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Survival of Hevea *Latex Bacteria in the Presence of a Composite Mixture of Tetramethyl Thiuram Disulphide and Zinc Oxide*

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Bacterial counts of Hevea *field latices treated with 0.025% TMTD/ZnO, after three days' storage, were consistent compared to the counts of latices treated with 0.01% and 0.02% TMTD/ZnO. Treatment with 0.025% TMTD/ZnO reduced the bacterial log count of* Hevea *field latex to less than 1 per millilitre after fifteen days' storage while 0.01% had no effect. Treatment with 0.025% TMTD/ZnO on the latex concentrate was able to inhibit the metabolic activities of the contaminated bacteria. However, there was indication that contaminated bacteria in high ammonia (0.7% ammonia) latex concentrate could develop resistant to the ammonia. The predominant bacteria isolated from* Hevea *field latex treated with TMTD/ZnO were* Streptococcus *sp.,* Corynebacterium *sp., Bacillus sp.,* Alcaligenes *sp.,* Micrococcus *sp. and* Pseudomonas *sp. Incorporation of 0.01% TMTD/ZnO completely killed* Streptococcus *sp. after 48 hours. For* Corynebacterium *sp., 0.02% TMTD/ZnO completely killed the bacterium after 24 hours. There was hardly any effect on* Pseudomonas *sp. even at 0.025% TMTD/ZnO.*

Fresh *Hevea* field latex contains high bacterial population[1]. Their presence in the latex is mainly through contamination[2]. These bacteria are originated from the bark of the tree, residual scrap rubbers formed during tapping and collection and the air. *Hevea* latex also contains an abundance of proteins, sugars, free amino acids and small amount of inorganic compound[3,4]. These substances promote the proliferation of bacteria in the latex[5,6]. Biochemical reactions brought about by these bacteria cause destabilisation of the latex[1,7,8]. These microbial activities need to be inhibited or reduced in order to keep the latex stable. In Malaysia, the current recommendation for the low ammonia (LA) latex concentrate production is a composite mixture of ammonia, tetramethyl thiuram disulphide (TMTD) and zinc oxide (ZnO)[9-11]. The ratio of TMTD to ZnO is 1:1 and they are applied in dispersion form. The recommended composite concentration of TMTD and ZnO to be applied to field and concentrate latices is 0.025% (weight/weight).

During the preparation of latex concentrate steps are usually taken to prevent contamination of the latex. However, accidental contamination can occur due to the handling of large volume of latex. In order to reduce cost of production, the producers might attempt to apply TMTD and ZnO at concentration below the recommended level. This paper provides information on the bacterial counts of field latex treated with TMTD/ZnO at levels below the recommended concentration and the growth of bacteria isolated from TMTD/ZnO-treated field latex at various TMTD/ZnO concentrations. This paper also reports the effect of contamination on the bacterial counts of field and concentrate latices treated with TMTD/ZnO.

MATERIALS AND METHODS

Bacterial Enumeration of Field Latices

Bacterial enumeration was on field latex treated with different levels of TMTDZnO

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(0.01% to 0.025% weight/weight). TMTD/ZnO is a composite mixture in the ratio of 1:1. In addition to TMTD/ZnO, 0.2% ammonia was added to the field latex. Enumerations were carried out by plating using Molasses Yeast Extract (MYE) agar[12].

Bacterial enumeration was also conducted on TMTD/ZnO-treated accumulated field latex. Accumulation was carried out by adding 10% TMTD/ZnO-treated field latex (three days old) into freshly collected field latex. Investigation was carried out with different levels of TMTD/ZnO treatments (0.01%–0.025%).

Isolation of Bacteria

The bacteria were isolated from field latex treated with 0.2% ammonia and TMTD/ZnO. The isolation and purification of the bacteria were carried out by plating using MYE agar. The purified bacterial strains were identified according to the method described by Cowan and Steel[13].

