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ORIGINAL PAPER

Carbon utilization profiles of bacteria colonizing the headbox water of two paper machines in a Canadian mill

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Abstract Forty-one bacterial strains isolated from the headbox water of two machines in a Canadian paper mill were associated with the genera *Asticcacaulis*, *Acidovorax*, *Bacillus*, *Exiguobacterium*, *Hydrogenophaga*, *Pseudomonas*, *Pseudoxanthomonas*, *Staphylococcus*, *Stenotrophomonas* based on the sequence of their 16S rRNA genes. The metabolic profile of these strains were determined using Biolog EcoPlate, and the bacteria were divided into four metabolic groups. Metabolic profiles of the bacterial communities colonizing the headbox water of two paper machines was also determined weekly over a 1 year period. The only compound that was not reduced by the bacterial community was 2-hydroxybenzoic acid. Utilization frequency of the other carbon sources in the Biolog EcoPlate ranged from 3 to 100%. The metabolic profiles of the bacterial community did not vary considerably between the two paper machines. However, the metabolic profile varied among the sampling dates.

Keywords 16S rDNA · Biolog · Metabolic profile · Microbial community · Principal component analysis

Introduction

The headbox of a paper mill is one of the main parts of a paper production system. The headbox water contains paper raw material slurry that is transported from the headbox to a forming wire. In spite of the use of biocides, several bacterial species contaminate headbox water of paper machines [1–3]. These microorganisms comprised sporulating and non sporulating aerobic bacteria, anaerobic bacteria and some fungi and algae [1, 3–6]. Desjardins and Beaulieu [2] determined that bacteria belonging to the genera *Pseudoxanthomonas*, *Pseudomonas*, *Bacillus* and *Leptothrix* were the main bacterial contaminants of the headbox water of a Canadian paper machine. Although microorganisms present in the paper machine environment have been characterized by several research groups, little is known about spatial and temporal variations within microbial communities that colonize paper machines.

Different techniques have been developed to study microbial communities in specific environments. Some rely on culture-based assays, whereas others are based on a biomolecular approach [7–9]. Dabert et al. [10] divided the biomolecular methods into two groups to monitor microbial community dynamics. The first group comprises techniques that are based on the use of molecular probes to target specific populations. The second analyzes the entire community rather than targeting specific microorganisms. It comprises techniques that are based on the analysis of the community DNA profile such as terminal restriction fragment length polymorphism [8], amplified ribosomal DNA restriction analysis [11, 12], denaturing gradient gel electrophoresis [13, 14], thermal gradient gel electrophoresis [15], single strand conformation polymorphism [16–19], and rRNA intergenic spacer analysis [20–22].

Determining the pattern of carbon source utilization at the community level is another approach that can be used to monitor population changes within bacterial communities [23, 24]. This approach is simple and can rapidly provide information on the functional aspects of microbial communities [20].

Biolog EcoPlate contain a triplicate of 31 carbon sources. These carbon sources, consisting of eight carbohydrates,

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eight carboxylic acids, four polymers, six amino acids, two amines and three miscellaneous substrates, are linked to a colorless tetrazolium dye. When microorganisms from the bacterial community oxidize a carbon source, the dye is reduced and turns purple. Color development in each well is measured to give a characteristic fingerprint of the bacterial communities. Biolog plates have been used in several studies for determining both functional diversity and community structure of microorganisms in different environments [25–27].

In this study, the Biolog EcoPlate system was used to compare the metabolic profiles of 41 bacteria that were isolated from the headbox water of two alkaline paper machines of a Canadian paper mill. Furthermore, Biolog EcoPlate were also used to monitor spatial and temporal changes in the composition of headbox bacterial communities.

Materials and methods

Isolation and identification of bacteria

Pulp of two alkaline paper machines of a Canadian paper mill were sampled periodically in 2003 and 2004. Serial dilutions of the samples were spread on plate count agar (5 g tryptone, 2.5 g yeast extract, 1 g glucose, 15 g agar per liter; pH 7.0) and the plates were incubated at 50°C for 48 h [2]. Pure cultures were obtained by a random process and serial passage of the colonies on plate count agar.

