Analysis of two additional signaling molecules in Streptomyces coelicolor and the development of a butyrolactone-specific reporter system

Hsiao, Nai-Hua; Nakayama, Satoshi; Merlo, Maria Elena; de Vries, Marcel; Bunet, Robert; Kitani, Shigeru; Nihira, Takuya; Takano, Eriko

Published in:
Chemistry & Biology

DOI:
10.1016/j.chembiol.2009.08.010

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher’s PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 20-04-2017
Supplemental Data

Analysis of Two Additional Signaling Molecules in *Streptomyces coelicolor* and the Development of a Novel Butyrolactone-Specific Reporter System

Nai-Hua Hsiao, Satoshi Nakayama, Maria Elena Merlo, Marcel de Vries, Robert Bunet, Shigeru Kitani, Takuya Nihira, and Eriko Takano

Primers used in this work

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB4F</td>
<td>aeggcggttcgggtacggttgctggccccggttctgggtcaattcgccggatatccgtcgcacccgtgggccgggcgttcttc</td>
</tr>
<tr>
<td>RB4R</td>
<td>tgctcagccggaacgcgggccccggacagctggtggtggtgtaagctgctggagctgttc</td>
</tr>
<tr>
<td>DDAR2</td>
<td>cccacgtggttgacctttctgg</td>
</tr>
<tr>
<td>scbA sec 2</td>
<td>ctaggatctcgccaccggcagcaaggcgcgg</td>
</tr>
<tr>
<td>Tnneo1</td>
<td>tcgaattcatgattgaacaaagatg</td>
</tr>
<tr>
<td>neo2</td>
<td>agcttaag</td>
</tr>
<tr>
<td>pkasO1</td>
<td>cagagctctctgctgtctg**</td>
</tr>
<tr>
<td>pkasO2</td>
<td>gcagagctcagcgca**</td>
</tr>
<tr>
<td>scbrtt2</td>
<td>gcaggtcctcgagaacagcggg</td>
</tr>
<tr>
<td>JGattB1-fwd</td>
<td>tctggaatactcgaaggg</td>
</tr>
<tr>
<td>JGattPint-rev</td>
<td>gtaagaccccggtacctgtgc</td>
</tr>
</tbody>
</table>

* The EcoRI site is underline.
** The SacI site is underline.

Supplemental Data LC-MS and tandem MS Data

The extracted ion chromatograms (EIC) of mass range 245.17-245.18 in which SCB1/SCB2 hydrogen adducts [(M+H)+] should be present show that they eluted late during the run. The synthesized SCB1 eluted at RT 40.33 min (Table 1). In the M145 ethyl acetate extract, the m/z 245.17 eluted at RT 39.30 min and RT 40.20 min (Table 1). In the mass spectra of wild type *S. coelicolor* M145 ethyl acetate extract and the synthesized SCB1 and SCB2 in correspondence to the highest peaks detected in the EICs, the following masses were detected: 245.17 (M+H)+, 227.16 (M+H-H2O)+ and 209.15 (M+H-H2O)+. The best matching chemical formula given by the Xcalibur 2.0.5 elemental composition tool software (Thermo Fisher Scientific) for these masses are: C_{13}H_{25}O_{4} (m/z 245.17), C_{13}H_{23}O_{3} (m/z 227.16) and C_{13}H_{21}O_{2} (m/z 209.15), which confirm the mono- and di-dehydration.

In the tandem MS spectrum of m/z 245.17, the most intense peaks correspond to the dehydrated structures (m/z 227.16 and m/z 209.15). Fragmentation of mass 227.16 (M+H-H2O)+ was also performed: major peaks at m/z 169.12, 155.11 and 141.09 were found and they represent the mono-dehydrated structures of SCB1 or SCB2 after beta-elimination on the acyl chain at different position. Minor peaks at m/z 191.14, 135.12, 133.10, 107.09, 95.09 and 81.07 were also detected at much lower intensity. Predicted formulas were also given for these masses: C_{10}H_{17}O_{2} (m/z 169.12), C_{9}H_{15}O_{2} (m/z 155.11), C_{8}H_{13}O_{2} (m/z 141.09), C_{10}H_{15} (m/z 135.12), C_{10}H_{13} (m/z 133.10), C_{8}H_{11} (m/z 107.09), C_{7}H_{14} (m/z 95.09) and C_{6}H_{9} (m/z 81.07).

