A Medium for Isolation and Cultivation of Hevea Latex Bacteria

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A medium (molasses yeast extract agar) consisting of 0.5% molasses, 0.5% yeast extract, 0.005% bromocresol purple and 1.25% Oxoid agar No. 3 was prepared for the evaluation of the bacterial population of fresh field latex, ammoniated field latex, ammoniated concentrate latex and pure cultures. The bromocresol purple adequately indicates acid production from the metabolic breakdown of substrates. The medium effectively suppresses the spreading habit of a number of bacteria commonly present in latex, enabling easier enumeration. Incorporation of 0.01% actidione into the medium inhibits the growth of mould contaminants, without adversely affecting the bacterial population.

Hevea latex contains large numbers of bacteria (TAYSUM, 1957) and yeast (JOHN AND TAYSUM, 1963), which proliferate mainly at the expense of the non-rubber substances and produce acids destabilising the latex (JOHN, 1966 a and b). Although early workers had some knowledge of the numbers and kinds of bacteria in the fresh latex (PRESCOTT AND DOELGER, 1927; CORBET, 1929 and 1930), their investigations were handicapped by the lack of a suitable medium. OVERTON (1954) used a latex serum agar medium. RHINES AND MCGAVACK (1954) used nutrient agar containing glucose and latex cream serum; TAYSUM (1956) used a medium containing twelve ingredients, closely resembling Kligler's iron agar. The earlier media gave variable results; the modified Kligler's iron agar gave good results but was found to be somewhat tedious to prepare. This paper describes a simple isolation and growth medium which permits an accurate evaluation of the bacterial population in fresh field latex, ammoniated field latex, ammoniated concentrate latex and pure cultures.

MATERIALS AND METHODS

Media

Constituents of twelve experimental media for comparison with Kligler's iron agar are given in *Table 1*. To each was added 1.25% Oxoid agar No. 3 and 0.005% bromocresol purple, the pH of the mixture being maintained at 7.8. Media were sterilised by autoclaving at 15 lb/in^2 pressure for 15 minutes. For supressing mould growth, actidione was incorporated at the rate of 0.01% on the medium (JOHN, 1968).

When latex serum was used, other ingredients of the medium were dissolved in the required quantity of the serum, which was obtained by coagulating *Hevea* latex with formic acid at a pH of 5.2. Molasses samples obtained from local refineries had a sugar content of about 50%. Beef and yeast extracts were obtained from Oxoid.

Inocula

Three latex systems were used as inocula: fresh field latex (FFL), ammoniated field latex (AFL) with 0.3% ammonia, and ammoniated concentrate latex (ACL) with 0.7% ammonia. The ammonia levels were based on the weight of latex. Pure cultures, isolated from *Hevea* latex, were of *Serratia marcescens*, *Bacillus subtilis*, *Micrococcus* sp. *Agrobacterium* sp. *Streptococcus faecalis*, and *Corynebacterium* sp.—grown in Oxiod nutrient broth for 24 hours at 30°C.

Enumeration of Bacteria

One ml inocula from serial tenfold dilutions of FFL, AFL, ACL and pure cultures were pour-plated, incubated at 30°C for three or four

TABLE 1. MEAN BACTERIAL COUNTS (IN LOG) OF LATEX SAMPLES AND PURE CULTURES WHEN GROWN ON DIFFERENT MEDIA

Medium	Inoculum			
	FFL	AFL	ACL	Pure culture
Yeast extract, 0.5%	6.61	6.49	5.06	8.42
Yeast extract, 1.0%	6.56	6.37	4.95	8.38
Molasses, 0.5%	6.32	6.22	4.69	8.24
Molasses, 0.5% + Latex serum	6.42	6.30	4.51	8.16
Molasses, 0.5% + Beef extract, 0.5%	6.60	6.57	5.01	8.60
Molasses, 0.5% + Yeast extract, 0.5%	6.66	6.60	5.02	8.40
Molasses, 0.5% + Yeast extract, 1.0%	6.62	6.50	4.90	8.35
Molasses, 1.0%	6.30	6.24	4.44	8.21
Molasses, 1.0% + Latex serum	6.40	6.40	4.32	8.12
Molasses, 1.0% + Beef extract, 0.5%	6.54	6.50	4.63	8.28
Molasses, 1.0% + Yeast extract, 0.5%	6.63	6.56	4.86	8.33
Molasses, 1.0% + Yeast extract, 1.0%	6.57	6.46	4.91	8.26
Kligler's iron agar (control)	6.68	6.63	5.07	8.38

S.E. within an inoculum ± 0.06 Min. sig. diff. 0.17 (P = 0.05)

days and the bacteria counted by the method of JOHN AND TAYSUM (1963).

Mould Contamination

To assess inhibition of mould contaminants in the presence of actidione, plates were prepared using two media with and without actidione; they were exposed for 30 minutes to trap moulds and incubated at 30°C for three days before the mould colonies were counted.

