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Formation of Polyhydroxyalkanoate in Aerobic Anoxygenic Phototrophic Bacteria and Its Relationship to Carbon Source and Light Availability▽

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Aerobic anoxygenic phototrophic bacteria (AAPB) are unique players in carbon cycling in the ocean. Cellular carbon storage is an important mechanism regulating the nutrition status of AAPB but is not yet well understood. In this paper, six AAPB species (*Dinoroseobacter* sp. JL1447, *Roseobacter denitrificans* OCh 114, *Roseobacter litoralis* OCh 149, *Dinoroseobacter shibae* DFL 12^T, *Labrenzia alexandrii* DFL 11^T, and *Erythrobacter longus* DSMZ 6997) were examined, and all of them demonstrated the ability to form the carbon polymer polyhydroxyalkanoate (PHA) in the cell. The PHA in *Dinoroseobacter* sp. JL1447 was identified as poly-beta-hydroxybutyrate (PHB) according to evidence from Fourier transform infrared spectroscopy, differential scanning calorimetry, and ^{1}H nuclear magnetic resonance spectroscopy examinations. Carbon sources turned out to be critical for PHA production in AAPB. Among the eight media tested with *Dinoroseobacter* sp. JL1447, sodium acetate, giving a PHA production rate of 72%, was the most productive carbon source, followed by glucose, with a 68% PHA production rate. Such PHA production rates are among the highest recorded for all bacteria. The C/N ratio of substrates was verified by the experiments as another key factor in PHA production. In the case of *R. denitrificans* OCh 114, PHA was not detected when the organism was cultured at C/N ratios of <2 but became apparent at C/N ratios of >3. Light is also important for the formation of PHA in AAPB. In the case of *Dinoroseobacter* sp. JL1447, up to a one-quarter increase in PHB production was observed when the culture underwent growth in a light-dark cycle compared to growth completely in the dark.

Polyhydroxyalkanoates (PHAs) are a group of biopolymers found in heterotrophic bacteria (31), archaea (16, 22), cyanobacteria (3, 15, 34, 39), and plants (41). PHAs include poly-(*R*)-3-hydroxybutyrate (PHB), a copolymer (PHBV) of (*R*)-3-hydroxybutyrate (3HB) and (*R*)-3-hydroxyvalerate (3HV), and a copolymer (PHBHHx) of 3HB and (*R*)-3-hydroxyhexanoate (3HHx) (10). Due to their thermal plasticity, PHAs have gained great attention for their replacement of conventional petrolic plastics, and it is thus of great importance to find organisms with a high capacity of PHA production for industrial purposes. Since they have the advantage of being cultivated easily for massive production, bacteria are the main targeted organisms for this purpose (10). In recent years, many marine bacteria have been reported to be able to produce PHAs (2, 13, 29, 30, 35). Some bacteria have been employed as sewerage scavengers (4). Different industrial wastes (malt, soy, sesame, molasses, bagasse, and pharmaceutical waste) have also been studied as cheaper substrates to minimize the cost of the PHA bio-industry (4).

Photosynthetic bacteria, which are able to utilize sunlight for energy, are naturally considered to be favorable for economic PHA production (24). Anaerobic phototrophic bacteria have thus been studied substantially with respect to PHA production, and multiple factors have been reported to influence PHA productivity. Functional genes are the primary controllers, e.g., a *phaC* deletion strain is not able to synthesize PHA on any carbon source, but *phaA* and *phaB* deletion strains are able to produce PHA. These results indicate that alternative routes for the synthesis of substrates of the synthase are present (23). Variability in the PHA production rate is also significant at the species level, e.g., *Rhodobacter sphaeroides* strains are recognized as the highest PHA producers (producing 3.5 g/liter PHA, a 60% PHA content [2 to 3 times higher than those of other photosynthetic bacteria]) (27). Carbon source is another determining factor, e.g., *Rhodobacter capsulatus* produces high levels of PHA when suitable carbon sources are provided (23).

