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ORIGINAL ARTICLE

Staphylococcus aureus promoter-*lux* reporters for drug discovery

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We describe a collection of antibiotic-activated *Staphylococcus aureus* promoter-*lux* reporter strains that can be used to discriminate among antibiotic classes on the basis of their light production response profile. We screened over 400 culture supernatants from previously uncharacterized actinomycetes from soil for the production of aminocoumarin-type compounds and DNA-damaging agents. Novobiocin production was determined in three isolates of *Streptomyces*, and streptonigrin, a DNA-damaging agent, together with several other bioactive compounds (oxopropaline D and G), was identified from a novel *Kitasatospora* isolate. This array provides an effective and specific whole-cell approach to search for classes of antimicrobial compounds in unfractionated culture broths.
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INTRODUCTION

Staphylococcus aureus, a cosmopolitan pathogen isolated from both hospital- and community-acquired infections, causes significant morbidity and mortality (upward of 100 000 deaths a year in the United States).[1,2] Methicillin-resistant *S. aureus*[1] and vancomycin-resistant *S. aureus*[3] are the most harmful (for reviews see De Leo and Chambers[4] and Fishbach and Walsh[5]), and there is an urgent need for creative screening methods to identify new antimicrobials to treat these infections.[5,6]

It has been argued that the decline in the number of new antimicrobials is due to past emphasis on molecules that were readily detected because they are produced in abundance, and that the supply of such easily accessible, 'low hanging fruit' is close to being exhausted.[7] A highly sensitive assay for the detection of bioactive compounds at low concentrations would be desirable. Bioluminescent reporters, such as *lux*, have varous applications, ranging from the detection of specific bacteria in foods[8] and model infections[9,10] to the detection of environmental contaminants.[11,12] These assays are extremely sensitive, accurately reflect real-time changes in gene expression and are well suited for monitoring the activity of promoters on an individual or a genome-wide level.[13–16]

We have used an array of promoter-*lux* clones in *S. aureus* for the purpose of detecting low concentrations of bioactive compounds produced by actinomycetes. The approach is based on the observation that almost all antibiotics, at subinhibitory concentrations (sub-MIC), alter the transcription of up to 5% of genes in bacteria.[17] This suggested that transcription modulation would provide a sensitive and informative way to test for the presence of biologically active natural products present at low concentrations.[14,18] Such an array would be extremely useful in natural product screening, when uncharacterized strains are grown on a small scale in generic media; moderately or even poorly productive isolates may be identified in routine fermentation conditions, and an indication of chemical class may be obtained, as transcriptional responses provide characteristic fingerprints.[19,20]

MATERIALS AND METHODS

Bacterial strains and growth conditions

S. aureus strains (Table 1) were grown at 37 °C on NYE[21] supplemented with chloramphenicol (10 μg ml^{-1}) (NYEC). *S. aureus* novobiocin-resistant derivative Nov^{r}I was selected by plating 500 μl of a stationary-phase culture of *S. aureus* RN4220 on NYE medium supplemented with novobiocin

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It is a pleasure to dedicate this little article to Arnie Demain. However, Arnie deserves more! He has had an enormous impact on streptomycete biology and on the entire antibiotic field. Arnie has written more encyclopedic articles on natural products than anyone I know; these reviews are always extremely useful and are widely quoted. In one respect, Arnie Demain is the self-appointed 'scribe' for the science of antibiotics! Arnie Demain is a fine human being and is one of the nicest people I have ever met; he is always friendly and courteous and I value his friendship enormously.
Thank you, Arnie for what you have done for all of us; stay cool!
Most sincerely,
Julian Davies
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($5\,\mu g\,ml^{-1}$). The MIC for Nov^{r}I was determined in NYE broth: an aliquot of a culture grown overnight from a single colony at 37 °C was diluted in water at a ratio of 1:100, and 10 µl was inoculated into 1.5 ml NYE broth in tubes containing novobiocin representing twofold serial dilutions starting from 20 $\mu g\,ml^{-1}$. The MIC value determined after incubation at 37 °C for 20 h was $10\,\mu g\,ml^{-1}$, or 30-fold higher than that for *S. aureus* RN4220. The *gyrB* genes of Nov^{r}I and RN4220 were sequenced to identify mutations as described previously.[22]

Table 1 *S. aureus* strains and clones of the promoter-*lux* array used in this study

