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Comparative Allostery of 3-Deoxy-D-*arabino*-Heptulosonate 7-Phosphate Synthetase as an Indicator of Taxonomic Relatedness in Pseudomonad Genera

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Recently, an analysis of the enzymological patterning of L-tyrosine biosynthesis was shown to distinguish five taxonomic groupings among species currently named *Pseudomonas*, *Xanthomonas*, or *Alcaligenes* (Byng et al., J. Bacteriol. 144:247-257, 1980). These groupings paralleled with striking consistency those previously defined by ribosomal ribonucleic acid-deoxyribonucleic acid homology relationships. The comparative allostery of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthetase has previously been shown to be a useful indicator of taxonomic relationship at about the level of genus. The comparative allostery of DAHP synthetase was evaluated in relationship to data available from the same pseudomonad species previously studied. Species of *Xanthomonas* and some named species of *Pseudomonas*, e.g., *P. maltophilia*, were unmistakably recognized as belonging to group V, having a DAHP synthetase sensitive to sequential feedback inhibition by chorismate. This control pattern is thus far unique to group V pseudomonads among microorganisms. Group V organisms were also unique in their possession of DAHP synthetase enzymes that were unstimulated by divalent cations. Group IV pseudomonads (*P. diminuta*) were readily distinguished by the retro-tryptophan pattern of control for DAHP synthetase. Activity for DAHP synthetase was not always recovered in group IV species, e.g., *P. vesicularis*. The remaining three groups exhibited overlapping patterns of DAHP synthetase sensitivity to both L-phenylalanine and L-tyrosine. Individual species cannot be reliably keyed to group I, II, or III without other data. However, each group overall exhibited a different trend of relative sensitivity to L-tyrosine and L-phenylalanine. Thus, although enzymological patterning of L-tyrosine biosynthesis alone can be used to separate the five pseudomonad groups, the independent assay of DAHP synthetase control pattern can be used to confirm assignments. The latter approach is, in fact, the easiest and most definitive method for recognition of group V (and often of group IV) species.

The presence in nature of variant enzyme steps and of alternative allosteric patterns of control, within a suitably complex biochemical pathway, has been postulated to reflect an ancient evolutionary event whose conserved nature is apt to offer a stable indication of taxonomic relatedness or divergence among microbial species (21). Some instances of metabolic variations that have previously been used as indicators of natural relationships are the β-ketoadipate pathway for the dissimilation of aromatic compounds (6), the arginine biosynthetic system (37), homocysteine biosynthesis (9), and lysine biosynthesis among lower fungi (38).

The most extensive studies have been carried out with the shikimate pathway, which is the common portion of the biochemical route to aromatic amino acid biosynthesis (15). The allosteric enzyme 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthetase is the first enzymatic step of this complex and multibranched biosynthetic pathway. DAHP synthetase has been the focus of many detailed enzymological studies in *Escherichia coli* (34, 36), *Salmonella typhimurium* (16, 26), and *Bacillus subtilis* (17, 20). The diversity of allosteric control for DAHP synthetase was initially appreciated with the recognition of six distinctive control patterns following a comprehensive survey of DAHP synthetase in many species spanning 32 genera of microorganisms (19). Jensen et al. (19) observed striking conservation of a particular pattern of allosteric control in member species of a given genus.

Pseudomonas species exhibit marked biochemical versatility (7), occupying a broad range

of ecological niches. A few species are mammaliar pathogens, whereas numerous plant pathogens are recognized. The natural relationships between a number of *Pseudomonas* species have been investigated and characterized by morphological and nutritional properties (12), DNA-DNA hybridization (25, 27, 31, 32), and rRNA-DNA hybridization (29). Recently five taxa defined by enzymological patterning of L-tyrosine biosynthesis (4) were shown to correspond strikingly with the five rRNA homology groups separated by rRNA-DNA hybridization. Reinforcement of the latter results in defining the systematic relationships and the hierarchical groupings of classification that relate named species of *Pseudomonas*, *Xanthonomas*, and *Alcaligenes* to one another is provided by data obtained about the allosteric patterning of DAHP synthetase. Substantial experience and background information obtained from the extensive analysis of aromatic amino acid enzymology in *P. aeruginosa* (18, 30) has provided a firm base of comprehensive enzymological characterizations for pseudomonad microorganisms.

MATERIALS AND METHODS

Microorganisms. All strains were obtained from the American Type Culture Collection (ATCC), and we refer to strains in the text by both name and ATCC catalog number (Table 1).

