GENOMICS APPLIED TO THE FERMENTED MEAT ISOLATE *STAPHYLOCOCCUS XYLOSUS* IMDO-S216: ANTIMICROBIAL COMPOUNDS AND COMPETITIVENESS FACTORS

A. Sosa-Fajardo[1*], C. Diaz-Muñoz[1], D. Van der Veken[1], I. Pradal[1], M. Verce[1], S. Weckx[1], F. Leroy[1]

[1]Vrije Universiteit Brussel (VUB); Faculty of Sciences and Bioengineering Sciences; Research Group of Industrial Microbiology and Food Biotechnology (IMDO)

*Corresponding author email: ana.sosa.fajardo@vub.be

I. INTRODUCTION

Staphylococcus xylosus, a species within the group of Gram-positive catalase-positive cocci (GCC), often plays a leading role in the process of meat fermentation, either as starter culture or as a member of the natural microbiota [1]. This can be ascribed to both its relatively high competitiveness in fermented meat matrices compared to other GCC and its range of biotechnological abilities that contribute to the development of sensory properties, in particular colour and flavour. In addition, there may be potential to improve the biosafety of fermented meat products based on the production of antimicrobial compounds. However, while such compounds have been studied for lactic acid bacteria, they remain largely unexplored in the GCC community [2].

Whereas the presence of genes encoding for metabolic functionalities within the genome of *S. xylosus* has been explored previously, a detailed look into the antimicrobial capabilities of the species is lacking [3]. Recently, the fermented meat isolate *S. xylosus* IMDO-S216 was shown to exert antibacterial action against related staphylococci, indicative of the production of an antimicrobial compound that remained, however, uncharacterised [4].

The aim of the present study, therefore, was to obtain a complete genome sequence and a detailed annotation for *S. xylosus* IMDO-S216, in particular with respect to the presence of antimicrobial potential and other competitiveness factors.

II. MATERIALS AND METHODS

Genomic DNA of *S. xylosus* IMDO-S216 was sequenced using a combination of long-read sequencing (MinION, Oxford Nanopore Technologies) and short-read sequencing (NovaSeq, Illumina). Next, the sequence reads were *de novo* assembled (Unicycler), and the resulting genome was manually curated and annotated using different software tools (antiSMASH, Prokka, blastp, and Artemis) for the identification of secondary metabolite biosynthesis gene clusters (BGCs). Procedures were similar to what has been previously described [5].

III. RESULTS AND DISCUSSION

A complete genome of *S. xylosus* IMDO-S216 was obtained containing one circular chromosome and eight plasmids. Ten biosynthetic gene clusters (BGCs), located on the chromosome, were identified. One of these BGCs was identified as bacteriocin-encoding, namely the bacteriocin lactococcin 972, for which all necessary genes were present (Fig. 1). In addition, five other genome clusters were found, encoding competitiveness factors. Based on functional annotation, these clusters were found to be involved in a diminished sensitivity to antimicrobial peptides and competing microorganisms (D-alanylation of lipoteichoic acids), to affect membrane fluidity at low temperatures (staphyloxanthin), to participate in iron uptake from the medium during environmental conditions of

iron limitation (staphyloferrin A), to be involved in the biosynthesis of isoprenoids (mevalonate pathway), and to serve as a quorum sensing system for Gram-positive bacteria (Agr system).

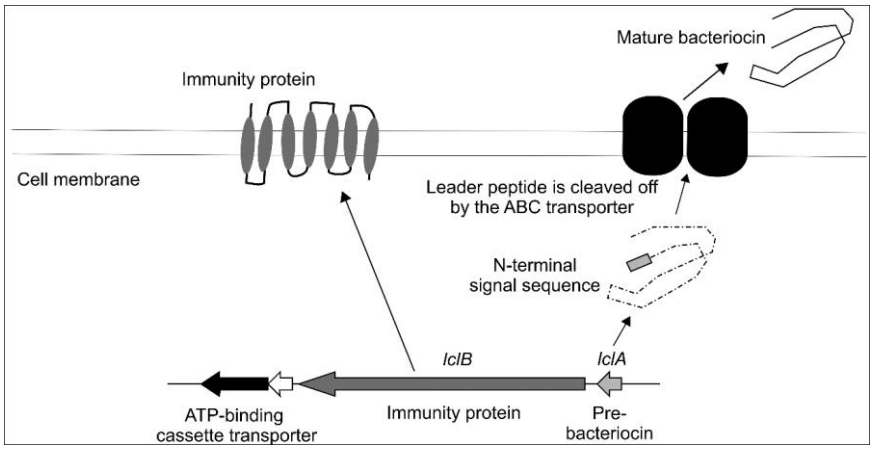

Fig. 1. Presumed biosynthesis pathway of lactococcin 972 from its annotated genome cluster [6].

IV. CONCLUSION

The combination of a bacteriocin gene cluster along with five other competitiveness factors encoded within the genome of *S. xylosus* IMDO-S216 suggested a contribution of this genomic toolbox to the fitness of the strain in a fermented meat environment. To further evaluate the importance of this genomic potential, the study of the expression of these gene clusters and the biosynthesis of their products is required.

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