Chapter 9
Bacterial Synthesis of Polyhydroxyalkanoates Using Renewable Resources

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9.1 Introduction

Synthetic plastics have replaced natural materials and become an integral part of the everyday life. They are being extensively used because of their desirable physical and chemical properties such as low cost, durability, lightweight, ease in processing and high resistance to chemical and biological degradation. The very properties of plastics that have contributed immensely towards their popularity are causing grave environmental and societal concerns. Their xenobiotic and recalcitrant nature could be attributed to their large molecular size and the inability of nature's existing in-built mechanisms to degrade these novel unfamiliar pollutants (Reddy et al. 2003). New enzymes capable of degrading synthetic polymers are yet to be evolved since their presence in nature has increased enormously only during the recent years (Mueller 2006; Reddy et al. 2003). Increasing environmental and societal concerns have put pressure on the development of sustainable and eco-friendly plastic materials. Biodegradable polymers, especially polyhydroxyalkanoates (PHA) are ideal candidates to replace petroleum-based synthetic plastics. They are a class of biopolyesters synthesized and accumulated by a wide range of microorganisms as reserve food material. These polymers are produced in response to the limitation of any essential nutrient required for cell growth and the presence of a generous supply of carbon (Anderson and Dawes 1990). PHA are gaining tremendous importance as these are the only plastics produced exclusively by microorganisms and hence are completely degraded to benign compounds (Anderson and Dawes 1990; Yu 2007). They are nonpolluting as they do not need catalysts or additives to promote their degradation. The common microflora present in soil, water, compost or sewage is capable of degrading this polymer. These polymers completely degrade to carbon dioxide and water when disposed in aerobic environments (Lee 1996; Luzier 1992).

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S. Borkar (ed.), *Bioprospects of Coastal Eubacteria,*
DOI 10.1007/978-3-319-12910-5_9

In anaerobic conditions, the degradation of PHA can result in methane formation which can be trapped and resold as fuel (Budwill et al. 1992). Biocompatibility of PHA and its manufacture from inexpensive resources are some other key features which have generated global interest in this polymer (Shah et al. 2008).

The market price of commercially available PHA (approximately 10–12 €/kg) is comparatively higher than that of conventional synthetic plastics (Castilho et al. 2009). The high manufacturing costs involved during the production of these polymers is mainly responsible for hampering the commercialization of biodegradable plastics. The most important factors affecting the overall economics of PHA production are PHA productivity, PHA content and PHA yield. These are dependent on the bacterial strains employed, raw materials used for production, fermentation strategies and recovery processes. The widespread use of this polymer is restricted due to its high production cost and finds limited applications in the medical field.

9.2 Use of Efficient Bacterial PHA-Producing Strains

In PHA production process, the final quality and quantity of the product greatly depends on the strain, metabolic pathway involved, fermentation parameters, PHA synthesis phase (either stationary or throughout growth), carbon source as well as nutrient limitation conditions necessary for PHA production (Somashekara et al. 2009).

Selection of an ideal bacterial strain is very crucial for PHA production. There are several factors that determine the suitability of the organism for large-scale production of the polymer such as the cell's ability to utilize an inexpensive carbon source, growth rate, polymer synthesis rate and the maximum extent of polymer accumulation (Khanna and Srivastava 2005). The factors that directly affect the productivity include growth rate, polymer synthesis rate and the maximum extent of polymer accumulation which should be as high as possible. Further, the cost of PHA can be significantly reduced using cheap carbon sources. In addition, the microorganisms that produce extracellular polysaccharides (EPS) besides PHA should be avoided since the cells not only use up the carbon source for their synthesis but they also make the recovery process inefficient (Lee and Chang 1995). Fermentation strategies targeting high productivity of PHA are absolutely essential in reducing the fermentation and purification costs of the process. Since PHA is synthesized and accumulated under unfavourable growth conditions, cultivation strategies that stimulate these conditions and allow efficient production of PHA should be employed (Lee and Chang 1995). Higher PHA content in the cells will allow more fermentation runs to be carried out for the given total annual operating time, thereby resulting in the reduction of the fermenter size and fixed cost (Choi and Lee 1997). Equipment-related costs are also reduced with increasing PHA content as smaller amount of cells needs to be produced to obtain the same amount of PHA.

High PHA content also reduces the amount of carbon substrate needed to produce PHA and hence the feedstock will not be wasted on other cellular materials

and/or metabolites. This will help in lowering the cost of the carbon substrate, a major contributor to the overall total raw material cost (Kim 2000). Higher productivity has a profound influence on the purity and yield of the PHA produced. High PHA yield not only reduces the recovery costs by allowing the processing of less amount of non-PHA cellular material to obtain the same amount of PHA, but also reduces the costs associated with waste disposal (Choi and Lee 1999).

PHA synthesis has been described in a large number of bacteria, but only a few bacteria have been employed for the production of PHA at an industrial scale. Based on the culture conditions required for PHA synthesis, these bacteria can be divided into two groups. The first group which includes bacteria such as *Cupriavidus necator* (formerly known as *Ralstonia eutropha*), *Pseudomonas oleovorans* and several methylotrophs require unbalanced growth conditions for PHA synthesis. The presence of excess carbon with the simultaneous limitation of an essential nutrient triggers the synthesis and accumulation of the polymer. PHA production is growth-associated in bacteria belonging to the second group (e.g., *Alcaligenes latus*, a mutant of *Azotobacter vinelandii* and recombinant *Escherichia coli*) and does not depend on nutrient limitation (Lee 1996; Khanna and Srivastava 2005). These characteristics have to be taken into consideration during strain selection for PHA production (Khanna and Srivastava 2005). In addition, the cyclic nature of PHA metabolism, synthesis and degradation also needs to be considered since prolonged metabolism inside the cells can result in PHA degradation by depolymerases (Jendrossek 2001).