Strain	*Genotypes*	*Source*
RN4220	Restriction-deficient derivate of 8325-4, rK$^-$mK$^+$	49
Cip^{r}I	*grlA* (GrlA(S80F))	18
Cip^{r}Ia	Insertion in *norA* promoter	18
Cip^{r}II	*grlA* (GrlA(S80Y)), *gyrA* (GyrA(E88K))	18
Cip^{r}IIb	grlA (GrlA(S80Y)), insertion in *norA* promoter[a]	18
Nov^{r}I	*gyrB* (GyrB(G85S))	This study
Clone[b]	*Relevant characteristics (gene/ID promoter-*lux*)*	*Source*
A	*tet-lux*	23
B	*recA-lux*	18
C	*recA-lux*	18
D	*recA-lux*	18
E	*recA-lux*	18
F	*recA-lux*	18
G	SAOUHSC_02631-*lux*[c]	This study
H	SAOUHSC_T0008-*lux*[a]	This study
I	SAOUHSC_01895-*lux*[a]	This study
J	SAOUHSC_00545-*lux*[a]	This study
K	SAOUHSC_00535-*lux*[a]	This study
L	SAOUHSC_00007-*lux*[a]	This study
M	SAOUHSC_00694-*lux*[a]	This study
N	SAOUHSC_01100-*lux*[c]	This study

[a]Promoters were cloned into pGYlux.
[b]A, B and G through N are in RN4220; C, D, E and F are in Cip^r derivatives, Cip^{r}I, Cip^{r}Ia, Cip^{r}II and Cip^{r}IIb, respectively, of RN4200.
[c]Promoters were cloned into pAmilux.

Actinomycete strains

Actinomycete strains named with a 'DCA' prefix were obtained from a collection of microorganisms isolated from lichens, bryophytes and soil in British Columbia. In brief, bacteria were washed from freshly collected materials with 0.001% Tween and plated on ISP4 agar containing nalidixic acid ($20\,\mu g\,ml^{-1}$) and the fungicides cycloheximide ($50\,\mu g\,ml^{-1}$) and benomyl ($20\,\mu g\,ml^{-1}$). After 7 days at 30 °C, individual colonies were isolated from the plates, purified and deposited into the DCA collection. Strains are not characterized further unless they are flagged as potentially interesting by a screening program. DCA3491 strain was isolated from a lichen sample collected from the University of British Columbia, Malcolm Knapp Research Forest, British Columbia. Reference strain *Streptomyces spheroides* NRRL 2449 was obtained from the Agricultural Research Collection, United States Department of Agriculture, Peoria, IL.

Construction of the *S. aureus* promoter-*lux* reporter clones

Partial *Sau*3AI digests of *S. aureus* genomic DNA and *Bam*HI-digested PCR amplicons (0.5–2 kb) of regions upstream of selected *S. aureus* genes were ligated using T4 DNA ligase into pGYlux and pAmilux, replicative *E. coli*/*S. aureus* shuttle plasmids containing a promoterless *luxABCDE* operon.[13] The recombinant plasmids were cloned initially in *E. coli* and then transformed into *S. aureus* RN4220 or its ciprofloxacin-resistant derivatives Cip^{r}I, Cip^{r}Ia, Cip^{r}II, Cip^{r}IIb as described previously.[18,23] The cloned regions in recombinant strains that exhibited a light response were sequenced.[13,18]

Disk diffusion and *lux* assays

A single colony of bioluminescent *S. aureus* from NYEC agar was resuspended in 200 µl of sterile water, diluted 1000-fold into 0.7% (w/v) agar and overlaid on NYEC plates. Antibiotic disks (Becton Dickinson, Mississauga, ON, Canada; Difco, Detroit, MI, USA) were placed on the overlay and the plates incubated at 37 °C. After 20 h, inhibition zones were measured and luminescence was detected with a luminograph LB980 photon camera (Berthold, Oak Ridge, TN, USA) (for example; see Figure 1).

