Amino Acid Metabolism in the Genus *Propionibacterium*[1] (36179)

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The unique metabolic feature of members of the genus *Propionibacterium* is the conversion of carbohydrate to propionate, with varying amounts of acetate, succinate, and carbon dioxide (1, 2). The enzymatic steps for propionate fermentation, deciphered in elegant detail by Wood and associates (2) and Allen *et al.* (3) are catalyzed by a biotin-dependent oxaloacetate transcarboxylase, a CoA transferase, a cobamide-dependent methylmalonyl-CoA isomerase, and a methylmalonyl-CoA racemase. Kaziro and Ochoa (4) have elucidated the partially similar enzymes for the reversed flow of carbon from propionate to succinate in animal tissues. The mechanism for the role of biotin and vitamin B_{12} coenzyme in these enzymatic reactions has received detailed attention recently (3–5).

Knowledge of these distinctive characteristics led to our earlier experimental inquiry on the identity of the pathway of valine–isoleucine biosynthesis in the genus *Propionibacterium* (6). Since *Propionibacterium shermanii, Propionibacterium arabinosum,* and *Propionibacterium pentosaceum* possessed a reductoisomerase and a dihydroxyacid dehydratase, it was concluded that the parallel pathway of biosynthesis of these two amino acids from pyruvate and α-ketobutyrate, respectively, was present here as in *Escherichia coli* (6). During this investigation, the paucity of information on the amino acid metabolism of these bacteria became apparent and required ascertaining their simplest defined medium. The propionic acid bacteria had earlier been shown to require pantothenic acid and biotin and to be stimulated by thiamine and/or *p*-aminobenzoic acid [7, 8) and earlier references cited therein]. Some strains thrived in the absence of amino acids and others grew with difficulty under these nutritional conditions (7). This report presents further information on amino acid nutrition, ammonia assimilation, and some areas of amino acid catabolism in the genus *Propionibacterium.*

Materials and Methods. Source of cultures and media. Lyophilized cultures of *P. shermanii,* ATCC 9614, *P. arabinosum,* ATCC 4965, and *P. pentosaceum,* ATCC 4875, were first grown on Delwiche's defined liquid medium (8) at 30°, and then maintained on tomato juice agar slants (9). In growth experiments, the bacteria were grown semianaerobically at 30° in stationary 13 × 100 mm culture tubes containing 5 ml of medium and closed with polyurethane plugs. Cell growth was measured as the turbidity at 660 nm in a Klett-Summerson photoelectric colorimeter. When the nitrogen source was changed, the casein hydrolysate, L-cystine, and L-tryptophan in Delwiche's medium (8) were replaced by an amino acid mixture (10) containing 700 mg of nitrogen/liter, or by an isonitrogenous amount of amino acid(s), or $(NH_4)_2SO_4$. Only α-nitrogen was taken into consideration when L-histidine, L-arginine, L-lysine, or L-tryptophan was used. The inocula for *P. shermanii* and *P. arabinosum*

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were grown on Delwiche's medium with casein hydrolysate or with $(NH_4)_2SO_4$ for *P. pentosaceum*. Each experimental medium (5 ml run in duplicate) was inoculated with 0.1 ml of a suspension of log phase cells which were washed twice in sterile 0.9% NaCl and resuspended in the same solution to give a Klett reading of 100.

