GLYCOPEPTIDE ANTIBIOTICS: A MECHANISM-BASED SCREEN EMPLOYING A BACTERIAL CELL WALL RECEPTOR MIMETIC

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The evolution of a highly targeted screening program for the discovery of antibiotics of the glycopeptide (vancomycin) class is described. A holistic approach was utilized which optimized not just screening techniques but also the selection of candidate producer cultures and their growth under conditions which enhanced production of target compounds. Two screen techniques were utilized; differential inhibition of a vancomycin-resistant strain and its susceptible parent, and a specific antagonism screen using the reversal of glycopeptide activity by a tripeptide analog of the glycopeptide receptor, diacetyl-L-lysyl-D-alanyl-D-alanine. The latter screen was 2- to 32-fold more sensitive to known glycopeptides than the former, and was absolutely specific, yielding no false positive responses. The use of the tripeptide antagonism assay, combined with optimized culture selection and growth conditions yielded novel glycopeptide antibiotics at a rate of 1 per 320 cultures screened. With a holistic approach to screening and properly optimized techniques, large numbers of cultures do not need to be examined in order to discover novel antibiotics.

The production of antimicrobial metabolites is a surprisingly common attribute of microorganisms, particularly the soil actinomycetes which have been favored for screening.

However, as the 'antibiotic era' has progressed, the rate of antibiotic discovery, as a proportion of the cultures screened has steadily declined[1,2]. If we assume that there is a large but finite number of antibiotics available for discovery, then random screening of the same population of producer cultures will lead to an ever increasing frequency of rediscovery of previously observed compounds, coupled to an exponentially decreasing rate of discovery of novel compounds. Thus, in screening during the year 1957, WOODRUFF, HERNANDEZ and STAPLEY[2] reported that from 21,830 cultures screened against four organisms, 6,464 produced antibiotics, 490 produced activities 'worthy of retest', 6 were selected for structure determination, and 2 novel compounds were discovered. During 1958, HAHN[3] reported that of 10,000 cultures screened, 2,500 produced antibiotics, 10 novel compounds were found, of which 1 was clinically useful. BETINA[4] in 1983 estimated that several hundred thousand cultures must be screened to discover one useful antibiotic.

The problem of rediscovery necessitates the development of techniques which are capable of discriminating known from novel antibiotics. Discrimination systems capable of dealing with simple screens for antibiotic activity are necessarily complex, multistage procedures utilizing a variety of chromatographic separations often using bioautographic detection[4,5], and activity against a battery of specifically antibiotic-resistant test strains[6], which make heavy demands on staff and time. Even with well designed discrimination systems it is not uncommon to recognize a known compound only at the stage of structure determination of the purified product.

Purely random screening is no longer a practical route for antibiotic discovery. Several tactics

may be employed to improve the rate of discovery of novel antibiotics from a given structural class or activity profile. These include the selection of producer cultures from unusual or under-exploited taxa[7], preselection of candidate producer cultures by characteristics which enhance their likelihood of producing the compound of interest[8], growth under conditions which enhance the production of the compounds of interest[9], and the use of highly sensitive and selective screens targeted to the compound or activity of interest[10]. While all of these techniques have been used to a greater or lesser extent by various groups in antibiotics discovery[11], the extent to which they have been combined in the cell wall-targeted screen described here provides unique insight into how the steps can be optimized to yield a high productivity of novel compounds.

Glycopeptide antibiotics, of which vancomycin is the prototype, are inhibitors of bacterial cell wall biosynthesis. They are selectively antibacterial due to a high affinity for cell wall receptors, terminating in L-lysyl-D-alanyl-D-alanine, that are incorporated into peptidoglycan[12]. The spectrum of activity of glycopeptide antibiotics is limited to Gram-positive bacteria due to their large size (>1,400 daltons), which precludes penetration of the Gram-negative outer membrane. Compounds of this class have been the subject of a recent revival of interest and clinical use, with the rise of multiply antibiotic-resistant Gram-positive bacteria[13]. We have previously reported the discovery of aridicins, novel lipid-containing glycopeptides[14,15] and now wish to report on the functionally-directed screen which was developed to discover additional new members of this antibiotic class. The potential of this screen was further optimized by techniques for the selection of candidate producer cultures, and their growth under conditions which enhanced the production of glycopeptide antibiotics.