In contrast to the industrial applications of the anaerobic anoxygenic phototrophic bacteria, another important group of anoxygenic phototrophic bacteria in natural oxic environments, namely, the aerobic anoxygenic phototrophic bacteria (AAPB), plays a significant role in carbon cycling in the ocean. AAPB are primarily heterotrophic, but they also possess the capability of harvesting light for energy and are present in all of the world's oceans (19, 20). AAPB are closely associated with phytoplankton to comprise the dissolved organic carbon (DOC) in marine environments (20, 48), and they grow much faster than other heterotrophic bacteria (26). Since they are 2 to 3 times larger in cell size than other heterotrophic bacteria (36), AAPB can be grazed upon more easily by predators (37), and thus they contribute a larger proportion of carbon to the upper trophic levels. On the other hand, high abundances of viruses are found in aquatic environments (6). AAPB cells can be lysed quickly by viruses, releasing DOC into the water. PHA can be a part of the DOC. Some of the bacterium-derived DOC is resistant to further biological degradation (14, 28), which contributes to carbon sequestration in the ocean ("microbial carbon pump" effects) (18). To date, there have been no systematic physiological and ecological studies of carbon

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polymers in AAPB, except for a few case observations of presumed PHA-like granules as evidence for species classification (45); little is known about conditions that promote accumulation or consumption of these cytoplasmic inclusions in AAPB. Therefore, it is of great interest to identify the observed polymers in AAPB and to elucidate their controlling mechanisms. In the present study, 6 common marine AAPB strains, including strains in the *Roseobacter* clade, which has been studied intensively for its abundance, diversity, and distribution in the world's oceans (19), were examined for a better understanding of the formation of PHAs and relevant ecological conditions.

MATERIALS AND METHODS

Experimental bacteria, media, and culture conditions. The following six marine AAPB strains were tested for PHA formation: *Dinoroseobacter* sp. JL1447, *Roseobacter litoralis* OCh 149, *Roseobacter denitrificans* OCh 114, *Dinoroseobacter shibae* DFL 12^T, *Labrenzia alexandrii* DFL 11^T, and *Erythrobacter longus* DSMZ 6997. *Dinoroseobacter* sp. JL1447, isolated from *Phaeodactylum tricornutum* Bohlin, was chosen for detailed study of PHA formation through physiological and analytical approaches, including transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. The 16S rRNA gene sequence of *Dinoroseobacter* sp. JL1447 (1,388 bp) (deposited in the NCBI GenBank sequence database under accession number nuc_1 HQ704891) is 100% identical to that of the model strain *Dinoroseobacter shibae* DFL 12^T, isolated from marine dinoflagellates (7). Strain OCh 114 was chosen for testing of the effects of different C/N ratios on PHA production. For preculture, artificial seawater (ASW) with 1 g liter^{-1} yeast extract and 1 g liter^{-1} tryptone was used in a 1.5% megaplate. For experimental cultivation, a defined, synthetic seawater medium (minimal medium) (12) was used and contained the following components per liter of medium: 4.0 g $NaSO_4$, 0.2 g KH_2PO_4, 0.25 g NH_4Cl, 20.0 g NaCl, 3.0 g $MgCl_2 \cdot 6H_2O$, 0.5 g KCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.19 g $NaHCO_3$, 1 ml trace element solution, and 10 ml vitamin solution. The trace element solution contained 2.1 g $Fe(SO_4) \cdot 7H_2O$, 13 ml 25% (vol/vol) HCl, 5.2 g $Na_2EDTA \cdot 2H_2O$, 30 mg H_3BO_3, 0.1 g $MnCl_2 \cdot 4H_2O$, 0.19 g $CoCl_2 \cdot 6H_2O$, 2 mg $CuCl_2 \cdot 2H_2O$, 0.144 g $ZnSO_4 \cdot 7H_2O$, and 36 mg $Na_2MoO_4 \cdot 2H_2O$ per liter. The vitamin solution contained the following components: 0.2 g biotin, 2.0 g nicotinic acid, and 0.8 g 4-aminobenzoic acid per liter. All solutions were sterilized separately and mixed at room temperature prior to inoculation. To test the PHA production ability of *Dinoroseobacter* sp. JL1447 with different carbon sources, sodium acetate, glucose, sodium glutamate, sodium pyruvate, and trisodium citrate were employed, with the dosage standardized to the carbon content of 1 g liter^{-1} glucose. To test the effects of C/N ratios on *R. denitrificans*, a medium containing glucose as the sole carbon source and NH_4Cl as the nitrogen source was used, with the concentration of NH_4Cl fixed at 0.25 g liter^{-1} and the glucose concentration adjusted to obtain C/N ratios of 1.8, 3.6, 7.2, and 18. The cultivations were carried out on orbital shakers at 180 rpm in 500-ml shaking flasks with a culture volume of 200 ml at 28°C. For illumination, two 40-W Osram lamps were mounted at a distance of 8 cm from the growth chamber wall. The resulting illuminance measured at the flasks was about 30 microeinsteins/m^2-s (40).

