Lack of A-factor production induces the expression of nutrient scavenging and stress-related proteins in *Streptomyces griseus*

Zsuzsanna Birkó¹, Magdalena Swiatek⁴, Emília Szájli², Katalin F. Medzihradszky²,³, Erik Vijgenboom⁴, András Penyige¹, Judit Keserű¹, Gilles P. van Wezel⁴*, and Sándor Biró¹,*

¹Department of Human Genetics, Faculty of Medicine, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen, Nagyerdei körút 98, Hungary.

²Proteomics Research Group, Biological Research Center, Hungarian Academy of Sciences, H-6726 Szeged, Temesvári körút 62, Hungary.

³Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 941582517.

⁴Microbial Development, Leiden Institute of Chemistry, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands.

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*Corresponding authors:

Sándor Biró, Department of Human Genetics, Faculty of Medicine, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen, Nagyerdei körút 98, Hungary.
Tel/FAX +36-52416531. E-mail: sbiro@med.unideb.hu

Gilles P. van Wezel, Microbial Development, Leiden Institute of Chemistry, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands. Tel. +31-715274310. FAX: +31-715274340. E-mail: g.wezel@chem.leidenuniv.nl
SUMMARY

The small γ-butyrolactone A-factor is an important autoregulatory signaling molecule for the soil-inhabiting streptomycetes. Starvation is a major trigger for development, and nutrients are provided by degradation of the vegetative mycelium via a process of programmed cell death, reusing proteins, nucleic acids and cell wall material. The A-factor regulon includes many extracellular hydrolases. Here we show via proteomic analysis that many nutrient scavenging and stress-related proteins are overexpressed in an A-factor nonproducing mutant (AFN) of *Streptomyces griseus* B2682. Transcript analysis shows that this is primarily due to differential transcription of the target genes during early development. The targets include proteins relating to nutrient stress, environmental stress and an orthologue of the *Bacillus* sporulation control protein SpoOM. The enhanced expression of these proteins underlines the stress that is generated by the absence of A-factor. Wild-type developmental gene expression is restored to AFN by the signaling protein Factor C, in line with our earlier observation that Factor C triggers A-factor production.

Keywords: *Streptomyces griseus*; A-factor; Factor C; Sporulation; Secretion; ABC transporter; Stress
INTRODUCTION

Bacteria of the Gram-positive filamentous *Streptomyces* are a well-known model system for the study of prokaryotic multicellular differentiation. They have a complex mycelial life cycle starting with a vegetative mycelium that develops into aerial mycelium, which then produces chains of spores at the ends of the hyphae (1). The onset of development is triggered by nutritional signals (2) and temporally relates to the production of antibiotics and other secondary metabolites (3). Autoregulatory molecules play a key role in controlling both the onset of cellular differentiation and secondary metabolism. The best studied autoregulator is A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone), a small microbial hormone-like molecule (243 Da) that induces both morphological and physiological differentiation in *Streptomyces griseus* (4, 5). The γ-butyrolactone regulatory system is widespread in Streptomycetes. Virginiae butanolids control virginiamycin production in *Streptomyces virginiae* (6) and SCB1 plays an important role in the control of actinorhodin and undecylprodigiosin biosynthesis and a cryptic, type I polyketide synthase (cpk) gene cluster in *Streptomyces coelicolor* (7, 8). In *Streptomyces griseus* binding of A-factor to its cellular receptor ArpA derepresses expression of the transcriptional activator AdpA. While initially identified as the activator of streptomycin production through *strR*, this protein acts as a central switch, and the AdpA regulon includes several important positive regulators of development (*ssgA, amfR, and adsA (bldN)*) and secreted proteases, reviewed in (9, 10). A-factor deficient mutants are neither able to sporulate nor to produce antibiotics (streptomycin).

Another interesting autoregulator is the secreted signaling protein Factor C (Mw 34.555 KDa), originally isolated from the culture fluid of “*Streptomyces griseus 45H*” (11) which was recently shown to be identical to a laboratory strain known as *Streptomyces flavofungini*, itself a member of the *Streptomyces albidoflavus* species group (12). The Factor
C producer strain like *S. griseus* readily sporulates in submerged culture (13). Similarly to A-factor, Factor C also plays a key role in cellular communication and cytodifferentiation.

