Transcriptomic analysis of *Ehrlichia ruminantium* by micro-arrays

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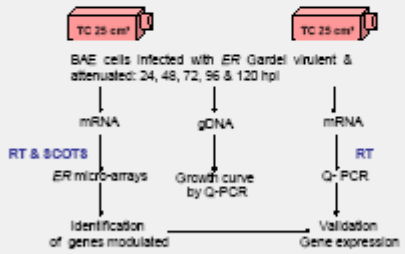
Introduction

Rickettsiale *Ehrlichia ruminantium* (*ER*), the causative agent of heartwater, is an obligate intracellular pathogen (1). This characteristic explains that little is known about the genetic determinants and molecular mechanisms of *ER* pathogenesis. Recently a method of selective capture of transcripts sequences (SCOTS) (2) was successfully adapted to *ER*. This step was essential for any future transcriptomic study on obligate intracellular pathogen. The annotation of 2 genomes of Gardel and Welgevonden strains revealed that 30% of the CDS had unknown functions (3). The objectives of this study was to do high throughput gene expression analysis through the development of *ER* microarrays and then identify genes involved in the pathogenesis and attenuation of *ER* strains and comprehension of these mechanisms. An *in vitro* model using both virulent and attenuated Gardel strains in bovine endothelial cells allowed us to compare for the first time *ER* gene expression . In this study, we reported a set of genes differentially expressed between virulent and attenuated strains of *ER* Gardel.

Experimental model & Strategy



Comparison of gene expression between virulent & attenuated *ER* strains at different stage of development

Growth kinetics of *ER* strains by Q-PCR

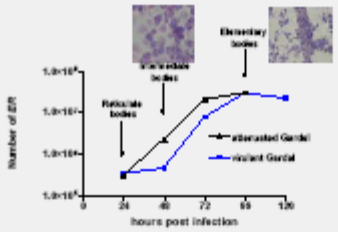


Figure 1: Q PCR of map1 gene on gDNA samples

Optical microscopy observations of *ER* development depending on time of collect & following up of *ER* growth by Q PCR. The kinetics of both strains showed typical bacteria growth curve and allowed us to chose the different stage of development in association with post infection time.

map1, *recA* and *rpoD* expression after SCOTS

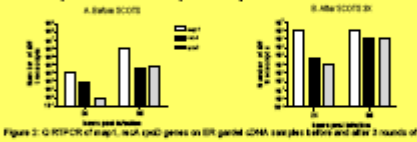


Figure 2: Q RTPCR of map1, recA rpoD genes on ER gardel cDNA samples before and after 3 rounds of capture

The level of expression was different depending on gene: 1 copies for *rpoD* and 10^3 copies for *map1* at 24hpi. SCOTS after 3 capture allowed to detect a higher number of copies for each time post infection. A difference of expression between genes was maintained after capture (1 log10).

ER Micro-arrays validation

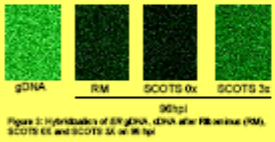


Figure 3: Hybridization of ER gDNA, cDNA after RM extraction (RM), SCOTS 0X and SCOTS 3X on 96 hpi

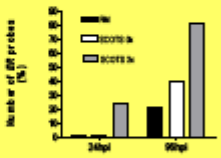


Figure 4: Detection of ER transcripts by micro-arrays

Hybridization on *ER* micro-arrays :
- ✓for gDNA: 99% of detected probes
- ✓for cDNA after removal of eucaryotic rRNA (RM): 1% at 24hpi & 20% at 96hpi
- ✓for cDNA without capture: <1% at 24hpi & 40% of detected probes at 96hpi
- ✓for cDNA after 3 captures: 24% at 24 hpi & 81% of detected probes at 96hpi

ER gDNA hybridization demonstrated the efficiency and specificity of *ER* micro-arrays. SCOTS method is crucial to increase the quantity of transcripts in order to measure gene expression at early stage of development. At late stage, the percentage of genes detected doubled after capture.

