Original Research

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Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/40/2/MRN-854

Use of bacterial endospore with longer shelf-life in improved retting of jute

Paper received: 06.02.2018 *Revised received*: 28.05.2018 *Re-revised received*: 27.06.2018 *Accepted*: 13.08.2018

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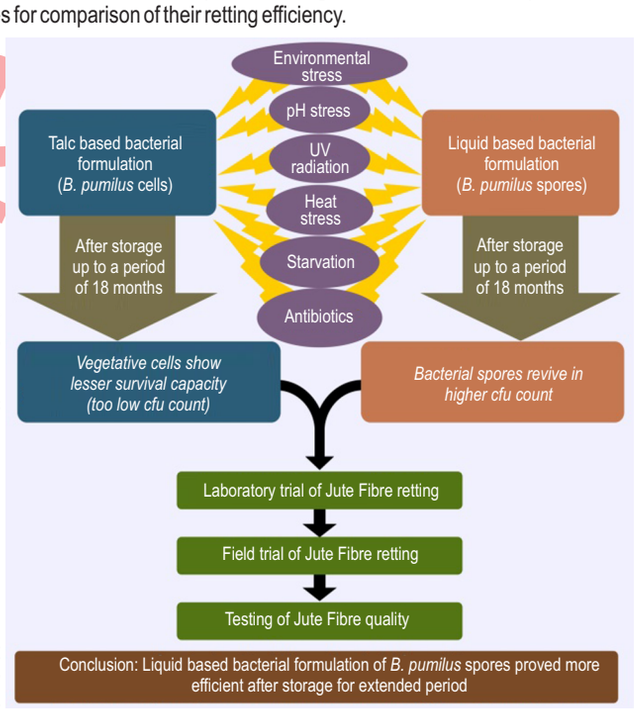
Abstract

Aim : The aim of the present study was to evaluate the retting of jute (*Corchorus olitorius* L. and *C. capsularis* L.) using the endospores of microbial consortium of three strains of *Bacillus pumilus* with extended shelf-life.

Methodology : Endospore and vegetative cells of *Bacillus pumilus* were tested for viability by introducing them into different temperature, pH, UV radiation and antibiotics. Laboratory, as well as field-trials of jute retting was performed with 6 and 18-months-old endospores and vegetative cells of *Bacillus pumilus* with estimation of enzymatic activities for comparison of their retting efficiency.

Results: Endospores of *Bacillus pumilus* recorded very high colony forming unit (10^9 to $10^8 ml^{-1}$) compared to their vegetative cells (10^6 to $10^4 ml^{-1}$) after 6 to 18 months of their preservation. Endospores also showed higher resistance to temperature, pH, UV irradiation and antibiotic than their vegetative forms. High colony forming unit and higher release of pectinolytic and xylanolytic enzymes during retting of jute by endospores resulted in complete of jute retting in 10 days with good quality jute fibre compared to talc based formulation.



Interpretation: It can be concluded from the study that endospores remained highly efficient in rejuvenating higher CFU and quantitatively larger pool of enzymes to accelerate retting of jute after prolonged preservation. Therefore, the endospores of *Bacillus pumilus* can be used cost effectively in place of their talc based formulation for higher shelf life of the product, faster retting and better fibre quality of jute.

Key words: *Bacillus pumilus,* Carrier based inoculants, Endospore, Jute retting, Shelf-life.

How to cite: Chattopadhyay, L., B. Majumdar, S.P. Mazumdar, A.R. Saha, R. Saha and S. Barai : Use of bacterial endospore with longer shelf-life in improved retting of jute. *J. Environ. Biol.*, **40**, 245-251 (2019).

