

Supplementary Information

Microbial cells reprogram energy and protein metabolisms in response to plastic exposure

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Supplementary Method 1. Transcriptomic data processing and analysis of *P. pastoris* GS115

RNA extraction and library preparation

Total RNA was extracted from *P. pastoris* GS115 cells using the OMEGA Yeast RNA Extraction Kit according to the manufacturer's instructions. RNA integrity and concentration were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA), and only samples with high RNA integrity were used for downstream analyses.

Poly(A)+ mRNA was enriched from total RNA using oligo(dT) magnetic beads and fragmented under divalent cation conditions. First-strand cDNA was synthesized using random hexamer primers, followed by second-strand cDNA synthesis. The resulting cDNA fragments were subjected to end repair, A-tailing, adaptor ligation, size selection, PCR amplification, and purification to generate the final sequencing libraries.

Library quality was assessed using a Qubit Fluorometer and an Agilent 2100 Bioanalyzer. Libraries with expected insert sizes and sufficient concentrations were pooled and sequenced on an Illumina platform using paired-end sequencing.

Quality control and read alignment

Raw sequencing reads were processed using fastp to remove adaptor sequences, low-quality reads, and reads containing ambiguous bases. Quality metrics, including Q20, Q30, and GC content, were evaluated, and all downstream analyses were conducted using high-quality clean reads.

Clean reads were aligned to the *P. pastoris* GS115 reference genome (ASM908v1) and corresponding gene annotation files obtained from NCBI using HISAT2 (v2.0.5). Transcript assembly and novel transcript prediction were performed using StringTie (v1.3.3b).

Gene expression quantification and differential expression analysis

Gene-level read counts were generated using featureCounts (v1.5.0-p3). To account for differences in sequencing depth and gene length, gene expression levels were normalized as fragments per kilobase of transcript per million mapped reads (FPKM).

Differential expression analysis between polypropylene- and glass-cultured cells was performed using the DESeq2 R package (v1.20.0). Raw P values were adjusted using the Benjamini–Hochberg method to control the false discovery rate. Genes with an adjusted P value ≤ 0.05 and an absolute $\log_2$ fold change ≥ 1 were considered significantly differentially expressed.

Functional enrichment analysis

Functional enrichment analysis of differentially expressed genes was conducted using clusterProfiler (v3.8.1). Gene Ontology (GO) enrichment analysis was performed with correction for gene length bias, and GO terms with adjusted *P* values < 0.05 were considered significantly enriched. KEGG pathway enrichment analysis was also conducted using clusterProfiler to identify metabolic and regulatory pathways affected by polypropylene exposure.

Supplementary Method 2. Transcriptomic data processing and analysis of *E. coli* BL21

RNA extraction and library preparation

Total RNA was extracted from *E. coli* BL21 cells using an OMEGA Bacterial RNA Isolation Kit according to the manufacturer's instructions. RNA integrity and concentration were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA), and only samples with high RNA integrity were used for downstream analyses.

Ribosomal RNA was removed prior to library preparation. Strand-specific cDNA libraries were constructed following standard protocols, including RNA fragmentation, first- and second-strand cDNA synthesis, end repair, adaptor ligation, size selection, PCR amplification, and purification. Library quality and insert size distribution were evaluated using Qubit fluorometry and an Agilent 2100 Bioanalyzer. Qualified libraries were pooled and sequenced on an Illumina platform using paired-end sequencing.

Quality control and read alignment

Raw sequencing reads were processed to remove adaptor sequences, low-quality reads, and reads containing ambiguous bases. All downstream analyses were performed using high-quality clean reads.

Clean reads were aligned to the *E. coli* BL21 reference genome (NC_000913.3) using Bowtie2, allowing up to two mismatches. Transcript assembly and identification of transcriptional features were performed using Rockhopper.

Gene expression quantification and differential expression analysis

Gene-level read counts were obtained using HTSeq (v0.6.1). Gene expression levels were normalized as fragments per kilobase of transcript per million mapped

reads (FPKM).

