THE PRODUCTION OF SEMDURAMICIN BY DIRECT FERMENTATION

Sir:

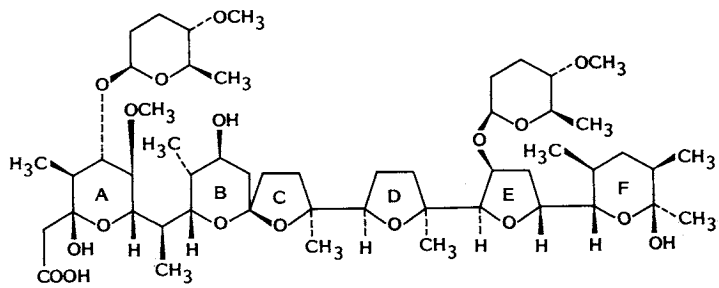
Shortcomings[1] experienced with the bisglycoside polyether ionophore UK-58,852[2] (Fig. 1) included high tissue residues in poultry. These difficulties were overcome through synthetic modification[1,3] leading to the monoglycoside ionophore, semduramicin (Fig. 1). Semduramicin was found to be both highly efficacious and very well tolerated, with an anticoccidal profile[4] comparable to the industry standard, salinomycin, yet more than twice as potent. While semduramicin can be synthesized in the laboratory through partially selective acidic hydrolysis, we sought a method which would allow economical large-scale production. This communication describes results of a program designed to obtain a mutant of *Actinomadura roseorufa* Huang sp. nov.[2] producing semduramicin directly by fermentation, presumably through inactivation of the A-ring glycosyl transferase.

Actinomadura roseorufa Huang sp. nov. culture N619-30 (ATCC 53666) produces UK-58,852 and no semduramicin.

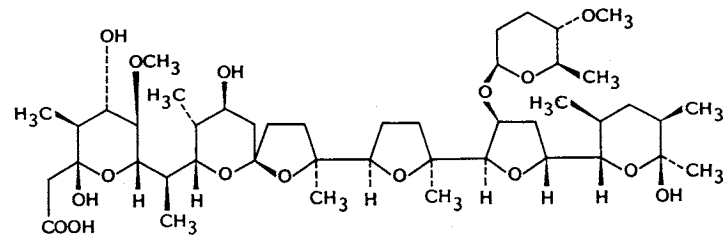
Mutagenesis was conducted as follows. Growth from a thawed frozen slant of culture N619-30, maintained on modified ATCC 172 medium (Table 1), was suspended in sterile water and a 5.0 ml aliquot was used to inoculate a 2.8-liter Fernbach flask containing 800 ml of growth medium (Table 1). After shaking 8 days at 28°C, a 50 ml aliquot was first homogenized and then sonicated. The fragmented cells were then transferred to a sterile 300-ml Erlenmeyer flask containing 50 ml of fresh modified ATCC 172 medium and incubated at 34°C in a reciprocating water bath shaker for two hours at 160 rpm. After this time the flask growth was washed free of medium and the pellet was suspended in 50 ml of Tris buffer, pH 9.0. Aliquots were then treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[5,6] at concentrations of 7.5 ~ 15.0 mg for one hour at 34°C. After treatment, the cells were washed free of the mutagen. The pellets were then brought to their original volumes with modified medium ATCC 172, and 2.0 ml of each suspension was used to inoculate 300-ml Erlenmeyer flasks containing 30 ml of the same medium and were shaken at 32°C and 200 rpm for three days.

After three days growth, the treated cells were homogenized and sonicated as before. Aliquots of the sonicate were serially diluted and plated onto solid modified ATCC 172 medium; plates were incubated at 28°C until colony-forming units were

Fig. 1. The structures of UK-58,852 and semduramicin.



