

PHD PROPOSAL FOR THE DOCTORAL SCHOOL

« Ecologie, Géosciences, Agronomie, Alimentation »

GENERAL INFORMATION

Thesis title: Action mechanisms of lactic acid bacteria bioprotective cultures against spoilage fungi in fermented dairy products
Acronym: TACTICIAN
Disciplinary field 1: Food sciences Disciplinary field 2: Ecology
Three keywords fungi; lactic acid bacteria; bioprotection
Research unit : LUBEM UR 3382
Name of the thesis director: Jérôme Mounier Email address of the thesis director : jerome.mounier@univ-brest.fr Name of the thesis co-supervisor 1 (if applicable): Emmanuel Coton Email address of the thesis co-supervisor 1 (if applicable): emmanuel.coton@univ-brest.fr Name of the thesis co-supervisor 2 (if applicable): Florence Valence Email address of the thesis co-supervisor 2 (if applicable): florence.valence-bertel@inrae.fr
Thesis grant (funding origin and amount): 100 % CDE
Contact(s) (mailing address and E-mail): Technopôle Brest Iroise 29280 Plouzané, lubem@univ-brest.fr
Recruitment process: Recruitment process depends on thesis funding. To select the corresponding recruitment process, please visit the EGAAL website here . This information is needed for proposal publication. <input type="checkbox"/> Doctoral school contest <input checked="" type="checkbox"/> Interview <input type="checkbox"/> Other (indicate) :

All sections must be filled. Once filled, please save the proposal form in pdf format using the following naming: Supervisor Name_Unit_Subject Acronym_EN.pdf

ED EGAAL

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SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context

Fungal spoilage is a major concern for the dairy industry, resulting in significant food losses and waste, and substantial economic losses at both the industrial and consumer levels. At the same time, consumers are more and more demanding natural solutions to ensure food quality during storage, while simultaneously reducing food waste. Among emerging strategies, biopreservation using lactic acid bacteria (LAB) bioprotective cultures is more and more used to protect dairy products against spoilage fungi. The mechanism and mode of action of antifungal LAB are still largely unknown and a better understanding of the latter would be helpful for strain selection and for defining efficient strain combinations against 'super spoiler' fungal species and strains.

Assumptions and questions

Until recently, and the discovery that competitive exclusion for Mn was an important antifungal mechanism in several dairy lactobacilli species, production of antifungal metabolites, acting by additive effects or in synergy, and pH decrease, were believed to be the main mechanisms involved in antifungal LAB activity. Nevertheless, it is still not perfectly clear how antifungal molecule synthesis and competition-exclusion interact together in different fungal species with various degree of resistance to bioprotective LAB. Thus, to better understand the mechanism and mode of action of antifungal LAB, in depth studies on selected antifungal LAB are necessary.

The PhD project aims to answer the following scientific questions:

- What are the key genes/metabolic pathways involved in the activity of selected antifungal LAB?
- What are the mechanisms involved in their antifungal activity?
- How do fungi respond to antifungal LAB?

The main steps of the thesis and scientific procedure & Methodological and technical approaches considered

i- Identification of key genes/pathways involved in LAB antifungal activity:

The first part will aim at identifying key genes/metabolic pathways i) encoding for the production of antifungal molecules well known (organic acids, fatty acids and volatile compounds) or suspected (short cyclic poly lactate, spermine) and for their antifungal activities and ii) involved in competition-exclusion phenomena (trace metals, vitamins) in dairy matrices. To reach this goal, genome study of antifungal LAB will be performed using bioinformatics (comparison with genome databank, antismash).

ii- Study of the action mechanisms involved in LAB antifungal activity:

The objective will be to identify the action mechanisms involved in LAB antifungal activity and their respective role in the observed antifungal activity in different fungi at the inter- and intraspecific level. The methodological and technical approach will consist in :

- Metabolite profiling of antifungal LAB in dairy matrices (GC-MS/ LC Q ToF, Nano-LC-MS)
- Utilization profile of trace-elements (metals, vitamins) of antifungal LAB and fungal targets in dairy matrices
- In vitro trials to validate hypotheses concerning action mechanism based on trace-elements and metabolite profiling / structure-function analysis

iii- Study of the response mechanism of fungi:

In this last part, the objective will be to investigate the physiological and metabolic responses of fungi to antifungal LAB using flow cytometry/microscopy as well as a transcriptomic approach.

