Strain specific, stress-modulating effects of probiotics: a systematic screening in a mouse model of chronic restraint stress.

Lotta K. Stenman[1], Elaine Patterson[1], Johann Meunier[2], Francois J. Roman[2], Markus J. Lehtinen[1]

[1]DuPont Nutrition & Biosciences, Sokeritehtaantie 20, 02460 Kantvik, Finland [2]Amylgen SAS, 2196 Boulevard de la Lironde, 34980 Montferrier-sur-Lez, France

INTRODUCTION: The gut-brain axis plays a key role in regulating the stress response, and the microbiota are essential in controlling the microbiota-gut-brain axis response to stress. Evidence suggests that gut microbiota influence stress-related behaviors, including those relevant to anxiety and depression (Foster et al., 2017). Probiotics are one such means of targeting the gut microbiota to deliver health benefits and influence brain function, physiology and behavior.

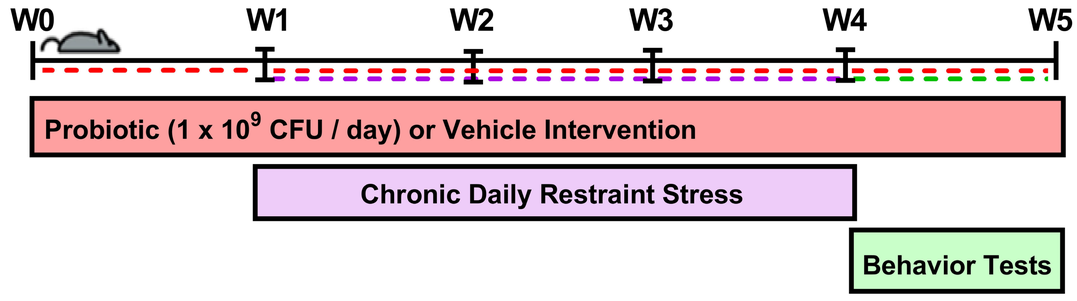
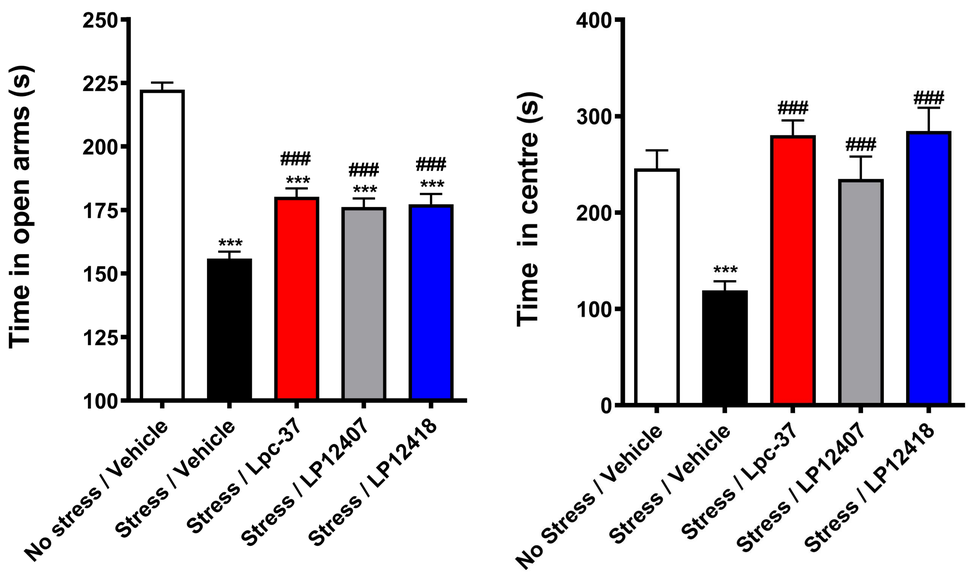


Fig. 1: Experimental schedule and timeline of procedures during the 5 weeks of probiotic intervention in all 5 experiments.