Bacteria were identified by a partial sequencing of the 16S rDNA as follows. Pure colonies were directly picked up and resuspended with a vortex in 100 µl of sterile and distilled water [2]. The Polymerase Chain Reaction (PCR) amplification of the 16S rDNA gene was carried out using 8/20 and 1,541/20 as forward and reverse primers, respectively (Table 1). The DNA amplification was carried out in 50 µl PCR buffer (Amersham Biosciences, Baie d'Urfé, Canada) containing 2 µl of the bacterial suspension (DNA template), 0.2 pmol/µl of primers, 0.25 µM dNTPs and 5U *Taq* DNA polymerase (Amersham Biosciences, Baie d'Urfé, Canada). PCR conditions were as follows: a 5 min hot start at 94°C followed by 30 cycles consisting of 30 s at 94°C, 30 s at the primers annealing temperature (55°C) and 2 min at 72°C.

The sequence reaction was performed on an ALFexpress sequencer using dideoxynucleotide chain termination methods and the Auto Cycle Sequencing Kit supplied by the manufacturer (Amersham Biosciences, Baie d'Urfé, Canada). The primers were the same as those used in PCR amplification. DNA sequence homology searches were performed using the BLAST program [28].

Extraction of the bacteria colonizing the headbox water

The metabolic profile of the headbox bacterial communities of paper machines #7 and #8 was determined weekly over a 1 year period. For each sampling date, two pulp samples were collected and analyzed. Bacterial cells present in the pulp of the headbox paper machines were extracted as follows. The first step of the extraction procedure consisted of a decantation of the pulp sample for 40 min at room temperature. The liquid fraction was then transferred into a new flask, whereas the solid material was mixed with an equivalent volume of NaCl 0.85%. This last suspension was centrifuged at 650 rpm at 4°C for 15 min to remove paper debris. The supernatant was added to the liquid that was recovered in the first step and the entire solution was centrifuged at 4°C at 4,200 rpm for 10 min to obtain a bacterial pellet. To recover a maximum of bacteria, a second extraction of bacteria was performed on the paper debris as described above. Bacterial pellets were pooled and washed three times with distilled water. The cells were finally resuspended in sterile water to a final optical density (OD) of 0.2 at 595 nm. These bacterial suspensions were used to inoculate Biolog EcoPlate (Biolog Inc., Hayward, CA).

Biolog assays

Two different assays were done with the Biolog system: one using pure cultures of the pulp isolates and another using bacterial extracts of the entire headbox community (see above). In each case, Biolog EcoPlate were inoculated with 150 µl of a bacterial suspension ($OD_{595\ nm} = 0.2$). These plates were placed in a plastic bag containing a humidified paper and incubated at 50°C for 4 days. After

Table 1 Oligonucleotide primers used for DNA amplification

Primer	Nucleotide sequence	Corresponding region of the 16S rDNA[a]
BSF 8/20	5′-AGAGTTTGATCCTGGCTCAG-3′[b]	8–27
BSR 1541/20	5′-AAGGAGGTGATCCAGCCGCA-3′[c]	1541–1522

[a] Numbers refer to the corresponding nucleotide positions of *Escherichia coli* 16S rDNA [46]

[b] Labeled at the 5′ end with cyanine

[c] Labeled at the 5′ end with biotin

incubation, the optical density (OD) of the Biolog EcoPlate wells was measured using the microplate reader FL 600 (Bio-Tek, VE). The OD of the control well was subtracted from the OD of all the other wells to correct for background activity. Positive (substrate oxidized) and negative wells (substrate not oxidized) were recorded. A well was scored as positive when its corrected OD was higher than 0.05. The Biolog assay was carried out in triplicate for each isolate and for the bacterial communities extracted from the two pulp samples.

The utilization frequency of a carbon source by the headbox water community was determined by establishing the ratio between the number of positive wells for a specific compound and the total number of readings for the same compound over a month or a year period.

Principle component analysis (PCA) was used to evaluate the differences in the carbon source utilization patterns of the bacterial communities between paper machines and among sampling dates [29]. PCA was done using SAS System Windows (version 6.12) statistical package.