Genes differentially expressed between virulent & attenuated strains

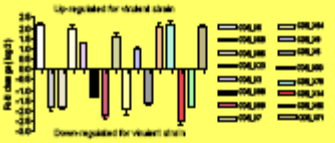


Figure 5: n-fold expression (log 2) of gene between virulent and attenuated Gardel strains by micro-arrays

ID	Ratio	function	
CDS_X8	2,16	ABC transfert	
CDS_X88	-1,81	33 kDa conserved protein	
CDS_X89	-1,82	Signal transduction mechanism	pathogenicity
CDS_X23	1,87	*	
CDS_X3	1,27	*	
CDS_X99	-1,38	*	
CDS_X89	-2,24	Hypothetical transcriptional regulator	
CDS_X7	1,55	Hypothetical intracellular trafficking, secretion & vesicular transport	
CDS_X98	1,32	Hypothetical map1 related protein	
CDS_X86	-1,56	Hypothetical putative cobalamin decrease	metabolism
CDS_X54	-1,87	Nucleotide transport and metabolism	
CDS_X9	1,61	*	
CDS_X7	1,64	*	
CDS_X5	-1,58	Secondary metabolite biosynthesis transport metabolism	
CDS_X27	1,64	Energy production and conversion	
CDS_X20	2,66	*	
CDS_X29	2,14	Unknown	hypothetical
CDS_X14	-2,51	*	
CDS_X39	-1,64	*	
CDS_X71	2,64	*	
CDS_X3	-2,17	*	
CDS_X45	-2,42	*	
CDS_X59	-1,77	*	

Table1. 23 Genes differentially expressed between virulent and attenuated strains of ER Gardel at 96hpi. Ratio is expressed in log2.

34 genes with modulated expression at 96hpi: 4 genes involved in virulence for intracellular pathogens *S. Enterica typhimurium*, *S. flexeneri*, *M. tuberculosis*; 3 genes potentially involved in pathogenicity; 19 genes with unknown function; 6 genes involved in the metabolism

Discussion & Perspectives

The adaptation of SCOTS method to our *in vitro* model, allowed to obtain a large amount of *ER* transcripts and do further transcriptomic study. For the first time, *ER* micro-arrays was validated by hybridization of *ER* gDNA and cDNA. SCOTS method allowed the detection of the lowest expressed genes such as *rpoD* or *recA*. There was a strong increase of detection of transcripts after SCOTS both demonstrated by Q RTPCR and micro-array analysis. We demonstrated that SCOTS method was crucial for expression analysis during early stationary phase of growth of *ER* (24 hpi) with 24% of transcripts detected compare to 1% of transcripts before capture. Preliminary results on *ER* microarrays at late stage of development demonstrated that 34 genes were strongly modulated depending on virulent or attenuated strains. 19 of the genes were significantly up-regulated for virulent strain. Through comparative genomic study, on 34 genes, 19 had unknown function and 6 were involved in metabolism pathway. Interestingly, 4 genes were identified to be virulent factors for other intracellular pathogens and 2 as hypothetical virulent factor specific of *ER*. Three of these genes should be strongly involved in the virulence (CDS_X23, CDS_X3, CDs_X7; up-regulated for virulent strain) and 3 involved in the attenuation (CDS_X86, CDS_X98, CDS_X99; down-regulated). The differential gene expression is under validation by Q RTPCR. Further analysis on *ER* earlier stage of development using SCOTS method are under process. This strategy paves the way for new insights in pathogenicity of obligate intracellular pathogens.

References

1. Bezuidenhout et al, 1985, Onderstepoort J Vet Res 52, 113-120. 2. Faucher et al., PNAS, 2006, Vol 103, n° 6, p1906-1911, 3. Frutos et al., Journal of Bacteriology, 2006, p2633-2642.

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