Supplement of

Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere

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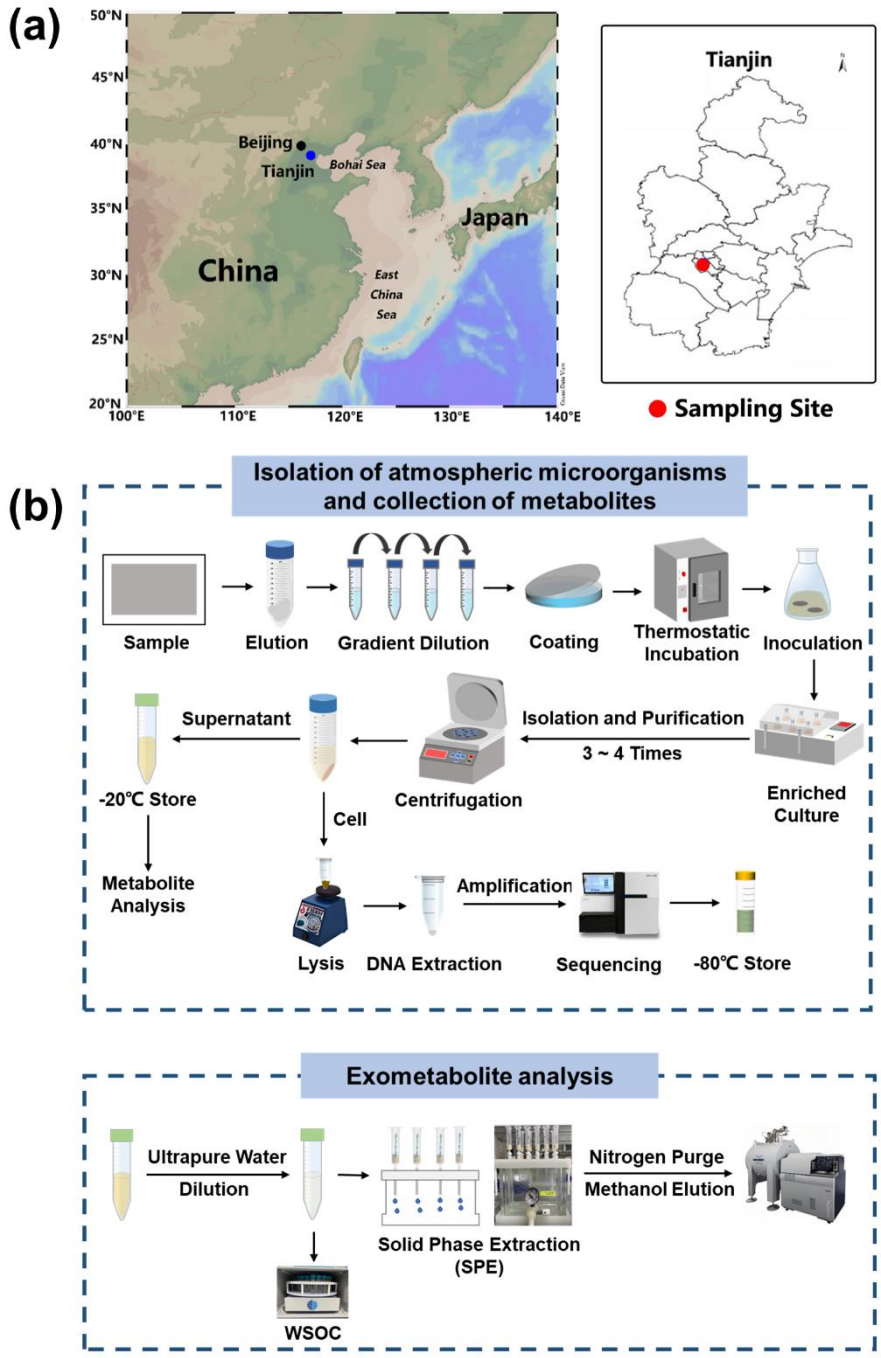

15 **Figure S1.** The location of culturable airborne microorganisms and experimental procedures. (a) The map shows the location of Tianjin city and the sampling site in the Nankai district, Tianjin (the map is from Ocean Data View). (b) Experimental procedures include the isolation of atmospheric microorganisms, collection of metabolites, and exometabolite analysis.

