

## Research Article

# Changes in the bacterial community structure and diversity during bamboo retting

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Microbial retting is a critical step in obtaining fiber bundles from bamboo culm using indigenous microorganisms. A cultivation-independent technique for monitoring the changes in bacteria community during bamboo retting was applied in this work. This technique involves genetic profiling of PCR-amplified small-subunit rRNA and the single-strand conformation polymorphism (SSCP) gel analysis of the PCR-amplified 16S rDNA fragments. The study revealed that both the structure and the diversity of investigated communities varied with the incubation periods and sample locations. The bacteria bands from SSCP gel profiles related to *Bacillus* sp. decreased in intensity, and *Phaeosporillum* sp. and *Azospirillum brasilense* completely disappeared during the 4<sup>th</sup> and 5<sup>th</sup> month of incubation, while the bands related to the *Sphingomonas japonica*, *Alphaproteobacterium Ellin335* and *Microbacterium* sp. increased. The bands closely related to *Sphingomonads*, *Brevundimonas brasilense*, *Pseudoclavibacter* sp., *Agrococcus jenensis* and *Oxalophagus oxalicus* remained dominant during the whole incubation period. This study showed that the use of PCR assay targeting 16S rRNA and SSCP profiling provided valuable information on monitoring the bacteria dynamic changes occurring in the bacteria community during bamboo retting, which is crucial for controlling the quality of the retting process and improving the retting efficiency, and thus benefits for fiber recovery.

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## 1 Introduction

Bamboo is one of the fastest growing and most abundant woody plants in tropical countries [1]. The rate of growth, up to 1 m per day, is very attractive for commercial applications. Therefore, bamboo is gaining increasing attention as an alternative crop with multiple utilizations. It is estimated that there are up to 1500 bamboo species having commercial potential [2]. Bamboo has recently at-

tracted a great deal of attention as a natural, green and eco-friendly new-type raw material for textile applications. The relatively long length of bamboo monofilaments (1.5–3.2 mm) [3] coupled with its antibacterial properties make it suitable for specialty textile materials such as sanitary napkins, bandages, surgical clothes and food-packing bags. Recently its potential value for textile yarn production has emerged [4–7].

Traditionally bamboo fiber is obtained through alkaline hydrolysis followed by multiple chemical bleaching steps; however, these processes are expensive and have negative effects on the environment. Therefore, alternative green technologies are

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**Abbreviation:** rDNA(RNA), ribosomal DNA(RNA)

**Colour online:** See the article online to view Fig. 1 in colour.

being investigated. Nevertheless, until now only a few studies have been dedicated to developing biological processes for bamboo fiber recovery [8–11], whereas extensive research has been conducted into enzymatic wood pulping [12–18]. Retting, based on enzymes (e.g., hemicellulases, cellulases, oxidoreductases, and pectinases) produced by an indigenous complex bacteria community (e.g., fermenting, homoacetogenic, syntrophic, and acetate-utilizing bacteria) to degrade cellular tissues and pectins, gums, etc., is gaining potential as a pretreatment step to obtain fibers from raw bast materials (e.g., jute, flax, hemp and kenaf). It facilitates separation of cellulose fiber bundles from the matrix [19–22] and has been shown to play an important role in determining the quality and yields of jute fiber [23]. Although retting is a crucial step for the production of fiber bundles, the available reports on the bacterial communities in the retting environments are scarce, with most literature coming from flax [24–27] and jute retting [20, 28]. The current knowledge of the microbial community is mainly based on culture-dependent studies [22, 25, 29–31]. However, cultivation-based approaches favor the growth of only certain community members, while a great part of the population is overlooked [32]. In fact, culture-based methods are reported to only account for 1% of the microorganisms present in an ecosystem and the remainder (99%) of the microorganisms can not be characterized using the traditional culture techniques [33, 34]. Culture-independent 16S rRNA gene-based studies of bacteria communities from various habitats have provided scientists with crucial information on the diversity and dynamics of these communities. For example, the potential applications of culture-independent studies (PCR assays targeting bacterium 16S rRNA genes) during the retting of jute provided insight into the changes in the bacteria communities during the retting process [23]. The use of such knowledge is expected to improve fiber bundle extraction in terms of quality and quantity. The present study was aimed at gaining an insight into the microbial communities and their changes during the retting of bamboo, making use of PCR assays targeting 16S rRNA genes and the single-strand conformation polymorphism (SSCP) gel analysis of the PCR-amplified 16S rRNA fragments. The SSCP profiling technique was chosen rather than denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) because of its simplicity, the fact that it did not require a gradient, the absence of GC clamps and the ability to provide valuable information on changes in bacteria communities [32, 35, 36]. To the best of our knowledge, the present

