

Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers

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In a recent clinical study, we demonstrated in the general population that *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (PF) taken in combination for 30 days decreased the global scores of hospital anxiety and depression scale (HADs), and the global severity index of the Hopkins symptoms checklist (HSCL-90), due to the decrease of the sub-scores of somatization, depression and anger-hostility spheres. Therefore, oral intake of PF showed beneficial effects on anxiety and depression related behaviors in human volunteers. From there, it is interesting to focus on the role of this probiotic formulation in the subjects with the lowest urinary free cortisol levels at baseline.

This addendum presents a secondary analysis of the effects of PF in a sub-population of 25 subjects with urinary free cortisol (UFC) levels less than 50 ng/ml at baseline, on psychological distress based on the percentage of change of the perceived stress scale (PSs), the HADs and the HSCL-90 scores between baseline and follow-up. The data show that PF improves the same scores as in the general population (the HADs global score, the global severity index of the HSCL-90 and three of its sub-scores, i.e., somatization, depression and anger-hostility), as well as the PSs score and three other sub-scores of the HSCL-90, i.e., “obsessive compulsive,” “anxiety” and “paranoid-ideation.” Moreover, in the HSCL-90,

the score of the Factor 1, related to anxiety and depression, is significantly improved over time in PF-treated subjects compared with controls.

Additional preclinical data showed that PF formulation does not induce side effects such as addiction or learning and memory impairments, and therefore displays a good safety profile.

Complementary hypothetical mechanisms of action are proposed to explain the functioning of the brain-gut axis, particularly the relationship between probiotics and stress-related psychopathologies, such as anxiety and depression.

Introduction

Animal studies have shown that experimental manipulations of the enteric microbiome can modify host's neural function, potentially leading to altered emotional and cognitive functions during adulthood. The first evidence came from studies comparing germ-free and gnotobiotic animals.^{1,2} Despite the intrinsic artificial nature of the experimental situation, it clearly demonstrated that gut microbiota alterations in pups can disturb the development of stress-related HPA-axis function.¹ In that study, germ-free mice presented an increased HPA-axis responsiveness to a psychological stress during adulthood compared to specific pathogen free mice and gnotobiotic mice associated with a single bacterium at the neonate stage. Germ-free adult mice also presented a decreased sensitivity to the

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negative feedback exerted by glucocorticoids on the HPA-axis.² The reversal of the HPA-axis alteration was possible only if bacterial colonization occurred before the mice reached six weeks of age, suggesting a role for enteric bacteria in the brain development with possible long-lasting effects. Moreover, in recent studies in germ free mice, it was suggested that the gut microbial colonization process induces neurochemical changes in the brain and initiates signaling mechanisms that affect neuronal pathways implicated in motor control and anxiety-like behaviour.^{3,4}

These changes were associated to significantly lower concentrations of brain-derived neurotrophic factor, nor-epinephrine and serotonin in the cortex and the hippocampus of these mice. The authors also reported a downregulation of the N-methyl-D-aspartate (NMDA) receptors, i.e., NR1 and NR2 subunits, in the cortex and the hippocampus.^{1,2} Since NMDA receptors in the hippocampus are implicated in long-term memory consolidation,⁵ these results are in good accordance with those of a study which recently demonstrated memory impairments in such germ-free mice.⁶ Interestingly, similar brain modifications have also been described in animals following stress exposure, along with depressive-like behavior and reduced memory abilities.⁷⁻⁹ Taken together, these results suggest that an impaired gut colonization during the early stages of life may dramatically contribute to an exaggerated stress response during adulthood, potentially facilitating the appearance of related pathologies such as anxiety and mood disorders. Regarding this hypothesis, it seems critical to better determine the extent to which gut microbiota composition in early life influences mental well-being during adulthood in animal models more connected to the human situation.