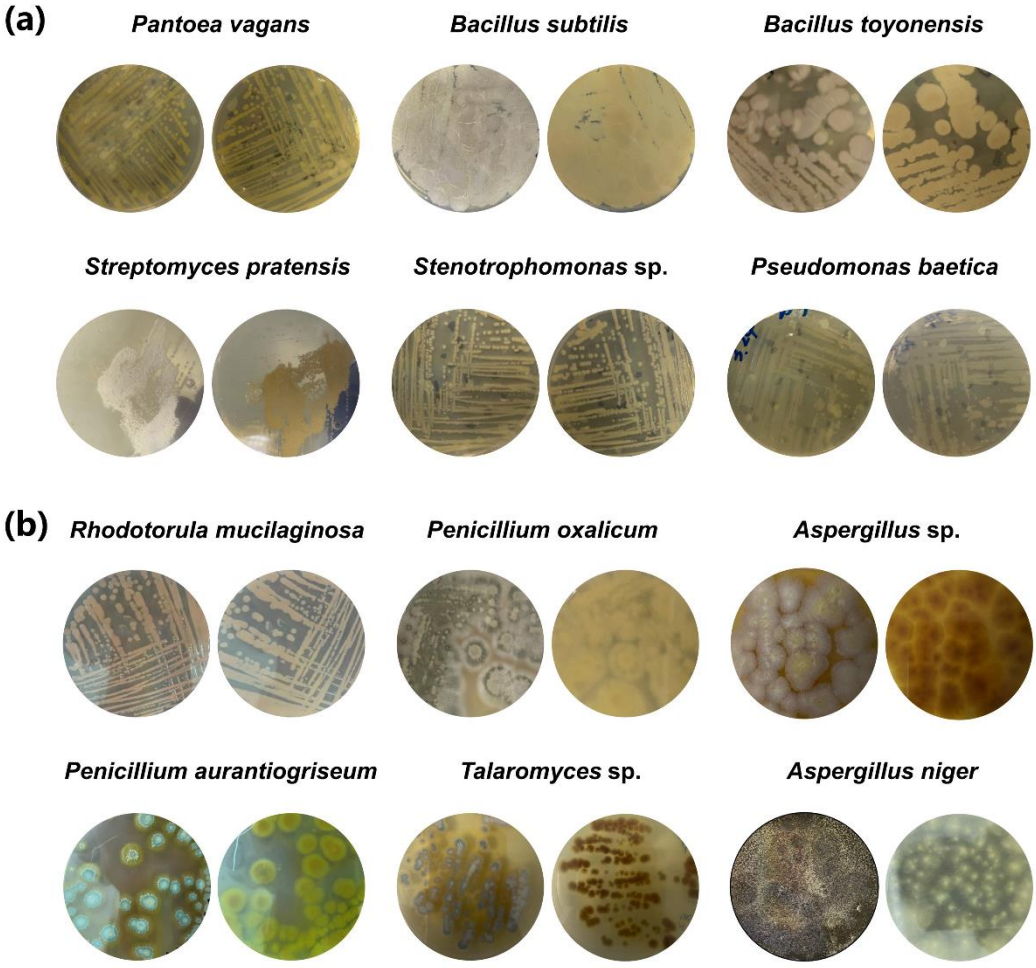

Figure S2. The representative bacterial (a) and fungal (b) strains isolated atmospheric microorganisms.

