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Detection of the cell wall-affecting antibiotics at sublethal concentrations using a reporter *Staphylococcus aureus* harboring *drp35* promoter - *lacZ* transcriptional fusion

Rajkrishna Mondal[1,#], *Palas K Chanda*[1,#], *Amitava Bandhu*[1], *Biswanath Jana*[1], *Chia Y Lee*[2] *& Subrata Sau*[1,*]

[1]Department of Biochemistry, Bose Institute, P1/12 - CIT Scheme VII M, Kolkata 700 054, West Bengal, India, [2]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, 4301 W Markham St, Little Rock, Arkanasas, USA

Previously, various inhibitors of cell wall synthesis induced the *drp35* gene of *Staphylococcus aureus* efficiently. To determine whether *drp35* could be exploited in antistaphylococcal drug discovery, we cloned the promoter of *drp35* (P_d) and developed different biological assay systems using an engineered *S. aureus* strain that harbors a chromosomally-integrated P_d - *lacZ* transcriptional fusion. An agarose-based assay showed that P_d is induced not only by the cell wall-affecting antibiotics but also by rifampicin and ciprofloxacin. In contrast, a liquid medium-based assay revealed the induction of P_d specifically by the cell wall-affecting antibiotics. Induction of P_d by sublethal concentrations of cell wall-affecting antibiotics was even assessable in a microtiter plate assay format, indicating that this assay system could be potentially used for high-throughput screening of new cell wall-inhibiting compounds. [BMB reports 2010; 43(7): 468-473]

INTRODUCTION

Staphylococcus aureus causes various diseases in primates and non-primates. Administration of several potent antibiotics (such as methicillin, oxacillin, rifampin, ciprofloxacin and tetracyclines) since 1940s has not brought the *S. aureus*-mediated infections under control primarily because of the emergence and dissemination of multiple antibiotic-resistant *S. aureus* strains and the non-availability of an effective vaccine (1, 2). The glycopeptide antibiotics, once found to be very effective against multi drug-resistant strains, could not be administered for long as *S. aureus* strains with resistance to vancomycin and the related antibiotics have emerged across the world lately (3). To date, staphylococcal resistances to linezolid, daptomycin and tigecycline are low but these compounds have some serious limitations (4). Recently, a few new compounds (e.g., ceftobiprole, telavancin, iclaprim, etc.) have been discovered that exhibit promising activity against most bacteria including *S. aureus* (4). As the new inhibitors are at various phases of development, additional antistaphylococcal compounds need to be screened or developed on a priority basis.

The *drp35* gene of *Staphylococcus aureus* encodes a cytoplasmic protein that possesses calcium-dependent lactonase activity (5, 6). Interestingly, *drp35* was induced by various cell wall-affecting antibiotics (such as β-lactams, bacitracin, fosfomycin and vancomycin) as well as by detergents that damage cell membrane (5, 7). Transcription from *drp35* promoter though seems to be induced by cell wall inhibitors has not been cloned and characterized yet.

Several recombinant bacterial strains have been constructed by fusing antibiotic-inducible promoters to reporter genes (for example, *lacZ*, *lux*, etc.) in the last two decades (8-16). Biological assay systems, developed with the above reporter strains, were suggested to be useful for screening novel compounds capable of inhibiting various macromolecular biosyntheses including cell wall biosynthesis. Bacterial enzymes involved in cell wall biosynthesis are considered attractive targets of drug discovery (17). Only a few *Staphylococcus aureus* reporter strains (14) have been constructed for screening cell wall-affecting compounds and the *drp35* promoter is yet to be utilized for this purpose. In this communication, we have reported the cloning of the *drp35* promoter from *Staphylococcus aureus* Newman and demonstrated its induction specifically by cell wall-affecting antibiotics. Our data also suggest that a 96-well microplate assay, developed with an engineered *Staphylococcus aureus* strain SAU1289 (harboring a chromosomally-integrated *drp35* promoter - *lacZ* transcriptional fusion), would be suitable for screening new cell wall inhibitors efficiently.

