Uncovering Biosynthetic Potential of Plant-Associated Fungi: Effect of Culture Conditions on Metabolite Production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*[1]

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In an attempt to uncover the biosynthetic potential of plant-associated fungi, the effect of culture conditions on metabolite production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii* was investigated. These studies indicated that the production of the major metabolites by *P. quadriseptata* differ when the water used to make the media was changed from tap water to distilled water. It resulted in the isolation of six new secondary metabolites, cytosporones F−I (**1**−**4**), quadriseptin A (**5**), and 5′-hydroxymonocillin III (**6**) together with monocillin III (**7**), a metabolite new to *P. quadriseptata*, in addition to monocillin I (**8**), a previously known metabolite from this organism. Aposphaerin B (**9**) encountered was suspected to be an artifact originating from cytosporone F (**1**). Incorporation of heavy metal ions to *P. quadriseptata* culture medium induced production of monocillin I (**8**) by this fungus. Cultivation of *C. chiversii* in liquid medium resulted in the isolation of chaetochromin A (**12**) as the major metabolite instead of radicicol (**10**), the major constituent of this organism when grown in a solid medium. Compounds **1**−**7** and **12** were evaluated for their potential to inhibit Hsp90 and antiproliferative activity toward the cancer cell lines NCI-H460, MCF-7, and SF-268. Only compounds **6**, **7**, and **8** exhibited significant activity in both assays.

Plant-associated microorganisms represent a largely untapped resource of small-molecule natural products, some with chemical structures that have been optimized by coevolution for biological and ecological relevance.[2] According to Bode et al.,[3] with more than 20 000 compounds described in the literature, microorganisms must be called metabolic artists superior to any metabolic diversity created by man. This ability of microorganisms combined with the potential to bring up a variety of new metabolites from a single strain by systematic alteration of its cultivation parameters, known as OSMAC (one strain many compounds) approach,[4] and the use of elicitors to induce or inhibit certain biosynthetic and/or signal transduction pathways[5] provide new opportunities to maximize chemical diversity of their metabolites. The OSMAC approach has resulted from the observation that very small changes in the cultivation conditions can completely shift the metabolic profile of many microorganisms. Thus, it represents a powerful tool to elucidate the secondary metabolome (the overall number of all secondary metabolites of one organism) of different microbes. This approach has recently been used to release the chemical diversity of a number of soil-borne fungi and actinomycetes[6] and a fungus of marine origin.[7]

In a study to uncover the chemical diversity of plant-associated microorganisms, we have investigated the influence of culture conditions on metabolite production of the fungal strains *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*. We have previously reported that the rhizosphere fungal strain *P. quadriseptata*, when cultivated in potato dextrose agar (PDA) and potato dextrose broth (PDB; a medium with a similar constitution to PDA but without any added agar) media made up in tap water, produced the C_{18} polyketide monocillin I (**8**) as the major metabolite,[8a] together with the minor isocoumarins paraphaeosphaerins A−C, biosynthetically related to **8**, aposphaerin C (**11**), eugenetin (**13**), 6-methoxymethyleugenin (**14**), and 6-hydroxymethyleugenin (**15**).[9] Similarly, the endophytic fungal strain *C. chiversii*, when cultivated in PDA medium made up in tap water, produced the corresponding metabolites radicicol (**10**)[8b] and chaetochiversins A and B.[9] In the present study, we have investigated the metabolite profiles of these organisms cultivated in PDB medium made up separately in tap water and distilled water, and herein we report the isolation of six new metabolites, cytosporones F−I (**1**−**4**), quadriseptin A (**5**), and 5′-hydroxymonocillin III (**6**), together with monocillin III (**7**), monocillin I (**8**), and aposphaerin B (**9**) from *P. quadriseptata* and the known metabolites chaetochromin A (**12**), eugenetin (**13**), and 6-methoxymethyleugenin (**14**) from *C. chiversii*. The octaketide metabolites structurally related to **1**−**4**, namely, cytosporones A−E and dothiorelone A, have previously been encountered in *Cytospora* sp. CR200 and *Diaporthe* sp. CR146,[10] and *Dothiorella* sp. HTF3,[11] respectively.

Results and Discussion

The rhizosphere fungus *P. quadriseptata*[8a] was cultivated for 14 days in PDB media made up separately with distilled water and tap water, the culture supernatants were extracted with EtOAc, and the resulting extracts were analyzed by HPLC. The HPLC profiles of the two extracts indicated that they contained almost the same metabolites but with significant differences in their major constituents (Figure 1). Thus, they were separately processed to isolate and characterize the major metabolites present in these extracts. Fractionation of the EtOAc extract of *P. quadriseptata* cultured in distilled water involving reversed-phase column chromatography and silica gel preparative TLC furnished cytosporones F (**1**) and G (**2**), quadriseptin A (**5**), monocillin I (**8**), and aposphaerin B (**9**). Cytosporone F (**1**) was determined to have the molecular formula $C_{18}H_{22}O_5$ by a combination of HRFABMS, ^{13}C NMR, and HSQC data and indicated eight degrees of unsaturation. The IR spectrum had absorption bands due to OH (3369 cm^{-1}), ester carbonyl (1732 cm^{-1}), and α,β-unsaturated ketone carbonyl (1724 cm^{-1}) groups. The ^1H NMR spectrum exhibited signals due to a chelated OH (δ 11.51), two aromatic protons at δ 6.31, a 2H singlet at δ 3.77 due to a CH_2 group sandwiched by an aromatic ring and an ester carbonyl, an $-OCH_2CH_3$ group [δ 4.16 (2H, q, $J = 7.1$ Hz) and 1.25 (3H, t, $J = 7.1$ Hz)], and four olefinic protons in a conjugated diene system [δ 7.26 (1H, dd, $J = 15.0$ and 10.1 Hz); 6.55 (1H, d, $J = 15.0$ Hz); 6.21 (2H, m)] (Table 1). The ^{13}C NMR spectrum of **1** when analyzed with the help of HSQC data displayed signals for 18 carbon atoms and indicated the presence of a ketone carbonyl (δ 195.1), an ester carbonyl (δ 171.1), six aromatic carbons of which

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