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Upgradation of Jute Retting by Pectinolytic Bacteria at Farmers' Level for the Improvement of Fibre Quality

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UPGRADATION OF JUTE RETTING BY PECTINOLYTIC BACTERIA AT FARMERS' LEVEL FOR THE IMPROVEMENT OF FIBRE QUALITY

THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
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BY
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B.Sc. (Hons.), M.Sc. (CHITTAGONG)

An enlarged view of growth of Z_1 (***Micrococcus lylae***) in Echandi medium.

CERTIFICATE

This is to certify that Md. Zulficquer Zaman, Research Fellow, Institute of Biological Sciences, University of Rajshahi , worked under our supervision for the award of degree of Doctor of Philosophy entitled "Upgradation of jute retting by pectinolytic bacteria at farmers' level for the improvement of fibre quality" since July, 1992. This thesis embodies the work done by him and has not been submitted before for any other degree.

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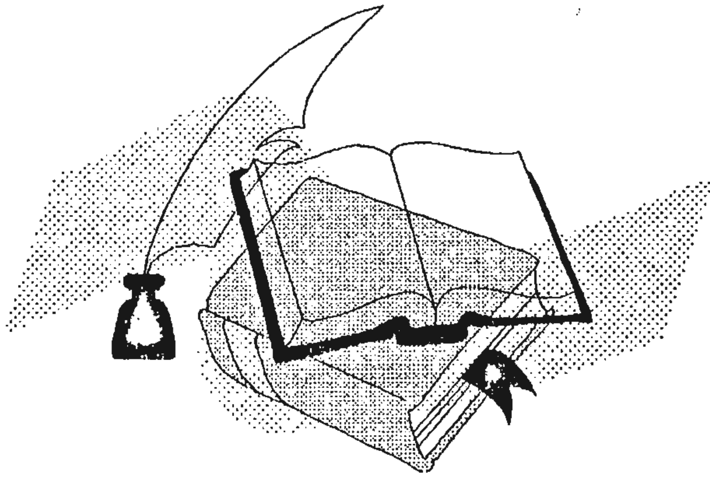

DEDICATED

TO

MY

PARENTS AND TEACHERS

WHO OPENED MY EYES OF KNOWLEDGE

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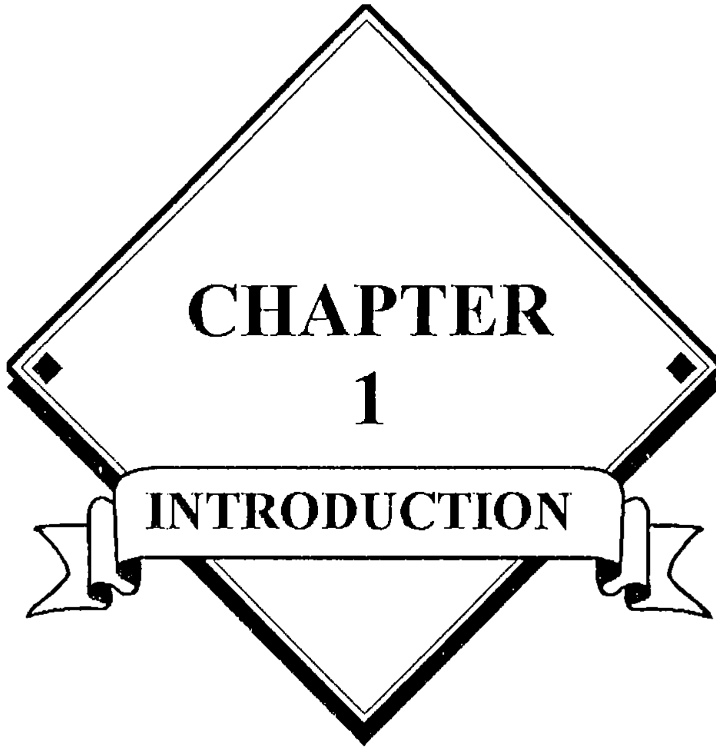
ABSTRACT

Fourteen bacterial colonies have been isolated from jute retting bacterial collections from farmers' retting tanks in the districts of Rajshahi and Pabna. Out of these fourteen bacterial colonies only three are found to be pectinolytic and non-cellulolytic. They grow moderate in Nutrient agar medium and well in Echandi medium containing Apple pectin as a source of carbon. These three bacterial strains are named by Z_1 , Z_4 and Z_{30} and are identified as ***Micrococcus lylae, M. luteus*** and ***Microbacterium laevaniformans,*** respectively.

In field trials pretreatment with single or mixed culture of Z_1 , Z_4 and Z_{30} on basal portions of green jute resulted in removing cuttings from jute. In Tossa jute removal of cuttings are made from 1.72 inches to 0.4 - 0.33 inches and in Deshi jute from 5.8 inches to 2.0 inches. Tensile strength, fineness and colour of jute fibre are also improved. These result in upgradation of jute by one to two grades over the control.


CHAPTER
1
INTRODUCTION

INTRODUCTION

Jute cuttings are the coarsest portion of jute both in white (***Corchorus capsularis*** L.) and Tossa (***Corchorus olitorius*** L). These are also obtained from mature jute plant and also after harvesting of ripe fruits for seeds. Retting period of these jute stems usually take longer period of over fortnight. However, the retting is not uniform from basal to the top. The top portions are over-retted whereas, the basal portions remain stiff and hard resulting is degradation of also top fibres. This is a big problem for the farmers. Bangladesh produces annually approximately 8 lakh metric tons of raw jute (Sobhan, 1992b) which constitute about 10-25% of cuttings (Mohiuddin ***et al.*** 1978). So, we obtain about 1-2 lakh metric tons of jute cuttings per year. Uniform retting could be a good use of these jute and also immense upgradation of jute in the process of jute retting.

Thermal degradation of jute cuttings in the mills and factories have long been used for upgradation of jute cuttings. In this process, sometimes temperature goes so high that the fibres are also degraded resulting in the loss of tensile strength of fibres. In the conventional mill practices jute cuttings are sprayed with a mineral water oil emulsion to 30% moisture content followed by piling in a large concrete bin and are left for 10-12 days during which temperature rises upto 70°C (Mohiuddin, 1985;1991). Therefore, biodegradation of cuttings was thought to be the best way of

preserving the tensile strength of fibre. It is known to all that the quality of jute depends on the strength, amount of cuttings, fineness, reed length, colour and lusture. All these qualities depend mostly on the proper period of retting. Over retting and under retting affect very much in the qualities of jute fibre. Pectin is the most cementing material between the cells. Many microbes take part in pectin degradation resulting is the release of fibre cells and freeing them from surrounding parenchymatus tissues of jute. During jute retting, pectinolytic bacteria predominantly the aerobic bacteria with the help of enzymes pectinase hydrolyses pectin substances from the middle lamella (Ali, 1957).

In usual retting practices, retting process is not uniform. Generally the basal portion of jute plant is thick, stiff and hard which take longer period for retting than the upper portion (Mohiuddin ***et al.*** 1978 & Mohiuddin ***et al.*** 1981). If the farmer prolongs the retting period to completely removing the bark of the basal portions then the fibre in the upper portions are over retted. Thus in usual retting practices the overmaturing basal portions remains under retted or unretted (Debsharma, 1946; Bose, 1966). It is known that hardness or stiffness of the jute cuttings is caused due to presence of higher amount of pectin (Ibid, 1981). Normal fibre contains 0.5-0.9% of pectin whereas in jute cuttings there are about 1.7-2.8% pectin (Mohiuddin, ***et al.*** 1981). These hard and stiff basal fibres are not suitable for spinning in jute mills and have little demand in both National and International markets.

CHEMISTRY OF JUTE:

Jute is a composite fibre containing approximately an intimate mixture of cellulose (58-63%), hemicellulose (21-24%) and lignin (12-14%). In addition, small quantities of other substances, such as, natural wax (0.4-0.8%), protein (0.8-1.5%), pectin (0.2-0.5%) traces of tanin and colour pigments (Abdullah, 1992a). According to Abdullah (1991) due to the presence of cellulose and lignin jute has some similarities with cotton and wood. Cellulose is mostly present in crystalline part whereas, non-crystalline amorphous part consists of lignin and hemicellulose.

BOTANICAL DESCRIPTION AND DISTRIBUTION OF JUTE:

The genus *Corchorus* has several species (Hooker, 1872; Walt, 1889; Kundu, 1951) of which only two *C. olitorius* (Tossa) and *C. capsularis* (White) are commercially used in our country. These two cultivated species have hundred of varieties (Kabir *et al.*, 1968).

Photograph I :
Ia White or Deshi Jute (*Corchorus capsularis*).
Ib Tossa Jute (*Corchorus olitorius*).

***C. capsularis* L. (White or Deshi)**

This species is 2.5 to 2.57M (8-10 ft) in height. Leaves are light green coloured and bitter to test for the presence of glucoside. Flowers are small in size and dark yellow in colour. Fruits are round capsules (Photograpg Ia).

C. capsularis is distributed throughout the country, although the extent of occurrence varies from region to region. It is predominant in all the divisions, intensive in north and central regions covering Tista flood plain, Brahmaputra alluvium and lower Jamuna flood plains. It is cultivated in high, medium high and medium low lands. The span of sowing time of white jute ranges from mid March to mid April in different regions based on the availability of pre-monsoon shower required for optimum soil moisture suitable for germination of seed (Sobhan, 1992c). The recommended varieties of Deshi jute in Bangladesh are CC-45 (JO pat), CVE-3 (Ashu pat), CVL-1 (Sabuj pat), D154 (2) (Anon., 1992).

***C. olitorius* L. (Tossa jute)**

The height of this species is 3.0 to 3.5 M. Leaves are dark green. Flowers are comparatively large and light yellow coloured. Fruits are long cylindrical capsules (Photograph Ib).

The major Tossa jute growing areas are Khulna division followed by Rajshahi, Dhaka and Chittagong divisions. The major concentration is in Gangetic alluvium and in the lower Jamuna flood plain. Higher concentration is in Gangetic alluvium and in the lower Jamuna flood plain.

Higher concentration of local types are found in Noagaon, Natore, Pabna, Sirajgong, Jhenidah, Magura, Narail and Jessore. In the eastern region mainly in Chittagong division Tossa jute is grown for culinary purpose (Sobhan, 1992c). The two recommended varieties of Tossa jute presently under cultivation are 0-9897 (Falguni Tossa) and 0-4 (Anon., 1992).

JUTE SITUATION AT PRESENT:

Jute plays a vital role in the economy of Bangladesh. Jute and jute goods are responsible for 65% of the export earning. Now-a-days, jute cultivation is alleged to be a loosing concern because of fall of business in the International market signaling a great threat to the economy of Bangladesh. (Asaduzzaman *et al.*, 1992). Annually approximately 8 lakh metric tons of raw jute fiber are produced from nearly 6 lakh hectares of land that offers cash income to 40 lakh farmers. About 8 lakh people are engaged in different phases of industry and trade of jute (Sobhan, 1992b). Jute agriculture is facing various problems in present days. The problems are socio-economic and agro-ecological types. Because of all these, jute is no more profitable crop for the farmers. Moreover synthetic fibres have replaced jute fibres in jute importing countries. We have to look for other uses of jute. Now-a- days raw jute or green jute is being used in paper mills. This is a very good alternative use of jute in Bangladesh.

ECONOMIC IMPORTANCE

Jute is the most important cash crop of Bangladesh since long. Its influence on ecology are significantly reflected on agro-ecological and the socio-economic life of the people (Sobhan, 1992b). In the article 'jute : Its Economic and Ecological Attributes' Abdullah (1991) described that one third of the foreign exchange was earned with jute and jute products. Both agriculture and industry are greatly associated with jute based activities. So, jute was called to be the backbone of Bangladesh economy.

Jute is second only to cotton as a textile fibre. As a coarse fibre it is mainly used as packaging materials in industries. This visco-elastic, mechanically quite stable fibre is used as a raw material for production of twine, hessian, gunny bag, carpet, blankets, wall covers, mat, rugs, various furnishing fabrics, felts and geotextiles, yearn and wool pack in industries. In small and cottage industries various fashionable decorative handicrafts are made from jute which are very attractive and popular in aboard. Not only fibre., jute leaves and sticks are also useful. The green leaves contain vitamins and proteins which are edible to human. Jute sticks are used in fencing and paper mills. In villages jute sticks are the light fuel materials. Now jute stickes are used as raw material in the 'partex' industries and these partex boards are very suitable in making light doors and furniture.

SCOPE AND AIM OF THE PRESENT WORK:

A very important process in jute fibre production is retting. Quality of jute greatly depends on proper retting. Research work on evolving effective method of retting of jute was started in laboratory condition very long ago in different Universities and Research Institutes. Although, many retting bacteria have been isolated but it has not yet been possible to use them in the practical retting in non-sterile water at farmers' level. The bacteria do not seem to be able to compete with other microbes in the retting water tanks in field condition (Ali, 1989).

The present work was undertaken to isolate, characterize and study aggressive pectinolytic bacteria from different retting tanks and their possible use for the improvement and upgradation of jute cuttings at farmers' level or their possible use for the improvement of jute cuttings at the retting stage in field condition.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

Bangladesh is the largest jute growing country in the world but in the recent years synthetic fibre has occupied the position of jute. Because of this jute research activities have been dwindled. Jute growers are still following conventional method of jute retting in the field. As a result the quality of jute is deteriorating. This and the emergence of synthetic fibre have created an adverse effect on jute in the world market.

