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Gene-Enzyme Relationships of Aromatic Acid Biosynthesis in *Bacillus subtilis*

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Mutants have been isolated which correspond to every step concerned with the biosynthesis of the aromatic amino acids in *Bacillus subtilis*. Each mutant has been characterized, and the lesion it bore was analyzed by deoxyribonucleic acid transformation and PBS-1 mediated transduction. The biochemical analysis revealed that each of the mutations appears to have affected a single enzyme, except for two groups of pleiotropic mutations. All *aroF* mutants (chorismic acid synthetase) lack dehydroquinic acid synthetase (*aroB*) activity. The gene that specifies *aroB* is closely linked to the gene coding for the *aroF* enzyme. Both genes are a part of the *aro* cluster. Mutants lacking chorismate mutase activity also lack D-arabino-heptulosonic acid-7-phosphate synthetase and shikimate kinase activity, presumably as a result of these three activities forming a multi-enzyme complex. Another mutant, previously undescribed, had been isolated. The affected gene codes for the tyrosine and phenylalanine aminotransferase activity. All of the mutations have been located on the *B. subtilis* genome except those in the genes specifying shikimate kinase activity and tyrosine-phenylalanine aminotransferase activity.

The aromatic biosynthetic pathway synthesizes the aromatic ring for the amino acids, phenylalanine, tyrosine, and tryptophan along with several growth factors, including *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, and various quinones and compounds concerned with iron transport. The regulation of this pathway has been reviewed recently by Gibson and Pittard (4) and Pittard and Gibson (19). In *Bacillus subtilis*, mutants with enzymatic alterations in the aromatic pathway have been isolated and the lesion has been mapped (15, 18). These studies have shown that many of the *aro* genes are closely linked to form the so-called *aro* cluster, which is represented near the terminus of the chromosome map. This cluster includes the tryptophan operon, at least one gene of histidine synthesis, and a number of loci of aromatic acid synthesis (7, 16). A portion of the genes of aromatic acid synthesis, the tryptophan operon and the gene concerned with histidine biosynthesis seem to form a unit of control under certain conditions (21). We have now isolated mutants which represent every step in the synthesis of the aromatic amino acids. In this more detailed analysis, the chromosomal location of these mutations has been delineated by means of deoxyribonucleic acid (DNA) transformation and PBS-1 transduction.

MATERIALS AND METHODS

Strains. All strains used in this study were derived from *Bacillus sbutilis* 168 (Table 1). It should be noted that all *B. subtilis* 168-derived strains are chorismate mutase deficient (see Results).

Auxotrophic mutants were obtained after ultraviolet irradiation or treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (13).

Genetic analysis. The preparation of PBS-1 lysates and methods for transduction have been described previously (5). Transformation was carried out by the method of Anagnostopoulos and Spizizen (1).

Enzyme assay. D-Arabino-heptulosonic acid-7-phosphate synthetase (DAHP synthetase) was assayed by the method of Jensen and Nester (11).

Dehydroquinate synthetase (DHQ synthetase) was assayed by the method of Srinivasan et al. (22), as modified by Jensen and Nester (10).

Dehydroquinase and dehydroshikimate reductase were assayed as in Nasser and Nester (15).

Shikimate kinase was determined as described by Nakasukasa and Nester. (14).

Enolpyruvyl shikimate-5-phosphate synthetase was assayed as described by Nasser and Nester (15).

