Investigations on Malic Acid Utilisation in *Schizosaccharomyces* species

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Abstract

The aims of this investigation were: to determine whether alterations in uptake, or metabolism, of glucose and malate was the cause of malate dependence; to determine the number of genes involved in malate dependence; and to clone the gene(s) involved. A malate dependent mutant, i.e. a mutant that requires both malate and glucose for growth, (mutant 11) of *Schizosaccharomyces malidevorans* 442 was characterised. Malic enzyme activity was increased almost ten-fold. The V_{max} of malate uptake was increased four-fold compared to *S. malidevorans* 442. Uptake of glucose was significantly lower in mutant 11 than the wild-type. The kinetics of glucose uptake by *S. malidevorans* 442 and mutant 11 suggested the presence of two glucose transporters, a high affinity and a low affinity transporter. Only the low affinity transport was apparently altered in mutant 11 compared to the wild-type.

Genetic analysis indicated that the malate dependent mutation is recessive and is the result of a single mutational event. Crosses involving derivatives of mutant 11 and *Schizosaccharomyces pombe* strains did not yield the expected segregation of markers. Tetrad analysis showed that the spore viability was very low. It was not possible, therefore, to determine linkage of the malate dependence locus and any other loci.

All malate dependent strains were apparently homothallic although linkage between the mating-type locus and malate dependence could not be established. The isolation of similar mutants from homothallic strains of *S. pombe*, but not from heterothallic strains,

provided strong support for the requirement of homothallism for malate dependence. The pulse field gel electophoresis karyotypes of mutant 11 and derivatives of mutant 11 suggested the presence of a large chromosomal rearrangement of chromosome 2 that co-segregated with malate dependence.

Malate dependent mutants were not obtained from homothallic *Saccharomyces cerevisiae* MD26.

A malate dependent mutant (WT 6) was isolated from *S. pombe* WT 4 and found to have characteristics similar but not identical to those of mutant 11. WT 6 demonstrated increased utilisation of malate and decreased utilisation of glucose. Malic enzyme activity was not altered in WT 6 compared to the wild-type. Malate uptake was not affected. The karyotype of WT 6 suggested that a chromosomal rearrangement had occurred, but it is not identical to the rearrangement in mutant 11.

The differences in the characteristics of mutant 11 and WT 6 suggested the mutations in these mutants may not be identical. The finding that mutant 11 and WT 6 belong to different complementation groups could explain these differences.

Although differences were found in the uptake of malate and glucose, the inability of malate dependent mutants to grow on glucose implicates a defect in glucose metabolism.

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For Hugh, Micah and Bowser

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