

1984

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Baxter, M., & Sieburth, J. McN. (1984). Metabolic and Ultrastructural Response to Glucose of Two Eurytrophic Bacteria Isolated from Seawater at Different Enriching Concentrations. *Appl. Environ. Microbiol.*, 47(1), 31-38. Retrieved from <http://aem.asm.org/content/47/1/31>. Available at: <http://aem.asm.org/content/47/1/31>

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Metabolic and Ultrastructural Response to Glucose of Two Eurytrophic Bacteria Isolated from Seawater at Different Enriching Concentrations

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Received 11 May 1983/Accepted 25 October 1983

Two marine bacteria, an *Acinetobacter* sp. (strain GO1) and a *Vibrio* sp. (strain G1), were isolated by extinction dilution and maintained in natural seawater supplemented with nitrogen, phosphorus, and glucose at 0.01 and 1.0 mg of glucose carbon per liter above ambient monosaccharide concentrations, respectively. After 3 days in unsupplemented natural seawater, growth in batch culture with glucose supplements was determined by changes in cell numbers and glucose concentration. The exponential growth of the *Acinetobacter* strain with added glucose was indistinguishable from that in natural seawater alone, whereas that of the *Vibrio* strain was more rapid in the presence of glucose supplements, suggesting that the *Acinetobacter* strain preferred the natural organic matter in seawater as a carbon source. The ultrastructure for both isolates was unaffected by glucose supplements during exponential growth, but there were marked changes in stationary-phase cells. The *Vibrio* strain formed polyphosphate at 10 mg of glucose carbon per liter, whereas poly- β -hydroxybutyrate formation occurred at 100 mg and became excessive at 1,000 mg, disrupting the cells. In contrast, the *Acinetobacter* strain elongated at 100 and 1,000 mg of glucose carbon per liter but failed to show poly- β -hydroxybutyrate formation. The diversity of responses shown here would not have been detected with a single concentration of substrate, often used in the literature to characterize both pure and natural populations of marine bacteria.

Jannasch and Jones (23) documented the marked discrepancy between the populations of marine bacteria estimated by cultural procedures and by direct microscopic counts. With increased detection and precision due to epifluorescence microscopy (18, 34), this discrepancy has become larger by two orders of magnitude. This discrepancy may be due in part to two apparently different bacterial populations, one requiring low nutrient concentrations (low K_s bacteria) and the other requiring high nutrient concentrations (high K_s bacteria) (21, 28). These hypothetical groups may be similar to the free-living planktonic bacteria and the surface associated epibacteria, respectively (37).

Isolation of low K_s bacteria has been attempted (1, 2, 10). These isolation procedures, however, included growth on agar media which contain utilizable polysaccharide (P. J. LeMay, M.S. thesis, University of Rhode Island, Kingston, 1979) at concentrations of several grams of carbon per liter (C/L) and would preclude finding the hypothetical low K_s bacteria which presumably would not grow under these eutrophic conditions (37). The extremes of dissolved organic carbon concentrations in the sea range from less than 1.0 mg/liter in bulk seawater to 3 g/liter in the organic film at the sea-air interface (39). Other sites which are potentially substrate rich are fouled surfaces (27, 36) and the aggregated organic matter of marine snow (11, 38). Perhaps most marine bacteria are eurytrophic, utilizing a wide range of dissolved organic matter (3), whereas the hypothetical low K_s bacteria may not exist.

The purpose of this study was to determine the effect of nutrient concentration in the culture medium used to isolate bacteria by the extinction dilution technique at glucose

additions one order of magnitude above (hypertrophic) and one order of magnitude below (hypotrophic) the ambient total monosaccharide (MCHO) concentration in subsurface seawater. Glucose, the most common MCHO in the sea (30), was used as the MCHO supplement because seawater concentrations remain consistently low (4-8, 25), possibly indicating rapid uptake to a threshold level by the indigenous bacterial populations. The two isolates studied which grew on the ambient dissolved organic matter in seawater, as well as on agar media, are eurytrophic and have similar growth curves regardless of their metabolic response to MCHO. Divergent metabolic and ultrastructural responses emerged, however, when the isolates were grown at a variety of glucose concentrations.

MATERIALS AND METHODS

Extinction dilution isolation. Seawater was collected on 15 September 1980 from the Graduate School of Oceanography pier in Narragansett Bay, R.I., and was transferred immediately to the laboratory in a 2-liter screw cap flask covered with black plastic to exclude light. The bacterial inoculum was prepared by passing the water through a 20- μ m Nytex screen (Sterling Marine Products, Montclair, N.J.) under gravity to remove large organisms and debris and then through a sterile 0.6- μ m Nuclepore filter (Nuclepore Corp., Pleasanton, Calif.) under a vacuum in an autoclaved 47-mm diameter Millipore filtration apparatus (Millipore Corp., Bedford, Mass.) with a stainless steel screen base.

