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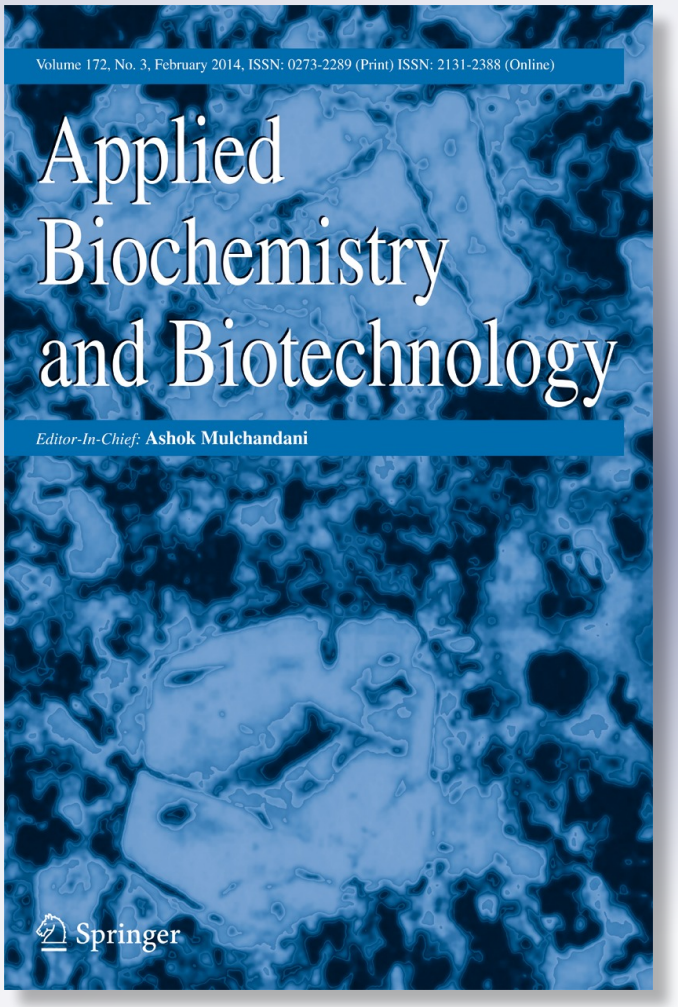

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Putative Role of a *Streptomyces coelicolor*-Derived α-Mannosidase in Deglycosylation and Antibiotic Production

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Abstract SCO0948 was found to be the single open reading frame annotated to encode an α-mannosidase (AM1) in *Streptomyces coelicolor* M145. To characterize the protein, we overexpressed SCO0948 in *Escherichia coli* BL21(DE3). Recombinant AM1, with a molecular weight of 110 kDa, exhibited α-mannosidase activity toward 4-nitrophenyl-α-D-mannopyranoside with a K_m of 4.61 mM, a V_{max} of 101.6 mM/min, and a specific activity of 47.96 U/mg. Treatment of ovalbumin, a glycoprotein, with AM1 resulted in partial deglycosylation, as assessed by glycostaining and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The *S. coelicolor* deletion mutant for SCO0948 failed to produce α-mannosidase activity, confirming AM1 as the only α-mannosidase in *S. coelicolor*

T. Rajesh · J.-M. Jeon · H. M. Seo · H.-J. Kim · D.-H. Yi · Y.-H. Kim · Y.-H. Yang
Department of Microbial Engineering, College of Engineering, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, South Korea

E. Song · H.-M. Park · K.-Y. Choi
School of Chemical and Biological Engineering, Bioengineering Institute, and Institute of Molecular Biology and Genetics, Seoul National University, Gwanak-gu, Seoul 151-742, South Korea

Y.-G. Kim
Chemical Engineering, Soongsil University, 511 Sangdo-dong, Seoul 156-743, Republic of Korea

H.-Y. Park
Bio-MAX Institute, Seoul National University, Gwanak-gu, Seoul 151-742, South Korea

Y. K. Lee
Arctic Research Center, Korea Polar Research Institute, KIOST, 26 Songdomirae-ro, Incheon 406-840, South Korea

Y.-H. Yang (✉)
Institute for Ubiquitous Information Technology and Applications (CBRU), Konkuk University, Seoul 143-701, South Korea
e-mail: seokor@konkuk.ac.kr

M145. Interestingly, the deletion mutant and a complementation strain produced lower levels of the antibiotics actinorhodin and undecylprodigiosin in glucose minimal media. The results indicate that AM1 as an α-mannosidase influences deglycosylation and antibiotic production in *S. coelicolor* M145.