UK-58,852

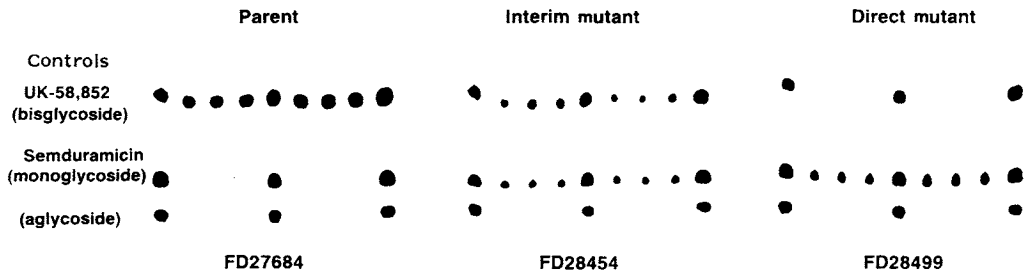


Monoglycoside semduramicin (UK-61,689)

Table 1. Media/parameters employed in maintaining/growing *Actinomadura roseorufa*.

Stage	Media		Parameters
1. Slants/plates	Modified ATCC No. 172	g/liter	pH 7.0
	NZ Amine type A (Humko Sheffield)	1.0	Sterilize by autoclaving
	Glucose	10.0	121°C for 30 minutes
	Soluble starch (Difco)	20.0	Incubate 10~14 days
	Yeast extract (Difco)	5.0	
	Calcium carbonate	1.0	
	Agar (Difco)	20.0	
2. Flask growth	Cerelose	10.0	Volume: 800 ml/2.8-liter Fernbach
	Corn starch	5.0	flask
	Corn steep liquor	5.0	Sterilization: Autoclave 121°C for
	NZ Amine YTT (Humko Sheffield)	5.0	30 minutes
	Cobalt chloride	0.002	
	pH adjusted to 7.0		
3. Flask production	Cerelose	45.0	Volume: 25 ml/300-ml
	Soybean flour	10.0	Erlenmeyer flask
	Corn steep liquor	15.0	Sterilization: Autoclave
	$MnSO_4 \cdot H_2O$	0.1	121°C for 30 minutes
	$MgSO_4 \cdot 7H_2O$	0.1	
	Cobalt chloride	0.002	
	Calcium carbonate	3.0	
	pH adjusted to 7.0		

Fig. 2. Thin layer chromatograms of polyethers produced by *Actinomadura roseorufa* parent and mutants.



of sufficient size for transfer to slants of the same medium. The inoculated slants were allowed to grow at 28°C for 10 to 14 days, after which time they were ready for use in fermentation.

Mutant testing was performed in 300-ml Erlenmeyer flasks containing 25 ml of flask production medium (Table 1). After sterilization, the flasks were inoculated with individual slant growth suspensions and incubated 28°C on a shaker for 7 days at 200 rpm.

After 7 days shaking, the production profiles of each isolate was determined by examining half volume methyl isobutyl ketone (MIBK) extracts. 10 μl of MIBK phase were pipetted onto thin layer plates and developed using 9 parts chloroform to 1 part methanol. Rf values of ~0.3 and ~0.65 were obtained for semduramicin and UK-58,852, respectively. The aglycone of UK-58,852 has an Rf of ~0.1 in this system. The compounds were visualized by spraying developed thin layer plates with a vanillin reagent (6.0 g vanillin in 100 ml ethanol and 3% concentrated H_2SO_4) and heating at 100°C in an oven for 5 minutes.

Alternatively, the dried TLC plates were overlayered with one-half strength Brain Heart Infusion Agar (Difco) seeded with *Bacillus subtilis*, to which 0.4 ml of a 5% solution of 2,3,5-triphenyl-2*H*-tetrazolium chloride had been added. The plates were incubated at 37°C for 16 hours; the ionophores were visualized as colorless areas against a red background.

The desired mutant was obtained in two steps (Fig. 2). After screening 2,300 isolates, an interim mutant (ATCC 53674), producing both UK-58,852 and semduramicin (~1:9 ratio) was detected and retrieved. In a second step, after screening 2,500

isolates of this mutant, the direct semduramicin producing mutant (FD28499) (ATCC 53664) was generated, free of any UK-58,852. Productivity of this mutant was ten-fold lower than that of N619-30 (semduramicin vs. UK-58,852). As reported elsewhere, structural identity of fermentation-produced semduramicin was verified by ^{13}C NMR and other techniques[7]. The mutant serves as a basis for an ongoing culture improvement program.

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† A part of this work was presented at the 2nd. S.I.M. International Conf. on Biotech. of Microbial Products: Novel Pharmacolog. and Agrobiol. Activities. Abstract No. P-13, p. 31, Sarasota, FL. Oct. 14～17, 1990.