Biochemical Systematics and Ecology 39 (2011) 163–165

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Endophytic fungus *Penicillium chrysogenum*, a new source of hypocrellins

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ARTICLE INFO

Article history:
Received 30 November 2010
Accepted 5 February 2011
Available online 1 March 2011

Keywords:
Endophytic fungus
Penicillium chrysogenum
Hypocrellin
Anti-fungal activity

1. Subject and source

Endophytic fungi have proven to be a rich source of novel secondary metabolites with interesting biological activities and a high level of chemical diversity (Schulz and Boyle, 2005; Zhang et al., 2006). In our ongoing screening for biologically active secondary metabolites from endophytic fungi (Zhang et al., 2008a, b, c, 2009), we investigated metabolites produced by the endophytic fungus *Penicillium chrysogenum*, isolated from *Fagonia cretica*, a zygophyllaceous plant of the semiarid coastal regions of Gomera, Spain. The fungus was selected for chemical and biological investigation since the crude ethyl acetate extract of its culture showed very good antifungal activity against *Microbotryum violaceum* and moderate algicidal and antibacterial activities.

The endophytic fungus was identified by Prof. Zhiyong Li, Shanghai Jiao Tong University. A voucher specimen (ZW66) is deposited in the culture collection of the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University.

2. Previous work

Penicillium chrysogenum is widely distributed in nature, and is often found to live on foods and in indoor environments. It is an important filamentous fungus because of its ability to produce large amounts of penicillin (Elander, 2003). The secondary metabolites of *P. chrysogenum* include various penicillins, chrysogine, xanthocillins, secalonic acids, sorrentanone, and PR-toxin (Hoog et al., 2000). Recently, Singh et al. (2003) reported two novel polyketides xanthoviridicatins E and F from endophytic strain of *P. chrysogenum* isolated from a leaf collected in Peru. Gerhard et al. (2005) reported three new sorbicillin-derived compounds sorbicillactones A, B and sorbivinetone, as well as known fungal metabolites oxosorbicillinol, sorbicillin,

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doi:10.1016/j.bse.2011.02.003

bisvertinolone, meleagrine and roquefortine C, from a sponge-derived *P. chrysogenum* strain. Lin et al. (2008) reported a new neuroprotective alkaloid chrysogenamide A together with cumdatin G, 2-[(2-hydroxypropionyl)amino] benzamide, 2′,3′-dihydrosorbicillin, (9*Z*,12*Z*)-2,3-dihydroxypropyl octadeca-9,12-dienoate from an endophytic fungus associated with *Cistanche deserticola* Y. C. Ma. Qi et al. (2009) reported the production of gallic acid from endophytic *P. chrysogenum* of Acer ginnala.

3. Present work

The endophytic fungus *P. chrysogenum* was isolated following surface sterilization from the plant *F. cretica*, and was cultivated on 12 L of 5% w/v biomalt solid agar medium at room temperature for 28 days (Schulz et al., 1995). The cultures were then extracted with ethyl acetate to afford 19.8 g of residue after removal of the solvent under reduced pressure. A portion of crude extract (1.5 g) was subjected to a silica column chromatography (CC) and separated into 10 fractions, using a gradient of petroleum and chloroform mixture (100:0 ~ 0:100). Fraction 9 (920 mg), mainly the mixture of hypocrellins B and C, was separated by Sephadex LH-20 eluted with chloroform: methanol (7:3) to give pure hypocrellins B (**1**, 880.0 mg) and C (**2**, 24.0 mg) (Kishi et al., 1991). The structures were elucidated on the basis of extensive spectroscopic analysis (1D and 2D NMR UV, IR, MS) and comparison with literature data. This is the first report of hypocrellins B and C from the fungus *P. chrysogenum*.

In *in vitro* antifungal bioassay, hypocrellin B showed strong activity against *M. violaceum* and *Trichophyton rubrum*, and moderate activity against *Cryptococcus neoformans, Candida albicans* and *Aspergillus fumigatus*. Hypocrellin C showed strong activity against *M. violaceum*, *C. albicans* and *T. rubrum*.

4. Ecological significance

It is commonly accepted that microorganisms associated with plants in nature are one of the richest sources of new compounds having phytotoxic and plant growth-regulating properties (Greaves, 1996; Tringali, 2001). Secondary metabolites of endophytic microorganisms play very important ecological roles as parasiticides, insect antifeedants, and pathogen inhibitors.

In the present research, the mixture of hypocrellins B and C were isolated as main metabolites of endophytic fungus *P. chrysogenum*, accounting for about 60% of the crude extract. The strong antifungal activity against phytopathogenic fungus *M. violaceum* of both hypocrellins B and C suggests that endophytic fungi *P. chrysogenum* in plant *F. cretica* may protect the host by producing the metabolites that may be toxic or even lethal to phytopathogens.

The hypocrellins have been only isolated so far from *Hypocrella bambusae* and *Shiraia bambusicola*, two typical fungi associated with *Fargesia spathacea* Franch, *Brachystachyum densiflorum*, and *B. keng*. The two fungi *H. bambusae* and *S. bambusicola* have been only found in some region of China, Japan and Sri Lanka. Hypocrellin A, the first member of the family, was discovered from *H. bambusae* in 1980 in China (Wan and Chen, 1980, 1981) with its structure being further confirmed by an X-ray diffraction analysis (Chen et al., 1981). In the years that followed, hypocrellin A was also repeatedly reported from *Shiraia* sp. (Kishi et al., 1991; Fang et al., 2006; Liang et al., 2009; Yang et al., 2009). Hypocrellins B-D and shiraiachromes A-C, other members in the family with the perylenequinone fused heptacycle carbon skeleton, were isolated from the fungi *H. bambusae* and *S. bambusicola* (Wan et al., 1985; Wu et al., 1989; Kishi et al., 1991; Fang et al., 2006). Since these metabolites have only been discovered in the two bamboos associated fungi *H. bambusae* and *S. bambusicola* in nature, they are commonly regarded as a significant taxonomic marker of the fungi and the plants in China. The isolation of hypocrellins from the endophytic fungus *P. chrysogenum* in the present work suggests that these metabolites may be produced by an additional endophytic fungus in plant *F. cretica*. Therefore, the significance of these compounds as a taxonomic marker of the plants species and associated fungi must be reconsidered. On the other hand, the high yield of these compounds from the fungi may give a new source in drug development as a photodynamic therapy agent in treatment of various skin diseases in clinical (Yang et al., 2009).

Acknowledgments

This research work was financially supported by Natural Science Foundation of China (No. 30873200, 41076082), STCSM Projects (No. 08JC1405600), Shanghai Leading Academic Discipline Project (B906), and Shanghai Pujiang Program (PJ2008). The authors are grateful to Kathrin Meier and Tatjana Stolz for excellent technical assistance.

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