

AN ORGANISM FOUND IN THE LATEX OF HEVEA BRASILIENSIS

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When an incision is made in the bark of the rubber tree (*Hevea brasiliensis*, Muell. Arg.), a somewhat viscous, creamy-white liquid, called latex, exudes. In the course of a few hours the liquid thickens and, later, a clot of rubber is formed which floats on the surface of a clear or milky serum. It is found that while the serum is acid, the surface layer is alkaline in reaction.

Much discussion has taken place concerning the biochemical changes occurring during the natural coagulation of Hevea latex. Whitby (1912) considered them to be due to enzyme activity while Eaton and Grantham (1915) advanced an explanation attributing them to the presence of bacteria. Both these views have received support from later workers and, more recently, de Vries and Beumée-Nieuwland (1924) have concluded that both bacteria and enzymes are responsible for the changes. It was recognised by Eaton and Grantham that precipitation of the rubber particles occurs as a result of the acid produced by bacterial activity but de Vries and Beumée-Nieuwland have shown that this precipitation is of the nature of flocculation and they consider that the subsequent coalescence of the particles (coagulation) is effected by a specific enzyme. Their views regarding the presence of an enzyme, however, have not met with general acceptance and Groenewege (1924), Belgrave (1925) and van Harpen (1929) offer a different explanation.

A bacteriological study of Hevea latex was carried out by Dernier and Vernet (1917) in Annam. These authors isolated 27 different species of bacteria of which one, "Bacillus No. 1,"

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BRIEF CHARACTERISATION	REMARKS
<p>Microscopic features; Form: 5 (rods) Endospores: 2 (excentric to terminal)</p>	<p>Some trouble was experienced in detecting endospores. The organism was not killed by maintaining cultures at 85°C. for 10 minutes. The spores were eventually found by Anjeszky's method using boiling carbol-fuchsin.</p>
<p>Flagella: 1 (peritrichic) Gram stain: 1 (positive)</p>	<p>The organism was found to be motile in agar culture. Flagella were observed by means of Loeffler's flagella stain. Organisms from cultures 3 to 5 days old were found to be Gram-positive by staining with gentian violet and counterstaining with carbol-fuchsin.</p>
<p>Vegetative cells: Diameter: 2 (between 0.5 and 1μ) Length: 2 (more than 2 diameters)</p>	<p>Measurements were made from agar cultures stained with carbol-fuchsin. The rods, which had slightly rounded ends, had an average length of 3.1μ and an average diameter of 0.9μ. The largest cell measured had a length of 4.6μ and a diameter of 1.2μ.</p>
<p>Chains: 1 (present)</p>	<p>Chains were not common but occasionally chains containing up to 9 cells were seen.</p>
<p>Capsules: 1 (present)</p>	<p>Capsules were detected by Hiss' method.</p>
<p>Spores: Shape: 2 (ellipsoid to cylindrical) Diameter: 2 (greater than diameter of rod)</p>	<p>The ellipsoid spores had an average length of 2.3μ and an average diameter of 1.4μ.</p>
<p>Miscellaneous biochemical reactions: Biologic relationship: U (undetermined) Relation to oxygen: 2 (facultative anaerobe) Gelatin liquefaction: 1 (positive) In nitrate media: 2 (nitrite but no gas)</p>	<p>Growth under anaerobic conditions similar to under aerobic conditions. Tests for nitrite were made by means of sulphanilic acid and α-naphthylamine in acetic acid. The nitrate used gave no reaction with this test.</p>

