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Distribution of an L-Isoaspartyl Protein Methyltransferase in Eubacteria

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A protein carboxyl methyltransferase (EC 2.1.1.77) that recognizes age-damaged proteins for potential repair or degradation reactions has been found in all vertebrate tissues and cells examined to date. This enzyme catalyzes the transfer of methyl groups from *S*-adenosylmethionine to the carboxyl groups of D-aspartyl or L-isoaspartyl residues that are formed spontaneously from normal L-aspartyl and L-asparaginyl residues. A similar methyltransferase has been found in two bacterial species, *Escherichia coli* and *Salmonella typhimurium*, suggesting that this enzyme performs an essential function in all cells. In this study, we show that this enzyme is present in cytosolic extracts of six additional members of the α and γ subdivisions of the purple bacteria: *Pseudomonas aeruginosa* (γ), *Rhodobacter sphaeroides* (α), and the γ enteric species *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Serratia marcescens*. DNA probes from the *E. coli* methyltransferase gene hybridized only to the chromosomal DNA of the enteric species. Interestingly, no activity was found in the plant pathogen *Erwinia chrysanthemi*, a member of the enteric family, nor in *Rhizobium meliloti* or *Rhodopseudomonas palustris*, two members of the α subdivision. Additionally, we could not detect activity in the four gram-positive species *Bacillus subtilis*, *B. stearothermophilus*, *Lactobacillus casei*, and *Streptomyces griseus*. The absence of enzyme activity was not due to the presence of inhibitors in the extracts. These results suggest that many cells may not have the enzymatic machinery to recognize abnormal aspartyl residues by methylation reactions. Since the nonenzymatic degradation reactions that generate these residues occur in all cells, other pathways may be present in nature to ensure that these types of altered proteins do not accumulate and interfere with normal cellular physiology.

In all vertebrate tissues examined to date, a cytosolic L-isoaspartyl/D-aspartyl protein carboxyl methyltransferase (EC 2.1.1.77) catalyzes the transfer of the methyl group from *S*-adenosylmethionine to L-isoaspartyl or D-aspartyl residues (3, 4, 14, 25, 27). These unusual residues can accumulate in all proteins via the spontaneous decomposition of normal L-aspartyl and L-asparaginyl residues (11, 33) and can inhibit the function of the modified proteins and/or the normal pathways for their enzymatic degradation (12, 16, 19, 22). The enzymatic methylation of these altered residues in vitro can lead to their conversion to L-aspartyl residues via a nonenzymatic repair pathway (9, 16, 17, 21).

A similar methyltransferase, characterized by its ability to methylate L-isoaspartyl-containing peptides, has also been detected in the cytosolic fraction of procaryotic cells such as *Salmonella typhimurium* (26) and *Escherichia coli* (8). The enzyme from the latter source has been purified, and the nucleotide sequence and chromosomal location of its structural gene *pcm* (59 min) have been determined (8). The deduced amino acid sequence of the *E. coli* enzyme is 31% identical to that of the human erythrocyte isozyme I (15), suggesting a rather high conservation of structure (and presumably function) in the large evolutionary gap between procaryotic and eucaryotic cells. This bacterial methyltransferase is distinct from the *cheR* gene product that encodes an L-glutamyl-specific methyltransferase that modifies membrane chemoreceptor proteins in *S. typhimurium* and *E. coli* (5, 23, 31) but is probably identical to the broad-methyl-accepting-specificity enzyme described in *E. coli* (18) and *S. typhimurium* (5) before the substrate specificity of the L-isoaspartyl/D-aspartyl methyltransferase was known.

The presence of a methyltransferase in bacteria highly similar to the vertebrate enzyme suggested that its function in recognizing damaged proteins may be essential to all cells in nature. It was therefore surprising when we did not detect methyltransferase activity in cytosolic extracts of the thermophilic bacterium *Bacillus stearothermophilus*, especially because these cells must survive at high temperatures that accelerate the rates of the spontaneous processes converting L-aspartyl and L-asparaginyl residues to L-isoaspartyl and D-aspartyl residues (11). We thus began to assay extracts from a variety of eubacterial species for this methyltransferase. We found that while enzyme activity was present in a variety of gram-negative cells, no activity was found in any of the gram-positive cells tested. These results suggest that alternative pathways may exist for the metabolism of proteins with altered aspartyl and asparaginyl residues.