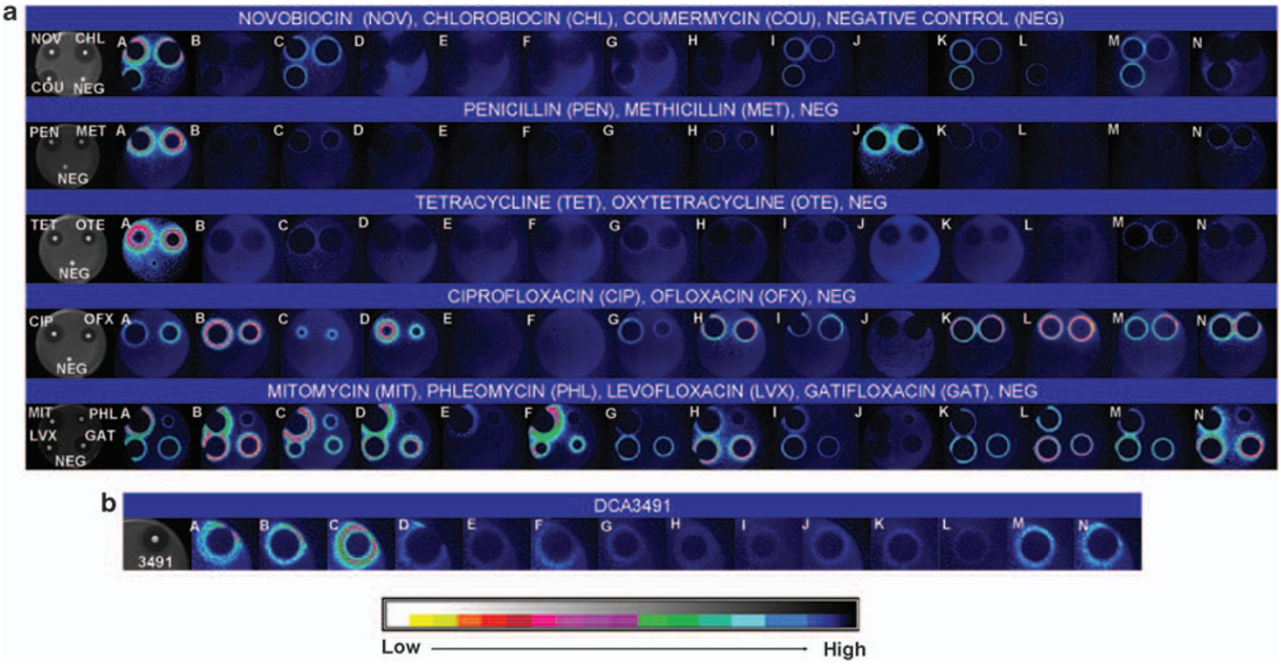

Figure 1 (**a**) Response of array to antibiotics (**b**) Response of array to DCA3491 supernatant. Clone name is in the upper left corner of each picture. A clear zone around a disk indicates inhibition of growth in the presence of high levels of antibiotic. At sub-MIC levels (periphery of the zone), changes in luminescence indicate altered transcription. The pseudocolor scale at the bottom indicates relative luminescence.

Antibiotics used to characterize *S. aureus* clones included cell-membrane damaging agents (daptomycin 30 μg, polymyxin B 100 μg), cell wall biosynthesis inhibitors (bacitracin 10 μg, fosfomycin 20 μg, imipenem 10 μg, penicillin G 10 μg, methicillin 10 μg, vancomycin 30 μg), protein synthesis inhibitors (clindamycin 2 μg, erythromycin 15 μg, gentamicin 10 μg, kanamycin 30 μg, neomycin 30 μg, pristinamycin I and II 10 μg, spectinomycin 100 μg, streptomycin 10 μg, tetracycline 30 μg, oxytetracycline 30 μg, tobramycin 30 μg), DNA-damaging agents (mitomycin C 5 μg and phleomycin 30 μg) and DNA replication inhibitors (ciprofloxacin 5 μg, ofloxacin 5 μg, levofloxacin 5 μg, gatifloxacin 5 μg, novobiocin 5 μg, chlorobiocin 5 μg and coumermycin 5 μg), a metabolic inhibitor (trimethoprim 5 μg) and an RNA polymerase inhibitor (rifampicin 10 μg). Antibiotics were obtained from Sigma (Oakville, ON, Canada) or from the laboratory collection.

Fermentation and preparation of samples for screening

Spores and hyphae of actinomycete isolates grown on ISP4 were inoculated into 96-deep-well plates, 24-well plates, 125 ml or 1 l flasks containing 1 ml (both types of plates), 30 ml or 250 ml fermentation medium, respectively, and shaken at 200 r.p.m. for 7 days at 30 °C. Medium JR2 was composed of 2.5% (w/v) soluble starch, 0.2% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) bacto peptone and 0.3% (w/v) $CaCO_3$, pH 7. JR4 was composed of 1% (w/v) glucose, 1.5% glycerol, 1.5% (w/v) soya peptone, 0.3% (w/v) NaCl, 0.5% (w/v) malt extract, 0.5% (w/v) yeast extract, 0.1% tween 80 and 2% MOPS, pH7. JR11 was composed of 2% (w/v) soluble starch, 1% (w/v) glucose, 1.5% (w/v) soybean meal, 0.5% (w/v) peptone and 0.3% (w/v) $CaCO_3$, pH7. Medium JR30 was similar to JR11, but contained 0.25% (w/v) animal-free peptone (Millipore Lucratone Pea, Celliance subsidiary of Serologicals Corporation, Milford, MA, USA) instead of 0.5% (w/v) peptone. JR2 and JR11 were used in 96-well plate fermentations; JR4 and JR30 were used for fermentation in 24-well plates and flasks. Except for 96-well plates, biomass was removed from cultures by centrifugation and the clarified broth was used directly or stored at −20 °C. Cultures in 96-well plates were passed through a frit to remove cells, and later through a >10 kD filter before use.