study is the first attempt to use culture-independent methods to monitor changes occurring in bacteria communities during the retting of bamboo. We are convinced this study will enable us to identify key microorganisms involved at every stage of the retting process. The universal bacteria community primers, as well as three other specific bacteria community primers (bacillus, actinomycete and alphaproteobacteria), which had been present in other retting systems [20, 23, 37, 38], were used. Furthermore, dominant bands were sequenced to provide information about the identity of the microorganism.

## 2 Materials and methods

### 2.1 Sampling

One-year old Moso bamboo (*Phyllostachy*) culms were harvested from bamboo gardens in Jiangxi Province, P. R. China. The culms from two different gardens were cut into small pieces and placed in two 2-L Erlenmeyer flasks with 1 L sterilized water as two independent retting systems. The flasks were incubated at 28°C with shaking at 150 rpm in a chamber with controlled humidity at 60%. The water lost due to potential evaporation was readjusted every 2 weeks. Samples (including bamboo and liquid) from the retting systems were withdrawn at different time intervals when observable changes of culm morphology were noted (i.e., after 1.5, 4 and 5 months).

### 2.2 Bacterial community analysis

**2.2.1 Extraction and purification of total genomic DNA**  
The bacterial communities for the bamboo retting process were analyzed by culture-independent tools based on the PCR amplification of the 16S rRNA fragments and subsequent partial sequencing.

The DNA of the microbial community of the bamboo retting system was extracted by mechanical disruption and homogenization of 2 g samples including retted bamboo and retting liquid using a FastPrep Instrument (BIO 101 Systems: Qbiogene, Carlsbad, CA, USA) for 30 s at speed 5.0 and 400 mg sample per time point. The total genomic DNA of bacterial-cell consortia was recovered using the FastDNA Spin Kit for Soil (Bio101, Carlsbad, CA) following the manufacturer's protocol and purified by the GENECLLEAN Turbo kit (MP Biomedicals, Illkirch, France) containing the special binding buffer guanidine thiocyanate for the removal of humic acids. The quality and concentration of extracted DNA were estimated by electrophoresis on a

0.8% agarose gel running at 90 V for 30 min at room temperature and analyzed after ethidium bromide staining under ultra violet (UV) emission.

### 2.2.2 Cultivation-independent analysis by SSCP gel

Fingerprinting of the Bamboo bacterial communities by SSCP was carried out as described by Schwieger and Tebbe [35] and using the Gene Ruler 1 kb DNA ladder, Fermentas, as standard. The reproducibility of the SSCP results was verified in two independent samplings at three time points (1.5, 4 and 5 months) during the retting period. Bacterial 16S rDNA fragments (positions 515–927 according to *Escherichia coli* 16S rRNA sequence numbering) were amplified by PCR with the primers Unibac-II-515f and Unibac-II-927r-P [39]. Alphaproteobacteria were selectively amplified by a nested PCR approach with primers ADF 681F/1492r (5'-AGT GTA GAG GTG AAA TT-3'/5'-TAC GGY TAC CTT GTT ACG ACT T-3') followed by a second PCR with the primers ADF 681F/Unibac-II-927r-P (5'-CCC GCT AAT TYM TTT GAC TT-3') [40]. Bacillus amplicons were obtained with BLS342f/BACr833-P (5'-CAG CAG TAG GGA ATC TTC-3'/5'-CTA ACA CTT AGC ACT CAT-3') primers [40, 41]. For specific patterns of actinomycetes, a nested PCR was applied with first PCR primer pair Eubac1/Eubac2 (5'-GAG TTT GAT CCT GGC TCA G-3'/5'-AGA AAG GAG GTG ATC CAG CC-3'), followed by a second PCR primer Actino243f / Actino513r-P (5'-GGA TGA GCC CGC GGC CTA-3' / 5'-CGG CCG CGG CTG GTG GCA CGT A-3') [42]. The PCR products were purified using a GeneClean Turbo Kit (Qbiogene, BIO101®), before exonuclease digestion and DNA single-strand folding according to Lieber et al. [43]. The amplicons were separated using the TGGE Maxi system (Biometra, Göttingen, Germany) at 400 V and 26°C. Eubacterial as well as bacillus-specific amplicons were separated in 8% acryl amide gels for 26 h. DNA fragments of the Alphaproteobacteria- and actinomycete-specific PCR were separated in 9% acryl amide gels for 16 h. The gels were silver-stained according to the procedure of Bassam [44] for subsequent visualization.

