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

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ABSTRACT

Pollen from wind-pollinated plants is the predominant cause of pollinosis which corresponds mainly to allergic rhinitis and conjunctivitis. Bacterial communities associated to leaves and roots surface were analysed for many years with both culture-dependent and independent methods. However, microbiota of pollen grains was rarely investigated and never with a molecular fingerprint technique. The aim of this study was to perform a preliminary analysis of the pollen microbiota by applying PCR-TTGE method. Pollen samples were collected from various anemophilous trees genera (Cephalotaxus, Cupressus, Pinus, Platanus and Quercus) in Montpellier (France) in 2013. Thorough pollen preparation was essential to a successful recovery of DNA from pollen. Regarding the results, pollen microbiota was tree genus-dependent. In addition, intra-tree genus variations were also observed. The Grammaproteobacteria 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 that 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, Piniaceae and mostly Cupressaceae pollen grains constitute most clinically relevant trees pollen (D’Amato et al., 2007; Yacél et al., 2014). 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 pollensomes) 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 (Bué 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 exudated 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. Colldahl and Carlsson (1968) isolated the Gram-negative Pseudomonas maltophilia (currently Stenotrophomonas maltophilia) from pollen samples. The presence of microorganisms on surface of pollen was then confirmed by scanning electron microscopy (Colldahl and Nilsson, 1973). Later, Spiewak et al. (1996a) highlighted the presence of a mixed microflora consisted of Gram-positive and Gram-negative mesophilic bacteria, thermophilic actinomycytes and fungi on allergenic pollen grains. Moreover, Spiewak et al. (1996b) showed that Gram negative bacteria such as Pantoea agglomerans endotoxin associated to pollen should be considered as a potential factor aggravating pollinosis. More recently, Heydenreich et al. (2012) reported that Gram positive pollen grains were colonized by Gram-negative bacteria as Acinetobacter lwoffii 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 syringae 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 (McCartney, 2002; Roudière et al., 2009; Michon et al., 2012), animals (Richards et al., 2005; Navarrete et al., 2012), plants (Lambais et al., 2014), foods (Ogier et al., 2004; Hamdouche 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 polyacylamide gels containing DNA denaturants according to a temperature gradient. Molecules with various sequences will have a different melting stranded DNA molecules.
polinosis 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 were 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 (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 Platania (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, pollen grains 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 dark. Quantification of bacteria in pollen presented in Table 1 was performed according to the protocol described below.

Table 1. Data concerning the pollen sampling. Quantification of bacteria in pollen 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 × 10³</td>
</tr>
<tr>
<td>P2</td>
<td>2013/04/10</td>
<td>Pinus</td>
<td>II</td>
<td>1.4 × 10⁵</td>
</tr>
<tr>
<td>P3</td>
<td>2013/04/10</td>
<td>Pinus</td>
<td>I</td>
<td>6.7 × 10⁴</td>
</tr>
<tr>
<td>P4</td>
<td>2013/04/14</td>
<td>Pinus</td>
<td>III</td>
<td>3.9 × 10³</td>
</tr>
<tr>
<td>P8</td>
<td>2013/04/22</td>
<td>Pinus</td>
<td>IV</td>
<td>1.9 × 10⁴</td>
</tr>
<tr>
<td>C1</td>
<td>2013/03/21</td>
<td>Cupressus</td>
<td>I</td>
<td>1.4 × 10³</td>
</tr>
<tr>
<td>C1b</td>
<td>2013/03/21</td>
<td>Cupressus</td>
<td>I</td>
<td>5.4 × 10³</td>
</tr>
<tr>
<td>C2</td>
<td>2013/02/23</td>
<td>Cupressus</td>
<td>V</td>
<td>2.0 × 10⁴</td>
</tr>
<tr>
<td>C3</td>
<td>2013/03/25</td>
<td>Cupressus</td>
<td>I</td>
<td>5.7 × 10⁴</td>
</tr>
<tr>
<td>Cx</td>
<td>2013/04/03</td>
<td>Cephalotaxus</td>
<td>VI</td>
<td>5.0 × 10³</td>
</tr>
<tr>
<td>Q</td>
<td>2013/04/18</td>
<td>Quercus</td>
<td>VI</td>
<td>7.0 × 10³</td>
</tr>
<tr>
<td>Pla2</td>
<td>2013/04/22</td>
<td>Platania</td>
<td>VII</td>
<td>5.7 × 10³</td>
</tr>
</tbody>
</table>

