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NOTE

Biosynthesis of Poly(3-Hydroxybutyrate-*co*-3-Hydroxyvalerate) Copolyesters with a High Molar Fraction of 3-Hydroxyvalerate by an Insect-Symbiotic *Burkholderia* sp. IS-01

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***Burkholderia* sp. IS-01 capable of biosynthesizing poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly(3HB-*co*-3HV)] copolyesters with a high molar fraction of 3HV was isolated from the gut of the adult longicorn beetle, *Moechotypa diphysis*. The strain IS-01 was relatively tolerant to high concentrations of levulinic acid and accumulated a poly(13.5 mol% 3HB-*co*-86.5 mol% 3HV) copolyester when cultivated on a mixture of gluconate (20 g/L) and levulinic acid (12.5 g/L). In this case, the content of the copolyester in the cells was approximately 60.0%. The compositions of the copolyesters were easily regulated by altering the molar ratio of gluconate and levulinic acid in the medium. The organism was found to possess a class I PHA synthase (PhaC) gene (1,881 bp) that encodes a protein with a deduced molecular mass of 68,538 Da that consists of 626 amino acids. The PhaC of this organism was most similar to that of *B. cenocepacia* PC184 (92% similarity).**

***Keywords*:** *Burkholderia*, insect-symbiotic microorganism, levulinic acid, polyhydroxyalkanoates (PHAs), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly(3HB-*co*-3HV)], PHA synthase

Poly(3-hydroxyalkanoates) (PHAs) are representative biodegradable polyoxoesters that can be produced from various renewable resources by microorganisms (Steinbüchel and Lütke-Eversloh, 2003; Kim *et al*., 2007). Because of their superior mechanical properties comparing to poly(3-hydroxybutyrate) [poly(3HB)] homopolyester, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly(3HB-*co*-3HV)] copolyesters have been drawing a great deal of attention in bio-industrial fields that require biodegradable and biocompatible materials. However, the production of poly(3HB-*co*-3HV) copolyesters with a wide range of 3HV contents can only be accomplished by certain microorganisms due to the toxicity of the cosubstrates that are usually used as the precursor molecule of 3HV, such as propionic, valeric, and levulinic acids (Rodrigues *et al*., 2000a; Chung *et al*., 2001; Choi *et al*., 2003; Keenan *et al*., 2004). Therefore, development of polyester-producing microorganisms tolerant to the cosubstrates is necessary to enable the production of various biomaterials with different 3HV contents that can be used for a wide range of target-specific applications.

Insects are the most abundant organisms in the world due to their ability to survive in various environments. Accordingly, insects have recently attracted interest from various industries as sources of a wide variety of culturable and non-culturable intestinal microorganisms that are expected to have novel genes encoding biocatalysts or new metabolic routes. However, although many studies to explore useful products, such as novel enzymes, biopolymers, and secondary metabolites, from insect-symbiotic microorganisms have been achieved to date, there is no report on the PHA biosynthesis by exo-symbiotic gut bacteria from insects. A longicorn beetle, *Moechotypa diphysis*, as a wood-attacking insect similar to a termite (Brune, 1998), contains many fibrolytic bacteria in its gut, which can aid in the digestion of woods (Park *et al*., 2007). Because of this, a variety of microorganisms capable of efficiently utilizing sugars and carboxylic acids, which are produced by the hydrolysis of cellulosic and hemicellulosic materials present in wood are also found in the digestive tract of longicorn beetles.