In general, pseudomonad organisms seem to lack repression control over the synthesis of biosynthetic enzymes (7). *P. aeruginosa* does not possess a repressible DAHP synthetase (18), and we found no evidence for influence of media composition upon specific activity or upon the regulatory pattern of DAHP synthetase in a representative sampling of strains used in this study. Hence, cells were cultured in complex medium as a convenient source of extract preparations. Organisms were grown and harvested at the ATCC facility and shipped to our laboratory as cell pellets maintained during transit at dry-ice temperature.

Preparation of extracts. Extracts were prepared by sonication, using a Lab-Line Ultra-tip sonicator. Whole-cell pellets were suspended in 50 mM potassium phosphate buffer (pH 7.0) containing 1.0 mM dithiothreitol, sonicated, and centrifuged for 1 h at 150,000 × *g* to remove cell debris. After centrifugation the extract was passed through a Sephadex G-25 column equilibrated in starting buffer to remove small molecules and endogenous amino acids that might interfere with enzyme assays.

DAHP synthetase. This enzyme activity was assayed by the method of Calhoun et al. (5). Since it was commonly found that DAHP synthetase activity required the addition of divalent metal ions, extracts were initially assayed in the presence of various metal ions in order to optimize enzyme activity. Stock solutions of cobalt sulfate, manganese sulfate, or magnesium sulfate in 50 mM potassium phosphate buffer (pH 7.0) were added to reaction mixtures to a final concentration of 1.0 mM. Subsequent assays to determine the regulatory pattern were carried out in the presence of the metal ion producing maximal activity. Both substrates were present in saturating concentrations (2 mM final concentration), and the activity was determined to be linear with respect to protein concentration. Possible allosteric effectors of DAHP synthetase were tested singly at a final concentration of 0.5 mM. To uncover multi-effector (22) regulatory patterns (e.g., additive, cumulative, or synergistic feedback inhibition), assays in the simultaneous presence of 0.5 mM L-phenylalanine, L-tyrosine, and L-tryptophan were also included. Appropriate blanks were included to insure against interference of the chemical assay by effector molecules (19).

Arogenate (39) was tested as a possible inhibitor of DAHP synthetase activity, but this inhibition pattern was not found after assaying more than half of the species within each group surveyed. One apparent exception was *P. alboprecipitans* (19860), which demonstrated a sharp sensitivity to arogenate. However, *P. alboprecipitans* possesses a very active arogenate dehydratase which converts arogenate to L-phenylalanine, also a potent inhibitor of DAHP synthetase in this species. We suspect that the latter activity in crude extracts provides a trivial explanation for apparent effector action of arogenate upon DAHP synthetase. Fractionation of crude extracts to separate these activities is necessary to establish an unambiguous interpretation.

Biochemicals and chemicals. Amino acids, erythrose 4-phosphate, phosphoenolpyruvate, and Sephadex G-25 were obtained from Sigma Chemical Co. Prephenate was prepared as the barium salt from culture supernatants of a tyrosine auxotroph of *S. typhimurium* (10) and was converted to the potassium salt with excess K_2SO_4 before use. Arogenate was prepared from culture supernatants of a triple auxotroph of *Neurospora crassa* (24). The purification and isolation was modified according to Zamir et al. (39). Chorismate was isolated from the accumulation medium of the triple auxotroph *Klebsiella aerogenes* 62-1 and purified as the free acid (14). All chemicals were of the best grade commercially available.

RESULTS

Group I. Group I species had a very tightly regulated tyrosine-sensitive DAHP synthetase (see Fig. 1 for summary of group patterns). At 0.5 mM concentrations, L-tyrosine strongly inhibited DAHP synthetase activity. Inhibition was extremely variable. It must be noted that detailed analysis of DAHP synthetase in *P. aeruginosa* revealed an additional sensitivity to inhibition by phenylpyruvate and L-tryptophan that was interpreted as part of a physiologically significant multi-effector pattern (18). However, particular assay conditions are required to recognize allosteric sensitivity to these additional effector molecules. With the standardized assay conditions adopted for this survey, inhibition of *P. aeruginosa* DAHP synthetase by L-tryptophan was not detected and only moderate inhibition by phenylpyruvate was discerned. Al-

TABLE 1. *Species examined among named Pseudomonas, Xanthomonas, and Alcaligenes*[a]

