

# BifiZen<sup>®</sup>

A gut-brain axis innovative  
range from Probiotal  
for cognitive health destined  
to the whole family



Probiotics like nobody else



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# BifiZen<sup>®</sup>

Probiotic product • Gut brain

A probiotic blend to reduce depressive mood state, fatigue and anger and improve the quality of sleep and of mood state in healthy individuals (1)

*Lactobacillus fermentum* LF16 (DSM 26956)  
*Lactobacillus rhamnosus* LR06 (DSM 21981)  
*Lactobacillus plantarum* LP01 (LMG P-21021)  
*Bifidobacterium longum* 04 (DSM 23233)

## The gut-brain axis

It is now well established that our gut microbiota and brain communicate in a mutual signalling network through the nervous, endocrine and immune systems. This interconnection is known as the gut-brain axis and it is emerging as a key factor in the stress-related body responses (2-4).

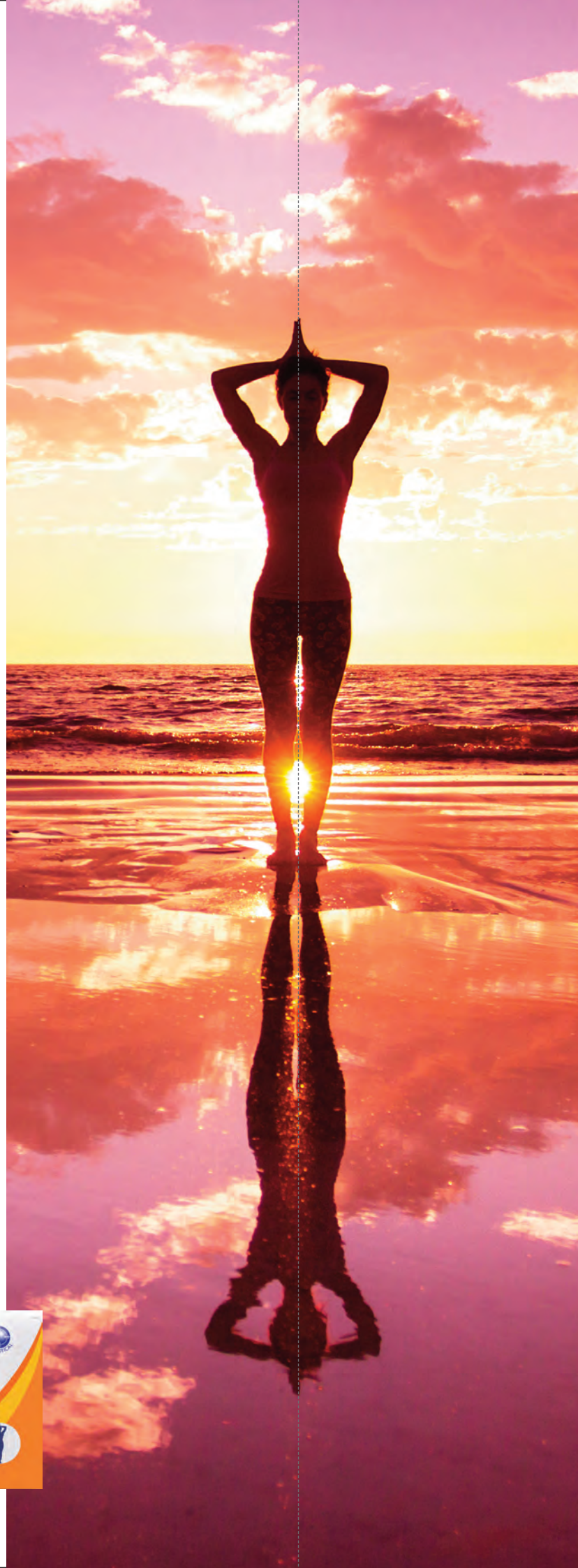
The intestinal microbiota can influence our mood and cognitive sphere by producing important metabolites that act as neurotransmitter precursors. By their impact on gut microbiota, intervention through probiotics intake demonstrated to improve and restore mood and cognitive functions.

## Functionality

BifiZen supplementation significantly improved mood, in a double-blind, placebo-controlled, randomized supplementation of 38 healthy subjects, according to a study by University of Verona, with a reduction in depressive mood state, anger and fatigue, as well as significant improvements in acceptance (which correlates with decreased depression sensitivity) and sleep quality. Interestingly, all these observed significant effects were maintained after 3 weeks of wash-out, showing prolonged benefits after cessation of probiotics intake (1).

## How to use

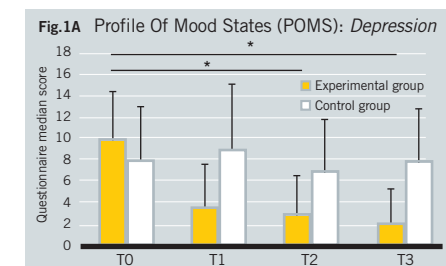
One dose per day for 6 weeks, available in different dosage forms including sachets, sticks or capsules.



