Specification of Sleep Aid Probiotics Capsule

1. Brief Introduction

Product Name: Sleep Aid Probiotics Capsule

Product Specification: 60 capsules/box, 500 mg/capsule

Directions: Take one(1) Sleep Aid Probiotics Capsule 30 min before sleep.

Caution: Consult physician if pregnant/nursing, taking medication, or have a medical condition. Keep out of reach of children. After taking the capsule, avoid watching electronic screens or light exposure, which may upset melatonin production in the body.

Storage: store in a cool(below 4℃), dry place to maintain potency.

Shelf Life: 24 months.

Potency: *Lactobacillus plantarum* N-1 (25 billion CFU/capsule), *Lactobacillus rhamnosus* PB-LR76 (15 billion CFU/capsule), *Lactobacillus paracasei* HH-LP58 (20 billion CFU/capsule)

Other ingredients: chamomile extract, lemon balm, passion flower, *Ziziphi Spinosae Semen*, galactooligosaccharide.

This probiotic formula is specially formulated for sleep health. This probiotic formula contains patented and scientifically proved probiotic strains *Lactobacillus plantarum* N-1 and *Lactobacillus rhamnosus* PB-LR76, which

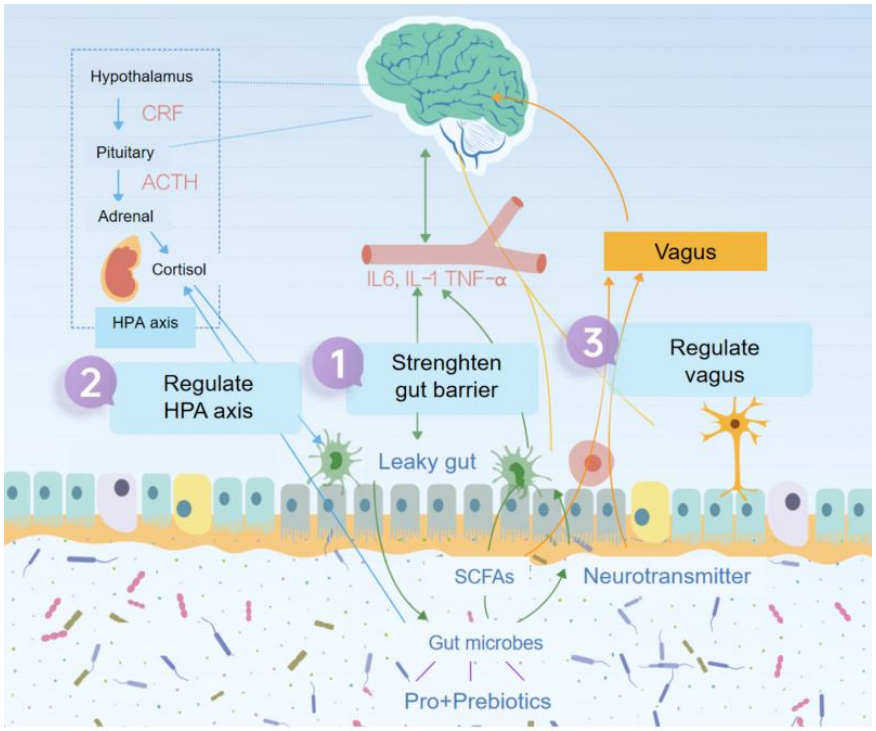
have the following functions:

√ Stress relieve

√ Anti- inflammation

2. Gut Microflora and Sleep Health

According to the research on the gut-brain axis mechanism in recent decades, scientists have found that gut microflora can affect sleep mainly through the following ways:



① Immune system pathway:

The structural components of gut microbes(such as LPS) releasing by phagocytosis, could stimulate the innate immune system to produce cytokines, resulting in a state of immune activation.

② Neuroendocrine pathway: The gut microbiota is directly involved in the production of several neurotransmitters, cytokines, and metabolites, such as 5-HT (5-hydroxytryptamine), dopamine, gamma-aminobutyric acid (GABA), SCFA (short-chain fatty acids), and melatonin. Some *Lactobacillus* and *Bifidobacterium* could produce GABA. Abnormal expression of GABA mRNA is often observed in patients with insomnia. Some *E. coli* could produce norepinephrine, serotonin, and dopamine; Some *Streptococcus* and *Enterococcus* could produce serotonin; *Bacillus* could produce norepinephrine and dopamine. Beneficial bacteria are involved in the production of hormones that regulate sleep.

