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Consistent production of penigequinolone A and B by *Penicillium scabrosum*

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1. Subject and source

P. scabrosum isolates: IBT 3740, 4030, 6635, 6637, 6815, 12258, 13671, 16088, 16106, 16246, 16395, 17219, 18318, 19284, 19286, 19422 were all obtained from the Culture Collection at the Department of Biotechnology (IBT), Technical University of Denmark.

2. Previous work

P. scabrosum was described as a new species by Frisvad et al. (1990). *P. scabrosum* resembles *P. atrovenetum* morphologically and physiologically, however, the two species share no secondary metabolites, and thus can be distinguished chemotaxonomically (Frisvad et al., 1990). *P. scabrosum* has previously been reported to produce cyclopenin, cyclopenol, viridicatin, fumagillin, all metabolites produced by several *Penicillium* species, as well as a large number of unknown metabolites (Frisvad et al., 1990). Recently, Smedsgaard (1997a) and Smedsgaard and Frisvad (1997) studied terverticillate penicillia by direct electrospray mass spectrometric profiling of

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crude fungal extracts and demonstrated that *P. scabrosum* have a unique mass profile among the terverticillate penicillia which allowed direct identification by database screening.

3. Present study

The present study reports the identification of two of the major unknown metabolites produced by *P. scabrosum* to be penigequinolone A and B (Fig. 1), when grown on Czapek Yeast Extract agar (CYA).

Mycelium from 2 l CYA medium was extracted twice with 500 ml EtOAc producing 500 mg crude extract. A fraction rich in penigequinolone alkaloids was obtained by vacuum liquid chromatography on Silica using stepwise elution with heptane, heptane-EtOAc, EtOAc-MeOH, MeOH (Coll and Bowden, 1986). The penigequinolone rich fraction was further purified on a Waters Prep Nova-Pak C18 cartridge (25×100 mm, 6-μm, 60 Å) with H_2O/CH_3CN (50 : 50) as mobile phase (20 ml/min) resulting in 4.0 mg penigequinolone A and B mixture, homogenous in HPLC.

Evaluation of the 400 MHz ^1H and 100 MHz ^{13}C NMR spectral data in $CDCl_3$, including 2D experiments (DEPT, HMBC, NOESY), mass spectroscopy data (probe EIMS showing M^+ at m/z 467) and elemental microanalysis ($C_{27}H_{33}O_6N$) established what was considered a single compound, to be identical to the diastereomerical mixture of penigequinolone A and B described by Kimura et al. (1996) and isolated from an unspecified *Penicillium* species. Kimura et al. (1996) found the two diastereomers to occur in a 1 : 2 ratio, while we observe a 1 : 1 ratio established by ^{13}C resonance intensity.

A total of 16 isolates of *P. scabrosum* were cultivated on CYA, and screened for secondary metabolite production using the plug extraction procedure and HPLC analysis as described by Smedsgaard (1997b). Furthermore mass profiles of the extracts were determined using direct electrospray mass spectrometry according to Smedsgaard and Frisvad (1996).

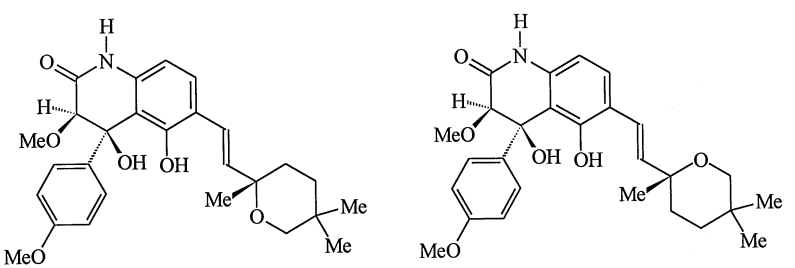

Fig. 1. Structures of penigequinolone A and B.

4. Chemotaxonomic significance

HPLC analysis demonstrated the metabolite profiles of the 16 isolates of *P. scabrosum* to be qualitatively very similar, each displaying penigequinolones A and B as major peaks together with cyclopenin, cyclopenol and viridicatin (Fig. 2, top).

