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Proteomes Induced by *S*-Adenosyl-L-Methionine in *Streptomyces coelicolor* A3(2)

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Abstract It was reported that an accumulation of *S*-adenosyl-L-methionine increases production of actinorhodin in *Streptomyces lividans* and induces antibiotic biosynthetic genes. We also obtained the same result in *Streptomyces coelicolor* A3(2). Therefore, in order to identify proteins changed by the addition of *S*-adenosyl-L-methionine in *S. coelicolor* A3(2), LC/MS/MS analyses were carried out. Thirteen proteins that were not observed in the control were found.

Key words: *S*-Adenosyl-L-methionine, *Streptomyces coelicolor*, tandem MS, proteome

It is known that Actinomycetes produces over 70% of known antibiotics, and therefore, it has been used as a model system to study antibiotic production [3]. Many proteins participating in the biosynthetic pathway to produce secondary metabolites, such as actinorhodin and undecylprodigiosin, have been reported [5, 9]. When a signal stimulates AfsK, the first serine/threonine kinase in *S. coelicolor*, it phosphorylates AfsR, and its binding ability to AfsS is then enhanced [11]. AfsS activates transcription of ActII-ORF4, which is a transcriptional activator. AfsS of *S. coelicolor* consists of 63 amino acids, which includes three repeats of TXDNHMPXPA, where X denotes undefined amino acid. AfsR of *S. coelicolor* is composed of 993 amino acids, and its disruption causes loss of actinorhodin production. It was found that AfsR is phosphorylated when incubated with ATP, suggesting the presence of a kinase; it was AfsK. AfsK consists of 799 amino acids, showing a high homology with eukaryotic serine/threonine kinases. Their N-terminals are well conserved. The catalytic domain between 1 and 311 residues of AfsK can autophosphorylate and phosphorylate AfsR [1, 4, 6, 7, 12].

Kim *et al.* [2] reported that an accumulation of *S*-adenosyl-L-methionine (SAM) increases production of actinorhodin in *S. lividans* and induces antibiotic biosynthetic genes. Therefore, it is highly possible that SAM can be a signal molecule to enhance production of antibiotics. The direct binding of SAM to AfsK was indicated by nuclear magnetic resonance spectroscopy (unpublished data). In the present study, in order to identify proteins changed by the addition of SAM in *S. coelicolor*, tandem mass spectrometry (MS/MS) was applied.

S. coelicolor A3(2) was purchased from ATCC (BAA-471D, American Type Culture Collection, Manassas, VA, U.S.A.) [13]. Spores were collected from the strain and precultured in LB medium with shaking at 150 rpm and 28°C for one day. One ml of precultured broth was inoculated onto 50 ml of LB medium and it was cultured for 7 days under the same condition as the preculture [SAM(−) sample]. To compare the effect of SAM on *S. coelicolor* A3(2), another 50 ml of LB medium was prepared. One ml of 1 mM SAM was added, and this LB medium was cultured under same condition as the control [SAM(+) sample]. The cultured broth was centrifuged at 8,000 rpm and resuspended with lysis buffer [0.1 M Tris-HCl (pH 7.5), 14 mM Mercapto-ethanol, 5 mM EDTA, 10% Glycerol]. It was sonicated for 2 min at 4°C. The supernatant was centrifuged for 90 min at 39,000 ×*g* and 4°C using a Micro-Ultra centrifuge (Hitachi, Tokyo, Japan). Protein analysis was carried out by the Bradford method, using bovine serum albumin as a standard. The concentration of proteins