Results

Identification of the pulp bacterial isolates

Forty-six bacterial strains were isolated from the pulp of paper machines. Forty-one of these bacterial isolates were identified by partial sequencing of the 16S rDNA gene (Table 2). Most bacteria belonged to the genera *Pseudomonas* and *Pseudoxanthomonas*. The other isolates comprised both Gram-positive (*Staphylococcus*, *Bacillus*, *Exiguobacterium*, *Asticcacaulis*) and Gram-negative bacteria (*Acidovorax*, *Hydogenophaga*). Five isolates could not be

Table 2 Nearest GenBank neighbors to the headbox bacterial isolates

Isolate	Accession number	Number of nucleotides sequenced	Nearest GenBank neighbors[a]	Accession number (homologs)	Similarity (%)
Ca8-2J04	EU177783	778	*Acidovorax* sp.	AY532541	95
Ca8-3J04	EU177786	779	*Asticcacaulis* sp.	AB016610	97
Ca7-3M04	EU177796	1,442	*Bacillus* sp.	DQ870698	99
Ca8-4J04	EU177784	779	*Exiguobacterium*	DQ870703	97
Ca7-3J04	EU177785	346	*Hydrogenophaga* sp.	EF532793	98
Ca7-1M04[b], Ca8-5M04, Ca8-1J04	EU177802	971	*Pseudomonas* sp.	AJ278108	97–99
Ca7-5M03	EU177787	548	*Pseudomonas citronellolis*	DQ113453	97
Ca7-5J04	EU177788	315	*Pseudomonas putida*	AY491973	96
Ca7-2J04[b], Ca7-4J04, Ca8-6J04, Ca8-2M04, Ca8-5J04, Ca8-3M04	EU177789	992	*Pseudomonas thermotolerans*	AJ311980	96–99
Ca7-5M04[b], Ca8-1M04	EU177790	750	*Pseudoxanthomonas* sp.	EU029560	99
Ca7-1J04[b], Ca7-2M04, Ca7-4M04	EU177792	1,146	*Pseudoxanthomonas* sp.	AB039330	99
Ca7-1J03[b], Ca7-3J03, Ca7-4J03, Ca7-1M03, Ca7-2M03, Ca7-3M03, Ca7-4M03, Ca8-1J03, Ca8-2J03, Ca8-3J03, Ca8-5J03, Ca8-7J03, Ca8-5J03, Ca8-4M03, Ca8-3M03, Ca8-5M03, Ca8-6M03, Ca8-7M03	EU177791	1,423	*Pseudoxanthomonas taiwanensis*	AJ864723	95–98.2
Ca8-4M04	EU177793	1,437	*Staphylococcus* sp.	AY486383	99
Ca7-6M04	EU177794	999	S*taphylococcus epidermidis*	AJ316320	97
Ca7-2J03	EU177795	238	*Stenotrophomonas maltophilia*	AY756730	96
Ca7-6J03	EU177798	487	S4-22	AY123961	99
Ca8-7J04	EU177797	482	Ca-13	AY123960	98
Ca7-7J03[c]	EU177799	779			
Ca8-1M03[c]	EU177800	618			
Ca7-6J04[c]	EU177801	559			

[a] Only the organisms with at least 95% similarity between their 16S rDNA were considered

[b] The same nearest GenBank neighbor was found for this group of isolates. Only the sequence of the first isolate of this group was submitted to GenBank

[c] No sequence sharing at least 95% similarity with the 16S rDNA of these isolates was found

identified. The 16S rDNA sequence of three of these isolates (Ca7-7J03, Ca8-1M03, Ca7-6J04) shared less than 95% similarity with sequences found in databases. The 16S rRNA gene of the two other unidentified isolates (Ca7-6J03, Ca8-7J04) exhibited 99% sequence similarity with the corresponding gene of two bacterial strains of unknown taxonomic identity that were previously isolated from the same paper mill [2].

Metabolic profiles of the pulp isolates

The Biolog EcoPlate were used to determine the metabolic profile of the bacteria isolated from the headboxes. A total of 41 isolates belonging to 9 genera (*Asticcacaulis*, *Acidovorax*, *Bacillus*, *Exiguobacterium*, *Hydrogenophaga*, *Pseudomonas*, *Pseudoxanthomonas*, *Staphylococcus*, *Stenotrophomonas*) were tested. Only 18 carbon sources (listed in Table 3) of the 31 on the Biolog EcoPlate were oxidized by at least one of the 37 isolates belonging to the genera *Acidovorax*, *Bacillus*, *Hydrogenophaga*, *Pseudomonas*, *Pseudoxanthomonas* and *Stenotrophomonas*.