## SCIENTIFIC REFERENCES

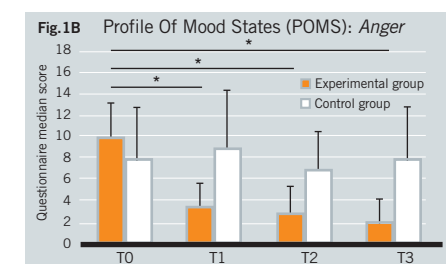
### Human clinical trials

1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

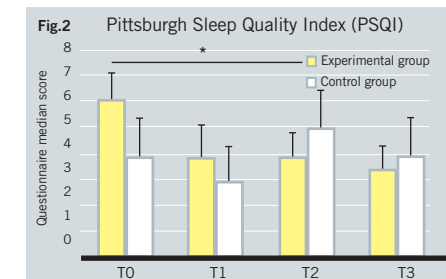
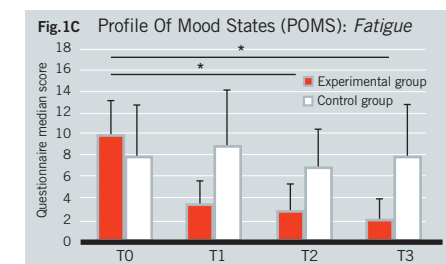


**Fig.1** Median POMS subscale score for the experimental and the control groups at all-time points.

(A) Depression subscale; (B) Anger subscale; (C) Fatigue subscale.



Error bars represent 95% confidence interval. Asterisks indicate within-groups significant differences (Bonferroni corrected  $p < 0.017$ ).



**Fig.2** Median PSQI score for the experimental and the control groups at all time points. Error bars represent 95% confidence interval. Asterisks indicate within-groups significant differences (Bonferroni corrected  $p < 0.017$ ).

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- Mayer EA, et al. Gut microbes and the brain: paradigm shift in neuroscience. J Neurosci. (2014) 34:15490–6.

## PLUS POINTS:

- Clean-label, safe and natural
- Allergen free (patented technology)
- Careful selection of the strains based on synergistic modes of action
- Available in different dosage forms
- Release in colony forming units (cfu) or active fluorescent units (afu) possible



# BifiZen® Stress

A natural, probiotic-based solution for stress management

## ■ Composition:

Bifizen®, Ashwagandha (*Withania somnifera*), Vitamin B8

## ■ Activity:

**BifiZen®** has been shown to reduce anger in healthy students (1).

**Ashwagandha** is documented for a reduction of cortisol and stress, anxiety and depression (2).

**Vitamin B8** (biotin) contributes to normal functioning of the nervous system and to normal psychological function.

*The ingredients have been tested for compatibility.*

## ■ Recommended use:

1 to 2 sachets per day for 8 weeks.

## ■ SCIENTIFIC REFERENCES

1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

2) Chandrasekhar K, Kapoor J, Anishetty S. A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. Indian J Psychol Med. 2012 Jul;34(3):255-62. doi: 10.4103/0253-7176.106022. PubMed PMID: 23439798; PubMed Central PMCID: PMC3573577.



# BifiZen® Focus

A natural, probiotic-based solution to improve attention and memory

## ■ Composition:

Bifizen®, Ashwagandha (*Withania somnifera*), Vitamin B5

## ■ Activity:

**BifiZen®**, through the improvement of sleep quality, mood, sensibility to depression, anger and fatigue, could improve focus and clear-thinking (1).

**Ashwagandha** was proven in a clinical study to increase memory, attention, executive functions and processes of information elaboration (2). Ashwagandha also improved significantly the quality of sleep and metnal alterntess in a recent study in elderly (3).

**Vitamin B5** (pantothenic acid) contributes to normal mental performance and to the reduction of tiredness and fatigue.

*The ingredients have been tested for compatibility.*

## ■ Recommended use:

1 to 2 sachets per day for 4 weeks.

## ■ SCIENTIFIC REFERENCES

1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

2) Choudhary D, Bhattacharyya S, Bose S. Efficacy and Safety of Ashwagandha (*Withania somnifera* (L.) Dunal) Root Extract in Improving Memory and Cognitive Functions. J Diet Suppl. 2017 Nov 2;14(6):599-612. doi: 10.1080/19390211.2017.1284970. Epub 2017 Feb 21. PubMed PMID: 28471731.

3) Kelgane S B, Salve J, Sampara P, et al. (February 23, 2020) Efficacy and Tolerability of Ashwagandha Root Extract in the Elderly for Improvement of General Well-being and Sleep: A Prospective, Randomized, Double-blind, Placebo-controlled Study. Cureus 12(2): e7083. DOI 10.7759/cureus.7083.



# BifiZen® Night

A natural, probiotic-based solution to sleep better

## ■ Composition:

Bifizen®, Melatonin, Vitamin B12

## ■ Activity:

**BifiZen®** has been shown to improve quality of sleep (1)

**Melatonin** contributes to the reduction of time to fall asleep and to the alleviation of subjective feelings of jetlag (2, 3).

**Vitamin B12** contributes to the reduction of tiredness and fatigue.

*The ingredients have been tested for compatibility.*

## ■ Recommended use:

1 sachet, stick or capsule half an hour before going to bed.

