GENETIC STRUCTURE OF LACTOSE-NEGATIVE VARIANTS OF Escherichia coli CARRYING MUTANT FACTOR F^1-LAC

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The genetic structure of lactose-negative variants of heterogenotes of Escherichia coli K-12, carrying mutant factor F^1-lac, characterized by partial loss of ability to transfer itself and to transfer the chromosomal segment, was investigated. Lac^- mutations within the chromosomal segment incorporated in mutant factor F^1-lac were shown to be functionally heterogeneous.

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Introduction of the "wild-type" factor F^1-lac (exogenote) into E. coli K-12 cells carrving the lac^- mutation on their chromosome is accompanied by the formation of heterogenotes (merodiploids) lac^-/F^1-lac^+, the phenotype of which is lac^+ [3]. A characteristic feature of these heterogenotes is that they are unstable, because cells incapable of fermenting lactose segregate from them. This is due either to loss of factor F^1-lac^+ with their conversion into haploids, or to the fact that the lac^+ locus included in the sex factor becomes a lac^- locus as the result of recombination between the exogenote and endogenote [4].

The object of the present investigation was to study the genetic structure of lactose-negative variants of heterogenotes possessing mutant factor F^1-lac^+, characterized by partial loss of their ability to transfer the factor itself and to transfer the chromosomal segment [1].

EXPERIMENTAL METHOD

Cultures isolated from lac^- colonies appearing in small numbers after seeding of E. coli 200 PS-74 and 200 PS-14 cells carrying mutant factor F^1-lac^+, on Endo's agar, were used as lactose-negative variants. The recipients were streptomycin-resistant (S^r) mutants of prototrophic strains E. coli 2320SF-lac_2^- and E. coli 200PSF-$lac\bar{y}$. The sensitivity of the lactose-negative variants to phage f2 was determined by the usual methods [2].

Complementation tests were carried out by crossing the investigated variants with each of the recipient strains mentioned above. For this purpose, 8-h broth cultures were mixed in the ratio 1 : 10, and after incubation of the mixtures at 37° for 2 h, the dilutions were seeded on minimal agar with lactose (1%) and streptomycin (250 units/ml). The lac^+S^s recombinants were planted after incubation of the cultures at 37° for 48 h.

Control experiments consisted of crossings of the same recipients with lac^- variants derived from heterogenotes carrying mutant factor F^1-lac^+, which had completely lost their ability to transfer the factor and to transfer the chromosomal segment (strains 200PS-21 and 200PS-22).

EXPERIMENTS RESULTS

By analogy with the properties of lactose-negative cells segregated by heterogenotes formed by "wild-type" factor F^1-lac^+, it could be postulated that the lac^- variants isolated from strains of heterogenotes possessing mutant factor F^1-lac^+ are either haploids or homozygotic merodiploids (homogenotes) lac^-/F^1-lac^-.

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TABLE 1. Functional Analysis of lac⁻ Mutations in Sexduction Experiments

lac⁻ variants investigated	Complementarity with different F-lac⁻ cells	
	2310S lac_z^-	200PS lac_y^-
200PS-74/F^1-lac⁻	–	–
200PS-14/F^1-lac⁻	+	–
200PS-21/F^1-lac⁻	–	–
200PS-22/F^1-lac⁻	–	–

In the initial experiments the tested lac⁻ variants including the controls were therefore tested for sensitivity to phage f2, which produces lysis only of cells carrying sex factor. The results of this test showed that lac⁻ cells of all the tested strains are sensitive to phage f2, i.e., that they are homogenotes and their genotype is lac⁻/F^1-lac⁻.

In the next experiments the fine genetic structure of the lac⁻ locus contained in mutant factor F^1 was analyzed. For this purpose complementation tests were used, in which mutant factor F^1-lac⁻ was introduced into cells (sexduction) carrying a lac⁻ mutation belonging to different functional groups (y⁻ and z⁻) on their chromosomes. Complementarity of the mutants was judged by the appearance of recombinant lac^+ colonies.

As Table 1 shows, only lac⁻ mutations on the chromosome of 2310SF⁻ cells and on the segment included in F^1 factor of lactose-negative variant 200PS-14/F^1-lac⁻ were found to be complementary.

From the functional point of view, not all lac⁻ mutants are identical. Some of these are lac_y^- mutants, incapable of synthesizing galactoside permease (acetylase), while others are lac_z^- mutants, incapable of synthesizing β-galactosidase, and finally, a third group are lac_o^0 mutants, unable to synthesize either permease or galactosidase. The lac_y^- mutants are complementary only to lac_z^- mutants, and the lac_z^- mutants are complementary to lac_y^- mutants, whereas lac_o^0 mutants are complementary neither to the lac_y^- nor the lac_z^- mutants.

To sum up the results, it can be concluded that the lac⁻ mutation within the chromosomal segment of the exogenote of variant 200PS-14/F^1-lac⁻ is a lac_y^- mutation. So far as the lac⁻ mutation in the chromosomal segment of the exogenotes of variant 200PS-74/F^1-lac⁻ is concerned, this is a lac_o^0 mutation, because it is complementary neither to the lac_y^- nor the lac_z^- mutations occurring on the chromosome of cells 2310SF⁻ and 200PSF⁻ (respectively).

The lac⁻ mutations within the chromosomal segment included in mutant factor F^1-lac are thus heterogeneous.

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