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ORIGINAL PAPER

Characterization of polyhydroxyalkanoate and the *phaC* gene of *Paracoccus seriniphilus* E71 strain isolated from a polluted marine microbial mat

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Abstract Polyhydroxyalakanote (PHA) was produced by the marine bacteria *Paracoccus seriniphilus* Strain E71. Three methods were used for screening PHA in this strain: (1) microscopic analysis, (2) specifically designed primers for amplify fragments of *phaC* gene from Gram negative bacteria, and (3) measurements using spectroscopy, calorimetry, thermogravimetry, and rheology. The polyhydroxyalkanoic acid synthase gene (*phaC*) sequence had 77% identity with the *phaC* gene of *P. denitrificans* PD1222 strain. Additionally, the translated sequence showed an 86% similarity with the amino acid sequence of the *phaC* gene N-terminal portion of the *P. denitrificans* PD1222 strain. Our *phaC* sequence was closely related to two *phaC* sequences that correspond to *P. denitrificans*; therefore, this is the first report of a sequence of *phaC* that codifies a poly-(3-hydroxyalkanoate) synthase class I, specifically a poly-beta-hydroxybutyrate polymerase from the marine bacteria *Paracoccus seriniphilus*. The polymer PHA of E71 melts at 167.86°C (T_m), which corresponded to the fusion of the crystalline polymer and thermally degrades at 296.52°C, indicating that the biopolymer has good thermal stability. Rheology showed that this polymer behaves as a nonNewtonian fluid. All these characteristics suggest that the E71 strain produces a PHA that corresponds to the crystalline thermoplastic polymer PHB type.

Keywords *Paracoccus seriniphilus* · E71 · Polyhydroxyalkanoates · *phaC* gene · Microbial mats · Physical properties

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Introduction

Numerous bacteria accumulate polyhydroxyalkanoates (PHA), especially poly-ß-hydroxybutyrate (PHB). However, very few of them can be used for industrial production purposes. As an industrial PHA production strain, the microorganism should satisfy the following requirements: rapid growth in cheap carbon sources, high PHA accumulation in the cells, high transformation efficiency of substrate in product, harmless to animals, humans, and the environment, large size to ease its separation, and easily lysed for PHA extraction (Chen 2002). Polymers of PHA show material properties that are similar to some common plastics, such as polypropylene (Suriyamongkol et al. 2007). The bacterial origin of PHA makes this polyester a natural material and indeed, many microorganisms have evolved the ability to degrade these macromolecules (Madison and Huisman 1999). The diversity of different monomers that can be incorporated into PHA, combined with a biological polymerization system that generates high molecular weight materials, has resulted in an enormous

range of new polymers that are potentially available and reason why has attracted commercial interest (Mothes et al. 2004). PHA are regarded as thermoplastics, elastomers, and rubbers, they can be used in a wide variety of products including containers, bottles, razors, materials for food packaging, implants, suture filaments, and osteosynthetic material. The latex of PHAs can be used to produce a water-resistant layer for paper, film or cardboard (Suriyamongkol et al. 2007). Different genes involved in PHA synthesis have been isolated and characterized from several kinds of bacteria (Kolibachuck et al. 1999; Kranz et al. 1997; Ueda et al. 1992, 1996). There are different PHA-synthesis systems, but in general are controlled by a three-enzyme pathway; ß-ketothiolase, acetoacetyl CoA reductase, and PHA synthase. These enzymes are encoded by three different genes, *phaA*, *phaB*, and *phaC*, respectively (Kalia et al. 2007).

Growing concern about environmental pollution has renewed interest in the development of PHA, which are completely biodegradable by bacteria in most environments (Khanna and Srivastava 2005). More important for the biological characteristics and biodegradability of PHA is the fact that their production could be based on renewable resources. First reports of strains of PHA producers from marine sediments were the genera *Beneckea* and *Vibrio* (Baumann et al. 1971; Oliver and Colwell 1973). Bacterial isolates from polluted marine microbial mats, belonging to the genera *Bacillus*, *Staphylococcus*, *Paracoccus*, and *Micrococcus* were detected and evaluated in vitro on their capability to accumulate poly-ß-hydroxybutyrate (PHB) (López-Cortés et al. 2008). Descriptions of marine *Paracoccus* species *P. seriniphilus*, *P. marinus*, *P. homiensis*, and *P. halophilus* have recently been published, however, their PHA production or *pha* gene sequencing were not determined (Pukall et al. 2003; Kim et al. 2006; Liu et al. 2008; Khan et al. 2008). Currently, microbial mats are considered as highly diverse and productive systems that produce high quantities of PHB under natural conditions, and bio-prospecting for PHB-producing bacteria in marine microbial mats has been done for the bulk of the community (López-Cortés et al. 2008; Rothermich et al. 2000). Microbial mats have been proposed as excellent sources for isolation of new PHA-producing strains with industrial applications (Berlanga et al. 2006; López-Cortés et al. 2008). Here, we report preliminary physico-chemical characterization of PHA and determination of a new partial sequence of the *phaC* gene in the marine bacterial strain, E71, which was identified as *Paracoccus seriniphilus*, based on the sequencing of 16S rRNA. This study describes rapid screening methods that permit discovery of a suitable industrial PHA production strain from many available bacterial strains.

