

FEAST OR FAMINE: NUTRIENT MEDIATED REGULATION OF DIFFERENTIATION IN *STREPTOMYCES GRISEUS*

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The genus *Streptomyces* comprises Gram+, soil-dwelling, filamentous bacteria. It shows complex morphological differentiation that relates to the production of secondary metabolites. This ability makes *Streptomyces* biotechnologically significant bacteria since they produce 75% of the known antibiotics. Extracellular autoregulatory molecules play a key role in controlling antibiotic production and morphological differentiation. In *Streptomyces griseus* A-factor (2-isocaprolyl-3R-hydroxymethyl- γ -butyrolactone) proved to be significant, whose synthesis depends on the *afsA* gene. Our aim is to understand the regulation of A-factor mediated differentiation and antibiotic production in *Streptomyces griseus*. We studied an A-factor negative (AFN) mutant to shed light on the genetic and physiological background of the AFN phenotype. Previous results showed that the *afsA* gene is functional and transcribed in the AFN strain. In this study the production of AfsA protein, aerial mycelium, spores, extracellular protease and antibiotics was followed on rich and minimal medium. The AfsA protein was detected both in the control and AFN strains by Western blotting in cultures growing on rich or minimal medium. The presence of AfsA in the AFN strain supposes that AfsA protein is not enough for sufficient A-factor production. Additional elements are also required that might be involved in the synthesis of A-factor or in the regulation of this process. This also repels the hypothesis that A-factor production would be regulated by the expression of *afsA* gene. Moreover our data suggest different regulation of morphological differentiation on rich and minimal medium. On rich medium the AFN strain did not form aerial mycelium due to the lack of A-factor but spores were formed from the substrate mycelium in old cultures. On minimal medium aerial mycelium emergence and sporulation were observed in AFN although the amount of spores was decreased compared to the control strain. These data suggest that on rich medium A-factor is essential for aerial mycelium but not for spore formation. On minimal medium other nutrient stress mediated pathways have primary role in the induction of morphological differentiation. The production of antibiotics and extracellular proteases was lower in AFN than in the control strain on both rich and minimal medium.

This confirms that A-factor is essential for antibiotic production either by direct gene activation or by the induction of enzymes (extracellular proteases) that provide nutrients for antibiotic synthesis. A crosstalk between A-factor and nutrient stress mediated pathways may contribute to sufficient sporulation and antibiotic production in *Streptomyces griseus*.

Understanding the details of this regulation pattern may lead to the development of antibiotic overproducing strains or to the identification of new type of antibiotics.

CEFOTAXIMASE (CTX-M) AND QUINOLONE RESISTANCE GENES (QNR) WITH ADDITIONAL ANTIMICROBIAL RESISTANCE MECHANISMS IN COMMENSAL *ESCHERICHIA COLI* FROM HEALTHY PIGS

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Concerning the importance of food producing animals as potential reservoirs of enteric bacteria with clinically relevant antimicrobial resistance traits, we tested the prevalence of extended-spectrum β -lactamase (ESBL)-producing and fluoroquinolone resistant *Escherichia coli* from pigs in order to identify multiple resistance mechanisms circulating in pig farms in Hungary and Croatia with special regards to plasmid mediated genes encoding cefotaximases (CTX-M) and quinolone resistance (*qnr*). For this purpose, faecal samples were collected from pigs representing three farms from Hungary and six farms from Croatia with 45 and 60 samples respectively. Farms were located in separate regions of the countries. Cefotaxime or nalidixic acid resistance were used as prime markers for the isolation of multiresistant *E. coli* strains. A second selection was based on resistance to additional antimicrobials (i.e. gentamicin) aiming to reduce the collection to isolates with representative multiresistance phenotypes. In several cases more than two different multiresistance phenotypes have been isolated from the same pig, which were considered as independent *E. coli* isolates. This collection of multidrug resistant *E. coli* contained 139 strains and was tested for the presence of *bla*CTX-M and *qnr* genes by PCR. Selected isolates carrying genes *bla*CTX-M and/or *qnr* are being subjected for confirmation and further typing of antimicrobial resistance genes by using the PCR-microarray AMR05. Cefotaxim resistant *E. coli* have been detected in one Hungarian and one Croatian farm representing 17% of all pigs tested. In majority of the strains, the plasmid-related resistance phenotypes such as ampicillin, cefotaxim, gentamicin and tetracycline occurred in multiple combinations. In 11% of the strains the coexistence of Ctx-Nal phenotypes was detected, together with the presence of the cefotaximase gene *bla*CTX-M.

E. coli strains with nalidixin resistance phenotype have been predominantly (70%) characterizing healthy pigs independently from the farm and country of isolation. Ciprofloxacin resistant strains occurred on one farm only. The plasmid-mediated fluoroquinolone resistance gene *qnrS* was identified in 11% of the strains, with or without the nalidixin-ciprofloxacin resistant phenotype, while genes *qnrA* and *qnrB* were absent. Our results lead to conclude that multiresistant commensal *E. coli* strains carrying plasmid-mediated CTX-M type cefotaximase and/or quinolone resistance genes in different combinations are widespread on some pig farms but much less on others, most likely reflecting differences in use of antimicrobials.

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