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RESEARCH PAPER

Biosynthesis of Poly(2-hydroxybutyrate-co-lactate) in Metabolically Engineered *Escherichia coli*

Cheol Gi Chae, You Jin Kim, Se Jin Lee, Young Hoon Oh, Jung Eun Yang, Jeong Chan Joo, Kyoung Hee Kang, Young-Ah Jang, Hyuk Lee, A-Reum Park, Bong Keun Song, Sang Yup Lee, and Si Jae Park

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Abstract We have previously reported *in vivo* biosynthesis of polyhydroxyalkanoates containing 2-hydroxyacid monomers such as lactate and 2-hydroxybutyrate in recombinant *Escherichia coli* strains by the expression of evolved *Clostridium propionicum* propionyl-CoA transferase (Pct_{Cp}) and *Pseudomonas* sp. MBEL 6-19 polyhydroxyalkanoate (PHA) synthase 1 ($PhaC1_{Ps6\text{-}19}$). Here, we report the biosynthesis of poly(2-hydroxybutyrate-co-lactate)[P(2HB-co-LA)] by direct fermentation of metabolically engineered *E. coli* strain. Among *E. coli* strains WL3110, XL1-Blue, and BL21(DE3), recombinant *E. coli* XL1-Blue strain expressing PhaC1437 and Pct540 produced P(76.4mol%2HB-co-23.6mol%LA) to the highest content of 88 wt% when it was cultured in a chemically defined medium containing 20 g/L of glucose and 2 g/L of sodium 2-hydroxybutyrate. When recombinant *E. coli* XL1-Blue strain expressing PhaC1437 and Pct540 was cultured in a chemically defined medium containing 20 g/L of glucose and varying concentration of sodium 2-hydroxybutyrate, 2HB monomer fraction in P(2HB-co-LA) increased proportional to the concentration of sodium 2-hydroxybutyrate added to the culture medium. P(2HB-co-LA)] could also be produced from glucose as a sole carbon source without sodium 2-hydroxybutyrate into the culture medium. Recombinant *E. coli* XL1-Blue strain expressing the *phaC1437*, *pct540*, *cimA3.7*, and *leuBCD* genes together with the *L. lactis* Il1403 *panE* gene, successfully produced P(23.5mol%2HB-co-76.5mol%LA)] to the polymer content of 19.4 wt% when it cultured in a chemically defined medium containing 20 g/L of glucose. The metabolic engineering strategy reported here should be useful for the production of novel copolymer P(2HB-co-LA)].

Keywords: PHA, 2-hydroxyacid, lactate, 2-hydroxybutyrate, poly(2-hydroxybutyrate-co-lactate), recombinant *E. coli*

Cheol Gi Chae, You Jin Kim, Si Jae Park*
Department of Environmental Engineering and Energy, Myongji University, Yongin 17058, Korea
Tel: +82-31-324-1337; Fax: +82-31-336-6336
E-mail: parksj93@mju.ac.kr

Se Jin Lee, Young Hoon Oh, Jeong Chan Joo, Kyoung Hee Kang, Young-Ah Jang, Bong Keun Song
Center for Bio-based Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 34114, Korea

Jung Eun Yang, Sang Yup Lee
Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Program), Center for Systems and Synthetic Biotechnology, and Institute for the BioCentury, KAIST, Daejeon 34141, Korea

Hyuk Lee, A-Reum Park
Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, Daejeon 34114, Korea

1. Introduction

Bio-based processes have been developed for the production of green chemicals, fuels, and polymers from renewable resources to solve the environmental problems such as global warming [1]. Natural microorganisms have been engineered as host strains for such bioprocesses to produce bio-based fuels, polymers and chemicals. Representative products from bio-based processes include ethanol, butanol, polyhydroxyalkanotes (PHAs), polylactic acid (PLA), 1,4-butanediol, 1,3-propanediol, and cadaverine [1].

