



Supplement of

Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere

Rui Jin et al.

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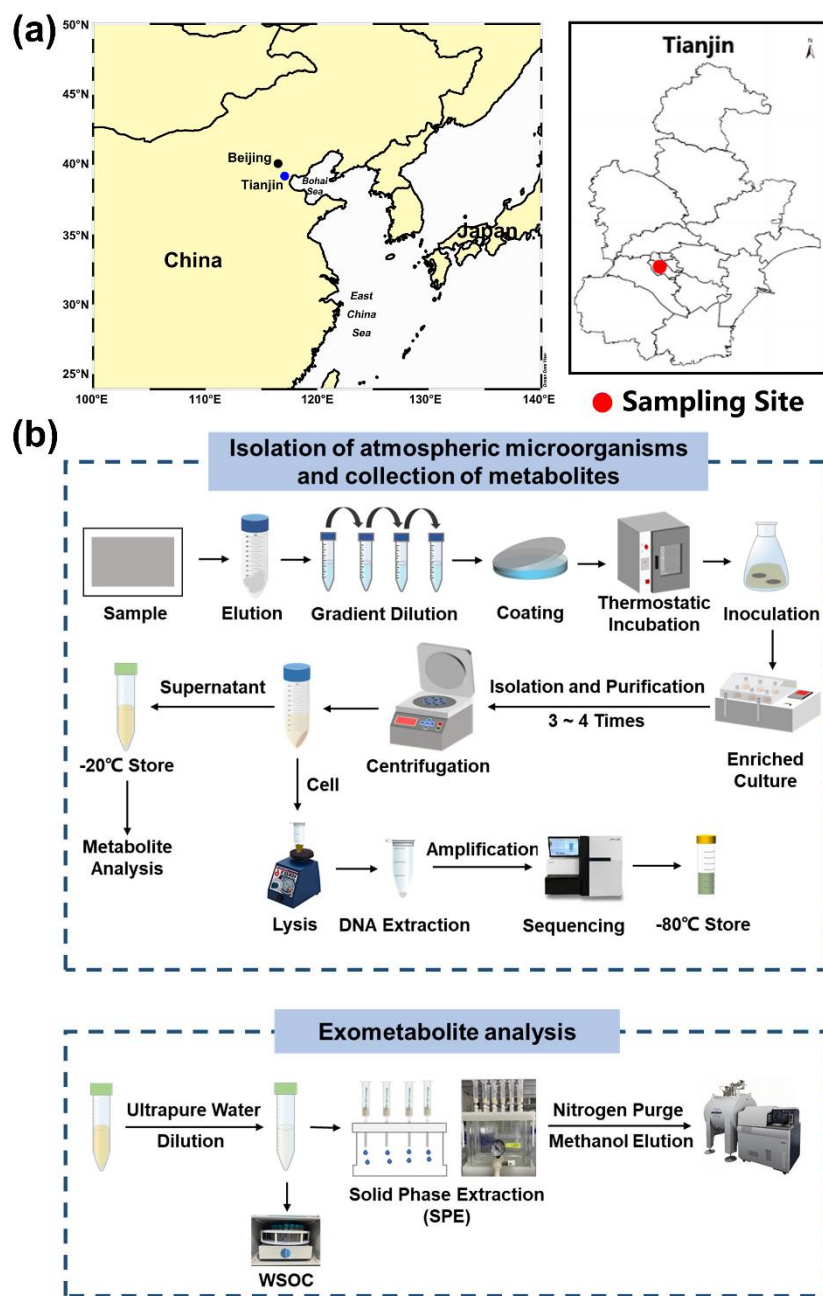


Figure S1. The location of culturable airborne microorganisms and experimental procedures. (a) The map shows the location of Tianjin city and the urban sampling site in the Nankai district, Tianjin (the map is from Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, 2024). (b) Experimental procedures include the isolation of atmospheric microorganisms, collection of metabolites, and exometabolite analysis.