Levulinic acid is an inexpensive carboxylic acid that can be cost-effectively prepared from various cellulosic biomasses for use as an alternative cosubstrate of 3HV (Cha and Hanna, 2002). Therefore, we attempted to isolate a polymer-producing microorganism that is relatively tolerant to levulinic acid at concentrations of greater than 10 g/L from the gut microorganisms of a wood-attacking longicorn beetle, *M.*

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Table 1. Results of shake-flask fermentations of SCL-PHA biosynthesis by *Burkholderia* sp. IS-01 grown with various carbon substrates

Carbon source (g/L)	Culture time (h)	Dry cell weight (g/L)	PHA content (wt%)	PHA (g/L)	Conversion of substrate to PHA (%)	PHA composition (mol%)	
						3HB	3HV
Xylose (20)	38	0.61	-	-	-	ND[a]	ND
Glucose (20)	36	0.62	20.9	0.13	0.7	100	-
Fructose (20)	80	2.05	54.1	1.10	5.5	100	-
Gluconate (20)	80	2.25	58.2	1.31	6.6	100	-
Valeric acid (5)	82	0.75	21.7	0.16	3.2	30.2	69.8
Valeric acid (10)	105	1.02	26.0	0.31	3.1	29.4	70.6
Levulinic acid (5)	66	2.28	27.3	0.62	12.4	-	100
Levulinic acid (10)	70	2.95	37.2	1.10	11.0	-	100
Gluconate (20) + levulinic acid (5)	72	4.32	60.4	2.61	10.4	53.1	46.9
Gluconate (20) + levulinic acid (10)	96	4.63	63.2	2.93	9.8	32.8	67.2

[a] Not determined

diphysis. Here, we describe the biosynthesis of poly (3HB-*co*-3HV) copolyesters with a high molar fraction of 3HV by an exo-symbiont, *Burkholderia* sp. IS-01, which was isolated from the gut of the adult *M. diphysis*. A PHA synthase (PhaC) gene of this organism was also cloned, sequenced, and analyzed.

To isolate the levulinic acid-tolerant microorganisms, the heterotrophic gut bacteria isolated from the gut of *M. diphysis* using a R2A agar medium (Scharlau Chemie S.A.) were cultivated on a solid mineral salt medium (Kim *et al.*, 2000) containing 10 g/L levulinic acid for 72 h at 30°C. Of the levulinic acid-tolerant microorganisms isolated, strain IS-01 showed relatively good growth, even when 15 g/L levulinic acid was used as the sole carbon source. This organism was also able to produce poly(3HV) homopolyester from the carbon substrate. Therefore, strain IS-01 was preferentially selected for further study. Sequence analysis of the 16S rRNA gene of the isolate was performed as previously described (Kim *et al*., 2003). The 16S rDNA sequences were aligned with those from strains of the genus *Burkholderia*, on the basis of similarities in the primary and secondary RNA structures using the PHYDIT program. The 16S rDNA sequence of strain IS-01 has been deposited in GenBank under accession no. EU438869.

The ability of the isolate to synthesize PHAs was routinely investigated using a mineral salt medium (Kim *et al*., 2000) containing the respective carbon substrate listed in Table 1. Shake flask cultures were carried out aerobically in 500 ml Erlenmeyer flask containing 100 ml of the mineral salt medium (pH 7.0) on a rotary shaker (250 rpm) at 30°C. Cells were harvested 2 h after their growth reached the stationary phase. Batch fermentations were conducted in a 7 L jar fermentor (Biotron Co. Ltd., Korea) with a working volume of 3.5 L. The medium was inoculated with a 1.5% (v/v) inoculum of an overnight culture in LB medium. The temperature and pH were automatically controlled at optimal values of 30°C and 7.0, respectively. The airflow rate was 0.5 vvm and agitation speed was 250 rpm. Cell cultivation was stopped approximately 2 h after their growth reached the stationary phase. Cells were harvested by centrifugation followed by lyophilization. Dry cell weight (DCW) was determined by measuring the weight of lyophilized cells. PHA was isolated and purified from lyophilized cells by extraction with hot chloroform using a Soxhlet apparatus. PHA content and its composition were determined by gas chromatography (Kim *et al*., 2000) using PHA standards containing known proportions of 3HB and 3HV monomers. Molecular weights of poly(3HB-*co*-3HV) copolyesters were determined using a gel permeation chromatography (GPC) system (Kim *et al*., 1998).

