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Authors
Cramer, CL
Davis, RH

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Screening for Amino Acid Pool Mutants of *Neurospora* and Yeasts: Replica-Printing Technique

CAROLE L. CRAMER AND ROWLAND H. DAVIS*

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

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A replica-printing technique has been developed for permeabilized colonies of *Neurospora* and yeast by which endogenous amino acid pools are visualized. By the use of cation-exchange paper, the method has detected *Neurospora* mutants unable to store the large pools of basic amino acids characteristic of wild type. Mutants of this sort were sought to study the function of the fungal vacuole.

Cells of *Neurospora* have an organelle, the "vesicle," which stores large amounts of basic amino acids (4). We are interested in mutations affecting the vesicle. Accordingly, we wished to have a screening technique to identify colonies of *Neurospora* which are deficient in basic amino acids. Selection schemes dependent upon the inability of the mutants to accumulate compounds from the medium result in a predominance of transport mutants. We describe here a method which yields a visual representation of endogenous basic amino acid pools of colonies growing on minimal agar plates. It is adaptable to yeast and can probably be adapted to screen for many other types of mutants.

Mutagenized conidia of *Neurospora* were plated (50 to 80 colonies per plate) on a nitrate minimal medium (2) containing 0.05% each fructose and glucose, 1.8% sorbose, and 2% agar. The plates were processed after 3 days of growth at 30°C, before the onset of conidation. A disk of carboxymethyl cellulose paper (Whatman CM-82) was placed over the colonies, and any air bubbles were removed. A permeabilizing solution (1.2 ml of toluene and ethanol, 1:3 vol/vol) was spread over the disk, and immediately a disk of Whatman no. 1 paper was placed on it. The disks were pressed to the plate for 3 min with a heavy replica block, then removed and oven dried (80°C). The CM-82 paper was dipped briefly into distilled water to remove the nonbasic amino acids, and excess water was quickly removed by suction drying in a Buchner funnel. After fully drying the CM-82 paper, both disks were stained by dipping in a solution of 0.25% ninhydrin in acetone and heating at 80°C for 5 min. For yeast, colonies were plated on yeast-nitrogen base and grown for 3 to 4 days at 30°C. The same replica-printing technique was used with several modifications: a disk of Whatman no. 1 paper was first placed over the colonies, 1.5 ml of the permeabilizing solution was added, and then both Whatman CM-82 and no. 1 test papers were added. The first disk prevents cells from adhering to the CM-82 paper.

Ninhydrin-stained disks were screened on a light box. Colonies yielding light or no prints on CM-82 paper (representing basic amino acids and polyamines), while yielding normal or slightly lighter prints on no. 1 paper, were identified. In both *Neurospora* and yeast, some cells of all colonies on the original plates remained viable, and presumptive mutants could be transferred to tubes or plates for analysis.

The ninhydrin spots on the papers were shown to be due to intracellular amino acids because cold 5% trichloracetic acid removed the colorigenic material (a test protein spot was not removed), and the material appeared only after permeabilization of cells. Reconstruction experiments with a *Neurospora* strain (UM-300) which contains very low amino acid pools (R. H. Davis, unpublished data) showed the method effective in identifying the mutant in a background of wild-type cells.

Of 12,000 colonies developed from mutagenized (wild-type) *Neurospora* conidia, 136 colonies were identified as having low levels of basic amino acids. Such colonies were transferred to agar tubes for growth, then spotted onto plates and screened again by the replica-printing technique. About one-fourth (35/136) were retained for further analysis. The basic amino acid pool sizes (in nanomoles per milligram, dry weight) were determined (1) from the trichloroacetic acid-soluble extracts of cultures growing exponentially in Vogel's minimal or arginine-supplemented liquid media containing ammonium as the primary nitrogen source. (Ammonium could not be used when applying the replica printing technique on *Neurospora* because it produces a ninhydrin-positive background.) Ten mutants had deficiencies of 20 to 70% for basic amino acids on this minimal medium. One mutant of

particular interest also failed to accumulate arginine when grown in arginine-supplemented medium. This mutant showed the most extreme deficiency as judged by its replica print on CM-82 paper. Thus, such mutants can be obtained more efficiently if one is very selective at the replica printing stage. The other mutants obtained had the phenotypic behavior expected of very leaky mutants in nitrate reductase (corrected by ammonium-base medium) and arginine synthesis (corrected by arginine supplementation), among others. No tests of mutagenized yeast were performed. However, yeast yields very dark prints (Fig. 1), and mutant isolation should be relatively straightforward.

The replica-printing method could be useful for screening for a variety of mutants. It requires only the soluble compounds be released by permeabilization and revealed by a suitable visual test. The following applications merit consideration: (i) the use of anion exchange paper for representing acidic compounds; (ii) the use of minimal ninhydrin concentrations in the color test to screen for elevated, rather than reduced, amino acid pools; (iii) the use of specific color tests, fluorescence, or UV absorption to identify variants for individual compounds; (iv) the use of the system for enzyme tests by flooding the plate with appropriate substrates together with the permeabilization solution (4). It should be noted that the replica-printing technique can be used for tests of genetic segregation in crosses and for complementation tests of mutants isolated by this method.

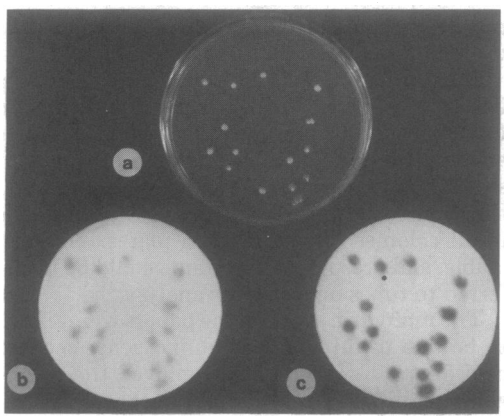

FIG. 1. *Replica prints of yeast. (a) Plated colonies of the wild-type strain X2180-1a. (b) Carboxymethylcellulose replica print. (c) Whatman no. 1 replica print.*

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