Most of the articles and research papers are of general in nature.Nandi and Bashu (1938) made a systematic study and analysis of jute retting. Cambium and soft bast tissues are first attacked by retting bacteria. This results in the separation of pericycle from the wood. The epidermal and subepidermal layers are not attacked by bacteria and therefore they remain intact. The primary , secondary and tertiary walls of the fibre cells are not attacked by bacteria due to their impervious nature and absence of intercellular spaces in the fibre tissue.

Patel and Ghosh (1943) isolated a rod-shaped and an oval-shaped bacteria from retted samples of jute.Ghosh (1943-44) reported that there were manifold increase in the population of aerobic micro-organisms in the beginning of retting and in later stages a general decrease of aerobes

followed by a partial replacement of anaerobic micro-organisms were noticed in the attainment of final retting.

Debsharma (1946) isolated five strains of ***Clostridium*** species, a group of anaerobic bacteria from the jute retting liquor. Two of these were characterized and named ***Clostridium felsine*** and ***C. bytricum*** and were claimed to ret jute stems.

Rouf (1955) reported that a large number of aerobic microbes took part in retting process and were independent of the necessity of presence or absence of anaerobes for the completion of retting.

Azmi (1955) reported, ***Micrococcus luteus*** as a jute retter.Ali (1958) studied several Bacilli of which only ***Bacillus polymyxa*** was found to be a jute retter.

Ahmed (1963) isolated some spore forming and non-spore forming bacteria and were claimed to be active retters. These were ***Bacillus brevis, B. alvei , B. sphearicus, B. laterosporus, B. macerans, B. polymyxa, B. subtilis, B. megaterium, B. cereus, Micrococcus varians, M. luteus var. liquefaciences*** and ***M. corchorus.***

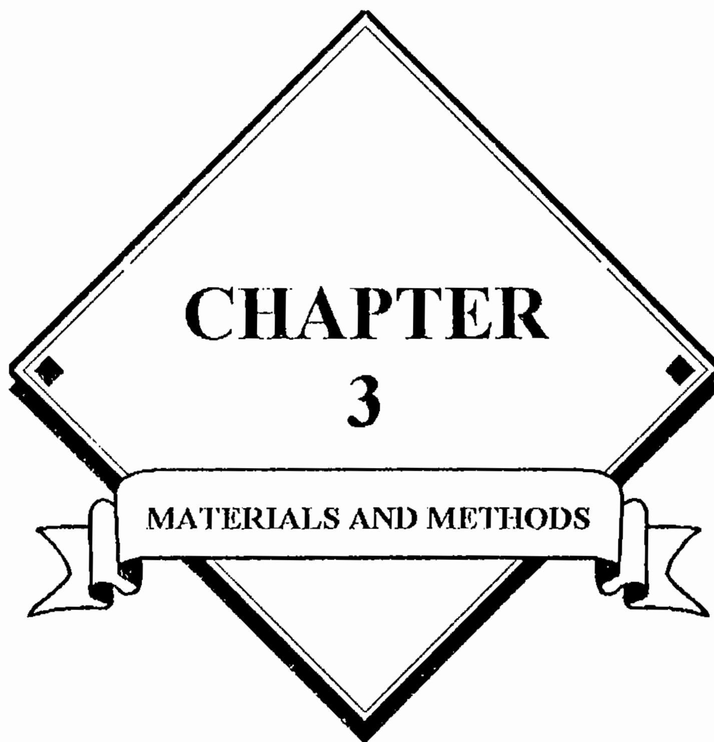
Biswas (1964) isolated several rod-shaped bacteria but only ***Bacillus cereus*** was found to be a jute retter. Jalaluddin (1965) reported ***Bacillus macerans, B. subtilis, B. cereus*** and ***B. megaterium*** as jute retters.

Alam (1970) studied retting activities of several aerobic and anaerobic bacteria on green jute but only a few were found to be jute retters. These are

Clostridium tertium, C. lacunarum, C. lactoacetophilum, C. histolyticum, Bacillus coagulans, B. circulans, B. polymyxa and ***B. megaterium***.

Retting process in jute depends on the secretion of pectinolytic enzymes by the micro-organisms to act on pectin, the cementing material between the fibre cells. Isolations of micro-organisms are usually made from the retting liquor or from the retting plant stems.Numerically by for the greatest number of bacteria were found to be associated with the stems (Chesson, 1978; Donaghy ***et al.***, 1990). Bacteria involved in retting process may originate primarily from soil adhering to root and from dust on the stems with minor contributions coming from the water and air. Pectin fermenting bacteria , ***Clostridium spp*** have been isolated from jute field soil by Rahmatullah ***et al.***, (1977). Entry of bacteria into stem seems to be through stem fissurs. This has been demonstrated in jute by Jalaluddin (1970).

Almeida and Rosemberg (1985) attempted to retting with introduced cultures of bacteria. Pure culture retting with bacteria has not been used for commercial fibre production so far, because of problems in removing the resident micro-flora. Addition of pure and mixed cultures of retting bacteria to jute rets has been attempted by Paul and Bhattacharya, (1978); Paul ***et al.***, (1987) and Basak ***et al.***, (1988). They found that the retting reached completion more rapidly with fibre yield and quality equal to or better than with the naturaly occuring micro-flora.


CHAPTER
3
MATERIALS AND METHODS

MATERIALS AND METHODS

1. SOURCE AND COLLECTION OF JUTE RETTING BACTERIA

Jute fibre are extracted from the jute plants by the process of retting which is brought about by the pectinolytic bacteria. The quality of commercial jute greatly depends on the entire process of retting and the type of bacteria involved in it.

Jute retting bacteria present in retting jute in field were collected at their different retting stages in retting tanks in Rajshahi and Pabna districts. Samples of retting jute were collected for the purpose of high quality jute production by selective pectinolytic bacteria from the nature.

2. SAMPLE COLLECTION PROCEDURE

Retted samples of jute were collected in sterilized test tubes from the retting heaps of jute in tanks. Before the collection, hands and weapons were sterilized with rectified spirit. Jute samples were cut into pieces with the help of sterile scissors and introduced in the sterile test tube after flaming the mouth over a spirit lamp. Then cotton plug was replaced. The samples were labelled giving date and collection number.

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3. STORING OF SAMPLES

Collected samples were taken to the laboratory and stored in a refrigerator (temperature 5-8°C). These samples were examined and used for isolation of pectinoltic bacteria under laboratory condition.

4. LSOLATION AND SELECTION OF PECTINOLYTIC BACTERIA.

Pectin degrading bacteria may not normally grow on synthetic media without pectin. Therefore it was necessary to select proper media for isolation of pectinolytic bacteria.

A. Media used.

Media are the synthetic mixture of nutritive substances. The micro-orgamisms expecially in this case bacteria are grown on the synthetic media. The media provide growth requirments and the viability. The composition of synthetic media are chemically pure salts, sugars, acids, proteins and nitrogen sources such as beef extract and peptone. Media may be liquid or solidified. The solidified media are produced by adding BDH agar powder to the liquid media. Agar is a complex mixture of carbohydrates and a small amount of protein which are not used as a source of nutrition.

The following media were used:

1) Nutrient Agar medium (NA)

2) Potato Dextrose Agar (PDA)

3) Echandi medium

4) Czapek's medium

Composition of media

1) Nutrient Agar medium

a) Beef extract - 3.0 ml

b) Peptone - 5.0 gm

c) Nacl - 1.0 gm

d) Agar - 15.0 gm

Distilled water to 1000 ml

2) Potato Dextrose Agar (PDA)

a) Potato - 200 gm

b) Dextrose - 16 gm

c) Agar - 16 gm

d) Distilled water to 1000 ml

3) Echandi broth mediam

a) Mg SO_4 - 0.5 gm

b) KH_2 PO_4 - 0.5 gm

c) K_2HPO_4 - 2.0 gm

d) Asparagine - 2.5 gm

e) Apple pectin - 10.0 gm powder

Distilled water to 1000 ml

In case of solidified medium 16 gm of powdered agar was added before autoclaving.

4). Czapek's medium

a) Amonium oxalate - 3.0 gm

b) K_2HPO_4 - 1.0 gm

c) Mg SO4, 7 H_2O - 0.05 gm

d) Carboxy methyl cellulose (CMC) - 16.0 gm

Distilled water to 1000 ml.

B. Method of isolation

1.0 gm of retting fibre of jute was cut into small pieces with a sterile scissor. These pieces were placed in sterilized test tube containing 9.0 ml of sterile water to prepare a mother suspension. The tubes were gently shaken. Ten fold dilution of suspension was prepared as shown bellow:

1) 1 ml of the mother suspension was added to the test tube containing 9.0 ml sterilized water.

2) 1 ml suspension from the 1st test tube was added to another test tube containing 9.0 ml sterile distilled water.

3) 1 ml suspension from the 2nd test tube was added to another test tube containing 9.0 ml of sterile distilled water.

4) 1 ml suspension from the 3rd tube was added to another test tube containing 9.0 ml of sterile distilled water and so on.

Thus serial dilution were prepared from mother suspension and were numbered 1st dilution, 2nd dilution and so on. Appropriat dilutions were used for further work. Samples from each of suspension numbered 1,2,3 and 4 were plated on Nutrient agar in petridishes to allow all bacteria to grow.

C. Cleaning of glasswares, test tubes, Petridishes and apparatus.

Fresh or used glasswares, test tubes, conical flax and petridishes were used for laboratory work. Cleaning, washing and drying of glassware and apparatus were done as follows:

a) The fresh glasswares and culture test tubes need no special treatment before being used, other then the normal washing up with tap water. Then, these were dried in the sun in the laboratory room and these were stored for experiments.

b) Used petridishes, test tube and other apparatus were firstly sterilized by autodaving at 120°C and 15 1bs/sq.inch above atmospheric pressure for 20 minutes to kill the discarded culture or contaminants. At this condition all solid media were liquified and then the all aparatus were washed with tap water to remove the media and the culture.

After the removal of the media or culture, the glasswares were soaked in Trix solution in dish for overnight. Then these were finally cleared with a brush and rinsed in water. These were dried in the sun or left on the laboratory bench for drying. The pipettes were washed by distilled water and same manner as in the case of other glass wares.

D. Sterilization

Sterilization is necessary to remove the unexpected microbes from the culture medium. To remove the microbes, all glasswares, culture media, and other requirments are necessary for sterilization. The sterilization procedures which were followed in this study are given bellow:

a) Sterilization by direct heat.

Inoculating needles, wire loops, scaples were sterilized by flaming on spirit lamp or after deeping them in the methylated spirit and flaming on the sprit lamp.

b) Sterilization by moist heat.

Before sterilization test tubes, flasks with or with out media were plugged with nonabsorbent cotton wool. The back side of the pipettes and petridished were wraped with a packing paper, before introducing in the autoclaves.

Firstly, the water level in the autoclave was checked and if necessary, water was poured to make the water level are to the mark. The prepared materials were put in the autoclave and the lid of the autoclave was filted by tightening the screw of the lid. Then the switch was placed on the on position. When the air inside the autoclave began to pass through the gas outlet in the form of water vapours, then the screw of the outlet was tightened. Gradually the temperature raised up to 120°C and the pressure of 15 lbs/sq. inch. At this situation the autoclave was left for 15 minutes. After

this, the suitch of the autoclave was put off. When the temperature and presure of the autoclave fell down at `O' Position as shown by the indicate needle, the out let of the nozzle was opened for releasing the pressure. Then the lid of the autoclave was made open and the materials were taken out. These were left of the laboratory table for colling and for use in the experiments.

E. Sowing of bacteria

Three petridishes, each with 0.5 ml of sample suspension were used for inoculating 12 -15 molten Nutrient agar and Potato Dextrose Agar medium at 40-45°C. Twenty plates were prepared from four different dilutions. Inoculated petridishes were incubated at 37°C temperature for 48 hours.

F. Isolation of bacterial colonies

During the incubation period, the bacteria multiplied and formed colonies. Individual colonies from the petridishes which form clearly spaced were isolated with the help of sterile wire loop and isolated colonies were transferred in fresh test tubes containing solidified Nutrient agar medium slants. The slants were then incubated at 37°C temperature for 48 hours.

G. Storing of bacterial slants culture

The slants culture tubes were than placed in the polyethylene bags, properly tied and stored as stock culture at 10°C. Occasional subculturing (3/4 weeks) was maintained to kept the culture in active condition.

H. Preliminary selection of pectinolytic bacteria

We are highly interested to with the pectinolytic bacteria. The bacteria which are able to grow in Echandi broth medium containing pectin as carbon source, were separated from stock and preserved.

I. Final selection

Presence of cellulolytic activity deteriorate the jute fibre quality. So, the primery selected bacteria were grown on Czapek's broth medium containing 1-6% of cellulose as carbon source. The bacteria which can not grow on cellulose medium were finally selected so that in jute retting the fibres are not damaged or destroyed.

5. SCREENING OF THREE BACTERIA FOR PECTINOLYTIC ACTIVITY.

Three bacterial strains were screened for their pectinolytic activity. For the *in vitro* production of pectinase by the isolates, the Echandi broth medium was used. The medium was dispensed at the rate of 50 ml in each 100 ml conical flask and were sterilized in autoclave at 120°C and 151b/ sq. inch pressure for 10-20 minutes. After cooling the flasks were incubated at 37°C for 48 hours. Then the culture media were filtered through whatman No-1 filter paper. The filtrates were. centrifuged at 6,000 r. p. m. in a refrigerated centrifuge (Model H-502 A type) for 30 minute. The supernatant was collected and used as crude enzyme. The enzyme was stored at 4°C with few drops of toluene to avoid bacterial contamination.