Growth of Isolated Bacteria in the Presence of TMTD/ZnO

Each bacterial isolate was grown for 24 h in 200 ml nutrient broth in 500 ml Erlenmeyer flask and incubated on a rotary shaker at 30°C before inoculating into fresh nutrient broth flasks containing a range of TMTD/ZnO concentrations (0%–0.025%) and 0.2% ammonia. 10 ml of the broth culture was used for the inoculation. The flasks were incubated on a rotary shaker at 30°C. At periodic intervals, samples were taken and plated for bacterial count using nutrient agar. The bacterial colonies were counted after five days' incubation at 30°C.

Bacterial Enumeration of Contaminated Latex Concentrates

Latex concentrate preserved with 0.025% TMTD/ZnO and 0.2% ammonia was inoculated separately with aged field and concentrate latices and a suspension of bacteria previously isolated from TMTD/ZnO-treated field latex. The ages of the field and concentrate latices used for the inoculation were two weeks and two months respectively. The bacterial log counts of the aged field and concentrate latices and the bacterial suspension were 6.6 per millilitre, less than 1 per millilitre and 12.1 per millilitre respectively. As comparison, latex concentrate preserved with 0.7% ammonia only was also inoculated in a similar manner. Bacterial enumerations of the contaminated latex concentrates were carried out by plating using MYE agar.

Investigation was also carried out to study the effect of sucrose addition (0.5%) as substrate for the bacterial suspension inoculated into 0.025% TMTD/ZnO-treated latex concentrate on the bacterial growth and volatile fatty acid formation. The bacterial log count of the suspension inoculated was 9.2 per millilitre.

RESULTS

Bacterial Enumeration of Field Latices

Tratment with 0.025% TMTD/ZnO was able to reduce the bacterial log count to less than 1 per millilitre after fifteen days' storage *(Table 1)*. Treatment with 0.01% had hardly any effect in reducing the bacterial population. The bacterial counts of the treated latices, irrespective of the concentration of the preservative, decreased with time.

TABLE 1. EFFECT OF TMTD/ZnO ON THE BACTERIAL COUNT OF FIELD LATEX

TMTD/ZnO (%)	Bacterial count (log/ml)		
	Day 0	Day 3	Day 15
0.01	6.24	6.13	6.08
0.02	6.08	5.07	3.88
0.025	6.74	4.36	<1.00

Table 2 shows the bacterial counts of field latices treated with TMTD/ZnO from different collections. Bacterial counts of field latices treated with 0.025% TMTD/ZnO, after three days of storage, were more consistent and had the least standard deviation compared to latices treated with 0.01% and 0.02% TMTD/ZnO.

TABLE 2. BACTERIAL COUNTS OF TMTD/ZnO FIELD LATICES FROM DIFFERENT COLLECTIONS

TMTD/ZnO (%)	Bacterial count (log/ml)	
	Day 0	Day 3
0.01	8.4	8.3
0.01	6.2	6.1
0.01	6.0	4.3
0.01	7.6	4.1
0.01	7.1	3.4
Mean		5.3
S.D.		1.98
Range		3.4 – 8.3
0.02	7.6	1.5
0.02	6.1	5.1
0.02	6.4	4.4
0.02	6.0	4.3
Mean		3.8
S.D.		1.59
Range		1.5 – 5.1
0.025	6.5	3.4
0.025	6.7	4.4
0.025	6.5	4.9
0.025	6.0	3.9
Mean		4.2
S.D.		0.65
Range		3.4 – 4.9

However, there were instances whereby treatments at 0.01% and 0.02% gave good bacterial counts reduction. Bacterial log counts of field latices treated with 0.025% TMTD/ZnO were all below 5.0 per millilitre. Field latices treated with 0.01% and 0.02% TMTD/ZnO showed bacterial log counts ranging from 3.4 to 8.3 per millilitre and 1.5 to 5.1 per millilitre respectively. Hence, it is safer to treat *Hevea* field latex with TMTD/ZnO at 0.025%.