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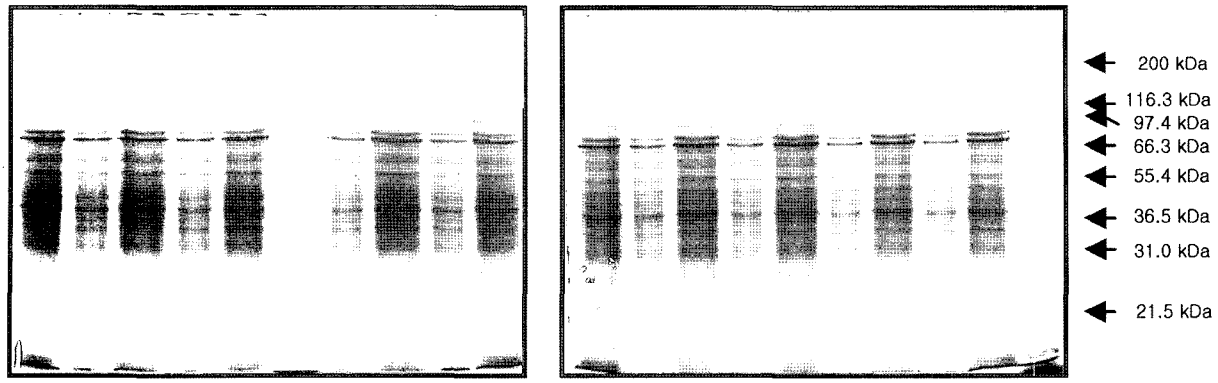

Fig. 1. SDS-PAGE of SAM(-) sample (left) and SAM(+) sample (right).
As molecular weight standards, myosin (200 kDa), β-galactosidase (116.3 kDa), phosphorylase B (97.4 kDa), BSA (66.3 kDa), glutamic dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31.0 kDa), and trypsin inhibitor (21.5 kDa) were used.

present in SAM(-) sample was 101 μg/10 μl and that in SAM(+) sample was 99 μg/10 μl. Therefore, the addition of SAM did not appear to affect the production of proteins. The determination of actinorhodin content and growth followed the method of Liao *et al.* [2, 8]. As published by Okamoto *et al.* [9] and Park *et al.* [10], treatment of *S. coelicolor* with SAM caused overproduction of actinorhodin, but it did not affect the growth. When SAM was not added during the growth of *S. coelicolor* A3(2) in LB medium, absorbance was 0.266. However, when 2 μM and 100 μM of SAM were added, absorbances were 0.702 and 1.716, respectively.

Because there are too many kinds of proteins in the sample to be detected by LC/MS/MS, they should first be separated using sodium dodecylsulfate polyacrylamide gel

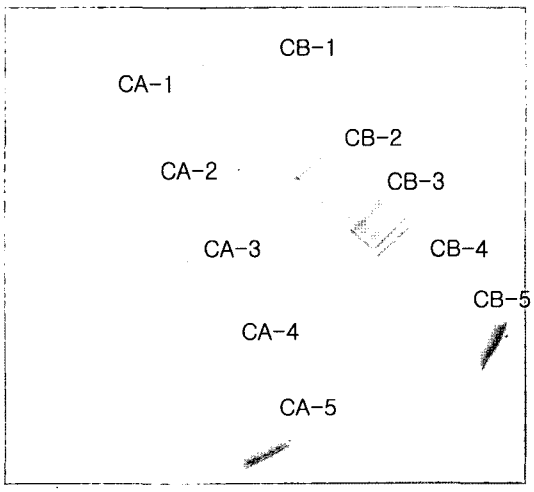

Fig. 2. Gel slices of SAM(-) sample (left: CA series) and SAM(+) sample (right: CB series).
The expected molecular masses are over 120 kDa for CA-1 and CB-1, 50–120 kDa for CA-2 and CB-2, 33–50 kDa for CA-3 and CB-3, 25–33 kDa for CA-4 and CB-4, and less than 25 kDa for CA-5 and CB-5.