To amplify a partial fragment of the PhaC gene from the genomic DNA of the IS-01 strain, degenerate oligonucleotides were designed based on two internal amino acid sequences (AHKTWD and EDHIVP) conserved in *Burkholderia* PHA synthases. The upstream primer was 5'-GCSCA CAAGACSTGGGAC-3' and the downstream primer was 5'-ACGGSACGATGTGRTCCTC-3'. The PCR reaction (50 µl) was conducted using a FastStart Taq DNA Polymerase System (Roche Diagnostics Gmbh) according to the manufacturer's instructions. The amplified product, which was 601 bp, was then extracted and purified using a HiYield™ Gel/PCR DNA Extraction kit (Real Biotech Corp.). Next, the purified PCR products were cloned into a pGEM-T easy vector (Promega), followed by transforming the recombinant plasmid DNA into *E. coli* DH5α, and the nucleotide sequence of the target fragment was analyzed. Cloning of the full PhaC gene was performed by repeated DNA walking and nested PCR methods using a DNA Walking *SpeedUp*™ Premix kit (Seegene). The nucleotide sequence of PhaC gene has been deposited in GenBank under accession number EU438870.

Strain IS-01 was a Gram-negative rod-shaped bacterium that produced a lipolytic enzyme when grown on a mineral salt medium that contained either tributyrin or olive oil as the sole carbon source. Phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene revealed that strain IS-01 was closely linked to *Burkholderia xenovorans* LB400^T, with the highest sequence similarity being 98.2%. However, strain IS-01 could not be conclusively identified to species level in the present study because its 16S rDNA sequence shared high sequence homologies (>97%) with those of other several *Burkholderia* species. Based on these results, the isolate was identified as *Burkholderia* species and deposited in the Korean Collection for Type Cultures under

code no. *Burkholderia* sp. IS-01 KCTC 22425.

Among the short-chain-length (SCL)-PHA-producing microorganisms, a threonine-overproducing mutant of *Alcaligenes* sp. SH-69 has been known to produce poly (3HB-*co*-3HV) copolyesters with high molar fractions (38∼77 mol%) of 3HV in the presence of propionic, valeric, or levulinic acid (each 10 mM) as the cosubstrate (Choi *et al*., 2003). Similarly, *Burkholderia* strains are often promising candidates capable of biosynthesizing substantial amounts of poly (3HB-*co*-3HV) copolyesters with a high molar fraction of 3HV. The formation of poly (3HB-*co*-3HV) copolyesters by *Burkholderia* species is generally observed when these organisms are cultivated on carbohydrate substrates, such as glucose, xylose, sucrose, and gluconate, in the presence of propionic, valeric or levulinic acids, which are well known to serve as the precursor substrates of 3HV (Keenan *et al*., 2004; Alias and Tan, 2005).

The ability of *Burkholderia* sp. IS-01 to biosynthesize PHAs was evaluated by cultivating it on various types of sugars and fatty acids (Table 1). When grown with carbon substrates such as glucose, fructose, and gluconate, *Burkholderia* sp. IS-01 produced poly(3HB) homopolyester, while no PHA was observed when the cells were grown on a medium containing xylose as the sole carbon source. The inability of this organism to biosynthesize poly(3HB) from xylose was comparable to the metabolic capability of *B. cepacia* that could produce poly(3HB-*co*-3HV) copolyesters from xylose in the presence of levulinic acid as the cosubstrate (Keenan *et al*., 2004). It is well known that the carbon availability and PHA biosynthetic ability of *Pseudomonas* strains for the same carbon substrates differ significantly, even though they belong to the same genus (Kim *et al*., 2000). Therefore, it is likely that the inability of *Burkholderia* sp. IS-01 to produce a polyester from xylose is related to its unique metabolic system, which may differ from that of *B. cepacia*. Of the simple carbohydrate compounds evaluated, gluconate was found to induce the best growth and highest poly(3HB) production by *Burkholderia* sp. IS-01. Specifically, the content of poly(3HB) in cells grown on a mineral medium supplemented with 20 g/L gluconate was 58.2 wt%, and the bioconversion yield of gluconate to the corresponding polyester was estimated to be 7.4%.