Differential expression analysis between polypropylene- and glass-cultured cells was performed using the DESeq2 R package. *P* values were adjusted using the Benjamini–Hochberg method to control the false discovery rate, and genes with adjusted *P* values < 0.05 were considered significantly differentially expressed.

Functional enrichment analysis

Functional enrichment analysis of differentially expressed genes was conducted using clusterProfiler (v3.8.1). Gene Ontology (GO) and KEGG pathway enrichment analyses were performed, with pathways and terms showing adjusted *P* values < 0.05 considered significantly enriched. KEGG pathway analysis served as the primary basis for functional interpretation in this study.

Supplementary Method 3. Untargeted metabolomic profiling of *P. pastoris* GS115

Untargeted metabolomic profiling of *P. pastoris* GS115 was performed on cells harvested at the physiological steady phase (36 h). Metabolites were extracted using methanol-based protocols and analyzed by UHPLC–MS/MS in both positive- and negative-ion modes on an Orbitrap-based platform at Novogene (Beijing, China).

Raw LC–MS/MS data were processed using XCMS for peak detection, alignment, and retention-time correction. Metabolite annotation was performed by matching accurate mass and MS/MS spectra against public databases, including KEGG, HMDB, and LIPID MAPS, supplemented by in-house libraries.

Multivariate analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), were conducted to assess global metabolic separation between polypropylene- and glass-cultured cells. Model robustness was evaluated by permutation testing.

Differential metabolites were identified based on $|\log_2\text{fold change}| \geq 1$ and a P value ≤ 0.05 . Pathway enrichment analysis was performed by mapping differential metabolites to KEGG pathways. In addition, metabolite set enrichment analysis (GSEA) was conducted separately for positive- and negative-ion datasets to capture coordinated pathway-level metabolic shifts independent of strict differential metabolite thresholds.

Supplementary Method 4. Untargeted metabolomic profiling of *E. coli* BL21

E. coli BL21 cells were harvested at the physiological steady phase (28 h). Cell pellets were collected by centrifugation and immediately quenched in liquid nitrogen. Metabolites were extracted using prechilled 80% methanol following standard protocols, including vortexing, ice incubation, sonication, and centrifugation to remove cellular debris. The resulting supernatants were dried and reconstituted prior to LC–MS/MS analysis.

Untargeted metabolomic profiling was performed by Novogene (Beijing, China) using a Vanquish UHPLC system (Thermo Fisher Scientific) coupled to an Orbitrap Q Exactive™ HF mass spectrometer, operated in both positive- and negative-ion modes. Metabolites were separated on a reversed-phase column using a linear gradient at a flow rate of 0.2 mL min⁻¹, and data were acquired in high-resolution full-scan mode under standard electrospray ionization conditions.

Raw LC–MS/MS data were processed using XCMS for peak detection, retention-time alignment, and peak integration. After background removal and quality filtering, peak intensities were normalized based on quality control samples. Metabolites were annotated by accurate mass and MS/MS spectral matching against public databases, including KEGG, HMDB, and LIPID MAPS, with a mass tolerance of ≤10 ppm.

Multivariate statistical analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), were conducted to evaluate global metabolic differences between polypropylene- and glass-cultured cells. Differential metabolites were identified based on univariate analysis using the criteria $|\log_2\text{fold change}| \geq 1$ and $P \leq 0.05$. KEGG pathway enrichment analysis was subsequently performed to identify metabolic pathways associated with polypropylene exposure.

Supplementary Method 5. Untargeted extracellular metabolomic profiling of *P. pastoris* GS115

Pichia pastoris GS115 was cultured in plastic or glass vessels under the conditions described in the main Methods, with three independent biological replicates per condition. Cells were removed after cultivation, and cell-free supernatants were collected. Aliquots (1 mL) were freeze-dried, resuspended in prechilled 80% methanol and vortexed thoroughly. Samples were incubated on ice for 5 min and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was diluted with LC-MS-grade water to a final methanol concentration of 53%, transferred to a fresh tube and centrifuged again under the same conditions. The resulting supernatant was used for LC-MS/MS analysis.