Bacteria could be divided in four groups according to their metabolic profiles (Table 3). Group 1 comprised four bacterial isolates belonging to the genera *Asticcacaulis*, *Exiguobacterium* and *Staphylococcus*. Bacteria from this group showed no metabolic activity on the substrates contained in the EcoPlate under the experimental conditions used in this study. Group 2 included all *Pseudoxanthomonas* isolates as well as *Stenotrophomonas* sp. isolate Ca7-2J03 and *Hydrogenophaga* sp. isolate Ca7-3J04. Isolates from this group oxidized pyruvic acid methylester, Tween 40, Tween 80, xylose and glycyl-L-glutamic acid. The third group comprised only *Bacillus* sp. isolate Ca7-3M04. This strain utilized nine compounds on the EcoPlate including the amino acids L-asparagine, L-serine and L-threonine. The fourth group included all *Pseudomonas* isolates and *Acidovorax* sp. isolate Ca8-2J04. Members of the fourth group showed metabolic activity on 4-hydroxybenzoic acid, D-malic acid, asparagine and putrecine.

No strain was able to utilize any of the 13 following products as a carbon source: D-cellobiose, D-lactose, β-methyl-D-glucoside, i-erythritol, D-mannitol, *N*-acetyl-D-glucosamine, D-glucosaminic acid, glucose-1-phosphate, D,L-glycerol phosphate, 2-hydroxybenzoic acid, L-arginine, L-phenylalanine, phenylethyl-amine.

Metabolic profile of the bacterial community of the headbox

To determine the profile of the bacterial community, the headboxes were sampled once a week for a 1 year period. Of the 31 carbon sources that are contained on the Biolog EcoPlate, 30 carbon sources were used at least once by some members of the bacterial community. However, some carbon sources such as D-lactose, i-erythritol, D-manitol, *N*-acetyl-D-glucosamine acid, D-glucosaminic acid, glucose-1-phosphate, D,L-glycerol phosphate, L-phenylalanine and phenylethylamine were not frequently utilized, whereas some other metabolites (pyruvic acid methyl ester, Tween 40, Tween 80, cyclodextrine, D-xylose, D-galacturonic acid, hydroxybutyric acid, L-arginine, L-asparagine, L-serine, glycyl-L-glutamic acid, putrecine) could be easily catabolized. A carbon source (2-hydroxybenzoic acid) was never used by the bacterial community during the 1 year investigation. Table 4 presents the utilization frequency of the 31 carbon sources by the headbox community of machine #7 during a 1 year period. This table shows that carbon utilization

Table 3 Metabolic groups of bacteria isolated from the machine headboxes of a Canadian paper mill

Metabolic group	Bacterial isolate	Carbon source
Group 1	*Asticcacaulis* isolate Ca8-3J04, *Exiguobacterium* isolate Ca8-4J04, *Staphylococcus* isolates Ca8-4M04, Ca7-6M04	No carbon source utilized
Group 2	*Pseudoxanthomonas* spp. isolates Ca7-3J03, Ca7-4J03, Ca7-1M03, Ca7-2M03, Ca7-3M03, Ca7-4M03, Ca8-1J03, Ca8-2J03, Ca8-3J03, Ca8-5J03, Ca8-7J03, Ca8-3M03, Ca8-4M03, Ca8-5M03, Ca8-6M03, Ca8-7M03, Ca7-5M04, Ca8-1M04, Ca7-1J03, Ca7-1J04, Ca7-2M04, Ca7-4M04, *Stenotrophomonas* isolate Ca7-2J03, *Hydrogenophaga* isolate Ca7-3J04	Pyruvic acid, methylester, Tween 40, Tween 80, xylose, glycyl-L-glutamic acid
Group 3	*Bacillus* isolate Ca7-3M04	Cyclodextrine, glycogen, D-galactonic acid lactone, D-galacturonic acid, hydroxybutyric acid, ketobutyric acid, L-asparagine, L-serine, L-threonine
Group 4	*Pseudomonas* isolates Ca7-5J04, Ca8-1J04, Ca7-1M04, Ca8-5M04, Ca7-5M03, Ca7-2J04, Ca7-4J04, Ca8-6J04, Ca8-2M04, Ca8-5J04, Ca8-3M04, *Acidovorax* isolate Ca82J04	4-hydroxybenzoic acid, D-malic acid, arginine, putrecine

Table 4 Utilization frequency of the EcoPlate carbon sources by the bacterial community associated with the headbox of paper machine #7 from April 2003 to March 2004

