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Detection of Antistaphylococcal and Toxic Compounds by Biological Assay Systems Developed with a Reporter *Staphylococcus aureus* Strain Harboring a Heat Inducible Promoter - *lacZ* Transcriptional Fusion

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Previously it was reported that promoter of *groES-groEL* operon of *Staphylococcus aureus* is induced by various cell-wall active antibiotics. In order to exploit the above promoter for identifying novel antistaphylococcal drugs, we have cloned the promoter containing region (P_g) of *groES-groEL* operon of *S. aureus* Newman and found that the above promoter is induced by sublethal concentrations of many antibiotics including cell-wall active antibiotics. A reporter *S. aureus* RN4220 strain (designated SAU006) was constructed by inserting the P_g-*lacZ* transcriptional fusion into its chromosome. Agarose-based assay developed with SAU006 shows that P_g in single-copy is also induced distinctly by different classes of antibiotics. Data indicate that ciprofloxacin, rifampicin, ampicillin, and cephalothin are strong inducers, whereas, tetracycline, streptomycin and vancomycin induce the above promoter weakly. Sublethal concentrations of ciprofloxacin and ampicilin even have induced P_g efficiently in microtiter plate grown SAU006. Additional studies show for the first time that above promoter is also induced weakly by arsenate salt and hydrogen peroxide. Taken together, we suggest that our simple and sensitive assay systems with SAU006 could be utilized for screening and detecting not only novel antistaphylococcal compounds but also different toxic chemicals.

Keywords: Heat shock promoters, antibiotics, Reporter *S. aureus* Strain, biological assay system

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Introduction

Staphylococcus aureus remains the most common cause of community-acquired and nosocomial infections in almost all countries primarily because of the emergence and dissemination of *S. aureus* strains which are resistant to virtually all potent antibiotics such as methicillin, oxacillin, rifampin, ciprofloxacin, tetracyclines etc. (Tenover and Gaynes, 2000). Most methicillin-resistant *S. aureus* (MRSA) strains are resistant to multiple antibiotics, including fluoroquinolones and macrolides (Projan, 2000; Fraimow and Courvalin, 2000; Picazo *et al*., 2006; Draghi *et al*., 2006; Feiz and Redline, 2007). Only prevalence of vancomycin-resistant or linezolid-resistant MRSA is still low. There are reports of casualties from the infections caused by the above multiple drug-resistant strains and in some cases prevention needed exorbitant prices. To eradicate these antibiotic-resistant strains, novel antistaphylococcal drugs are to be discovered on a priority basis.

Bacterial heat and cold shock genes are induced by different antibiotics and toxic chemicals too (Van Bogelen and Neidhardt, 1990; Jiang *et al*., 1993; Ng *et al*., 2003; Shaw *et al*., 2003; Yamaguchi *et al*., 2003; Qiu *et al*., 2005; Lin *et al*., 2005). Bianchi and Baneyx (1999) constructed some reporter *E. coli* strains using heat and cold inducible promoters and showed that above promoters could be induced by sub-MIC concentrations of different antibiotics. Simple assay systems developed with the above strains were shown to be useful for screening promising antibacterial compounds efficiently. Such whole cell-based assay systems involving heat/cold inducible promoters and reporter gene have not been developed so far with other bacterial species.

Heat shock genes of *Staphylococcus aureus* were cloned and characterized at length (Qoronfleh *et al*., 1990; Ohta *et al*., 1993; Kuroda *et al*., 1999). It was shown that promoter of *groES-groEL* operon is transcriptionally regulated and induced not only by the elevated growth temperatures but also by

vancomycin, D-cycloserine, cephalothin, bacitracin, and oxacillin (Ohta *et al*., 1993; Singh *et al*., 2001). Here we report the agarose-based as well as the microplate-based assay systems with an engineered *S. aureus* strain harboring chromosomally-integrated *groES -groEL* promoter (P_g)-*lacZ* reporter cassette. It was demonstrated that P_g in the recombinant *S. aureus* strain is induced by sublethal concentrations of different classes of antibiotics. Additional experiments have shown that P_g is also induced by an arsenate salt and hydrogen peroxide.

