Technical Report

Significance of *Lactobacillus plantarum* in CanXida Restore (Formula RST)

*"In CanXida Restore (Formula RST), **Lactobacillus plantarum** antimicrobial, anti-inflammatory & wound healing properties, offering a potent solution for microbial restoration and Candida spp. control.*

1 Contents

Executive Summary

Lactobacillus plantarum is a versatile probiotic bacterium with diverse functional properties and a strong safety profile. *L. plantarum* exhibits antimicrobial, antioxidant, and anti-inflammatory properties, promoting microbial balance, and enhancing gut barrier function. It has the potential of restoring vaginal health, preventing gastrointestinal disorders, and managing allergies.

It effectively targets various bacterial and fungal species, including *Bacillus*, *Clostridium*, *Pseudomonas*, *Aspergillus*, and *Candida spp*. The inclusion of *L. plantarum* in the CanXida Restore formula leverages these benefits, optimizing its efficacy through synergies with other ingredients. The versatility of *L. plantarum* makes it a valuable asset in promoting microbial restoration and eliminating pathogens*.

1. Introduction

L. plantarum is known for its presence in diverse ecological habitats, including vegetables, fermented foods, and the healthy human intestinal mucosa, making it one of the most well-known *Lactobacillus* species. It is classified under the phylum Firmicutes, which is one of the two primary phyla that are prevalent in the intestinal microbiota (Yilmaz et al., 2022). There have been reports of more than 186 strains of *L. plantarum* (Le & Yang, 2018).

L. plantarum is a type of lactobacilli that is Gramme positive, has a short-rod shape, requires low levels of oxygen, can tolerate acidic conditions, does not form spores or engage in respiration, and is hetero-fermentative. It is commonly used in the food industry as a starter culture and preservative (Valan Arasu et al., 2013). It is also a non-spore forming bacteria

L. plantarum has garnered significant attention from researchers due to its extensive range of applications in the medical field. It has been found to possess various beneficial properties, including antioxidant, antiproliferative, anti-inflammatory, antiproliferative, antidiabetic and anti-obesity effects (Arasu et al., 2016).

L. plantarum thrives in the stomach and other bile salt secretions found in humans and other mammals, which have low buffering capacity. In addition to its use in the food industry, *L. plantarum* also has extensive applications in the pharmaceutical industry, making significant contributions to human medicine without causing any adverse effects.

2. Functional Properties of *Lactobacillus plantarum*:

Lactobacillus plantarum contributes significantly to gut health through various mechanisms, such as

2.1 Antimicrobial Property

L. plantarum produces antimicrobial substances that inhibit the growth of pathogenic bacteria in the gut. This protective effect helps prevent infections and maintains a healthy microbial balance (Dempsey & Corr, 2022).

L. plantarum produces different antimicrobial agents, such as

- Organic acids

- Hydrogen peroxide
- Diacetyl
- Bacteriocins
- Antimicrobial peptides

2.2 Antioxidant Property

L. plantarum has been found to exhibit antioxidant activity, helping to neutralize harmful free radicals in the body (Feng & Wang, 2020). Antioxidants play a role in protecting cells from oxidative stress.

The antioxidant compounds produced by *L. plantarum* include (Garcia-Gonzalez et al., 2021):

- Folate
- Butyrate
- Glutathione
- Enzymes
- Extracellular Polymeric Substances (EPS)

2.3 Anti-inflammatory Property

Chronic inflammation is associated with various gut disorders, and the presence of beneficial bacteria can mitigate inflammatory responses. The anti-inflammatory properties of *L. plantarum* can help reduce inflammation in the gastrointestinal tract (Garcia-Gonzalez et al., 2021), such as

- By reducing IL-1β and IL-6, acting as anti-inflammatory agents.
- By inhibiting NF-κB and AP1, modulating immune response*,
- TLR-dependent immunoregulation by inducing cytokines (IL-12, IL-10, IL-13, IFN-γ) for immune modulation*.
- By blocking NF-κB activation.

2.4 Maintaining Microbial Balance:

L. plantarum helps maintain a balanced and diverse gut microbiota (Linninge et al., 2019; Xie et al., 2016). *L. plantarum* is known for its ability to enhance the growth of beneficial bacteria in the gut (Echegaray et al., 2023), making it an effective probiotic. This process aids in the displacement of detrimental microorganisms, creating a more favorable environment for gut health.

