Electronic Supplementary Information

Micro-Spray-Based High-Throughput Screening System for Bioplastic-Degrading Microorganisms

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Experimental Section

Materials

PLA was purchased from Natureworks (Minnetonka, MN, USA). PBS was purchased from Ankor Bioplastics Co., Ltd. (Wonju, Gangwon-do, Republic of Korea). PBAT was purchased from BASF (Ludwigshafen, Rheinland-Pfalz, Germany). PHA was purchased from Cheiljedang Co. (Seoul, Republic of Korea). PS was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, tetrahydrofuran, and ethyl acetate were purchased from JT Baker (Phillipsburg, NJ, USA). Dichloromethane was purchased from Burdick & Jackson (Muskegon, MI, USA). LB broth and bacto agar were purchased from BD (Franklin Lakes, NJ, USA).s

Basal solid agar media preparation

LB agar media was prepared by dissolving 25 g L^{-1} of LB broth and 15 g L^{-1} agar in distilled water. Basal carbon-free agar media was prepared with modified M9 minimal media consisting of 1 × M9 salt (Sigma-Aldrich, St. Louis, MO, USA), 2×10^{-2} M $MgSO_4$, 1×10^{-4} M $CaCl_2$, 0.2% casamino acids, 2 mL L^{-1} trace mineral supplement (ATCC MD-TMS), and 15 g L^{-1} agar.

Plastic-containing screening plate preparation

The air-blowing spray system consisted of an air compressor, pressure regulator, syringe pump, and coaxial core-sheath nozzle. The inner and outer diameters and gauge of the core nozzle were 0.26 mm, 0.50 mm, and 25 mm, respectively. Those of the sheath nozzle were 0.86 mm, 1.26 mm, and 18 mm, respectively. The PLA (0.5, 1, 2, and 3 wt%), PBS (1 wt%), PBAT (1

wt%), and PHA (1 wt%) solutions for the air sprays were prepared with chloroform. The plastic solution was injected into the core nozzle by a syringe pump at a rate of 12 mL h^{-1}, and the air was supplied into the sheath nozzle at a pressure of 20 psi. The distance between the solid agar plate and nozzle was 20 cm. The solid medium plate was sprayed for 5 min. The diameter data of the sprayed particles are the mean and standard deviation of 30 measurements.

Agar plate preparation with PLA powder and film

10 g of PLA powder, with particles smaller than 1 mm in diameter, was added to 1 L of molten LB agar, and mixed thoroughly. After that, 15 mL of mixed media was poured into Petri dishes. In the case of media with the PLA film, the film was prepared by the hot-pressing method. The hot-pressed film was cut to a diameter of 6 cm. The thickness of the films was 130 μm, and the weight of the films was 438 ± 4.08 mg.

Viscosity measurement

The viscosity of the PLA solution (0.5, 1, 2, and 3 wt%) was measured with an oscillatory rheometer MCR 302 (Anton Paar, Graz Austria). Oscillatory single frequency sweep was performed at 1 rad s^{-1} of angular frequency and 5% of shear strain.

Microorganism inoculation and screening

An airbrush air sprayer was used for inoculation of biological samples on the plastic-coated agar plates. The sprayer consisted of an air compressor, pressure regulator, and airbrush with a 0.50 mm tip installed (BBA-GP50, YAMATO COMP, Seoul, Republic of Korea). The

inoculum solution was sprayed at an air pressure of 10 psi. In the environmental sample screening, activated sludge was collected from a sewage treatment plant (Ulsan, Republic of Korea). The sludge was serially diluted, and 100 μL of each sample was inoculated on a plastic-coated plate through spraying. The inoculated plates were incubated at 30 °C until a clear zone appeared due to bioplastic-degrading activity. The bacteria isolated from the screening plate were characterized by 16S rRNA gene sequence analysis. Sequencing was performed by Cosmogenetech (Seoul, Republic of Korea), and nucleotide similarity searches were performed using BLAST.

SEM imaging of sprayed plastic particles and bacterial cells

The morphologies of plastic particles and bacterial cells were examined by FE-SEM (Tescan MIRA3, Brno, Czech Republic). The specimens for SEM were prepared by spraying plastic and/or bacteria on a silicon wafer. For the biological samples, specimens were prepared by chemical fixation with a 2% glutaraldehyde aqueous solution to stabilize the structure of the cells. The fixed specimens were then dehydrated with ethanol. The ethanol concentration was gradually increased from 50% to 100%. Prior to SEM observation, all the dried samples were coated with platinum for 7.0 nm.

