

The role of limosilactobacillus reuteri strains on human health

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Master's thesis / Diplomski rad

2023

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, School of Medicine / Sveučilište u Zagrebu, Medicinski fakultet**

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:105:228147>

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UNIVERSITY OF ZAGREB SCHOOL OF MEDICINE

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**The Role of *Limosilactobacillus reuteri*
Strains on Human Health**

GRADUATION PAPER



Zagreb, 2023

This graduate thesis paper was completed at the Centre for Translational and Clinical Research, University of Zagreb School of Medicine during the academic year of 2022/2023. Special thanks to Ph.D. Mihaela Perić, Senior Research Associate and Ph.D. Mario Matijašić, Research Associate, for their mentorship.

Acronyms and Abbreviations

AhR	Aryl hydrocarbon receptor
ASD	Autism spectrum disorder
C-section	Cesarean section
CNS	Central nervous system
EPS	Extracellular polymeric substances
GI	Gastrointestinal
GMSCs	Gingiva mesenchymal stem cells
HMOs	Human milk oligosaccharides
ILCs	Innate lymphoid cells
MHFD	Maternal high fat diet
MSCs	Mesenchymal stem cells
MUBs	Mucus-binding proteins
NK cell	Natural killer cell
PPI	Proton-pump inhibitor
RANKL	Receptor activator of nuclear factor kappa beta ligand
RCT	Randomized control trial
TNFα	Tumor necrosis factor- α
Tregs	Regulatory T lymphocytes
3-HPA	3-Hydroxypropionaldehyde

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Abstract

In recent years the human gastrointestinal microbiota has proven to play a critical role in host health and disease. The majority of human microbiota resides in the large intestine and is composed of a diverse array of microorganisms, including bacteria, fungi, and archaea. Among these, *Limosilactobacillus reuteri* (formerly known as *Lactobacillus reuteri*), a gram-positive, heterofermentative lactic acid bacterium has gained attention as a probiotic due to its potential health benefits. Several strains of *L. reuteri* have been identified, each with its unique set of functional genes contributing to its probiotic effects. The beneficial properties include gastrointestinal microbiota modulation, pathogen colonization resistance, cobalamin synthesis, as well as positive endocrine, musculoskeletal, integumentary effects, anti-inflammatory, and neurodevelopmental effects.

Many chronic diseases have been associated with states of microbiota imbalance called dysbiosis. In contrast to the conventional pharmaceutical management of chronic diseases, probiotics such as *L. reuteri* could serve as important treatment options to rehabilitate perturbed microbial ecosystems and its functionally active metabolites, thus improving the safety and efficacy of current clinical practices. With more data gathered, *L. reuteri* could become a valuable tool for treating chronic conditions and restoring human health.

Key words: *Limosilactobacillus reuteri*, probiotic, gastrointestinal microbiota, dysbiosis, treatment

Sažetak

Posljednjih godina istraživanja su pokazala da mikrobiota probavnog trakta igra ključnu ulogu u zdravlju i bolesti ljudi. Većina ljudske mikrobiote nalazi se u debelom crijevu i sastoji se od raznolikog niza mikroorganizama, uključujući bakterije, gljivice, i arheje. Među njima se ističe gram-pozitivna, heterofermentativna bakterija mliječne kiseline *Limosilactobacillus reuteri* (ranije poznata kao *Lactobacillus reuteri*) koja je privukla pozornost kao probiotik zbog svojih potencijalnih zdravstvenih koristi. Identificirano je nekoliko sojeva *L. reuteri*, koji svaki sa svojim jedinstvenim skupom funkcionalnih gena pridonose specifičnim probiotičkim učincima. Tu spadaju modulacija gastrointestinalne mikrobiote, otpornost na kolonizaciju patogena, sinteza kobalamina te pozitivni učinci na endokrini, mišićno-koštani, pokrovni i imuni sustav te na neurorazvoj.

Mnoge kronične bolesti povezuju se sa stanjima neravnoteže mikrobiote koja se nazivaju disbiozom. Za razliku od konvencionalnog farmaceutskog liječenja kroničnih bolesti, probiotici poput *L. reuteri* mogli bi poslužiti kao važne terapijske opcije u oporavku narušenih mikrobnihi ekosustava i njegovih funkcionalno aktivnih metabolita te tako unaprijediti sigurnost i učinkovitost trenutnih kliničkih praksi. S više prikupljenih podataka, *L. reuteri* bi mogao postati vrijedan alat za liječenje kroničnih stanja kao i za unapređenje ljudskog zdravlja.

