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Microbial degumming of decorticated ramie and its fibre characteristics

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Decorticated bast fibres from ramie [*Boehmeria nivea* (L) Gaud] have been treated for different time periods using the bacterial and fungal cultures of *Bacillus subtilis*, bacterial isolates of $BCJF_1$ and $BCJO_1$, *Curvularia* sp., *Aspergillus* sp. and their mixed cultures. The residual gum of the fibres decreases to 10.4% and 9.7% after 4 and 6 days treatment respectively with bacterial and fungal strains. Treatment for 1-8 days of ramie bast fibres with crude supernatant of mixed cultures shows a correlation between incubation period and fibre separation. It is observed that the microbial consortium of fungi and bacteria plays an important role in the degradation of residual gum and is found to be more efficient for degumming purpose as compared to single culture treatment. It can be applied in large scale for cost effective fibre production from ramie bark.

Keywords: Bast fibres, Degumming, Fibre characteristics, Microbial consortium, Ramie

Ramie [*Boehmeria nivea* (L) Gaud], a strongest natural textile fibre, is obtained from the plant *Boehmeria nivea* (L) Gaud belonging to the nettle family Urticaceae mainly grown in temperate and tropical areas[1, 2]. The fibre, composed of long single cells fixed together in bundles by gums and pectins, is embedded in the cells of the bast. However, despite excellent properties and diverse applications, ramie has failed to become a major textile crop mainly due to difficulties in processing of the fibre[3]. One of the most important problems associated with the ramie fibre is its proper degumming. The decorticated ramie fibre contains nearly 19-30% gum[4], depending upon the variety, condition of cultivation and other factors. The gum content of the fibre should be reduced to 7-9% for good spinnability[5] and yarn properties[6] as well as for the durability of the products. The degumming of ramie fibre can be carried out by two methods, namely microbial degumming and chemical degumming using various alkali[7, 8]. The degumming of ramie fibres by microorganisms or their enzymes has attracted wide attention and, several reports are available[9,10]. Though various microbial methods have been developed for degumming, it does not appear that the microorganisms are in common use for degumming. Bacteria occurring naturally on the fibre have the ability to degum fresh decorticated fibre under alkaline unsterile condition and the quality of biologically degummed fibres seems to be better than the quality of chemically treated fibre[1-10]. As the conventional degumming process with a solution of NaOH not only has high consumption of chemicals and energy, it also causes environmental pollution. The preliminary results showed that the alkalophilic bacteria have potential value in the degumming of ramie fibres. Considering the status of ramie fibre as a scare commodity in the international as well as national fibre trade, better scope exists in India for the commercialization of this potential fibre and fabrics. Review of literature indicated that no work has been done regarding degumming of ramie with microbial consortium or mixed microbial cultures. Therefore, an attempt has been made to study the effect of single and consortium culture capable of removing residual gum for better processing of ramie fibre.

Fifteen bacterial and fungal cultures from cow dung, retted ramie stem and rhizosphere soils were isolated and screened for degumming of ramie to yield fibre. Isolates of *Curvularia* sp. and *Aspergillus* sp. were extracted from the rhizosphere soil of ramie plant and maintained in potato dextrose agar (PDA) media (potatoes: infusion form, 200g; dextrose, 20 g; agar, 30 g; distilled water, 1000 mL and *p*H, 5.6 ± 0.2) at 28° ± 2°C. Nutrient agar (NA) media (beef extract, 3g; peptone, 5g; agar, 15 g; distilled water, 1000 mL and *p*H, 6.8-7.2 ± 0.2) was used to isolate bacteria, viz. *Bacillus subtilis*, *Bacillus* sp.,

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$BCJF_1$ and $BCJO_1$ from cow dung and retted ramie stem and were maintained at 36° ± 2°C. Bacterial strains were found alkalophilic in nature and their alkalophilic characters were ascertained[11].

Ramie varieties, viz. R-1452 and R-1412, were collected from Ramie Research Station (ICAR), Sorbhog, Assam (India) and cultivated in the experimental fields of North East Institute of Science and Technology (CSIR), Jorhat, Assam (India). Ramie stem samples of 60 days maturity were collected after harvesting and decorticated in decorticating machine of the 3HP capacity.

