Addressing the relationship between *Staphylococcus aureus* abundance in the human skin microbiome and Atopic Dermatitis: a metagenomics approach

Rafaela Zoé Carvalho Tavares
Instituto Superior Técnico, October 2018

Abstract: The increased interest in the relationship between human microbiome and disease has contributed to the development of lower-cost sequencing techniques and software for the analysis of metagenomes. Several studies revealed a relationship between the severity of the disease Atopic Dermatitis and the bacterium *Staphylococcus aureus*. This thesis makes use of a metagenomics-driven approach to determine the extent to which this interaction affects the composition of the total microbiome of the human skin. To determine whether microbial community structure shifts according to higher *S. aureus* abundance in the skin microbiome, an *in silico* survey was performed on 183 samples representing various degrees of *S. aureus* incidence. Principal Coordinates Analysis (PCoA), revealed, in general, significant dissimilarities for skin samples with different percentage values of *S. aureus* and from different body sites, namely Volar Forearm, Antecubital and Popliteal creases. *Propionibacterium acnes*, *Corynebacterium* sp., *Staphylococcus* sp. *and Staphylococcus succinus* were revealed to be among the most responsive taxa to *S. aureus* incidence regardless of skin location. This study revealed a thus far unknown, positive correlation between *S. aureus* and *S. succninus* abundances in the skin microbiome. Genome-wide analyses performed *in silico* revealed that, although these species possess several genes in common, there are significant diferences in the number and types of genes involved in virulence, disease and defense, suggesting similar physiological aptitude along with divergent virulence strategies adopted by both species to dwell in the human skin microbiome.

Keywords: Metagenomics, Atopic Dermatitis, Human Skin Microbiome, *Staphylococcus aureus*, *Staphylococcus succinus.*

Introduction

The human skin is considered to be one of the largest organs in the human body and has many functions, such as to serve as a physical barrier that protects our body from toxic substances and foreign organisms. (Grice & Segre, 2013) The human skin contains multiple microorganisms - a density up to 10^7 per cm^2 with diverse communities of bacteria, fungi, mites and viruses (Cooper, Weyrich, Dixit, & Farrer, 2015), where the majority are harmless or even beneficial to their host. (Grice & Segre, 2013) The skin surface has a range of microenvironments with distinct features, like temperature, pH, and topography. Other aspects that define different microenvironments are the density of hair follicles, glands, skin thickness and folds. (Kong & Segre, 2015) Because of this heterogeneity across the human skin, a wide range of bacterial communities with different composition and diversity colonize the human body. (Grice, 2014) Nevertheless, since the skin is continuously exposed to the environment, it becomes a struggle to identify

what species are transient or residents of the community. (Grice & Segre, 2011) Therefore, it is challenging to define what constitutes a "healthy" bacterial community. (Fierer et al.,2008) The perception of the skin ecosystem is very important to understand the relationship between host and microorganisms. (Grice & Segre, 2011)

It has been already acknowledged that there are different bacterial phyla on normal human skin. However, there are a few types that have larger quantities than others, such as: *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* (Thomas et al., 2017). Bacterial taxa, that is frequently found on skin surfaces, has an intra- and inter-individual variability: intra-individual variation in microbial communities is less than inter-individual variation. (Grice & Segre, 2011)

The Metagenomics approach has the ability to identify and characterize all sorts of microorganisms independently of cultivation. Some of its advantages consist in the ability to reduce costs and time, while it increases sensitivity to discover new pathogens that cannot be detected so easily by the culture-based methods. It is a promising approach for the microbial diagnostics field. (Mulcahy-O'Grady & Workentine, 2016)

There are several benefits that can be withdrawn from metagenomics data sets in order to improve clinical applications, for example, fast and easy identification of pathogens. Moreover, this method may be also beneficial to other applications, such as detection of antibiotic-resistance genes and diagnosis of many diseases associated with bacterial, viral and fungal microbiomes. Metagenomics also can support the study of more complex phenotypes that are correlated with disorders and disruptions of the skin microbiome. Even though some disorders do not correlate with single pathogens, they emerge from the relationships of the microbiota present in the samples. (Mulcahy-O'Grady & Workentine, 2016)

Atopic Dermatitis (AD), also known as atopic eczema, is a chronic skin disorder that has many different causes such as skin barrier dysfunction, decreased immune responses and microbial skin colonization. (Kim et al., 2017) It is a cyclical disease, with flare periods that induce cutaneous phenotypes and non-flare periods where the skin has no signs of infections. (Chng et al., 2016) To measure AD severity, it is common to use the SCORing Atopic Dermatitis (SCORAD) index (Kong, 2012)

