An Assessment of Variability of Pulp Mill Wastewater Treatment System Bacterial Communities using Molecular Methods

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The DNA fingerprinting techniques, 16S-restriction fragment length polymorphism (16S-RFLP), ribosomal intergenic spacer analysis (RISA) and repetitive extragenic palindrome PCR (Rep-PCR), were used for analyzing the bacterial communities of seven pulp and paper wastewater treatment systems. All three methods generate DNA fingerprints that can be compared using the computer-assisted program, Gelcompar®. Community similarity coefficients were based on quantitative determinations of both the positions of the DNA bands and the band intensities in order to compare the relative differences in the populations. Unique 16S-RFLP DNA fingerprints were observed for each mill suggesting that individual mills contained phylogenetically different communities. However this method was not sensitive enough to detect differences within a mill treatment system from different locations or from different sampling times. The RISA method, which generated more complex fingerprints than 16S-RFLP, could, for some mills, discern differences between samples. The Rep-PCR technique, however, showed the highest degree of resolution and produced not only distinct patterns for each mill but also distinct fingerprints for the temporal and spatial samples from some of the treatment systems. The sensitivity of this method might potentially be used to monitor the stability of the bacterial community within a secondary treatment system.

Key words: 16S-RFLP, RISA, Rep-PCR, diversity, secondary treatment, pulp mills

Introduction

To treat pulp mill effluents, secondary wastewater treatment systems have been used for decades for the reduction of BOD (biochemical oxygen demand), COD (chemical oxygen demand), AOX (adsorbable organic halogens), and resin acids in the final effluent (Lindstrom and Mohamed 1988; Bryant and Berkley 1991; Kostyal et al. 1997). The two main types of biotreatment processes employed by the pulp and paper industry are the
aerated lagoon (aerated stabilization basin) and the activated sludge system. Regardless of the design, both these secondary wastewater treatment systems are engineered systems that employ a complex community of microorganisms to process vast quantities of wastewater everyday, and to degrade or transform toxic and complex compounds into simple and harmless products that can be discharged into receiving waters. There are relatively few studies of the microbial communities within pulp and paper treatment systems. In 1992, Liss and Allen (1992) surveyed three Northern Ontario mills and showed that the culturable community was dominated by aerobic and facultative anaerobic heterotrophs. Fulthorpe et al. (1993) showed that the culturable heterotrophic bacteria from one of the mills were dominated by populations of *Ancylobacter aquaticus*, *Pseudomonas*, and a methylotrophic group (later identified as *Xanthobacter*). Mohn et al. (1999) isolated several bacterial species capable of resin acid degradation from pulp mill treatment systems including *Sphingomonas*, *Zoogloea* and *Pseudomonas*. The presence and importance of these bacterial groups in all mill treatment systems however, is questionable since culturable isolates have been found to comprise less than 10% of the bacterial population in complex communities and environmental samples (Ward et al. 1992; Wagner et al. 1993; Amann et al. 1995; Torsvik et al. 1996, 1998). Therefore, community level approaches that take into account both the culturable and non-culturable microbes in the treatment system are important in the analysis of the community structure and composition.

Phenotypic analysis on whole communities has been carried out using carbon substrate utilization profiles. Victorio et al. (1996) and Schneider et al. (1997) demonstrated the utility of GN and MT Biolog plates for investigating various wastewater samples. They were able to generate phenotypic fingerprints of communities and demonstrate their superiority over traditional microbial assays for identifying shifts in and comparing mill biotreatment microbial communities. However such growth-dependent phenotypic fingerprints do not seem to reflect the in situ functional capabilities nor the identity of the community (Smalla et al. 1998).

Genotypic analysis methods are invaluable for the identification and classification of microbes. They are independent of culture techniques, do not rely on gene expression and can be applied to the whole community. Each of these methods permits a certain level of phylogenetic resolution from genera to species to strain when used to look at pure cultures. Although these methods were developed with pure cultures they have been used to monitor phylogenetic changes in simple communities (Massol-Deya et al. 1997; Matheson et al. 1997) and have the potential to be useful in characterizing complex microbial communities (Grey and Herwig 1996; Smit et al. 1997; Yu and Mohn 2001).

