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Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere

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Abstract. The interactions of metabolically active atmospheric microorganisms with cloud organic matter can alter the atmospheric carbon cycle. Upon deposition, atmospheric microorganisms can influence microbial communities in surface Earth systems. However, the metabolic activities of cultivable atmospheric microorganisms in settled habitats remain less understood. Here, we investigated exometabolites produced by typical bacterial and fungal species isolated from the urban atmosphere to elucidate their biogeochemical roles. Molecular compositions of exometabolites were analyzed using ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry. Annotation through the Kyoto Encyclopedia of Genes and Genomes database helped identify metabolic processes. Results showed that bacterial and fungal strains produce exometabolites with lower H/C and higher O/C ratios than consumed and resistant compounds. CHON compounds constituted over 50% of the identified formulas of exometabolites. Bacterial exometabolites contained more abundant CHONS compounds (25.2%), while fungal exometabolites were rich in CHO compounds (31.7%). These microbial exometabolites predominantly comprised aliphatic/peptide-like and carboxyl-rich alicyclic molecules (CRAM-like). Significant variations in metabolites were observed among different strains. Bacteria showed a performance for amino acid synthesis, while fungi were more active in transcription and expression processes. Lipid metabolism, amino acid metabolism, and carbohydrate metabolism varied widely among bacterial strains, while fungi exhibited marked differences in carbohydrate metabolism and secondary metabolism. This comprehensive examination of metabolite characteristics at the molecular level for typical culturable airborne microorganisms enhances our understanding of their potential metabolic activities at air-land/water interfaces. These insights are pivotal for assessing the biogeochemical impacts of atmospheric microorganisms following their deposition.

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

1 Introduction

Bioaerosols are an important component of aerosols and are involved in various ecological processes (Fröhlich-Nowoisky et al., 2016; Smets et al., 2016). Broadly speaking, bioaerosols include microorganisms (e.g., viruses, bacteria, fungi, archaea), various propagules (e.g., spores and pollen), biological debris, and biological metabolites (Després et al., 2012; Zhai et al., 2018). These components are essential substructures to implications for climate change, hydrological, and biogeochemical cycles (Xie et al., 2021b; Kanakidou et al., 2018; Morris et al., 2011). Atmospheric microorganisms are usually freely floating or attached to the surface of particulate matter and can be transported over long distances by wind (Hu et al., 2020; Ruiz-Gil et al., 2020). Bacteria are emitted into the atmosphere from Earth's surfaces through aerosolization. Due to the increased environmental stress, some bacteria develop stress resistance to survive and degrade low molecular weight organic matter in the atmospheric environment (Šantl-Temkiv et al., 2022; Joly et al., 2015; Ariya and Amyot, 2004). This resilience gives atmospheric microorganisms a potential role in atmospheric chemistry and physics, driving chemical reactions at environmental interfaces (e.g., the land-air interface).

Bacteria and fungi in the atmosphere can maintain metabolic activity due to specific growth characteristics, such as spore production capacity, UV resistance, drought resistance, or through extracellular secretions (Matulová et al., 2014; Huang and Hull, 2017; Bryan et al., 2019). For example, *Aspergillus niger*, a common fungus in the atmosphere, has pigmented spores that facilitate it to resist damage from high-intensity UV light (Cortesao et al., 2020). Similarly, *Sphingomonas aerolata* NW12 isolated from the air can increase rRNA content and produce proteins while aloft (Krumins et al., 2014). Atmospheric microbial activity can be affected by freeze-thaw and condensation/evaporation cycles. *Bacillus* sp. 3B6 efficiently metabolizes sugar, and its exopolymeric substances (EPSs) help protect cells from osmotic shocks (Matulová et al., 2014; Joly et al., 2015). Additionally, a large number of active microorganisms in the atmosphere and cloud water are involved in atmospheric chemical processes.

Atmospheric microorganisms play a dual role in atmospheric biogeochemistry. Not only do they directly metabolize organic carbon, but they can also reduce the availability of radical sources, thereby decreasing the oxidative capacity of cloud systems (Zhang et al., 2019; Lallement et al., 2018a; Vaitilingom et al., 2013). At the beginning of the 21st century, studies demonstrated that some bacteria isolated from cloud water could degrade small molecule organic matter (e.g., phenol, formaldehyde, carboxylic acids, and methanol), affecting the oxidative capacity and organic carbon budget in clouds (Vaïtilingom et al., 2010; Amato et al., 2007; Vaïtilingom et al., 2011; Lallement et al., 2018b; Jaber et al., 2020). For example, *Rhodococcus enclensis*, a highly active strain isolated from clouds, biodegrades catechol approximately ten times faster than phenol (Jaber et al., 2020). It was observed that 93% of 145 bacterial strains isolated from clouds over Puy de Dôme could degrade phenol, as demonstrated through metatranscriptomic analysis and laboratory incubation experiments (Lallement et al., 2018b). Microcosm laboratory experiments with or without simulated sunlight, along with varying pH values in artificial cloud

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

water, indicated that these conditions influenced the biodegradation of organic acids by bacteria (Liu et al., 2023b). The ability of bacteria to degrade organic matter in clouds implies that atmospheric microorganisms may contribute to natural remediation and cloud processes in the atmosphere.

On the other hand, microorganisms can interact with oxidants in clouds, such as iron (Fe), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2). Hydroxyl radicals are the main oxidizing agents in cloud water and are also present in the atmosphere, driving the oxidizing capacity of clouds (Lallement et al., 2018a). H_2O_2 can influence the energy metabolism of cloud microbiota, potentially regulating the biotransformation rates of carbon compounds (Wirgot et al., 2017). In the presence of H_2O_2, *Pseudomonas graminis* exhibited a distinct metabolome compared to unexposed cells, with notable changes in the metabolisms of carbohydrates, glutathione, lipids, peptides, and amino acids (Wirgot et al., 2019). Therefore, atmospheric microorganisms can potentially impact atmospheric and cloud chemistry processes and biogeochemical cycling.

Atmospheric microorganisms carried by wet and dry deposition are a crucial biological source to the ocean and freshwater surfaces, and participate in ocean surface biogeochemical cycling processes, which is especially significant in oligotrophic aquatic systems. Many studies have emphasized the impact of atmospheric deposition on the biogeochemical cycling of nitrogen (N) in the marine surface and mixed layer. In mesocosm experiments, the addition of Saharan dust rapidly (within 30 h), intensely (2–4 times), and steadily (over 6 days) increased the N_2 fixation rates, contributing 3–8% to primary productivity (Rahav et al., 2016b). Atmospheric aerosol (especially dust) deposition can be a potential source of a wide array of microorganisms; for example, viable airborne diazotrophs may boost dinitrogen (N_2) fixation in marine areas with high aerosol loads (Rahav et al., 2016a; Rahav et al., 2018). Dust aerosols also deliver active bacteria to the ocean surface, altering the structure of bacterial communities and reducing the diversity of marine bacterial populations (Na et al., 2023). These results reveal the potential impact of the atmospheric deposition of microbes carried by atmospheric aerosols on autotrophic and heterotrophic productivity in surface seawater.

Only rare studies have focused on the impact of atmospheric microbial deposition on freshwater microbial ecosystems. For example, based on cultivation experiments, when rainwater affected by Saharan dust was added to sterilized high-altitude lake water, bacterial concentrations increased from approximately 3×10^3 cells mL^{-1} to between 3.6×10^5 and 11.1×10^5 cells mL^{-1} within 4–5 days (Peter et al., 2014). The low-abundance species in the initial sample became the dominant bacterial community, suggesting that microbes carried by Saharan dust may be an important source of rare bacteria in freshwater. Despite these insights, few studies have investigated the biotransformation and degradation of small molecule organic matter by atmospheric microorganisms, making it challenging to accurately assess their metabolic processes and potential impacts on atmospheric chemistry and biogeochemical cycles. Therefore, further research is needed to explore the metabolome of atmospheric microorganisms to understand better the essential processes that influence the Earth's atmosphere and ecosystems.

Metabolomics provides a direct output of the functional activity in a system (Bauermeister et al., 2021). The metabolome encompasses the small molecule products of enzyme-catalyzed metabolic reactions, offering functional and phenotypic information (Baidoo and Teixeira Benites, 2019). The endometabolome refers to all the metabolites within a cell, while the exometabolome comprises all the metabolites released into the extracellular environment (León et al., 2013). For example,

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

marine phytoplankton generate large amounts of exometabolites, forming a carbon pool known as extracellular release or dissolved primary production (Moran et al., 2022). Recently, a framework for atmo-ecometabolomics, from sampling to data analysis, was demonstrated to characterize the chemical composition of atmospheric aerosol particles measured by liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) (Rivas-Ubach et al., 2019). This study sheds light on how aerosol chemical compositions impact ecosystem structure, function, and biogeochemistry. However, previous studies have not considered the effects of metabolically active microorganisms in the atmosphere.

Mass spectrometers with different mass analyzers, including time-of-flight, Orbitrap, and FT-ICR, are favored in many metabolomics applications (Bauermeister et al., 2021). Their high mass resolution and precision allow accuracy below parts per million for thousands of molecules in complex mixtures (Moran et al., 2022). FT-ICR-MS is a tool for obtaining high-resolution metabolomic fingerprints and a deeper understanding of potential chemical transformations in various types of samples, such as landfill leachate, algal products, seawater, lake water, soil, and aerosols (Yuan et al., 2017; Gonsior et al., 2019; Bahureksa et al., 2021; Xie et al., 2021a; Qi et al., 2022). Since FT-ICR MS determines potent polarity molecules with molecular weight (MW) from 100 Da to 1000 Da, pigments or extracellular enzymes secreted by bacteria or fungi can be detected and identified.