Preparation of the cell-free extracts. For larger scale growth, the propionic acid bacteria were incubated at 30° semiaerobically in 1 liter of medium in a plugged 2-liter Erlenmeyer flask. After 24 to 48 hr incubation at 30°, the log phase cells were harvested by centrifugation at 3,500*g* for 15 min at 3°, washed twice with 0.1 *M* Tris–HCl, (pH 7.4) or 0.1 *M* potassium phosphate buffer (pH 7.4). The usual yield of cells was 0.9 to 1.5 g of wet cells/liter. The cells were resuspended in 0.1 *M* Tris or phosphate buffer (pH 7.4) to give a 10 or 20% (w/v) suspension and ruptured by ultrasonic oscillation at 4° (Branson Sonifier Model S-125, Branson Instruments, Inc., Danbury, CT). The time for maximum release (*i.e.*, highest specific activity) for another enzyme from *P. pentosaceum, P. shermanii,* and *P. arabinosum* was 10, 5 and 5 min, respectively (6); these sonication times were used in the present study. The ruptured cell suspension was centrifuged at 18,000*g* or 39,000*g* for 20 min at 3° as specified below. The supernatant was kept at 3° and used the same day or was frozen at —20° in small vials. For the pellet suspension, which was prepared by a modification of the method by Wagner *et al.* (11), the ruptured cell suspension was centrifuged at 500*g* for 15 min in the cold to remove unbroken cells and cell debris. The supernatant was then centrifuged at 39,000*g* for 1 hr at 3°. The pellet was washed once in 0.1 *M* phosphate buffer (pH 7.4) and dispersed (5% w/v) in the same buffer by used of a teflon homogenizer.

Enzyme assays. Several enzymes were measured spectrophotometrically: L-glutamate dehydrogenase (EC 1.4.1.4) at 340 nm for NADPH formation (12), L-alanine dehydrogenase (EC 1.4.1.1) at 340 nm for NADH oxidation in the presence of NH_4Cl and pyruvate (13), and aspartase (EC 4.3.1.1) at 240 nm using L-aspartate as substrate (14). For the assay of L-serine dehydratase (EC 4.2.1.13) and L-threonine dehydratase (EC 4.2.1.16), the incubation mixture contained 0.1 *M* potassium phosphate buffer (pH 8.0), 10 μg of pyridoxal phosphate, 40 m*M* L-serine or L-threonine, 3 m*M* GSH,[3] and 3 m*M* AMP (15). The log phase cells of *P. pentosaceum* were washed twice with 0.1 *M* phosphate buffer (pH 7.4), and resuspended in the same buffer containing 3 m*M* each of GSH and AMP (15), ruptured by ultrasonic oscillation for 10 min at 4°, and centrifuged at 39,000*g*. The enzyme assays were performed the same day with fresh extracts, or with whole cells in some experiments. After a 30 min incubation at 37°, the protein was removed by adding 2 ml of 5% (w/v) trichloroacetic acid and centrifuging. The keto acid was determined as the hydrazone in alkaline solution by a modification (16) of a colorimetric method (17). Protein was determined by the method of Lowry *et al.* (18) with crystalline bovine serum albumin as the standard. After subtraction of values from appropriate controls (*i.e.*, assay tubes deficient in substrate for Tables V–VII), the results were expressed as specific activity in units of micromoles of keto acid enzymatically formed per hour per milligram of protein. Amino acids, nucleotides, coenzymes, and cofactors were purchased from Nutritional Biochemicals Corp., Cleveland, OH, or Sigma, St. Louis, MO.

Results. Vitamin requirements of Propionibacterium. The vitamin requirements of the propionic acid bacteria were determined with two nitrogen sources: Delwiche's medium (8) containing either casein hydrolysate, L-cystine, and L-tryptophan, or a mixture of 18 amino acids (10). Pantothenic acid was essential for the growth of all three organisms, whereas *p*-aminobenzoic acid and thiamine were stimulatory. The first trial with biotin-deficient media showed moderate growth, but when washed biotin-deficient

[3] Abbreviations used in this paper include: reduced glutathione, GSH; adenosine-5′-monophosphate, AMP; adenosine-5′-diphosphate, ADP; and the usual abbreviations for the amino acids.

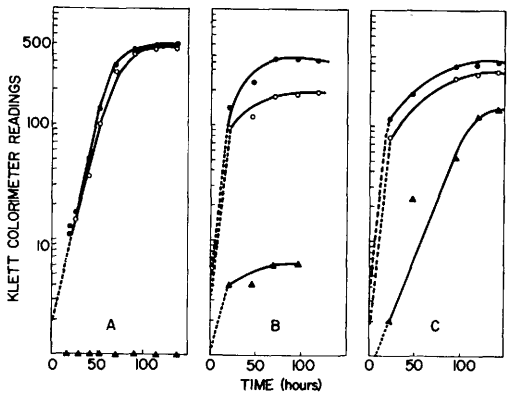

FIG. 1. Growth curves of members of the genus *Propionibacterium*: The change in turbidity for *P. shermanii* (A); *P. arabinosum* (B); and *P. pentosaceum* (C); with casein hydrolyzate, L-cystine, and L-tryptophan (●—); mixture of 18 amino acids (○—); and $(NH_4)_2SO_4$ (▲—) as the main nitrogen source during a 30° semianaerobic, incubation.

cells were transferred to fresh, biotin-free media, there was no growth with either of the two nitrogen sources. Thus the vitamin requirements were identical for both nitrogen sources and remained the same as that reported earlier (7, 8).

