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Poly-β-Hydroxybutyrate Accumulation as a Measure of Unbalanced Growth of the Estuarine Detrital Microbiota†

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The procaryotic endogenous storage material poly-β-hydroxybutyrate (PHB) can be induced to accumulate in the estuarine detrital microbiota under conditions which suggest unbalanced growth, such as limitation of a critical factor(s) in the presence of carbon and energy sources. Changes in PHB-to-lipid phosphate ratios detected in field samples can be mimicked in the laboratory with common estuarine stresses. Acute anoxia or low pH induces conditions of no growth with depression of both the synthesis and catabolism of PHB without change in the lipid phosphate. Balanced growth induced by nutrients increases the lipid phosphate, depresses PHB synthesis, and stimulates PHB catabolism, resulting in a low ratio of PHB to lipid phosphate. Unbalanced growth induced to a small extent by high salinity or much more readily by dark upland runoff water results in rapid accumulation of PHB and slowing of PHB catabolism with little change in lipid phospate. Unbalanced growth conditions result in high PHB-to-lipid phosphate ratios in the detrital microbiota.

The nutritional status of vertebrates with metabolic defects such as diabetes mellitus can often be monitored by the rates of formation and catabolism of the endogenous storage material triglyceride. Is it possible that the uniquely procaryotic endogenous storage material poly-β-hydroxybutyrate (PHB) could serve a similar function in helping to understand the nutritional history of the portion of the complex detrital microbiota that can produce it?

Previous work from our laboratory has shown that the estuarine detrital and aerobic sedimentary microbiota form PHB and that the PHB formed endogenously or added to the environmental samples can be recovered quantitatively and assayed either after incorporation of ^{14}C from sodium [1-^{14}C]acetate or by its absorbance at 235 nm (5). An indirect measure of microbial mass possibly less affected by the nutritional history of the detrital microbes can be estimated with the extractable lipid phosphate. Bacteria contain a high proportion of phospholipid which is associated with membranes (6, 8).

The extractable lipid phosphate content of several bacterial monocultures has been shown to be a direct measurement of the dry weight of the bacteria (10, 15, 16). The rates of phospholipid synthesis are directly related to the detrital mass (14), and in both detritus and sediments the extractable lipid phosphate content parallels the adenosine 5'-triphosphate (ATP) content with linear correlations of $r^2 = 0.89$ (detritus) and $r^2 = 0.71$ (sediments). The phospholipid has an active metabolism in the detrital microbiota (7). Extractable ATP has been shown to be related to the muramic acid, respiratory activity, and several enzyme activities (2, 13).

The lipid phosphate, muramic acid, and enzymatic activities of the estuarine detrital microbiota have been shown to depend on the nature of the detrital material (2, 7). The facts that essentially nondegradable materials still support a significant microbiota (2) and that there is significant uptake of nutrients from the water column of various materials (4) suggest that the microbiota are also affected by the composition of the suspending water.

In this paper the effects of changing the composition of the water column on the metabolism of PHB in the estuarine detrital microbiota have been examined.

MATERIALS AND METHODS

Materials. Analytical-grade solvents (Mallinckrodt or Fisher) were used without further purification. Reagents used in purification were described previously (7, 12).

Detritus. Oak leaves (*Quercus virginiana*) were collected after abscission and stored dried. They were placed in bags and incubated in Apalachicola Bay, Fla., for 3 to 8 weeks as described (11). Disks 6.5 mm in diameter were punched with a paper hole punch from randomly assorted leaves (14). Samples of mixed

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hardwood leaves (primarily oak) collected from the dry flood plain, *Vallisneria americana,* and polyethylene sheets cut to resemble oak leaves were incubated for 4 weeks in two areas, one river dominated and the other local runoff dominated. They were then treated the same as the oak leaves. The characteristics of this estuary and the two stations have been documented (9).

PHB assay. PHB was extracted from the detrital microbes in chloroform after release by incubation in 5% sodium hypochlorite for 30 min at 37°C. PHB isolated from the detrital microbes was then purified by successive extractions with water, ethanol, and diethyl ether and assayed as [^{14}C]PHB on paper disks or spectrophotometrically as described (5).

Phospholipid assay. Phospholipids were extracted by the Bligh and Dyer (1) method, and the lipid phosphate was determined colorimetrically or by measurement of the lipid ^{14}C or ^{32}P as described (7).

