ULTURE, MINISTRY OF AGRICULTURE

Y DEVELOPMENT, MINISTRY OF INTERIOR

OFFICE OF ACCELERATED RURAL DEVELOPMENT, OFFICE OF THE PRIME MINISTER

DEPARTMENT OF FOREIGN TRADE, MINISTRY OF ECONOMIC AFFAIRS

DEPARTMENT OF LAND DEVELOPMENT, MINISTRY OF NATIONAL DEVELOPMENT

KASETSART UNIVERSITY, OFFICE OF THE PRIME MINISTER

DEPARTMENT OF VOCATIONAL EDUCATION, MINISTRY OF EDUCATION

ROYAL FOREST DEPARTMENT, MINISTRY OF AGRICULTURE

ASIAN INSTITUTE FOR ECONOMIC DEVELOPMENT AND PLANNING, UNITED NATIONS

ECONOMIC COMMISSION FOR ASIA AND THE FAR EAST

UNITED STATES OPERATIONS MISSION TO THAILAND

CHECCHI AND COMPANY

THAI JUTE ASSOCIATION

APPLIED SCIENTIFIC RESEARCH CORPORATION OF THAILAND

COOPERATIVE RESEARCH PROGRAMME NO. 1

PRODUCTION, PROCESSING, AND UTILIZATION OF

KENAF AND ALLIED FIBRES

RESEARCH PROJECT NO. 1/11

MICROBIOLOGY OF KENAF RETTING

# REPORT NO. 2

# *BACILLUS SUBTILIS*, A POTENT KENAF RETTER

BY

SMARN VARDHANABHUTI

CHIRAPORN CHOUVALIT

VEERACHAI GRIVAPHAN

NAIYANA NIYOMWAN

ASRCT, BANGKOK 1969

not for publication

DEPARTMENT OF AGRICULTURE, MINISTRY OF AGRICULTURE
DEPARTMENT OF COMMUNITY DEVELOPMENT, MINISTRY OF INTERIOR
OFFICE OF ACCELERATED RURAL DEVELOPMENT, OFFICE OF THE PRIME MINISTER
DEPARTMENT OF FOREIGN TRADE, MINISTRY OF ECONOMIC AFFAIRS
DEPARTMENT OF LAND DEVELOPMENT, MINISTRY OF NATIONAL DEVELOPMENT
KASETSART UNIVERSITY, OFFICE OF THE PRIME MINISTER
DEPARTMENT OF VOCATIONAL EDUCATION, MINISTRY OF EDUCATION
ROYAL FOREST DEPARTMENT, MINISTRY OF AGRICULTURE
ASIAN INSTITUTE FOR ECONOMIC DEVELOPMENT AND PLANNING, UNITED NATIONS
ECONOMIC COMMISSION FOR ASIA AND THE FAR EAST
UNITED STATES OPERATIONS MISSION TO THAILAND
CHECCHI AND COMPANY
THAI JUTE ASSOCIATION
APPLIED SCIENTIFIC RESEARCH CORPORATION OF THAILAND

COOPERATIVE RESEARCH PROGRAMME NO. 1
PRODUCTION, PROCESSING, AND UTILIZATION OF
KENAF AND ALLIED FIBRES

RESEARCH PROJECT NO. 1/11
MICROBIOLOGY OF KENAF RETTING

# REPORT NO. 2
# *BACILLUS SUBTILIS*, A POTENT KENAF RETTER

BY
SMARN VARDHANABHUTI
CHIRAPORN CHOUVALIT
VEERACHAI GRIVAPHAN
NAIYANA NIYOMWAN

ASRCT, BANGKOK 1969
not for publication

# BACILLUS SUBTILIS, A POTENT KENAF RETTER

By Smarn Vardhanabhuti,* Chiraporn Chouvalit,*
Veerachai Grivaphan,* and Naiyana Niyomwan+

## SUMMARY

An aerobic bacterium isolated from retting kenaf bark was identified as Bacillus subtilis (Cohn 1872) practically identical with B. subtilis ATCC 6633. The two strains were tested and compared for their retting ability on sterilized fresh kenaf ribbon, fresh kenaf stem, dry kenaf ribbon, and dry kenaf stem. Both would ret well all the four types of kenaf materials. The local isolate was used in bacteriological retting of dry kenaf ribbon and also in bacteriological batching of retted kenaf fibres. It was found to be of some advantage in both cases.