Amino acid requirements of Propionibacterium. Growth of the 3 *Propionibacterium* species on casein hydrolysate or on a mixture of 18 amino acids was comparable (Fig. 1). Of the 3 *Propionibacterium*, only *P. pentosaceum* grew appreciably on $(NH_4)_2SO_4$ as the main source of nitrogen (50% of the control amino acid mixture). When amino acids were deleted one by one from the control mixture of 18 amino acids, *P. shermanii* showed growth depression (90% of the control or lower values) for 11 out of 18 amino acids; the greatest depression was given by glutamate (51%), alanine (53%), tryrosine (44%), and leucine (32% of the control). For *P. arabinosum*, growth was depressed upon omission of 14 of the 18 amino acids although to a lower extent, since only aspartate (58%) and methionine (55%) reached the lower levels. The growth of *P. pentosaceum* was decreased from the control for 8 of the 18 amino acids, with only isoleucine (54% of control) being at the lower values. Evidently the biosynthetic ability of *P. pentosaceum* is greater than *P. arabinosum*, which in turn, is greater than *P. shermanii*.

With this background, groups of amino acids were deleted from the control mixture of amino acids (Table I). These groups are based on the known routes for amino acid biosynthesis from a common precursor in *E. coli*, *i.e.*, the histidine-, aromatic amino acid-, serine-, branched chain amino acid-, aspartate-, and glutamate families (19). The data in Table I show that the main growth decrease was observed with the omission of the glutamate family (glutamate, arginine, and proline), and, to a lesser extent, by the lack of aspartate family (aspartate, threonine, lysine, methionine, and isoleucine). Evidently

TABLE I. Multiple Amino Acid Deletions from Medium for *Propionibacterium*.

Omissions from amino acid mixture[a]	Growth by Klett reading at 96 hr	
	P. shermanii	*P. arabinosum*
None	379	262
His	280	217
Phe + Tyr + Trp	260	253
Ser + Gly + Cys	171	202
Ala + Val + Ile + Leu	210	299
Asp + Thr + Lys + Met + Ile	144	34
Glu + Arg + Pro	21	13

[a] The propionic acid bacteria were grown in Delwiche's medium with 18 amino acids, or the same medium deficient in a group of amino acids. The amino acids were grouped according to their biosynthetic route of formation. The results are means of duplicate experiments with readings at 0, 48, 96, and 144 hr.

TABLE II. Substitutions for Aspartate in Medium for *P. shermanii*.

Additions to deficient medium[a]	Growth by Klett reading	
	96 hr	144 hr
None	151	360
Succinate	134	211
Fumarate	94	187
Malate	105	204
Aspartate	321	431
Asparagine	382	475

[a] *P. shermanii* was grown in Delwiche's medium with 13 of the usual 18 amino acids as the nitrogen source (*i.e.*, deletion of aspartate, threonine, lysine, methionine, and isoleucine in the deficient medium).

these two *Propionibacterium* could synthesize the other four amino acid families at a faster rate than the glutamate and aspartate groups.

To study the possible precursors of the aspartate family in *P. shermanii*, a medium deficient in aspartic acid, threonine, lysine, methionine, and isoleucine was used as the control (Table II). Addition of succinate, fumarate, or malate to the deficient medium did not improve the bacterial growth, but aspartate or asparagine increased their growth. These observations suggest an impaired conversion of citric acid cycle intermediates to aspartate, or an inadequate transport of these intermediates into the cell. A similar experiment was performed with deletion of the glutamate family from the usual medium (Table III). Addition of α-ketoglutarate did not give a growth response. Perhaps the cells degraded the α-ketoglutarate, or were unable to transport, or transaminate this keto acid. The marked increase in growth upon addition of glutamate, ornithine, citrulline, or arginine is consistent with the presence of the ornithine–urea cycle among these amino acids. Proline was only partially utilized under these experimental conditions. Hydroxyproline was not available here for the formation of the other five-carbon amino acids, which is consistent with the irreversible formation of hydroxyproline in other biological systems (20).