Materials and Methods

Antibiotics, salts, enzymes, kits, chemicals, and oligonucleotides. Antibiotics, chemicals and salts were purchased from Sigma, EMerck and HiMedia. Stock solutions of chloramphenicol, tetracycline and erythromycin were prepared in ethanol. Rifampicin was dissolved in methanol. Ampicillin, streptomycin sulfate, ciprofloxacin hydrochloride, bacitracin, spectinomycin, cephalothin sodium salt and vancomycin were dissolved in double-distilled water. Trimethoprim was dissolved in DMSO (SRL). All antibiotic stock solutions (each at concentration of 10 mg per ml) were filter sterilized. All salts (e.g., Na_2HAsO_4, As_2O_3, $Cu(SO_4)$, $Pb(NO_3)_2$, $CdCl_2$, $HgCl_2$, $NiCl_2$, $SbCl_3$, and $ZnCl_2$) solutions (each at final concentration of 1 M/N) were prepared in double-distilled water and sterilized by 0.2 μm filters (Millipore).

All restriction and modifying enzymes, PCR kit, T vector cloning kit were purchased from Genei, Fermentas and Roche. All fine chemicals including X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), 4-methyl umbelliferone (4-MU), and MUG (4-methylumbelliferyl-β-D-galactopyranoside) were purchased either from Sigma or from local companies. Stock solutions (0.1%) of MUG and 4-MU were prepared in 10% DMSO and DMSO, respectively.

Oligonucleotides P1 (5'CTGCAGATGCCCATAATCTTGTC), P2 (5'GGATCCGTCCAGTTCTCACTGC), P3 (5'TAGTCAGTTCTATGCCAC), and P4 (5'TCGCTATTACGCCAGC TG) (Fig. 2A) were purchased from Genei (India). The underlined sequences in P1 and P2 primers are the recognition sites of *Pst*I and *Bam*HI restriction enzymes, respectively. P1 and P2 were designed on the basis of sequence of *S. aureus* N315 (http://genolist.pasteur.fr/aureolist) *groES* and its upstream region. P3 sequence is located at further upstream of P1 sequence. P4 was designed from sequence encoding N-terminal end of *E. coli* (http://genolist.pasteur. fr/colibri) β-galactosidase reporter protein.

Bacterial strains and plasmids. All bacterial strains and plasmids used in this study were listed in Table 1. *Escherichia coli* DH5α used as a host strain for plasmid constructions was grown in Luria-Bertani broth (HiMedia, India). All *S. aureus* strains namely, Newman, RN4220, CYL316, and SAU006 were cultivated in trypticase soy broth (TSB, Difco) supplemented with/without appropriate antibiotic. Strain SAU006 was also grown in nutrient broth (Difco). Strains RN4220 and CYL316 were used as recipients of plasmid electrotransformation.

Minimum inhibitory concentrations (MICs) of different antibiotics/salts for RN4220 were measured by a modified method of Shapiro and Baneyx (2002). Briefly, aliquots containing 2×10^5 cells were inoculated to 4 ml of TSB /NB supplemented with serial dilutions of an antibiotic. Cultures were grown for 24 h at 37°C followed by the determination of their optical densities at 590 nm (OD_{590}). Concentration of an antibiotic that almost inhibited the growth of culture ($OD_{590} < 0.01$) was taken as its MIC. All experiments were carried out at least in duplicate (data not shown).

Molecular biological techniques. All basic molecular biological techniques such as plasmid isolation, restriction enzyme digestion, agarose gel electerophoresis, DNA ligation, competent *E. coli* cells preparation, plasmid DNA transformation, labeling of DNA fragment by radioactive [α-^{32}P] dATP (purchased from BARC), Southern hybridization, polymerase chain reaction (PCR), protein estimation, etc. were performed by standard procedures (Ausubel *et al*., 1998; Sambrook and Russell, 2001). Chromosomal DNAs from *S. aureus* Newman, RN4220 and SAU006 were isolated by a standard method (Sau *et al*., 1997). Electroporation of plasmids to *S. aureus* were carried out by a standard procedure (Schenk and Laddaga, 1992). A DNA fragment was amplified by PCR using RN4220 genomic DNA as template and oligonucleotides P1 and P2. It was labeled with [α-^{32}P] dATP and used as probe in Southern hybridization experiment.

