Electronic Supplementary Material

Triggering and identifying the polyurethane and polyethylene-degrading machinery of filamentous fungi secretomes

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1.1 Fungi identification

1.1.1 Molecular identification using

For molecular identification, each fungal strain was grown in liquid cultures containing 1 % (w/v) yeast extract, 2 % (w/v) peptone, and 2 % (w/v) dextrose (YPD) for 4 days, at 27 °C and 120 rpm agitation. Afterward, fungal biomass was harvested using a sterile cloth and washed with PBS solution, until the flow through was clear. Washed biomass was powdered using a mortar and a pestle, under cryogenic conditions (liquid nitrogen). The powdered biomass was used for the isolation of genomic DNA, according to the instructions of NucleoSpin, Plant II Kit, DNA, RNA, and protein purification (Macherey-Nagel, Germany). The entire ITS region was PCR amplified, using ITS1 and ITS4 PCR primers and the purity of PCR products was confirmed by gel electrophoresis on 1 % agarose gel. Finally, the PCR products were cloned in the pCR™ Blunt plasmid vector, according to the protocol of Zero Blunt® PCR Cloning Kit.

1.2 Proteomic analysis

1.2.1 Sample preparation

For the secretome analysis, fungal strains were initially grown in 50 mL MM/Impranil, MM/hexadecane and MM/lcAlk at 27 oC, under continuous stirring at 120 rpm for 4 days. The preculture was used for the inoculation (10% v/v) of a 500 mL sterilized growth medium, which was incubated under the same conditions for 3 days, when the enzymatic activity is maximized. Subsequently, the culture broth was centrifuged at 4,000 x g for 15 min at 4 °C, sterilized using a 0.22 um filter, and concentrated 10-fold using an Amicon ultrafiltration apparatus (exclusion size, 10 kDa; Amicon chamber 8400 with membrane Diaflo PM10, Millipore, Billerica, MA). The concentrated protein extract was dialyzed overnight against a 20 mM Tris-HCl buffer (pH 7.0), freeze-dried under vacuum, and analyzed by LC-MS/MS. Afterwards, the freeze-dried

protein extracts were dissolved in 5 % SDS in 50 mM triethyl ammonium bicarbonate (TEAB) to a final concentration of 1 µg µl-1. To remove sugars, an aliquot from each sample containing 100 µg of protein was filtered with a 3 kDa Nanosep filter (Pall) according to the manufacturer's guidelines. Proteins retained on the filter membrane were re-dissolved in 300 µl 5 % SDS in 50 mM TEAB and reduced by the addition of 15 mM DTT and incubation for 30 minutes at 55 °C followed by alkylation by addition of 30 mM iodoacetamide and incubation for 15 min in the dark. Phosphoric acid was added to a final concentration of 1.2 % to lower the pH to 1, followed by addition of 7 volumes of 90 % methanol in 100 mM TEAB at pH 7.55. Samples were loaded on mini S-trap columns (Protifi) in aliquots of 400 µl centrifuged each time for 30 seconds at 4000 xg, followed by 3 washing steps with 400 µl 90 % methanol in 100 mM TEAB. Then, 1 µg of MS-grade trypsin (Promega) in 125 µl 50 mM TEAB was added onto the filter and incubated overnight at 37 °C to digest the proteins. Finally, peptides were eluted from the S-trap columns in 3 centrifugation steps of 1 minute at 4000 x g, first with 80 µl 50 mM TEAB, then with 80 µl 0.2 % formic acid and finally with 80 µl 50 % acetonitrile. The eluted peptides were transferred into an MS vial, dried completely by vacuum drying and re-dissolved in 0.1% TFA in water/ACN (98:2, v/v)) for an additional purification step on Omix C18 tips (Agilent). The resulting purified peptides were dried completely by vacuum drying.

1.2.2 LC-MS/MS analysis

Peptides were re-dissolved in 20 µl loading solvent A (0.1 % TFA in water/ACN (98:2, v/v)) and firstly loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) × 20 mm, 5 µm beads C18 Reprosil-HD, Dr. Maisch, Ammerbuch-Entringen, Germany). Then, they were separated on a 50 cm µPAC™ GEN2 column and eluted by a stepped gradient from 98 % solvent A (0.1 % formic acid in water) to 30 % solvent B (0.1% formic acid in

water/acetonitrile, 20/80 (v/v)) in 135 min up to 50 % MS solvent B in 15 min, followed by a 5 min wash reaching 95 % solvent B, all at a constant flow rate of 250 nL/min. The mass spectrometer (Ultimate 3000 RSLC nano- LC (Thermo Fisher Scientific, Bremen, Germany) in-line connected to a Q Exactive mass spectrometer (Thermo Fisher Scientific)) was operated in data-dependent, positive ionization mode, automatically switching between MS and MS/MS acquisition for the 5 most abundant peaks in a given MS spectrum. The source voltage was set to 3.0 kV, and the capillary temperature was 275 °C. One MS1 scan (m/z 400−2,000, AGC target 3 ×E6 ions, maximum ion injection time 80 ms), acquired at a resolution of 70,000 (at 200 m/z), was followed by up to 5 tandem MS scans (resolution 17,500 at 200 m/z) of the most intense ions fulfilling predefined selection criteria (AGC target 50.000 ions, maximum ion injection time 80 ms, isolation window 2 Da, fixed first mass 140 m/z, spectrum data type: centroid, intensity threshold 1.3xE4, exclusion of unassigned, 1, 5-8, >8 positively charged precursors, peptide match preferred, exclude isotopes on, dynamic exclusion time 12 s). The HCD collision energy was set to 25 % Normalized Collision Energy and the polydimethylcyclosiloxane background ion at 445.120025 Da was used for internal calibration (lock mass). QCloud was used to control instrument longitudinal performance during the project (Chiva et al., 2018).