Single nitrogen sources for the Propionibacterium. In contrast to the previous experiments, the ability of the propionic acid bacteria to grow on various single nitrogen sources (*i.e.*, other than the vitamins in the medium) was tested next (Table IV). *P. shermanii* failed to grow on all tested substances, except glutamate; this finding is consistent with the observation in Fig. 1 and Table I. *P. arabinosum* grew significantly only on aspartate, again in concord with the data in Table I. *P. arabinosum* grew slowly on histidine, arginine, methionine, phenylalanine, serine, leucine, and glutamate. *P. pentosaceum* was more versatile with its growth on $(NH_4)_2SO_4$ (54% of the control amino acid mixture), glutamate (83%), aspartate (52%), and to a lesser extent with other amino acids.

Some enzymes are repressed by the presence of glucose or its catabolic products in the growth medium (21). Since the growth experiments with *P. pentosaceum* contained 1% glucose and 0.8% acetate (Fig. 1 and Table IV), the effectiveness of carbon sources (*i.e.*, 1% glucose, 0.8% acetate, 1% glycerol, or only the amino acid) to support growth with several single nitrogen sources (*i.e.*, $(NH_4)_2SO_4$, glutamate, histidine, serine, or arginine) was tested. The growth of *P. pentosaceum* with glucose as a carbon source was the same as the glucose plus acetate medium for each of the 5 nitrogen sources. When

TABLE III. Substitutions for Glutamate in Medium for *P. shermanii*.

Additions to deficient medium[a]	Growth by Klett reading	
	96 hr	144 hr
None	17	45
α-Ketoglutarate	36	121
Glutamate	181	325
Ornithine	105	281
Citrulline	291	356
Arginine	333	382
Proline	61	204
Hydroxyproline	11	12

[a] *P. shermanii* was grown in Delwiche's medium with 15 of the usual amino acids as the nitrogen source (*i.e.*, deletion of L-glutamate, arginine, and proline).

TABLE IV. Single Nitrogen Sources in Medium for *Propionibacterium*.

Main nitrogen source[a]	Growth by Klett reading (hr):		
	P. shermanii 144	*P. arabinosum* 96	*P. pentosaceum* 96
18 Amino acids	398	218	348
$(NH_4)_2SO_4$	0	8	188
L-Glutamate	102	72	290
DL-Aspartate	9	208	181
DL-Alanine	0	0	122
L-Arginine	0	107	138
L-Proline	0	38	79
DL-Valine	0	60	142
DL-Isoleucine	0	31	74
L-Leucine	0	75	161
DL-Phenylalanine	0	85	89
L-Glutamine	0	7	10
L-Asparagine	0	32	28
DL-Serine	0	84	111
DL-Threonine	0	12	58
DL-Histidine	0	115	144
Glycine	0	3	12
DL-Methionine	0	97	82
L-Lysine	0	63	73

[a] The propionic acid bacteria were grown in Delwiche's medium with one of the indicated nitrogen sources at a concentration of 0.07% (w/v) α-amino nitrogen. The results are the means of 3 separate experiments.

acetate was the carbon source tested with each of the above amino acids as the nitrogen source, growth was absent. In the test for single amino acids as the sole carbon source [*i.e.*, $(NH_4)_2SO_4$ present], or as the sole carbon and nitrogen source, the cells failed to grow. With glycerol as the carbon source, there was no growth with $(NH_4)_2SO_4$ as the nitrogen source, and reduced growth for each of the amino acids (0–45% of the growth in the glucose + acetate medium). These results suggest that glucose, or its degradation products, did not repress the first enzyme for the catabolism of these amino acids to the extent of retardation of bacterial growth.