Furthermore, quantitative comparisons of microbial communities using molecular methods can be analyzed using computer-assisted software packages that can quantify differences in DNA fingerprints (Rademaker and de Bruijn 1997; Rademaker et al. 1999). The software is capable of either basing similarities on the absence or presence of com-
mon DNA bands in the profiles or basing similarities on both band position and intensity. Similarities calculated based on band position alone tend to overestimate the similarity between populations and therefore to compare relative similarities between communities, band intensity should be included in the analysis (Yang and Crowley 2000; Yu and Mohn 2001).

In this paper, three molecular methods based on polymerase chain reaction (PCR) amplification of target sites were used. The first method involved the amplification of the 16S rRNA gene followed by restriction analysis (16S-RFLP; restriction fragment length polymorphism) (Moyer et al. 1996). The second method involved the amplification of the intergenic spacer region between the 16S and 23S rRNA genes (RISA) (ribosomal intergenic spacer analysis) (Borneman and Triplett 1997). The last method involved the amplification of repetitive extragenic palindromic sequences (Rep-PCR) (de Bruijn et al. 1996; Fulthorpe et al. 1998; Versalovic et al. 1991). The first two methods are based on DNA fingerprints generated from a single gene or region of the genome while the third technique generates a DNA fingerprint based on sequences throughout the whole genome. The DNA patterns that are generated were compared using computer-assisted cluster analysis of both band position and intensity to estimate the genotypic relatedness of the profiles.

Although a few studies have reported on the bacterial community structure in individual aerated lagoon or activated sludge systems used in the pulp and paper industry (Yu and Mohn 2001; Muttray et al. 2001), no studies exist that have directly compared the bacterial community composition of several systems to each other. The aim of this study was to characterize the bacterial communities from seven pulp and paper mill secondary wastewater treatment systems using DNA-based methods to determine how genotypically similar they were. Furthermore, we examined whether those differences could be related to differences in wood furnish (the type of wood used for pulping) or the type of effluent treatment process (aerated lagoon versus activated sludge). We also compared spatial and temporal bacterial community profiles within several of the mill biotreatment systems.

**Experimental Procedures**

**Sample Collection**

More than eighty treatment system effluent samples were received from seven bleach kraft pulp and paper mills from Brazil, New Zealand, U.S.A. and Canada (Table 1). Mills were assigned codes based on the wood furnish, S for softwood, H for hardwood and M for mills using a mixture of both hardwood and softwood. The temporal samples were collected approximately one-third of the way through the treatment system at all mills. Spatial samples were collected for analysis at additional sites in three mills (H1, H2 and M1). All samples were grab samples (1 litre) collected by mill personnel. The samples were shipped on ice and arrived in the lab...
Table 1. List of mills sampled

<table>
<thead>
<tr>
<th>Mill code&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Location</th>
<th>No. of times sampled</th>
<th>No. of locations sampled</th>
<th>Wood furnish</th>
<th>Effluent treatment process</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Iowa, U.S.A.</td>
<td>6</td>
<td>1</td>
<td>Pine</td>
<td>Aerated lagoon – single cell</td>
</tr>
<tr>
<td>S2</td>
<td>New Zealand</td>
<td>8</td>
<td>1</td>
<td>Radiata pine</td>
<td>Aerated lagoon – four cells</td>
</tr>
<tr>
<td>H1</td>
<td>Brazil</td>
<td>4</td>
<td>6</td>
<td>Eucalyptus</td>
<td>Aerated lagoon – six cells</td>
</tr>
<tr>
<td>H2</td>
<td>Alberta</td>
<td>3</td>
<td>2</td>
<td>Poplar/aspen</td>
<td>Activated sludge – extended aeration</td>
</tr>
<tr>
<td>M1</td>
<td>Ontario</td>
<td>3</td>
<td>3</td>
<td>Mixed hard and soft</td>
<td>Aerated lagoon</td>
</tr>
<tr>
<td>M2</td>
<td>Ontario</td>
<td>3</td>
<td>1</td>
<td>Mixed hard and soft</td>
<td>Aerated lagoon</td>
</tr>
<tr>
<td>M3</td>
<td>Quebec</td>
<td>4</td>
<td>1</td>
<td>Mixed hard and soft</td>
<td>Activated sludge</td>
</tr>
</tbody>
</table>

<sup>a</sup> S for softwood furnish, H for hardwood furnish and M for a mixture of soft and hardwood furnish.
within 48 hours. Biomass was immediately pelleted (5,000 x g for 10 minutes at 4°C) for DNA extraction and frozen at -20°C until needed.

