GENETIC ANALYSIS OF AROMATIC MUTANTS OF *SALMONELLA TYPHIMURIUM*¹

Y. NISHIOKA, M. DEMEREC² AND A. EISENSTARK

Brookhaven National Laboratory, Upton, N. Y., and Department of Bacteriology,
Kansas State University, Manhattan, Kansas 66502³

Received March 13, 1967

AN aromatic (*aro*) mutant of *Escherichia coli* may demand for its growth tyrosine, phenylalanine, tryptophan, para-aminobenzoic acid, para-hydroxybenzoic acid and a sixth unknown factor, or only the first two, three, four or five of these (DAVIS 1952a). Biochemical analyses of the aromatic pathway by several workers have indicated the possible presence of seven sequential steps leading to chorismic acid biosynthesis in *E. coli* (UMBARGER and DAVIS 1962; GIBSON and GIBSON 1964). Chorismic acid is then converted to the above aromatic end products by individual pathways (COTTON and GIBSON 1965; COX and GIBSON 1964; GIBSON 1964; GIBSON, GIBSON and COX 1964).

The genetic analysis of 89 aromatic mutants of *Salmonella typhimurium* presented here has shown that they represent mutations in five structural genes involved in chorismic acid biosynthesis. The mutants of each gene consist of those which require for growth two, three, four or five of the aromatic acids, as is the case in *E. coli aro* mutants (DAVIS 1952a; PITTARD and WALLACE 1966); also the variety of requirements among allelic mutants is related to mutational position within an aromatic gene. In addition to the *aro* mutants, phenylalanine-specific (*phe*) and tyrosine-specific (*tyr*) mutants were examined; only one locus was found for each of these classes.

MATERIALS AND METHODS

Bacteria and bacteriophages: Eighty-nine *aro* (multiple requiring), 20 *tyr* (tyrosine requiring) and 14 *phe* (phenylalanine requiring) mutants of *Salmonella typhimurium* used in this study were isolated by several investigators from LT-2 and LT-7 strains as spontaneous mutants or after the strains were treated with 2-amino purine, ultraviolet light, diethylsulphate, 5-bromodeoxyuridine or X rays (Table 1).

Phage stocks were derived from the temperate strain H1 of PLT22. Its mutant, H4, was used to obtain nonlysogenic progeny from transductant clones. The procedure was to streak each transductant colony for single colony isolation and then to test each progeny clone for lysogeny. Another mutant phage, H5, which is normally virulent but is capable of transducing cells that have been lysogenized with H1, was also used.

¹ A portion of this work was done at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission, and it was continued at Kansas State University with the support of National Science Foundation Research Grant GB 4702. Contribution No. 443, Department of Bacteriology, Agricultural Experiment Station, Kansas State University, Manhattan.

² Deceased April 12, 1966.

³ Present address of first and third authors.

TABLE 1

Classification of aro, phe and tyr mutants according to their nutritional requirement, mutational origin, revertibility and gene loci

Nutritional requirement	Mutational origin	Very few or no spontaneous reversions		
		Very few or no reversions by diethyl sulfate	Revertible by diethyl sulfate	Revertible spontaneously and by diethyl sulfate
TYR + PHE + TRY + PABA + PHBA TYR + PHE + TRY + PABA	Spontaneous	<i>aroD166</i>		<i>aroA15*</i> , 20, 89 <i>aroD21</i>
	Spontaneous	<i>aroA1</i>		<i>aroA62</i> , 71 <i>aroB50</i> , 66, 76 <i>aroC32</i> , 65, 79 <i>aroD37</i> , 58, 73 <i>aroE41</i> , 68 <i>aroC92</i> <i>aroD86</i>
	2-Aminopurine	<i>aroA64</i> , 67 <i>aroB74</i>	<i>aroA40</i> <i>aroB34</i> , 49, 51 <i>aroC36</i> <i>aroD35</i>	
	Diethylsulfate			
	X rays	<i>aroA148</i>		<i>aroA9*</i> , 10*, 13*, 18*, 19*, 72, 201 <i>aroB8*</i>
TYR + PHE + TRY	Spontaneous			<i>aroD11*</i> , 17*, 22, 23 <i>aroA70</i> , 81 <i>aroB39</i> , 75 <i>aroC36</i> , 52 <i>aroD28</i> , 38, 45, 57, 77, 78, 100
	2-Aminopurine		<i>aroA42</i> , 53, 95 <i>aroB25</i> , 33, 47 <i>aroD44</i> , 96	
	Ultraviolet light		<i>aroD6</i>	
	Diethylsulfate		<i>aroA84</i> <i>aroD90</i>	<i>aroE85</i> , 88
	5-Bromodeoxyuridine			<i>aroA101</i>

