Q. Jl. Rubb. Res. Inst. Ceylon (1971) 48, 182—192.

BACTERIAL COAGULATION OF LATEX*

By

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INTRODUCTION

Hevea latex while in the latex vessel is sterile and if collected in a sterile condition remains stable for a considerable period of time (McMullan, 1951). During tapping as the latex flows down the tapping cut and collects in the cup, it becomes contaminated with micro-organisms like bacteria and yeast. These micro-organisms in the process of growth metabolise the non-rubber constitutents of the latex and bring about coagulation. This is referred to as natural coagulation or autocoagulation. Taysum (1958) has reported that in a field where precoagulation was rare, the field latex at routine collection contained an initial population of 8×10^6 bacteria/ml. When the population increased to 10^9 bacteria/ml the latex began to thicken. At coagulation the colony count varied between 4×10^9 at the minimum and $6 \cdot 8 \times 10^9$ bacteria/ml at the maximum.

Taysum (1957) has also reported that the bacterial population in latex is composed of a number of species of bacteria which vary according to particular conditions. Altman (1947) thought that the different species of bacteria present in latex could be classified into two groups namely, (1) anaerobic bacteria able to accelerate the coagulation by transforming the sugars and other carbohydrates in latex into acids, and (2) aerobic bacteria, repressing the coagulation by degrading the proteins to give alkaline products. Kendall (1912 — 1913) has indicated that "fermentation takes precedence over putrifaction". This means, as Altman (1947) has suggested that, if both sugars and proteins are present, the bacteria will affect the sugars first, causing fermentation and afterwards the proteins, producing putrifaction products ; or that fermentation processes proceed more rapidly than putrification processes. Hence, it follows that, natural coagulation cannot be controlled, as much depends on the strains of bacteria present in the latex. Natural coagulation time may vary from 24 to 48 hours or more and even then coagulation is often incomplete. Apart from this, John (1966) has reported that the rubber produced is relatively unsatisfactory in ageing properties and has an offensive odour. He believed that these disadvantages could be overcome by speeding up the process of coagulation.

This could be achieved by two or three different methods of approach :

(1) by increasing the carbohydrates available for microbial breakdown to acidic derivatives in the latex ;

(2) by seeding the latex with suitable strains of bacteria so as to increase the population of efficient acid producers. These bacteria would then hasten the breakdown of the carbohydrates present in the latex to acidic derivatives ;

(3) by seeding the latex with suitable strains of bacteria and also adding carbohydrates to latex. This would be necessary only if there is insufficient carbohydrates in the latex for microbial breakdown, to give the required levels of acids for coagulation.

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* Patent applied for.

The first method, now referred to as assisted biological coagulation (or ABC process) was demonstrated by Eaton & Grantham (1915); they showed that by adding sugars to latex, the fermentative activity of bacteria could be encouraged to give complete coagulation. Recent work carried out at the Rubber Research Institute of Malaya has also shown that this method gives encouraging results (John, 1966). A near complete coagulation of 99·5% was obtained by adding sugar molasses (containing 50% sugar) or pineapple juice (containing 8 — 10% sugar) to undiluted latex. The time of coagulation was then reduced to 16 hours.

This paper gives some preliminary observations and results of experiments directed towards the coagulation of latex by the aid of suitable strains of bacteria, after they were cultured in a coconut water medium.

The experimental work is divided into two parts :

(1) the isolation and selection of bacteria on their ability to coagulate latex;

(2) the physical and technological properties of rubber obtained by bacterial treatment.

MATERIALS AND METHODS

Different species of bacteria were isolated from (*a*) rubber serum collected from latex collecting cups after autocoagulation in the field, (*b*) bucket coagulum, where the latex had precoagulated in the bucket, before it was taken for processing in the factory, and (*c*) coconut water which had been freely exposed to natural contamination.

All isolations were done by the dilution plate method using nutrient agar and incubation at room temperature (28±2° C) for 24 — 48 hours. All coloured colonies of bacteria were discarded and the predominantly white colonies were selected for study.

The selected bacteria were sub-cultured on plates of nutrient agar and transferred to tubes containing nutrient agar or peptone water agar, for stock culture. The stock cultures were then screened for their ability to coagulate sterile latex.

