STUDY OF THE SPECIES OF CLOSTRIDIA INVOLVED IN RETTING OF JUTE

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Several anaerobic bacteria were isolated from retted jute fibres (*Corchorus capsularis L.*). Of the several isolates, only *Clostridium Sp1* and *Clostridium Sp2* retted jute stem in the laboratory in relatively much shorter time releasing better fibres.

Introduction

Jute fibre is extracted by steeping jute plants in water to allow certain microorganisms in water to decompose the various gummy and pectic substances cementing the fibres, and thereby release the fibres. This process of extracting jute fibres is known as 'biological retting'. Under natural conditions, biological retting takes about 15-18 days for complete retting.

Various workers have reported different microorganisms involved in the retting of jute. Nandi and Basu[1] claim that microorganisms attack the cambium and secondary phloem and secrete some enzymes which cause the decomposition of parenchymatous tissues. Fibre quality depends on the duration of retting and also on the environmental conditions.[2-3] Hauman[4] and Beherens[5] have reported a few strains of fungi which influence the process of retting of jute, flax and hemp under natural conditions. Kayser and Delavel,[6] Katagiri and Makahama,[7] and Debsarma[8] have isolated a few anaerobic bacteria which can be used for retting these materials. Active aerobic jute retting bacterium (*Bacillus polymyxa*) has also been reported by Ali.[9]

The present work was undertaken to isolate a few anaerobic bacteria which can ret jute in relatively shorter time, producing quality fibres.

Materials and Methods

The isolations were made from the naturally retted jute fibres collected from various districts of East Pakistan. The cultures were always kept at 37°C. and isolations were made in anaerobic culture jars. After incubation at 37°C. for at least 10 days, the cultures were heat-shocked at 80°C. for 20 minutes to destroy all the nonspore forming vegetative cells so that only the spores of the spore forming survive (spores can withstand heat at 80°C.). They were plated in agar medium (Peptone 5 g., yeast extract 5 g., glucose-D 5 g., L-Cystine 0.2 g. and agar 20 gm./1000 ml. of distilled water), and were placed in anaerobic jar and incubated at 37°C. for 8 days. The different types of colonies obtained were isolated in Peptone-yeast extract broth with agar (0.75 g./litre) and calcium carbonate (only a pinch) which gave better growth and sporulation. After sporulation all the isolates were heat-shocked at 80°C. for 20 minutes. Again they were isolated in broth and liquid paraffin was poured in the broth to attain anaerobiosis.

Sterile jute stem tubes, prepared with slight thioglycolate acid in distilled water, were inoculated with the bacterial cultures under investigation. After inoculation, molten paraffin wax was poured in the tubes to cut off air. The tubes were put inside the anaerobic jars and kept at 37°C. pH of the media was adjusted at 7.2. The media were sterilised for 20 minutes at 15 psi.

Results

The spore forming rod bacteria under anaerobic conditions were species of *Clostridia* (*Clostridium Sp1* and *Clostridium Sp2*) (Figs. 1-2). These bacteria were the most active types and completely retted the jute stem under laboratory conditions in 5 days. These species were closely related to *Clostridium novyii* and *Clostridium hemolyticum*, respectively according to Bergey's manual but with slight variations in their physiological activities. Cultural and Physiological characters of the bacteria are given in Tables 1 and 2.

Discussion

The study of the microorganisms of jute retting tanks in East Pakistan comprised three genera: *Bacillus*, *Micrococcus* and *Clostridium*. In the present investigation four types of isolates were observed. Some of them were complete retters, some were partial retters, some were only bubblers and others were inactive. Only the active and complete retters were chosen for further study. Two species of Clostridia (*Clostridium Sp1* and *Clostridium Sp2*) were isolated and found to be the most active retters of jute. The activity of the isolates were optimum at pH 7.2 and decreased with the decrease of pH value. Whether the retting phenomenon is purely a chemical process or symbiotic

