The candididin gene cluster from *Streptomyces griseus* IMRU 3570

Ana Belén Campelo and José A. Gil

A 205 kb DNA region from *Streptomyces griseus* IMRU 3570, including the candididin biosynthetic gene cluster, was cloned and partially sequenced. Analysis of the sequenced DNA led to identification of genes encoding part of a modular polyketide synthase (PKS), genes for thioesterase, macrolactone ring modification, mycosamine biosynthesis and attachment to the macrolide ring, candididin export and regulatory proteins. It represents the first extensive genetic characterization of an aromatic polyene macrolide antibiotic biosynthetic gene cluster. Of particular interest is the presence of the CanP1 loading domain (the first described as responsible for the activation of an aromatic starter unit) and the polypeptide CanP3 (carrying modules for the formation of five out of seven conjugated double bonds). Disruption of the *pabAB* gene that encodes the starter unit of candididin abolished its production [which was restored when exogenous *p*-aminobenzoic acid (PABA) was supplied to the culture] and resulted in an enhanced production of another antifungal compound that is barely detected in the wild-type.

**Keywords**: aromatic polyene antibiotic, type I PKS, genetic disruption

**INTRODUCTION**

Polyketides are a large group of complex natural products produced mainly by species of *Streptomyces* and related filamentous bacteria that include antibacterial, antifungal, anticancer, antiparasitic and immunosuppressant compounds. Despite their structures being strikingly diverse, they share a common pattern of biosynthesis in which simple carboxylic acid units are condensed onto the growing chain by a polyketide synthase (PKS) in a process resembling fatty acid biosynthesis (Hopwood & Sherman, 1990; Katz & Donadio, 1993). Each unit contributes two carbon atoms to the assembly of the linear chain, of which the β-carbon always carries a keto group. After each condensation, the β-keto group of the growing chain may remain unchanged or be reduced to a hydroxyl, enoyl or methylene group. The second carbon atom donated by each unit to the growing polyketide chain carries different substituents depending on its origin: only hydrogen in the case of acetate residues; a methyl or ethyl group for propionate or butyrate; and others for the rarer building units. The structural diversity of naturally occurring polyketides arises from the choice of building units, the extent of β-ketoreduction, the possibility of chirality at one or more carbon atoms and the total length of the chain. Further variety is produced by functionalization of the polyketide chain by the action of glycosylases, methyltransferases and oxidative enzymes.

Macrolide polyketides are formed through the action of type I modular PKSs. These enzymes usually consist of several large polypeptides housing modules (sets) of enzymic activities, each module containing the enzymes required to catalyse a particular round of elongation (Cortès et al., 1990; Donadio et al., 1991; Bevitt et al., 1992; Swan et al., 1994; Schwecke et al., 1995; Aparicio et al., 1996, 1999, 2000; Brautaset et al., 2000). The modules are arranged in the multienzyme polypeptides in the order in which they are used, but the order of the genes encoding them may or may not be co-linear with the order of action of the polypeptides.

Polyene macrolides are a group of polyketides with lactone rings of 20–44 members. They contain a chromophore formed by a system of three to eight conjugated double bonds in the macrolactone ring that...
contributes to the interaction with sterols present in the membrane of fungi leading to formation of transmembrane pores and cell death (Gil & Martin, 1995).

Although polyene antibiotics are usually described as antifungal agents, some of them have antibacterial, antiviral and immunostimulating activities (Gil & Martin, 1995). CANDICIDIN is an aromatic polyene (heptaene) antibiotic produced by Streptomyces griseus IMRU 3570 (Fig. 1). It was first described by Lechevalier et al. (1953) and named antibiotic C135, although it was renamed candicidin because of its strong activity against species of Candida.