Untargeted metabolomic profiling was performed by Novogene Co., Ltd. (Beijing, China) using a Vanquish UHPLC system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer (Thermo Fisher Scientific), operated in positive- and negative-ion modes. Metabolites were separated on an ACQUITY UPLC BEH Amide column (100 × 2.1 mm, 1.7 µm) at a flow rate of 0.2 ml min⁻¹ over a 12-min gradient. Mobile phase A was 5 mM ammonium acetate in 90% acetonitrile and mobile phase B was 5 mM ammonium acetate in 50% acetonitrile. The gradient was 2% B for 1.5 min, 2-100% B from 1.5 to 7 min, 100% B until 9 min, 100-2% B from 9 to 9.1 min and 2% B until 12 min. Data were acquired under the standard electrospray-ionization settings of the platform. A pooled quality-control sample was prepared by mixing equal volumes of all samples in the analytical batch. Three quality-control injections were performed before sample acquisition, and further quality-control samples were interspersed throughout the run.

Raw files were converted to mzXML format using ProteoWizard and processed using XCMS for peak detection, alignment, retention-time correction and peak-

area integration. Features missing from more than 50% of samples within a group were removed, and remaining missing values were imputed using the k-nearest-neighbour method. Blank-derived background ions were excluded. Peak areas were normalized by total-signal scaling, and compounds with coefficients of variation greater than 30% in pooled quality-control samples were removed. Metabolites were identified by matching precursor-ion information and MS/MS spectra against the NovoMetDB in-house spectral database using a mass tolerance of 10 ppm and were annotated using KEGG, HMDB and LIPID MAPS.

Principal component analysis was performed using normalized peak areas. Fold change was calculated as the ratio of the mean abundance in plastic to that in glass, and statistical significance was assessed using a two-sided t-test. Metabolites with fold change greater than 2 or less than 0.5 and $P < 0.05$ were classified as differentially abundant for volcano-plot visualization. Benjamini-Hochberg-adjusted P values for the complete dataset are provided in the associated source data. Differentially abundant annotated metabolites were mapped to KEGG pathways for exploratory enrichment analysis.

Supplementary Method 6. Untargeted extracellular metabolomic profiling of *E. coli* BL21

Escherichia coli BL21 was cultured in plastic or glass vessels under the conditions described in the main Methods, with three independent biological replicates per condition. Cells were removed after cultivation, and cell-free supernatants were collected. Aliquots (1 ml) were freeze-dried, resuspended in prechilled 80% methanol and vortexed thoroughly. Samples were incubated on ice for 5 min and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was diluted with LC-MS-grade water to a final methanol concentration of 53%, transferred to a fresh tube and centrifuged again under the same conditions. The resulting supernatant was used for LC-MS/MS analysis.

Untargeted metabolomic profiling was performed by Novogene Co., Ltd. (Beijing, China) using a Vanquish UHPLC system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer (Thermo Fisher Scientific), operated in positive- and negative-ion modes. Metabolites were separated on an ACQUITY UPLC BEH Amide column (100 × 2.1 mm, 1.7 µm) at a flow rate of 0.2 ml min⁻¹ over a 12-min gradient. Mobile phase A was 5 mM ammonium acetate in 90% acetonitrile and mobile phase B was 5 mM ammonium acetate in 50% acetonitrile. The gradient was 2% B for 1.5 min, 2-100% B from 1.5 to 7 min, 100% B until 9 min, 100-2% B from 9 to 9.1 min and 2% B until 12 min. Data were acquired under the standard electrospray-ionization settings of the platform. A pooled quality-control sample was prepared by mixing equal volumes of all samples in the analytical batch. Three quality-control injections were performed before sample acquisition, and further quality-control samples were interspersed throughout the run.

