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PRELIMINARY PCR-TTGE ANALYSES OF BACTERIAL COMMUNITIES ASSOCIATED WITH POLLEN FROM ANEMOPHILOUS TREES: POTENTIAL IMPACTS ON PLANTS AND HUMAN HEALTH

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Preliminary PCR-TTGE results, pollen microbiota was tree genus dependent. In addition, intra-tree genus variations were also observed. The Grammoproteobacteria class was the most represented in pollen samples whatever the tree genus. Among this class, some bacteria were recognized as phytopathogens (Pseudomonas, Erwinia) but also opportunistic human pathogens (Pseudomonas, some enterobacteria). PCR-TTGE is a suitable tool to analyse the microbiota associated with pollen responsible for phytopathogenicity or affecting the human respiratory tract.

Keywords: pollinosis; anemophilous trees; pollen microbiota; health; PCR-TTGE

INTRODUCTION

Pollen grains represent a category of Primary Biological Aerosol Particles (PBAP) in addition to bacteria, virus, fungal spore, algae, cyanobacteria (Després et al., 2012). During pollination, pollen grains come into contact with human respiratory or conjunctival mucosa and could be responsible for pollinosis which correspond mainly to allergic rhinitis and conjunctivitis. In France, pollinosis affect from 10 to 15% of the general population and are increasing in urban areas (Ravault et al., 2005). In some cases, small pollen grains can induce asthma attacks (Heydenreich et al., 2012). Allergic rhinitis triggered by the pollen grains of some seasonal plants is commonly named "hay fever", and appears mostly during haying season. However, atopic patients with seasonal allergies present hay fever throughout the year. The pollen grains which provoke hay fever change between individuals and from region to region. The hardly visible pollen of wind pollinated plants such as trees, grasses, and weeds are yet the predominant cause (D’Amato et al., 2007). In Mediterranean region, Pinaceae and mostly Cupressaceae pollen grains constitute most clinically relevant trees pollen (D’Amato et al., 2007; Yalcin et al., 2013). Moreover, pollen is regarded as a source of not only allergens but also immunomodulatory molecules, which present major roles in sensitization and/or the exacerbation of allergies (Kamijo et al., 2009). Recently, nanovesicles containing allergens (named pollenosomes) released during in vitro germination of pollen grains were described to contribute to allergic reaction (Prado et al., 2015).

For many years, bacterial communities associated to plants were analysed particularly root-inhabiting (Buée et al., 2009; Bulgarelli et al., 2012), leaf-surface inhabiting (Yashiro et al., 2011; Izhaki et al., 2013) and less frequently flower-inhabiting (Aleklett et al., 2014) bacterial microbiota. Both culture-dependent and independent methods were performed to explore bacterial microbiota. Since the diameter of pollen grains from anemophilous species varies from 17 to 58 μm (Després et al., 2012), pollen grains may represent a support for bacteria. Moreover, many exuded compounds of pollen grains are known to be attractive for bacterial colonizers (Aleklett et al., 2014).

Bacterial communities associated to pollen called bacterial pollen microbiota were rarely investigated. Coldhald and Carlsson (1968) isolated the Gram-negative Pseudomonas multilatima (currently Stenotrophomomas maltophilia) from pollen samples. The presence of microorganisms on surface of pollen was then confirmed by scanning electron microscopy (Coldhald and Nilsson, 1973). Later, Spiewak et al. (1996a) highlighted the presence of a mixed microflora consist of Gram-positive and Gram-negative mesophilic bacteria, thermophilic actinomyces and fungi on allergic pollen grains. Moreover, Spiewak et al. (1996b) showed that Gram negative bacteria such as Pantoea agglomerans endotxin associated to pollen should be considered as a potential factor aggravating pollinosis. More recently, Heydenreich et al. (2012) reported that grass pollen grains were colonized by Gram-negative bacteria as Acinetobacter lwoffi and Gram-positive bacteria belonging to the genus Bacillus which presents adjuvant activity inducing inflammatory T cell responses. Few studies on plant protection have been carried out in order to detect bacteria responsible for plant disease as Pseudomonas syringa pv actinidiae on the kiwifruit pollen grains (Vanneste et al., 2011). All these studies were investigated with culture-dependent method based on the isolation of bacteria on different agar media. Molecular methods were never used. Currently, bacterial communities associated with humans (McCarty, 2002; Roudriére et al., 2009; Michon et al., 2012), animals (Richards et al., 2005; Navarrette et al., 2012), plants (Lambais et al., 2014), foods (Ogier et al., 2004; Handhouche et al., 2015) or environment (Lyautey et al., 2005) are analysed by culture-independent approaches such as genetic fingerprinting methods (PCR-DGGE, PCR-TTGE). The 16S rRNA gene is the most frequently gene used for amplification. Separation of PCR products in TTGE is based on the decrease of the electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels containing DNA denaturants according to a temperature gradient. Molecules with various sequences will have a different melting behaviour and will stop migrating at distinct position in the gel (Ogier et al., 2002). The aim of this study was to perform a preliminary analysis of the bacterial communities associated with pollen isolated from plants responsible for
pollinosis by applying PCR-TTGE method. A PCR anchored on the bacterial 16S rRNA gene followed by a TTGE analysis was carried out on DNA extracts from several pollen grain samples collected in Mediterranean region. On the one hand, methodological aspects were developed including a comparative study of different pollen preparation methods. On the other hand, bacterial diversity of a set of samples was analysed with a 16S rDNA based PCR-TTGE approach.