Scientific and technical skills required by the candidate

The candidate will be graduated from an engineering school or hold a master 2 degree, with a 6-month internship related to microbiology or microbial ecology. The candidate must have skills in molecular biology and microbiology. He/She must show ability to take initiative and work independently as well as in a team environment.

THESIS SUPERVISION¹

Unit name: LUBEM UR 3882	Team name: LUBEM site de Plouzané
Unit director name: Prof. Emmanuel Coton	Team director name: Prof. Jérôme Mounier
Mailing address of the unit director: emmanuel.coton@univ-brest.fr	Mailing address of the team director: jerome.mounier@univ-brest.fr
<p>Thesis director</p> <p>Surname, first name: Mounier, Jérôme</p> <p>Position: Professor</p> <p>Obtained date of the HDR (Habilitation thesis to supervise research): 12/2012</p> <p>Employer: UBO</p> <p>Doctoral school affiliation: ED EGAAL</p> <p>Rate of thesis supervision in the present project (%): 40%</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 140%</p> <p>Number of current thesis supervisions/co-supervisions: 3</p>	
<p>Thesis co-supervisor 1 (if applicable)</p> <p>Surname, first name: Coton, Emmanuel</p> <p>Position: Professor</p> <p>Habilitation thesis to supervise research <input checked="" type="checkbox"/> yes <input type="checkbox"/> no If yes, date diploma received: 22/12/2008</p> <p>Employer: UBO</p> <p>Doctoral school affiliation: ED EGAAL</p> <p>Rate of thesis supervision in the present project (%): 30%</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 130%</p> <p>Number of current thesis supervisions/co-supervisions: 3</p>	

¹ In EGAAL Doctoral School, if only one scientist in thesis supervision = 100% of supervision rate; if 2 people involved in thesis supervision = from 50% to 70% of supervision rate for the director; if 3 people involved in thesis supervision = 40% / 30% / 30% of supervision rate distribution among supervisors.

Thesis co-supervisor 2 (if applicable)

Surname, first name: Valence, Florence

Position: Research Engineer

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer: INRAE

Doctoral school affiliation: EGAAL

Rate of thesis supervision in the present project (%): 30 %

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 30%

Number of current thesis supervisions/co-supervisions: 1

Private partner (if CIFRE funding, private funding,...)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

International partner (if Cotutelle thesis)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)

Please provide the following information for each PhD students supervised

Surname, first name: Franciosa Irene

Date of PhD beginning and PhD defence: 01/09/2017- 01/03/2021

Thesis supervision: Jérôme Mounier (50%)/Luca Cocolin (50%)

Professional status and location: Post-doc, Université de Turin

Contract profile (post-doc, fixed-term, permanent): post-doc CDD

List of publications from the thesis work:

1. Franciosa, I., Alessandria, V., Dolci, P., Rantsiou, K., & Cocolin, L. Sausage fermentation and starter cultures in the era of molecular biology methods. *Int J Food Microbiol.* 2018, 279, 26-32.
2. Franciosa, I., Coton, M., Ferrocino, I., Corvaglia, M. R., Poirier, E., Jany, J. L., ... & Mounier, J. Mycobiota dynamics and mycotoxin detection in PGI Salame Piemonte. *J Appl Microbiol.* 131, 2336-2350.
3. Franciosa I., Ferrocino I., Giordano M., Mounier J., Rantsiou K., Cocolin C. Specific metagenomic asset drives the spontaneous fermentation of Italian sausages. *Food Res Int*, 110379, 20121.

Surname, first name: Quéro Laura

Date of PhD beginning and PhD defence: 01/12/2015- 21/12/2018

Thesis supervision: Jérôme Mounier

Professional status and location: Ingénieur Application, bioMérieux, Rennes

Contract profile (post-doc, fixed-term, permanent): CDI

List of publications from the thesis work:

1. Quéro L, Courault P, Cellière B, Lorber S, Jany JL, Puel O, Girard V, Vasseur V, Nodet P, Mounier J. Application of MALDI-TOF MS to species complex differentiation and strain typing of food related fungi: Case studies with *Aspergillus* section *Flavi* species and *Penicillium roqueforti* isolates. *Food Microbiol.* 2020. 86:103311.
2. Quéro L, Girard V, Pawtowski A, Tréguer S, Weill A, Arend S, Cellière B, Polsinelli S, Monnin V, van Belkum A, Vasseur V, Nodet P, Mounier J. Development and application of MALDI-TOF MS for identification of food spoilage fungi. *Food Microbiol.* 2019. 81:76-88.