③Hypothalamic-pituitary-adrenal axis pathway: sleep, in particular deep sleep, has an inhibitory influence on the hypothalamic-pituitary-adrenal (HPA) axis, whereas activation of the HPA axis can lead to arousal and sleeplessness..

④ The vagus pathway: The gut microbiota is the most abundant and diverse microbiota in the human body and the vagus nerve is the most widely distributed and complex nerve in the body, both of them are essential in maintaining homeostasis. The vagus nerve pathway is activated when sensory neurons of the myenteric plexus are stimulated by contact with gut microbiota. Having a high concentration of pathogenic bacteria in the microbiome can lead to an excess of neurotoxic metabolites entering the CNS via the vagus nerve. This impacts on sleep cycles and stress response.

3. Mechanism of probiotics function

①Participate in immune regulation： Probiotics can produce various cytokines and chemokines in the body, changing peripheral inflammatory markers and immune environment. The gut microbiota interacts with immune cells in the brain, thereby affecting the levels of cytokines, cytodynamic response factors and prostaglandin E2 levels, affecting the immune system of the host, and thereby regulating brain activity to achieve the effect of sleep aid.

② Participate in endocrine secretion: Probiotics can regulate central neurotransmitters by altering their precursor levels. Probiotics can also directly synthesize and release the neurotransmitter gamma-aminobutyric acid (GABA), which is an important inhibitory neurotransmitter in the central nervous system of the human body, and can inhibit the excitation of the sympathetic nervous system, play a role in hypnosis, sedation and so on.

③Probiotics and prebiotics can act on gut microbes to modulate nerve signals on the hypothalamic-pituitary-adrenal (HPA) axis via cortisol.

4. About N-1

Origin

The strain was isolated from traditional yak cheese in Qinghai-Tibet Plateau, China.

Culture Deposit No.: CGMCC No.15463

Acid and Bile Salt Tolerance

Acid and bile tolerance are the most crucial properties for probiotic bacteria, as it determines its ability to survive in the small intestine, and consequently its capacity to play its functional role as a probiotic. N-1 exhibited a very good survival at low pH and high concentration of bile salt.

Strain	Survival Rate(%)			
	pH 3.5	pH 2.5	0.1% Bile Salt	0.2% Bile Salt
N-1	98.0	60.6	99.1	73.9

Adhesion Property

Adhesion ability to the host is a classical selection criterion for potential probiotic bacteria. It is considered to be associated with colonization, pathogen inhibition, immune interations, and barrier function enhancement. Adhesion test result showed that the adhesion number of N-1 to Caco-2 cells was 6.04×10^6 CFU/mL, and the adhesion rate was 4.03%.

Sleep Aid Effect

In an animal study, 50 mice(body weight: 18-22g) were randomly divided into 5 groups with 10 mice(5 male, 5 female) in each group. The probiotic treatment group 1-3 were fed with 2g/kg probiotics via oral gavage; positive control group was fed with 2g/kg *Ziziphi Spinosae Semen;* Blank control group was fed with saline. The oral gavage was conducted daily for 1 week. Then, the sleep rates and levels of GABA and 5-HT were recorded and analyzed. The results showed that probiotic treatment could increase GABA and 5-HT levels in mice brain and improve their sleep health.