Besides the possible long-lasting delayed influence of microbiota on the brain, animal studies demonstrated that gut bacteria can affect mood and behavior in an acute way. It was demonstrated that mice orally treated with an enteric pathogen were more anxious than control mice.^{10,11} Indeed, infected mice presented no evidence that bacteria reached blood circulation and no elevation of circulating

pro-inflammatory cytokines was detected at this time, suggesting a role for the vagus nerve in the modulation of behavior. The authors then reproduced a similar anxiogenic-like effect in mice in several studies, investigating the neuronal pathways implicated in the processing of the viscerosensory information ultimately leading to behavioral response.¹²⁻¹⁴ They also showed that such an anxiogenic effect occurred at the early stages of the infection, 7–8 hours post-colonization.^{14,15} The overall results clearly demonstrate that this bottom-up gut-brain axis can produce, in a few hours, anxiety-related behaviors supposed to be appropriate for coping with threats associated with future sickness.¹⁶

Along with these results which indicate that the gut microbiota status can negatively affect the mental well-being of the host, appears the possibility of novel prophylactic or therapeutic approaches to stress-related disorders such as depression and anxiety. One of these approaches may consist in oral administrations of selected commensal bacteria, usually referred to as probiotics, in order to restore or improve the gut microbiome. Recently, such a strategy has been successfully applied and evidence from various preclinical studies suggests that probiotics or microbes could be used to reduce stress, and therefore some of its related pathologies. A preparation containing *Lactobacillus rhamnosus* and *Lactobacillus helveticus* has been demonstrated to improve colonic function and colonization, and to normalize corticosterone levels in rat pups submitted to maternal separation stress.¹⁷ In the same way, it was reported that oral treatment with *Bifidobacterium infantis* reduced depressive-like behavior of rats in the forced swimming test¹⁸ and that *Bifidobacterium longum* prevented anxiogenic-like effects of a chronic infection with the gut parasite *Trichuris muris* in mice.¹⁹ In a recent article, it was reported unpublished data indicating that mice treated with *Lactobacillus reuteri* exhibited reduced plasmatic corticosterone levels after stress exposure and a decreased anxiety-like behavior in the elevated plus-maze.²⁰

Preclinical and clinical trials have shown that a variety of psychological and physiological stressors can impair normal intestinal microbiota in turn,²¹⁻²³ and most

notable are stress-induced reductions in lactobacillus and bifidobacteria species. Thus, gut bacteria and the brain seem to interact in a close relationship using various positive and negative feedbacks. The value of probiotics as preventive and/or adjuvant therapeutics in stress-related diseases seems sound.

Psychotropic-Like Effects of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 Formulation in the General Population and in a Sub-Population with Low Urinary Free Cortisol Levels

In our recent study performed in the general population,²⁴ *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (PF) (Institut Rosell-Lallemand, Blagnac, France), taken daily in combination for 30 days, decreased over time the global score of the hospital anxiety and depression scale (HADs) and the global severity index (GSI) of the Hopkins symptoms checklist-90 (HSCL-90), due to the decrease of the sub-scores of the “somatization”, “depression” and “anger-hostility” spheres. The results suggest that in the general population, PF formulation is beneficial in reducing anxiety and stress response, as well as improving mood in moderately stressed human subjects.

In the light of the results of a clinical trial involving the administration of *Lactobacillus casei* strain Shirota to healthy adults showing that subjects with the lowest score in the depressed/elated dimension at baseline had significant improvement in mood scores after taking the probiotic,²⁵ we wondered what could be the effects of PF in subjects who have the lowest urinary free cortisol (UFC) levels at baseline? Since our goal was to focus on less stressed subjects, we determined the median of the UFC in the studied population (50 ng/ml) and chose to include in the exploratory secondary analysis only subjects with UFC levels lower than that median value at baseline. Thus, this analysis of the data was conducted in a sub-population of 25 subjects, 10 PF and 15 placebo, with UFC levels between 10 and 50 ng/ml at

baseline, on psychological distress based on the percentage of change of the perceived stress scale (PSs), the HADs and the HSCL-90 scores between baseline and follow-up. It was shown that PF improved the same scores as in the general population (see above), as well as the PSs score (Fig. 1), and three other sub-scores of the HSCL-90, i.e., “obsessive compulsive”, “anxiety” and “paranoid-ideation” (Fig. 2). Moreover, in the HSCL-90, the score of the Factor 1, related to anxiety and depression,²⁶ was significantly improved over time in PF-treated subjects compared with controls (Fig. 3).

