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Development of a Bacterial Consortium for Coir Retting

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The development of a consortium comprising indigenous flora of the coconut husks is reported. This consortium which could be grown on husk leachates has been used as inoculum for the retting process under laboratory conditions. The consortium has been developed on husk leachates with salinity adjusted to 6.5 ppt after an acclimation period of ninety days with three subcultures. When plated on nutrient agar, 10 different bacterial cultures could be isolated among which gram negative coccobacillary forms have been the most predominant. The consortium is grown on HLM (Husk leachate medium) and inoculated on coconut husks steeped for retting in 5 and 10 per cent concentrations in tanks. It is found that the consortium could reduce the retting period to three months. The environmental parameters such as pH, salinity and temperature have been monitored for a period of three months. It is observed that the fibre could be separated from the husks as early as 3 months, as compared to the control husk. The fibre from these husks is observed to possess a light-fastness rating equivalent to the traditionally 9-11 month retted fibre.

Introduction

The mesocarp of coconut, also known as coconut "husk" is the main source of the coir fibre which is extracted from the husk by the "retting" process. The export of coir fetches over 100 crore rupees annually and although the production of coconuts in India is 12,355 million nuts[1], less than 30 per cent of the husks are utilized for coir extraction. It is envisaged that this is due to non-availability of proper retting conditions. The coir extraction as an industry is confined to Kerala in South India, although coconut production prevails in Goa, Andhra Pradesh, Tamil Nadu, Karnataka, Orissa, West Bengal and Assam. The natural saline backwaters in Kerala provide ideal "retting" conditions for coir extraction. The process of retting has been reported to mainly involve the degradation of polyphenols and pectins which bind the fibre in the husk[2]; the efficiency of retting therefore depends on the rate and extent of degradation of these binding components. Inoculation of selected strains bacterial cultures in substandard retting areas has resulted in improving the quality of fibre[3].

One of the important observations made in the retting of coconut husks is that polyphenols from the husks get constantly leached out into the surrounding steep liquors. The high percentage of such polyphenols in coconut husks and the steep liquors appears to influence significantly the retting process, resulting in a delay in the extraction of the fibre[4]. An analysis of the phenolic compounds leached out during the retting of coconut husks in a natural system has confirmed the presence of resorcinol, pyrogallic acid, catechol as some of the main phenolic compounds[5]. These phenolic compounds can be degraded by an anaerobic bacterial consortium[6-8]. It was therefore envisaged to develop a consortium of indigenous bacteria in coconut husk growing on husk leachates and study the retting of husks with this consortium under laboratory conditions.

Materials and Methods

(A) Development of the Consortium

One sample of husk was steeped in 1.5 L of estuarine water of salinity 6 ppt in a 5-L beaker. To this 1.5 L of distilled water was added and the salinity was adjusted to 6 ppt with sodium chloride. The mixture was allowed to stand for 30 d, to form the first subculture of the organism in husk leachates.

1.5 L of this subculture with the husk was transferred to another beaker and was supplemented with 1.5 L distilled water, the salinity was adjusted to 6 ppt and kept for 30 d to yield the second subculture. This subculture was also subjected to the above treatment to give a third subculture of the consortium growing on husk leachate. The viable count of the first, second and third subcultures were taken on nutrient agar and resorcinol mineral medium (Table 1).

Table 1 — Influence of subcultures on total count

No. of subculture	Media	
	Nutrient agar	Resorcinol agar
1	23×10^2	43×10^2
2	85×10^2	73×10^2
3	120×10^3	141×10^2

(B) Identification of a Suitable Substrate for Growth of the Consortium

To find a suitable substrate for mass culturing of the consortium, 5% of the consortium was inoculated into one litre each of nutrient broth (N.B), 0.05% resorcinol mineral medium and husk leachate medium (HLM). The husk leachate medium (HLM) was prepared by adding one surface sterilized husk into one litre sterile mineral medium comprising per litre of K_2HPO_4-12.6% , KH_2PO_4-18.2%, $(NH_4)_2SO_4$-10%, $MgSO_4$-1%, $MnSO_4$-0.6% $Na_2MoO_4.2H_20$- 0.6%, $CaCl_2.2H_20$-1%, $FeSO_4$-0.12 g and allowed to stand for 48 h at room temperature to facilitate leaching out of the polyphenolic compounds from the husk into the medium. The husk was aseptically removed and the medium inoculated with 50 mL of the consortium and incubated at room temperature. The viable count in each of the three media was determined by drawing out samples at zero, twenty-four and forty-eight hours of incubation and plated using spread plate techniques on Nutrient Agar (Table 2).