Materials and Methods

Isolation of Candidate Producer Cultures

Actinomycete cultures were isolated from soil samples by serial dilution onto isolation media. Plates were incubated for four weeks at 28°C, and selected colonies inoculated onto agar slants. Slant cultures were incubated for two weeks, and used to inoculate fermentation media.

All media contained actidione (50 μg/ml) and nystatin (100 units/ml) as antifungal agents. Media tested were designated: Arginine glycerol salts (AGS)[16]; glycerol asparagine (GA)[17]; oatmeal soil extract [oatmeal agar (ISP 3), 22 g; soil extract, 500 ml (500 g soil plus 1,200 ml tap water – autoclave 30 minutes – cool and filter); deionized water, 500 ml; OSE]; starch casein nitrate (SCN)[18]; SCN + vitamins (vitamins; thiamine hydrochloride, 0.5 mg; riboflavin, 0.5 mg; niacin, 0.5 mg; pyridoxine hydrochloride, 0.5 mg; inositol, 0.5 mg; calcium pantothenate, 0.5 mg; *p*-aminobenzoic acid, 0.5 mg; biotin, 0.25 mg; per liter) (SCNV); soluble starch casein[19] plus $MgSO_4$ anhydrous 0.05% (SC).

Growth of Producer Cultures

Candidate producer cultures were grown in 10 ml of liquid media in 23×200 mm culture tubes on a reciprocating shaker (300 strokes/minute) at 28°C. Samples were removed for assay at three and five days incubation. During screen development, a variety of fermentation media were evaluated using known glycopeptide producing strains from different genera.

Plate Assay Techniques

All screening assays were performed as disc diffusion assays. *Staphylococcus aureus* 209P and its vancomycin-resistant progeny were maintained as frozen stock cultures. Seed cultures were grown in Mueller-Hinton broth (Difco) for 5 hours at 37°C on a reciprocating shaker (150 strokes/minute). Vancomycin, 50 μg/ml, was included in seed cultures of the vancomycin-resistant strain. Mueller-Hinton agar at 45°C was inoculated with a 1: 100 dilution of seed culture and plates poured as above. *Bacillus subtilis* strain 6633 was maintained as a washed spore suspension. Penassay agar (Difco) was

prepared, cooled to 45°C, and seeded with 1×10^9 spores per ml. Plates (150 mm) were poured with 15 ml seeded agar to give a depth of 1 mm.

All control antibiotics were applied to plates in 20 μl aliquots on 6.5-mm filter paper discs. When nonaqueous solvents were used, discs were air dried prior to placing on the agar surface. Culture broths were applied by dipping the disc briefly into the whole broth suspension. This absorbed approximately 30 μl of broth onto the disc, however, the volume applied by this technique was somewhat dependent on broth viscosity. Discs for the co-administration of glycopeptide antagonist (tripeptide, see below) were prepared by pipetting 20 μl of a 5 mg/ml solution of diacetyl-L-lysyl-D-alanyl-D-alanine in H_2O-acetonitrile (1: 1) onto standard discs. Discs were then air dried at room temp. The tripeptide-loaded discs were used for comparison with plain discs to determine the differential inhibition zones obtained with culture broths.

Determination of Antibiotic Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC, μg/ml) were determined by the broth micro-dilution technique[20] in Mueller-Hinton broth (*Staphylococcus* strains) or Penassay broth (*B. subtilis*). Microtiter plates were inoculated with 10^4 cfu/ml, and incubated at 37°C for 24 hours prior to determining the MIC.