Table 3 shows the bacterial counts of accumulated field latex treated with TMTD/ZnO (0.01%–0.025%). Bacterial counts of field latices of the second accumulation *(i.e. Treatment C)* showed an increase after three days of storage irrespective of the concentration of TMTD/ZnO applied. Hence, continuous accumulation of TMTD/ZnO-treated field latex should be avoided. However, bacterial counts of field latices of the first accumulation *(i.e. Treatment B)* showed a decrease after three days of storage but treatment with 0.01% TMTD/ZnO did not give satisfactory bacterial reduction (bacteria log count greater than 6.00 per millilitre). Accumulation of TMTD/ZnO-treated field latex should only be carried out once and the concentration of TMTD/ZnO applied is greater than 0.02%.

Bacteria Isolated from Field Latex Treated with TMTD/ZnO

The predominant bacteria isolated from TMTD/ZnO-treated field latex were identified as *Streptococcus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Alcaligenes* sp. and *Corynebacterium* sp.

Growth of Isolated Bacteria

Incorporation of 0.02% and 0.025% TMTD/ZnO into the nutrient broth completely killed *Corynebacterium* sp., after 24 h *(Figure 1a)*. The viable count was reduced to half after 24 h in the presence of 0.01% and there was no further reduction after 72 hours. Treatment with 0.01% TMTD/ZnO reduced the Streptococcal population to half after 24 h and completely killed after 48 h *(Figure 1b)*. *Bacillus* sp. count was reduced to half after 72 h in the presence of 0.01% TMTD/ZnO and there was no improvement when the concentration was increased to 0.025% *(Figure 1c)*. *Alcaligenes* sp. population was reduced to half in the presence of 0.02% TMTD/ZnO after 72 h *(Figure 1d)*. Increasing the dosage to 0.025% did not give further reduction. Treatment with 0.025% gave only slight reduction of *Micrococcus* sp. *(Figure 1e)* and hardly any effect on *Pseudomonas* sp. *(Figure 1f)* after 72 hours.

Bacterial Enumeration of Contaminated Latex Concentrates

Contamination of 0.025% TMTD/ZnO preserved latex concentrate with aged field and concentrate latices and bacterial suspension did