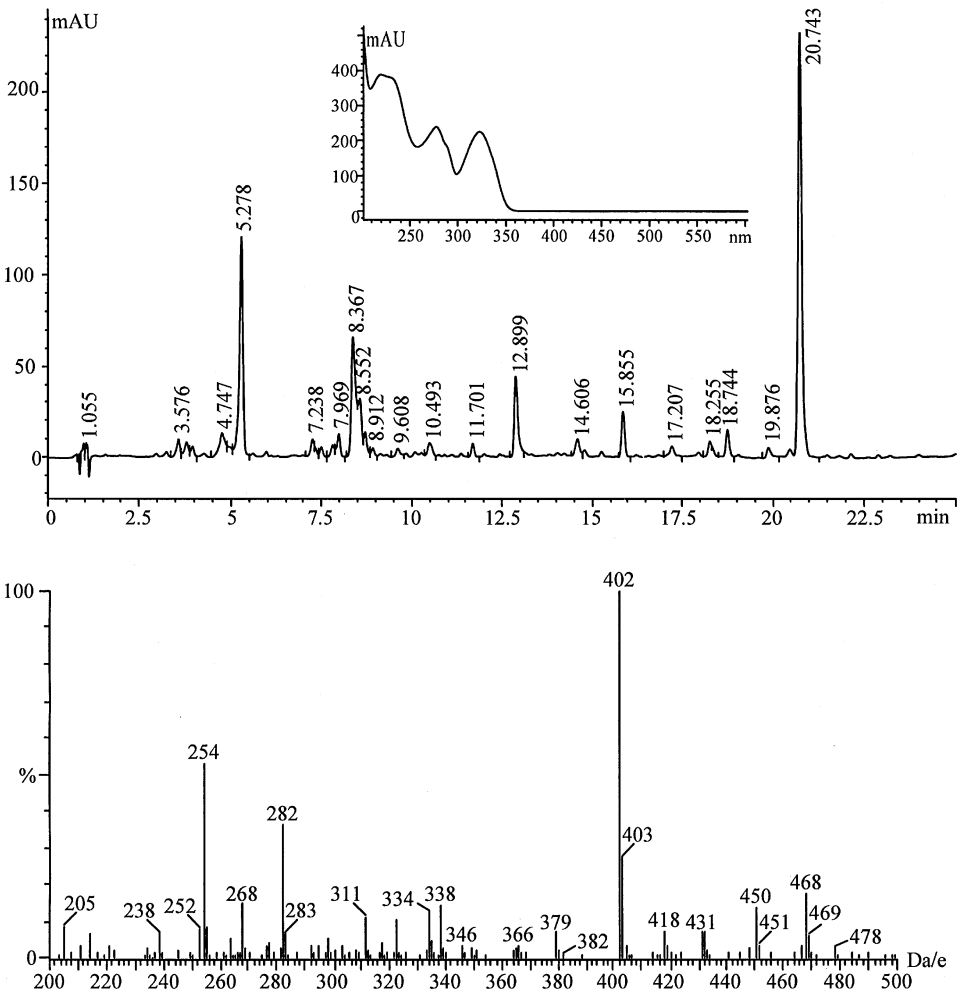

Fig. 2. 210 nm chromatographic profile (top) and mass profile (bottom) from a typical *P. scabrosum* isolate (IBT 16088) cultivated on CYA. The following metabolites can be seen in the profiles (with retention time and protonated molecular mass in brackets): viridicatin (12.88 min, 238 Da); 3-methoxy-viridicatin (12.93 min, 252 Da); viridicatol (no standard available, 254 Da); de-hydro-cyclopeptin (no standard available, 278 Da); cyclopeptin (9.61 min, 281 Da); cyclopenin (8.55 min, 295 Da); cyclopenol (4.75 min, 311 Da) penigequinolones (20.73 min, 468 Da). The UV spectrum of the peniquinolones is shown at top (λ_{max} (EtOH) nm (log ε) 217 (2.88), 279 (2.58), 324 (2.54)). Note that the sodiated molecular ions can be observed from all these compounds as well.

Cyclopenin, cyclopenol and viridicatin are biosynthetically related and are found in several *Penicillium* species e.g. in the *P. aurantiogriseum* "complex" (Lund and Frisvad, 1994). Several other metabolites are known from the cyclopenin-viridicatin pathway and are likely candidates for some of the unknown metabolites seen in the profiles from *P. scabrosum* (Fig. 2, top). The co-occurrence of cyclopenin-viridicatin-pathway metabolites and the penigequinolones is unique to *P. scabrosum* and there are thus excellent chemical indicators for the growth of *P. scabrosum*.

In agreement with Smedsgaard (1997a) and Smedsgaard and Frisvad (1997) the penigequinolones can be observed as ions at m/z 468 ($M + H^+$) in the ES-MS analysis of the extracts (Fig. 2, bottom), thus contributing to the unique mass profile of *P. scabrosum* when compared to other penicillia.

Acknowledgements

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