RESULTS

Enumeration of Bacteria on Growth Media

Six samples each of FFL, AFL, ACL and pure cultures were pour-plated using each of the thirteen media and bacterial colonies were counted after four days (*Table 1*). Of the two levels of molasses tried, 0.5% gave higher bacterial counts than 1.0%. Additions of beef extract or yeast extract to molasses resulted in still higher counts and, of the two, yeast extract generally gave marginally higher counts. When tested in the presence or absence of molasses, 0.5% yeast extract gave by and large better counts than 1.0%. The addition of latex serum to molasses did not improve the counts markedly. Thus, of the twelve experimental media tested, molasses (0.5%) yeast extract (0.5%) agar was found to be the best medium which compared favourably with Kligler's iron agar.

Comparison of Molasses Yeast Extract Agar with Kligler's Iron Agar

Acid production. FFL, AFL, ACL and pure cultures were plated on molasses yeast extract agar and on Kligler's iron agar. Plates were incubated at 30°C and acid production observed, using bromocresol purple indicator. Acid was produced in molasses yeast extract agar, in many cases more than in Kligler's iron agar.

Spreading habit. Platings were made from FFL, AFL, ACL and pure cultures on molasses yeast extract agar and Kligler's iron agar to observe the spreading habits of bacterial colonies on these media. Molasses yeast extract agar effectively restricted the spreading of most of the colonies giving rise to nearly isolated and discrete colonies, whereas colonies spread moderately or profusely on Kligler's iron agar *(Figure 1)*.

Effect of actidione on mould contaminants. Plates of molasses yeast extract agar and Kligler's iron agar, with and without actidione, were exposed for 30 minutes and incubated at 30°C; mould colonies were counted after three days. Actidione effectively inhibited the moulds on both media.

Effect of actidione on bacterial growth. Six samples each of FFL, AFL, ACL and pure cultures were plated on molasses yeast extract agar with and without actidione and on Kligler's iron agar plates, incubated at 30°C for four days and bacterial colonies counted *(Table 2)*. The 0.01% actidione added effectively controlled mould contamination without adverse effect on the bacteria counts on molasses yeast extract agar.

TABLE 2. MEAN LOG BACTERIAL COUNTS OF LATICES AND PURE CULTURES

Inoculum	Medium		
	Kligler's iron agar	Molasses yeast extract agar (M)	M + actidione
FFL	7.11	7.06	7.09
AFL	6.73	6.78	6.75
ACL	4.42	4.37	4.38
Pure cultures	7.65	7.64	7.64
		±0.025 (0.07)	
Mean	6.48	6.46	6.46
S.E. of Mean		±0.012	
Min. sig. diff. (P = 0.05)		0.03	

DISCUSSION AND CONCLUSIONS

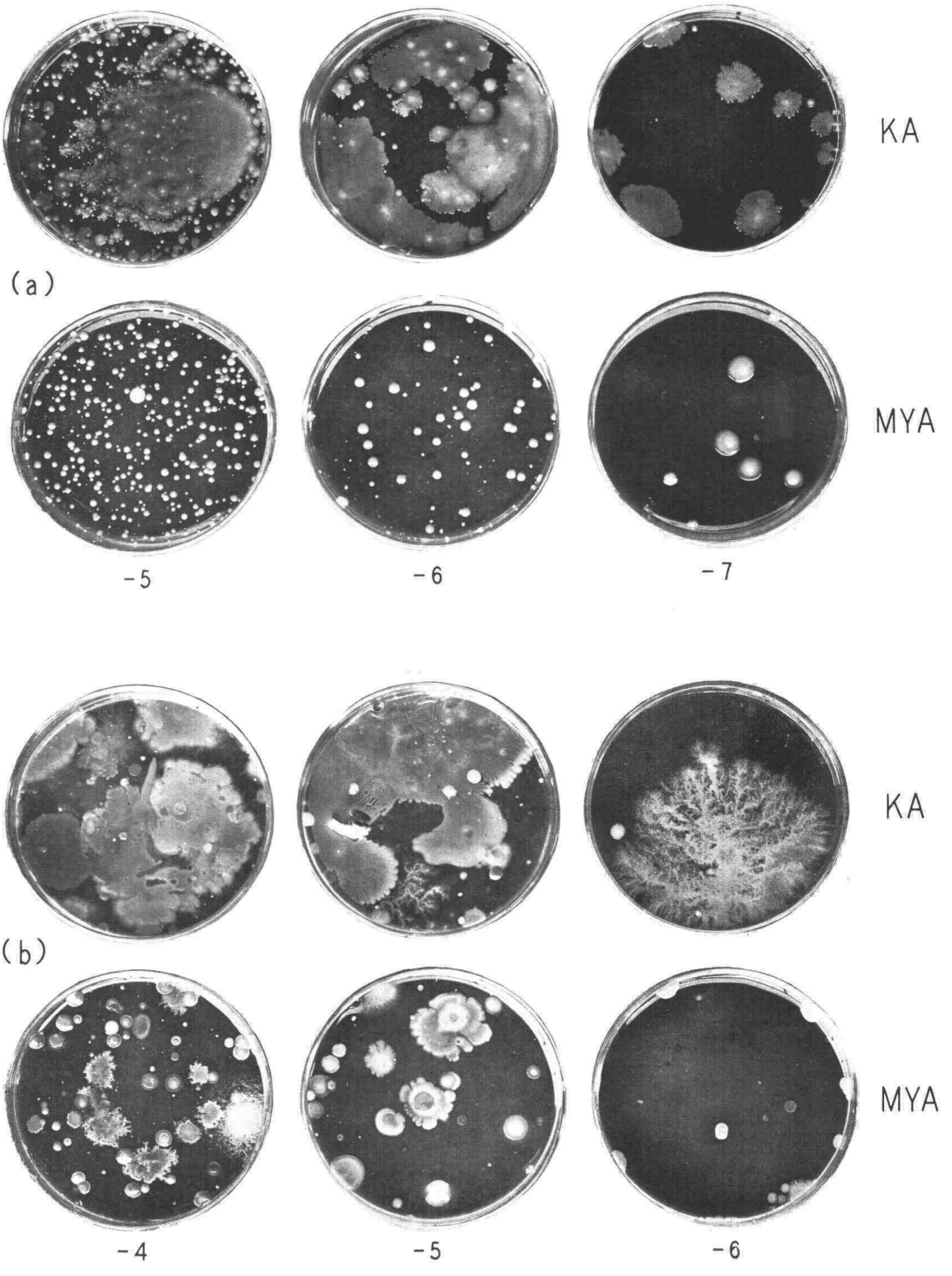
Hevea latex is a complex biological system containing a wide variety of non-rubber substances—carbohydrates, inositols, proteins, lipids, resins, carotenoids, mineral salts and enzymes (ARCHER *et al.*, 1963). Its bacterial population is highly diverse. A medium for isolating latex bacteria should be similarly complex. Though Kligler's iron agar meets these demands, it is tedious to prepare.