9.2.1 The Genus Bacillus and PHA Production

Currently, Gram-negative microorganisms such as *C. necator*, *A. latus* and recombinant *E. coli* are used for commercial polymer production (Valappil et al. 2007a). The lipopolysaccharide (LPS) tainted polymers extracted from these bacteria require additional purification procedures before they can be safely used in mammalian systems, thereby increasing production costs (Chen and Wu 2005).

Studies have shown the genus *Bacillus* to be one of the most versatile PHA producers since it can accumulate PHA from a variety of substrates (Table 9.1). These Gram-positive bacteria have an added advantage as potential candidates for industrial scale PHA production due to the lack of LPS layer. Members of this genus are also known to grow rapidly, possess various hydrolytic enzymes and produce copolymers from structurally unrelated carbon sources (Halami 2008; Valappil et al. 2007b). These very characteristics of *Bacillus* spp. can be exploited for the production of PHA with desirable material properties from various low-cost agricultural feedstocks.

Table 9.1 Studies on PHA production carried out using various *Bacillus* spp. and low-cost feedstocks

Organism	Carbon source	PHA content (%)	Reference
Bacillus cereus M5	Beet molasses	73.8	Yilmaz and Beyatli (2005)
B. cereus (5 strains)	Pea-shell waste	47.0–72.0	Kumar et al. (2009)
Bacillus megaterium ATCC 6748	Molasses	35.0	Chaijamrus and Udpuay (2008)
B. megaterium BA-019	Molasses	55.5	Kulpreecha et al. (2009)
B. megaterium	Dairy waste + sea water	11.3	Pandian et al. (2010)
Bacillus sphaericus	Cornflour	3.3	Ramadas et al. (2009)
	Wheat bran	6.8	
	Cassava bagasse	6.4	
	Jackfriut seed powder	46.0	
	Potato starch	47.0	
B. sphaericus	Sesame oil cake	14.6	Ramadas et al. (2009)
	Groundnut oil cake	18.7	
Bacillus thuringiensis R1	Molasses	23.1	Rohini et al. (2006)
	Table sugar	28.2	
B. thuringiensis EGU45	Pea-shell waste	55.0	Kumar et al. (2009)
Bacillus sp. JMa5	Molasses	35.0	Wu et al. (2001)
Bacillus sp.	Soytone	25.4	Full et al. (2006)
Bacillus sp. 256	Mahua flower extract	51.0	Anil Kumar et al. (2007)
Bacillus sp. COL1/A6	Hydrolysed coconut oil cake	41.92	Santimano et al. (2009a)
Bacillus sp. COL1/A6	Hydrolysed wafer residue	62.4	Santimano et al. (2009b)
	Cane molasses	54.7	
	Hydrolysed citrus pulp	47.5	

9.3 PHA-Producing *Bacillus* Strains Isolated from Marine and Coastal Ecosystems of Goa

Sixteen bacterial strains belonging to the genus *Bacillus* previously isolated and identified in the laboratory were used for the study (Prabhu 2010). These strains were isolated from sediment samples collected from diverse econiches in and around Goa. Preliminary investigations revealed the isolates to be potential candidates for PHA production. Kumar et al. (2009) have also observed that among the different bacteria belonging to various genera, *Bacillus* species were found to possess high PHA-producing ability.

Submerged cultivation of the different *Bacillus* species used in the present study revealed that these isolates grew rapidly on glucose as sole carbon source (Fig. 9.1). The

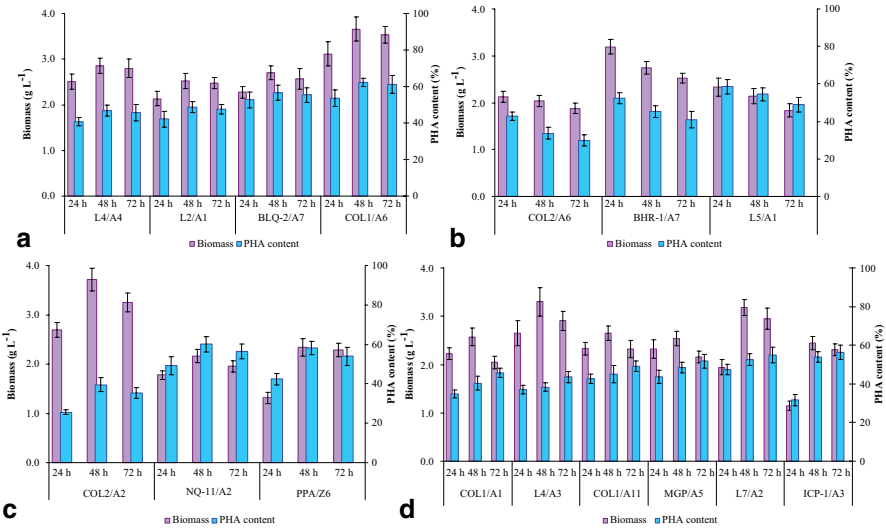

Fig. 9.1 Temporal variation in PHA-accumulating ability among different *Bacillus* species using glucose as sole carbon source. **(a)** Consistent PHA yield after maximum accumulation, **(b)** maximum PHA yield at 24 h, **(c)** maximum PHA yield at 48 h, **(d)** maximum PHA yield at 72 h

PHA content accumulated by these isolates, which is calculated as the ratio between the polymer extracted and the cell biomass, both in terms of dry cell weight (DCW) ranged between 39.6% (COL2/A2) to 62.3% (COL1/A6). Generally, members of the genus *Bacillus* are known to produce PHA content ranging from 6.53 to 48.2% (Aslim et al. 2002; Chen et al. 1991; Shamala et al. 2003). Recently, few researchers have reported higher amounts of accumulated PHA in different *Bacillus* spp. such as *Bacillus megaterium* NQ-11/A2 (61% DCW), *Bacillus thuringiensis* R1 (64.1% DCW), *Bacillus mycoides* RLJ B-017 (69.4% DCW) and *Bacillus* sp. CL1 (90% DCW) (Borah et al. 2002; Full et al. 2006; Prabhu et al. 2009, 2010; Rohini et al. 2006).

