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Bacteria in the water from the snow from the area Ružomberok

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Abstract. The aim of our study was to investigate the possible environmental pollution by the Mondi SCP pulp mill in Ružomberok. Bacterial communities were examined by T-RFLP analysis of the 16S rRNA gene. This gene was subjected to restriction digestion by using the restriction enzyme MspI. Terminal restriction fragments (T-RFs) from 60-924 bp were evaluated. An experimental size of fragments was compared to in silico sizes T-RF from the database of 16S rDNA sequences by using the MiCA program. Amplification and sequencing of 16 rDNA from DNA isolated from bacteria in the snow samples indicate the presence of bacteria. It was established three categories of bacteria. The first representated the dominant phyla of 5 to 25 % (*Proteobacteria* 19.5 %, *Firmicutes* 18.2 %, *Actinobacteria* 13.4 %, *Bacteroidetes* 11.1 %, and *Chloroflexi* 10.1 %). The second group has a relatively low percentage of bacterial phyla 1 to 5 % (*Planctomycetes, Acidobacteria, Tenericutes, Planctomycetes, Acidobacteria, Tenericutes, Spirochaetes, Cyanobacteria, Deinococcus-Thermus, Gemmatimonadetes, Fusobacteria, Nitrospirae*). In the last one, the percentage of bacterial phyla ranged from 0 to 1% (*Defferibacteres, Chlorobi, Verrucomicrobia, Synergistes, Fibrobacteres, Thermotogae, Caldiserica, Thermodesulfobacteria*).

Key words: snow-bacteria, mg DNA, pulp mill, Mondi SCP Ružomberok, T-RFLP

Introduction

We chose to study bacteria in snow melt surrounding the Mondi SCP mill near Ružomberok to show what type of bacteria occur in this environment. It is known that paper and pulp processing results in the production of many highly toxic chlorinated organic compounds. Of prime concern are chlorinated phenols, guaiacols, catechols, furans, dioxins, aliphatic hydrocarbons and highly mutagenic agents. In addition, two natural wood resin acids, neoabietic and 7-oxodehydroabietic acids, exhibit mutagenic activity (Murray 1992). Recent research on bacteria in the snow and their influence show that an abundance of bacteria are closely related to dust (Chuvochina *et al.* 2011; Yamaguchi *et al.* 2012). Bacterial concentration positively correlates with the distribution of dirty layers (Zhang *et al.* 2008). Other reports suggest that microorganisms impact composition, abundance of nutrients, and the dynamics and it seems bacteria have a role in governing redox conditions and also play a part in Fe, S, N, and P cycling (Hodson *et al.* 2008). It is likely that they are also responsible for the metabolism and transformation of environmental contaminants such as mercury (Barkay and Poulain 2007).

Our aim was to collect snow samples around Mondi SCP in Ružomberok and isolate the DNA of bacteria in samples of melted snow. For monitoring we chose to use Terminal restriction fragment length polymorphism analysis (T-RFLP), which is a cultivation independent method of microorganism identification (Marsh 1999).

Psychrophilic bacteria covered an extensive range of phylogenetic diversity (Bowman *et al.* 1997). They represente important functional groups such as methanotrophic organisms (Bowman *et al.* 1997), sulphate-reducing (Knoblauch *et al.* 1999), and methanogenic organisms (Franzmann *et al.* 1997).

Terminal restriction fragment length polymorphism

T-RFLP analysis is a technique used to study complex microbial communities based on variation in the 16S rRNA gene (Osborn *et al.* 2000). In this way in is possible to determine if new microbes are present in a particular environment. The length of the bacterial 16S rRNA gene is approximately 1540 base pairs. This is why it can be quickly and inexpensively copied and sequenced (www.microbeworld.org 2015).

Total DNA is first extracted from the microbial community and the16S rRNA gene is isolated from samples using fluorescently-labelled forward and reverse primers. Next, the PCR product is purified and subjected to restriction enzyme digestion with enzymes that have 4 base pair recognition sites. This step generates fluorescently labelled terminal restriction fragments. The digested products are then separated and detected on an appropriate electrophoresis platform. For a given sample the terminal fragments will contain a fluorescent label at the 5'- end and will therefore be detected.

The output will be a series of peaks (fragments) of various sizes and heights that represents the profile of that sample (Osborn *et al.* 2000).