Introduction

Jute retting involves controlled decomposition of the non-fibrous materials (pectin, hemicelluloses etc.) with the help of enzymes secreted by aquatic microbes. Although jute retting is better in slow moving soft water, only 10 % of jute growers of India have access to this and the remaining 90% ret their harvest in stagnant water bodies like lakes, ditches and ponds. Use of microbial inoculants to hasten the jute retting in stagnant water retting has been reported by several workers (Banik *et al.,* 2007; Das *et al.,* 2012; Das *et al.,* 2015; Das *et al.,* 2018). The major drawback in using the carrier based microbial inoculants either as biofertilizer/ biopesticides or bioformulation is their short shelf life, as the microbial cells cannot withstand environmental stress for prolonged period. In most of the cases, the shelf life of solid carrier based inoculant is about six months,which can be extended up to two years by making liquid formulation (Mahdi *et al.,* 2010; Pindi and Satyanarayana, 2012).

Endospores of various Bacillus species are formed during sporulation, a process that is generally induced by reduced levels of nutrients, high mineral composition, neutral pH, temperature and high cell density in the environment (Sella *et al.,* 2014). Sporulation generates a non-reproductive, dormant structure which can survive for extended periods with little or no nutrients. Endospores have very different structural and chemical composition than that of a growing cell (Mohammadi and Sohrabi, 2012). They have thick spore like structure with exosporium, spore coat, cortex and core wall, and exhibit a high degree of resistance to ionizing (γ) radiation, UV radiation, dry and wet heat and various chemicals that can cause DNA damage. These properties are achieved because of their low water content, presence of dipicolinic acid (5-15% of spore dry weight) and a group of small acid soluble spore proteins (SASP of α/β type). Bacterial spores, despite being metabolically dormant, have remarkable capacity to detect nutrients and other molecules in their environment through a biochemical sensory apparatus which can trigger spore germination to vegetative cells within minutes of exposure of germinants (Thirumurugan and Asha, 2010).

Use of talc based pectinolytic microbial formulation was successfully demonstrated for faster retting of jute and fibre quality improvement across the jute growing states of India (Majumdar *et al.,* 2013; Majumdar and Satpathy, 2014). The talc based microbial formulation consisted of three strains of *Bacillus pumilus* viz., *Bacillus pumilus* IMAU80221; *Bacillus pumilus* GVC 11 and *Bacillus pumilus* SYBC-W (Das *et al.,* 2015). In the present study, an attempt was made to increase the shelf life of microbial consortium by using their endospores for accelerating the retting process and fibre quality improvement.

Materials and Methods

Endospore preparation: Endospores were prepared by cultivating three strains of *Bacillus pumilus. Bacillus pumilus* IMAU80221 (MTCC 5573), *Bacillus pumilus* GVC 11 (MTCC 5574) and *Bacillus pumilus* SYBC-W (MTCC 5575) cells were taken separately in 500 ml Erlenmeyer flasks with 100 ml sporulation broth medium (pH-7.6). The sporulation broth medium composed of sporulation broth 15.8 g l^{-1} (Hi-Media, Mumbai, India), 1 ml each of 0.05% $MgSO_4$. $7H_2O$, 0.2% KCl and 1M Ca $(NO_3)_2$, 0.01M $MnCl_2$ and 1mM $FeSO_4$. Batch cultures were incubated at 34˚C in rotary shaker at 150 rpm.

Bacterial growth was monitored via optical density measurements at 600 nm, using UV-visible spectrophotometer and sporulation was checked by a phase contrast microscope (EVOS XL CORE, Thermo Fisher Scientific). Endospores were harvested when they accounted for at least 90% of total population (generally on the 4th day), and stored at 4˚C in sterile distilled water (Nicholson and Setlow, 1990). To germinate the bacterial spores into vegetative cells, pectin agar medium (pH 8) was used containing 1% citrus pectin, 0.5% peptone, 0.5% NaCl, 0.02% $MnSO_4$, 0.04% KH_2PO_4 and 2% agar powder.

