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ORIGINAL ARTICLE

Fungal endophytes isolated from *Protium heptaphyllum* and *Trattinnickia rhoifolia* as antagonists of *Fusarium oxysporum*

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Abstract Control of fungal pathogens is mainly addressed by the use of chemically synthesized fungicides which result in environmental pollution, developing resistance after prolonged use. In this context, endophytes have been recognized as potential biocontrollers, and also as a promising source of antifungal metabolites. Therefore, as part of our research on phytopathogen controllers, 355 fungal endophytes were isolated from *Protium heptaphyllum* and *Trattinnickia rhoifolia* (Burseraceae), both ethnobotanically important tree species that produce secondary metabolites of agronomic and industrial interest. Endophytes were tested by *in vitro* dual culture against *Fusarium oxysporum*, a phytopathogen of agronomic importance. Five endophytes exerted at least 40% inhibition on *F. oxysporum* growth. Ethyl acetate (EtOAc) extracts were obtained from the most active antagonistic fungi, after growing them in three different liquid media. The extracts were tested against a conidial suspension of *F. oxysporum* by direct bioautography. Two extracts derived from fungi identified as *Chaetomium globosum*, F211_UMNG and *Meyerozima* sp. F281_UMNG showed inhibition of pathogen growth. Isolate *C. globosum*, F211_UMNG was selected for a chemical analysis by RP-HPLC-DAD-ESI-MS and antifungal molecules such as cladosporin, chaetoatrosin A and chaetoviridin A were annotated and identified based on their MS data.

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PALABRAS CLAVE
Endófitos;
Burseraceae;
Chaetomium;
Metabolitos

Endófitos fúngicos aislados de *Protium heptaphyllum* y *Trattinnickia rhoifolia* como antagonistas de *Fusarium oxysporum*

Resumen El control de patógenos fúngicos se basa principalmente en el uso de fungicidas de síntesis química, los que pueden dar lugar a la contaminación del medio ambiente y el desarrollo de resistencia después de un uso prolongado. En este contexto, los endófitos han sido reconocidos como potenciales biocontroladores y también como fuentes prometedoras de metabolitos secundarios antifúngicos. En el marco de nuestra investigación sobre controladores de fitopatógenos, se aislaron 355 hongos endófitos de *Protium heptaphyllum* y *Trattinnickia rhoifolia* (Burseraceae), especies arbóreas de valor etnobotánico que producen metabolitos secundarios de interés agronómico e industrial. Los endófitos fueron evaluados *in vitro* en cultivos duales frente a *Fusarium oxysporum*, un fitopatógeno de importancia agronómica. Cinco endófitos mostraron al menos un 40% de inhibición en el crecimiento de *F. oxysporum*. Una vez determinados los hongos más activos, estos se cultivaron en 3 medios líquidos diferentes y a partir de ellos se preparó una serie de extractos solubles en acetato de etilo. Los extractos fueron probados contra una suspensión de conidios de *F. oxysporum* por bioautografía directa. Dos extractos derivados de los hongos identificados como *Chaetomium globosum* (F211_UMNG) y *Meyerozima* sp. (F281_UMNG) mostraron inhibición del crecimiento del patógeno. En el extracto derivado del hongo *C. globosum* se anotaron e identificaron los compuestos antifúngicos cladosporina, chaetoatrosina A y chaetoviridina A mediante el análisis por RP-HPLC-DAD-ESI-MS.

Introduction

Using chemically synthesized fungicides has been the first line strategy to control phytopathogenic fungi[31]. However, secondary effects, such as environmental pollution and resistance development due to the use of these products, has led to a growing reluctance to use hazardous fungicides in agriculture. Thus, an enhanced trend in searching new control strategies involving environment-friendly alternatives in the management of plant pathogens has arisen[20]. In the search for such control strategies, naturally-occurring chemical entities have become potential alternatives for the industry to replace synthetic products[23]. In this context, microorganisms constitute a rich source of compounds with useful properties[48] for several applications in the agrochemical and pharmaceutical industries[11,23].

For several decades, the interaction between fungal endophytes and their hosts has attracted the researchers' attention, mainly because of the advantageous characteristics they confer to their host. Among these characteristics we can mention enhanced stress tolerance, plant growth factor production, herbivore repellency and protection against pathogens[18]. The latter characteristic is partly due to the fact that endophytes compete with other microorganisms for a specific niche, which could be achieved by the production of antibiotic-like secondary metabolites, along with other strategies[4]. As a consequence of their repellent properties, endophytes have been proposed as biocontrollers and as a promising source of antifungal metabolites against phytopathogens of agronomic importance[18].

Based on our ongoing search for biologically active secondary metabolites from endophytic fungi, the objective of this work was to explore the diversity of endophytes isolated from *Protium heptaphyllum* and *Trattinnickia rhoifolia* (Burseraceae) form Casanare, Colombia. These tree species, have been traditionally used by indigenous communities to treat several ailments[12], and their complex chemical repertory has provided useful compounds having industrial, pharmaceutical and agronomic potential[38,43]. Furthermore, endophytes have been isolated from a species of the Burseraceae family, such as *Muscodor yucatensis*[25], with potential for controlling phytopathogens. Therefore, the aim of this work was to test *in vitro* the abilities of endophytes to inhibit the mycelial growth of *Fusarium oxysporum*, by metabolite production. *F. oxysporum* is a pathogen of many plant species that represent a major threat for the production of several agriculturally important crops, such as banana, carnation, chickpeas, dates, lentils, tomato, and others[27]. The active component or components, responsible for the antifungal activity were partially characterized following a bioassay-guided fractionation test of the liquid culture-derived crude extract from the most antagonistic endophyte, to be incorporated in the future to control management programs for plant pathogen *F. oxysporum*.

Methods

Recovery of endophytes and isolation

A total of two individuals from *P. heptaphyllum* and two from *T. rhoifolia* were collected in the foothill of the west Colombian Andes mountains in Aguazul, Casanare, Colombia (N 05°13′47.89″, W 072°30′31.38″), a transition ecosystem between the savanna and the high Andean ecosystems. Botanical specimens of *P. heptaphyllum* (Aubl.) Marchand (COL573961) and *T. rhoifolia* (Aubl.) Marchand (COL573962)

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