A. Pectinase enzyme action on potato discs maceration.

The technique used introduced by Brown (brown, 1915). For this purpose thin potato discs of standard dimension (1 mm thick, 2 mm diam.) were placed in enzyme solution and incubate at 37°C for one hour. Pectinolytic activity was estimated by determining coherence of potato discs with the help of dissecting needles. The results was expressed using a scale of maceration range from + (just) to ++++(complete) maceration. Cells in the tissue remain bound together mostly with the middle lamella made of pectic substances. If the pectinase enzymes can work on these pectic substancas the cells are loosely separated and lost coherence. The tissue maceration method of studying pectic enzymes is based on this principle.

B. Determination of Enzyme pectinase activity

The pectinase activity was measured following the method of Mohadevan and sridhar (1982). Apple pectin was used as substrate (1.0%) in 100 mm sodium Phosphate buffer, pH 7.5 pectinase activity was measured by detecting the release of reducing sugars from the used substrate. The amount of reducing sugar released in pectinase assay after incubation was measured spectrophotometrically with dinitrosalicylic acid (DNS) using Miller method (Miller, 1972). Pectinase activity was calculated and expressed by amount of glucose released/ml of enzyme extract/ unit time.

Reagents:

a) Sodium phosphate buffer pH 7.5.

b) 1.0% of pectin solution:- 1.0 gm of Apple pectin was dissolved in 100 ml of 100 mM sodium phosphate buffer (pH 7.5), keeping in a blender. blend for 3-5 minutes at low speed. Stair the content with a glass rod and again blend at high speed for 2-3 minutes; Filter through whatnan No-1 filter paper.

c) Dinitrosalicylic acid (DNS): Simultaneously dissolved 1.0 gm of dinitrosalicylic acid, 200 mg of crystal phenol and 50 mg of sodium sulphite and placed in a beaker with 100 ml of 1% solution of NaOH by stirring. The reagent was stored in a stoppered bottle at 4°C . The reagent deteriorates during storage due to atmospheric oxidation of the sulphite present. When required to store, reagent was prepared with out sulphite. However, in case of storing sulphite was not added to save this from deteriotion. In this case sulphite was freshly added before use.

d) 40% Solution of Rochelle salt (Sodium potassium tartrate).

Measurement:

Four ml of Apple pectin suspension (1.0%) was taken in a test tube. Then 1 ml of sodium phosphate buffer pH 7.5 and 2 ml of enzyme was added. The tubes were incubated at 37°C in three sets for 15,16, and 20 minutes, respectively. After incubation, 3 ml oliqoit of the extract was taken

into a test tube and 3 ml DNS reagent was added. The mixture was incubated for 3 - 5 minutes in a boiling water bath. After the colour have developed, 1 ml of 40 percent Rochelle salt was added when the contents of the tubes are still warm. The contents in the tubes were cooled under a running tap water in room temperature and the amount of reducing sugar was a calculated using standard preparation of glucose. A control experiment was also run at the same time.

6. PROCEDURE OF RETTING OF JUTE STEM BASAL PORTION BY PECTINOLYTIC BACTERIAL CULTURES AT FARMES' LEVEL.

Corchorus Olitorius (Tossa) jute plants at floowering stage and *C. Capsularis* (White) at seed-cut crop age were used for field trials.

A. Bacterial culture:

This contained pectinolytic bacteria which were capable of utilizing pectin, the binding materials of jute fibre as a source of carbon. These bacteria were able to ret jute stem under laboratory condition.

B. Multiplication of mother culture:

Bacterial culture were multiplied at farmers' level in plastic containers with capasity of about 20 litres. 20-25 pieces of stems of green jute plants of 3 inches long were mixed in 20 litres of tube wel water and heated to boiling temperature for an hour. After cooling at room temperature (28-30°C), the

mother culture was added and incubated for 7 days at room temperature. The bacterial culture produced in this container was shaken and diluted 1-2 times with clean tubewell water and were poured in a vat containg water. These were throughly stirred. The basal portions of jute stems were put in the vat for soaking with bacterial culture.

C. Inoculation of bacteria in jute.

The basal portions (approximately six inch) of green jute plants after 4-5 days of harvesting when the stems are defoliated, were slightly hammered by a wooden hammer. This was necessary for good absorption of bacterial culture. Thus prepared basal portions of jute were soaked in bacterial culture for 5-10 minutes for inoculation of bacteria. Then these were taken out from the bacterial culture vat. After an hour these jute were again put in bacterial culture vat. In this way third treatment was given so that bacteria were fully inoculated in the jute plants. After this the stems were made bundles. These jute bundles were put under 6 inch water for retting for 13-17 days. After completation of retting, fibres from culture treated and untreated jute plants were extracted, washed in water and dried under sunlight.

7. FIBRE QUALITY ASSESSMENT AFTER RETTING.

Fibres obtained from bacteria inoculated and uninoculated jute were examined and tested for quality assessment for cutting, reed length, strength, fineness, colour etc. Cuttings, reed length and colour was determined by

visual observation. Fibre strength and fineness was determined by 'Pressly Bundals Strength Tester', kissokey, Japan in the Physics Division of the laboratory of Bangladesh Jute Research Institute, Dhaka.

A. Statistical analysis of cuttings and reed length of culture treated and untreated jute samples.

The biometrical technique of analysis developed by Mather (1949) based on mathematical model of Fisher *et. al* (1932) was followed. Assessment for cuttings from treated and untreated retting jute of 100 stem fibre samples at random, was made. Mean value of each 20 cutting was taken and in such a way 5 mean values were found from 100 stem. Measurement of total reed length from treated plants from 9 bundles of 3 mean values were taken and the mean lenght of reed was found out. Similarly, the measurement of total reed length and mean length were also taken from untreated plants of 9 bundles.

Analysis of variance

For two-way:

$$F(\text{Treatment}) = \frac{\text{Treatment MS}}{\text{Error MS}}$$

$$F\ (\text{Replication}) = \frac{\text{Replication MS}}{\text{Error MS}}$$

Where, F = Variance ratio

MS = Mean square

$$MS = \frac{SS}{df}$$

Where, MS = Mean square

SS = Sum of square

df = Degree of freedom

Total $SS = \Sigma x^2 - CF$

Where, CF = Correction factor

X = Value per variable

$$\text{Treatment SS} = \frac{\Sigma x^2}{N} - CF$$

Where, X = Summation of per treatment for all replication.

Where, N = Number of replications

Cf = Carrection factor

$$\text{Replication SS} = \frac{\Sigma x^2}{N} - Cf$$

Where, X = Summation per replication for all treated samples.

N = Number of treatments

CF = Correction factor

Error SS = Total SS - (Treatment SS+ Replication SS)

$$CF = \frac{(\Sigma x)^2}{N}$$

Where, X = Variable

N = Number of Variables.

Total df = Total No. of variable - 1

Treatment df = Total No. of Treatement - 1

Replication df = Total No. of replication - 1

Errror df = Treatment df x Replication df

B. Grading of the culture treated and untreated jute fibre.

Bangladesh Standard Testing Institute (B.S.T.I.) presented a grading system which was used by Bangladesh Jute Research Institute, was followed in our studies for grading of jute fibres. Depending on the qualities of fibres, namely, (I) Strength of fibre (ii) Smothness. (iii) amount of cuttings present (iv) cleanness, (v) length etc. The Raw jute fibres are graded as follows:

(1) Top ; (2) Middle ; (3)B - bottom ; (4) C - bottom and (5) Cross - bottom.

This method is also is vogue in local market to determine the price of jute as per qualities by Jute Inspectors.

DESCRIPTIONS OF ROW TOSSA AND DESHI (WHITE) JUTE FIBRE ON THEIR GRADES:

DESHI (WHITE) JUTE:

1) Deshi (White) Top:

Very strong and long fibre. white or creamy white colour fibre, with glaze, very clean fibre without any defect and have not more than 20% cuttings by weight. When the cuttings are discarded from the total length of fibre, it is called the pucca class jute fibre or Bangladeshi special jute.

2) Deshi (White) Middle:

Strong and long fibre. Light creame colour to creame colour with glaze and without any defects in fibre and cuttings not more than 20% by weight. When the cuttings are discarded from the total length of fibre, it is called the pucca class Bangladeshi 'A' class jute.

3) Deshi (White) B - bottom:

Strong and long fibre. Light ash or light cream to straw colour with glaze but without any defect in fibre and have not more than 25% cuttings by weight. When the cuttings are discarded from the total length of fibre, it is called the pucca classes Bangladeshi B - bottom jute.

4) Deshi (White) C - bottom:

Generally strong and long fibre. It may be any colour but in general glaz in apperence, clean fibres and without any defect. The amount of cuttings shall not be more than 33% by weight. When the cuttings are discarded, it is called pucca class Bangladesh C - bottom jute.

5) Deshi (White) Cross - bottom:

This may be of any colour and of any strength. These may be dried back or dirt or defect in fibre. The amount of cuttings shall not be more than 40%. After discarding the cutting, these fibre are called in pucca Class Bangladeshi - D jute.

TOSSA JUTE:

1) Tossa Top:

Very strong and long fibre, golden to redish golden in colour. very glazy, clean and defectless fibre, not more than 15% cuttings by weight. In pucca Classification, this is called Bangladesh Tossa special.

2) Tossa Middle.

Strong and long fibre. Light golden to ash colour fibre, clean. Very glazy and defecless fibre, not more than 15% cuttings by weight. In pucca Class jute, it is called Bangladesh Tossa - A jute.

3) Tossa B - bottom:

Strong and long fibre, redish or Ash- redish to copper colour fibre, glazy and completely defectless and cuttings not more than 20% by weight. In Pucca class jute, it is called Bangladesh Tossa - B jute.

4) Tossa C- bottom:

Generally strong fibre and may be of any colour, defectless clean fibre and not more than 20% cuttings by weight. In pucca class jute, it is called Bangladesh Tossa - C jute.

5) Tossa Cross - bottom:

Fibres may be of any strength or of any colour, may be with some dried bark or pieces of jute stick and with not more than 25% cuttings by weight. This in Pucca Jute Classification is known as Bangladesh Tossa - D or E jute.

8. MORPHOLOGICAL STUDIES FOR THE IDENTIFICATION OF THE THRE PECTINOLYTIC BACTERIAL STRAINS:

With a view to identify the selected strains the following morphological characters were studied.

A. NON-MICROSCOPIC:

Agar colony:

The bacterial colonies grown on plating medium were studied for their morphological characters such as size, shape, edge elevation, opacity, colour, weather grown inside, at the bottom or on the surface of the medium and rate of the growth.

Agar slant:

The modes of bacterial growth on slants such as rhizoidal, spreading, adherent or slimy etc. were studied.

Potato slant:

Potato digestion rate, nature of growth and pigmentation were studied.

Motility test:

Mannitol motility medium was used for the determination of motility of bacterial cells. Stabbing inoculation on incubation shows rhizoidal growth of cells are motile.

Broth culture:

Production of turbidity, sedimentation and surface growth (Flocculent, Ring, Pellicle and membranous) in nutrient broth was observed and noted.

B. MICROSCOPIC:

The sizes and shapes of the vegetative cells were determined. The arrangement of the cells whether present singly. in chains or in clusters, were also observed. Sporulation characters were studied. The length and breadth of cells and spores were measured by standardizing the ocular micrometer by means of a stage micrometer.

Preparations for light microscopic examination:

a. Hanging drop method:

Hanging drop preparations were made by placing a drop of the bacterial suspension on a cover slip and inverting it over the concave area of a hollow ground slide (Pelczer and Ried 1965). By this method organisms were studied in a living motile condition suspended in water.

b. Fixed stained smears:

The techniques was used for the observation of the morphological characters of bacteria.

For this purpose much importance was given to the slides. For good staining, slide should be extremely clean.

c. Cleaning of the slide:

New slides were rubbed with a piece of clean grease free cloth and then washed with sodium carbonate followed by washing with tap water. The sleds were then immersed in chromic acid which was prepared by dissolving 10 gms of potassium dichromate in 100 ml of concentrated sulfuric acid. They were kept for 3 to 4 days. Then the slides were taken out

and washed with tap water. followed by boiling with sodium carbonate. Finally after washing them with distilled water they were kept ready for use in a solution of 95% ethanol acidified with concentrated HCl when using, a slide was taken out, dried in air and then heated over a spirit lamp. The slide was then allowed to cool and was taken on a filter paper keeping the heated surface on the upside.

PREPARATION OF FIXED STAINED SMEAR:

i. Preparation of the smear:

Bacteria were grown on nutrient agar slants for 48 hours. A portion of bacterial culture taken out by a sterilized loop was suspended in sterilized distilled water. The suspension was made sufficiently dilute. A drop of the suspension was taken on a slide and a very thin film was made which was allowed to dry in air. This method was followed in almost all types of staining except flagella staining, where a slightly different method was used.

ii. fixation of the smear: The smear was fixed by slightly heating the slide over a spirit lamp.

iii) Application of stains: The fixed smears were stained by three different methods.

(a) Simple staining (b) Differential staining (gram, spore and acid fast staining), (c) Flagella staining.

(a) Simple staining:

For this purpose 5% aqueous solution of basic stains such as methylene blue, crystal violet, mercurochrome etc. were used.

