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Bioluminescence Screening In Vitro (Bio-*Siv*) Assays for High-Volume Antimycobacterial Drug Discovery

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Bioluminescence-based assays to indicate antimicrobial susceptibility have been developed and validated for recombinant strains of *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, *Mycobacterium avium*, and *Mycobacterium intracellulare* expressing an integrated eukaryotic luciferase gene. MICs determined with these bioluminescence assays for several antimycobacterial agents, including isoniazid, ethambutol, rifampin, amikacin, streptomycin, ciprofloxacin, and clarithromycin, compared favorably with traditional BACTEC methods and visual estimations of the inhibitory end point. Assay methodology has been optimized for the analysis of large numbers of novel compounds and is simple, inexpensive, and labor efficient. The availability of these four recombinant mycobacteria has permitted a strategy for drug discovery employing the nonpathogenic BCG strain for mass screening purposes with subsequent confirmation of activity against the pathogenic mycobacteria. Furthermore, evidence suggests that the BCG-based screen may allow the direct identification of bactericidal agents.

Despite renewed interest among academic and industrial researchers, tuberculosis continues to be a major worldwide cause of morbidity and mortality in humans, accounting for more adult deaths worldwide than any other single infectious agent (17). Latest estimates from the World Health Organization forecast 88 million new cases of tuberculosis and 30 million associated deaths within this decade (7). Problems associated with the disease have been exacerbated by the emergence of isolates resistant to commonly used therapeutic agents. Pulmonary disease associated with strains resistant to both isoniazid and rifampin is more recalcitrant to treatment, the cure rate being reduced to less than 60% compared with nearly 100% for pan-sensitive strains (9). Worthy of note is the fact that rifampin, the last highly efficacious drug aimed specifically at the treatment of tuberculosis, was introduced to the marketplace over 20 years ago (1a). In addition, the emergence of the *Mycobacterium avium-Mycobacterium intracellulare* (MAC) complex as a major cause of disseminated infection in immunocompromised patients (10) has had a profound effect on perceptions of other mycobacterial species as opportunistic pathogens.

Traditional methods for antimycobacterial drug susceptibility testing are time-consuming and can be expensive, excluding themselves from high-throughput drug discovery applications. In the modified proportion method used in many clinical laboratories (15), the antimicrobial agent is incorporated into agar medium which is subsequently inoculated with the test organism. Results are available only after 21 days of incubation. The BACTEC 460TB system represents significant savings in time, with results generally being available within 7 days. However, the test uses vials containing a radioactive broth medium whose unit price is substantial, with associated costs for disposal of radioactive liquids. Newer methodologies have been proposed, some of which are in the process of evaluation. Others suffer from the unavailability of suitable equipment or expense incurred for high-throughput use. A new generation of BACTEC instruments for the detection of mycobacteria in pathological specimens is under clinical evaluation and employs a fluorescence quenching-based oxygen sensor impregnated in silicone rubber to monitor for the presence of viable mycobacteria (2). The application of this technology to susceptibility testing is under investigation. A similar principle using visual determination of fluorescence has been extended to the detection of multidrug-resistant strains (13). Measurements of differential light scattering have also been applied to susceptibility testing of mycobacteria (4), although this technique requires specialized instrumentation incorporating a polarized laser light source and would be cumbersome if large numbers of samples were involved.

Bioluminescence assays have employed two different strategies, each assessing the metabolic activity of a culture by measurement of ATP levels. Earlier methods concentrated on direct measurement of ATP extracted from cells and proved useful for both rapid assessment of mycobacterial cell numbers and susceptibility testing (3, 16). More recently, the luciferase gene from *Photinus pyralis*, the American firefly, has been introduced by transformation and transfection into mycobacterial cells and methods have been described to assess antimicrobial susceptibilities (5, 11, 18). These studies have been insightful and have provided access to technology which is appropriate for adaptation to high-throughput drug discovery efforts. For this application, we have developed bioluminescent strains of different mycobacterial species and have optimized methodology for in vitro evaluation of antimicrobial agents. The validity of results obtained with these new assays has been verified by comparison with the more traditional BACTEC and macrodilution broth methods. We also outline the genesis of a strategy for discovery of novel antimycobacterial drugs employing recombinant strains of *Mycobacterium bovis* BCG, *Mycobacterium tuberculosis*, *M. intracellulare*, and *M. avium*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The following strains were obtained from the American Type Culture Collection: *M. tuberculosis* $H_{37}Rv$ (ATCC 27294), *M. bovis* BCG Connaught (ATCC 35745), and *M. intracellulare* ATCC 35761. *M. avium* MAC101 and A5 were obtained from M. Cynamon (State University of New York, Syracuse) and R. Cooksey (Centers for Disease Control