Extraction of PHAs. Bacterial cells were harvested at stationary phase (50 to 70 h after incubation, depending on culture conditions), centrifuged at 8,000 × *g* for 10 min, and washed twice with a 10 mM Tris buffer. The pellet was then frozen at −20°C and lyophilized. Dried frozen cells recovered from the pellet were ground in a mortar, and the powder was put into a triangular flask, mixed with 50 ml chloroform, and rotated forcefully at 50°C for 5 h. The PHA-containing chloroform was concentrated and extracted once with water to remove residual solid particles. During the organic phase, the medium containing PHA polymers was evaporated and washed with cold methanol and acetone to remove the pigment. The resulting crude extract was preserved for further analyses (38).

FTIR spectroscopy to record IR spectra. Potassium bromide (KBr) pellets were prepared by using dry cells of *Dinoroseobacter* sp. JL1447, PHAs were extracted from *Dinoroseobacter* sp. JL1447, and a PHB standard (Sigma) and the other 5 bacterial strains' dry cells were cultured in corresponding media. A PerkinElmer Spectrum GX FTIR spectrometer was used with a spectral range of 400 to 4,000 cm^{-1} to record the IR spectra. The spectrum peaked at about 1,728 to 1,738 cm^{-1}, which was taken as the diagnostic signal for PHAs (21).

NMR. ^{1}H NMR spectra were acquired by dissolving the polymer in deuterochloroform ($CDCl_3$) at a concentration of 10 mg/ml and were analyzed on a Bruker Avance III 500 spectrometer at 25°C, with a 13.85-ms pulse width (30° pulse angle), 2-s pulse repetition, 10,330-Hz spectral width, and 65,536 data points. Tetramethylsilane was used as an internal shift standard.

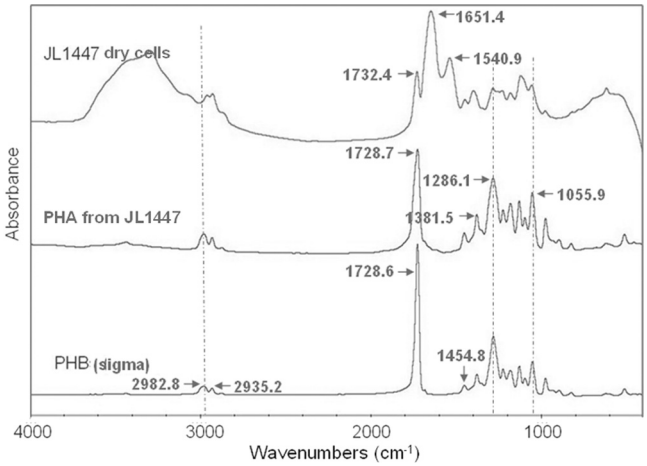

FIG. 1. FTIR spectra of PHB standard (bottom) and of dry cells (top) and PHA extract (middle) of *Dinoroseobacter* sp. JL1447 cultured with glucose as the carbon source. The spectra indicate that the target signal peak (1,728.7 cm^{-1}) of the PHA extract of *Dinoroseobacter* sp. JL1447 is almost identical to the C═O vibration absorption peak of the PHB standard (1,728.6 cm^{-1}) and that the target signal of the dry cells of *Dinoroseobacter* sp. JL1447 is also similar (1,732.4 cm^{-1}).

