Journal of Cell and Molecular Research (2012) 4 (1), 28-33

The effect of pH and temperature on spatial variation of *Acidobacteria* /*Actinobacteria* communities from Alpine soil

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Received 30 April 2012 Accepted 11 June 2012

Abstract

Bacteria play a major role in environmental processes. However, the spatial and seasonal variations and environmental impact factors on different bacterial groups have been poorly studied. In the present study, we compared the spatial and seasonal variations of two bacterial groups (*Acidobacteria*, *Actinobacteria*) from Early Snow Melt and Late Snow Melt locations in Alpine tundra by CE-SSCP method. We examined correlation between the two groups and environmental factors. The results revealed that pH of soil is the essential factor for structure of two bacterial groups. The SSCP pattern of *Acidobacteria* is very similar to the overall bacterial communities in our previous study, while both bacterial communities are highly influenced by seasonal variations with an independent pattern.

***Keywords*:** Alpine soil, Single Strand Conformation Polymorphism (SSCP), Acidobacteria, Actinobacteria, pH

Introduction

Bacteria play a key role in carbon biogeochemical cycle; therefore it is important to identify their origins and functions in different environments such as Alpine soils. In the recent decades, developments in molecular technology provide an increase in literature. These studies indicate that environmental variables can be influenced by bacterial communities. It is known that presence or absence of different plant species provide different conditions for bacterial groups in soil (Harris and Tibbles, 1997; Kowalchuk et al., 2002; Yergeau et al., 2007). Plants can structure bacterial communities through the root exudation or their litter. In addition, the quality or quantity of the root exudates may vary with plants and physiological conditions or age of plants can influence the bacterial communities (Eviner and Chapin, 2003, Graystone et al., 1996; Maloney et al., 1997). Certain studies showed that soil properties, for example, soil texture, pH, and organic carbon/nitrogen have strong effects on soil bacteria (Marschner et al., 2003; Sessitsch et al., 2001; Girvan et al., 2003). However, impacts of these environmental conditions on different bacterial groups are poorly investigated.

Alpine ecosystems present a high spatial heterogeneity and its topography is characterized by the absence of trees. In fact, topographic context leads to differential snow deposition with the concomitant changes in soil characteristics, lengthening of seasonal vegetation, diversity in plant species composition, vegetation and microbial communities (Olear and Seastedt, 1994; Korner, 1995; Litaor et al., 2001; Choler, 2005). These ecosystems are the best models to investigate the effect of environmental conditions on the bacterial community.

In this framework, we selected two locations in the Alpine ecosystem. Despite their vicinity to each other topographically, they are different in the duration of snow-cover, type of vegetation, Soil Organic Matter (SOM) content, soil texture, and pH. The Early Snow Melt (ESM) location presents a weakly snow cover, in which the cycles of freezing and thawing are accruing. In contrast, the soils of Late Snow Melt (LSM) location are protected in winter with a snowpack. These regimes strongly influence the snow cover and consequently generate a mosaic plant cover typical for Alpine ecosystems.

Recent studies using PLFA (Phospholipid Fatty Acid), 16s rRNA and Single Strand Conformation Polymorphism (SSCP) demonstrated some spatial

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and seasonal variations in Alpine ecosystems (Bjork et al., 2008; Lipson et al., 2002; Lipson and Schmidt, 2004; Zinger et al., 2009). In a previous work (Zinger et al., 2009), we found that all of bacterial communities were submitted to spatial and temporal variations. In present work, we investigate how changes over space/seasons can drive *Acidobacteria* and *Actinobacteria* groups that were the most bacterial groups presented in our locations and second to investigate the environmental conditions causing the pattern similar between these bacterial groups.

Materials and Methods

Site characterization and sample collection

The study area is located in the Grand Galibier massif (south-western Alps, France, 45°05' N, 06°37' E). The sampling site is a topographical gradient comprising two neighboring habitats: an Early Snow Melt and Late Snow Melt location, with less than 100 m^2. Table 1 shows the soil characteristics for each location. The slow-growing, stress-tolerant plants (*Kobresia myosuroides, Dryas octopetala, Carex curvula All. subsp. rosae)* dominate ESM, while LSM is covered by fast-growing species (*Carex foetida, Salix herbacea L., Alopecurus alpinus Vill., Alchemilla pentaphyllea L.*). Summer length and growing season is longer in ESM than LSM. In winter harsh condition resulted from snow-cover in LSM plays a protective role for bacterial communities and keeps soil temperature approximately 0°C in LSM. Conversely, the soils from ESM are under freeze-thaw condition because of absence of snow-cover. Five soil samples from each location were collected in June (spring), August (top of growing season), October (after litter-fall), and in May (late winter). During May (latest days of the winter in our site) at a course of 10 days the mean soil temperature of ESM became above 0°C and those of LSM got around 0°C. The LSM soil was covered by >2 m of snow pack, but already waterlogged. The sampled soils were immediately transported to the laboratory in a sterile condition before DNA extraction. Soil pH was measured after mixing 5 grams of it with 12.5 ml of distilled water (adapted from Yan et al., 1996). Soil organic matter content (SOM) was determined by loss-on-ignition according to Schulte and Hopkins (1996).

