CAN MICROBES ON SKIN HELP LINKING PERSONS AND CRIMES?

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INTRODUCTION

Linking persons to crimes through DNA analysis is well-established approach for more than 25 years. While enormous numbers of cases all over the world have been solved based on DNA, there is still need for additional tools for improvement in physical evidence choice and collection (Jeffreys 2005). Unfortunately not all samples collected from crime scenes, are suitable for linking persons to crimes due to the quantity and quality of the human DNA collected. Recent studies have shown personality of bacterial community on human body surface that may open new perspectives for forensics (Fierer et al 2010).

The aim of the study was evaluation of variability of bacterial communities on skin of palm and fingers between and within individuals as well as transfer of bacterial DNA during contact to the object and persistence of community parameters during storage.

RESULTS

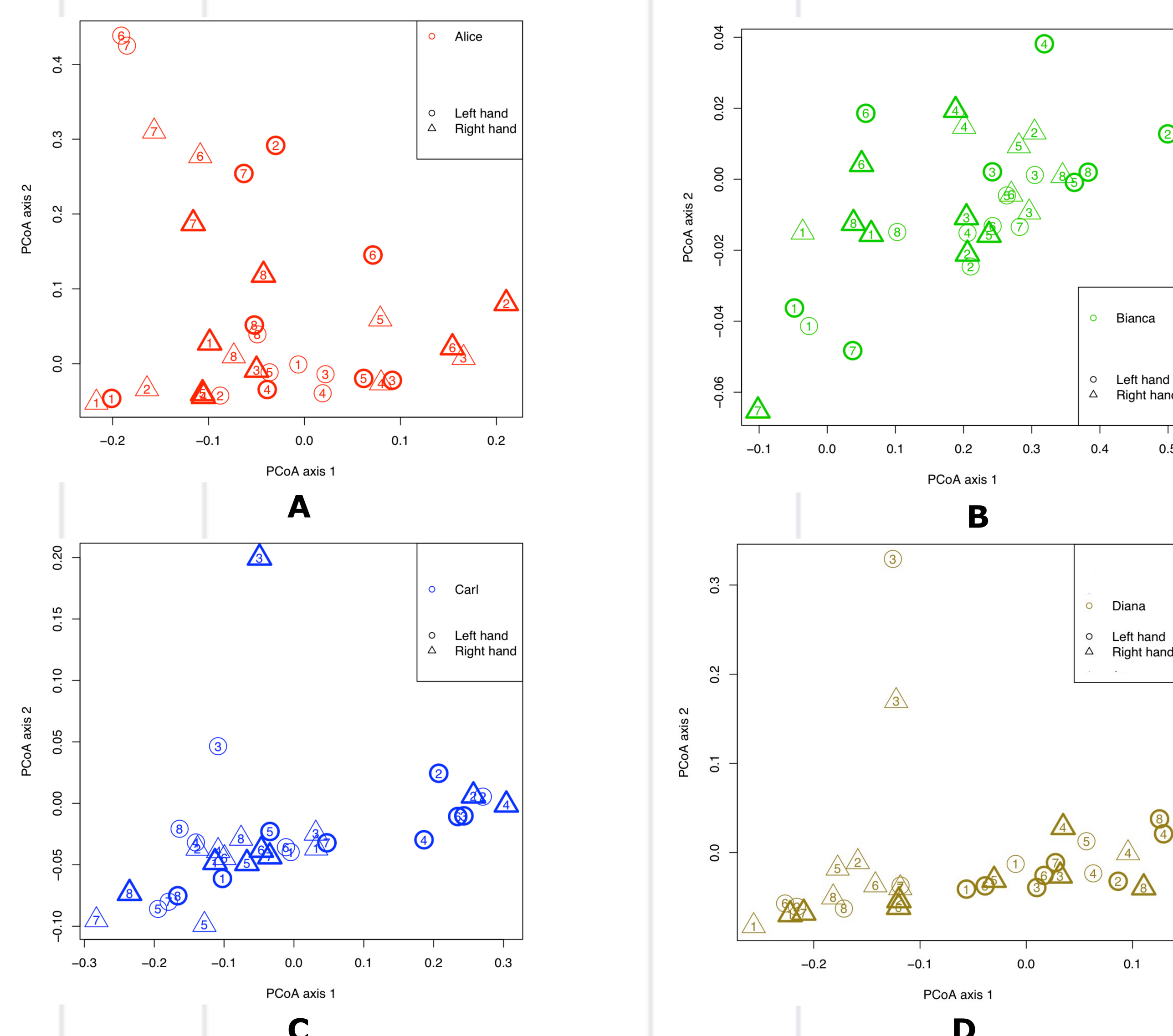

Figure 1. PCoA (Principal Coordinate Analysis) for OTU based distribution of samples taken from the left hand and right hand of four persons during sampling course. Colours denote persons; samples taken in the afternoon are in bold; numbers denote days of sampling: days 1-5, day 8 is denoted by 6, day15 by 7 and day 22 by 8. Microbial communities of samples from individuals shown on plots **A** and **B** are more heterogeneous than of individuals shown on plots **C** and **D**. The reason could be related their lifestyle and work: **A**: bank teller dealing with money; **B**: police officer, having close contacts with many people/public facilities; **C** and **D**: working at determined space.

