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Screening of Marine Bacteria To Synthesize Polyhydroxyalkanoate from Lignin: Contribution of Lignin Derivatives to Biosynthesis by *Oceanimonas doudoroffii*

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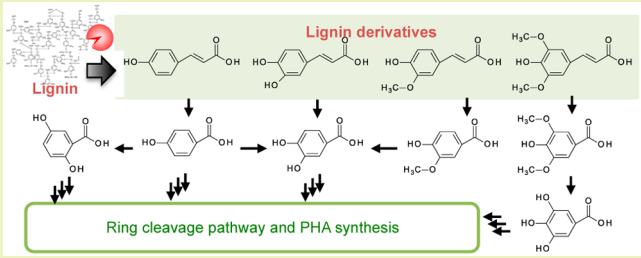
Supporting Information

ABSTRACT: To convert lignin and lignin derivatives to biopolyesters, six types of marine bacterial strains were investigated with mineral salt media containing lignin and lignin derivatives. Our results showed that *Oceanimonas doudoroffii*, which was isolated from an area of the sea polluted with allantoin, directly synthesized polyhydroxyalkanoate (PHA) using lignin or several lignin derivatives as sole carbon sources. Based on the PHA accumulation data, the conversion from lignin to PHA can be carried out mainly via sinapinic acid and syringic acid rather than other lignin derivatives. This is the first study to show the direct microbial conversion from lignin to biopolyester, which will open the door for lignin-based biorefinery applications.



KEYWORDS: *Polyhydroxyalkanoate, marine bacteria, lignin, lignin derivatives, Oceanimonas doudoroffii*

INTRODUCTION

Poly(hydroxyalkanoate)s (PHAs) are biodegradable polyesters that are biosynthesized by various bacteria as intracellular storage of carbon, and their physical and biological characteristics have been studied extensively.[1−3] The mechanical properties of PHAs can be improved by means of drawing techniques.[4] These improved mechanical properties are comparable to those of industrial plastics. PHAs are therefore an attractive polymeric material due to decreased carbon dioxide emissions, resulting in a more positive environmental impact than those of other plastics. The biosynthesis mechanism of PHA has been studied using many kinds of soil bacteria.[1−3] However, owing to culture conditions containing high concentrations of salt, marine bacteria have not been investigated widely for PHA production. The advantages of biosynthesizing PHA under marine conditions include avoiding contamination from bacteria that lack salt-water resistance, as well as the ability to use filtered seawater as a culture medium, which would enable large-scale industrial production of PHA. Furthermore, marine bacteria have recently drawn attention as candidates for the production of practical materials from marine ecosystems, including the oceanic carbon dioxide cycle.[5−7] The marine environment is a typical oligotrophic condition where the local bacteria need to metabolize a wide variety of carbon sources, including lignin and its degradation products. To date, a few kinds of marine bacteria have been investigated for the production of PHA under marine conditions,[8−10] and the compositions of these marine PHAs have been characterized in detail.[11,12]

Lignin is an abundant biomass resource from plant cell walls as well as an aromatic biopolymer consisting of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol as its fundamental structural components.[13] However, lignin is not used as a raw material in the industrial process because of the thermal and biological stability and cytotoxicity of lignin monomers. One exception is that lignin has successfully been used as a precursor for vanillin and dimethyl sulfoxide synthesis.[14] To enable the application of lignin as a raw material, its degradation needs to be performed by physical, chemical, and/or biological methods.[14−16] In nature, lignin is degraded by lignin peroxidases, manganese peroxidases, and laccase from white and brown rot fungi.[16] The degradation of lignin produces derivatives such as *p*-coumaric acid, caffeic acid, ferulic acid, and sinapinic acid, which can be metabolized by various bacterial strains.[17−20] Under marine conditions, most of lignin is from plants on land via biodegradation process.[21] Recently, we successfully converted lignin derivatives to PHA by using the PHA-accumulating soil bacterial strain, *Ralstonia eutropha* H16.[22] This bacterial strain synthesized PHA from 4-hydroxybenzoic acid (4-HBA), 2,5-dihydroxybenzoic acid (2,5-DHBA), and 3,4-dihydroxybenzoic acid (3,4-DHBA). On the basis of these data, we suggested that the conversion of lignin derivatives into intermediates such as 4-HBA, 2,5-DHBA, 3,4-DHBA, and vanillic acid represents the major bottleneck in the synthesis of PHA from lignin derivatives. New mircroorganisms must be assayed in order to achieve efficient PHA production from untreated lignin. Some marine bacteria can degrade and metabolize lignin and lignin derivatives