Material and Methods

Study area and sample collecting

Collection of samples was conducted during snowy periods in January-February 2015 the Mondi SCP mill in Ružomberok (N: 49° 4´ 50.18", E: 19° 19´ 35.44").

The sample collection period was chosen based on predicted snowfall (during the winter 2014/2015). Samples were collected from two localities. The first sampling location was in Lisková which is located to the east and 2 km away from Mondi SCP Ružomberok (N: 49° 5´ 27.93"; E: 19° 21´ 0.45V). The second control location was in Hrboltová which is located to the west and 6,5 km away from Mondi SCP Ružomberok (N: 49° 6´ 1.14"; E: 19° 14´ 40.58").

The first sample of snow was collected on January 8. Samples were collected from both sampling sites once per day. Due diligence during sampling processes was important to ensure minimal contamination. Snow was melted slowly to 5° C (up to 48 h) to avoid stress to the bacteria during melting. Depending on the snow texture, melted snow resulted in 500 - 850 ml melt water, which could be compared to previous observations (Ferrari *et al.* 2004). All of the samples were collected between 7:30 am and 6:30 pm, but most of them between 12:00 - 4:00 pm. During sampling we recorded the time of sampling, temperature and weather. A total of 61 samples were collected (Table 1).

T-RFLP analysis

DNA isolation

Melted snow was filtered by a vacuum filtration system (KNF Neuberger, Inc., USA). Until further processing in laboratory, the filters were stored at a temperature of 4° C, and filled with 50 ml of water to avoid drying out the filters. Following the collection of all the samples, DNA was isolated using the PowerWater® DNA Isolation Kit. DNA was isolated according to the manual.

Following DNA isolation, the concentration and purity of DNA was measured by the NanoPhotometer P330 (Implen, Germany) device and isolated DNA was stored at -20° C.

Agarose gel electrophoresis

DNA extracts were verified by gel electrophoresis, using 1 % Agarose gel (1 g of Agarose and 100 ml 1xTBE). They were observed under UV light with the addition of ethidium bromide (50 µg for 100 ml of gel).

PCR

16S rRNA genes were amplified by PCR (polymerase chain reaction). This step was carried out using universal primers for bacteria 8F (Edwards *et. al.* 1989) and 926R (Muyzer *et al.* 1995). The forward primer was fluorescently labelled at the 5'- end with Cyanine5 (Cy5'). For PCR were prepared with master mix in 50 µl volumes. The PCR master mix contained reagents with the following final concentrations: 1x PCR buffer, 1.5 mM $MgCl_2$, 200 mM of dNTPs, 0.1 µM of each primer, 2.5 U Fire Pol DNA Polymerase.

Date	Age of snow in days	Number of samples	Lisková		
			Time	T [° C]	Actual weather
3.12.2014	0	I	14:00	-1	overcast
8.1.2015	4	III	13:15	-7	overcast
10.1.2015	6	1	14:20	4	overcast
22.1.2015	18	3	16:00	4	overcast
25.1.2015	1	5	15:10	-3	snowing
26.1.2015	2	7	13:45	1	snowing
27.1.2015	-	-	-	-	-
28.1.2015	4	11	14:00	1	overcast
29.1.2015	0	13	13:15	1	snowing
30.1.2015	0	15	12:45	4	overcast
31.1.2015	0	17	14:00	-3	overcast
1.2.2015	1	19	15:34	-2	overcast
2.2.2015	2	21	13:30	1	overcast
3.2.2015	3	23	13:30	0	overcast
4.2.2015	0	25	12:10	2	snowing
5.2.2015	1	27	10:15	-1	sunny
6.2.2015	2	29	12:30	2	sunny
7.2.2015	3	31	14:00	-2	sunny

continued...