Carbon source	Frequency of utilization (%)												
	April	May	June	July	August	September	October	November	December	January	February	March	Mean
Pyruvic acid methyl ester[a]	66	100	100	100	100	100	100	94	100	100	100	100	97
Tween 40[a]	83	100	100	100	100	86	100	100	100	100	100	100	97
Tween 80[a]	25	100	100	100	100	86	100	100	100	100	100	100	93
Cyclodextrin[b]	0	0	17	90	100	86	94	5	100	100	73	100	64
Glycogen[b]	0	8	5	95	58	94	78	0	78	67	60	100	54
D-Cellobiose	0	8	11	57	42	14	0	0	28	11	13	0	15
D-Lactose	0	0	0	17	17	0	0	0	0	0	0	0	3
β-Methyl-D-Glucoside	0	0	0	0	42	0	0	0	17	0	0	0	5
D-Xylose[a]	33	92	83	100	100	86	100	89	100	100	100	100	90
i-Erythritol	0	0	0	90	0	0	0	0	0	0	0	0	8
D-Mannitol	0	0	0	33	67	0	0	0	0	0	0	0	8
N-Acetyl-D-Glucosamine	0	0	5	52	67	0	0	0	0	0	0	0	10
D-Glucosaminic acid	0	0	0	52	0	0	0	0	0	0	0	0	4
Glucose-1-Phosphate	0	0	0	33	0	0	0	0	0	0	0	0	3
D,L-Glycerol phosphate	0	0	5	48	67	0	0	0	0	0	0	0	10
D-Galactonic acid lactone[b]	0	75	28	70	50	14	33	67	100	72	13	33	46
D-Galacturonic acid	8	92	72	100	83	53	83	67	100	94	60	33	70
2-Hydroxybenzoic acid	0	0	0	0	0	0	0	0	0	0	0	0	0
4-Hydroxybenzoic acid[c]	0	100	33	100	50	28	50	17	100	61	47	100	57
Hydroxybutyric acid[b]	0	10	89	100	67	86	100	67	100	100	100	100	77
Itaconic acid	25	16	39	100	33	0	11	17	33	28	7	33	29
Ketobutyric acid[b]	0	0	5	100	8	0	0	0	83	33	7	78	26
D-Malic acid[c]	0	83	28	90	42	0	61	33	94	61	20	33	45
L-Arginine[c]	75	100	100	100	100	62	72	67	100	83	100	100	86
L-Asparagine[b]	75	100	100	100	100	67	83	94	100	100	100	100	93
L-Phenylalanine	0	0	22	18	17	0	5	0	50	0	0	0	9
L-Serine[b]	9	100	94	100	100	86	94	89	100	100	100	100	89
L-Threonine[b]	0	16	0	100	50	0	5	5	33	33	0	33	23
Glycyl-L-Glutamic acid[a]	33	75	50	100	75	86	89	61	100	100	53	55	73
Phenylethyl-amine	0	0	0	48	8	0	0	0	0	0	0	0	5
Putrecine[c]	0	100	61	95	67	57	67	50	100	89	100	100	74

[a] Compound oxidized by the metabolic Group 2

[b] Compound oxidized by the metabolic Group 3

[c] Compound oxidized by the metabolic Group 4

varied among months. In April, the bacterial populations were able to metabolize only 10 carbon sources, whereas the bacterial populations sampled in July and August utilized over 20 of the carbon sources.

Population dynamics

The monitoring of metabolic profiles of the bacterial communities in the two headboxes was done to estimate temporal and spatial variation in the headbox communities of two machines at the same paper mill. Figure 1 presents the principal component analysis carried out to compare the metabolic profiles of the bacterial community of machine #7 over time, from April 2003 to March 2004. Each point of the graph represents the mean of all samples collected during a specific month, whereas the bars represent the standard deviations. Figure 1 shows that most points were clustered in the same area of the graph and that several standard deviation bars intersected. Only the point associated with the month of July clearly diverged from the others.