Antibiotics and Other Reagents

Diacetyl-L-lysyl-D-alanyl-D-alanine was obtained from Vega Biochemicals. Vancomycin and ristocetin were obtained from Sigma; actaplanin, A-477 and A-35512B from Eli Lilly and Co.; actinoidin A from Dr. G. F. GAUSE, Institute of New Antibiotics, Moscow; avoparcin and LL-AM-374 from Lederle Laboratories; teicoplanin from Lepetit; aridicin A and OA-7653 were isolated in our laboratories. Other test antibiotics were obtained from various sources.

Results

The screening program consisted of two phases, period A where the *S. aureus* 209P VAN^R (vancomycin-resistant) prescreen was used and period B when the tripeptide antagonism screen was used in parallel with *S. aureus* 209P VAN^R. During period B the strain isolation and culture conditions were directed to improving the isolation and productivity of glycopeptide producing bacteria.

Glycopeptide Screening Technique

Preparation of Vancomycin-resistant *S. aureus* Strain

A variant of *S. aureus* 209P highly resistant to glycopeptide antibiotics was generated by UV treatment of the vancomycin susceptible parent strain. An overnight culture of *S. aureus* 209P (vancomycin MIC 1.6 μg/ml) was diluted to 10^4 cells/ml and 0.1 ml was spread onto Mueller-Hinton agar containing vancomycin, 100 μg/ml. Plates were irradiated with UV light for 30 minutes; (sufficient to give 99% killing of the initial inoculum). Plates were incubated at 37°C for 40 hours and surviving colonies were picked onto isolation plates containing the same vancomycin concentration. The resultant colonies were streaked for single colonies, and the cloned isolates evaluated for vancomycin resistance by broth micro-dilution assay. *S. aureus* 209P VAN^R (vancomycin MIC >100 μg/ml) was at least 60-fold more resistant to vancomycin than was the parent strain.

Sensitivity of *S. aureus* 209P VAN^R to Known Antibiotics

A positive response was defined as ≥ 5 mm difference in the diameter of the inhibition zones on assay plates of the parent and the vancomycin resistant variant. The differential inhibition of the parent and the resistant variant was tested with pure antibiotics of known structure or activity classes

Table 1. Sensitivity of glycopeptide screens to known antibiotics.

Antibiotic class[a]	Number tested	*S. aureus* VAN^R screen	Tripeptide reversal screen
Glycopeptides	11	7 (64%)[c]	11 (100%)[d]
Other cell wall active antibiotics	19	5 (26%)	0 (0%)
Protein synthesis inhibitors	45	17 (38%)	0 (0%)
Nucleic acid inhibitors	30	9 (30%)	0 (0%)
Other mechanisms[b]	71	18 (25%)	0 (0%)
Total of non-glycopeptides	165	49 (30%)	0 (0%)

[a] Antibiotics were assayed at 2 μg/disc (100 μg/ml).
[b] Includes membrane active agents, energy poisons, and antibiotics with undefined mechanism of action.
[c] Two of 7 also detected at 0.5 μg/disc (25 μg/ml).
[d] Nine of 11 also detected at 0.5 μg/disc (25 μg/ml).

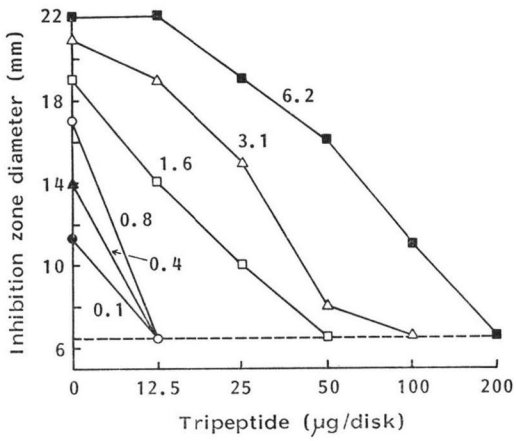
Table 2. Sensitivity of screens to known glycopeptide antibiotics.