Materials and methods

Sampling and isolation of bacteria

Samples of marine coastal sediment were collected from a site polluted with cannery discharge (24°46′56.4″N, 112°06′21.2″W) at Puerto San Carlos, BCS, Mexico. The bacterial Strain E71 was isolated from the microbial mat using diazotrophic (AZ) medium composed of (in g l^{-1} distilled water): glucose (5), mannitol (5), $CaCl_2{\cdot}2H_2O$ (0.1), $MgSO_4{\cdot}7H_2O$ (0.1), $Na_2MoO_4{\cdot}2H_2O$ (0.005), K_2HPO_4 (0.9), KH_2PO_4 (0.1), $FeSO_4{\cdot}7H_2O$ (0.01), $CaCO_3$ (5), agar (15) (López-Cortés et al. 2008). Further growth was done using peptone–yeast marine medium (PYM) composed of (in g l^{-1} half concentration of synthetic seawater). Since the Strain E71 was retrieved from marine microbial mats, half concentration of synthetic seawater (1/2× SSW) was used to satisfy the salt requirements for optimum growth and cell yields and was formulated as follow: NaCl (200 mM), KCl (10 mM), $MgSO_4{\cdot}7H_2O$ (50 mM), $CaCl_2{\cdot}2H_2O$ (10 mM) and Tris HCl buffer 1.0 M pH 7.5 (50 mM) (Baumann and Baumann 1981). The other components of PYM were (g l^{-1} 1/2× SSW), peptone (5), yeast extract (3), glucose (20), agar (15) (López-Cortés et al. 2008). Colonies from AZ medium were transferred to PYM medium, which allows best growth with PHA accumulation. Inoculated media were routinely incubated at 30°C.

Marine salt requirement for growth

To determine the requirements of NaCl and other salts representative of the seawater bacterial Strain E71 were grown in Basal Medium (BM) composed of (g l^{-1} distilled water) glucose (20), NH_4Cl (0.5), $K_2HPO_4{\cdot}3H_20$ (0.076), $FeSO_4{\cdot}7H_2O$ (0.028), which was used as negative control. A buffer solution (50 ml l^{-1}) of Tris–HCl, 1.0 M, pH 7.5 (Baumann and Baumann 1981) was added to maintain pH. BM was complemented with NaCl (9.34 g l^{-1}) or without NaCl and with NaCl + $MgSO_4{\cdot}7H_2O$ (9.88 g l^{-1}). Marine Basal Medium (MBM) contained the same ingredients as BM, but was complemented with 1/2× SSW (Baumann and Baumann 1981), and enriched medium PYM, cited above. Inoculated media were incubated at 35°C and agitated at 100 rpm. Growth was measured with help of a spectrophotometer (Jenway 6505-UV-vis) and measured optical density at 580 nm at 0, 24, and 48 h.

Detection of PHA in E71 bacterial strain with microscopy

Two microscopic techniques were used to detect PHA in E71. A drop (100 µl) of cell culture from PYM was put in

glass slide with cover slip and visualized with a phase contrast microscopy for the detection of brightly refractile cytoplasmic inclusions interpreted as PHA inclusions. The lipophilic stain Sudan Black was used to identify PHA with bright field microscopy. Sudan Black was performed on heat-fixed samples and prepared as 0.3 g Sudan Black B dissolved in 75 ml 95% ethanol, bringing it to 100 ml with distilled water. Samples were stained with Sudan Black solution for 10 min, dried with filter paper, and clarified with xylene drops, dried again with filter paper, and counterstained with 0.5% aqueous safranine for 5 s (López-Cortés et al. 2008).

Chemical extraction of PHA

Chemical extraction of PHA was conducted as previously reported (Law and Slepecky 1961). E71 was grown in 1-l flasks with 300 ml PYM medium in each flask and incubated for 48 h under orbital agitation at 100 rpm. To harvest the cells, 285 ml of the culture was centrifuged at 4,700*g* for 20 min. The cell pellet was re-suspended in a volume of NaOCl equal to the original volume of culture (285 ml). After 1 h at 37°C, the lipid granules (PHA) were centrifuged, washed with water, and then washed with acetone and ethanol. The PHA polymer was dissolved by extraction with three small portions of boiling chloroform, the chloroform solution was filtered, and the filtrate was used for further analyses. Simultaneously, 15 ml of the culture were centrifuged and lyophilized to determined dry weight of cells.

Spectrophotometric identification of PHA

A subsample of 1 g PHA from the chemical extraction was processed to evaporate the excess chloroform; 10 ml concentrated H_2SO_4 was added and then heated for 10 min at 100°C in a water bath to convert PHA to crotonic acid. PHA was determined as crotonic acid and quantified by spectrophotometry at 235 nm (Law and Slepecky 1961). A standard curve with commercial DL-β hydroxybutyric acid (Sigma, H6501) was made to determine PHA quantities in our samples.

IR spectroscopy

A subsample of 16 g PHA from the chemical extraction was used in IR spectroscopic analyses. Casting films were prepared and analyzed in a spectrometer (Nicolet Magna 550) in the wave number range from 400 to 4,000 cm^{-1} and a resolution of 4 cm^{-1} for 25 scans. Films were dried in a vacuum oven for 24 h at 80°C before making the recordings.

Differential scanning calorimetry

The calorimetric measurement of biopolymer samples were carried out using a DuPont thermal analyzer fitted with a Differential scanning calorimetry (DSC) cell. Dried samples were first heated to 250°C in a N_2 atmosphere and then cooled to 0°C. In the second heating cycle, samples were heated from 25 to 250°C at a heating rate of 10°C min^{-1}. The enthalpy and the melting temperature of each sample were estimated in the second heating cycle.

Thermogravimetry

TGA measurements were made with a DuPont thermal analyzer fitted with a TGA cell. Dried samples were heated from 25 to 600°C under N_2 flow of 10 ml^{-1} and scan rate of 10°C min^{-1}.

Rheology studies

An Instron capillary rheometer was used to characterize flow behaviour, samples were run at 190°C in a shear rate range from 1 to 3 reciprocals s^{-1}.

