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Microbial consortium: A new approach in jute retting of preserved dry ribbons

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Abstract

The present research was taken to formulate bacterial consortium as whole cell biocatalyst for retting of dry jute ribbons. The bacteria were obtained from different sources of jute retting water, enriched on nutrient broth medium. Microbial consortium was constructed from 7 (seven) selected isolated bacteria to become 7 (seven) combination culture which exhibited remarkable retting efficacy due to the induction of different enzymes activities. The enzymatic as well as biochemical activity of these bacteria were tested. The strains were selected based on the criteria that they were able to display good zone of inhibition. Formulations showed good potential as candidates for microbial consortium. In the two combination treatment with water (5 ml), microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b) and (OMEW4b+10DTW2b) were found better for all the cases. Again, in three combinations treatment with water (5 ml d.H_2O), fineness, brightness and smoothness/softness, all were found higher in microbial consortia of (3PRRF5b+4DTF1b+10DTW2b), which is a unique findings. This research is on-going and need to optimize these consortiums with different parameters and also carry out retting analysis.

Keywords: Jute; Dry Ribbon; Formulation; Microbial inoculums; Consortia

1 Introduction

The process of separation and extraction of fibers from non-fibrous tissues and woody part of the stem through dissolution and decomposition of pectins, gums and other mucilaginous substance which called retting, is a biochemical process carried out by the action of various retting microbes. Usually retting is best carried out in slow moving soft water and more than 90% of jute growers ret the jute and mesta plants in stagnant water following conventional method of retting. The repeated retting of jute and mesta in the stagnant water of same natural retting tank lead to the production of inferior quality fiber unless the tank is recharged with fresh water after each retting. Moreover, during retting, some area of Bangladesh particularly in north Bengal faces drying or water scarcity. That's why farmers experiences acute crisis of jute retting during the season. If green ribbon of jute dry and preserve for retting in next time, the problems can be solved to some extent. If dried barks yield same quality fiber then retting of dried or preserved fiber could be helpful for the farmers. To overcome this problem, use of microbial inoculums/consortia can be an alternative retting technique in order to obtain fibers. Inoculums formulation and development is the process of preparing a population of important microorganisms from a dormant stock culture to a population of microorganisms that can be used for inoculating a final productive stage [1]. The objectives of inoculums development were rapid growth rates and high biomass concentration at the beginning of a fermentation process. Singha et al [2] claimed a microbial consortium for fast retting of bastfibre comprising three *Bacillus pumilus* strains containing *Bacillus pumilus* IMAU 80221, *Bacillus pumilus* GVC 11 and *Bacillus pumilus* SYBC-W mixed in a ratio of 1:2:1. However, the most important objective is to obtain pure culture of microbial population for the purpose of fermentation. This concept applies not only to different species in the inoculums but also to variants within the pure culture itself [3]. Attempt was taken to identify the best microbial teams that can be a powerful alternative to stacking or mixing individually identified microbes,

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resulting in products with higher efficacy, pre-selected for their ability to colonize in dry jute ribbon together. Microbial consortia are elements of diverse communities of bacteria and fungi found naturally throughout the biosphere. Microbes naturally colonize plants, providing beneficial, harmful, or neutral interactions. Microbial population can be obtained from the environment directly or by sub-culturing from an available stock culture. These microorganisms may come from various sources, depending on their activities that lead to their use in a particular issue. Microbial inoculums/consortia on the basis of their enzyme production ability can be formulated. These inoculums may be applied on dried or preserved bark. The microbial formulation may be found suitable not only for the reduction of retting duration but also for the improvement in fiber quality by at least two to three grades. For this reason present study has been taken for developing retting/fiber extraction process of preserved or dried jute bark using microbial consortia. The present study was taken to formulate microbial inoculums for fiber extraction and to develop method of retting in suitable time not to be following obligatory seasonal retting. This study will help to develop novel technology for fiber extraction of preserved bark of jute.

2 Material and methods

Present study was conducted in Bangladesh Jute Research Institute (BJRI), Head office, Dhaka, Bangladesh using the equipments, chemicals and raw materials where all the chemicals and media were reagent grade (Sigma). Nutrient agar media (Sigma) was used through-out the study for isolation and growth purposes of microorganisms; Carboxy-Methyl-Cellulose (CMC), pectin and xylan agar media has been used for primary screening.