2.5 Modulating the Immune System:

L. plantarum has immunomodulatory properties, meaning it can influence the activity of the immune system*. It may

stimulate the production of certain immune cells and cytokines, contributing to a well-regulated immune response in the gut (Arasu et al., 2016; Garcia-Gonzalez et al., 2021).

2.6 Enhancing Gut Barrier Function:

L. plantarum plays a role in maintaining the integrity of the gut barrier. It helps strengthen the tight junctions between intestinal cells, preventing the leakage of harmful substances and pathogens into the bloodstream (Echegaray et al., 2023).

2.7 Production of Short-Chain Fatty Acids:

L. plantarum participates in the fermentation of dietary fibers, producing short-chain fatty acids such as butyrate. SCFAs provide energy for the cells lining the colon and contribute to a healthy colonic environment (Markowiak-Kopeć & Śliżewska, 2020).

2.8 Digestive Enzyme Production:

L. plantarum can produce enzymes that aid in the digestion of complex carbohydrates. This may enhance nutrient absorption and support overall digestive function (Liu et al., 2023).

2.9 Resilience to Environmental Stresses:

This strain exhibited tolerance to a wide variety of salts, including NaCl and bile salts. It was also able to thrive in a pH range of 4.0 to 8.0 and at temperatures ranging from 28 to 45 °C. The optimal conditions for cell growth were found to be a temperature of 37 °C and a pH of 7.0. It has successfully withstood numerous biological barriers, including acidic conditions, lytic enzymes, and bile salts in the upper gastrointestinal tract (Vijayakumar et al., 2015).

3. Health Benefits of *Lactobacillus plantarum*

L. plantarum offers several health benefits, primarily through its probiotic properties and contributions to gut health (Echegaray et al., 2023). Here are some key health benefits associated with *L. plantarum*

3.1 Restoration of microbial balance:

L. plantarum contributes to the maintenance of a balanced and diverse gut microbiota, promoting a healthier microbial community.

It helps in the prevention of dysbiosis, which is an imbalance in the gut bacteria associated with various digestive disorders (Kaźmierczak-Siedlecka et al., 2021; Y. Liu et al., 2021; Zhang et al., 2022).

3.2 Mental health

There is emerging research on the gut-brain axis, suggesting a connection between gut health and mental well-being. Probiotics, including *L. plantarum*, may influence this axis and potentially have positive effects on mood and mental health (Gupta et al., 2024).

3.3 Vaginal Health

L. plantarum can contribute to preventing and managing vaginal infections, such as bacterial vaginosis and yeast infections (Liu et al., 2023) It does so by producing lactic acid, which helps maintain an optimal pH level in the vagina.

3.4 Gastrointestinal Disorder Prevention:

The presence of *L. plantarum* in the gut may help prevent or alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome, inflammatory bowel diseases, and diarrhea (Lee & Bak, 2011; Yang et al., 2021).

3.5 Allergy Management:

Some studies suggest that *L. plantarum* may have a role in managing allergies by modulating the immune response and reducing inflammation (Duan et al., 2023).

3.6 Wound Healing Property:

L. plantarum, primarily known for its role in gut health, has shown potential relevance in wound healing and burn infections. While direct studies on its impact are limited, *L. plantarum* anti-inflammatory and immune-modulating properties could indirectly aid in the wound healing process by promoting a balanced inflammatory response. It also shows wound healing properties due to proliferation of fibroblast (Dubey et al., 2021).

Table 1: *Clinical trials that have used Lactobacillus plantarum for treatment or prevention are detailed here. Source: clinicaltrials.gov*

Clinical trial ID	Health Condition	Status
NCT03975569	Candidiasis, Vulvovaginal	*Completed*
NCT04461782	Vaginal Infection	*Completed*
NCT05587283	• Vaginal Disease • Bacterial Infections • Bacterial Vaginosis • Infection, Bacterial • Vaginitis	*Recruiting*
NCT05176535	• Dysbiosis • Fertility Issues • Microbial Colonization	*Unknown status*
NCT03940612	Vaginal Diseases	Completed
NCT03116789	Bacterial Vaginosis	Completed
NCT05060029	Bacterial Vaginosis	*Recruiting*
NCT01456767	Gastro Intestinal Infection	*Completed*
NCT04950296	Irritable Bowel Syndrome with Diarrhea	*Completed*
NCT00355810	• Functional Gastrointestinal Disorders • Irritable Bowel Syndrome	*Completed*
NCT05523427	• Irritable Bowel Syndrome with Diarrhea • Irritable Bowel Syndrome	*Completed*
NCT03237078	• Inflammation • Major Depressive Disorder	*Unknown status*

