DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Ecologie, Géosciences, Agronomie, ALimentation »

GENERAL INFORMATION

Thesis title: Champagne grape microbiota contribution to fresh mushroom off-flavours and aroma quality
Acronym: MAGNUM (Microbiota ACF on Grapes and Natural Useful Microbiota)
Disciplinary field 1: Food sciences **Disciplinary field 2:** Ecology
Three keywords: Grape microbiota, volatile compounds, fungal interactions
Research unit : LUBEM UR3882
Name of the thesis director: COTON Monika **Email address of the thesis director :** monika.coton@univ-brest.fr **Name of the thesis co-supervisor 1 (if applicable): PICOT Adeline** **Email address of the thesis co-supervisor 1 (if applicable):** adeline.picot@univ-brest.fr **Name of the thesis co-supervisor 2 (if applicable): PENSEC Flora** **Email address of the thesis co-supervisor 2 (if applicable):** flora.pensec@univ-brest.fr
Thesis grant (funding origin and amount): 100% CIFRE
Contact(s) (mailing address and E-mail): Laboratoire de Biodiversité et Écologie Microbienne (EA 3882), Parvis Blaise Pascal, Technopole Brest-Iroise, 29280 Plouzané. monika.coton@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. ☐ **Doctoral school contest** ☐ **Interview** ☒ **Other (indicate) :** **Application submission before July 18th** **Scientific and technical interviews From July 24th – by visioconference** **Interview with MCHS Human Resources From August 3rd – on-site**

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

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DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context:

Current changes in climate and agricultural practices have impacted the frequency and intensity of fungal contaminations in vineyards. This shift in microbiota may lead to organoleptic defects that appear after fermentation and for specific vintages. One example is the so-called “fresh mushroom” off-flavours that have been observed over the past ten years. This sensorial defect is not detected on berries or in must but rather appears in the months following the end of the fermentation. Professionals in the wine industry hypothesize that this fresh mushroom off-flavour is related to hot and warm vintages and the macroscopic status of grape berries.

In spite of this, no scientific study has yet been undertaken to compare the microbiota of grape berries from « healthy » and « sensitive » vineyards. The objective of this PhD project thus aims at characterizing the diversity and composition of Champagne grape associated microbiota from these different vineyards and pinpointing its potential role in the fresh mushroom defect in wines.

Assumptions and questions:

The Champagne wine-growing region is located in Northern France and benefits from both oceanic and continental climatic influences. The grapes in these vineyards grow in both wet and extreme temperature conditions. The main grape varieties are Pinot Noir, Meunier and Chardonnay. This diversity, together with the diversified terroirs encountered, contribute to the typical and characteristic wines produced here.

In Champagne, like other vineyards across France, wine quality is highly linked to the characteristics of the raw materials used. The influence of pedoclimatic conditions is already well followed and documented, however, the Champagne grape microbiota has yet to be fully characterized and described.

In this context, the first objective of this study is to dynamically identify and compare the microbiota associated with the main stages of grape development on berries from « healthy » versus « sensitive » vineyards used for Champagne production. These « sensitive » vineyards have been previously linked to undesirable fresh mushroom off-flavours after fermentation. To do so, complementary culture-dependent and -independent approaches will be used. Global data analyses will be carried out by combining the obtained results with all available data related to climatic conditions and vine and grape growing practices to determine which factors might impact the microbial ecosystem. In parallel, microbial co-occurrence networks and patterns will be explored using metagenetics data. This should provide deeper understanding about species interactions within the different vineyards and potentially linked to undesirable off-flavours and point out potential « suppressive » species that may prevent unwanted fungal species development. The « suppressive » effect will also be studied using small scale biological tests directly on the matrix. Finally, fungal development, for a selection of strains and species, will be evaluated by testing multiple abiotic factors linked to typical climatic conditions using a high throughput laser nephelometry method. Off-flavour production will also be monitored at key time points for the same samples using biochemical methods. Using the results obtained, cardinal growth values will be calculated using secondary growth models. Overall, these results will provide novel information about the most favorable conditions leading to the described undesirable off-flavours.

The main steps of the thesis and scientific procedure:

Task 1: ***Microbiota of Champagne grape berries.*** This task will focus on dynamically characterizing the Champagne related microbiota of grape berries from different vineyards described as “healthy” or “sensitive to off-flavours” using culture-dependent and -independent (metagenetics) approaches. According to the identified fungal species diversity, those capable of producing undesirable fresh mushroom off-flavours will be determined and conserved.

Task 2: ***Interactions study.*** Possible key species interactions, potentially linked to off-flavour development, will be studied by analyzing co-occurrence networks and patterns between samples based on the obtained metagenetics data. This analysis should enable us to pinpoint the key taxa that negatively or positively impact off-flavour

development. Small scale biological tests will also be carried out to confirm the potential "suppressive" effect by inoculating microorganism(s) individually or in combinations in the presence of the unwanted fungal species.