Keywords *Streptomyces coelicolor* · α-Mannosidase · Deglycosylation · Antibiotic production

Introduction

α-Mannosidases, members of the glycosyl hydrolase family of enzymes, process glycans and complex oligosaccharides. Based on their glycosyl hydrolase (GH) domain, they are classified as GH38, GH47, GH76, and GH92 [1, 2]. GH38 and GH47 enzymes, alternatively named class I and class II α-mannosidases, respectively, have been reported and studied in prokaryotes and eukaryotes [3]. To date, there is only a single report available on the characterization of a GH92 family α-mannosidase [4], and no reports are available regarding the characterization of prokaryotic GH76. Class I α-mannosidases (GH47) specifically cleave the α-(1–2) glycosidic bonds in $Man_9GlcNAc_2$. This family of enzyme has been reported to play a role in the maturation of N-glycans in eukaryotes and in the production of $Man_5GlcNAc_2$ [4]. The GH92 family α-mannosidase has been shown to play a role in the degradation of N-glycans from dietary glycoproteins.

Class II or GH38 family α-mannosidases from prokaryotic and eukaryotic sources have been characterized in detail. The class II α-mannosidases consist of multidomains and occur as Golgi α-mannosidases or lysosomal α-mannosidases in eukaryotes [5]. Golgi α-mannosidases represent attractive clinical targets. The best-studied member of this class, dGMII, is from *Drosophila melanogaster*. The reaction mechanism, active sites, and 3D structure of dGMII have been identified and reported in detail [6, 7]. Members of the GH38 family have also been studied in archaea and bacteria. Several reports have characterized GH38 family α-mannosidases from *Escherichia coli*, *Mycobacterium tuberculosis*, *Bacillus* sp., *Thermotoga maritima*, and *Streptococcus pyogenes* [8–12]. One report has characterized a GH38 enzyme from an archaeal source, *Picrophilus torridus* [13].

α-Mannosidases from *Streptomyces* sp. were characterized a few decades ago; they have not been described recently [14]. α-Mannosidase activity and its role in antibiotic (streptomycin) production have been described only in *Streptomyces griseus* [15]. Recently, a single β-mannosidase was reported in *Streptomyces* sp. S27 [16]. Enzymes involved in the glycosylation of antibiotics or specific proteins have been reported in *Streptomyces galilaeus*, *Streptomyces hygroscopicus*, *Streptomyces venezuelae*, *Streptomyces lividans*, *Streptomyces olivaceus*, and *Streptomyces ambofaciens* [17–22]. In *Mycobacteria*, glycosylation has been reported in *M. tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium smegmatis* [23–27]. The glycosylated targets in *Streptomyces coelicolor* include proteins encoded by SCO2664, SCO3150, SCO0638, SCO3546, PstS, and BldKB [28–30].

In this context, we sought to identify and characterize proteins that perform deglycosylation in *S. coelicolor*. In the present study, we studied SCO0948, a single open reading frame (ORF) annotated to encode an α-mannosidase (AM) from *S. coelicolor* M145. Characterization of the encoded enzyme, AM1, is of particular interest, given the recent reports of glycosylation in members of *Streptomyces* and *Mycobacteria*. Characterization of recombinant AM1 revealed that the protein exhibited α-mannosidase activity and played a role in deglycosylation. Our results also suggest the possible involvement of AM1 in antibiotic production and glycosylation in *S. coelicolor*.

