World J Microbiol Biotechnol (2009) 25:1751–1756
DOI 10.1007/s11274-009-0072-9

ORIGINAL PAPER

Screening of bacteria to produce polyhydroxyalkanoates from xylose

Mateus Schreiner Garcez Lopes · Rafael Costa Santos Rocha · Sandra Patricia Zanotto · José Gregório Cabrera Gomez · Luiziana Ferreira da Silva

Received: 19 February 2009 / Accepted: 14 May 2009 / Published online: 2 June 2009
© Springer Science+Business Media B.V. 2009

Abstract Although xylose is a major constituent of lignocellulosic feedstock and the second most abundant sugar in nature, only 22% of 3,152 screened bacterial isolates showed significant growth in xylose in 24 h. Of those 684, only 24% accumulated polyhydroxyalkanoates after 72 h. A mangrove isolate, identified as *Bacillus* sp. MA3.3, yielded the best results in literature thus far for Gram-positive strains in experiments with glucose and xylose as the sole carbon source. When glucose or xylose were supplied, poly-3-hydroxybutyrate (PHB) contents of cell dry weight were, respectively, 62 and 64%, PHB yield 0.25 and 0.24 g g^{-1} and PHB productivity (P_{PHB}) 0.10 and 0.06 g l^{-1} h^{-1}. This 40% P_{PHB} difference may be related to the theoretical ATP production per 3-hydroxybutyrate (3HB) monomer calculated as 3 mol mol^{-1} for xylose, less than half of the ATP/3HB produced from glucose (7 mol mol^{-1}). In PHB production using sugar mixtures, all parameters were strongly reduced due to carbon catabolite repression. PHB production using Gram-positive strains is particularly interesting for medical applications because these bacteria do not produce lipopolysaccharide endotoxins which can induce immunogenic reactions. Moreover, the combination of inexpensive substrates and products of more value may lead to the economical sustainability of industrial PHB production.

M. S. G. Lopes · R. C. S. Rocha · J. G. C. Gomez · L. F. Silva (✉)
Departamento de Microbiologia, Universidade de São Paulo, Avenida Professor Lineu Prestes, 1374, Edifício Biomédicas II, Cidade Universitária, São Paulo, SP 05508-900, Brazil
e-mail: lukneif@icb.usp.br; lukneif@usp.br

S. P. Zanotto
Mestrado em Biotecnologia, Universidade do Estado do Amazonas, Av. Carvalho Leal, 1777, Cachoeirinha, Manaus, AM 69065-170, Brazil

Keywords Xylose · Sugar mixtures · Polyhydroxyalkanoates · *Bacillus* · Poly-3-hydroxybutyrate · Catabolite repression

Introduction

Polyhydroxyalkanoates (PHAs) represent a large family of intracellular bacterial storage polyesters with wide range of material properties permitting applications as biodegradable and biocompatible thermoplastics and elastomers (Reddy et al. 2003). The most extensively studied and frequently found member of PHAs, the homopolymer PHB is very promising as a biodegradable plastic because of its material properties, which are comparable to those of polypropylene (Tokiwa and Ugwu 2007).

One major limiting factor for the industrial production of this natural polyester is the expense associated with the carbon source (Keenan et al. 2006), which can account for up to 29% of its overall production cost even when integrated to sugarcane mills (Nonato et al. 2001). For that reason, efforts have been made to utilize low cost substrates as renewable lignocellulosic materials. Two platforms are considered for production of PHAs from agricultural byproducts and forest residues: (1) cellulosic and hemicellulosic hydrolysates which generate a mixture of sugars including mainly glucose, xylose and arabinose (Silva et al. 2004) or (2) hemicellulosic hydrolysates as a xylose-rich substrate for PHAs production (Keenan et al. 2004).

Xylose is a major constituent of the raw material in both approaches and the second most abundant sugar in nature. Nevertheless its efficient utilization presents a technical barrier for several bioprocesses (Saha 2003), including ethanol production (Jeffries and Jin 2004) and PHA

production (Silva et al. 2004). For this reason, the scope of this work was to search different environments for bacterial isolates capable of producing PHAs efficiently from xylose and sugar mixtures. For the first time a large scale screening to isolate Gram-positive and Gram-negative PHB producing bacteria from xylose is described.