DSC. The thermal properties of *Dinoroseobacter* sp. JL1447 polyesters, the glass transition temperature (T_g), the melting point (T_m), the heat of fusion (ΔH_m), and the decomposition temperature (T_d) were examined by DSC. Samples (less than 10 mg) were exposed to a temperature profile of −30°C to 400°C, with a heating rate of 10°C min^{-1}.

Ultra-thin-section TEM. Samples were taken at the exponential phase of precultures and at early stationary phase of the sole carbon source cultures, cooled on ice, and centrifuged at 6,000 × *g*. The pellets were resuspended in phosphate-buffered saline (PBS; 50 mM, pH 7.4) and then chemically fixed in PBS containing 0.3% (vol/vol) glutaraldehyde for 1.5 h at 4°C. Embedding in Lowicryl K4M resin was performed as previously described (30). Resin sections of about 80 nm in thickness were cut with glass knives. The sections were stained with phosphotungstic acid solution (3% [wt/vol], pH 7.0) for 3 min. Electron microscopy was carried out with a Jeol JEM CX-100 II instrument at 120 kV.

Cellular PHA contents under different cultivation conditions. The AAPB strain *Dinoroseobacter* sp. JL1447 was cultured with glucose as the sole carbon source either in the dark or with a 12-h–12-h dark-light cycle. The cells were harvested at early stationary phase of the culture for transmission electron micrographs. The relative content (% a/a) of PHA was estimated based on the micrographs. The % a/a value was calculated by dividing the number of pixels of the PHA area by the total number of pixels of the whole cell area. Sixty-two counts for the dark culture and 27 counts for the dark-light cycle cultures were taken for statistical analysis by the average difference inspection method.

RESULTS

Identification of PHA produced by AAPB strain *Dinoroseobacter* sp. JL1447. Both *in vivo* cell samples of *Dinoroseobacter* sp. JL1447 and its PHA extract were analyzed along with a PHB standard (Sigma) through the FTIR approach. The FTIR spectra of the PHB standard (Fig. 1, bottom spectrum) and the dry cells (top spectrum) and extract (middle spectrum) of *Dinoroseobacter* sp. JL1447 cultured with glucose as the carbon source show peaks at similar positions (Fig. 1). The peak absorption band of the PHB standard, at about 1,728.6 cm^{-1}, corresponds to the ester carbonyl group of PHB. The peak of *Dinoroseobacter* sp. JL1447, at about 1,732.4 cm^{-1}, represents

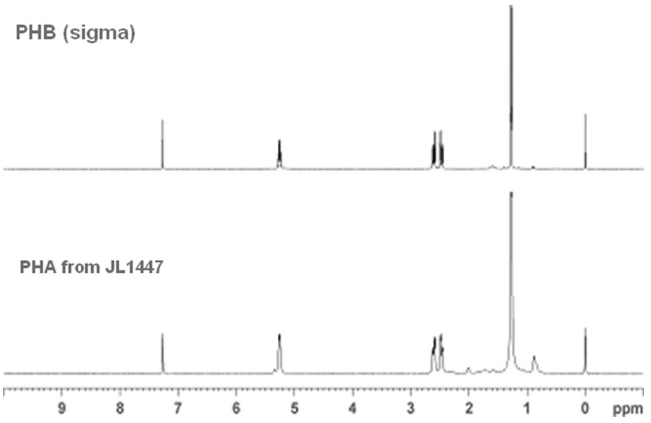

FIG. 2. ^{1}H NMR spectra of PHB standard and PHA produced by *Dinoroseobacter* sp. JL1447. The spectra show peaks of HC═CH bonds at 5.30 ppm, CH_2—COOH bonds at 2.50 ppm, a -CH_2 group in the saturated lateral chain at 1.28 ppm, and a terminal -CH_3 group at 0.9 ppm.