Nutritional requirement	Mutational origin	Very few or no spontaneous reversions		
		Very few or no reversions by diethylsulfate	Reversible by diethylsulfate	Reversible spontaneously and by diethylsulfate
TYR + PHE	Spontaneous	<i>aroA16*</i>		<i>aroA3</i> <i>aroB12*</i> <i>aroD5</i> <i>aroA29, 43, 55, 98, 102</i> <i>aroC56</i> <i>aroD80</i> <i>aroA54</i> <i>aroA87, 91</i>
Leaky	2-Aminopurine		<i>aroA46, 97</i>	
	Ultraviolet light			
	Diethylsulfate			
	Spontaneous	<i>aroA48†</i>		
	2-Aminopurine	<i>aroD103†</i>		
	Diethylsulfate	<i>aroD93†</i>		
TYR	Spontaneous	<i>tyrA11</i>	<i>tyrA7*</i>	<i>tyrA1, 3, 5, 8*, 9*, 10*, 12, 15, 16</i> <i>tyrA19, 23, 24, 25, 31, 32, 33, 35, 36</i>
PHE	2-Aminopurine			<i>pheA3, 5, 6, 7*, 8*, 9, 10</i>
	Spontaneous			<i>pheA14, 15, 17, 18, 20, 21</i>
	2-Aminopurine			<i>pheA16</i>
	Ultraviolet light			

* LT-7 strains. Others are LT-2.

† Revertibility is not clear.

Media: The minimal medium contained K_2HPO_4 (10 mg/ml), KH_2PO_4 (4.5 mg/ml), $(NH_4)_2SO_4$ (1 mg/ml), sodium citrate (0.5 mg/ml), $MgSO_4 \cdot 7H_2O$ (0.05 mg/ml), and glucose (4 mg/ml). Enriched minimal medium was made by adding 0.01% (single enriched) and 0.02% (double enriched) dehydrated nutrient broth. For plate cultures both media contained 1.5% agar. For testing growth response of the mutants L-tryptophan (TRY), L-tyrosine (TYR), L-phenylalanine (PHE), *p*-aminobenzoic acid (PABA), and *p*-hydroxybenzoic acid (PHBA) were used. When the medium for transduction was supplemented with these compounds, each final concentration was 15 μ g/ml in the case of amino acids and 1.5 μ g/ml in the case of vitamins.

Nutritional requirement tests: One-tenth ml of each mutant grown overnight in nutrient broth was spread on a minimal agar plate and one drop (about 0.03 ml) of the combined supplement solution (amino acid 2 mg/ml, vitamin 0.2 mg/ml) was placed on the seeded agar. Each *aro* mutant was designated as either quintuple (TYR + PHE + TRY + PABA + PHBA), quadruple (TYR + PHE + TRY + PABA), triple (TYR + PHE + TRY), or double (TYR + PHE) according to the least number of compounds required for maximum growth response during 72 hours of incubation at 37°C. Under certain testing conditions (e.g. different media, different length of incubation), phenotypic expression may vary.

An aqueous solution of *p*-hydroxyphenylpyruvic acid (HPP) after autoclaving was found to be much less active than the crystal form in stimulating growth of *tyr* mutants; in such cases, crystals of aromatic compounds were added directly to agar without previous dissolution.

The following compounds were kindly supplied by Dr. D. B. SPRINSON, Columbia University: 3-deoxy D-arabino heptulosonic acid 7-phosphate (DAHP), 5-dehydroquinic acid (DHQ), 5-dehydroshikimic acid (DHS), 5-phosphoshikimic acid (SP), 3-enolpyruvylshikimate 5-phosphate (EPSP), and chorismic acid (CA). These chemicals and shikimic acid (SA) were placed directly on a minimal agar plate seeded with a mutant bacterial culture for growth response tests.

