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Selection of *AraB* and *AraC* Mutants of *Escherichia coli* B/r by Resistance to Ribitol

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The growth of strain *araC^c67*, which produces the enzymes of the *ara* operon constitutively, is inhibited by the addition of ribitol. Isolation of strains resistant to ribitol yields mutants of either the *araB* or *araC* genes. A model to account for the inhibition by ribitol is discussed.

During the course of a study in which compounds related to L-arabinose were examined for their possible effect upon the expression of the *ara* operon in *Escherichia coli* B/r (*see* Fig. 1), it was noted that ribitol produced a marked effect upon the growth of an *ara* constitutive mutant, *araC^c67*. Addition of ribitol to a growing culture of strain *araC^c67* resulted in a drastic reduction in the growth rate, from a doubling time of 45 min in the absence of ribitol to a doubling time of 186 min in its presence (Fig. 2A). The growth rate of the wild-type strain, however, was unaffected by the presence of ribitol.

When the strain *araC^c67* is plated on 2,3,5-triphenyltetrazolium chloride (TTC)-ribitol tryptone medium, light-pink colonies, less than 1 mm in diameter, are observed after 24 hr. After an additional 24 hr, however, large red colonies begin to arise. A ribitol-resistant mutant, *RR500*, was isolated and purified, and its growth in casein hydrolysate medium, with and without ribitol, was examined. Its growth rate was only slightly affected by the presence of ribitol (Fig. 2B).

One-hundred independent spontaneous ribitol-resistant mutants were isolated from strain *araC^c67* on TTC-ribitol tryptone plates. These were purified once on homologous media and screened for the presence of *ara* mutations in a complementation test on mineral arabinose plates employing various homogenotes which possessed *araA*, *araB*, *araC*, or *araD* mutations (5). The presence of the *araC^c67* allele in each of the resistant strains was also examined in a complementation test with the homogenote *F'araC5/araC5* on mineral arabinose D-fucose plates. [The presence of the dominant *C^c67* allele results in fucose resistance (2).] It was found that 59 of the 100 resistant mutants screened contained mutations in the *araB* gene by failing to complement only the homogenotes which carried *araB* muta-

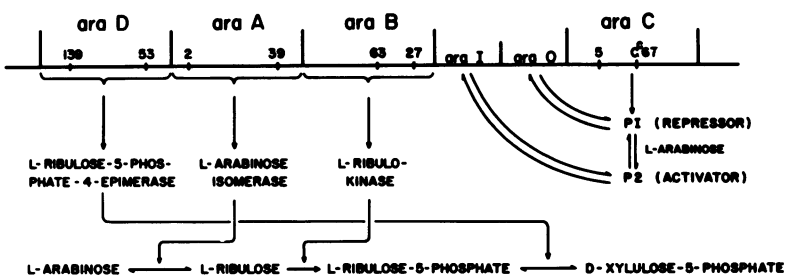

FIG. 1. *L-Arabinose gene-enzyme complex. The structural genes, araA, araB, and araD, code for the enzymes which convert L-arabinose to D-xylulose-5-phosphate. AraC is the regulatory gene of the operon (2, 5). Its product, P1, functions as a repressor by binding to araO, the operator site (4). In the presence of arabinose, P1 is converted to P2, the activator, which binds to the initiator region, araI, and stimulates expression of the structural genes (3). Mutant sites are defined by a number. The strain containing the mutation C^c67 can produce the enzymes in the absence of arabinose (2).*

