MICROBIOLOGY

Bacterial Count and Cell Differentiation in Milk

A METHOD has been developed in this laboratory which has been found to be superior to the Breed-type smears such as are routinely used for assessing the bacteriological quality of cow's milk. The technique not only stains the bacteria, but also the somatic cells in such a way that the various cell-types may be satisfactorily differentiated and classified. Granular cytoplasmic inclusions which might be released on cell-degeneration are easily distinguished and not mistaken for the coccoid form of micro-organisms.

The use of 0·2 ml. of sample instead of 0·01 ml. (as in the Breed-method and its modifications), even distribution and low microscopic factor improves the precision of the count.

The fact that the cells are differentiated will help the investigator who is not only concerned with a total count to distinguish between pathological and normal conditions of the udder. The micro-organisms and the cells present in the milk sample are stained in a siliconed serological tube. This stained preparation is filtered, by vacuum filtration, through a membrane filter (M.F. Corp. plain white D.A.; diam., 25 mm; pore size, 0·65μ). This filter, after being dried, is made transparent with immersion oil and the bacteria and cells can then be counted and studied with a light microscope.

The procedure is as follows: 2 ml. 'Triton *X*-100', 0·1 per cent solution, *p*H 7·0 (prepared with double distilled water and filtered through a membrane filter), is placed in a serological tube. 2–3 drops 0·5 per cent solution $CuSO_4 5H_2O$ is added. Three drops benzidine–peroxide solution is added (benzidine 0·2 g/200 ml. distilled water; to which is added 4 drops of H_2O_2, 3 per cent). The milk sample (0·2 ml.) is added, and the mixture allowed to stand for 3 min. One drop of May–Grünwald stain (BDH standard stain in solution) is added, with 2 drops of Giemsa stain (improved Giemsa stain, Gurr *R*66 in solution). The tube is stood for 20 min in a water bath at 48° C, then poured into a filter funnel; two rinsings of the serological tube with 'Triton' solution at 48° C are also poured into the funnel. Vacuum is applied to pull the emulsion through the membrane filter. Rinsing is accomplished by flushing about 7 ml. 'Triton' solution (at 48° C) through the filter. The membrane filter is removed and dried on a glass slide at 35° C for about 10 min; immersion oil is added until the membrane filter becomes transparent. It is then covered with a cover slip. Another drop of immersion oil is placed on the cover slip, and the preparation is examined under the microscope.

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Formation of Protocatechuic from Quinic Acid by Fluorescent Pseudomonads

ROGOFF[1] described two fluorescent pseudomonads he electively isolated from soil which could oxidize quinic acid with the formation of protocatechuic acid as an intermediate.

Fifty-two isolates, assignable to the genus *Pseudomonas* and producing pyoverdine in the medium of King, Ward and Raney[2], have been examined for their ability to aromatize quinic acid by this pathway. The strains were isolated from infected hen-eggs, surface waters, chicken carcases, chicken droppings, beef and milk, using ordinary peptone media containing no quinate.

Protocatechuate formation by the isolates was investigated in the following medium, adapted from that of Rogoff[1]: *Solution A*. NH_4Cl, 0·4 g; $MgSO_4.7H_2O$, 0·05 g; $MnCl_2.4H_2O$, 0·01 g; $FeSO_4.7H_2O$, 0·005 g; $CaCO_3$, 0·2 g; yeast extract (Difco), 0·05 g; agar No. 3 (Oxoid), 0·05 g; distilled water, 50 ml., *p*H 7·4. *Solution B*. K_2HPO_4, 0·1 g; quinic acid, 1·0 g; distilled water, 50 ml., *p*H 7·4 (adjusted with 10 per cent (w/v) NaOH). The two solutions were mixed, dispensed in 2-ml. amounts into test-tubes (100 mm × 10 mm) and autoclaved for 15 min at 15 lb. pressure. One drop from broth cultures of the 52 strains was used to inoculate the quinate medium. On incubation at 25°, protocatechuate was readily detected by the formation of a purple colour below the bacterial surface growth due to the action of ferrous ions on this compound. In most such cases, a green coloration also appeared at the medium surface: this may have been due to the reaction of protocatechuate with ferric ions, formed at the medium surface by oxidation of ferrous ions (Soloway and Rosen[3]), but since this zone fluoresced strongly it may simply have been pyoverdine produced on the surface of the medium. A drop of medium removed from the purple portion of each culture did, however, form a deep green colour on the addition of a drop of aqueous 0·5 per cent (w/v) $FeCl_3.6H_2O$. By these simple tests, protocatechuate was discernible in the cultures within 2–3 days; after a week or so, the purple colour slowly disappeared and no ferric chloride reaction could be demonstrated.

Of the 52 fluorescent pseudomonads, 49 produced protocatechuate. The three negative strains (from river water, chicken droppings and spoiling beef respectively) grew more sparsely on the medium, utilizing the yeast extract as a carbon source, presumably. An interesting biochemical characteristic these three strains shared was the ability to decarboxylate L-glutamic acid to γ-amino butyric acid; one of the other strains was also able to carry out this reaction but it possessed in addition an L-aspartic acid decarboxylase.

Manometric experiments with washed cell suspensions gathered after 60 h growth on the quinate medium (solidified with 1·5 per cent w/v agar) demonstrated no oxygen uptake by the three strains in the presence of sodium quinate (*p*H 7·4, 30°), whereas representatives of the other 49 strains rapidly oxidized this substrate.

It would seem that fluorescent pseudomonads commonly possess the ability to oxidize quinic acid via protocatechuic acid but that some strains are unable to attack it by this or any other pathway. Since the formation of protocatechuate can be easily demonstrated in growing cultures by its reactions with ferrous and ferric ions, and since a significant number of strains do not effect its production, the test should prove a useful one for inclusion in taxometric methods for the sub-grouping of the *Pseudomonas*. It has been found that the use of a semi-solid medium favours the accumulation of the protocatechuate formed in the region below the surface growth and prevents its too rapid oxidation.

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[2] King, E. O., Ward, M. K., and Raney, D. E., *J. Lab. Clin. Med.*, **54**, 301 (1954).
[3] Soloway, S., and Rosen, P., *Anal. Chem.*, **25**, 595 (1953).

VIROLOGY

Rubella Virus Complement-fixation Test

METHODS in present use for the serological diagnosis of rubella consist of the neutralization[1,2] and the indirect fluorescent-antibody[3] techniques. The former is expensive and laborious and requires usually 7–10 days to carry out, while the latter, although it can give an answer within a matter of hours, is a difficult technique for routine