Valorization of agrifood residues for the development of highly active and environmentally friendly biofungicides

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The PhD research project aims at the development of novel and highly active antifungal agents to be used as biofungicides for crop protection, overcoming the emergency of resistant strains and limiting the use of harmful chemicals. A particular emphasis will be placed on the valorization of value-added bioactives present in agrifood residues by mild and efficient technologies. Scaffold optimization strategies, to increase both the activity and the bioavailability of the natural compounds, will be exploited taking advantage of green chemoenzymatic approaches.

Valorizzazione di residui della filiera agroalimentare per lo sviluppo di biofungicidi ad elevata attività e rispettosi dell’ambiente

Il progetto di ricerca mira allo sviluppo di nuovi antifungini ad elevata attività da utilizzare come biofungicidi per la protezione delle colture, per contrastare l'emergenza causata dall’insorgenza di ceppi resistenti e limitare l'uso di sostanze chimiche dannose. Particolare attenzione sarà posta al recupero e alla valorizzazione dei bioattivi presenti nei residui della filiera agroalimentare, mediante l’impiego di tecniche innovative. Partendo dalle molecole recuperate, al fine di aumentarne l'attività e la biodisponibilità, si procederà a cicli di ottimizzazione strutturale, anche mediante approcci chemoenzimatici.

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

Fungi are among the greatest biotic threats to agricultural and food security. Fungal diseases cause between 10% and 23% of crop losses every year, plus a 10-20% of post-harvest leakage. All the five most important crops in the world (rice, wheat, maize, soya beans and potatoes) are affected by fungal infections. The pressure that a growing human population and global warming have on the food system, and the devastating impact fungi have and will keep having on the world’s food supply, represent unprecedented challenges to food production. Fungi are highly infective and persistent, also because of the production of spores, and show an impressive plasticity and genetic variability. The increasingly widespread use of antifungal treatments, in addition to intensive monoculture cropping, provide an ideal environment for the emergence in fungal pathogens of fungicide-resistant strains. The current situation urges the discovery and development of novel, highly effective antifungal compounds (Stukenbrock *et al*., 2023).

In the past decade, the agrochemical industry has refocused its priorities moving towards the use of biological control agents (BCAs) including naturally occurring substances. The use of natural products from plant extracts as biofungicides has received increasing attention because of their high diversity and versatility. Especially in the context of sustainable agricultural development, research on the transformation of agrifood waste into high-value-added extracts or molecules has been intensified. Recently, it has been demonstrated that the production of numerous natural compounds by plants can be favored by adverse conditions, e.g. fungal infections. These secondary metabolites are usually acids or amides, enzymatically obtained as a self-defense mechanism. Under the stress conditions created during the infections, and because of the higher level of free reactive oxygen species, an increased formation of radicals is observed. Being highly unstable, radicals can react with other molecules and recombine their structure, resulting in the formation of new products such as dimers and trimers. These derivatives, having higher molecular weights, different spatial orientation and three-dimensional structure, are often responsible for a more specific and effective interaction with their biological target (Morimoto *et al*., 2018).

Cinnamic acids (ferulic, sinapic, caffeic, *p*-coumaric) are commonly found in plant cell walls of forage plants, or in cereals, vegetables, and fruits. They could be extracted as free acids, esterified with arabinoxylans and pectin, or as cinnamoylamides. Moreover, they could be recovered from agrifood waste and residues to be employed as such or as starting materials for the preparation of more complex derivatives. Cinnamic acids are often involved in dimerization reactions, due to the formation of reactive radical species. Ferulic acid and its derivatives, including dimers, have been recently reported as a promising class of antifungal agents. In particular, the poacic acid dimer is known to directly bind β-1,3-glucan, an important component of the cell wall, acting on the formation of glucan fibrils, and causing cell leakage. This mode of action is distinct from that of other antifungal agents targeting the cell wall, such as echinocandins. Furthermore, *in vivo* studies showed a significant decrease in the β-1,3-glucan synthesis after treatment with poacic acid. Other ferulic acid derivatives, belonging to the class of Hordatines (dimers of hydroxycinnamic acid amides) showed *in vivo* inhibition of the spore germination in early growth stages of several fungal pathogens. Little is known about further functionalization of these compounds, or about their use in combination with other antifungals (Piotrowskia *et al*., 2015; Kohyama *et al*., 2013; Jia *et al*., 2018; Djande *et al.*, 2022).

# **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:

A1) **Extraction, purification, and characterization of cinnamic acids** from agrifood waste.

A2) **Synthesis of a first collection of dimers from various cinnamic acids** by green chemistry approaches and biocatalyzed reactions.

A3) **Design and setup of a first series of biological test for the evaluation of the antifungal activity on various fungal strains (**in collaboration with plant pathologists). Tests will be an important part of my project since their result will lead to the identification of the most promising scaffolds and will guide the successive synthetic steps.

A4) **Identification of the lead compound and functionalization of the structure:** the result of the biological screening will allow to identify the most promising molecules for further investigation. Different functionalization will be introduced on the natural skeleton to obtain a larger collection of compounds hopefully endowed with high activity and/or bioavailability. In this part, it could also be considered the idea of developing molecules with a dual mode of action by functionalization of the lead structure with other antifungal moieties.

A5) **Optimization of the synthetic process and further investigation on the mechanism of action:** to obtain the most effective synthetic pathway and to better understand the molecular target and mechanism of action of the selected compounds.

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Extraction, purification and characterization of cinnamic acids*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Synthesis of a first collection of dimers from various cinnamic acids*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Design and setup of a first series of biological test for the evaluation of the antifungal activity on various fungal strains*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Identification of the lead compound and functionalization of the structure*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Optimization of the synthetic process and further investigation on the mechanism of action*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Writing and Editing*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# \*If the synthetized molecules will show a low or no activity after the biological screening (A3), a new series of natural compound, known in literature to be active as fungicides, will be taken into consideration.

# **3. Selected References**

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