DEMONSTRATION OF MISSING MEMBRANE PROTEINS IN DELETION MUTANTS OF E. COLI K12

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Deletion mutants extending from tol A,B to chl A of E. coli K12 were isolated and characterized. Physiological studies indicate that these mutants show sensitivity to a wide range of antibiotics and detergents, indicating an alteration of the cell envelope. Analysis by polyacrylamide gel electrophoresis demonstrated the loss of membrane proteins in the deletion mutants of this genetic region.

The cell membrane is a complex cell organelle, both structurally and functionally. An understanding of its organization would thus be facilitated not only by looking at the cell membrane biochemically and physiologically, but genetically as well. A genetic approach requires the isolation of mutants, and a physiological and biochemical comparison of mutants and the wild type. This paper describes the isolation and characterization of a deletion mutant of E. coli K12 that is demonstrably missing several membrane proteins.

MATERIALS AND METHODS

E. coli KA56 (Hfr, gal-D, gal lethal, obtained from Dr. J. Pittard) was used as the parent strain in the isolation of deletion mutants. Strain KA56 is sensitive to the colicins E1, E2, E3 and K. However,

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mutations leading to resistance or 'tolerance' to all of these colicins occur at the gene tol A,B which is located near gal on the E. coli chromosome (1). The parent strain was plated at 10^{10} cells/ml on eosine-methylene blue (EMB) plates containing galactose and the colicins E2, E3 and K. These plates were incubated at 37°C for 48 hours, and the surviving colonies were purified by restreaking on EMB plates before being tested to see if they were deletion mutants.

The extent of the deletions was determined by testing for the presence or absence of various genes located near gal (Fig. 1). For genes nic A and bio A, this was determined by plating cells on minimal agar plates with or without nicotinic acid or biotin, using glucose as the carbon source. The ability to utilize galactose as a carbon source was tested by plating the mutants on minimal agar plates containing nicotinic acid, biotin, and galactose. To determine whether mutations had occurred in the tol A,B locus, colicins El, E2, E3 and K were spotted onto a lawn of the mutant being tested. Mutants in the tol A,B locus can still adsorb these colicins to their cell surface (2, 3). To determine whether the mutants still possessed the receptor for each colicin, they were tested for their sensitivity to the bacteriophages BF23 and T6, which share the same receptor site as the above colicins (4). In addition, direct tests of adsorption of these colicins to the mutants were performed (3).

Loss of the gene uvr B was examined by determining the number of surviving colonies after different doses of ultraviolet (UV) light (6); host-cell reactivation (HCR) properties were determined by measuring the plating efficiency of lambda phage irradiated with different doses of UV light (7).

Presence or absence of the gene chl D was tested by examining whether the mutants could grow on nutrient agar plates containing 2% chlorate under anaerobic conditions. Cells wild type for chl D are

unable to grow under the above conditions, while point mutations or deletions of chl D enable the cell to grow (8).

The sensitivity to various antibiotics was determined by placing Dispens-O-Discs (Difco) of different antibiotics on a lawn of the strain to be tested.

Membrane proteins were prepared for polyacrylamide gel electrophoresis by growing cells overnight in nutrient broth with vigorous aeration at 37°C. Cells were harvested by low speed centrifugation and washed with 0.1 M Tris-HCl buffer, pH 8.1, three times before disruption in an Aminco French pressure cell at 15,000 p.s.i. The disrupted cell homogenate was then centrifuged at 5,000 r.p.m. for 20 minutes to remove whole cells. The supernatant was centrifuged at 17,500 r.p.m. (37,000 g) for 60 minutes. The pellet was resuspended in the Tris-HCl buffer, and washed 3 times by centrifuging at 17,500 r.p.m. for 60 minutes. Lipid from the final membrane pellet was extracted with 10 ml of ether-ethanol (1:3) solvent for 15-18 hours. The extracted membrane was centrifuged at 17,500 r.p.m. for 60 minutes. The organic solvent was carefully decanted and the membrane pellet was solubilized with phenol-acetic acid - water (2:1:0.5, w/v/v). The solubilized membrane protein was centrifuged at 17,500 r.p.m. for 60 minutes. The supernatant is the solubilized membrane protein fraction.

The gels contained 5% (w/v) acrylamide, 0.1% bisacrylamide in 25% (v/v) acetic acid, and 2.5 M urea. The gels were run in 10% (v/v) acetic acid, 1 M urea, at 3.3 mA/tube for 4 hours. The proteins in the gel were stained with Amido Black 10 B (9).

RESULTS AND DISCUSSION

By plating KA56 on EMB-galactose-colicin plates, a large number of deletion mutants were obtained. One of these mutants, KB5, will

TABLE 1.

Characteristics of the Bacterial Strains KA56 and KB5.

Phenotype	Bacterial Strains	
	KA56 Parent	KB5 Deletion Mutant
Colicin sensitivity	Sensitive	Resistant
Ability to produce nicotinic acid	+	-
Ability to use galactose	Killed by galactose addition	Unable to use galactose
Ability to reduce chlorate under anaerobic conditions	+	-
Multi-drug resistance	Resistant	Sensitive
Ability to lysogenize with λ phage	High frequency	Low frequency
Ability to produce biotin	+	-
UV sensitivity	Resistant	Sensitive
HCR	+	-
Utilization of nitrate as an electron acceptor	+	-

be considered in this paper. The characteristics of KB5 and its parent, KA56, are shown in Table 1. KB5 grows on minimal agar plates only if nicotinic acid, biotin, and a carbon source are provided. Glucose, acetate, and succinate can all serve as carbon sources whereas galactose can not. Strain KB5 is therefore $\underline{suc}^+$, $\underline{nic\ A}^-$, $\underline{gal}^-$, and $\underline{bio}^-$.

