Research Fellowship Report

"How indoor environment can shape human microbiome: culture-independent approaches"

Name of the fellow: Inês Ribeiro Paciência

Type of fellowship: Short Term Research Fellowship

Home Institution: Faculty of Medicine of the University of Porto, Portugal

Host Institution: Institute of Biotechnology of University of Helsinki, Finland

Project tittle: How indoor environment can shape human microbiome: culture-independent approaches

Duration: January-April

Introduction

What questions were addressed and why? What was the nature of the research?

Urban lifestyle has had a considerable impact on our life during the past decades. Cites have fewer green areas and people spend more time indoors. As a consequence, human being might be losing the opportunity to contact with environmental microbiota with which we used to coevolve, affecting human microbiota composition. We spend more and more time indoors, where several factors such as temperature, humidity, type and condition of ventilation, cleaning and disinfection by products, have contributed to impoverished microbial diversity [1]. In turn, those microbial disturbances have been associated with a lower development and maintenance of barrier function and immunological tolerance [2] and, therefore with the increasing prevalence of many chronic diseases including allergies, and asthma [3-7].

Although the impact of the environment on human microbiome, and consequently in health, has long been recognized [8], a comprehensive analysis of human microbiota is still needed to understand how environment can shape human microbiome and the mechanisms by which the microbiome is involved in human health and disease. In the recent past, studies on microbiome were mainly based on culture methods, which have provided a valuable but incomplete information of the vast diversity found in the human microbiome [9]. Most of microbial species have not yet been successfully isolated, cultured studied or quantified, probably due to the inability to reproduce necessary growth conditions. Thus, culture-independent approaches have been developed, providing more detailed information on the composition and diversity of the microbiome and a baseline for further research into the impacts of the microbiome on human health [10, 11]. Comparisons across multiple body sites in individuals exposed to different environments will be fundamental to understand how environmental factors may affect the composition of the human

microbiome. Therefore, the overall objective was to analyze and evaluate the differences on gut, oral, nasal and skin microbiome of swimmers compared with non-water competitive athletes, assessing the impact of indoor environment on the children's microbiome.

Work performed during the fellowship

What was the results?

During the first month of my stay in Institute of Biotechnology of University of Helsinki I was involved on the methodology of microbiome analysis including sample collection, storage, DNA extraction and how to analyze and interpret the results. During this month, I also realized how important and determinant is the whole process (from sample collection to data analysis) in microbiome characterization.

During the last months, I have learned and analyzed the skin, oral, nasal and gut samples from 29 swimmers and 34 non-water competitive athletes, using a culture-independent approach. Skin, oral and nasal samples were collected in two different moments: before (T0) and after 2 hours of swimming training (T1). Regarding to non-water competitive athletes, samples were collected in one moment: before training (T0). Stool sample were collected by each participant on the same day that the other samples were collected (within 24 hours).

I was involved in DNA extraction, PCR and sequencing. Sample DNA was extracted using FastDNA SPIN Kit for Soil according to manufacturer's instructions. Each extraction batch included a blank with no template DNA. PCR amplification was carried out in a PTC-225 thermal cycler. PCR amplification of the V3-V4 region of the 16S rRNA gene was performed in two steps. The first step was run with 2x25 µL technical replicates of each sample. The PCR products were purified with purified with Exonuclease I and FastAP (Figure 1a). A second PCR was performed with TruSeq and Index 8bp primers. DNA extraction kit blank and PCR blanks (also with no DNA template) were amplified and sequenced for identification of potential contaminating DNA (Figure 1b).