The media were prepared by adding 4% NaCl and 4% sucrose in pond water, boiled for half an hour, and *p*H was adjusted at 7.5 ± 0.2. Spore suspension of fungal and bacterial cultures was inoculated at 28° ± 2°C and 36° ± 2°C respectively for proper growth of the microorganisms.

Altogether 15 isolates of bacteria and fungi were extracted, characterized and screened, but only 5 of them were selected for degumming on the basis of percentage of gum removal. Degumming of ramie bast fibres was carried out in Erlenmeyer flasks (3000 mL) containing sterilized fibres (10g) of 30 cm. A loop of each selected strain was inoculated into Erlenmeyer flask and suitable condition was maintained for growth. Fibres were inoculated in cell-free supernatants (21 days old) of *Curvularia* sp., *Aspergillus* sp., *Bacillus subtilis*, $BCJF_1$, $BCJO_1$ and their mixed culture respectively. Flasks were kept at room temperature (25° ± 2°C) and the contents were stirred regularly for thorough mixing. The bundles were taken out one after another at the interval of 1- 8 days (T_1-T_8 days). The bundles were washed in running water followed by distilled water to remove the loosened barks from the fibre and finally sun dried.

The gum content of the fibres after experiment was determined gravimetrically by sequential degumming of the fibres with hot sodium hydroxide (NaOH) solutions of increasing concentrations[4,12].

The fibres were bleached with 1.5% hydrogen peroxide solution and a *p*H of 7.0 ± 0.2 was maintained, as the higher *p*H causes deposits. The fibres were kept in bleaching solution for 60 min with intermittent checking along with 0.2 mL Tween–80 surfactant to enhance the fibre brightness. The fineness of bleached ramie fibre was determined as per the standard method [13]. The brightness of the degummed and bleached ramie fibre was determined using EIL digital reflectance spectrophotometer and the results are expressed on the basis of MgO=100[8].

The degummed, combed and bleached fibres were subjected to quality tests to evaluate the best quality fibre resulting from various microbial treatments. The tensile strength of the fibre was measured on a Stelometer by taking a bundle of fibres. The values were calculated according to Booth [14] using the following relationship:

$$\text{Tensile strength (g/tex)} = \frac{\text{Breaking load (kg)} \times \text{Specimen length(mm)}}{\text{Specimen mass(mg)}}$$

Degummed fibre exhibits weight loss and a decrease in moisture content as compared to un-degummed fibre. A correlation between incubation period applied to ramie bast fibres and the resulting degumming effect is shown in Fig.1. It is found that the gum content of fibres during degumming with cell-free supernatants of the microbes grown on ramie fibres reduces gradually over the time. $BCJO_1$ and mixed culture reduce gum content up to 50% after 4 days of treatment, i.e. 17.20% and 10.40% respectively. Remarkable decrease in residual gum content is observed in mixed culture treatment after 72 -192 h (3-8 days) treatment. The percentage of residual gum content after 72-192 h microbial consortium treated fibre is recorded as 13.50%, 10.40%, 10.0%, 9.70%, 8.20% and 7.10% respectively (Fig. 1).

Tensile strength reduces with the increase in period of treatment. Tensile strength and breaking load of the fibre in mixed culture treatment as well as in other monoculture treatment decrease gradually, may be due to multiple enzyme activities of the microbes [14-18]. The fibre treated with *Curvularia* sp. is found to have greater tensile strength after 8 days of treatments followed by bacterial isolates *Bacillus subtilis* and