## INTRODUCTION

It was reported earlier from this laboratory‡ that a strain of aerobic bacterium isolated from retting kenaf bark was found to possess quite marked kenaf retting ability. It would ret well sterilized fresh kenaf ribbon, fresh kenaf stem, dry kenaf ribbon, and dry kenaf stem. This micro-organism has now been identified and preliminary attempts were made for its utilization in kenaf industries. This report presents the results of these investigation.

---

* Chemical Technology Group, Technological Research Institute, ASRCT.

+ Physics and Engineering Group, Technological Research Institute, ASRCT.

‡ "Preliminary studies on microbial flora of kenaf retting" by Smarn Vardhanabhuti, Chiraporn Chouvalit, Naiyana Nopakun, and Pranee Jutasirivongse. Report No. 1 on Research Project No. 1/11 (Microbiology of kenaf retting). ASRCT unpublished report.

## MATERIALS AND METHODS

Microscopic morphology of the isolate was studied by Gram stain of smears from various cultures. Its aerobic growth characteristics were studied on nutrient agar (Difco), nutrient agar containing 4 per cent glucose, Simmon's citrate agar (Difco), Phenol red tartrate agar (Difco), in 0.1 per cent potassium nitrate in nutrient broth, and in 7 per cent sodium chloride in peptone (1 per cent) broth containing 4 per cent glucose. Its anaerobic growth was tested in dextrose broth (Difco) and in nutrient broth containing 0.1 per cent potassium nitrate and 0.1 per cent glucose, the pH of the broth being adjusted to 7.8 with 0.1 N sodium hydroxide. Anaerobic cultivation was carried out in Torbal anaerobic jar, Model AJ-2 (The Torsion Balance Co., Chicago) in an atmosphere of hydrogen. The motility was tested in motility test medium (Difco) as well as by microscopic observation. The abilities to produce indol and urease were tested in 1 per cent peptone broth and Christensen's urea agar (Christensen 1946) respectively. Smith's medium (Conn et al. 1957) and M.R.-V.P. medium (Difco) were used for the M.R.-V.P. reactions. Liquefaction of gelatin was tested in stab-culture in nutrient gelatin prepared from Oxoid gelatin, Batch No. 181, containing 0.3 per cent bactobeef extract (Difco) and 0.5 per cent bacto-peptone (Difco). Starch hydrolysis was tested in 4 per cent soluble starch containing Andraide's indicator, 1.0 per cent peptone, 0.5 per cent sodium chloride, and 0.3 per cent disodium phosphate. Hydrolysis of starch was indicated by acid production and failure to develop blue colour on addition of iodine solution. Fermentation studies were carried out in liquid media containing Andraide's indicator for acid production and Durham tube for the detection of gas produced. These studies included the fermentations of dextrose, maltose, mannite, saccharose, lactose, dulcite, xylose, arabinose, rhamnose, sorbitol, salicin, and adonitol. They were tested both in conventional medium and in medium with 0.1 per cent ammonium sulphate added.

Retting ability was tested by the method of Allen (1946) as modified by Lanigan (1951). Sterilized fresh kenaf ribbon, fresh kenaf stem, dry kenaf ribbon, and dry kenaf stem were used as the test materials. Tests were done in duplicate. Results were read after 10 days

of incubation at 35°C. Complete separation of fibres and complete disintegration of the bark was recorded as 4+, while ~~good~~ not quite complete separation of fibre, but ~~not quite~~ with complete disintegration of the bark as 3+, moderate separation of fibre and partial disintegration of the bark as 2+, and softening of the bark with minimal separation of the fibre as 1+. Bacillus subtilis ATCC 6633* was also concurrently studied in all the above tests.