Crossfeeding test: Nutrient broth cultures from pairs of *S. typhimurium* mutant strains were streaked near each other on double enriched minimal agar, and feeding response was observed after 24, 48, and 72 hours of incubation at 37°C. When feeding was positive, heavy growth was oriented at the edge of the streak closest to the other (feeder) strain.

Transduction: Recipient bacteria grown overnight in nutrient broth were infected with phage grown previously on the donor bacteria, and the mixture was plated on a suitable medium. The titer of the overnight cultures was about 2×10^9 cells/ml, and 0.1 or 0.2 ml of the culture was spread on each agar plate. The multiplicity of infection was approximately five plaque forming units (pfu) per bacterial cell. Colonies were scored after 24 to 48 hours of incubation at 37°C. The technique to detect suppressors by transduction was adapted from that of YURA (1956). This procedure includes infecting the suppressed strain by a H4 phage culture grown on a wild-type strain, and then finding the recombinants of prototrophic phenotype that possess a suppressor marker but not an auxotroph marker.

Abortive transduction: For the complementation test, 0.2 to 0.4 ml of an overnight nutrient broth culture was spread on a minimal agar plate and a drop of a phage suspension (10^{10} to 5×10^{10} pfu/ml) grown on donor bacteria was placed on the seeded recipient cells. After 24 hours incubation at 37°C, abortive transductants were observed under a low power microscope at $7\times$ to $30\times$ magnification. If the two mutations complemented each other, minute colonies of the abortive transductants appeared. The complementation was confirmed by disturbing several of the minute colonies as a group directly on the agar surface of the transduction plate, using saline and a micro-platinum loop, then incubating the plate overnight at 37°C. If they were the abortive transductants, the minute colonies appeared again at the disturbed area. If they were not the abortives, only the confluent growth took place. All of the *phe* mutants had a heavy background on minimal agar after 24 hours of incubation and the minute abortive transductant colonies were outgrown. To avoid this for the *phe* mutants, the cells were centrifuged and resuspended in saline to make 0.1 the original volume. Plating 0.1 or 0.2 ml of this suspension on a minimal agar plate successfully reduced background growth.

RESULTS

Transduction of the *aro*, *tyr*, and *phe* mutants separated them into seven groups. Five groups with aromatic multiple requirements were designated as the *aroA*, *aroB* . . . *aroE* loci. Two additional loci were designated *tyrA* and *pheA*. Numbers of stable transductants were invariably lower in crosses between alleles of the same locus than in crosses between mutants of different loci. Transduction frequencies of representative mutant $\times$ mutant crosses are shown in Table 2.

Joint transductions were detected in a cross between *pheA* and *tyrA* mutants. When a mutant *tyrA3* was used as a recipient and a mutant *pheA3* as donor on transduction plates supplemented with PHE, 21 out of 1341 tested colonies did not grow on minimal replica plates. They were confirmed to be *pheA3* mutants by backcrossing. The reciprocal cross, *pheA3* $\times$ *tyrA3*, on TYR supplemented plates showed that 12 out of 1104 tested colonies were donor type recombinants, *tyrA3*. Any other possible combinations among representatives from the seven loci, however, failed to show joint transduction although the number of colonies tested were at least 200 for each combination. Those loci which were mapped as neighbors by conjugation (SANDERSON and DEMEREC 1965) were tested with larger numbers of transductants; 1290 colonies for *aroC* $\times$ *aroB*; 2946 colonies for *aroE* $\times$ *aroA*, 388 colonies for *aroD* $\times$ *pheA* and 567 colonies for *aroD* $\times$ *tyrA*. The recipient *aro* mutants used in the tests required TRY + TYR + PHE + PABA and the donor *aro* mutants required TYR + PHE.

The *aro*, *phe* and *tyr* mutants were tested for complementation at first with several representative mutants from each of the seven loci. As a result, every mutant showed abortive transduction with the representatives of the other loci but failed to do so with those of its own locus. Then all *aro* mutants were tested for the complementation within each locus. No complementation was observed

TABLE 2

Transduction tests among mutants of different loci 0.1 ml of bacteria + 0.05 ml of phage
(10^{10} — 10^{11} /ml) One plate was observed for each cross