Cloning of promoter of *S. aureus groES-groEL operon* into multiple and single copy vectors. The heat-inducible promoter (P_g) expressing *groES* and *groEL* genes of *S. aureus* strain Newman was cloned by a standard procedure. Briefly, a 341 bp DNA fragment carrying the upstream region of *groES* gene was amplified by *Pfu* polymerase (Qiagen, Germany) using *S. aureus* Newman chromosomal DNA as template and primers P1 and P2. The resulting DNA fragment was modified with Taq polymerase & dATP and cloned into T vector (Genei, India) according to the manufacturer's protocol. On the basis of restriction enzyme digestion and DNA sequencing analyses (data not shown), one of the recombinant T vectors, which carries P_g sequence with no mutation was selected and named p1126. The 341 bp *Bam*HI-*Pst*I DNA fragment of p1126 which carries P_g was subcloned at the upstream of *xylE* reporter gene (encoding catechol 2,3-dioxygenase) of pLL38 (Table 1) in order to generate p1139. The plasmid p1139 was transformed to *S. aureus* RN4220 and a healthy transformant which carried the above plasmid and turned yellow after catechol spray (data not shown) was chosen for further study.

To study the expression from P_g in single copy environment, a vector p1251 was constructed by ligating 4149 bp *Xba*I-X*mn*I fragment (carrying *lac*Z reporter gene) of pAZ106 (donated by Dr Anne Moir, University of Sheffield, UK; Kemp *et al*., 1991) with *Xba*I-*Sma*I double digested pLL26 (Table 1). Next the 341 bp *Pst*I-*Bam*HI DNA fragment of p1139 was subcloned to P*st*I and *Bam*HI double digested p1251. The newly constructed plasmid vector was designated p1252 (Table 1). A RN4220 derivative namely SAU006 (Table 1) was constructed using p1252 (see below for details).

XylE assay. To determine xylE activities in RN4220 (p1139) (Table 1) cells, a log phase RN4220 (p1139) cell culture was divided into 2 equal parts. Specific amount (equivalent to 1/2 MIC) of an antibiotic was added to one part and both parts were continued to grow at 37°C. At different time intervals, equal

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
S. aureus RN4220	8325r$^-$	Kreiswirth *et al.* (1983)
S. aureus Newman	CP5 strain	Laboratory stock
CYL316	A cm^r RN4220 derivative	Lee *et al.* (1991)
E. coli DH5α	*supE*44Δ*lac*U169 (Φ80*lacZ*Δ M15) *hsd*R17 *recA1 end*A1 *gyr*A96 *thi*-1 *rel*A1	Laboratory stock
SAU006	RN4220 (*groES* :: *lacZ*) tet^r cm^s	This study
RN4220 (p1139)	RN4220 carrying plasmid p1139, tet^r	This study
Plasmids		
T vector	Cloning vector	Genei (India)
pAZ106	An *E. coli-B. subtillis* shuttle vector	Kemp *et al.* (1991)
pLL38	A pLL35 (Luong and Lee, 2006) derivative constructed by replacing *blaZ* gene with *xylE* reporter gene	This study
pLL26	A single copy integration vector for *S. aureus* harboring L54a *attP* site	This study
p1126	T vector harboring *S. aureus groES/EL* promoter (P_g)	This study
p1139	pLL38 carrying P_g	This study
p1251	pLL26 plus *lacZ* gene from pAZ106	This study
p1252	p1251 derivative carrying P_g-*lacZ* fusion	This study

volumes of culture aliquots were withdrawn from each culture and xylE levels in all aliquots were measured by the procedure as described earlier (Zukowski *et al.*, 1983).

