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Climatic variations influence the dynamic of epiphyte bacteria of baby lettuce

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ABSTRACT

The objective of the present work was to study the relationship between the dynamic of epiphyte bacteria and the climatic variations in three varieties of pigmented baby lettuce (*Lactuca sativa*) harvested during 16 consecutive weeks, between February and May, which corresponded with the most important growing period for this crop in the south of Spain. Bacterial counts of the main groups (aerobic bacteria, *Pseudomonas* and coliforms) were enumerated by plating on the appropriate culture media. Concurrently, characterization of the culturable bacterial community present on the leaf surface was performed by partial sequence analysis of the 16S rRNA gene. To relate bacteria counts with weather variation first the main weather modes by principal component analysis (PCA) of climatic variables were characterized. Then, multiple linear regression models, with bacteria count as the dependent variable and the PCA factors as the independent variables, were built. The results obtained showed that the diversity and abundance of the epiphytic community of baby lettuce were affected by the climate variations. However, other factors such as the environment and the agricultural practices may play a role because the R^2 obtained was relatively low. Thus, the harvest week as well as the variations in radiation, rainfall and relative humidity (RH) explained part of the observed differences. *Pseudomonas* growth was influenced by the variations in radiation, rainfall, wind and temperature, the latter being the most relevant variable. Coliform growth was influenced by variations in temperature and RH. *Bacillus* and *Pseudomonas* were the most predominant genera identified among the isolates, their growth showing a negative correlation ($P < 0.01$) through the weeks of the study in two of the three lettuce varieties evaluated. Given the diverse functions of phyllospheric microbes, understanding the variations of specific communities such as *Pseudomonas* spp. due to the climate change could help to understand the different susceptibilities of crops to be affected by spoilage or pathogenic bacteria.

1. Introduction

Leafy greens cultivated in open field, such as baby lettuces, are grown and harvested all year round under a wide range of climatic conditions (FAO/WHO, 2008). Microbial epiphytes are daily exposed to climatic variations (Vorholt, 2012), which might play an important role in the seasonal variations of bacterial population present in the phyllosphere. Changes in weather/climatic conditions include among others: wide fluctuations in temperature, humidity changes due to the presence and absence of free moisture from rain and dew, and variations in solar radiation. Consequently, the phyllosphere is an ecosystem characterized by environmental stress conditions and limited availability of nutrients (Zwilehner, Handschur, Michaelsen, Irez, Demel, et al., 2008). Previous studies in peanut have demonstrated that solar UV-B radiation alters phyllosphere bacterial community composition and that UV tolerance is a prevalent phenotype late in the season (Jacobs & Sundin, 2001). Sufficient water availability is a crucial parameter, but fluctuations in water availability also result in osmotic stress that affects abundance and diversity of bacterial population (Axtell & Beattie, 2002; Vorholt, 2012). The weather/climatic variations might affect not only the abundance and variety of bacteria population that is consistently present, but also the spoilage and pathogenic bacterial communities that might be present. For instance, the concentration of bacteria such as *Pseudomonas* spp. is particularly important, because members of this genus have been described as producing enzymes associated with spoilage (Babic, Roy, Watada, & Wergin, 1996). Furthermore, previous studies have concluded that changes in the climatic conditions under which plants are grown affect not only the phyllosphere population but also the ability of foodborne pathogens to colonize and survive in the plant tissue (López-Velazco, Tydings, Boyer, Falkinham, & Ponder, 2012; Strawn, Fortes, Bihn, Nightingale, Gröhn & Worobo, 2013). The risk of foodborne diseases is directly related to the prevalence and the concentration of pathogenic bacteria in the tissue. The likelihood of leafy green contamination and the possible presence of associated pathogens in relevant concentrations for public health are strongly related to

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environmental and climatic conditions (Liu, Hofstra, & Franz, 2013). Some members of epiphytic microbiota have been described to have an antagonist effect against pathogens (López-Velazco et al., 2012). However, most of the studies to improve the shelf-life of leafy greens have been focused on postharvest processing steps, highlighting the importance of low bacteria contamination when leaves are harvested (Gorny, 1997). Scarce literature about changes in microbial population of baby leaves during preharvest is available. Few studies have recently focused on the identification of the phyllosphere microbiota based on the use of molecular techniques, from different plant species, geographical locations, environmental regimes and postharvest handling (López-Velazco, Welbaum, Boyer, Mane, & Ponder, 2011; Rastogi, Sbodio, Tech, Suslow, Coaker & Leveau, 2012; Williams, Moyne, Harris, & Marco, 2013). However, the impact of the climatic variability during the growing period on the dynamic of epiphytic bacterial abundance and diversity has to be investigated. Understanding microbial ecology and dynamics might bring new possibilities in the search of effective strategies to control spoilage and pathogenic bacteria. Thus, the objective of this study was to determine the climatological factors associated with the seasonal dynamic of epiphyte bacteria in baby lettuce phyllosphere. The study was focused on the estimation of those climatic variations that affected the bacterial count of baby lettuce as well as the culturable bacterial diversity present in the phyllosphere of the leaves. Hence, three pigmented baby lettuces were sampled during 16 consecutive weeks from February to May, which correspond with the most important growing period in the south of Spain.