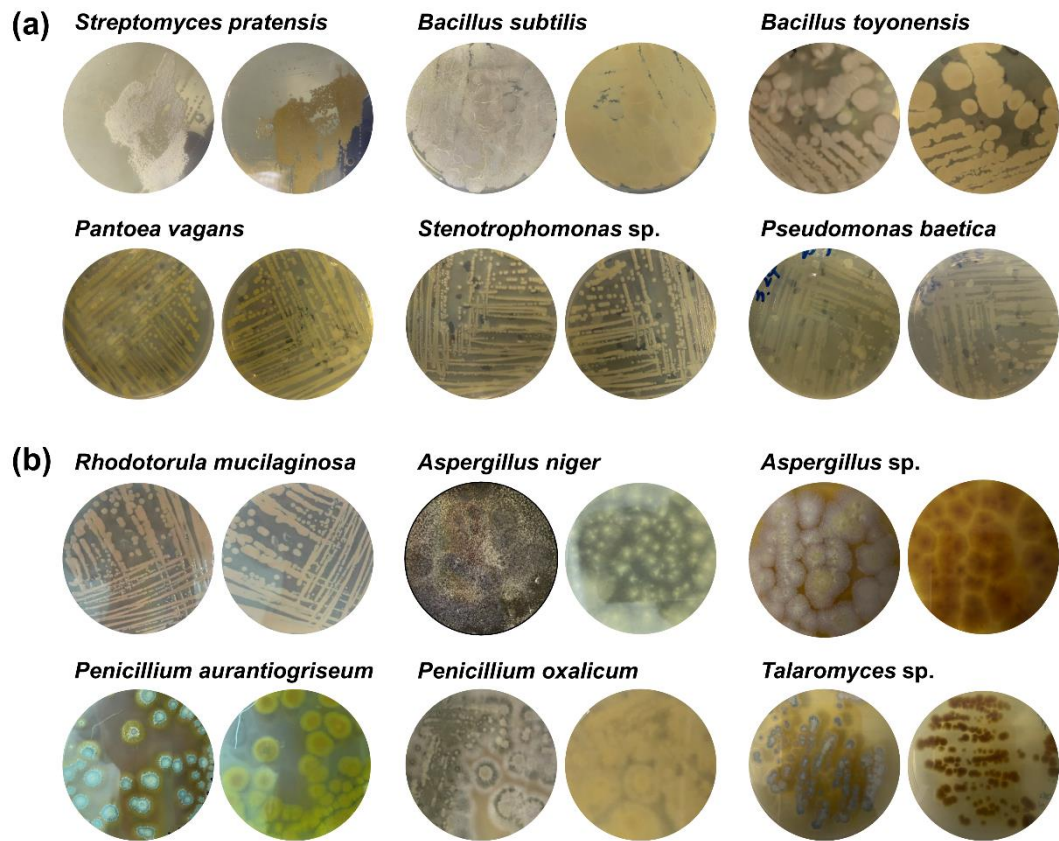


Figure S2. The representative bacterial (a) and fungal (b) strains isolated atmospheric microorganisms.

