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Macromycetes metabolites to fight multidrug resistant bacteria

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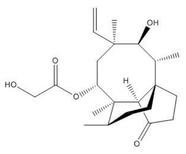
Introduction

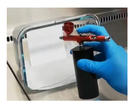
T. versicolor



Pleuromutilin

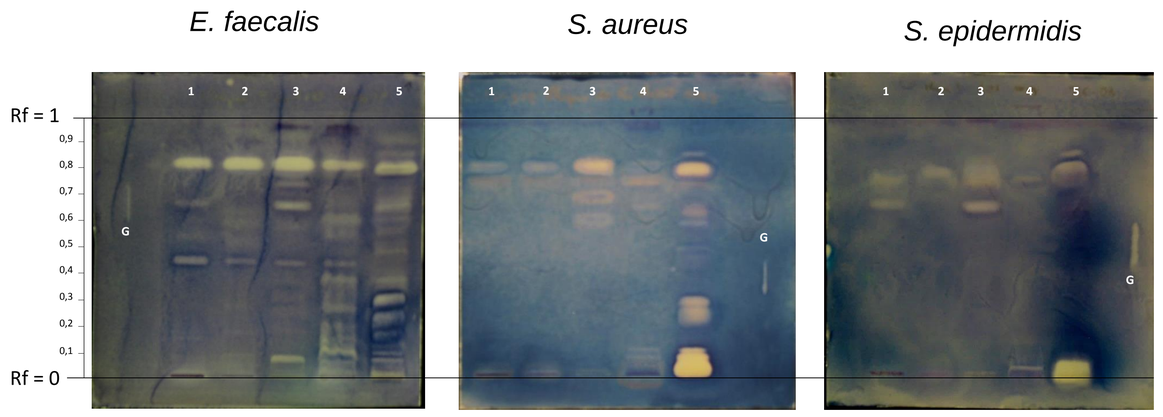
The emergence of numerous resistance mechanisms in human pathogenic bacteria has become a major concern of medical and pharmaceutical research. The World Health Organization reckons that the number of deaths due to antibiotic resistance could reach 10 million by 2050 if new effective therapies are not rapidly developed [1]. Only recently, pleuromutilins from Basidiomycota mushrooms have joined the antibiotic arsenal [2]. However, although the literature indicates the potential of mushroom compounds for antibacterial treatments, macrofungi represent a yet largely unexplored source in the field of anti-infectious agents [3]. We will present here the methodology to screen a bank of macromycetes extracts and the identification of active metabolites against multidrug resistant (MDR) bacteria.

Screening by direct bioautography



Since 2014, the sporocarps of 70 macromycetes species have been collected in the Alsatian region. After an identification of macro and microscopic characters, the identity of the species was confirmed by genetic sequencing. Following the identification, an extract library was constituted by successive macerations of the dried fungi in solvents of increasing polarity (cHex, EtOAc, MeOH and H_2O). The first challenge of this work was to apply a rapid screening technique for this extract library in order to asses the antibacterial activity. The direct bioautography (TLC-DB) was chosen for the screening using 5 wild type bacterial strains: *E. faecalis*, *S. epidermidis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The technique used for the application of the bacteria and the MTT on the chromatographic support has been optimized with the use of an airbrush. Antibacterial activity can be visualized by whitish clear zone on the antibiograms contrasting with a homogenous blue-purple background has it is presented below.

Result of the preliminary screening on wild type bacteria (Gram +)

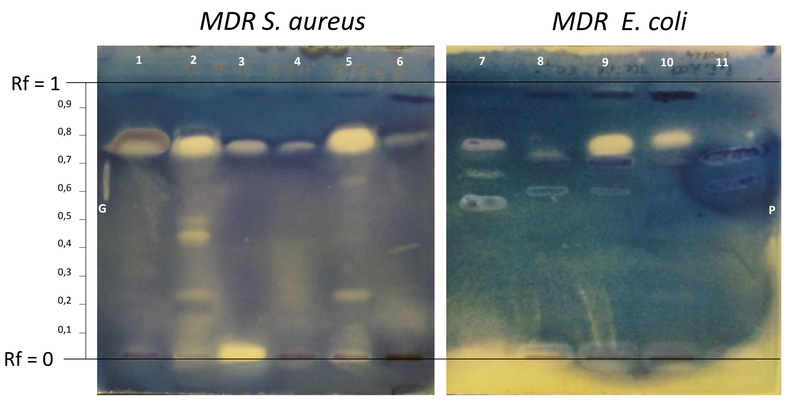


Examples of bioautograms of 5 EtOAc mushroom extracts
1: *N. erythropus*, 2: *P. vesiculosa*, 3: *S. citrinum*, 4: *A. muscaria* and 5: *F. pinicola*
Stationary phase: silica gel 60 (F_{254}) and mobile phase: MTBE-THF-cHex (5:1:4 v/v/v)