Exposure to chronic stress could disrupt the balance of intestinal microbiota and induce various diseases. Therefore, whatever the stress level, daily intake of probiotics could prevent such an imbalance, and the improvement in symptoms among subjects with low to mild stress levels suggests the value of prophylactic intake of probiotics in terms of digestive comfort and general well-being.

The risks of addiction and memory impairment are common expected side-effects encountered with psychoactive drugs. For this reason, these risks should be assessed as part of the safety evaluation of all new nutritional or pharmacological neuroactive agents. For this purpose, to assess the safety profile of PF in relation with addiction and learning and memory disorders, the conditioned place preference paradigm (CPP),²⁷ and the passive avoidance test (PAT),²⁸ were performed respectively in rats using methods derived from those described by Messaoudi et al.²⁹ The results demonstrated that PF did not have rewarding properties in the CPP, suggesting no abuse potential compared with morphine (Fig. 4), and does not induce learning and memorization deficit in the PAT as is the case with diazepam (Fig. 5). Therefore, these results reinforce the idea that consumption of selected probiotics may represent a safe and natural prophylactic approach against some stress-related psychological disorders.

Despite the fast growing interest in the impact of probiotics on stress, anxiety and depression, the underlying physiological mechanisms by which probiotics might influence mental health remain largely unknown and hypothetical.

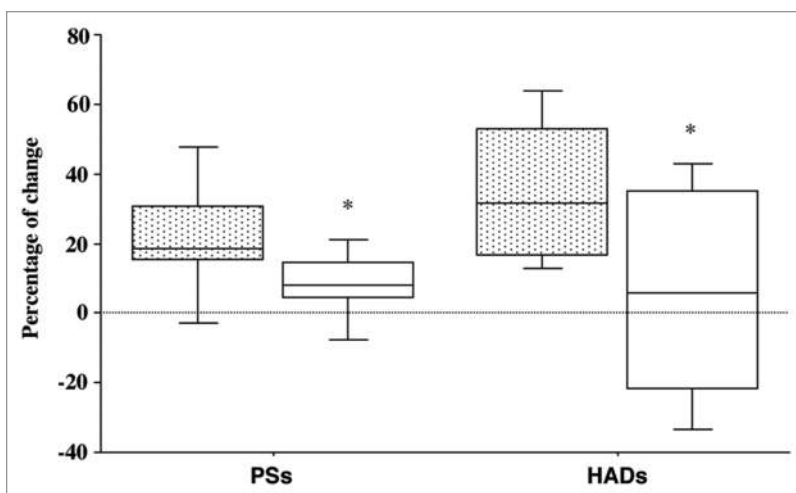


Figure 1. Effects of PF (dashed box plot) (n = 10) and placebo (white box plot) (n = 15) on the percentage of improvement of the perceived stress scale (PSs) and the hospital anxiety and depression scale (HADs) scores between baseline and follow-up in subjects with low urinary cortisol levels at baseline. Mann-Whitney U-test: *p < 0.05 (PF vs. placebo). Data are the 10th, 25th, 50th (median), 75th and 90th percentiles.

If the probiotics reduce stress and its related disorders through gut-brain signaling, will they interact with antidepressant and anxiolytic agents, inhibiting their effects or enhancing their effects and lowering the drug concentrations needed? Further studies are needed to evaluate the effects of coadministration of probiotics with psychotropic drugs in terms of interaction.