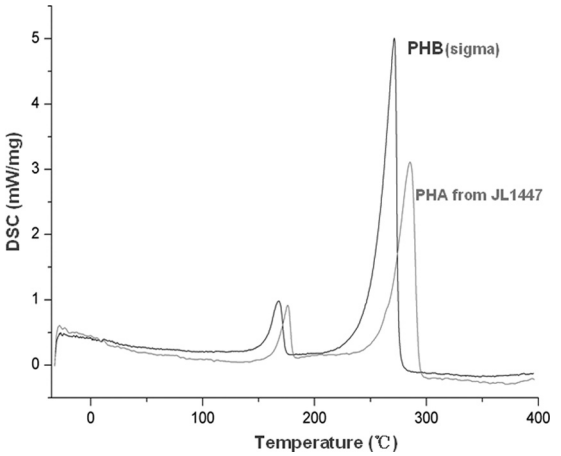

FIG. 3. DSC spectra of PHB standard and PHA produced by *Dinoroseobacter* sp. JL1447. The T_m, ΔH_m, and T_d values of the sample are 175.8°C, 51.87 J g^{-1}, and 285°C, respectively, which are very close to those of the PHB standard (167.8°C, 58.3 J g^{-1}, and 272°C).

the vibration of the C═O bond of ester functional groups, primarily from lipids, fatty acids, and PHA (17, 47). The exact position of PHA absorbance depends on the degree of crystallinity of the PHA (21). The spectra of the polymer extract from *Dinoroseobacter* sp. JL1447 and the PHB standard (Sigma) are almost identical. The peaks at 2,982 and 2,935 cm^{-1} belong to the C—H stretching vibration of methyl and methylene (25). At about 1,650 cm^{-1} is the vibration peak of the C═O bond of amides associated with proteins, usually called the amide I band, which may also contain contributions from C═C stretches of olefinic and aromatic compounds. At about 1,540 cm^{-1}, the deformation vibration peak of the N—H bond of amides associated with proteins becomes apparent. It is usually called the amide II band and may also contain contributions from C═N stretches (21). The two peaks at about 1,650 and 1,540 cm^{-1} have disappeared in the sample spectrum. At about 1,455 cm^{-1}, the asymmetric stretching peak of -CH_3 and -CH_2 groups (46) emerges. The positions of these assignments could vary with contributions from PHA (21). The peak at about 1,382 cm^{-1} is the symmetric deformation peak of -CH3 and -CH_2 groups and the symmetric stretch of C—O bonds of COO^- groups (46); the positions of these assignments could also vary with contributions from PHA (46). The peaks between 1,200 cm^{-1} and 900 cm^{-1} are due to the vibration of C—O—C bonds (43, 46), with contributions from PHA (21).

In addition, the bulk culture sample of *Dinoroseobacter* sp. JL1447 also shows a similar FTIR spectrum (Fig. 1).

The ^{1}H NMR spectra of the PHAs extracted from *Dinoroseobacter* sp. JL1447 and the PHB standard (Sigma) (Fig. 2) show the following resonance signals: HC═CH bond at 5.30 ppm, CH_2—COOH bond at 2.50 ppm, a high signal at 1.28 ppm that belongs to the hydrogen of methylene in the saturated lateral chain, and a terminal -CH_3 group at 0.9 ppm. The ^{1}H NMR spectra of the sample and the standard are almost identical.

DSC spectrum analysis provided the thermal characteristics of the PHA produced by *Dinoroseobacter* sp. JL1447 (Fig. 3). The T_m of the PHA from *Dinoroseobacter* sp. JL1447 was 175.8°C, the ΔH_m was 51.87 J g^{-1}, and the T_d was 285°C. The T_m of the PHB standard was 167.8°C, the ΔH_m was 58.3 J g^{-1}, and the T_d was 272°C. The differences between the peaks were probably due to the different extraction methods and the treatment temperatures.

All this evidence from FTIR, DSC, and ^{1}H NMR spectra suggests that the PHA produced by *Dinoroseobacter* sp. JL1447 is PHB.

Investigation of PHA in common AAPB strains. In addition to *Dinoroseobacter* sp. JL1447, the PHA formation of 5 other marine AAPB strains was investigated through the FTIR approach. In order to obtain evident results, these strains were cultured in respective appropriate media: *Labrenzia alexandrii* DFL 11^T and *Roseobacter litoralis* OCh 149 were cultured in complex medium (ASW with 1 g liter^{-1} peptone and 1 g liter^{-1} yeast extract), *Erythrobacter longus* DSMZ 6997 was cultured in complex medium plus 2.5 g liter^{-1} glucose, and *Roseobacter denitrificans* OCh 114 and *Dinoroseobacter shibae* DFL 12^T were cultured in medium with glucose as the sole carbon source. All of the FTIR spectra show a peak for a C═O bond from the PHA at about 1,730 cm^{-1} (Fig. 4).