PHAs are naturally synthesized biopolyesters that are accumulated in many bacteria as carbon and reducing power storage material, the monomers of which are generated through inherent intermediates of metabolic pathways of

host strains [2,3]. The key enzyme for the synthesis of PHAs is the PHA synthase that uses various hydroxyacyl-CoAs (HA-CoAs) as its substrates. Up to date, more than 150 kinds of hydroxycarboxylic acids have been identified as monomer constituents of PHAs [4]. Since PHA synthase only accepts (*R*)-HA-CoAs as its substrate if HA-CoAs contain a chiral center at the carbon position of hydroxyl group, PHA monomers are all in (*R*)-configuration. Among various HA-CoAs, natural PHA synthases prefer 3-hydroxyacyl-CoAs (3HA-CoAs) along with 4-, 5- and 6-hydroxyacyl-CoAs [2,3], however, it hardly accepts 2-hydroxyacyl-CoAs such as lactate (LA) and 2-hydroxybutyrate (2HB) as substrates due to much low or negligible activities towards 2-HA-CoAs compared with other HA-CoAs [5-7]. Thus, there has been no report on the *in vivo* biosynthesis of PHAs containing 2-hydroxyacid monomer by natural PHA producing bacteria.

Recently, we have developed metabolically engineered *Escherichia coli* strains able to produce PHAs containing 2-hydroxyacid monomers such as lactate and 2HB by employing evolved PHA synthase and evolved propionyl-CoA transferase as two key enzymes, in which engineered *Clostridium propionicum* propionyl-CoA transferase (Pct_{Cp}) converts (*D*)-lactate and (*D*)-2HB into (*D*)-lactyl-CoA and (*D*)-2HB-CoA, respectively, using acetyl-CoA as a CoA donor and (*D*)-lactyl-CoA and (*D*)-2HB-CoA are polymerized into PHAs by engineered *Pseudomonas* sp. 6-19 PHA synthase ($PhaC1_{Ps6\text{-}19}$) [5,8-16]. Also, similar strategies have been reported for 2-hydroxyacid containing PHAs [17-19]. Even though lactate and 2HB could be incorporated into PHA copolymer, its monomer fraction was still low due to low substrate specificity of engineered $PhaC1_{Ps6\text{-}19}$ towards lactyl-CoA and 2HB-CoA. Also, 3HB-CoA, the most favorable substrate of PHA synthase was needed for the efficient incorporation of lactate and 2HB monomers into PHA [5,8-16].

Since incorporation of 2-hydroxybutyrate monomer, analogous to lactate monomer, into polylactic acid (PLA) backbone, has been suggested to confer a soft, pliable, and stretchy material properties on rigid PLA polymer [19], thus, in this study, recombinant *E. coli* strains were further examined for the efficient production of PHAs containing 2-hydroxyacids such as lactate and 2HB as major monomers without incorporation of 3HB monomer.

Here, we examined the biosynthesis and characterization of novel copolymer, poly(2-hydroxybutyrate-co-lactate) [P(2HB-co-LA)] by metabolically engineered *E. coli* strains, in which sodium 2-hydroxybutyrate was added to the culture medium as a direct precursor for 2HB monomer in [P(2HB-co-LA)] while lactate was synthesized by inherent synthesis pathway from glucose. Furthermore, metabolic pathway for *in vivo* synthesis of P(2HB-co-LA) directly from glucose as a sole carbon source was developed in recombinant *E. coli* strain, in which 2HB and lactate were synthesized through recombinant and inherent metabolic pathways from glucose and utilized for the synthesis of P(2HB-co-LA).

2. Materials and Methods

2.1. Bacterial strains and plasmids

All bacterial strains and plasmids used in this study are listed in Table 1. For the production of P(2HB-co-LA), recombinant *E. coli* XL1-Blue, WL3110 [17], BL21(DE3) strains were used as host strains. *E. coli* XL1-Blue *ldhA* mutant, XLdh strain [10], was also used as a host strain. Plasmid p619C1437-pct540, which expresses the *Pseudomonas* sp. 6-19 PHA synthase gene containing quadruple mutations of E130D, S325T, S477G, and Q481K (PhaC1437) and the *C. propionicum* propionyl-CoA transferase mutant gene containing V193A and four silent nucleotide mutations of T78C, T669C, A1125G, and T1158C (Pct540) under the *Ralstonia eutropha* PHA biosynthesis operon promoter, has been previously described [5]. pKM22-PanE that expresses the *Lactococcus lactis subsp. lactis* Il1403 *panE* gene and

