Conjunctive analysis of genomics and proteomics data: an innovative approach to screen potential targets for the genetic improvement of *Salmonella* resistance in pigs

RP Martins[1], A Moreno[1], R Bautista[2], MG Claros[2], A Carvajal[3], JJ Garrido[1*]

[1]Grupo de Genómica y Mejora Animal, Universidad de Córdoba. Departamento de Genética, Facultad de Veterinaria, Universidad de Córdoba, Campus de Rabanales, Edif. C5 1ª planta, 14071 Córdoba, Spain.
[2]Plataforma Andaluza de Bioinformática, Universidad de Málaga
[3]Departamento de Sanidad Animal, Universidad de León
*Corresponding autor

ABSTRACT

In this study we employed an in vivo approach coupled to 2D-DIGE and microarrays analysis to explore the response of porcine mesenteric lymph nodes (MLN) to S. Typhimurium infection at genomic and proteomic levels. MLN samples were collected from four control and twelve infected pigs (at 1, 2 and 6 days post infection) for protein and RNA purification. Protein was screened by differential in gel analysis and changes in transcriptome were assessed by Bayesian Analysis of Time Series. Afterwards, bioinformatic tools were employed to analyse and integrate data from both strategies. Comparison of genomic and proteomic data uncovered unique enriched mechanisms from each methodology. However, common functional changes were also observed, highlighting “Antigen processing and presentation” as the most significantly altered pathway in both approaches. Since both methodologies confirmed the induction of this mechanism in porcine MLN upon *S.* Typhimurium infection, we stress molecules taking part in this route as candidates for studies focusing on the genetic improvement of *Salmonella* resistance in pigs.

KEY WORDS: *Salmonella* Typhimurium, microarrays, 2D-DIGE, bioinformatics, pigs.

INTRODUCTION

Salmonella is asserted as the most frequent cause of food-borne outbreaks in Europe (EFSA, 2011). Among more than 2000 serovariants, the host-generalist *Salmonella enterica* serovar Typhimurium (herein *S.* Typhimurium) is reported as the predominant serotype isolated from humans and pigs are inferred to be the most important source of infection with this serotype (Boyen et al, 2008).

Recently, comparative genome and proteome analyses have been used to generate more detailed understanding of molecular mechanisms behind diseases (Bendixen et al, 2010). In fact, *in vivo* models combined with technological advances in functional genomics represent unprecedented means of characterizing host-pathogen interactions (Zhang et al, 2005). This approach enables a systematic identification and classification of host mechanisms involved in infection, providing potential targets for disease prevention and treatment strategies.

Aiming to identify molecules of interest for the genetic improvement of the resistance to porcine salmonellosis, in the current study we employed a model of *in vivo* experimental infection followed by genomic and proteomic data mining to elucidate the molecular mechanisms undergone by swine porcine mesenteric lymph nodes (MLN) upon *S.* Typhimurium infection.

MATERIAL AND METHODS

Experimental infection

Experimental infection design was described elsewhere (Collado-Romero et al, 2010). Briefly, sixteen weaned piglets were randomly allocated to control (4 piglets) or infected groups (12 piglets). Control (0 day post-infection - dpi) pigs were necropsied two hours before the experimental infection and those ones belonging to the infected groups were orally challenged with 10^8 cfu of *S.* Typhimurium phagetype DT104. Afterwards, infected pigs were randomly sampled and necropsied at 1, 2 and 6 dpi (four animals at each time point). Mesenteric lymph nodes from all experimental animals were collected after necropsies and immediately frozen in liquid nitrogen for RNA and protein isolation.

Proteomic, transcriptomic and bioinformatic analysis

For proteome analysis, 2D-DIGE coupled to MALDI-TOF/TOF MS was used to screen for differentially expressed proteins (for details refer to Martins et al, 2012). Changes in transcriptome were assessed by microarray analysis employing Affymetrix technology (GeneChip Porcine genome array). Genes differently expressed along time were identified by Bayesian Analysis of Time Series (Angelini et al, 2008). Afterwards, bioinformatic tools (IPA – Ingenuity® Systems and ArrayUnlockTM – Integromics) were employed to analyse and integrate data from both strategies. Differently expressed genes and proteins were listed to generate interaction networks as well as identify enriched signalling pathways and biological functions.

