

# Global and targeted complexome analysis of the cytosolic proteome of Enteropathogenic *Escherichia coli* using Native-PAGE combined with nLC-LTQ Orbitrap MS

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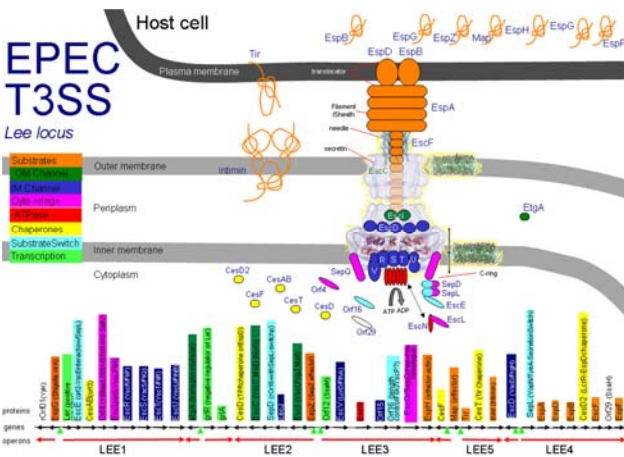
## 1. Overview - Introduction

### Overview

- Global and targeted (T3SS) complexome analysis of EPEC was performed in an attempt to identify putative functional protein complexes.
- ~1300 polypeptides were identified participating in >200 known and putative cytosolic protein complexes.
- 15/54 predicted T3SS related proteins were identified.

### Introduction

- The molecular pathogenesis of the Enteropathogenic *Escherichia coli* (EPEC) is still poorly understood.
- The type III secretion system (T3SS) is a central element of the pathogenesis mechanism.
- A pathogenicity island called the locus of enterocyte effacement (LEE) encodes 41 T3SS proteins [1, 2]. Another 13 T3SS proteins are encoded in non-LEE loci [2].
- We seek to elucidate the EPEC complexome especially within T3SS proteins.



### Bibliography

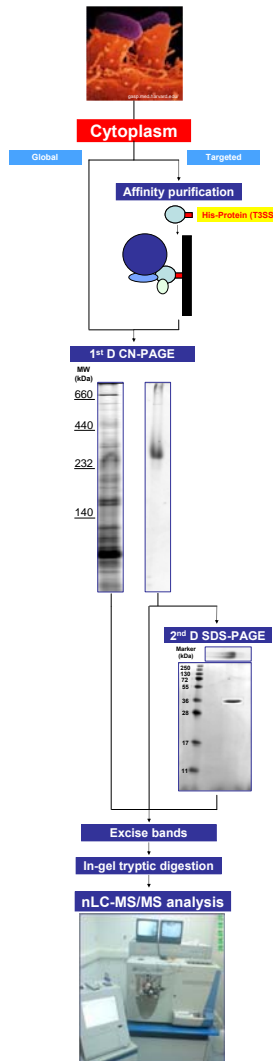
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### Acknowledgements

The research leading to these results has received funding from the Greek General Secretariat of Research and the European Regional Development Fund (PENED03ED623). The proteomics facility of the IMBB was established through the European Community's Seventh Framework Programme agreement 229823 Capacities-FP7-REGPOT-2008-1/ project «ProFI».

## 2. Methods

### EPEC (E2348/69)



### Protein identification / characterization

Proteome Discoverer 1.1-Seqest (Thermo Scientific)  
Mascot server 2.2.0 (Matrix Science)

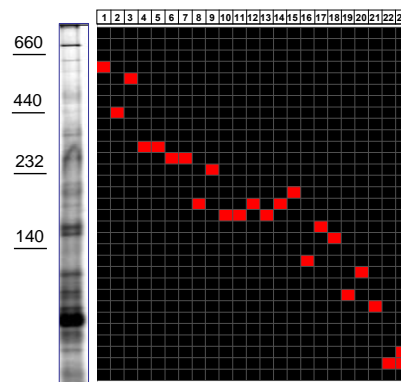
### Data evaluation / Complexome analysis

Scaffold 3.0 (Proteomics Software)  
Spotfire DecisionSite 8.0 (TIBCO)

