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ORIGINAL PAPER

# Complete genome sequence of *Aquitalea pelogenes* USM4 (JCM19919), a polyhydroxyalkanoate producer

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## Abstract

Polyhydroxyalkanoate (PHA) is a type of biopolymer produced by most bacteria and archaea, resembling thermoplastic with biodegradability and biocompatibility features. Here, we report the complete genome of a PHA producer, *Aquitalea* sp. USM4, isolated from Perak, Malaysia. This bacterium possessed a 4.2 Mb circular chromosome and a 54,370 bp plasmid. A total of 4067 predicted protein-coding sequences, 87 tRNA genes, and 25 rRNA operons were identified using PGAP. Based on ANI and dDDH analysis, the *Aquitalea* sp. USM4 is highly similar to *Aquitalea pelogenes*. We also identified genes, including acetyl-CoA (*phaA*), acetoacetyl-CoA (*phaB*), PHA synthase (*phaC*), enoyl-CoA hydratase (*phaJ*), and phasin (*phaP*), which play an important role in PHA production in *Aquitalea* sp. USM4. The heterologous expression of *phaC1* from *Aquitalea* sp. USM4 in *Cupriavidus necator* PHB$^{-}$4 was able to incorporate six different types of PHA monomers, which are 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 4-hydroxybutyrate (4HB), 5-hydroxyvalerate (5HV), 3-hydroxyhexanoate (3HHx) and isocaproic acid (3H4MV) with suitable precursor substrates. This is the first complete genome sequence of the genus *Aquitalea* among the 22 genome sequences from 4 *Aquitalea* species listed in the GOLD database, which provides an insight into its genome evolution and molecular machinery responsible for PHA biosynthesis.

**Keywords** Complete genome sequence · *Aquitalea* · Polyhydroxyalkanoate · PHA biosynthetic genes · PHA monomers

## Introduction

Polyhydroxyalkanoates (PHAs) is a biopolyester that can be produced naturally by various bacteria and is biodegradable in soil and marine environment (Lopez-Llorca et al. 1993; Doi et al. 1995; Sudesh et al. 2000). Its naturally occurring, biodegradable, and environment-friendly properties have made PHA a good alternative as a bioplastic compared to petroleum-based plastics, which take years to degrade, in addition to the petroleum depletion concern.

PHAs are accumulated in the cytoplasm as their energy reserve and carbon source in response to stress, for example, under nutrient limiting and excess carbon conditions (Anderson and Dawes 1990). Many different carbon sources, such as palm oil, sludge palm oil, crude palm kernel oil, sugar, and many more, were reported to be fed to bacteria for PHA production (Riedel et al. 2012; Hassan et al. 2013; Thinagaran & Sudesh 2019). Among the different carbon sources, fatty acids are preferable as they contain more carbon content compared to sugar. Besides that, there are also reports on waste like frying oil used as feedstock for PHA production using *Pseudomonas fluorescens* S48 (Gamal et al. 2013). The utilisation of waste as feedstock for PHA production not only reduces the production cost of PHA, but also converts these wastes into useful biodegradable plastics (Lee & Choi 1999; Chee et al. 2010). Depending on the carbon sources, different PHA will be accumulated in the bacteria. For instance, poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate), P(3HB-*co*-3HHx) will be produced when the bacteria are fed with oil, while only poly(3-hydroxybutyrate), P(3HB) will be accumulated when fed with sugar such as fructose and sucrose (Lee et al. 2008; Ng and Sudesh 2016).

Among the different types of PHAs, polyhydroxybutyrate (PHB) is the most common type of polymer produced by

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bacteria. However, its stiffness, brittle, low thermal stability and high crystallinity properties of PHB have limited its potential application. Therefore, the incorporation of other monomers such as 3-hydroxyhexanoate (3HHx) and 4-hydroxybutyrate (4HB) to produce copolymers such as P(3HB-*co*-3HHx) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate), P(3HB-*co*-4HB) is crucial to improve its thermostability, flexibility and biocompatibility to replace the conventional single-used plastics and widen its potential application, especially in the medical field (Jendrossek and Handrick 2002; Chee et al. 2010).

Each bacterium has its unique PHA biosynthetic genes, including *phaA*, *phaB*, *phaC*, *phaJ,* and *phaP*. These PHA biosynthetic genes are closely associated with synthesising PHA and significantly affect the amount and types of PHA synthesised in bacteria. For example, PHA synthase (PhaC) is responsible for polymerising monomeric hydroxyalkanoate (HA) substrates into PHA, and its substrate specificity determines the types of PHAs produced by the bacteria (Steinbüchel & Valentin 1995; Rehm 2003). In brief, the discovery of each new biosynthetic gene provides us with a better insight and understanding of the PHA biosynthesis mechanism in bacteria, which may aid us in improving and producing a better and wider application of bio-based plastics.

The genus *Aquitalea* is a member of Betaproteobacterium in the family *Chromobacteriaceae*. To date, *Aquitalea magnusonii*, *Aquitalea denitrificans*, *Aquitalea pelogenes,* and *Aquitalea aquatilis* are the four members of this genus that have been reported (Lau et al. 2006; Lee et al. 2009; Sedláček et al. 2016; Ngo et al. 2020). Compared to the genus *Chromobacterium*, *Aquitalea* can be considered understudied as there are very few studies on its production of bio-products such as PHA (Steinbüchel et al. 1993; Brito et al. 2004; Bhubalan et al. 2011; Ling et al. 2011), since the first *Aquitalea* species was only reported in 2006 (Lau et al. 2006). According to GOLD database, a total of 22 *Aquitalea* genome sequences are publicly available, for example *A. magnusonii* H3, *Aquitalea* sp. strain MWU14-2217, *A. pelogenes* CCM 7557 and *A. aquatilis* THG-DN7.12, but the ability to produce PHA has not been reported (Sedláček et al. 2016; Ishizawa et al. 2017; Ebadzadsahrai & Soby 2018; Ngo et al. 2020) (the whole-genome sequences or whole-genome shotgun sequences of *Aquitalea* strains are available in NCBI and BV-BRC and their accession numbers are listed in Table S2).

To further analyse the potential of the *Aquitalea* species in PHA production, we found that some of the *Aquitalea* strains, for example, *A. denitrificans* strain 5YN1-3, *A. aquatilis* strain THG-DN7.12 and *A. magnusonii* strain H3, do have genes that are necessary for PHA synthesis such as *phaA*, *phaB*, *phaP*, *phaJ*, and *phaC*, but neither has been reported to produce PHA. *Aquitalea* sp. USM4 was first isolated by Ng and Sudesh (2016) from freshwater in Perak, Malaysia, and is the first *Aquitalea* strain reported to produce PHA. Its PhaC ($PhaC_{As}$) exhibits high activity (863 U/g protein) (Ng & Sudesh 2016). The ability to produce PHA by *Aquitalea* sp. USM4 has triggered our interest in elucidating its whole genome, especially its PHA biosynthetic genes, as previously isolated *Aquitalea* strains are non-PHA producers. Hence, this study provides insight into the first whole genome of *Aquitalea* sp. USM4 and its PHA biosynthetic genes for future research on *Aquitalea* species.

