# SENSITIVITY OF CHEESE STARTER CULTURES TO OXYTETRACYCLINE AND STREPTOMYCIN

Trouble experienced by some Queensland cheese factories from considerable numbers of slow vats has led to increased work by Departmental field and laboratory officers to detect inhibitory factors in milk. Once detected, penicillin can be identified by the enzyme penicillinase, but identification of other, newer antibiotics requires a large sample and long chemical procedures. Much work has been reported in the literature of the effects on cheesemaking of residual penicillin, but few studies have been reported on streptomycin, the tetracyclines, and other antibiotics which are used for infusion into the udder. The increasing use of antibiotics other than penicillin for the treatment of mastitis in cattle (Marth 1961*a*, 1961*b*), and their possible and obvious effects on cheese manufacture, have led to the present investigation.

## Methods

The experiments were carried out with the collection of single-strain starter cultures kept in the Dairy Research Branch in Brisbane for distribution to cheese factories as required. These consisted of 22 strains of *Streptococcus cremoris,* 2 strains of *S. lactis,* and 3 strains of *S. diacetilactis.* A series of tests was made with these cultures, using varying concentrations of oxytetracycline and streptomycin.

*Starter Propagation.*—All strains were subcultured daily in sterile skim-milk and incubated for 18 hr at 24°C. All clotted during incubation. Stock cultures from which they were prepared were subcultured weekly in sterile chalk litmus milk and after clotting were stored in the refrigerator.

*Antibiotics.*—Crystalline oxytetracycline hydrochloride in 500-mg quantities (Pfizer "Terramycin") and crystalline streptomycin sulphate in 1 gm quantities (Glaxo "Streptomycin") were used. Serial dilutions of each of these were made.

*Disc Assays.*—The cultures were diluted to 1/10. Then 5-ml amounts of melted and cooled tryptone yeastrel lactose beef extract agar were seeded with 1-ml amounts of the suspensions, poured into petri dishes, and allowed to set and dry. Two sizes of discs were used: 1 cm and ¼ in. The smaller ones were no less sensitive than the others and were easier to form. The discs were cut from Schleicher and Schuell No. 601 paper and were used in pairs as double discs to increase the size of zones (Naylor 1960). The discs were dipped into the antibiotic solutions, the surplus was allowed to drain off, and the discs were applied to the agar surface. The plates were incubated for 24 hr at 30°C and the sizes of zones recorded.

*Activity Tests.*—Activity tests followed the method of Anderson and Meanwell (1942). Amounts of 1 ml of diluted antibiotic were added to the 20-ml quantities of sterile skim-milk (autoclaved at 15 lb pressure momentarily) to give known final concentrations from 100 μg/ml to 0·0001μg/mg. Controls were prepared using sterile diluent. The milk was inoculated with 0·4 ml (2 per cent.) of a clotted starter culture and incubated at 30°C. Uninoculated controls were set up in parallel. After 6 hr, 9-ml samples were titrated, using 0·1N NaOH and phenolphthalein.

*Vitality Tests.*—Vitality tests were done by the method of Whitehead and Cox (1932). Dilutions of antibiotic were added to the milk before inoculation to give final concentrations as used in the activity test and 6 ml of starter was used. Acidities and the rise in the final hour were recorded, and compared with controls.

## Results

*Effect of Oxytetracycline.*—No starter appeared more resistant than another to oxytetracycline. Typical results, for starter KH, a strain of *S. cremoris,* are shown in Table 1.

**TABLE 1**

EFFECT OF OXYTETRACYCLINE ON KH

| Concentration of Terramycin μg/ml. | Disc Assay | Activity Test | | Vitality Test | |
|---|---|---|---|---|---|
| | Figures are diameters of zones measured in cm. Discs were 1 cm. | Figures are acidity percentages | | Figures represent the total acidity percentage reached and the rise in the final hour | |
| | | Blank | Test | Total Acidity | Rise |
| 100·0 | 2·8 | .. | .. | .. | .. |
| 10·0 | 2·3 | .. | .. | .. | .. |
| 1·0 | 1·5 | 0·21 | 0·25 | 0·11 | 0·01 |
| 0·1 | no zone | 0·20 | 0·37 | 0·11 | 0·0 |
| 0·01 | no zone | 0·19 | 0·45 | 0·42 | 0·18 |
| 0·001 | no zone | 0·22 | 0·50 | 0·48 | 0·20 |
| Control | no zone | 0·20 | 0·50 | 0·53 | 0·22 |

With each of the three tests, 1 μg/ml of oxytetracycline was the lowest level which caused total inhibition of all the starter cultures. However, inhibition of acid production to a lesser degree occurred in vitality and activity tests with levels of antibiotics as low as 0·01 μg/ml. No zones appeared in the disc assay plates with those lower concentrations of antibiotic.

*Effect of Streptomycin*: Reactions were more variable than those with oxytetracycline, and typical results are shown in Table 2 for KH.

**TABLE 2**
EFFECT OF STREPTOMYCIN ON KH

| Concentration of Streptomycin $\mu$g/ml. | Disc Assay | Activity Test | | Vitality Test | |
|---|---|---|---|---|---|
| | Figures are diameters of zones measured in cm. Discs were 1 cm. | Blank | Test | Total Acidity | Rise |
| 1000·0 | 2·3 | .. | .. | .. | .. |
| 100·0 | 1·65 | .. | .. | .. | .. |
| 10·0 | Faint narrow zone | 0·18 | 0·22 | 0·11 | 0·0 |
| 1·0 | No zone | 0·21 | 0·37 | 0·115 | 0·0 |
| 0·1 | No zone | 0·18 | 0·46 | 0·535 | 0·255 |
| 0·01 | No zone | 0·18 | 0·50 | 0·615 | 0·29 |
| Control | No zone | 0·18 | 0·52 | 0·605 | 0·265 |

## Remarks

Streptomycin appeared to be less effective and results were more variable. The lowest level of antibiotic which caused any decrease in acid production was 0·1 $\mu$g/ml. A level of 10 $\mu$g/ml was needed for total inhibition of acid production of all starters, while 100 $\mu$g/ml was the lowest level causing inhibitory zones for all starters. Faint narrow zones for 12 of the 27 starters were produced by 10 $\mu$g/ml of streptomycin.

## Discussion

The results agree with those of Mattick (1955), who found 0·1 $\mu$g/ml oxytetracycline to cause slight inhibition of acid production, while 0·9 $\mu$g/ml was required for total inhibition. He found the corresponding levels of streptomycin to be 0·2 $\mu$g/ml and 1 $\mu$g/ml respectively. The results also agree with those reported by Feagan (1962), who performed sensitivity tests in milk.

Both vitality and activity tests appeared more sensitive than the disc assay method. Since vitality tests are performed under conditions similar to those experienced in a cheese vat, results from these may be of more use than those of activity tests. Also, the change from no effect to total inhibition of acid production was more clearly defined in the vitality tests.

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