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Evolution of the Regulatory Isozymes of 3-Deoxy-D-*Arabino*-Heptulosonate 7-Phosphate Synthase Present in the *Escherichia coli* Genealogy

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The evolutionary history of isozymes for 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase has been constructed in a phylogenetic cluster of procaryotes (superfamily B) that includes *Escherichia coli*. Members of superfamily B that have been positioned on a phylogenetic tree by oligonucleotide cataloging possess one or more of four distinct isozymes of DAHP synthase. DAHP synthase-0 is insensitive to feedback inhibition, while DAHP synthase-Tyr, DAHP synthase-Trp, and DAHP synthase-Phe are sensitive to feedback inhibition by L-tyrosine, L-tryptophan, and L-phenylalanine, respectively. The evolutionary history of this isozyme family can be deduced within superfamily B by using a cladistic methodology of maximum parsimony (R. A. Jensen, Mol. Biol. Evol. 2:92–108, 1985). DAHP synthase-0 was found in *Acinetobacter* species and in *Oceanospirillum minutulum*, organisms that also possess DAHP synthase-Tyr. These two isozymes were apparently present in a common ancestor that predated the evolutionary divergence of contemporary superfamily B sublineages. DAHP synthase-0 is postulated to have been the evolutionary forerunner of DAHP synthase-Trp. The newly evolved DAHP synthase-Trp is postulated to have possessed sensitivity to feedback inhibition by chorismate as well as by L-tryptophan, chorismate sensitivity having been retained in rRNA group I pseudomonads (minor sensitivity), group V pseudomonads (very sensitive), and *Lysobacter enzymogenes* (ultrasensitive). Organisms constituting the enteric lineage of the phylogenetic tree (including a cluster of four *Oceanospirillum* species) have all lost the chorismate sensitivity of DAHP synthase-Trp. The absence of DAHP synthase-Phe in the *Oceanospirillum* cluster of organisms supports the previous conclusion that DAHP synthase-Phe evolved recently within superfamily B, being present only in *Escherichia coli* and its close relatives.

Ever since advancement of the idea (28) that macromolecules might be reliable documents of evolutionary history, a progression of techniques for analytical comparison of nucleic acids has been under development for the purpose of grouping related bacteria. These have included guanine and cytosine contents of DNA (2), DNA-DNA hybridization (9, 10) and DNA-rRNA hybridization (18). An historical and broadly based perspective on the impact of these techniques upon the classification of pseudomonad bacteria, a large and diverse group of prokaryotes having a classic place within the microbiological literature, can be obtained from references 18 and 24. The advent of oligonucleotide cataloging has led to progress so dramatic that phylogenetic trees, once thought impossible to derive, are being constructed at a rapid pace (11, 12, 24–27).

Given the new availability of phylogenetic trees, it is now possible to deduce the evolutionary history of biochemical pathways (16). The biosynthetic pathway for aromatic amino acids is complex and varies widely in nature (5). Variable features include alternative biochemical steps, presence of distinctive isozymes, differing patterns of allosteric control, specificity of aromatic-pathway dehydrogenases for pyridine nucleotide cofactors, and presence or absence of multifunctional proteins. A given biochemical feature will be shared by a cluster of organisms whose phylogenetic breadth is unpredictable because the initial evolutionary event was fortuitous. For example, the contemporary *Escherichia coli* contains a pleasing symmetry of bifunctional proteins that constitute the first two steps of phenylalanine biosynthesis (P protein) and the first two steps of tyrosine biosynthesis (T protein). These would appear to be equivalent types of evolutionary changes; yet, as shown earlier (16), the P protein evolved early and is a reliable characteristic of the entire superfamily B assemblage (Fig. 1) while the T protein is found only in a narrow range of the enteric bacteria that are most closely related to *E. coli*.

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase has been studied in more detail in superfamily B organisms than in any other comparable grouping of procaryotes. Four patterns for DAHP synthase distinguish the four major lineages shown in Fig. 1. (i) *Acinetobacter* species possess an allosterically insensitive isozyme (DAHP synthase-0) and a tyrosine-sensitive isozyme (DAHP synthase-Tyr) of DAHP synthase (G. S. Byng, A. Berry, and R. A. Jensen, Arch. Microbiol., in press). (ii) Group I pseudomonads are similar except that DAHP synthase-0 has been replaced with a tryptophan-sensitive isozyme (DAHP synthase-Trp) (4). It has been postulated that DAHP synthase-Trp evolved from DAHP synthase-0 (16). (iii) Group V pseudomonads possess only DAHP synthase-Trp (21). (iv) *E. coli* (1) and certain near relatives (16) possess three regulated isozymes: DAHP synthase-Tyr, DAHP synthase-Trp, and a phenylanine-sensitive isozyme (DAHP synthase-Phe).

With respect to the evolution of basic isozyme number (i.e., one enzyme, two isozymes, or three isozymes), the assumption was presented as the simplest possibility (16) that a common superfamily B ancestor possessed two iso-

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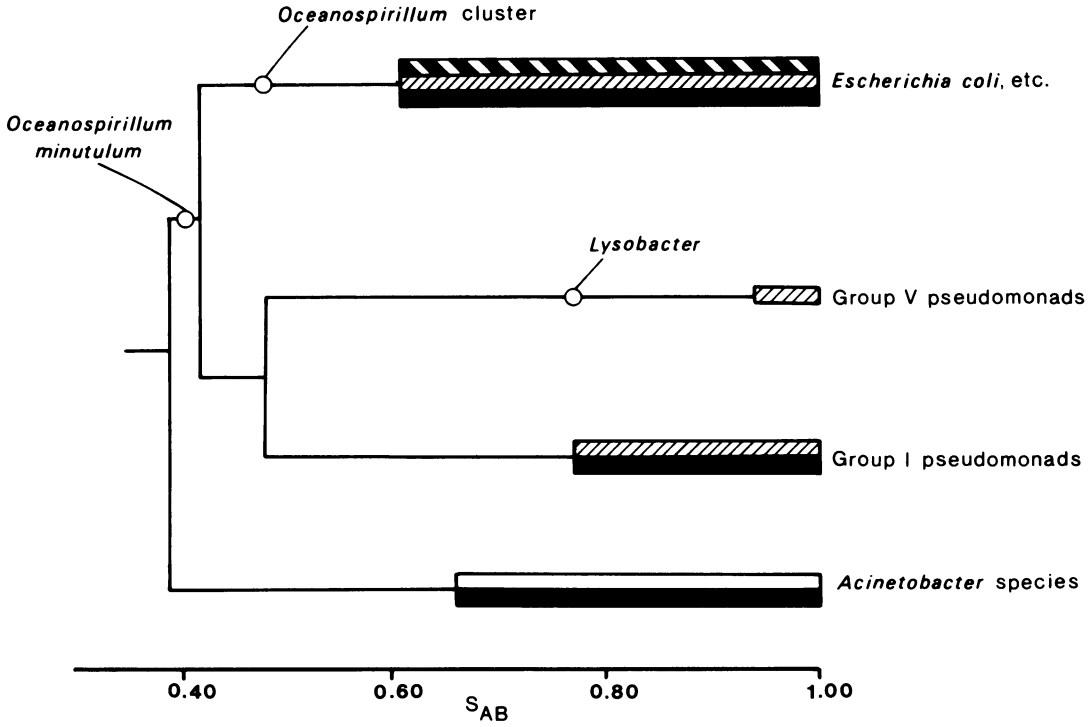

