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A beauvericin hot spot in the genus *Isaria*

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ARTICLE INFO

Article history:
Received 24 February 2009
Received in revised form
21 August 2009
Accepted 25 August 2009
Available online 18 September 2009
Corresponding Editor:
Joseph W. Spatafora

Keywords:
Beauvericin
Chemotaxonomy
Entomogenous fungi
Isaria

ABSTRACT

Beauvericin is a naturally occurring cyclohexadepsipeptide originally described from *Beauveria bassiana* but also reported from several *Fusarium* species as well as members of the genus *Isaria*. Twenty-six isolates of *Isaria* species and its *Cordyceps* teleomorph, and ten taxonomically close strains including *Beauveria*, *Nomuraea* and *Paecilomyces* species were sequenced and tested for beauvericin production. Trees using ITS rDNA and β-tubulin sequence data were constructed and used to infer the phylogenetic distribution of beauvericin production. A group comprising *Isaria tenuipes* and its known teleomorph *Cordyceps takaomontana*, *Isaria cicadae* and its *Cordyceps* teleomorph, *Isaria japonica* and *Isaria fumosorosea*, showed positive beauvericin production which correlated well with combined ITS rDNA and β-tubulin phylogenies. The results suggested that beauvericin can serve as a chemotaxonomic marker for these limited species of the *I. tenuipes* complex.

Introduction

Beauvericin is a cyclohexadepsipeptide, composed of alternate linkages of three N-methyl-L-phenylalanine and three D-2-hydroxyisovaleric acid (Fig 1). Beauvericin has been considered most recently as a mycotoxin of the enniatin family produced by several hypocrealean *Fusarium* species (*Fusarium moniliforme*, *Fusarium proliferatum*, *Fusarium semitectum*, *Fusarium subglutinans*) infecting maize, rice and wheat (Bottalico *et al.* 1995; Logrieco *et al.* 1998; Plattner & Nelson 1994). However, it was first reported from the hypocrealean invertebrate pathogen *Beauveria bassiana* on the basis of its toxicity to brine shrimp (Hamill *et al.* 1969). It was later found from another invertebrate-pathogenic fungus *Isaria fumosorosea* (Bernardini *et al.* 1975) and was recently also isolated from *Isaria tenuipes* BCC 1614 and evaluated for its anti-tuberculous and anti-malarial activity (Nilanonta *et al.* 2000, 2002).

As part of a biodiversity research programme at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand, the mycology laboratory has worked extensively on the diversity of Thai hypocrealean insect fungi. These isolates have been studied by fermentation, bioassay and chemistry laboratories in an ongoing search for bioactive fungal secondary metabolites as sources of drug leads (Isaka *et al.* 2005). These results are now being linked with parallel studies on the phylogenetics of the insect fungi. With the discovery of beauvericin in *I. tenuipes* BCC 1614 this study was initiated to determine if beauvericin was produced by other species of *Isaria* as well as phylogenetically related taxa and some morphologically related taxa.

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doi:10.1016/j.mycres.2009.08.017

