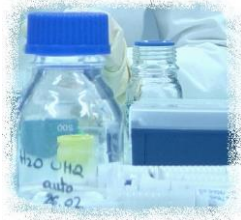


MICROBIOME ACTIVE INGREDIENT STUDY

- 1 - FOREWORD
- 2 - WORKFLOWS
- 3 - LC-MS/MS PROTEOMICS OUTPUT
- 4 - METAGENOMICS OUTPUT
- 5 - CLAIMING POTENTIAL



1-FOREWORD

Principle

A quantitative comparison between ingredient treated samples vs placebo treated samples

Why a new approach based on proteins:

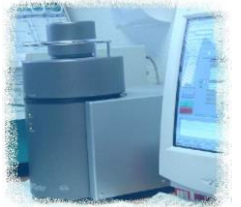
Proteins are the molecules directly in charge of cell functions and structures, nucleic acid are not. High resolution LC-MS/MS allows to identify and quantify thousands of proteins. Huge amounts of data are generated by high resolution LC-MS/MS and each protein has to be included in the bioinformatics analysis in order to know its impact in a potential effect.

Output: Events occurring on biological processes, molecular functions and cellular components

Why a new approach based on DNAs:

Metagenomics refers to the study of genomic DNA obtained from microorganisms that cannot be cultured in the laboratory. This represents the vast majority of terrestrial microorganisms. By amplicon sequencing, an informative marker—such as the 16S rRNA gene—is amplified by polymerase chain reaction (PCR) and sequenced

Output: Abundance, diversity and identity of microbes in a sample. Taxonomic composition and phylogenetic structure of a microbial community expressed as OTUs (Operational taxonomic units)



2-WORKFLOWS

DNA: extraction-purification, amplification, library preparation, sequencing, data processing

Moist, sebaceous areas swabs:
retroauricular crease, antecubital
fossa, anterior nares, forehead..

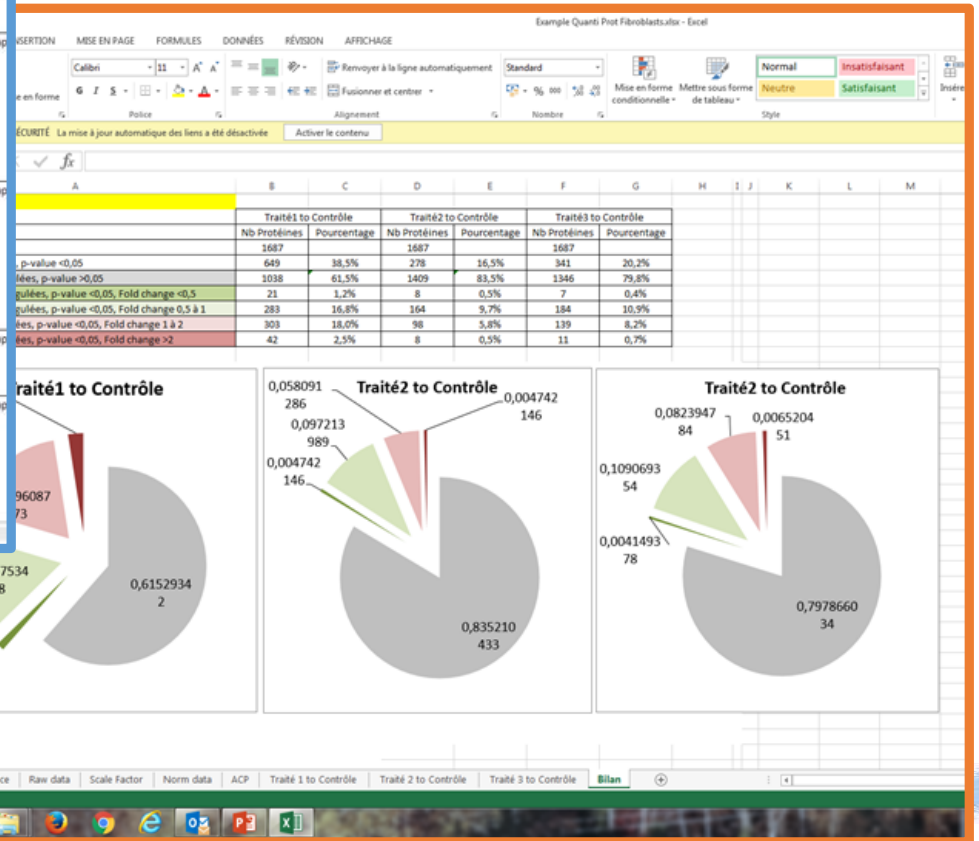


Proteins: extraction, reduction, alkylation, digestion, nanoLC-MS/MS, data processing