Table 1. Lists of bacterial strains and plasmids used in this study

Plasmid	Relevant characteristics	Reference or Source
Strains		
E. coli XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^R)]	Stratagene
E. coli XLdh	XL1-Blue *ΔldhA*	[10]
WL3110	W3110 *ΔlacI*	[20]
BL21(DE3)	F−*ompT hsdS*$_B$ (*r*^-_B *m*^-_B) *gal dem (DE3)*	Novagene
Plasmids		
p619C1437-pct540	pBluescript KS II(+) derivative; *R. eutropha* PHA biosynthesis promoter, the *phaC1437*$_{Ps6\text{-}19}$ gene, the *pct540*$_{Cp}$ gene; Ap^r	[5]
pKA32CimALeuBCD	pKA32-MCS derivative; the *M. jannaschii cimA3.7* gene, the *E. coi leuBCD* gene; Cm^r	[10]
pKM22PanE	pKM22-MCS derivative; $P_{LlacO\text{-}1}$ promoter, the *L. lactis subsp. lactis* Il1403 *PanE* gene; Km^r	[10]

pKA32-CimALeuBCD that expresses the *M. jannaschii cimA3.7* gene and the *E. coli leuBCD* genes have been previously described [10].

2.2. Culture condition

For the synthesis of P(2HB-co-LA), recombinant *E. coli* XL1-Blue, WL3110, BL21(DE3) strains were cultured in MR medium supplemented with 20 g/L of glucose and desired concentrations of 2HB at 30°C in a rotary shaker at 250 rpm for 96 h. Recombinant *E. coli* XLdh strain was also cultured in the same culture condition. The MR medium (pH 7.0) contains (per liter) 6.67 g KH_2PO_4, 4 g $(NH_4)_2HPO_4$, 0.8 g $MgSO_4{\cdot}7H_2O$, 0.8 g citric acid, and 5 mL trace metal solution. The trace metal solution contains (per liter of 0.5 M HCl) 10 g $FeSO_4{\cdot}7H_2O$, 2 g $CaCl_2$, 2.2 g $ZnSO_4{\cdot}7H_2O$, 0.5 g $MnSO_4{\cdot}4H_2O$, 1 g $CuSO_4{\cdot}5H_2O$, 0.1 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 0.02 g $Na_2B_4O_7{\cdot}10H_2O$. Glucose, $MgSO_4{\cdot}7H_2O$, sodium 2-hydroxybutyrate (Acros organics, Geel, Belgium) were sterilized separately. Sodium 2-hydroxybutyrate was added into the culture medium to different concentrations. Recombinant *E. coli* XL1-Blue harboring p619C1437-pct540, pKM22-PanE and pKA32-CimALeuBCD was grown to the OD_{600} of 0.5 before induction with 1 mM of IPTG for the expression of the *cimA*, *leuBCD, phaAB* and *panE* genes. Ampicillin (Ap, 50 μg/mL), kanamycin (Km, 30 μg/mL) and chloramphenicol (Cm, 34 μg/mL) were added to the medium depending on the resistance marker of the employed plasmid.

2.3. Polymer analysis

The content and monomer composition of the synthesized polymer were determined by gas chromatography (GC) [21]. Polymers were purified from the cells by the solvent extraction method [22]. The structure was determined by nuclear magnetic resonance (NMR) spectroscopy as previously described [5,16].

3. Results and Discussion

3.1. Biosynthesis of P(2HB-co-LA) in recombinant *E. coli* strains by adding sodium 2-hydroxybutyrate into the culture medium

We have previously reported the production of PHAs containing 2HB monomer in recombinant *E. coli* XL1-Blue strain expressing PhaC1437 and Pct540 by introduction of 3HB monomer as the second monomer to facilitate the incorporation of 2HB monomer into PHA in recombinant *E. coli* since 3HB-CoA, most favorable substrate of PHA synthase, could act as an initiator for 2-hydroxyacid containing polymer synthesis [10].