Table 1
Variations in the bacterial count (log CFU/g) of baby Batavia (B1 and B2) and Red oak leaf (R) lettuce varieties during 16 weeks of sampling.

Sample	Aerobic bacteria	*Pseudomonas* spp.	Coliforms
B1	3.7 ± 0.0–5.7 ± 0.0	3.4 ± 0.1–5.9 ± 0.1	LD–3.7 ± 0.7
B2	3.6 ± 0.0–5.5 ± 0.1	3.0 ± 0.1–5.7 ± 0.3	LD–3.5 ± 0.3
R	3.7 ± 0.3–5.6 ± 0.0	2.5 ± 0.6–5.3 ± 0. 1	LD–2.8 ± 0.3

Values are the mean of three replicates ± standard deviation. LD: limit of detection = 2 log CFU/g.

2. Materials and methods

2.1. Plant material

Three pigmented baby lettuce cultivars, two Batavia cultivars (B1 and B2) and one Red oak leaf (R), were cultivated under commercial conditions in Fuente Alamo (Murcia, Spain) by a commercial grower (Langmead, Spain). The growing fields were located within an area of 20 km^2 (Fig. 1). The type of soil in the selected fields was mainly clay and clay loam. Sowing was performed directly from seeds on elevated beds using a density of 700 plants m^{-2}. Baby leaves at the commercial maturity stage (10–12 cm) were mechanically harvested during 16 consecutive weeks from February (week 5) to May (week 20) in 2012. At each sampling time, 9 samples of baby lettuce were randomly collected from different locations in the field. Supplementary information regarding the dates of plantation and harvesting, and crop cycle schedule are

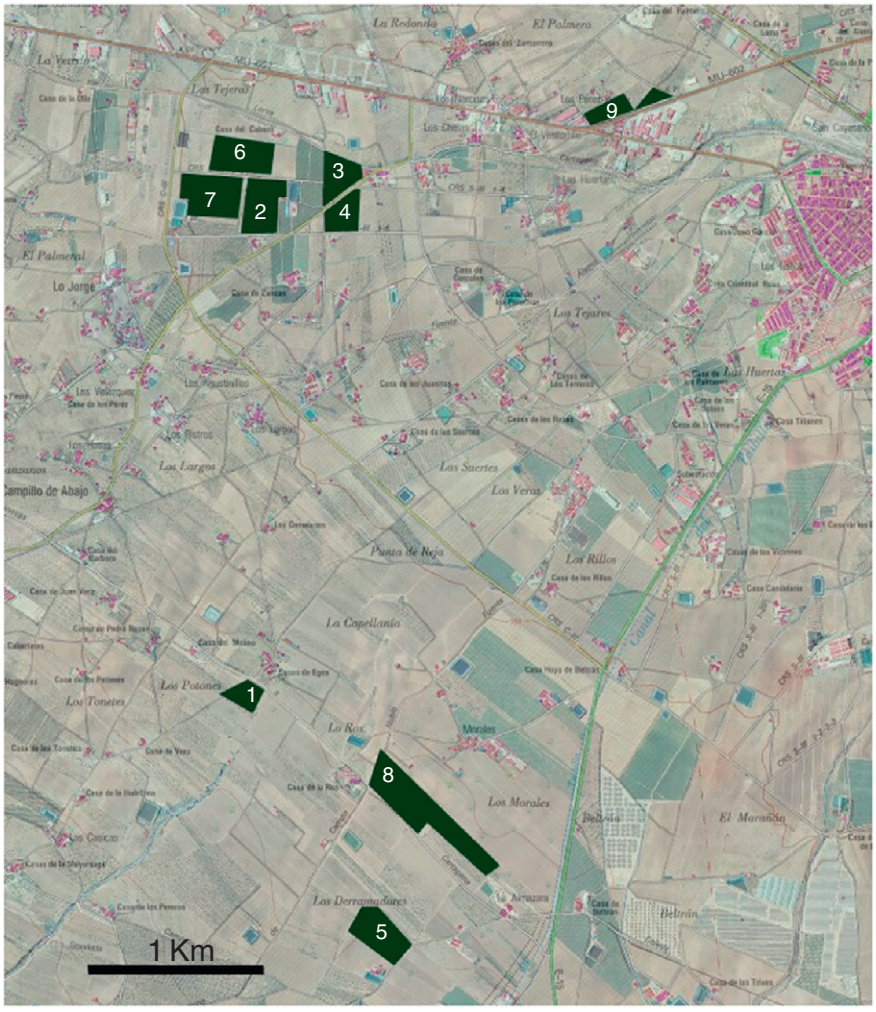

Fig. 1. Aerial view showing the location of the fields in Fuente Alamo, Murcia (Spain). http://sigpac.mapa.es/fega/visor/. Numbers in the squares indicate the names of the plantation areas: 1: High; 2, 6, 7: Campero; 3, 4: Inicio; 5, 8: Los Ro; 9: Campero II.