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and Prevention, Atlanta, Ga.), respectively. Routine bacterial growth of wild-type strains was performed at 37°C on Middlebrook 7H11 agar (Remel, Lenexa, Kans.) or in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.5% (vol/vol) glycerol and 10% (vol/vol) ADC enrichment (BBL, Becton Dickinson & Co., Cockeysville, Md.). In certain experiments, ADC enrichment was made as described by Jacobs et al. (12) or was purchased from Difco.

Construction of vectors. The construction of shuttle vector pMV361-*lux* expressing the firefly luciferase gene (*lux*) has been described previously (8). One additional vector, termed pMH46, was created by inserting the MOP/*lux* expression cassette from pMH30 (8) into pJEB381 (a gift from J. Burlein, MedImmune Corporation, Gaithersburg, Md.) 3′ to the *Escherichia coli rho*-independent *rrn*AB T1T2 terminator (19) between the *Not*I and *Sal*I restriction sites. All constructions were verified by restriction enzyme mapping, and *lux* gene expression was confirmed by luminescence assay as described below.

Transformations. Conditions employed for the electroporation of purified plasmid DNA into mycobacteria and the selection of recombinants were essentially as described by Stover et al. (21). Single colonies appearing after incubation on plates containing 20 μg of kanamycin per ml were confirmed to express the luciferase gene by transferring the colony to 0.2 ml of Middlebrook 7H9 broth, sonicating for 20 s at ambient temperature in a Vibracell cup horn sonicator (Sonics and Materials Inc., Danbury, Conn.) at a 20% amplitude with a 0.1-s on-off pulse cycle, and measuring luminescence as detailed below.

Antimicrobial agents. Isoniazid, ethambutol, streptomycin, amikacin, viomycin, capreomycin, cycloserine, *p*-aminosalicylic acid (all from Sigma Chemical Co., St. Louis, Mo.), and ciprofloxacin (Miles Inc., West Haven, Conn.) were prepared in sterile deionized water. Rifampin, clofazimine, ethionamide (all from Sigma), and clarithromycin (Abbott Laboratories, Abbott Park, Ill.) were prepared in dimethyl sulfoxide (Sigma). 4,4′-Diaminodiphenyl sulfone (dapsone; Sigma) was prepared in methanol. All stock solutions were made to a concentration of 640 μg/ml. Further twofold dilutions were performed to obtain final concentrations of each antibiotic in a range above, below, and at the MIC reported for wild-type strains.

Luminometry. Assays for luminescence were performed either in white opaque 96-well microtiter plates (MicroLite 1, Dynatech Inc., Chantilly, Va.) in an Autolumat 96P microtiter format luminometer or in Falcon 2054 polystyrene tubes in an AutoLumat 953B tube luminometer (both from Wallac Instruments, Gaithersburg, Md.). A sample volume of 0.1 ml was used in both cases. Aliquots of 1 mM luciferin (R & D Systems, Minneapolis, Minn.) in 0.1 M trisodium citrate (pH 5.1) were stored at −20°C and were allowed to reach room temperature before use. The luciferin was injected automatically in a 0.1-ml volume into the reaction well or tube and luminescence, determined as relative light units (RLUs), was measured for 15 s.

BACTEC and Bio-*Siv* assays. Strains were cultivated at 37°C in Middlebrook 7H9 broth supplemented with 10% ADC and 0.05% Tween 80 to obtain cells in the logarithmic phase of growth. After brief sonication as described above, the culture was assayed spectrophotometrically at 540 nm and diluted to yield two stock bacterial suspensions containing ~4×10^6 and ~1×10^5 CFU/ml. The lower-concentration suspension was distributed into Falcon 2027 test tubes in 4-ml volumes for the Bio-*Siv* assay. Controls containing recombinant cells in the absence of the antibiotic were included. The high-concentration suspension was injected in 0.1-ml volumes into BACTEC 12B vials (Becton Dickinson, Sparks, Md.) to obtain a final bacterial concentration of 1×10^5 to 5×10^5 CFU/ml. BACTEC vials and Bio-*Siv* assay tubes were incubated at 37°C in a water-jacketed incubator. Radiometric readings, expressed as growth index (GI) units, were obtained for each BACTEC vial by using the BACTEC 460TB instrument (Becton Dickinson). Luminescence readings were obtained by assay of 0.1-ml aliquots from each tube as described above.