Bacteriological retting of dry kenaf ribbon was carried out in aerobic retting tanks made of steel and lined with sealed plastic sheet. Each tank has a surface area of 0.60 m x 1.16 m. Two tanks were used, one for the control uninoculated ret, and the other for the inoculated ret. Six bundles of dry kenaf ribbon, each weighing 500 grammes were used in each tank with a ribbon-water ratio of 1:24 (wt./vol.). The kenaf ribbons were weighted down with bricks and with this ribbon-water ratio, there was a depth of about 6 cm of water above the top of the ribbons and a total depth of about 11 cm. Both tanks were heated with electric bulbs placed at the bottom of the tank, and the temperature was controlled by a thermostat to be between 34° and 35°C.

Tap water was used to soak the ribbons in each tank for one night. The following morning the water in each tank was siphoned off and fresh tap water was added to the original level.

For the inoculated ret, crude ammonium sulphate (fertilizer grade) was added to make 0.2 per cent (wt./vol.) and crude sodium sulphate (fertilizer grade) to make 0.05 per cent (wt./vol.). The pH was not adjusted, bacause after the addition of the salts it was found to be 7.2. Enough 18 hour-old culture of the isolate in potato-dextrose broth (made up of infusion from 200 grammes of potato, 20 grammes of dextrose, and water q.s. ad 1 litre) was added to make 0.5 per cent by volume inoculum. The retting liquor was not changed, but fresh water was added daily to replace the amounts lost by evaporation.

Retting liquor in the control tank was completely siphoned off every morning and fresh tap water was added up to the original level. Care was taken not to disturb the retting ribbons and to retain as much of the scums formed as possible.

---

* Obtained from U.S. FDA by Mrs. Sumana Vardhanabhuti.

The temperature of each retting tank was recorded daily. The pH of the retting liquor of the control tank was measured daily by a Beckman pH-meter, Model GS, both before and after changing of the water, while that of the inoculated tank was measured once every morning.

One bundle of retting fibre was taken out each day from the inoculated tank, from day 7 to day 11, day 1 being the day of the inoculation. The fibres were washed and dried in air and later sent for the estimation of per cent of retting and the strength of the retted fibre.

One bundle was taken out each day from the uninoculated tank on day 9, day 12, day 14, day 16, day 23, and day 26, and they were washed and similarly tested.

Per cent of retting was estimated by visual observation based on the amount of the bark retained on the ribbons. Fibre strength was determined by Avery ballistic strength tester, Type 6702, and the average of ten 2-g samples 24 inches in length was recorded.

Batching experiments were carried out with the cooperation of the Bangkok Jute Mill, Ministry of Finance. Three grades of fibre (B, C, and rejected), 160 kg each, were used. Fibres of each grade were divided into two lots, one for the control batching, and the other for the inoculated batching.

Control fibres were batched through a batching machine with 64 pairs of fluted rollers, using batching emulsion made up of 20 per cent by volume batching oil, 0.5 per cent by volume emulsifier, and 0.3 per cent wt./vol. urea.

Inoculated fibres were batched similarly using the same batching emulsion with the addition of 5 per cent by volume of 18-hour old culture of the isolate in potato-dextrose broth.

Control fibre of each grade were arranged together in one bin, and the inoculated fibres together in another bin. Both bins were covered with canvas sheets and left undisturbed for three nights, during which time heat was slowly built up. They were taken out on the fourth day to be carded, and the carded fibres examined for softness and amounts of shives left.

## EXPERIMENTAL RESULTS

### Identification of the isolate

The isolate was aerobic, but facultatively anaerobic.

Colonies on nutrient agar, when freshly isolated, were large, smooth, soft, yeast-like, opaque, and white. On subsequent transfers they became rough, opaque, dull-looking, spreading, and greyish white. The isolate formed tough, wrinkled, waxy surface pellicle on liquid media. Growth was better at 35°C than at room temperature (28-32°C).

Microscopic examination revealed Gram positive rods, 0.4-1.0 $\mu$ x 1.5-4.7 $\mu$, not encapsulated, not in chains except in liquid media where long chains were formed. It was motile. Old cells were Gram negative.