## ■ SCIENTIFIC REFERENCES

1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

2) Xie Z et al. A review of sleep disorders and melatonin. Neurol Res. 2017 Jun;39(6):559-565.

3) Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol. 2018 Aug;175(16):3190-3199.

4) Tardy AL et al. Vitamins and Minerals for Energy, Fatigue and Cognition: A Narrative Review of the Biochemical and Clinical Evidence. Nutrients. 2020 Jan 16;12(1). pii: E228





## BifiZen<sup>®</sup> AgeWell

A natural, probiotic-based solution against age-related cognitive decline and to improve memory, focus and attention

### ■ Composition:

Bifizen<sup>®</sup>, Alpha-GPC, Zinc

### ■ Activity:

**BifiZen<sup>®</sup>** is useful to improve mood, fatigue, anger and sensibility to depression (1), and will be further tested in a population with major depression.

**Alpha-GPC** is a precursor of phosphatidylcholine, which increases the release of acetylcholine, the neurotransmitter involved in cognitive functions such as memory and attention. It significantly improved mental and cognitive function in patients with Alzheimer (2) and showed neuroprotective action in animals (3).

**Zinc** contributes to normal cognitive functions.

*The ingredients have been tested for compatibility.*

### ■ Recommended use:

1 sachet, stick or capsule per day.

### ■ SCIENTIFIC REFERENCES

(1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

(2) De Jesus Moreno Moreno M. Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. Clin Ther. 2003 Jan;25(1):178-93. PubMed PMID: 12637119.

(3) Traini E, Bramanti V, Amenta F. Choline alfoscerate (alpha-glyceryl-phosphoryl-choline) an old choline- containing phospholipid with a still interesting profile as cognition enhancing agent. Curr Alzheimer Res. 2013 Dec;10(10):1070-9. Review. PubMed PMID: 24156263.



## BifiZen<sup>®</sup> Kids

A natural, probiotic-based solution for Kids mental and cognitive health

### ■ Composition:

Bifizen<sup>®</sup>, *L. rhamnosus* GG (ATCC 53103), Passiflore extract (*Passiflora incarnata*)

### ■ Activity:

**BifiZen<sup>®</sup>** is useful to improve mood, anger, sensibility to depression, fatigue and quality of sleep (1).

***L. rhamnosus* GG** early administration may reduce the risk of developing autism and Attention Deficit Hyperactivity Disorder (ADHD) (2).

***Passiflora incarnata*** was shown as effective in decreasing (by more than half) symptoms of ADHD (Attention Deficit Hyperactivity Disorder) as the standard of care, with much less side effects (3).

*The ingredients have been tested for compatibility.*

### ■ Recommended use:

1 sachet, stick or capsule per day.

### ■ SCIENTIFIC REFERENCES

(1) Marotta A. et al., Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality, 2019 Frontiers in Psychiatry.

(2) Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr Res. 2015 Jun;77(6):823-8. doi: 10.1038/pr.2015.51. Epub 2015 Mar 11. PubMed PMID: 25760553.

(3) Akhondzadeh S, Mohammadi MR, Momeni F. Passiflora incarnata in the treatment of attention-deficit hyperactivity disorder in children and adolescents. Therapy. 2005;2(4):609-614.

BifiZen<sup>®</sup> Stress

BifiZen<sup>®</sup> Night

BifiZen<sup>®</sup>

Focus

BifiZen<sup>®</sup> AgeWell

BifiZen<sup>®</sup> Kids

A new innovative range from Probiotal for cognitive health destined to the whole family