(b) 1. Gram staining:

This is one of the most important and widely used differential staining technique. Here Hucker and Conn's (1923) modified method was followed. The fixed smear was treated with ammonium oxalate crystal violet solution for 30 seconds (Frobisher. 1957). This was gently rinsed off and an iodine solution was applied for 30 seconds. This in term was drained off. Ethyl alcohol, (95%) was then applied for 20 seconds to decolonize the stain. Finally saffranine was used as a counter stain for 10 seconds. Then the slide

was gently rinsed off with water and blotted dry. The result was recorded as gram positive and gram negative.

(b) 2. Acid fast staining:

Some bacteria specially the genus Mycobacterium have an abundance of a particular waxy material in the cell, so that once they get stained, retain the dye even when decolourized with acid alcohol. These organisms are known as acid fast organisms. If not they are termed as non acid fast.For the acid fast staining, Zeihl- Neelson method was followed (1885) In using this stain, a dried and fixed smear was flooded with a solution of carbol fuchsi and heated to steaming over a hot copper plate for five minutes. After washing the excess dye. the smear was treated for five minutes (Frobisher. 1957) with cold 95% alcohol containing 5 to 10% HCI. Methylene blue was than applied as a counter stain.

(b). 3. Spore staining:

Cauklin's (1934) modification of Wiretz's (1908) method was followed for spore staining.

Here 15 to 24 hours old culture was used. The fixed scear was flooded with 5% aquous solution of malachite green and heated to steaming for about 10 to 15 minutes. Boiling was strictly avoided. When necessary malachite green was added from time to time. The excess dye was then washed off and 5% aqueous solution of mercurochrome was then applied as a counter stain for about one and half minute. The slide was washed, dried and examined. Morphology of the spore and sporangia were studied under 100 x 10x using oil. The results were recorded.

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* [Zeihl- Neelson method (1983) = In; principles of Bacteriology by Brayan, 1962.]
* [Wiretz's (1908) = In; plant science formulae by McLean, R.C.and Cook, W.R.I., 1965, MacMillan and Co. Ltd., London.]

9. PHYSIOLOGICAL STUDIES FOR THE IDENTIFICATION OF THE THREE PECTINOLYTIC BACTERIAL STRAINS:

The following studies were made on the physiological activities of the organisms.

1. Catalase reaction:

The enzyme catalase is capable of decomposing hydrogen peroxide into water and molecular oxygen.

For this purpose, nutrient broth tubes were inoculated with 48 hours old culture and incubated at 37°C for 48 hours.

After incubation one ml of hydrogen peroxide solution was added to each of the tubes. production of bubbles indicated the positive result.

2. Deep glucose agar test:

With the help of this test organisms can be classified with relation to free oxygen as strict aerobes, facultative anaerobes, microaerophile and strict anaerobes.

For this test a tube of deep agar medium (8 to 9 ml column of medium containing 2.6% glucose) was inoculated while in fluid condition at 45°C.

The tube was rotated to mix the inoculum with the medium and then the inoculated medium was transferred into another sterile tube to ensure through mixing. After incubation at 37°C for 48 hours strict aerobes were found to grow on the surface and in the upper layer of the medium, microaerophile grew a few millimeters below the surface, facultative anaerobes grew through out the medium and strict anaerobes grew deeper in the medium.

3. Glucose broth:

Glucose broth tubes were inoculated and incubated for 48 hours. Growth on the surface, formation of turbidity or sedimentation were observed.

4. Indole test:

Indole is produced by the action of microbes on the amino acid tryptophane. For indole test the following method was followed.

Tubes of tryptophane broth in duplicates were inoculated with 48 hours nutrient broth cultures. In one set of the tubes the oxalic acid test paper stripes were suspended from the mouths of the tubes were then

incubated for two days at 37°C. After incubation, Kovac's solution was added only to the tubes which did not contain the oxalic acid paper. The tubes were shaken vigorously for one minutes.

Turning of oxalic acid paper into pink in the test tubes of the first set and deep cherry red colour in the tubes of the second set indicated the formation of indole.

Kovac's solution:

Para-dimethyl-amino-benzaldehyde	5 gms.
Butyl alcohol	75 ml.
Hydrochloric acid (37%)	25 ml.

Para-dimethyl-amino-benzaldehyde was first dissolved in the butyl alcohol and then hydrochloric acid was added.

5. Test for nitrate:

Nitrate in reduced to nitrite by certain microorganisms proving that they contain the enzyme nitrate reductase. The following reagent were required for this test.

Reagent:

A. Sulfanilic acid	8.0 gms.
5N acetic acid	1.0 litre.

(1 part chemically pure acetic acid to 2.5 parts distilled water).

Sulfanilic acid dissolved in acetic acid and stored in brown glass bottle.

B. Dimethyl a-napthalamine	6.0 ml.
5N acetic acid	1.0 litre.

Stored in brown bottle.

C. Zinc dust.

48 hours old culture were used to inoculate the nitrate broth tubes (ISP 8); then incubated at 37°C for 2 days. After incubation a few drops of solution and equal volume of solution B was added and shaken well. Formation of red to pink colour indicates the reduction of nitrate to nitrite.

A small piece of Zn dust added to the tubes where solution A & B was already added by remaining nitrate (in case) would be reduced to nitrite resulting red to pink colour.

6. Growth in synthetic media:

Synthetic medium was inoculated with very small amount of 48 hours old culture and incubated for 2 days at 37°C. Frequent observation of the tubes were made. Appearance of turbidity showed the utilization of synthetic medium.

7. Inorganic salt test:

Inorganic salt medium was inoculated and allowed to incubate at 37°C for 2 days. Growth showing turbidity indicated utilization of inorganic salt.

8. Citrate utilization:

Citrate medium was inoculated with a very small amount of 48 hours old culture and incubated at 37°C for 48 hours. Appearance of turbidity indicated the utilization of citrate.

9. Gelatin Liquefaction:

Many organisms posses the enzyme gelatinase which is capable of hydrolysing gelatin. As a result it loose the property to gell. This test indicates the presence or absence of the enzyme gelatinase in the tested organism.

Duplicate tubes of gelatin medium were inoculated with 48 hours old culture and incubated at 37°C for 48 hours. After incubation, the experimental tubes along with control tubes were kept in the refrigerator. In a few minutes the medium in the control tube become solidified. Solidification of the inoculated tubes indicated the absence of the enzyme gelatinase in that organism. The liquification of the inoculated tubes indicated the hydrolysis of gelatin and hence the presence of enzyme gelatinase.

10. Production of hydrogen sulphide:

The properties of hydrogen sulphide to give sulphite of lead (black in colour) with lead acetate has been utilized in the demonstration of its production in the peptone iron agar medium (ISP VI) (Tresner & Danga-1958).

Three inches long filter paper strips were soaked in lead acetate solution and then dried. Peptone iron agar medium was inoculated and lead acetate paper was introduced in each of the tubes by holding them between the plug and the wall of the test tube. The tubes were then incubated at 37°C. The production of hydrogen sulphide was indicated by blackening of the load acetate paper.

11. Urease test:

Urea medium was inoculated with 48 hours old culture for 2 days. After incubation at 37°C for 3 days, a glass rod moistened with dilute HCl was inserted into each test tube. The white fumes and the smell of ammonia indicated the formation of ammonia and hence the presence of the enzyme urease in the organisms.

12. Proteolysis test:

This test indicated the presence or absence of the enzyme proteinase in the organisms. This enzyme is capable of hydrolysis of protein.

For the test, coagulated egg albumen in nutrient broth was inoculated and incubated at 37°C for 6-8 days. Disintergation of coagulated egg while during growth, indicated proteolytic activity.

13. Casein Hydrolysis:

One ml of sterilized milk was taken in a sterilized petridish. Molten milk agar medium was poured in to the petridish, mixed thoroughly and allowed to solidify. Then inoculated by streak method and incubated at 37°C for 48 hours. A clear transparent zone along with the streak indicated the hydrolysis of casein.

14. Starch hydrolysis:

Organisms capable of hydrolysing starch to maltose the enzyme amylase. By this test the presence or absence of this enzyme in the organisms can be detected. For the test, starch agar plates were inoculated by streak method, incubated at 37°C for 48 hours. After the growth iodine solution was added to each of the plate.

Development of clear white to bluish brown colour indicated the complete and partial hydrolysis of the starch respectively, hence the presence

of enzyme amylase. Development of deep blue colour indicated that starch had not been hydrolysed.

15. Voges-Proskaur test (V.P. test):

V.P. medium was inoculated with 48 hours old culture and incubated at 37°C for 48 hours. After growth 3 ml naphthol solution was added to each of the test tubes followed by one ml of potassium hydroxide-creatine solution.

a Naphthol solution:

a Naphthol	5 gms.
Ethanol (95%)	100 c.c.

Potassium Hydroxide Creatine solution:

KOH	40 gms.
Creatine	300 mgm.
Distilled water	100 c.c.

The tubes were then shaken vigorously for 1 to 2 minutes. Appearance of a crimson ruby colour in the indicated the positive result.

16. Methyl red reaction:

V.P. medium was inoculated and incubated at 37°C for 48 hours. After incubation a few drops of methyl red solution were added in each tube. A distinct red colour indicated methyl red positive and yellow colour indicated methyl red negative.

Methyl red solution:

Methyl red	100 mgm.
Ethanol (95%)	300 ml.
Distilled water	200 ml.

17. Nitrogen utilization:

Micro-organisms are selective; some prefer free N_2, some NO_3, some NO_2 and some NH_4 etc. Glucose Asparagine medium with 0.1% of nitrogen combined in different nitrogen sources (KNO_2, KNO_3 and NH_4SO_4) was included with 48 hours old culture and incubated at 37°C for 48 hours. Frequent observation was made. Appearance of turbidity indicated the utilization of nitrogen sources that compared with the control and among the nitrogen sources.

18. Temperature tolerance:

To find out the maximum, minimum and optimum temperature for growth, the nutrient broth and nutrient agar plates inoculated (Plates by streak method) with 48 hours old culture and were allowed to grow at different temperature. Then their growth in different temperature were compared with the control.

19. pH requirments:

To find out the maximum, minimum and optimum pH for growth of the selected pectinolytic bacteria, Echandi broth medium was adjusted from pH 5-10 by adding 0.1N HCl and 0.1N NaOH.

20. Salt tolerance:

Nutrient agar slant containing different concentration of sodium chloride were inoculated and incubated at 37°C for 48 hours. The growth at different concentration of NaCl were then compared with the control.

21. The fermentation test:

Fermentation test is of considerable significance in the identification and classification of bacteria. The microorganisms differ in their ability to ferment different carbohydrates. Some of them, upon fermentation of carbohydrates produce both acid and gas, some produce only acid, no gas; some produces only alkali and still others which can not ferment carbohydrate at all. In this test following carbohydrates and sugar alcohol were used.

Monosaccharides:

i) Pentoses: Arabinose, Rhamnnose, Xylose.

ii) Hexoses: Glucose, Fructose, Galactose.

Disaccharides: Source, Lactose.

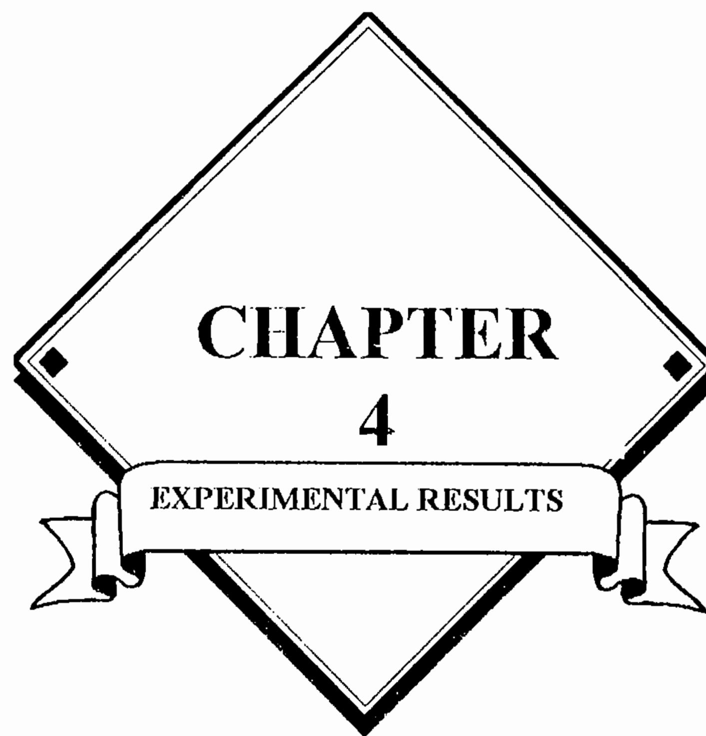
Polysaccharides: Raffinose, Inulin, Cellulose, Starch.

Sugar alcohol: Mannitol, Glycerol.

To perform these tests Bromothymol blue was used as indicator. One Durham tube was used in each of the fermentation tubes. The tubes were

then inocubated in duplicates with 48 hours old culture, incubated at 37°C for 48 hours.

The change of colour of indicator from colourless or yellow indicated production of acid. Blue colour indicated alkalinity. Carbondioxide if form would be collected in the Durham tubes.


CHAPTER
4
EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

Jute cuttings are hard and coarse. They may be of use after chemical treatment but are expensive. Therefore pretreatment of basal portions with proper bacteria before retting the jute was thought to be economic, maintaining the tensile strength of fibre intact. Therefore experiments were designed to search for pectin degrading bacteria from retting stems in field conditions.