Enrichment cultures were prepared in test tubes with a basal medium of inorganically supplemented seawater (see below) containing either 0.01 or 1.0 mg of glucose C/L. The medium (9 ml) was inoculated with 1 ml of the filtrate, and 1 ml from each tube was aseptically transferred to 9 ml of fresh medium every other day for 1 week. Pure cultures were obtained from the enrichment cultures by extinction dilu-

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tion. A serial decimal dilution series was made and 1-ml portions of the 10^{-5} through the 10^{-8} dilutions were used to inoculate 9 or 10 replicate tubes of the seawater medium. After 48 h of incubation at 17°C, 3 ml from each tube was examined with epifluorescence microscopy (see below), and one tube showing growth from the dilution series which had both negative and positive tubes was used for the next dilution series. After three or more dilution series, a subculture was maintained by regular transfer. These were pure cultures since plating on OZR agar (35) yielded single colonies that had bacteria with a uniform cell morphology by phase contrast microscopy as well as by transmission electron microscopy (TEM). The pure culture isolated at 1.0 mg of glucose C/L was labeled G1 and that isolated at 0.01 mg of glucose C/L was labeled GO1. Isolates were maintained at 17°C and transferred to fresh media every other day.

Media. The basal medium was prepared by filtering natural seawater through a 0.22- μ m Millipore filter, adding NH_4Cl and FePO_4 to 10 and 1 mg/liter, respectively, and autoclaving. For maintenance (0.01 and 1.0 mg of glucose C/L) and experimental media, the basal medium was supplemented with a sterile glucose stock solution. Glucose agar plates were made with the basal medium, 1.5% agar, and either 0.01 or 1.0 mg of glucose C/L. OZR plates were made with 0.22- μ m Millipore-filtered seawater, 1.5% agar, 0.1% yeast extract, and 0.1% Trypticase (35).

Growth experiment. Isolates G1 and GO1 were inoculated into six 2-liter flasks containing 1 liter of basal medium with glucose supplements ranging from 0.2 to 425 mg of glucose C/L. A seventh flask containing only basal medium served as a control. Exponential-phase cells for the growth experiment were prepared by transferring isolates G1 and GO1 into fresh basal medium daily for 3 days. This inoculum added to the flasks yielded ca. 10^3 cells per ml. The flasks were incubated at 17°C and sampled after 0, 6, 7.5, 9, 10.5, 12, 15, 18, 21, and 24 h for 4',6-diamidino-2-phenylindole cell counts, as preliminary observations showed that the exponential phase occurred between 6 and 12 h. Sampling at 1.5-h intervals during exponential growth permitted the calculation of uptake rates during this period. Before sampling, the flasks were swirled for at least 30 s. Samples for carbohydrate analysis were removed at 0, 7.5, 9, 10.5, 12, and 24 h.

Cell counts. Samples (5 to 10 ml) were aseptically obtained, fixed with Formalin (1%, vol/vol), and stored in the dark at 4°C. Bacterial populations were estimated by epifluorescence direct count (18) with 4',6-diamidino-2-phenylindole (Sigma Chemical Co., St. Louis, Mo.) (34) at a final concentration of 0.27 μ g of 4',6-diamidino-2-phenylindole per ml. Twelve to twenty fields were enumerated, for a final count of at least 600 cells per sample (12) with an Olympus Vanox epifluorescence microscope (Olympus, Inc., Tokyo, Japan) at 1,000 \times .

Microscopy. Large-volume cultures similar to the growth experiment were made to obtain sufficient cells for TEM. The exponential-phase cultures were grown on 70 mg of glucose C/L, whereas the stationary-phase cultures were grown at glucose concentrations ranging from 1 to 1,100 mg of glucose C/L for strain G1 and at concentrations ranging from 0.5 to 1,000 mg of glucose C/L for strain GO1. Cells were fixed by adding glutaraldehyde to the medium at a final concentration of 1% and were prepared for thin sectioning (26), examined, and photographed in a Zeiss EM9S-2 transmission electron microscope. The cultures were also examined for the presence of poly- β -hydroxybutyrate (PHB) and polyphosphate (PP) after being stained with Sudan black B and toluidine blue, respectively (14), and were observed by

using bright-field microscopy with a Zeiss Photomicroscope I.

Uptake. Uptake of carbohydrate carbon per cell per hour was determined for the exponential phase during the first 12 h and for the stationary phase between 12 and 24 h. Since the exponential phase essentially occurred between 6 and 12 h, both cell counts and carbohydrate analyses were made at 1.5-h intervals during this period to account for short-interval differences in population.

Yield. By using the cell length and diameter determined from the micrographs of cells grown on 1 mg of MCHO C/L and by estimating the grams per cell (dry weight) for both isolates (16), cell yield (grams of cells produced per gram of MCHO used) was calculated for both 12 and 24 h.

Taxonomy. The isolates were subjected to numerical taxonomic analysis (13) and were identified by R. R. Colwell (University of Maryland, College Park, Md.). Isolate GO1 was identified as an *Acinetobacter* sp. and isolate G1 as a *Vibrio* sp.