Plastic degradation tests with film

Isolated single colonies from screening plates were transferred into test tubes containing 3 mL of liquid LB media. The test tubes were incubated at 30 °C overnight (only bacterial strain H4 for 48 h) in a shaking incubator at 200 rpm. The cultures were diluted 100-fold with a carbon-free M9 liquid culture media containing 1 × M9 salt, 2×10^{-2} M $MgSO_4$, 1×10^{-4} M $CaCl_2$,

0.2% casamino acids, 2 mL L^{-1} trace mineral supplement, and 50 μL of the diluted cultures were spread individually on LB agar plates and carbon-free M9 agar plates. Then, corresponding plastic films (1 cm × 1 cm) were mounted on the surface of the inoculated media. All plates except for PHA films were incubated at 30 °C for 2 week. The PHA films were incubated for 1 week. After incubation, the films were retrieved and washed in 1% sodium dodecyl sulfate for 30 min, followed by washing with distilled water and vacuum-drying overnight. The dried films were then observed by SEM, contact angle, and XPS. The water contact angle was measured by drooping 1 μL of deionized water to the suface of the film using a contact angle analyzer (DSA25 Basic, KRUSS, Germany). XPS was conducted using a Kratos AXIS NOVA spectrometer (Kratos Analytical, UK).

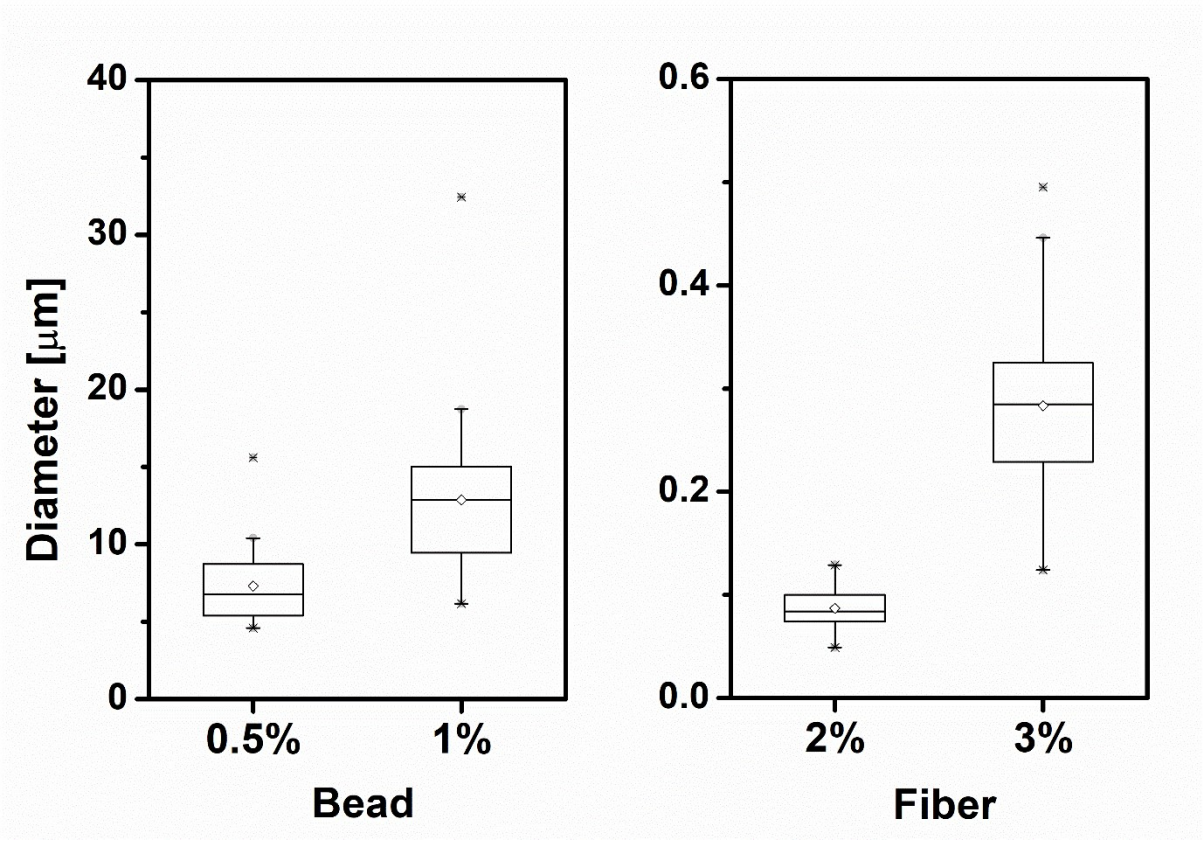

Fig. S1. Size distribution of PLA by varying the solution concentration. Left, Micro-plastic particle diameters of 0.5 and 1% (w/w) PLA solution (n=30). Right, fiber diameters of 2 and 3% (w/w) PLA (n=30). Box-and-whisker plots represent the interquartile range with outliers (●) and mean (◇). Middle line inside the box represents the median. Whiskers extend to the furthest point within 1.5 times the interquartile range.