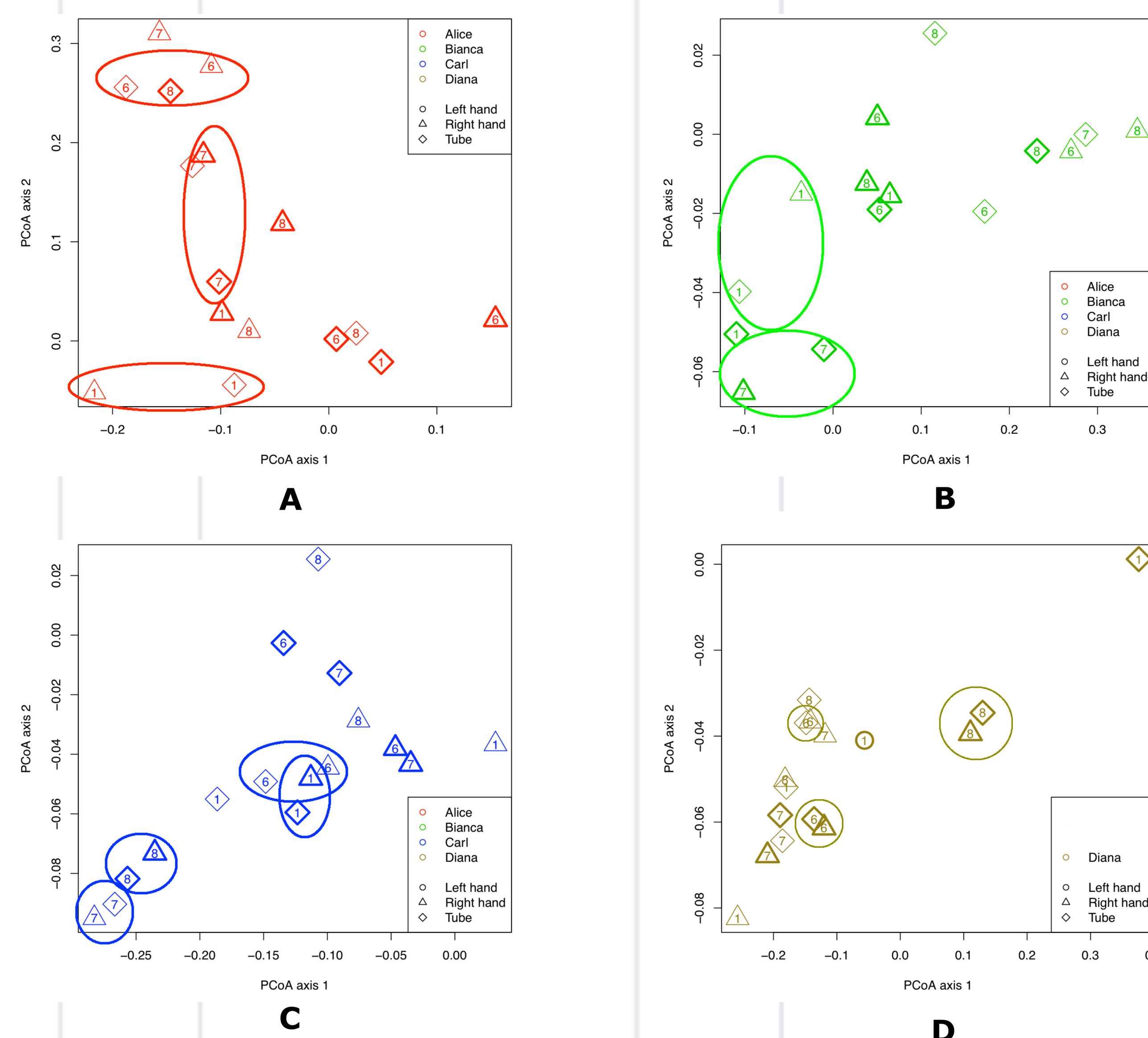

Figure 2. PCoA for OTU based distribution of samples taken from the right hand of four persons and from tubes during sampling course. Colours denote persons; samples taken in the afternoon are in bold; numbers denote days of sampling: days 1-5, day 8 is denoted by 6, day 15 by 7 and day 22 by 8. samples taken from the tube and corresponding hand at the same time are circled.

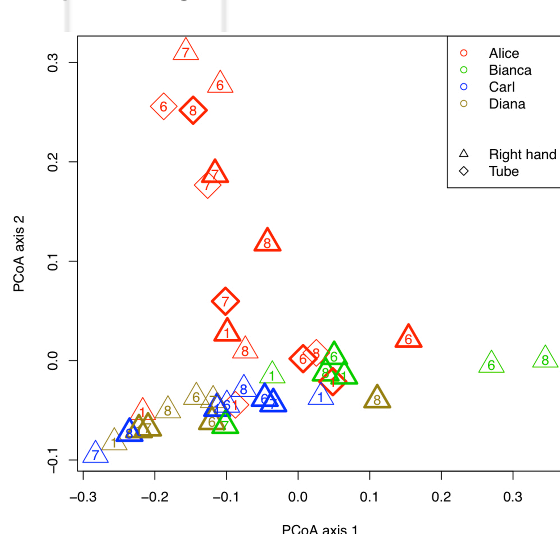

Figure 3. PCoA for OTU based distribution of samples taken from the right hand of four persons and from tubes during sampling course. Colours denote persons; samples taken in the afternoon are in bold; numbers denote days of sampling: days 1-5, day 8 is denoted by 