In this study, recombinant *E. coli* strains expressing

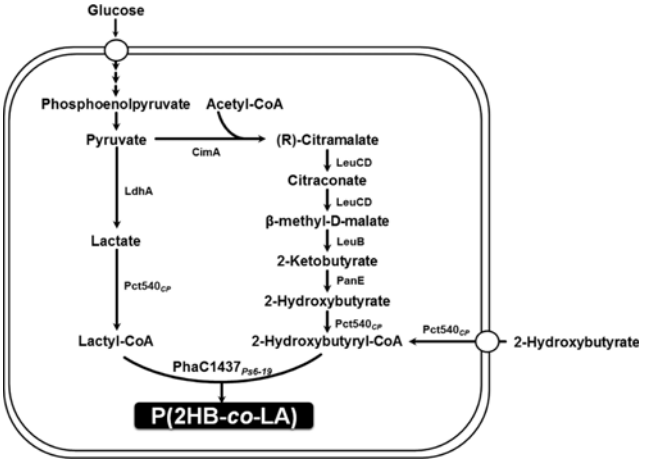

Fig. 1. Metabolic pathways for the production of P(2HB-co-LA) developed in this study. The overall metabolic pathways consisting of inherent metabolic pathways and introduced metabolic pathways for the production of P(2HB-co-LA) in *E. coli* are shown. Citramalate pathway is constructed by the expression of the evolved *M. jannaschii cimA3.7* gene and *E. coli leuBCD* genes to synthesize 2-hydroxybutyrate from glucose.

PhaC1437 and Pct540 were further examined whether they could synthesize PHAs containing 2HB monomer without assimilation of 3HB monomer since polylactate (PLA) homopolymer, the polymer consisting of only lactate, the representative 2-hydroxyacid monomer, could be produced by enhancing the metabolic capacity of host strain to supply more lactate into polymer synthesis pathway without incorporation of 3HB monomer [16]. Even though, the PLA content achieved in this system was as low as 4 wt%, it was reasoned that PHAs containing only 2-hydroxyacid monomers might be more efficiently synthesized if much monomer precursors are directly provided with polymer synthesis pathway. Metabolic pathways constructed in recombinant *E. coli* strains were described in Fig. 1. Three *E. coli* strains XL1-Blue, WL3110, and BL21(DE3) harboring p619C1437-pct540 were cultured in a chemically defined medium containing 20 g/L of glucose and 2 g/L of sodium 2-hydroxybutyrate, respectively. Inherently synthesized lactate was used as precursor for lactyl-CoA that is used up for the synthesis of P(2HB-co-LA) as previously reported [10,16]. All the recombinant *E. coli* strains expressing PhaC1437 and Pct540 were able to produce P(2HB-co-LA) with different polymer contents as shown in Fig. 1A, in which the highest P(2HB-co-LA) content of 88 wt% was achieved by recombinant *E. coli* XL1-Blue. 2HB monomer fraction of P(2HB-co-LA) was different depending on host strains ranging from 63.7 to 91.3 mol% (Fig. 2A). The highest 2HB monomer fraction was obtained by recombinant *E. coli* WL3110, but rather low polymer content of 34.1 wt% compared with that of recombinant *E. coli* XL1-Blue was