Agarose-based assay. Agarose-based assay was performed according to the modified procedure of Osburne *et al.* (1993). Briefly, 200 µl of saturated SAU006 cells culture (grown in NB) was mixed well with 3 ml of molten top agarose (nutrient broth medium + 0.6% agarose) and poured onto nutrient broth hard agarose. Sterile paper (Whatman 3 MM) disks (each having diameter of 5 mm) were placed on the solidified top agarose and an appropriate volume of antibiotic/salt solution was added to each disk aseptically. After overnight incubation at 37°C, disks were removed and 10 ml of 0.6% molten agarose containing 1 ml of 0.1% MUG solution was poured onto the plate. After 30 min incubation in dark at room temperature, fluorescent ring around the zone of inhibition was investigated under long wave-length UV light and photographed by a digital camera.

β-Galactosidase assay. To see the heat induction of β-galactosidase in SAU006 cells, a log phase SAU006 cell culture was divided into 2 equal parts. One part was grown at 42°C and another part was continued to grow at 37°C. At different time intervals, equal volumes of culture aliquots were withdrawn from each culture and β-galactosidase levels in all aliquots were determined by using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate (Miller, 1972).

The β-galactosidase levels in small volumes of SAU006 cell cultures were determined by a modified MUG (fluorogenic substrate for lacZ) assay method of Youngman (1990). Briefly, SAU006 cells culture was grown at 37°C in nutrient broth to mid-log phase (OD_{620} ≈ 0.5) and 100 µl cell aliquots were added to the wells of a 96 well black F96 microtiter plate (Nunc, Denmark), each of which already contained 2-3 µl of appropriate antibiotic or salt. After 3 h [18 h for arsenate salt] of incubation at 37°C (with occasional shaking), 5 µl of 0.1% MUG solution was added to each well and the plate was shifted to dark room. After 1 h incubation at room temperature, fluorescence intensity at each well was measured by a fluorescence plate reader (PolarStar Optima, BMG LabTechnologies, Germany) using excitation wavelength of 355 nm and fluorescence wavelength of 460 nm. Immediately 100 µl cell culture was withdrawn from each well and its optical density measured at 620 nm. Fluorescence levels of known concentrations of 4-methyl umbelliferone and SAU006 grown in absence of antibiotics were also determined by above plate reader. From standard curve of fluorescence of 4-Methyl umbelliferone versus its concentration, concentration of hydrolyzed MUG (catalyzed by SAU006) in each well was estimated. The specific activity of β-galactosidase was determined by dividing concentration of hydrolyzed MUG with the cell density (at OD_{620}) in a well.

Results and discussion

***S. aureus groES-groEL* promoter (P_g) is induced by various antibiotics.** To study the effects of different antibiotics on *S. aureus groES-groEL* promoter P_g, plasmid p1139 carrying P_g -*xylE* transcriptional fusion was constructed by the procedure as described in Materials and Methods. It was found that xylE levels in *S. aureus* RN4220 (p1139) cells were increased as expected when cells were grown separately with sublethal concentrations (equivalent to 1/2 MIC) of ampicillin and vancomycin (data not shown) or when cells were shifted to 42°C from 37°C (Fig. 1). To see the effect of other antibiotics on P_g, above RN4220 cells were grown separately with sub-inhibitory concentrations of ciprofloxacin, streptomycin, and trimethoprim, and xylE levels in all cell aliquots withdrawn at

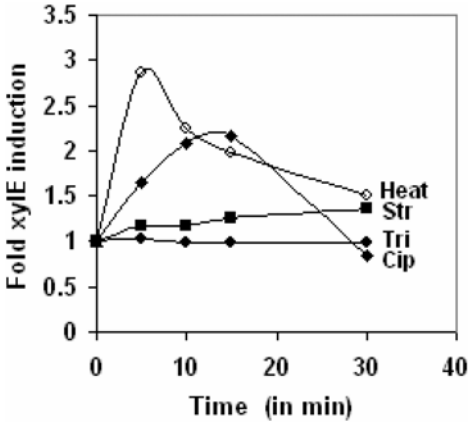

Fig. 1. Induction kinetics of promoter P_g by various antibiotics. The xylE levels in RN4220 (p1139) cell aliquots were measured by the procedure as described in Materials and Methods. Fold of P_g induction was determined by dividing the xylE level in antibiotic-treated or 42°C grown culture aliquot with xylE level in antibiotic-untreated or 37°C grown culture aliquot collected at the same time point. Abbreviations: cip, ciprofloxacin; Tri, trimethoprim; Str, streptomycin. The xylE levels in antibiotic/ heat induced cultures were measured 3-4 times. One representative data for each inducer was presented here.