### 2.2.3 Analysis of SSCP profiles

Evaluation of SSCP gel profiles of the bacterial communities in the bamboo-retting liquid was carried out using the GelCompar program (version 4.1; Applied Maths, Kortrijk, Belgium). Digitized gel images were obtained by scanning the silver-stained SSCP gels (Epson perfection 4990 Photo, Japan) for the community-fingerprint comparison. Individual SSCP patterns were normalized and the background was subtracted as recommended by the

manufacturer. The band-based Dice similarity coefficient was applied for similarity matrix calculation [45]. The profiles generated were processed by UPGMA (Unweighted Pair Group Method with arithmetic Average) analysis and a dendrogram was deduced from the matrix of similarities.

### 2.2.4 Calculation of diversity indices

A diversity index based on the Shannon-Wiener diversity index ( $H'$ ) was adopted to characterize the differences in the genetic diversity of the various bacterial communities, and calculated as [46]:

$$H' = -\sum_{i=1}^S \left[ \left( \frac{n_i}{N} \right) \ln \left( \frac{n_i}{N} \right) \right] \quad (1)$$

where,  $n_i$  is the relative abundance of a single taxon (intensity of a single SSCP band);  $N$  is the total abundance of all taxa (accumulated band intensity);  $S$  is the total number of abundant species (total number of bands).

The diversity of the SSCP bands was calculated using the same GelCompar program mentioned above. The different peaks of a densitometric curve of each SSCP lane were analyzed.

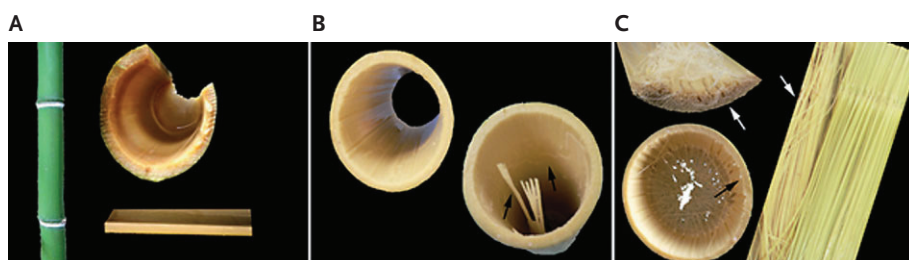
### 2.2.5 Sequence analysis

Dominant bands corresponding to the different bacterial communities were excised from SSCP gels and the DNA recovered by elution according to Schwieger and Tebbe [35]. The gel-extracted DNA was re-amplified and sequenced. Bacteria were then identified by comparing the obtained sequences with sequences available in the GenBank database, using the NCBI BLAST program at <http://www.ncbi.nlm.nih.gov/BLAST> [47].

## 3 Results

### 3.1 Bamboo retting process

Morphological studies showed that retting resulted in the separation of fiber bundles from bamboo culm, regardless of bamboo source. The general process of bamboo retting is summarized in Figs. 1A–C. At the initial stage (Fig. 1A), the bamboo culms were intact, while with prolonged incubation the culms started to be loosen innerly (Fig. 1B). Further incubation led to the untwisting of the fiber bundles at the node level and separation of the bundles from the culm since the materials surrounding the fiber bundles were degraded (Fig. 1C). Microscopic inspection (Fig. 2) confirmed that a single bamboo bundle in a hollow structure