Carbohydrate analysis. Samples were aseptically removed from the experimental flasks with acid-cleaned glass syringes. Twelve milliliters of sample was filtered through an acid-cleaned Swinnex filter holder (Millipore) with a 0.2- μ m Nuclepore membrane to remove the cells. The first 2 ml of filtrate were discarded, and the remainder was stored in chemically cleaned and combusted (450°C for 12 h) sample bottles at -20°C until analysis. Monomeric carbohydrates were analyzed with the 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) assay of Johnson and Sieburth (25) as modified by Johnson et al. (24) with a Hitachi Perkin-Elmer Coleman 111 spectrophotometer with a model 68 digital concentration readout and a model 80 printer (A. H. Thomas, Philadelphia, Pa.). Precision for the MBTH analysis was 2.6%. The MBTH assay does not measure glucose specifically, but rather all MCHOs in solution. Thus, in the controls (basal medium only) MBTH determinations represent the total ambient MCHOs and the glucose supplement.

RESULTS

Growth characteristics. Glucose supplements always accelerated the growth rate of isolate G1 (*Vibrio* sp.); the slope ($x = 0.240$) of the logarithmic growth curve in the control flask was significantly lower ($P < 0.05$) than the slopes ($\bar{x} = 0.306 \pm 0.02$) obtained in any of the experimental flasks (Fig. 1A). However, the growth rate of isolate GO1 (*Acinetobacter* sp.) in the basal medium (Fig. 1B) was not influenced by glucose supplements; the slope of the logarithmic growth curve in the control flask did not significantly differ ($P < 0.05$) from the slopes ($\bar{x} = 0.247 \pm 0.02$) in any of the experimental flasks to which glucose was added. The log-phase generation times were 1.44 ± 0.12 h and 1.57 ± 0.16 h for the G1 and GO1 isolates, respectively. For both isolates, the final cell populations at stationary phase were controlled by the initial substrate concentration (S_i) up to a concentration of 20 mg/liter. A leveling off of the final cell count with increasing S_i in Fig. 1 may be due to a substrate saturation effect of glucose or to the exhaustion of a growth-limiting factor.

Carbohydrates. Carbohydrate decrease in batch culture experiments is presumed to be due to bacterial uptake. The percent loss of carbohydrate from solution by isolates G1 and GO1 over a series of initial MCHO concentrations for two time intervals is summarized in Table 1 and plotted in Fig. 2. Table 1 shows that the rate of MCHO utilization

