RELATIONSHIP BETWEEN ANTIBIOTIC RESISTANCE AND ANTIBIOTIC PRODUCTIVITY IN ACTINOMYCETES WHICH PRODUCE AMINOGLYCOSIDE ANTIBIOTICS

Sir:

We have reported that most actinomycete strains which produce aminoglycoside antibiotics (AGs) show different multiple AG resistance patterns[1]. This suggested that actinomycete isolates showing multiple AG resistance might be candidates for AG production, and that specific AG resistance phenotypes would correlate with strain specific production of AGs. In this paper, evidence supporting this prediction is provided.

Approximately 200 actinomycetes isolated from soil samples were selected at random[1] and cultivated at 27°C for 5 and 8 days on a rotary shaker in a medium consisting of potato starch 1.0%, glucose 0.1%, soy bean meal 1.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1% and NaCl 0.3%. As shown in Table 1, AG resistant actinomycetes had a higher probability of antibiotic production than AG-susceptible isolates. The wider the resistance range, the higher the probability of antibiotic production. Filtrates of the cultured broths with antibiotic activity were passed through Amberlite IRC50 (NH_4^+ or Na^+ type). Antibiotic activities were eluted with 1 N NH_4OH or 1 N HCl and characterized by high voltage paper electrophoresis[2], PPC[3] and TLC[4] in comparison with authentic antibiotic samples. If necessary, antibiotics were purified and characterized for their physico-chemical properties. Water soluble basic antibiotics (mainly AGs) except streptothricins were found exclusively in AG resistant isolates. It was noted that AG production was recognized exclusively among AG resistant isolates, and that actinomycete isolates with cross-resistance to 4~7 AGs ("middle" resistance group in Table 1) provided the widest variety of AGs. Thus, multiply AG resistant actinomycete strains are often capable of producing AGs.

The AG resistance patterns of the above strains were compared with those of known AG producing organisms as shown in Table 2. It was noted that resistance patterns were generally distinct depending on the AG produced and the color of surface growth. For the producers of spectinomycin, istamycins, gentamicins and neomycins, strains producing the same antibiotic and the same surface growth showed the same AG resistance pattern. Streptomycin producing strains had a similar resistance pattern although

Table 1. Antibiotic productivity of AG resistant actinomycete isolates.

Resistance range group*	Isolates tested (A)	Antibiotic producers				Antibiotics found***
		Total (B)	B/A (%)	WSB** (C)	C/A (%)	
Wide	34	26	76.5	5	14.7	Ristocetin (3) Spectinomycin (2)
Middle	66	48	72.2	20	30.3	Amicetin (3) 2-Aminotrehalose (1) 4-Aminotrehalose (1) Gentamicins (1) Istamycins (3) Negamycin (3) Neomycins (3) Ribostamycin (1) Streptothricins (4)
Narrow	69	34	49.3	7	10.1	Streptomycin (5) Streptothricins (1) Viomycin (1)
Susceptible	33	14	42.4	3	9.1	Streptothricins (3)

* Grouping actinomycete isolates was based on range of AG resistance described previously[1].

** Antibiotics showing water soluble and basic property.

*** Numbers in the bracket refer to the numbers of producing organisms. Underlined antibiotics were aminocyclitol or AG.

Table 2. AG Resistance patterns in AG-producing actinomycetes.

Antibiotic produced	Organism	Aerial mass color*	Resistance** to 50 μg/ml of SM	KM	DK	GM	RM	BT	NM	PR	LV	NE	IS
Spectinomycin	*Streptomyces* sp. 3 strains	Red	●	○	●	○	●	●	●	●	●	●	●
	S. spectabilis ISP5512	〃											
Istamycin	*S. tenjimariensis* SS-939	Blue		●	●		●	●				●	●
	〃 6 strains	〃											
Gentamicin	*Micromonospora* sp. 4 strains	—		●	●	●					○		●
	M. purpurea KCC-0074***	—											
Streptomycin	*S. griseus* ISP5236	Yellow	●										
	S. streptomycinii ISP5200	〃											
	Streptomyces sp. 5 strains	〃											
	Streptomyces sp. 4 strains	Gray	●				○					○	
Neomycin	*S. fradiae* ISP5063	Red					●		●	●		●	
	Streptomyces sp. 5 strains	〃											
Ribostamycin	*S. lavendulae* SS-1364	Red					●		●	●		●	
Paromomycin	*Streptomyces* sp. MC604	Yellow					●		●	●		●	
	S. catenulae ISP5258	Gray					●			●		●	
	Streptomyces sp. SS-1914	〃											
	S. chrestomyceticus ISP5545	White	●	●	●	○	●	○	●	●	●	●	●
4-Aminotrehalose	*Streptomyces* sp. SS-1227	Red		●	●		●					●	●
2-Aminotrehalose	*Streptomyces* sp. SS-1281	Red	●				●					●	●

* Aerial mass color based on 8th edition of BERGEY's Manual for Determinative Bacteriology.