1. COLLECTION OF RETTING SAMPLES OF JUTE:

In the present study on pectinolytic bacteria sixtyfour samples were collected from different areas of North Bengal in the districts of Rajshahi and Pabna (Table-1)

2. ISOLATION OF BACTERIA:

Collected samples were examined and bacteria were isolated for identification of any bacterium causing pectin degradation. Different media were used for screening of bacteria which were isolated from retting jute. Fourteen colonies were isolated to test them for pectinolytic activity (Table-2)

3. PRELIMINARY SELECTION OF PECTINOLYTIC BACTERIA:

In order to examine pectinolytic activities, 14 isolated bacterial strains were cultured and grown on Echandi medium containing 1% pectin as a source of carbon at pH 7.5 and at 37°C in a rotary water bath. Among the 14 strains,

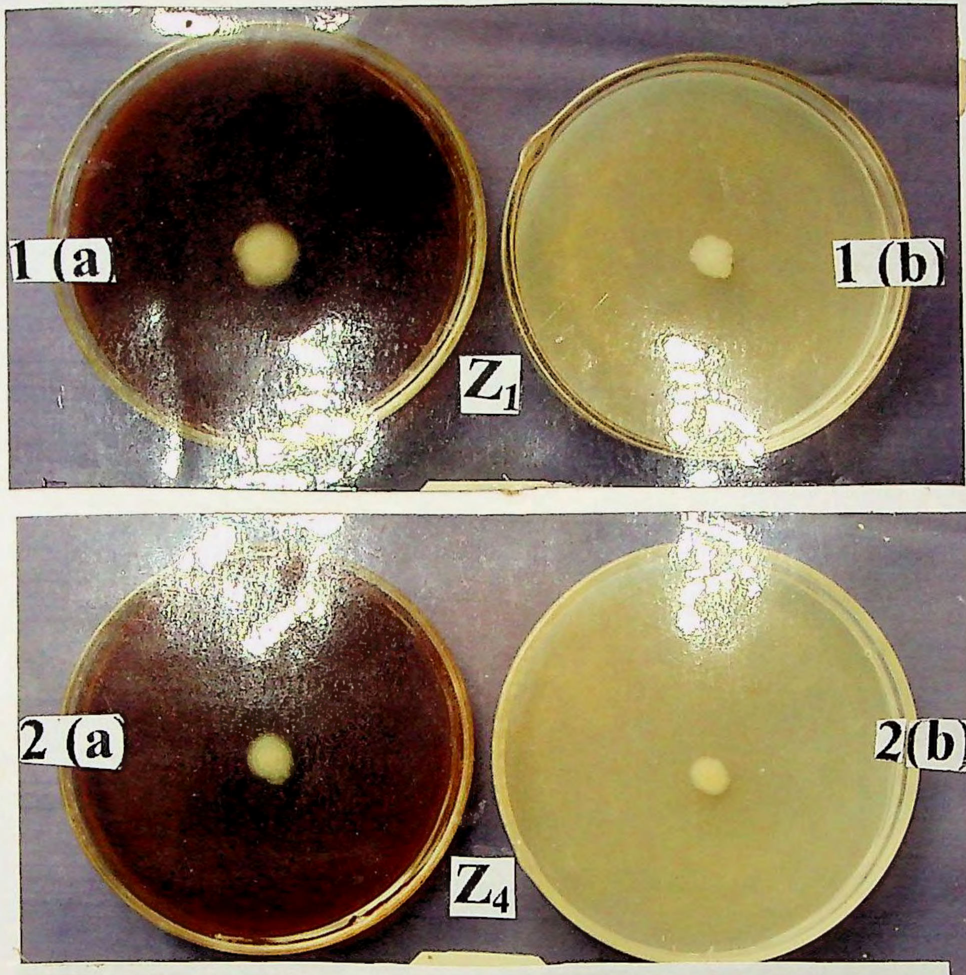
only three bacterial strains were found to be highly pectin degrading and therefore they were primarily selected for further studies (see Table-2).

4. FINAL SELECTION OF PECTINOLYTIC BACTERIA:

For final selection three primarily selected strains were tested for cellulose utilizing ability, because the presence of cellulolytic activity deteriorates the fibre quality. For these purpose three bacterial strains were grown in Czapeck's medium containing 1.6% of cellulose, at pH 7.5 and at 37°C. The bacterial strains Z_1 ,Z_4 and Z_{30} did not show any cellulolytic activity. Therefore these were finally selected for further studies (Table-3).

5. GROWTH STUDY OF THREE SELECTED PECTINOLYTIC BACTERIA:

Finally selected 3 bacterial strains Z_1, Z_4 and Z_{30} were grown on different media for the study of growth in colony diameter. For these purpose, Nutrient agar and Echandi agar media containing pectin were used. Bacterial strains were plated on both the media at pH 7.5 and incubated at 37°C for 48 hours. It was found that the growth and colony diameter were more in apple pectin medium than on Nutrient medium. In apple pectin medium, colony diameter ranged from 11-15mm whereas in Nutrient agar medium bacterial colony diameter ranged only from 7-9mm (Table-4, photograph - II).



Photograph II :
Colony diameter of bacterial strain Z_1 (***Micrococcus lylae***).
1(a) in Echandi medium.
1(b) in Nutrient Agar medium.

Colony diameter of bacterial strain Z_4 (***Micrococcus luteus***).
2(a) in Echandi medium.
2(b) in Nutrient Agar medium.

6. GROWTH STUDY OF PECTINOLYTIC BACTERIA IN ECHANDI LIQUID MEDIUM WITH DIFFERENT CONCENTRATION OF PECTIN.

In order to find out maximum growth of pectinolytic bacteria on Echandi broth medium, experiments on pectin concentration were made using 1-4% of pectin in Echandi broth medium. The medium pH was 7.5 and incubation was done at 37°C for 48 hours. The bacterial strain Z_1 was found to produce heavy growth in 4% of pectin in the medium. The bacterial strain Z_4 was found to produce moderate growth in 4% of pectin. Where as the strain Z_{30} produced desire growth in 2% of pectin (Table-5).

7. MACERATION OF POTATO DISCS AND PECTINOLYTIC ACTIVITY BY PECTINOLYTIC BACTERIAL STRAINS.

The three bacterial strains were allowed to grow in Echandi medium for the determination of pectinolytic activity. The culture supernatant of the three bacterial Strains Z_1 Z_4 and Z_{30} were tested for enzyme activities on potato discs. All the three bacterial strains macerated potato discs within an hour of incubation at 37°C and therefore showed pectinolytic activity (Table -6). Pectinase activity in O. D. of three bacterial strains is also shown in Table-6. Strain Z_1 showed maximum pectinolytic activity of 75µg /ml/ minute, Z_4 and Z_{30} showed a moderate activity of 58 and 49 µg / ml / minute, respectively. But in a control experiment there was no pectinolytic activity (Table -6) .

Table 1. Collection of retting samples of jute.

Retted jute samples	Variety	Place of collection	Date of Collection
1-13	Tossa *(C. olitorius)*	Chatmohor, Pabna	August' 92
14-30	White *(C. capsularis)*	Chatmohor Pabna	August' 92
31-45	Tossa *(C. olitorius)*	Bhangoora, Pabna	August' 93
46-50	Tossa (*C. olitorius*)	Jamalpur Village, Rajshahi	September' 93
5-9-64	Tossa (*C. olitorius*)	University campus, Rajshahi	September' 93

Table 2. Preliminary selection of pectinolytic bacteria.

Serial No	Strains isolated with isolation number	Growth in Nutrient medium	Growth in Pectin medium
1.	Z_1	++	++++
2.	Z_2	++++	-
3.	Z_3	+++	+
4.	Z_4	++	+++
5.	Z_5	+++	++
6.	Z_{14}	+++	+++
7.	Z_{20}	+++	+++
8.	Z_{23}	+++	+
9.	Z_{24}	++++	-
10.	Z_{27}	++++	-
11.	Z_{28}	+++	++
12.	Z_{30}	++	+++
13.	Z_{34}	+++	+
14.	Z_{35}	++++	-

Note: - = No growth,

+ =Scanty growth, ++= Small growth

+++= Moderate growth ++++= Heavy growth.

Table 3. Test results for cellulolytic activity.

Strains	Growth in pectin medium	Growth in cellulose medium
Z_1	++++	-
Z_4	+++	-
Z_{30}	+++	-

Note : - = No growth.

+++ = Moderate growth, ++++ = Heavy growth.

Table 4. Growth of bacteria in colony diameter in mm on different media.

Strains	Growth in diameter in mm on Nutrient agar.	Growth in diameter on Echandi medium (1.0% of apple pectin)
Z_1	9.0 mm	15.0 mm
Z_4	7.0 mm	12.0 mm
Z_{30}	8.0 mm	11.0 mm

Table 5. Effect of pectin concentration on the growth of bacteria.

Strains	Pectin concentration			
	1%	2%	3%	4%
Z_1	+	++	+++	++++
Z_4	+	++	++	+++
Z_{30}	++	++++	+++	++

Note: + = Scanty growth, ++ = small growth

+++ = Moderate growth, ++++ = heavy growth.

Table 6. Maceration of potato discs and pectinolytic activity by pectinolytic bacterial strains.

Strains	Potato discs maceration (one hour incubation) at 37°C	Pectinase activity µg/ml/min measured in O.D.
Z_1	++++	75
Z_4	+++	58
Z_{30}	+++	49
Control	–	0

Note :+++ = moderately macerated.

++++ = completely macerated.

8. A FIELD OBSERVATION OF JUTE RETTING.

A field study was taken about jute retting condition available at the farmers' level in Pabna. The retting water pH range was found to be form 6.5-7.9 and control water (ditch) pH ranged from 6.9-7.6. Jute bundle temperature at retting condition was found to be range from 29-32°C and control (air) temperature ranged from 30-34°C in July-August (Table-7).

9. JUTE RETTING AT FARMERS' LEVEL WITH PECTINOLYTIC BACTERIAL CULTURE.

At farmers' level, jute retting was conducted in the village Kalkati near Bhangoora in the district of Pabna in north Bengal. The specific three retting bacteria Z_1, Z_4 and Z_{30} were added as inoculum in the retting tank. After 4-5 days of harvesting, the green jute plants were defoliated. Then the basal portions of green, jute plants were slightly hammered and soaked with pectinolytic bacterial culture for 2-3 times with in 24 hours and then dipped in tank water. It was found that in this treatment the retting of jute was uniform from the basal to top portion. The upgradation of jute quality was due to substantial reduced of cuttings in addition of further improvement of reed length, tensile strength and colour due to treatment with single culture of Z_1 and mixed culture of Z_1,Z_4 and Z_{30} (Table-8,9; photograph III, IV, V & VI).

Photograph III :
1. Tossa Jute plants after defoliation.
2. White Jute plants after defoliation.

Photograph IV :

1. Hammering of Jute basals.
2. Soaking in bacterial culture.
3. Jute retting in JAK .
4. Separation of fibres from Jute.

Table 7. A Field observation of jute retting condition available at the farmers' level in Pabna.

Field	Retting ditches No.	Retting water pH	Retting bundle temperature (°C)	Air temperature
Chatmohor, Pabna	1	6.68	29°C	31°C
Do	2	7.90	31°C	34°C
Do	3	6.50	30°C	33°C
Do	4	7.92	31°C	35°C
Do	5	7.29	32°C	33°C
Do	6	7.29	32°C	34°C
Do	7	7.20	30°C	31°C
Bhangoora, Pabna	8	6.65	29°C	33°C
Do	9	7.45	30°C	33°C
Do	10	7.9	30°C	31°C
		Average pH 7.27	Average Temperature30.4°C	Average Temperature32.7°C

* Control (ditch) water pH range 6.95 - 7.60 Average pH = 7.27

Table 8. Effect of inoculation at basal portions of green jute plants with single bacterial culture of Z_1 and mixed bacterial culture of Z_1, Z_4 and Z_{30} at fermers' level.

Location of the trial	Jute	Age of the plants	Treatment	Retting Period (day)	Retting type (top to bottom)
village- Kalkati Thana - Bhangoora Dist.- Pabna North Bengal	Tossa (*C. olitorius*)	Flowering stage	Control (without treatment)	13	Non-uniform with 1.72 inch coarse bottom
	Do	Do	Single culture of Z_1	Do	Uniform with 0.4 inch coarse bottom
	Do	Do	Mixed culture of Z_1, Z_4 and Z_{30}	Do	Uniform with 0.33 inch coarse bottom
	White (*C. capsularis*)	Seed-cut	Control (without treatment)	17	Non-uniform with 5.8 inch coarse bottom
	Do	Do	Single culture of Z_1	Do	Uniform with 2.0 inch coarse bottom

Photograph V :
Pectinolytic bacterial culture treated and untreated Tossa Jute produced at farmers' level.
T_1 = After single bacterial culture (Z_1) treatment.
T_2 = After mixed bacterial culture ($Z_1 + Z_4 + Z_{30}$) treatment.
Control = Without treatment.

Photograph VI :
Pectinolytic bacterial culture treated and untreated White Jute produced at farmers' level.
W_1 = After single bacterial culture (Z_1) treatment.
Control = Without treatment.