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Table 1. Marine Bacteria Strains Used in the Present Study and Their PHA Accumulation Results by Single Step Cultivation with 0.1 wt % Lignin as a Sole Carbon Source

marine bacteria strain	original marine condition	original marine area	optimal temperature (°C)	dry cell weight (g/L)	PHA content (wt %)	PHA composition (mol %)[a]	
						3HB	3HV
Muricauda olearia	crude-oil-contaminated seawater	the west coast of Korea	30	–[b]	–[b]	–[b]	–[b]
Pesudoalteromonas piscicida	Red-tide seawater	South Florida, USA	30	–[b]	–[b]	–[b]	–[b]
Pseudomonas taeanensis	crude oil-contaminated seawater	the Taean area of Korea	30	0.12 ± 0.10	–[b]	–[b]	–[b]
Oceanimonas doudoroffii	allantoin-rich seawater	the coast of Oahu, HI, USA	25	0.13 ± 0.12	0.52 ± 0.11	100	0
Vibrio nereis	propanol-rich seawater	Oahu, HI, USA	25	–[b]	–[b]	–[b]	–[b]
Listonella pelagia	succinate-rich seawater	the coast of Oahu, HI, USA	25	0.35 ± 0.18	–[b]	–[b]	–[b]

[a]Determined by ^{1}H NMR spectroscopy. [b]Not determined.

due to lack of fungi present the marine condition.[23] Hence, the aim of the present study was to find a marine bacterial strain to convert lignin to PHA directly. To investigate the pathway to synthesize PHA from lignin, we used lignin and 11 lignin derivatives as well as hydroxybenzoic acids, including the intermediates from lignin derivatives, as sole carbon sources. On the basis of the present results, we identified one marine bacterial strain, *Oceanimonas doudoroffii*, that is capable of synthesizing PHA directly from lignin and lignin derivatives.

EXPERIMENTAL SECTION

Bacterial Strains. Six marine bacterial strains, *Muricauda olearia* (JCM15563T), *Pesudoalteromonas piscicida* (JCM20779T), *Pseudomonas taeanensis* (LCM16046T), *Oceanimonas doudoroffii* (JCM21046T), *Vibrio nereis* (JCM21190T), *Listonella pelagia* (JCM21191T), were obtained from the Japan Collection of Microorganisms (RIKEN, Saitama, Japan) and used in this study (Table 1). *M. olearia* was collected in crude-oil-contaminated seawater from the west coast of Korea. *P. piscicida* was collected in Red-tide seawater from South Florida, USA. *P. taeanensis* was collected in crude oil-contaminated seawater in the Taean area of Korea. *O. doudoroffii* was collected from seawater off the coast of Oahu, HI, USA, by enrichment with allantoin. *V. nereis* was collected from seawater enriched with propanol in Oahu, HI, USA. *L. pelagia* was collected in seawater off the coast of Oahu, HI, USA, by enrichment with succinate.

PHA Production. The culture condition was modified slightly from previous studies.[11,22] In a single-phase culture, preculture of the strain was performed in marine broth medium (Difco, BD, Franklin Lakes, NJ) in a 5 mL tube with constant shaking at 140 rpm for 8 h.[12] PHA production was carried out in mineral salt medium (MM) with either lignin (Tokyo Chemical Industry Co. Ltd. Tokyo, Japan) or a lignin derivative, namely, *p*-coumaric acid, caffeic acid, ferulic acid, sinapinic acid, 3-HBA, 4-HBA, 2,5-DHBA, 3,4-DHBA, vanillic acid, syringic acid, and gallic acid (these chemicals were purchased from Tokyo Chemical Industry Co. Ltd.) as a sole carbon source in a 500 mL shaking flask containing 100 mL of medium at optimal temperatures with constant shaking at 140 rpm for 48 h under aerobic conditions (Table 1). Lignin powder was suspended in the culture medium at 5 times higher concentration than the final concentration. The lignin derivatives were prepared as reported previously.[22] The composition of MM was identical to a previous report.[12] The culture condition without nitrogen (nitrogen-limited condition) was selected for PHA production, according to our previous study.[22] After cultivation, the collected cells were washed with Milli-Q water three times to remove the remaining carbon sources and medium salts, then lyophilized. In a two-phase culture, a preculture of the strain was performed in marine broth medium in a 5 mL tube with constant shaking at 140 rpm for 8 h, and then 1 mL of preculture was transferred into a 500 mL shake flask containing 100 mL of marine broth medium and the cells were grown for 24 h with constant shaking at 140 rpm.[12] The cells were then harvested under sterile conditions by centrifugation at 15900*g* for 10 min and suspended in sterilized water. The cells were centrifuged once again at 15900*g* for 10 min, resuspended, and transferred aseptically into 100 mL of MM medium containing either lignin or lignin derivatives as a carbon source. The final concentration of those lignin derivatives in the medium was 0.1 wt %. The cell cultures were incubated for 48 h at an appropriate temperature (see Table 1), harvested by centrifugation, and then lyophilized.[24]

