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Isolation and Characterization of an *Escherichia coli* K-12 Mutant Defective in Tyrosine- and Phenylalanine-Specific Transport Systems

MARGARET J. WHIPP, DOROTHY M. HALSALL,† AND A. J. PITTARD*

Department of Microbiology, University of Melbourne, Parkville, Victoria, 3052, Australia

A mutant strain of *Escherichia coli* K-12 that is defective in both the tryosine-specific and phenylalanine-specific transport systems was isolated. The defects in these systems were shown to be due to mutations in two distinct loci, *tyrP* and *pheP*, respectively.

Studies of Brown (4) have indicated that the aromatic amino acids are transported into *Escherichia coli* K-12 by a number of transport systems. The common aromatic amino acid transport system transports all of the three aromatic amino acids, and three additional systems each transport a single aromatic amino acid, either tyrosine, phenylalanine, or tryptophan. In addition to these four systems there is also an inducible system for transporting tryptophan (3, 6). The existence of the common transport system and tryptophan-specific transport system has been confirmed by the isolation of mutants defective in each of these systems (4, 11, 13). In this paper we describe a selection designed to isolate mutants defective in the tyrosine-specific transport system. The characterization of a mutant strain isolated by this method is presented.

MATERIALS AND METHODS

Organisms. Strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1.

Chemicals. The chemicals used were obtained commercially and were not further purified. L-[*U*-^{14}C]tyrosine (400 and 430 mCi/mmol) and L-[*U*-^{14}C]phenylalanine (374 and 436 mCi/mmol) were purchased from Commissariat à l'Energie Atomique, France; L-[*methylene*-^{14}C]tryptophan (51.8 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, England. The isotopes were diluted appropriately with nonradioactive amino acids for use.

Growth medium. The minimal medium used was half-strength medium 56 described by Monod et al. (12), supplemented with glucose, thiamine, and required growth factors. For growth of cells for the preparation of cell extracts, medium 56 was used with 0.5% glucose.

Buffers. Sodium phosphate buffers used were prepared by the method of Dawson and Elliott (8).

Mutagenesis. Cells were treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg et al. (1).

Growth of cells for transport assays. Cultures were grown in a rotary shaker at 37°C, using as an inoculum an unwashed 16-h culture in the same medium. The cells were grown for at least two mass doublings. Turbidity was monitored by using a Klett-Summerson photoelectric colorimeter with a no. 54 filter (0.21 mg [dry weight] of cells per ml = 100 Klett units). Cells were harvested in the mid-exponential phase of growth by centrifugation, washed twice in an equivalent volume of half-strength 56 buffer, and resuspended in this buffer supplemented with glucose (0.2%), chloramphenicol (80 μg/ml), and specific growth requirements. The cells were incubated at 37°C for 10 min and then stored at 4°C until used.

Transport assay. The transport assay used was described previously (17).

Growth of cells and preparation of cell extracts for enzyme assay. The method used for growing cells and preparing cell extracts for assay was that described by Camakaris and Pittard (7).

Assay of DAHP synthetase. To assay 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthetase, we used the method of Doy and Brown (9) with the modification of Camakaris and Pittard (7).

Transduction. The method used for P1 transductions was that used by Camakaris and Pittard (7).

Conjugation. Cultures of male and female strains were grown to early exponential phase in Luria broth, then mixed in a ratio of one male to nine female cells (usually the total volume of mating mixture was 10 ml). After the mixture was swirled gently for several minutes to allow for the formation of mating pairs, the culture was incubated without agitation at 37°C. For the transfer of an F', incubation was usually for 30 min; for uninterrupted matings, incubation was for 2 h. A sample was then removed and blended vigorously in a Vortex mixer to disrupt mating pairs. Samples of dilutions were then plated to selective medium. Samples of both parents were also plated to check for reversion.

Cross-feeding. The method used to detect cross-feeding between strains was based on the general method of Gibson and Jones (10).