Readings were obtained with both systems until definitive MICs were calculable with the BACTEC method. For *M. tuberculosis* and *M. bovis* BCG, the BACTEC MIC was defined as the lowest concentration of the antibiotic for which the change in GI units was less than or equal to the change in GI units for a control vial inoculated with a 1:100 dilution of the BACTEC inoculum. This determination was made on the day the GI reading for the 1:100 control reached or exceeded 30 U. In the case of *M. avium* and *M. intracellulare*, the MIC end point for the BACTEC system was determined essentially as described by Siddiqi et al. (20). For the Bio-*Siv* assay, the MIC was defined as the lowest concentration of the antibiotic which gave an RLU reading less than or equal to 1/100 the value obtained for the antibiotic-free growth control.

In some studies, the MIC was also estimated by visual inspection of the Bio-*Siv* assay tubes at the end of the experiment, comparing growth of the bacterial strain in the presence of the antibiotic with that observed in the antibiotic-free control. MIC was defined as the lowest concentration of the antibiotic which inhibited visible bacterial growth.

To ensure reproducibility, standardized susceptibility protocols which used stocks of mycobacterial cells harvested at early logarithmic phase of growth and frozen at −80°C were developed. For *M. tuberculosis*, the frozen cells were thawed at 37°C, added to fresh Middlebrook 7H9 broth, and incubated at 37°C for 72 h, with subsequent dilution to achieve an inoculum concentration of ~1×10^5 CFU/ml. MICs or screening end points were determined 8 days thereafter. For BCG and *M. intracellulare*, the frozen cells were cultivated for 48 h before the screen was set up and inhibitory activity was ascertained after 6 or 4 days, respectively.

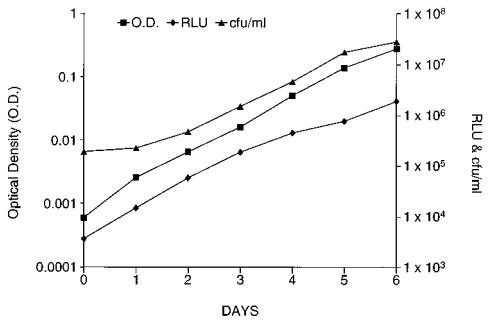

FIG. 1. Optical density, RLU, and CFU data for a culture of the recombinant *M. bovis* BCG strain transformed with pMV361-*lux*.

Assessment of bactericidal activity. An estimation of the bactericidal concentration was made by plating a 25-μl aliquot from each Bio-*Siv* assay tube or BACTEC vial onto 7H11 agar. Such estimations were made on the day that MICs were determined. Plates were incubated for 12 days when *M. avium* or *M. intracellulare* was tested and for 21 days when the test organism was *M. tuberculosis* or *M. bovis* BCG. The lowest concentration of antibiotic which resulted in growth of less than 100 colonies per 25-μl aliquot, representing approximately 1% of the starting inoculum, was defined as the bactericidal concentration. An antibiotic with an MBC/MIC ratio of 4 or less was considered bactericidal.

RESULTS

Transformants of *M. bovis* BCG, *M. tuberculosis*, and *M. intracellulare* carrying the integrated luciferase gene from pMV361-*lux* were readily identified since all colonies appearing on kanamycin-containing plates after electroporation expressed the luciferase gene and the occurrence of spontaneous resistance from control electroporation experiments containing no vector was negligible. However, this was not true for attempts to isolate transformants of the two *M. avium* strains. Spontaneous background resistance was very high, with the number of colonies being unaffected by whether the cells were electroporated in the absence or presence of vector. Consequently, colonies had to be picked at random and assessed for bioluminescence. All attempts at introducing the luciferase gene into either MAC101 or the A5 strain using the pMV361-*lux* vector proved ineffective. Even with pMH46, transformants expressing the luciferase gene were rare. The discrepancies observed within the MAC group were not unexpected in light of the demonstrated resistance of *M. avium* and the relative sensitivity of *M. intracellulare* to kanamycin (22). Early observations indicated that expression of the luciferase gene from our vectors was constitutive in a growing mycobacterial culture, with the increase in RLU values closely mimicking the increase in optical density and number of viable bacteria (Fig. 1), making RLU determinations an ideal indicator for susceptibility protocols.