A-factor mutants fail to develop aerial hyphae and spores and are therefore classified as bald mutants. Expression of *facC* from a low-copy plasmid in a spontaneous A-factor-deficient bald mutant of *S. griseus* NRRL B-2682 restored its A-factor production as well as aerial mycelium and spore formation on solid media (14). The wild-type strain itself does not produce Factor C, as shown by immunoblotting (15) and by DNA hybridization studies (16). Our previous results (14) indicate a connection between two highly divergent types of signaling molecules and possible interplay between their regulatory networks. In preliminary experiments we observed characteristic differences between the extracellular proteomes of the strains that prompted detailed further analysis, facilitated by the currently available DNA sequence of the genome of *S. griseus* IFO13350 (17). Here we showed that the bald AFN mutant overexpressed several ABC transporter solute-binding proteins and stress response proteins compared to the wild type *S. griseus* B2682 strain or to the *facC* transformant of the AFN mutant in an effort to supply the cells with nutrients.
EXPERIMENTAL PROCEDURE

Strains and preparation of extracellular protein fractions

Strains of *Streptomyces griseus* were grown on R2YE agar plates (18) covered with a PCTE membrane (Poretics 0.2 μm pore size). The strains were *S. griseus* NRRL B-2682 (parental strain; in short B2682), its A-factor non-producing bald mutant *S. griseus* NRRL B-2682 AFN (AFN) and a transformant of AFN (designated AFN/pSGF4) that harbors *facC* on the pHJL401-based low-copy-number plasmid pSGF4 (16). Protein extracts were prepared from spent agar of surface-grown cultures by crumbling the solid medium and passing it through a syringe with frits at 4 °C by centrifugation. Samples of approximately 300 μg protein (measured using the Coomassie Protein Assay Reagent, Pierce) were purified using the ReadyPrep™ 2-D Cleanup Kit (Bio-Rad) according to the instructor's manual, and dissolved in Rehydration Buffer (8M urea, 2% CHAPS, 50 mM dithiothreitol (DTT), 0.2% 100x Bio-Lyte 3/10 (or 4/7) ampholyte, 0.002% Bromophenol Blue).

2D gel electrophoresis and image analysis

Separation of protein extracts (approximately 300 μg) in the first dimension was performed by isoelectric focusing using 17 cm long Immobiline DryStripsGels (IPG) in the pH range of 3-10 or 4-7 (Bio-Rad) on a Protean IEF cell (Bio-Rad). Samples were focused at 250 V for 15 minutes followed by an increase to 8000 V over 2.5 h and kept at this voltage for 45000 V-h. Focused strips were separated on the basis of relative molecular weight in the second dimension on 13% SDS-polyacrylamide gels in a Protean II XL vertical gel system (Bio-Rad). For quantitative comparison of extracellular protein profiles gels were stained with colloidal Coomassie G-250 (19). Gels were scanned using a GS-800 Imaging Densitometer (Bio-Rad) and images were analyzed with PDQuest™ software (Bio-Rad). Histograms
comparing spot quantity were generated with this software. Two-fold differences between the mutant and the parental B2682 were considered as significant changes. The data below are from a single representative experiment but at least two additional, biological replicas were performed and they showed similar results.

**In-gel digestion**

Gel slices containing 2D PAGE-separated proteins were cut and diced and then washed with 25 mM NH₄HCO₃ in 50% (v/v) acetonitrile/water. After reduction with 20 μl 10 mM DTT (30 min at 56 °C) and alkylation with 25 μl 55mM iodoacetamide (30 min at room temperature, in the dark) the proteins were digested with side-chain-protected porcine trypsin (Promega, Madison, WI, U.S.A.) at 37 °C for 4 h. Tryptic digests were extracted into 1%FA/50%ACN/H₂O solution and desalted on C18 ZipTips (Millipore, Bedford, MA, U.S.A.). Mass spectrometric analysis of the unfraccionated tryptic digests was performed in positive-ion, reflectron mode, on a Reflex III MALDI-TOF mass spectrometer (Bruker, Karlsruhe, Germany), using 2,5-dihydroxybenzoic acid as the matrix. Two-point external calibration was applied; this guarantees a mass accuracy within 200 ppm. The peak lists were generated with X-Tof (version 5.1.5) software. Mascot database search software (http://www.matrixscience.com/) was used to identify proteins in the full NCBI database (2007.11.16, 5633163 sequences). Searches also were performed using an in-house Mascot server (v2.2.04), and a *Streptomyces griseus* database (2008, 7138 sequences) downloaded from http://streptomyces.nih.go.jp/gview/download.cgi?molecule= TG&item=sgr_cds_am). Protein scores greater than 80 (full NCBI database search) or 51 (S. griseus database) were considered significant (P < 0.05).

