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Genetic and proteomic comparison of *Staphylococcus aureus* isolates causing gangrenous or subclinical intramammary infections in a dairy sheep flock

Le Maréchal, C.^{1,2}, M. Chevalier², G. Jan¹, J. Jardin¹, S. Even¹, J.A. McCulloch¹, F.R. Rosado¹, V. Azevedo³, R. Thiéry², E. Vautor² and Y. Le Loir¹.

¹INRA, Agrocampus Ouest, UMR1253 STLO, Rennes Cedex, France ²Unité de pathologie des ruminants, AFSSA Sophia Antipolis, France ³ICB, Federal University of Minas Gerais, Belo Horizonte, Brazil
caroline.le-marechal@rennes.inra.fr / yves.leloir@rennes.inra.fr

Introduction

Staphylococcus aureus is a major causative agent of mastitis in ruminants worldwide. Infection acuteness ranges from subclinical to lethal gangrenous mastitis. The genomic and proteomic profiles of 2 strains isolated from ewe mastitis (1 subclinical and 1 gangrenous) were compared.

S. aureus strains analyzed and approaches used

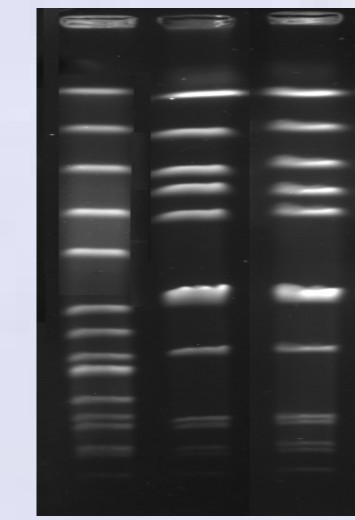
***S. aureus* O11:** isolated from a ewe gangrenous mastitis in South East of France, november 2002



***S. aureus* O46:** isolated from a ewe subclinical mastitis in South East of France, january 2002



Genotyping of O11 and O46



Macrorestriction of the chromosome (pulse field gel electrophoresis): O11 and O46 belong to the same pulsotype

Spa type: O46 (Spa type 3568) and O11 (Spa type 524) belong to the same Spa clonal complex (1173)