On Fig. 2, data from machines #7 and #8 were compiled. Data from the headbox of machine #8 are presented as

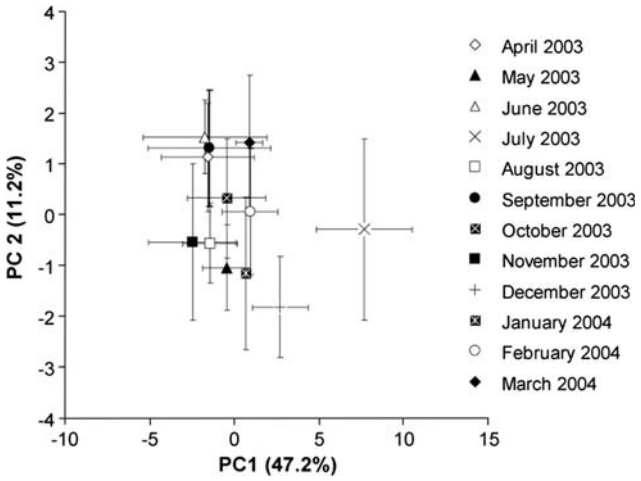

Fig 1 Principal component analysis (PCA) of carbon source utilization patterns of the microbial communities of paper machine #7 generated with Biolog EcoPlate. PC1, principal component 1; PC2, principal component 2. *Vertical* and *horizontal lines* represent standard errors (SE) of the mean ($n = 3$); data points represent the months

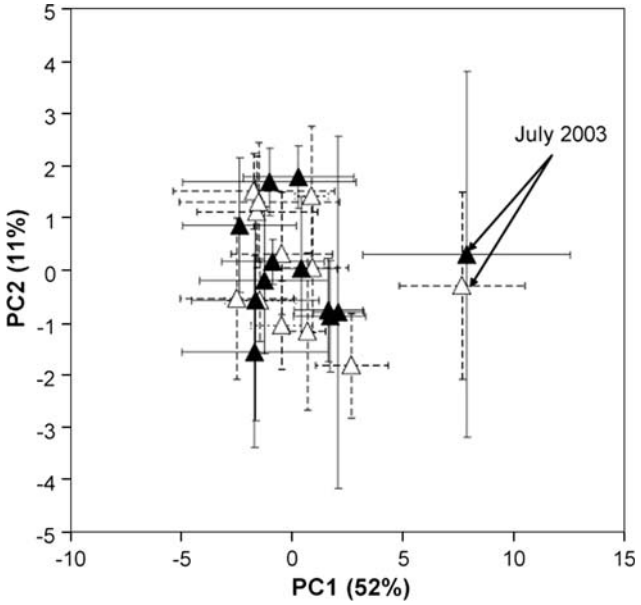

Fig 2 Principal component analysis (PCA) of carbon source utilization patterns of the microbial communities of paper machines #7 and #8 generated with Biolog EcoPlate. PC1, principal component 1; PC2, principal component 2. *Vertical* and *horizontal lines* represent standard errors (SE) of the mean ($n = 3$); *dotted lines* are associated with data of the paper machine #7, whereas *continuous lines* are associated with data of paper machine #8. Data points represent the months; the two points that are not clustered with the others were associated with the month of July 2003

continuous lines, whereas dotted lines are associated with machine #7. No clear separation was observed within points associated with machines #7 and #8. However, the month of July clearly differed from other groups of data in both machines.

Discussion

The monitoring of population dynamics has been carried out in different environments and different countries using Biolog plates [30–32]. To our knowledge, no study has been carried out to analyze the changes of the microbial community associated with the headbox of a paper machine using the Biolog microplate approach. However, molecular techniques have been used to study the dynamics of bacterial communities associated with pulp mill effluent treatment systems [22, 33, 34], and several studies have reported the isolation of microorganisms from paper machines [1–6]. For example, Desjardins and Beaulieu [2] carried out a microbiological study at the same Canadian paper mill that we examined in this study. They have, periodically over a 1 year period, isolated bacteria from a paper machine to obtain a global representation of the bacterial communities associated with the pulp of the headbox and the slimes colonizing the machine surface.

Desjardins and Beaulieu [2] most often isolated paper machine bacteria belonging to the genera *Bacillus*, *Pseudomonas*, *Microbacterium* and *Pseudoxanthomonas*. Other studies on the identification of bacteria contaminating paper mills that were conducted in USA [1, 5, 35] have also demonstrated that *Pseudomonas*, *Bacillus* and *Microbacterium* were among the paper mill bacterial community. Our results confirmed the work of Desjardins and Beaulieu [2] suggesting that *Pseudoxanthomonas*, a bacterial genus associated with various environments such as polluted soil and hot springs [36, 37] was a common inhabitant of the warm water of two different Canadian paper machines. In this study, we isolated the same bacterial genera that were previously isolated from headboxes by Desjardins and Beaulieu [2] from the same paper machines. However, strains of *Leptothrix* spp. which were isolated in the headbox by Desjardins and Beaulieu [2], were not found in this study, whereas the genera *Asticcacaulis* and *Exiguobacterium* were isolated in the present study but not in the previous one [2]. The differences observed between these studies might reflect a temporal change of the bacterial community or it could simply result from the low number of bacteria that have been identified in these studies.

