*Metabolism of Quebrachitol and other Carbohydrates by Hevea Latex Bacteria**

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A study was made of the breakdown of quebrachitol (methyl-l-inositol) and common carbohydrates glucose, galactose and fructose by bacteria isolated in Malaya from Hevea *latex. Twelve cultures belonging to ten genera were tested for their ability to ferment quebrachitol; among them, only two organisms* (Aerobacter aerogenes *and* Achromobacter delicatulus) *metabolised quebrachitol, the former producing acid and gas and the latter producing only acid. Both organisms followed the oxidative metabolic pathway in acid production. Strain variation in the ability to metabolise quebrachitol was also observed; of the two strains of* A. aerogenes *included in the study, only one fermented quebrachitol. The importance of quebrachitol as a substrate for the production of acids destabilising* Hevea *latex is discussed.*

In addition to the two organisms used in quebrachitol investigations the breakdown of common carbohydrates was carried out using Micrococcus luteus *and a different strain of* A. aerogenes *both isolated from latex. All four organisms utilised glucose, galactose and fructose as sources of carbon and all except* M.luteus *metabolised the carbohydrates fermentatively;* M. luteus *metabolised glucose and fructose fermentatively but galactose oxidatively. Experimental evidence presented shows that these carbohydrates are important substrates for the development of volatile fatty acids (VFA) and non-volatile acids (NVA) in latex, leading to its spoilage. The relative ability of the four organisms and the three carbohydrates to bring about the spoilage of latex is discussed.*

Besides containing dispersed rubber hydrocarbon particles, *Hevea* latex has also diverse non-rubber substances. Amino acids, proteins, quebrachitol and carbohydrates are important substances maintaining the quality of *Hevea* latex. In an earlier paper it has been shown that a number of amino acids present in latex are decomposed in the presence of latex bacteria resulting in the production of a substantial quantity of non-volatile acid (JOHN, 1966). Latex contains up to 2% quebrachitol and smaller concentrations of sucrose, glucose, galactose, fructose and two pentoses which, together, form the major constituents among soluble materials of latex (SMITH, 1954; LOWE, 1961). The role of quebrachitol as an important substrate for acid formation in latex has been controversial (PHILPOTT AND SEKAR, 1953; LOWE, 1961). However, the part played by conventional carbohydrates is better understood. This study on the bacterial breakdown of quebrachitol, glucose, galactose and fructose was therefore conducted to assess their importance as substrates for acid production in latex leading to spoilage.

MATERIALS AND METHODS

Organisms

The organisms used were *Brevibacterium insectiphilium*, *Flavobacterium aquatilis*, *Bacillus megaterium*, *Kurthia Zopfii*, two strains of *Streptococcus* spp:, *Pseudomonas* sp:, *Serratia* sp:, *Aerobacter aerogenes* (J_1), *Achromobacter delicatulus* (J_2), *Micrococcus luteus* (J_3), and another strain of *Aerobacter aerogenes* (J_4). These twelve organisms, isolated and maintained on modified Kligler's iron agar medium

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(TAYSUM, 1956), formed a fair cross section of the bacterial population of the Malayan *Hevea* latex samples used in the study. Investigations on the metabolic breakdown of quebrachitol and carbohydrates, though extensive, were confined to the last four organisms; others were tested solely for their ability to ferment quebrachitol in a typical biochemical reaction.

Carbohydrates

In the present investigation, quebrachitol was considered as a major carbon source derived from methyl-l-inositol; this is in contrast to the common practice of regarding inositols as vitamins required in traces. Quebrachitol used in this study was prepared from *Hevea* latex serum by using a modification of the U.S. Rubber Company's method, and purified by repeated crystallisation (JOHN, 1964). The remaining carbohydrates were obtained from the Kerfoot range of bacteriological grade sugars. Glucose was prepared as a 20% (w/v) solution, sterilised at 10 lb/in^2 for 10 min and later aseptically diluted to give a final solution of 1% concentration for final acid estimation. Quebrachitol, galactose and fructose were prepared directly as a 1% (w/v) solution in distilled water and sterilised at 10 lb/in^2 for 10 min.