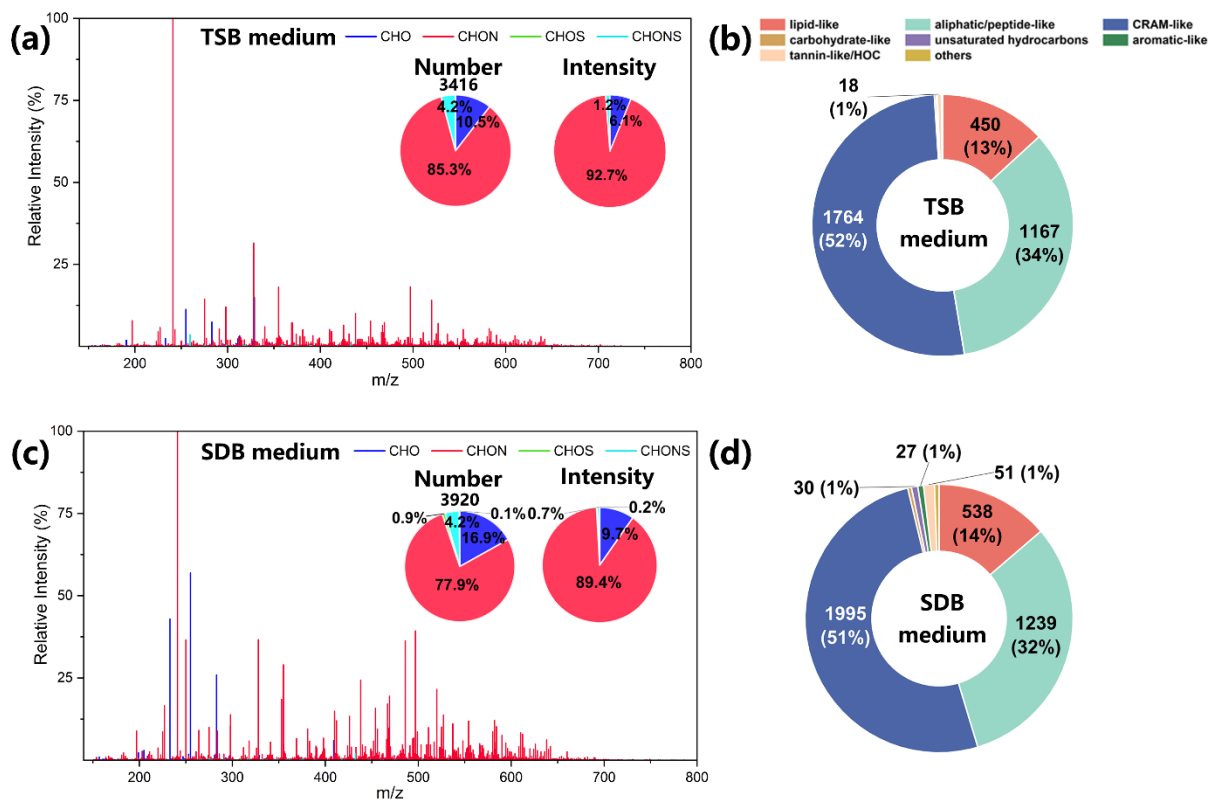


Figure S3. Mass spectra and different categories of organic compounds in the initial culture broths. (a-b) The mass spectrum and elemental composition of TSB used for bacterial growth (a), and the formula numbers of different categories of organic matter for TSB (b). (c-d) The mass spectrum and elemental composition of SDB used for fungal growth (c), and the formula numbers of different categories of organic matter for SDB (d).

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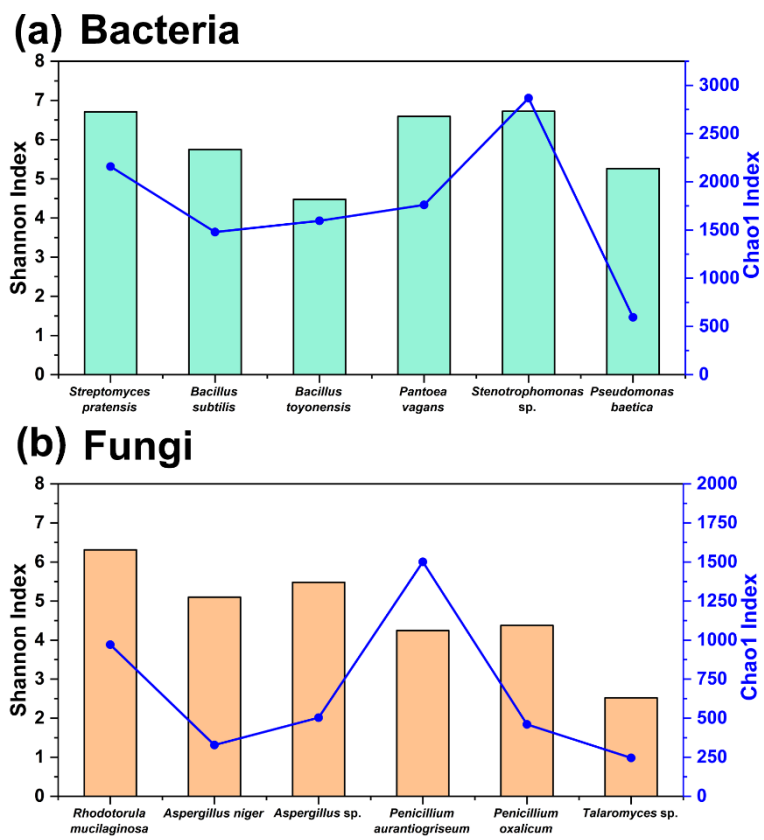
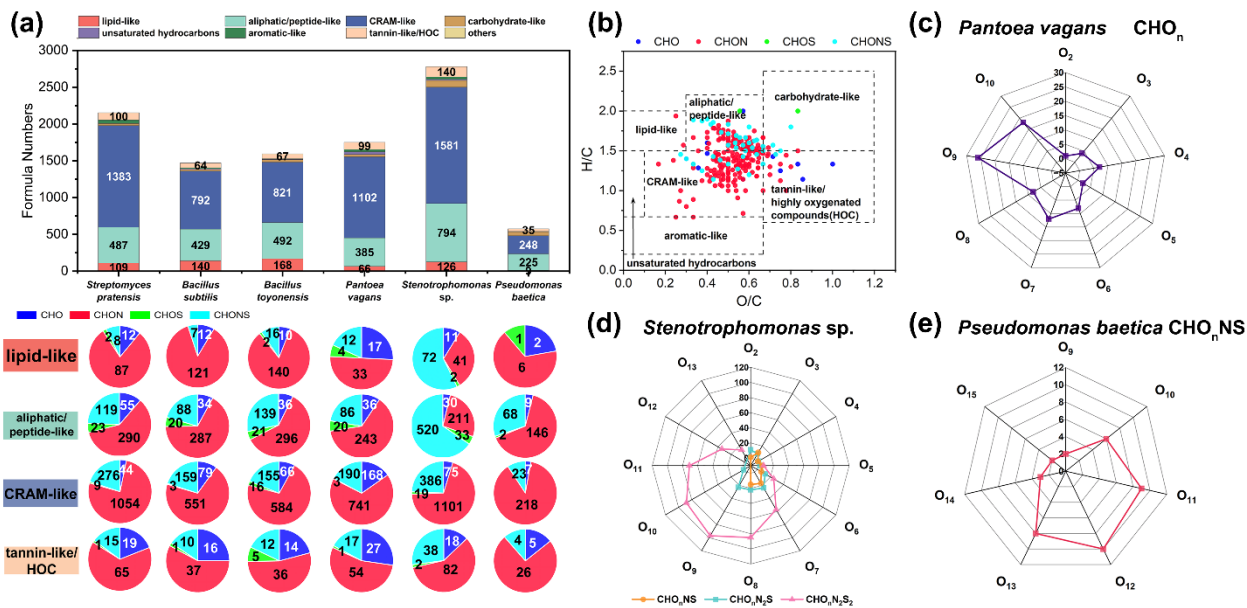