**MATERIAL AND METHODS**

**Pollen grains sampling**

Pollen samples were collected from various anemophilous trees (Tab 1) in Montpellier, France (GPS data = 43.6° N & 3.9° W) between February and April 2013, in accordance with the pollinic bulletins of the French aerobiology network RNA (Réseau National de Surveillance Aérobiologique). Collected pollen samples belonged to the genera Pinus (x = 5), Cupressus (x = 4), Cephalotaxus (x = 1), Quercus (x = 1), and Platanus (x = 1). Data concerning the samples figured in Table 1. According to the type of male inflorescence, pollens were either directly collected or spikes were cut with sterile scalpel. In both cases, pollens were aseptically collected in sterile storage bags after shaking and sieving through sterile gauze. Careful consideration was given to collect the pollen grains in order to avoid mixed pollen samples from different plants. Pollen samples were stored at 4°C in the dark. Quantification of bacteria in pollen presented in Table 1 was performed according to the protocol described below.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Date of sampling</th>
<th>Plant genus</th>
<th>Geographic location</th>
<th>Enumeration number of bacteria / g pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>2013/04/10</td>
<td>Pinus</td>
<td>I</td>
<td>4.0 \times 10^2</td>
</tr>
<tr>
<td>P2</td>
<td>2013/04/10</td>
<td>Pinus</td>
<td>II</td>
<td>1.4 \times 10^3</td>
</tr>
<tr>
<td>P3</td>
<td>2013/04/10</td>
<td>Pinus</td>
<td>I</td>
<td>6.7 \times 10^3</td>
</tr>
<tr>
<td>P4</td>
<td>2013/04/14</td>
<td>Pinus</td>
<td>III</td>
<td>3.9 \times 10^3</td>
</tr>
<tr>
<td>P8</td>
<td>2013/04/22</td>
<td>Pinus</td>
<td>IV</td>
<td>1.9 \times 10^3</td>
</tr>
<tr>
<td>C1</td>
<td>2013/03/21</td>
<td>Cupressus</td>
<td>I</td>
<td>1.4 \times 10^3</td>
</tr>
<tr>
<td>C1b</td>
<td>2013/03/21</td>
<td>Cupressus</td>
<td>I</td>
<td>5.4 \times 10^3</td>
</tr>
<tr>
<td>C2</td>
<td>2013/02/23</td>
<td>Cupressus</td>
<td>V</td>
<td>2.0 \times 10^3</td>
</tr>
<tr>
<td>C3</td>
<td>2013/03/25</td>
<td>Cupressus</td>
<td>I</td>
<td>5.7 \times 10^3</td>
</tr>
<tr>
<td>Cx</td>
<td>2013/04/03</td>
<td>Cephalotaxus</td>
<td>VI</td>
<td>5.0 \times 10^3</td>
</tr>
<tr>
<td>Q</td>
<td>2013/04/18</td>
<td>Quercus</td>
<td>VI</td>
<td>7.0 \times 10^3</td>
</tr>
<tr>
<td>Pia2</td>
<td>2013/04/22</td>
<td>Platanus</td>
<td>VII</td>
<td>5.7 \times 10^2</td>
</tr>
</tbody>
</table>

Legend: 1 Faculty of Sciences campus, II Faculty of arts campus, III campus, IV Garden of plants, VII Albert 1st square.