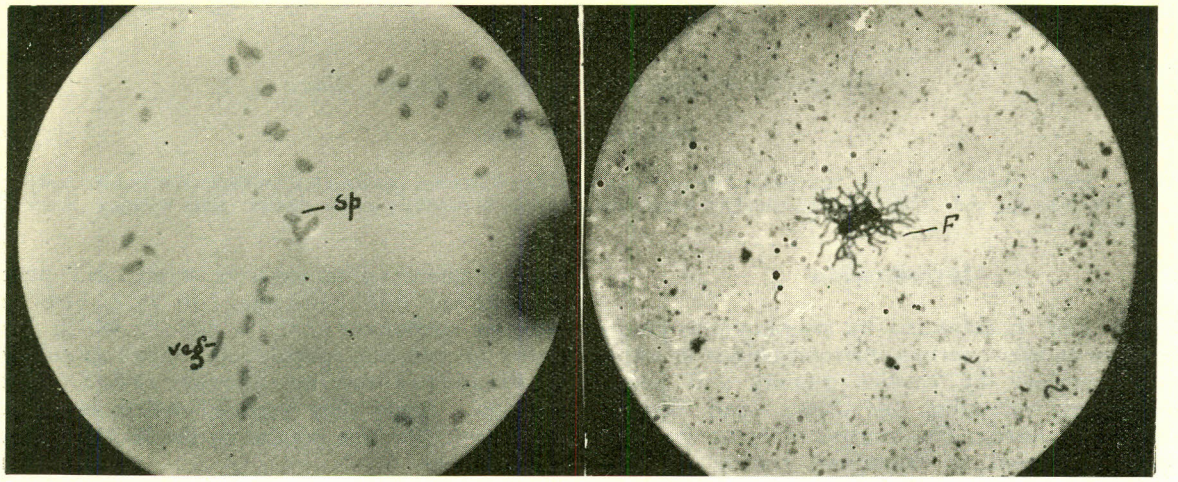

Fig. 1.—*Clostridium Sp 1.* (Veg, Vegetative cell; sp, Sporangium; F, Flagella) x 1,440.

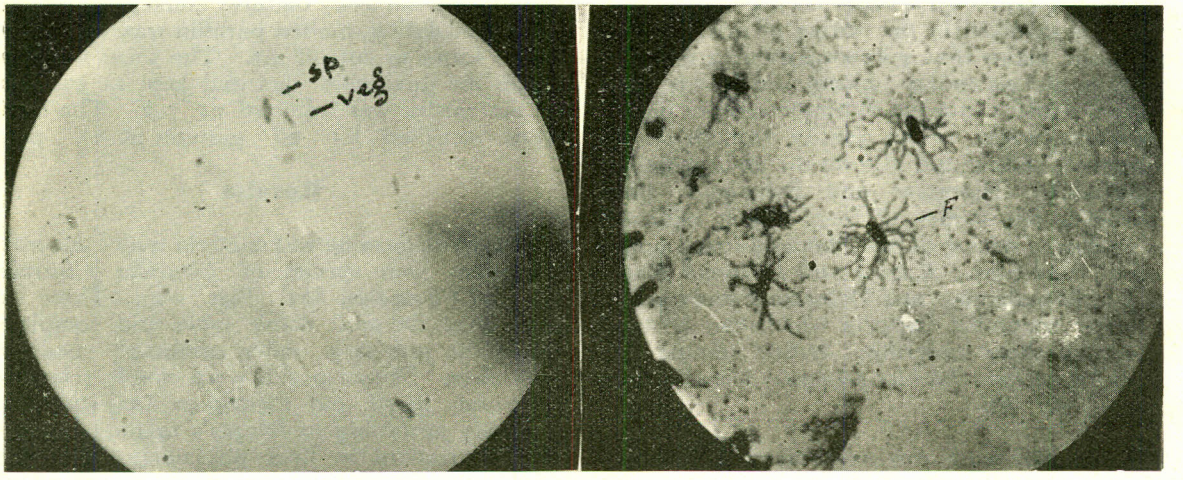

Fig. 2.—*Clostridium Sp* 2. (Veg, Vegetative cell; Sp, Sporangium; F, Flagella) x1,440

Table 1.