Figure S4. The molecular diversity of bacterial (a) and fungal (b) exometabolites.



20 **Figure S5.** The formula categories of 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
 25 exometabolites from *Stenotrophomonas* sp. (d) and *Pseudomonas baetica* (e).

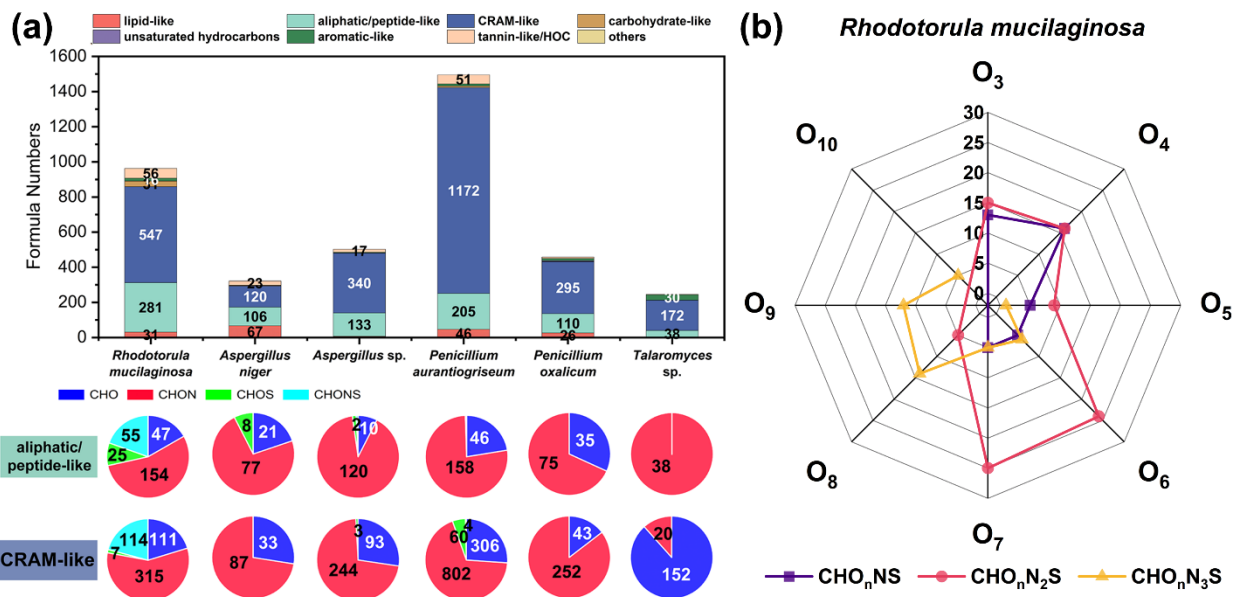


Figure S6. The formula categories of fungal exometabolites (a) and the distribution of oxygen number for unique CHONS compounds in exometabolites from *Rhodotorula mucilaginosa* (b). Stacked bars illustrate formula categories based on H/C and O/C ratios in fungal exometabolites, and pie charts illustrate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

