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Mapping of the 5-Methyltryptophan Resistance Locus in *Bacillus subtilis*

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The 5-methyltryptophan resistance locus (*mtr*) in *Bacillus subtilis*, which leads to constitutive production of the tryptophan enzymes, has been mapped on the chromosome. The order of loci is *ser-1—mtr—aroF—aroB—trp—hisB*.

Two methyltryptophan resistance loci have been reported in *Bacillus subtilis*. The first locus, designated *trpS*, codes for the tryptophanyl-transfer ribonucleic acid synthetase and was mapped between *argC* and *metA* by Steinberg and Anagnostopoulos (9). The second locus, designated *mtr*, is linked to the tryptophan operon and was initially described by Nester et al. (5). The latter class of mutants results in constitutive synthesis of the tryptophan enzymes and tryptophan excretion (3). Derepression of the tryptophan operon, either due to tryptophan limitation or to an *mtr* mutation, also results in the derepression of several genes to the right of the tryptophan cluster, namely, imidazolylacetolphosphate aminotransferase (*hisB*), prephenate dehydrogenase (*tyrA*), and possibly enolpyruvylshikimate-5-phosphate synthetase (*aroE*); this sequence of genes has been termed a supraoperon (6). If the *mtr* locus in *B. subtilis* is analogous to the *trpR* locus in *Escherichia coli* (4), it may code for the repressor molecule which controls the transcription of the genes in the vicinity of the tryptophan operon. However, the *mtr* locus may also be an operator locus. In either case, the *aro* gene cluster may also include genes to the left of the tryptophan operon; two genes were known to be closely linked to the *trp* region, *aroB* coding for dehydroquinate synthetase, and *aroF*, coding for chorismate synthetase (2). The position of *aroB* was of particular interest because it did not appear to be derepressed by the *mtr* mutation (6). If the enzymes encoded by the *aroF* and *aroB* loci are not constitutively produced in *mtr* mutants and if the *mtr* locus maps to the left of these two loci, then the *mtr* mutations would appear not to be perturbations of the operator-promotor region.

Previous attempts to pinpoint the *mtr* locus by two- and three-factor transformation crosses by several groups had been unsuccessful. Whitt and Carlton (8) reported recombination values between *mtr* and *trpE* (anthranilate synthase) ranging from 40 to 99%. This problem was partially resolved with the discovery that the *mtr* marker could result in a partial requirement for phenylalanine, depending on the level of chorismate mutase in the strain (3). Recombination values between *mtr* and *trpE* were then calculated from 48 to 61% with *mtr* strains isolated in three separate laboratories (3). However, this same scoring system with a phenylalanine supplement did not give reproducible results when two-factor crosses between *mtr* and *aroB* or *aroF* were attempted.

The complex interrelationships already known to exist between enzymes of the aromatic and histidine pathways suggested that the mapping difficulties might be attributable to nutritional requirements other than just phenylalanine. The screening procedure for these requirements was effected in liquid culture with Spizizen minimal medium (7) containing 0.5% glucose and the desired supplements at a concentration of 50 μg/ml. Strains to be tested were grown up in Penassay broth, harvested, and suspended in minimal medium; a 1% inoculum (vol/vol) was grown overnight at 37 C with shaking. Such a procedure was simple and quantitative. The results indicated that phenylalanine, tyrosine, histidine, arginine, and proline (designated supplement) simulated growth in medium containing 0.05% acid-hydrolyzed casein (AHC). The supplement is included in all plates unless otherwise indicated. (Earlier experiments had already shown that AHC could not be used for reproducible scoring on plates containing 5-fluorotryptophan, presumably because of changes in colony morphology attributed to the AHC. 5-Fluorotryptophan is used to score for the *mtr* marker [3].)

Contemporary studies by other laboratories agreed that the *mtr* mutations were closely linked to the *aroB* mutations, but due to the scoring difficulties outlined above, a consistent

TABLE 1. *Two-factor transformation crosses to locate the mtr locus*

Donor (genotype)	Recipient (genotype)	Classes[a] (phenotype)	No.	Recombination (%)
mtr-222[b] .	*ser-1*	Ser$^+$ f1-Trp^R	19	81
		Ser$^+$ f1-Trp^S	81	
mtr-264 ..	*ser-1*	Ser$^+$ f1-Trp^R	17	83
		Ser$^+$ f1-Trp^S	83	
mtr-264 ..	*aroB584*	Aro$^+$ f1-Trp^R	60	40
		Aro$^+$ f1-Trp^S	40	
aroF888 ..	*ser-1*	Ser$^+$ Aro$^+$	87	87
		Ser$^+$ Aro$^-$	13	

[a] Conditions are as follows. The first two crosses were selected for Ser$^+$ on plates containing tryptophan and supplement. Individual colonies were streaked and replicated to plates containing 4 μg of tryptophan per ml, 500 μg of 5-fluorotryptophan per ml, and supplement to score for f1-Trp^R. The third cross was selected for Aro$^+$ on plates containing supplement and scored for f1-Trp^R on plates containing 500 μg of 5-fluorotryptophan per ml and supplement. The fourth cross was selected for Ser$^+$ on plates containing phenylalanine, tyrosine, and tryptophan. Individual colonies were streaked and replicated to plates containing tryptophan to score for Aro$^+$.