Thin-layer chromatography (TLC)

A volume of 1 ml of culture supernatant was extracted with two volumes of EtOAc, and the dried organic phase was dissolved in 50 μl EtOAc. An aliquot (1 μl) was analyzed by TLC using dichloromethane–methanol–formic acid (45:2:1)[24] and visualized under short ultraviolet light. Novobiocin (5 μg) was used as standard.

Structure elucidation

The culture broth (3.4 l) of strain DCA3491 in medium JR11 was extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* to afford 243 mg of residue, which was subjected to column chromatography on Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden) and to elution with MeOH to yield 19 fractions. The combined fractions (Fr.1, Fr.2–4, Fr 5–9, Fr.10–18 and Fr.19) were used for *lux* assays and susceptibility tests in *S. aureus*. Fractions 10–18 contained active compounds and were repeatedly chromatographed over Sephadex LH-20, eluting with MeOH to yield six subfractions. Active compounds from subfraction 5 were purified by TLC using $CHCl_3$-MeOH (9:1) yielding three compounds: compound 1 (4.1 mg, R_F=0.26), compound 2 (3.5 mg, R_F=0.54) and compound 3 (2.1 mg, R_F=0.74). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra of these compounds were recorded on a Bruker AV-400 MHz and AV-600 MHz NMR spectrometer (Bruker Corporation, Billerica, MA, USA) with TMS as internal standard, respectively.

16S rRNA analysis

The 16S rRNA gene was amplified from heat-lysed cells of DCA3491 by PCR using primers 7F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1491R (5′-CGGACTCCTTGTTA-CGACTTC-3′) and a program consisting of two cycles at 94 °C for 15 s, 35 cycles at 94 °C for 40 s, 55 °C for 1 min 30 s and at 72 °C for 2 min, and one cycle at 72 °C for 10 min. The amplicon was sequenced using these or additional primers as needed, and a similarity search was conducted using BLASTN in the NCBI database. The partial 16S rRNA gene sequence of DCA3491 (GenBank Accession GU599919) was compared with those of the 23 recognized *Kitasatospora* species[25] using alignment and tree-building functions of MacVector (MacVector, Inc., Cary, NC, USA). Automatically generated alignments were manually adjusted slightly after secondary structure consideration. All available 16S rRNA gene sequence accessions for each *Kitasatospora*-type strain were assessed, and a best sequence was identified (for example, on the basis of absence of ambiguities or missed bases).

RESULTS

Development of promoter-*lux* array

Two approaches were used to obtain reporter plasmids for the array. An initial shotgun cloning step of a *Sau*3AI partial digest of *S. aureus* DNA in *E. coli* was performed, followed by transformation of individually purified plasmids back into *S. aureus* RN4220. Of a sample of 35 distinct *S. aureus* transformants obtained in this manner, three luminescent clones were responsive to sub-MIC antibiotics. This showed the feasibility of recovering antibiotic-responsive promoter fragments by random cloning, and more clones were obtained in this manner. In parallel, promoter fragments for *recA and lexA* genes,[18] and for other selected genes,[18,23] were cloned and introduced into *S. aureus* RN4220 and its ciprofloxacin-resistant (Cip^r) derivatives.[18,23] A total of 53 distinct luminescent promoter-*lux*-bearing strains (11 from random cloning, 42 with specifically cloned promoters) were tested in disk diffusion assays against a set of 22 antibiotics of different chemical classes and modes of action, and 14 clones with distinct response profiles were finally selected (Table 1).

The light responses can be correlated to a particular class of compounds (Figure 1a). For example; aminocoumarins (novobiocin, chlorobiocin and coumermycin) activated clones A, C, I, K and M, whereas β-lactam antibiotics (penicillin G and methicillin) activated A and J. Tetracyclines (tetracycline and oxytetracycline) activated only clone A. Fluoroquinolones (ciprofloxacin and ofloxacin) activated all, except E, F and J; it is interesting that the newer fluoroquinolones (levofloxacin and gatifloxacin) also activated F. Two classes of DNA-damaging antibiotics were also tested: mitomycin C (an aziridine compound) activated all but clone J, whereas phleomycin (a glycopeptide) activated A, B, C and N.