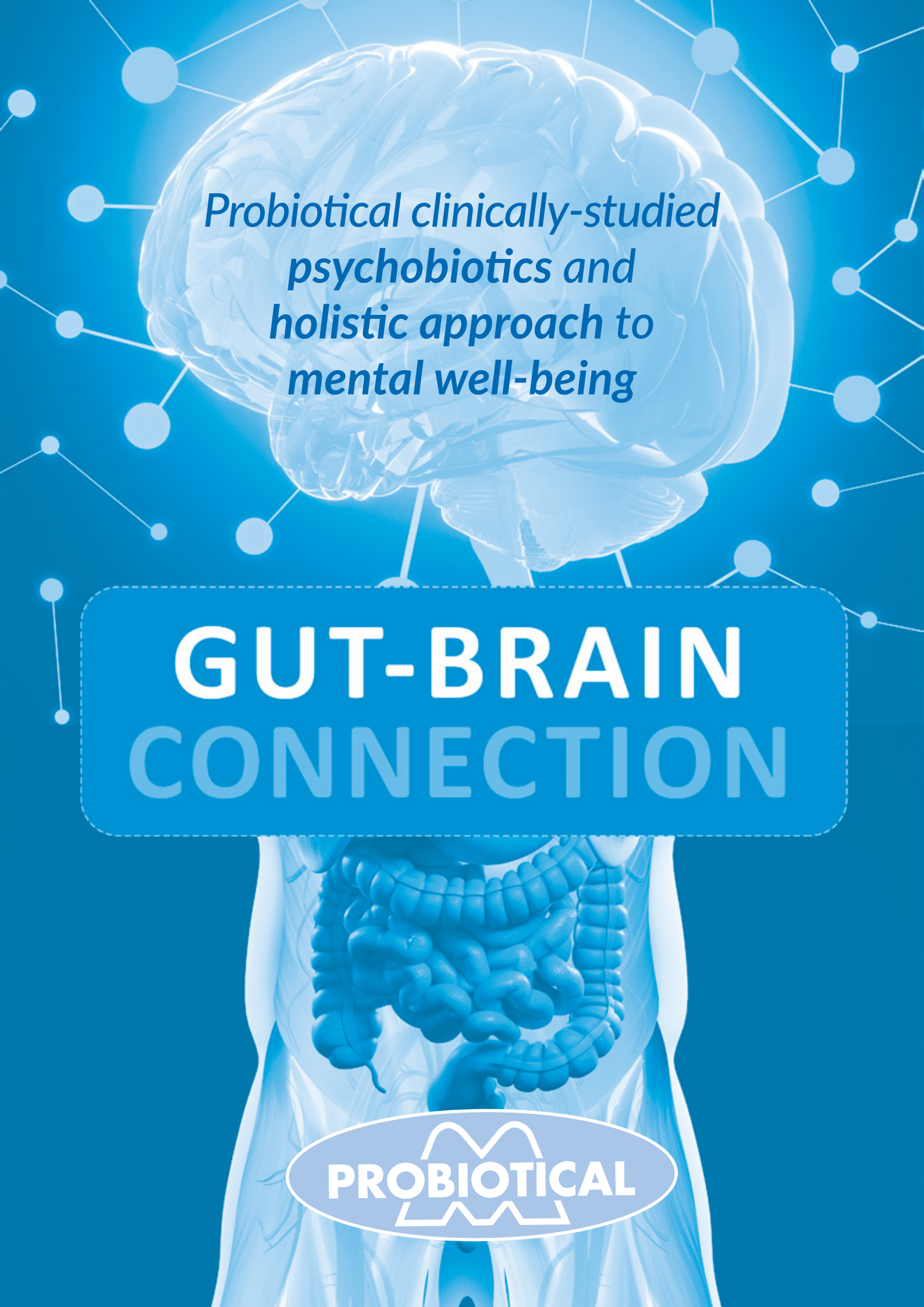
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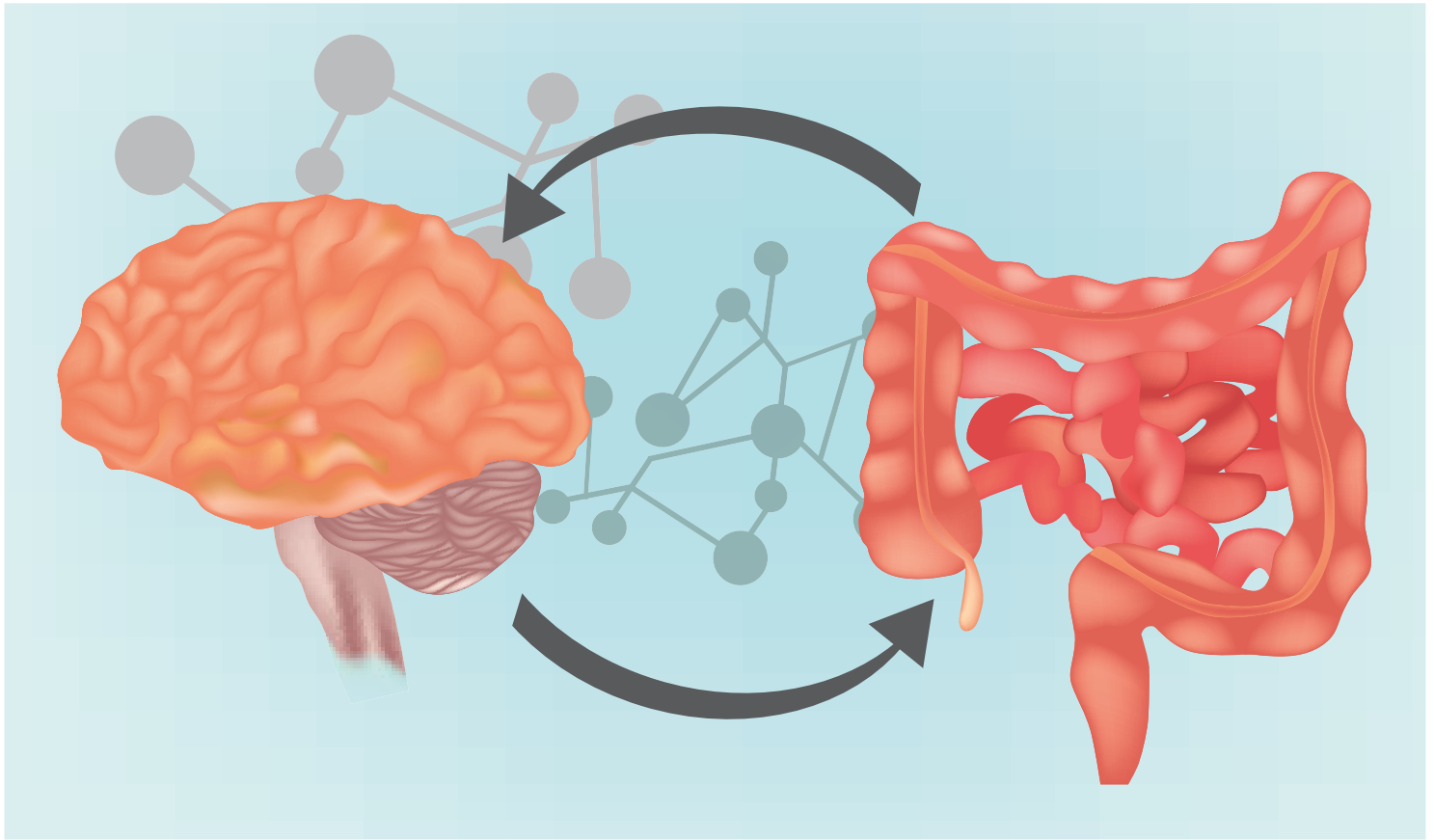