Many researchers believe that the emergence of AD is linked to a balance of the microbial community. Therefore, it is needed a clear understanding how AD may influence the microbiome and which microorganisms influence its severity in order to decide which is the "best" treatment. (Thomas et al., 2017) Currently, there is no established "best" treatment to AD, although studies of the skin microbiome may help in the pursuit of alternative therapies. (Grice, 2014)

To better understand the characteristics of microbial communities associated with AD, several scientists measured their diversity using for instance the Shannon diversity index, a measure tool that considers not only the richness (i.e., the total number of bacterial types), but also its evenness, the relative proportion of these bacterial types. It was concluded that there is a strong association between an increase in

severity of AD and a lower diversity of the microbial community. (Kong, 2012)

In several studies, *Staphylococcus aureus* has been revealed to be associated with AD, because it is usually found in lesional and nonlesional AD skin. The *S. aureus* bacterium is especially abundant during AD flares. However its colonization on the host skin has not been yet clarified as being a cause or effect. (Grice, 2014) Nevertheless, It is known that there is a correlation between an increase of *S. aureus*, the emergence of AD and a decrease in microbial diversity. (Kong, 2012) A decrease in *S. aureus* is, usually, followed by an increase in *Propionibacterium*, *Streptococcus* and *Corynebacterium,* which can be used as an indicator of the recovery from deterioration of AD. (Kim et al., 2017) It has also been proven that patients with more severe cases have a higher percentage of *S. aureus* strains whereas less severe cases display a more heterogeneous *S. epidermidis* strain community. (Gallo, 2016) (Otto, 2009) Researchers have came up with the conclusion that *S. epidermidis* is not causing the disease but rather keeping a relationship with the host. It is also believed that these bacteria may be beneficial during infection. (Otto, 2009) However, questions still remain to be answered on this relationship. (Byrd et al., 2017)

Materials and methods

The aim of this study was to address the relationship between *Staphylococcus aureus* abundance and shifts in the structure of the human skin microbiome, with implications to our understanding about Atopic Dermatitis, using a metagenomic approach. To this end, three datasets collected from the EBI-Metagenomics (MGnify) database were analysed to deliver Phylum-level Abundance profiles, OTU-level Taxonomic profiles and Functional (IPR) profiles of the human skin microbiome across gradients of *S. aureus* abundance in the samples, a major indicator of the emergence of AD flares in skin. The datasets had samples collected from Antecubital (Ac), Popliteal (Pc) Creases and Volar forearm from patients with Atopic Dermatitis in several stages.

The dataset can be downloaded from the MGnify platform using the following code: MGYS00000604. The file names are respectively from phylum level taxonomies, taxonomic assignments and InterPro matches for a functional analysis: SRP002480_phylumtaxonomy_abundances_v2.0; SRP002480_taxonomy_abundances_v2.0 and SRP002480_IPR_abundances_v2.0. All of the files are built upon the same samples using only 16S RNA gene sequencing for taxonomic assignments at phylum (phylum taxonomies table) and Operational Taxonomic Units (OTUs, a proxy for bacterial species determined at 97% 16S rRNA gene similarity) levels, plus a functional table derived from untargeted shotgun sequencing containing InterPro IPR entries functional assignments.

As a consequence of the selection of only those samples characterized by total metagenome sequencing and of the filtering employed to discard samples with less than 1,000 rRNA gene reads from these samples, a total of 183 skin microbiome samples were left. The resulting contingency tables (phylum- and OUT-level taxonomies and IPR assignments) were prepared for downstream statistical analyses as explained below.

Phylum, OTU and IPR, contingency tables were normalized using the Hellinger Transformation in order to have an improvement of the proportional abundance data, thus correcting for a possible overweight of highly abundant IPR entries, OTUs or phyla when determining the most differentiating taxonomic groups and functions across skin samples with different abundances of *S. aureus*.

Taxonomic and Functional analysis

In order to determine which phylum/ OTU/IPR displayed the largest shifts in abundance according to increasing levels of *S. aureus* abundance in the skin, Past v3 (PAleontological STatistics) was used. Past is a free software available for scientific data analysis, that integrates spread-sheet-type data with functions for data manipulation, plotting, univariate and multivariate statistics, ecological analysis, time series and spatial analysis, morphometrics and stratigraphy. (Ø. Hammer, Harper, & Ryan, 2001)

To test for shifts in microbiome structure and identify phyla/OTUs/IPRs most responsible for these shifts, the following resources within PAST were used: PCoA with the Bray-Curtis Dissimilarity index, PERMANOVA with Bonferroni corrections to test the validity of clusters formed using PCoA and SIMPER (similarity percentage) analysis.

