

# Complexome dynamics during Type III protein secretion

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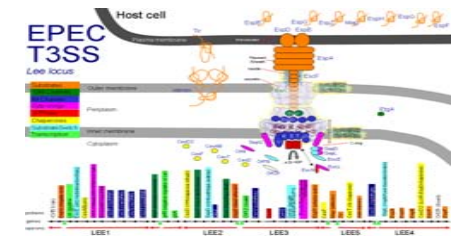
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## Introduction

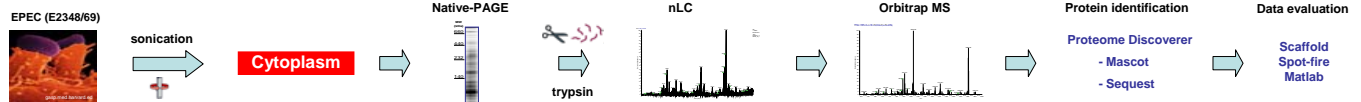
- The type III secretion system (T3SS) is a highly specialized bacterial protein secretion pathway.
- T3SS plays an essential role in the pathogenesis of many Gram-negative bacteria including *Yersinia*, *Shigella*, *Salmonella*, *Pseudomonas*, and *Enteropathogenic E. coli* (EPEC).
- A pathogenicity island called the locus of enterocyte effacement (LEE) encodes 41 T3SS proteins which are used to inject both LEE and non-LEE effectors into the eukaryotic host cell, where these effectors modulate host key cellular processes [1, 2].
- Although a plethora of studies have expanded our knowledge on the structure and function of T3SS and its effectors, the precise mechanism of type III protein secretion and translocation and their co-ordination remain poorly understood.
- Towards a better understanding of these mechanism a comprehensive analysis of the protein complexes during type III protein secretion is required.

## LEE-proteins

- 4 transcription factors
- 5 chaperones
- 13 "injectisome" proteins
- 7 effectors
- 12 proteins with unknown function



## Experimental

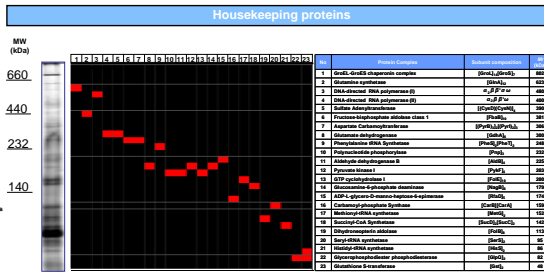
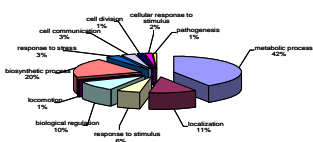


## Complexome analysis

1305 Identified proteins

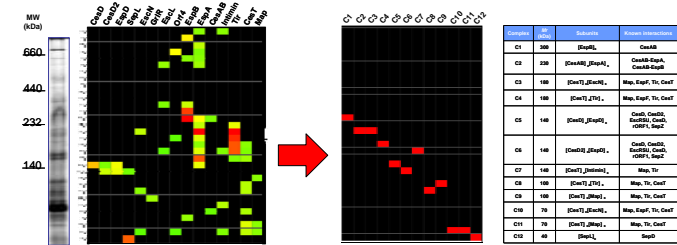


Biological Processes according to GO



More than 200 known protein complexes were identified in the present study with apparent MW in agreement with the bibliographic data. 23 selected known protein complexes are shown in the above figure and table.

T3SS proteins

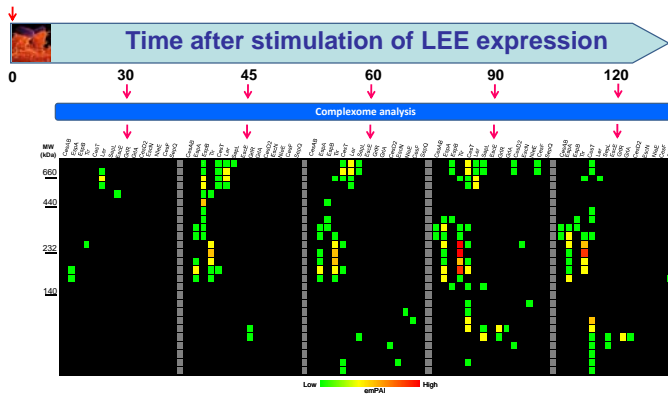


15 T3SS proteins were identified from the cytoplasm of EPEC after CN-PAGE.

12 putative protein complexes of T3SS proteins were determined.

## Complexome Kinetics

Stimulation of LEE expression



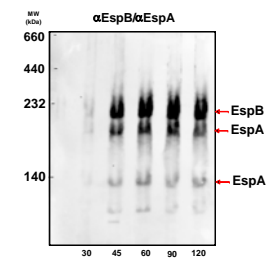
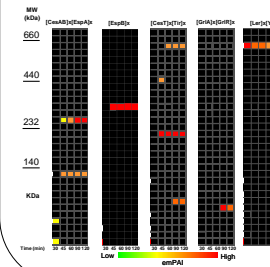
The differentiation of the T3SS-complexome during the early stages of Type III protein secretion was studied.

## Complexes Validation

I. Literature-Manual

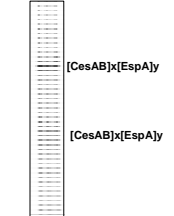
II. Western Blot

III. Affinity purification



Pull-down HisEspA

Native-PAGE



The identified protein complexes were validated using 3 separate approaches: Manual validation using literature data, western blot analysis using T3SS-antibodies, and affinity purification under native conditions of the protein complexes using His-tagged T3SS-proteins.

## Literature

- Iguchi A. et al. *J. Bacteriology*, 191, 347-354 (2009)
- Dean P. & Kenny B. *Current Opinion in Microbiology*, 12:101-109 (2009)

## Acknowledgements

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