HOKKAIDO UNIVERSITY

Title	Microbial degradation of the artificial polyhydroxyalkanoate poly(2-hydroxybutyrate-co-3-hydroxybutyrate) with random and block sequences using soil bacteria
Author(s)	Hachisuka, Shin-ichi; Iguchi, Ami; Kikukawa, Hiroshi et al.
Citation	Journal of applied microbiology, 136(6), lxaf123 https://doi.org/10.1093/jambio/lxaf123
Issue Date	2025-06
Doc URL	https://hdl.handle.net/2115/97913
Rights	This is a pre-copyedited, author-produced version of an article accepted for publication in Journal of Applied Microbiology following peer review. The version of record Shin-ichi Hachisuka, Ami Iguchi, Hiroshi Kikukawa, Hiroya Tomita, Toshihiko Ooi, Ken'ichiro Matsumoto, Microbial degradation of the artificial polyhydroxyalkanoate poly(2-hydroxybutyrate-co-3-hydroxybutyrate) with random and block sequences using soil bacteria, Journal of Applied Microbiology, Volume 136, Issue 6, June 2025, lxaf123 is available online at: https://doi.org/10.1093/jambio/lxaf123.
Type	journal article
File Information	JAMICRO-2024-1140.R2_Supplementary data_Revised250516.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Supplementary Data

Microbial degradation of the artificial polyhydroxyalkanoate poly(2-hydroxybutyrate-*co*-3-hydroxybutyrate) with random and block sequences using soil bacteria

Short running head: Microbial degradation of P(2HB-*co*-3HB)

Shin-ichi Hachisuka[1]*, Ami Iguchi[2]*, Hiroshi Kikukawa[1], Hiroya Tomita[1], Toshihiko Ooi[1], and Ken'ichiro Matsumoto[1#]

[1]Division of Applied Chemistry, Faculty of Engineering, Hokkaido University, N13W8, Kita-ku, Sapporo 060-8628, Japan

[2]Graduate School of Chemical Sciences and Engineering, Hokkaido University, N13W8, Kita-ku, Sapporo 060-8628, Japan

*The authors contributed equally to this study.

[#]Corresponding author: Ken'ichiro Matsumoto

Tel.: (+81)-11-706-6610; Fax: (+81)-11-706-6610

E-mail: mken@eng.hokudai.ac.jp

Table S1. Monomer composition and molecular weight of the copolymers used in this study.

Polymer	PHA composition (mol%)		Molecular weight		
	2HB	3HB	M_w	M_n	M_w/M_n
P(2HB-*ran*-3HB)	86	14	6.1×10^4	4.1×10^4	1.5
P(2HB)-*b*-P(3HB)	78	22	4.4×10^5	2.2×10^5	2.0

Note: These values were determined at the time of the initial synthesis.

Table S2. Diameter sizes of the clear zones are shown in Figure 3.

Polymer	Strain	Incubation time		
		3 days	7 days	14 days
P(3HB)	A25i	17	34	52
	A60i2	16	26	40
	T1	20	33	52
	YM1609	25	38	52
P(2HB)	A25i	−	−	−
	A60i2	−	−	−
	T1	−	−	−
	YM1609	−	−	−
Random	A25i	6	7	11
	A60i2	7	10	18
	T1	−	−	−
	YM1609	7	11	22
Block	A25i	−	−	−
	A60i2	−	−	−
	T1	−	−	−
	YM1609	?	?	16

Note: A minimum value is shown if the zone was not a regular circle; an estimated value from the radius was shown if it protruded from the plate. −, not detected; ?, too faint to estimate.

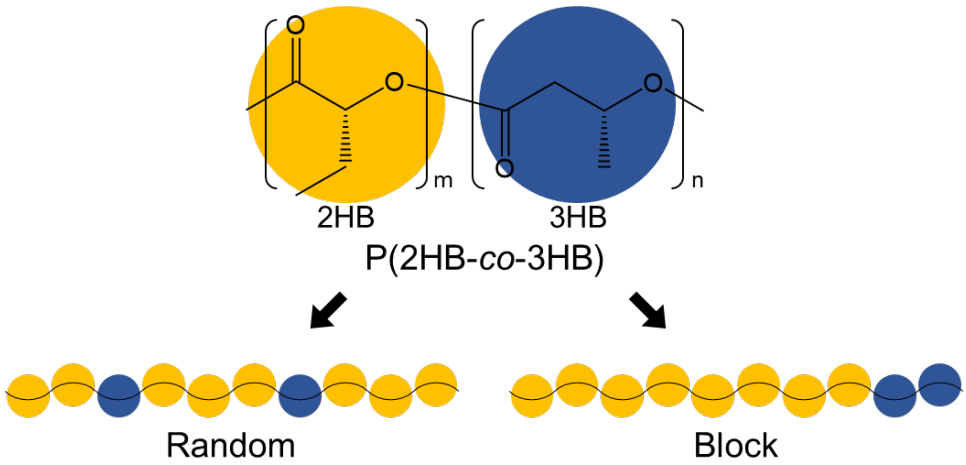


Figure S1. Conceptual models of random- and block-sequenced P(2HB-*co*-3HB) in this study.

