Proteomic response to the thaxtomin phytotoxin elicitor cellobiose and to the deletion of the cellulose utilization regulator CebR in *Streptomyces scabies*

Sören Planckaert[1], Samuel Jourdan[2], Isolde M. Francis[3], Benoit Deflandre[2], Sébastien Rigali[2,†,*], and Bart Devreese[1,†,*]

[1] Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, B-9000, Ghent, Belgium

[2] InBioS – Center for Protein Engineering, University of Liège, Institut de Chimie, B6a, B-4000, Liège, Belgium

[3] Department of Biology, California State University Bakersfield, CA 93311-1022, Bakersfield, USA

† These authors jointly supervised this work and are joint corresponding authors.

Bart Devreese

Laboratory for Microbiology,

Ghent University

K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Bart.devreese@ugent.be

Sébastien Rigali

Center for Protein Engineering

University of Liège

Institut de Chimie B6a, B-4000 Liège, Belgium

srigali@uliege.be

Abstract

Streptomyces scabies is responsible for common scab disease on root and tuber vegetables. Production of its main phytotoxin thaxtomin A is triggered upon transport of cellulose byproducts cellotriose and cellobiose, which disable the repression of the thaxtomin biosynthesis activator gene *txtR* by the cellulose utilization regulator CebR. To assess the intracellular response under conditions where *S. scabies* develops a virulent behavior, we performed a comparative proteomic analysis of wild-type *S. scabies* 87-22 and its *cebR* null mutant (hyper-virulent phenotype) grown in the absence or presence of cellobiose. Our study revealed significant changes in abundance of proteins belonging to metabolic pathways known or predicted to be involved in pathogenicity of *S. scabies*. Among these, we identified proteins of the cello-oligosaccharide-mediated induction of thaxtomin production, the starch utilization system required for utilization of the carbohydrate stored in *S. scabies*'s hosts, and siderophore synthesis utilization systems which are key features of pathogens in order to acquire iron once they colonized the host. Thus, proteomic analysis supported by targeted MS-based metabolite quantitative analysis revealed the central role of CebR as a regulator of virulence of *S. scabies*.

Keywords

Common scab disease, thaxtomin, proteomics, multiple reaction monitoring, siderophore, pyochelin, concanamycin, plant pathogen, CebR.

Introduction

Streptomyces species are Gram-positive, filamentous bacteria with a complex developmental life cycle involving the production of desiccation-resistant spores. The vast majority of *Streptomyces* spp. are soil saprophytes recycling nutrients in the environment. They are well known for the production of pharmaceutically and agriculturally important secondary metabolites such as antibiotics, anti-tumor agents, immunosuppressants, insecticides and pesticides.[1-2] However, some *Streptomyces* species are recognized as plant pathogens amongst which *Streptomyces scabies* is the best characterized species, being responsible for an important plant disease called common scab (CS) via the production and secretion of the phytotoxin thaxtomin A as the main virulence factor.[3] Thaxtomin A induces the formation of corky lesions on the surface of root and tubers crops, like potato, radish, beet and peanut reducing the marketability of these crops.[4] Thaxtomins are cyclic dipeptides (2,5-diketopiperazines formed by the condensation of L-4-nitrotryptophan and L-phenylalanine residues). The 4-nitroindole moiety is unique amongst microbial metabolites and is essential for virulence.[5] The thaxtomin biosynthetic genes (*txt*) reside on a toxicogenic region of a pathogenic island (PAI) within the genome, while other putative virulence genes such as *nec1* and *tomA* that are required for colonization of the plant host and detoxification of plant antimicrobial compounds, respectively, reside on a colonization region.[6-8] Recent studies demonstrated that the mobilization of this PAI is responsible for newly emerged pathogenic species.[7, 9]

Different triggers originating from plant material are associated with thaxtomin A production, i.e. xylan degradation products [10]; suberin, a lipidic plant polymer [11]; and degradation products of cellulose, cellobiose and cellotriose.[12] So far, only the cello-oligosaccharide mediated pathway of thaxtomin A production has been detailed at the molecular level. The ATP-binding cassette (ABC) transporter system CebEFG-MsiK triggers virulence by transporting cellobiose and cellotriose into the cell.[13] Cellobiose and, to a lesser extent, cellotriose interact with the cellulose utilization repressor CebR, which leads to the release of the repressor from its binding sites within the thaxtomin biosynthetic cluster.[14] The loss of the interaction of CebR with its *cis*-acting sequences allows the expression of *txtR,* which encodes the AraC/XylS family transcriptional activator allowing the expression of the thaxtomin A biosynthetic (*txt*) genes. Binding of cello-oligosaccharides to TxtR may increase the DNA-binding ability of TxtR and therefore the activation of the expression of *txt* genes resulting in the production of the phytotoxin.[15] Finally, we recently showed that the *bglC* gene downstream of the *cebEFG* operon encodes for a beta-glucosidase that releases glucose from both cellobiose and cellotriose. Deletion of *bglC* strongly decreased the levels of thaxtomin production.[16]

Earlier proteomics work assessed the global response of *S. scabies* grown under conditions eliciting pathogenesis, such as in the presence of suberin, or inhibiting pathogenesis, in the Δ*tatC* mutant. [17-20] Two-dimensional (2D)-PAGE coupled with N-terminal sequencing or tandem mass spectrometry showed the overproduction of proteins linked to bacterial stress response, specific metabolic pathways, and proteins known to be involved in secondary metabolism and morphological differentiation due to the presence of suberin.[20] Comparative

secretome analysis also demonstrated the production of a range of glycosyl hydrolases and other extracellular enzymes, possibly involved in the degradation of cellulose and suberin.[19] The comparative proteomic study of a *S. scabies* mutant of the TAT secretion system (Δ*tatC*) showed impaired virulence behavior and revealed novel proteins/genes essential for pathogenicity, again dominated by glycosyl hydrolases.[18]

In this work, we completed data analysis of a previously reported label-free proteomics dataset[13] and performed complementary targeted proteomics and metabolite analysis using multiple reaction monitoring (MRM) to investigate the cello-oligosaccharide mediated pathway of the virulence behavior of *S. scabies* 87-22. More precisely, we assessed how the presence of the critical elicitor cellobiose and/or how the inactivation of the global regulator CebR influenced - separately or together – the response of the intracellular "virulome", *i.e.*, proteins associated with the pathogenic lifestyle of *S. scabies*.

Experimental procedures

Bacteria, growth conditions

Streptomyces scabies 87-22 [21] and *Streptomyces scabies* Δ*scab57761* (Δ*cebR*) [14] were used in the following experiments. *Streptomyces* strains were routinely grown at 28°C on International *Streptomyces* Project medium 4 (ISP-4, BD Biosciences). Cellobiose was purchased from Megazyme (Ireland). *S. scabies* 87-22 and its *cebR* null mutant were grown on ISP-4 plates with or without a 0.7% cellobiose supply as previously described.[15] Growth curves were obtained by collecting the mycelium of *S. scabies* wild-type and Δ*cebR* strains inoculated on ISP-4 agar plates (with or without 0.7% cellobiose) covered with cellophane membranes (10^7 spores per plate).

Discovery proteomic analysis

Methodology for sample preparation, tryptic digestion and data collection for the discovery proteomics analysis are reported in supplementary method file 1. Actually, we report here the detailed analysis and validation of a dataset that was already briefly described in supplementary information of previous work[13]. Herein, the 2D LC-MS^E data were no part of the discussion, as it entirely focused on the cellobiose import system. Identifications of the different biological replicates and the relative quantification levels are listed in Table S1 and S2, respectively.

The $LCMS^E$-based proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE[22] partner repository with the dataset identifier PXD007820.

Protein functions were derived from the UniProt and KEGG databases.

Targeted proteomic analysis

Novel protein extracts were prepared as described above, from three biological replicates per condition. Protein extracts were spiked with Bovine Serum Albumin (MS-grade protein standard) (1:250 concentration ratio to protein extract) and digested as described above. The resulting peptide solutions were dissolved in 0.1% HCOOH in H_2O. The LC-MS/MS system consisted of a Waters NanoAcquity M-Class UPLC and an IonKey source connected to a Waters Xevo TQ-S triple quadrupole mass spectrometer. The IonKey source contained a 150 µm x 100 mm, 1.8 µm HSS T3, iKey separation device.

Briefly, 1 µl of each sample (0.1 µg) was injected and trapped for 5 min, 15 µl/min on a 300 µm x 50 mm, 5 µm, 100 Å Acquity UPLC M-Class Symmetry C18 Trap Colum (Waters). The peptides were separated on the iKey using a 20 min gradient of 3-50% acetonitrile (ACN) at a flow rate of 1 µl/min. The mobile phase consisted of 0.1% HCOOH in H_2O (Biosolve) as (A), and 0.1% HCOOH in ACN (Biosolve) as (B). The strong and weak solutions used to wash the auto-sampler were 0.1% HCOOH in H_2O and 0.1% HCOOH in acetonitrile/water/isopropanol (50:25:25, v/v/v), respectively.

The separated peptides were introduced into the Waters Xevo TQ-S mass spectrometer for quantification of the analytes in positive ion mode (ESI+). Detection was obtained by MRM mode with transitions of selected precursor ions at a set cone voltage and different collision energies and dwell times for each precursor (Table S3). Selection of precursors was based on predictions performed using Skyline.[23] Peptides were checked for proteotypicity using Unipept.[24] The following ESI-MS/MS parameters were set: capillary voltage, 3.6 kV; cone voltage, 35 V; source

temperature, 120°C. The collision gas argon was introduced into the collision cell at a flow rate of 0.19 ml/min. Data acquisition was performed by MassLynx 4.1 software and processed in Skyline. The imported data were subjected to a Savitsky-Golay Smoothing. The total area under the curve (AUC) for each proteotypic peptide was calculated and normalized to the spiked BSA standard. A student's t-test (two-tailed, homoscedastic) was executed to evaluate the differential protein abundance between the different conditions in a statistical way. The MRM results for each protein are documented in Table S4.

The MRM data have been deposited to Peptide Atlas[25] with the dataset identifier PASS01103.

Metabolite extraction and analysis

After 72 h and 96 h of growth at 28 °C, agar was collected, crushed and incubated with the same volume of methanol. After overnight incubation at 4°C under agitation, 20 ml of each supernatant was collected and filtered through a 0.22 µm polyvinylidene difluoride (PVDF) filter. The different methanol extracts were first diluted ten times in 0.1% HCOOH in H_2O. The same LC-MS set-up was used as described above (Targeted proteomic analysis). 0.5 µl of each diluted methanol extract was injected and trapped for 5 min, 15µl/min on a 300 µm x 50 mm, 5 µm, 100 Å Acquity UPLC M-Class Symmetry C18 Trap Colum (Waters). The metabolites were separated on the iKey using a 5 min gradient of 3-70% ACN at a flow rate of 3 µl/min. Both the cone voltage and the collision voltage were set at 40 V. The precursor mass 325.06 $[M+H]^+$ of pyochelin and its fragment ions 189.98 m/z, 172 m/z, 145.95 m/z, 127.96 m/z and 99.86 m/z, and the precursor mass 888.51 $[M+Na]^+$

of concanamycin A and its fragment ions 679.42 m/z, 515.3 m/z, 396.2 m/z and 378.19 m/z were used to selectively quantify these metabolites. Selection of fragments were based on information in the GNPS public spectral library and our own optimization experiments. The other MS parameters were identical as in the targeted proteomic analysis. A student's t-test was performed on the AUC of the metabolite of interest in the different conditions. The performance of the M-class UPLC - Xevo TQ-S system was verified before and after the analysis. Therefore, the AUC of three enolase peptides (MassPREP Enolase Digestion Standard (50 fmol) (Waters) was checked. The AUC of the peptides NVNDVIAPAFVK (1350697 and 1348857), AADALLLK (2755420 and 2484611) and VNQIGTLSESIK (558514 and 538685) remained constant (data not shown).

Results and Discussion

Global proteome analysis

We analyzed the effect of cellobiose, an elicitor of thaxtomin A production, on the cellular proteome of *S. scabies* 87-22 on solid media. Agar plates were chosen because it allows monitoring the effect on sporulation, which is tightly linked to the production of many specialized metabolites. Arguing that inhibition of the DNA-binding ability of the cellulose utilization repressor CebR is responsible for cellobiose-mediated transcriptional regulation, we also studied the effect of a *cebR* deletion on the intracellular proteome of *S. scabies* 87-22 (wild-type). However, it should be stressed that the deletion of *cebR* does not simply result in the same cellular conditions as obtained by the external cellobiose supply. Indeed, the

strength of the interaction between a transcription factor and its *cis*-acting element, and therefore transcriptional control, is variable and, in this case, depends on the rate of cellobiose uptake and its intracellular consumption. Moreover, cellobiose is also a positive allosteric effector presumed to improve the transcriptional activation by binding to TxtR.[15, 26] Finally, the glucose generated from cellobiose hydrolysis and related molecules resulting from subsequent catabolic and anabolic pathways are expected to influence the proteomic response by acting as nutrients.

Growth of *S. scabies* 87-22 wild-type and its *cebR* null mutant (Δ*cebR*) on ISP-4 medium in the presence or absence of 0.7 % cellobiose are presented in Figure 1. With or without cellobiose supply, the Δ*cebR* mutant revealed a much faster growth compared to the wild type strain (Figure 1A). The addition of cellobiose enhanced the accumulation of biomass in both strains (Figure 1A) and inhibited the observed early morphological differentiation (sporulation) in Δ*cebR* (Figure 1B). The accelerated sporulation in strain Δ*cebR* further confirms the idea that the inactivation of *cebR* and the addition of cellobiose do not necessarily imply identical physiological responses. For our proteomic analysis, mycelia of both the wild-type and the Δ*cebR* strain were collected after two days i.e. when both strains are still at the exponential phase and did not initiate sporulation. After protein extraction and digestion, the peptides were separated with 2D-UPLC (high pH – low pH RPLC) and eluted peptides were analyzed online with a SYNAPT^{TM} HDMS Q-TOF mass spectrometer (Waters) via MS^{E}. A total of 451 proteins were identified from the crude extract of the wild-type mycelium grown on the ISP-4 medium in the absence of cellobiose, considering only the proteins that were found in at least two of the

three biological replicates. Of these, 338 proteins met the filtering criteria, *i.e.* at least two peptides per protein were identified and the identification passed the Green autocurate threshold (ProteinLYNX GLOBAL Server (PLGS)). Similarly, the analysis of the wild-type mycelium grown in the presence of cellobiose resulted in the identification of 482 proteins of which 345 proteins passed the filtering criteria. When the Δ*cebR* mutant was grown under the same culture conditions (ISP-4 with or without cellobiose 0.7%), 482 and 496 proteins were identified amongst which 360 and 345 proteins, respectively, passed the Green autocurate threshold and were identified by at least two peptides.

Table 1-3 and S3 list the proteins that displayed a 1.5-fold difference in abundance (Progenesis software, Waters) as a result of cellobiose supply and/or as a consequence of the gene deletion of *cebR*. These proteins were divided into functional groups based on their known or putative functions. A total of 53 proteins were significantly more abundant in the *cebR* knockout mutant alone and 29 proteins were solely affected positively by cellobiose, while 16 proteins were more abundant in both conditions. The fact that only 16 proteins out of the 98 proteins, which displayed higher abundance patterns, were shared between the two conditions studied suggests again that the absence of the cellobiose-responsive repressor CebR is not similar to the presence of cellobiose. This is further confirmed by the identification of 10 proteins that were more abundant in Δ*cebR*, yet less expressed in the presence of cellobiose, while 7 proteins displayed the opposite pattern (Figure 2). The diminished abundance of certain proteins due to the deletion

of the CebR repressor suggests that this transcription factor has a regulon that extends beyond cellulose utilization genes and might control other regulatory proteins.

Response of proteins composing the cello-oligosaccharide mediated pathway leading to thaxtomin A production

Prior to a discussion of the global response of the "virulome" of *S. scabies* upon sensing of cellobiose and/or as a consequence of the *cebR* deletion, we first analyzed the fold-change in the abundance of proteins known to be part of the thaxtomin A production pathway. The proper experimental set-up is confirmed by the increased abundance of peptides from the thaxtomin synthetases TxtA and TxtB in both tested conditions (Table 2). Indeed, as previously demonstrated at the transcriptional level[14-15], expression of thaxtomin synthetase A (TxtA), and thaxtomin synthetase B (TxtB) is upregulated in the presence of cellobiose, and in the *cebR* mutant (Table 2). This was confirmed by the LC-MRM experiment. Two proteotypic peptides for TxtA, LLGATDDPSDVALR and SVGLVLGR, and three proteotypic peptides for TxtB, LGGGTDIVVGTAAAAR, SDIEPELPAEAASR and TALDDVVAR were selected and quantitatively analyzed. Indeed, all TxtA and TxtB selected peptides were significantly more abundant in the *cebR* mutant (with or without cellobiose supply) and in the wild-type (87-22 strain) cultivated in the presence of cellobiose compared to the wild-type (Figure 2). In addition, higher abundance of TxtC (cytochrome P450-type monooxygenase) and TxtE (L-tryptophan-nitrating cytochrome P450) peptides were also observed under the tested conditions (Table 2) confirming that the thaxtomin cluster is indeed under negative control of CebR and requires cellobiose

as environmental elicitor. It has to be noted that, in this case, the abundance of TxtA, TxtB, TxtC, and TxtE peptides was always higher in the presence of cellobiose compared to their abundance as a result of *cebR* deletion, suggesting that thaxtomin production is improved by the binding of cellobiose to the thaxtomin biosynthesis activator TxtR.[15]

Primary metabolism - Carbohydrate metabolism

As mentioned in the introduction and discussed in a recent opinion article[27], the utilization of cellobiose as an elicitor of pathogenicity is intriguing as this molecule is also the most abundant carbon source released from decomposing lignocellulose that will directly provide glucose for the central carbon metabolic pathways. Consumption of cellobiose and its further catabolism into glucose could therefore provide sufficient energy for *S. scabies* to simply behave as a saprophyte without concomitant triggering of a pathogenic lifestyle. In fact, the very first step towards glycolysis, *i.e.* the conversion of glucose units - generated by beta-glucosidase (Bglc) activity[16] into glucose-6-phosphate by the glucose kinase GlkA (SCAB_67551) seemed to be reduced in the presence of cellobiose as suggested by the 1.91-fold decrease of GlkA peptides. This effect would be mediated by cellobiose itself, without implying CebR, as the deletion of the transcription factor did not affect the negative effect of the carbohydrate on GlkA intracellular accumulation (Table 1). The fact that the first step towards glycolysis is impaired by cellobiose is possibly a key event for *S. scabies* to perceive this molecule as a signal inducing its pathogenic lifestyle instead of using it as a carbon source. In addition, the decreased abundance of the glucose kinase would be beneficial for cellobiose uptake as GlkA is

the protein that imposes carbon catabolite repression on the utilization of alternative and more complex carbon sources in streptomycetes.[28] Its reduced abundance would allow prioritization of import of di-and oligosaccharides such as those derived from cellulose and starch.