DNA manipulation

Total bacterial genomic DNA was isolated according to the protocol described in Sambrook and Russell (2001) with some modifications. The modifications consist of mechanical maceration using glass beads (0.2 mm) and chemical cell lysis by the addition of lysozyme (20 mg ml^{-1}). The DNA was analyzed in a 1.2% agarose gel electrophoresis at 100 V for 30 min and stained with ethidium bromide to determine its quality. The DNA extracted was used for taxonomic identification and partial *phaC* gene amplification assays.

16S rRNA sequencing for the identification of bacterial strain E71

Bacterial DNA of strain E71 was amplified with a thermal cycler (Gen Cycler, Bio-Rad) and Primer A 5′ AGA GTT TGA TCM TGG CTC AG 3′ and Primer B: 5′ AAG GAG GTG ATC CAN CCR CA 3′ (Giovannoni 1991) to selectively amplify the sequence of the 16S rRNA gene. Partial amplification of the 16S rRNA gene was done with the following reaction mixture: Each 25 µl reaction mixture contained 25 mM $MgCl_2$, 2.5 mM dNTP, 250 ng μl^{-1} of

each primer, 180 ng μl^{-1} of DNA, and 1 U GoTaq® Flexi DNA polymerase (Promega). The PCR amplification protocol consists of an initial denaturation step of 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and a final cycle extension of 10 min at 72°C. Products of sequencing reactions were done commercially (Macrogen, Korea).

Detection of *phaC* gene

To obtain a prime region of *phaC* gene from strain E71, two degenerated primers were designed from a multiple alignment of 10 *phaC* sequences of Gram-negative bacteria. The flanking consensus regions (upstream and downstream) among the multiple alignments were used to design primers that amplified a region of at least 500 bp (Table 1).

The primers were used to amplify a region belonging to the *phaC* according to the following conditions. The PCR reaction mixture contained 1× PCR amplification buffer containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl and 3.5 mM $MgCl_2$, 0.2 mM dNTP, 2 μM each primer, 10–50 ng DNA, and 1 U *Taq* polymerase (Invitrogen) in 12.5 μl of PCR reaction mixture. The thermal cycler (GeneAmp PCR system 9700, Perkin-Elmer), program consisted of a hot start at 94°C for 5 min, 35 cycles at 94°C for 1 min, at 52°C for 1 min, at 72°C for 1 min, and a final cycle extension at 72°C for 10 min. The PCR products were run in a 0.8% agarose gel electrophoresis and stained with ethidium bromide. The resulting bands were cloned with the TOPO TA Cloning Kit (Invitrogen) according the manual protocol and positive colonies were finally sent to Macrogen Inc. (Korea) for sequencing.

Sequence analyses

Bacterial gene sequences, 16S rRNA as well as *phaC* from Strain E71 were compared with those available from GenBank, by using BLAST tools (Altschul et al. 1990; Altschul et al. 1997).

Nucleotide sequence accession numbers

The sequences of the studied bacteria were deposited in GenBank, accession numbers were: FJ798706 for 16S rRNA and FJ619574 for *phaC* genes.

Table 1 Primers designed to amplify *phaC* of Gram negative bacteria

Primer name	Sequence
PHACGNF	5′ CCYRGATCAACAAGTTCTAC 3′
PHACGNR	5′ TTCCAGAACAGMAGGTCGAAGG 3′

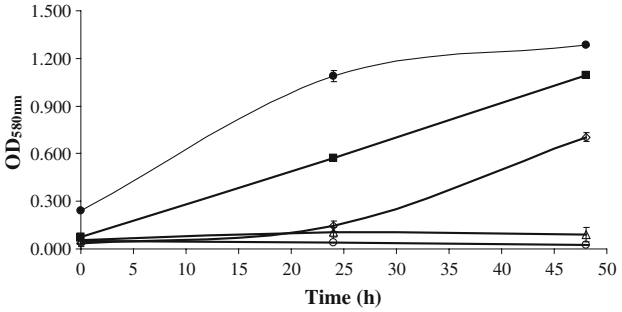


Fig. 1 Effect of marine salts on growth of Strain E71: basal medium, this was used as negative control (*empty circle*), basal medium NaCl (*empty triangle*), basal medium NaCl + $MgSO_4$ (*empty rhomboid*), marine basal medium (*filled square*), peptone–yeast extract marine medium (*filled circle*)

Results and discussion

Marine nature

Strain E71, after 72 h incubation in BM, with and without NaCl, showed incipient or no growth, respectively. BM with NaCl + $MgSO_4 \cdot 7H_2O$ showed growth; this suggests that this strain requires Na^+, and Mg^{2+}, and $SO_4{}^{-2}$. In media with seawater salts, MBM and PYM showed significant difference in growth when compared with BM complemented with NaCl + $MgSO_4 \cdot 7H_2O$. PYM, which contained peptone and yeast extract and seawater salts, resulted in the best medium for the growth of Strain E71 (Fig. 1), which suggests that the strain needs seawater ions such as Na^+, Mg^{2+}, K^+, Ca^{2+}, and $SO_4{}^{-2}$, a stable attribute of marine bacteria to grow and, therefore E71 must be consider a true marine bacteria (Baumann and Baumann 1981).

