Electronic Supplemental Information

Experimental Section

Materials. Spectinabilin producer *Streptomyces spectabilis* NRRL ISP-5512 and *S. lividans* 66 were obtained from the United States Department of Agriculture Agricultural Research Service Culture Collection (Peoria, IL). The plasmids pJK050, pAE4, pAE5, and *E. coli* strains WM3608, WM4489 were kindly provided by W. Metcalf.[1] Authentic spectinabilin was purchased from Bioaustralis (Smithfield, Australia).

Construction of a Library of Genomic DNA of *Streptomyces spectabilis*. A genomic library of *Steptomyces spectabilis* was prepared using genomic DNA obtained by the method of Eliot *et al.*[1] Briefly, a single colony of *Streptomyces spectabilis* was grown in GYM broth (1% malt extract, 0.4% yeast extract, 0.4% dextrose, pH 7.2) for 48 hr at 30 °C, and 3 ml of this culture was transferred into 150 ml S medium (4 g/l peptone, 10 g/l glucose, 4 g/l yeast extract, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 4 g/l K_2HPO_4, 2 g/l KH_2PO_4, 0.75% (w/v) glycine) in a 1 liter flask baffled with a coiled string and grown for 50 hr at 30 °C. The cells were harvested and homogenized with a sterile ground glass tissue homogenizer and washed with 10 ml TE25S buffer (25 mM Tris-HCl, 25 mM EDTA, 0.3 M sucrose, pH 8.0). The cells were then resuspended in 10 ml TE25S buffer containing 2 mg/ml lysozyme. After incubation at 37 °C for 20 min, the protoplasted cells were treated with 0.15 mg/ml proteinase K and incubated at 50 °C for 30 min, and sodium dodecyl sulfate was added to 0.5% (w/v) final concentration. After 5 min at room temperature, the lysate was washed with an equal volume of a 25:24:1 mixture of buffer saturated phenol, chloroform, and isoamyl alcohol. After centrifugation at 22,500 × *g* for 45 min, the aqueous layer was removed, and washed with an equal volume of a 24:1 mixture of chloroform and isoamyl alcohol. Genomic DNA was precipitated from the aqueous layer by addition of 0.1 volume 5 M NaCl and 0.7 volume isopropanol. The precipitated DNA was spooled onto a glass rod, washed three times with 70% ethanol and once with 100% ethanol, dried, and re-suspended in 2 ml TE buffer (pH 8.0).

The prepared genomic DNA was partially digested with Sau3A1. The fraction with ~ 20-60 kb fragments as determined by field inversion gel electrophoresis (FIGE) was purified for DNA ligation. The vector pJK050 was prepared by sequential NheI digestion, shrimp alkaline phosphatase (SAP) treatment and BamHI digestion. The genomic DNA fraction was ligated with prepared pJK050 overnight at 4 °C, followed by ethanol precipitation and packaging into lambda phage using the MaxPlax packaging extract according to the manufacturer's instructions (Epicenter Biotechnologies, Madison, WI). *E. coli* WM4489 cells were transfected with the packaged library and plated on TYE medium (1% (w/v) tryptone, 0.5% (w/v) yeast extract, 0.8% (w/v) NaCl) plus 12.5 μg/ml

chloramphenicol (Cm) agar plates. Individual colonies were picked into standard 96-well plates with each well containing 200 µl LB plus 12.5 µg/ml Cm and grown overnight at 37 °C.