Screening crude culture supernatants

An initial trial of the array to identify potential hits used a group of six actinomycete strains (DCA2237, DCA2286, DCA2510, DCA2641, DCA2865 and DCA3488), including some suspected to produce novobiocin. Culture supernatants from small-scale fermentations were tested against a subset of five reporter clones: A, B, H, J and K. Supernatants from four strains (DCA2237, DCA2286, DCA2865 and DCA3488) exhibited an aminocoumarin-like profile (only clones A and K were induced) (Figure 2). Results from subsequent testing of these supernatants, as well as the one obtained from *S. spheroides* NRRL 2449, a known novobiocin producer, against the full 14-clone array indicated the presence of novobiocin or novobiocin-like compounds (only clones A, C, I, K and M were induced) (Figure 2a). This was consistent with the finding that extracts inhibited growth of *S. aureus* RN4220, but not of Nov^rI, a novobiocin-resistant derivative carrying a mutation in the *gyrB* gene (Tables 1 and 2). TLC analysis of EtOAc extracts of the culture supernatants of DCA2286, DCA2865, DCA3488 and *S. spheroides* revealed ultraviolet fluorescent spots comigrating with a reference sample of pure novobiocin (R_F=0.36 ± 0.05) in all extracts (Figure 2b). Metabolites from DCA2237 have been studied elsewhere, and novobiocin was determined to be one of the components.[26]

In another test, 400 culture supernatants obtained by fermentation of 240 newly isolated, uncharacterized actinomycetes in media JR11 and JR2 were investigated. About 17% of the broths (22% of the

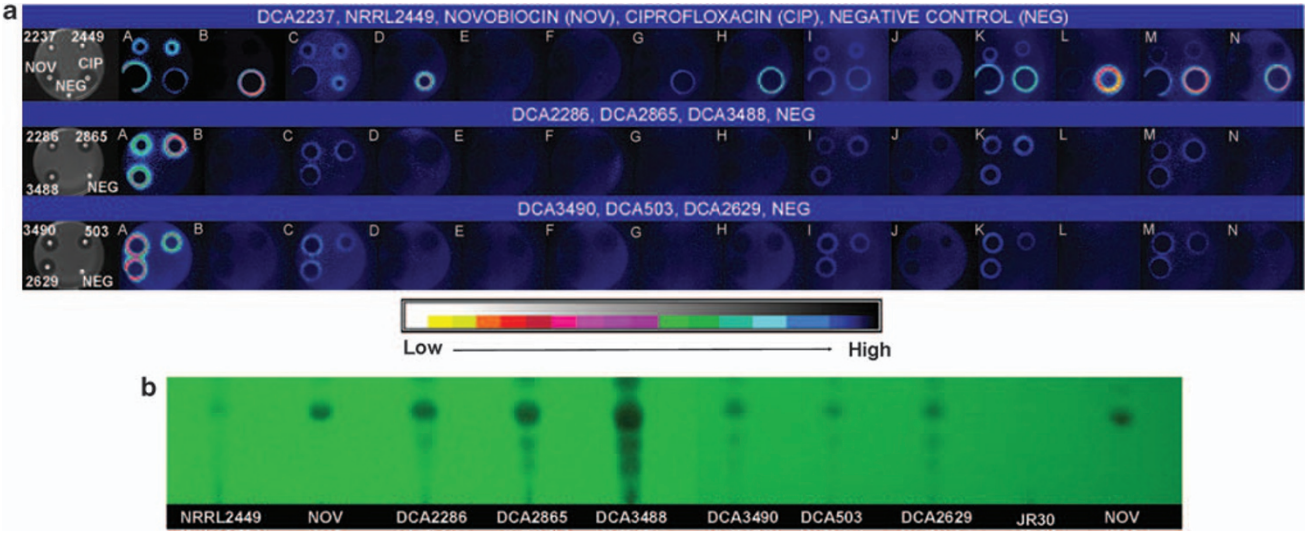

Figure 2 (**a**) Screening of novobiocin-like compounds. The pseudocolor scale at the bottom indicates relative luminescence. (**b**) TLC of EtOAc extracts of culture media and novobiocin standard (5 μg).

Table 2 Inhibition of *S. aureus* RN4220 and Nov^{r}I by novobiocin and culture broths

	Inhibition zone (standard deviation) in mm[a]	
Antibiotic/streptomycete broths	S. aureus *RN4220*	S. aureus *Nov^{r}I*
Novobiocin (5 μg per disk)	29 (1.9)	12 (3.7)
NRRL2449 (20 μg per disk)	12 (0)	0
DCA2237 (20 μl per disk)	12 (0.7)	0
DCA2286 (20 μl per disk)	16 (0.9)	0
DCA2865 (20 μl per disk)	20 (0.4)	0
DCA3488 (20 μl per disk)	20 (0.4)	0
DCA503 (20 μl per disk)	18 (2.6)	0
DCA2629 (20 μl per disk)	20 (0)	0
DCA3490 (20 μl per disk)	21 (0)	0

[a]Each experiment was repeated three times.

strains) had some level of inhibitory activity against *S. aureus*. Three strains (DCA503, DCA2629 and DCA3490) were identified as possible novobiocin producers (Figure 2a), and subsequent tests showed that their antibiotic activity against *S. aureus* RN4220 was abolished in Nov^{r}I (Table 2). TLC analysis of EtOAc extracts of the culture supernatants from these strains showed spots consistent with authentic novobiocin (Figure 2b). Partial 16S rRNA gene sequences indicated that, even though these strains were isolated from separate environmental samples collected in different years, they were closely related to each other, and to *S. spheroides*. The 16S rRNA gene sequences of DCA503 (1315 nucleotides) and DCA2629 (1314 nucleotides) were identical to that of *S. spheroides* (*Streptomyces caeruleus,* GenBank Acc. FJ406110), whereas the gene sequence from DCA3490 (1314 nucleotides) had three conservative ambiguities, but was otherwise also identical to the others. Together, these experiments show that a *lux*-based screen can be effectively applied to identify bioactive compounds in crude form, and, furthermore, may facilitate dereplication of samples and strains.

