AGRICULTURAL UNIVERSITY OF ATHENS
Department of Food Science and Human Nutrition
Laboratory of Microbiology and Biotechnology of Foods

Metabolomic Analysis of *Salmonella enterica* cells *in vitro* and *in situ*

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AIM

Salmonella enterica
- ❖ Important human pathogen
- ❖ Ability to form biofilm
- ❖ Persistent in the plant environment

+

Raw plant tissues
- ❖ Consumption has been associated with the risk of foodborne diseases
- ❖ cross contamination

➡

Study
The different metabolic compounds of *Salmonella* during the growth on either abiotic or plant surfaces needs to be further studied

MATERIALS and METHODS

Rocket extract preparation

Rocket

1:1 dH_2O

4h at 4°C

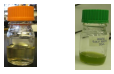
Autoclave (presence or absence of agar)

Inoculation

Solid

LB, rocket agar or Rocket (10x10cm)

Broth



LB or Rocket Extract

10-15 cfu/mL or cm² *Salmonella* Typhimurium (CDC 6516-60)

20 ℃

Microbiological analysis

Inoculated LB, Rocket Extract, Rocket

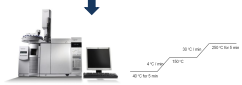
Serial Dilutions

Counts

GC - MS analysis

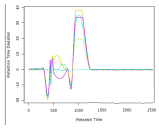
Cell free supernatant

SPME

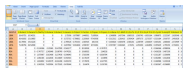


Agilent 7890A gas chromatograph coupled to an Agilent 5973C mass spectrometer

Metabolomic analysis



GC-MS Spectra



148 compounds

Metaboanalyst 2.0

RESULTS

- ❖ Final population of S. Typhimurium was
  - ca. 8 log cfu/mL on rocket extract
  - ca. 5 log cfu/cm² on tissue
  - ca. 9 log cfu/mL on LB
- ❖ Heat sterile rocket extract was sub-cluster in the same branch with rocket tissue, while rocket extract agar was more related
- ❖ The different metabolic compounds were associated with
  - The growth on the different media
  - Sampling points
  - The inoculation on the surface or stomata of tissue

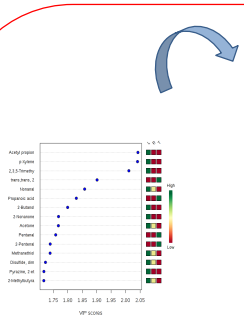


Fig 1. Box plots and kernel density plots before and after normalization. Data scaling: Autoscaling

Fig 5. Important features identied by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study

Metaboanalyst results

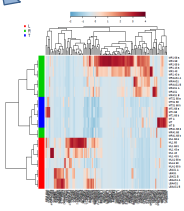


Fig 2. Clustering result shown as heatmap (distance measure using pearson, and clustering algorithm using ward)

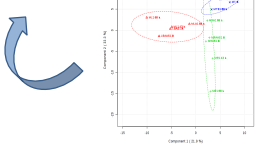


Fig 4. Score plot between the selected PCs

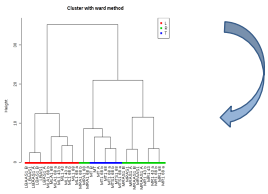


Fig 3. Clustering result shown as dendrogram (distance measure using pearson, and clustering algorithm using ward)

CONCLUSION

The correlation of these metabolites with the different growth conditions of the microorganism could be fundamental for understanding the possible actions to be taken for controlling the probability of survival on food chain or food processing environments

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METABOLOMIC ANALYSIS OF *SALMONELLA ENTERICA* CELLS *IN VITRO* AND *IN SITU*

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Current trends indicate an increase in produce-based outbreaks caused by e.g., *Salmonella* spp., while their persistent in the plant environment is due to biofilm formation either on or within the plants. In the present study a comparison of metabolomics, on laboratory medium, on rocket extract, of *S.* Tymphimurium (*ST*) CDC 6516-60, as well as on the developed biofilm on rocket tissue was investigated. This pathogen grew on Luria – Bertani (LB) growth media and extract from rocket, and the samples were incubated at 20°C. The metabolomic analyses with GC-MS resulted in a great number of compounds for LB, rocket extract either with or no biofilm formation. The compounds 2-butanodiol, 2,3,5-trimethylpyrazine, pyrazine, 2-ethyl-5-methyl-, acetyl propionyl were high associated with LB, while amyl alcohol, 2-Hexen-1-ol(trans), butanamide, N-methyl-4-(methylthio)-2-(2,2-dimethylpropylidene)amino-, Pyrazine,2,5-dimethyl- and 2-Penten-1-ol,(Z) were associated with rocket extract without biofilm formation. In the case where biofilm has been developed the following compounds were evident Sulfide, dimethyl, Toluene, Isobutylaldehyde, 2 Hexen-1-ol (trans) and Heptyl alchohol. An open sources platform (the web server Metaboanalyst 2.0) was used to analyze the data derived from these analyses. The knowledge of the different metabolic compounds and the correlation with the different growth conditions of the microorganism could be fundamental for understanding of its growth and the possible actions to be taken for controlling the probability of survival on food chain or food processing environments.

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