Legend: *1 F- 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). 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 1 x 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 1 x 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 mg / ml ethidium bromide in 1x TAE buffer, rinsed for 45 min in 1x TAE buffer and then photographed on a UV transilluminator.

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Preparation of pollen samples

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![Preparation of pollen samples](image)

**Figure 1** Presentation of the different protocols used to prepare pollen samples before DNA extraction. For protocols C.pca and C.tsh, an additional culture on PCA medium and TSH medium were performed respectively before extraction with the MasterPure™ DNA purification Kit (Epicentre).

The primary objective was to highlight the best protocol to extract effectively all bacterial DNA associated to pollen grains. For protocol A, 30 mg of pollen sample was suspended directly in 150 μl of Tris-EDTA (TE) buffer with 1 μl of lysozyme (Sigma) and incubated at 37°C for 18 h. For protocol B, 30 mg of pollen sample were centrifuged (12,000 g for 10 min) thrice in 150 μl of TE buffer. Then, the final pellet was suspended in 150 μl of TE buffer with 1 μl of lysozyme and incubated at 37°C for 18 h. The protocol C was based on the method described by Vanneste et al. (2011) including few modifications as follows: 30 mg of dry pollen suspended in 1 ml of sterile distilled water were sonicated for 5 min. Suspension was shaken with a rotary shaker for 60 min at 120 rpm. After settling, the supernatant was filtered through sterile gauze and centrifuged at 10,000 g for 20 min at 6°C. The supernatant was discarded and the pellet was rehydrated with 1 ml of sterile distilled water. This solution is referred to as the Final Concentrate (FC). From this FC, three different variations to protocol C called C.adn, C.pca and C.tsh were performed in order to optimise the recovery of DNA. For C.adn, DNA was directly extracted from 500 μl of FC. For C.pca, 100 μl of the FC were spread onto Plate Count Agar (PCA) (Bio-Mérieux) and incubated at 30°C for 48 h. For C.tsh, 100 μl of the FC were spread onto Tryptic Soy agar supplemented with 5 % of horse blood (TSH) (Bio-Mérieux) and incubated at 30°C for 48 h. For C.pca and C.tsh, DNA was extracted from cultures collected at the surface of PCA or TSH plates respectively. The last step of protocol C consists of re-suspension in 150 μl of TE buffer with 1 μl of lysozyme and incubation at 37°C for 18 h.

Bacterial enumeration

For protocol C, three decimal dilutions were carried out in saline serum from Final Concentrate (FC). Then 100 μl of FC and each dilution were plated in duplicate on PCA agar and incubated at 30°C for 18 h.

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 HD1A1F-GC and HDA2 (Oger 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 1 x 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 1 x 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 mg / 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 \( S_D = \frac{2N_c}{N_a + N_b} \) (Heyndrickx et al., 1996) where \( N_a \) represented the number of bands detected 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).

![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).](image)

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. Figure 3 presents TTGE patterns 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 3: Bacterial 16S rDNA PCR-TTGE profiles obtained from pollen samples of five different Pinus samples, prepared according to four protocols.](image)

For C1b, one band was detected for protocol C.adn while 12 and 16 bands were respectively obtained 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 and the second cluster comprised patterns obtained from protocols A and C.adn (Fig 4).