	• Probiotics	
NCT01886781	Irritable Bowel Syndrome	*Completed*
NCT03330678	Probiotics	*Completed*
NCT01456767	Gastro Intestinal Infection	*Completed*
NCT00355810	• Functional Gastrointestinal Disorders • Irritable Bowel Syndrome	*Completed*
NCT04375475	Intestinal Health	*Completed*
NCT04375475	• Diarrhea • Irritable bowel syndrome	*Completed*
NCT05836155	Dysbiosis	*Recruiting*

4. Biosafety Profile of *Lactobacillus plantarum*

Similar to the majority of lactobacilli, this particular strain has also been included in the EFSA's list of microorganisms with Qualified Presumption of Safety and is generally recognized as safe by FDA.

Lactobacillus plantarum has been extensively studied for its potential medicinal applications. It has been tested by **DRUG BANK**, with the accession **ID DB11360**.

L. plantarum has a well-established track record of being safe for consumption. Over the course of many years in the field of food and clinical practice, there have been limited instances of infections attributed to *L. plantarum*. Certain strains of *L. plantarum* have the potential to impact the health of elderly individuals or those with compromised immune systems. This can lead to an increased risk of blood clotting, which in turn causes the aggregation of human platelets in laboratory settings (Le & Yang, 2018).

Nevertheless, certain studies have provided evidence that *L. plantarum* does not induce infection when given orally or through intravenous injection in mice. In addition, the post-market surveillance study did not find any evidence of bacteremia caused by L. plantarum (Le & Yang, 2018).

Recently, there has been a growing interest in the use of *L. plantarum*, specifically in its probiotic properties and its various applications in fermented foods and beverages. This includes the fermentation of dairy products such as cheese, kefir, sauerkraut, silage, and wine. In addition, there are fermented meat products and fermented vegetables (Amro Abdelazez et al., 2018).

The biosafety profile of *L. plantarum* is generally considered favorable, especially in the context of its widespread use in food fermentation and its potential applications as a probiotic (Behera et al., 2018).

5. Effective Targets of *Lactobacillus plantarum*

L. plantarum is a probiotic bacterium known for its versatility in interacting with various microbial targets in different environments. *L. plantarum* effectively acts upon various microbial targets (Amro Abdelazez et al., 2018), some of which include

5.1 Bacterial Species

***Bacillus*:** Various species can produce toxins causing food poisoning; Bacillus anthracis causes anthrax.

***Clostridium*:** *Clostridium* difficile causes antibiotic-associated diarrhea.

***Pseudomonas**:* Opportunistic pathogen causing infections.

***Helicobacter pylori*:** Major cause of peptic ulcers.

***Campylobacter jejuni*:** Common cause of bacterial gastroenteritis, leading to diarrhea, abdominal pain, and fever.

***Klebsiella*:** It is capable of causing urinary tract infections, pneumonia, and other

***Salmonella*:** Causes Salmonellosis, characterized by diarrhea, fever, and abdominal cramps.

Shigella: Causes Shigellosis, leading to severe diarrhea, fever, and stomach cramps.

***Escherichia coli*:** Various pathogenic strains cause foodborne illnesses, urinary tract infections, and other health issues.

5.2 Fungal Species

***Aspergillus*:** Typically, not linked to gut health; instead, it is more commonly associated with respiratory risks and concerns about mycotoxins in food.

***Candida spp.*:** It has the potential to impact gut health, as overgrowth has been associated with conditions such as candidiasis, which can affect digestion and overall well-being.

6 Significance of *Lactobacillus plantarum* in CanXida Restore Formula

The inclusion of *Lactobacillus plantarum* in the CanXida Restore Formula holds significant therapeutic value, leveraging the versatile functional properties of this probiotic.

L. plantarum contributes to gut health by inhibiting pathogenic bacteria through its antimicrobial properties, producing organic acids, hydrogen peroxide, and bacteriocins. Its antioxidant and anti-inflammatory capabilities are crucial for mitigating oxidative stress and reducing inflammation in the gastrointestinal tract. The probiotic aids in maintaining microbial balance by promoting the growth of beneficial bacteria, modulating the immune system, and enhancing gut barrier function. Additionally, its role in producing short-chain fatty acids supports a healthy colonic environment.