Search parameters were as follows: mass accuracy: 200 ppm; only tryptic cleavages were permitted; and 2 missed cleavages were considered. Carbamidomethylation of Cys-residues
was a fixed modification; methionine oxidation, protein N-acetylation and pyroglutamic acid formation from N-terminal Gln residues (and Glu), and Me esterification of Asp and Glu residues (CBB-staining side reaction proven by PSD (20) were the considered variable modifications. Esterified peptides were only accepted when the peptide was also detected without the modification. Protein identification was confirmed by sequence information obtained from MS/MS (post source decay) spectra, acquired in 10-12 steps, lowering the reflector voltage by 25% in each step, then stitching the data together. Some MS/MS experiments were performed on an Agilent XCT Plus 3D ion trap equipped with an atmospheric pressure MALDI source using 4-OH-α-CN-cinnamic acid as the matrix. Search parameters for MS/MS data: Mass accuracy 200 ppm for the precursor ion and 1 Da for the fragment ions. Cleavage specificity and covalent modifications were considered as described above. MS/MS spectra are shown in supplementary Figures S1-S10. Instead of the accession number the SGR Locus number is listed in Table 1., except for protein ID#14 which is not present in the sequenced S. griseus IFO 13350 strain.

RNA isolation and RT-PCR

For transcript analysis, RNA was isolated from surface-grown cultures of S. griseus B2682 or its A-factor nonproducing derivative AFN. Mycelium was grown on R2YE agar plates and harvested after 36 hrs (onset of aerial growth) or 54 hrs (sporulation). RNA isolation and semi-quantitative RT-PCR was carried out twice, as described previously (21). 200 ng of RNA was used for each reaction (concentration was assessed using a Nanodrop® spectrophotometer). Oligonucleotides used for RT-PCR are presented in Supplementary material Table S1.
RESULTS AND DISCUSSION

Extracellular proteome analysis

The extracellular proteomes of *S. griseus* B2682, of its A-factor non-producing spontaneous mutant (AFN) and of AFN complemented with a plasmid expressing Factor C (called AFN/pSGF4; (16)) were studied. AFN has a nonsporulating (bald) phenotype, and introduction of Factor C-expressing plasmid pSGF4 restores wild-type levels of sporulation to this strain (16). The choice for the extracellular proteome is a logical consequence of our focus on extracellular signaling mechanisms, with many of the proteins that are part of the A-factor/AdpA response regulon extracellularly localized.

To obtain an initial assessment of the protein expression profiles in the different strains, SDS-PAGE was performed, which revealed that several protein bands varied strongly in intensity. Considering the low separative capacity of 1D-gels the samples were analyzed further by 2D-proteome analysis, and initial analysis with a pI range of 3-10 revealed that the vast majority of the proteins appeared in the pI range of 4-7, which was then used for all experiments. Over 200 detectable protein spots were identified on each gel by colloidal Coomassie staining (Figure 1). In total 42 spots differed significantly between the parent and the mutants and these were analyzed by MALDI-TOF mass spectrometry. In a previous study (14) we showed that Factor C acts by restoring A-factor production and normal sporulation to *S. griseus* AFN. Restoration of A-factor production also resulted an increase in the expression of several A-factor responsive secreted proteases to wild-type levels. However, at the time detailed analysis of the proteomes of AFN and AFN/pSGF4 was severely hampered by the lack of adequate genome information of *S. griseus*. Though our data now indicate (see below) that most proteins studied are highly conserved in *S. coelicolor* and *S. avermitilis* a single amino acid substitution in the peptides will prevent protein identification. This significant
hurdle was overcome once the full genome sequence of *S. griseus* had become publicly available (17).