<p>Chromogenesis: 0</p> <p>Carbohydrate reactions:</p> <p>Diastatic action: 0</p> <p>From d-glucose: 2 (acid without gas)</p> <p>From lactose: 1 (acid and gas)</p> <p>From sucrose: 1 (acid and gas)</p> <p>Cultural features:</p> <p>Agar stroke</p> <p>Growth: 2 (moderate)</p> <p>Lustre: 2 (dull)</p> <p>Surface: 1 (smooth)</p> <p>Agar colonies: 1 (punctiform)</p> <p>Gelatin colonies: 1 (punctiform)</p> <p>Relation of growth to reaction of medium:</p> <p>Milk:</p> <p>Acid: 1 (sufficient for curdling)</p> <p>Rennet curd: 1 (present)</p> <p>Peptonisation: 0 (absent)</p> <p>Indol production: 1 (positive)</p> <p>Additional reactions:</p> <p>Ammonia production: (positive)</p> <p>Hydrogen sulphide production: (negative)</p> <p>Albumen digestion: (positive)</p>	<p>A very pale straw colour was obtained with growth on agar.</p> <p>Litmus was completely decolourised in 2 to 3 days.</p> <p>The agar stroke was opaque, without chromogenesis or odour and no visible changes occurred in the medium.</p> <p>Agar colonies smooth and white, edge irregular. Growth slow.</p> <p>Gelatin colonies white, edge smoother than in the case of agar colonies. Growth slow.</p> <p>The organism grew in a dilute aqueous solution of egg albumen containing small amounts of mineral salts between pH = 4.0 and pH = 10.0. Good growth, however, only occurred between pH = 4.75 and pH = 9.0. It was difficult to delimit the pH range accurately as growth became very irregular as the end-points were approached.</p> <p>Complete coagulation of milk occurred 2 days after inoculation.</p> <p>When heated with magnesium oxide, the vapour from a 13 day old culture containing peptone and potassium nitrate turned litmus blue.</p> <p>Only a very slight darkening of lead acetate agar occurred after 12 days.</p> <p>Egg albumen was partially digested by cultures of the organism, the solution became alkaline and ammonia was evolved.</p>
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CHARACTERISATION OF ORGANISM—*Concluded*

BRIEF CHARACTERISATION	REMARKS
<p>Reactions with Hevea latex products: Latex: (floculated)</p> <p>Latex serum: (acid without gas) Latex "carbohydrate": (acid without gas)</p>	<p>Solutions were prepared by adding 1 part of latex to 9 parts of boiling water and heating the mixture at 15 pounds pressure for 15 minutes. After inoculation with the organism, the latex floculated, the surface layer being alkaline while the serum was acid. In most cases the further stage of coalescence was reached after 2 or 3 weeks. Similar results were obtained [with more concentrated solutions of latex but coagulation occurred more readily.</p> <p>The latex "carbohydrate," presumably methyl-l-inositol (see Pickles and Whitfield, 1911), was prepared by evaporating latex serum to small bulk, taking up with alcohol, and evaporating the alcoholic solution to dryness. After inoculation of aqueous solutions of this substance with the organism, acid was produced without gas evolution.</p>

All work was carried out at the laboratory temperature which ranged between 26° and 30°C.

was present in all samples examined. "Bacillus No. 1" was described as an aerobic, motile, Gram-negative organism, producing acid with "dambose" (the latex "carbohydrate"), lactose, and sucrose, effecting coagulation of milk and liquefaction of gelatin but unable to digest albumen.

The writer has isolated a bacillus from Hevea latex and a detailed study of its characteristics has been made. It was found in large numbers in all samples of latex examined from Selangor, Perak and Johore so that it appears to be distributed throughout the Malay Peninsula. It was invariably the predominant feature in samples which were coagulating and there can be no doubt that it is responsible for the important change occurring during the natural coagulation of Hevea latex.

The characteristics and reactions of the organism are given in detail in the manner required by the Committee on Bacteriologic Technic of the Society of American Bacteriologists (1924).

From the reactions given it will be seen that the organism in question can effect the precipitation of rubber from Hevea latex. The actual mechanism of the change is discussed in another paper (Corbet, 1929).

The organism differs from the "Bacillus No. 1" of Dernier and Vernet only in being Gram-positive and in its ability to digest albumen, so that the two may be identical.

The organism is a member of the genus *Bacillus* of the family Bacillaceae (see Bergey, 1926). It resembles closely several species of *Bacillus* which occur in the soil but does not appear to be identical with any one of them. It is most nearly allied to *Bacillus tumescens* (Zopf) and *B. silvaticus* (Neide) but differs from them in several important respects. The rapid decolorisation of litmus, which is so noticeable with the latex organism, would surely have been recorded had it been observed and no mention of this point is made in connection with *B. tumescens* or *B. silvaticus*.

As the organism does not appear to have been described previously, I propose to denominate it *Bacillus pandora*.

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