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Diverse Enzymological Patterns of Phenylalanine Biosynthesis in Pseudomonads Are Conserved in Parallel with Deoxyribonucleic Acid Homology Groupings

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L-Tyrosine biosynthesis in nature has proven to be an exceedingly diverse gestalt of variable biochemical routing, cofactor specificity of pathway dehydrogenases, and regulation. A detailed analysis of this enzymological patterning of L-tyrosine biosynthesis formed a basis for the clean separation of five taxa among species currently named *Pseudomonas*, *Xanthomonas*, or *Alcaligenes* (Byng et al., J. Bacteriol. **144**:247-257, 1980). These groupings paralleled taxa established independently by ribosomal ribonucleic acid/deoxyribonucleic acid (DNA) homology relationships. It was later found that the distinctive allosteric control of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in group V, a group dominated by most named species of *Xanthomonas* (Whitaker et al., J. Bacteriol. **145**:752-759, 1981), was the most striking and convenient criterion of group V identity. Diversity in the biochemical routing of L-phenylalanine biosynthesis and regulation was also found, and phenylalanine patterning is in fact the best single enzymatic indicator of group IV (*Pseudomonas diminuta* and *Pseudomonas vesicularis*) identity. Enzymological patterning of L-phenylalanine biosynthesis allowed discrimination of still finer groupings consistently paralleling that achieved by the criterion of DNA/DNA hybridization. Accordingly, the five ribosomal ribonucleic acid/DNA homology groups further separate into eight DNA homology subgroups and into nine subgroups based upon phenylalanine pathway enzyme profiling. (Although both fluorescent and nonfluorescent species of group I pseudomonads fall into a common DNA homology group, fluorescent species were distinct from nonfluorescent species in our analysis.) Hence, phenylalanine patterning data provide a relatively fine-tuned probe of hierarchical level. The combined application of these various enzymological characterizations, feasibly carried out in crude extracts, offers a comprehensive and reliable definition of 11 pseudomonad subgroups, 2 of them being represented by species of *Alcaligenes*.

Useful criteria for the reliable discrimination of taxa require (i) a degree of variability that is appropriate to distinguish the number of groups to be sorted at a given hierarchical level, and (ii) a high degree of conservation of the variable characters employed. The steadily increasing documentation of variant pathways for amino acid biosynthesis has been reviewed by Jensen (16). An interpretation of major lines of evolution in fungi was based upon the presence of two variant biochemical pathways for lysine biosynthesis in nature (24, 39). Six basic pathway patterns leading to tyrosine and phenylalanine synthesis alone have been described by Byng et al. (G. S. Byng, J. F. Kane, and R. A. Jensen, Crit. Rev. Microbiol., in press; also see references 14, 19, 22, 30, 37, 38). The most extensive studies that document the conserved nature of multiple patterns of allosteric control which oversee complex biochemical pathways have been with 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase (17, 18, 20, 21). Each distinctive allosteric control pattern for DAHP synthase was found to be a highly conserved trait among member species of a given genus. The array of metabolic and regulatory interactions that may account for the conservatism of the different enzymological patterns which exist for sufficiently complex biochemical pathways has been discussed (20).

Pseudomonad bacteria comprise a diverse representation of soil and water species, being

generally noted for biochemical versatility (10). Only a few species are mammalian pathogens, whereas a broad range of plant pathogens are known. Enzymological patterning (biochemical route, cofactor specificity, and allosteric pattern of regulation) of L-tyrosine biosynthesis separated five distinct taxonomic groups from member species of the named genera *Pseudomonas*, *Xanthomonas*, and *Alcaligenes* (7). Although the study was less comprehensive and did not include named *Alcaligenes* species, a previous approach of rRNA/DNA hybridization also separated five distinct groupings (29). Each strain common to the two studies fell into the same group. These completely independent approaches therefore sort taxa at virtually the same hierarchical level. The pattern of allosteric control for DAHP synthase provided confirmatory data in support of many pseudomonad strain groupings (40). Group V (and often group IV) species are in fact grouped most definitively by DAHP synthase assay. Data consistent with the aforementioned pseudomonad groupings have also been obtained through immunological comparison of glutamine synthetases (5).