Identification and phylogeny of Strain E71 by 16S rRNA sequencing

Strain E71 was isolated from a marine microbial mat subject to environmental stress of organic waste from a fish cannery that adds unbalanced nutrients. Sequence analysis of 16S rDNA of E71 (FJ798706) was affiliated with the genus *Paracoccus* of the α-subclass of the *Proteobacteria*. The 16S rRNA sequence of E71 (850 bp) was closely related to *Paracoccus* sp. Strain QDHT-17 (FJ210812) isolated from seawater, with 100% similarity. The sequence of *P. seriniphilus* strain MBT-A4 (AJ428275) from marine bryozoa had 99% similarity. The sequences of *P. homiensis* Strain DD-R11 (DQ342239) from sea sand and *P. marinus* Strain KKL-B9 (AB185959) from seawater had 98% identity. *P. kamogawaensis* Strain 4SFL3 (AB275604), *P. zeaxanthinifaciens* Strain R-1506 (AF461159), and *P. denitrificans* Strain E4 (EF 186005) had 97% similarity (Fig. 2).

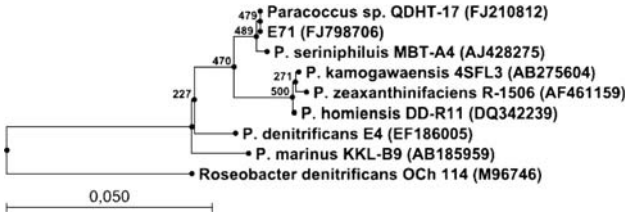


Fig. 2 Phylogentic tree based on 16S rRNA sequences (850 bp), constructed with CLC v 5.0.1 software using neighbor-joining. Accession numbers appear in *parenthesis*. *Numbers* at nodes represent bootstrap values (500 times re-sampling analysis); only values >200% are presented. *Roseobacter denitrificans* was used as the outgroup. The *bar scale* corresponds to five changes per 100 positions

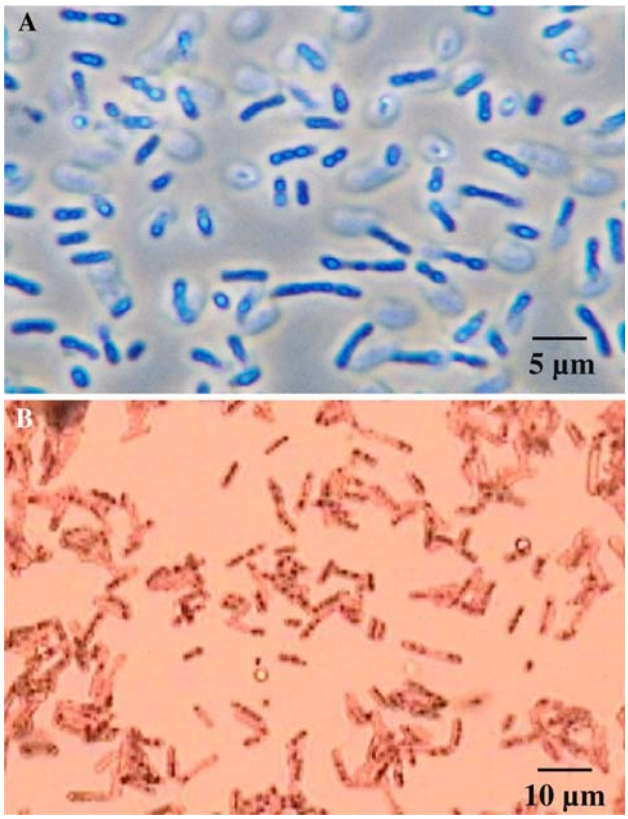


Fig. 3 Phase contrast micrograph showing: **a** brightly refractile cytoplasmic inclusions (*RCI*) and **b** dark inclusions obtained with Sudan black stain. Both correspond to polyhydroxyalkanoate-PHAs of the Strain E71

Microscopic detection of PHA

Strain E71 shown positive results from the two microscopic techniques used to detect PHA (Fig. 3). After 48 h incubation in PYM medium, E71 accumulated 53% PHA by dry cell weight, which is competitive, in comparison with *P. denitrificans* which accumulated 57% (Ueda et al. 1992).