Raw files were converted to mzXML format using ProteoWizard and processed using XCMS for peak detection, alignment, retention-time correction

and peak-area integration. Features missing from more than 50% of samples within a group were removed, and remaining missing values were imputed using the k-nearest-neighbour method. Blank-derived background ions were excluded. Peak areas were normalized by total-signal scaling, and compounds with coefficients of variation greater than 30% in pooled quality-control samples were removed. Metabolites were identified by matching precursor-ion information and MS/MS spectra against the NovoMetDB in-house spectral database using a mass tolerance of 10 ppm and were annotated using KEGG, HMDB and LIPID MAPS.

Principal component analysis was performed using normalized peak areas. Fold change was calculated as the ratio of the mean abundance in plastic to that in glass, and statistical significance was assessed using a two-sided t-test. Metabolites with fold change greater than 2 or less than 0.5 and $P < 0.05$ were classified as differentially abundant for volcano-plot visualization. Benjamini-Hochberg-adjusted P values for the complete dataset are provided in the associated source data. Differentially abundant annotated metabolites were mapped to KEGG pathways for exploratory enrichment analysis.

Supplementary Method 7. Untargeted LC-MS/MS profiling of uninoculated YPD medium

Uninoculated YPD medium was dispensed into plastic or glass vessels and incubated under the same conditions and for the same duration as GS115 cultures. Three independent samples were prepared per condition. Aliquots (1 ml) were freeze-dried, resuspended in prechilled 80% methanol and vortexed thoroughly. Samples were incubated on ice for 5 min and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was diluted with LC-MS-grade water to a final methanol concentration of 53%, transferred to a fresh tube and centrifuged again under the same conditions.

The YPD samples were analysed in the same LC-MS/MS batch and using the same chromatographic, mass-spectrometric, quality-control, data-processing and annotation procedures as the GS115 culture supernatants described in Supplementary Method 5.

Principal component analysis was performed using normalized peak areas to compare the chemical profiles of YPD medium incubated in plastic and glass vessels. Fold change was calculated as the ratio of the mean abundance in plastic to that in glass, and statistical significance was assessed using a two-sided t-test. Features with fold change greater than 2 or less than 0.5 and $P < 0.05$ were classified as differentially abundant for volcano-plot visualization. Benjamini-Hochberg-adjusted P values for the complete feature dataset are provided in the associated source data.

Supplementary Method 8. Untargeted LC-MS/MS profiling of uninoculated LB medium

Uninoculated LB medium was dispensed into plastic or glass vessels and incubated at 37 °C with shaking at 180 rpm for 14 h. Three independent samples were prepared per condition. Aliquots (1 ml) were freeze-dried, resuspended in prechilled 80% methanol and vortexed thoroughly. Samples were incubated on ice for 5 min and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was diluted with LC-MS-grade water to a final methanol concentration of 53%, transferred to a fresh tube and centrifuged again under the same conditions.

The LB samples were analysed in the same LC-MS/MS batch and using the same chromatographic, mass-spectrometric, quality-control, data-processing and annotation procedures as the BL21 culture supernatants described in Supplementary Method 6.

Principal component analysis was performed using normalized peak areas to compare the chemical profiles of LB medium incubated in plastic and glass vessels. Fold change was calculated as the ratio of the mean abundance in plastic to that in glass, and statistical significance was assessed using a two-sided t-test. Features with fold change greater than 2 or less than 0.5 and $P < 0.05$ were classified as differentially abundant for volcano-plot visualization. Benjamini-Hochberg-adjusted P values for the complete feature dataset are provided in the associated source data.