*Corresponding author. Tel: 91-33-2355-9416; Fax: 91-33-2355-3886; E-mail: subratasau@yahoo.co.in, sau@bic.boseinst.ernet.in
[#]These authors have contributed equally to work.

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RESULTS AND DISCUSSION

Cloning of the promoter of *S. aureus drp35*

The region immediately preceding the coding region of *drp35* might harbor its promoter (designated P_d) as *Staphylococcus aureus* carries *drp35* gene and its upstream gene in the divergent orientation (5). To confirm the above hypothesis, we generated a plasmid, p1288 (Fig. 1A), by cloning the putative P_d carrying region at the upstream of promoterless *lacZ* gene (encoding β-galactosidase) in p1287 (see Materials and Methods for details). SAU1287 and SAU1288 strains, constructed by transforming *S. aureus* RN4220 with p1287 and p1288, respectively, were grown on trypticase soy agar supplemented with MUG (4-methylumbelliferyl-β-D-galactopyranoside). The cell colonies if express β-galactosidase from the resident plasmid would generate 4-methylumbelliferone from MUG (18). As 4-methylumbelliferone fluoresces in the presence of UV light, colonies synthesizing this compound would appear as fluorescent colonies under UV light. Upon exposure to UV light, colonies of SAU1288 were indeed fluorescent, whereas, SAU1287 colonies did not fluoresce under identical conditions (Fig. 1B), indicating the presence of a promoter at the upstream of the *drp35* coding region.

