



HAL
open science

Searching for Synergistic Bacterial Partners to Improve Lentil Flour Fermentation by *Pleurotus ostreatus*

Isa Hollopp, Eric Dugat-Bony, Pascal Bonnarme, Lukas Y Wick, Vincent Hervé

► To cite this version:

Isa Hollopp, Eric Dugat-Bony, Pascal Bonnarme, Lukas Y Wick, Vincent Hervé. Searching for Synergistic Bacterial Partners to Improve Lentil Flour Fermentation by *Pleurotus ostreatus*. FOODSIM'2026, EUROSIS, Apr 2026, Ghent, Belgium. ⟨hal-05549963⟩

HAL Id: hal-05549963

<https://hal.science/hal-05549963v1>

Submitted on 12 Mar 2026

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-SA 4.0 - Attribution - Non-commercial use - ShareAlike - International License

SEARCHING FOR SYNERGISTIC BACTERIAL PARTNERS TO IMPROVE LENTIL FLOUR FERMENTATION BY *PLEUROTUS OSTREATUS*

Isa Hollopp¹, Eric Dugat-Bony¹, Pascal Bonnarne¹, Lukas Y. Wick², Vincent Hervé¹

¹Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood
22 Place de l’Agronomie
91120 Palaiseau
France
E-mail: isa.hollopp@inrae.fr

²Department of Applied Microbial Ecology (AME)
Helmholtz Centre for Environmental Research – UFZ
Permoserstr. 15
04318 Leipzig
Germany

KEYWORDS

Basidiomycota, bacterial-fungal interaction, pulses, Hidden Markov Models

ABSTRACT

Edible basidiomycetes, such as *Pleurotus ostreatus*, are increasingly used to ferment cereals and pulses, with the aim of improving their nutritional quality. However, naturally fermented foods commonly involve a mixed microbial community, consisting of fungi and bacteria. The fermentation process can be shaped by synergistic interactions between those microorganisms, including niche modification, cross-feeding or bacterial dispersal along fungal hyphae. Since basidiomycetes are not commonly used in food fermentation, information about their interaction with food-associated bacteria is limited. This work presents preliminary experiments aiming at identifying possible synergistic partnerships between *P. ostreatus* and food-associated bacteria, using a combination of *in silico* screening and *in vitro* co-culture on green lentil flour medium.

INTRODUCTION

Pulses, *i.e.*, the dried seed of leguminous plants (*Fabaceae*), provide a good source of plant proteins, with an average protein content ranging from 15-30% dry weight depending on the species. Additionally, pulses are rich in complex carbohydrates, fiber, minerals, and vitamins (Hall et al., 2017). However, they also contain a variety of antinutrients, including phytic acid, oxalic acid, trypsin inhibitors, lectins, and galactooligosaccharides (GOS), requiring processing prior to consumption (Kumar et al., 2022). Fermentation is a cost- and energy-efficient strategy for removing antinutrients, with the added benefit of introducing new beneficial molecules and flavor compounds. While traditionally cultured for their fruiting bodies, edible members of the *Basidiomycota*, such as *Pleurotus ostreatus*, have received increased attention regarding their potential in fermenting pulses (Asensio-Grau et al., 2020; Espinosa-Páez et al., 2017). Being saprotrophic filamentous fungi growing on decaying plant material, they possess an extensive set of extracellular enzymes, some of which can target antinutrients present in pulses (Hu et al., 2016; Sánchez-García et al., 2022) or result in the release of pleasant aromatic volatile compounds (Lomascolo et al., 1999). Synergistic microbe-microbe interactions, such as cross-feeding, are often crucial for food

fermentation processes (Driessen et al., 1982), but are currently understudied with regard to edible basidiomycetes. This study aims to identify bacterial candidates that engage in synergistic interactions with *P. ostreatus* to create novel lentil-based fermented food products with improved nutritional and organoleptic properties.

