Electronic Supplementary Information

Polystyrene nanoplastics foster *Escherichia coli O157:H7* growth and antibiotic resistance with a stimulating effect on metabolism

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Text S1

Headspace SPME methodology. Based on our previous studies [1-2], a novel hydrophobic-hydrophilic balanced SPME probe was used to realize the high-coverage capture to in vivo volatile metabolites in this work. Before each extraction, the SPME probe was conditioned at 250 °C in the GC injection port under nitrogenous atmosphere in order to avoid any contamination or carryover. The bacterial suspension (1 mL) of *E. coli O157:H7* and 7 mL of the treated or untreated *E. coli O157:H7* cultures were inoculated into a 20 mL glass vial sealed with PTFE cap. The incubation condition was set as 37 °C with agitation at 150 r/min for overnight. For each sampling procedure, the probe was inserted into the glass vial for 20 mm to conduct headspace extraction. Other extraction conditions of in vivo volatile metabolites were set as follows: balance temperature, 37 °C; balance time, 60 min; extraction temperature, 37 °C; extraction time, 60 min; desorption temperature, 250 °C; desorption time, 5 min.

GC-QTOFMS analysis and data processing. GC-QTOFMS analysis was performed using an Agilent 7890B GC instrument (Agilent Technologies, Shang Hai, China) with a 7250 accurate-mass QTOFMS instrument (mass resolution 20,000 and a mass accuracy specification of 3 ppm) (Agilent Technologies, Shang Hai, China). In our work, a HP-5 MS column (30 m long, 250 μm i.d., with a 0.25-μm-thick 5% phenyl-95% dimethylpolysiloxane film; Agilent Technologies) was used as the 1D column. The samples were introduced by a split/splitless injector (SSL) system with an autosampler (PAL RSI 120, CTC Technologies). The MS parameters were set as follows: electron impact ionization, 70 eV; transfer line temperature, 280 °C; ion source temperature, 200 °C and MS quadrupole temperature, 150 °C.

The MS system was routinely set in a scan mode. The carrier gas was helium, and the constant flow rate was 1 mL/min. For analysis of volatile metabolites generated from *E. coli O157:H7*, the oven temperature program started at 50 °C (held for 1 min), and then increased at 5 °C/min to 260 °C (held for 2 min). The total analysis time was 45 min.

In vivo volatile metabolites generated by *E. coli O157:H7* were identified according to our previous work [3]. Briefly, the detected metabolites were identified based on (1) spectral similarity of the data available in the National Insititute of Standards and Technology (NIST) library (match factor threshold >70), (2) comparison with accurate mass of molecular ions (within 40 ppm), if they existed, and (3) retention index (RI, within 20 units). RI was calculated by a series of n-alkanes (C_9 - C_{25}) analyzed on a HP-5 MS column under the same chromatographic conditions. Semi-quantitative information for the volume fractions of the identified compounds (mean of five replicate trials) were obtained using peak area normalization, which was shown as below: on the assumption of equal response factor, the sum of the peak areas of all the compounds was treated as 1, and then the percentage of the peak area of each component to the total peak area was calculated as component content.

Text S2

RNA extraction. RNA extraction was performed by using bacteria total RNA isolation kit (Sangon Biotech (Shanghai) Co., Ltd., China) following the instructions. After overnight cultivation in response to 0.05-μm, 0.2-μm and 0.6-μm PS microbeads with a certain concentration of 1 mg/mL, *E. coli O157:H7* suspension (1mL) within the logarithmic phase was harvested and centrifuged at 8000 rpm for 1 min at 4 °C. After discarding the supernatant, *E. coli O157:H7* cells were washed three times with sterile deionized water, followed by 100