Surname, first name: Nguyen Van Long, Nicolas

Date of PhD beginning and PhD defence: 01/10/2014- 11/10/2017

Thesis supervision: Jérôme Mounier

Professional status and location: Chef de projet, ADRIA Quimper

Contract profile (post-doc, fixed-term, permanent): CDI

List of publications from the thesis work:

1. Nguyen Van Long, N., Rigalma, K., Coroller, L., Dadure, R., Debaets, S., Mounier, J., Vasseur, V. Modelling the effect of water activity reduction by sodium chloride or glycerol on conidial germination and radial growth of filamentous fungi encountered in dairy foods. *Food Microbiol.* 2017. 68: 7-15.
2. Nguyen Van Long, N.N., Vasseur, V., Couvert, O., Coroller, L., Burlot, M., Rigalma, K., Mounier, J. Modeling the effect of modified atmospheres on conidial germination of fungi from dairy foods. 2017. *Frontiers in Microbiol.*, 8: art. no. 2109. 13.
3. Nguyen Van Long N, Vasseur V, Coroller L, Dantigny P, Le Panse S, Weill A, Mounier J, Rigalma K. Temperature, water activity and pH during conidia production affect the physiological state and germination time of *Penicillium* species. *Int J Food Microbiol.* 2017. 241: 151-160.

Surname, first name: Garnier, Lucille

Date of PhD beginning and PhD defence: 01/10/2014- 2/10/2017

Thesis supervision: Jérôme Mounier / Florence Valence

Professional status and location: Coordinatrice pédagogique, Dax

Contract profile (post-doc, fixed-term, permanent): CDI

List of publications from the thesis work:

1. Garnier L, Salas ML, Pinon N, Wiernasz N, Pawtowski A, Coton E, Mounier J, Valence F. Technical note: High-throughput method for antifungal activity screening in a cheese-mimicking model. *J Dairy Sci.* 2018. pii: S0022-0302(18)30275-3.
2. Garnier, L., Valence, F., Mounier, J. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. *Microorganisms.* 2017. 5: 42-52.
3. Garnier L, Valence F, Pawtowski A, Auhustsinava-Galerie L, Frotté N, Baroncelli R, Deniel F, Coton E, Mounier J. Diversity of spoilage fungi associated with various French dairy products. *Int J Food Microbiol.* 2017. 241: 191-197.
4. Coton, M., Lebreton, M., Leyva Salas, M., Garnier, L., Navarri, M., Pawtowski, A., Le Blay, G., Valence, F., Coton, E., Mounier, J. Biogenic amine and antibiotic resistance profiles determined for lactic acid bacteria and a propionibacterium prior to use as antifungal bioprotective cultures *Int Dairy J.* 2018. 1: 21-26.
5. Garnier L, Mounier J, Lê S, Pawtowski A, Pinon N, Camier B, Chatel M, Garric G, Thierry A, Coton E, Valence F. Development of antifungal ingredients for dairy products: From in vitro screening to pilot scale application. *Food Microbiol.* 2019.81:97-107.
6. Péter G, Mounier J, Garnier L, Soós D, Dlauchy D. *Cutaneotrichosporon suis* sp. nov., a lipolytic yeast species from food and food-related environment. *Int J Syst Evol Microbiol.* 2019. 69:2367-2371.
7. Garnier, L., Penland, M., Thierry, A., Maillard, M. B., Jardin, J., Coton, M., ... & Mounier, J. (2020). Antifungal activity of fermented dairy ingredients: identification of antifungal compounds. *Int J Food Microbiol*, 322, 108574.

Surname, first name: VISCONTI Vincent

Date of PhD beginning and PhD defence: du 02/05/2018 au 17/12/2021

Thesis supervision: DANTIGNY Philippe/ RIGALMA Karim/COTON Emmanuel

Professional status and location: Formation enseignant

Contract profile (post-doc, fixed-term, permanent):

Liste des publications issues de ce travail de thèse :

- Visconti V, Rigalma K, Coton E, Dantigny P. 2020. Impact of intraspecific variability and physiological state on *Penicillium commune* inactivation by 70% ethanol. *Int J Food Microbiol.* 332:108782
- Visconti V, Rigalma K, Coton E, Dantigny P. 2021. Impact of the physiological state of fungal spores on their inactivation by active chlorine and hydrogen peroxide. *Food Microbiol.* 100:103850
- Visconti V, Rigalma K, Coton E, Dantigny P. 2022. Impact of temperature application and concentration of commercial sanitizers on inactivation of food-plant fungal spores. *Int J Food Microbiol.* 2022 366:109560

