Journal of Industrial Microbiology, 13 (1994) 53–62

Published by The Macmillan Press Ltd

The distribution of antibiotic resistance patterns within streptomycetes and their use in secondary metabolite screening

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(Received 31 August 1993; accepted 23 November 1993)

Key words: Streptomycete ecology; Metabolite screening; Cluster analysis; Antibiotic resistance

SUMMARY

A collection of 169 streptomycetes representing natural isolates and type strains were examined for resistance phenotypes to 11 antibiotics. A total of 84 profiles were obtained with 18 patterns being repeated in two or more strains. The most common pattern was resistance to penicillin in an otherwise sensitive phenotype and accounted for 51 strains. This data was used to cluster the strains and groups defined were examined for correlation with bioactivity. Antibiotic producers were found in clusters 1, 2, 4 and 5–10. Certain strains in these areas were highly bioactive and typically had multiple resistances. Almost half of the collection of strains examined grouped in cluster 3, and were characterized as having a sensitive phenotype and virtually no biological activity in agrochemical screens.

INTRODUCTION

Antibiotic resistance patterns within the genus *Streptomyces* have been used both for speciation [17] and selective isolation [18]. Characterization by resistance to a wide variety of macrolide and aminoglycoside antibiotics has shown that many streptomycete strains have multiple resistances. Fujisawa and Weisblum [6] reported finding a diverse range of resistance phenotypes to macrolide, lincosamide and streptogramin antibiotics, which were not limited to strains producing these compounds. Hotta et al. [7] showed that certain aminoglycoside resistance patterns correlated with the production of specific aminoglycoside antibiotics.

A number of studies have reported that biosynthesis and resistance genes are genetically linked [2,10,11]; producers of auto-toxic compounds must have a self-defence mechanism [3]. Strains which produce identical compounds can be grouped together on the basis of resistance to antibiotics of the same chemical family [1,6,7]. There is, however, evidence that novel compounds can be found by selecting strains with unusual resistance profiles. Indolizomycin was discovered during protoplast fusion experiments because the resistance patterns of the producing strain differed from those of the two parental strains [22].

There may be a link between unusual resistance profiles and the production of specific secondary metabolites. If so, these profiles could be used to select strains capable of producing certain classes of biologically active compounds from natural populations. Our aim was to test this hypothesis and try to evaluate the phenotypic diversity of streptomycete populations based on antibiotic resistance profiles.

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MATERIALS AND METHODS

Strains

Strains were chosen to represent a heterogenous group of streptomycetes from different geographical locations and which were obtained using a variety of isolation methods (Table 1) [18]. A further 46 reference strains were chosen to represent a selection of known antibiotic producers (Table 2).

TABLE 1

Natural isolates selected for study of antibiotic resistance patterns

Strain series	Source method	Isolation	Medium	No. of strains
A	Italy	Chemostat	Starch	7
B	Warwick	Chemostat	C32	1
C	Warwick	Pour Plate	AGS	62
D	Warwick	Pour Plate	C32	26
E	Italy	Chemostat	C32	6
F	Warwick	Pour Plate	C32	2
MM	Martin Mere	Pour Plate	C32	5
RB	Italy	Pour Plate	AGS	3
JHCC	Various	Various	Various	4
JHCC	Various	Various	Various	7*
Total				123

* = Not used in cluster analysis. Refer to '*The use of resistance profiles to aid the selection of strains for screening*'.

