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Supplementary Information

Identification of an extracellular poly(3-hydroxybutyrate) depolymerase in the genus *Alteromonas* and its phylogenetic distribution among *Alteromonas* species

Kyogo Iseki^a, Shin-ichi Hachisuka^{b#}, Hiroshi Kikukawa^b, Takeharu Tsuge^c, and Ken'ichiro Matsumoto^b

^aGraduate School of Chemical Sciences and Engineering, Hokkaido University, N13W8, Kita-ku, Sapporo 060-8628, Japan

^bDivision of Applied Chemistry, Faculty of Engineering, Hokkaido University, N13W8, Kita-ku, Sapporo 060-8628, Japan

^cDepartment of Materials Science and Engineering, Institute of Science Tokyo, 4259 Nagatsuta, Midori-ku, Yokohama 226-8502, Japan

[#]Corresponding author: Shin-ichi Hachisuka (s-hachisuka@eng.hokudai.ac.jp)

Table S1. Distribution of PhaZ homologs in *Paraglaciecola* and *Salinimonas* spp.

Genus	Strain	PhaZ homolog
<i>Paraglaciecola</i>	<i>Paraglaciecola agarilytica</i> NO2	-
	<i>Paraglaciecola arctica</i> BSs20135	-
	<i>Paraglaciecola chathamensis</i> 4H-3-7+YE-5	-
	<i>Paraglaciecola chathamensis</i> S18K6	-
	<i>Paraglaciecola hydrolytica</i> S66	-
	<i>Paraglaciecola marina</i> D3211	-
	<i>Paraglaciecola mesophila</i> KMM 241	-
	<i>Paraglaciecola mesophila</i> PLSV	-
	<i>Paraglaciecola polaris</i> LMG 21857	-
	<i>Paraglaciecola psychrophila</i> 170	+
<i>Salinimonas</i>	<i>Salinimonas chungwhensis</i> DSM 16280	+
	<i>Salinimonas iocasae</i> KX18D6	-
	<i>Salinimonas lutimaris</i> DPSR-4	-
	<i>Salinimonas sediminis</i> IO390401	+
	<i>Salinimonas sediminis</i> N102	+

Note: The PhaZ from *Alteromonas* sp. D210916BOD_24 (BFT29534.1) was used as the query for the protein BLAST. The sequences with an E value less than 1e-100 were considered homologs. These also apply to Table S2.

23 **Table S2. Distribution of PhaZ homologs in *Alcanivorax* and *Alloalcanivorax* spp.**

Genus	Strain	PhaZ homolog
<i>Alcanivorax</i>	<i>Alcanivorax borkumensis</i> 97CO-5	-
	<i>Alcanivorax borkumensis</i> 97CO-6	-
	<i>Alcanivorax borkumensis</i> E4	-
	<i>Alcanivorax borkumensis</i> NBRC 101098	-
	<i>Alcanivorax borkumensis</i> NBRC 102028	-
	<i>Alcanivorax borkumensis</i> SK2	-
	<i>Alcanivorax hongdengensis</i> A-11-3	-
	<i>Alcanivorax jadensis</i> T9	-
	<i>Alcanivorax nanhaiticus</i> 19-m-6	-
	<i>Alcanivorax profundus</i> EVN1	-
	<i>Alcanivorax profundus</i> MD8A	-
	<i>Alcanivorax profundus</i> MTEO17	-
	<i>Alcanivorax sediminis</i> PA15-N-34	-
<i>Alloalcanivorax</i>	<i>Alloalcanivorax dieselolei</i> B5	+
	<i>Alloalcanivorax gelatiniphagus</i> MEBiC 08158	+
	<i>Alloalcanivorax mobilis</i> MT13131	+
	<i>Alloalcanivorax mobilis</i> N3-7A	+
	<i>Alloalcanivorax venustensis</i> DSM 26295	-
	<i>Alloalcanivorax venustensis</i> G9-1	-
	<i>Alloalcanivorax xenomutans</i> 43B_GOM-46m	+
	<i>Alloalcanivorax xenomutans</i> EA2	+
	<i>Alloalcanivorax xenomutans</i> JC109	+
	<i>Alloalcanivorax xenomutans</i> KS-293	+
	<i>Alloalcanivorax xenomutans</i> KX64203	+
	<i>Alloalcanivorax xenomutans</i> P2S70	+
	<i>Alloalcanivorax xenomutans</i> P40	+