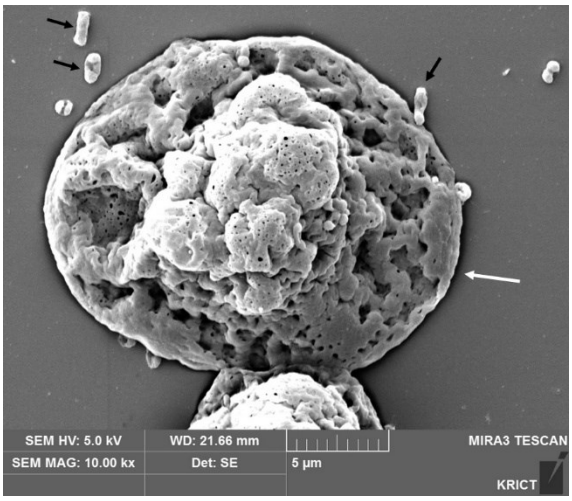

Fig. S2. SEM images of *Escherichia coli* cells and PLA particles. The black arrows indicate bacterial cells and the white arrow indicates the micro-sprayed PLA particle.

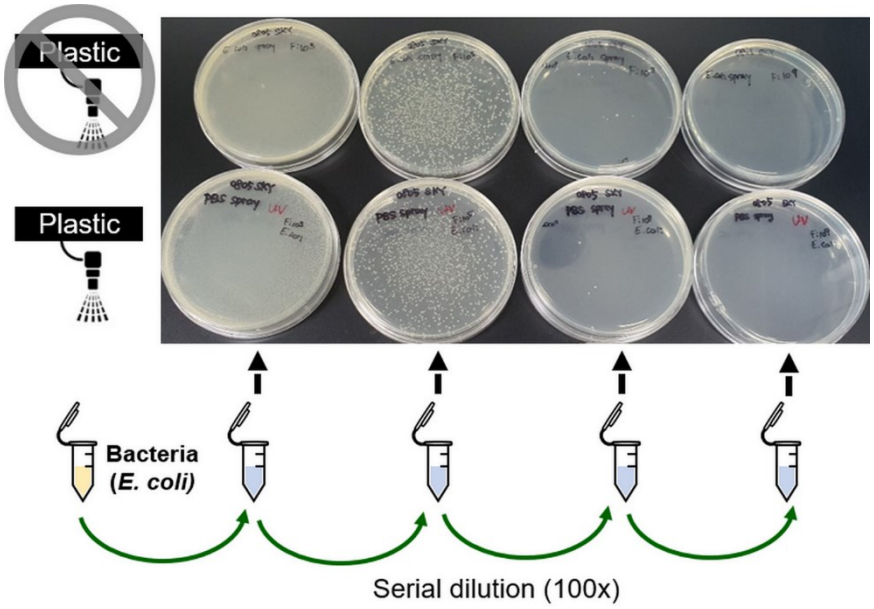

Fig. S3. Growth inhibition test with Gram-negative ~~bacteira~~ bacteria *E. coli.* Serial dilutions of bacterial culture inoculated on the agar plate were prepared without and with PLA (representative plate images).

