Bacterial Culture Media from Hevea Latex

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The availability is indicated of two bacterial growth media, at present rejected in large quantities by the natural latex industry in Malaya: details for the preparation of these for routine laboratory use are given. Some of the bacteria which have been cultured on these media are listed.

THE RAPID GROWTH OF BACTERIA in fresh hevea latex suggests that reject materials derived from this latex may serve as bacterial culture media. It is known that the activities of the fresh latex bacteria are confined, in main, to components present in the serum and are not associated with the decomposition of the dispersed hydrocarbon. Two sera are readily available from the factory; the first is 'sheet serum' formed on the (formic) acid coagulation of diluted field latex, the second is 'skim serum'. Both of these materials are good culture media for bacteria. It is the purpose of this paper to indicate the extreme utility of both as general culture media for microorganisms and to describe their preparation for routine laboratory use.

The production of latex concentrate from hevea latex by centrifuging is accompanied by the accumulation of large quantities of the low polyisoprene content dispersion known as 'skim'. The coagulation of this material is usually accomplished by lowering the pH, either by the direct addition of mineral acid or by the progressive action of bacteria principally of the genera *Streptococcus* Rosenbach, and *Propionibacterium* Orla-Jensen. In the former case there results a skim serum the pH of which varies between 4 and 5 but which otherwise possesses all the properties of a first class bacteriologic medium. This serum is, at present, rejected as a useless by-product of natural rubber production.

EXPERIMENTAL METHODS

Skim serum rejected in this way from the factory is bulked, its pH is adjusted roughly to 7, and it is autoclaved at a pressure of 10 lb per sq. in. for 10 minutes. On cooling, the pH is accurately adjusted to 7.0 ± 0.1 by the addition of 2N aqueous sodium hydroxide. The medium is then filtered through paper pulp and sterilised at a pressure of 15 lb per sq.in. for 10 minutes. A solid medium can be obtained by the addition of agar agar; the agar is added after the rough adjustment of pH and the initial autoclaving serves to melt the agar into solution — accurate adjustment again follows on filtration through paper pulp. The final medium is a golden brown in appearance, not unlike normal (lab' lemco) nutrient agar.

The initial autoclaving serves to precipitate any hydrocarbon or protein not coagulated by the factory addition of mineral acid. The necessity for this procedure depends upon the time allowed after coagulation for the separation of serum from coagulum. It is general factory practice to allow the acidified serum to stand overnight and if this procedure is adopted, no further precipation occurs on autoclaving.

Sheet serum may be prepared for laboratory use in an exactly analogous fashion.

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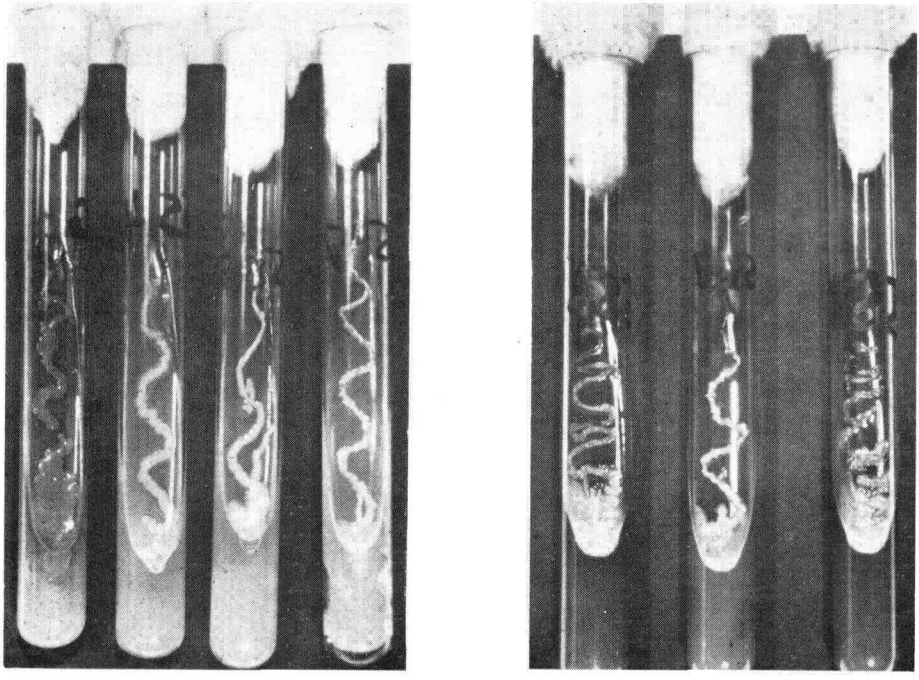

Strains of bacteria growing aerobically on (right) skim serum agar slopes, and on (left) sheet serum agar slopes.

RESULTS

Bacteria known to grow well on these media include *Serratia marcescens* Bizio, *Bacterium mycoides* Grotenfelt, *Chromobacterium violaceum* Schroeter *Mycobacterium phlei* Lehmann & Neumann, the whole of the coliform group of organisms, staphylococci, streptococci, propionibacteria, microbacteria, bacilli, micrococci, some corynebacteria, and flavobacteria and a large variety of unidentified organisms derived from hevea latices and Malayan soils. In addition the growth of species of streptomyces has been observed. There seems no reason why the media should not be equally useful for the cultivation of fungi (*Eumycetes*).

DISCUSSION

The media contain a wide variety of amino acids principally alanine, valine, tyrosine and proline, carbohydrates mainly the polyhydric alcohols myo-inositol and quebrachitol (methyl-l-inositol), an exceedingly diverse series of proteins of widely varying molecular weights probably extending to the nuclear proteins and various plant growth factors.

Since sera are rejected from latex factories in Malaya at the rate of thousands of gallons per day, this material is available for the large scale production of antibiotics and growth factors. One example may be of value: the tripyrryl methene pigment of *Serratia marcescens* is active in vitro against various pathogenic fungi (Harned 1954, Wier et alii 1952); its production depends upon the availability of cheap culture media

for the growth of this organism. Both skim and sheet sera are excellent media for the cultivation of *S. marcescens* although no doubt the amount of pigment produced per cell can be increased by the addition of further carbohydrates.

The utilisation of sera would prevent their pollution of local river water supplies. In these, at present large numbers of bacteria are cultivated under almost ideal conditions and constitute a continual danger to the latex producer who frequently uses these contaminated waters in factory washing. The processing of sera (and factory waste in general) would thus both remove a danger and provide an asset.

Under present unhygenic factory conditions, sheet serum, despite its further dilution, has proved the better culture medium. Growth on skim serum is less luxuriant. This position could be reversed, but the reversal is unlikely under present economic conditions.

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