Table 9. Effect of inoculation of green jute plants (basal portion) with three pectinolytic bacterial culture on the quality of jute. (for details see appendix I)

Jute	Treatment	Quality of jute fiber					Grade
		Cuttings (inches)	Reed length (feet)	Tensile strength (g/tex)	Fineness (width in microns)	Fibre colour	
C. olitorius (Tossa)	Control (without treatment)	1.72	8.58	56.40	46.71	Reddish ash	Tossa B-Bottom
Do	Single culture of Z_1	0.40	9.27	52.99	45.09	Light golden	Tossa Middle
Do	Mixed culture of Z_1, Z_4 and Z_{30}	0.33	9.40	52.11	45.54	Light golden	Tossa Middle
C. capsularis (White)	Control (without treatment)	5.80	7.50	40.75	49.46	Light ash to straw	Deshi C-Bottom
Do	Single culture of Z_1	2.00	8.63	43.28	43.46	Light creamy white	Deshi Middle

10. FIBRE QUALITY ASSESMENT AFTER RETTING.

A. Amount of cuttings after treatment with bacterial culture.

In *C. olitorius* (Tossa), the amount of cuttings obtained by single culture treatment was 0.4 inch and that of mixed culture treated samples was 0.33 inch. Cuttings from control (untreated) samples was 1.72 inch. In *C. capsularis*, the amount of cuttings by treated single bacterial culture of Z_1 was 2.0 inch. Whereas cuttings from control (untreated) samples were 5.8 inch (Table-9).

B. Amount of reed length after bacterial culture treatment and untreated jute.

In *C. olitorius* (Tossa), the amount of reed length by treated single culture of Z_1 was 9.27 feet. After treatment with mixed culture of Z_1, Z_4 and Z_{30}, the reed length was 9.40 feet. In control (untreated) the reed length was found to be 8.58 feet. In *C. capsularis* (White jute), the amount of reed length after treatment with single culture of Z_1, the reed length was 8.63 feet. In control (untreated) samples, the reed length was found to be 7.5 feet (Table 9).

C. Analysis of variance for jute cuttings

Analysis of varience for cuttings obtained after bacterial treatment in case of *C. olitorius* was calculated. The varience ratio for treatment was found highly significant (at 0.01 level), whereas that for application was non-significant indicating a big difference from the bacterial treatment (Table - 10).

Further the result revealed that the treatment significantly improved the retting of jute basal portion in *C. oliorius.*

Analysis of variance for cuttings obtained after bacterial treatment in case of ***C. capsularis*** was also calculated. The variance ratio for treatment was also found highly significant (at 0.1 level), whereas that replication was not significant (Table -11). The results indicated that bacterial pre-treatment significantly improved the retting of jute basal region also in *C. capsularis,* thereby retaining no or little cuttings.

D. Analysis of variance of reed length.

Analysis of variance for reed length obtained after bacterial treatment in case of ***C. olitorius*** was calculated. The variance ratio for treatment was found highly significant (at 0.01 level), whereas that for replication was not significant indicating a big difference from the bacterial treatment. Further the result revealed that the treatment significantly improved the retting of jute from basal to top portion and increase the reed length of *C. olitorius* (Table -12).

Analysis of variance for reed length obtained after bacterial treatment in case of ***C. capsularis*** was also calculated. The variance ratio for treatment was also found significant (at 0.01 level), whereas that for replication was non significant indicating a difference from the bacterial treatment. Further the result revealed that the treatment significantly improved the retting of jute from basal to top portion and increase the reed length of *C. capsularis* (Table - 13).

Table 10. Two way analysis of variance for cuttings of *C. olitorius* after bacterial treatment and without treatment.

Analysis of variance

Sources of variation	df.	S.S	M.S.	F
Treatment	2	6.1866	3.09	618.66**
Replication	4	0.016	0.004	0.80^{NS}
Error	8	0.043	0.005	
Total	14	2.245		

NS = Non siguni facant, ** = Significant at 0.01 level.

Table 11. Two way analysis of variance for cuttings of *C.capsularis* after bacteral treatment and without treatment.

Analysis of variance

Sources of variation	df.	S.S	M.S.	F
Treatment	1	36.1	36.1	2062.86***
Replication	4	0.09	0.0225	1.2857^{NS}
Error	4	0.07	0.0175	
Total	9	36.26		

NS = Non significant, *** = Significant at 0.01 level.

Table 12. Two way analysis of variance for reed length of *C. olitorius* after bacterial treatment and without treatment.

Analysis of variance

Sources of variation	df.	S.S.	M.S.	F
Treatment	2	2.3749	1.1875	36.6512**
Replication	2	0.0017	0.00085	0.026^{NS}
Error	4	0.0594	0.0324	
Total	8			

NS = Non-significant, ** = Significant at 0.01 level.

Table 13. Two way analysis of variance for reed length of *C. capsularis* after bacterial treatment and without treatment.

Analysis of variance

Sources of variation	df.	S.S.	M.S.	F
Treatment	1	2.34	2.34	26.00*
Replication	2	0.03	0.015	0.17^{NS}
Error	2	0.19	0.09	
Total	5	2.56		

NS = Non-significant, * = Significant at 0.01 level.

E. Determination of tensile strength of the bacterial treated and untreated jute fibre.

In *C. olitorius* (Tossa), the tensile strength of single culture of Z_1 treated fibre was 52.99 g/tex. After treatment with mixed culture of Z_1, Z_4 and Z_{30}, the tensile strength of fibre was 52.11 g/tex. In control (untreated) fibre strength was 56.40 g/tex indicating a little harmful effect on the fibre in pre-treated samples (Table - 9).

In *C. capsularis* (White), the tensile strength of single culture of Z_1 treated fibre was 43.28 g/tex.Control (untreated) samples fibre strength was 40.75 g/tex indicating increased the tensile strength in the bacterial culture treatment (Table - 9).

F. Determination of finenesss of the bacterial treated and untreated jute fibre.

In *C. olitorius* **(Tossa)**, the fineness of single culture of Z_1 treated fibre was 45.09 width in microns. The fineness of mixed culture of Z_1, Z_4 and Z_{30} treated fibre was 45.54 width in microns. In contral (untreated) fibre fineness was 46.71 width in microns indicating an improvement of fibre in the bacterial treatment (Table - 9).

In *C. capsularis* **(White)**, the fineness of single culture of Z_1 treated fibre was 43.46 width in microns. In control (untreated) fibre fineness was 49.46 width in microns indicating a big difference from the bacterial treatment. Further the result revealed that the bacterial treatment improved the fineness of jute fibre (Table -9).

G. Fibre colour of bacterial culture treated and untreated jute.

In *C. olitorius* (Tossa), the fibre colour of single culture of Z_1 treated jute was light golden and bright. The fibre colour of mixed culture of Z_1, Z_4 and Z_{30} treated jute was light golden and brigth. In control (untreated) jute fibre colour was reddish ash indicating a great difference from the bacterial treatment (Table -9, photograph -V). Further the result revealed that the treatment improved the fibre colour in *C. olitorius.*

In *C. capsularis* (White), the fibre colour of single culture Z_1 treated jute was light creany white. In control (untreated) jute fibre colour was light ash to straw colour indicating a big difference from the bacterial treatment (Table-9, photograph - VI). Further the result revealed that the treatment improved colour in *C. capsularis.*

H. Grading of bacterial culture treated and untreated jute fibre.

In *C. olitorius* (Tossa), the fibre grade of single culture of Z_1 treated jute was Tossa Middle. The fibre grade of mixed culture of Z_1, Z_4 and Z_{30} treated jute was Tossa Middle. In control (untreated) jute fibre grade was Tossa B-Bottom indicating a difference from the bacterial treatment (Table -9). Further the result revealed that the treatment upgrade the jute fibre in *C. olitorius.*

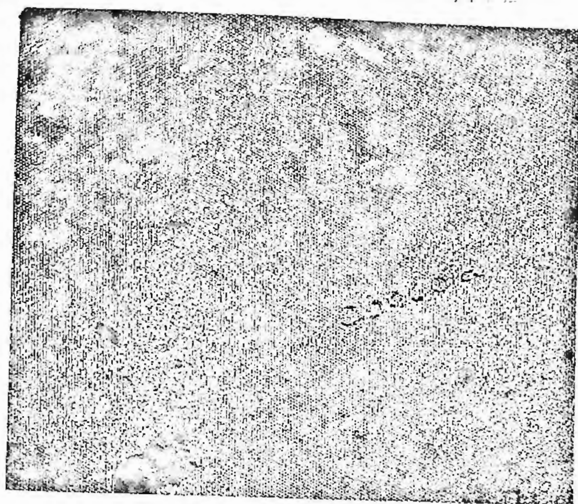
In *C. capsularis* (White), the fibre grade of single culture treated of Z_1 jute was Deshi Middle. In control (untreated) jute fibre grade was Deshi C - Bottom indicating a difference from the bacterial treatment (Table -9). Further the result revealed that the treatment upgrade the jute fibre in *C. capsularis.*

An attempt was made to identify the 3 finally selected pectinolytic bacterial strains Z_1, Z_4 and Z_{30} on the basis of their morphological, cultural and biochemical characters.

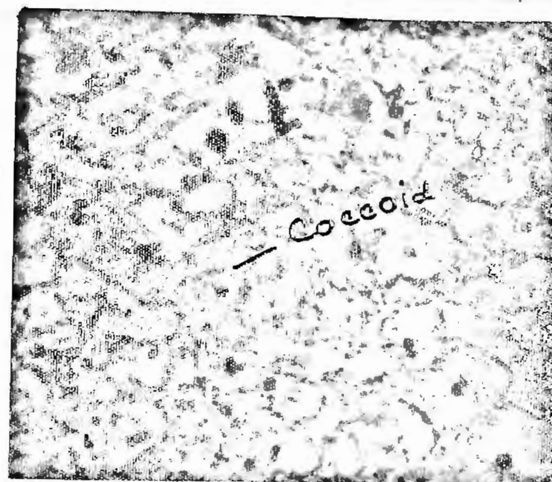
Among the 3 bacterial strains, The strain Z_1 was identified as ***Micrococcus lylae***. The strain Z_4 was identified as ***Micrococcus luteus*** and the strain Z_{30} was identified as ***Microbacterium leavaniformans***. The detail results of study (identifying features) is given bellow:-

11. CHARACTERIZATION OF THE THREE PECTINOLYTIC BACTERIAL STRAINS.

1.	Strain No	:	$\mathbf{Z_1}$
	Place of collection	:	Kajla, Rajshahi
	Vegetative cells	:	Coccoid, 0.5-2.0 μm in diameter (Photograph VII)
	Spores	:	Non spore farmer
	Gram stain	:	Gram positive
	Acid first strain	:	Not acid first
	Motality test	:	Non motile
	Agar colonies	:	Circular to irregular
	Agar slant	:	Echinulate growth, cream white



Z_1



Z_1

Photograph VII :
Vegetative cells of strain Z_1 (***Micrococcus lylae***) under 1250X power of light Microscope.

Starch agar plate	: Hydrolysed (moderately)								
Acetyl methyl carbinol	: Negative								
Methyl red	: Negative								
Growth in	KNO_3	KNO_2	$(NH_4)_2SO_4$						
	++	+	++						
Growth at :	0°C	10°C	20°C	30°C	32-37°C	40°C	45°C	50°C	
	-	+	++	+++	++++	++	+	-	
Growth at pH (5-10)	5	6	7	8	9	10			
	-	++	+++	++++	++	+			
Growth at salt conc.(%)	Nosalt	1	2	3	4	5	6	7	
	+++	+++	+++	++	++	+	+	–	

Fermentation test : **Acid and gas form:** Arabinose, Glucose, Starch, Fructose, Sucrose, Galactose, Xylose, Raffinose, Mannitol, Inulin.

Acid and no gas form : Rhammnose

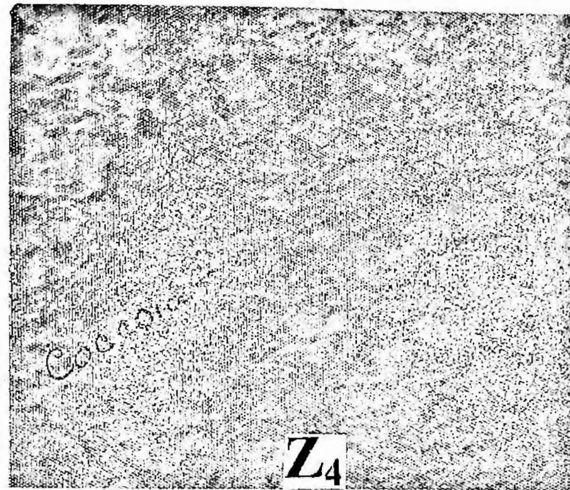
Alkali form : Lactose, Glycerol, cellulose

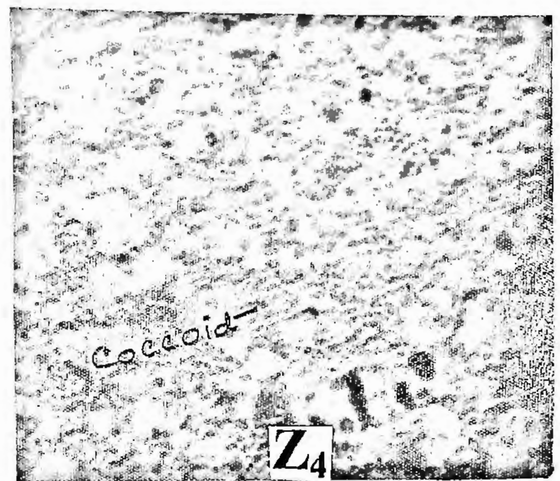
Identification : Provisionally identified as ***Micrococcus lylae.*** It differed with described strain from growth in citrate medium and glycerol fermentation

Note : – =No growth, + =Scanty growth, ++ = Small growth, +++ = Moderate growth, ++++ = Heavy growth.