The approach of DNA/DNA homology partitions taxa at a finer level and separates nine species clusters from the five rRNA/DNA groups (25, 27, 32). We found that enzymological patterning of L-phenylalanine synthesis sorts at a finer hierarchical level than does enzymological patterning of L-tyrosine synthesis. Groupings defined by phenylalanine patterning paralleled almost exactly the DNA/DNA homology groupings. These independent defining criteria of pseudomonad groupings are summarized in Table 1.

MATERIALS AND METHODS

Microbial species. A collaborative arrangement with the American Type Culture Collection (ATCC) provided authentic strains of known pedigree and enduring status. Our rationale emphasizes the importance for all stocks to be generally available for distribution and to be maintained indefinitely under conditions that maximize unaltered preservation of properties. Cultures were grown in a rotary shaker at 1,000 rpm in media and at temperatures recommended in the ATCC catalog of strains (1). All cell populations were grown and harvested at the ATCC facility, where authenticity and purity of cultures were established before shipment to New York in dry ice. The cell pellets were maintained in New York at −80°C until extract preparation. The total species surveyed can be compiled from Tables 2 through 4.

Preparation of extracts. Cell suspensions were sonicated in 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM dithiothreitol, followed by high-speed centrifugation at 150,000 × *g* for 1 h. Extracts were subsequently passed through a Sephadex G-25 column to remove small molecules before analysis.

Enzyme assays. Assay conditions of proportionality with respect to protein concentration and time at saturating substrate concentrations as optimized for *Pseudomonas aeruginosa* (30) were employed with all enzyme extracts.

Prephenate dehydratase. Reaction mixtures (200 μl) contained 1 mM prephenate, 50 mM potassium phosphate (pH 7.0), and enzyme. After incubation at 37°C for 20 min, 800 μl of 2.5 N NaOH was added, and phenylpyruvate was measured spectrophotometrically at 320 nm (30). Inhibition or activation of prephenate dehydratase was determined in the presence of 0.5 mM L-phenylalanine or 0.5 mM L-tyrosine, respectively. Due to the occasional presence of transamination of L-tyrosine to 4-hydroxyphenylpyruvate by contaminating aminotransferase activity, each tube containing 0.5 ml of L-tyrosine was scanned from 300 to 340 nm to determine the wavelength of peak absorbance. Activation activity was correlated to peak activities at 320 nm, and transamination was correlated to peak activities at 331 nm, the wavelength used to measure 4-hydroxyphenylpyruvate.

Arogenate dehydratase. Reaction mixtures (150 μl) contained 50 mM potassium phosphate (pH 7.5), 1 mM arogenate, and enzyme. These were assayed by the method of Shapiro et al. (35). An attempt to compare the possible variation in regulatory properties of arogenate dehydratase by L-phenylalanine was too cumbersome for this survey owing to the increased fluorescence obtained upon addition of L-phenylalanine. The possibility of L-tyrosine activation was tested in representative strains from each subgroup (see Table 1). Evidence of enzyme activation was not seen in any species tested.

Other analytical techniques. Protein concentrations were estimated by the method of Bradford (6) as described in Bio-Rad Technical Bulletin 1051.

Biochemicals and chemicals. Amino acids and Sephadex G-25 were obtained from the Sigma Chemical Co. Barium prephenate was prepared from culture supernatants of a tyrosine auxotroph of *Salmonella typhimurium* (11) and was converted to the potassium salt with excess K_2SO_4 before use. L-Arogenate was prepared from the culture supernatants of a triple auxotroph of *Neurospora crassa* (23), the purification and isolation being modified according to Zamir et al. (41).