FIG. 1. Distribution of DAHP synthase isozymes in major lineages of superfamily B organisms. Abscissa values showing phylogenetic proximities are S_{AB} values (12). Each lineage shown possesses one, two, or three isozymes. Circles indicate the phylogenetic positions of *O. minutulum*, *L. enzymogenes*, and a cluster of *Oceanospirillum* species. The *Oceanospirillum* cluster includes *O. japonicum*, *O. beijerinckii*, *O. maris*, and *O. linum*. Symbols: ▭ DAHP synthase-0; ■, DAHP synthase-Tyr; ▨, DAHP synthase-Trp; ▩, DAHP synthase-Phe.

zymes. Two character-state changes would then yield the evolutionary results shown in Fig. 1, i.e., gain of a third isozyme (DAHP synthase-Phe) in the enteric lineage and loss of an isozyme (DAHP synthase-Tyr) to give the one-enzyme pattern found in the pseudomonad group V lineage. An alternative evolutionary scenario assumes a Superfamily B ancestor having all three isozymes. In this case, three character-state changes could have given the arrangement shown in Fig. 1 (two independent losses of DAHP synthase-Phe: in the group V-group I pseudomonad lineage and in the *Acinetobacter* lineage, as well as further loss of DAHP synthase-Tyr in the group V pseudomonad lineage). Additional evidence in favor of one of these possibilities (nearly equally probable) can be obtained by studying other organisms whose points of dendrogram divergence lie between the known three-isozyme and two-isozyme systems. The divergence position of a cluster of *Oceanospirillum* species is ideal for this purpose (Fig. 1).

Since group V pseudomonads exhibit a unique single-enzyme type of DAHP synthase pattern (21) within superfamily B, the point of dendrogram divergence for *Lysobacter enzymogenes* was also of interest, being between known two-isozyme and one-enzyme organism types. Divergence of the *Oceanospirillum minutulum* lineage at a deep dendrogram position near that of the *Acinetobacter* lineage was of particular interest with respect to whether *O. minutulum* would prove to possess DAHP synthase-O.

This study illustrates how judicious selection of previously unstudied organisms having established positions on a phylogenetic tree can produce an ever more firm basis for deducing the ancestral states of biochemical pathways that must have existed in a retrogressive series of more and more ancient ancestors.

MATERIALS AND METHODS

Organisms and growth conditions. *O. minutulum* (ATCC 19193), *Oceanospirillum japonicum* (ATCC 19191), *Oceanospirillum beijerinckii* (ATCC 12754), *Oceanospirillum maris* (ATCC 12749), and *Oceanospirillum linum* (ATCC 11336) were kindly provided by N. Krieg, Virginia Polytechnic Institute and State University, Blacksburg, Va. The organisms were grown at 30°C as described by Hylemon et al. (15). *L. enzymogenes* (ATCC 27796) was obtained from the American Type Culture Collection, Rockville, Md., and was grown as described by Christensen and Cook (7). The cells were harvested by centrifugation, washed twice with buffer A (50 mM potassium phosphate buffer [pH 7.0], 1 mM dithiothreitol), and stored at −80°C until used.

Preparation of cell extracts and enzyme assay. Cell pellets were suspended in buffer A and disrupted by sonication, and the crude cell extracts (free of small molecules) were prepared exactly as described previously (22).

DAHP synthase activity was assayed as described by Calhoun et al. (6). The reaction mixture (final volume, 0.2 ml) contained buffer A, a suitable amount of enzyme, 1 mM cobaltous chloride, 1 mM D-erythrose 4-phosphate (E4P; sodium salt), and 1 mM trisodium phosphoenolpyruvate (PEP). Incubation at 37°C was carried out for 20 min, and then the reaction was terminated by the addition of 0.05 ml of 20% (wt/vol) trichloroacetic acid.

Since DAHP synthase-Trp is more sensitive to inhibition at pH 6.5 by L-tryptophan (unpublished observations), all inhibition studies with L-tryptophan were carried out at pH 6.5. Protein in the crude extracts was assayed by the method of Bradford (3) with bovine serum albumin as the standard protein.