On comparing the PHA yield produced for three consecutive days of incubation, it was observed that the time of incubation influenced PHA production. It was interesting to note that these *Bacillus* species could be categorized into four groups based on the PHA content produced.

Most of the isolates showed a reduction in the accumulated PHA content on prolonged incubation whereas only about one third of the isolates were capable of consistently maintaining the maximally accumulated PHA till the termination of the experiment which included isolates BLQ-2/A7, L2/A1, L4/A4 and COL1/A6 (Fig. 9.1a). With a further incubation till 72 h, the intracellular PHA content accumulated by these strains was found to be stable, indicating that the PHA depolymerase enzyme of these strains remains inactive over a longer period of time. *Bacillus cereus* SPV is also `reported to exhibit similar pattern for PHA production (Philip et al. 2009; Valappil et al. 2007c).

The isolates belonging to the second group, namely, BHR-1/A7, L5/A1 and COL2/A6 produced maximum PHA at 24 h of incubation (Fig. 9.1b). Further

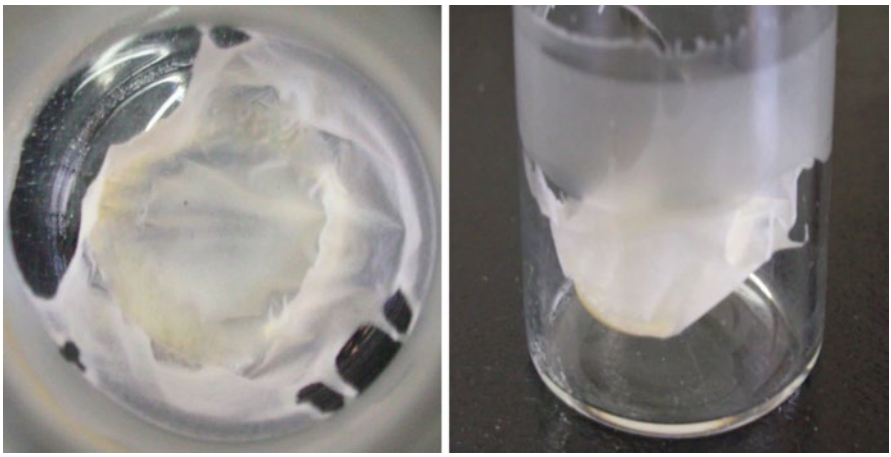

Fig. 9.2 PHA polymer extracted from isolate COL1/A6 after 72 h of growth

increase in the incubation time reduced the PHA content in the cells suggesting that the accumulated PHA was possibly utilized for growth or spore formation as observed by Wu et al. (2001). A slightly different trend was observed with isolate NQ-11/A2, PPA/Z6 and COL2/A2 (belonging to the third group) which exhibited maximum PHA content at 48 h and further incubation resulted in a decrease in the accumulated PHA content (Fig. 9.1c). These findings were consistent with that reported by Shamala et al. (2003). They observed maximal production of PHA at 24 h (or 48 h in case of *B. megaterium*) and the amount of accumulated PHA decreased on further incubation in fermentations employing various *Bacillus* species. The amount of PHA accumulated in six isolates belonging to the fourth group namely, ICP-1/A3, L4/A3, L7/A2, MGP/A5, COL1/A1 and COL1/A11 increased with increase in the incubation time (Fig. 9.1d). Halami (2008) has also reported a similar observation using *B. cereus* CFR06 wherein the PHA content increased on prolonged incubation of 72 h. This trend could be attributed to the decline in the cell biomass consequently resulting in increased PHA content. The polymer extracted from isolate COL1/A6 after 72 h of growth is shown in Fig. 9.2.

9.4 Use of Alternative Substrates for Microbial PHA Production

In the manufacturing process, the raw material cost, especially the carbon source greatly influences the overall cost of the final product. Synthesis of PHA using expensive carbon sources such as glucose renders the process economically nonviable. Therefore, for economical PHA production inexpensive substrates that can be used as carbon sources for bacterial strains to synthesize large quantities of intracellular PHA are necessary. By-products such as molasses, straw, bagasse generated in the agricultural sector are available abundantly and are generally used as cattle feed

since they have little economic value. Their use as a carbon feedstock for PHA production can contribute to as much as 40–50 % reduction in the overall manufacturing cost (Kim 2000). These agricultural residues are rich in carbohydrates and the use of such materials for the synthesis of value-added products can be advantageous and also contribute significantly to the reduction of their disposal costs (Thomsen 2005; Castilho et al. 2009).

Members of the genus *Bacillus* are ubiquitous in nature and possess innate ability to produce various hydrolytic enzymes that metabolize complex residues present in the surrounding environment. Therefore, such native *Bacillus* strains are now being explored industrially for economic PHA production from complex residues, e.g. agroindustrial by-products (Gouda et al. 2001; Kumar et al. 2009; Kulpreecha et al. 2009; Santimano et al. 2009a, b).

After the preliminary evaluation of various inexpensive and easily available agroindustrial residues, the polymer-producing ability of the selected *Bacillus* isolates on these carbon substrates was quantified by cultivation under submerged conditions. These isolates were specifically selected based on the maximum intensity of fluorescence exhibited by them on the respective substrates (Kitamura and Doi 1994).