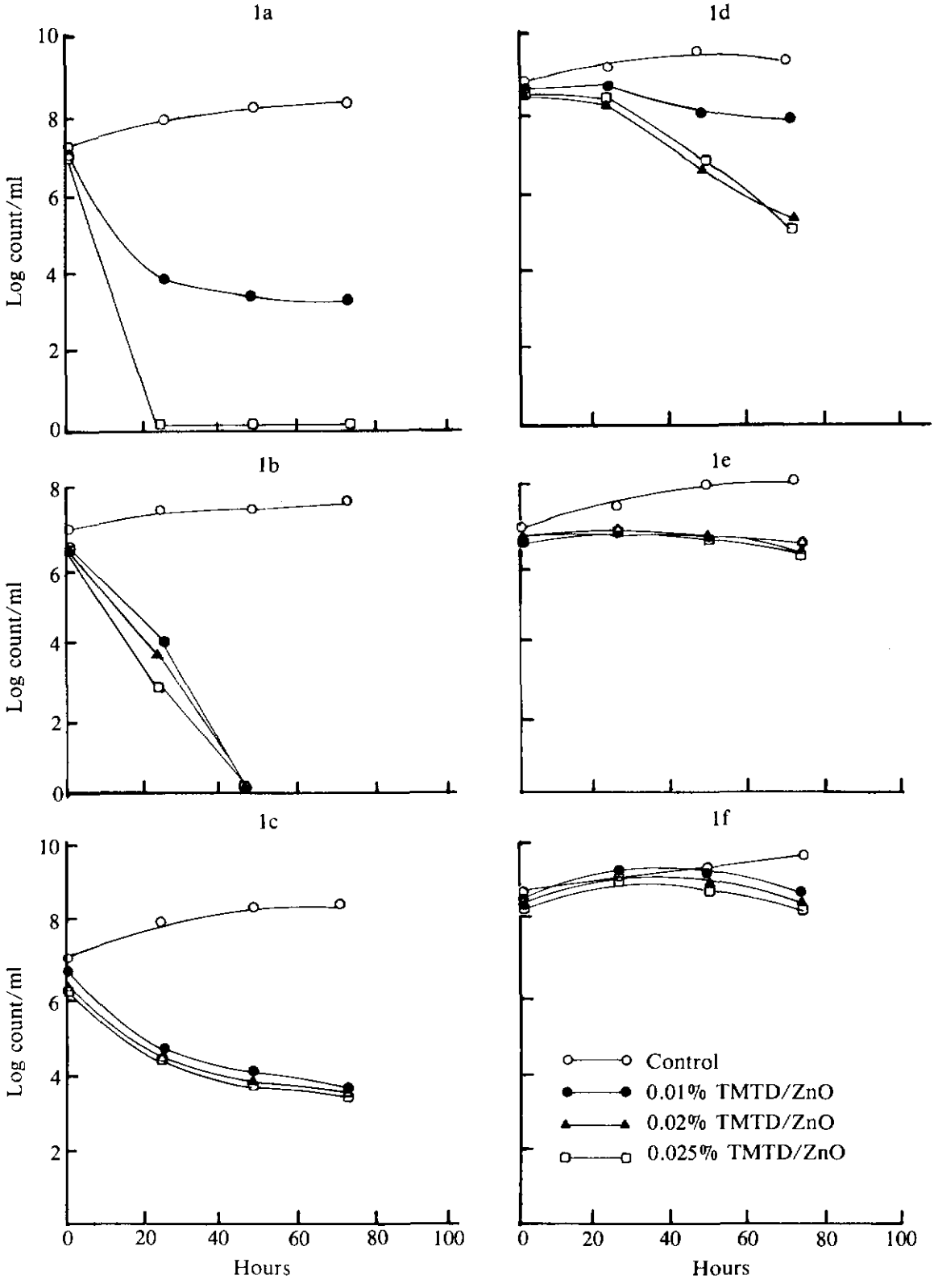

Figure 1. Growth of Corynebacterium *sp. (1a),* Alcaligene *sp. (1b),* Streptococcus sp. *(1c),* Micrococcus *sp. (1d),* Bacillus *sp. (1e), and* Pseudomonas *sp. (1f), in the presence of TMTD/ZnO.*

TABLE 3. BACTERIAL COUNTS OF ACCUMULATED FIELD LATEX TREATED WITH TMTD/ZnO (0.01%–0.025%)

Treatment			Bacterial count (log/ml) Day 0	Day 3
1.	(A)	0.01% TMTD/ZnO	6.02	4.30
	(B)	0.01% TMTD/ZnO + 10% latex from (A)	6.81	6.20
	(C)	0.01% TMTD/ZnO + 10% latex from (B)	6.16	7.60
2.	(A)	0.02% TMTD/ZnO	6.04	4.30
	(B)	0.02% TMTD/ZnO + 10% latex from (A)	5.95	4.85
	(C)	0.02% TMTD/ZnO + 10% latex from (B)	6.11	7.31
3.	(A)	0.025% TMTD/ZnO	6.04	3.93
	(B)	0.025% TMTD/ZnO + 10% latex from (A)	5.83	5.20
	(C)	0.025% TMTD/ZnO + 10% latex from (B)	6.56	7.58

Treatment (A) is treatment of fresh field latex without contamination.

Treatment (B) is carried out three days after *Treatment (A)*. Field latex consisted of 90% fresh field latex and 10% three-day-old field latex.

Treatment (C) is carried out three days after *Treatment (B)*. Field latex consisted of 90% fresh field latex and 10% three-day-old field latex.

not increase the bacterial population of the latex *(Table 4)*. Treatment with 0.025% TMTD/ZnO was effective in killing the bacterial contaminant in the latex concentrate. Contamination with 5% aged latex concentrate showed bacterial log count of less than 1 after one day of storage because the inoculum had bacterial log count of less than 1 per millilitre.