RESULTS

Isolation of a strain defective in the tyrosine-specific transport system. The first step of the aromatic amino acid biosynthetic pathway is carried out by three DAHP synthe-

† Present address: C.S.I.R.O., Plant Industry, Canberra, 2601, A.C.T., Australia.

TABLE 1. *E. coli K-12 strains used in this work*

Strain no.	Relevant genotype[a]	Source or reference
KB1366	*aroG aroH aroP argE his proA* (P1*[b])	K. D. Brown
JP1099	*aroG aroH aroP tyrR391 tyrP474 pheP367 argE his proA* (P1*)	By mutation; tyrosine-resistant derivative of KB1366
JP1100	*aroG aroH aroP tyrP474 pheP367 argE his proA* (P1*)	By transduction with P1*kc*; *tyrR*$^+$ derivative of JP1099
JP2648	*aroG aroH aroP tyrP474 pheP367 argE his proA rpoB359* (P1*)	By mutation; rifamycin-resistant derivative of JP1100
JP2636	*aroG aroH aroP tyrP474 pheP367 argE his proA nalA376* (P1*)	By mutation; nalidixic acid-resistant derivative of JP1100
JP2369	*aroG aroH aroP tyrP474 pheP367* (P1*)	By transductions (P1*kc*); *his*$^+$ *arg*$^+$ *pro*$^+$ derivative of JP1100
JP2392	F16 *aroG aroH aroP tyrP474 pheP367* (P1*)	By conjugation; F16 derivative of JP2369
JP2547	F142 *aroG aroH aroP tyrP474 pheP367* (P1*)	By conjugation; F142 derivative of JP2369
JP2424	F123 *aroG aroH aroP tyrP474 pheP367* (P1*)	By conjugation; F123 derivative of JP2369
JP2365	*aroG aroH aroP argE his proA rpoB352* (P1*)	By mutation; rifamycin-resistant derivative of KB1366
JP2403	*aroG aroH aroP tyrP474 pheP367 argE rpoB352* (P1*)	By conjugation; *proA*$^+$ *pheP tyrP* derivative of JP2365
JP2404	*aroG aroH aroP pheP367 argE his rpoB352* (P1*)	By conjugation; *proA*$^+$ *pheP* derivative of JP2365
JP2405	*aroG aroH aroP tyrP474 argE proA rpoB352* (P1*)	By conjugation; *his*$^+$ *tyrP* derivative of JP2365
JP2684	*aroG aroH aroP his* (P1*)	By transductions; *argE*$^+$ *proA*$^+$ derivative of KB1366
KL98	Hfr[c] (see Fig. 2) *aroH*$^+$ *aroP*$^+$ *tyrP*$^+$ *his*$^+$	B. J. Bachmann
PK191	Hfr (see Fig. 2) *aroP*$^+$ *tyrP*$^+$ *his*$^+$	B. J. Bachmann
AT2471	*tyrA4*	A. L. Taylor
AT2022	*pheA1*	A. L. Taylor
JP2310	*his-29 tyrR370*	By transduction (P1*kc*); *trp*$^+$ *tyrR370* derivative of JP2140 (7)
AB2332	HfrC (see Fig. 2)	E. A. Adelberg

[a] Symbols are as described by Bachmann et al. (2).

[b] Strains designated as P1* are presumed to be P1 defective lysogens. They do not spontaneously release P1, are resistant to P1 infection, and have the P1 restriction-modification system.

[c] When crosses involved P1 defective lysogens as recipients, Hfr strains were first lysogenized with P1*kc*.

tase isoenzymes. Each of these enzymes is feedback inhibited, and its synthesis is repressed by one of the end products of the pathway, either tyrosine, phenylalanine, or tryptophan (15). The wild-type strain, which possesses the three isoenzymes, grows readily in the presence of tyrosine. However, strain KB1366, in which only the DAHP synthetase (Tyr) isoenzyme is functional, is unable to grow in the presence of tyrosine because feedback inhibition and repression of this enzyme result in starvation for phenylalanine, tryptophan, and aromatic vitamins. In addition, KB1366 is defective in the common aromatic amino acid transport system due to a mutation in the gene *aroP*. One mechanism by which resistance to growth inhibition by tyrosine could arise in this strain would involve the loss of a functional tyrosine-specific transport system.