μL lysis solution (400 μg/ml) addition for 3-5 min. Later, 900 μL Buffer Rlysis-B was added into the above solution system with an intensive mixing, and the whole solution was in incubation for 3 min at room temperature. Then, 200 μL chloroform was fully mixed into the material and the suspension was centrifuged again at 12,000 rpm for 5 min at 4°C, sampling the supernatant. Anhydrous ethanol (1/3 of the supernatant volume) was subsequently added and the mixture was placed at room temperature for 3 min, followed by a new centrifugation at 12,000 rpm for 5 min at 4 °C and all sediments acquisition, which would be washed by 75% ethanol prepared in DEPC-treated ddH_2O (700 μL) twice. Finally, the sediments were inverted at room temperature for 10 min to get rid of the remaining ethanol, dissolved by DEPC-treated ddH_2O (30-50 μL), and stored at -70°C for a long time. The RNA samples were treated with RQ1 DNase (Ambion AM 1906), following the manufacturer's instructions. RNA purity and concentration were measured using the Nanodrop ND-1000.

Reverse transcriptase (RT). RT reactions were conducted by PrimeScript™II 1st Strand cDNA Synthesis Kit (Takara, Japan), using 5 μg of total RNA, 1 μL Oligo dT Primer (50 μM) and 1 μL dNTP Mixture (10 mM each). Prior to cDNA synthesis, denaturation was performed for 5 min at 65 ℃ and then rapidly placed on ice for cooling. After this step, 10 μL of denatured reaction liquid above, 4 μL of 5× PrimeScript II Buffer, 20 U of RNase Inhibito (40 U/μL) and 200 U of PrimeScript II RTase (200 U/μL) were added to each reaction to a final volume of 20 μL by RNase Free ddH_2O. The reaction conditions were 10 min at 30 ℃, 30-60 min at 42 ℃ and 5 min at 95 ℃.

Relative quantification of gene expression using RT-PCR. After obtaining the cDNA, real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) was

performed using the 7500 Real-Time PCR System. The reactions were performed in a final volume of 20 μL, containing 0.8 μL of each primer, 0.4 μL of 50× ROX Reference Dye, 2 μL of cDNA synthesis, 10 μL of 2× TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) and a small amount of sterile water. The reaction procedure was as following: the divided stage one was for pre-degeneration at 95 ℃ for 30 s, and then stage two was for PCR reaction at 95 ℃ for 5 s firstly and at 60 ℃ for 30-34 s. Each treatment was tested with 4–6 biological replicates with each biological replicate consisting of three technical replicates each. The primers used for RT-PCR analysis referred to previous studies [4-5] and were listed in supplementary table S1.

Supplementary Tables

Table S1. TaqMan® primers and probes.

Primers and probes	Primers sequences (5′ to 3′)
16srRNA-Forward	CAGCCACACTGGAACTGAGA
16srRNA-Reverse	GTTAGCCGGTGCTTCTTCTG
tolC-Forward	CTGAAAGAAGCCGAAAAACG
tolC-Reverse	CTGGCCCATATTGCTATCGT
acrA-Forward	AGCCCTAACAGGATGTGACG
acrA-Reverse	GCTTCGATGTCGCTACCTTC
acrB-Forward	GATTACCATGCGTGCAACAC
acrB-Reverse	TCTGCAAGCAACTGGTTACG
oxyR-Forward	CATTCATTGAAGTGCCGTTG
oxyR-Reverse	CGCGGAAGTGTGTATCTTCA
soxR-Forward	GTATCCGTAACAGCGGCAAT
soxR-Reverse	CATTGGGACGAAAGCTGTTT
soxS-Forward	TTATCGCATGGATTGACGAG
soxS-Reverse	ACATAACCCAGGTCCATTGC

Table S2. In vivo volatile metabolites acquired from the untreated and PS-treated *E. coli O157:H7.*

