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Biological tools for microbial control: *Leuconostoc mesenteroides* and *Lactiplantibacillus plantarum* strains isolated from Apulian honeys as promising antifungal agents

Ester Presutto[1*], Maria Lucia Valeria de Chiara[2], Vittorio Capozzi[2], Giuseppe Spano[1], Mariagiovanna Fragasso[1]

[1]Department of Agriculture Food Natural Science Engineering, University of Foggia, Via Napoli 25, 71122, Foggia, Italy

[2]Institute of Sciences of Food Production, National Research Council (CNR), c/o CS-DAT, Via Michele Protano, Foggia, Italy

*Corresponding author: ester.presutto@unifg.it

Università di Foggia

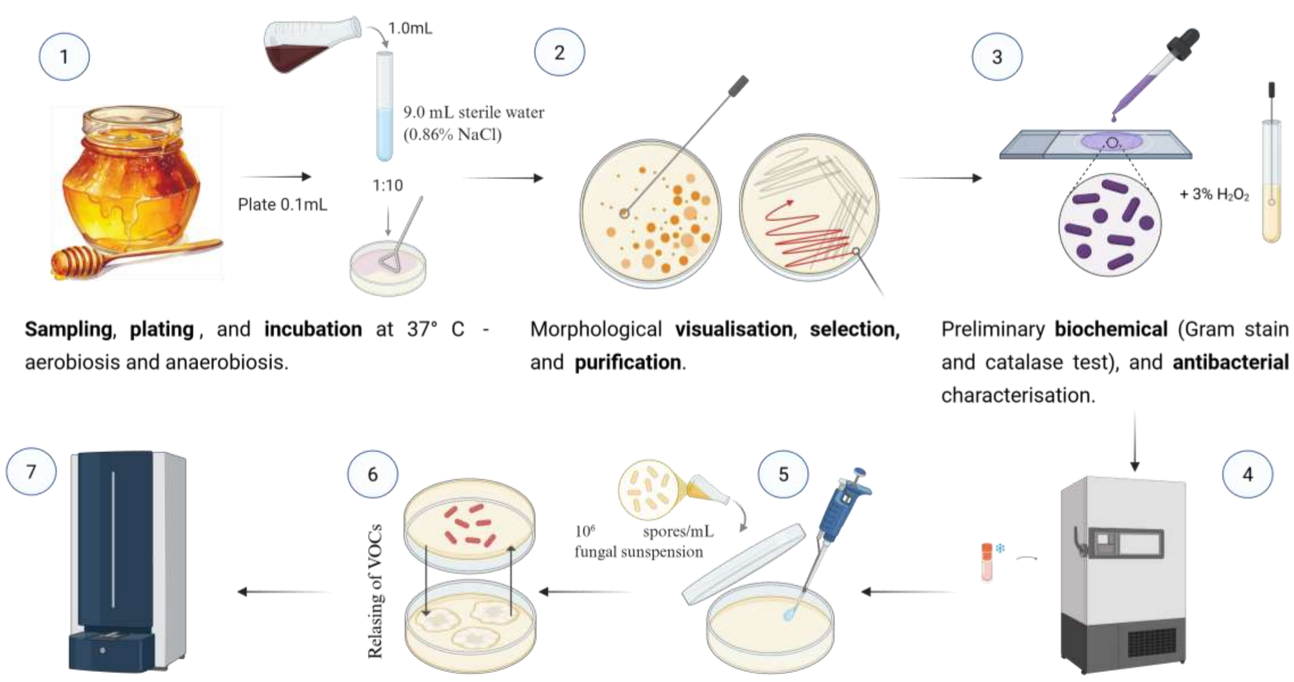
Consiglio Nazionale delle Ricerche ISPA ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

INTRODUCTION & AIM

Lactic acid bacteria (LAB) are emerging as valuable agents for microbial biocontrol, due to their ability to produce broad-spectrum antagonistic compounds, including organic acids, bacteriocins, bioactive peptides, short-chain fatty acids, and volatile organic compounds (VOCs).