With this in mind, cells of strain KB1366 were treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and clones were selected which were able to grow in the presence of 5×10^{-4} M tyrosine. Any mutants that were able to grow because of reversion at either the *aroG* or *aroH* loci to give functional DAHP synthetase (Phe) or functional DAHP synthetase (Trp) were identified by growth inhibition tests and discarded (see reference 16). The remaining strains were not further examined for any mutations affecting either the repression or feedback inhibition of the DAHP synthetase (Tyr) enzyme but were assayed directly for tyrosine uptake. One strain, JP1099, was identified which was defective in tyrosine-specific uptake as determined by its inability to transport [^{14}C]tyrosine. A more detailed examination of this strain showed that it also carried a mutation in the *tyrR* locus which resulted in the synthesis of DAHP synthetase (Tyr) being no longer subject to repression (data not shown). A *tyrR*$^+$ derivative of this strain, JP1100, was obtained by in-

troducing a wild-type *tyrR* allele by P1 transduction. Strain JP1100 was still defective in tyrosine-specific uptake (Fig. 1), but the synthesis of DAHP synthetase (Tyr) in this strain showed normal repression by the aromatic end products (see Table 2). It could also be seen that when JP1100 was grown in the absence of aromatic end products, this strain had higher levels of DAHP synthetase (Tyr) than did the parent strain KB1366. This will be discussed later in this paper. The growth responses of the mutant strains JP1099 and JP1100 and parent strain KB 1366 in the presence of tyrosine and the tyrosine analogs 4-amino-phenylalanine (APA) and 3-fluorotyrosine (3FT) are given in Table 3.

Genetic analysis of strain JP1100. A difficulty in determining the map location of the mutant allele responsible for the defect in tyrosine-specific transport was that the available screening for this allele, namely, growth resistance to tyrosine and tyrosine analogs, is only manifested in a strain which relies entirely on DAHP synthetase (Tyr) to carry out the first reaction of aromatic biosynthesis. In addition, this strain must lack a functional common aromatic transport system. As previously mentioned, introduction of the *aroG*$^+$ or *aroH*$^+$ alleles automatically confers resistance to growth inhibition by tyrosine. For this reason initial mapping was carried out using F′ factors to mobilize chromosome from the tyrosine-specific transport mutant back into the parent strain (both strains possessing identical mutations in *aroG*, *aroH*, and *aroP*).

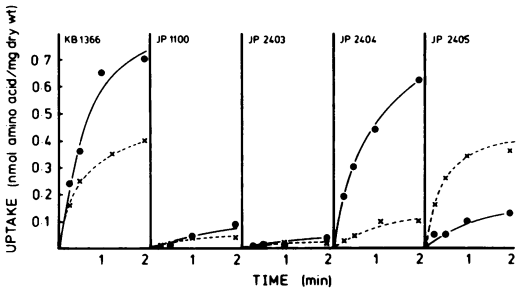

FIG. 1. *Tyrosine and phenylalanine uptake of strains KB1366 and JP1100 and recombinants JP2403, JP2404, and JP2405 from the mating JP2392 × JP2365. These strains all carry the aroP allele, and hence the uptake in these strains is due to the relevant specific transport systems. The cells used in these assays were grown to mid-exponential growth phase in minimal medium. Symbols: Uptake of tyrosine (●); uptake of phenylalanine (×).*

TABLE 2. *Specific activity of DAHP synthetase (Tyr) in KB1366, JP1099, and JP1100*

Strain	Sp act of DAHP synthetase (Tyr)[a]	
	MM	MMA
KB1366	0.118	0.005
JP1099	0.464	0.327
JP1100	0.313	0.009

[a] In each case DAHP synthetase (Tyr) activity was inhibited 96 to 98% by 0.5 mM tyrosine. MM, Cells grown in minimal medium. MMA, Cells grown in minimal medium supplemented with the aromatic end products.