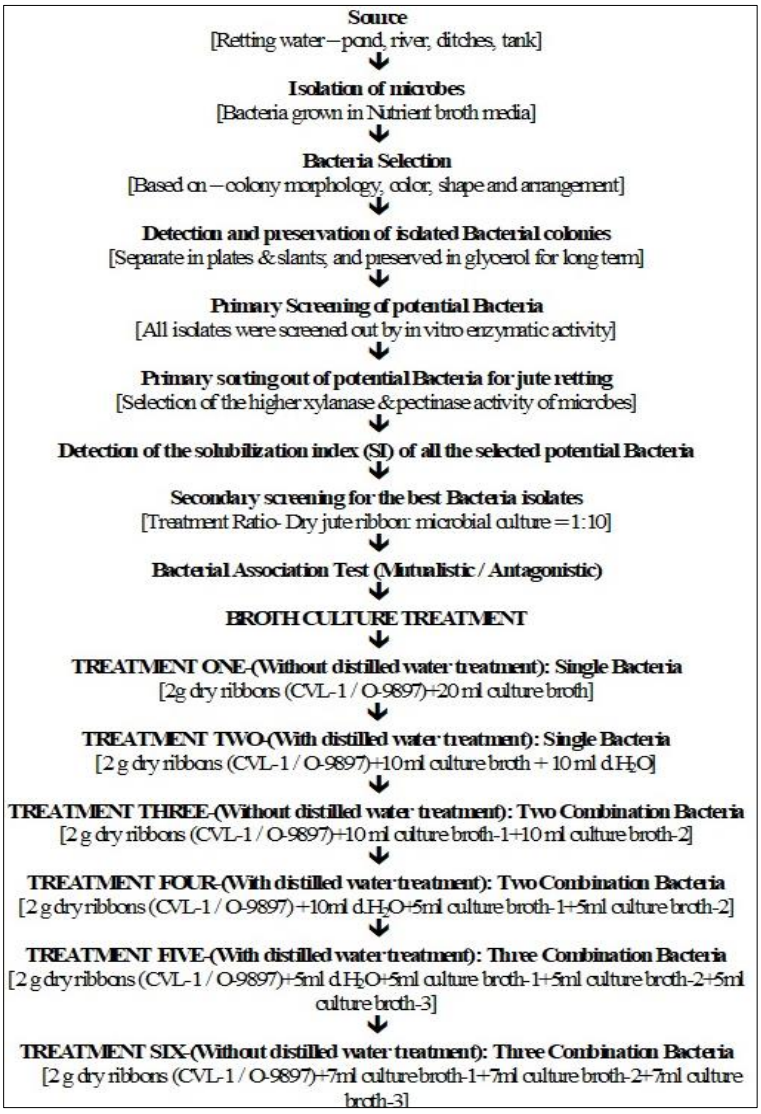


Figure 1 Flow diagram of work plan

A sequential work plan was generated - (a) study of enzymatic (cellulose/ pectinase/ xylanase) study of individual microbes (that are screened out and isolated from various jute retting sources), (b) study the preparation of consortia among the best microbes, and (c) study the application of consortia for retting of preserved jute ribbon/bark. Figure 1 shows the flow diagram of research plan.

2.1 Screening of microbes

The bacterial isolates were inoculated separately in 50ml nutrient broth medium (in 250 ml conical flasks) prepared for pectinases / xylanase screening. Each isolate was inoculated in 50ml nutrient broth media (pH 7.0). All cultures were cultivated at 37°C and 200 rpm for 48 h. Quantitative determination of pectinase and xylanase activities, in duplicates, as described below:

2.1.1 Screening for pectinase production

Screening for pectinase production was performed as described by Soares et al [4].

2.1.2 Screening for xylanase production

Xylanase production by the isolates was evaluated as described by Horikoshi [5] and Dhawale et al [6].

The efficiency of the pectinases and xylanases to solubilize pectin and starch respectively, and thus form halos around the colonies during the screening processes above, was determined qualitatively in terms of solubilization index (SI) value for each isolate calculated using the formula [7]:

$$SI = \frac{\text{Mean diameter of halo (Dh) (mm)}}{\text{Mean diameter of colony (Dc) (mm)}}$$

The obtained mean SI values were used to rank the isolates as excellent producers of the enzymes when the colonies presented halo sizes > 3.0; very good producers when the halos were 2.0 < SI ≤ 3.0; good producers when halos were 1.0 < SI ≤ 2.0; weak producers when halos were 0 < SI ≤ 1.0 (or not clear); producers of minor and unquantifiable enzymes when halo sizes cannot be determined and poor producers when no halos were observed.

2.2 Preparation of Bacterial consortia

Out of the collected isolates from different locations of jute retting water in different periods of time, 11 (eleven) isolates were selected for this experiment. Selected bacteria for consortium microbes were grown separately in nutrient broth (NB) as single culture and were manipulated in the following manners (Table 1):

Table 1 Different parameters used in present study

Dry ribbons	**CVL-1 jute**
Age of the plant	122 days
Weight of dry ribbons (*Corcorus capsularis* CVL-1)	2 g
Length of ribbon for retting	3 inch (middle part)
Incubation for retting	5 days
Retting temperature	40°C
Retting ratio	Dry ribbon : d.H_2O = 1 : 10
Optical Density (OD)	600 nm
Microbial selected samples (2 days grown)	15 DTW2b, OMEW4b, 3PPRF5, 4DTF1b, 10DTW2b, OMPW4b and 4DTW7b
Combinations of microbial consortia	Single isolate, two combinations of the isolates and three combinations of the isolates

A control was set up, consisting of distilled water instead of the inoculums. Three replicates were maintained under same condition.

