THE USE OF β-GALACTOSIDASE GENE FUSIONS TO SCREEN FOR ANTIBACTERIAL ANTIBIOTICS

D. R. KIRSCH[a], M. H. LAI[a], J. MCCULLOUGH and A. M. GILLUM[b]

Department of Microbial Biochemistry and Genetics,
Squibb Institute for Medical Research,
P.O. Box 4000,
Princeton, New Jersey 08543-4000, U.S.A.

(Received for publication September 4, 1990)

The desirable features for a screening assay to detect antibacterial antibiotics include 1) high specificity for the desired antibiotic type 2) high sensitivity 3) lack of interference by other compounds likely to be associated with the antibiotic of interest and 4) ease of operation to allow a large number of samples to be tested. These characteristics are largely found in screens employing strains carrying fusions between antibiotic induced promoters and the structural genes for *Escherichia coli* β-galactosidase. Screens were designed based upon fusions with three antibiotic induced promoters: the tetracycline induced *tet*A/*tet*R promoter from transposon Tn10, the erythromycin induced promoter from the *Staphylococcus aureus erm*C erythromycin-resistance gene and the chloramphenicol induced promoter from the *S. aureus cat*86 chloramphenicol-resistance gene. Because there have been no reports of vancomycin induced resistance determinants, a Tn903 random gene fusion pool was screened to isolate a vancomycin induced gene fusion. This gene fusion was induced fairly specifically by glycopeptide antibiotics and the fusion was used as the basis for a glycopeptide screen.

Several years ago, ELESPURU and collaborators[1,2] described a screening protocol for antitumor agents. This method employed a strain of *Escherichia coli* which contained a λ phage lysogen carrying the *E. coli* gene for β-galactosidase. Lysogens of phage λ are induced by a wide variety of compounds which interact with DNA and interfere with DNA synthesis. The induction of phage λ had been developed over several years as a method to detect such activities (ref cited in 1). However, the use of a λ lysogen carrying the β-galactosidase gene made the assay faster, easier and more sensitive than previously described λ lysogen induction methods. This is due to the fact that β-galactosidase can be assayed very easily on plates with the use of chromogenic substrates. Subsequently, a related method (the SOS chromotest) to detect genotoxic agents was described. The SOS chromotest employs a gene fusion between the gene for β-galactosidase and the *E. coli sfi*A locus which is one of the genes of the SOS induction pathway and is known to be induced by a wide variety of genotoxic treatments.[3] The SOS chromotest is similar in sensitivity and selectivity to the *Salmonella*-microsome assay developed by AMES and collaborators[4] and is easier to perform.

More recently, a gene induction assay was described by SYKES and WELLS to screen for β-lactam antibiotics.[5] This method exploited a strain of *Bacillus licheniformis* which carries an inducible β-lactamase gene. In this strain, little or no β-lactamase is produced under uninduced conditions and high levels of β-lactamase are produced in the presence of β-lactam antibiotics. β-Lactam antibiotics are detected in this assay *via* the induction of β-lactamase which can be conveniently monitored through the use of a chromogenic substrate such as nitrocephin. The assay is also extremely sensitive and as little as 1 ng of

Present address: [a]American Cyanamid, Agricultural Research Division, P.O. Box 400, Princeton, N. J. 08543-400, U.S.A. [b]Sterling Research Group, 25 Great Valley Parkway, Great Valley, Pa. 19355, U.S.A.

benzylpenicillin can be detected. Also, similar to the induction assays described above, the β-lactamase induction assay shows high specificity, employs simple manipulations and can be performed rapidly.

Inducible drug resistance determinants have been described for a variety of clinically useful antibiotic classes. However, except for resistance determinants to β-lactam antibiotics, the products of these inducible systems are difficult to assay and none of these assays are adaptable for rapid detection on Petri plates. Therefore, it seemed reasonable to try to combine promoters induced by compounds of interest with structural genes whose products could be readily assayed on Petri plates. In several instances, studies of the mechanism of gene induction have utilized fusions between the structural gene for β-galactosidase and promoters induced by clinically useful antibiotics such as tetracycline,[6] erythromycin[7,8] and chloramphenicol.[9] This report will describe the use of such gene fusions in the development of screens to detect novel antibiotics related to agents in current clinical usage.