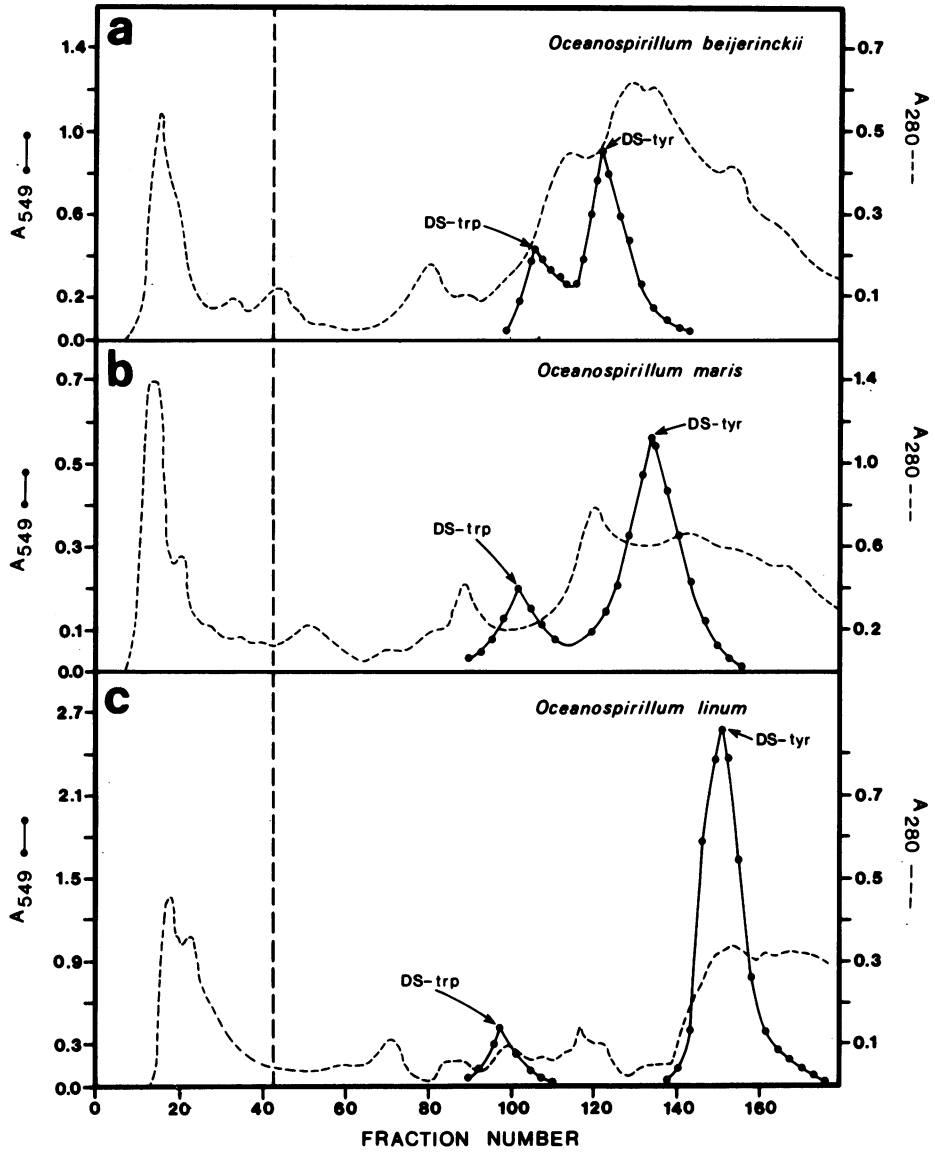

FIG. 2. Elution profiles from DE52 columns of DAHP synthase-Tyr and DAHP synthase-Trp isozymes from *O. beijerinckii* (a), *O. maris* (b), and *O. linum* (c). DE52 chromatography was performed as described in Materials and Methods. Vertical dashed lines indicate the onset point of gradient elution. DAHP synthase activity is expressed as A_{549}. The distribution of protein as measured by A_{280} is shown by dotted lines.

DE52 column chromatography. Approximately 100 mg of crude extract protein was applied to a DEAE-cellulose (DE52) column (1.5 by 20 cm) equilibrated in buffer A. The column was washed with two bed volumes of the equilibration buffer, and then bound proteins were eluted with 300 ml of a linear gradient of KCl (0 to 0.4 M) in buffer A. Fractions (2.2 ml) were collected and were assayed for A_{280} and DAHP synthase activity. Fractions containing the enzyme activity were tested for sensitivity to potential inhibitor compounds as indicated.

Biochemicals and chemicals. Amino acids, E4P, PEP, and Sephadex G-25 were obtained from Sigma Chemical Co., St. Louis, Mo. DE52 was purchased from Whatman, Inc., Clifton, N.J. Prephenate was prepared as the barium salt from culture supernatants of a tyrosine auxotroph of *Salmonella typhimurium* (8) and was converted to the potassium salt before use. Chorismate was isolated from the accumulation medium of the triple auxotroph *Klebsiella pneumoniae* 62-1 and purified as the free acid (13). All other chemicals were of the best grade commercially available.

RESULTS

DAHP synthase in the major *Oceanospirillum* cluster. Two similar isozymes of DAHP synthase were found in all four species of *Oceanospirillum* that form the cluster diverging at a similarity coefficient (S_{AB}) value of about 0.48 from the uppermost of the four major lineages shown in Fig. 1. Eluate profiles were obtained by DEAE-cellulose chromatography of extracts prepared from *O. beijerinckii*, *O. maris*, and *O. linum* (Fig. 2). In each case, DAHP synthase-Trp eluted as a minor leading peak followed by a major trailing peak of DAHP synthase-Tyr. Essentially similar results were obtained from *O. japonicum* (data not shown). In the latter case, DAHP synthase-Tyr eluted in the salt gradient as a

