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Proteomics and pig intestinal research: love at first sight

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Introduction:

Intestinal health promotion is essential in **pig** industry to maximize **production** and keep public health. Furthermore, pigs are ideal **biomodels** for human intestinal research. Hence, there is a wide interest in increasing the knowledge in intestinal pathophysiology in this species. Recently, we have applied proteomics to compare and complete the genomic, transcriptomic and immunologic data repository that we have obtained while investigating the host response to different challenges. Here we describe the proteomic response of the pig small intestine to a transient challenge with the enteropathogen enterotoxigenic *Escherichia coli* (**ETEC**) as explored with **UPLC-HDMS^E**.

Material and Methods:

Two groups (n=4) of weaned piglets were orally challenged with PSB (control) or a single dose of ETEC (5x10⁹ CFU). After 24h, animals were euthanized and the absence of ETEC in the intestine was checked. Mucosal scrapings were taken and proteins were then extracted, quantified, reduced, alkylated, trypsin-digested, purified and spiked with MassPREP Digest Standards 1 internal standards. Peptides were separated in a **nanoAcquity UPLC** system and sprayed into the **HDMS^E SYNAPT G2 mass spectrometer**. Spectral data processing, protein identification (SwissProtKB *Sus Scrofa* database), quantification and statistics (threshold: 1.3-fold \pm 0.3 natural log scale) were performed with **ProteinLynx Globalserver v2.3**. Functional annotation was defined for uncharacterized proteins with a combination of BLASTp, Gene Ontology annotation and literature search. Pig proteins were mapped to human orthologs by identifying reciprocal-best-BLAST hits. Then, protein IDs were uploaded into different systems biology tools such as **PantherDB, String, Ingenuity Pathway Analysis and InnateDB**.

Results and discussion:

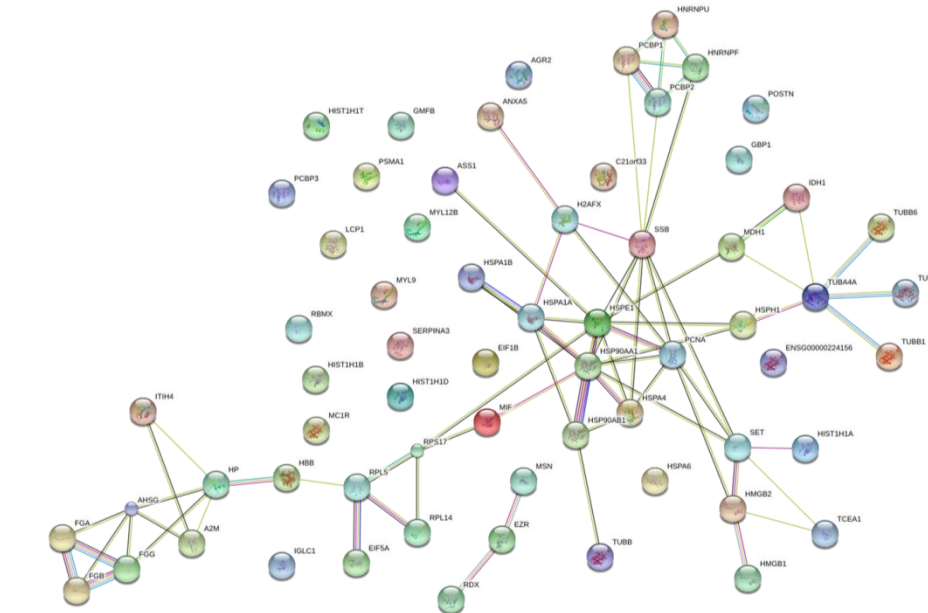
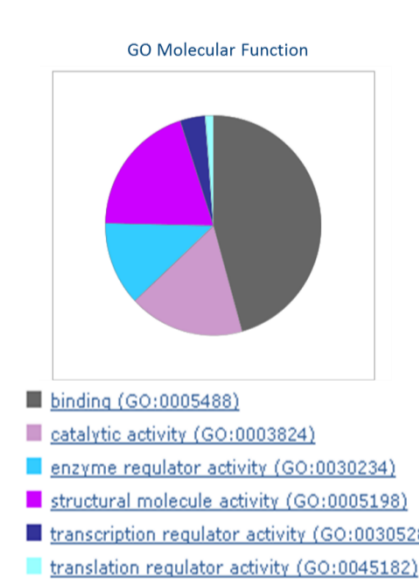
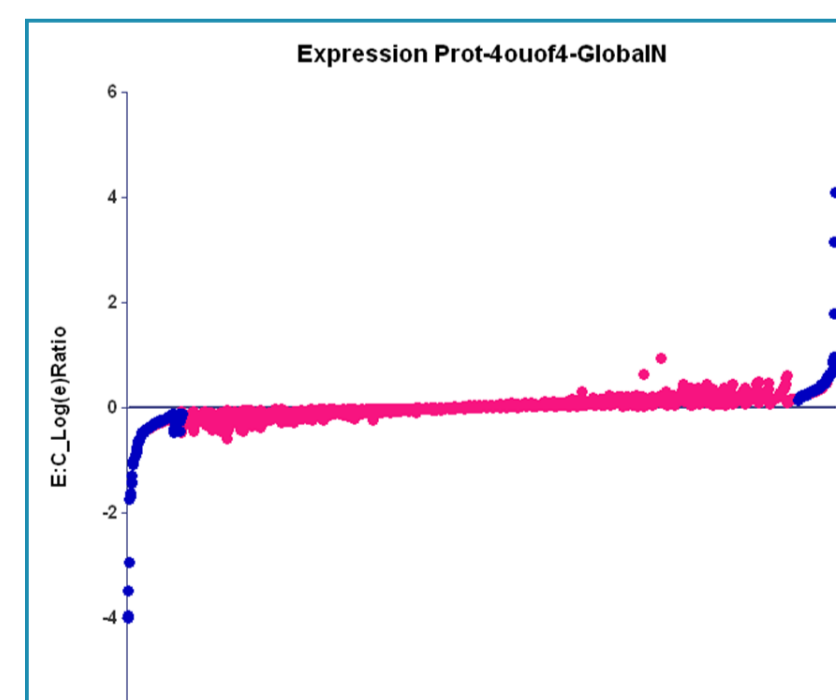
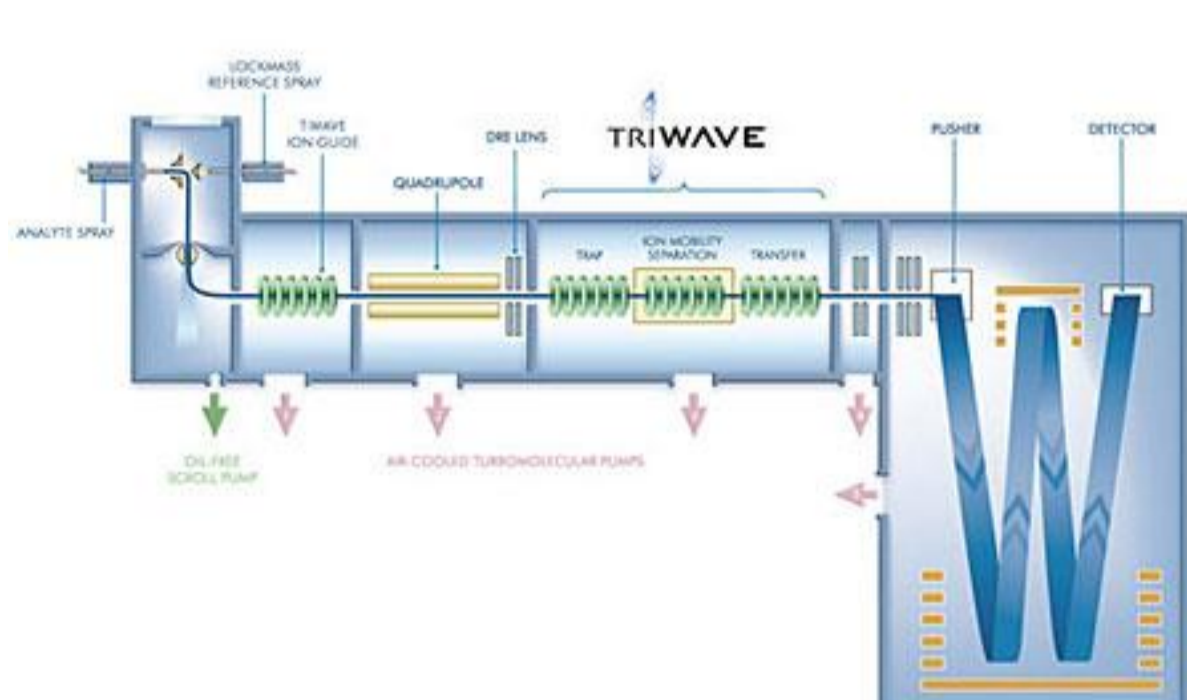
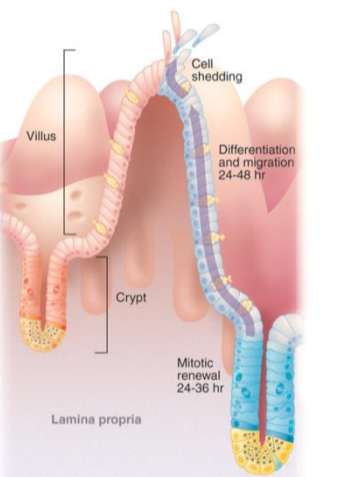
More than 1500 proteins were identified per sample, of which 124 and 101 were down- and up-regulated, respectively. In agreement with previous DNA microarray and qPCR results, systems biology analysis of results indicated that the intestinal response to a transient challenge with ETEC is characterized by the following events:

-An activation of the **crypt cycle**: increased cell apoptosis and cell shedding together with enhanced proliferation at the crypts and cell differentiation/mobility along the villi.

-Activation of the **innate immune response**.

-Activation of **a local antimicrobial response**: leukocytes and enterocytes seem to be involved in the production and secretion of different antimicrobial proteins, some of them not well explored in pigs.

-Possible **transmigration of leukocytes** to the intestinal lumen as a consequence of ETEC non invasive infection.



Conclusions and future studies:

We found that proteomics is a highly valuable tool to explore host responses alone or in combination with other -omics platforms. In the present study, we identified the cellular events related with an uncomplicated ETEC infection, which can be useful in search for new preventive or therapeutic intervention pathways. Furthermore, we identified a local antimicrobial response relatively unexplored in the pig small intestine that seems to play a pivotal role as a first line of defense. Additionally, different proteins were strongly regulated under the studied conditions, which could be considered as candidate intestinal health biomarkers. The present results will be verified by mRNA expression and gel-based proteomics complementary approaches, and further validated in alternative infection models in the future.