TABLE 2

Type species selected for study of antibiotic resistance patterns

Culture collection reference no.	Name	Taxonomic identity[a]	Bioactive product	Antibiotic resistance[c]
KCC S-0446	*S. albidoflavus*	1		NOPS
ATCC27416	*S. annulatus*	1	Antibacterial Antifungal	TNeSNiPE
DSM40106[C1]	*S. azureus*	18	Thiostrepton	TP
DSM40598	*S. bacillians*	1		EKNeNiOPSTV
ATCC11062	*S. bikiniensis*	64	Streptomycin	TNeVSPOE
DSM40419[C1]	*S. caesius*	21		NoNiPOB
KCC S-0731[C1]	*S. caesius*	21		NoNiPB
ISP 5442	*S. coeliatus*	1		NiP
A3(2)[C1]	*S. coelicolor*	21	Methylenomycin Undecyl-prodigiosin actinorhodin Polyamines	SPB
ISP 5233	*S. coelicolor*	21	Polyamines	KTNoVSNiPE
ISP 4213[C1]	*S. endus*	32	Endomycins	VNiPO
DSM 40323	*S. flavogriseus*	1		NoVS
ATCC23907	*S. fluorescens*	1	Actinomycin-X	TNoVSNiPOE
DSM40236[C5]	*S. griseus*	1	Streptomycin	VSP
ATCC12760	*S. humidus*	19	Dihydro-streptomycin	NeVSNiP
KCC S-0772[C2]	*S. hygroscopicus*	32	Hygromycins	PO
NRLL3664[C4]	*S. hygroscopicus*	32	Scopofungin	NoVNiP
ATCC14607	*S. hygroscopicus*	32	Bluensomycin	TNeSPB
ATCC21705[C7]	*S. hygroscopicus*	32	Bialaphos	S
JHCC1002[C8]	*S. hygroscopicus*	32	Milbemycin	B
AM3672[C4]	*S. hygroscopicus*	32	Herbimycins Nigericin	NoNi
NRRL3602[C4]	*S. hygroscopicus*	32	Geldanamycin Nigericin	TNoVnIP
ISP 5550	*S. katraie*	61	Streptothricin Polyenes	NiPO
DSM40069[C1]	*S. lavendulae*	61	Streptothricin Polyenes	NiPB
ATCC3331	*S. lipmanii*			TNeNoVSPE
KCC S-0783	*S. lividans*	21		
KCC S-0785[C1]	*S. lusitanus*	44	Tetracycline Chlor-tetracycline	
KCC S-0495[C4]	*S. melanosporofaciens*	32	Melanosporium Elaiophylin	P
DSM40508	*S. naraensis*	1	Naramycins	TSNiPO
DSM40023	*S. nitrosporeus*	1	Nitrosporin	NoSNi
ATCC25481	*S. ornatus*	1	Ornamycin	TVSnIPOE
DSM40077	*S. rutgersensis*	1	Camphomycin	VSPO
DSM40445	*S. subrutilus*	61	Hydroxy-streptomycin	NeVSOK

Continued over

TABLE 2

Continued

Culture collection reference no.	Name	Taxonomic identity[a]	Bioactive product	Antibiotic resistance[c]
KCC S-0519[C1]	*S. thermotolerans*	19	Carbomycin	NiOP
ISP 5515[C5]	*S. vinaceus*	6	Viomycin	VPE
DSM40438[C1]	*S. violaceolatus*	21		TOPB
KCC S-0850[C4]	*S. violaceoniger*	32		Ni
DSM40049	*S.* sp.	1		TSNi
EMW463	*S.* spp.	1		TNeNiO
JHCC1319[C5]	*S.* spp.		Actin	TVSPEB
JHCC1390[C5]	*S.* spp.		Blasticidin	TSPE
KCC S-0331[C3]	*Stv. hachijoensis*	55	Trichomycin	P
ATCC27441	*Stv. ladakanum*	SMC[b]	5-Azacytidine	TNoNiPO
ATCC23934	*Stv. mashuensis*	55	Streptomycin	NiO
EMW746	*S.* spp.			KNeNiPS
EMW736	*S.* spp.			ENeNiNoOPSTV

Total number of strains = 46

[a] Species defined by Williams et al. [21].
[b] SMC = single member cluster group.
[c] Antibiotic resistance to B, blasticidin; E, erythromycin; K, kanamycin; Ne, neomycin; Ni, nigericin; No, novobiocin; O, oxytetracycline; P, penicillin G; S, streptomycin; T, thiostrepton; V, viomycin.
Culture collections, AM, Upjohn Co., Kalamazoo, MI, USA; ATCC, American Type Culture Collection, Rockville, MD, USA; DSM, German Collection of Microorganisms, Braunschweig, Germany; EMW, Elizabeth M. Wellington Culture Collection, Warwick University, UK; ISP, International *Streptomyces* Project (terminated); JHCC, Jealotts Hill (ICI) Culture Collection, Bracknell, UK; KCC, Kaken Culture Collection, Kaken Pharmaceutical Company Ltd, Tokyo, Japan; NRRL, Northern Regional Research Laboratories, Peoria, IL, USA.
[C1–C10] Present in clusters 1–10 respectively.

Media

Prior to antibiotic extraction, strains were maintained on oatmeal agar [20]. Spore suspensions were stored at −20 °C in 20% (v/v) glycerol. Strains were grown in 30 ml of A37 broth ($CaCO_3$ 2 g, Corn Steep Liquor 10 g, Glucose 5 g, NaCl 5 g, Soya Bean Meal 10 g, Starch 15 g, deionized distilled water up to 1 L, pH 7.0) in 200-ml Erlenmeyer flasks for 6 days at 28 °C and 200 r.p.m.

Bioactivity

Data summarizing the bioactivity of type-strains in the study were collated from published work (Table 2). Bioactivity data for natural isolates were from two sources; firstly, biological screens for activity against a wide variety of crop pests (insects, weeds and plant pathogenic fungi) were supplied by ICI Agrochemicals plc, Jealotts Hill Research station, Bracknell, UK (screening protocols not included). Secondly, data were obtained in the present study as detailed in the following section.