Cell wall-affecting antibiotics induce P_d

To see whether P_d in SAU1288 retained the antibiotic- in-

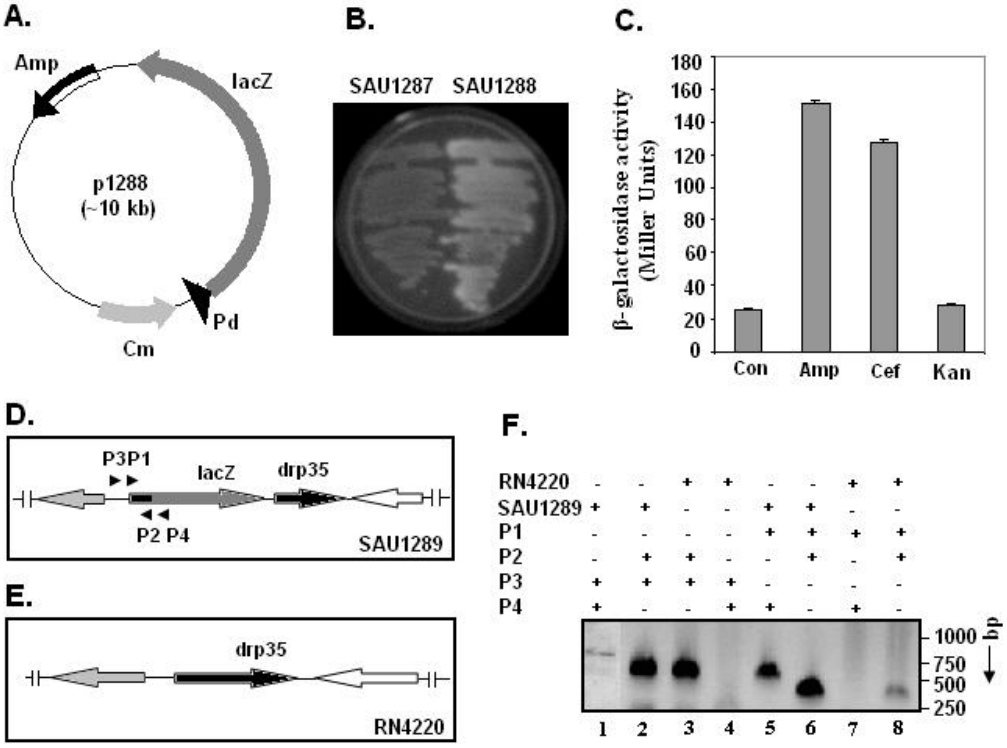

Fig. 1. Cloning and characterization of the *S. aureus drp35* promoter. (A) Physical map of plasmid p1288. Construction of p1288, which carries the *drp35* promoter-*lacZ* transcriptional fusion, is described in Materials and Methods. Abbreviations: Amp, ampicilin resistance gene; *lacZ*, β-galactosidase encoding gene, P_d, *drp35* promoter and Cm, chloramphenicol resistance gene. (B) Photograph shows the growth of SAU1287 and SAU1288 strains on trypticase soy agar supplemented with MUG and chloramphenicol. See text for details. (C). Estimation of β-galactosidase levels in SAU1288 under different conditions. SAU1288 cells were grown in nutrient broth (containing chloramphenicol) to log phase. Culture aliquots were exposed separately to 0.5 MIC equivalents of ampicillin (Amp), cefalothin (Cef) and kanamycin (Kan) for 30 min followed by the estimation of β-galactosidase levels in all aliquots by a standard method (19) using ONPG (*o*-nitrophenyl-β-D-galactopyranoside) as the substrate. 'Con' indicates β-galactosidase level in the antibiotic-untreated SAU1288 culture aliquot. The error bars indicate standard deviations (n=2). Schematic maps of the *drp35* locus and neighboring regions in SAU1289 (D) and in RN4220 (E) are presented. The genes immediately upstream and downstream of *drp35*, and *lacZ* are represented by arrows. The black bar at the end of *lacZ* denotes P_d. Different primers (P1-P4) including their locations are indicated by arrowheads. Primers were used to confirm the insertion of *lacZ* in *drp35* locus of SA1289 (see below). Maps were not drawn according to scale. (F) Analysis of the PCR-made DNA fragments. Amplification reactions were carried out using SAU1289 or RN4220 chromosomal DNA as template and primer pairs P1 & P2, P1 & P4, P3 & P2, and P3 and P4. The resulting DNA fragments were analyzed by 1% agarose gel electrophoresis. Lanes 1-8 contain the DNA fragment(s) those were amplified from chromosomal DNAs of the indicated strains and the primer pairs. Sizes of marker DNA fragments (in bp) are listed to the right of the figure.

ducible property, we exposed SAU1288 culture aliquots to subinhibitory concentrations of ampicillin, cefalothin, and kanamycin, separately, for 30 min followed by measuring β-galactosidase levels in the aliquots as described by Miller (19). The levels of β-galactosidase in the ampicillin- and cefalothin-treated aliquots were found about ~5-6 fold higher than those in the kanamycin-treated or antibiotic-untreated cultures (all P values are < 0.006; Fig. 1C), indicating that P_d is induced by ampicilin and cefalothin but not by kanamycin.

Construction of a recombinant *S. aureus* strain harboring single copy P_d-*lacZ* transcriptional fusion

To study the effects of different antibiotics on *drp35* promoter more precisely, a recombinant *S. aureus* strain (designated SAU1289; Fig. 1D) was constructed by transforming p1289 (see Materials and Methods for details) to *S. aureus* RN4220 (Fig. 1E) according to the standard procedure (16). The P_d-*lacZ* transcriptional fusion in p1289 was expected to be integrated into the RN4220 chromosome as this plasmid lacks an *S. aureus*-specific origin of replication. To determine whether P_d-*lacZ* cassette was integrated into the *drp35* locus by homologous recombination, a comparative analysis was made among the PCR-generated DNA fragments from SAU1289 and RN 4220 chromosomal DNAs using primer pairs P1 & P2, P1 & P4, P3 & P2, and P3 & P4. As shown in Fig. 1F, ~800 bp (lane 1) and ~ 620 bp (lane 5) DNA fragments were amplified from SAU1289 DNA using P3 & P4 and P1 & P4, respectively, whereas no PCR product was produced from RN4220 DNA by the same primer pairs. In contrast, DNA fragments of equal sizes were generated from both SAU1289 and RN4220 DNAs by the other primer pairs (lanes 2, 3, 6, and 8). Amplification of the above types of DNA fragments from SAU1289 and RN4220 indicates that former strain carries *lacZ* at the downstream of the *drp35* promoter. Additional studies revealed that SAU1289 grows similarly to RN4220 and stably maintains the P_d - *lacZ* cassette in the absence of tetracycline (data not shown). SAU1289 also formed blue colonies on trypticase soy agar supplemented with X-Gal and exhibited induced expression of β-galactosidase in the presence of ampicillin (data not shown).

