THE DISTRIBUTION OF DNA SEQUENCES HYBRIDIZING WITH ANTIBIOTIC PRODUCTION AND RESISTANCE GENE PROBES WITHIN TYPE STRAINS AND WILD ISOLATES OF *Streptomyces* SPECIES

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Total DNA preparations from 74 antibiotic-producing type strains and 102 natural *Streptomyces* isolates were examined by dot blots for homology to 6 antibiotic production and resistance genes. Pattern diversity of hybridizations decreased as stringency increased from 65% to 85%. There were 146 unique profiles at 65% stringency with 13 repeated patterns, whilst there were only 14 unique and 11 repeated profiles at 85% stringency. Most of the strains which hybridized at 85% reacted with one or two probes although a few strains showed multiple homologies. This data was used to cluster strains and the groups defined were examined for phenotypic antibiotic resistance. Producers of certain classes of antibiotics clustered to specific groups and some gene homologies were more common amongst strains which produced similar antibiotics.

Traditionally *Streptomyces* taxonomy has grouped species by phenotype[1], but these species-groups are heterogeneous both at the DNA level[2] and as determined by 16S rRNA analysis[3]. Although selected *Streptomyces* species have distinctive antibiosis and resistance patterns[4], there is generally little correlation between antibiotic production and taxonomic groupings.

Resistance to a specific antibiotic is not limited to known producers[5] and different resistance determinants for the same antibiotics have been described. Kanamycin resistance can be conferred by ribosomal methylation in *Streptomyces kanamyceticus* and *Streptomyces tenebrarius* or by acetylation of the drug by both the producing strain and *Streptomyces griseus*[6]. Some resistance determinants confer resistance to more than one antibiotic in the same chemical family[6]. Other resistance genes confer resistance to compounds which belong to different chemical classes but they have similar modes of action; for example the *erm* gene confers resistance to antibiotics from three chemical classes of antibiotic[5,7,8].

Several studies have shown that many streptomycetes have multiple resistance patterns, which can be correlated with the production of antibiotics in the same chemical family[5,9,10]. The clustering of antibiotic production and resistance genes on the streptomycete chromosome has been clearly demonstrated for a

range of species[11~13] and in several cases resistance genes have a role in biosynthesis. However, the distributions of antibiotic resistance mechanisms and their genetic determinants amongst populations of streptomycetes has not been well studied.

Previous work has shown a dichotomy in the phenotypic expression of secondary metabolism in streptomycete natural isolates[10]. The aim of this study was to investigate the use of DNA probes, derived from antibiotic resistance and biosynthesis genes, for further analysis of antibiotic resistance profiles amongst natural populations of streptomycetes. The strains studied were already grouped on the basis of antibiotic production, resistance and conventional taxonomy.

Materials and Methods

Strains

74 type strains were chosen to represent a selection of producers of 46 known and 5 unclassified antibiotic compounds and 23 non-producing strains (Tables 1, 6, 7). An additional 102 natural *Streptomyces*

Table 1. *Streptomyces* type species selected for study of antibiotic resistance patterns.

Culture collection reference No.	Name	Taxonomic identity[a]	Bioactive product
DSM40260	*Streptomyces albofaciens*	C42	Oxytetracycline
DSM40106	*S. azureus*	C18	Thiostrepton
KCC S-0459	*S. bottropensis*	C19	Bottromycin
DSM40419	*S. caesius*	C21	
KCC S-0731	*S. caesius*	C21	
A3 (2)	*S. coelicolor*	C21	Methylenomycin Undecylprodigiosin Actinorhodin Polyamines
ISP 5233	*S. coelicolor*	C21	Polyamines
KCC S-0313	*S. coralus*	C19	
ISP 4213	*S. endus*	C32	Endomycins
NRLL3664	*S. hygroscopicus*	C32	Scopofungin
ATCC21705	*S. hygroscopicus*	C32	Bialaphos
JHCC1002	*S. hygroscopicus*	C32	Milbemycin
AM3672	*S. hygroscopicus*	C32	Herbimycins Nigericin
NRRL3602	*S. hygroscopicus*	C32	Geldanamycin Nigericin
ATCC3331	*S. lipmanii*	C1	
KCC S-0783	*S. lividans*	C21	
KCC S-0785	*S. lusitanus*	C44	Tetracycline Chlortetracycline
KCC S-0495	*S. melanosporofaciens*	C32	Melanosporium Elaiophylin
DSM40268	*S. rimosus*	C42	Oxyteracycline
ISP 5515	*S. vinaceus*	C6	Viomycin
DSM40438	*S. violaceolatus*	C21	
KCC S-0850	*S. violaceoniger*	C32	
JHCC1319	*Streptomyces* sp.		Actin
JHCC1390	*Streptomyces* sp.		Blasticidin
JHCC1233	*Streptomyces* sp.		Bialaphos
JHCC1234	*Streptomyces* sp.		Cycloheximide
KCC S-0331	*Streptoverticillium hachijoensis*	C55	Trichomycin
Total			49 Strains