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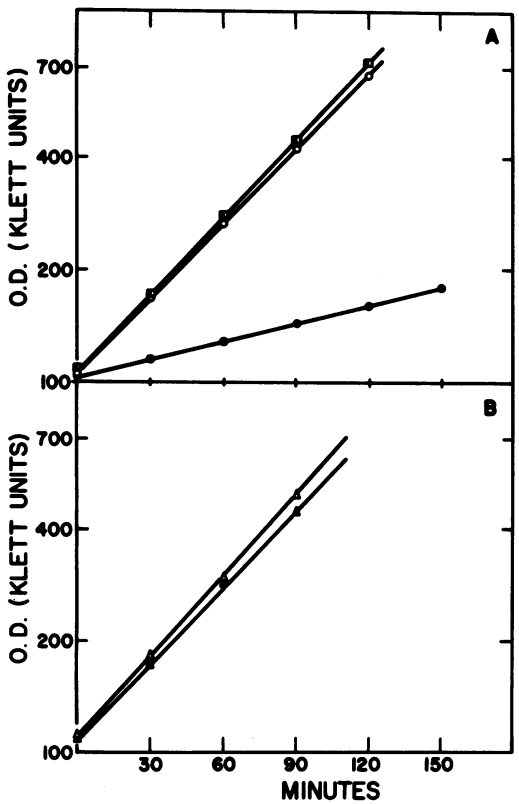

FIG. 2. *Effect of ribitol upon growth. To cells, growing in 1% casein hydrolysate medium (2), either no addition or addition of ribitol to a final concentration of 0.4% was made at time zero. Optical density at 420 nm was measured on a Klett-Summerson colorimeter (1 unit = 2.5 × 10⁶ viable bacteria per milliliter). (A) Strain araC^c67 grown in casein hydrolysate (○) and casein hydrolysate plus ribitol (●). Wild-type strain grown in casein hydrolysate (□) and casein hydrolysate plus ribitol (■). (B) Ribitol-resistant mutant, RR500, grown in casein hydrolysate (△) and casein hydrolysate plus ribitol (▲).*

tions. Similarly, 24 of the resistant strains contained *araC* mutations and 8 contained deletions from at least the *araB63* mutant site to the *araC5* mutant site (*see* Fig. 1). Every single *Ara*⁻ ribitol-resistant mutant was found to complement both *araA* and *araD* gene mutants. Only 1 of the 76 strains which had not mutated to C^- was found to have reverted to the wild-type allele, C^+.

What is perhaps the most interesting class is represented by eight resistant mutants which appear to be similar to the *araC^c67* strain used for the selection. These strains grow as well as the parent strain on mineral arabinose or mineral arabinose fucose media but are resistant to ribitol. The basis for the resistance to ribitol has not yet been determined.

The ribitol inhibition of the *araC^c67* strain closely resembles the inhibition by arabinose of *araD* gene mutants described by Englesberg et al. (1). They demonstrated that a deficient L-ribulose-5-phosphate-4-epimerase results in the accumulation of the phosphorylated intermediate, L-ribulose-5-phosphate, which is inhibitory to growth. Mutation to resistance involves a mutation which results in the prevention of the accumulation of the phosphorylated compound; either $araD^-A^-$, $araD^-B^-$, or $araD^-C^-$.

In attempting to explain the ribitol effect, we note the fact that ribitol is a substrate of the L-ribulokinase (N. Lee, *personal communication*). It is proposed that ribitol is converted by the enzyme L-ribulokinase to the phosphorylated, nonmetabolizable compound, ribitol phosphate, which accumulates in the cell, thereby inhibiting growth. Mutation to resistance involves prevention of accumulation of the phosphorylated compound by either loss of kinase activity (*araB* mutation) or cessation of its synthesis (*araC* mutation). The observation that a double mutant, *araA39C^c67*, is sensitive to ribitol and the failure to find any *araA* or *araD* mutants among the resistant strains are consistent with this interpretation. Furthermore, because the wild-type strain and the $araC^+$-resistant revertant are refractory to the ribitol effect, it is further argued that the kinase must be produced constitutively for the inhibition by ribitol to occur. This model is also in agreement with the earlier finding that ribitol is not an inducer of the L-arabinose operon in the wild-type strain.

Two types of mutations, not previously described in the *ara* system, can account for the resistance to ribitol in the strains which appear to be similar to the *araC^c67* parent strain. Mutation in the *araB* gene may result in the alteration of the structure of the L-ribulokinase such that it is no longer capable of binding or converting ribitol to the phosphorylated compound. A loss of ability to bind ribitol need not be accompanied by the loss of ability to bind the natural substrate, L-ribulose. Alternatively, mutation in the *araC* gene may result in the loss of constitutivity without loss of fucose resistance. Experiments are in progress to understand the nature of resistance to ribitol in these strains.

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