MATERIALS AND METHODS

Bacterial strains and growth. The bacterial strains used in this study and their sources are listed in Table 1. *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* were grown under aerobic conditions, and cell pastes were kindly supplied by Mark Moore and Samual Kaplan (University of Texas, Houston). Other bacteria were grown to late log phase in LB medium (Difco) except for *Lactobacillus casei*, which was grown in lactobacilli MRS broth (Difco). Cells were grown under aerobic conditions at 37°C with the following exceptions: *Erwinia chrysanthemi*, *Serratia marcescens*, and *Enterobacter aerogenes* were grown at 28°C, *Rhizobium meliloti* and *Streptomyces griseus* were grown at

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TABLE 1. Bacterial strains

Organism	Strain	Source
Escherichia coli	JA200	B. Bachmann
Salmonella typhimurium	M1074	P. Bernard
Klebsiella pneumoniae	M1084	P. Bernard
Enterobacter aerogenes	ATCC 13048	ATCC[a]
Erwinia chrysanthemi	EC16	N. Keen
Serratia marcescens	ATCC 13880	ATCC
Proteus vulgaris	ATCC 13315	ATCC
Pseudomonas aeruginosa	M1033	P. Bernard
Rhizobium meliloti	1021	A. Hirsch
Rhodopseudomonas palustris	1e5	M. Moore and S. Kaplan
Rhodobacter sphaeroides	2.4.1	M. Moore and S. Kaplan
Bacillus subtilis	M1004	P. Bernard
Bacillus stearothermophilus	M1091	P. Bernard
Lactobacillus casei	ATCC 7469	P. Bernard
Streptomyces griseus	ATCC 10971	P. Bernard

[a] ATCC, American Type Culture Collection.

30°C, and *B. stearothermophilus* was grown at 55°C. The identities of the enteric species and some of the nonenteric species were checked after each culture by Enterotube II (Hoffmann-La Roche Inc.) analysis.

Preparation of bacterial cell extracts for enzyme assays. All steps were performed at 0 to 4°C. Cells were harvested by centrifugation and washed twice with buffer A (5 mM sodium phosphate, 5 mM EDTA, 15 mM β-mercaptoethanol, 25 μM phenylmethylsulfonyl fluoride, 10% glycerol, pH 7.0). Cells were resuspended in 1.5 ml of buffer A per g of wet cell pellet and were broken by French press treatment or sonication. The lysate was centrifuged at 20,000 × *g* for 15 min to sediment the cell debris, and the supernatant was recentrifuged at 100,000 × *g* for 90 min to obtain a cytosolic fraction that was stored at −20°C for analysis. Protein concentrations were determined by a modified Lowry assay (2) following precipitation of the protein with 10% trichloroacetic acid; bovine serum albumin was used as a standard.

Enzyme assays. L-Isoaspartyl protein carboxyl methyltransferase activity was measured by the incorporation of base-labile volatile radioactivity from *S*-adenosyl-L-[*methyl*-^{14}C]methionine into methyl-accepting substrates. Unless otherwise indicated, the final concentration of *S*-adenosyl-L-[*methyl*-^{14}C]methionine (50 mCi/mmol; ICN) was 10 μM. Methyl-accepting substrates included an L-isoaspartyl-containing peptide at concentrations of 100 and 500 μM (L-Lys-L-Ala-L-Ser-L-Ala-L-isoAsp-L-Leu-L-Ala-L-Lys-L-Tyr [19]) as well as several proteins at concentrations of 40 mg/ml (hen egg white ovalbumin [Sigma grade V], bovine serum albumin [Sigma fraction V], bovine gamma globulin [Sigma Cohn fraction II], and pig skin gelatin [Sigma]). The assay mixtures (final volume with extract is 40 μl) were buffered in 0.1 M sodium citrate (pH 6.0). For extracts that showed no isopeptide-dependent methyltransferase activity at this pH, additional assays were performed in 0.1 M Tris-HCl buffer at pH 8.0, where the activity with endogenous substrates was greater in some cases. The reaction was incubated at 37°C (55°C for *B. stearothermophilus*) for 5 to 60 min and was then quenched by adding an equal volume (40 μl) of 0.2 N NaOH–1% (wt/vol) sodium dodecyl sulfate (SDS) for ester hydrolysis and [^{14}C]methanol determination as described previously (13). One unit of activity is defined as 1 pmol of methyl groups transferred per min.