[a] Clusters defined by WILLIAMS *et al.* (1983a).

isolates were chosen to represent strains exhibiting multiple resistance or sensitivity to a selection of antibiotics, resistance to an antibiotic for which there was a gene probe, and a range of antibiotic producers. DNA from *Streptomyces lividans* 66 (John Innes 1326), *Erwinia carotovora* (SCR1193) and salmon sperm (Boehringer) was selected as negative controls. Strains were maintained as previously reported[10].

Antibiotic Resistance

Resistance was defined by reference to sensitivity of a large population of streptomycetes (196 strains). This was achieved by testing the population using gradient plates composed of arginine glycerol salts medium (AGS); glycerol 12.5 g, arginine 1 g, KH_2PO_4 1 g, NaCl 1 g, K_2SO_4 0.5 g, agar (Oxoid No 1) 15 g in 1 liter tap water, streaked with 75 μl of 10^7 spore/mycelium cfu/ml in a line from one end of the gradient to the other. Gradients were poured in Nunc bioassay dishes (22.5 cm × 22.5 cm). The first layer (150 ml AGS) was poured as a 0~0.5 mm slant, whilst the top layer (150 ml AGS plus antibiotic) was poured onto the base layer to give a flat surface. Growth across a gradient was measured in cm and since strains did not grow where an antibiotic had reached an inhibitory level the measurement was related to minimum inhibitory concentration. The population of strains tested showed a linear decline in numbers versus distance grown along gradients; resistance was defined when this response became limited to a constant fraction of the population. The distances at which population response became constant were as follows: Thiostrepton (2.25 cm, where the range tested was 0~50 μg/ml), neomycin (4.5 cm for a range from 0~10 μg/ml), novobiocin (4.5 cm for a range of 0~100 μg/ml), viomycin (4.5 cm for a range of 0~30 μg/ml), streptomycin (7.88 cm for a range of 0~10 μg/ml), erythromycin (9 cm for a range of 0~100 μg/ml), oxytetracycline (10.13 cm for a range of 0~100 μg/ml), kanamycin (0.5 cm for a range of 0~100 μg/ml), nigericin (19.85 cm for a range of 0~17 μg/ml), blasticidin (0.5 cm for a range of 0~100 μg/ml) and penicillin G (21.38 cm for a range of 0~100 μg/ml). Strains with growth on gradients equal to or greater than the above values for each antibiotic, were classified as resistant. Antibiotics were obtained from Sigma Chemical Company Ltd., except for viomycin, which was kindly supplied by the Pfizer Ltd. and blasticidin, which was a gift from ICI Agrochemicals plc.

DNA Probing

Total DNA was isolated using the rapid small scale method of *Streptomyces* total DNA[14]. Standardization of DNA was achieved by spectrophotometry and 1 μg DNA was used for each sample. This was denatured by adding an equal volume of 0.5 M NaOH with 1.5 M NaCl and incubated at room temperature for 10 minutes. The reaction was neutralized with four volumes of 0.5 M HCl, 0.5 M Tris-HCl (pH 7.4) and 1.5 M NaCl, 20 × SSC (175.32 g/liter NaCl + 88.23 g/liter Na-citrate), mixed as a 1 : 1 : 2 ratio. Hybond nylon filters were prewetted in 10 × SSC and placed in a blot manifold (Hybri-Dot Manifold-1050MM, Bethesda Research Laboratories). Samples were put into the wells and transferred under vacuum onto the nylon filter, which was removed from the manifold and dried. DNA was fixed by UV cross-linking on a longwave transilluminator for 4 minutes. Filters were subjected to prehybridization using the method of HOPWOOD *et al.*[14], but in a Hybaid oven (Hybaid) overnight at 70°C using 15 ml and with two filters per tube.

