Streptomyces griseus 45H, a producer of the extracellular autoregulator protein factor C, is a member of the species Streptomyces albidoflavus

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Streptomyces griseus strain 45H, isolated in 1960 during a mutagenesis programme on the industrial streptomycin producer S. griseus 52-1, encodes an extracellular, pleiotropic autoregulatory signalling protein, factor C, which stimulates sporulation of S. griseus 52-1 in submerged culture. The facC gene, which codes for factor C, is present in very few streptomyces and is not present in S. griseus 52-1. Based on 16S rRNA gene sequencing and other molecular data, S. griseus 45H, the factor C producer, is here shown to be related to the original laboratory strain of Streptomyces flavofungini, which was being studied in the same laboratory in 1960, and to Streptomyces albidoflavus. Southern blotting revealed that three out of four independently isolated strains of S. albidoflavus possess facC. Both the original strain of S. flavofungini and S. griseus 45H are therefore identified as members of the species Streptomyces albidoflavus, and we propose that S. griseus 45H should be renamed Streptomyces albidoflavus 45H.

It has been known for a long time that antibiotic production is restricted to a specific short period of the complex developmental cycle of the producer streptomycetes and that the regulation of antibiotic production is intimately interconnected with morphogenesis (Chater, 1998). This connection underlines the continuing interest in studying the morphological differentiation of many Streptomyces strains.

Streptomyces griseus 45H was isolated during a mutagenesis programme aimed at the isolation of morphological mutants (non-sporulating and hypersporulating) of the industrial streptomycin producer strain Streptomyces griseus 52-1 (Szabó et al., 1960, 1961). S. griseus 45H has been described as a strain that sporulates well both on solid medium and in liquid culture, the latter being a rather rare property of Streptomyces strains. In addition, the strain secreted a substance, named factor C, into the culture medium that also induced cytodifferentiation of S. griseus 52-1 and stimulated sporulation in liquid culture.

The activity of factor C was shown to be due to a protein (Biró et al., 1980). The cloning and sequencing of the facC gene (Birkó et al., 1999), which encodes this extracellular signalling protein, revealed a gene with no close relatives in the database at that time. The identity and mode of action of factor C, the morphological differentiation of S. griseus 45H and the effect of factor C on the differentiation of S. griseus 52-1 have been the subject of many publications (for a complete list see http://web.dote.hu/~sbiro/).

Interaction between factor C and the regulon of S. griseus controlled by the best known γ-butyrolactone autoregulator, A-factor (Ohnishi et al., 2005), has been described recently (Birkó et al., 2007).

Since the two S. griseus strains (52-1 and 45H) differed in several substantial properties (Table 1), the origin of S. griseus 45H has often been questioned (Fehér & Szabó, 1978). Unquestionable evidence that the strain S. griseus 45H could not be a descendant of S. griseus 52-1 was obtained when the cloned factor C gene (facC) did not hybridize to the genomic DNA of S. griseus 52-1 (Birkó et al., 1999). The same experiment also showed that (i) the restriction digestion patterns of S. griseus 52-1 and S. griseus 45H are different and (ii) the restriction digestion and hybridization patterns of S. griseus 45H and our laboratory strain of Streptomyces flavofungini (referred to here as the Szabó lab strain) are highly similar. These observations prompted us to study the relatedness of these
Table 1. Properties that differ between S. griseus strains 52-1 and 45H

<table>
<thead>
<tr>
<th>Property</th>
<th>52-1</th>
<th>45H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyene antibiotic</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A factor</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Factor C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sporulation in liquid culture</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

strains by 16S rRNA gene sequence comparison, which has proved to be successful in the differential identification of members of the genus Streptomyces (Stackebrandt et al., 1997; Mehling et al., 1995).