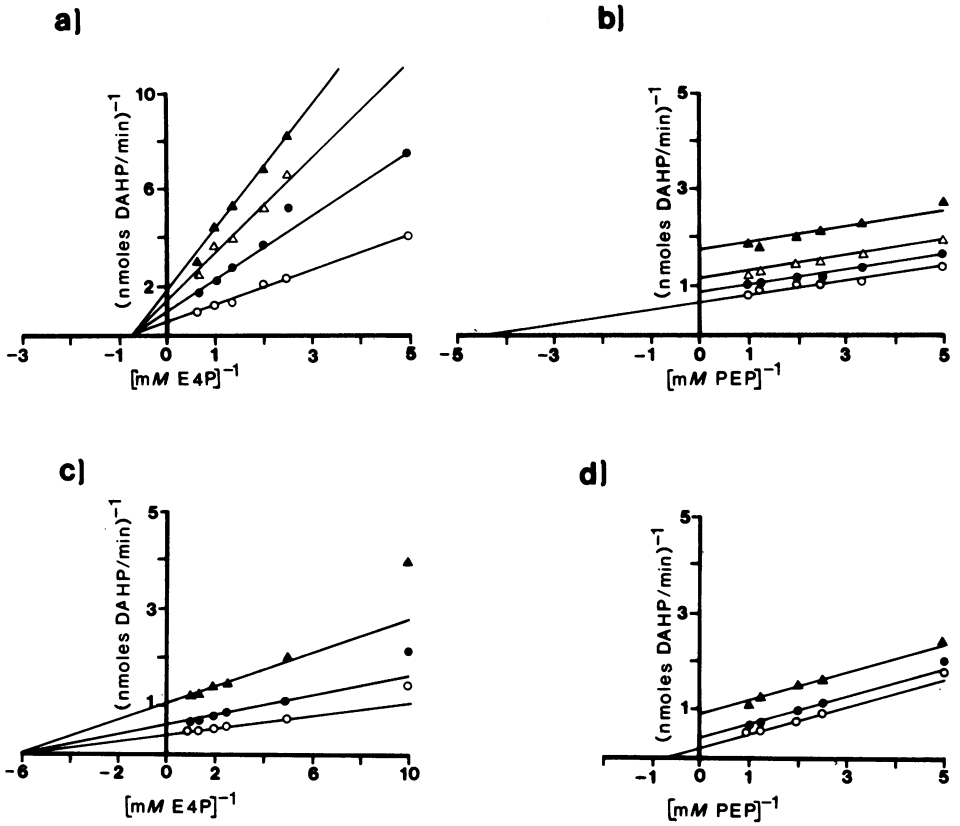

FIG. 3. Double-reciprocal plots of DAHP synthase-Trp (a and b) and of DAHP synthase-Tyr (c and d) from *O. linum*. Each velocity (expressed as nanomoles of DAHP formed per minute) is plotted as a function of the concentration of E4P (a and c) or of PEP (b and d). PEP concentrations were fixed at 0.4 mM for panel a and at 0.8 mM for c. E4P concentrations were held constant at 1.0 mM for panel b and at 0.8 mM for d. L-Tryptophan concentrations for panel a were 0 μM (○), 10 μM (●), 25 μM (△), and 50 μM (▲); those for panel b were 0 μM (○), 5 μM (●), 10 μM (△), and 20 μM (▲). L-Tyrosine concentrations for panels c and d were 0 μM (○), 10 μM (●), and 20 μM (▲). DAHP synthase-Trp and DAHP synthase-Tyr were recovered by DE52 column chromatography (Fig. 2).

major leading peak with a trailing shoulder of activity which proved to contain DAHP synthase-Trp.

More detailed studies of the two isozymes were carried out in *O. linum*, which was selected as a representative species of the *Oceanospirillum* species cluster. Double-reciprocal plots were made of substrate saturation data obtained for both DAHP synthase-Trp and DAHP synthase-Tyr in the presence and absence of inhibitor molecules (Fig. 3). Both isozymes were inhibited uncompetitively with respect to PEP and noncompetitively with respect to E4P. Kinetic constants obtained for the two *O. linum* isozymes are listed in Table 1.

DAHP synthase in *O. minutulum*. The chromatographic resolution of two isozymes from *O. minutulum*, an organism that is far outside the phylogenetic cluster defined by the other *Oceanospirillum* species (Fig. 1), is shown in Fig. 4. In this case, a peak of DAHP synthase-0 activity washed through the column bed without retardation. The major peak of activity recovered in the salt gradient was DAHP synthase-Tyr. Inhibition of DAHP synthase-Tyr by L-

TABLE 1. Properties of DAHP synthase isozymes isolated from two diverse species of *Oceanospirillum*[a]

Species of Oceanospirillum	Isozyme	Substrate	K_m (mM)	Inhibitor	K_i (μM)	Type of inhibition
O. linum	DAHP synthase-Trp	E4P	1.33	L-Tryptophan	15	Noncompetitive
		PEP	0.23	L-Tryptophan	11	Uncompetitive
	DAHP-synthase-Tyr	E4P	0.16	L-Tyrosine	11	Noncompetitive
		PEP	1.66	L-Tyrosine	5	Uncompetitive
O. minutulum	DAHP synthase-0	E4P	0.25	None		
		PEP	0.30	None		
	DAHP synthase-Tyr	E4P	0.27	L-Tyrosine	60	Noncompetitive
		PEP	0.67	L-Tyrosine	75	Noncompetitive

[a] K_m values, K_i values, and the modes of inhibition were determined from the data in Fig. 3 and 4. K_i values were determined from slope and intercept replots.