DNA Extraction and Purification

Community DNA was extracted from 1.5-mL aliquots of effluent according to the method of Zhou et al. (1996). The sample was spun down and 1 mL of DNA extraction buffer (100 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM Na phosphate [monobasic], 1.5 M NaCl, 1% cetyltrimethylammonium bromide [CTAB]) was added. The sample was vortexed to resuspend the pellet and 4 L of proteinase K (20 mg/mL) was added. The sample was mixed and rotated continuously at 37°C for 30 min. Then, 150 µL of 20% sodium dodecyl sulfate (SDS) was added and the sample was incubated at 65°C for 2 hours with shaking. The sample was then centrifuged at 6000 g for 10 min and the supernatant removed to a clean tube. To the pellet, 1 mL DNA extraction buffer and 150 µL of 20% SDS were added, vortexed for 10 sec, heated to 65°C for 10 min, centrifuged and the supernatant added to the previous supernatant. This last step was repeated once more. An equal volume (~3 mL) of chloroform/isoamyl alcohol (24:1) was added to the combined supernatants and mixed well. The sample was centrifuged (3000 x g) for 1 min and the aqueous layer (top) transferred to a clean tube. To this, 0.6 volume (~1.8 mL) of cold isopropanol was added, mixed and left on ice for 1 hour or overnight at −20°C. The sample was centrifuged (16,000 x g) for 20 min and the isopropanol poured off. The pellet was washed with 1 mL of ice-cold 70% ethanol, dried completely and dissolved in 100 to 200 µL of TE (Tris 10 mM, EDTA 0.1 mM).

Humic materials were coextracted with DNA in all samples so further purification of community DNA was carried out on polyvinylpyrrolidone (PVPP) spin columns as described by Berthelet et al. (1996). This procedure effectively removed all coloured material from all samples. Purified DNA was dissolved in TE at a final concentration of 100 ng/mL and stored at 4°C.

Amplification of DNA

DNA samples were amplified in triplicate in independent reactions with each of the primer sets unless otherwise noted. 16S ribosomal RNA genes were amplified using the universal eubacterial primers fD1, 5′-AGA GTT TGA TCC TGG CTC AG-3′ and rD1, 5′-AAG GAG GTG ATC CAG CC-3′ (Weisburg et al. 1991). PCR reaction mixtures (100 µL) for 16S-RFLP analysis contained 1X PCR buffer (Roche), 250 µM of each deoxynucleotide triphosphate (dNTP) (Pharmacia), 2.5 µM of primer, 3 U of Taq polymerase (Roche), and 50 ng of template DNA. The amplification program used was: 94°C for 1 min, 50°C for 1 min, 72°C for 1 min for 36 cycles with an initial denaturation step at 94°C for 5 min and a final extension step at 72°C for 5 min. The PCR products were digested with RsaI overnight at 37°C (Moyer et al. 1996) and digestion products were separated on a 2% agarose gel in 0.5X Tris-borate-EDTA buffer at 100 volts for 6 hours.
Ribosomal intergenic spacer analysis was carried out using 1406F universal rRNA small subunit primer (5'-TGYACACACCGCCCGT-3') and the 23SR bacterial 23S rRNA large subunit primer (5'-GGGTTBCCCATTCRGRG-3') (Borneman and Triplett 1997). PCR reaction mixtures (100 L) for 16S-RISA analysis contained 1 X PCR buffer (Roche), 250 µM of each dNTP (Pharmacia), 2.5 M of primer, 3 U of Taq polymerase (Roche), and 50 ng of template DNA. The amplification program used was: 94°C for 15 sec, 56°C for 15 sec, 72°C for 30 sec, for 30 cycles with an initial denaturation step at 94°C for 2 min and a final extension step at 72°C for 1 min. The PCR products were separated on a 2% agarose gel in 0.5X Tris-borate-EDTA buffer at 100 volts for 8 hours.