## Materials and methods

### Whole-genome sequencing

Paired-end and mate-pair libraries were prepared from the bacterial genomic DNA using Illumina TruSeq DNA PCR-free and Nextera mate-pair sample prep kit, respectively. One paired-end library (insert size, 600 bp; read length, 250 bp; total, 1.3 Gb) and three mate-pair libraries (insert size, 3 kb, 6 kb, 6.5 kb; read length, 250 bp; total, 912 Mb) were sequenced by Illumina MiSeq sequencer. Adaptor sequences and low-quality regions in raw reads were trimmed using Platanus_trim (version 1.0.7; http://platanus.bio.titech.ac.jp/pltanus_trim), and de novo assembly was done with Platanus (version 1.2.1) (Kajitani et al. 2014), resulting in three scaffolds (size > 1 kb). One scaffold (size, 5,518 bp) was excluded as the PhiX genome was used as a control for Illumina sequencing. The others were considered as a chromosome and a plasmid. Finally, the gap-close module of MetaPlatanus (pre-release version) (Kajitani et al. 2021) was additionally applied, and all gaps were filled. The bacterial genome and the protein-coding sequences (CDSs) were predicted and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016; Haft et al. 2018).

### Genome analysis

The whole-genome-based pairwise average nucleotide identity (ANI) was done between *Aquitalea* sp. USM4 and other *Aquitalea* genomes. Different ANI calculating tools were used independently to calculate the ANI, including the ANI calculator (http://enve-omics.ce.gatech.edu/ani/index), EzGenome (http://www.ezbiocloud.net/ezgenome/ani) and Jspecies (http://www.imedea.uib.es/jspecies) where the genome sequences were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) and BV-BRC (https://www.bv-brc.org/) (Richter et al. 2015; Yoon et al. 2017). ANI values obtained were based on either BLASTn (ANIb) or MUMMER software (ANIm) (Richter & Rosselló-Móra 2009). The EzGenome was used to calculate OrthoANIb, which is ANI by

orthology and calculated using the BLASTn program (Lee et al. 2016). The ANI between *Aquitalea* sp. USM4 and two other strains from the same clique cluster, *A. pelogenes* CCM7557 and *A. magnusonii* SM6, were also analysed using Genomes OnLine Database (GOLD) v.8 (Chen et al. 2020; Mukherjee et al. 2020). Digital DNA–DNA hybridisation (dDDH) values and confidence intervals were calculated using the recommended settings of the GGDC3.0 (https://www.dsmz.de/services/online-tools/genome-to-genome-distance-calculator-ggdc) paired with the BLAST + alignment tool and formula 2 (Meier-Kolthoff et al. 2013).

## Bacterial strain and plasmids

Bacterial strains and plasmids utilised in this study are listed in Table 1. Strains of *Aquitalea* sp. USM4 and *Cupriavidus necator* were routinely cultured on nutrient-rich agar (NR) containing 10 g/L peptone (HiMedia, India), 10 g/L meat extract (HiMedia, India), and 2 g/L yeast extract (HiMedia, India) at pH 7.0 and 30 ℃. *Escherichia coli* strains were grown in Luria–Bertani (LB) (HiMedia, India) medium at 37℃. For the cultivation of recombinant strains with the plasmid, the medium was supplemented with 50 µg/mL of kanamycin (Sigma Aldrich).

## Cloning of PHA synthase genes from *Aquitalea* sp. USM4

In this study, *phaC1* of *Aquitalea* sp. USM4 ($phaC1_{As}$) *was* amplified using polymerase chain reaction (PCR). The sequences of two of the oligonucleotide primers used in this study are ATCAAGCTTAGGAGGAGGCATGTTAAG GAGATGTACCTTGA ($PhaC_{As}$_*Hin*dIII_RBS_Forw) and ATCGGGCCCATTTAAATTTATTGCAGGCTGGCTACC GTGCT ($PhaC_{As}$_*Apa*I_*Swa*I_Rev).

The PCR products were digested using FastDigest restriction enzymes (Thermo Scientific, USA) and ligated with the digested plasmid using DNA Ligation Kit Ver.2.1 (Takara Bio Inc. Japan) in accordance with the manufacturer's protocol. The broad-host-range pBBR1MCS-2 plasmid (Kovach et al. 1995) was used and transformed into *C. necator* PHB4 through transconjugation with *E. coli* S17-1 (Friedrich et al. 1981). Colony PCR with plasmid-specific primers and DNA sequencing were used to verify the successful transformants.

## PHA biosynthesis

PHA accumulation in *C. necator* PHB$^{-}$4 harbouring $phaC1_{As}$ was carried out through one-stage shake flask cultivation as described by Budde et al. (2011). The ability of the *Aquitalea* sp. USM4 to incorporate different PHA monomers was studied by adding structurally related carbon sources or precursors into the PHA biosynthesis medium. In this work, 10 g/L of crude palm kernel oil (CPKO) or fructose was used as the carbon source. To examine the substrate specificity of the PhaC, a total 10 g/L of fructose was added as the carbon source with 2 g/L of sodium 3-hydroxyvalerate (Na3HV), sodium 4-hydroxybutyrate (Na4HB), sodium 5-hydroxyvalerate (Na5HV), sodium 3-hydroxyhexanoate (Na3HHx) and 1 g/L of isocaproic acid (3H4MV) as structurally related precursors.

After 48 h of incubation, the culture was harvested by centrifugation at 8000 rpm for 10 min at 4℃ and then lyophilised for 2 days. Then the cells were further analysed using gas chromatography (GC) (Braunegg et al. 1978). The P(3HB-*co*-3HHx) copolymer was extracted and further verified using proton nuclear magnetic resonance ($^{1}$H-NMR).

