Development of real-time PCR targets for the detection of *Tenebrio molitor* and *Hermetia illucens*

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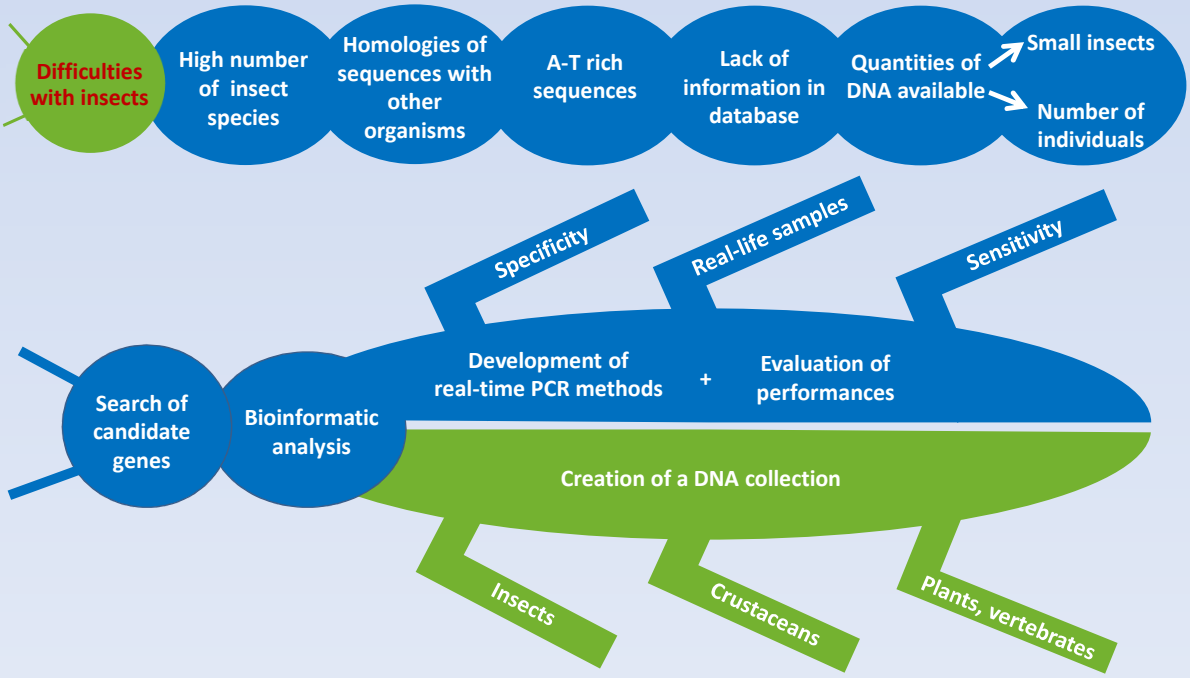
Context

Insects are rich in proteins and can be an alternative source of proteins to feed animals. Numerous companies started the production of insects for feed purposes. A lot of business models for feed production are based on the mealworm (*Tenebrio molitor*) or the black soldier fly (*Hermetia illucens*). In Europe, the processed animal proteins obtained from seven insect species have been authorized since the 1st of July 2017 for aquaculture by EU regulation 2017/893. Methods of authentication are required to check the conformity of the products. In this study, we propose a real-time PCR method for the specific detection of *T. molitor* and *H. illucens*.

Development of methods

Targets focused on insects (target common to all insects) and targets specific to particular insect species are required. Real-time PCR methods are developed at CRA-W in this way. At this stage, methods were only considered for qualitative purposes.



Results

Two targets for the detection of ***Tenebrio molitor***, based on the *wingless* and *cadherin* gene, and one target for the detection of ***Hermetia illucens***, based on mitochondrial COX3 gene, were proposed.

The specificity of each target was tested against a minimum of 34 insect species but also checked against plant and other animal species as crustaceans, mammals and birds. The target based on the *wingless* gene does not show aspecificities with the non-target DNA tested. For the second *T. molitor* target and for the *H. illucens* PCR test, the signals were observed with 3 insect species. These aspecificities should not cause problems because the signals are late and for the most part not reproducible.

The limit of detection of these three targets was estimated under 20 copies through the AFNOR XP V03-020-2 standard approach, reaching the recommended performance criteria.

The applicability of the tests was proved through the analysis of real-life processed samples (industrial meals).

Moreover, for the *H. illucens* PCR assays, the efficiency and robustness were also successfully tested.

This is a first step for the authentication of insects derived products. Tests for other insect species are under study.