Identification of DNA–damaging agents

As it is known that DNA-damaging agents activate *recA*,[18,27,28] *S. aureus* clone B was used to screen the 400 supernatants for these agents. Five percent of the strains activated light responses, and two, DCA3491 and DCA3492, were investigated further. When tested against the complete array, supernatants from these strains activated clones A, B, C, M and N. This pattern did not correspond to any of the original compounds profiled using the array. The 16S rRNA gene analysis indicated that DCA3491 was a strain of *Kitosatospora,* with 1435/1446 identities to *Kitasatospora atroaurantiaca* comb. nov., NRRL B-24282[25] (Figure 3). DCA3492 had the same *lux* response profiles and was subsequently found to have the same partial 16S rRNA sequence as DCA3491.

Fractionation and purification of metabolites from a 3.4-l culture of DCA3491 in medium JR11 resulted in the identification of three compounds. The ^{1}H and ^{13}C NMR data of the compounds are shown in Table 3.

Compound 1 (dark-brown powder) had the molecular formula of $C_{25}H_{22}N_4O_8$ as deduced from NMR spectra and electrospray ionozation mass spectrum having *m/z* 507 $[M+H]^+$. Its ^{1}H NMR spectrum suggested the presence of two 1,2,3,4-tetra-substituted benzene rings with signals at δ_H 8.36 (1H, d, *J*=11 Hz), 9.00 (1H, d, *J*=11.0 Hz), 6.73 (1H, d, *J*=9.0 Hz), 6.70 (1H, d, *J*=9.0 Hz), three methoxy groups (δ_H 3.85, 3.81, 3.75 (each 3H, s)) and one aromatic methyl (δ_H 2.17, 3H, s). Its ^{13}C NMR spectrum showed the presence of one methyl, three oxymethyls, 18 aromatic carbons between δ_C 160 and δ_C 104, two carbonyl carbons (δ_C 180.3 and 176.0) and one carboxyl carbon (δ_C 167.1). These ^{1}H and ^{13}C NMR data of compound 1 showed the characteristic signals of streptonigrin.[29–31] By comparison of mass and ^{1}H and ^{13}C NMR spectral data with published values, compound 1 was identified as streptonigrin.

Compound 2 (pale-yellow powder; $[\alpha]_D^{20}$=+30 (*c* 0.1)) had the molecular formula of $C_{15}H_{14}N_2O_3$ as deduced from NMR spectra and electrospray ionozation mass spectrum having *m/z* 271 $[M+H]^+$. Its ^{1}H NMR spectrum suggested the presence of 1,2-disubstituted benzene rings (δ_H 8.27 (1H, d, *J*=8.0 Hz), 7.35 (1H, dd, *J*=8.0, 7.0 Hz), 7.61 (1H, dd, *J*=8.2, 7.0 Hz), 7.74 (1H, d, *J*=8.2 Hz), an aromatic singlet proton (δ_H 8.30, 1H, s), one aromatic methyl (δ_H 2.94, 3H, s), one oxymethine (δ_H 5.59, 1H, t, *J*=4.1 Hz) and one oxymethylene (δ_H 4.10, 1H, d, *J*=4.1 Hz, H-12)). Its ^{13}C NMR spectrum showed the presence of one methyl, 11 aromatic carbons between δ_C 143 and δ_C 113, an α,β-unsaturated ketone group (δ_C 202.2), one oxymethine

