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NOTES

Detection, with the Dye Phloxine B, of Yeast Mutants Unable to Utilize Nitrogenous Substances as the Sole Nitrogen Source

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Yeast mutants unable to degrade certain nitrogen compounds produce characteristic small red colonies on an agar medium containing the red dye phloxine B, galactose, the test nitrogen compound, and a small amount of ammonium chloride.

The dye phloxine B (tetrabromotetrachlorofluorescein, also named acid red 92 or magdala red) has been incorporated by some authors (2, 6) into agar media to facilitate detection of yeast mutants. Nagai (6) used the dye for the detection of respiratory-deficient mutants. Horn and Wilkie (2) found that Nagai's phloxine B indicator medium is suitable for the detection of yeast mutants unable to synthesize purine or pyrimidine bases or amino acids. On indicator medium respiratory-deficient and auxotrophic yeast mutants produce small colonies that accumulate the red dye, whereas wild-type colonies are bigger and pink or pale. Preliminary experiments revealed that nitrogen-limited growth on glucose ammonium agar also causes phloxine B accumulation, optimal visual contrast between red colonies and pink medium being observed with 2.5 mM ammonium chloride. This observation prompted us to develop a phloxine B medium suitable for the detection of yeast mutants unable to grow on certain nitrogenous substances as the sole nitrogen source.

The wild-type strain of *Saccharomyces cerevisiae*, α-Σ1278b, was used, as well as two mutant strains derived from it: α-HP100 (α-*his*, *ura*), an auxotrophic mutant requiring histidine and uracil; and α-R79, ornithine transaminaseless, not utilizing arginine and ornithine as the sole nitrogen source. Complex media (YEPG and YEPE) contained, per liter of tap water: 20 g of peptone, 10 g of Oxoid yeast extract, and 20 g of glucose (YEPG) or 20 ml of 96% ethanol (YEPE). Minimal media contained, per liter of demineralized water: 40 g of glucose or 20 g of galactose, nitrogen source (40 mM assimilable N), 200 mg of histidine, and 200 mg of uracil if required, 1 g of magnesium sulfate, trace elements and vitamins as described previously (4), and 0.1 M potassium phosphate, pH 5.5. If ammonium chloride was the nitrogen source, 40 mM potassium sodium tartrate was added as a buffer substance. Culture media were solidified by the addition of 20 g of Oxoid agar per liter. The media were sterilized at 120°C for 15 min. Sugars were sterilized separately. Labile nitrogenous substances and phloxine B (color index no. 45410, Koch-Light, Colnbrook, England) were sterilized by Seitz filtration. Ethanol was added aseptically. The phloxine B medium was identical to minimal medium except it contained, per liter: 10 mg of phloxine B, 20 g of galactose, 2.5 mM ammonium chloride, and 40 mM test nitrogen compound. Yeast mutants were obtained by treatment with ethyl methane sulfonate (1). Mutants unable to assimilate a nitrogenous substance as the sole nitrogen source were enriched by nystatin treatment (7, 8).

A series of phloxine B medium plates of 11 cm in diameter was inoculated with 100 to 300 colony-producing units/plate. After 5 to 10 days at 30°C small red colonies were transferred to YEPG plates and, after 2 days at 30°C, replicated on glucose minimal medium plates with different nitrogen sources to screen the presumed mutants for the properties desired. Mutant strains were purified by streaking on YEPG. Respiratory-deficient mutants were recognized by their inability to grow on YEPE plates and were discarded.

Attempts to detect mutants unable to utilize arginine as the sole nitrogen source were successful if a mutagen-treated yeast suspension was plated on phloxine B agar containing 10 mM L-arginine in addition to 2.5 mM ammonium chloride as the nitrogen source and galac-

TABLE 1. *Isolation of yeast mutants unable to utilize arginine as the sole nitrogen source*

Determination	No.
Colonies on 100 phloxine B plates[a]	27,000
Small red colonies tested	743
Respiratory-deficient small red colonies	19
Strains not growing on arginine or ornithine	149
Arginaseless	10
Ornithine transaminaseless	98
argR (1 class)	39
Unidentified	2

[a] Strain α-Σ1278b was treated with ethyl methane sulfonate. The mutants desired were enriched by nystatin treatment and detected on phloxine B, galactose, 10 mM arginine, 2.5 mM ammonium chloride medium.

tose as the carbon source (Table 1). With glucose as the carbon source, however, most of the small red colonies were respiratory deficient. Galactose is known to be not, or very slowly, assimilated by respiratory-deficient yeast mutants (5).

From Table 1 it can be seen that about 20% of the small red colonies tested were unable to degrade arginine. Pale or white colonies, irrespective of the colony size, yielded arginine-degrading strains. The number of respiratory-deficient colonies was very small. The arginine-negative mutants belonged to five different genetic classes: arginaseless, ornithine transaminaseless, regulatory mutant *argR* (3) uninducible for both of these enzymes (only one of three known *argR* classes was isolated in this experiment), and two different, as yet unidentified mutants unable to grow with arginine but growing normally on ornithine minimal medium. The variety of the mutants obtained is surprisingly high, especially in view of the fact that all mutants had arisen from one mutagenic treatment.

Our method has been applied successfully for the isolation of mutants unable to degrade allantoin. Moreover, it gave good results in obtaining mutants of α-HP100 showing impaired growth on ammonium, glutamate, or urea as the sole nitrogen source. Established enzyme defects include anabolic and katabolic glutamic dehydrogenase and allantoinase.

Phloxine B is not growth inhibiting either in batch or continuous culture but promotes death under conditions of nitrogen limitation (unpublished data). Its accumulation by the dead cells constitutes most of the staining of nitrogen-limited yeast colonies growing on phloxine B indicator medium.

LITERATURE CITED

1. **Fink, G. R.** 1970. The biochemical genetics of yeast, p. 59–78. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 17A. Academic Press Inc., New York.
2. **Horn, P., and D. Wilkie.** 1966. Use of magdala red for the detection of auxotrophic mutants of *Saccharomyces cerevisiae*. J. Bacteriol. 91:1388.
3. **Messenguy, F., and J. M. Wiame.** 1969. The control of ornithinetranscarbamylase activity by arginase in *Saccharomyces cerevisiae*. FEBS Lett. 3:47–49.
4. **Middelhoven, W. J.** 1970. Induction and repression of arginase and ornithine transaminase in baker's yeast. Antonie van Leeuwenhoek J. Microbiol. Serol. 36:1–19.
5. **Moat, A. G., J. J. Barnes, and E. McCurley.** 1966. Factors affecting the survival of auxotrophs and prototrophs of *Saccharomyces cerevisiae* in mixed populations. J. Bacteriol. 92:297–301.
6. **Nagai, S.** 1963. Diagnostic color differentiation plates for hereditary respiration deficiency in yeast. J. Bacteriol. 86:299–302.
7. **Snow, R.** 1966. An enrichment method for auxotrophic yeast mutants using the antibiotic nystatin. Nature (London) 211:206–207.
8. **Thouvenot, D. R., and C. M. Bourgeois.** 1971. Optimisation de la sélection de mutants de *Saccharomyces cerevisiae* par la nystatine. Ann. Inst. Pasteur Paris 120:617–625.