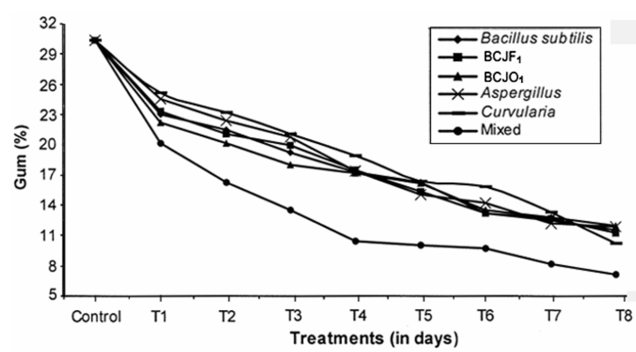

Fig.1— Gum loss of ramie fibres after incubation with the strains [values given are the means of three replicates ± standard deviation]

Table 1— Variation in breaking load, fibre fineness and brightness characteristics of ramie fibre after bleaching

[Bleaching conc. 2.0%, and Bleaching period 60 min]

Physical properties	Microbial isolates	Treatment period, days							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Average breaking load kg	Bacillus subtilis	5.91±0.20	5.79±0.01	5.59±0.07	5.46±0.064	5.22±0.06	5.11±0.03	4.67±0.11	4.16±0.03
	$BCJF_1$	5.82±0.09	5.61±0.04	5.53±0.03	5.39±0.07	5.15±0.04	5.01±0.05	4.59±0.04	4.09±0.06
	$BCJO_1$	5.88±0.06	5.68±0.03	5.48±0.08	5.35±0.05	5.12±0.02	4.96±0.04	4.56±0.03	3.99±0.27
	Aspergillus sp.	5.98±0.03	5.78±0.08	5.56±0.04	5.30±0.02	5.13±0.05	4.72±0.11	4.58±0.06	3.98±0.07
	Curvularia sp.	6.01±0.06	5.85±0.09	5.69±0.07	5.26±0.05	5.12±0.06	4.70±0.13	4.57±0.06	4.20±0.01
	Mixed culture	5.78±0.06	5.60±0.06	5.55±0.03	5.16±0.07	5.07±0.01	4.63±0.12	4.26±0.09	3.91±0.02
Fineness, µ	*Bacillus subtilis*	6.45±0.05	6.38±0.02	6.21±0.02	6.11±0.03	5.86±0.06	5.70±0.10	5.65±0.05	5.16±0.06
	$BCJF_1$	6.43±0.11	6.43±0.02	6.25±0.05	6.13±0.02	5.95±0.05	5.83±0.02	5.55±0.05	4.88±0.08
	$BCJO_1$	6.48±0.08	6.41±0.02	6.31±0.02	6.10±0.1	5.90±0.05	5.85±0.05	5.53±0.06	5.15±0.13
	Aspergillus sp.	6.35±0.05	6.33±0.06	6.18±0.03	6.13±0.05	5.85±0.05	5.75±0.05	5.55±0.05	5.33±0.06
	Curvularia sp.	6.48±0.03	6.43±0.06	6.36±0.02	6.16±0.06	5.81±0.03	5.68±0.08	5.58±0.02	5.50±0.05
	Mixed culture	6.45±0.05	6.31±0.02	6.25±0.05	6.05±0.05	5.88±0.07	5.83±0.06	5.65±0.05	5.38±0.07
Brightness, %	*Bacillus subtilis*	61.33±1.52	64.00±1.00	65.66±2.51	71.33±2.08	75.33±1.15	79.66±1.73	84.33±0.57	86.00±1.00
	$BCJF_1$	58.66±0.57	60.66±0.57	63.66±1.15	67.00±1.00	71.66±1.15	76.33±1.52	78.33±1.15	82.66±1.52
	$BCJO_1$	60.33±3.05	63.66±1.52	65.66±3.21	67.33±0.57	72.66±1.52	74.33±1.52	78.00±1.00	81.33±2.51
	Aspergillus sp.	57.66±1.52	61.66±1.52	63.33±1.54	65.66±0.57	69.00±1.00	72.33±0.57	75.00±1.00	79.66±0.57
	Curvularia sp.	56.66±2.51	60.00±2.64	63.33±1.15	65.00±2.64	68.33±2.08	72.00±2.64	76.66±1.52	82.00±1.00
	Mixed culture	64.33±1.52	68.00±1.00	71.00±2.00	74.00±1.00	79.00±1.00	85.00±1.15	87.00±1.15	90.00±1.73

T_1-T_8 are the days 1-8 respectively.