The biosynthetic gene clusters for two non-aromatic polyene antibiotics, pimaricin (Aparicio et al., 1999, 2000) and nystatin (Brautaset et al., 2000), have been analysed so far. In both cases a type I PKS is involved in the biosynthesis of the macrolactone ring. Here, we report the cloning and partial analysis of a large gene cluster from S. griseus IMRU 3570 involved in the biosynthesis of candicidin.

**METHODS**

**Bacterial strains, plasmids and culture conditions.** The bacterial strains and plasmids used are listed in Table 1. Streptomyces griseus IMRU 3570 was cultured in SPG medium which supported high antibiotic production. SPG medium is 25 g soy peptone 1\(^{-1}\), 60 g glucose 1\(^{-1}\) and 0.143 g zinc sulfate 1\(^{-1}\). Cultures were incubated at 32°C in triple-baffled flasks containing 100 ml SPG in a rotary shaking incubator operating at 220 r.p.m. The production flasks were inoculated with 10 ml of a 24 h inoculum grown in YED medium (10 g glucose 1\(^{-1}\) and 10 g yeast extract 1\(^{-1}\)). The production medium SPG was supplemented, when required, with p-aminobenzoic acid (PABA), o-aminobenzoic acid (anthraminic acid), p-coumaric acid and p-aminosalicylic acid at final concentration of 0.3 mM.

**CANDICIDIN determination and detection.** CANDICIDIN was extracted from liquid cultures with n-butanol and measured spectrophotometrically as described previously (Gil et al., 1980) or spotted onto silica gel plates. Chloroform/methanol/20% NH\(_2\)OH (2:2:1, by vol.) was the solvent used for thin-layer chromatography of the antibiotic. CANDICIDIN was also tested by bioassay against Candida utilis CECT 1061.

**DNA isolation, manipulation and characterization.** Total DNA isolation from S. griseus and recombinant DNA techniques in Streptomyces and Escherichia coli were as described by Hopwood et al. (1985) and Sambrook et al. (1989). For the construction of the genomic library, S. griseus genomic DNA was partially digested with Sau3AI and fragments in the range of 35–40 kb were cloned into SuperCos 1 (Stratagene) digested with BamHI and XbaI. The library was initially screened using the 4.6 kb BamHI fragment of S. griseus.

**Table 1.** Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Properties</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptomyces griseus IMRU 3570</strong></td>
<td>Wild-type, candicidin producer</td>
<td>IMRU*</td>
</tr>
<tr>
<td><strong>Streptomyces griseus ABC28</strong></td>
<td>S. griseus IMRU 3570 derivative with pabAB disrupted, candicidin non-producer</td>
<td>This work</td>
</tr>
<tr>
<td><strong>Escherichia coli XL1-Blue MR</strong></td>
<td>Host for maintaining the cosmid library</td>
<td>Stratagene</td>
</tr>
<tr>
<td><strong>Escherichia coli DH5</strong></td>
<td>General cloning host</td>
<td>BRL</td>
</tr>
<tr>
<td><strong>Escherichia coli ET12567(pUZ8002)</strong></td>
<td>Strain for intergeneric conjugation</td>
<td>Flett et al. (1997)</td>
</tr>
<tr>
<td><strong>Candida utilis CECT 1061</strong></td>
<td>Strain for candicidin bioassay</td>
<td>CECT*</td>
</tr>
<tr>
<td><strong>SuperCos 1</strong></td>
<td>Cosmid used in the construction of the S. griseus IMRU 3570 library</td>
<td>Stratagene</td>
</tr>
<tr>
<td><strong>pOJ260</strong></td>
<td>ColEl replicon, oriT, Am(^{\text{R}}), 3.5 kb</td>
<td>Biernert et al. (1992)</td>
</tr>
<tr>
<td><strong>pCAB</strong></td>
<td>pOJ260 derivative containing the 4.6 kb BamHI DNA fragment with the pabAB::tsr</td>
<td>This work</td>
</tr>
<tr>
<td><strong>pIJ486</strong></td>
<td>Source of thiostrepton resistance gene (tsr)</td>
<td>Ward et al. (1986)</td>
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* CECT, Spanish Type Culture Collection (Colección Española de Cultivos Tipo), Universidad de Valencia, 46100 Burjasot (Valencia), Spain; IMRU, Institute of Microbiology Rutgers University, The State University, New Brunswick, NJ, USA.
griseus containing the *pabAB* gene (Gil & Hopwood, 1983; Criado et al., 1993).