Ključne riječi: *Limosilactobacillus reuteri*, probiotik, crijevna mikrobiota, disbioza, liječenje

1. Introduction

The World Health Organization defines probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (1). *Lactobacillus reuteri*, recently renamed *Limosilactobacillus reuteri* (*L. reuteri*), is a well-studied microorganism known to induce numerous probiotic effects in its host. In addition, *L. reuteri* has fulfilled the necessary safety and efficacy criteria to be termed probiotic. These criteria include the capacity to survive in the gastrointestinal (GI) tract, a high resistance to gastric acids, the lack of any transferable antibiotic resistance genes, and the capacity to exert clear benefits to the host (2). Multiple studies have demonstrated *L. reuteri* supplementation to be safe, even at doses of as high as $2,9 \times 10^9$ colony-forming units (CFU)/day, in adults, children, infants, and even in the HIV-infected demographic (3–7).

L. reuteri has been shown to be an indigenous member of the human microbiota (8) and is found in multiple body sites, including the gastrointestinal tract, urinary tract, skin, and breast milk (9). However this beneficial species is becoming exceedingly rare in humans (10), probably due to the microbiome disrupting factors such as a low fiber diet, ultra-processed foods, antibiotics, oral contraceptives, etc. Although microbiota are not genetically transmitted, they still follow a hereditary pattern since offspring microbiota are maternally derived via vaginal delivery amongst other mechanisms. In this manner the loss of microbial diversity could be compounded over subsequent generations (11).

The human body is actually a super-organism composed of an approximately 1:1 ratio of microbial cells and human cells (12) with over 99% of the unique genes in human genome are bacterial and less than 1% are human (13). The emerging fields of metagenomics, meta-transcriptomics, and metabolomics are beginning to elucidate the intimacies between probiotic organisms and host physiology. A central understanding in the area of microbiota research is that prebiotics are metabolized by probiotics to yield postbiotics. Postbiotics are defined as “soluble factors, metabolic products or by-products, secreted by live bacteria, or released after bacterial lysis providing physiological benefits to the host” (14). For example, 90% of serotonin and 50% of dopamine is produced in the gastrointestinal tract by probiotic organisms (15).

In the past, microbiota research has yielded inconsistent results. This is largely attributed to the failure to identify microorganisms on the strain level. Therefore, current probiotic research ensures to differentiate microbes on a strain level. The aim of this review will be to amalgamate the beneficial effects of various *L. reuteri* strains on human physiology, by examining both animal and human studies.

2. *Limosilactobacillus reuteri* Probiotic Characteristics

2.1 Gastrointestinal Tract Adherence and Colonization

In addition to the commonly known functions of the gastrointestinal tract, such as digestion of food and absorption of nutrients, the gastrointestinal tract also has the function of killing pathogenic microbes entering with food. These mechanisms include low pH of the stomach and the action of bile salts in the proximal small bowel. Multiple strains of *L. reuteri* were found to be resistant to acidity and bile salts. Researches attributed this to *L. reuteri*'s ability to form biofilms (16), as well as, a gene cluster for cytoplasmic urease enzyme (17). *H. pylori* uses cytoplasmic urease to convert urea into carbon dioxide and ammonia. Ammonia neutralizes gastric acid upon entering the outer bacterial membrane, thus averting acidification at the inner membrane (18). The presence of cytoplasmic urease in the genome of *L. reuteri* is believed to serve the same function.

The majority of commercial probiotics on the market today exert a transient effect. Meaning they are unable to adhere and colonize the gastrointestinal tract and hence their presence in host microbiota is transitory (19). Specific strains of *L. reuteri* exhibit colonization effect in specific vertebrates, including humans (20). It is believed that microbe host adherence is achieved through bacterial surface proteins binding elements present in host mucus layer. The *L. reuteri* genome contains genes coding for clusters of orthologous protein adherence mediators, or so-called adhesins, also called mucus-binding proteins (MUBs) and MUB-like proteins (21). These bacterial proteins bind elements in mucus secreted by enterocytes of the gastrointestinal tract, facilitating the first stage of colonization, adherence.

