Lysogenic *Klebsiella pneumoniae* Strains of Capsular Serotype 2(B)

Lysogenic strains of *Klebsiella pneumoniae* (*Aerobacter aerogenes*) of capsular serotypes 1(A) and 2(B) have been reported by PARK[1] and CIUCA et al.[2]. These lysogenic strains released phages active on other strains of *K. pneumoniae*. The present communication reports the finding of four other lysogenic strains, of capsular serotype 2(B).

Stab inocula were made from overnight nutrient broth cultures of *K. pneumoniae* strains into nutrient agar plates bearing a soft agar overlay seeded with the prospective indicator strain. This method is similar to that employed by PAPAVASSILIOU[3] to detect lysogeny in *Bacterium anitratum* strains. *K. pneumoniae* strains which gave zones of inhibition on the plates were tested further. Aged nutrient broth cultures and broth cultures of ultra-violet irradiated bacteria were centrifuged and the supernatants examined for the presence of phage. These supernatants were sterilized by shaking with chloroform or by treatment with streptomycin. In the latter case the tests for phage were made on a plate seeded with a streptomycin-resistant mutant of the bacterial indicator strain.

Type 2(B) strains B7380, 2.2, 2.5, and 2.12 gave turbid zones of inhibition when tested against a type 2 strain obtained from Dr. B. H. PARK. Strains 2.5 and 2.12 are derivatives of N.C.T.C. strains 7761 and 9620 respectively. PARK[1] reported his type 2 strain itself to be lysogenic, but none of the 48 capsulate and non-capsulate *K. pneumoniae* strains in my collection was sensitive to the phage released by the PARK strain. Tests of culture supernatants and dilutions showed that strains B7380, 2.2 and 2.5 are indeed lysogenic, discrete phage plaques being obtained on lawns of PARK's type 2 strain.

Résumé. L'auteur décrit quatre souches de *Klebsiella pneumoniae* du type capsulaire B lysogènes pour les bactériophages actifs pour une autre souche du même type.

C. H. CLARKE[4]

Institute of Animal Genetics, West Mains Road, Edinburgh (Scotland), August 22, 1963.

[1] B. H. PARK, Virology *2*, 711 (1956).
[2] M. CIUCA, S. STAMATESCO-EUSTATZIOU, C. BARBER, M. VOINEA, and G. TULPAN, Arch. Roum. Pathol. exp. Microbiol. *18*, 347 (1959).
[3] J. PAPAVASSILIOU, J. Bacteriol. *80*, 138 (1960).
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Isolation of 2-Acetyl-2-decarboxamidotetracycline from Cultures of *Streptomyces psammoticus*

VIRGILIO and HENGELLER described *Streptomyces psammoticus* as a new species of streptomyces capable of producing only tetracycline, even in the presence of considerable quantities of chloro-ions in the fermentation medium[1]. On the basis of the analytical methods which they employed, VIRGILIO and HENGELLER concluded that neither chlorotetracycline nor oxytetracycline were detectable in *S. psammoticus* cultures. On the other hand, HOCHSTEIN et al.[2] and MILLER and HOCHSTEIN[3] found that strains of streptomyces which produce either 7-chlorotetracycline or 5-oxytetracycline also synthesize 2-acetyl-2-decarboxamidotetracyclines. These authors pointed out that the capacity of *S. rimosus* and *S. aureofaciens* to synthesize such products is indicative of a biogenetic relationship between the tetracycline and the class of the 'indicator' antibiotics which are characterized by a considerably unsaturated chromophoric nucleus of naphthacene structure.

It seemed worthwhile to investigate whether also *Streptomyces psammoticus* cultures could synthesize small quantities of 2-acetal-2-decarboxamidotetracycline together with tetracycline. Fermentations with *S. psammoticus* were carried out at 28°C in glass jars of 4 l capacity, stirred at 750 r.p.m. and aerated at 0.75 litre/litre of medium/min. The fermentation medium consisted of: corn steep 20 g; $(NH_4)_2SO_4$ 9 g; glucose 60 g; $MgSO_4 . 7H_2O$ 1 g; $CaCO_3$ 9 g; tap water 1l; pH, before sterilization 6.8. After 96 h the concentration of the antibiotic produced was 2030 μg/ml.

The fermentation broth was acidified to pH 2.0, filtered, and extensively extracted with *n*-butanol after addition of 15% w/v of sodium chloride. The combined extracts were evaporated under reduced pressure to a small volume, the precipitated sodium chloride removed by filtration and the tetracycline was reextracted in water by addition of petroleum ether to the butanol.

Reextraction of the tetracycline in butanol and addition of ethyl ether to the concentrated butanolic extract yielded a precipitate of crude tetracycline hydrochloride with a microbiological activity of 550 μg/mg. A 1.82 g sample of this crude product (corresponding to 1 g of pure tetracycline hydrochloride) was fractionated by countercurrent distribution. The fundamental procedure described by CRAIG was employed, using 10 ml per tube of each phase of a solvent system consisting of 0.01 *N* hydrochloric acid and *n*-butanol; 230 transfers were carried out. When the distribution was complete, a sample (1 ml of each phase) of every fifth tube was analysed by determining the extinction at 360 mμ after dilution with methanol 0.01 *N* in hydrochloric acid. The results are given in the Figure.

In addition to the main peak, which was at the 66th tube (K = 0.40) and which corresponds to tetracycline, a second small maximum was found at about the 109th tube (K = 0.9). Spectrophotometric analysis of the 100th to the 120th tubes showed maxima at 276 mμ and 360 mμ, whereas tetracycline gives maxima at 268 mμ and 360 mμ.

In order to isolate an amount of the substance K = 0.9 for identification purposes, the countercurrent distribu-

[1] A. VIRGILIO and C. HENGELLER, Il Farmaco, Ed. Sci. *15*, 164 (1960).
[2] F. A. HOCHSTEIN, M. SCHACH VON WITTENAU, F. W. TANNER, and K. MURAI, J. Amer. chem. Soc. *82*, 5934 (1960).
[3] M. W. MILLER and F. A. HOCHSTEIN, J. org. Chem. *27*, 2525 (1962).