Materials and Methods

Screen for Tetracyclines and Tetracycline-like Compounds

E. coli strain CB215 (BECK *et al.*[6]) which carries a *tet*R::*lac*Z fusion is grown overnight to saturation at 37°C in LB medium (Bacto-tryptone 1%, Bacto-yeast extract 0.5%, NaCl 0.5%). This culture is used at 2% to inoculate TTC indicator agar (agar 1.5%, K_2HPO_4 1.4%, KH_2PO_4 0.6%, protease peptone 2.0%, $MgSO_4 \cdot 7H_2O$ 0.1%, lactose 0.2%, 2,3,5-triphenyltetrazolium chloride (TTC) 0.005%) and plates are poured. Test samples are dried into 6 mm-disks which are placed on top of the solidified media and the plates are incubated at 37°C overnight. Positives are detected by the production of red colored zones. Strain CB46 (which is identical with strain CB215 but lacks the *tet*R::*lac*Z fusion)[6] is used in place of strain CB215 to control for artifacts.

Screen for Macrolide-lincosamide-streptogramin (MLS) Antibiotics and MLS-like Antibiotics

Bacillus subtilis strain BD927 (BD170 (pBD246), GRYCZAN *et al.*[7]) which carries an *erm*C::*lac*Z fusion is grown overnight to saturation at 37°C in LB medium plus 5 μg/ml chloramphenicol (to insure plasmid maintenance). This culture is used at 1% to inoculate LB medium which is solidified with agar. Test samples are dried onto 6 mm-disks which are placed on the top of the solidified medium and the plates are incubated at 37°C overnight. β-Galactosidase is detected with the chromogenic reagent 6-bromo-2-naphthyl-β-D-galactopyranoside (BNG) as follows. BNG 60 mg/ml and Fast blue RR salt 10 mg/ml are dissolved in DMSO which is added at a 1:30 ratio to 0.5% agar held at 50°C. Approximately 2~3 ml of this solution is used to overlay the test samples and the plates are incubated at room temperature for 20 minutes. Positives are detected by the production of a red ring. Strain BD170[7] lacks the β-galactosidase fusion and can be used in place of strain BD927 to control for certain types of artifacts.

Screen for Chloramphenicol and Chloramphenicol-like Antibiotics

B. subtilis strain 1A422 (pPL31acZ) (MONGKOLSUK *et al.*[9]) which carries a *cat*86::*lac*Z fusion is grown at 37°C overnight in LB medium to saturation. This culture is used at 2% to inoculate melted LB medium containing agar which is held at 50°C and is used to pour plates. Beyond this step, the protocol is identical to the assay for MLS antibiotics.

Isolation of a Vancomycin Induced Gene Fusion in *B. subtilis*

A pool of random *B. subtilis* chromosome insertions was generated in *B. subtilis* with plasmid pTV53 using a slight variation of the method of PERKINS and YOUNGMAN[10] (P. J. YOUNGMAN; personal communication). Plasmid pTV53 carries a modified version of transposon Tn917 into which promoter-less copies of *lac*Z and *cat* have been inserted. Plasmid pTV53 carries a temperature sensitive mutation in replication function such that plasmid loss occurs at the restrictive temperature, 47°C. Plasmid loss can be quickly verified by scoring tetracycline resistance, a plasmid encoded marker. Cells in which a

transposition event has occurred were isolated by selecting for erythromycin-resistance at 47°C. The erythromycin-resistant transposition pool was screened by replica plating onto agar solidified antibiotic assay broth (AAB) media either containing or lacking a subinhibitory concentration of vancomycin. β-Galactosidase activity was detected by overlaying the plates with a 50-μg/ml solution of methyl-umbelliferyl-β-D-galactoside (MUG) (a fluorescent reagent for β-galactosidase) and observing the plates under UV light. Positives were selected which produced β-galactosidase on the vancomycin containing plate and not on the plate without vancomycin. A strain specifically induced by glycopeptide antibiotics was identified and retained as strain SGB499. A control strain, SGB317 was isolated as described above using mitomycin C in place of vancomycin.