**Table 1** List of plasmids and bacterial strains used in this study

| Strain or plasmid | Description | Reference or source |
|---|---|---|
| Strain | | |
| *Aquitalea* sp. USM4 | Wild type; PHA-producing bacterium isolated from freshwater | JCM 19,919 |
| *C. necator* strains | | |
| PHB$^{-}$4 | A PHA-negative mutant of wild-type *C. necator* H16 | DSM 541 |
| *E. coli* strains | | |
| DH5α | F$^{-}$ l$^{-}$ deoR supE44 hsdR17(rK$^{-}$ mK$^{+}$) phoA *rec*A1 *end*A1 *gyr*A96 *thi*-1 relA1 D(*lac*ZYA-*arg*F) U169 f80d*lac*ZDM15 | Toyobo |
| S17-1 | *rec*A, *tra* genes of plasmid RP4 integrated into the chromosome, auxotrophic for proline and thiamine | Simon et al. (1983) |
| Plasmids | | |
| pBBR1MCS2-$C_{As}$ | pBBR1MCS-2 harbouring *C. necator phaC*1 promoter and *phaC1* from *Aquitalea* sp. USM4 at the *Hin*dIII and *Apa*I sites | This study |

### Nucleotide sequence accession number

The genomic sequence of chromosome and plasmid of *Aquitalea* sp. USM4 has been submitted in GenBank with accession numbers NZ_CP029539 and NZ_CP029540, respectively. The strain is accessible through the Japan Collection of Microorganisms (JCM) with accession number JCM 19919.

## Results and discussion

### The genome attributes of *Aquitalea* sp. USM4

The *Aquitalea* sp. USM4 genome was sequenced from 600 bp paired-end library and 3 kb, 6 kb, and 6.5 kb mate-pair libraries using Illumina MiSeq sequencer, yielding 1.3 Gb and 912 Mb of sequence data, respectively. A complete genome of *Aquitalea* sp. USM4 was obtained after the sequenced data were trimmed and assembled as contigs by performing de novo assembly. The *Aquitalea* sp. USM4 genome consisted of one circular chromosome and one plasmid. The size of the circular chromosome is 4,291,790 bp, with a G + C content of 59.4% (Table 2), whereas the plasmid size is 54,370 bp, and its G + C content is 56.3%.

The bacterial whole-genome assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). There is a total of 87 tRNAs, 25 rRNA operons, and 3952 protein-coding sequences (CDSs) predicted in *Aquitalea* sp. USM4. The distribution proportion of Clusters of Orthologous Groups (COGs) functional categories of *Aquitalea* sp. USM4 were analysed using eggNOG (Table S1). *Aquitalea* sp. USM4 possesses an exceptionally high number of genes classified by COG as "poorly characterised", which includes "function unknown" (19.21%) and "general function prediction only" (4.17%), indicating that there are many genes and mechanisms in *Aquitalea* remain largely unknown. The higher proportion of known protein-coding genes are categorised under the "metabolism" group, especially "amino acid transport and metabolism" (9.14%) and "energy production and conservation" (6.62%). It is followed by "transcription" (7.13%) and "signal transduction mechanisms" (6.54%) under the group "Information storage and processing" and "cellular processes and signalling".

**Table 2** Genome features of *Aquitalea* sp. USM4

| Features | *Aquitalea* sp. USM4 |
|---|---|
| Length (bp) | 4,346,160 |
| G + C content (%) | 59.4 |
| Genes (total) | 4,067 |
| CDS (coding) | 3,952 |
| tRNA genes | 87 |
| rRNA genes | 25 |
| ncRNA genes | 3 |

Previously, the 16S rRNA phylogenetic analysis showed that *Aquitalea* sp. USM4 was closely related to the *A. magnusonii* TRO-001DR8(T) and *A. denitrificans* 5YN1 (Ng and Sudesh 2016). To further investigate the phylogeny relationship of *Aquitalea* sp. USM4 with other *Aquitalea* sp., we performed ANI and dDDH analysis using the whole genome of *Aquitalea* sp. USM4. The ANI values were calculated using different tools, including the ANI calculator, EzGenome, Jspecies and GOLD database. The ANI result showed *Aquitalea* sp. USM4 has higher similarity (97.64–98.01%) against *A. pelogenes* CCM7557 (supplementary data, Table S2). The recommended threshold ANI value for the demarcation of species is 96.5% when using complete or nearly complete genomes (Varghese et al. 2015). In addition to that, the classical standard species delineation threshold dDDH value is 70%, from the dDDH analysis result showed 81.6% between *Aquitalea* sp. USM4 and *A. pelogenes* CCM7557 which is above the threshold value (supplementary data, Table S2) (Goris et al. 2007; Richter & Rosselló-Móra 2009). Hence, the ANI and dDDH values showed that the *Aquitalea* strain isolated indicated that it belongs to the *A. pelogenes* species and can be referred to as *Aquitalea pelogenes* USM4.

### PHA biosynthesis-related genes in *Aquitalea* sp. USM4

Genes involved in PHA biosynthesis were identified from the whole genome of *Aquitalea* sp. USM4. There are a total of 19 genes encoded for proteins have been identified (Table 3): three acetyl-CoA C-acyltransferase (*phaA*), acetoacetyl-CoA reductase (*phaB*), three phasin family proteins (*phaP*), seven enoyl-CoA hydratase (*phaJ*), two MaoC family dehydratase, repressor protein (*phaR*), and two PHA synthase (*phaC*).

The biosynthetic pathway of P(3HB) consists of three main enzymatic reactions that are catalysed by *phaA*, *phaB*, and *phaC*. These three genes are usually clustered together in the bacterial genome and form the *phaCAB* operon generally found in the PHA-accumulating bacteria, as it promotes the synthesis of SCL-PHA (Rehm and Steinbüchel 1999; Reddy et al. 2003). In the *Aquitalea* genome, we found the *phaCA* operon, while the *phaB* is located elsewhere in the chromosome. However, this does not affect its ability to produce PHA, as similar gene arrangements can also be found in *Chromobacterium violaceum* and *Jeongeupia* sp. USM3 (Kolibachuk et al. 1999; Zain et al. 2020). This is contrary to the *C. necator,* which is the model organism in PHA studies, where the *phaC*, *phaA,* and *phaB* are clustered together (Peoples and Sinskey 1989). Interestingly, two PhaCs, $PhaC1_{As}$ and $PhaC2_{As}$ were identified in *Aquitalea* sp. USM4. Class I PhaC is known to incorporate SCL-PHA monomer only;