Number	Compound	RT	Match fators	CAS	RI_{exp}	RI_{lib}	M_{cal} (amu)	M_{exp} (amu)
1	disulfide, dimethyl	7.212	90.8	624-92-0	763	746±6	93.9911	93.9906
2	dimethyl trisulfide	14.3606	99.3	3658-80-8	984	970±7	125.9632	125.9626
3	2-nonanone	17.8958	94.6	821-55-6	1094	1092±2	142.1358	142.1353
4	benzyl nitrile	19.5804	89	140-29-4	1149	1144±5	117.0578	117.0575
5	3-decanone	20.8193	73.6	928-80-3	1190	1187±3	156.1514	156.1499
6	2-decanone	20.9656	80.1	693-54-9	1195	1193±2	156.1514	156.1505
7	octylcyclopropane	23.2345	87.7	1472-09-9	1273	1095±20	154.1722	--
8	2-undecanone	23.9177	93	112-12-9	1297	1294±2	170.1671	170.1668
9	2-undecanol	24.0495	89.2	1653-30-1	1302	1307±4	172.1827	--
10	indole	24.3083	99	120-72-9	1311	1295±7	117.0578	117.0575
11	2-dodecanone	26.6498	87.6	6175-49-1	1398	1396±9	184.1827	184.1825
12	cyclodecanol	27.043	94	1502-05-2	1413	--	156.1514	156.1548
13	cubenene	27.9174	75.2	29837-12-5	1447	1532±1	204.1878	204.1884
14	cis-thujopsene	28.399	83.8	470-40-6	1466	1433±8	204.1878	204.1886
15	9-Decen-1-ol, methyl ether	28.9931	84.9	1000352-65-1	1489	--	170.1671	--
16	2-tridecanone	29.2396	94.9	593-08-8	1499	1497±4	198.1984	198.1982
17	2-tridecanol	29.3587	90	1653-31-2	1504	1510±0	200.2140	200.2091
18	2,4-di-tert-butylphenol	29.7134	91	96-76-4	1519	1519±6	206.1671	206.1668
19	(R)-1-methyl-4-(1,2,2-trimethylcyclopentyl)-benzene	30.2027	85.1	16982-00-6	1539	1505±4	202.1722	202.1727
20	1,1,4,5,6-pentamethyl-2,3-dihydro-1h-indene	30.4602	87	16204-67-4	1549	1523±N/A	188.1565	188.1559
21	1-methyl-7-(1-methylethyl)-naphthalene	31.4786	78.2	490-65-3	1592	1603±25	184.1252	184.1252

Number	Compound	RT	Match fators	CAS	RI_{exp}	RI_{lib}	M_{cal} (amu)	M_{exp} (amu)
22	hexadecane	87.1	87.1	544-76-3	1601	1600	226.2661	226.2661
23	2,4,6-tri-tert-butyl-phenol	89.8	89.8	732-26-3	1619	1627±N/A	262.2297	262.2293
24	cedrol	78.4	78.4	77-53-2	1647	1598±3	222.1984	222.1950
25	3-methyl-hexadecane	90.6	90.6	6418-43-5	1673	1671±5	240.2817	--
26	2-pentadecanone	88.3	88.3	2345-28-0	1703	1698±4	226.2297	226.2292
27	methyl myristoleate	80.2	80.2	56219-06-8	1707	1715±0	240.2089	240.2095
28	methyl tetradecanoate	90.6	90.6	124-10-7	1726	1725±2	242.2246	242.2248
29	4-methyl-heptadecane	90.7	90.7	26429-11-8	1759	1758±1	254.2974	--
30	octadecane	89.6	89.6	593-45-3	1800	1800	254.2974	254.2990
31	Pentadecanoic acid, methyl ester	74.1	74.1	7132-64-1	1826	1820±4	256.2402	256.2395
32	1,4-diphenyl-1,3-butadiene	89.8	89.8	886-65-7	1835	1738±N/A	206.1096	206.1095
33	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	88.3	88.3	84-69-5	1884	1870±4	278.1518	--
34	nonadecane	94.9	94.9	629-92-5	1900	1900	268.3130	268.3170
35	1-(phenylmethylene)-1h-indene	89.9	89.9	5394-86-5	1904	--	204.0939	204.0932
36	hexadecanoic acid, methyl ester	81.5	81.5	112-39-0	1927	1926±2	270.2559	270.2565
37	eicosane	95.3	95.3	112-95-8	2000	2000	282.3287	282.3264
38	3,4-dihydro-4-phenyl-1(2h)-naphthalenone	80	80	1000337-94-1	2044	--	222.1045	222.1036
39	docosane	92.3	92.3	629-97-0	2200	2200	310.3600	310.3578
40	tricosane	81	81	638-67-5	2300	2300	324.3756	324.3775