Supplementary Table 1. Primers sequences for qPCR

Primer	Sequences (5'-3')
GS115	
RT- <i>tdh3</i> -F	TTGACGGTCCATCCCACAAG
RT- <i>tdh3</i> -R	ACGGTCAAGTCAACAACGGA
RT- <i>fbp1</i> -F	TCCGTAACGGTGTCGTCAAG
RT- <i>fbp1</i> -R	CACCCTCACGAACCCAAACT
RT- <i>pfk1</i> -F	TTCGTGGTGGAAGTCATGGG
RT- <i>pfk1</i> -R	TGGTCTTCCTGCGTCCTTTC
RT- <i>pfk2</i> -F	TGAGTCTGATGTACCAAGGCG
RT- <i>pfk2</i> -R	GCTTGCGGCATTTATTGTTGG
RT- <i>pgm2</i> -F	AGCCGATGATGTCCGTTTTG
RT- <i>pgm2</i> -R	ATGCTACTGCCCGTCTTTGG
RT- <i>tpi1</i> -F	ATGGGAGGTCGGAGAAACAG
RT- <i>tpi1</i> -R	GGAGCAGGAGGAGCAATCAC
RT- <i>pdh1</i> -F	GCTAGTACACAACAATGGCTGAA
RT- <i>pdh1</i> -R	TTTCCTGCCCATCTCATGCC
RT- <i>eno1</i> -F	TGTCTTCTTGGTTGAAACACAC
RT- <i>eno1</i> -R	GTAGAAGCACCGGAAGGAACA
RT- <i>ald4</i> -F	GGGTTACTTCGTCCAGCCAA
RT- <i>ald4</i> -R	GATACCAGCAGCAAGACCGT
RT- <i>pgk1</i> -F	AAGCGTGTCTTCATCCGTGT
RT- <i>pgk1</i> -R	TTGACCTCTCCGTTTGGACG
RT- <i>gpm1</i> -F	TACGAGTTGGACGAGCGTTT
RT- <i>gpm1</i> -R	TCCCCAAGATGTGTTGTGTT
RT- <i>bms1</i> -F	TCACGGTACGCCTCTTCAAC
RT- <i>bms1</i> -R	CCGCCTCTTTAACGGCTTCT

RT- <i>nog1</i> -F	GACAGACGAAGTGACGGTGT
RT- <i>nog1</i> -R	ACCACGCTTACCTGCAATCA
RT- <i>nog2</i> -F	GAAGCCGCCAAAAATGCTGA
RT- <i>nog2</i> -R	TCAAGATTCCCTTCCACATTAAAC
RT- <i>nop1</i> -F	GTCGTTGGACCAGAAGGTGT
RT- <i>nop1</i> -R	GGCGACATCTGCGAAAACAA
RT- <i>rip1</i> -F	CGGTTGGGATGCAATCAAGG
RT- <i>rip1</i> -R	TGTCATCCTCAAAGCCAGCA

BL21

RT- <i>rpoB</i> -F	CGTGGTGGTGTCTGTCAGTA
RT- <i>rpoB</i> -R	AGAGACACACACGGCATCTG
RT- <i>pgm</i> -F	TGCGACTTCCGCACAAAAAG
RT- <i>pgm</i> -R	AACCAGCCGTTGTCAGTCAT
RT- <i>aceE</i> -F	GTGAACGCTGGAACATGCTG
RT- <i>aceE</i> -R	GACCGAAGCCATCAGTACCC
RT- <i>pckA</i> -F	AACCCGCAGTGGAAGAACA
RT- <i>pckA</i> -R	CCGAAGAACACCGCAACATC
RT- <i>sucA</i> -F	GCGTGAAGCGCGTAGTAATG
RT- <i>sucA</i> -R	TCACGGAAATGATGCTGGCT
RT- <i>icd</i> -F	GGAGCAAACATCGGTGACGA
RT- <i>icd</i> -R	GGCTTCAGTCCAGCCCAT
RT- <i>mdh</i> -F	CGGCATCGTGAAAAACCTGG
RT- <i>mdh</i> -R	ATATCCAGCGTGGTAACGCC
RT- <i>aceF</i> -F	AGTGAAAACCTGGCTCGCTGA
RT- <i>aceF</i> -R	GTCGCGTGAACATAAGCGTC
RT- <i>acs</i> -F	CGGTAAGCCAAAAGGTGTGC
RT- <i>acs</i> -R	GCCTTCAAACATCAGCGTGG