Materials and methods

Bacterial strains and culture conditions

The bacterial isolates used in this study were obtained from environmental samples and *B. sacchari* IPT101 (LMG 19450) was used as a control in all experiments due its ability to produce PHA from glucose, xylose, and arabinose. Nutrient broth (NB) was used to prepare inocula in the experiments and for the isolation of bacterial strains. Erlenmeyer flasks (500 ml, 30°C, 200 rev min^{-1}) with mineral salt medium (MM) were used in the experiments for bacterial growth (3 g l^{-1} of ammonium sulphate) and PHB production (1 g l^{-1} of ammonium sulphate). However, instead of sucrose, as described by Rocha et al. (2008), other carbohydrates were supplemented as carbon source.

Isolation procedure

Most environments were chosen for their high content of organic mater in decomposition or potential biodiversity. The location of sampling sites were the mangrove of Itanhaém River (Brazil, S24°0.9′34.4″/W46°48′17.7″), a sewage treatment plant (Brazil, S23°32′33″/W46°18′39”), the King George Island (Antartic, S62°05′23.8″/W58°24′20.3″), the Amazon Rainforest (Brazil, S62°05′23.8″/W58°24′20.3″), and the Atlantic Rainforest (Brazil, S23°46′41″/W46°18′16″).

Approximately 5 g of soil samples with 50 ml salt solutions (0.85% NaCl) were incubated in a rotary shaker (200 rev min^{-1}, 30°C) for 1 h. Samples of different serial dilutions were spread in nutrient agar (NA: NB plus agar 20 g l^{-1}) containing cycloheximide (0.06 g l^{-1}). Gram positive and Gram negative isolates were transferred to solid MM with xylose (15 and 1 g l^{-1} of ammonium sulphate). After 24 h of incubation at 30°C the isolates were classified as Xyl^+ or Xyl^-, and, after 72 h incubation they were stained with Sudan Black B and classified as either PHA^+ or PHA^- (Schlegel et al. 1970).

Phylogenetic characterization of the isolates

Genomic DNA was extracted according to the method described by Ausubel et al. (1992). The primers used for 16S rDNA gene amplification were 27F (Lane 1991) 5′AGA GTT TGA TCM TGG CTC AG 3′ and 1401R (Heuer et al. 1997) 5′CGG TGT GTA CAA GAC CC 3′. Polymerase chain reaction and sequencing procedures are described by Piza et al. (2004). The Molecular Evolutionary Genetics Analysis, Version 3.1 (Kumar et al. 2004) was used for sequence alignment and phylogram design. The morphological and physiological properties of the isolates (MA3.3) were investigated according to Slepecky and Hemphill (2006).

Analytical methods

Bacterial growth was evaluated spectrophotometrically at 600 nm. Cell dry weight (CDW) was determined gravimetrically, carbohydrates by HPLC (Silva et al. 2004) and PHA amount and composition by gas chromatography of propyl-esters (Gomez et al. 1996). The experiments were run in duplicate with very good reproducibility (less than 10% variation in $Y_{PHB/C}$). Kinetic parameters were calculated as indicated by Pirt (1975).

Results and discussion

Initial screening

A total of 3,152 isolates were screened on solid MM with xylose for a strain capable of growing in xylose in 24 h and producing PHA after 72 h (Fig. 1). Only 684 (22% of the total) grew in 24 h; indicating that, although xylose is the second most abundant sugar in nature, it is generally not rapidly metabolized by bacteria. Proportionally, more Xyl^+ organisms were found in the mangrove (32%), Atlantic Rainforest (31%) and Amazon Rainforest (26%) in comparison to the brut sewage (19%), activated sludge (10%) and Antarctic (14%). This indicates that xylose-degrading bacteria are more likely to be found in natural environments with a high content of decomposing organic matter. In a smaller sample, Silva et al. (2004) studied fifty-five Gram-negative bacterial isolated from sugarcane crop soil and a total of fifty-four grew on solid MM with xylose, approximately 100% of the strains.

