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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Objective: The leading hypothesis of this work is to use omics approach, histological and immunohistochemistry, and RT-qPCR as tools to investigate the possible role and mechanism of SLAb51 formulation in the mitigation of Bisphenol A toxicity at different biological levels, focusing on reproduction, liver metabolism, intestine microbial composition, immune system, and brain health.

Experimental Design

Reproduction

Materials and Methods: in order to investigate if the selected probiotic formulation was able to mitigate the toxicity of BPA at reproductive level, male and female gonads were analyzed through histology and transcript analysis of key genes involved in reproductive functions.

a)

Testis	C	BPA	BPA+P	P
fshr	10.77 ± 2.22 (a)	2.82 ± 0.27 (b)	7.3 ± 1.82 (a)	9.72 ± 4.67 (a)
lhcgr	6.9 ± 0.69 (a)	3.92 ± 0.94 (b)	3.45 ± 1.45 (b)	5.6 ± 2.14 (a,b)
ar	5.15 ± 0.76 (a)	1.88 ± 0.69 (b)	3.57 ± 2.10 (a,b)	2.81 ± 1.64 (a,b)
esr1	8.41 ± 0.61 (a)	8.57 ± 1.20 (a)	3.4 ± 1.12 (b)	3.03 ± 1.10 (b)
esr2a	5.31 ± 0.84 (a)	8.11 ± 1.52 (b)	2.05 ± 0.70 (c)	2.12 ± 1.41 (c)
esr2b	6.17± 1.23 (a)	4.03 ± 1.2 (a,b)	2.05 ± 0.41 (b,c)	1.1 ± 0.32 (c)
pgrmc1	5.60 ± 1.37 (a,b)	7.96 ± 2.06 (a)	5.52 ± 1.89 (a,b)	2.63 ± 0.93 (b)
pgrmc2	7.34 ± 2.06 (a)	3.99 ± 1.50 (b)	5.64 ± 1.14 (a,b)	3.46 ± 1.17 (b)

b)

Class III Follicles	C	BPA	BPA+P	P
fshr	1.85 ± 0.80 (a)	5.15 ± 0.85 (b)	4.03 ± 0.12 (b)	1.18 ± 0.25 (a)
lhcgr	3.39 ± 0.82 (a)	5.63 ± 0.19 (b)	3.32 ± 0.4 (a)	1.41 ± 0.57 (c)
pgrmc1	4.95 ± 0.07 (a)	6.91 ± 0.20 (b)	6.33 ± 1.19 (a,b)	11.70 ± 0.02 (c)
pgrmc2	40.04 ± 5.60 (a)	19.85 ± 1.54 (b)	38.75 ± 1.90 (a)	1.25 ± 0.36 (c)

c)

Class IV Follicles	C	BPA	BPA+P	P
fshr	1.72 ± 0.68 (a)	4.43 ± 0.77 (b)	4.05 ± 0.24 (b,c)	3.02 ± 0.49 (a,c)
lhcgr	4.30 ± 0.27 (a)	6.65 ± 0.36 (b)	4.44 ± 0.29 (a)	6.01 ± 0.47 (b)
pgrmc1	3.41 ± 0.77 (a)	4.22 ± 0.69 (a)	1.65 ± 0.92 (b)	4.11 ± 0.84 (a)
pgrmc2	33.21 ± 1.82 (a)	24.79 ± 4.68 (b)	15.37 ± 0.66 (c)	16.45 ± 0.35 (c)

Table 1. mRNA expression values of genes regulating a) spermatogenesis in the testis and b-c) follicle growth and maturation in class III and IV follicles respectively of the different experimental groups. Data are reported as means ± SD. Different letters indicate statistically significant variations among the groups (one-way ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$).