TABLE 3. *Growth resistance of KB1366, JP1099, and JP1100 to tyrosine and tyrosine analogs*[a]

Addition to minimal medium	Growth response		
	KB1366	JP1099	JP1100
Tyrosine			
1×10^{-6} M	+++	+++	+++
5×10^{-6} M	−	+++	+++
5×10^{-4} M	−	+++	+
1×10^{-3} M	−	−	−
APA, 1×10^{-4} M	−	+++	+++
3FT, 1×10^{-4} M	−	+++	+++

[a] Growth response is signified as follows: +++, strong growth; +, slight growth; −, no growth. Growth on solid media was assessed after 24 h of incubation at 37°C.

The existence of two mutations contributing to tyrosine sensitivity. The F-merogenote F16 (14) mobilizes chromosome in a counterclockwise direction with a point of origin in the *ilv* region (see Fig. 2). Strain JP2392, which is an F16 derivative of JP1100, was mated with strain JP2365 (a rifamycin-resistant derivative of strain KB1366). After 2 h at 37°C, samples were plated for the selection of His$^+$ or Pro$^+$ recombinants. These recombinants were purified and then examined for their ability to grow in the presence of APA, 3FT, and tyrosine. Strain JP2392 grew on each of these media, whereas strain JP2365 grew on none of them. To our surprise, approximately half of the His$^+$ recombinants exhibited a new phenotype, different from that of either parent. These recombinants, referred to as class 2 recombinants, could grow on APA but not on tyrosine or 3FT. Similarly, class 2 recombinants were found among the Pro$^+$ recombinants, where in addition there were also some recombinants (class 1) which exhibited the growth response of the donor strain JP2392 (Table 4). The simplest explanation for this finding was that strain JP2392 and almost certainly its parent strain JP1100 possessed two separate mutations, both of which would seem to contribute to the tyrosine resistance of these strains.

Studies on inhibition of the specific aromatic transport systems by Brown (4) had shown that of 22 amino acids tested, only phenylalanine

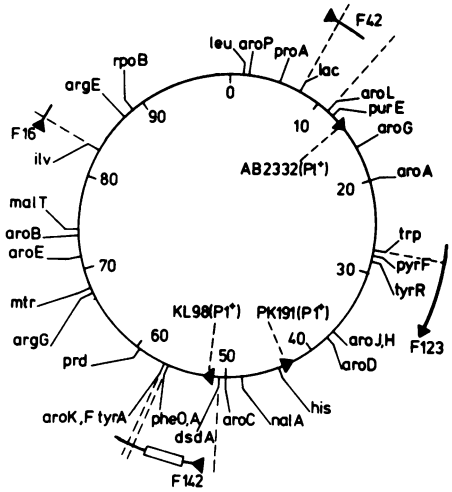

FIG. 2. *Genetic linkage map of chromosome of E. coli K-12. Gene symbols are as specified by Bachmann et al. (2). The approximate location of the Hfr points of origin and the approximate chromosomal regions carried by F' factors used in these studies are shown. The F' factors are represented by an arc which has an arrowhead drawn to represent the point of origin of the ancestral Hfr strain.*

TABLE 4. *Growth responses of parent and recombinant strains obtained in the mating JP2392 × JP2365*

Addition to minimal medium (concn)	Response of strain[a]:			
	Parent		Recombinant	
	JP2392 Donor	JP2365 Recipient	JP2403 Class 1	JP2405 Class 2
APA (10^{-4} M)	+++	−	+++	+++
APA (5×10^{-4} M)	+++	−	+++	+++
Tyrosine (10^{-4} M)	+++	−	+++	−
3FT (10^{-4} M)	+++	−	+++	−