8.2.2015	0	33	11:20	-1	snowing
9.2.2015	0	35	12:30	1	snowing
10.2.2015	1	37	18:30	2	overcast
11.2.2015	2	39	15:00	4	overcast
12.2.2015	3	41	13:30	7	overcast
13.2.2015	4	43	16:00	4	sunny
15.2.2015	6	45	15.30	3	sunny
17.2.2015	8	47	13:30	2	sunny
18.2.2015	9	49	13:30	1	sunny
19.2.2015	10	51	13:30	2	sunny
20.2.2015	11	53	13:30	6	sunny
23.2.2015	14	55	15:30	7	overcast
24.2.2015	15	57	9:00	-1	overcast
6.3.2015	3	59	9:30	4	overcast

			Hrboltová		
			Time	**T [˚ C]**	**Actual weather**
3.12.2014	0	II	14:30	-1	overcast
8.1.2015	4	IV		0	overcast
10.1.2015	6	2	15:00	0	overcast
22.1.2015	18	-	-	-	-
25.1.2015	1	6	14:20	-3	snowing
26.1.2015	2	8	17:00	0	snowing
27.1.2015	-	10	13:05	0	snowing
28.1.2015	4	12	12:15	-1	overcast
29.1.2015	0	14	12:15	-6	snowing
30.1.2015	0	16	14:15	2	overcast
31.1.2015	0	18	14:30	-3	overcast
1.2.2015	1	20	14:40	-2	overcast
2.2.2015	2	22	12:00	0	snowing
3.2.2015	3	24	11:30	0	overcast
4.2.2015	0	26	10:30	1	overcast
5.2.2015	1	28	8:00	-4	sunny
6.2.2015	2	30	14:40	-4	sunny
7.2.2015	3	32	14:40	-2	sunny
8.2.2015	0	34	12:00	-1	snowing
9.2.2015	0	36	13:30	-1	snowing
10.2.2015	1	38	16:30	0	overcast
11.2.2015	2	40	14:00	2	overcast
12.2.2015	3	42	14:00	3	overcast
13.2.2015	4	44	13:15	4	sunny
15.2.2015	6	46	16:10	2	sunny
17.2.2015	8	48	14:00	3	sunny
18.2.2015	9	50	14:00	0	sunny
19.2.2015	10	52	14:00	2	sunny
20.2.2015	11	54	14:00	4	sunny
23.2.2015	14	56	14:15	8	overcast
24.2.2015	15	58	7:30	2	overcast

Table 1. Database of samples with characterization of time of sampling, temperature and weather.

The PCR reactions were run over in the thermocycler (Standart PCR, Bio-Rad C1000 TOUCH) by using PCR program (Table 2).

Used primers: **8F** 5' Cy5-GAGTTTGATCCTGGCTCAG 3' and **926R** 5' CCGTCAATTCCTTTRAGTTT 3'.

PCR conditions were optimized, and the first PCR test was performed on 10 samples. The chosen samples were exposed to PCR amplification.

PCR reactions were verified by electrophoresis in 1 % agarose gel (1 g of Agarose and 100 ml 1 × TBE). It was visualised under UV light and addition of ethidium bromide (50 µg for 100 ml of gel).

Temperature profile:			
Initial denaturation	95° C	3 min.	
Denaturation	94° C	30 sec.	
Annealing	56° C	45 sec.	35 cycles
Extension	72° C	2 min.	
Final Extension	72° C	3 min.	

Table 2. PCR program for amplification of 16S rRNA gene.

Ethanol precipitation

PCR products were precipitated according to Ondreičková *et al.* (2014). A volume of 1/10 volume 3M sodium acetate (pH 5.2) was added to the PCR products. Followed by three times the total volume of chilled 96 % ethanol. The mixture was thoroughly mixed and incubated for 20 minutes at -80° C, then centrifuged at 13 500 rpm for 20 minutes at 4° C. The supernatant was carefully poured down after centrifugation, as the pellet must not be removed. 70 % ethanol (250 µl) was added, and then the sample was centrifuged at maximum speed for 5 minutes at 4° C. Following this, supernatant was again carefully poured down. The pellet was left to dry at room temperature and then dissolved in sterile water.

Restriction digest

The purified PCR products were subjected to restriction by the following procedure:

A reaction mixture was prepared of 10 µl mg DNA, 2 µl of 10x buffer for a given restriction enzyme, and 20 units appropriate restriction endonucleases. Distilled water was added to the reaction mixture to a volume of 20 µl and allowed to incubate for 3 hours at 37° C. The restriction enzyme MspI (New England Biolabs, USA) was used. The digested PCR products were precipitated by ethanol. It was added 1/10 volume of 3M sodium acetate (pH 5.2) to the PCR products. Then it was added 3 multiple of the volume of chilled 96 % ethanol. The mixture thoroughly mixed and incubated for 20 minutes at -80° C. Centrifuged at 13 500 rpm, 20 min at 4° C. Supernatant was carefully poured down after centrifugation, because pellet has to be not removed. It was added 70 % ethanol (250 µl). Centrifuged at maximum speed for 5 min, 4° C. Subsequently carefully poured down the supernatant. Pellet was left to dry at room temperature and then the pellet resolved in the sterile water.

