

Summary of the Ph.D thesis No., 17891. . **Bacteriology**, Faculty of Veterinary Medicine, Urmia University.

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Title: Genomic search of *Melissococcus (Streptococcus) pluton* in honey bee larvae in Takab city.

Abstract:

European locust is a disease affecting honeybee larvae and is considered one of the dangerous diseases for bee colonies. It is caused by the Gram-positive bacterium *Melissococcus plutonius*. This disease is sporadically distributed worldwide, and recent advances in molecular technology have made the identification of the bacterium much easier. Nucleic acid detection technology is rapidly replacing traditional microbiological methods. Most studies conducted in Iran have aimed to establish the PCR method for the rapid and reliable diagnosis of this disease. European locust is endemic in many regions and causes seasonal epidemics. The method involved setting up the standard bacterial strain using specific PCR primers. European locust in bees is a seasonal disease, primarily emerging from mid to late spring, coinciding with the breeding season. Like American locust, it is present in most parts of the world. The disease agent was discovered in 1949 by Mrs. Alexandrowa and was initially named *Streptococcus pluton*, later renamed *Melissococcus pluton*. The causative agent is a saprophytic or opportunistic bacterium. During outbreaks, other bacteria such as *Bacillus alvei* and *Bacterium eurdice* also act as secondary agents. Unlike American locust, European locust does not have uniform and consistent symptoms. It is not confined to Europe but is widespread in all countries where the *Apis mellifera* species is extensively bred. The disease is more prevalent in spring and early summer and, in some populations, appears with less intensity in autumn. Its damage is less severe compared to American locust. The infectious cycle of European locust begins when larvae consume food contaminated with *Streptococcus plutonius*, which establishes itself in the midgut of the bee. Most larvae die at four to five days old, but some may resist European locust and develop into adult bees. The causative agent of European locust is *Melissococcus pluton* (*Streptococcus pluton*), a Gram-positive, non-spore-forming bacterium that appears in clusters. The present study aims to explore the genome of *Melissococcus pluton* and assess the prevalence of infection in honeybee larvae in apiaries across different regions of Takab County. It also seeks to sequence the *16SrRNA* gene of *Melissococcus pluton* and construct its phylogenetic tree. Given the unknown level of infection and prevalence of *Melissococcus (Streptococcus) pluton*, and considering an eight percent prevalence rate in Takab County, 40 samples of honeybee larvae and newly born bees were collected from apiaries in Takab County. The collected samples were immediately transferred on ice to the bacteriology laboratory of the Faculty of Veterinary Medicine. The genome of *Melissococcus pluton* in the collected larval samples was extracted using the DNA extraction method by Dong and colleagues. Out of 120 larval samples collected from apiaries in Takab County, PCR was performed based on the *16SrRNA* gene, identifying the genus *Melissococcus pluton*, and positive samples were obtained.

For phylogenetic analysis, the positive samples were sent to Pishgam Company for Nested-PCR sequencing. The obtained sequences were then registered in the GenBank (NCBI), and the isolated sequences were subjected to a BLAST test in the GenBank.

Keywords: *Melissococcus pluton*, larvae, PCR.