MMMGLGKGFRLTLFAGNSVLACIVCLFTSLGANAGSWQQNVAMGGFNNVHIYTPDTQSP
 IGQGRALLVVLHGCTQSINAYLTANLEEAENYGMVIAVPDAMNKAGFSCWSYWQGVKS
 RTAGDYANLVSLANSLANSNYGIDADQVYIAGLSSGAAFANTTACLAPDVFAGMGISAGP
 SIGTSSSGAIGSCEYADVTTTCQQYAGSYSASFDDQITSIAHGDAITTVDTCTYNRQNAEG
 MAGVYGVSEEPGTTIIGSGSRTAEELWQDGRVSMWLNLGVDHAWSGGAGASGSYINGS
 GVNYANYLGQFFVDNNKRVDNRQPPELSGVTVQSSNGQLIVQGSAVDQEGSVQINVL
 SDNAGSTFQYSTITQPDDTFSITSAVLADALYVSVTAVDNAAVSAETILTARVGPPIPIA
 PVLSNVIATVSGQCATVSGDVYDDNEDLANVIVSYSTGDVTATLDGLSFTASACDLPGGQ
 QSIVITANDAGGLSTNTQISVVIDAGVATLSEHISAGRLDYATATCYLEYSTNAFKLTETV
 QTDGNCTWQDDDGSCNGPAQVCSGGGNSGGDNPTTCVEFTTSNYYHKVAGRAYSTG
 NVFAPDYFANGSDNAMPGSTWGTNTLYSNDDTLWSVGSCP

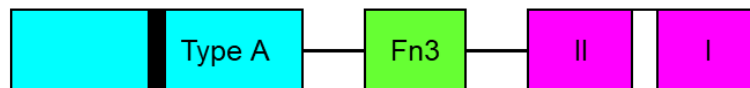


Fig. S1 Sequence analysis of the PhaZ homolog with a Type A CD from *Alteromonas* sp. D210916BOD_24 and its domain organization. Gray, light blue, light green, and magenta-highlighted sequences indicate SP, CD, LD, and SBD, respectively. The red and dark blue-highlighted residues in the CD sequence represent the catalytic triad and the conserved histidine residue for the oxyanion hole, respectively. For the disruption of *phaZ* homolog, *kanR* and its promoter were inserted into the region just after the dark yellow-highlighted Gln residue. The model diagram showing the domain organization is represented in the same style as Fig. 1c. The SP was predicted using SignalP 6.0 (Teufel et al. 2022), while the other domains and residues were predicted by comparison with the previously reported sequences (Behrends et al. 1996; Hisano et al. 2006; Kasuya et al. 2003; Ma et al. 2011; Schöber et al. 2000; Sung et al. 2016).