	Clostridium Sp 1	*Clostridium Sp* 2
(1) Catalase activity	Negative	Negative
(2) Deep glucose agar test (in test tube)	Colonies throughout the medium and only at the bottom with increasing dilution. No colony on the upper surface	Colonies always throughout the medium. No colony on the upper surface
(3) Spores	Oval, excentric, 1.12μ–1.80μ × 1.70μ–2.25μ	Oval, central, 1.12μ–2.2μ5 × 3.15μ–4.50μ

Table 1.—*Continued.*

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(4) Sporangia	Excentric, thick-walled on one side and thin on the other; swollen at sporulation	Central, boat-shaped, bulged on sporulation. Spore formed more to one side of the cell so that a thick sporangial wall is seen on one side whereas none is seen on the other side. Spores usually not shed even after one month
(5) Vegetative cells	Single, some being long at the young stage which break later on, 2.25μ—3.75μ × 1.12μ–1.50μ. g. positive, not acid fast, motility present with peritrichous flagella. Liquefied gelatine stab, spreading smooth colonies formed above as well as below the agar. Very finely echinulate on agar slant and scanty growth seems to be whitish dull on potato slant	Single, 3.70μ—6.10μ × 1.20μ—1.50μ. g. positive, not acid fast, motility present with peritrichous flagella. Liquified gelatine stab, whitish punctiform colonies formed in the middle of the agar. Echinulate on agar slant. Creamy, glistening, abundant growth on potato slant
(a) Nutrient broth	Cloudy growth, presence of sediment, no surface growth, no bubbling	Pellicle, clear, no sediment
(b) Litmus milk	Not curdled, pinkish violet, slightly clear area on the top. Litmus not changed	Not curdled, pinkish violet, medium not cleared, Litmus not changed
(c) Milk-agar plate	Not hydrolysed	Not hydrolysed
(d) Nitrate	Reduced	Reduced
(e) Glucose broth	Cloudy growth, no sediment, no gas. pH 6.0	Cloudy, no surface growth, no gas, presence of sediment. pH 5.5
(f) Acetyl Methyl Carbinol	Produced	Not produced
(g) Citrate	Utilised	Utilised
(h) Urease	Produced	Produced
(i) Indole	Not formed	Not formed
(j) Coagulated egg albumen	Not proteolysed	Not proteolysed
(k) H_2S	Formed	Not formed
(l) Synthetic medium	Not utilised	Growth present (slimy growth on the upper surface)
(m) Inorganic salts medium	Not utilized	Not utilized
(n) Blood agar	Haemolysed (24 hours)	Haemolysed (48 hours)
(o) Brain medium	Growth present, no change	Growth present, no change
(p) Cellulose	Not igdested	Not digested

Table 1.—*Continued*

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(q) Alkaline Pyrogallic acid	Scanty growth	Scanty to moderate growth
(r) Growth at pH 6.0	Present	Present
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(s) Fermentation tests	Acid and no gas from mannitol, glucose, lactose, laevulose, D (–) mannose, dextrine, maltose, glycerol, raffinose and inuline. No acid and no gas from sucrose, pectine, rhamnose, galactose, starch and arabinose	Acid and gas from sucrose: Acid and no gas from glucose, lactose, laevulose, maltose, galactose, glycerol, raffinose and inulin. No acid and no gas from mannitol pectin, starch, arabinose and rhamnose
(t) Jute stem	Retted jute stem in 5 days	Retted jute stem in 5 days

Table 2.—Showing the Retting Behaviour of the Two Strains.

	*Clostridium Sp*1	*Clostridium Sp*2
1st day	Inoculation on the jute stem	Inoculation on the jute stem
2nd day	Turbidity and presence of blisters on the stem. No bubbles	Few bubbles, turbidity and presence of blisters on the stem
3rd day	,, ,,	No bubbles
4th day	Bursting of blisters showing separation of fibres	Bursting of blisters showing separation of fibres
5th day	Retting completed	Retting completed

one was not ascertained. However, it is reported that certain chemicals accelerate the process of retting and reduce the retting period (Chatterjee, Nodder and Sarker[10], Baruah and Baruah[11]). It was observed that under laboratory conditions these two species completed the process of retting within 5 days. So far, there are no such *Clostridia* reported earlier which can ret jute in such a short time. The two species under investigation differ from the *Clostridia* in their morphological and physiological characters. The morphological and physiological characters and the retting behaviour of these two species have been described in Table 1 and 2, respectively.

It is interesting that these two retting bacteria completed retting under laboratory conditions in a short time. If these results can be reproduced under field conditions, they should prove very useful to growers.

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