

Cloning and Expression of Some Iron-Regulated Genes of Bovine Mastitis *Staphylococcus Aureus*

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Mastitis due to *Staphylococcus aureus* is difficult to control with antibiotics. In order to develop alternatives to antibiotherapy, new cellular targets specific to *S. aureus* must be discovered. The characterization of the iron-acquisition mechanisms of *S. aureus* could lead to development of new strategies to efficiently control this bacterium. Fourteen *S. aureus* isolates from bovine mastitis cases as well as four isolates from food (milk and cheese) and four reference strains were used to evaluate the ability of *S. aureus* to grow on iron-depleted media. All strains were found to produce siderophores and to tolerate 0.64 mM of the iron chelator 2,2'-dipyridyl in their culture medium except one strain (GP-31) which tolerated only 0.32 mM. The iron related *sirA* (SACOL0099), *sirB* (SACOL0098), *sirC* (SACOL0097), *fhuG* (SACOL0706), *fhuD2* (SACOL2277), *srtB* (SACOL1145) and *harA* (SACOL1781) genes were detected in all strains, whereas the *fhuD1* (SACOL2010) gene was not detected in three strains (two mastitis and one food strains). Cloning of the *sirA*, *fhuD2*, *srtB* and *harA/isdH* genes, followed by the expression and purification of the corresponding recombinant proteins specifically revealed the strong immunogenic character of the HarA protein resulting in a high antibody titer in rabbits. Our results confirm that *S. aureus* is well equipped to survive in an iron stressed environment using different iron uptake strategies. This study also shows that *S. aureus* iron related proteins can induce variable degrees of immunological responses. The potential use of these proteins as antigens for the development of effective vaccines against this pathogen must be carefully evaluated.

Implication for the dairy industry. Our data confirms that *S. aureus* is a versatile pathogen that can obtain iron for growth from various sources using several iron related genes well conserved among strains from different origins. We were able to clone and express four most iron regulated genes as recombinant proteins, among which, HarA induced the strongest antibody response in rabbits suggesting that this protein might be a useful candidate for the development of efficient control strategies against *S. aureus* in mastitis and dairy products.