

Use of Actidione to Control Mould Contaminants in Hevea Latex Systems Containing Yeasts and Bacteria

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*Accurate enumeration of bacteria in Hevea latex, using Kligler's iron agar, is made difficult by the profuse overgrowth of moulds which often obscure the bacterial colonies. Kligler's iron agar containing 0.01% actidione (cycloheximide) inhibited the growth of moulds, except some *Aspergillus* spp., which were not affected even by 0.05%. Enumeration of bacteria in fresh latex, ammoniated field latex, ammoniated concentrate latex or pure cultures, using Kligler's iron agar, was not adversely affected by the presence of actidione in the medium; indeed, if anything, it improved the counts, particularly from ammoniated latex concentrate and pure cultures. When latices containing bacteria and yeasts were cultured on Kligler's iron agar, bacterial cells grew to form colonies, but only exceptionally were yeasts' colonies obtained.*

The use of modified Kligler's iron agar (TAYSUM, 1956) for the enumeration of bacteria from latex samples is often complicated by the presence of moulds (PEPPER, 1961) and yeasts (JOHN AND TAYSUM, 1963). Moulds in the growth medium develop rapidly during incubation and often obscure most of the bacterial colonies; some synthesise anti-bacterial substances. For an accurate enumeration of latex bacteria it is thus necessary to prevent growth of moulds and yeasts in the medium.

MATERIALS AND METHODS

Inocula were fresh field latex (FFL), ammoniated field latex (AFL), ammoniated concentrate latex (ACL), or pure cultures of *Staphylococcus aureus*, *Micrococcus* sp., *Lactobacillus* sp., *Serratia marcescens*, *Xanthomonas* sp. and *Bacillus* sp. isolated locally from *Hevea* latex.

The basic medium was modified Kligler's iron agar (TAYSUM, 1956). Actidione was added to the melted basal medium as a filtered sterilised 1% solution in distilled water, to give the required level. Bacteria and yeasts in latices were enumerated using Kligler's iron agar and modified Martin's medium (JOHN AND TAYSUM, 1963) respectively.

RESULTS

Presence of Yeasts in Cultures from Latices
Latex samples were surface plated using

0.5 ml inocula from serial ten-fold dilutions. Plates without much crowding were selected for microscopic examination of each colony using negative staining with nigrosin. Kligler's iron agar, with or without 0.1% actidione, inhibited yeast growth (Table 1). However, on modified Martin's medium, mean values of 8.8×10^3 and 4.0×10^3 yeasts per ml of inoculum was obtained from FFL and AFL respectively. No yeasts were obtained from ACL inocula.

TABLE 1. TOTAL NUMBER OF BACTERIAL AND YEAST COLONIES PRESENT ON KLIGLER'S IRON AGAR WITH AND WITHOUT ACTIDIONE

Inoculum	No. of samples tested	Medium			
		Kligler's iron agar (K)		K+0.01% actidione	
		Bacteria	Yeasts	Bacteria	Yeasts
FFL	8	216	2	224	0
AFL	8	155	0	187	0
ACL	5	27	0	32	0
Total	21	398	2	443	0