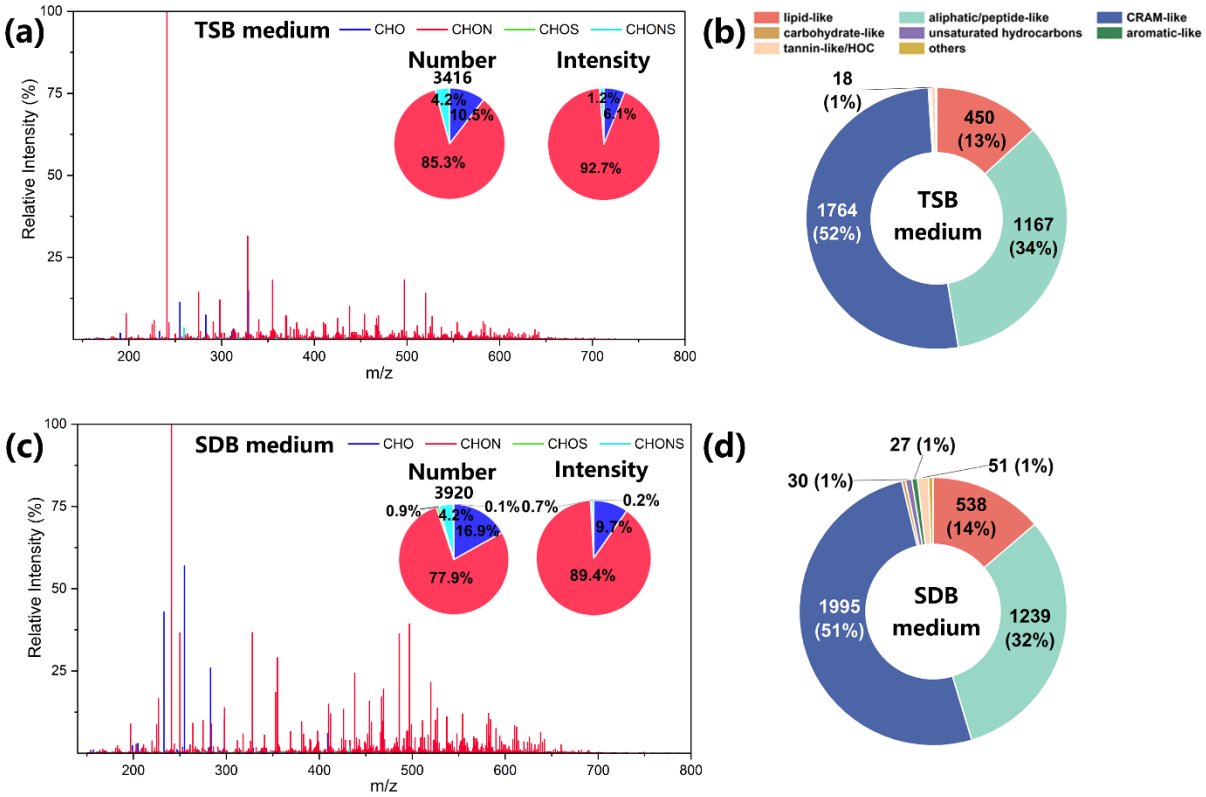

Figure S3. Mass spectra and different categories of organics in the initial medium. The subfigures on the left illustrate the mass spectra and elemental compositions of TSB (a) and SDB (c) media. The right subfigures display the formula numbers of different categories of organic matter for TSB (b) and SDB (d) media.

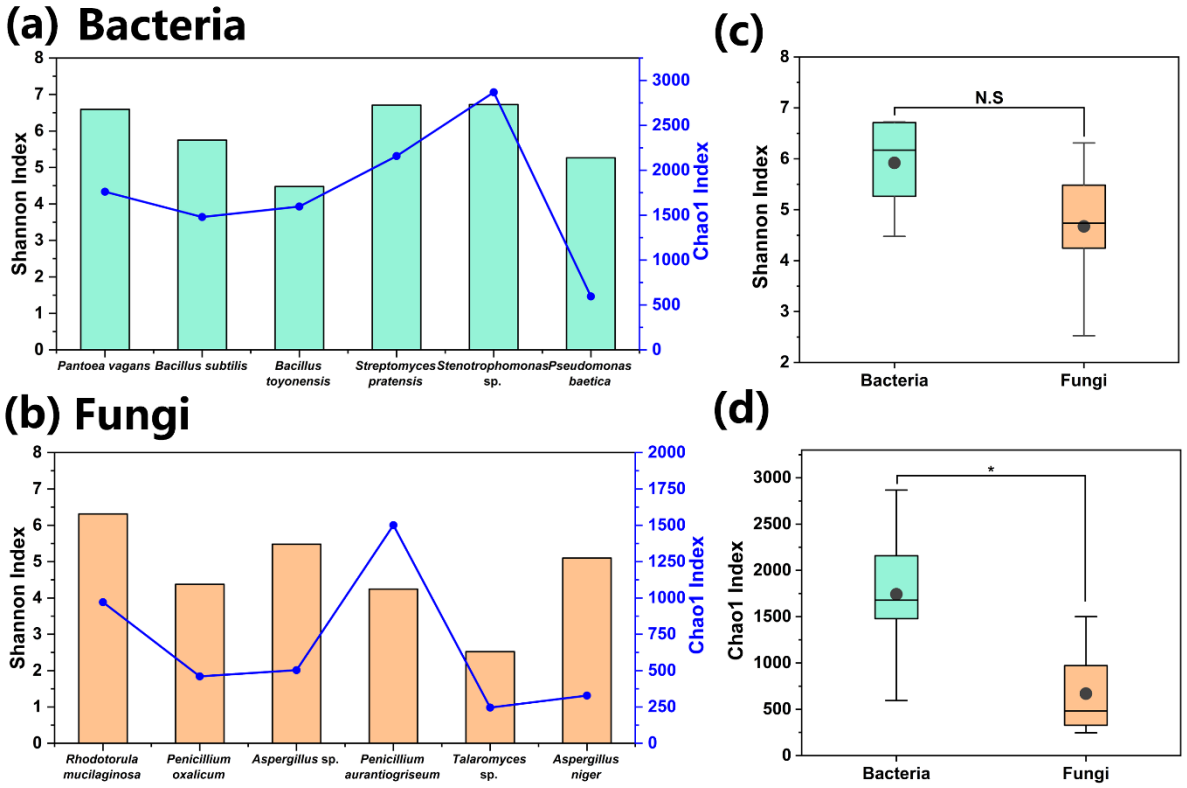

Figure S4. The molecular diversity of bacterial and fungal exometabolites. The two subfigures on the left illustrate the molecular diversity of bacterial (a) and fungal (b) exometabolites and compare the Shannon index (c) and Chao1 index (d).