Other assays. ATP, alkaline phosphatase, and respiratory activity were measured as in previous work (2, 11).

Growth experiments. In growth experiments, plastic containers were filled with 500 ml of estuarine water and the pH was adjusted to 6.7 with HCl or NaOH unless otherwise stated. To each of these were added 100 disks 6.5 mm in diameter cut from oak leaves that had been incubated in the bay for 3 or 8 weeks. Leaf disks taken from the bay were aerated with compressed air at a rate of 0.5 liters/min for 3 days at 25°C. Where indicated, carbon sources were added to 0.1 mM final concentration. Sodium dihydrogen phosphate and ammonium nitrate were added where indicated to a final concentration of 0.01 mM each. Tannin water was collected from pine plantation drainage ditches. It had a pH of 4.1 and an absorbance of 0.06 at 400 nm. In the estuarine water no absorbance maxima were detectable between 750 and 300 nm, but there was an exponential increase in absorbance with decreasing wavelength when tannin water was added. The ocean salts were made to 4‰ by adding Instant Ocean powder (Aquarium Systems Inc., East Lake, Ohio). At the termination of the experiments the disks were lyophilized and weighed, the PHB was extracted and determined spectrophotometrically (5), the lipids were extracted, and the phosphate was determined colorimetrically (7). Data reported are averages of triplicate determinations.

Synthesis experiments. In experiments to test the rate of PHB synthesis, 100 oak leaf disks 6.5 mm in diameter were cut from leaves incubated in the bay for 4 weeks. The disks were then incubated in the laboratory in estuarine water, to which various components were added, in 16-mm test tubes with100 μCi of [*U*-^{14}C]glucose and 1 mCi of $H_3{}^{32}PO_4$ for 3 h with agitation at 25°C. The disks were removed, the PHB was extracted as described (5) or the phospholipid was extracted (7), and the radioactivity was determined by scintillation spectrometry.

Catabolism experiments. To test the catabolism of [^{14}C]PHB, pulse-chase experiments were performed. In these experiments the disks were incubated under the conditions described in the presence of 100 to 300 μCi of sodium [1-^{14}C]acetate and 1 mCi of $H_3{}^{32}PO_4$ at a specific activity maintained by periodic additions of ^{14}C and ^{32}P. After 3 to 5 h the disks were washed several times and incubated in the absence of radioactivity. At specified intervals 1- to 4-mg dry portions of the disks were removed, and the [^{14}C]PHB or ^{32}P-labeled lipid phosphate and dry weight were determined.

RESULTS

Relationship between PHB and lipid phosphate in the field. Measurements of the endogenous storage material PHB and the lipid phosphate of the detrital microbes in two stations in Apalachicola Bay, Fla., revealed differences in the ratios of PHB to biomass measured as the extractable phospholipid (Table 1). The water at one station was predominantly from the Apalachicola River, and that at the other station was subjected to periodic influxes of acidic brown runoff water from local pine plantations. The detrital microbiota from the runoff-dominated station showed a significantly higher PHB content in both *Vallisneria* (t = 3.2, 10 df; P = 0.005) and hardwood (t = 2.0, 10 df; P = 0.02) than at the river-dominated station. This was reflected in the higher PHB-to-lipid phosphate ratios.

Stimulation of PHB synthesis. To determine if this higher level of PHB could reflect the nutritional history of a population essentially similar in lipid phosphate, a series of experiments was initiated. From studies of bacterial monocultures, PHB accumulated under conditions where carbon and energy sources were adequate but growth was limited by lack of some critical requirement (3). In an attempt to stimulate PHB formation, microcosms containing about 300 mg of oak leaf detritus in 500 ml of water from the estuary were aerated gently at 25°C for 3 days. Potential substrates (0.1 mM), potential rate-limiting factors (0.01 mM NaH_2PO_4 and NH_4NO_3), and combinations of the substrate and potential rate-limiting factors were added to the incubation mixtures, and the PHB and lipid phosphate were determined. Addition of ammonium nitrate or sodium phosphate to the experiment had no effect on either the lipid phosphate or the PHB content (as shown in Table 2, experiment 2). From the data of Table 2 the addition of a 0.1 mM concentration of a series of carbohydrates or glutamate increased the PHB-to-lipid phosphate ratio two- to threefold above the control and had much less effect on the lipid phosphate.