The main question we sought to address this time is what proteins are expressed or overexpressed in the AFN mutant to compensate for the lack of extracellular hydrolases that normally supply the developing colonies with nutrients. 16 out of 42 spots fulfilled the selection criteria (for details see the Materials and Methods section) and they were unambiguously identified (Table 1). The expression profiles of all 16 protein spots were very similar in the 72 and 96 hr cultures, with the majority completely absent in wild-type cultures and in the *facC* complemented AFN strain. A few proteins were visible in B2682 and AFN/pSGF4 but with strongly reduced intensity (Fig. 1A-F. and Fig. 2A-B.). The 16 spots represented 10 different proteins, namely SGR1460 (#1), SGR2245 (#2), SGR3109 (#3-4), SGR1498 (#5-8), SGR5704 (#9), SGR2237 (#10), SGR5275 (#11) SGR1737 (#12-13), SGR5280 (#15-16) and SodA (#14). Interestingly, SodA or manganese superoxide dismutase is absent in the sequenced *S. griseus* strain IFO 13350. All proteins were overexpressed in both 72 h and 96h surface-grown cultures (Figure 2 A and B). Detailed information regarding the identification is provided in Table 1.

Interestingly, all proteins with the exception of SodA are well conserved in *S. avermitilis* and *S. coelicolor* (Table 1), which suggests that these proteins play a universal role in the *Streptomyces* life-cycle. In fact, the proteins are conserved (more than 50% aa identity for the gene products) in the sequenced genomes of *Streptomyces* species *S. clavuligerus* ATCC 27064, *Streptomyces* sp. Mg1, *S. sviceus* ATCC 29083, *S. pristinaspiralis* ATCC 25486, *Streptomyces* sp. SPB74, and *S. scabies* (data not shown). All identified proteins relate to stress, and may primarily be subdivided in environmental stress and nutrient stress (*i.e.* starvation) proteins. One exception is perhaps SGR5704, an orthologue of the *Bacillus* sporulation control protein Spo0M. However, sporulation results in
the autolytic breakdown of the vegetative mycelium and in fact it is a major stress mechanism in bacteria (22, 23).

The environmental stress control proteins are SGR1460, SGR2245 and SodA. SGR1460 is a putative ATP/GTP binding protein and a homologue of the *E. coli* osmotically inducible OsmC protein that is involved in osmotic stress response. Superoxide dismutase SodN (SGR2245) and SodA are involved in the control of oxidative stress. While these are predicted cytoplasmic proteins, SodF (SGR4906) is known to be autotransported as a leaderless protein (14, 24) and our experiments suggest that the same is true for SGR1460, SodN and SodA.

Several of the proteins with enhanced expression in AFN are involved in supplying nutrients to the growing colonies, and the strongly enhanced expression in AFN suggests that this strain suffers from nutrient deprivation. SGR3109 is an orthologue of the phosphoserine aminotransferase SerC of *Mycobacterium tuberculosis* (Accession Z73101; 66% aa identity), an aminotransferase that plays an important role in the amino acid and vitamin biosynthesis, notably of glycine, serine, threonine and vitamin B6. SGR1498 is an ABC transporter solute binding protein that is known to be involved in xylose uptake (25). SGR2237 is a predicted arginine/ornithine binding protein and likely involved in the transport of polar amino acids. SGR5275 is MalE or maltose-binding protein which is the solute binding protein belonging to the maltose uptake system in streptomycetes (26, 27). SGR1737 is a glutamate ABC transporter substrate-binding protein and likely involved in sensing and processing of environmental information. SGR1498, SGR1737, SGR2237 and SGR5275 all have an amino-terminal secretion signal sequence and a conserved prokaryotic membrane lipoprotein lipid attachment site with a cystein residue that is most likely a site for ADP-ribosylation.

Finally, SGR5704 is an orthologue of the well-studied sporulation control protein Spo0M of *Bacillus subtilis* and most likely in an operon with the phospholipid binding
protein SGR5703. Mutation/deletion in spo0M or overexpression of spoOM in *B. subtilis* results in impaired sporulation (28).

The identified proteins are all overexpressed in the AFN mutant, which is deficient in A-factor production. A-factor as a pleiotropic autoregulator in *S. griseus* during normal development induces the biosynthesis of several secreted hydrolases whose function is to digest biopolymers and supply the differentiating colony with nutrients (29, 30, 31). In the AFN mutant these hydrolases are not secreted (14), therefore the supply of small molecule nutrients is probably scarce.

