Discovery of inhibitors of the ferredoxin-NADP+ reductase from the Xanthomonas citri subsp. citri phytopathogen

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Plant type Ferredoxin-NADP(H) reductases (FNRs, EC 1.18.1.2) constitute a family of FAD containing enzymes that deliver NADPH or low potential one-electron donors to redox-based metabolisms in plastids and bacteria. Based on phylogenetic analysis, FNRs present in most prokaryotes (collectively known as FPRs) have been classified into two subclasses represented by the *Azotobacter vinelandii* (subclass I) and the *Escherichia coli* (subclass II) [1]. *Xanthomonas axonopodis citri* subsp. *citri* (*Xcc*) is a Gram-negative bacterium responsible for citrus canker, a disease that affects most commercial citrus crops and has economic impact worldwide [2]. Its *fpr* gene encodes a subclass I FPR (*Xcc*FPR). The role of *Xcc*FPR nor its potential substrate have been elucidated, but its involvement in the oxidative stress response of *X. citri* via interaction with ferredoxin XAC1762 has been proposed [3]. Therefore, *Xcc*FPR is relevant for the pathogen survival and the inhibition of its activity might represent an effective treatment against citrus canker. We started performing a high-throughput screening (HTS) of a library of 11120 druglike compounds to find inhibitors of *Xcc*FPR based on its diaphorase activity. We selected 43 HTS hits and narrowed them down to 5 primary hits that showed IC_{50} values in the low micromolar range and successfully abolished the activity of *Xcc*FPR. The four best inhibitors were assayed *in vivo* on plate cultures, and two of them showed bacterial growth inhibition. Based on the best primary hit, secondary hits were selected and one of them improved the characteristics of the primary one. Type of inhibition of this hit was determined and its effect on plastidic type FNR. This work is in progress but represents a promising pathway for the development of phytosanitary compounds against citrus canker propagation.

[1] E. A. Ceccarelli et al. BBA, 1698, 155-165, 2004.
[2] J. H. Graham et al. Mol Plant Pathol, 5, 1-15, 2004.
[3] M. L Tondo et al. PLoS One, 6, e27124, 2011.