Addition of glucose substantially increased the level of PHB with minimal effects on the lipid phosphate of the entire population.

Higher levels of PHB occurred when glucose was supplemented with either ammonium nitrate or sodium phosphate. During most of these

TABLE 1. *Recovery of PHB and lipid phosphate from the detrital microbiota from river-dominated and local runoff-dominated stations in Apalachicola Bay, Fla.*[a]

Sample	Lipid phosphate (μmol/g of detritus)		PHB (μmol/g of detritus)		Ratio PHB/lipid phosphate	
	River	Runoff	River	Runoff	River	Runoff
Vallisneria	6.0 ± 1.6	9.4 ± 2.6	26.1 ± 20.6	69.2 ± 24	3.9 ± 3.1	8.3 ± 4.9
Hardwood	1.6 ± 0.4	1.6 ± 0.3	8.2 ± 6.6	24.2 ± 18.9	4.7 ± 2.6	15.8 ± 14.2

[a] Uprooted *V. americana* and dried, mixed hardwood leaves collected from the flood plains to the river were incubated in the estuary for 4 weeks in cages (11) over an 8-week period, September to November 1977, and sampled biweekly. Each value was determined from three samples in duplicate and is expressed as the mean ± standard deviation.

TABLE 2. *Effect of carbon sources, nitrate, and phosphate on the formation of PHB by the estuarine detrital microbiota*

Expt	Substrate	Lipid phosphate (μmol/g of detritus)	PHB (μg/g of detritus)	Ratio PHB/lipid phosphate
1[a]	Control	0.45	4.5	10
	Galactose	0.56	9.5	17
	Glucosamine	0.54	9.8	18
	Lactate	0.52	15.5	30
	Acetate	0.50	19.1	38
	Ribose	0.59	6.6	11
	Erythrose	0.52	16.3	31
	Glutamate	0.58	17	29
	Glutamate + PO_4	0.87	3.4	3.9
	Glucose	0.63	82	130
	Glucose + NH_4	0.52	152	292
	Glucose + PO_4	0.77	338	439
	Glucose + PO_4 + NH_4	0.83	31	37
2[b]	Control	0.68	1.6	2.4
	Control + NH_4	0.80	1.9	2.4
	Glycolate	0.66	9.3	14
	Glucose	0.69	106	154
	Glucose + PO_4 + NH_4	0.95	4.8	5.1

[a] Lipid phosphate and PHB were determined in duplicate on oak leaf detritus incubated in the estuary for 3 to 6 weeks and then incubated in 500 ml for 3 days.

[b] Substrates were added to a 0.1 mM final concentration, and ammonium nitrate and sodium dihydrogen phosphate were added each at 10 μM final concentrations where indicated. 1 and 2 indicate different batches of oak leaves.

experiments there was also an increase in the extractable lipid phosphate.

Addition of both ammonium nitrate and sodium phosphate to glucose-amended experiments consistently resulted in a decrease in PHB recovery and an increase in extractable lipid phosphate.

The substantial increase in PHB content of the detrital microbiota, with little change in the lipid phosphate in the field (Table 1) and in the laboratory (Table 2), led to study of possible estuarine stresses.

Anoxia. When the estuarine detrital microbiota developed on oak leaves suspended in bay water was suddenly purged with argon, a number of measures showed slight changes in biomass and activity. The alkaline phosphatase activity remained a constant 2 ± 0.15 mmol (mean ± standard deviation) of *p*-nitrophenol released per g (dry weight) of detritus per 30 min over a 24-h period. This was measured at 3-h intervals for 12 h and then twice daily for 72 h after aeration with air or argon was initiated. In the anaerobic vessel, the extractable ATP remained a relatively stable 9.5 ± 3 μg of ATP per g (dry weight) for 9 h, after which the ATP level fell to 6 μg of ATP per g (dry weight). In the aerated control the ATP contents rose to 27 μg/g (dry weight) and remained at that level for the first 9 h, after which it fell slowly to the anaerobic ATP level.

The respiratory activity measured as the rate of oxygen utilization showed little change in the first 6 h and then fell by half in the next 78 h in the aerated detrital microbiota. The potential respiratory activity in the anaerobic detrital microbiota showed a progressive fivefold increase during the experiments. The potential respiratory activity was tested when the samples were transferred to aerated water and the oxygen utilization was measured (Fig. 1A).