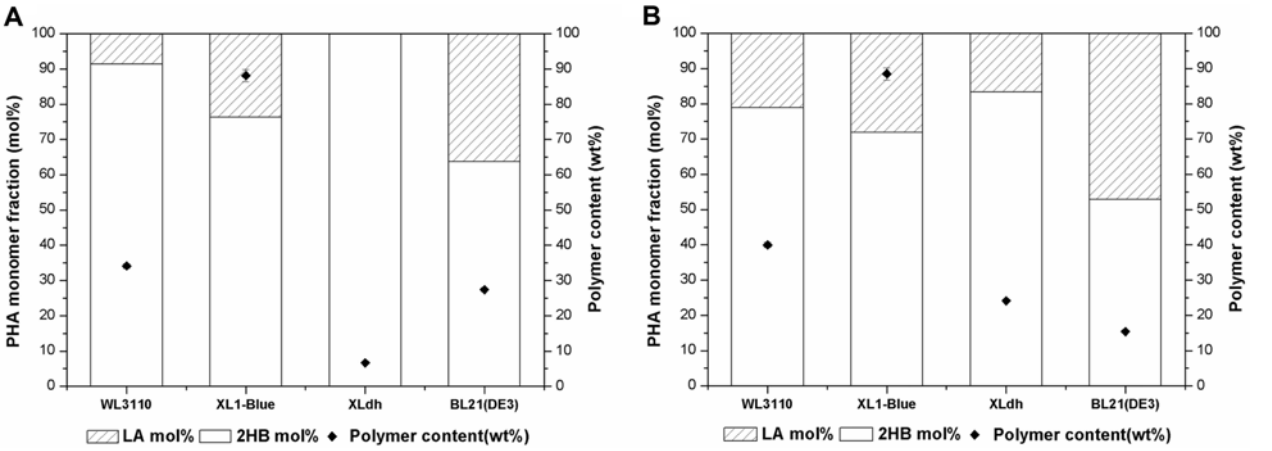

Fig. 2. Biosynthesis of P(2HB-co-LA) in recombinant *E. coli* strains harboring p619C1437-pct540 by adding sodium 2-hydroxybutyrate and sodium lactate into the culture medium. Recombinant *E. coli* XL1-Blue, WL3110, BL21(DE3) strains were examined for the synthesis of P(2HB-co-LA) in different culture medium as follows: (A) 2 g/L of sodium 2-hydroxybutyrate was added to the culture medium (B) 2 g/L of sodium 2-hydroxybutyrate and 2 g/L of sodium lactate were added to the culture medium. (Symbols are: striped bar, lactate monomer fraction in polymer; open bar, 2HB monomer fraction in polymer; filled diamond, polymer content).

achieved (Fig. 2A).

Since lactate is also the co-monomer of P(2HB-co-LA), the effect of supplementation of lactate into the culture medium was examined in recombinant *E. coli* strains expressing PhaC1437 and Pct540. When these *E. coli* strains were cultured in a chemically defined medium containing 20 g/L of glucose, 2 g/L of sodium 2-hydroxybutyrate, and 2 g/L of sodium lactate, increase of lactate monomer fraction in P(2HB-co-LA) was obtained, the extent of which was different depending on host strains (Fig. 2B). In recombinant *E. coli* WL3110, lactate monomer fraction was increased from 8.7 to 21.2 mol% by the supplementation of sodium lactate into the culture medium, but in the other strains, the increase of lactate monomer fraction was not significant (Fig. 2B). These results might be due to that metabolic capacity of inherent synthesis of lactate is different from each host strain.

E. coli XLdh strain, in which the *E. coli ldhA* gene has been completely deleted from the chromosome of *E. coli* XL1-Blue was also examined as host strain for P(2HB-co-LA). When it was cultured in a chemically defined medium containing 20 g/L of glucose and 2 g/L of sodium 2-hydroxybutyrate, P(2HB) homopolymer was synthesized to the polymer content of 6.7 wt% (Fig. 2A). When 2 g/L of sodium lactate was added to the culture medium, P(83.3mol%2HB-co-16.7mol%LA) was produced to to the polymer content of 24.2 wt% (Fig. 2B). It was interesting to note that XLdh strain could not produce PHAs consisting of only 2-hydroxyacid monomers such as lactate and 2HB in our previous report [10], however, in present study, it could support the production of P(2HB) homopolymer and P(2HB-co-LA). This may be resulted from the advance of detection procedure for 2-hydroxyacid methyl ester by GC. Since it is very hard to detect monomers of PHAs that are accumulated in host strains lower than 4 wt%, recently, we usually employed much amount of dried cells for methanolysis to prepare samples for GC analysis when recombinant host strains used for PHAs containing 2-hydroxyacid monomers were found to accumulate small amount of PHAs.

3.2. Biosynthesis of P(2HB-co-LA) in recombinant *E. coli* XL1-blue strain

Since *E. coli* XL1-Blue supported highest production of P(2HB-co-LA), it was further examined for the production of P(2HB-co-LA) having different 2HB monomer fraction in a culture medium containing different concentration of sodium 2-hydroxybutyrate. As shown in Fig. 3, as sodium 2-hydroxybutyrate concentration was increased from 0.2 to 1.5 g/L, 2HB monomer fraction in P(2HB-co-LA) was increased from 22.1 to 64.4 mol% along with the increase of P(2HB-co-LA) content from 2.4 to 51.6 wt%. Successful synthesis of P(2HB-co-LA) could be confirmed by NMR analysis (Fig. 4). It has been suggested that the material properties of P(2HB-co-LA) are in between of those of P(2HB) and PLA, the tensile strength of which is higher than that of P(2HB) and the elongation at break of which is smaller than that of P(2HB) [19]. Thus, P(2HB-co-LA) synthesized by incorporation of 2HB monomer into PLA backbone may be a soft, flexible, and elastic PLA-based copolymer, which has different application area compared with rigid PLA [19].