**Preparation of pollen samples**

Pollen samples were prepared by five different protocols (A, B, C.adn, C.pca and C.tsh) before DNA extraction (Fig 1).

**DNA extraction**

DNA was extracted from the five previous protocols of pollen preparation, following the recommendations of the MasterPure™ DNA purification Kit (Epicentre). Quality of DNA extracts was evaluated using UV spectrophotometry (rate 260 nm / 280 nm) and diluted to obtain a final concentration of 50 μg/ml.

**PCR – Temporal Temperature Gradient Gel Electrophoresis (TTGE) analysis**

A 199 bp-fragment (from position 338 to position 536, Escherichia coli numbering) overlapping the V3 variable region of 16S rDNA (position 338 to position 534, *E. coli* numbering) (Sundquist et al., 2007) was amplified using primers HDA1F-GC and HDA2 (Ogier et al., 2002). A 40-bp GC-clamp was added to the forward primer. PCR was performed in a final volume of 50 μl containing 200 μM each dNTP (Fermentas), 200 nm each primer (Sigma), 2.5 U of Fast Start Taq DNA Polymerase (Roche) in the appropriate 1x reaction buffer with 1.8 mM MgCl2 and 1 μl of the DNA extract. Amplification program was carried out as follows: an initial denaturation step at 95 °C for 2 min followed by 30 cycles of 95 °C for 1 min, 62 °C for 30 s, 72 °C for 1 min and 72 °C for 7 min for the final extension. PCR products were checked using conventional electrophoresis in 1.5 % (w/v) agarose gel with 1x Tris Borate EDTA (TBE) buffer and then submitted to TTGE analysis using a Dcode Universal Mutation Detection System (Bio-Rad). PCR products (1 μl) were loaded into 8% (w/v) bisacrylamide (37.5:1), 7 M urea, 40 μl TEMED and 0.1 % (w/v) ammonium persulfate gels. Migrations were performed in 1x Tris Acetate EDTA (TAE) buffer with additional magnetic shaking in the electrophoresis compartment. A pre-migration for 15 min at 63 °C and 20 V was followed by migration for 16 h at 46 V with an initial temperature of 63 °C and a final temperature of 70 °C corresponding to an increase of 0.4 °C / h. Gels were stained for 15 min with 0.5 μg / ml ethidium bromide in 1x TAE buffer, rinsed for 45 min in 1x TAE buffer and then photographed on a UV transilluminator.
TTGE bands analysis and sequencing

Dominant bands were cut from TTGE gels, rinsed twice with molecular biology grade water and eluted overnight in 10 mM Tris buffer (pH 8.5) at 37 °C. Extracted DNA was re-amplified using primers HDA1 and HDA2 without GC-clamp as previously described (Michon et al., 2010). Then PCR products were sequencing on an applied automatic sequencer (Cogenics) by using the forward primer HDA1. DNA sequences were visualized and analysed with BioEdit program version 7.0.9 (Hall, 1999). The 16S rRNA gene sequences were screened using GenBank’s Blast program (Altschul et al., 1990).

Fingerprinting and statistical analysis

The TTGE gel images were analyzed using Image Quant TL software v. 2003 (Amersham Biosciences). Individual lanes of gel images were aligned which permits detection and record of the relative position of each DNA band. TTGE patterns were manually scored by presence and absence of co-migrating bands between lanes. Pairwise community similarities were quantified using the Dice similarity coefficient ($S_D = 2 N_c / N_a + N_b$ (Heyndrickx et al., 1996)) where $N_a$ represented the number of bands in sample A, $N_b$ represented the number of bands in sample B, and $N_c$ represented the number of bands common to both samples. A cluster analysis was carried out using the similarity matrix to group pollen samples according to their similarity index. A Principal Component Analysis (PCA) was also used as a multivariate technique for exploratory data analysis using Statistica (version 7) software (StatSoft, USA).