Materials and Methods

Bacterial Strains, Plasmids, Media, and Culture Conditions

The bacterial strains, primers, and plasmids used in this study are listed in Table 1. *S. coelicolor* A3(2) M145 was obtained from the Korean Collection for Type Cultures (KCTC, Korea). *E. coli* DH5α and *E. coli* BL21(DE3), used for general transformation and protein overexpression, respectively, were from laboratory stock. *S. coelicolor* A3(2) M145 was grown in $R5^-$ medium composed of 103 g of sucrose, 0.25 g of K_2SO_4, 10.12 g of $MgCl_2{\cdot}6H_2O$, 10 g of glucose, 0.1 g of Difco Casamino Acids, 2 mL of trace element solution (comprised of $ZnCl_2$, 40 mg; $FeCl_3{\cdot}6H_2O$, 200 mg; $CuCl_2{\cdot}2H_2O$, 10 mg; $MnCl_2{\cdot}4H_2O$, 10 mg; $Na_2B_4O_7{\cdot}10H_2O$, 10 mg; and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 10 mg in 1 L of deionized water), 5 g of yeast extract, 5.73 g of TES buffer, and 7 mL of 1 N NaOH in 1 L of distilled water [31]. $R5^-$ fructose and $R5^-$ mannose media were prepared by replacing 10 g of glucose with 10 g of fructose or 10 g of mannose, respectively. The liquid cultures were grown at 30 °C with shaking at 200 rpm in a Hanbaek shaker for 5 days (Hanbaek Scientific Co., Korea). *E. coli* strains were routinely cultured in nutrient agar and/or liquid broth composed of 3 g of beef extract, 5 g of peptone, and 15 g of agar in 1 L of distilled water. For expression analysis, *E. coli* cultures were grown at 37 °C with shaking at 220 rpm. Antibiotics, such as 30 μg/mL of thiostrepton dissolved in DMSO for selection of *S. coelicolor* transformants, 50 μg/mL of

Table 1 List of bacterial strains, plasmids, and primers used in this study

Strain/primer/plasmid	Relevant information	Source/ reference
Bacterial strains		
E. coli strains		
DH5α	F^-ϕ80*lacZ* M15 *endA recA hsdR*($r_k^-m_k^-$) *supE thi gyrA relA* Δ(*lacZYA-argF*)U169	Laboratory stock
BL21(DE3)	F^-*ompT hsdS*$_B$($r_B^-m_B^-$) *gal dcm*	Novagen
JM110	*dam*$^-$, *dcm*$^-$	Laboratory stock
S. coelicolor M145	$SCP1^-$, $SCB2^-$, Pgl^+	KCTC
*S. coelicolor*Δ*0948*	Deletion mutant of *S. coelicolor* M145 for SCO0948	This study
*S. coelicolor*Δ*0948*::*0948*	*S. coelicolor* carrying SCO0948 in pIBR25 (p250948)	This study
Primers		
28-0948 F	5′-CGCGCATATGGACCGCCTCGACAACAC-3′	This study
28-0948 R	5′-TATAAAGCTTTCAGACGCGCACGGTGGCC-3′	This study
25-0948 F	5′-GCCGGAATTCCATATGCTCGACGAATCGCT CCTCGACG-3′	This study
25-0948 5	5′-GCCGAAGCTTTCAGGCTCCCGAGGCGAGCG-3′	This study
Plasmids		
pET24ma	p15A replication origin, T7 lac promoter, C-terminal His-tag coding, kan^R	[43]
pIBR25	pWHM3 carrying *ermE** promoter from *Saccharopolyspora erythraea*	[44]
p280948	pET28ma carrying PCR product of SCO0948from *S. coelicolor*	This study
p250948	pIBR25 carrying PCR product of SCO0948 from *S. coelicolor*	This study

kanamycin, and 100 μg/mL of ampicillin for transformation of *E. coli*, were added when required to select transformants and maintain plasmids.

Gene Cloning and Protein Expression

S. coelicolor strains were cultivated by following standard procedures [31]. Conventional plasmid DNA preparation, restriction enzyme digestion, ligation, and transformation of *E. coli* were performed essentially as reported [32]. The entire SCO0948 sequence was obtained by polymerase chain reaction (PCR) using the primer pair 28-0948 F/28-0948 R and chromosomal DNA as a template, followed by cloning at the *Nde*I and *Hin*dIII sites of plasmid pET28ma. The resulting recombinant plasmid p280948 was transformed into *E. coli* BL21(DE3). For complementation analysis, SCO0948 from *S. coelicolor* was amplified using the primer pair 25-0948 F/25-0948 R and cloned at the *Eco*RI and *Hin*dIII sites of pIBR25 to produce recombinant plasmid p250948. The recombinant plasmids were initially transformed into *E. coli* JM110, re-isolated, and then used to transform *S. coelicolor* M145.