Detection limit is the lowest concentration (μg/ml) giving 5 mm differential zone.

Antibiotic	VAN^R screen	Tripeptide screen
Actaplanin	>100	25
Actinoidin	100	3.1
Aridicin A	25	12.5
Avoparcin	100	3.1
A-477	>100	100
A-35512B	>100	25
OA-7653	100	50
LL-AM-374	100	6.25
Ristocetin	>100	12.5
Teicoplanin	50	25
Vancomycin	25	3.1

Fig. 1. Antagonism of vancomycin by tripeptide.

Vancomycin (0.1~6.2 μg/disk) was added to 6.5 mm discs impregnated with diacetyl-L-lysyl-D-alanyl-D-alanine (12.5~200 μg/disk). The assay organism was *Bacillus subtilis* 6633.



(Table 1). Of the 11 glycopeptide antibiotics tested, 7 were positive in the assay at 100 μg/ml (Table 2). There was, however, a considerable incidence of false-positives. Thus of 165 known antibiotics tested, 49 or 30%, were detected by the VAN^R test at 100 μg/ml.

Development of the Tripeptide Antagonism Screen

The documented capability of vancomycin to bind to synthetic peptides which are structural analogs of the peptide terminus of bacterial cell wall murein[12] encouraged us to examine whether one such peptide analog, diacetyl-L-lysyl-D-alanyl-D-alanine (tripeptide), could be used to specifically reverse the activity of vancomycin against susceptible bacteria. For this assay *B. subtilis* was chosen since it was 2- to 32-fold more sensitive to different glycopeptide antibiotics than the *S. aureus* 209P strain used previously (Table 2). Cross-titration of a range of vancomycin concentrations with a range of tripeptide concentrations (Fig. 1), showed marked antagonism of vancomycin activity by tripeptide. Thus, 25 μg of tripeptide on the antibiotic disc completely reversed the activity of 0.78 μg of vancomycin, 100 μg completely reversed 3.1 μg of vancomycin, and 200 μg of tripeptide completely reversed 6.2 μg of vancomycin. For routine screening a disc loading of 100 μg of tripeptide was chosen.

Sensitivity of the Tripeptide Antagonism Screen to Known Antibiotics

The tripeptide reversal assay was tested with the same library of known antibiotics which were used for evaluation of the VAN^R screen (Table 1). The tripeptide screen was capable of detecting the 11 known glycopeptides tested at 100 μg/ml, and all but 2 (A-477 and OA-7653) at 25 μg/ml (Table 2).

Contrastingly, none of the other 146 antibiotics tested was detected by the tripeptide reversal assay. While this was a limited antibiotic library, in our experience the tripeptide antagonism screen was found to be entirely specific for glycopeptide antibiotics. A positive response was defined as a 5 mm or greater reduction in zone size in the presence of tripeptide. Glycopeptides, unless present at very high concentration (>200 μg/ml), were completely reversed (*i.e.*, no inhibition zone) by tripeptide at the standard 100 μg/disc loading.

Glycopeptide Screening Performance

Performance of the VAN^R Screen with Producer Broths

During the first period of screening for glycopeptide antibiotics, a total of 2,457 cultures were screened using the VAN^R screen test (Table 3). Of 2,457, 344 cultures (14%) were scored as positive (5 mm of greater zone differential). From this group, 27 (1.1%) were selected in the secondary screening (using the tripeptide antagonism test), and 5 (0.2%) glycopeptide producers were subsequently verified. The 22 cultures not shown to produce glycopeptides had marginal activity, gave incomplete reversal (<5 mm decrease in zone size) in the tripeptide assay, or failed to reproduce the initial activity.