RESULTS AND DISCUSSION

Proteome analysis by 2D-DIGE

Differential in gel analysis uncovered 54 spots exhibiting significant abundance changes as a consequence of the bacterial challenge (**Figure 1A**). Paired analyses between infected and control groups detected 16, 43 and 16 differently changed spots at 1, 2 and 6 dpi respectively. Moreover, a predominance of spots that were significantly more abundant in relation to controls was observed in all infected groups (**Figure 1B**).

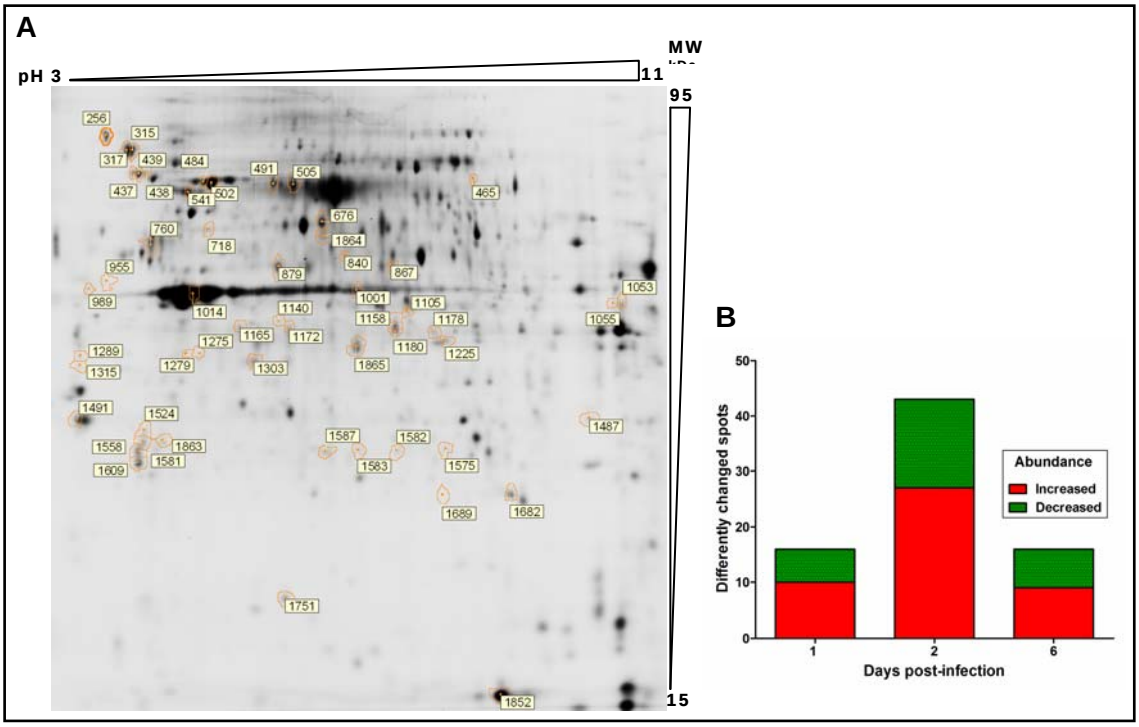

Figure 1 – DIGE analysis of mesenteric lymph nodes proteins from controls and Salmonella Typhimurium infected pigs. (A) Spots showing significant changes in abundance are indicated by numbers. (B) Bars demonstrate the number of spots exhibiting significant increase or decrease of abundance at 1, 2 and 6 days post-infection (dpi).