The quality of the bacterial inoculum was a parameter in need of standardization to ensure consistency of results between experiments. Initial studies with the recombinant BCG strain were performed in glycerol-supplemented Middlebrook 7H9 broth, and, although susceptibility endpoints were clearly discernible, clumping of bacterial cells was noticeable and introduced a problematic variable. Replacing glycerol with Tween 80 in the medium resulted in more homogenous bacterial suspensions throughout the course of an experiment. As expected, the presence of Tween 80 had a significant effect on the MIC for rifampin (23) but did not alter the activities of other antimicrobial agents tested. Mild sonication of the culture prior to dilution for preparation of the inoculum was

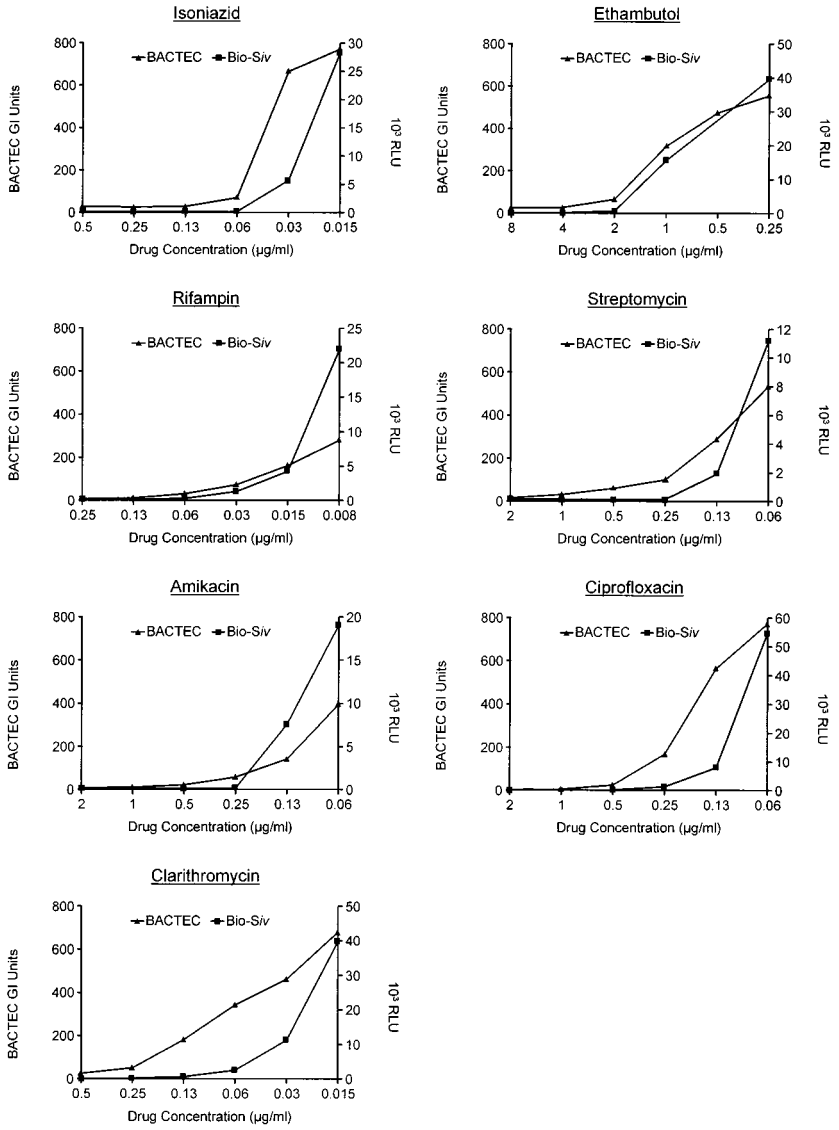

FIG. 2. Comparison of BACTEC GI and Bio-*Siv* RLU data for seven antimycobacterial agents tested against the recombinant *M. bovis* BCG strain. The data were obtained 5 days after inoculation.

found to dissipate any aggregated cells. At the instrument settings used, sonication had no effect on cell viability assessed by quantitative plating on agar medium. In addition, growth curves based on RLU values were identical for sonicated and nonsonicated portions of the same culture (data not shown).