The solid ISP-4 medium was chosen as it contains starch as the main carbon source, which is also the main storage polymer found in the tubers and roots of the hosts of *S. scabies*. It can therefore be assumed that cellobiose could act as the eliciting molecule, while starch would be used as most available carbon source after colonization of the plant host. Interestingly, the addition of cellobiose to the growth medium of *S. scabies* resulted in a 1.65-fold increase of the starch/maltose utilization repressor MalR.[29-30] This response seemed to involve CebR as the *cebR* deletion also resulted in a 1.87-fold increased production of MalR. This overproduction of MalR has no significant effect on the abundance of proteins belonging to the MalR regulon in the *cebR* mutant grown with or without cellobiose (Table 1). Instead, the higher abundance of MalR resulted in a decreased accumulation of the ABC-type maltose/maltodextrin-binding protein MalE (-3.8-fold), the ABC transporter membrane component MalG (-2.65-fold), the intracellular alpha-glucosidase AglA (-3.03-fold) when wild-type *S. scabies* was grown in the presence of cellobiose. To validate this important observation - as it connects virulence signaling to access of the food reservoir within the host - we performed a targeted proteomics experiment using MRM. The tryptic peptides ANLGIATVPAGTSGK, SEVGWTPAFAK and ITVNSPEAK were selected as the proteotypic peptides of MalE, while GYFDTIPFEIDEAGR and VDGLSPFGTFAR served as the ones of MalG. IFVAEAWTPVER

and LVLDEYAGER were picked as proteotypic peptides of the intracellular alpha-glucosidase AglA. The targeted proteomic experiments show that those proteins are indeed significantly less abundant when *S. scabies* 87-22 was grown in the presence of cellobiose (Figure 3). This would suggest that as long as cellobiose is available and incorporated as a signal for pathogenicity, it would postpone the utilization of starch available within tubers or roots by *S. scabies*. Interestingly, in the *cebR* null mutant, we observed a strong increase (4.06-fold) of peptides from the alpha-1,4 glucan phosphorylase (maltodextrin phosphorylase) GlgP (SCAB_27951) possibly involved in the phosphorolytic degradation of starch, while cellobiose itself had only a minor negative effect on the accumulation of GlgP. The fact that the deletion of *cebR* and cellobiose supply do not induce a similar proteomic response is counterintuitive as cellobiose is known to relieve the repression of CebR and constitutes the first example of a CebR controlled gene (directly or indirectly) for which induction is not dependent on cellobiose as an allosteric effector to prevent binding of CebR to its target *cis*-acting elements. The multiple ABC-type sugar importer ATPase MsiK involved in the uptake of tens of carbohydrates including cellulose and starch derived by-products[31-32] as well as three enzymes of the pentose phosphate pathway (ZWF3, Tal, and TktA) also displayed a CebR-repressed but cellobiose-independent production pattern (Table 1). Finally, Enzyme I of the phosphoenol-pyruvate phosphotransferase system (PTS) displayed a 3.03-fold decrease in the presence of cellobiose. This enzyme is not only required for the PTS-dependent uptake of N-acetylglucosamine[33-34] and fructose[35], but its inactivation has been shown to severely affect morphogenesis and metabolite production in *S. coelicolor.*[36-37] Its

much lower abundance in the presence of cellobiose could therefore drastically affect the lifestyle of *S. scabies* well beyond its role in carbohydrate uptake.

Primary metabolism - Amino acids metabolism

The flux in amino acid metabolic pathways and the appropriate balance between anabolic and catabolic routes are predicted to be intricately linked to the pathogenic lifestyle of *S. scabies*. Indeed, the amino acids L-tryptophan and L-phenylalanine are the building blocks of thaxtomin phytotoxins. Nitric oxide for nitration of L-tryptophan in 4-nitro-L-tryptophan intermediate biosynthesis is provided by L-arginine by the nitric oxide synthase TxtD. While the addition of tryptophan to the culture medium reduced the amount of thaxtomin A produced[38], feeding with 4-nitro-L-tryptophan instead enhanced its production.[39] Moreover, L-tryptophan is also used to generate the plant hormone indole-3 acetic acid (IAA, auxin), which in some plant pathosystems contributes to the severity of host disease symptoms by increasing colonization and growth *in planta* through 'manipulation' of the hormone balance within the plant.[40] Tryptophan degradation is also able to provide anthranilate and chorismate, which are important intermediates of siderophore biosynthesis. Accordingly, enzymes associated with tryptophan, phenylalanine and tyrosine metabolism are the amino acid-related pathways where the highest number of proteins displayed significant abundance variation (Table 1). Tryptophan synthase beta chain TrpB (SCAB_68501) generates tryptophan from serine and has been found more abundantly in both the *cebR*-null mutant and the wild-type grown with cellobiose (1.52 and 1.57-fold, respectively). Two other enzymes associated with tryptophan metabolism were also more present in both tested conditions, namely

the tryptophan 2,3-dioxygenase KynA (2.31 and 6.18-fold, respectively) that will generate N-formylkynurenine, and the kynureninase KynU (2.19 and 2.30-fold, respectively) that will convert the latter into formylanthranilate or L-kynurenine in anthranilate.[41] Finally, the chorismate mutase AroQ is 1.72-fold more abundant in the presence of cellobiose emphasizing again that the CebR regulon influences the pool of metabolites used for siderophore biosynthesis.

Additionally, three out of the four proteins involved in enzymatic reactions of the S-adenosyl-L-methionine (SAM) and L-homocysteine metabolic circuit (MetH, MetK, SahH) are more abundant either in the presence of cellobiose or in the *cebR* mutant (Table 1). SAM is an important methyl donor required for thaxtomin synthesis but it is also a second messenger involved in the synthesis of different antibiotics in streptomycetes.[42-46] Alterations in the betaine/sarcosine pathway can be linked to SAM production as betaine can serve as substrate for methionine/SAM recycling from homocysteine.

Most other amino acid biosynthetic pathways seem to be repressed. In fact, overall protein translation is affected (see below). The cellobiose/CebR regulon might therefore be intricately associated with the production of specialized metabolites required for virulence while general protein synthesis is suppressed.

Genetic Information Processing – Housekeeping functions

All proteins identified of the 30S and the 50S ribosomal subunits that displayed >1.5 fold change in any of the experiments were less abundant in the *cebR* mutant

(average 1.7-fold less) as well as when cellobiose was externally supplied, although to a lesser extent. Except the elongation factor Tuf3, all other proteins involved in the translational process, GatB, GlyS, InfB, InfA, InfC and AviX1, were less abundant in the *cebR* mutant. Two proteins involved in peptide degradation, TldD and PrcB, were more abundant in the *cebR* knockout mutant, respectively 1.68-fold and 1.62-fold (Table 3). Three chaperone proteins are also listed in Table 3, GroL1 (-1,77-fold) and GroEL2 (-1,67-fold) were negatively affected by Δ*cebR*, while cellobiose had a negative effect on DnaK (-1.53-fold). These findings can be related to the growth curves, since both addition of cellobiose and deletion of the *cebR* gene result in faster growth and those cells are approaching the later exponential phase explaining reduced requirements to produce biomass.

The physiological and proteomic study of the role the twin arginine protein transport (TAT) showed that deletion of *tatC* abolished the virulence of *S. scabies*, demonstrating that key proteins for the onset of its pathogenic life style are secreted via the TAT pathway.[18] Interestingly, the TatA component of the TAT pathway, (SCAB73591) is positively affected by cellobiose (2.07-fold change) therefore globally stimulating the secretion of TAT-dependent virulence associated proteins. Although our proteomics study involved soluble cellular protein extracts, few extracellular and membrane bound proteins secreted through the TAT pathway were identified i.e., i) the zinc-binding lipoprotein ZnuA (SCAB61981) (1.85-fold change upon supply of cellobiose), ii) a BldKB-like solute-binding component of an ABC transporter (SCAB31461), and iii) a secreted solute-binding protein (SCAB57661), the latter instead were 2.61 and 1.64-fold negatively affected by cellobiose, respectively.

Specialized, secondary metabolism

The discovery proteomics experiment revealed that action of cellobiose and the role of the cellulose utilization repressor CebR are not limited to thaxtomin A production but also affect the abundance of several enzymes involved in the biosynthesis of different secondary (or specialized) metabolites that could be involved in the virulence of *S. scabies* (Table 2). Among specialized metabolites, siderophores are not only known to be essential to obtain iron for a series of housekeeping functions, but have also been demonstrated to be key features in animal and plant pathogens.[47-48] Indeed, the concentration of soluble iron within the host is known to be extremely low[49] which makes the ability to steal iron a vital strategy for any pathogen. In line with this need to acquire iron, the DesE protein presents one of the most overexpressed profiles upon addition of cellobiose with 4.79- and 13.15-fold increase in the wild-type and the *cebR* mutant, respectively (Table 2). DesE is an ABC-type ferri-siderophore binding protein, which in streptomycetes, is not only able to import their homemade siderophores but also xenosiderophores.[50-51] The induced production of DesE upon transport of cellobiose would maximize the chance of *S. scabies* to acquire iron from the most diverse sources in its environment. As siderophore synthesis is a costly process, acquisition of iron-carriers from the host or produced by other organisms would save energy for *S. scabies* and preserve building blocks for the construction of other specialized metabolites required for its pathogenic life-style. Also, deletion of DesE[52] as well as the reduction of siderophore biosynthesis[37, 53-55] resulted in the arrest of the sporulation process and/or altered metabolite production in streptomycetes suggesting a tight link between iron-chelator uptake and the onset of the developmental program.

Next to DesE, CebR mutation has a negative impact on DmdR1 production (Figure 3). DmdR1 acts as a global regulator of iron homeostasis and is a negative regulator of desferrioxamine production providing an additional path to control siderophore production.[56]

Surprisingly, unlike thaxtomin, the other identified proteins related to clusters of secondary metabolites displayed a dissimilar response to cellobiose supply compared to the inactivation of *cebR* (Table 2). This is for instance the case for the three identified proteins of the pyochelin biosynthetic gene cluster (PBGC), an AMP-binding NRPS ligase (SCAB1411) involved in the production of pyochelin, the ATP-binding subunit of an ABC transporter (SCAB1431) and its membrane component (SCAB1451) both suggested to import ferric-pyochelin[57]. While cellobiose did not affect SCAB1411 and SCAB1431, and only weakly induced SCAB1451, the deletion of *cebR* resulted in a 4.21, 2.24, and 18.23-fold increase in abundance of these three proteins, respectively. Pyochelin is another siderophore, important to cope with the low availability of iron[58]. This siderophore is best known from studies in *Pseudomonas aeruginosa* where it is a virulence factor.[59-60] Its functional biosynthetic pathway was recently identified in the genome of *Streptomyces scabies* 87-22, but a mutant unable to produce pyochelin still displayed pathogenicity on excised potato tissues and radish seedlings.[57] To further validate these results, we performed targeted analysis of selected tryptic peptides of these enzymes by label-free liquid chromatography-multiple reaction monitoring LC-MRM mass spectrometry. Of the pyochelin-related proteins, respectively, three (TSFTEDGYFR, SLTFGALDLAADR and TTDGNLEVVGR), three (ETDLIAFLDGGR,

LPLGWFTPR and LPDGWATR) and two (EGAPAEPSGQAGLPATEVR and LLGALSAK) proteotypic peptides were used to quantify the protein levels. Figure 3 shows the negative correlation between the expression of CebR and three proteins from the large operon of the PBGC. All peptides of these proteins mentioned above are significantly more abundant in the Δ*cebR* mutant, both in the presence or absence of cellobiose. To confirm the production of the metabolite itself, pyochelin, we also performed a quantitative mass spectrometry assay to have direct evidence of the effect of CebR on its production. The selected precursor mass 325.06 $[M+H]^+$ and its fragment ions 189.98 m/z, 172 m/z, 145.95 m/z, 127.96 m/z and 99.86 m/z were obtained from the GNPS Public Spectral Library and experimental evidence. The areas under the curves of these peaks are summarized in Figure 4 and are indicating a significant greater production of pyochelin in the *cebR* mutant, both in the presence or absence of cellobiose, when the cells were grown for 96 hours. The involvement of the cellulose utilization repressor CebR in the control of pyochelin production somehow suggests indeed that pyochelin is linked to pathogenesis.

A similar CebR-repressed pattern was observed for products of genes encoding a carbamoyltransferase (SCAB84021) and an O-methyltransferase (SCAB84051) which are believed to be part of the concanamycin A biosynthesis gene cluster.[61] Concanamycins are polyketide macrolides, which function as vacuolar-type ATPase inhibitors with antifungal and anti-neoplastic activity.[62-63] Both proteins did not respond to the presence of cellobiose but were more abundant in the *cebR* mutant compared to the wild-type with a 2.02 and 5.74-fold increased abundance, respectively. These results were validated by a targeted proteomic experiment (LC-MRM). AELYELWQQR, SADPTADLDGLVPR and VQVVDPAANER were selected as

proteotypic peptides for the carbamoyltransferase, while EDDLLLELR, VTDPQFTDADTAGIR and AGVDGLIDLR served as those for the O-methyltransferase. This experiment confirmed the greater abundance of both transferases in the knockout mutant (Figure 3). Similar to pyochelin, concanamycin A was targeted in the secretome of cells grown under the different conditions. The precursor mass 888.51 $[M+Na]^+$ and its fragment ions 679.42 m/z, 515.3 m/z, 396.2 m/z and 378.19 m/z were selected for the MRM experiment based on information from the GNPS Public Spectral Library and experimental evidence. Concanamycin A could only be detected in the *cebR* mutant and solely after 96 hours of cultivation (Figure 4). Recently, the necrosis-inducing activity of concanamycin A and its synergistic effect with thaxtomin A was demonstrated in *S. scabies* 87-22[64] which could possibly explain why they are both negatively controlled by CebR.

Two proteins that are part of the coronafacoyl phytotoxin biosynthetic pathway, the type-II fatty acid dehydratase (SCAB79611, Cfa2) and acyl-CoA ligase (SCAB79671, CFL)[65], were found to be less abundant in the *cebR* mutant as well as when the wild-type was grown on ISP-4 medium supplemented with cellobiose (Table 2, Figure 3). The coronafacoyl compounds cause hypertrophy of potato tuber tissue and consist of amino acids (or amino acid derivatives) linked via an amide bond to the polyketide coronafacic acid (CFA)[66]. Coronafacoyl-L-isoleucine is the most abundant form in *S. scabies* and exhibits bioactivity similar to that of coronatine in *Pseudomonas syringae.*[67] In this case, both *cebR* deletion and cellobiose altered the protein abundance in the same way (decrease) suggesting that CebR would directly or indirectly control the expression of a repressor of coronafacoyl phytotoxin

biosynthesis. SCAB79611 (Cfa2) and SCAB79671 (CFL) were targeted via LC-MRM through their proteotypic peptides AEVSGRPVAMTR and ILDYEPGK, and AAAEPAPDLTDTR, SQWLASGDIVR and FGWIGGAPTTYR, respectively. These data confirmed that both proteins were significantly more abundant in the total protein extracts of the wild-type strain compared to the *cebR* mutant (Figure 3). The lower abundance of proteins involved in the biosynthetic pathway of the coronafacoyl phytotoxins in both the presence of cellobiose and the *cebR* null mutant could reveal a competitive production between CFL and metabolites that are produced upon cellobiose induction. Thus, the PAS-LuxR family regulator CfaR is probably not the only protein responsible for the regulation of coronafacoyl phytotoxin production.[66] We searched for the presence of different coronafacoyl phytotoxins like coronafacoyl-L-isoleucine (CFA-L-Ile) and coronafacoyl-L-valine (CFA-L-Val) in the extracellular medium, but their presence in the secretome could not be confirmed.

Lastly, the inactivation of *cebR* also led to a higher abundance of two enzymes of which the corresponding genes reside in two unknown secondary metabolite clusters, though their responses towards CebR deletion and cellobiose addition were different. A TetR-family transcriptional regulator (SCAB72821) and a polyketide cyclase (SCAB72851) displayed a 2.06- and 2.64-fold change respectively in the CebR mutant. Like the proteins involved in concanamycin A and pyochelin biosynthesis, CebR apparently represses them, while cellobiose supply did not change their abundance. The other cluster of genes predicted to be involved in the synthesis of a secondary metabolite displayed opposite response patterns as a result

of cellobiose supply or the inactivation of *cebR*. Indeed, cellobiose significantly increased the abundance of four proteins of the scab3211–scab3361 locus.

Proteins globally involved in the developmental process of streptomycetes

As the deletion of *cebR* and/or the presence of cellobiose altered the abundance profiles of numerous proteins of secondary metabolite biosynthesis clusters without obvious CebR-binding sites in their vicinity (as predicted by PREDetector 3.1.[69], see supplementary file Table S5), we postulated that the observed effect might involve proteins known to globally affect the developmental program of *Streptomyces* species. Apart from PTS EI and MetK discussed in the sections above, at least seven other proteins (AfsK, BldKB, BldN, CutR, EshA, NsdB, and NusG) with significant altered abundance, are known to impact morphological and physiological differentiation in streptomycetes. Among them, SCAB_51861 presents the highest increase in abundance (14.83-fold) in the presence of cellobiose. This protein is the orthologue of AfsK, a serine/threonine kinase that phosphorylates serine and threonine residues of AfsR, a two-component system response regulator that plays an important role in signal transduction of metabolite production as well as in morphogenesis, polar growth and hyphal branching of several *Streptomyces* species.[67-68] Autophosphorylation of AfsK has been shown to be increased by S-adenosyl-L-methionine (SAM). As discussed earlier, different proteins involved in SAM synthesis displayed a higher abundance in the presence of cellobiose and/or as a result of the deletion of *cebR* which could increase the level of phosphorylation of AfsK, and in turn the activity of its associated transcription factor AfsR. Based on the current data deduced from other model *Streptomyces* species, SAM/AfsK-dependent activation of AfsR could globally affect the secondary metabolism of *S. scabies*.

Among the other key *Streptomyces* developmental proteins, our proteomic analysis identified BldN and BldKB, which showed a 2.19 and 2.61-fold abundance decrease in the *cebR* null mutant and in the presence of cellobiose, respectively. Bld/*bld* (bald) proteins/genes in streptomycetes refer to genes which, when inactivated, block the sporulation process at the vegetative state and thus also lead to severe consequences in metabolite production. BldKB is the extracellular component of an ABC-type oligopeptide transporter (BldKABCDE) which upon gene deletion arrests the signaling cascade leading to the erection of aerial hyphae at its earliest stage.[69] BldKB is thought to bind and transport the earliest extracellular signal triggering the formation of aerial hyphae. Interestingly, expression of the BldK transporter is induced by SAM[703] and the study of the proteomic response of *S. scabies* to suberin also identified components of this oligopeptide transporter among the proteins displaying an increased variation abundance.[20] BldN is a sigma factor required for expression of the chaplin and rodlin hydrophobic proteins that cover the surface of the *Streptomyces* spores.[71-72] Interestingly, *bldN* and *bldkB* are *bld* genes that have been shown to be tightly related to iron homeostasis. Expression of *bldN* is highly induced by iron[73] and iron is able to restore aerial hyphae formation in the *bldKB* mutant.[37] The fact that these two known iron-associated *bld* genes have been highlighted by our proteomic approach is possibly a consequence or a cause of the drastic effect of cellobiose and/or *cebR* deletion on the abundance of proteins involved in siderophore biosynthesis (pyochelin, DmdR1) and ferri-siderophore transport (DesE). It further emphasizes the close link between the onset of pathogenicity and iron homeostasis. Both proteins involved in triggering sporulation are down regulated under conditions that trigger thaxtomin production, which could

explain why their respective deletion did not significantly affect the virulence of *S. scabies.*[74] It seems logical that once *S. scabies* senses the proper conditions for the onset of its pathogenic life style it down regulates BldK and BldN, which are essential proteins to enter the dormancy state (sporulation) while it overexpresses AfsK, a developmental protein required for activation of hyphal apical growth and branching.

Cellobiose also induced the abundance of the tetratricopeptide repeat (TPR) domain-containing protein NsdB (2.41-fold), the two-component system regulator CutR (1.81-fold), and the ScbR-like γ-butyrolactone binding regulator SlbR (1.98-fold). The inactivation of these three genes resulted in increased antibiotic production in *S. lividans* and/or *S. coelicolor*, suggesting that they both negatively affect the onset of secondary metabolism.[78-80] Their overexpression in the presence of cellobiose could participate in balancing the production levels of the different metabolites produced by *S. scabies*.

Finally, the inactivation of *cebR* resulted in a 1.54-fold decrease of EshA, a cyclic-nucleotide binding-domain protein essential for morphogenesis and streptomycin production in *S. griseus*[75-76], and for actinorhodin production in *S. coelicolor.*[77] EshA accentuates the accumulation of deoxynucleoside triphosphates[76] and of the alarmone guanosine tetraphosphate ppGpp[78] involved in the stringent response inhibiting RNA synthesis and decreasing translation under shortage of amino acids. The decreased abundance of EshA in the *cebR* mutant therefore does not correlate with the observed decreased abundance of ribosomal proteins and proteins involved translation initiation and transcription elongation and termination factors (Table 3).

Proteins with unknown function

Finally, supplementary Table S6 lists 74 uncharacterized proteins with no or partial information on their biological function with significant (±1.5-fold, and p-value < 0.05) altered abundance under one or more of the tested conditions and strains. Among them, ten encode for transcription factors, alternative sigma factors, anti-sigma factors, and putative DNA-binding proteins, which further highlight the reach of the CebR/cellobiose regulon. The large majority of other proteins displayed high homology to hypothetical proteins conserved in streptomycetes and other Actinobacteria, while others seemed to be more specific to *S. scabies*. Among these uncharacterized proteins we observed some of the most important responses to either *cebR* deletion or cellobiose supply suggesting that the "virulome" of *S. scabies* would certainly imply novel key protagonists than those currently described in the literature.