Characterization of PHA. The lyophilized cells were weighed, and the accumulated PHA was extracted with chloroform from the dry cells for 72 h at 70 °C. The chloroform extracted PHA was purified by precipitation with hexane (1.0 mL chloroform/9.0 mL hexane). The resultant PHA was dissolved into 1.0 mL of chloroform and purified by precipitation with 9.0 mL of methanol. The PHA content was quantitatively determined based on the weight of the purified PHA and the dry cell weight. The polymer was analyzed by proton nuclear magnetic resonance (^{1}H NMR) (JNM-Excalibur 270; JEOL, Ltd., Tokyo, Japan) to determine its chemical structure, and the concentration of sample used was approximately 4 mg/mL in $CDCl_3$ with 0.05 v/v % of tetramethylsilane (TMS) (Wako Pure Chemical Industries, Ltd., Osaka, Japan). To determine the PHA composition, dry cells taken before the PHA extraction were subjected to methanolysis with a solution consisting of 1.7 mL of methanol, 0.3 mL of 98% sulfuric acid, and 2.0 mL of chloroform at 100 °C for 140 min to convert the constituents to their methyl esters. The addition of 1 mL of water to the reaction mixture induced phase separation. The lower chloroform layer was used for gas chromatography (GC) analysis on a Shimadzu GC-17A system equipped with Neutra Bond-1 capillary column (30 m × 0.25 mm) and a flame ionization detector. The PHA content and monomer composition were determined based on a calibration curve employing various concentrations of (*R*)-3-hydroxybutyric acid (3HB) and (*R*)-3-hydroxyvaleric acid (3HV) methyl esters. The molecular weight of PHA was determined by gel-permeation chromatography (GPC) (RI-2031, PU-2086, AS-2055, CO-2056; JASCO, Tokyo, Japan) with a Shodex K-806M, K802 and K-G column at 40 °C. Chloroform was used as the mobile phase at a flow rate of 0.8 mL/min and the concentration of the sample used was approximately 1 mg/mL. The molecular weight of the polymer was estimated by comparison with polystyrene standards of the following molecular weights: 1.32×10^3, 3.25×10^3, 1.01×10^4, 2.85×10^4, 6.60×10^4, 1.56×10^5, 4.60×10^5, 1.07×10^6, and 3.15×10^6, respectively.

Statistical Analysis. The significance of differences in cell growth with different lignin concentrations were determined by unpaired *t*-tests with a two-tailed distribution. Differences were considered statistically significant at $p < 0.05$.

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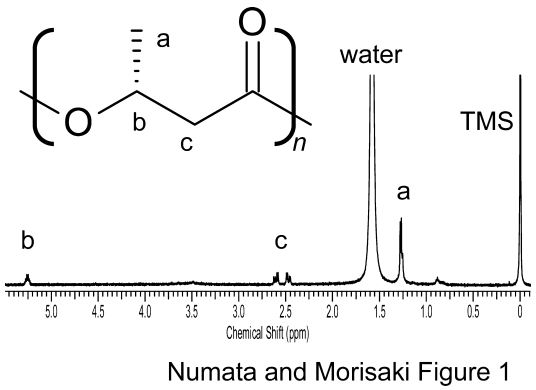
RESULTS AND DISCUSSION

