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SHORT COMMUNICATION

Environmental context shapes the bacterial community structure associated to *Peltigera* cyanolichens growing in Tierra del Fuego, Chile

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Abstract The structure of the associated bacterial community of bipartite cyanolichens of the genus *Peltigera* from three different environmental contexts in the Karukinka Natural Park, Tierra del Fuego, Chile, was assessed. The sampling sites represent different habitat contexts: mature native forest, young native forest and grassland. Recently it has been determined that the bacterial community associated to lichens could be highly structured according to the mycobiont or photobiont identities, to the environmental context and/or to the geographic scale. However, there are some inconsistencies in defining which of these factors would be the most significant on determining the structure of the microbial communities associated with lichens, mainly because most studies compare the bacterial communities between different lichen species and/or with different photobiont types (algae vs. cyanobacteria). In this work bipartite lichens belonging to the same genus (*Peltigera*) symbiotically associated with cyanobacteria (*Nostoc*) were analyzed by TRFLP to determine the structure of the bacterial community intimately associated with the lichen thalli and the one present in the substrate where they grow. The results indicate that the bacterial community intimately associated differs from the one of the substrate, being the former more influenced by the environmental context where the lichen grows.

Keywords Lichen · *Peltigera* · *Nostoc* · Bacterial community structure · Environmental context · TRFLP

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Introduction

Lichens are pioneers in the colonization of terrestrial habitats and are found in a variety of environments, such as rocks, soil and living on plants as epiphytic organisms (Petrini et al. 1990). Although the diversity of the symbiotic components of lichens has been extensively studied, the research on the diversity of the bacterial community associated with them is very recent and limited. Lichens are able to grow and survive in nutrient-poor substrates, therefore it has been suggested that associated bacterial communities would give them a source of nutrients for the development of lichen thalli in these environments (Gonzáles et al. 2005; Cardinale et al. 2008; Liba et al. 2006; Selbmann et al. 2010).

The new DNA fingerprinting techniques permit the in situ characterization of the microbial communities using specific biomarkers (Cardinale et al. 2008; Grube et al. 2009). Using these techniques Cardinale et al. (2008) concluded that the structure of bacterial communities in lichens was not correlated with the host-species and gave evidence that some bacterial communities associated with lichens would rather be an extension of those found in the soil where these organisms grow. However, Grube et al. (2009) determined that the bacterial composition of certain groups associated with lichens would be species-specific and may even be considered as another member of the symbiosis.

More recently, massive sequencing tools provided a higher resolution for the determination of these bacterial communities, however until now it has not been clearly elucidated the best descriptor of the structure of the lichen associated microbial communities. Bates et al. (2011) state that there are differences between the bacterial communities of different species of lichens, concluding that these

microbial communities would be an important part of the lichen symbiosis and could play a specific role in this association. However, Hodkinson et al. (2012), found that the geographic scale, together with the type of photobiont (cyanobiont or chlorobiont), are important factors in determining the structure of the microbial community associated with lichens, while the mycobiont identity would have less effect on such determination.

Therefore, currently there are discrepancies to define which would be the most influential factor shaping the structure of the microbial community associated with lichens, mainly because studies compare the bacterial communities of different lichen species and/or with different types of photobionts (algae vs. cyanobacteria). To minimize these differences, and to determine the influence of the environmental context, in this work lichens belonging to the same genus (*Peltigera*), in bipartite symbiosis with cyanobacteria (*Nostoc*), from three habitats of the Karukinka Natural Park, Tierra del Fuego (Chile) were analyzed. It is proposed that there will be a differentiation in the structure of the bacterial communities associated with these cyanolichens in different environmental contexts, because for example of the lack of tree cover and the increased exposure to direct sunlight in the grassland in comparison to the forest contexts (Waring 2008).

Materials and methods

The *Peltigera* lichen samples and their associated substrates were collected in the locality of Vicuña (54°08′19″S–68°42′17″W), Karukinka Natural Park, Tierra del Fuego, Chile. The climate of this region is cold temperate with no dry season. Annual precipitations vary between 400 and 620 mm and most of these fall as snow. The samples were taken on January 2011 from three different contexts at the Park: mature-forest of *Nothofagus pumilio* (M); young-forest of *N. pumilio* (Y) and grassland (G) adjacent to the mature-forest. In each environment representatives of the different species present were collected: *P. rufescens* (M, Y and G), *P. fuscopraetextata* (M and Y), *P. ponojensis* (M and G), *P. extenuata* (Y), *P. frigida* (M), and *P. hymenina* (M).

