Selective Media for the Detection of Revertants in Cultures of Glycogen-deficient Mutants

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Glycogen-deficient yeast mutants grew more slowly than wild-type cells on certain nonfermentable carbon substrates. Revertant colonies could be detected on these lines.

Cultures of certain strains of Saccharomyces cerevisiae contain about 0.5% of spontaneous glycogen-deficient mutants (1, 2). Mutant colonies growing on nutrient agar can be detected by their failure to give the usual brown staining reaction of glycogen with iodine solution. Mutants were maintained by subculture on nutrient agar slopes, and from time to time the stability of the cultures was tested by plating cells on agar and staining the colonies with iodine solution. Some mutant strains were much less stable than others, and brown-staining revertant colonies appeared among the mutants.

We found that the mutants grow much more slowly than normal cells on such carbon substrates as lactate, gluconate, fumarate, succinate, and pyruvate, despite the fact that their rate of respiration with glucose as substrate is about 75% of that of the parent yeast. This permitted the preparation of selective media for revertant cells, based on the control medium of Ogur and St. John (3), modified by the substitution of one of the above carbon substrates (final concentration, 1%) for the glucose. When mutant cultures were plated on these media, a small proportion of the colonies grew much faster than the rest. Cultures obtained from isolates of these larger colonies replated on glucose-containing nutrient agar gave a positive glycogen staining reaction with iodine. Figure 1 shows large revertant colonies among the smaller mutants growing on a lactate medium. The revertants could be detected on the plates after growth for 2 days, but the mutant colonies could not be seen until at least 5 days after inoculation. (The petri dishes were sealed to prevent the agar from drying out.)

A diagnostic medium with lactate as the carbon substrate was used originally by Ogur and St. John to identify respiration-deficient cells. Respiration-deficient cells do not grow on carbon sources which can be utilized only by oxidative degradation. This property has been of particular value in the present work, because glycogen-deficient cultures may contain up to 9% of respiration-deficient cells (1). Media of the type discussed in this paper can thus be useful for distinguishing between respiration-deficient cells, glycogen-deficient cells, and revertants from glycogen-deficiency.

LITERATURE CITED