MATERIALS AND METHODS: **(1)** The efficacy of 12 candidate probiotic strains from 10 species / sub-species of *Bifidobacterium* and *Lactobacillus* were tested using a systematic screening on behavioral and physiological outcomes of chronically stressed male C57BL/6J mice (n=12 per group). **(2)** The strains were tested in four screening experiments and compared with non-stressed and chronically stressed vehicle groups. **(3)** The chronic restraint stress procedure was initiated 1 week after intervention and continued for 90 min/day, 5 days/week, for 3 weeks. The 3 most efficacious strains were re-tested in a follow-up experiment to validate the results. **(4)** Mice were administered 1 x 10^9 CFU / day of selected candidate probiotic or saline solution for 5 weeks in total, according to the schedule in Fig. 1.

RESULTS: The results presented are from the validation experiment. The groups in the validation experiment were: **Group 1:** No stress / Vehicle **Group 2:** Stress / Vehicle **Group 3:** Stress / *Lactobacillus paracasei* Lpc-37 (DGCC4981; DSM 32661) **Group 4:** Stress / *Lactobacillus plantarum* LP12407 (DGCC12407; DSM 32654) **Group 5:** Stress / *Lactobacillus plantarum* LP12418 (DGCC12418; DSM 32655)

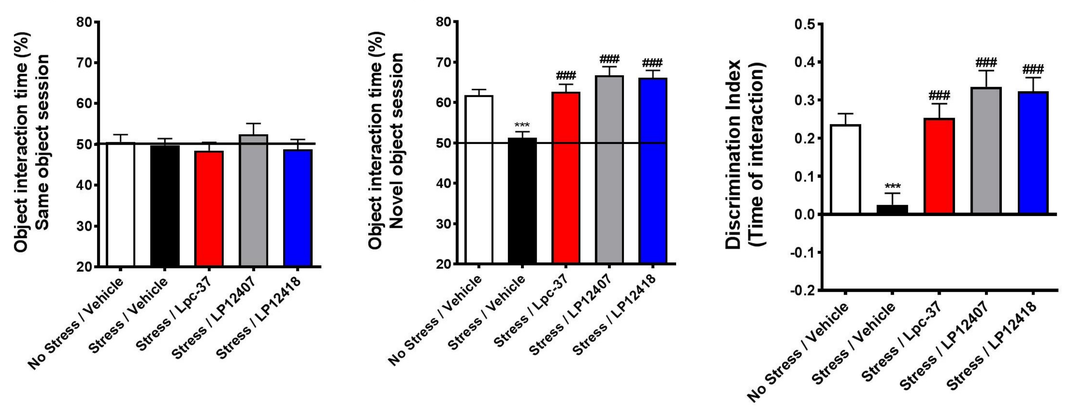
ANXIETY-RELATED BEHAVIOR:



*** P < 0.001, vs No stress / Vehicle. ### P < 0.001, vs Stress / Vehicle

Fig. 2: **(A)** Time spent in the open arms of the elevated plus maze (EPM) test. **(B)** Time spent in the centre of the arena in the open field (OF) test.

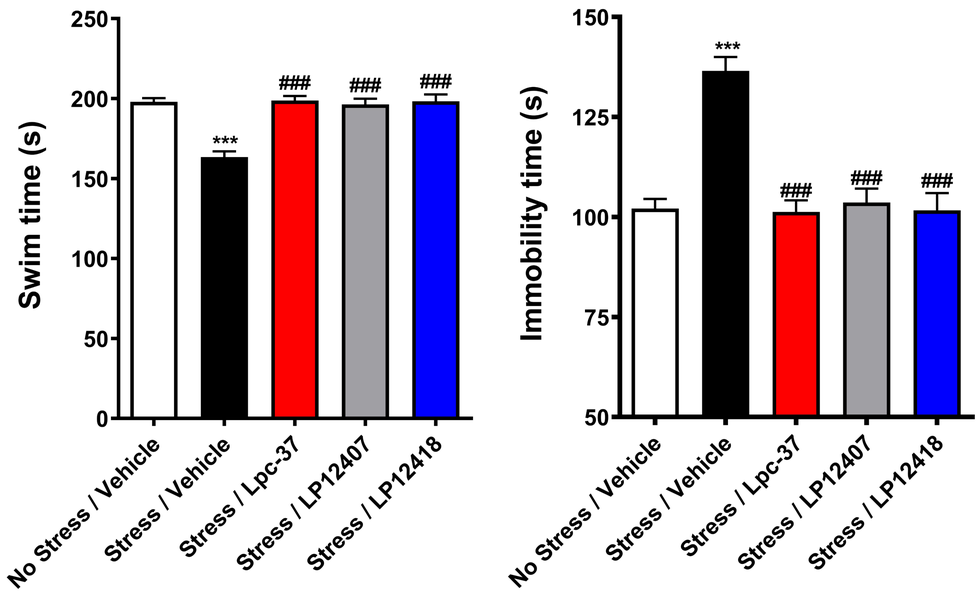
COGNITIVE FUNCTION:



*** P < 0.001, vs No stress / Vehicle. ### P < 0.001, vs Stress / Vehicle

Fig. 4: **(A)** Time spent interacting with the same object during Day 2 of the novel object recognition (NOR) test. **(B)** Time spent interacting with the novel object during Day 3 of the NOR test. **(C)** Discrimination index.

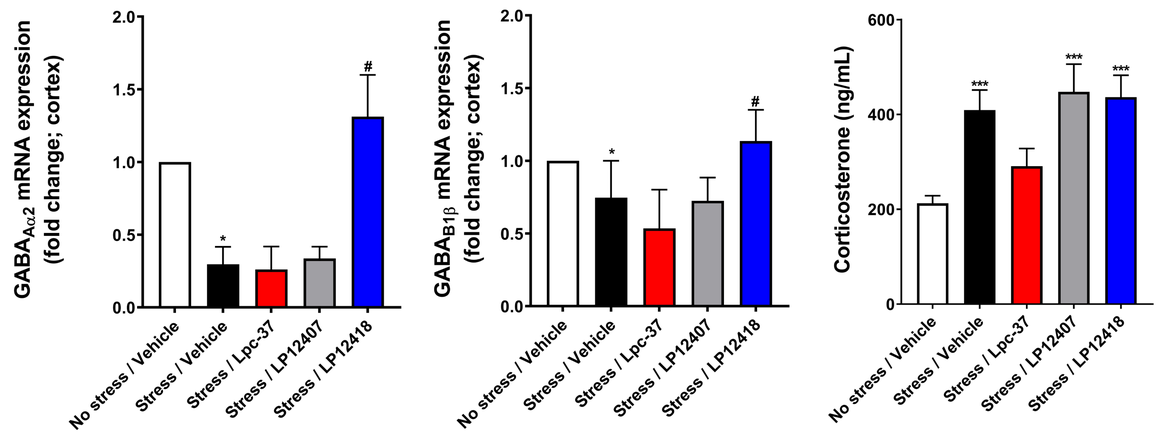
DEPRESSION-RELATED BEHAVIOR:



*** P < 0.001, vs No stress / Vehicle. ### P < 0.001, vs Stress / Vehicle

Fig. 3: **(A)** Total time spent swimming in the forced swim test (FST). **(B)** Total time spent immobile in the FST.

GABA-RECEPTOR EXPRESSION AND CORTICOSTERONE:



* P < 0.05, *** P < 0.001 vs No stress / Vehicle. # P < 0.05, vs Stress / Vehicle

Fig. 5: **(A)** GABA$_{A\alpha2}$ and **(B)** GABA$_{B1\beta}$ receptor expression in the prefrontal cotrex. **(C)** Plasma corticosterone.

CONCLUSION: The study results demonstrated that Lpc-37, LP12418 and LP12407 prevent stress-related behaviors from developing in mice and each strain had a unique mechanistic profile related to the HPA axis and prefrontal cortex GABA expression.

DuPont Nutrition & Biosciences
Sokeritehtaantie 20, 02460 Kantvik, Finland
Tel: +358 40 4827626
elaine.patterson@dupont.com
www.dupontnutritionandhealth.com

***Conflict of interest statement:** EP and MJL are employed by DuPont Nutrition & Biosciences. At the time the research was undertaken, LKS was also employed by DuPont Nutrition & Biosciences. JM and FJR are employed by Amylgen SAS, which provides research services in the field of cognitive health.

Reference: Foster JA, Rinaman L, Cryan JF. Stress & the gut-brain axis: Regulation by the microbiome. Neurobiology of Stress. 2017;7:124-136. doi:10.1016/j.ynstr.2017.03.001.