Characterization of the *in vitro* gene response of chicken cells to *Salmonella* Enteritidis

Ama Szmolka[1], Marta Matulova[2], Zoltán Wiener[3], Béla Nagy[1], Ivan Rychlik[2]

[1] Institute for Veterinary Medical Research, Centre for Agricultural Research, HAS, Budapest, Hungary
[2] Veterinary Research Institute, Brno, Czech Republic
[3] Semmelweis University, Department of Genetics, Cell- and Immunobiology, Budapest, Hungary

Introduction and objectives

Salmonella Enteritidis (SE) is one of the most frequently reported causative agent of human gastroenteritis, originating mainly from poultry. Pathogenesis of SE infection in poultry is well-elucidated, but the complexity of the host cell response, and its relation to differring pathogenic potential of various strains is much less understood.

To provide a genome-wide comparative characterization of the gene expression profiles of chicken embryo fibroblast (CEF) cells as a response to infection with wild strains and virulence-defective mutants of *Salmonella* Enteritidis.

Materials and methods

Chicken embryo fibroblast (CEF) cells were used to measure the *in vitro* invasiveness of wild type strains SE 147, SE 11 and non-motile (virulence defective) mutants of SE 11: SE 2102 (ΔfliD) and SE Δ155 (ΔfliD, virulence plasmid-free) (**Table 1**).
The gene expression profile of CEF cells was determined by Agilent custom 8×15K microarray to model gene response of young chickens to Salmonella infection, with emphasis to those related to the immune response.
Significant gene expression changes with fold change ≥3 (in total of 31 genes) were verified by real-time PCR.

Functional analysis and verification of *Salmonella*-induced *in vitro* gene expression changes

Functional clustering of overlapping genes within groups infected by the wild strains (**Fig. 1B**) resulted in five large functional groups as listed in **Fig. 2**.