RESULTS

Parallel clustering of species by DNA homology and dehydratase patterning. Representative species previously assembled into groups I to V by the dual and independent criteria of rRNA/DNA homology (29) and enzymological patterning of L-tyrosine biosynthesis (7) are shown in Table 2. These further split into eight subgroups, as defined by DNA homology, and correlate perfectly with subgroups defined by enzymological patterning of L-phenylalanine biosynthesis (except that DNA homology group 1 corresponds with two subgroups defined by enzyme patterning). Although

TABLE 1. *Correspondence of pseudomonad groupings discriminated by nucleic acid hybridization or by enzymological patterning*

Allosteric effectors of DAHP synthase[a]	Tyrosine branchlet patterning profile[b]	rRNA homology group[c]	Species[d]	DNA homology group[e]
L-Tyrosine	[PPA:NAD] [AGN:NAD]	I	*P. stutzeri* *P. mendocina* *P. alcaligenes* *P. aeruginosa* *P. putida* *P. fluorescens* *P. syringae* *P. cichorii*	1
L-Tyrosine + L-phenylalanine	PPA:NAD/NADP [AGN:NAD/NADP]	II	*P. cepacia* *P. marginata* *P. pseudomallei* *P. mallei* *P. caryophylli*	2a
			P. pickettii *P. solanacearum*	2b
L-Tyrosine + L-phenylalanine	PPA:NAD/NADP AGN:NAD/NADP	III	*P. acidovorans* *P. testosteroni*	3a
			P. delafieldii *P. facilis*	3b
			P. saccharophila	3c
L-Tryptophan	PPA:NAD/NADP	IV	*P. diminuta* *P. vesicularis*	4
Chorismate	[PPA:NAD] AGN:NAD	V	*P. maltophilia* *X. campestris*	5

[a] As shown in reference 41.
[b] As defined in reference 7. Abbreviations: PPA:NAD denotes the presence of NAD-linked prephenate dehydrogenase; AGN:NAD denotes the presence of NAD-linked arogenate dehydrogenase; NAD/NADP refers to enzyme reactivity with either cofactor. Enclosure within brackets indicates that the enzyme activity shown is sensitive to inhibition by L-tyrosine; otherwise the indicated activity is insensitive to inhibition.
[c] As defined by rRNA/DNA hybridization (29).
[d] Named species of *Pseudomonas* or *Xanthomonas*.
[e] As defined in references 25, 27, and 32.

specific activities are given in Table 2, no significance is attached to variations in specific activity values between species, and no significance is attached to the variation in ratio of prephenate dehydratase to arogenate dehydratase. Only presence or absence of activity was interpretable. On the other hand, factor ranges of tyrosine activation [1, 2–3, 3–4, 6–12, >15] were interpretable.

Phenylalanine patterning groups Ia and Ib correspond to obvious physiological groups, namely, nonfluorescent species (Ia) and fluorescent species (Ib). Subgroup Ia lacks arogenate dehydratase, apparently depending upon the phenylpyruvate route as the sole pathway to L-phenylalanine (see Fig. 1). Subgroup Ib possesses dual enzyme sequences to L-phenylalanine, a pattern studied in considerable detail with *P. aeruginosa* (9, 30, 31). Prephenate dehydratases of group I species uniformly lack sensitivity to activation by L-tyrosine.

All group II species possessed both prephe-

TABLE 2. *Enzymological patterning of* L-*phenylalanine biosynthesis in pseudomonad species representing groups I through V*