Biolog plates have frequently been used for testing the ability of microorganisms or populations to oxidize compounds from a preselected panel of carbon sources [23]. It is a valuable and rapid tool for microbial community analysis, but it has some limitations [29, 38]. For example, some of the bacteria that were isolated from the headbox machine did not show any metabolic activity on substrates contained in the Biolog EcoPlate. Prévost et al. [29] have also reported that Biolog EcoPlate could not be used to determine the metabolic profile of bacterial species such as *Streptomyces* spp.

In this study, strains belonging to a same taxonomic genus showed the same metabolic profile, but some genera could not be distinguished from each other by their metabolic patterns. Similarity in metabolic abilities might be, in the case of *Pseudomonas* and *Acidovorax*, explained by a taxonomic proximity [39]. As bacteria belonging to a same metabolic group exhibited similar biochemical properties, one could expect that they also share similar ecological niches. Bacterial genera belonging to the same metabolic profiles have been co-isolated not only from paper machines but also from other environments such as mineral oil hydrocarbon-contaminated soils [40], arterial walls of aortic aneurysms [41], mushroom compost [42], and biofilters [43].

All together, bacteria belonging to the genera *Acidovorax*, *Bacillus*, *Hydrogenophaga*, *Pseudomonas*, *Pseudoxanthomonas* and *Stenotrophomonas* oxidized only 18 carbon sources of the 31 contained in the Biolog EcoPlate. However, 16 out of these 18 compounds were the carbon sources for which the utilization frequency by the headbox communities was the highest (from 45 to 97%). Since the utilization of a carbon source depends not only on the microbial efficiency but also on the population size [44], our data suggest that these six genera were important colonizers of the headbox paper machines. Furthermore, the utilization frequency of 11 of the 13 compounds that could not be oxidized by the pure cultures was relatively low (3–15%), which suggests once again that the bacteria isolated in this study represented an important fraction of the bacterial community.

Although D-galacturonic acid and itaconic acid were quite frequently metabolized by the headbox bacterial communities species (70 and 29%, respectively), no pure culture oxidized these compounds, indicating that other bacterial groups well adapted to the paper machine environment still need to be isolated. Furthermore, the variation observed in the utilization frequency of compounds associated with a metabolic group also indicated that other bacterial species, which were not isolated in this study, contributed to the metabolic profiles of the microbial community. For example, although the utilization frequency by the headbox bacterial community of ketobutyric acid and threonine, two compounds that could be only oxidized by *Bacillus* sp. isolate Ca7-3M04, was of about 25%, the utilization frequency of other carbon sources catabolized by the *Bacillus* sp. isolate Ca7-3M04 was considerably higher, i.e., from 46 to 93%.

The principal component analysis of the metabolic profiles of the headbox communities from paper machines #7 and #8 shows little variation in the metabolic abilities of the bacterial communities of the two machines. Our results suggest that paper machines from the same location and under the same operation procedures will be associated with comparable microbial communities. As for other industrial process [34], the microbial communities the headbox paper machines appeared to be quite stable, even if the metabolic abilities of the headbox bacterial communities varied among sampling dates. The bacterial community recovered in July especially diverged from the ones of the other sampling dates. The discrepancy observed in July in regard to the metabolic profiles of the bacterial community is still unexplained but could be due to the high microbial diversity of the water entering the system. Indeed, Ainsworth and Goulder [45] have suggested that viable bacterial population densities increased significantly from spring to summer in rivers of a temperate country.

This study shows that Biolog system could be useful not only to monitor environmental ecosystems but also to study the microbial structure of communities associated with industrial processes. The metabolic patterns generated by the Biolog analysis could be useful in correlating the structural changes of the paper machine communities with other factors in the paper making process. It would also be interesting to expand this study to other paper mills and paper making processes.

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