**Table 3** Putative PHA-associated genes in the genome of *Aquitalea* sp. USM4 with its function and closest organism match based on BLASTx

| Enzymes (gene) | Gene locus tag | Accession number | Size (bp) | Function | Reference |
|---|---|---|---|---|---|
| Acetyl-CoA C-acyltransferase (*phaA*) | DKK66_RS00305 | WP_131354427.1 | 1182 | Catalyses the two-step condensation reaction of acetyl-CoA to acetoacetyl-CoA | (Slater et al. 1988) |
| | DKK66_RS13935 | WP_131359136.1 | 1188 | | |
| | DKK66_RS00085 | WP_131354339.1 | 1203 | | |
| Acetoacetyl-CoA reductase (*phaB*) | DKK66_RS18155 | WP_045844985.1 | 741 | Catalyses the reduction of acetoacetyl-CoA to form 3-hydroxybutyryl-CoA | (Reddy et al. 2003) |
| Polyhydroxyalkanoate synthesis repressor (*phaR*) | DKK66_RS14750 | WP_045845152.1 | 567 | Regulates the expression of *phaP* and *phaC* | (McCool & Cannon 2001; Maehara et al. 2002) |
| Phasin family protein (*phaP*) | DKK66_RS16180 | WP_131359719.1 | 1638 | Control of the surface properties of PHA granules | (Ushimaru et al. 2014) |
| | DKK66_RS03265 | WP_045845588.1 | 573 | | |
| | DKK66_RS14745 | WP_045845153.1 | 546 | | |
| Enoyl-CoA hydratase (*phaJ*) | DKK66_RS00090 | WP_131354342.1 | 2376 | Catalyses the (*R*)-specific hydration of the β-oxidation intermediate 2-trans-enoyl-CoA to (*R*)-3-hydroxyacyl-CoA | (Tsuge et al. 2000) |
| | DKK66_RS01725 | WP_207389837.1 | 798 | | |
| | DKK66_RS13920 | WP_131359121.1 | 1122 | | |
| | DKK66_RS13925 | WP_062790491.1 | 783 | | |
| | DKK66_RS14470 | WP_045845199.1 | 786 | | |
| | DKK66_RS17195 | WP_131360045.1 | 813 | | |
| | DKK66_RS19275 | WP_131360655.1 | 780 | | |
| MaoC family dehydratase | DKK66_RS04340 | WP_131355813.1 | 420 | Catalyses the (*R*)-specific hydration of the β-oxidation intermediate 2-trans-enoyl-CoA to (*R*)-3-hydroxyacyl-CoA | (Wang et al. 2013) |
| | DKK66_RS15015 | WP_131359405.1 | 456 | | |
| PHA synthase (*PhaC*) | DKK66_RS14295 | WP_014086688.1 | 1770 | Catalyses the polymerisation of (*R*)-3-hydroxybutyryl-CoA monomers into PHAs | (Huisman et al. 1989; Fiedler et al. 2002) |
| | DKK66_RS16180 | WP_131359719.1 | 1638 | | |

however, our PhaC1$_{As}$ is classified into a special class of class I PhaC where it is capable of incorporating both SCL-PHA and MCL-PHA (Rehm and Steinbüchel 1999; Neoh et al. 2022). This characteristic of PhaC is similar to previously isolated PhaCs, such as PhaC$_{Cs}$ from *Chromobacterium* sp. USM2, PhaC$_{Ac}$ from *Aeromonas caviae* and PhaC isolated from mangrove soil metagenome (PhaC$_{BP\text{-}M\text{-}CPF4}$) which are also class I PhaC that are able to incorporate the MCL-PHA monomer (Doi et al. 1995; Bhubalan et al. 2011; Foong et al. 2017).

PhaJ and MaoC family dehydratase were the enzymes involved in supplying monomers for PHA biosynthesis through the β-oxidation pathway (Tsuge et al. 2000; Wang et al. 2013). PhaJ plays a vital role in 3HHx accumulation, as it creates a pathway for supplying (*R*)-3-hydroxyhexanoate-CoA, [(*R*)-3HHx-CoA] monomer units from fatty acid β-oxidation (Fukui & Doi 1997). The co-expression of *phaJ* and *phaC* can enhance the accumulation and incorporation of 3HHx into the P(3HB-*co*-3HHx) (Budde et al. 2011; Wang et al. 2013; Tan et al. 2020). The PhaP is a group of amphiphilic proteins that consists of both hydrophobic and hydrophilic surface that binds to the surfaces of PHA granules accumulated in the form of inclusion body in the bacterial cells (Fukui et al. 2001; Zhao et al. 2016).

## Production of PHA

Our previous research has shown that *Aquitalea* sp. USM4 can accumulate up to 1.5 g/L of PHA (Ng and Sudesh 2016) when cultivated in MM (Doi et al. 1995). Depending on the type of carbon sources and precursors added into the culture, the PHA copolymers accumulated were composed of 3HB, 3HV, 4HB, and 3H4MV.

In this study, PhaC1$_{As}$ was cloned into plasmid pBBR1MCS-2 with the *phaC1* promoter from *C. necator* and transconjugated into *C. necator* PHB$^{-}$4 (Foong et al. 2017). The transformant was cultured in MM to induce PHA production (Budde et al. 2010). Compared to the previous result from Ng and Sudesh (2016), there is an improvement in the dry cell weight of the culture. The transformant increased in dry cell weight from 2.7 to 3.8 g/L when cultivated using fructose co-fed with sodium 3-hydroxyvalerate. The transformant also showed increments of 4.4–4.6 g/L in dry cell weight when CPKO was used as the carbon source.

The marked improvement is probably due to the effect of the promoter derived from *phaC1* of *C. necator* (P$_{phaC1}$) exhibiting better expression of *phaC*$_{As}$ for PHA biosynthesis as compared to the *lacZ* promoter (P$_{lacZ}$). Fukui et al. (2011) reported that different promoters could affect the expression

**Table 4** PHA biosynthesis *C. necator* transformant harbouring pBBR1MCS2-$C_{As}$ using different carbon sources and precursors in shake flask experiments

| Carbon source | Dry cell weight (g/L) | PHA content (wt. %) | Monomer composition (mol %) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3HB | 3HV | 4HB | 5HV | 3HHx | 3H4MV |
| 10 g/L fructose | 3.7 ± 0.2 | 43.0 ± 1.1 | 100 | – | – | – | – | – |
| 10 g/L fructose + 2 g/L sodium 3-hydroxyvalerate | 3.8 ± 0.1 | 49.0 ± 5.3 | 95 ± 0 | 5 ± 0 | – | – | – | – |
| 10 g/L fructose + 2 g/L sodium 4-hydroxybutyrate | 4.4 ± 0.2 | 49.1 ± 4.2 | 89 ± 3 | – | 11 ± 3 | – | – | – |
| 10 g/L fructose + 2 g/L sodium 5-hydroxyvalerate | 4.7 ± 0.0 | 49.5 ± 7.1 | 74 ± 4 | – | – | 26 ± 4 | – | – |
| 10 g/L fructose + 2 g/L sodium 3-hydroxyhexanoate | 2.8 ± 0.2 | 43.1 ± 2.1 | 98 ± 0 | – | – | – | 2.2 ± 0 | – |
| 10 g/L fructose + 1 g/L isocaproic acid | 2.6 ± 0.0 | 26.9 ± 6.2 | 75 ± 5 | – | – | – | – | 25 ± 5 |
| CPKO | 4.6 ± 0.2 | 68.8 ± 2.7 | 97 ± 0 | – | – | – | 3.3 ± 0 | – |

Data shown are the means ± standard deviation of triplicate results.