Conclusions

Our comparative proteomics analysis confirmed the role of cellobiose via the cellulose utilization repressor CebR in the production of thaxtomin A, the main virulence factor of *S. scabies*. Considering that thaxtomin A is generated from the condensation of L-tryptophan and L-phenylalanine, we observed important modifications of metabolic fluxes in pathways related to biosynthesis of the building blocks of the phytotoxin. Our work also highlights close connections between the cellulose/CebR regulon and pathways predicted to play an important role in the pathogenic lifestyle of *S. scabies* such as the acquisition of iron through DesE for the import various types of ferri-siderophores. The wide impact of *cebR* deletion and/or cellobiose supply on clusters involved in secondary metabolite production suggests that CebR does not only control thaxtomin A production but is most likely a global regulator of the virulence of *S. scabies*, possibly through direct or indirect regulation of key developmental proteins such as BldKB, BldN, MetK, PTS EI, and AfsK, amongst others. In conclusion, our work through the combination of discovery proteomics, targeted proteomics, and targeted metabolomics revealed complex regulatory networks and pathways associated with the cellobiose/CebR-mediated pathogenic lifestyle of *Streptomyces scabies*, also providing new insights on possible novel key proteins triggering virulence.

Acknowledgments

This work is supported in part by the Belgian program of Interuniversity Attraction Poles initiated by the Federal Office for Scientific Technical and Cultural Affairs (PAI no. P7/44), by the PRODEX program initiated by the same Office, and by the FNRS (research project RFNRS.3342-T.0006.14-PDR [FRFC]). S.J.'s work is supported by an Aspirant grant from the FNRS. And S.R. is FRS-FNRS research associate.

Table legends

Table 1. List of proteins with significant change in abundance involved in general primary metabolism. Proteins were regarded as significant more/less abundant compared to the wild-type with ANOVA values of $p < 0.05$, regulation of ±1.5-fold, power > 0.8 and q-values < 0.05. The measured increase (in green) or decrease (in red) of the abundance of the detected proteins is illustrated by a color scale. $(Glc)_2$: cellobiose.

Table 2. List of proteins with significant change in abundance involved in specialized secondary metabolism and development. Proteins were regarded as significant more/less abundant compared to the wild-type with ANOVA values of $p < 0.05$, regulation of ±1.5-fold, power > 0.8 and q-values < 0.05. The measured increase (in green) or decrease (in red) of the abundance of the detected proteins is illustrated by a color scale. $(Glc)_2$: cellobiose.

Table 3. List of proteins with significant change in abundance involved in transcriptional and translational processes. Proteins were regarded as significant more/less abundant compared to the wild-type with ANOVA values of $p < 0.05$, regulation of ±1.5-fold, power > 0.8 and q-values < 0.05. The measured increase (in green) or decrease (in red) of the abundance of the detected proteins is illustrated by a color scale. $(Glc)_2$: cellobiose.

Figure legends

Figure 1. Growth and development of *S. scabies* 87-22 and its *cebR* deletion mutant. A. Growth curves presenting the mycelium collected from both strains grown on ISP4 with or without 0.7% cellobiose. **B.** Early onset of sporulation in the *cebR* null mutant grown in ISP4 covered with cellophane membranes. The addition of cellobiose inhibits the accelerated sporulation in strain Δ*cebR*. Pictures were taken after 72 h of growth.

Figure 2. Number of proteins significantly increased/diminished in the presence of cellobiose and/or the *cebR* deletion mutant compared to the wild-type strain *Streptomyces scabies* 87-22. Figure was adapted from Venny 2.0 (BioinfoGP, ES) output.

Figure 3. Relative abundance of proteins involved in a carbohydrate and secondary metabolism in response to *cebR* deletion and/or cellobiose (CB) supply determined by targeted proteomics (LC-MRM). Proteins were extracted from *S. scabies* 87-22 and its *cebR* mutant strain grown in the presence/absence of cellobiose for 48h on ISP-4 agar. The plot displays the average normalized area under the curve for each peptide used as a marker for the different proteins. These results show significant normalized quantitative peptide abundances ($p < 0.05$) compared to the wild-type strain grown on ISP-4 agar without cellobiose (^{1}), with cellobiose (^{2}) and/or the *cebR* null mutant grown without cellobiose (^{3}). A statistical two-sided student's t-test (homoscedastic) was performed. The error bars plot the standard deviation (SD) of the three biological replicates.

Figure 4. Abundance of pyochelin and concanamycin A in response to *cebR* deletion and/or cellobiose (CB) supply determined by targeted metabolomics (LC-MRM). Metabolites were extracted from *S. scabies* 87-22 and its *cebR* mutant strain grown in the presence/absence of cellobiose for 72h and 96h on ISP-4 agar. The plot displays the area under the curve for each metabolite. These results show significant

quantitative abundances ($p < 0.05$) compared to the wild-type strain grown on ISP-4 agar without cellobiose ([1]), with cellobiose ([2]) and/or the *cebR* null mutant grown without cellobiose ([3]). The significant difference between 72h and 96h is shown by ([*]). A statistical two-sided student's t-test (homoscedastic) was performed. The error bars plot the SD of the three biological replicates.

SUPPORTING INFORMATION:

The following supporting information is available free of charge at ACS website http://pubs.acs.org

Contents of Supporting Information File

Method S1. Detailed label-free proteomic methodology.

Table S1. Separate multi-tabbed excel file containing protein identifications from the different biological replicates of the discovery proteomics experiment.

Table S2. Separate multi-tabbed excel file containing relative quantification levels of the different comparisons.

Table S3. Lists of transitions selected for MRM validation of proteins with significant change in abundance upon *cebR* deletion or cellobiose induction.

Table S4. Separate multi-tabbed excel file containing MRM results for each protein.

Table S5. Prediction CebR regulon in *Streptomyces scabies.*

Table S6. List of unknown and uncharacterized proteins with significant change in abundance.

References

1. Procopio, R. E.; Silva, I. R.; Martins, M. K.; Azevedo, J. L.; Araujo, J. M., Antibiotics produced by Streptomyces. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases* **2012,** *16* (5), 466-71.

2. Berdy, J., Bioactive microbial metabolites. *The Journal of antibiotics* **2005,** *58* (1), 1-26.

3. Loria, R.; Bignell, D. R.; Moll, S.; Huguet-Tapia, J. C.; Joshi, M. V.; Johnson, E. G.; Seipke, R. F.; Gibson, D. M., Thaxtomin biosynthesis: the path to plant pathogenicity in the genus Streptomyces. *Antonie van Leeuwenhoek* **2008,** *94* (1), 3-10.

4. King, R. R.; Calhoun, L. A., The thaxtomin phytotoxins: sources, synthesis, biosynthesis, biotransformation and biological activity. *Phytochemistry* **2009,** *70* (7), 833-41.

5. Barry, S. M.; Kers, J. A.; Johnson, E. G.; Song, L.; Aston, P. R.; Patel, B.; Krasnoff, S. B.; Crane, B. R.; Gibson, D. M.; Loria, R.; Challis, G. L., Cytochrome P450-catalyzed L-tryptophan nitration in thaxtomin phytotoxin biosynthesis. *Nature chemical biology* **2012,** *8* (10), 814-6.

6. Huguet-Tapia, J. C.; Badger, J. H.; Loria, R.; Pettis, G. S., Streptomyces turgidiscabies Car8 contains a modular pathogenicity island that shares virulence genes with other actinobacterial plant pathogens. *Plasmid* **2011,** *65* (2), 118-24.

7. Zhang, Y.; Bignell, D. R.; Zuo, R.; Fan, Q.; Huguet-Tapia, J. C.; Ding, Y.; Loria, R., Promiscuous Pathogenicity Islands and Phylogeny of Pathogenic Streptomyces spp. *Molecular plant-microbe interactions : MPMI* **2016,** *29* (8), 640-50.

8. Bignell, D. R.; Huguet-Tapia, J. C.; Joshi, M. V.; Pettis, G. S.; Loria, R., What does it take to be a plant pathogen: genomic insights from Streptomyces species. *Antonie van Leeuwenhoek* **2010,** *98* (2), 179-94.

9. Zhang, Y.; Loria, R., Emergence of Novel Pathogenic Streptomyces Species by Site-Specific Accretion and cis-Mobilization of Pathogenicity Islands. *Molecular plant-microbe interactions : MPMI* **2017,** *30* (1), 72-82.

10. Wach, M. J.; Krasnoff, S. B.; Loria, R.; Gibson, D. M., Effect of carbohydrates on the production of thaxtomin A by Streptomyces acidiscabies. *Archives of microbiology* **2007,** *188* (1), 81-8.

11. Lerat, S.; Simao-Beaunoir, A. M.; Wu, R.; Beaudoin, N.; Beaulieu, C., Involvement of the plant polymer Suberin and the disaccharide cellobiose in triggering thaxtomin A biosynthesis, a phytotoxin produced by the pathogenic agent streptomyces scabies. *Phytopathology* **2010,** *100* (1), 91-6.

12. Johnson, E. G.; Joshi, M. V.; Gibson, D. M.; Loria, R., Cello-oligosaccharides released from host plants induce pathogenicity in scab-causing Streptomyces species. *Physiological and Molecular Plant Pathology* **2007,** *71* (1), 18-25.

13. Jourdan, S.; Francis, I. M.; Kim, M. J.; Salazar, J. J.; Planckaert, S.; Frere, J. M.; Matagne, A.; Kerff, F.; Devreese, B.; Loria, R.; Rigali, S., The CebE/MsiK Transporter is a Doorway to the Cello-oligosaccharide-mediated Induction of Streptomyces scabies Pathogenicity. *Scientific reports* **2016,** *6*, 27144.

14. Francis, I. M.; Jourdan, S.; Fanara, S.; Loria, R.; Rigali, S., The cellobiose sensor CebR is the gatekeeper of Streptomyces scabies pathogenicity. *mBio* **2015,** *6* (2), e02018.

15. Joshi, M. V.; Bignell, D. R.; Johnson, E. G.; Sparks, J. P.; Gibson, D. M.; Loria, R., The AraC/XylS regulator TxtR modulates thaxtomin biosynthesis and virulence in Streptomyces scabies. *Molecular microbiology* **2007,** *66* (3), 633-42.

16. Jourdan, S.; Francis, I. M.; Deflandre, B.; Tenconi, E.; Riley, J.; Planckaert, S.; Tocquin, P.; Martinet, L.; Devreese, B.; Loria, R.; Rigali, S., Contribution of the beta-glucosidase BglC to the onset of the pathogenic lifestyle of Streptomyces scabies. *Molecular plant pathology* **2018,** *19* (6), 1480-1490.

17. Padilla-Reynaud, R.; Simao-Beaunoir, A. M.; Lerat, S.; Bernards, M. A.; Beaulieu, C., Suberin Regulates the Production of Cellulolytic Enzymes in Streptomyces scabiei, the Causal Agent of Potato Common Scab. *Microbes Environ* **2015,** *30* (3), 245-53.

18. Joshi, M. V.; Mann, S. G.; Antelmann, H.; Widdick, D. A.; Fyans, J. K.; Chandra, G.; Hutchings, M. I.; Toth, I.; Hecker, M.; Loria, R.; Palmer, T., The twin arginine protein transport pathway exports multiple virulence proteins in the plant pathogen Streptomyces scabies. *Molecular microbiology* **2010,** *77* (1), 252-71.

19. Komeil, D.; Padilla-Reynaud, R.; Lerat, S.; Simao-Beaunoir, A. M.; Beaulieu, C., Comparative secretome analysis of Streptomyces scabiei during growth in the presence or absence of potato suberin. *Proteome Sci* **2014,** *12*, 35.

20. Lauzier, A.; Simao-Beaunoir, A. M.; Bourassa, S.; Poirier, G. G.; Talbot, B.; Beaulieu, C., Effect of potato suberin on Streptomyces scabies proteome. *Molecular plant pathology* **2008,** *9* (6), 753-62.

21. Loria, R.; Bukhalid, R. A.; Creath, R. A.; Leiner, R. H.; Olivier, M.; Steffens, J. C., Differential Production of Thaxtomins by Pathogenic Streptomyces species in Vitro. *Phytopathology* **1995,** *85*, 537-541.

22. Vizcaino, J. A.; Csordas, A.; del-Toro, N.; Dianes, J. A.; Griss, J.; Lavidas, I.; Mayer, G.; Perez-Riverol, Y.; Reisinger, F.; Ternent, T.; Xu, Q. W.; Wang, R.; Hermjakob, H., 2016 update of the PRIDE database and its related tools. *Nucleic acids research* **2016,** *44* (D1), D447-56.

23. MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J., Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **2010,** *26* (7), 966-8.

24. Mesuere, B.; Van der Jeugt, F.; Devreese, B.; Vandamme, P.; Dawyndt, P., The unique peptidome: Taxon-specific tryptic peptides as biomarkers for targeted metaproteomics. *Proteomics* **2016,** *16* (17), 2313-8.

25. Desiere, F.; Deutsch, E. W.; King, N. L.; Nesvizhskii, A. I.; Mallick, P.; Eng, J.; Chen, S.; Eddes, J.; Loevenich, S. N.; Aebersold, R., The PeptideAtlas project. *Nucleic acids research* **2006,** *34* (Database issue), D655-8.

26. Yang, J.; Tauschek, M.; Robins-Browne, R. M., Control of bacterial virulence by AraC-like regulators that respond to chemical signals. *Trends Microbiol* **2011,** *19* (3), 128-35.

27. Jourdan, S.; Francis, I. M.; Deflandre, B.; Loria, R.; Rigali, S., Tracking the Subtle Mutations Driving Host Sensing by the Plant Pathogen Streptomyces scabies. *mSphere* **2017,** *2* (2), e00367-16.

28. Kwakman, J. H.; Postma, P. W., Glucose kinase has a regulatory role in carbon catabolite repression in Streptomyces coelicolor. *Journal of bacteriology* **1994,** *176* (9), 2694-8.

29. van Wezel, G. P.; White, J.; Young, P.; Postma, P. W.; Bibb, M. J., Substrate induction and glucose repression of maltose utilization by Streptomyces coelicolor A3(2) is controlled by malR, a member of the lacI-galR family of regulatory genes. *Molecular microbiology* **1997,** *23* (3), 537-49.

30. Schlosser, A.; Weber, A.; Schrempf, H., Synthesis of the Streptomyces lividans maltodextrin ABC transporter depends on the presence of the regulator MalR. *FEMS microbiology letters* **2001,** *196* (1), 77-83.

31. Hurtubise, Y.; Shareck, F.; Kluepfel, D.; Morosoli, R., A cellulase/xylanase-negative mutant of Streptomyces lividans 1326 defective in cellobiose and xylobiose uptake is mutated in a gene encoding a protein homologous to ATP-binding proteins. *Molecular microbiology* **1995,** *17* (2), 367-77.

32. Schlosser, A.; Kampers, T.; Schrempf, H., The Streptomyces ATP-binding component MsiK assists in cellobiose and maltose transport. *Journal of bacteriology* **1997,** *179* (6), 2092-5.

33. Nothaft, H.; Dresel, D.; Willimek, A.; Mahr, K.; Niederweis, M.; Titgemeyer, F., The phosphotransferase system of Streptomyces coelicolor is biased for N-acetylglucosamine metabolism. *Journal of bacteriology* **2003,** *185* (23), 7019-23.

34. Nothaft, H.; Rigali, S.; Boomsma, B.; Swiatek, M.; McDowall, K. J.; van Wezel, G. P.; Titgemeyer, F., The permease gene nagE2 is the key to N-acetylglucosamine

131 sensing and utilization in Streptomyces coelicolor and is subject to multi-level
132 control. *Molecular microbiology* **2010,** *75* (5), 1133-44.

133 35. Nothaft, H.; Parche, S.; Kamionka, A.; Titgemeyer, F., In vivo analysis of HPr
134 reveals a fructose-specific phosphotransferase system that confers high-affinity
135 uptake in Streptomyces coelicolor. *Journal of bacteriology* **2003,** *185* (3), 929-37.

136 36. Rigali, S.; Nothaft, H.; Noens, E. E.; Schlicht, M.; Colson, S.; Muller, M.; Joris,
137 B.; Koerten, H. K.; Hopwood, D. A.; Titgemeyer, F.; van Wezel, G. P., The sugar
138 phosphotransferase system of Streptomyces coelicolor is regulated by the GntR-
139 family regulator DasR and links N-acetylglucosamine metabolism to the control of
140 development. *Molecular microbiology* **2006,** *61* (5), 1237-51.

141 37. Lambert, S.; Traxler, M. F.; Craig, M.; Maciejewska, M.; Ongena, M.; van
142 Wezel, G. P.; Kolter, R.; Rigali, S., Altered desferrioxamine-mediated iron utilization is
143 a common trait of bald mutants of Streptomyces coelicolor. *Metallomics : integrated*
144 *biometal science* **2014,** *6* (8), 1390-9.

145 38. Legault, G. S.; Lerat, S.; Nicolas, P.; Beaulieu, C., Tryptophan regulates
146 thaxtomin A and indole-3-acetic acid production in Streptomyces scabiei and
147 modifies its interactions with radish seedlings. *Phytopathology* **2011,** *101* (9), 1045-
148 51.

149 39. Johnson, E. G.; Krasnoff, S. B.; Bignell, D. R.; Chung, W. C.; Tao, T.; Parry, R. J.;
150 Loria, R.; Gibson, D. M., 4-Nitrotryptophan is a substrate for the non-ribosomal
151 peptide synthetase TxtB in the thaxtomin A biosynthetic pathway. *Molecular*
152 *microbiology* **2009,** *73* (3), 409-18.

153 40. Spaepen, S.; Vanderleyden, J., Auxin and plant-microbe interactions. *Cold*
154 *Spring Harb Perspect Biol* **2011,** *3* (4), a001438

155 41. Zummo, F. P.; Marineo, S.; Pace, A.; Civiletti, F.; Giardina, A.; Puglia, A. M.,
156 Tryptophan catabolism via kynurenine production in Streptomyces coelicolor:
157 identification of three genes coding for the enzymes of tryptophan to anthranilate
158 pathway. *Applied microbiology and biotechnology* **2012,** *94* (3), 719-28.

159 42. Kim, D. J.; Huh, J. H.; Yang, Y. Y.; Kang, C. M.; Lee, I. H.; Hyun, C. G.; Hong, S.
160 K.; Suh, J. W., Accumulation of S-adenosyl-L-methionine enhances production of
161 actinorhodin but inhibits sporulation in Streptomyces lividans TK23. *Journal of*
162 *bacteriology* **2003,** *185* (2), 592-600.

163 43. Pang, A. P.; Du, L.; Lin, C. Y.; Qiao, J.; Zhao, G. R., Co-overexpression of lmbW
164 and metK led to increased lincomycin A production and decreased byproduct
165 lincomycin B content in an industrial strain of Streptomyces lincolnensis. *Journal of*
166 *applied microbiology* **2015,** *119* (4), 1064-74.

167 44. Jin, Y. Y.; Cheng, J.; Yang, S. H.; Meng, L.; Palaniyandi, S. A.; Zhao, X. Q.; Suh, J.
168 W., S-adenosyl-L-methionine activates actinorhodin biosynthesis by increasing

autophosphorylation of the Ser/Thr protein kinase AfsK in Streptomyces coelicolor A3(2). *Bioscience, biotechnology, and biochemistry* **2011,** *75* (5), 910-3.

45. Zhao, X.; Wang, Q.; Guo, W.; Cai, Y.; Wang, C.; Wang, S.; Xiang, S.; Song, Y., Overexpression of metK shows different effects on avermectin production in various Streptomyces avermitilis strains. *World journal of microbiology & biotechnology* **2013,** *29* (10), 1869-75.

46. Butler, A. R.; Gandecha, A. R.; Cundliffe, E., Influence of ancillary genes, encoding aspects of methionine metabolism, on tylosin biosynthesis in Streptomyces fradiae. *The Journal of antibiotics* **2001,** *54* (8), 642-9.

47. Franza, T.; Expert, D., Role of iron homeostasis in the virulence of phytopathogenic bacteria: an 'a la carte' menu. *Molecular plant pathology* **2013,** *14* (4), 429-38.

48. Ratledge, C.; Dover, L. G., Iron metabolism in pathogenic bacteria. *Annu Rev Microbiol* **2000,** *54*, 881-941.

49. Chu, B. C.; Garcia-Herrero, A.; Johanson, T. H.; Krewulak, K. D.; Lau, C. K.; Peacock, R. S.; Slavinskaya, Z.; Vogel, H. J., Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. *Biometals* **2010,** *23* (4), 601-11.