Internal fragments of the following genes were used; streptomycin phosphotransferase (*aphD*) excised from pCKL719 (originally cloned from *S. griseus* ATCC12475)[15]; dimethyl phosphinothricin acetyl transferase (*bar*) excised from pIJ4104 (origin *Streptomyces hygroscopicus* ATCC21705)[13]; viomycin phosphotransferase (*vph*) excised from pIJ673 (origin *Streptomyces vinaceus* ATCC11861)[16]; neomycin phosphotransferase (*aph*) excised from pIJ680 (origin *Streptomyces fradiae* ATCC1074520)[16]; an rRNA methylase conferring resistance to thiostrepton (*tsr*) excised from pIJ680 (origin *Streptomyces azureus* ATCC14921)[17]; a novobiocin resistance determinant (Nb^r) excised from pGL103 (origin *Streptomyces niveus* ATCC19793)[18]. Single stranded DNA was labelled using ^{32}P dCTP by random primed labelling (Molecular Biology Boehringer Mannheim). Unincorporated DNA was removed using a Sephadex G50 column[14]. The hybridization was carried out in a Hybaid Oven at 70°C, using the procedure of HOPWOOD *et al.*[14]. Filters were washed twice, in a volume of 50 ml, for 30 minutes at each of three levels of stringency. These were 3 × SSC and 0.1% SDS, 1 × SSC and 0.1% SDS and 0.2 × SSC and 0.1% SDS; at a temperature of 70°C equivalent to 65%, 75% and 85% homology assuming a G+C content of 73%. Autoradiography was carried out at each stringency; X-ray film (Fuji X-Ray) was exposed to hot filters at

−70°C. Depending on the specific activity of each probe varying times of exposure were used, but in general these were one week after the first wash, two weeks after the second and four weeks after the third. The Phosphorimager (Molecular Dynamics Ltd.) was also used to record positive hybridizations and so exposure times were greatly reduced by use of direct counting. Positive hybridizations were scored only on filters where the negative controls did not hybridize and positive controls (1 ng isolated gene, and 1 μg DNA from the source organism) hybridized. Positives at each stringency were identified where the dot blot signal was significantly greater than background as illustrated in Fig. 1. The negative control gave readings of 1186 (*S. lividans*, 1326), 735 (salmon sperm DNA) and 251 (SCR119) compared with a positive control reading of 96865. Dots giving a reading greater than 2000 were taken as positive. When such filters were exposed to X-ray film for autoradiography, results were recorded and compared to counting data. Dots taken as positives, therefore, showed a range of strengths in hybridization signals.

Data Handling

Cluster analysis was achieved using NTSYS-pc (Exeter Publishing, Ltd.). Relationships between gene homology and antibiotic production were deduced using either interval data or binary data relating to the homology each strain had with the gene probes. Similarities were calculated in SIMINT usig Euclidian distances and clusters defined by UPGMA.

Results

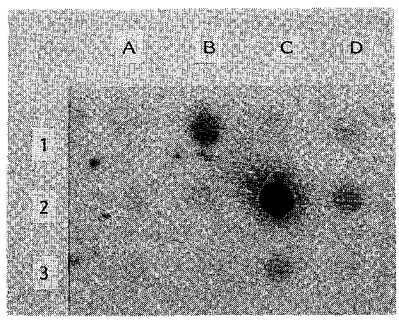
The Distribution of Sequences Hybridizing with Probes

Positive signals from dot blot hybridizations declined as stringency increased from 65 to 85% homology (Table 2). At high stringency the most common hybridizations were to the streptomycin and neomycin phosphotransferase gene probes and the least common was to the bialaphos resistance determinant (*bar*) probe.

The diversity of profiles of homology at high stringency is given in Table 3. Theoretically there are 720 combinations (6!) of 6 gene probes; this contrasts with the 25 profiles which were observed amongst 176 different strains. Homology to either or both the streptomycin and neomycin phosphotransferases was common. Multiple hybridizations were rare and confined only to one or two strains in each of the pattern types illustrated in Table 3. Examination of homology patterns at lower levels of stringency revealed greater diversity; at 65% homology there were 13 profiles repeated amongst 30 strains and 146 which were unique.

Fig. 1. Dot blot produced using the Phosphorimager.