Viability count of talc-based microbial formulation and endospores: After six and eighteen months of preparation, the viability of spores in suspension and bacterial cells in carrier-based microbial formulation was checked by serial dilution method. Microbial formulation and spore suspensions were serially diluted in sterile distilled water and plated on pectin agar plates, incubated for 48 hr at 34°C, and then the CFUs were counted using a digital colony counter.

Monitoring of pH resistance of bacterial cells and endospores: To monitor the degree of pH resistance, pectin agar media plates with pH values 3, 8, 10 and 12 were prepared. A 100µl of three different types of endospores and their respective bacterial cells were plated on pectin agar plates with varying pH and incubated for 48 hr at 34°C. After incubation, CFUs were counted with a digital colony counter.

Monitoring temperature resistance: To study the temperature resistance, 1ml each of bacterial cells and endospores were incubated at 60, 80 and 100°C, respectively, for 15 min using water bath. The bacterial cells and endospores were then incubated for 48 hr at 34°C on pectin agar plates. After completion of incubation, CFUs were counted with a digital colony counter.

Monitoring viability in UV radiation: To monitor the resistance to UV radiation, a 100µl each of bacterial cells and endospores were placed on pectin agar plates and exposed under UV light (254nm) for 10, 15, 20 and 30 min, respectively. The UV exposed plates were then incubated for 48 hr at 34°C. After completion of incubation, CFUs were counted with a digital colony counter.

Antibiotic sensitivity test: The antibiotics *viz.,* streptomycin, tetracycline, ampicillin and amoxicillin were used at concentrations of 5, 10, 30, 60, 120 and 240 µgml^{-1}. To detect the antibiotic susceptibility of endospores, 1ml each of the spore suspension was centrifuged and the pellet was re-suspended again with 1ml each of the said concentrations of

antibiotics and then incubated overnight at 4°C. After incubation, a 100µl of each spore suspension were spread over pectin agar plates to view the endospore population. The susceptibility of bacterial cells was also checked with antibiotic strips (Hi Media) of the said concentrations.

Small scale retting: Six month old spore suspensions and their vegetative cells in talc based formulation with a combination of 1:2:1 v/v for MTCC 5573, MTCC 5574 and MTCC 5575 were used for retting of jute plants, as this combination had been reported previously for highest activities of polygalacturonase, pectin lyase and xylanase enzymes (Das *et al.,* 2015). For each set, one kilogram of whole jute plant was immersed into 10 l sterile pond water. In Set 1, 4 ml of spore formulation (1:2:1 v/v with a CFU $10^9 ml^{-1}$) and in Set 2, 2 ml (1:2:1 v/v with a CFU $10^9 ml^{-1}$) of spore formulation was added. Set 3 contained 2 g of regular talc-based microbial formulation. The study was replicated thrice for statistical validation.

Large scale retting: After preservation of bacterial spores and talc based formulation for 18 months, a large scale field trial was carried out in triplicate with whole jute plants, grown under standard field conditions, in a set of three large concrete retting tanks. Whole jute plants, after harvesting, were kept for leaf shedding and then 450 kg of it was immersed in each retting tank containing 4500 l of underground water. Bacterial spore suspensions were used with a combination same as applied in laboratory scale study. A 1.8 l and 900 ml of spore suspension was used in tank I and tank II, respectively, while 900 g of talc based formulation was used in tank III.

Enzyme activity assays of retting liquor: Polygalacturonase and xylanase activities were measured and CFU counts were monitored throughout the retting process after collection of retting liquor samples from small and large scale retting trials. Extracellular polygalacturonase was measured by DNS method (Phutela *et al.,* 2005) and that of xylanase by reducing sugar estimation method using 3, 5 dinitro salicylic acid (Manisha *et al.,* 2009).

Retting water samples after serial dilution, pour-plated on pectin agar plates to monitor the growth of pectinolytic bacteria. Similarly, xylanolytic isolates were estimated by plating retting water samples in xylan agar (0.5%, xylan, 0.5% yeast extract, 0.5% NaCl, 2% agar, at pH 8.0). The plates were incubated for 72 hrs at 34°C and then colonies were counted by a digital colony counter.