Group I							
P. agarici	25941	*P. cichorii*	14120	*P. putida*	795	*P. syringae*	19872
P. agarici	25943	*P. fluorescens*[b]	13525	*P. putrefaciens*	8071	*P. syringae*	10205
P. alcaligenes	14909	*P. fragi*	4973	*P. stutzeri*	17591	*P. syringae*	19866
P. angulata	13453	*P. marginalis*	10844	*P. stutzeri*[b]	17588	*P. syringae*	13523
P. antirrhini	19871	*P. marina*	25374	*P. synxantha*	9890	*P. syringae*	19306
P. asplenii	10855	*P. mendocina*[b]	25411	*P. syringae*	12273	*P. syringae*	10862
P. aureofaciens	13986	*P. nautica*	27132	*P. syringae*	9005	*P. taetrolens*	4683
P. caryocyanae	19373	*P. panici*	19875	*P. syringae*	9004		
P. chlororaphis	17810	*P. perfectomarinus*	11405	*P. syringae*	8727		
P. cichorii	10858	*P. putida*[b]	12633	*P. syringae*	19322		
Group II				Group III			
P. caryophilii	25418	*P. marginata*	19302	*P. acidovorans*	17475	*P. saccharophila*[b]	15946
P. cepacia	10856	*P. methanolica*	21074	*P. alboprecipitans*	19860	*P. testosteroni*	17510
P. cepacia	17460	*P. pickettii*[b]	27511	*P. andropogonis*	23060	*A. faecalis*	15173
P. cepacia	25609	*P. pyrocinia*	15958	*P. delafieldii*[b]	17505	*A. faecalis*	15554
P. marginata[b]	10248	*P. solanacearum*	10692	*P. facilis*	11228	*A. faecalis*	8750
P. marginata	25417	*A. eutrophus*	17697	*P. palleronii*	17724	*A. paradoxus*	17713
P. marginata	10854			*P. palleronii*	17728		
Group IV		Group V					
P. diminuta[b]	11568	*P. gardeneri*	19865	*P. maltophilia*	17445	*X. campestris*	9924
P. diminuta	19146	*P. geniculata*	19374	*P. maltophilia*[b]	13637	*X. campestris*	8721
P. diminuta	13184	*P. hibiscicola*	19867	*X. albilineans*	12785	*X. campestris*	9563
P. vesicularis[b]	11426	*P. maltophilia*	17860	*X. axonopodis*	19312	*X. campestris*	12612

[a] Group assignments are based upon enzymological analysis of L-tyrosine biosynthesis (4). All species are listed with their corresponding ATCC number. Specific activities of DAHP synthetase ranged from a low of 0.054 nmol/min per mg for *P. syringae* (19872) to a high of 11.12 nmol/min per mg of *P. pickettii* (27511).

[b] Exact species used by Palleroni et al. (29) for rRNA-DNA hybridizations.

though routine assay conditions used in screening are thus illustrated not to necessarily identify all effector molecules, the high sensitivity to L-tyrosine inhibition nevertheless provides a reliable control pattern that characterizes member species of group I.

Several species assigned to the group I nomenspecies by DNA homology studies (27, 31) lacked significant DAHP synthetase activity but were assigned to group I on the basis of the other enzymological analyses (4; R. J. Whitaker et al., submitted for publication). It seems unlikely that repression of enzyme synthesis would be responsible for the observed low level of DAHP synthetase activity (7).

Group II. When species assigned to group II on the basis of other criteria (4, 29) were analyzed with respect to DAHP synthetase, certain trends of DAHP synthetase patterning were recognized. Compared with group I, group II species exhibited DAHP synthetases which were distinctly less sensitive to inhibition by L-tyrosine, less sensitive to the phenylalanine-tyrosine-tryptophan combination, rarely inhibited by phenylpyruvate, but noticeably more sensitive to inhibition by L-phenylalanine (Fig. 1). A tendency for inhibition by L-phenylalanine and L-tyrosine to be additive may suggest the presence of two regulatory isoenzymes.