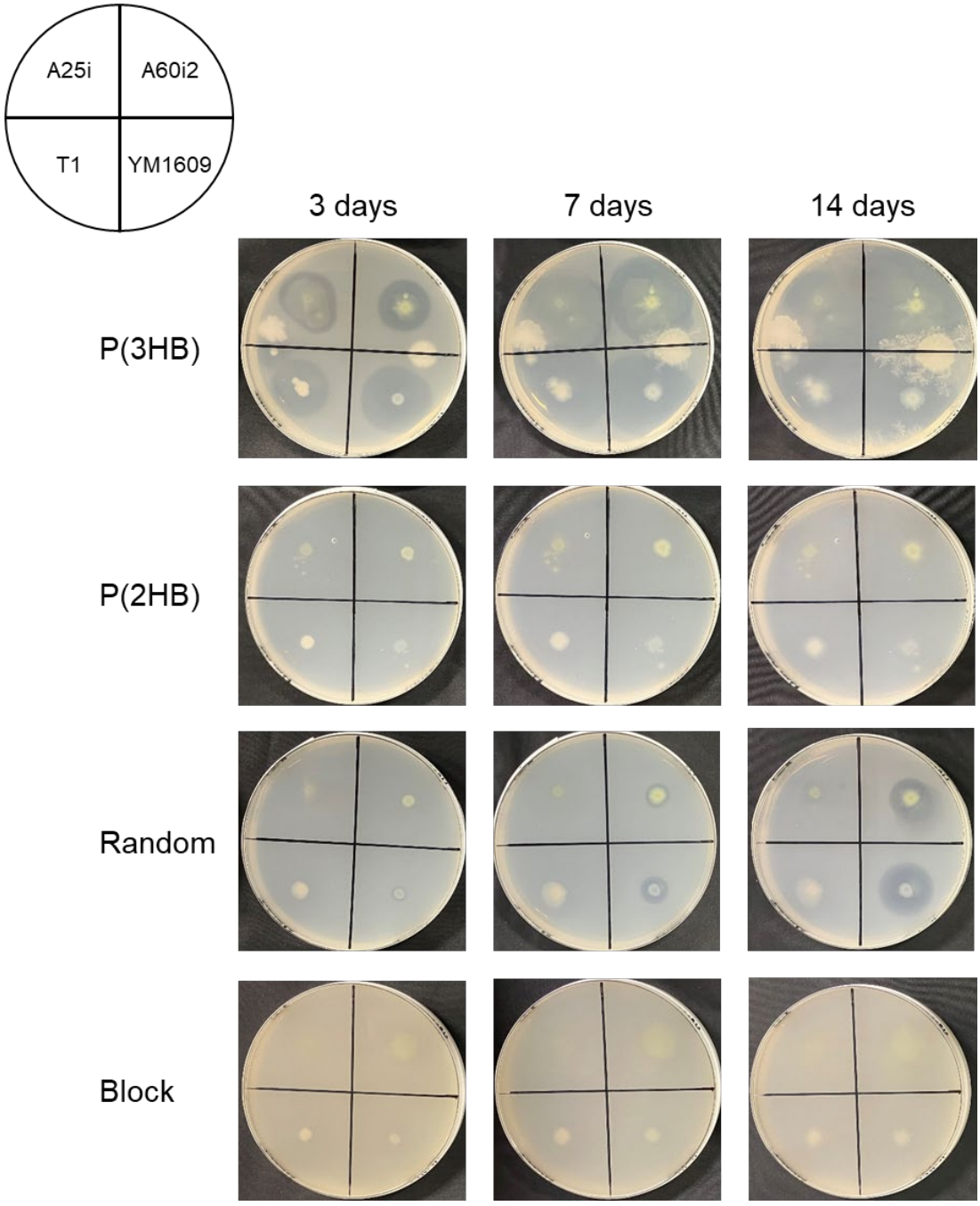


Figure S2. An experiment under the same condition as Figure 3 performed on a different occasion.