electrophoresis (SDS-PAGE) prior to LC/MS/MS analysis. Thus, a mini polyacrylamide gel system, Ready Gel (BioRad, Hercules, CA, U.S.A.), with 5% (w/v) stacking gel and 12.5% (w/v) running gel was used. A mixture of 10 μl of the sample and the same volume of loading buffer was loaded. As shown in Fig. 1, proteins were well resolved according to their molecular weights. Each gel was cut into 5 slices (Fig. 2): The molecular weights of CA-3 and CB-3 were expected to range between 33 kDa and 50 kDa, since the molecular masses of the proteins participating in the production of antibiotics, such as ecr proteins, belong to this range. The selected slice was washed with distilled water and methanol, and transferred into an eppendorf tube. It was washed again with washing buffer (50 mM ammonium bicarbonate:acetonitrile=1:1) until decoloring with Coomassie blue used for SDS-PAGE. The washed slice was dehydrated with acetonitrile (HPLC grade), which was dried using a Speed-vac (Maxi Dry Lyo, Heto-Holten, Denmark). The dried gel was incubated in trypsin solution (30 μl of Promega trypsin buffer, 570 μl of 50 mM ammonium bicarbonate) at 37°C for 16 h. Peptides extracted from the gel slice was dried with a Speed-vac. It was then dissolved in the solution A (0.05% formic acid in water) used for LC/MS/MS analysis. LC/MS/MS analysis was performed on a linear ion-trap mass spectrometer, LTQ (Thermo Finnigan, San Jose, CA, U.S.A.) mounted with a Surveyor HPLC system and electrospray interface. Data process and library search were carried out using Xcalivar 1.3 software and TurboSEQUEST program, respectively. For the peptides identified with specific protein sequences, the Xcorr values were at least 2.0, 2.5, and 3.3 for singly, doubly, and triply charged ions, respectively. The identified MS/MS spectra were manually validated. The electrospray voltage was 2 kV. For HPLC, gradient eluents (solution A: 0.05% formic acid in water; solution B: 0.05% formic acid in acetonitrile, 0% to 40% of solution B during 60 min) were applied at a flow rate of 300 nl/min on