Group III. The allostery of DAHP synthetase in species clearly belonging in group III based on other taxonomic evidence (4, 29, 32) was studied. Generally, a trend to increased sensitivity to inhibition by L-phenylalanine was seen with a concomitant decreased inhibition by L-tyrosine in group III organisms. Individual inhibitions did not appear to be additive (as with group II) when combinations of L-tyrosine and L-phenylalanine were used. That comparative allostery of DAHP synthetase alone does not unambiguously establish group placement is illustrated by *P. saccharophila* (15946), which demonstrated high levels of L-tyrosine inhibition (88%) and low levels of L-phenylalanine inhibition (15%). Although *P. saccharophila* is clearly within group III (4, 29), its pattern of DAHP synthetase regulation most resembles that of a group I species.

Currently named species of *Alcaligenes*, a genus of questionable taxonomic status (12), were also studied (Table 2). Interestingly, the allosteric pattern exhibited by species of *Alcaligenes* was very similar to the pattern described for group III pseudomonads. Indeed, *A. faecalis* (15173) demonstrated a cumulative pattern of L-tyrosine and L-phenylalanine feedback inhibition much in the same manner as *P. acidovorans* (17476), a member species of group III.

Group IV. The representatives of the fourth group of pseudomonads are listed in Table 3. The member species of this group, *P. diminuta* and *P. vesicularis* (1), have been shown by DNA-DNA hybridization and rRNA-DNA hybridization to constitute a distinct homology

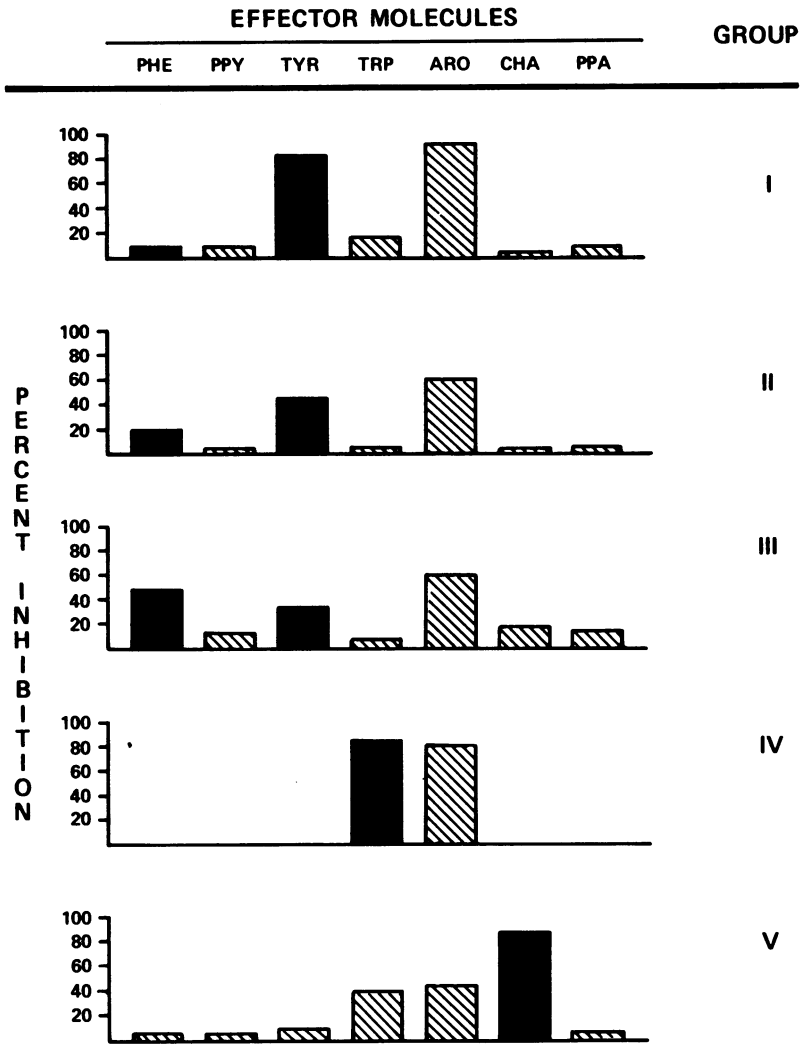

FIG. 1. *Comparative allostery of DAHP synthetase in member species of groups I through V. The levels of inhibition shown in the histogram represent the average inhibition calculated by using all species included in this study that were assigned to a given group. Inhibition by molecules of practical use in distinguishing the five groups are represented by solid blocks. Abbreviations for effectors are as in Table 2.*