rRNA homology group[a]		ATCC No.	Species	DNA homology group[b]	Phenylalanine patterning group[c]	Specific activity[d] AGN	PPA	Tyrosine activation factor[e]
I	*	17588	*P. stutzeri*	1	Ia	0	27.1	0.7
		17591	*P. stutzeri*			0	35.5	0.7
		14909	*P. alcaligenes*			0	6.6	1.1
	*	25411	*P. mendocina*			0	13.1	1.0
			P. aeruginosa[f]		Ib	0.3	9.8	0.8
	*	12633	*P. putida*			1.2	11.9	1.3
	*	13525	*P. fluorescens*			0.3	16.3	1.1
		10862	*P. syringae*			0.1	2.6	1.0
		19872	*P. mori*			0.8	5.1	1.0
		10205	*P. aptata*			0.2	12.1	1.0
		10857	*P. cichorii*			1.5	16.0	1.1
II		10856	*P. cepacia*	2a	IIa	0.2	7.1	3.0
		17760	*P. cepacia*			1.4	10.8	1.7
	*	19248	*P. marginata*			0.2	3.2	2.1
		10854	*P. marginata*			0.2	4.3	3.8
		19302	*P. marginata*			0.2	5.9	2.5
		25418	*P. carophylli*			0.1	3.1	2.0
	*	27511	*P. pickettii*	2b	IIb	0.1	9.6	1.0
		10692	*P. solanacearum*			7.1	15.6	1.1
III		17476	*P. acidovorans*	3a	IIIa	0	2.1	6.3
		17510	*P. testosteroni*			0	5.9	11.5
	*	17505	*P. delafieldii*	3b	IIIb	0.9	1.9	3.4
		11228	*P. facilis*			0.2	1.6	4.3
	*	159-6	*P. saccharophila*	3c	IIIc	0.9	0.9	19.7
IV	*	11568	*P. diminuta*	-	IV	0.5	0	
		1318-	*P. diminuta*			0.9	0	
	*	11426	*P. vesicularis*			0.6	0	
V	*	13637	*P. maltophilia*	5	V	0.7	0.4	1.0
		17806	*P. maltophilia*			0.3	0.8	1.3
		8721	*X. campestris*			0.1	5.9	1.0

[a] Groups defined by rRNA/DNA homology techniques. Identical groupings have been made independently by enzymological analysis of tyrosine patterning (7). The symbol * denotes absolute strain synonymy with those used by Palleroni et al. (29). The remaining strains can be regarded as representative on the following bases. *P. acidovorans* ATCC 17476 was identical to the Palleroni strain ATCC 15668, and *P. testosteroni* ATCC 17510 was identical to Palleroni strain ATCC 11996 on the criterion of hybridization against rRNA from *Janthinobacterium lividum* NCTC 9796 (12). *P. facilis* ATCC 11228 (the type strain) and ATCC 17695 (the Palleroni strain) were also identical after DNA hybridization to *J. lividum* rRNA. Species of *P. cepacia* were Stanier isolates, and all exhibit phenotypic properties and guanine plus cytosine base ratios similar to those of Palleroni strain ATCC 17759.

[b] DNA homology groups are documented in references 2, 3, 26, 28, 32, 33, and 36.

[c] Group defined by data contained in this paper.

[d] Specific activities of arogenate (AGN) dehydratase and prephenate (PPA) dehydratase are expressed as nanomoles of product per minute per milligram of protein. Values of 0 are <0.01 and <0.06 for AGN dehydratase and PPA dehydratase, respectively.

[e] The activity of prephenate dehydratase in the absence of L-tyrosine was assigned a relative activity of 1.0 in comparison with otherwise identical assays in the presence of 0.5 mM L-tyrosine.

[f] Strain 1 (15) obtained from B. Holloway.

nate dehydratase and arogenate dehydratase. Subgroups were recognized on the criterion of sensitivity of prephenate dehydratase to activation by L-tyrosine. Subgroup IIa enzymes are activated by a factor of 2 to 3, whereas prephenate dehydratase enzymes of subgroup IIb are invulnerable to activation by tyrosine. *P. solanacearum* (ATCC 10692), a phytopathogen of considerable economic importance (27), was assigned to section III pseudomonads (group III) in *Bergey's Manual* (13). The proper niche of this misplaced species within group II is mutually confirmed by rRNA/DNA homology (29) and our previous enzymological analysis (7). The location of *P. solanacearum* in a DNA homology group different from *P. marginata* and *P. cepacia* fits our independent identification of *P. solanacearum* in subgroup IIb. Species of *P. solanacearum* and *P. cepacia* were also cleanly separated from one another on the basis of comparative starch-gel electrophoresis of 11 enzyme activities (4). Furthermore, *P. pickettii* (ATCC 27511), a group II member by rRNA/DNA homology (29), was placed in a common DNA homology group with *P. solanacearum* (34).