**DNA sequencing and analysis.** Sequencing clones were constructed with the Erase-a-Base kit (Promega), converted into ssDNA by standard procedures (Sambrook et al., 1989) and sequenced by the dideoxynucleotide chain-termination method of Sanger et al. (1977) using Sequenase (Amersham). Deazanucleotides were used to eliminate compression problems. Sequence analysis was performed with DNASTAR, database similarity searches were done at the BLAST and BLITZ public server (EBI), and multiple alignments of sequences were done by using CLUSTAL W (EBI).

**RESULTS AND DISCUSSION**

**Cloning of the candicidin biosynthetic gene cluster**

The candicidin biosynthetic gene cluster was identified by genomic walking using an *S. griseus* IMRU 3570 cosmid library and a 4-6 kbp BamHI DNA fragment that contains the *pabAB* gene of *S. griseus* involved in biosynthesis of the starter unit for this antibiotic (Gil & Hopwood, 1983; Criado et al., 1993). Two cosmids (Cos R62 and Cos G33) were isolated and their inserts were hybridized with the labelled *eryAII* gene of *Saccharopolyspora erythraea* (Tuan et al., 1990; Bevitt et al., 1992). This probe was chosen because it contains β-ketoacyl ACP synthase (KS), acyltransferase (AT), dehydratase (DH), enoylreductase (ER), β-ketoreductase (KR) and acyl carrier protein (ACP) domains. Only cosmid Cos G33, but not Cos R62, gave positive hybridization with the *eryAII* gene. Preliminary DNA sequence analysis of the insert of cosmid Cos G33 showed that a part of it encodes a type I PKS. Therefore, the rest of the putative PKS should be located downstream from the DNA cloned in cosmid Cos G33. The 3′ end of cosmid Cos G33 was used as probe to find more cosmids that hybridized with the *eryAII* gene. After several rounds of hybridizations with the 3′ end of the *eryAII*-hybridizing cosmids, a total of 205 kbp of contiguous DNA was cloned on 11 cosmids (Fig. 2a), which were mapped with BamHI, KpnI, NotI, PaeI and SacI.

**Overall organization of the region involved in candicidin biosynthesis**

Restriction fragments from the 11 cosmids were probed with the labelled *eryAII* gene and about 126 kbp of continuous DNA was found to hybridize (Fig. 2a). This result is in agreement with the expectation for the 21-step condensation process required for the biosynthesis of candicidin and the previous results of Hu et al. (1994) for the gene cluster of the heptaene macrolide FR-008. The ends of this hybridizing DNA segment are the 4-6 kbp BamHI DNA fragment used as the starting point for chromosome walking and a 1-2 kbp PaeI DNA fragment from Cos S54. Sometimes larger DNA fragments that hybridized covered small DNA fragments which did not hybridize to the PKS probe.

**Fig. 2.** Organization of the candicidin biosynthetic gene cluster. (a) Inserts from the overlapping recombinant cosmids encompassing the cloned region. NotI restriction sites are shown. Shading indicates the cloned region hybridizing with probe *eryAII* from *Saccharopolyspora erythraea*. (b) Genes identified within the analysed sequence (Cos G33, Cos I80 and 1.2 kbp PaeI DNA fragment from Cos S54). The arrowheads indicate transcription direction. Dashed lines indicate incomplete genes.
Table 2. Deduced functions of the putative genes identified in the candicidin biosynthetic gene cluster

