

M2 INTERNSHIP SUBJECT -6 months

TITLE: Investigating the effect of abiotic factors on the production of mycotoxins by *Penicillium camemberti* and *roqueforti*

CONTEXT:

Plant-based cheese is a dairy alternative made from a variety of plant-based milks. During the last decade, the market of dairy alternatives has grown significantly due to an increased number of flexitarian or vegan consumers, as well as a rise in the number of citizens concerned by animal welfare and environmental issues. In addition to be tasty alternatives, plant-based products must also be safe and reliable. Among safety issues related to these products, the issue of mycotoxins is of high concern. Indeed, the use of *Penicillium roqueforti* and *Penicillium camemberti*, two well-known producers of mycotoxins, in alternatives to blue cheese or to soft cheese could raise safety concerns. While, in dairy cheese, it is acknowledged that mycotoxins of inoculated *Penicillium species* pose no significant health risk to consumers as they are produced at very low levels or are quite unstable, very few is known regarding the production and stability of these mycotoxins in plant matrices. Besides the knowledge on the regulation of the yield of these mycotoxins is currently very restricted and too incomplete to allow anticipating what could happen when *P. camemberti* or *P. roqueforti* are inoculated in plant matrices.

OBJECTIVES :

The aim of this internship is to acquire the lacking knowledge on the effect of key abiotic factors (temperature, water activity/*aw*, pH) on the yield of mycotoxins by *P. camemberti* (cyclopiazonic acid) and *P. roqueforti* (roquefortine C, andrastin A, mycophenolic acid).

METHODS :

Task1- Definition of culture conditions and analytical methods

First assays in YES medium, using a set of strains of each targeted *Penicillium* species will be done to adjust the inoculum concentration, the duration and sampling times of the kinetics, the protocols to analyze mycotoxins.

Fungal biomass will be quantified by weighing dried mycelia - Mycotoxins will be quantified by liquid chromatography coupled with a diode array or mass detector.

Task2- Kinetics of growth and mycotoxin yields in different environmental conditions

Growth and mycotoxins yield of the set of considered strains will be followed after inoculation in YES medium and incubation under different environmental conditions (3 temperatures, 5 pH, that are characteristics of the plant-based cheese production context and storage). Besides 3 *aw* conditions will be studied, using YES media supplemented with glycerol.

Task3- Transcriptional regulation of the mycotoxin yield

For one strain of each targeted species, the expression of key biosynthetic genes will be compared between the different culture conditions applied in task2 (Rt-qPCR).

PREREQUISITES :

Skills in microbiology, analytical chemistry, molecular biology, statistics

KEYWORDS (5): filamentous fungi, mycotoxins, biosynthesis, environmental parameters, plant alternatives to animal products

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