50. Arias, A. A.; Lambert, S.; Martinet, L.; Adam, D.; Tenconi, E.; Hayette, M.-P.; Ongena, M.; Rigali, S., Growth of desferrioxamine-deficient Streptomyces mutants through xenosiderophore piracy of airborne fungal contaminations. *FEMS microbiology ecology* **2015,** *91* (7), fiv080.

51. Patel, P.; Song, L.; Challis, G. L., Distinct extracytoplasmic siderophore binding proteins recognize ferrioxamines and ferricoelichelin in Streptomyces coelicolor A3(2). *Biochemistry* **2010,** *49* (37), 8033-42.

52. Tierrafria, V. H.; Ramos-Aboites, H. E.; Gosset, G.; Barona-Gomez, F., Disruption of the siderophore-binding desE receptor gene in Streptomyces coelicolor A3(2) results in impaired growth in spite of multiple iron-siderophore transport systems. *Microbial biotechnology* **2011,** *4* (2), 275-85.

53. Traxler, M. F.; Watrous, J. D.; Alexandrov, T.; Dorrestein, P. C.; Kolter, R., Interspecies interactions stimulate diversification of the Streptomyces coelicolor secreted metabolome. *mBio* **2013,** *4* (4), e00459-13.

54. Craig, M.; Lambert, S.; Jourdan, S.; Tenconi, E.; Colson, S.; Maciejewska, M.; Ongena, M.; Martin, J. F.; van Wezel, G.; Rigali, S., Unsuspected control of siderophore production by N-acetylglucosamine in streptomycetes. *Environ Microbiol Rep* **2012,** *4* (5), 512-21.

55. Yamanaka, K.; Oikawa, H.; Ogawa, H. O.; Hosono, K.; Shinmachi, F.; Takano, H.; Sakuda, S.; Beppu, T.; Ueda, K., Desferrioxamine E produced by Streptomyces

griseus stimulates growth and development of Streptomyces tanashiensis. *Microbiology* **2005,** *151* (Pt 9), 2899-905.

56. Tunca, S.; Barreiro, C.; Coque, J. J.; Martin, J. F., Two overlapping antiparallel genes encoding the iron regulator DmdR1 and the Adm proteins control siderophore [correction of sedephore] and antibiotic biosynthesis in Streptomyces coelicolor A3(2). *FEBS J* **2009,** *276* (17), 4814-27.

57. Seipke, R. F.; Song, L.; Bicz, J.; Laskaris, P.; Yaxley, A. M.; Challis, G. L.; Loria, R., The plant pathogen Streptomyces scabies 87-22 has a functional pyochelin biosynthetic pathway that is regulated by TetR- and AfsR-family proteins. *Microbiology* **2011,** *157* (Pt 9), 2681-93.

58. Guerinot, M. L., Microbial iron transport. *Annu Rev Microbiol* **1994,** *48*, 743-72.

59. Cornelis, P.; Dingemans, J., Pseudomonas aeruginosa adapts its iron uptake strategies in function of the type of infections. *Front Cell Infect Microbiol* **2013,** *3*, 75.

60. Takase, H.; Nitanai, H.; Hoshino, K.; Otani, T., Requirement of the Pseudomonas aeruginosa tonB gene for high-affinity iron acquisition and infection. *Infect Immun* **2000,** *68* (8), 4498-504.

61. Yaxley, A. M., Study of the complete genome sequence of Streptomyces scabies (or scabiei) 87.22. *PhD dissertation* **2009**.

62. Kinashi, H.; Someno, K.; Sakaguchi, K., Isolation and Characterization of Concanamycin-a, Concanamycin-B and Concanamycin-C. *J Antibiot* **1984,** *37* (11), 1333-1343.

63. Seki-Asano, M.; Okazaki, T.; Yamagishi, M.; Sakai, N.; Hanada, K.; Mizoue, K., Isolation and characterization of new 18-membered macrolides FD-891 and FD-892. *The Journal of antibiotics* **1994,** *47* (11), 1226-33.

64. Natsume, M.; Tashiro, N.; Doi, A.; Nishi, Y.; Kawaide, H., Effects of concanamycins produced by Streptomyces scabies on lesion type of common scab of potato. *Journal of General Plant Pathology* **2017,** *83* (2), 78-82.

65. Bignell, D. R.; Seipke, R. F.; Huguet-Tapia, J. C.; Chambers, A. H.; Parry, R. J.; Loria, R., Streptomyces scabies 87-22 contains a coronafacic acid-like biosynthetic cluster that contributes to plant-microbe interactions. *Molecular plant-microbe interactions : MPMI* **2010,** *23* (2), 161-75.

66. Cheng, Z.; Bown, L.; Tahlan, K.; Bignell, D. R., Regulation of coronafacoyl phytotoxin production by the PAS-LuxR family regulator CfaR in the common scab pathogen Streptomyces scabies. *PloS one* **2015,** *10* (3), e0122450.

67. Hempel, A. M.; Cantlay, S.; Molle, V.; Wang, S. B.; Naldrett, M. J.; Parker, J. L.; Richards, D. M.; Jung, Y. G.; Buttner, M. J.; Flardh, K., The Ser/Thr protein kinase AfsK

regulates polar growth and hyphal branching in the filamentous bacteria Streptomyces. *Proceedings of the National Academy of Sciences of the United States of America* **2012,** *109* (35), E2371-9.

68. Umeyama, T.; Lee, P. C.; Horinouchi, S., Protein serine/threonine kinases in signal transduction for secondary metabolism and morphogenesis in Streptomyces. *Applied microbiology and biotechnology* **2002,** *59* (4-5), 419-25.

69. Nodwell, J. R.; McGovern, K.; Losick, R., An oligopeptide permease responsible for the import of an extracellular signal governing aerial mycelium formation in Streptomyces coelicolor. *Molecular microbiology* **1996,** *22* (5), 881-93.

70. Park, H. S.; Shin, S. K.; Yang, Y. Y.; Kwon, H. J.; Suh, J. W., Accumulation of S-adenosylmethionine induced oligopeptide transporters including BldK to regulate differentiation events in Streptomyces coelicolor M145. *FEMS microbiology letters* **2005,** *249* (2), 199-206.

71. Bibb, M. J.; Domonkos, A.; Chandra, G.; Buttner, M. J., Expression of the chaplin and rodlin hydrophobic sheath proteins in Streptomyces venezuelae is controlled by sigma(BldN) and a cognate anti-sigma factor, RsbN. *Molecular microbiology* **2012,** *84* (6), 1033-49.

72. Bibb, M. J.; Molle, V.; Buttner, M. J., sigma(BldN), an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in Streptomyces coelicolor A3(2). *Journal of bacteriology* **2000,** *182* (16), 4606-16.

73. Traxler, M. F.; Seyedsayamdost, M. R.; Clardy, J.; Kolter, R., Interspecies modulation of bacterial development through iron competition and siderophore piracy. *Molecular microbiology* **2012,** *86* (3), 628-44.

74. Bignell, D. R.; Francis, I. M.; Fyans, J. K.; Loria, R., Thaxtomin A production and virulence are controlled by several bld gene global regulators in Streptomyces scabies. *Molecular plant-microbe interactions : MPMI* **2014,** *27* (8), 875-85.

75. Kwak, J.; McCue, L. A.; Trczianka, K.; Kendrick, K. E., Identification and characterization of a developmentally regulated protein, EshA, required for sporogenic hyphal branches in Streptomyces griseus. *Journal of bacteriology* **2001,** *183* (10), 3004-15.

76. Saito, N.; Matsubara, K.; Watanabe, M.; Kato, F.; Ochi, K., Genetic and biochemical characterization of EshA, a protein that forms large multimers and affects developmental processes in Streptomyces griseus. *The Journal of biological chemistry* **2003,** *278* (8), 5902-11.

77. Kawamoto, S.; Watanabe, M.; Saito, N.; Hesketh, A.; Vachalova, K.; Matsubara, K.; Ochi, K., Molecular and functional analyses of the gene (eshA) encoding the 52-kilodalton protein of Streptomyces coelicolor A3(2) required for antibiotic production. *Journal of bacteriology* **2001,** *183* (20), 6009-16.

78. Saito, N.; Xu, J.; Hosaka, T.; Okamoto, S.; Aoki, H.; Bibb, M. J.; Ochi, K., EshA accentuates ppGpp accumulation and is conditionally required for antibiotic production in Streptomyces coelicolor A3(2). *Journal of bacteriology* **2006,** *188* (13), 4952-61.

Table 1. Metabolism

Protein assignation	Gene assignation	Name	Known or Putative function	Fold change (vs wild-type)		
				Δ*cebR*	**+ $(Glc)_2$**	**Δ*cebR* + $(Glc)_2$**
Carbohydrate metabolism						
Glycolysis and Gluconeogenesis						
C9Z433	SCAB_42161	Fba	Putative fructose 1.6-bisphosphate aldolase	1.59	1.02	1.55
C9YTW6	SCAB_67551	GlkA	Glucokinase	1.05	-1.91	-1.71
TCA cycle						
C9YVY6	SCAB_21101	SacA	Aconitate hydratase	1.44	-1.71	-
C9ZF68	SCAB_33551	FumB	Fumarate hydratase class I	1.79	-	-
C9YUT6	SCAB_35791	SucD	Succinyl-CoA ligase [ADP-forming] subunit alpha	-	2.09	-
Pentose phosphate pathway						
C9Z545	SCAB_11811	ZWF3	6-phosphogluconate dehydrogenase	1.57	1.38	1.94
C9YY12	SCAB_54161	PrsA2	Ribose-phosphate pyrophosphokinase	-1.85	2.33	1.27
C9YY83	SCAB_69911	Tal	Transaldolase	1.65	1.38	2.19
C9YY84	SCAB_69921	TktA	Transketolase	1.54	1.04	1.64
Simple. oligo- and complex carbohydrates utilisation						
Cellulose, cello-oligosaccharides and cellobiose utilization						
C9Z448	SCAB_57721	BglC	Beta-glucosidase	3.00	2.69	6.83
C9Z449	SCAB_57731	CebG	Cellobiose/cellotriose ABC-transporter permease	3.03	2.21	3.83
C9Z451	SCAB_57751	CebE	cellobiose/cellotriose ABC-transporter binding	7.69	4.70	7.33
Starch, maltodextrine, maltose utilization						
C9Z6V6	SCAB_27951	GlgP	Alpha-1.4 glucan (maltodextrin) phosphorylase	4.06	-1.15	3.06
C9ZHD4	SCAB_66571	MalR	Starch/Maltose operon transcriptional repressor	1.87	1.65	2.01
C9ZHD5	SCAB_66581	MalE	Maltose ABC-transporter binding protein	1.54	-3.80	-1.32
C9ZHD7	SCAB_66601	MalG	Maltose ABC-transporter membrane component	1.07	-2.65	-2.23
C9ZHD8	SCAB_66611	AglA	Alpha-glucosidase	-1.32	-3.03	-1.31
C9Z1A6	SCAB_71771	-	GH15 family glucoamylase and related GHs	-2.28	-1.73	-3.01
Xylose utilization						
C9YVX8	SCAB_21021	XylF	D-xylose ABC-transporter binding protein	-	-1.62	-
Multiple carbohydrate pathway transporter/enzyme						
C9Z435	SCAB_42181	-	Sugar mutarotase (aldose epimerase family)	1.88	1.06	1.58
C9ZH37	SCAB_50161	MsiK	Multiple sugar ABC transporter ATP-binding subunit	1.99	1.06	1.73
C9Z9C1	SCAB_76201	PTS E1	PEP phosphotransferase system E1 (Fru and GlcNAc)	-	-3.11	-
C9ZFY0	SCAB_79811	ThcA	Aldehyde dehydrogenase	2.05	-1.43	2.44
Amino sugar and nucleotide sugar metabolism						

C9YWG2	SCAB_53131	GtaB	UTP-glucose-1-phosphate uridylyltransferase	-1.84	1.98	-
Amino acids metabolism						
Tryptophan, phenylalanine, and tyrosine metabolism						
C9Z8D6	SCAB_28641	-	3-hydroxybutyryl-coA dehydrogenase	1.58	-	1.73
C9Z429	SCAB_42121	KynU	Kynureninase	2.19	2.30	2.40
C9Z430	SCAB_42131	KynA	Tryptophan 2.3-dioxygenase	2.31	6.18	2.77
C9ZC21	SCAB_46571	PheA	Prephenate dehydratase	-	-1.64	-
C9Z5T6	SCAB_58971	AroQ	Chorismate mutase	-	1.79	-
C9YTX6	SCAB_67661	AroH	Phospho-2-dehydro-3-deoxyheptonate aldolase	-1.59	-	-
C9YVG1	SCAB_68361	HisC	Histidinol-phosphate aminotransferase	-2.16	-	-
C9YVH5	SCAB_68501	TrpB	Tryptophan synthase beta chain	1.52	1.57	2.01
Valine, leucine, and, isoleucine Biosynthesis						
C9Z555	SCAB_26601	LeuD	3-isopropylmalate dehydratase small subunit	-1.56	-1.50	-1.62
C9Z5A1	SCAB_27071	IlvN	Acetolactate synthase small subunit	-1.51	-	-
C9ZAQ8	SCAB_61851	LeuA	2-isopropylmalate synthase	-2.77	-1.76	-2.45
Methionine and cysteine metabolism						
C9ZGT9	SCAB_34371	MetB	Cystathionine gamma-synthase	-1.66	-1.35	-1.79
C9YZJ1	SCAB_55181	SahH	Adenosylhomocysteinase	2.03	2.23	3.35
C9Z309	SCAB_73321	MetH	Methionine synthase	1.14	1.58	1.62
C9Z7L0	SCAB_75311	MetK	S-adenosylmethionine synthase	1.61	-	1.94
Glutamate metabolism						
C9Z204	SCAB_24891	GluB	Glutamate uptake system binding subunit	-	-1.85	-1.56
C9ZHG5	SCAB_66881	GlnA	Glutamine synthetase	-	-1.55	-
Lysine biosynthesis						
C9Z234	SCAB_25191	DapA1	4-hydroxy-tetrahydrodipicolinate synthase	-	-1.52	-1.42
C9ZBW7	SCAB_31231	DapE	Succinyl-diaminopimelate desuccinylase	1.55	-1.25	-
Glycine, serine and threonine metabolism						
C9Z3M2	SCAB_25841	-	Betaine-aldehyde dehydrogenase	-	2.84	-
C9Z599	SCAB_27051	SerA	D-3-phosphoglycerate dehydrogenase	-1.61	-	-
C9ZH61	SCAB_50401	ThrC3	Threonine synthase	-1.23	1.51	-
C9YTK4	SCAB_51101	SerC	Phosphoserine aminotransferase	-1.36	-1.54	-1.63
C9YXA3	SCAB_6921	SoxG	Sarcosine oxidase gamma subunit	1.80	-2.48	-
C9YXA4	SCAB_6931	SoxA	Sarcosine oxidase alpha subunit	3.03	-2.01	-
C9YXA7	SCAB_6961	-	Putative dehydrogenase	1.59	-1.99	-1.13
Arginine biosynthesis						
C9Z5Y7	SCAB_74301	ArgH	Argininosuccinate lyase	-1.81	-	-1.50
Metabolism of other amino acids						
Glutathione metabolism						
C9YV75	SCAB_52361	-	Glutathione peroxidase	2.13	-	-
Lipid metabolism						
Fatty acid metabolism						
C9YVX2	SCAB_20961	-	Fatty acid oxidation complex alpha-subunit	-3.30	-	-2.41
C9YY49	SCAB_54571	FadA2	Acetyl-CoA C-acyltransferase	1.87	-1.40	1.74
C9Z9F5	SCAB_76541	FabG	3-oxoacyl-[acyl-carrier protein] reductase	1.96	-1.34	1.56
Steroid metabolism						
C9Z6Y6	SCAB_28271	Che	Cholesterol esterase	-	-3.66	-2.92
Nucleotide metabolism						
Purine & pyrimidine metabolism						
C9YUW0	SCAB_36051	GuaA	GMP synthase	-1.58	-1.51	-1.65
C9YWT5	SCAB_69391	PsuG	Pseudouridine-5'-phosphate glycosidase	-	-1.77	-
C9Z7K7	SCAB_75271	Gmk	Guanylate kinase	-1.71	-1.30	-1.88
C9ZB78	SCAB_78051	PurB	Adenylosuccinate lyase	-1.84	-	-
C9YU37	SCAB_82101	YagT	Xanthine dehydrogenase iron-sulfur-binding subunit	-1.74	-	-
Energy metabolism						
Oxidative phosphorylation						
C9YXV4	SCAB_38011	NuoI	NADH-quinone oxidoreductase subunit I	1.83	-2.32	-2.08
C9YXV6	SCAB_38031	NuoG	NADH-quinone oxidoreductase	1.45	-1.78	-1.50
C9YXV7	SCAB_38041	NuoF	NADH dehydrogenase subunit	1.64	-1.96	-1.48
C9YXV9	SCAB_38061	NuoD	NADH-quinone oxidoreductase subunit D	1.50	-1.38	-1.18
C9YXW0	SCAB_38071	NuoC	NADH-quinone oxidoreductase subunit C	1.96	-	-
C9YTU0	SCAB_67291	CtaC	Cytochrome c oxidase subunit II	-1.31	-1.64	-
Sulfur metabolism						
C9YUJ7	SCAB_19771	-	Nitrite/sulphite reductase	-1.79	-1.47	-
C9ZH62	SCAB_50411	-	Sulfur metabolism protein	-2.22	1.43	-
Metabolism of cofactors and vitamins						
Nicotinate and nicotinamide metabolism						
C9ZBR9	SCAB_30741	-	NUDIX hydrolase	1.64	-	-

Porphyrin metabolism						
C9YZ88	SCAB_38711	HemL	Glutamate-1-semialdehyde 2.1-aminomutase	-1.63	-	-
C9ZHA5	SCAB_66281	-	Heme oxygenase	1.80	-	-
Thiamine metabolism						
C9ZBZ9	SCAB_46341	ThiC	Thiamine biosynthesis protein	-1.67	-	-
Xenobiotics biodegradation and metabolism						
C9Z1F7	SCAB_72331	-	Carboxymethylenebutenolidase	1.62	1.30	2.34
Metabolism of terpenoids and polyketides						
Biosynthesis of enediyne antibiotics						
C9ZBT1	SCAB_30871	-	NDP-hexose 4-ketoreductase	-1.90	-1.55	-3.51

294

295

296 **Table 2. Specialized metabolism and other proteins involved in *Streptomyces* development**