Only 5% of the isolates (total of 164) grew in xylose in 24 h and were classified as PHA^+ after 72 h of incubation. Fifty of the isolates that exhibited promising results compared to IPT101 (colony size and staining) were selected for growth experiments in MM with xylose (15 g l^{-1}). Specific growth rates were calculated from the exponential growth data (Pirt 1975) and the isolates showed a specific growth rate (μ_{Xmax}) range of 0.15–0.62 h^{-1}, an average lag phase of 2.5 h and after 20 h of incubation an average of 5.12 Abs. Thirteen selected strains showed μ_{Xmax} average of 0.37 h^{-1}, with an average lag phase of 2.5 h, and

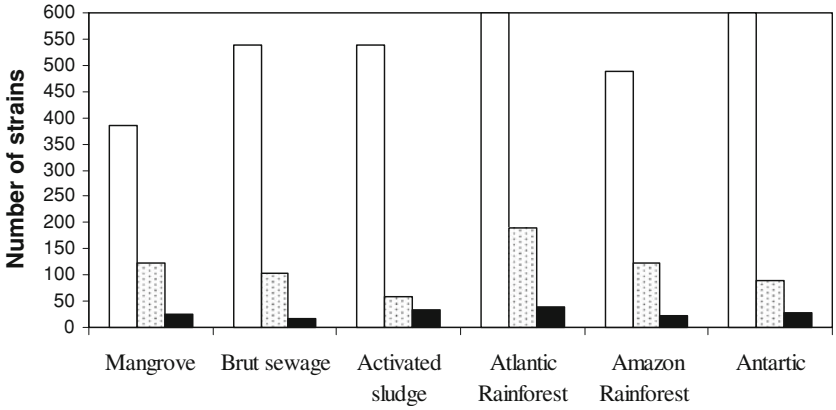

Fig. 1 Results from the screening of solid mineral media with xylose (15 g l^{-1}). The *columns* represent the total number of strains (*white*), the strains that grew in xylose in 24 h (*dotted*) and strains that grew in xylose in 24 h and accumulated PHA in 72 h (*black*)

reached about 6.84 Abs after 20 h of incubation. This μ_{Xmax} average is similar to several microorganisms grown in glucose (Silva et al. 2000).

PHAs production experiments

The 13 isolates were analyzed in MM with xylose and all strains accumulated PHB as anticipated by Sudan Black B results. The average PHB yield from xylose ($Y_{PHB/C}$), PHB volumetric productivity (P_{PHB}) and PHB content of the cell dry weight (%PHB) were 0.07 g g^{-1}, 0.02 g l^{-1} h^{-1}, and, 30%, respectively (Table 1). *B. sacchari* and the mangrove isolate MA3.3 showed the best results: 55% of PHB content, P_{PHB} of 0.06 g l^{-1} h^{-1} and Y_{PHB} from xylose of 0.24 and 0.21 g g^{-1}, respectively.

B. sacchari and MA 3.3 were analyzed in further experiments in single, dual and triple sugar mixtures (Table 2; Fig. 2). In the single sugar experiments cultures showed similar values of %PHB and $Y_{PHB/C}$, although P_{PHB} for both strains in xylose were about 40% lower than in glucose (Table 2). This could be related to the energetic balance of those sugars. After xylose is converted to glyceraldehyde-3-phospate by the pentose-phosphate pathway, it is metabolized to pyruvate through the Embden–Meyerhof–Parnas-Pathway. After that, two acetyl-CoA moieties are condensed to acetoacetyl-CoA which is then reduced to (*R*)-3-hydroxybutyryl-CoA. The last reaction is the polymerization of (*R*)-3-hydroxybutyryl-CoA into PHB with monomers of (*R*)-3-hydroxybutyryl (HB). The overall reaction is presented:

$$6C_5H_{10}O_5 + 16NAD^+ + 5NADPH \rightarrow 5C_4H_6O_2 + 15NADH + 5NADP^+ + 10CO_2$$

Supposing that there is an active transhydrogenase, the $NADP^+$ could be regenerated:

$$5NADP^+ + 5NADH + 5ATP \rightarrow 5NAD^+ + 5NADH + 5ADP$$

The combination of both equations is:

Table 1 Production of PHB by different soil bacteria in mineral media containing xylose (15 g l^{-1})