MATERIAL AND METHODS

In Silico Screen of Target Functions

Bacterial genomes were collected from previous sequencing projects or retrieved from NCBI (Benson et al., 2013; see Web References). Contamination and completeness of the genomes were estimated with CheckM2 v1.1.0 (Chklovski et al., 2023). Eight Hidden Markov Models (HMM) were collected from the KOfam database (Aramaki et al., 2020) and queried against the 41 genomes using *hmmsearch* from HMMER v.3.4 (Eddy, 2011) (Table 1). Genomes were ranked by the number of positive hits across all queried HMMs. This allowed to select six strains across the three represented phyla (*Actinomycetota*, *Bacillota* and *Pseudomonadota*), so that each target function was at least covered once.

Table 1: Hidden Markov Models collected from KOfam Database used to screen for Target Functions in Bacteria

KEGG	Protein	Description	Threshold
K08177	OxlT	MFS transporter, OFA family, oxalate/formate antiporter	229.83
K01577	Oxc	oxalyl-CoA decarboxylase	738.93
K07749	Frc	formyl-CoA transferase	429.73
K01093	AppA	4-phytase/acid phosphatase	375.03
K01083		3-phytase	155.3
K07407	GalA/RafA	alpha-galactosidase	68
K07406	MelA	alpha-galactosidase	359.5
K01505	AcdS	1-aminocyclopropane-1-carboxylate deaminase	373.43

Cultivation of Fungal and Bacterial Strains

Pleurotus ostreatus DSM11191 was routinely cultivated on malt agar at 25°C under aerobic conditions. One week prior to confrontation assays, a one-cm disk taken from the outer edges of the growing mycelium was plated on 15% green lentil medium (see below). For the bacterial inoculum used in the confrontation assays, *Bacillus subtilis* DSM10, *B. licheniformis* C03, *Brevibacterium linens* ATCC9172, *Hafnia alvei* ATCC13337 and *Pseudomonas helleri* AOP29-B2-16 were grown in tryptic soy broth overnight at 28°C with agitation at 150 rpm. *Lactobacillus acidophilus* CNRZ204 (= ATCC4356) was grown in MRS broth at 30°C without agitation. Overnight cultures were centrifuged at 4,000 × g for 5 minutes and washed twice using a 0.9% NaCl solution. The pellet was resuspended in 0.9% NaCl solution and the inoculum volume was calculated using previously established OD (optical density at 600 nm) to CFU (colony-forming unit) conversion.

In Vitro Bacterial-Fungal Confrontations

Green lentil flour was mixed with Milli-Q water to a final concentration of 15% (w/w) flour in water and stirred until homogeneous. The medium was autoclaved at 121°C for 20 minutes. Once the medium was cold enough to handle, it was homogenized using a T25 digital ULTRA-TURRAX® equipped with the S25N-25F Dispersion tool (IKA®-Werke, Germany) at medium-low speed. Using a sterile spoon, 25 g of medium was distributed into 60 ml crystallizing dishes. Bacteria were inoculated at a final concentration of 10⁶ cells per gram medium. A one-cm disk taken from the outer edges of the mycelium pre-culture was placed in the center of the dish. The dishes were incubated at 25°C in the dark for 14 days. A dish of water was placed in the incubator to prevent the medium from drying out.

RESULTS

Genome Screening of Targeted Bacterial Functions

A total of 41 bacterial genomes from an in-house strain collection were selected to assess their functional potential *in silico* using Hidden Markov Models (HMM). The selected strains were isolated from fermented foods or type strains of species known to be associated with fermented foods. The selection contained 18 *Actinomycetota*, 15 *Bacillota* and eight *Pseudomonadota*; it was composed of strains with known functional roles during fermentation as well as spoilage bacteria. Target functions (8 HMMs, Table 1) included oxalotrophy, phytic acid degradation and GOS degradation, covering important functions related to the removal of antinutrients present in the flour medium or produced by the fungus. The enzyme degrading the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), ACC deaminase, was included since ACC-degrading bacteria can have a growth-promoting effect on fungal hyphae (Chen et al., 2013).