RT- <i>gltA</i> -F	TCACCTTTGACCCTGGCTTC
RT- <i>gltA</i> -R	AGTTAGAATCGGTCGCCAGC
RT- <i>sucC</i> -F	ACGTGTAACCGAAGCGTTCA
RT- <i>sucC</i> -R	TTCGGCGTTGTTACCTTCCA
RT- <i>yihX</i> -F	GCTTTCCAATACCAACCGCC
RT- <i>yihX</i> -R	ATTCGTGCTTCAGGTTTGCG
RT- <i>adk</i> -F	GACGTACCGGACGAACTGAT
RT- <i>adk</i> -R	CAGACGTTTACGCACGGTTT
RT- <i>metC</i> -F	GTTTCTGGAATCGCCAGGCT
RT- <i>metC</i> -R	TGGTGGCGGCTTGAATAGAA
RT- <i>nrdD</i> -F	AGCAAGGTGATTCCAACCCA
RT- <i>nrdD</i> -R	CGGGAAGAACGGCGAGTAA
RT- <i>pyrD</i> -F	ACGCCGTATTACAGGAACG
RT- <i>pyrD</i> -R	TCAATGCACTCCCCGTCTTT
RT- <i>sdhB</i> -F	TCTGGCCTGTATTACCCCGA
RT- <i>sdhB</i> -R	GCTGGCGGATTTTGTCCATT
RT- <i>fumB</i> -F	CAGGTGACGGATGCCTGTAA
RT- <i>fumB</i> -R	TGGCTTCCATTCCCAGTTCC

Supplementary Table 2. Growth Retardation of GS115 and BL21

Group	$X_{\max}$	$\mu_{\max}$ (h ⁻¹)	λ (h)
GS115-Plastic	2.522	0.192	2.1
GS115-Glass	2.634	0.211	1.8
BL21-Plastic	2.488	0.123	10.1
BL21-Glass	2.215	0.231	6.2

Supplementary Dataset

Supplementary Table 3. Reference set of PP-associated leachable compounds (separate file)

Spreadsheet containing a literature-curated reference set of PP-associated leachable compounds, including 693 compounds and their corresponding source information. For each compound, the table provides the compound name and the title and DOI of the reference from which its association with polypropylene leaching was identified. This reference set was used to match compounds detected in the extracellular LC–MS/MS datasets and to assess the potential contribution of PP-associated chemical leaching to the observed microbial phenotypes.

Data S1. (separate file)

Spreadsheet of transcriptome-wide information in *P. pastoris* GS115 strain, including gene identification list, list of differentially expressed genes (DEGs), KEGG enrichment results of all DEGs, KEGG enrichment results of down-regulated DEGs, KEGG enrichment results of up-regulated DEGs, and GO enrichment results of all DEGs.

Data S2. (separate file)

Spreadsheet of transcriptome-wide information in *E. coli* BL21 strain, including gene identification list, list of differentially expressed genes (DEGs), KEGG enrichment results of all DEGs, KEGG enrichment results of down-regulated DEGs, KEGG enrichment results of up-regulated DEGs, and GO enrichment results of all DEGs.

Data S3. (separate file)

Spreadsheet of metabolome-wide information in *P. pastoris* GS115 strain,

including metabolite identification list, list of differentially accumulated metabolites (DAMs), KEGG enrichment results, and GSEA results.

Data S4. (separate file)

Spreadsheet of metabolome-wide information in *E. coli* BL21 strain, including metabolite identification list, list of differentially accumulated metabolites (DAMs), KEGG enrichment results, and GSEA results.

Data S5. (separate file)

Spreadsheet of extracellular metabolome-wide information in GS115, including metabolite identification list, list of differentially accumulated metabolites (DAMs), and KEGG enrichment results.

Data S6. (separate file)

Spreadsheet of extracellular metabolome-wide information in BL21, including metabolite identification list, list of differentially accumulated metabolites (DAMs), and KEGG enrichment results.

Data S7. (separate file)

Spreadsheet of metabolite identification results from untargeted LC–MS/MS profiling of uninoculated YPD medium incubated in plastic and glass vessels.

Data S8. (separate file)

Spreadsheet of metabolite identification results from untargeted LC–MS/MS profiling of uninoculated LB medium incubated in plastic and glass vessels.