Principal Coordinates Analysis (PCoA) is a multivariate statistic, an ordination method used to explore and to visualize similarities or dissimilarities of data; to visualize individual and/or group differences. (Øyvind Hammer, Harper, & Ryan, 2001) (Gail, Krickeberg, Samet, Tsiatis, & Wong, 2007) PCoA is typically applied when it is needed a reduction and interpretation of large multivariate data sets with some underlying linear structure.

Permutational multivariate analysis of variance (PERMANOVA) is a geometric partitioning of variation across a multivariate data, defined explicitly in the space of a chosen dissimilarity measure, in response to one or more factors in an analysis of variance design. Statistical inferences are made in a distribution-free setting using permutational algorithms. (Anderson, 2005)

SIMilarity PERcentages decompose the similarities into the contributions from each species, mostly used as a post hoc test in multivariate abundance, in other words, it finds the average contributions from each species with Bray-Curtis dissimilarity. It is commonly used to answer the question 'in which taxa is this difference most evident?'. (Warton, Wright, & Wang, 2012)

From the top 10 SIMPER analyses, we aimed to further investigate what were the relationships between those taxa and *Staphylococcus aureus*. Therefore, several linear regressions were made to understand those relationships. *Staphylococcus aureus and Staphylococcus succinus* were revealed to be among the most responsive taxa regardless of skin location. Therefore, the RAST annotation pipeline was used to compare four *S. succinus* genomes with one genome representing a multi-resistant *S. aureus* strain. The strains used for the comparison were *S. aureus* USA300 and *S. succinus* SNUC1280, 14BME20, DSM15096 and DSM14617.

RAST, Rapid Annotations using Subsystems Technology, is an automatic annotation free server, built upon the framework provided by the SEED system. (Overbeek et al., 2014) It returns an analysis of

the genes and subsystems of the genome in question, as supported by comparative and other forms of evidence. (Glass & Meyer, 2011)

Discussion and Results

Analysis of Taxonomic Abundance

Phylum Taxonomic Abundance analysis (Figure 1) demonstrated that the most abundant phyla were: *Actinobacteria*, *Firmicutes*, followed by *Proteobacteria* and *Bacteroidetes*, reflecting what is referred to in the literature. (Kong, 2012)

In order to understand the relationship between *S. aureus* and the microbial community, the response of phylum taxonomic abundances according to the percentage of *S. aureus* in the Ac, Pc and Vf samples was evaluated. The samples were divided in five groups according to the percentage of *S. aureus*: A (0-1%), B (1-2%), C (2-5%), D (5-10%) and E (≥10%). This analysis demonstrated that, although the major phyla were the same, there were significant dissimilarities in relative frequency between Ac, Pc and Vf and also, that increases in the relative abundance of *S. aureus* (*Firmicutes*) induces a decrease in all other phyla, and therefore, a reduction of microbial diversity.