Table 5 shows the bacterial population of 0.7% ammonia only preserved latex concentrate contaminated with aged field and concentrate latices and bacterial suspension after one day and fourteen days of storage. Contamination of the latex concentrate with aged latex concentrate did not affect the latex. Bacterial

TABLE 4. BACTERIAL COUNTS OF LATEX CONCENTRATE (0.025% TMTD/ZnO) INOCULATED WITH AGED FIELD AND CONCENTRATE LATICES AND BACTERIAL SUSPENSION

Inoculum	Bacterial count (log/ml) Day 1	Day 14
Aged field latex (5% weight/weight)	4.47	<1.0
Aged latex concentrate (5% weight/weight)	< 1.00	<1.0
Bacterial suspension	3.47	1.86
Control (uninoculated)	<1.00	<1.00

TABLE 5. BACTERIAL COUNTS OF LATEX CONCENTRATE (0.7% AMMONIA) INOCULATED WITH AGED FIELD AND CONCENTRATE LATICES AND BACTERIAL SUSPENSION

Inoculum	Bacterial count (log/ml) Day 1	Day 14
Aged field latex (5% weight/weight)	4.48	<4.61
Aged latex concentrate (5% weight/weight)	<1.00	<1.00
Bacterial suspension	1.89	2.12
Control (uninoculated)	<1.00	<1.00

count of the latex concentrate contaminated with bacterial suspension after one day of storage was lower compared to the bacterial count of TMTD/ZnO-treated latex concentrate *(Table 5)* which was similarly contaminated. This could be due to the high concentration of ammonia (0.7%) in the latex concentrate which the bacteria had not encountered before. The bacteria were previously isolated from TMTD/ZnO-treated field latex. Bacteria counts of the latex concentrate contaminated with aged field latex and bacterial suspension after fourteen days' storage were slightly higher than after one day's storage. This could be due to the development of resistance by some of the bacteria in the latex to ammonia.

Table 6 shows that the contamination of 0.025% TMTD/ZnO-preserved latex concentrate with bacterial suspension did not increase the volatile fatty acid concentration in the latex although 0.5% sucrose was added as substrate to the bacteria. Concentrations of volatile fatty acid in the contaminated latices were similar to that of the control indicating the absence of metabolic activites by the inoculated bacteria. Treating the latex concentrate with 0.025% TMTD/ZnO was able to inhibit totally the metabolic activity of the contaminated bacteria.

DISCUSSION

Treatment of field latex at 0.025% TMTD/ZnO had shown to give consistent bacterial reduction and to kill a wider spectrum of bacteria in the field latex. Although instances had shown good bacterial reduction with treatment at TMTD/ZnO concentration lower than 0.025%, the reductions were inconsistent and the variations were very wide which were unsafe for latex concentrate preparation. There was inconsistency in bacterial counts of field latices collected from different sources although treated with same level of TMTD/ZnO. This inconsistency was due to the variability in the bacteria surviving in the field latices. There was also variability in bacteriacidal action of TMTD/ZnO on the bacteria. Different bacteria required different levels of TMTD/ZnO and periods of exposure to kill them completely; *Streptococcus* sp. was completely killed at 0.01% after 24 h but *Corynebacterium* sp. required the dosage to be increased to 0.02%.

Accumulation of field latex treated with TMTD/ZnO should be avoided because the accumulation caused the ineffectiveness of TMTD/ZnO to reduce the bacterial counts of the field latex. Latex concentrate treated with 0.025% TMTD/ZnO was not affected by contamination. Preservation of latex concentrate with 0.025% TMTD/ZnO was able to inhibit the metabolic activities of the contaminated bacteria in the latex. There was indication in the study that contaminated bacteria in the high ammonia (0.7%) preserved latex concentrate could develop resistant to ammonia. Shum and Wren[14] also observed the bacteria counts of 0.7% ammonia-preserved latex concentrate contaminated with aged field latex decreased initially during the first week but later showed an increase. Rhines and McGavack[5] reported the presence of ammonia resistant bacteria in latex. Hence, producers of high ammonia