Automated separation of T-RFs

The purified terminal restriction fragments were separated by capillary electrophoresis by an automated sequencer (GenomeLab GeXP, Beckman-Coulter, U.K.). The separation of the fragments is carried out in polyacrylamide gel during denaturing conditions. Composition of the mixture to fragment analysis was: SLS (38.5 µl, AB Sciex, Canada), standard MapMarker 1000 labelled with D1 (0.5 µl Bioventures, Inc., USA) and 1 µl of DNA.

Evaluation of T-RFs profile and statistical analyses

Terminal restriction fragments (T-RFs) were defined as those between 60 and 924 bp. Using by GenomeLab GeXP software. We compared these fragments with in silico sizes T-RF from the 16S rDNA sequence database using the MiCA 3 program (Microbial Community Analysis III; Shyu *et al.* 2007).

To compare bacterial communities in the samples, T-RFLP profiles were evaluated based on the presence of T-RF. Size T-RF were expressed as present (1) or absent (0) in the samples. Due to this constellation of binary system PCA (Principal Component Analysis) by the Statistica 8 program was used.

Our spreadsheet also includes the age of snow, defined by three categories. The first category was snow 0 to 1 days old, followed by the second category; snow 2 to 4 days old. The third category was snow older than 5 days.

The month in which samples were collected was also recorded. Months 1 includes the first half of winter and Months 2 the second half. Three categories of weather were recorded: overcast, sunny, or snowing. The last set of data were temperatures, either below ° C or above 0° C. These data were analysed by One-way ANOVA and factorial ANOVA.

Results

We collected 61 samples. DNA concentration level varied from 4 to 64 ng/µl. The ratio of absorbance at 260 nm and 280 nm is used to assess DNA purity. If the ratio is considerably lower than 1.8, it may indicate the presence of protein, phenol or other contaminants. The purity of PCR products after precipitation ranged from 1.4 - 2.3.

The second measured of purity was (A260/230). Expected values of A260/230 are usually in the range of 2.0 - 2.2. If it is considerably lower, it may also indicate the presence of contaminants. The purity of PCR products after precipitation was ranged 0.24 – 4.93.

To define the genetic diversity of the bacterial community, we chose the 16S rRNA gene. This gene isolated in the DNA by universal primers 8F and 926R (Fig. 1). The first primer was fluorescently labelled at the 5'end with Cyanine -5 (Cy-5').

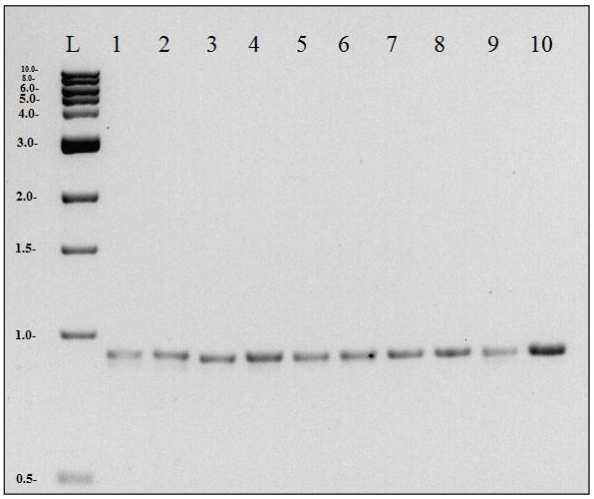

Fig. 1. PCR products - 16S rRNA gene of 10 chosen samples. L - 1kb DNA ladder. 1-10 - number of samples putted to lane in gel.

Conclusion T-RFLP analysis

Using T-RFLP analysis, fragments of digestion were compared with the in silico T-RF sizes. We determined relative abundance phyla. We created three groups According to the percentage of bacterial phyla. The first group includes a representation of the dominant phylum of 5 to 25 %. The second group had a relatively low percentage of bacterial phylum (1-5 %). In the last group, the phylum of the minority representation was up to 1 % (Table 3).