[a] +++, Good growth; +, slight growth; −, no growth. Growth on solid media was assessed after 24 h of incubation at 37°C.

showed any significant ability to inhibit tyrosine uptake by the tyrosine-specific system, and, similarly, only tyrosine showed any significant ability to inhibit phenylalanine uptake via its specific system. It seemed possible, therefore, that strain JP1100 may have defects in both the phenylalanine-specific and tyrosine-specific transport systems. To test this proposition, the uptake of radioactively labeled phenylalanine and tyrosine was assayed in KB1366, in JP1100, and in recombinants from the mating of JP2392 and JP2365. Representative results are shown in Fig. 1. It can be seen that strain JP1100 is defective in both phenylalanine- and tyrosine-specific uptake, whereas KB1366 is defective in neither. Strain JP2392 gave results identical to JP1100, and JP2365 gave the same response as strain KB1366 (data not shown). The recombinants that were resistant to APA, 3FT, and tyrosine, such as strain JP2403, were also defective in both phenylalanine- and tyrosine-specific transport, whereas the recombinants that were only resistant to APA, such as JP2404 or JP2405, were defective in either phenylalanine-specific transport or tyrosine-specific transport (see Fig. 1). The genes in which the putative mutations affecting each of these systems have occurred have been termed *tyrP* and *pheP*, respectively.

The map location of *tyrP*. In the mating involving the F16 donor it was observed that His^+ recombinants were of two types only, namely, $tyrP^+$ $pheP^+$ (able to transport both phenylalanine and tyrosine) like the recipient, or *tyrP474* $pheP^+$ with a specific defect in the tyrosine transport system. Strains exhibiting tyrosine resistance and presumably being both *pheP* and *tyrP* were only found among the Pro^+ recombinants. To more closely identify the chromosomal location of *tyrP* and *pheP*, derivatives of JP1100 were made that were prototrophic for markers such as *arg* and *pro* and which carried either the F-merogenote F142 or F123 (see Fig. 2 for description of these F-merogenotes). The recipient JP2404 that was used in each case was $tyrP^+$ *pheP367*, and recombinants that were obtained were examined for their ability to resist growth inhibition by tyrosine. When the F' donor carried F142, selection was made for His^+, and when it carried F123, Arg^+ was the selected phenotype. In the cross involving F142, the *tyrP* donor allele (*tyrP474*) was coinherited with his^+ at a frequency of 70%. On the other hand, in the mating with F123 in which the arg^+ allele was selected, no transfer of the *tyrP* locus was detected. These results indicate that the *tyrP* locus lies between the point of origin of F142 and that of F123. In a further attempt to localize the *tyrP* locus more precisely, crosses were carried out in which the $tyrP^+$ allele was transferred by Hfr strains into a *tyrP474 pheP367* recipient (JP2648). The points of origin of both Hfr strains PK191 and KL98 are shown in Fig. 2. In the cross PK191 × JP2648, His^+ recombinants were selected after 30 and 40 min of mating. When examined for tyrosine sensitivity, 40 of 40 were shown to retain their tyrosine resistance, indicating that $tyrP^+$ did not lie between the origin of PK191 and *his*. It also indicates that $tyrP^+$ is not transferred as an early marker by PK191. In the cross involving KL98, 16 of 80 His^+ recombinants were now tyrosine sensitive. Five of these were assayed for common aromatic- and

tyrosine-specific transport and, as expected, were shown to have regained the tyrosine-specific but not the phenylalanine-specific common transport system. Because KL98 also transfers *aroH*$^+$ as an early marker and all *aroH*$^+$ strains will automatically be tyrosine resistant, the actual coinheritance of *tyrP* and *his* is probably greater than 16 of 80. On the basis of these results, therefore, one can say that *tyrP* lies between the points of origin of the Hfr strain PK191 and the point of chromosome mobilization of F' F123. Recent results (Kasian and Pittard, unpublished data) show that *tyrP* is cotransducible at a high frequency with the *uvrC* locus.