different time intervals were estimated by standard method. It was found that different antibiotics regulated xylE expression from P_g by distinct manner (Fig. 1). While xylE expression was induced to about 2 fold within 15 min of addition of ciprofloxacin, streptomycin induced the xylE level slowly with time. The order of P_g induction with antibiotics is ciprofloxacin > ampicillin > vancomycin > streptomycin. Trimethoprim (Fig. 1), spectinomycin and chloramphenicol (see below) on the other hand did not affect transcription from P_g significantly. None of the above antibiotics induces xylE expression like that of heat which showed ~3 fold induction within 5 min of shifting of RN4220 (p1139) cell culture from 37°C to 42°C. The data suggest that P_g is not only induced by cell-wall antibiotics but also by the antibacterial agents belonging to quinolones, aminoglycosides, etc.

Construction of a recombinant *S. aureus* strain harboring single copy P_g-*lacZ* fusion. Promoter of *groES-groEL* operon is induced by various antibiotics indicating that it could be exploited in developing *S. aureus*-based biological assay system for screening novel antistaphylococcal compounds. To make *S. aureus* RN4220 extremely sensitive for screening drugs, plasmid p1252 was transformed to *S. aureus* CYL316 (Lee *et al*., 1991; Table 1) for facilitating integration of its P_g-*lacZ* cassette into the lipase gene of above strain by site-specific recombination. To our surprise, none of the tetracycline resistant chloramphenicol sensitive transformants were lipase negative (data not shown) though they all carried P_g-*lacZ* cassette (as assessed by blue coloration of the transformants on trypticase soy agar supplemented with X-gal and 0.5% yeast extract). The data indicates that the above plasmid possibly might have been integrated into the *gro* locus of CYL316 by homologous recombination. If this happens, then *gro* locus of the above transformants might have a genetic map similar to that presented in Fig. 2A. To reveal the real architecture of *gro* locus in the transformants, PCR was carried out with chromosomal DNA from one transformant (designated SAU006) and primer pair P3 and P4 (Fig. 2A). As expected, a ~590 bp DNA fragment was amplified from SAU006 DNA by P3 and P4 (Fig. 2B, lane 1). In contrast, no fragment was generated when RN4220 DNA was amplified by above primers (Fig. 2B, lane 2). On the other hand, both RN4220 and SAU006 DNAs yielded DNA fragments of identical sizes when amplified with primers P1 and P2 (Fig. 2B, lanes 4 and 5). The data suggest that SAU006 carries *lacZ* at the downstream of P_g.

Additional analysis shows ~4.8 kb and ~1.29 kb DNA fragments when *Hinc*II-digested SAU006 DNA fragments were hybridized with ^{32}P labeled DNA fragment carrying P_g (Fig. 2C, lane 2). In contrast, only one ~1.48 kb DNA fragment appeared when *Hinc*II-digested RN4220 DNA fragments were hybridized with the above probe (Fig. 2C, lane 1). The data confirm that SAU006 was indeed generated by insertion of *lacZ* gene at the downstream of P_g by a single crossover event and its gene architecture at *gro* locus is identical to that presented in Fig. 2A. Additional study indicates that P_g in single copy is also induced by heat significantly (Fig. 2D). Further studies reveal that SAU006 grew similarly to that of RN4220 and did not loose its P_g-*lacZ* insert significantly even after 100 generation of growth in absence of tetracycline (data not shown).