2.2.1 Inoculums Preparation

The inoculums were prepared by inoculating one loopful of individual bacterial isolates, separately in 50 ml of sterilized nutrient broth. Inoculated broths were incubated in a shaker incubator at 40ºC for 24 h so as to obtain actively growing mother cultures. These mother cultures (1000 ml) were used for sub-culturing.

2.2.2 Formulation designing

Formulation design of consortium microbe using 7 (seven) selected bacteria. The microorganism that has the highest degradation potential was inoculated into minimal media broth. The minimal media broths were incubated for 48 h in shaker incubator.

2.2.3 Treatment

Bacterial broth was inoculated in dry ribbon of *C. capsularis* CVL-1 jute in the ratio of 1:10 in 500 ml conical flask. In order to check the activity of the single isolate under same conditions, blank containing only distilled water was paced. All the flasks were incubated in a shaker incubator at 40°C and 70 rpm for 48 h.

2.2.4 Consortia

Each consortium consists of two or three bacteria. Formulation of consortia was done by following permutation combination in which repetition was not allowed and order of the bacteria does not matter. Consortia were formulated by using the following formula:

$$\frac{n}{(n-r) \times r}$$

Where, n = total number of bacteria and r =bacteria in each consortia.

The isolates were grown separately in MB medium and processed to yield separate suspensions with an absorbance reading of 0.5 [8]. Control used in this study is ASW medium containing only 1% crude oil. Bacterial consortium was prepared by formulating 10 (ten) selected bacteria into 30 (thirty) combination. List of bacterial consortia is given in Table 2.

Table 2 Formulation of diiferent combinations of microbial inoculum

Treatments	**Mixture**	**Ratio (H_2O: Fiber wt.)**
Single microbe (without water)	20 ml microbial broth+2 g dried fiber = 20 ml	10:1
Single microbe (with water)	10 ml d.H_2O+10 ml microbial broth+2 g dried fiber (2 times) = 20 ml	10:1
	15 ml d.H_2O+5 ml microbial broth + 2 g dried fiber (4 times) = 20 ml	10:1
Combination of microbes (with water)	10 ml d.H_2O+(5 ml + 5 ml) microbial broth+ 2 g dried fiber = 20 ml (2 combination)	10:1
	5 ml d. H_2O+(5 ml + 5 ml+5 ml) microbial broth+ 1 g dried fiber = 20 ml (3 combination)	10:1
Combination of microbes (without water)	10 ml microbial broth-1 + 10 ml microbial broth-2+ 2 g dried fiber = 20 ml (2 combination)	10:1
	5 ml microbial broth-1 + 5 ml microbial broth-2 +5 ml microbial broth-3+2 g dried fiber = 20 ml (3 combination)	10:1

2.2.5 Application of Consortia

Application of inoculums on preserved jute bark was carried out as shown in the Table 2.

2.3 *In-vitro* study of synergistic or mutalisticeffect of consortia

These promising isolates were applied in all probable combination for antagonism and synergism by using standard method [9]. *In vitro* test was performed using dual culture method. Each bacterial consortium was grown on nutrient agar (NA) medium incubated for 24 h. One loopful of bacterial colony was streaked onto NA plate, then one test microbe was placed 3 cm away at the opposite of other bacteria. The treatments were arranged with three replications, consisted of treatments labeled -A1 (5DTW2b–4DTW7b–10DTW2b), A2 (4DTW7b–4DTF1b–OMEW4b), A3 (10DTW2b–4DTF1b-OMEW4b), A4 (OMEW4b–15DTW2b–3PPRF5b), A5 (4DTF1b-15DTW2b–3PPRF5b), A6 (3PPRF5b-4DTW7b–10DTW2b), A7 (OMPW4b-OMEW4b–3PPRF5b), A8 (15DTW2b-4DTW7b–10DTW2b) and A9 (4DTF1b-OMPW4b-3PPRF5b). Untreated control was prepared using water. The bacterial cells were harvested NA plates for 24 h. The inhibition growth was compared with the untreated controls. The inhibition percentage was determined using the following equation [9]:

$$Inhibition\ (\%) = \frac{C\text{-}T}{C}\ x100$$

Where, C = the colony diameter of the bacteria on the control plate; T = the colony diameter of the bacteria on the treatment plate

3 Results and discussion

The jute fiber was obtained from the dry ribbons of CVL-1 variety of *C. capsularis* by engineering microbial consortia (Fig 2). Amicrobial consortium is a group of different species of microorganisms that act together as a community. They are more resistant to environmental shock, andcan better compete and survive in the complex environment than single microorganism.