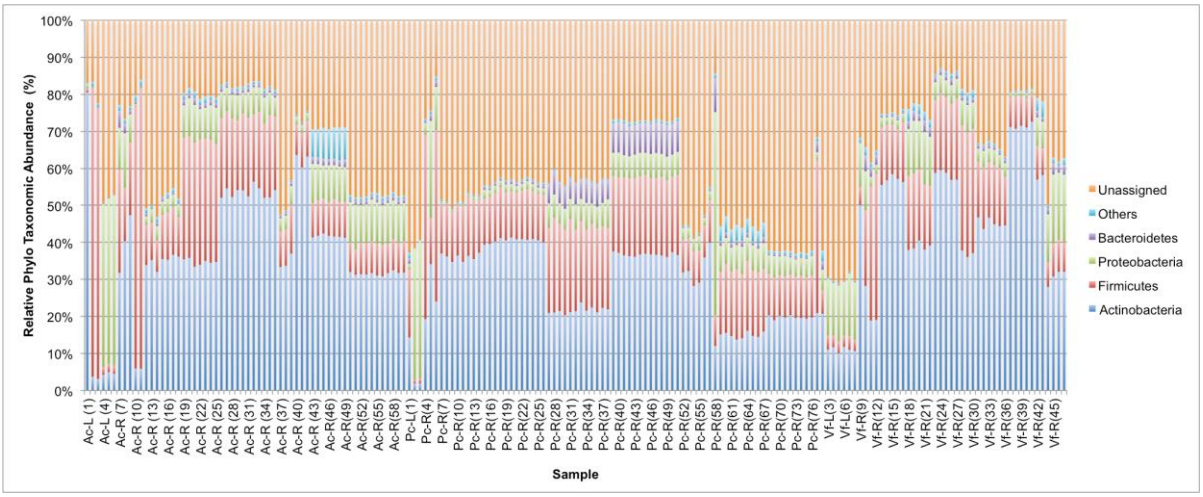


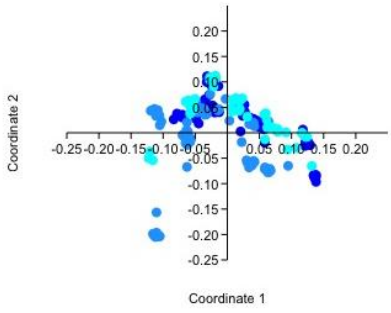
Figure 1 | Overview of Taxonomic Abundance at Phylum Level per Sample. Relative Frequency of the Abundance of Phylum per Sample. Ac- Antecubital Crease; Pc – Popliteal Crease, Vf – Volar Forearm. (R) . right side; (L), left side. Chart for all the 183 samples analysed in this study are show. Sample lables for only about 1/3 of the total amount of samples are shown for the sake of simplicity.

Taxonomic Analysis at the OTU level

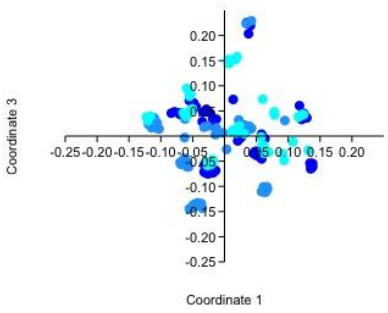
Taxonomic analysis revealed that the microbiome of samples with the same relative abundance of *S. aureus* were more similar to one another than those with lower *S. aureus* abundances, revealing convergence in structure and reduced complexity in skin microbiomes with high *S. aureus* incidence, as expected. Through multivariate statistics using Principal Coordinates Analysis (PCoA), the formation of clusters along with the percentage of *S. aureus* in the samples was examined. Since the ordination diagrams obtained from the PCoAs did not allow for a clear visual distinction between sample groups as it can be

seen in Figure 2, One-way PERMANOVA tests were necessary to precisely test for significant differences in skin microbiome structure among the studied groups. There were statistical significant dissimilarities between all areas, and within areas also except for group A and B in Ac, between group E and A, B and C in Pc and between group B and C in Vf. These results reinforce the fact that the bacterial community varies not only between sites but also according to the abundance of *S. aureus*. To identify the bacterial taxa that contribute to the major dissimilarities between the microbiomes according to site of origin or different abundances of *S. aureus*, SIMPER tests were performed at the OTU (Operational Taxonomic Unit) level. Results from the SIMPER analysis of all samples can be observed in Table 1. After identifying the major taxa that contributed to the dissimilarity of the microbiome, it was sought to understand if there would be any correlation with *S. aureus*. It was found that there were positive correlations between *S. aureus* and *Staphylococcus* sp., *S. succinus* and *S. epidermidis* (Figure 3). *S. succinus* was a surprise since it demonstrated the best regression coefficient and it was never mentioned before in skin disorders and with *S. aureus*. *Propionibacterium acnes*, *Corynebacterium* spp., *Streptococcus* spp., among others, demonstrated in almost all cases that in the presence of a higher abundance of *S. aureus* there is a decrease to low or null abundance levels of these species (Figure 3). Some exceptions, relatively to *Corynebacterium* spp., were identified in samples from Ac and in Vf samples, *P. acnes* demonstrated a negative linear correlation with *S. aureus*.

a)



b)