3-LC-MS/MS PROTEOMICS OUTPUT

Use	t-value	p-value	Fold Change	Log (Fold Change)	Entry	Entry name	Protein names	Gene names	Organism
True	9,40242679	0,0007	1,11	0,047159143	Q09666	AHNK_HUMAN	Neuroblast differentiation-associated protein AHNAK (Desmoyokine)	AHNAK PM227	Homo sap
True	2,92439754	0,0808	1,20	0,080225376	Q15149	PLEC_HUMAN	Plectin (PCN) (PLTN) (Hemidesmosomal protein 1) (HD1) (Plectin-1)	PLEC PLEC1	Homo sap
True	5,47351411	0,0054	1,04	0,016029271	P21333	FLNA_HUMAN	Filamin-A (FLN-A) (Actin-binding protein 280) (ABP-280) (Alpha-filamin) (Endothelial actin-binding protein) (Filamin-1) (Non-muscle filamin)	FLNA FLN FLN1	Homo sap
True	-1,92286258	0,1269	0,97	-0,012705877	P35579	MYH9_HUMAN	Myosin-9 (Cellular myosin heavy chain, type A) (Myosin heavy chain 9) (Myosin heavy chain, non-muscle IIA) (Non-muscle myosin heavy chain A) (NMMHC-A) (Non-muscle myosin heavy chain IIA) (NMMHC II-a) (NMMHC-IIA)	MYH9	Homo sap
True	2,97787474	0,0408	1,06	0,023561103	Q14204	DYH1C1_HUMAN	Cytoplasmic dynein 1 heavy chain 1 (Cytoplasmic dynein heavy chain 1) (Dynein heavy chain, cytosolic)	DYH1C1 DHCL1 DNCH1 DNCL DNECL DYHC KIAA0325	Homo sap
True	4,25609507	0,0131	1,05	0,023020182	P49327	FASN_HUMAN	Fatty acid synthase (EC 2.3.1.85) [Includes: [Acyl-carrier-protein] 5-acetyltransferase (EC 2.3.1.88); [Acyl-carrier-protein] 5-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Oleoyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	FASN FAS	Homo sap



Thousands of proteins identification and relative quantification between treated sample and control sample (Id, Fold Change, p-value)