Spores measured 0.4-1.1 $\mu$ x 1.0-2.0 $\mu$. They were ellipsoidal to cylindrical, central or paracentral. They showed bipolar and peripheral staining. The vegetative coating was only slightly swollen, if at all.

The biochemical reactions of the isolate as well as those of *B. subtilis* ATCC 6633 which was tested concurrently are given in Table 1.

Sugar reactions with conventional media and with the same media containing added 0.1 per cent ammonium sulphate agreed closely. Salicin was fermented better with added ammonium sulphate than without it.

### Test for retting ability

The results of the retting ability test of the isolate and those of *B. subtilis* ATCC 6633 are given in Table 2.

### Bacteriological retting of dry kenaf ribbons

The results of bacteriological retting of dry kenaf ribbons and those of the control uninoculated ret are given in Table 3.

### Batching experiments

After three days of batching and subsequent carding, inoculated grades B, C, and rejected fibres were found to be definitely softer

TABLE 1

BIOCHEMICAL REACTIONS OF BACILLUS SUBTILIS ATCC 6633 AND OF ASRCT ISOLATE

| Type of test | B. subtilis ATCC 6633 | ASRCT isolate |
|---|---|---|
| Growth in dextrose agar | Very good, 24 h | Very good, 24 h |
| Growth in 7% sodium chloride | 1+, 24 h | 1+, 48 h |
| Citrate utilization | +, 48 h | +, 48 h |
| Tartrate utilization | +, 24 h | +, 24 h |
| Nitrite production from nitrate | +, 48 h | +, 48 h |
| Indol production | -, 24 h + 48 h | -, 24 h + 48 h |
| M.R.-V.P. reaction | +, 48 h | +, 48 h |
| Urease production | -, 120 h | -, 120 h |
| Motility | + | + |
| Acid production from dextrose | 1+, 48 h | 1+, 120 h |
| Acid production from maltose | -, 7 days | -, 7 days |
| Acid production from mannite | 3+, 120 h | 2+, 48 h |
| Acid production from saccharose | 4+, 48 h | 3+, 48 h |
| Acid production from lactose | -, 7 days | -, 7 days |
| Acid production from dulcite | -, 7 days | -, 7 days |
| Acid production from xylose | -, 7 days | -, 7 days |
| Acid production from arabinose | ±, 48 h | ±, 48 h |
| Acid production from rhamnose | -, 7 days | -, 7 days |
| Acid production from sorbitol | ±, 48 h | -, 7 days |
| Acid production from salicin | 4+, 48 h | 4+, 120 h |
| Acid production from adonitol | -, 7 days | -, 7 days |
| Anaerobic growth in dextrose broth | - | - |
| Anaerobic growth in alkaline nitrate solution | Gas not produced, nitrite not formed | Gas not produced, nitrite not formed |
| Liquefaction of gelatin | + | + |
| Hydrolysis of starch | + | + |
| Growth in litmus milk | Acid and coagulated, 72 h | Acid and coagulated, 72 h |
| Gas production in carbohydrate fermentation media | - | - |

TABLE 2

KENAF RETTING ABILITY OF BACILLUS SUBTILIS ATCC 6633 AND OF ASRCT ISOLATE

| Type of kenaf material used | B. Subtilis ATCC 6633 | | ASRCT isolate | | Control (uninoculated) | |
|---|---|---|---|---|---|---|
| | Thai kenaf | Cuban kenaf | Thai kenaf | Cuban kenaf | Thai kenaf | Cuban kenaf |
| Sterilized fresh ribbon | 4+, 3+ | 3+, 3+ | 4+, 4+ | 4+, 4+ | ± | ± |
| Sterilized fresh stem | 2+, 2+ | 2+, 3+ | 2+, 1+ | 4+, 4+ | 0 | 0 |
| Sterilized dry ribbon | 4+, 4+ | 3+, 4+ | 4+, 4+ | 4+, 4+ | ± | 0 |
| Sterilized dry stem | 1+, 0 | 2+, 3+ | 3+, 2+ | 4+, 4+ | 0 | 0 |