This study conducted the non-targeted screening of microbial exometabolomes formed during the growth of atmospheric culturable microorganisms using FT-ICR MS coupled to negative ion mode electrospray ionization (ESI–). This study aimed to reveal the characteristics of active atmospheric microbial metabolites and underline the important connection between atmospheric microbes and subsurface environments. The specific objectives of this study were to (i) explore the molecular characteristics of exometabolites from typical atmospheric culturable bacterial and fungal strains, (ii) elucidate the central metabolic processes and the differences between different strains, and (iii) unravel the potential metabolic capacities of these typical bacteria and fungi, providing data to support the roles of atmospheric microorganisms in affecting atmospheric organic matter and biogeochemical cycling processes after deposition onto the ocean or freshwater surfaces.

2 Materials and methods

2.1 Samples collection and microbial isolation

The sampling site was located on the roof of Building No.19 on the Weijin Road campus of Tianjin University (39.11°N, 117.16°E, about 21 m above the ground level) in an urban area (Fig. S1a), about 500 m from the main road. Total suspended particulate matter (TSP) samples were collected onto quartz fiber filters (10 in. × 8 in.) at a flow rate of 1.05 m^3 min^{-1} from 15:00 to 19:00 on 5th, 8th, and 11th January 2022. All samples were stored at –20°C until subsequent treatment. Before sampling, quartz fiber filters were heated in a muffle furnace at 450°C for 6 h to remove organic matter.

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

Tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) media were used for bacterial and fungal isolation, respectively. The media compositions are shown in Table S1. All media were sterilized in an autoclave at 115°C for 20 min, and then solid culture plates were prepared under aseptic conditions. Under sterile conditions, one-eighth of each filter sample was cut and placed in a 50 mL centrifuge tube with 40 mL of 1×phosphate buffer saline (PBS) solution. Microorganisms on the filter were detached using low-power ultrasonication in an ice bath for 5 min and then centrifuged at 250 rpm for 30 min. Next, 100 µL of the well-mixed suspension was spread-plated onto the two types of solid media. The plates were incubated at a constant temperature of 37°C for 48 h for bacteria and 28°C for 72 h for fungi. Single colonies were picked and re-streaked from each plate at least three times to isolate individual strains. After purification, the strain was identified and stored at –80°C with 25% sterile glycerol.

2.2 DNA extraction and microbial identification

Liquid cultures were inoculated with single colonies and grown to the exponential phase. DNA was extracted from 2 mL liquid cultures using the Universal Genomic DNA Extraction Kit (Solarbio, Beijing, China). The DNA was then stored at –80°C. The 16S rRNA gene region was amplified using 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers. The PCR reaction volume was 25 µL, containing 1 µL genomic DNA, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 13 µL 2×Taq PCR Mix (Vazyme, Nanjing, China), and 9 µL ddH2O. The PCR program was: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The fungal internal transcribed spacer (ITS) regions were amplified by PCR (94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; final extension at 72°C for 10 min) using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR reaction system for fungi was the same as that for bacteria. The PCR products were checked by 1% agarose gel electrophoresis and then purified by Universal DNA Purification Kit (TIANGEN, Beijing, China). The sequences of the purified gene fragments were determined by Sanger sequencing (BGI Genomics Co., Ltd, Beijing, China). Taxonomic assignments were determined from 16S rRNA gene sequences or ITS sequences using the BLAST program at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3 Microbial growth conditions and metabolite collection

Six representative bacterial strains and six typical fungal strains were selected from the obtained atmospheric microorganisms for subsequent exometabolome studies. Single colonies of all bacterial strains were picked and inoculated in 50 mL tryptic soy broth (TSB) medium and incubated at 37°C, 200 rpm for 12 h to obtain the primary seed solution. The primary seed solution was transferred to a 100 mL flask containing 50 mL of sterilized liquid medium at an inoculum level of 3 mL (6%) and incubated for 12 h at 37°C with shaking to obtain the secondary seed solution. The secondary seed solution was also transferred to 50 mL sterilized liquid medium at an inoculum level of 3 mL (6%) and incubated for 7 days at 37°C, 200 rpm. Cultures

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

were then centrifuged at 4500 rpm for 20 min at 4°C. The metabolic product supernatant was transferred into a new centrifuge tube and stored at –20°C.

For all fungi strains, spore suspensions were prepared by eluting spores from fungal plates incubated at 28°C for 7 days with sterile PBS buffer. The spore suspension concentration was calculated using a hemocytometer. Then, about 10^8 cells were inoculated into 50 mL Sabouraud dextrose broth (SDB) medium and incubated for 15 days at 200 rpm in a 28°C shaker. The method of obtaining and preserving the metabolic products was identical to that used for bacterial cultures.

2.4 FT-ICR MS analysis

The metabolic products filtered by 0.22 μm pore membranes were acidified to pH 2 using high-pressure liquid chromatography (HPLC) grade hydrochloric acid (HCl). Dissolved organic matter (DOM) was extracted using a solid phase extraction (SPE) cartridge (200 mg, Oasis HLB, 6cc, Waters, U.S.) to remove salts (Chen et al., 2022; Han et al., 2022). After extraction, the cartridges were dried by flushing with high-purity N_2. Finally, 2 mL of HPLC-grade methanol (Sigma-Aldrich) was used to elute the extracted DOM. The eluent was analyzed with a 7.0 T superconducting magnet Bruker Solarix FT-ICR MS (SolariX 2xR, Bruker, Germany) equipped with an electrospray ionization (ESI) source in the negative ion mode. The samples were directly injected into the device at a continuous flow rate of 150 μL h^{-1} with a capillary voltage of 5000 V and an ion accumulation time of 0.028 s. The signal acquisition process included 256 cumulative scans and a 4M transient to obtain a higher signal-to-noise ratio (S/N) to resolve the sample fully. More detailed instrument parameter information is available in previous studies (Su et al., 2021). The complete experimental procedure is shown in Fig. S1b.

2.5 Data processing and statistical analysis

The raw datasets were calibrated using the DataAnalysis (ver. 5.0, Bruker Daltonics). The elemental compositions for all recalibrated peaks with S/N ≥ 4, using a mass allowance of ±1.0 ppm, were assigned using Composer (Sierra Analytics, USA) software. The molecular formulas were screened according to the criteria: $C_{0\text{-}50}H_{0\text{-}100}O_{0\text{-}50}N_{0\text{-}10}S_{0\text{-}3}P_{0\text{-}3}$, 0.3 ≤ H/C < 2.5, O/C < 1.2, N/C < 0.5, S/C < 0.2 (Chen et al., 2021; Yu et al., 2019). Based on the H/C and O/C ratios of the molecular formulas, all formulas were classified into seven categories: lipid-like (1.5 < H/C ≤ 2.0, 0 ≤ O/C ≤ 0.3), aliphatic/peptide-like (1.5 < H/C ≤ 2.2, 0.3 < O/C ≤ 0.67), carbohydrate-like (1.5 < H/C ≤ 2.5, 0.67 < O/C < 1.0), unsaturated hydrocarbons (0.67 < H/C ≤ 1.5, O/C < 0.1), carboxyl-rich alicyclic molecules (CRAM-like) (0.67 < H/C ≤ 1.5, 0.1 ≤ O/C < 0.67), aromatic-like (0.2 ≤ H/C ≤ 0.67, O/C < 0.67), and tannin-like/highly oxygenated compounds (HOC) (0.6 < H/C ≤ 1.5, 0.67 ≤ O/C ≤ 1.0) (Bianco et al., 2018).

Depending on the elemental composition of the compound, the molecular formulas were divided into CHO, CHON, CHOS, CHONS, and CHONSP compounds. The potential metabolic processes of microorganisms were annotated at a molecular level and analyzed using the MetaboDirect pipeline, and the annotation of metabolic pathways mainly relied on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Ayala-Ortiz et al., 2023; Ogata et al., 1999). The molecular mass-based transformation was analyzed by calculating the differences between a pair of m/z for all peaks in each sample and

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

comparing them to the list of pre-defined masses of common metabolic reactions (biochemical transformations key). The KEGG database contains three levels of metabolic pathways: 7 classes of primary pathways, 59 classes of secondary pathways, and 563 classes of tertiary pathways (Kanehisa et al., 2017). The Shannon and Chao1 index were calculated in R using the "vegan" (v2.6–4) packages. All statistical analyses were performed in R Studio for R version 4.2.1 and OriginPro 2023b.