The use of LAB as natural preservatives aligns with current trends toward sustainable and eco-friendly food production systems, offering an alternative to synthetic chemical preservatives. The aim of this study was to assess the antifungal potential of 10 LAB strains belonging to the species *Leuconostoc* (*Leuc*.) *mesenteroides* and *Lactiplantibacillus plantarum*, isolated from five varieties of Apulian honey: i) honeydew honey, ii) French honeysuckle (Sulla) honey, iii) wildflower honey, iv) coriander honey, and v) *Eucalyptus* honey. Antifungal activity was investigated through a sequential assay combining the agar *overlay* method and the 'Plate-on-Plate' technique, tested against *Aspergillus* (*A*.) *niger*, *Penicillium* (*P*.) *crustosum*, *P*. *roqueforti*, *Aureobasidium* (*Au*.) *pullulans* and *Geotrichum* spp.

METHODS



Sampling, **plating**, and **incubation** at 37° C - aerobiosis and anaerobiosis.

Morphological **visualisation**, **selection,** and **purification**.

Preliminary **biochemical** (Gram stain and catalase test), and **antibacterial** characterisation.

Molecular **identification** of the most promising strains (**MALDI-ToF-MS**).

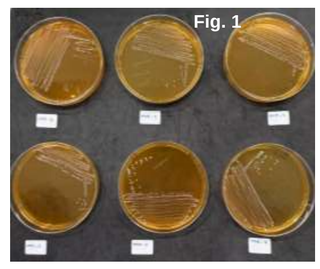
VOCs production evaluation ("***Plate-on-Plate***" assay).

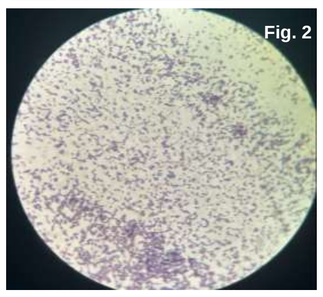
First antifungal screening using a double-layer ("***overlay***") agar assay.

Preservation of selected putative LAB strains at −80 °C for long-term storage.

Tab. 1

ID strains	Isolation source	Species
MME-1	Honeydew honey	*Leuconostoc mesenteroides*
MME-2	Honeydew honey	*Leuconostoc mesenteroides*
MME-3	Honeydew honey	*Leuconostoc mesenteroides*
MME-4	Honeydew honey	*Leuconostoc mesenteroides*
MME-9	Honeydew honey	*Lactiplantibacillus plantarum*
MM1	Wildflower honey	*Lactiplantibacillus plantarum*
MM9	Wildflower honey	*Lactiplantibacillus plantarum*
MS1-CB	Sulla honey	*Lactiplantibacillus plantarum*
ME-4	*Eucalyptus* honey	*Lactiplantibacillus plantarum*
MC-1	Coriander honey	*Lactiplantibacillus plantarum*





LAB strains were preliminarily selected based on morphological and biochemical characterisation (Figs. 1–2). The most promising isolates were then identified at the species level by Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry (MALDI-ToF MS).

CONCLUSIONS

The results support the application of LAB from non-conventional matrices as bioprotective agents in food and packaging systems, promoting the development of 'green' solutions in line with the principles of sustainability and the circular bioeconomy.