Protein Purification and Enzyme Assay

E. coli BL21(DE3)/p280948 was grown at 37 °C with shaking at 220 rpm in 100 mL of nutrient broth containing 50 mg/L of kanamycin. The cells were grown to an OD of 0.6 at 600 nm, after which 0.1 mM IPTG was added to the growth medium to induce recombinant protein expression. Following induction, the cells were harvested after 12 h of incubation at 16 °C. For enzyme preparation, the harvested cells were washed and suspended in 5 mL of 50 mM Tris–HCl buffer (pH 7.5) containing 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.01 % (*v*/*v*) 2-mercaptoethanol, and 1 mM 1,4-dithiothreitol. Subsequently, the cells were subjected to ultrasonication for 5 min with 10 cycles consisting of 15 s on and 15 s off at 4 °C (Vibra-Cell™, Sonics Scientific, Inc.). The supernatant solution containing the soluble proteins was prepared by centrifugation (17,000×*g*, 20 min). For purification of His-tagged proteins, soluble proteins were applied to Ni^{2+}-NTA beads pre-equilibrated with base buffer (50 mM NaH_2PO_4, 0.05 % NaCl, 0.05 % Tween 20, pH 8.0). Following a 2-h binding reaction, unbound proteins were removed by washing four times with 4 mL of 20 mM imidazole. Purified enzyme was eluted three times from the column with 500 μL of 250 mM imidazole. Each purification step was individually analyzed by 12 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

α-Mannosidase activity was determined according to previous methods, with slight modifications [1]. The reaction mixture consisted of 2.5 mM of 4-nitrophenyl-α-D-mannopyranoside (4-NP-α-D-Man) and 2 μg of enzyme in a final volume of 0.2 mL. The assay was performed in 50 mM sodium phosphate buffer (pH 6.5) at 65 °C. After incubation, the reaction was terminated at specific time points by the addition of 0.8 mL of ice-cold 1 M Na_2CO_3 solution, and the absorbance was measured at 420 nm. The enzyme unit was calculated based on the amount of enzyme catalyzing the conversion of 1 μmol of substrate into product in 1 min at reaction conditions. Kinetic parameters for AM1 with 4-NP-α-D-Man as a substrate were measured under standard conditions with substrate concentrations ranging from 0.5 to 50 mM.

Construction of the SCO0948 Deletion Mutant

SCO0948 was disrupted using the plasmid pSuperCos1 to deliver the apramycin resistance gene cassette. Oligonucleotide primers with 5′-ends overlapping the upstream (36 bp) and

downstream (36 bp) regions of the SCO0948 ORF were designed to amplify the apramycin resistance cassette from pIJ773 and the corresponding cosmid. All constructs were delivered into *S. coelicolor* M145 by conjugation with *E. coli* ET12567. Conjugation between *E. coli* and the *Streptomyces* sp. was performed as previously described, with minor modifications [33]. The transformants (*S. coelicolorΔ0948*) were selected on R5$^-$ agar plates overlaid with thiostrepton and nalidixic acid.

Complementation and Protein Expression in *S. coelicolor*

Plasmid p250948 was transformed with *S. coelicolorΔ0948* by the protoplast-mediated method. Briefly, *S. coelicolorΔ0948* was cultured in 25 mL of YEME medium for 48 h at 30 °C with shaking at 200 rpm. Cells were harvested by centrifugation at 1,000×*g* for 10 min. The cell pellet was then resuspended in 15 mL of 10.3 % sucrose and centrifuged as above. This washing step was repeated twice. After washing, the mycelium was resuspended in 4 mL of P buffer [31] containing 1 mg/mL of lysozyme. The tubes were incubated at 30 °C for 45 min, after which 6 mL of P buffer was added. The prepared protoplasts were filtered using cotton wool, transferred to 15-mL centrifuge tubes, and centrifuged at 3,000×*g* for 10 min. The supernatant was discarded, and the protoplasts in the pellet fraction were resuspended in 50 mL of P buffer. To the prepared protoplasts, 12 μL of plasmid DNA was added, followed by the immediate addition of 200 μL of T buffer [31] and 500 μL of P buffer. The contents in the tubes were mixed gently, plated on R5$^-$ agar medium, and incubated overnight at 30 °C. The next day, the plates were overlaid with 1 mL of P buffer containing thiostrepton. Positive transformants (*S. coelicolorΔ0948*::*0948*) were segregated twice and used for further analysis.