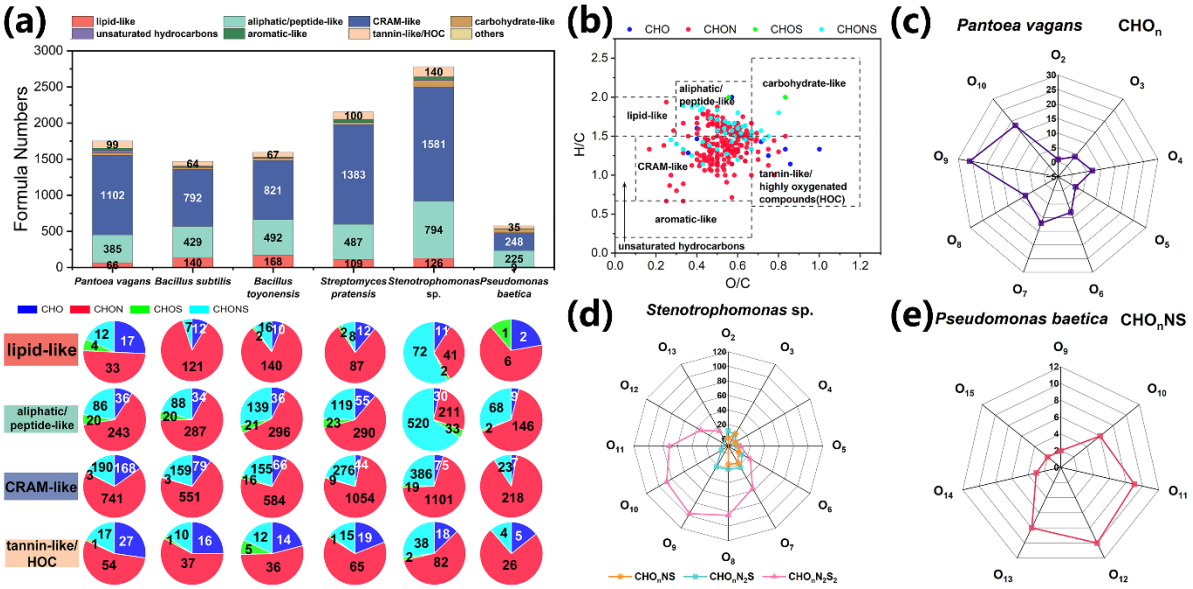

Figure S5. The formula categories in bacterial exometabolites and differences in the molecular composition of shared and unique exometabolites among bacterial strains. (a) Stacked bars illustrate formula categories based on H/C and O/C ratios in bacterial exometabolites, and pie charts illustrate the elemental compositions of high abundance categories. (b) van Krevelen diagrams illustrate the shared molecules. Radar maps show the distribution of oxygen number of unique CHO compounds in exometabolites from *Pantoea vagans* (c) and the distribution of oxygen number of unique CHONS compounds in exometabolites from *Stenotrophomonas* sp. (d) and *Pseudomonas baetica* (e).

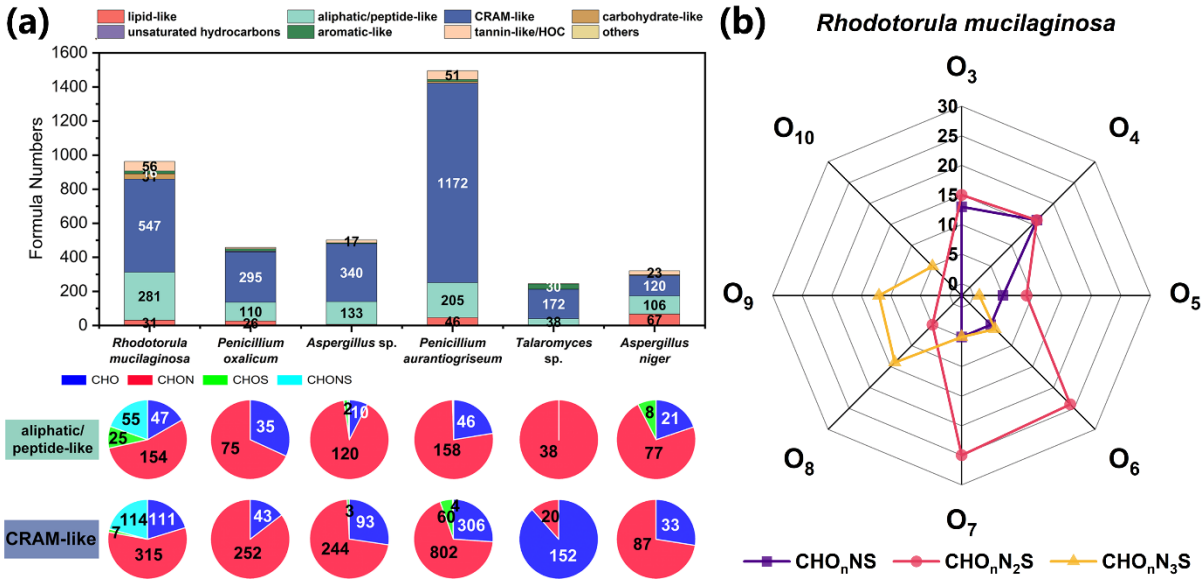

Figure S6. The formula categories in fungal exometabolites (a) and the distribution of oxygen number of unique CHONS compounds in exometabolites from *Rhodotorula mucilaginosa* (b). Stacked bars illustrate formula categories based on H/C and O/C ratios in fungal exometabolites, and pie charts illustrate the elemental compositions of high abundance categories.

