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IDENTIFICATION OF BACTERIA LIVING IN THE CHRYSOLINA HERBACEA GUT BY CULTURE-BASED APPROACHES

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Chrysolina herbacea (the mint beetle), is a phytophagous beetle that establishes specific relationship with the plant *Mentha aquatica* (the watermint). In particular, the ability of *C. herbacea* to use volatiles emitted by undamaged *M. aquatica* plants as attractants and the plant's response to herbivory involving the production of deterrent molecules, have been recently described (1). Moreover, there is evidence that some *Chrysolina* species produce deterrents to natural enemies from plant-derived compounds (2-4).

The objective of the present study was to examine the gut microbial flora by using culture-based approach. A total of 10 single insect pools (male or from female *C.herbacea*) were placed in a sterile physiological solution (0,9% NaCl). Each insect pool suspension was decanted and rinsed 3x10ml of sterile 0,9% NaCl solution. Then, the rinsed insect pool was treated with 3% H_2O_2 for 20s, and finally rinsed with 70% ethanol and rapidly flamed. The throat of each insect was cut with a sterile scalpel and the head was removed. Pressing on the paunch of the cutted insects the total intestine was collect in 5ml LB broth containing 2-3g of sterile glass beads (ϕ 0,5mm) , heavily vortexed for 4-5min and left to elute overnight at 4°C. To remove gut debris, samples were centrifuged at 2000 g for 1min. Then appropriate dilutions of the supernatant were transferred on the surface of NA, LB and YEPD solid media, respectively, and incubated at 30°C. The bacterial titers by plating on the upper different solid media were: on NA plates 10^7cell/ml ; LB plates 10^7 cell/ml ; YEPD plates 10^5 cell/ml . After 24 h incubation at 30°C , a number of colonies, from each agar plate media, were picked up randomly and streaked onto fresh plates. Only plates with colony numbers ranging from 50 to 200 were used for isolation of pure cultures. Pure cultures were checked by microscopy, and were stored either in LB agar slants or in LB broth plus 60% glycerol at -20°C.

By using culture-based approach we have analyzed more than 200 bacterial isolates from the digestive tract of *C. herbacea*. Most bacterial isolates belong to three species of the genera *Serratia*, *Pseudomonas* and *Sphyngomonas* with marked quantitative differences between male and female

individuals. Furthermore, Head-Space analysis by GCxGC qMS revealed the differential emission of hydroxylated cineole derivatives between male and female faeces (frass) Volatile Organic Compounds (VOCs). The hypotheses that these bacteria may be involved in metabolism of plant-derived compounds and that biotransformed metabolites may be used as recruitment/sex pheromones by female insects will be discussed. Because *C. herbacea* is quite diffuse in mint fields and herbivore feeding alters the aromatic profile of essential oil-producing plants like *M. aquatica*, the issue is not only ecologically but also economically relevant.

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