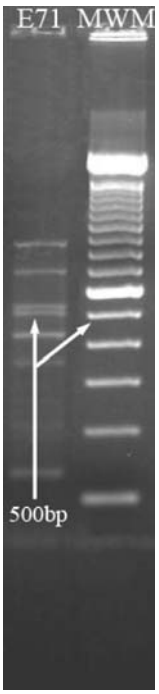
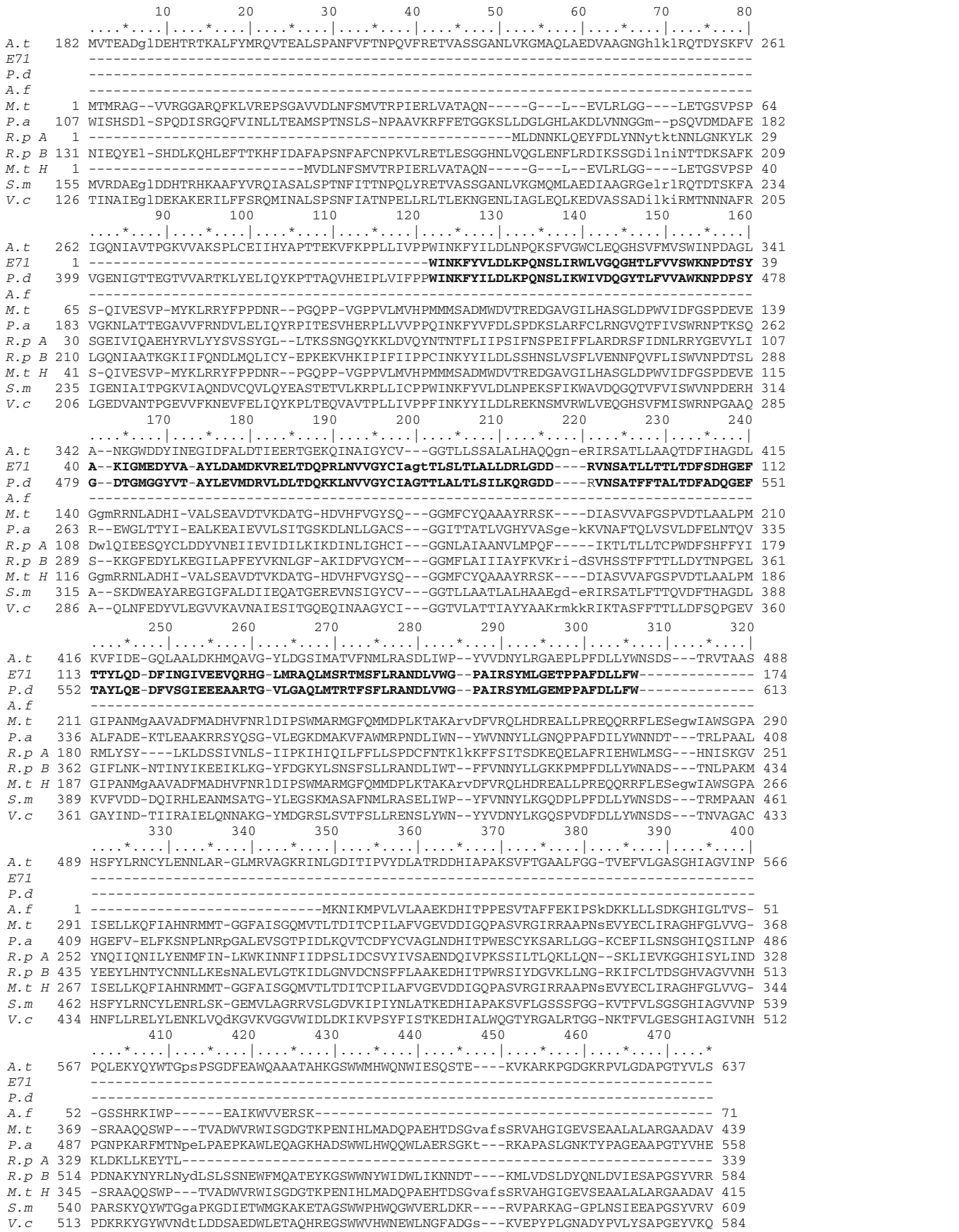


Fig. 4 PCR amplification of Strain E71 with the primers PHACGNF and PHACGNR. MWM = molecular weight marker of 100 bp DNA Ladder from Invitrogen (15628-050). Only the fragment of ~500 bp was cloned and sequenced

phaC partial-gene isolation

The primers designed did generate several products of different sizes in E71 (Fig. 4) ranged from 100 to 900 bp. We used as standard, DNA of a strain from the genus *Paracoccus* sp. Strain E33 reported as PHA producer (López-Cortés et al. 2008). The DNA of Strain E33 was used as template in PCR using primers of Table 1. PCR products were sequenced and confirmed that is related with *pha*C gene (unpublished results). Values of similarity of strain E71 <90% with amino acid sequence of the *phaC* gene of *P. denitrificans* Strain PD1222 were interpreted as a new sequence. Only the bands of ~500 bp and that of 900 bp from strain E71 were recovered from the agarose gel by the Qiaquick Gel extraction Kit (QIAGEN) and cloned in the pCR-2.1 TOPO vector (TOPO TA cloning kit, Invitrogen). From the clones obtained, we perform PCR and sequencing for three clones, which presented a fragment of 500 bp. The same procedure was used for the band of 900 bp.

Only the fragment of ~500 bp was related with *pha*C gene. This sequence had 526 bp, and was deposited in GenBank (FJ619574). This was compared with a BLAST-nucleotide (Altschul et al. 1990) that had 77% identity with the *phaC* gene of *P. denitrificans* Strain PD1222 (AB017045). Additionally, the sequence was translated to amino acids and compared using the putative conserved domains search service (RSA-BLAST). An ORF that codify 174 residues was obtained, having an identity of 74% and a similarity of 86% against the amino acid



◀ **Fig. 5** Amino acid sequence alignment of partial poly(*R*)-hydroxyalkanoic acid synthase (*phaC*), class I from *Paracoccus seriniphilus* Strain E71. The sequence was aligned using the PSI-BLAST algorithm and compared with the putative conserved domains search service (RSA-BLAST). Residues in *bold* show significant alignment among *Paracoccus denitrificans phaC* gene (Accession no. ZP-00633085) and E71 putative *phaC* gene. Identities = 130/174 (74%); positives = 151/174 (86%); gaps = 0/174 (0%). *A.t.* = *Agrobacterium tumefaciens*; *P.d.* = *Paracoccus denitrificans*; *A.f.* = *Archaeoglobus fulgidus*; *M.t.* = *Mycobacterium tuberculosis*; *P.a.* = *Pseudomonas aeruginosa*; *R.p.* A and *R.p.* B = *Rickettsia prowazekii*; *M.t.* H = *Mycobacterium tuberculosis* H; *S.m.* = *Sinorhizobium meliloti* and *V. c.* = *Vibrio cholerae*

sequence deduced of the *phaC* gene N-terminal portion of *P. denitrificans* Strain PD1222 (Fig. 5). This result is relevant, since our sequence of *phaC* is the second deposit in GenBank from species of the genus *Paracoccus* that is different from *phaC* sequences corresponding to *P. denitrificans* strains. In the case of the fragment of 900 bp the sequence obtained did not present homology with the *phaC* gene (data not shown).