RESULTS

Bacterial communities were analysed on pollen samples from various wind-pollinated trees in order to evaluate the microbiota associated to pollen. The comparison of microbial ecology of pollen grains from various anemophilous trees was performed by PCR-TTGE analysis.

Optimization of preparation of pollen samples and bacterial numeration

Five protocols were evaluated to prepare the pollen samples. Figure 2 shows TTGE patterns obtained from pollen samples of five Pinus specimens, i.e., P1-4 and P8 (Tab 1) prepared according to four different protocols (A, C.adn, C.pca and C.tsh).

For P8 sample, one band was detected for protocols A and C.adn while 16 bands were detected for protocols C.pca and C.tsh. Thus, both protocols C.pca and C.tsh highlight the highest number of detected bands whatever the sample.

The cluster analysis of TTGE patterns obtained from pollen samples of the five Pinus specimens prepared according to four protocols showed two main dissimilar clusters: the first cluster grouped patterns obtained from protocols C.pca and C.tsh while the second cluster comprised patterns obtained from protocols A and C.adn (Fig 4).

The Principal Component Analysis performed on the same TTGE patterns showed 70% of the variability of samples according to four protocols with or without a step of culture before DNA extraction (Fig 5).

Figure 2 Bacterial 16S rDNA PCR-TTGE profiles obtained from pollen samples of five different Pinus (P1, P2, P3, P4 and P8), prepared according to four different protocols (A, C.adn, C.pca and C.tsh).

Figure 3 Bacterial 16S rDNA PCR-TTGE profiles obtained from pollen samples of Cupressus (C1, C1b, C2 and C3), Cephalotaxus (Cx), Quercus (Q) and Platanus (Pla2) prepared according to five protocols (A, B, C.adn, C.pca, C.tsh).

Figure 4 Cluster analysis of 16S rDNA PCR-TTGE profiles obtained from pollen samples of five Pinus samples, prepared according to four protocols.

Figure 5 Principal Component Analysis (PCA) of 16S rDNA PCR-TTGE profiles obtained from pollen samples of five different Pinus samples, prepared according to four protocols.
Two distinct groups could be discriminated: the first group contained samples obtained from protocols with step culture (C.pca and C.tsh), a second group included samples prepared without step culture (protocols A and Caud.).

Bacterial enumeration from PCA medium varied from 1.4x10^4 to 4.0x10^4 CFU / g pollen and from 2.0x10^4 to 5.7x10^4 CFU / g pollen for Pinus and Cupressus, samples, respectively (Table 1). Bacterial numeration ranged from 7.0x10^4 to 5.7x10^4 CFU / g pollen for Quercus, Cephalotaxus and Platanoa.

**Bacterial communities associated to pollen samples**

The objective of this study was to evaluate the bacterial communities associated to pollen grains from different tree genera, i.e., Pinus, Cupressus, Cephalotaxus, Quercus, and Platanoa using PCR-TTGE.

Concerning pollen samples from Pinus, a total of 53 DNA bands were observed in total of the samples (Fig 2). Among the 53 observed bands, 46 bands were cut and sequenced. DNA sequences obtained from cut bands varied from 147 to 172 bp compared to the 199 bp of the V3 region. First, we demonstrated that several sequences corresponded to chloroplast DNA. Presence of chloroplast DNA was not unexpected because of the high similarity between bacterial and chloroplast 16S rRNA gene sequence in the context of endosymbiotic origin of chloroplast (McFadden 2001). As a result, bacterial but also chloroplast 16S rRNA genes could be amplified with primers used in this study. Then, we detected a majority of bacteria belonging to the Enterobacteriaceae family such as Erwinia sp., Pantoaea sp., Enterobacter sp., Klebsiella sp., and Providencia sp. but also to Pantoea sp., Dickeya sp., and Trabulsiella sp. concerning the genus Bacillus. Within the genus Pinus, the five TTGE patterns corresponding to C.pca protocol were very different from one tree to another. Indeed, we observed that bacterial communities associated to pollen seemed to vary among the genus Pinus. Only bacterial strains belonging to Pseudomonas genus seemed to be present in four Pinus samples from five.

