Cloning and Location of the *dgsA* Gene of *Escherichia coli*

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The *dgsA* locus of *Escherichia coli* was isolated on plasmids obtained from the library of L. Clarke and J. Carbon (Cell 9:91–99, 1976). Restriction fragment analysis and further subcloning demonstrated that the gene is located at kilobase 425 on the Bouché physical map of the terminus region (J. P. Bouché, J. Mol. Biol., 154:1–20, 1982). This corresponds to 35.2 min on the Bachmann genetic map (B. J. Bachmann, Microbiol. Rev. 47:180–230, 1983).

The *dgsA* locus of *Escherichia coli* has been mapped at 35 min of the genetic map by bacteriophage P1 transduction (9). In addition to its role in the uptake of sugars by the phosphoenolpyruvate:sugar phosphotransferase system, this locus is of interest since it provides one of the very few gene, and determined its location on the physical map constructed by Bouché (2).

The *dgsA* locus is most readily studied in strains containing a mutant *ptsG* locus (9), which inactivates transport of glucose and mannose by the III^{glc}/IIB' system of the
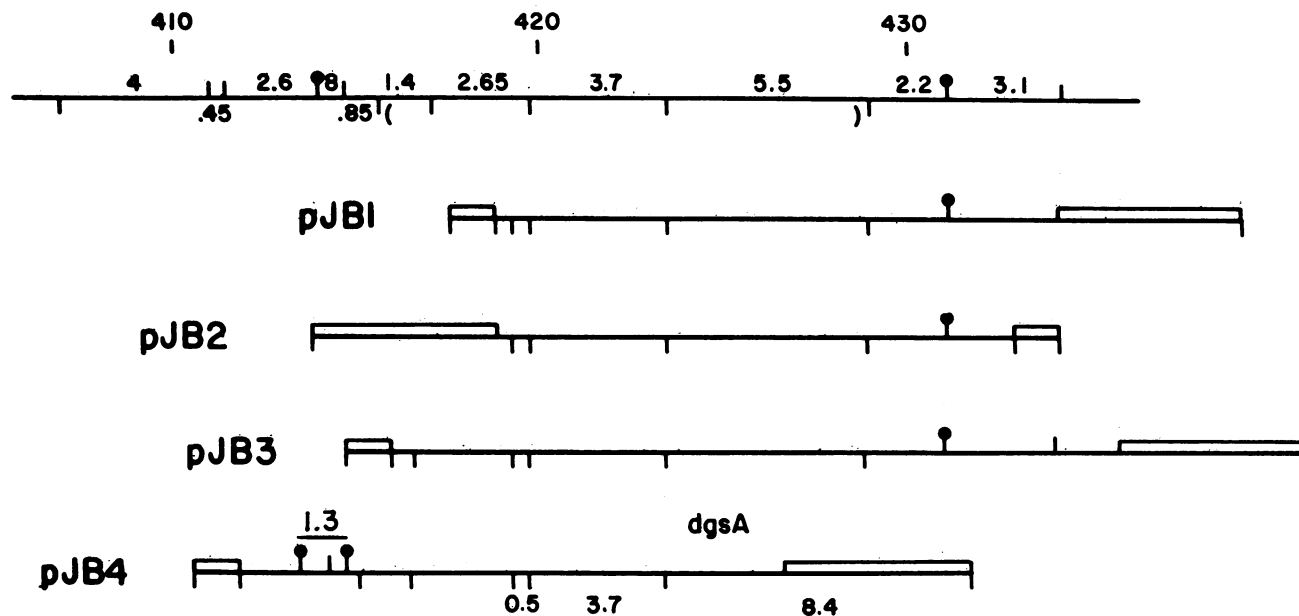