<table>
<thead>
<tr>
<th>Gene designation</th>
<th>Product</th>
<th>Proposed function</th>
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<tbody>
<tr>
<td>orf1 (incomplete)</td>
<td>Transcriptional activator</td>
<td>Regulation</td>
</tr>
<tr>
<td>orf2</td>
<td>Transcriptional activator</td>
<td>Regulation</td>
</tr>
<tr>
<td>orf3</td>
<td>Transcriptional activator</td>
<td>Regulation</td>
</tr>
<tr>
<td>canG</td>
<td>Glycosyltransferase</td>
<td>Attachment of mycosamine</td>
</tr>
<tr>
<td>canA</td>
<td>Aminotransferase</td>
<td>Mycosamine biosynthesis</td>
</tr>
<tr>
<td>canC</td>
<td>Cytochrome P450</td>
<td>Oxidation of a methyl group at C-18</td>
</tr>
<tr>
<td>canF</td>
<td>Ferredoxin</td>
<td>Electron transfer in P450 system</td>
</tr>
<tr>
<td>canT</td>
<td>TE</td>
<td>Release of polyketide chain from PKS</td>
</tr>
<tr>
<td>pabAB</td>
<td>PABA synthase</td>
<td>Starter unit for candicidin biosynthesis</td>
</tr>
<tr>
<td>canP1</td>
<td>Type I PKS</td>
<td>Candicidin PKS</td>
</tr>
<tr>
<td>canRA</td>
<td>ABC transporter</td>
<td>Efflux of candicidin</td>
</tr>
<tr>
<td>canRB</td>
<td>ABC transporter</td>
<td>Efflux of candicidin</td>
</tr>
<tr>
<td>canP3</td>
<td>Type I PKS</td>
<td>Candicidin PKS</td>
</tr>
<tr>
<td>canP2 (incomplete)</td>
<td>Type I PKS</td>
<td>Candicidin PKS</td>
</tr>
<tr>
<td>canPF (incomplete)</td>
<td>Type I PKS</td>
<td>Candicidin PKS</td>
</tr>
<tr>
<td>canM (incomplete)</td>
<td>GDP-mannose-4,6-dehydratase</td>
<td>Mycosamine biosynthesis</td>
</tr>
</tbody>
</table>

Fig. 3. Functional organization of the candicidin PKS proteins described in this work, including CanP1, CanP2 (incomplete) and CanP3. Each domain is represented by a rectangle whose length is proportional to the size of the domain. The dashed line indicates an incomplete protein.

To start the analysis of the cloned region, the inserts from cosmids Cos G33, Cos I80 and the 1-2 kb PaeI DNA fragment from Cos S54 were subcloned in E. coli plasmids and sequenced. Computer-assisted analysis of the sequenced region led to identification of the genes shown in Fig. 2(b) and listed in Table 2. They include genes for a modular PKS as well as genes for chain-termination, modification, mycosamine biosynthesis, export and regulation.

PKS genes involved in the first steps of candicidin biosynthesis

Four genes, canP1, canP3, canP2 (incomplete) and canPF (incomplete), encoding parts of a type I PKS were identified. canP1 is transcribed convergently to canP3 and canP2. The canPF 5'-end is contained in the last restriction fragment (a 1-2 kb PaeI DNA fragment from Cos S54) that hybridizes with the eryAII gene, establishing one end of the PKS gene cluster. The functional features of the deduced products encoded by these genes were determined by comparing them to the well known type I erythromycin PKS and are shown in Fig. 3.

Analysis of CanP1 reveals a loading module in its N-terminal region that contains a putative ATP-dependent carboxylic acid:CoA ligase (CoL) and an ACP domain. Such an activating domain has been identified in the rapamycin (Schwecke et al., 1995) and pimaricin (Aparicio et al., 1999) biosynthetic gene clusters and is involved in the initiation of aglycone biosynthesis. The loading domain is fused with the first condensing module that includes KS, AT and ACP domains, leaving the β-keto group unreduced.