A1, DSM4112 (2493); A2, ISP 5060 (3095); A3, ISP 5174 (2104); B1, ISP 5196 (17338); B2, ISP 5556 (4340); B3, ISP 5329 (1401); C1, DSM 40232 (6793); C2, ATCC 12475 (96865); C3, ISP 5246 (5679); D1, DSM 40419 (3338); D2, DSM 40438 (6576); D3, DSM 40455 (2271).



Sample DNA was hybridized with a streptomycin resistance gene probe (*aphD*), overnight at 70°C with in hybridization solution and washed twice with 3×SSC and 0.1% SDS for 30 minutes (equivalent to 65% homology, assuming G+C=73%), DNA from the indicated strains was probed and the Phosphorimager signals (arbitrary units) are given in parenthesis.

Table 2. The distribution of sequences hybridizing with antibiotic resistance gene probes.

Gene	65% Stringency No. strains	73% Stringency No. strains	85% Stringency No. strains
aphD	122	99	56
aph	79	45	35
tsr	99	21	16
vph	105	48	13
Nb^r	48	25	15

Table 3. Patterns of hybridization (85%).

Pattern	Streptomycin	Neomycin	Viomycin	Thiostrepton	Novobiocin	Bialaphos	No. strains
1[a]	−	−	−	−	−	−	76
2	+	−	−	−	−	−	28
3[b]	−	−	−	−	−	−	24
4	+	+	−	−	−	−	12
5	−	+	−	−	−	−	9
6	−	−	−	+	−	−	3
7	−	−	−	−	−	+	3
8	+	+	+	+	+	+	2
9	+	+	+	+	+	−	2
10	+	−	+	+	+	−	2
11	+	+	+	−	+	−	2
12	+	+	−	+	+	−	1
13	+	+	+	−	−	+	1
14	+	+	+	+	−	−	1
15	+	+	−	−	+	−	1
16	−	+	−	+	+	−	1
17	+	+	+	−	−	−	1
18	+	+	−	+	−	−	1
19	−	−	−	+	+	−	1
21	−	−	+	+	−	−	1
22	+	−	−	+	−	−	1
23	+	−	−	−	+	−	1
24	−	−	−	−	+	−	1
25	−	+	+	−	+	+	1
No. strains	56	35	13	16	15	7	176

[a] These strains did not hybridize at 85%, but did show binding at lower stringency.
[b] These strains did not hybridize at any of the stringencies used.

Table 4. The distribution of antibiotic resistance amongst strains probed with resistance gene probes.

Antibiotic	Number of strains (total = 176)			
	Resistant[a] and hybridized[b]	Resistant and non-hybridized	Sensitive and hybridized	Sensitive and non-hybridized
Streptomycin	19	31	39	87
Neomycin	6	17	29	126
Viomycin	9	33	4	132
Thiostrepton	13	53	3	109
Novobiocin	8	38	7	125

[a] Refers to phenotypic resistance.
[b] Refers to hybridization with antibiotic resistance gene probes at 85% stringency.

Correlation between Phenotypic Resistance and Hybridization with Gene Probes

Strains in the study were classified into 4 groups based on resistance phenotypes and probe hybridizations at 85% stringency (Table 4). Around 20~30% of strains classified as resistant to any of the antibiotics tested hybridized with probes used in this study. Sensitive, non-homologous strains formed the largest group, but the distributions amongst the other two categories varied (Table 4). *aphD* and *aph* probes hybridized with significantly more sensitive strains than did *tsr*, *vph* and Nb^r, which may suggest that the two former probes gave more non-specific binding. There were 16 strains with 3 or more homologies, representing 48.5% of all positives observed at high stringency and two fifths of these had resistant

Table 5. The clustering of strains hybridizing with antibiotic resistance gene probes.

No. of Homologies[a]	Type strains		Natural isolates	
	No expression	Expression[b]	No expression	Expression
0	2	12	11	17
1	0	5	3	10
2	0	0	1	6
3	0	1	0	1
4	0	3	0	1
5	0	1	0	1
6	0	0	0	0
Cummulative No. positives	0	25	5	34
Total No. strains	2	22	15	36

[a] Hybridization at 85% stringency, assuming a G:C ratio of 73%.
[b] Refers to expression of antibiotic production and/or resistance.

Table 6. Hybridization patterns with *aphD* and *aph* gene probes amongst aminoglycoside producers.