Group III species were the most diverse group

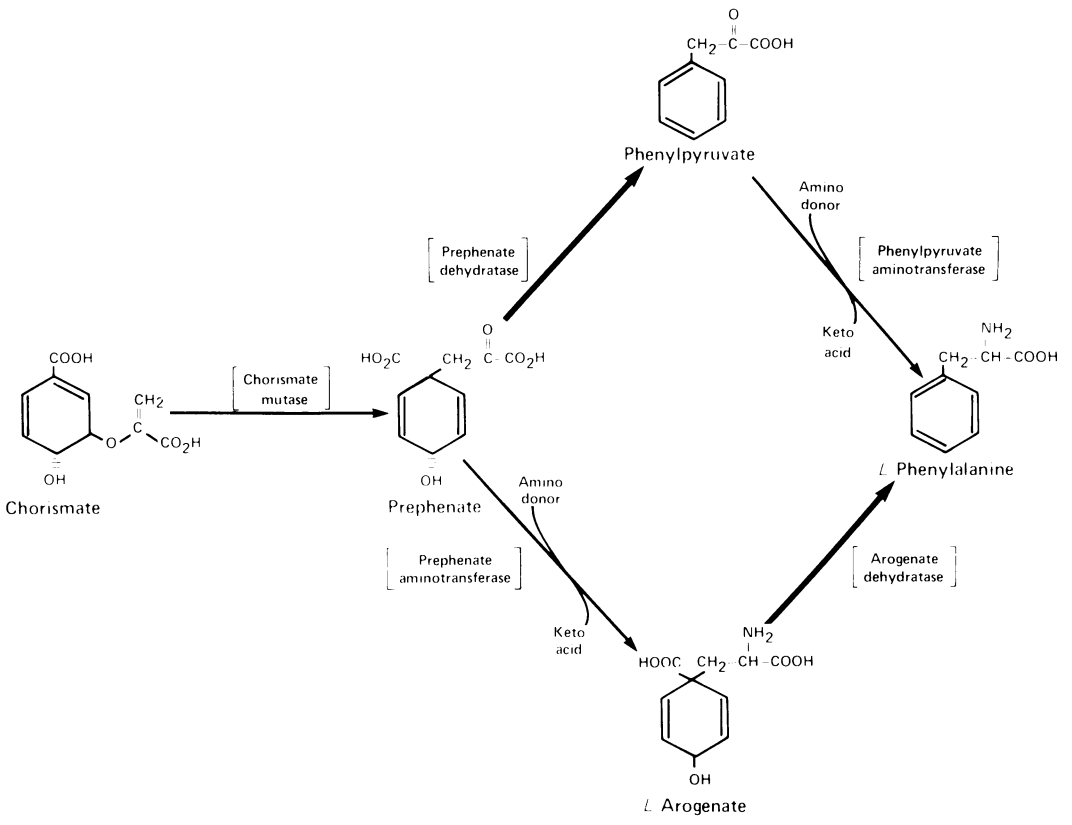

FIG. 1. *Diversity of biochemical routing to* L-*phenylalanine. Dehydratase patterning depends upon the presence or absence of prephenate dehydratase and arogenate dehydratase, denoted with heavy arrows. An additional variable feature has been the sensitivity of prephenate dehydrase to activation by* L-*tyrosine.*

in terms of DNA homology (33), a finding also paralleled by enzymatic profiling (Table 2). Members of subgroup IIIa lacked arogenate dehydratase activity. The remaining group III subgroups possessed both dehydratase activities. *P. saccharophila*, the sole representative species of subgroup IIIc, possessed a prephenate dehydratase of extreme hypersensitivity to L-tyrosine activation (20-fold). Subgroup IIIb species displayed a three- to fourfold activation of prephenate dehydratase by L-tyrosine. Subgroup IIIa also possessed a tyrosine-activated enzyme (6- to 12-fold). Note that although subgroups Ia and IIIa lack arogenate dehydratase in common, they may be distinguished by assessment of presence or absence of tyrosine-activatable prephenate dehydratase.