9.4.1 PHA Production Using Submerged Cultivation

A. Molasses Molasses served as an excellent source for growth as well as polymer production. The growth of the isolates was highest on molasses (3.58–6.23 g L^{-1}) as compared with any other substrate. A PHA content as high as 68.56 % DCW was achieved with ICP-1/A3 (Fig. 9.3). All the isolates grown under these conditions accumulated more than 50 % DCW as PHA.

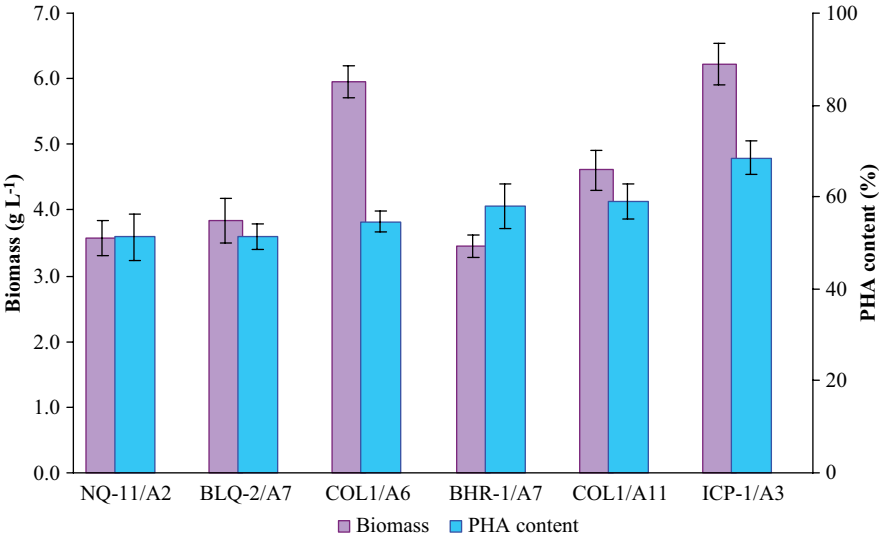

Fig. 9.3 Production of PHA in various *Bacillus* species using sugarcane molasses

The enhanced growth and PHA production of the isolates can be attributed to the additional nutrients such as vitamins and minerals found in molasses which function as growth factors (Kulpreecha et al. 2009; Oliveira et al. 2004). The PHA content accumulated by *B. megateruim* BA-019 improved significantly when molasses rather than sucrose were used as a carbon source. This isolate was able to accumulate 55.46% DCW as PHA when cultivated on molasses and urea (Kulpreecha et al. 2009). Gouda et al. (2001) reported maximum PHA production using *B. megaterium* with cane molasses and glucose as sole carbon sources (40.8 and 39.9% DCW, respectively). These authors demonstrated that higher molasses concentration (3% w/v) resulted in increased growth whereas 2% molasses yielded maximum PHA content. *B. thuringensis* R1 cells were found to accumulate 22.95 and 31.36% DCW as PHA in the presence of molasses and table sugar, respectively (Rohini et al. 2006). Wu et al. (2001) demonstrated that under fed-batch conditions, *Bacillus* sp. JMa5 could accumulate 25–35% PHA during fermentation using molasses as a sole carbon source.

B. Starch-Based Residue Even though the isolates were capable of hydrolysing starch with the enzyme amylase, acid hydrolysis of the wafer residue could not be avoided since the insoluble starch particles interfered during downstream processing.

The resulting hydrolysate of wafer residue was hence used as a carbon source for PHA production. The majority of the isolates exhibited bright fluorescence indicating excellent PHA-producing ability of these isolates on wafer hydrolysate. Isolates able to produce PHA on all the 3 days were further selected for submerged cultivation. Luxuriant growth of the isolates was observed with isolate L7/A2 producing maximum biomass of 5.24 g L^{-1} (Fig. 9.4). The PHA content accumulated in the various isolates ranged between 56.4 and 62.4% DCW.

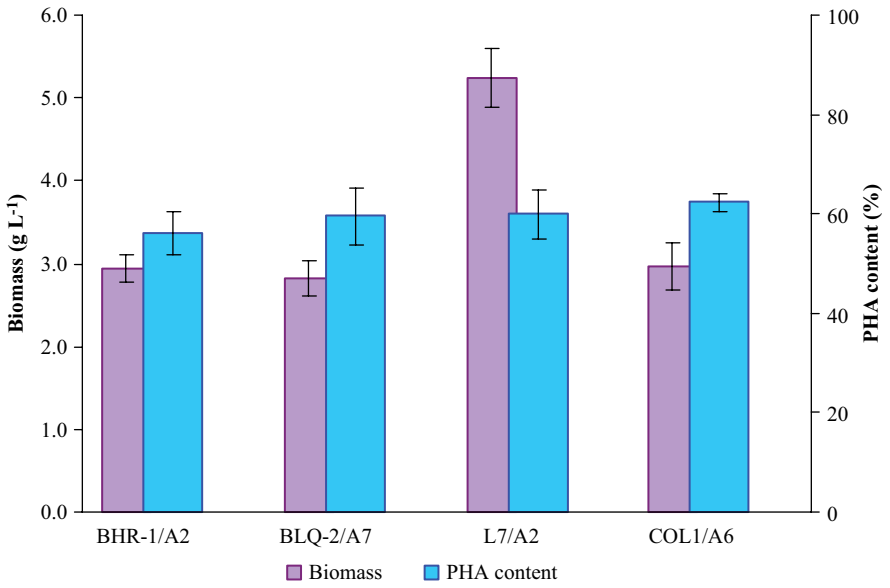

Fig. 9.4 Production of PHA in various *Bacillus* species using wafer residue hydrolysate