2. Strain No. : Z_4

Place of collection	:	Kajla, Rajshahi
Vegetative cells	:	Coccoid, Size : 0.5-1.90 in diameter (Photograph VIII)
Spores	:	Non spore former
Gram stain	:	Gram positive
Acid fast stain	:	Not acid fast
Motility test	:	Non motile
Agar slant	:	Filiform growth
Nutrient broth	:	Turbid growth with little sedimentation
Glucose broth	:	Heavy flocculant growth with little sedimentation
Asparagine broth	:	Sediment growth
Potato slant	:	Moderate growth
Catalase activity	:	Catalase present (moderately)
Deep glucose agar	:	Turbid growth
Oxygen relationship	:	Aerobic
Indole test	:	Not produced
Nitrate test	:	Nitrate produced
Synthetic medium	:	Sediment growth
Inorganic salt medium	:	Not growth
Citrate medium	:	Membranous growth
Gelatine liquefaction	:	Liquefied (weakly)
Hydrogen sulphide	:	Produced (weakly)

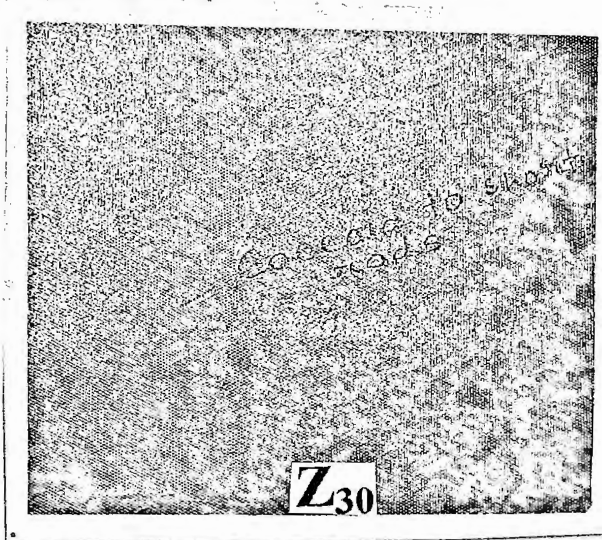




Photograph VIII :
Vegetative cells strain Z_4 (***Micrococcus luteus***) under 1250 X power of light Microscope.

3.

Strain No	:	Z_{30}
Place of collection	:	Chatmohor, Paban.
Vegetative cells	:	Coccoid to short rod, some times 'V'form. Size:-0.4-0.75 μm in diameter and 1.0-3.8 μm in length (Photograph IX).
Spores	:	Non spore former.
Gram stain	:	Gram positive
Acid fast stain	:	Not acid fast.
Motality test	:	Non motile
Agar colonies	:	Circular, light yellow coloured.
Agar slant	:	Echinulate growth.
Nutrient broth	:	Pellicle growth with little sedimentation.
Glucose broth	:	Moderate pellicle growth.
Asparagine broth	:	Turbid growth with little sedimentation.
Potato slant	:	Moderate growth.
catalase activity	:	Catalase Present
Deep glucose agar	:	Heavy surface growth.
Oxygen relationship	:	Aerobic
Indole test	:	Not produced.
Nitrate test	:	Not produced.
Synthetic medium	:	Moderate sediment growth.
Inorganic medium	:	Scanty growth
Citrate medium	:	Membranous growth with little sedimentation.



Photograph IX :
Vegetative cells of strain Z_{30} (***Microbacterium laevaniformans***) under 1250X power of light Microscope.

Gelatin liquefaction	:	Liquefied
Hydrogen sulphide	:	Produced (weakly)
Urease	:	Ammonia produced (Weakly)
Coagulated egg albumen	:	Proteolysed (moderately)
Milk agar plate	:	Hydrolysed (moderately)
Starch agar plate	:	Hydrolysed (moderately)
Methyl red	:	Negative.

Growth :

KNO3	KNO2	(NH4)2SO4
++	+	+

Growth at :

0°	10°C	20°C	30°C	32-37°C	40°C	45°	50°C
-	+	++	+++	++++	++	+	-

Growth at pH (5-10) :

5	6	7	8	9	10
+	++	++++	+++	++	+

Growth at salt cone (%) :

No salt	1	2	3	4	5	6	7
+++	+++	++	++	+	+	+	-

Fermentation test : Acid and gas form: Glucose, Fructose, Sucrose, Xylose, Raffinose, Inuline and Manitol.

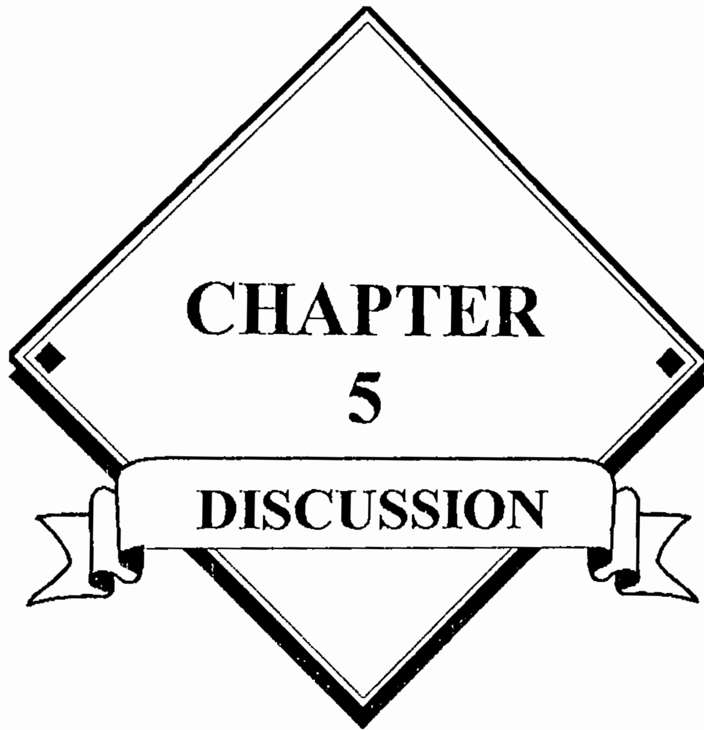
Acid and no gas form: Rhammnose, starch.

Alkali for: Arabinose, Lactose, Glycerol and Cellulose.

Identification

Provisionally identified as

Microbacterium laevaniformans. It differed with described strain from urease production


CHAPTER
5
DISCUSSION

DISCUSSION

In the course of a search for pectinolytic bacteria, sixtyfour retting samples of jute were collected from different retting heaps in tanks at different localities of North Bengal in the districts of Rajshahi and Pabna. Collected samples were studied and bacteria were isolated by plating them on selective media for identification of any bacteria causing pectin degradation. Throughout the study fourteen bacterial strains were isolated. These isolated strains were screened for pectin degradation. Among the fourteen strains only three bacterial strains were found to be highly pectin degrading. These three strains were primarily selected for further studies. The presence of cellulolytic activity of bacteria deteriorates fibre quality. So, three primary selected strains were tested for cellulose utilizing ability. The bacterial strains Z_1, Z_4 and Z_{30} did not show any cellulolytic activity. Therefore these were finally selected for further studies in order to use them for jute retting.

All these three selected bacterial strains were tested for their morphological, cultural, biochemical and colony pigmentation. On the basis of these characters, they were compared with the standard description in the Bergey's Mannual of Systematic Bacteriology Vol-2, 1986. The strain Z_1 was found to be closely related to the ***Micrococcus lylae***. The strain Z_4 was found to be closely related to the ***Micrococcus luteus*** and the strain Z_{30} was found to be closely related to ***Microbacterium laevaniformans***. But all

of them differed from the standard in some biochemical and cultural characters like glycerol fermentation and growth in citrate medium and others (see page 69, 71 & 73).

When the finally selected three bacterial strains Z_1, Z_4 and Z_{30} were grown on different media for growth study in colony diameter it was found that the growth and colony diameter was more in apple pectin than on Nutrient medium. When they were grown on Echandi broth medium with different pectin concentration (from 1-4%) it was observed that the strain Z_1 was found to produce high growth in 4% pectin in the medium, whereas the strain Z_4 was found moderate in growth on 4% pectin. The strain Z_{30} produced poor growth in 4% pectin but incredibly produced high growth in 2% pectin. High concentration of pectin seems to be toxic to this strain.

Degradation of pectin materials is a complex process requiring participation by a number of enzymes. Comparative study of enzymes of the three bacterial strains Z_1, Z_4 and Z_{30} were tested for pectinolytic activity. All the strains macerated potato discs and showed pectinolytic activity. Among the tree strains the strain Z_1 completely macerated the potato discs which were in accordance with findings of other workers (Mohiuddin ***et al.***, 1978). Comparative study of pectinase production in O.D. by the three bacterials strains showed that the strain Z_1 imparted maximum pectinolytic activity of 75µg/ml/minute. The strain Z_4 and Z_{30} showed a moderate activity of 58 and 49µg/ml/minute, respectively.

A very important phase of jute production is retting. But in conventional retting practices basal portion of overmatured jute plant remain

underretted resulting in hard and stiff fibre (Debsharma,1946; Bose,1966). This impart low grading of jute fibre (Mohiuddin, 1978). Research work was undertaken by many people on evolving effective method of jute retting (Ahmed, 1963; Alam, 1970; Rahmatullah *et al.*,1977). Although many retting bacteria have been isolated at the laboratories of Bangladesh Jute Reseach Institute and Dhaka University where retting was done in sterile water in test tubes in 3-8 days reducing the normal retting time of 15-20 days, it has not yet been possible to use them in the farmers field retting in non-sterile water. Bacteria which ret in sterile water cannot ret in non-sterile water are unable to compete with other microbes of the retting tank in field condition (Ali, 1989).

Recently different workers tried to upgrade jute retting by using different methods with non-sterile water at the farmers' level to improve the jute cuttings(basal hard portion). Basak *et al* (1988) sprayed bacterial culture suspension on the basal portion (about 2 feet) of jute plants along with 0.5% urea applied on basal portion of the jute plants before placing them for JAK in the retting water. Paul *et al.*,(1987) hammered green jute basal portions and soaked them in 5% urea solution before placing them in retting tanks.

In our experiments first the jute stems were defoliated. Then the basal portions were gently hammered and soaked in pectinolytic bacterial suspensions in locally made earthen "CHARIS" called vats for 5-10 minutes. Then these were put in JAK in normal water retting tank. Results of trial jute retting at farmers' level by pectinolytic bacterial culture conducted in village Kalkati, in the district of Pabna have been presented in Table-9. It was observed that in the pectinolytic bacterial culture treated samples, retting was uniform from the base to the top portion.

In the case of *C. olitorius* (Tossa) by single culture treatment jute fibre was improved by one grade from **Tossa B-bottom** to **Tossa middle** and by mixed culture treatment the fibre quality was improved but not above the grade as obtained in single culture treatment. The upgradation over the untreated sample was due to great reduction in cuttings (unretted or partially retted basal portions), increase the reed length, in addition to further improvement of fineness and colour due to treatment with single and mixed bacterial culture. But it was found that in both the single and mixed bacterial culture treatment, fibre strength was little deteriorated. It was observed that in single culture of Z_1 treatment, cuttings was reduced from 1.72 to 0.4 inch and in mixed culture of Z_1, Z_4 and Z_{30} treatment jute cuttings was reduced from 1.72 to 0.33 inch. These reduction are statistically highly significant as compared to the untreated control. The reed length was found to be increased from 8.58 to 9.27 feet by single culture treatment and in mixed culture treatment it was found to be increased from 8.58 to 9.40 feet. These results are also statistically highly significant. However, fibre strength, fineness, and colour were found to be similar both in single and mixed culture treatment. From the above results, it may be calculated that mixed culture treatments are superior to single culture treatments. This is conformed with those of Paul *et al.* (1987).

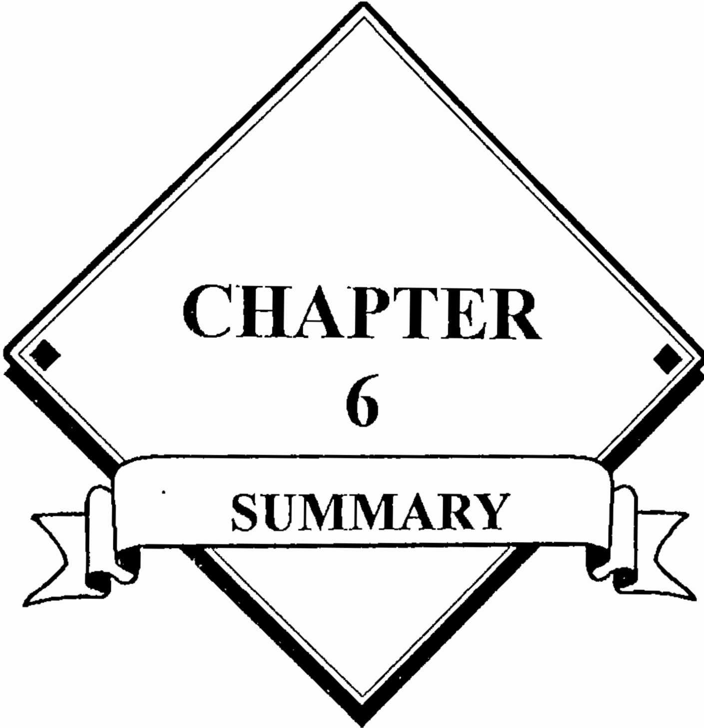
In case of *C. capsularis* (White) by treatment with single culture of Z_1, jute fibre was improved by two grades from **Deshi-C-Bottom** to **Deshi Middle**. The upgradation over the untreated samples was due to great reduction of cuttings (from 5.8-2.0 inch) and increase of reed length(from 7.5-8.63feet), these results are statistically significant. In addition, there was

further improvement of tensile strength (from 40.75-43.28g/tex), fineness (from49.46-43.46 width in microns) and colour (from light ash to creamy white). Therefore, from our observation and studies the pretreatment of basal portions with pectinolytic bacterial cultures, the upgradation of jute cuttings resulted in the improvement in jute qualities at farmers' level.