(C) Preparation of Inoculum for Laboratory Scale Retting of Husks Using the Consortium

The husk leachate medium (HLM) which allowed maximum growth of the consortium after nutrient medium, was selected as the substrate for growing the consortium. 50 g of surface sterilized husk was taken in each of the four 5L round bottom flasks containing 2L of sterile mineral medium and allowed to stand for 48 h. The medium was decanted aseptically into sterile flasks and was inoculated with 5 % of the consortia and incubated at room temperature for 24 h.. This was used as inoculum for the laboratory scale retting experiment.

(D) Laboratory Scale Retting Using Consortia

Mature coconut husks from 11-month-old nuts, which are normally utilized for coir extraction, were used for the laboratory scale study. Three tanks A, B and C were set up with 10 husks immersed in tap water. After 24-h of soaking tanks A and B were inoculated with the consortium (grown in HLM) in concentrations of 5 and 10 per cent, respectively. Tank C was maintained as the untreated control. In all the three sets the final husks : liquor ratio was maintained as 1:5. A periodic flushing of the water in all the three tanks was carried out by removal of the steep liquor and refilling with tap water at fortnightly intervals. This was done to simulate the flushing action in the environment which had a brightening effect on the fibre. To supplement the loss of organisms due to flushing, tanks A and B were inoculated after 30 d with the consortium in the concentration of 5 and 10%, respectively. Husks were drawn out from each of the three tanks every month to monitor the progress in retting. On completion of three months of soaking, the husks in the three sets were drawn out and the fibre extracted. The fibre thus obtained from the three sets were subjected to the Xenotest for assessing the light-fastness property which determines the quality of the fibre. Xenotest is a uniform specification for rating the light-fastness and weather-fastness of materials more quickly than naturally. It has a 1500 W Xenon arc lamp as a source of radiation; the filtered spectrum of this lamp when used in the Xenotest is the same as sunlight. The samples were subjected to alternate periods of light and darkness. This mimics conditions of day and night approximately. A test time of 24 h in the Xenotest was roughly equivalent to the radiation received over 10 days in the open air averaged throughout the year. Samples fading within 80 min of test exposure were rated as Grade I and those after 80 min, as Grade II.

Table 2 — Growth pattern of the consortium on different substrates

Incubation period (days)	Cells/mL ($\times 10^2$) Media		
	Husk leachate	Resorcinol	Nutrient
0	48	–	38
1	287	141	300
2	20	14	3
3	3	5	3

Table 3 — Time bound pH, temperature and salinity profile in retting tanks

Incubation period days	Parameters								
	pH			Salinity, ppt Tank			Temperature, °C		
	A	B	C	A	B	C	A	B	C
1	6.5	6.5	6.8	1	1	1	31	31	31
5	5.5	5.7	5.8	1	1	0	31	31	31
12	5.8	5.0	4.4	3	2	1	30	30	30
15	5.0	6.0	4.7	2	2	2	29	29	29
20	6.0	6.2	4.8	1	4	2	30	30	30
33	6.3	6.5	4.8	1	2	3	30	30	30
40	6.7	6.7	5.8	0.5	0.5	2	30	30	30
47	6.8	6.9	6.2	1.5	1.5	2.5	30	30	30
55	6.9	7.0	6.5	1.0	1.0	2.5	31	31	31
62	7.0	7.0	6.5	1.0	2.0	4.0	31	31	31
70	7.1	7.1	7.2	0.5	0.5	0.5	30	30	30

Results and Discussion

Analysis of the steep liquor from all the three tanks at periodic intervals revealed that the pH dropped initially in all the three tanks from 7.2 to 6.5. The tanks A and B on inoculation showed an increase in pH within 41 days (less than 2 months), the pH in the control set rose to 7.2 only on the 63rd day (after two months). The salinity of the steep liquor in all the three tanks ranged from 0.5 to 4.0 throughout the study. The husks drawn out from the inoculated tanks were softer and the exocarp could be peeled off easily, indicating completion of retting after 90 days. Although there is no scientific criterion for determining the completion of retting, the softening of the husk, ease in separation of the exocarp (which peels off without exertion), fibre of length between 15-20 cm and soft texture devoid of extraneous pith material in bright colour were taken as the main indications of completion of retting.

A significant difference was observed with the husks drawn out from the control (untreated tank) which exhibited hard nature and the exocarp would not peel off easily, indicating incomplete retting.