Strain	CDW (g l^{-1})	PHB (%)	$Y_{PHB/C}$ (g g^{-1})	P_{PHB} (g l^{-1} h^{-1})
IPT101	5.48	54.61	0.24	0.06
MA3.3	4.16	54.84	0.21	0.06
MA3.10	5.53	19.98	0.07	0.02
MA10.3	1.49	23.16	0.03	0.00
MA11.15	2.77	10.17	0.03	0.01
SE7.5	3.54	29.34	0.09	0.01
AM1.11	3.02	32.25	0.08	0.04
AM2.16	3.33	12.15	0.03	0.01
AM4.15	3.07	15.53	0.04	0.01
AM6.16	1.28	32.76	0.03	0.02
AN2.16	2.74	39.41	0.06	0.01
AN2.25	2.30	46.67	0.06	0.01
AN2.9	4.62	24.84	0.10	0.05
AN5.5	2.80	42.60	0.07	0.02

Cell dry weight (CDW), PHB content of the cell dry weight (%PHB), PHB yield from carbon source ($Y_{PHB/C}$), and PHB volumetric productivity (P_{PHB}) are presented

$$6C_5H_{10}O_5 + 10NAD^+ + 5ATP \rightarrow 5C_4H_6O_2 + 10NADH + 5ADP + 10CO_2$$

The overall equation for 3HB monomers from glucose is:

$$6C_6H_{12}O_6 + 6ADP + 6Pi + 18NAD \rightarrow 6C_4H_6O_2 + 6ATP + 18NADH + 12CO_2$$

Since PHB production is an aerobic process through oxidative phosphorylation, it is possible to produce ATP from NADH. Admitting the production of 2 ATP per NADH, the theoretical ATP/3HB monomer ratio is 3 mol mol^{-1} for xylose, which is less than half of the ATP produced from glucose (7 mol mol^{-1}). The same energetic issue is observed in anaerobic fermentation of xylose in *Escherichia coli* to produce ethanol (Tao et al. 2001). In sugar mixtures experiments with *B. sacchari* and MA3.3 after

24 h and 36 h, respectively, increased PHB accumulation (Fig. 2) is probably due the nitrogen exhaustion (data not shown). The strains did not consume all sugars simultaneously; rather, they preferentially utilized glucose and arabinose, delaying the use of xylose. This result is due to carbon catabolic repression. But it is not only related with sugar assimilation as demonstrated in Table 2, but also for CDW, %PHB, $Y_{PHB/C}$ and P_{PHB} values. Both strains declined their performance when dual and triple sugar mixtures were utilized. Moreover, for MA3.3 the effect is clearly stronger than to IPT101.

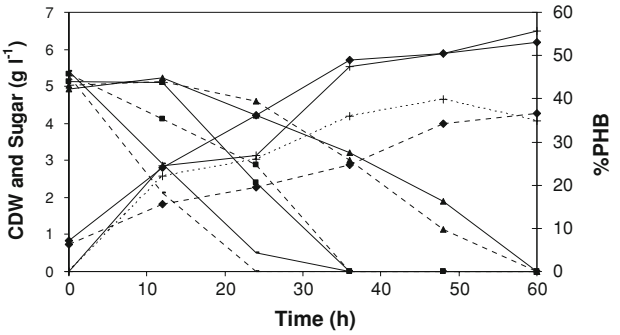

Fig. 2 Production of PHB by *B. sacchari* IPT101 (*solid line*) and *Bacillus* sp. MA 3.3 (*dashed line*) in sugar mixtures: (-) glucose, (■) arabinose, (▲) xylose, (◆) cell dry weight (CDW) and (+) PHB content of the dry cell weight (%PHB)

Identification of the strains

Sequences of the bacterial 16S rDNA were compared to the sequences deposited in the GenBank of the National Center of Biotechnology Information (NCBI) using the program BLASTN 2.2.14 (Altschul et al. 1997). Letters in the strain's name represent the sampling site: (MA) mangrove, (SE) brut sewage, (SL) activated sludge, (AT) Atlantic Rainforest, (AN) Antarctic and (AM) Amazon Rainforest.

