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The Stable Phylogenetic Distribution of the Recently Evolved L-Phenylalanine-inhibited Isozyme of 3-Deoxy-D-*Arabino*-Heptulosonate 7-Phosphate Synthase in Enteric Bacteria[1]

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Abstract. *Escherichia coli* and some other enteric bacteria possess three regulatory isozymes of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase, each of which is inhibited by one aromatic amino acid. The L-phenylalanine-sensitive isozyme of DAHP synthase has evolved most recently since it is absent in all other members of the Gram-negative cluster that contains enteric bacteria as a subcluster. A comprehensive survey of enteric genera was carried out to determine whether the newly evolved isozyme is a stable, conserved trait. The results obtained show that all the genera of the contemporary *Enterobacteriaceae* family possess the recently evolved phenylalanine-sensitive isozyme in addition to the tyrosine- and tryptophan-sensitive isozymes of DAHP synthase. However, physiological manipulation was usually necessary to derepress the tryptophan-sensitive DAHP synthase in order to demonstrate its presence.

The biosynthetic pathway for aromatic amino acids is complex and varies widely in nature [7]. Distinctive features include alternative biochemical steps, presence of distinct isozymes, different patterns of allosteric control, varied specificity of dehydrogenase enzymes for co-factor, and presence or absence of multifunctional proteins [11]. The enzymological diversity of character states within the aromatic pathway has been successfully exploited to deduce the evolutionary history of the key enzymes of the biosynthetic sequence among clusters of closely related microorganisms within phylogenies defined by 16S rRNA homology [1, 4, 11].

The Gram-negative assemblage of prokaryotes designated as Superfamily B includes enteric bacteria, an *Oceanospirillum* cluster, Group I pseudomonads (e.g., *Pseudomonas aeruginosa*), Group V pseudomonads (e.g., *Xanthomonas campestris*), and *Acinetobacter* species. The evolutionary events that generated the three regulatory isozymes of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase (E.C. 4.1.2.15) [phenylalanine-sensitive DAHP synthase (DS-phe), tyrosine-sensitive DAHP synthase (DS-tyr), and tryptophan-sensitive DAHP synthase (DS-trp)] in *Escherichia coli* have been proposed recently [2, 4]. The L-phenylalanine-sensitive isozyme of DAHP synthase (DS-phe) is the most recently evolved isozyme in Superfamily-B organisms. Since DS-phe is absent throughout Superfamily B except within the enteric lineage (defined as the traditional enteric bacteria and the *Aeromonas* and *Alteromonas* genera) [4], it has been proposed that DS-phe evolved prior to the divergence of *Alteromonas* (branches at the deepest level within the enteric lineage) from the enteric bacteria and after divergence of the *Oceanospirillum* sublineage [5] (the next closest cluster to enteric bacteria).

The enteric lineage, defined by species that diverged at a phylogenetic depth (S_{AB} = 0.58) [17], has been evolutionarily dynamic for aromatic biosynthesis [2]. The enteric lineage gained two new character states after its divergence from the *Oceanospirillum* lineage, namely, the bifunctional T-protein of tyrosine biosynthesis (chorismate mutase-T:cyclohexadienyl dehydrogenase) and DS-

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phe. Several other character states of aromatic biosynthesis, namely, the presence of a monofunctional chorismate mutase (CM-F, F for free of association with other enzyme activities) and broad-specificity cyclohexadienyl dehydratase (CDT), vary considerably within the enteric bacteria [2]. All the genera making up the enteric bacteria were screened for the presence of DS-phe isozyme to determine whether this newly acquired isozyme is uniformly distributed throughout the enteric bacteria or whether any genera have lost this character state.

Materials and Methods

Organisms and growth conditions. *Shigella dysenteriae* ATCC 11456a, *Salmonella enteritidis* ATCC 13076, *Klebsiella pneumoniae* ATCC 13883, *Citrobacter fruendii* ATCC 29935, *Enterobacter cloacae* ATCC 13047, *Enterobacter aerogenes* ATCC 13048, *Enterobacter agglomerans* ATCC 29915, *Serratia rubidaea* ATCC 27614, *Kluyvera ascorbata* ATCC 33433, *Cedecea divisae* ATCC 33431, *Morganella morganii* ATCC 25830, *Edwardsiella tarda* ATCC 15947, *Yersinia enterocolitica* ATCC 9610, *Hafnia alvei* ATCC 13337, *Providencia alcalifaciens* ATCC 9886, and *Proteus vulgaris* ATCC 29905 were obtained from American Type Culture Collection (Rockville, Maryland) *Salmonella enteritidis* (37°C), *K. pneumoniae* (37°C), *C. fruendii* (37°C), *E. cloacae* (30°C), *E. aerogenes* (30°C), *E. agglomerans* (26°C), and *S. rubidaea* (26°C) were grown on M9 medium, as described by Winkler and Stuckman [16]. *Kluyvera ascorbata* (37°C), *C. devisae* (26°C), *Y. enterocolitica* (30°C), *P. vulgaris* (37°C), *H. alvei* (30°C), and *P. alcalifaciens* (37°C) were grown on M9 medium containing 1 mg/l each of nicotinamide, *p*-aminobenzoate, D-biotin, calcium pantothenate, and thiamine. *Shigella dysenteria* (37°C), *M. morganii* (37°C), and *E. tarda* (37°C) were grown on M9 medium containing the above-mentioned vitamins and supplemented with 0.1% (wt/vol) acid-hydrolyzed casein (Difco). Where indicated, the minimal medium was supplemented with 0.5% (wt/vol) acid-hydrolyzed casein to derepress DS-trp. The organisms were grown to the late exponential phase of growth, harvested by centrifugation, washed once with 50 m*M* K-phosphate buffer (pH 7.0) containing 1 m*M* dithiotreitol (DTT) (buffer A), and stored at −80°C until used.