3 Results and Discussion

3.1 Composition of atmospheric culturable microorganisms

Twenty-four bacterial and sixteen fungal strains were isolated from the TSP samples (Tables S2 and S3). Figure 1 illustrates the dominant taxa of culturable bacteria and fungi at the phylum and genus levels. The culturable bacterial community primarily consisted of Proteobacteria (45.8%), Firmicutes (37.5%), and Actinobacteria (16.7%). The dominant genera were *Bacillus* (37.5%), *Pseudomonas* (25%), and *Streptomyces* (16.7%). The isolated bacteria represent the major taxa commonly found in the atmosphere (Yan et al., 2021; Lee et al., 2017; Calderon-Ezquerro et al., 2021). *Pantoea* is a biological control agent that produces a variety of antibiotics (Kamber et al., 2012; Stockwell et al., 2010). *Bacillus subtilis* is highly adaptable to the environment, allowing it to survive in diverse extreme conditions and be widely distributed (Losick, 2020; Liu et al., 2018). *Pseudomonas* can act as ice nuclei to participate in cloud formation and is an important bacterium for assessing bacterial gene abundance and contribution (Huffman et al., 2013; Ruiz-Jimenez et al., 2021).

Ascomycota (87.5%) was overwhelmingly dominant in the culturable fungal community. The most abundant fungal genera were *Aspergillus* (37.5%) and *Penicillium* (25%), collectively accounting for more than 50% of the isolated strains. Ascomycota has been identified as the most abundant fungal phylum in previous studies, under different pollution levels and across seasonal observations (Abd Aziz et al., 2018; Drautz-Moses et al., 2022; Cáliz et al., 2018; Pyrri et al., 2020).

The spores of *Aspergillus* and *Penicillium* isolated in this study have pigments, and most fungi produce secondary metabolites also containing multiple pigments. Previous studies have demonstrated that many of the isolated microorganisms derived from atmospheric aerosols and rainwater were highly pigmented, potentially aiding their survival under intense UV exposure and photo-oxidative stress (Tong and Lighthart, 1997; Fahlgren et al., 2010; Fahlgren et al., 2015; Dassarma and Dassarma, 2018; Ziegelhoffer and Donohue, 2009). Many culturable halotolerant ice-nucleating bacterial and fungal strains isolated from coastal precipitation and aerosols could also produce pigments (Beall et al., 2021). To investigate the potential influence of bacterial and fungal secondary metabolites on atmospheric processes, we selected representative bacterial and fungal strains (Table S4 and Fig. S2) for further study, focusing on their exometabolome to determine and analyze the metabolic capacity of atmospheric microorganisms.

3.2 Changes of organic molecules in culture media after microbial incubation

The nutrients in the initial culture media supply the growth and reproduction of microorganisms. Through their diverse metabolic processes, microorganisms consume a significant amount of bioavailable organic matter and produce a variety of

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

secondary metabolites. The medium components, before and after microbial incubation, were characterized using FT-ICR MS. Organic molecules were grouped into three categories: (1) those present in the initial medium but absent after incubation, indicating consumed organic matter; (2) those absent in the initial medium but present in the metabolites, representing organic matter produced by microorganisms, they were considered to be biologically-controlled, define as exometabolites; (3) those present in both the initial medium and the metabolites, indicating resistant organic matter. Due to limitations in FT-ICR MS techniques, isomers could not be considered in this study.

Molecular changes during the metabolic processes of typical atmospheric bacteria (Fig. 2) and fungi (Fig. 3) are demonstrated using van Krevelen diagrams and stacked bar charts, the formula numbers as depicted in Table S5. In the bacterial initial medium (TSB medium), a total of 3416 formulas were detected, with CHON compounds dominating both in number (85.3%) and intensity (92.7%) (Fig. S3a). Furthermore, lipid-like compounds accounted for 13%, aliphatic/peptide-like compounds for 34%, and CRAM-like compounds for 52%, collectively representing 99% of the total number of molecules (Fig. S3b). Bacterial products had a lower H/C ratio and higher O/C ratio (Fig. 2a). They are more oxidized than consumed and resistant molecules. These bacteria consumed 413–1198 formulas, primarily in the categories of lipid-like (19–37%), aliphatic/peptide-like (27–43%), and CRAM-like (31–44%) compounds (Fig. 2b). *Stenotrophomonas* sp. and *Pseudomonas baetica* consumed more aliphatic/peptide-like and CRAM-like compounds (in terms of formula number) than other strains. CRAM-like compounds dominated resistant organic matter, accounting for 54–60% of the total resistant molecules. All bacterial strains, except for *Pseudomonas baetica*, showed that CRAM-like compounds constituted 51–64% of the produced organic molecules. CRAM-like compounds are an essential component of marine DOM. They are produced and consumed by heterotrophic bacteria that transform recalcitrant DOM, as demonstrated in laboratory studies of the model diatom *Skeletonema dohrnii* (Liu et al., 2023c; Liu et al., 2021). Specific bacterioplankton lineages with oxidative enzymes can catabolize complex CRAM-like molecular structures, e.g., long-chain aliphatics, cyclic alkanes, and carboxylic acids (Liu et al., 2020). The presented study confirms that CRAM-like compounds are significant exometabolites of the typical atmospheric bacteria. A long-term macrocosm experiment has also demonstrated that bacteria and archaea play a vital ecological role in producing refractory DOM, whose central part is CRAMs (He et al., 2022). Rainwater from coastal storms, after 30 days of dark incubation, produced molecules mainly in the form of aliphatic/peptide-like and CRAM-like compounds (Mitra et al., 2013), similar to the products of bacterial strains in this study. These results suggest that atmospheric bacteria could contribute to the biotransformation of refractory DOM as it settles to the ocean surface.

The fungal initial medium (SDB medium) had 3920 formulas, with CHON compounds dominating both in number (77.9%) and intensity (89.4%) (Fig. S3c). Furthermore, lipid-like compounds accounted for 14%, aliphatic/peptide-like compounds for 32%, and CRAM-like compounds for 51%, collectively representing 97% of all molecules in terms of number (Fig. S3d). The formula elemental composition and category of fungal initial medium is similar to that of bacterial initial medium. Molecules consumed by fungi were mostly compounds with low O/C ratios and high H/C ratios (Fig. 3a). The products had elevated O/C and reduced H/C, which also suggests that airborne microorganisms have the potential to influence atmospheric oxidative capacity and organic carbon budget.

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

The six typical fungi consumed 811–2531 formulas during incubation, with key categories including lipid-like (16–34%), aliphatic/peptide-like (25–32%), and CRAM-like (32–53%) compounds (Fig. 3b). Unlike bacteria, fungi consumed unsaturated hydrocarbons almost exclusively for their growth, with no unsaturated hydrocarbons in the resistant molecules or products. Notably, *Talaromyces* sp. consumed 2531 formulas, more than any other fungal strains, indicating highly diverse consumption compared to resistant (1389 formulas) and produced (246 formulas) compounds (Table S5). The resistant compounds for fungal strains contained a high proportion of aliphatic/peptide-like (31–37%) and CRAM-like (50–61%) compounds. The product composition of *Aspergillus niger* was significantly different from those of the other five fungal species, with its product containing 20% lipid-like molecules, much larger than the products of the other fungi.

Aspergillus niger is rich in genetic and metabolic diversity. In its natural growth state, *Aspergillus niger* possesses large cryptic biosynthetic gene clusters (BGCs), which synthesize a wide range of extracellular enzymes to degrade special biopolymers in the environment, thus allowing the fungus to obtain nutrients (Yu et al., 2021; Romsdahl and Wang, 2019). Lipids and lipid-like molecules are always the main metabolites of *Aspergillus niger* in different fermentation systems. For example, 69 lipids and lipid-like molecules were significantly different in tea leaves fermented by *Aspergillus niger* (Ma et al., 2021). Furthermore, in rare earth element bioleaching, 56 (14.2%) lipids and lipid-like molecules showed significant differences with the involvement of *Aspergillus niger* (Zhou et al., 2023). Additionally, *Aspergillus niger* serves as an important biosynthetic enzyme factory, represented in lipase synthesis (Li et al., 2020; Mhetras et al., 2009). For example, an optimized solid-state fermentation method uses agro-industrial waste as a substrate for large-scale production of *Aspergillus niger* lipase (Putri et al., 2020). In this study, the lipid-like compounds produced by Aspergillus niger were closely related to its high lipase activity.

3.3 Molecular characteristics of exometabolites from typical cultivable bacteria and fungi

3.3.1 Molecular diversity of bacterial and fungal exometabolites

As mentioned above, 651–2868 formulas and 246–1501 were produced for bacterial and fungal strains (Table S5), providing a basis for exometabolomic analysis. The molecular diversity of bacterial and fungal exometabolites is illustrated in Fig. S4. For bacterial stains, *Pantoea vagans*, *Streptomyces pratensis*, and *Stenotrophomonas* sp. showed higher molecular diversity in the exometabolites (Fig. S4a). For fungal strains, *Rhodotorula mucilaginosa* and *Aspergillus* demonstrated higher molecular diversity of exometabolites (Fig. S4b). Comparing the molecular diversity between bacteria and fungi, the Shannon index of bacterial exometabolites was higher than that of fungi, and the Chao 1 index of bacteria was significantly higher than that of fungi (Fig. S4c and S4d). This indicates the greater environmental adaptability and more diverse metabolic processes of atmospheric bacteria than fungi.