Development of an agarose-based assay with SAU1289

To demonstrate the antibiotic-mediated induction of P_d directly, we developed an agarose-based assay with SAU1289 and MUG according to a standard procedure (see Materials and Methods for details). Under the assay conditions, SAU1289 cells immediately around the zone of inhibition will appear as a 'fluorescent ring' if inhibitory antibiotic induces β-galactosidase expression. In SAU1289 background, fluorescent rings were indeed observed around the zones of inhibition formed by ciprofloxacin, rifampicin, ampicillin, vancomycin, bacitracin, and cefalothin (Fig. 2A). In contrast, no prominent fluorescent rings were detected around the zones of inhibition produced by trimethoprim and chloramphenicol. Erythromycin,

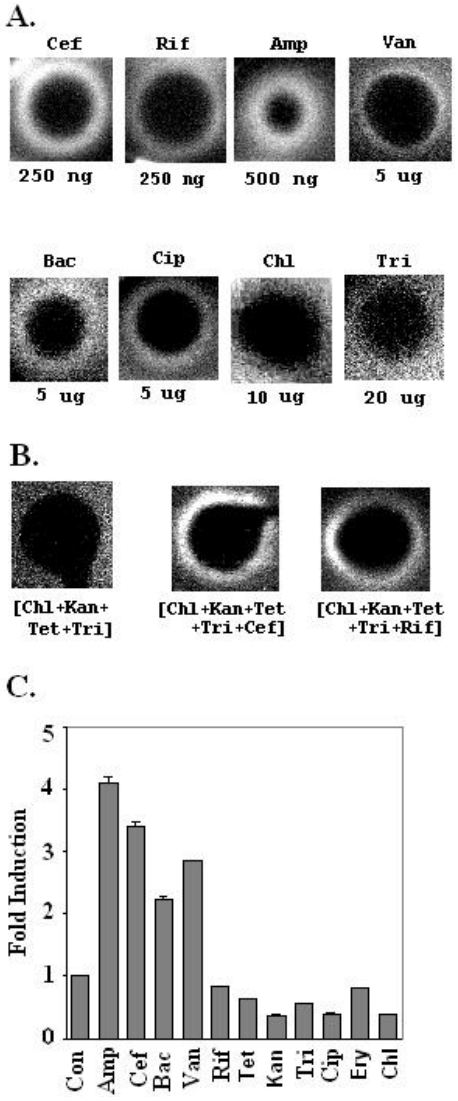

Fig. 2. Expression of β-galactosidase in SAU1289. (A) Agarose-based assay. The assay was developed with SAU1289 and performed according to the procedure described in the text. The amount of antibiotic added to paper disc is shown at the bottom of each picture. Abbreviations: Amp, ampicillin; Bac, bacitracin; Chl, chloramphenicol; Cip, ciprofloxacin, Cef, cefalothin; Rif, rifampicin; Tet, tetracycline; Tri, trimethoprim; and Van, vancomycin. (B) Agarose-based assay in the presence of multiple antibiotics. Assay was performed as described above except that 4 to 5 antibiotics (indicated) were added together to a paper disc. Five hundred nanogram of rifampicin or cefalothin along with 1 μg each of the other antibiotics were added to the paper disc. Abbreviations used for antibiotics are the same as described above. (C) Estimation of β-galactosidase levels in SAU1289. The β-galactosidase level in each of the indicated antibiotic-treated SAU1289 culture aliquots was determined by a standard method (19) using ONPG (*o*-nitrophenyl-β-D-galactopyranoside) as the enzyme substrate. 'Con' indicates β-galactosidase level in the antibiotic-untreated SAU1289 culture aliquot. Fold induction was measured by dividing the β-galactosidase level in the antibiotic-treated culture with the β-galactosidase level in the control culture. The error bars indicate standard deviations ($n \geq 2$).