A total of 28 strains contained at least one of the eight targeted proteins. The majority of strains had a positive hit for the oxalate transporter (OxIT), but only *Hafnia alvei* ATCC13337 and *Lactobacillus acidophilus* CNRZ204 had positive hits for oxalyl-CoA decarboxylase (Oxc) and formyl-CoA transferase

(Fre), required for oxalate degradation. Interestingly, *L. acidophilus* CNRZ204 did not match for OxIT, while the strain has shown to degrade oxalic acid *in vitro* (Karamad et al., 2020). Positive hits for ACC deaminase (AcdS) were identified in all tested strains belonging to the genus *Brevibacterium* (*B. aurantiacum* and *B. linens*). For *Pseudomonas fluorescens* ATCC13525 and *P. helleri* AOP29-B2-16, the HMM used did not match for AcdS, but both strains carry another *acdS* gene and *Pseudomonas* spp. are known to express ACC deaminase (Chen et al., 2013; Nascimento et al., 2014). Regarding α -galactosidase, MeIA was detected in *Bacillus* spp. and *Staphylococcus equorum* CIP103502, while GalA/RafA was detected in members of the lactic acid bacteria and *Micrococcales* (*Microbacterium* spp. and *Brachybacterium tyrofermentans* ATCC700068) as well as *H. alvei* CIRM-BIA2718. *Bacillus subtilis* strains and *B. licheniformis* C03 had positive HMM hits for 3-phytase, while *H. alvei* strains and *P. helleri* AOP29-B2-16 had a positive hit for 4-phytase. Based on these findings, six strains with different functional profiles were selected to assess their impact on *Pleurotus ostreatus* DSM11191 growth *in vitro* on lentil-flour medium.

Effects of Bacterial-Fungal Co-Cultivation on Hyphal Growth

Six bacterial strains, predicted to degrade oxalic acid, phytic acid, GOS or ACC, were grown on solid lentil flour medium in co-culture with *Pleurotus ostreatus* DSM11191 to assess their impact on fungal growth. When the fungus was inoculated without any bacteria, it covered the surface of the dish after seven days, in a dense layer of mycelium growing in concentric ripples (Figure 1, 'F'-column). All six bacterial strains successfully colonized the medium, as evidenced by a change in color and texture of the medium, as well as an impact on fungal growth (Figure 1, 'NF'-column). *Bacillus subtilis* DSM10 completely inhibited mycelium extension. *Bacillus licheniformis* C03 did not inhibit fungal growth as dramatically, as the mycelium covered almost the entire surface of the dish after seven days of fermentation. However, the hyphae grew less densely compared to the control. *Brevibacterium linens* ATCC9172, *Hafnia alvei* ATCC13337 and *P. helleri* AOP29-B2-16 initially affected fungal growth in a similar manner. After seven days, hyphal extension was slowed down compared to the control, but the mycelium was dense, although strain-specific effects on mycelial morphology could be observed. After 14 days, the mycelium reached the edges of the dish when co-cultured with *H. alvei* ATCC13337 and *P. helleri* AOP29-B2-16, but not with *B. linens* ATCC9172. *Lactobacillus acidophilus* CNRZ204 was the only bacterial strain showing a growth-promoting effect on fungal mycelium. The hyphae extended in dense, evenly concentric ripples and covered the dish surface after seven days, similar to the control. After 14 days, orange droplets started to appear on the mycelium surface.