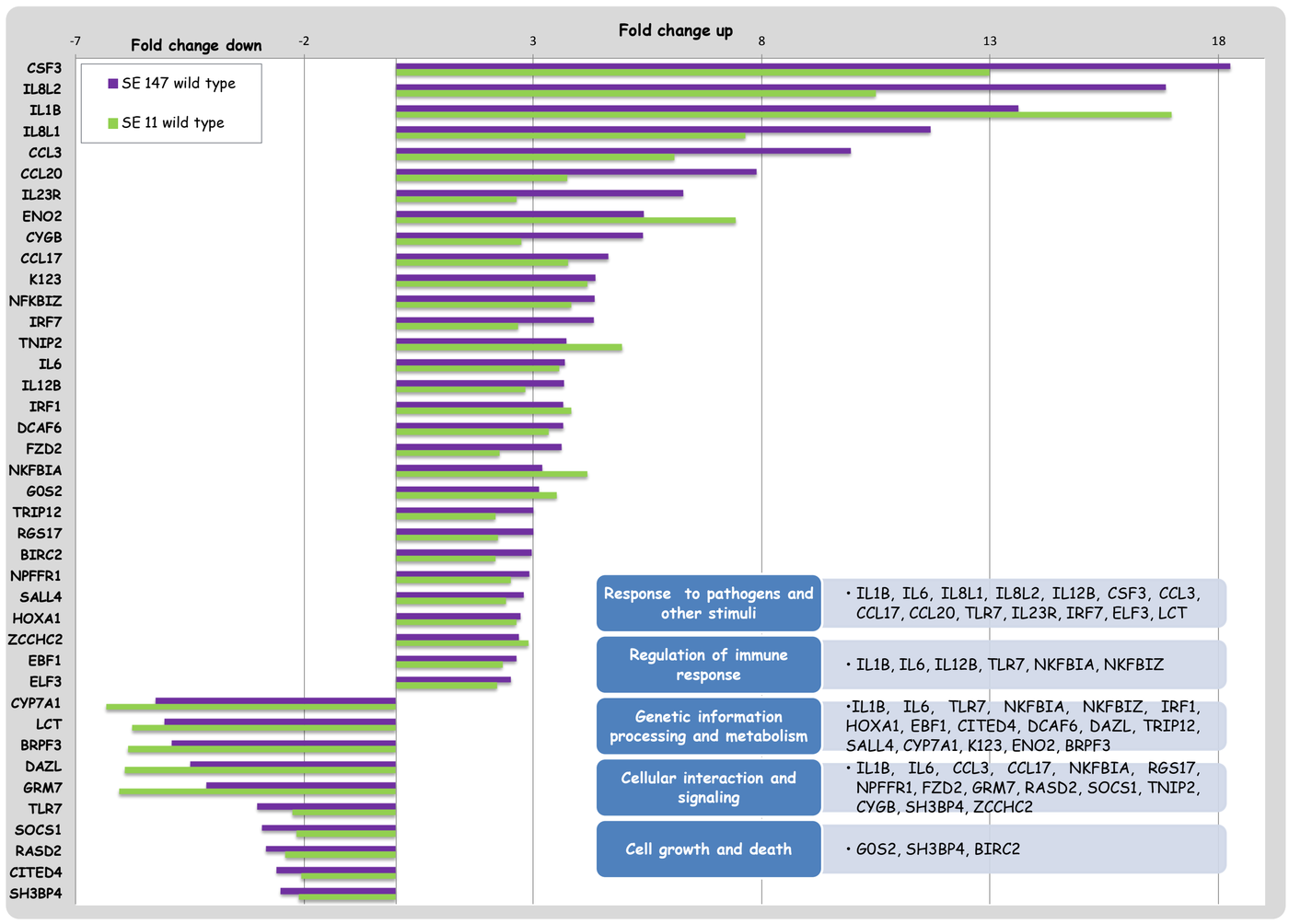

Fig. 2. Fold changes and rough functional clustering of annotated genes highly expressed as a response to both wild strains of *Salmonella* Enteritidis

Results

Invasiveness of *Salmonella* Enteritidis wild strains and mutants for chicken embryo fibroblast cells

The invasion comparison of wild strains of poultry S. Enteritidis revealed, that strain SE 147 invaded CEF cells 1.12 times higher than strain SE 11, although this difference was not significant (**Table 1**).

The invasiveness of the virulence defective (ΔfliD) non-motile mutant SE 2102 became 3.19 times lower than that of the parental strain SE 11. The elimination of the serovar specific virulence plasmid in strain SE Δ155 (ΔfliD, plasm-) did not cause further reduction of invasiveness (**Table 1**).

Table 1. Experimental setup and invasiveness of *S.* Enteritidis wild strains (wt) and mutants for CEF) cells

	Salmonella strains and mutants			
$\log_{10}$ CFU/ml	SE 147 wt	SE 11 wt	SE 2102 *ΔfliD*	SE Δ155 *ΔfliD*, plasm-
Inoculation	6.54	6.72	6.52	6.69
Co-incubation (2 hrs)	7.52	7.39	7.52	7.51
CEF lysate	4.48	3.99	1.53	1.25
Fold change (* signif.)	1,12	2,60*	0,81	3,19*

Quantifiation of the gene expression changes derived by the microarray analysis of CEF cells

The comparison of infected and non-infected (NI) groups resulted 314 significant misregulations induced by the wild strain SE 147, while only 135 genes were up- or down-regulated as a sult to SE11 infection (**Fig. 1A**).

However, infection with virulence-defective mutants (ΔfliD and/or plasm-) did not cause any significant change in host gene expression.

There were 100 genes overlapped in the host response significantly induced by the two wild strains SE 147 and SE 11 (**Fig 1B**)