*Probiotal clinically-studied  
psychobiotics and  
holistic approach to  
mental well-being*

# GUT-BRAIN CONNECTION

  
**PROBIOTAL**

## Rising demand for cognition and mental well-being supplements: a role to play for the microbiota and clinically-studied probiotics



The intuition of a **link between the gut and our feelings** has been with us for a long time - deep down, we have always known about gut feelings and butterflies in the stomach, and we have understood since over 20 years that the **gut is our second brain**<sup>1</sup> and that some microorganisms are able to alter completely their host's behavior in their own interest, as is the fascinating case of *Toxoplasma gondii* turning the natural aversion of mice to cats into an attraction, leading to increased chance of being caught and eaten, a boon for the parasite in order to finish its life cycle in the feline's gut<sup>2, 3</sup>. Today, **the science is catching up on the intuition, and the market is flourishing with foods and supplements to support our mental well-being.**

### A STRONG MARKET DEMAND FOR PSYCHOBOTICS

**"The rise of psychobiotics"**, a recent report by Lumina Intelligence<sup>4</sup> looked at **associations between certain bacterial groups in the microbiome and mental conditions** including depression, Attention Deficit Hyperactivity Disorder, autism, Alzheimer's, Multiple Sclerosis and anorexia nervosa.

It evaluated the online market of the gut-brain axis food supplements category, which has received over 16 000 reviews by January 2020, and is overall rated at 4.47 stars out of 5, positioning the category as a one showing growing interest and consumer satisfaction.

Stress and depression in particular have become a public health concern. In 2008, the WHO ranked major depression as the third cause of burden of disease worldwide and projected it to become the first one by 2030<sup>5</sup>. This year, the WHO estimated that over 264 million people are suffering from depression<sup>6</sup>. Right before the Covid-19 pandemic, a survey from Euromonitor<sup>7</sup> covering 21 000 participants from 21 countries highlighted that **over 50% of global consumers declare to suffer from moderate, high or extreme stress - a figure exceeding 60% in generations of working age.**

This trend is expected to grow at an even faster trend as a result of the pandemic, and consumers are wary to take drugs like SSRIs and monoamine oxidase inhibitors because of their significant side effects.

A recent review<sup>8</sup> confirmed that **psychobiotics**, which are defined as live organisms that, when ingested in adequate amounts, produce a health benefit **in patients suffering from psychiatric illness, show promise as an alternative therapy in human mental**, neurodegenerative and neurodevelopmental disorders and a second review by Noonan and colleagues on **prebiotic and probiotic interventions in the treatment of anxiety and depression in adults also concluded positively**<sup>9</sup>.

### THE GUT-MICROBIOTA-BRAIN AXIS: MULTIPLE ROADS OF COMMUNICATION

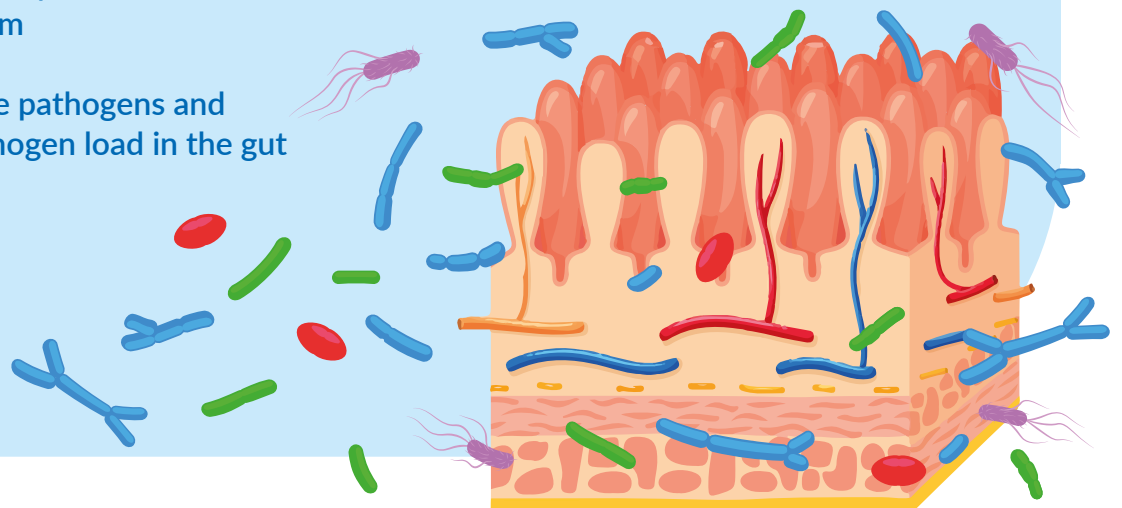
The mechanisms of action **between the bidirectional cross-talk between the gut**, the microbiome and the brain are still being elucidated. They include a direct communication through the vagus nerve, signaling molecules comprising neurotransmitters and their precursors, immune signals such as cytokines that are able to cross the blood-brain barrier, as well as endocrine signals.

