

INTRODUCTION

- Most Common Food borne illness are often caused by pathogenic *Escherichia coli* strains
- Certain *E. coli* strains are drug-resistant while others are not!
- *E. coli* Strain differentiation is vital for the development of effective medical countermeasure
- Non-O157:H7 *E. coli* strains, O111, O145, O26, O103, O45 and O121 referred as big Six group reported to be involved in the recent outbreaks
- We have applied isobaric tag-based TMT labeling combined with high-resolution Fourier transform mass spectrometry to study the secreted and extra cellular proteome of these *E. coli* big six strains.

METHODS

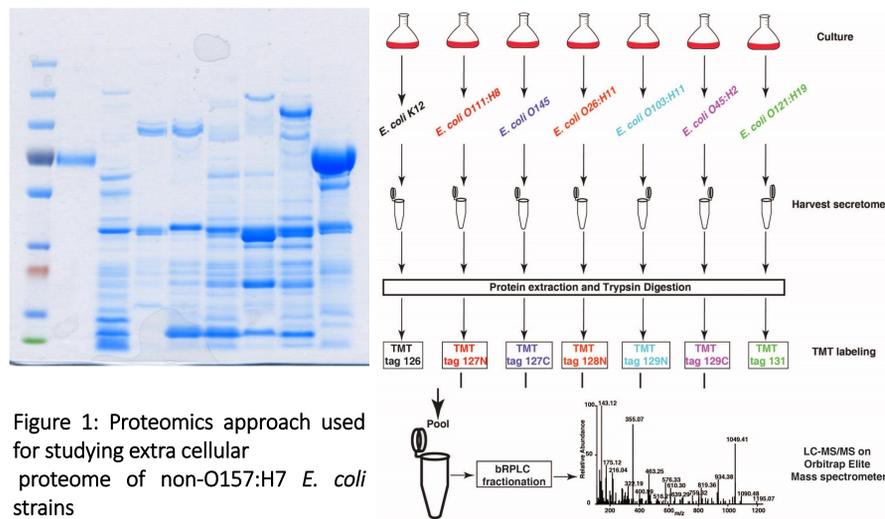
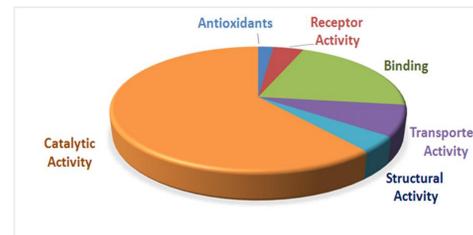


Figure 1: Proteomics approach used for studying extra cellular proteome of non-O157:H7 *E. coli* strains

A single colony of each *E. coli* strain was inoculated in a pre LB medium and was allowed to grow over night until the OD600 reaches to 1.0. The pre-inoculum was sub-cultured in a 250 ml of DMEM medium and was agitated at 180 rpm at 37°C until the OD 600 reaches to 0.8. The culture was centrifuged at 2500 rpm for about 10 min to pellet down the bacterial cells and the supernatant, secretome was filtered through 0.22 um (Millipore filter) to get rid of any bacteria and the filtrate was further concentrated using 3 kDa cut-off filters (Millipore) to collect the secreted proteins. Protein amount was measured using BCA assay. and stored in -80 deg freezer until the further analysis. Equal amounts of protein 100 ug from each condition was reduced and alkylated using 5mM DTT and 10mM iodoacetamide. The samples were then subjected to the FASP and digested In-solution using trypsin. Tandem mass tag labeling (TMT) was carried out as per the manufacturer instructions with minor modifications. Labeled peptides were pooled and fractionated using basic reverse-phased liquid chromatography (bRPLC) and the fractions were concatenated and analyzed on LTQ-Orbitrap Elite mass spectrometer.

Approved for Public Release

Molecular Function



Cellular component

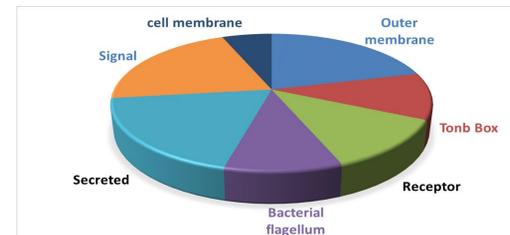


Figure 2: Gene Ontology analysis representing the distribution of extracellular proteome of non O157:H7 *E. coli* strains

Protein description	O111:H8 /K12	O145/K12	O26:H11 /K12	O103:H1 1/K12	O45:H 2/K12	O121:H1 9K12	Function
Secreted protein EspA	0.3	0.5	0.6	0.6	22.4	1.9	EspA is a core member of type III secretion system. This protein is essential for attaching and effacing lesion formation
Secreted protein B	0.6	0.8	3.0	9.3	21.0	0.8	This protein known to be involved in EPEC pathogenesis
T3SS effector protein NieH	0.2	0.3	0.7	0.4	5.5	0.4	A member of the type III secretion system and plays a role in injection of effector proteins into host cells
EspP	0.5	1.6	5.7	8.9	0.7	4.5	A member of type IV secretion system and is an auto transporter domain containing protein
Universal stress protein UP12	0.02	0.4	0.05	0.04	0.05	0.03	Member of universal stress protein and is been shown as overexpressed during growth inhibitory conditions that are induced by heat shock
IutA ferric aerobactin receptor protein	11.9	4.5	3.1	1.4	0.5	0.7	TonB dependent ligand gated channel protein. Known to involve in <i>E. coli</i> colonization and infection
Colicin I receptor	0.3	0.3	0.2	0.1	0.4	2.1	Colicin Ia is a channel forming bactericidal protein and it uses the outermembrane protein