Recipients	Donors							
	Wild type	<i>aroA1</i>	<i>aroB33</i>	<i>aroC36</i>	<i>aroD35</i>	<i>aroE88</i>	<i>tyrA3</i>	<i>pheA3</i>
<i>aroA1</i>	229	0	744	934	842	321	284	173
<i>aroA46</i>	206	145	840	1056	944	556	312	180
<i>aroB33</i>	340	2472	0	1244	1740	1248	1228	780
<i>aroB74</i>	226	2196	9	1144	1118	754	580	576
<i>aroC36</i>	227	972	632	0	644	492	291	235
<i>aroC79</i>	264	150	217	6	96	427	638	740
<i>aroD5</i>	421	2826	1330	1314	0	754	738	310
<i>aroD35</i>	310	2940	1316	654	0	876	1036	277
<i>aroE85</i>	118	808	288	359	336	0	198	101
<i>aroE88</i>	111	1256	512	508	640	0	177	108
<i>tyrA3</i>	740	3528	2572	2492	3200	2916	1	1026
<i>pheA3</i>	913	1275	983	981	2255	2055	1331	0

FIGURE 1.—Complementation maps for *aroD* and *tyrA*. Numbers refer to mutant stock numbers. Maps show that mutants of any two groups do not complement if their groups overlap in the map. Any two mutants of the same group do not complement each other.

between any two mutants within each locus, except that some mutants of *aroD* and *tyrA* showed also, with those of their own loci, the abortive transductant colonies which were smaller than those found in nonallelic transductions. The complementation maps of the *aroD* and *tyrA* loci are shown in Figure 1.

The growth requirement of *aro* mutants was satisfied by (1) TYR + PHE; (2) TYR + PHE + TRY; (3) TYR + PHE + TRY + PABA; (4) TYR + PHE + TRY + PABA + PHBA (Table 1). None of the *aro* mutants grew on the supplements of the presumed aromatic precursors DAHP, DHQ, DHS, SA, SP, EPSP. The precursor CA stimulated the growth of only three mutants (*aroA54*, *aroA55*, and *aroD5*), all of which could also grow on TYR + PHE.

No crossfeeding was observed among mutants *aroA3*, *aroB25*, *aroC32*, *aroD28*, and *aroE85* whereas many *aro* mutants were fed by the three mutants (*pheA3*, *tyrA3*, and *tryA52*) which might be secreting CA (Table 3).

Each of the *aroC* mutants was found to excrete a blue-green pigment on minimal agar medium or in liquid minimal culture supplemented with required amino acids and vitamins. This might be due to the location of the metabolic

TABLE 3

Feeding of aroA, B and D mutants by mutants pheA3, tryA52 and tyrA3

<i>aro</i> mutants to be fed		Feeders		
Type of mutants	Total number examined	<i>pheA3</i>	<i>tryA52</i>	<i>tyrA3</i>
Duadruple-requiring Mutants	21	21* (100)†	2 (9.5)	0 (0)
Triples	27	27 (100)	17 (63)	9 (33)
Doubles	11	11 (100)	11 (100)	5 (45)

* Number of mutants showing a positive response.

† Percentage of the total.

block imposed by *aroC* mutation. Mutants of no other *aro* loci accumulated pigment except for slight accumulations by *aroA1*, 18, 20, 42, 53, 70, and 81. The intensities of the accumulations suggest that *aroA* mutations block a biosynthetic step following the block imposed by *aroC* mutations. Enzyme analysis supports this contention (GOLLUB, ZALKIN, and SPRINSON 1966).

Each of the *tyr* mutants tested grew well on TYR supplement. TRY or phenylpyruvic acid (PPA) could not be substituted for TYR, whereas the single addition of PHE stimulated slight growth of the *tyr* mutants. The growth response to HPP varied from slight to full depending on the particular *tyr* mutant.

In contrast to the nutritional varieties among the *aro* and *tyr* mutants, all *phe* mutants grew well on either PPA or PHE. Neither TYR, HPP nor TRY could be substituted for them in the growth of the *phe* mutants. Each of the *phe* mutants grew heavily on minimal agar after prolonged incubation. This is analogous to the *E. coli phe* mutants which were studied by KATAGIRI and SATO (1953) and DAVIS (1953). They reported that prephenic acid was spontaneously converted to PPA during prolonged incubation.

In the *aroA* locus, six quadruple-, two triple-, and four double-requiring mutants were mapped by transduction tests (Figure 2). Although the number of mutants examined is small, those with similar requirements are clustered; this suggests that single one-step mutations can produce different phenotypes, depending on which part of the *aroA* gene is mutant.

