POSSIBLE CONTROL OF FORMATION OF AERIAL MYCELIUM AND ANTIBIOTIC PRODUCTION IN *STREPTOMYCES* BY EPISOMIC FACTORS

Sir:

In general, actinimycetes reduce the formation of aerial mycelium and productivity of antibiotics after repeated sequential cultures. According to OKAMI (personal communication), productivity of kasugamycin of *Streptomyces kasugaensis*[1] markedly decreases after cultivation on an agar slant at higher than 30°C. These phenomena could be explained, if episomic factors or plasmids played a role in determining these properties. GREGORY and his collaborators reported that tyrosinase inheritance in *S. scabies* is controlled by a plasmid which replicates faster than the other genomes. They reached this conclusion from an analysis of the progeny of heterocaryons obtained from genetically marked strains[2], and from the fact that acriflavin caused the loss or alteration of the genetic material for tyrosinase production[3]. On the other hand, YOKOTA *et al.* found that R-factors controlling kanamycin resistance in *Escherichia coli* and *Salmonella typhimurium* were eliminated from their host cells by incubation at 43°C[4].

In this paper sudies are reported on the effect of acriflavin and high temperature which cause an increased incidence of progenies in *Streptomyces* lacking the ability to form aerial mycelium and to produce kasugamycin and aureothricin.

In order to eliminate plasmids from the mycelium, each culture was grown in an appropriate liquid medium (cf. footnotes of Table 2) in L-tubes at the highest temperature permitting growth or incubated at 27°C in the presence of acriflavin. After 4 days of the incubation each culture was treated for 1 minute by a 10-KC sonic oscillator to cut the mycelia and this was inoculated onto selective media (Table 1) to observe the production of melanin and formation of aerial mycelium. The mycelium was also inoculated onto PRIDHAM's medium[5] containing glucose as the sole carbon source to observe the properties of each colony.

In order to test antibiotic production, each colony was transferred onto the antibiotic-producing agar medium (Table 1) and incubated for 4 days at 27°C. The antibiotic productivity of each colony grown on this medium was assayed by transferring a cylinder of agar bearing the colony (diameter 5 mm) on an antibiotic assay plate. *Bacillus sphaericus* NIH-B 143 and *Bacillus cereus* NIH-B 310 were used as test organisms for kasugamycin and aureothricin, respectively. The spectrum of sugar utilization was tested by the velveteen replica-plating techniques transferring the colonies on PRIDHAM's media containing various sugars. The characteristics of strains which had lost the abilities of aerial mycelium formation and production of antibiotics were also studied using the agar slants of the same composition as the selective media.

The highest temperatures permitting

Table 1. Composition of selective media and antibiotic-producing media

Medium	Purpose used	Composition (g/liter)
M	Melanin formation in *S. scabies* and *S. venezuelae*	glucose 2.0, yeast extract 10.0, L-tyrosine 1.0, NaCl 3.0, agar 15
GAA	Aerial mycelium formation in *S. scabies*	glucose 10, sodium asparaginate 1.0, asparagine 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCO_3$ 0.5, K_2HPO_4 1.0, agar 15
YGAA	Aerial mycelium formation in *S. venezuelae*	glucose 10, sodium asparaginate 1.0, asparagine 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCO_3$ 0.5, K_2HPO_4 0.5, yeast extract 1.0, agar 15
BENNETT	Aerial mycelium formation in *S. kasugaensis*	glucose 10, Polypeptone 2.0, meat extract 1.0, yeast extract 1.0, agar 15
KSM	Kasugamycin production in *S. kasugaensis*	Prorich 10, maltose 15, agar 15
ATM	Aureothricin production in *S. kasugaensis*	glycerol 10, meat extract 5, Polypeptone 5, NaCl 5, agar 15

Table 2. Effect of preincubation at high temperature on abilities of streptomycetes to form melanin, develop aerial mycelium and produce antibiotics