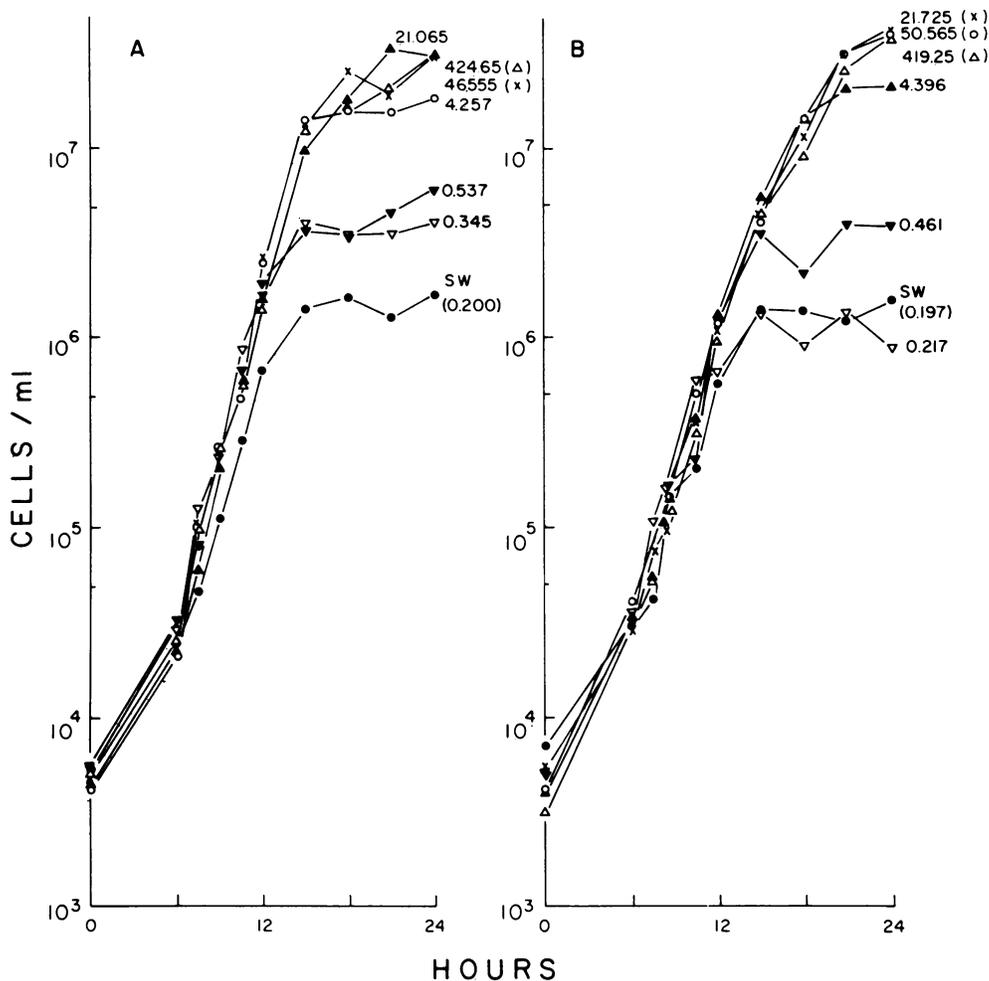


FIG. 1. Initial populations and growth curves of isolates obtained above (strain G1; panel A) and below (strain GO1; panel B) ambient MCHO concentrations in seawater when grown in natural seawater (SW) with and without glucose additions. The initial MCHO concentrations of all flasks are shown in mg of C/L.

during the log phase (12 h), as determined by the percent MCHO uptake, was maximal for isolate G1 in flask 2 ($S_i = 0.537$ mg of MCHO C/L; glucose added = 0.35 mg of glucose C/L), and for isolate GO1 in the control flask ($S_i = 0.198$ mg of MCHO C/L). By the stationary phase at 24 h, the uptake

curves for both isolates were nearly identical (Fig. 2). The rate of MCHO uptake per cell was positively correlated ($P < 0.01$, $n = 6$) with increasing additions of glucose for both isolates, although for isolate GO1 this correlation is obvious only at very high levels of glucose addition (flasks 10 to 12). MCHO uptake per cell after 24 h was nearly identical in both isolates. Yield (efficiency) generally decreased with increasing S_i in isolate G1 but sharply increased with increasing S_i in isolate GO1 (flasks 7 to 9) during exponential growth. In the stationary phase, yield varied inversely with S_i for both isolates.

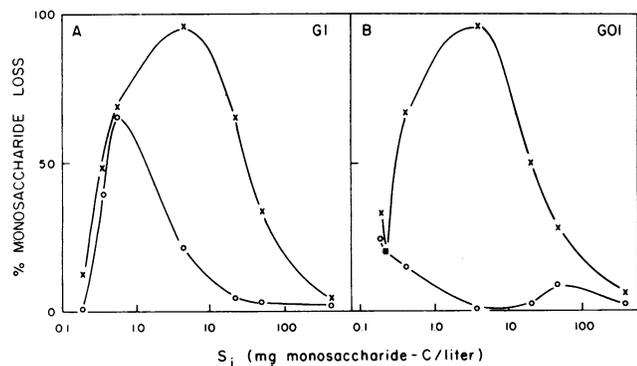


FIG. 2. Comparison of the different patterns of monosaccharide loss with the time in cultures of isolates G1 (A) and GO1 (B) grown on a series of glucose concentrations added to the ambient MCHO in seawater. Symbols: O, 12-h loss; X, 24-h loss.

Microscopy. TEM of thin sections of both isolates grown on 70 mg of MCHO C/L revealed similar morphologies of typical gram-negative rods during exponential-phase growth (Fig. 3A and 3B). Stationary-phase growth, however, was characterized by markedly different morphologies (Fig. 3C and 3D). Isolate G1 was found to have granules of electron-dense PP and electron-transparent PHB. Isolate GO1, which did not form PHB, had only a relatively small number of PP deposits and was characterized by elongation of the cells to several micrometers in length. To further observe the ultrastructural changes occurring in isolate G1, it was grown on four concentrations of glucose and harvested in the stationary phase (Fig. 4). A typical gram-negative cellular morphol-

TABLE 1. Summary of the measured MCHO uptake, percent uptake, uptake per cell, and the yield for isolates G1 and GO1 grown in basal medium with (flasks 1 to 12) or without (control) glucose supplements during the log (12-h)^a and stationary (24-h) growth phases

Isolate and flask	MCHO (mg of C/L)			MCHO uptake % of S _i		Uptake (mg of MCHO carbon per cell per h)		Yield ^b	
	S _i	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
G1									
Control	0.198	g ^c	0.7		12.5	g	0.12	g	331.0
1	0.345	0.21	0.18	38.6	47.8	1.41	0.33	58.5	127.0
2	0.537	0.19	0.17	64.6	68.0	2.94	0.50	28.0	83.3
3	4.257	3.36	0.24	21.1	94.2	6.18	1.82	13.4	22.8
4	21.065	20.02	7.41	5.0	21.1	11.12	3.74	7.4	11.1
5	46.555	45.40	30.80	2.4	33.8	7.62	4.04	10.8	10.3
6	424.65	415.75	407.15	2.1	4.1	104.1	4.64	0.8	8.9
GO1									
Control	0.198	0.15	0.13	24.9	33.5	1.42	0.35	96.6	200.0
7	0.217	0.17	0.17	20.7	21.2	1.17	0.42	118.3	164.3
8	0.461	0.39	0.15	15.6	66.6	0.96	0.66	146.1	106.1
9	4.396	4.36	0.21	0.8	95.2	0.49	1.65	288.2	42.8
10	21.725	21.04	10.80	3.1	50.3	9.49	2.25	14.7	31.3
11	50.565	46.16	36.70	8.7	27.4	67.73	2.69	2.1	26.2
12	419.25	411.85	397.75	1.8	5.1	127.8	4.78	1.1	14.7

^a Based on data obtained at 1.5-h intervals during exponential growth that occurred between 6 and 12 h.