Use of soluble starch without hydrolysis has been reported by Halami (2008) and Kim (2000). *B cereus* strain described by Halami (2008) was able to accumulate a PHA content of 48% DCW using starch-based medium, whereas 46% DCW was obtained using *Azotobacter chroococcum* (Kim 2000). Lillo and Rodriguez-Valera (1990) have reported soluble starch as the ideal carbon substrate for *Haloferax mediterranei* growth and polymer synthesis. Koutinas et al. (2007) have demonstrated the use of wheat hydrolysate and fungal extract (as carbon and nitrogen source, respectively) for PHA production in *C. necator*. Under these conditions, a PHA content of 70% DCW was achieved. Production of PHA from inexpensive extruded rice bran (ERB) and extruded corn starch (ECS) employing *H. mediterranei* was investigated by Huang et al. (2006). Repeated fed-batch fermentation with ERB resulted in a PHA content of 38.7% DCW and on using a mixture of ERB and ECS resulted in a PHA content of 55.6% DCW. However, *B. sphaericus* NCIM 5149 grown on hydrolysates of cornflour and wheat bran was able to produce only 3.3 and 6.8% DCW of PHA content, respectively. It was observed that wheat bran hydrolysate favored cell growth rather than PHA synthesis (Ramadas et al. 2009). Using waste potato starch hydrolysate as the chief carbon source, Rusendi and Sheppard (1995) have reported PHA production with a yield of 77% DCW employing *R. eutropha*. *Halomonas boliviensis* LC1 attained a PHA content of 34% DCW when grown on wheat bran hydrolysate (Van-Thuoc et al. 2008).

C. Citrus Fruit Waste Exploiting the ability of the various *Bacillus* species to grow and produce PHA from citrus pulp waste resulted in a PHA content ranging from 38.87% DCW (BLQ-2/A7) to 48.86% DCW (NQ-11/A2) (Fig. 9.5). Citrus pulp waste promoted biomass production with maximal growth observed in case of isolate BLQ-2/A7 (4.5 g L^{-1}) followed by COL1/A6 (4.13 g L^{-1}).

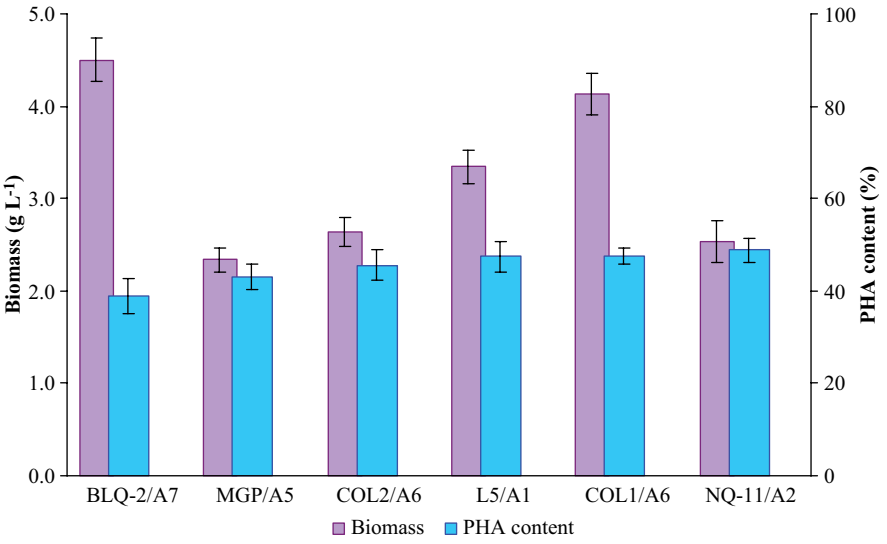

Fig. 9.5 Production of PHA in various *Bacillus* species using citrus pulp waste hydrolysate

Till date, no studies have been published reporting the use of citrus pulp waste as a carbon source. However, a number of studies are conducted using organic matter from wastes as an alternative to produce the polymer from inexpensive sources. These studies also highlight the necessity of incorporating a hydrolysis step prior to inoculation (Rebah et al. 2009). Polymer production from mahua flower extract employing fermentations with *Bacillus* sp. 256 resulted in a PHA content of 51 % DCW (Anil Kumar et al. 2007). Kumar et al. (2009) have evaluated PHA production from pea-shell waste with the help of different *Bacillus* strains. They have reported higher yields (22–65 % DCW) with the enzyme-hydrolysed substrate as compared to the unhydrolysed waste.

D. Coconut Oil Cake Preliminary evaluation of coconut oil cake, an agroindustrial residue suggested it to be a potential carbon source for PHA production. Further, studies involving submerged fermentation were also carried out. Isolates exhibiting good fluorescence intensity on staining with Nile blue A were selected for these studies. Among the five isolates tested, isolate COL1/A6 exhibited maximal biomass and PHA of 3.75 and 1.58 g L^{-1}, respectively (Fig. 9.6) whereas the highest PHA content was observed with isolate COL1/A11 (42.4 % DCW). The removal of adherent fatty acids from the coconut oil cake hydrolysate was achieved by a brief hexane wash thereby facilitating the quantification process (Lee et al. 2000; Santimano et al. 2009a).