Chorismic acid synthetase was assayed by the

TABLE 1. *Bacillus subtilis strains*

Strain	Genotype	Nutritional requirements of mutants bearing *aro*⁻ lesions	Source
GSY384	*argA11 leu-1*	None	C. Anagnostopoulos
GSY1070	*phe-1 trpC2*	None	C. Anagnostopoulos
60154	*lys-1*	None	E. Freese
BD25	*purA16 leu-8 metB5 nic-38*	None	D. Dubnau
JH22	*spoA12 trpC2*	None	This study
SB-5	*hisA1 trpC2 ura-1*	None	This study
WB2281b	*aroA2281*	Shikimic acid or phenylalanine, tyrosine and tryptophan	This study
SB138	*aroB138 hisH32*	Shikimic acid or phenylalanine, tyrosine and tryptophan	This study
SB121	*aroC121 trpC2*	Shikimic acid or phenylalanine, tyrosine and tryptophan	This study
SB120	*aroD120 trpC2*	Shikimic acid or phenylalanine, tyrosine and tryptophan	This study
WB906	*aroI906*	Phenylalanine, tyrosine, and tryptophan	This study
SB130	*aroE130 hisH32*	Phenylalanine, tyrosine, and tryptophan	This study
WB888	*aroF888*	Phenylalanine, tyrosine, and tryptophan	This study
WB2030	*aroF2030*	Phenylalanine, tyrosine, and tryptophan	This study
WB2188	*aroF2188*	Phenylalanine, tyrosine, and tryptophan	This study
WB2201	*aroF2201*	Phenylalanine, tyrosine, and tryptophan	This study
WB932	*aroG932*	Phenylalanine, tyrosine, and tryptophan	This study
WB3550	*aroJ3550 hisH32*	Phenylalanine, tyrosine, and tryptophan	This study
SB672[a]	*aroH* prototroph	None	This study

[a] This strain is a derivative of the original strain 23 and as such is $aroH^+$. All of the other strains in this table are considered $aroH^-$ since they were originally derived from strain 168. The $aroH^-$ allele does not confer a nutritional requirement but causes a loss of the chorismate mutase isoenzyme $CM_{1,2}$.

method of Nasser and Nester (15).

Chorismate mutase was assayed as described by Nakasukasa and Nester (14).

The substrate DAHP was prepared by incubating a highly purified DAHP synthetase-chorismic acid mutase enzyme preparation from *B. subtilis* with erythrose-4-phosphate and phosphoenol pyruvate at 37 C. The reaction mixture was incubated until there was no further increase in DAHP as assayed by the periodate-thiobarbituric assay of Srinivasan and Sprinson (23). When the reaction was complete, 12 N HCl was added until the pH reached 2.0. The precipitated protein was removed by centrifugation, and the pH of the supernatant solution was adjusted to 7.0. This preparation was used directly as substrate for the assay of DHQ synthetase or it was further purified by passage through a Dowex IX8 (CL^- form) column by the method of Nasser and Nester (15).

Tyrosine-phenylalanine aminotransferase activity was assayed according to a modification of the procedure of Pittard and Wallace (20). The concentration of the amino acids in the reaction vessel was increased to 5×10^{-3} M, and the concentration of alpha-ketoglutarate was increased to 5×10^{-2} M. Phosphate buffer (pH 7.5, 5×10^{-2} M) was routinely employed, and the final readings were made at 320 nm for phenylalanine and at 330 nm for tyrosine.

RESULTS

Biochemical characterization of mutants. In Fig. 1 the enzymatic steps emphasized in this paper for the biosynthesis of aromatic amino acids are depicted, and Table 1 lists the classes of mutants which lack one or more of the enzymes for this pathway. Table 2 shows enzyme levels and growth characteristics for a representative of each class of mutants. Mutants that are defective in the first four steps of the pathway leading to shikimic acid have been described in previous publications (11, 15). Mutations in either the *aroA*, *aroB*, *aroC*, or *aroD* genes lead to the loss of only one enzymatic activity characteristic of the gene, and the mutants studied appear to have single-step,