*C. necator* PHB$^{-}$4 transformant was cultivated for 48 h, at 30℃ and 200 rpm in MM supplemented with 10 g/L of carbon sources or carbon source with precursors, 0.54 g/L of urea, and 50 µg/mL kanamycin.

of *phaC* gene, as they reported that the strain with $P_{phaC1}$ had better P(3HB) accumulation when cultured using fructose and soybean oil compared to the strain with $P_{lacZ}$. A better expression of $PhaC_{As}$ using $P_{phaC1}$ probably allows the *C. necator* PHB$^{-}$4 to accumulate more PHA and better dry cell weight. Furthermore, the addition of ribosome-binding site (RBS) [AGGAGG] is also suspected to increase the expression of $PhaC_{As}$ and, hence, increase the concentration of $PhaC_{As}$ in bacterial cells for better PHA accumulation. A conceptually similar work was also carried out by Arikawa and Matsumoto (2016) using the RBS from *C. necator* [AGAGAGA] with trc promoter ($P_{trc}$), lacUV5 promoter ($P_{lacUV5}$), and trp promoter ($P_{trp}$), which showed remarkably improved synthase activity due to better gene expression, hinting at the importance of RBS in the expression of PhaC.

Compared to the previous result by Ng and Sudesh (2016), there is a stark improvement in terms of substrate specificity of the $PhaC_{As}$, which is the ability to incorporate 3HHx and 5HV in the copolymer produced when supplemented with CPKO, fructose with Na3HHx, and fructose with Na5HV, respectively. A total of 2.2 mol% and 3.3 mol% of 3HHx monomer were successfully

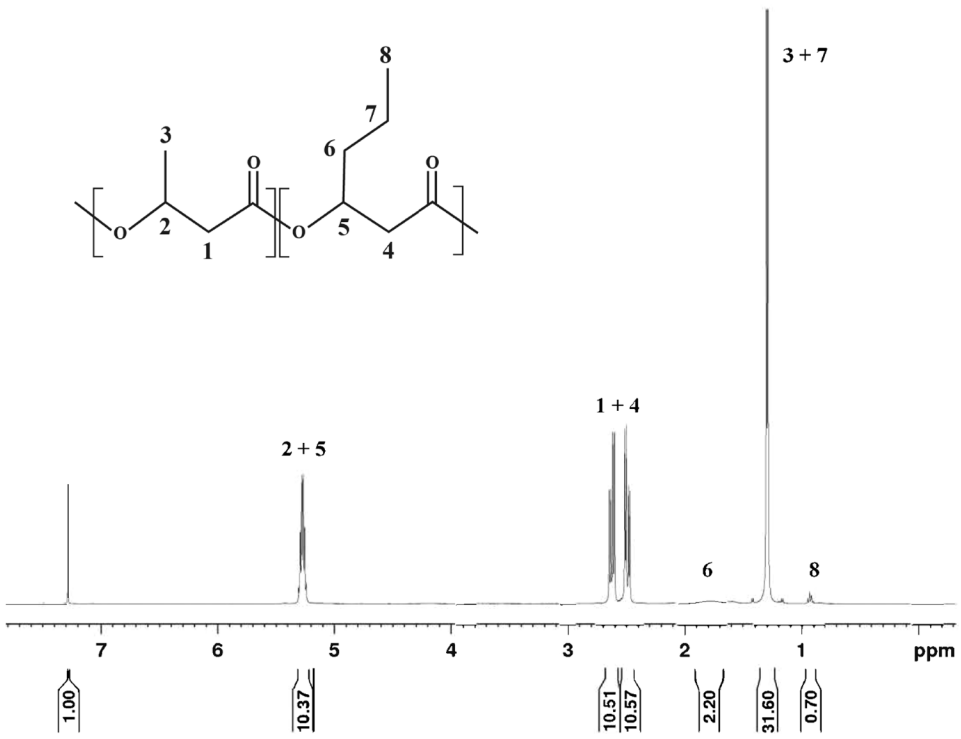


**Fig. 1** Proton nuclear magnetic resonance spectroscopy ($^{1}$H NMR) spectrum of P(3HB-*co*-3HHx) copolymer consisting of 98 mol% 3HB and 2.2 mol% 3HHx synthesised by *C. necator* PHB$^{-}$4 transformant harbouring pBBR1MCS2-$C_{As}$ from 10 g/L fructose and 2 g/L sodium 3-hydroxyhexanoate

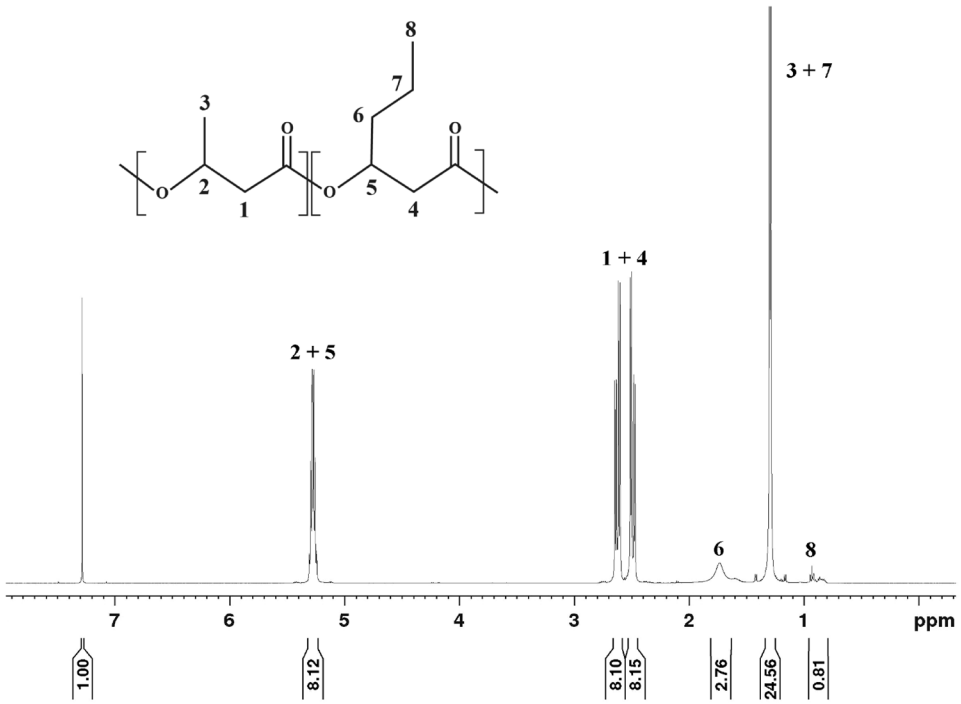


**Fig. 2** Proton nuclear magnetic resonance spectroscopy (.$^{1}$H NMR) spectrum of P(3HB-*co*-3HHx) copolymer consisting of 97 mol% 3HB and 3.3 mol% 3HHx synthesised by *C. necator* PHB$^{-}$4 transformant harbouring pBBR1MCS2-$C_{As}$ from 10 g/L crude plum kernel oil (CPKO)

incorporated when the transformant was fed with fructose with Na3HHx and CPKO, respectively, whereas 26 mol% of 5HV monomer was successfully incorporated into P(3HB-*co*-5HV) copolymer (Table 4). The P(3HB-*co*-3HHx) copolymer was then subjected to additional verification by $^{1}$H NMR (Figs. 1 and 2) to confirm the GC result for 3HHx in the P(3HB-*co*-3HHx) copolymer. The results were consistent as compared to the GC result, so it proved that PhaC1$_{As}$ could incorporate 3HHx as well.