Fibre quality testing: After completion of retting, fibre samples were extracted, washed with clean water and then dried under the sun to record the dry weight of fibre. The fibre samples were processed and then the bundle fibre strength was measured following the method of Roy *et al.* (2009), and fibre fineness by airflow method of Bandyopadhyay and Sinha (1968).

Statistical analysis: The data were analysed by using Sigma plot 11.0 for estimating the standard deviations.

Results and Discussion

The endospores of three strains of *Bacillus pumilus* maintained their CFU number after 6 months of storage at 4°C. The CFU of endospores ranged between 11 to 20 x $10^9 ml^{-1}$ after 6 months compared to their initial CFU of 25 to 32 x $10^9 ml^{-1}$. The CFU count decreased marginally after 18 months of storage to 16 to 18 x $10^8 ml^{-1}$. On the other hand, the CFU count of the vegetative forms of *Bacillus pumilus* in talc based formulation decreased drastically from initial CFU of 12 x $10^{10} ml^{-1}$ to 14 x $10^6 ml^{-1}$ after 6 months and 23 x $10^4 ml^{-1}$ after 18 months of storage, respectively. The difference in structural and chemical composition and over all, the metabolic state of the spores from corresponding vegetative cells provided them the distinct resistance power against environmental stress (Sella *et al.,* 2014) which eventually helped them to survive for longer period of time as in this case. Higher shelf life of spores in hydrated condition was also reported by Stelow (2007) and Leggett *et al.* (2012).

Endospores of *B. pumilus* also showed higher level of pH tolerance compared to their vegetative forms (Table 3). After incubation, endospores showed higher growth at pH 8 and pH10, moderate growth at pH 3 and pH 12; whereas, their vegetative forms grew well only at pH ranging between pH 8 to 10, while no growth was observed for vegetative cells at very low pH (pH 3) and very high pH (12). The inner membrane of endospores composed of lipids that acted as impermeable layer to small molecules, including water, which might have protected the core from various chemicals (Paredes-Sabja *et al.,* 2011; Leggett *et al.,* 2012). This in turn also helped the spores to survive at a wider pH range.

The data in Table 3. clearly indicates that bacterial endospores were quite able to withstand higher temperatures (moist heat) than their vegetative counterparts. For all three bacterial spores, the number of colonies per plate was >300 even after 15 min of incubation at 100°C, whereas their vegetative cells could only withstand lower temperatures (60°C and 80°C). Bacterial cells did not propagate suitably when introduced into boiling water bath, and counted less than 10% of the spores at 100°C. The addition of divalent ions like Ca^{2+}, Mg^{2+} and Mn^{2+} in the sporulation media might have increased the wet heat resistance of the endospores of *B. pumilus* by affecting the peptidoglycan composition and core dehydration (Atrih and Foster, 2001; Cazemier *et al.,* 2001; Hornstra *et al.,* 2009) compared to their vegetative cells. Occurrence of large amounts of SASPs in the core of endospores resulted in binding of these proteins to the DNA of endospores and these SASPs prevented the breakdown of DNA at high temperature by physical shielding (Nicholson *et al.,* 2000).