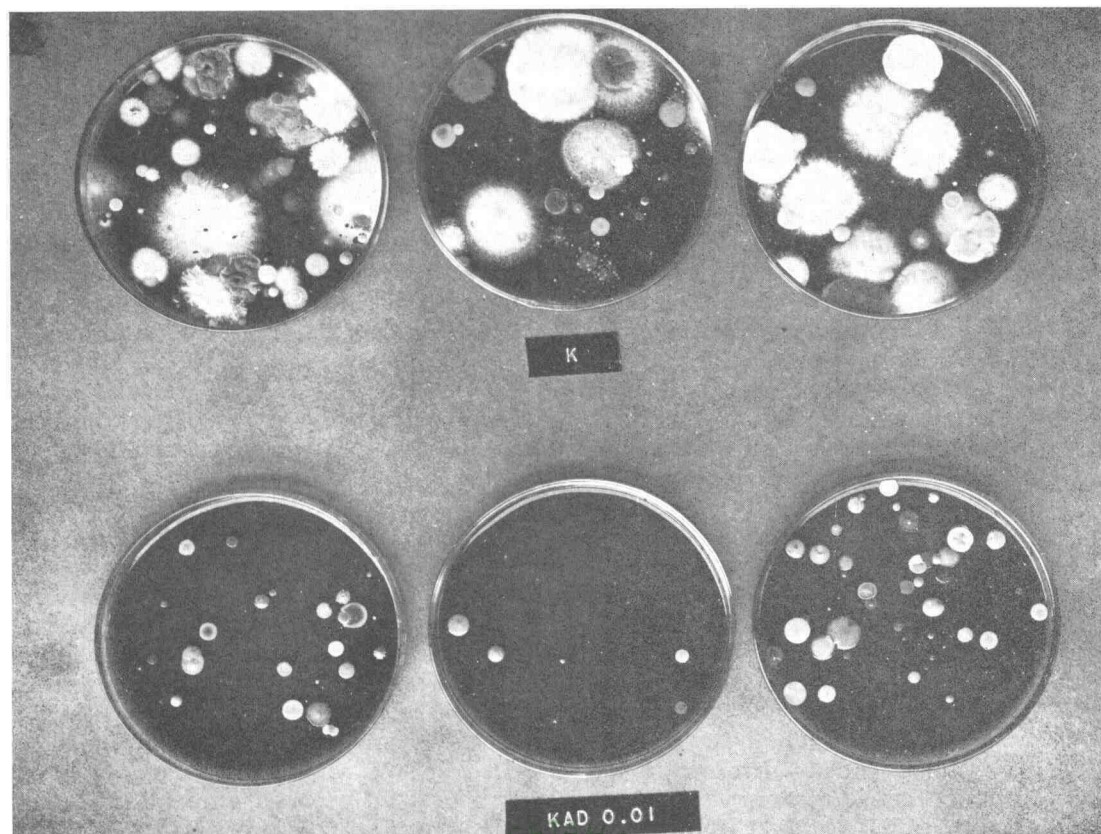


Figure 1. Effect of actidione on moulds. Kligler's iron agar plates containing 0.01% actidione (KAD) inhibited the mould growth while they grew profusely in its absence (K).

Effect of Actidione on Mould Contaminants

Kligler's iron agar plates with and without actidione were exposed in the laboratory for 25 minutes, covered, incubated at 30°C for three days and the mould colonies counted. The operation was repeated on ten days.

Common laboratory contaminants grew profusely on the medium without actidione, obscuring the bacterial growth, but even the lowest of the five levels of actidione used in this study effectively inhibited almost all the moulds (Figures 1 and 2); the addition of 0.01% actidione reduced the mean count of colonies per plate from 8.1 to 0.4. Higher concentrations of actidione did not give a statistically significant further reduction. The few resistant moulds

were mostly species of *Aspergillus*, producing small and discrete colonies.

Effect of Actidione on Bacteria

Poured plates were made with 1 ml inoculum from serial ten-fold dilution series. Six samples each of FFL, AFL, ACL and pure cultures were plated and the bacteria counted after three days (Table 2). The higher levels of actidione gave significantly higher counts than the control.

DISCUSSION

Though yeast colonies infrequently appeared when FFL was plated, AFL and ACL samples yielded none; thus yeasts do not interfere when Kligler's iron agar is used to enumerate bacteria in latex. With added 0.01% actidione

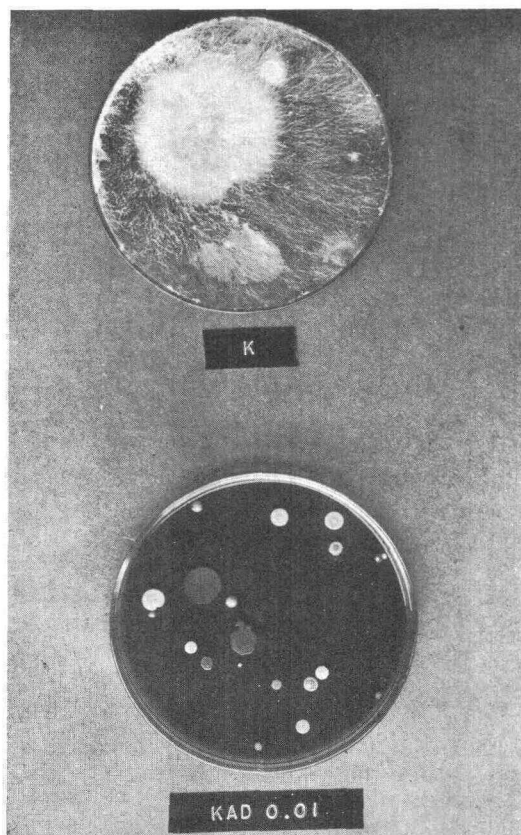


Figure 2. Effect of actidione on *Monilia*. K=Kligler's iron agar; KAD 0.01=Kligler's iron agar + 0.01% actidione.

no yeast colony survived, most being inhibited at 0.0025% or below (WHIFFEN, 1948). Further, most contaminating moulds are inhibited by 0.01% actidione. Higher bacterial counts obtained with actidione added to the Kligler's iron agar (Table 2) can be attributed to the suppression of inhibitory growth.

WHIFFEN (1948), PHILLIPS AND HANEL (1950) and JEFFERS (1954), who among them tested the action of actidione against 58 bacteria in 22 genera, found none to be inhibited by 0.01% actidione. In the present study, actidione concentrations up to 0.04% did not adversely affect the bacteria present in latex.

It is concluded that yeasts do not interfere with the enumeration of latex bacteria using

TABLE 2. BACTERIAL COUNTS (LOGARITHMIC MEANS) ON LATEX SAMPLES AND PURE CULTURES OF BACTERIA IN KLIGLER'S IRON AGAR WITH AND WITHOUT ACTIDIONE

Actidione concentration %	Inoculum				Mean
	FFL	AFL	ACL	Pure cultures	
0.00	6.78	6.66	3.87	8.11	6.36
0.01	6.79	6.68	3.97	8.11	6.39
0.02	6.81	6.68	3.95	8.12	6.39
0.03	6.81	6.67	3.96	8.15	6.40
0.04	6.83	6.68	3.97	8.16	6.41
S.E.	±0.016				±0.008
Min. sig. diff.*	0.04				0.02

* $P=0.05$

Kligler's iron agar and that an accurate enumeration can still be made on samples containing or contaminated with moulds, by incorporating 0.01% actidione.

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