Malate dehydrogenase was assayed in 0.23 mM *cis*-oxalacetic acid, 0.13 mM NADH, and 10 mM sodium phosphate buffer (pH 7.5) at 22°C by measuring the decrease in A_{340}. One unit is defined as 1 μmol of NADH oxidized per min.

Slot blot hybridization of bacterial DNA. DNA from various bacterial strains was prepared by using a modification (8) of the protocol of Silhavy et al. (30). DNA concentration was determined by the A_{260}, assuming that an A_{260} of 1.0 corresponds to 50 μg of double-stranded DNA per ml. Chromosomal DNA (50 μl) was denatured by heating at 100°C for 10 min and then incubated with 50 μl 0.5 N NaOH–1.5 N NaCl at room temperature for 10 min and neutralized with 400 μl of 1.5 N NaCl–0.5 M Tris-HCl–1 mM EDTA (pH 7.2). Samples of this DNA were applied to Immobilon-N (Millipore) membranes, using a MilliBlot-S apparatus. After air drying for 1 h, the membrane was baked for 2 h at 80°C under vacuum. ^{32}P-labelled DNA probes (specific activity, >5 × 10^8 cpm/μg) were prepared by using [α-^{32}P]dCTP (3,000 Ci/mmol; ICN) and a Prime-It random primer kit (Stratagene). A 0.32-kb *Eco*RI-*Cla*I fragment, containing nucleotides +263 to +572 of the *E. coli pcm* L-isoaspartyl methyltransferase gene, was prepared from pJF3 as the template (8). Prehybridization was carried out at 65°C for 3 h in 5× SSPE–5× Denhardt's solution (29)–0.5% SDS–100 μg of salmon sperm DNA (Sigma) per ml and was followed by hybridization with the denatured probe in fresh solution at 65°C overnight. The membrane was washed twice in 2× SSPE–0.1% SDS for 10 min at room temperature and once in 1× SSPE–0.1% SDS for 15 min at 65°C. The wet membrane was put in a heat-sealable bag and autoradiographed at −80°C for 1 to 5 days, using Kodak XAR film. The intensities of the bands were quantitated by using an LKB Ultrascan XR densitometer.

RESULTS

L-Isoaspartyl protein methyltransferase activity in *E. coli* and *S. typhimurium*. Previous studies had identified L-isoaspartyl methyltransferase activity in the cytosolic fraction of *S. typhimurium* (26) and *E. coli* (8). To provide a baseline for comparative studies of other eubacterial strains, we assayed soluble extracts of *E. coli* and *S. typhimurium* with a variety of methyl-accepting substrates. Figure 1 shows the incorporation of methyl groups into endogenous substrates, an isoaspartyl peptide, or ovalbumin as a function of incubation time. The latter substrates showed enhanced activity over the endogenous levels, with the highest activities for the peptide substrate, as was previously seen for *E. coli* (8). Methyl-accepting activity was also seen with gamma globulins and gelatin, while little activity was seen with bovine serum albumin (Table 2).

L-Isoaspartyl methyltransferase activity in other enteric bacteria. Cell extracts from *Klebsiella pneumoniae* and *Enterobacter aerogenes* demonstrated a lower level of methyltransferase activity with the L-isoaspartyl peptide substrate than did the related species *E. coli* and *S. typhimurium* (Fig. 1; Table 2). Little or no activity with any protein substrates was seen in *E. aerogenes* extracts, while marginal activity was seen with gelatin in *K. pneumoniae* extracts (Fig. 1; Table 2). Other enteric bacteria tested (*Serratia marcescens*, *Proteus vulgaris*, and *Erwinia chrysanthemi*) exhibited no methyltransferase activity above endogenous levels when the L-isoaspartyl peptide was used as a methyl-accepting substrate at a concentration of 100 μM, although some activity was seen in extracts of *S. marcescens* and *P.*

