JOURNAL OF BACTERIOLOGY, Jan. 1995, p. 459–461
0021-9193/95/$04.00+0

Vol. 177, No. 2

NOTES

D-Serine Deaminase Is a Stringent Selective Marker in Genetic Crosses

WERNER K. MAAS,* RENATA MAAS, AND ELIZABETH MCFALL

Department of Microbiology, New York University School of Medicine, New York, New York 10016

Received 12 September 1994/Accepted 7 November 1994

The presence of the locus for D-serine deaminase (*dsd*) renders bacteria resistant to growth inhibition by D-serine and enables them to grow with D-serine as the sole nitrogen source. The two properties permit stringent selection in genetic crosses and make the D-serine deaminase gene an excellent marker, especially in the construction of strains for which the use of antibiotic resistance genes as selective markers is not allowed.

The gene for the enzyme D-serine deaminase, *dsd*, provides for a dual selection in genetic crosses, because its presence not only renders bacteria resistant to the growth-inhibitory action of D-serine but also permits bacteria to use D-serine as a sole nitrogen or carbon source (8). The simultaneous selection for D-serine resistance and D-serine utilization prevents the outgrowth of practically all spontaneous D-serine-resistant mutants. This stringent selection by D-serine is especially useful in the construction of live vaccines, for which the use of antibiotic resistance genes is not allowed. Such constructions usually involve the introduction of a plasmid carrying one or more antigen- producing genes into a suitable host strain by transformation, and because the transformation frequencies are usually very low, elimination of background spontaneous mutants is essential to the selection.

D-Serine deaminase is an inducible enzyme that converts D-serine to pyruvate and ammonia (8). The *dsd* locus, comprising the structural gene *dsdA* and a positive regulatory gene, *dsdC*, is located at min 51 of the *Escherichia coli* linkage map. A 4-kb *Sal*I-*Eco*RI fragment containing the locus has been cloned into a pUC8 vector and was denoted pMM1 (9).

The usefulness of D-serine deaminase as a stringent selective marker is illustrated in a transformation experiment with pMM1, described in Table 1. The recipient strain, 770011 (serotype O157:H43), is a wild-type Dsd^- *E. coli* strain isolated from a pig (14). It grows well in minimal medium A (3) with ammonia as a nitrogen source and is inhibited by D-serine. As shown in Table 1, with selection on ordinary minimal medium agar with D-serine, there are significant numbers of D-serine-resistant colonies on the control plates with only the recipient bacteria (no DNA). With selection on minimal medium agar with D-serine as a nitrogen source, there are no colonies on the no DNA control plates.

Plasmid pMM1 has a high copy number, and this property may prevent its use for the cloning of genes whose overexpression interferes with growth. We have therefore cloned the *dsd* operon in a low-copy-number cloning vector derived from replicon RepFIC, one of the replicons present in IncFI plasmids (12). This replicon has a copy number of two to three (6). We ligated a *Pst*I-*Eco*RI fragment of pMM1 that contains the *dsd* operon to a PCR-generated fragment containing replicon RepFIC. The latter fragment was synthesized with the compatible *Eco*RI and *Nsi*I sites at either end. An internal *Bam*HI site was retained to be used for subsequent insertions. We obtained many transformant clones from this ligation, one of which was retained for further work and was denoted pWM45.

We carried out further transformation experiments with pWM45, using as recipient strains 770011 and 6082, a nonreverting *dsdA* mutant of *E. coli* K-12 (1, 9). The following results were obtained with strain 6082. There were 68 transformants obtained with 100 μl and 8 transformants obtained with 10 μl of transformed cells plated on the selective medium (medium A-N [modified medium A in which potassium sulfate is substituted for ammonium sulfate] plus 1,000 μg of D-serine, 25 μg of threonine, and 25 μg of leucine per ml). The conditions of transformation were the same as those described in Table 1, and the plates were scored after 2 days of incubation at 37°C. The no DNA control plates had no colonies. Results with strain 770011 were similar to those obtained with plasmid pMM1.