It should be noted that *Burkholderia* sp. IS-01 biosynthesized poly(3HV) homopolyester from levulinic acid when it was used as the sole carbon source because bacterial PHAs produced from levulinic acid usually contain 4HV and 3HB in addition to 3HV as constituents (Steinbüchel and Lütke-Eversloh, 2003). The use of 5 or 10 g/L of valeric acid as the sole carbon source supported the formation of poly (3HB-*co*-3HV) copolyesters with approximately 70 mol% 3HV units by *Burkholderia* sp. IS-01. A similar observation was reported during the synthesis of poly(3HB-*co*-3HV) copolyester from valeric acid by *B. cepacia* FLP1; however, that polyester contained 93 mole% 3HV as the major constituent (Alias and Tan, 2005). Comparatively, it has been reported that *B. cepacia* IPT 64 synthesizes a terpolyester consisting of 73.4 mol% 3HB, 23.0 mol% 3HV, and 3.7 mol% 3-hydroxy-4-pentenoate from valeric acid (Rodrigues *et al*., 2000b). Taken together, the results of this study and those of previous studies indicate that the ability of SCL-PHA-producing microorganisms to generate (*R*)-3-hydroxyacyl-CoAs from certain substrate and the substrate specificities of their SCL-PHA synthases differ significantly among these organisms.

The data shown in Table 1 also indicate that levulinic acid is less toxic to *Burkholderia* sp. IS-01 than valeric acid at the same concentration. Indeed, the growth of this organism was better and the PHA production yield was 3-fold greater when it was cultivated in the presence of 10 g/L levulinic acid than when it was cultured in the presence of 10 g/L valeric acid. Conversely, when *Burkholderia* sp. IS-01 was grown in the presence of propionic acid alone (2 g/L), almost no PHA biosynthesis was observed, indicating that this substrate was not suitable for use as the precursor molecule of 3HV. Studies evaluating the biosynthesis of medium-chain-length (MCL)-PHA by *Burkholderia* sp. IS-01 using octanoic, decanoic, and oleic acids were also performed, but no SCL-PHAs or MCL-PHAs were detected in cells grown with the respective substrate (5 g/L) solely given. These results suggest that *Burkholderia* sp. IS-01 possesses only a SCL-PHA biosynthetic system. However, *B. caryophylli* AS 1.2741 was found to produce both SCL-PHAs and MCL-PHAs because it has the two distinct PHA synthases, $PhaC1_{Bc}$, and $PhaC2_{Bc}$, which exhibited substrate specificities to SCL-3-hydroxyacyl-CoAs and MCL-3-hydroxyacyl-CoAs, respectively (Hang *et al*., 2002). It has been also found that the *B. cepacia* PHA biosynthetic system contains at least two putative SCL-PHA synthases, which likely have different substrate specificities (Rodrigues *et al*., 2000a).

Compared to other known SCL-PHA-producing microorganisms, the organism was found to be highly tolerant to levulinic acid solely given, even at concentrations as high as 15 g/L, but that its growth rate decreased gradually, as the concentration of levulinic acid increased (data not shown). Based on the above results, fermentation strategies that

Table 2. Compositions and molecular masses of poly(3HB-*co*-3HV) copolyesters produced by batch fermentations of *Burkholderia* sp. IS-01 using various molar mixtures of gluconate (GA) and levulinic acid (LA) in a 7 L jar fermentor