Performance of Tripeptide Antagonism Screen with Fermentation Broths

During screening period B, the tripeptide reversal screen was run as a primary screen in parallel with the VAN^R screen (during screening period A a limited supply of tripeptide precluded its use in primary screening). Of 1,936 cultures screened, 57 (2.9%) were positive, of which, 41 (2.1%) were identified as glycopeptide leads; in parallel testing with the VAN^R screen, 492 (25%) were identified as primary positive (Table 3). It should be noted that only 30 of the cultures ultimately identified as glycopeptide producers were detected by the VAN^R screen test, missing 12 additional leads detected with the tripeptide screen. This demonstrates that the enhaced sensitivity of the tripeptide antagonism screen with pure compounds (Table 2) translated to enhanced detection of glycopeptide antibiotics from fermentation broths.

Table 3. Performance of glycopeptide screens.

	Period A	Period B[a]	
	VAN^R	VAN^R	Tripeptide
Cultures screened	2,457	1,936[b]	1,936[b]
Primary positive	344 (14%)	492 (25%)	57 (2.9%)
Secondary positive	27 (1.1%)	46 (2.4%)	NA[c]
Glycopeptide leads	5 (0.2%)	30 (1.6%)	42 (2.2%)
Novel glycopeptides	1 (0.04%)	3 (0.15%)	6 (0.3%)

[a] During period B, the screen performance was enhanced by the use of improved strain selection and antibiotic production conditions.

[b] The same cultures were tested in both pre-screens during period B.

[c] NA: Not applicable.

Optimization of Screen Input

Selection of Candidate Producer Cultures

During screening period B, a variety of primary isolation media were evaluated for the isolation of glycopeptide producers from soils. Also, the use of selective antibiotics in the isolation media was evaluated. While these studies were empirical in nature, distinct trends emerged (Table 4). As might be expected, the presence of vancomycin in isolation media lead to enhanced isolation rates of glycopeptide producers, although its use led to overgrowth of isolation plates by bacteria. Certain

Table 4. Performance of culture isolation media for the discovery of glycopeptide producers during screening period B.

Medium	Selective agent[a]	Cultures screened	Glycopeptide leads	%
SCNV	None	220	3	1.4
SCNV	D-Cycloserine (5 μg/ml)	156	1	0.6
SCNV	Gentamicin (2.5 μg/ml)	115	2	1.8
SCNV	Vancomycin (1 μg/ml)	383	13	3.4
SCN	Vancomycin+polymyxin (25 μg/ml+5 μg/ml)	178	6	3.4
SC	Novobiocin (10 μg/ml)	161	7	4.4
AGS	None	329	7	2.1
GA	Polymyxin B (5 μg/ml)	85	2	2.4
OSE	Vancomycin (25 μg/ml)	26	1	3.8
Others[b]		435	0	0
Total		2,088	42	2.0

[a] Antibiotic incorporated into the isolation agar media.
[b] Other media numbered 13.

Table 5. Productivity of glycopeptide producer cultures on selected growth media.

Medium	Producer culture					
	Vancomycin *Streptomyces orientalis*		Teicoplanin *Actinoplanes teichomyceticus*		Aridicin *Kibdelosporangium aridum*	
	Bs[a]	*Bs*/tri[b]	*Bs*	*Bs*/tri	*Bs*	*Bs*/tri
E1	24	9	17	9	19	12
G1	23	0	14	8	15	13
I1	11	9	12	10	15	12
E2	18	0	14	13	15	14
G2	23	10	20	13	19	12
I2	17	9	16	10	17	10
E4	19	0	11	10	18	12
G4	23	0	8	8	18	11
I4	15	8	12	9	18	12

[a] Zone size (mm) on *Bacillus subtilis*.
[b] Zone size on *Bacillus subtilis* using tripeptide loaded discs.

other antibiotics, notably novobiocin, also gave enhanced isolation rates. Other compounds, such as gentamicin and D-cycloserine, were much less effective in selecting glycopeptide producers.