Phylum	%	Percentage in Lisková (1)	Percentage in Hrboltová (2)
Dominant phyla 5-27 %			
Proteobacteria	19.5	19.7	19.3
Firmicutes	18.2	18.2	18.1
Actinobacteria	13.4	13.1	13.6
Bacteroidetes	11.1	11.7	10.6
Chloroflexi	10.1	10.1	10.1
Percentage of phyla 1-5%			
Planctomycetes	4.7	5.1	4.4
Acidobacteria	4.5	4.6	4.4
Tenericutes	3.9	3.7	4.1
Spirochaetes	2.4	2.4	2.4
Cyanobacteria	2.4	2.2	2.5
Deinococcus-Thermus	1.8	1.5	2.1
Gemmatimonadetes	1.6	1.6	1.6
Fusobacteria	1.2	1.0	1.4
Nitrospirae	1.0	0.9	1.0
Percentage of phyla to 0-1%			
Defferibacteres	0.9	1.0	0.9
Chlorobi	0.8	0.8	0.8
Verrucomicrobia	0.8	0.6	1.0
Synergistes	0.7	0.6	0.7
Fibrobacteres	0.5	0.6	0.5
Thermotogae	0.2	0.2	0.2
Caldiserica	0.2	0.3	0.1
Thermodesulfobacteria	0.1	0.0	0.2

Table 3. Relative abundance phyla compared by the sizes of T-RFs obtained by digestion with the enzyme MspI with the in silico T-RF sizes from the database of the 16S rDNA sequences.

T-RFs were detected in the samples in various amounts. T-RFs were detected in 61 samples from 2 localities. The most T-RFs were detected in sample number 12 (with 182 fragments), and the least in sample number 28 (with 6 fragments). The average value of fragments of all samples collected was 59. To compare bacterial communities in the samples T-RFLP profiles were evaluated using the presence of T-RF. Due to this constellation of binary system PCA (Principal Component Analysis) with a statistical program was used.

The complex of data used in the statistical program included records of the age of the snow, its temperature, month collected, and locality. We determined a correlation between these and the presence of T-RF. We focused on the 3 most significant of 7 tested principal components (factors).

Under the principal component Factor 3, correlation between localities and months was found. It shows that during the first months of winter, composition of bacterial diversity differs from the localities, though it is not statistically significant (Fig. 2)

A correlation between localities and samples shows that there are not significant differences between localities of bacterial diversity in the individual samples (Fig. 3). We compared fragments from samples 18, 42, and 44 (representing the Hrboltová locality) as well as fragments of samples 17, 23, and 25 (represent the Lisková locality) with the remaining samples.

Discussion

Amplification and sequencing of 16 rDNA from DNA isolated from bacteria in our snow samples indicate the presence of bacteria. We organized these into three categories. The first includes the representation of the dominant phyla of 5 to 25 %. The second group has a relatively low percentage of bacterial phyla (1 to 5 %). In the last one, the percentage of bacterial phyla ranged from 0 to 1 %. We devoted the most attention to bacterial phyla in the first category. This catecory includes *Proteobacteria* (19.5 %), *Firmicutes* (18.2 %), *Actinobacteria* (13.4 %), *Bacteroidetes* (11.1 %), and *Chloroflexi* (10.1 %). First we searched what type of bacteria are typical in cold environments, including psychrophilic microorganisms, which are restricted to permanently cold habitats. Several studies include psychrophilic