** Streptomycin sulfate (SM), kanamycin A sulfate (KM), dibekacin (DK), gentamicin C complex sulfate (GM), ribostamycin (RM), butirosin A sulfate (BT), neomycin B sulfate (NM), paromomycin (PR), lividomycin A sulfate (LV), neamine (NE), and istamycin A sulfate (IS) were used for resistance test as described previously[1]. Solid circle, open circle and empty refer to good growth, variable growth and no growth, respectively.

*** Gentamicin-producing strains were tested for their AG resistance in ISP No. 2 medium.

Table 3. AG Resistance *in vitro* of strains which produce neomycin group antibiotics.

Antibiotic (100 μg/ml)	Resis-tance* (μg/ml)	Poly (U)-directed *in vitro* polyphenylalanine synthesis** (%) Ribosome / S150	5063 / 5063	1365 / 1365	1364 / 1364	MC604 / MC604	5063 / 5236	1365 / 5236	1364 / 5236	MC604 / 5236
None	—		100.0 (8248)	100.0 (8302)	100.0 (2694)	100.0 (7733)	100.0 (6217)	100.0 (6486)	100.0 (21264)	100.0 (11792)
Neamine	200~500		86.3	79.0	67.0	31.9	23.4	26.7	20.9	4.9
Ribostamycin	500~1,000		86.8	75.7	51.7	72.6	31.3	16.1	14.5	6.0
Neomycin B	200~500		64.8	39.5	54.9	48.2	15.2	18.0	10.1	5.4
Paromomycin	500		109.5	82.4	85.0	88.5	29.6	8.4	11.9	7.4
Butirosin A	25~50		24.0	11.4	11.0	13.3	28.9	19.5	13.5	5.6
Kanamycin A	25~50		22.3	9.6	7.6	11.8	22.2	13.0	11.5	5.2

* Upper limit of resistance in Tryptic Soy Broth (Difco).

** Ribosomes and S150 fractions were prepared from neomycin producing *S. fradiae* ISP5063 and *S. lavendulae* SS-1365, ribostamycin producing *Streptomyces* SS-1364 and paromomycin producing *Streptomyces* sp. MC604 and combined to synthesize polyphenylalanine (left-half). S150 fractions from these strains were exchanged with *S. griseus* ISP5236 which is susceptible to the antibiotics examined (right-half). Polyphenylalanine syntheses in the presence of each antibiotic were expressed as % control. Numbers in the bracket refer to incorporation counts (dpm) of [^{14}C]phenylalanine into TCA insoluble fraction was counted after 60-minute incubation at 37°C.

they consisted of two surface color groups. Only in the case of paromomycin producers, three different resistance patterns were recognized on the basis of their surface growth.

A common resistance pattern was observed in strains which produced the neomycin group antibiotics such as neomycin, ribostamycin and paromomycin (Table 2). These strains belonged to three different species and were highly resistant to neamine, ribostamycin, neomycin and paromomycin, but relatively susceptible to butirosin A and kanamycin A (Table 3).

Using *in vitro* polyphenylalanine synthesizing systems prepared according to the method reported previously[5], the resistance mechanisms have been characterized (Table 3). Consistent with the resistance of intact cells, polypeptide synthesis was inhibited by butirosin A and kanamycin A. The resistance in extracts proved to be associated with supernatant (S150) fractions, because polyphenylalanine syntheses were strongly inhibited by all antibiotics tested when the S150 fraction of each strain was substituted with that of *Streptomyces griseus* ISP5236; the latter strain is susceptible to all antibiotics in this study. In the S150 fractions of these producing-organisms, both phosphotransferase and acetyltransferase activities were detected when examined according to the method reported in a previous paper[6]. Inactivating enzymes such as an aminoglycoside phosphotransferase, APH(3′)[7~9] and an aminoglycoside acetyltransferase, AAC(3)[7,10] are most probably involved in the mechanisms of multiple AG resistance of these strains.

It seems very likely an actinomycete strain found to produce a new AG would have a pattern of AG resistance characteristic for the new antibiotic. One can predict the capability of strains of actinomycetes to produce certain AGs on the basis of their AG resistance patterns and the color of their surface growth.

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