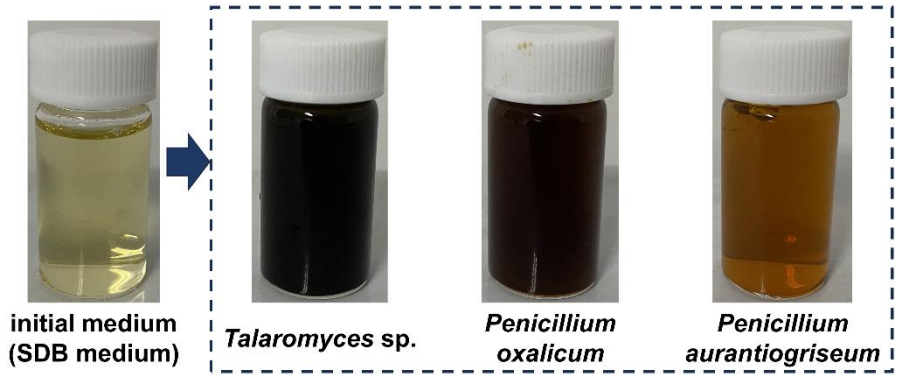

Figure S7. Changes in the supernatant of the genera *Penicillium* and *Talaromyces* culture medium after 14 days.

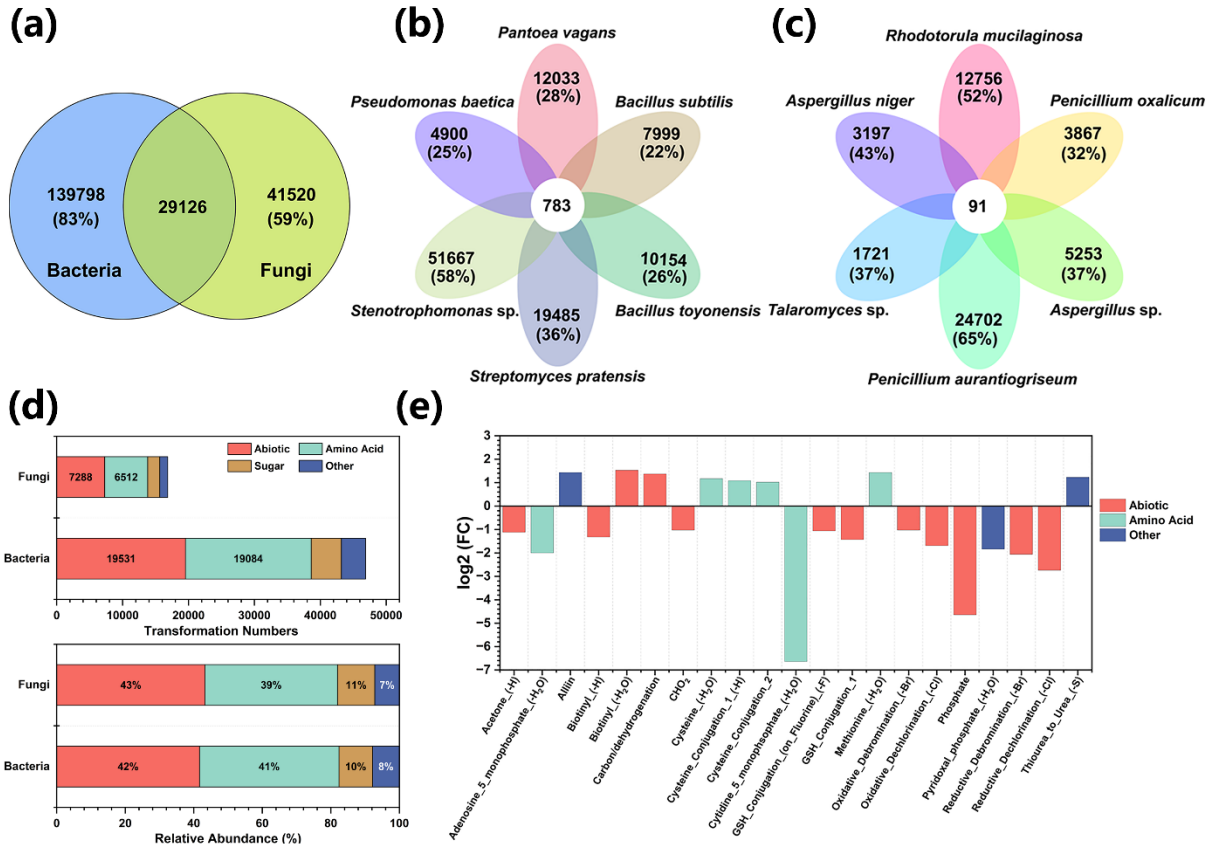

Figure S8. The differences in molecular transformations of bacteria and fungi. Venn diagrams display the numbers and percentages of shared and unique potential molecular transformations of bacteria and fungi (a), typical bacteria (b), and typical fungi (c). The percentages in the graph represent the ratio of unique transformations to the total number of transformations of the strain. (d) Bar diagrams show the grouped distribution of the transformation numbers and the relative abundance of bacteria and fungi. (e) Bar diagram illustrates the distribution of transformation types with significant differences (Fold Change (FC) ≥ 2 or ≤ 0.5) between bacteria and fungi. Log2 (FC) ≥ 0 indicates that this transformation type is more significantly represented in bacteria.