CanP2 has not been fully studied and the N-terminal region remains unknown. So far, we have identified three modules. All of them have methylmalonate-specific AT (mAT) domains. The last module of CanP2 contains KS, mAT, KR and ACP domains, processing the β-carbon up to a hydroxyl group in the polyketide chain. The adjacent module includes extra DH and ER domains that account for the formation of a methylene group. The last known module of CanP2 houses mAT, KR and ACP domains; a KS is most probably situated upstream from the mAT domain. If so, this module would process the β-carbon up to a hydroxyl group in the growing polyketide chain.
CanP3 consists of five identical modules with KS, AT, DH, KR and ACP domains, responsible for the formation of five (out of seven) conjugated double bonds.

CanPF was identified by sequencing the 1.2 kb Paet DNA fragment from Cos S54. Two incomplete ORFs were determined; one of them displays the C-terminal region of a protein similar to GDP-mannose-4,6-dehydratases and the other corresponds to the N-terminal region (KS domain) of a modular PKS polypeptide (CanPF). Therefore, CanPF presumably represents one end of the PKS gene cluster.

We propose that candicidin aglycone biosynthesis starts on CanP1, a polypeptide with two modules (a loading domain and module 1) and continues on CanP2 (modules 2–4) and CanP3 (modules 5–9). Candicidin is an aromatic molecule and the chain starter unit is PABA. Chain initiation might require activation of PABA by the loading domain, which would be followed by the first elongation step catalysed by the adjacent module (module 1). The nascent chain would be transferred by the ACP of module 1 to the KS of the first module of CanP2 (module 2). Taking into account the co-linearity of the order of modules within each polypeptide with the order of their utilization during biosynthesis in type I PKSs (Donadio et al., 1991; Yu et al., 1999) and the structure of the candicidin molecule, it is very probable that complete sequencing of CanP2 would not reveal any modules beyond the three described here. CanP2 would be responsible for the incorporation of three molecules of methylmalonyl-CoA as extender units into the growing polyketide chain, which is in good agreement with candicidin structure. After the three elongation steps carried out by CanP2 the chain would be transferred to CanP3, which would catalyse formation of most of the chromophore (five out of seven conjugated double bonds). The presence of a single polypeptide responsible for the formation of five conjugated double bonds is unprecedented.

It is notable that all domains that have been studied so far contain the following active site motifs: GPXX-XXXTACSS and the two invariant His residues located 135 and 175 aa on the C-terminal side of the active site Cys for KS domains (Aparicio et al., 1996); GHSXG ‘complemented’ with three conserved active site residues Gln, Arg and His for AT domains (Aparicio et al., 1996; Serre et al., 1995); HXXXGXXXXP for DH domains (Bevitt et al., 1992; Aparicio et al., 1996); the potential NADP(H) binding GXGXGXXXA for KR and ER domains (Scrutton et al., 1990); and LGXDSLXXVE for ACP domains.

Genes for chain-termination and aglycone modification

The thioesterase (TE) gene (canT) included in the 4.6 kb BamHI DNA fragment described previously (Criado et al., 1993) has been located in this work. The deduced product (256 aa) shows a percentage of identity much higher to the TE encoded by independent genes in the pikromycin (pikAV), tylosin (tylO), nystatin (nysE) and pimaricin (pimI) biosynthetic gene clusters of Streptomyces venezuelae, Streptomyces fradiae, Streptomyces noursei and ‘Streptomyces natalensis’, respectively (Xue et al., 1998; Merson-Davies & Cundliffe, 1994; Brautaset et al., 2000; Aparicio et al., 2000) than to the TE domains located at the C termini of many modular PKSs. All the above biosynthetic clusters include two TE activities, one of them embedded in the PKS and the other one in a discrete gene. It has been proposed that the separate additional TE proteins might be essential to unblock (‘proof read’) the PKS complex after blockage with aberrant incomplete polyketide chains (Butler et al., 1999; Tang et al., 1999). If so, the candicidin biosynthetic gene cluster probably contains another TE activity located in the PKS and responsible for chain-termination and ring closure to form the candicidin aglycone.