Rep-PCR primers for REP sequences were, 1R, (5'-IIIICgICgICATCIggC-3'), and REP 2I, (5'-ICgICTTATCIggCCTAC-3') (Versalovic et al. 1991). The PCR reaction mixture (25 L) contained 1X Gitschier buffer (83 mM [NH₄]₂SO₄, 335 mM Tris-HCl, pH 8.8, 33.5 mM MgCl₂, 33.5 µM EDTA, 150 mM -mercapto-ethanol and 800 g/mL BSA), 4 mg BSA, 10% DMSO, 1.25 mM of each dNTP (Pharmacia), 0.3 µg of each primer, 2 U of Taq polymerase (Roche) and 50 ng of template DNA (Rademaker et al. 1997, 1998). The amplification program used with the REP primers was: 94°C for 1 min, 40°C for 1 min, 65°C for 8 min for 35 cycles with an initial denaturation step at 95°C for 7 min and a final extension step at 65°C for 16 min.

The Rep-PCR products were separated on a 2% NuSieve agarose gel in 0.5X Tris-borate-EDTA buffer and run at a constant voltage of 100 for approximately 16 hours. All gels were stained with ethidium bromide (0.5 µg/mL) and photographed with DS-34 fixed focal length Polaroid camera (Bio/Can Scientific). The molecular weight marker (100 base pair ladder) was purchased from Gibco/BRL and used as a reference in all the gels.

Computer-Assisted Analysis of the DNA Fingerprint Patterns

All three molecular methods generated complex DNA fingerprints and therefore computer assistance was essential for analysis. Polaroid pictures of gels were scanned using a BioRad (GS 700) scanner and stored as TIFF files. The images were converted, normalized against the 100 bp molecular weight marker (Gibco/BRL) and analyzed with GelCompar® software (version 4.0; Applied Maths, Kortrijk, Belgium). The “rolling disk” background subtraction method was applied. Hierarchic clustering of the whole community patterns were based on similarity and grouped using the clustering algorithm UPGMA (unweighted pair group method using arithmetic averages). Jaccard similarity coefficients were used when only DNA band positions were considered, however these may overestimate community similarities because banding intensities are ignored. Therefore we also compared patterns using Pearson correlation coefficients based on both band position and intensity (the relative areas under each of the corresponding bands). The latter have been shown to be use-
ful in community comparisons (Yang and Crowley 2000; Yu and Mohn 2001; Rademaker et al. 1999).

It should be noted that Polaroid film and subsequent digital scanning does not accurately capture the full dynamic range of the fluorescent intensities of the bands, so similarities might be overestimated when intense bands saturate or underestimated when faint bands are simply not captured. The use of state of the art broad dynamic range CCD cameras rather than film will allow more faithful calculation of fingerprint similarities in the future. The general trends we see, however, particularly between fingerprint methods, are not likely to differ.

**Results and Discussion**

**Intra-sample (Inter- aliquot) Fingerprint Variability**

DNA fingerprints generated in independent PCR reactions for the same sample were 95 to 100% similar for all three methods. As shown below, independent extractions of the same samples also generated highly similar fingerprints (see Rep-PCR fingerprint comparisons below). We therefore felt confident that the degree of similarity seen between samples within and between mills reflected actual community similarity values and not differences in amplifications.

All similarity values reported below were calculated by comparing the full densitogram of the fingerprint, i.e., both band and intensity were taken into account. Replicate sample similarities were always higher when only band positions were used, since some information is ignored.

**16S-RFLP Fingerprint Comparisons**

The 16S ribosomal RNA gene is highly conserved, and different 16S-RFLP patterns are generally accepted to define different genera but may not be divergent enough to distinguish species of the same genus (Ludwig et al. 1998). It however can be useful as a phylogenetic marker to characterize microbial communities. In this study the 16S-RFLP analysis of the bacterial communities from seven pulp and paper wastewater treatment systems were performed and analyzed with Gelcompar©. A dendogram was generated for all mills. In general, samples from each mill formed individual clusters. Similarities with and between mills were derived from this dendogram, i.e., the similarity value of the node where all samples from one mill converge defines within-mill similarity, the similarity value of the node where two mills converge defines between-mill similarities. Samples from individual mills either from multiple locations within the treatment systems or from different time points were also found to generate a very high degree of similarity in the 16S-RFLP patterns (from 82–96%) suggesting that the genera within individual mills changed very little over space and time at this level of resolution. For example, in Mill H1’s treatment system that consists of 6 lagoons in tandem, samples were collected both from the edge and the middle of all
6 basins and subjected to 16S-RFLP analysis. The 16S-RFLP patterns generated from these samples differed by less than 15% from one basin to the next with the most prominent change between basins 3 and 4 (Fig. 1). This transition between basin 3 and 4 corresponds to a significant drop in both BOD and microbial numbers between basins 1 to 3 and basins 4 to 6 (mill personnel, pers. comm.). Furthermore, samples from mill S2 taken on 8 different occasions gave almost identical 16S-RFLP patterns over time with a pattern similarity of 85% (Fig. 2). This suggests that the genera were relatively stable over time within the treatment system. The comparison of the profiles between systems indicated only a 38% to 83% similarity (Table 2).