[b] The strains used in these crosses were: GSY222, *mtr-222*; GSY264, *mtr-264*; WB888, *aroF888*; BR148, *ser-1 trpC2*; and SR584, *aroB584*.

order could not be established (C. Anagnostopoulos, personal communication). The order of loci in the relevant region is *ser-1—aroF—aroB—trp—hisB* (2). With the *mtr-222* and *mtr-264* mutations in donor deoxyribonucleic acid, transformation experiments confirmed a close linkage to *aroB* and, furthermore, showed that both *aroF* and *mtr* mutations could be linked to *ser-1* (Table 1). Since *ser-1* and *trp* markers do not co-transform (J. Hoch, personal communication), the two-factor crosses indicate that *aroB*, *aroF*, and *mtr* are between *ser-1* and *trp*. The position of *mtr* among the two *aro* loci was the subject of three-factor crosses.

Three-factor crosses were performed with *mtr-264* containing donor deoxyribonucleic acid and with *aro*$^-$ recipients carrying either *trpC2* or *hisB2* as the outside marker. The outside marker was selected and *mtr* and *aro* were scored among the recombinants. The results of this study (Table 2) imply the order *ser-1—mtr—aroF—aroB—trp—hisB*. The com-

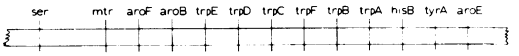

FIG. 1. *A portion of the linkage map of B. subtilis. The map is not drawn to scale. The hisB locus is also known as the hisH locus (6).*

TABLE 2. *Ordering of mtr marker by three-factor transformation*

Cross	Donor (genotype)	Recipient (genotype)	Classes[a] (phenotype)	No.	Order implied
1	*mtr-264*[b]	*aroB137, trpE24*	Trp$^+$ Shik$^+$ f1-Trp^R	60	*mtr—aroB—trpE*
			Trp$^+$ Shik$^+$ f1-Trp^S	32	
			Trp$^+$ Shik$^-$ f1-Trp^R	2	
			Trp$^+$ Shik$^-$ f1-Trp^S	6	
2	*mtr-264*	*aroB138, hisB2*	His$^+$ Aro$^+$ f1-Trp^R	58	*mtr—aroB—hisB*
			His$^+$ Aro$^+$ f1-Trp^S	20	
			His$^+$ Aro$^-$ f1-Trp^R	7	
			His$^+$ Aro$^-$ f1-Trp^S	15	
3	*mtr-264*	*aroF117, trpC2*	Trp$^+$ Aro$^+$ f1-Trp^R	29	*mtr—aroF—trpC*
			Trp$^+$ Aro$^+$ f1-Trp^S	29	
			Trp$^+$ Aro$^-$ f1-Trp^R	18	
			Trp$^+$ Aro$^-$ f1-Trp^S	24	
4	*mtr-264*	*aroF3112, hisB2*	His$^+$ Aro$^+$ f1-Trp^R	47	*mtr—aroF—hisB*
			His$^+$ Aro$^+$ f1-Trp^S	18	
			His$^+$ Aro$^-$ f1-Trp^R	8	
			His$^+$ Aro$^-$ f1-Trp^S	26	

[a] Conditions were as follows. Cross 1 was selected for Trp$^+$ on plates containing minimal medium plus shikimate and supplement. Individual colonies were scored for Aro$^+$ on minimal plus supplement, and for f1-Trp^R on minimal plus shikimate, supplement and 5-fluorotryptophan. Crosses 2 and 4 were selected for His$^+$ on Trp, Phe, Tyr, Arg, and Pro. Individual colonies were scored for Aro$^+$ on minimal plus Arg and Pro, and for f1-Trp^R on minimal plus Trp, Phe, Tyr, Arg, Pro, and 5-fluorotryptophan. Cross 3 was selected for Trp$^+$ on minimal plus anthranilate and supplement. Individual colonies were scored for Aro$^+$ on minimal plus His, Arg, and Pro, and for f1-Trp^R on minimal plus anthranilate, supplement, and 5-fluorotryptophan.

[b] The strains used in these crosses were: GSY264, *mtr-264*; BS66, *aroB137 trpE24*; SB138, *aroB138 hisB2*; SB117, *aroF117 trpC2*; and WB3112, *aroF3112 hisB2*.

plete linkage map for this region of the *B. subtilis* genome is seen in Fig. 1.

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