Some enzymes for amino acid metabolism in Propionibacterium. Since *P. pentosaceum* was the one species observed to grow with $(NH_4)_2SO_4$ as the nitrogen source (Fig. 1), a search for the enzyme responsible for ammonia assimilation [*i.e.*, glutamate dehydrogenase, alanine dehydrogenase or aspartase, cf. (22)] was conducted. Cells of *P. pentosaceum* grown on an $(NH_4)_2SO_4$ medium were harvested in the log phase, ruptured, and centrifuged at 39,000*g* to give a supernatant extract and a pellet. The supernatant extract had glutamate dehydrogenase activity (1.28 nmoles of NADPH oxidized/min; sp act, 1.07 nmoles/min/mg of protein), low NAD-glutamate dehydrogenase activity, and no detectable activity for the other 2 enzymes. The resuspended pellet was devoid of activity for NADP- or NAD-specific, glutamate dehydrogenase, alanine dehydrogenase and aspartase. Therefore the reaction for ammonia assimilation in *P. pentosaceum* is catalyzed by NADP-specific glutamate dehydrogenase.

P. pentosaceum was shown earlier to degrade serine and threonine (Table IV). Preliminary assays showed low serine and threonine dehydratase activity in the supernatant extracts of *P. pentosaceum* grown on serine or threonine. This observation led to tests for activities in the resuspended pellet as well as

TABLE V. Enzymes for Serine and Threonine Degradation in Extracts of *P. pentosaceum*.

Enzyme assayed	Source of nitrogen for cell growth[a]	Sp act (μmoles of keto acid formed/hr/mg of protein)	
		Supernatant extract	Resuspended pellet
L-Serine dehydratase	L-Serine	0.40	2.46
	L-Threonine	0.70	3.14
	L-Glutamate	2.90	3.48
L-Threonine dehydratase	L-Serine	4.16	2.48
	L-Threonine	3.14	0.10
	L-Glutamate	8.65	3.08

[a] *P. pentosaceum* was grown on the indicated amino acid as the nitrogen source, harvested in the log phase and ruptured by sonic oscillation. The suspension was centrifuged at 500*g* for 15 min and then at 39,000*g* for 60 min. The values for the corrected enzyme assays on the supernatant extract and resuspended pellet are the means of duplicate experiments.

the supernatant extract (Table V). Although the cells had been treated by sonic oscillation for 10 min, a time demonstrated to give the maximum release of dihydroxyacid dehydratase (6), the serine dehydratase had a greater activity and specific activity in the pellet than the supernatant. The location of the threonine dehydratase activity and specific activity had the reverse pattern, which suggested two separate enzymes for these activities. Hence in the next experiment, intact cell suspensions with the addition of a surface active agent were used with these polar substrates (Table VI). The addition of cetyltrimethylammonium bromide or toluene increased each dehydratase activity, but toluene was more effective. Again the range of ratios of threonine dehydratase/serine dehydratase activities (5, 2, and 1 in the toluene column of Table VI) provided another clue for separate enzymes in *P. pentosaceum*.

The results (Table V and VI) also show that higher L-threonine dehydratase activities were observed in L-serine- and L-glutamate-grown *P. pentosaceum* than for L-threonine-grown cells. This finding is inconsistent with a constitutive enzyme and suggests a complex pattern of regulation.

TABLE VI. Assay of Cell Suspensions of *P. pentosaceum* for Serine and Threonine Dehydratases.

Enzyme assayed	Source of nitrogen for cell growth[a]	Sp act (μmoles of keto acid formed/hr/mg of dried cells); surface active agent added		
		None	Cetavlon	Toluene
L-Serine dehydratase	L-Serine	0.40	0.68	1.08
	L-Threonine	0.28	0.42	0.68
	L-Glutamate	0.00	0.42	2.80
L-Threonine dehydratase	L-Serine	0.82	1.22	6.40
	L-Threonine	0.54	0.68	1.22
	L-Glutamate	0.00	0.88	2.80

[a] *P. pentosaceum* was grown as before (Table V), harvested, washed, and resuspended in buffer [10% (w/v)]. One tenth milliliter of this suspension was added to the usual assay components (final vol, 1.0 ml) along with, in some cases, 1.0 μmole of cetyltrimethylammonium bromide (Cetavlon), or 1 drop of toluene. The above results are corrected by subtraction of the trace of keto acids found in control tubes deficient in substrate and are the means of duplicate experiments.

