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Imaging mass spectrometry-guided fast identification of antifungal secondary metabolites from *Penicillium polonicum*

Jing Bai[1] · Peng Zhang[1,2] · Guanhu Bao[2] · Jin-Gang Gu[3] · Lida Han[1] · Li-Wen Zhang[1] · Yuquan Xu[1]

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Abstract

The discovery of antibiotics from microorganisms using classic bioactivity screens suffers from heavy labor and high re-discovery rate. Recently, largely uncovered biosynthetic potentials were unveiled by new approaches, such as genetic manipulation of "silent" biosynthetic gene clusters, innovative data acquisition, and processing methods. In this work, a fast and efficient antibiotic identification pipeline based on the MALDI-TOF imaging mass spectrometry was applied to study the antifungal metabolites during the confrontation of two fungal species, *Penicillium polonicum* and wilt-inducing fungus *Fusarium oxysporum*. By visualizing the spatial distribution of metabolites directly on the microbial colony and surrounding media, we predicted the antifungal candidates before isolating pure compounds and individually testing their bioactivity, which subsequently guided the identification of target molecules using classic chromatographic methods. Via this procedure, we successfully identified two antifungal metabolites, fructigenine A and B, which belong to indole alkaloid class and were not reported for antifungal activity. Our work assigned new bioactivity to previously reported compounds and more importantly showed the efficiency of this approach towards quick discovery of bioactive compounds, which can help study the vast unexploited synthetic potential of microbial secondary metabolites.

Keywords MALDI-TOF imaging mass spectrometry · *Penicillium polonicum* · Antifungal secondary metabolite · Indole alkaloid

Introduction

Fungi such as *Penicillium* species have served as a rich source of bioactive secondary metabolites (SMs) of medicinal and agricultural importance, e.g., the treatment of infectious diseases and cancer, and the prevention of crop damage (Bladt et al. 2013). Genomics studies have revealed that many microorganisms have far greater potential to produce SMs than what was estimated from classic bioactivity screens (Rutledge and Challis 2015). This underestimation is partially due to the fact that many metabolite biosynthetic gene clusters (BGCs) are silent or expressed at very low levels in laboratory growth conditions, and consequently, their products can easily escape the standard identification procedure for bioactive compounds. Recent attempts have set up productive approaches to uncover this synthetic potential via (1) bypassing the strict genetic regulation of the host species with manipulation of epigenetic or BGC-specific regulatory units, or using appropriate heterologous expression systems; (2) innovating the methods of measurement and data processing; and (3) using combinatory -omics (Clevenger et al. 2017; Fan et al. 2017; Kersten et al. 2011; Lin et al. 2016; Nielsen et al. 2017; Yaegashi et al. 2014). These studies have contributed new specific metabolites of various bioactivities and, more importantly, paved the way to better understand microbial SMs.

In our study, we applied a fast identification pipeline to discover antifungal metabolites by visualizing the chemical communication between two species. This procedure was

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✉ Li-Wen Zhang
zhangliwen@caas.cn

[1] Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, People's Republic of China

[2] Natural Products Laboratory, International Joint Lab of Tea Chemistry and Health effects, State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei 230036, People's Republic of China

[3] Agricultural Culture Collection of China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China

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