TABLE 2. *Allosteric pattern for DAHP synthetase in Alcaligenes*

ATCC No.	Species	% Inhibition by effectors[a]						
		PHE	PPY	TYR	TRP	ARO	CHA	PPA
8750	*A. faecalis*	62	0	0	0	69	0	0
15173	*A. faecalis*	25	18	32	11	50	21	20
17697	*A. eutrophus*	16	22	5	0	55	17	10
17713	*A. paradoxus*	49	10	10	0	69	39	9

[a] Final concentration of each effector was 0.5 mM. PHE, Phenylalanine; PPY, phenylpyruvate; TYR, tyrosine; TRP, tryptophan; ARO, phenylalanine, tyrosine, and tryptophan; CHA, chorismate; PPA, prephenate.

group that is only distantly related to the other pseudomonad groups (29). The group IV pseudomonads had a regulatory pattern clearly distinct from the other patterns of allosteric control that characterized the remaining four pseudomonad groups. Allosteric control was accomplished solely by a retro-tryptophan pattern of unimetabolite inhibition.

Group V. Group V species (Table 4) were easily identified and distinguished from other pseudomonad species by virtue of their unique allosteric control pattern for DAHP synthetase. Chorismate, a branch-point metabolite of the shikimate pathway, was the primary regulatory molecule for DAHP synthetase in this group of organisms. Neither L-phenylalanine or L-tyrosine exerted much inhibition, but L-tryptophan exhibited substantial inhibitor action. The xanthomonad species listed in Table 4 are inseparable from species of *P. maltophilia*, using the regulatory pattern for DAHP synthetase as the criterion for separation. Using the technique of segmental DNA homology, Murata and Starr concluded that the overlap of *Pseudomonas* species and *Xanthomonas* species is minimal except for that of *P. maltophilia* and *Xanthomonas* (25).

Table 4 also includes *P. geniculata* (19374), *P. gardeneri* (19864), *X. albilineans* (12785), and *X. axonopodis* (19312). Our enzymological results place these four species within group V. It is interesting in this context that *P. geniculata* (19374) was originally deposited at the ATCC as a *maltophilia* strain, and a recent analysis revealed that *P. geniculata* possesses *maltophilia* characteristics (R. Gherna, personal communication). *P. gardeneri* has not been extensively studied, but this species has been described as possessing characteristics of *Xanthomonas* (Gherna, personal communication).

TABLE 3. *Allosteric pattern for DAHP synthetase in group IV species P. diminuta*[a]

ATCC No.	% Inhibition by effectors[b]						
	PHE	PPY	TYR	TRP	ARO	CHA	PPA
11568	0	0	0	85	76	0	0
19146	0	0	0	87	87	0	0

[a] *P. vescicularis* (11426) did not possess levels of DAHP synthetase activity adequate for determination of inhibition sensitivities.

[b] See Table 2.

Metal ion stimulation. Representative species of the five enzymological groups outlined in this paper are listed in Table 5 along with their relative activities in the presence of different divalent cations. Occasional instances of metal ion activation of DAHP synthetase have been previously documented (36). Generally, we found a degree of specificity in that only one particular metal ion provided maximal stimulation in any one species. When metal ions were found to be activators of DAHP synthetase, either cobalt or manganese produced the observed activation; in most cases these metal ions were not interchangeable.

The activation of DAHP synthetase by divalent cations provided a clear distinction between the group V pseudomonads and the other four pseudomonad groups. All of the species tested in this survey required a divalent cation for maximum activity except those species belonging to group V.

DISCUSSION

Five pseudomonad groupings recognized via molecular criteria. It has been shown that the chromosomal segment coding for rRNA is highly conserved relative to other segments of the bacterial genome (11, 13). The conservative nature of rRNA makes it an ideal taxonomic probe for delineating natural relationships. Indeed, Palleroni et al. (29) described five distinct homology groups which were as distantly related

TABLE 4. *Allosteric pattern for DAHP synthetase in group V*

ATCC No.	Species	% Inhibition by effectors[a]						
		PHE	PPY	TYR	TRP	ARO	CHA	PPA
13637	*P. maltophilia*	0		0	64	36	88	2
17445	*P. maltophilia*	15	9	7	4	30	72	13
9563	*X. campestris*	2	7	11	4	42	90	1
9924	*X. campestris*	0		0	71	53	96	5
8721	*X. campestris*	0	0	6	33	36	81	1
12612	*X. campestris*	0		0	70	57	96	0
19045	*X. campestris*	0	0	0	17	40	93	0
19864	*P. gardeneri*[b]	0	11	0	21	47	64	0
19374	*P. geniculata*[b]	27		20	56	46	81	16
12785	*X. albilineans*[b]	16	0	24	52	39	93	19
19312	*X. axonopodis*[b]	9	10	11	31	40	91	6
29074	*X. ampelina*[c]	99		94	56	99	63	49

[a] See Table 2.