Strain KB5 is also multi-tolerant to the colicins E1, E2, E3, K and A, indicating a mutation in the tol A,B locus which was confirmed

by phage P1 transduction. Like other tol A,B mutants (3, 5), KB5 is sensitive to the detergents sodium dodecyl sulfate, sodium deoxycholate, Triton X-100 and the antibiotics vancomycin and bacitracin. KB5 is also sensitive to the antibiotics sulfadiazine, sulfamerazine, sulfathiazole, gantrisin, madribon, actinomycin D and rifampin. This sensitivity is due to mutations in the gene mdr^+ (multi-drug resistance, 10) which may be identical or closely linked to the gene blue (11). Clearly, these detergent and antibiotic sensitivities both reflect changes in the structure of the cell envelope of strain KB5.

Mutant KB5 can grow on nutrient agar plates containing 2% potassium chlorate under anaerobic conditions. Thus, KB5 is $chl\ D^-$, and possibly $chl\ A^-$. Furthermore, it cannot utilize nitrate as an electron acceptor under anaerobic conditions. The nitrate respiratory system is membrane-bound in E. coli (12) and thus chl D and chl A either directly or indirectly affect some enzyme system or structural components involved in the nitrate respiratory system (13, 14, 15, 16).

Strain KB5 is very sensitive to UV-irradiation. This UV sensitivity can be reversed by lysogenizing the mutant with transducing phage λpbiouvr-B (unpublished observations). This provides strong evidence that KB5 is $uvr\text{-}B^-$. Thus mutant KB5 is $tol\ A,B^-$, $nic\ A^-$, gal^-, $chl\ D^-$, mdr^-, bio^-, $uvr\text{-}B^-$, and $chl\ A^-$. The fact that

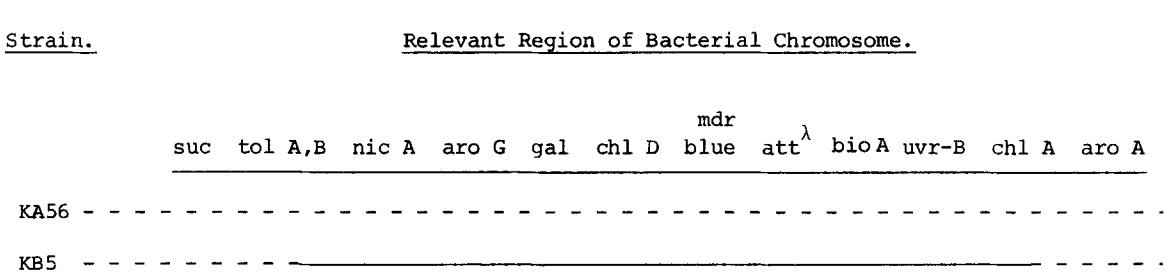

Figure 1. Relevant bacterial genes on the E. coli chromosome modified after Taylor and Trotter (1). The solid line represents the deleted genes. The precise left and right end points have not been determined.

all these genes are contiguous, and no revertants leading to colicin sensitivity have been found, provides strong evidence that strain KB5 is a deletion mutant, missing the genes indicated above (Fig. 1). Furthermore, several of these missing genes appear to affect membrane structural organization and its function.

Polyacrylamide gel electrophoresis (MATERIALS AND METHODS) demonstrates that strain KB5 is missing several membrane proteins, when compared with its parent KA56 (Fig. 2). It is now possible to

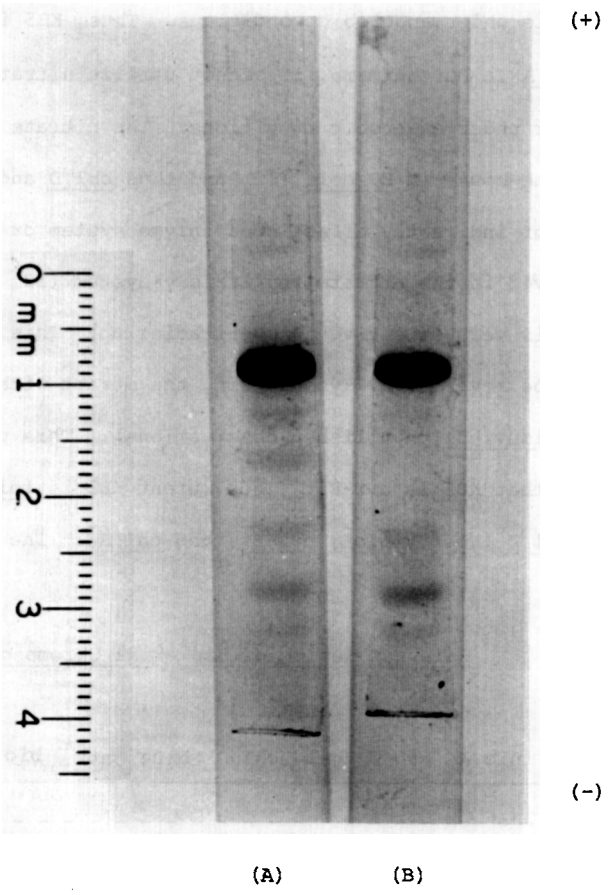

Figure 2. Polyacrylamide gel electrophoresis of membrane proteins of parent strain, KA56 (A), and its deletion mutant, KB5 (B).

ask which genetic loci code for these proteins by genetically reconstructing the deletion mutant with either λ transducing phages or by P1 phage transduction. Comparative studies of membrane-bound enzyme systems will lead us to an understanding of the function of these membrane proteins which are missing in the deletion mutants, and their relationship to the cell membrane organization in general.

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