Six marine bacterial strains, *M. olearia*, *P. piscicida*, *P. taeanensis*, *O. doudoroffii*, *V. nereis*, *L. pelagia*, isolated from various areas of polluted seawater, were selected for this study due to potential higher chemical resistance and wider uptake pathways (Table 1). Production of PHAs by those marine bacteria was examined in 4 mL of MM medium supplemented with 0.1 wt % lignin as the sole source of carbon. Although *P. taeanensis*, *O. doudoroffii*, and *L. pelagia* showed cell growth with 0.1 wt % lignin, only *O. doudoroffii* accumulated poly[(*R*)-3-hydroxybutyric acid] [P-(3HB)]. The standard deviations of dry cell weight were very high (Table 1), which could be due to the heterogeneous components of lignin. Its P(3HB) content was 0.52% ± 0.11% (Table 1). The chemical structure of the synthesized P(3HB) was confirmed by ^{1}H NMR (Figure 1). The number-average



Numata and Morisaki Figure 1

Figure 1. ^{1}H NMR spectra of poly[(*R*)-3-hydroxybutyric acid], P(3HB), synthesized by *Oceanimonas doudoroffii* using 0.1 wt % lignin as a sole carbon source.

molecular weight and polydispersity index of the synthesized P(3HB) were determined by GPC and were approximately 2000 g/mol and 1.1. *O. doudoroffii* is a marine γ-proteobacterium and is reported to have at least three different enzymes that can cleave dimethylsulfoniopropionate, one of the most abundant products of marine phytoplankton.[25,26] This quality is similar to some Ascomycete fungi, which can degrade a wide variety of biomass, and also might contribute to the degradation and uptake of lignin. According to our previous report, some *Pseudomonas* spp. metabolize lignin derivatives but cannot accumulate PHA by using lignin and its derivatives due to their metabolic pathways relation to β-oxidation.[22] A growth curve of *O. doudoroffi* utilizing lignin as a sole carbon source showed a doubling time of 1.5 h (Figure S1, Supporting Information). Lignin is an aromatic compound and shows cytotoxicity to bacterial cells. Therefore, the effect of lignin concentration on the growth of *O. doudoroffii* was studied, as shown in Figure 2, demonstrating that 0.05 and 0.1 wt % of lignin was an appropriate concentration for the cell growth. When using over 0.5 wt % of lignin, the cell growth decreased. The effect of lignin concentration on the PHA content of *O. doudoroffii* was also characterized (Table S2, Supporting Information), indicating that 0.1 and 0.5 wt % of lignin provide similar PHA contents. On the basis of the effects of lignin concentrations on the cell growth and PHA content, we concluded that 0.1 wt % of lignin was an appropriate concentration for bacterial culture without significant cytotoxicity.

To enhance the productivity of PHA, two-step cultivations of *O. doudoroffii* with 0.1 wt % lignin or lignin derivatives (*p*-

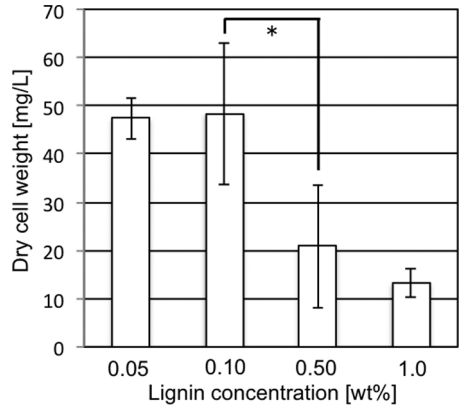


Figure 2. Effect of lignin concentration on cell-growth of *Oceanimonas doudoroffii*. Error bars represent the standard deviations of samples (n = 3). An asterisk indicates significant difference between two groups at p < 0.05.

coumaric acid, caffeic acid, ferulic acid, sinapinic acid, 3-HBA, 4-HBA, 2,5-DHBA,3,4-DHBA, vanillic acid, syringic acid, and gallic acid) were performed (Table 2). The PHA contents of the