Different groups of bacteria were identified and more than 75% belong to the genera *Bacillus* and *Burkholderia*. Isolates AN2.16, AN2.25, AN2.9, AN5.5 and AM4.15 showed the highest similarity to *Burkholderia cepacia* (97–99%), MA3.3, AM1.11, AM.2.16, AM6.16 to *Bacillus megaterium* (97–98%), and MA3.10 to *Bacillus cereus* (99%). *B. cepacia*, *B. megaterium*, and *B. cereus* are well known producers of PHA (Young et al. 1994; Gouda et al. 2001; Valappil et al. 2008).

MA11.15 and SE7.5 showed 96 and 94% similarity to *Klebsiella pneumoniae and Klebsiella oxytoca*, respectively. The production of PHA in different *Klebsiella* species has been described only in a recombinant strain harboring the operon *phaCAB* of *Ralstonia eutropha* (Zhang et al. 1994). Lastly, MA3.1 presented the greatest similarity to *Paenibacillus amylolyticus* (94%), even though it had also never been described as a bacterium that accumulates PHB. Interestingly, MA11.15, MA3.1, and SE7.5 presented a lower identity percentage in 16S rDNA analyses. Therefore, these isolates deserve further investigation concerning their taxonomic identity.

The phylogenetic analysis of the 16S rDNA sequence of isolate MA3.3 (Fig. 3) obtained by the neighbor-joining method showed ≥99% identity to the 16S rDNA of *Bacillus megaterium* indicating that these species are taxonomically closely related.

This result is corroborated by the microbiological properties of isolate MA3.3. The cells were straight, rod-shaped, and formed heat-resistant endospores. The isolate grew at temperatures ranging from 20 to 50°C, but not at 65°C. MA3.3 produces cytochrome oxidase and amylase, but it neither was able to produce acetoin from pyruvate in Voges–Proskauer tests nor produced acid and gas from glucose. This observation, together with the data obtained by 16S rRNA gene analyses, indicates that the isolate is probably a *Bacillus megaterium* and was named *Bacillus* sp. MA3.3.

Table 2 Production of PHB by *B. sacchari* IPT101 and *Bacillus* sp. MA3.3 in mineral medium containing glucose (Glu, 15 g l^{-1}), xylose (Xyl, 15 g l^{-1}), glucose and xylose (Glu + Xyl, 7.5 g l^{-1} each), and glucose, xylose and arabinose (Glu + Xyl + Ara, 5 g l^{-1} each) in shaken-flask experiments

Strain/Isolate	Carbon source	CDW (g l^{-1})	PHB (%)	Time (h)	$Y_{PHB/C}$ (g g^{-1})	P_{PHB} (g l^{-1} h^{-1})
IPT101	Glu	6.37	63.14	36	0.29	0.11
IPT101	Xyl	5.53	58.07	48	0.26	0.07
IPT101	Glu + Xyl	5.82	53.42	36	0.25	0.09
IPT101	Glu + Xyl + Ara	5.72	47.49	36	0.22	0.08
MA3.3	Glu	5.76	62.15	36	0.25	0.10
MA3.3	Xyl	5.54	64.36	60	0.24	0.06
MA3.3	Glu + Xyl	3.86	38.16	24	0.14	0.06
MA3.3	Glu + Xyl + Ara	3.99	39.89	48	0.11	0.03

Cell dry weight (CDW), PHB content of the cell dry weight (%PHB), PHB yield from carbon source ($Y_{PHB/C}$), and PHB volumetric productivity (P_{PHB}) are presented