Soil DNA extraction and Capillary Electrophoresis-SSCP analysis of Acido- and Actionbactria community

Extraction of total soil DNA has already been described (Zinger et al., 2009). Briefly, three replicates 250 mg of soil from each sample were extracted using Power Soil™ Extraction Kit (MO BIO Laboratories, Ozyme, St Quentin en Yvelines, France) according to manufacturer's instructions. To minimize the location heterogeneity, DNA derived from the five replicates was pooled to obtain one sample location/date. The V3 of 16S rRNA genes was amplified with specific primer pA (5'-GCCTGAGAGGGCRC-3') (Barn 1999) and W104-FAM (5'-TTACCGCGGCTGGCAC-3') (Delbes 1998) for *Acidobacteria* whereas F243 (5'-GGATGAGCCCGCGGCCA-3') and R513-FAM (5'-CGGCCGCGGCTGCTGGCACGTA-3') (Heuer, 1997) for *Actinobacteria*. PCRs were performed with 2.5 mM $MgCl_2$, 0.1 mM each dNTP, 0.2 mM of each primer, 1 U AmpliTaq Gold™ polymerase, 10X buffer provided by the manufacturer, 20g.l^{-1} of bovine serum albumin and 10 ng of DNA template. The thermal PCR profile was as following: initial denaturation at 95°C for 10 min, 30 cycles of amplification: denaturation at 95°C for 30 s, annealing at 59°C (for *Acidobacteria*) or 56°C (for *Actinobacteria*) for 15s and extension 72°C for 15s, and a final elongation at 72°C for 7 min. PCRs were carried out by triplicating to limit the influence of PCR biases.

PCR products were checked on a 1.5% agarose gel. CE-SSCP conditions were performed on an ABI Prism 3130 XL genetic analyzer (Applied Biosystems, Courtaboeuf, France), as previously described in (Zinger et al., 2008). For analysis, initially, an informatics tool was applied that allow retrieving the digital data of obtained CE-SSCP profiles. In a second step, these dates were normalized in order to reduce the variations of fluorescence intensity among profiles. Finally, standardization of data allows us to calculate a distance matrix by Edwards' distance between the different profiles and a dendrogram constructed by Neighbor-Joining with 1000 bootstrap replications (package ade4). These analyses were carried out with the R software (The R Development Core Team, 2007).

Linking bacteria composition with environmental parameters

We performed mantel tests to exam if the pattern similarity between bacterial communities were associated with two environmental variations (soil temperature and pH). This test carried out using the Spearman rank correlation method (999 permutations) that allowed examining correlation between distance obtained from each group bacterial community and distance of environmental factors.

Results

Variation of Acidobacteria and Actinobacteria with CE-SSCP

At the previous work (Zinger et al., 2009), we found that bacterial communities from ESM and LSM are different. The *ssu* sequence data (see more information in Zinger et al., 2009) indicated that ESM location is co-dominated by *Acidobacteria*, *Acitnobacteria* and *α-proteobacteria*, while *Acidobacteria* is dominant in LSM location. In order to test if seasonal variations of *Acidobacteria* and *Actinobacteria* are periodic over the years, and if environmental variation has the same effect for different bacterial communities, we performed SSCP analysis using primers specific of these two phyla and measured significance among environmental variations and bacteria communities. As shown in figure 1, two bacterial communities were separated from ESM and LSM. This discrepancy was noticed for all sampling date for two bacterial communities. During the growing season (June/August) and plant senescence period (October), ESM *Acidobacteria* were grouped together (figure 1a), while in LSM location *Acidobacteria* communities were grouped in all seasons. During May ESM community showed a shift toward the LSM community. The obtained profiles by *Acidobacteria* populations exhibited same pattern presented for total bacteria (Zinger et al., 2009). Thus, the largest distance between ESM and LSM was observed in June and August and decreased from October to May. Present SSCP pattern by *Actinobacteria* from ESM and LSM location displayed mirror to *Acidobacteria*. Similar to *Acidobacteria*, the *Actinobacteria* dendrogram (figure 1b) during May showed a convergence between two locations with tendency of *Actinobacteria* from LSM to ESM location. However, the lowest distance between two locations from each bacterial community was pronounced during May, though the pattern observed from bacterial communities was not similar.