Table 2. PHA Accumulation by Two-Step Cultivation of *Oceanimonas doudoroffii* with Lignin and Its Derivatives as a Sole Carbon Source

carbon source	dry cell weight (g/L)	PHA content (wt %)	PHA composition (mol %)[a] 3HB	3HV
marine broth (control)	0.45 ± 0.09	–[b]	–[b]	–[b]
lignin	0.48 ± 0.11	0.2 ± 0.1	100	0
p-coumaric acid	0.20 ± 0.02	0.2 ± 0.1	100	0
caffeic acid	0.38 ± 0.03	0.2 ± 0.1	100	0
ferulic acid	0.36 ± 0.04	0.4 ± 0.1	100	0
sinapinic acid	0.41 ± 0.02	1.9 ± 0.1	100	0
3-HBA	0.27 ± 0.19	0.4 ± 0.1	52 ± 19	48 ± 19
4-HBA	0.62 ± 0.26	0.8 ± 0.1	46 ± 3	54 ± 3
2,5-DHBA	0.37 ± 0.06	0.2 ± 0.1	73 ± 12	27 ± 12
3,4-DHBA	0.42 ± 0.16	0.4 ± 0.1	29 ± 10	71 ± 10
vanillic acid	0.27 ± 0.06	0.3 ± 0.1	100	0
syringic acid	0.40 ± 0.01	2.7 ± 0.3	100	0
gallic acid	0.33 ± 0.06	0.9 ± 0.1	58 ± 3	42 ± 3

[a]Determined by gas chromatography. [b]Not determined.

bacteria were characterized by GC as proportions of the weights of the dry cells. The PHA content and dry cell weight were also normalized by carbon content of each aromatic carbon source (see Table S2 of the Supporting Information), indicating the similar tendency to the PHA content and dry cell weight normalized by overall weight of carbon source shown in Table 2. To clear the PHA synthesis from the residual preculture, PHA content with marine broth was investigated as a control. No PHA production in marine broth culture was detected by GC, indicating that the residual preculture did not contribute to PHA production in the two-step cultivations. Four carbon sources, namely *p*-coumaric acid, 3-HBA, vanillic acid, and gallic acid, significantly inhibited the cell growth, based on the decrease in dry cell weight from the control. The two-step cultivations of *O. doudoroffii* with 0.1 wt % aromatic carbon sources produced a

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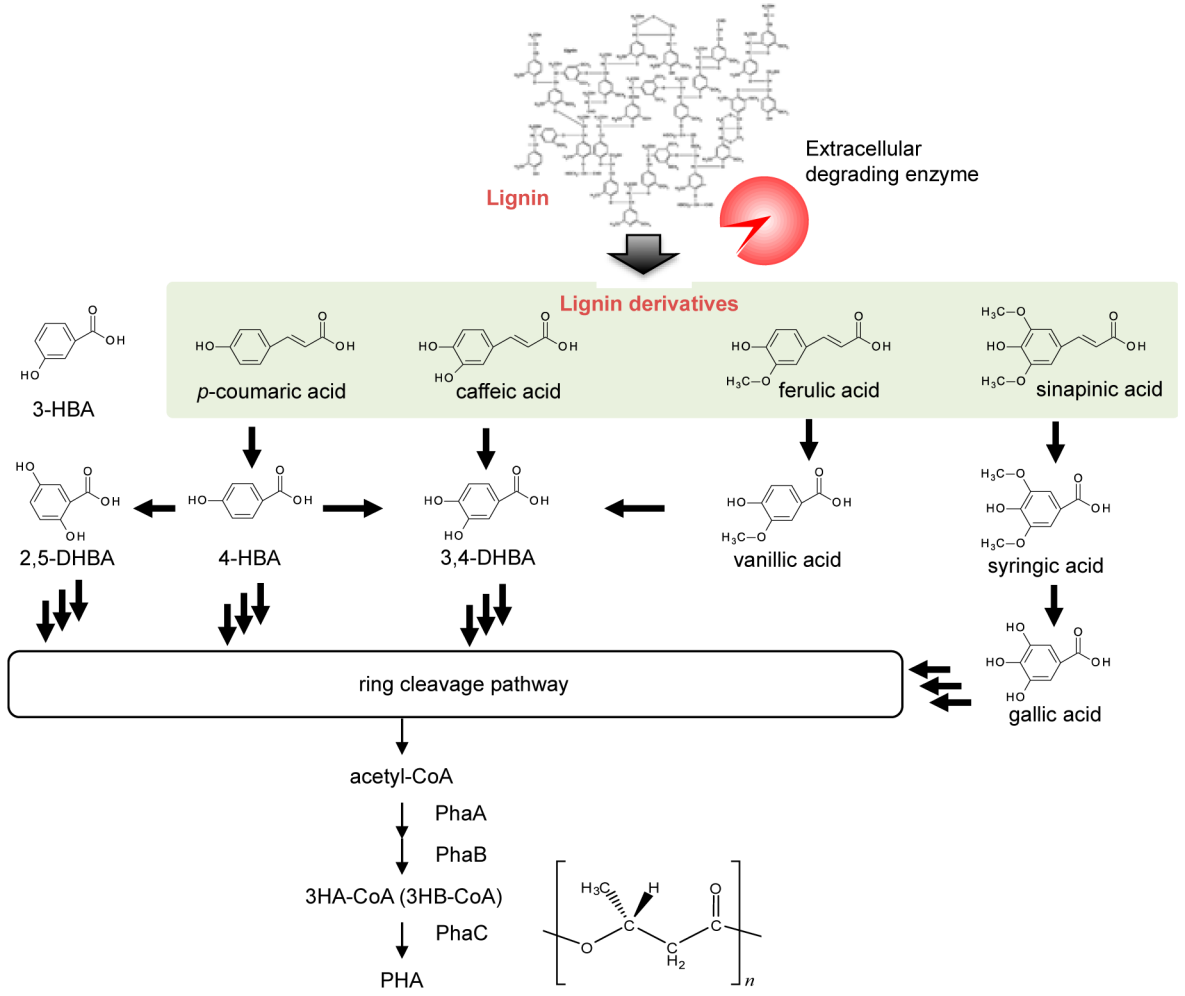