tetracycline, and kanamycin also did not induce P_d (data not shown). The data together suggest that the above agarose-based assay involving SAU1289 and MUG may be useful for screening new antistaphylococcal agents including cell wall-affecting antibiotics, quinolones and rifampicin. Additional agarose-based assay indeed revealed that cefalothin or rifampicin, when mixed with tetracycline, kanamycin, trimethoprim and chloramphenicol, can also induce the formation of similar fluorescent rings by SAU1289 (Fig. 2B).

Sublethal concentrations of cell wall-affecting antibiotics induce P_d

Induction of P_d by rifampicin and ciprofloxacin (as described above) was quite surprising as previous workers noticed the induction of *drp35* only with cell wall-affecting antibiotics (7). This might have happened due to the longer exposure of the SAU1289 cells to the lethal concentrations of antibiotics in the agarose-based assay. To confirm the above hypothesis, we exposed aliquots of a nutrient broth-grown SAU1289 culture to 0.5 MIC equivalents of eleven representative antibiotics (mentioned above) separately, for 30 min followed by the estimation of the β-galactosidase levels in all aliquots according to Miller (19). The β-galactosidase levels in the ampicillin, cefalothin, vancomycin and bacitracin-treated cells were found to be ~2-5 fold higher than those in the rifampicin, ciprofloxacin, and trimethoprim-treated cells or in cells grown in the absence of any antibiotic (all P values are < 0.025; Fig. 2C). Exposure of protein synthesis inhibitors also did not induce β-galactosidase expression in the liquid medium-grown SAU1289 cells. The data together indicate that cell wall-affecting antibiotics specifically induce the *drp35* promoter.

Development of a microtiter plate assay with SAU1289

To determine whether SAU1289 could be employed in the large scale screening of antistaphylococcal compounds, we developed a microtiter plate-based assay according to a standard procedure (see Materials and Methods for details) using 100 μl SAU1289 cell aliquots and MUG as substrate. As shown in Fig. 3, β-galactosidase levels in SAU1289 cells were increased about 5-9 fold when the cells were exposed to 0.25 MIC equivalents of ampicillin, cefalothin, vancomycin or bacitracin (all P values are < 0.0003). Reporter enzyme levels were enhanced gradually in the presence of higher MICs of most cell wall-affecting antibiotics. Levels of β-galactosidase became nearly static at antibiotic concentrations greater than 1 MIC for all antibiotics. Contrary to the above, sublethal concentrations of rifampicin, chloramphenicol, erythromycin, and ciprofloxacin only marginally induced P_d in the microtiter plate format. The data together suggest that the above microtiter plate assay could be potentially used for the high-throughput screening of new cell wall-affecting compounds if the concentrations of these agents in the compound library are equivalent to 0.25 MIC and higher. Despite this restriction, strain SAU1289 appears to be more sensitive than the previously reported *S. aureus* reporter strains (14) constructed for similar purpose.