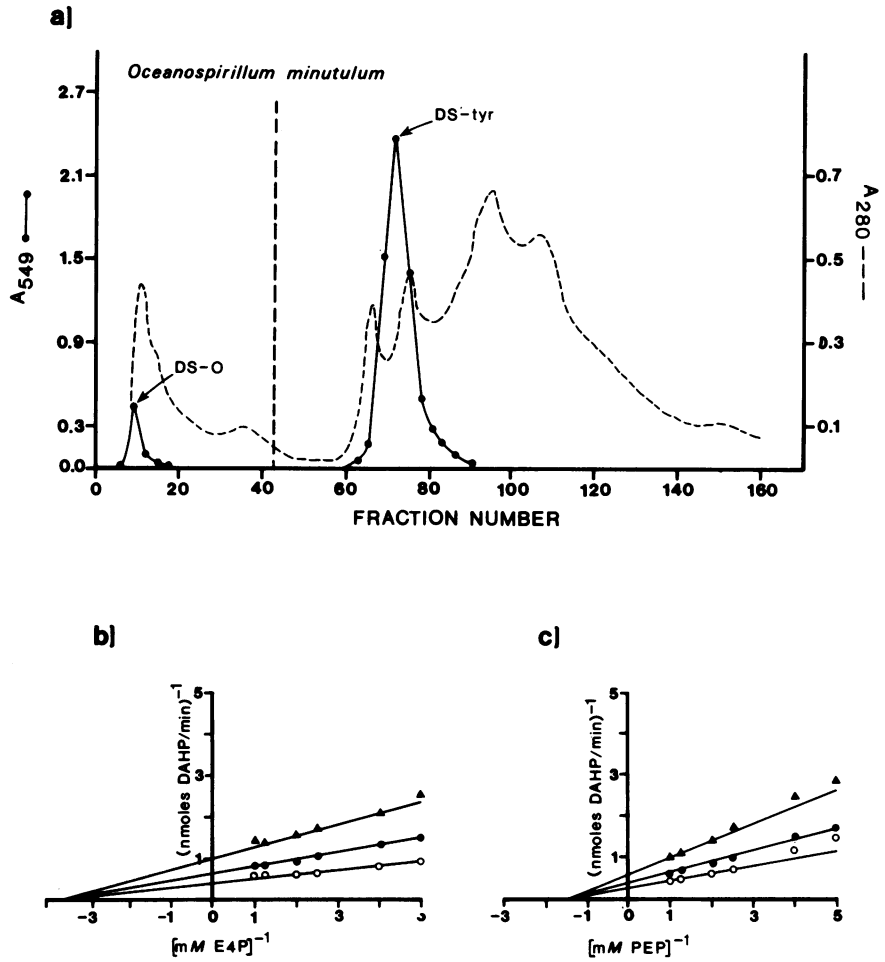

FIG. 4. (a) Elution profiles of DAHP synthase-0 (DS-0) and DAHP synthase-Tyr (DS-Tyr) isozymes from *O. minutulum*, showing their resolution by DE52 chromatography, performed as described in Materials and Methods. The vertical dashed line indicates the onset point of gradient elution. DAHP synthase activity is expressed as A_{549}. The distribution of proteins eluted (expressed as A_{280}) is shown by dotted lines. (b and c) Double-reciprocal plots of DAHP synthase-Tyr from *O. minutulum*. Each velocity on the ordinate scale is plotted as a function of E4P concentration (b) or PEP concentration (c). PEP concentrations for panel b were held constant at 0.5 mM and E4P concentrations for panel c were held constant at 0.8 mM. L-Tyrosine concentrations for both panels were 0 μM (○), 50 μM (●), and 100 μM (▲). DAHP synthase-Tyr was recovered by DE52 column chromatography (Fig. 2).

tyrosine proved to be noncompetitive with respect to either substrate. A summary of kinetic characterizations is given in Table 1.

None of the DAHP synthase isozymes isolated from the five species of *Oceanospirillum* proved to be sensitive to inhibition (at K_m levels of substrate) by chorismate, prephenate, phenylpyruvate, or L-phenylalanine.

DAHP synthase in *L. enzymogenes*. A large, single peak of DAHP synthase activity was eluted in the salt gradient after DEAE-cellulose chromatography of crude extracts prepared from *L. enzymogenes* (Fig. 5a). This activity had apparent K_m values of 1.42 mM for E4P and 0.83 mM for PEP. The enzyme was sensitive to inhibition by L-tryptophan, an allosteric effector that inhibited noncompetitively with respect to E4P (Fig. 5b) or to PEP (Fig. 5c).

As did DAHP synthase-Trp isozymes isolated from group I pseudomonads and group V pseudomonads, DAHP synthase-Trp isolated from *L. enzymogenes* proved to be sensitive to inhibition by chorismate. In fact, chorismate proved to be far more effective than L-tryptophan as an inhibitor of DAHP synthase-Trp. Leading and trailing fractions defining the eluate peak were compared for sensitivity to chorismate and to L-tryptophan to ensure that different overlapping enzyme species were not present. Identical results were obtained. Table 2 contains K_i values obtained for both chorismate and L-tryptophan. In *L. enzymogenes*, the K_i value of DAHP synthase-Trp for chorismate was 20- to 40-fold lower than the K_i value for L-tryptophan. Chorismate inhibited noncompetitively with respect to E4P (Fig. 5d) and uncompetitively with respect to PEP (Fig. 5e). Inhibition by chorismate in *L. enzymogenes* was greater, compared with that in *Xanthomonas campestris* and *Pseudomonas aeruginosa*, by one and two orders of magnitude, respectively.

DISCUSSION

DAHP synthase-0. *Oceanospirillum minutulum*, but not other species of *Oceanospirillum*, was found to possess DAHP synthase-0. The only other superfamily B member found to possess DAHP synthase-0 so far has been *Acinetobacter* (Byng et al., in press). This result is consistent with the phylogenetic position of *O. minutulum*, whose den-