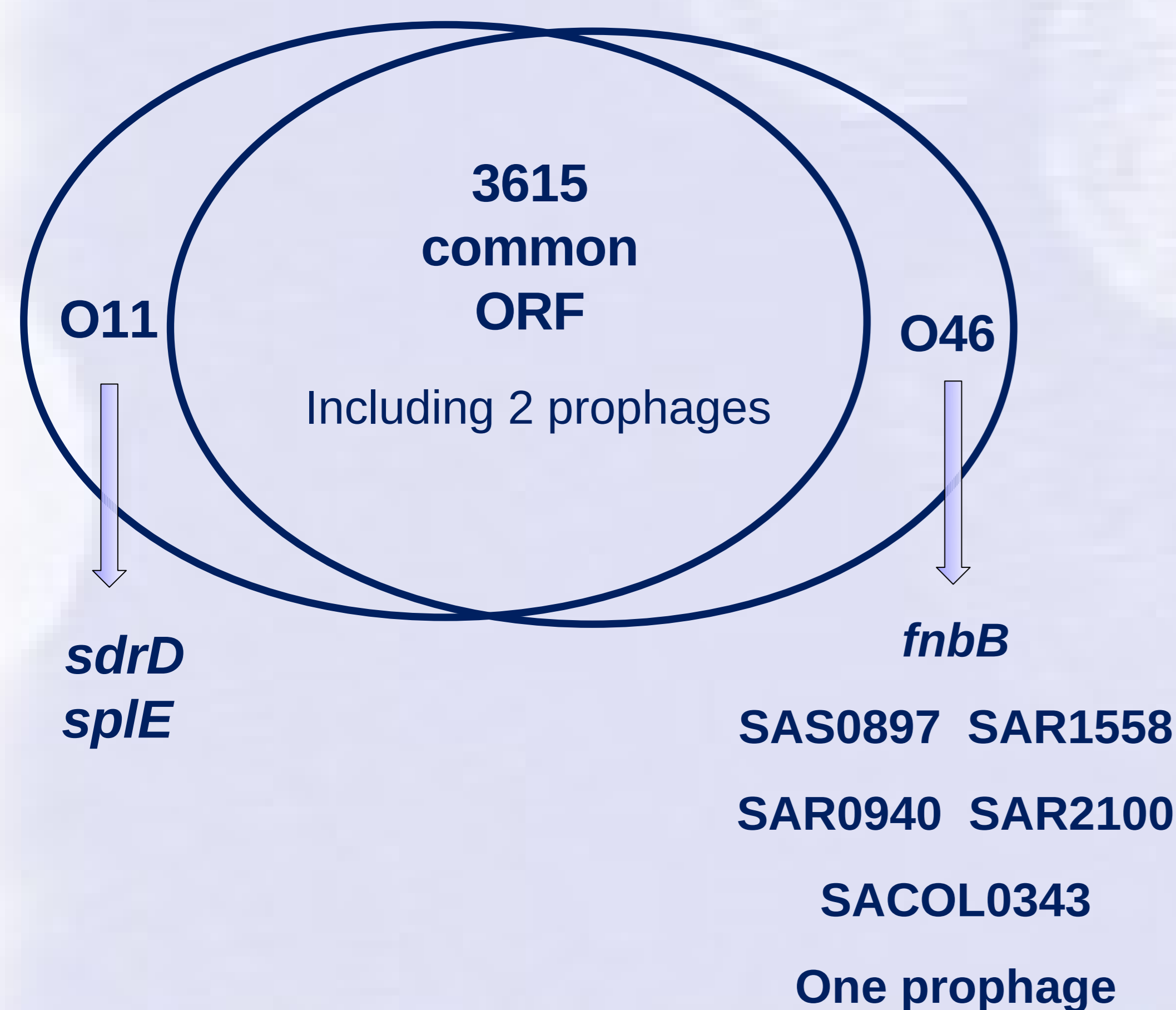
MLST: O11 (6-33-6-2-7-12-7/18) and O46 (6-2-6-2-7-14-2/5) belong to the same Sequence Type (ST130)

- Strain characterisation
 - Multiple techniques used for O11 and O46 genotyping
- Strain comparison
 - Comparative genomics: DNA-DNA hybridization on a pangenomic microarray
 - Comparative proteomics: 2D gels on samples prepared from culture on iron depleted RPMI (without aeration)

→ *S. aureus* O11 and *S. aureus* O46 are genotypically closely related. These results were reinforced by other genotyping techniques (MLVA, RAPD, Sas Typing)