TABLE 3

RESULTS OF BACTERIOLOGICAL RETTING
OF DRY KENAF RIBBONS IN AEROBIC RETTING TANK

| Day of retting | Inoculated ret | | | | Control ret | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temp. °C | pH | % retting | Fibre strength of retted fibres g.cm.Tex | Temp. °C | pH | | % retting | Fibre strength of retted fibres g.cm.Tex |
| | | | | | | Before water change | After water change | | |
| 1 | 35 | 7.20 | | | 34 | 4.85 | 6.79 | | |
| 2 | 35 | 6.53 | | | 35 | 5.11 | 6.78 | | |
| 3 | 35 | 6.55 | | | 34.5 | 4.93 | 6.74 | | |
| 4 | 35 | 6.30 | | | 35 | 5.79 | 6.79 | | |
| 5 | 35 | 5.65 | | | 35 | 6.19 | 6.89 | | |
| 6 | 35 | 6.18 | | | 34 | 6.85 | 7.01 | | |
| 7 | 35 | 6.31 | 65 | 7.6 | 34 | 6.60 | 6.95 | | |
| 8 | 35 | 6.69 | 70 | 5.4 | 34.5 | 6.91 | 6.92 | | |
| 9 | 35 | 7.11 | 80 | 6.7 | 35 | 6.91 | 7.15 | 40 | 8.46 |
| 10 | 35 | 7.22 | 75 | 6.2 | 34.5 | 7.01 | 6.95 | | |
| 11 | 35 | 7.55 | 85 | 5.0 | 34 | 7.08 | 6.95 | | |
| 12 | 35 | 7.59 | | | 34 | 7.22 | 7.20 | 60 | 6.76 |
| 13 | 35 | 7.55 | | | 34 | 7.28 | 7.13 | | |
| 14 | 35 | 7.41 | | | 34.5 | 7.23 | 7.14 | 55 | 6.74 |
| 15 | 35 | 7.38 | | | 34.5 | 7.41 | 6.88 | | |
| 16 | 35 | 7.46 | 80 | 4.30 | 34.5 | 7.42 | 6.96 | 65 | 6.96 |
| 17 | | | | | 34.5 | - | - | | |
| 18 | | | | | 34.5 | - | - | | |
| 19 | | | | | 34.5 | 7.22 | 7.16 | | |
| 20 | | | | | 34.5 | 7.54 | 7.02 | | |
| 21 | | | | | 34.5 | 7.35 | 7.38 | | |
| 22 | | | | | 34.5 | 7.68 | 8.11 | | |
| 23 | | | | | 34.5 | 6.95 | - | 75 | 4.58 |
| 24 | | | | | - | - | - | | |
| 25 | | | | | - | - | - | | |
| 26 | | | | | - | - | - | 85 | 4.60 |

than uninoculated fibres of the same grade.

For rejected grade fibre, inoculated fibres after 3 days of batching compared favourably in their softness with similar fibres batched conventionally without urea for 6 days. Moreover, the inoculated fibres appeared to contain less shives.

## DISCUSSION

From the results of various identification tests, the isolate conforms to the descriptions of Bacillus subtilis (Cohn 1872) in Bergey's Manual of Determinative Bacteriology (Breed et al. 1957). This strain, however, does not ferment xylose, even when 0.1 per cent ammonium sulphate was added to the medium. It appears to be identical with B. subtilis ATCC 6633. This strain also does not ferment xylose.

As early as 1932, enzymes from members of the then-called Bacteria subtilis-mesentericus group were claimed to digest substances surrounding the cellulose fibres of such plants as flax, ramie, jute, sisal, hemp, etc., and liberate those cellulose fibres for industrial use (Hollander and Del Plaine 1932).

Love (1958) also found Bacillus subtilis, as well as B. macerans, B. polymyxa, B. cereus, an Aerobactor sp., and two species of Pseudomonas able to ret sterilized dry kenaf ribbons. Under Cuban conditions, however, B. cereus, the Aerobactor sp., and the two Pseudomonas spp. produced fast rets when inoculated on unsterilized kenaf ribbons and were able to maintain themselves as the dominant organism (50 per cent or more of the bacterial population in the retting liquor). He isolated these various micro-organisms from soil and from retting liquors, not from retting bark.

