ALDAUTOX

Project title: Biosynthesis and biological activity of aldaulactone, a fungal toxin involved in the pathogenicity of Alternaria douci, the main causal agent of carrot leaf blight

Acronym: Aldautox

Project duration: 24 months - Start date: 01/01/2019 End date: 31/12/2020

Key-words: polyketides, fungal toxin, plant cell cultures, yeast transformation, labelled analogues.

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Summary:

Context
The pathogenicity of Alternaria douci, the main fungal necrotrophic pathogen causing leaf blight on carrot, is at least partly based on toxin production (Lecomte et al., 2014). These toxins, such as aldaulactone, a polyketide recently uncovered by Courtial et al. (submitted), are used by the fungus to kill the plant cells and grow inside the plant. Toxin biosynthetic pathways and their mode of action are currently unknown.

Goals
The aims of this project are to characterize the biosynthetic pathway of aldaulactone and to study its mode of action in the plant cell. Aldaulactone is one of the main toxin produced by A. douci and is, in that way, a crucial element ta uncover how the pathogen attacks carrot leaves, leading to a reduction of root production or seed formation.

Methodology
Polyketide synthase {PKS) genes, selected from a transcriptomic analysis previously performed in FungiSem, will be used to transform the Pichia pastoris yeast. The toxicity of yeast extracts after transformation with PKS genes will be tested on carrot cell cultures by comparison with toxicity assays performed with pure aldaulactone. Chemical analysis of metabolites found in (or isolated from) taxie extracts produced by the transformed yeast will allow identifying and defining the synthetic pathway of aldaulactone. Toxin mode of action will be studied through a multi-step strategy. First semisynthetic modifications of the substitution pattern of aldaulactone will lead to new analogues whose toxicity on carrot cells will be evaluated. lt will help us identifying both the structural moieties of aldaulactone that can be transformed without any decrease of its toxicity and the moieties that support its biological activity. Then new analogues, either bearing a fluorescent probe or a reactive linker for click chemistry reactions or exhibiting 13C isotopie enrichment, will be semisynthesized. These derivatives are chemical tools that could be used to track the toxin and/or its metabolites.