Reference:

Chiva, C., Olivella, R., Borràs, E., Espadas, G., Pastor, O., Solé, A., & Sabidó, E. (2018). QCloud: a cloud-based quality control system for mass spectrometry-based proteomics laboratories. *PLoS ONE*, *13*(1). https://doi.org/10.1371/JOURNAL.PONE.0189209

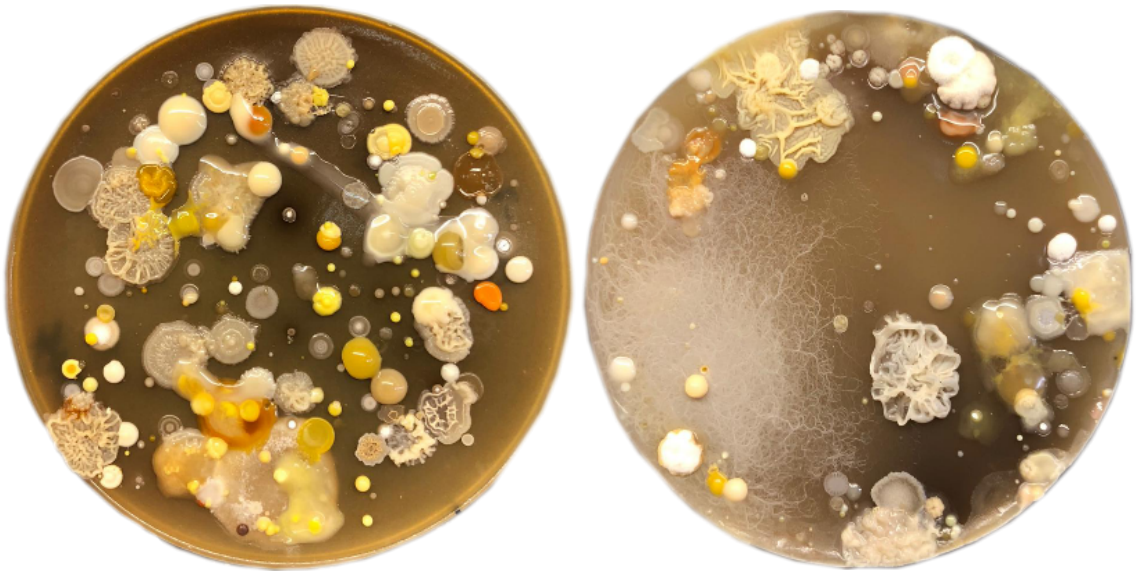

Figure S1: YPDA plates inoculated with the isolated microorganisms

Table S1: Source and codes of fungal strains isolated from variety of environmental samples

Strain Number	Strain Code	Source
1	SSB1	Soil from uncontrolled landfill (Hrtkovci, Serbia)
2	MM41	Dead isopods
3	BPOP17	Plastic contaminated river sediment (Brzece, Serbia)
4	IA1	Decaying PE plastic bag
5	BPBP23	Soil around PE plastic buried for 30 years (Athlone, Ireland)
6	SS1	Contaminated plate with BHET as sole carbon source
7	IA2	Soil from uncontrolled dump site (cemetery, Belgrade, Serbia)
8	MM36	Dead isopods
9	BPBP22	Soil around PE plastic buried for 30 years (Athlone, Ireland)
10	BPOP18	Plastic contaminated river sediment (Brzece, Serbia)
11	SJR2	Soil from uncontrolled landfill (Ruma, Serbia)
12	SeS2	Decaying oak log (Kosutnjak, Serbia)
13	SJR1	Soil from uncontrolled landfill (Ruma, Serbia))

Table S2: Protein content (mg/ml) after concentrating the culture supernatant of each strains

Time (h)	Protein content (mg/ml supernatant)
0	0.0 ± 0.0
24	20.0 ± 1.4
48	30.0 ± 0.3
72	100.0 ± 1.1
96	100.0 ± 0.5
120	110.0 ± 1.3

Table S3: % $\overline{M_n}$ and $\overline{M_w}$ decrease after treating PU powder with the concentrated extracellular fraction of each time point

	F. oxysporum BPOP18		*Fusarium* sp. IA2	
Time (h)	% Mn decrease	% Mw decrease	% Mn decrease	% Mw decrease
0	0.0 ± 0.0	0.0 ± 0.0	0 ± 0.0	0.0 ± 0.0
24	0.3 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.4 ± 0.1
48	9.2 ± 0.0	4.5 ± 0.3	3.9 ± 0.0	3.1 ± 0.2
72	20.4 ± 1.6	9.3 ± 1.0	8.2 ± 1.0	4.2 ± 0.8
96	5.2 ± 0.9	5.5 ± 0.3	2.8 ± 0.4	2.3 ± 0.3
120	1.1 ± 0.4	6.5 ± 0.8	0.2 ± 0.0	0.8 ± 0.2