Screen for Glycopeptides and Glycopeptide-like Antibiotics

Strain SGB499 is grown overnight to saturation at 37°C in AAB medium containing erythromycin 10 μg/ml and lincomycin 10 μg/ml. This culture is used at 1% to inoculate melted AAB medium containing 1.5% agar held at 50°C and is used to pour plates. Samples on disks are placed on top of the solidified medium and the plates are incubated overnight at 37°C. At this stage the plates are overlayed with melted 0.5% agar containing MUG 50 μg/ml, are incubated at room temperature for approximately 20 minutes and are screened under UV light. Positives are detected by the production of a fluorescent ring. Strain SGB317 can be used as a control in place of strain SGB499. β-Galactosidase in strain SBG317 is non-specifically induced by a wide variety of antibiotics but not by glycopeptides.

Results

General Considerations

A variety of methods exist for the detection of β-galactosidase including MACCONKEY's medium, TTC indicator media and chromogenic substrates including BNG, MUG and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). From a practical standpoint, these methods differ in their applicability to Gram-positive or Gram-negative bacteria and in the lower limit of β-galactosidase which can be detected. For each fusion screening strain, a detection system was chosen which would not detect the basal level produced by the fusion strain, but which (for maximum sensitivity) would detect a small increase in β-galactosidase level. In addition, inoculum size, incubation period and media composition were optimized for sensitivity and reproducibility.

To obtain a rough estimation of the specificity of fusion induction, a small panel of antibiotics was chosen which represented a variety of structural types and mechanisms of action. This panel is shown in Table 1. In each case, a quantity of antibiotic was used which was suffcient to produce a zone of inhibition in order to insure that the compound was adequately tested. Induction zones, when they appear, surround the zone of inhibition. It was not possible in each case to obtain a zone of inhibition (for example, when antibiotics having a Gram-positive spectrum were tested on Gram-negative fusion strains). In addition to this standard list of compounds, each fusion was tested with a group of compounds structurally related to the prototype compound upon which the assay was based. The prototype compound was used in each case to determine the limit of detection of the assay.

Table 1. Antibiotics used for assay characterization.

Actinomycin D, amikacin, ampicillin, aztreonam, bacitracin, cephalothin, clindamycin, colistin, cephaloridine, chloramphenicol, erythromycin, gentamicin, kanamycin, lincomycin, methicillin, mitomycin C, nalidixic acid, nitrofurantoin, novobiocin, oxacillin, oxalinic acid, benzylpenicillin, polymyxin B, rifampin, sulfachlorophyridazine, tetracycline, tobramycin, streptomycin, vancomycin

Experiments were also performed to assess the potential for, and means for dealing with, assay interference. While it is difficult to prospectively

study negative assay interference (the suppression of a positive response by components of the test samples), many of the potential causes of positive assay interference (the production of a positive response by undesired compounds) can be easily anticipated and controlled. One type of artifact is the production of colored or fluorescent compounds or the production of β-galactosidase in the fermentation. In addition, the "tetracycline" screening protocol utilizes a detection method in which the presence of a fermentable carbon source in the test sample produces a positive. Such artifacts can be eliminated by a variety of means. In the "tetracycline" screening protocol, an isogenic strain which lacks the β-galactosidase fusion can be used to control for artifacts since such compounds should be positive for both strains. A β-galactosidase negative *Bacillus* strain can be used in a similar fashion in the other screens. The MLS and chloramphenicol screens can be used to control for one another as artifacts will be positive in both assays while compounds of interest will be positive only in one of the two assays. In the glycopeptide screen, an isogenic strain carrying a different fusion is used as control. This second fusion strain is not induced by glycopeptides but is likely to be positive for most or all compounds which are artifacts with the screening strain.