Ac Pc Vf

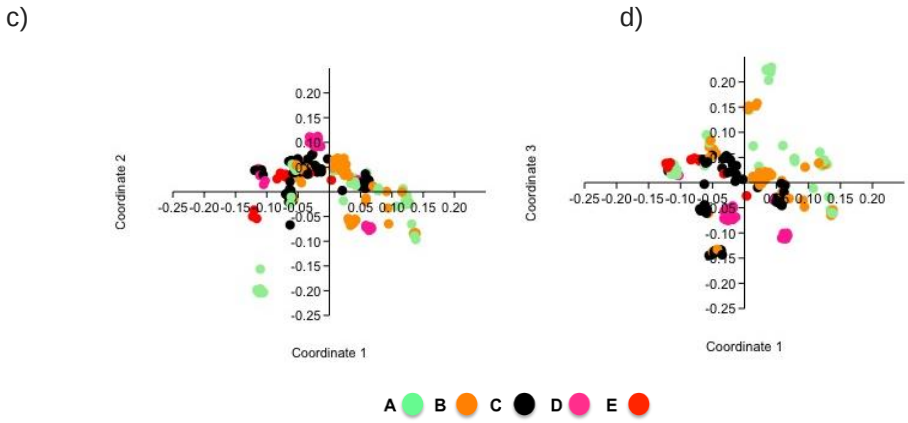


Figure 2 | Principal Coordinates Analysis (PCoA) of OTU profiles retrieved for All Samples (N = 183). PCoA performed on Bray-Curtis dissimilarity index calculated from Hellinger transformed data. (a) PCoA of Taxonomic microbial community between the three areas: Antecubital Creases (Ac), Popliteal Creases (Pc) and Volar Forearm (Vf); Coordinate 1 versus Coordinate 2. (b) PCoA of Taxonomic microbial community between the three areas: Antecubital Creases (Ac), Popliteal Creases (Pc) and volar forearm (Vf); Coordinate 1 versus Coordinate 3. (c) PCoA of Taxonomic microbial community according to the relative percentage of *S. aureus* in the samples: A (0-1%), B (1-2%), C (2-5%), D (5-10%) and E (More than 10%), Coordinate 1 versus Coordinate 2. (d) PCoA of Taxonomic microbial community according to the relative percentage of *S. aureus* in the samples; Coordinate 1 versus Coordinate 3.

Table 1 | Results from SIMPER analysis of all samples. SIMPER analysis identifying the percentage contribution of each OTU according to the Bray Curtis dissimilarity metric between Ac, Pc and Vf.

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Ac	Mean Pc	Mean Vf
Root;k__Bacteria;**p__Actinobacteria;**c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;**g__Propionibacterium;s__acnes**	1.459	2.606	2.606	0.486	0.241	0.508
Root;k__Bacteria;**p__Actinobacteria**;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae;**g__Corynebacterium;s__**	1.077	1.923	4.53	0.347	0.486	0.394
Root;k__Bacteria;**p__Proteobacteria**;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;**g__Pseudomonas;s__**	0.601	1.074	5.603	0.0992	0.0705	0.119
Root;k__Bacteria;**p__Firmicutes**;c__Bacilli;o__Bacillales;f__Staphylococcaceae;**g__Staphylococcus;s__aureus**	0.5512	0.9848	6.588	0.18	0.149	0.148
Root;k__Bacteria;**p__Firmicutes**;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;**g__Streptococcus;s__**	0.5197	0.9284	7.517	0.194	0.125	0.187
Root;k__Bacteria;**p__Firmicutes**;c__Bacilli;o__Bacillales;f__Staphylococcaceae;**g__Staphylococcus;s__**	0.4258	0.7608	8.277	0.175	0.166	0.151
Root;k__Bacteria;**p__Firmicutes**;c__Bacilli;o__Bacillales;f__Staphylococcaceae;**g__Staphylococcus;s__succinus**	0.3794	0.6778	8.955	0.136	0.119	0.115
Root;k__Bacteria;**p__Firmicutes**;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];**g__Anaerococcus;s__**	0.3438	0.6142	9.569	0.068	0.125	0.0843
Root;k__Bacteria;**p__Actinobacteria**;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;**g__Propionibacterium;s__granulosum**	0.3357	0.5998	10.17	0.107	0.052	0.117
Root;k__Bacteria;**p__Firmicutes**;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];**g__Peptoniphilus;s__**	0.3316	0.5925	10.76	0.0414	0.0961	0.0468
(...)						
Root;k__Bacteria;**p__Firmicutes;**c__Bacilli;o__Bacillales;f__Staphylococcaceae;**g__Staphylococcus;s__epidermidis**	0.2486	0.4442	15.67	0.0897	0.0692	0.0747

Similarity percentage analysis of the OTU differences between the different sites Ac, Pc and Vf. The first column identifies the OTU explained by that row, the second column represents the average of dissimilarity, the third column shows the % dissimilarity explained by that OTU, the fourth column is related to the cumulative Bray-Curtis dissimilarity metric for the OTU thus far represented in the table and the last three show mean abundance at site Ac, mean abundance at site Pc and mean abundance at site Vf.