Endospores and bacterial cells of *B. pumilus* were exposed to UV radiation, respectively, for 10, 15 and 20 min. The result presented in Table 3. clearly states that the UV radiation had affected the vegetative cells and spores, but severe damage was noticed on vegetative cells compared to their corresponding

Table 1 : Changes in pectinolytic and xylanolytic bacterial population and polygalacturonase and xylanase enzyme activities in laboratory scale study of jute retting

Days of retting	Day 1	Day 5	Day 7	Day 10	Day 12
			Pectinolytic bacteria (CFU ml^{-1})		
Set 1	33 x 10^3	180 x 10^7	54 x 10^{10}	147 x 10^{12}	52 x 10^{13}
Set 2	28 x 10^3	55 x 10^5	34 x 10^5	21 x 10^8	46 x 10^9
Set 3	32 x 10^3	51 x 10^5	11 x 10^7	10 x 10^8	25 x 10^9
			Xylanolytic bacteria (CFU ml^{-1})		
Set 1	25 x 10^3	44 x 10^7	58 x 10^9	90 x 10^{11}	67 x 10^{13}
Set 2	41 x 10^3	48 x 10^5	78 x 10^6	92 x 10^8	16 x 10^{10}
Set 3	23 x 10^3	20 x 10^5	11 x 10^7	25 x 10^8	72 x 10^{10}
			Polygalacturonase activity (U ml^{-1})		
Set 1	0	20.8±0.612	48.55±1.65	93.64±0.343	104.04±1.09
Set 2	0	12.71±0.49	28.09±1.22	50.86±0.52	58.95±1.8
Set 3	0	3.46±0.494	11.56±0.598	21.96±1.3	31.21±0.37
			Xylanase activity (U ml^{-1})		
Set 1	0	10.4±0.524	18.49±0.88	36.99±0.68	50.86±0.88
Set 2	0	4.62±0.26	8.09±0.15	20.81±1.61	30.06±0.79
Set 3	0	1.16±0.09	3.47±0.21	8.09±0.14	12.72±0.66

Values are mean ± SD in each row for particular set

Table 2 : Effect of endospores and talc based formulation of *Bacillus pumilus* on retting duration, fibre recovery, fibre strength and fibre fineness of jute

Test set	Fibre strength (gtex^{-1})	Fibre fineness (tex)	Retting duration (days)	Fibre recovery (%)
		Laboratory scale (After 6 months of storage)		
Set 1	27.80 ± 2.21	2.40± 0.11	10	7.2
Set 2	25.71 ± 0.59	2.54 ± 0.44	12	6.8
Set 3	25.11 ± 0.94	2.61± 0.05	15	6.2
		Field study (After 18 months of storage)		
Set 1	27.60± 1.65	2.51± 0.16	10	7.3
Set 2	25.60± 1.38	2.62± 0.13	12	6.9
Set 3	21.80± 1.22	2.84± 0.10	21	5.5

Values are mean ± SD in each row for particular set

spores. The population of vegetative cells reduced drastically after 20 min of exposure to UV radiation, but at the same time the spore count was15 times higher than their vegetative cells. Reduction in population of vegetative cells under UV radiation was mainly due to the interference of UV radiation with the transcription and translation of nucleic acid. Higher resistance (5 to 50 times) of spores of various *Bacillus* species against UV radiation compared to their vegetative cells was also reported by Stelow (2001). The increasing UV resistance in spores is primarily because of difference in the UV photochemistry of DNA in spores, and the efficient and relatively infallible repair of the novel photoproduct formed by UV light in spore DNA (Nicholson *et al.,* 2000). The prime UV photoproduct formed in DNA of vegetative cells and spores by 254-nm UV irradiation is a *cis* (*syn*-cyclobutane type thymine dimer) and SP (thymine adduct 5-thyminyl-5, 6-dihydrothymine), respectively (Nicholson *et al.,* 2000). This unique structure formed in spores provides the resistance power to the spores towards UV radiation.