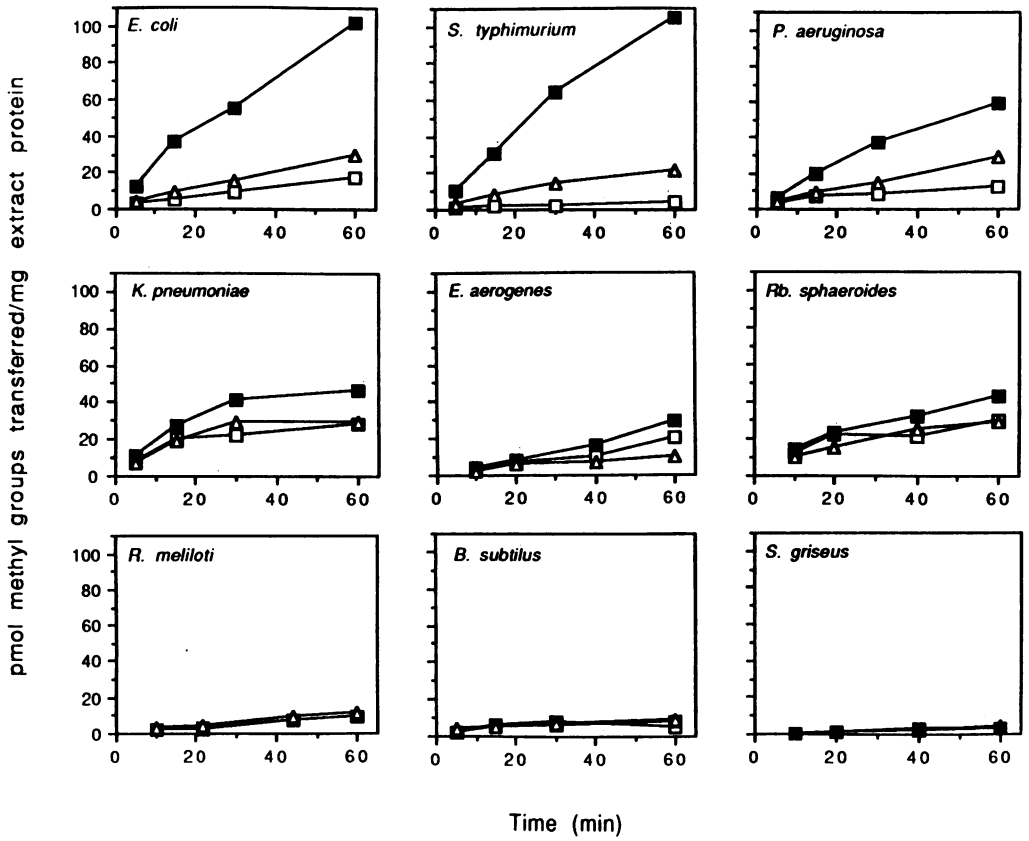

FIG. 1. Carboxyl methylation of L-isoaspartyl peptides, ovalbumin, and endogenous substrates catalyzed by bacterial cytosolic extracts. Enzyme activity was assayed as described in Materials and Methods. Reaction mixtures contained either no added methyl-accepting substrates (open squares), 100 μM L-Lys-L-Ala-L-Ser-L-Ala-L-isoAsp-L-Leu-L-Ala-L-Lys-L-Tyr (solid squares), or 40 mg of ovalbumin per ml (open triangles). Assays were performed in 0.1 M sodium citrate buffer (pH 6.0) except for the extracts from *B. subtilis* and *S. griseus*, which were performed in 0.1 M Tris-HCl buffer (pH 8.0). For each point, a small background in the absence of added enzyme has been subtracted. The average activity of duplicate samples is shown.

vulgaris extracts when the peptide concentration was raised to 500 μM (Table 2).

L-Isoaspartyl methyltransferase activity in other purple bacteria. *E. coli* and other enteric species belong to the γ subdivision of purple bacteria, which is a major division of eubacteria (36, 37). Cytosolic extracts of another γ purple bacterium, *Pseudomonas aeruginosa*, showed methyltransferase activities toward both the L-isoaspartyl peptide and protein substrates at levels similar to those seen with *E. coli* (Fig. 1; Table 2). We also tested three species in the α subdivision of purple bacteria: *Rhizobium meliloti*, *Rhodobacter sphaeroides*, and *Rhodopseudomonas palustris*. Extracts of *R. sphaeroides* gave methyltransferase activities similar to those of *K. pneumoniae* or *E. aerogenes*, with some methylation of the L-isoaspartyl peptide but no detectable action on ovalbumin or the other protein methyl acceptors (Fig. 1; Table 2). However, no methyltransferase activity with any of the substrates could be detected in cytosolic extracts of either *R. meliloti* or *R. palustris*.

