Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Protective role of Probiotics in reducing the harmful effects of environmental contaminations.

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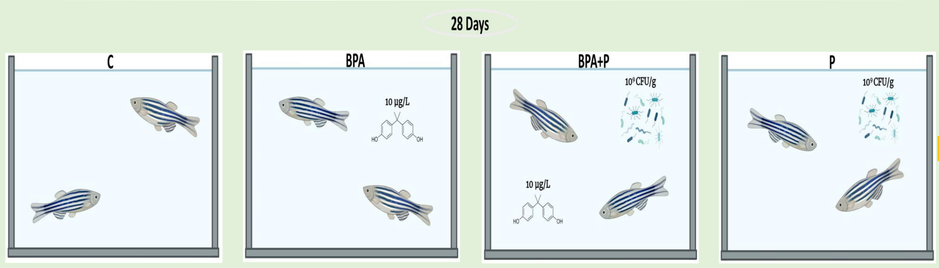
EXPERIMENT 1

OBJECTIVES:

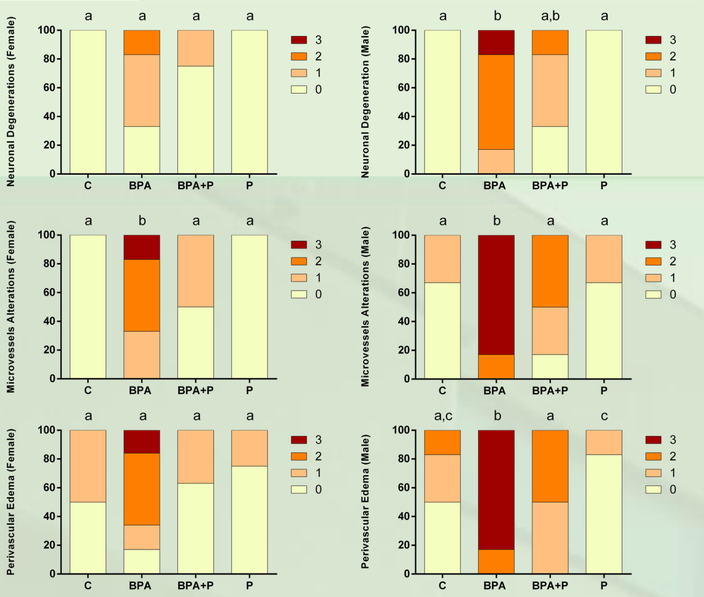
The aim of this experiment is to study the ability of the commercial probiotic formulation selected, SLAB51, already observed to ameliorate gastrointestinal disease and neurodegenerative disorder in mice[1], to counteract the well-known BPA toxicity[2] at brain, gut and spleen levels in both female and male adult zebrafish through a multidisciplinary approach.

MATERIALS and METHODS:

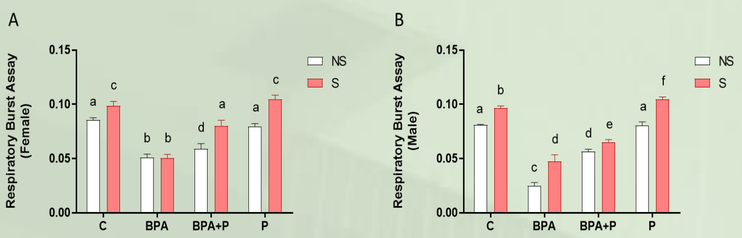
A chronic exposure was conducted using an environmentally relevant concentration of BPA (10 µg/L) with or without administration of SLAB51 (10^9 CFU/g). Brain, gut and spleen of female and male fish were analysed at the end of the trial (28 days)