3.3.2 Molecular composition of exometabolites from typical bacterial strains

There are significant differences in metabolites between bacteria, and Figure 4 shows the molecular characteristics of bacterial exometabolites. The spectral peaks of the exometabolites displayed an uneven distribution across different bacterial species

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Preprint. Discussion started: 25 June 2024

(Fig. 4a). Overall, CHON compounds accounted for over 50% of the total formula numbers. The exometabolites of *Stenotrophomonas* sp. and *Pseudomonas baetica*, which metabolized and synthesized large quantities of CHONS compounds (55–62% of intensity), largely differed from those of the other four bacteria strains (only 3.5–22% of intensity). The exometabolites of *Stenotrophomonas* sp. and *Pseudomonas baetica* also exhibited higher amounts of high molecular weight compounds (m/z >500) (Table S6), likely representing extracellular polymeric substances (EPS), e.g., polysaccharides, extracellular enzymes, and cellular debris (Vandana et al., 2023; Moradali and Rehm, 2020).

The bacterial exometabolites predominantly consisted of lipid-like, aliphatic/peptide-like, CRAM-like, and tannin-like/highly oxygenated compounds (Fig. 4b). *Pantoea vagans* produced a higher proportion of nitrogenous unsaturated molecules (e.g., $C_{12}H_{10}N_2O$, $C_{15}H_{12}N_2O$, $C_{13}H_{12}N_2O$). CHON compounds were the primary constituents of these high abundance categories of bacterial exometabolites (Fig. S5a). Microbes can convert bioavailable lipid-like and protein-like N-containing compounds into more oxygenated, unsaturated (more refractory), and N-containing CRAM/lignin-like compounds (Osborne et al., 2013). The interactions between live bacteria (*Bacillus subtilis*, *Pseudomonas putida*, and *Enterobacter hormaechei*) and ·OH radicals in clouds contributed a significant amount of CHON compounds, which accounted for more than 50% in the water-soluble compounds (Liu et al., 2023a). Moreover, CHONS compounds dominated lipid-like and aliphatic/peptide-like molecules in exometabolites from *Stenotrophomonas* sp., distinguishing it from other bacterial strains (Fig. 4b). In cloud water incubation experiments, the compounds produced by the microorganisms were mainly distributed in lipid-like (12.1–16.5%), aliphatic/peptide-like (14.3–24.9%), and CRAM-like (42.2–44.7%) compounds, which were similar to the results of the present study (Bianco et al., 2019).

Comparing the unique molecules in the exometabolites of different strains can provide insights into their distinct metabolic processes. It can help to understand the impacts of atmospheric microbial deposition on microbial metabolic activity and the biogeochemical cycle in aquatic ecosystems. A total of 253 common molecules existed among the six typical bacteria, predominantly CHON compounds concentrated in aliphatic/peptide-like and CRAM-like compounds (Fig. 4c and S5b). The unique molecules in exometabolites of *Pantoea vagans* mainly consisted of CHO compounds (Fig. S5c), with the highest number of O_9 class molecules, dominated by $C_{18}H_{28}O_9$ and $C_{13}H_{16}O_9$, indicating a potential for high oxidizing properties. *Stenotrophomonas* sp. had the highest percentage of unique molecules, indicating more diverse metabolic processes. Certain *Stenotrophomonas* species can produce polyamines, indole-3-acetic acid ($C_{10}H_9NO_2$), and cytokinins (e.g., $C_{10}H_9N_5O$, $C_9H_8N_4OS$, $C_{17}H_{19}N_5O_5$), which contribute to their ability to protect plants and promote their growth (Peleg and Abbott, 2015; Zhao et al., 2024).

The elemental compositions of unique molecules varied across different bacteria, with those strains dominated by CHON and CHONS (Fig. 4c). Most CHON metabolites had an O/N ratio ≤ 3 (Fig. 4d), suggesting they may be associated with amino and amide groups and N-heterocyclic-containing combinations. It was further inferred that the metabolism of *Pantoea vagans* yielded unsaturated hydrocarbons compounds containing amino or amide groups. The oxygen numbers of CHONS compounds from *Stenotrophomonas* sp. were mainly concentrated in the range of O_7 to O_{11} (Fig. S5d), suggesting a higher level of oxidation, potentially due to the presence of $–OSO_3H$ and $–ONO_2$ groups, or other oxygen-containing groups like hydroxyl or

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Preprint. Discussion started: 25 June 2024

carboxyl groups. Some *Stenotrophomonas* spp. synthesize biologically active compounds, including various antibiotic enzymes (e.g., β-lactams, aminoglycosides, and macrolides), chitinases, lipases, and proteases (Ryan et al., 2009; Peleg and Abbott, 2015; Wang et al., 2018). It is inferred that many of the CHONS compounds in the exometabolites are likely proteases or their hydrolysates. All CHONS compounds specific to Pseudomonas baetica were CHO_nNS (Fig. S5E), and the vast majority were $CHO_{10–13}NS$ compounds. These results suggest that these compounds were highly oxidized and can act as indicator molecules among different bacteria.

3.3.3 Molecular composition of exometabolites from typical fungal strains

The number of formulas in fungal exometabolites was lower than in bacteria (Table S6). CHONS compounds appeared only in *Rhodotorula mucilaginosa* exometabolites, accounting for approximately 19% of the total formulas in terms of number (Fig.5a), indicating the diversity of sulfur-related metabolic processes for *Rhodotorula*. *Rhodotorula mucilaginosa*, as an essential biotechnological yeast, is utilized for producing carotenoids, lipids, enzymes, and other functional bioproducts using low-cost agricultural wastes due to its strong metabolism and resilience (Li et al., 2022; Sundaramahalingam and Sivashanmugam, 2023; Ju et al., 2023). The exometabolites from the genera *Penicillium* (*Penicillium oxalicum* and *Penicillium aurantiogriseum*) and *Talaromyces* (*Talaromyces* sp.) were dominated by CHO compounds, with over 50% intensity (Fig.5a). The formulas with the highest intensities were $C_{15}H_{10}O_6$, $C_{12}H_{16}O_6$, and $C_{15}H_8O_7$. The compositions of exometabolites from *Talaromyces* sp. were even more specific, with 98% intensities being CHO compounds, representing 74% of formula numbers. Fungal exometabolites were mainly aliphatic/peptide-like and CRAM-like compounds (Fig. 5b). *Talaromyces* sp. produced aliphatic/peptide-like molecules consisting exclusively of CHON compounds. In contrast, CRAM-like compounds contained mainly CHO compounds (Fig. S6a). The highest intensity compounds, $C_{15}H_8O_7$ and $C_{16}H_{16}O_5$, belong to aromatic-like and CRAM-like compounds, respectively. *Talaromyces* can produce many bioactive secondary metabolites, with $C_{15}H_8O_7$ and $C_{15}H_{10}O_6$ identified as emodic acids and catenarin (Lei et al., 2022). These compounds are among the leading products from the exometabolites of *Talaromyces* sp. revealed in this study, which are also the main products of *Talaromyces avellaneus* and *Talaromyces stipitatus* (Zhai et al., 2016).

Among the exometabolites of the six selected fungi, only 12 molecules were common, including eight CHO compounds and four CHON compounds (Fig. 5c). This number is considerably smaller compared to the common molecules observed in bacteria, highlighting the tremendous difference of fungal exometabolites. There are significant variations in the composition of unique molecules among exometabolites from different fungi. *Rhodotorula mucilaginosa* exhibited distinctive molecules characterized by a substantial presence of CHONS compounds (Fig. 5c). A relatively high percentage of these compounds possess an oxygen number greater than five (Fig. S6b), indicating a high degree of oxidation, with possible functional groups such as amino, amide, sulfuryl, and nitro groups. Most of the unique molecules were CHON metabolites in these six typical fungi, with an O/N ratio ≤ 6, mainly in aliphatic/peptide-like and CRAM-like classes (Fig. 5d), with the possible presence of amino, amide, and nitro groups in the products. *Aspergillus*, *Penicillium*, and *Talaromyces* exhibited the most inventive chemodiversity in metabolites among all fungi, producing a wide range of secondary metabolites (Frisvad, 2015; Zhai et al.,

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

2016; Morales-Oyervides et al., 2020). These three genera share similarities in exometabolites such as proteins, carbohydrates, lipids, and morphological features. They are holophyletic in a cladistic sense and polythetic in an anagenetic or functional purpose (Frisvad, 2015; Adelusi et al., 2022).

Significantly different from the bacteria, the genera of *Penicillium* and *Talaromyces* generated very distinctive pigmentation during cultivation, and some of these pigment molecules were classified into CRAM-like compounds (Fig. S7). In recent years, *Penicillium* and *Talaromyces* have been recognized as potential strains with the ability to produce natural pigments, similar to those pigments from Monascus, which are thermally stable, pH stable, and light stable (Ugwu et al., 2021; Akilandeswari and Pradeep, 2016). These two strains have potential applications in the food, pharmaceutical, nutritional, and textile industries (Mapari et al., 2010; Velmurugan et al., 2010; Morales-Oyervides et al., 2020). Compared to the molecular formulas of pigments resolved in previous studies (Morales-Oyervides et al., 2020; Contreras-Machuca et al., 2022), 10 and 8 typical pigment molecules in metabolites from *Penicillium* and *Talaromyces*, respectively, were identified in this study (Table S8).