(a) CVL-1 dry ribbons

(b) Fibers obtained from dry ribbon treatment

Figure 2 Jute fiber (a) dry ribbon before the treatment (b) fiber after the treatment

As the first step, collection was screened out and formulates different possible combinations. Therefore, selected microbes were grown on CMC, pectin and xylan media where the zone of inhibition was measured accordingly in order to determine the enzymatic activity of these microbes. In primary screening and determination of enzymatic activity from retting bacteria, highest pectinase activity was found in 4DTW2b followed by OMPW4b and 4DTF1b; xylanase activity in 3PRRF2b followed by 10DTW3b and 10DTW2b; and cellulase activity in 4DTF1b followed by 10DTW2b and 10DTW3b (Fig. 3).

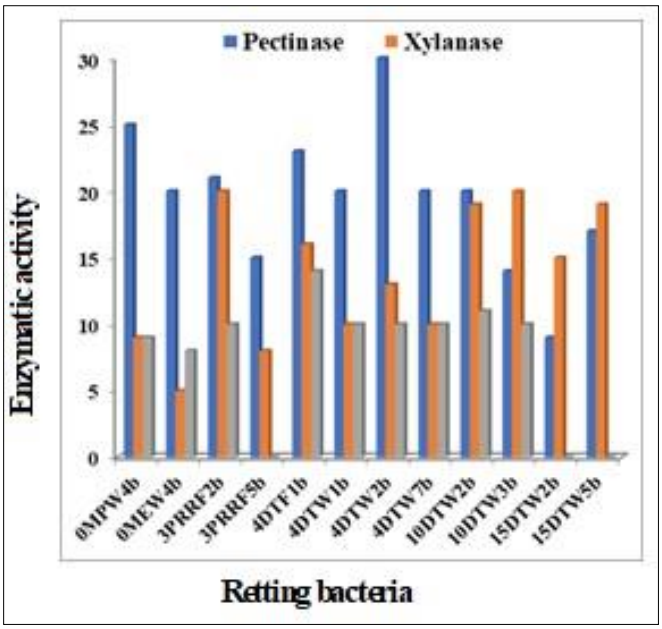


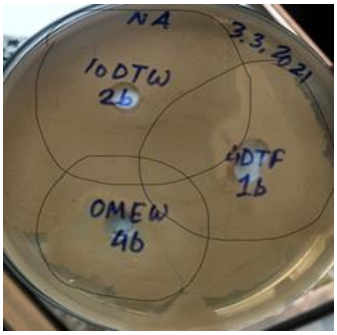
Figure 3 Determination of enzymatic activity (Solubilization index, SI) of retting microbes

In *in-vitro* study of bacterial consortia for their interrelationship effect on treatment, it was found that most of the combination of microbial consortia was mutalistic except, (15DTW2b+4DTW7b+10DTW2b), (15DTW2b+ OMEW4b+3PPRF5b) and (3PPRF5b+10DTW2b+4DTW7b) (Table 3, Fig 4). In a microbial consortium the organisms work together in a complex system where all are benefited from the activities of others in a community.The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have been widely demonstrated. It could be attributed to the effects of synergistic interactions among members of the association. It is possible that one species removes the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that the second species are able to degrade compounds that are partially degraded by the first [3]. Sarkar et al [10] also worked on effective degradation of organic kitchen wastes using microbial consortium. Yadi et al [11] worked on the effect interrelationship of bacterial consortia.

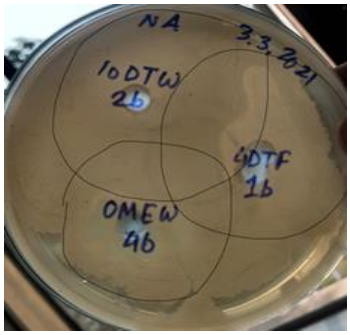
Table 3 Study of *in-vitro* effect of interrelationship among bacterial consortia

COMBINATIONS	SYNERGISTIC / MUTALISTIC
15DTW2b+4DTW7b+10DTW2b	Synergistic
4DTW7b+4DTF1b+OMEW4b	Mutalistic
4DTF1b+OMPW4b+3PPRF5b	Mutalistic
10DTW2b+4DTF1b+OMEW4b	Mutalistic
4DTF1b+3PPRF5b+15DTW2b	Mutalistic
15DTW2b+OMEW4b+3PPRF5b	Synergistic
3PPRF5b+10DTW2b+4DTW7b	Synergistic
OMPW4b+3PPRF5b+OMEW4b	Mutalistic
4DTF1b+4DTW7b+OMEW4b	Mutalistic
15DTW2b+10DTW2b+4DTW7b	Synergistic

