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Phospholipid Composition and Cardiolipin Synthesis in Fermentative and Nonfermentative Marine Bacteria

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Twenty biochemically distinct isolates of marine bacteria, comprising a collection of gram-negative, motile, straight and curved rod-shaped organisms, were separated into fermentative and nonfermentative groups. The isolates were analyzed for phospholipid composition and the activities of the enzymes, cardiolipin synthetase, and a phospholipase were determined. The phospholipid compositions of all isolates were generally similar. Phosphatidylethanolamine and phosphatidylglycerol were the major phospholipid classes detected. The absence of cardiolipin in most of the nonfermentative isolates was the most striking observation noted. This was verified chromatographically and by the absence of cardiolipin synthetase activity. In isolates which had cardiolipin, it apparently was synthesized by the condensation of two molecules of phosphatidylglycerol, a mechanism similar to that observed in terrestrial bacteria. Possible correlations between the presence of cardiolipin and Mg^{2+} requirements for growth are discussed.

Significant differences in the composition and structure of the cell envelope, resulting from genetic alterations, may have allowed microorganisms to adapt to diverse ionic environments. Bacterial species which are normally found in seawater can provide excellent experimental systems to determine the kinds of effects a highly concentrated ionic environment may have on the membrane. Because phospholipids are essential components of biological membranes and also have been used as a parameter of taxonomic significance (17, 29), a comparative study of phospholipid composition, metabolism, and properties of similar enzyme systems, in marine and terrestrial bacterial species, which survive and grow under radically different environmental conditions, could lead to an increased understanding of the nature of membrane organization and evolution.

Although the phospholipid composition of many terrestrial bacterial species has been determined, there have been relatively few analyses of marine species even though the sea is the largest and perhaps the most important biological environment on earth. A comparison of the phospholipid composition of several marine and terrestrial bacteria has been reported in a recent paper by Oliver and Colwell (22). In addition they have reviewed much of the work in this area.

In this study the phospholipid composition of 20 marine isolates was determined and the activities of the enzymes cardiolipin synthetase and a phospholipase were analyzed. In addition to their intrinsic interest, these data were analyzed in an attempt to ascertain the degree of uniformity of phospholipid composition among biochemically distinct marine isolates. In this manner, future intensive studies of the cell membranes of an atypical organism with unique peculiarities could be avoided and experimental models chosen from more generally representative isolates.

MATERIALS AND METHODS

Bacterial cultures. Twenty isolates were chosen at random from a collection of 100 (MB series) originally isolated from the North Florida Atlantic seashore (28). These bacteria are gram-negative, straight and curved, rod-shaped organisms, motile by means of flagella, and originally were selected for their inability to grow on nutrient media without the addition of seawater. All cultures required Na^+ and were shown to be unique isolates which were different by at least one biochemical characteristic. For comparison, the marine strains *Vibrio parahaemolyticus* (ATCC 17802) and B16 (*Pseudomonas* sp. ATCC 19855) were included for phospholipid analysis. Stock cultures have been maintained on semisolid medium made with artificial seawater (ASW) and stored under mineral oil at 15 to 18 C. ASW was prepared as a 5× solution containing 2.0 M NaCl, 0.25 M $MgSO_4 \cdot 7H_2O$, and 0.05 M KCl in distilled water. Stock culture medium consisted of 0.1% Trypticase, 0.01% yeast extract (BBL), 0.3% Ionagar no. 2 (Oxoid) and 20% (vol/vol) 5× ASW. The medium was ad-

justed to pH 7.4 with tris(hydroxymethyl)aminomethane (Tris) base (Sigma).

Fermentation of glucose and other biochemical tests. Glucose fermentation was determined using a modification of the O/F test medium of Leifson for marine bacteria (19). The nutrient base consisted of 0.1% casein hydrolysate (vitamin and salt free) (Nutritional Biochemicals Co.), 0.01% yeast extract (BBL), 0.2% Ionagar no. 2 (Oxoid), 0.5% of a solution of 0.24% phenol red adjusted to pH 7.4 with Tris base, 20% 5× ASW, and water added to produce 90% of the final volume and then was sterilized. To this base medium was added 10% of a filter-sterilized 10% solution of D-glucose. After inoculation, the tubes were sealed by the addition of 2 ml of 2% Ionagar. Fermentation was considered positive when the phenol red indicator had a complete color change in both open and sealed tubes after 48 h of incubation.