Cell motility, Anti-apoptosis, Protein folding and Rho GDP-dissociation inhibitor activity were among the top enriched biological processes and molecular functions related to the differently abundant proteins. Furthermore, data mining identified the following pathways among the most enriched ones: Antigen processing and presentation, MAPK signalling, 14-3-3 mediated and protein ubiquitination pathways. In summary, our proteomic study indicated changes in essential host functions such as phagocytes infiltration and cytoskeleton remodelling, induction of cell death and modulation of host second line of defence upon S. Typhimurium infection.

Transcriptional profiling by microarray analysis

Changes in expression were observed for 288 genes, arranged in ten groups according to their expression pattern along the studied time course (**Figure 2**). Regulated genes were associated to biological functions such as positive regulation of Tumor necrosis factor biosynthetic process, Antigen processing and presentation, Cell death, cell-to-cell signalling and interaction. Results of pathway analysis highlighted the enrichment of different mechanisms related to endocytic internalization of particles, such as Clathrin mediated endocytosis signalling, Macropinocytosis and CTLA4 signalling in cytotoxic T lymphocytes. Furthermore, the observed up-regulation of genes coding for MHC molecules and CD86 suggests an increment of mechanisms resulting in maturation of dendritic cells.

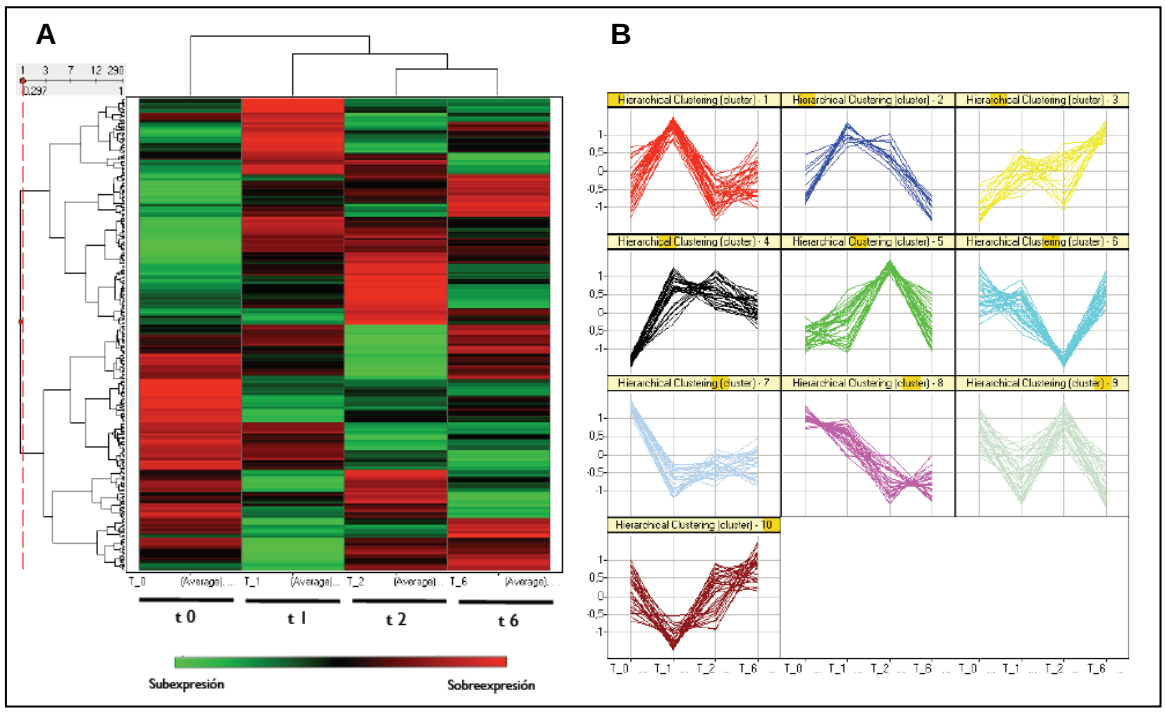

Figure 2 – Expression pattern of differently expressed genes in porcine MLN after *S.* Typhimurium infection. (A) Heat map, (B) Hierarchical clustering along time.