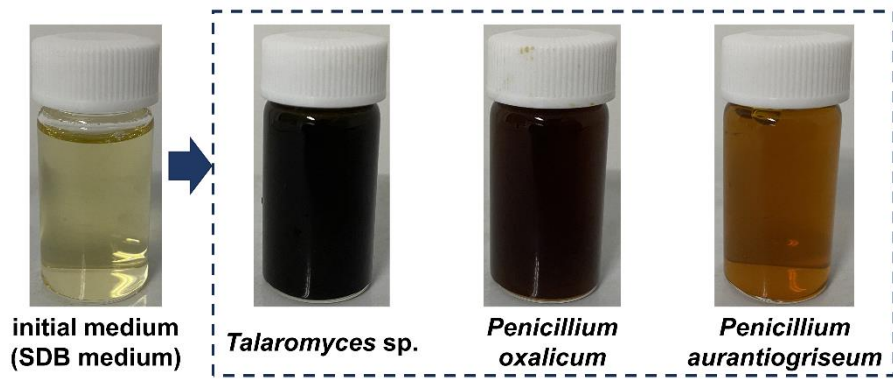
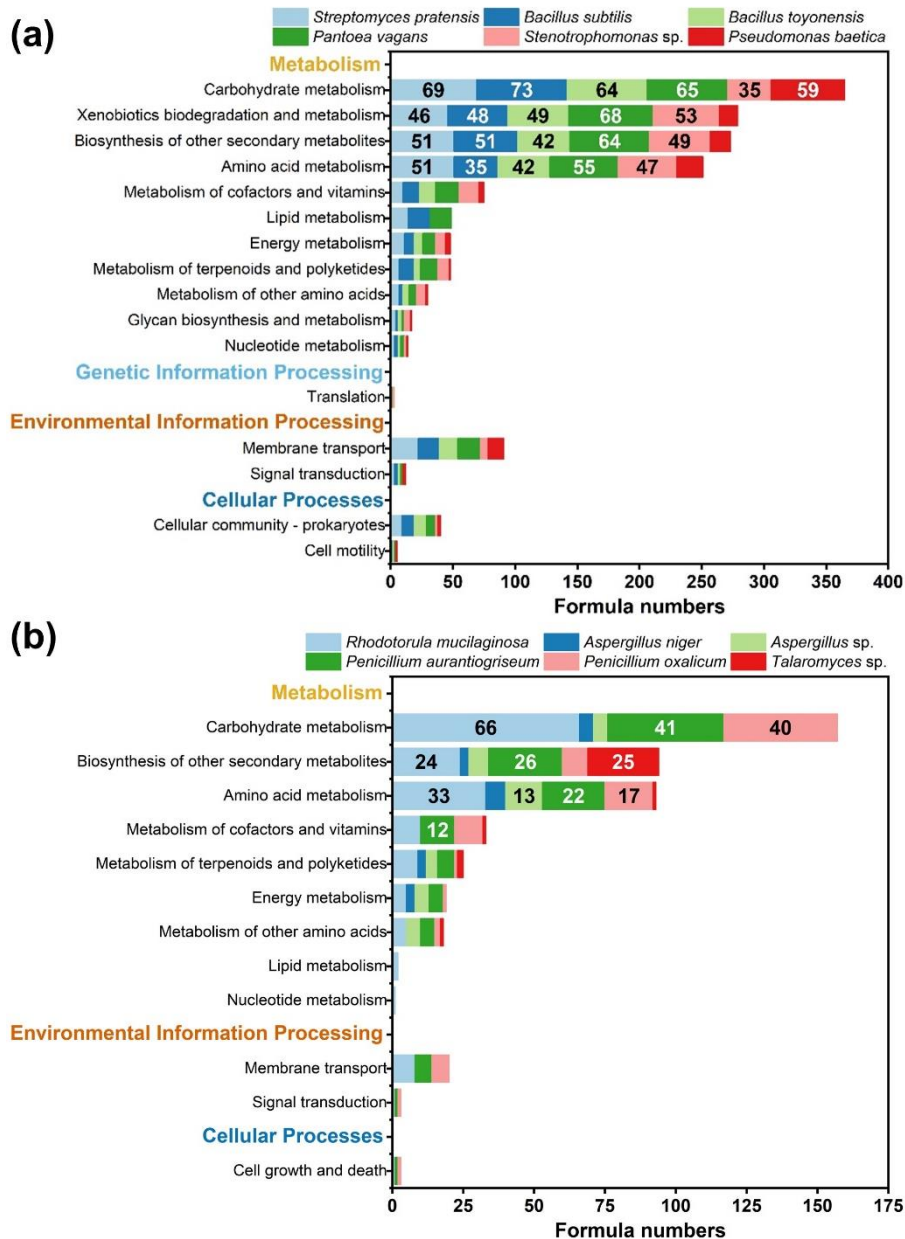


Figure S7. Changes in the supernatant of the culture media for the genera *Penicillium* and *Talaromyces* after 14 days.