Similarity between 16S-RFLP profiles from mills was partially correlated to wood furnish. The highest pattern similarity (85.3%) between mills was between M1 and M3, both of which processed a mixture of hardwood and softwood and between H1 and H2, both of which processed hardwood. The S1 and S2 mills which both processed softwood were as different from each other (43% similar) as they were to all the other mills (38–43.5% similar). Furthermore, no correlation between DNA profiles and the type of treatment process was noted.

RISA Fingerprint Comparisons

The RISA analysis consisted of the amplification of the intergenic spacer region between the 16S and 23S rRNA genes. The intergenic spacer region is known to contain tRNA genes in various copy numbers and exhibits length polymorphisms among bacterial genera and even within species. The variability of this region should be reflected in the complexity of the DNA banding patterns and therefore allow a finer degree of resolution than the 16S-RFLP method. Figure 3 shows several of the RISA patterns generated from 6 of the mills, some with multiple samples. Intra-mill similarity ranged from 37.3 to 92.3% while inter-mill similarity ranged from 26.2 to 65.8% (Table 2). Although RISA profiles were often very similar from different locations (Fig. 3, lanes 1–3) or from different times (lanes 10 and 11) within the same treatment system, they were sensitive enough to reflect differences (lanes 9 and 12 or lanes 14 and 15) within some of the mills. Most interestingly, mill H2, where multiple samples from the same locations (lane 4 and 6) from different time points were highly similar, showed a significantly different DNA profile from a third sample after a mill perturbation, a black liquor spill into the secondary treatment system (lane 8). This suggests that the level of resolution of the RISA analysis was sensitive enough to detect a bacterial composition shift after a shock to the system. At this level of resolution the wood furnish made little difference—there was as much dissimilarity between the mixed furnish mills as between softwood and hardwood/mixed mills and again the softwood mills (S1 and S2) were not at all similar. The type of treatment process also had no correlation to the DNA profiles obtained.
Fig. 1. 16S-RFLP fingerprint patterns. a) Dendogram showing the clustering of 16s-RFLP DNA fingerprints from samples from 3 mills, S1, S2 and H2; b) Samples were taken from 12 different locations within the biotreatment system of Mill H1. Lane M: Molecular weight marker; Lane 1: centre of cell 1; Lane 2: edge of cell 1; Lane 3: centre of cell 2; Lane 4: edge of cell 2; Lane 5: centre of cell 3; Lane 6: edge of cell 3; Lane 7: centre of cell 4; Lane 8: edge of cell 4; Lane 9: centre of cell 5; Lane 10: edge of cell 5; Lane 11: centre of cell 6; Lane 12: edge of cell 6.
The third method, Rep-PCR, is based on primers that are complementary to naturally occurring, highly conserved, extragenic, repetitive DNA sequences throughout the genome of most bacteria. Rep sequences are found associated with 30% of bacterial operons. Amplification of DNA between Rep sites led to highly reproducible fingerprints with single isolates (Rademaker et al. 1997). Rep fingerprints of entire communities have also been found to be highly reproducible (Farhana et al. 1997; Kong et al. 2001; Matheson et al. 1997). In this study Rep fingerprints produced more complex DNA fingerprints than either 16S-RFLP or RISA patterns and allowed a greater degree of discrimination between samples. Rep-PCR amplifications from replicate samples produced highly similar fingerprints (Fig. 4a). DNA fingerprints from different samples from the same mill were more similar (75–96%) when only DNA band position was taken into account, as has been reported by other studies (Lindström 1998; Murray et al. 1998), than when both band position and intensity were considered (43–78%) (Table 2). Although similarity appears to vary considerably with this method, fingerprints among the different mill samples were still less similar than fingerprints from the same mill. When the Rep-PCR DNA patterns from the 7 different mills were compared, the percent similarity ranged from 12 to 34% (Table 2). No correlation could be seen between the fingerprints and either treatment process type or wood furnish. With Rep-PCR, samples from different locations within the same
mill or from different time points showed a lesser degree of similarity in the DNA profiles (43–78%) than with the other two methods. For example, the Rep-PCR patterns from Mill S2 from 8 different time points (Fig. 4b) showed a distinct change in the DNA pattern between the August and September samples which is in sharp contrast to the 16S-RFLP patterns generated from the same samples (Fig. 2). This difference can be attributed to the ability of the Rep-PCR method to differentiate bacteria down to the strain level, a degree of resolution not capable with the 16S-RFLP method. Whether this DNA profile change correlated to mill process para-