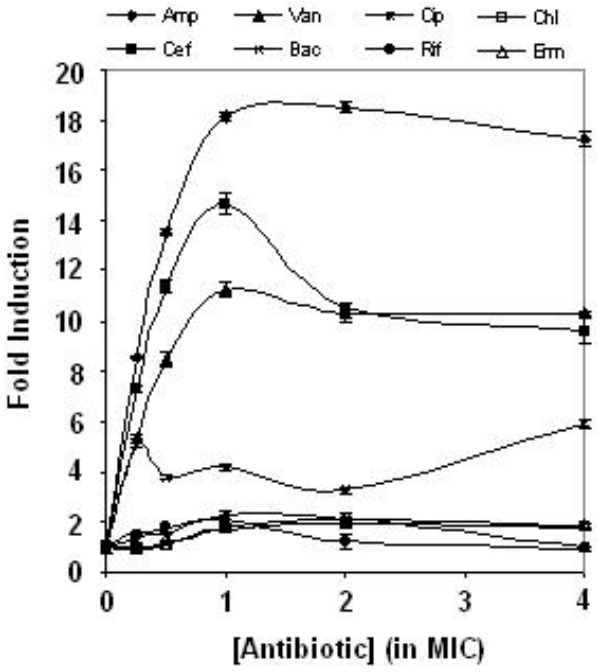

Fig. 3. Microtiter plate assay. SAU1289 culture aliquots were grown in the wells of a 96-well plate containing different MIC equivalents of ampicillin, cefalothin, vancomycin, bacitracin, rifampicin, ciprofloxacin, erythromycin (Erm), and chloramphenicol (Chl) followed by the estimation of β-galactosidase levels in all the aliquots according to Chanda *et al.* (16). The β-galactosidase level in the antibiotic-untreated culture aliquot was determined by a similar manner. Fold induction was estimated by the same way as described in Fig. 2C. Error bars indicate standard deviations of induction ratios measured from 3 wells.

MATERIALS AND METHODS

Bacterial strains and plasmids

Most plasmids and bacterial strains used here were reported previously (13). All *S. aureus* strains were grown in trypticase soy broth or nutrient broth. *Escherichia coli* DH5α was grown in Luria-Bertani broth. Antibiotics were added to growth media whenever needed. Minimum inhibitory concentrations (MICs) of different antibiotics for RN4220 were measured by a standard method (13).

Molecular biological techniques

All basic molecular biological techniques such as plasmid isolation, restriction enzyme digestion, agarose gel electerophoresis, DNA ligation, competent *E. coli* cell preparation, plasmid DNA transformation, polymerase chain reaction (PCR), chromosomal DNA isolation from *S. aureus*, electroporation of plasmids to *S. aureus*, etc. were performed using the standard

procedures (13, 20, 21).

Construction of plasmids and strains

The putative *drp35* promoter region was amplified by *Pfu* polymerase (Qiagen, Germany) using *S. aureus* Newman chromosomal DNA as the template and primers P1 (5′CTGCAGTT GATAGTTCATAGG) and P2 (5′GGATCCGATCAAAGTTCAA TCC). The resulting 409 bp DNA fragment was cloned into a T vector (Genei, India) after treatment with *Taq* polymerase and dATP (20). One of the recombinant T vectors, carrying no mutations in the cloned DNA insert, was selected and named p1284. The 409 bp *Bam*HI-*Pst*I DNA fragment from p1284 that carries the putative *drp35* promoter was subcloned into p1287 [a pLI50 (22) derivative harboring the promoterless *lacZ* gene from pAZ106 (23)] to generate p1288 (Fig. 1A). *S. aureus* SAU1287 and SAU1288 were generated by transforming p1287 and p1288 to *S. aureus* RN4220 separately.

The plasmid p1289 was generated by subcloning the 409 bp *Bam*HI-*Pst*I DNA fragment of p1284 into the identical sites of p1251 (13). Using p1289, a *S. aureus* RN4220 derivative (designated SAU1289; Fig. 1C) was constructed according to Chanda *et al*. (13). The insertion of the P_{σ}-*lacZ* transcriptional fusion into the SAU1289 chromosome was confirmed by PCR using primers P1, P2, P3 (5′CATCGGCATGCAT ATGTG) and P4 (5′TCGCTATTACGCCAGCTG). Based on the sequence of *NWMN_2586* gene (annotated as *drp35*) of *S. aureus* Newman (NCBI, USA) and its upstream region, the oligonucleotides P1, P2, and P3 were designed. P4 was designed based on the N-terminal end sequence of the *E. coli* (http://genolist.pasteur.fr/colibri) *lacZ* gene.

