

Investigations on the gut microbiota of the Maltese honeybee (*Apis mellifera ruttneri*)

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In the last decade, the fast development of next generation sequencing (NGS) techniques has enhanced the study of microbial populations in different ecological niches. Honeybees, due to their important role in the pollination of economically important crops, have been a favourite object of NGS studies.

In 1997, a new subspecies of honeybee, *Apis mellifera ruttneri* was described as endemic to the Maltese Islands. It belongs to the African honeybee subgroup, and is more closely related to *Apis mellifera intermissa* and *Apis mellifera sicula* rather than other subspecies. Maltese honeybees show peculiar characteristics of adaptation to drought, heat and strong wind. These characteristics were developed after a long period of isolation, presumably during the Pleistocene.

In this study, the gut microbiota of *Apis mellifera ruttneri* was studied through microbial dependent and independent techniques, hypothesising particular distinctive differences from the rest of the European honeybees. Twenty bees for culture dependent techniques and 30 bees for microbial culture independent techniques were sampled from three different beehives of Maltese honeybees located in Għargħur, Msida and Żejtun. Tenfold serial dilution of gut content and culturing on Man Rogosa Sharpe Agar (MRS), on Tryptone, Phytone, Yeast extract Agar (TPY) and on Trypticase Soy Agar (TSA) plates was carried out. This led to the isolation of 43 microbial species. Genomic DNA was extracted from honeybee gut, and the V3-V4 regions of 16S rRNA were amplified, run in denaturing gradient gel electrophoresis (DGGE) and sequenced on the MiSeq Illumina platform with an average sequence yield of 200.000 seq. per bee. Sequencing results were compared with those of other studies on different honeybee subspecies.

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