Screen for Tetracycline and Tetracycline-like Molecules

Regulation of tetracycline-resistance in Tn10 is produced by a repressor protein. This protein binds to operator sequences and represses both its own synthesis and the synthesis of a membrane protein which prevents the accumulation of tetracycline in the cell.[6] Binding of tetracycline to the repressor protein blocks operator sequence binding and thus induces tetracycline-resistance. One might expect that there would be similarities between the ribosome binding site for tetracyclines and the tetracycline binding site on the Tn10 repressor protein. If this is true, repressor binding could be used to identify inhibitors of protein biosynthesis. It has been reported that the Tn10 resistance determinant is induced by a variety of semi-synthetic and naturally occurring tetracyclines.[11]

A screening assay was therefore developed based upon a strain carrying a *tet*R/*lac*Z fusion in which β-galactosidase was shown to be induced by tetracycline.[6] The properties of this assay are summarized in Table 2. The antibiotics listed in Table 1 were tested as a crude measure of the specificity of this assay and only tetracycline produced a positive. A number of naturally occurring tetracyclines were also tested in the assay (7-chlorotetracycline, 7-chloro-6-demethyltetracycline (demeclocycline), 6-demethyltetracycline, 5-hydroxytetracycline, 7-chloro-8-methoxytetracycline (Sch34164) and all were positive. A compound related to the tetracyclines, β-chelocardin (cetotetrine) however was negative. Sensitivity of this assay to tetracycline was determined by applying a dilution series of tetracycline on disks. The lowest quantity of compound detected was 10 ng. In comparison, 80 ng was required to produce detectable zone of inhibition on *Staphylococcus aureus* FDA 209P. This indicates that a significant increase in sensitivity could be

Table 2. Screen for tetracycline and tetracycline-like compounds.

Knowns detected in screening of pure compounds:	Tetracycline, 7-chlorotetracycline, 7-chloro-6-demethyltetracycline (demeclocycline), 6-demethyltetracycline, 5-hydroxytetracycline, 7-chloro-8-methyltetracycline (Sch34164)
Limit of detection of tetracycline in screen:	10 ng
Limit of detection of tetracycline with *Staphylococcus aureus* FDA 209P:	80 ng
Knowns detected in screening natural products:	Thiobistropolone, safracin A

anticipated through the use of this assay while reasonable specificity is retained.

A pilot screen of several thousand cultures was therefore run. In addition to the identification of known tetracyclines as anticipated, two previously reported antibacterials, thiobistropalone[12] and saframycin A[13] were also detected. It is arguable that these compounds bear some structural resemblance to tetracyclines. In summary, these data indicate that this assay shows a high degree of sensitivity and a relatively high degree of specificity for compounds which structurally resemble tetracyclines. While this manuscript was in preparation, an analogous assay for tetracyclines based upon a β-galactosidase gene fusion was published by CHOPRA and co-workers[14] who reported results similar to those described in this paper.

Screen for MLS and MLS-like Antibiotics

Regulation of the induction of the *erm*C gene, a ribosomal RNA methylase which produces erythromycin-resistance, is produced by a translational attenuation mechanism.[15,16] Messenger RNA sequences upstream from the *erm*C methylase initiation codon exist in a hairpin base-paired configuration such that the ribosome binding site for methylase synthesis is occluded. Stalling of the ribosome, produced by the presence of erythromycin, leads to the translation of a short, 19 amino acid open reading frame within the base-paired region. This causes the opening of the mRNA structure which leads to the initiation of methylase synthesis. Induction by erythromycin is also seen for β-galactosidase synthesis in *erm*C/*lac*Z fusions.[7,8] One would therefore expect that antibiotics which produce ribosome stalling in a fashion similar to erythromycin could be readily detected *via* the induction of an *erm*C/*lac*Z fusion. The properties of the screen developed based upon this fusion are summarized in Table 3.