TABLE 6. BACTERIAL COUNTS AND VOLATILE FATTY ACIDS (VFA) NUMBER OF LATEX CONCENTRATE INOCULATED WITH BACTERIAL SUSPENSION AND 0.5% SUCROSE

Treatment	Bacterial count (log/ml)			VFA no.		
	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30
0.025% TMTD/ZnO (control)	<1.00	<1.00	<1.00	0.02	0.02	0.03
0.025% TMTD/ZnO + 0.5% sucrose	<1.00	1.15	<1.00	0.02	0.02	0.03
0.025% TMTD/ZnO + 0.5% sucrose + bacterial suspension	5.75	5.04	3.43	0.02	0.03	0.03
0.025% TMTD/ZnO + bacterial suspension	5.38	5.18	3.60	0.02	0.03	0.03

(0.7%) latex concentrate should be cautious of the possible undesirable effect of bacterial contamination of their latex concentrate.

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REFERENCES

1. TAYSUM, D.H. (1958) The Numbers and Growth Rates of Bacteria in *Hevea* Latex, Ammoniated Field Latex and Ammoniated Latex Concentrates. *J. appl. Bact.*, **21**, 161.
2. JOHN, C.K. (1977) Microbiological Studies on *Hevea* Latex with Particular Reference to Its Keeping Quality and Coagulation. D.Sc. (Agric.) Thesis, University of Gent, Belgium.
3. COCKBAIN, E.G. AND PHILPOTT, M.W. (1963) Colloidal Properties of Latex. *The Chemistry and Physics of Rubber-like Substances (Bateman, L. ed)*. London: Maclaren and Sons Ltd.
4. ARCHER, B.L., AUDLY, B.G., McSWEENEY, G.P. AND TAN, C.M. (1969) Studies on Composition of Latex Serum and Bottom Fraction Particles. *J. Rubb. Res. Inst. Malaya*, **21(4)**, 560.
5. RHINES, C.E. AND McGAVACK, J. (1954) The Ammonia Resistant Bacteria Associated with Latex Deterioration. *Rubb. Age*, **75(b)**, 852.
6. JOHN, C.K. (1966) Biological Coagulation of *Hevea* Latex Using Waste Carbohydrate Substances. *J. Rubb. Res. Inst. Malaya*, **19**, 286.
7. JOHN, C.K. (1966) Breakdown of Amino acids by *Hevea* Latex Bacteria. *J. Rubb. Res. Inst. Malaya*, **19(4)**, 29.
8. JOHN, C.K. (1966) Metabolism of Quebrachitol and Other Carbohydrates by *Hevea* Latex Bacteria. *J. Rubb. Res. Inst. Malaya*, **19(4)**, 219.
9. JOHN, C.K., NADARAJAH, M., RAMARAO, P.S., LAU, C.M. AND NG, C.S. (1975) A Composite Preservative System for *Hevea* Latex. *Proc. Int. Rubb. Conf. 1975 Kuala Lumpur*, **4**, 339.
10. JOHN, C.K., NADARAJAH, M. AND LAU, C.M. (1976) Microbiological Degradation of *Hevea* Latex and Its Control. *J. Rubb. Res. Inst. Malaysia*, **24(5)**, 261.
11. RAMA RAO, P.S., JOHN, C.K., NG, C.S., SMITH, M.G. AND ROBERT, C.F. (1976) Commercial Exploitation of TMTD/ZnO Preservative System. *Proc. Rubb. Res. Inst. Malaysia Plrs' Conf. Kuala Lumpur 1976*, 324.
12. JOHN, C.K. (1968) A Medium for Isolation and Cultivation of *Hevea* Latex Bacteria. *J. Rubb. Res. Inst. Malaya*, **20(5)**, 285.
13. COWAN, S.T. AND STEEL, K.J. (1965) *Manual for Identification of Medical Bacteria*. London Cambridge University Press.
14. SHUM, K.C. AND WREN, W.G. (1977) Observations on Bacterial Activity in Natural Rubber Latex Plate Counts of Latex Bacteria on a Supplemented Medium. *J. Rubb. Res. Inst. Malaysia*, **25(2)**, 81.