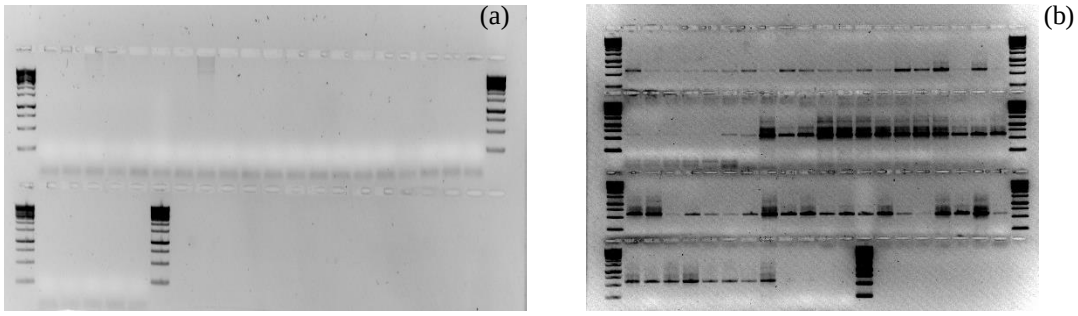

Figure 1. Gel electrophoresis from (a) first PCR and (b) second PCR. (a) corresponded to skin samples; (b) correspond to skin and stool samples.

The final PCR products were purified and pooled (Figure 2). All samples were sequenced in a single run on MiSeq.

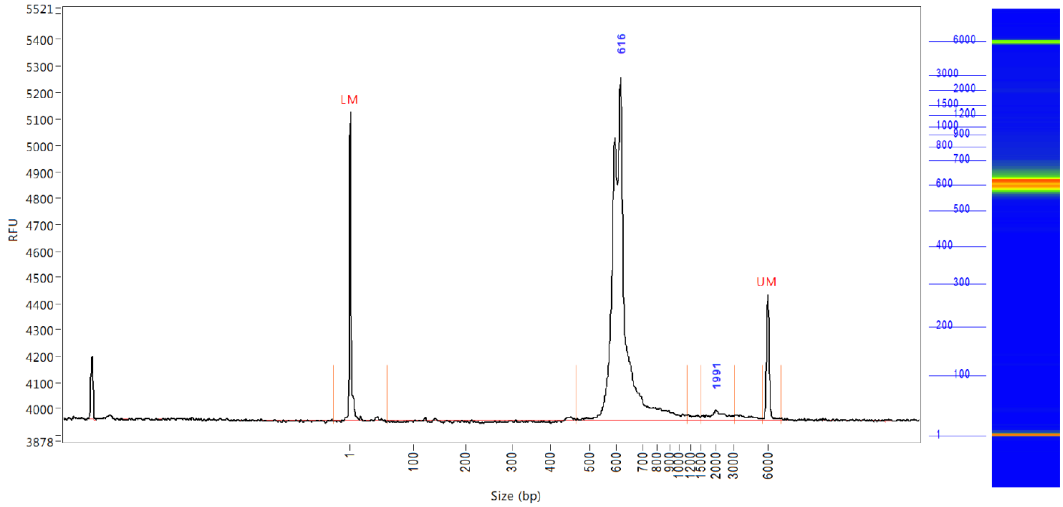

Figure 2. Fragment analyzer run from stool pool

During the research fellowship period, we aimed to provide information on the composition and diversity of human microbiome as proposed in the fellowship application, as well as, characterize microbiome of swimmers and non-water athletes. However, some data analyses still have to be done and the results analyzed and, thus, these results cannot be included in this report.

Conclusions

How will the findings impact future research?

The results of my fellowship will provide a more detailed information on the composition and diversity of the microbiome and also a comparison across multiple body sites in individuals exposed to different environments, being fundamental to understand how environmental factors may affect the composition of the human microbiome.

I am extremely grateful for the opportunity given by the EAACI Research Fellowship. I have learned new techniques based on culture-independent approaches on the methodology of microbiome analysis. This fellowship also provided and promoted the exchange of knowledge and an opportunity to me to integrate a research team with more experience and knowledge in microbiome and to return to my home institution and share my experience and knowledge, allowing the opportunity to implement what I have learned in another project that is taking place in healthcare units. This knowledge and experience will also allow to make a comprehensive characterization of the human microbiome and to understand how the microbiome affects human health and disease. With this opportunity and experience, I will move forward with the study of the role of environmental factors on childhood health, namely the impact of indoor environment on the children's microbiome.

