NOTES

ANTIBIOTICS FROM *MYCOPLASMA*. I *ACHOLEPLASMA LAIDLAWII* B

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In the course of our studies on mechanisms of antibiotic resistance in *Mycoplasma*[1,2] we noted that under certain conditions *Acholeplasma laidlawii* B produced an antibiotic substance[3]. Further investigation of conditions favoring the production of this material and the identity of the substance(s) was relatively inconclusive as *A. laidlawii* B was erratic in producing this antibiotic.

However, the tetracycline-resistant mutant mentioned earlier[2] appeared to be more consistent in antibiotic production even though 95% of the fermentations did not show any antibiotic potency by the bioassay methods used.

Best results were obtained when the *A. laidlawii* B mutant was grown in Difco PPLO broth (without crystal violet) supplemented with bovine serum, sodium acetate, and Difco yeast extract. The serum level could be varied from 0.1 to 10% without variation in antibiotic production, and swine and horse serum could be substituted for the bovine serum component. Best results were obtained when static culture was used (100 ml/250 ml cotton-plugged Erlenmeyer of 500 ml/1,000 ml Erlenmeyer) and the cultures were incubated at 37°C. No correlation between antibiotic production and growth was noted (SMITH's method was used for measuring growth, *e. g.* increase in colony forming units[4]), and antibiotic production was independent of incubation period. In the 'active' preparations, antibiotic production phase lasted for less than 24 hours.

Antibiotic production was measured by agar-diffusion assays using the inhibition of *Bacillus subtilis* Marburg. One unit of antibiotic activity has been defined as that amount of material required to produce an inhibition zone of 22 mm in diameter when the antibiotic solution is placed in a 10 mm well in a 100 mm Petri dish containing 10 ml of Penassay[R] agar seeded with *B. subtilis* spores. The most potent fermentation contained 4 units per 100 ml of broth.

Recovery of the antibiotic material was accomplished by lyophilizing the fermentation and resuspending the solids in 1/20 of the original volume of 50% ethanol. The undissolved solids were discarded, and the solution concentrated *in vacuo* and passed over columns of Dowex 50×2 and then Dowex 1×2 with NH_4OH as eluting solution for the first and water for the second column. This procedure resulted in about 8-fold purification and loss of about 50% of the activity.

The purified material contained 3 bioactive substances as shown by paper chromatography (n-BuOH - EtOH - H_2O, 2 : 1 : 1) followed by bioautography against *B. subtilis*. The bioactive mixture was stable to heating at 100°C for 15 minutes over the pH range 2 to 9. It was not inactivated by Nagarse[R] (1 mg enzyme/unit of antibiotic) at pH 7.5 with an incubation period of 3 days at 37°C.

The bioactivity was found to be limited to certain Gram-positive bacteria including *B. subtilis* Marburg (spores and vegetative cells), *Bacillus megaterium*, *Staphylococcus aureus* FDA 209P, and *S. aureus* 1206 (a tetracycline-resistant clinical isolate). No inhibition was observed at levels tested of *Sarcina lutea*, *Escherichia coli*, *Saccharomyces cerevisiae*, *A. laidlawii* B, or mammalian cells in tissue culture (EAGLE's KB, EARLE's L_{929}, or GEY's HeLa). A *B. subtilis* mutant with induced resistance (25 times m.i.c.) was found as sensitive as the parent culture to: ampicillin; penicillin G; streptomycin; tetracycline; erythromycin; neomycin; novobiocin; lincomycin; capreomycin; mikamycins A+B; staphylomycins S+M; and chloramphenicol.

The mechanism of action of the *A. laidlawii* B antibiotics appears to involve inhibition of protein synthesis as determined by studies involving the incorporation of L-leucine-^{14}C, uridine-5-^{3}H and thymidine-methyl-^{3}H by whole cells of *B. megaterium*, and cell-free protein synthesis using *E. coli* B ribosomes with polyuridylic acid as messenger. The antibiotics are bacteriostatic at the m.i.c. for *B. subtilis* (0.066 units/ml).

Further evaluation of the antibiotics *in vitro* and *in vivo* will depend upon availability of active fermentations.

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References

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