Expression of Heme Related Genes in *Staphylococcus Aureus*

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Heme uptake by Staphylococcus aureus play important role in virulence and is mediated by S. aureus surface determinants coded by several isd genes. In this study we compared the expression of some heme-uptake genes in 16 S aureus SHY97-3906 mutants obtained by using 1-Methyl-3-nitrosoguanidine mutagenesis. Identification of factors that affect surface related virulence and iron-acquisition functions may lead to develop new control strategies.

Biofilm production was determined in vitro using crystal violet staining and hemolysin production was determined using TSA-blood plates. RNA was extracted and reverse-transcribed to obtain cDNAs for quantitative PCR of isdG, isdA, isdD, isdC, srtB, hlb, and icaD genes. The ability to colonize the mouse mammary glands was also assessed. **Results:** Biofilm production was reduced in all but three mutants when compared to the parental strain. Five mutants showed reduced hemolysis activity. Important differences in the expression of the studied genes were observed among the 16 mutants. One mutant (MSD17) was found to down regulate all isd genes as well as the hemolysin hlb gene. In another mutant (MSD15), all genes but isdA and isdC were down regulated and MSD02 was found to significantly down regulate isdG involved in the iron release from heme. At 12 hrs post infection, approximately 1-Log_{10} decrease of viable bacterial counts (CFU/gram of gland, $P < 0.05$) was observed for mutant MSD15 compared to the parent strain, demonstrating the reduced capacity of this mutant to colonize the mammary gland. **Conclusion:** Further investigation is warranted for mutants MSD02, MSD15 and MSD17 that have apparently acquired mutations in genes important for heme uptake and mammary gland colonization. In vivo, synergistic actions may exist between hemolysins and S. aureus cell surface proteins that offer an advantage in evading host immune defenses.

Implications. Characterization of molecular events underlying the iron acquisition and assimilation by S. aureus is important and allows understanding basic mechanisms of its interaction with its host. The study of mutants gives opportunity to gain access to such knowledge. These data could lead to the development of control measures against S. aureus infections in dairy cows.