Task 3: ***Physiological study and conditions necessary for off-flavour production.*** Growth of key fungal species, individually or combined, and involved in the production of undesirable off-flavours will be determined by varying abiotic conditions mimicking climate change. A risk prediction model for growth and off-flavour production will be proposed.

Methodological and technical approaches considered

Task 1: A minimum of 12 vineyards will be included to study berry microbiota dynamics using both metagenetic and culture-dependent analyses. Sampling will be performed during 6 key stages of berry development (pre- and post-harvest). A working collection of fungal strains of interest will also be created. The ability of a preselected number of these strains and species to produce undesirable volatile compounds leading to fresh mushroom off-flavours will be determined using biochemical techniques.

Task 2: Comparison of co-occurrence networks, constructed using metagenetics data, between sensitive and non-sensitive vineyards to spoilage, will be performed to pinpoint putative taxa positively and negatively contributing to this defect. To validate whether these taxa can suppress spoilage, small scale biological tests will be set up based on confrontation between a selection of spoilage fungal strains and putative suppressive microorganisms, covering a large range of phenotypic and/or genetic diversity. Fungal growth will be monitored by qPCR to demonstrate whether these taxa of interest reduce the target fungal growth.

Task 3: A high through-put screening method based on laser nephelometry will be used to follow fungal growth, for preselected strains, according to various abiotic factors. Primary growth models will first be used to calculate growth rates while cardinal growth values will be obtained with a secondary model. These data will be correlated to the ability of strains to produce undesirable volatile compounds, under the same conditions. Altogether, a risk prediction model estimating the likelihood of growth and production of undesirable volatile compounds will be proposed.

Scientific and technical skills required by the candidate

The PhD candidate requirements:

- Master's Degree or equivalent Degree **in microbiology, molecular biology,** and have experience in data analyses related to their specialty (culture-dependent and –independent approaches, PCR, metagenetics, statistics...)
- Good lab experience with the ability to work autonomously and learn new techniques
- **Well-organized, rigorous and a good team player**, the candidate will be required to work with a team and do regular progress reports
- Be capable of adapting and simplifying a scientific discussion according to the context
- Good communication and written skills (protocols, reports, synthesis…), good experience with Office Pack and statistical analyses
- **Fluency in English** (both spoken and written) and be able to present their research in International conferences
- **An interest for wine science**, fermented beverages and biotechnology as well as experience in biochemistry is an asset.

The candidate should also be interested in **hands-on work in a winery setting** and will be required to be on-site during the pre-harvest and harvest periods (3 months/yr).

DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

THESIS SUPERVISION[1]

Unit name: Laboratoire de Biodiversité et Écologie Microbienne (EA 3882)	**Team name:** Ecosystèmes à composante fongique
Unit director name: COTON Emmanuel	**Team director name:** MOUNIER Jérôme
Mailing address of the unit director: emmanuel.coton@univ-brest.fr	**Mailing address of the team director:** jerome.mounier@univ-brest.fr

Thesis director

Surname, first name: **COTON Monika**

Position: **Assistant professor**

Obtained date of the HDR (Habilitation thesis to supervise research): **2012**

Employer: **UBO**

Doctoral school affiliation: **ED EGAAL**

Rate of thesis supervision in the present project (%): **40**

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): **80**

Number of current thesis supervisions/co-supervisions: **2**

Thesis co-supervisor 1 (if applicable)

Surname, first name: **PICOT Adeline**

Position: **Assistant professor**

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

Employer: **UBO**

Doctoral school affiliation: **ED 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**

Thesis co-supervisor 2 (if applicable)

Surname, first name: **PENSEC Flora**

Position: **Assistant professor**

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

[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.**

DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

Employer: **UBO**

Doctoral school affiliation: **ED 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: **BREVOT Marc – LE GUILLOU Romain - HERVE Marion**

Position: **Directeur R&D – Directeur Vignoble - Chef de projet R&D**

Employer: **MHCS**

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: **GILLOT Guillaume**

Date of PhD beginning and PhD defence: **01.10.12-30.09.15**

Thesis supervision: **Emmanuel COTON, co-supervision Monika COTON et Jean-Luc JANY**

Professional status and location: **Project Manager, ADRIA Développement, QUIMPER**

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

List of publications from the thesis work:

1. Gillot, G., Jany, J. L., Dominguez-Santos, R., Poirier, E., Debaets, S., Hidalgo, P. I., Ullan, R. V., Coton, E., & Coton, M. (2017). Genetic basis for mycophenolic acid production and strain-dependent production variability in *Penicillium roqueforti*. Food Microbiol, 62, 239-250.
2. Gillot, G., Jany, J. L., Poirier, E., Maillard, M. B., Debaets, S., Thierry, A., Coton, E., & Coton, M. (2017). Functional diversity within the *Penicillium roqueforti* species. Int J Food Microbiol, 241, 141-150.
3. Gillot, G., Jany, J. L., Coton, M., Le Floch, G., Debaets, S., Ropars, J., Lopez-Villavicencio, M., Dupont, J., Branca, A., Giraud, T., & Coton, E. (2015). Insights into *Penicillium roqueforti* Morphological and Genetic Diversity. PLoS One, 10, e0129849.
4. Ropars, J., Lopez-Villavicencio, M., Dupont, J., Snirc, A., Gillot, G., Coton, M., Jany, J. L., Coton, E., & Giraud, T. (2014). Induction of sexual reproduction and genetic diversity in the cheese fungus *Penicillium roqueforti*. Evol Appl, 7, 433-441.

Surname, first name: **LEGRAND Fabienne**

Date of PhD beginning and PhD defence: **01.05.2014 – 16.10.2017**

Thesis supervision: **Gaétan LE FLOCH, co-encadrante Adeline PICOT**

Professional status and location: **ANSES, Paris**

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

DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

Liste des publications issues de ce travail de thèse :

1. Legrand, F., Chen, W., Cobo-Díaz, J. F., Picot, A. and Le Floch, G. 2019. Effects of agronomic practices and soil properties in soil fungistasis against *Fusarium graminearum*. FEMS Microbiology Ecology. **95**:fiz056
2. Legrand, F., Picot, A., Cobo-Díaz, J.F., Carof M., Chen, W. and Le Floch, G. 2018 Effect of tillage and static abiotic soil properties on microbial diversity. Applied Soil Ecology 132 :135-145
3. Legrand, F., Picot, A., Cobo-Díaz, J. F., Delaunois, B., Cor, O., Barbier, G. and Le Floch, G. 2018 Development of qPCR assays to monitor the ability of *Gliocladium catenulatum* J1446 to reduce the cereal pathogen *Fusarium graminearum* inoculum in soils. European Journal of Plant Pathology 152:285–295.
4. Legrand, F., Picot, A., Cobo-Díaz, J. F., Chen, W. and Le Floch, G. 2017 Challenges facing the biological control strategies for the management of Fusarium Head Blight of cereals caused by *F. graminearum*. Biological Control 113, 26–38.

Surname, first name: **DUBRULLE Guillaume**

Date of PhD beginning and PhD defence: **01.10.2016 – 30.09.2019**

Thesis supervision: **Gaétan LE FLOCH, co-encadrantes Adeline PICOT & Flora PENSEC**
Professional status and location: **unemployed**
Contract profile (post-doc, fixed-term, permanent):

Liste des publications issues de ce travail de thèse :

1. Dubrulle, G., Pensec, F., Picot, A., Rigalma, K., Pawtowski, A., Nicolleau, S. et al. 2019. Phylogenetic diversity and effect of temperature on pathogenicity of *Colletotrichum lupini* Plant Disease 104, 938-950.

Five main recent publications of the supervisors on thesis subject:

1. Legrand F, Chen W, Cobo-Díaz Jf, Picot A, Le Floch G. (2019). Co-occurrence analysis reveal that biotic and abiotic factors influence soil fungistasis against *Fusarium graminearum*. FEMS Microbiol. Ecol. 95, fiz056
2. Coton M, Hymery N, Piqueras J, Poirier E, Mounier J, Coton E, Picot A. (2019). *Monascus* spp. used in wheat kernel solid-state fermentations: growth, extrolite production and citrinin cytotoxicity. *World Mycotoxin Journal*. 12, 223-232. doi : 10.3920/WMJ2018.2425
3. Cobo-Díaz Jf, Baroncelli R, Le Floch G, Picot A. (2019). Combined metabarcoding and co-occurrence network analysis to profile the bacterial, fungal and *Fusarium* communities and their interactions in maize stalks. Frontiers in Microbiology.
4. Gillot, G., Jany, J. L., Poirier, E., Maillard, M. B., Debaets, S., Thierry, A., Coton, E., Coton, M. (2017). Functional diversity within the *Penicillium roqueforti* species. Int J Food Microbiol, 241, 141-150.
5. Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., Fall, A., Daube, G., & Coton, E. (2017). Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. FEMS Microbiol Ecol, 93.

DOCTORAT ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

THESIS FUNDING

Origin(s) of the thesis funding: 100% CIFRE
Gross monthly salary: Not specified
Thesis funding state : Acquired
Funding beginning date/Funding ending date: 1.11.2020, 36 months

Date: 26/6/2020

Name, signature of unit director: COTON Emmanuel

Name, signature of team director: MOUNIER Jérôme

Name, signature of thesis project director: COTON Monika