OMICS- Based Identification of Molecular Effects of Fungicidal Active Substances in Zebrafish Embryo

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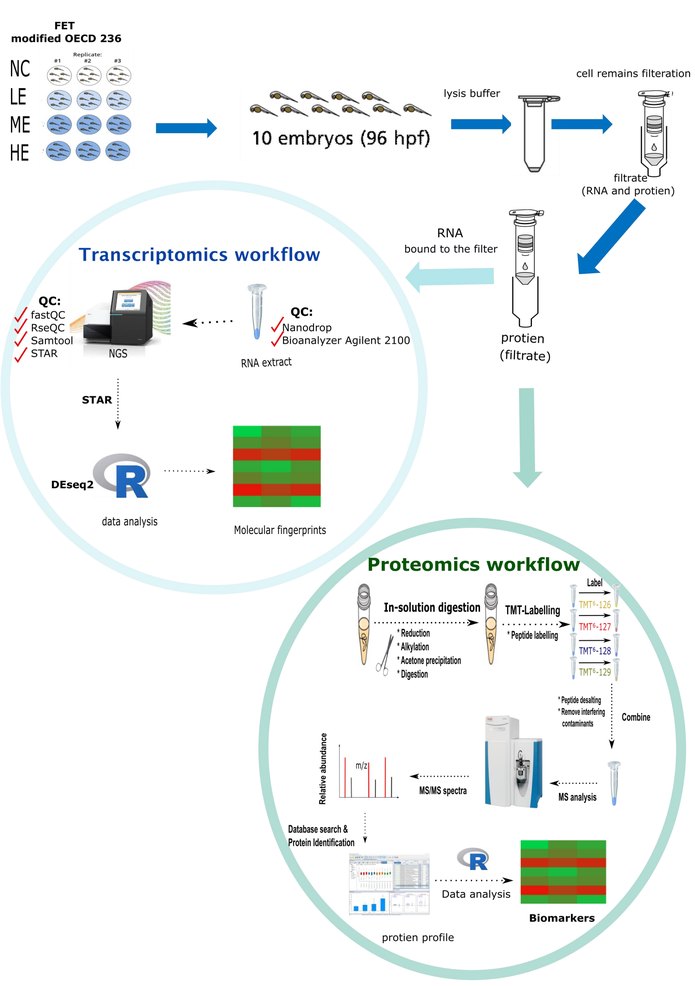
Abstract

This project aims at the identification of molecular changes induced by a number of FRAC (Fungicide Resistance Action Committee)-classified fungicidal active substances in zebrafish embryo in order to predict the adverse environmental effects and global gene expression changes induced by these substances. Zebrafish embryos were exposed to sub-lethal concentrations of fungicidal active substances (Difenoconazole and Carbendazim) for 96 hrs followed by simultaneous RNA and protein extraction from respective samples in order to analyse further effects induced by those substances in transcriptomic and proteomic profiles by applying RNA-Seq (transcriptome) and HPLC-Mass-Spec (proteome) analyses. The resulting unique molecular fingerprints will provide the basis for a mode-of-action (MoA)-specific screening of active substance precursors under development and monitoring of environmental samples in order to assess the environmental load of fungicide active substances.

Background

Active substances of fungicides introduced to the ecosystem in a targeted manner can exert adverse effects on non-target organisms, in particular populating soil and the aquatic ecosystem. Currently, the regulatory authorities prescribe a number of studies for the assessment of environmental side effects, including the analysis of bioaccumulation, environmental fate and toxicity in different model organisms such as earthworm, algae, water flea or fish. However, these studies do not cover substance-induced effects at the molecular level. The application of recent OMICs-technologies allows for a sensitive and global identification of molecular changes at the level of RNA (transcriptomics) and proteins (proteomics).

Materials and Methods:



Results and discussion

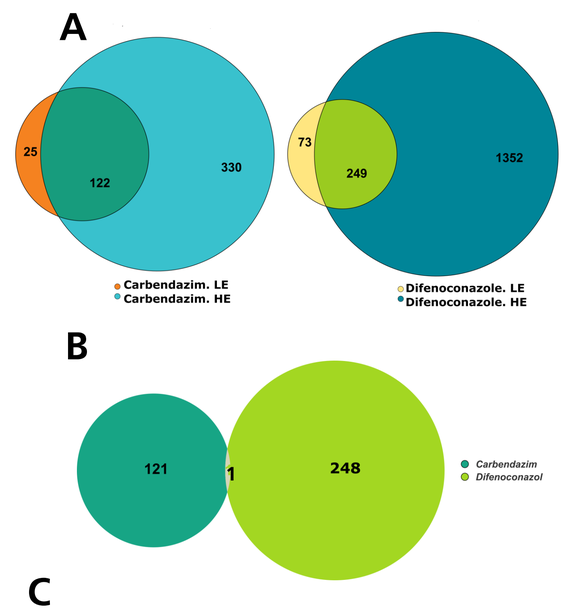
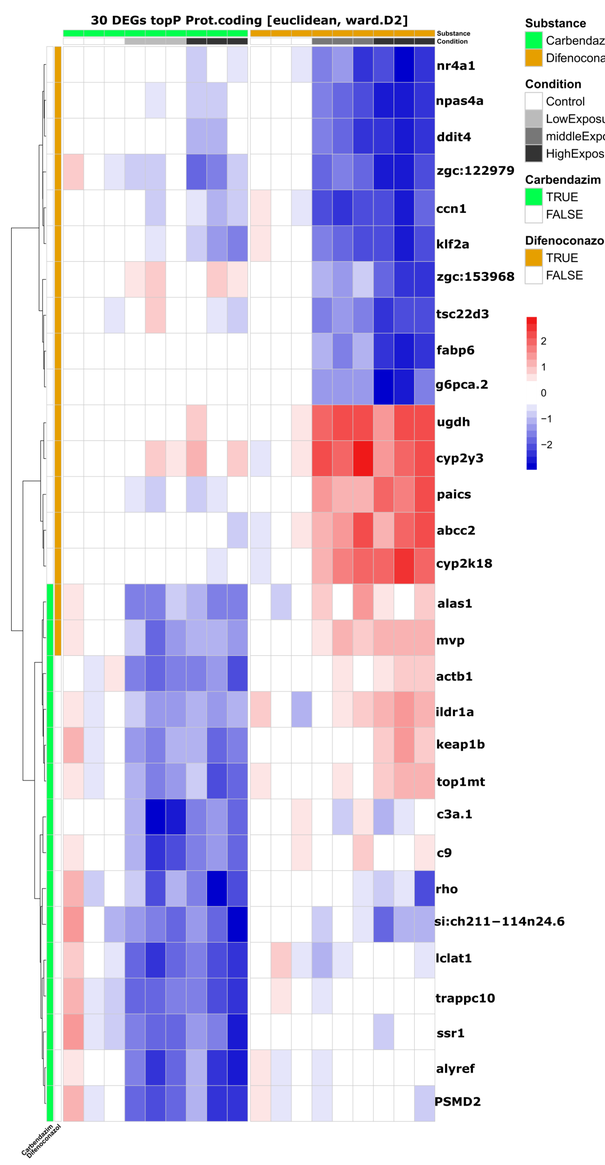


Fig.1. **(A)** Venn diagram showing differentially expressed genes (DEGs) after exposure to 125 µg/L (LE) and 500 µg/L (HE) Carbendazim and to 500 µg/L (LE) and 1000 µg/L (HE) Difenoconazole as compared to the non-treated controls Displayed ellipses and intersection sizes correspond to the numbers of DEGs. **(B)** Venn diagram shows the overlap for the different core DEG sets between the common DEG set of the two fungicides (core DEG). **(C)** Heatmap containing the top 30 most significantly core DEG according to P value. As compared to the mean of each non-treated control, an enhanced expression is indicated in red and a suppressed expression is indicated in blue.

Transcriptome response of zebrafish embryos to sublethal concentrations of Carbendazim and Difenoconazole at 96 hpf indicated consistent number of differentially expressed genes for both tested fungicides (Fig. A). Accordingly, the genes in the common set between HE and LE were significantly differentially expressed in both LE and HE, showing a strong positive correlation for both Carbendazim (R = 0.95) and Difenoconazole (R = 0.91). common DEG subset of LE and HE could be then considered substance-specific gene expression signature in *Danio rerio*.

For assessing specificity at the gene expression level, we compared the identified gene expression signatures for both test substances (Fig. B). While the common subsets of LE and HE of each single substance were highly significant, there was one gene in the common subset of the LE treatments of both substances but in contrasting direction of regulation. Consequently, the resulting substance-specific signatures are unique (Fig. C).



Conclusion and future remarks

- Deep knowledge about molecular changes induced by active substances with different (MoA) and the linkage with phenotypic and population effects will significantly facilitate the classification of active substances related to their environmental side effects.
- The generated substance- specific molecular signatures are will lead us to Establishment of molecular indications (fungicide- induced molecular responses) for toxicity in zebrafish embryo.
- Establishment of mode of action (MoA) - specific biomarker panels for environmental monitoring and for the development of screening approaches for environmental risk prediction of fungicidal substance precursor under development.

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Bibliography:
OECD, 2013. Test Guideline 236: Fish Embryo Acute Toxicity (FET) Test.