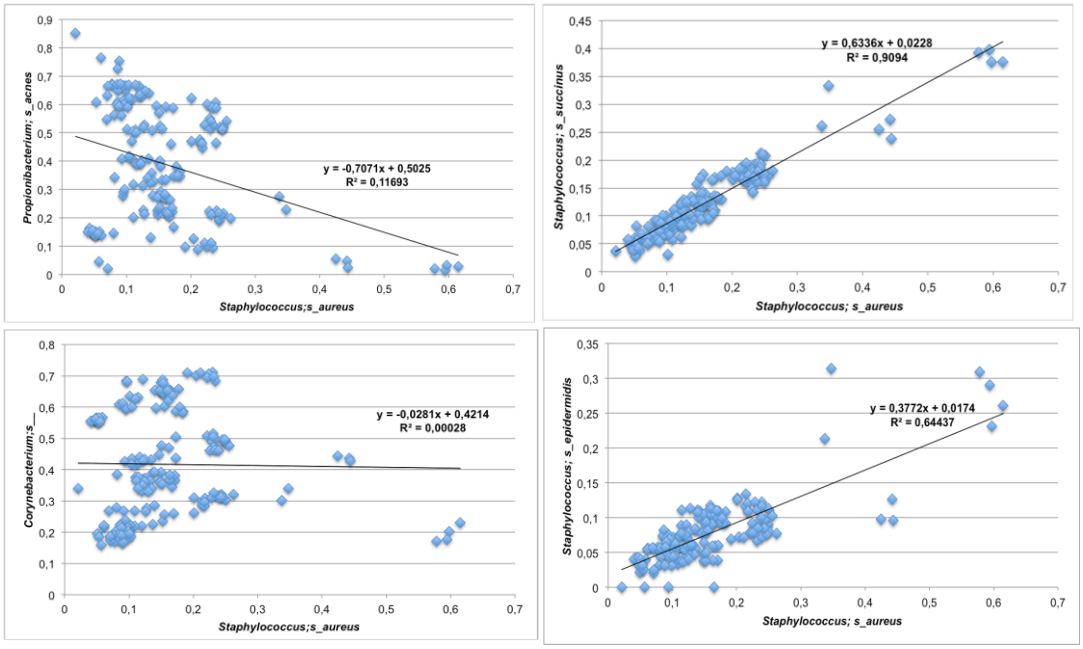


Figure 3| Linear Regression between the abundance of four of the ten most site-differentiating OTUs revealed by SIMPER analysis and *S. aureus*. The data used for this linear regressions were Hellinger transformed**.**

Functional Analysis

For Functional analysis at the IPR level, the same multivariate analysis and tests mentioned above were performed. Scatter plots derived from PCoA were inconclusive, since the samples were very dispersed. Results from One-Way PERMANOVA test were more conclusive and revealed that there was no significantly difference in functional profiles across skin sites or *S. aureus* abundance groups, only between Ac and Vf and groups A and B, considering all the samples. Within the Ac site, it was demonstrated that groups B and C were too similar; there were also no significant differences between groups B and D, E and A, B, C and D in Pc samples; and for Vf there were no significant between A and B,C and group B with C and D and finally witg group E, C and D. To rank all IPRs that contributed the most for the total variation of the dataset SIMPER analyses were performed, revealing four IPRs that were common in all analyses undertaken for differences among *S. aureus* abundance groups: Transposase, L1; Transposase, mutator type; TonB-dependent receptor, beta-barrel; and EAL domain. All of these IPRs are related to pathogenesis factors that may contribute to AD or responses to pathogenesis at different degrees.

Analysis of *S. succinus* genomes

After linear regression analysis was carried out, based on the results obtained previously using the SIMPER test, a strong and positive correlation between *S. aureus* and *S. succinus* was revealed. Unfortunately, until now, there is no information about the precise molecular interactions, eventual syntrophic behaviour and niche partitioning underlying the relationship between *S. aureus* and *S. succinus* in the human skin. Therefore, to explore the abovementioned issues making use of currently-available genomic information, a comparative analysis of four *S. succinus* genomes with one genome representing a

multi-resistant *S. aureus* strain was carried out using the freely-available server RAST.

Alignments based on aminoacid sequence homologies inferred from the corresponding genome sequences of *Staphylococcus aureus* (MSRA) strain USA 300 and *Staphylococcus succinus* strains 14BME20, DSM14617, DSM15096 and SNUC1280 were performed (Figure 4). The alignments were executed in the RAST server using the "sequence-based comparison" tool with the aim of a general and faster analysis to overcome possible similarities between *S. aureus* and *S. succinus*.

