Pakistan J. Sci. Ind. Res., **12**, 229–231 (1969)

JUTE RETTING BACTERIA FROM CERTAIN RETTING DITCHES OF EAST PAKISTAN

MUHAMMAD SHAFIQUL ALAM*

University of Dacca, Dacca 2

(Received July 7, 1967; revised December 3, 1968)

The action of different aerobic and anaerobic bacteria on green jute was studied and only *Clostridium tertium*, *Cl. lacunarum*, *Cl. lactoacetophilum*, *Cl. histolyticum*, *Bacillus coagulans*, *B. circulans*, *B. polymyxa*, and *B. megaterium* retted jute. Their retting properties and physiological characters are described.

Introduction

Jute refers to two species of plant, *Corchorus capsularis*, and *Corchorus olitorious* as well as the fibre derived from them. Retting constitutes a very important part in jute fibre production and is commonly carried out in East Pakistan by steeping jute plants under water of ditches and rivers for about 15–20 days, during which enzymes of microorganisms, mostly bacteria, enter into the plant tissues, hydrolyse the pectin, cementing the fibre strands and release the fibre for mechanical separation.

Different workers isolated various retting microorganisms from vegetable fibres to identify them and to study their retting properties under controlled conditions. Kayser and Delavel[1] isolated five aerobic bacteria which retted flax and hemp. Patel and Ghose[2] isolated some and rod shaped bacteria which retted jute. Debsarma[3] isolated seven species of rod bacteria of which only *Bacillus subtilis*, *B. macentericus*, *B. macerans* could ret jute. Ali[4] studied *Bacillus polymyxa*, *B. subtilis*, *B. pumilus*, *B. lentus* and *B. sphaericus* of which only *Bacillus polymyxa* retted jute. Ahmad[5] isolated some spore forming and non-spore forming bacteria which were *B. brevis*, *B. alvei*, *B. sphaericus B. laterosporus*, *B. macerans*, *B. polymyxa*, *B. subtilis*, *B. megaterium*, *B. cereus*, *Micrococcus varians*, *M. luteus* var. *liquefaciens*, *M. corchorus* and claimed them as active jute retters. Jalaluddin[6] reported *B. macerans*, *B. subtilis*, *B. cereus*, and *Bacillus megaterium* as retters.

It appears that the retting organisms are plentiful and of various nature. With further studies it is likely that more effective and useful retting bacteria might be isolated, which might have some prospect of utilization in natural retting condition. This investigation was therefore undertaken to isolate more jute retting bacteria from certain retting ditches of East Pakistan and to study their characteristics *in vitro*

*Present address: Microbiology Division, Jute Research Institute, Dacca 15.

Materials and Method

For the isolation of retting bacteria samples of retting water and retted fibre were collected from retting ditches from the districts of Bogra, Rangpur, Dacca and Mymensingh of East Pakistan. Small pieces of fibre from the retting jute plants together with some of the retting water squeezed from the fibre were taken in sterilized 250 ml conical flasks and immediately plugged maintaining strict aseptic condition. From these samples bacteria were isolated both aerobically and anaerobically. A small piece of fibre and 1 ml retting liquor from each of the sample were transfered to peptone yeast extract broth, PY, (peptone 5g, yeast extract 5g, dist. water 1000 ml). For anaerobic culture, when sufficient growth was observed in PY broth, the cultures were heated in water-bath for 20 min at 80°C to limit the study to spore-forming anaerobes only, and then plated out on nutrient agar by using a diluted culture (1 in 104). Representative colonies appearing after 7-day incubation were transferred to nutrient broth. Unless stated otherwise all cultures of anaerobic organisms were incubated at 37°C in specially made anaerobic jars (BTL anaerobic jar). For aerobic culture, PY broth cultures of all the samples were directly plated without prior heating and incubated at 37°C in presence of air. For testing the retting ability of the bacteria, autoclaved sterile jute stem pieces in test tubes in distilled water were inoculated with the bacteria and incubated at 37°C. A bacterium was taken as retter if it could completely separate the fibre of the jute stem in the test tube in 10 days.

Physiological and morphological characters of these bacteria were studied to key out the bacteria using Bergey's *Manual of Determinative Bacteriology* (7th edition). Any variation from the characters of these bacteria as reported in the manual were recorded.

Result

Of the 256 different spore-forming bacteria isolated only eight of them retted jute at 37°C

within a shorter period of 3–7 days (normal retting period 18–20 days). They were flagellated, motile, Gram positive, non-acid fast and belong to both aerobic and anaerobic groups. Anaerobic strains were identified as *Clostridium tertium*, *Cl. lacunarum*, *Cl. lactoacetophilum*, and *Cl. histolyticum*. The aerobic strains were identified as *Bacillus circulans*, *B. coagulans*, *B. polymyxa*, and *B. megaterium*. The physiological characters in which these bacteria differed from those reported in Bergey's manual were in respect of milk coagulation, rhamnose and inulin fermentation, nitrate reduction, growth in potato slant, gelatin liquification and manitol fermentation, (Table 1).

