STUDY OF AN ACTIVE AEROBIC JUTE RETTING BACTERIUM

A.C. Biswas

Jute Research Institute, Tejgaon, Dacca

(Received June 16, 1966; revised September 5, 1968)

Among different types of rod-shaped, spore forming bacteria isolated from jute retting water *Bacillus sphaericus* showed retting ability under laboratory conditions. Its physiological-cultural behaviour and ctaion on jute stem has been studied in detail.

Introduction

The usual practice of retting of jute and other fibre crops by microorganisms is known to the scientific world. This produces biochemical action on the plant which is the basis of fibre separation. During the process fibre bundles from the cortex and wood are separated and a partial digestion of the pectic substances cementing the fibre cells with one another is effected. But the problem connected with the process causes enormous difficulties in retting by unusual delay, producing fibre of low quality etc. The breakdown of plant tissues by bacteria is generally believed to be due to the action of enzymes secreted by the organisms; pectic substances in the middle lamella of the parenchymatous cells by bacterial enzymes known as pectinase. Delay caused in retting may be due to the slow and insufficient production of enzymes or interference of different types of enzymes produced by different types of bacterial flora present in retting water. The microorganisms are known to produce at first protopectase which hydrolyses the protopectin to pectin and this pectin is further broken down by pectinase to galacturonic acid. Ali and Islam[2] reported pectic enzyme from *Penicillium frequentans* which retted jute stem hydrolysing the pectic substances.

Attempts have been made by many microbiologists to isolate such bacteria which are capable of retting jute and other fibre plants. That both aerobic and anaerobic bacteria are capable of retting jute plants have been reported by many workers. Previous investigators have established that the agents responsible in water retting are spore forming, anaerobic, microaerophillic and aerobic bacteria. Biswas[3] isolated two species of anaerobic clostridia both of which retted jute stem in 5 days under laboratory conditions. An anaerobic Bacillus species was isolated by Katagiri and Makahama[10] which was capable of retting jute. Active aerobic jute retting bacteria *Bacillus polymyxa* and *Bacillus cereus* were also isolated by Ali[1] and Biswas[4] respectively. Kayser and Delavel[9] isolated some aerobic and anaerobic bacteria which were capable of retting flax and hemp. *Bacillus substilis*, *Bacillus macentricus* and *Bacillus macerans* isolated by Debsarma[7] from retted jute stem showed retting ability. It has been demonstrated by Behrens,[5] Rossi,[13] and Rossi and Carbone[14] that some species of Bacillus can ret flax under aerobic conditions.

It is evident from the evidences that, although, according to some author only members of the genus clostridia are the responsible agents in water retting of fibre plants, possibility of proper retting by aerobic bacteria could not be ruled out. On the other hand, isolation of pure aerobic bacteria and their maintenance is much easier than those of anaerobes. So it would be of much importance if active aerobic bacteria could be isolated and utilized in retting jute plants. Present investigation led to the isolation of an aerobic rod-shaped, spore forming bacterium which was an active retter of jute under laboratory conditions and it properly retted jute stem in much shorter time.

Materials and Methods

The isolation was made from the water of a jute-growing area in the Mymensingh District of East Pakistan in full jute retting period. Collected water sample was kept in incubator at 37°C for 7 days and then plated in agar medium (glucose 5.00 g, yeast extract 5.00 g, peptone 5.00 g, L-cystine 0.2 g, and agar 20.00 g/l of distilled water) to isolate the microorganisms present in retting water. Bacterial isolates taken into account and tested for retting ability, inoculating in the jute stem in test tubes immersed in sterile ditilled water. The inoculated jute stem tubes were kept at 37°C and were observed daily to ascertain the ability of individual isolates.

The isolate showing vigorous retting ability giving bubbles, swelling and bursting of epidermal layer and exposing the fibre strands was considered as active retter. This was reisolated for purification and again tested for retting ability. The bacterium concerned was then confirmed as active retter of jute. The media used for isolation and physiological studies were sterilized for 20 min at 15 lb pressure and the pH adjusted at 7.2.

Results

Among the different spore-forming aerobic rod-shaped bacteria found and identified some were non-retters and some were retters. But this strain was considered to be an active aerobic jute retting bacterium. This was found to ret jute stem under laboratory condition in 6 days. During retting period the strain showed no change in the liquor on the first day of inoculation. On the second and third day the retting liquor became turbid and some bubbles were observed. On the fourth day there were blisters on the stem. On the fifth day there was bursting of blisters showing the fibre strands. On the sixth day there was separation of fibres and retting was considered complete. Observations of cultural and physiological behaviours of the strain are given in Table 1. From the study of the characteristic of the isolate it was identified as a strain of *Bacillus sphaericus* (Fig. 1).

TABLE 1

Spores—spherical, thick-walled, ramnants of sporangium sometimes found adhered to the spore, dia of the spore 1.12–2.25μ (Fig. 1).

Sporangia—terminal, swollen spherical, subterminal at immature stage (Fig. 1).

Vegetative body—rod, occurring singly; 1.12–1.5μ by 3.12–7.8μ. motile with peritrichous flagella, gram positive, no acid fast (Fig. 2).

Gelatin stab—liquefied.

Agar colonies—small, spreading, formed above the surface of the medium, translucent.

Agar slant —echinulate, spreading, becoming yellowish brown.

Potato slant — scanty to moderate growth, gradually becoming yellowish, colour of potato unchanged.

Alkaline pyrogallic acid—scanty, thin growth.