Concerning pollen samples from Cupressus, Cephalotaxus, Quercus, and Platanoa, a total of 99 DNA bands were observed in total of the samples which permitted to obtain 81 DNA sequences. From Cupressus samples, we found bacteria belonging to Enterobacteriaceae family such as Erwinia sp., Pantoaea sp., Enterobacter sp., Klebsiella sp., and Providencia sp. but also to Pantoea sp., Dickeya sp., and Trabulsiella sp. Concerning the genus Bacillus. Within the genus Pinus, the five TTGE patterns corresponding to C.pca protocol were very different from one tree to another. Indeed, we observed that bacterial communities associated to pollen seemed to vary among the genus Pinus. Only bacterial strains belonging to Pseudomonas genus seemed to be present in four Pinus samples from five.

Bacteria belonging to Gammaproteobacteria class represented 73% of the 16S rDNA sequences obtained and were detected in most of samples. These results are in accordance with those of Wetz et al. (2010) which showed that 81.2% of bacterial species isolated from edible flowers belonged to the class Gammaproteobacteria. This result is in accordance with the intraintragenic and intraspecific heterogeneity of the 16S rDNA gene, it is very difficult to obtain identification at the species level and even at the genus level. It is a classical disadvantage of the PCR-TTGE method (Michon et al., 2012). Some Enterobacteriaceae species are commonly present in plant. We found DNAs of Erwinia sp. and Pantoea sp. in Pinus and Cupressus samples. Erwinia is a well-known bacterial genus associated to plants. Some Erwinia species could lead to damages of plant structure, wilting, dieback, yellowing or rot (Thomson et al., 1999). The main phytopathogenic Erwinia species are E. amylovora and E. carotovora responsible for the fire blight of Rosaceae especially apple and pear (Piqeu et al., 2015), and soft rot diseases respectively (Parent et al., 1996). Pantoea agglomerans (formerly Erwinia herbicola) could be opportunistic pathogen in cases of weakness of the plant (Dutkiewicz et al., 2016). Dickeya sp., Enterobacter sp. and Klebsiella sp. DNA sequences were also detected in pollen samples. The genus Dickeya comprises several species particularly D. chrysanthemi (formerly Erwinia chrysanthemi) known to be phytopathogenic (Toth et al., 2011). Enterobacter asburiae, Enterobacter cloacae and Enterobacter cowanii were already recovered from plants (Wetz et al., 2010; Humann et al., 2011). E. asburiae was considered as a Plant Growth Promoting Rhizobacteria (PGPR) particularly for Citrus reticulata (Thokhom et al., 2014) whereas E. cloacae is showed to be phytopathogenic for numerous plants such as Quercus sp. (Providencia sp.) (Koth et al., 2007) and Daucus carota (Trabulsiella sp.) as Klebsiella oxytoca were reported to cause wilt in many plants (Sarkar and Chaudhuri, 2015). K. oxytoca, Enterobacter sp., Erwinia sp., Tatumella pylowe etc. were also dominant taxa in a Greek vineyard (Nisiotta et al., 2011). To conclude on the Enterobacteriaceae species present on pollen grains, we observed that they were usually present in other parts of the plants where they potentially phytopathogenic.

The genus Pseudomonas which also belongs to the Gammaproteobacteria class presents various species associated to plants, e.g., P. syringae (Kennelly et al., 2007), P. rhizoxinae (Peix et al., 2003) and P. panacis (Park et al., 2005). Thus Pseudomonas sp. could be considered as phytopathogenic, entomopathogenic or PGPR (Vanneste et al., 2011; Noori and Saud, 2012; Mansfield et al., 2012). The genus Xanthomonas with X. campestris and X. oryzae causes also significant damage in a range of crops too (Ryan et al., 2011). The Enterobacteriaceae is a well-known family of bacteria which constitute human intestinal microbiota but several species or serotypes can also be pathogenic to human. Most of them are recognized as opportunistic pathogen and causes respiratory symptoms. In particular, Pantoea strains are responsible for respiratory infections in immunocompromised patients (Flores Popoca et al., 2012; Kursun et al., 2012; Walterson and Stravrinatedes, 2015; Dutkiewicz et al., 2016). Enterobacter asburiae was also found in human samples particularly in sputa (Koth et al., 2012). Klebsiella cause various infections in humans including community-acquired pneumonia and nosocomial infections. Moreover, Tatumella pylowe strains were notified to be responsible for tracheal aspirate of a patient suffering from pulmonary infections (Leneveu-Jenvrin et al., 2013). Regarding the phylum Firmicutes which represents 14% of the 16S rDNA sequences obtained, we underscored the presence of Bacillus strains in particular Bacillus cereus which is well-known as volatile human pathogen (Bottone, 2010), and Staphylococcus pasteuri responsible for a few cases of infections in immunocompromised patients (Morfin-Otero et al., 2012). Pollen samples from Cephalotaxus, Quercus and Platanoa also presented also Gammaproteobacteria such as Enterobacteriaceae and Pseudomonas strains.