Preparation of Washed Cells

Bacterial cells grown in nutrient broth were harvested, washed and standardised (JOHN, 1966). All cultures except organism J_2 were grown in the presence of 1% (w/v) glucose in the nutrient broth; the enzymic activities of organism J_2 were found to be inhibited when grown in the presence of glucose.

Fermentation Reaction

The solution used for the test contained 1% (w/v) peptone, 1% (w/v) carbohydrate and 0.1% (w/v) bromocresol purple; the solution was sterilised at 10 lb/in^2 for 10 min. A 24-hour culture was used to inoculate the carbohydrate solution which was then incubated at 30°C and read at various time intervals.

Oxidation or Fermentation Test

Organisms were investigated for their ability to produce acid aerobically and anaerobically from the carbohydrates using the method of HUGH AND LEIFSON (1953). Carbohydrates were sterilised as 20% solutions and added aseptically to the sterile melted basal agar to give a final concentration of 1% (w/v). Tubes incubated at 30°C were examined daily for up to 14 days.

Utilisation of Sugars

Auxanographic tests (LODDER AND KREGER-VAN RIJ, 1952) were carried out using all the four organisms. The basal medium designed for this purpose had the composition: NaCl, 0.15g; $MgSO_4$. $7H_20$, 0.05g; KH_2PO_4, 0.75g; Na_2HPO_4, 1.5g; Agar No. 3 (Oxoid), 15.0g; yeast extract, 400 mg; distilled water, 1000 ml. The medium was adjusted to pH 7.4 and sterilised at 10 lb/in^2 for 15 min. The plates were incubated at 30°C and the results recorded after 48 hours.

The growth of these organisms was further examined in liquid medium, the composition of which was similar to that described above save that it contained no agar. The required amount of sterile carbohydrate was added aseptically to the sterile base to give a final concentration of 1%. The sugars were inoculated with a 24-hour culture of each of the organisms and incubated at 30°C. The growth at various intervals was estimated by light scattered in a nephelometer.

Estimation of Acids

Volatile, non-volatile and total acids were estimated (JOHN, 1966); volatile fatty acids were identified by paper chromatograms (REID AND LEDERER, 1951).

RESULTS

Quebrachitol

Of the two *A. aerogenes* strains included, only J_4 fermented quebrachitol, producing acid and gas. Among the other organisms tested, only J_2 fermented quebrachitol producing only acid. Thus organisms J_2 and J_4 were selected for further investigations.

