WHAT IRON SOURCES IN MEAT ARE PREFERENTIALLY USED BY STAPHYLOCOCCUS XYLOSUS?

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Abstract – *Staphylococcus xylosus* is a ubiquitous bacterium frequently isolated from food of animal origin. This species is one of the major starter cultures used for meat fermentation. Meat is particularly rich in hemic and non-hemic iron. We have characterized the behaviour of *S. xylosus* towards the different sources of iron in meat. The growth rate of the *S. xylosus* strain C2a was depended of the iron source. Among these sources, ferritin was one of the preferential iron sources. Ferritin is a storage protein able to capture of large quantities of iron. This protein is resistant to microbial attack and few microorganisms can use it as an iron source. Our preliminary data from a microarray study in *S. xylosus* indicated that a cluster of three genes was highly up-expressed under ferritin condition and could be implicated in iron acquisition from ferritin. The corresponding deletion mutant showed a decrease in its ability to acquire iron from ferritin. This study characterized for the first time the capacity of a *Staphylococcus* to use iron from ferritin.

Key Words – acquisition of iron, ferritin, starter culture

I. INTRODUCTION

Staphylococcus xylosus belongs to the group of coagulase-negative staphylococci and historically this species has been defined as a non-pathogenic bacterium. It is a commensal of skin and mucous membranes of human and animal. This ubiquitous bacterium is usually isolated from food of animal origin and food processing environment. *S. xylosus* is one of the major starter cultures used for the fermentation of meat products. *S. xylosus* has the capacity to grow on meat, to survive during the steps of meat processing and to remain viable for long periods of time in fermented meat products, such as dry sausages [1, 2]. The ability of *S. xylosus* to use meat substrates contributes to its adaptation to this environment.

Meat is an iron-rich substrate but this element lacks of bioavailability, being complexed within the heme as a cofactor for myoglobin or hemoglobin, or bound within ferritin or transferrin [3]. Iron is a key element, required for survival and for many cellular processes for almost all bacteria [4]. The whole-genome sequencing of the *S. xylosus* reference strain C2a (LN554884) has revealed that this species has many iron-related genes encoding proteins that could be involved in either transport or binding.

The behaviour of *S. xylosus* towards the different sources of iron in meat was characterized. *S. xylosus* was able to grow in presence of ferritin as sole source of iron which has been described in only few bacteria. This iron source is abundant in meat and we have identified in *S. xylosus* a system implicated in ferritin-iron uptake.

II. MATERIALS AND METHODS

To investigate whether *S. xylosus* has source-dependent iron preference, the C2a strain was first serially passaged in an iron-deficient medium to deplete iron store. Then we cultured the C2a iron-starved strain in iron-free chemically defined medium supplemented with $FeSO_4$ or the different sources of iron found in meat: ferritin, myoglobin, hemoglobin and transferrin. Cells were grown at 30°C in 100-wells microtiter plate in a Bioscreen C plate reader (Labsystems). Every 30 min, the OD600 nm was measured. Three independent experiments were done for each condition.

To inactive the cluster of three genes potentially implicated in ferritin-iron uptake, we made a deletion of part of the coding sequence and an insertion of an erythromycin resistance gene using the temperature-sensitive vector pBT2 as described Brückner [5]. The resulting mutant strain was cultured in presence of ferritin or $FeSO_4$ as described above.

III. RESULTS AND DISCUSSION

The growth of *S. xylosus* C2a was measured using Bioscreen C plate reader on different iron sources: $FeSO_4$, myoglobin, hemoglobin, ferritin and transferrin. The growth curves were compared and the maximum specific growth rates (µmax) were calculated (Table 1). The growth of C2a strain was very weak in the iron-free chemically defined medium. Its growth was enhanced in the presence of iron sources and was dependant of the nature and the concentration of those. The addition of ferritin as sole iron source significantly increased the growth of *S. xylosus* C2a and a maximal specific growth rate was reached with 5 µM of ferritin (Table 1).

Table 1 Effect of the iron source on maximum specific growth rate (µmax) of *S. xylosus* C2a

Iron source	µmax (h^{-1})
FeSO4 (50 µM)	0.23
Myoglobin (40 µM)	0.13
Hemoglobin (20 µM)	0.23
Ferritin (5 µM)	0.38
Transferrin (80µM)	0.23

To determine if the cluster of three genes highly up-expressed under ferritin condition in *S. xylosus* C2a was implicated in an uptake system contributing to the specific acquisition of this iron source, we constructed a deletion mutant strain. No difference of growth was observed between the corresponding mutant and the wild type strain after growth in the iron-free chemically defined medium supplemented with $FeSO_4$. A significant difference of growth between the mutant and the wild type strain was observed after growth of the strains with ferritin as sole iron source (Fig. 1).

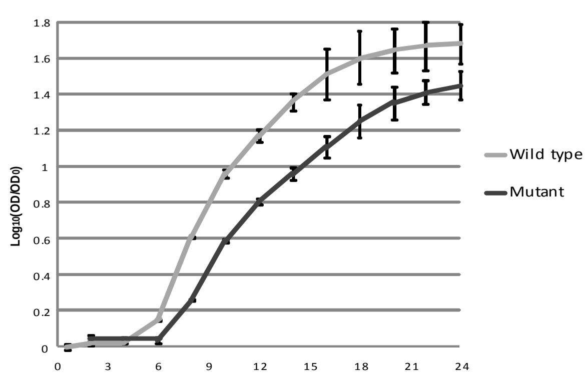

Figure 1. Growth of *S. xylosus* C2a *vs* mutant in presence of ferritin 0.5 µM as sole iron source

IV. CONCLUSION

Depending on the iron sources, the growth of the *S. xylosus* was more or less promoted. We revealed that one of its preferential sources of iron is ferritin which is abundant in meat.

This study characterised for the first time the capacity of a *Staphylococcus* to acquire iron from ferritin. This capacity could give to *S. xylosus* an advantage to growth or survive in meat. Moreover, we identified a cluster of three genes coding a system specifically involved in the ferritin-iron uptake.

REFERENCES

1. Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R. & Parente, E. (2004). Diversity and dynamics of communities of coagulase-negative staphylococci in traditional fermented sausages. Journal of Applied Microbiology 97, 271-284.
2. Corbiere Morot-Bizot, S., Leroy, S. & Talon, R. (2006). Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. International Journal of Food Microbiology 108, 210-217.
3. Linder, M. C., Nagel, G. M., Roboz, M. & Hungerford, D. M., Jr. (1981). The size and shape of heart and muscle ferritins analyzed by sedimentation, gel filtration, and electrophoresis. The Journal of Biological Chemistry 256, 9104-9110.
4. Wandersman, C. & Delepelaire, P. (2004). Bacterial iron sources: From siderophores to hemophores. Annual Review of Microbiology 58, 611-647.
5. Brückner, R. (1997). Gene replacement in *Staphylococcus carnosus* and *Staphylococcus xylosus*. FEMS Microbiology Letters 151, 1-8.