

DISCOVERY AND DEVELOPMENT OF NEW ANTIBACTERIAL AGENTS AS AGROCHEMICALS FOR SUGARCANE CULTURES: BIOLOGICAL SCREENING, MEDICINAL CHEMISTRY AND STRUCTURAL BIOLOGY

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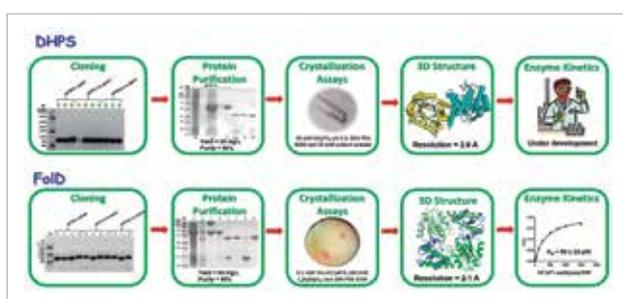


Figure 1. Structural molecular biology strategies toward the determination of the 3D structural of key molecular targets from *X. albilineans*

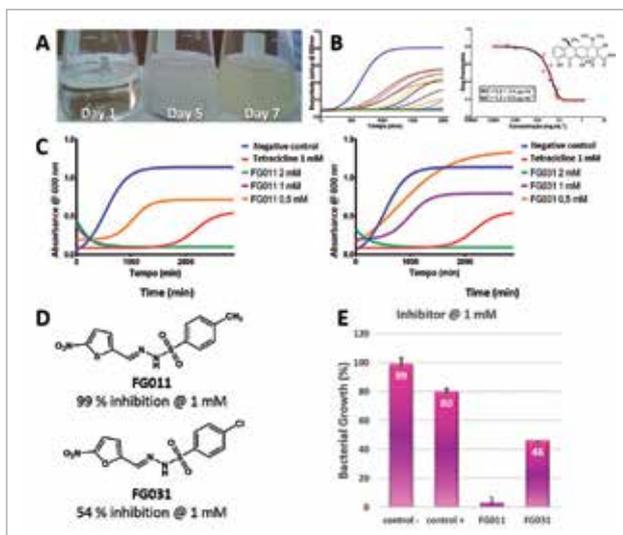


Figure 2. (A) *X. albilineans* growth in XAS culture medium. (B) Tetracycline as positive control for *X. albilineans* inhibition. (C) *X. albilineans* phenotypic assay for screening compounds as agrochemical candidates. (D) Representative example of nitro derivatives as *X. albilineans* inhibitors. (E) Inhibitory activity of nitro derivatives against *X. albilineans*

Sustainable development exhibits as main feature the use of scientific knowledge to generate technological innovation to meet human needs while preserving the environment. The environmental benefits obtained from the production and use of sugarcane derivatives boost the development of methods and strategies to enhance the generation of bioenergy. However, the occurrence and severity of plant diseases, such as leaf scald, hinder the productivity of sugarcane crops. Sugarcane leaf scald is a widespread and devastating disease caused by the bacteria *Xanthomonas albilineans*. The disease has a dramatic impact on crop productivity, including reduced yields and drop in quality of the juice. Currently, there is no chemical or biological treatment for disease control. Therefore, there is an urgent need for new effective and selective agrochemicals with low cost and environmental impact. This work aims at identifying and developing new molecules with antibacterial activity on the basis of the screening of natural and synthetic compounds. Additionally, a combination of state of the art methods and strategies in structural molecular biology and medicinal chemistry is employed in the design of the bioactive compounds. This work relies on the multidisciplinary organization, which led to the establishment of effective collaboration. In addition, the project provided the opportunity for the creation of a new field research in medicinal chemistry focused on the discovery and development of new agrochemicals for tropical agriculture. The new field of research expands the scope and adds value to the set of projects conducted in Center for Research and Innovation in Biodiversity and Drugs (CIBFar/CEPID) and offers the possibility of our group contribute to the sustainable development of Brazil through effective scientific actions in the strategic areas of agriculture and bioenergy.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Leaf scald is a serious sugarcane disease and the absence of chemical or biological agents against the phytopathology boosts the research of bioactive molecules as effective agrochemicals. *X. albilineans* produces a family of phytotoxins (albicidins), which play an important role in pathogenesis. Mutants of *X. albilineans* lacking the production of albicidins fail to cause any disease symptoms. Hence, enzymes involved in the biosynthesis of albicidins are attractive molecular targets for disease control. Phosphopantetheinyl transferase (PPT) and benzoate-coenzyme A ligase (BCL) play key roles on the biosynthesis of albicidins. Because of that, they were selected as molecular targets for agrochemical design. In addition, we selected several key enzymes of the folate biosynthesis as alternative targets. For instance, N5, N10 - methylenetetrahydrofolate dehydrogenase-cyclohydrolase (FolD) and dihydropteroate synthase (DHPS) act on folate metabolism and are crucial to biosynthesis of thymidine, purines and amino acids. The enzymes were successfully cloned and soluble expressed in large scale. The purification by chromatography led to proteins with high degree of purity (>95%). Enzyme characterization revealed affinities for the respective substrates in the mid-micromolar range. Crystallization assays identified attractive conditions to XaPPT, XaBCL, XaFolD and XaDHPS crystallization. We solved the 3D structure of XaFolD and XaDHPS at high resolution and on the basis of the structural knowledge new inhibitors can be designed (*Figure 1*). Simultaneously, we developed and standardized a phenotypic assay against *X. albilineans* for screening compounds as agrochemical candidates (*Figure 2*). Active compounds from several chemical classes have been identified. In sum, pure protein is crucial to the development of structural biology and structure-based drug design strategies. Additionally, the functional characterization of the targets and the development of a whole-cell method for screening compounds provide the basis for the rational discovery and design of inhibitors as novel agrochemical candidates for leaf scald control.

MAIN PUBLICATIONS

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