Many of the proteins overexpressed in AFN are specific ligand-binding proteins associated to the transmembrane translocator component of the ABC transporters (31). It is likely that the overproduction of these nutrient scavenging proteins is a response to try and compensate for the reduced availability of nutrients. All of the ABC transporter substrate binding proteins have an amino-terminal secretion signal sequence and a properly positioned conserved prokaryotic membrane lipoprotein lipid attachment site with a cysteine residue. Secretion of these proteins is regulated by ADP-ribosylation. When the cystein is ADP-ribosylated these proteins cannot be exported to the surface and are therefore nonfunctional in transport (32). ADP-ribosylation-mediated inactivation of substrate binding proteins most likely takes place particularly during normal growth and development, when the supply of nutrients is plenty.

**Transcriptional analysis of the overexpressed proteins**

To our surprise, many of the overexpressed proteins detected extracellularly are predicted lipoproteins whose final destination is the cell surface, and their amount in the secretome therefore does not necessarily reflects their gene expression. To analyze if the enhanced
accumulation of proteins in the AFN mutant was at least in part due to enhanced transcription, RNA was isolated from surface-grown cultures of *S. griseus* B2682 and its AFN mutant derivative. For this, mycelium was grown on R2YE agar plates and harvested at the time when aerial mycelium was formed as well as during sporulation. We used *rpsI* (SGR2801, encoding ribosomal protein S9) as positive control, and reactions without reversed transcriptase step were performed to confirm the lack of DNA contamination in the samples. As shown in Figure 3, while *rpsI* transcript levels were the same in wild-type and mutant and did not vary in time, transcription of SGR1460, 1498, 1737, 2237, 2245, 3109, 5275, 5280 and 5704 was strongly upregulated in the AFN mutant during early aerial growth. This corresponds to the critical phase in the *Streptomyces* life-cycle when programmed cell death is initiated to provide the cells with nutrients. The transcriptional analysis shows that the enhanced protein levels observed in the proteome of AFN (Figures 1-2) is at least in part due to enhanced gene expression in the absence of A-factor. Interestingly, differences in transcript levels were far less pronounced during sporulation, with only SGR1460 and SGR3109 upregulated at this time point, while SGR5704 was lower in the mutant than in the parent. This strongly suggests that transcriptional upregulation is specific for the time corresponding to programmed cell death.

Interestingly, we also observe elevated protein levels for the stress-related proteins in the AFN mutant during sporulation, which is not explained by differences at the transcriptional level alone. A likely explanation is provided by the fact that A-factor activates a multitude of extracellular proteases (10, 14), and hence secreted proteins are expected to be much more stable in the A-factor nonproducing mutant AFN, further contributing to the accumulation of proteins in the mutant.

*ABC transporters and the control of Streptomyces development*
Streptomycetes are soil-dwelling bacteria that are able to utilize almost all naturally occurring polymers, such as starch, cellulose, chitin and agar. These polymers are degraded by extracellular hydrolases and subsequently imported (mainly as monomers or dimers) via specific permeases, either via ABC transporters or via the phosphotransferase system PTS (34, 35, 36, 37). Permeases that are involved in sensing and transport therefore substantially influence their metabolism and morphogenesis. An in silico survey (25) identified 81 ABC transporters in *S. coelicolor* that are involved in the uptake of sugars, oligopeptides and other nutrients. This large number of transporters and secreted hydrolases perfectly reflects the lifestyle of these microbes. In a recent analysis of the membrane-associated proteome (38) in *S. coelicolor* it was noted that a large proportion of these characterized proteins related to ABC transporter systems, including SCO5776, SCO2231 and SCO6009 whose orthologues we found in this study, which was considered as a snapshot of the nutritional requirement of the organism.

Interestingly, recent studies revealed that in fact several sugar transporters play a crucial role in the control of development, and in particular those that relate to N-acetylglucosamine transport and metabolism. These include the universal PTS components EI, EIIA\(^{Crr}\) and HPr (36, 2) as well as components of the DasABC transporter. DasABC transports chitobiose, the dimer of N-acetylglucosamine, and DasA is a lipoprotein that functions as the chitobiose binding protein (39). Besides in the transport of chitobiose DasA also plays a role in nutrient sensing, perhaps by interacting with the chiRS two-component system (34). The *dasABC* operon is developmentally regulated and plays a role in the control of sporulation in an A-factor independent manner in *S. griseus* (40, 41), and deletion of *dasA* results in a non-sporulating (bald) phenotype in *S. coelicolor* (34). Other oligomeric transporters that relate to the onset of development are for example the BldK oligopeptide transporter of *S. coelicolor* that is essential for sporulation on rich and glucose-containing media.
media (42) and a second developmentally regulated ABC transporter - SCO7167 - involved in morphogenesis of *S. coelicolor* (43).