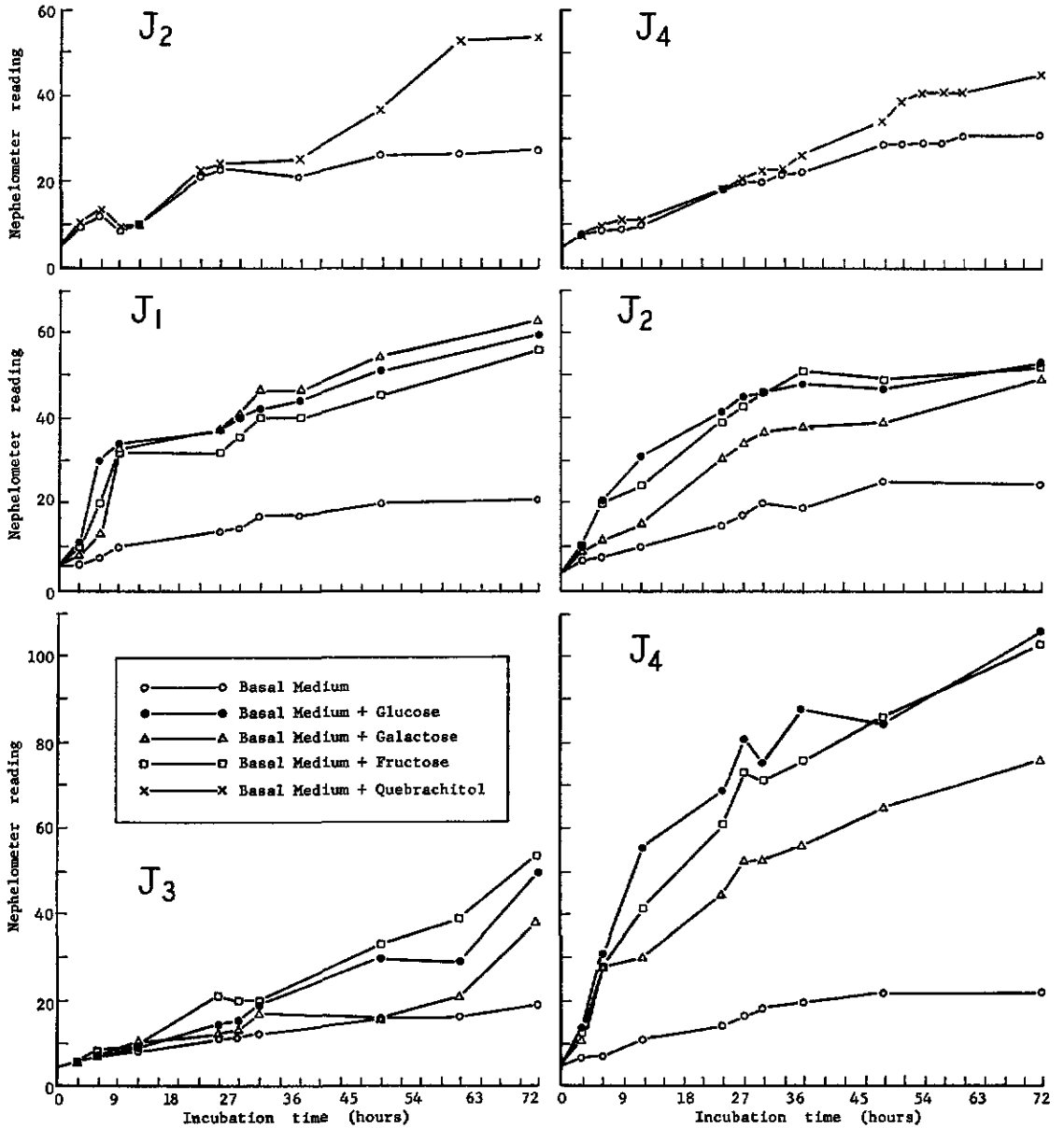

Figure 1. Growth of organisms J_2 and J_4 in quebrachitol and J_1, J_2, J_3 and J_4 in other carbohydrates.

Auxanographic tests using these organisms showed zones of heavy growth around the regions where quebrachitol had been added; it was found that the quebrachitol was metabolised oxidatively. Examination of growth in liquid medium, with quebrachitol as the main carbon source, showed that quebrachitol was utilised by both organisms (*Figure 1*). This may have important bearing on its function in metabolism of the latex system of *Hevea brasiliensis* (ATLANTIC RESEARCH CORPORATION, 1956).

Some nitrogenous materials were inevitably transferred with the cells as the inocula were from broth cultures. This might account for the growth during the first 36 hours in the basal medium which was otherwise very low in nitrogen. Such phenomenon is clearly exhibited by organism J_2 where there is a second phase of active growth beginning at the 36th hour. A similar picture is obtained with organism J_4 but with the effect of quebrachitol less marked.

The acid production from quebrachitol was also estimated. It was found that organisms J_2 and J_4 produced only small quantities of acid and that the volatile acids formed were not enough to be estimated by titration (*Table 1*). However, paper chromatographic separation of the steam distillate of the reaction mixture showed trace quantities of acetic and/or formic acid, the quantity of which increased with prolonged incubation. However, neither could be identified positively. Gas chromatography was of no help either.