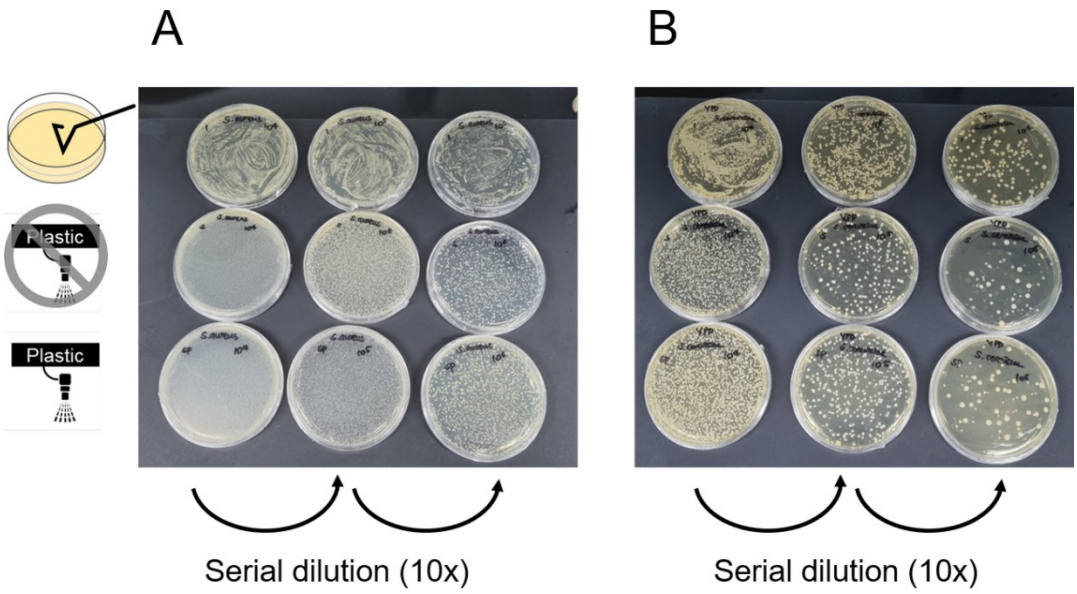

Fig. S4. Growth inhibition test with A) Gram-positive bacteria (*S. aureus)* and B) yeast (*S. cerevisiae*). Serial dilutions of culture inoculated on the agar plate by standard spread method (top) and spray method (middle and bottom). PLA solution was applied on the inoculated plate (bottom) by spraying method.

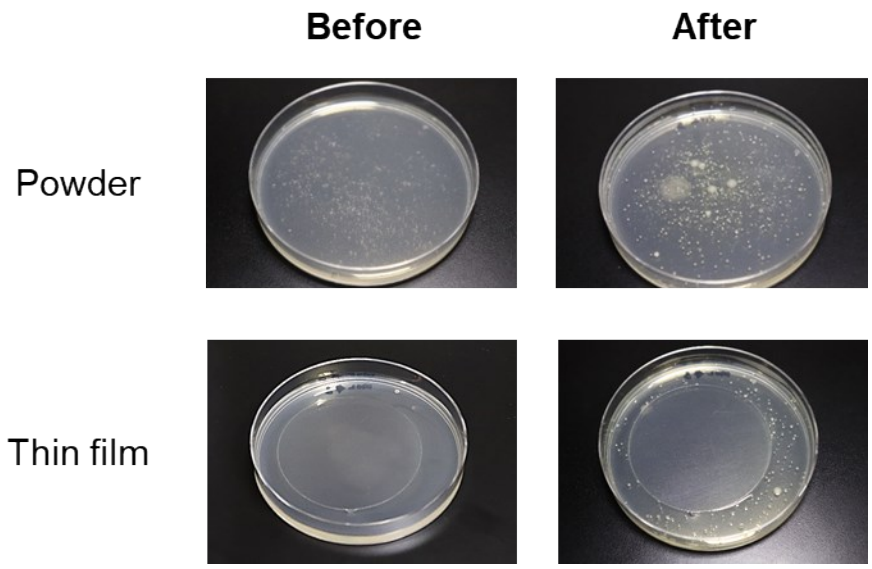

Fig. S5. Activated sludge inoculum cultured on an LB plate with PLA powder and film.