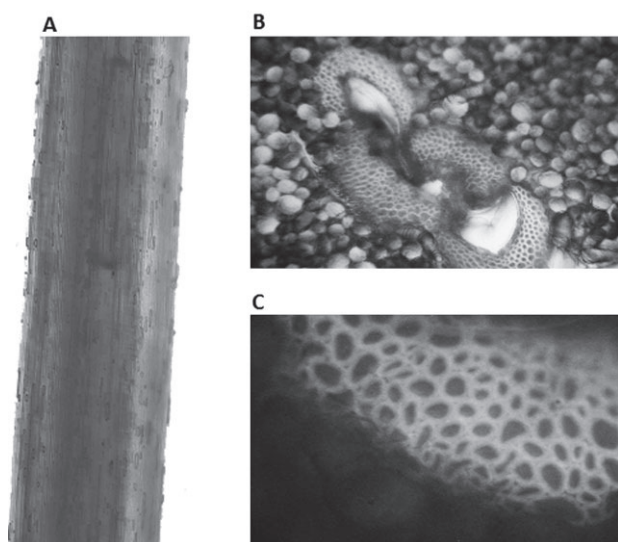
**Figure 1.** Morphological changes of bamboo culms during the retting process. (A) Initial stage where the bamboo is intact. (B) Bamboo fiber loosening and de-pilling. (C) Fibers separating from each other and falling into the lumen of the culm.

contained many monofilaments with lumen and smooth surface.

### 3.2 Bacterial community analysis

#### 3.2.1 Universal bacteria community analysis

SCCP profiles of universal bacteria community in both retting systems showed high reproducibility within replicates (Fig. 3), indicating that DNA sam-



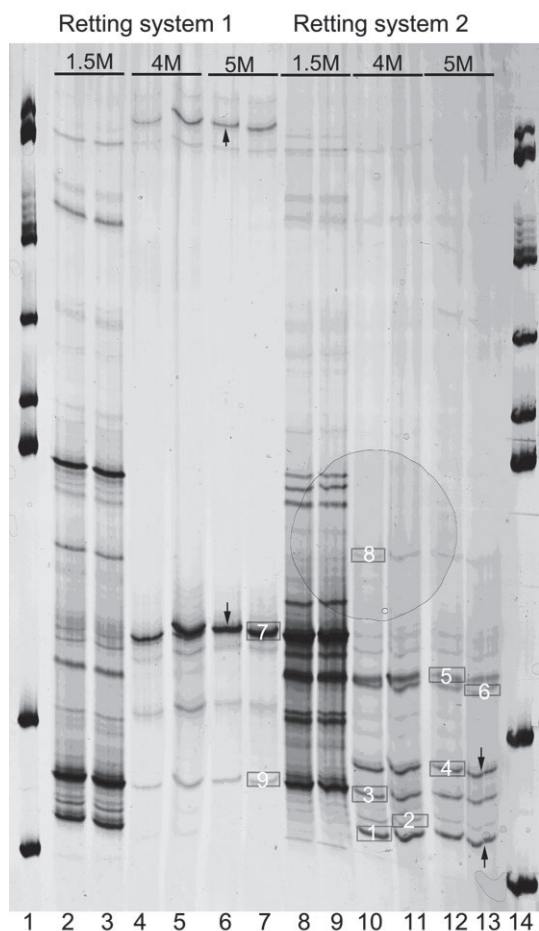
**Figure 2.** Morphology of a single bamboo fiber bundle (Nikon Eclipse 400, in transmission mode). (A) Vertical section ( $\times 40$ ); (B) cross-section of a single bundle ( $\times 100$ ); (C) cross-section of bamboo bundle ( $\times 400$ ).

ples were of high quality for further analysis. The partial sequence analyses and tentative close relations of specified SSCP bands are summarized in Table 1, showing a more predominant uncultured population in this category. The information of the uncultured bacteria population normally can not be obtained by the traditional culture-dependent method, i.e., the screening and isolation of the strains with various media. The sequences mainly correlated with uncultured *Sphingobacterium* sp. (bands 7 and 9) in retting system 1. In retting system 2, the sequences closely related to uncultured *Paludibacter* sp. (band 1), *Candidatus Sulcia muelleri* (band 2), uncultured *Pedobacter* sp. (band 3), *Flavobacterium* sp. (band 4), uncultured *Porphyrobacter* sp. (bands 5 and 6) and uncultured *Planctomycete* (band 8) showed dominant signals during the whole incubation period (Fig. 3). Interestingly, the intensity change of some specific bands differed with the samples from different regions. For example, bands 7 and 9, with strong density in retting system 1, decreased or disappeared along with the prolongation of the incubation time in retting system 2. However, bands 1, 2 and 8, which completely disappeared during months 4 and 5 in retting system 1 remained strong in retting system 2. In general, visual inspection of SSCP banding patterns for universal bacteria communities (Fig. 3) revealed a gradual decrease in intensity and subsequent disappearance of some bands after 4 to 5 months of incubation in both retting systems