The panel of antibiotics shown in Table 1 was tested to crudely determine the specificity of *lac*Z induction in this assay. All of the MLS antibiotics in the panel as well as a group of other MLS antibiotics were positive. This is somewhat unexpected since only erythromycin and oleandomycin induce *erm*C mediated MLS-resistance.[15,16] However, in order to produce resistance, a fairly rapid rate of ribosome methylation must be induced relative to the protein synthesis inhibitory effect of the antibiotic. Most MLS antibiotics may induce a rate too low to produce resistance. The low level of induction may, however, be sufficient for the detection of β-galactosidase activity since this can be measured even at extremely low levels on plates. In addition, weak apparent, β-galactosidase induction was observed with several other compounds in the panel and especially with β-lactam antibiotics. The simplest explanation for this result is that a low basal level of β-galactosidase in the cell is detected in the presence of such compounds because they lower permeability barriers to the substrate. This allows an increased reaction rate between enzyme within the cell and exogenously added chromogenic substrate. Irrespective of the mechanism, these agents are also weakly positive with the "chloramphenicol" assay (see below) and are thus eliminated as non-specific artifacts (no MLS antibiotics are positive in the chloramphenicol assay). Other artifacts can also be

Table 3. Screen for MLS and MLS-like antibiotics.

Knowns detected in screening of pure compounds:	Erythromycin, carbomycin, clindamycin, josamycin, lincomycin, oleandomycin, megalomycin, tylosine, veramycin B
Limit of detection of erythromycin in screen:	2 ng
Limit of detection of erythromycin with *Staphylococcus aureus* FDA 209P:	200 ng
Knowns detected in screening natural products:	Berninamycin A

eliminated with an isogenic strain lacking the β-galactosidase fusion.

The sensitivity of this assay was determined by testing disks carrying aliquots of a dilution series of erythromycin. The lowest quantity of erythromycin detected was 2 ng while 200 ng of erythromycin was the lowest level detected by the production of a zone of inhibition against *S. aureus* FDA 209P. This indicates that this assay should be significantly more sensitive than conventional tests. A pilot screen of several thousand microbial fermentations was performed to determine the practical utility of the assay. No attempt was made to distinguish novel MLS antibiotics because an insufficient number of characterization standards was available. In addition to presumptive MLS antibiotics, the previously reported antibiotic berninamycin A was identified in the process of screening. Berninamycin A is an inhibitor of protein biosynthesis which, like macrolide antibiotics, acts by binding to the 50S ribosomal subunit.[17] It is possible that the activity of berninamycin A in some ways mimics the translational stalling produced by the macrolides and this results in β-galactosidase induction. It is expected that this assay, coupled with a good characterization scheme for MLS antibiotics, could lead to the discovery of novel compounds of the MLS type.

Screen for Chloramphenical and Chloramphenicol-like Antibiotics

The expression of the *cat*86 gene is regulated at the level of translation and this regulation is not dependent upon the presence of the transcriptional or translational products of *cat*86.[9,18] Dependence upon chloramphenicol for expression is conferred by a 144-base pair fragment from the 5′ end of the gene. This sequence will produce chloramphenicol dependent regulation when placed upstream of the *E. coli lac*Z gene. Data describing the behavior of a screening assay based upon this gene fusion are summarized in Table 4. The panel of antibiotics listed in Table 1 were tested to crudely determine the specificity of the assay. Only chloramphenicol gave a distinct positive response. In addition, several β-lactam antibiotics were weakly positive. The β-lactam antibiotics can be easily eliminated as artifacts since they are also weakly positive with the "MLS" screen.

The sensitivity of this assay was determined with test disks carrying aliquots of a dilution series of chloramphenicol. As little as 0.5 ng of chloramphenicol could be detected while 700 ng of chloramphenicol was required to produce a zone of inhibition against *S. aureus* FDA 209P. These results suggest that the assay should have significantly increased sensitivity relative to inhibition based tests. A pilot screen of several thousand microbial fermentations was performed to judge the practical applicability of the assay. In this screen, chloramphenicol was detected as well as a fermentation containing corynecins A, B and C. This is not surprising since the corynecins are close structural relatives of chloramphenicol. Presumably it might be possible to discover additional structural analogues of chloramphenicol with further implementation of this screen.

Screen for Glycopeptide and Glycopeptide-like Antibiotics

Each of the previously described screens was based upon an inducible resistance determinate. No inducible resistance determinate has been reported for glycopeptide antibiotics. Vancomycin, the prototype

Table 4. Screen for chloramphenicol and chloramphenicol-like antibiotics.

