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An experimental strategy towards optimising directed biosynthesis of communesin analogues by *Penicillium marinum* in submerged fermentation

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ABSTRACT

It was previously demonstrated that a fungus producing communesin alkaloids, subsequently identified as *Penicillium marinum*, could also accept 6-fluoro analogues of tryptophan or tryptamine to form mono-fluoro-communesin analogues in addition to communesins. A strategy to increase the relative yield of analogues by mutation to impair decarboxylation of tryptophan has been studied. Four mutants with much reduced activity of tryptophan decarboxylase, and other phenotypic change, were selected from 1500 colonies from spores that survived a 99 % kill treatment with N-methyl N-nitro N-nitrosoguanidine. Tlc assessment of cell-associated products from standard submerged fermentations showed that one non-sporing mutant apparently produced little or no communesins, but productivity was restored when grown in a medium supplemented with glutamine. However, more sensitive mass spectrometric analysis detected both communesins A and B in mycelium grown on a rich, yeast extract–sucrose agar, showing that deletion of communesin biosynthesis was not absolute. It was concluded that mutagenesis had generally achieved its objective, but that new literature on a putative role of aurantioclavine in communesin biosynthesis presented an additional challenge to integrate the prenylation of tryptophan before its decarboxylation, which is a characteristic of ergot alkaloid biosynthesis.

Introduction

Communesins were first described as metabolites of a fungus assigned to *Penicillium commune* (Numata *et al.* 1993). Concurrently, another fungus, studied at the Pfizer laboratories because of its anthelminthic activity, produced the same compounds, and was subsequently used to explore biosynthesis of communesin alkaloids (Wigley *et al.* 2006). However, according to the morphological characteristics used in the taxonomy of *Penicillium* as per Pitt (1980), the fungus could not be assigned to *P. commune* (Wigley 1995), although conidiophores were similarly terverticillate. However, it is now clear that the fungus is the recently described species *P. marinum* (Frisvad & Samson 2004). *P. marinum* is slower growing than the closely related *P. expansum*, but unlike *P. expansum* does not cause rot in apples. Another concurrent study of a biverticillate *Penicillium*, undertaken at the UK Central Science Laboratory, Slough (K. Scudamore & M. Hetmanski, pers. comm.), isolated compounds that were toxic to brine shrimps. The electron impact mass spectrometry (EIMS) and ^{1}H-nuclear magnetic resonance (NMR) spectra of the compounds corresponded to those of communesins A and B (Wigley 1995),

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but the fungus in question is now described as another distinct species, *P. buchwaldii* (Frisvad & Samson 2004). Similarly, a fungal metabolite, named nomofungin (Ratnayaka *et al.* 2001, 2003), produced by an unidentified endophytic fungus obtained from the bark of *Ficus microcarpa*, is now instead recognised as communesin B (May *et al.* 2003).

Subsequently, communesins have been recognised as typical metabolites of the ubiquitous *P. expansum* and as occasional contaminants in fruit juices (Andersen *et al.* 2004), confirming the wide occurrence of communesin biosynthesis within *Penicillium*, and elevating communesins as potential contaminants for food and fruit juices.

The potential for directed biosynthesis of communesin alkaloids by administration of indolic precursor analogues was demonstrated with respect to 6-fluoro derivatives of tryptophan and tryptamine (Wigley 1995; Wigley *et al.* 2006). It was notable that, although two molecules of tryptophan are used in communesin biosynthesis as the source of the tryptamine

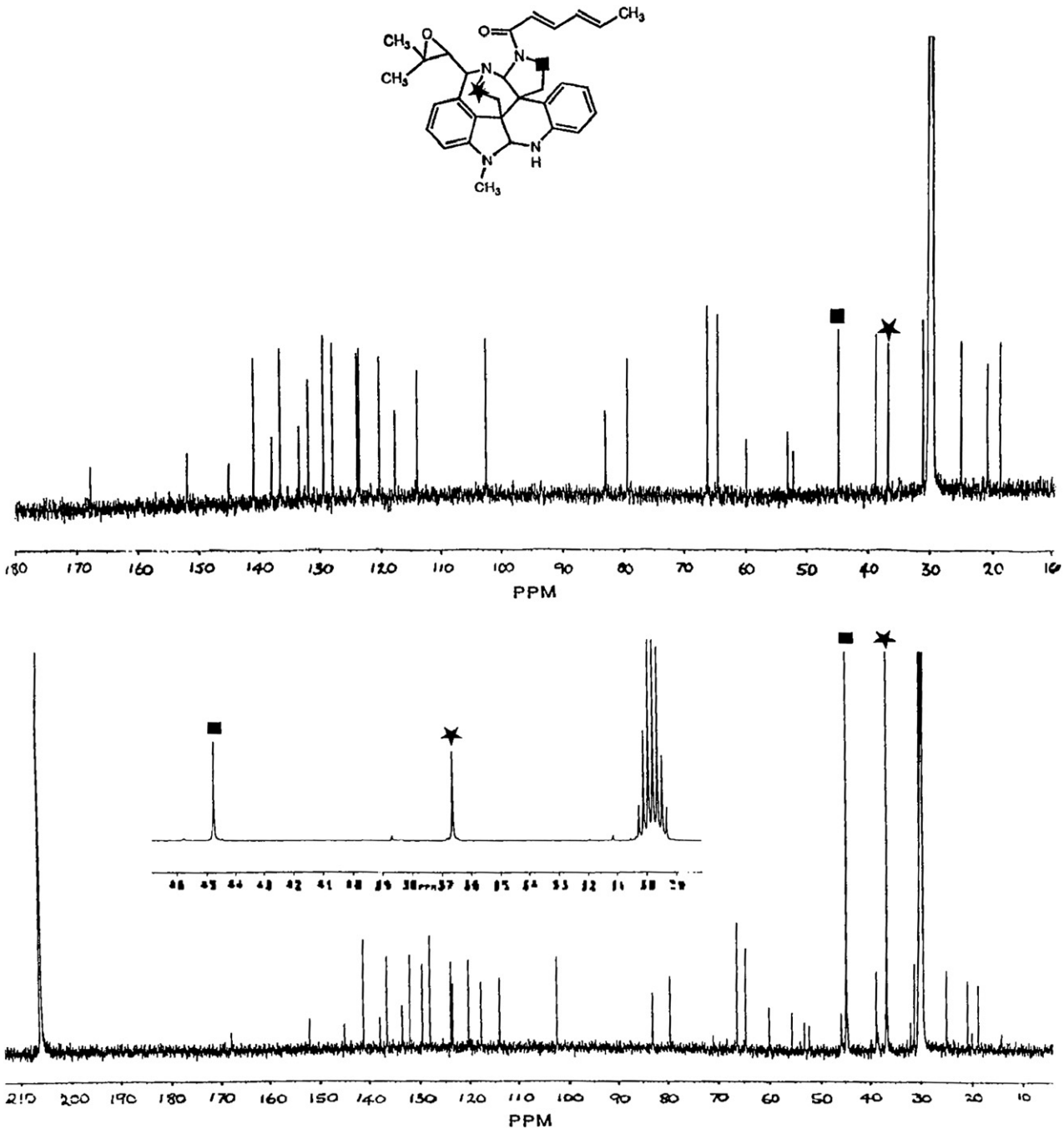

Fig 1 – ^{13}C NMR spectra of communesin B. Above, natural abundance spectrum; below, enriched spectrum showing the location of the carbon atom derived from 2-^{13}C tryptophan, similarly enriching two positions in communesin B.