Discussion

All the bacteria isolated were not retters. Of the 256 bacteria isolated from the retting liquor only 4 aerobic and 4 anaerobic bacteria retted jute completely. Others were non-retters. None of the Clostridia reported in this paper were previously known as jute retters. When the aerobic organisms isolated in the present work were compared with the aerobic bacteria reported by those who studied jute retting, they were found to be more or less similar. *Bacillus polymyxa* was reported by Ali[4] and Ahmad.[5] *B. circulans* was also reported by earlier workers like Hakim,[7] Hussain,[8] and Salil[9] as active jute retter. Allen[10] and Southgate[11] held that the actual retting of flax in England is done by *Cl. tertium*. The work presented in this paper indicated that *Cl. tertium* is also not uncommon in the retting of jute. This is also the first published report that strains of *B. coagulans* is capable of retting. The strain *B. megaterium* as described here differed from the one reported by Ahmad[5] and Jalaluddin[6] in retting time which was 9 days as against 7 days.

It is interesting to note that while the natural process of retting takes about 18–20 days at about 30°C–35°C the strains reported in this paper retted jute in 3–7 days at about 37°C. The use of active strains of bacteria in pure culture, therefore, offers the possibility of greatly reducing the retting period. *Bacillus comessii*, a sporeforming bacteria, has been utilized industrially in Italy, France and Germany for the retting of flax and hemp. Such a method could be adapted in jute industry of Pakistan also. With active pure strain, the possibility exists of increasing the quality and uniformity of the fibre and so the utility.

Of the various retting bacteria reported in this paper only *B. ciculans* fully conformed to the standard strain described in Bergey's manual. Most other differed from the standard strains. They may, however, be varieties of the standard strains. It is evident, therefore, that the retting bacteria exhibit physiological characteristics quite different from those of the standard strains.

As for the presence of large number of non-retters and their possible role in the process of retting it may be speculated that condition during retting are obviously favourable for the development of the non-retting flora. The soluble constituents of the jute plants may be good source of simpler carbohydrates and related substances for them. Consequently by their action on the

TABLE 1.—DIFFERENCES IN THE PHYSIOLOGICAL CHARACTERS OF THE STRAINS FROM THE STANDARD AND THEIR RETTING TIME

Culture	Characters noted in the study	Characters reported in Bergey's manual	Retting time in days
Clostridium lancunarum	Slight coagulation in milk	Milk not coagulated	4
Clostridium lactoaecetophilum	Rhamnose and Inulin not fermented	Rhamnose, Inulin fermented	3
Clostridium histolyticum	No action in milk	Softly coagulated, then slowly digested	3
Clostridium tertium	Nitrites not produced	Nitrites produced	4
Bacillus coagulans	Heavy growth in potato slant	Growth erratic, not distinct	5
Bacillus polymyxa	Gelatin not liquified	Slow liquification	4
Bacillus megaterium	Manitol not fermented	Manitol fermented	7

soluble constituents of the jute stem, they may be expected to contribute appreciably to the total acidity of the retting liquor. It is tempting to speculate whether or not these non-retting microflora are of assistance to retting species by their synergistic action. It appears that the true retting organisms constitute a relatively small part of the total bacterial flora of the retting water of jute and, consequently, it might well be anticipated that their activity would be influenced to some extent at least by variation in the composition of the non-retting flora. As far as is known, however, the possibility has not been investigated. Thus, the inter-relationship of various non-retting organisms and retting bacteria may prove a fruitful field for future investigation.

Acknowledgement.—The author wishes to express his thanks to Dr. Majeed Ahmed, Head of the Department of Botany, to Mr. Mahbubur Rahman, Lecturer, University of Dacca, East Pakistan, and to Dr. Myser Ali, Microbiologist, Jute Research Institute, Dacca, for their assistance in the course of work as well as in the preparation of the manuscript.

References

1. E. Kayser and H. Delavel, Bull. Soc. Ecour. Ind. Natl., **132,** 214 (1920).
2. J.S. Patel and S.S. Ghosh, Indian Central Jute Committee Agri. Res. Mem., No. 2 (1943).
3. G.D. Debsharma, Indian J. Agr. Sci., **16,** 453 (1946).
4. M.M. Ali, Appl. Microbiol., 6, No. 2, U.S.A. 87 (1958).
5. M. Ahmad, J. Appl. Bacteriol., **26,** (2), 117–126 (1963).
6. M. Jalaluddin, Econ. Bot., **19,** 384 (1965).
7. A. Hakim, Jute Retting Bacteria, Thesis, Dacca University, (1955).
8. M. Hussain, Bacterial Retting of Jute, Thesis, Dacca University (1959).
9. K. Salil, Jute Retting Bacteria, Thesis, Dacca University (1964).
10. L.A. Allem, J. Soc. Chem. Ind. Lond., **65,**365 (1946a).
11. B.A. Southgate, Wat. Pollut. Res. Lab. Tech. Pap. No. 10, 10(1948).