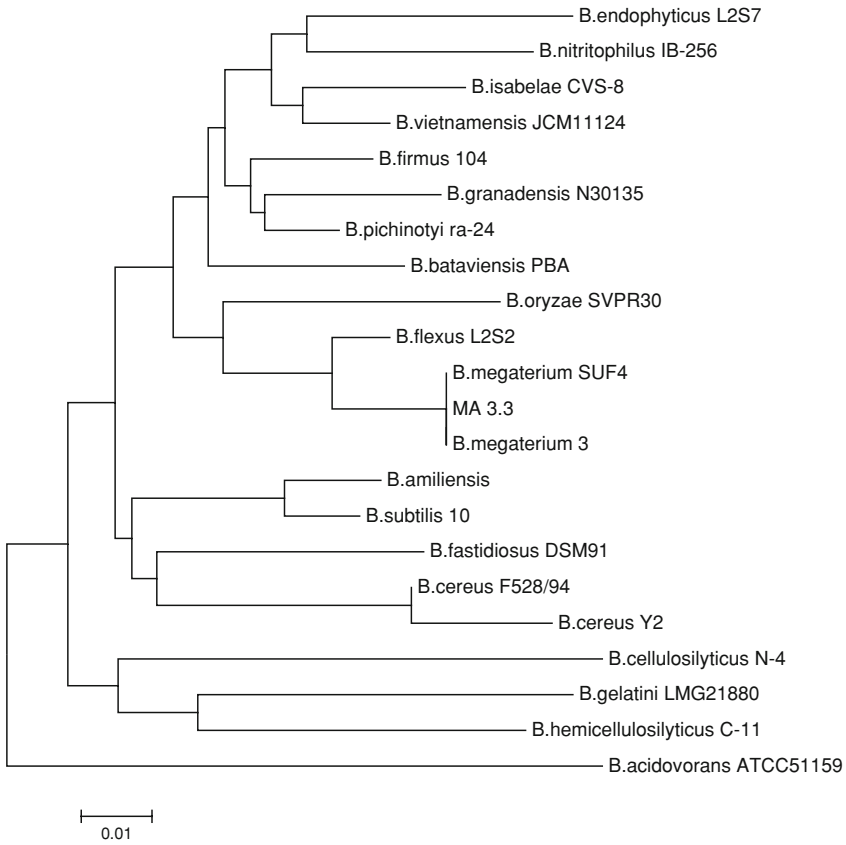

Fig. 3 Unrooted phylogram obtained by neighbor-joining of the 16S rDNA sequence from the *Bacillus* ssp. strains type obtained from the GenBank and MA3.3. The scale represents the number of changes in the nucleotide sequences

Conclusions

Gram-negative bacteria, such as *Escherichia coli* and *B. sacchari*, contain outer membrane lipopolysaccharide (LPS) endotoxins, which may contaminate PHAs during the extraction process and may induce immunogenic reactions undesirable in biomedical application (Lee et al. 1999; Chen and Wu 2005). For that reason, Gram-positive microorganisms, such as *Bacillus* sp. MA3.3, are more suitable to produce PHAs for medical applications. Also, the present findings of PHB production represent the best results when compared with other *Bacillus* ssp. in literature (Valappil et al. 2007). Therefore, the combination of cheaper raw materials, such as sugar mixtures originated from lignocellulosic feedstock, and the production of materials with more added values, such as medical materials, may provide more economically sustainable production of PHAs.

The carbon catabolite repression observed in the presented experiments also affects other biotechnological processes that use lignocellulosic hydrolysates. Therefore, the repression of this mechanism would increase the efficiency of industrial microbial processes (Gosset 2005). Consequently, the construction of *Bacillus* sp. MA3.3 with abolished carbon catabolite repression for PHB production in sugar mixtures is already in progress in our laboratory.

Acknowledgments The authors would like to thank to Dr. Vivian Pelizzari for providing Antarctic soil samples and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support.

References

Altschul SF, Madden TL, Schaffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389

Ausubel E, Brent R, Kingston RE et al (1992) Short protocols in molecular biology. Wiley, New York

Chen GQ, Wu Q (2005) The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials 26:6565–6578. doi:10.1016/j.biomaterials.2005.04.036

Gomez JGC, Rodrigues MFA, Alli RCP et al (1996) Evaluation of soil gram-negative bacteria yielding polyhydroxyalkanoic acids from carbohydrates and propionic acid. Appl Microbiol Biotechnol 45:785–791. doi:10.1007/s002530050763

Gosset G (2005) Improvement of *Escherichia coli* production strains by modification of the phosphoenolpyruvate:sugar phosphotransferase system. Microb Cell Fact 4(1):14. doi:10.1186/1475-2859-4-14

Gouda MK, Swellam AE, Omar SH (2001) Production of PHB by a *Bacillus megaterium* strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. Microbiol Res 156:201–207. doi:10.1078/0944-5013-00104

Jeffries TW, Jin YS (2004) Metabolic engineering for improved fermentation of pentose by yeasts. Appl Microbiol Biotechnol 63:495–509. doi:10.1007/s00253-003-1450-0