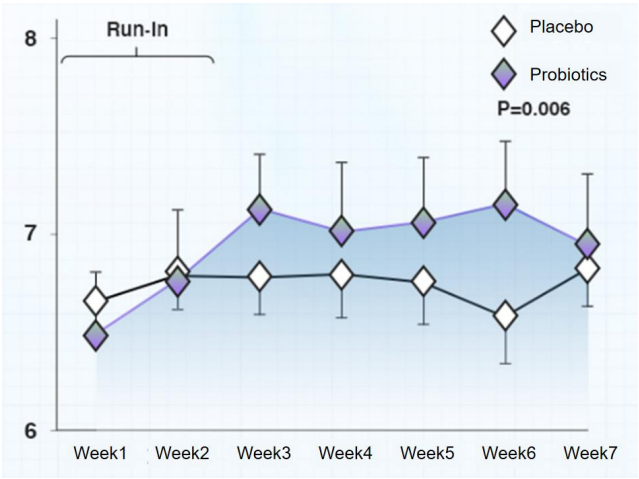
Group	Number of asleep mice 30min after oral gavage	Asleep rate
Blank control	4	40
Positive control	8	80
Probiotics 1	9	90
Probiotics 2	10	100
Probiotics 3	10	100

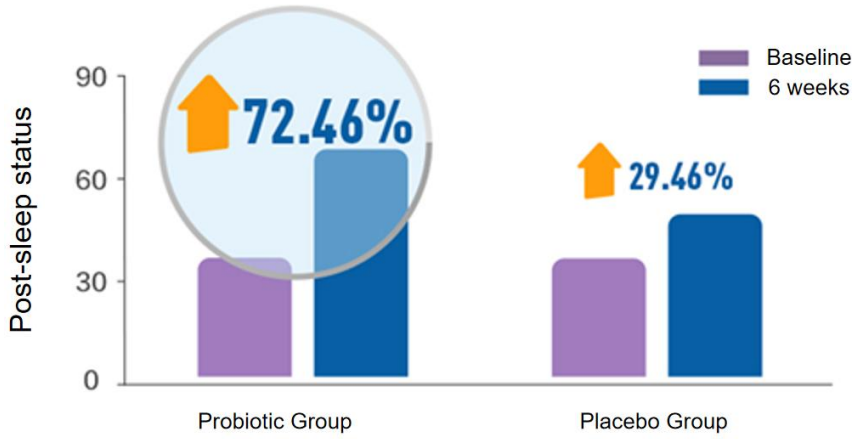
Group	Level of 5HT(ng/mL)	Level of GABA(μmol/L)
Blank control	2213.22±324.51	0.05±0.01
Positive control	3378.17±452.61	0.13±0.04
Probiotics 1	3488.12±425.72	0.15±0.05
Probiotics 2	3495.71±449.17	0.17±0.09
Probiotics 3	3504.16±461.29	0.18±0.07

Post Sleep Status Assessment

Thirty melancholia model mice were randomly divided into two groups: the control group was fed ordinary diet, while the experimental group was fed probiotic-containing diet, and the other feeding conditions were the same. The post-sleep mental scores of the two groups were measured for 7 weeks to simulate the post-sleep recovery of people with high chronic stress. The weekly

sleep duration and falling asleep time of the following 6 weeks were compared and evaluated. The results showed that probiotic treatment improved their post-sleep health and recover after sleep. In addition, the sleep latency was shortened by 18.8%, while the sleep duration was increased by 7.2%





Sleep Quality Assessment

In an animal study, N-1 intervention was able to significantly improve the anxiety, stress and depression-like behavior of autistic mice.

5. Publications and Patents

[1] Qiu Z, et al. (2023) *Lactiplantibacillus plantarum* N-1 improves autism-like behavior and gut microbiota in mouse. Frontiers in Microbiology. DOI

10.3389/fmicb.2023.1134517

6. Other ingredients

Chamomile extract: Chamomile is widely recognized as a mild tranquilizer and sleep-inducer. It is thought that chamomile may increase brain neurotransmitter activity(serotonin, dopamine, and noradrenaline) and thereby have positive effects on mood and anxiety.

Lemon balm: lemon balm may be effective in improving anxiety and depressive symptoms.

Passion flower: works by increasing levels of GABA for treating anxiety and insomnia.

***Ziziphi Spinosae Semen*:** *Ziziphi Spinosae Semen* (ZS) has a long history as an effective traditional Chinese medicine. It has been used to treat palpitations, insomnia, dizziness, headache, nausea and vomiting, coughs, depression, anxiety, and other diseases. More than 150 compounds have been isolated

from ZS, including terpenoids, alkaloids, flavonoids, fatty acids, volatile oils, polysaccharides, and some inorganic compounds .