## 3. Results - Conclusions

### Global Complexome

#### Identified known Protein Complexes-Proof of Principle



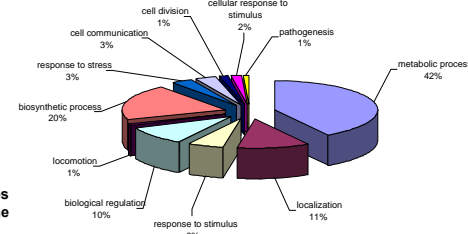
| No | Protein Complex                           | Subunit composition          | #P (kDa) |
|----|---|------------------------------|----------|
| 1  | GroEL-GroES chaperonin complex            | [GroL]14[GroS]7              | 802      |
| 2  | Glutamine synthetase                      | [GlnA]12                     | 623      |
| 3  | DNA-directed RNA polymerase (I)           | $\alpha 2\beta\beta' \omega$ | 480      |
| 4  | DNA-directed RNA polymerase (II)          | $\alpha 2\beta\beta' \omega$ | 400      |
| 5  | Sulfate Adenylyltransferase               | [(CysD)(CysN)]4              | 390      |
| 6  | Fructose-bisphosphate aldolase class 1    | [FbaB]10                     | 381      |
| 7  | Aspartate Carbamoyltransferase            | [(PyrB)2](PyrI)23            | 306      |
| 8  | Glutamate dehydrogenase                   | [GdhA]6                      | 300      |
| 9  | Phenylalanine RNA Synthetase              | [PheS]2[PheT]2               | 248      |
| 10 | Polynucleotide phosphorylase              | [Pnp]3                       | 232      |
| 11 | Aldehyde dehydrogenase B                  | [AldB]4                      | 226      |
| 12 | Pyruvate kinase I                         | [PyrK]4                      | 203      |
| 13 | GTP cyclohydrolase I                      | [FolE]10                     | 200      |
| 14 | Glucosamine-6-phosphate deaminase         | [NagB]6                      | 179      |
| 15 | ADP-L-glycero-D-manno-heptose-6-epimerase | [RfaD]5                      | 174      |
| 16 | Carbamoyl-phosphate Synthase              | [CarB][CarA]                 | 159      |
| 17 | Methionyl-tRNA synthetase                 | [MetG]2                      | 152      |
| 18 | Succinyl-CoA Synthetase                   | [SucD]2[SucC]2               | 142      |
| 19 | Dihydropyrimidinase                       | [FolB]8                      | 113      |
| 20 | Seryl-tRNA synthetase                     | [SerS]2                      | 95       |
| 21 | Hisidyl-tRNA synthetase                   | [HisS]2                      | 86       |
| 22 | Glycerophosphodiester phosphodiesterase   | [GlpD]2                      | 82       |
| 23 | Glutathione S-transferase                 | [Gst]2                       | 48       |

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

#### 1305 Identified proteins

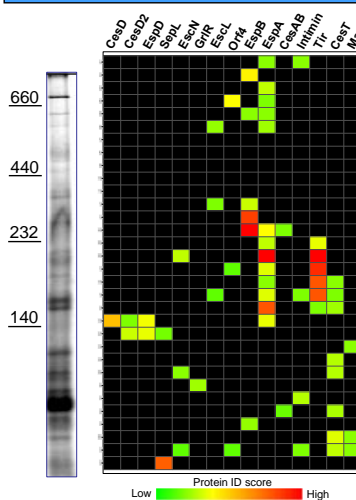


#### Biological Processes according to GO



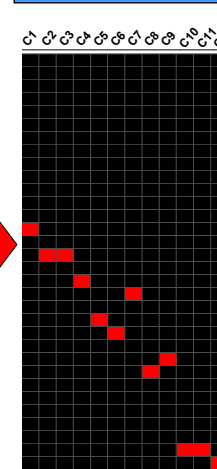
## T3SS Complexome

### Identified T3SS proteins



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

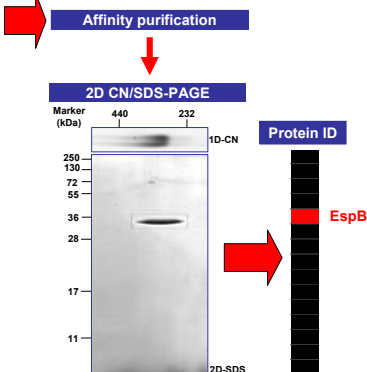
### Validation



12 putative protein complexes of T3SS proteins were determined.

### Single Complex Analysis

| Complex | #P (kDa) | Subunits                                   | Known interactions                     |
|---------|----------|--|--|
| C1      | 300      | [EspB] <sub>1</sub>                        | CesAB                                  |
| C2      | 230      | [CesA] <sub>1</sub> [EspA] <sub>1</sub>    | CesAB-EspA, CesAB-EspB                 |
| C3      | 180      | [CesT] <sub>1</sub> [EscN] <sub>1</sub>    | Map, EspF, Tir, CesT                   |
| C4      | 180      | [CesT] <sub>1</sub> [Tir] <sub>1</sub>     | Map, EspF, Tir, CesT                   |
| C5      | 140      | [CesD] <sub>1</sub> [EspD] <sub>1</sub>    | CesD, CesD2, EscRSU, CesD, rORF1, SepZ |
| C6      | 140      | [CesD2] <sub>1</sub> [EspD] <sub>1</sub>   | CesD, CesD2, EscRSU, CesD, rORF1, SepZ |
| C7      | 140      | [CesT] <sub>1</sub> [Intimin] <sub>1</sub> | Map, Tir                               |
| C8      | 100      | [CesT] <sub>1</sub> [Tir] <sub>1</sub>     | Map, Tir, CesT                         |
| C9      | 100      | [CesT] <sub>1</sub> [Map] <sub>1</sub>     | Map, Tir, CesT                         |
| C10     | 70       | [CesT] <sub>1</sub> [EscN] <sub>1</sub>    | Map, EspF, Tir, CesT                   |
| C11     | 70       | [CesT] <sub>1</sub> [Map] <sub>1</sub>     | Map, Tir, CesT                         |
| C12     | 40       | [SepL] <sub>1</sub>                        | SepD                                   |



EspB was the only protein identified in the purified protein complex of an apparent molecular mass of ~300 kDa.