No L-isoaspartyl methyltransferase activity is detected in four gram-positive species. *Bacillus subtilis*, *Lactobacillus casei*, and *Streptomyces griseus*, representing three branches of the gram-positive bacteria, were examined for their methyltransferase activity. Reactions with extracts of these cells failed to show any activity with the L-isoaspartyl peptide or ovalbumin (Fig. 1). Additionally, no activity was detected either with the peptide substrate at higher concentrations or with various protein substrates (Table 2). It is interesting that we also could find no evidence for the presence of this enzyme in a gram-positive thermophile, *B. stearothermophilus*. Since this organism grows at elevated temperatures, an increased rate of spontaneous protein damage might result in a rapid accumulation of racemized and isomerized aspartyl residues that would need to be metabolized. Nevertheless, we were unable to detect any methyltransferase activity with the isoaspartyl peptide (Table 2). We did find an activity with ovalbumin as a methyl acceptor that appears to be distinct from that of the L-isoaspartyl methyltransferase; the products of this reaction have not been characterized.

Controls to validate the apparent absence of methyltransferase activity in some species. The simplest explanation for the results presented above is that some bacterial species have little or no L-isoaspartyl protein methyltransferase activity. However, the fact that this enzyme had previously been detected at comparable levels in all cells and tissues examined suggested that its apparent absence in several procaryotes might be an experimental artifact. We explored this possibility by testing whether the cell disruption techniques used were effective. We found that the protein concentrations measured for the various bacterial cytosolic fractions were within a range (8 to 50 mg/ml) that is compat-

TABLE 2. Comparison of protein carboxyl methyltransferase activity in various bacterial cell extracts with protein and peptide methyl acceptors

Bacterial strain	Methyltransferase activity (pmol/min/mg of extract protein)[a]							Malate dehydrogenase activity (μmol/min/mg of protein)
	Endogenous substrates	L-isoAsp peptide		Ovalbumin	Gamma globulin	BSA	Gelatin	
		100 μM	500 μM					
Escherichia coli	0.41	3.26	3.58	0.71	0.65	0.50	1.23	0.42
Salmonella typhimurium	0.08	2.13	3.04	0.48	0.29	0.17	0.89	ND[b]
Klebsiella pneumoniae	1.04	1.05	2.25	1.27	0.78	0.76	1.60	0.24
Enterobacter aerogenes	0.20	0.46	0.76	0.17	0.17	0.22	0.28	0.12
Erwinia chrysanthemi	0.05	0.05	0.06[c]	0.05	0.05	0.04	0.05	0.83
Serratia marcescens	0.57	0.50	0.81	0.30	0.40	0.38	0.43	0.46
Proteus vulgaris	0.28	0.31	0.58	0.32	0.40	0.39	0.35	0.49
Pseudomonas aeruginosa	0.49	1.44	3.22	0.69	0.54	0.48	1.32	0
Rhizobium meliloti	0.13	0.15	0.12[c]	0.20	0.17	0.13	0.12	0.34
Rhodopseudomonas palustris	0.08	0.17	0.08[c]	0.10	0.05	0.07	0.16	0.46
Rhodobacter sphaeroides	1.51	1.50	1.82	1.38	1.03	1.11	0.99	ND
Bacillus subtilis	0.08	0.13	0.09[c]	0.10	0.19	0.19	0.04	0.39
Bacillus stearothermophilus	0.08	0.07	0.08					ND
Lactobacillus casei	0.04	0.06	0.05					0.05
Streptomyces griseus	0.00	0.00	0.00					0.68

[a] Activities were determined as described in Materials and Methods within the linear range of reaction time and enzyme amount. Endogenous substrates represent methyl acceptors present in the extract itself. The L-isoAsp peptide utilized was L-Lys-L-Ala-L-Ser-L-Ala-L-isoAsp-L-Leu-L-Ala-L-Lys-L-Tyr. The methyl-accepting protein concentration was 40 mg/ml in each case. BSA, bovine serum albumin. All points represent the average of duplicate values.

