TEMPERATURE DEPENDENCE OF
LINCOMYCIN PRODUCTION
BY THERMODURIC
STREPTOMYCES ESPINOSUS
STRAINS

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In the course of our antibiotic screening program several lincomycin-producing *Streptomyces spinosus* strains were isolated. These strains show a remarkable temperature dependence regarding the production of lincomycin. At incubation temperatures up to 28°C little or no lincomycin is produced despite good mycelial growth. Incubation above 28°C results in an up to hundred-fold increase of antibiotic titers. Hence these organisms possess a drastic and sharp incubation temperature threshold (>28°C) above which significant antibiotic synthesis is initiated.

The strains used in this study were characterized as *S. spinosus* Dietz sp. n. biotypes UC 5371, 5372, 5408 and 5498 respectively. All fermentations described in Table 1 were conducted under submerged culture conditions in 500-ml Erlenmeyer flasks containing 100 ml of

medium. Seed cultures were prepared in a medium containing in g/liter; glucose monohydrate 25, Pharmamedia (Traders Oil Mill, Fort Worth, Texas) 25. The pH was adjusted to 7.2 before sterilization. The seed flasks were inoculated from a frozen agar plug and incubated on a rotary shaker (250 rpm, 6 cm stroke) at 28°C for 3 days. The fermentation medium contained in g/liter; black strap molasses 20, dextrin 30, fish meal 15, and Pharmamedia 15. This medium was adjusted to pH 7.2 before sterilization. The fermentation flasks were inoculated with 5 ml of seed culture and incubated on a rotary shaker. Lincomycin was assayed by the standard disc-plate agar diffusion method using *Micrococcus luteus* UC 130 as the indicator organism. For the temperature shift studies where cell masses were determined a clear medium was needed. Its composition was in g/liter; Tryptone (Difco) 10, yeast extract (Difco) 5, NaCl 5. All other fermentation conditions were as described above. Cell masses were determined by centrifugation of 10 ml broth aliquots in a clinical centrifuge for 10 minutes. The volume of the wet pellets was then recorded in ml and corresponds to % wet mycelial mass.

The temperature dependence of lincomycin production with the four strains was studied at 28°C and 45°C. The results indicate that all strains produced none or trace amounts when incubated at 28°C but three strains produced up

Table 1. Lincomycin production by thermotolerant *S. spinosus* strains.

Strain	Incubation temp (°C)	Day	pH	Lincomycin (µg/ml)	Strain	Incubation temp (°C)	Day	pH	Lincomycin (µg/ml)
UC 5371	28	2	6.6	0	UC 5408	28	2	6.6	Trace
		3	7.8	0			3	8.1	Trace
		4	8.3	0			4	8.4	Trace
		5	8.5	Trace			5	8.7	Trace
		2	8.3	920		45	2	8.5	340
	45	3	8.6	430			3	8.7	360
		4	8.8	350			4	8.7	480
		5	8.7	450			5	8.6	570
UC 5372	28	2	6.5	0	UC 5498	28	2	6.8	5
		3	7.5	0			3	7.8	6
		4	8.4	0			4	8.3	7
		5	8.6	0			5	8.5	8
		2	8.2	20		45	2	8.2	600
	45	3	8.5	20			3	8.5	600
		4	8.7	20			4	8.6	730
		5	8.7	20			5	8.7	690

Table 2. Effect of temperature shifts on *S. spinosus* strain UC 5498.

Incubation temp (°C)	Day	pH	Mycelial wet mass (%)	Lincomycin (μg/ml)	Incubation temp (°C)	Day	pH	Mycelial wet mass (%)	Lincomycin (μg/ml)
25	1	8.0	0.7	0	37	1	8.2	0.9	1
25	2	8.4	1.0	1	37	2	8.5	0.9	72
25	3	8.7	1.0	5.5	37	3	8.8	0.8	100
25	4	8.8	1.0	5	37	4	8.8	0.9	100
25	5	8.8	1.0	5	37	5	8.8	1.0	100
25	1	8.0	0.7	0	37	1	8.2	0.9	1
25	2	8.4	1.0	1	37	2	8.5	0.9	72
37	3	8.8	1.0	17	25	3	8.8	0.9	80
37	4	8.9	1.0	90	25	4	8.8	1.0	80
37	5	8.8	0.9	90	25	5	8.8	1.0	82

to 500~900 μg of lincomycin per ml when incubated at 45°C (Table 1). Peak titers were attained in 2~3 days.

Temperature shift experiments were done with *S. spinosus* strain UC 5498 in a clear medium to assess mycelial mass in addition to other fermentation parameters such as pH and antibiotic titers. Peak titers in this clear medium were lower than in the regular medium and amounted to 100 μg of lincomycin per ml if the culture was continually incubated at 37°C and approximately 5 μg/ml if the culture was incubated at 25°C (Table 2). If the culture was incubated at 25°C for 2 days followed by a shift to an incubation temperature of 37°C, the strain commenced to synthesize larger amounts of lincomycin. Peak titers nearly equaled the ones observed in cultures continually kept at 37°C. Conversely if the strain was incubated first at 37°C for 2 days and then shifted to 25°C for several additional days,

titers peaked about 10% above the titers present at the time of the temperature shift. In terms of growth as reflected by the mycelial wet mass, the strain grew equally well at 25°C or 37°C.

In a detailed temperature dependence study with strain UC 5498 it was found that only low levels of lincomycin were produced at incubation temperatures up to 28°C. Incubation above this temperature and covering a range from >28 to 45°C resulted in a drastic increase of lincomycin production and the peak titers were equal regardless of the specific incubation temperature within this range. The strain grew well at 50°C but ceased to produce lincomycin at that incubation temperature.

The temperature-dependent lincomycin production by these strains offers thus a system whereby antibiotic synthesis is inducible or suppressible by simply changing the incubation temperatures of the cultures.