International Biodeterioration & Biodegradation xxx (2017) 1–8

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

First evaluation of the microbiome of built cultural heritage by using the Ion Torrent next generation sequencing platform

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ARTICLE INFO

Article history:
Received 23 October 2016
Received in revised form
30 January 2017
Accepted 30 January 2017
Available online xxx

Keywords:
Next generation sequencing (NGS)
Ion Torrent™ sequencing
Biodeterioration
Historic materials
Halophilic microorganisms
Primer bias

ABSTRACT

The aim of this study was to detect the community structure of Bacteria and Archaea, especially halophilic microorganisms, involved in the biodeterioration of historic building materials, as brick and paint coating. The Ion Torrent™ sequencing technology platform was used for the first time to analyse these materials. Amplicons were generated by using universal, bacteria- and archaea-specific primers, all flanking the V4 hypervariable region of the 16S rDNA, in order to compare the sensitivity of taxon detection.

Results showed that the Ion Torrent™ sequencing platform has an enormous potential to evaluate the microbiome of historic building materials in a fast and easy way. The brick sample displayed a total of 1178 genera belonging to 27 bacterial and 3 archaeal phyla, while the paint coating showed 145 genera belonging to 11 bacterial phyla. Members of the *Actinobacteria* were dominant in both samples. Halophiles were detected in both materials showing to be important colonizers when salty micro-niches are available in the surfaces of the buildings.

Nevertheless, analyses showed to be influenced by the primer choice, and bacteria- and archaea-specific primers confirmed the occurrence of primer bias, as they enabled the unspecific detection of Archaea and Bacteria. The results derived from universal and archaeal-specific primers were comparable, except for *Nitrospirae*, detected only using universal primers. These data clearly showed the impact of primer choice on the structure of the microbial communities, pointing out the need of a carefully choice of primers. Therefore, we recommend using, in addition to the universal primers, bacteria- and archaea-specific primers in order to cover a higher taxonomic diversity.

1. Introduction

The variety of biodeterioration phenomena observed on historic building materials is determined by chemical, physical and biological factors. These materials contain different hygroscopic salts, that migrate with capillary water, dry out and precipitate forming efflorescences on the surfaces (Stryszewska, 2014). These salty deposits offer suitable ecological niche for halophilic microorganisms (Saiz-Jimenez and Laiz, 2000; Piñar et al., 2009). Several studies have shown that halophiles are responsible for aesthetic damages on surfaces by the formation of coloured biofilms. Moreover, their growth can contribute to structural destabilization of materials (Piñar et al., 2009, 2014; Ettenauer et al., 2014). Therefore, the precise determination of the microbial community structure inhabiting historic objects has become an important research field both for conservators and for microbiologists.

Microbial diversity analyses have been traditionally assessed by cultivation techniques. However, the proportion of culturable microorganisms can be low, especially for highly specialized species like halophiles. For this reason, molecular techniques have been increasingly used in most recent studies assessing the structure of halophilic microorganisms on cultural assets (Piñar et al., 2013; Otlewska et al., 2015).

Ongoing development of next-generation sequencing (NGS) technologies allows in-depth sequencing and data analysis of various types of environmental samples (Takahashi et al., 2014;

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http://dx.doi.org/10.1016/j.ibiod.2017.01.040
0964-8305/

Chimienti et al., 2016; Kraková et al., 2016). Nowadays, several types of high-throughput sequencing platforms are widely used. One of the first was the Roche 454 employing pyrosequencing, which allows long read length and relatively high speed (Petrosino et al., 2009; Di Bella et al., 2013). The Illumina platform uses bridge amplification for polony generation and sequencing by synthesis. The major advantages of this method are the relatively inexpensive price per base and the comparatively high sequencing depth, despite short length reads (Klindworth et al., 2012; Gupta and Gupta, 2014). An alternative sequencing approach is the Ion Torrent™ technology platform, which also uses sequencing by synthesis strategy, but in the contrast to other technologies, it directly detects hydrogen ions when bases are incorporated into the growing strand (Di Bella et al., 2013). The attractiveness of this method lies in the fast sequencing and relatively low cost (Rothberg et al., 2011; Lu et al., 2015). Independently from the type of applied NGS technology employed, 16S ribosomal DNA is the most commonly sequenced gene as it is found in all prokaryotes. 16S rRNA gene contains nine hypervariable regions (V1–V9) which demonstrate significant sequence diversity among different bacteria and archaea (Shah et al., 2011). Usually one or more of the 16S rRNA hypervariable regions (e.g. V1–V3, V1–V4, V2–V3, V4, V5–V6, V6–V7) are amplified and sequenced. It is however still unclear which combinations of regions and primer pairs allow to precisely describe the taxonomic composition (specificity) and to fully assess the population diversity (sensitivity) (Fouhy et al., 2016). Inappropriately designed or selected primers may cause receiving incorrect and questionable results (Marchesi et al., 1998). Several studies have suggested that the use of universal 16S rDNA primer sets is the best choice to investigate the significant fraction of Bacteria and Archaea in environmental specimens (Baker et al., 2001, 2003; Schleper et al., 2005; Galand et al., 2009; Gantner et al., 2011; Zanardini et al., 2016). Nevertheless, 16S rRNA primer pairs designed and described in the bibliography to be universal are not really universal and thus, cannot detect some mismatched species (Baker et al., 2006).