**Table 1.** Partial sequence analyses and tentative close relations of SSCP bands for universal bacteria community

Band no.	Microhabitat most closely related sequence(s)	SI <sup>a)</sup>	GenBank accession no.
1	Uncultured <i>Paludibacter</i> sp.	91	EU809705.1
2	<i>Candidatus Sulcia muelleri</i>	91	DQ066640.1
3	Uncultured <i>Pedobacter</i> sp.	90	GQ287498.1
4	<i>Flavobacterium</i> sp. SRS18	98	AY621158
5	Uncultured <i>Porphyrobacter</i> sp.	94	EF662624.1
6	Uncultured <i>Porphyrobacter</i> sp.	97	EF662624.1
7	Uncultured <i>Sphingobacterium</i> sp.	94	FJ756565.1
8	Uncultured <i>planctomycete</i>	85	DQ870157.1
9	Uncultured <i>Sphingobacterium</i> sp.	92	FJ756565.1

a) SI, similarity index: for isolates identified by 16S rDNA sequencing ranging from 0 to 100%.



**Figure 3.** SSCP profiles showing the universal bacteria communities during bamboo retting in the retting system 1 and 2 at different sampling times [1.5 months (1.5M), 4 months (4M) and 5 months (5M)]. The resolved bands in retting system 1 and 2 were very similar during the incubation period. Bands indicated by numbers (1–8) were purified and sequenced (for results, see Table 1); Gene Ruler 1-kb DNA ladder (Fermentas) was used as standard.

despite the fact that some bands showed an increase in density with long-term incubation (Fig. 3, arrows).

The highest diversity represented by the Shannon-Wiener index  $H'$  was observed at the first sampling time ( $H' = 3.17 \pm 0.02$  and  $3.35 \pm 0.00$  for retting system 1 and 2, respectively). In the following 3–4-month period, the complexity of the SSCP profiles decreased, revealing a reduction of diversity ( $H' = 2.00 \pm 0.01$  and  $2.78 \pm 0.01$  for retting system 1 and 2 after 4 months, respectively, and  $H' = 1.85 \pm 0.00$  and  $2.56 \pm 0.02$  for retting system 1 and 2 after 5 months, respectively) and an enrichment of specialized bacteria. The changes were also reflected in the dendrograms (Fig. 4A), clearly showing a higher similarity between the bacterial communities after 4 and 5 months (about 87% for both retting systems) than between the profiles after 1.5 months and the later sampling points (44% for retting system 1 and 64% for retting system 2).

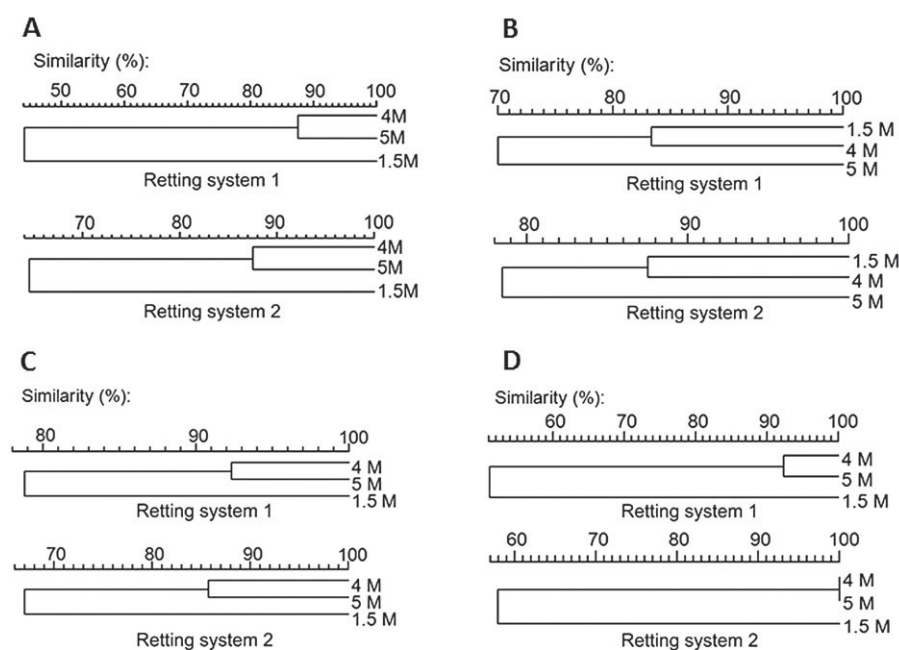
### 3.2.2 Alphaproteobacteria community analysis