Figure 3. Degradation pathways of lignin and its derivatives for PHA synthesis.

small amount of PHA, and then the yields ranged from 0.2 to 2.7 wt %.

A hypothetical lignin to PHA metabolic pathway derived from lignin degrading pathways of *Sphingomonas paucimobilis* SYK-6 and *Pseudomonas putida* KT2440 (Figure S2, Supporting Information) is shown in Figure 3.[17,27] Lignin must be degraded by extracellular depolymerase and then the degradation products, namely, various low-molecular weight lignin, were metabolized via lignin derivative (*p*-coumaric acid, caffeic acid, ferulic acid, sinapinic acid). Sinapinic acid and syringic acid were converted to PHA more efficiently, even though sinapinic acid is one of the major lignin derivatives. Syringic acid is an intermediate compound from sinapinic acid to acetyl-CoA; therefore, both sinapinic acid and syringic acid could be the major intermediates for PHA production from lignin (Figure 3). On the other hand, another intermediate compound from sinapinic acid, gallic acid, did not show comparable PHA yield to sinapinic acid and syringic acid. The lower yield using gallic acid may be a result of a more cytotoxic nature, due to a relatively high number of hydroxyl groups.[28] The other major lignin components, *p*-coumaric acid, caffeic acid and ferulic acid, showed smaller PHA accumulation, suggesting that the PHA synthesis from lignin resulted from the pathway of sinapinic acid to acetyl-CoA. The synthesized PHAs contained not only a 3HB unit but also a (*R*)-3-hydroxyvalerate (3HV) unit, which was up to 71 mol %, based on the GC measurements (Table 2). It is unknown how the organism converts 3-HBA, 2,5-DHBA, 4-HBA, 3,4-DHBA, and gallic acid to 3HV.

CONCLUSION

In the present study, we performed screening from pollute marine environment in order to discover a strain to convert lignin to PHA, resulting that *O. doudoroffii* demonstrated PHA synthesis from 0.1 wt % lignin and its derivatives, especially, sinapinic acid and syringic acid. To our knowledge, *O. doudoroffii* is the first strain to synthesize PHA using lignin and its derivatives as a sole carbon source. The metabolic pathways including lignin degradation of *O. doudoroffii* needs to be estimated, because the genome sequence of *O. doudoroffii* has not been clarified. By consideration of the lignin degradation pathways of *S. paucimobilis* SYK-6 and *P. putida* KT2440, the major pathway from lignin to PHA via sinapinic acid is estimated from the current results on the PHA synthesis from sinapinic acid and syringic acid.