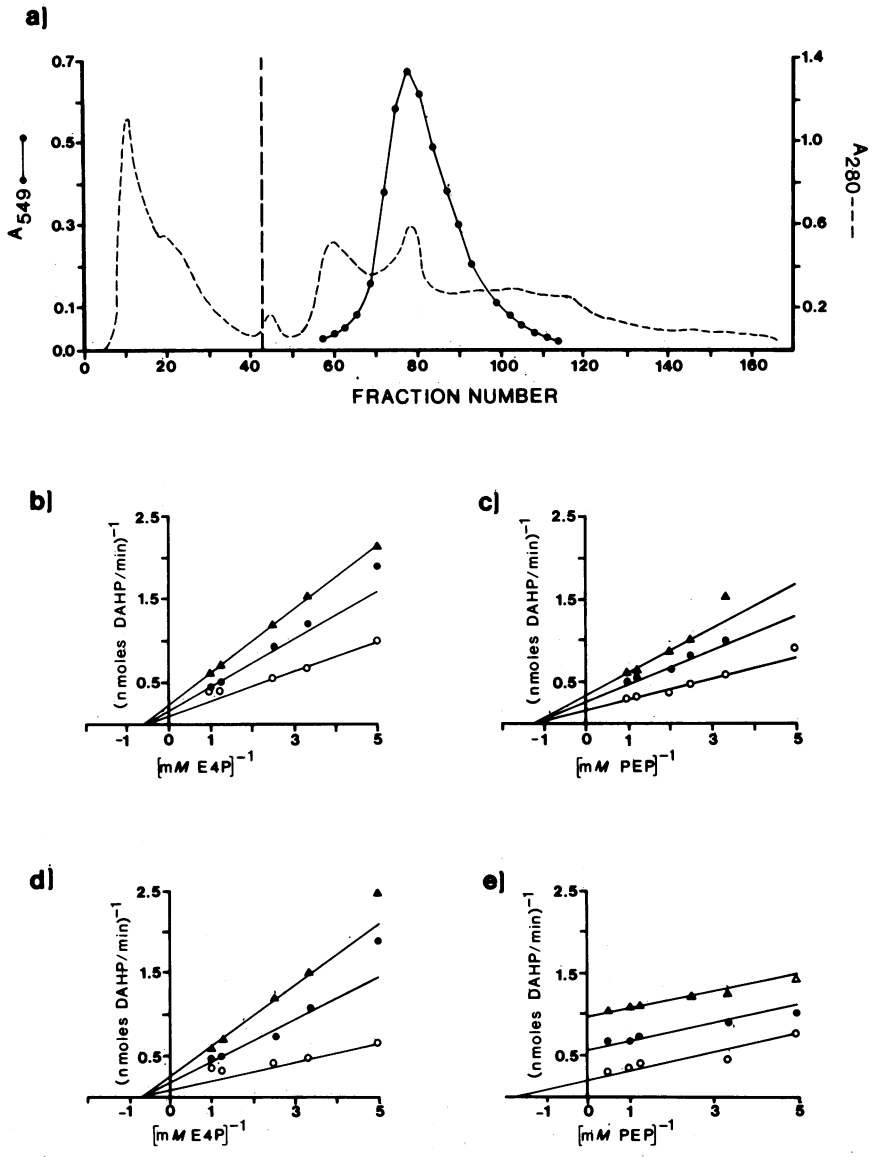

FIG. 5. (a) Profile of DAHP synthase-Trp from *L. enzymogenes* after elution from a DE52 chromatography column (see Materials and Methods). The vertical dashed line indicates the onset point of gradient elution. DAHP synthase activity is expressed as A_{549}. The distribution of proteins eluted (expressed as A_{280}) is shown by dotted lines. (b through e) Double reciprocal plots of DAHP synthase-Trp from *L. enzymogenes*. Each velocity is plotted as a function of the concentration of E4P (b and d) or of PEP (c and e). PEP concentrations were fixed at 0.5 mM for panel b and at 1.0 mM for panel d. E4P concentrations were fixed at 0.5 mM for panels c and e. L-Tryptophan concentrations for panel b were 0 mM (○), 0.2 mM (●), and 0.5 mM (▲); those for panel c were 0 mM (○), 0.1 mM (●), and 0.2 mM (▲). Chorismate concentrations for panels d and e were 0 μM (○), 10 μM (●), and 20 μM (▲). DAHP synthase-Trp was recovered from DE52 column fractions (a).

drogram assignment on the criterion of oligonucleotide cataloging (12, 24) places it nearer to *Acinetobacter* spp., pseudomonad rRNA group I, and pseudomonad rRNA group V than to other species of *Oceanospirillum* (11, 12, 24, 25). *O. minutulum* exemplifies an organism carrying a generic name that is inappropriate to its phylogenetic position, a circumstance that has become apparent in many cases (11, 20, 24, 25).

DAHP synthase-Trp. The suggestion (Byng et al., in press) that DAHP synthase-Trp evolved from DAHP synthase-0 was based upon the correlation of the disappearance of DAHP synthase-0 with the appearance of DAHP synthase-Trp in superfamily B organisms. This event occurred sometime between the divergence of *O. minutulum* (S_{AB} = 0.41) and the major part of superfamily B, with a point of divergence defined by S_{AB} = 0.42.

DAHP synthase-Trp isozymes found in superfamily B organisms fall into two distinct classes: (i) isozymes sensitive to allosteric inhibition only by L-tryptophan, and (ii) isozymes sensitive to allosteric inhibition by both L-tryptophan and chorismate. All organisms diverging from the upper section of the tree shown in Fig. 6 (i.e., the enteric lineage and the *Oceanospirillum* cluster diverging at an S_{AB} value of 0.48) possess the first class of DAHP synthase-Trp. On the other hand, all organisms belonging to the group V pseudomonad-group I pseudomonad-*Lysobacter* spp. lin-

TABLE 2. Comparison of allosteric properties of DAHP synthase-trp from *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *L. enzymogenes*

Organism	K_i (mM)			
	L-Tryptophan		Chorismate	
	PEP	E4P	PEP	E4P
P. aeruginosa[a]	0.04	0.005	1.35	2.25
X. campestris[b]	0.38	0.46	0.40	0.10
L. enzymogenes	0.17	0.42	0.007	0.01

[a] Data shown are from Whitaker et al. (23). Results are representative of other group I pseudomonads.

[b] Data shown are from Whitaker et al. (21). Results are representative of other group V pseudomonads.

eage (also diverging at S_{AB} = 0.48 in Fig. 6) possess a DAHP synthase-Trp sensitive to both inhibitors. Within this dendrogram section, the sensitivity of DAHP synthase-Trp to chorismate varies over 100-fold (Table 2). In rRNA group I pseudomonads, L-tryptophan is well over an order of magnitude more effective than chorismate as an inhibitor. The opposite is true in *L. enzymogenes*. In rRNA group V pseudomonads, L-tryptophan and chorismate are about equally effective as inhibitors. We have speculated (21) that allosteric sites for the two inhibitors could have originated from an ancestral anthranilate synthase as the result of a single gene fusion event. A pattern of overall pathway control by sequential feedback inhibition has been described in group V pseudomonads in which chorismate plays a crucial regulatory role (21). Data given here (Table 2) suggest that *L. enzymogenes* may exemplify this regulatory pattern even more dramatically.

A correlation seems to exist between the presence of DAHP synthase-Tyr and a dominating or exclusive role of L-tryptophan as an inhibitor of DAHP synthase-Trp. Thus, the cluster containing enteric bacteria and all species of *Oceanospirillum* (except for *O. minutulum*) possess DAHP synthase-Tyr and DAHP synthase-Trp lacking sensitivity to inhibition by chorismate. In contrast, the group V pseudomonad-*Lysobacter* spp. cluster lacks DAHP synthase-Tyr in correlation with a DAHP synthase-Trp of the type with minimal sensitivity to L-tryptophan and en-