Sensitivity of the marine isolates to polymyxin B was determined using Sensi-Discs (BBL) and a modified Kirby-Bauer spreading technique (4) on agar medium containing 1% Casitone, 0.01% yeast extract, 1% Ionagar no. 2, and 20% 5× ASW adjusted to pH 7.4 with Tris base. The effect of the vibriostatic agent 0/129 (2,4-diamino-6,7-di-isopropyl pteridine, Calbiochem) was demonstrated by using the method of Bain and Shewin (1).

Indole formation was determined according to Conn (7) after 48 h of incubation in 1% tryptone broth (Difco) dissolved in ASW.

Nitrate reduction was tested by growing cultures for 7 days in tubes of nitrate broth (Difco) made up in ASW and sealed with the addition of sterile paraffin oil. The presence of nitrite was determined by adding 0.5 ml each of 4% sulfanilic acid in 5 N acetic acid and 3% dimethyl-α-naphthylamine in 5 N acetic acid (4). The production of a pink color was taken as an indication of nitrate reduction.

Salt requirements. Requirements for augmented amounts of Na^+, K^+, Mg^{2+}, and Ca^{2+} were demonstrated by the absence of growth after 24 h in a medium lacking a specific ion compared with controls containing the complete ion mixture. It should be noted that low concentrations of all ions were always present as contaminants of media constituents. The media contained 0.05% casein hydrolysate (vitamin and salt free), 0.025% yeast extract, and salts to give a final concentration of 0.4 M NaCl, 0.01 M KCl, 0.028 M $MgSO_4 \cdot 7H_2O$ and 0.01 M $CaCl_2 \cdot 2H_2O$. Individual salts were omitted to test for growth with different salt combinations. The media were adjusted with Tris base to pH 7.5 before autoclaving.

Preparation of labeled cells and phospholipid analysis. All cultures used for phospholipid analysis were grown in broth medium (BM) containing 2% Casitone (Difco), 0.005% yeast extract (BBL), 0.05% $(NH_4)_2SO_4$, and 0.05% Tris in ASW. The pH of the medium was adjusted with NaOH to 7.5 and the medium was autoclaved. Inocula for production of cells and for tests were obtained by inoculating BM from stock cultures and incubating for 16 to 24 h at ambient temperature. Labeled phospholipids were prepared by inoculating 50 ml of BM in a 125-ml flask to which ^{32}P-orthophosphate (approximately 2 μCi/ml) had been added. The flasks were incubated for 16 h at 30 C a water bath with shaking. The cultures, which were in the stationary phase of growth, were harvested by centrifugation, washed once with cold ASW, and extracted with chloroform-methanol (1 + 2 vol/vol) following the procedure of Bligh and Dyer (5). The total lipid extract was stored in chloroform at −90 C. The lipid extracts were chromatographed in two dimensions on silica gel loaded paper (Whatman SG 81) at ambient temperature using solvents modified from those of Wuthier (30). The solvent for direction 1 contained chloroform, methanol, 2,6-dimethyl-4-heptanone (practical), acetic acid, water in ratios of 90:30:60:40:8 (vol/vol). The solvent for direction 2 contained chloroform, methanol, 2,6-dimethyl-4-heptanone, pyridine, 0.5 M ammonium chloride-hydrochloride buffer, pH 10.4, in ratios of 60:20:60:70:10 (vol/vol). The chromatograms were washed, stained with rhodamine 6G, dried, and tested for the presence of amino groups (18). Autoradiograms of each chromatogram were made using Dupont Cronex 2 X-ray film to localize the phospholipids. The phospholipid spots were cut out and counted in a gas flow planchet counter, and relative percentages were calculated.

Identification of some phospholipids was accomplished by chromatographic comparison with available commercial standards and the phospholipids of other microorganisms (8, 9). The identification of bisphosphatidic acid is described in a separate paper (21).