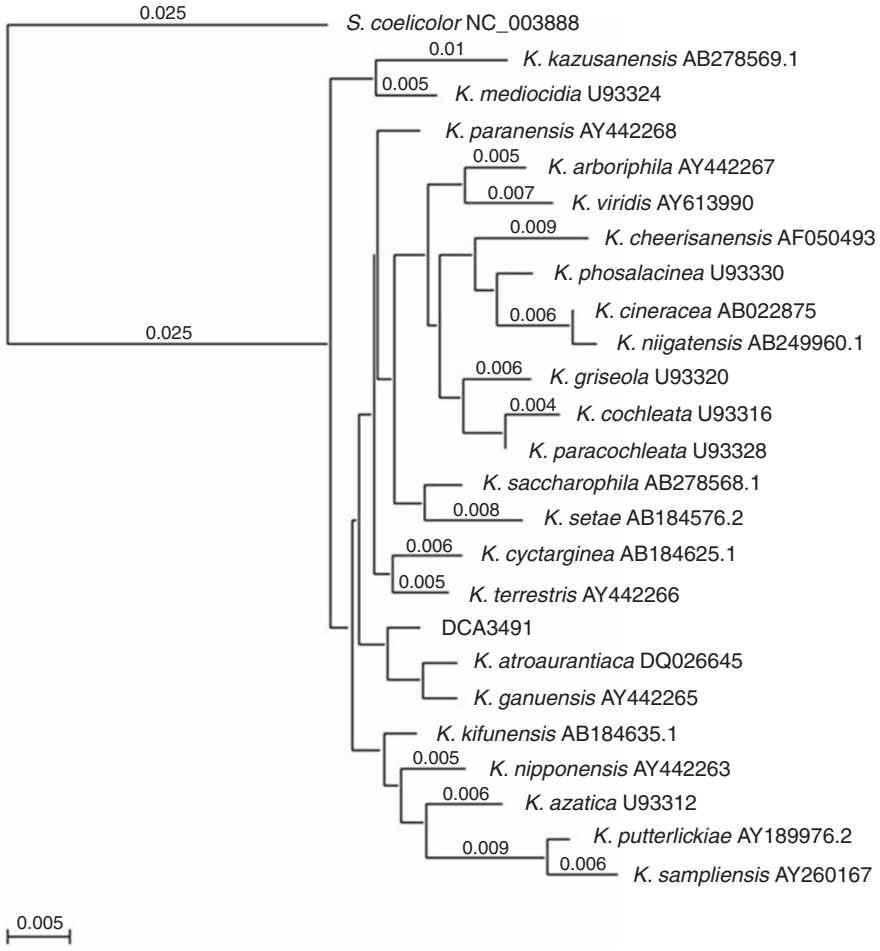

Figure 3 Partial 16S rRNA gene sequence-based dendrogram showing the similarity of DCA3491 and all recognized *Kitasatospora* species; *S. coelicolor* was used as an outgroup.

(δ_C 76.3) and one oxymethylene (δ_C 66.1). These data showed that two had a β-carboline chromophore. By comparison of mass and ^1H and ^{13}C NMR spectral data with published values,[32,33] compound 2 was identified as oxopropaline D.

Compound 3 (pale-yellow powder, optically inactive) had the molecular formula of $C_{15}H_{14}N_2O_2$ as deduced from NMR spectra and electrospray ionozation mass spectrum having *m/z* 255 $[M+H]^+$. Its ^1H and ^{13}C NMR spectral data were very similar to those of compound 2 with the only obvious difference that one methylene instead of one oxymethine was observed in compound 3. By comparison of mass and ^1H and ^{13}C NMR spectral data with published values,[32,33] compound 3 was identified as oxopropaline G.

DISCUSSION

The demand for new antibiotics against resistant and emerging pathogens mandates that innovative antimicrobial screening efforts be increased.[34] We describe a cell-based *S. aureus* promoter-*lux* array as a sensitive and predictive method to identify antimicrobials from natural products against *S. aureus* and other bacteria. Similar promoter-*lux* systems have been used to study the multiple effects of subinhibitory levels of antibiotics, individually or in combination, on gene expression in *Salmonella typhimurium* and *S. aureus*;[14,17–19,23] the sensitive and specific nature of transcription responses to bioactive compounds make them useful as a convenient and robust whole-cell screening system for antimicrobials. The light response readout of a *lux*-based system has an advantage over firefly luciferase (*luc*) or β-galactosidase (*lacZ*) reporter systems for this purpose[35–37] in that additional substrates are not required for the assay. Promoter–reporters have been used for the discovery of antimicrobials active against *Bacillus subtilis*[20,35,36,38] and *E. coli*,[37,39–41] but not against *S. aureus* until now.

The array of *S. aureus* promoter clones used in this study includes 14 bioluminescent *S. aureus* strains carrying promoter-*lux* constructs[23] selected for their response to a variety of known antibiotics. Most promoters in the array were obtained after transfer of individual clones of a partial *Sau*3AI digest library from *E. coli* into *S. aureus* because direct cloning into the latter was inefficient and did not result in a sufficiently large number of luminescent clones.[21,42] Francis *et al.* (2000)[43] recovered 73 clones in a different *lux* vector with at least moderate luminescence out of 20 000 *S. aureus* transformants, and selected only the six brightest for use in *in vivo* tracking. In our study, 53 luminescent clones were generated. We included a combination of selectively cloned (*recA, tet*) and randomly cloned/functionally selected promoters to provide a broader base for the array. The response profiles of promoter-*lux* plasmids are affected by host backgrounds; for example, *recA-lux* plasmids in *S. aureus* RN4220 and Cip^r mutants respond differently to DNA-damaging agents, novobiocin and rifampcin.[18] Combining reporter plasmids and specific antibiotic resistances in the host expands the screening range, and can exclude