Group-specific primer systems were used to selectively amplify 16S rDNA fragments of Alphaproteobacteria. The dominant and individual bands with strong intensity and clear shape were excised from SSCP gels for DNA recovery. The partial sequence analyses and tentative close relations of the bands are listed in Table 2. Bands closely related to *Alphaproteobacterium Ellin335* (band 3 in both retting systems and band 4 in retting system 2) and *Sphingomonas japonica* (band 7 in retting system 2) increased in intensity during months 4 and 5, while bands corresponding to *Phaeospirillum* sp. (band 6 in both retting systems) and *Azospirillum brasilense* (band 1 in retting system 1) disappeared after 4 months. Interestingly, the sequence related to *Brevundimonas diminuta* (band 2) remained dominant and showed a remarkable intensity during the whole incubation time in both retting systems. Band 5 (in retting system 1) and band 8 (in retting system 2) corresponding to *Kaistia* sp. and uncultured *Sphingomonas* sp., respectively, were relatively weak in density throughout the whole incubation time.

**Table 2.** Partial sequence analyses and tentative close relations of SSCP bands for the alphaproteobacteria community

Band no.	Microhabitat most closely related sequence(s)	SI <sup>a)</sup>	GenBank accession no.
1	<i>Azospirillum brasilense</i>	96	AB480703.1
2	<i>Brevundimonas diminuta</i>	95	FJ843099.1
3	<i>Alphaproteobacterium Ellin335</i>	97	AF498717.1
4	<i>Alphaproteobacterium Ellin335</i>	91	AF498717.1
5	<i>Kaistia</i> sp.	93	FJ719344.1
6	<i>Phaeospirillum</i> sp.	94	FJ529718.1
7	<i>Sphingomonas japonica</i>	91	AB428568.1
8	Uncultured <i>Sphingomonas</i> sp.	98	AM936457.1

a) SI, similarity index: for isolates identified by 16S rDNA sequencing ranging from 0 to 100%.



**Figure 4.** Constructed dendrogram based on amplified fragments using different community analysis primers. (A) Universal bacteria community; (B) alphaproteobacteria community; (C) actinomycete community; (D) bacillus community.

The diversity of alphaproteobacteria community in both retting systems decreased with increasing incubation time. The highest diversity represented by the Shannon-Wiener index  $H'$  was observed at the first sampling time ( $H' = 1.74 \pm 0.02$  and  $2.00 \pm 0.06$  for retting system 1 and 2, respectively). In the following 3–4 months there was a reduction in diversity ( $H' = 1.70 \pm 0.01$  and  $1.81 \pm 0.04$  for retting system 1 and 2, respectively, after 4 months and  $H' = 1.06 \pm 0.04$  and  $1.56 \pm 0.06$  for retting system 1 and 2, respectively, after 5 months).

The dendrogram in Fig. 4A clearly reveals that in comparison with the universal bacteria community analysis, the similarity of alphaproteobacteria community was higher in both retting systems (above 80%) before 4 months. The similarity remained high at around 70% in retting system 1 and 78% in retting system 2 after 5 months of retting.

### 3.2.3 Actinomycete community analysis

Generally, SSCP patterns of actinomycete populations revealed only minor changes up to month 4 of

the retting process. Thus, the Shannon-Wiener's diversity index remained stable at  $1.75 \pm 0.17$  and  $1.91 \pm 0.14$  for retting system 1 and 2, respectively. The dominant and representative bands were excised from SSCP gel. The recovered DNA was sequenced and results are presented in Table 3. Bands with the same partial 16S rRNA gene as *Pseudoclavibacter* sp. (band 3) and *Agrococcus jenensis* (band 5) were amazingly dominant during the whole incubation period in both retting systems. Bands closely related to *Rhodococcus erythropolis* (band 1) and *Demequina aestuarii* (band 4) decreased in density when assayed after 4 months of incubation, while bands mainly concerned with *Microbacterium* sp. (band 2) increased intensity after 4 months of incubation in both retting systems. Further, in comparison with the universal bacteria and alphaproteobacteria community analysis, BLAST sequence analysis of the actinomycete community showed a higher maximum identity index >98% and identified sequences of predominant bands were related to cultivable microbes.