Two genes in the analysed sequence, canC and canF, encode proteins possibly involved in modification of the candicidinolide. CanC (393 aa) is a P450 monoxygenase and shows a high percentage of identity along its full length with TylH1 (40.6%), RapJ (35.9%) and EryF (33.2%), which are P450 enzymes involved in the biosynthesis of tylosin, rapamycin and erythromycin, respectively (Fouces et al., 1999; Molnár et al., 1996; Haydock et al., 1991). By analogy with a similar oxidation in the biosynthesis of nystatin (Brautaset et al., 2000), CanC would catalyse the oxidation of a methyl group at C-18. This is based on the assumption that a propionate residue is introduced in the 13th elongation step.

The finding of a ferredoxin gene (canF) immediately downstream of the P450 monoxygenase gene strongly indicates that the deduced protein is the in vivo electron transport protein physiologically involved with the haem protein. CanF is a small acidic protein (64 aa) very similar to ferredoxins containing [3Fe–4S] clusters, such as PimF (Aparicio et al., 2000), SuaB (O’Keefe et al., 1991), SubB (O’Keefe et al., 1991), TylH2 (Fouces et al., 1999) and RapO (Molnár et al., 1996).

Genes for mycosamine biosynthesis and attachment

The cluster includes at least three genes possibly involved in biosynthesis and transfer of the candicidin deoxysugar moiety mycosamine. canG and canA are located on one side of the PKS gene cluster and canM (incomplete) is on the other side.

CanG (458 aa) shows up to 27% identity with eukaryotic enzymes that belong to the UDP-glycosyltransferase family. Therefore, this enzyme might be involved in the attachment of mycosamine to the candicidin aglycone at C-21. The deduced product of canA (352 aa) shows considerable similarity to proteins that belong to the EryCl family. The members of this family are part of a large group of proteins involved in carbohydrate metabolism in either antibiotic or outer-cell-wall biosynthesis, which appear to be aminotransferase or DH enzymes dependent on pyridoxal phosphate or pyridoxamine phosphate as a cofactor, respectively (Kim et al., 1998).
CanA includes the conserved active sites Asp (aa 154) and Lys (aa 176) that are important for pyridoxal phosphate binding. The fact that mycosamine contains an amino group suggests that CanA is an amino-transferase involved in mycosamine biosynthesis that requires pyridoxal phosphate as cofactor. CanM reveals high similarity to GDP-mannose-4,6-dehydratases and it is plausible that it is involved in one of the initial steps in mycosamine biosynthesis.

Because the candicidin biosynthetic gene cluster has not been fully sequenced, further analysis might reveal other genes involved in mycosamine biosynthesis. However, no other mycosamine biosynthesis genes different from those described in this work have been reported to be present in the completely sequenced nystatin and pimaricin biosynthetic gene clusters (Brautaset et al., 2000; Aparicio et al., 2000), although both of these metabolites contain mycosamine. This suggests that the rest of the enzymes required for deoxysugar formation could be recruited from different metabolic pathways.

**Regulatory region and export genes**

The deduced products of *orf1, orf2* and *orf3* show significant sequence similarity to the transcriptional regulators found in the upstream region of the cholesterol oxidase/cytochrome P450 operon of *Streptomyces* sp. SA-COO (Molnár & Murooka, 1993). These genes lie upstream of the region containing *canG-canA-canC-canF-canT-pabAB-canP1-canRA-canRB* genes (*pabAB* sequenced by Criado et al., 1993) and are transcribed in the opposite direction. Such putative regulatory genes have also been identified in the nystatin biosynthetic gene cluster where disruption of one of them abolished nystatin production (Brautaset et al., 2000). In candicidin biosynthesis, the deduced products of *orf1, orf2* and *orf3* might have a regulatory function, although experimental confirmation is required.