Indeed, we detected in pollen grains from anemophilous (deciduous and coniferous) trees various bacterial genera, i.e., Pseudomonas, Erwinia and Enterobacter well-known as responsible for a wild range of human diseases and plant infections.

**DISCUSSION**

In the present study, we analysed bacterial communities associated to twelve pollen samples collected from five anemophilous tree genera at different locations in Montpellier (France). We used a molecular fingerprint technique called PCR-TTGE which was already applied to determine bacterial communities associated to a number of edibles plants sampling (Vanneste et al., 2012; Navarrete et al., 2012). Five pollen preparation protocols were evaluated. Preparation of pollen samples before DNA extraction was a critical step of the PCR-TTGE analyses since it appeared decisive to obtain the totality of bacterial communities associated to a sample. As concerns the number of detected DNA bands for each TTGE profile, mean values varied according to the pollen preparation protocol. Thus, the best results were obtained with the protocol adapted from Vanneste et al. (2011) followed by a culture dependent step on a non-selective medium (PCA medium) or enriched medium (TSH medium) before DNA extraction, amplification and TTGE analyses. Indeed, the previous mentioned preparation of pollen comprising sonication and a 1-hour shaking allowed to optimally recovery bacteria from exine pollen wall. Then, the culture-dependent step permitted to concentrate bacteria. We obtained TTGE patterns with a largest number of DNA bands corresponding to main but also less abundant bacteria presenting in a sample. Indeed, pollen grains from Pinus and for the four pollen samples from Cupressus. Bacteria belonging to the class Gammaproteobacteria (Enterobacteriaceae, Pseudomonadaceae, Xanthomonadaceae...) were the most represented in pollen samples before bacteria belonging to the phylum Firmicutes, whatever the plant genovesing pollen samples were. Yet, the highest bacterial diversity observed by Spiewak et al. (1996a) which highlighted in particular mixed microflora consisting of Gram-positive and Gram-negative mesophilic bacteria on allergenic pollen grains.

**CONCLUSION**

In this present study, we analysed for the first time bacterial communities associated to twelve pollen samples collected from five anemophilous tree genera at different locations in Montpellier (France). We used a molecular fingerprint technique called PCR-TTGE which was already applied to determine bacterial communities associated to a number of edibles plants sampling (Vanneste et al., 2012; Navarrete et al., 2012). Five pollen preparation protocols were evaluated. Preparation of pollen samples before DNA extraction was a critical step of the PCR-TTGE analyses since it appeared decisive to obtain the totality of bacterial communities associated to a sample. As concerns the number of detected DNA bands for each TTGE profile, mean values varied according to the pollen preparation protocol. Thus, the best results were obtained with the protocol adapted from Vanneste et al. (2011) followed by a culture dependent step on a non-selective medium (PCA medium) or enriched medium (TSH medium) before DNA extraction, amplification and TTGE analyses. Indeed, the previous mentioned preparation of pollen comprising sonication and a 1-hour shaking allowed to optimally recovery bacteria from exine pollen wall. Then, the culture-dependent step permitted to concentrate bacteria. We obtained TTGE patterns with a largest number of DNA bands corresponding to main but also less abundant bacteria presenting in a sample. Indeed, pollen grains from Pinus and for the four pollen samples from Cupressus. Bacteria belonging to the class Gammaproteobacteria (Enterobacteriaceae, Pseudomonadaceae, Xanthomonadaceae...) were the most represented in pollen samples before bacteria belonging to the phylum Firmicutes, whatever the plant genovesing pollen samples were. Yet, the highest bacterial diversity observed by Spiewak et al. (1996a) which highlighted in particular mixed microflora consisting of Gram-positive and Gram-negative mesophilic bacteria on allergenic pollen grains.