Nutrient broth—no surface growth, turbid, sediment present.

Litmus milk — no change in colour, not curdled, milk gradually digested from top downwards.

Milk agar with casein—casein hydrolysed.

Nitrate—reduced to nitrite.

Glucose broth —cloudy growth, no gas, presence of sediment.

Starch—not hydrolysed.

Acetyl methyl carbinol—not produced.

Citrate—utilised.

Urease—formed.

H_2S—not produced.

Indole—not formed.

Coagulated egg albumen—proteolysed.

Blood agar—haemolysed.

Brain medium—presence of growth, no change.

Synthetic medium—not utilized.

Growth in inorganic salt—present (weak).

Cellulose—not digested.

Growth at pH 6.00—present.

Optimum temperature of growth—35–37°C.

Growth at 50°C—scanty.

Fermentation test—acid and gas from rhamnose. Acid but no gas from glucose and dextrine. No acid and gas from mannitol, lactose, sucrose, laevulose, D(−)mannose, pectin, maltose, galactose, starch, arabinose, glycerole, raffinose and inuline.

Oxygen relationship—aerobic facultative.

Action on jute stem—retted jute stem in 6 days.

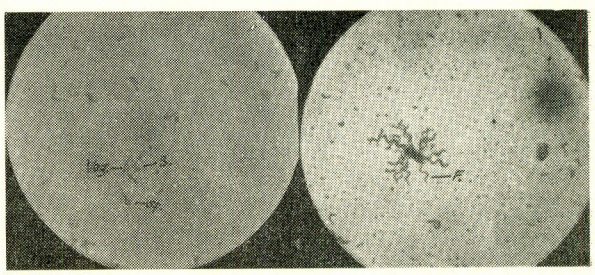

Fig. 1.—Vegetative cell (veg.), sporanguim (sp) and spores (s) of *Bacillus sphaericus*. ×1440.

Fig. 2.—Peritrichous flagella (F) of *Bacillus* sphaericus. ×1440.

For identification, physiological-cultural study and staining of vegetative body and flagella, literature [6,15] were consulted and techniques of Gray,[8] Leifson[11] and Maneval[12] were used as guides.

Discussion

Previous records showed that some spore-forming aerobic rod-shaped bacteria also take active part in the retting of jute. Retting period of jute plant under natural conditions usually varies from 15 to 18 days [3] whereas in the present observation the organism which was identified as strain of *Bacillus sphaericus* retted jute stem under laboratory conditions in 6 days. This species was not previously reported as retter of jute and any other fibre crop. It showed slightly different physiological characteristic from the original strain described in Bergey's manual in its ability to utilize citrate, reduction of nitrate and liquefaction of gelatine. However, such differences were negligible and it might be a strain of the original race of *Bacillus sphaericus* which was capable of retting jute. Possibility of existence of different strain of original race of jute retting aerobic bacterium, *Bacillus cereus* was indicated by Biswas.[4] *Bacillus subtilis* isolated by Ali[1] could not ret jute whereas the same species isolated by Debrma[7] was an active retter of jute. Debsarma[7] also reported *Bacillus cereus* as non-retter of jute. *Bacillus sphaericus* was reported[6] to have been isolated from rotting cypress and oak wood which also suggested its potential retting ability. The present experiment was conducted at a controlled temperature of 37°C as the temperature of natural jute-retting water during the proper jute season varies between 32–37°C. The optimum growth of the isolate was also recorded between 35–37°C. The pH value of all the media tried were maintained at 7.2. Decrease in pH value was marked with the gradual decrease in growth of the strain in different media and jute stem. Since microbial retting of jute plants is a very complex biochemical process, detailed biochemical study of such active retting agent is of much importance which could ret jute plants in a definite and shorter period of time for producing quality fibre of commerce.

Acknowledgement.—The author expresses his thanks to Professor Majeed Ahmed, Head of the Department of Botany, Dacca University, who showed interest and suggested the problem.

References

1. M. Ali, Appl. Microbiol, **6**, 87 (1958).
2. M. Ali, and M. A. Islam, Pakistan J. Sci. Ind. Res., **8** 47 (1965).
3. A.C. Biswas, Pakistan J. Sci. Ind. Res., **7** 51 (1964).
4. A.C. Biswas, Pakistan J. Sci. **16** 232 (1964).
5. J. Behrens, Zbl. Bakt. II, **10**, 524 (1903).
6. R.S. Breed, E.G.D. Murray, and A.P. Hitchens, Bergey's *Manual of Determinative Bacteriology* (The Williams & Wilkins Co. Baltimore, Maryland, 1948) sixth edition.
7. G.D. Debsarma, Ind. J. Ag. Sci., **16** 453 (1946).
8. P.H. Gray, J. Bacteriol, **12,** 273 (1926).
9. E. Kayser, and H. Delavel, Bull. Soc. Encour. Ind. Natl., **132,** 214 (1920).
10. Katagiri and Makahama, J. Agr. Chem. Soc. Japan, **15,** 832 (1940).
11. E. Leifson, J. Bacteriol, **20,** 203 (1930).
12. W.E. Maneval, J. Bacteriol, **12,** 300 (1930).
13. G. Rossi, Ann. Sci. Agr. Portici, **5** (1904).
14. G. Rossi and D. Carbone, Ann. Sci. Agr. Portici, **9** (1909).
15. Society American Bacteriologists, *Manual of Methods for Pure Culture Study of Bacteria* (Biotech. Publ. Geneva, N.Y. 1948) ninth edition.