The last two genes that are transcribed in the same direction as *canG, canA, canC, canF, canT, pabAB* and *canP1* are *canRA* and *canRB*. Their deduced products display high degrees of identity to proteins belonging to the ATP-dependent ABC transporter superfamily and might be involved in candicidin export. CanRA (335 aa) exhibits up to 49% identity to ATP-binding proteins belonging to the ABC transporter superfamily, particularly several from *Streptomyces* species involved in the efflux of antibiotics. All these proteins, including CanRA, display single Walker A and Walker B motifs (Walker et al., 1982) and loop 3 (Hyde et al., 1990). CanRB (268 aa) is very similar to transmembrane proteins described for members of the ABC transporter.
Candicidin synthase in *S. griseus*

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**Disruption experiments**

It has been suggested that *S. griseus* IMRU 3570 is not a suitable host for molecular genetic manipulation (Martin & Gil, 1984). However, we decided to use new vectors that had worked in other strains with the same problems to attempt disruption of some genes in the candicidin cluster of *S. griseus* IMRU 3570. After several attempts to disrupt *pabAB*, *canP1* and *canG*, we obtained one positive result: disruption of *pabAB*. PabAB is responsible for the formation of PABA, which is the immediate precursor of the aromatic moiety of candicidin (Liu *et al*., 1972; Martin & Liras, 1976; Gil *et al*., 1980). The disrupted strain (*S. griseus* ABC28) was obtained in a conjugation experiment between *E. coli* ET12567(pUZ8002) (Flett *et al*., 1997) transformed with pCAB and *S. griseus* IMRU 3570. pCAB was constructed by cloning the 4-6 kb *BamHI* DNA fragment containing *pabAB*::tsr in the *BamHI* site of the conjugative suicide vector pOJ260 (Bierman *et al*., 1992). *S. griseus* ABC28 is the result of a double homologous recombination between the chromosomal *BamHI* fragment (containing *pabAB*) and the pCAB insert (containing *pabAB*::tsr) (Fig. 4).

*S. griseus* ABC28 and *S. griseus* IMRU 3570 were grown in a production medium and the presence of candicidin was assayed by spectrophotometry and TLC. No candicidin production was observed in the tested trans-conjugant as compared to the wild-type. However, when *S. griseus* ABC28 and *S. griseus* IMRU 3570 supernatants were bioassayed against *C. utilis*, haloes of antifungal activity were obtained in both cases. Auto-biography confirmed that *S. griseus* ABC28 did not synthesize candicidin, directing its metabolism to synthesize an antifungal compound whose presence in the wild-type is minimal (Fig. 5a).

PABA and some of its structural analogues were added to *S. griseus* ABC28 cultures at a final concentration of 0.3 mM. Candicidin production was restored only with the addition of PABA (Fig. 5b), suggesting that the PKS involved in candicidin biosynthesis is particularly specific for the natural substrate (PABA). Since the data shown in Fig. 5(b) are quantitative, it is clear that *p*-aminosalicylic acid inhibits the production of the new antifungal compound.

**Candicidin biosynthetic pathway in *S. griseus* IMRU 3570**

A partial analysis of the candicidin biosynthetic gene cluster led to the identification of four PKS genes and 11 more genes, all of them with a plausible role in candicidin biosynthesis. It is very probable that synthesis starts with PABA activation by the loading domain of CanP1, followed by a first-chain extension cycle catalysed by the module located in the C terminus of CanP1 and CanRB constitute an ABC transporter, structurally formed by two homodimeric elements that might be involved in ATP-dependent efflux of candicidin.
Fig. 6. Proposed biosynthetic intermediates for the first chain elongation steps in candidin biosynthesis.

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REFERENCES


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