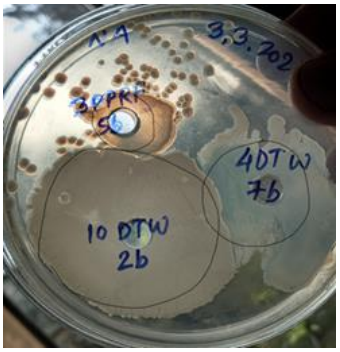
4DTF 1b-3PPRF 5b-15DTW2b



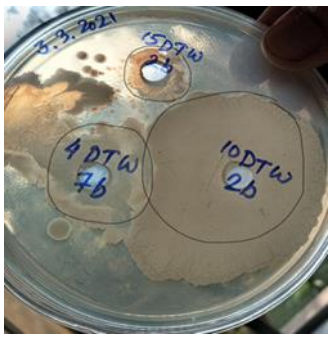
10DTW2b-OMEW4b-4DTF1b



4DTF1b-OMEW4b-10DTW2b

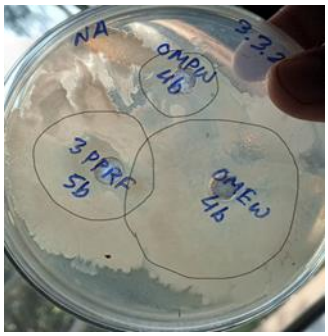


3PPRF5b-10DTW2b-4DTW7b

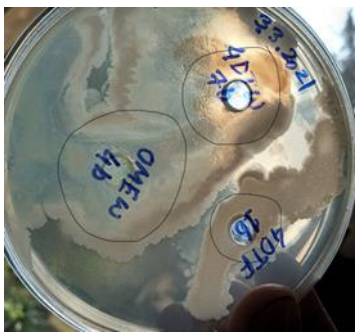


4DTW7b-10DTW2b-15DTW2b

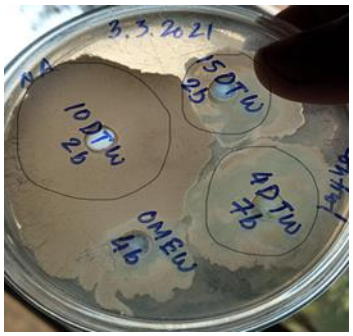
4DTF 1b-OMEW4b-3PPRF5b



3PPRF5b-OMEW4b-OMPW4b



OMEW4b-4DTF1b-4DTW7b



OMEW4b-4DTW7b-15DTW2b
10DTW2b

OMEW4b-3PPRF5b-15DTW2b

Figure 4 Synergistic and mutalistic relationship among different microbial consortia

In treatment with selected microbial broth (20 ml) without water (single microorganism) of preserved CVL-1 dry fiber, fineness was almost same in all the isolates but brightness and smoothness/ softness, both were found higher in OMEW4b, 3PRRF5b & 4DTF1b (Fig 5 a).