A wide difference was observed in the minimum inhibitory concentration (MIC) of antibiotics for the vegetative cells of *B. pumilus* compared to their respective endospores (Table 3). The vegetative cells of *B. pumilus* could withstand very little concentration of antibiotics; therefore; the corresponding MIC value was low. Among the strains of *B. pumilus,* MTCC 5574 recorded moderately higher MIC value in amoxicillin (30 µg ml^{-1}) and streptomycin (60 µg ml^{-1}), whereas MTCC 5575 recorded comparatively higher MIC values in ampicillin (8 µg ml^{-1}) and tetracycline (10 µg ml^{-1}). On the other hand, at the highest concentrations (240 µg ml^{-1}) used for each of the antibiotics under

study, the growth of endospores of *B. pumilus* was found unaffected, indicating the fact that the spore formation facilitated the life form regardless of the presence or absence of antibiotics. This might be due to less permeability of the outer and inner membranes of spores (Paredes-Sabja *et al.,* 2011; Leggett *et al.,* 2012).

In laboratory trial, it was observed that spores germinate into their vegetative forms under favourable condition and secrete extracellular pectinolytic and xylanolytic enzymes, indicated by higher CFU counts on the pectin agar as well as xylan agar plates (Table 1) during the entire retting period. The test sets (Set 1 and Set 2) were inoculated with endospores. The viable cell count increased significantly and reached up to 10^{10} cells ml^{-1} (Set 1) and 10^{8} cells ml^{-1} (Set 2) within one week whereas in talc-based set (Set 3) the CFU count was 10^{7} ml^{-1} (Table 1). Higher CFU in Set 1 and Set 2 compared to Set 3 was due to the higher germination of endospores of *Bacillus pumilus* to their vegetative cells immediately after getting the suitable environment in terms of nutrients etc. Higher germination of *Bacillus* spp. in response to nutrients was also reported by Wang *et al.* (2015). The CFU counts of pectinolytic and xylanolytic bacteria in Set 1 after 12 days of retting were 52 x 10^{13} and 67 x 10^{13} per ml of retting water, respectively, which was significantly higher as compared to Set 2 and Set 3 at the same time (Table 1). The presence of higher CFU in Set 1 was also reflected in its corresponding rise in polygalacturonase and xylanase enzyme activities compared to that of Set 2 and Set 3. On day 7, Set 1 showed significantly higher polygalacturonase (48.55 U ml^{-1}) and xylanase (18.49 U ml^{-1}) enzyme activity over Set 2 (28.09 U ml^{-1} and 8.09 U ml^{-1}) and Set 3 (11.56 U ml^{-1} and 3.47 U ml^{-1}). The higher polygalacturonase and xylanase activities in Set 1 on day 7 might have been due to the persistence of significantly larger copy number in spore suspensions after six months of preparation. The Set 1 also showed highest polygalacturonase and xylanase activity on day 12, which was 104.04 U ml^{-1} (1.76 and 3.33 times higher than Set 2 and Set 3) and 50.86 U ml^{-1}, respectively (1.69 and 3.99 times higher than Set 2 and Set 3).

In large scale field study with 18-month-old endospores and talc based formulation, the CFU number of pectinolytic bacteria reached up to 25 x $10^{14}$$ml^{-1}$ in Set 1 and 9 x $10^{13}$$ml^{-1}$ in Set 2 after 12 days of retting from the initial value of 15 x $10^{3}$$ml^{-1}$ and 12 x $10^{3}$$ml^{-1}$, respectively. Similarly, the CFU count of xylanolytic bacteria reached up to 17 x $10^{9}$$ml^{-1}$ and 9 x $10^{7}$$ml^{-1}$ from the initial value of 32 x 10^{2} and 27 x $10^{2}$$ml^{-1}$, respectively, after 12 days of retting in Set 1 and Set 2. However, in Set 3, the CFU number of pectinolytic and xylanolytic bacteria reached only up to 13 x 10^{8} and 15 x $10^{6}$$ml^{-1}$ after 12 days of retting because of their significantly lower initial CFU counts. This was also reflected in the polygalacturonase and xylanase activity of three sets (Fig 1a, 1b). There was a sharp increase in polygalacturonase and xylanase activity throughout the retting period in Set 1 and 2 compared to