In the CanXida Restore Formula, *L. plantarum* offers health benefits such as restoring microbial balance, supporting vaginal health, preventing gastrointestinal disorders, and managing allergies. It potentially aids in wound healing that can help recovery from candidiasis. *L. plantarum*, supported by numerous studies, demonstrates beneficial outcomes when combined with other probiotics. In CanXida Restore, its inclusion is optimized through synergies with carefully chosen ingredients*.

References

Amro Abdelazez, Abdelmotaal Heba, Zong-Tao Zhu, Jia Fang-Fang, Rokayya Sami, Lu-Ji Zhang, Abdel Rahman Mohammad Al Tawaha, & Xiang-Chen Meng. (2018). Potential benefits of Lactobacillus plantarum as probiotic and its advantages in human health and industrial applications: A review. *Advances in Environmental Biology*, *12*(1). https://doi.org/10.22587/AEB.2018.12.1.4

Arasu, M. V., Al-Dhabi, N. A., Ilavenil, S., Choi, K. C., & Srigopalram, S. (2016). In vitro importance of probiotic Lactobacillus plantarum related to medical field. *Saudi Journal of Biological Sciences*, *23*(1), S6. https://doi.org/10.1016/J.SJBS.2015.09.022

Behera, S. S., Ray, R. C., & Zdolec, N. (2018). Lactobacillus plantarum with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/9361614

Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for Gastrointestinal Health: Current and Future Perspectives. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.840245

Duan, C., Ma, L., Yu, J., Sun, Y., Liu, L., Ma, F., Li, X., & Li, D. (2023). Oral administration of Lactobacillus plantarum JC7 alleviates OVA-induced murine food allergy through immunoregulation and restoring disordered intestinal microbiota. *European Journal of Nutrition*, *62*(2), 685–698. https://doi.org/10.1007/S00394-022-03016-5/FIGURES/10

Dubey, A. K., Podia, M., Priyanka, Raut, S., Singh, S., Pinnaka, A. K., & Khatri, N. (2021). Insight Into the Beneficial Role of Lactiplantibacillus plantarum Supernatant Against Bacterial Infections, Oxidative Stress, and Wound Healing in A549 Cells and BALB/c Mice. *Frontiers in Pharmacology*, *12*, 728614. https://doi.org/10.3389/FPHAR.2021.728614/BIBTEX

Echegaray, N., Yilmaz, B., Sharma, H., Kumar, M., Pateiro, M., Ozogul, F., & Lorenzo, J. M. (2023). A novel approach to Lactiplantibacillus plantarum: From probiotic properties to the

omics insights. *Microbiological Research*, *268*, 127289. https://doi.org/10.1016/J.MICRES.2022.127289

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*, *12*(1). https://doi.org/10.1080/19490976.2020.1801944

Garcia-Gonzalez, N., Battista, N., Prete, R., & Corsetti, A. (2021). Health-Promoting Role of Lactiplantibacillus plantarum Isolated from Fermented Foods. *Microorganisms 2021, Vol. 9, Page 349*, *9*(2), 349. https://doi.org/10.3390/MICROORGANISMS9020349

Gupta, N., Agarwal, A. K., Al-Dossari, M., & Abd EL-Gawaad, N. S. (2024). Psychobiotic potential of Lactiplantibacillus plantarum: Current perspective in neurodegeneration and geriatric therapies. *Brain Behavior and Immunity Integrative*, *5*, 100038. https://doi.org/10.1016/J.BBII.2023.100038

Kaźmierczak-Siedlecka, K., Daca, A., Folwarski, M., Witkowski, J. M., Bryl, E., & Makarewicz, W. (2021). The role of Lactobacillus plantarum 299v in supporting treatment of selected diseases. *Central European Journal of Immunology*, *45*(4), 488–493. https://doi.org/10.5114/CEJI.2020.101515

Le, B., & Yang, S. H. (2018). Efficacy of Lactobacillus plantarum in prevention of inflammatory bowel disease. *Toxicology Reports*, *5*, 314–317. https://doi.org/10.1016/J.TOXREP.2018.02.007

Lee, B. J., & Bak, Y. T. (2011). Irritable Bowel Syndrome, Gut Microbiota and Probiotics. *Journal of Neurogastroenterology and Motility*, *17*(3), 252. https://doi.org/10.5056/JNM.2011.17.3.252