^b (Grams of cell carbon produced/grams of MCHO carbon utilized) × 100.

^c g, MCHO increase.

ogy (fibrillar nuclear material in the center surrounded by ribosome-rich cytoplasm, both contained by the wavy cell envelope) was apparent at 1 mg of MCHO C/L (Fig. 4A). At 10 mg of MCHO C/L, the cells were enlarged and contained numerous granules of PP (Fig. 4B). At 100 mg of MCHO C/L, light microscopy as well as TEM (Fig. 4C) showed a further increase in cell size due to an accumulation of PHB. At 1,000 mg of MCHO C/L, isolate G1 became so swollen that the cells actually burst (Fig. 4D). When grown on increasing concentrations of glucose as above, isolate GO1 elongated, with the accumulation of some PP but without PHB formation as shown in Fig. 3D.

Taxonomy. The different metabolic and ultrastructural responses shown above for the two isolates reflect not only their different conditions of isolation but their taxonomic differences. The hypotrophic isolate GO1, enriched at a glucose concentration 1/10 that of MCHO in Narragansett Bay, was a nonflagellated, gram-negative rod (0.5 by 1.5 μm) that oxidized glucose and hydrolyzed starch but was otherwise biochemically inactive and had a 45.1% guanine plus cytosine base ratio, which presumptively identifies it as an *Acinetobacter* sp. The hypertrophic isolate G1, enriched at a glucose concentration 10-fold that of MCHO in Narragansett Bay, was a flagellated, gram-negative coccobacillary form (0.5 by 1.0 μm) that was biochemically active, fermentative, inhibited by 0/129, and had a 42.1% guanine plus cytosine base ratio, which presumptively identifies it as a *Vibrio* sp.

DISCUSSION

Carlucci and Shimp (10) isolated a bacterial colony growing on unsupplemented seawater agar inoculated with seawater that grew on the ambient dissolved organic matter in natural seawater and was assumed to be a low K_s isolate. The use of unenriched or low nutrient media containing agar to isolate "oligotrophic" or low K_s bacteria (1, 10) and to enumerate "low-nutrient" bacteria obtained from chemostats (17, 21) may result, instead, in the isolation of high K_s bacteria or those with multiphasic uptake kinetics (3). The high concentration of contaminating polysaccharides present

in agar precludes isolation of the hypothetical low-nutrient bacteria (37). In an attempt to avoid this problem, we grew marine bacteria in inorganically supplemented natural seawater with glucose additions 1/10 and 10× the ambient MCHO levels of subsurface water and obtained pure cultures with extinction dilution. Despite this precaution, both isolates GO1 and G1 grew on unsupplemented and supplemented agar media, indicating their eurytrophic nature. These isolates were found to differ greatly, however, in their strategies for glucose utilization. As identified by numerical taxonomy, the hypotrophic isolate GO1 is an *Acinetobacter* sp., and the hypertrophic isolate G1 is a *Vibrio* sp. The *Vibrio* populations in Narragansett Bay range from 0 to 38% of the CFU on agar, averaging 14% (35). *Vibrio* spp. are commonly associated with rich organic surfaces, which is in agreement with the more rapid growth of isolate G1 with glucose additions (Fig. 1A, Table 1). *Acinetobacter* spp. are considered oligotrophic bacteria and are widely distributed in soil and water. This genus has not been found to accumulate intracellular reserve materials such as PHB, which is in agreement with the observations on isolate GO1 (Fig. 3).

Neither isolate G1 nor GO1 represents the theoretical low K_s bacteria, as both grow on agar at MCHO concentrations three orders of magnitude greater than the ambient concentrations in bulk seawater. Isolate G1 exhibited normal cell morphology at 10× the ambient MCHO of subsurface water and formed PHB to such an extent that the cells were greatly enlarged at 1,000× the ambient MCHO and burst at 10,000× the ambient MCHO. The latter carbohydrate concentration (1 g of MCHO C/L) is approximately that of the MCHO of surface films (39) and one-third that of the contaminating carbohydrates in agar gels and the organic nutrients added to most marine agar media. The inhibition of growth of natural bacterial populations at above-ambient levels of added carbon (33) may be the result of an imbalance in metabolism by the overproduction of one compound in a pathway that may block a step in another pathway (15).