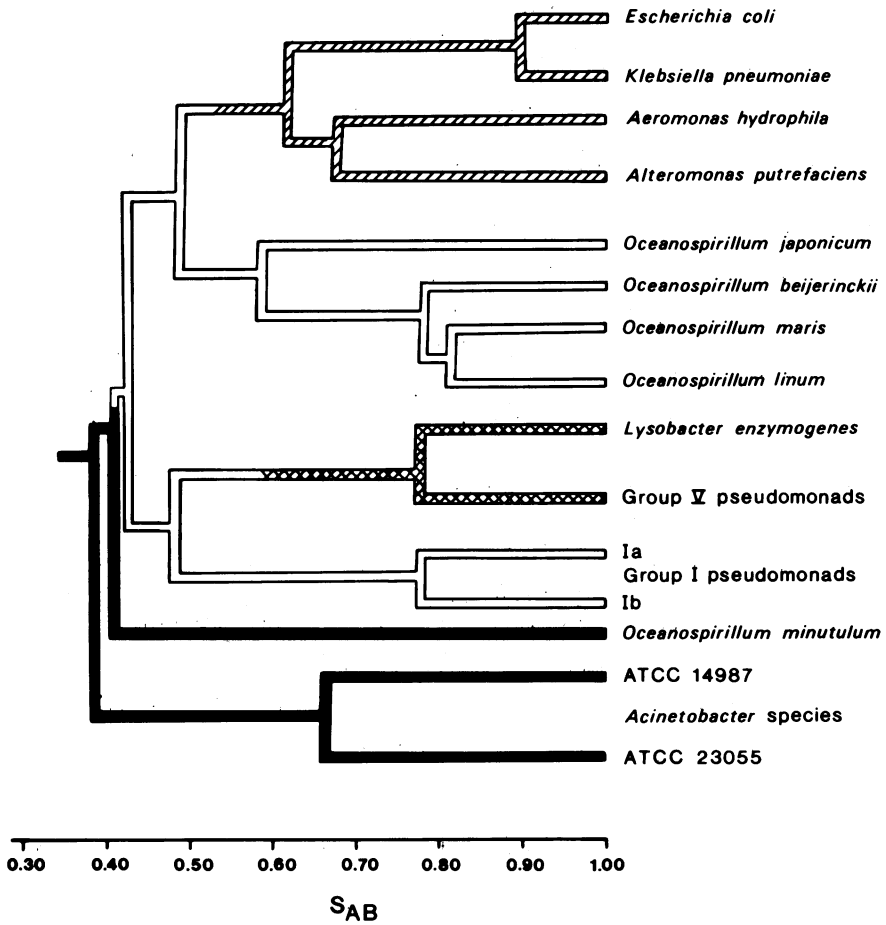

FIG. 6. Evolution of DAHP synthase isozymes in superfamily B procaryotes. The dendrograms are based upon oligonucleotide cataloging of superfamily B organisms (12, 24) in correlation with the evolution of DAHP synthase isozymes. The putative common ancestor possessed two isozymes of DAHP synthase, DAHP synthase-0 and DAHP synthase-Tyr. DAHP synthase-Trp may have evolved from an ancestral DAHP synthase-0. DAHP synthase-Phe was acquired in both the enteric lineages (presumably by gene duplication), while DAHP synthase-Tyr was lost in both group V pseudomonads and *Lysobacter* spp. Symbols: ▨, three isozymes (DAHP synthase-Tyr, DAHP synthase-Trp, and DAHP synthase-Phe); ▭, two isozymes (DAHP synthase-Tyr and DAHP synthase-Trp); ■, two isozymes (DAHP synthase-Tyr and DAHP synthase-0); ▩, one enzyme (DAHP synthase-Trp).

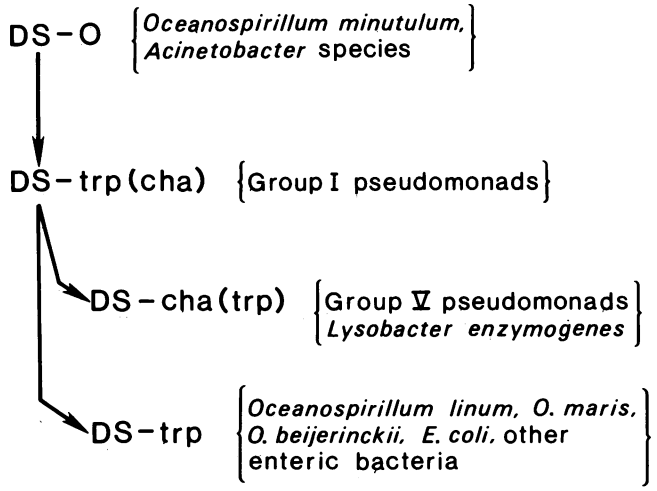

FIG. 7. Evolution of specialized DAHP synthase-Trp isozymes from the unregulated DAHP synthase (DS-0) in superfamily B organisms. This isozyme acquired, during the course of evolution, sensitivity to L-tryptophan as well as weak sensitivity to chorismate [DS-trp(cha), as found in group I pseudomonads]. This later evolved into an enzyme with much greater sensitivity to chorismate than to L-tryptophan [DS-cha(trp), as found in group V pseudomonads and *L. enzymogenes*] or lost the chorismate sensitivity and became a specialized DAHP-synthase-Trp isozyme (DS-trp, as found in the *Oceanospirillum* cluster and enteric bacteria).

hanced sensitivity to chorismate. Of superfamily B members retaining DAHP synthase-Tyr, only group I pseudomonads possess a DAHP synthase-Trp that is sensitive to inhibition by chorismate. An evolutionary trend toward the loss of this weak sensitivity to chorismate may be under way in this lineage (Table 2). A progression of evolutionary events, in which DAHP synthase-Trp having minor sensitivity to chorismate originated from DAHP synthase-0, is shown in Fig. 7. This progression gave rise to (i) an enzyme having enhanced sensitivity to chorismate and (ii) DAHP synthase-Trp. We had previously speculated (16) that chorismate sensitivity of DAHP synthase-Trp was lost after the new acquisition of DAHP synthase-Phe produced a balanced three-isozyme system that was directly responsive to all three end products (as in *E. coli*). However, this must not be correct since the *Oceanospirillum* cluster of species diverging at S_{AB} = 0.58 (Fig. 6) all possessed a chorismate-insensitive DAHP synthase-Trp but lacked DAHP synthase-Phe.

DAHP synthase-Tyr and DAHP synthase-Phe. The DAHP synthase-Tyr isozyme is distributed throughout superfamily B and appears to have evolved before the divergence of superfamily B sublineages. So far, only the *L. enzymogenes*-group V pseudomonad lineage lacks DAHP synthase-Tyr, and this character state must have been lost in a common ancestor of these organisms after divergence from rRNA group I pseudomonads.