Recently, a few studies have been conducted using oil cakes such as sesame, groundnut, mustard, palm under submerged fermentation and babassu, soy cake using solid-state fermentation (Ramadas et al. 2009; Singh and Mallick 2009; Oliveira

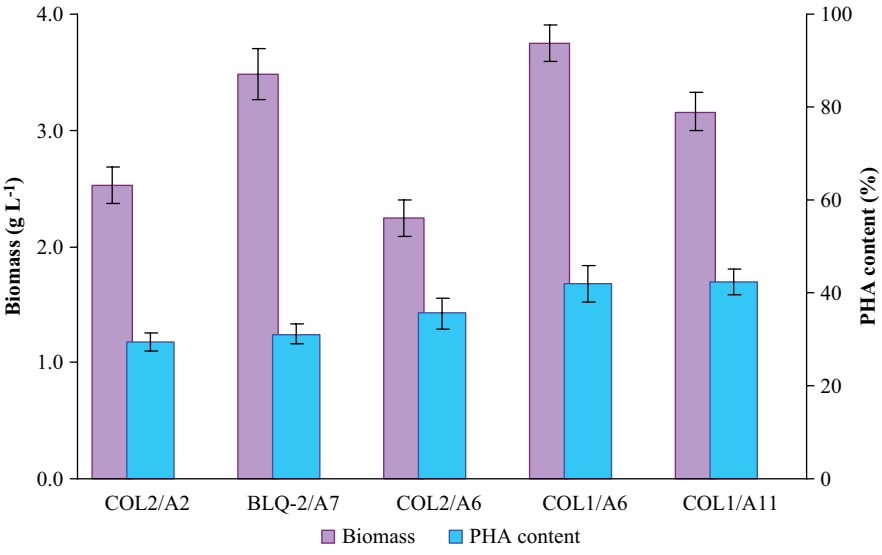

Fig. 9.6 Production of PHA by various *Bacillus* species using coconut oil cake hydrolysate

et al. 2004). The PHA content accumulated using these oil cakes varied from 14.0 to 39.2 % DCW. As seen from these studies, species belonging to the genus *Bacillus* are able to utilize oil cakes for growth better than Gram-negative organisms.

E. Polymeric Substrates Bacterial strains belonging to the genus *Bacillus* were evaluated for their PHA-producing ability using the simplest and most easily metabolizable monomeric sugar, glucose. Since these isolates exhibited excellent PHA production on glucose, polymers of glucose were then tested as sole carbon substrates. Among these, starch proved to be an excellent source for PHA production. All the *Bacillus* isolates (except isolates PPA/Z6 and COL1/A1) were able to synthesize PHA on the majority of the polymers tested. All the 16 isolates were unable to utilize bagasse and rice chaff as raw material for polymer production.

Quantitation of PHA using submerged cultivation of selected isolates was achieved using hydrolysate of wafer residue. Hydrolysis of these by-products using dilute acid not only improved the ability of the isolates to assimilate the released fermentable sugars as PHA, but also avoided the interference caused by the insolubles present in the wastes during downstream processing when grown under submerged cultivation conditions. Under these conditions, high PHA accumulation ranging from 56.14 to 62.41 % DCW was obtained.

9.5 Conclusions and Future Prospects

The present study deals with PHA production by selected *Bacillus* spp. and indicates their potential as PHA producers. These *Bacillus* spp. isolated from diverse marine and coastal econiches, exhibited temporal variation in their PHA accumulation pattern. Based on the PHA content accumulated within the cells, the isolates could be categorized into four groups. Isolates (BLQ-2/A7, COL1/A6, L2/A1 and L4/A4) belonging to one of these groups were capable of consistently maintaining the accumulated PHA without significant intracellular degradation during the entire experimentation time. This property is crucial in large-scale PHA production as variation in the time of harvest will not adversely affect the product yield.

Ability of the different *Bacillus* isolates to utilize diverse agroindustrial by-products revealed substrates such as molasses, wafer residue, citrus pulp and coconut oil cake to be potential carbon feedstock for PHA production. The majority of the isolates were capable of producing PHA using these substrates. Using molasses as the carbon source, all the isolates tested were able to accumulate PHA ranging between 51.23 % and 68.56 % DCW. Wafer residue was also efficiently utilized by the isolates as more than 55 % DCW PHA was produced within the cells. Citrus pulp was able to support PHA content ranging between 38.87 % and 48.86 % DCW in the selected *Bacillus* spp. Coconut oil cake also served as a potential carbon source for PHA production with a highest PHA content of 42.4 % DCW.

These studies emphasize the potential of *Bacillus* spp. to produce value-added product (PHA polymer) from diverse renewable low-cost feedstocks at substantially reduced production costs. The isolates were able to unequivocally produce PHA ranging from 29.45% DCW (COL2/A2 on coconut oil cake hydrolysate) to 68.56% DCW (ICP-1/A3 on molasses) using low-cost agroindustrial residues. Further characterization of the produced polymer is necessary as this genus is known to produce PHA with different monomer compositions from a wide variety of substrates (Valappil et al. 2007a). Based on these investigations further studies may be directed towards:

- PHA production and high-cell density fermentation with intensive studies on fed-batch mode of operation involving pH, dissolved oxygen (DO)-regulated system and glucose supplementation
- The use of solid-state fermentation (SSF) for the production of PHA

The high-cell density is vital for the economic viability of the production process while SSF is an attractive strategy that allows the use of inexpensive feedstock such as agroindustrial residues. The advantage of employing such fermentation strategies is that, they provide solutions to the disposal of agro-based residues with simultaneous production of value-added products. These residues can be directly incorporated in the fermentation media without any pretreatment unlike submerged fermentation (SMF). The fermented solids containing PHA products can be used directly without downstream processing to prepare composite materials of increased biodegradability.

References

Anderson, A. J., & Dawes, E. A. (1990). Occurrence, metabolism, metabolic role and Industrial uses of bacterial polyhydroxyalkanoates. *Microbiological Reviews, 54,* 450–472.

Anil Kumar, P. K., Shamala, T. R., Kshama, L., Prakash, M. H., Joshi, G. J., Chandrashekar, A., Latha Kumari, K. S., & Divyashree, M. S. (2007). Bacterial synthesis of poly(hydroxybutyrate-co-hydroxyvalerate) using carbohydrate-rich mahua (*Madhuca* sp.) flowers. *Journal of Applied Microbiology, 103,* 204–209.

