O 25 EXPRESSION STUDY BY REAL-TIME QUANTITATIVE RT-PCR OF THE *SALMONELLA* TYPHIMURIUM *mntH* GENE

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Summary: The aim of our study was to compare the *mntH* expression of different *Salmonella* Typhimurium strains and other *Salmonella* serovars with real-time RT-PCR. Following the *mntH* expression in function of the growth showed that the *mntH* expression of *S.* Typhimurium is growth dependent. A strong decrease of the *mntH* expression is noticed when the growth reaches 1.78 10^8 CFU/ml. After induction with EDTA or H_2O_2, variations between different *S.* Typhimurium strains were observed. For some *S.* Typhimurium strains a 10 to 20 times higher *mntH* expression was noticed after H_2O_2 induction. The EDTA induction was for most strains lower (5 to 10 times) but also variations between different strains were observed. The other *Salmonella* serovars were strongly induced after H_2O_2 but not after EDTA induction.

Introduction: The NRAMP (natural resistance-associated macrophage protein) family of divalent-metal transporters was first identified by studies in mice on the genetic basis of susceptibility to intracellular parasites. The NRAMP family is widespread and highly conserved with homologues in other animals, insects, worms and plants. In bacteria an orthologue of the eucaryotic *Nramp*1 gene is described, the *mntH* gene (H^+-coupled manganese transport). In the phagosome the bacterial and the eucaryotic NRAMP would compete for the transport of bivalent cations. The competition between both transport systems would determine the intracellular survival of the pathogenic bacterium (Agranoff et al, 1999). Differences in the activity of the *mntH* gene could be important for the pathogenicity of the strain and the capacity to survive in the host. In our study the expression of the *mntH* gene was compared from different pig related *Salmonella* Typhimurium strains after induction with EDTA and H_2O_2.

Materials and methods: 30 pig related *S.* Typhimurium strains of different origin, an *mntH* deletion mutant of *S.* Typhimurium MB 2534 and 2 human *S.* Typhimurium strains were selected for this study. To compare with other *Salmonella* serovars a *S.* Enteritidis, *S.* Kedougou, *S.* Havana, *S.* Ohio, *S.* Livingstone, *S.* Derby, *S* Goldcoast and *S.* Brandenburg were included. *Determination of the growth curve*: For 3 *S.* Typhimurium strains MB 2150, MB 2486, MB 2109 and an *S.* Derby strain 821175a the growth curve was determined in BHI at 37°C. Starting from 1.0 10^6 CFU/ml, every 30 minutes a 1 ml sample was taken and total RNA was extracted using the RNeasy Mini kit (Qiagen) as described by the manufacturer. Residual DNA was digested using a DNase treatment. The amount of *mntH,* 16S rRNA and *rpoD* mRNA was measured. For the induction experiment strains were grown to OD 0.050. To the bacteria culture H_2O_2 (100_M) or EDTA (1 mM) was added. After H_2O_2 induction the sample was taken directly (T0) whereas for EDTA induction a sample after 15 and 45 minutes incubation at 37°C was taken. Reverse transcription was carried out with random hexamers and Multiscribe Reverse Transcriptase (Applera) with 1 _l RNA as template. 5 _l of the cDNA was used in the real-time PCR amplification mixture containing 1x SyberGreen I master mix (Applera) and 300 nM of the forward and reverse primer. As control genes the 16S rRNA and the *gmk* (guanylate kinase gene) were analyzed.

Results: The *mntH* expression of *S.* Typhimurium is growth dependent. Figure 1 presented the expression in function of the growth in BHI for *S.* Typhimurium strain MB 2486. The same results were obtained with the other strains (results not shown). A strong decrease of the *mntH* expression is noticed when the OD_{610} of the culture is greater than 0.5. The expression of 16S rRNA remains constant during growth and a decrease in *rpoD* expression is noticed when the plateau phase is reached.

After induction with EDTA or H_2O_2, variations between different *S.* Typhimurium strains were observed. For some *S.* Typhimurium strains a 10 to 20 times higher *mntH* expression was noticed after H_2O_2 induction. The EDTA induction was for most strains lower (5 to 10 times) but also variations between different strains were observed. The other *Salmonella* serovars were strongly induced after H_2O_2 but not after EDTA induction. In the *mntH* mutant no detectable level of *mntH* mRNA could be measured after a 40 cycle PCR.
For the relative quantification *rpoD* could not be used as control gene because it turned out to be induced by H_2O_2. The relative expression of the *mntH* gene normalized against both control genes 16S and *gmk* was comparable.

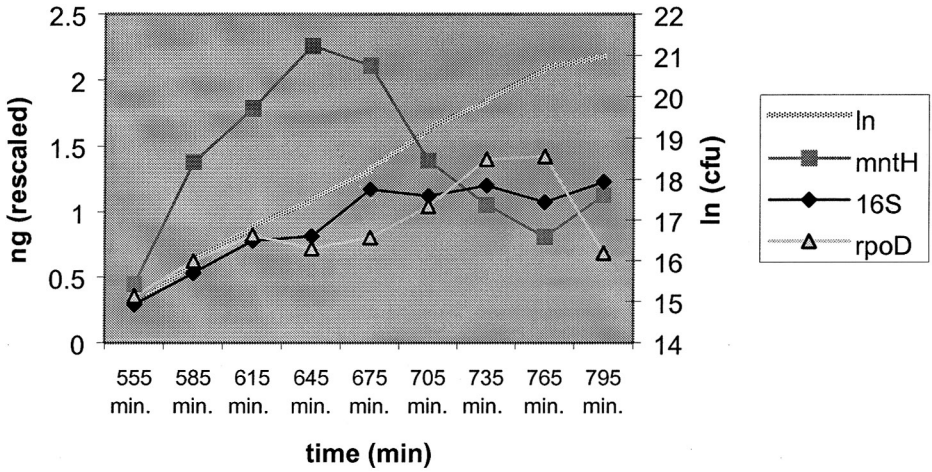

Figure 1: *The expression of mntH, 16S rRNA and rpoD in function of the growth in BHI for S. Typhimurium MB 2486.*

Conclusion: Real time PCR is an efficient tool to study the relative quantitative expression of different genes. By these technique differences in the *mntH* expression could be observed between the different *S.* Typhimurium strains.

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References:

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