Ours results allow us to determine that the partial *phaC* sequence of E71 is a poly-(3-hydroxyalkanoate) synthase class I, specifically a poly-beta-hydroxybutyrate polymerase. This result match with the microscopic detection and physico-chemical properties obtained from the polymer described below. Review of the description of the marine species, *P. seriniphilus*, *P. marinus*, *P. homiensis*, and *P. halophilus*, has not included the determination of PHB production (Pukall et al. 2003; Kim et al. 2006; Liu et al. 2008; Khan et al. 2008); therefore, this is the first report of sequence of *phaC* from marine species of *Paracoccus*.

Physico-chemical properties of PHA from E71

IR spectrum of the polymer extracted is shown in Fig. 6, namely that two intense absorption bands, 1726.78 and 2,980 cm^{-1} corresponding to aliphatic ester carbonyl C=O of RCOA and C–H stretch, respectively. Other absorption bands were at 3449.40, 2,920 cm^{-1}, representing the presence of O–H bonding and C–H bond of aliphatic compounds, respectively (Pal and Paul 2002). The most prominent marker (ester carbonyl) band for PHB was at 1,740 cm^{-1} by Helm and Naumann (1995) and 1724.03 cm^{-1} for pure PHB by Misra et al. (2000). Band absorption between 1,724 and 1,740 cm^{-1} is typical of PHA (Shamala et al. 2003). In these polymers, the carboxyl group of one monomer forms an ester bond with the hydroxyl group of the neighboring monomer (Madison and Huisman 1999). The thermogram obtained by differential scanner calorimetry showed a melting enthalpy (ΔH_m) of 60.51 J g^{-1}, similar at those reported for PHB of *Azotobacter chroococcum* Strain MAL-201 (Pal and Paul 2002) and a melting temperature of 167.86°C (T_m) that corresponds to the fusion of the crystalline polymer (Fig. 7). This T_m was very close to values reported for polyhydroxybutyrate-PHB at 174.73–177°C and polypropylene