**Table 3.** Partial sequence analyses and tentative close relations of SSCP bands for the actinomycete community

Band no.	Microhabitat most closely related sequence(s)	SI <sup>a)</sup>	GenBank accession no.
1	<i>Rhodococcus erythropolis</i>	99	AP008957.1
2	<i>Microbacterium</i> sp.	99	FJ654436.1
3	<i>Pseudoclavibacter</i> sp.	98	EU086820.1
4	<i>Demequina aestuarii</i>	99	DQ010160.1
5	<i>Agrococcus jenensis</i>	99	FJ482044.1

a) SI, similarity index: for isolates identified by 16S rDNA sequencing ranging from 0 to 100%.

**Table 4.** Partial sequence analyses and tentative close relations of SSCP bands for the bacillus community

Band no.	Microhabitat most closely related sequence(s)	SI <sup>a)</sup>	GenBank accession no.
1	<i>Oxalophagus oxalicus</i>	99	Y14581.1
2	<i>Oxalophagus oxalicus</i>	94	Y14581.1
3	<i>Bacillus</i> sp. NSJ-14	98	FJ941090.1
4	<i>Bacillus</i> sp. th9	87	EU814517.1
5	<i>Bacillus</i> sp. BD-94	88	AF169523.1
6	<i>Oxalophagus oxalicus</i>	99	Y14581.1

a) SI, similarity index: for isolates identified by 16S rDNA sequencing ranging from 0 to 100%.

The dendrogram in Fig. 4C show that community similarity was higher between 4 and 5 month (above 85% in both retting systems), indicating a great change occurring between 1.5 and 4 months, and then became tempered in the following month. This change trend was in accordance with universal bacteria community.

#### 3.2.4 *Bacillus* community analysis

SCCP gel profiles of bacillus community for both retting systems were identical and showed a similar pattern and band intensity. The visible and dominant bands were excised and DNA was recovered. The sequence results are listed in Table 4. Bands with the same partial 16S rRNA gene as *Oxalophagus oxalicus* (bands 1, 2 and 6) were dominant during the whole incubation period in both retting systems. Bands related to *Bacillus* sp. NSJ-14 (band 3) and *Bacillus* sp. th9 (band 4) showed slightly decreased intensity after 1.5 months of incubation in both retting systems, while the band related to *Bacillus* sp. BD-94 (band 5) dramatically decreased in intensity and finally disappeared after 4 months of incubation.

The Shannon-Wiener index  $H'$  of both retting systems decreased with increasing incubation time. For retting system 1,  $H'$  declined from  $2.7 \pm 0.04$  at 1.5 months to  $1.70 \pm 0.02$  at 4 and 5 months, while in retting system 2,  $H'$  reduced from  $2.37 \pm 0.01$  at 1.5 months to  $1.85 \pm 0.02$  at 4 and 5 months. Therefore, the huge diversity change also occurred between 1.5 and 4 months and then maintained relatively constant from 4 to 5 months. The dendrogram (Fig. 4D) reveals that the community at 4 and 5 months gained a higher similarity (above 92%) for two retting systems, in line with the diversity analysis.

## 4 Discussion

Culture-independent molecular biology based techniques were used to investigate changes of the microbial community during bio-retting of bam-