Preparation of cell extracts and enzyme assay. Cell pellets were suspended in buffer A, disrupted by sonication, and centrifuged at 150,000 *g* for 1 h. The resulting supernatant was passed through a Sephadex G-25 column (1.5 × 20.0 cm) equilibrated in 10 m*M* K phosphate (pH 7.0) containing 1 m*M* DTT (buffer B). The protein-containing fractions were pooled and are termed crude extract.

DAHP synthase activity was assayed by the method of Srinivasan and Sprinson [14] as modified by Jensen and Nester [13]. The reaction mixture in a final volume of 0.2 ml contained buffer A, a suitable amount of enzyme, 1 m*M* cobaltous chloride (which gave maximal activity among the metal ions tested), 1 m*M* sodium D-erythrose 4-phosphate (E4P), and 1 m*M* trisodium phosphoenolpyruvate (PEP). Incubation at 37°C was carried out for 20 min, and the reaction was terminated by the addition of 0.05 ml of 20% (wt/vol) trichloroacetic acid. After removal of precipitated proteins by centrifugation, DAHP was estimated in the supernatant. The absorbance was read in a thermoregulated cuvette at 55°C in a spectrophotometer at 549 nm. Protein in the crude extracts was assayed by the method of Bradford [6], with bovine serum albumin as the standard reference protein.

DE52 column chromatography. Approximately 100 mg of crude extract protein was applied to a DEAE–cellulose (DE52) column (1.5 × 20.0 cm) equilibrated in buffer B. The column was washed with two bed volumes of the equilibration buffer, and then the bound proteins were eluted with 300 ml of a linear gradient of KCl (0.0–0.35 *M*) contained in the equilibration buffer. Fractions of 2.2 ml were collected and assayed for absorbance at 280 nm and for DAHP synthase activity. Fractions containing enzyme activity were tested for sensitivity to potential inhibitor compounds, as indicated.

Biochemicals. Amino acids, E4P, PEP, DTT, bovine serum albumin and Sephadex G-25 were obtained from Sigma Chemical Company (St. Louis, Missouri). DE52 was purchased from Whatman, Inc. (Clifton, New Jersey). Prephenate was prepared as the barium salt from culture supernatants of a tyrosine auxotroph of *S. typhimurium* [8] and was converted to the potassium salt before use. Chorismate was isolated from the accumulation medium of *K. pneumoniae* 62-1 and purified as the free acid [9]. All chemicals were of reagent grade.

Results and Discussion

DAHP synthase isozymes in enteric bacteria. Two peaks of DAHP synthase were eluted following DE52 column chromatography of crude extracts from a *Citrobacter fruendii* culture (Fig. 1a). The major leading peak fractions (DS-phe) were found to be sensitive to inhibition by L-phenylalanine (Table 1), while the minor trailing peak (DS-tyr) was sensitive to L-tyrosine (Table 2). The latter peak fractions were also sensitive to inhibition by L-phenylalanine owing to cross-contamination by DS-phe, and, as expected, a combination of aromatic amino acids (Aro) produced more inhibition than did either alone (Table 2). Similar results were obtained from *Shigella dysenteriae* (data not shown).

Two peaks of DAHP synthase activity were also eluted following chromatography of crude extracts prepared from *Salmonella enteritidis* (Fig. 1b) and *Klebsiella pneumoniae* (Fig. 1c). These proved to be DS-phe (Table 1) and DS-tyr (Table 2). DS-tyr was the major isozyme in *S. enteritidis*, while DS-phe and DS-tyr were expressed in a relatively balanced ratio in *K. pneumoniae*.

Two peaks of DAHP synthase activity also eluted following DE52 column chromatography of crude extracts prepared from cultures of *Enterobacter aerogenes* (Fig. 2a) and *E. cloacae* (Fig. 2b). Again these proved to be DS-phe (Table 1) and DS-tyr (Table 2). In each case, DS-phe eluted as a minor leading peak of activity while DS-tyr was the

Table 1. Inhibition of DAHP synthase-phe isozymes in the presence of aromatic amino acids

Bacterial species	Fraction number[a]	% Inhibition[b] by				% Inhibition[c] by			
		Phe	Tyr	Trp	Aro	Phe	Tyr	Trp	Aro
Citrobacter fruendii	126	70	0	0	67	92	0	1	91
Salmonella enteritidis	138	56	6	3	55	82	3	1	81
Klebsiella pneumoniae	133	76	5	0	70	91	0	0	89
Enterobacter aerogenes	119	67	0	2	66	80	0	1	80
Enterobacter cloacae	111	58	0	0	54	81	0	2	80
Hafnia alvei	116	22	1	0	16	56	4	0	50
Proteus vulgaris	125	27	0	2	17	75	0	0	74
Edwardsiella tarda	95	40	0	0	29	62	0	2	53
Kluyvera ascorbata	116	60	0	0	63	81	3	0	78
Yersinia enterocolitica	145	72	0	0	69	86	4	1	88

[a] Refers to column profiles shown in Figs. 1 (a,b,c), 2 (a,b), 3 (a,b,c), and 4 (a,b).
[b] The final concentration of L-phenylalanine (Phe), *L*-tyrosine (Tyr), *L*-tryptophan (Trp), or the combination of all three (Aro) was 0.1 m*M*.
[c] The final concentration of Phe, Tyr, Trp or Aro was 0.5 m*M*.