3.4 Metabolic processes of typical isolated bacterial and fungal strains

3.4.1 Biochemical transformation of bacterial and fungal strains

Microorganisms can selectively uptake substrates and undergo diverse transformations during their metabolism. There were 168924 and 70646 potential molecular transformations during bacterial and fungal growth, respectively, with 139798 (83%) transformations unique to bacteria and 41520 (59%) to fungi (Fig. S8a). Among the bacterial transformations, *Stenotrophomonas* sp. had 5167 unique transformations, which accounted for 58% of the total number of transformations for this strain, and had a higher diversity of metabolite interconversions (Fig. S8b). *Penicillium aurantiogriseum* had the most unique transformations, with 24702 transformations, accounting for 65% of this strain's total number of transformations. In contrast, *Penicillium oxalicum* had fewer unique transformations, with 3867 (32%) transformations (Fig. S8c). This result shows that the potential metabolic pathways vary greatly among *Penicillium* spp., making it all the more critical for follow-up studies to characterize the metabolism in different species of typical *Penicillium*.

According to the classification rules of Ayala-Ortiz et al. (2023), 95 and 99 transformation types were identified for bacterial and fungal strains, respectively. Methylation and oxidation/hydroxylation were the most prevalent potential transformations observed during microbial metabolism (Fig. 5a and 5b). Interestingly, this finding aligns with the results obtained from a marine phage-host model system involving *Pseudoalteromonas* and two contrastingly different infecting phages (podovirus HP1 and siphovirus HS2), methylation and oxidation/hydroxylation were also the main transformation types in this culture system (Ayala-Ortiz et al., 2023). Amino acid metabolism differed markedly among bacteria, with alanine, isoleucine, and leucine transformation types being more prominent for *Bacillus subtilis* and glutamic acid transformation being more abundant for *Stenotrophomonas* sp. (Fig. 5a). This result is consistent with the transformation response of peatland microorganisms to moss leachate (Fudyma et al., 2021). The transformation types of carboxylation, glyoxylate, and erythrose accounted for the most prominent proportion of *Talaromyces* sp., significantly different from the other fungi (Fig. 5b).

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

There were significant differences in transformation numbers between bacteria and fungi, with abiotic and amino acid transformations dominating (Fig. S8d). Comparing the transformation types of bacteria and fungi, cysteine and methionine transformations related to amino acids were significantly higher in bacteria than in fungi (Fig. S8e). An abundance of putative amino acid transformation involving bacteria is also vital in surface river waters (Bridoux et al., 2023). The transformation types associated with phospholipid, biotin, and cytidine are more highly represented in fungi. Therefore, it's hypothesized that bacteria were more biased towards amino acid synthesis, whereas fungi were more active in transcription and expression.

The transformation networks are frequently used to analyze the metabolic state of organisms since the underlying biochemical reactions are well-known (Plamper et al., 2023). The main organic molecules involved in transforming the pigments of *Penicillium aurantiogriseum* and *Talaromyces* sp. were CRAM-like and aliphatic/peptide-like compounds (Fig. 5c) and CRAM-like and aromatic-like compounds (Fig. 5d), respectively. The transformation network showed interactions within a cluster of CRAM-like metabolites. Therefore, these fungi can efficiently convert certain peptides into stable pigment molecules, which may have industrial applications.

3.4.2 Metabolic pathways of typical isolated bacterial and fungal strains

There were 96 and 53 KEGG metabolic pathways annotated from typical bacterial and fungal exometabolites, respectively. Sixteen secondary pathways were annotated for bacteria, and carbohydrate metabolism, xenobiotics biodegradation and metabolism, biosynthesis of other secondary metabolites, and amino acid metabolism were the major pathways for bacterial exometabolites (Fig. S9a). Twelve secondary pathways were annotated for fungi, with major pathways similar to bacteria (Fig. S9b), but xenobiotics biodegradation and metabolism were not annotated for fungi. *Rhodotorula mucilaginosa* and *Penicillium* were predominantly involved in carbohydrate metabolism, *Talaromyces* sp. primarily participated in the biosynthesis of other secondary metabolites, with a large number of pigment molecules present in the exometabolites being secondary metabolites of *Talaromyces* sp. (Morales-Oyervides et al., 2020; Contreras-Machuca et al., 2022; Venkatachalam et al., 2018). Secondary metabolites of fungi and Actinomycetes have been a hot topic in extracellular metabolite research, which requires further studies.

Further, the primary KEGG Level 3 pathways varied widely among different bacteria (Fig. 7a). *Pantoea vagans* and *Bacillus subtilis* were mainly annotated to arachidonic acid metabolism (KO00590) of lipid metabolism. In comparison, *Bacillus toyonensis* and *Pseudomonas baetica* were more oriented towards carbohydrate metabolism, with the main pathways including fructose and mannose (KO00051), inositol phosphate (KO00562), ascorbate and aldarate (KO00053), and galactose metabolism (KO00052). Amino acid metabolism of tyrosine metabolism (KO00350) and histidine metabolism (KO00340) were more enriched in *Stenotrophomonas* sp.. Bacteria and fungi shared 16 pathways, mainly carbohydrate metabolism, energy metabolism, and membrane transport. These pathways are essential metabolic processes that sustain cell growth and reproduction.

The six typical fungal strains can be classified into three groups according to the KEGG Level 3 pathways (Fig. 7b). *Rhodotorula mucilaginosa* and *Penicillium* were mainly involved in fructose and mannose metabolism (KO00051), galactose

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

metabolism (KO00052), ABC transporters (KO02010), and diterpenoid biosynthesis (KO00904). Aflatoxin biosynthesis (KO00254) was dominant in the metabolism of *Talaromyces* sp.. *Aspergillus* metabolism was dominated by methane metabolism (KO00680) and lysine biosynthesis (KO00300).

Rhodotorula mucilaginosa is a typical platform strain that can produce many functional bioproducts (Li et al., 2022). Most of the high-value products detected in this study, like myristic acid ($C_{14}H_{28}O_2$), stearic acid ($C_{18}H_{36}O_2$), palmitic acid ($C_{16}H_{32}O_2$), linoleic acid ($C_{18}H_{32}O_2$), and linolenic acids ($C_{18}H_{30}O_2$). Ju et al. showed that the co-culture of *Rhodotorula mucilaginosa* with *Monascus purpureus* increased the production of carotenoids and *Monascus* pigments (Ju et al., 2023). The natural pigments of microbial synthesis are classified into various groups, including flavonoids, isoprenoids, porphyrins, N-heterocyclics, polyketides (e.g., anthraquinones and naphthoquinones), and others (Contreras-Machuca et al., 2022; Morales-Oyervides et al., 2020; Venkatachalam et al., 2018).

Aflatoxin biosynthesis is the predominant metabolic process annotated for *Talaromyces* sp. exometabolites, and this result is closely related to the synthesis of pigment molecules in *Talaromyces* secondary metabolites. Aflatoxin is a difuranocoumarin derived from a polyketide intermediate, and a significant amount of anthraquinones are synthesized during its biosynthesis (Mund and Čellárová, 2023; Mapari et al., 2010). Anthraquinones synthesized by fungi in symbiosis with plants protect host plants from insects or other microorganisms (Etalo et al., 2018). This study combines the molecular composition of the exometabolites with metabolic pathways, enriching the primary data for untargeted studies of microbial exometabolites using FT-ICR MS.

4 Conclusions

This study unveiled the characteristics of the molecular composition of exometabolites from atmospheric culturable typical microorganisms at the molecular level using FT-ICR MS. Vital metabolic processes for various bacteria and fungi were identified through mass-based transformation network analysis and KEGG enrichment analyses. The atmospheric culturable bacteria were primarily *Pseudomonas* and *Bacillus*, and the predominant fungi were *Aspergillus* and *Penicillium*. Bacterial and fungal strains produce exometabolites with lower H/C ratios and higher O/C ratios; this indicates that airborne microorganisms have the potential to influence atmospheric oxidative capacity and the organic carbon budget. The typical bacteria largely contribute to CHON and CHONS compounds in their metabolism, whereas fungi mainly produce CHO compounds as exometabolites. The microbial exometabolites predominantly consisted of aliphatic/peptide-like and CRAM-like compounds. The unique molecules of each typical bacterial strain were highly oxidizable and can serve as indicator molecules for various bacteria. Lipid metabolism and amino acid metabolism varied widely among different bacteria; Carbohydrate metabolism and secondary metabolism differed markedly among fungi. *Talaromyces* sp. exhibited a unique metabolic process with exometabolites dominated by CHO compounds, undergoing primarily carboxylation and glyoxylate transformations. Aflatoxin biosynthesis was identified as the central metabolic pathway of *Talaromyces* sp., along with the biosynthesis of other secondary metabolites.

https://doi.org/10.5194/egusphere-2024-1880
Preprint. Discussion started: 25 June 2024

These findings reveal the molecular composition of exometabolites from typical atmospheric microorganisms and provide data supporting potential metabolic processes that could occur if atmospheric microorganisms were deposited onto the oceans or inland waters. Future research should focus more on the deposition and activity of atmospheric microorganisms in natural environments and on building a database of source profiles for typical atmospheric microbial exometabolites at the molecular level.

Data availability. The dataset for this paper is available upon request from the corresponding author (huwei@tju.edu.cn; fupingqing@tju.edu.cn).

Author contribution. RJ, WH, and PF designed the entire study and the experiments, visualized the data, and determined the structure of the article. RJ, MN, and PD executed experiments. RJ, MS, DL, and ZH analyzed the data. RJ and WH wrote the draft manuscript with input from all of the authors. All of the authors discuss and edit the manuscript. All of the authors read and approved the final manuscript.

Competing interests. The authors declare that they have no conflict of interests.