Crucially, **neuroinflammation has been identified as key in the pathogenesis of multiple psychiatric conditions**, of which **anxiety** and major **depressive disorders**<sup>10-15</sup>.

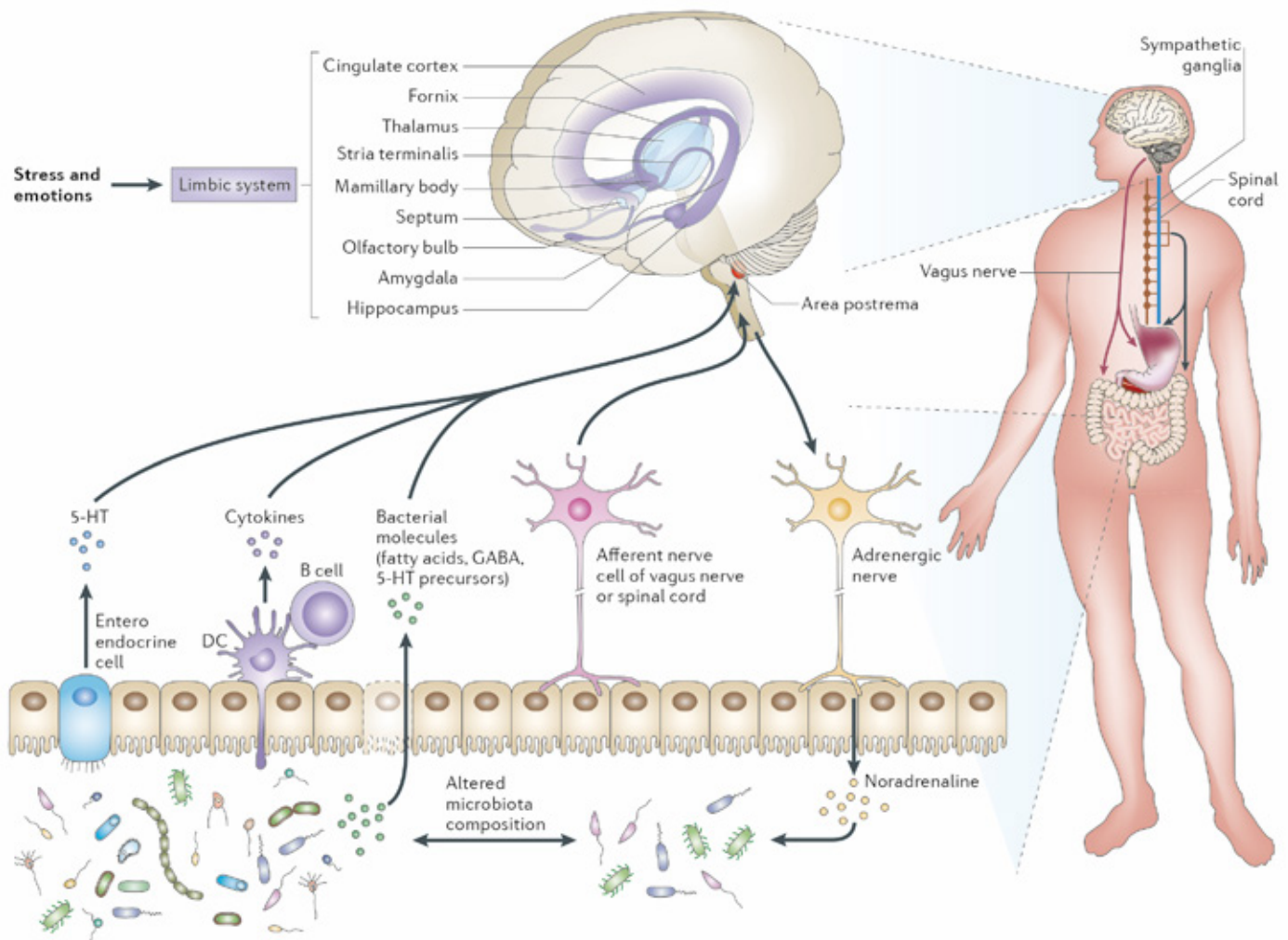
**Probiotics can act** on these different pathways **of the gut-brain axis**, and in particular on inflammation, directly and indirectly.

