**Antifungal activity of bioactive molecules isolated from agricultural waste against rice blast fungus**

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This Ph.D. project aims to search for antifungal molecules, which will be extracted from agricultural waste to control rice blast pathogen, *Pyricularia oryzae* Cavara*.* As strobilurin-fungicide resistance is becoming an urgent problem in rice blast management, both the strobilurin-resistant as well as sensitive strains will be evaluated.Moreover, the project aims to get an insight into the molecules’ mode of action, to identify new potential molecular targets for the development of innovative and environment-friendly fungicides, which could control also the strains that developed resistance to currently used synthetic fungicides.

**Attività antifungina di molecole bioattive isolate da scarti della filiera agricola, nei confronti del brusone del riso**

Questo progetto di dottorato mira ad identificare molecole con attività antifungina nei confronti delbrusone delriso, *Pyricularia oryzae* Cavara, estratte da scarti della filiera agricola. Poiché resistenza alle strobilurine sta diventando un problema urgente nella gestione del brusone del riso, sarà valutata l’ attività delle molecole selezionate sia su ceppi sensibili che su ceppi resistenti alle strobilurine. Inoltre, nell’ambito del progetto verrà studiato il meccanismo d’azione delle molecole ottenute, per identificare nuovi potenziali target molecolari per lo sviluppo di fungicidi innovativi e eco-compatibili, che potrebbero essere attivi anche nei confronti dei ceppi che hanno sviluppato resistenza ai fungicidi sintetici attualmente utilizzati.

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

Fungicide resistance in plant pathogens is spreading mainly because of monoculture cropping and extensive use of synthetic fungicides. It is a global threat to crop security, urgently requiring research focusing on identification and development of environmentally friendly compounds with novel modes of action (Steinberg *et al*., 2020; Piotrowski *et al*., 2015; Pinna *et al*., 2023). One of the most destructive fungal pathogen is the *Pyricularia oryzae* Cavara, a complex pathogen having different host-specialized pathotypes, which infect different important crops (Gladieux *et al.,* 2018). The most common is the rice-pathotype, causing severe blast disease in rice. Other crops, such as wheat, are also affected by its host-specialized pathotypes. Wheat blast has been reported in South America and South Asia. *P. oryzae* is also responsible for the grey leaf spot disease of turf grasses.

In rice, the disease causes annually 10-30% losses (Kunova *et al.,* 2021). The pathogen can infect rice plant at all growth-stages, but the major damage is caused by infection of the last internode (neck blast) and the panicle. Moreover, secondary metabolites such as pyriculols, nectriapyrones, tenuazonic acid (TeA) are produced by *P. oryzae,* of whichTeA is the most toxic, showing acute toxicity to mammals and also has inhibitory activity of photosynthesis (Motoyama *et al.*, 2020).

Rice blast management is based on integrated management, but fungicides still represent the most common approach. Strobilurins are well-consolidated fungicides and among the most widely used, especially in the European risiculture. Their almost exclusive use, and a single-site mode of action are important factors for quick development of resistance in pathogen populations (Kunova *et al.,* 2021). The scarcity of alternative fungicides effective against rice blast highly increases the risk of QoI resistance development in Europe, and in particular in Italy. Molecular studies of the cytochrome b (cytb) – the target of QoI fungicides – identified the presence of the G143A mutation responsible for the QoI resistance also in Italian isolates (Tenni *et al*., 2021) accelerating the urgency of the development of novel means for the disease management . Recent research indicates that often the extract obtained from agricultural waste contain a set of bioactive molecules with antifungal activity. Extract from agro-industrial wastes of unripe grapes showed antifungal properties against multiple strains of *Candida* spp. and other dermatophytes (Simonetti *et al*., 2019). Extracts from agricultural matrices such as grapevines were reported to be endowed with bioactive molecules with high antifungal activity, e.g. pterostilbene or trans-resveratrol were shown to be active against *Plasmopora viticola* and *Botrytis cinerea* (Guerrero et al., 2016).

These studies indicate that agricultural wastes are a rich source of bioactive molecules with antifungal activity and with novel mechanisms of action.

1. **PhD Thesis Objectives and Milestones:**

Objectives in the context of this ongoing PhD thesis can be achieved by certain activities subdivided below through the Gantt diagram. A1, A2, and a part of A3 are not included in the Gantt chart as these have been performed in the 1st year.

**A1. Extraction from agricultural waste**: Different matrices of agricultural wastes were collected followed by extraction by aqueous and organic solvents (e.g. methanol).

**A2. Characterization of the extracted molecules:** Molecules present in the extract were characterized by HPLC, Mass-spectrometry, possibly using the University of Milan COSPECT platform.

**A3. In vitro evaluation of biological activity of extracted molecules**: Mycelium inhibition test and spore germination inhibition test was performed to evaluate the efficacy of the crude extracts and purified molecules. Eventually, molecules with the highest biological activity were subjected to enzymatic assays to measure e.g. the mitochondrial activity.

 **A4. In vivo evaluation of biological activity of the extracted molecules:** The preventive andpost-inoculation application of the compounds will be evaluated in leaf-disk assay, in greenhouse, and possibly in a field trial to assess the reduction of disease symptoms.

**A5. Investigation of the mode of action of the molecules through novel tools:** To get an insight into the mode of action of the extracted molecules, transcriptomic analysis will be done to conclude this study. Pathogen treated or not with the compound will be subjected to RNA extraction and then differential gene expression will be analysed to get the hints about the mode of action of the new molecules.

**A6. Thesis and Paper Writing:** Paper and thesis writing will take place according to the progress of the work.

***Table 1***Gannt chart 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** |
| A3) | ***Enzymatic assay*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A4) | ***In vivo trials***  |   |   |  |   |  |  |  |   |   |   |  |  |   |   |   |  |  |   |   |   |  |  |  |  |
|  | 1) leaf-disk assays |  |   |   |  |  |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 2) greenhouse trials |   |  |   |   |   |   |   |  |  |  |   |   |   |  |  |  |  |  |   |   |   |   |  |  |
|  | 3) field trials |   |  |   |  |  |   |  |   |   |  |  |   |   |   |   |  |  |  |   |   |  |  |   |  |
| A5) | ***Mode of action studies*** |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |   |  |   |  |  |  |  |  |  |  |
| A6) | ***Thesis and Paper Preparation*** |   |   |   |  |   |  |  |   |   |   |  |  |   |   |   |  |  |  |  |  |   |   |   |   |

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