The marked ultrastructural differences between isolates G1 and GO1 were not manifested by the nearly identical

growth curves (Fig. 1). During exponential growth, *Acinetobacter* sp. strain GO1 grew at the same rate at a wide variety of glucose concentrations, which indicates that this isolate grows maximally on the dissolved organic substances present in natural seawater. This is consistent with the oligotrophic nature of *Acinetobacter* spp. and implies that MCHO is being used as an energy source rather than as a carbon source. Indeed, for isolate GO1, glucose supplements of <20 mg of C/L were associated with yields exceeding 100%. At

supplements of >20 mg of C/L, however, sharply lower yields were observed during exponential growth (Table 1). Glucose supplements increased the final populations attained during the stationary phase (Fig. 1B). Because yield (grams of cells produced per gram of substrate used) is a measure of efficiency and because conversion efficiency (9, 19, 32) in bacteria during exponential growth is ca. 60%, yields greater than 70% (Table 1) represent more cell carbon production than MCHO utilization. These data indicate that

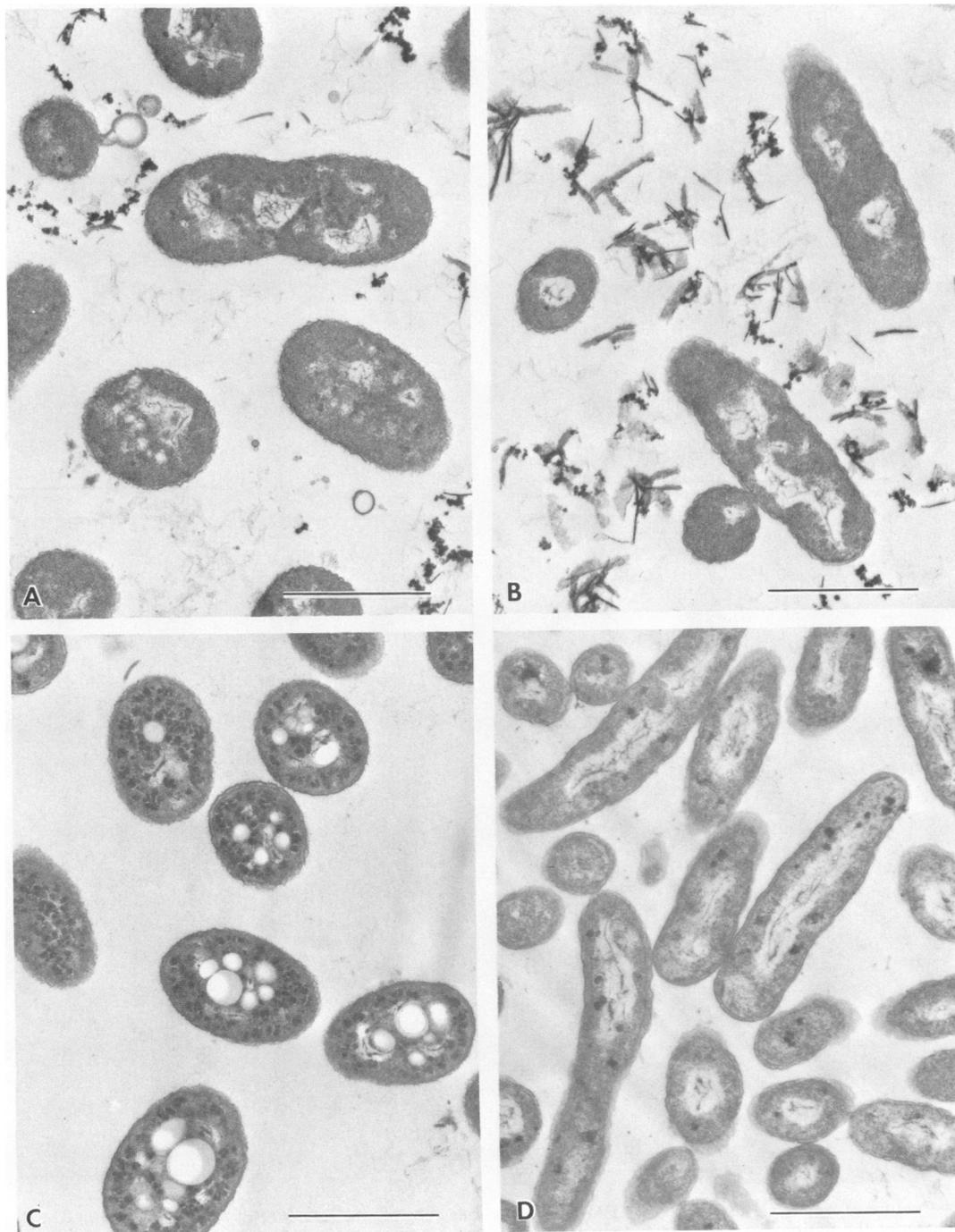


FIG. 3. Transmission electron micrographs of thin sections of isolates G1 (A, C) and GO1 (B, D) when grown on 70 mg of MCHO C/L, showing the ultrastructure during exponential-phase growth at 12 h (A, B) and stationary-phase growth at 24 h (C, D). The light inclusion bodies in isolate G1 at the stationary phase (C) are PHB granules. Bars = 1.0 μ m.