### PROBIOTICS CAN OPPOSE DIRECTLY OR INDIRECTLY INFLAMMATION:

- By direct induction of anti-inflammatory cytokines
- By reinforcing the gut barrier and limiting pro-inflammatory pathogen and LipoPolySaccharides (LPS) translocation into the bloodstream
- By antagonizing the pathogens and decreasing the pathogen load in the gut







From Collins et al., 2012. Nature Reviews Microbiology

## CAREFULLY SELECTED PROBIOTIC STRAINS FOR BENEFITS IN MENTAL HEALTH

Based on these perspectives, **Probiotal selected high-potential strains for mental well-being**, built on a pre-clinical, in silico and in vitro evaluation<sup>16-19</sup> to target strains with anti-inflammatory properties, anti-oxidant activity, antagonism towards different classes of pathogens, protection and restoration of membrane integrity and presenting genes coding for the production of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the human central nervous system.

## BIFIZEN® PROVED EFFECTIVE IN MOOD AND SLEEP IMPROVEMENT IN A CLINICAL STUDY

The four chosen strains, ***L. rhamnosus* LR06 (DSM 21981)**, ***L. plantarum* LP01 (LMG P-21021)**, ***B. longum* 04 (DSM 23233)** and ***L. fermentum* LF16 (DSM 26856)** (together branded as **Bifizen®**) were subsequently tested in a double-blind placebo-controlled trial involving 38 healthy students<sup>20</sup>.

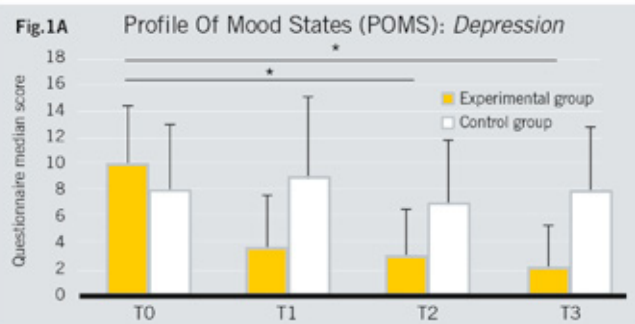
The probiotic group received a daily dosage of 1 billion cfu/afu per strain and per day, in sachets, for 6 weeks, while the control group received a placebo. Participants compiled a battery of psychological validated questionnaires evaluating their mood, personality and sleep quality at baseline, 3 weeks into supplementation, 6 weeks into supplementation, and after 3 weeks of wash-out.

Their fecal microbiota was also sampled for subsequent analysis and will be the object of a second publication expected within 2020.

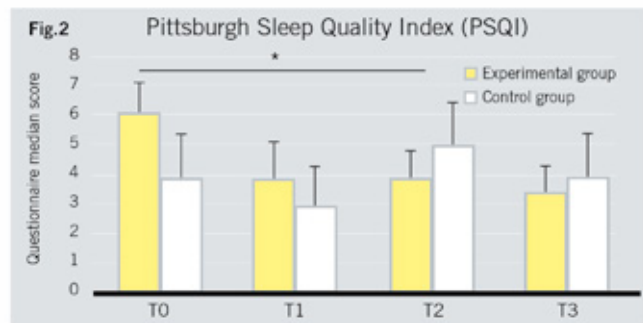
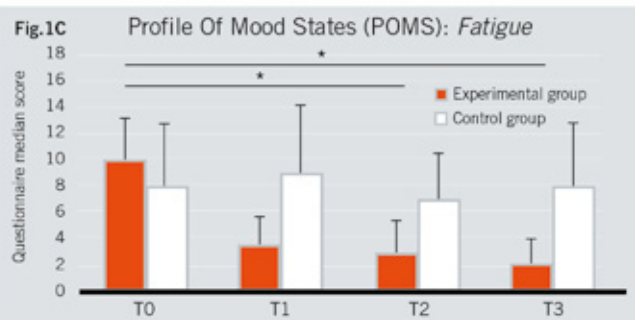
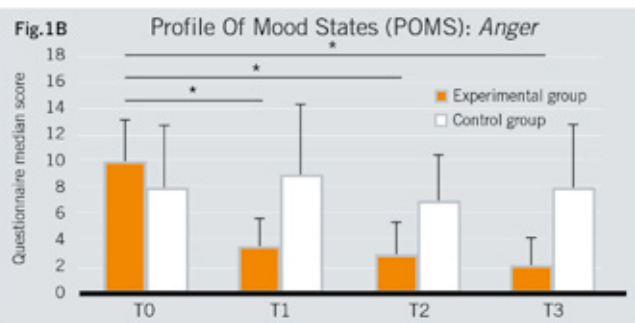
The **probiotics supplementation** significantly improved mood, with a **reduction in depressive mood state, anger and fatigue**, as early as 3 weeks of supplementation and even more profound after 6 weeks, as well as significant improvements in **sleep quality** measured by the Pittsburgh Sleep Quality Index. It also showed an increase in acceptance, which correlates with decreased depression sensitivity<sup>21</sup>.