Protein assignation	Gene assignation	Name	Known or Putative function	Fold change (vs wild-type)		
				Δ*cebR*	**+ $(Glc)_2$**	**Δ*cebR* + $(Glc)_2$**
Specialized (secondary) metabolism						
Pyochelin						
C9ZDM0	SCAB_1411	-	AMP-binding NRPS ligase (Pyochelin)	4.21	-	3.94
C9YTN2	SCAB_1431	-	ABC transporter ATP-binding subunit (Pyochelin)	2.24	-	2.41
C9ZCY5	SCAB_1451	-	Transporter membrane component (Pyochelin)	18.23	1.66	19.00
Thaxtomin A						
C9ZDB8	SCAB_31761	TxtC	Cytochrome P450 monooxygenase	3.76	4.83	6.44
C9ZDC0	SCAB_31781	TxtB	Thaxtomin synthetase B	1.71	2.29	2.62
C9ZDC1	SCAB_31791	TxtA	Thaxtomin synthetase A	2.61	3.53	3.60
C9ZDC6	SCAB_31831	TxtE	L-tryptophan-nitrating cytochrome P450	2.42	2.77	2.47
Coronafacoyl phytotoxins						
C9ZED0	SCAB_79611	Cfa2	Polyketide dehydratase component	-1.86	-2.41	-5.90
C9ZED6	SCAB_79671	CFL	CoA ligase	-1.98	-	-2.77
Concanamycin A						
C9YYJ3	SCAB_84021	-	Carbomoyltransferase	2.02	-	-
C9YYJ6	SCAB_84051	-	O-methyltransferase	5.74	-	4.12
Unknown 1						
C9ZG90	SCAB_3221	-	NRPS multienzyme	-79.31	35.43	-
C9ZG91	SCAB_3231	-	Oxidoreductase	-	1.80	-
C9ZG92	SCAB_3241	-	Thioesterase	-	6.56	3.90
C9ZG96	SCAB_3281	-	NRPS-associated AMP-binding protein	-	3.26	2.00
Unknown 2						
C9Z2W2	SCAB_72821	-	TetR-family transcriptional regulator	2.06	-	1.57
C9Z2W5	SCAB_72851	-	Putative polyketide cyclase	2.64	-	2.58
Development associated proteins						
C9Z0H0	SCAB_24081	CutR	Two-component response regulator	-	1.81	-
C9ZD97	SCAB_31531	BldKB	BldKB-like ABC oligopeptide-binding transport	-	-2.61	-1.92
C9YZC9	SCAB_39121	BldN	AdsA-like sigma factor	-2.19	-	-1.71
C9YUD3	SCAB_5051	EshA	Cyclic nucleotide-binding protein	-1.54	-1.38	-
C9YV27	SCAB_51861	AfsK	Membrane protein	-	14.83	-1.56
C9Z060	SCAB_84991	NsdB	Tetratricopeptide repeat (TPR) domain protein	-1.36	2.41	-
C9Z393	SCAB_86731	SlbR	ScbR-like γ-butyrolactone binding regulator	-	1.98	-
Stress response						
C9Z719	SCAB_43471	F40	Cold shock protein	-1.76	-	-
C9YWU0	SCAB_69441	TerZ	Putative stress-induced export associated protein	-	-2.08	-1.73
C9ZHS9	SCAB_81661	TerD	Tellurium resistance protein	-	-1.55	-
Metal homeostasis						
Iron homeostasis						
C9ZCY1	SCAB_47401	DesE	(Xeno)siderophore-interacting protein	-1.22	4.79	10.76
C9ZCY3	SCAB_51401	DmdR1	Iron-dependent repressor	-2.59	1.56	-
C9ZAS1	SCAB_67681	Bfr	Bacterioferritin	1.45	-3.75	-
Zinc homeostasis						
C9YTX8	SCAB_61981	ZnuA	Metal-binding lipoprotein	-	1.85	3.06

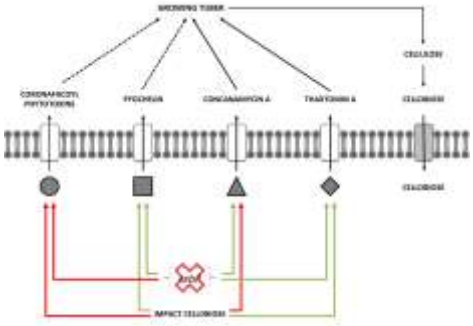
297

298

299 **Table 3. Genetic Information Processing**

Protein assignation	Gene assignation	Name	Known or Putative function	Fold change (vs wild-type)		
				Δ*cebR*	+ $(Glc)_2$	Δ*cebR* + $(Glc)_2$
Transcription						
mRNA biogenesis						
C9Z3S7	SCAB_26391	Rnc	Ribonuclease 3	1.96	1.07	1.52
Transcription termination						
C9Z8G5	SCAB_28931	Rho	Transcription termination factor	-2.10	-	-1.47
RNA polymerase-associated proteins						
C9YWA8	SCAB_37251	NusG	Transcription antiterminator	-	1.76	-
Transcription elogation						
C9ZGT4	SCAB_34321	GreA	Transcription elongation factor	-1.73	-1.19	-1.61
Translation						
Ribosomal proteins						
C9YW47	SCAB_36631	RplM	50S ribosomal protein L13	-1.71	-1.50	-1.67
C9YW52	SCAB_36681	RpsK	30S ribosomal protein S11	-1.69	-1.30	-
C9YW61	SCAB_36771	RpsE	30S ribosomal protein S5	-1.68	-1.35	-1.50
C9YW63	SCAB_36791	RplF	50S ribosomal protein L6	-1.55	-1.48	-
C9YW66	SCAB_36821	RplE	50S ribosomal protein L5	-1.51	-1.64	-1.62
C9YW68	SCAB_36841	RplN	50S ribosomal protein L14	-1.63	-1.44	-
C9YW71	SCAB_36871	RplP	50S ribosomal protein L16	-1.75	-1.44	-1.53
C9YW76	SCAB_36921	RplW	50S ribosomal protein L23	-1.60	-1.37	-1.33
C9YW77	SCAB_36931	RplD	50S ribosomal protein L4	-1.63	-1.42	-
C9YW79	SCAB_36951	RpsJ	30S ribosomal protein S10	-1.74	-1.41	-
C9YW94	SCAB_37111	RspG	30S ribosomal protein S7	-1.51	-1.38	-1.41
C9YWA3	SCAB_37201	RplL	50S ribosomal protein L7/L12	-1.90	-	-
C9YWA6	SCAB_37231	RlpA	50S ribosomal protein L1	-1.79	-	-
C9ZAN3	SCAB_46101	RpsF	30S ribosomal protein S6	-1.54	-	-
C9ZAN6	SCAB_46131	RplI	50S ribosomal protein L9	-1.52	-	-1.20
C9YY11	SCAB_54151	RplY	50S ribosomal protein L25	-1.80	1.47	-
C9Z7H2	SCAB_60151	RplU	50S ribosomal protein L21	-1.54	-1.43	-1.40
C9Z7H3	SCAB_60161	RpmA	50S ribosomal protein L27	-1.68	-1.47	-
C9Z4H9	SCAB_73971	RpmI	50S ribosomal protein L35	-1.56	-	-
C9Z7K6	SCAB_75261	RpsM	30S ribosomal protein S13	-2.48	-	-1.67
Aminoacyl-tRNA biosynthesis						
C9Z5B3	SCAB_27201	GatB	Aspartyl/glutamyl-tRNA amidotransferase subunit B	-1.31	-1.55	-
C9ZAS2	SCAB_62001	GlyS	Glycine-tRNA ligase	-2.12	1.85	-
C9Z1L3	SCAB_85511	-	Methionyl-tRNA formyltransferase	-	2.13	3.42
Translation factors						
Initiation factors						
C9Z250	SCAB_25351	InfB	Translation initiation factor IF-2	-1.98	-	-
C9YW55	SCAB_36711	InfA	Translation initiation factor IF-1	-2.38	-1.43	-1.97
C9Z4H8	SCAB_73961	InfC	Translation initiation factor IF-3	-2.05	-	-1.60
Elongation factors						
C9ZB41	SCAB_77661	Tuf3	Elongation factor Tu	1.55	-	-
Maturation factors						
C9ZF57	SCAB_33441	AviX1	Ribosome-binding ATPase	-1.80	2.78	-
Folding, sorting and degradation						
Chaperones and folding catalysts						
Chaperones						
C9Z5G9	SCAB_42541	DnaK	Chaperone protein	-	-1.53	-
C9YUY4	SCAB_36291	GroL1	60 kDa chaperonin	-1.77	-	-
C9ZH64	SCAB_50441	GroEL2	60 kDa chaperonin	-1.67	-	-
C9Z7E9	SCAB_59931	Tig	Cell division trigger factor (PPIase)	-2.08	1.08	-1.14
Protein export						
C9Z4E2	SCAB_73591	TatA	Sec-independent protein translocase protein	-	2.07	2.48
Peptide degradation						
C9YZV3	SCAB_71311	TldD	Inhibitor of DNA gyrase	1.68	-	-
C9Z4D0	SCAB_73471	PrcB	Proteasome subunit beta	1.62	1.48	-
Replication and repair						
DNA replication						
C9YWP5	SCAB_68981	PolA	DNA polymerase I	1.66	1.39	2.17

300 **Table of contents graphic**

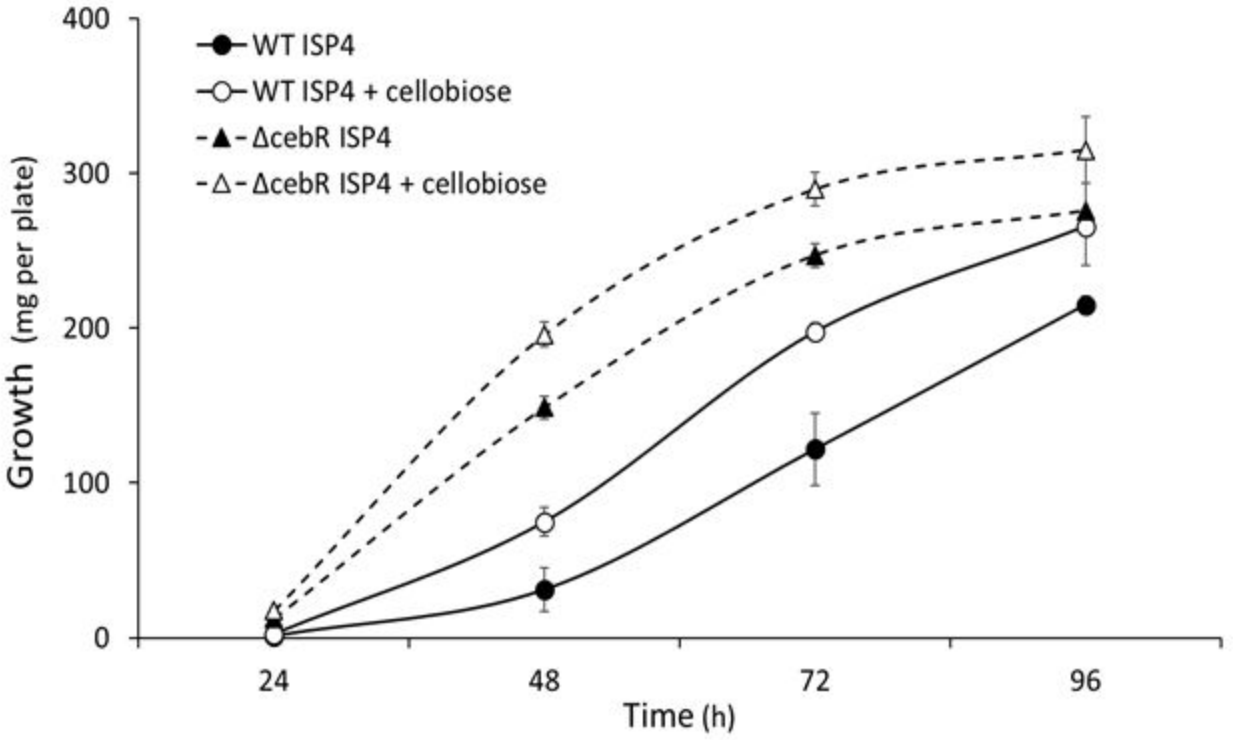


301

302

Figure 1

A


WT ISP4
WT ISP4 + cellobiose
ΔcebR ISP4
ΔcebR ISP4 + cellobiose
Growth (mg per plate)
400
300
200
100
0
24
48
72
96
Time (h)

B

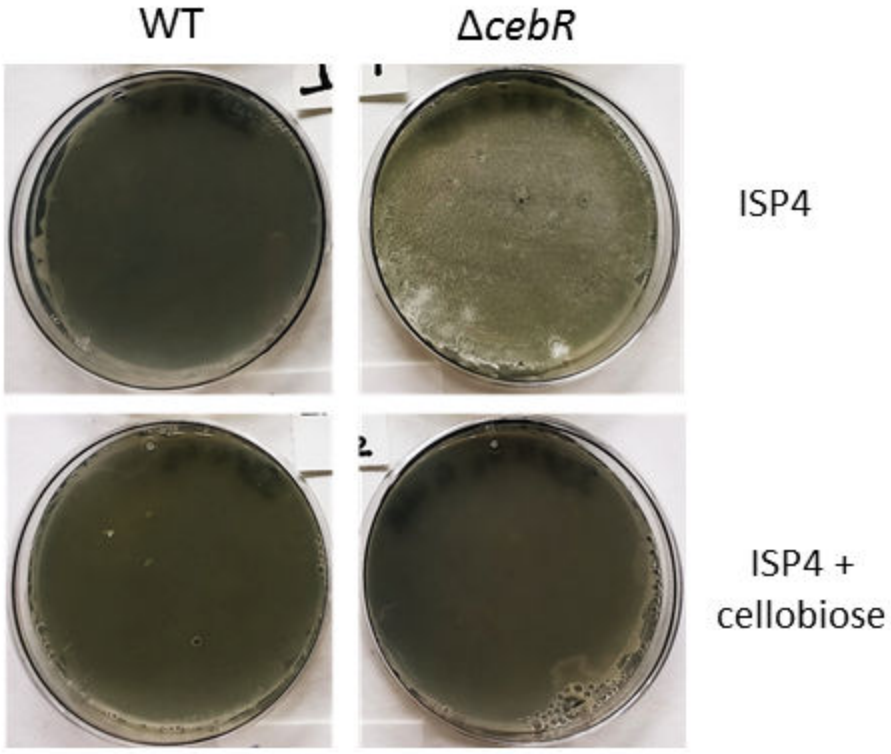
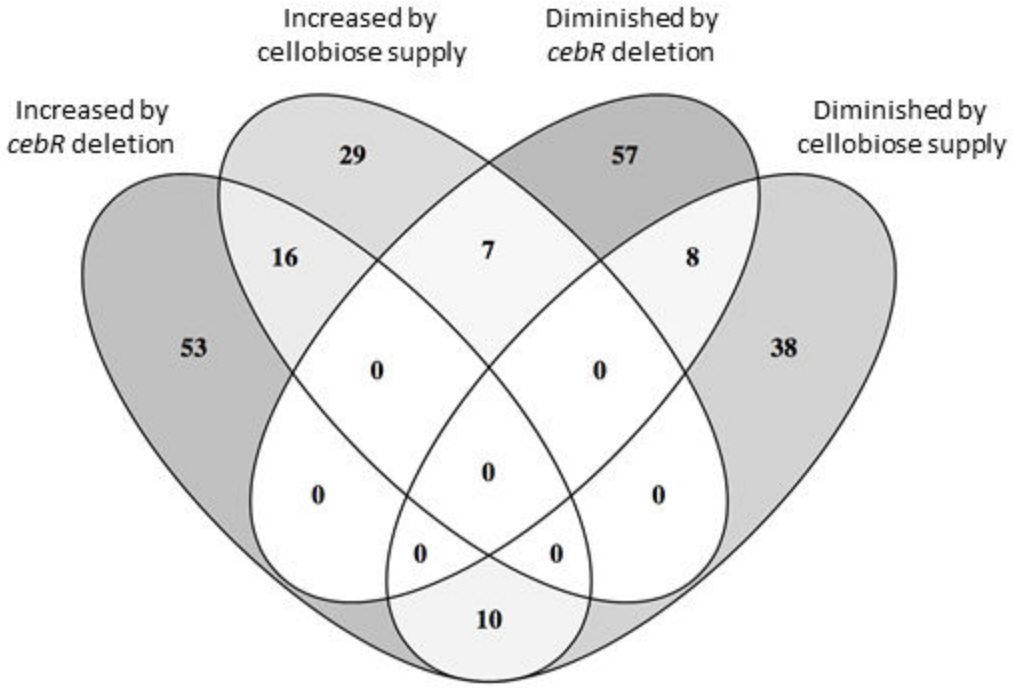

WT
ΔcebR
ISP4
ISP4 +
cellobiose

Figure 2


Increased by cellobiose supply
Diminished by *cebR* deletion
Increased by *cebR* deletion
Diminished by cellobiose supply
29
57
16
7
8
53
0
0
38
0
0
0
0
0
10

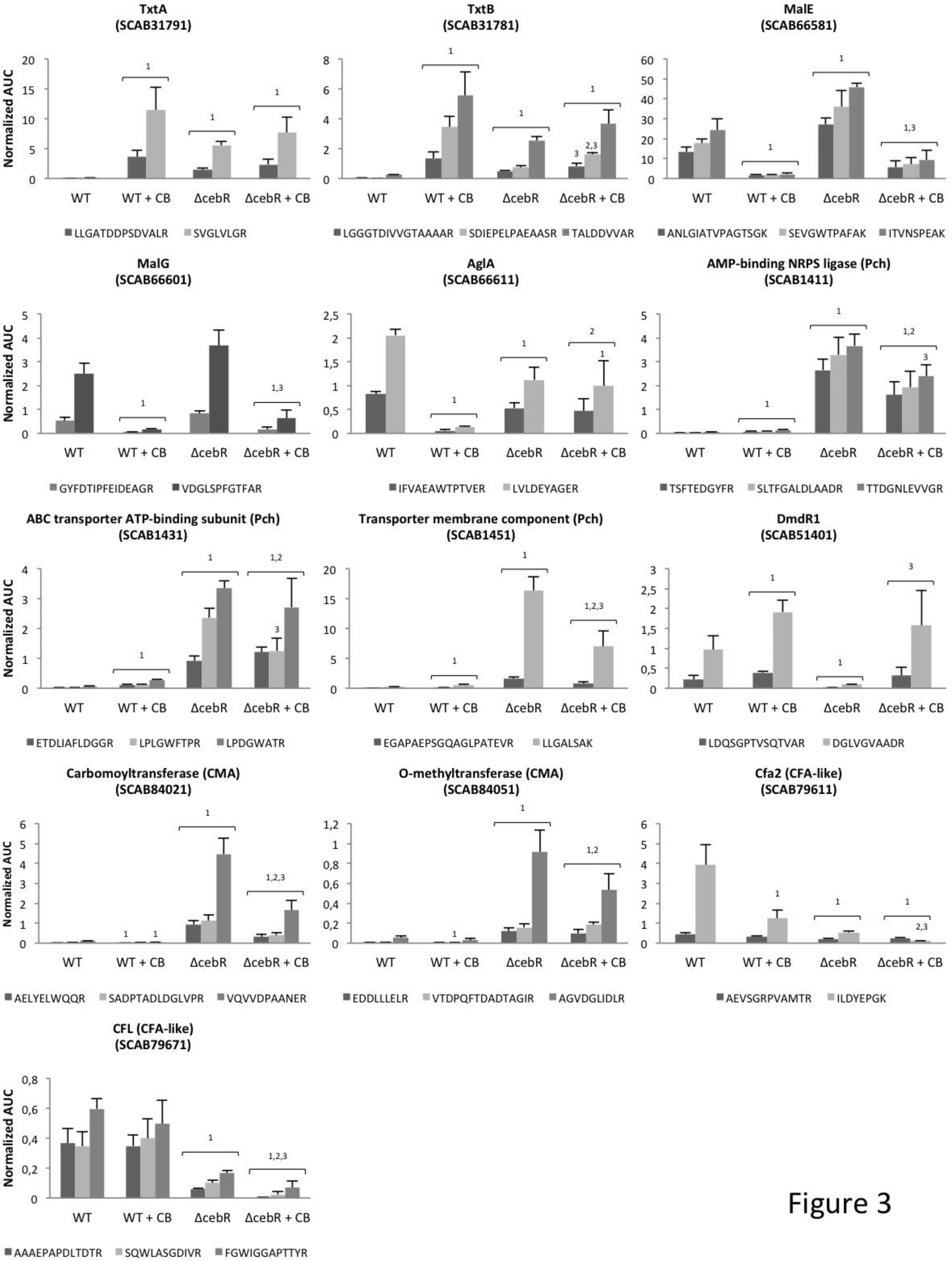
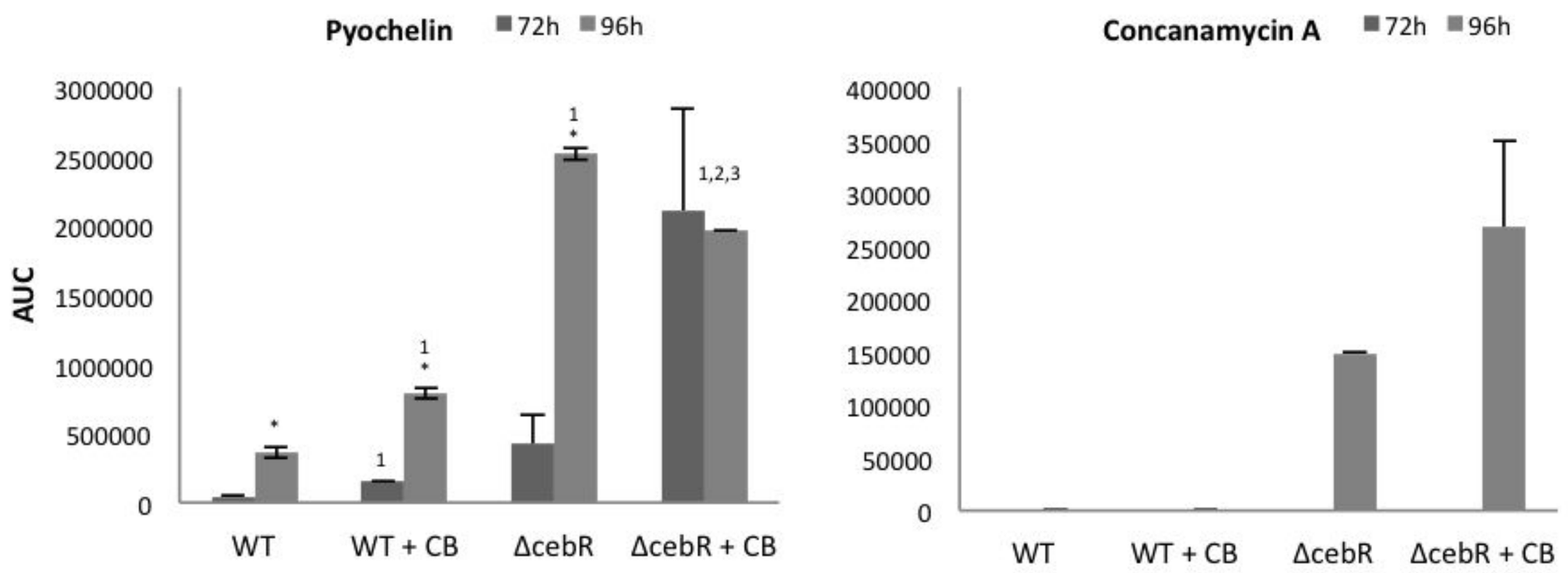