Personal reflection on what you have learned and how you can improve for the future

This experience has been incredible and has enriched me both personally and professionally. The research team, particularly Research director Petri Auvinen and laboratory engineer Lars Paulin, Pedro Pereira, Velma Aho, Ursula Lönnqvist, Eeva-Marja Turkki, Eevakaisa Vesanen and Sergei Belanov, was very important for my integration and success of this fellowship. I would like to thank them for being very kind with me in every moment. Since the first day in the laboratory, I felt as one more in the group, and I would like to appreciate all the essential help during these months there. I came back with the satisfaction of learning a new technique based on culture-independent approach on the methodology of microbiome analysis. I would like to mention the role of this research team, and also Professor André Moreira and Professor Tari Haahtela, for following my progress closely, teaching me and supporting me. The knowledge and experience of my supervisors were and still are essential for my professional progress, being fundamental for success of this fellowship.

During these months I have also made really good friends, met very intelligent and amazing people and lived in an incredible country. For all these reasons, I strongly recommend this country, institute and this group for future research students. The Institute of Biotechnology is at the global forefront of research on

microbial genomics and maintains research infrastructures that constitute Finland's premier technology platforms for sequencing, genomics and biocomputing. The laboratory has multiple platforms available for DNA sequencing, aiming at providing the best, most cutting-edge combination of sequencing approaches.

Finally, I would like to thank to European Academy of Allergy and Clinical Immunology (EAACI) this opportunity.

References

1. Stamper CE, Hoisington AJ, Gomez OM, *et al*. The Microbiome of the Built Environment and Human Behavior: Implications for Emotional Health and Well-Being in Postmodern Western Societies. *Int Rev Neurobiol*. 2016;131:289-323. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27793224.

2. Lasse R, Jenni L, Antti K, *et al.* Holistic View on Health: Two Protective Layers of Biodiversity. *Annales Zoologici Fennici*. 2017

3. Haahtela T, Holgate S, Pawankar R, *et al.* The biodiversity hypothesis and allergic disease: world allergy organization position statement. *World Allergy Organ J*. 2013;6(1):3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23663440.

4. Riiser A. The human microbiome, asthma, and allergy. *Allergy Asthma Clin Immunol*. 2015;11:35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26664362.

5. Ege MJ, Mayer M, Normand AC, *et al*. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364(8):701-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21345099.

6. Lynch SV, Wood RA, Boushey H, *et al.* Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. *J Allergy Clin Immunol*. 2014;134(3):593-601 e12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24908147.

7. Hanski I, von Hertzen L, Fyhrquist N, *et al.* Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci U S A*. 2012;109(21):8334-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22566627.

8. von Hertzen L, Beutler B, Bienenstock J, *et al.* Helsinki alert of biodiversity and health. *Ann Med*. 2015;47(3):218-25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25904094.

9. Fouhy F, Ross RP, Fitzgerald GF, *et al*. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes*. 2012;3(3):203-20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22572829.

10. Yuan S, Cohen DB, Ravel J, *et al.* Evaluation of methods for the extraction and purification of DNA from the human microbiome. *PLoS One*. 2012;7(3):e33865. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22457796.

11. Aho VT, Pereira PA, Haahtela T, *et al.* The microbiome of the human lower airways: a next generation sequencing perspective. *World Allergy Organ J*. 2015;8(1):23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26140078.

12. von Hertzen L, Hanski I, Haahtela T. Natural immunity: Biodiversity loss and inflammatory diseases are two global megatrends that might be related. *EMBO Reports*. 2011;12(11):1089-93. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3207110/.

13. Golding J. Determinants of child health and development: the contribution of ALSPAC--a personal view of the birth cohort study. *Arch Dis Child*. 2010;95(5):319-22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20457699.

14. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9(5):313-23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19343057.

15. von Hertzen L, Hanski I, Haahtela T. Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. *EMBO Rep*. 2011;12(11):1089-93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21979814.

16. Butkus M. All Health is Local: Biodiversity, Ethics, and Human Health. *Ethics, Policy & Environment*. 2015;18(1):1-15. Available from: http://dx.doi.org/10.1080/21550085.2015.1016969.

17. Karjalainen E, Sarjala T, Raitio H. Promoting human health through forests: overview and major challenges. *Environ Health Prev Med*. 2010;15(1):1-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19568838.

18. Cavaleiro Rufo J, Madureira J, Paciencia I, *et al.* Indoor fungal diversity in primary schools may differently influence allergic sensitization and asthma in children. *Pediatr Allergy Immunol*. 2017. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28208225.