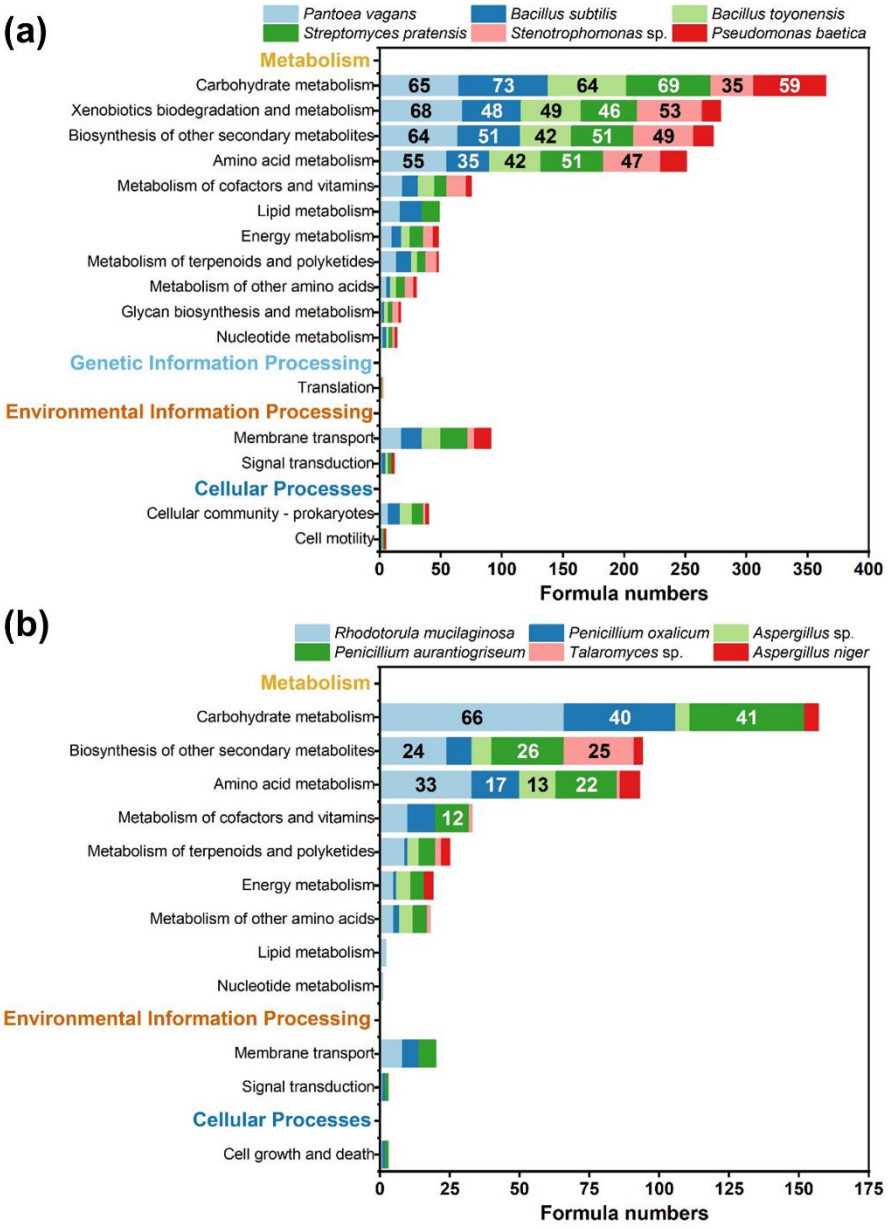

Figure S9. The KEGG metabolic pathways of typical bacterial (a) and fungal (b) strains enriched and analyzed based on their exometabolites, including both primary and secondary pathways.

Table S1 Compositions of the media used for isolation of culturable bacteria and fungi in the urban atmosphere.

Component	Tryptic soy agar (TSA)	Sabouraud dextrose agar (SDA)
Tryptone	15 g	-
Soy peptone	5 g	-
NaCl	5 g	-
Peptone	-	10 g
Glucose	-	40 g
Agar	15 g	20 g
Double distilled water	1000 mL	1000 mL

Table S2 Identities of cultivable bacteria derived from aerosol samples.