TxtA
(SCAB31791)
TxtB
(SCAB31781)
MalE
(SCAB66581)
Normalized AUC
WT
WT + CB
ΔcebR
ΔcebR + CB
LLGATDDPSDVALR
SVGLVLGR
LGGGTDIVVGTAAAAR
SDIEPELPAEAASR
TALDDVVAR
ANLGIATVPAGTSGK
SEVGWTPAFAK
ITVNSPEAK
MalG
(SCAB66601)
AglA
(SCAB66611)
AMP-binding NRPS ligase (Pch)
(SCAB1411)
GYFDTIPFEIDEAGR
VDGLSPFGTFAR
IFVAEAWTPTVER
LVLDEYAGER
TSFTEDGYFR
SLTFGALDLAADR
TTDGNLEVVGR
ABC transporter ATP-binding subunit (Pch)
(SCAB1431)
Transporter membrane component (Pch)
(SCAB1451)
DmdR1
(SCAB51401)
ETDLIAFLDGGR
LPLGWFTPR
LPDGWATR
EGAPAEPSGQAGLPATEVR
LLGALSAK
LDQSGPTVSQTVAR
DGLVGVAADR
Carbomoyltransferase (CMA)
(SCAB84021)
O-methyltransferase (CMA)
(SCAB84051)
Cfa2 (CFA-like)
(SCAB79611)
AELYELWQQR
SADPTADLDGLVPR
VQVVDPAANER
EDDLLLELR
VTDPQFTDADTAGIR
AGVDGLIDLR
AEVSGRPVAMTR
ILDYEPGK
CFL (CFA-like)
(SCAB79671)
AAAEPAPDLTDTR
SQWLASGDIVR
FGWIGGAPTTYR

Figure 3

Figure 4


Pyochelin
72h
96h
AUC
3000000
2500000
2000000
1500000
1000000
500000
0
*
1
*
1
*
1
1,2,3
WT
WT + CB
ΔcebR
ΔcebR + CB
Concanamycin A
72h
96h
400000
350000
300000
250000
200000
150000
100000
50000
0
WT
WT + CB
ΔcebR
ΔcebR + CB

Supporting Information: Proteomic response to the thaxtomin phytotoxin elicitor cellobiose and to the deletion of the cellulose utilization regulator CebR in *Streptomyces scabies*

Sören Planckaert[1], Samuel Jourdan[2], Isolde M. Francis[3], Benoit Deflandre[2], Sébastien Rigali[2,†,*], and Bart Devreese[1,†,*]

[1] Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, B-9000, Ghent, Belgium
[2] InBioS – Center for Protein Engineering, University of Liège, Institut de Chimie, B6a, B-4000, Liège, Belgium
[3] Department of Biology, California State University Bakersfield, CA 93311-1022, Bakersfield, USA
† These authors jointly supervised this work and are joint corresponding authors.

Correspondence should be addressed to B.D. (bart.devreese@ugent.be) and S.R. (srigali@uliege.be)

Contents of Supporting Information File

Method S1. Detailed label-free proteomic methodology.

This methodology is identical to the one used in Jourdan et al. (2016) doi:10.1038/srep27144

The protein lysate was mixed with 100% (w/v) TCA solution (Sigma-Aldrich) (4:1). The extract was then centrifuged at 16,000 x g for 30 min at 4°C. The supernatant was discarded and the pellet was washed twice with ice-cold acetone (Sigma-Aldrich). After each washing step the pellet was recovered by centrifugation at 16,000 x g for 30 min at 4°C. The resulting pellet was solubilized in 50 mM ammonium bicarbonate (Sigma-Aldrich) containing 2 M urea (GE Healthcare). The protein concentration was assessed by Bradford analysis using the Pierce™ Coomassie Protein Assay Kit. Protein solutions (10 µg) were subsequently reduced, alkylated and digested with trypsin (Promega) overnight (1:50 w/w). Digested samples were dried and dissolved in 50 µl 20mM ammonium formate. This procedure was performed for three biological replicates.

Peptide mixtures (0.2 µg/µl in 20 mM ammonium formate, pH 10) were separated on a NanoAcquity UPLC® system (Waters Corporation) in 2D mode. For the first dimension (high pH) the sample (1 µg) was loaded onto an XbridgeTM BEH130 C18 column (300 µm × 50 mm, 5 µm; Waters) at 3% solvent B1 (A1 & B1: 20 mM ammonium formate in water and ACN respectively, pH 10) at 2 µl/min. Peptides were eluted from the first dimension column in 5 fractions (11.1%, 14.5%, 17.4%, 20.8%, and 45.0% of solvent B1), and fractions were trapped on a Symmetry® C18 trapping column (180 µm × 20 mm, 5 µm; Waters). For the second dimension (low pH) each fraction was separated on a HSS T3 C18 analytical column (75 µm × 250 mm, 1.8 µm; Waters) at 40°C at 250 nl/min by increasing the acetonitrile concentration from 5 to 50% B2 (A2 & B2: 0.1% formic acid in water and ACN respectively, pH 2) over 60 min. The outlet of the column was directly connected to a PicoTip™ Emitter (New Objective) mounted on a Nanolockspray source of a SYNAPT™ G1 HDMS mass spectrometer (Waters). The time- of-flight (TOF) analyser was externally calibrated with MS/MS fragments of human [glu1]-fibrinopeptide B (Glu-fib) from m/z 72 to 1285, and the data were corrected post-acquisition using the monoisotopic mass of the doubly charged precursor of Glu-fib (m/z 785.8426) (lock mass correction). Accurate mass data were collected in a data independent positive mode of acquisition (MS^E) by alternating between low (5 V) and high (ranging from 15 to 35 V) energy scan functions (Geromanos et al., 2009). The selected m/z range was 125–2000 Da. The capillary voltage was set to 3.0 kV, the sampling cone voltage was 26 V and the extraction cone voltage 2.65 V. The source temperature was set at 65°C.

The acquired spectra (Waters.raw) were loaded to the Progenesis software (version 4.1, Nonlinear) for label free quantification. Peaks were then modelled in non-noisy areas to record their peak m/z value, intensity, area under the curve (AUC) and m/z width. After selecting a quality control sample (mixture of all samples) as a reference, the retention times of all other samples within the experiment are aligned to maximal overlay of the 2D feature maps. After alignment and feature exclusion, samples were divided into the appropriate condition. The identification was performed by ProteinLynx Global SERVER v2.5 Identity^{E} search engine (PLGS, Waters Corporation) using *Streptomyces scabies* 87-22 database (uniprot-streptomyces+AND+scabies, 27/05/2015, 8722 sequences). The precursor and fragment ion tolerance were determined automatically. The default protein identification criteria used included a maximal protein mass of 250,000 Da, a detection of minimal of three fragment ions per peptide, minimal seven fragment ions per protein and minimal two peptides per protein. Carbamidomethyl-cysteine (fixed) and methionine oxidation (variable) were selected as modifications. Maximally one missed cleavage and a false positive rate of 4% was allowed. After normalization of the raw abundances of all features and statistical analysis the different fractions were combined. For quantification, all unique peptides of an identified protein were included and the total cumulative abundance was calculated by summing the abundances of all peptides allocated to the respective protein. ANOVA values of $p < 0.05$, regulation of 1.5-fold or 0.66-fold, power > 0.8 and q-values < 0.05 were regarded as significant for all further results.

Table S3. Lists of transitions selected for MRM validation of proteins with significant change in abundance upon *cebR* deletion or cellobiose induction.

	Protein name	Peptide sequence	Precursor (m/z)	Product ion (m/z)	Collision energy (V)
Pyochelin	Salicyl-AMP ligase (SCAB1411)	SLTFGALDLAADR	675.356616	1048.542185	24
				901.473771	
				773.415194	
		TSFTEDGYFR	611.772385	887.389373	22
				786.341694	
				657.299101	
		TTDGNLEVVGR	580.798934	958.495235	20
				843.468292	
				672.403901	
	ABC transporter permease/ATPase (SCAB1431	LPLGWFTPR	543.808376	876.472649	19
				763.388585	
				706.367121	
		LPDGWATR	458.237784	802.384228	16
				705.331464	
				590.304521	
		ETDLIAFLDGGR	653.835516	848.462478	23
				735.378414	
				664.341300	
	Major facilitator superfamily protein (SCAB1451)	LLGALSAK	386.749996	659.408652	13
				546.324588	
				418.266010	
		EGAPAEPSGQAGLPATEVR	918.957954	141.171758	33
				128.267499	
				1098.590198	

	Protein name	Peptide sequence	Precursor (m/z)	Product ion (m/z)	Collision energy (V)
	Iron-dependent repressor (SCAB1401)	LDQSGPTVSQTVAR	729.880987	958.531620	26
				861.478856	
				760.431178	
		DGLVGVAADR	486.759080	687.378414	17
				588.310000	
				531.288536	
		HLELTEEGR	542.275095	833.399937	19
				704.357344	
				591.273280	
Concanamycin A	Carbamoyltransferase (SCAB84021)	VQVVDPAANER	599.314751	970.495235	21
				871.426821	
				657.331464	
		SADPTADLDGLVPR	713.862262	955.520721	25
				884.483607	
				769.456664	
		AELYELWQQR	668.338226	1135.589470	24
				1022.505406	
				859.442077	
	O-methyltransferase (SCAB84051)	EDDLLLELR	558.300778	871.524744	20
				756.497801	
				643.413737	
		AGVDGLIDLR	514.790381	801.446494	18
				686.419551	
				516.314023	
		VTDPQFTDADTAGIR	803.889009	1066.516364	29
				919.447950	
				818.400272	

Protein name		Peptide sequence	Precursor (m/z)	Product ion (m/z)	Collision energy (V)
Thaxtomin A	Thaxtomin synthetase A (SCAB31791)	SVGLVLGR	400.753070	614.398421	14
				557.376957	
				444.292893	
		LLGATDDPSDVALR	721.877913	987.474165	26
				872.447222	
				757.420279	
	Thaxtomin synthetase B (SCAB31781)	SDIEPELPAEAASR	742.865002	1040.537100	26
				943.484336	
				814.441743	
		TALDDVVAR	480.261456	787.430844	17
				674.346780	
				559.319837	
		LGGGTDIVVGTAAAAR	714.893897	928.557441	25
				815.473377	
				716.404963	
	Polyketide dehydratase component (SCAB79611)	ILDYEPGK	467.747650	821.403960	16
				708.319896	
				593.292953	
		AEVSGRPVAMTR	637.337703	887.487981	23
				830.466518	
				674.365407	
	CoA ligase (SCAB79671)	FGWIGGAPTTYR	663.335487	822.410442	23
				765.388979	
				708.367515	

Protein name		Peptide sequence	Precursor (m/z)	Product ion (m/z)	Collision energy (V)
	CoA ligase (SCAB79671)	AAAEPAPDLTDTR	664.328056	888.442137	24
				817.405023	
				720.352259	
		SQWLASGDIVR	616.325119	830.473043	22
				717.388979	
				646.351865	
				693.320230	
				564.277637	
	MalR (SCAB66571)	AQGVPFVLVDGFSPK	780.924666	1205.656487	28
				961.535309	
				862.466895	
		VQAPFISPDDR	622.817127	1017.499986	22
				946.462872	
				849.410108	
		IGLALGPK	384.752538	655.413737	13
				598.392273	
				485.308209	
	MalE (SCAB66581)	SEVGWTPAFAK	596.803488	976.525078	21
				877.456664	
				634.355888	
		ITVNSPEAK	479.763831	845.436323	17
				744.388644	
				645.320230	

Protein name		Peptide sequence	Precursor (m/z)	Product ion (m/z)	Collision energy (V)
	MalE (SCAB66581)	ANLGIATVPAGTSGK	678.877716	1058.584050	24
				888.478522	
				817.441408	
	MalG (SCAB66601)	GYFDTIPFEIDEAGR	865.407034	1362.653586	31
				1146.578964	
				1033.494900	
		VDGLSPFGTFAR	633.827494	1052.552356	22
				882.446828	
				795.414800	
	α-amylase (SCAB66611)	LVLDEYAGER	582.798403	952.437051	20
				839.352987	
				595.283451	
		IFVAEAWTPTVER	759.901191	1159.574213	27
				959.494506	
				888.457393	
	BSA (P02769)	AEFVEVTK	461.747650	722.408317	16
				575.339903	16
				476.271489	16
		QTALVELLK	507.813324	785.513117	18
				714.476003	18
				601.391939	18

Table S5. Prediction CebR regulon in *Streptomyces scabies*

PREDetector Regulon prediction report

Analysis Summary

Date: 2018-06-13

Template sequence

- Streptomyces scabiei 87.22 strain=87.22 [NC_013929_1]
- Source: ncbi [2018-06-13]
- Taxid: 680198

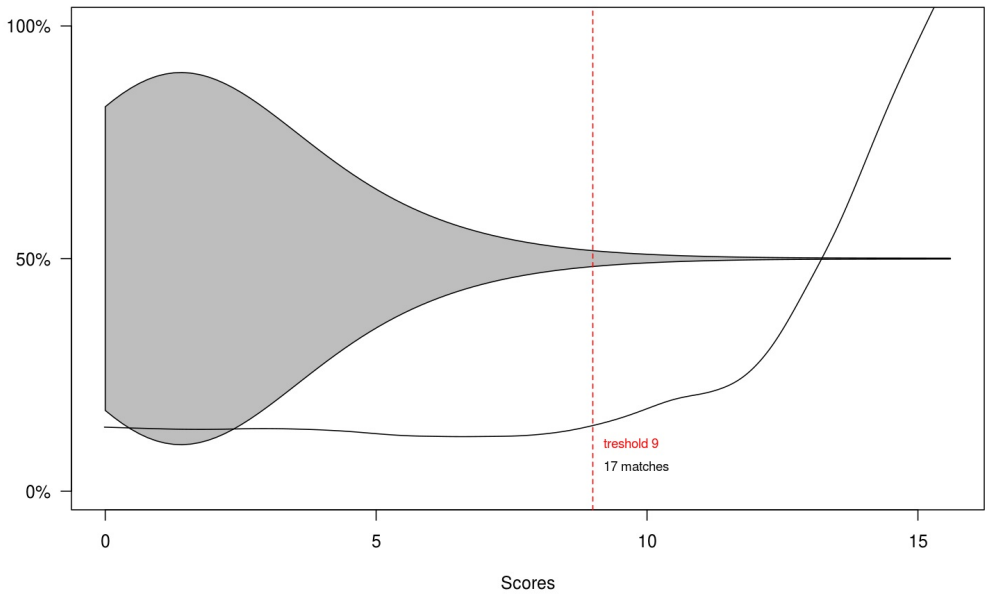
Matrix sequences

- CebR_Sina
- 16 sequences.
- 2018-06-13

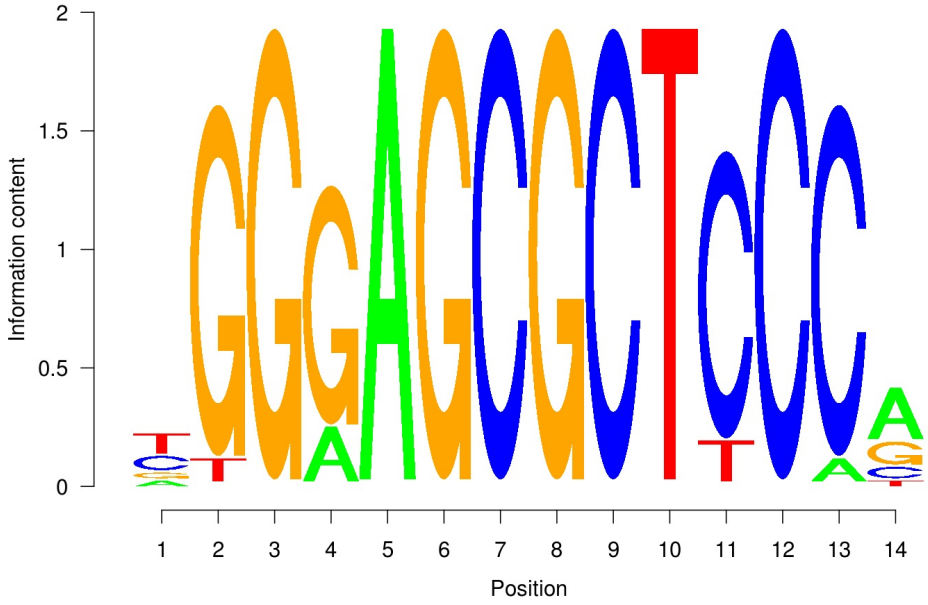
Parameters

- Cutoff score: 9.00
- Selected regions: Upstream Regulatory Coding
- Co-transcribed genes: -50 40
- Regulatory bounds: -350 to 50

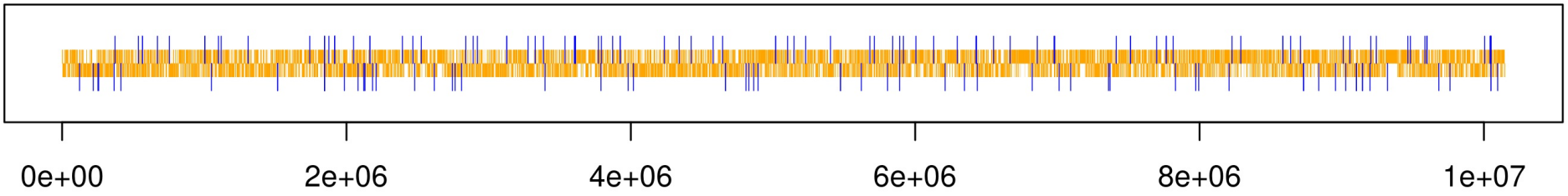
Cut-off plot



Matrix Logo



Genomic Context



Prediction results

Gene	Product	Sequence	Position	Score	Co-transc. genes	Co-transc. products
SCAB_RS02300 [SCAB_4971] (SCAB_RS02300)	hypothetical protein	TGGGAGCGCTCCCA	-158	15.6	NA	NA
SCAB_RS02295 [SCAB_4961] (SCAB_RS02295)	glucuronoxylanase xynC	TGGGAGCGCTCCCA	-66	15.6	NA	NA
SCAB_RS02775 [SCAB_5981] (SCAB_RS02775)	glycosyl hydrolase family 5	TGGGAGCGCTCCCA	-82	15.6	NA	NA
SCAB_RS04175 [SCAB_8871] (SCAB_RS04175)	glycosyl hydrolase family 5	TGGGAGCGCTCCCA	-90	15.6	NA	NA
SCAB_RS04180 [SCAB_8891] (SCAB_RS04180)	signal transduction histidine kinase	TGGGAGCGCTCCCA	-926	15.6	NA	NA
SCAB_RS07750 [SCAB_16431] (SCAB_RS07750)	endoglucanase	TGGGAGCGCTCCCA	-113	15.6	NA	NA
SCAB_RS07745 [SCAB_16421] (SCAB_RS07745)	LacI family transcriptional regulator	TGGGAGCGCTCCCA	-200	15.6	NA	NA
SCAB_RS08030 [SCAB_17011] (SCAB_RS08030)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGCTCCCA	-82	15.6	NA	NA
SCAB_RS08025 [SCAB_17001] (SCAB_RS08025)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGCTCCCA	-311	15.6	NA	NA
SCAB_RS09970 [SCAB_21081] (SCAB_RS09970)	chitin-binding protein	TGGGAGCGCTCCCA	-237	15.6	NA	NA
SCAB_RS24385 [SCAB_51081] (SCAB_RS24385)	endoglucanase	TGGGAGCGCTCCCA	-54	15.6	NA	NA
SCAB_RS27575 [SCAB_57751]	sugar-binding protein	TGGGAGCGCTCCCA	-130	15.6	SCAB_RS27570 [SCAB_57741] (SCAB_RS27570); SCAB_RS27565 [SCAB_57731] (SCAB_RS27565)	sugar ABC transporter permease; carbohydrate ABC transporter permease