Extraction of bioactive compounds from soil isolates

Ethyl acetate (10 ml) was added to each culture (1:3 v/v) and the flasks were stoppered with rubber bungs to prevent the solvent from evaporating. These were shaken (200 r.p.m.) at room temperature for 1 h. The flask contents were transferred to centrifuge tubes and spun at 14000 *g*, 10 °C for 15 min. The solvent phase was removed, placed in a universal container and vacuum dried in a desiccator. The dried extracts were resuspended in 200 μl ethyl acetate and used for thin layer chromatography.

Thin layer chromatography (TLC)

TLC was carried out on 0.25-mm silica gel plates with fluorescent indicator (UV_{254}). The baseline was 2 cm from the bottom of each plate. Each sample (20 μl) was loaded as a 1-cm line along the baseline with a 1-cm interval between each sample and a 1.5 cm margin at the edge of the plate. Plates were run until the solvent front was 2 cm from the top of the plate. The following solvent systems were used: ethyl acetate: n-hexane: dichloromethane: methanol (v/v 9:6:1:1) to detect ansamycin antibiotics; geldanamycin (R_F = 0.14, 0.41, 0.62), herbimycins A (R_F = 0.37) and C (R_F = 0.31); dichloromethane: methanol (v/v 9:1) to detect nigericin (R_F = 0.72). Standards were run on all plates, the ansamycins were observed under UV light and nigericin identified as a scarlet spot visualized by spraying with 0.3% vanillin in ethanol plus 0.5% H_2SO_4 and then developed at 100 °C for 10 min. TLC plates were also used for bioautography.

Bioautography

TLC plates, prepared as above, were placed onto 200 ml base layer of nutrient agar in bioassay dishes (Nunc, Roskilde, Denmark). These were left for 30 min to allow the metabolites to diffuse into the medium, the plates were then removed and a seeded overlay (45 ml) containing 200 μl log phase *B. subtilis* cells (1×10^6) was poured evenly onto the base layer. These dishes were incubated at 37 °C overnight. For antifungal bioautograms TLC plates were sprayed with *Aspergillus niger* spores (1×10^5) in glycerol salts solution (7 ml per plate). These were placed in bioassay dishes and suspended above wet filter paper to provide a humid environment for 3–7 days at 30 °C. Zones of inhibition for both types of bioautography were compared with spots on replicate TLC plates.

Gradient plates

150 ml arginine glycerol salts medium (AGS) [19] was poured as a 0–5 mm slant in a bioassay dish (22 × 22 cm). A further 150 ml AGS, containing the prescribed antibiotic was poured on top of the base layer to give a flat surface. The plates were poured immediately prior to use.

Determination of resistance

A spore or mycelial suspension (75 μl of 1×10^7 CFU ml^{-1}) was inoculated in an even line, using a Finn pipette, across an AGS gradient plate containing one of the following antibiotics and incubated for 6 days at 30 °C. The antibiotics tested were blasticidin (0–100 $\mu g\ ml^{-1}$), erythromycin (0–100 $\mu g\ ml^{-1}$), kanamycin (0–100 $\mu g\ ml^{-1}$), neomycin (0–10 $\mu g\ ml^{-1}$), nigericin (0–17 $\mu g\ ml^{-1}$), novobiocin (0–100 $\mu g\ ml^{-1}$), oxytetracycline (0–100 $\mu g\ ml^{-1}$), penicillin G (0–100 $\mu g\ ml^{-1}$), streptomycin (0–10 $\mu g\ ml^{-1}$), thiostrepton (0–50 $\mu g\ ml^{-1}$) and viomycin (0–30 $\mu g\ ml^{-1}$). These tests were done in duplicate for each strain studied and results were only taken when control plates, containing no antibiotic, showed growth.

Antibiotic concentrations at which strains showed resistance were extrapolated from the gradients as follows: resistance was measured as length of line of growth (mm)/ total length of gradient (mm) × the maximum antibiotic concentration ($\mu g\ ml^{-1}$) = level of antibiotic resistance ($\mu g\ ml^{-1}$) (Figs 1 and 2).

Antibiotics were obtained from Sigma Chemical Company Ltd, Poole, UK, except for geldanamycin, which was kindly supplied by the Upjohn Co., Kalamazoo, MI, USA and blasticidin, which was a gift from ICI Agrochemicals plc, Bracknell, UK.

Data handling

Foxbase was used as a data base (Fox Software Inc., Perrysberg, OH, USA). Cluster analysis was achieved using NTSYS-pc (Exeter Publishing Ltd, Setauket, NY, USA). To deduce any relationship between resistance and bioactivity, the resistance data were first converted to binary form as described in Fig. 1. Test error for these data was 4.8% (Table 3) and within the 5% level recommended by Sneath and Johnson [14].