Isolate ID	Isolate	BLAST Identity	% Identity	Phylum	Class	Order	Family
B1	*Bacillus* sp. B1	*Bacillus* sp.	99.93%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B2	*Pantoea vagans* B1	*Pantoea vagans*	100.00%	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B3	*Streptomyces thermoviolaceus* B1	*Streptomyces thermoviolaceus*	100.00%	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
B4	*Bacillus subtilis* B1	*Bacillus subtilis*	99.17%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B5	*Streptomyces thermoviolaceus* B2	*Streptomyces thermoviolaceus*	99.93%	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
B6	*Bacillus* sp. B2	*Bacillus* sp. Y1(2012)	99.93%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B7	*Bacillus subtilis* B2	*Bacillus subtilis*	100.00%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B8	*Erwinia* sp. B1	*Erwinia* sp.	100.00%	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B9	*Erwinia* sp. B1	*Erwinia* sp.	100.00%	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B10	*Streptomyces pratensis* B1	*Streptomyces pratensis*	99.12%	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
B11	*Streptomyces* sp. B1	*Streptomyces* sp. SYP-A7193	99.93%	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
B12	*Bacillus* sp. B3	*Bacillus* sp. 210_62	99.72%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B13	*Pseudomonas* sp. B1	*Pseudomonas* sp.	99.93%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B14	*Pantoea vagans* B2	*Pantoea vagans*	99.32%	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B15	*Pseudomonas* sp. B2	*Pseudomonas* sp. B14-6	100.00%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B16	*Stenotrophomonas* sp. B1	*Stenotrophomonas* sp.	99.79%	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
B17	*Pseudomonas fluorescens* B1	*Pseudomonas fluorescens*	100.00%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B18	*Bacillus toyonensis* B1	*Bacillus toyonensis*	100.00%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B19	*Pseudomonas* sp. B3	*Pseudomonas* sp.	99.71%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B20	*Bacillus halotolerans* B1	*Bacillus halotolerans*	100.00%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B21	*Bacillus proteolyticus* B1	*Bacillus proteolyticus*	100.00%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B22	*Bacillus safensis* B1	*Bacillus safensis*	100.00%	Firmicutes	Bacilli	Bacillales	Bacillaceae
B23	*Pseudomonas baetica* B1	*Pseudomonas baetica*	100.00%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B24	*Pseudomonas* sp. B4	*Pseudomonas* sp.	99.85%	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae

Table S3 Identities of cultivable fungi derived from aerosol samples.

Isolate ID	Isolate	BLAST Identity	% Identity	Phylum	Class	Order	Family
F1	*Trametes elegans* F1	*Trametes elegans*	100.00%	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae
F2	*Talaromyces* sp. F1	*Talaromyces* sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae
F3	*Aspergillus niger* F1	*Aspergillus niger*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F4	*Aspergillus* sp. F1	*Aspergillus* sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F5	*Aspergillus* sp. F2	*Aspergillus* sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F6	*Aspergillus nidulans* F1	*Aspergillus nidulans*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F7	*Penicillium oxalicum* F1	*Penicillium oxalicum*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F8	*Sarocladium terricola* F1	*Sarocladium terricola*	100.00%	Ascomycota	Sordariomycetes	Hypocreales	Sarocladiaceae
F9	*Penicillium sumatraense* F1	*Penicillium sumatraense*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F10	*Penicillium aurantiogriseum* F1	*Penicillium aurantiogriseum*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F11	*Aspergillus* sp. F3	*Aspergillus* sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F12	*Aspergillus carneus* F1	*Aspergillus carneus*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F13	*Cladosporium parahalotolerans* F1	*Cladosporium parahalotolerans*	100.00%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae
F14	*Rhodotorula mucilaginosa* F1	*Rhodotorula mucilaginosa*	100.00%	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae
F15	*Penicillium cinnamopurpureum* F1	*Penicillium cinnamopurpureum*	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F16	*Cladosporium* sp. F1	*Cladosporium* sp.	99.42%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae

Table S4 The bacterial and fungal isolates used for exometabolomic studies.

Kingdom	Isolate ID	Isolate	Type
Bacteria	B2	*Pantoea vagans*	Gram-negative bacteria
	B4	*Bacillus subtilis*	Gram-positive bacteria
	B18	*Bacillus toyonensis*	Gram-positive bacteria
	B10	*Streptomyces pratensis*	Gram-positive bacteria
	B16	*Stenotrophomonas* sp.	Gram-negative bacteria
	B23	*Pseudomonas baetica*	Gram-negative bacteria
Fungi	F14	*Rhodotorula mucilaginosa*	yeast
	F7	*Penicillium oxalicum*	mold
	F5	*Aspergillus* sp.	mold
	F10	*Penicillium aurantiogriseum*	mold
	F2	*Talaromyces* sp.	mold
	F3	*Aspergillus niger*	mold

Table S5 Changes in the number of formulas after incubation for bacteria and fungi.

Sample	Culture nutrients	Consumed	Resistant	Produced	Total
Before incubation					
TSB	-	-	-	-	3416
SDB	-	-	-	-	3920
After incubation					
Pantoea vagans	TSB	413	3003	1761	4764
Bacillus subtilis	TSB	477	2939	1479	4418
Bacillus toyonensis	TSB	708	2708	1596	4304
Streptomyces pratensis	TSB	689	2727	2158	4885
Stenotrophomonas sp.	TSB	1198	2218	2868	5086
Pseudomonas baetica	TSB	1122	2294	651	2945
After incubation					
Rhodotorula mucilaginosa	SDB	811	3109	971	4080
Penicillium oxalicum	SDB	1362	2558	460	3018
Aspergillus sp.	SDB	2049	1871	503	2374
Penicillium aurantiogriseum	SDB	1262	2658	1501	4159
Talaromyces sp.	SDB	2531	1389	246	1635
Aspergillus niger	SDB	1689	2231	328	2559

Table S6 The molecular weight distribution of the typical bacterial and fungal exometabolites.

