APENDIX A. SUPPLEMENTARY DATA

A sensitive pH indicator-based spectrophotometric assay for PHB depolymerase activity on microtiter plates

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Nomenclature

U = units of enzyme activity

for turbidimetric technique (PHBt) one U corresponds to 1 μg of PHB hydrolyzed per minute,

for PHB suspended with pH indicators (PHBs) one U corresponds to 1 μmol of 3-hydroxybutyric acid released per minute,

for PHB coated adding indicators (PHBc) one U corresponds to 1 μmol of 3-hydroxybutyric acid released per minute.

OD = optical density.

$\Delta OD/min_R$ = reaction signal determined by linear regression of time vs absorbance of the catalyzed reaction.

$\Delta OD/min_B$ = background signal determined by linear regression of time vs absorbance of the background signal.

$\Delta OD/min$ = reaction rate measured as the difference of reaction signal and background signal.

s = slope value of standard curve of 3-hydroxybutyric acid (mM^{-1}).

DF = dilution factor.

Z = Z-factor.

σ_R = standard deviation of reaction signal.

σ_B = standard deviation of the background signal.

μ_R = mean of reaction signal.

μ_B = mean of background signal.

λ_{max} = maximum wavelength (nm).

V_{max} = maximum velocity (OD/min).

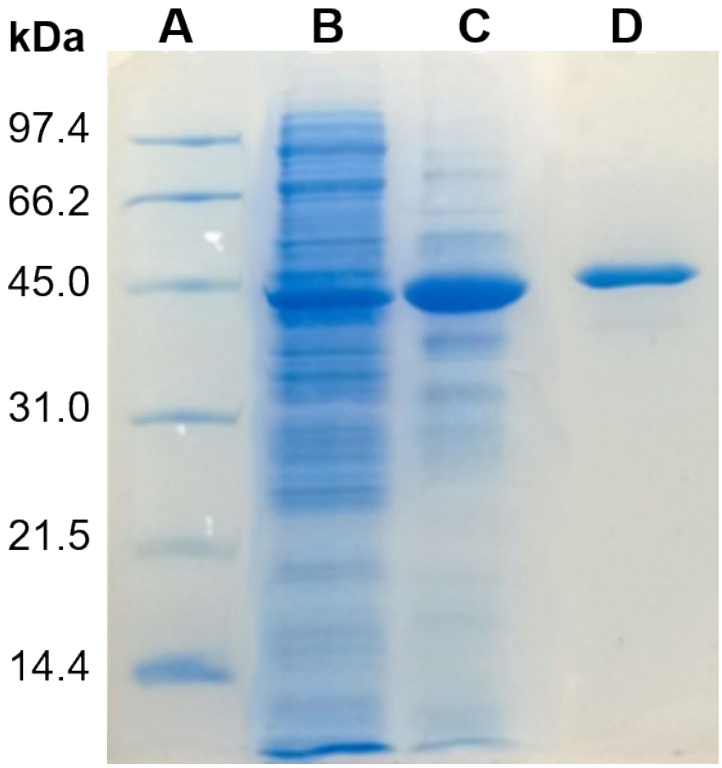

Fig. S1. rPl-PhaZ1 purity analysis by SDS-PAGE (12%). A. Molecular weight markers (Low range, BioRad). B. Lysate from *E. coli*. C. Concentrated pool from affinity HisTrap FF column (GE healthcare). D. Concentrated pool from gel filtration column (Superdex G-200 10/300 GL). The samples were prepared under reducing conditions and Coomassie brilliant blue was used to stain the proteins.