Strain	Species	Taxonomic cluster	Antibiotic product	*aphD* Stringency	*aph* Stringency
DSM40236	*Streptomyces griseus*	1	Streptomycin	85	85
ATCC12760	*S. humidus*	19	Dihydrostreptomycin	85	65
ATCC14607	*S. hygroscopicus*	32	Bluensomycin	85	<65
KCC S-0772	*S. hygroscopicus*	32	Hygromycins	85	85
DSM40455	*S. subrutilis*	61	Hydroxystreptomycin	65	<65
ISP 5550	*S. katrae*	61	Streptothricin	73	65
DSM40069	*S. lavendulae*	61	Streptothricin	73	<65
ATCC23934	*Streptoverticillium mashuense*	55	Streptomycin	73	73
ATCC11062	*Streptomyces bikiniensis*	64	Streptomycin	85	65
KCC S-0133	*S. fradiae*	68	Neomycin	nd[a]	85
ATCC27441	*Streptoverticillium ladakanum*	Single member cluster	5-Azacytidine	73	<65

[a] nd: Not done.

phenotypes. Only 1.1% of strains hybridized with all six probes at high stringency.

Relationship between Antibiotic Production and Resistance

Previous studies provided evidence for a dichotomy in the distribution of antibiotic resistance phenotypes amongst natural isolates and type strains[10]. Half of the population expressed multiple antibitic resistances and many produced antibiotics, whilst the remainder showed only a limited resistance phenotype. It was clear that the population of natural isolates and type strains which produced bioactive secondary metabolites contained higher numbers of strains hybridizing with the gene probes (Table 5). While this is not definitive evidence that such strains contain the same gene it does appear to correlate well with the distribution of resistance phenotypes.

Eleven type strains, which produce aminoglycosides similar to streptomycin, were examined for homology to *aphD* (Table 6). Six strains hybridized with *aphD* at high stringency (85%) and one, a dihydroxystreptomycin producer, at low stringency (65%). Two aminoglycoside-producing streptoverticillia and 2 streptothricin producers hybridized at 73%. Table 8 shows how this compared to the hybridization pattern of all strains in the study. Slightly more aminoglycoside producers hybridized to the *aphD* probe compared

Table 7. Hybridization to *aphD* and Nb^r gene probes in cluster 1 streptomyces.

Strain	Name	Antibiotic product	*aphD* Stringency	Nb^r Stringency
DSM40236	*Streptomyces griseus*	Streptomycin	85	85
LIV 463	*Streptomyces* sp.	NP	85	85
ATCC25481	*S. omatus*	Ornamycin	85	73
DSM40508	*S. naraensis*	Naramycins	85	65
ATCC23907	*S. fluorescens*	Actinomycin	85	<65
KCC S-0446[a]	*S. albidoflavus*	NP	73	65
LIV 049	*Streptomyces* sp.	NP	73	<65
DSM40598	*S. bacillans*	NP	73	<65
DSM40232	*S. baarnensis*	NP	73	<65
ATCC27416	*S. anulatus*	Antibacterial/antifungal	73	<65
DSM40023	*S. nitrosporeus*	Nitrosporin	73	<65
ATCC3331[c]	*S. lipmanii*	NP	65	85
NCIB 9219	*S. niveus*	Novobiocin	<65	85
DSM40323	*S. flavogriseus*	NP	<65	<65
DSM40077[a]	*S. rutgersensis*	Camphomycin	<65	<65

NP: No known product.
[a] *S. albidoflavus*, sub-cluster 1a.
[c] *S. halstedii*, sub-cluster 1c.
Strains with no superscript belong to sub-cluster 1b, *S. anulatus*.

Table 8. Hybridization of *aphD* gene probe in aminoglycoside producers and cluster 1 streptomycetes.

Stringency	Average (%) to *aphD* (all strains)	Aminoglycoside producers		Cluster 1's	
		% hybridizing to *aphD*	% hybridizing to *aph*	% hybridizing to *aphD*	% hybridizing to Nb^r
85%	32.8	55.0	20.0	33.0	26.4
73%	24.4	36.0	10.0	40.0	6.6
65%	13.1	9.1	30.0	6.7	13.2
<65%	30.7	0.0	40.0	13.3	52.8
No. strains	176	11	10	15	15

with the whole population of strains screened. In contrast to this data there was no correlation between aminoglycoside production and homology with *aph* (Table 8).