Similarity values were calculated for the data matrix using SIMQUAL in conjunction with either the Simple Matching [15] or Dice coefficient [4]. A variety of clustering algorithms were then used to cluster the strains. Phenograms were created in SAHN using the unweighted pair group method with arithmetic averages (UPGMA) [15], single [7] and complete linkage [10]. Groups of related strains were obtained at 51% SDICE similarity level (Fig. 3).

A computer program was written to calculate the key resistances diagnostic for each group of strains and investigate specific bioactivities in each group. The significance of this information was examined using standard deviations from the average values of the whole sample set. The goodness of fit between similarity matrices and cophenetric matrices (calculated from the phenograms using COPH) was done in the MXCOMP program for matrix comparison.

RESULTS

The distribution of antibiotic resistance in soil isolates

The distribution of resistances to ten antibiotics amongst the collection of soil isolates is presented in Figs 1 and 2. Resistance to kanamycin was found in only one strain, which was included in the phenogram, so data for this antibiotic are not presented. Certain strains which were not included in the phenogram (Types in Table 2) also had kanamycin resistance. Two forms of distribution were found for antibiotic resistance in the population: type A where increasing concentrations of the antibiotic elicited an exponential decrease in numbers of strains resistant followed by change in gradient (elbow of the curve) where the resistant population numbers became constant. This allowed identification of resistant strains as those able to grow at the antibiotic concentration beyond the elbow of the curve. The other type of resistance distribution, type B, occurred when the population of strains showed a more gradual variation of phenotype. It was difficult to define resistant and sensitive populations for this type of distribution and so for the purposes of clustering, a somewhat arbitrary value was assigned by selection of a cut-off point when the resistance level in the population declined less markedly. Consequently the data for resistance to these antibiotics showed a greater probability of an erroneous result (Table 3).

Resistance to nigericin, oxytetracycline and penicillin was common (Figs 1 and 2) and populations showed a gradual decline with concentration of the type B. Although there are 4×10^7 theoretical antibiotic resistance profiles (11!), only 18 patterns were found amongst 169 different strains (Table 4). Sixty-five strains exhibited unique resistance profiles and all of the profiles, except three, contained at least one of the more common antibiotic resistances. Twenty-five percent of isolates showed a sensitive phenotype with resistance only to penicillin.

Characteristics of clusters, defined by antibiotic resistance patterns

The average linkage method [15] with the DICE coefficient [4] allowed delimitation of ten clusters at 51% similarity

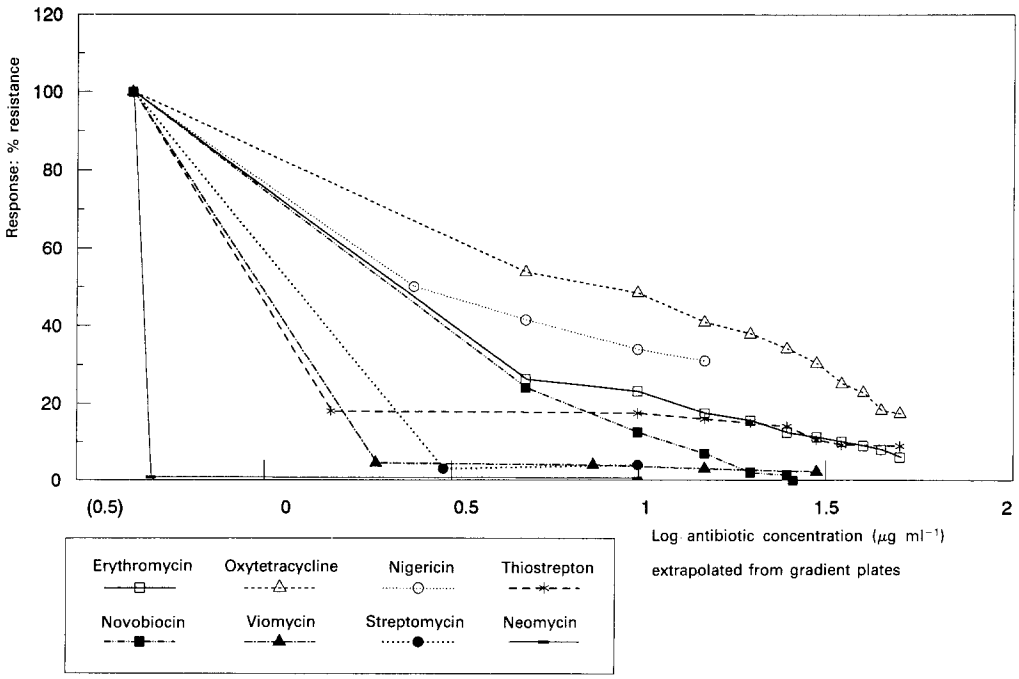

Fig. 1. Growth profiles for a random selection of *Streptomyces* strains along antibiotic concentration gradients. Resistance levels (x-axis) extrapolated from the gradient plates as described in Materials and Methods; y-axis, percent of population able to grow. The elbow of each curve was used to denote the antibiotic concentration where growth indicated a resistant phenotype for binary coding.