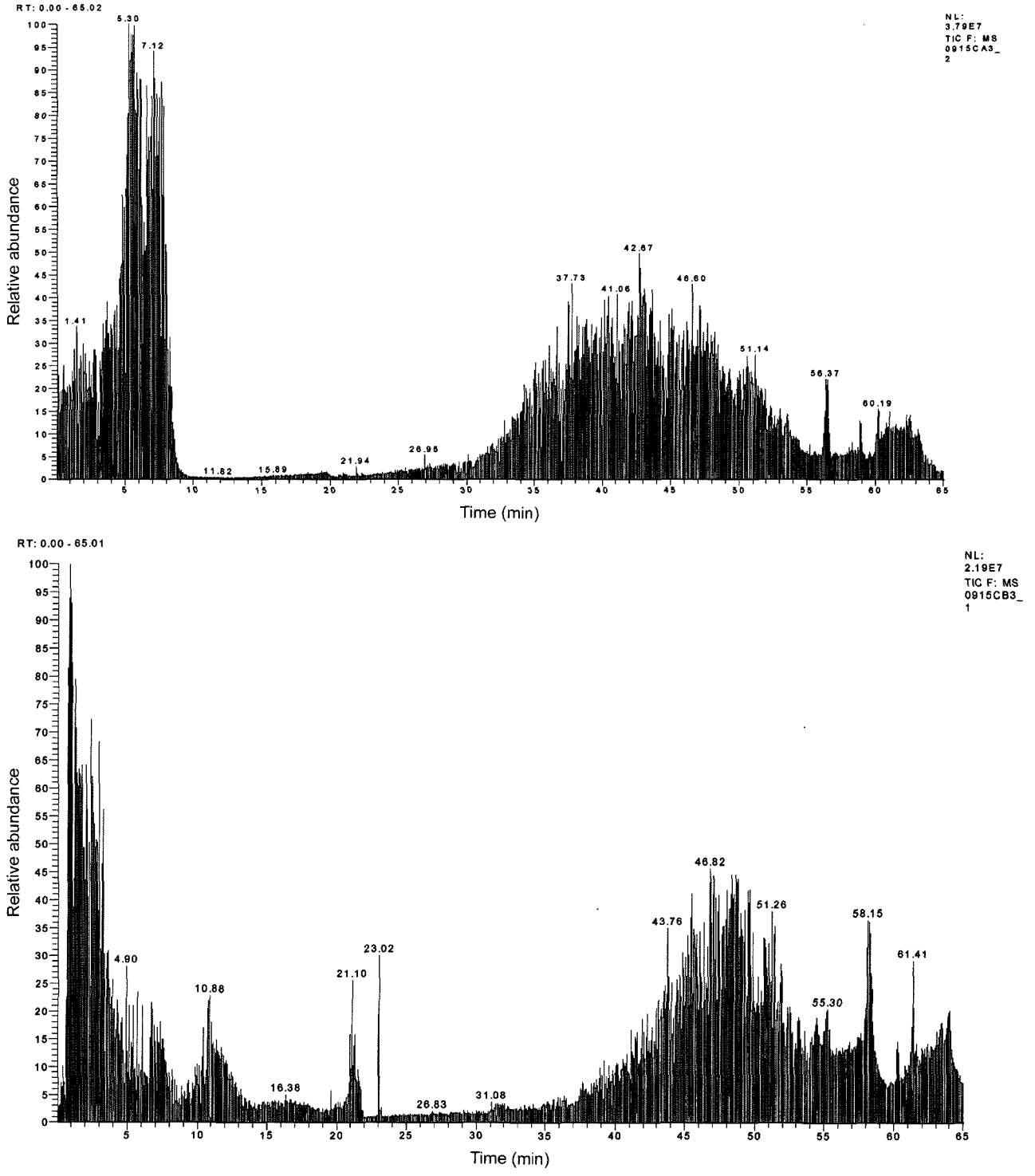

Fig. 3. Total ion current chromatogram of CA-3 (upper) and CB-3 (lower).

a capillary column. The MS/MS acquisition rate was 1 spectrum/sec. Additional MALDI TOF-MS/MS analysis was performed on an APPLIED Biosystems 4700 Proteomics Analyzer (Framingham, MA, U.S.A.). For library search, Mascot program was used.

As shown in the total ion current (TIC) chromatograms of CA-3 and CB-3 (Figs. 3A and 3B), different patterns were observed. Whereas 21 proteins were identified from SAM(−), 15 proteins were observed from SAM(+). They are listed in Table 1. Two proteins were found in both samples. As a

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NP_733728     MGRAHVSTHELVAGRYRLFEVVQRETNRVCWSGEDATTGRPCLVTRIELPEGRAGEAARR 60
P54741|AFSK   MVDQLTQHDPRRIGPFEVLGRLGAGGMGLVYLARSASGRR--VAIKTVRTELAEDQLFRV 58

NP_733728     APGRVIRTGETMASLCPGRIAPVLDAVVADGMLWTVTEWVAGVPLGDLLDRRGAFGCARA 120
P54741|AFSK   RFTREVEAARAVSGFYT---AAVVDADPRAAVPWLATAYVPAPSLEEIVNECGPMPAQAV 115

NP_733728     ARVGLELLAVLEAAHTHGVTHGELSPGQVFVREEGSVLVTGFGLAGATLAPRLTAP---- 176
P54741|AFSK   RWLAAGVAEALQSIHGAGLVHRDLKPSNVLVVEDGPRVID-FGIASGVSNTRLTMTNVAV 174

NP_733728     ---AYASPEQARDER-IGPAADLWTLGAILYTMVEGRPPFRDRGRPEATLKGVDRLPLRT 232
P54741|AFSK   GTPAYMSPEQAKDSRSVTGASDVFSLGSMLVFAATGHPPFHGANPVETVFM--------- 225