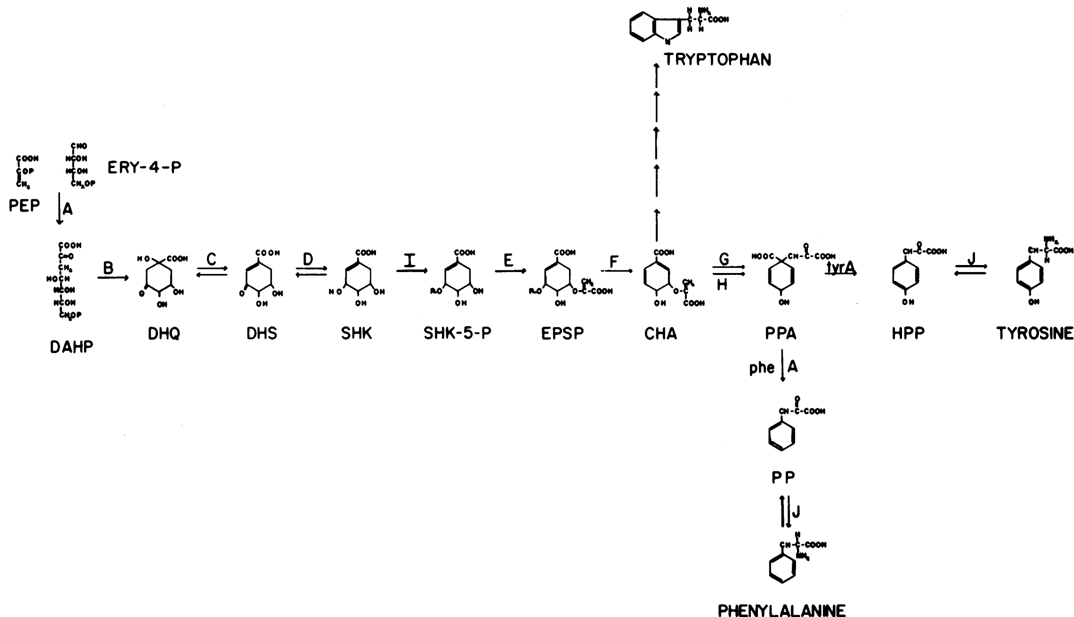

FIG. 1. *Aromatic amino acid biosynthetic pathway in B. subtilis. Enzymes: A, DAHP (3-deoxy-D-arabino heptulonsonic acid 7-phosphate) synthetase; B, DHQ (dehydroquinate) synthetase; C, dehydroquinase; D, DHS (dehydroshikimate) reductase; I, SHIK (shikimate) kinase; E, EPSP (3-enolpyruvylshikimate-5-phosphate) synthetase; F, CHA (chorismate) synthetase; G and H, chorismate mutase; J, tyrosine-phenylalanine aminotransferase; tyrA, PPA (prephenate) dehydrogenase; pheA, PPA dehydratase.*

TABLE 2. *Enzyme levels and growth characteristics of mutants*

Strain	Enzyme step[a]	Aromatic enzyme defect	Sp act[b] in mutant	Sp act of enzyme in wild-type strain
WB2281b	A	DAHP synthetase	<0.001	12 nmol of DAHP formed per min per mg of protein
SB138	B	DHQ synthetase	<0.01	2 nmol of DAHP removed per min per mg of protein
SB121	C	Dehydroquinase	<0.1	10 nmol of DHS formed per min per mg of protein
SB120	D	DHS reductase	<0.01	3 nmol of TPNH formed per min per mg of protein
WB906	I	Shikimate kinase	<0.01	0.7 nmol of shikimate-5-PO4 formed per min per mg of protein
SB130	E	EPSP synthetase	<0.1	15 nmol of anthranilate formed per min per mg of protein
WB888	F	Chorismate synthetase	<0.1	11 nmol of anthranilate formed per min per mg of protein
WB932	G	Chorismate mutase	<0.01	2.8 nmol of prephenate formed per min per mg of protein
		DAHP synthetase	Low	See reference 14
		Shikimate kinase	Low	See reference 14
SB672	H	$CM_{1,2}$ and CM_3	12.5	2.8 nmol of prephenate formed per min per mg of protein
WB3550	J	Phenylalanine and tyrosine aminotransferase	0.009	4 nmol of phenylpyruvate formed per min per mg of protein

[a] As designated in Fig. 1.
[b] Specific activity of mutants relative to wild type set equal to 1.0.

revertable lesions. All of these mutants respond both to the full complement of aromatic amino acids and to shikimic acid as the source of the amino acids. The next step in the pathway, shikimate kinase, is coded for by a gene designated *aroI* (14). A number of revertable and transformable mutants lacking only this enzyme activity have been isolated.

Mutants defective in the next step of the pathway, catalyzed by 3-enolpyruvylshikimate-5-phosphate synthetase, have been characterized biochemically and shown to have lesions in a single locus designated *aroE* (15). Chorismate synthetase-deficient mutants, bearing *aroF* mutations, have been isolated, and all of these mutants isolated so far also lack the *aroB* activity, dehydroquinate synthetase. If one reverts the *aroF* mutants for the aromatic requirement, the *aroB* activity is simultaneously regained. The biochemical basis for this pleiotropic mutation will be considered in a subsequent publication (Ahmed, Montoya, and Nester, manuscript in preparation). Thus, the key intermediate, chorismic acid, is synthesized by seven enzymatic steps, and seven mutant classes corresponding to these steps have been found. The only apparent discrepancy is the *aroF* mutation which results in the loss of the *aroB* activity.