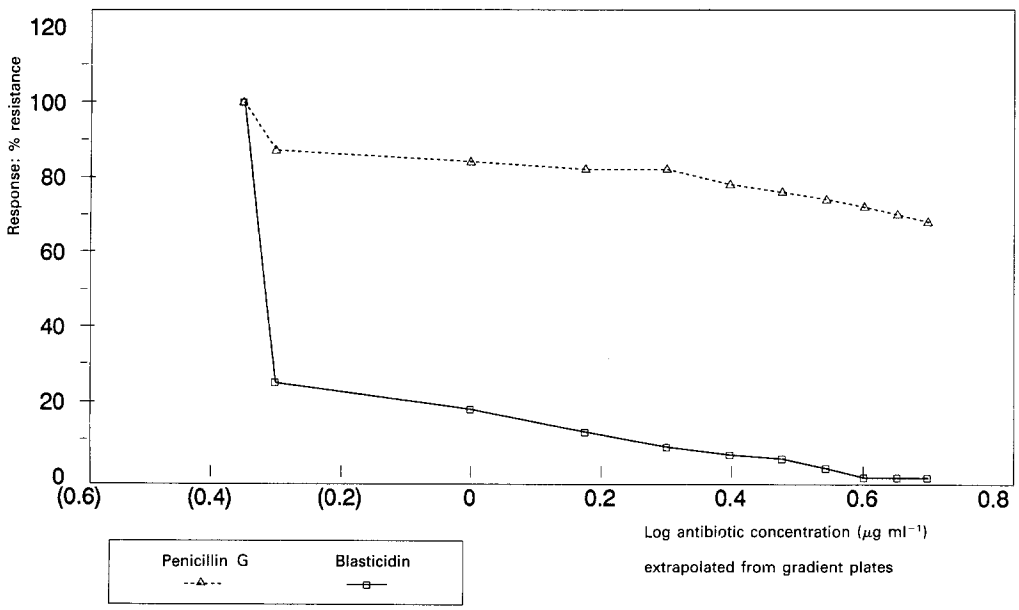

Fig. 2. Growth profiles for *Streptomyces* strains along concentration gradients of penicillin G and blasticidin, x and y-axis as for Fig. 1.

(Fig. 3). Each cluster in the phenogram owes its identity to a unique combination of resistances. The characteristics of the clusters were examined with respect to biological activity and these data are presented as a stacked bar chart in Fig. 4 (giving cumulative percentages for each category). The stacked bars represent the proportion of strains with resistant and bioactive phenotypes within each of the clusters defined in Fig. 3. Cluster 3 comprised 37% of all strains in the study; there were only three bioactive strains in this large cluster, one produced geldanamycin and nigericin, another

TABLE 3

Test error[a] estimated for determination of resistance to various antibiotics

Resistance to	*P* error[a]
Thiostrepton	3.8
Neomycin	1.3
Novobiocin	3.1
Viomycin	1.2
Streptomycin	1.7
Nigericin	9.6
Oxytetracycline	6.8
Erythromycin	6.8
Blasticidin	6.8
Kanamycin	0.3
Penicillin G	12.6

[a] *P* is the probability of an erroneous result calculated using the formula of Sneath and Johnson [14], the average *P* value for all resistance tests was 4.8%.

was an actin producer and the third a trichomycin producer. Other strains in cluster 3 showed no activity on any of the screens.

The remaining 63% of strains were in the major clusters 1, 2 and 4 (>20 strains) and minor clusters 5–10. These included a wide range of known antibiotic producers from a variety of chemical classes with diverse modes of action (Table 2). There were biologically active natural isolates, including producers of ansamycins, polyethers and unknown antibacterial and antifungal compounds. Agrochemically inactive strains were also present in these clusters. Major clusters 1 and 2 contained 27% and 11% respectively of the bioactive strains; cluster 4 had 34% and minor clusters 5 to 10, 14%. Similar results were observed with other phenograms, obtained using different clustering algorithms and similarity coefficients (data not presented).

Distribution of resistance phenotype in the major clusters

Table 5 illustrates how groups of strains in cluster 3 of the phenogram were phenotypically less resistant than the other strains. The main resistance exhibited by strains in this cluster was penicillin, which was the most commonly observed character throughout the data (Figs 1, 2 and Table 5). Although resistance versus sensitivity to this antibiotic was not a clearly defined trait for the population studied (Fig. 2), most of the population could be classified as resistant.