Many *aro* mutants with a requirement for TYR + PHE + TRY + PABA spontaneously gave rise to less exacting progenies which could be isolated on agar medium supplemented with TYR + PHE or TYR + PHE + TRY. These isolates were named by adding numbers at the end of the parent number (e.g. *A1-1* and *A1-2* derived from *A1*). These modified characters could be transduced into the original quadruple-requiring mutants, though three exceptional cases (*A1-2*, *A62-1* and *A81-1*) did not show the transduction (Table 4).

Tests by transduction showed that the secondary mutations in the cases of *aroA1-1* and *aroD86-1*, were linked to the original mutational sites. Nine phenotypically wild-type recombinants for each were isolated from transduction tests with wild-type donor. Phage prepared on these recombinants lost the modifying

FIGURE 2.—Approximate positions of *aroA* mutant sites from the results of two-point transduction tests.

TABLE 4

List of modified mutants

Original mutants		Modified mutants		Transduction tests*
Name	Requirement for maximum rate of growth	Name	Requirement for maximum rate of growth	Recombinants (modified phenotype)†
<i>aroA1</i>	TYR+PHE+TRY+PABA	<i>A1-1</i>	TYR+PHE	+
		<i>A1-2</i>	TYR+PHE+TRY	—
<i>A62</i>	TYR+PHE+TRY+PABA	<i>A62-1</i>	TYR+PHE+TRY	—
<i>A81</i>	TYR+PHE+TRY	<i>A81-1</i>	TYR+PHE	—
<i>A89</i>	TYR+PHE+TRY+PABA	<i>A89-1</i>	TYR+PHE+TRY	+
<i>B49</i>	TYR+PHE+TRY+PABA	<i>B49-1</i>	TYR+PHE+TRY	+
		<i>B49-2</i>	TYR+PHE	+
<i>B76</i>	TYR+PHE+TRY+PABA	<i>B76-1</i>	TYR+PHE	+
<i>C32</i>	TYR+PHE+TRY+PABA	<i>C32-1</i>	TYR+PHE	+
<i>C65</i>	TYR+PHE+TRY+PABA	<i>C65-1</i>	TYR+PHE	+
<i>D37</i>	TYR+PHE+TRY+PABA	<i>D37-1</i>	TYR+PHE	+
<i>D86</i>	TYR+PHE+TRY+PABA	<i>D86-1</i>	TYR+PHE+TRY	+

1st and 2nd columns are original *aro* mutants. 3rd and 4th columns are modified mutants derived from the originals. 5th column shows transduction between the original mutant and its modified mutant (as donor).

* On selective media; original cells X phage grown on the modified cells.

† +: recombinants (modified phenotype) appeared; —: no recombinants appeared.

effect when used further as donor, indicating that the *aro* and *aro*-modified were allelic or closely linked (cf. YURA 1956).

One mutant stock *aroB75* was found to contain a suppressor (*sup*⁻) mutation that reduced the requirement of *aroB49*, *aroB76*, *aroC32*, and *aroC65*. The *sup*⁻ could not affect *aroB34*, *aroB50*, *aroB51*, *aroB74*, *aroC79* nor the mutants of other loci; *aroA1*, *aroA64*, *aroA71*, *aroD37*, and *aroD86*. When this *aroB75-sup*⁻ was transduced by the phage grown on wild type, *aroB75+sup*⁺, both true wild-type and suppressor-type, *aroB75+sup*⁻, recombinants were found. Thus, the *sup*⁻ marker is linked with *aroB75* but its site is not identical with the *aroB75* site. Bacteria carrying *sup*⁻ were prototrophic when isolated through reversion or transduction of *aroB75-sup*⁻ to *aroB75+sup*⁻. The continued presence of the *sup*⁻ mutation was discerned by using phage grown on the phenotypically wild isolates as donors and a suppressible mutant, *aroC65*⁻, as recipient. Two types of recombinants were found: *aroC65+sup*⁺ and *aroC65-sup*⁻. The suppressed cells (*aroC65-sup*⁻) could grow on TYR + PHE, whereas the original *aroC65*⁻ could grow on TYR + PHE + TRY + PABA.