The present work indicates that a simple medium capable of meeting the demands of the latex bacteria can be prepared from 0.5% molasses and 0.5% yeast extract. Molasses, apart from its carbohydrates, contains a wide variety of materials which are generally utilised by bacteria as growth substances (EPPS, 1966). Yeast extract is a potent source of growth substances. FFL, AFL, ACL and pure cultures plated on molasses yeast extract agar made as good growth as on Kligler's iron agar.

Any medium employed in the enumeration of latex bacteria should indicate acid production. Bromocresol purple added to molasses yeast extract agar adequately indicates acid formation.

Spreading of colonies on Kligler's iron agar makes counting difficult when dealing with mixed cultures. Molasses yeast extract agar has a marked tendency to prevent the spreading of bacterial colonies *(Figure 1)*.

Incorporation of 0.01% actidione into Kligler's iron agar which effectively prevents the


KA
(a)
MYA
-5
-6
-7
KA
(b)
MYA
-4
-5
-6

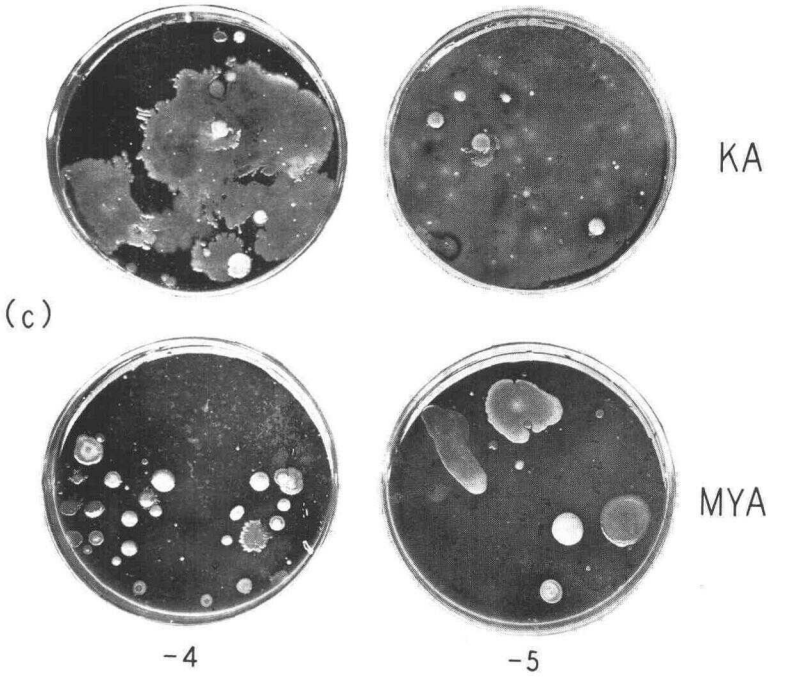


Figure 1. Bacterial colonies are seen here spreading profusely on Kligler's iron agar (KA), whereas their spread is restricted on molasses yeast extract agar (MYA). Pour-plates are from:

(a) pure cultures;
(b) ammoniated field latex; and
(c) fresh field latex.

growth of moulds without adversely affecting bacterial counts (JOHN, 1968) is equally effective in suppressing mould growth in molasses yeast extract agar.

It is concluded that molasses yeast extract agar equals Kligler's iron agar as an isolation and growth medium for the bacteria of *Hevea* latex and excels it in being far easier to prepare and more efficient in preventing the spreading of bacterial colonies.

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