Table 2. Percent average inter-sample similarities of the DNA fingerprints within and between each of the mill secondary treatment system samples using 16S-RFLP, RISA and Rep-PCR techniquesa

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>H1</th>
<th>H2</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>85.0</td>
<td>43</td>
<td>43.5</td>
<td>38</td>
<td>43.5</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>S2</td>
<td>96.0</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
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<tr>
<td>H1</td>
<td>88.0</td>
<td>73.8</td>
<td>61.7</td>
<td>59.6</td>
<td>61.7</td>
<td>61.7</td>
<td>61.7</td>
</tr>
<tr>
<td>H2</td>
<td>92.1</td>
<td>61.7</td>
<td>59.6</td>
<td>59.6</td>
<td>52.0</td>
<td>52.0</td>
<td>41.8</td>
</tr>
<tr>
<td>M1</td>
<td>96.0</td>
<td>61.0</td>
<td>59.6</td>
<td>59.6</td>
<td>52.0</td>
<td>52.0</td>
<td>41.8</td>
</tr>
<tr>
<td>M2</td>
<td>82.2</td>
<td>59.6</td>
<td>37.3</td>
<td>37.3</td>
<td>28.8</td>
<td>28.8</td>
<td>15.0</td>
</tr>
<tr>
<td>M3</td>
<td>83.6</td>
<td>83.6</td>
<td>83.6</td>
<td>83.6</td>
<td>83.6</td>
<td>83.6</td>
<td>83.6</td>
</tr>
</tbody>
</table>

a The first line in each box represents the 16S-RFLP similarities, the second line represents the RISA similarities and the third line represents the Rep-PCR similarities.

b NA; Not available, rep-PCR reactions were not performed with the M1 sample.

c ND; Not determined, only one rep-PCR reaction was performed with the M2 and M3 samples and therefore a comparison of averages from samples could not be determined.
meters is not known since the authors are not aware of any change in mill parameters during the time that the samples were taken.

Conclusions

All three methods allowed the quantification of similarity in pairwise comparisons of bacterial communities based on DNA band position and band intensity from electrophoresis of PCR products using computer-assisted pattern analysis. The 16S-RFLP method however was not sensitive enough to discern bacterial community differences within a particular treatment system although different treatment systems could be differentiated. The RISA method was sensitive enough to detect some shifts in community composition within a mill treatment system. However the Rep-PCR method, which can resolve down to the strain level, showed differences in DNA patterns from one sampling time to the next that could not be resolved with either of the other two methods.

This study has shown that the bacterial composition of pulp mill treatment systems can differ substantially from mill to mill—even at the
coarse level of resolution afforded by the 16S-RFLP analysis. One wonders then why some manufacturers market uniform industrial inocula products for these divergent systems and calls into questions the value of studying individual species from single mills in great detail if they are unlikely to be important in other systems. However, this study has also shown that at this level of resolution one treatment system remains fairly stable, resisting large genera changes. RISA and Rep-PCR are useful for studying the compositional changes associated with intra-mill shocks because these high resolution fingerprints have more highly variable targets relative to the ribosomal RNA genes. They can detect more subtle

Fig. 4. Rep-PCR fingerprint patterns. a) Duplicate Rep-PCR fingerprints generated from independent PCR reactions from Mill S1 (lanes 1 and 2) and from Mill M2 (lanes 3 and 4); b) Rep-PCR fingerprint patterns from the Mill S2 bacterial community from 8 different time points (monthly samples over a 9-month period). Lane 1: April; Lane 2: May; Lane 3: July; Lane 4: August; Lane 5: September; Lane 6: October; Lane 7: November; Lane 8: December.
species shifts. Ultimately, these tools may enable operators to be aware of community shifts that could potentially lead to sub-optimal degradation conditions and the eventual discharge of toxic effluent.

References