Ahmad (1963) found Bacillus subtilis to be the most common among the aerobes and facultative anaerobes isolated from samples of retted jute stems collected from 12 districts of East Pakistan, but claimed that B. macerans, B. polymyxa, Micrococcus corchorus, and Pseudomonas aeruginosa were the most active retting agents in that country. However, B. subtilis was still classified by him as one of the complete retters of jute.

Scott (1964) also listed Bacillus subtilis as one of the predominant species in the retting liquor of kenaf ribbons, but he followed Lanigan (1951) not Love (1958) in assigning the major role in kenaf retting to anaerobic organisms while the aerobic activity serves to create conditions suitable for multiplication of the anaerobes.

In our previous work* studies were made with 30 isolates, 21 (10 aerobes, 11 anaerobes) from retting barks, and 9 (all aerobes) from retting liquor during retting of green kenaf stems. Of the 9 aerobes from retting liquor, only one isolate (a Gram negative rod) was found to possess some retting activity. It could ret well only sterilized fresh kenaf ribbon, but not the sterilized fresh kenaf stem, dry kenaf ribbon, and dry kenaf stem. Of the 10 aerobic isolates from retting barks, 4 were found to have retting ability. These include 2 strains of yeasts, one strain of a Neisseria sp., and the isolate being reported in this paper. One of the yeasts could ret well only the fresh kenaf ribbon, while the other strain moderately retted fresh ribbon, and slightly retted the other three sterilized kenaf materials. The Neisseria sp. slightly retted fresh kenaf ribbon, dry kenaf ribbon, and fresh kenaf stems. Only the isolate being reported (already identified as Bacillus subtilis) was found to ret well all the four types of sterilized kenaf materials.

Among the anaerobes isolated from retting barks (10 Clostridia, and 1 Streptococcus), only three strains of the genus Clostridium showed some retting activity. This was slight, however. Two strains could ret well only fresh kenaf ribbon, and did not ret the other types of sterilized kenaf materials. The other strain could slightly ret only fresh kenaf ribbon and dry kenaf stem.

From comparative study of the retting ability of Bacillus subtilis ATCC 6633 and of the ASRCT isolate (see Table 2), both strains were found to be good kenaf retters. The ASRCT strain possesses somewhat greater activity, however.

---

* "Preliminary studies on microbial flora of kenaf retting" by Smarn Vardhanabhuti, Chiraporn Chouvalit, Naiyana Nopakun, and Pranee Jutasirivongse. Report No. 1 on Research Project No. 1/11 (Microbiology of kenaf retting). ASRCT unpublished report.

The results of bacteriological retting of dry kenaf ribbon shown in Table 3 clearly indicated that inoculation with Bacillus subtilis, ASRCT strain, was of definite advantage in tank retting of this type of kenaf material. It took only 10 days with inoculation to get 75-80 per cent of retting without any loss of strength, while it took 23 days for the control uninoculated ret to obtain the same degree of retting. Moreover inoculation produced cleaner and softer fibre than without it.

Thai kenaf usually takes a much longer time to ret than African and Cuban kenaf. This is because Thai farmers customarily cut their kenaf when the plant starts to bloom or has bloomed. By this time the plant is usually more than six months old. The bark of the lower portion of the stem has almost completely lignified and is almost impossible to ret. The dry kenaf ribbons used in our experiment were of this type and they were kept in the dry state for over one year.

Over-retting usually does not occur in kenaf retting. It did occur with the control ribbons because the retting liquor was completely changed daily throughout the major part of the retting period. This was done on purpose to obtain the shortest possible retting period of the control ret to compare with that of the inoculated ret. This is based on the experience of Scott (1964) who reported that the tank receiving only 20 per cent of its volume of fresh water in 24 hours developed a pH of 4.2 after 3 to 4 days whereafter retting virtually ceased, while the tank receiving a purge of 100 per cent tank replacements maintained a pH above 4.6 and retted to completion.

Scott (1963) also mentioned that over-retting of kenaf had been shown experimentally to be due to the aerobic condition associated with a plentiful supply of fresh water. This was essentially the condition in our control tank where the retting liquor was completely changed daily with fresh water.