(SCAB_RS27575)						
SCAB_RS27580 [SCAB_57761] (SCAB_RS27580)	LacI family transcriptional regulator	TGGGAGCGCTCCCA	-499	15.6	SCAB_RS27585 (SCAB_RS27585)	DUF2191 domain-containing protein
SCAB_RS37770 [SCAB_78881] (SCAB_RS37770)	beta-mannosidase	TGGGAGCGCTCCCA	-85	15.6	NA	NA
SCAB_RS42910 [SCAB_89741] (SCAB_RS42910)	chitin-binding protein	TGGGAGCGCTCCCA	-116	15.6	NA	NA
SCAB_RS43070 [SCAB_90061] (SCAB_RS43070)	hypothetical protein	TGGGAGCGCTCCCA	-138	15.6	NA	NA
SCAB_RS43080 [SCAB_90081] (SCAB_RS43080)	glycosyl hydrolase family 5	TGGGAGCGCTCCCA	-60	15.6	NA	NA
SCAB_RS43085 [SCAB_90091] (SCAB_RS43085)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGCTCCCA	-66	15.6	NA	NA
SCAB_RS43090 [SCAB_90101] (SCAB_RS43090)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGCTCCCA	-761	15.6	NA	NA
SCAB_RS27560 [SCAB_57721] (SCAB_RS27560)	beta-glucosidase	TGGAAGCGCTCCCA	-13	15.1	NA	NA
SCAB_RS39425 [SCAB_82441] (SCAB_RS39425)	esterase	TGGGAGCGCTTCCA	-117	15.1	NA	NA
SCAB_RS39420 [SCAB_82421] (SCAB_RS39420)	pectate lyase	TGGGAGCGCTTCCA	-351	15.1	SCAB_RS39415 [SCAB_82411] (SCAB_RS39415)	pectate lyase
SCAB_RS15090 [SCAB_31801] (SCAB_RS15090)	cellobiose-dependent regulator TxtR	CGGGAGCGCTCCCA	-801	14.4	NA	NA
SCAB_RS15085 [SCAB_31791] (SCAB_RS15085)	non-ribosomal peptide synthetase	CGGGAGCGCTCCCA	-899	14.4	NA	NA
SCAB_RS37830 [SCAB_79011] (SCAB_RS37830)	acetylxylan esterase	TGGGAGCGCTCCCG	-94	14.4	NA	NA
SCAB_RS39240 [SCAB_82041] (SCAB_RS39240)	pectate lyase	TGGGAGCGCTCCCG	-285	14.4	NA	NA
SCAB_RS39235 [SCAB_82031] (SCAB_RS39235)	cellulose-binding protein	TGGGAGCGCTCCCG	-40	14.4	NA	NA
SCAB_RS43090 [SCAB_90101] (SCAB_RS43090)	cellulose 1%2C4-beta-cellobiosidase	CGGGAGCGCTCCCA	-192	14.4	NA	NA
SCAB_RS43085 [SCAB_90091] (SCAB_RS43085)	cellulose 1%2C4-beta-cellobiosidase	CGGGAGCGCTCCCA	-635	14.4	NA	NA
SCAB_RS07750 [SCAB_16431] (SCAB_RS07750)	endoglucanase	TGGAAGCGCTTCCA	-128	14.1	NA	NA
SCAB_RS07745 [SCAB_16421] (SCAB_RS07745)	LacI family transcriptional regulator	TGGAAGCGCTTCCA	-185	14.1	NA	NA
SCAB_RS16490 [SCAB_34801] (SCAB_RS16490)	DNA-binding response regulator	TGGGAGCGCTCCAA	-35	14	NA	NA
SCAB_RS16485 (SCAB_RS16485)	SigE family RNA polymerase sigma factor	TGGGAGCGCTCCAA	-268	14	NA	NA

SCAB_RS30100 [SCAB_63031] (SCAB_RS30100)	hypothetical protein	CGGAAGCGCTCCCA	458	13.9	NA	NA
SCAB_RS07750 [SCAB_16431] (SCAB_RS07750)	endoglucanase	TGGGAGCGCTTCCG	-264	13.9	NA	NA
SCAB_RS07745 [SCAB_16421] (SCAB_RS07745)	LacI family transcriptional regulator	TGGGAGCGCTTCCG	-49	13.9	NA	NA
SCAB_RS15080 [SCAB_31781] (SCAB_RS15080)	non-ribosomal peptide synthetase	GGGGAGCGCTCCCA	1507	13.6	NA	NA
SCAB_RS34495 [SCAB_72101] (SCAB_RS34495)	hypothetical protein	TGGGAGCGCTCCCC	417	13.6	NA	NA
SCAB_RS01525 (SCAB_RS01525)	non-ribosomal peptide synthetase	CGGGAGCGCTTCCA	1410	13.5	NA	NA
SCAB_RS23415 [SCAB_49071] (SCAB_RS23415)	hypothetical protein	GGGGAGCGCTTCCA	-226	12.8	NA	NA
SCAB_RS23420 [SCAB_49091] (SCAB_RS23420)	hypothetical protein	GGGGAGCGCTTCCA	-501	12.8	NA	NA
SCAB_RS41050 [SCAB_85841] (SCAB_RS41050)	XRE family transcriptional regulator	AGGGAGCGCTCCCG	-34	12.8	SCAB_RS41055 [SCAB_85851] (SCAB_RS41055)	DUF397 domain-containing protein
SCAB_RS41045 [SCAB_85831] (SCAB_RS41045)	hypothetical protein	AGGGAGCGCTCCCG	-284	12.8	NA	NA
SCAB_RS27620 [SCAB_57831] (SCAB_RS27620)	AraC family transcriptional regulator	GGGGAGCGCTCCCG	818	12.2	NA	NA
SCAB_RS40580 [SCAB_84861] (SCAB_RS40580)	penicillin amidase	CGGGAGCGCTCCCC	264	12.2	NA	NA
SCAB_RS15910 [SCAB_33591] (SCAB_RS15910)	serine/threonine protein phosphatase	TGGGATCGCTCCCA	1718	11.8	NA	NA
SCAB_RS28585 [SCAB_59891] (SCAB_RS28585)	serine/threonine-protein phosphatase	TGGGATCGCTCCCA	694	11.8	NA	NA
SCAB_RS00510 [SCAB_1121] (SCAB_RS00510)	NADP oxidoreductase	GGGGAGCGCTTCCT	298	11.6	NA	NA
SCAB_RS11705 [SCAB_24801] (SCAB_RS11705)	hypothetical protein	GGGGAGCGCTTCCG	349	11.6	NA	NA
SCAB_RS20725 [SCAB_43591] (SCAB_RS20725)	bile acid:sodium symporter	GGGGAGCGCTTCCG	465	11.6	SCAB_RS20720 [SCAB_43581] (SCAB_RS20720)	hypothetical protein
SCAB_RS35515 [SCAB_74201] (SCAB_RS35515)	N-acetyl-gamma-glutamyl-phosphate reductase	TGGGAGCGCTTCTA	327	11.4	SCAB_RS35520 [SCAB_74211] (SCAB_RS35520); SCAB_RS35525 [SCAB_74221] (argB); SCAB_RS35530 [SCAB_74231] (SCAB_RS35530); SCAB_RS35535 [SCAB_74241] (SCAB_RS35535)	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase; acetylglutamate kinase; aspartate aminotransferase family protein; arginine repressor
SCAB_RS39685 [SCAB_82971] (SCAB_RS39685)	cysteine synthase	TTGGAGCGCTTCCC	579	11.4	NA	NA
SCAB_RS11540 [SCAB_24441] (SCAB_RS11540)	RNA polymerase sigma factor	CGGGAGCGCTTCCC	-393	11.4	SCAB_RS44705 (SCAB_RS44705)	IS5/IS1182 family transposase
SCAB_RS09230 [SCAB_19511] (SCAB_RS09230)	riboflavin biosynthesis protein RibD	TGGGAGAGCTTCCA	531	11.3	NA	NA
SCAB_RS08030						

[SCAB_17011] (SCAB_RS08030)	cellulose 1%2C4-beta-cellobiosidase	TGGAACCGCTCCCA	-235	11.3	NA	NA
SCAB_RS08025 [SCAB_17001] (SCAB_RS08025)	cellulose 1%2C4-beta-cellobiosidase	TGGAACCGCTCCCA	-158	11.3	NA	NA
SCAB_RS07380 (SCAB_RS07380)	MarR family transcriptional regulator	CGGGAGCGCTCCAG	139	11.1	SCAB_RS07385 [SCAB_15641] (SCAB_RS07385)	IclR family transcriptional regulator
SCAB_RS21630 [SCAB_45471] (SCAB_RS21630)	DUF2662 domain-containing protein	CGGGAGCGCTCCAG	97	11.1	SCAB_RS21635 [SCAB_45481] (SCAB_RS21635)	FHA domain-containing protein
SCAB_RS37425 [SCAB_78171] (SCAB_RS37425)	hypothetical protein	CTGGAGCGCTCCAA	201	11.1	NA	NA
SCAB_RS08740 [SCAB_18481] (SCAB_RS08740)	hypothetical protein	TGGGAGCGCACCCA	387	10.9	NA	NA
SCAB_RS40530 [SCAB_84761] (SCAB_RS40530)	ABC transporter substrate-binding protein	TTGAAGCGCTTCAA	-42	10.8	NA	NA
SCAB_RS10215 [SCAB_21571] (SCAB_RS10215)	amino acid permease	ACGGAGCGCTCCCA	1036	10.7	SCAB_RS10220 [SCAB_21581] (SCAB_RS10220)	glutamine synthetase
SCAB_RS23015 [SCAB_48261] (SCAB_RS23015)	phage portal protein	CGGGAGCGCTGCCA	12	10.7	NA	NA
SCAB_RS08905 [SCAB_18841] (SCAB_RS08905)	DNA-binding response regulator	GGGAAGCGCTTCCG	-326	10.7	NA	NA
SCAB_RS08900 [SCAB_18831] (SCAB_RS08900)	glycoside hydrolase family 68 protein	GGGAAGCGCTTCCG	-72	10.7	NA	NA
SCAB_RS11840 [SCAB_25101] (rimO)	30S ribosomal protein S12 methylthiotransferase RimO	AGGAAGCGCTCCAG	1062	10.6	SCAB_RS11835 [SCAB_25091] (pgsA); SCAB_RS11830 [SCAB_25081] (SCAB_RS11830)	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase; CinA family protein
SCAB_RS16255 [SCAB_34281] (SCAB_RS16255)	ATP-binding protein	CGGGACCGCTCCCA	2277	10.6	NA	NA
SCAB_RS06435 [SCAB_13531] (SCAB_RS06435)	hypothetical protein	TGAGAGCGCTCCCT	-98	10.6	NA	NA
SCAB_RS06430 [SCAB_13521] (SCAB_RS06430)	LacI family transcriptional regulator	TGAGAGCGCTCCCT	-70	10.6	NA	NA
SCAB_RS10915 [SCAB_23091] (SCAB_RS10915)	carbonic anhydrase	TGAGAGCGCTCCCG	-167	10.6	NA	NA
SCAB_RS13025 [SCAB_27461] (gcvT)	glycine cleavage system protein T	CGGGAGAGCTCCCA	-262	10.6	NA	NA
SCAB_RS13020 [SCAB_27451] (SCAB_RS13020)	ATP-binding protein	CGGGAGAGCTCCCA	-232	10.6	NA	NA
SCAB_RS05530 [SCAB_11651] (SCAB_RS05530)	Fpg/Nei family DNA glycosylase	CGGGAGCGCTCCAC	657	10.5	NA	NA
SCAB_RS43290 [SCAB_90571] (SCAB_RS43290)	oxidoreductase	GTGGAGCGCTCCCG	669	10.5	NA	NA
SCAB_RS19680 [SCAB_41391] (SCAB_RS19680)	WhiB family transcriptional regulator	GTGGAGCGCTCCCG	161	10.5	NA	NA
SCAB_RS19675 [SCAB_41381] (SCAB_RS19675)	anion-transporting ATPase	GTGGAGCGCTCCCG	347	10.5	NA	NA
SCAB_RS33755	pectate lyase	TGGAAGCGCTTCGA	804	10.3	SCAB_RS33760 [SCAB_70561] (SCAB_RS33760); SCAB_RS33765 [SCAB_70571] (SCAB_RS33765);	pectinesterase; pectinesterase; hydrolase; pectate lyase

[SCAB_70551] (SCAB_RS33755)	pectate lyase	TGGAACCGCTTCCA	804	10.3	SCAB_RS33770 [SCAB_70581] (SCAB_RS33770); SCAB_RS33775 [SCAB_70591] (SCAB_RS33775)	pectinesterase; pectinesterase; hydrolase; pectate lyase
SCAB_RS28095 [SCAB_58881] (SCAB_RS28095)	hypothetical protein	AGGAAGCGCTGCCA	102	10.2	NA	NA
SCAB_RS31795 [SCAB_66591] (SCAB_RS31795)	sugar ABC transporter permease	TGGCAGCGCTTCCG	708	10.2	SCAB_RS31800 [SCAB_66601] (SCAB_RS31800)	ABC transporter permease
SCAB_RS39705 [SCAB_83021] (SCAB_RS39705)	sporulation protein	AGGAAGCGCTCCAT	2178	10.2	NA	NA
SCAB_RS40115 [SCAB_83891] (SCAB_RS40115)	polyketide synthase	TGGCAGCGCTTCCG	4311	10.2	NA	NA
SCAB_RS24510 [SCAB_51351] (SCAB_RS24510)	isopenicillin N synthase family oxygenase	AGGAAGCGCTCCGA	-402	10.2	NA	NA
SCAB_RS01065 [SCAB_2361] (SCAB_RS01065)	peptidoglycan-binding protein	TGGGAGCGTTTCCT	138	10.1	SCAB_RS01070 [SCAB_2371] (SCAB_RS01070)	XRE family transcriptional regulator
SCAB_RS07865 [SCAB_16671] (SCAB_RS07865)	TatD family hydrolase	CGGAAGGGCTCCCA	165	10.1	NA	NA
SCAB_RS30210 [SCAB_63281] (SCAB_RS30210)	secondary metabolite biosynthesis protein	TGGGAGCCCTTCCG	1017	10.1	NA	NA
SCAB_RS32000 [SCAB_67011] (SCAB_RS32000)	DUF4240 domain-containing protein	CGGAAGCGTTCCCA	498	10.1	NA	NA
SCAB_RS38850 [SCAB_81171] (SCAB_RS38850)	N-methyl-L-tryptophan oxidase	TGGGAGCGTTCCAA	189	10.1	NA	NA
SCAB_RS40975 [SCAB_85671] (SCAB_RS40975)	cyclase	TTGAAGCGCTCCAG	381	10.1	NA	NA
SCAB_RS41830 [SCAB_87491] (SCAB_RS41830)	sugar ABC transporter substrate-binding protein	TGGGAGCGTTTCCG	863	10.1	NA	NA
SCAB_RS43700 [SCAB_18181] (SCAB_RS43700)	hypothetical protein	AGGAAGCGGTCCCA	1290	10.1	NA	NA
SCAB_RS31755 [SCAB_66511] (SCAB_RS31755)	pyridoxamine 5'-phosphate oxidase family protein	TGGGATCGCTTCCT	-13	10.1	NA	NA
SCAB_RS13640 [SCAB_28761] (atpD)	F0F1 ATP synthase subunit beta	AGGAAGCGCTCCAC	1233	10	NA	NA
SCAB_RS24905 [SCAB_52201] (SCAB_RS24905)	TPR repeat protein	GTGGAGCGCTTCCT	1140	10	NA	NA
SCAB_RS25795 [SCAB_54081] (SCAB_RS25795)	glycosyltransferase family 4 protein	GGGGAGCGCTGCCA	1256	9.9	NA	NA
SCAB_RS00890 [SCAB_1991] (SCAB_RS00890)	FMN-dependent NADH-azoreductase	TGTGAGCGCTCCCC	60	9.8	NA	NA
SCAB_RS25415 [SCAB_53281] (SCAB_RS25415)	peptidase S41	CGGTAGCGCTTCCA	588	9.8	NA	NA
SCAB_RS29445 [SCAB_61611] (SCAB_RS29445)	peptidase S41	TGGAAGCGCTACCG	471	9.8	NA	NA
SCAB_RS32430 [SCAB_67881]	transglutaminase	GGTGAGCGCTCCCA	133	9.8	NA	NA

(SCAB_RS32430)						
SCAB_RS16870 [SCAB_35601] (trpS)	tryptophan--tRNA ligase	TGGGTGCGCTCCCG	870	9.7	NA	NA
SCAB_RS25065 [SCAB_52561] (SCAB_RS25065)	hypothetical protein	GTGAAGCGCTCCAA	186	9.7	NA	NA
SCAB_RS37840 [SCAB_79041] (SCAB_RS37840)	hypothetical protein	TGGGTGCGCTCCCG	576	9.7	NA	NA
SCAB_RS39840 [SCAB_83301] (SCAB_RS39840)	LuxR family transcriptional regulator	AGGGCGCGCTCCCA	2481	9.7	NA	NA
SCAB_RS18720 [SCAB_39371] (SCAB_RS18720)	DNA integrity scanning protein DisA	CGGGAGCGCCCCCA	-35	9.7	NA	NA
SCAB_RS10275 [SCAB_21711] (SCAB_RS10275)	secondary metabolite protein	TGCGAGCGCTTCAA	100	9.6	NA	NA
SCAB_RS10540 [SCAB_22251] (SCAB_RS10540)	ATP-binding protein	TGGAAGCTCTCCCG	1173	9.6	NA	NA
SCAB_RS16730 [SCAB_35301] (SCAB_RS16730)	succinate dehydrogenase iron-sulfur subunit	CGCGAGCGCTTCCA	438	9.6	NA	NA
SCAB_RS21810 [SCAB_45901] (SCAB_RS21810)	chromosome partitioning protein ParB	TGGAAGCGATCCCG	451	9.6	NA	NA
SCAB_RS25290 [SCAB_53041] (SCAB_RS25290)	hypothetical protein	CTGAAGCGCTTCCT	141	9.6	NA	NA
SCAB_RS25295 [SCAB_53051] (SCAB_RS25295)	large conductance mechanosensitive channel protein MscL	TTGAAGCCCTCCCA	27	9.6	NA	NA
SCAB_RS33530 [SCAB_70111] (SCAB_RS33530)	QacE family quaternary ammonium compound efflux SMR transporter	AGTGAGCGCTTCCA	-132	9.6	SCAB_RS33535 [SCAB_70121] (SCAB_RS33535)	DNA-binding transcriptional regulator
SCAB_RS33525 [SCAB_70101] (SCAB_RS33525)	alpha-L-arabinofuranosidase	AGTGAGCGCTTCCA	-231	9.6	NA	NA
SCAB_RS14105 [SCAB_29731] (lon)	endopeptidase La	CTGGAGCGCTCCAG	831	9.4	NA	NA
SCAB_RS44785 (SCAB_RS44785)	restriction endonuclease subunit S	TGGATGCGCTTCCA	34	9.4	SCAB_RS13860 [SCAB_29211] (SCAB_RS13860); SCAB_RS13865 [SCAB_29221] (SCAB_RS13865); SCAB_RS44790 [SCAB_29231] (SCAB_RS44790)	type I restriction endonuclease subunit R; M48 family peptidase; helicase
SCAB_RS43085 [SCAB_90091] (SCAB_RS43085)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGGTTCCC	-209	9.3	NA	NA
SCAB_RS43090 [SCAB_90101] (SCAB_RS43090)	cellulose 1%2C4-beta-cellobiosidase	TGGGAGCGGTTCCC	-618	9.3	NA	NA
SCAB_RS08325 [SCAB_17621] (ccrA)	crotonyl-CoA carboxylase/reductase	TGGAAGCGCTTCGG	882	9.2	SCAB_RS08330 [SCAB_17631] (SCAB_RS08330); SCAB_RS08335 [SCAB_17641] (SCAB_RS08335); SCAB_RS08340 [SCAB_17651] (SCAB_RS08340); SCAB_RS08345 [SCAB_17661] (SCAB_RS08345)	protein meaA; CoA ester lyase; MaoC family dehydratase; acyl-CoA dehydrogenase
SCAB_RS11465 [SCAB_24271] (SCAB_RS11465)	insulinase family protein	TTGGTGCGCTCCCA	237	9.2	SCAB_RS11460 [SCAB_24261] (SCAB_RS11460)	insulinase family protein
SCAB_RS24075 [SCAB_50451] (SCAB_RS24075)	12-oxophytodienoate reductase	TGGGAGCGCACCAA	564	9.2	NA	NA
SCAB_RS27180 [SCAB_56971] (SCAB_RS27180)	hypothetical protein	TGGGTGCGCTTCCT	255	9.2	SCAB_RS27185 [SCAB_56981] (SCAB_RS27185)	hypothetical protein
SCAB_RS33235 [SCAB_69521]	amino acid ABC transporter permease	CGGTAGCGCTCCCG	102	9.2	NA	NA

