This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713714.

11TH PROBIOTICS, PREBIOTICS & NEW FOODS, NUTRACEUTICALS AND BOTANICALS for NUTRITION & HUMAN and MICROBIOTA HEALTH
2ND SCIENCE & BUSINESS SYMPOSIUM
ROME, SEPTEMBER 12-14 2021
UNIVERSITÀ URBANIANA

MODULATION OF THE ENDOCANNABINOID SYSTEM BY PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* IMC513 IN ZEBRAFISH EXPOSED TO ENDOCRINE DISRUPTORS

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ABSTRACT

Environmental pollutants, used as plasticizers in food packaging and in thousands of everyday products, have become harmful for their impact on human health. Among them, phthalates have been recognized as emerging endocrine-disrupting compounds (EDs), that can induce toxic effects leading to different animals and humans health disorders (1), including a dysregulation of the endocannabinoid system (ECS).

Few studies have been carried out to evaluate the effects in *in vivo* animal models of di-n-hexyl phthalate (DnHP), commonly used in a wide variety of everyday products for the manufacturing of plastic care products and food packaging (2), whereas no studies have been conducted to determine the effect of DnHP exposure on the ECS of adult zebrafish. Moreover, the interest in dietary interventions (i.e. probiotic bacteria), that are able to positively affect human health, is relevant in the scientific community. Among all the beneficial effects that probiotics may have, their ability to counteract the toxicity of environmental chemicals, such as EDs, is also emerging (3).

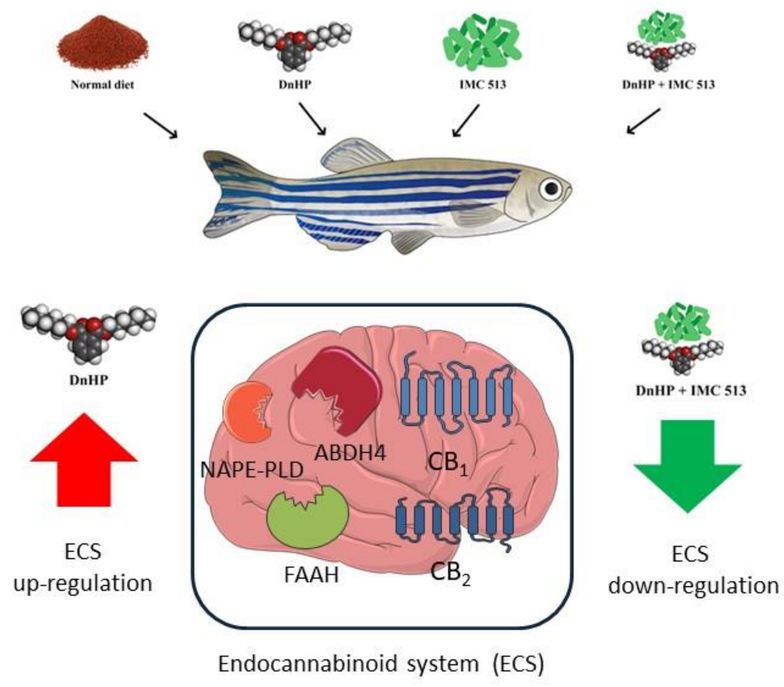
The probiotic strain *Lpb. plantarum* IMC513 was used as dietary intervention. This strain was previously characterized for several properties, including the antigenotoxic activity, the ability to survive the gastrointestinal conditions (4) and to interact with intestinal cell models (5-7). In addition, this strain was selected for this experimental trial, based on its ability to inhibit the genotoxicity of DnHP in a preliminary *in vitro* study (2).

In this perspective, the present study was aimed at evaluating the potential neuroprotective impact of the probiotic strain *Lactiplantibacillus plantarum* IMC513) on the physiology of zebrafish exposed to DnHP, with a focus on the modulation of the ECS in the brain.

EXPERIMENTAL DESIGN

- *Lpb. plantarum* IMC513 (10^9 CFU/g) was administrated by directly adding to the water freeze-dried cells together with the standard diet. *Lpb. plantarum* IMC513 was daily delivered for six months either alone (probiotic group) or as a combined treatment (probiotic + 1mg/kg DnHP group).
- After 6 months of treatment, all fishes (n= 12/group) were euthanized using tricaine and then dissected.
- Total length and wet weight of each fish as well as wet weight of each organ (gut and brain) were measured immediately upon sampling.
- The presence of key genes of the ECS was tested at the transcriptional level by qRT-PCR analysis in the brain of zebrafish. The gene levels of the toll-like receptor 9 (TLR9), a key molecule involved in recognition of microbes in the gut, were measured in the intestine samples.
- The levels of two main endocannabinoids (eCBs), anandamide (AEA) and 2-arachidononyl-glycerol (2-AG), and the eCB-like mediator palmitoylethanolamide (PEA) were detected through UHPLC/MS-MS in zebrafish brain samples.