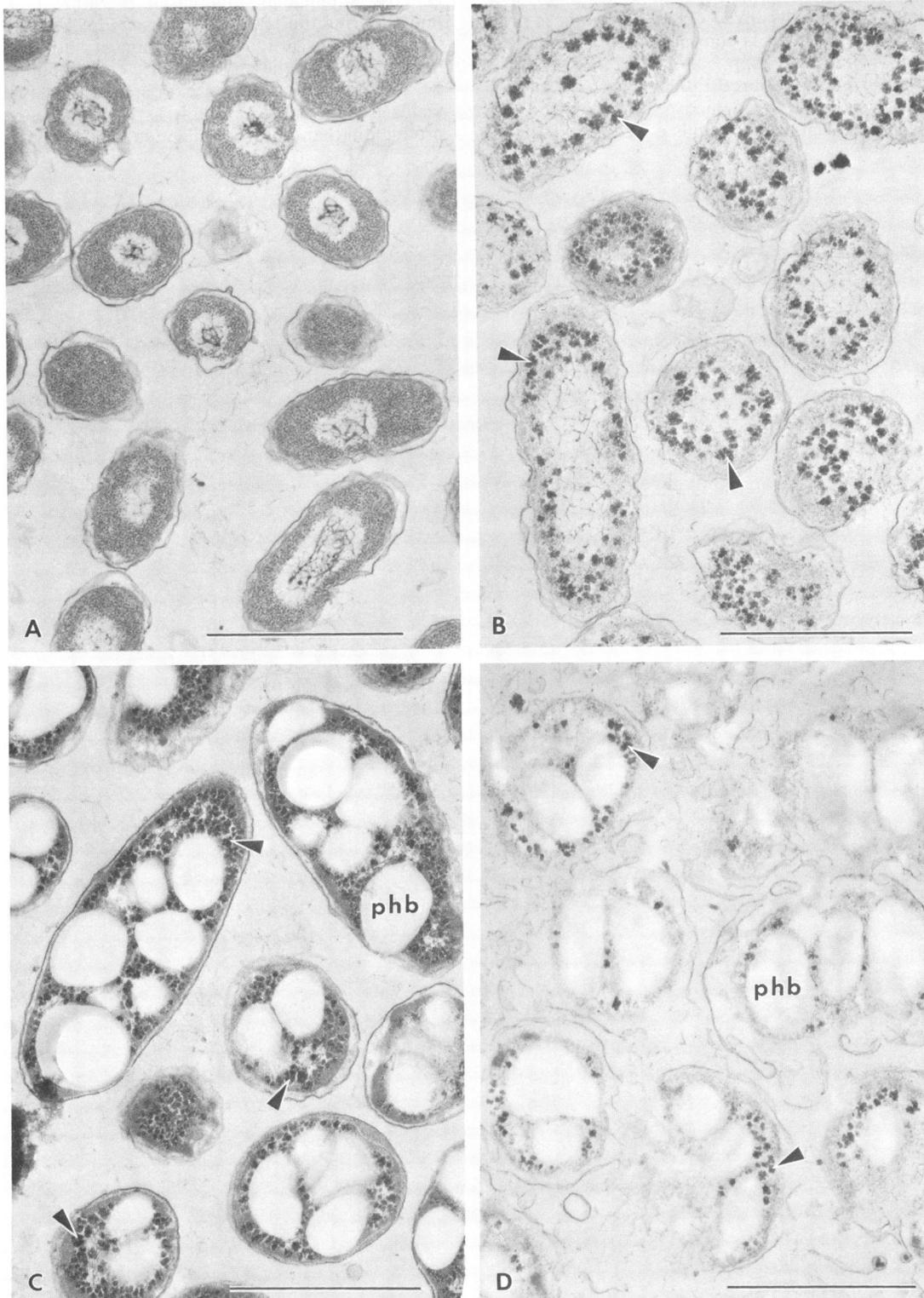


FIG. 4. Ultrastructure of isolate G1 as shown by TEM of thin sections showing the influence of four concentrations of glucose in basal medium after 36 h during the stationary phase. (A) At 1 mg of C/L, the bacteria appear normal, with small, evenly distributed ribosomes and a central nucleic acid region; (B) at 10 mg of C/L, the cells have increased in size, with the formation of PP (arrows); (C) at 100 mg of C/L, the cells are swollen with PP (arrows) and PHB; and (D) at 1,000 mg of C/L, the cells appear to have burst, with only residual cell wall material, PP (arrows), and PHB present. Bars = 1.0 μm .

isolate GO1 must be preferentially using organic substrates other than MCHO in natural seawater to satisfy its need for carbon. Glucose supplements (>20 mg of C/L) appear to be characterized by increased MCHO uptake per cell as well as decreased yield in both log- and stationary-growth phases (Table 1). These data, and the higher cell counts with increasing S_i (Fig. 1B), suggest that there is a switchover to MCHO as a carbon source after the exhaustion of the preferred substrate.