We get nearly 6.0" (six) inches to one foot cuttings in Deshi and only one to two inches cuttings in Tossa variety. Therefore cutting problem is more acute in **Deshi** (White) jute than in **Tossa** jute.

It is easier to hammer the basal portions (The place of occurrence of cuttings) of Deshi jute because of their comparatively soft nature, whereas the basal portion of Tossa jute are stiff and thereby hard to hammer and take more time and in doing so the fibres get disrupted. In case of Deshi jute, mild hammering is sufficient for the absorption of bacterial suspension for retting purpose and the cuttings are almost removed after retting, whereas in Tossa jute the cuttings are not completely removed and the strength of fibres is lost due to hard hammering.

Therefore it is more beneficial to apply the method of pre-soaking technique in Deshi jute basals with pectinolytic bacteria in order to make them cutting free than those of Tossa jute basals from the commercial point of view.


CHAPTER
6
SUMMARY

SUMMARY

A search was made for jute retting bacteria in North Bengal districts of Rajshahi and Pabna. Best pectin degrading bacteria were thought to reduce cutting in mature and in seed bearing jute plants. Keeping this in mind collections of samples were made from jute retting tanks in field conditions. These samples were analysed in the laboratory and fourteen colonies of diferent types on their growth and morphological characteristics were selected and cultured in different media such as, Nutrient agar, Potato dextrose agar (PDA) and Echandi medium with different concentrations of pectin. Not only the pectinolytic activity, non-cellulolytic activities of jute retting bacteria were also considered for selection because cellulolytic activity could damage the fibre conditions.

Out of the fourteen initially selected bacterial colonies from jute retting samples, only three were finally selected on the basis of high pectinolytic and non-cellulolytic activities. These were named by Z_1 , Z_4 and Z_{30} after the name of the author, Zulficquer Zaman. These were also called bacterial strains. All of them grew well in Apple pectin medium.

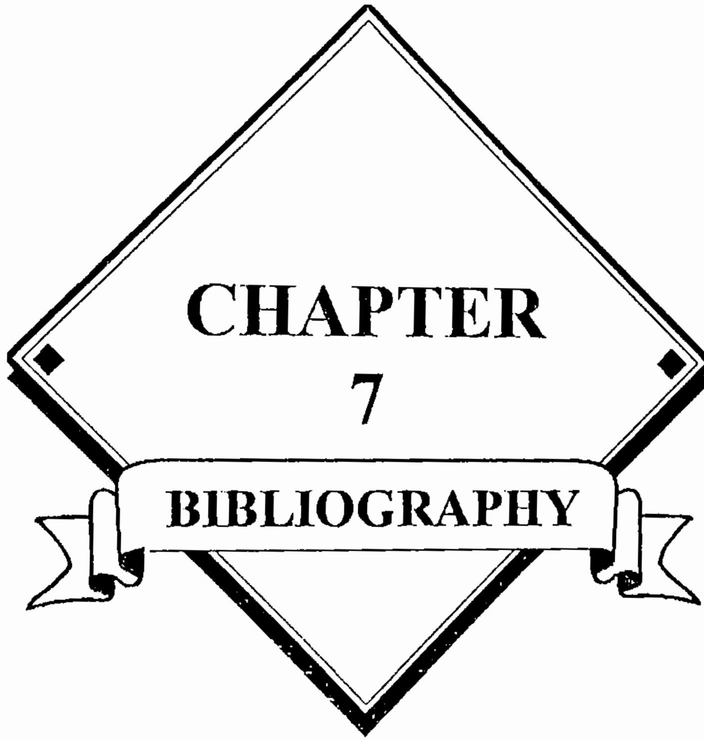
The bacterial strains Z_1 was found to be similar to ***Micrococcus lylae*** in morphological, physiological and biochemical characteristics. This strain was found to give high growth in 4% of pectin as compared to other two strains Z_4 and Z_{30}. The strain Z_4 and Z_{30} were found to be similar to ***Micrococcus luteus*** and ***Microbacterium laevaniformans***, respectively.

The strain Z_1 (identified as ***Micrococcus lylae***) was found to be highly pectinolytic as on potato disc maceration and in pectinase production of 75 µg/ml/min. compared to Z_4 (identified as ***Micrococcus luteus***) and Z_{30} (identified as ***Microbacterium laevaniformans***) in pectinase production of 58µg/ml/min. and 49 µg/ml/min. , respectively.

On field trial experiments at farmers' level pretreatment with jute retting pectinolytic bacteria on the basal portions removed jute cuttings to different grades from mature and seed bearing jute plants after retting. Therefore, fibre quality was upgraded. The range of upgradation was estimated and varied from one to two grades. Defoliated green jute both ***Corchorus olitorius*** and ***Corchorus capsularis*** when their basal portions were gently hammered with a wooden hammer and pre-soaked in pectinolytic bacterial suspensions prior to retting in field condition resulted in complete removal of cuttings from matured and seed bearing jute plants.

Mixed bacterial culture treatment showed better results than single culture prior to retting in field conditions in removing cuttings from the jute. Upgradation of jute fibre was found to be owing to reduction or complete removal of cuttings from 1.72 inches to 0.4 - 0.33 inches in ***Corchorus olitorius*** (Tossa) jute and from 5.8 inches to 2.0 inches in ***Corchorus capsularis*** (White , also called Deshi) jute.

Tensile strength, reed length, fineness and characteristic colour (from reddish to light golden in Tossa and from ash to creamy white in Deshi) were also found to improved significantly in pretreated samples than in control (untreated) experiments.


CHAPTER
7
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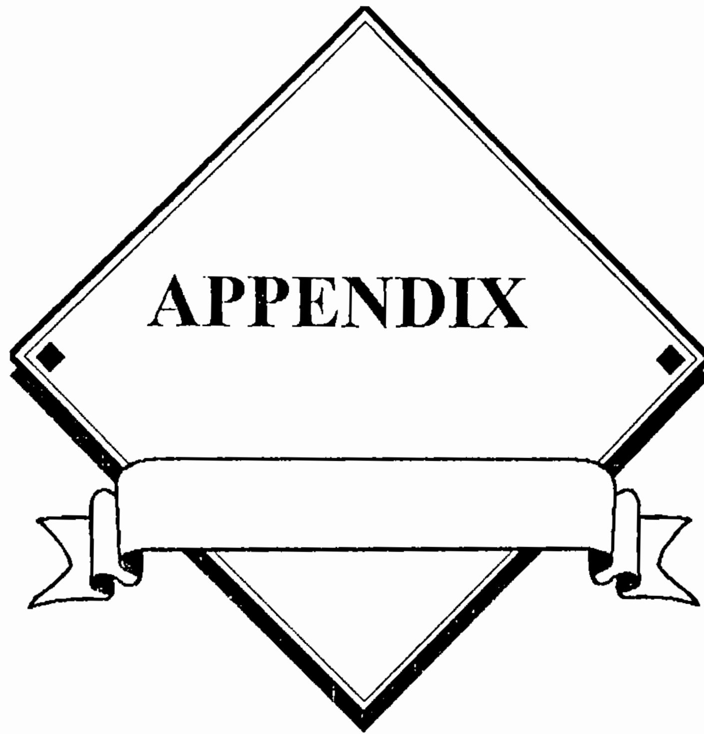
APPENDIX

APPENDIX

QUALITY OF JUTE FIBRE :

Table No. 14 Tensile strength (Gram per Tex)

Sample code	T_0	T_1	T_2	W_0	W_1
Bottom	$\bar{X}$ =57.4	$\bar{X}$ =50.44	$\bar{X}$ =51.62	$\bar{X}$ =41.70	$\bar{X}$ =40.55
C.V% Range	38.48=78.95	35.91=73.75	35.80=72.31	29.48=59.87	34.30=57.88
Middle	$\bar{X}$ =55.69	$\bar{X}$ =55.56	$\bar{X}$ =55.26	$\bar{X}$ =42.83	$\bar{X}$ =47.65
C.V% Range	36.98=83.44	31.08=82.70	39.29=76.49	37.70=48.94	31.36=54.77
Top	$\bar{X}$ =56.12	$\bar{X}$ =52.95	$\bar{X}$ =49.47	$\bar{X}$ =37.73	$\bar{X}$ =41.64
C.V% Range	34.95=87.31	35.38=67	37.03=58.64	25.46=53.06	32.43=56.60
Mean value Gram / Tex	56.40	52.99	52.11	40.75	43.28

Table No. 15 Fineness (width in microns)

Sample	Mean fibre width in mecrons	S.D	C.V%
T_o	46.71	10.35	22.16%
T_1	45.09	10.79	23.92%
T_2	45.54	4.37	9.61%
W_0	49.46	8.45	17.08%
W_1	43.46	3.17	7.29%

Note : To = Tossa control (Without treatment)
T_1 = Tossa treatment with single culture of Z_1
T_2 = Tossa treatment with mixed cilture of Z_1, Z_4 and Z_{30}
W_0= White control (without treatment)
W_1= White treatment with single culture of Z_1

COMPOSITIONS OF SOME OF THE MEDIA USED IN THIS COURSE OF WORK:

Citrate medium: Sodium ammonium phosphate - 1.5gm, Magnesium sulphate - 0.2gm, Sodium citrate - 3gm, Distilled water to 1000 ml.

Coagulated egg albumen : The white portion of hard boiled egg was cut into small pieces. One piece was introduced in each nutrient broth tubes and sterilize.

Czapek's medium: Ammonium oxalate -3gm, K_2HPO_4 -1gm, $MgSO_4,7H_2O$-0.05gm, CMC -16gm, Agar - 20gm, Distilled water to 1000ml.

Deep glucose agar : Beef extract - 3 gm, Peptone - 5gm, glucose - 2.5gm, Agar - 20gm, Distilled water to 1000ml.

Echandi medium : $MgSO_4$ -0.5gm, KH_2PO_4 - 0.5gm, K_2HPO_4 - 2.0gm, Asparagine - 2.5gm , Apple pectin-10gm (powder), Agar-15gm, Distilled water to 1000ml.

Glucose broth: Peptone - 5gm, Glucose - 5gm, Beef extrnct - 3gm, Distilled water to 1000ml.

Inorganic salt medium: Sodium nitrite - 1gm, Sodium carbonate - 1gm, Dipotassium phosphate - 5gm, Distilled water to 1000ml.

Motility medium : Peptone - 20 gm, Mannitol - 2 gm, KNO_3 - 1 gm, 1% phenol red solution - 4 ml, Agar - 4 gm, Distilled water to 1000 ml.

Nutrient Agar (NA): Beef extract - 3gm, Peptone - 5gm, NaCl - 1gm, Agar-16gm, Distilled to water 1000 ml.

Nutrient gelatins : Beef extract - 3gm, Peptone - 5gm, Gelatin - 150gm, Distilled water to 100ml.

Peptone iron agar medium (Test for H_2S): Peptone - 20gm, Ferric ammonium citrate - 0.5gm, K_2HPO_4 - 1gm, Sodium thiosulphate - 80mg, Agar - 20gm, Distilled water to 1000 ml.

Potato Dextrose Agar (PDA): Potato- 200gm, Dextrose - 18gm , Agar- 16gm Distilled water to 1000 ml .

Synthetic medium: Ammonium dihydrogen phosphate-1gm, KCI - 0.2gm, $MgSO_4$ - 0.2gm, Glueose - 10 gm, Distilled water to 1000 ml.

Tryptophane broth: Thyptophane-10 gm, Beef extract- 3 gm, Distilled water to 1000 ml.

THE BACTERIAL STAINS WERE PREPARED ACCORDING TO THE FORMULAE GIVEN BELOW :

1. **Gram stains** (Hucker's modification) :-

   Ammonium oxalate crystal violet : Crystal violet - 2 gm, Ethyl alcohol -20 ml, Ammonium oxalate -0.8 gm, Distilled water - 80 ml,

   Iodine solution : Iodine - 1gm, Potassium iodide- 2gm,Distilled water-300ml.

2. **Acid fast stain** : Zeihl's carbol fuchsin : Basic fuchsin - 3gm, Ethyl alcohol- 10ml, Phenol -5gm, Distilled water - 95 ml.

   Methylene blue solution: Methylene blue - 3 gm, Ethyl alcohol - 30 ml, dilute KOH -1000ml. (1: 10,000).

   Decolourizing agent: Ethyl alcohol -(95%)- 97 ml, conc.HCL- 3 ml.

3. **Spore strain** : Aq. malachite green : Malachite green- 5gm, Distilled water -100 ml.

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