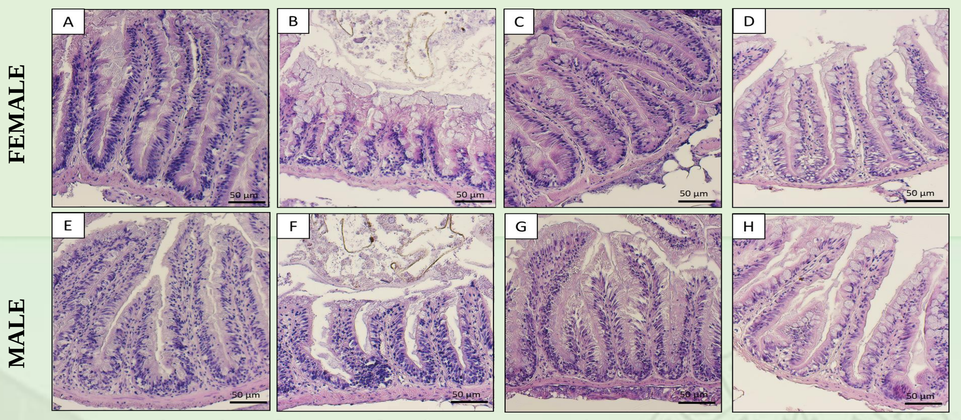
RESULTS:



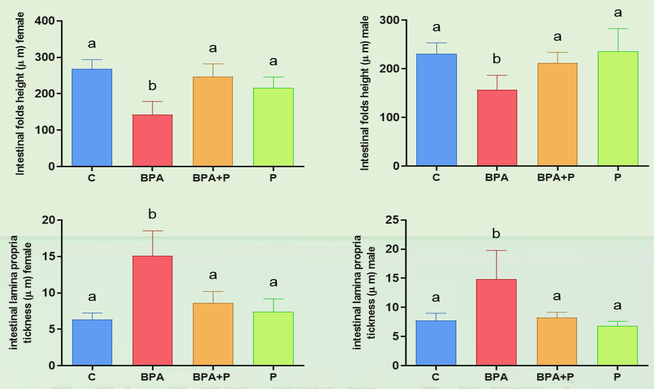
Histopathological analysis of brain sections

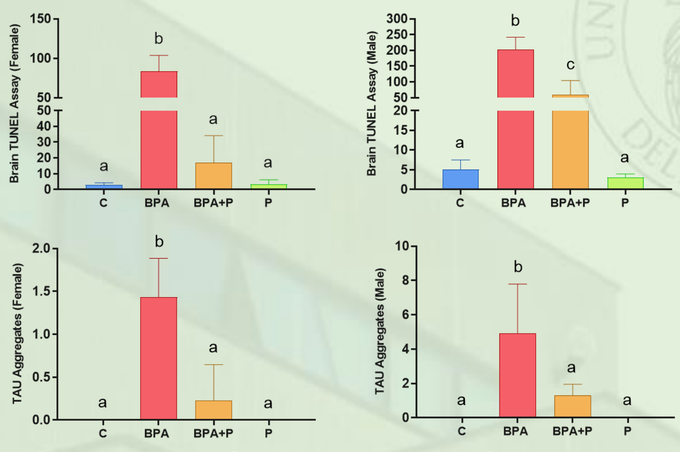


Spleen Respiratory Burst Assay



Representative microphotographs of gut 4 µm sections stained with Hematoxylin and Eosin of A and E) C, B and F) BPA, C and G) BPA+P and D and H) P and statistical analysis of biomarkers analyzed in gut histology.





Brain TUNEL analysis and TAU aggregates immunoreaction

CONCLUSIONS:

- **BPA toxicity at intestinal level was not sex dependent and was mitigated by SLAB51 co-administration in both sexes.**
- **Regarding brain, male were more affected by the contaminant which increased all the biomarker analyzed, while female were affected in a lesser extent and the probiotic antagonization was more evident in these specimens compared to male.**
- **At spleen level, BPA caused a reduction of ROS production but interestingly, it affected mostly female by decreasing ROS production both before and after the stimulation with PMA. The co-administration of SLAB51 ameliorated the BPA-induced immune system toxicity in both sexes.**
- **Overall, these results suggest the possible use of SLAB51 to counteract BPA toxicity at different levels. Due to *Danio rerio* characteristics as an animal model, these results can be easily transferred to human. This last aspect, together with the lack of probiotic side effects open to the possible use of SLAB51 as treatment mainly for the working categories which are mostly exposed to this class of contaminant, in order to reduce its toxicity.**