Development of agarose-based assay with SAU006. Using *E. coli* or *B. subtilis* reporter strains, several hard agarose-based assay systems were developed for screening as well as determining the modes of actions of antibiotics (Kirsch *et al*., 1991; Osburne *et al*., 1993; Ulijasz *et al*., 1996; Bianchi and Baneyx, 1999). To see the effects of different antibiotics on promoter P_g easily, an agarose-based assay was also developed with SAU006 and MUG (see Materials and Methods for details). MUG was preferred over chromogenic lacZ substrate X-gal as former gave very less background on hard agarose. The lacZ catalyzed hydrolysis product of MUG (i.e., 4-Methylumbelliferone) fluoresces in presence of UV light. So if an antibiotic induces the expression of *lacZ* from P_g in SAU006, a fluorescent ring would be seen around its zone of inhibition under UV light. Photographs presented in Fig. 3 indeed show beautiful fluorescent rings around the zones of inhibitions formed by different classes of antibiotics on SAU006 background. While antibiotics like ciprofloxacin, rifampicin, ampicillin, and cephalothin were required in nanogram quantities to see fluorescent rings, microgram quantities of vancomycin, tetracycline, and streptomycin were needed to observe the same. This indicates that former set of antibiotics induce P_g strongly but later set of antibiotics are weak inducers.

