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Genetic and Biochemical Studies on Mannose-Negative Mutants That Are Deficient in Phosphomannose Isomerase in *Escherichia coli* K-12

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Two mannose-negative mutants of *Escherichia coli* K-12 have been isolated. These mutants are deficient in the ability to synthesize phosphomannose isomerase and capsular polysaccharide when grown on glucose-containing media. Interrupted mating experiments to determine the kinetics of genetic transfer show that the two mannose-negative mutations map together between the histidine and tryptophan regions of the *E. coli* chromosome.

The capsular polysaccharide of *Escherichia coli* K-12 contains glucuronic acid, D-galactose, D-glucose, and L-fucose in the approximate molar ratios of 1:2:1:2 (11, 14, 17). The disaccharide 3-0-β-D-glucuronosyl-D-galactose has been identified in acid hydrolysates of the polysaccharide (15), but the remainder of the structure has not been elucidated. Synthesis is controlled by two separate regulator genes, *capR* and *capS* [previously designated R_1 and R_2 (11, 14)]. The wild-type strain (*capR*$^+$, *capS*$^+$) produces clones that are nonmucoid at 37 C, but certain wild-type strains produce clones that are partially mucoid at 23 C. Mutations at either *capR or capS* give rise to clones that are mucoid at all temperatures between 37 and 23 C (11, 14). A postulated biosynthetic scheme for the polysaccharide is presented in Fig. 1. Most of these reactions have been demonstrated to occur in vitro but not necessarily in strains of *E. coli* K-12. Phosphomannose isomerase (PMI) [1], guanosine diphosphate (GDP)-mannose hydro-lyase [4], GDP-L-fucose synthetase [5], and uridine diphosphate (UDP)-galactose-4-epimerase [9] are elevated in crude extracts of *capR* but not in *capS* mucoid mutants (11, 13). However, phosphoglucose isomerase (PGI) [6] and glucose-6-phosphate (G6P) dehydrogenase are not elevated in *capR* or *capS* mucoid mutants (11, 13). Another study demonstrated that low concentrations of *p*-fluorophenylalanine (FPA) induced mucoidness in the wild type at 37 C, and three of the above-mentioned enzymes ([1], [5], and [9]) were elevated in these mucoid FPA-phenocopies (8). Studies of dominance of *capR* alleles also demonstrated that *capR* is a regulator gene (11, 14). Other genetic studies showed that the *capR* gene controls ultraviolet (UV) radiation sensitivity (3, 4, 6, 12; Uretz and Markovitz, *in preparation*) as well as polysaccharide synthesis. Ochre suppressors, a class of suppressors that function at the level of translation of messenger ribonucleic acid into protein, are capable of suppressing UV sensitivity and mucoidness (12). Together, these results indicate that the *capR* gene product is a protein that may be called a repressor (7).

On the basis of the postulated biosynthetic pathway of polysaccharide synthesis (Fig. 1) and the additional assumption that absence of any one nucleotide sugar precursor prevents polysaccharide synthesis, it is predicted that any of 11 structural gene mutations could preclude polysaccharide synthesis. This would be dependent on the carbon and energy source in some cases. For example, a mutant lacking PMI should be nonmucoid when grown on glucose or fructose. It might also be unable to grow on mannose as a sole source of carbon and energy. We have isolated two mannose negative mutants and shown that they are missing detectable PMI when grown on glucose and are also deficient in polysaccharide synthesis. Time of entry experiments demonstrated that the mannose-negative mutations (designated *man-1* and *man-2*) map in the same region of the *E. coli* chromosome. *Man* maps at a distance from the probable structural genes for

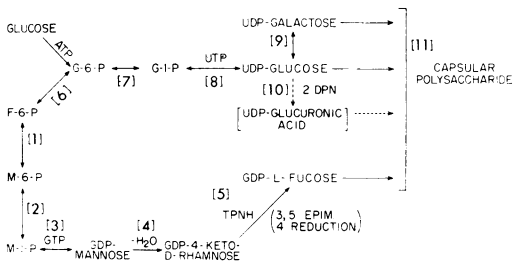

FIG. 1. *Postulated biosynthesis of capsular polysaccharide in Escherichia coli K-12.*

UDP-galactose-4-epimerase (*gal*) (19) and UDP-glucose pyrophosphorylase (*galE*) (18), and also separate from the regulator genes, *capR* and *capS* (11, 13, 14), as shown in Fig. 2 [taken in part from Taylor and Thoman (21)].