For the recombinant BCG strain, there was excellent correlation between the BACTEC and Bio-*Siv* assays for all antimicrobial agents tested. This is well illustrated in Fig. 2, in which the effects of different concentrations of each drug, assessed by BACTEC GI units and RLU determinations, clearly demonstrate similar trends. For BACTEC, an experimental end point was reached in five days (Table 1), dictating the day on which drug MICs were determined with this system. For assay by bioluminescence, MICs were identical on days 4 and 5 postinoculation for all drugs examined. Assessments of inhibitory activity at earlier time points were discordant with results obtained with the radiometric method. A number of other drugs tested with the bioluminescence system alone established clear end points 5 days after incubation and permitted easy determination of MICs (Table 1). A noteworthy observation made early in our investigations indicated the importance of the source of ADC enrichment used in bioluminescence susceptibility assays for the mycobacteria. In several experiments using ADC made at our facility or purchased from Difco, the MIC determined for isoniazid against the recombinant BCG strain ranged from 0.06 to 0.13 μg/ml, whereas a value of 0.5 μg/ml was consistently obtained when ADC from BBL was used. This effect seemed to be limited only to susceptibility tests performed with isoniazid and was not observed with other antimicrobial agents tested.

Experiences with the BCG system were then used to devise assay regimens for *M. tuberculosis*. BACTEC and Bio-*Siv* assays revealed similar inhibitory activities for streptomycin, amikacin, rifampin, and ciprofloxacin (Table 2). BACTEC end points became available 7 days postinoculation. For these four drugs, MICs were determinable with the Bio-*Siv* assay after only 4 days and remained unchanged for a further 4 days thereafter. Again, the MIC for rifampin was found to be lower

TABLE 1. Comparison of BACTEC and Bio-*Siv* assay data for seven antimycobacterial agents tested against the recombinant BCG strain carrying pMV361-*lux*

Antimicrobial agent	MIC (μg/ml)[a]		Bio-*Siv* MBC[b] (μg/ml)
	BACTEC	Bio-*Siv*	
Isoniazid	0.06	0.06	0.13
Ethambutol	2.0	4.0	4.0
Streptomycin	0.25	0.25	0.25
Amikacin	0.13	0.25	0.25
Ciprofloxacin	0.25	0.5	0.5
Rifampin	0.03	0.06	0.13
Clarithromycin	0.25	0.25	>0.5
Viomycin		2.0	2.0
Capreomycin		1.0	1.0
Cycloserine		8.0	16.0
Clofazimine		0.13	0.25
para-Aminosalicylic acid		8.0	8.0
Dapsone		>128	>128

[a] After 5 days of incubation at 37°C.
[b] Estimate determined by plating a 25-μl aliquot from each tube onto 7H11 agar.

in the presence of Tween 80 than in glycerol-supplemented medium. Clarithromycin proved inactive at the highest concentration tested in BACTEC (2 μg/ml) but did show activity at 4 to 8 μg/ml in the bioluminescence assay. The activity of ethambutol determined by BACTEC analysis was 1 to 2 μg/ml. With a standard 1% resistance cutoff criterion, the MIC for ethambutol by bioluminescence assay was >8 μg/ml when tested on days 4 through 7, and only on day 8 was an acceptable MIC achieved (4 μg/ml). However, a plot of RLU versus concentration for day 7 clearly indicated a cutoff at 2 to 4 μg/ml, and this is calculable from the raw data if the cutoff criterion is extended from 1 to 2%. Isoniazid results for the bioluminescence method were eightfold higher than those determined with BACTEC, a difference attributable to the use of ADC obtained from BBL. MICs for all drugs determined by bioluminescence assay were in complete agreement with visual estimations of the inhibitory end point. In separate experiments, clear end points were observed within 7 days for the Bio-*Siv* assay in susceptibility tests against the recombinant *M. tuberculosis* strain for cycloserine, ethionamide, viomycin, and clarithromycin. These demonstrated MICs of 16, 4, 2, and 16 μg/ml, respectively.