Penicillin resistance was present in large numbers of strains in clusters 1, 3 and 5 (Table 5). However, the fact that no strain clustering to the minor clusters 6, 9 and 10 was penicillin resistant showed that sensitivity was useful in identifying unusual strains. Neomycin and kanamycin resistances were very rare and had no effect on the clustering.

Clusters containing bioactive strains were distinguished from cluster 3 by having isolates with resistances other than

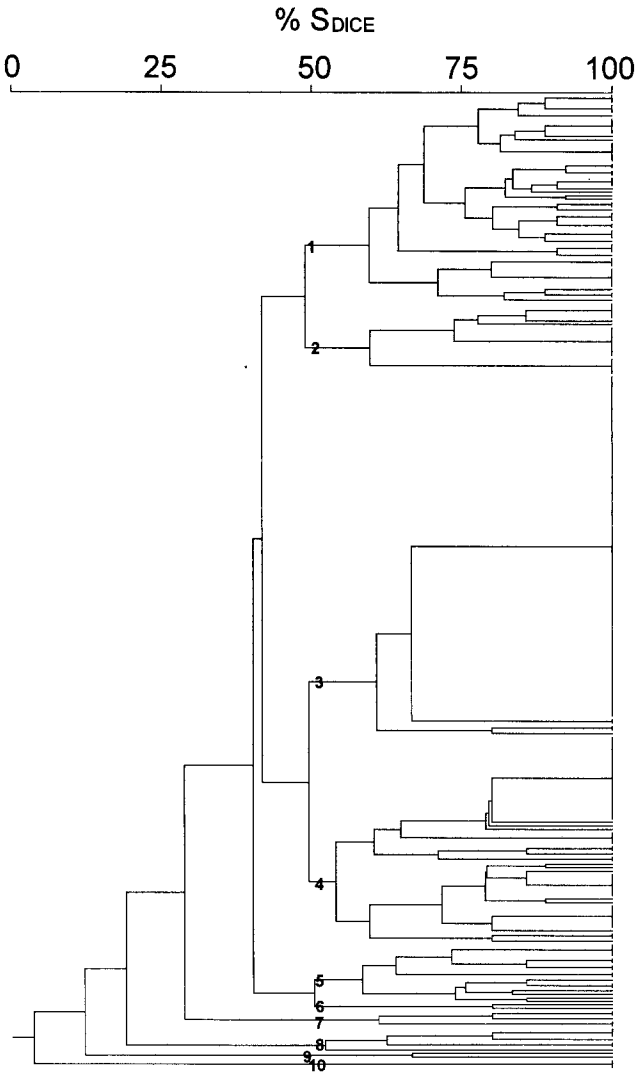

Fig. 3. Phenogram derived from clustering antibiotic resistance profiles. Similarity calculated using Dice coefficient with binary data and clustered by UPGMA. Ten clusters were defined at 51% similarity.

penicillin. The five minor clusters (6–10) comprised smaller ranges of resistances than clusters 1, 2, 4 and 5 and contained strains with the most unusual profiles. The milbemycin producer (JHCC1002) was the only producer of a xenotoxic compound in the study. It grouped to cluster 8, showing it had a very different resistance profile.

Use of resistance profiles to aid selection of strains for screening

Four of the strains included in the study had individual activity spectra that indicated the presence of potential novel compounds and which warranted chemical characterization. This level of interest was typical for the sample size and the screens used. In order to test the hypothesis that antibiotic resistance profiles could help select strains for screening, a selection of seven new strains were put through the clustering

TABLE 4

Distribution of resistance patterns

Antibiotic resistance	Pattern type 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19–83
Blasticidin	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	Various
Erythromycin	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	
Kanamycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Neomycin	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
Nigericin	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	
Novobiocin	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	
Oxytetracycline	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	
Penicillin G	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Streptomycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	
Thiostrepton	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	1	1	1	
Viomycin	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	
Number of strains	51	7	5	5	5	4	3	3	3	2	2	2	2	2	2	2	2	2	65

Total no. strains = 169.
Total no. combinations, which are possible with 11 antibiotics = 4×10^7.