ASSOCIATED CONTENT

Supporting Information

Growth curve of *Oceanimonas doudoroffii* in marine broth medium, potential degradation pathways of lignin derivatives based on the KEGG (Kyoto Encyclopedia of Genes and Genomes), PHA contents of *Oceanimonas doudoroffii* as a function of lignin concentration, and chemical formula and

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carbon content of lignin and lignin derivatives used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

^{1}H NMR = proton nuclear magnetic resonance
3HV = (*R*)-3-hydroxyvalerate
DHBA = dihydroxybenzoic acid
GC = gas chromatography
GPC = gel-permeation chromatography
HBA = hydroxybenzoic acid
MM = mineral salt media
P(3HB) = poly[(*R*)-3-hydroxybutyric acid]
PHA = polyhydroxyalkanoate
TMS = tetramethylsilane

REFERENCES

(1) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990.

(2) Doi, Y.; Steinbüchel, A. *Biopolymers*; Wiley-VCH: Weinheim, Germany, 2001; Vol. 3.

(3) Lenz, R. W.; Marchessault, R. H. Bacterial polyesters: Biosynthesis, biodegradable plastics and biotechnology. *Biomacromolecules* **2005**, *6* (1), 1−8.

(4) Iwata, T.; Aoyagi, Y.; Fujita, M.; Yamane, H.; Doi, Y.; Suzuki, Y.; Takeuchi, A.; Uesugi, K. Processing of a strong biodegradable poly[(*R*)-3-hydroxybutyrate] fiber and a new fiber structure revealed by microbeam X-ray diffraction with synchrotron radiation. *Macromol. Rapid Commun.* **2004**, *25* (11), 1100−1104.

(5) Karl, D. M. Microbial oceanography: Paradigms, processes and promise. *Nat. Rev. Microbiol.* **2007**, *5* (10), 759−769.

(6) Moran, M. A.; Miller, W. L. Resourceful heterotrophs make the most of light in the coastal ocean. *Nat. Rev. Microbiol.* **2007**, *5* (10), 792−800.

(7) Umani, S. F.; Del Negro, P.; Larato, C.; De Vittor, C.; Cabrini, M.; Celio, M.; Falconi, C.; Tamberlich, F.; Azam, F. Major inter-annual variations in microbial dynamics in the Gulf of Trieste (Northern Adriatic Sea) and their ecosystem implications. *Aquat. Microb. Ecol.* **2007**, *46* (2), 163−175.

(8) Gonzalez-Garcia, Y.; Nungaray, J.; Cordova, J.; Gonzalez-Reynoso, O.; Koller, M.; Atlic, A.; Braunegg, G. Biosynthesis and characterization of polyhydroxyalkanoates in the polysaccharide-degrading marine bacterium *Saccharophagus degradans* ATCC 43961. *J. Ind. Microbiol.* **2008**, *35* (6), 629−633.

(9) Wang, Q.; Zhang, H. X.; Chen, Q.; Chen, X. L.; Zhang, Y. Z.; Qi, Q. S. A marine bacterium accumulates polyhydroxyalkanoate consisting of mainly 3-hydroxydodecanoate and 3-hydroxydecanoate. *World J. Microbiol. Biotechnol.* **2010**, *26* (6), 1149−1153.

(10) Lopez, N. I.; Pettinari, M. J.; Stackebrandt, E.; Tribelli, P. M.; Potter, M.; Steinbuchel, A.; Mendez, B. S. *Pseudomonas extremaustralis* sp. nov., A poly(3-hydroxybutyrate) producer isolated from an Antarctic environment. *Curr. Microbiol.* **2009**, *59* (5), 514−519.

(11) Numata, K.; Doi, Y. Biosynthesis of polyhydroxyalkanaotes by a novel facultatively anaerobic *Vibrio* sp. under marine conditions. *Mar. Biotechnol.* **2012**, *14* (3), 323−331.

(12) Numata, K.; Morisaki, K.; Tomizawa, S.; Ohtani, M.; Demura, T.; Miyazaki, M.; Nogi, Y.; Deguchi, S.; Doi, Y. Synthesis of poly- and oligo(hydroxyalkanoate)s by deep-sea bacteria, *Colwellia* spp., *Moritella* spp., and *Shewanella* spp. *Polym. J.* **2013**, *45* (10), 1094−1100.