Preparation of cell fractions for enzymatic analyses. Cultures were prepared by inoculating 100 ml of BM in 250-ml flasks. The flasks were incubated at 30 C in a water bath with shaking. Cells were harvested by centrifugation, washed once with cold ASW, suspended up to 2 ml with cold ASW, and subjected to sonic treatment in an ice bath at maximum power for approximately 2 min with 15-s intervals (Sonic Dismembrator, Quigley-Rochester, Inc.). The suspension was centrifuged at 3,000 × *g* for 15 min to remove unbroken cells, and the supernatant fluid was centrifuged at 30,000 × *g* for 30 min. The resulting pellet was suspended in ASW and used without further treatment for enzyme assays. Protein was determined by the method of Lowry et al. (20) using bovine serum albumin as the standard.

Assays for cardiolipin synthetase and phospholipase. The presence of cardiolipin synthetase in cell fractions of marine bacteria was detected as described previously by De Siervo and Salton (9). The assay mixture contained 0.1 mM ^{32}P-phosphatidylglycerol (isolated from *Micrococcus lysodeikticus*), 200 mM Tris-hydrochloride buffer, pH 7.0, and 0.25% Triton X-100. Cell fractions were added to produce a final volume of 0.1 ml. Samples were incubated at 30 C for 15 min.

Phospholipase activity was measured as the decrease in the total amount of labeled phospholipids in the chloroform phase of the lipid extract and the appearance of label, as water-soluble products, in the aqueous phase. In the absence of any phospholipase activity there was no loss of total phospholipid label from the chloroform phase. The total radioactivity

was accounted for by the label found in cardiolipin and phosphatidylglycerol.

RESULTS

Fermentation and salt requirements. Twenty isolates of marine bacteria were separated into two groups on the basis of their ability to ferment glucose. Nine isolates fermented glucose with acid and no gas and 11 isolates were nonfermentative. The ability to ferment glucose also correlates with several other biochemical characteristics which are listed in Table 1. All the fermentative cultures were resistant to 50 U of polymyxin B, whereas the nonfermentative cultures were sensitive, with the exception of MB 37. The vibriostatic agent 0/129 inhibited the fermentative cultures with the exception of MB 21 and MB 74, but was not effective against the nonfermentative cultures with the exception of MB 6 and MB 37. Only the fermentative isolates produced indole. Nitrate was reduced to nitrite in all the fermentative isolates and only one, MB 37, of the nonfermentative isolates.

All isolates examined required sodium for growth. Requirements for additional potassium, magnesium, or calcium ions are listed in Table 1. Only one fermentative isolate required Mg^{2+} (MB 28), whereas it was required by seven of the 11 nonfermentative isolates. Only isolate MB 24 required the addition of Ca^{2+} for growth. A diagram of a composite two-dimensional chromatogram of all the phospholipids detected in all the isolates is shown in Fig. 1, and their R_f values are given in Table 2. Only two phospholipids were ninhydrin positive, phosphatidylethanolamine (PE) and lysophatidylethanolamine (LPE). The acidic phospholipids, cardiolipin (CL), phosphatidylglycerol (PG), and phosphatidic acid (PA), were identified in many marine isolates. PA produced two spots in the acid system. Bisphosphatidic acid (BPA) has recently been identified in MB 45 (21) and was found in most cultures examined. The identity of phospholipids labeled 1 through 7 is not known although phospholipid 1 corresponds chromatographically to acylphosphatidylglycerol, which has been found in other gram-negative bacteria (6, 23). Autoradiograms of a representative nonfermentative marine isolate, MB 8, and fermentative isolate, MB 14, are shown in Fig. 2. No CL was detected in MB 8, whereas it represented a large proportion (13.9%) of the phospholipids of MB 14. The

TABLE 1. *Biochemical characteristics of fermentative and nonfermentative marine isolates*[a]