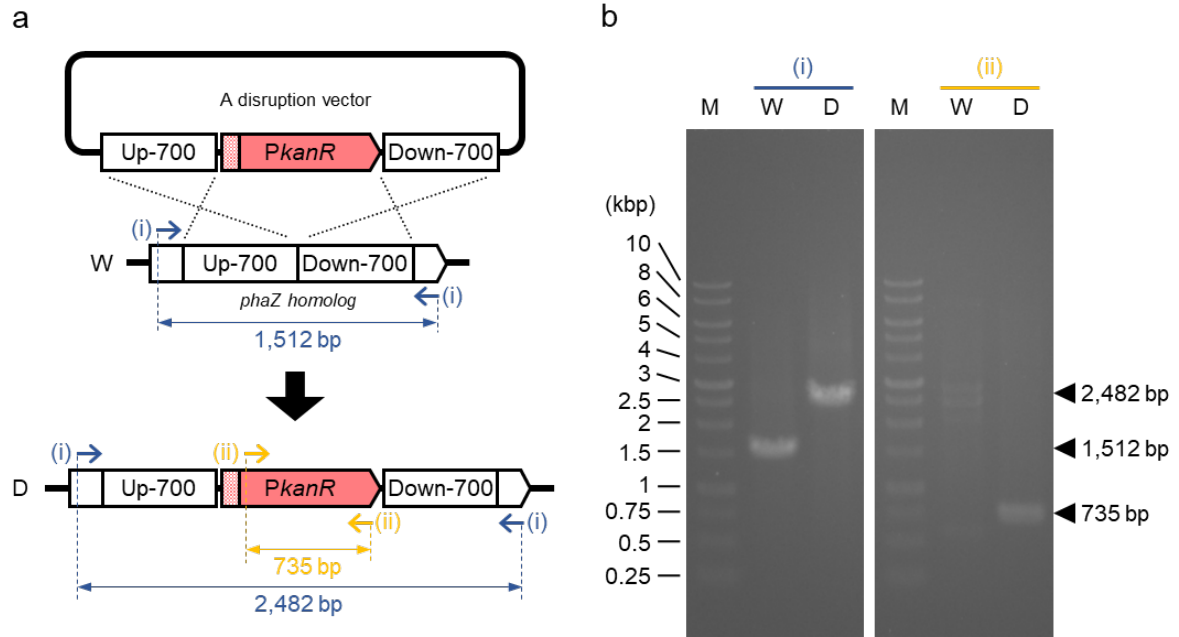


Fig. S2 Construction of the *phaZ* homolog-disruption strain. (a) The procedure for constructing the disruption strain by homologous recombination is shown. (b) The wild-type (W) and disruption (D) strains were analyzed by PCR. The primer sets used in (b), (i) and (ii) are illustrated in (a). *PkanR*, *kanR* and its promoter; M, DNA size marker.

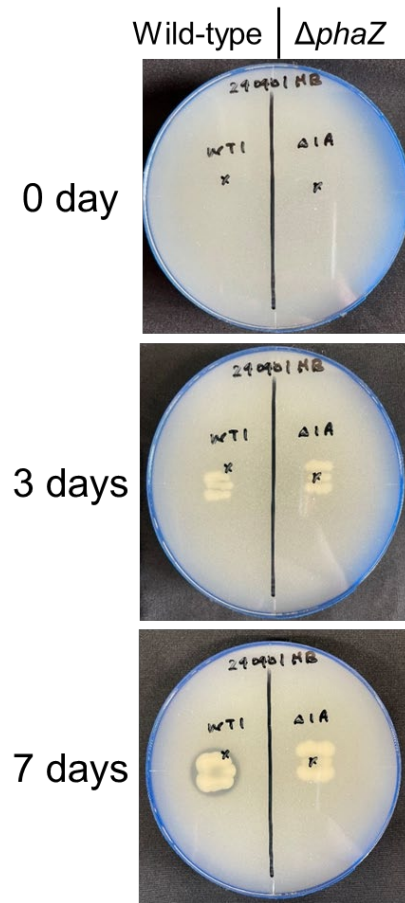


Fig. S3 P(3HB) degradation assay on a MB-2 g/L P(3HB) agar plate. The same conditions as in Fig. 2 were used, except for the concentration of MB in the plate medium.