For the MAC strains, results became available much sooner,

TABLE 2. BACTEC and Bio-*Siv* assay comparison for six antimycobacterial agents tested against the recombinant *M. tuberculosis* strain carrying pMV361-*lux*

Antimicrobial agent	MIC (μg/ml)[a]		MBC[a,b] (μg/ml)	
	BACTEC	Bio-*Siv*	BACTEC	Bio-*Siv*
Isoniazid	0.06	0.5	0.13	0.5
Ethambutol	1.0	>8.0[c]	4.0	4.0
Streptomycin	0.5	0.5	1.0	0.5
Amikacin	0.5	1.0	1.0	1.0
Ciprofloxacin	0.5	0.5	1.0	0.5
Rifampin				
7H9 broth + glycerol	0.13	0.13	0.5	0.25
7H9 broth + Tween		0.03		0.03

[a] On day 7.
[b] Estimate determined by plating a 25-μl aliquot from each Bio-*Siv* tube or BACTEC vial onto 7H11 agar.
[c] On day 8, the MIC was 4.0 μg/ml.

TABLE 3. BACTEC and Bio-*Siv* assay comparison for six antimycobacterial agents tested against the recombinant *M. intracellulare* strain carrying pMV361-*lux*

Antimicrobial agent	MIC[a] (μg/ml)		MBC[a,b] (μg/ml)	
	BACTEC	Bio-*Siv*	BACTEC	Bio-*Siv*
Isoniazid	2.0	8.0	>32	16.0
Ethambutol	2.0	4.0	>32	16.0
Streptomycin	2.0	1.0	8.0	16.0
Amikacin	4.0	2.0	8.0	16.0
Kanamycin[c]	>32	>32	>32	>32
Rifampin	0.03	0.06	8.0	8.0
Clarithromycin	0.13	0.13	8.0	16.0

[a] On day 4.
[b] Estimate determined by plating a 25-μl aliquot from each Bio-*Siv* tube or BACTEC vial onto 7H11 agar.
[c] MICs for kanamycin were high since the pMV361-*lux* vector carries a kanamycin resistance gene.

with end points being reached within 3 to 4 days. BACTEC and bioluminescence assay MICs for ethambutol, rifampin, amikacin, streptomycin, and clarithromycin showed good correlation for the *M. intracellulare* strain, with not more than a twofold difference in values for each drug (Table 3). Isoniazid MICs for the BACTEC (2 μg/ml) and bioluminescence (8 μg/ml) methods differed by a factor of 4 but were still considered to be within an acceptable range. With the recombinant *M. avium* A5 strain, the two assay systems again indicated similar inhibitory activities for all agents tested except isoniazid, and these results were further confirmed by visual estimations of the MIC (Table 4). *M. avium* MAC101 also had concordant results with the BACTEC and Bio-*Siv* methods. MICs for clarithromycin, streptomycin, amikacin, and ciprofloxacin obtained with BACTEC were 0.5, 2, 2, and 2 μg/ml, respectively. The corresponding values for these agents obtained with the Bio-*Siv* assay were 1, 2, 4, and 2 μg/ml, respectively.

Our findings with the estimation of bactericidal concentrations revealed an interesting phenomenon. MICs and MBC estimations determined from the Bio-*Siv* assay against the *M. intracellulare* strain were clearly different (Table 3), reflecting the bacteriostatic or bactericidal effects of different antimicrobial agents against these organisms. However, for every agent tested against the BCG strain, values for the MIC and MBC estimations were always equivalent (Table 1). This was true for all of the commercially available therapeutic drugs examined and was independent of the type of ADC enrichment used. The same effect was observed with the *M. tuberculosis* strain,

TABLE 4. BACTEC and Bio-*Siv* assay comparison for six antimycobacterial agents tested against the recombinant *M. avium* A5 strain carrying pMH46

Antimicrobial agent	MIC (μg/ml)		
	BACTEC[a]	Bio-*Siv*	Visual estimation[b]
Isoniazid	1.0	8.0	8.0
Ethambutol	2.0	4.0	2.0
Streptomycin	0.5	0.5	0.5
Amikacin	1.0	2.0	1.0
Ciprofloxacin	0.25	0.25	0.13
Rifampin	≤0.13	0.5	0.13
Clarithromycin	≤0.13	0.25	0.13

[a] On day 3.
[b] On day 5.

although we have examined only six drugs to date for comparisons of MICs and MBC estimations (Table 2).