PHA synthesis ability of *R. denitrificans* OCh 114 in media with different C/N ratios. The FTIR spectra of OCh 114 grown in media with different C/N ratios show different PHA yields (Fig. 5). The PHA feature peak of the C═O bond at 1,729.71 cm^{-1} is almost invisible in the spectrum for cells cultured in the medium with a C/N ratio of 1.8 but increases gradually with increasing C/N ratios. The height of the FTIR PHA signal of OCh 114 cultured with a C/N ratio of 18 is roughly 5-fold higher than that of the strain cultured with a C/N ratio of 1.8 (Fig. 5).

PHB synthesis ability of *Dinoroseobacter* sp. JL1447 with different carbon sources and light conditions. As seen from ultra-thin-section electron micrographs (Fig. 6), the ability of *Dinoroseobacter* sp. JL1447 to synthesize PHB varied with different culture media or carbon sources. In the case of a complex medium (ASW with 1 g liter^{-1} peptone and 1 g liter^{-1} yeast extract), no PHB granules were observed. However, PHB synthesis became visible when glycerol (2% [wt/wt]) or glucose

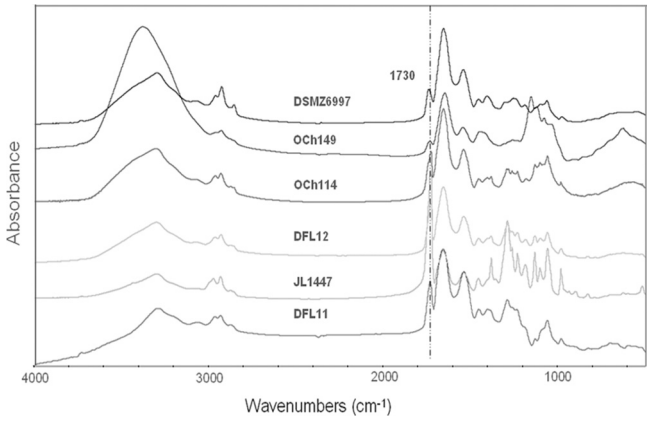

FIG. 4. FTIR spectra of six marine AAPB: *Erythrobacter longus* DSMZ 6997, *Roseobacter litoralis* OCh 149, *Roseobacter denitrificans* OCh 114, *Dinoroseobacter shibae* DFL 12^T, *Dinoroseobacter* sp. JL1447, and *Labrenzia alexandrii* DFL 11^T. All spectra show a peak for a C═O bond at about 1,730 cm^{-1}, indicating the existence of the PHA.

(1 g $liter^{-1}$) was added to the medium (Fig. 6B; Table 1). PHB granules were formed tremendously when a simple carbon compound, such as sodium acetate, glucose, sodium glutamate, sodium pyruvate, or sodium citrate, was used as the sole carbon source (Fig. 6C and D; Table 1). This phenomenon occurred especially when sodium acetate or glucose was provided as the sole carbon source, with PHB granules in the *Dinoroseobacter* sp. JL1447 cells accounting for about 70% of the cell dry weight (Table 1).

The effects of light on PHB production were tested with cultures of *Dinoroseobacter* sp. JL1447 in medium with 1 g $liter^{-1}$ glucose. The relative cellular PHB content (% a/a) accounted for 59.21% ± 8.39% of the whole cell area in the case of dark cultures ($n = 62$), while the value went up to 73.54% ± 3.90% for cultures under dark-light cycle conditions ($n = 27$) ($P < 0.001$).