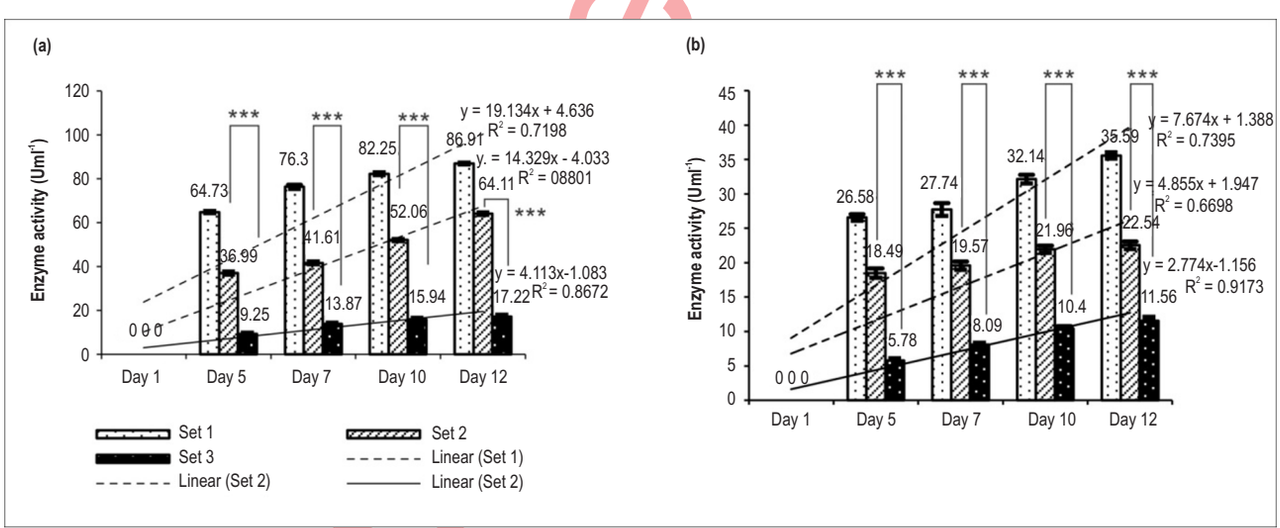


Fig. 1 : **(a)** Changes in polygalacturonase activity during large scale retting: Test results were recorded on 5th, 7th, 10th and 12th day after inoculation. Although in all three sets a gradual increase in enzyme activity had been observed as days passed, but the most steep increase was detected in case of set 1, as perceived from the straight line equation (slope, m = +19.134). The slowest change in enzyme activity was detected in set 3 (slope, m = +4.113). The set 2 scored much closer to the set 1 (slope, m = +14.329). Set 2 was found to show significantly higher (*** = p<0.001) polygalacturonase activity as compared to set 3. From this, it was also evident that set 1 would definitely bear a significant difference with set 3;

(b) Changes in xylanase activity during large scale retting: A similar pattern of enzyme activity increment with time could be observed in xylanase activity assay as well. Here also, the most steep increase was detected in case of set 1 (slope, m = +7.674) and the slowest change in xylanase activity was noticed in set 3 (slope, m = +4.855). Though Set 2 slope was much lesser than set 1, the set 2 was found to show significantly higher (*** = p<0.001) polygalacturonase activity as compared to set 3. Here also it was evident from the graph that set 1 would definitely bear a significant difference with set 3. Data used as mean ± SD of enzyme activity (Uml^{-1}).