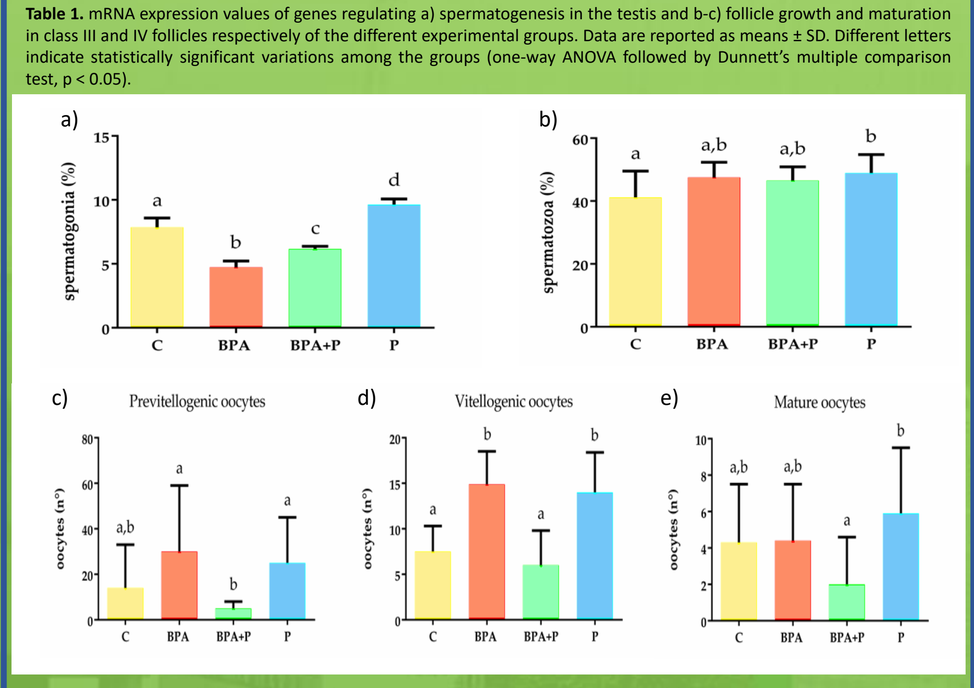

Figure 1. Percentage of zebrafish testicular area occupied by a) spermatogonia and b) spermatozoa and c-e) number of different follicle classes quantified in C (yellow), BPA (orange), BPA+P (green) and P (light blue), using section of gonads stained with Eosin and Mayer's Hematoxylin. Data reported as means ± SEM. Different letters denote statistically significant differences among experimental groups (one-way ANOVA, $p < 0.05$, Dunnett's multiple comparison test).

Results and conclusions: in fish exposed to the mix of BPA and probiotic, most of the key signals regulating spermatogenesis in testis and folliculogenesis in two classes of oocytes were closer to C and/or P levels, supporting the hypothesis that SLAb51 could antagonize BPA reproductive toxicity. In addition, SLAb51 positively interact with spermatogenesis, increasing the number of spermatogonia and spermatozoa respect to C. The reduction of spermatogonia induced by BPA was mitigated by the co-administration of probiotic. Regarding oocyte growth and maturation, further investigation are needed to clarify the interaction between bacteria, contaminant and gonadal tissue.

Giommi C, Habibi HR, Candelma M, Carnevali O, Maradonna F. Probiotic Administration Mitigates Bisphenol A Reproductive Toxicity in Zebrafish. Int J Mol Sci. 2021 Aug 27;22(17):9314. doi: 10.3390/ijms22179314.

Metabolism

Materials and Methods: liver histopathology and immunohistochemistry were conducted to evaluate the hepatic health status, coupled with UHPLC-MS metabolomic approach to investigate changes in metabolite concentration and RT-qPCR of genes involved in metabolism and inflammation, while the intestine microbioma was analyzed with a metagenomic approach.

