Proteomes of gut commensal bacterial extracellular vesicles (BEVs):

Search for consistent patterns in extensive heterogenicity

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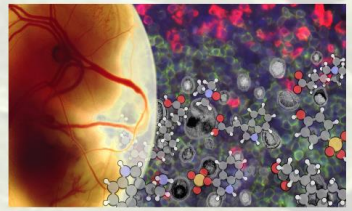
Effects of maternal gut microbiota on fetal immune system: competence, tolerance, etc.



Gut commensal bacteria produce a myriad of metabolites, proteins and extracellular vesicles (BEVs).

BEVs are loaded with proteins, polysaccharides, nucleic acids, and metabolites and can act as mediators in a variety of physiological dialogues affecting intestinal epithelium homeostasis, immune resistance and tolerance, metabolic processes, etc. BEVs can enter the mucin layer, directly interact with epithelial cells, and bind their pattern recognition receptors, thereby modulating immune responses.

May gut microbiota-derived BEVs be present in diverse biological fluids of a host organism, *including blood plasma? Amniotic fluid? Be transferred across placenta to a fetus?*

??? Are there any available instruments to detect BEVs in host tissues ???

Principal goal of the project: ***to analyse BEV proteomes originating from a list of taxonomically distant common bacterial species of mammalian gut microbiota to establish universal conservative epitope sequences for antibody elaboration***

Wet lab

Taxonomically distant species highly abundant in mammalian gut bacteriome: *Akkermansia*, *Bacteroides*, *Enterobacter* etc.

In vitro culturing

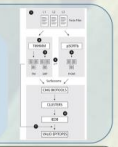
BEV purification

MS-based proteome characterisation

Dry lab

EpitoCore: a bioinformatic strategy that integrates surfaceome and subcellular localization prediction to pangenomic characterization, and further defines conserved epitopes in core proteins (Fiuza et al., 2020). *Front. Immunol.*

Prediction conservative epitopes for antibody elaboration



Statistical analysis

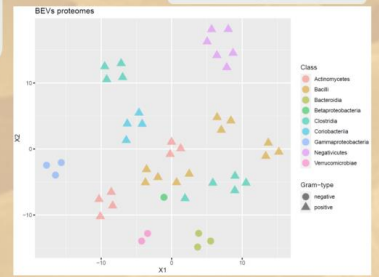
Protein alignment based on sequence, clustering based on domain structure, compositional proteome comparison, analysis correlations of proteome composition with taxonomy, metabolic characteristics, cell surface features, vesicle size, etc.

Revealing significant predictors for BEV proteome composition, most conservative proteins across bacterial species, most abundant proteins in BEV proteomes, etc.

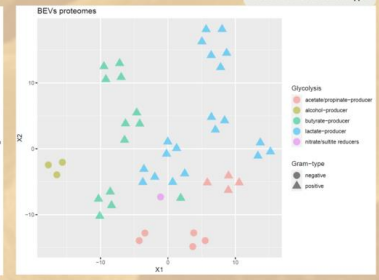
Result_1: no clear predictors

Proteomes vary strongly in terms of both composition and richness;
No detectable correlation with taxonomy, shape, size, etc.

t-SNE ordination
Based on taxonomy (class level)

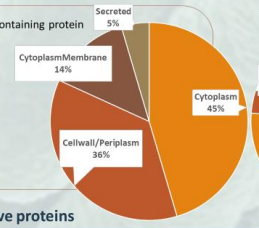


t-SNE ordination
Based on metabolism type

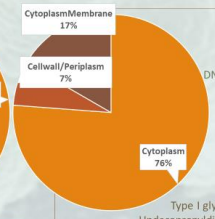


Adundant protein localization

Twin-arginine translocation signal domain-containing protein
CAMP factor family pore-forming toxin
ABC transporter
Solute-binding protein
Cell wall-binding repeat-containing protein
F0F1 ATP synthase subunit beta
ABC transporter substrate-binding protein
DUF3869 domain-containing protein
Porin OmpA [Enterobacteriaceae]
SLAP domain-containing protein
Phosphopyruvate hydratase [Enterococcus]



Conservative protein localization



30S ribosomal protein S10
50S ribosomal protein L20
DEAD/DEAH box helicase
DNA-directed RNA polymerase subunit beta
recombinase RecA
Preprotein translocase subunit SecA
Signal peptidase I
Signal recognition particle protein
Translation initiation factor IF-2
Peptidylprolyl isomerase
Chaperonin GroEL #1
EF-Tu/IF-2/RF-3 family GTPase
Type I glyceraldehyde-3-phosphate dehydrogenase
Undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase phosphopyruvate hydratase
F0F1 ATP synthase subunit alpha

Result_2: there are conservative proteins

Among proteins most widely distributed across different proteomes ae mainly evolutionarily conservative ones with cytosolic localization (ribosomal proteins, translation initiation factors, chaperons). These widely distributed proteins often were not the most abundant ones.
In general, the most abundant in BEV proteins tended to be different between species, and usually associated with cell surface (ABC transporters, porins, etc.)