6, day 15 by 7 and day 22 by 8. The figure shows samples taken from the right hand of all four individuals and from tube held by Alice (individual with heterogeneous microbial community).

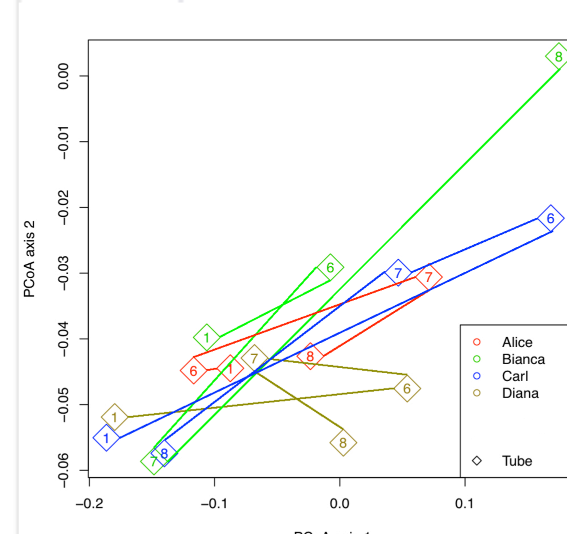

Figure 4. PCoA for OTU based distribution of samples taken from the tubes stored at room temperature. Colours denote persons who held the tube; numbers denote days of sampling: day 1, day 8 is denoted by 6, day 15 by 7 and day 22 by 8. Samples taken from the same tube are connected by lines. Further studies needed to explain this heterogeneity; possible reasons could be different contact areas between tube and palm/fingers that could behave differently (different transfer of biological material).