RESULTS AND DISCUSSION

Collection of sterile latex: Sterile, disposable, plastic syringes of 10 ml capacity were used to collect sterile latex direct from the tree. Latex collection was always carried out from virgin bark above the tapping cut.

A small area of the outer bark was scraped off to expose the underlying stone cell layer and a hole was bored, with a 21 gauge sterile needle down to the wood. The needle was removed and the hypodermic needle of the syringe quickly inserted into the hole ; the plunger of the syringe was kept at the zero mark. The latex, under pressure in the bark slowly filled the syringe barrel pushing the plunger up.

The latex from each syringe (5 ml) was transferred to a sterile McCartney bottle and inoculated with the bacterial suspension in sterile distilled water, using a platinum needle. The remaining latex from each syringe served as controls. After 48 hours the uncoagulated sterile latex and the serum from the coagulated latex were plated out as described earlier (Figs. 1a and 1b).

The results of the experiments are summarised in Table 1, which confirmed the following :—

(1) Sterile latex which remained stable was free of any micro-organisms ;

(2) The bacterial species introduced into the latex were responsible for the coagulation of latex to which they were added ;

(3) That these bacteria were capable of growth and multiplication in latex ;

(4) Some bacteria were more efficient than others in coagulating latex ;

(5) Some did not discolour the coagulum while others did ;

(6) Some gave malodourous coagulum while some did not.

TABLE 1

EFFICIENCY OF BACTERIAL ISOLATES ON COAGULATION OF STERILE LATEX

CLONE PB 86

Isolates	Time of coagulation in hours	Coagulum	
		Colour	Odour
CA	21	White	Not offensive
C2	64	White	Not offensive
C5	36	White	Not offensive
C6	21	White	Not offensive
RS 1	24	Cream	Not offensive
RS 2	36	White	Not offensive
RS 4	48	Cream	Unpleasant
RS 6	18	White	Not offensive
RS 7	24	White	Not offensive
RS 8	36	Pinkish	Unpleasant
BC 2	21	White	Not offensive
L 52	60	Yellowish	Unpleasant
M	21	White	Not offensive
Control	Uncoagulated at end of observation period — five days		

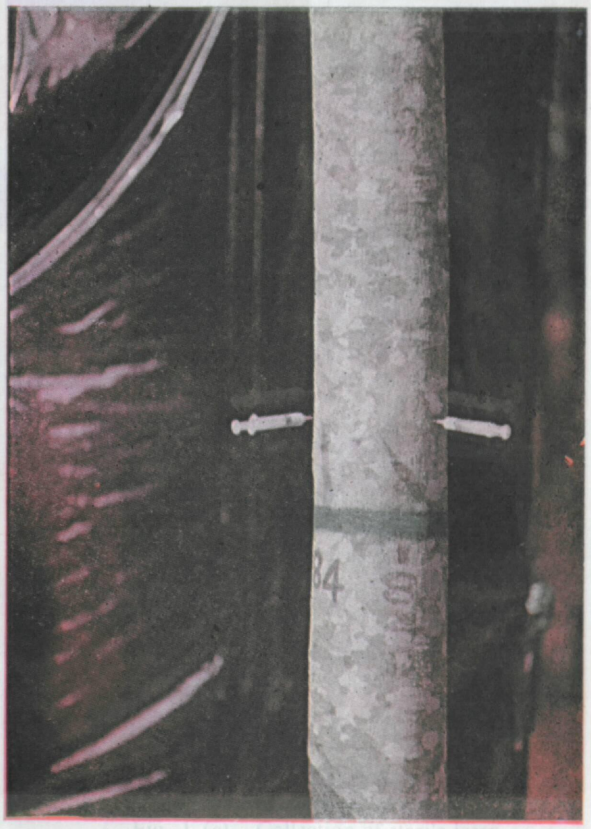

Fig. 1 (*a*). Collection of sterile latex.