Aslim, B., Yüksekdağ, Z. N., & Beyatli, Y. (2002). Determination of PHB growth quantities of certain *Bacillus* species isolated from soil. *Turkish Electronic Journal of Biotechnology Special Issue,* 24–30.

Borah, B., Thakur, P. S., & Nigam, J. N. (2002). The influence of nutritional and environmental conditions on the accumulation of poly-β-hydroxybutyrate in *Bacillus mycoides* RLJ B-017. *Journal of Applied Microbiology, 92,* 776–783.

Budwill, K., Fedorak, P. M., & Page, W. J. (1992). Methanogenic degradation of poly(3-hydroxyalkanoates). *Applied Environmental Microbiology, 58,* 1398–1401.

Castilho, L. R., Mitchell, D. A., & Freire, D. M. G. (2009). Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. *Bioresource Technology, 100,* 5996–6009.

Chaijamrus, S., & Udpuay, N. (2008). Production and Characterization of Polyhydroxybutyrate from Molasses and Corn Steep Liquor produced by *Bacillus megaterium* ATCC 6748. Agricultural Engineering International: the CIGR Ejournal. Vol. X. Manuscript FP 07 030.

Chen, G.-Q., & Wu, Q. (2005). The application of polyhydroxyalkanoates as tissue engineering materials. *Biomaterials, 26,* 6565–6578.

Chen, G.-Q., König, K.-H., & Lafferty, R. M. (1991). Occurence of poly-D(-)-3-hydroxyalkanoates in the genus *Bacillus*. *FEMS Microbiology Letters, 84,* 173–176.

Choi, J., & Lee, S. Y. (1997). Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Engineering, 17,* 335–342.

Choi, J., & Lee, S. Y. (1999). Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. *Applied Microbiology and Biotechnology, 51,* 13–21.

Full, T. D., Jung, D. O., & Madigan, M. T. (2006). Production of poly-β-hydroxyalkanoates from soy molasses oligosaccharides by new, rapidly growing *Bacillus* species. *Letters in Applied Microbiology, 43,* 377–384.

Gouda, M. K., Swellan, A. E., & Omar, S. H. (2001). Production of PHB by a *Bacillus megaterium* strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. *Microbiological Research, 156,* 201–207.

Halami, P. (2008). Production of polyhydroxyalkanoate from starch by the native isolate *Bacillus cereus* CFR06. *World Journal of Microbiology and Biotechnology, 24,* 805–812.

Huang, T.-Y., Duan, K.-J., Huang, S.-Y., & Chen, C. W. (2006). Production of polyhydroxyalkanaotes from inexpensive extruded rice bran and starch by *Haloferax mediterranei*. *Journal of Industrial Microbiology and Biotechnology, 33,* 701–706.

Jendrossek, D. (2001). Microbial degradation of biopolyesters. *Advances in Biochemical Engineering/Biotechnology, 71,* 294–325.

Khanna, S., & Srivastava, A. K. (2005). Recent advances in microbial polyhydroxyalkanoates. *Process Biochemistry, 40,* 607–619.

Kim, B. S. (2000). Production of poly(3-hydroxybutyrate) from inexpensive substrates. *Enzyme Microbial Technology, 27,* 774–777.

Kitamura, S., & Doi, Y. (1994). Staining method of poly(3-hydroxyalkanoic acid) producing bacteria by Nile Blue. *Biotechnology Techniques, 8,* 345–350.

Koutinas, A. A., Wang, Y. X., & Webb, C. (2007). Polyhydroxybutyrate production from a novel feedstock derived from a wheat-based biorefinery. *Enzyme Microbial Technology, 40,* 1035–1044.

Kulpreecha, S., Boonruangthavorn, A., Meksiriporn, B., & Thongchul, N. (2009). Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of *Bacillus megaterium*. *Journal of Bioscience and Bioengineering, 107,* 240–245.

Kumar, T., Singh, M., Purohit, H. J., & Kalia, V. C. (2009). Potential of *Bacillus* sp. to produce polyhydroxybutyrate from biowaste. *Journal of Applied Microbiology, 106,* 2017–2023.

Lee, S. Y. (1996). Bacterial polyhydroxyalkanoates. *Biotechnology and Bioengineering, 49,* 1–14.

Lee, S. Y., & Chang, H. N. (1995). Production of poly(hydroxyalkanoic acid). *Advances in Biochemical Engineering, 52,* 28–58.

Lee, S. Y., Wong, H. H., Choi, J., Lee, S. H., Lee, S. C., & Han, C. S. (2000). Production of medium-chain-length polyhydroxyalkanoates by high-cell-density cultivation of *Pseudomonas putida* under phosphorus limitation. *Biotechnology and Bioengineering, 68,* 466–470.

Lillo, J. G., & Rodriguez-Valera, F. (1990). Effects of culture conditions on poly(β-hydroxybutyric acid) production by *Haloferax mediterranei*. *Applied Environmental Microbiology, 56,* 2517–2521.

Luzier, W. D. (1992). Materials derived from biomass/biodegradable materials. *Proceedings of the National Academy of Science USA, 89,* 839–842.

Mueller, R. J. (2006). Biological degradation of synthetic polyesters-enzymes as potential catalysts for polyester recycling. *Process Biochemistry, 41,* 2124–2128.

Oliveira, F. C., Freire, D. M. G., & Castilho, L. R. (2004). Production of poly(3-hydroxybutyrate) by solid-state fermentation with *Ralstonia eutropha*. *Biotechnology Letters, 26,* 1851–1855.