DAHP synthase-Phe is the most recent isozyme to have emerged in superfamily B. The absence of DAHP synthase-Phe in the *Oceanospirillum* cluster places the origin of DAHP synthase-Phe somewhere between S_{AB} = 0.48 and S_{AB} = 0.61. The gene for DAHP synthase-Phe probably originated from a duplication of the gene for DAHP synthase-Tyr or DAHP synthase-Trp (17). DAHP synthase-Trp is the likeliest candidate since Shultz et al. (19) showed more sequence homology and secondary structure similarities (in *E. coli*) between DAHP synthase-Trp and DAHP synthase-Phe than between either and DAHP synthase-Tyr.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Baumann, L., P. Baumann, M. Mandel, and R. D. Allen.** 1972. Taxonomy of aerobic marine bacteria. J. Bacteriol. **110**: 402–429.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Byng, G. S., A. Berry, and R. A. Jensen.** 1983. A pair of regulatory isozymes for 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase is conserved within group I pseudomonads. J. Bacteriol. **156**:429–433.
5. **Byng, G. S., J. F. Kane, and R. A. Jensen.** 1982. Diversity in the routing and regulation of complex biochemical pathways as indicators of microbial relatedness. Crit. Rev. Microbiol. **9**:227–252.
6. **Calhoun, D. H., D. L. Pierson, and R. A. Jensen.** 1973. Channel-shuttle mechanism for the regulation of phenylalanine and tyrosine synthesis at a metabolic branch point in *Pseudomonas aeruginosa*. J. Bacteriol. **113**:241–251.
7. **Christensen, P., and F. D. Cook.** 1978. *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int. J. Syst. Bacteriol. **28**:367–393.
8. **Dayan, J., and D. B. Sprinson.** 1970. Preparation of prephenic acid. Methods Enzymol. **17**:559–561.
9. **Doi, R. H., and R. T. Igarashi.** 1965. Conservation of ribosomal and messenger ribonucleic acid cistrons in *Bacillus* species. J. Bacteriol. **90**:384–390.
10. **Dubnau, D., I. Smith, P. Morell, and J. Marmur.** 1965. Genetic conservation in *Bacillus* species and nucleic acid homologies. Proc. Natl. Acad. Sci. USA **54**:491–498.
11. **Fowler, V. J., N. Pfennig, W. Schubert, and E. Stackebrandt.** 1984. Towards a phylogeny of phototrophic purple sulfur bacteria—16S rRNA oligonucleotide cataloging of 11 species of *Chromatiaceae*. Arch. Microbiol. **129**:382–387.
12. **Fox, G. E., E. Stackebrandt, R. B. Hespell, J. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. A. Tanner, L. J. Magrum, L. B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Leuhrsen, K. N. Chen, and C. R. Woese.** 1980. The phylogeny of prokaryotes. Science **209**: 457–463.
13. **Gibson, F.** 1964. Chorismic acid. Purification and some chemical and physical studies. Biochem. J. **90**:256–261.
14. **Gibson, J., E. Stackebrandt, L. B. Zablen, R. Gupta, and C. R. Woese.** 1979. A phylogenetic analysis of the purple photosynthetic bacteria. Curr. Microbiol. **3**:59–64.
15. **Hylemon, P. B., J. S. Wells, Jr., N. R. Krieg, and H. W. Jannasch.** 1973. The genus *Spirillum*: a taxonomic study. Int. J. Syst. Bacteriol. **23**:340–380.
16. **Jensen, R. A.** 1985. Biochemical pathways in prokaryotes can be traced backward through evolutionary time. Mol. Biol. Evol. **2**:92–108.
17. **Jensen, R. A., and G. S. Byng.** 1981. The partitioning of biochemical pathways with isozyme systems. Curr. Top. Biol. Med. Res. **5**:143–174.
18. **Palleroni, N. J.** 1983. The taxonomy of bacteria. Bioscience **33**:370–377.
19. **Shultz, J., M. A. Hermodson, and K. M. Herrmann.** 1981. A comparison of the amino-terminal sequences of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase isoenzymes from *Escherichia coli*. FEBS Lett. **131**:108–110.
20. **Stackebrandt, E., and C. R. Woese.** 1981. The evolution of prokaryotes, p. 1–32. *In* M. J. Carlile, J. F. Collins, and

B. E. B. Moseley (ed.), Molecular and cellular aspects of microbial evolution. 32nd Symposium Soc. General Microbiol. Cambridge University Press, Cambridge.

21. **Whitaker, R. J., A. Berry, G. S. Byng, M. J. Fiske, and R. A. Jensen.** 1985. Clues from *Xanthomonas campestris* about the evolution of aromatic biosynthesis and its regulation. J. Mol. Evol. **21**:139–149.
22. **Whitaker, R. J., G. S. Byng, R. L. Gherna, and R. A. Jensen.** 1981. Comparative allostery of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase as an indicator of taxonomic relatedness in pseudomonad genera. J. Bacteriol. **145**:752–759.
23. **Whitaker, R. J., M. J. Fiske, and R. A. Jensen.** 1982. *Pseudomonas aeruginosa* possesses two novel regulatory isozymes of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase. J. Biol. Chem. **257**:12789–12794.
24. **Woese, C. R., P. Blanz, and C. M. Hahn.** 1984. What isn't a pseudomonad: the importance of nomenclature in bacterial classification. System. Appl. Microbiol. **5**:179–195.
25. **Woese, C. R., P. Blanz, R. B. Hespell, and C. M. Hahn.** 1982. Phylogenetic relationships among various helical bacteria. Curr. Microbiol. **7**:119–124.
26. **Woese, C. R., E. Stackebrandt, W. G. Weisburg, B. G. Paster, M. T. Madigan, V. J. Fowler, C. M. Hahn, P. Blanz, R. Gupta, K. H. Nealson, and G. E. Fox.** 1984. The phylogeny of purple bacteria: the alpha subdivision. System. Appl. Microbiol. **5**:315–326.
27. **Woese, C. R., W. G. Weisburg, B. G. Paster, C. M. Hahn, R. S. Tanner, N. R. Kreig, H. P. Koops, H. Harms, and E. Stackebrandt.** 1984. The phylogeny of purple bacteria: the beta subdivision. System. Appl. Microbiol. **5**:327–336.
28. **Zuckerkandl, E., and L. Pauling.** 1965. Molecules as documents of evolutionary history. J. Theor. Biol. **8**:357–366.