(13) Vanholme, R.; Demedts, B.; Morreel, K.; Ralph, J.; Boerjan, W. Lignin biosynthesis and structure. *Plant Physiol.* **2010**, *153* (3), 895−905.

(14) Wu, G.; Heitz, M.; Chornet, E. Improved alkaline oxidation process for the production of aldehydes (vanillin and syringaldehyde) from steam-explosion hardwood lignin. *Ind. Eng. Chem. Res.* **1994**, *33* (3), 718−723.

(15) Sawada, T.; Nakamura, Y.; Kobayashi, F.; Kuwahara, M.; Watanabe, T. Effects of fungal pretreatment and steam explosion pretreatment on enzymatic saccharification of plant biomass. *Biotechnol. Bioeng.* **1995**, *48* (6), 719−724.

(16) Tuomela, M.; Vikman, M.; Hatakka, A.; Itävaara, M. Biodegradation of lignin in a compost environment: A review. *Bioresour. Technol.* **2000**, *72* (2), 169−183.

(17) Masai, E.; Katayama, Y.; Fukuda, M. Genetic and biochemical investigations on bacterial catabolic pathways for lignin-derived aromatic compounds. *Biosci. Biotechnol. Biochem.* **2007**, *71* (1), 1−15.

(18) Vicuña, R.; González, B.; Mozuch, M. D.; Kirk, T. K. Metabolism of lignin model compounds of the arylglycerol-β-aryl ether type by *Pseudomonas acidovorans* D3. *Appl. Environ. Microbiol.* **1987**, *53* (11), 2605−2609.

(19) Jiménez, J. I.; Miñambres, B.; Garcia, J. L.; Díaz, E. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. *Environ. Microbiol.* **2002**, *4* (12), 824−841.

(20) Ohta, Y.; Nishi, S.; Haga, T.; Tsubouchi, T.; Hasegawa, R.; Konishi, M.; Nagano, Y.; Tsuruwaka, Y.; Shimane, Y.; Mori, K. Screening and phylogenetic analysis of deep-sea bacteria capable of metabolizing lignin-derived aromatic compounds. *Open J. Mar. Sci.* **2012**, *2* (4), 177−187.

(21) Pailleret, M.; Haga, T.; Petit, P.; Prive-Gill, C.; Saedlou, N.; Gaill, F.; Zbinden, M. Sunken wood from the Vanuatu Islands: Identification of wood substrates and preliminary description of associated fauna. *Mar. Ecol. (Berlin, Ger.)* **2007**, *28* (1), 233−241.

(22) Tomizawa, S.; Chuah, J. A.; Matsumoto, K.; Doi, Y.; Numata, K. Understanding the limitations in the biosynthesis of polyhydroxyalkanoate (PHA) from lignin derivatives. *ACS Sustainable Chem. Eng.* **2014**, *2* (5), 1106−1113.

(23) Gonzalez, J. M.; Mayer, F.; Moran, M. A.; Hodson, R. E.; Whitman, W. B. *Sagittula stellata* gen. nov, sp. nov, A lignin-transforming bacterium from a coastal environment. *Int. J. Syst. Bacteriol.* **1997**, *47* (3), 773−780.

(24) Kunioka, M.; Kawaguchi, Y.; Doi, Y. Production of biodegradable copolyesters of 3-hydroxybutyrate and 4-hydroxybutyrate by *Alcaligenes eutrophus*. *Appl. Microbiol. Biotechnol.* **1989**, *30* (6), 569−573.

(25) Curson, A. R. J.; Fowler, E. K.; Dickens, S.; Johnston, A. W. B.; Todd, J. D. Multiple DMSP lyases in the gamma-proteobacterium *Oceanimonas doudoroffii*. *Biogeochemistry* **2012**, *110* (1−3), 109−119.

(26) Todd, J. D.; Curson, A. R. J.; Dupont, C. L.; Nicholson, P.; Johnston, A. W. B. The dddP gene, encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. *Environ. Microbiol.* **2009**, *11* (6), 1376−1385.

(27) Jimenez, J. I.; Minambres, B.; Garcia, J. L.; Diaz, E. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. *Environ. Microbiol.* **2002**, *4* (12), 824−841.

(28) Borges, A.; Ferreira, C.; Saavedra, M. J.; Simoes, M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.* **2013**, *19* (4), 256−265.

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