Data integration

Correlation of changes in gene expression and protein abundance with alteration in cell and tissue function is expected to derive insight into a broad range of biological processes (Waters et al, 2006). In view of this, an approach based on the analysis of common functional changes observed in both genomic and proteomic levels, is proposed by us as a strategy to select target molecules for programs focused on breeding for improved disease resistance in domestic animals.

As depicted in **Figure 3,** comparison of genomic and proteomic data uncovered unique enriched mechanisms from each methodology, confirming that exploring host response in both mRNA and protein levels provides a broader view of host response to infection.

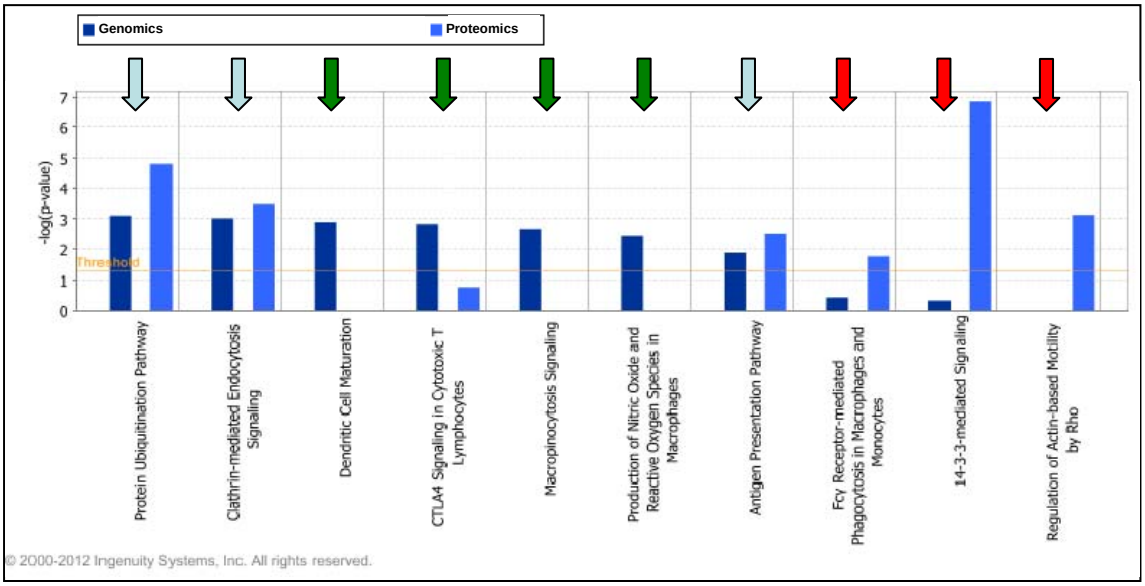

Figure 3 – Enriched canonical pathways in genomic and proteomic datasets.

Interestingly, “Antigen processing and presentation” was the most significantly altered pathway in both analysis, reinforcing the relevance of this mechanism in the response of porcine MLN to *S*. Typhimurium (**Figure 4**). “Protein ubiquitination” and “Clathrin mediated endocytosis signalling” were also enriched in both approaches. It is also relevant to highlight that proteome and transcriptome analysis produced complimentary data, since different molecules from the same route were identified in each data set.