MATERIALS AND METHODS

Bacteria and bacteriophage. All strains of bacteria were streptomycin-resistant derivatives of *E. coli* K-12. Strain AB 311 (kindly supplied by E. Adelberg) is a bacteriophage T6-sensitive, threonine- and leucine-requiring Hfr strain with the following order of entry of markers: origin-histidine (*his*)-tryptophan (*trp*)-galactose (*gal*) (20). Strain MC117 is a recently isolated histidine-requiring mutant of strain MC116 [previously designated 156-2 (11)] obtained by the penicillin method after mutagenesis with UV light. Strain MC117 requires leucine (*leu*), proline (*pro*), purine (*pur*), tryptophan, and histidine for growth, is not able to grow on lactose, and is bacteriophage T6-resistant, bacteriophage P1-immune, and F^-. Its genotype is *leu-1*, *proC*, *purE*, *try-1*, *his-1*. Both strains AB311 and MC117 are nonmucoid and contain the wild-type alleles ($capR^+$, $capS^+$) that control mucoidness (capsular polysaccharide synthesis) (11, 13, 14). Lysates of bacteriophage T6 that contained 2×10^{10} to 4×10^{10} plaque-forming units per ml were prepared with strain AB 311 as a host.

Media. M-9 minimal medium (1) was supplemented with 2.5×10^{-3} M $CaCl_2$ and 10 μg of thiamine/ml. HCl and 0.6% glucose was used for liquid cultures and was solidified with 1.5% agar for plating experiments. Streptomycin was present in all M-9 medium at a final concentration of 200 μg/ml. The following supplements were present at the indicated final concentrations (mg/liter): L-leucine, 50; L-proline, 50; adenine, 50; DL-tryptophan, 100; and L-histidine HCl, 50. Mannose was used at a concentration of 0.6% and, in some conjugation experiments, at 0.2% in place of glucose. The concentration of L-proline also was reduced in some experiments to 15 mg/liter. The lower concentrations of mannose and proline reduced the background growth in recombination experiments involving the *man-2* mutation. Penassay Broth (PA; Difco) was used for conjugation experiments (except where otherwise indicated) and L-broth (9) for growth of phage T6 and strain MC117 prior to treatment with the mutagen, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (NTG; Aldrich Chemical Co. Milwaukee, Wis.). The tris(hydroxymethyl)aminomethane-maleic (TM) buffer has been described (2). L-agar is L-broth solidified with 1.1% agar. Bacteria were grown aerobically by means of a reciprocal shaker (Psycrotherm, New Brunswick Scientific Co., New Brunswick, N. J.).

Isolation of mannose-negative mutants. Strain MC 117 was grown in L-broth and treated with 1 mg of freshly prepared NTG per ml as described by Adelberg et al. (2). Approximately 10^8 treated cells were inoculated into L-broth for two to three divisions. The suspension was then serially diluted, plated on L-agar plates, and incubated at 37 C for 24 hr. Suitable plates were replicated onto M-9 with glucose and M-9 with mannose as carbon sources and incubated at 37 C. Two mannose-negative clones (designated strains MC118, *man-1*; and MC119, *man-2*) were isolated by this technique from 150 clones examined. It is noteworthy that no mannose-negative clones were detected from approximately 600 clones when the initial plating was on M-9 with glucose followed by replication to M-9 with mannose.

Mating conditions and time of entry experiments. The procedure follows that of deHaan and Gross (5) and of Taylor and Thoman (21). Exponentially growing donor (AB 311) and recipient (MC117 or mannose-negative derivatives) bacteria were mixed to give a final concentration of 2×10^7 donors and 4×10^8 recipients per ml. This mixture was incubated without agitation for 5 min at 37 C and then diluted 1:100 into prewarmed M-9 that contained all the growth requirements of the recipient strain. Samples (0.5 ml) were removed at 5-min intervals and mixed with 10^{10} T6 in 0.5 ml of L-broth. This mixture was shaken on a Vortex Junior mixer for 0.5 min and placed in a water bath (37 C) for 10 min to allow phage absorption; then 0.1-ml portions were plated in duplicate on appropriate selective media. Growth of any T6-resistant mutants of the donor was prevented by omitting threonine from the selective media.