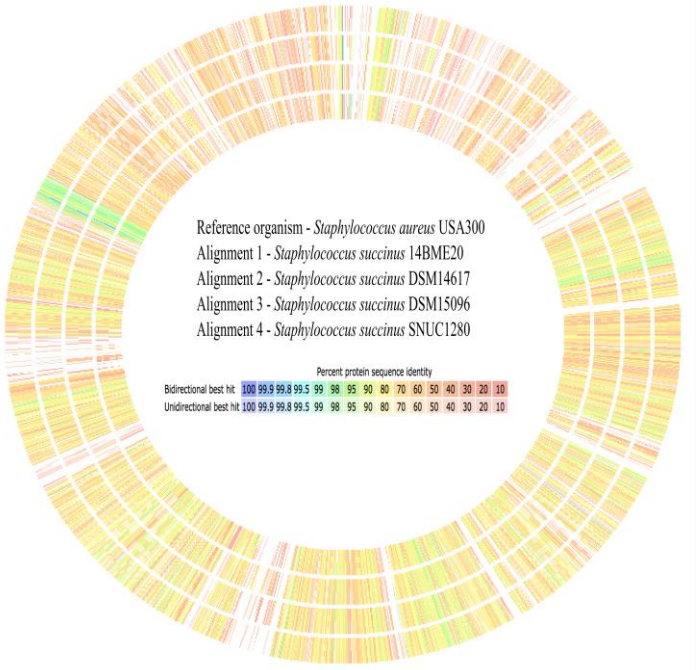


Figure 4| Sequence-based, genome-wide alignment between the multi-resistant *Staphylococcus aureus* (MSRA) strain USA 300 and *Staphylococcus succinus* strains 14BME20 (outermost ring), DSM14617, DSM15096 and SNUC1280 (innermost ring). Alignments were performed on RAST based on aminoacid sequence homologies inferred from the corresponding genome sequences. Several genes are common to S. aureus and *S. succinus* strains, but usually they display only moderate levels of aminoacid sequence homology.

Observing the alignment, although there are several genes in common between *S. aureus* and *S. succinus*, they exhibit only moderate levels of aminoacid sequence homology.

The RAST sever provides some general data and subsystems feature counts about the species under analysis. *S. aureus* and *S. succinus* share several genes across all RAST subsystems. Although they share the same subsystems as expected, there are some fluctuations between the number of genes. The number of genes varies more in the following subsystems: Virulence, Disease and Defense; Membrane Transport; Iron Acquisition and Metabolism and Carbohydrates.

Since, in the previous comparison, it was found that there is a substantial difference between *S. aureus* and *S. succinus* and even between *S. succinus* strains in the subsystem of Virulence, Disease and Defense, deeper research was made into this subsystem. The main difference between the subsystem Virulence, Disease and Defense comparing *S. aureus* and *S. succinus* strains is in the subgroup of adhesion. *S. aureus* has 23 genes classified as "Adhesins in *Staphylococcus*" and

S. succinus strains do not have any gene. Secondly, whereas all *S. succinus* strains are potentially able to produce bacteriocins, this gene cluster was not found in multi-resistant *S. aureus* USA300. Altogether, these results suggest that, while *S. aureus* may be a more competent skin/host colonizer through the action of adhesins, *S. succinus* may possess the ability to outcompete other bacteria in the skin microbiome through the biosynthesis of bacteriocins. Provided that *S. aureus* strains can cope with bacteriocin production by *S. succinus* (what is thus far not clear), both strains could engage in a synergistic interaction where *S. aureus* modifies the physical-chemistry of the skin microbiome, favouring both strains to thrive (given their similarities, for instance, in nutrient acquisition) whereas *S. succinus* deters growth of potential competitors through the biosynthesis of inhibitory secondary metabolites. Such hypotheses must be addressed in the future through dedicated studies if we are to illuminate the positive relationships usually observed among *Staphylococcus* species during the emergence of AD. Further, a subsystem in which several differences between *S. succinus* and *S. aureus* strains were found was “Resistance to antibiotics and toxic compounds”. There are genes for Methicillin resistance in *Staphylococci* in strains SNUC1280 and DSM14617 but the other strains have no genes.

Conclusion

In conclusion, although is already being recognized the importance of the study of the relationship between *S. aureus* and other microorganisms, further research is needed particularly to elucidate its relationship with other Staphylococci during AD emergence, and whether or not these species act as disease causing agents or their increased frequency is a consequence of a physico-chemically altered skin microniche under AD. The field of metagenomics is improving, and has been demonstrating a huge potential in addressing diseases from the taxonomic and functional standpoints.

Acknowledgments

I would like to thank Prof. Dr. Isabel Sá Correia and Prof. Dr. Rodrigo Costa and Dr. Tina Keller Costa for the advices and instructions during this journey. I would also like to thank Prof. Dr. Carmo Fonseca, Prof. Dr. João Ferreira and Prof. Dr. Paulo Leal Filipe from FMUL for sharing their knowledge with me, in order to complement my critical opinion on Atopic Dermatitis.

Last but not least, I would like to thank my family for the support during, not only this period, but also during my journey in Instituto Superior Técnico.

