

Structural and functional insights into the ‘giant’ adhesin protein from *Apilactobacillus kunkeei*

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Apilactobacillus kunkeei, a prevalent bacterial species in the gut of honey bees (*Apis mellifera*), plays a crucial role in pollination and agriculture. Known for its probiotic properties, it colonizes the honey gut and produces the antibiotic kunkecin, which targets *M. plutonius*. Our genome analysis revealed a set of orphan genes encoding Giant proteins, characterized by their extensive size and secretion, hinting at a potential adhesion function. Using AlphaFold2, an AI-based structure prediction tool, we generated high-confidence models for these Giant proteins, overcoming the absence of structural homologs. Our predictions unveil a structural organization consisting of a beta-solenoid N-terminal domain, repeated long alpha-helices, and a SH3-like C-terminal domain. To dissect the structural and functional roles of these Giant proteins, we engineered recombinant constructs representing truncated N and C terminal domains. Employing SEC-SAXS, we anticipated their low-resolution dimensions and structural configuration. Additionally, single-particle cryo-EM analysis yielded a 2.5Å resolution map, validating our AlphaFold2 predictions. Further investigations involved a screen kit (with a diverse array of sugars), mainly sugars in honey and commercial honey samples to probe the interaction with the N-terminal construct, employing DSF thermal shift assays. Additionally, for future binding experiments, GFP-fused constructs of the N and C terminal domains were engineered.

Through a comprehensive integration of structural and biophysical data, our project aims to elucidate the molecular mechanisms underlying the probiotic effect of *Apilactobacillus kunkeei* in the honey bee gut.