P(3HB-*co*-3HHx) is a PHA copolymer reported to have a close resemblance to commercial polypropylene (PP) and low-density polyethylene (LDPE) (Doi 1990). This suggests that P(3HB-*co*-3HHx) is capable of replacing single-use petrochemical plastic. Besides that, P(3HB-*co*-3HHx) is also reported to be applied in the field of tissue engineering, where it can be moulded into a scaffold for bone tissue engineering (Ang et al. 2020). As for P(3HB-*co*-5HV), the copolymers or terpolymers with 5HV monomers were reported to have the potential as biomaterial (Chuah et al. 2013; Lakshmanan et al. 2019). Lakshmanan et al. (2019) reported that lipases could degrade co- and terpolymer of 5HV. Chuah et al. (2013) also reported that terpolymer of 5HV was less cytotoxic and good for cell proliferation.

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**Author contributions** JHW and LMN were involved in conceptualization. JHW, LMN, and RK designed and performed the experiment. JHW, LMN, and SZN were involved in formal analysis and wrote the original draft. KS and SK provided supervision. KS provided the funding. All authors reviewed and edited the manuscript.

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**Data availability** The datasets generated during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare no conflict of interest regarding the publication of this article.

**Ethics approval and consent to participate** This manuscript does not report data collected from humans or animals.

**Consent for publication** Not applicable.

## References

Anderson AJ, Dawes EA (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol Mol Biol Rev 54(4):450–472. https://doi.org/10.1128/mr.54.4.450-472.1990

Ang SL, Shaharuddin B, Chuah J-A, Sudesh K (2020) Electrospun poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)/silk fibroin film is a promising scaffold for bone tissue engineering. Int J Biol

Macromol 145:173–188. https://doi.org/10.1016/j.ijbiomac.2019.12.149

Arikawa H, Matsumoto K (2016) Evaluation of gene expression cassettes and production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) with a fine modulated monomer composition by using it in *Cupriavidus necator*. Microb Cell Fact 15(1):184. https://doi.org/10.1186/s12934-016-0583-7

Bhubalan K, Chuah JA, Shozui F, Brigham CJ, Taguchi S, Sinskey AJ, Rha C, Sudesh K (2011) Characterization of the highly active polyhydroxyalkanoate synthase of *Chromobacterium* sp. strain USM2. Appl Environ Microbiol 77(9):2926–2933. https://doi.org/10.1128/aem.01997-10

Braunegg G, Sonnleitner B, Lafferty R (1978) A rapid gas chromatographic method for the determination of poly-*β*-hydroxybutyric acid in microbial biomass. Eur J Appl Microbiol Biotechnol 6(1):29–37. https://doi.org/10.1007/BF00500854

Brito C, Carvalho CB, Santos F, Gazzinelli RT, Oliveira SC, Azevedo V, Teixeira S (2004) *Chromobacterium violaceum* genome: molecular mechanisms associated with pathogenicity. Gen Mol Res 3(1):148–161

Budde CF, Mahan AE, Lu J, Rha C, Sinskey AJ (2010) Roles of multiple acetoacetyl coenzyme A reductases in polyhydroxybutyrate biosynthesis in *Ralstonia eutropha* H16. J Bacteriol 192(20):5319–5328. https://doi.org/10.1128/JB.00207-10

Budde CF, Riedel SL, Willis LB, Rha C, Sinskey AJ (2011) Production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from plant oil by engineered *Ralstonia eutropha* strains. Appl Environ Microbiol 77(9):2847–2854. https://doi.org/10.1128/AEM.02429-10

Chee JY, Yoga SS, Lau N-S, Ling SC, Abed RM, Sudesh K (2010) Bacterially produced polyhydroxyalkanoate (PHA): converting renewable resources into bioplastics. Curr Res Technol Educ Top Appl Microbiol Microb Biotechnol 2:1395–1404

Chen I-MA, Chu K, Palaniappan K, Ratner A, Huang J, Huntemann M, Hajek P, Ritter S, Varghese N, Seshadri R, Roux S, Woyke T, Eloe-Fadrosh EA, Ivanova NN, Kyrpides NC (2020) The IMG/M data management and analysis system v.6.0: new tools and advanced capabilities. Nucl Acid Res 49(D1):D751–D763. https://doi.org/10.1093/nar/gkaa939

Chuah J-A, Yamada M, Taguchi S, Sudesh K, Doi Y, Numata K (2013) Biosynthesis and characterization of polyhydroxyalkanoate containing 5-hydroxyvalerate units: effects of 5HV units on biodegradability, cytotoxicity, mechanical and thermal properties. Polym Degrad Stab 98(1):331–338. https://doi.org/10.1016/j.polymdegradstab.2012.09.008

Doi Y (1990) Microbial polyesters: VCH, New York

Doi Y, Kitamura S, Abe H (1995) Microbial synthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate). Macromolecules 28(14):4822–4828. https://doi.org/10.1021/ma00118a007

Ebadzadsahrai G, Soby S (2018) Draft genome sequence of *Aquitalea* sp. strain MWU14–2217, isolated from a wild cranberry bog in Provincetown, Massachusetts. Microbiol Resour Announ 7(21):e01493-e11418. https://doi.org/10.1128/MRA.01493-18

Fiedler S, Steinbüchel A, Rehm BH (2002) The role of the fatty acid β-oxidation multienzyme complex from *Pseudomonas oleovorans* in polyhydroxyalkanoate biosynthesis: molecular characterization of the *fadBA* operon from *P. oleovorans* and of the enoyl-CoA hydratase genes *phaJ* from *P. oleovorans* and *Pseudomonas putida*. Arch Microbiol 178(2):149–160. https://doi.org/10.1007/s00203-002-0444-0

Foong CP, Lakshmanan M, Abe H, Taylor TD, Foong SY, Sudesh K (2017) A novel and wide substrate specific polyhydroxyalkanoate (PHA) synthase from unculturable bacteria found in mangrove soil. J Polym Res 25(1):23. https://doi.org/10.1007/s10965-017-1403-4