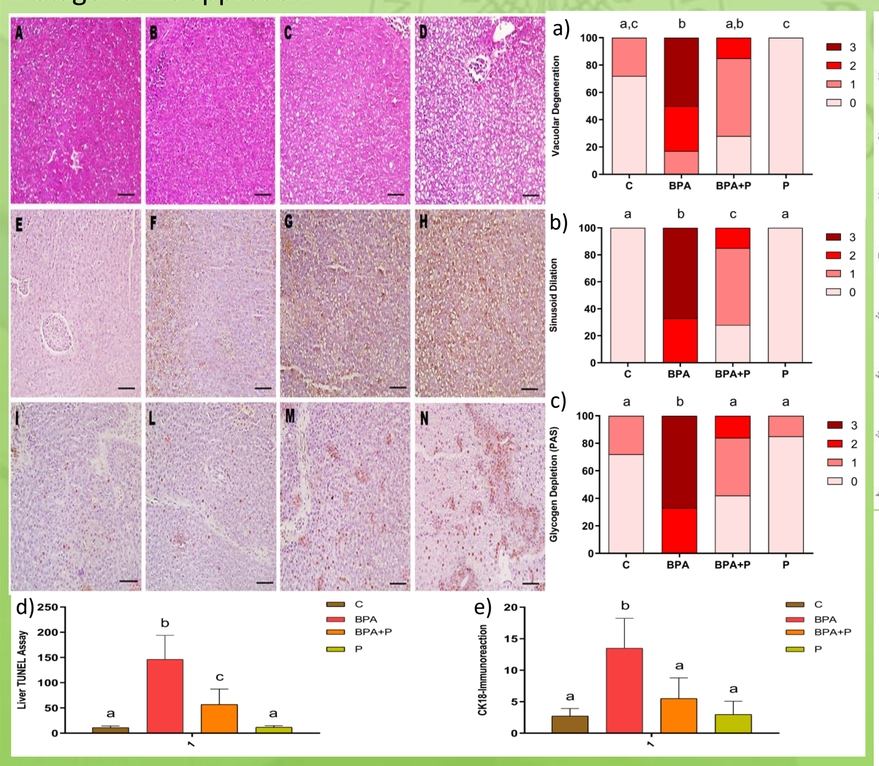

Figure 2. Histopathological and immunohistochemistry evaluation of liver samples. (A-D) Hematoxylin and eosin staining, (E-H) cleaved CK-18 cytokeratin immunoreaction and (I-N) TUNEL assay of C, P, BPA+P and BPA respectively. a) Vacuolar Degeneration, b) Sinusoid Dilation and c) Glycogen Depletion of zebrafish liver, with 0 = no abnormalities, 1 = mild abnormalities, 2 = moderate abnormalities, and 3 = severe abnormalities. Different letters indicate statistically significant differences (FDR < 0.05) among experimental groups (Exact Fisher's Test corrected using Benjamini & Hochberg procedure). d) TUNEL assay and e) CK-18 Immunoreaction quantification in zebrafish liver. Different letters indicate statistically significant differences ($p < 0.05$) among groups (one-way ANOVA followed by Dunnett's multiple comparison test).

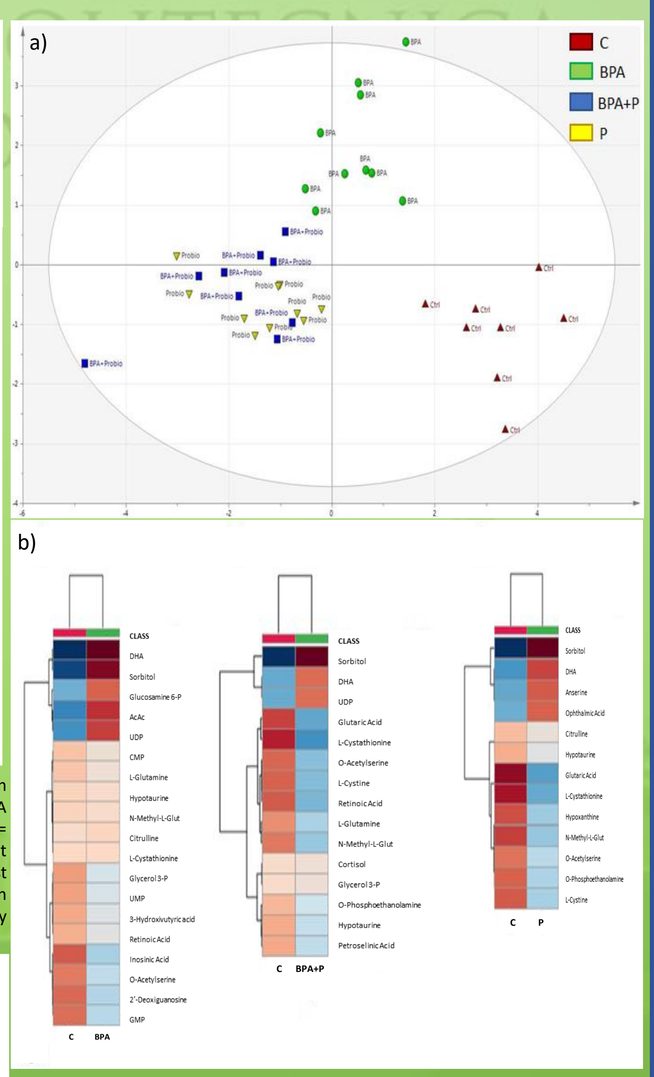

Figure 4. a) OPLS-DA analysis of metabolic phenotype and b) heatmaps summurizing metabolite concentration changes of treated groups against C.