Table 3 : Effect of pH, temperature, UV radiation and antibiotics on endospores and vegetative cells of *B. pumilus*

Sample	pH 3	pH 8	pH 10	pH 12
MTCC 5573	0	>300	147 ± 6	0
MTCC 5574	0	>300	152 ± 5	0
MTCC 5575	0	>300	159 ± 8	0
MTCC 5573 spore	>300	Lawn	Lawn	>300
MTCC 5574 spore	>300	Lawn	Lawn	>300
MTCC 5575 spore	>300	Lawn	Lawn	>300
Sampie	**60°C**	**80°C**		**100°C**
MTCC 5573	TNTC	145 ± 6.06		25 ± 1.83
MTCC 5574	TNTC	152 ± 2.58		21 ± 1.41
MTCC 5575	TNTC	137 ± 4.69		18 ± 2.58
MTCC 5573 s.pore	Lawn	Lawn		>300
MTCC 5574 spore	Lawn	Lawn		>300
MTCC 5575 spore	Lawn	Lawn		>300
Sample	**Time of exposure**			
	10 min	**15 min**		**20 min**
MTCC 5573	247 ± 5.35	192 ± 7.83		18 ± 6.48
MTCC 5574	238 ± 6.22	173 ± 5.83		17 ± 5.6
MTCC 5575	256 ± 5.48	212 ± 6.98		15 ± 4.69
MTCC 5573 spore	TNTC	>300		272 ± 11.22
MTCC 5574 spore	TNTC	>300		259 ± 10.49
MTCC 5575 spore	TNTC	>300		282 ± 7.61
Sample	**Antibiotics (MIC µg ml^{-1})**			
	Ampicillin	**Amoxycillin**	**Tetracycline**	**Streptomycin**
MTCC 5573	2	0.01	0.01	10
MTCC 5574	2	30	0.1	60
MTCC 5575	8	0.01	10	30
MTCC 5573 spore				
MTCC 5574 spore	**Growth was up to 240 µg ml^{-1}**			
MTCC 5575 spore				

Values are mean of replicate ± SD; TNTC= Too numerous to count, MIC = Minimum inhibitory concentration

Set 3, and the enzymatic activities were higher several times compared to Set 3 at different sets of time. Higher polygacturonase and xylanase in Set 1 and Set 2 was due to higher copy number of pectinolytic and xylanolytic microbes at every stage of retting, compared to Set 3.

In laboratory scale study, with 6-month-old endospores and talc based formulation, the retting of jute was completed in 10 and 12 days for Set 1 and Set 2, whereas the retting of Set 3 was accomplished in 15 days (Table 2). The fibre recovery was also higher in case of endospores than talc based formulation. In Set 1, fibre recovery was 7.2% which was 16.1% higher over Set 3. The fibre strength was very high in case of Set 1 (27.8 gtex^{-1}) which was 10.75% higher over Set 3 (25.11 gtex^{-1}). In case of large scale field study with 18-month-old endospores and talc based formulation, the retting of jute was completed in 10 and 12 days with two different doses of endospores and in 21 days with talc based formulation (Table 2). The fibre recovery was 7.3% in case of Set 1, while it was only 5.5% in case of talc based formulation. The significantly lower CFU counts and enzymatic activities during retting (Fig. 1a, b) in case of talc based formulation led to higher retting duration, and subsequently lower fibre recovery compared to endospores. This was also reflected in strength and fineness of fibre.

The lower the retting duration, the higher was the fibre recovery, and consequently higher fibre strength was observed in case of retting with endospores as compared to the talc based formulation.The reason behind this might be the sustenance of higher copy number (refers to the CFU count), significantly higher polygalacturonase and xylanase activities during retting with endospores as shown in Table 1 and Fig. 1a, b. In a comparative

study of jute retting, use of talc based microbial formulation resulted in higher CFU count, polygalacturonase and xylanase activities compared to conventional retting without any microbial formulation (Das *et al.,* 2018).

It can be concluded from the study that in future endospores of *Bacillus pumilus* can be used in place of talc based microbial formulation for higher shelf life of the product, faster retting and better fibre quality of jute. The use of endospores would also reduce the production cost and facilitate the use of left out formulation in the succeeding years for faster retting because of its resistance to environmental stresses.

Acknowledgment

Authors are highly grateful to the Director, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata for providing necessary facilities and financial support for the study.

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