# Chemical mapping and bio-guided fractionation of macromycetes for the discovery of new and bioactive natural products

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**Résumé**

Throughout history, natural products have been a cornerstone of drug discovery(1), with the origins of many modern medications traceable to compounds isolated from plants, marine organisms, bacteria, or fungi. Antibiotics are a striking example, as most are derived from natural products-primarily of bacterial or fungal origin. During the so-called "golden era of antibiotics" (1950–1970), industrial high-throughput screening of natural extract libraries was widespread. However, this approach eventually yielded diminishing returns in terms of novel scaffolds, leading to a shift in focus toward synthetic chemistry(2). Simultaneously, the widespread and often inappropriate use of antibiotics-such as prophylactic use in the agri-food industry or inadequate treatment in human medicine-has contributed to the alarming rise of multidrug-resistant bacteria(3). Today, more than ever, there is an urgent need for new scaffolds with original modes of action to combat bacterial infections.
Macromycetes, commonly referred to as mushrooms, have been relatively underexplored as potential sources of antibiotics compared to filamentous fungi like *Penicillium* and *Fusarium*, despite preliminary evidence of promising antimicrobial activity(4). These organisms share ecological niches with plants, bacteria, animals, and micromycetes, yet have distinct resource requirements. As a result, they produce a range of unique compounds likely involved in signaling or defense, including antimicrobial agents. Research has largely focused on traditional medicinal mushrooms from Chinese pharmacopoeia(5)-such as *Ganoderma lucidum*, *Cordyceps militaris*, *Lentinula edodes*, and *Hericium erinaceus*-while few European species have been historically recognized for medicinal properties(6), with most attention limited to edible or toxic varieties.

Alsace is a region characterized by a diverse landscape, dominated by a calcareous alluvial plain nestled between two mountain ranges (the Vosges in France and the Black Forest in Germany). Approximately 4000 mushroom species have been recorded in this area, the

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majority of which remain chemically unstudied. To assess the potential of macromycetes as antibiotic sources, a mycotheque of 150 wild mushroom species was assembled. Fungal extracts were subjected to bioautography(7) against members of the pathogenic ESKAPE family-specifically *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-as well as *Serratia marcescens* and the fungal pathogen *Candida albicans*. These microorganisms are known for their ability to develop multidrug resistance and evade conventional antibiotics.

Preliminary screenings identified several extracts and compounds with antimicrobial activity. Additionally, UHPLC-MS/MS analysis coupled with data processing via the MZMine-GNPS pipeline allowed us to visualize the chemical diversity of the extracts as a molecular network, facilitating the identification of rare versus ubiquitous compounds. The integration of chemical and biological data guided the selection of promising extracts for fractionation and further characterization of active constituents. Two such extracts will be discussed in this talk to illustrate the effectiveness of the described approach.

**References: (1)** Atanasov et al., *Nat Rev Drug Discov* **2021 (2)** Hutchings et al., *Current Opinion in Microbiology* **2019 (3)** Bharadwaj et al. *Biomed Res Int* **2022 (4)** Hamers et al., *Planta Med* **2021 (5)** Ray et al., *Pharmacological Research - Modern Chinese Medicine* **2024 (6)** Gründemann et al., *Phytomedicine* **2020 (7)** Huguet et al., *Applied Sciences* **2022**