TABLE VII. Effect of Cofactors and L-Isoleucine on L-Threonine Dehydratase Activity(s) of *P. pentosaceum*.

Additions to the assay mixture	Sp act (μmoles of keto acid formed/hr/mg of protein); nitrogen source for growth of cells[a]		
	L-Serine	L-Threonine	L-Glutamate
None	2.00	2.12	10.00
GSH + AMP	2.80	2.30	10.76
GSH + ADP	2.72	2.36	10.76
L-Isoleucine	0.44	0.22	1.10
GSH + AMP + L-isoleucine	1.36	0.72	4.94
GSH + ADP + L-isoleucine	1.36	0.94	4.06

[a] *P. pentosaceum* was grown on the nitrogen sources indicated above, harvested and ruptured. The assay conditions are the same as that described in the Methods section, except for the deletion of GSH and AMP in line 1. Where indicated, 3.0 μmoles of AMP or ADP and 4.0 μmoles of L-isoleucine were added to the assay system (final vol, 1.0 ml). Similar results were observed in a separate, parallel experiment.

The nucleotide requirements of threonine dehydratase varies from AMP to ADP according to the microbial source; a biosynthetic threonine dehydratase is known, as well as the catabolic threonine dehydratase. Hence, threonine dehydratase was studied further with respect to its cofactors and L-isoleucine (Table VII). The addition of AMP or ADP with GSH caused a mild increase in the enzyme activity, even though they were included in the preparation of the extract. Isoleucine (line 4, Table VII) inhibited 78, 90, and 89% of the control threonine dehydratase activity (line 1). However, the inhibition by isoleucine in the presence of GSH and AMP (line 5) was only 32, 66, and 51% of the control; similar results were observed in the presence of ADP. The difference in the two levels is due to the catabolic threonine dehydratase activity, which as a crude estimate was 49, 36, and 42% of the total activity (values in line 5 + 6 × 100 ÷ values in lines 2 + 3, Table VII).

Discussion. To distinguish between the amino acids required for growth and those synthesized by the cells, amino acids were deleted singly or by biosynthetic groups. *P. shermanii* had the lowest ability to synthesize amino acids, particularly glutamate and aspartate (Tables I, II, III). *P. arabinosum* had an intermediate biosynthetic ability; whereas *P. pentosaceum* had the highest synthetic ability in these experiments. The growth results (Table III) suggested a metabolic interconversion among the five carbon amino acids, *i.e.*, glutamate → glutamate-γ-semialdehyde → ornithine → citrulline → arginine → ornithine (20). At this time, it is unknown whether the pathways for ornithine formation occurs by *N*-acetyl intermediates or nonacetylated intermediates (19). A similar alternative utilization of ornithine, citrulline, or arginine has been reported for several lactic acid bacteria (23). However, the pathway for proline catabolism (20), *i.e.*, proline → Δ′—-pyrroline-2-carboxylate → 2-keto-5-aminovalerate → L-Δ′-pyrroline-5-carboxylate → L-glutamate-γ-semialdehyde → glutamate, was inefficient in *P. shermanii* (Table III and IV), but more effective in the other 2 propionic acid bacteria (Table IV).

Confirmation of the above order of biosynthetic ability was found in the experiment (Fig. 1), where *P. s hermanii* and *P. arabinosum* failed to grow on $(NH_4)_2SO_4$, in contrast to the positive growth of *P. pentosaceum*. With regard to degradative ability, as demonstrated by growth with single amino acids as the main nitrogen source, *P. shermanii* could grow only on L-glutamate, whereas *P. pentosaceum* could grow on many amino acids, as well as $(NH_4)_2SO_4$ (Table IV). The ability of *P. arabinosum* to grow on

single nitrogen sources was between that of *P. shermanii* and *P. pentosaceum*. Wood *et al.* (7) reported growth experiments with *P. shermanii* 52W and *P. pentosaceum* 49W leading to the observation that amino acids were beneficial, but not essential for the propionic acid bacteria. Using quite different test groups of amino acids and no single amino acid deletions, they concluded that no single group of amino acids was essential.