Pandian, S. R., Deepak, V., Kalishwaralal, K., Rameshkumar, N., Jeyaraj, M., & Gurunathan, S. (2010). Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by *Bacillus megaterium* SRKP-3. *Bioresource Technology, 101,* 705–711.

Philip, S., Sengupta, S., Keshavarz, T., & Roy, I. (2009). Effect of impeller speed and pH on the production of Poly(3-hydroxybutyrate) using *Bacillus cereus* SPV. *Biomacromolecules, 10,* 691–699.

Prabhu, N. N. (2010). Polyhydroxyalkankaote (PHA) synthases in selected *Bacillus* spp. PhD dissertation, Goa University

Prabhu, N. N., Santimano, M. C., & Garg, S. (2009). Studies on polyhydroxyalkanote production by a marine *Bacillus* sp. NQ-11/A2 isolated from continental shelf sediment. *Journal of Current Science, 14*(1), 265–273.

Prabhu, N. N., Santimano, M. C., Bhosle, S. N., Mavinkurve, S., & Garg, S. (2010). Native granule associated SCL-PHA synthase from a marine derived *Bacillu*s sp. NQ-11/A2. *Antonie van Leeuwenhoek, 97,* 41–50.

Ramadas, N. V., Singh, S. K., Soccol, C. R., & Pandey, A. (2009). Polyhydtoxybutyrate production using agro-industrial residue as substrate by *Bacillus sphaericus* NCIM 5149. *Brazilian Archives of Biology and Technology, 52,* 17–23.

Rebah, F. B., Prévost, D., Tyagi, R. D., & Belbahri, L. (2009). Poly-beta-hydroxybutyrate production by fast-growing Rhizobia cultivated in sludge and in industrial wastewater. *Applied Biochemistry and Biotechnology, 158,* 155–163.

Reddy, C. S. K., Ghai, R., Rashmi, Kalia, V. C. (2003). Polyhydroxyalkanoates: An overview. *Bioresource Technology, 87,* 137–146.

Rohini, D., Phadnis, S., & Rawal, S. K. (2006). Synthesis and characterization of poly-β-hydroxybutyrate from *Bacillus thuriengiensis* R1. *Indian Journal of Biotechnology, 5,* 276–283.

Rusendi, D., & Sheppard, J. D. (1995). Hydrolysis of potato processing waste for the production of poly-β-hydroxybutyrate. *Bioresource Technology, 54,* 191–196.

Santimano, M. C., Prabhu, N. N., & Garg, S. (2009a). A simple method for PHA production by *Bacillus* sp. COL1/A6 utilizing waste coconut oil cake. *Journal of Current Science, 14,* 274–282.

Santimano, M. C., Prabhu, N. N., & Garg, S. (2009b). PHA production using low-cost agronomic wastes by *Bacillus* sp. strain COL1/A6. *Research Journal of Microbiology, 4,* 89–96.

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances, 26,* 246–265.

Shamala, T. R., Chandrashekar, A., Vijayendra, S. V. N., & Kshama, L. (2003). Identification of polyhydroxyalkanoate (PHA)-producing *Bacillus* spp. using the polymerase chain reaction (PCR). *Journal of Applied Microbiology, 94,* 369–374.

Singh, A. K., & Mallick, N. (2009). Exploitation of inexpensive substrates for production of a novel SCL-LCL-PHA co-polymer by *Pseudomonas aeruginosa* MTCC 7925. *Journal of Industrial Microbiology and Biotechnology, 36,* 347–354.

Somashekara, D. M., Rastogi, N. K., & Ramachandriah, S. T. (2009). A simple kinetic model for growth and biosynthesis of polyhydroxylaknaote in *Bacillus flexus*. *New Biotechnology, 26,* 92–98.

Thomsen, M. H. (2005). Complex media from processing of agricultural crops for microbial fermentation. *Applied Microbiology and Biotechnology, 68,* 598–606.

Valappil, S. P., Peiris, D., Langley, G. J., Hernimann, J. M., Boccaccini, A. R., Bucke, C., & Roy, I. (2007a). Polyhydroxyalkanoate (PHA) biosynthesis from structurally unrelated carbon sources by a newly characterized *Bacillus* spp. *Journal of Biotechnology, 127,* 475–487.

Valappil, S. P., Boccaccini, A. R., Bucke, C., & Roy, I. (2007b). Polyhydroxyalkanoates in Gram-positive bacteria: Insights from the genera *Bacillus* and *Streptomyces*. *Antonie van Leeuwenhoek, 91,* 1–17.

Valappil, S. P., Misra, S. K., Boccaccini, A. R., Keshavarz, T., Bucke, C., & Roy, I. (2007c). Large-scale production and efficient recovery of PHB with desirable material properties, from the newly characterized *Bacillus cereus* SPV. *Journal of Biotechnology, 132,* 251–258.

Van-Thuoc, D., Quillaguaman, J., Mamo, G., & Mattiason, B. (2008). Utilization of agricultural residues for poly(3-hydroxybutyrate) production by *Halomonas boliviensis* LC1. *Journal of Applied Microbiology, 104,* 420–428.

Wu, Q., Huang, H., Hu, G., Chen, J., Ho, K. P., & Chen, G.-Q. (2001). Production of poly-3-hydroxybutyrate by *Bacillus* sp. JMa5 cultivated in molasses media. *Antonie van Leeuwenhoek, 80,* 111–118.

Yilmaz, M., & Beyatli, Y. (2005). Poly-β-hydroxybutyrate (PHB) production by a *Bacillus cereus* M5 strain in sugarbeet molasses. *Zuckerindustries, 130,* 109–112.

Yu, J. (2007). Microbial production of bioplastics from renewable resources. In S.-T. Yang (Ed.), *Bioprocessing for value-added products from renewable resources* (pp. 585–610). Amsterdam: Elservier B.V.