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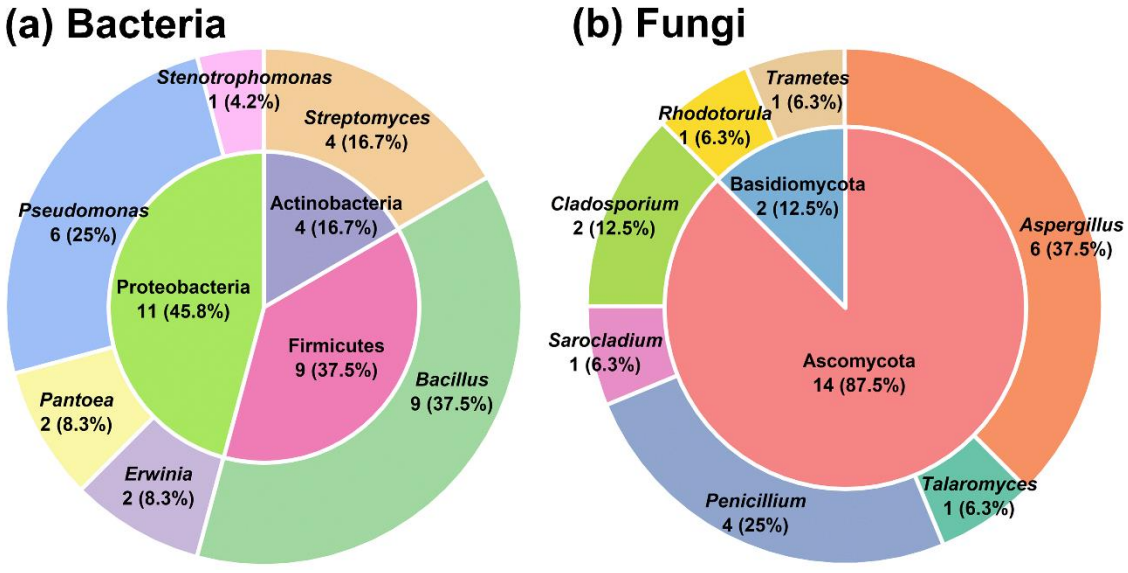

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Figure 1. The distribution of culturable bacterial (a) and fungal (b) taxa at the phylum (inner pie charts) and genus (external pie charts) levels.

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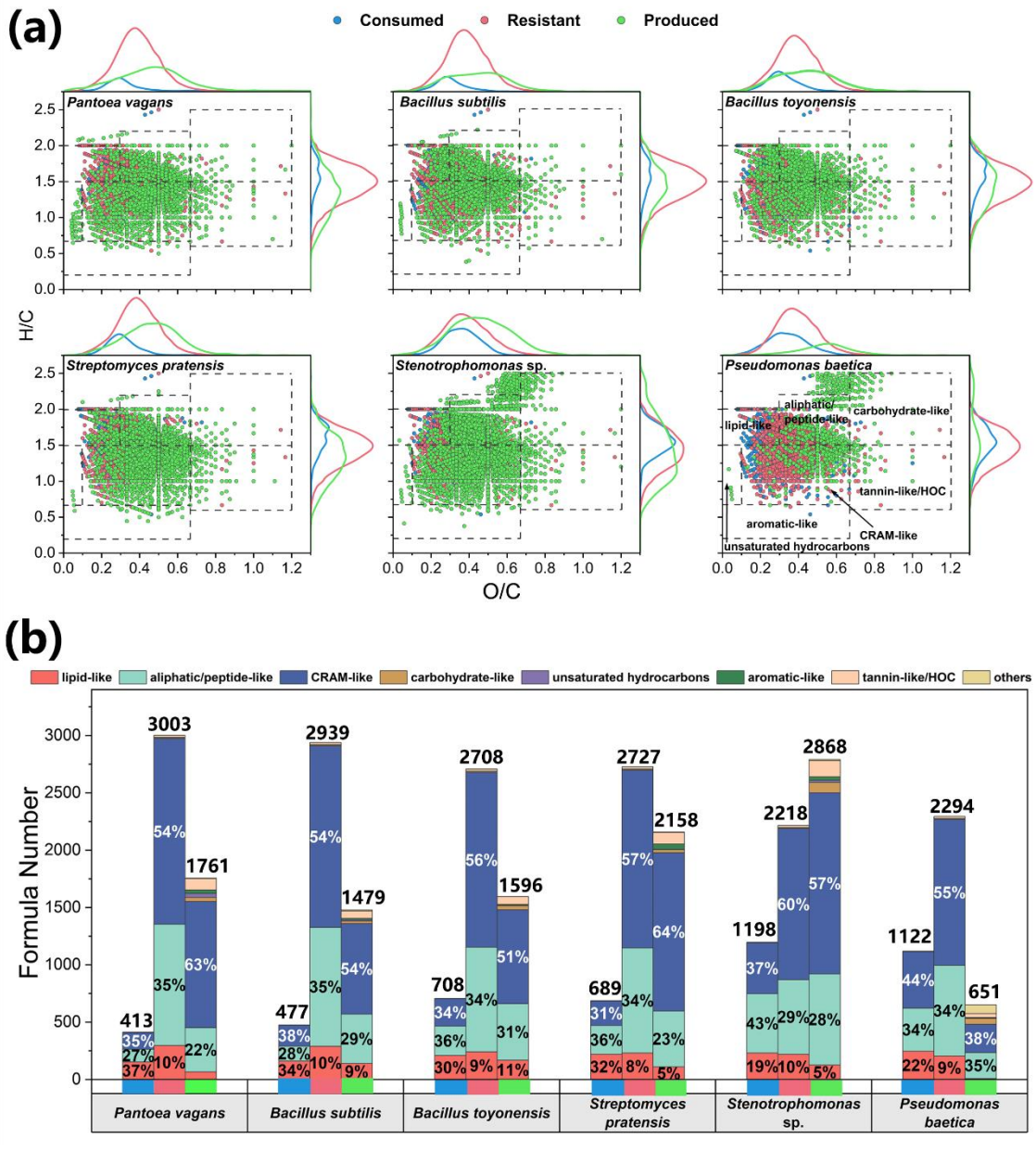

Figure 2. Changes in molecular compositions of culture media during the metabolic processes of six typical atmospheric culturable bacteria. (a) Van Krevelen diagrams illustrate changes in different categories of organics in the media after incubation. (b) The stacked bar charts indicate the formula numbers of different categories of consumed, resistant, and produced organic matter.

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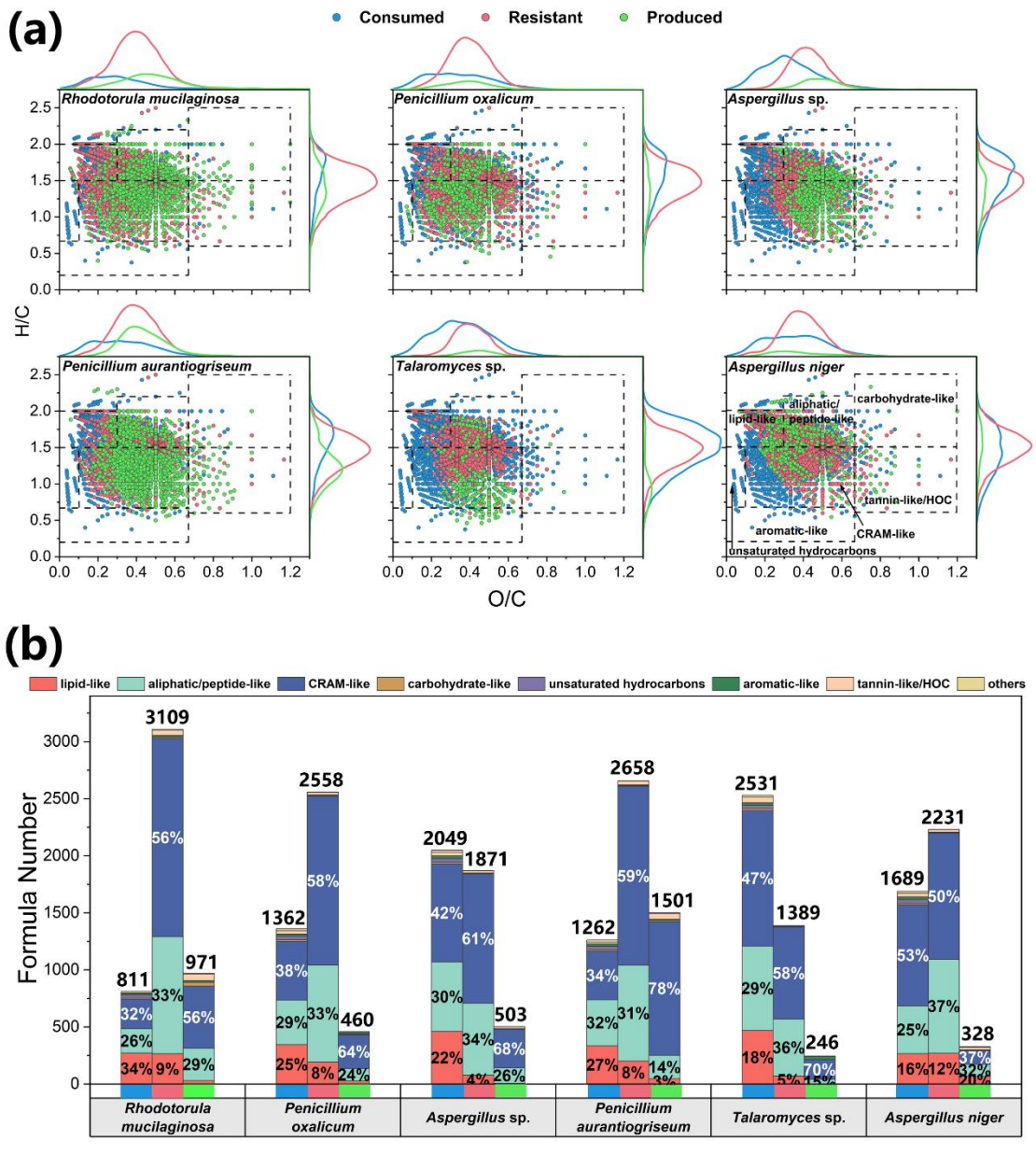

Figure 3. Changes in molecular compositions of culture media during the metabolic processes of six typical atmospheric culturable fungi. (a) Van Krevelen diagrams illustrate changes in different categories of organics in the media after incubation. (b) The stacked bar charts indicate the formula numbers of different categories of consumed, resistant, and produced organic matter.