Carbon substrate (g/L)	Culture time (h)	Dry cell weight (g/L)	PHA content (wt%)	PHA composition (mol%)		M_n[a] (kDa)	M_w[b] (kDa)	M_w/M_n[c]
				3HB	3HV			
GA (20) + LA (5)	55	6.6	60.5	52.7	47.3	127	149	1.17
GA (20) + LA (7.5)	67	6.6	57.2	42.3	57.7	120	147	1.23
GA (20) + LA (10)	82	5.9	58.0	33.8	66.2	144	162	1.13
GA (20) + LA (12.5)	120	5.9	61.6	13.5	86.5	124	148	1.19

[a] The number average molecular mass
[b] The weight average molecular mass
[c] Polydispersity index

employed various molar mixtures of gluconate and levulinic acid to produce poly(3HB-*co*-3HV) copolyesters with a high molar fraction of 3HV in an increased yield was designed and applied to the polyester production by *Burkholderia* sp. IS-01. The results of shake-flask experiments revealed that *Burkholderia* sp. IS-01 was able to produce a poly(53.1 mol% 3HB-*co*-46.9 mol% 3HV) copolyester with the content of 60.4 wt% in the cells from a mixture of gluconate (20 g/L)

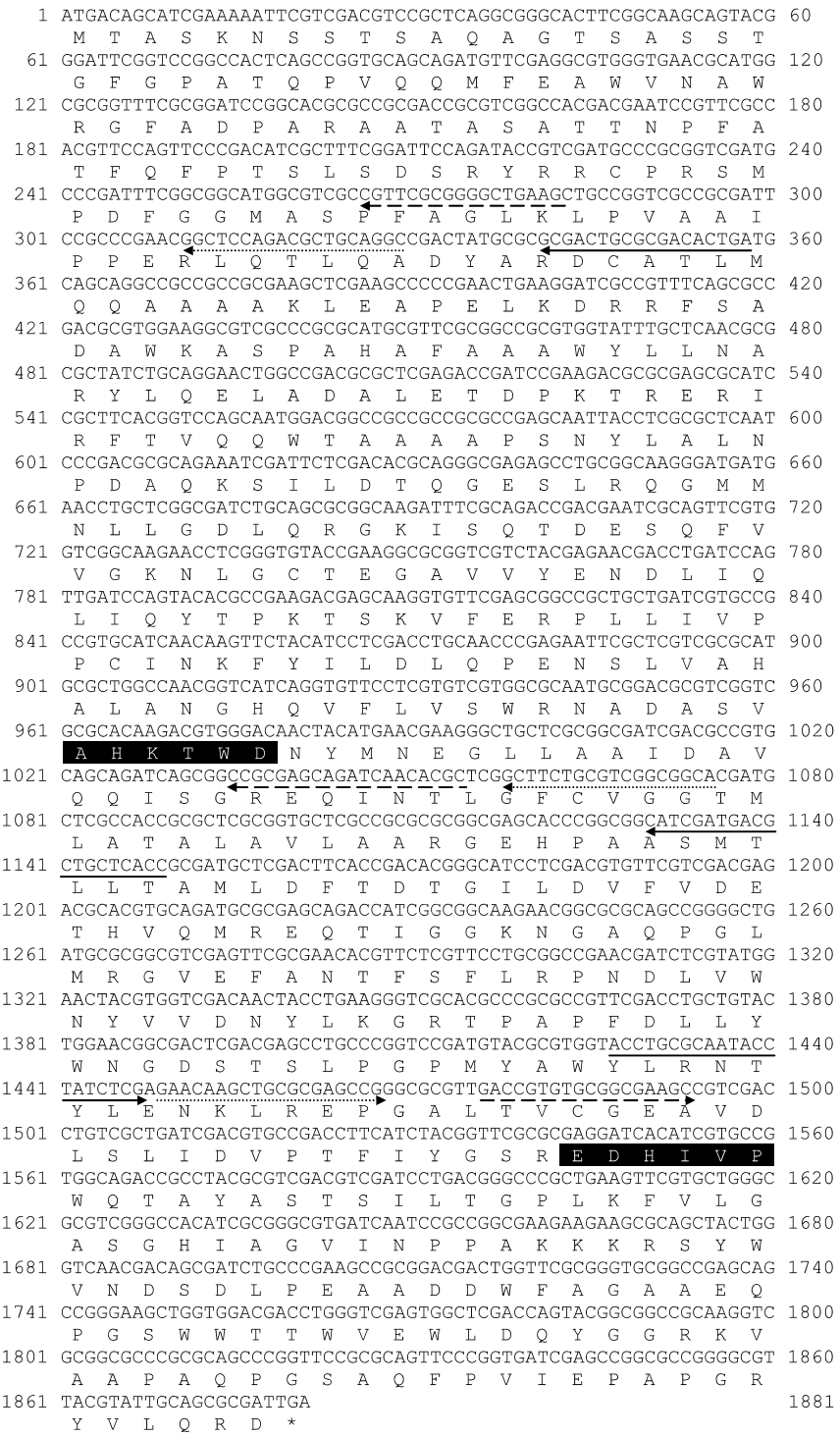

Fig. 1. Nucleotide sequence of the PhaC gene of *Burkholderia* sp. IS-01. Amino acids deduced from the nucleotide sequence are indicated by standard one-letter abbreviations. The solid boxes represent the conserved region used in design of the PCR primers to clone the partial PHA synthase gene fragment. The oligonucleotide sequences used in DNA walking are shown by solid arrows and those used in 1st and 2nd nested PCRs are indicated by dotted and dashed arrows, respectively.

and levulinic acid (5 g/L) (Table 1). In this case, approximately 10.4% of the total substrates (25 g/L) provided were found to have been converted to the copolyester. Another experiment showed that 3HV units could be incorporated by 67.2 mol% into the growing-copolyester chain when *Burkholderia* sp. IS-01 was grown with a mixture of gluconate (20 g/L) and levulinic acid (10 g/L). In that case, only 9.8% of the two substrates that were consumed by the organism were used in the biosynthesis of poly(32.8 mol% 3HB-*co*-67.2 mol% 3HV) copolyester.