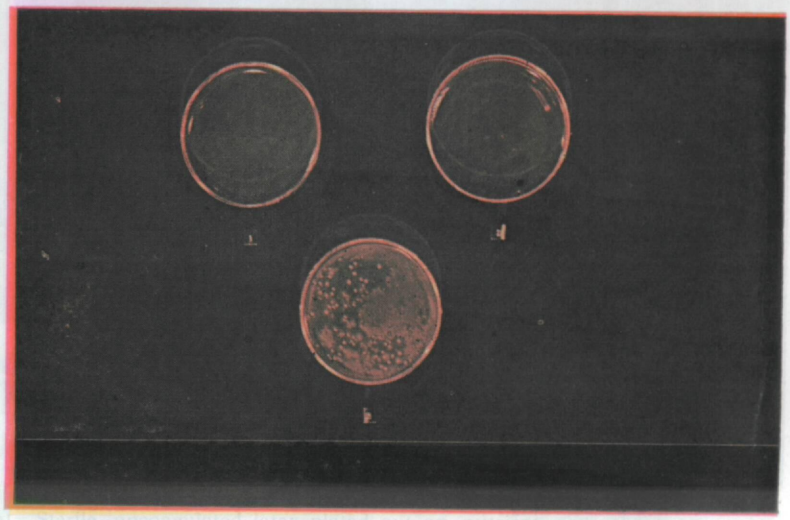

Fig. 1 (*b*). Sterile, uncoagulated latex plated out on nutrient agar shows no contamination (top row) and serum from bacteria-coagulated latex shows the presence of bacteria (bottom row).

All those bacteria which coagulated the latex within 24 hours and which in addition did not discolour the coagulum or produce an offensive odour were selected for further studies with field latex.

These bacteria were then cultured in 100 ml of sterile coconut water in 150 ml Erlenmeyer flasks. A pure coconut water medium supported good growth of all the bacteria except one. Some of these cultures grown in coconut water, which were used in the present study are shown in Fig. 2.

Coagulation of field latex: Undiluted field latex from clones PB 86 and RRIC 52 was coagulated by stirring in two to four-day old cultures in the ratio of one of culture to ten of latex by volume, in 3-litre plastic boxes, or 1 gal or 10 gal aluminium pans or tanks. The results are given in Table 2.

TABLE 2

EFFICIENCY OF ISOLATES ON COAGULATION OF FIELD LATEX

CLONE PB 86

Treatment or isolate	Final pH	Gelling rating			Coagulation		Coagulum	
		4 hours	7 hours	10 hours	* %	Time taken	Colour	Odour
CA	5·4	1	4	8	99·5	12	Yellowish	Not offensive
C6	5·4	3	8	10	99·4	10	Off white	Not offensive
RS6	5·2	3	8	10	99·9	10	Off white	Not offensive
M	5·4	1	6	10	97·3	10	Off white	Not offensive
BC2	5·4	4	8	10	96·5	10	Yellowish	Not offensive
L52	5·8	0	3	5	90·2	28	Yellowish	Unpleasant
Acid	4·5	10	—	—	100		White	
Assisted coagulation	5·2	1	3	5	98·2	24	Off white	Not offensive
Autocoagulation	5·8	0	2	4	88	48	Yellowish brown	V. unpleasant

Gelling rating 0 — Fluid (no change)
1 — Floculation
3 — Gel line
5 — Gel (well set)
10 — Firm coagulum

* Coagulation calculated as percentage of acid coagulation

Compared with acid coagulation taken as 100%, autocoagulation was incomplete, resulting in 88% coagulation after 48 hours. Near complete coagulation ranging from 96% to 99·9% was obtained in 10 hours with different strains of bacteria used. Assisted coagulation, by the addition of sterile coconut water to field latex gave 98% coagulation in 24 hours. The coagulum was processed into blanket crepe without difficulty. The colour of the crepe varied from pale yellow to light brown (Fig. 3).

The physical appearance and blown up nature of the coagulum due to gas production by the bacteria is shown in Fig. 4. The bacteria-treated rubber is more spongy than the autocoagulated rubber, as is evident from the increase in volume of the bacteria-treated rubber which is more than that of the autocoagulated rubber (Fig. 4). In the new process rubber manufacture, a porous coagulum could be processed and dried easily.

Technological properties: Some technological properties of the rubber obtained using a few isolates from the original selection are given in Table 3. The coagulum was processed about 22 hours after addition of cultures, that is about 12 — 14 hours after complete coagulation by bacteria. Autocoagulation was incomplete at this stage.