Antibiotic Quantification Assays

To quantify α-mannosidase activity and antibiotics from *S. coelicolor* M145, *S. coelicolorΔ0948* and *S. coelicolorΔ0948*::0948, grown on R5$^-$ thiostrepton plates, were inoculated in 5 mL of R5$^-$ fructose medium without antibiotics and grown for 24 h. They were then washed twice with sterilized water and diluted to have the same wet weight in the same amount of water. Exactly 20 mg (wet weight) of cells was inoculated and cultured in a 250-mL baffled flask with 50 mL of R5$^-$ containing different carbon sources. The cultures were grown at 30 °C with shaking at 200 rpm for 5 days. One milliliter of each sample, obtained at the specified time intervals, was centrifuged in a micro-centrifuge for 10 min, and 900 μL of the supernatant was sampled for further analysis according to previously reported procedures [34]. The antibiotics actinorhodin (ACT) and undecylprodigiosin (RED) were determined spectrophotometrically at 633 and 530 nm, respectively, in the treated samples (150 μL) using a 96-well plate and multiscanner (Thermo Electron Corp., Finland).

Protein Deglycosylation Studies

For protein deglycosylation studies, ovalbumin was used as a substrate. Ovalbumin at a concentration of 20 μg was treated with 2.5 μg of AM1 in 10 mM sodium acetate buffer (pH 6.5) for 4 h at 65 °C. The control reaction contained all the above except AM1. Following the reaction, the samples were desalted by passage through membrane filters. A portion of the test sample and control sample was analyzed by SDS-PAGE and glycostained (Pierce Inc.,

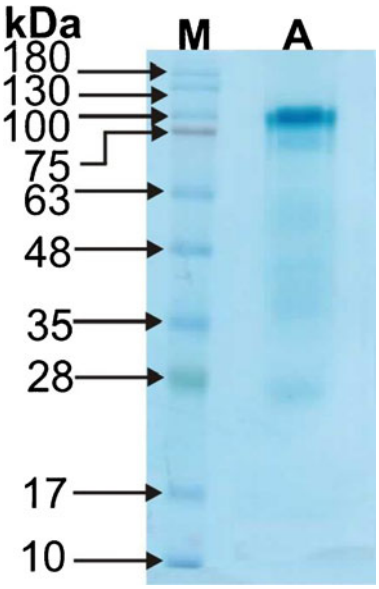


Fig. 1 Purification of AM1 from *E. coli* BL21 (DE3)/p280948. Ni-NTA purified AM1 (110.8 kDa) is shown (**a**). *Lane M*: Molecular weight protein marker

USA). The rest of the sample was analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) to measure the change in molecular mass.

Derivatization with Girard's Reagent T

Girard's reagent T (GT) derivatization of oligosaccharides was performed following a previously described report with minor modifications [35]. Ten microliters of sample solution in 50/50 (*v*/*v*) methanol/water was mixed with 100 μL of GT solution (5 nmol/μL in 1/99 (*v*/*v*) acetic acid/methanol). The solution was incubated at room temperature for 4 h. The GT-derivatized sample was analyzed by MS without further purification steps.

MS Analysis

Sample solution (0.5 μL) was mixed with 0.5 μL of 2,5-dihydroxybenzoic acid (DHB) solution (30 mg/mL in 30/70 (*v*/*v*) water/acetonitrile). One microliter of the mixture was

Table 2 α-Mannosidase activity in *S. coelicolor* M145 wild type, deletion mutant, and complementation strain

Sample no.	Strain	α-Mannosidase activity (U/mg)	
		Glucose	Mannose
1	*S. coelicolor* WT	10.65	65.7
2	*S. coelicolorΔ0948*	ND	ND
3	*S. coelicolorΔ0948::0948*	15.6	144.1

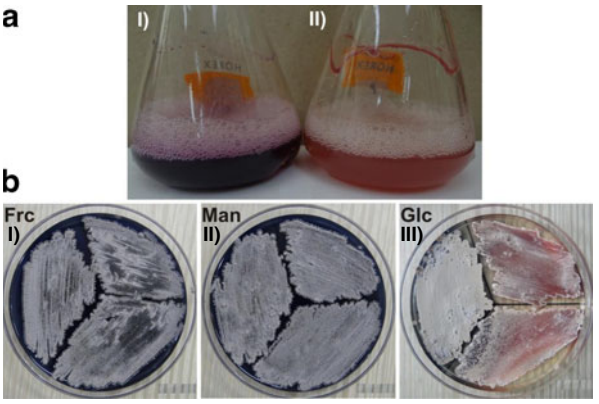


Fig. 2 Phenotypic assessment of antibiotic production pattern in wild-type and other strains of *S. coelicolor*. **a** Antibiotic production in *S. coelicolor* WT (*I*) and *S. coelicolorΔ0948* (*II*) grown on R5⁻ glucose medium as observed visually on day 5 are shown. **b** Antibiotic production in *S. coelicolor* WT (*I*), *S. coelicolorΔ0948* (*II*), and *S. coelicolorΔ0948::0948* (*III*) grown on R5⁻ fructose (Frc), mannose (Man), and glucose (Glc) as observed visually on day 5 is shown