We have previously reported the production of PHAs containing 2HB monomer *via* native amino-acid pathways

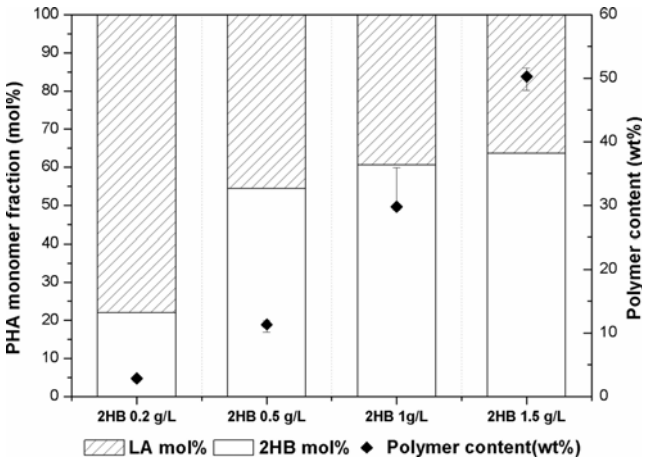

Fig. 3. Biosynthesis of P(2HB-co-LA) in recombinant *E. coli* XL1-Blue strain harboring p619C1437-pct540 cultured in chemically defined medium containing different concentration of sodium 2-hydroxybutyrate. Sodium 2-hydroxybutyrate was added to the culture medium to support the synthesis of 2HB-CoA by Pct540. Lactate, synthesized by inherent lactate synthesis pathway from glucose, was converted into lactyl-CoA by Pct540. 2HB-CoA and lactyl-CoA were polymerized into P(2HB-co-LA) by PhaC1437. (Symbols are: striped bar: lactate monomer fraction in polymer; open bar: 2HB monomer fraction in polymer; filled diamond, polymer content.

in microorganisms, which can provide 2-ketobutyrate, the possible precursor for 2HB [10]. The metabolic pathway for the production of 2-ketobutyrate was constructed by employing citramalate pathway constructed by the expression of the evolved *M. jannaschii cimA3.7* gene and *E. coli leuBCD* genes. 2-ketobutyrate was further converted into 2HB by (*D*)-2-hydroxyacid dehydrogenase encoded by the *L. lactis subsp. lactis* Il1403 *panE* gene [10]. The metabolically engineered *E. coli* XL1-Blue strain expressing the *phaC1437*, *pct540*, *cimA3.7*, and *leuBCD* genes along with the *L. lactis* Il1403 *panE* gene successfully produced P(23.5mol%2HB-co-76.5mol%LA) to the polymer content of 19.4 wt% when it was cultured in a chemically defined medium containing 20 g/L of glucose. To our knowledge, this is the first report about the production of P(2HB-co-LA) in recombinant *E. coli*, in which all the monomers 2HB and LA are synthesized from recombinant and inherent metabolic pathways developed in host strain using glucose as a sole carbon source.

4. Conclusion

In the present study, we have developed recombinant *E. coli* strains for *in vivo* synthesis of P(2HB-co-LA). P(2HB-co-LA) having different monomer composition could be synthesized by these recombinant *E. coli* strains by varying concentrations of sodium 2-hydroxybutyrate added to the culture medium as a direct precursor for 2HB monomer in P(2HB-co-LA). Furthermore, *in vivo* synthesis of P(2HB-co-LA) could be achieved for the first time from structurally unrelated carbon source, glucose by metabolically engineered *E. coli* strains. The strategies developed here would be useful