Generic Identity of Glycopeptide Lead Cultures

Among cultures assignable to recognized genera, glycopeptide antibiotics were discovered from only single representatives of *Nocardia, Actinomadura, Actinoplanes*, and *Streptomyces* (Table 6). Seven producers were found to belong to the new genus *Kibdelosporangium*[14], however, the largest number of cultures were unclassifiable, falling into two groups. Group 1 contained 28 cultures with long spore chains of no classifiable morphology. Within this group two cell wall chemotypes were recognized; type III, which are not readily accommodated in any described genus, and type IV, which probably belong to the mycolate-less *Nocardia*. Group 2 included 8 cultures which did not produce sporulating aerial mycelia, and could not be classified by conventional criteria.

Optimization of Antibiotic Production Conditions

A systematic study was undertaken of the effects of carbon, nitrogen, and phosphorus sources on the production of glycopeptides by known producer cultures. Six carbon sources and nine nitrogen sources were examined, forming an array of 54 media. Production of glycopeptide (as assayed by tripeptide reversal assay) was investigated using 14 producer cultures from four genera. Representative data for five producer cultures grown on media utilizing three nitrogen sources and three carbon sources is shown in Table 5. From these studies, three media were chosen for both the maximum production of glycopeptide and minimum production of other antibiotics (*i.e.*, tripeptide non-reversible activity).

Effect of Improved Isolation and Production Techniques

The use of the improved culture isolation techniques and of the selected production media was implemented at the beginning of screening period B. While it is not possible to dissect out the effect

Table 6. Glycopeptide antibiotics discovered during screening periods A and B.

Glycopeptide	Number	Genera
Aridicins	4	Unidentified actinomycete — Group 2 (1) *Kibdelosporangium* (3)
LL-AM-374	3	Unidentified actinomycete — Group 1 (2) *Streptomyces* (1)
A47934	2	Unidentified actinomycete — Group 2 (2)
Actinoidin B	1	Unidentified actinomycete — Group 1 (1)
Ristocetin	6	*Kibdelosporangium* (2) Unidentified actinomycete — Group 1 (4)
Vancomycin	13	*Actinoplanes* (1) *Nocardia* (1) Unidentified actinomycete — Group 1 (9) Unidentified actinomycete — Group 2 (2)
Novel	14	*Actinomadura* (1) *Kibdelosporangium* (1) Unidentified actinomycete — Group 1 (10) Unidentified actinomycete — Group 2 (2)
Not yet identified/ discriminated	4	*Kibdelosporangium* (1) Unidentified actinomycete — Group 1 (2) Unidentified actinomycete — Group 2 (1)

of these two parameters, the combined effect is clearly visible (Table 3). During screening period A, 0.3 percent of the cultures screened were found to produce glycopeptides; during screening period B, with the improved culture input and growth procedures, 1.6 percent of the cultures screened using the VAN^R screen were found to produce glycopeptides, a 5-fold increase in the discovery rate. Factoring in the improved screen, based on tripeptide reversal, the improvement in detection rate during period B was 7-fold (2.2%).

Antibiotics Identified in SK&F Screening

The identity of glycopeptide antibiotics produced by 47 producer cultures are shown in Table 6. Of previously described glycopeptide antibiotics, by far the most common was vancomycin, which accounted for 28% of the isolates. Ristocetin was also fairly common, accounting for 13% of isolates. Other known glycopeptides were less frequently encountered. Aridicins, first described by this group, accounted for 4 (8%) of the isolates during this period. Two other aridicin producers, including the prototype AAD-216 producer culture, were identified prior to the initiation of glycopeptide-directed screening. Thirty percent of the isolates produced novel compounds, a total of eight distinct compounds or complexes. A small number, 4, remain to be identified as known or novel. A description of the discrimination techniques will be published separately.