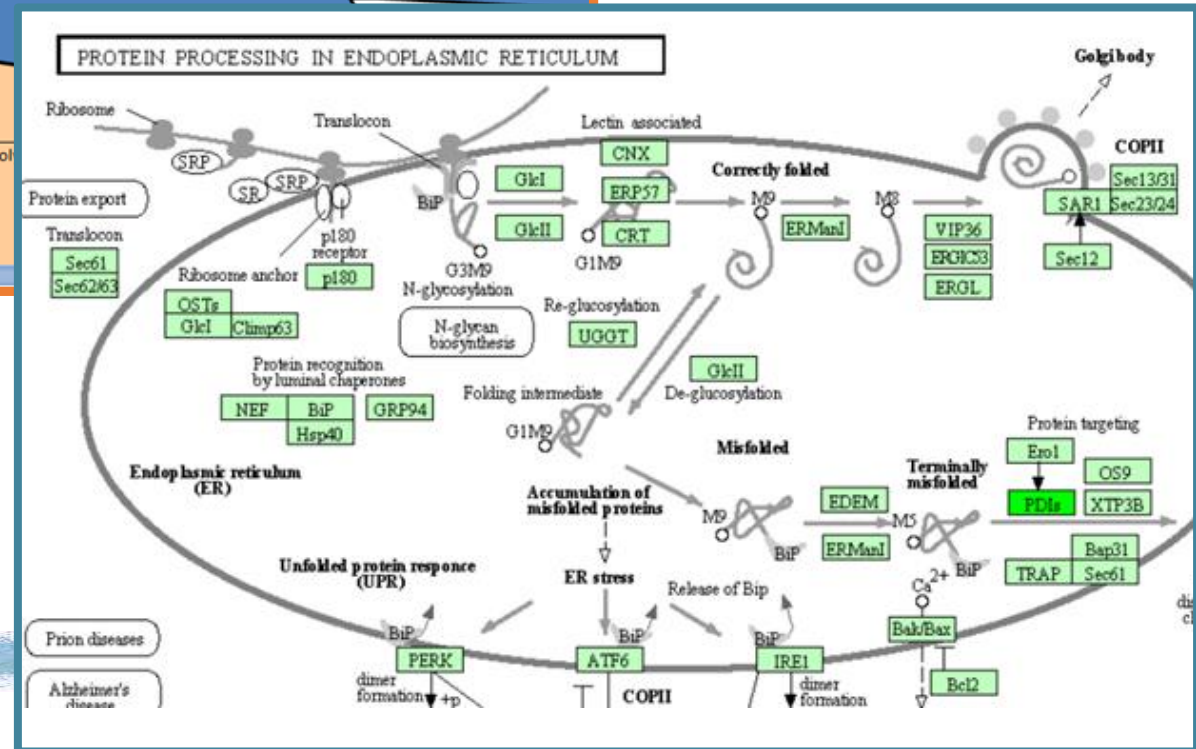


3-LC-MS/MS PROTEOMICS OUTPUT

L	M	N	O	P	Q
Delt	p-value	Fold Change	Protein names	Log (Fold Change)	Entry
07	2,58E+07	0,0146	1,45	0,16	D2JYH5
06	1,50E+08	0,0001	2,25	0,35	D3DTX7
07	2,66E+08	0,0000	2,17	0,34	P02452
			Collagen, type III, alpha 1		
			Collagen, type I, alpha 1, isoform CRA_a		
			Collagen alpha-1(I) chain (Alpha-1 type I collagen)		
05	1,57E+06	0,0474	1,12		
			Gelsolin (AGEL) (Actin-depol		

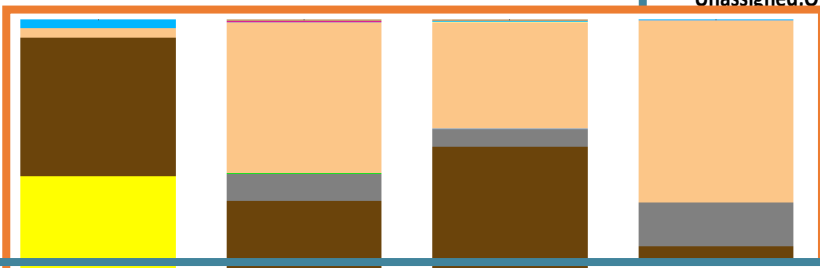
1287 identified proteins, fold change, p-value

Events occurring on biological processes, molecular functions and cellular components

