238), with various carbon sources failed to fulfill the requirements of the selective medium, since no growth inhibition of *A. nidulans* strain R21 (Armitt *et al.* 1976 J. Gen. Microbiol. **92**:263-282) occurred in the presence of 3-AT. Addition of several components to this medium was tried to design a suitable selective medium. Addition of 1% NaCl and 0.5% yeast extract was necessary to satisfy the requirements of the selection medium (SM). The growth behavior of *A. nidulans* R21 on this selection medium with different carbon sources, with and without 3-AT is shown in Table 1. Solid SM containing 0.5% starch plus 0.03% lauric acid - 1% Tergitol NP-40 (Sigma) as carbon sources proved to be a good medium for the selection of *olu* mutants. In addition to the nutritional requirement of *A. nidulans* R21 (1 µg/ml p-aminobenzoic acid), histidine (50 µg/ml) had to be added to the medium, since *A.*

nidulans seems unable to synthesize this amino acid in the presence of 3-AT as reported in *S. cerevisiae* (Kanazawa et al. 1988 Mol. Cell Biol. **88**:664-673).

Inoculum optimization trials showed a value of 5 x 10⁶ viable spores per dish as the maximum to be employed in this positive selection method. Higher spore concentrations were unsuitable because they resulted in very dense growth.

From 18 experiments, using ultraviolet light mutagenesis (LD90) and involving 5 x 10⁶ viable spores per experiment, 816 colonies resistant to 3-AT were obtained. After subculturing them for two generations, 423 stable 3-AT resistant mutants remained. Stable mutants were tested for their ability to grow on MM with 6 mM oleic acid - 1% Tergitol NP-40 (Sigma) as sole carbon source, from which a total of 40 oleate non-utilizing (*olu*) mutants were isolated (Table 2). The *olu* mutants were unable to grow on acetate as sole carbon source, suggesting that their lesions did not occur in the -oxidation pathway. These 40 mutants were able to grow like the R21 parent on a wide range of

Table 1.- Growth behavior of *Aspergillus nidulans* R21 on selective medium (SM) with different carbon sources in the presence and absence of 3-aminotriazole (3-AT).

CARBON SOURCE	3-AT	+3-AT (50 mM)
0.5% Starch	+++	+++
2% Glucose	+++	++ (1)
6 mM Oleic acid	++	+(1)
0.03% Lauric acid	+	(+)
2% Glucose + 0.03% Lauric acid	+++	(+)
0.5% Starch + 0.03% Lauric acid	+++	

(+), +, ++, +++, are increasing amounts of growth. , no growth. (1) no conidiation.

Table 2.- Recovery of mutants unable to utilize oleate for growth

MUTANTS	NUMBER	PERCENTAGE
PUTATIVE	816	100%
STABLE	423	51.8%
OLEATE () MALTOS E (+) GLYCEROL (+)	40	4.9%

carbon sources e.g. maltose, glucose, glycerol, L-glutamate, L-proline, which do not require functional peroxisomes to be metabolised.

Mutants of *A. nidulans* unable to utilise oleate or acetate for growth have also been obtained by selecting for inability to use tributyrin as sole carbon source. Genetic analysis showed that they were allelic with already known *acu* (*acuD*, *acuF* and *acuE*) mutants (Kawasaki et al. 1995 Exp. Mycol. **19**:81-85).

Complementation analysis by means of heterokaryon tests showed that our *olu* mutants could be placed in five different complementation groups. The phenotypes of our *olu* mutants (growth with glycerol, but no growth with oleate - see Table 2) indicates that the *olu* mutants are not allelic with *acuA*, *facB*, *acuC* or *acuG* (Armitt et al. 1976 J. Gen. Microbiol. **92**:263-282). Work is in progress to determine whether the *olu* mutants are allelic with any of the remaining known *acu* mutants of *A. nidulans*. Preliminary studies indicate that mutants in at least two complementation groups are not allelic with any of the *acu* mutants described previously (Apirion 1965 Genet. Res. **6**:317-329; Armitt et al. 1976 J. Gen. Microbiol. **92**:263-282; Sealy-Lewis 1994 Curr. Genet. **25**:47-48).

Compared with the results obtained with *S. cerevisiae* using the same positive selection method, there is a two-fold higher yield of oleate non-utilizing mutants of *A. nidulans*. In addition, the use of this positive selection method in *A. nidulans* enabled us to obtain mutants which could not utilize oleate or acetate for growth. Unfortunately, the mutants of *S. cerevisiae* were not tested for growth on acetate. It is possible that lesions in our mutants are concerned with the biogenesis of peroxisomes, and work is in progress to determine whether the mutant cells carry intact or aberrant peroxisomes.

Despite the difficulty of finding an adequate selection medium, this report demonstrates that resistance to 3-AT provides one strategy to the isolation of oleate/acetate non-utilizing mutants of *A. nidulans*. Extension of this method to other filamentous fungi should be possible.

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