	Temp. of preincubation (°C)	Melanin formation		Aerial mycelium formation		Antibiotic production	
		No. of colonies tested	FD [d] (%)	No. of colonies tested	FD [d] (%)	No. of colonies tested	FD [d] (%)
S. scabies [a]	27	143	0	151	6.0	Not produced	
	35.5	128	5.9	458	7.2		
S. venezuelae [b]	27	115	0	316	0	Not produced	
	42	78	12.8	245	7.3		
S. kasugaensis [c]	27	Not produced		383	0	KS [e]: 135	0
						AT [f]: 96	0
	35			393	8.9	KS: 97	6.2
						AT: 112	5.4

a) *S. scabies* was incubated using medium Z containing glucose 15 g, Polypeptone 8 g, yeast extract 4 g, NaCl 3 g, and distilled water 1 liter.
b) *S. venezuelae* was incubated using medium YGA containing glucose 10 g, sodium asparaginate 1.0 g, asparagine 1.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 0.5 g, K_2HPO_4 0.5 g, yeast extract 1 g and distilled water 1 liter.
c) *S. kasugaensis* was incubated using medium B containing glucose 10 g, Polypeptone 4 g, yeast extract 2 g, meat extract 1 g and distilled water 1 liter.
d) Frequency of progeny colonies deficient in the property cited
e) Kasugamycin
f) Aureothricin

Table 3. Effect of preincubation in the presence of acriflavin on abilities of streptomycetes to form melanin, develop aerial mycelium and produce antibiotics

	Acriflavin concentration (μg/ml)	Melanin formation		Aerial mycelium formation		Antibiotic production	
		No. of colonies tested	FD (%)	No. of colonies tested	FD (%)	No. of colonies tested	FD (%)
S. scabies	0	312	0	Not determined		Not produced	
	5	243	10.7				
S. venezuelae	0	477	0	383	0	Not determined	
	5	273	9.1	445	7.0		
S. kasugaensis	0	Not produced		128	0	KS: 77	0
						AT: 489	0
	20			342	0	KS: 108	4.6
						AT: 255	2.4 [g]

Abbreviations are the same as in Table 2.
g) Experiments on the elimination of aureothricin used acridine orange instead of acriflavin.

growth were 35.5°C for *S. scabies* At-382, 42.0°C for *S. venezuelae* At-40, and 35.0°C for *S. kasugaensis* At-534. As a result of preincubation at high temperature (Table 2), colonies arose which were deficient in production of melanin, formation of aerial mycelium or production of antibiotics. However, the spectrum of sugar utilization was not changed in all strains. In the case of *S. scabies*, the incidence of colonies lacking an aerial mycelium was not significantly increased by incubation at the high temperature. In this strain, 6.0% of the progeny colonies failed to form an aerial mycelium on incubation at 27°C after they had twice been purified by sequential single-colony isolations.

The minimum concentrations of acriflavin allowing growth of the test cultures were 5 μg/ml with *S. scabies* and *S. venezuelae* and 20 μg/ml with *S. kasugaensis*. In agreement with GREGORY's reports[3], following treatment with acriflavin, melanin non-producing colonies appeared with frequencies of 10.7% in *S. scabies* and 9.1% in *S. venezuelae*. The ability to form an aerial

mycelium in *S. venezuelae* was lost with a frequency of 7.0% but acriflavin did not affect this characteristics of *S. kasugaensis*. Inabilities to produce kasugamycin and aureothricin appeared at 4.6% and 2.4%, respectively (Table 3). The spectra of sugar utilization in progenies of all species tested did not show any detectable change following treatment with acriflavin.

No correlation was found among deficiencies in the characteristics studied but deficiencies in two kinds of characteristics of *S. kasugaensis*, that is, production of kasugamycin and aureothricin appeared simultaneously in a very few progeny colonies following treatment with either high temperature or acriflavin.

From the results described above, it seems probable that the production of melanin in *S. scabies* and *S. venezuelae*, the formation of aerial mycelium in *S. venezuelae* and *S. kasugaensis*, and the production of kasugamycin and aureothricin in *S. kasugaensis* would be controlled by plasmids. The fact that the formation of aerial mycelium in *S. kasugaensis* was not eliminated by acriflavin treatment does not necessarily mean that the ability to form aerial mycelium in this strain is not controlled by a plasmid since it is known that several kinds of plasmids are not eliminated by acriflavin treatment.[6,7] The fact that preincubation at a high temperature resulted in an increased incidence of colonies deficient in aerial mycelium sugguests that the formation of aerial mycelium in *S. kasugaensis* might also be controlled by a plasmid. However, the possibilities that high temperature and acriflavin may act to favor the growth of strains lacking in abilities to form aerial mycelium or to produce antibiotics, or to cause mutation, have not yet been eliminated.

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