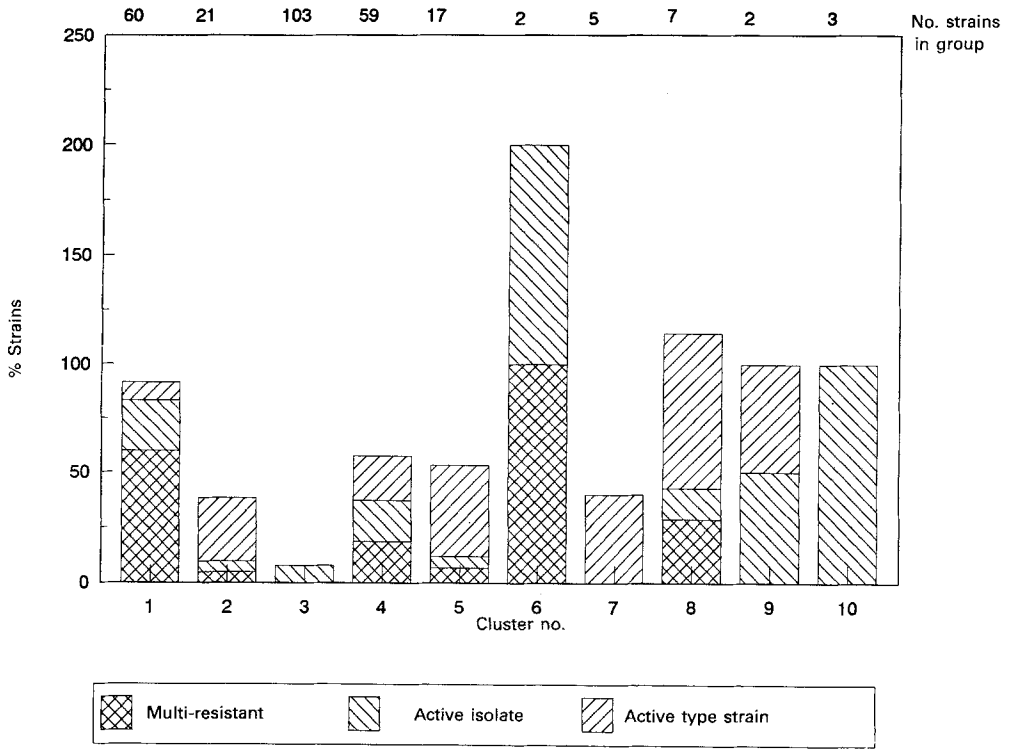

Fig. 4. Cluster attributes based on antibiotic resistance (to three or more antibiotics) termed multi-resistant and bioactivity, determined by the production of one or more detectable antibiotics, termed active for wild isolates and type strains. *Y*-axis represents the cumulative percentages of strains in each cluster which were multi-resistant and/or active and *x*-axis indicates the cluster. Number of strains includes 116 soil isolates (Table 1) and 22 type cultures (Table 2). 135 strains were duplicated and three were triplicated, giving a total of 279 data sets.

system. These strains also produced compounds of possible novel agrochemical activity. Each time a new resistance profile is added the clustering is slightly altered. The resulting phenogram showed that all of the seven new strains and the four original strains had multiple patterns of between four and seven antibiotic resistances (average = 5.7). Consequently all of these strains exhibit resistance characteristics which would place them in an area of the phenogram

TABLE 5

The distribution of antibiotic resistance in the major and minor clusters

Cluster (no.)	Antibiotic resistance										
	Blasticidin	Erythromycin	Kanamycin	Neomycin	Nigericin	Novobiocin	Oxytetracycline	Penicillin G	Streptomycin	Thiostrepton	Viomycin
1 (60)	88.3	33.3	0.0	0.0	76.7	23.3	71.7	76.7	10.0	35.0	11.7
2 (21)	0.0	0.0	0.0	0.0	0.0	100.0	0.0	33.3	71.4	4.8	0.0
3 (103)	0.0	0.0	0.0	0.0	1.0	1.9	0.0	100.0	0.0	0.0	2.9
4 (59)	0.0	3.4	1.7	1.7	86.4	13.6	5.1	83.1	1.7	35.6	3.4
5 (17)	35.3	100.0	0.0	0.0	17.7	0.0	11.8	100.0	5.9	41.2	11.8
6 (2)	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0
7 (5)	40.0	40.0	0.0	0.0	0.0	0.0	20.0	60.0	100.0	20.0	0.0
8 (7)	28.6	28.6	0.0	0.0	0.0	0.0	14.3	42.9	71.4	14.3	0.0
9 (2)	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	100.0	0.0
10 (3)	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0
Mean	24.0	13.4	0.4	1.4	40.4	16.5	15.8	87.8	5.0	19.0	6.8
SD	36.6	26.7	0.7	0.5	39.5	25.6	28.6	23.3	4.2	18.7	4.3

Mean values were calculated from the % strains which were resistant within each cluster and took into account the number of strains present in each group.

which would reinforce their selection as candidates for screening. Resistance profiles for additional reference strains (Table 2) were also completed and this data reinforced the theory that there is a correlation between antibiotic production and resistance. Strains with multiple resistances were more likely to produce bioactive metabolites. Seventy-four percent of the known antibiotic producers (Table 2) had three or more resistances, whilst two of the remaining type strains had a resistance profile consistent with those found in cluster 3.