RT, retention time;

RI_{exp}, a series of alkanes (C_9 - C_{25}) was used to calculate the retention index; RI_{lib}, the retention index in the NIST library;

M_{cal}, accurate molecular mass of theory; M_{exp}, accurate molecular mass of the actual measurement.

Supplementary Figures

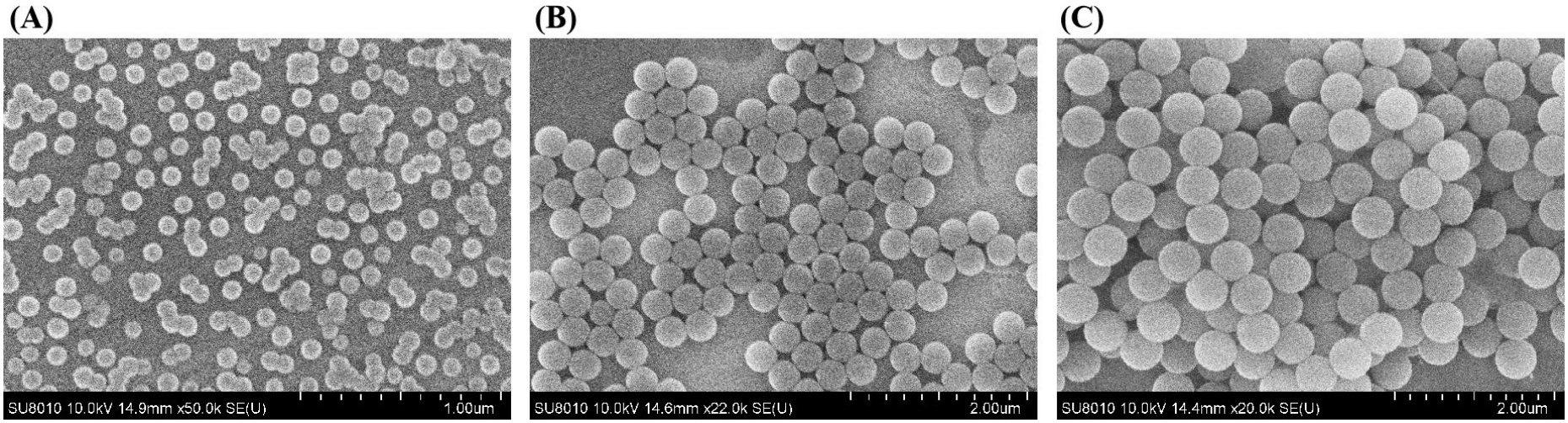

Fig. S1. Polystyrene nanoplastics (PS-NPs) used in this study (A, 0.05-0.1 μm PS-NPs; B, 0.2-0.5 μm PS-NPs; C, 0.6-0.9 μm PS-NPs).