The known enzymes for ammonia assimilation (glutamate dehydrogenase, alanine dehydrogenase, and aspartase) vary with the microorganism [*cf.* (22)]. For *P. pentosaceum*, the only enzyme present for ammonia incorporation into an amino acid was a NADP-specific glutamate dehydrogenase.

The α,β-elimination reactions from α-amino-β-hydroxy acids are catalyzed by several different enzymes, many of which have been shown to be pyridoxal phosphate dependent (24). Initially the L-serine- and L-threonine dehydratase activities were believed to be catalyzed by one enzyme in *E. coli* (25) and *N. crassa* (26), but more recent evidence indicates that 2 or more entities are present in *E. coli* (27–29) and in *Clostridium tetanomorphum* (30). L-Serine dehydratase was quite unstable (27, 29) and specific for L-serine (29). The catabolic L-threonine dehydratase in *E. coli* had cofactors of pyridoxal phosphate, GSH, and AMP (25, 31–34); whereas this enzyme from *C. tetanomorphum* was activated by pyridoxal phosphate, reducing agents and ADP (35–38). The biosynthetic L-threonine dehydratase had an enzymatic role in isoleucine biosynthesis and was subject to end product inhibition by isoleucine in *E. coli* (39, 40), *Salmonella typhimurium* (41) and *Bacillus subtilis* (42) and had a somewhat different pattern in *Rhodopseudomonas spheroides* (43) and *Saccharomyces cerevisiae* (44, 45). The D-serine dehydratase activities of *E. coli* had inherent, although lower activity for D-threonine (24, 27). The catalytic mechanism for these enzymes is based on a Schiff's base intermediate (24) and their mode of regulation by isoleucine, AMP, or ADP has received recent attention (31–44). The L-serine- and D-serine dehydratases have been assayed in toluene-treated cell suspensions (21, 27). D-Serine dehydratase was induced solely by D-serine in *E. coli*, whereas L-serine dehydratase had a more complex pattern of induction than merely L-serine as an inducer (27).

From the present series of experiments, *P. pentosaceum* also had an unstable dehydratase activity; required toluene for assays with cell suspensions (Table VI); probably had separate L-serine dehydratase and L-threonine dehydratase (Table V); had a GSH plus adenine nucleotide-stimulated, L-threonine dehydratase activity (Table VII); and had an L-isoleucine-inhibited, L-threonine dehydratase (Table VII). The main difference from the above results and those in the literature (31–38) was the ambivalence between AMP and ADP for the catabolic threonine dehydratase activity from *P. pentosaceum*. With their varying levels in Tables V, VI, and VII, L-serine dehydratase and catabolic L-threonine dehydratase were apparently not constitutive enzymes, but the mode of their regulation appeared to be complex and was not deciphered in the reported experiments. The results establish the presence of a L-serine dehydratase, a catabolic L-threonine dehydratase and a biosynthetic L-threonine dehydratase in *P. pentosaceum*.

Summary. *Propionibacterium shermanii, P. arabinosum,* and *P. pentosaceum* can synthesize many amino acids as determined by growth in the absence of a single amino acid or a group of biosynthetically related amino acids. *P. shermanii* and *P. arabinosum* were distinctive in their limited ability to synthesize glutamate and aspartate. When examining amino acids and $(NH_4)_2SO_4$ as possible single nitrogen sources, *P. shermanii* had the most limited capacity and grew only on glutamate. *P. arabinosum* was able to utilize several amino acids, but not $(NH_4)_2SO_4$, as the main nitrogen source. For *P. pentosaceum*, glutamate dehydrogenase was demonstrated for the function of ammonia assimilation, L-serine dehydratase for L-serine degradation, and an AMP- or ADP-dependent L-threonine dehyratase for threonine catabolism. A biosynthetic, L-isoleucine-inhibited, L-threonine dehydratase was observed in *P. pentosaceum*.

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