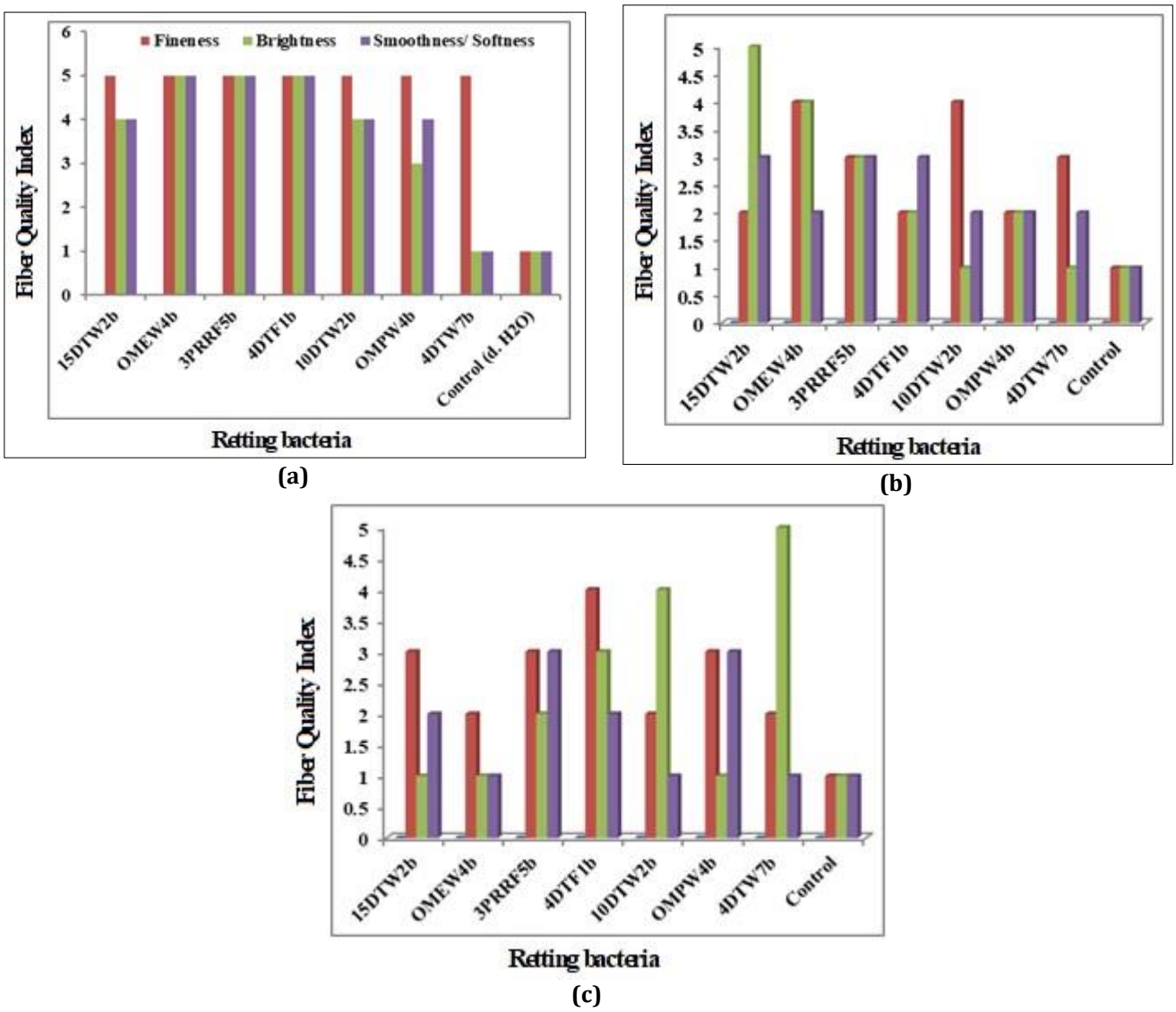


Figure 5 Treatment of single microbial broth with dry jute ribbon- (a) 20ml microbial broth without water; (b) 10 ml microbial broth with water; (c) 5ml microbial broth with water. Soft & touch method: 1= Poor, 2=Fair, 3=Good, 4= Very good, 5=Excellent

On the other hand, in case of treatment with selected microbial broth (10 ml) with water (10 ml d.H_2O+10 ml microbial broth+2 g dried fiber (single microorganism), fineness was found higher in 10DTW2b & OMEW4b, brightness was found higher in 15DTW2b followed by OMEW4b and smoothness/ softness was found higher in 15DTW2b, 3PRRF5b, 4DTF1b (Fig 5b). Moreover, in case of treatment with selected microbial broth (5 ml) with water (15 ml d. H_2O+5 ml microbial broth+2 g dried fiber), fineness was found higher in 4DTF1b followed by 15DTW2b and 3PPRF5b, brightness was found higher in 4DTW7b followed by 10DTW2b, and smoothness/ softness was found higher in 3PRRF5b and OMPW4b (Fig 5c). In treatment with selected microbial broth (5 ml+5 ml) of two combinations with water (10ml d. H_2O+ (5 ml+5 ml) microbial broth+ 2 g dried fiber), fineness was found higher in microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b) and (OMEW4b+10DTW2b); brightness was found higher in microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b), (OMEW4b+10DTW2b) and (3PRRF5b+4DTF1b); and smoothness/softness was found higher in microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b) and (OMEW4b+10DTW2b) (Fig. 6).