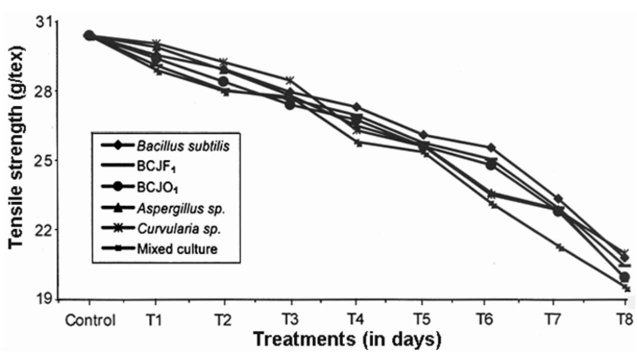

Fig. 2— Tensile strength of ramie fibres after incubation with the strains [values given are the means of three replicates ± standard deviation]

Table 2— Effect of temperature on degumming of ramie fibre after 96 h of incubation

Microbial isolates	Per cent removal of gum				
	Ambient temp. (33°C)	25°C	30°C	35°C	40°C
Bacillus subtilis	50.62	48.43	49.66	50.12	50.40
$BCJF_1$	50.32	48.82	49.24	50.30	50.33
$BCJO_1$	53.50	48.52	49.12	50.25	50.30
Aspergillus sp.	50.64	50.42	50.56	49.62	49.94
Curvularia sp.	50.23	49.45	50.12	48.74	48.25
Mixed Culture	52.24	51.48	53.86	53.02	52.21
Control	1.98	1.82	1.92	1.95	1.98

Table 3— Effect of *p*H on degumming of ramie fibre after 96 h of incubation

Microbial isolates	Per cent removal of gum				
	5.5 *p*H	6.5 *p*H	7.5 *p*H	8.5 *p*H	9.0 *p*H
Bacillus subtilis	36.94	47.32	50.65	42.20	35.32
$BCJF_1$	38.31	48.18	50.30	41.18	37.28
$BCJO_1$	35.93	47.28	52.23	41.50	36.65
Aspergillus sp.	37.01	47.40	50.62	42.80	35.52
Curvularia sp.	38.87	48.08	50.20	41.62	36.08
Mixed culture	39.94	50.24	52.36	51.64	38.20
Control	1.62	1.72	1.80	1.75	1.70

$BCJF_1$ treatment. On the other hand, mixed culture shows lesser tensile strength as compared to monoculture, might be due to multiple enzyme action (Table 1 and Fig. 2).

Fibre fineness and brightness characteristics are found to be independent of concentration of bleaching solution and bleaching period. Fibre fineness characteristic decreases with the increase in treatment period and brightness of the fibre increases with the increase in treatment period. It is observed that the fineness and brightness characteristics of the fibre is better for 96 h (T_4) treatment period, might be due to higher removal of gum (Table 1).

It has been observed that the 35°C is the optimum temperature and 7.5 is the optimum *p*H for the growth and development of the bacterial isolates after 96 h (Tables 2 and 3).

Degumming process could not be continued beyond seven days as the fibre quality starts deteriorating due to the growth of cellulose decomposing microorganisms during uncontrolled microbial degumming. T_4, T_5 and T_6 days microbial consortium treated fibres are found to be better when fibre quality is considered and can be suggested as standard degumming methods. It is found that the microbial consortium of fungi and bacteria plays an important role in the degradation of residual gum and are therefore efficient for degumming purpose as compared to single culture treatment (Fig 2 and Tables 1-3).

Industrial Importance: The easy, cost effective, viable degumming is strongly correlated with commercialization and marketing of the fibre product. Only effective and economically viable degumming process can be entrusted to some other agencies like established textile concern or Government marketing and 'Industrial Organizations'. This technical efficiency must be combined with the economic efficiency for economic viability of the fibre. This easier extraction of ramie fits well with the concept of village industry where the ingenuity can be marred to local resources for expansion of its cultivation and industrialization.

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