[b] ND, not determined.

[c] Measured in a separate experiment and normalized to the relative endogenous substrate activity.

ible with efficient membrane lysis. Additionally, we quantitated the level of a cytosolic citric acid cycle enzyme, malate dehydrogenase. Extracts from strains apparently lacking methyltransferase activity were found to have malate dehydrogenase activity comparable to that in extracts from strains containing methyltransferase activity (Table 2). These results indicated that the methods used to generate cell extracts were suitable for assays of cytosolic enzymes.

Another possible explanation for the absence of detectable activity is that methyltransferase inhibitor(s) or peptidase(s) were present in the bacterial extracts and interfered with the enzyme assay. We tested for the presence of such factors by assaying the related human erythrocyte L-isoaspartyl/D-aspartyl methyltransferase in the presence of the bacterial cell extracts. Because the K_m of the erythrocyte enzyme for the peptide L-Lys-L-Ala-L-Ser-L-Ala-L-isoAsp-L-Leu-L-Ala-L-Lys-L-Tyr (0.52 μM [19]) is about 100 times lower than that for the enzyme from *E. coli* (8), it was possible to assay at a low concentration of peptide (2.5 μM) where most of the methylation would be due to the erythrocyte enzyme. The results of such experiments indicated that 10 μl of the bacterial extracts in a total volume of 40 μl does not significantly affect the erythrocyte methyltransferase activity (range, 60 to 140%) with the possible exception of the extract from *R. meliloti*, in which only 28% of the erythrocyte activity was seen in the presence of 10 μl of extract (data not shown). Thus, the absence of detectable methyltransferase activities in the bacterial extracts in Fig. 1 and Table 2 was probably not due to inhibiting or degrading factors present in the extracts.

Genes homologous to the *E. coli pcm* gene in other bacteria. To determine whether an isoaspartyl methyltransferase gene might be present in some bacteria but not expressed, we measured the extent of hybridization of a fragment of the *E. coli* isoaspartyl methyltransferase gene to the chromosomal DNA of other species. This fragment contains DNA sequences that encode the conserved region I and region II amino acid sequences present in a variety of methyltransferases (15) and that would be expected to be most similar in different organisms. We find hybridization only in the enteric bacterial species (Fig. 2). Interestingly, we see no hybridization to DNA of *P. aeruginosa*, although it is clear that this bacterium contains active methyltransferase. It thus appears that the nucleotide sequences of these enzymes have diverged to the point that we could not detect the gene from nonenteric species under our conditions. However, within the enteric group of bacteria, we detected hybridization to all species tested with the exception of *E. chrysanthemi*. These results confirm the identification of the methyltransferase in species such as *S. marcescens* and *P. vulgaris*, in which low enzymatic activities were detected, and support the absence of the enzyme in *E. chrysanthemi*.

DISCUSSION

It has been proposed that the vertebrate L-isoaspartyl/D-aspartyl methyltransferase functions in the recognition and metabolic processing of proteins damaged by spontaneous aging reactions in these generally long-lived organisms (3, 4, 27). Enzymatic methylation may set the stage for spontaneous reactions that can reverse the damage (9, 16, 17, 21) or may prepare the altered protein species for degradation (22). It was thus initially hard to rationalize the presence of a similar activity in bacteria that are capable of rapid cell growth and division. However, it is clear that in the natural setting, nutrient deprivation can be the rule and cells thus need to survive for potentially long periods of time in the absence of growth (20, 28). Under these circumstances, altered proteins containing isomerized and racemized aspartyl residues can accumulate. The type of L-isoaspartyl protein methyltransferase identified in *E. coli* (8) and *S. typhimurium* (26) may be important for survival under such restrictive growth conditions, and it is therefore not surprising that we find this enzyme activity in a number of enteric