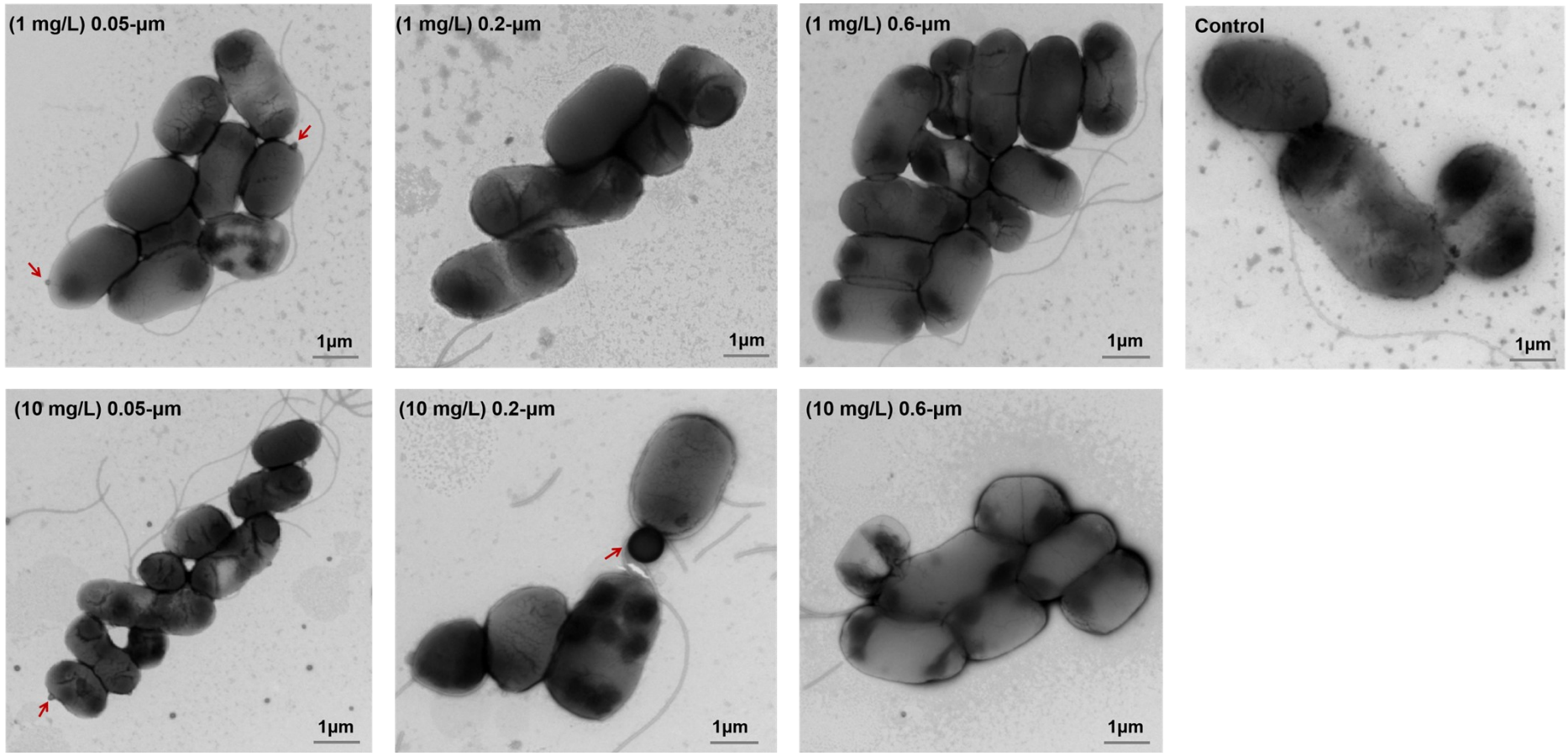

Fig. S2. *E. coli O157:H7* morphologies after PS-NPs exposures in three sizes at the concentration of 1 mg/mL and 10 mg/mL.