The fibre extracted from tanks A and B exhibited less pith content as compared to the fibre from tank C, which was comparatively inferior having a dull colour (Figure 1). A light-fastness rating of Grade II in the Xenotest has been reported for the conventionally retted fibre[5]. The fibres from the three sets were subjected to the Xenotest and the fibre from the consortia- treated husks showed a rating of Grade II whereas the fibre from the untreated husk showed a rating of Grade I. The study could establish the fact that retting of coconut husks using the consortia could be carried out in tanks to yield fibre of retted quality. Such fibre could then be used as a raw material for the coir industry.

The potential of the husks in coconut growing regions of India like Andhra Pradesh, Assam, Goa, Karnataka, Tamilnadu and West Bengal has not been utilized fully due to non-existence of adequate retting facilities. The possibility of extraction of coir by economical means using consortium of bacterial cultures would lead to the establishment of coir extraction in these non-traditional coir producing states. This would not only provide an additional income through the raw material production but will also lead to generation of employment in the spinning and weaving sectors for manufacture of coir products.

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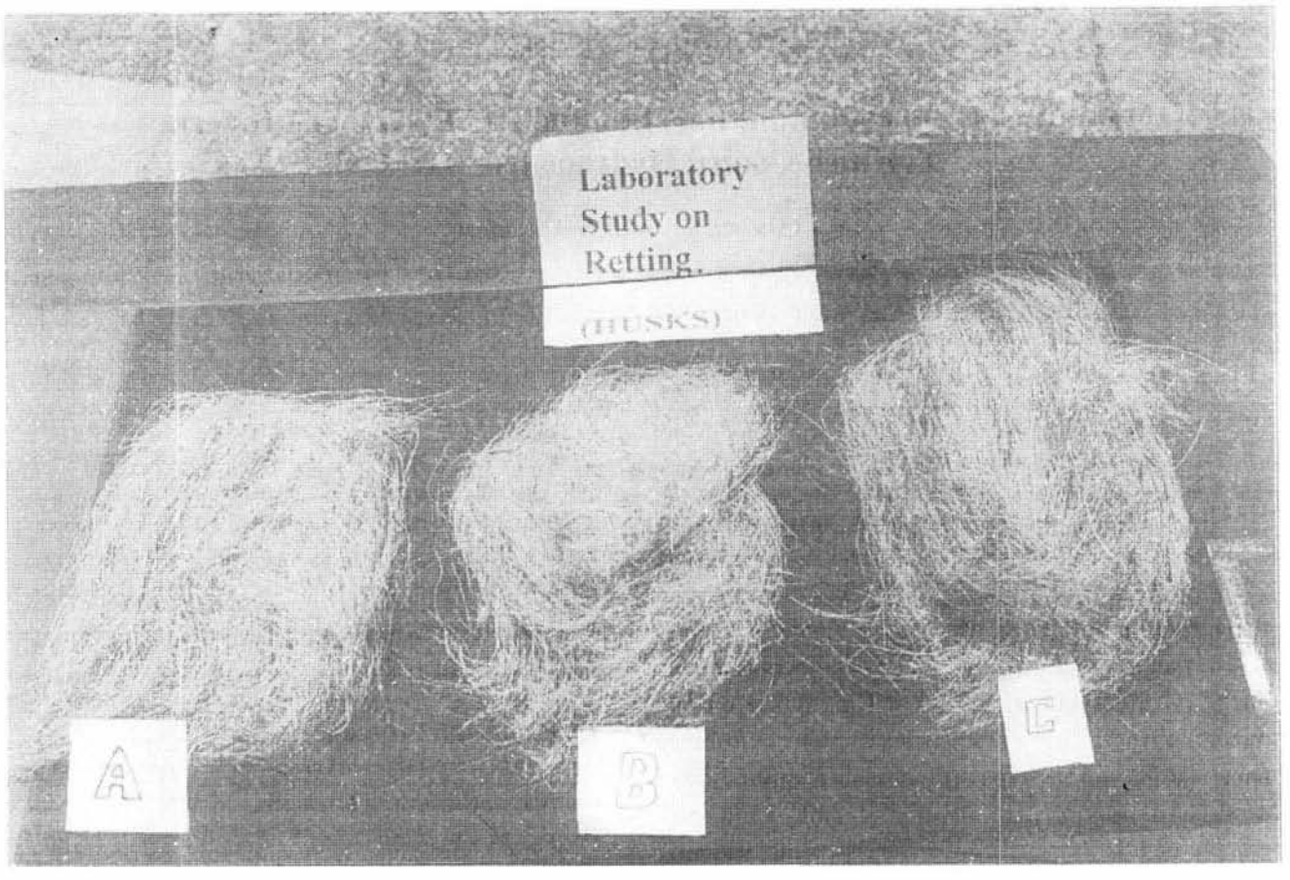

Figure 1 — A comparison of retting study at laboratory scale

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