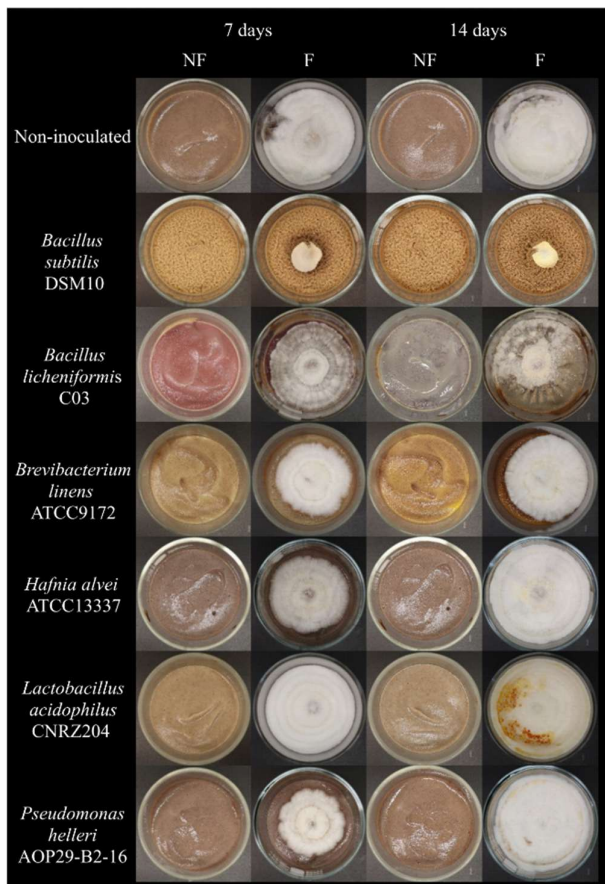


Figure 1: Bacterial-Fungal Co-Cultures on Lentil Flour Medium after seven and 14 days at 25°C. NF: Control without Fungus, F: *Pleurotus ostreatus* DSM11191

DISCUSSION

The HMM analysis identified a range of bacterial strains with target functions related to the degradation of antinutrients and the promotion of fungal growth. However, all but one of the six bacterial strains impacted hyphal growth *in vitro* negatively. The interaction between fungi and bacteria are highly strain and growth-condition dependent. For example, while *B. subtilis* strongly inhibited the growth of *P. ostreatus* in this study, another *B. subtilis* strain had a growth-promoting effect on *Aspergillus nidulans* through the action of thiamine (Abeyasinghe et al., 2020). *Bacillus velezensis*, known for its antifungal activity, enters in a mutualistic relationship with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (Anckaert et al., 2024), while its antagonistic effect on *Trichoderma guizhouense* depends on the growth environment (Xie et al., 2024). It is important to stress that the bacteria were inoculated at a relatively high load in our experiment, as done for starter culture inoculation in fermented foods production. While ultimately, the fungus succeeded in colonizing the entire surface in most conditions, only the co-culture with *L. acidophilus* CNRZ204 seemed to have a positive effect on hyphal growth. The growth-promoting effect of lactic acid bacteria on hyphal growth has been previously observed on the edible basidiomycetes *Laetiporus sulphureus* and *Wolfiporia cocos* (Todorov et al., 2024). Future work will include repeating the co-cultivation

experiments on a larger scale, collecting additional data such as pH, bacterial viable cell counts, fungal biomass, and the production of volatile organic compounds. Furthermore, changes in antinutrient composition will be assessed to test whether fungal-bacterial co-cultures can improve the composition of the matrix compared to the fungal strain alone.

CONCLUSION

This study identified bacteria potentially improving lentil flour fermentation via synergistic interactions with the filamentous fungus *P. ostreatus* DSM11191. The lactic acid bacterium *L. acidophilus* CNRZ204 positively impacted fungal growth. More in-depth analyses will further assess the effects of bacterial-fungal interaction on antinutrient concentrations and aroma profiles of fermented food.

ACKNOWLEDGEMENT

This work is part of the FAIROmics (FAIRification of multiOmics data to link databases and create knowledge graphs for fermented foods) project, funded by the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 101120449.

REFERENCES

- Abeyasinghe, G., Kuchira, M., Kudo, G., Masuo, S., Ninomiya, A., Takahashi, K., Utada, A. S., Hagiwara, D., Nomura, N., Takaya, N., Obana, N., & Takeshita, N. (2020). Fungal mycelia and bacterial thiamine establish a mutualistic growth mechanism. *Life Science Alliance*, 3(12), e202000878.
- Anckaert, A., Declerck, S., Poussart, L.-A., Lambert, S., Helmus, C., Boubsi, F., Steels, S., Argüelles-Arias, A., Calonne-Salmon, M., & Ongena, M. (2024). The biology and chemistry of a mutualism between a soil bacterium and a mycorrhizal fungus. *Current Biology*, 34(21), 4934–4950.
- Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., & Ogata, H. (2020). KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics*, 36(7), 2251–2252.
- Asensio-Grau, A., Calvo-Lerma, J., Heredia, A., & Andrés, A. (2020). Enhancing the nutritional profile and digestibility of lentil flour by solid state fermentation with *Pleurotus ostreatus*. *Food & Function*, 11(9), 7905–7912.
- Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. *Nucleic Acids Research*, 41(D1), D36–D42.
- Chen, S., Qiu, C., Huang, T., Zhou, W., Qi, Y., Gao, Y., Shen, J., & Qiu, L. (2013). Effect of 1-aminocyclopropane-1-carboxylic acid deaminase producing bacteria on the hyphal growth and primordium initiation of *Agaricus bisporus*. *Fungal Ecology*, 6(1), 110–118.
- Chklovski, A., Parks, D. H., Woodcroft, B. J., & Tyson, G. W. (2023). CheckM2: A rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nature Methods*, 20(8), 1203–1212.
- Driessen, F., Kingma, F., & Stadhouders, J. (1982). Evidence that *Lactobacillus bulgaricus* in yogurt is stimulated by