Microbial communities and environmental conditions

In order to test the significance relation between two bacterial communities and soil abiotic factors we examined the effects of soil temperature and pH on bacteria communities. *Acidobacteria* displayed strong correlation with soil pH ($p \leq 0.001$) and temperature ($p \leq 0.004$), while *Actionbacteria* were only correlated with soil pH ($p \leq 0.009$) (table 2).

Table 1. Characteristic of soil samples gathered from Early and Late Snow Melt locations. Soil temperatures are average from May 1999 to May 2007 in same months of sampling date.

Sample	Date	pH (H_2O)	Soil Temp. (°C)	Granulometry (%) Clay (<2µm) Silt (2-50 µm) Sand (50-2000) µm	SOM (%)	Summer length (d)
ESM	June	6.74 ± 0.16^a	9.45 ± 1.86	9.7 (0.5) 41.4 (1.0) 48.6 (1.2)	15.7 ± 4.7	170 ± 3
	August	6.5 ± 0.32^a	10.88 ± 1.58			
	October	6.7 ± 0.16^a	3.66 ± 1.63			
	May	5.2 ± 0.1^b	3.49 ± 2.18			
LSM	June	5.56 ± 0.09^c	5.34 ± 2.32	26.4 (2.6) 61.7 (2.0) 11.9 (4.5)	8.7 ± 2.5	125 ± 3
	August	5.33 ± 0.2^c	11.04 ± 1.33			
	October	5.45 ± 0.09^c	3.07 ± 1.58			
	May	5.96 ± 0.1^d	0.98 ± 2.67			

Table 2. Correlation between bacterial communities (*Acido- and Actinobacteria*) with environmental conditions. Correlation significances were assessed with 1000 permutations and are indicated as follows: * P<0.05, ** P<0.01, *** P<0.001.

Bacterial groups	Environmental conditions Spearman rank ρ values (Significance)	
	Soil pH	Temperature
Acidobacteria	0.7(0.001)***	0.2(0.004)**
Actinobacteria	0.2(0.009)**	0.02(0.3)