spotted on a stainless steel MALDI plate and dried. MS analysis was performed using MALDI-TOF MS with an autoflex™ system from Bruker Daltonics (Bruker, Bremen, Germany). The analysis parameters were as follows: positive-ion and reflectron mode, detector gain=3.9, and laser power=70 %. One thousand different spots were scanned to acquire mass spectra data. The intensity of each peak was obtained by integrating the area from the first to third isotopic peaks. Data acquisition and processing were performed with flexAnalysis 2.4 software (Bruker, Bremen, Germany).

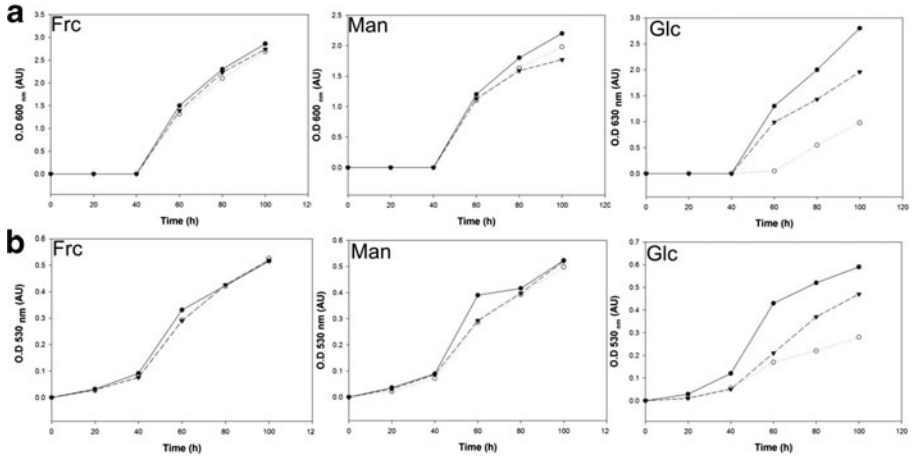


Fig. 3 Quantification of antibiotic production by various strains of *S. coelicolor*. Levels of antibiotics ACT (**a**) and RED (**b**) in *S. coelicolor* WT (*filled circles*), *S. coelicolorΔ0948* (*empty circles*), and *S. coelicolorΔ0948::0948* (*filled triangles*) grown on R5⁻ fructose (Frc), mannose (Man), and glucose (Glc) for 5 days are shown. Values obtained are average of three independent experiments, and error bars are intentionally omitted to provide better clarity

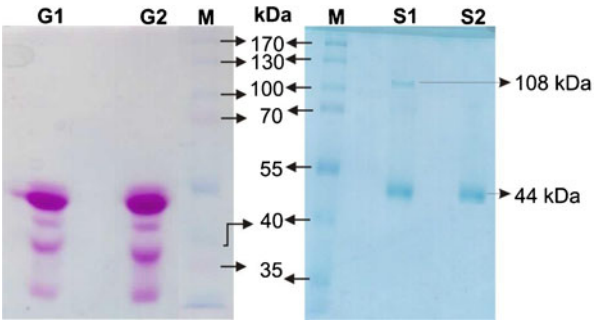


Fig. 4 SDS-PAGE and glycostaining analysis of AM1-treated and untreated ovalbumin. *G1* and *S1* represent glycostained and Coomassie Brilliant Blue-stained ovalbumin treated with AM1, respectively. *G2* and *S2* represent untreated samples. AM1 of 108 kDa and ovalbumin of 44 kDa are also shown