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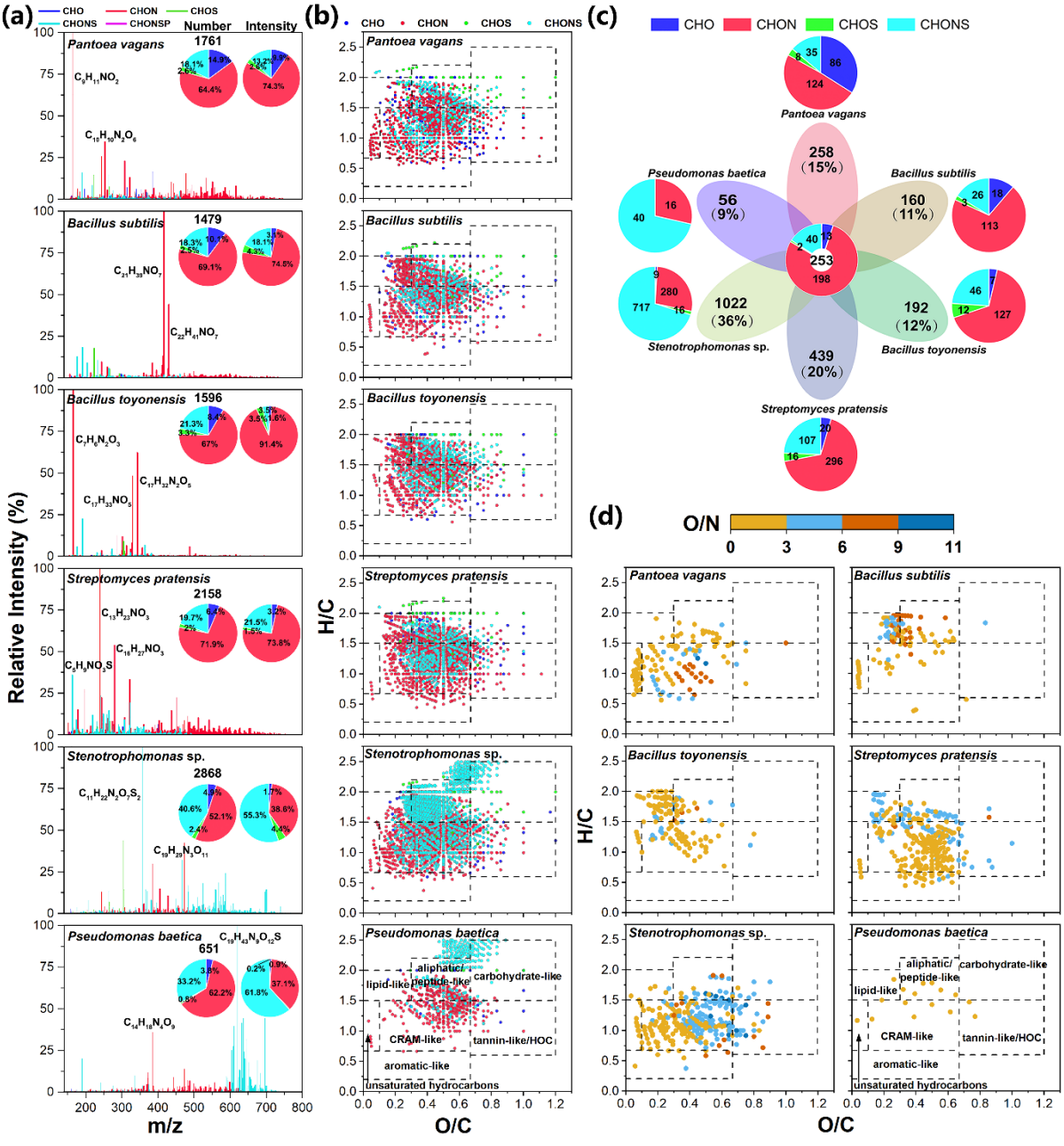

Figure 4. Characteristics of the molecular composition of bacterial exometabolites. (a) Mass spectra and pie chart show the distribution of m/z and elemental compositions of bacterial exometabolites. (b) Van Krevelen diagrams show the molecular distribution of bacterial exometabolites classified by elemental composition (CHO, CHON, CHOS, and CHONS). (c) Venn diagram displays the numbers and percentages of shared and unique molecules for six typical bacteria, and the donut diagram in the center and pie charts show the elemental compositions of shared and unique molecules for each bacterial strain. The percentages in the graph represent the ratio of unique molecules to the total molecule numbers of the strain. (d) Van Krevelen diagrams reveal the distribution of unique CHON compounds with different O/N ratios for each bacterial strain.

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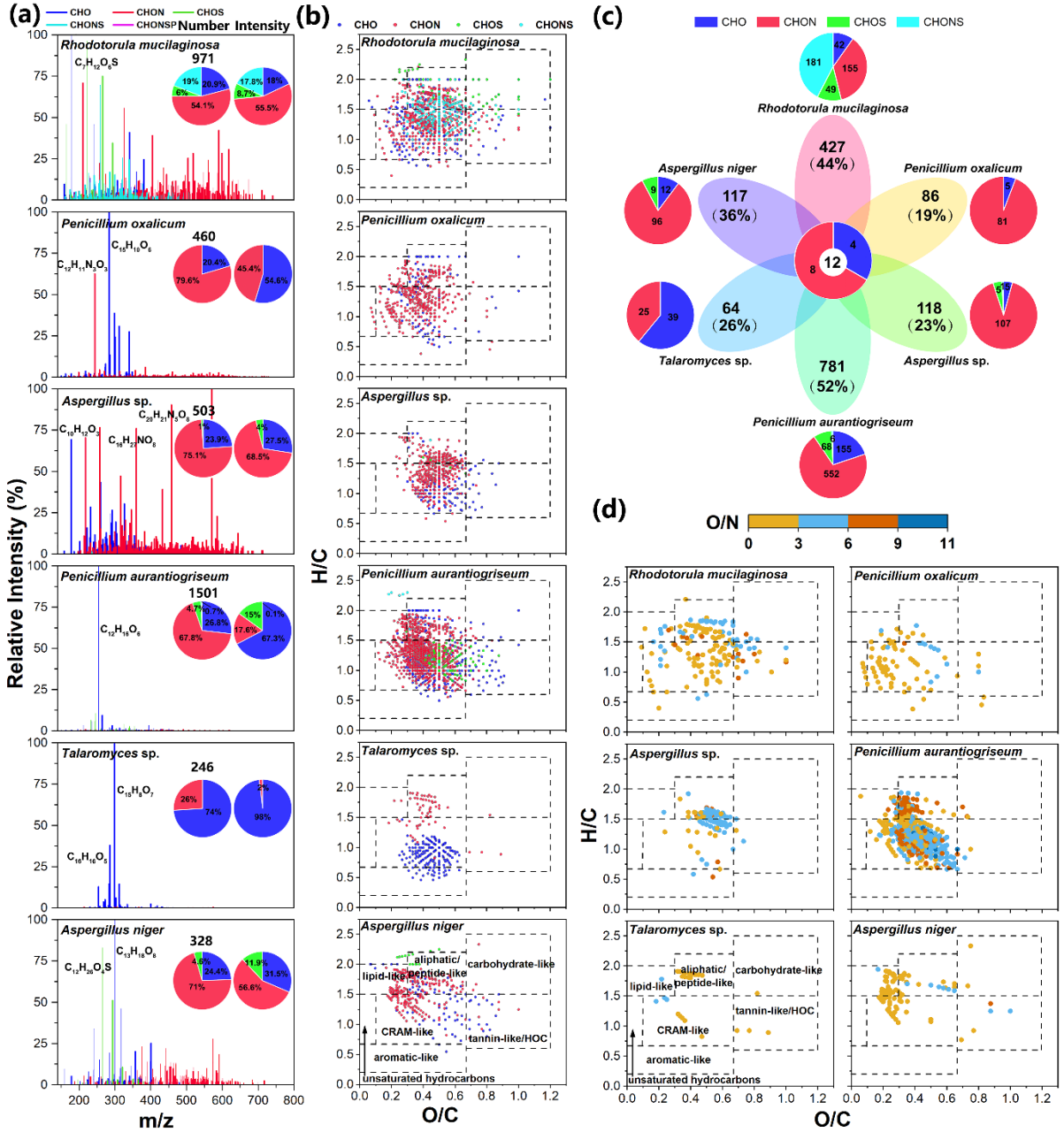

Figure 5. Characteristics of the molecular composition of fungal exometabolites. (a) Mass spectra and pie chart show the distribution of m/z and elemental compositions of fungal exometabolites. (b) Van Krevelen diagrams show the molecular distribution of fungal exometabolites classified by elemental composition (CHO, CHON, CHOS, and CHONS). (c) Venn diagram displays the numbers and percentages of shared and unique molecules for six typical fungi, and the donut diagram in the center and pie charts show the elemental compositions of shared and unique molecules for each fungal strain. The percentages in the graph represent the ratio of unique molecules to the total molecule numbers of the strain. (d) Van Krevelen diagrams reveal the distribution of unique CHON compounds with different O/N ratios for each fungal strain.