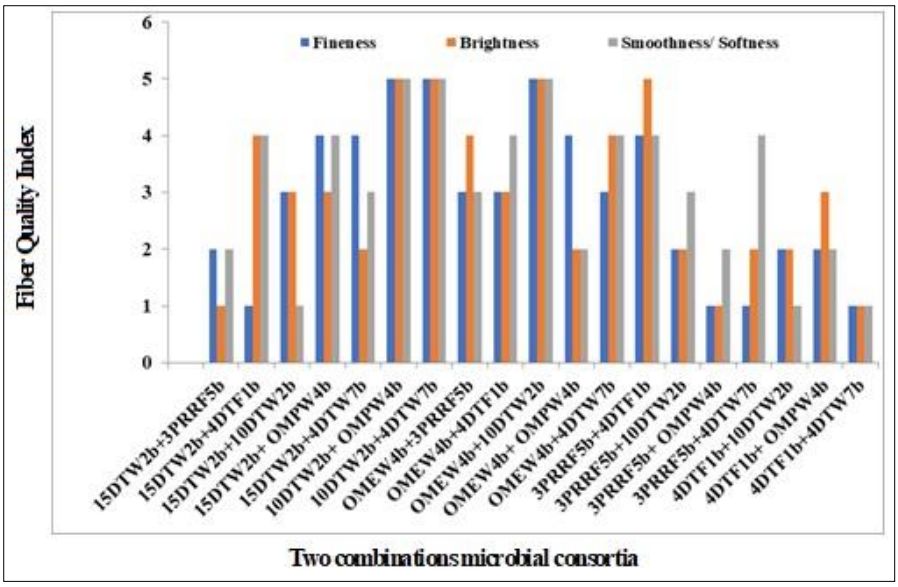
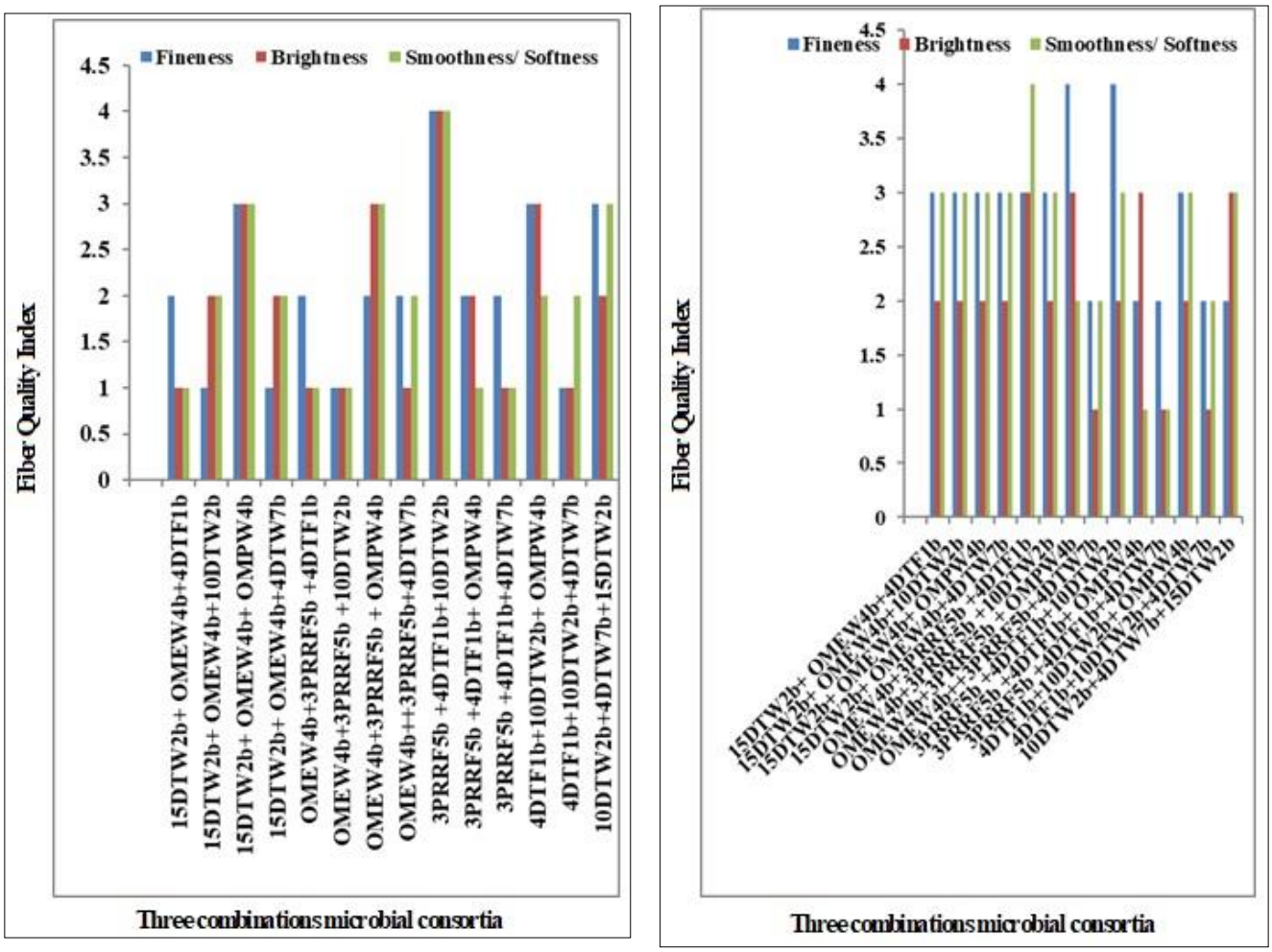


Figure 6 Treatment of two combinations microbial broth with dry jute ribbon. Soft & touch method: 1=Poor, 2=Fair, 3=Good, 4= Very good, 5=Excellent

It is noted that the microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b) and (OMEW4b+10DTW2b) were found better for all the cases. In treatment with selected microbial broth (5 ml + 5 ml + 5 ml) of three combinations with water (5 ml d. H_2O + (5 ml microbial broth-1+5 ml microbial broth-2 + 5 ml microbial broth-3) + 2 g dried fiber), fineness, brightness and smoothness / softness, all were found higher in microbial consortia of (3PRRF5b+4DTF1+10DTW2b) followed by (15DTW2b+OMEW4B+OMPW4b) (Fig. 7a), which is a unique findings. In treatment of dry fiber with selected microbial broth of three combinations without water (6.5 ml microbial broth-1 + 6.5 ml microbial broth-2 + 6.5 ml microbial broth-3 + 2 g dried fiber), fineness was found higher in microbial consortia of (OMEW4b+3PRRF5b+OMPW4b) and (3PRRF5b+4DTF1b+10DTW2b); in case of brightness, four microbial consortia of (OMEW4b+3PRRF5b+4DTF1b), (OMEW4b+3PRRF5b+OMPW4b), (3PRRF5b+4DTF1b+OMPW4b) and (10DTW2b+4DTW7b+15DTW2b) were equally showed higher results.