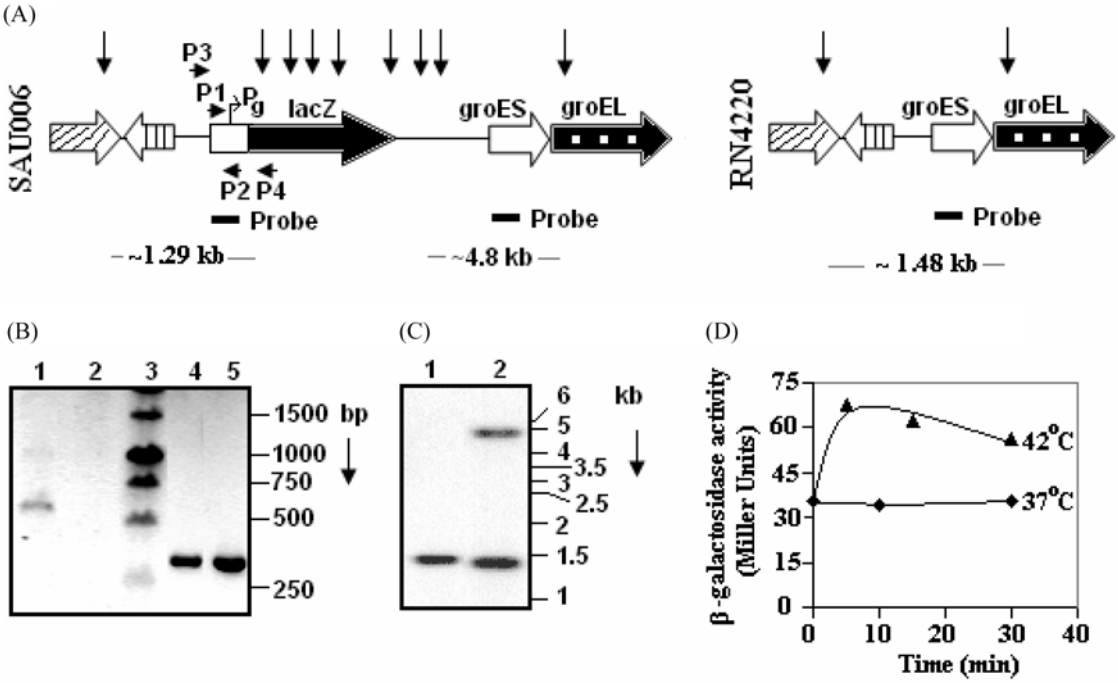

Fig. 2. Construction of *S. aureus* SAU006 carrying single copy P_g-*lacZ* fusion. SAU006 was constructed using p1252 (Table 1). (A) Schematic maps of *gro* loci and neighboring regions in SAU006 and in RN4220. The *gro* locus of SAU006 was confirmed by PCR and Southern hybridization (see below). Downward arrows indicate *Hinc*II restriction enzyme sites. Positions of *groES*, *groEL*, P_g, *lacZ*, two upstream genes of *groES* (represented by divergent arrows) are shown. Maps were not drawn according to scale. (B) PCR analysis. Amplification reactions were carried out using SAU006/RN4220 chromosomal DNA as template and primer pairs P4 & P3 or P1 & P2 and resulting DNA fragments were analyzed by 1% agarose gel electrophoresis. Lanes 1-2 contain DNA fragment (s) amplified from SAU006 and RN4220 DNA with primer pair P4 & P3, respectively. Lane 3 contains a marker DNA. Sizes of marker DNA fragments (in bp) are mentioned at the right side of gel picture. Lanes 4-5 contain DNA fragment (s) amplified from SAU006 and RN4220 DNA with primer pair P1 & P2, respectively. (C) Southern hybridization analysis. Hybridization was carried with a [^{32}P] labeled DNA probe (shown in panel A) according to the standard method as described above. Lanes 1-2 contain *Hinc*II-digested chromosomal DNAs from RN4200 and SAU006, respectively. At the right side of autoradiogram, sizes of maker DNA fragments (in kb) are shown. Locations of ~1.29 kb and ~4.8 kb SAU006 DNA fragments and ~1.48 kb RN4220 DNA fragment which hybridized with the above labeled probe are shown in panel A. (D) Heat induction kinetics of β-galactosidase in SAU006. The β-galactosidase activity versus time plot shows that there was instantaneous induction of β-galactosidase level when SAU006 culture was shifted from 37°C to 42°C. The whole experiment was performed 3 times. Data of one representative set was shown here.

Spectinomycin and chloramphenicol on the other hand did not induce P_g to a detection level. The data suggest that agarose-based assay system involving SAU006 and MUG may be useful for screening and detecting novel antistaphylococcal agents related to quinolones, cell-wall antibiotics, tetracyclines, aminoglycosides., etc.

Developing microtitre plate-based β-galactosidase assay with SAU006. In order to make SAU006 amenable to the microtitre-based screens capable of high-throughput screening of the antistaphylococcal compounds, we determined β-galactosidase levels in 100 µl aliquots of SAU006 cells culture according to the method as described in Materials and Methods. As shown in Fig. 4, there is about 6 fold induction of β-galactosidase level in SAU006 cells in presence of 1 MIC of ciprofloxacin. Reporter enzyme level in SAU006 cells also increases to nearly 12 fold at half MIC of ampicillin. In contrast, spectinomycin did not induce P_g in microtiter plate format too. Taken together, we suggest that above microtiter plate assay system could be utilized for screening and detecting novel antistaphylococcal compounds in high-throughput manner. The important characteristics of this assay system are that it may detect the antistaphylococcal compound even if present in very low concentration in compound library and provide information about the bioavailability of any potential antistaphylococcal compound. Although our assay system is unable to give any clue about the mode of action of any putative antistaphylococcal compound, it must be more sensitive than the traditional growth inhibition assay for screening antistaphylococcal compounds. Several such assay systems were developed in order to screen antimycobacterial compounds in high-throughput manner (Shawar *et al*., 1997; Collins *et al*., 1998; Deb *et al*., 2000; Maisetta *et al*., 2001; Zafer *et al*., 2001).