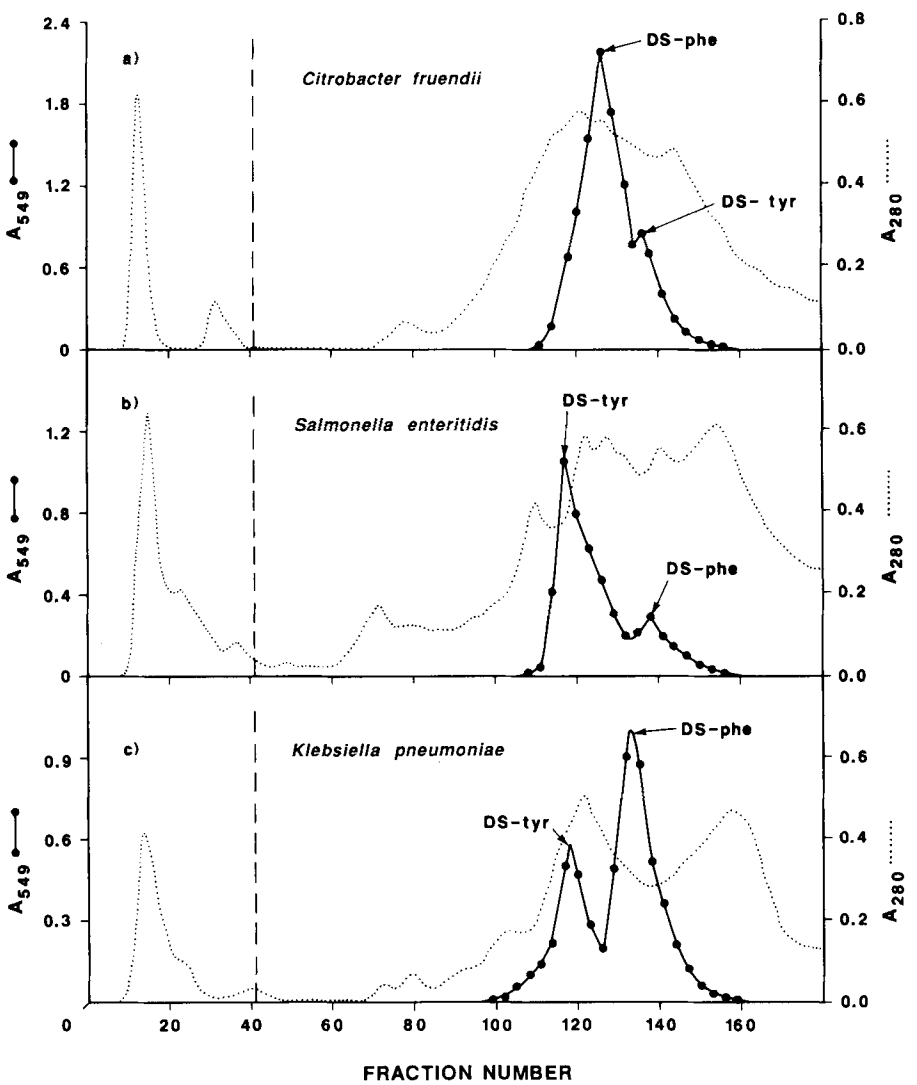

Fig. 1. Elution profiles of DAHP synthase-phe and DAHP synthase-tyr resolved by DE52 chromatography with extracts prepared from cultures of *C. fruendii* (a), *S. enteritidis* (b), and *K. pneumoniae* (c) grown in minimal medium. The column chromatography was performed as described under 'Methodology'. The vertical dashed line indicates the onset point of gradient elution. DAHP synthase activity is expressed as A_{549}. An absorbance value of 1.0 at 549 nm corresponds to 2.8 nmoles of DAHP formed per min. The distribution of protein eluted (expressed as A_{280}) is shown by dotted lines.

