Forensic Science International: Genetics Supplement Series 7 (2019) 161–163

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

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

High resolution melting analysis (HRM) based on 16SrRNA as a tool for personal identification with the human oral microbiome

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ARTICLE INFO

Keywords:
HRM
Personal identification
Oral microbiome

ABSTRACT

Personal identification plays an important role in the forensic practice. The conventional methods of personal identification including STR, SNP and InDel are focusing on the molecular characteristics of the human cell. Recently, researchers pay attention to human microbiome by the reason that the human microbiome is rich in amount and variable among people. The purpose of this study is to apply the human oral microbiome to forensic personal identification. We designed one general primer pair: 518 F&806R, which targeted the 16SrRNA. We conducted the high-resolution melting analysis (HRM) with the one primer pairs. The results indicated that oral microbiome from different people could be distinguished by using HRM based on 16SrRNA. This study showed that human oral microbiome could be a promising marker for the forensic personal identification application.

1. Introduction

Human oral cavity owes the second most abundant microbiota after gastrointestinal tract [1], and the human oral microbiome is influenced by many factors, such as gene, diet, and hygiene etc. which cause the composition of human oral microbiome is unique among the individuals. Human oral microbiome potentially applies in forensic personal identification. Recently, the potential of human microbiome applies in the forensic personal identification, most of these studies use the next generation sequence to acquire the sequence information of the human microbiome for the bioinformatic analysis [2]. The cost of the next generation sequencing is a problem to study with large amounts of samples. An accurate, rapid and low-cost method should be applied before the sequencing. The researchers had used the High-Resolution Melting Analysis of 16SrRNA gene variable region for studying the comparison of bacteria community composition in soil sample [3] and the rapid differentiation of bacterial community in tadpole digestive tract and feces samples [4]. So, the High-Resolution Melting Analysis can be a tool to demonstrate the discrepancy of human oral microbiome.

2. Materials and methods

Five healthy volunteers participated the study, two males and three females respectively. The five volunteers did not treat with antibiotics in the past three months. The volunteers were required to not eat food or drink water at least one hour before sampling. Totally, five saliva samples and five oral swab samples were collected. These samples were named as Sa1, Sa2, Sa3, Sa4, Sa5, Os1, Os2, Os3, Os4, Os5, respectively. The genomic DNA was extracted using QIAmp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was quantified by Nanodrop™ 2000 spectrophotometer (Thermo Scientific,Wilmington, NC, USA). Forward primer and reverse primer were 518 F CCAGCAGCCGCGGTAAT and 806R GGACTACCAG GGTATCTAATCCTGTT respectively, targeting at 16S rRNA V4 region of bacteria [5]. The reaction mixture with a final volume of 10 µl contained: 0.2 µM forward primer and reverse primer, 10 ng genomic DNA, 5 µl 2 × Light Cycler® 480 High Resolution Melting Master Mix (Roche, Mannheim, Germany), 1.2 µl 25 Mm $MgCl_2$ and DNase/RNase free water. The touch down PCR and High-resolution melting analysis was performed on the Light Cycler 480(Roche, Mannheim, Germany). The condition of touch down PCR was 95°C for 10 min, 55 cycles of 94°C for 10 s, annealing at 65°C for 10 s with a 0.5°C decrease in temperature after each cycle down to 53°C, and extension at 72°C for 10 s. HRM analysis was performed at the end of PCR protocol under the following condition: 95°C for 1 min, 40°C for 1 min, temperature continuously increased from 75°C to 95°C at a 0.2°C/s to acquire sample fluorescence, 40°C for 10 s to cool. The result of the HRM was analyzed according to

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https://doi.org/10.1016/j.fsigss.2019.09.063
Received 6 September 2019; Accepted 24 September 2019
Available online 25 September 2019
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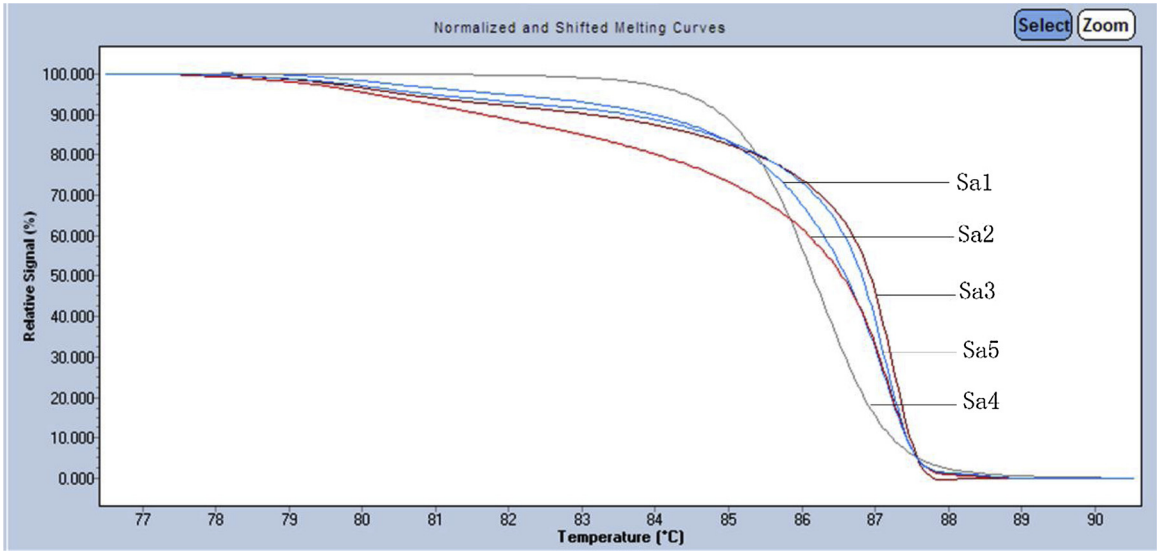

