Mutants of *Escherichia coli* K12 Sensitive to Acidic pH

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Four mutants of *Escherichia coli* K12 were isolated on the basis of their sensitivity to pH 5.4. Under non-permissive conditions their growth was reversibly inhibited. At pH 7.0 these mutants showed a highly pleiotropic phenotype, which included altered phage and detergent sensitivities and leakage of periplasmic proteins. The findings suggest a defect in the outer membrane, perhaps in lipopolysaccharide. Two mutants mapped in or near the *rfa* locus, while the other two were remote from this region.

INTRODUCTION

The cell envelope of Gram-negative bacteria is composed of three layers. The outer and inner (cytoplasmic) membranes are separated by a layer of peptidoglycan. Mutants with defects in their outer membranes have been isolated by different selection techniques: increased sensitivity to small hydrophobic molecules, such as detergents, dyes and drugs (Dame & Shapiro, 1976; Coleman & Leive, 1979), resistance to specific phages (Prehm *et al.*, 1976; Picken & Beacham, 1977), or leakage of periplasmic proteins (Lopes *et al.*, 1972).

In this paper we report the isolation and partial characterization of *Escherichia coli* mutants sensitive to low pH. The results suggest defect(s) in the outer membrane of the mutants.

METHODS

Organisms. *Escherichia coli* K12 strain KMBL 49 (Rörsh) was used for mutant isolation. Recipient strains for transduction are described in Table 1. The R-core specific bacteriophages used were T3 and T4. Phage K3 has its receptor site in outer membrane protein A. The transducing phage was P1.

Mutant isolation. KMBL 49 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described by Miller (1972). The bacteria were plated on complete LB medium (Miller, 1972), pH 7.0. The colonies were subsequently replicated to LB plates pH 5.4. The initial pH of the medium was adjusted by adding appropriate amounts of 1% (v/v) HCl. The plates were incubated at 37 °C for 24 h. Those colonies that failed to grow at the acidic pH were selected for further tests. They were next grown aerobically at 37 °C with rotary shaking in LB medium. Bacterial growth was monitored at 540 nm at both pH 7.0 and 5.4.

Bacteriophage and detergent sensitivities. For phage sensitivity the conventional cross-streak test was used. Detergent sensitivity was estimated in plates, pH 7.0, containing 800 µg of sodium dodecyl sulphate l⁻¹, a concentration that did not kill the wild-type.

Leakage of ribonuclease I. The method described by Lopes *et al.* (1972) was used.

Genetic analysis. Transduction with phage P1 was performed as described by Willetts *et al.* (1969) after preliminary mapping of crosses with different F' donors, which corrected the defect of pH-sensitive recipients.

RESULTS AND DISCUSSION

Four independently isolated mutants that did not grow at pH 5.4 were: KMBL 49-3, KMBL 49-30, KMBL 49-51 and KMBL 49-63. At pH 7.0 the growth of these mutants was normal. The effect of pH on the growth of the wild-type and two mutants is shown in Fig. 1.

Fig. 1. Growth at pH 5.4 and pH 7.0 of the wild-type strain KMBL 49, and mutants sensitive to acidic pH. Wild-type strain, pH 7.0 (●) and pH 5.4 (○); strain KMBL 49-3, pH 7.0 (▲) and pH 5.4 (△); strain KMBL 49-51, pH 7.0 (■) and pH 5.4 (□). The arrow indicates the time that the pH was adjusted from 7.0 to 5.4.

Table 1. *Genetic mapping of mutations conferring sensitivity to acidic pH by P1-mediated transduction*

Phage P1 was grown on the pH-sensitive mutants and used to transduce the indicated pH-resistant strains. The transductants, after being purified by single colony isolation, were tested on LB plates, pH 5.4.

Donor	Recipient	Relevant genotype	Map position	Selected marker	Number scored	Frequency of cotransduction of pH sensitivity (%)
KMBL 49-3	CRT 46 (Hirota)	<i>ilvB</i>	82	Ilv ⁺	600	4.1
KMBL 49-30	CRT 46 (Hirota)	<i>ilvB</i>	82	Ilv ⁺	800	10.0
KMBL 49-51	AB 1157 (Clark)	<i>galE</i>	17	Gal ⁺	800	4.1
KMBL 49-63	JC 5484 (Clark)	<i>argE</i>	89	Arg ⁺	660	19.0

The effect of low pH on growth was reversed after readjusting the pH to 7.0. All the four mutants had the following properties: (1) they were resistant to phages T3 and T4, while retaining their original sensitivity to phage K3; (2) they had become sensitive to the anionic detergent, sodium dodecyl sulphate; (3) they had started to excrete the periplasmic enzyme, ribonuclease I, into the medium. This combination of properties suggested that they might belong to a group of deep rough mutants defective in their lipopolysaccharide (LPS). However, sensitivity to acidic pH has not been described previously for LPS mutants.

Preliminary genetic analysis of the mutants, done by mating with different F' donors, indicated that three distinct chromosomal locations were involved: the 70–89 min interval for mutations in the strains KMBL 49-3 and KMBL 49-30, the 14–19 min interval for strain KMBL 49-51 and the 87–95 min interval for strain KMBL 49-63.

For more accurate mapping we used phage P1 transduction (Table 1). The mutations in strains KMBL 49-3 and KMBL 49-30 were cotransducible with *ilvB* at the approximate map position of 82 min. This is the same region as that of the *rfa* genes (81 min), which are involved in LPS core biosynthesis (Coleman & Leive, 1979; Bachmann & Low, 1980). The

pH sensitivity of the mutant KMBL 49-51 was weakly linked with *galE* (at 17 min) and that of KMBL 49-63 with *argE* (at 89 min). There have been no previous reports on mutations at the last two positions which would show changes in the outer membrane structure and function.

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