Study and comparison of ivermectin metabolism and bioavailability in drug-sensitive and drug-resistant strains of the nematode model *Caenorhabditis elegans*.

Supervisors : Mélanie Alberich (melanie.alberich@inrae.fr), Anne Lespine (anne.lespine@inrae.fr)**.**
Unit/Area/Location: INRAE, Intheres Unit "Therapeutic Innovations and Resistances", Antiparasitic Axis, Ecole Nationale Vétérinaire de Toulouse (France).

Context : The project of the INRAE host team is part of the global context of the rational use of anti-parasitic drugs to ensure the efficient and sustainable control of parasites. The performance of anthelmintic drugs is challenged by the development of drug resistance in target organisms (e.g. nematodes). Faced with the lack of new active agents, a reasonable option is to preserve the efficacy of the most widely used anthelmintics today: the macrocyclic lactones (MLs). For this, it is essential to know the molecular determinants that prefigure the development of resistance to these drugs in the parasite target. In particular, drug detoxification systems in the pathogen contribute to the elimination of the active principle from the target, thus limiting its action. These systems are relevant targets for improving the therapeutic efficacy of MLs. The team showed an overexpression of genes involved in drug biotransformation and export, e.g. cytochromes P450 and ABC transporters, in the nematode model *Caenorhabditis elegans* strains resistant to MLs[1].

Objectives: The first objective is to study and compare the metabolism of a major anthelmintic, ivermectin, in sensitive and resistant strains in the nematode model *C. elegans*. The second objective is to evaluate if the resistance phenotype is correlated to a differential bioavailability in the nematodes. Several aspects will be explored:

(i) Search for drug metabolite(s): Initially, the student will search for the presence of the drug metabolite(s) by HPLC. The identification of these metabolites will then be conducted by mass spectrometry. The comparison of the metabolic profile of the drug in susceptible and resistant strains will allow to evaluate if the resistance leads to a differential production of metabolite(s) or not.

(ii) Quantification of drug metabolite(s): The student will quantify the metabolite(s) characterized in the different strains by HPLC. Comparison of the amount of metabolite(s) produced in susceptible and resistant strains will allow evaluating if the drug resistance comes from a differential production of metabolite(s).

(iii) Study of the bioavailability of the drug: The student, by comparing confocal microscope images of susceptible and resistant strains in the presence of the fluorescent drug, will assess the impact of resistance on drug localization and bioavailability.

(iv) Contribution of detoxification genes to drug fate: By using the fluorescent drug, the student will evaluate the bioavailability of the drug in parental strains, hypersensitive[2], resistant to the drug and/or deficient in proteins of interest (cytochromes, transporters). This will allow evaluating the specific role of these actors in the fate of the drug.

Methodologies: The student will grow and maintain different strains of *C elegans* under different experimental conditions, chosen according to the question posed. He/she will use RNA interference (RNAi) to modulate the expression of genes of interest. He/she will perform HPLC analysis and measurement of drug and residue concentration. This approach will assess the ability of detoxification systems to metabolize ivermectin. He/she will use confocal imaging to localize and study drug bioavailability. This study will provide relevant support and predictive tools to search for compounds capable of interacting with resistance processes, particularly by targeting nematode P450 cytochromes.

[1] C. Ménez, M. Alberich, D. Kansoh, A. Blanchard, A. Lespine, Acquired tolerance to ivermectin and moxidectin after drug selection pressure in the nematode Caenorhabditis elegans, Antimicrob. Agents Chemother. 60 (2016) 4809–4819. https://doi.org/10.1128/AAC.00713-16.

[2] C. Ménez, M. Alberich, E. Courtot, F. Guegnard, A. Blanchard, H. Aguilaniu, A. Lespine, The transcription factor NHR-8: A new target to increase ivermectin efficacy in nematodes, 2019. https://doi.org/10.1371/journal.ppat.1007598.