Macromycetes species selection

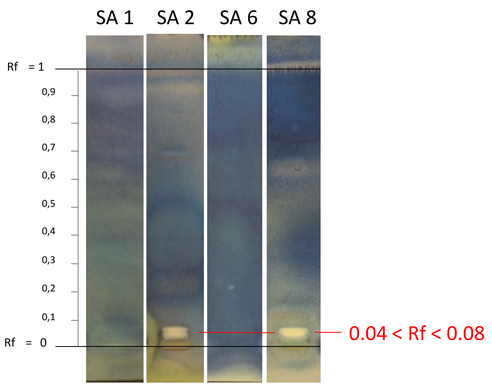
After the screening of 72 extracts (EtOAc and MeOH) from 31 mushroom species by TLC-DB, 8 species were subjected to a second TLC-DB screening against 4 different multidrug resistant (MDR) strains of *S. aureus* and 4 other MDR strains of *E. coli*.

Result of the preliminary screening on MDR bacteria



Exemples of bioautogramms of 5 EtOAc mushroom extracts
1: *L. piperatus*, 2: *C. nebularis*, 3: *H. fasciculare*, 4: *L. helvus*, 5: *P. ostreatus*, 6: *R. lepida*, 7: *F. pinicola* and 7 – 11: *S. citrinum*
Stationary phase: silica gel 60 (F_{254}) and mobile phase: MTBE-THF-cHex (5:1:4 v/v/v)

This second screening allows us to confirm our antibacterial activity against MDR bacteria. Moreover, we have detected specific activity from *H. fasciculare* MeOH extract towards two *S. aureus* MDR strains. This information would not have been detectable by traditional screening techniques on complex natural extracts.

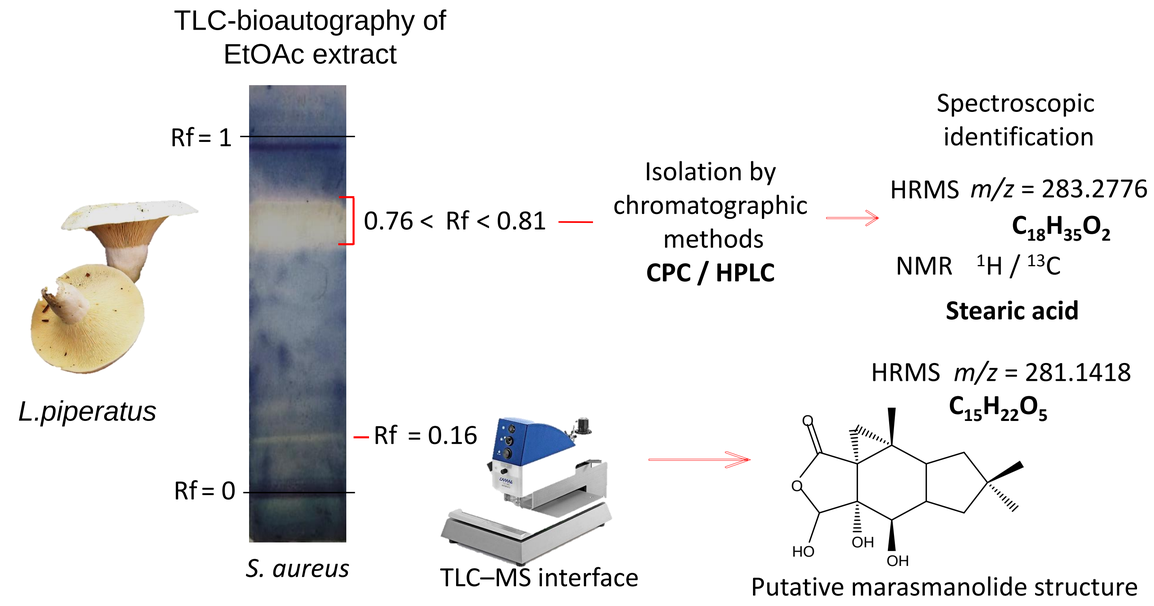


Bioautograms of *H. fasciculare* MeOH extract against 4 MDR *S. aureus* strains
Stationary phase: silica gel 60 (F_{254}) and mobile phase: MTBE-THF-cHex (5:1:4 v/v/v)