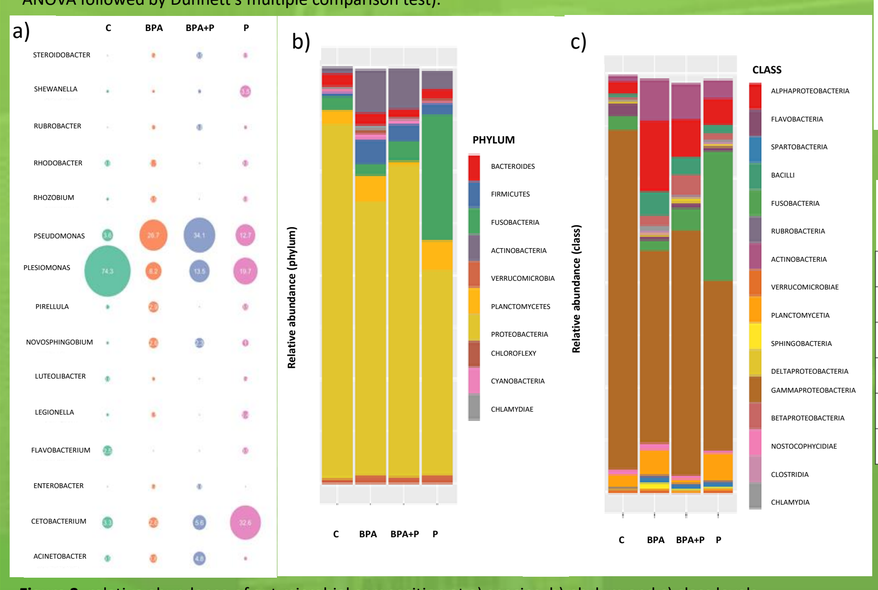

Figure 3. relative abundance of gut microbial composition at a) species, b) phylum and c) class levels.

	C	BPA	BPA+P	P
pparα	7.88 ± 1.64 (a)	11.55 ± 1.75 (b)	7.06 ± 3.16 (a)	7.51 ± 3.30 (a)
pparγ	26.72 ± 3.30 (a)	34.67 ± 5.24 (b)	30.38 ± 8.59 (a,b)	18.20 ± 5.22 (c)
ptgs2a	7.78 ± 1.62 (a)	13.32 ± 3.71 (b)	7.42 ± 5.59 (a)	8.39 ± 1.55 (a)
mboat4	31.74 ± 8.49 (a)	44.24 ± 7.79 (b)	23.83 ± 10.52 (a,c)	18.59 ± 6.23 (c)
nucb2a	2.89 ± 0.54 (a,b)	2.71 ± 1.08 (a)	3.66 ± 1.28 (a,b)	4.22 ± 0.96 (b)
cebpα	4.62 ± 1.41 (a)	8.74 ± 2.49 (b)	4.29 ± 1.84 (a)	5.99 ± 1.45 (a)

Table 2. mRNA expression values of genes involved in metabolism, appetite control and inflammation in the different experimental groups. Data are reported as mean ± standard deviation (SD). Different letters indicate statistically significant change among groups (n = 10). $p < 0.05$ (one-way ANOVA followed by Dunnett's multiple comparison test) was considered statistically significant.

Results and conclusions: Liver metabolomic analysis showed BPA+P clustering with P, suggesting a positive role of the probiotic formulation, shifting BPA+P metabolic phenotype from BPA to P. Further investigation through univariate analysis showed a reduction of retinoic acid level in BPA+P group, suggesting an increase of BPA detoxification. Histopathology and immunohistochemistry demonstrated the capacity of the formulation selected to reduce alterations or completely restore the C conditions in the BPA+P group. At transcriptional level, the expression of genes involved in lipid metabolism, inflammation and appetite regulation (*pparα*, *pparγ*, *ptgs2a* and *mboat4*) was restored to C levels. In the intestine, co-treatment of P and BPA reduced the presence of pathogenic bacteria such as *Chlamidiae* and *Bacilli* and increased those of *Cetobacterium,* a vitamin B12 producing bacteria.