Skin Metaproteomics : Understanding function of our microbial part

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Overview

•Purpose: Profiling skin proteome simultaneously with microbial proteome environment.

•Methods: Offline multidimensional reverse phase chromatography of isobaric labelled proteomes analyzed by high resolution accurate mass spectrometry.

•Results: 2500 human proteins and 975 microbial proteins from skin swabbing samples were identified and quantified.

Introduction

•Skin is the largest organ from human body. Skin's primary role is affording protection against environmental variations to maintain homeostasis regulation. While it is sterile at birth, skin is quickly colonized by numerous microorganisms. These microorganisms (Also known collectively as microbiome) may help protecting their host and also training innate immunity though symbiotic mechanisms. Colonization is driven by both endogenous host's and environmental factors and creates an homeostatic balance between host and flora. Driven by large **16S ribosomal RNA** (16S) and **metagenomic** sequencing efforts, numerous studies have described stereotypical changes in microbiota composition which reflect various states of health and pathology such as atopic dermatitis or psoriasis. However, the understanding of these microbial population fluctuation and how these changes impact **host physiology remain poor/or limited** due to **metagenomics bacterial taxon centered approach**. Mass spectrometry based proteomics of an host and its microbiome, also called **metaproteomics, can even decipher subtle functional and signaling mechanisms that would escape other 'omics' measurements**. Identification and quantification of proteins also allows to **highlight functional pathways, and to simultaneously perform fungi and bacteria related measurements**.

•Analysis of skin surface (*stratum corneum*) proteome and associated microbial flora presents **some analytical challenges**. First, dynamic range is very large and dominated by structural proteins like keratins, which make up more than 90% of *stratum corneum* total proteins content . Second, biomass of microorganisms is limited in comparison of human biomass, which accentuate the dynamic range problem. To try and **overcome these limitations**, we use **prefractionation** at peptides level coupled with **isobaric labeling** for keeping **accurate relative quantitation** and gaining depth of proteome coverage. Third, flora diversity isn't known a priori, while such knowledge is useful to improve analytical efficiency by restricting MS peaks attribution space and identification accuracy, as well as processing time. To overcome this limitation, we performed iterative processing to restrict search space.

Methods

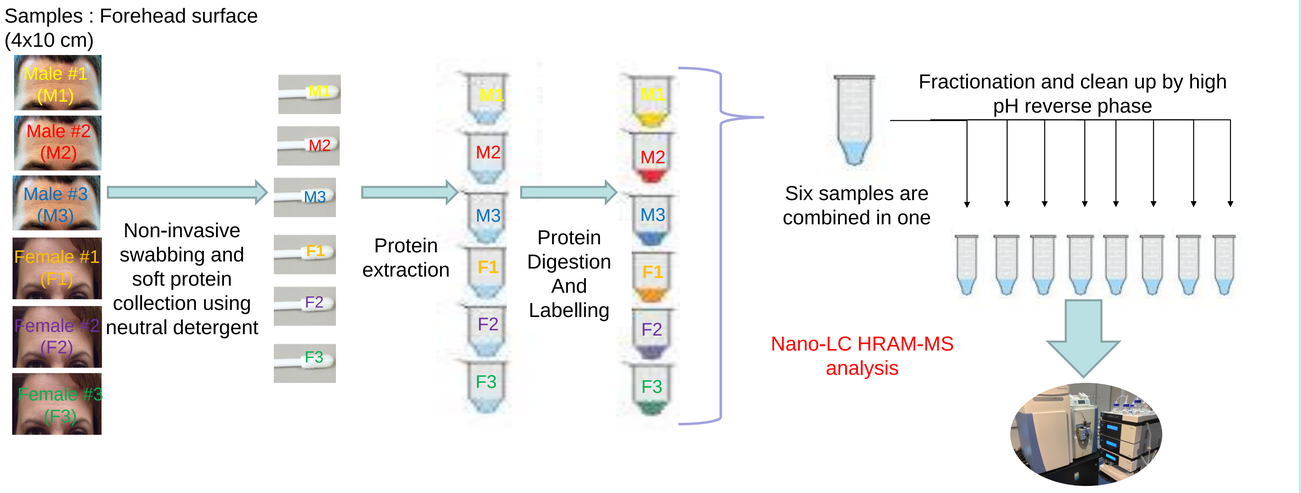

FIGURE 1. Schematic worflow of assay: Sample collection and processing: Volunteer panel was constituted of 3 healthy women and 3 healthy men. *Stratum corneum* from forehead corresponding at 40 cm^2 were collected with cotton swabs swell by soft detergent solution. Proteins from swab were extracted with chaotropic buffer, containing one step reduction/alkylation reagents and sequentially boiled and sonicated. Denatured proteins were sequentially digested by LysC and trypsin. Each peptide digest was labeled by TMT isobaric tag and combined to the others. The combined extract is then separated into 8 fractions. **Mass spectrometry**: Each fraction (500 ng) was trapped onto precolumn cartridge in backflush mode with PepMap Easy-spray 50 cm RSLC using Ultimate 3000 RSLC. Peptides were separated using linear gradient of acetonitrile 0.1 % formic acid at a flow rate of 300nL/min. Mass spectra were acquired on a Q Exactive Plus throughout the chromatographic run (200 minutes), FTMS scans at 70000 resolution following each FTMS scan HCD was carried out on 10 of the most intense ions from each FTMS scan then put on a dynamic exclusion list for 25secs (10 ppm m/z window).