Results

Features of SCO0948 and Characterization of AM1

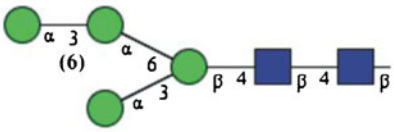
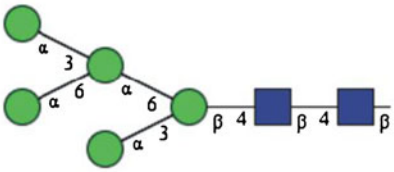
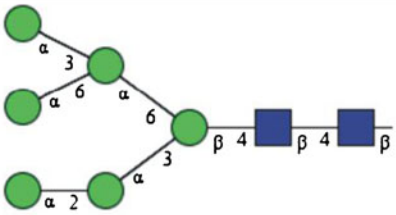
When analyzing the genome of *S. coelicolor*, we identified SCO0948 as a single ORF annotated to encode an α-mannosidase. Further sequence similarity analysis using BLASTN revealed that similar putative α-mannosidases were present in almost all of the available *Streptomyces* genomes. Further, the glycosyl hydrolase family domain GH38 was predicted to be present in AM1, the enzyme encoded by SCO0948. Considering the conserved nature of AM1 and its homologs in other *Streptomyces*, SCO0948 was chosen for further analysis. Cloning and heterologous expression of SCO0948 at 30 °C initially resulted in the formation of inclusion bodies. To obtain the recombinant protein in the soluble fraction, protein was expressed at a lower temperature (16 °C). His-tagged AM1 was purified, and SDS-PAGE analysis revealed a single protein band of the expected molecular weight (110 kDa), suggesting that the protein is a monomer (Fig. 1). With the synthetic substrate 4-NP-α-D-Man, the specific activity obtained with AM1 was 47.96 U/mg. In kinetic studies with the same substrate, the K_m was 4.61 mM and the V_{max} was 101.6 mM/min. Comparing the kinetic parameters with those of other α-mannosidases indicated that AM1 exhibited a low level of activity (Table 2). This could be due to the large size of the protein and its poor expression in *E. coli*. With respect to metal ions, none of the metal ions tested (Ca^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}) affected or enhanced the activity of AM1 (data not shown).

Deletion of SCO0948 Abolishes α-Mannosidase Activity

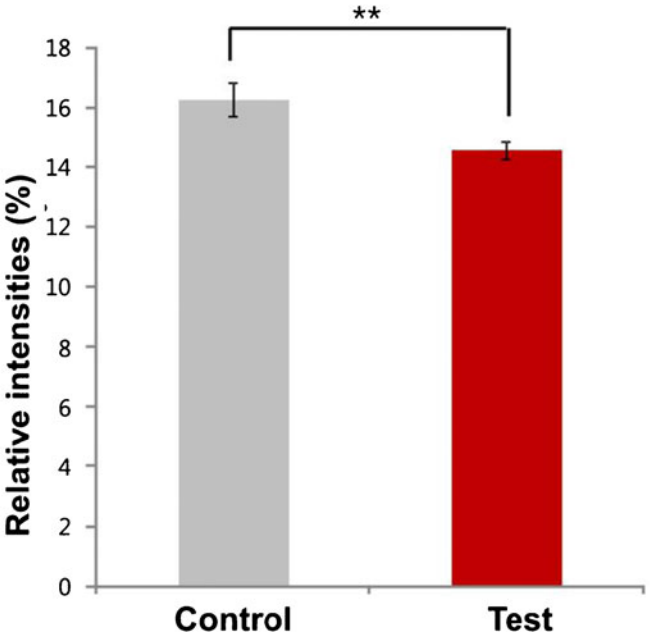
To confirm the absence of other α-mannosidases in *S. coelicolor* M145, the deletion mutant for SCO0948, *S. coelicolorΔ0948*, was constructed. No α-mannosidase activity was observed in soluble protein fractions from *S. coelicolorΔ0948* collected on five consecutive days (Table 2). However, the complementation strain *S. coelicolorΔ0948::0948* produced α-mannosidase activity comparable to that of *S. coelicolor* M145 (Table 2). These results confirmed the presence of a single α-mannosidase (AM1) in *S. coelicolor* M145.