[b] Species not yet described in terms of rRNA-DNA hybridization (29) that conform to the allosteric pattern of group V.

[c] Does not conform to the regulatory profile described for other xanthomonad species (see text).

TABLE 5. *Metal ion stimulation of DAHP synthetase*

ATCC No.	Species	Group	% Activity[a]			
			None	Mn^{2+}	Mg^{2+}	Co^{2+}
14120	*P. cichorii*	I	18	76	3	100
13525	*P. fluorescens*		15	53	6	100
12633	*P. putida*		4	100	57	69
17591	*P. stutzeri*		4	100	8	86
19872	*P. syringae (mori)*		7	65	2	100
10205	*P. syringae (aptata)*		23	74	14	100
19866	*P. syringae (helianthi)*		20	100	17	43
25418	*P. caryophilii*	II	19	1	6	100
25417	*P. marginata*		71	87	79	100
17476	*P. acidorovans*	III	16	100	10	16
17724	*P. palleronii*		75	100	66	57
15946	*P. saccharophila*		51	36	26	100
11568	*P. diminuta*	IV	14	36	18	100
19374	*P. geniculata*	V	100	89	91	75
17445	*P. maltophilia*		98	91	77	100
12785	*X. albilineans*		100	32	57	80
9924	*X. campestris*		100	72		
12612	*X. campestris*		100	66	56	69

[a] Maximum activity was arbitrarily assigned a value of 100.

to one another as to *E. coli* based upon data from rRNA-DNA hybridization. Palleroni et al. further suggested that each homology group eventually might be elevated to generic level, thus assembling five genera from the two current genera, *Pseudomonas* and *Xanthomonas*.

Very recently, the enzymological patterning of L-tyrosine biosynthesis has been shown to be an approach which yields strikingly parallel results to those provided by rRNA-DNA hybridization, accordingly segregating out the same five groups. The tyrosine pathway analysis depends upon a gestalt of characteristics in which the base of variability includes differences in dehydrogenase cofactor specificity, in the pathway itself, and in regulation of prephenate dehydrogenase or arogenate dehydrogenase.

Comparative allostery of DAHP synthetase. DAHP synthetase is a regulatory enzyme that is subject to the most diverse number of distinctly different patterns of feedback inhibition known for a single enzyme in microorganisms (22). A given pattern is remarkably constant at about the hierarchical level of genus, judging from the uniformity of DAHP synthetase allostery pattern in member species within genera. It has been suggested that, once established, a given control pattern may be highly conserved because of its complex integration with control circuits in other pathways, i.e., metabolic interlock (23). According to this thesis an alteration of DAHP synthetase, even though seemingly advantageous per se, may perturb overall metabolism sufficiently to constitute a selective disadvantage. The appreciation in recent years that the enzymatic arrangement of the aromatic pathway itself is variable provides another level of explanation to account for the observed diversity of DAHP synthetase allostery in nature. The independent approaches of enzymological patterning of tyrosine biosynthesis and of rRNA-DNA homology can separate five groups within *Pseudomonadaceae*. The analysis of DAHP synthetase alone cannot reliably be used to separate these groups. However, our results show that application of comparative allostery of DAHP synthetase provides useful data that complement the data provided by the former two approaches.

When a given strain which had been placed into one of the five subgroups on the basis of nucleic acid homology or tyrosine pathway patterning was evaluated for allosteric pattern of DAHP synthetase, each group was seen to differ as shown in Fig. 1. Group V and group IV species exhibit distinctive patterns. On the other hand, groups I, II, and III exhibit overlapping patterns. Although the ratio of sensitivity to inhibition by L-tyrosine to inhibition by L-phenylalanine is distinctly greatest in the order group I > group II > group III (ratios are about 8:2:1), these are group trends. Individual species of a given group may produce data characteristic overall of a different group. Species within group I always yielded a ratio greater than 3, but ratios greater than 3 were obtained with occasional species

belonging to groups II and III. Although group I species are usually recognized by ratios obtained, such data secured from group II and group III species were too similar to offer predictive value in analysis of unknown species. Separation of groups II and III is readily accomplished through the comparative allostery of arogenate-NADP dehydrogenase, however (4). Group V species not only are distinctive in allosteric control pattern for DAHP synthetase, but also differ from species in all other groups in having DAHP synthetase enzymes that uniformly are not stimulated by divalent cations.