*Superoxide dismutases in Streptomyces*

Superoxide dismutases are a ubiquitous part of the cellular defense system against oxidative stress. They convert the toxic superoxide to hydrogen peroxide which is further degraded by the enzyme catalase. So far in *Streptomyces* two classes of superoxide dismutases have been described, namely the nickel containing SodN and the iron-zinc containing SodF. We detected an additional superoxide dismutase, which has high similarity to the *Bacillus pumilus* SAFR-032 manganese superoxide dismutase SodA (YP_001487459). This protein so far has not been described in *Streptomyces* and may therefore represent a third class. In *S. pristinaespiralis* the expression of SodF is regulated by the SpbR protein, an analogue of the *S. griseus* ArpA and thus relating to the γ-butyrolactone regulon (44). It is also hypothesized that SodF is involved in adaptation to oxidative stress generated by metabolic shifts during colony development (44). In further support of this hypothesis, a catalase is also developmentally regulated and required for aerial mycelium formation in *S. coelicolor* (45). Previously (14) we identified SodF in *S. griseus* as upregulated in mutant strain AFN relative to the parental strain *S. griseus* B2682, suggesting that SodF is negatively controlled by AdpA. In this work we identified SodN as a protein upregulated in the AFN mutant. Interestingly, the presence of nickel in a micromolar concentration in the growth media induced the expression of SodN in *S. coelicolor*, whereas the expression of SodF was repressed (46). The differential expression of superoxide dismutases seem to suggest that a strict control of the level of SOD is of great importance to cellular defenses against oxidative stress.
The enhanced expression of the nutrient and environmental stress proteins in a strain that fails to produce (significant amounts of) A-factor underlines the stress that is generated by the absence of this signaling molecule.

The A-factor nonproducing strain of *S. griseus* detailed in this work is a valuable asset in the search for genes that control the production of A-factor and the response it elicits. The *afn* locus does not correspond to any of the known A-factor biosynthetic genes or regulatory genes, such as *afsA*, *adpA*, or *arpA*. Considering the fact that the *afn* mutant is able to produce A-factor under some conditions (such as after addition of the signaling protein Factor C) we anticipate that the mutation lies in a gene involved in the regulation of A-factor biosynthesis. The nature of the *afn* mutation and its role in the control of A-factor production is currently under investigation in our laboratories.

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Figures legends

Figure 1.
2D-gel electrophoresis maps of secreted proteins extracted from 72 h old surface-grown cultures of (a) B2682, (b) AFN, (c) AFN/pSGF4, and from 96 h old surface-grown cultures of (d) B2682, (e) AFN, (f) AFN/pSGF4. Numbers correspond to the ID number of proteins in Table 1.

Figure 2.
Quantitative image analysis of the proteins performed by PDQuest™ 2-D Analysis Software (Bio-Rad) in 72h old (2A) and 96 h old (2B) cultures. Numbers underneath each plot correspond to the protein ID # in Figure 1. and Table 1. The bars represent the relative amount of the different proteins in the strains in the order of B2682 (a), AFN (b) and AFN/pSGF4 (c).

Figure 3.
Transcriptional analysis by semi-quantitative RT-PCR. Samples were harvested from R2YE agar-grown mycelium of *S. griseus* B2682 and its AFN mutant. Time points: A, aerial growth (36 hrs); S, sporulation (54 hrs). No RT, the same as for RT-PCR with rpsI but without cDNA synthesis (control for absence of DNA contamination). Transcripts are indicated below the panels.
Table 1. Proteins with enhanced expression in *S. griseus* B2682 AFN. Match: peptides identified/masses searched; Sec* presence of a secretion signal. * SCO, *S. coelicolor* database; SAV, *S. avermitilis* database; % age between brackets refers to aa identity between the predicted SGR and SCO proteins.