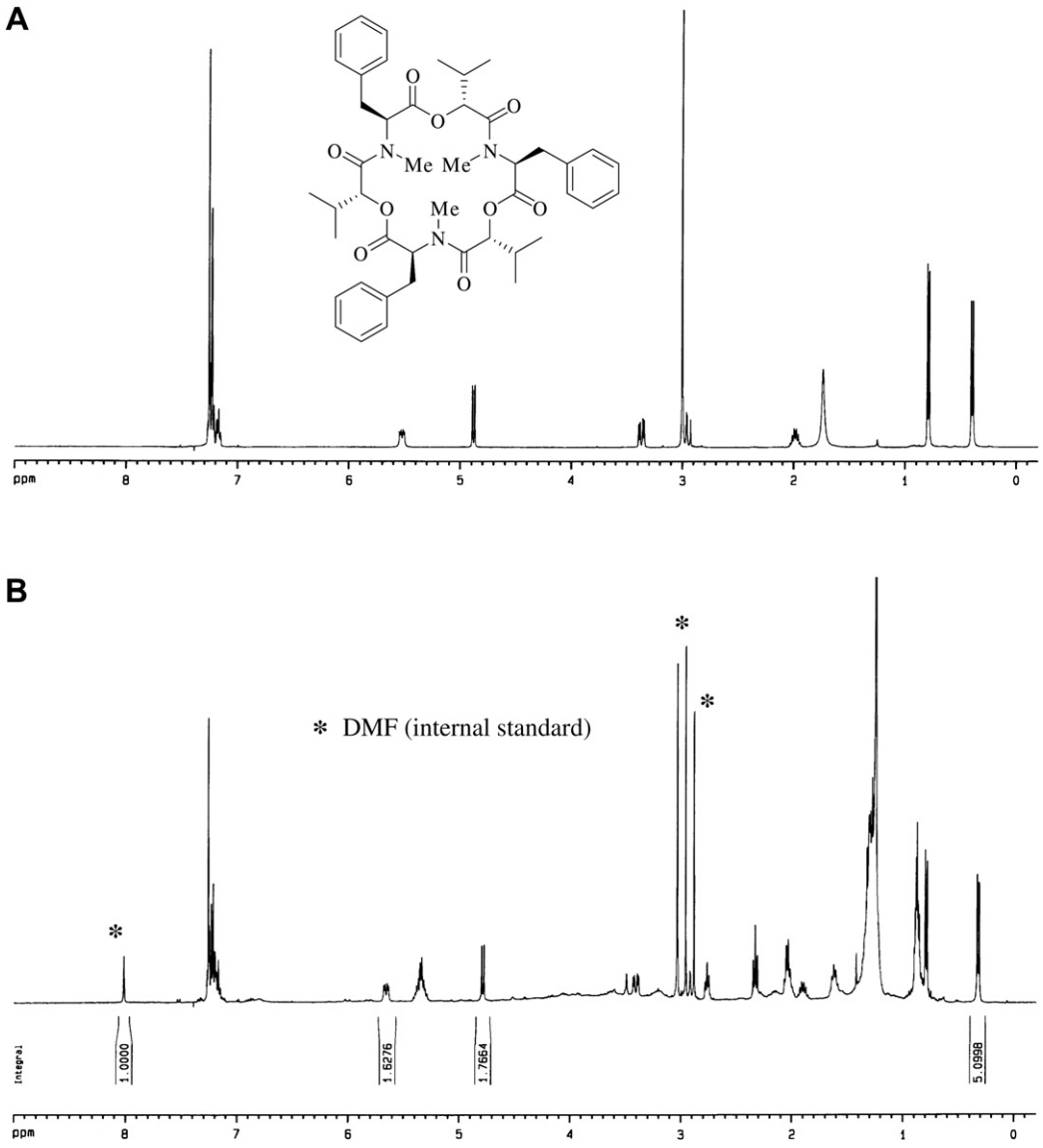

Fig 1 – ^{1}H NMR (400 MHz, $CDCl_3$) spectra. (A) Beauvericin (standard sample). (B) Mycelial extract from *Isaria tenuipes* ARSEF 2488 with dimethylformamide (DMF, 10 μmol).

Materials and methods

Fungal samples

Thirty-six hypocrealean isolates comprising six *Isaria* species and their purported *Cordyceps* teleomorphs; the phylogenetically related *Beauveria* and morphologically related *Paecilomyces sensu lato* and *Nomuraea* species were sequenced and tested for beauvericin production (Table 1). The strains used in this study were obtained from CBS, ARSEF and BIOTEC.

Cultivation of fungi for molecular work

For DNA extraction purposes, all fungal samples were grown in Sabouraud Dextrose Broth (Difco) for 7–14 d at 25 °C, mycelia were then harvested by filtration and washed with sterile distilled water several times. Mycelia were lyophilised and DNA was extracted as previously described (Luangsa-ard *et al.* 2004, 2005).

PCR amplification and purification

Amplification was done in 50 μl volume consisting of 1× PCR buffer, 200 μM of each of the four dNTPs, 2.5 mM $MgCl_2$, 1 U SuperTaq polymerase (HT Technologies; Cambridge, UK) and 0.5 μM of each primer using primers ITS4 and ITS5 (White *et al.* 1990) and Bt2a and Bt2b (Glass & Donaldson 1995). A GeneAmp PCR System 9700 (Applied Biosystems, Singapore) was used with the following parameters for ITS: 2 min at 96 °C; denaturation 1 min at 96 °C, annealing 1 min at 55 °C, extension 2 min at 72 °C (35 cycles) and 8 min at 72 °C; and for the β-tubulin gene: 3 min at 94 °C; denaturation 1 min at 94 °C,