Discussion

There have been relatively few full descriptions of screening techniques used for the discovery of novel antibiotics. ŌMURA has described the screen for cell wall active antibiotics which led to the discovery of azureomycin, a novel inhibitor of the peptidoglycan synthesis[6,21]. Other reports include the β-lactam-targeted screens used at the Beecham, Takeda, and Fujisawa Research Laboratories[22~24]. These reports and others on antibiotic screening have tended to be limited to the assay procedures. The value of a holistic approach to antibiotic screening has previously been emphasized[11]. In this report we have demonstrated that optimization of strain selection, culture conditions and the screening system can markedly enhance the discovery rate.

One aspect of this holistic approach is the optimization of culture input. Techniques for the selection of antibiotic producer cultures have been previously described[8]. In our hands the use of antibiotics for the selection of glycopeptide producers was particularly effective. While firm conclusions are difficult to form, the results suggest that glycopeptide producers were favored by high molecular weight antibiotics such as vancomycin, novobiocin, and polymixin, whereas low molecular weight antibiotics such as gentamicin and D-cycloserine were less effective.

Glycopeptides were elaborated by a wide range of genera of actinomycetes, including *Nocardia*, *Actinomadura*, *Actinoplanes*, *Kibdelosporangium* and *Streptomyces* species. Eight strains were unclassifiable due to the absence of aerial mycelia and 28 strains produced long spore chains with cell wall type IV or type III. The last group probably cannot be accommodated in described genera and promises to provide descriptions of new genera of the *Actinomycetales*. The generic diversity of the cultures discovered and the large number of novel leads found substantiates the hypothesis that the utilization of a library of diverse and unusual organisms will enhance discovery.

One of the trends of antibiotic discovery programs has been toward a more rational approach to screening. Rather than using screens which detect any antimicrobial activity (open screening), researchers have recognized the value of using targeted screens to detect only inhibitors of a metabolic, biosynthetic, or structural target which is known or predicted to be essential to the microorganism[10]. Thus, the bacterial cell wall has been recognized as a highly desirable target for antibacterial intervention. The screen of ŌMURA[21] combined prescreening, by differential activity against mycoplasma, with secondary screening, by incorporation of radio-labeled cell wall precursors. Screens targeted to β-lactam structures, have been based on β-lactam hypersensitive strains of *Escherichia coli*[24], *Pseudo-*

monas aeruginosa[23], and *Bacillus megaterium*[25], or on the use of β-lactamase and carboxypeptidase inhibition[11]. In the case of the glycopeptide-targeted screen, we initially used a partially selective vancomycin-resistant test strain (*S. aureus* VAN^R) and subsequently used the highly specific tripeptide antagonism assay. The latter assay resembles receptor binding systems.

In contrast to the hypersensitive assays, the glycopeptide screens were not highly sensitive, the tripeptide antagonism assay having detection limits of 3.1 to 100 μg/ml for the different known glycopeptides. Despite the lack of high sensitivity, the high discovery rate demonstrates that the production media used were capable of enhancing expression of producer cultures to detectable levels. Thus, the enhancement of antibiotic expression can obviate the need for hypersensitive detection systems, although ideally they should be used synergistically.

The initial screen (period A) detected novel glycopeptide antibiotics at a rate of 1: 2,500 cultures screened whereas the optimized holistic screen (period B) detected novel antibiotics at a rate of 1: 320 cultures screened. These rates of discovery stand in considerable contrast to the commonly quoted figures of 1 novel antibiotic from 10,000 to 1: 100,000 cultures screened[21], or the 'over 1 million' cultures screened at Squibb to discover 6 novel β-lactones[25]. Indeed, glycopeptide antibiotics are common actinomycete secondary metabolites (1: 45 cultures screened), and novel antibiotics in general may be surprisingly common. Thus, the use of highly targeted screens, combined with techniques to optimize culture input to the screen, can be coupled to give dramatic reductions in the time and labor required to discover new therapeutic compounds. The extent to which such highly targeted screens can also be coupled to extremely rapid and facile discrimination of known compounds, and isolation and purification of lead compounds will be reported subsequently.

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