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Monitoring the Growth of *Salmonella enterica* serovar Typhimurium *in silico* and *in situ* With a View in Gene Expression

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AIM

***Salmonella enterica* and plant tissues**
❖ Important human pathogens
❖ Consumption of plants has been associated with the risk of salmonellosis

Pathogenicity
Several factors have to be checked as infection requires the expression of genes not only coding virulence factors but also physiological processes such as stress response and adaptation

Study
The ability of *Salmonella* Typhimurium to develop a biofilm community on rocket tissue *in silico* and *in situ*

MATERIALS and METHODS

Rocket extract preparation
Rocket
1:1 dH_2O
4h at 4°C
Autoclave

Inoculation
LB or Rocket or Rocket Extract
10-15 cfu/mL or cm² *Salmonella* Typhimurium (CDC 6516-60)
20 °C

Microbiological analysis
Inoculated LB, Rocket Extract, Rocket
Serial Dilutions
Counts

RNA extraction and cDNA synthesis
Frozen cells (-80°C) stored in RNA later
Total RNA extraction (The PureLink® RNA Kit, Ambion)
cDNA synthesis (Primescript Reverse Transcriptase, Takara)
-20 °C

Real time PCR and data analysis
cDNA
SYBR green Master Mix (Kapa Biosystems)
Primers (Table 1)
2-ΔΔCt Function , $\Delta\Delta Ct = (Ct_{target} - Ct_{reference})_{test} - (Ct_{target} - Ct_{reference})_{control}$
(Livak & Schmittgen, 2001)

Table 1. Target genes and primer synthesis

Gene	Function	Primer sequence F	Primer sequence F	Reference
dps	starvation, stress response	ATGCGCGGTGCTAACTTTAT	AAGTGATCCTGCACGTTATG G	In this study
dppA	adaptation to nutrient deficiency	TTGACCGTCTGGTCTTCTCC	GACATCATCCAGCGGTTTTT	In this study
rpoH	Starvation	CGTTAAAGTTGCAACCACGA	CCATCTCACGCACGTCTTTA	In this study
osmY	Osmotic stress	GTCACCCTGAGCGGCTTTGT	CTTCACTGGTCGTGGCCGT A	In this study
csgB	Attachment	AGTGCCAGAGTACGCCAGGA	ACCGTAAGCGCTTTGCGATA	In this study
sidA	Cell division control, quorum sensing	ACGCGCAATGTTGTTACGC	ACCCACGCCGGAGGATAAG T	In this study
sspH2	Pathogenicity	GCACAACTGGCTGAAGA TGA	CGTATTGCCCTTTTTCT GC	In this study
rrsG	Reference gene	GTTACCCGCAGAAGAAGCAC	CACATCCGACTTGACAGAC C	Zheng et al 2011

RESULTS

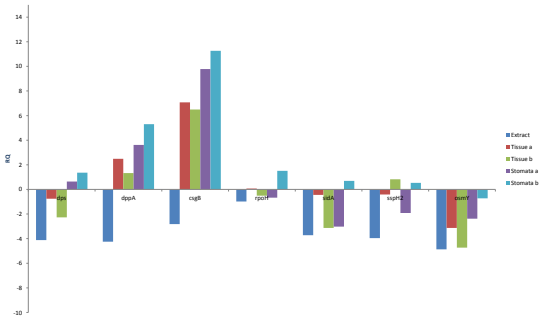

Figure 1. Relative expression level of different genes associated with stress (dps, dppA, csgB, rpoH, sidA, sspH2, osmY, Table 1) of *Salmonella* Typhimurium cultured on rocket extract and tissue 20°C. A 42h cultures on LB broth at 20°C was used as reference, while rrsG gene was used as housekeeping gene.

❖ The final population of *Salmonella* Typhimurium was affected from the rocket extract and rocket tissue as a difference of 1 log cfu/mL and 4 log cfu/cm² was observed regarding the laboratory medium

❖ Regarding seven studied genes i.e. starvation, stress response, adaptation to nutrient deficiency, attachment, pathogenicity and quorum sensing, the differential expression was more associated with the planktonic and biofilm grown cells.

❖ *Salmonella* reacts as exposed to different types of stress when inoculated to a heat sterile plant extract and plant tissue

❖ dps gene expression was found to be also affected when the pathogen was inoculated on stomata

Table 2. Total population (log cfu/mL or cm²) of the examined samples at 20°C

Samples	Hours	Population (log cfu/mL or cm²)
LB	43	9.07
Rocket extract	68	8.27
Tissue a	68	5.09
Tissue b	68	4.53
Stomata a	68	5.08
Stomata b	68	5.07

CONCLUSION

➢ The findings of the present study could show that *Salmonella* Typhimurium reacts as exposed to different types of stress when inoculated to a heat sterile plant extract at lower temperature

➢ Further studies are needed to better determine the survival and / or growth of the pathogen as "real" biofilm cells on plant tissues

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MONITORING THE GROWTH OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM *IN SILICO* AND *IN SITU* WITH A VIEW IN GENE EXPRESSION

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Salmonella is an important human pathogen capable of causing a diverse array of diseases, while it is recognized to be the one of the most related microorganism for foodborne diseases. However, several factors have to be evaluated for better understanding of bacterial pathogenesis as infection is a process which requires the expression of genes not only coding virulence factors but also physiological processes such as stress response and adaptation.
In the present study, the ability of *S.* Typhimurium to develop a biofilm community on rocket tissue was investigated at 20°C. The differences on expression of genes associated with several functional roles during growth of *S.* Typhimurium on rocket extract and rocket tissue regarding a laboratory growth medium (Luria – Bertani broth, LB) was also monitored. The final population of *S.* Typhimurium was affected from the rocket extract (ca. 8 log cfu/mL) and tissue (ca. 5 log cfu/cm^2) as difference was observed regarding LB (ca. 9 log cfu/mL). Regarding seven studied genes i.e. starvation, stress response, adaptation to nutrient deficiency, attachment, pathogenicity and quorum sensing, the differential expression was more associated with the planktonic and biofilm grown cells. The findings of the present study could show that *Salmonella* reacts as exposed to different types of stress when inoculated to a heat sterile plant extract and plant tissue. However, further studies are needed to better determine the survival and / or growth of these as “real” biofilm cells on plant tissues.

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