Surname, first name: SAVARY Océane

Date of PhD beginning and PhD defence: du 01/10/2018 au 16/12/2021

Thesis supervision: COTON Emmanuel / JANY Jean-LUC/ COTON Monika

Professional status and location: Recherche d'emploi

Contract profile (post-doc, fixed-term, permanent):

Liste des publications issues de ce travail de thèse :

- Savary O, Coton M, Jany JL, Coroller L, Coton E. *Int J Food Microbiol.* 2022 Effect of abiotic factors and culture media on the growth of cheese-associated Nectriaceae species. *Mar* 2;364:109509. doi: 10.1016/j.ijfoodmicro.2021.109509
- Savary O, Coton M, Frisvad JC, Nodet P, Ropars J, Coton E, Jany J-L (2021) Unexpected Nectriaceae species diversity in cheese, description of *Bisifusarium allantoides* sp. nov., *Bisifusarium penicilloides* sp. nov., *Longinectria* gen. nov.

lagenoides sp. nov. and *Longinectria verticilliforme* sp. nov. Mycosphere 12(1): 1077–1100 Doi 10.5943/mycosphere/12/1/13

Surname, first name: LEYVA-SALAS Marcia

Date of PhD beginning and PhD defence: du 01/11/2015 au 06/11/2018

Thesis supervision: COTON Emmanuel / THIERRY Anne

Professional status and location: Enseignante-chercheure ENSAIA Nancy

Contract profile (post-doc, fixed-term, permanent): CDI

Liste des publications issues de ce travail de thèse :

-Leyva Salas M, Mounier J, Maillard MB, Valence F, Coton E, Thierry A. Identification and quantification of natural compounds produced by antifungal bioprotective cultures in dairy products. Food Chem. 2019 301:125260. doi: 10.1016/j.foodchem.2019.125260. Epub 2019 Jul 25.

-Ouiddir M, Bettache G, Leyva Salas M, Pawtowski A, Donot C, Brahimi S, Mabrouk K, Coton E, Mounier J. Selection of Algerian lactic acid bacteria for use as antifungal bioprotective cultures and application in dairy and bakery products. Food Microbiol. 2019 82:160-170. doi: 10.1016/j.fm.2019.01.020. Epub 2019 Feb 2.

-Leyva Salas M, Thierry A, Lemaître M, Garric G, Harel-Oger M, Chatel M, Lê S, Mounier J, Valence F, Coton E. Antifungal Activity of Lactic Acid Bacteria Combinations in Dairy Mimicking Models and Their Potential as Bioprotective Cultures in Pilot Scale Applications. Front Microbiol. 2018; 9:1787. doi: 10.3389/fmicb.2018.01787. eCollection 2018.

-Leyva Salas M, Mounier J, Valence F, Coton M, Thierry A, Coton E. Antifungal Microbial Agents for Food Biopreservation-A Review. Microorganisms. 2017; 5(3). pii: E37. doi: 10.3390/microorganisms5030037.

- Garnier L, Salas MI, Pinon N, Wiernasz N, Pawtowski A, Coton E, Mounier J, Valence F. Technical note: High-throughput method for antifungal activity screening in a cheese-mimicking model. J Dairy Sci. 2018; 101(6):4971-4976. doi: 10.3168/jds.2017-13518.

Surname, first name: SMITH Marie-Caroline

Date of PhD beginning and PhD defence:: du 01/10/2014 au 03/11/2017

Thesis supervision: COTON Emmanuel / HYMERY Nolwenn /MADEC Stéphanie

Professional status and location: Scientific sales representative, cell culture products chez STEMCELL

Contract profile (post-doc, fixed-term, permanent): CDD

Liste des publications issues de ce travail de thèse :

- Smith MC, Timmins-Schiffman E, Coton M, Coton E, Hymery N, Nunn BL, Madec S. Differential impacts of individual and combined exposures of deoxynivalenol and zearalenone on the HepaRG human hepatic cell proteome. J Proteomics. 2018 ; 173:89-98.