boo. Due to the fact that cultivatable microbes comprise a minority (approximately 1%) of total community diversity [33, 48] previous studies on microbial retting based on cultivation may have overlooked a major part of the microbial community. As evidenced by the Shannon-Wiener  $H'$  index, the diversity of the total bacteria in the microbial retting environment investigated in this study tended to decrease with increasing incubation time. This was also confirmed by the analysis of SSCP profiles obtained with universal bacteria, alphaproteobacteria and bacillus community primers showing a general decrease in number of bands and/or decrease in intensity when compared to the bands profiles obtained at 1.5 months, although fluctuating changes were observed for the actinomycete community. Strong correlations between changes in microbial succession patterns (taxonomic and functional diversity) during plant cell wall degradation and the sequential utilization of nutrients have been reported [49]. Similarly, in this study, the bands intensity, for example, closely related to *Bacillus* sp., *Rhodococcus erythropolis*, *Sphingobacterium* sp. and *Demequina aestuarii* decreased, while, in particular, *Phaeospirillum* sp. and *Azospirillum brasilense* disappeared completely between 4 and 5 months of incubation. A few bands related to *Alphaproteobacterium Ellin335*, *Sphingomonas japonica* and *Microbacterium* sp. increased intensity from 1.5 to 4 months. For other lignocellulosic materials, the early phases of retting are known to be dominated by organisms that can attack the easily accessible polysaccharides, like pectins, monosaccharides and gums [49]. Similarly, in this study, the initial phase of bamboo retting proved to be the most dynamic part of the process, and it is assumed that each bacterial population at this stage plays a special role in trying to change the chemical environment to favor for its own survival [50]. However, as more complex substrates (hemicellulose interlinked with lignin) remained, only those microorganisms with a complex enzymatic system were able to survive. The disappearance of certain microorganisms during the retting process at 4 and

5 months, confirmed by SSCP profiles, indicates sequential metabolism of simple substrates followed by degradation of complex materials. Indeed, organisms identified during the latter phase are well known for the production of extracellular enzymes (including oxidases) that degrade complex materials. For example, *Sphingomonas* sp. are able to degrade complex substrates like lignin, and *Sphingomonas paucimobilis* SYK-6 has been shown to be able to degrade a wide variety of dimeric lignin compounds, including  $\beta$ -aryl ether, biphenyl, and diarylpropane [51, 52]. Many *Bacillus* sp. are also widely reported to produce enzymes of industrial application in paper industry [53–60] and bast fiber degumming [61, 62]. Consequently, pretreatment with the combined *Sphingomonas paucimobilis* and *Bacillus* strains improved the hydrolysis of office paper from municipal wastes [63], justifying the observed presence of both organisms during the incubation period. Interestingly, although members of the *Actinomycetes* genus have been reported to develop more slowly than most other microorganisms, and are comparatively ineffective competitors under high-nutrient conditions [64], in this study bacteria with the same partial 16S rRNA gene as *Pseudoclavibacter* were quite dominant during the whole incubation period and bacteria related to *Microbacterium* greatly increased in band density at 4 months. This is not surprising since a *Microbacterium* sp. was recently isolated for its ability to degrade xylan [65]. We also tried to analyze the pseudomonas community (data not shown), and two bands closely related to *Pseudomonas* sp. T8 and *Pseudomonas* sp. T7 (100% by max ident) were dominant during the whole incubation period. Similarly, in previous jute-retting studies, *Pseudomonas* sp. was also shown to be dominant and was attributed to their ability to secrete a set of enzymes that enable the degradation of plant cell wall components [20]. *Pseudomonas* sp. are widely distributed in the environment and are known to participate in  $N_2$  fixation, denitrification and degradation of pollutants [66–68]. Although little is known about *Oxalophagus oxalicus*, the survival and dominance of bands closely related to it during the whole incubation period requires special attention since its high frequency may suggest a significant role during the retting process. As clearly indicated by the SSCP profile of universal bacteria community, the band patterns differed between the two retting systems. This was mainly attributed to the fact that the samples originated from two different gardens, which may have had different environmental conditions (e.g., temperature, humidity, air, or soil quality) for microorganisms, and finally led to the difference in bacterial community. The fungal com-

munity studies in the retting systems based on 18S rRNA fungus-specific primers were not successful. This may be due to the culture conditions (liquid system), since fungi prefer moisture and the observation are in line with previous studies with flax- [21, 24] and jute- [20] retting systems.

This study showed that the cultivation-independent technique for genetic profiling of PCR-amplified small-subunit rRNA and the SSCP gel analysis of the PCR-amplified 16S rDNA fragments can be used for monitoring the changes in bacterial communities occurring during the retting of bamboo. The SSCP profile analysis revealed that the bacterial community changed during the retting of bamboo, leading to a general decrease in diversity. Most of the DNA recovered from the dominant bands were sequenced and classified as uncultivable microorganisms confirmed by previous reports. The obtained information can be exploited in various ways, including identifying the microorganisms and isolating key microorganisms involved in during the retting process. Such pure cultures could then be bioaugmented to enhance the retting process. On-going studies are aimed at investigating the natural retting system of bamboo obtained from different geographical regions. This study forms a novel basis on which such future studies can be built.

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