TABLE 3

TECHNOLOGICAL PROPERTIES OF BACTERIALLY-COAGULATED RUBBER

	Acid	Auto	Bacteria (RS6)
Nitrogen %	0·448	0·33	0·42
Acetone extract %	3·1	1·79	3·55
PRI	86·5	78·8	83·8
*Strain (technical class)	88·0	72·0	71·0
*Wallace (BS)	38·0	37·0	37·5
*Resilience (Lupke pendulum)	77·5	76·5	75·5
*Raw Mooney V/C at 212°F	64·0	63·5	62·5

*ACS 1 mix cured for 40 min at 140°C.

The technological properties as enumerated in Table 3, indicate that the bacterially-coagulated rubbers were in no way inferior to that obtained by formic acid coagulation of latex at initial concentration. The rubbers also possessed fast curing characteristics in the ACS 1 mix, indicating that the cure accelerating substances naturally present in latex had been mostly conserved in the coagulum.

In some further experiments two to four-day old cultures were centrifuged to separate the bacteria from the liquid medium and the bacteria added as a suspension in distilled water. The results obtained were similar to those with liquid cultures. It was also observed that when the amount of bacteria added was increased either as liquid culture or bacterial suspension in distilled water, the time of coagulation was further reduced. The minimum time required was 4 — 5 hours.

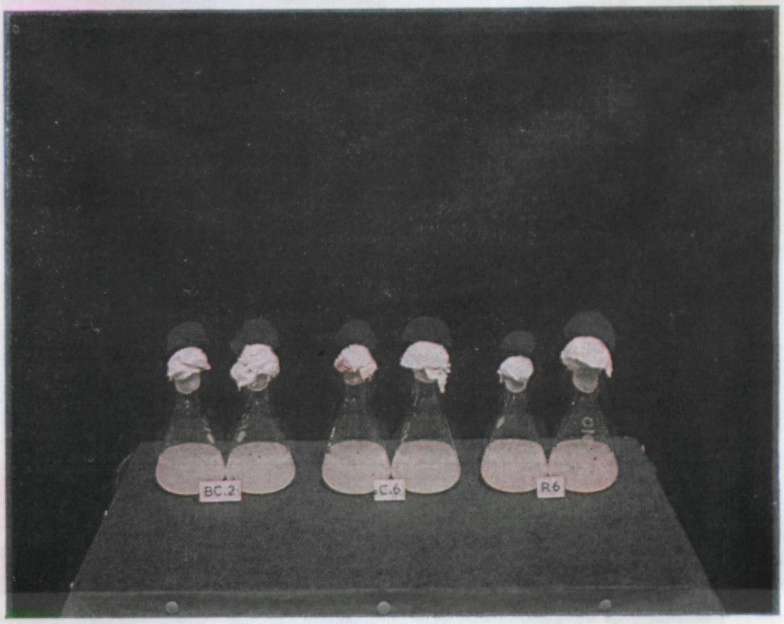

Fig. 2. Bacterial cultures in coconut water medium.