Agarose-based assay

Agarose-based assay was performed according to Chanda *et al*. (13). Briefly, a mixture of nutrient broth-grown SAU1289 cell culture and molten top agarose (nutrient broth medium + 0.6% agarose) was poured onto nutrient broth hard agarose. Sterile paper disks (diameter 0.5 cm) were placed on the solidified top agarose and soaked with an appropriate volume of antibiotic solution. The disks were removed after 16-18 h incubation of the plate at 37°C followed by flooding the plate with a solution containing molten agarose and MUG. After 30 min incubation in the dark at 25°C, plates were exposed to the long wave-length UV light and photographed.

ONPG assay

To study the effects of antibiotics on the β-galactosidase expression in SAU1288 or SAU1289, nutrient broth-grown cultures ($OD_{590} \approx 0.6$) were divided into several 5 ml aliquots. One aliquot was grown continuously in the absence of antibiotic at 37°C, whereas, each of the remaining aliquots were grown in the presence of 0.5 MIC of a specific antibiotic at the same temperature. After 30 min of growth, β-galactosidase levels in all culture aliquots were determined by a standard procedure (19) using ONPG (o-nitrophenyl-β-D-galactopyranoside) as the substrate.

Microtiter plate assay

Using MUG as the substrate, the β-galactosidase levels in a 96-well (black) microtiter plate-grown SAU1289 cell cultures were determined according to a standard procedure (13). Briefly, 100 μl aliquots of nutrient broth-grown SAU1289 culture ($OD_{620} \approx 0.5$) were added to wells containing an appropriate amount of antibiotic. After 3 h growth at 37°C, 5 μl of 0.1% MUG solution was added to each well and the plate was incubated in the dark for an hour. Using a fluorescence plate reader (PolarStar Optima, BMG LabTechnologies, Germany), the fluorescence intensity in each well was measured at 460 nm after excitation at 355 nm. Immediately, culture was pulled out from each well followed by the determination of its optical density at 620 nm. Similarly, fluorescence intensity and the optical density of SAU1289 cells grown in the absence of antibiotic were measured. Fluorescence of growth medium and MUG were deducted from the fluorescence values recorded above. Using the fluorescence values of different concentrations of 4-Methyl umbelliferone, the concentration of hydrolyzed MUG (catalyzed by SAU1285) as well as the specific activity of β-galactosidase in each well was determined as previously reported (16).

Statistical analysis

The β-galactosidase enzyme levels determined from the ONPG or microplate assay were analyzed by MS Excel using a paired Student's *t* test. The P values less than 0.05 were considered significant.

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REFERENCES

1. Draghi, D. C., Sheehan, D. F., Hogan, P. and Sahm, D. F. (2006) Current antimicrobial resistance profiles among methicillin-resistant *Staphylococcus aureus* encountered in the outpatient setting. *Diagn. Microbiol. Infect. Dis.* **55**, 129-133.
2. Feiz, V. and Redline, D. E. (2007) Infectious scleritis after pars plana vitrectomy because of methicillin-resistant *Staphylococcus aureus* resistant to fourth-generation fluoroquinolones. *Cornea.* **26**, 238-240.
3. Howden, B. P., Davies, J. K., Johnson, P. D., Stinear, T. P. and Grayson, M. L. (2010) Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and