Keenan TM, Tanenbaum SW, Stipanovic AJ et al (2004) Production and characterization of poly-β-hydroxyalkanoate copolymers from *Burkholderia cepacia* utilizing xylose and levulinic acid. Biotechnol Prog 20:1697–1704. doi:10.1021/bp049873d

Keenan TM, Nakas JP, Tanenbaum SW (2006) Polyhydroxyalkanoate copolymers from forest biomass. J Ind Microbiol Biotechnol 33:616–626

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163. doi:10.1093/bib/5.2.150

Lee SY, Choi JI, Song JY (1999) Removal of endotoxin during purification of poly(3-Hydroxybutyrate) from Gram-negative bacteria. J Appl Microbiol 65:2762–2764

Nonato RV, Mantelato PE, Rossel CEV (2001) Integrated production of biodegradable plastic, sugar and ethanol. Appl Biotechnol 57:1–5. doi:10.1007/s002530100732

Pirt SJ (1975) Principles of microbe and cell cultivation. Blackwell Scientific Co, London (ISBN-0-63208150-3)

Piza FF, Prado PI, Manfio GP (2004) Investigation of bacterial diversity in Brazilian tropical estuarine sediments reveals high actinobacterial diversity. Antonie Van Leeuwenhoek 86:317–328. doi:10.1007/s10482-005-0162-0

Reddy CSK, Ghai R, Rashmi VC (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87:137–146. doi:10.1016/S0960-8524(02)00212-2

Rocha RCS, Silva LF, Taciro MK et al (2008) Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) P(3H-co-3HV) with a broad range of 3HV content at high yields by *Burkholderia sacchari* IPT 189. World J Microbiol Biotechnol 24:427–431. doi:10.1007/s11274-007-9480-x

Saha BC (2003) Hemicellulose bioconversion. Microbiol Biotechnol 30:279–291. doi:10.1007/s10295-003-0049-x

Schlegel HG, Lafferty R, Krauss I (1970) The isolation of mutants not accumulating poly-β-hydroxybutyric acid. Arch Mikrobiol 38:209–222. doi:10.1007/BF00422356

Silva LF, Gomez JG, Oliveira MS et al (2000) Propionic acid metabolism and poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate (P3HB-co-3HV) production by *Burkholderia* sp. J Biotechnol 76:165–174. doi:10.1016/S0168-1656(99)00184-4

Silva LF, Taciro MK, Michelin MER et al (2004) Poly-3-hydroxybutiyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolisate. Ind Microbiol Biotechnol 31:245–254

Slepecky RA, Hemphill HE (2006) The genus *Bacillus*—non-medical. In: Dworkin M (ed) The prokaryotes: a handbook on the biology of bacteria, vol 4, 3rd edn. Springer, New York, pp 462–530

Tao H, Gonzalez R, Martinez A et al (2001) Engineering a homoethanol pathway in *Escherichia coli*: increased glycolytic flux and levels of expression of glycolytic genes during xylose fermentation. J Bacteriol 183:2979–2988. doi:10.1128/JB.183.10.2979-2988.2001

Tokiwa Y, Ugwu CU (2007) Biotechnological production of (*R*)-3-hydroxybutyric acid monomer. J Biotechnol 132:264–272. doi:10.1016/j.jbiotec.2007.03.015

Valappil SP, Boccaccini AR, Bucke C et al (2007) Polyhydroxyalkanoates in Gram-positive bacteria: insights from the genera Bacillus and Streptomyces. Antonie Van Leeuwenhoek 91:1–17. doi:10.1007/s10482-006-9095-5

Valappil SP, Rai R, Bucke C et al (2008) Polyhydroxyalkanoate biosynthesis in *Bacillus cereus* SPV under varied limiting conditions and an insight into the biosynthetic genes involved. J Appl Microbiol 104:1624–1635. doi:10.1111/j.1365-2672.2007.03678.x

Young FK, Kastner JR, May SW (1994) Microbial production of poly-β-hydroxybutyric acid from D-xylose and lactose by *Pseudomonas cepacia*. Appl Environ Microbiol 60:4195–4198

Zhang H, Obias V, Gonyer K et al (1994) Production of polyhydroxyalkanoates in sucrose-utilizing recombinant *Escherichia coli* and *Klebsiella* strains. Appl Environ Microbiol 60:1198–1205