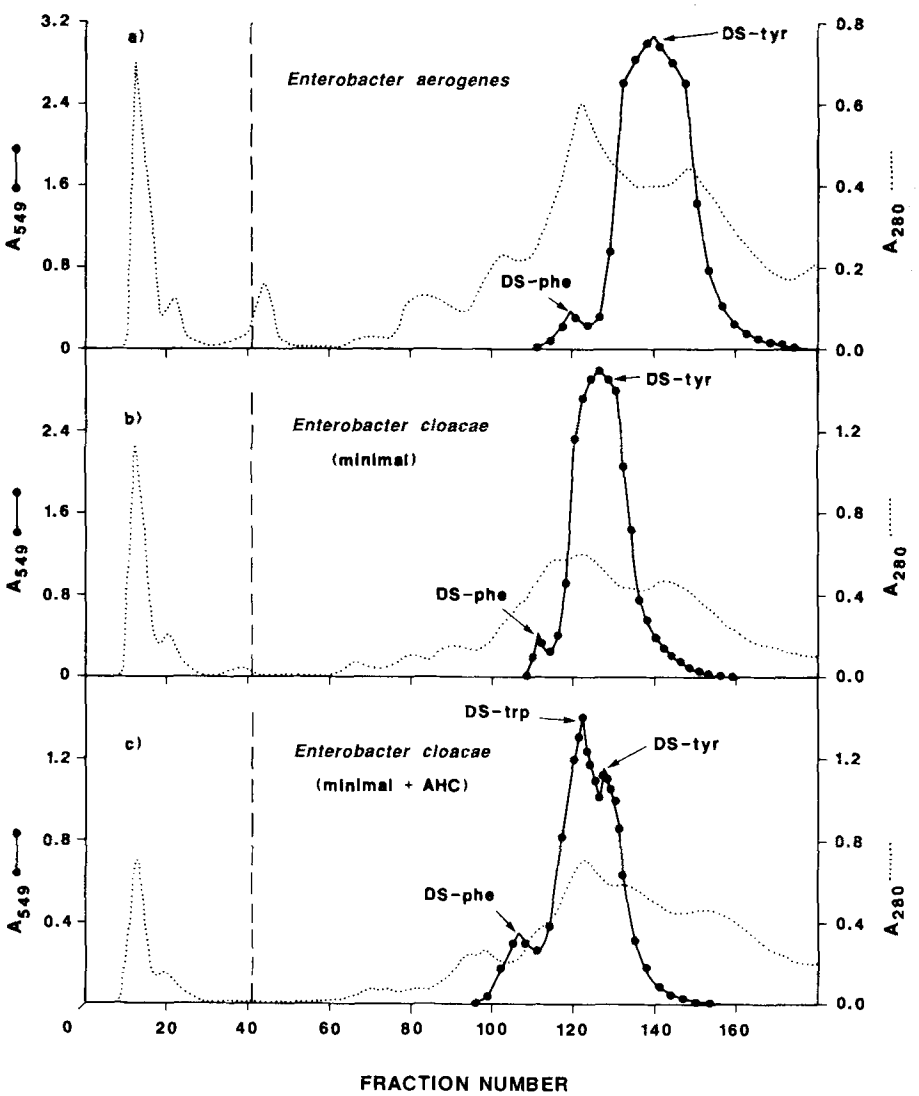

Fig. 2. Elution profiles of DAHP synthase-phe and DAHP synthase-tyr resolved by DE52 column chromatography with extracts prepared from cultures of *E. aerogenes* (a) and *E. cloacae* (b). The profiles shown in Fig. 3c were obtained when crude extracts prepared from cultures of *E. cloacae* grown in minimal medium supplemented with acid-hydrolyzed casein (AHC) were used for DE52 chromatography and show the resolution of all three DAHP synthase isozymes. Nearly two times more extract protein was used in assays to obtain the profiles in *c* than in *b* owing to the repressibility of DAHP synthase-phe and DAHP synthase-tyr. However, even when the amount of extract used for assays done in *a* was increased, no DAHP synthase-trp isozyme profile was detected.

major peak of activity. Similar results were obtained from *E. agglomerans* and *Serratia rubidaea* (data not shown).

No DAHP synthase-trp was detected in any of the organisms listed above after growth in minimal medium. In *Escherichia coli* cultures grown in the minimal medium, DS-phe is the major isozyme, DS-tyr constitutes a minor fraction of the total activity, while DS-trp is maximally repressed [12]. Growth of *E. coli* on acid-hydrolyzed casein results in derepression of tryptophan-pathway enzymes (including DS-trp), since the faster growth rate and the presence of all amino acids except L-tryptophan makes tryptophan rate-limiting to growth. Application of this nutritional manipulation to *E. cloacae* did indeed result in the appearance of a third peak of DAHP synthase activity when crude extracts prepared from cultures grown in the presence of acid-hydrolyzed casein were used for DE52 column chromatography (Fig. 2c). The new peak of activity proved to be sensitive to L-tryptophan (DS-trp) (36% and 51% inhibition by 0.1 and 0.5 m*M* L-tryptophan respectively). In addition, the DS-tyr isozyme was repressed about five- to sixfold, whereas the DS-phe activity was barely affected.

Two isozymes of DAHP synthase also eluted after DE52 chromatography of crude extracts prepared from *Halfnia alvei* (Fig. 3a), *Proteus vulgaris* (Fig. 3b), and *E. tarda* (Fig. 3c). In each case one peak of activity proved to be sensitive to L-phenylalanine (DS-phe) (Table 1), while the second peak of activity was sensitive to L-tryosine (DS-tyr) (Table 2). In each case DS-tyr constituted the major peak of activity, while DS-phe eluted as

Table 2. Inhibition of DAHP synthase-tyr isozymes in the presence of aromatic amino acids

Bacterial species	Fraction number[a]	% Inhibition[b] by				% Inhibition[c] by			
		Phe	Tyr	Trp	Aro	Phe	Tyr	Trp	Aro
Citrobacter fruendii	136	37	42	0	72	46	54	2	91
Salmonella enteritidis	117	0	70	0	77	4	88	1	92
Klebsiella pneumoniae	118	2	25	0	24	0	55	2	53
Enterobacter aerogenes	138	0	68	0	65	0	88	0	87
Enterobacter cloacae	126	0	80	0	77	1	94	2	94
Hafnia alvei	129	0	59	0	65	0	92	0	93
Proteus vulgaris	108	0	53	0	51	0	93	0	93
Edwardsiella tarda	107	0	66	0	69	0	86	0	90
Kluyvera ascorbata	132	2	34	0	40	4	82	0	84
Yersinia enterocolitica	119	4	75	0	70	0	91	2	95

[a] Refers to column profiles shown in Figs. 1 (a,b,c), 2 (a,b), 3 (a,b,c), and 4 (a,b).
[b] The final concentration of Phe, Tyr, Trp or Aro was 0.1 m*M*.
[c] The final concentration of Phe, Tyr, Trp or Aro was 0.5 m*M*.