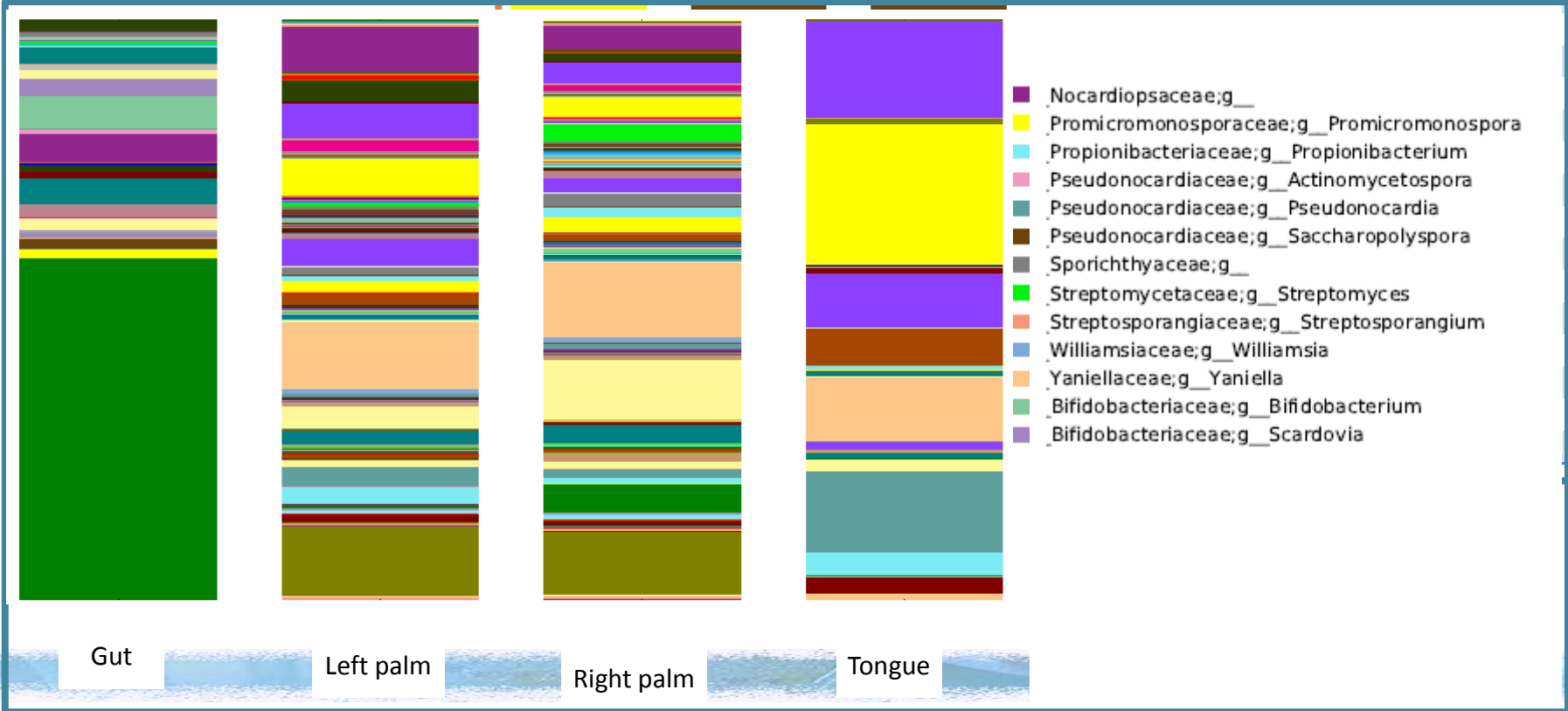


4-METAGENOMICS OUTPUT

Percentages at phylum and genus level



Phylum	Sample type			
	gut	left palm	right palm	tongue
Unassigned:Other	0,01	0,04	0,05	0,02
	0,00	0,02	0,03	0,00
	0,00	0,04	0,08	0,00
	0,04	15,79	15,00	4,03
etes	0,00	0,00	0,01	0,00
	63,39	10,98	11,92	20,39
	0,00	0,04	0,04	0,00
	0,01	2,14	3,21	0,91
			0,00	0,00
			40,08	21,70
			4,14	10,35
			0,00	0,00
			0,02	0,00
			24,89	42,56
			0,02	0,01
			0,09	0,01
			0,08	0,01
			0,00	0,01
			0,07	0,00
			0,16	0,00
			0,08	0,00