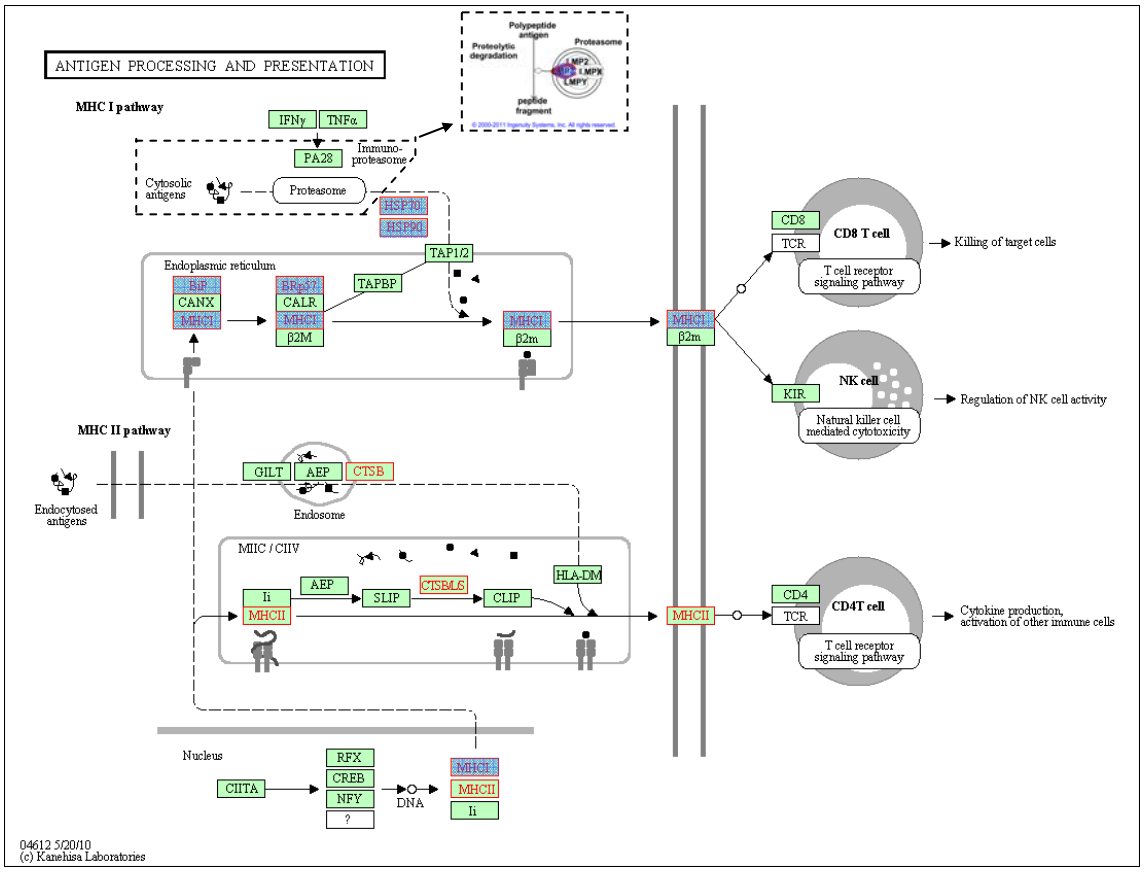

Figure 4 – Antigen processing and presentation pathway. Differently abundant proteins and differently expressed genes are highlighted in blue and red respectively. Dotted line indicates a pathway detail from Ingenuity Pathway Analysis.

As expected, infection resulted in an increment molecules related to antigen presentation via MHC class II. However, we also observed an enrichment of antigen presentation via MHC class I. Antigenic peptides presented via MHC-I are produced through cytosolic degradation of intracellular proteins by proteasome (Lattanzi et al, 2011). Since proteasomal activity is dependent on the ubiquitination of its targets, the significant enrichment of the “Protein ubiquitination pathway” uncovered by IPA analysis gives an additional evidence of the enhancement of protein degradation by immunoproteasome as a result of infection. We speculate that cross-presentation, a mechanism in which professional antigen-presenting cells present exogenous peptides via their own MHC-I molecules to CD8+ T cells (Houde et al, 2003), could explain the clear indications of adaptive immunity induction via MHC-I upon S. Typhimurium infection.

In conclusion, a conjunctive analysis of genomics and proteomics data indicated that as well as classical processing and presentation of exogenous antigens via MHC-II, *S.* Typhimurium induces host response via MHC-I upon infection. Since antigen

processing and presentation pathway plays a key role in the induction of immune response and its enrichment was corroborated by both transcriptomic and proteomic level, we stress molecules taking part in this mechanism as candidates for studies focusing on the genetic improvement of the resistance to Salmonella in pigs.

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