ADAPTATION OF STAPHYLOCOCCUS XYLOSUS TO PORK MEAT BATTER DURING THE FERMENTATION STEP

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Abstract – ***Staphylococcus xylosus* is used as starter culture for sausage fermentation for a long time but the molecular mechanisms for its adaptation in meat remained unknown. A global transcriptomic approach was carried out to determine these molecular mechanisms. *S. xylosus* modulated the expression of 38 to 48 % of the total genes during its growth and survival in the meat model. The expression of many genes encoding enzymes involved in glucose and lactate catabolism up to the respiratory chain and in cofactors synthesis necessary to their activities was up regulated. In parallel, genes encoding transport of peptides and peptidases that could furnish amino acids were up expressed and thus concomitantly a lot of genes involved in amino acids synthesis were down regulated. Finally *S. xylosus* responded to salt added in the meat model by over expressing genes involved in transport and synthesis of osmoprotectants, Na^+ and H^+ extrusion and in production of energy through the F_0F_1-ATPase.**

Key Words – microarray, starter culture, transcriptome

I. INTRODUCTION

Staphylococcus xylosus is a commensal of the skin and the mucous membranes of animals. Consequently, it is naturally present in food of animal origin and frequently isolated from meat products. It is one of the major starter cultures used for sausage fermentation. Its physiology has been extensively studied in laboratory conditions highlighting its role in flavour and colour development.

The development of genomics has allowed studying their metabolism *in situ* in the food matrix. As the molecular mechanisms involved in the adaptation of bacteria in meat product remained little known, we analysed the transcriptome of *S. xylosus* in a meat model incubated until 72 hours at 22°C in conditions that mimic the fermentation step. The *in situ* response of *S. xylosus* was analyzed *versus* the *S. xylosus* culture used as inoculum. A microarray developed for *S. xylosus* C2a strain was used to determine how the gene expression profile was modified during the fermentation step growth in meat by comparison to the inoculum.

II. MATERIALS AND METHODS

The *S. xylosus* C2a strain was cultured overnight at 30°C under agitation in a minimal medium. The culture was centrifuged and part of the cell pellet constituted the inoculum and was immediately frozen in liquid nitrogen. Another part of the cell pellet was inoculated in a pork meat batter and incubated at 22°C during 24, 48 and 72 h. Aliquots of 200 mg meat samples at the different times were taken and immediately frozen in liquid nitrogen. Three independent experiments were done.

The RNA extraction and labeling of *S. xylosus* either from the inoculum or directly in meat at the different times were carried out as described by Vermassen et al. [1]. The different steps of the protocol were schematized in Figure 1.

Microarray data were analyzed and validated as described [1]. A gene was considered to be differentially expressed if at least 50 % of the corresponding probes were differentially expressed and if the ratio of expression was upper than 2 or lower than 0.5.

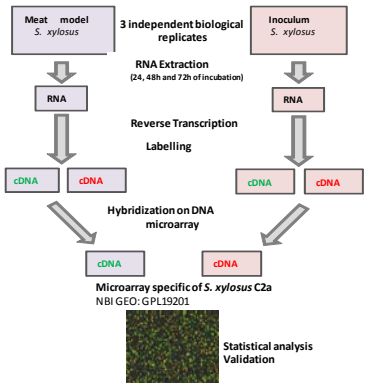

Figure 1: The different steps of the transcriptomic experiment

III. RESULTS AND DISCUSSION

Growth of S. xylosus and transcriptome profile

The growth of *S. xylosus* in the pork meat batter was exponential until 24 h, reached 9.0 log CFU/g and remained almost at this level of population until the end of the experiment (72h).

The *in situ S. xylosus* response revealed a global change in the gene expression during its survival in the meat model by comparison with the inoculum. There were 1278, 1258 and 993 genes differentially expressed at 24, 48 and 72 h, respectively. Noteworthy, 782 genes out off the 2634 genes were differentially expressed at the three times of incubation. To have a global view, we focused specifically on the 782 common genes with 356 up- and 426 down -regulated. These common genes have been classified into different functional categories; the most represented being the information storage and processing, the cellular processes and the metabolism (Figure 2). These genes differentially expressed between the inoculum and the culture in meat indicate that major transcriptional changes have occurred at 24 h and lasted during all the incubation time (72 h).

Carbohydrate catabolism

The expression of many genes encoding enzymes involved in glucose and lactate catabolism through the Embden-Meyerhof-Parnas pathway up to the respiratory chain and in cofactors synthesis necessary to their activities was up regulated. These results suggested that *S. xylosus* could simultaneously catabolize the glucose added to the batter and the lactate present in the pork meat.

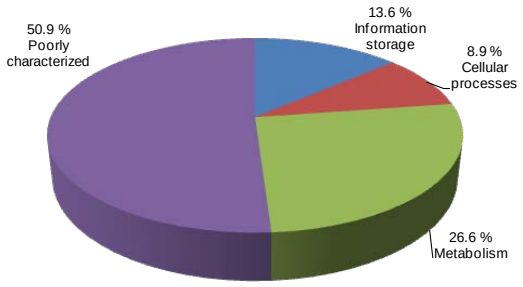

Figure 2: Percentage of genes differentially expressed in the pork meat batter at 24, 48 or 72 hours of incubation by comparison with the inoculum

Peptides, amino acids metabolism

Genes encoding transport of peptides and peptidases that could furnish amino acids were up expressed and thus concomitantly a lot of genes involved in amino acids synthesis were down regulated. The meat is rich in amino acids and peptides and *S. xylosus* can transport and use them. Several genes encoding degradation of glutamine to glutamate were highly up regulated. Glutamate served as the major amino group donors for all nitrogen-containing compounds, as link to nitrogen and carbon metabolism.

Response to osmotic stress

S. xylosus responded to salt added in the meat model by over expressing genes involved in transport and synthesis of osmoprotectants in particular glycine betaine, a powerful osmoprotectant, Na^+ and H^+ extrusion and in production of energy through the F_0F_1-ATPase.

IV. CONCLUSION

To our knowledge, this study is the first characterization of the adaptation of the starter culture *S. xylosus* in meat. It revealed a global change in the gene expression of this bacterium in this environment. It increased the understanding of how *S. xylosus* can grow and survive in meat.

REFERENCES

1. Vermassen, A., de La Foye, A., Loux, V., Talon, R. & Leroy, S. (2014). Transcriptomic analysis of *Staphylococcus xylosus* in the presence of nitrate and nitrite in meat reveals its response to nitrosative stress. Frontiers in Microbiology, 5:691. doi: 10.3389/fmicb.2014.00691.