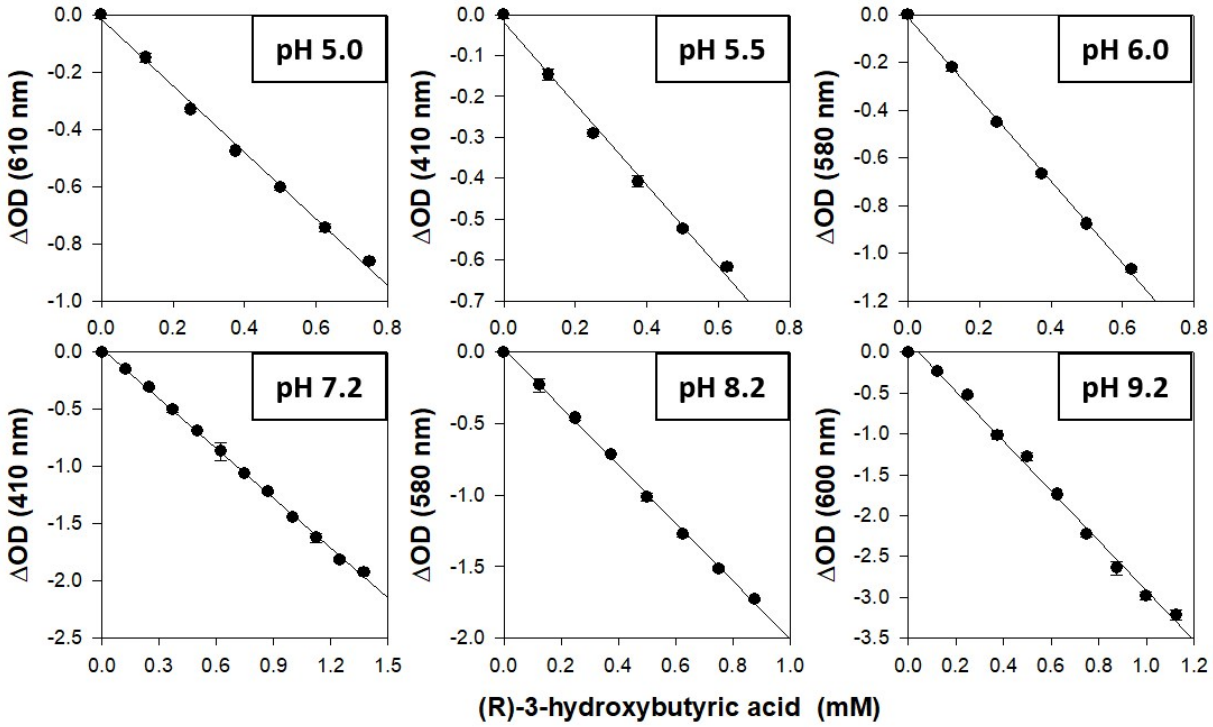

Fig. S2. Standard curves of hydroxybutyric acid (HBA) at different pH values. Assays were performed using different pH indicator-buffer pair for each one of pH values as described in Materials and Methods. Results are given as means ± S.D. for three independent assays. Linear regressions with a R^2 value of 0.99 are shown.