Table 3 ^{1}H (400 MHz) and ^{13}C (150 MHz) NMR data for compounds 1–3

	1		*2*		*3*	
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1				134.8 C		135.5 C
2		159.9 C				
3	9.00 (d, 11.0)	125.9 CH	8.30 (s)	140.0 CH	8.26 (s)	139.8 CH
4	8.36 (d, 11)	133.4 CH		134.8 C		134.1 C
4a		126.7 C		131.4 C		131.3 C
4b				122.0 C		122.3 C
5		176.0 C	8.27 (d, 8.0)	124.7 CH	8.25 (d, 8.0)	124.5 CH
6		135.8 C	7.35 (dd, 8.0, 7.0)	122.3 CH	7.34 (dd, 8.0, 7.0)	121.8 CH
7		141.6 C	7.61 (dd, 8.2, 7.0)	129.9 CH	7.59 (dd, 7.0, 7.0)	129.7 CH
8		180.3 C	7.74 (d, 8.2)	113.6 CH	7.72 (d, 7.0)	113.5 CH
8a		144.2 C		143.4 C		143.4 C
9	3.81 (s)	59.8 CH_3		127.4 CH		127.4 CH
9a				136.5 C		136.2 C
10				202.2 C		203.3 C
11			5.59 (t, 4.1)	76.3 CH	3.54 (t, 6.2)	41.9 CH_2
12			4.10 (d, 4.1)	66.1 CH_2	4.08 (t, 6.2)	66.1 CH_2
13			2.94 (s)	17.8 CH_3	2.92 (s)	18.0 CH_3
2'		134.0 C				
3'		136.9 C				
4'		134.6 C				
5'		145.7 C				
6'		129.6 C				
7'	2.17 (s)	17.0 CH_3				
8'		167.1 C				
1"		114.9 C				
2"		148.1 C				
3"		136.2 C				
4"		153.1 C				
5"	6.70 (d, 9.0)	104.4 CH				
6"	6.73 (d, 9.0)	124.7 CH				
7"	3.85 (s)	60.3 CH_3				
8"	3.85 (s)	55.7 CH_3				
NH2	6.90 (s)	134.8 CH		134.8 CH		134.8 CH
OH	8.90 (s)	32.7 CH_2		32.7 CH_2		32.7 CH_2

Compounds 1–3 were determined in DMSO-*d*6, CD_3OD and CD_3OD, respectively; chemical-shift values δ were in ppm and coupling constant values *J* in Hz.

detection of commonly found antibiotics in the same screen. Clones not used in the current array remain a resource for the future, as they may give distinctive responses to new compounds added to expand the capabilities of the array. Promoters identified through recent microarray-based studies[27,44–47] of transcriptional changes in *S. aureus* in response to sub-MIC antibiotics may be other candidates for inclusion.

Exposure of the array to bioactive compounds elicits a response from each clone, with the set of responses forming a characteristic profile or fingerprint for that source: potentially new compounds are consequently highlighted by novel profiles. The ability to use crude broths, a key consideration during the development of the array, makes it an economical and robust assay with high information value that can be applied at the earliest stages of a natural product screening program. We identified potential novobiocin producers among 240 uncharacterized strains in a single screen using only crude broth, allowing efficient execution of confirmation studies (TLC and inhibition assays against Nov^{r}1). This showed the value of *lux* array, not only for early elimination of known compounds but also for dereplication of duplicate strains.

The screening array is valuable for the prioritization of hit candidates and amenable to customization by including clones that recognize additional antibiotics or classes of antibiotics based on structure or mode of action, such as clones that respond to macrolides and aminoglycosides, as well as those that distinguish among structural classes of DNA-damaging agents. Streptonigrin, an antitumor agent discovered in *Streptomyces flocculus*,[48] has been observed in *Micromonospora* sp.,[31] and oxopropalines D and G have been reported from microorganisms, such as *Streptomyces* sp. G324.[33] The producing strain, *Kitasatospora* sp. DCA3491, is a distinct taxon from *Kitasatospora* sp. MJM383, a strain that was also recently reported to produce streptonigrin and oxopropaline G.[30] The latter strain was noted as being most similar to *K. azatica*, and belongs to a different clade.[30]

In conclusion, we have used the sensitive and specific relationships between antibiotics and transcription modulation in bacteria to develop a promoter–reporter-based *S. aureus* array for screening bioactive compounds in crude broths from microbial fermentations. Additional combinations of host strains and promoter-*lux* constructs, including downstream promoters, will expand the screening potential in the future.

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