Possible Underlying Mechanisms

In addition to the assumptions made in our recent article in reference 24, other hypothetical mechanisms are proposed to explain the beneficial effects of probiotics in psychopathological disorders such as anxiety and depression.

Pro-inflammatory cytokines could act as mediators in case of gut infection to induce anxious behavior in mice,¹⁹ and the involvement of inflammation in the depressive syndrome is well-known.³⁰

Various cytokine receptors have been found on peripheral nerves including the vagus nerve and spinal nerves that may signal inflammation to the brain, potentially evoking anxiety and depression.³¹ However, even if vagotomy does not always prevent the anxiogenic and depressant effects of inflammation, cytokines could enter the brain via circumventricular organs by volume diffusion³²

or via cytokine transporters at the blood-brain barrier where pro-inflammatory cytokines could gain access to the brain through these saturable transport systems.³³ Another pathway may consist in the activation of interleukin-1 receptors, located on perivascular macrophages and endothelial cells of brain venules, by circulating cytokines inducing local production of prostaglandin E₂.^{34,35} Interestingly, several studies demonstrated that inflammatory responses in rodents are attenuated by lactobacilli and bifidobacterium strains,^{30,36,37} while antidepressant-like properties of oral administration of *infantis* in stressed animals were associated with anti-inflammatory effects.¹⁸ Very recently, we have shown that a formulation containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 in combination with *Lactobacillus rhamnosus* R0011, reduced the serum levels of pro-inflammatory cytokines interleukin-1 α , interleukin-6, interferon- γ and tumor necrosis factor- α following an *Escherichia coli* infection in rats.³⁸ In addition, probiotic-treated rats showed a significant increase of serum levels of anti-inflammatory cytokines interleukin-4 and interleukin-10 in comparison with controls. Thus, the beneficial psychological effects of a chronic oral treatment with *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175

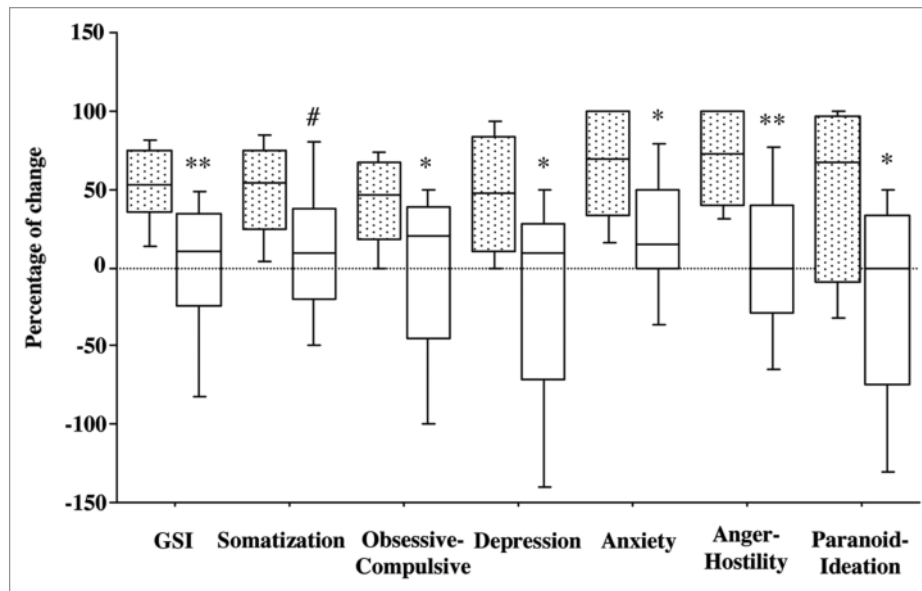


Figure 2. Effects of PF (dashed box plot) (n = 10) and placebo (white box plot) (n = 15) on the percentage of improvement of HSC-90 global severity index (GSI) and some sub-scores between baseline and follow-up in subjects with low urinary cortisol levels at baseline. Mann-Whitney U-test: *p < 0.10; **p < 0.05, **p < 0.01 (PF vs. placebo). Data are the 10th, 25th, 50th (median), 75th and 90th percentiles.