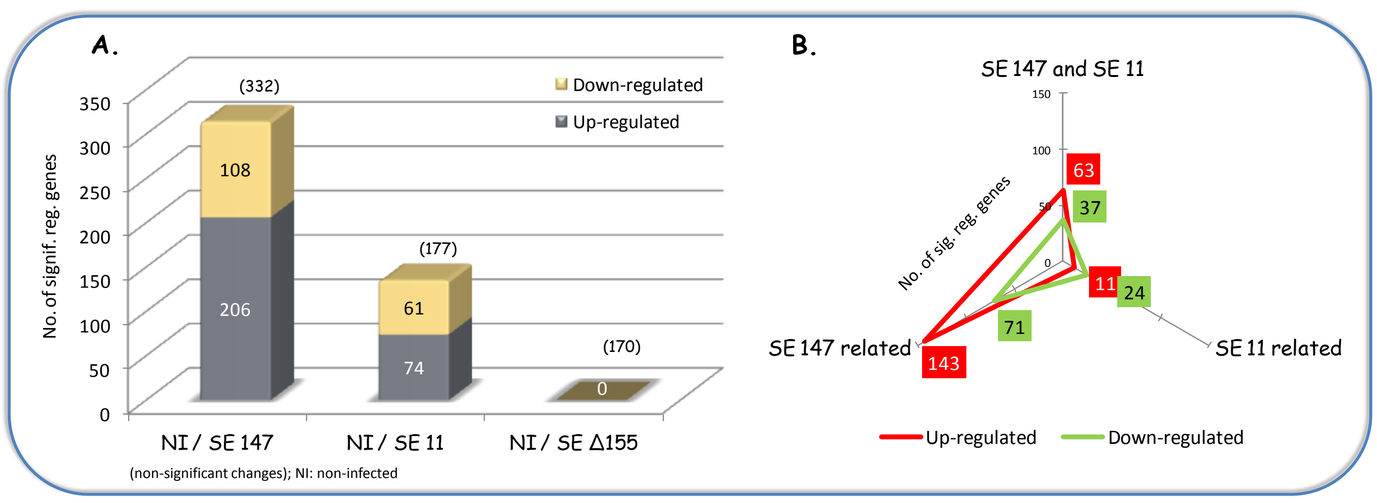

Fig. 1. Number of gene changes detected in comparison with the non-infected control (A) and those showing overlapping or strain-related expression (B)

In general, the most invasive wild strains were the prime inducers for most tested genes, however the cell cycle gene GOS2 (G0/G1 switch) and the metabolism gene ENO2 (enolase) were highly induced by the mutant strains only (**Fig.3**).

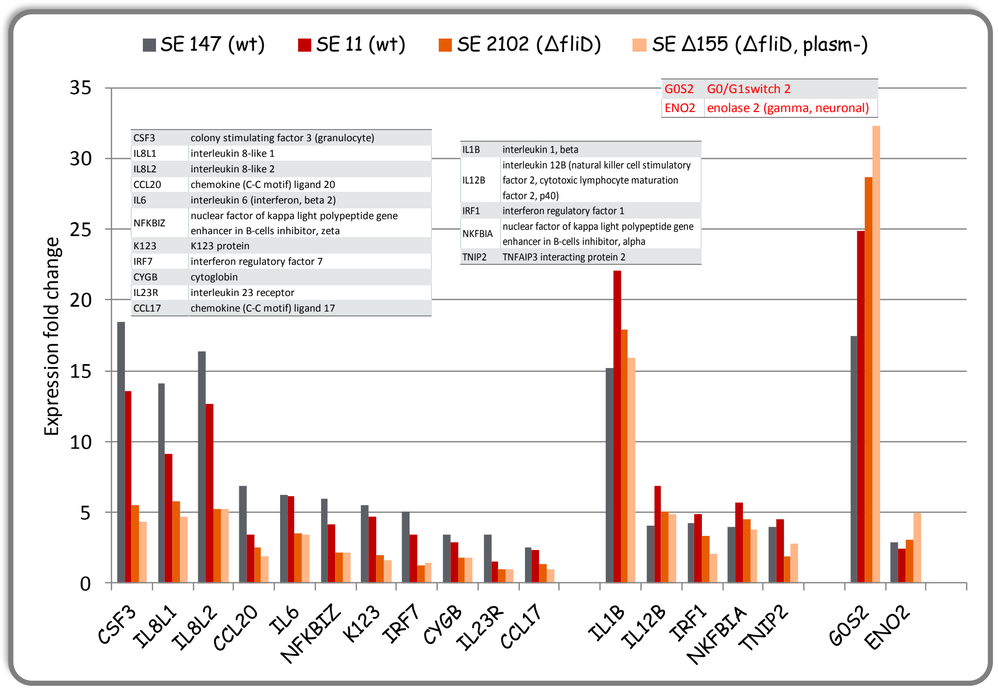

Fig. 3. Real-time PCR verification of the major up-expressions

Conclusion

Virulence gene *fliD* plays a key role in the invasiveness of *Salmonella*, and could be considered as an important modulator of the chicken response to *Salmonella* infection.

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