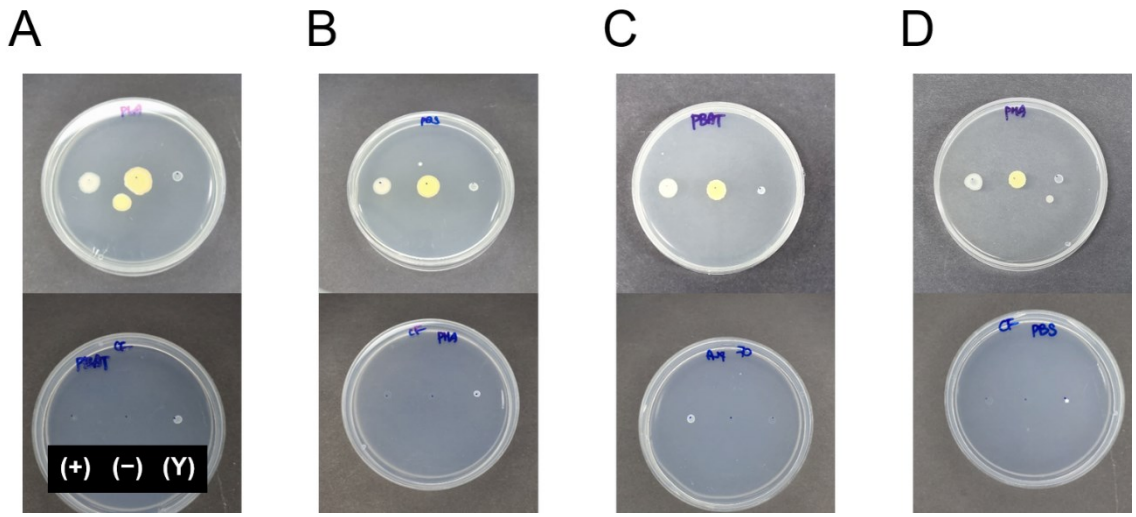

Fig. S6. Cultivation of Gram-negative bacteria *E. coli* (-), Gram-positive bacteria *S. aureus* (+), and yeast *S. cerevisiae* (Y) as negative control on screening plate of A) PLA, B) PBS, C) PBAT, and D) PHA. Basal media of the plates are LB agar (top), and carbon-free agar (bottom).

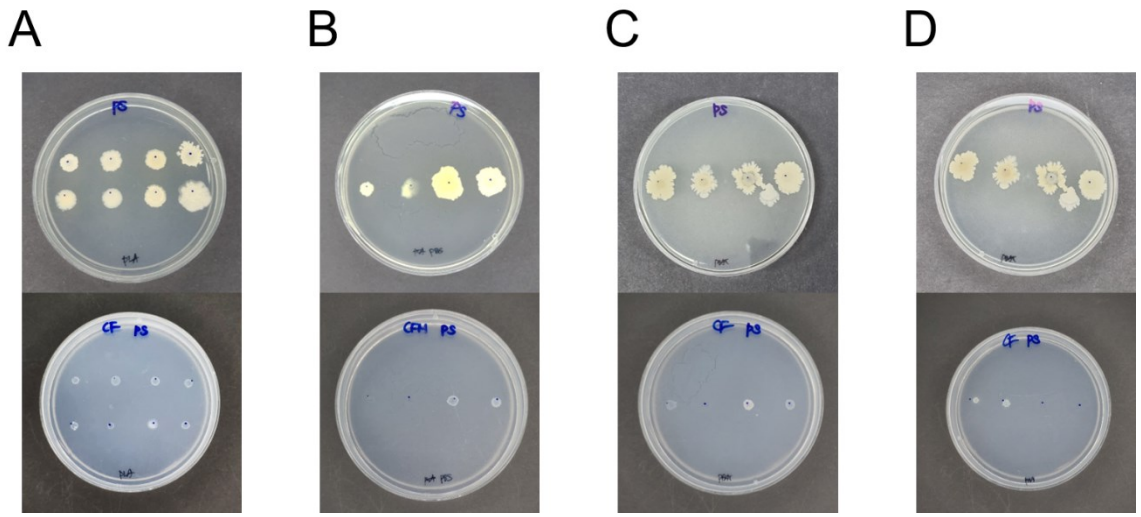

Fig. S7. Cultivation of selected bacteria from screening plate of A) PLA, B) PBS, C) PBAT, and D) PHA on PS sprayed plates. Basal media of the plates are LB agar (top), and carbon-free agar (bottom).

Table S1. List of isolated bacteria from screening plates.

Plastic	Abbreviations	Strains
PLA	L1	*Bacillus velezensis* BP-L1
	L2	*Bacillus subtilis* BP-L2
	L3	*Bacillus* sp. BP-L3
	L4	*Bacillus altitudinis* BP-L4
	L5	*Bacillus amyloliquefaciens* BP-L5
	L6	*Bacillus stratosphericus* BP-L6
	L7	*Bacillus licheniformis* BP-L7
	L8	*Bacillus licheniformis* BP-L8
PBS	S1	*Marinomonas sp.* BP-S1
	S2	*Marinomonas sp.* BP-S2
	S3	*Marinomonas primoryensis* BP-S3
	S4	*Pseudomonas migulae* BP-S4
PBAT	T1	*Marinomonas primoryensis* BP-T1
	T2	*Bacillus* sp. BP-T2
	T3	*Aeromonas media* BP-T3
	T4	*Pseudomonas* sp. BP-T4
PHA	H1	*Pseudomonas stutzeri* BP-H1
	H2	*Pseudomonas* sp. BP-H2
	H3	*Acidovorax facilis* BP-H3
	H4	*Vibrio* sp. BP-H4

Movie S1. Screening procedure with the micro-spray system.