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

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Table S2 Identities of cultivable bacteria derived from aerosol samples.

Isolate ID	Isolated strains	BLAST Identity	% Identity	Phylum	Class	Order	Family
B1	<i>Bacillus</i> sp. TJB1	<i>Bacillus</i> sp.	99.93%	Bacillota	Bacilli	Bacillales	Bacillaceae
B2	<i>Pantoea vagans</i> TJB2	<i>Pantoea vagans</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B3	<i>Streptomyces thermoviolaceus</i> TJB3	<i>Streptomyces thermoviolaceus</i>	100.00%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B4	<i>Bacillus subtilis</i> TJB4	<i>Bacillus subtilis</i>	99.17%	Bacillota	Bacilli	Bacillales	Bacillaceae
B5	<i>Streptomyces thermoviolaceus</i> TJB5	<i>Streptomyces thermoviolaceus</i>	99.93%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B6	<i>Bacillus</i> sp. TJB6	<i>Bacillus</i> sp. Y1(2012)	99.93%	Bacillota	Bacilli	Bacillales	Bacillaceae
B7	<i>Bacillus subtilis</i> TJB7	<i>Bacillus subtilis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B8	<i>Erwinia</i> sp. TJB8	<i>Erwinia</i> sp.	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B9	<i>Erwinia</i> sp. TJB9	<i>Erwinia</i> sp.	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B10	<i>Streptomyces pratensis</i> TJB10	<i>Streptomyces pratensis</i>	99.12%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B11	<i>Streptomyces</i> sp. TJB11	<i>Streptomyces</i> sp. SYP-A7193	99.93%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B12	<i>Bacillus</i> sp. TJB12	<i>Bacillus</i> sp. 210_62	99.72%	Bacillota	Bacilli	Bacillales	Bacillaceae
B13	<i>Pseudomonas</i> sp. TJB13	<i>Pseudomonas</i> sp.	99.93%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B14	<i>Pantoea vagans</i> TJB14	<i>Pantoea vagans</i>	99.32%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B15	<i>Pseudomonas</i> sp. TJB15	<i>Pseudomonas</i> sp. B14-6	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B16	<i>Stenotrophomonas</i> sp. TJB16	<i>Stenotrophomonas</i> sp.	99.79%	Pseudomonadota	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
B17	<i>Pseudomonas fluorescens</i> TJB17	<i>Pseudomonas fluorescens</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B18	<i>Bacillus toyonensis</i> TJB18	<i>Bacillus toyonensis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B19	<i>Pseudomonas</i> sp. TJB19	<i>Pseudomonas</i> sp.	99.71%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B20	<i>Bacillus halotolerans</i> TJB20	<i>Bacillus halotolerans</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B21	<i>Bacillus proteolyticus</i> TJB21	<i>Bacillus proteolyticus</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B22	<i>Bacillus safensis</i> TJB22	<i>Bacillus safensis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B23	<i>Pseudomonas baetica</i> TJB23	<i>Pseudomonas baetica</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B24	<i>Pseudomonas</i> sp. TJB24	<i>Pseudomonas</i> sp.	99.85%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae

Table S3 Identities of cultivable fungi derived from aerosol samples.

Isolate ID	Isolated strains	BLAST Identity	% Identity	Phylum	Class	Order	Family
F1	<i>Trametes elegans</i> TJF1	<i>Trametes elegans</i>	100.00%	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae
F2	<i>Talaromyces</i> sp. TJF2	<i>Talaromyces</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae
F3	<i>Aspergillus niger</i> TJF3	<i>Aspergillus niger</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F4	<i>Aspergillus</i> sp. TJF4	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F5	<i>Aspergillus</i> sp. TJF5	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F6	<i>Aspergillus nidulans</i> TJF6	<i>Aspergillus nidulans</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F7	<i>Penicillium oxalicum</i> TJF7	<i>Penicillium oxalicum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F8	<i>Sarocladium terricola</i> TJF8	<i>Sarocladium terricola</i>	100.00%	Ascomycota	Sordariomycetes	Hypocreales	Sarocladiaceae
F9	<i>Penicillium sumatraense</i> TJF9	<i>Penicillium sumatraense</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F10	<i>Penicillium aurantiogriseum</i> TJF10	<i>Penicillium aurantiogriseum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F11	<i>Aspergillus</i> sp. TJF11	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F12	<i>Aspergillus carneus</i> TJF12	<i>Aspergillus carneus</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F13	<i>Cladosporium parahalotolerans</i> TJF13	<i>Cladosporium parahalotolerans</i>	100.00%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae
F14	<i>Rhodotorula mucilaginosa</i> TJF14	<i>Rhodotorula mucilaginosa</i>	100.00%	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae
F15	<i>Penicillium cinnamopurpureum</i> TJF15	<i>Penicillium cinnamopurpureum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F16	<i>Cladosporium</i> sp. TJF16	<i>Cladosporium</i> sp.	99.42%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae