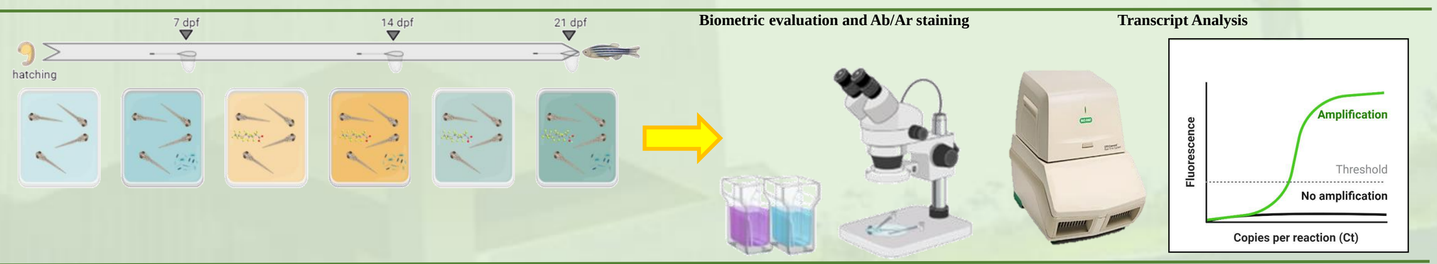
EXPERIMENT 2

OBJECTIVES:

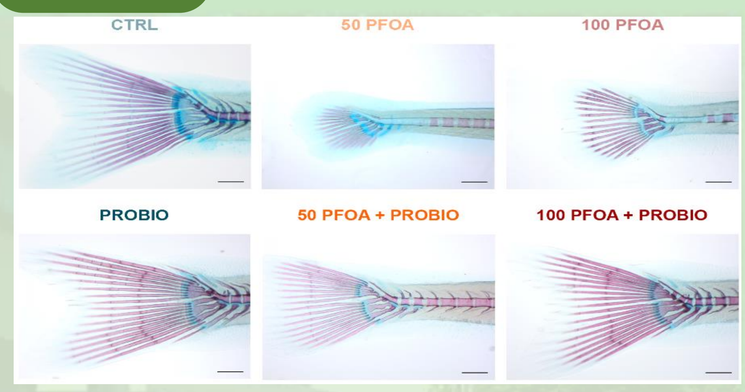
The aim of this second experiment is to study the ability of the probiotic strain *Bacillus subtilis natto*, selected based on previous study demonstrating its ability to improve zebrafish larval bone development[3], to counteract Perfluorooctanoic Acid (PFOA) toxicity on zebrafish larvae development[4] and ossification at 7, 14 and 21 days post fertilization (dpf).

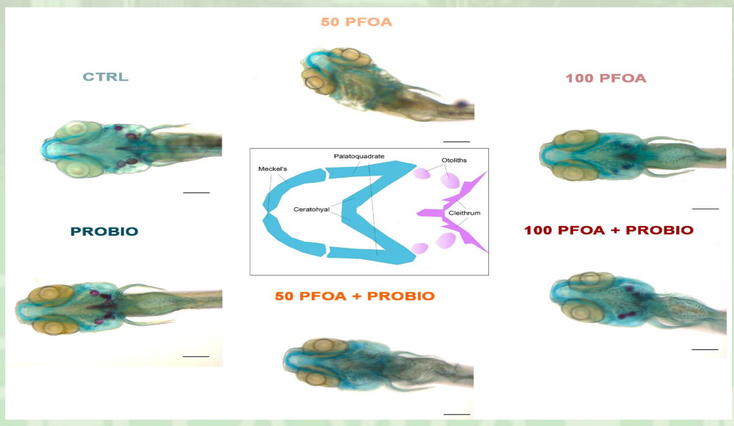
MATERIALS and METHODS:

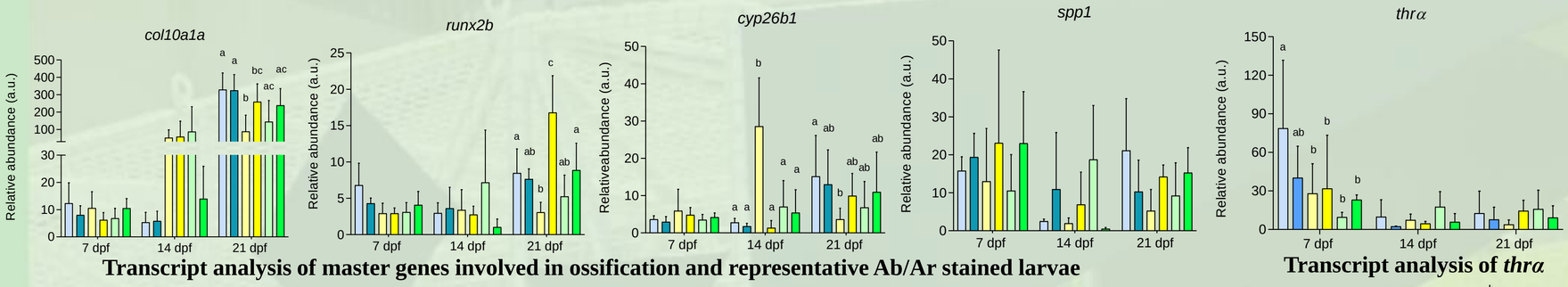
A 21 days chronic exposure was conducted exposing zebrafish embryos to two PFOA concentrations (50 and 100 mg/L) with or without the administration of *Bacillus subtilis natto* (10^7 CFU/g). Larvae were analysed at 7, 14 and 21 dpf through biometric evaluations, transcript analysis and craniofacial malformations by Alcian blue/Alizarin red (Ab/Ar) staining.



RESULTS:

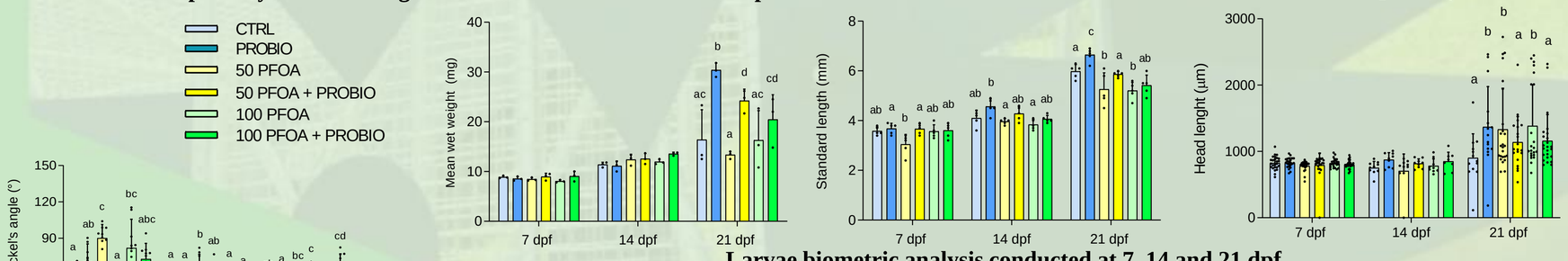






Transcript analysis of master genes involved in ossification and representative Ab/Ar stained larvae

Transcript analysis of *thrα*



Meckel's-Meckel's angle analysis in Ab/Ar stained larvae and respectively representative microphotographs

Larvae biometric analysis conducted at 7, 14 and 21 dpf.

CONCLUSIONS:

- **At 7 and 14 dpf, the mitigation of PFOA effect is evident surprisingly, at 21 dpf, probiotic exacerbate the PFOA damage on Meckel's-Meckel's angle.**
- **PFOA at 7 dpf decrease the *thrα* level and this may cause biometric alterations observed at 21 dpf.**
- ***Bacillus subtilis natto* mitigated PFOA detrimental effects as highlighted by the results of the biometries and ossification genes transcription conducted at 21 dpf, the most impacted endpoint.**

References: 1 Bonfili et al., 2017, *Sci. Rep.* doi: 10.1038/s41598-017-02587-2; 2 Ni et al., 2021, *Chemosphere.* doi: 10.1016/j.chemosphere.2021.130952; 3 Sojan et al., 2022, *Int J Mol Sci.* doi: 10.3390/ijms23094748; 4 Wang et al., 2020, *Environ Int.* doi:10.1016/j.envint.2019.105317.