Friedrich B, Hogrefe C, Schlegel HG (1981) Naturally occurring genetic transfer of hydrogen-oxidizing ability between strains of *Alcaligenes eutrophus*. J Bacteriol 147(1):198–205. https://doi.org/10.1128/jb.147.1.198-205.1981

Fukui T, Doi Y (1997) Cloning and analysis of the poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) biosynthesis genes of *Aeromonas caviae*. J Bacteriol 179(15):4821–4830. https://doi.org/10.1128/jb.179.15.4821-4830.1997

Fukui T, Kichise T, Iwata T, Doi Y (2001) Characterization of 13 kDa granule-associated protein in *Aeromonas caviae* and biosynthesis of polyhydroxyalkanoates with altered molar composition by recombinant bacteria. Biomacromol 2(1):148–153. https://doi.org/10.1021/bm0056052

Fukui T, Ohsawa K, Mifune J, Orita I, Nakamura S (2011) Evaluation of promoters for gene expression in polyhydroxyalkanoate-producing *Cupriavidus necator* H16. Appl Microbiol Biotechnol 89(5):1527–1536. https://doi.org/10.1007/s00253-011-3100-2

Gamal RF, Abdelhady HM, Khodair TA, El-Tayeb TS, Hassan EA, Aboutaleb KA (2013) Semi-scale production of PHAs from waste frying oil by *Pseudomonas fluorescens* S48. Braz J Microbiol 44(2):539–549. https://doi.org/10.1590/S1517-83822013000200034

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities 57(1): 81–91. https://doi.org/10.1099/ijs.0.64483-0

Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR (2018) RefSeq: an update on prokaryotic genome annotation and curation. Nucl Acids Res 46(D1):D851–D860. https://doi.org/10.1093/nar/gkx1068

Hassan MA, Yee L-N, Yee PL, Ariffin H, Raha AR, Shirai Y, Sudesh K (2013) Sustainable production of polyhydroxyalkanoates from renewable oil-palm biomass. Biomass Bioenerg 50:1–9. https://doi.org/10.1016/j.biombioe.2012.10.014

Huisman GW, de Leeuw O, Eggink G, Witholt B (1989) Synthesis of poly-3-hydroxyalkanoates is a common feature of fluorescent pseudomonads. Appl Environ Microbiol 55(8):1949–1954. https://doi.org/10.1128/aem.55.8.1949-1954.1989

Ishizawa H, Kuroda M, Ike M (2017) Draft genome sequence of *Aquitalea magnusonii* strain H3, a plant growth-promoting bacterium of duckweed (Lemna minor). Genom Announ. https://doi.org/10.1128/genomeA.00812-17

Jendrossek D, Handrick R (2002) Microbial degradation of polyhydroxyalkanoates. Annu Rev Microbiol 56:403–432. https://doi.org/10.1146/annurev.micro.56.012302.160838

Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H (2014) Efficient *de novo* assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 24(8):1384–1395. https://doi.org/10.1101/gr.170720.113

Kajitani R, Noguchi H, Gotoh Y, Ogura Y, Yoshimura D, Okuno M, Toyoda A, Kuwahara T, Hayashi T, Itoh T (2021) MetaPlatanus: a metagenome assembler that combines long-range sequence links and species-specific features. Nucl Acids Res 49(22):e130–e130. https://doi.org/10.1093/nar/gkab831

Kolibachuk D, Miller A, Dennis D (1999) Cloning, molecular analysis, and expression of the polyhydroxyalkanoic acid synthase (*phaC*) gene from *Chromobacterium violaceum*. Appl Environ Microbiol 65(8):3561–3565. https://doi.org/10.1128/AEM.65.8.3561-3565.1999

Kovach ME, Elzer PH, Steven Hill D, Robertson GT, Farris MA, Roop RM, Peterson KM (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166(1):175–176. https://doi.org/10.1016/0378-1119(95)00584-1

Lakshmanan M, Foong CP, Abe H, Sudesh K (2019) Biosynthesis and characterization of co and ter-polyesters of polyhydroxyalkanoates containing high monomeric fractions of 4-hydroxybutyrate and 5-hydroxyvalerate via a novel PHA synthase. Polym Degrad Stab 163:122–135. https://doi.org/10.1016/j.polymdegradstab.2019.03.005

Lau H-T, Faryna J, Triplett EW (2006) *Aquitalea magnusonii* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from a humic lake. Int J Syst Evolut Microbiol 56(4):867–871. https://doi.org/10.1099/ijs.0.64089-0

Lee SY, Choi J-i (1999) Production and degradation of polyhydroxyalkanoates in waste environment. Waste Manage 19(2):133–139. https://doi.org/10.1016/S0956-053X(99)00005-7

Lee W-H, Loo C-Y, Nomura CT, Sudesh K (2008) Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3-hydroxyvalerate precursors. Biores Technol 99(15):6844–6851. https://doi.org/10.1016/j.biortech.2008.01.051

Lee C-M, Weon H-Y, Kim Y-J, Son J-A, Yoon S-H, Koo B-S, Kwon S-W (2009) *Aquitalea denitrificans* sp. Nov., isolated from a Korean wetland. Int J Syst Evolut Microbiol 59(5):1045–1048. https://doi.org/10.1099/ijs.0.002840-0

Lee I, Ouk Kim Y, Park S-C, Chun J. (2016). OrthoANI: an improved algorithm and software for calculating average nucleotide identity. 66(2), 1100–1103. https://doi.org/10.1099/ijsem.0.000760

Ling SC, Tsuge T, Sudesh K (2011) Biosynthesis of novel polyhydroxyalkanoate containing 3-hydroxy-4-methylvalerate by *Chromobacterium* sp. USM2. J Appl Microbiol 111(3):559–571. https://doi.org/10.1111/j.1365-2672.2011.05084.x

Lopez-Llorca L, Valiente MC, Gascon A (1993) A study of biodegradation of poly-$\beta$-hydroxyalkanoate (PHA) films in soil using scanning electron microscopy. Micron 24(1):23–29. https://doi.org/10.1016/0968-4328(93)90012-P

Maehara A, Taguchi S, Nishiyama T, Yamane T, Doi Y (2002) A repressor protein, PhaR, regulates polyhydroxyalkanoate (PHA) synthesis via its direct interaction with PHA. J Bacteriol 184(14):3992–4002. https://doi.org/10.1128/JB.184.14.3992-4002.2002

McCool GJ, Cannon MC (2001) PhaC and PhaR are required for polyhydroxyalkanoic acid synthase activity in *Bacillus megaterium*. J Bacteriol 183(14):4235–4243. https://doi.org/10.1128/JB.183.14.4235-4243.2001

Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14(1):60. https://doi.org/10.1186/1471-2105-14-60