Isolate G1, on the other hand, differs from isolate GO1 by showing yields near 60% at glucose supplements of <20 mg of C/L during the exponential phase. These data, coupled with the rapid initial uptake (Table 1) and the significantly greater growth rate on glucose-supplemented basal medium (Fig. 1A), suggest that glucose was more readily used as a carbon source by isolate G1. These responses probably reflect the physiological differences responsible for the isolation of isolates GO1 and G1 at very different levels of glucose enrichment. At high levels of glucose addition (>20 mg of C/L), the decreasing yields contrasted with higher cell counts, and increased uptake per cell for both isolates in both growth phases shows that in addition to the energy or carbon source, some other mechanism was available to deal with large excesses of glucose in the environment.

For low-nutrient bacteria (isolate GO1) cultured in excess carbon, polymeric carbohydrate production (29, 41, 42) and accumulation (31) has been shown to be a survival mechanism to dispose of the excess MCHO taken up. Bacteria which can alter their metabolism to do this may have an advantage over those bacteria that cannot. Indeed, isolate GO1 may possess multiphasic uptake kinetics with separate low K_s and high K_s systems, as hypothesized by Azam and Hodson (3). The low K_s system appears to be constitutive and saturable, whereas the high K_s system may result from facilitated diffusion coupled with extracellular polymeric carbohydrate production, allowing isolate GO1 to survive at substrate concentrations far above the ambient concentration, as would occur on organically rich surfaces. Isolate G1 differs by showing the formation of PP and PHB granules at high glucose concentrations (Fig. 3 and 4). The formation of storage products may serve as an overflow mechanism for bacteria unable to produce extracellular polymeric carbohydrate, but only up to concentrations which cause the bursting of cells.

In the three flasks with the lowest S_i , isolates GO1 and G1 reduced the MCHO concentration to a mean final concentration of 0.17 and 0.15 mg of C/L, respectively, at 23 h (Table 1). These low levels correspond to the concentration of MCHO found in Narragansett Bay in summer (4). Although extrapolation from pure cultures to in situ mixed populations may not be valid, it is possible that our isolates belong to a group of bacteria capable of utilizing substrate only above a threshold level (20–22, 30, 40).

Glucose supplementation one order of magnitude above and below the environmental MCHO concentrations in subsurface seawater apparently allowed the selection of two eurytrophic marine bacteria exhibiting divergent strategies for growth. Increasing glucose concentrations increased the rate of exponential growth of *Vibrio* sp. (strain G1) over that in natural seawater but did not change the exponential growth rate of *Acinetobacter* sp. (strain GO1). Both isolates G1 and GO1 grew at or near maximal growth rates of 1.44 and 1.56 h, respectively, in inorganically supplemented natural seawater, increasing from starting populations of 10^3 to final cell populations of 10^6 or 10^7 cells per ml. These isolates are probably representative of estuarine planktonic

bacteria, which are maintained at populations of 10^6 cells per ml in Narragansett Bay, and may have similarly rapid growth rates in situ. Natural populations, limited by predation, phototoxicity, and inhibitory substances, may be growing close to their maximum rate, as evidenced by the numbers of bacteria consistently observed. The different metabolic strategies for energy overflow, which are PHB formation in the *Vibrio* sp. strain G1 (Fig. 3 and 4) and possibly extracellular polymeric carbohydrate production in the *Acinetobacter* sp. strain GO1, allow for high uptake rates with low assimilation efficiencies. The disruption of the *Vibrio* sp. strain G1 in late-stationary phase due to excessive PHB formation suggests that not all survival strategies may be successful. Stationary-phase growth, however, is a product of batch incubation and may not be observed in nature except where organic matter accumulates and remains somewhat constant, such as in the surface film at the sea-air interface (39). PHB inclusions have been observed in 8% (25 of 315) of the bacterial cells observed from the nutrient-rich surface sediments of sea grass beds (D. W. J. Moriarty, personal communication), and inclusions suggestive of PHB are present in many of the microcolonies developing at the sea-air interface (38). Perhaps the survival strategies observed in this laboratory study may also occur in the marine environment.

ACKNOWLEDGMENTS

This paper is from the master's thesis of M.B., who acknowledges Curtis M. Burney for assistance with the MBTH assay, Paul W. Johnson for electron microscopy and photomicrography, Norris P. Wood and Michael E. Q. Pilsen for their critical review, Kenneth M. Johnson for his most helpful discussions and aid in manuscript preparation, and R. R. Colwell for the generic identification of the isolates.

This work was supported by the Biological Oceanography Program of the National Science Foundation through grants OCE-7681779, OCE-7826388, and OCE-8121881.

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