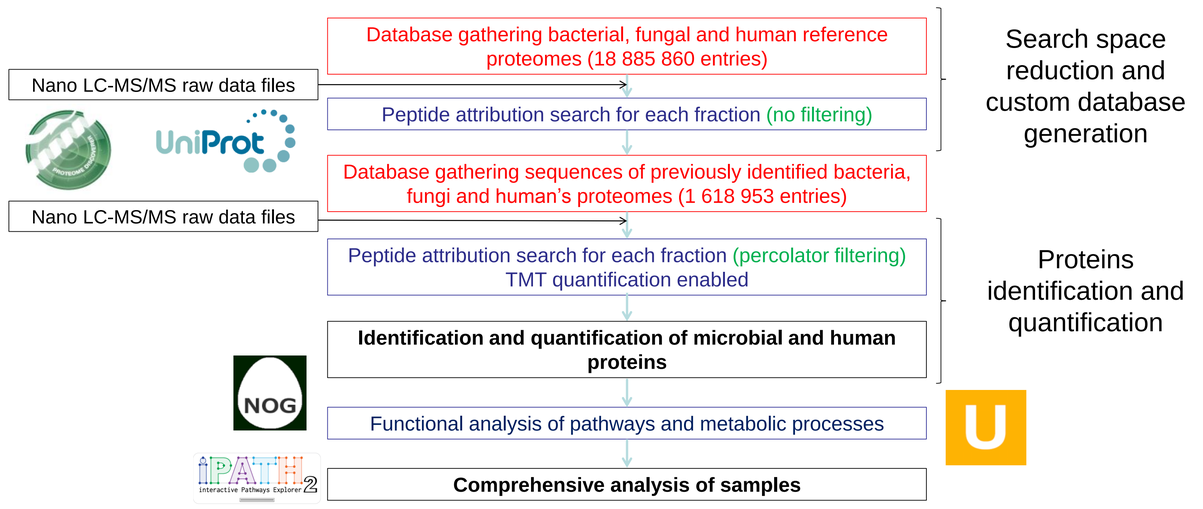

FIGURE 2. Schematic workflow of data processing: Data analysis: Mass spectrometry results (24 files) were analyzed by Proteome Discoverer 2.1 using 2 iterative steps: first step was identification only against all bacteria, fungi and human reference proteomes mined from UniProt KB (18 885 860 entries) with no filter applied in order to create a custom Fasta file for reducing search space (1 618 953 entries). This custom database was used for identification and reporter quantification with quality filter criteria (Percolator FDR calculation). For statistical analysis of results, normalized and scaled reporter abundances were used to perform PCA analysis (Pareto scaling) and t-test to evaluate fold changes' statistical significance between groups. **Functional analysis**: Taxonomic and functional annotations were performed using our customize pipeline MicroXplore. This pipeline combines taxonomic classification of the peptide sequences by Unipept, Functional annotation using Eggnog and iPath to map proteins into metabolic pathways.

Results

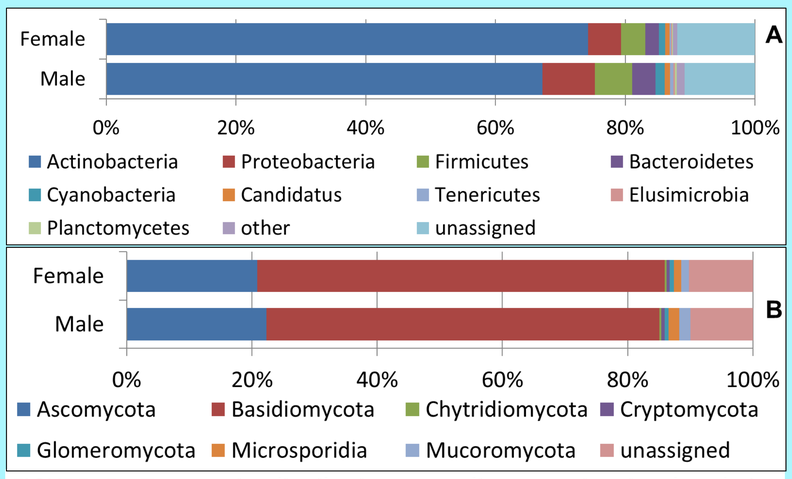

FIGURE 5. Taxonomic distribution according protein signal: relative abundance of each taxon (namely phylum) is represented as the sum of reporter ions for all the unique peptides found with Unipept. A bacterial phylum B fungi phylum.