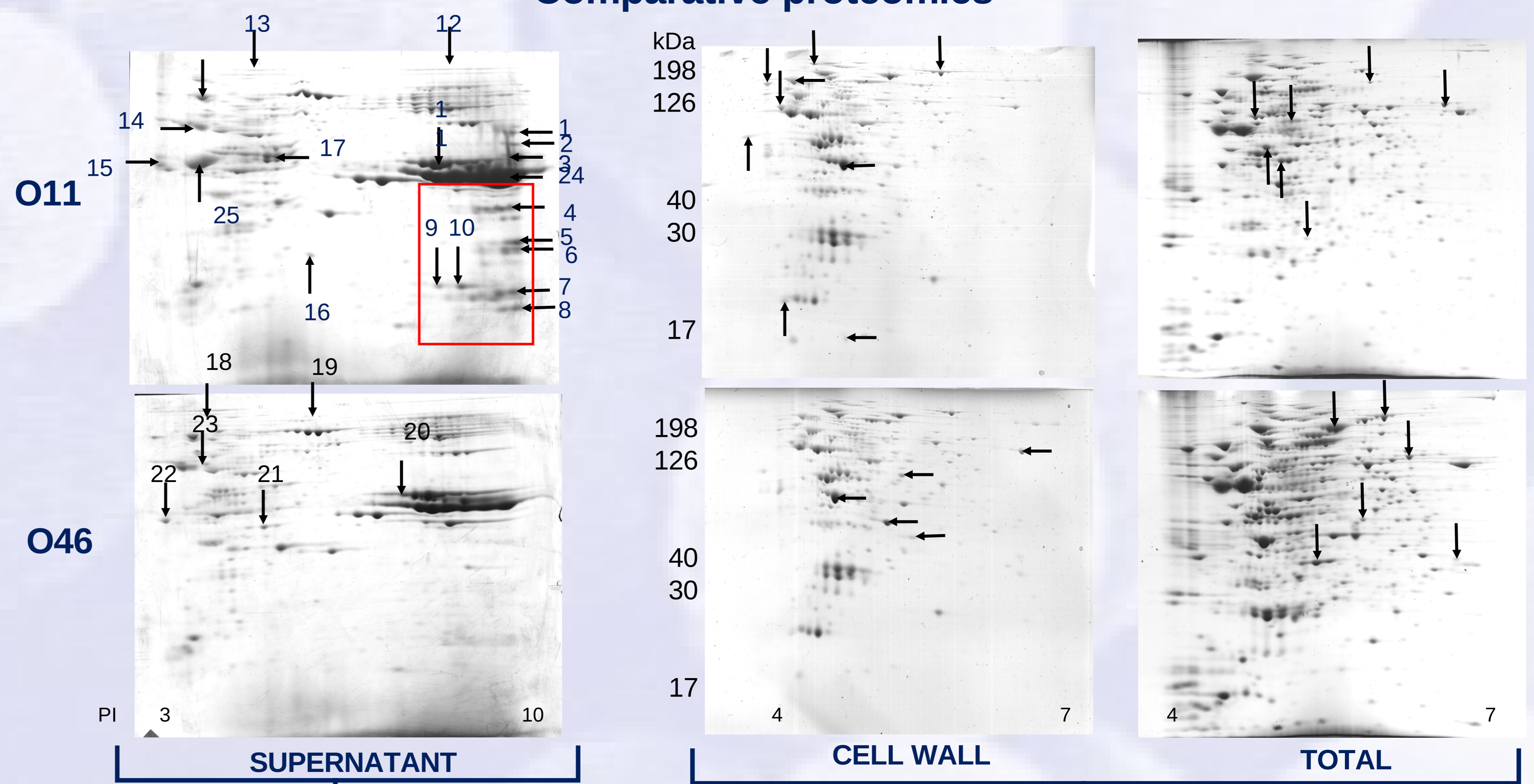
Genomic and Proteomic comparison of O11 and O46

Comparative genomics



→ Gangrenous mastitis isolate (O11):
- variant versions of several surface proteins (*sdrD* and *spIE*)
- missing *fnbB* and a prophage.

Comparative proteomics



→ Overproduction of secreted proteins by O11 (especially those indicated in the red square)

→ Very few differences between O11 and O46 (indicated by arrows)

- Immunoglobulin-binding protein *sbi* (1,7,24)
- Alpha-hemolysin, Gamma-hemolysin (12,6,24)
- LukF-PV, LukM, uncharacterized leukocidin-like protein1/2 precursor (12,6,7,24,11,7,8,2)

- Serine protease *spIE*, F, B, C, (25,4)
- Staphopain A, B (4)
- Phospholipase C (24,11,12)
- Iron-regulated surface determinant protein B, H, A (13,12,7,4)

Conclusions & Perspectives

- Despite opposite pathogenicity degrees, O11 and O46 are closely related as shown by both genotyping and CGH.
- CGH approach revealed that gangrenous mastitis isolate (O11) carried variant versions of several surface protein genes (*sdrD* and *spIE*) and was missing *fnbB* and a prophage.
- Cell wall and cytoplasmic proteomes were almost similar whereas secreted protein pattern were clearly different: O11 overproduces toxins including LukM/F'PV (important factor in the development of gangrenous mastitis in ruminants?).
- Serine protease E (encoded by *spIE*) together with other serine proteases were oversecreted by O11. Association between secretion of serine proteases and mastitis acuteness remains unclear.