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

Kingdom	Isolate ID	Isolated strains	Species	Category
Bacteria	B10	<i>Streptomyces pratensis</i> TJB10	<i>Streptomyces pratensis</i>	Gram-positive bacteria
	B4	<i>Bacillus subtilis</i> TJB4	<i>Bacillus subtilis</i>	Gram-positive bacteria
	B18	<i>Bacillus toyonensis</i> TJB18	<i>Bacillus toyonensis</i>	Gram-positive bacteria
	B2	<i>Pantoea vagans</i> TJB2	<i>Pantoea vagans</i>	Gram-negative bacteria
	B16	<i>Stenotrophomonas</i> sp. TJB16	<i>Stenotrophomonas</i> sp.	Gram-negative bacteria
	B23	<i>Pseudomonas baetica</i> TJB23	<i>Pseudomonas baetica</i>	Gram-negative bacteria
Fungi	F14	<i>Rhodotorula mucilaginosa</i> TJF14	<i>Rhodotorula mucilaginosa</i>	yeast
	F3	<i>Aspergillus niger</i> TJF3	<i>Aspergillus niger</i>	mold
	F5	<i>Aspergillus</i> sp. TJF5	<i>Aspergillus</i> sp.	mold
	F10	<i>Penicillium aurantiogriseum</i> TJF10	<i>Penicillium aurantiogriseum</i>	mold
	F7	<i>Penicillium oxalicum</i> TJF7	<i>Penicillium oxalicum</i>	mold
	F2	<i>Talaromyces</i> sp. TJF2	<i>Talaromyces</i> sp.	mold

Note: The species names instead of the strain names are used in the figures and tables to facilitate the description of the different isolates.

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

Media / Species	Culture nutrients	Consumed			Resistant			Produced			Total
		Number	H/C	O/C	Number	H/C	O/C	Number	H/C	O/C	Number
Before incubation											
TSB	-	-			-			-			3416
SDB	-	-			-			-			3920
After incubation											
<i>Streptomyces pratensis</i>	TSB	689	1.60	0.31	2727	1.46	0.39	2158	1.32	0.46	4885
<i>Bacillus subtilis</i>	TSB	477	1.56	0.30	2939	1.47	0.39	1479	1.43	0.44	4418
<i>Bacillus toyonensis</i>	TSB	708	1.58	0.32	2708	1.46	0.39	1596	1.44	0.44	4304
<i>Pantoea vagans</i>	TSB	413	1.58	0.30	3003	1.47	0.39	1761	1.32	0.45	4764
<i>Stenotrophomonas</i> sp.	TSB	1198	1.57	0.36	2218	1.44	0.39	2868	1.42	0.46	5086
<i>Pseudomonas baetica</i>	TSB	1122	1.53	0.34	2294	1.47	0.39	651	1.65	0.53	2945
Mean ± SE		768±133	1.57±0.01	0.32±0.01	2648±133	1.46±0.00	0.39±0.00	1752±301	1.43±0.05	0.46±0.01	4400±314
After incubation											
<i>Rhodotorula mucilaginosa</i>	SDB	811	1.56	0.29	3109	1.45	0.40	971	1.39	0.48	4080
<i>Aspergillus niger</i>	SDB	1689	1.43	0.36	2231	1.50	0.39	328	1.53	0.40	2559
<i>Aspergillus</i> sp.	SDB	2049	1.50	0.34	1871	1.43	0.43	503	1.32	0.49	2374
<i>Penicillium aurantiogriseum</i>	SDB	1262	1.55	0.33	2658	1.43	0.40	1501	1.26	0.44	4159
<i>Penicillium oxalicum</i>	SDB	1362	1.52	0.35	2558	1.44	0.40	460	1.33	0.39	3018
<i>Talaromyces</i> sp.	SDB	2531	1.48	0.36	1389	1.45	0.41	246	1.06	0.44	1635
Mean ± SE		1617±250	1.51±0.02	0.34±0.01	2303±250	1.45±0.01	0.41±0.01	668±196	1.32±0.06	0.44±0.02	2971±406