The total amount of PHB recovered from the detrital samples in the argon atmosphere showed an almost constant 20 ± 5 μg/g of detritus throughout the experiment. The PHB recovered from the aerated detrital microbiota showed an initial 30% increase in the first 2 h followed by a decrease in the anaerobic content by the fourth hour. This is illustrated in Fig. 1B.

The incorporation of sodium [1-^{14}C]acetate into PHB showed a linear increase over a 3-h period of 1.07×10^6 dpm of ^{14}C/g of detritus per h in the aerated sample and 2.76×10^4 dpm of ^{14}C/g of detritus per h in the absence of oxygen at 25°C. The degradation of [^{14}C]PHB can be measured with a pulse-chase experiment (Fig. 1C). The loss of ^{14}C from PHB can be expressed as a biphasic line, using the exponential least-square expression, $y(\text{dpm}) = 5.91 \times 10^6 e^{-1.149(h)}$

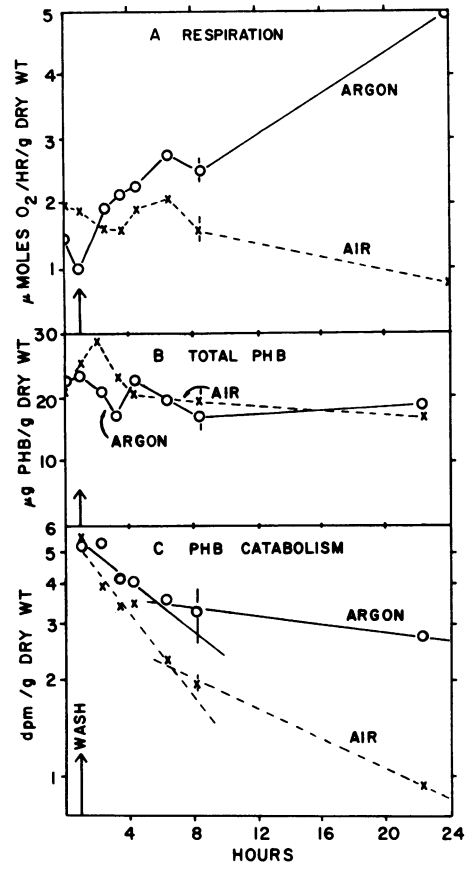

FIG. 1. *Effect of anoxia on the respiratory activity (A), total PHB (B), and catabolism of PHB (C) of the detrital microbiota. Leaves incubated in Apalachicola Bay for 7 weeks were recovered, randomly cut into 5,000 6.5-mm disks, and then incubated in 500 ml of bay water for 3 h in the presence of 100 μCi of sodium [1-^{14}C]acetate at 25°C with aeration. The ^{14}C in the filtered bay water was maintained within 10% of the initial value by periodic addition of ^{14}C. The disks were then washed and suspended in bay water not containing ^{14}C, and the oxygen utilization was measured polarographically, the total PHB was measured spectrophotometrically, and the [^{14}C]-PHB was measured after purification. The standard deviation is indicated by the bars on the 8.1-h sample. Symbols: (×) measurements from disks in aerobic water; (○) measurements from disks in water sparged with argon.*

$+ 3.12 \times 10^6 e^{-0.054(h)}$, for the aerobic samples. The correlation coefficients (r) were −0.91 for the fast component and −0.996 for the slow component. The 50% turnover time for the rapid phase was 4.6 h; the 50% turnover time for the slow phase was 12.7 h. The anaerobic samples, expressed as y(dpm) $= 5.91 \times 10^6 e^{-0.009(h)} + 3.83 \times 10^6 e^{-0.015(h)}$, gave correlation coefficients of −0.85 and −0.98 for the fast and slow components with 50% turnover times of 7.6 and 45.5 h, respectively.

Acidity. Storm runoff water entering the estuary from pine plantations has a low pH. Acidity had little effect on the biomass measured as the lipid phosphate and did not result in the stimulation of PHB formation (Table 3, experiment 1). Under conditions where growth would be expected (Table 3, experiment 2), there was an increase in the lipid phosphate and an accumulation of PHB that was inhibited at pH 4.4 and 7.7. The salinity of the estuarine water was 4‰.