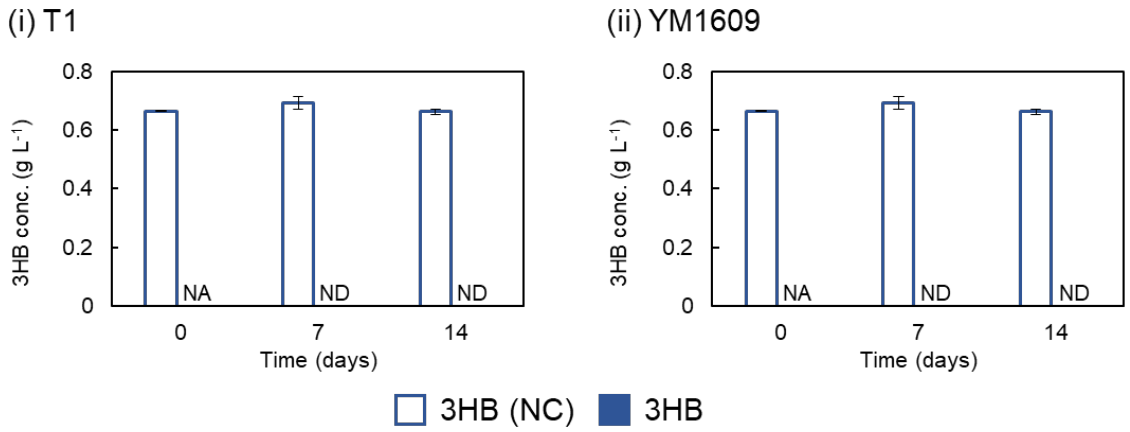


Figure S3. P(3HB) assimilation assays of *Cupriavidus* sp. T1 and *Acidovorax* sp. YM1609. NCs indicate uninoculated samples. Three independent samples for each condition were analyzed. Error bars indicate the standard error of the mean. “NA” and “ND” mean not available and not detected, respectively.

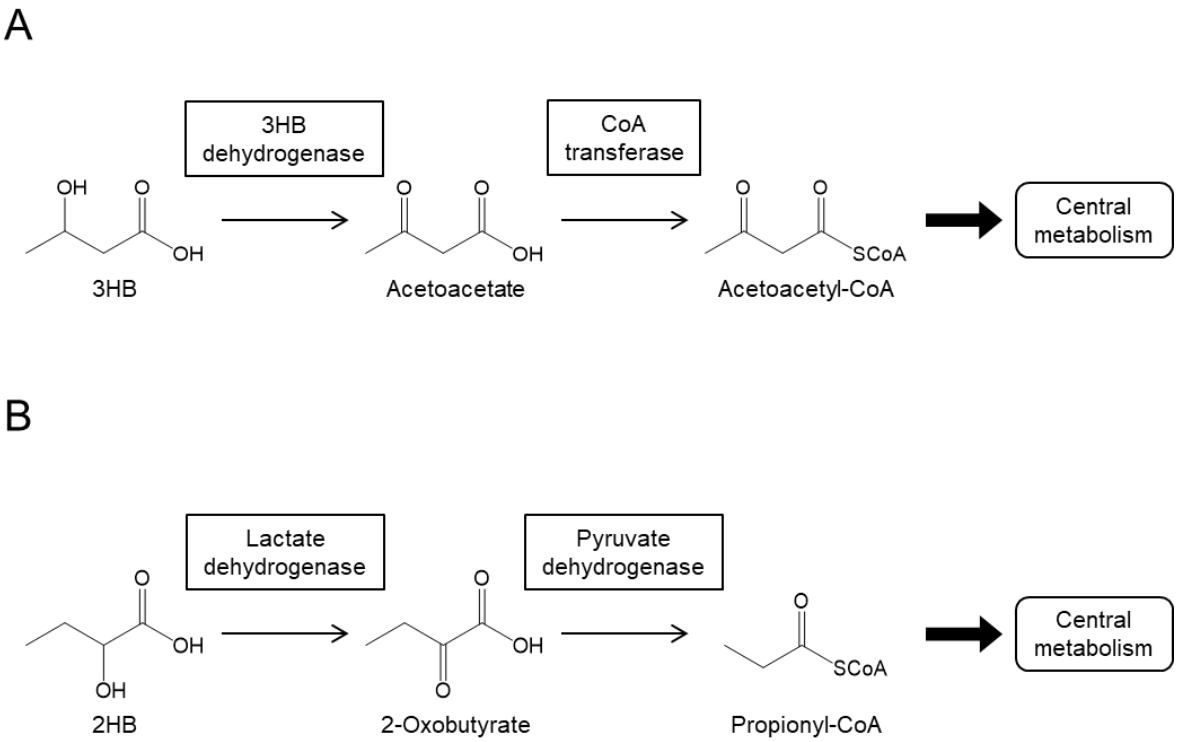


Figure S4. Metabolic pathways of 2HB and 3HB. The general 3HB metabolic pathway in PHA-degrading bacteria (A) and the predicted 2HB metabolic pathway (B) are shown.