On the other hand, smoothness / softness was found almost same in all the microbial consortia except (3PRRF5b+4DTF1b+OMPW4b) and (3PRRF5b+4DTF1b+4DTW7b) (Fig. 7b). As higher pectinase and xylanase activity is required in this experiment, so 3PRRF2b, 10DTW3b, 10DTW2b, 4DTW2b, OMPW4b and 4DTF1b are suitable for the preparation of microbial consortia. Again, from the results of interrelationship effect on treatment, 10DTW2b and OMPW4b found antagonistic effects. It is noted that in the two combination treatment with water (5 ml), microbial consortia of (10DTW2b+OMPW4b), (10DTW2b+4DTW7b) and (OMEW4b+10DTW2b) were found better for all the cases. Again, in three combinations treatment with water (5 ml d.H_2O), fineness, brightness and smoothness / softness, all were found higher in microbial consortia of (3PRRF5b+4DTF1b+10DTW2b), which is a unique findings. Yetti et al [12] formulated a bacterial consortium for degradation of oil compounds where they constructed microbial consortium from 4 (four) selected marine oil bacteria to become 15 (twelve) combination culture and in which, the strains were selected based on the criteria that they were able to display goodgrowth in crude oil containing media; they found that five bacterial formulations showed good potential as candidates for microbial consortium. Microorganism found in retting water is postulated to have the potential to degrade proteins and starch in the organic material found in dry jute ribbon. This on-going research opined that these breakthrough findings will help to further the knowledge on the unique microbial retting process in jute and will accentuate the improvement in this microbial formulation. For example, the genes for degrading pectin, hemicellulose and other non-cellulosic materials can be altered for enhanced retting efficiency and shortening the retting duration with minimal water usage. It is believed that this will also open up an avenue to characterize the enormous diversity of retting microbial population at the metagenome scale and incorporate other strains to complement the consortium. This will establish a correlation between the microbial diversities and regional differences in fiber. In inoculums development, the methods must aims at minimizing the loss of viable microorganisms during recovery from dormancy stage, obtaining a genotypically identical copy of the population that was stored, increase biomass and the development of culture to a physiological state suitable for performance in the final production stage. Many researchers reported that even though microorganisms are highly adapted specific microbial types are associated with different niches or samples within a variety of ecosystem. Various reports are available on the study of microbial consortia for their biotechnological application. Sarunyou et al [13], showed degradation of lignocellulosic agro-industrial residues by means of complex microbial community and had shown it as

a promising approach providing efficient biomass decomposition for subsequent conversion to value added products. In previous studies, it was found that mixed bacterial consortium showed more degradation than individual strains [8, 14, 15]. Puentes-Téllez and Salles [16] constructed minimal effective consortia from environmental samples with optimal degradation levels as the ones found in consortia built with a higher number of community members. Vats et al [17] extracted blend of enzymes from microbial consortia and showed degradation of lignocellulosic biomass. Fungus and bacteria in combination were also used by some researchers to increase the degradation rate. Microorganisms that were isolated from different location of sampling will be different from one another and microorganism that is isolated from one location may not be able to ret jute from another location, owing much too environmental factors such as humidity, temperature etc. An example of cellulolytic and amylolytic bacteria that had been successfully isolated and screened for its ability to degrade cellulose and amylase is the *Bacillus amylolique*/ *adens*. This bacterium had been isolated from sago pith waste. It has both the cellulolytic and amylolytic activities in the decomposition of sago pith residue [18].



(a) **(b)**

Figure 7 Treatment of three combinations of microbial broth (a) with 5ml water; (b) without water. Soft & touch method: 1=Poor, 2=Fair, 3=Good, 4=Very good, 5=Excellent

The drawback of above mentioned studies was the time taken to complete the process which directly affects the total cost of the treatment. But the present experiment took less time to degrade jute ribbon and separate the fiber. The bacterial strains are also non-toxic and thus the retting water with microbial strains can successfully be used for irrigation purpose. This is on-going research and has potential future. The use of consortium as inoculants is an efficient approach to obtain jute fiber with less amount of water. The role of consortium exerts beneficial effects on fiber extraction and development. Further studies of in-depth genomic analysis are needed. It has a long way future plan to introduce microbial consortia package which can be an added market value.

4 Conclusion

This is on-going research which has positive indication and potential future. It has a long way future plan to introduce microbial consortia package which can be an added market value and solve the retting water problem for jute retting.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no competing interests.

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