The biosynthesis of prephenic acid and, ultimately, phenylalanine and tyrosine is catalyzed by chorismate mutase. It has been shown previously that two functional chorismate mutase enzymes exist in *B. subtilis* (13). One of these, *aroH*, is present in the 23 strain of *B. subtilis*, but not in the 168 strain. Mutants lacking the second chorismate mutase can be isolated from the 168 strain, and these have been designated *aroG*. These mutants simultaneously lose DAHP synthetase and shikimate kinase activity in addition to chorismate mutase. This behavior is due to the fact that these enzymes form a multienzyme complex in vivo, and mutations in the chorismate mutase gene invariably result in the loss of activity of the other two members of the complex (14). Mutations affecting the enzymes converting prephenate to either tyrosine (prephenate dehydrogenase, *tyrA*) or phenylalanine (prephenate dehydratase, *pheA*) have been described and mapped previously (15).

The ultimate step in the conversion of prephenate to either tyrosine or phenylalanine is an aminotransferase reaction involving the corresponding alpha-keto acids, *p*-hydroxyphenylpyruvate and phenylpyruvate, respectively. A mutant has been isolated that lacks about 90% of both tyrosine and phenylalanine aminotransferase activity of the parent strain, which is a mutant lacking imidazoleacetolphosphate:L-glutamate aminotransferase activity (*hisH*). The detailed analysis of the nutritional and biochemical characterization of this mutant will be reported elsewhere. It is not yet known whether the tyrosine-phenylalanine aminotransferase activity is also involved in other biosynthetic pathways.

Genetic mapping of mutants. Two new classes of *aro* mutations were found to be linked to the *aro* cluster previously mapped by Nester et al. (18). One class, the *aroF* mutants, can be shown to be linked by transformation; the other, *aroC*, requires transduction by PBS-1 for a demonstration of its linkage to the *aro* cluster. This cluster was previously identified as consisting of *trp*, *aroB*, *aroE*, *aroH* genes, the *tyrA* locus, and one *his* locus. In two-factor transformation crosses, we found *aroF888* was linked to *trpC2*. Further crosses revealed that *aroF888* was weakly linked to the *tyrA* locus, suggesting that *aroF888* was to the left of the tryptophan operon and close to *aroB*. To order *aroF888* with respect to *aroB* and the *trp* genes, a three-factor analysis was undertaken. Reciprocal transformation crosses between strains WB888 and SB138 were performed with aro^+ as the selected marker, and the segregation of *hisH32* among the recombinants was scored. The results of these analyses (Table 3) were consistent with an order *aroF888-aroB138-hisH32*. As a check on this order, advantage was taken of the fact that *aroB* strains respond to shikimic acid, whereas *aroF* strains do not. Donor DNA from strain SB138 was used to transform strain WB888, and aro^+ was selected on minimal plates containing shikimic acid and histidine. The segregation of shikimate and histidine phenotypes among the aro^+ recombinants confirmed the order (Table 3). Similar analyses using strains bearing *aroF2030*, *aroF2188a*, and *aroF2201* as recipients showed these mutant sites were also to the left of aroB138 as represented in Fig. 2. A recombination index analysis between the *aroB138* and the *aroF* lesions gave recombination values from 10 to 30%, suggesting that the *aroF* locus is distinct from the *aroB* locus. Since the *aroF* mutations readily revert and none of them fails to recombine with *aroB138*, the possibility that the lack of *aroB* enzymatic activity in *aroF* mutants is a result of deletion is remote. None of the *aroF* mutations nor *aroB138* is suppressed by *sup-3* or *sup-13* suppressors (5).

The *aroC121* mutation was unlinked to the *aro* cluster by transformation but linked by PBS-1 transduction to *trpC2*. Two-factor PBS-1

TABLE 3. *Three-factor transformation crosses to order aroF888*

Recipient (genotype)	Donor (genotype)	Class (phenotype)	No.	Order implied
aroF888	*aroB138 his32*	Aro+His+	161	
		Aro+His-	36	*aroF888-aroB138-his32*
aroB138 his-32	*aroF888*	Aro+His+	83	
		Aro+His-	110	
aroF888	*aroB138 his32*	Aro+Shi+His+	16	
		Aro+Shi+His-	3	*aroF888-aroB138-his32*
		Aro+Shi-His+	127	
		Aro+Shi-His-	54	