It needs to be mentioned that with bacteriological retting of dry kenaf ribbons, the retting must be terminated at the right moment. Prolonged retting will result in over-retting with rapid loss of strength of the retted fibres. As shown in Table 3, fibre strength dropped from 6.2 g cm/tex at day 10 to 5.0 g cm/tex on day 11.

Even though batching experiments showed that inoculation of Bacillus subtilis into batching emulsion was of some advantage, i.e. softer and cleaner fibres after carding, and probably would shorten the batching time of the more difficult to batch (rejected grade) kenaf fibres, it was not possible to bring the experiments to the spinning stage whereby the spun threads could be evaluated for their quality and strength. To do so would require treating at least two tonnes of kenaf fibre and the factory was not in a position to do this. It is hoped that this work can be repeated in the laboratory in the future when small units for batching, carding, finishing, drawing, and spinning of kenaf fibres may be set up so that such a large amount of kenaf fibres would not be required for assessment.

## CONCLUSIONS

(1) A potent aerobic kenaf retter isolated from retting bark has been identified as Bacillus subtilis (Cohn 1872), practically identical with B. subtilis ATCC 6633.

(2) Both ATCC and ASRCT strains showed good in vitro retting ability on sterilized kenaf ribbon, fresh kenaf stem, dry kenaf ribbon, and dry kenaf stem. The ASRCT strain was a little better in this respect, however.

(3) Bacillus subtilis could be used with advantage in bacteriological retting of dry kenaf ribbon and in bacteriological batching of kenaf fibres. More work is needed to demonstrate the utility of this technique in processing kenaf industrially.

## ACKNOWLEDGEMENTS

The authors wish to thank Flight Lieutenant Prapai Keonil, Acting Director of the Instrument Repair and Calibration Centre, and Mr. Siri Nanthasri, Head, Workshop and Maintenance Unit, and their respective staffs for designing, building, and operating the retting tanks used in the retting experiments. They also wish to thank Mr. Vallop Rajavejbhisal, Chief Engineer of the Bangkok Jute Mill, and his staff for their co-operation in the batching experiments.

Thanks are also due to various supporting staff of ASRCT who have helped in one way or another in various investigations reported in this paper.

## REFERENCES

AHMAD, M. (1963).—Studies on jute retting bacteria. J.appl.Bact. 26 (2): 117-126.

ALLEN, L.A. (1946).—Recent developments in the microbiology of retting. Part II. The part played by bacteria in retting the flax and in purifying the liquor. J.Soc.chem.Ind., Lond. 65: 373-376.

BREED, R.S., MURRAY, E.G.D., and SMITH, N.R. (1957).—"Bergey's Manual of Determinative Bacteriology." 7th ed. pp. 620-621. (E & S Livingstone Ltd.: Edinburgh and London.)

CHRISTENSEN, W.B. (1946).—Urea decomposition as a means of differentiating Proteus and Paracolon from each other and from Salmonella and Shigella types. J.Bact. 52: 461-466.

CONN, H.J., JENNISON, M.W., and WEEKS, O.B. (1957).—Routine tests for the identification of bacteria. In: Society of American Bacteriologists "Manual of Microbiological Methods." p. 163. (McGraw-Hill Book Company, Inc.: New York.)

HOLLANDER, C.S., and DELPLAINE, P.H. (1932).—Process of retting fibres. U.S. Patent Office No. 1, 842, 024.

LANIGAN, G.W. (1951).—The bacterial flora of Australian flax retting. Austr.J.scient.Res. Ser. B. 4: 461-485.

LOVE, H.T. (1958).—Kenaf retting studies. In: Proceedings of the World Conference on Kenaf. pp. 141-153. (International Co-operation Administration: Washington 25, D.C.)

SCOTT, K.J. (1963).—An experimental study of kenaf retting. J.Text. Inst. 54: 128-137.

SCOTT, K.J. (1964).—The physical and chemical changes accompanying the water retting of kenaf ribbons. S.Afr.J.agric.Sci. 7: 681-706.