TABLE 1. VOLATILE FATTY ACIDS (VFA), NON-VOLATILE ACIDS (NVA) AND TOTAL ACID PRODUCTION FROM CARBOHYDRATES[a]

Substrate	Organism	6 hours*			24 hours*			% of VFA to total acid (24 h)
		VFA	NVA	Total	VFA	NVA	Total	
Quebrachitol	J_2	Nil	—	Nil	Nil	—	11.8	
	J_4	Nil	—	Nil	Nil	—	6.3	
					Nil		18.1	—
Glucose	J_1	19.6	—	172.5	25.5	149.0	178.6	
	J_2	43.7	—	146.0	48.9	137.5	179.8	
	J_3	Nil	—	Nil	30.7	14.1	46.1	
	J_4	12.4	—	72.2	96.7	25.2	126.1	
					201.8	325.8	530.6	38.0
Galactose	J_1	26.7	37.7	67.2	47.1	50.2	92.6	
	J_2	Nil	26.6	29.3	Nil	26.6	29.9	
	J_3	Nil	Nil	Nil	30.3	9.5	37.8	
	J_4	Nil	Nil	Nil	16.3	5.7	23.9	
					93.7	92.0	184.2	50.9
Fructose	J_1	5.0	3.3	9.0	61.8	6.6	67.1	
	J_2	3.8	3.2	6.6	38.0	14.2	53.8	
	J_3	Nil	3.8	3.8	82.3	19.0	104.5	
	J_4	3.3	3.3	6.6	43.2	5.0	48.6	
					225.3	44.8	274.0	82.2

[a] Expressed as ml of 0.02 N barium hydroxide per 100 ml of bacterial suspension.

* Incubation time

Common Carbohydrates

Fermentation and utilisation. Organisms J_1, J_2 and J_4 readily fermented glucose, galactose and fructose, J_1 and J_4 producing acid and gas and J_2 producing only acid. Organism J_3 fermented glucose and fructose in about 48 hours but galactose was not fermented until about 10 days. All organisms except J_3 metabolised glucose, galactose and fructose fermentatively; J_3 metabolised glucose and fructose fermentatively but galactose oxidatively. Using the auxanographic technique, it was further observed that organisms J_1, J_2 and J_4 assimilated these carbohydrates, but at different rates. J_3 gave distinct zones of growth with glucose and fructose indicating their utilisation, but the zone of growth around galactose was poor. The above observations confirmed the delayed fermentation reaction recorded on galactose. When the growth of these organisms was further examined in liquid medium with the individual carbohydrate as the main carbon source it was observed that glucose was more readily utilised than fructose and galactose by J_1, J_2 and J_4 (*Figure 1*). No evidence of utilisation of any of the carbohydrates by J_3 was observed until after 48 hours. Even then, such utilisation was confined only to glucose and fructose, galactose utilisation being apparent only after 72 hours.

Acids. Estimations of volatile fatty acid, non-volatile acid and total acid was made (*Table 1*).

(i) *Glucose.* It was observed that all the four organisms produced volatile and non-volatile acids from glucose, the quantity dependent on the organism. Except for J_3, all the organisms produced large quantities of acid during the first 6 hours and the quantity increased with longer incubation periods. Organism J_3 required more than 6 hours to produce reasonable quantity of acid. The proportion of VFA to the total acid after 24 hours was highest with organism J_4 (77%), followed by J_3 (67%), J_2 (27%) and J_1 (14%).

(ii) *Galactose.* Organism J_1 was more active than others in producing a substantial quantity of acid with VFA accounting for approximately 40 and 50% during the first 6 and 24 hours respectively. With organism J_2 no titratable quantity of VFA was observed at any stage of the experiment and there was no appreciable increase in the total acid with prolonged incubation. The acid production by organisms J_3 and J_4 was rather slow but a substantial quantity of acid was produced on prolonged incubation with VFA accounting for 80 and 68% respectively.