In this study, the Personal Genome Machine (PGM, Ion Torrent™) was used for the first time to investigate the microbial community structure on historic building materials. With this background, the study was aimed to: (a) compare the diversity of the microbial communities dwelling historic building materials (brick, and paint coating) using the Ion Torrent™ technology platform, and (b) compare the sensitivity of taxon detection depending on type and number of primer pairs. To this end, three sets of primers were selected (universal, bacteria- and archaea-specific primers) to detect the unique composition of Bacteria and Archaea involved in biodeterioration processes, with a special focus on halophilic microorganisms.

2. Materials and methods

2.1. Site description and sampling

Brick and paint coating samples were collected from the outer and inner walls, respectively, of the historic 19th century townhouse in Lodz (Poland). The building showed visible symptoms of deterioration, such as cracking, detachment, material loss and salt efflorescence on the surface (Fig. 1). The total salt content in percent of sample mass for brick and paint coating was 1.61% and 0.72%, respectively. Each sample was scraped with sterilized scalpels up to the depth 1–5 mm, and immediately placed in sterile plastic tubes.

2.2. DNA extraction

Brick and paint coating fragments were grounded employing sterile mortar and pestle. The complete microbial DNA was isolated directly from the powdered material (0.5 g of brick powder; 0.25 g of paint coating powder) using the FastDNA SPIN Kit for soil (MP Biomedicals, Illkrich, France) as recommended by the manufacturers. The DNA yield and purity (A260/A280 ratio) were assessed using a NanoDrop ND-1000 spectrophotometer (peqLabBiotechnologie GmbH, Linz, Austria).

2.3. PCR amplification

Primers sets used for the amplification of the 16S rDNA were targeting exactly the same V4 region of bacterial and archaeal rDNA. The primer sequences, the amplicon size and coverage rates are listed in Table 1. All PCR reactions were carried out in a BioRad C1000 Thermal Cycler using the 1 × PCR Master Mix from Promega (Vienna, Austria) [50 units ml^{-1} of TaqDNA Polymerase supplied in an appropriate reaction buffer (pH 8.5), 400 μM dNTP's, 3 mM $MgCl_2$]; in addition, 12.5 pmol μl^{-1} of each primer (stock: 50 pmol μl^{-1}, VBC-Biotech, Austria) and 400 μg ml^{-1} BSA (stock: 20 mg ml^{-1}; Roche, Diagnostics GmbH, Germany) were added. Each reaction contained 3.5 μl of template DNA. Independently of the primer set used, PCR reactions were performed under the same thermal conditions: initial denaturation at 95 °C for 30 s, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min; and a final extension cycle at 72 °C for 5 min. To exclude the possibility of cross-contamination, in each PCR reaction a negative control was included (no DNA template). The duplicate amplicons of each sample (2 × 50 μl) were pooled and 7 μl visualized on 2% (w/v) agarose gels, stained in an ethidium bromide solution (10 mg ml^{-1}) and documented using an UVP documentation system (BioRad Transilluminator, Universal Hood). The remaining PCR reaction was purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and the concentration of the purified amplicons was assessed with the Qubit 2.0 Fluorometer (Invitrogen Corporation).

2.4. Library construction and quantification

DNA libraries were constructed using the Ion Plus Library Kit for AB Library Builder System (Thermo Fisher Scientific) following the library preparation protocol for short amplicons, provided by the manufacturers. The amplicons derived from 16S rDNA universal primers were processed as non-barcoded library, by using the adapters (P1-A) from the Ion Plus Fragment adapters Kit, while the amplicons obtained from the archaea-specific and bacteria-specific primers were processed as barcoded libraries, by using the P1 adapter and the barcoded A adapters provided in the Ion Xpress Barcode Adapter Kit. The adapters were diluted according to the amount of DNA input, as recommended by the manufacturers. The resulting DNA libraries were subsequently quantified by qPCR using the Ion Library Quantitation Kit (Thermo Fisher Scientific) as described by the manufacturers, to calculate the dilution factor of each library for a final concentration of 50 pmol. After individual quantitation, the barcoded libraries (amplified with archaea- and bacteria-specific primers) were pooled in equimolar amounts to ensure equal representation of each barcoded library in the sequencing run.

2.5. Template preparation and sequencing

Template preparation was performed by the Ion Chef System (Thermo Fisher Scientific) using the Ion PGM Hi-Q Chef Kit and the sequencing by using the Ion Personal Genome Machine (PGM) using the Ion PGM Hi-Q Sequencing Kit with the Ion 314 Chip v2 following the instructions of the manufacturers (Thermo Fisher Scientific).

Please cite this article in press as: Adamiak, J., et al., First evaluation of the microbiome of built cultural heritage by using the Ion Torrent next generation sequencing platform, International Biodeterioration & Biodegradation (2017), http://dx.doi.org/10.1016/j.ibiod.2017.01.040