(SCAB_RS33235)						
SCAB_RS38930 [SCAB_81381] (SCAB_RS38930)	membrane protein	TGGGTGCGCTTCCT	105	9.2	NA	NA
SCAB_RS30085 (SCAB_RS30085)	hypothetical protein	CGGCAGCGCTCCCG	-51	9.2	SCAB_RS30090 [SCAB_63011] (SCAB_RS30090)	alpha/beta hydrolase
SCAB_RS04620 [SCAB_9771] (SCAB_RS04620)	cobaltochelatase subunit CobN	CGGTAGCGCTCCAA	2526	9.1	NA	NA
SCAB_RS04690 [SCAB_9921] (SCAB_RS04690)	hypothetical protein	AGGAAGAGCTTCCA	465	9.1	NA	NA
SCAB_RS12110 [SCAB_25621] (SCAB_RS12110)	hypothetical protein	ATGGAGCGCTGCCA	609	9.1	SCAB_RS12105 [SCAB_25611] (SCAB_RS12105); SCAB_RS12100 [SCAB_25601] (SCAB_RS12100)	sugar ABC transporter permease; carbohydrate ABC transporter permease
SCAB_RS14145 [SCAB_29811] (SCAB_RS14145)	hypothetical protein	TGGAAGGGCTTCCT	207	9.1	SCAB_RS14150 [SCAB_29821] (SCAB_RS14150)	PadR family transcriptional regulator
SCAB_RS15825 [SCAB_33421] (SCAB_RS15825)	hypothetical protein	CGGAAGGGCTTCCA	336	9.1	NA	NA
SCAB_RS19350 [SCAB_40651] (SCAB_RS19350)	hypothetical protein	CGGAACCGCTTCCA	1449	9.1	NA	NA
SCAB_RS19595 [SCAB_41221] (SCAB_RS19595)	hypothetical protein	AGGAAGCTCTTCCA	1230	9.1	NA	NA
SCAB_RS20310 [SCAB_42651] (SCAB_RS20310)	hypothetical protein	TGGCAGCGCTCCAG	622	9.1	NA	NA
SCAB_RS26950 [SCAB_56491] (SCAB_RS26950)	DUF2017 domain-containing protein	TCGAAGCGCTCCCC	13	9.1	NA	NA
SCAB_RS32435 [SCAB_67891] (SCAB_RS32435)	DUF58 domain-containing protein	AGGGAGCGCTGCCG	606	9.1	NA	NA
SCAB_RS35910 [SCAB_74971] (SCAB_RS35910)	DUF349 domain-containing protein	CGGAAGCGGTTCCA	738	9.1	NA	NA
SCAB_RS38170 [SCAB_79741] (SCAB_RS38170)	alpha/beta hydrolase	GAGGAGCGCTTCCA	229	9.1	NA	NA
SCAB_RS09065 [SCAB_19181] (SCAB_RS09065)	gfo/Idh/MocA family oxidoreductase	AGAAAGCGCTTCCA	-44	9.1	NA	NA
SCAB_RS09060 [SCAB_19171] (SCAB_RS09060)	carbohydrate ABC transporter substrate-binding protein	AGAAAGCGCTTCCA	-35	9.1	SCAB_RS09055 [SCAB_19161] (SCAB_RS09055); SCAB_RS09050 [SCAB_19151] (SCAB_RS09050); SCAB_RS09045 [SCAB_19141] (SCAB_RS09045); SCAB_RS09040 [SCAB_19131] (SCAB_RS09040)	sugar ABC transporter permease; carbohydrate ABC transporter permease; hypothetical protein; ankyrin repeat domain-containing protein
SCAB_RS01040 [SCAB_2311] (SCAB_RS01040)	XRE family transcriptional regulator	GGGGAGCGGTTCCA	1	9	NA	NA
SCAB_RS01525 (SCAB_RS01525)	non-ribosomal peptide synthetase	CCGGAGCGCTCCCG	6963	9	NA	NA
SCAB_RS01675 [SCAB_3621] (SCAB_RS01675)	serine/threonine protein kinase	CGGGAGGGCTCCCG	1879	9	NA	NA
SCAB_RS02185 [SCAB_4741] (SCAB_RS02185)	AraC family transcriptional regulator	CTGGAGCGCTCCGA	816	9	NA	NA
SCAB_RS03175 [SCAB_6821] (SCAB_RS03175)	ABC transporter substrate-binding protein	AGGGAGCGCTCGCG	1482	9	SCAB_RS03170 [SCAB_6811] (SCAB_RS03170)	ABC transporter permease
SCAB_RS04410						

[SCAB_9351] (SCAB_RS04410)	transposase	CGGGATCGCTCCCG	1153	9	NA	NA
SCAB_RS08925 [SCAB_18881] (SCAB_RS08925)	MBL fold metallo-hydrolase	CGGGAGCGGTCCCG	366	9	NA	NA
SCAB_RS09135 [SCAB_19321] (SCAB_RS09135)	ATP-binding protein	CGGGACCGCTCCCT	17	9	NA	NA
SCAB_RS12010 [SCAB_25411] (SCAB_RS12010)	kinase	TGGAAGGGCTCCCC	103	9	NA	NA
SCAB_RS15905 [SCAB_33581] (SCAB_RS15905)	DUF402 domain-containing protein	GGGGACCGCTTCCA	99	9	NA	NA
SCAB_RS17865 [SCAB_37621] (SCAB_RS17865)	hypothetical protein	AGGGAGCGGTCCCG	341	9	NA	NA
SCAB_RS18370 [SCAB_38701] (SCAB_RS18370)	histidine phosphatase family protein	AGGGAGCGCTCACG	670	9	NA	NA
SCAB_RS20585 [SCAB_43271] (SCAB_RS20585)	monooxygenase	CGGGAGCGGTCCCG	1618	9	NA	NA
SCAB_RS22160 [SCAB_46631] (SCAB_RS22160)	hypothetical protein	AGGGAGCGTTCCCG	129	9	NA	NA
SCAB_RS26290 [SCAB_55121] (SCAB_RS26290)	DUF3499 domain-containing protein	TGGAAGCGCTCGCC	280	9	NA	NA
SCAB_RS26565 [SCAB_55671] (SCAB_RS26565)	serine/threonine protein kinase	CGGGAGCGCTCCGT	195	9	NA	NA
SCAB_RS29605 [SCAB_61931] (SCAB_RS29605)	isoprenyl transferase	TGGAAGCGCTCGCC	273	9	SCAB_RS29610 [SCAB_61941] (SCAB_RS29610)	hypothetical protein
SCAB_RS30545 [SCAB_64011] (SCAB_RS30545)	MarR family transcriptional regulator	GGGGAGCGGTTCCA	160	9	SCAB_RS30540 [SCAB_64001] (SCAB_RS30540)	N-acetyltransferase
SCAB_RS33825 [SCAB_70691] (SCAB_RS33825)	sigma factor	CGCGAGCGCTCCCT	387	9	NA	NA
SCAB_RS34575 [SCAB_72271] (SCAB_RS34575)	membrane protein	TCGGAGCGCTCCAG	408	9	NA	NA
SCAB_RS35615 [SCAB_74401] (SCAB_RS35615)	methionine ABC transporter ATP-binding protein	AAGGAGCGCTCCCG	372	9	SCAB_RS35620 [SCAB_74411] (SCAB_RS35620)	ABC transporter permease
SCAB_RS38640 [SCAB_80731] (SCAB_RS38640)	phospholipid carrier-dependent glycosyltransferase	CGGGAGCGGTCCCG	783	9	NA	NA
SCAB_RS39070 [SCAB_81681] (SCAB_RS39070)	hypothetical protein	CGGGAGCGCTCCGG	186	9	SCAB_RS39075 [SCAB_81691] (SCAB_RS39075)	membrane protein
SCAB_RS41450 [SCAB_86661] (SCAB_RS41450)	LacI family transcriptional regulator	GGCGAGCGCTTCCA	796	9	NA	NA
SCAB_RS45095 [SCAB_42902] (SCAB_RS45095)	hypothetical protein	GGCGAGCGCTTCCA	151	9	NA	NA
SCAB_RS14800 [SCAB_31161] (SCAB_RS14800)	O-methyltransferase	AGGGAGGGCTCCCG	617	9	NA	NA
SCAB_RS21270	hypothetical protein	CGGGAGCGCTCGCG	-44	9	NA	NA

[SCAB_44752] (SCAB_RS21270)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SCAB_RS21265 [SCAB_44751] (SCAB_RS21265)	hypothetical protein	CGGGAGCGCTCGCG	-217	9	NA	NA
SCAB_RS37170 [SCAB_77641] (SCAB_RS37170)	TVP38/TMEM64 family protein	CCGGAGCGCTCCCG	11	9	NA	NA
SCAB_RS37165 [SCAB_77631] (SCAB_RS37165)	cupin	CCGGAGCGCTCCCG	12	9	NA	NA

Matrix sequences

>cebEFG_SCAB_RS27575(57751)_RS27570(57741)_RS27565(57731)_[S.scabiei_87.22]
TGGGAGCGCTCCCA
>bglC_SCAB_RS24385(51081)_[S.scabiei_87.22]
TGGAAGCGCTCCCA
>txtA_SCAB_RS15085(31791)_[S.scabiei_87.22]
TGGGAGCGCTCCCG
>txtR_SCAB_RS15090(31801)_[S.scabiei_87.22]
CGGGAGCGCTCCCA
>txtB_SCAB_RS15080(31781)_[S.scabiei_87.22]
GGGGAGCGCTCCCA
>SCAB_RS16485(34791)_sigma-factor_[S.scabiei_87.22]
TTGGAGCGCTCCCA
>afsQ1-2_SCAB_RS16490-RS16495(34801-34811)_[S.scabiei_87.22]
TGGGAGCGCTCCAA
>SCAB_RS41045(85831)_hypothetical-protein_[S.scabiei_87.22]
CGGGAGCGCTCCCT
>SCAB_RS41050(85841)_transcriptional-regulator_[S.scabiei_87.22]
AGGGAGCGCTCCCG
>SCAB_RS23415(49071)_MFS-transporter-sporulation-associated_[S.scabiei_87.22]
TGGAAGCGCTCCCC
>SCAB_RS23420(49091)_hypothetical-protein_[S.scabiei_87.22]
GGGGAGCGCTTCCA
>SCAB_RS07745(16421)_LacI-family-transcriptional-regulator_[S.scabiei_87.22]
CGGAAGCGCTCCCA
>CelA1_SCAB_RS07750(16431)_[S.scabiei_87.22]
TGGGAGCGCTTCCG
>bglC_SGR_RS23585(4738)_[S.griseus_subsp.griseus_NRBC13350]
AGGGAGCGCTCCCA
>SGR_RS00890(199)_chitin-binding_protein_[S.griseus_subsp.griseus_NRBC13350]
CGGGAGCGCTCCCC
>SGR_RS16785(3391)_N-acyltransferase_[S.griseus_subsp.griseus_NRBC13350]
CGGGAGCGCTCCCG

Table S6. List of unknown and uncharacterized proteins with significant change in abundance. Proteins were regarded as significant more/less abundant compared to the wild-type with ANOVA values of $p < 0.05$, regulation of ±1.5-fold, power > 0.8 and q-values < 0.05. The measured increase (in green) or decrease (in red) of the abundance of the detected proteins is illustrated by a color scale. $(Glc)_2$: cellobiose.

Protein assignation	Gene assignation	Name	Putative or predicted function	Fold change (vs wild-type)		
				ΔcebR	+ $(Glc)_2$	*ΔcebR* + $(Glc)_2$
Hypothetical proteins and proteins with unknown function						
C9ZBB0	SCAB_0241	-	Uncharacterized protein	2.94	-	1.70
C9ZCZ1	SCAB_1511	-	Putative carbamoyltransferase	3.14	1.23	6.92
C9ZD03	SCAB_1641	-	Hypothetical protein	3.98	-	30.42
C9ZEK6	SCAB_2511	-	Putative secreted protein	2.51	-	2.32
C9Z898	SCAB_13691	-	Conserved membrane protein	-	2.13	1.53
C9ZD54	SCAB_16471	-	Putative DNA-binding protein	1.91	-	1.29
C9ZGJ8	SCAB_18411	-	Putative secreted protein	-	-4.77	-3.53
C9YT46	SCAB_19381	-	Conserved hypothetical protein	2.66	2.06	4.93
C9YUS2	SCAB_20571	-	Conserved hypothetical protein	1.55	-	-
C9YZ06	SCAB_23481	-	Putative nucleotide sugar epimerase/dehydratase	2.34	-	-
C9Z0H5	SCAB_24131	-	FAD-dependent oxidoreductase	2.53	-	-
C9Z569	SCAB_26751	RarB-like	Dynein regulation protein LC7	-1.78	-2.77	-3.12
C9Z5D4	SCAB_27411	-	Oligopeptide-binding transport system protein	1.76	-1.92	-
C9ZAB5	SCAB_30041	-	Crp/Fnr family transcriptional regulator	-1.76	-1.36	-2.39
C9ZBX1	SCAB_31271	FdxA1	Ferredoxin	-	-1.60	-
C9ZBY9	SCAB_31461	-	BldKB-like transport system extracellular solute-binding protein	1.39	1.80	2.46
C9YT92	SCAB_34981	-	BMP family ABC transporter substrate-binding protein	-	-1.71	-1.31
C9YTD0	SCAB_35361	-	Putative secreted protein	2.10	-1.87	-
C9YUZ0	SCAB_36351	-	Transcription regulation protein	5.95	-	14.12
C9YZD3	SCAB_39161	BdtA	DNA-binding protein (BldD target)	1.61	-1.41	-
C9Z0R8	SCAB_39511	-	Lsr2-like (surface antigen) protein	-	-1.63	-
C9Z0X0	SCAB_40041	-	Putative lipoprotein	-1.33	-1.60	-2.75
C9Z3Z7	SCAB_41791	-	Hypothetical Nucleoid-associated protein	-1.46	2.26	-
C9Z8Q6	SCAB_44691	-	Putative secreted protein	2.08	-1.73	-1.17
C9Z8S1	SCAB_44831	-	Conserved hypothetical protein	1.55	-	-
C9ZAI3	SCAB_45561	-	Penicillin-binding kinase	-3.96	-	-
C9ZAJ3	SCAB_45661	-	Possible nosiheptide resistance regulator	1.65	-	2.15
C9ZC44	SCAB_46811	-	Protease	-	1.94	-
C9ZGX7	SCAB_49521	-	Two-component system response regulator (CopR-like)	-	4.61	3.71
C9ZH08	SCAB_49851	-	Conserved hypothetical protein	-	2.04	2.11
C9ZH23	SCAB_50001	-	Hypothetical protein	-	-2.29	-3.15
C9ZH71	SCAB_50511	-	Putative membrane protein	-26.24	-	-15.67
C9ZH85	SCAB_50651	-	Conserved hypothetical protein	-	-1.58	-
C9YTI6	SCAB_50921	-	Putative secreted protein	2.96	-1.41	2.14
C9YTK3	SCAB_51091	-	Secreted peptidase	1.83	-1.30	-
C9YTN9	SCAB_51491	-	Conserved hypothetical protein	-	-1.57	-
C9YTP4	SCAB_51541	-	Putative calcium binding protein	-	-1.70	-1.73
C9YV95	SCAB_52551	-	Sigma factor	-3.70	-3.22	-6.07
C9YV96	SCAB_52561	-	Conserved membrane protein (possibly associated with metal resistance)	-4.10	1.42	-3.70
C9YY32	SCAB_54381	-	Putative lipoprotein	-	-1.66	-
C9YZG8	SCAB_54951	-	Putative membrane protein	-	-1.63	-
C9YZK6	SCAB_55341	-	Conserved hypothetical protein	-1.57		-1.56
C9Z120	SCAB_56051	-	Putative oxidoreductase	1.51	-	-
C9Z2I9	SCAB_56741	-	Putative secreted protein	-	-1.90	-1.96
C9Z2N1	SCAB_57151	-	Preprotein translocase subunit	1.74	1.16	-
C9Z2T0	SCAB_57661	-	Secreted solute-binding protein		-1.64	
C9Z481	SCAB_58071	-	Conserved hypothetical protein	1.78	-	1.85

C9Z4C1	SCAB_58491	-	Anti-sigma regulatory factor (AbaA-like)	-1.74	-	-
C9Z5W8	SCAB_59311	-	Putative ABC transporter ATP-binding subunit	-	1.53	-
C9Z7C5	SCAB_59701	-	Aminopeptidase	2.23	-1.19	-
C9Z7C6	SCAB_59711	-	Conserved hypothetical protein	3.54	-	3.06
C9ZAQ7	SCAB_61831	-	Secreted metalloendopeptidase	1.80	-2.86	-1.85
C9ZDZ7	SCAB_64231	-	Putative oxidoreductase	1.50	1.90	3.07
C9ZE05	SCAB_64311	-	Conserved hypothetical protein	1.03	2.15	-
C9ZFP0	SCAB_65281	-	Putative O-methyltransferase	1.57	1.52	2.41
C9YTY7	SCAB_67771	-	Putative transcriptional regulator	-3.19	-	-
C9YVG8	SCAB_68431	-	Conserved hypothetical TIGR03085 protein	2.21	-	2.07
C9YWP0	SCAB_68931	-	Putative secreted branched chain amino acid binding protein	1.45	-1.83	-
C9YY97	SCAB_70061	-	ABC transporter ATP-binding subunit	-2.20	1.34	-
C9YZU8	SCAB_71261	-	Conserved hypothetical protein	1.78	1.52	-
C9YZX7	SCAB_71571	-	Putative sporulation protein	-	-2.05	-2.00
C9Z2Z5	SCAB_73171	-	Conserved hypothetical protein	2.10	1.46	2.71
C9Z4H0	SCAB_73871	-	Conserved hypothetical protein	1.22	-1.94	-
C9Z613	SCAB_74581	-	Putative thioredoxin-like protein	-1.51	-1.40	-
C9Z7Q9	SCAB_75831	-	Conserved membrane protein	-	3.41	1.83
C9Z9D0	SCAB_76291	-	Conserved hypothetical protein	1.44	1.92	-
C9YYP6	SCAB_7641	-	Conserved hypothetical protein	1.96	-	1.44
C9YYR3	SCAB_7821	-	Ferredoxin/ferredoxin-NADP reductase	-	2.04	-
C9ZCN6	SCAB_78601	-	Putative mur ligase family	-1.85	-	-
C9YWX5	SCAB_82771	-	Peptidase	7.90	-1.25	-
C9YX10	SCAB_83121	-	Mut-like domain signature (antimutator)	-3.46	2.01	-2.73
C9Z393	SCAB_86731	-	Regulatory protein	-	1.98	-
C9Z7Z8	SCAB_89681	-	Putative TetR-family transcriptional regulator	-1.64	-	-
C9Z9U4	SCAB_90901	-	Putative secreted protein	1.38	-1.56	-1.54