Enzymatic analyses of E. coli K-12. Conditions, reagents, and chemicals for the assays of G6P dehydrogenase, PGI, and PMI have been described (8). Bacteria for enzyme assays were grown and assayed at room temperature (23 C).

Mannose-6-phosphate was purchased from British Drug House Ltd., Poole, Dorset, England, and contained low levels of G6P and fructose-6-phosphate which were eliminated by reaction with the added G6P dehydrogenase and PGI before the PMI assay. Transduction was carried out as described by Lennox (9), and protein was measured by the method of Lowry et al. (10).

RESULTS

General characteristics of mannose-negative strains. No striking differences in growth rates were noted between the wild-type and mannose-negative mutants when they were grown in M-9 minimal medium at from 23 to 37 C with glucose, gluconate, or fructose as sole sources of carbon and energy. However, MC118 (*man-1*) did not

grow in mannose M-9 at 37 C and grew extremely slowly in this medium at 30 and 23 C (generation time of over 40 hr). Strain MC119 (*man-2*) grew very slowly in mannose M-9 at 37 C (generation time of 22 hr) and at 30 and 23 C grew at rates that changed gradually from a generation time of between 9 and 11 hr to a generation time of 40 to 50 hr. Both MC118 and MC119 produced nonmucoid clones when streaked on glucose M-9 agar at all temperatures, but MC119 yielded mucoid clones when both 0.3% glucose and 0.3% mannose were included in M-9 agar at 23 C. In contrast, MC118 appeared nonmucoid when it was streaked on the glucose-mannose mixture and incubated at 23 C.

Mapping of man-1 and man-2 by time of entry. Conjugation experiments with several Hfr strains [AB259, AB311, and AB312 (21)] indicated that *man-1* was in the *his-try* region of the chromosome. Figure 2 shows the average time of entry of *his*$^+$, *man*$^+$, and *try*$^+$ (average of five experiments) in crosses with Hfr strain AB311 and the F$^-$ strain MC118 (*man-1*). The time of entry of the *his*$^+$ and *try*$^+$ markers (4 and 19 min, respectively) was determined by extrapolation of the straight-line plots to the time axis; these values agree with those reported by Taylor and Thoman (21). The time of entry of *man*$^+$ was 13.5 min. Similar crosses were carried out with the same donor strain and the F$^-$ strain MC119 (*man-2*). The time of entry of *man-2*$^+$ was 13 min. These results indicate that the two mutations *man-1* and *man-2* are closely linked and could be in the same operon or cistron.

Capsular polysaccharide synthesis and enzymatic analyses of strains MC117, MC118, and MC119. Table 1 presents the results of measurements of polysaccharide synthesis and enzyme assays on strain MC117, MC118, and MC119. When grown on glucose, both mannose-negative strains synthesized little polysaccharide and contained no detectable PMI, but contained normal amounts of PGI and G6P-dehydrogenase. Extracts of the mannose-negative strains did not inhibit the PMI of strain MC117.

Strains MC118 and MC119 were transduced to *man*$^+$ by use of P1 grown on a wild-type strain. The *man*$^+$ transductants were assayed for PMI, PGI, and G6P-dehydrogenase, and had specific enzymatic activities similar to strain MC117. Thus, transduction back to *man*$^+$ leads to synthesis of PMI. The *man*$^+$ transductants of strains MC118 and MC119 also appeared to produce more polysaccharide (50 to 60 μg of nondialyzable methylpentose per ml per optical density unit at 600 mμ) than the two mannose-negative mutants but somewhat less than the original *man*$^+$ strain (Table 1). Variations in polysaccharide synthesis between 50 and 200 μg of nondialyzable methylpentose per ml per optical density unit at 600 mμ among wild-type strains (*capR*$^+$ *man*$^+$) grown under these conditions are not unusual (8, 11).

DISCUSSION

The present results demonstrate that two mannose-negative mutants lack detectable PMI when grown with glucose as a source of carbon and energy at 23 C, and are also deficient in the synthesis of capsular polysaccharide. Since NTG is likely to cause more than one mutation in an organism, it is pertinent to ask whether or not the mannose-negative, PMI-negative, and reduced polysaccharide-synthesizing phenotype is the result of a single mutation. Transduction of the mannose-negative mutants to *man*$^+$ restores their ability to synthesize PMI and capsular polysaccharide. Thus, it is likely that all three changes are the result of a single mutation. Similarly, Rosen et al. (16) showed that a mannose-negative, PMI-deficient strain of *Salmonella typhimurium* produced an incomplete cell wall lipopolysaccharide when grown in complex medium.