Antibacterial metabolites isolation and identification

• *Lactifluus piperatus*

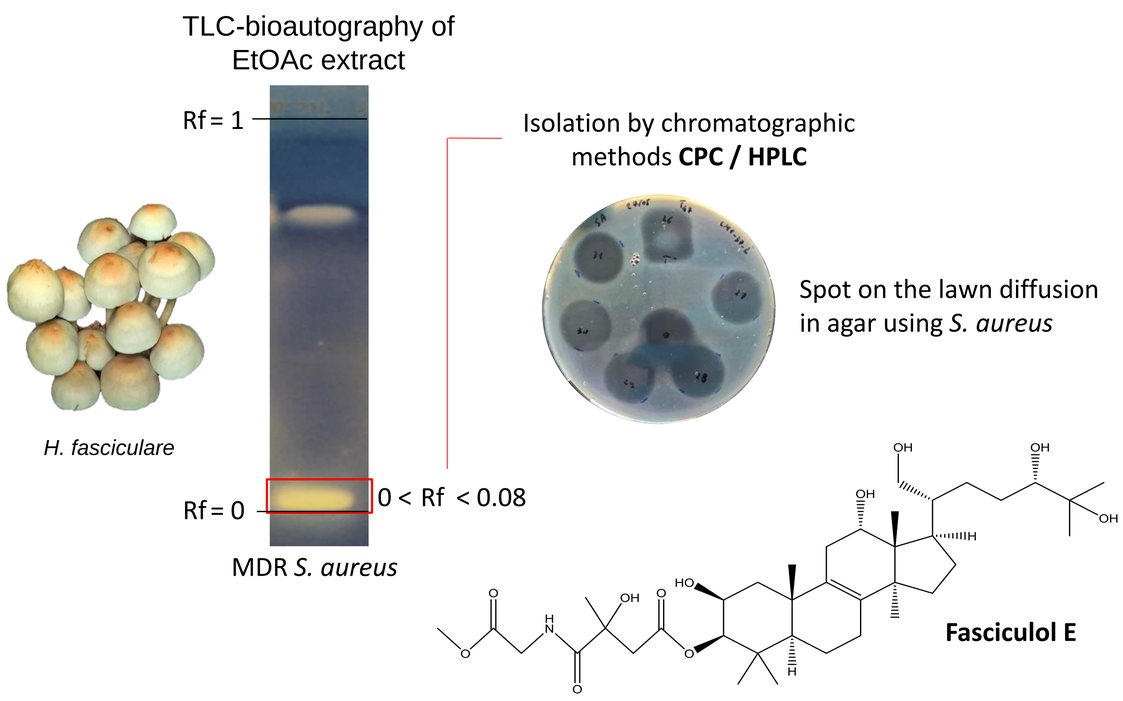
We have chosen this species to isolate and characterize 2 antibacterial metabolites. One of this metabolite was active against several bacteria (wild type and resistant phenotype) and the Rf of activity was common with other mushroom species between 0.76 and 0.81. Another strategy was applied using the TLC/MS interface in order to collect spectroscopic data for an antimicrobial compound with a Rf of 0.16.



The apolar active compound on our bioautogrtaphy (0.76 < Rf < 0.81) was undoubtedly identified as stearic acid. On the other hand, we need more spectroscopic information to describe the polar antibacterial compound (Rf = 0.16). According to our first hypothesis, it seems that it is a sesquiterpene belonging to the marasmanolid family.

• *Hypholoma fasciculare*

After bioautography screenings, we observed a strong zone of inhibition of our bacterial models (wild type an multidrug resistant) just above the deposit line on the bioautography of the EtOAc extract from *H. fasciculare*. Due to the strong polarity of the molecules and the weak separation with the applied TLC system, the bioguided purification was carried out by the technique of spot on the lawn on agar.



Four compounds have been successfully isolated and one of them has been characterized as fasciculol E based on HRMS and NMR datas. The other metabolites appear to be analogues of this compound.

Conclusion and perspectives

- **8 macromycetes species were selected after a preliminary screening and were actives against MDR bacterial strains on bioautography.**
- **Macromycetes are a source of antibacterial molecules of various chemical nature. This suggests original mechanisms of action for the development of new antibiotics.**
- **This is the first time that the antibacterial action of fasciculol E has been described against pathogenic bacteria.**
- **Further investigations must be carried out on the action of these fungal molecules (mechanism, antibacterial spectrum, MIC) as well as the proof of their harmlessness on eukaryotic cells.**

References

[1] de Kraker ME *et al.* Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? *PLoS Medicine* **13**(11):e1002184 (2016)
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[3] Hamers V *et al*. Antibacterial Compounds from Mushrooms: A Lead to Fight ESKAPEE Pathogenic Bacteria? *Planta Medica* **87**(5):351-367 (2021)