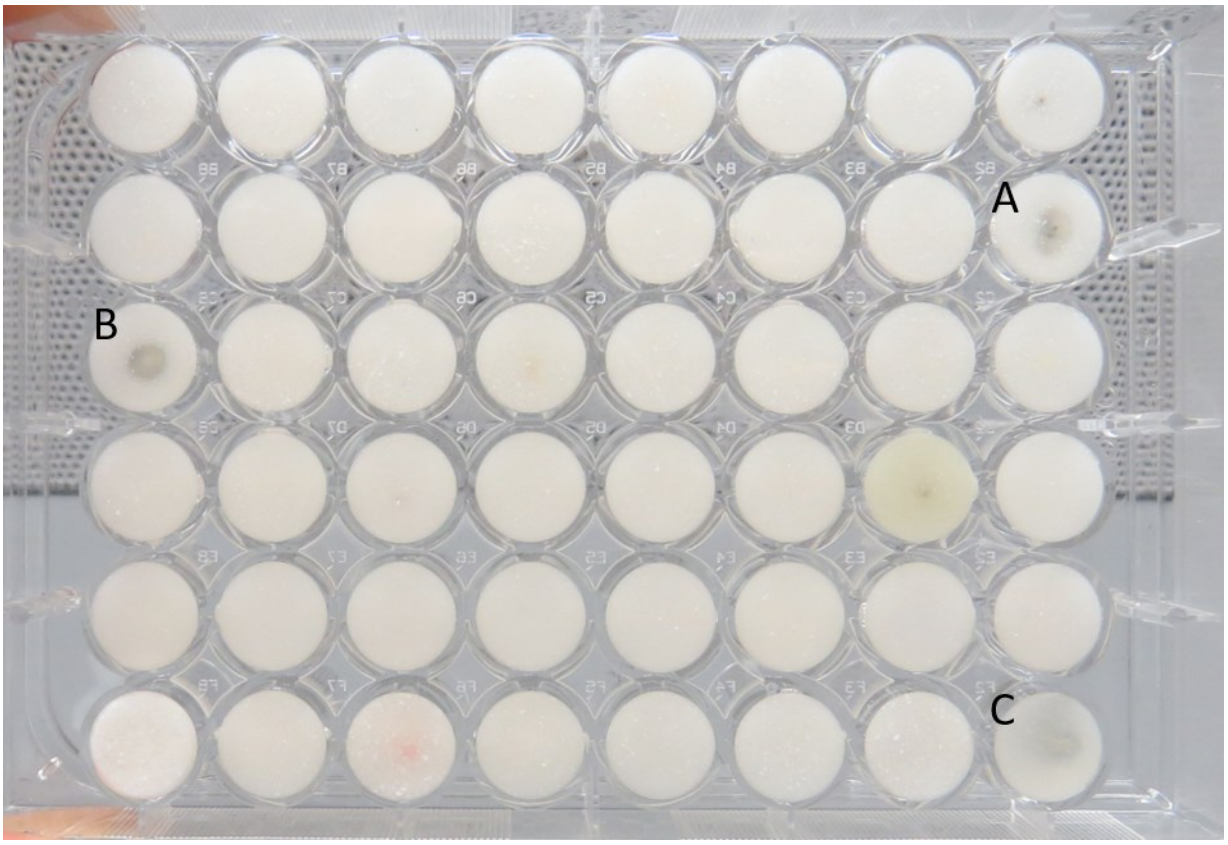

Fig. S3. Example of primary screening of PHB depolymerase strains producers. Assay was carried out using agar cultures with PHB as the sole carbon source. A halo of hydrolysis was observed in strains ACTXRF-5 (A), ACSJRF-3 (B) and AC3-5 (C). The photography was taken of the bottom of the plate.

Table S1. Repeatability of techniques.

Technique	N	Mean (U/mg)	Standard deviation	Confidence Interval* (95%)	Total error (%)
PHBt	15	7433	883	(6945, 7922)	±6.6
PHBc	15	10.40	1.26	(9.71, 11.10)	±6.7
PHBs	15	29.12	3.2	(27.33, 30.89)	±6.1

*t-distribution was used to construct confidence intervals (α=0.05).

The enzymatic activity of a series of enzyme dilutions corresponding to the linear range was measured. Each test was performed in triplicate under repeatability conditions. All efforts were made to keep conditions constant by using the same instrument and operator, and repeating the measurements during a short time period. The assays were performed with 50 µg of PHB per microwell at 37°C and pH 7.2. The enzymatic activity was expressed in U/mg.

Table S2. One-way ANOVA for reproducibility of PHBc technique. Effect of human-associated random error.

Analyst	N	Mean (U/mg)	Standard deviation	Confidence Interval (95%)	Total error (%)	p-value*
A	9	9.51	1.01	(8.65, 10.37)	±9.05	0.363
B	9	8.97	1.40	(8.11, 9.84)	±9.59	

*p-value was determined by One-way ANOVA with 95% of confidence.

Each test was performed under repeatability conditions. The assays were performed with 0.5 µg of rPI-PhaZ1 and 50 µg of PHB per microwell at 37°C and pH 7.2. The enzymatic activity was expressed in U/mg.