Marine isolates	Characteristics				
	Sensitivity to polymyxin B (50 units)	Sensitivity to 0/129	Indole formation	Nitrate reduction	Salt requirement in addition to sodium
Fermentative					
MB 3	R	S	+	+	K^+
MB 14	R	S	+	+	K^+
MB 19	R	S	+	+	– –
MB 21	R	R	+	+	– –
MB22	R	S	+	+	K^+
MB 25	R	S	+	+	K^+
MB 28	R	S	+	+	K^+, Mg^{2+}
MB 39	R	S	+	+	K^+
MB 74	R	R	+	+	– –
Nonfermentative					
MB 2	S	R	–	–	Mg^{2+}
MB 6	S	S	–	–	K^+
MB 8	S	R	–	–	K^+, Mg^{2+}
MB 24	S	R	–	–	Mg^{2+}, Ca^{2+}
MB 29	S	R	–	–	K^+, Mg^{2+}
MB 35	S	R	–	–	K^+, Mg^{2+}
MB 37	R	S	–	+	K^+
MB 41	S	R	–	–	– –
MB 45	S	R	–	–	K^+, Mg^{2+}
MB 51	S	R	–	–	K^+
MB 59	S	R	–	–	K^+, Mg^{2+}

[a] Abbreviations: R, Resistent; S, sensitive; – –, no additional salt requirements; +, positive; –, negative.

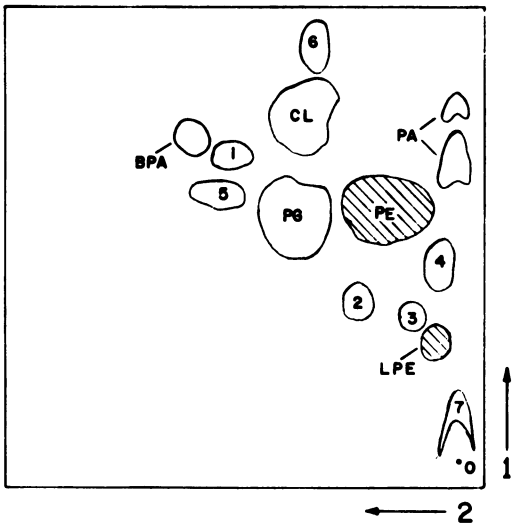

FIG. 1. *Diagramatic representation of a two-dimensional chromatogram of all the phospholipids detected in the marine bacteria analyzed in this study. There were no individual marine isolates in which all the phospholipids were detected. Phospholipids 1 to 7 were not identified. Cross-hatching indicates a ninhydrin-positive reaction. Solvents for directions 1 and 2 are given in Materials and Methods.*

TABLE 2. *R_f values of the phospholipids found in marine bacterial isolates*

Phospholipids[a]	Acid solvent[b]	Basic solvent[c]
PE	0.55	0.17
PC	0.55	0.37
CL	0.76	0.34
LPE	0.26	0.06
PA	0.67, 0.76	0.01
BPA	0.71	0.58
1	0.66	0.50
2	0.34	0.22
3	0.31	0.10
4	0.43	0.04
5	0.58	0.53
6	0.91	0.31
7	0.11	0

[a] Phospholipids 1 to 7 are unidentified.

[b] Chloroform, methanol, 2,6-dimethyl-4 heptanone (practical), acetic acid, water in ratios of 90:30:60:40:8:(vol/vol).

[b] Chloroform, methanol, 2,6-dimethyl-4-heptanone (practical), acetic acid, water in ratios of 90:30:60:40:8 (vol/vol).

relative percentages of the phospholipids of the fermentative isolates and, for comparison, *V. parahaemolyticus*, grown in an identical manner, are given in Table 3. The major phospholipids in all fermentative strains were PE, PG, and CL. PE was the phospholipid present in the largest quantities (60 to 82%) in all marine cultures analyzed. The relative percentages of phospholipid detected in the nonfermentative isolates, and B 16, grown in an identical manner for comparison, are listed in Table 4. PE and PG were the major phospholipids found except in B 16 which also had a large amount of CL. CL was not detected in 9 of the 11 nonfermentative isolates tested. Low levels of CL (<1%) were found in MB 37 and MB 41.