The 16S rRNA gene sequences of S. griseus 45H, our laboratory strain of S. flavofungini and S. griseus 52-1 were determined by direct sequencing of the 16S rRNA gene PCR product as described by Kim et al. (2000) and a 16S rRNA gene sequence-based phylogenetic tree of these strains, related streptomycetes and representative strains of the genus Streptomyces was constructed (Fig. 1). The 16S rRNA gene sequences of S. griseus 45H and the laboratory strain of S. flavofungini were identical, and different from the sequence of S. griseus 52-1 (96.0% similarity). The sequence for S. griseus 52-1 matches the database entries for S. griseus strain 2247 rRNA operons rRNA–E (GenBank accession numbers AB030567–AB030572) and other S. griseus strains (Fig. 1b).

The 16S rRNA gene sequence for the descendent of the original laboratory strain of S. flavofungini ['Actinomycetes flavofungini' (U´ri and Békési) Szabó and Preobrazhenskaya 1962; Gause et al., 1986] does not match the recently determined sequences for the deposited type strain of S. flavofungini, NRRL B-12307 (GenBank accession no. AY999792) and NBRC 13371 (AB184359). These sequences also differ from the sequence for our laboratory strain of S. flavofungini (97.21 and 97.40% similarity). Instead, the sequences for S. griseus 45H and our laboratory strain of S. flavofungini are identical to the 16S rRNA gene sequences of members of the Streptomyces albidoflavus species group (GenBank accession numbers DQ855477, DQ978978 and others).

S. griseus 45H and S. flavofungini from our culture collection are clearly identified as members of the same species. At the time when S. griseus 45H was isolated, research was being undertaken in the same laboratory with S. flavofungini, the producer of a potential antifungal antibiotic flavofungin (U´ri & Békési, 1958). Considering the techniques available in the 1960s for strain identification and the classification of S. griseus and S. albidoflavus together, as grey-spored series, e.g. classified in cluster 1 in the numerical taxonomic classification of Williams et al. (1983), our recent molecular data indicate that S. griseus 45H was probably picked up as a laboratory contaminant of the strain of S. flavofungini.

The identification of strain 45H as a member of the species S. albidoflavus suggested that other members of this species group should carry the facC gene. When four members of the S. albidoflavus species group were tested for facC by Southern blotting, three (Streptomyces canescens ICSSB 1001, Streptomyces odorifer ICSSB 1004 and Streptomyces sampsonii ICSSB 1011) gave strong signals. Only the type strain of S. albidoflavus Rossi Doria 1891 (ICSSB 1006), which has been in laboratory culture for a long time and is relatively distinct from the rest of the members of the species group (Ferguson et al., 1997), did not give a signal (Fig. 2). Since previous studies have indicated that facC is absent from many Streptomyces genomes, including the completely sequenced genomes of Streptomyces coelicolor A3(2) and Streptomyces avermitilis MA-4680, it may be specifically associated with S. albidoflavus and related species. Indeed, Southern blotting with 21 further Streptomyces strains (S. Biró and others, unpublished) revealed homologous DNA in just one, Streptomyces albus 391. On the other hand, recent genome sequencing has revealed related, but somewhat diverged genes in strains of Streptomyces scabies, Saccharopolyspora erythraea (formerly Streptomyces erythraeus) and Streptomyces ambofaciens. The facC-like genes in S. scabies and S. ambofaciens are located near to a terminus of the linear chromosomes, a region that is frequently involved in deletion, rearrangements and the acquisition of DNA by horizontal gene transfer. This could...
account for the sporadic occurrence of facC genes in diverse Streptomyces strains.

Based on the above data we propose that, in future, S. griseus 45H, the producer of the extracellular pleiotropic autoregulatory protein factor C, is identified as Streptomyces albidoflavus and should be named Streptomyces albidoflavus 45H.

Note added in proof

After submitting this manuscript, the Streptomyces griseus IFO 13350 genome database became available for BLAST search. As expected, S. griseus IFO 13350 does not contain a factor C gene.

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References