Fig. 1. The melting curve profiles of five individuals' saliva samples.

the Light Cycler 480 software.

3. Results and discussion

The result of HRM revealed the presence of distinct microbial communities in different human oral cavity. HRM analysis of the 16S rRNA V4 region showed the amplicon melting curve profiles had a discrepancy in different saliva samples. Five saliva samples from different individuals were divided into four groups (shown in Fig. 1). Sa1 and Sa5 were divided into a same group: blue. The reason may be that the two persons lived in the same circumstances. Their diet was similar, and they shared same environment, resulting in small difference in their oral microbial communities. The melting curve profiles of Sa5 was in accordance with Os5 (shown in Fig. 2). Sa5 and Os5 were divided into a same group, and the melting curves were similar. Another melting curve profiles of saliva samples and oral swab samples, which cannot match well. It may explain by the saliva samples contained not only the oral microbiome, but also the microbiome of throat.

4. Conclusion

According the primary study, we demonstrated there existed discrepancy in different human oral microbiome. This discrepancy provides the basis of personal identification. We can apply some more accurate method like sequencing to further analysis the discrepancy. The human oral microbiome has the potential to be a promising marker for the forensic application.

Declaration of Competing Interest

None.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NSFC, No.81772030 and81701866), the Postdoctoral Fund of Sichuan University (No. 2017SCU12049), and the Outstanding Youth Fund of Sichuan University (No. 2016SCU04A07).

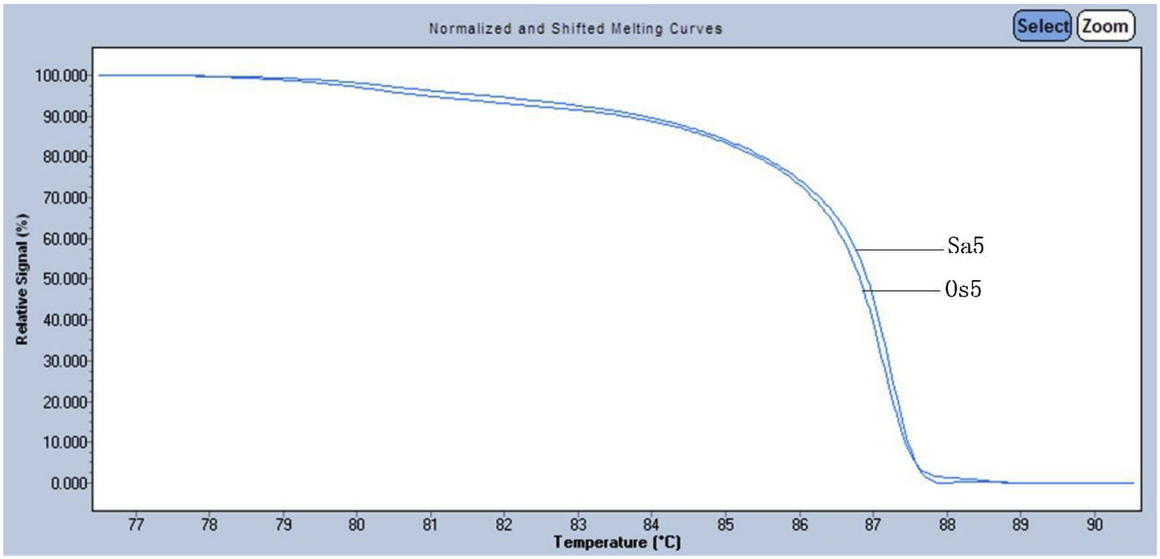

Fig. 2. The melting curve profiles of one individual's saliva sample and oral swab sample.

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