(iii) *Fructose.* A large quantity of acid was produced during the first 24 hours by all the organisms despite near inactivity during the first 6 hours. J_3 was particularly active in metabolising fructose compared to its rather sluggish performance in glucose and galactose.

Examination of the overall production of acid from glucose, galactose, and fructose shows that in terms of the total acid, organism J_1 produced more acid followed by J_2, J_4 and J_3 in that order, but in terms of VFA, this order was not maintained. The four organisms produced acid from glucose, fructose and galactose in the approximate proportion of 6 : 3 : 2.

Paper chromatography of VFA produced from carbohydrates indicated that they consisted essentially of acetic and/or formic acid. Gas chromatography did not give different indications.

DISCUSSION

A. aerogenes (J_4) and *A. delicatulus* (J_2) produced volatile and non-volatile acids from quebrachitol, though the quantity of volatile acid produced was too little to be estimated by titration. Small amounts of acid are produced when quebrachitol is metabolised oxidatively. It has been found that only some organisms are capable of metabolising quebrachitol. However, as latex contains a substantial quantity of quebrachitol—roughly one order greater than the concentration of any other carbohydrate—and as the diversity of bacterial infection in latex is also great, quebrachitol may be considered a possible substrate for acid production. The rate of production of VFA in latex is much higher when quebrachitol is added.

TAYSUM (1954) found that only coliforms fermented quebrachitol; the present work has however shown that an organism (*A. delicatulus*) outside the 'coliform' group also ferments quebrachitol; of two strains of *A. aerogenes* tested, only one fermented quebrachitol.

Hevea latex is known to contain carbohydrates other than quebrachitol, but only glucose, galactose and fructose were considered in this study on the role of the common carbohydrates as substrates for acid formation in latex.

Substantial quantities of acids were formed from glucose, galactose and fructose. Adding the acid production from the four organisms used in this investigation gives the relative amounts from glucose, fructose and galactose as 6 : 3 : 2 approximately. About half is VFA; it is approximately 38% of the total acid from glucose, 51% from galactose and 82% from fructose. Thus in terms of the quantity of VFA produced, fructose assumes more importance than glucose and galactose. However, the relative importance of each of these carbohydrates in relation to the spoilage of latex cannot be carried further unless more knowledge is obtained of the quantity of each of these caprohydrates present in latex *per se* free of all bacterial decomposition.

It is a matter of doubt whether the importance of a substrate in this context should be assessed on the basis of the quantity of total acid or VFA produced from it. It has been claimed (PHILPOTT AND SEKAR, 1953) that the VFA estimation is a more sensitive test than the total acid estimation as a measure of latex degradation. However, more recent experience has not entirely supported their contention. Non-volatile acids (lactic acid for example) are quite as damaging to latex stability as their volatile counterparts. Secondly, processes such as proteolysis remove stabilising protein layers from the surface of the hydrocarbon but are not monitored by the VFA test —proteolytic organisms often produce remarkably little volatile acid. In latex concentrate preserved with ammonium borate in the presence of free ammonia, bacterial degradation is often encountered and manifests itself as a strong increase in KOH number (fixed acid value) but not of steam-volatile acids. On the other hand, the defect in the fixed acid test is that it fails to discriminate between long chain stabilising acids (C_{10} and above) capable of exerting a detergent action in the presence of ammonia and the destablising acids of lower molecular weight. Its strength lies in that the latter are far more abundant quantitatively. JORDAN (1938) held that the KOH number could be influenced by deliberate addition of fixed alkalis or soaps; but, this is not sustained since this effect, which extends to borate ion, can be measured and allowed for in monitoring subsequent decomposition. It is hence felt that at least three groups of acids —the volatile fatty acids, the lower destabilising but non-volatile acids and the long chain fatty acids—must be assayed to estimate the extent of microbial breakdown of non-rubber constituents of latex leading to spoilage.

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