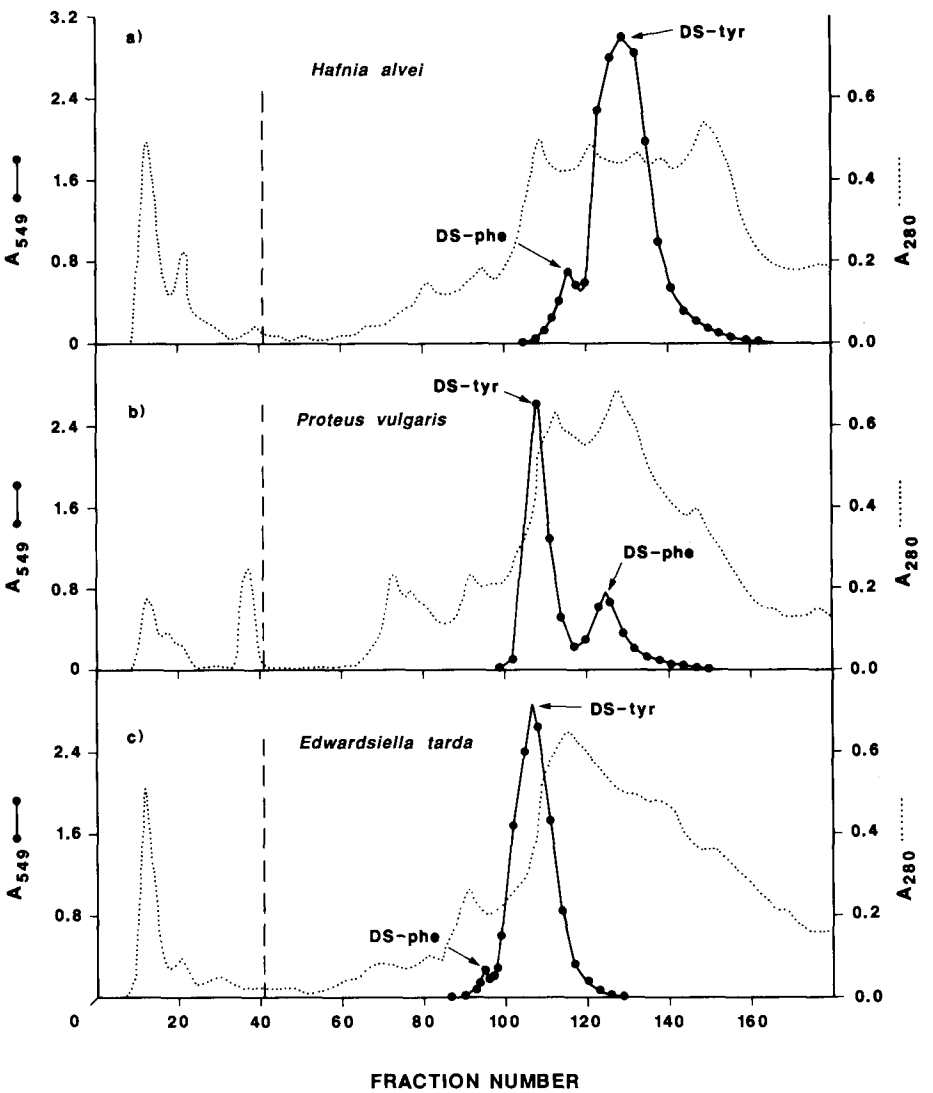

Fig. 3. Elution profiles of DAHP synthase-phe and DAHP synthase-tyr resolved by DE52 chromatography of crude extracts prepared from cultures of *H. alvei* (a), *P. vulgaris* (b), and *E. tarda* (c) grown in the minimal medium.