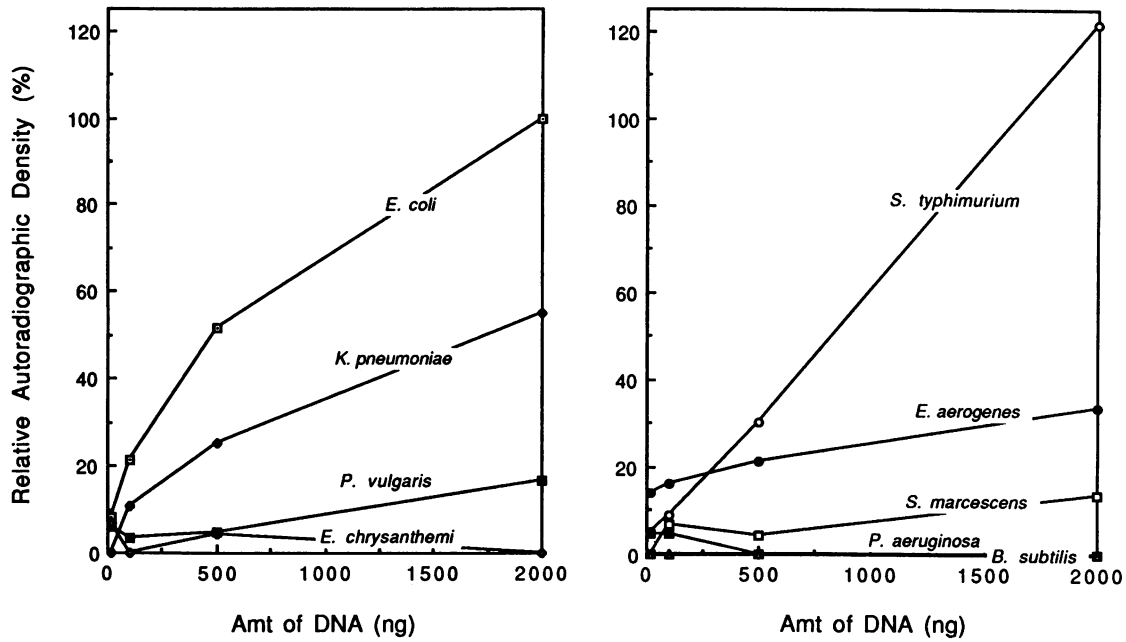

FIG. 2. Slot blot hybridization of chromosomal DNA (20 ng, 100 ng, 0.5 μg, and 2 μg) of various bacteria with a ^{32}P-labelled probe from the isoaspartyl methyltransferase gene (*pcm*) of *E. coli*. Experiments were performed as described in Materials and Methods. The intensity of the radioautographic signal from 2 μg of *E. coli* chromosomal DNA was defined as 100%.

and other bacterial species. What is surprising, however, is the apparent absence of this enzyme (at least under the conditions of cell growth and enzyme assay used here) in at least one enteric species, two out of the three examples of bacteria from the α purple bacteria, and all four gram-positive species tested (Fig. 3).

It was of interest to find that the only bacterium in the enteric family tested that lacked methyltransferase activity