To determine the structure of the bacterial community associated to these cyanolichens, a TRFLP analysis of the bacterial communities of the lichens and their associated substrate was performed. Eighty to 100 mg from each lichen thallus and 250 mg of the intimately associated soil were used for DNA extraction with the PowerSoil™ DNA Isolation kit (MoBio Laboratories Inc.) according to the manufacturer's instructions. The V1–V3 variable region of the 16S rRNA gene was amplified by PCR (Kumar et al. 2011), the amplicons were hydrolyzed with the enzyme *Alu*I (Fermentas) and terminal restriction fragments were separated on an automated Genetic Analyzer ABI3730 (Applied Biosystems; Macrogen).

The analysis included only fragments larger than 50 bp to discard the signals derived from the primers. To normalize the data from different samples, an iterative procedure was performed to standardize the fluorescence units (Dunbar et al. 2001), and the signals that accounted for less than 10 % of the total fluorescence were eliminated. Additionally, the profiles were manually aligned to avoid misidentification of the fragments due to the expected shift in size by electrophoresis. Using the corrected data, the relative fluorescence of each TRF was determined. The relationship between the profiles of the different bacterial communities (lichen thalli and associated substrate) was assessed by an analysis of similarities (ANOSIM).

In addition, using the relative fluorescence of each TRF obtained from the lichens and the substrates separately, principal component analyses (PCAs) were performed to determine if the similarity pattern of the samples was influenced by the symbiotic components and/or the environmental contexts. Convex hulls, which are the smallest convex polygon that contains all related points, were plotted. Finally, the Similarity Percentages (SIMPER) analysis was performed, by means of which was determined the contribution of the TRFs to the observed dissimilarity between samples. The ANOSIM, PCAs and SIMPER analyses were performed in the tool PAST v2.16 (Hammer et al. 2001).

Results

The similarity analyses for both types of samples (lichen thalli and associated substrates) indicate that the profiles obtained from the associated substrates are more similar between them than those obtained from the lichen thalli (ratio between the two types of samples is 0.55 ($p = 0.0001$), indicating dissimilarity between the compared groups). On average about 8 fragments were obtained from the samples intimately associated with the lichen thalli and about 6 from the substrate, while no major differences in the number of TRFs were observed between samples of each type obtained from the different environmental contexts.

Analyzing the different types of samples separately, a multivariate PCA was performed, obtaining in both cases an explanation of 100 % of the variance in the first two components (Fig. 1). In the case of the profiles of the bacterial communities obtained from the lichen thalli, a clear separation between the samples from the three environmental contexts was observed, being the samples of the