-Smith MC, Gheux A, Coton M, Madec S, Hymery N, Coton E. *In vitro* co-culture models to evaluate acute cytotoxicity of individual and combined mycotoxin exposures on Caco-2, THP-1 and HepaRG human cell lines. Chem Biol Interact. 2018 ; 281:51-59

- Smith MC, Hymery N, Troadec S, Pawtowski A, Coton E, Madec S. Hepatotoxicity of fusariotoxins, alone and in combination, towards the HepaRG human hepatocyte cell line. Food Chem Toxicol. 2017 ; 109(Pt 1):439-451

- Smith MC, Madec S, Pawtowski A, Coton E, Hymery N. Individual and combined toxicological effects of deoxynivalenol and zearalenone on human hepatocytes in *in vitro* chronic exposure conditions. Toxicol Lett. 2017 ; 280:238-24

- Smith MC, Madec S, Troadec S, Coton E, Hymery N. Effects of fusariotoxin co-exposure on THP-1 human immune cells. Cell Biol Toxicol. 2017; doi: 10.1007/s10565-017-9408-7

- Smith MC, Madec S, Coton E, Hymery N. Natural Co-Occurrence of Mycotoxins in Foods and Feeds and Their *in vitro* Combined Toxicological Effects. Toxins. 2016 ; 8(4):94.

Surname, first name: Lucas von Gastrow

Date of PhD beginning and PhD defence: Thesis supervision: 01/09/2018 – 30/11/2021

Thesis supervision: Delphine Sicard/ Florence Valence

Professional status and location: Engineer in independent brewer company, Montpellier

Contract profile (post-doc, fixed-term, permanent): CDI

List of publications from the thesis work:

1. von Gastrow L, Madec M-N, Chuat V, Lubac S, Morinière C, Lé S, Santoni S, Sicard D, Valence F. Microbial Diversity Associated with Gwell, a Traditional French Mesophilic Fermented Milk Inoculated with a Natural Starter. *Microorganisms*. 2020; 8(7):982. <https://doi.org/10.3390/microorganisms8070982>
2. von Gastrow L, Amelota R, Segonda D, Guézennec S, Valence F., Sicard D. Microbial community dispersal in sourdough. *Molecular Ecology*. In revision
1. von Gastrow L. Madec M-N., Xu Y-X, Sicard D., Valence F., Dynamics of fermented milk microbial communities under fluctuating environments. Submitted

Five main recent publications of the supervisors on thesis subject:

Garnier, L., Mounier, J., Lê, S., Pawtowski, A., Pinon, N., Camier, B., et al. (2019). Development of antifungal ingredients for dairy products: From in vitro screening to pilot scale application. *Food Microbiology* 81, 97–107. doi:10.1016/j.fm.2018.11.003.

Garnier, L., Penland, M., Thierry, A., Maillard, M.-B., Jardin, J., Coton, M., et al. (2020). Antifungal activity of fermented dairy ingredients: Identification of antifungal compounds. *International Journal of Food Microbiology* 322. doi:10.1016/j.ijfoodmicro.2020.108574.

Leyva Salas, M., Mounier, J., Maillard, M.-B., Valence, F., Coton, E., and Thierry, A. (2019). Identification and quantification of natural compounds produced by antifungal bioprotective cultures in dairy products. *Food Chemistry* 301. doi:10.1016/j.foodchem.2019.125260.

Mieszkin, S., Hymery, N., Debaets, S., Coton, E., Le Blay, G., Valence, F., et al. (2017). Action mechanisms involved in the bioprotective effect of *Lactobacillus harbinensis* K.V9.3.1.Np against *Yarrowia lipolytica* in fermented milk. *International Journal of Food Microbiology* 248, 47–55. doi:10.1016/j.ijfoodmicro.2017.02.013.

Salas, M. L., Thierry, A., Lemaître, M., Garric, G., Harel-Oger, M., Chatel, M., et al. (2018). Antifungal activity of lactic acid bacteria combinations in dairy mimicking models and their potential as bioprotective cultures in pilot scale applications. *Frontiers in Microbiology* 9. doi:10.3389/fmicb.2018.01787.

THESIS FUNDING

Origin(s) of the thesis funding: 100% CDE

Gross monthly salary: 1770 euros

Thesis funding state : Acquired

Funding beginning date/Funding ending date: November 1st 2022, 3 years

Date: September 2nd 2022

Name, signature of unit director: Emmanuel Coton



Name, signature of team director: Jérôme Mounier

A handwritten signature in black ink, appearing to read 'Mounier', written in a cursive style.

Name, signature of thesis project director: Jérôme Mounier

A handwritten signature in black ink, appearing to read 'Mounier', written in a cursive style.