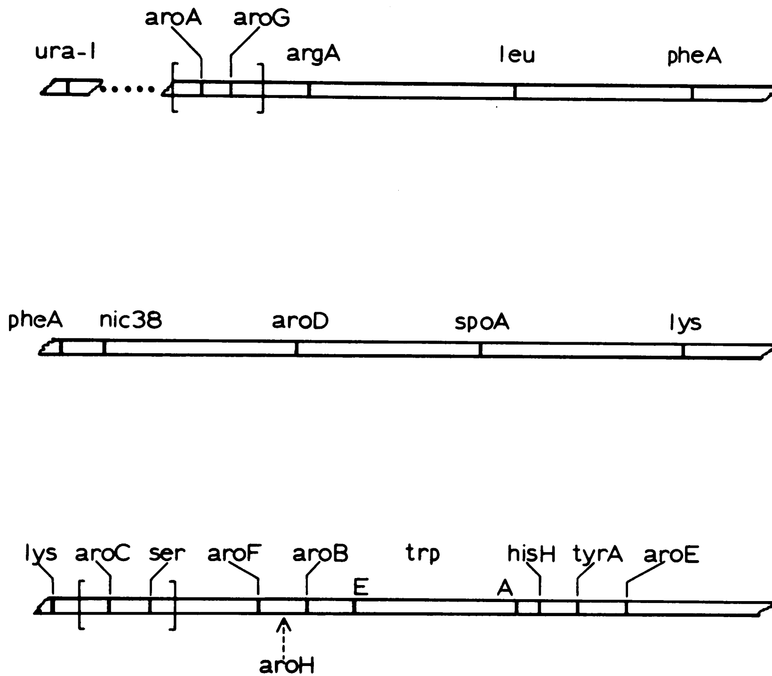

FIG. 2. *Genetic maps showing the location of aro loci on the Bacillus subtilis chromosome. The maps are not drawn to scale.*

transduction crosses suggested that *aroC121* was to the left (Fig. 2) of the *aro* cluster and close to *lys-1*. A three-factor cross in which donor phage grown on strain 60154 was used to transduce strain SB121 established the order *lys-1-aroC121-trpC2* (Table 4). Two-factor transformation crosses gave 50% recombination between *aroC121* and a serine auxotroph, *ser-1*. The position of *ser-1* with respect to outside markers was not determined. A weak linkage was also found in transformation crosses between *aroC121* and *aroF888*. The *aroC121* mutation cannot be considered a part of the *aro* cluster on the basis of these results.

Another gene that was previously identified as being a part of the *aro* cluster is *aroH*, which codes for one of the isozymes of chorismate mutase, $CM_{1,2}$ (13). This locus was located, tentatively, to the right (Fig. 2) of the trypto-

TABLE 4. *Three-factor PBS-1 transduction cross to order aroC121*

Donor (genotype)	Recipient (genotype)	Classes (phenotype)	No.	Implied order
lys-1	*trpC2 aroC121*	Trp+Shi+Lys+	24	
		Trp+Shi+Lys-	90	*lys-1 aroC121 trpC2*
		Trp+Shi-Lys+	69	
		Trp+Shi-Lys-	0	

phan operon (13). Since strains derived from *B. subtilis* 168 are deficient in this isozyme, we used strain SB672, derived from *B. subtilis* 23, as DNA donor in two-factor crosses with strains bearing mutations in the *aro* cluster. Recombinants were picked and purified, and the presence of $CM_{1,2}$ was determined by in vitro assay of lysates. With strain SB672 as donor, the ability to form $CM_{1,2}$ was co-transformed 50 and 83% with *hisH32* and *trpC2*, respectively. The same donor gave 67% co-transformation with *aroB138* and 36% co-transformation with *hisH32* of the SB138 strain (Table 5). The present results suggest that *aroH* is located to the left (Fig. 2) of the *trp* operon rather than to the right as previously mapped (13).