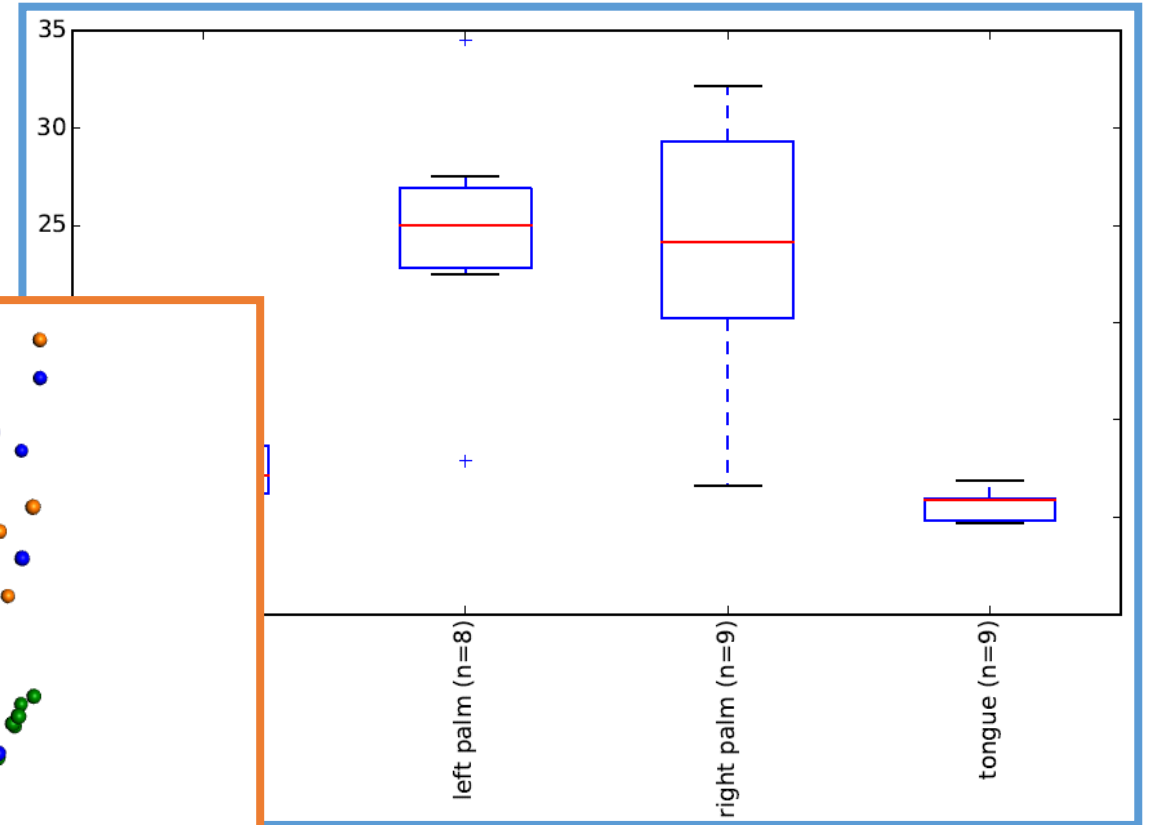
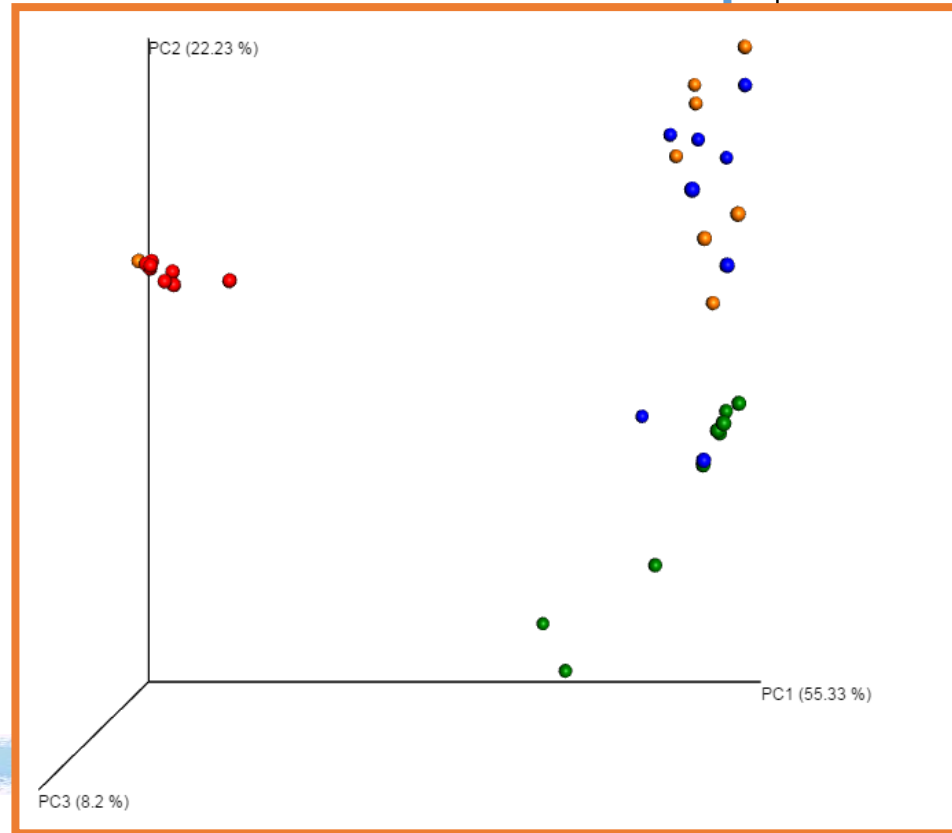


- _Nocardiopsaceae;g_
- _Promicromonosporaceae;g_Promicromonospora
- _Propionibacteriaceae;g_Propionibacterium
- _Pseudonocardiaceae;g_Actinomycetospora
- _Pseudonocardiaceae;g_Pseudonocardia
- _Pseudonocardiaceae;g_Saccharopolyspora
- _Sporichthyaceae;g_
- _Streptomycetaceae;g_Streptomyces
- _Streptosporangiaceae;g_Streptosporangium
- _Williamsiaceae;g_Williamsia
- _Yaniellaceae;g_Yaniella
- _Bifidobacteriaceae;g_Bifidobacterium
- _Bifidobacteriaceae;g_Scardovia

Gut Left palm Right palm Tongue

4-METAGENOMICS OUTPUT

Intra sample and inter samples diversity



5- CLAIMING POTENTIAL

Microbiome preservation

Microbiome diversity enhancement

Pathogen reduction

Metabolic host-bacteria interactions : sensitization and inflammation

Pathogenic and virulence mechanisms management

Skin diseases pathogeny