GRAPHICAL ABSTRACT



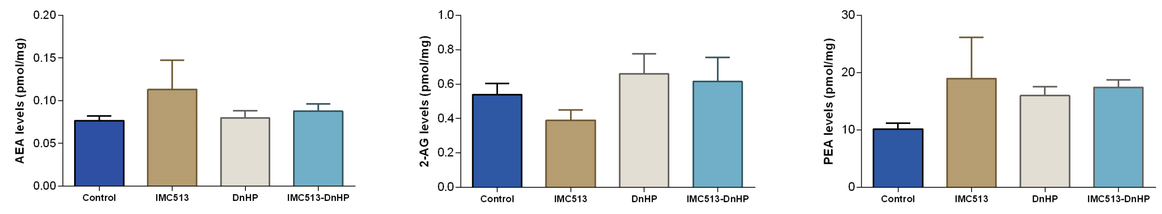
MORPHOMETRIC DATA

Samples	Lenght (mm)	Weight (mm)	Brain (g)	Gut (g)
Control	23.25±1.08	0.212±0.043	0.0044±0.0009	0.0158±0.0035
IMC513	23.79±0.66	0.223±0.036	0.0044±0.0008	0.0141±0.0031
DnHP	23.33±1.17	0.226±0.118	0.0041±0.0007	0.0126±0.0023
IMC513+DnHP	23.08±1.84	0.218±0.066	0.0042±0.0007	0.0165±0.0067

Values are expressed as mean ± SD (n=12) for each treatment group. No significant differences were found by One-way ANOVA analysis (p>0.05).

During the 6 months treatment period, no fish died in all the treated groups including the control. Both DnHP and probiotic administration did not affect total length, neither total body weight nor in terms of brain and gut weight.

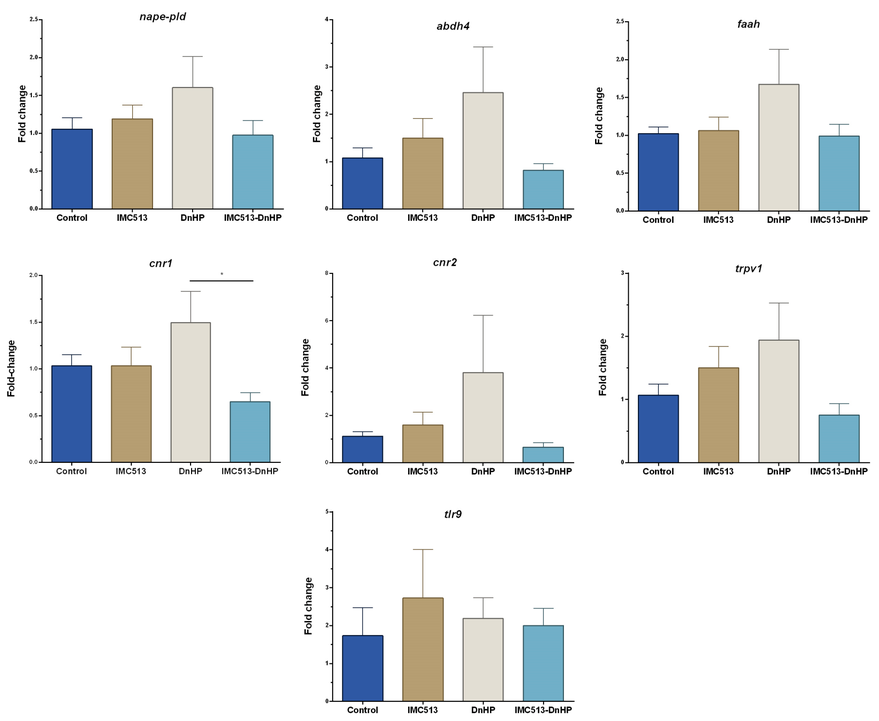
MODULATION OF eCBs LEVELS BY IMC513



Although the statistical analysis did not revealed significant changes, 2-AG and PEA levels result increased in zebrafish exposed to DnHP.

The levels of AEA; 2-AG and PEA were modulated by the daily intake of *Lpb. plantarum* IMC513, suggesting the ability of probiotic to interact at central level.

EFFECTS OF IMC513 ON THE ECS



The exposure to DnHP significantly upregulated the mRNA levels of *CNR*1 and *CNR*2 in the brain, whilst the gene expression of cannabinoid receptors resulted downregulated in the brain of zebrafish fed with probiotic *Lpb. plantarum* IMC513. mRNA levels of TLR9 were increased by the 6-months administration of the probiotic *Lpb. plantarum* IMC513 with respect to the control samples, showing an upregulation of the intestinal expression of the gene encoding for TLR9 in response to the probiotic presence in the gut. Overall, these results reveal a promising protective role of probiotic through a downregulation of the ECS by restoring the gene expression of ECS elements to the control level.

CONCLUDING REMARKS

- *Lpb. plantarum* IMC513 showed a potential ability to modulate, at the level of the central nervous system, some ECS components in zebrafish.
- These findings suggest the use of probiotics as innovative dietary strategy to counteract the emerging health risk of EDs, which is worth to be further investigated..

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ACKNOWLEDGEMENTS

The experimental procedures on zebrafish were approved by the Committee for Animal Care (OPBA) of Teramo University and by the Italian Ministry of Health (authorization number: 529/2018 -PR). The authors gratefully acknowledge Synbiotec s.r.l. (Camerino, Italy) to kindly provide the probiotic strain Lpb. plantarum IMC513.