DISCUSSION

We have described antibiotic screening protocols for mycobacteria utilizing recombinant organisms and appropriate methodology to assay for the gene product luciferase. Results obtained with these bioluminescence-based methods have been corroborated by parallel determinations made by BACTEC analyses and visual estimations of the inhibitory end point. The undoubted appeal of the Bio-*Siv* assay is its suitability for antibiotic discovery programs for which a high throughput is required. We have also shown the technique to work in a microtiter format, making it an attractive option for robotic instrumentation. The assay itself is straightforward, and the low cost of reagents makes it highly amenable to the testing of large numbers of drugs. Unlike the radiometric methods, in which the vials have to be run through the BACTEC 460TB instrument at approximately the same time every day to establish differences between daily GI readings, our standardized Bio-*Siv* assays need to be run only on one defined day after incubation with the test substance, affording considerable savings in personnel time and effort.

We are currently making use of these Bio-*Siv* assays in our approaches to identify new antibiotics to combat pathogenic mycobacteria. A few modifications have been necessary for routine screening applications. Long-term continuous subculture of a test strain to serve as the inoculum for an in vitro assay is fraught with problems such as alterations in susceptibility patterns and culture contamination. To avert such outcomes, stocks of bacterial cells in early logarithmic phase of growth have been stored at −80°C and regimens have been designed for the subsequent use of these frozen cells to initiate cultures for inoculum preparation. Also, both of the vectors described in this study integrate into the host genome, obviating any potential problems associated with changes in copy number or loss of an extrachromosomal vector carrying the luciferase gene. For all strains, we have standardized culture conditions to obtain cells in the early phase of logarithmic growth prior to commencing a screening experiment. In studies with standard antimycobacterial drugs, we saw no appreciable differences in MICs when the inoculum was derived from a continuously passaged strain or from a culture seeded with the frozen stock (data not shown).

The Bio-*Siv* assay does not require the mycobacterial cells to be lysed prior to bioluminescence analysis, so the only steps at the end of a susceptibility test involve the transfer of samples to tubes or a microtiter plate and automatic injection of luciferin by the luminometer. Our recombinant strain of *M. avium* A5 proved to be sensitive to amikacin at 2 μg/ml, in contrast to the findings reported by Cooksey et al. which indicated their strain to be resistant to amikacin concentrations as high as 100 μg/ml (6). The *M. avium* MAC101 and *M. intracellulare* recombinant strains also proved sensitive to similar levels of amikacin. These disparities are difficult to explain since the kanamycin resistance determinant in our vectors and that in the pLUC10 shuttle plasmid used by Cooksey et al. (5, 6) were derived from the same vector.

The BCG and *M. tuberculosis* strains proved to have identical susceptibilities to nearly all of the antimicrobial agents examined. Of the two exceptions, amikacin demonstrated only fourfold higher MICs against *M. tuberculosis* whereas clarithromycin showed the greatest disparity, with an MIC for BCG of 0.25 μg/ml compared with 16 μg/ml for *M. tuberculosis*. This relative concordance in susceptibility profiles has prompted us to employ the BCG strain for all routine screening analyses performed on uncharacterized or novel compounds. BCG, originally derived from *M. bovis*, demonstrates considerable genetic identity with *M. tuberculosis* (14), so BCG and *M. tuberculosis* would also be expected to share many common drug targets. The activity of any agent showing appreciable inhibition of the BCG strain can then be confirmed against the recombinant *M. tuberculosis*. In adopting this strategy, we can test and eliminate the great majority of compounds inactive against the mycobacteria without ever entering a biosafety level III environment. In addition, since MICs and MBC estimations have always proven to be very similar for BCG, this screening system may permit the identification and prioritization of agents exhibiting the greatest bactericidal activity. We then proceed to ascertain the utility of any active compounds against the MAC complex by first testing them against the *M. intracellulare* isolate and then against one of the *M. avium* strains. Recombinant mycobacteria expressing the luciferase gene have formed the core of our strategy to expedite all facets of the drug discovery process. Interesting compounds detected with our in vitro assays can rapidly be tested for antimycobacterial efficacy in animal models (8) and ex vivo in macrophages (1). In these cases, the use of bioluminescence replaces laborious and often lengthy procedures involving the plating of samples on solid media for CFU determinations.

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