A number of *aro* mutations could not be linked to the *aro* cluster by PBS-1 transduction. Among these mutations, the *aroD120* mutation was found to be linked very weakly to *lys-1*. Further crosses with the *spoA12* mutation, known to be to the left of *lys-1* (5), gave a stronger linkage of 79% recombination. Since this position of *aroD120* placed it in the "gap" of the chromosome replication map of Dubnau et al. (3), an attempt was made to link *aroD120* to the next-earlier-replicating linkage region. Two-factor PBS-1 transduction crosses with *phe-1* and *nic-38* gave a stable weak linkage to *aroD120* (Table 6). Thus, the order of markers across the gap is *phe-1-nic-38-aroD120-spoA12-lys-1*. The *aroD120* marker is not co-transformed above congression values (i.e., the amount of co-transformation of unlinked markers due to simultaneous uptake of independent DNA molecules) with either *nic-33* or *spoA12*. In extensive crosses with diverse auxotrophs, no further linkage has been found to *aroD120* (Hoch and Mathews, unpublished data). Thus, this region of the chromosome appears to be "silent" in terms of auxotrophic loci.

A second group of mutations linked to each other, but unlinked to the *aro* cluster or *aroD*, are the *aroA* and *aroG* mutants. A search of the known transducing segments revealed that this group of mutations was linked to the *argA11* marker. Two-factor PBS-1 transduction crosses revealed a strong linkage to *argA11* and weak linkage to *leu-1* (Table 7). From these results the order *aroA(G)-argA11-leu-1* is predicted. This result again placed an *aro* mutation in a gap of the chromosome map of Dubnau et al. (3). The situation with respect to this gap is unclear, however. Assuming congression does not occur in the system, transduction data in all cases have revealed a weak linkage of *ura-1* and *argA11* via *recA1* (6) and phosphatase (12). The *aroG932* mutation and *ura-1* are rarely co-transduced (Table 6). Thus, the order of loci from *argA11* to *ura-1* is uncertain. The possibility exists that the gap is an artifact of the transduction system or of the markers involved.

The *aroI906* marker represents a genetically distinct class from any of the preceding. It is unlinked by either transformation or transduction to the *aro* cluster, *aroD120*, or *aroG932*. Utilizing PBS-1 transduction has not revealed linkage of *aroI906* to any known auxotrophic marker whether *aroI906* was used as recipient or donor. It is probable that *aroI906* resides in one of the yet unfilled gaps of the chromosome. An independently isolated *aroI* mutation is linked

TABLE 5. *Two-factor transformation crosses to locate aroH*

Donor	Recipient	Classes	No.	Recombination (%)
SB672	SB138	$aroB^+hisH^{\pm}aroH^+$	16	33
		$aroB^+hisH^{\pm}aroH^-$	8	
		$hisH^+aroB^{\pm}aroH^+$	9	64
		$hisH^+aroB^{\pm}aroH^-$	19	

TABLE 6. *Two-factor PBS-1 transduction crosses with aroD120*

Recipient (genotype)	Donor (genotype)	Class (phenotype)	No.	Recombination (%)
aroD120	*nic-38*	Aro^+Nic^+	772	93
		Aro^+Nic^-	58	
aroD120	*spoA12*	Aro^+Spo^+	1,383	79
		Aro^+Spo^-	373	
aroD120	*phe-1*	Aro^+Phe^+	690	99
		Aro^+Phe^-	10	
aroD120	*lys-1*	Aro^+Lys^+	347	99
		Aro^+Lys^-	3	

TABLE 7. *Two-factor PBS-1 transduction crosses with aroA and aroG mutants*

Recipient (genotype)	Donor (genotype)	Class (phenotype)	No.	Recombination (%)
argA11	*aroG932*	Arg^+Aro^+	111	40
		Arg^+Aro^-	184	
argA11	*aroA2281*	Arg^+Aro^+	77	46
		Arg^+Aro^-	91	
argA11	*aroA2191*	Arg^+Aro^+	115	30
		Arg^+Aro^-	265	
leu-1	*aroA2191*	Leu^+Aro^+	304	87
		Leu^+Aro^-	46	
aroG932	*ura-1*	Aro^+Ura^+	212	99
		Aro^+Ura^-	1	
aroA2281	*ura-1*	Aro^+Ura^+	207	100
		Aro^+Ura^-	0	

by transformation to *aroI906* so the results are not site specific. Further studies by density transfer experiments are needed to locate these markers on the chromosome.