Fig. 5 Mass spectrometric analysis of AM1-treated ovalbumin. **a** Structures of the *N*-linked glycans in ovalbumin as analyzed by mass spectra. *Circle* is mannose unit and *square* is *N*-acetylglucosamine unit. **b** Quantification of the *N*-linked glycans from untreated and treated samples of ovalbumin (*p* value <0.01)

a

m/z [M+GT]+	Glycan structure
1186.7	
1348.8	
1511.0	

b



Deletion of SCO0948 Affects Antibiotic Production in *S. coelicolor*

Antibiotic production in *S. coelicolor* M145, *S. coelicolorΔ0948*, and *S. coelicolorΔ0948::0948* was studied to analyze the effect of SCO0948 deletion on antibiotic production. In $R5^-$ glucose medium, *S. coelicolorΔ0948* produced less ACT and RED than *S. coelicolor* M145 (Fig. 2a). However, on solid agar plates containing fructose or mannose as a carbon source, the normal antibiotic production pattern was observed in all three strains (Fig. 2b). As observed with the liquid cultures, in the phenotypic assessment of *S. coelicolorΔ0948* and *S. coelicolorΔ0948::0948* on $R5^-$ glucose medium, antibiotic production was reduced (Fig. 2b). Quantitative assessment of ACT and RED production confirmed the above results, clearly indicating that AM1 as an α-mannosidase influences antibiotic production (Fig. 3).

Role of AM1 in Glycoprotein Deglycosylation

To test whether AM1 can deglycosylate glycoproteins, ovalbumin, a well-known glycoprotein, was tested. Ovalbumin was treated briefly with AM1, and the protein samples were analyzed by SDS-PAGE and glycostaining. AM1 treatment reduced the level of glycosylated ovalbumin, compared to control treatment (Fig. 4). Because the native molecular weights of ovalbumin (42. 7 kDa) and ovalbumin with carbohydrate and phosphate modifications (44.3 kDa) are very similar, the difference in migration on SDS-PAGE could not be detected. To assess the extent of deglycosylation, AM1-treated ovalbumin was analyzed by MALDI-TOF. The results indicated that AM1 treatment of the glycoprotein resulted in partial deglycosylation (Fig. 5). Although we detected differences in glycosylation by glycostaining and MALDI-TOF, the deglycosylation activity was weak, perhaps because ovalbumin is not a natural substrate of AM1 in *S. coelicolor*.

Discussion

The function of α-mannosidases in eukaryotes is mainly to modify glycoproteins in cellular organelles such as Golgi bodies, the endoplasmic reticulum, and lysosomes. In cytosol, α-mannosidases are presumed to be involved in protein signaling [36]. Prokaryotic GH38 family α-mannosidases have a wide range of functions. In *Bacillus* sp., they are involved in the degradation of xanthan for subsequent use as a carbon source [11]. In the pathogenic bacteria *M. tuberculosis*, they are involved in the breakdown of cell wall glycoproteins and glycolipids, which could in turn function as virulence factors [10]. Our characterization of AM1, a GH38 family α-mannosidase, clearly indicated a role in antibiotic production by *S. coelicolor* in glucose medium. Apart from pathway-specific and global regulators, several other proteins have been shown to influence antibiotic production in *S. coelicolor*. Notably, members of two-component systems, noncoding RNAs, cell division-related proteins, membrane proteins, and quorum sensing molecules have also been shown to influence antibiotic production [30, 37, 38]. With regard to metabolic enzymes, malic enzymes, phosphopantetheinyl transferase, and phosphomannose isomerase have been shown to affect antibiotic production in *S. coelicolor* [39–41].

The role of AM1 in antibiotic production seems to be new and unique. In *S. coelicolor*, the role of mannose metabolic pathway-related enzymes (ManA and ManB) has already been reported [40, 42, 43]. Carbon catabolite repression based on glucose has also been identified. Both the systems influence antibiotic production. In this context, it could be possible that carbon catabolite repression is activated in the presence of glucose, or the enzyme (AM1) by itself could act on similar systems to reduce antibiotic production.

Few reports describe the enzymatic deglycosylation of glycoproteins. Trifluoromethanesulfonic acid (TFMS)-mediated deglycosylation is the most extensively applied method for the removal of sugar molecules from glycoproteins. Because deglycosylating enzymes have less access to the glycoprotein core and because the structure of the glycoprotein influences the binding of enzymes, enzymatic deglycosylation remains a challenging task. Enzymes of the *N*-glycanase family have been reported to deglycosylate glycoproteins; they require denaturing agents to facilitate the process. Our preliminary results on AM1 deglycosylation of a glycoprotein hold promise for further studies. Our glycostaining and MALDI-TOF analyses provide evidence for the partial deglycosylation of ovalbumin. The precise targets for AM1 in the *S. coelicolor* proteome remain to be identified.

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