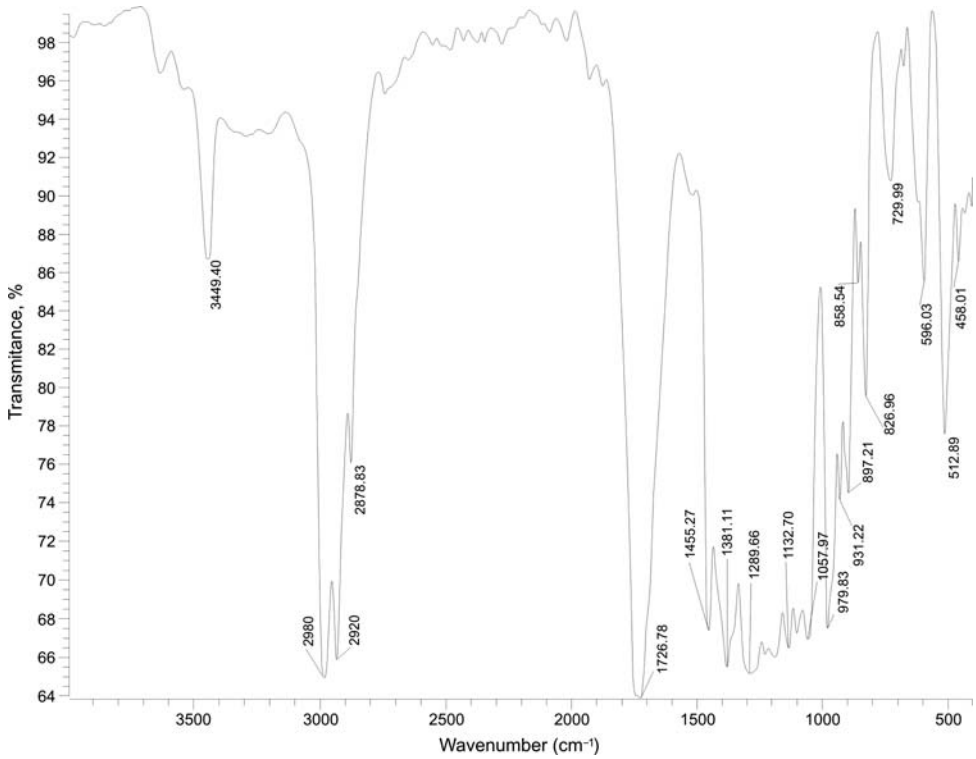


Fig. 6 Infra-red spectrum of the PHB polymer extracted from Strain E71

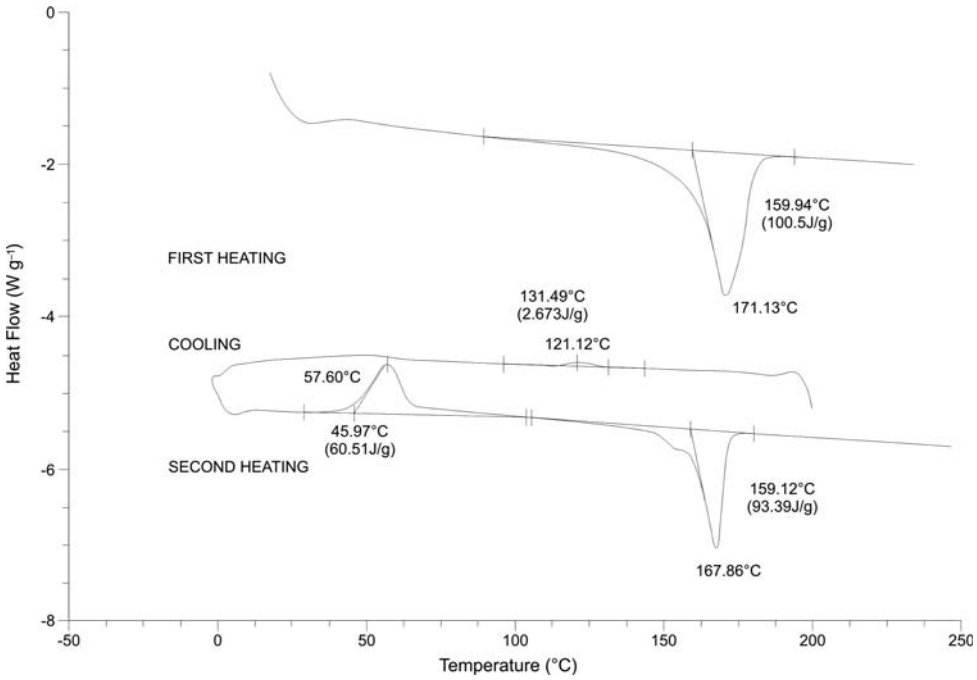


Fig. 7 DSC curve contains a melting enthalpy (ΔH_m) of 60.51 J g^{-1} and melting temperature (T_m) of 167.86°C

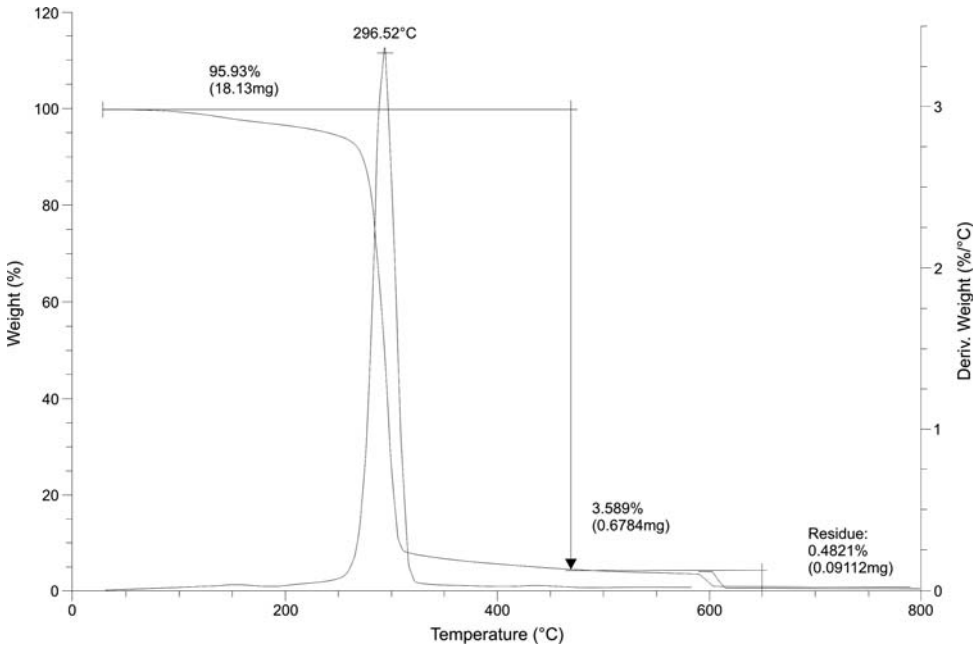


Fig. 8 The thermogravimetric analysis showed that thermal degradation occurred at ~296.52°C, with a single weight-loss step

at 176°C (Pal and Paul 2002; Madison and Huisman 1999). Crystallinity and molecular weight have marked effect on the mechanical properties of polymers. Thermogravimetric analysis showed that this polymer is degraded at 296.52°C (Fig. 8), indicating that the biopolymer has good thermal stability. These results allowed establishment of the conditions for rheological characterization. Rheology results at 190°C showed that this polymer behaves as a nonNewtonian fluid (Fig. 9). It was determined that, if the residence time inside the rheometer is too long (more than 5 min), the polymer starts to degrade. All these characteristics suggest that Strain E71 produced a PHA that corresponded to a crystalline thermoplastic polymer.

Conclusion

Overall the results confirm that PHA biopolymer were produced, 53% dry cell weight, by *Paracoccus seriniphilus* strain E71, a bacterium with requirements of seawater salts

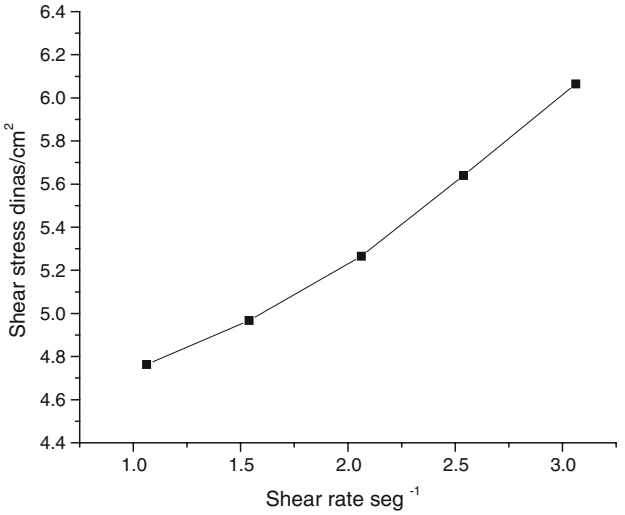


Fig. 9 Rheology of PHA polymer from Strain E71

for growth, and characterized by, IR, DSC, TGA, and rheology, confirmed that this biopolymer is a crystalline thermoplastic polymer PHB type whose partial sequence of *phaC* gene codify to poly-(3-hydroxyalkanoate) synthase class I, specifically a poly-beta-hydroxybutyrate polymerase, this strain has industrial production potential, since alternative strains are currently not available. This study is useful for microbial biotechnology because offer an alternative strain that satisfy several demands for industrial production purposes such as fast growth, being able to utilize cheap carbon source and high PHA accumulation in the cells (Chen 2002).