The map location *pheP*. In the cross in which JP2392 was the donor, none of the His$^+$ clones selected at early times had received the *pheP367* allele. In the same experiment, however, some Pro$^+$ clones selected after a longer period of mating were shown to have inherited *pheP367*. This result suggested that the gene *pheP* would be found somewhere in the chromosomal region between *his* and *proA* or a point beyond *proA*. In a second experiment in which the donor was an F' strain carrying the F-genote F42 (see Fig. 2), neither *proA*$^+$ nor *argE*$^+$ recombinants had inherited *pheP* (data not shown). This indicated that *pheP* must be in the region between *his* and the origin of F42. In another experiment, F123 was introduced into strain JP2684, which is an *arg*$^+$ *pro*$^+$ derivative of strain KB1366 and which still retains the *aroP* mutant allele. This donor was mated with strain JP2636 (a nalidixic acid-resistant derivative of JP1100), and the mating was interrupted at 10-min intervals, selection being made for *Pro*$^+$ recombinants. These were obtained in samples which had been interrupted at 50, 60, and 70 min and, after purification, were tested for the loss of resistance to growth inhibition by tyrosine. With this particular cross, the introduction of *pheP*$^+$ allele from the donor strain was expected to render the recipient tyrosine sensitive. A fraction (3 of 10) of the earliest Pro$^+$ recombinants that were obtained in the 50-min sample was found to be tyrosine sensitive. Pro$^+$ recombinants obtained at 60 and 70 min also contained tyrosine-sensitive recombinants (8 of 30 and 20 of 40, respectively). Twenty recombinants were taken from these 50-, 60-, and 70-min samples and tested for their ability to transport phenylalanine. In every case, the phenotype of tyrosine sensitivity (inhibition of growth by 10^{-4} M tyrosine) was found, as expected from earlier studies, to be associated with a fully functional phenylalanine-specific transport system (data not shown). In another experiment it was shown that the Hfr donor AB2332 did not transfer *pheP*$^+$ as an early marker (data not shown).

From these results it can be concluded that the gene *pheP* is located in the region between the point of chromosome mobilization of F123 and the point of origin of Hfr AB2332. Because of the difficulties encountered in direct selection for PheP$^+$ or PheP phenotypes, we have not yet succeeded in locating the *pheP* gene any more precisely.

Cross-feeding of tyrosine and phenylalanine and auxotrophs by transport mutants. As indicated earlier in this paper, after growth in minimal medium, JP1100 (*aroP tyrP pheP*) had higher levels of the enzyme DAHP synthetase (Tyr) than the parent strain, KB1366 (*aroP*) (Table 1). If this was due to the mutant *tyrP* and *pheP* alleles, it could indicate that cells of JP1100 were unable to maintain tyrosine pools at the same level as cells of KB1366. This possibility was investigated by testing the ability of the transport mutants to cross-feed auxotrophs requiring either tyrosine or phenylalanine. The results in Table 5 show that the tyrosine auxotroph is cross-fed by JP1100 (*aroP tyrP pheP*) and, to an extent, by JP2405 (*aroP tyrP*). Similarly, the phenylalanine auxotroph is cross-fed by JP1100 and to a lesser extent by JP2404 (*aroP pheP*). KB1366 (*aroP*) did not visibly cross-feed any of the auxotrophs. Brown (4) also could not detect excretion of any of the aromatic amino acids with an *aroP* strain which was wild type for the three DAHP synthetase isoenzymes. No valid comparison can be made between the cross-feeding responses of the two auxotrophs because differences may be partly due to strain background differences and differences in ability to respond to each aromatic amino acid. The cross-feeding by the transport mutant strains is very specific and corresponds with the loss of the relevant specific transport systems.