Library Screening. Cell culture from 48 clones of the library was pooled together, boiled in water, and used as a template for PCR reactions. Subsequent row and column pooling allowed positive clones to be identified. Partial clusters including possible pABA synthase sequence sharing 88% sequence identity with AurG from the aureothin biosynthetic gene cluster were screened using the degenerate primers PABAF1 and PABAR1.[1] Fosmid DNA was isolated from single positive clones grown overnight in 5 ml LB plus 12.5 □g/ml Cm plus 0.2% rhamnose using a Qiagen Miniprep kit (Qiagen, Valencia, CA). The resulting fosmids were sequenced from the junction points within the fosmid vector pJK050 using primers that anneal to the T7 promoter on one side of the vector and the T3 promoter on the other side of the vector. The following screening primers were designed after partial sequences of the spectinabilin gene cluster were obtained by junction sequencing: SpnG-Fwd (5'-CTC GGC TTC TGC CAG GAG G-3') and SpnG-Rev (5'-GCG TTC GCC GGT GGC GTC-3') for the putative pABA synthase SpnG, SpnF-Fwd (5'-CCG AGG TCA TGG ACG AGA-3') and SpnF-Rev (5'-TCA TCC GCT CGT ACA GCT C-3') for the putative *p*-aminobenzoate *N*-oxygenase SpnF, and SpnC-Fwd (5'-CGG GCC AGT GGC TGT C-3') and SpnC-Rev (5'-CGG TGG ACC TGC GCA AC-3') for the putative polyketide synthase AurC. These primers and further junction sequencing were used to increase the probability of isolating the entire spectinabilin gene cluster.

In order to transfer the spectinabilin biosynthetic gene cluster into the heterologous host *Streptomyces lividans* 66, the purified fosmids were individually recombined *in vitro* with pAE4 using a BP clonase kit according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The reaction mixtures were transformed into *E. coli* WM3608, and successfully recombined plasmids were selected on LB plus 12.5 µg/ml Cm plus 50 µg/ml apramycin (Apr) agar plates. These transformants were then used as donors for conjugal transfer of the fosmids to *S. lividans* 66 following a previously described high-throughput protocol with the exception that the entire *E. coli*/*S. lividans* mixture was spotted on R2 no-sucrose media in 2.5 µl aliquots.[2] After 16-20 hr at 30 °C, plates were flooded with 2 ml of a mixture of 1 mg/ml nalidixic acid (Nal), 50 µg/ml Apr and 12.5 µg/ml Cm, and incubated at 30 °C for additional 5 days, at which point *S. lividans* exconjugants were picked and restreaked on ISP2 plus 50 µg/ml Apr plus 12.5 µg/ml Cm agar plates and allowed to grow for several days.

DNA Sequencing. Sequencing of fosmid 79C was carried out by creating a library of transposon insertions and remaining gaps were filled in by further sequencing using

specifically designed primers. Potential ORFs were identified using BLAST analysis,[2] GLIMMER,[3] and visual inspection. DNA sequencing was carried out at the Biotechnology Center at the University of Illinois at Urbana-Champaign.

HPLC Analysis. To check for the production of spectinabilin, wild type and recombinant strains were grown on solid- or liquid-phase GYM broth (1% malt extract, 0.4% yeast extract, 0.4% dextrose, pH 7.2) for 4 days at 30°C with exclusion of light and extracted by methanol and ethyl acetate. The extract was concentrated *in vacuo*, and finally dissolved in methanol for LC-MS/MS analysis. Spectinabilin was identified from concentrated extracts by comparison of retention times and mass spectra to that of the authentic spectinabilin. The LC-MS/MS was performed using an Agilent 1100 series LC/MSD XCT plus ion trap spectrometer (Agilent, Palo Alto, CA). Mass spectra were acquired in ultra scan mode using electrospray ionization (ESI) with negative polarity. The LC was carried out on a ZORBAX SB-C18 column (Agilent). HPLC parameters were as follows: 25°C; solvent A: 1% acetic acid in water; solvent B: methanol; gradient: 10% B for 1 min; then from 10% B to 100% B over 20 min; 100% B for 10 min; flow rate: 0.5 ml/min; UV absorbance detection: 367 nm.

References

1. A. C. Eliot, B. M. Griffin, P. M. Thomas, T. W. Johannes, N. L. Kelleher, H. Zhao and W. W. Metcalf, *Chem Biol*, 2008, **15**, 765-770.
2. S. F. Altschul, W. Gish, W. Miller, E. W. Myers and D. J. Lipman, *J Mol Biol*, 1990, **215**, 403-410.
3. A. L. Delcher, D. Harmon, S. Kasif, O. White and S. L. Salzberg, *Nucleic Acids Res*, 1999, **27**, 4636-4641.