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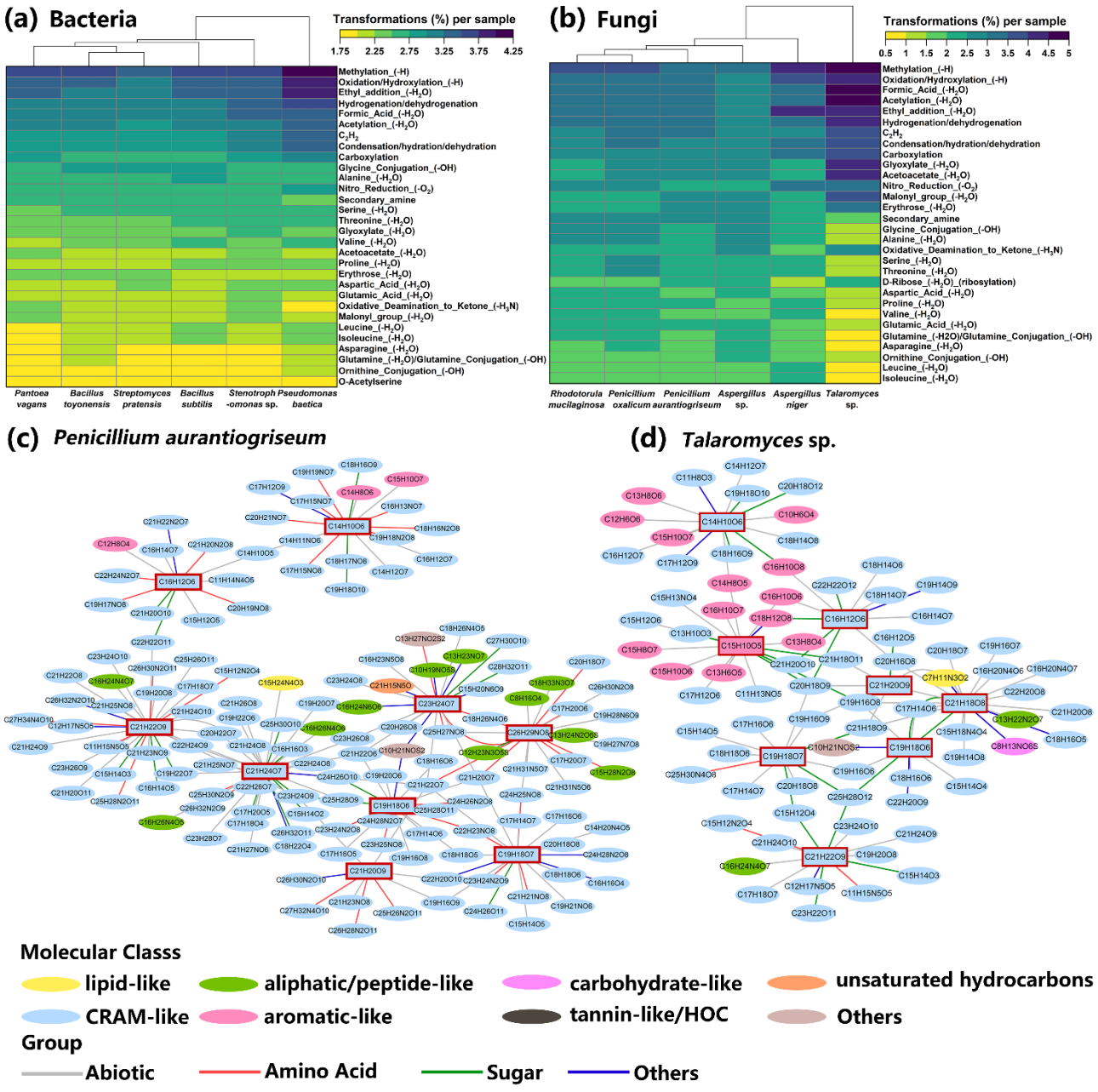

Figure 6. The types and networks of biochemical transformations for bacteria and fungi. Heatmaps show the top 30 transformation types in terms of relative abundance for bacteria (a) and fungi (b). Molecular networks illustrate the transformations for the major pigment formulas (Table S8) of *Penicillium aurantiogriseum* (c) and *Talaromyces* sp. (d).

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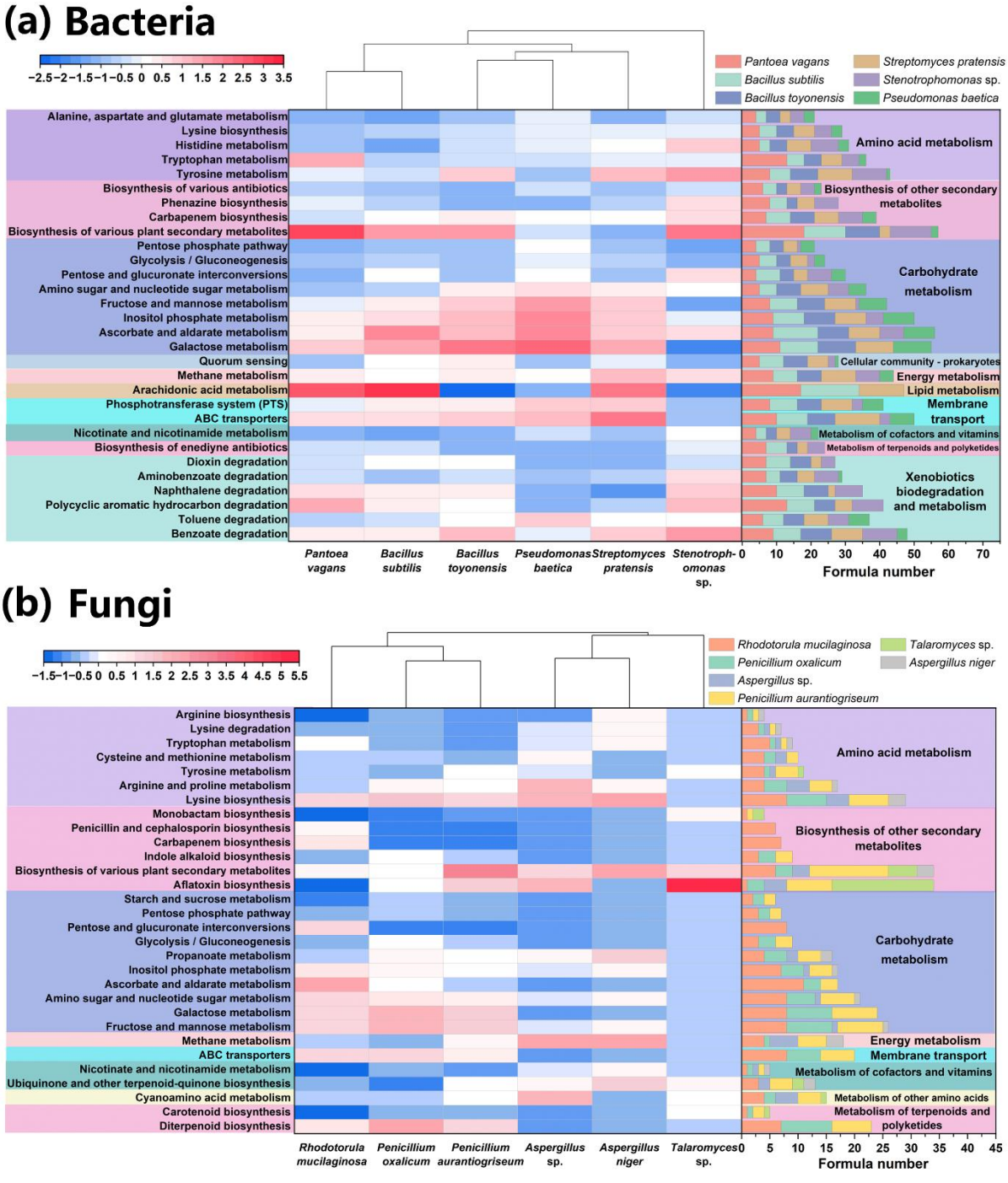

820 **Figure 7.** The KEGG metabolic pathways for typical airborne bacterial (a) and fungal (b) strains. Heatmaps illustrate the normalized relative abundance (top 30) of KEGG tertiary pathways of bacterial and fungal strains. Stacked bar diagrams show the formula numbers annotated to microbial exometabolites in each pathway.