- carbon dioxide produced by *Streptococcus thermophilus*. *Netherlands Milk and Dairy Journal*, 36(2), 135–144.
- Eddy, S. R. (2011). Accelerated Profile HMM Searches. *PLOS Computational Biology*, 7(10), e1002195.
- Espinosa-Páez, E., Alanís-Guzmán, M. G., Hernández-Luna, C. E., Báez-González, J. G., Amaya-Guerra, C. A., & Andrés-Grau, A. M. (2017). Increasing Antioxidant Activity and Protein Digestibility in *Phaseolus vulgaris* and *Avena sativa* by Fermentation with the *Pleurotus ostreatus* Fungus. *Molecules*, 22(12), 2275.
- Hall, C., Hillen, C., & Garden Robinson, J. (2017). Composition, Nutritional Value, and Health Benefits of Pulses. *Cereal Chemistry*, 94(1), 11–31.
- Hu, Y., Tian, G., Geng, X., Zhang, W., Zhao, L., Wang, H., & Ng, T. B. (2016). A protease-resistant α -galactosidase from *Pleurotus citrinopileatus* with broad substrate specificity and good hydrolytic activity on raffinose family oligosaccharides. *Process Biochemistry*, 51(4), 491–499.
- Karamad, D., Khosravi-Darani, K., Hosseini, H., Tavasoli, S., & Miller, A. W. (2020). Assessment of the Process Variables for Degradation of Oxalate by *Lactobacillus acidophilus* ATCC 4356 Using Simulated Rumen Fluid Media and Tea. *Applied Food Biotechnology*, 7(4), 195–204.
- Kumar, Y., Basu, S., Goswami, D., Devi, M., Shivhare, U. S., & Vishwakarma, R. K. (2022). Anti-nutritional compounds in pulses: Implications and alleviation methods. *Legume Science*, 4(2), e111.
- Lomascolo, A., Stentelaire, C., Asther, M., & Lesage-Meessen, L. (1999). Basidiomycetes as new biotechnological tools to generate natural aromatic flavours for the food industry. *Trends in Biotechnology*, 17(7), 282–289.
- Nascimento, F. X., Rossi, M. J., Soares, C. R. F. S., McConkey, B. J., & Glick, B. R. (2014). New Insights into 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Phylogeny, Evolution and Ecological Significance. *PLOS ONE*, 9(6), e99168.
- Sánchez-García, J., Asensio-Grau, A., García-Hernández, J., Heredia, A., & Andrés, A. (2022). Nutritional and antioxidant changes in lentils and quinoa through fungal solid-state fermentation with *Pleurotus ostreatus*. *Bioresources and Bioprocessing*, 9(1), 51.
- Todorov, S. K., Orban, A., Hammer, A., Oberpaul, M., Back, C., Jansen, C. L., Hobley, T. J., Rühl, M., & Bang-Berthelsen, C. H. (2024). Interactions between two strains of lactic acid bacteria and *Laetiporus sulphureus* strain FH24 and FH319, and *Wolfiporia cocos* strain FH9 mycelium. *LWT*, 197, 115891.
- Xie, J., Sun, X., Xia, Y., Tao, L., Tan, T., Zhang, N., Xun, W., Zhang, R., Kovács, Á. T., Xu, Z., & Shen, Q. (2024). Bridging the Gap: Biofilm-mediated establishment of *Bacillus velezensis* on *Trichoderma guizhouense* mycelia. *Biofilm*, 8, 100239.

WEB REFERENCES

www.ncbi.nlm.nih.gov/datasets/genome/