Supplementary Information

Identification and Characterisation of the Nybomycin Gene Cluster from the Marine Strain Streptomyces albus subsp. chlorinus NRRL B-24108

Marta Rodríguez Estévez 1, Maksym Myronovskiy 1, Nils Gummerlich 1, Suvd Nadmid 1, Andriy Luzhetskyy 1,2,*

1 Pharmazeutische Biotechnologie, Universität des Saarlandes, Saarbrücken 66123, Germany; marta.rodriguezestevez@uni-saarland.de (M.R.E.); m.myronovskiy@googlemail.com (M.M.); nils.gummerlich@uni-saarland.de (N.G.)
2 Helmholtz-Institut für Pharmazeutische Forschung Saarland, Saarbrücken 66123, Germany
* Correspondence: a.luzhetskyy@mx.uni-saarland.de; Tel.: +49-0681-70223

Tables

Table S1. 1H-NMR (500 MHz, d-TFA) data for isolated and standard nybomycin.

<table>
<thead>
<tr>
<th>Position</th>
<th>Isolated nybomycin δH</th>
<th>Standard nybomycin δH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6.77 s</td>
<td>6.77 s</td>
</tr>
<tr>
<td>5</td>
<td>7.09 s</td>
<td>7.09 s</td>
</tr>
<tr>
<td>6'</td>
<td>2.81 s</td>
<td>2.81 s</td>
</tr>
<tr>
<td>7</td>
<td>7.67 s</td>
<td>7.67 s</td>
</tr>
<tr>
<td>8'</td>
<td>5.52 s</td>
<td>5.52 s</td>
</tr>
<tr>
<td>9</td>
<td>8.10 s</td>
<td>8.10 s</td>
</tr>
<tr>
<td>11'</td>
<td>4.47 s</td>
<td>4.47 s</td>
</tr>
</tbody>
</table>
**Table S2.** Bacterial strains and BACs used in this work.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Features</th>
<th>Reference/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces albus</em> subsp. chlorinus NRRL B-24108</td>
<td><em>S. albus</em> subspecies strain harboring nybomycin biosynthetic gene cluster</td>
<td>[1]</td>
</tr>
<tr>
<td><em>Streptomyces albus</em> Del14</td>
<td>Wild-type strain</td>
<td>[2]</td>
</tr>
<tr>
<td><em>Streptomyces albus</em> 4N24</td>
<td><em>S. albus</em> strain with BAC 4N24 insertion</td>
<td>This work</td>
</tr>
<tr>
<td><em>Streptomyces albus</em> 4M14</td>
<td><em>S. albus</em> strain with BAC 4M14 insertion</td>
<td>This work</td>
</tr>
<tr>
<td><em>Streptomyces albus</em> 6M11</td>
<td><em>S. albus</em> strain with BAC 6M11 insertion</td>
<td>This work</td>
</tr>
<tr>
<td><em>Streptomyces lividans</em> TK24</td>
<td>Wild-type strain</td>
<td>[3]</td>
</tr>
<tr>
<td><em>Streptomyces lividans</em> 4N24</td>
<td><em>S. lividans</em> strain with BAC 4N24 insertion</td>
<td>This work</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ET12567 pUB307</td>
<td>Donor strain for intergeneric conjugation</td>
<td>[4]</td>
</tr>
<tr>
<td><em>Escherichia coli</em> DH10β</td>
<td>General cloning strain</td>
<td>[5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BACs</th>
<th>Features</th>
<th>Reference/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSMART</td>
<td>AmR; BAC vector</td>
<td>Lucigen (USA)</td>
</tr>
<tr>
<td>4N24/4M14/6M11</td>
<td>BACs containing full or partial nybomycin gene cluster</td>
<td>This work</td>
</tr>
</tbody>
</table>
Figures

**Figure S1.** UV chromatograms of crude extracts from *S. albus* 4N24 and *S. albus* Del14. The new peak found in *S. albus* 4N24 crude extract is indicated with an asterisk (*). The profiles correspond to wavelength 285 nm.

**Figure S2.** Comparison of LC-MS chromatograms at $t_R = 4.1$ min of *Streptomyces albus* 4N24 crude extract and a nybomycin standard. (A) Extracted ion chromatograms (299.10 ± 0.1 Da) of crude extract from *S. albus* 4N24 supplemented with 0.05 mg/ml of pure nybomycin, crude extract from *S. albus* 4N24 broth culture, a 0.05 mg/ml nybomycin solution in methanol, and crude extract from *S. albus* Del14 broth culture. (B) Mass spectra associated to the peak at $t_R = 4.1$ min from the three upper chromatograms displayed in (A).
Figure S3. $^1$H-NMR (500 MHz, d-TFA) spectra of a nybomycin standard and nybomycin isolated from S. albus 4N24. $^1$H-NMR spectra of nybomycin standard (A) and the isolated nybomycin (B) are identical.
Figure S4. Extracted ion chromatograms of S. albus 4M14, S. albus 6M11, and S. albus 4N24 crude extracts. Nybomycin appears only in S. albus 4N24 crude extract, which shows a peak at $t_R = 4.7$ min (indicated with an asterisk) comprising an [M+H]$^+$ of 299.102 m/z (mass spectrum not shown).

References