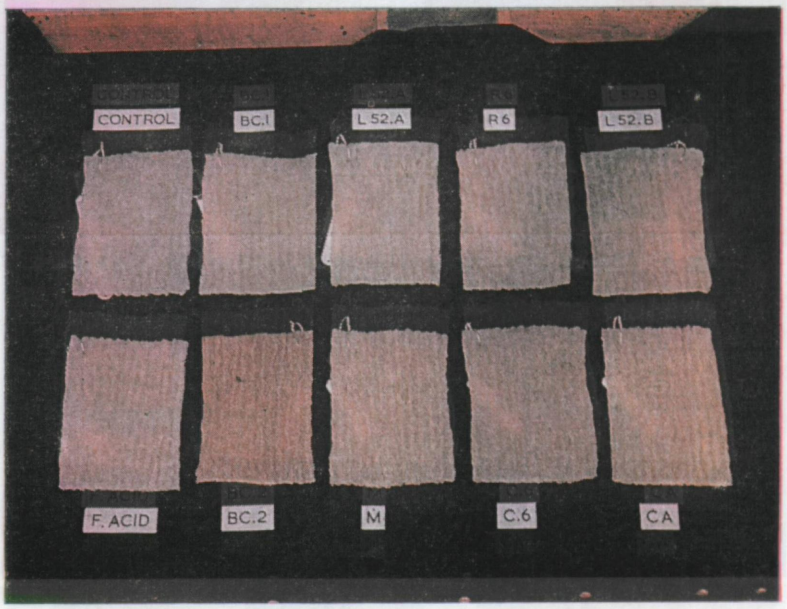

Fig. 3. Blanket crepe obtained by bacterial coagulation using different strains of bacteria (BC 1 ; L 52 A ; R 6 ; L 52 B ; M ; C 6 and CA) compared with acid coagulation (formic acid) and autocoagulation (control). Autocoagulation was incomplete at the time of creping. hence the light colour.

Fig. 4. Physical appearance and blown-up nature of bacteria-coagulated rubber.

This clearly established that coagulation could be controlled by seeding latex with selected bacterial strains to give a coagulum with good physical and technological properties.

Kluyver & Houwink (1954) have shown that a reduction in vulcanization rate of rubber can be obtained by cultivating in latex bacteria which have been selected on the basis of their ability to break down vulcanization accelerators present among non-rubber components of latex. They attempted to produce a uniform slow curing rubber. In our studies we have tried to achieve similar results by the use of selected bacteria to produce a uniform fast curing rubber.

However, if this process is to be economically feasible, then the most important pre-requisite is the mass culture of bacteria in a medium which will not only support good growth and multiplication of the bacteria but also one which is inexpensive and readily available. Coconut water seems to fulfil these requirements ideally. It is a good culture medium for micro-organisms. It contains sugars, vitamins, amino acids, growth substances and minerals (Child & Nathanael, 1947; Tulecke *et al.*, 1961). It supported good growth and multiplication of many of the isolates used in the present study. The possibility of using coconut water as a mass culture medium for growing yeast — *Rhodoturula pilimanae* as a cheap protein supplement in food, has been well established by the work of Hipolito *et al.* (1965) and Aliwalas *et al.* (1968) in the Philippines.

Coconut water ferments on standing to produce an acid liquor corresponding to about ½% of acetic acid (0·5 g in 100 ml) in four to five days. Such fermented coconut water has been used for coagulating rubber in times of shortage of formic and acetic acids. The transport of such a dilute acid solution is not economical, but during the last war it has been used in Ceylon for coagulating rubber in areas where rubber and coconut are grown on adjoining lands (Anon, 1940; Child & Nathanael, 1947). Approximately one bottle (1/6 gal) of fermented coconut water is required to make 1½ lb of sheet rubber, that is 1 gal to produce 9 lb of sheet rubber (O'Brien, 1939). Compared with this, if the coconut water is first used to culture selected, efficient bacteria and the bacteria used for the coagulation, then 30 lb or more of rubber could be made with 1 gal of coconut water; with the further possibility of using the serum of such coagulated rubber for subsequent coagulation, because the serum itself serves as a good growth medium for the bacteria. (This aspect is discussed in our next paper).

In Ceylon, coconut water is readily available and is a waste product in copra curing and in the desiccated coconut industry.

CONCLUSION

The sheet rubber produced by smallholdings (less than 10 acres) and medium holdings (10 — 100 acres) amounts to one hundred and thirty five million pounds annually. It is hoped that this amount will be converted to block rubbers by 1980. If the conventional acid coagulation method is adopted, the cost of acids and anti-coagulants will amount to Rs. 1,350,000 or more in foreign exchange every year. Bacterial coagulation involves no foreign exchange expenditure. The significant savings in foreign exchange that could possibly be achieved by this process should alone make it worthy of consideration and further study.

ACKNOWLEDGEMENTS

The authors would like to thank the Director, Dr. O. S. Peries, for encouragement ; Mr. M. Nadarajah, Head, Rubber Chemistry Department ; Mr. S. W. Karunaratne, Rubber Chemist, for helpful discussions ; Mr. I. Amarasinghe for technical assistance and Messrs. A. S. Dekumpitiya, D. D. Medagama, T. M. Ahamadeen and M. D. C. Seneviratne for testing the properties of the rubber.

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