![Figure 4: Cluster analysis of 16S rDNA PCR-TTGE profiles obtained from pollen samples of five Pinus samples, prepared according to four protocols.](image)

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 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.](image)
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 Platanus 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 Pseudomonas aeruginosa. In addition, we observed a band matching the genus Bacillus. Within the genus Pinus, the five TTGE patterns corresponding to C.pca protocol were very different from one 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 Platanus, 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 Pseudomonas aeruginosa. Erwinia sp., derived from Cupressus sp., a new species isolated in 2011 (Otero et al. 2012), was reported to cause pulpitis in human teeth (Otero et al. 2014). Erwinia sp. DNAs were also detected in our pollen samples. Concerning Platanus pollen sample, the TTGE pattern comprises bacteria from Enterobacteriaceae (Tatumella sp. and Erwinia sp.), Pseudomonas sp. and Stenotrophomonas sp.

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 based on PCR-TTGE which was already applied to determine bacterial communities associated to pollen samples from various plant genera (Parent et al. 1996, Trabulsi 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 present on pollen samples. However potential viable but nonculturable (VBNC) bacteria could not be detected. The culture-independent protocol highlighted only the main bacteria. The bacterial enumeration of pollen samples varied from 7.0 10^3 to 5.7 10^4 CFU/g pollen. These results were concordant with those obtained by Spiewak et al. (1996a) for different trees. The bacterial communities associated to pollen samples varied among two plant genera (inter- genera variation) and also inside a same genus (intra-genus variation). Indeed, we observed very different TTGE profiles for the five pollen samples 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 genus. Numbers of pollen samples were even less represented by Proteobacteria (1996a) which highlighted in particular mixed microflora consisting of Gram-positive and Gram-negative mesophilic bacteria on allergenic pollen grains. 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 Wetzel et al. (2010) which showed that 81.2% of bacterial species isolated from edible flowers belonged to the class Gammaproteobacteria. Gammaproteobacteria comprises several species particularly D. chrysanthemi (formerly Erwinia chrysanthemi) known to be phytopathogenic (Toth et al. 2011). Enterobacter asburiae, Enterobacter cloacaе and Enterobacter cowanii were already reported to cause and even to accumulate in many plants (Sarkar and Chaudhuri, 2015). K. oxytica, Enterobacter sp., Erwinia sp., Tatumella ptyseos etc. were also dominant taxa in a Greek vineyard (Nisiotou 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 are 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. rhizosphere (Peix et al., 2003) and P. panacis (Park et al., 2005). This Pseudomonas species could be considered as phytopathogen, biological agent or PGPR (Vanneste et al., 2011; Noori and Saud, 2012; Mansfield et al., 2012). The genus Xanthomonas with X. campestris and X. orzyae 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, Pantoaea strains are responsible for respiratory infections in immunocompromised patients (Flores Popoca et al., 2012; Kursun et al., 2012; Walterton and Stravrinides, 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 ptyseos strains were notified to be responsible for human tracheobronchial/pulmonary infections (Costa et al., 2008). For the Pseudomonadaceae family, Pseudomonas aeruginosa is likely to be responsible for hospital-acquired pneumonia (Park et al., 2005). In many cases, hospital-acquired. For P. aeruginosa is also recovered from respiratory tracts of cystic fibrosis patients (Winstanley et al., 2016). Indeed, P. aeruginosa should be taken into account as a potential human pathogen which was especially isolated from tracheal aspirate of a patient suffering from pulmonary infections (Leneveu-Janvri 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 pasteure responsible for a few cases of infections in immunocompromised patients (Morfín-Otero et al., 2012). Pollen samples from Cephalotaxus, Quercus and Cupressus included also Gammaproteobacteria such as Enterobacteriaceae and Pseudomonadaceae 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 wide range of human diseases and plant infections.

CONCLUSION

In this present study, we analysed for the first time bacterial communities associated to pollen grains collected from Pinus, Cupressus, Cephalotaxus, Quercus and Platanus by a molecular method. PCR-TTGE is a suitable tool since bacterial microbiota was determined for each pollen samples. Bacterial communities varied inside a plant genus and from genus to genus. We highlighted here the potential presence of phytopathogens and also human potential opportunistic bacteria on allergenic pollens. Indeed, pollen grains

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