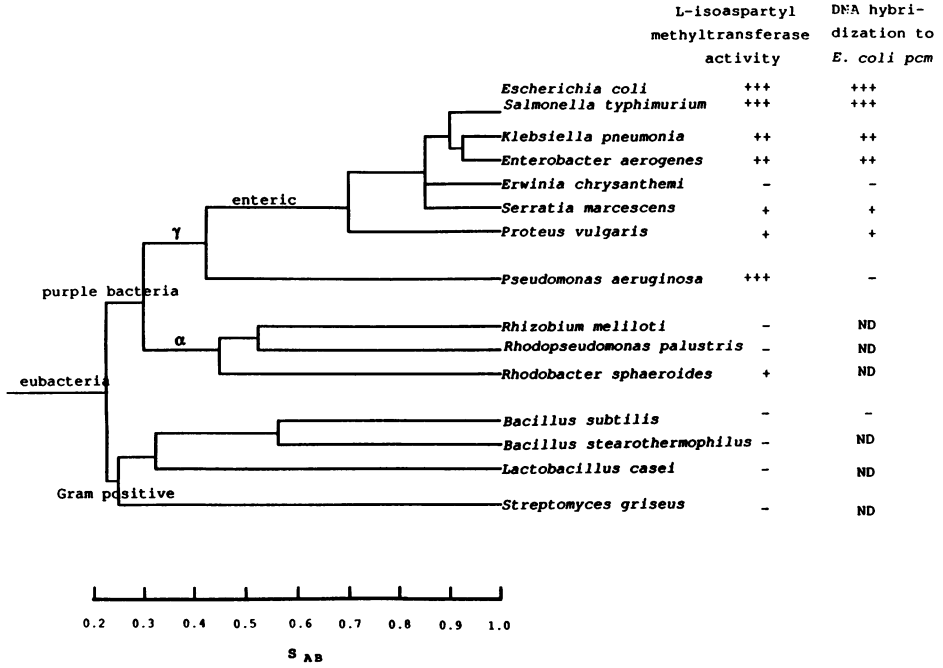

FIG. 3. Distribution of L-isoaspartyl methyltransferase activity in bacteria. This dendrogram is based on nucleotide sequence data from 16S rRNAs (7, 32, 36, 37). *Erwinia* is positioned according to the DNA:rRNA hybridization results given in reference 1. The binary association coefficient (S_{AB} value) is defined as the ratio of twice the sum of bases in oligonucleotides (length greater than five) common to two catalogs A and B to the sum of all bases in the two catalogs (7). Isoaspartyl methyltransferase levels are summarized from Fig. 1 and Table 2; DNA hybridization data are from Fig. 2. ND, not determined.

was the plant pathogen *E. chrysanthemi*. Based on DNA:rRNA hybridization, the genus *Erwinia* is evolutionarily closer to *Escherichia* than to *Proteus* or *Serratia* (1). However, DNA hybridization of *E. chrysanthemi* chromosomal DNA to the *E. coli pcm* methyltransferase gene probe was not detected under conditions in which both *P. vulgaris* and *S. marcescens* DNA showed cross-reactivity (Fig. 2). Although it is possible that *E. chrysanthemi* does not go through a "feast and famine" cycle, distinct mechanisms to deal with altered proteins may have evolved in this and other species lacking the methyltransferase (see below). Alternatively, the methylation pathway may be a relatively recent arrival in the bacterial world. All of the other enteric bacteria tested have animal hosts for at least part of their life cycle, and one might speculate that the gene for the isoaspartyl methyltransferase was passed by horizontal means from animal to bacterium (34). Mechanisms for transferring genes between bacterial species have been well described (24) and could account for the spread of a gene once introduced to a single species. On the other hand, the similarity of the *E. coli* and human methyltransferases on an amino acid level is 31%, a figure similar to that of highly conserved metabolic enzymes such as glycolytic components (6). Additional evidence against a recent origin of methyltransferase genes in bacteria is provided by the apparent divergence of the DNA sequences for the *E. coli* and *P. aeruginosa* methyltransferase genes: we see no cross-hybridization in the experiment shown in Fig. 2.

What possible mechanisms might protect cells against the accumulation of altered proteins containing isomerized and racemized aspartyl residues in organisms that lack the L-isoaspartyl methyltransferase? In *Bacillus* and other sporulating species, the loss of water in a dehydrated spore may inhibit the spontaneous reactions that result in altered aspartyl residues. Alternatively, bacteria that are not capable of differentiation may remove altered proteins by a strictly proteolytic mechanism. For example, it is conceivable that a protease exists with specificity for isoaspartyl and other altered residues. Such a mechanism could remove damaged proteins as effectively as the methylation system, although repair could not occur. The advantage of being able to repair altered proteins by methylation may be important in cells in which protein synthesis is more limited under starvation conditions. Finally, it is possible that the methyltransferase has a novel function not presently appreciated that is important in some but not all cells.

From our work, the L-isoaspartyl protein methyltransferase appears to be limited to members of the purple bacteria (Fig. 3). We should stress, however, that we have not attempted to make a comprehensive survey of procaryotic cells, and this enzyme may in fact have a wider distribution. There have been two other reports in the literature on protein carboxyl methylation in species other than *E. coli* and *S. typhimurium*. In vitro assays of homogenates of *Caldariella acidophila*, a thermophilic and acidophilic archaebacterium, demonstrated a methyltransferase activity of endogenous proteins, but the methyl acceptor specificity of this enzyme was not established (10). *Mycobacterium smegmatis* cells have been shown to produce methanol from *S*-adenosylmethionine, and at least a part of this product appears to be formed by the hydrolysis of protein methyl esters (35). Further work will be needed to determine whether either of these activities reflects the presence of an L-isoaspartyl protein methyltransferase.

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