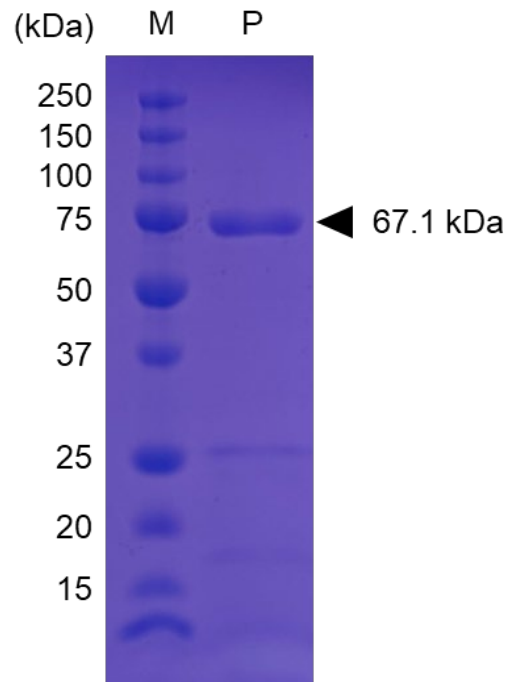


Fig. S4 SDS-PAGE of the purified His-tagged PhaZ homolog. A black arrowhead denotes the band corresponding to the predicted molecular weight from the amino acid sequence. Four micrograms of the purified protein were flowed. M, protein size marker; P, the purified protein.

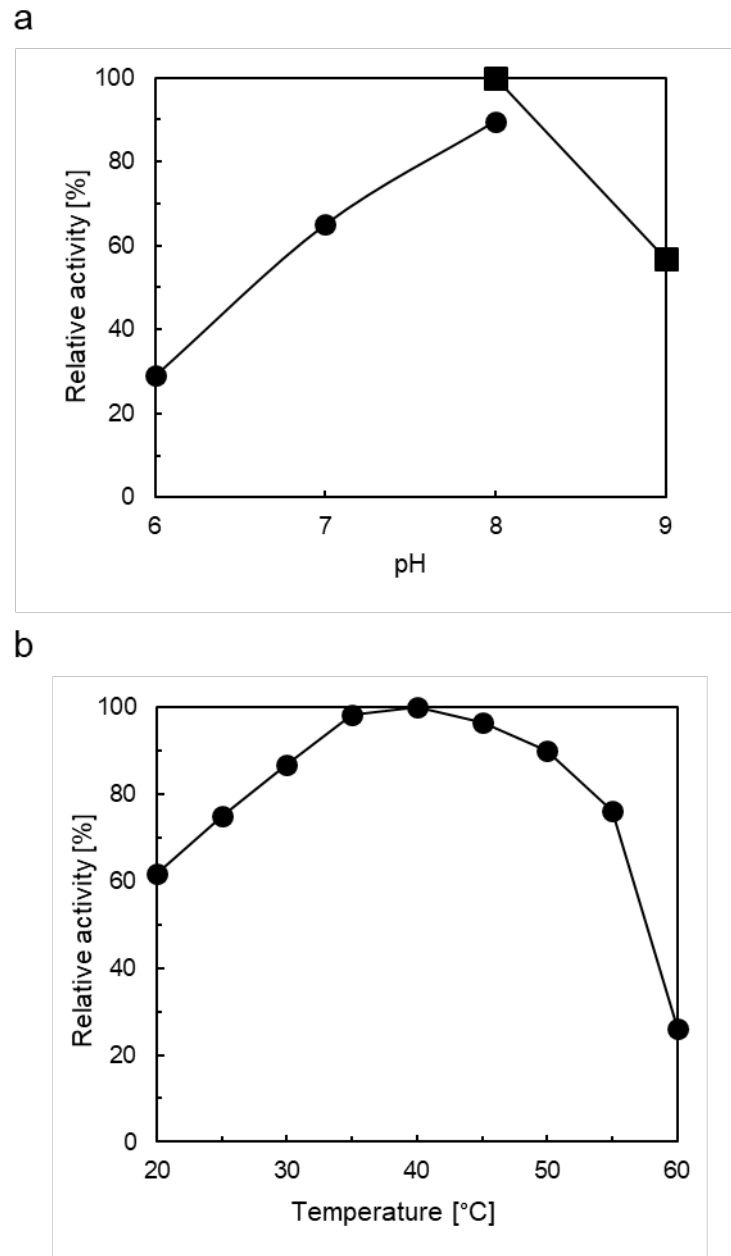


Fig. S5 Assay of the esterase activity of the PhaZ homolog toward *p*NPB. The effects of pH (a) and temperature (b) on the esterase activity were examined. The filled circles and squares indicate results using potassium phosphate and Tris-HCl buffers, respectively.

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