Ocean salts. Increasing salinity of the water shows a reproducible depression of the respiratory activity of the detrital microbiota. If the microbiota are exposed to increasing concentrations of saline water, there seems to be a loss of lipid phosphate (biomass) with little stimulation of PHB synthesis, except possibly at 32‰ (Table 4, experiment 1). During conditions of stimulation in the presence of glucose, ammonium ni-

TABLE 3. *Effect of acidity on the formation of PHB by the detrital microbiota*

Expt	pH	Lipid phosphate (μmol/g of detritus)	PHB (μg/g of detritus)	Ratio PHB/lipid phosphate
1[a]	4.4	0.62	0.7	1.1
	5.6	0.60	3.7	6.2
	6.7	0.60	2.3	3.8
	7.7	0.65	4.4	6.8
2[b]	4.4	0.86	253	249
	5.6	0.80	808	1,010
	6.7	0.85	818	962
	7.7	0.81	116	143

[a] Experiments were performed as in Table 1.
[b] The estuarine water was amended with glucose, ammonium nitrate, and phosphate.

TABLE 4. *Effect of salinity on the formation of PHB by the detrital microbiota*

Expt	Salinity (‰)	Lipid phosphate (μmol/g of detritus)	PHB (μg/g of detritus)	Ratio PHB/lipid phosphate
1[a]	0	0.60	2.3	3.8
	8	0.59	2.4	4.1
	16	0.58	1.2	2.1
	32	0.45	8.0	17.8
2[b]	0	0.65	818	1,258
	8	0.75	114	152
	16	0.74	169	228
	32	0.58	98	169

[a] Experiments were performed as in Table 1.
[b] The estuarine water was amended with glucose, ammonium nitrate, and phosphate.

trate, and sodium phosphate, the synthesis of PHB induced in freshwater was depressed by increasing the proportion of salinity (Table 4, experiment 2). The total growth of the community as evidenced by an increase in lipid phosphate was not stimulated in freshwater as much as in the moderate salinities typical of this estuary. These experiments were performed at pH 6.7.

Brown storm runoff water. The brown runoff water as it enters the estuary usually has a much lower pH than the receiving water. Comparison of the rates of synthesis of PHB and phospholipid at different pH values in the presence of brown runoff water shows little difference between estuarine water and brown runoff water on the rate of phospholipid synthesis (Fig. 2B). The rate of phospholipid synthesis increased with increasing pH for both types of water. There was a significant stimulation of PHB synthesis by the brown runoff water that was essentially unaffected by pH (Fig. 2A). The water contained 4‰ salinity.

Additions of brown water found in drainage ditches from upland pine plantations (after adjustment of the pH to 6.7 and the salinity to 4‰) to the detrital microbiota resulted in increased levels of PHB with little change in the levels of extractable lipid phosphate in experiments similar to those described in Table 2. Addition of brown runoff water amended with glucose, ammonium nitrate, and phosphate resulted in a progressive increase in the level of PHB formed by the detrital microbiota with little change in the extractable lipid phosphate. This is illustrated in Fig. 3.

The effects of salinity, brown runoff water, and nutrient enrichment on the catabolism of [^{14}C]PHB was examined after a pulse-chase experiment. The catabolism of [^{14}C]PHB was accelerated somewhat by nutrient enrichment but was slowed by increasing the salinity to 20‰ and further slowed by the presence of brown upland runoff water. This is illustrated in Fig. 4. The slopes of calculated linear regressions of the loss of [^{14}C]PHB were −3.9 (r = 0.98) for brown runoff water, −6.2 (r = 0.93) for 20‰ salinity,