Strain 6082 requires threonine and leucine for growth. These amino acids were added to the selective medium, medium A with D-serine as the sole nitrogen source, at low enough concentrations to prevent them from contributing significantly to the total nitrogen requirement and to overcome D-serine toxicity (2) yet still high enough to fulfill the specific

TABLE 1. Transformation of pMM1 DNA into strain 770011[a]

Amt (μl) of transformation mixture plated	No. of transformants with:			
	Medium A with D-serine (500 μg/ml)		Medium A-N[b] with D-serine (1,000 μg/ml)	
	pMM1 DNA	No DNA	pMM1 DNA	No DNA
100	158	48	95	0
10	12	19	10	0

[a] Transformation was carried out by electroporation (4). After the electric shock, the bacteria were incubated in SOC medium for 3 h before plating. During the last hour, D-serine (500 μg/ml) was added to the growth medium. The plates were incubated for 2 days at 37°C before scoring for transformant colonies.

[b] Medium A-N is modified medium A in which potassium sulfate is substituted for ammonium sulfate.

* Corresponding author. Phone: (212) 263-5322. Fax: (212) 263-8276.

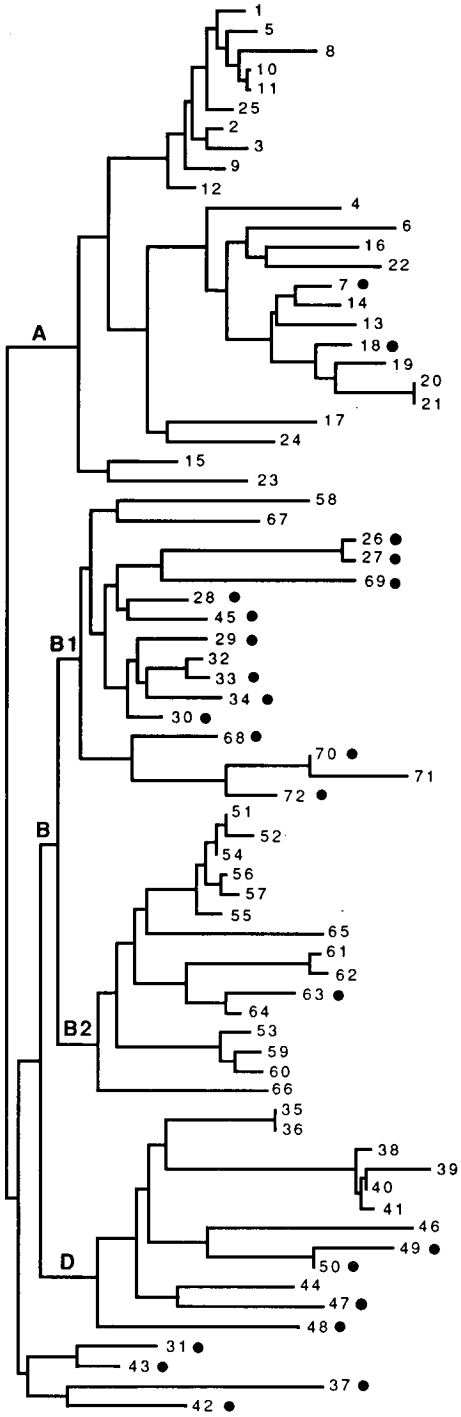

FIG. 1. Phylogenetic distribution of Dsd$^-$ and Dsd$^+$ *E. coli* strains of the ECOR collection (11). Dsd$^-$ strains are denoted by solid circles. The dendrogram is adapted from the paper by Herzer et al. (5).

requirement for protein synthesis (25 μg/ml). We picked 10 transformant colonies to test for D-serine deaminase. Single-colony isolates from these transformants were grown to (2 to 5) $\times$ 10^8 cells per ml in Luria-Bertani medium and exposed to D-serine at a final concentration of 500 μg/ml for 40 min to induce synthesis of the enzyme. Enzyme assays were performed on induced and uninduced cultures, as described previously (7). Of the 10 transformants, 9 produced high levels of enzyme: 6 after induction only and 3 constitutively. Presumably, the latter had undergone a mutation after the transfer of the *dsd* operon. After 2 days of incubation at 37°C, the no DNA control plates had no colonies, which matches the result for strain 6082.