Status of *Alcaligenes*. The enzymological patterning of L-tyrosine biosynthesis suggested that species currently placed within *Alcaligenes* can be reclassified within either group II or group III. *A. eutrophus* (17697) was the sole representative of group II among the relatively few strains of *Alcaligenes* studied. An interesting approach using an immunological analysis of glutamine synthetase by Baumann and Baumann (3) yielded results consistent with our conclusions about organisms currently classified as *Alcaligenes*. Although the present analysis of DAHP synthetase does not separate *A. eutrophus* from group III species of *Alcaligenes*, the overall results support the synonymy of *Alcaligenes* with group II or III. Data clinching the validity of reclassifying *Alcaligenes* as above should be obtained via rRNA-DNA homology studies (J. Johnson, manuscript in preparation).

New allosteric control pattern in group V. The inhibitory pattern demonstrated by group V species is thus far unique in nature. The effectiveness of chorismate as a regulatory molecule for DAHP synthetase would indicate an allosteric pattern denoted sequential feedback inhibition (19). This mode of regulation implies end-product sensitivity at the initial enzymatic step of each of the three branchlets leading from the branch-point metabolite, chorismate, whose accumulation in the presence of end products would then sequentially inhibit the first step of the common pathway, DAHP synthetase. A more detailed enzymological analysis of DAHP synthetase allostery in *X. campestris* (12612) will be presented separately (R. J. Whitaker, G. S. Byng, M. Fiske, R. A. Jensen, in preparation). Our results are consistent with the nucleic acid hybridization studies of Palleroni et al. (29) and the segmental homology studies of Murata and Starr (25), which suggest the similarity of *Xanthomonas* species and *P. maltophilia*. The clear-cut results obtained by analysis of DAHP synthetase, together with our previous enzymological results, leave little doubt that most species of *Xanthomonas* and some pseudomonads (e.g., *P. maltophilia*) belong to a common taxon.

Possible synonymy of *Acetobacter* and group IV. Phenotypic similarities of *Acetobacter* species and *P. diminuta* (group IV) have been previously noted (1). In this context it is suggestive that the retro-tryptophan pattern of control for DAHP synthetase found in group IV species was previously reported in *Acetobacter suboxydans* (19). Analysis of tyrosine pathway patterning and rRNA-DNA homology are clearly important in confirming or denying the validity of this synonymy.

Miscellaneous species of uncertain taxonomic status. The comparative allostery of DAHP synthetase has provided data to assist difficult taxonomic assignments. *P. solanacearum* (10692), a phytopathogenic species of considerable economic importance (28), was assigned to section III pseudomonads (group III) in *Bergey's Manual* (12), primarily due to inability to use arginine and betaine as sole carbon sources. However, rRNA-DNA hybridization studies have placed *P. solanacearum* in group II (29). Within group II, DNA-DNA hybridization experiments (28) (an apparently more finely tuned methodology) showed that *P. cepacia* and *P. solanacearum* are not closely related. The enzymological patterning of L-tyrosine biosynthesis further supports the assignment of *P. solanacearum* (10692) to group II. Although not compelling per se, the allosteric profile of DAHP synthetase for *P. solanacearum* (10692) is indeed typical of a group II pseudomonad (Fig. 1).

P. pickettii (27511) has been shown to be similar to *P. solanacearum* on the basis of DNA homology (33) and to belong in group II on the criterion of rRNA-DNA homology (29). The enzymological patterning of tyrosine biosynthesis (4) and the analysis of DAHP synthetase (this paper) all support assignment of *P. pickettii* (27511) to group II.

Among the xanthomonads examined in this survey, *X. ampelina* (29074) does not conform to the characteristic regulatory pattern established for these organisms (Table 4). In addition to the expected inhibition by chlorismate and L-tryptophan, there is almost complete inhibition of DAHP synthetase activity by both L-phenylalanine and L-tyrosine. In addition, *X. ampelina* (29074) requires the addition of cobalt for maximum DAHP synthetase activity whereas all other group species demonstrate no such requirement. Starr et al. (35) have questioned the inclusion of *X. ampelina* among the named xanthomonads because it does not form pigments typical of these organisms.

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