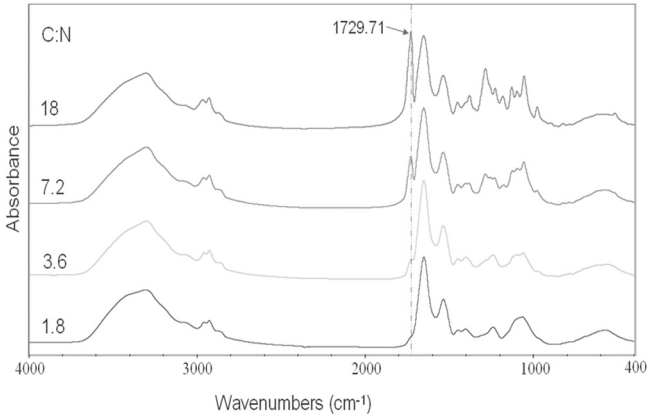

FIG. 5. FTIR spectra of *R. denitrificans* OCh 114 cultured in media with different C/N ratios. The medium was adjusted with glucose at different concentrations, with NH_4Cl at a fixed concentration of 0.25 g $liter^{-1}$, and the spectra indicate the increase of the PHA signal with increasing C/N ratios.

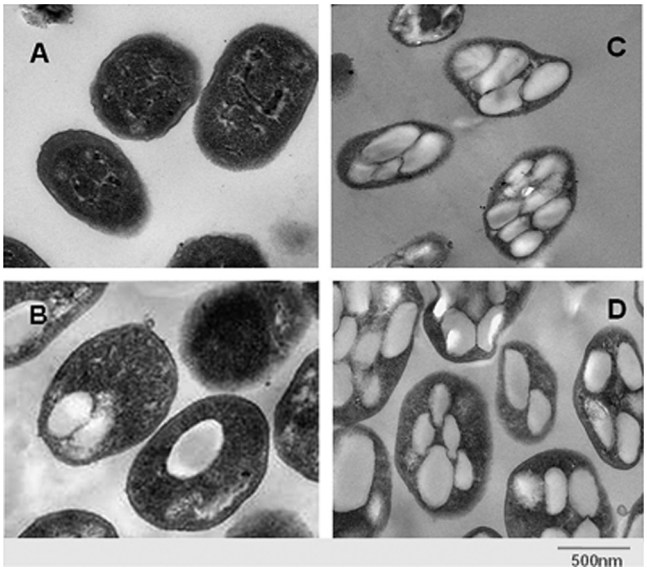

FIG. 6. Transmission electron micrographs of ultrathin sections of *Dinoroseobacter* sp. JL1447 cultivated with different carbon sources. (A) Complex medium (artificial seawater with yeast extract at 1 g $liter^{-1}$ and tryptone at 1 g $liter^{-1}$). (B) The same complex medium as in panel A but with 2% (wt/wt) glycerol. (C) Culture with glucose as the sole carbon source. (D) Culture with sodium acetate as the sole carbon source.

DISCUSSION

FTIR spectroscopy is a robust method for the identification of PHAs, with the C═O double bond acting as the diagnostic signal. For PHB, one type of PHA, the C═O FTIR peak is at about 1,728 cm^{-1}, and this has been used for PHB identification and physiological studies of *Escherichia coli* and other bacteria (21). In the present study, we applied this method as well as DSC and NMR approaches for the detection of PHA formation in AAPB and revealed that the PHA in the tested AAPB strain *Dinoroseobacter* sp. JL1447 is PHB.

Some nonphototrophic marine bacteria have been demonstrated to be producers of PHAs, such as *Pseudomonas guezennei* sp. nov. cultured in sea salt broth with 20 g $liter^{-1}$ glucose, in which PHA granules can fill up about 80% to 90% of the cellular volume (38). *Saccharophagus degradans* ATCC 43961 was also cultured in sea salt broth with 20 g $liter^{-1}$ glucose, and the PHA in this strain accounted for about 17% of

TABLE 1. PHB production by *Dinoroseobacter* sp. JL1447 with various carbon sources

Carbon source[a]	Cell dry wt (g)	PHB wt (g)	Yield (%)
Sodium acetate	0.1112	0.0804	72.30
Glucose	0.0702	0.0480	68.35
Sodium glutamate	0.0848	0.0183	21.60
Sodium pyruvate	0.0736	0.0140	19.02
Trisodium citrate	0.0785	0.0146	18.60
CM	0.1035	0	
CM with glycerol	0.2691	0.0472	17.54
CM with glucose	0.1830	0.0251	13.72