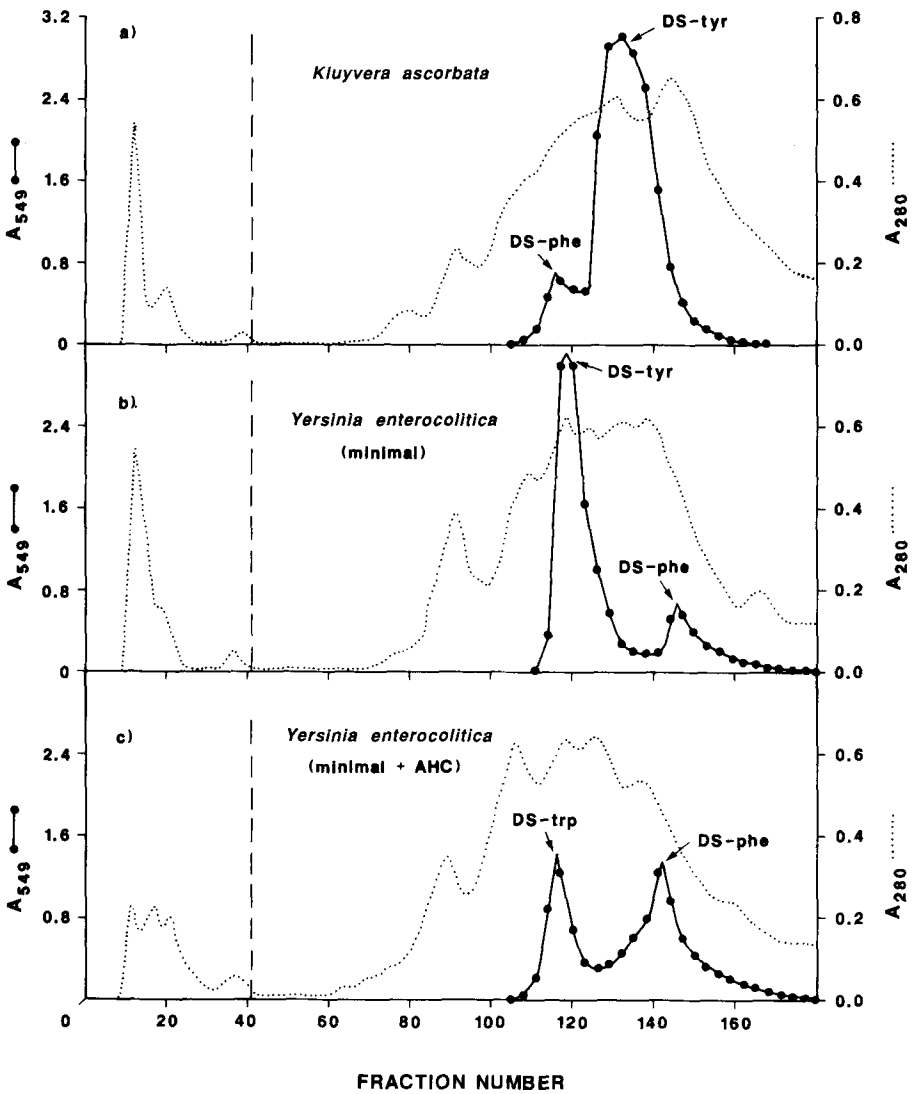

Fig. 4. DE52 chromatography showing elution profiles of DAHP synthase-phe and DAHP synthase-tyr from crude extracts of cultures of *K. ascorbata* (a) and *Y. enterocolitica* (b) grown in minimal medium and of DAHP synthase-trp and DAHP synthase-phe from crude extracts of cultures of *Y. enterocolitica* (c) grown in the minimal medium supplemented with acid-hydrolyzed casein (AHC). Even though roughly two times as much extract protein was used in assays to obtain the profiles in c as in b owing to the repressibility of DAHP synthase-tyr and DAHP synthase-phe, no DAHP synthase-tyr profile was obtained. No column fractions exhibiting DAHP synthase activity were sensitive to inhibition by L-tyrosine.

the minor peak of activity. Similar results were obtained from *C. devisae*, *P. alcalifaciens*, and *Morganella morganii*, except that in *P. alcalifaciens* and *M. morganii*, DS-phe constituted the major peak of activity (data not shown).

Two isozymes of DAHP synthase eluted after DE52 chromatography of crude extracts prepared from *K. ascorbata* (Fig. 4a) and *Yersinia enterocolitica* (Fig. 4b). In each case, the minor peak of activity proved to be sensitive to L-phenylalanine (DS-phe) (Table 1), while the major peak of activity was inhibited by L-tyrosine (DS-tyr) (Table 2). When crude extracts prepared from cultures of *Y. enterocolitica* grown in the minimal medium supplemented with acid-hydrolyzed casein were used for column chromatography, again two peaks of activity were recovered. However, the leading peak of activity proved to be sensitive to inhibition only by L-tryptophan (15% and 27% by 0.1 and 0.5 m*M* L-tryptophan, respectively), while the second peak of activity (DS-phe) was inhibited by L-phenylalanine only (inhibition data not shown). Thus, DS-tyr was almost completely repressed, and DS-trp was derepressed under these growth conditions. The level of DS-phe was nearly unaffected. To demonstrate the presence of DS-trp in other enteric bacteria studied in this investigation, the organisms were grown in minimal medium supplemented with acid-hydrolyzed casein. The sensitivity of DAHP synthase activity in crude extracts prepared from these cultures to aromatic amino acids was studied, and the results are presented in Table 3. The data show that DS-trp was derepressed in each organism, as evidenced by the inhibition of DAHP synthase activity in crude

Table 3. Inhibition of DAHP synthase activity[a] in the presence of aromatic amino acids

Bacterial species	% Inhibition[b] by						
	Phe	Tyr	Trp	Phe + Tyr	Phe + Trp	Tyr + Trp	Phe + Tyr + Trp
Citrobacter fruendii	78	9	17	80	82	18	83
Salmonella enteritidis	80	4	7	81	84	9	85
Klebsiella pneumoniae	75	14	16	78	81	21	89
Enterobacter aerogenes	13	85	7	92	14	87	95
Enterobacter cloacae	53	19	16	66	66	24	81
Enterobacter agglomerans	46	29	16	63	51	37	77
Kluyvera ascorbata	60	35	11	82	63	39	87
Hafnia alvei	50	6	25	51	67	26	68
Edwardsiella tarda	8	1	38	9	46	38	46
Yersinia enterocolitica	70	2	14	71	82	14	83

[a] DAHP synthase activities were assayed in crude extracts prepared from cell cultures grown in the presence of acid-hydrolyzed casein.

[b] The concentration of Phe, Tyr, or Trp alone or in various combinations was 0.5 m*M*.

extracts by tryptophan. The DAHP synthase activity in crude extracts prepared from cell cultures grown in minimal medium showed no inhibition by tryptophan (data not shown). The results presented here, together with earlier observations [5, 12, 15], suggest that DS-trp constitutes only a minor fraction of the total DAHP synthase activity in enteric bacteria grown in minimal medium. However, the enteric bacteria uniformly possess a mechanism for derepression of DS-trp in response to L-tryptophan starvation. The DS-trp isozyme elsewhere within Superfamily B is not known to be under repression control [4]. None of the DAHP synthase isozymes from any enteric organism were inhibited or activated by pathway intermediates such as chorismate, prephenate, or phenylpyruvate.

Relative expression levels of DAHP synthase isozymes in enteric bacteria. In *E. coli* the three DAHP snthase isozymes are subject to differential repression control [12]. The fractional contributions of each isozyme to total DAHP synthase activity presumably reflect the endogenous pool sizes of each aromatic amino acid. In cultures of *E. coli* grown in the minimal medium, DS-phe constitutes the major portion of total DAHP synthase activity (denoted dominant isozyme) [12], whereas DS-tyr is the dominant isozyme in *S. typhimurium*. DS-trp constitutes less than 1% of total activity in cultures of *E. coli* [15] and *S. typhimurium* [10] grown in the minimal medium. The results obtained in the present study together with earlier observations show that DS-tyr is the dominant isozyme in most of the enteric bacteria. DS-tyr was consistently much more repressible than DS-phe when both L-phenylalanine and L-tyrosine were present in the growth medium in organisms that possessed DS-tyr as the dominant isozyme (as illustrated by experiments carried out with *E. cloacae* and *Y. enterocolitica*).