clinical implications. *Clin. Microbiol. Rev.* **23**, 99-139.
4. Stryjewski, M. E. and Corey, G. R. (2009) New treatments for methicillin-resistant *Staphylococcus aureus*. *Curr. Opin. Crit. Care.* **15**, 403-412.
5. Murakami, H., Matsumaru, H., Kanamori, M., Hayashi, H. and Ohta, T. (1999). Cell wall-affecting antibiotics induce expression of a novel gene, *drp35*, in *Staphylococcus aureus*. *Biochem. Biophys. Res. Commun.* **264**, 348-351.
6. Tanaka, Y., Morikawa, K., Ohki, Y., Yao, M., Tsumoto, K., Watanabe, N., Ohta, T. and Tanaka, I. (2007) Structural and mutational analyses of *drp35* from *Staphylococcus aureus*: a possible mechanism for its lactonase activity. *J. Biol. Chem.* **282**, 5770-5780.
7. Morikawa, K., Hidaka, T., Murakami, H., Hayashi, H. and Ohta. T. (2005) Staphylococcal *drp35* is the functional counterpart of the eukaryotic PONs. *FEMS. Microbiol. Lett.* **249**, 185-190.
8. Osburne, M. S., Maiese, W. M. and Greenstein, M. (1993) An assay for the detection of bacterial DNA gyrase inhibitors. *J. Antibiot.* **46**, 1764-1766.
9. Ulijasz, A. T, Grenader, A. and Weisblum, B. (1996) A vancomycin-inducible *lacZ* reporter system in *Bacillus subtilis*: induction by antibiotics that inhibit cell wall synthesis and by lysozyme. *J. Bacteriol.* **178**, 6305-6309.
10. Bianchi, A. A. and Baneyx, F. (1999) Stress responses as a tool to detect and characterize the mode of action of antibacterial agents. *Appl. Environ. Microbiol.* **65**, 5023-5027.
11. Tenhami, M., Hakkila, K. and Karp, M. (2001) Measurement of effects of antibiotics in bioluminescent *Staphylococcus aureus* RN4220. *Antimicrob Agents Chemother.* **45**, 3456-3461.
12. Shapiro, E. and Baneyx, F. (2002) Stress-based identification and classification of antibacterial agents: second-generation *Escherichia coli* reporter strains and optimization of detection. *Antimicrob. Agents. Chemother.* **46**, 2490-2497.
13. Chanda, P. K., Ganguly, T., Das, M., Lee, C. Y., Luong, T. T. and Sau, S. (2007) Detection of antistaphylococcal and toxic chemicals by biological assay systems developed with a reporter *Staphylococcus aureus* strain harboring a heat shock promoter - *lacZ* fusion. *J. Biochem. Mol. Biol.* **40**, 936-943.
14. Steidl, R., Pearson, S., Stephenson, R. E., Ledala, N., Sitthisak, S., Wilkinson, B. J. and Jayaswal, R. K. (2008) *Staphylococcus aureus* cell wall stress stimulon gene-*lacZ* fusion strains: potential for use in screening for cell wall-active antimicrobials. *Antimicrob. Agents. Chemother.* **52**, 2923-2925.
15. Mesak, L. R., Miao, V. and Davies, J. (2008) Effects of sub-inhibitory concentrations of antibiotics on SOS and DNA repair gene expression in *Staphylococcus aureus*. *Antimicrob. Agents. Chemother.* **52**, 3394-3397.
16. Chanda, P. K., Mondal, R., Sau, K. and Sau, S. (2009) Antibiotics, arsenate and H_2O_2 induce the promoter of *Staphylococcus aureus cspC* gene more strongly than cold. *J. Basic Microbiol.* **49**, 205-211.
17. Sau, S., Chattoraj, P., Ganguly, T., Chanda, P. K. and. Mandal, N.C (2008) Inactivation of bacterial indispensable proteins by early/delayed early proteins of bacteriophages: implication in antibacterial drug discovery. *Curr. Protein Pept Sci.* **9**, 284-290.
18. Berg, J. D. and Fiksdal, L. (1988) Rapid detection of total and fecal coliforms in water by enzymatic hydrolysis of 4-methylumbelliferone-beta-D-galactoside. *Appl. Environ. Microbiol.* **54**, 2118-2122.
19. Miller, J. M. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor. N. Y.
20. Sambrook, J. and Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press, CSH, New York, USA.
21. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1998) *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc., USA.
22. Lee, C. Y. and Iandolo, J. J. (1986) Integration of staphylococcal phage L54a occurs by site-specific recombination: structural analysis of the attachment sites. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5474-5478.
23. Kemp, E. H., Sammons, R. L., Moir, A., Sun, D. and Setlow, P. (1991) Analysis of transcriptional control of the *gerD* spore germination gene of *Bacillus subtilis* 168. *J Bacteriol.* **173**, 4646-4652.