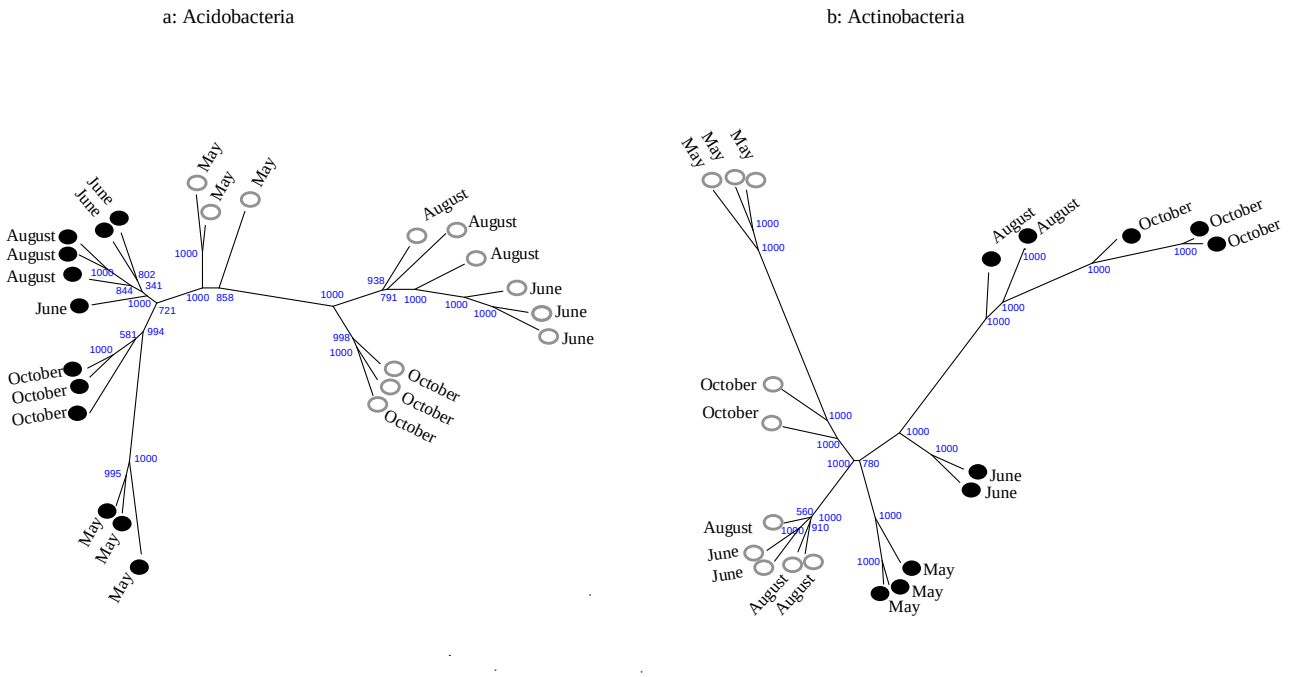

Figure 1. Obtained Dendrograms from a) *Acido* and b) *Actinobacteria* show seasonal variation in two different locations (Early and Late Snow Melt). These dendrograms were obtained from the molecular profiles of bacterial communities as described in Material and Methods. The ESM locations are in open symbols and LSM in filled symbols. Bootstraps values (1000) are indicated by blue.

Discussion

At the previous work, we found snow cover effects on microbial communities by CE-SSCP method and *ssu* sequences (Zinger et al., 2009) and bacterial phylogenetic structure (Shahnavaz et al., 2012). *Actinobacteria* (co-dominant in ESM location) are active in decomposition of organic material in soil such as lignin and other recalcitrant polymers (Crawford, 1988). The members of this phylum are sources of antibiotics and enzymes (Srinivasan et al., 1991; Trujillo, 2008; Stach et al., 2003). They are dominant in any ecosystems (Axelrood et al., 2002) and cold ecosystems (Tian et al., 2007; Bai et al., 2006). These bacteria are widely distributed in soil so that the resulted probability shows the importance of their ecological role in recycling nutrient and degradation of xenobiotic compounds (Trujillo, 2008).

The Phylum of *Acidobacteria* is one of the most distributed bacterial species in a wide variety of environment including different types of soil, sediments, fresh water and marine ecosystems (Hugenholtz et al., 1998; Janssen, 2006; Barn et al., 2007; Kleinsteuber et al., 2008). It is very difficult to cultivate and isolate members of the phylum *Acidobacteria* in the laboratory. They are often distributed in low pH soils (Sait et al., 2006; Lauber et al., 2008). Thus the knowledge of their morphological and biological properties, their consequences as well as their ecological roles is poorly known. Although certain studies showed that members of this phylum are metabolically active in rhizosphere soil (Lee et al., 2008), many authors have been presented that soil chemical and physical properties e.g. water content, pH, texture, nutrient and mineral availability and its creation of different ecological niches lead to different distribution of bacterial communities (Ulrich and Becker, 2006). The obtained patterns by *Acidobacteria* phylum were very similar to other bacteria indicating the abundance of this group of bacteria in both locations. Thus, in *Acidobacteria* phylum the largest distance between ESM and LSM in the growing season can be corresponded with plant cover. The root exudation from different plant species that vary with plant age and root zone (Graystone et al., 1996; Maloney et al., 1997) can form the bacterial community. LSM and ESM location display different plant cover that can recruit different bacterial phylotype. A correlation between plant species and bacterial groups in Alpine tundra (Yergeau et al., 2007a; Yergeau et al., 2007b) and other ecosystems (Buyer et al., 2002) has been known. This pattern was observed for *Actiobacteria* phylum except in LSM location, where the samples collected in June were far from

those gathered in August and October. It has been suggested that *Actionbacteria* phylotypes gathered in May are not very different from those gathered in June and new phylotypes adapted to plant cover do not appear. In any of bacterial groups, the lowest distance between bacterial groups was observed in May (end of winter in Alpine soil) according to Zinger et al. observations (2009) for overall bacterial groups. As described in the previous studies for overall bacterial communities, the low pH in ESM, weak temperature and absence of plant during May lead to *Acidobacteria* tendency from ESM to LSM and a convergence of *Actinobacteria* in LSM to ESM. Again, this pattern supports that behaviour of two bacterial groups is a little different in a given environment.

In this study, we attempted to examine impacts of abiotic factors (soil pH and temperature) on different bacterial groups (*Acido-* and *Actinobacteria*) by fingerprinting method. Consistent with other studies (Fierer and Jackson 2006; Lauber et al., 2008), our results showed that soil pH acts as main driver of two bacterial phyla though temperature can significantly affect *Acidobacteria* (Bryant et al., 2008). Differences in reactions of bacterial phyla to environmental factors may refer to their different morphological and physiological characteristics, although our information about *Acidobacteria* characteristic is somehow little.

Acknowledgment

I appreciate Lucie Zinger, Abderrahim Bouasria for statistical works. Philippe Choler and the staff of Station Alpine J. Fourier for providing logistic facilities during the fieldwork.

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