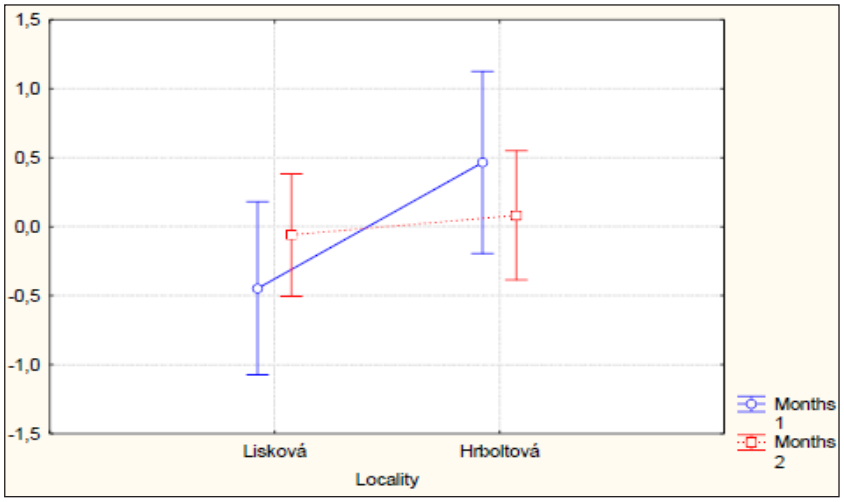

Fig. 2. The effects of increase or decrease bacterial diversity correlation between localities and months. (One – way ANOVA F (1,53)= 1.9220, p = 0.1714).

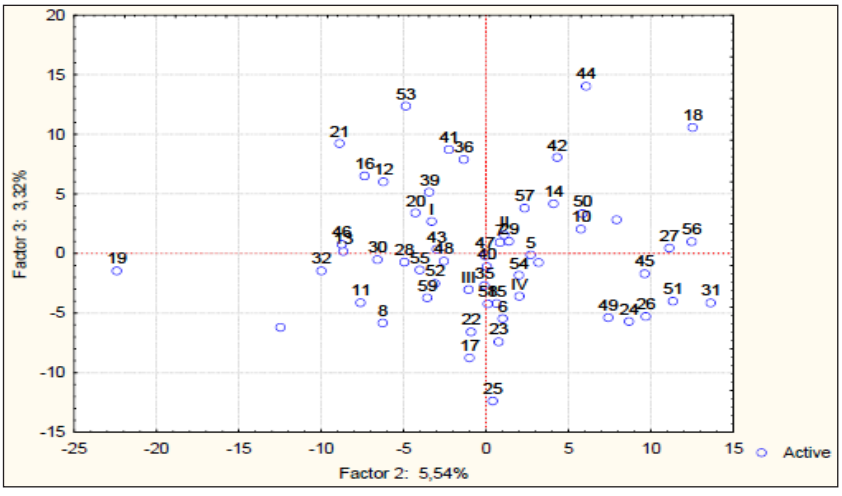

Fig. 3. The effects of increase or decrease bacterial diversity correlation between samples and Factor 3.

bacteria of phyla such as: *Proteobacteria, Bacteroidetes*, and *Firmicutes* (Gounot 1986). We compared studies researching bacteria found in snow. Bacterial diversity in snow varied among different glaciers. For example, members of *Bacteroidetes*, α, β, γ – *Proteobacteria, Actinobacteria*, and *Firmicutes* were dominant in studies of glaciers by Liu *et al.* (2009). In another study, they induct the most dominant group of α, β, γ – *Proteobacteria*, *Actinobacteria, Firmicutes, Verrucomicrobia, Cyanobacteria*, and *Flavobacterium* (Larose *et al.* 2010). This is similar to other research. In the study by Liu *et al.* (2009), *Proteobacteria, Bacteroidetes*, and *Cyanobacteria* dominated, though *Actinobacteria* and *Firmicutes* were also abundant. In the deepest snow layer, large percentages of *Firmicutes* and *Fusobacteria* can be found. In freshwater, *Bacteroidetes, Actinobacteria* and *Verrucomicrobia* were the most abundant phyla while and relatively few *Proteobacteria* or *Cyanobacteria* were present (Møller *et al.* 2011). We can see that phyla in the studies are sonsistent with our dominant phyla with the exception of *Chloroflexi*. This difference was the topic of our discussion.

The production of paper is the main source of halogen materials, which are used in whitening silks of cellulosic. In this procedure we can see the reaction of chlorine with organic material resulting in chlorinated organic materials, mainly polychlorinated dibenzodioxins and polychlorinated biphenyls (PCB) (Raclavská *et al.* 2008).