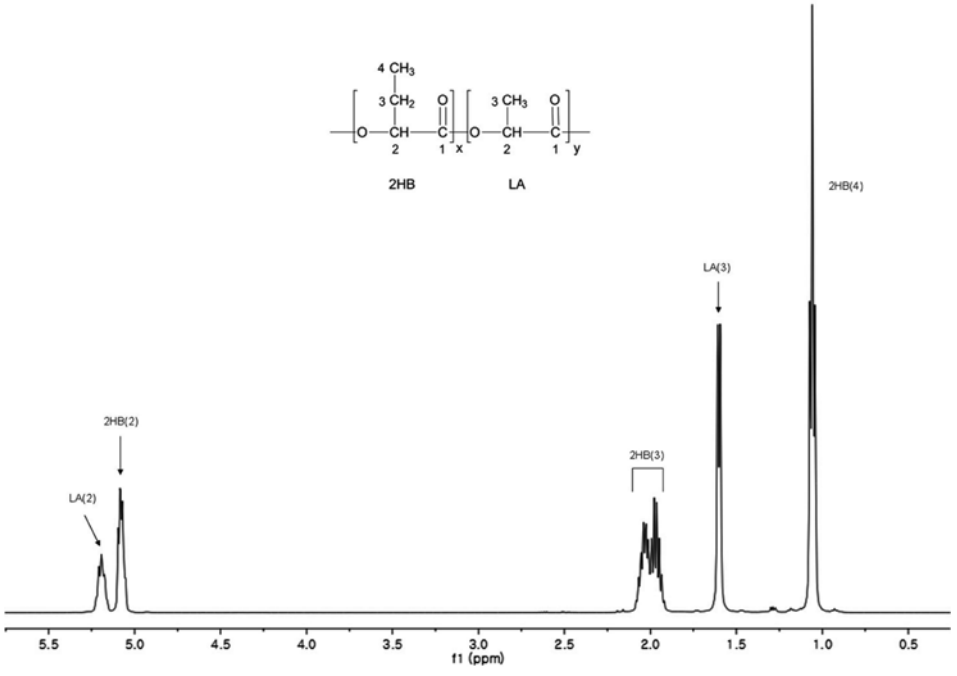

Fig. 4. NMR analysis (1D ^{1}H) of P(2HB-co-LA) synthesized in recombinant *E. coli* XL1-Blue (p619C1437-pct540).

for the development of recombinant microorganisms for the production of novel copolymer P(2HB-co-LA) consisting of only 2-hydroxyacid monomers by direct microbial fermentation from renewable resources.