Batch fermentations of *Burkholderia* sp. IS-01 using a 7 L jar fermentor were also conducted to evaluate the production patterns of various poly(3HB-*co*-3HV) copolyesters with different 3HV contents. When cultivated on mixtures of gluconate (20 g/L) with levulinic acid (5 to 12.5 g/L) as the cosubstrate, *Burkholderia* sp. IS-01 generally accumulated the copolyesters at concentrations that were up to approximately 60 wt% of the dry cell weight (5.9 to 6.6 g/L); however, relatively long culture times of approximately 55 to 120 h were required, depending on the concentration of levulinic acid in the mixed carbon substrates. The compositions of the poly(3HB-*co*-3HV) copolyesters isolated from the cells are shown in Table 2. In fermentor experiments, *Burkholderia* sp. IS-01 biosynthesized poly(52.7 mol% 3HB-*co*-47.3 mol% 3HV) and poly(33.8 mol% 3HB-*co*-66.2 mol% 3HV) copolyesters from mixtures that contained 20 g/L gluconate amended with 5 or 10 g/L levulinic acid, respectively. These compositions of copolyesters were similar to those of the copolyesters produced in cells obtained from the shake-flask experiments. A poly(3HB-*co*-3HV) copolyester consisting of greater than 80 mol% 3HV could be also prepared when the organism was grown on a medium containing 20 g/L gluconate supplemented with 12.5 g/L levulinic acid as the cosubstrate. These results suggest that the content of 3HV in the copolyesters being biosynthesized by *Burkholderia* sp. IS-01 could be easily controlled by altering the proportion of gluconate to levulinic acid in the carbon substrate mixtures. Poly(3HB-*co*-3HV) copolyesters with high 3HV content are not necessarily more useful, but the ability to alter the 3HV content in poly(3HB-*co*-3HV) is desirable from an industrial viewpoint, as this offers the possibility of producing a range of different thermoplastics with varying degrees of flexibility and toughness (Choi *et al*., 2003).

