

## Extending probiotic science beyond human health: Design and application of a novel spray-based formula for sustainable disease management in California honey bees

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### Abstract

Managed honey bee (*Apis mellifera*) populations play a crucial role in supporting adequate pollination of food crops but are facing unsustainable colony loss as the result of rampant disease spread within agricultural environments. Antibiotics have failed to resolve the issue so far, whereas mounting evidence from *in vitro* experiments suggest that select lactobacilli strains (some of which are symbionts in honey bees) can inhibit a broad range of important pathogens via multifaceted mechanisms. Importantly, there has been very little validation at the field-level potentially due to the fact that delivery methods for applying viable lactobacilli to the hive are lacking. Here, we compare how different delivery systems (standard pollen patty and a novel spray-based formula) influence the efficacy of a three-strain lactobacilli consortium (LX3) in reducing overall bacterial and fungal disease burden within a pathogen-dense region of California post-almond harvest. Hives were supplemented for 4-weeks, followed by a 20-week monitoring period. Results reveal long-lasting beneficial effects of LX3 in delivery-dependent and -independent manners. The most striking finding was that spray-based LX3 supplementation led to >100-fold reduction in *Ascosphaera apis* (deadly fungal agent of Chalkbrood disease), whereas patty-based LX3 showed unique nutritional benefits. In addition, spray-based LX3 was also highly active against many well-known opportunistic plant pathogens in the hive suggesting this method may be especially useful for reducing honey bee vectoring of plant diseases. The collective scope of this work is expansive and broadly relevant to microbial disease management in terrestrial ecosystems.