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Characterization of PlyPl23, an endolysin from a Paenibacillus larvae bacteriophage

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Bee's pollination presents a great impact in a wide range of terrestrial ecosystems. The estimated world value for the contribution of pollinators, especially bees, to the production of crops used directly for human food was estimated in €153 billion. Yet, beekeeping is facing many challenges, as changes in agricultural practices, extensive use of pesticides and the presence of pathogens. One of the most widespread and the most destructive of the honeybee brood diseases is the American foulbrood (AFB), caused by Paenibacillus larvae, a Gram-positive bacterium wherein the spore is the infectious form. In most situations P. larvae is highly virulent and when contracted will kill a complete colony. This represents an economic threat to commercial beekeepers and apiculture worldwide. The European regulation limits the presence of antibiotics in honey, excluding its use for therapy.

The antibacterial features of (bacterio)phages and endolysins were considered for the development of a therapeutic product for this disease. A P. larvae phage, phiIBB_Pl23, belonging to the Siphoviridae family was isolated and its genome has been sequenced and analyzed. Annotation of the phiIBB_Pl23 genome identified gp21 as an endolysin protein, designated as PlyPl23, which was further characterized and is presented herein. In silico analysis revealed that PlyPl23, is a single domain globular protein, which is a rare feature of Gram-positive endolysins. The catalytic domain was identified as an N-acetylmuramoyl-L-alanine amidase (pfam Amidase_2 family) with a molecular weight of 25.8 kDa and isoelectric point of 5.74. Activity tests were performed at 37oC, which is similar to the hive temperature (about 34-35ºC) and revealed that this is a broad-spectrum endolysin for P. larvae with an optimal pH for reactivity between 3 and 5. This matches the pH levels of honey, nectar, pollen, and royal jelly (pH 3 to 4). The enzyme also displays a high antimicrobial activity between 5 and 7 when supplemented with NaCl, matching also the intestinal pH of bee larvae (pH 6.8) and adult bees (pH 5.6 to 6.3). Furthermore, tests performed with honeybee larvae juice revealed a higher bacterial decrease reduction, which may be a good indicator for the in vivo effectiveness of the endolysin.

The present work describes, to our knowledge, the first characterization of an endolysin from a P. larvae phage. Moreover, characterization of endolysin PlyPl23 showed that it presents high potential in the development of a commercial product to control the problematic AFB.