DISCUSSION

Biochemical analysis of *S. typhimurium aro* mutants indicates that mutants of each of the five *aro* loci lack single, different enzyme activities (GOLLUB, ZALKIN, and SPRINSON 1966):

PITTARD and WALLACE (1966) recently reported biochemical and genetical

analyses of *E. coli* aromatic mutants. They identified six *aro*, one *tyr* and one *phe* genes. Although the gene symbols *aroA*—*E* do not correspond between the two organisms, distribution of the five *aro* gene functions on the chromosome maps are similar (SANDERSON and DEMEREC 1965; PITTARD and WALLACE 1966). There is no evidence of linkage in transduction tests among the *aro* genes of either organism, in contrast to many other cases of gene clustering (c.f. SANDERSON and DEMEREC 1965; DEMEREC 1964). However, in *Neurospora crassa*, there appears to be an aromatic operon for five enzymes (GILES, CASE and PARTRIDGE 1965).

A mutant involving DAHP synthetase was not found until PITTARD and WALLACE obtained a tyrosine sensitive *aro* mutant of *E. coli* which was still able to grow on minimal medium. The absence of a nutritional requirement in *E. coli* and *S. typhimurium* could be explained by the presence of a series of isozymes for the first step of the aromatic pathway (SMITH *et al.* 1962). It is not known, however, why no mutant has been isolated in *E. coli* or in *Salmonella* blocked in the conversion of shikimic acid to 5-phosphoshikimic acid.

Although SA was shown by DAVIS (1951) to substitute for the quintuple requirement of *E. coli* aromatic auxotrophs, none of the *S. typhimurium aro* mutants grew on SA. The lack of growth response to externally supplied precursors may be due to the permeability barriers against phosphorylated forms (DAVIS and MINGIOLI 1953) and to metabolite inhibition (DAVIS 1952b) in the case of *aroA* and *aroC* mutants, respectively.

The *aro* mutants of *S. typhimurium* exhibit a characteristic nutritional behavior in that each of the five loci include the mutants of varying requirements which are uniformly (TYR + PHE + TRY + PABA), (TYR + PHE + TRY), and (TYR + PHE), respectively. These phenotypes are analogous to those found in the study of *E. coli* aromatic multiple auxotrophs (DAVIS 1952a). According to DAVIS, this phenomenon seems to be caused by the incomplete nature of many genetic blocks and by preferential conversions of chorismic acid. In the *aroA* locus of *S. typhimurium* mutant sites eliciting similar phenotype were clustered, indicating that different mutations in certain localized regions of the gene similarly affect the catalytic activity of EPSP synthetase. In this locus, sites representing more complete biochemical blocks, except *aroA67*, are in the central portion of the locus, whereas those representing less complete blocks are at the two ends. This kind of gradient in the arrangement of sites has also been found in *athA* (adenine-thiamine) locus by OZEKI (DEMEREC *et al.* 1956). Since missense mutations may reduce the enzyme activities to various degrees, the *aro* mutants with double or triple requirements may have the missense mutations rather than nonsense or shift mutations which would drastically alter the enzyme structure.

Secondary mutations were found that shifted the requirements so that fewer aromatic acids were needed. In each case the secondary mutations were located very close to the original site of mutation. The secondary mutations of *aroA1-1* and *D86-1* were linked to *aroA1* and *D86* mutations, respectively. They had no effect on other loci. The *sup⁻* mutation in the *aroB75* stock was linked with the *aroB* locus; the effect of the *sup⁻* mutation was exerted only on the functions of

the *B* and *C* loci. These loci map very closely by conjugation (SANDERSON and DEMEREC 1965), but influence quite different steps of the biosynthetic pathway (GOLLUB, ZALKIN and SPRINSON 1966).

The authors wish to thank Drs. P. E. HARTMAN and H. OZEKI for critical reading of the manuscript.

SUMMARY

Complete and abortive transduction tests show the presence of at least seven loci; five (*aroA*, *B*, *C*, *D*, *E*) having multiple requirements, one (*pheA*) requiring phenylalanine, and one (*tyrA*) requiring tyrosine. Joint transductions show that the five *aro* loci are not linked, but *tyrA* and *pheA* loci are linked. The *aroD* and *tyrA* loci exhibit interallelic complementation. The nutritional behavior of the *aro* mutants is complex. Requirements vary among alleles of each locus, from quintuple (tyrosine + phenylalanine + tryptophan + *p*-aminobenzoic acid + *p*-hydroxybenzoic acid) to quadruple (tyrosine + phenylalanine + tryptophan + *p*-aminobenzoic acid) to triple (tyrosine + phenylalanine + tryptophan) to double (tyrosine + phenylalanine). Within the *aroA* locus, mutational sites of each phenotype (quadruple, triple or double requiring) tend to cluster. Quadruple requiring strains can mutate into triple or double requiring strains by secondary mutations.