Knowns detected in screening pure compounds:	Chloramphenicol
Limit of detection of chloramphenicol in screen:	0.5 ng
Limit of detection of chloramphenicol with *Staphylococcus aureus* FDA 209P:	700 ng
Knowns detected in screening natural products:	Corynecin complex

Table 5. Screen for glycopeptide and glycopeptide-like antibiotics.

Knowns detected in screening pure compounds:	Vancomycin, ristocetin, actinoidin, A35512B, teicoplanin
Limit of detection of vancomycin in screen:	200 ng
Limit of detection of vancomycin with *Staphylococcus aureus* FDA 209P:	700 ng
Knowns detected in screening natural products:	None

for the glycopeptide antibiotics, has recently become much more widely used for the treatment of multiple drug resistant Staphylococci.[19] Vancomycin therapy is, however, associated with a number of adverse reactions. It was therefore of interest to devise screening strategies for new compounds related in mechanism of action to vancomycin which might show lowered toxicity. There have been recent reports of systems in which large numbers of a random *lacZ* fusions can be rapidly generated.[10,20] It seemed reasonable to screen a pool of randomly generated fusions for a strain which would be specifically induced by vancomycin (it is also possible that this could provide a general approach to devise screens for any type of compound of interest which could generate a biological response).

Randomly generated fusions were initially screened by replica plating onto media containing or lacking a subinhibitory level of vancomycin. Positives were then tested with the panel of antibiotics shown in Table 1 for specificity. One fusion which showed induction by and specificity for vancomycin was selected and retained as strain SGB499. The fusion pool was also replica plated onto media containing or lacking a subinhibitory level of mitomycin C and one colony was selected which, in secondary tests with the panel of antibiotics, was positive with a variety of antibiotics but not with vancomycin. This fusion can be used to control for artifacts.

Data obtained from the use of a screening protocol based upon this strain are summarized in Table 5. Strain SGB499 was tested with the four glycopeptide antibiotics in our antibiotic bank. As shown in Table 5, the strain was induced by actinoidin, ristocetin, A35512B and weakly induced by teicoplanin. This suggested that this assay should detect a wide veriety of glycopeptide antibiotics. The sensitivity of this assay was determined using disks carrying various quantities of vancomycin. As little as 200 ng could be detected with the fusion while 700 ng of vancomycin was required to produce a zone of inhibition with *S. aureus* FDA 209P. Therefore, although this assay has reasonably good specificity, there was no significant improvement in sensitivity. A pilot screen of several thousand microbial fermentations was run to judge the practical utility of this assay. Since characterization standards were not available to distinguish known glycopeptide antibiotics, glycopeptides were crudely identified using a peptide reversal assay similar to the method described by RAKE *et al.*[21] A variety of glycopeptides were identified by this method in the pilot screen. However, no other known antibiotics were detected. Therefore, this assay is likely to be a valid screen for glycopeptide antibiotics and could potentially detect other agents having a related cell wall synthesis inhibition mechanism of action.

Discussion

These four assays demonstrate that β-galactosidase gene fusions can be exploited in the design of screens for antibacterial agents. Such screens are simple and rapid to use, and can show greatly increased specificity for and sensitivity to certain compounds. They can be used individually as primary screens and/or as a group to aid characterization. It is possible that the application of these and related assays

to mass screening could result in the identification of novel and potentially useful compounds.

Acknowledgments

We would like to thank CAROL RICCA and SCOTT WELLS for assistance in performing pilot screens and EDWARD MEYERS, FRANK PILKIEWICZ and ADRIENNE TYMIAK for characterization studies. BRENDA PAPPANO and CAROLE KAMRAD are acknowledged for outstanding secretarial services. We would also like to thank RICHARD SYKES for encouragement and stimulating discussions.