It should be noted here that *P. aeruginosa* (and other group I pseudomonads) and *Oceanospirillum* species possess two non-repressible isozymes of DAHP synthase (DS-tyr and DS-trp), DS-tyr being the major isozyme [4]. Thus, the ubiquitous dominance of DS-tyr may reflect the ancestral state. It may also be that after the generation of the third DAHP synthase isozyme (DS-phe), the pre-existing DS-tyr and DS-trp isozymes evolved mechanisms of repression control, since the existence of repression control for DAHP synthase isozymes appears to correlate with the existence of DS-phe [5].

Character states and phylogenetic relationships. Figure 5 illustrates most of the major sublineages that comprise the enteric bacteria and their close relatives in Superfamily B. The dendrogram shown in Fig. 5 is based upon the oligonucleotide cataloging results obtained by Woese and co-workers [18]. The bifunctional P-protein (chorismate mutase-P:prephenate dehydratase) is present throughout both Superfamily B and Superfamily A and exemplifies a character state that existed in their common ancestor [1]. On the other hand, the bifunctional T-protein of tyrosine biosynthesis is present only within the enteric lineage of Superfamily B [5]. The *Oceanospirillum* species possess two regulatory isozymes of DAHP synthase (DS-tyr and DS-trp).

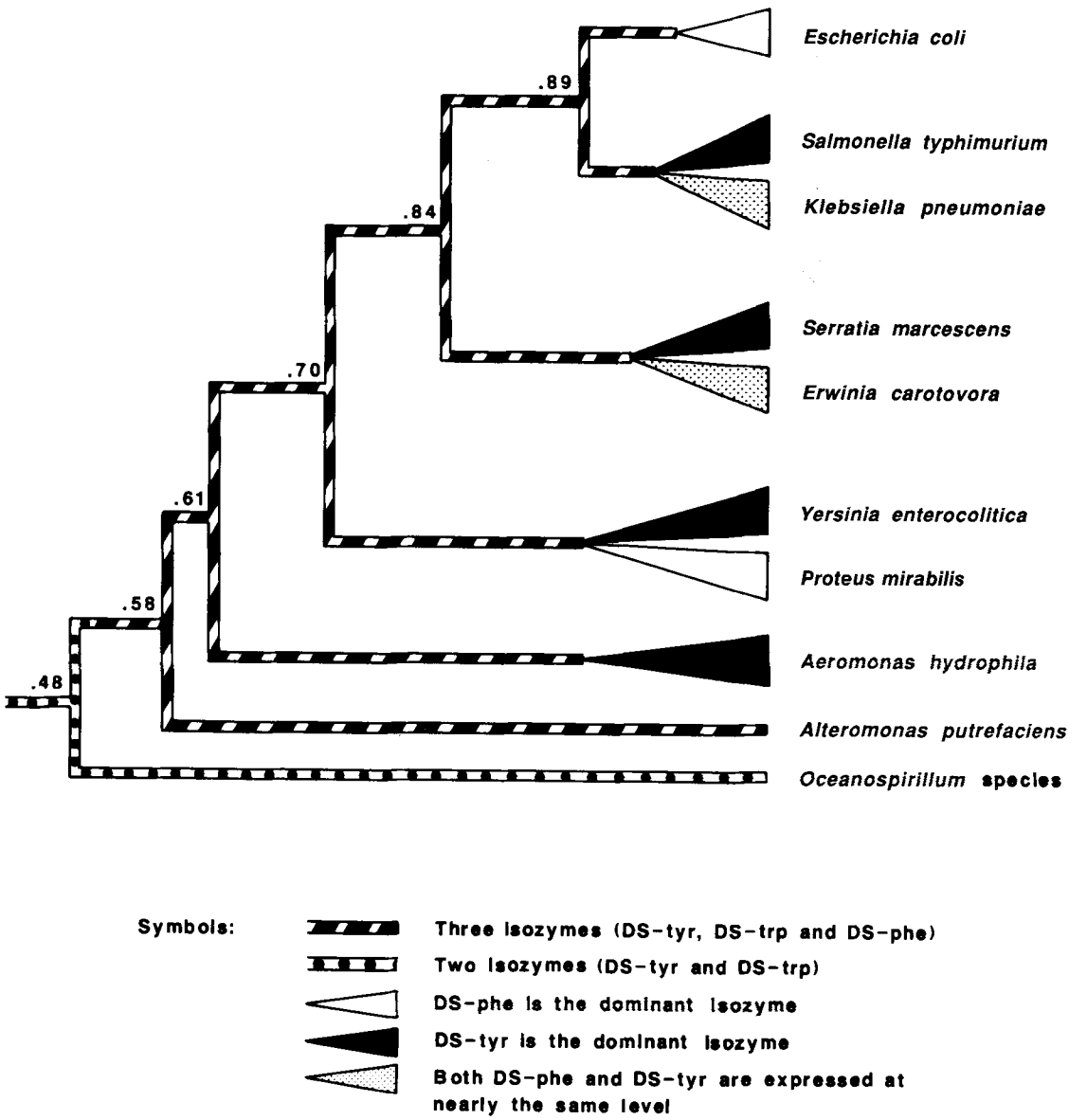

Fig. 5. Phylogenetic distribution of the three regulatory isozymes of DAHP synthase within the enteric lineage of bacteria. The dendrogram is based upon oligonucleotide-cataloging data (numbers shown are S_{AB} values) obtained by Woese and his coworkers. The next closest relatives of enteric bacteria, the *Oceanospirillum* species (as well as the remainder of Superfamily B), do not possess the phenylalanine-sensitive DAHP synthase isozyme and contain only two isozymes (DAHP synthase-tyr and DAHP synthase-trp). Of the three isozymes of DAHP synthase, the relative expression levels of these isozymes vary (as shown in the figure) from organism to organism grown in the minimal medium, though in no case was DAHP synthase-trp the dominant isozyme. The organisms shown in most cases represent a cluster of organisms. For example, *E. coli* also includes *S. dysenteriae* and *C. fruendii*; *S. typhimurium* also includes *S. enteritidis*, *E. cloacae*, *E. aerogenes*, and *E. agglomerans*; *S. marcescens* also includes *S. rubidiae*, *E. herbicola*, and *E. amylovora*; *Y. enterocolitica* also includes *H. alvei*, *P. vulgaris*, *E. tarda*, *K. ascorbata*, and *C. devisae*; and *P. mirabilis* also includes *M. morganii* and *P. alcalifaciens*.

DS-phe is the most recently evolved isozyme, being present only within the members of the enteric lineage. The results of the present study together with earlier observations [17] clearly indicate that DS-phe is another reliable indicator of the enteric lineage within Superfamily B. Since all genera belonging to the enteric bacteria, *Aeromonas* and *Alteromonas* possess DS-phe, the earlier conclusion that this isozyme evolved in the common ancestor of the enteric lineage after divergence of the

Oceanospirillum lineage is further supported. The relative expression levels of the DAHP synthase isozymes can also be followed in the major sublineages. Thus, DS-phe is the dominant isozyme in the sublineage leading to *E. coli/S. dysenteriae/C. fruendii* and *P. mirabilis/P. alcalifaciens/M. morganii*, whereas DS-tyr is the major isozyme in most other major sublineages. These sublineages are arranged as shown on the basis of shared character states of aromatic biosynthesis as presented in references [2] and [3] (and Ahmad, unpublished data).