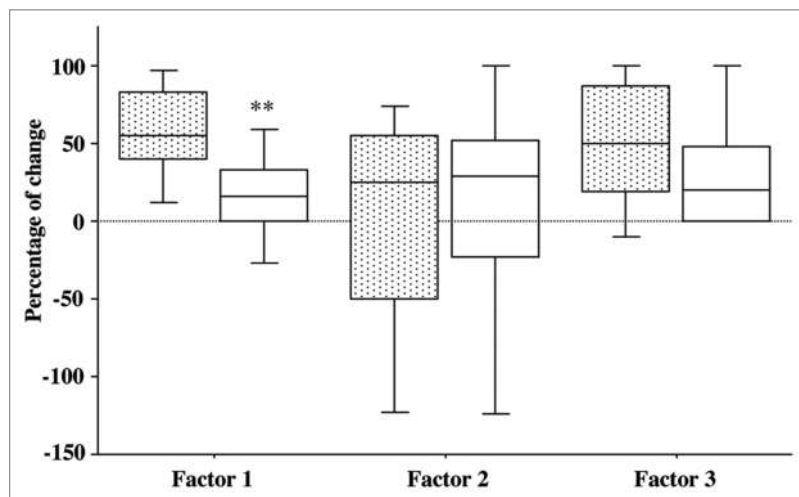


Figure 3. Effects of PF (dashed box plot) (n=10) and placebo (white box plot) (n=15) on the percentage of improvement of HSC-90 sub-scores of Factor 1 (Anxiety-Depression), Factor 2 (Paranoid-Psychotic) and Factor 3 (Panic-Phobia) between baseline and follow-up in subjects with low urinary cortisol levels at baseline. Mann-Whitney U-test: **P<0.01 (PF vs. placebo). Data are the 10th, 25th, 50th (median), 75th and 90th percentiles.

in humans may be explained, at least in part, by the anti-inflammatory properties of these bacteria.

Probiotics could also exert their effects by modulating the activity of brain structures involved in the processing of emotions related to anxiety, mood and aggression, including the septum, the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the

periaqueductal gray, via the vagus nerve independently of inflammation.¹⁶ In the same way, probiotics can reduce the activation/sensitization of neuronal circuits involved in stress-induced visceral hyperalgesia both at the spinal and supraspinal levels in the spinal cord, the paraventricular nucleus and the medial nucleus of the amygdala.³⁹ However, such a mediator between bacteria and neurons has not

been demonstrated yet. One hypothesis to consider is that probiotics could produce neurotransmitters to act directly or indirectly on specific targets in the central nervous system, since it was demonstrated that some bacteria are able to produce acetylcholine or gamma-aminobutyric acid.^{40,41}

Another mechanism whereby probiotic bacteria might have an influence on mood is via regulation of glycemic control. Indeed, emerging research shows that the intestinal microbiota contributes to the glucose tolerance⁴² and that epidemiological studies have made associations between glucose intolerance and risk of depression.^{43,44}

Otherwise, probiotic strains might be able to act on the reduction of substance P in the gut,⁴⁵ since it was shown that alterations to the normal gut microbiota can increase substance P release in the nervous system and promote behaviors reflective of anxiety, depression and aggression.^{46,47}

Conclusion

In our recent study, preliminary results indicate that oral administration of selected bacteria may have some beneficial properties in the treatment of gastrointestinal and psychological stress-related disorders. Nevertheless, further studies are

needed to confirm the potential therapeutic effects of probiotics in different subsets of subjects, and to better understand their underlying mechanisms of action. Then it would be of particular interest to determine in patients prone to anxiety and depression, if a probiotic preparation could potentiate chemotherapeutic agents and could therefore be used as an adjuvant therapy, or could replace them in part.