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Mol Biol 215:403–410

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Baumann P, Baumann L, Mandel M (1971) Taxonomy of marine bacteria: genus *Benekea*. J Bacteriol 107:268–294

Baumann P, Baumann L (1981) The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes: a handbook on habitats, isolation, and identification of bacteria, vol 2, 1st edn. Springer, New York, pp 1302–1331

Berlanga M, Montero MT, Hernández-Borrel J, Guerrero R (2006) Rapid spectrofluorometric screening of poly-hydroxyalkanoate-producing bacteria from microbial mats. Int Microbiol 9:95–102

Chen GQ (2002) Production and applications of microbial polyalkanoates. In: Chiellini E, Solaro R (eds) Biodegradable polymers and plastics. Springer, New York, pp 155–166

Giovannoni S (1991) The polymerase chain reaction. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 177–203

Helm D, Naumann D (1995) Identification of some bacterial cell components by FT-IR spectroscopy. FEMS Microbiol Lett 126:75–80

Kalia VP, Lal S, Cheema S (2007) Insight in to the phylogeny of polyhydroxyalkanoate biosynthesis: horizontal gene transfer. Gene 389:19–26

Khan ST, Takaichi S, Harayama S (2008) *Paracoccus marinus* sp. nov., an adonixanthin diglucoside-producing bacterium isolated from coastal seawater in Tokyo Bay. Int J Syst Evol Microbiol 58:383–386

Khanna S, Srivastava AK (2005) Recent advances in microbial polyhydroxyalkanoates. Process Biochem 40:607–619

Kim BY, Weon HY, Yoo SH, Kwon SW, Cho YH, Stackebrandt E, Go SJ (2006) *Paracoccus homiensis* sp. nov., isolated from a sea-sand sample. Int J Syst Evol Microbiol 56:2387–2390

Kolibachuck D, Miller A, Dennis D (1999) Cloning, molecular analysis, and expression of the Polyhydroxyalkanoic acid synthase (*phaC*) gene from *Chromobacterium violaceum*. Appl Envion Microbiol 65:3561–3665

Kranz RG, Gabbert KK, Locke TA, Madigan MT (1997) Polyhydroxyalkanoate production in *Rhodobacter capsulatus*: genes, mutants, expression, and physiology. Appl Envion Microbiol 63:3003–3009

Law JH, Slepecky RA (1961) Assay of poly-ß-hydroxybutyric acid. J Bacteriol 82:33–36

Liu ZP, Wang BJ, Liu XY, Dai X, Liu YH, Liu SJ (2008) *Paracoccus halophilus* sp. nov., isolated from marine sediment of the South China Sea, China, and emended description of genus *Paracoccus* (Davis 1969). Int J Syst Evol Microbiol 58:257–261

López-Cortés A, Lanz LA, García MJQ (2008) Screening and isolation of PHB-producing bacteria in a polluted marine microbial mat. Microb Ecol 56:112–120

Madison LL, Huisman GW (1999) Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. Microbiol Mol Biol Rev 63:21–53

Misra AK, Thakur MS, Srinivas P, Karanth NG (2000) Screening of polyhydroxybutyrate-producing microorganisms using Fourier transform infrared spectroscopy. Biotechnol Lett 22:1217–1219

Mothes G, Ackermann JU, Babel W (2004) Mole fraction control of poly([R]-3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) synthesized by *Paracoccus denitrificans*. Eng Life Sci 4:247–251

Oliver JD, Colwell R (1973) Extractable lipids of gram-negative marine bacteria: phospholipid composition. J Bacteriol 114:897–908

Pal S, Paul AK (2002) Physico-chemical characteristics of poly(3-hydroxybutyric acid) isolated from *Azotobacter chroococcum* MAL-201. Curr Sci 83:1565–1568

Pukall R, Laroche M, Kroppenstedt RM, Schumann P, Stackebrandt E, Ulber R (2003) *Paracoccus seriniphilus* sp. nov., an L-serinedehydratase-producing coccus isolated from the marine bryozoan Bugula plumose. Int J Syst Evol Microbiol 53:443–447

Rothermich MM, Guerrero R, Lenz RW, Goodwin S (2000) Characterization, seasonal occurrence, and diel fluctuation of poly (hydroxyalkanoate) in photosynthetic microbial mats. Appl Environ Microbiol 66:4279–4291

Sambrook J, Russell DW (2001) Molecular cloning. A laboratory manual, vol 1. CSHL, Plymounth, p 2100

Shamala TR, Chandrashekar A, Vijayendra SVN, Kshama L (2003) Identification of polyhydroxyalkanoate (PHA)-producing *Bacillus* spp. using the polymerase chain reaction (PCR). J Appl Microbiol 94:369–374

Suriyamongkol P, Weselake R, Narine S, Moloney M, Shah S (2007) Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants—a review. Biotechnol Adv 25:148–175

Ueda S, Matsumoto S, Takagi A, Yamane T (1992) Synthesis of poly (3-hydroxybutyrate-co-3-hydroxyvalerate from methanol and *N*-amyl alcohol by themethylotrophic bacteria *Paracoccus denitrificans* and *Methylobacterium extorquens*. Appl Environ Microbiol 58:3574–3579

Ueda S, Yabutani T, Maehara A, Yamane T (1996) Molecular analysis of the poly(3-hydroxyalkanoate) synthase gene from a methylotrophic bacterium, *Paracoccus denitrificans*. Journal of Bacteriology 178:774–779