**Characterization and quantification of *Fusarium* emerging mycotoxin(s) to improve food safety and quality.**

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This PhD thesis research project is aimed at predicting the risk of mycotoxin production by food transmitted fungal species by exploring their genomes and at developing innovative non disruptive mycotoxin(s) quantification methods to identify and quantify mycotoxins.

Caratterizzazione e quantificazione di micotossine emergenti prodotte da Fusarium per migliorare qualità e sicurezza dei prodotti alimentari

Questo progetto di ricerca ha lo scopo di predire il rischio di produzione di micotossine da parte di specie fungine emergenti esplorando il loro genoma e di sviluppare metodi innovativi e non distruttivi per la quantificazione di micotossine emergenti

# **1. State-of-the-Art**

The increasing world population requires safe food and increased productivity, but the worldwide contamination of cereals and cereal products by mycotoxins, secondary metabolites produced by filamentous fungi, is of potential concern for human and animal health and food safety. Fusarium toxins, produced by various *Fusarium spp.*, are most frequently present as mixtures (Rodriguez and Naher, 2012). They include the well-known trichothecenes, fumonisins and zearalenone, but also the enniatins (ENNs), beauvericin, moniliformin and fusaproliferin, which are frequently referred as emerging mycotoxins (Jestoi, 2018). Emerging mycotoxins that are frequently present in cereals, especially wheat, barley, rye, and oats. These emerging mycotoxins have been poorly studied, despite their potential concern for human health. The presence of emerging mycotoxins in a wide range of cereal grains and their by-products, along with their co-contamination with other mycotoxins, has raised concerns about the lack of regulatory limits for these compounds. The European Food Safety Authority (EFSA) recognizes the potential chronic exposure risks but has not conducted a risk assessment due to the limited toxicity data available (Oueslati et al., 2011). Currently, the quantification of mycotoxins, relies on high-performance liquid chromatography-mass spectrometry (HPLC-MS). Although HPLC-MS is precise, it has drawbacks such as the use of chemicals, time consumption, high instrument costs, and sample disruption. Therefore, the development of alternative methods for mycotoxin identification and quantification is essential for improved monitoring in the food chain and for preventive risk assessment.

Exploring emerging mycotoxin risk can start from the ability of the strains to produce specific secondary metabolites. To estimate the potential risk, genomic information can guide the search for secondary metabolites likely produced by emerging threats in the food chain. For this purpose, the exploration of the genomes of *F. musae* and the investigation of secondary metabolite gene clusters with bioinformatic approaches will be investigated to assess the potentiality to produce emerging mycotoxin by this emerging food and health threatening species (Valenti et al., 2022).

Aptamers, which are short single-stranded DNA or RNA molecules, offer a promising alternative to conventional quantification methods. Aptamers can bind to target ligands with high affinity, similar to antibodies, and their specificity can be exploited in array-based diagnostics. Aptamers are synthesized through an in vitro evolution procedure called SELEX (Systematic Evolution of Ligands by EXponential Enrichment). This iterative selection process generates aptamers with the desired function, sensitivity, and selectivity. Aptamer-based quantification can be performed using a simple qPCR machine, making it accessible to most research laboratories (Rowe et al., 2009).

Near-infrared spectroscopy (NIR) is another alternative method for mycotoxin detection in food. NIR offers a simple, fast, non-destructive, and chemical-free approach, making it environmentally friendly. It provides information about molecular vibrations in the tested object and has been used for quantifying some mycotoxins. Hyperspectral imaging (HSI), a novel approach that improves upon conventional NIR devices, provides spectral information from each pixel of the captured image. It is particularly useful for analysing heterogeneous samples. Diffuse reflectance and push broom imaging are the preferred techniques for HSI measurement in grain evaluation (Orina et al., 2017; Fox and Manley, 2014).

In this scenario, the project aims to develop and validate novel methods for mycotoxin quantification and to assess genomic information from emerging Fusarium species to assess the toxigenic potential of the species.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

**WP1**: **Genome analysis:** (1.1) Entire genome of Fusarium pathogens will be sequenced following standard and assembled (1.2) combining short and long reads technology and using software such as Galaxy and Geneious, Functional Analysis (1.3) on obtained assembly will add gene function after annotation, identifying secondary metabolite potential production.

**WP2**: **Sampling:** Greenhouse experiment (2.1) conduced on Wild Type and esyn1 KO transformed strains of *F. avenaceum* and field data (2.2) from different fields will produce the collection of wheat on which mycotoxins will be quantified using standard quantification methods (2.3) and will produce the reference for the two methods under development.

**WP3**: **Aptamer:** Selecton of Aptamer (3.1) using SELEX process will be the first step followed by the set up step (3.2) in which will be carried out sensitivity selectivity and robustness of the method and then tested (3.3) on the obtained collection in previous WP

**WP4**: **HIS-NIR:** Hardware setting (4.1) for continuous photo collection that will be used for the set up phase (4.2) in which will be carried out the sensitivity and selectivity, final test on the collection (4.3) will be the final step before validation.

***Table 1***Gantt diagram for this PhD thesis project.



# **3. Selected References**

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