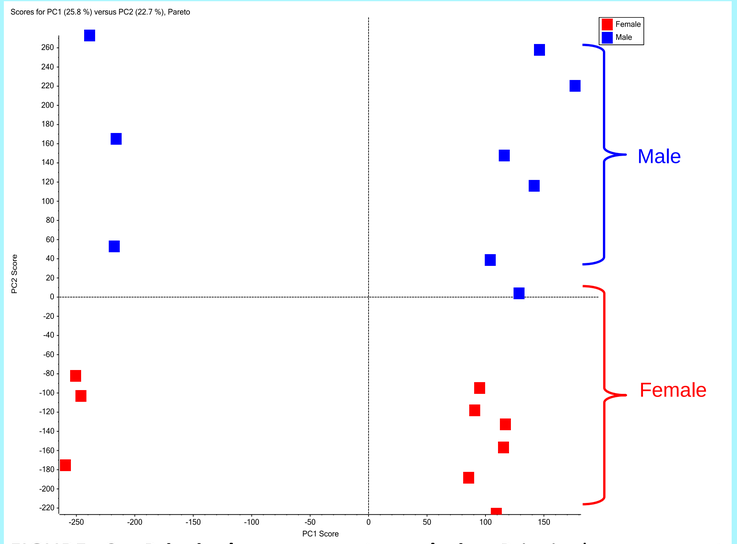

FIGURE 3. Principal component analysis: Principal component analysis showed panel could be separated depending on gender, thus relative quantitation between male and female are performed.

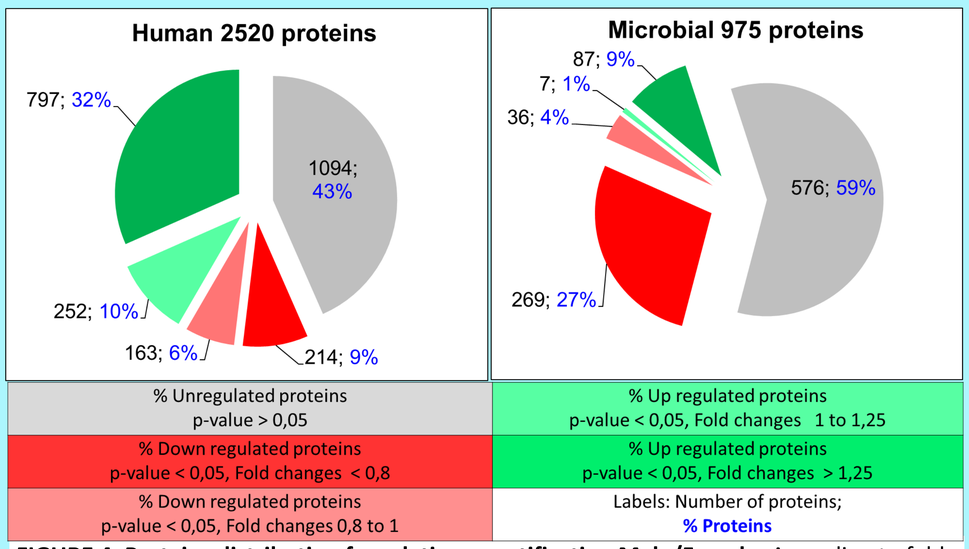

FIGURE 4. Proteins distribution for relative quantification Male/Female: According to fold changes and p-values, statistical variation of abundances was shown for 1011 human proteins and 356 microbial proteins (including fungi and bacteria).

Accession	Description	Ratio: (M) / (F)
Lipid degradation Human proteins		
P49748	Very long-chain specific acyl-CoA dehydrogenase	0,85
P42765	3-ketoacyl-CoA thiolase, mitochondrial	0,62
P33121	Long-chain-fatty-acid--CoA ligase 1	0,74
P11310	Medium-chain specific acyl-CoA dehydrogenase	0,69
Q9NZ01	Very-long-chain enoyl-CoA reductase	0,9
Lipid synthesis Bacteria proteins		
D4HAF0	Methylmalonyl-CoA carboxyltransferase 12S subunit *P.acnes*	0,55
E6D5I9	Methylmalonyl-CoA epimerase *P.acnes*	0,61
F1UEF3	Methylmalonyl-CoA carboxyltransferase 1.3S subunit *P.acnes*	0,53
D1YD03	Acyl carrier protein *P.acnes*	0,35
E4HHT9	Methylmalonyl-CoA mutase, small subunit *P. humerusii*	0,69
D1YBL3	Methylmalonyl-CoA mutase large subunit *P.acnes*	0,65
A0A0E1YIU8	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) *P.acnes*	0,66
D4HDE4	Acyl transferase domain protein *P.acnes*	0,58
D1YD01	Acyl transferase domain protein *P.acnes*	0,64
E4HJA7	Acyl-CoA dehydrogenase, C-terminal domain protein *P. humerusii*	0,74
E4HGK7	Uncharacterized protein *P. humerusii*	0,53