LPE was detected in most of the fermentative cultures and in three of the nonfermentative

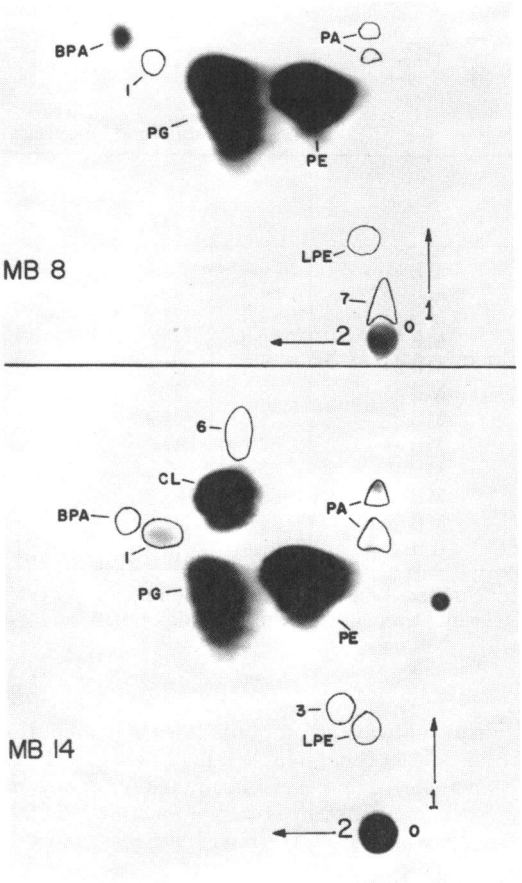

FIG. 2. *Autoradiograms of two-dimensional chromatograms of ^{32}P-labeled phospholipids of a representative nonfermentative isolate, MB 8, and fermentative isolate, MB 14. Minor phospholipids which were detected on the autoradiograms but which may not show up in the photograph are circled. Solvents for directions 1 and 2 are given in Materials and Methods.*

TABLE 3. *Phospholipid composition of fermentative marine bacteria*

Strain	% Total lipid phosphorus[a]											
	PE	PG	CL	LPE	PA	BPA	1	2	3	4	6	7
MB 3	65.7	7.0	19.3	0.7	4.4	0.3	1.0	0.3	0.5	—[b]	—	0.8
MB 14	70.8	13.0	13.9	0.2	0.8	0.2	0.7	—	0.2	—	0.3	—
MB 19	79.9	5.7	7.6	0.6	3.9	0.1	0.7	0.5	0.4	0.6	—	—
MB 21	75.9	17.3	5.8	0.5	—	—	0.4	—	—	—	—	—
MB 22	63.8	14.9	17.0	0.3	1.0	0.3	1.0	—	0.2	1.2	0.3	—
MB 25	68.4	18.7	9.8	0.4	1.4	0.3	1.0	—	—	—	—	—
MB 28	66.7	12.9	14.2	0.9	1.5	0.6	2.0	0.2	0.4	0.8	—	—
MB 39	64.0	9.9	15.9	0.9	2.0	1.5	4.0	0.2	0.8	0.8	—	—
MB 74	75.6	19.4	4.2	0.3	0.1	Tr[c]	0.3	—	—	—	—	—
Vibrio parahaemolyticus	82.7	5.1	11.5	—	0.6	—	Tr	—	—	—	—	—

[a] No corrections in percentages were made for cardiolipin which is known to have two phosphate groups per molecule. Unidentified phospholipids 1, 2, 3, 4, 6, 7 correspond to those in Fig. 1 and Table 2.
[b] —, Not detected
[c] Tr, Trace (<0.1%).

