

## **Study of the sporulation, pathogenicity and taxonomic identification of the factor C producer *Streptomyces griseus* 45H strain**

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**Doktori Iskola: Molekuáris Sejt- és Immunbiológia**

The secreted signalling protein factor C was isolated from the culture fluid of a strain originally identified as *Streptomyces griseus* 45H. Our recent molecular data in line with previous findings indicates that *S. griseus* 45H is not a descendant of *S. griseus* 52-1 but was probably picked up as a laboratory contaminant of the strain of *S. flavofungini*. Based on 16S rRNA sequences and Southern hybridization, *S. griseus* 45H and *S. flavofungini* from our culture collection are clearly identified as the same and belong to the *S. albidoflavus* group. Therefore *S. griseus* 45H should be named as *Streptomyces albidoflavus* 45H in the future.

*S. griseus* 45H has been described as a strain that sporulates well both on solid medium and in liquid culture. A null mutant of *S. griseus* 45H was designed by replacing the chromosomal copy of factor C gene by its *in vitro* inactivated form. The candidate gene replacement mutants showed the expected *bald* phenotype; however, instead of the expected homologous recombination probably induced genome rearrangement happened that resulted in deletion to part of the factor C gene as it was revealed by Southern hybridization. The resulting *bald* phenotype and its complementation by the cloned factor C gene confirmed that factor C plays a key role in controlling cellular differentiation.

Southern hybridization and database searches revealed only very few *Streptomyces* strains with homologous genes and proteins. One of them was the potato scab causing *S. scabies*. *Nec1* gene was used as an indicator of the presence of the large pathogenicity island (PAI) that confers pathogenicity to different *Streptomyces* strains. Using PCR reaction and Southern hybridization the *nec1* gene and some part of PAI was detected in *S. europaeiscabiei* CFBP4501, *S. griseus* 45H and its null mutant but not detected in *S. griseus* 52-1.

A good correlation was found between the production of a zinc-induced heat-stable extracellular esterase and the pathogenicity of the strains mentioned above. Both the pathogenicity and esterase activity was decreased but not lost in null mutant which suggest that factor C has a possible regulatory role in the production of extracellular esterase; however the change in enzyme activity can not be attributed to codeletion of factor C gene and the esterase gene. Regarding to the possible interaction between Factor C and A-factor regulon

and characteristics of extracellular enzymes regulated by Factor C as well as the fact that the protein binding site located upstream of extracellular esterase gene is highly similar to the AdpA consensus binding site, we assume that Factor C plays a regulatory role in the production of extracellular hydrolases.

It can be concluded that signalling protein Factor C secreted by *S. albidoflavus* 45H has a key role in morphological differentiation and probably regulates the production of different extracellular hydrolases including a zinc-induced, heat-stable esterase which is involved in scab development, furthermore, the *nec1* gene, another important virulence factor, is also present in that strain.

**Tárgyszavak:** *Streptomyces*, C faktor, 16S rRNS, null mutáns, *nec1*, extracelluláris észteráz

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