[a] CM, complex medium (artificial seawater with 1 g $liter^{-1}$ peptone and 1 g $liter^{-1}$ yeast extract); CM with glycerol, CM with 2% (wt/wt) glycerol; CM with glucose, CM with 1 g $liter^{-1}$ glucose.

the cell dry weight (13). In *Halomonas hydrothermalis* using 1, 2, 5, and 10% (wt/vol) *Jatropha* biodiesel by-product (containing 95% glycerol), the highest PHA accumulation can reach up to 70% of the dry cell mass (35). In the case of *Vibrio* sp. strains from marine sediments cultured in medium containing 4% sodium chloride as the carbon source, PHB accumulated to 41% of cell dry weight (11). Compared to these studies, the concentration of the carbon source used in the present study is the lowest one seen, but the PHA production rate is among the highest rates observed.

Furthermore, we studied the effects of carbon sources, the C/N ratios of substrates, and light on the production of PHA in some typical AAPB strains. The carbon source turned out to be critical for PHA production in AAPB. In the case of *Dinoroseobacter* sp. JL1447, among the eight media tested, sodium acetate as the sole carbon source yielded the most PHA production (72%), followed by glucose (68%). Such PHA production rates are among the highest recorded for all bacteria so far. Acetic acids, sugars, fatty acids, and butyric acids are all good carbon sources for AAPB to produce PHA. Acetyl-coenzyme A (acetyl-CoA) is an important precursor of the PHA production process (31): the quantity and production rate of acetyl-CoA can affect PHA synthesis, and this may explain why sodium acetate and glucose are the best tested carbon sources for production of PHB by *Dinoroseobacter* sp. JL1447.

The C/N ratio of substrates was verified to be another key factor in PHA production. In the case of *R. denitrificans* OCh 114, PHA was not detected at C/N ratios of <2 but was distinct at C/N ratios of >3. This occurrence is similar to the case for some psychrophilic or psychrotrophic crude oil-utilizing marine bacteria, which accumulate lipid storage compounds in the cytoplasm under nitrogen-limiting conditions when the C/N ratio becomes high (1).

Light is an important ecological factor for phototrophic bacteria. A diel pattern of PHA production in the microbial mats of anaerobic bacteria was reported previously (42). With external carbon sources such as acetate, lactate, and propionate, PHA production by anaerobic purple sulfur bacteria growing with light could be two to five times higher than that in the dark (32). That is, when additional exogenous carbon sources are available, PHA production can be stimulated by light. A similar case is also found in the anaerobic phototrophic bacterium *Rhodobacter sphaeroides*, which can form more PHA during anaerobic light cultivation with strong illumination than during aerobic dark cultivation (8, 27). This phenomenon is particularly evident when cells are under nitrogen limitation and provided with a sole carbon source, and it results in PHA accumulation increasing with increasing light illumination, up to 10 millieinsteins/m^2-s (8). It has been proposed that a mechanism may exist to regulate the photosynthesis and respiration processes (5). Such a mechanism can also be explained by assuming that PHA is a redox regulator in cellular metabolism (33). This validation is demonstrated by the effects of oxygen limitation on production of PHA by *Azotobacter beijerinckii*, which show that low oxygen causes an increase of the reducing power of NADH that then results in an increase of PHA synthesis (44). In our case, for the aerobic phototrophic bacteria, light was demonstrated to play a positive role in PHB production in *Dinoroseobacter* sp. JL1447. As an AAPB strain, *Dinoroseobacter* sp. JL1447 can utilize light for additional energy to add to its heterotrophic metabolism, since the photosynthetic process shares the cellular respiration electron transfer system: with light, the NADH-dependent respiration is actually restrained, and thus NADH is saved (5, 45), creating high NADH/NAD and PHB formation rates.

Our study suggests that PHA formation in AAPB may play an important role in regulating their photoheterotrophy. Until now, all studies on marine bacterial PHAs were based on isolated strains. Future studies should make more efforts to study *in situ* bacterial PHA dynamics to lead to a better understanding of its role in carbon cycling in the ocean.

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