| ID# | SSP | Accession | Protein name | Match | Seq. Cov. | Mr (kDa) | Sequence confirmed by PSD or CID | Sec* | ADP-ribosylation consensus | Orthologue in SCO/SAV * |
|-----|-----|-----------|--------------|-------|-----------|---------|----------------------------------|------|---------------------------|---------------------|
| 1   | 3002| SGR1460   | OsmC-like ATP/GTP-binding protein | 4/9   | 24%       | 14.5    | *VAHTNWEGNLIEGK*297              | no   | no                        | SCO6531 (83%), SAV1862 (84%) |
| 2   | 0004| SGR2245   | superoxide dismutase (SodN)     | 11/15 | 32%       | 15      | *YPELHQIINDTLK*297               | no   | no                        | SCO5254 (87%), SAV2988 (86%) |
| 3   | 4603| SGR3109   | putative aminotransferase       | 31/43 | 51%       | 39.4    | *ADIQIPADIKPADGR*10               | no   | no                        | SCO4366 (87%), SAV3883 (87%) |
| 4   | 4507| SGR3109   | putative aminotransferase       | 13/29 | 37%       | 39.4    | *ADIQIPADIKPADGR*10, *HIPEFSSLPTAIDNLSK*242 | no   | no                        | SCO4366 (87%), SAV3883 (87%) |
| 5   | 1502| SGR1498   | xylose ABC transporter solute-binding protein | 32/34 | 56%       | 38.8    | 1-27 *LAACG*25                   | 1-27 | *LAACG*25                 | SCO6009 (78%), SAV2247 (77%) |
| 6   | 2402| SGR1498   | xylose ABC transporter solute-binding protein | 22/27 | 39%       | 38.8    | *IGLLLPENQTAR*32, *GAGISPLPPVTQGDAELAGVQR*25 | 1-27 | 21LAACG*25                | SCO6009 (78%), SAV2247 (77%) |
| 7   | 2405| SGR1498   | xylose ABC transporter solute-binding protein | 41/49 | 56%       | 38.8    | *IGLLLPENQTAR*32, *GAGISPLPPVTQGDAELAGVQR*25 | 1-27 | 21LAACG*25                | SCO6009 (78%), SAV2247 (77%) |
| 8   | 2404| SGR1498   | xylose ABC transporter solute-binding protein | 31/41 | 44%       | 38.8    | *IGLLLPENQTAR*32, *GAGISPLPPVTQGDAELAGVQR*25 | 1-27 | *LAACG*25                 | SCO6009 (78%), SAV2247 (77%) |
| 9   | 8202| SGR5704   | putative SpoOM-type sporulation-control protein | 16/24 | 29%       | 28.5    | *IOGGSVAQHIEGLNVGLQAR*27, *GLHQVQELTVFADDR*205 | 1-17 | no                        | SCO1793 (81%), SAV6485 (83%) |
| 10  | 4305| SGR2237   | putative arginine/ornithine binding protein | 34/47 | 69%       | 33.5    | *SIEDLCGKPAAVOR*101, *SGGSGAVNYPVAVDLAR*249, *EAVIDAIIADGSYQK*299 | 1-25 | 29LTACG33                 | SCO5260 (58%), SAV2982 (58%) |
| 11  | 6701| SGR5275   | Maltooligosaccharide binding protein MalE | 21/45 | 43%       | 44.8    | *YVNPVFGDAQNK*24, *NAAQAGDGAPDVI*100 | 1-29 | 18ATACG22                | SCO2231 (48%), SAV5977 (48%) |
| 12  | 1307| SGR1737   | Glutamate ABC transporter substrate-binding protein | 33/35 | 55%       | 33      | *ADQPYLFEDQATK*30, *OQFAGPYK*158, *KPEYGASVELAK*209 | 1-22 | 19ATACG23                | SCO5776 (40%), SAV2485 (55%) |
| 13  | 1303| SGR1737   | Glutamate ABC transporter substrate-binding protein | 35/38 | 56%       | 33      | *ADQPYLFEDQATK*30, *OQFAGPYK*158, *KPEYGASVELAK*209 | 1-22 | 19ATACG23                | SCO5776 (40%), SAV2485 (55%) |
|   |   | manganase superoxide dismutase SodA |   | 9/14 | 56% | 22.4 | FKSDFAAAAAAGR | no | no |
|---|---|------------------------------------|---|------|-----|------|----------------|----|----|
| 15 | 2702 | SGR5280 | putative alpha-amylase | 21/25 | 27% | 60.7 | SAVFDNHDTER | 1-35 | no | SCO7020 (78%) | SAV5981 (73%) |
| 16 | 1803 | SGR5280 | putative alpha-amylase | 25/46 | 44% | 60.7 | SAVFDNHDTER | 1-35 | no | SCO7020 (78%) | SAV5981 (73%) |
Figure 1.
Figure 3