Sample	Low Molecular Weight (LMW) (150 – 300 Da)	Medium Molecular Weight (MMW) (300 – 500 Da)	High Molecular Weight (HMW) (500 – 800 Da)	Total
		Bacteria		
Pantoea vagans	617 35.04%	837 47.53%	307 17.43%	1761 100%
Bacillus subtilis	460 31.10%	731 49.43%	288 19.47%	1479 100%
Bacillus toyonensis	540 33.83%	774 48.50%	282 17.67%	1596 100%
Streptomyces pratensis	665 30.82%	1034 47.91%	459 21.27%	2158 100%
Stenotrophomonas sp.	721 25.14%	1244 43.38%	903 31.49%	2868 100%
Pseudomonas baetica	123 18.89%	252 38.71%	276 42.40%	651 100%
		Fungi		
Rhodotorula mucilaginosa	318 32.75%	518 53.35%	135 13.90%	971 100%
Penicillium oxalicum	100 21.74%	222 48.26%	138 30.00%	460 100%
Aspergillus sp.	78 15.51%	299 59.44%	126 25.05%	503 100%
Penicillium aurantiogriseum	187 12.46%	929 61.89%	385 26.65%	1501 100%
Talaromyces sp.	54 21.95%	164 66.67%	28 11.38%	246 100%
Aspergillus niger	55 16.77%	155 47.26%	118 35.98%	328 100%

Table S7 The elemental compositions of the typical bacterial and fungal exometabolites.

Sample	CHO	CHON	CHOS	CHONS	CHONSP	Total
			Bacteria			
Pantoea vagans	262 14.88%	1131 64.22%	45 2.56%	318 18.06%	5 0.28%	1761 100%
Bacillus subtilis	150 10.14%	1022 69.10%	37 2.50%	270 18.26%	0 0.00%	1479 100%
Bacillus toyonensis	134 8.40%	1070 67.04%	52 3.26%	340 21.30%	0 0.00%	1596 100%
Streptomyces pratensis	137 6.35%	1552 71.92%	43 1.99%	426 19.74%	0 0.00%	2158 100%
Stenotrophomonas sp.	140 4.88%	1494 52.09%	69 2.41%	1165 40.62%	0 0.00%	2868 100%
Pseudomonas baetica	25 3.84%	405 62.21%	5 0.77%	216 33.18%	0 0.00%	651 100%
			Fungi			
Rhodotorula mucilaginosa	203 20.91%	525 54.07%	58 5.97%	185 19.05%	0 0.00%	971 100%
Penicillium oxalicum	94 20.43%	366 79.57%	0 0.00%	0 0.00%	0 0.00%	460 100%
Aspergillus sp.	120 23.86%	377 74.95%	5 0.99%	1 0.20%	0 0.00%	503 100%
Penicillium aurantiogriseum	402 26.78%	1017 67.75%	71 4.73%	10 0.67%	1 0.07%	1501 100%
Talaromyces sp.	182 73.98%	64 26.02%	0 0.00%	0 0.00%	0 0.00%	246 100%
Aspergillus niger	80 24.39%	233 71.04%	15 4.57%	0 0.00%	0 0.00%	328 100%

Table S8 The formulas of possible pigments in exometabolites from *Penicillium* and *Talaromyces*.

Genus	Strain	Pigment	Formula
Penicillium	*Penicillium oxalicum*	Carviolin	$C_{16}H_{12}O_6$
		Phoenicin	$C_{14}H_{10}O_6$
		Naphthalic anhydride	$C_{21}H_{22}O_9$
	Penicillium aurantiogriseum	Carviolin	$C_{16}H_{12}O_6$
		Phoenicin	$C_{14}H_{10}O_6$
		Atrovenetin	$C_{19}H_{18}O_6$
		PP-O	$C_{23}H_{24}O_7$
		N-glutarylrubropunctamine	$C_{26}H_{29}NO_8$
		Purpurquinone-A	$C_{21}H_{20}O_9$
		Northerqueinone	$C_{19}H_{18}O_7$
		Xanthomonasin A	$C_{21}H_{24}O_7$
		Trihydroxymethyl-antraquinone	$C_{15}H_{10}O_5$
Talaromyces	*Talaromyces* sp.	Phoenicin	$C_{14}H_{10}O_6$
		Carviolin	$C_{16}H_{12}O_6$
		Naphthalic anhydride	$C_{21}H_{22}O_9$
		Purpurquinone-A	$C_{21}H_{20}O_9$
		Northerqueinone	$C_{19}H_{18}O_7$
		Mitorubrinol	$C_{21}H_{18}O_8$
		Atrovenetin	$C_{19}H_{18}O_6$