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

Species	Low Molecular Weight (LMW) (150 – 300 Da)	Medium Molecular Weight (MMW) (300 – 500 Da)	High Molecular Weight (HMW) (500 – 800 Da)	Total
Bacteria				
<i>Streptomyces pratensis</i>	665 30.8%	1034 47.9%	459 21.3%	2158 100%
<i>Bacillus subtilis</i>	460 31.1%	731 49.4%	288 19.5%	1479 100%
<i>Bacillus toyonensis</i>	540 33.8%	774 48.5%	282 17.7%	1596 100%
<i>Pantoea vagans</i>	617 35.1%	837 47.5%	307 17.4%	1761 100%
<i>Stenotrophomonas</i> sp.	721 25.1%	1244 43.4%	903 31.5%	2868 100%
<i>Pseudomonas baetica</i>	123 18.9%	252 38.7%	276 42.4%	651 100%
Fungi				
<i>Rhodotorula mucilaginosa</i>	318 32.7%	518 53.4%	135 13.9%	971 100%
<i>Aspergillus niger</i>	55 16.8%	155 47.3%	118 35.9%	328 100%
<i>Aspergillus</i> sp.	78 15.5%	299 59.4%	126 25.1%	503 100%
<i>Penicillium aurantiogriseum</i>	187 12.5%	929 61.9%	385 26.6%	1501 100%
<i>Penicillium oxalicum</i>	100 21.7%	222 48.3%	138 30.0%	460 100%
<i>Talaromyces</i> sp.	54 21.9%	164 66.7%	28 11.4%	246 100%

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

Species	CHO	CHON	CHOS	CHONS	CHONSP	Total
Bacteria						
<i>Streptomyces pratensis</i>	137 6.4%	1552 71.9%	43 2.0%	426 19.7%	0 0.0%	2158 100%
<i>Bacillus subtilis</i>	150 10.1%	1022 69.1%	37 2.5%	270 18.3%	0 0.0%	1479 100%
<i>Bacillus toyonensis</i>	134 8.4%	1070 67.0%	52 3.3%	340 21.3%	0 0.0%	1596 100%
<i>Pantoea vagans</i>	262 14.9%	1131 64.2%	45 2.6%	318 18.1%	5 0.2%	1761 100%
<i>Stenotrophomonas</i> sp.	140 4.9%	1494 52.1%	69 2.4%	1165 40.6%	0 0.0%	2868 100%
<i>Pseudomonas baetica</i>	25 3.8%	405 62.2%	5 0.8%	216 33.2%	0 0.0%	651 100%
Fungi						
<i>Rhodotorula mucilaginosa</i>	203 20.9%	525 54.1%	58 6.0%	185 19.0%	0 0.0%	971 100%
<i>Aspergillus niger</i>	80 24.4%	233 71.0%	15 4.6%	0 0.0%	0 0.0%	328 100%
<i>Aspergillus</i> sp.	120 23.9%	377 74.9%	5 1.0%	1 0.2%	0 0.0%	503 100%
<i>Penicillium</i>	402 26.8%	1017 67.7%	71 4.7%	10 0.7%	1 0.1%	1501 100%
<i>aurantiogriseum</i>	94 20.4%	366 79.6%	0 0.0%	0 0.0%	0 0.0%	460 100%
<i>Penicillium oxalicum</i>	182 74.0%	64 26.0%	0 0.0%	0 0.0%	0 0.0%	246 100%

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

Genus	Species	Pigment	Formula		
<i>Penicillium</i>	<i>Penicillium oxalicum</i>	Carviolin	C ₁₆ H ₁₂ O ₆		
		Phoenicin	C ₁₄ H ₁₀ O ₆		
	<i>Penicillium aurantiogriseum</i>	Naphthalic anhydride	C ₂₁ H ₂₂ O ₉		
		Carviolin	C ₁₆ H ₁₂ O ₆		
		Phoenicin	C ₁₄ H ₁₀ O ₆		
		Atrovenetin	C ₁₉ H ₁₈ O ₆		
		(10Z)-12-carboxymonascorubin (PP-O)	C ₂₃ H ₂₄ O ₇		
		N-glutaryl rubropunctamine	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		
		<i>Talaromyces</i>	<i>Talaromyces</i> sp.	Trihydroxymethyl-antraquinone	C ₁₅ H ₁₀ O ₅
				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				