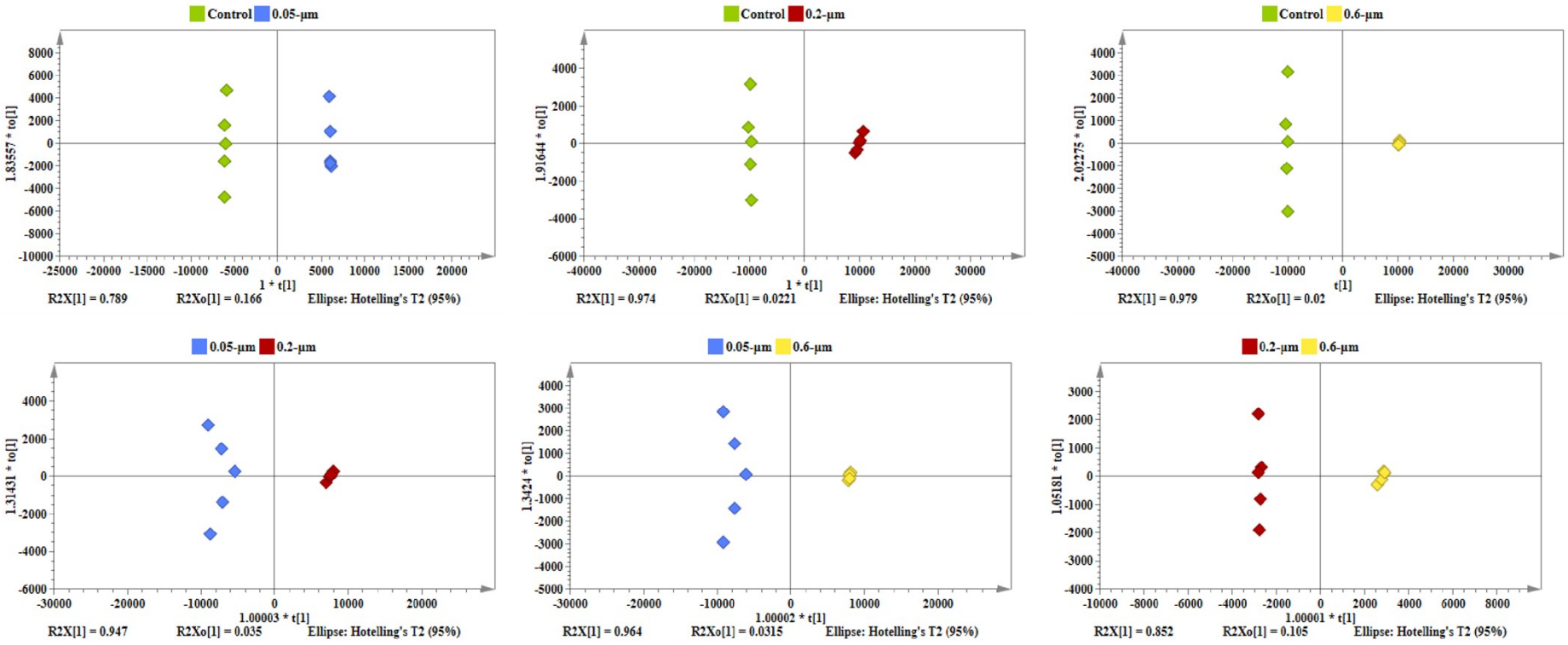

Fig. S3. OPLS-DA plots of in vivo volatile metabolites in pairwise comparison

Supplementary References

[1] Liu S, Fang S, Huang Y, Xiang Z, Ouyang G (2020). A heterogeneous pore decoration strategy on a hydrophobic microporous polymer for high-coverage capture of metabolites. Chemical Communications, 56, 7167-7170.

[2] Huang Y, Fang S, Xiang Z, Liu S, Ouyang G (2022). In vivo environmental metabolomic profiling via a novel microextraction fiber unravels sublethal effects of environmental norfloxacin in gut bacteria. Science of the Total Environment, 845, 157335.

[3] Fang S, Liu S, Song J, Huang Q, Xiang Z (2021). Recognition of pathogens in food matrixes based on the untargeted in vivo microbial metabolite profiling via a novel SPME/GC×GC-QTOFMS approach. Food Research International, 142, 110213.

[4] Fu L, Wei Z, Hu K, Hu L, Li Y, Chen X, Han Z, Yao G, Zhang H (2018). Hydrogen sulfide inhibits the growth of *Escherichia coli* through oxidative damage. Journal of Microbiology, 56, 238–245.

[5] Telke A, Olaitan A, Morand S, Rolain J (2017). SoxRS induces colistin hetero-resistance in Enterobacter asburiae and Enterobacter cloacae by regulating the *acrAB-tolC* efflux pump. Journal of Antimicrobial Chemotherapy, 72(10), 2715-2721.