TABLE 4. *Phospholipid composition of nonfermentative marine bacteria*

Strain	% Total lipid phosphorus[a]									
	PE	PG	CL	LPE	PA	BPA	1	4	5	7
MB 2	76.1	21.7	—[b]	—	0.5	0.2	0.3	–	1.3	–
MB 6	66.9	30.8	—	—	0.5	0.8	0.2	–	–	0.7
MB 8	72.9	25.8	—	0.1	0.2	0.7	0.2	–	–	0.2
MB 24	76.0	22.2	—	—	0.5	0.7	0.6	–	–	–
MB 29	73.6	24.1	—	—	0.8	1.1	0.5	–	–	–
MB 35	68.0	27.7	—	0.2	0.2	2.9	0.4	–	–	0.6
MB 37	76.6	21.9	0.4	0.1	0.3	Tr[c]	0.4	–	0.3	–
MB 41	67.7	19.1	0.3	—	6.0	0.3	0.5	4.2	–	4.2
MB 45	67.8	27.9	—	—	0.8	1.9	0.7	–	–	1.0
MB 51	68.6	28.7	—	—	0.3	2.0	0.4	Tr	–	–
MB 59	71.5	22.9	—	—	2.0	3.3	0.3	–	–	–
B 16	61.6	16.8	14.1	—	2.2	3.9	0.9	–	0.6	–

[a] No corrections in percentages were made for cardiolipin which is known to have two phosphate groups per molecule. Unidentified phospholipids 1, 4, 5, and 7 correspond to those in Fig. 1 and Table 2.
[b] —, Not detected.
[c] Tr, Trace (<0.1%).

cultures, always in amounts less then 1% of the total lipid, although large amounts of LPE have been reported in other marine bacterial strains (22). These differences in the amounts of LPE among marine isolates may have reflected differences in growth conditions or age of cells.

It was observed that in all the nonfermentative cultures, except MB 37, BPA was present in similar or considerably larger quantities than phospholipid 1. Both phospholipids were detected in all nonfermentative isolates (Table 4). However, in all the fermentative isolates, phospholipid 1 was found in significantly larger amounts than BPA (Table 3). If phospholipid 1 is acylphosphatidylglycerol, these differences in the two groups may have reflected differences in the amounts or activities of acylating enzymes.

Unidentified phospholipids 2 and 3 were detected only in the fermentative isolates and phospholipid 5 was detected only in three of the nonfermentative isolates.

CL synthetase and phospholipase activity. To determine if the absence of CL in nine nonfermentative marine isolates was due to very low levels of CL, the cultures were examined for CL synthetase activity. Since the mechanism of CL synthesis in marine bacteria had not been ascertained, the CL-containing

isolates were also tested to provide positive controls. It was found that all CL-containing strains, except MB 25, contained CL synthetase activity (Table 5). The CL synthetase activity of MB 25, which contains 9.8% CL, was presumably masked by a phospholipase, also present in this isolate, which degraded the PG substrate. The strains in which CL could not be detected chromatographically did not show CL synthetase activity. CL synthetase activity was observed in the nonfermentative isolates MB 37 and MB 41, which contained only 0.4% and 0.3% CL, respectively. A comparison of the specific activities of CL synthetase with the amount of CL found in the cells indicated no apparent relationship between enzyme activity and CL concentration.

Three of the fermentative isolates, MB 3, MB 14, and MB 25, and one nonfermentative isolate, MB 37, also had phospholipase activity which resulted in the breakdown of PG to water-soluble products (Table 5). The nature of this phospholipase is presently being investigated.

TABLE 5. *Specific activities of cardiolipin synthetase and phospholipase in marine bacteria*

Marine isolates	μg of protein/ 0.1 ml	CL synthetase[a]	Phospholipase[b]
Fermentative			
MB 3	139	4.0	34.7
MB 14	93	6.5	68.8
MB 19	87	20.7	0
MB 21	118	42.5	0
MB 22	144	13.4	0
MB 25	196	0[c]	49.9
MB 28	177	6.8	0
MB 39	157	9.6	0
MB 74	193	23.2	0
Vibrio parahaemolyticus	122	18.9	0
Nonfermentative			
MB 2	92	0	0
MB 6	124	0	0
MB 8	179	0	0
MB 24	66	0	0
MB 29	112	0	0
MB 35	108	0	0
MB 37	171	0.6	36.4
MB 41	277	22.9	0
MB 45	182	0	0
MB 51	126	0	0
MB 59	134	0	0
B 16	190	4.6	0

[a] Nanomoles of PG converted to CL per milligram of protein in 15 min.

[b] Nanomoles of PG breakdown per milligram of protein in 15 min.