AKNOWLEDGMENTS

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RESULTS & DISCUSSION

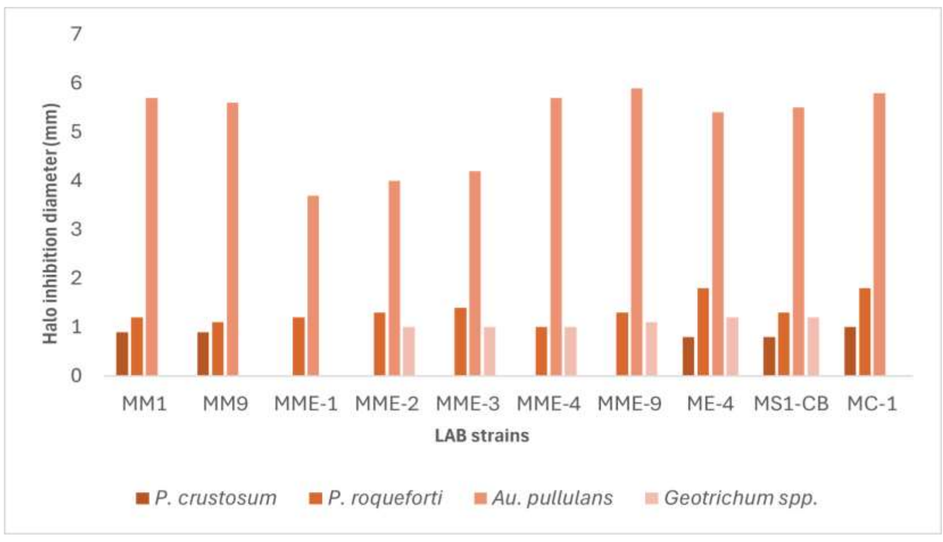


Fig. 3. Halo inhibition diameter (mm) of LAB strains using the *overlay* assay.

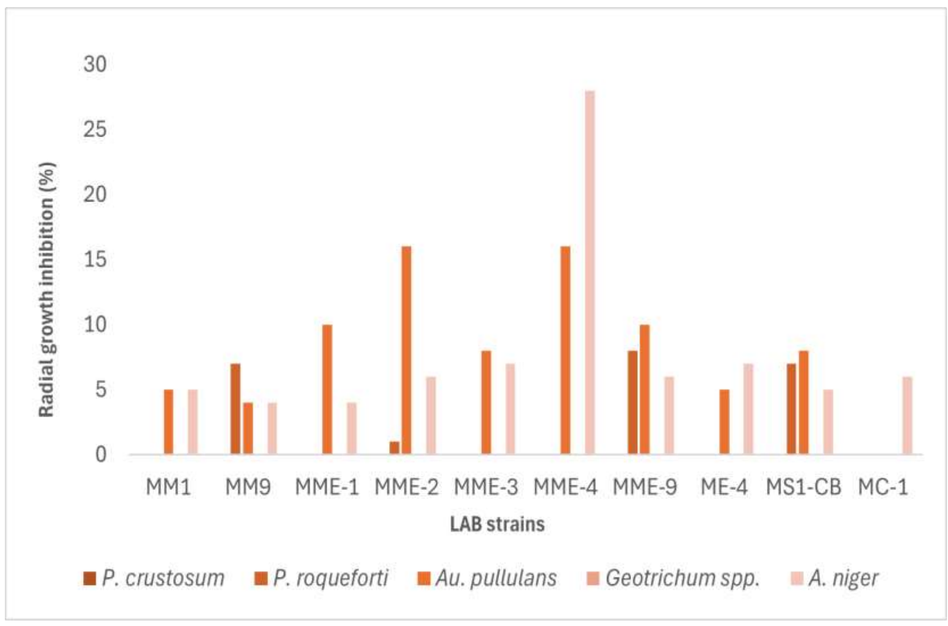
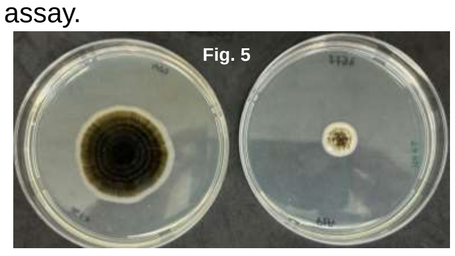
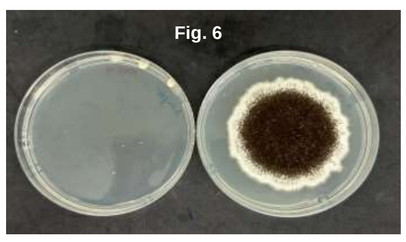


Fig. 4. Radial growth inhibition (%) by LAB VOCs using the 'Plate-on-Plate' assay.





In both cases, all strains exhibited moderate to high inhibitory activity against *P. roqueforti* and *Au. Pullulans* (Fig. 3-4). *Leuc. mesenteroides* MME-2 exhibited clear inhibition halos against *Au. pullulans* (Fig. 5). Complete inhibition of *A. niger* mycelial growth was observed exclusively for *Leuc. mesenteroides* MME-4 in the '*Plate-on-Plate*' assay, suggesting the involvement of bioactive VOCs within the shared headspace (Fig. 6).

FUTURE WORK / REFERENCES

- Integrate omic approaches to link genotype and antimicrobial phenotype;
- Validate selected LAB strains in real food/packaging systems;
- Assess honey-derived LAB in sustainable food preservation and biocontrol system.

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