Group IV species possess a distinctive enzymological profile among pseudomonad species, lacking prephenate dehydratase and possessing arogenate dehydratase. This appears to be the most convenient and reliable enzymological indicator of group IV identity, i.e., easier than tyrosine patterning (7) or allostery of DAHP synthase (40).

Group V species all possessed both dehydratase enzymes and showed no significant activation of prephenate dehydratase by L-tyrosine. Thus member species of group V, subgroup Ib, and subgroup IIb cannot be distinguished on the basis of enzymological patterning of L-phenylalanine biosynthesis alone. In the latter cases a prior evaluation by tyrosine patterning, rRNA homology, or DAHP synthase allostery must be done to establish the major group.

Tentative group placement of other species. Table 3 contains a listing of other species, many of which have not yet been grouped by rRNA/DNA homology, but which have been placed by enzymological patterning of L-tyrosine biosynthesis (7) and, in some cases, by comparative allostery of DAHP synthase (40). Our provisional subgroup assignments are based upon the evaluation of data as presented in Table 2.

Species of *Alcaligenes*. Named species of *Alcaligenes* fall into groups II and III by tyro-

TABLE 3. *Enzymological patterning in phenylalanine biosynthesis for strains not yet grouped by nucleic acid hybridization*[a]

Subgroup assignment	Species	ATCC No.
Ia	Pseudomonas agarici	25943
	Pseudomonas fragi	4973
	Pseudomonas agarici	25941
	Pseudomonas taetrolens	6683
Ib	*Pseudomonas syringae	12273
	*Pseudomonas syringae	19322
	*Pseudomonas syringae	19304
	*Pseudomonas syringae	8727
	*Pseudomonas putida	790
	*Pseudomonas cichorii	14120
	Pseudomonas panici	19875
	Pseudomonas angulata	13453
	Pseudomonas antirrhini	19871
	Pseudomonas synxantha	9098
	Pseudomonas aureofaciens	13986
	Pseudomonas chlororaphis	17810
IIa	*Pseudomonas cepacia	25609
	*Pseudomonas marginata	25417
IIb	Pseudomonas pyrocinia	15958
	Pseudomonas papaveris	13011
	Pseudomonas huttiensis	14670
IIIa	Pseudomonas palleroni	17728
	Pseudomonas palleroni	17724
IIIb	Pseudomonas alboprecipitans	19860
	Pseudomonas andropogonis	23068
IV	Pseudomonas diminuta	19146
V	*Pseudomonas maltophilia	17445
	*Xanthomonas campestris	12612
	*Xanthomonas campestris	19316
	*Xanthomonas campestris	9563
	Pseudomonas gardneri	19865
	Pseudomonas geniculata	19374
	Xanthomonas axonopodis	19312
	Xanthomonas albilineans	12785

[a] Major groupings were previously established by enzymological patterning of L-tyrosine biosynthesis (7) and by comparative allostery of DAHP synthase (40). Groups I, II, and III were then subdivided by enzymological patterning of L-phenylalanine biosynthesis as illustrated by data shown in Table 2. * symbols denote species that have been grouped on the criterion of nucleic acid hybridization but were not included in Table 2. Numbers following specific epithets are ATCC numbers. Data arranged as in Table 2 are available upon request. A number of species included in our study would have fit into group Ia on the criteria of DAHP synthase and phenylalanine patterning, but were not successfully established as group I species by tyrosine patterning (7). These were *P. putrefaciens* ATCC 8071, *P. nautica* ATCC 27132, *P. perfectomarinus* ATCC 14405, and *P. perlurida* ATCC 490. Similarly, *P. marina* ATCC 23574 could pass for a group Ib species on the criteria of DAHP synthase and phenylalanine patterning, but did not screen as a group I species on the basis of tyrosine patterning.

sine pathway patterning (7) and by immunological analysis of glutamine synthetase (6). The available data are summarized in Table 4. The phenylalanine patterns of group II *Alcaligenes* and group III *Alcaligenes*, although identical with one another, are different in patterning from all other group II and group III species. Thus, *A. eutrophus* is defined by the two leftward criteria (Table 4) as group II and is assigned to subgroup IIx on the criterion of its unique phenylalanine patterning (i.e., lack of arogenate dehydratase) among group II species. A similar basis supports the assignment of the remaining *Alcaligenes* species to subgroup IIIx.