[c] Cardiolipin synthesis was not observed in MB 25 presumably because 97.8% of the phosphatidylglycerol substrate was broken down by the phospholipase in 15 min.

DISCUSSION

Although taxonomy of gram-negative, marine bacteria has become quite complex, they have been divided into two general groups: fermentative strains which ferment glucose with the production of acid and no gas and placed in genera which include *Beneckea*, *Aeromonas*, and *Vibrio* (2); and nonfermentative or aerobic strains placed in genera which include *Alteromonas*, *Alcaligenes*, and *Pseudomonas* (3). In this study the 22 marine bacterial cultures including *Vibrio parahaemolyticus* and B16, which has been classified as *Alteromonas haloplanktes* (25), were separated into fermentative and nonfermentative groups and analyzed for phospholipid composition. All of the fermentative isolates had considerable amounts of CL, whereas in nine of the 12 nonfermentative strains CL could not be detected. The absence of CL in these strains was corroborated by the absence of CL synthetase activity. No specific references regarding the absence of CL in marine bacteria have been found; however, a recent report by Diedrick and Cota-Robles (13) on the phospholipid composition of *Pseudomonas* BAL-31 does not indicate if CL was present. Presumably it was absent. Other investigations of the phospholipid content of gram-negative bacteria have reported the presence of CL both in marine (12, 14, 22) and terrestrial bacteria (24). The phospholipid composition of *V. parahaemolyticus* was similar to that obtained by Oliver and Colwell (22) except for the relative amounts of CL and PG. However, the combined amounts of these two phospholipids were very similar. These differences are understandable since PG is the precursor of CL and the relative amounts of these lipids have been shown in both *Escherichia coli* (8) and *Micrococcus lysodeikticus* (10) to be highly variable with respect to culture age and growth conditions.

Two mechanisms for CL synthesis have been described. In animal systems it occurs by the reaction of PG with cytidine 5'-diphosphate-diglyceride (16, 27), whereas in bacterial cells two molecules of PG combine to form one molecule of CL (9, 15, 26). The synthesis of CL in marine bacteria in the absence of added or detectable endogenous cytidine 5'-diphosphate-diglyceride and with PG as the only added

substrate strongly suggests that the mechanism of CL synthesis in marine bacteria is similar to that found in terrestrial species.

The absence of CL in several nonfermentative isolates appears to be correlated to the requirement for additional magnesium because seven of the nine strains which lacked CL required Mg^{2+}. None of the strains in which CL was dectected, with the exception of MB 28, required additional magnesium. One of the functions of both the required Mg^{2+} and CL may be the stabilization of the cell membranes. It is possible that the absence of CL necessitates a requirement for Mg^{2+} for membrane stability and conversely the presence of CL eliminates the need for large amounts of Mg^{2+}. Whereas a stabilizing role for Mg^{2+} in marine bacteria has been suggested (11), a similar role for CL has not. However, the structure of CL, with two diglyceride moieties separated by glycerol and two phosphate groups, may allow the bridging of independent lipoprotein structures and result in increased stability of the membrane.

In general, the separation of marine bacteria into fermentative and nonfermentative groups correlated well with other parameters such as sensitivity to polymyxin B and the vibriostatic agent 0/129, indole formation, and nitrate reduction (Table 1). This correlation was also apparent in the phospholipid composition of these groups (Tables 3 and 4). Although all the marine isolates were similar to most gram-negative bacteria in having PE as the major phospholipid and large amounts of PG, the nonfermentative groups was unusual because of an absence of CL in 75% of the isolates studied. A larger group of bacterial isolates shown to be devoid of CL and CL synthetase activity has not been reported previously. The large number of isolates found suggests that marine species without CL may represent a significant percentage of the marine population. This absence of CL also indicates that in these isolates CL is not a necessary component for membrane function. Whether the ionic environment found in seawater had led to the evolution of unique bacterial species which do not require CL or whether corresponding terrestrial groups also exist remains to be seen. In either case, comparative studies of bacterial species which do not have CL with those which do can lead to a greater understanding of membrane organization and biosynthesis.

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