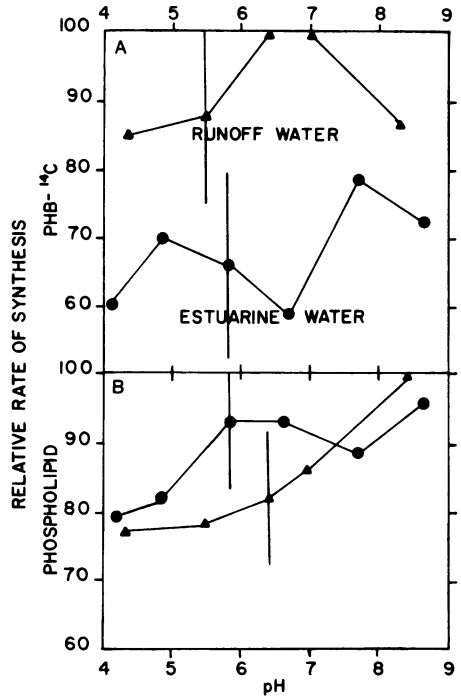

FIG. 2. *Effect of pH on the synthesis of PHB (A) and phospholipid (B) in the presence of brown runoff water. In these experiments 100 disks 6.5 mm in diameter were incubated with agitation at 25°C in 16-mm test tubes with 15 ml of bay water (●) or brown runoff water (▲) in the presence of 100 μCi of [U-^{14}C]glucose for PHB and 1 mCi of $H_3{}^{32}PO_4$ for lipid phosphate. The pH was adjusted with sodium hydroxide or HCl. After 3 h the [^{14}C]PHB and ^{32}P-labeled lipids were extracted, and the radioactivity was determined. The error bars on the third sample indicate the standard deviation of these measurements.*

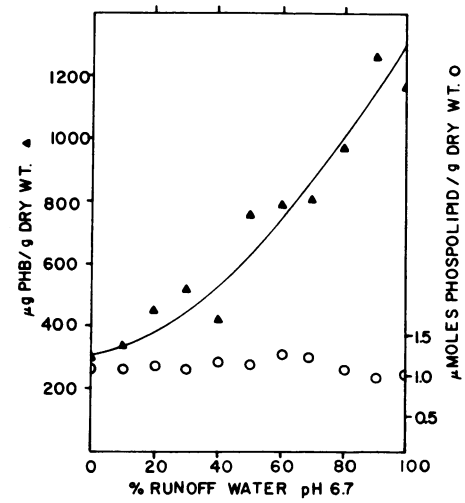

FIG. 3. *Effect of adding brown runoff water made to pH 6.7 on the formation of PHB (▲) and the level of phospholipid (○) formed by the detrital microbiota. One hundred 6.5-mm-diameter disks of oak leaves that had been incubated in the bay for 3 weeks were added to estuarine water containing 0.1 mM glucose, 0.01 mM sodium phosphate, and 0.01 mM ammonium nitrate to which increasing proportions of brown runoff water were added. The suspension was incubated with gentle aeration for 3 days at 25°C, and the PHB and lipid phosphate were determined.*

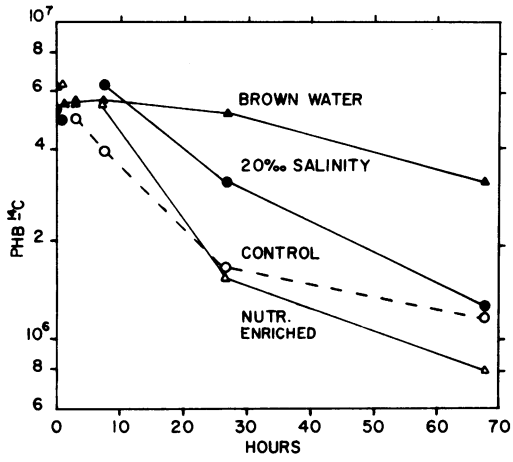

FIG. 4. *Effect of brown runoff water, salinity, and nutrient enrichment on the loss of [^{14}C]PHB in pulse-chase experiments. Oak leaf detritus was incubated in estuarine water (pH 6.7; salinity, 4‰), containing 300 μCi of sodium [1-^{14}C]acetate, for 4 h at 25°C. The specific activity was maintained by periodic additions of sodium [1-^{14}C]acetate. The detritus was rinsed in estuarine water several times and resuspended in estuarine water (control)* (○); *brown runoff water (pH 7.6), absorbance of 0.4 at 400 nm and 4‰ salinity* (▲); *saline water, 20‰, salinity* (●); *and nutrient-enriched estuarine water containing 0.01 mM sodium phosphate and 0.01 mM ammonium nitrate* (△). *Samples were taken at various times, and the radioactivity was determined.*

−8.3 ($r = 0.91$) for the nutrient-enriched sample, and −7.0 ($r = 0.85$) for the control incubated in estuarine water.