MS analysis was also performed to confirm the presence of SCB3 [(M+H)+...
259.19] in the M145 ethyl acetate extract. The synthesized SCB3 and IM-2 C9-i were used as reference standards. The extracted ion chromatograms (EICs) of mass range 259.18-259.20 in which SCB3 hydrogen adduct should be detected show that the synthesized SCB3 eluted at RT 44.21 min, and the synthesized IM-2 C9-i was eluted at RT 49.35 min and 57.57 min which may correspond to a racemic mixture of IM-2 C9-i and with the M145 ethyl acetate extract, the m/z 259.18 eluted at RT 43.97 min (Table 1).

In the MS spectrum of M145 at the highest peak in the mass range 259.18-259.20 at RT 43.97 min, the following masses are detected: m/z 259.19 (M+H)+, which is SCB3 hydrogen adduct, 241.18 (M+H-H2O)+ and 223.17 (M+H-H2O)+ which are the mono- and di-hydrated structure of SCB3. The same masses are also found in the reference standards spectra. The best matching chemical formula given by the Xcalibur elemental composition tool for these masses are: C14H27O4 (m/z 259.19), C14H25O3 (m/z 241.18) and C14H23O2 (m/z 223.17), which are the mono- and di-dehydrated structures.

In the tandem MS spectrum of mass 259.19 the most intense peaks correspond to the dehydrated structures (m/z 241.18 and m/z 223.17). Fragmentation of mass 241.18 (M+H-H2O)+ shows as major peaks with predicted formula m/z 223.17 (C14H22O2), m/z 169.12 (C10H17O2) and m/z 155.11 (C9H15O2), which represent the mono-dehydrated structures of SCB3 after beta-elimination on the acyl chain at different positions and m/z 205.16 (C14H21O1) which is a dehydrated form of the ring-opened lactone. Minor peaks of m/z 187.15, m/z 95.09 and m/z 67.05 are visualised at lower intensity. Predicted formulas for these masses are: C14H19O (m/z 187.15), C7H14 (m/z 95.09) and C5H7 (m/z 67.05). As negative control, SMMS ethyl acetate extract and ΔscbA, a mutant which does not produce any γ-butyrolactones, ethyl acetate extract were injected. No precursor ions and no fragment ions corresponding to SCB1, SCB2 and SCB3 were detected in these MS and MS/MS analysis.

![Figure S1.](https://example.com/figure-s1.png)

**Figure S1.**

Chemical structures of the γ-butyrolactones isolated previously from *S. coelicolor* from Anisova et al., (1984).
Figure S2. 400MHz $^1$H-NMR spectrum of SCB2
The predicted structure of SCB2 is shown in the inset. The number above each signal indicates the corresponding proton in SCB2. The signals at 4.43 and 3.99 ppm corresponded to methylene protons at C-4, and those at 3.76 and 3.70 ppm to hydroxymethylene protons at C-5. The signals at 4.02, 2.78 and 2.66 ppm corresponded to three methine protons at C-1’, C-3 and C-2, respectively. The remaining signals at 1.26 - 1.30 (10 protons) seemed to come from 5 methylene groups of alkyl side chain at C-2. However, the triplet signal at 0.89 ppm corresponded to three protons of one methyl group and indicated the presence of unbranched side chain at C-2, which contrasted to the terminal isopropyl containing side chain in SCB1. The coupling constant between H-2 and H-3 ($J_{2,3} = 9.2$ Hz) was different from that of VB-A ($J_{2,3} = 7.4$ Hz)(Yamada et al., 1987), but agreed well with that of IM-2 ($J_{2,3} = 9.3$ Hz) (Sato et al., 1989), suggesting that SCB2 possesses an IM-2-type stereochemistry for C-2, 3 and 1’.