Hybridizations with *aphD* amongst Cluster 1a and 1b Streptomycetes

The *aphD* gene probe was isolated from *S. griseus*. This strain identified with the *Streptomyces albidoflavus* cluster 1 and specifically to 1b *Streptomyces anulatus*. Fifteen cluster 1 streptomycetes were probed with *aphD* in order to ascertain any relationship between the gene and taxonomic identity (Tables 7 and 8).

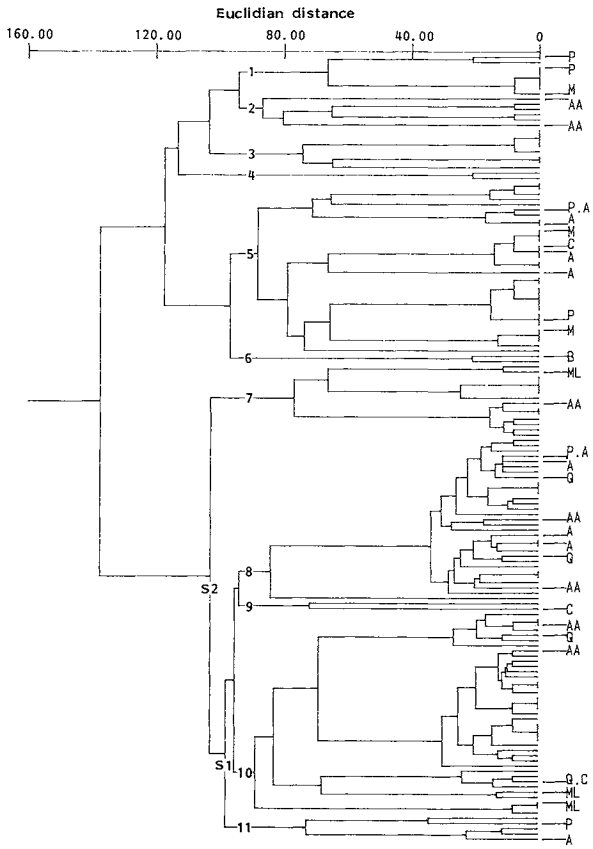
Members of the *S. anulatus* sub-group did not give significantly more hybridizations than the total population of strains probed (Table 8).

The novobiocin resistance determinant was also isolated from a cluster 1b streptomycete, *Streptomyces niveus*. However there was no correlation between homology to this probe and taxonomic identity (Tables 7 and 8).

Clustering of Hybridization Data and its Relationship to Antibiotic Production

The phenogram derived from Euclidian distances (Fig. 2) showed 11 groups were defined at a Eu-

Fig. 2. Phenogram derived from hybridization data at three levels of stringency for antibiotic resistance gene probes.



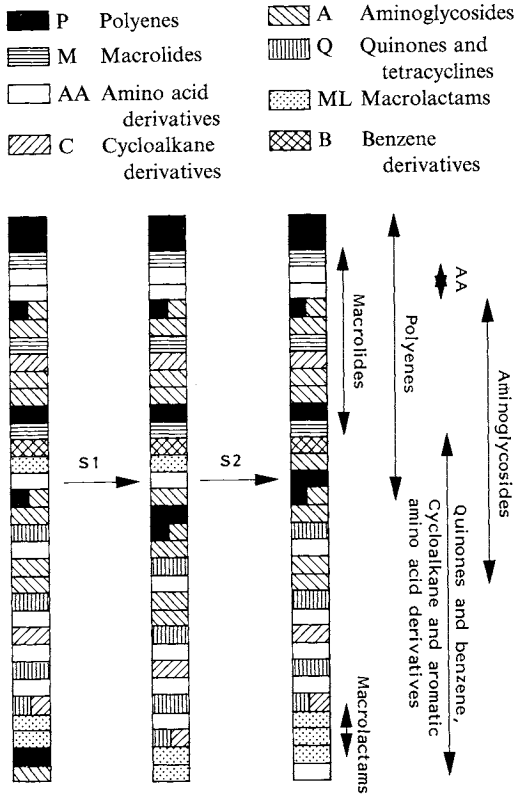
S1 and S2 denote positions where clusters were rotated. Key to letters given in Fig. 3, these indicate the position of strains producing that antibiotic. Interval data was coded at three levels of homology and dissimilarity calculated using Euclidean distance, clustering was achieved by average linkage (UPGMA).