In experiments similar to those illustrated in Fig. 2, the effects of brown runoff water on the accumulation of PHB by the detrital microbiota were similar to those induced by the chelator ethylenediaminetetraacetic acid in the presence of glucose, phosphate, and ammonium nitrate. Ethylenediaminetetraacetic acid at concentrations of 100, 10, and 1 μM induced PHB accumulation to 485, 354, and 697 μg/g of detritus under conditions where the extractable lipid phosphate was 1.19, 1.13, and 1.10 μmol/g of detritus. The control microbiota and control plus glucose, ammonium nitrate, and phosphate showed 31 and 298 μg of PHB per g of detritus and 1.08 and 1.04 μmol of lipid phosphate per g of detritus.

DISCUSSION

In particular bacterial monocultures the accumulation of PHB occurs with changes in the environment that lead to cyst or spore formation, antibiotic release, or limitation of oxygen, nitrogen, sulfur, or potassium with adequate carbon and energy sources (3, 5). In the detrital microbiota, supplementing the nutrients in the water column with carbohydrates, especially glucose, induces a rapid accumulation of PHB in the absence of changes in the lipidphosphate, which is taken as a measure of the total microbial biomass (Table 2). The PHB accumulation can be stimulated 2- to 300-fold by adding either supplemental sodium phosphate or ammonium nitrate. Adding glucose and both ammonium nitrate and sodium phosphate sharply reduces PHB accumulation. With this reduction in PHB content there was nearly always a detectable increase in the total detrital microbial lipid phosphate. Considering the complexity of this microbial assembly, which includes the microeucaryotes, and the fact that only a portion of the organisms form PHB, the increase in lipid phosphate by the stimulus of glucose and nutrients suggests that PHB accumulation in the detrital bacterial community represents the same type of unbalanced growth as documented in monocultures. The fact that adding nutrient supplements which cause lipid phosphate increases also increases the rate of [^{14}C]PHB catabolism (Fig. 4) helps strengthen this supposition.

The accumulation of PHB or its catabolism can be influenced by changes in the water chemistry in which the detrital community is suspended. Anoxia slows both accumulation and catabolism (Fig. 1). Extreme acidity to pH 4.4 depresses growth and PHB formation independently of the stimulus to grow, but otherwise shows little effect (Table 3, Fig. 2). Surprisingly, the estuarine detrital microbiota apparently do not relish oceanic water, as the respiratory rate and the rate of catabolism of PHB both are depressed with increasing addition of ocean salts. With increasing salinity the rate of PHB synthesis is slowed in the presence of glucose, ammonium nitrate, and sodium phosphate (Table 4). The brown runoff water induces an accumulation of PHB by stimulating synthesis and depressing catabolism (Fig. 3 and 4). This effect is independent of pH (Fig. 2) and much like that of a chelator such as ethylenediaminetetraacetic acid. The estuarine detrital microbes containing PHB formers apparently follow at least three scenarios resulting in different PHB-to-biomass ratios (Table 5). Analysis of the PHB-to-biomass ratio could reflect the recent nutritional history of the PHB-forming portion of the detrital microbiota.

This estuarine detrital microbial community is a complex assemblage: the analysis of ^{14}C-labeled fatty acids derived from the lipids shows both high proportions of typical bacterial fatty acids and a very small proportion of eucaryotic

TABLE 5. *Summary of the nutritional status of the detrital microbiota as determined by change in the metabolism of PHB in the detrital microbiota*

Condition	Total microbial mass	PHB synthesis	PHB catabolism	Ratio PHB/lipid phosphate	Stresses
No growth	Unchanged	Depressed	Depressed	Low-high[a]	Anoxia
Balanced growth	Increased	Depressed	Stimulated	Low	Nutrients plus carbon source
Unbalanced growth	Unchanged	Stimulated	Depressed	High	Brown runoff water Incomplete nutrient supplementation Increased ocean salts[b]

[a] Ratio depends upon prior nutritional history.
[b] Increased ocean salts primarily depress PHB catabolism.

derived long-chain polyenoic fatty acids. A particular subset of the detrital bacteria can be induced to form or utilize PHB under conditions readily observed in the estuary (Table 5). An examination of the changes in the ratio of PHB to the total extractable lipid phosphate (an indirect measure of biomass) could reflect the recent history of the PHB-forming bacterial component of the assembly.

Two situations in the estuary have been shown to lead to PHB accumulation: growth on a living as opposed to a dead plant surface (Table 1), and forced aeration of anaerobic sediments (5). These situations could very well represent unbalanced growth where lack of sufficient amounts of critical nutrients induced PHB accumulation without detectable effect on the total microbial biomass.

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