The Aro$^-$ phenotype of the mutant lacking tyrosine and phenylalanine aminotransferase activity (*aroJ*) is only expressed in strains bearing the *hisH* mutation (Nester and Montoya, manuscript in preparation). Thus, tests for linkage to the *aroJ3550* mutation were carried out with various auxotrophic donors bearing a *hisH* mutation. Auxotrophic donors with a *his*$^+$ genotype were also used to transduce the *hisH32*, *aroJ3550* mutant and the *his*$^+$ among the *aro*$^+$ transductants were excluded from consideration. By these means we were unable to demonstrate linkage of the aminotransferase mutation to any of our *aro* linkage groups. Moreover, no linkage was found to any of the auxotrophic mutations employed. Thus, the location of this mutation on the chromosome map is unknown.

DISCUSSION

The genetic mapping results have revealed that the genes for the enzymes for the conversion of phosphoenolpyruvate and erythrose-4-phosphate to the key branch point intermediate, chorismic acid, comprise five distinct linkage groups. The linkage groups and gene clusters do not have arrangements in common with either the arrangement in *Escherichia coli* (4), or *Neurospora crassa* (2). In both *B. subtilis* and *N. crassa*, enzyme aggregates of the enzymes of the aromatic pathway have been discovered (2, 17). In the case of *N. crassa*, the genes that code for the five enzymes in the multi-enzyme aggregate are closely linked to each other in the *arom* gene cluster and correspond to our designations *aroB*, *aroC*, *aroD*, *aroI*, *aroE*. It can be seen from Fig. 2 that this clustering is completely different from that in *B. subtilis*.

The existence of the enzyme aggregate in *B. subtilis* most likely explains the pleiotropic mutations which we have designated *aroG*. These mutants lack DAHP synthetase, chorismate mutase, and shikimate kinase and are most likely due to the disruption of the enzyme aggregate and subsequent loss of activities. The *aroG* and *aroA* mutations are closely linked, and studies are currently aimed at determining whether they represent one or two genes. The second group of pleiotropic mutations is the *aroF* mutations which simultaneously lose *aroB* activity. Again, the *aroF* and *aroB* loci are closely linked but most probably are distinct loci. The results in this paper expand the *aro* cluster to include one more locus.

Although the *aroC* mutation lies close to the *aro* cluster by PBS-1 transduction, it is not a part of this cluster since the linkage to *aroF* is only about 1% in transformation analysis. Another locus that seems to stand alone is the *aroD* locus. Mutations in this locus were found to lie between *pheA* and *spoA* in one of the less well-studied gaps of the chromosome (24). Ionesco et al. (9) have shown that a number of spore mutations can be mapped between the *pheA* and *lys-1* markers, and some of these markers can be weakly linked to both *pheA* and *lys-1*. The *aroA*, *aroG* markers again extend into a gap from the *argA* locus. In two-factor transduction crosses, we have found only slight and variable linkage of either *aroA* or *aroG* to the *ura-1* mutation in the next-earlier-replicating group.

Finally, *aroI* presents a special case. In two-factor PBS-1 transduction crosses we have not been able to link this marker to any of the known auxotrophic markers on the chromosome map. This situation is not unique to the aromatic pathway since a class of sporulation markers (Hoch and Mathews, in press) is also unlinked to the auxotrophic mutations known to comprise the chromosome map. Furthermore, the *aroI* and sporulation markers cannot be linked to each other by PBS-1 transduction or transformation. The reason for this behavior is not clear, but may indicate that the chromosome map contains large areas where no auxotrophic markers except these have been found.

Recent results by Farrand and Tabor (Bacteriol. Proc., p. 68, 1970) and by ourselves have shown that one can isolate aromatic mutants of *B. subtilis* that require shikimic acid in addition to the normal aromatic amino acids and known vitamins. Thus, the normal supplement given *aro* mutants is sufficient if the mutation is leaky enough to make enough shikimic acid for chorismate biosynthesis. Therefore, all of the mutants described in this study most likely are of the missense type since most were isolated as aromatic mutants in the absence of shikimate or quinones. Consistent with this conclusion is the fact that none of the aromatic mutations respond to presumed amber suppressors.

The one gene in the *aro* cluster which has not been mapped precisely is *aroH*. The gene product, chorismate mutase, is demonstrable in strain 23 and its derivatives as an isoenzyme of the enzyme coded by *aroG*. This functioning of *aroH* is apparently not obligatory to tyrosine and phenylalanine synthesis, although it does serve to channel chorismate to these latter two amino acids and away from tryptophan (8).

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