TABLE 5. *Cross-feeding of various aromatic auxotrophs by transport mutant strains*

Strain tested for excretion	Growth response[a] of indicator strain:	
	Tyrosine auxotroph (AT2471)	Phenylalanine auxotroph (AT2022)
KB1366 (*aroP*)	−	−
JP1100 (*aroP tyrP pheP*)	++	++
JP2404 (*aroP pheP*)	−	+
JP2405 (*aroP tyrP*)	+	−

[a] Growth response of the auxotrophs is signified as follows: ++, strong; +, slight; −, none. Test was carried out as described in the text.

DISCUSSION

The mapping data for the *tyrP* and *pheP* loci show that these loci are widely separated from each other on the *E. coli* chromosome and that they are distinct from the *aroP* locus, which is involved in the common aromatic transport system (4), and from the *mtr* locus, which is involved in the tryptophan-specific transport system (Yanofsky, as cited in reference 13).

Our data indicate that the *tyrP474* and *pheP367* alleles result in a defective tyrosine-specific transport system and a defective phenylalanine-specific transport system, respectively. However, since we have shown that these alleles are also associated with excretion of tyrosine and phenylalanine, it is opportune to point out a number of factors which indicate that the reduced transport of tyrosine and phenylalanine seen by us in our mutants is not in fact due to a dilution of labeled substrate by excreted amino acid.

(i) In a strain already blocked by mutation in the common transport system, introduction of the *tyrP474* allele results only in tyrosine excretion; similarly, introduction of the *pheP367* allele results only in phenylalanine excretion. Mutations in loci which theoretically might be expected to give rise to such an excretion pattern (mutations in the operator loci of the *aroF tyrA* operon and the *pheA* gene, or mutation to feedback resistance of the enzymes coded for by *tyrA* and *pheA*) do not map near the *tyrP* or *pheP* loci.

(ii) If the *tyrP474* and *pheP367* alleles simply result in overproduction of tyrosine and phenylalanine, then in a strain carrying either of these mutant alleles and the wild-type *aroP* allele, tyrosine or phenylalanine transport by the common transport system would be expected to be correspondingly reduced. This is not the case (data not shown).

(iii) A strain, JP2310, which is derepressed for both phenylalanine- and tyrosine-controlled DAHP synthetases and the enzymes of the tyrosine terminal pathway, is found to give qualitatively the same amount of tyrosine cross-feeding as strain JP1100, suggesting excretion of a similar amount of tyrosine (data not shown). However, tyrosine- and phenylalanine-specific uptake are unimpaired in strain JP2310 (17).

(iv) Growth of cells in media supplemented with phenylalanine results in a significant increase in tyrosine-specific uptake. This increase in uptake does not occur in a strain carrying the *tyrP474* allele (17).

The raised levels of DAHP synthetase (Tyr) in minimally grown JP1100 compared with levels in the parent strain, KB1366 (Table 2), together with cross-feeding results (Table 5), are consistent with the hypothesis that the strains carrying the *tyrP474* and *pheP367* alleles are not capable of retaining high internal tyrosine and phenylalanine pools, respectively. One explanation for this is that the mutation in the *aroP* locus or the mutations in the *tyrP* and *pheP* loci, or all three, have resulted in the respective membrane carriers being energy uncoupled. In such a case the carrier protein would be postulated to be intact or partially intact such that rapid equilibration of the substrate could be effected between inside and outside the cell, but the cell would not be capable of significant accumulation of substrate. Such mutants have been isolated in the *lac* transport system of *E. coli* ML and K-12 strains (18, 19).

The original mutant strain, JP1099, isolated from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine–mutangenized cells of strain KB1366 on the basis of growth resistance to tyrosine (5×10^{-4} M), carried mutations in both the tyrosine-specific and phenylalanine-specific transport systems and also in the *tyrR* locus. All three mutations appear to have been necessary to give growth resistance to the high level of tyrosine used in the original selection. From the data obtained in this study using mutants possessing only the *tyrP474* or *pheP367* mutation, it should now be possible to devise a more precise selection for strains that are defective in either tyrosine- or phenylalanine-specific transport systems. The isolation of additional mutants will help to elucidate the number of components involved in each system.

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