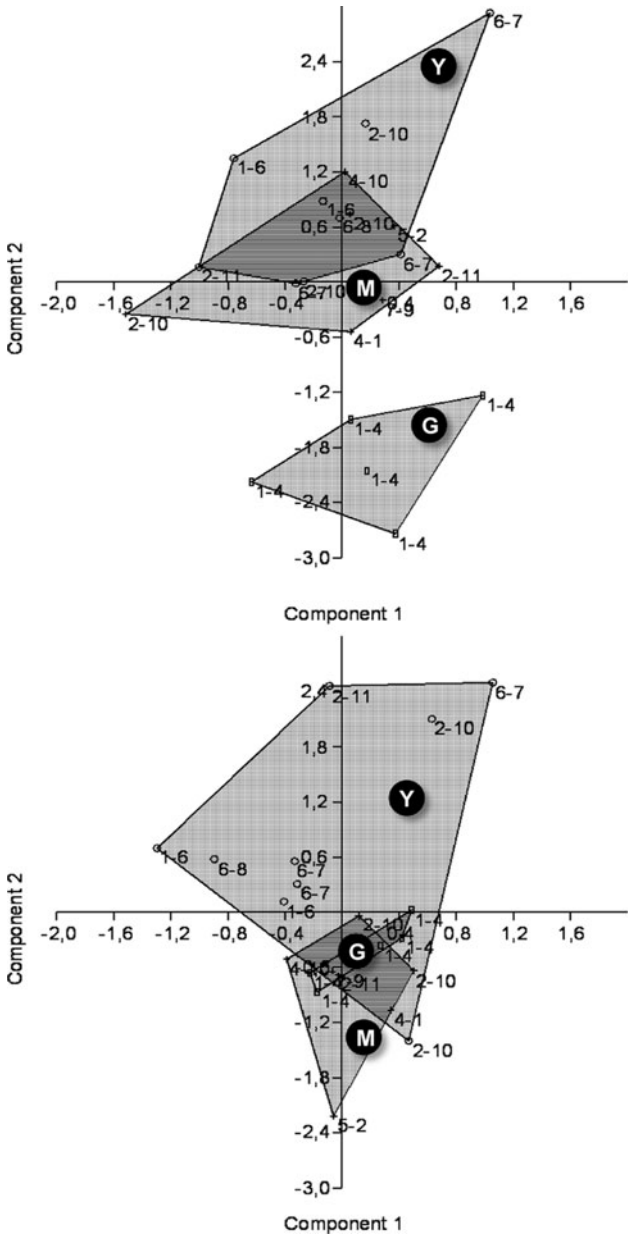

Fig. 1 Principal component analyses of the TRFLP profiles of the V1–V3 variable region of the 16S rRNA gene amplified from samples of lichen (*upper panel*) and the associated substrate (*lower panel*). *M* mature-forest samples, *Y* young-forest samples, and *G* grassland samples. In both cases the first two components explain 100 % of the variance of the data, reaching the first component about 80.0 %

grassland the most distant, and the samples from forest contexts (young and mature) more similar to each other. In this case there was not a large difference in the sizes of the convex hulls determined for each environmental context, being that of the grassland samples slightly smaller (Fig. 1, upper panel). In the case of the community profiles obtained from the substrate associated to each thallus, no separation was observed as clear as in the previous case in relation to the environmental context, being the convex hull of the grassland samples the smallest and superimposed to those of the forest contexts (Fig. 1, lower panel). As determined by the SIMPER analysis, the TRFs which contribute to at least 10 % of the dissimilarity between samples were the 58 bp (21.3 %) and the 88–92 bp (17.84 %).

Discussion

There are apparent contradictions on the most important factors that determine the structure of the microbiota associated with lichens, finding as potential predictors the biogeography (e.g. Cardinale et al. 2008; Hodkinson et al. 2012), the type of photobiont (e.g. Hodkinson et al. 2012) and/or the mycobiont identity (e.g. Grube et al. 2009; Bates et al. 2011). In order to evaluate whether the environmental context influences the bacterial community structures related to cyanolichens of the genus *Peltigera* from the Karukinka Natural Park, the structure of the bacterial community closely related to lichens and the one present in the associated substrate in three different habitats was determined by TRFLP.

In the TRFLP profiles obtained directly from the lichen thallus, there was not only the expected TRF from the associated cyanobiont, but also other TRFs. These data strengthen the fact that there is a bacterial community intimately associated with the tallus and are consistent with previous studies where highly structured microbial assemblages were found forming a biofilm on the lichen (Grube et al. 2009), which mainly correspond to Alphaproteobacteria (Bates et al. 2011; Cardinale et al. 2008; Grube et al. 2009; Hodkinson et al. 2012).

The profiles obtained from the lichen thalli were more heterogeneous, which could be explained if the identities of the symbiotic components influence the context in which microorganisms are intimately associated with the lichen thallus, enhancing or inhibiting the presence of certain bacterial groups (Karagoz et al. 2009). Instead, the soil samples adjacent to the lichens collected from the three environmental contexts were similar to each other, suggesting that the chemical environment provided by the lichen thallus would have less effect on the community of the adjacent substrate. This could be because in the genus *Peltigera* the absence of diffusible secondary metabolites was reported for several of the species included in this study (Martínez 1999).

In both PCAs, independently of the fraction of the bacterial community analyzed (lichen thalli or associated substrates), the environmental context was the best factor explaining the grouping of the profiles. In both cases, the area occupied by the profiles present in the grassland was smaller than the areas occupied by the profiles of the forest

contexts, which would account for a lower diversity of the microbiota associated with lichens from the grassland. This lower diversity could be related with the environmental conditions found in this context (Waring 2008), but also could be related indirectly to the decrease of diversity of lichen species in the grassland.

In addition, a higher similarity of the community profiles of the substrate adjacent to lichens compared with those closely associated with them was observed in the grassland context. This result could be explained if interactions between the lichens and their bacterial partners would require a close proximity, then the lichen could exert a protective role on the diversity of their associated bacterial community providing nutrients and protection (Ahmadjian 1993; Nash 2008), and thus maintaining a more stable bacterial community in more diverse environmental conditions.

These data support a previous work in which it was concluded that lichens might influence their associated microbiota; however there is not a single factor that determines the structure of this bacterial community (Hodkinson et al. 2012); instead, it seems to be a complex interaction which depends on multiple factors such as the identity of both symbionts and the environmental context in which they are located.

As determined by other authors (Hartman et al. 2008; Wu et al. 2013), more detailed studies about which climatic and environmental factors influence the structure of these bacterial communities are needed; along with identifying, using massive sequencing for example, which bacterial groups allow the observed separation of the samples.

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