Conclusions

The DS-phe isozyme evolved in the common ancestor of the enteric lineage after divergence from an ancestor possessing the DS-tyr and DS-trp isozymes. DS-phe is a stable character state that is uniformly distributed throughout the enteric bacteria. Thus, all members of enteric bacteria possess three regulatory isozymes of DAHP synthase, each regulated by one aromatic amino acid. In cultures grown in the minimal medium, DS-trp is expressed at very low levels and is easily overlooked. DS-tyr is the major (dominant) isozyme in all major sublineages except in the sublineage leading to *E. coli/S. dysenteriae/C. fruendii* and *P. mirabilis/M. morganii/P. alcalifaciens*.

ACKNOWLEDGMENT

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Literature Cited

1. Ahmad S, Jensen RA (1986) The evolutionary history of two bifunctional proteins that emerged in the purple bacteria. Trends Biochem Sci 11:108–112
2. Ahmad S, Jensen RA (1988) New prospects for deducing the evolutionary history of metabolic pathways in prokaryotes: aromatic biosynthesis as a case-in-point. Orig Life 18:41–57
3. Ahmad S, Jensen RA (1988) Phylogenetic distribution of components of the overflow pathway to L-phenylalanine within the enteric lineage of bacteria. Curr Microbiol 16:295–302
4. Ahmad S, Rightmire B, Jensen RA (1986) Evolution of the regulatory isozymes of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase present in the *Escherichia coli* genealogy. J Bacteriol 165:146–154
5. Ahmad S, Johnson JL, Jensen RA (1987) The recent evolutionary origin of the phenylalanine-sensitive isozyme of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in the enteric lineage of bacteria. J Mol Evol 25:159–167
6. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
7. Byng GS, Kane JF, Jensen RA (1982) Diversity in the routing and regulation of complex biochemical pathways as indicators of microbial relatedness. Crit Rev Microbiol 9:227–252
8. Dayan J, Sprinson DB (1970) Preparation of prephenic acid. Methods Enzymol 17A:559–561
9. Gibson F (1970) Preparation of chorismic acid. Methods Enzymol 17A:362–364
10. Gollub E, Sprinson DB (1969) A regulatory mutation in tyrosine biosynthesis. Biochem Biophys Res Commun 35:389–395
11. Jensen RA (1985) Biochemical pathways in prokaryotes can be traced backward through evolutionary time. Mol Biol Evol 2:92–108
12. Jensen RA, Nasser DS (1968) Comparative regulation of isoenzymic 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetases in microorganisms. J Bacteriol 95:188–196
13. Jensen RA, Nester EW (1966) Regulatory enzymes of aromatic amino acid biosynthesis in *Bacillus subtilis*. I. Purification and properties of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetase. J Biol Chem 241:3365–3372
14. Srinivasan PR, Sprinson DB (1959) 2-Keto-3-deoxy-D-*arabino*-heptonic acid 7-phosphate synthase. J Biol Chem 234:716–722
15. Wallace BJ, Pittard J (1967) Genetic and biochemical analysis of the isozymes concerned in the first reaction of aromatic biosynthesis in *Escherichia coli*. J Bacteriol 93:237–244
16. Winkler UK, Stuckman M (1979) Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J Bacteriol 138:663–670
17. Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271
18. Woese CR, Weisburg WG, Hahn CM, Paster BJ, Zablen LB, Lewis BJ, Macke TJ, Ludwig W, Stackebrandt E (1985) The phylogeny of purple bacteria: the gamma subdivision. Syst Appl Microbiol 6:25–33