Table 1: Shows a partial list of differentially regulated proteins in non O157:H7 *E. coli* strains

- From this analysis, we identified a total of 1,223 proteins across non O157:H7 *E. coli* strains using TMT based quantitative proteomic approach
- We identified more than 200 proteins with signal sequences as predicted by SignalP across the big six strains
- We also identified several known T3SS secretory proteins. In addition, we have identified several hypothetical proteins as secreted and extra cellular proteins and these could be likely targets for studying *E. coli* pathogenicity
- Quantitative proteomic approach demonstrated strain level differentiation of several proteins across the studied *E. coli* strains
- Gene Ontology analysis revealed several identified proteins with catalytic activity, receptor and binding activity

RESULTS

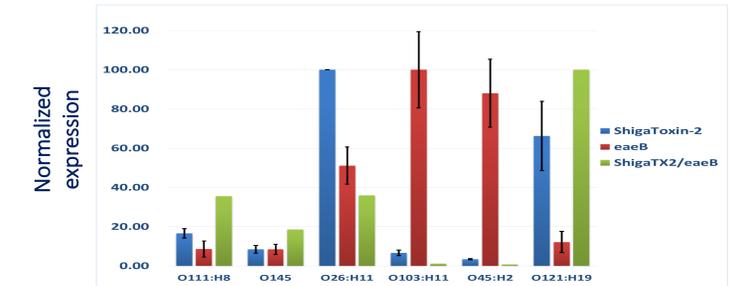
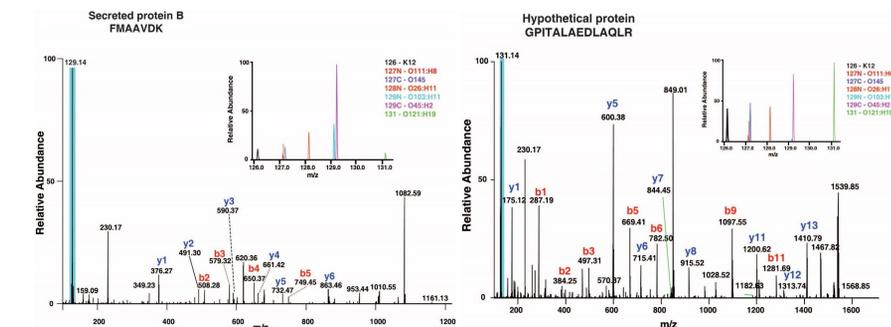
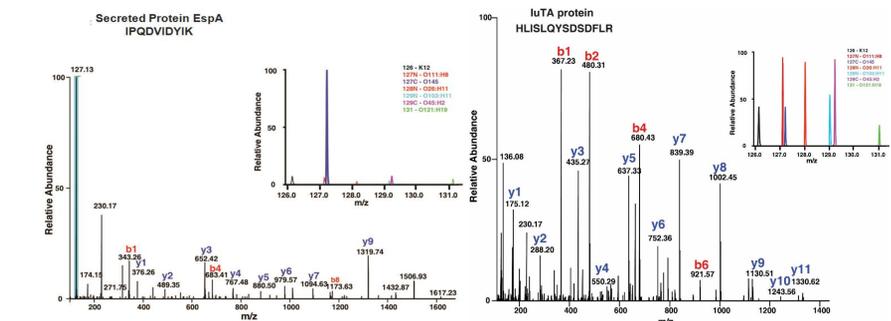


Figure 3: Relative expression of Shiga toxin and EaeB proteins across non O157:H7 *E. coli* strains



Representative MS/MS spectra of identified proteins from Secreted protein B and Hypothetical protein



Representative MS/MS spectra of identified secreted proteins EspA and IuTA

CONCLUSIONS

- We demonstrated quantitative proteomic analysis of secreted and extracellular proteome of non O157:H7 shiga toxin producing *E. coli* strains. Our study is the comprehensive quantitative TMT based approach for studying big six *E. coli* strain
- We identified several known secreted proteins and hypothetical proteins across the big six *E. coli* strains
- Unique 7 plex TMT based quantitative proteomic approach coupled with high resolution Fourier transform mass spectrometry enabled us to identify 1,200 proteins across the *E. coli* strains.
- Our results would enable researchers to understand the pathogenicity and would enable strain level differentiation for the development of effective medical countermeasures
- Our proteomic data could also be used to develop targeted MRM assays for various secreted and virulence factors