For the construction of *E. coli* vaccine strains for veterinary purposes, it is advantageous to use animal *E. coli* isolates as hosts for plasmids carrying antigen-producing genes, because such strains are good colonizers of the host's intestine. To determine the prevalence of D-serine deaminase-forming ability among such *E. coli* isolates, we tested the representative ECOR collection of strains (11) for the presence of the enzyme after exposure to D-serine. The results are presented in Fig. 1. Twenty-three of the 72 strains failed to form detectable amounts of the enzyme (Dsd$^-$). Within the collection, 44% of the animal strains are Dsd$^-$ and 22.5% of the human strains are Dsd$^-$. The distribution of the Dsd phenotype (and presumably the corresponding *dsd* genotype) is strongly clustered. Most of the strains in groups B1 and D are Dsd$^-$, and most of the strains in groups A and B2 are Dsd$^+$ (Fig. 1). There is a strong correlation between Dsd$^-$ and raffinose utilization (Raf$^+$) (13). There is also a strong correlation between the data obtained by multiple locus enzyme electrophoresis (13) and DNA sequencing of the *E. coli* genome (10).

The data show that Dsd$^-$ strains suitable for the construction of vaccine strains are readily available. With the use of *dsd*$^+$ as a stringent selective marker gene, it is not necessary to modify such strains genetically in order to make them suitable recipients for the introduction of antigen-producing genes.

Nucleotide sequence accession number. The accession number for the sequence of RepFIC in GenBank is M16167.

This work was supported by Public Health Service grant GM06048 from the National Institute of General Medical Sciences and by a grant from Ambico, Inc., Dallas Center, Iowa. W.K.M. is the holder of Public Health Service Research Career Award GM-15129 from the National Institute of General Medical Sciences.

The excellent technical assistance of Frank Alessandro is gratefully acknowledged.

REFERENCES

1. **Cosloy, S. D., and E. McFall.** 1973. Metabolism of D-serine in *Escherichia coli* K-12: mechanism of growth inhibition. J. Bacteriol. **114:**685–694.
2. **Davis, B. D., and W. K. Maas.** 1949. Inhibition of *E. coli* by D-serine and the production of serine-resistant mutants. J. Am. Chem. Soc. **71:**1865.
3. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60:**17–28.
4. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6145.
5. **Herzer, P. J., S. Inouye, M. Inouye, and T. S. Whittam.** 1990. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. **172:**6175–6181.
6. **Maas, R., S. Saadi, and W. K. Maas.** 1989. Properties and incompatibility behavior of miniplasmids derived from the bireplicon plasmid pCG86. Mol. Gen. Genet. **218:**190–198.
7. **McFall, E.** 1975. *Escherichia coli* K-12 mutant forming a temperature-sensitive D-serine deaminase. J. Bacteriol. **121:**1074–1077.
8. **McFall, E.** 1987. The D-serine deaminase operon, p. 1520–1526. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
9. **McFall, E., S. S. Nikam, and S. Palchaudhuri.** 1991. Effects of structural changes in the *dsdA-dsdC* intergenic region on D-serine deaminase synthesis. J. Bacteriol. **173:**1161–1167.
10. **Milkman, R., and M. McKane Bridges.** 1993. Molecular evolution of the *Escherichia coli* chromosome. IV. Sequence comparisons. Genetics **133:**455–468.
11. **Ochman, H., and R. K. Selander.** 1984. Standard reference strains of *Escherichia coli* from natural populations. J. Bacteriol. **157:**690–693.

12. **Saadi, S., W. K. Maas, D. F. Hill, and P. L. Bergquist.** 1987. Nucleotide sequence analysis of RepFIC, a basic replicon present in IncFI plasmids P307 and F, and its relation to the RepA replicon of IncFII plasmids. J. Bacteriol. **169:**1836–1846.
13. **Selander, R. K., D. A. Caugant, and T. S. Whittam.** 1987. Genetic structure and variation in natural populations of *Escherichia coli*, p. 1625–1648. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
14. **Whittam, T. S., and R. A. Wilson.** 1988. Genetic relationships among pathogenic *Escherichia coli* of serogroup O157. J. Bacteriol. **56:**2467–2473.