MATERIALS AND METHODS

For current study four healthy volunteers were recruited: Alice – female, bank teller; Bianca – female, police officer; Carl – male, engineer; and Diana – female, office manager (names amended). Volunteers asked to follow their normal lifestyle during the sampling course. Samples collected during five days (1-5) and afterwards once per week during 1 month (days 8, 15 and 22) in the morning and in the afternoon by swabbing of palm and fingers. On days 1, 8, 15 and 22 samples from dominant hand were taken after holding sterile 50 ml Falcon tube for 1 min with person's dominant hand. The samples were also collected from the handled objects at the same sampling time, except on the first day, when surface of the tube was quartered and sampled by quarter zone with one-week interval (on days 1, 8, 15 and 22). The tubes were stored at room temperature.

Chelex 100 (Bio-Rad Laboratories) was used for DNA extraction. V2-V3 hypervariable regions of 16S rRNA genes (16S rDNA) were amplified with universal 8F and 357R primers (Armougom and Didier 2008). Primer sequences were following:
8F-5'-TTGGCAGTCTCAGNNNNNNNN**AGTTTGATCCTGGCTCAG**-3'
and
357R-5'-GTCTCCGACTCAGNNNNNNNN**CTGCTGCCTYCCGTA**-3',
16S rDNA sequences 8F and 357R. The 8-bp barcode within primer 8F and 357R is denoted by 8 Ns. They refer to a unique sequence tag to barcode each sample (Hamady et al 2007). PCR amplificaton was performed using Maxima Hot Start PCR Master Mix (Fermentas). Cycling parameters were 15 min at 95 °C, followed by 3 cycles of 30 s at 95 °C , 30 s at 50 °C and 60 s at 72 °C , then 28 cycles of 30 s at 95 °C , 30 s at 65 °C and 60 s at 72 °C with a final extension at 72 °C for 10 min. Primer extention was carried out with full sequencing adapters (Primers A 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3' and B 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3'). Cycling parameters were 15 min at 95 °C, followed by 5 cycles of 30 s at 95 °C, 30 s at 62 °C and 60 s at 72 °C , then 20 cycles of 30 s 95 °C and 60 s at 72 °C with a final extension at final extension at 72 °C for 10 min. Amplicons with full sequencing adapter sequences were purified with QIAquick Gel Extraction kit (Qiagen) and sequenced with GS Junior Sequencing System (454 Life Sciences, Roche) with GS Junior emPCR reagents (Lib-L) Kit according to manufacturers protocol.

Sequences in the initial pretrimmed dataset were checked for chimeric artifacts. Pyrosequencing noise and non-unique sequences were removed. Reference sequences of aligned 16S rDNA were obtained from SILVA ribosomal RNA database. Taxonomic a and OTU frequencies were performed with R 2.13.1.

DISCUSSION AND CONCLUSIONS

To obtain more information for linking persons and objects we performed metagenome analysis of 16S rRNA gene V2-V3 hypervariable region using massive parallel sequencing approach. We found some clustering of deriving from individual person's left and right hand in different time points, however more genetic information (higher number of sequences per sample, information about other species (e.g. fungi)) should be obtained for better discrimination. Results of "storage experiment" suggest that more than one sample per object have to be analyzed for reliable clustering of samples. Samples taken from tubes resembled the samples taken from hand of respective holder as shown by the distribution of bacterial communities.

Our preliminary results are promising, however we foresee a need for more elaborative studies to be able to implement this approach into the routine practice. Currently we are carrying out additional analysis that includes expanded set of independent markers and more comprehensive statistical modeling of microbial communities.

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