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 streptomycetes 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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *S. griseus* strains 52-1 and 45H and *S. flavofungini* Szabó lab strain are EF571001–EF571003, respectively.

Table 1. Properties that differ between *S. griseus* strains 52-1 and 45H

Property	52-1	45H
Production of:		
Streptomycin	+	–
Polyene antibiotic	–	+
A factor	+	–
Factor C	–	+
Sporulation in liquid culture	–	+
Utilization of:		
Citrate	+	–
Arabinose	–	+

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* [*Actinomyces flavofungini*? (Úri 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^T (GenBank accession no. AY999792) and NBRC 13371^T (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 (Úri & 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 1002^T, *Streptomyces odorifer* ICSSB 1004^T and *Streptomyces sampsonii* ICSSB 1011^T) gave strong signals. Only the type strain of *S. albidoflavus* Rossi Doria 1891 (ICSSB 1006^T), 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

Fig. 1. Phylogenetic positions of *S. griseus* 45H (a) and *S. griseus* 52-1 (b) within the *Streptomyces albidoflavus* and *Streptomyces griseus* 16S rRNA gene sequence phylogenetic clades. The phylogenetic trees were generated using Jukes & Cantor (1969) distances and the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values (Felsenstein, 1985) expressed as percentages were calculated from 1000 replicates.

Fig. 2. Southern blot analysis of representative strains of the *S. albidoflavus* group using the *facC* gene as a probe. Left panel: SacII restriction digests of chromosomal DNA. Right panel: hybridization patterns. Hybridization was carried out as described in Birkó *et al.* (1999). Lanes: λ , HindIII digest of phage λ DNA; 1, *S. albus* 391; 2, *S. griseus* 45H; 3, *S. flavofungini* Szabó lab strain; 4, *S. canescens* ICSSB 1002^T; 5, *S. odorifer* ICSSB 1004^T; 6, *S. albidoflavus* ICSSB 1006^T; 7, *S. sampsonii* ICSSB 1011^T.

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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