LITERATURE CITED

- COTTON, R. G. H., and F. GIBSON, 1965 The biosynthesis of phenylalanine and tyrosine; enzymes converting chorismic acid into prephenic acid and their relationships to prephenate dehydratase and prephenate dehydrogenase. *Biochim. Biophys. Acta* **100**: 76-88.
- COX, G. B., and F. GIBSON, 1964 Biosynthesis of vitamin K and ubiquinone. Relation to the shikimic acid pathway in *Escherichia coli*. *Biochim. Biophys. Acta* **93**: 204-206.
- DAVIS, B. D., 1951 Aromatic biosynthesis. I. The role of shikimic acid. *J. Biol. Chem.* **191**: 315-325. — 1952a Aromatic biosynthesis. IV. Preferential conversion, in incompletely blocked mutants, of a common precursor of several metabolites. *J. Bacteriol.* **64**: 729-748. — 1952b Aromatic biosynthesis. V. Antagonism between shikimic acid and its precursor, 5-dehydroshikimic acid. *J. Bacteriol.* **64**: 749-763. — 1953 Autocatalytic growth of a mutant due to accumulation of an unstable phenylalanine precursor. *Science* **118**: 251-252.
- DAVIS, B. D., and E. S. MINGIOLI, 1953 Aromatic biosynthesis. VII. Accumulation of two derivatives of shikimic acid by bacterial mutants. *J. Bacteriol.* **66**: 129-136.
- DEMEREK, M., 1964 Clustering of functionally related genes in *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. U.S.* **51**: 1057-1060.
- DEMEREK, M., H. MOSER, R. C. CLOWES, E. L. LAHR, H. OZEKI, and W. VIELMERRER, 1956 Bacterial Genetics. Carnegie Inst. Wash. Year Book **55**: 301-315.
- GIBSON, F., 1964 Chorismic acid: Purification and some chemical and physical studies. *Biochem. J.* **90**: 256-261.
- GIBSON, M. I., and F. GIBSON, 1964 Preliminary studies on the isolation and metabolism of an intermediate in aromatic biosynthesis: chorismic acid. *Biochem. J.* **90**: 248-256.
- GIBSON, F., M. GIBSON, and G. B. COX, 1964 The biosynthesis of *p*-aminobenzoic acid from chorismic acid. *Biochim. Biophys. Acta* **82**: 637-638.

- GILES, N. H., M. E. CASE, and C. W. H. PARTRIDGE, 1965 Evidence for an aromatic operon in *Neurospora crassa*. (Abstr.) *Genetics* **52**: 444.
- GOLLUB, E., H. ZALKIN, and D. B. SPRINSON, 1966 Enzymes associated with unlinked genes of the aromatic pathway in *Salmonella*. *Fed. Proc.* **25**: 337.
- KATAGIRI, M., and R. SATO, 1953 Accumulation of phenylalanine by a phenylalanineless mutant of *Escherichia coli*. *Science* **118**: 250-251.
- PITTARD, J., and B. J. WALLACE, 1966 Distribution and function of genes concerned with aromatic biosynthesis in *Escherichia coli*. *J. Bacteriol.* **91**: 1494-1508.
- SANDERSON, K. E., and M. DEMEREC, 1965 The linkage map of *Salmonella typhimurium*. *Genetics* **51**: 897-913.
- SMITH, L. C., J. M. RAVEL, S. R. LAX, and W. SHIVE, 1962 The control of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthesis by phenylalanine and tyrosine. *J. Biol. Chem.* **237**: 3566-3570.
- UMBARGER, H. E., and B. D. DAVIS, 1962 Pathways of amino acid biosynthesis. pp. 167-252. *The Bacteria*, Vol. 3. Edited by I. C. GUNSALES and R. Y. STANIER, Academic Press, New York.
- YURA, T., 1956 Suppressor mutations in purine-requiring mutants of *Salmonella typhimurium*. *Carnegie Inst. Wash. Publ.* **612**: 77-86.