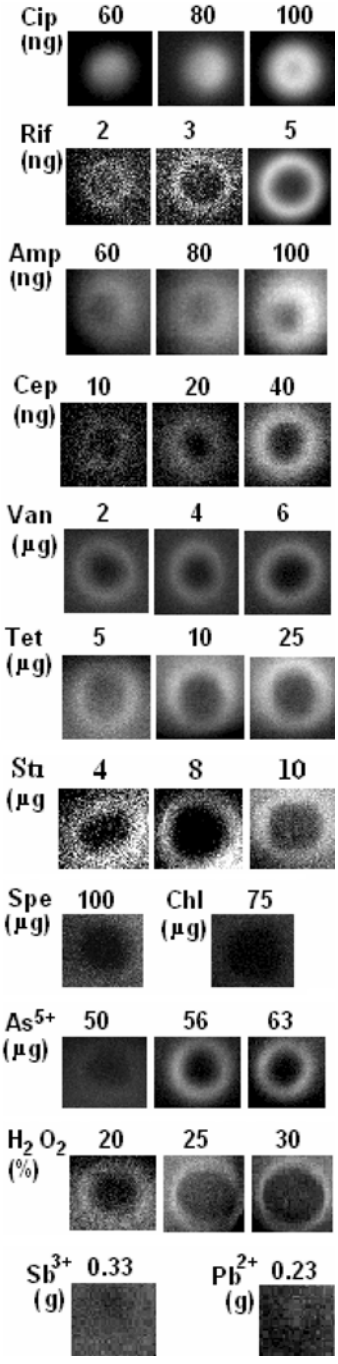

Fig. 3. Expression of lacZ in SAU006 grown on nutrient agar. The agarose-based assay procedure was described in Materials and Methods. Amount of antibiotic or salt added on paper disc is shown on the top of each picture. Fluorescent rings seen around the zone of inhibitions are formed by inducible antibiotics or toxic chemicals on SAU006 back ground. Spectinomycin, chloramphenicol, antimony chloride, and lead nitrate though inhibited growth of SAU006 did not induce P_g. Abbreviations: As^{5+}, sodium arsenate; Amp, ampicillin; Chl, chloramphenicol; Cip, ciprofloxacin, Cep, cephalothin; H_2O_2, hydrogen peroxide; Pb^{2+}, lead nitrate; Rif, rifampicin; Sb^{3+}, antimony chloride; Spe, spectinomycin; Tet, tetracycline, Str, streptomycin, and Van, vancomycin. See text for details.

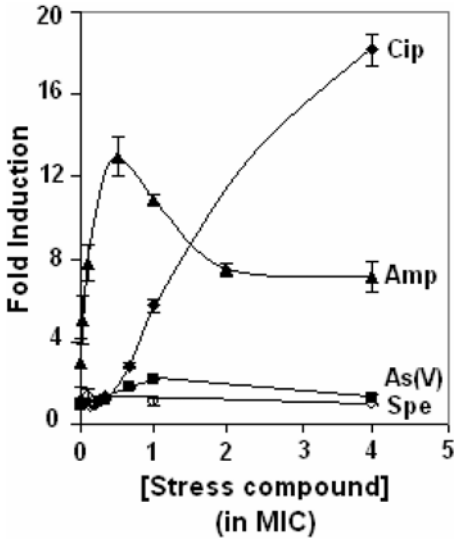

Fig. 4. Microtiter plate-based assay with SAU006. Aliquots (100 µl) of SAU006 cells culture (OD_{620} 0.5) were grown in wells with different MIC equivalents of antibiotic/salt or no antibiotic/salt and β-galactosidase levels in all aliquots were measured with MUG according to the method as described in Materials and Methods. Fold of P_g induction was determined by dividing the β-galactosidase activity in antibiotic/salt-treated culture aliquot with β-galactosidase activity in antibiotic/salt-untreated culture aliquot. Error bars correspond to the standard deviations of induction ratios measured from 3 wells.

Promoter P_g is also induced by arsenate salt and hydrogen peroxide. To see the effects of toxic chemicals on P_g, SAU006 cells were grown in presence of various salts followed by detection and quantification of β-galactosidase levels by the assay methods described above. As shown in Fig. 3, there are fluorescent rings around the zones of inhibition formed by microgram amounts of arsenate salt (As^{5+}) or higher concentrations of hydrogen peroxide on SAU006 background. The data indicate that both the toxic agents induce P_g weakly. Interestingly arsenite salt (As^{3+}) did not induce P_g to a detection level even at very high concentration (data not shown). Most other salts including lead nitrate and antimony chloride also did not elevate lacZ level in SAU006.

Significant induction of reporter enzyme was also seen when 100 µl aliquots of SAU006 cells culture were grown in microtitre plate with 1 MIC equivalent of arsenate salt (Fig. 4). The data therefore suggest that our assay systems developed with SAU006 could be utilized to detect hazardous toxic chemicals as well.

Conclusions

Simple and sensitive assay systems developed with an engineered S. *aureus* strain SAU006 here could be utilized for

screening and detecting novel antistaphylococcal compounds in high-throughput manner. In addition, these assay methods could also be used to detect hazardous chemical agents like arsenic and hydrogen peroxide.

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