As shown in Table 2, the number average molecular mass (M_n) of poly(3HB-*co*-3HV) copolyesters biosynthesized was found to be distributed in ranges from approximately 120 to 144 kDa, which are lower than those (>300 kDa) of poly (3HB-*co*-3HV) copolyesters prepared by different microorganisms (Park *et al*., 1997; Savenkova *et al*., 2000; Keenan *et al*., 2004). In addition, polydispersity indices (M_w/M_n) of the poly(3HB-*co*-3HV) copolyesters were evaluated to be in the range of 1.13 to 1.23, indicating that the length of copolyester chains in the respective copolyester is significantly similar. Taken together, these findings suggest that poly(3HB-*co*-3HV) copolyesters produced by *Burkholderia* sp. IS-01 are new biomaterials, which may have different mechanical properties compared to the known poly(3HB-*co*-3HV) copolyesters with a high molecular mass (>300 kDa) and a high polydispersity index (>2.0).

Burkholderia strains have been reported to possess a class I PHA synthase (PhaC) that catalyzes the conversion of SCL (*R*)-3-hydroxyacyl CoAs to the corresponding polyesters. However, only few studies on the molecular characterization of *Burkholderia* PhaC genes and the role of the related enzymes have been conducted to date (Rodrigues *et al*., 2000a; Hang *et al*., 2002), even though the nucleotide sequences of some putative *Burkholderia* PhaC genes that have been identified through genome survey are available in the NCBI database. The PhaC gene of *Burkholderia* sp. IS-01 contained an 1,881 bp open reading frame (ORF), which encodes a polypeptide of 626 amino acids with a deduced molecular mass of 68,538 Da and a calculated *p*I of 5.45 (Fig. 1). When the PhaC sequence of *Burkholderia* sp. IS-01 was aligned with other SCL-PHA synthases available in the NCBI database, it was found to have a molecular structure similar to that of class I PHA synthases obtained from other *Burkholderia* strains. The PhaC of strain IS-01 was most similar with the putative PhaC of *B. cenocepacia* PC184, with the highest sequence identity of 92%. In addition, it showed a high sequence similarity of 89% with the PhaC of *Burkholderia* sp. DSMZ 9242, which is known to be responsible for the biosynthesis of poly(3HB) homopolyester (Rodrigues *et al*., 2000a). However, the primary structure of the *Burkholderia* sp. IS-01 PhaC was only 72% similar with that of the PhaC of *B. xenovorans* LB400 that was the closest relative phylogenetically.

In conclusion, the results of this study indicate that *Burkholderia* sp. IS-01, an exo-symbiotic organism isolated from the gut of the adult *M. diphysis*, is a promising candidate for the biotechnological production of poly(3HB-*co*-3HV) copolyesters with a high molar fraction of 3HV. Further, the compositions of poly(3HB-*co*-3HV) copolyesters produced by *Burkholderia* sp. IS-01 can be easily regulated to some extent by altering the molar ratio of a principle carbon substrate to a cosubstrate: therefore, this organism will be useful for the preparation of various types of thermoplastics with different material properties.

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References

Alias, Z. and I.K.P. Tan. 2005. Isolation of palm oil-utilizing, polyhydroxyalkanoate (PHA)-producing bacteria by an enrichment technique. *Bioresour. Technol.* 96, 1229-1234.

Brune, A. 1998. Termite guts: the world's smallest bioreactors. *Trends Biotechnol*. 16, 16-21.

Cha, J.Y. and M.A. Hanna. 2002. Levulinic acid production based on extrusion and pressurized batch reaction. *Ind. Crops Prod*. 16, 109-118.