Mukherjee S, Stamatis D, Bertsch J, Ovchinnikova G, Sundaramurthi Jagadish C, Lee J, Kandimalla M, Chen I-MA, Kyrpides NC, Reddy TBK (2020) Genomes OnLine Database (GOLD) vol 8: overview and updates. Nucl Acids Res 49(D1):D723–D733. https://doi.org/10.1093/nar/gkaa983

Neoh SZ, Chek MF, Tan HT, Linares-Pastén JA, Nandakumar A, Hakoshima T, Sudesh K (2022) Polyhydroxyalkanoate synthase (PhaC): the key enzyme for biopolyester synthesis. Curr Res Biotechnol 4:87–101. https://doi.org/10.1016/j.crbiot.2022.01.002

Ng LM, Sudesh K (2016) Identification of a new polyhydroxyalkanoate (PHA) producer *Aquitalea* sp. USM4 (JCM 19919) and characterization of its PHA synthase. J Biosci Bioeng 122(5):550–557. https://doi.org/10.1016/j.jbiosc.2016.03.024

Ngo HT, Kim H, Trinh H, Yi T-H (2020) *Aquitalea aquatilis* sp. Nov., isolated from Jungwon waterfall. Int J Syst Evolut Microbiol 70(9):4903–4907. https://doi.org/10.1099/ijsem.0.004351

Peoples OP, Sinskey AJ (1989) Poly-beta-hydroxybutyrate (PHB) biosynthesis in *Alcaligenes eutrophus* H16: identification and characterization of the PHB polymerase gene (*phbC*). J Biol Chem 264(26):15298–15303. https://doi.org/10.1016/S0021-9258(19)84825-1

Reddy C, Ghai R, Kalia VC (2003) Polyhydroxyalkanoates: an overview. Biores Technol 87(2):137–146. https://doi.org/10.1016/S0960-8524(02)00212-2

Rehm BHA (2003) Polyester synthases: natural catalysts for plastics. Biochem J 376(1):15–33. https://doi.org/10.1042/bj20031254

Rehm BHA, Steinbüchel A (1999) Biochemical and genetic analysis of PHA synthases and other proteins required for PHA synthesis. Int J Biol Macromol 25(1):3–19. https://doi.org/10.1016/S0141-8130(99)00010-0

Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci 106(45):19126–19131. https://doi.org/10.1073/pnas.0906412106

Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J (2015) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32(6):929–931. https://doi.org/10.1093/bioinformatics/btv681

Riedel SL, Bader J, Brigham CJ, Budde CF, Yusof ZA, Rha C, Sinskey AJ (2012) Production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) by *Ralstonia eutropha* in high cell density palm oil fermentations. Biotechnol Bioeng 109(1):74–83. https://doi.org/10.1002/bit.23283

Sedláček I, Kwon S-W, Švec P, Mašlaňová I, Kýrová K, Holochová P, Černohlávková J, Busse H-J. (2016). *Aquitalea pelogenes* sp nov, isolated from mineral peloid. Int J Syst Evolut Microbiol 66(2), 962–967. https://doi.org/10.1099/ijsem.0.000819

Simon R, Priefer U, Pühler A (1983) A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. Nat Biotechnol 1(9):784–791

Slater SC, Voige WH, Dennis DE (1988) Cloning and expression in *Escherichia coli* of the *Alcaligenes eutrophus* H16 poly-$\beta$-hydroxybutyrate biosynthetic pathway. J Bacteriol 170(10):4431–4436. https://doi.org/10.1128/jb.170.10.4431-4436.1988

Steinbüchel A, Valentin HE (1995) Diversity of bacterial polyhydroxyalkanoic acids. FEMS Microbiol Lett 128(3):219–228. https://doi.org/10.1111/j.1574-6968.1995.tb07528.x

Steinbüchel A, Debzi E-M, Marchessault RH, Timm A (1993) Synthesis and production of poly(3-hydroxyvaleric acid) homopolyester by *Chromobacterium violaceum*. Appl Microbiol Biotechnol 39(4):443–449. https://doi.org/10.1007/BF00205030

Sudesh K, Abe H, Doi Y (2000) Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci 25(10):1503–1555. https://doi.org/10.1016/S0079-6700(00)00035-6

Tan HT, Chek MF, Lakshmanan M, Foong CP, Hakoshima T, Sudesh K (2020) Evaluation of BP-M-CPF4 polyhydroxyalkanoate (PHA) synthase on the production of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from plant oil using *Cupriavidus necator* transformants. Int J Biol Macromol 159:250–257. https://doi.org/10.1016/j.ijbiomac.2020.05.064

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucl Acids Res 44(14):6614–6624. https://doi.org/10.1093/nar/gkw569

Thinagaran L, Sudesh K (2019) Evaluation of sludge palm oil as feedstock and development of efficient method for its utilization to produce polyhydroxyalkanoate. Waste Biomass Valorization 10:709–720. https://doi.org/10.1007/s12649-017-0078-8

Tsuge T, Fukui T, Matsusaki H, Taguchi S, Kobayashi G, Ishizaki A, Doi Y (2000) Molecular cloning of two (*R*)-specific enoyl-CoA hydratase genes from *Pseudomonas aeruginosa* and their use for polyhydroxyalkanoate synthesis. FEMS Microbiol Lett 184(2):193–198. https://doi.org/10.1111/j.1574-6968.2000.tb09013.x

Ushimaru K, Motoda Y, Numata K, Tsuge T, Parales RE (2014) Phasin proteins activate *Aeromonas caviae* polyhydroxyalkanoate (PHA) synthase but not *Ralstonia eutropha* PHA synthase. Appl

Environ Microbiol 80(9):2867–2873. https://doi.org/10.1128/AEM.04179-13

Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati A (2015) Microbial species delineation using whole genome sequences. Nucl Acids Res 43(14):6761–6771. https://doi.org/10.1093/nar/gkv657

Wang H, Zhang K, Zhu J, Song W, Zhao L, Zhang X (2013) Structure reveals regulatory mechanisms of a MaoC-like hydratase from *Phytophthora capsici* involved in biosynthesis of polyhydroxyalkanoates (PHAs). PLoS ONE 8(11):e80024. https://doi.org/10.1371/journal.pone.0080024

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110(10):1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Zain N-AA, Ng L-M, Foong CP, Tai YT, Nanthini J, Sudesh K (2020) Complete genome sequence of a novel polyhydroxyalkanoate (PHA) producer, *Jeongeupia* sp. USM3 (JCM 19920) and characterization of its PHA synthases. Curr Microbiol 77(3):500–508. https://doi.org/10.1007/s00284-019-01852-z

Zhao H, Wei H, Liu X, Yao Z, Xu M, Wei D, Wang J, Wang X, Chen G-Q (2016) Structural insights on PHA binding protein PhaP from *Aeromonas hydrophila*. Sci Rep 6(1):39424. https://doi.org/10.1038/srep39424