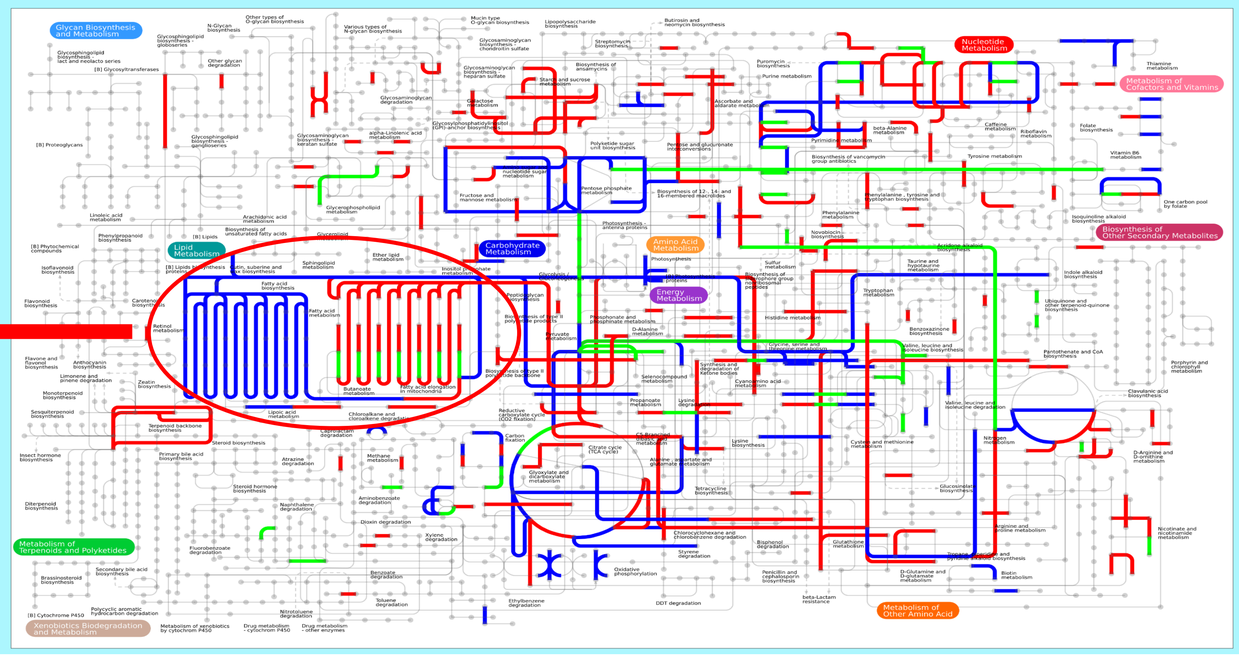

FIGURE 6. regulated metabolic pathway analysis of skin metaproteome: Left: Regulated proteins involved in lipid metabolism, an up-regulation of bacterial lipid synthesis and human lipid degradation was observed for female group. Right: distribution of the regulated proteins belonging to the Bacteria super kingdom (in blue), human species (in red) and overlapping regulations (in green) into metabolic pathways using iPATHv2. Study of this metabolic map shows bacterial human interplay for lipid metabolism

Conclusion

•This method **based on proteins** with **non-invasive sampling** allows to **reach the functions** in charge of cell working which cannot be done with genomics approaches. It allowed to overcome the challenging dynamic range of stratum corneum and to quantify more than 2500 human proteins and near 1000 microbial proteins.

• The taxonomic profile of forehead skin is largely dominated by phylum *Actinobacteria* for bacteria super-kingdom and Basidiomycota for fungi kingdom. We also observed slight variations of taxonomic profile between male and female, with for instance increased actinobacteria signal in female group. The functional analysis of regulated proteins also shows an interplay between bacteria and human in lipid metabolism.

•Microbial associated dermatological mechanisms is an important topic to study to improve health care consumers and dermatological patients management. Obtaining a higher knowledge on various interactions between microbial communities and their host is essential to improve next generation's care products and patient treatment. As a result, this proof of concept study shows that mass spectrometry based proteomics is **already a powerful** and probably up to now **unique tool** able to understand skin diseases for fundamental and applied research, and also to evaluate ingredients or cosmetics products effects **at skin and microbiota level simultaneously.**

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