Choi, G.G., M.W. Kim, J.Y. Kim, and Y.H. Rhee. 2003. Production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with high molar fractions of 3-hydroxyvalerate by a threonine-overproducing mutant of *Alcaligenes* sp. SH-69. *Biotechnol. Lett*. 25, 665-670.

Chung, S.H., G.G. Choi, H.W. Kim, and Y.H. Rhee. 2001. Effect of levulinic acid on the production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by *Ralstonia eutropha* KHB-8862. *J. Microbiol*. 39, 79-82.

Hang, X., G. Zhang, G. Wang, X. Zaho, and G.Q. Chen. 2002. PCR cloning of polyhydroxyalkanoate biosynthesis genes from

Burkholderia caryophylli and their functional expression in recombinant *Escherichia coli*. *FEMS Microbiol. Lett*. 210, 49-54.

Keenan, T.M., S.W. Tanenbaum, A.J. Stipanovic, and J.P. Nakas. 2004. Production and characterization of poly-β-hydroxyalkanoate copolymers from *Burkholderia cepacia* utilizing xylose and levulinic acid. *Biotechnol. Prog*. 20, 1697-1704.

Kim, D.Y., H.W. Kim, M.G. Chung, and Y.H. Rhee. 2007. Biosynthesis, modification, and biodegradation of bacterial medium-chain-length polyhydroxyalkanoates. *J. Microbiol.* 45, 87-97.

Kim, H.J., D.Y. Kim, J.S. Nam, K.S. Bae, and Y.H. Rhee. 2003. Characterization of an extracellular medium-chain-length poly (3-hydroxyalkanoate) depolymerase from *Streptomyces* sp. KJ-72. *Antonie Van Leewenhoek* 83, 183-189.

Kim, D.Y., Y.B. Kim, and Y.H. Rhee. 1998. Bacterial poly(3-hydroxyalkanoates) bearing carbon-carbon triple bonds. *Macromolecules* 31, 4760-4763.

Kim, D.Y., Y.B. Kim, and Y.H. Rhee. 2000. Evaluation of various carbon substrates for the biosynthesis of polyhydroxyalkanoates bearing functional groups by *Pseudomonas putida*. *Int. J. Biol. Macromol*. 28, 23-29.

Park, D.S., H.W. Oh, W.J. Jeong, H. Kim, H.Y. Park, and K.S. Bae. 2007. A culture based study of the bacterial communities within the guts of nine longicorn beetle species and their exoenzyme producing properties for degrading xylan and pectin. *J. Microbiol*. 45, 394-401.

Park, S.K., K.T. Lee, Y.B. Kim, and Y.H. Rhee. 1997. Biosynthesis of polyhydroxybutyrate and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by *Bacillus thuringiensis* R-510. *J. Microbiol*. 35, 127-133.

Rodrigues, M.F.A., H.E. Valentin, P.A. Berger, M. Tran, J. Asrar, K.J. Gruys, and A. Steinbüchel. 2000a. Polyhydroxyalkanoate accumulation in *Burkholderia* sp.: a molecular approach to elucidate the genes involved in the formation of two homopolymers consisting of short-chain-length 3-hydroxyalkanoic acids. *Appl. Microbiol. Biotechnol*. 53, 453-460.

Rodrigues, M.F.A., E.J. Vicente, and A. Steinbüchel. 2000b. Studies on polyhydroxyalkanoate (PHA) accumulation in a PHA synthase I-negative mutant of *Burkholderia cepacia* generated by homogenotization. *FEMS Microbiol. Lett*. 193, 179-185.

Savenkova, L., Z. Gercberga, I. Bibers, and M. Kalnin. 2000. Effect of 3-hydroxyvalerate content on some physical and mechanical properties of polyhydroxyalkanoates produced by *Azotobacter chroococum*. *Proc. Biochem*. 36, 445-450.

Steinbüchel, A. and T. Lütke-Eversloh. 2003. Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms. *Biochem. Eng. J*. 16, 81-96.