FIG. 1. Restriction map of region surrounding the *dgsA* locus. The top line shows the map obtained by Bouché (2), with his coordinates. kb 425 corresponds approximately to 35.2 min on the genetic map (1). Subsequent lines show restriction maps of plasmids obtained in this study. Restriction sites are *Eco*RI (⊥), *Hind*III (⊥), and *Pst*I (T). The open line designates ColE1 DNA (6.4 kb). Our data for the location of *Pst*I and *Eco*RI sites in ColE1 and plasmids obtained by cloning into the *Eco*RI site (4) were similar to those obtained by Edlund et al. (5). We did not attempt to resolve, however, the *Pst*I sites of ColE1 which are separated by ca. 150 bp and which are adjacent to the *Eco*RI cloning site. The *Eco*RI site of ColE1 was lost when deoxyribosyladenine-deoxyribosylthymine tailing was used to insert fragments into that site (4).

selectable genetic markers in the terminus region of the chromosome (1). To facilitate further studies of this region, we isolated plasmids containing the *dgsA* gene from the library constructed by Clarke and Carbon (4), subcloned the

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phosphoenolpyruvate:sugar phosphotransferase system (6, 8). Transport of these compounds, as well as glucosamine and 2-deoxyglucose, is then dependent on the IIA/IIB system (6, 8, 10). Under anaerobic conditions, *ptsG* derivatives of a number of *E. coli* strains are unable to grow on glucose, mannose, or glucosamine due to lack of expression of the IIA/IIB system. As demonstrated by Roehl and Vinopal (9), *dgsA* mutations increase the activity of the IIA/IIB system and confer the ability to grow anaerobically on these compounds.

It is worth mentioning that both *dgsA* and *dgsA*⁺ can be used as selectable markers in genetic studies. Growth of *dgsA* strains is inhibited by 2-deoxyglucose, and these conditions can be used to select *dgsA*⁺ recombinants (9). Conversely, most *ptsG dgsA*⁺ strains do not grow anaerobically on glucose, mannose, or glucosamine, and these conditions can be used to select *dgsA* recombinants. This is not always an effective selection, however, due to the high frequency at which some strains produce spontaneous mutants that grow anaerobically on these compounds (unpublished data).

Plasmids containing the *dgsA* gene were isolated from the library constructed by Clarke and Carbon (4), which contains sheared *E. coli* DNA inserted by means of deoxyribosyladenine-deoxyribosylthymine tailing into the *EcoRI* site of ColE1 DNA. A random collection of 500 clones from this library was mated by means of replica plating with strain RR313, which contained a deletion of the *dgsA* gene (9). Transconjugants that were *dgsA*⁺ were selected by aerobic growth in the presence of 10⁻³ M 2-deoxyglucose with 0.2% glycerol as carbon source. Four different plasmids were obtained, which were characterized by standard procedures for isolation of plasmid DNA, electrophoretic analysis of products of restriction enzyme digestions, transformation, and DNA ligation (7).

The restriction maps of the plasmids are shown in Fig. 1. For comparison, the figure also shows the part of the Bouché map of the terminus region (2) that would be expected to contain the *dgsA* gene. This estimation is based on *dgsA* being located 0.5 min to the left of *manA* (9), 1 min in the terminus region being ca. 44 kilobases (kb), and *manA* being located at kb 446 (3). There is a close correspondance between the plasmid and Bouché maps. The distribution of the *PstI* fragments in the various plasmids allowed an unambiguous placement of the 1.4-, 2.7-, 3.7-, and 5.5-kb fragments, which was not possible in the study of Bouché. Analysis of the plasmids also allowed the detection of *HindIII* (at kb 415) and *PstI* (at kb 419) sites not present in the Bouché map.

To locate the *dgsA* gene, pJB4 was digested with *PstI*, and the fragments were ligated with pBR322 that had also been digested with *PstI*. RR313 was transformed with this DNA, and transformants were obtained by selecting for resistance to tetracycline (Tcʳ). Plasmids containing inserts in the *PstI* site of pBR322 were then identified by scoring for sensitivity to ampicillin (Apˢ). All of these clones were then tested for growth in the presence of 2-deoxyglucose.

Several different plasmids that were Tcʳ Apˢ DgsA⁺ were obtained. All of these contained the 8.4-kb *PstI* fragment of pJB4, either by itself or in combination with other fragments. Since the 8.4-kb fragment also contained 5.1 kb of ColE1 DNA, this locates *dgsA* in the 3.3-kb region centered at kb 425.

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