NP_733728     PVRAGPLAQAVTGLLRKNSRERPTRPVVRAALARALAEDPGTAVTEVTTGPGVRGGYAAA 292
P54741|AFSK   LLREGPDLEGLPDELR----------PLIESCMQMEATGRPNPADLQAQLAPHLFG------ 271

NP_733728     RTAGRDRSRRTVAAGTALAVVTVAVAVLADLAVTDGLPGRGDGAAAGARQRPSASAAPPT 352
P54741|AFSK   --SGSDDS-------GTASAWLPERAVGLIE-GRRNGRPAVKPATTAGGR-----GHGHGPS 318

NP_733728     GSAPAAASPGSPSSPAGRPSASASPS-KPGVPAPAGFRRYDAPEGFSVALPEGWRRLDTA 411
P54741|AFSK   GARAPVHAPPLPPPPAHDPVVPAPPAHVPAVPAPVG------APDGGPVRLPGAAVPIGPG 373

NP_733728     SAPGGAYRVVFGASGDPRTLAVTYSRRAGADPVVVWRDDVEPGLARSDGYRRIGEIRSTT 471
P54741|AFSK   PRVADMRAAAVAAPPPESALAASWSR---PRPGVNGADPAVPAPAPAPPEASPAGWRPWR 430

NP_733728     YRGRAAADMEWLVRDDGTRLRTFGRGFLLGGGRSFSLRWTTPAGDWDDSANERALAAFLG 531
P54741|AFSK   FRMSNDVWGTPRVAEDLVYVTSFEVHALDVATG--RRRFKTRDVAWSMAVADGRIHASDG 488

NP_733728     TFRDGAA 538
P54741|AFSK   PT------ 490
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Fig. 4. Sequence alignments of putative serine/threonine protein kinase (NP_733728) and AfsK (P54741).

result, 13 proteins ranged between 33 kDa and 50 kDa appeared in *S. coelicolor* A3(2) by the addition of SAM. Specifically, a putative serine/threonine protein kinase (32141327) found in CB-3 showed 19% sequence homology with the N-terminal of AfsK. Their sequence alignments are shown in Fig. 4. As mentioned above, proteins such as AfsK, AfsR, and AfsS participate in the production of actinorhodin. The question of whether the putative serine/threonine protein kinase discovered in this study is related to the biosynthesis of actinorhodin remains to be clarified by future work.

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Table 1. Proteins identified by LC/MS/MS. The numbers in parenthesis denote the GenBank accession number.

CA-3	CA-3 & CB-3	CB-3
ecrE2 (21902164)	Putative acyl-CoA carboxylase complex A subunit (32141294)	ecrE1 (21902163)
ecrF (21902165)	Putative oxidoreductase (32141253)	Putative phospholipase C (32141312)
Hypothetical protein (21234326)		Putative oxidoreductase (21234083)
Putative secreted hydrolase (32141249)		Guanosine pentaphosphate synthetase (32141272)
Hypothetical protein (21234196)		Putative ATP/GTP-binding protein (21234142)
Transcription termination factor Rho (32141261)		Putative TetR-family regulator (21234309)
Putative TetR-family transcriptional regulator (32141296)		Spore-associated protein (21234301)
Phosphoenolpyruvate-utilizing enzyme (32141277)		Hypothetical protein (21234193)
Putative acetolactate synthase (32141318)		Insertion element IS466S transposase (21234275)
Putative ATP/GTP-binding protein (21234165)		Hypothetical protein (21234099)
Hypothetical protein (21234117)		Hypothetical protein (21234277)
Putative peptide transport system ATP-binding (32141254)		Putative serine/threonine protein kinase (32141327)
Hypothetical protein (21234319)		Putative integral membrane protein (32141299)
Putative 4-hydroxy-2-oxovalerate aldolase (21234299)		
Putative flavin-binding protein (32141333)		
Hypothetical protein (21234199)		
Histidine kinase (32141257)		
Hypothetical protein (21234154)		
Putative oxidoreductase (32141335)		

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