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## **Post-milking application of a *Lacticaseibacillus paracasei* strain on the teat skin as a new microbial strategy to prevent bovine mastitis**

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### Post-milking application of a *Lacticaseibacillus paracasei* strain on the teat skin as a new microbial strategy to prevent bovine mastitis

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Bovine mastitis is an inflammation of the mammary gland generally due to an intramammary infection. Prophylactic treatments mostly rely on post-milking teat disinfection with chemical products such as iodine. However, the latter are questioned due to a possible irritation of the teat skin but also dissemination of residues in milk and environment. Besides, the antibiotics commonly used to treat mastitis are unfortunately not entirely effective and may contribute to the risk of dissemination of antimicrobial resistance, inviting us to explore alternative solutions. Here, a microbial strategy aiming to reinforce the barrier effect of the microbiota naturally present on the teat through the application of *Lacticaseibacillus paracasei* CIRM BIA 1542 (Lp1542), was explored on 20 Holstein cows in mid-lactation. Treatment (Lp1542, iodine or no treatment) was applied post-milking twice a day on the 4 teats of healthy animals for 15 days. Blood and milk samples, and teat skin (TS) swabs were collected at day D1, D8, D15 and D26 before morning milking and at D15 before evening milking (D15E) to evaluate Lp1542 impact at the microbial, immune and physiological levels. Lp1542 treatment resulted in a higher lactic acid bacteria and total microbial populations on TS and in foremilk (FM) at D15(E) than with iodine treatment. Metabarcoding analysis revealed changes in the composition of TS and FM microbiota, beyond a higher *Lacticaseibacillus* abundance. In addition, Lp1542 treatment did not trigger any major inflammatory response in the mammary gland, except interleukin 8 production and expression which tended to be slightly higher in Lp1542-treated cows compared with the others. Finally, Lp1542 treatment had no impact on the mammary epithelium functionality (milk yield and composition) and integrity (epithelial cell exfoliation into milk and milk Na<sup>+</sup>/K<sup>+</sup> ratio). Altogether, these results indicate that a topical treatment with *L. paracasei* CIRM BIA 1542 is safe with regard to mammary gland physiology and immune system, while impacting its microbiota, inviting us to further explore its effectiveness for mastitis prevention.