Folate Deficient Mutants of *Saccharomyces cerevisiae*

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A screening for folate deficient mutants has been undertaken in connection with biochemical studies on the biosynthesis of riboflavin [1]. The biosynthetic pathways of both riboflavin [2] and folic acid [3] start from a guanine-type compound, and may possibly share one or more common intermediates [4]. The present paper describes the isolation of folate deficient mutants. To our knowledge, mutants of this kind have not been described hitherto.

Methods

Saccharomyces cerevisiae S 288 C was treated with ethyl methane sulfonate as described previously [5]. The cells were plated on complete medium (yeast extract, 10 g; peptone, 10 g; glucose, 20 g; folic acid, 20 mg; riboflavin, 20 mg; deion. water, 1 *l*). Colonies were streaked or replica plated on minimal medium [5] and on minimal medium supplemented with folic acid (20 mg/*l*) and riboflavin (20 mg/*l*).

Results

From 53 000 colonies tested, a total of 5 folate deficient mutants, 42 riboflavin deficient mutants and 13 *p*-aminobenzoic acid deficient mutants were isolated. *p*-Aminobenzoic acid mutants were not obtained in replica plating experiments. The folate deficient mutants require a high concentration of folate for growth (fig. 1). They do not grow with *p*-aminobenzoic acid. Three mutants grow slightly with methionine.

In an attempt to reduce the quantitiy of folate required by the mutants, one of them (HK 875) was serially transferred into minimal medium (250 ml, inoculum 1 ml) supplemented with successively decreasing quantities of folic acid (10, 3, 1, 0,3 mg/*l*). Growth response to folate was frequently tested. After 23 transfers a strain designated HK 875-1 was isolated, this one exhibiting enhanced growth on folic acid as shown in fig. 1.

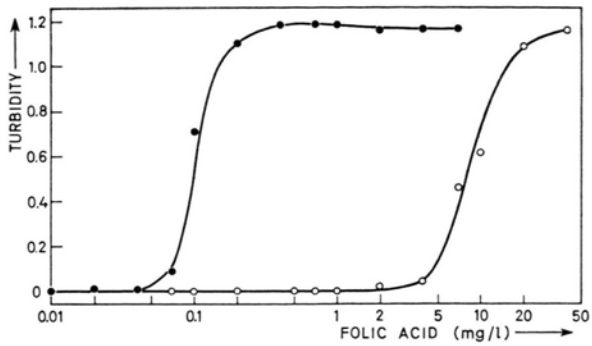

Fig. 1. Growth of strain HK 875 (open circles) and HK 875-1 (closed circles); 4 days, 30 °C.

Discussion

Folate deficient mutants of *S. cerevisiae* can only poorly utilize exogeneous folate. The poor growth effect may be due to the lack of a sufficiently active folate reductase or to difficulty in permeation or both. Folate utilization was greatly improved by serial transfer on limiting folate concentration, most probably involving selection of spontaneous mutants.

Some folate mutants can grow slightly on methionine. As a folate coenzyme is involved in the biosynthesis of methionine, it seems reasonable that methionine should exert a folate sparing effect.

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