DISCUSSION

Hierarchical level of enzymological patterning. Our previous study (7) of tyrosine pathway dehydrogenase patterning delineates taxa at a broader level than does phenylalanine pathway dehydratase patterning (this study). It is intriguing that: (i) the totally distinct approaches of dehydrogenase patterning and rRNA/DNA homology discern taxa at an equivalent hierarchical level; (ii) the five rRNA/DNA homology groups can be further separated into eight subgroups by DNA/DNA hybridization; (iii) the five dehydrogenase patterning groups can be separated into nine subgroups by dehydratase patterning; and (iv) DNA homology groups and dehydratase groups are virtually synonymous.

In one case dehydratase patterning separated two groups which were grouped together on the basis of DNA homology. Whereas group I species have been considered to be an exceptionally large overlapping DNA homology complex (25, 28, 29, 36), dehydratase patterning cleanly separates fluorescent species from nonfluorescent species within group I pseudomonads.

TABLE 4. *Enzymological patterning of* L*-phenylalanine biosynthesis in named species of Alcaligenes*

Tyrosine patterning group[a]	Gln syn immunology group[b]	Phenylalanine patterning group	ATCC no.	Species	Sp act[c]		Tyrosine activation factor[d]
					AGN	PPA	
II	II	IIx	17697	*A. eutrophus*	0	6.7	2.7
III		IIIx	15173	*A. faecalis*	0	3.8	2.9
III		IIIx	8750	*A. faecalis*	0	6.3	2.7
III	III	IIIx	17713	*A. paradoxus*	0	6.2	2.0

[a] As per reference 7.

[b] As per reference 5 with glutamine synthetase (gln syn).

[c] Specific activities of arogenate dehydratase (AGN) and prephenate dehydratase (PPA), expressed as nanomoles of product per minute per milligram of protein.

[d] The activity of prephenate dehydratase in the absence of L-tyrosine was assigned a relative activity of 1.0 in comparison with otherwise identical assays in the presence of 0.5 mM L-tyrosine.

Status of *Alcaligenes*. Pseudomonad groups II and III are known to be the most proximal of the five groupings. Results given in Table 4 are consistent with group II *Alcaligenes* and group III *Alcaligenes* representing the extreme boundaries of their groupings and therefore occupying an intermediate position between groups II and III. As shown in Table 5, a feasible continuum of subgroups can be extended further into group II and group III. Thus, *A. eutrophus* (IIx) is closer to IIa than to IIb on the criterion of activation of prephenate dehydratase. *A. paradoxus* (IIIx) is most like *P. acidovorans* (IIIa) on the criterion of lack of arogenate dehydratase. These interpretations predict that *A. eutrophus* will form a separate DNA homology group (2x) within group II, and that the remaining species of *Alcaligenes* will form at least one DNA homology group (3x) within group III. The results of Baumann and Baumann (5) suggest that at least one DNA homology group within group III *Alcaligenes* exists that is distinct from *A. paradoxus*.

Possible expansion of group IV species. Group IV species are unique among pseudomonads in the absence of prephenate dehydratase, and this assay offers perhaps the best single indicator of group IV identity. It seems likely that named species of *Acetobacter* belong to this group. Phenotypic similarities of *Acetobacter* species to *P. diminuta* (group IV) have been noted (2). The DAHP synthase allostery in *Acetobacter suboxydans* is the retro-tryptophan pattern (20), a distinctive characteristic of group IV (see Table 1). If pending studies employing rRNA/DNA homology and tyrosine patterning confirm the proper placement of *Acetobacter* within group IV, it seems quite possible that phenylalanine patterning may sort out subgroups existing at a finer hierarchical level.