Figure S3. The schematic map of pTE134 used for the kanamycin bioassay
pTE134 is constructed with \textit{scbR} (open arrow) with its own promoter region, \textit{scbRp} (black arrow) and a \textit{cpkO} promoter, \textit{cpkOp} (black arrow) coupled with a promoterless kanamycin resistant gene (\textit{neo}, solid arrow). The light gray arrow indicates \textit{hyg} (omega hygromycin resistance gene), the hatched arrow represents \textit{oriT} (\textit{RP4} origin of single-stranded DNA transfer), the deep gray arrow represents \textit{int} (\textit{phiC31} integrase), the shaded arrow indicates a partial coding region (204 bp) of \textit{scbB} and the \textit{attP} site (\textit{phiC31 attP} site) is indicated by a vertical black line. The 200 bp length is indicated below and the restriction enzymes used for cloning are indicated by vertical black lines (not all \textit{SacI} and \textit{EcoRI} sites are represented in the map).

**Fig. 4**

![Image](image.png)

**Figure S4. Kanamycin bioassay using A-factor**
Different concentrations of A-factor were spotted onto a lawn of LW16::pTE134 on DNAgar plates containing 5 \( \mu \text{g/ml} \) kanamycin, respectively. The plates were incubated at 30 °C for 72 hours. The concentration where a growth halo is observed indicates the ability for A-factor to bind to ScbR.
Figure S5.
Kanamycin bioassay of the SCB1 isomers, SCB2 and SCB3
SCB1 isomers, SCB2 and SCB3: the natural SCB1(-)-SCB1 [2R,3R,1’R]), its
isomers: (+)-SCB1 [2S,3S,1’S], VB type (-)-SCB1 [2R,3R,1’S] and VB type (+)-SCB1
[2S,3S,1’R], the racemic SCB2 (IM-2 C₈) and the racemic SCB3 (IM-2 C₉-sec) were
spotted onto a plate with different amounts. The bold represent the natural SCBs
identified from S. coelicolor. The name of the compound is indicated above. The
amounts of the analogues are indicated at the left. The plates were incubated at 30 °C
for 72 hours.
Figure S6. Kanamycin bioassay of the IM-2 analogues
IM-2 type series: racemic analogous possessing a C-1’-β-hydroxyl group with a different length of linear C-2 side chain were spotted with different concentrations.

Figure S7. Kanamycin bioassay of the VB analogues
VB type series: racemic analogous possessing a C-1’-α-hydroxyl group with a different length of linear C-2 side chain were spotted with different concentrations. The bold represents the natural γ-butyrolactone VB-D identified from \textit{S. virginiae}. 

Figure S6

Figure S7
Figure S8. Kanamycin bioassay of the branched IM-2 and VB analogues
The racemic IM-2 and the racemic VB analogues with 1'-hydroxy-7'-methylloctyl or 1'-hydroxy-6'-methylloctyl side chain or 1'-hydroxy-5'-methylheptyl side chain (VB C9-i, IM-2 C9-i, VB C9-sec, IM-2 C9-sec, VB C8-sec or IM-2 C8-sec) were spotted with different concentrations.

Figure S9. Kanamycin bioassay of three antibiotic producers from the Tübingen collection
*S. antibioticus*, *S. olivaceus ssp. Atratus* and *S. mediteranei* were grown on SMMS for 3 days at 30 °C (upper pictures) and extracted by ethyl acetate (see Experimental procedures) and spotted onto lawns of LW16:pTE134 on DNAgar plates containing 5 µg/ml kanamycin which were incubated at 30 °C for 6 days. Only the example for *S. antibioticus* is shown for those strains that did not grow well. Details listed in Table 3.