References

Anderson, M. J. (2005). PERMANOVA Permutational multivariate analysis of variance. *Austral Ecology*, 1–24. https://doi.org/10.1139/cjfas-58-3-626

Byrd, A. L., Deming, C., Cassidy, S. K. B., Harrison, O. J., Ng, W.-I., Conlan, S., … Kong, H. H. (2017). *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. *Science Translational Medicine*, *9*(397), eaal4651. https://doi.org/10.1126/scitranslmed.aal4651

Chng, K. R., Tay, A. S. L., Li, C., Ng, A. H. Q., Wang, J., Suri, B. K., … Nagarajan, N. (2016). Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nature Microbiology*, *1*(9). https://doi.org/10.1038/nmicrobiol.2016.106

Cooper, A. J., Weyrich, L. S., Dixit, S., & Farrer, A. G. (2015). The skin microbiome: Associations between altered microbial communities and disease. *Australasian Journal of Dermatology*, *56*(4), 268–274. https://doi.org/10.1111/ajd.12253

Fierer, N., Hamady, M., Lauber, C. L., & Knight, R. (2008). The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Sciences*, *105*(46), 17994–17999. https://doi.org/10.1073/pnas.0807920105

Gail, M., Krickeberg, K., Samet, J., Tsiatis, A., & Wong, W. (2007). *Analysing Ecological Data*.

Gallo, R. L. (2016). S. epidermis Influence on Host Immunity: More Than Skin, *17*(2), 143–144. https://doi.org/10.1016/j.chom.2015.01.012.S.

Glass, E. M., & Meyer, F. (2011). The Metagenomics RAST Server: A Public Resource for the Automatic Phylogenetic and Functional Analysis of Metagenomes. *Handbook of Molecular Microbial Ecology I: Metagenomics and Complementary Approaches*, *8*, 325–331. https://doi.org/10.1002/9781118010518.ch37

Grice, E. A. (2014). The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. *Seminars in Cutaneous Medicine and Surgery*, *33*(2), 98–103. https://doi.org/10.12788/j.sder.0087

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*(4), 244–253. https://doi.org/10.1038/nrmicro2537

Grice, E. A., & Segre, J. A. (2013). The skin microbiome. *Nat Rev Microbiol*, *9*(4), 244–253. https://doi.org/10.1038/nrmicro2537.The

Hammer, Ø., Harper, D. A. T. a. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, *4(1)*(1), 1–9. https://doi.org/10.1016/j.bcp.2008.05.025

Hammer, Ø., Harper, D., & Ryan, P. (2001). PAST-PAlaeontological STatistics, ver. 1.89. *University of Oslo, Oslo*, (1999), 1–31. Retrieved from http://www.researchgate.net/publication/228393561_PASTPalaeontological_statistics_ver._1.89/file/32bfe5135d45cd6b3b.pdf

Kim, M.-H., Rho, M., Choi, J.-P., Choi, H.-I., Park, H.-K., Song, W.-J., … Pyun, B. Y. (2017). A Metagenomic Analysis Provides a Culture-Independent Pathogen Detection for Atopic Dermatitis. *Allergy, Asthma & Immunology Research*, *9*(5), 453. https://doi.org/10.4168/aair.2017.9.5.453

Kong, H. H. (2012). Temporal shifts in the skin

microbiome associated with disease flare and treatment in children with atopic dermatitis. *Genome Research*, 850–859. https://doi.org/10.1101/gr.131029.111

Kong, H. H., & Segre, J. a. (2015). *Biogeography and individuality shape function in the human skin metagenome* (Vol. 514). https://doi.org/10.1038/nature13786.Biogeography

Mulcahy-O'Grady, H., & Workentine, M. L. (2016). The challenge and potential of metagenomics in the clinic. *Frontiers in Immunology*, *7*(FEB), 1–8. https://doi.org/10.3389/fimmu.2016.00029

Otto, M. (2009). Staphylococcus epidermidis—the'accidental'pathogen. *Nature Reviews Microbiology*, *7*(8), 555–567. https://doi.org/10.1038/nrmicro2182.Staphylococcus

Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., … Stevens, R. (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Research*, *42*(D1), 206–214. https://doi.org/10.1093/nar/gkt1226

Thomas et al., 2017. (2017). The microbiome and atopic eczema: More than skin deep. *Australasian Journal of Dermatology*, *58*(1), 18–24. https://doi.org/10.1111/ajd.12435

Warton, D. I., Wright, S. T., & Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods in Ecology and Evolution*, *3*(1), 89–101. https://doi.org/10.1111/j.2041-210X.2011.00127.x