Examination of test error

Of the 4.8% total test error shown in Table 3, 60% was due to duplicate strains falling either side of the cut-off point used to score resistance as a binary character. The extent to which these conflicting results affect the clustering was examined in the work described above and found to be negligible. The remaining anomalous results comprised absolute differences in resistance, possibly due to differential gene expression. It was discovered that a subset of nine strains were highly variable in resistance phenotype, causing 22% of all discrepancies observed (three discrepant results each). These strains are currently under further study. They comprise a small percentage of all strains (4.5%) studied and their presence in Fig. 1 did not distort any of the trends observed.

Clustering of specific antibiotic producers

One of the problems of drug discovery is that novel compounds can be produced in conjunction with known broad spectrum antibiotics. This can mean that the novel compounds remain unobserved if their spectrum of activity is similar to that of the known antibiotic. For example nigericin is a potent polyether antibiotic produced by many streptomycetes; 75% of nigericin producers in this study synthesized other bioactive compounds. Nigericin producers did not cluster to a specific region in any of the phenograms studied because each nigericin-producing strain had a unique antibiotic resistance profile.

In order to use clustering position in a predictive way, clusters which contained more than 2 SD above the mean number of nigericin producers were used for diagnosis. Seventeen strains (64% of nigericin producers in the study) were detected by using both single and complete linkage with the DICE coefficient. In addition 12 strains which did not produce nigericin were diagnosed as being possible nigericin producers. An alternative means of identifying the producers of specific broad spectrum compounds would be to use resistance profiles based on a single subfamily of compounds as in the work of Hotta et al. [7]. Resistance profiles to polyether antibiotics might be very specific and only predictive in terms of that class of compounds. Nigericin resistance was not useful in predicting production because although 15% of strains produce it, 40% of strains were resistant to it. Also not all of the strains which were confirmed as nigericin producers showed resistance to this compound above the cut-off point (Fig. 1). This may reflect a difference in the type of resistance mechanism used by nigericin-producing strains compared to non-producing strains.

DISCUSSION

Examination of the resistance and bioactivity data of the total collection of strains has given a clear indication that there are two populations; one with multiple resistance and the other with multiple sensitive phenotypes. This may be a reflection of the natural population and so provides an insight into the distribution of antibiotic resistance and biosynthesis genes in the environment. The clustering data also supports the observations of others that a correlation exists between multiple resistance and antibiotic production. Resistance profiles obtained using a range of diverse antibiotics were useful for predicting isolates likely to be bioactive but this may be a reflection only of the phenotype rather than the genotype. However, it was also observed that resistance was more widespread than production confirming the observations of Fujisawa and Wiesblum [6] who noted that phenotypic antibiotic resistance may not be confined to producers of these compounds. Blasticidin resistance, for example, was not an indicator of blasticidin production nor of any other nucleotide antibiotic. Of 39 blasticidin-resistant strains analyzed, only one strain was a blasticidin producer although some strains exhibiting resistance may possess a silent form of the antibiotic production genes.

Resistance may be linked with production of a closely related compound; for example Skeggs et al. [13] have shown that single aminoglycoside resistance determinants can confer resistance to several different 2-deoxystreptamine derivatives, namely kanamycin, apramycin and gentamycin. Resistance may be linked to another class of compound, perhaps with a similar mode of action. An example of this is the *erm* gene, which encodes an rRNA methylase and simultaneously confers resistance to a selection of antibiotics from three antibiotic chemical families [6,8,23].

Exceptions to the trends shown in this study might be better understood by considering the role of antibiotics in the natural environment. Certain antibiotic resistances in some strains may be present solely to confer protection against the metabolites of other streptomycetes. For example organisms which produce beta-lactams and nigericin are commonly isolated from the soil. The production of these compounds in the natural environment would provide a selective pressure for antibiotic resistance in streptomycetes. The large numbers of penicillin and nigericin-resistant strains observed in this study provides evidence for this hypothesis. Alternatively a gene conferring resistance could do so fortuitously, having an entirely different function in the host. For example some streptomycetes have two pathways of ammonium assimilation; these organisms are resistant to the herbicide bialaphos, the target of which is glutamine synthetase. Some resistance genes may not be expressed under laboratory conditions. No correlation was found between taxonomic status and clustering by resistance profiles, suggesting that antibiotic resistance is strain specific.

Streptomyces species show heterogeneity at the species level based on DNA homology [9] and 16S rRNA analysis [16] so it is also possible that there are many different genetic determinants and mechanisms for resistance. Conventional taxonomy is based on the phenotype including morphology and physiology relating to primary metabolism, which appears to have little in common with antibiotic production and resistance. Numerical taxonomy should not therefore be relied upon as a means of selecting potential antibiotic producers.

ACKNOWLEDGEMENTS

We acknowledge the support of the Science and Engineering Research Council for the award of a CASE studentship (LP) and ICI for financial support. We are grateful to Dorothy Sanders for strain identifications, Michael Sackin for advice on clustering and Barry Joyce for help with Foxbase.

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