clidian distance of 92. The distribution of 33 antibiotic-producing strains has been superimposed on the phenogram. Preliminary observations indicate that the producers of certain classes of antibiotics group to specific clusters. All of the macrolide producers cluster to group 5 and the macrolactam producers to groups 6 and 10. Polyene producers were observed in groups 1 and 5 and those which produced aminoglycosides in 5 and 8. Quinones and benzene and cycloalkane derivatives were found in the latter half of the phenogram (groups 6, 8, 9 and 10), compared to macrolides and polyenes, which were in the first half. Interestingly, producers of peptide antibiotics clustered to either side of the phenogram depending upon whether they were derived from aromatic amino acids. Two groups in the phenogram were rotated at positions S1 and S2 and a diagram was drawn to illustrate the positions of antibiotic producers across the phenogram (Fig. 3).

Discussion

At present only a small proportion of the antibiotic biosynthesis pathways and resistance mechanisms in nature are known. Virtually nothing is known about the distribution of production and resistance genes

Fig. 3. Diagram to illustrate the clustering position of antibiotic-producing type strains down the phenogram following rotation of clusters at S1 and S2.



within natural streptomycete populations in soil. The present study indicated that a sizeable proportion (~30%) of strains with phenotypic resistance might contain known, characterized resistance genes. However, the majority of strains classified as resistant did not hybridize with the gene probes used, this was particularly evident for thiostrepton resistance.

S. azureus and *Streptomyces laurentii* produce thiostrepton and can methylate rRNA for self-defence[19]. These determinants also confer resistance to other peptide antibiotics with the same mode of action such as siomycin, sporangiomycin, nosiheptide and thiopeptin[19,20]. Producers of these compounds were resistant to thiostrepton. However, a recent report has suggested that *S. azureus* may contain a further copy of an rRNA methylase which was not linked to the thiostrepton biosynthetic gene cluster, but was in a pIJ101-like plasmid integrated into the chromosome[20]. This has lead WOODMAN *et al.*[20] to hypothesize that these genes were fortuitous acquisitions obtained after the original resistance gene, which evolved alongside the biosynthetic gene cluster. Further analysis of the resistant strains found in soil is needed to determine if there is more evidence for the mobility of resistance genes within streptomycete populations.

A correlation was found between aminoglycoside production and hybridization with the *aphD* probe for streptomycin resistance. Streptomycin resistance genes were found to be clustered with biosynthetic genes[21,22] and comparison of two genes from taxonomically diverse strains revealed 75% homology[21]. This supports our observation that streptomycin-producing strains other than *S. griseus* showed homology with *aphD*. However, many aminoglycoside-resistant strains failed to hybridize with either *aphD* or *aph* and these strains may contain other genes known to confer resistance, for example *aphE*[23] or genes for acetyltransferases such as those found in *S. fradiae*[24] and *S. griseus*[25].

The *aphD* showed the highest number of strains hybridizing at each level of stringency. The probe was derived from regions B, C and D with small portions of A and E, where B and D were conserved between phosphotransferases[22]. WALKER[26] suggested that there has been a selection pressure for the evolution of the streptomycin biosynthetic pathway. Guanidino inositol derivatives could serve as a nutrient reserve, which is readily utilised during starvation and differentiation, this would give organisms a selective advantage for developing this portion of the pathway. An alternative proposal for the function of streptomycin exploits its polycationic character, as an accelerator of cell lysis[27]. Only a small proportion of the isolates hybridized to probes for viomycin and novobiocin resistance determinants although the proportion of phenotypic resistance was similar for each of the antibiotics tested. The only known producer of viomycin is *S. vinaceus*, which has three resistance genes including *vph*[28].

The mechanism of the novobiocin resistance determinant used in this study was not known and a further, uncharacterized resistance gene has been cloned from *S. niveus*[18]. However CUNDLIFFE and co-workers have cloned a resistance determinant with DNA gyrase activity from another producer *Streptomyces spheroides*[29]. It is possible that some of the strains with alternative novobiocin resistance mechanisms in this study also contain such an enzyme.

Studying the diversity of streptomycete populations may help in understanding the evolution and

ecological importance of antibiotic production and its relationship to antibiotic resistance. This study reported a novel approach to the analysis of resistance data and exploited taxonomic methodology for pattern recognition. It was shown that groups of strains producing similar antibiotics may be delimited by analysing their patterns of homology to antibiotic resistance gene probes. These patterns appeared to be more related to antibiotic production than they were to the taxonomy of the relevant organisms.

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