Linninge, C., Xu, J., Bahl, M. I., Ahrné, S., & Molin, G. (2019). Lactobacillus fermentum and Lactobacillus plantarum increased gut microbiota diversity and functionality, and mitigated

Enterobacteriaceae, in a mouse model. *Https://Doi.Org/10.3920/BM2018.0074*, *10*(4), 413–424. https://doi.org/10.3920/BM2018.0074

Liu, C., Ma, N., Feng, | Yue, Zhou, M., Li, | Huahui, Zhang, X., Ma, | Xi, & Ma, X. (2023). From probiotics to postbiotics: Concepts and applications. *Animal Research and One Health*, *1*(1), 92–114. https://doi.org/10.1002/ARO2.7

Liu, P., Lu, Y., Li, R., & Chen, X. (2023). Use of probiotic lactobacilli in the treatment of vaginal infections: In vitro and in vivo investigations. *Frontiers in Cellular and Infection Microbiology*, *13*. https://doi.org/10.3389/FCIMB.2023.1153894

Liu, Y., Yu, X., Yu, L., Tian, F., Zhao, J., Zhang, H., Qian, L., Wang, Q., Xue, Z., Zhai, Q., & Chen, W. (2021). Lactobacillus plantarum CCFM8610 Alleviates Irritable Bowel Syndrome and Prevents Gut Microbiota Dysbiosis: A Randomized, Double-Blind, Placebo-Controlled, Pilot Clinical Trial. *Engineering*, *7*(3), 376–385. https://doi.org/10.1016/J.ENG.2020.06.026

Markowiak-Kopeć, P., & Śliżewska, K. (2020). The Effect of Probiotics on the Production of Short-Chain Fatty Acids by Human Intestinal Microbiome. *Nutrients*, *12*(4). https://doi.org/10.3390/NU12041107

Valan Arasu, M., Jung, M. W., Ilavenil, S., Jane, M., Kim, D. H., Lee, K. D., Park, H. S., Hur, T. Y., Choi, G. J., Lim, Y. C., Al-Dhabi, N. A., & Choi, K. C. (2013). Isolation and characterization of antifungal compound from Lactobacillus plantarum KCC-10 from forage silage with potential beneficial properties. *Journal of Applied Microbiology*, *115*(5), 1172–1185. https://doi.org/10.1111/JAM.12319

Vijayakumar, M., Ilavenil, S., Kim, D. H., Arasu, M. V., Priya, K., & Choi, K. C. (2015). In-vitro assessment of the probiotic potential of Lactobacillus plantarum KCC-24 isolated from Italian rye-grass (Lolium multiflorum) forage. *Anaerobe*, *32*, 90–97. https://doi.org/10.1016/J.ANAEROBE.2015.01.003

Xie, Q., Pan, M., Huang, R., Tian, X., Tao, X., Shah, N. P., Wei, H., & Wan, C. (2016). Short communication: Modulation of the small intestinal microbial community composition over short-term or long-term administration with Lactobacillus plantarum ZDY2013. *Journal of Dairy Science*, *99*(9), 6913–6921. https://doi.org/10.3168/JDS.2016-11141

Yang, B., Yue, Y., Chen, Y., Ding, M., Li, B., Wang, L., Wang, Q., Stanton, C., Ross, R. P., Zhao, J., Zhang, H., & Chen, W. (2021). Lactobacillus plantarum CCFM1143 Alleviates Chronic Diarrhea via Inflammation Regulation and Gut Microbiota Modulation: A Double-Blind, Randomized, Placebo-Controlled Study. *Frontiers in Immunology*, *12*, 746585. https://doi.org/10.3389/FIMMU.2021.746585/BIBTEX

Yilmaz, B., Bangar, S. P., Echegaray, N., Suri, S., Tomasevic, I., Lorenzo, J. M., Melekoglu, E., Rocha, J. M., & Ozogul, F. (2022). The Impacts of Lactiplantibacillus plantarum on the Functional Properties of Fermented Foods: A Review of Current Knowledge. *Microorganisms*, *10*(4). https://doi.org/10.3390/MICROORGANISMS10040826

Zhang, S., Wang, T., Zhang, D., Wang, X., Zhang, Z., Lim, C., & Lee, S. (2022). Probiotic characterization of Lactiplantibacillus plantarum HOM3204 and its restoration effect on antibiotic-induced dysbiosis in mice. *Letters in Applied Microbiology*, *74*(6), 949–958. https://doi.org/10.1111/LAM.13683