Fine-tuned discrimination of subgroups. The enzymological patterning analysis in phenylalanine biosynthesis entails the presence or absence of two dehydratase activities and different ranges of sensitivity of prephenate dehydratase (if present) to activation by L-tyrosine. A summary of dehydratase patterning arrangements is given in Table 6 to show their utility in separating out the subgroups shown.

Many of the subgroups are sufficiently distinctive to be recognized without prior placement in one of the five major groups by rRNA/DNA hybridization or by dehydrogenase patterning. On the other hand, dehydratase patterning alone does not separate member species of subgroups Ib and IIb from group V; likewise, subgroups IIx and IIIx are not discriminated by dehydratase patterning alone. However, once the five major group assignments are unambiguously established by rRNA/DNA homology, DAHP synthase allostery, dehydrogenase patterning, or any combination of these, then each subgroup can be equated to a pattern that is unique within the major group.

Biochemical routing of aromatic amino acid biosynthesis in nature. Byng et al. (in press) have outlined the diverse patterns that lead to biosynthesis of L-phenylalanine and L-tyrosine in nature. *Euglena gracilis* represents one extreme where only arogenate dehydratase and arogenate dehydrogenase are used (8), so that L-arogenate (41) (rather than prephenate, as in *Escherichia coli* or *Bacillus subtilis*) is the metabolic branch-point. Of the half-dozen general patterns (Byng et al., in press), pseudomonad organisms currently exemplify much of the pathway diversity known. Thus, *P. diminuta* (group IV), employing only the arogenate route to phenylalanine and only the 4-hydroxyphenylpyruvate route to tyrosine, exemplifies a unique pattern. Other pseudomonad species, most documented in *P. aeruginosa* (30, 31), possess simultaneously present dual pathways to both phenylalanine and tyrosine.

TABLE 5. *Species of Alcaligenes as a continuum bridge between pseudomonad groups II and III*

Tyrosine patterning group[a]	Gln syn immunology group[b]	DNA homology group[c]	Phenylalanine patterning group[d]	Representative species	Presence (+) or absence (−) of:		Tyrosine activation factor[e]
					Arogenate dehydratase	Prephenate dehydratase	
II		2b	IIb	*Pseudomonas pickettii*	+	+	1
II	II	2a	IIa	*Pseudomonas cepacia*	+	+	2-3
II	II	(2x)	IIx	*Alcaligenes eutrophus*	−	+	2-3
III	III	(3x)	IIIx	*Alcaligenes paradoxus*	−	+	2-3
III	III	3a	IIIa	*Pseudomonas acidovorans*	−	+	6-12
III		3b	IIIb	*Pseudomonas facilis*	+	+	3-4
III		3c	IIIc	*Pseudomonas saccharophila*	+	+	>15

[a] As defined in reference 7.
[b] As defined in reference 5 with glutamine synthetase (gln syn).
[c] As established in references 2, 3, 26, 28, 32, 33, and 36.
[d] As established in Tables 2 and 4.
[e] Range of activation factors for stimulation of prephenate dehydratase by 0.5 mM L-tyrosine.

TABLE 6. *Pseudomonad subgroup profiles of phenylalanine patterning*

Subgroup designation[a]	Prephenate dehydratase[b]	Arogenate dehydratase[b]	Tyrosine activation factor[c]
Ia	+	−	1
Ib	+	+	1
IIb	+	+	1
IIa	+	+	2-3
IIx	+	−	2-3
IIIx	+	−	2-3
IIIa	+	−	6-12
IIIb	+	+	3-4
IIIc	+	+	>15
IV	−	+	
V	+	+	1

[a] See Tables 4 and 5 regarding the tentative status subgroups IIx and IIIx.
[b] Presence or absence of activity denoted + or −, respectively.
[c] Sensitivity of prephenate dehydratase to L-tyrosine-mediated activation, as in Table 2.

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