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ABSTRACT

To discover microorganisms that naturally possess chemical weapons against the phytopathogen *Fusarium oxysporum*, the biological and chemical diversity of plant leaf endophytes was investigated. Endophytes were isolated from the palm tree *Astrocaryum sciophyllum* collected in pristine forests of French Guiana. Several Xylariaceae inhibited the growth of *F. oxysporum* and were further explored. Antifungal specialized metabolites were isolated from the Xylariaceae BSNB-0294 strain in confrontation with phytopathogen and led to the identification of undescribed compounds, *i.e.,* two depsipeptides named xylariaceins, two metabolites containing a 3-imidazolinone moiety, and four new compounds including a nitro-phenylpropanamide and three phenylalanine analogs named xylariains A-D. In parallel, chemical investigation of the phytopathogen during the coculture led to the identification of an unknown compound, which we named focicin. The production of focicin was exacerbated during the competition. MALDI-TOF MS imaging of the competition between BSNB-0294 (endophytic strain) and *F. oxysporum* f.sp. *ciceris* (phytopathogen) highlighted time-dependent chemical interactions between the two microorganisms.

Fusarium oxysporum is a fungus widespread in soils. The species includes both nonpathogenic and economically important plant pathogens. Pathogenic strains of *F. oxysporum* have been intensively studied; the host range of these fungi is broad and includes animals from arthropods to humans, as well as plants.[1-3] Nevertheless, individual isolates usually cause disease only in a narrow range of plant species. This observation has led to the idea of a "special form" or *formae specialis* in *F. oxysporum*. These special forms indicate the specialization of the fungus towards the infected species or even plant cultivar. Exhaustive host range studies have been conducted for relatively few *formae speciales* of *F. oxysporum.*[4] The best-known and most feared form in agronomy is *F. oxysporum* f. sp. *cubense* causing fusarium wilt.[5] To date, 106 other fungal special forms have been characterized, and many are attacking crops such as cotton (f.sp. *vasinfectum*), soybeans (f.sp. *wisteria*), tomatoes (f.sp. *lycopersici*), melon (f.sp. *niveum*), spinach (f.sp. *betae*) or chickpea (f sp. *ciceris*). In addition to banana (f.sp. *cudenis*), this phytopathogen can attack different species of palm trees (Araceae family plants), such as the oil palm tree (f.sp. *elaeidis*), the date palm (f.sp. *albedinis*) as well as decorative species (f.sp. *canariensis* and *palmarum*).[6-7]

Once the infection is initiated by *F. oxysporum* on plants, the regulation of this pathogen is difficult to handle. Indeed, the contamination of soils by *F. oxysporum* implies severe treatments to avoid its dissemination. Most often, a chemical treatment is carried out, rending the soil sterile and noncultivable for several seasons.[8-10] The pathogenicity is due to fungal progression into vascular tissue, which may elicit an immediate host response, successfully restricting the invader; an otherwise ineffective or delayed response reduces the vital water-conducting capacity and induces wilting.[11] On the other hand, the plant might be able to tolerate limited fungal growth within xylem vessels provided that it was initially colonized by an endophyte.[12] In this case, any further changes in the host or parasite could disturb the relationship in a way that fungal activities or a host response would induce disease symptoms.

Faced with these infections, *F. oxysporum* biocontrol approaches have been investigated, showing the beneficial impact of environmental microorganisms in defense of the plant against this phytopathogen. Jie *et al.* suggested the preponderant role of the endophytic community in the regulation of *F. oxysporum.*[13]

Endophytes are microorganisms living over a period of their life cycle between plant cells without causing apparent damage. Specialized metabolites from plant endophytes, such as peptides or ergosterol derivatives, showed antagonist activity against phytopathogens, including *F. oxysporum.*[14-17] Although endophytes seem to be possible research resources for anti-phytopathogen compounds, the major difficulty in natural product research is the isolation of already known compounds during the study of active strains. As an example, it has been estimated that only 10-15% of the biosynthetic capacity of actinomycetes and fungi is expressed under standard laboratory conditions.[18] Most secondary pathways are silent due to the absence of natural living conditions when a pure strain is cultured on synthetic standard media. These silent pathways can be turned on by different stimuli: agitation, pH, temperature, the addition of enzyme inhibitors, or dual cultures, for example. Bode *et al.* defined the term OSMAC (one strain many compounds) to designate that one strain could potentially provide different metabolite profiles depending on the culture parameters.[19-20] By expending the biosynthetic potential of strains, their chemical diversity and thus the probability of discovering original metabolites are increased. In the same vein, new metabolites can be efficiently isolated from cocultures because biotic stress mimed with cocultures can induce chemical defense metabolites such as antimicrobial compounds.[21-22]

In this study, a collection of 197 endophytes isolated from the leaflets of the palm tree *Astrocaryum sciophilum* (Miq.) Pulle growing in the understory part of Amazonian forest[23] was screened by a confrontation test against the phytopathogen *F. oxysporum* f. sp. *ciceris* and *F. oxysporum* f.sp. *albedinis*. *A. sciophilum* is a large undergrowth palm in the Asteraceae family

from the primary forests of French Guiana. In this Asteraceae, the stems of young plants are generally underground, but in older plants, they grow more above the ground.[24] Its small size in tropical forests makes it a receptor for plant matter from surrounding trees that traps between its leaves. This palm is thus in contact with the ground but is also very exposed to phytopathogenic microorganisms from the environment.[25] Consequently, it can be affected by pathogens of the *Fusarium* genus in the same way as other Asteraceae. However, it presents great longevity (age of maturation estimated at 170 ± 70 years), and it is found in all undergrowths of South American primary forests; this ecological success may be due to symbiosis with competitive endophytes. Two endophytic strains were selected based on activity, and their chemical diversity was explored to identify active specialized metabolites. Time-dependent production of these chemical compounds was studied by matrix-assisted laser desorption/ionization coupled to time-of-flight (MALDI-TOF) imaging.[26,27] The methodological approach of this study and details of the experimental procedures are summarized in scheme 1.