These observed significant effects were maintained **after 3 weeks of wash-out, pointing to prolonged benefits after the discontinuation of probiotics intake.** (results on figure1 and 2)

## DOUBLE-BLIND, PLACEBO-CONTROLLED RANDOMIZED STUDY ON HEALTHY STUDENTS



**Fig.1** Median POMS subscale score for the experimental and the control groups at all-time points. (A) Depression subscale; (B) Anger subscale; (C) Fatigue subscale. Error bars represent 95% confidence interval. Asterisks indicate within-groups significant differences (Bonferroni corrected  $p < 0.017$ ).



**Fig.2** Median PSQI score for the experimental and the control groups at all time points. Error bars represent 95% confidence interval. Asterisks indicate within-groups significant differences (Bonferroni corrected  $p < 0.017$ ).

From Marotta et al., 2019, Frontiers in Psychiatry



**Marco Pane**, co-author of the study and R&D director at Probiotical commented that **these are strong and exciting findings since the probiotics intake managed to significantly and consistently improve several aspects of mood and sleep in a young and healthy cohort**, which is among the most resilient population possible.

He expects these results could translate to clinical benefits in a population with mental disorders and plans to test **Bifizen®** in patients with major depression.



As mentioned by Lumina<sup>4</sup>, personalization is a macro-trend that applies also to probiotic and psychobiotic supplements, and Euromonitor highlighted that consumers are increasingly seeking a holistic approach to healthy living<sup>7</sup>.

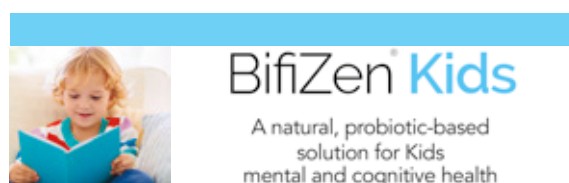
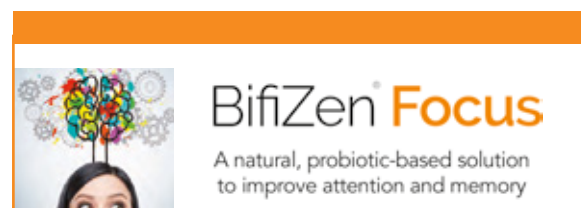
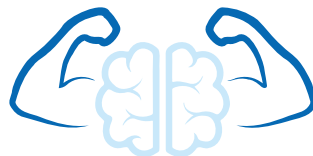
For these reasons, Probiotal team developed a range of products associating **BifiZen®** strains to well-established and clinically-backed actives including botanical extracts, vitamins and minerals, to offer a range of 5 products to address in complete, integrated formulations, different mental health benefits and focus on the different needs of each specific age category, as well as to allow claims in Europe. The added-value of Probiotal combinations is that **we tested the compatibility** between the strains and all the tested actives and can ensure the stability, in contact with these ingredients, for 24 months at 25°C.

For **Stress and Focus** targeted to working age generations, our laboratory team validated the compatibility with the King of adaptogens: Ashwagandha, and with group B vitamins to support the nervous system and mental performance.

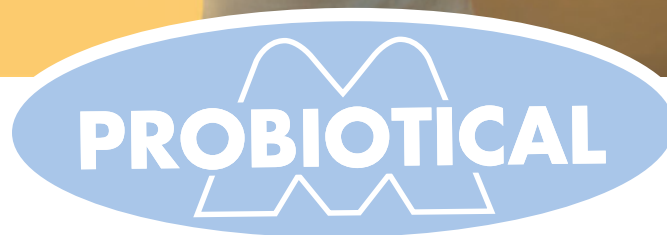
For **better nights**, our tested association combines the benefits of a better sleep quality thanks to **Bifizen®** while melatonin and vitamin B12 contribute to the reduction of tiredness, fatigue and time to fall asleep.

Our **Healthy Aging formulation** supports mood and sensibility to depression and includes actives showing neuroprotective action: alpha-GPC and zinc. It can claim to contribute to normal cognitive functions, including in patients with Alzheimer.

As **for Kids**, we combined **Bifizen®** with a well-established probiotic strain in pediatrics, that has demonstrated to significantly reduce the risk of developing autism and attention deficit hyperactivity disorder<sup>22</sup>, and with a natural botanical *passiflora incarnata* extract as effective in reducing the symptoms of hyperactivity as the standard of care, without the side effects.



CONTACT US FOR MORE INFORMATION ON OUR HOLISTIC SOLUTIONS FOR MENTAL WELL-BEING!



Probiotics like nobody else



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**Probiotal** is an Italian family company born in 1985 and ever since dedicated completely to the **research, development** and **production of probiotic strains** and **finished products**.

With a strains catalogue of over **50 different probiotic bacteria** studied in over 20 different functionalities ranging from immunity and digestive health to the vaginal flora, allergy and sports performance to name a few, **Probiotal** is able to **customize formulas** and dosage forms to fit the expectations of each customer, developing a real partnership.

Our pharmaceutical quality culture and guarantees of stability has put us among the world leaders in probiotics, and one of the main drivers of innovation in the sector.



Our team is passionate about the potential of our microscopic friends to improve people's health and quality of life, and is looking forward to answering your questions!

Thank you for reading and contacting us!

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- (6) <https://www.who.int/news-room/fact-sheets/detail/depression>
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**Probiotics like nobody else**