On the basis of the secondary analysis of our data, results suggest that the efficiency of PF may differ depending on the stress state of individuals. Thus, a chronic use of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 formulation could also contribute to mental well-being of subjects with low levels of stress, and could represent a prophylactic strategy against stress-related diseases in the population subject to the constraints of daily life.

Disclosure of Potential Conflict of Interest

This clinical trial was funded by Rosell-Lallemand SAS, France. None of the authors has any financial relationship with the funding sponsor and no interest in sales of this product.

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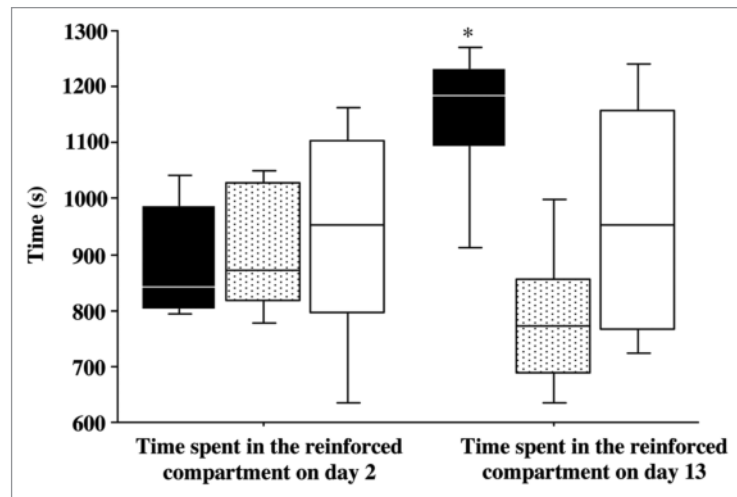


Figure 4. Effects of PF (250 mg, PO) (dashed box plot) (n = 8), morphine (4 mg/kg, PO) (black box plot) (n = 7) and vehicle (white box plot) (n = 8) on time spent in the reinforced compartment on day 2 and day 13 in the conditioned place preference test in rats. Wilcoxon test: *p < 0.05 (day 2 vs. day 13). Data are the 10th, 25th, 50th (median), 75th and 90th percentiles.

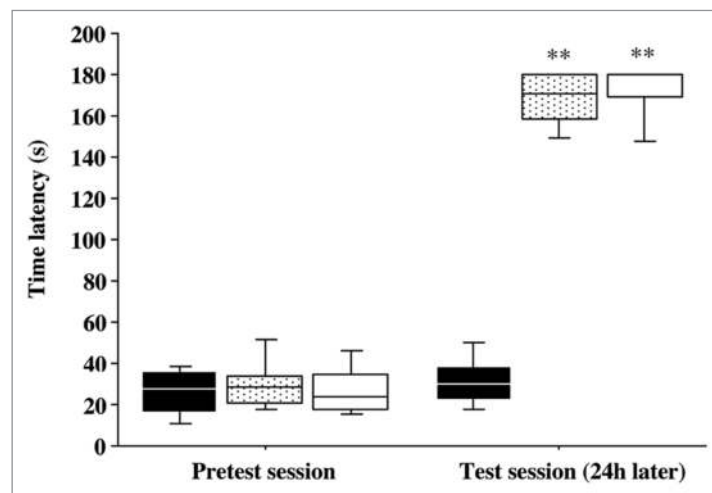


Figure 5. Effects of PF (500 mg, PO) (dashed box plot) (n = 8), diazepam (6 mg/kg, PO) (black box plot) (n = 8) and vehicle (white box plot) (n = 8) on time latency to enter the dark compartment during pretest and test sessions in the passive avoidance paradigm in rats. Wilcoxon test: **p < 0.01 (pretest session vs. test session). Data are the 10th, 25th, 50th (median), 75th and 90th percentiles.

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