References

1) ELESPURU, R. K. & M. B. YARMOLINSKY: A colorimetric assay of lysogenic induction designed for screening potential carcinogenic and carcinostatic agents. Environ. Mutagen. 1: 65~78, 1979
2) ELESPURU, R. K. & R. J. WHITE: Biochemical prophage induction assay: A rapid test for antitumor agents that interact with DNA. Cancer Res. 43: 2819~2830, 1983
3) QUILLARDET, P.; O. HUISMAN, R. D'ARI & M. HOFNUNG: The SOS chromotest: Direct assay of the expression of gene *sfiA* as a measure of genotoxicity of chemicals. Biochimie 64: 797~801, 1982
4) AMES, B. N.; J. MCCANN & E. YAMASAKI: Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31: 347~164, 1975
5) SYKES, R. B. & J. S. WELLS: Screening for β-lactam antibiotics in nature. J. Antibiotics 38: 119~121, 1985
6) BECK, C. F.; R. MUTZEL, J. BARBÉ & W. MÜLLER: A multifunctional gene (*tetR*) controls Tn*10*-encoded tetracycline resistance. J. Bacteriol. 150: 633~642, 1982
7) GRYCZAN, T. J.; M. ISRAELI-RECHES & D. DUBNAU: Induction of macrolide-lincosamide-streptogramin B resistance requires ribosomes able to bind inducer. Mol. Gen. Genet. 194: 357~361, 1984
8) KIRSCH, D. R. & M. H. LAI: Regulation of a macrolide resistance-β-galactosidase (*ermC-lacZ*) gene fusion in *Escherichia coli*. J. Bacteriol. 159: 381~384, 1984
9) MONGKOLSUK, S., N. P. AMBULOS, Jr. & P. S. LOVETT: Chloramphenicol-inducible gene expression in *Bacillus subtilis* is independent of the chloramphenicol acetyltransferase structural gene and its promoter. J. Bacteriol. 160: 1~8, 1984
10) PERKINS, J. B. & P. J. YOUNGMAN: Construction and properties of Tn917-lac, a transposon derivative that mediates transcriptional gene fusions in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 83: 140~144, 1986
11) TRAUB, B. & C. F. BECK: Resistance to various tetracyclines mediated by transposon Tn10 in *Escherichia coli* K-12. Antimicrob. Agents Chemother. 27: 879~881, 1985
12) KLAUS, G.; W. MOLLS, H. U. SEIHL, J. STRAHLE & C. WESTPHAL: Struktur einer nuen schwefelhaltigen Tropolonverbindung aus *Pseudomonas cepacia*. Liebigs Ann. Chem. 1982: 1006~1011, 1982
13) ARAI, T.; K. TAKAHASHI & A. KUBO: New antibiotics, saframycins A, B, C, D and E. J. Antibiotics 30: 1015~1018, 1977
14) CHOPRA, I.; K. HACKER, Z. MISULOVIN & D. M. ROTHSTEIN: Sensitive biological detection method for tetracyclines using a *tetA-lacZ* fusion system. Antimicrob. Agents Chemother. 34: 111~116, 1990
15) DUBNAU, D.: Translational attenuation: The regulation of bacterial resistance to the macrolide-lincosamide-streptogramin B antibiotics. CRC Crit. Rev. Biochem. 16: 103~132, 1984
16) WEISBLUM, B.: Inducible resistance to macrolides, lincosamides, and streptogramin type-B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression. *In* Gene Function in Procaryotes. *Ed.*, J. BECKWITH *et al.*, pp. 91~121, Cold Spring Harbor Laboratory, 1983
17) THOMPSON, J.; E. CUNDLIFFE & M. J. R. STARK: The mode of action of berninamycin and the mechanism of resistance in the producing organism, *Streptomyces bernensis*. J. Gen. Microbiol. 128: 875~884, 1982
18) DUVALL, E. J. & P. S. LOVETT: Chloramphenicol induces translation of the mRNA for a chloramphenicol-resistance gene in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 83: 3939~3943, 1986
19) KIRBY, W. M. M.: Vancomycin therapy of severe staphylococcal infections. J. Antimicrob. Chemother. 14 (Suppl. D): 73~78, 1984
20) CASADABAN, M. J. & S. N. COHEN: Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: *In vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. 76: 4530~4533, 1979
21) RAKE, J. B.; R. GERBER, R. J. MEHTA, D. J. NEWMAN, Y. K. OH, C. PHELEN, M. C. SHEARER, R. D. SITRIN & L. J. NISBET: Glycopeptide antibiotics: A mechanism-based screen employing a bacterial cell wall receptor mimetic. J. Antibiotics 39: 58~67, 1985