Man-1 and *man-2* map between *his* and *trp* on the *E. coli* chromosome. The probable structural

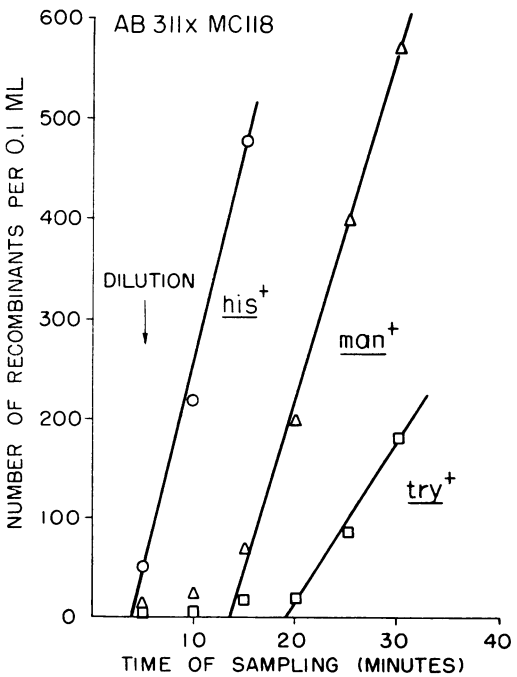

FIG. 2. *Transfer curves for his*$^+$, *man*$^+$, *and try*$^+$ *markers in a cross between donor strain AB311 and recipient strain MC118.*

TABLE 1. *Synthesis of capsular polysaccharide and enzymes by mannose-negative and wild-type strains*

Strain	Genotype	Final growth[a]		Specific enzymatic activity of[c]		
		Optical density at 600 mμ	Capsular[b] polysaccharide	PMI	PGI	G6P dehydrogenase
MC117	*capR*+ *man*+	0.50	140	2.26	12.8	7.02
MC118	*capR*+ *man-1*	0.33	11	<0.03[d]	7.59	6.14
MC119	*capR*+ *man-2*	0.21	32	<0.03[d]	10.9	6.08

[a] Final growth is the optical density at which portions were taken to measure the quantity of capsular polysaccharide and were not the cultures on which enzymatic activities were measured.
[b] As micrograms of nondialyzable methylpentose per milliliter per unit of optical density at 600 mμ.
[c] Expressed as micromoles of reduced nicotinamide adenine dinucleotide per hour per milligram of protein.
[d] No enzymatic activity was detected.

genes for UDP-galactose-4-epimerase (*gal*) (19), PMI (*man*), and UDP-glucose pyrophosphorylase (*gal E*) (18) are all separate (Fig. 3). Of the two regulator genes, *capS* is close to UDP-glucose pyrophosphorylase (*galE*), since *capS* maps close to *try* (13) and so does UDP-glucose pyrophosphorylase (18). *CapR* is separate from all of these (11; Fig. 3). UDP-galactose-4-epimerase, PMI, and UDP-glucose pyrophosphorylase also participate in reactions not necessarily connected with capsular polysaccharide synthesis. Thus, other structural genes more exclusively associated with capsular polysaccharide synthesis may yet be found in a "capsular polysaccharide operon."

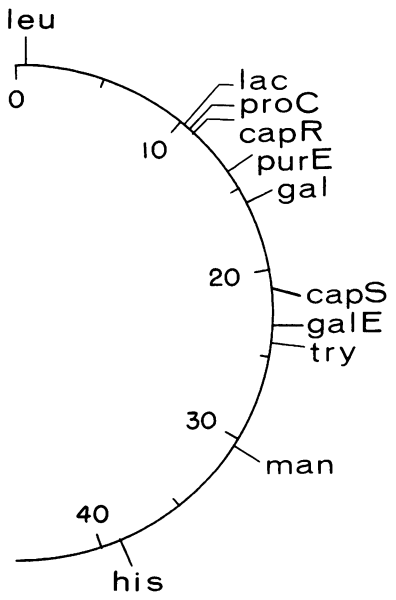

FIG. 3. *Partial genetic map of Escherichia coli K-12.*

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