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References

1. Oh, Y. H., I. Y. Eom, J. C. Joo, J. H. Yu, B. K. Song, S. H. Lee, S. H. Hong, and S. J. Park (2015) Recent advances in development of biomass pretreatment technologies used in biorefinery for the production of bio-based fuels, chemicals and polymers. *Korean J. Chem. Eng.* 32: 1945-1959.
2. Lee, S. Y. (1996) Bacterial polyhydroxyalkanoates. *Biotechnol. Bioeng.* 49: 1-14.
3. Madison, L. L. and G. W. Huisman (1999) Metabolic engineering of poly(3-hydroxyalkanoates): From DNA to plastic. *Microbiol. Mol. Biol. Rev.* 63: 21-53.
4. Steinbüchel, A. and H. E. Valentin (1995) Diversity of bacterial polyhydroxyalkanoic acids. *FEMS Microbiol. Lett.* 128: 219-228.
5. Yang, T. H., T. W. Kim, H. O. Kang, S. H. Lee, E. J. Lee, S. C. Lim, S. O. Oh, A. J. Song, S. J. Park, and S. Y. Lee (2010) Biosynthesis of polylactic acid and its copolymers using evolved propionate CoA transferase and PHA synthase. *Biotechnol. Bioeng.* 105: 150-160.
6. Yuan, W., Y. Jia, J. Tian, K. D. Snell, U. Muh, A. J. Sinskey, R. H. Lambalot, C. T. Walsh, and J. Stubbe (2001) Class I and III polyhydroxyalkanoate synthases from *Ralstonia eutropha* and *Allochromatium vinosum*: Characterization and substrate specificity studies. *Arch. Biochem. Biophys.* 394: 87-98.
7. Zhang, S., M. Kamachi, Y. Takagi, R. W. Lenz, and S. Goodwin (2001) Comparative study of the relationship between monomer structure and reactivity for two polyhydroxyalkanoate synthases. *Appl. Microbiol. Biotechnol.* 56: 131-136.
8. Yang, T. H., Y. K. Jung, H. O. Kang, T. W. Kim, S. J. Park, and S. Y. Lee (2011) Tailor-made type II *Pseudomonas* PHA synthases and their use for the biosynthesis of polylactic acid and its copolymer in recombinant *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 90: 603-614.
9. Yang, J. E., S. Y. Choi, J. H. Shin, S. J. Park, and S. Y. Lee (2013) Microbial production of lactate-containing polyesters. *Microb. Biotechnol.* 6: 621-636.
10. Park, S. J., T. W. Lee, S. C. Lim, T. W. Kim, H. Lee, M. K. Kim, S. H. Lee, B. K. Song, and S. Y. Lee (2012) Biosynthesis of polyhydroxyalkanoates containing 2-hydroxybutyrate from unrelated carbon source by metabolically engineered *Escherichia coli*. *Appl. Microbiol. Biotech.* 93: 273-283.
11. Park, S. J., S. Y. Lee, T. W. Kim, Y. K. Jung, and T. H. Yang (2012) Biosynthesis of lactate containing polyesters by metabolically engineered bacteria. *Biotechnol. J.* 7: 199-212.
12. Park, S. J., T. W. Kim, M. K. Kim, S. Y. Lee, and S. C. Lim (2012) Advanced bacterial polyhydroxyalkanoates: Towards a versatile and sustainable platform for unnatural tailor-made polyesters. *Biotechnol. Adv.* 30: 1196-1206.
13. Park, S. J., Y. A. Jang, H. Lee, A. R. Park, J. E. Yang, J. Shin, Y. H. Oh, B. K. Song, J. Jegal, S. H. Lee, and S. Y. Lee (2013) Metabolic engineering of *Ralstonia eutropha* for the biosynthesis of 2-hydroxyacid-containing polyhydroxyalkanoates. *Metab. Eng.* 20: 20-28.
14. Park, S. J., K. H. Kang, H. Lee, A. R. Park, J. E. Yang, Y. H. Oh, B. K. Song, J. Jegal, S. H. Lee, and S. Y. Lee (2013 Propionyl-CoA dependent biosynthesis of 2-hydroxybutyrate containing polyhydroxyalkanoates in metabolically engineered *Escherichia coli*. *J. Biotechnol.* 165: 93-98.
15. Park, S. J., Y. A. Jang, W. Noh, Y. H. Oh, H. Lee, Y. David, M.G. Baylon, J. Shin, J. E. Yang, S. Y. Choi, S. H. Lee, and S. Y. Lee (2014) Metabolic engineering of *Ralstonia eutropha* for the production of polyhydroxyalkanoates from sucrose. *Biotechnol. Bioeng.* 112: 638-643.
16. Jung, Y. K., T. Y. Kim, S. J. Park, and S. Y. Lee (2010) Metabolic engineering of *Escherichia coli* for the production of polylactic acid and its copolymers. *Biotechnol. Bioeng.* 105: 161-171.
17. Taguchi, S., M. Yamada, K. Matsumoto, K. Tajima, Y. Satoh, M. Munekata, K. Ohno, K. Kohda, T. Shimamura, H. Kambe, and S. Obata (2008) A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. *Proc. Natl. Acad. Sci. USA.* 105: 17323-17327.
18. Ochi, A., K. Matsumoto, T. Ooba, K. Sakai, T. Tsuge, and S. Taguchi (2013) Engineering of class I lactate-polymerizing polyhydroxyalkanoate synthases from *Ralstonia eutropha* that synthesize lactate-based polyester with a block nature. *Appl. Microbiol. Biotechnol.* 97: 3441-3447.
19. Matsumoto, K, S. Terai, A. Ishiyama, J. Sun, T. Kabe, Y. Song, J. M. Nduko, T. Iwata, and S. Taguchi (2013) One-pot microbial production, mechanical properties, and enzymatic degradation of isotactic P[(*R*)-2-hydroxybutyrate] and Its Copolymer with (*R*)-Lactate. *Biomacromol.* 14: 1913-1918.
20. Park, J. H., K. H. Lee, T. Y. Kim, and S. Y. Lee (2007) Metabolic engineering of Escherichia coli for the production of L-valine based on transcriptome analysis and in silico gene knockout simulation. *Proc. Natl. Acad. Sci. USA.* 104: 7797-7802.
21. Braunegg, G., B. Sonnleitner, and R. M. Lafferty (1978) A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass. *Eur. J. Appl. Microbiol. Biotechnol.* 6: 29-37.
22. Choi, J. and S. Y. Lee (1999) Efficient and economical recovery of Poly(3-hydroxybutylate) from recombinant *Escherichia coli* by simple digestion with chemicals. *Biotechnol. Bioeng.* 62: 546-553.