The available information indicates that the ring structures of dibenzo-p-dioxin, dibenzofuran (DF), and related compounds are degraded by aerobic bacteria containing aromatic hydrocarbon dioxygenases having broad substrate specificity. In addition to this type of biodegradation, another process of dioxin biotransformation has been recognized; reductive de-chlorination by anaerobic microorganisms (Yoshida *et al.* 2005). This trait, halorespiration - the use halogenated organics as energy sources - is found in some species belonging to three major phyla: *Proteobacteria, Chloroflexi* and *Firmicutes*. Whereas bacteria belonging to the phylum *Proteobacteria* (i.e. *Anaeromyxobacter, Desulfomonile, Desulfuromonas, Desulfovibrio* and *Geobacter*), and the phylum *Firmicutes* (i.e. *Desulfitobacterium*) are facultatively dehalorespiring bacteria. Strictly dehalorespiring microorganisms are limited to only two bacterial genera: *Dehalobacter* (*Firmicutes*) and *Dehalococcoides* (*Chloroflexi*) (Hiraishi 2008). As mentioned by Yoshida *et al.* (2005) aerobic bacteria of the abovementioned phyla are likely responsible for the oxidative degradation of dechlorinated products in the microcosm. Members of the "*Dehalococcoides*" group were reported to be putative de-chlorinators in anaerobic enrichment cultures capable of dechlorinating. The detection of *Dehalococcoides* and its relatives in polluted environments and dechlorinating enrichment cultures indicates that members of the *Dehalococcoides* group are widely distributed in nature and play major roles in the transformation of environmental organohalide pollutants (Yoshida *et al.* 2005). According to Japanese scientists, the detection of *Dehalococcoides* would provide more direct evidence for the involvement of the organism in de-chlorination, and for the presence of this group of bacteria worldwide in organohalide-polluted environments.

We endeavoured to explain the rising representation of steam *Chloroflexi* as well as *Proteobacteria* in the localities we sampled. In these localities we can assume contamination is caused by organic chlorinated materials. The steam *Chloroflexi* was the 5th most occurring steam, and its occurrence is generally atypical in snowy conditions. When taking into consideration the genre *Dehalococcoides* and its characteristic occurrence in organohalide-polluted environments we must consider the potential contamination caused by the nearby pulp and paper mill. Indeed 32 of 61 samples were contaminated. The organic halogens which were present in our samples could be a result of contamination by proximity to this facility. They may serve as culture medium for the increase of steam *Chloroflexi*, as well as more notably, *Dehalococcoides*. It is assumed that naturally occurring chlorophenols are candidates for the original substrates for the *Dehalococcoides* (Hiraishi 2008). A connection might also be made between *Dehalococcoides* and the *Deltaproteobacteria* found in the T-RFs profiles of samples 135, 512, 513, called facultatively dehalorespiring bacteria.

The usage of polychlorinated biphenyls is also interesting; as they are no longer used in the machines used for production of paper in the paper mill SCP Ružomberok.

The SCP paper mill in Ružomberok purchased a product called Delotherm DH which contains PCB. We are not aware of the quantity of this product but the usage of PCB is well know in the production of copy paper, which did not contain the carbon. (Murín *et al.* 2003).

PCBs are materials that inflict long lasting destruction, their concentration rises when the temperatures are low, and they can be carried over long distances (Kimáková 2009). They may also play a role in the increasing of the steam *Chloroflexi*. Bacteria like those from the group *Dehalococcoides* are able to consume PCB, so it's presence may increase their prevalence as the chlorine in PCB molecules acts as a metabolic fuel. Scientists from Rensselaer Polytechnic Institute lead by Donn Bedard proved that these bacteria are abundant in locations where there is desensitization caused by chloric disincorporation as well as polychlorinated biphenyls (DeMarco 2007). For this reason we began to investigate the influence of paper mill on the presence of *Dehaloccoides*, and the phylum of *Chloroflexi*, in our samples.

The correlation between localities and samples, which can be seen in Fig. 3, represents a diverse percentage of phyla in the remaining samples; particularly in fragments of samples number 18, 42, and 44 (represent locality Hrboltová), and fragments of samples number 17, 23, and 25 (represent locality Lisková). In the case of sample 44, differences could be a result of the prevalence of phylum *Chloroflexi* (12.4 %), since the remaining samples show percentages of phylum *Chloroflexi* between 8-10 %. Differences shown by sample 25 include most notably a decrease in the most abudant phylum; *Proteobacteria* (14.8 %), while the percentage of phylum *Proteobacteria* in redundant samples is around 20 %.

More attention should be paid toward the chemical cycle of halogenated compounds in which anaerobic dehalogenating microorganisms may play important ecological roles. Thsee organism could be the key to developing methods that help detoxify commercial PCB compounds.

Acknowledgments

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