Due to the anatomy and physiology of the gastrointestinal tract, different parts of the GI tract have different environments in terms of acidity, bile acids, digestive enzymes, oxygen availability, epithelia types, and competitive effect of native host microbiota. Due to these topographical

differences, individual microbiota have evolved to colonize their regional niche. When examining the microbiota topographically, *L. reuteri* is found in the proximal digestive tract of the host (22). In fact, host GI epithelia are able to differentiate between strains of the same microbial species, further supporting the notion that vertebrates and their microbial symbionts are highly coevolved and exclusive. Once a microbe has successfully adhered to host mucus, biofilm formation ensues, ensuring the stability of the colony.

2.2 Biofilm Activity

A biofilm is defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (23).

The biofilm formation activity of *L. reuteri* has been studied in a variety of contexts, including in the gut, on dental surfaces, and on medical devices. The ability of *L. reuteri* to form biofilms on dental surfaces was studied and the researchers found that *L. reuteri* was able to adhere to dental surfaces and form a biofilm, which was characterized by the presence of EPS and the ability to inhibit the growth of pathogenic bacteria (24).

On a mechanistic level, *L. reuteri* produces several proteins and enzymes that are involved in biofilm formation, including aggregation substance, cell surface proteins, and extracellular surface protein. These proteins and enzymes work together to initiate the production of EPS and to form the mature biofilm structure (25).

Recently, *L. reuteri* was delivered as a biofilm on microsphere and such delivery was found to promote the adherence of *L. reuteri* to intestinal epithelium and enhance its probiotic property (26,27). This system of using a probiotic organism's endogenously produced biofilm as a capsule in order to better survive hostile host conditions, mainly acidity and bile acids, has been termed fourth generation probiotics (16). When compared to non-bond (planktonic) state probiotics, biofilm delivered probiotics have demonstrated significantly more pronounced short and long term probiotic effects in animal models (28).

Besides *L. reuteri*'s ability to form its own biofilm, it also has the ability to infiltrate biofilms of other bacterial species. In one study regarding vaginal flora, uropathogenic *Escherichia coli* enveloped in a mature biofilm was exposed to *L. reuteri* RC-14. The study reported that *L. reuteri* was able to first integrate its own biofilm into the pre-existing *E. coli* biofilm, then penetrate to inner *E. coli* colony, followed by significantly reducing *E. coli* bacterial counts inside the biofilm (29). This antimicrobial activity of *L. reuteri* has been well documented and is attributed to its metabolite production profile. A particularly potent antimicrobial compound secreted from *L. reuteri* is called reuterin.

2.3 Production of Bacteriocins

Bacteriocins are proteinaceous or peptic toxins produced by bacteria in order to inhibit the growth of select microorganisms. Reuterin (3-hydroxypropionaldehyde, 3-HPA, is an organic compound containing both hydroxy and aldehyde functional groups exhibiting a wide range of antimicrobial activity against foodborne pathogens and spoilage microorganisms (Figure 1) (30).

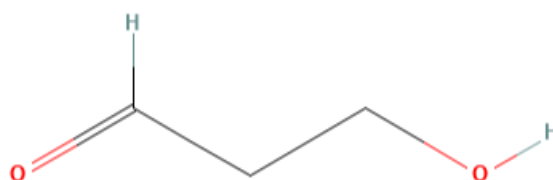


Figure 1. Chemical Structure of Reuterin (31)

The name reuterin is derived from *L. reuteri*, which produces reuterin biosynthetically from glycerol as a broad-spectrum antibiotic (bacteriocin) (32). Reuterin is an intermediate in the metabolism of glycerol to 1,3-propanediol catalysed by a coenzyme B12-dependent diol dehydrase (32).

In terms of anti-microbial spectrum, reuterin was found to kill a range of enteropathogenic organisms including yeasts, fungi, protozoa and viruses, without exerting effect on commensal gastrointestinal flora species (33). One study found that gram negative bacteria were especially susceptible to reuterin (33). Another study found that reuterin extracts were effective in decontaminating meat contaminated with of *E. coli* O157:H7 MRK 1452 and *L. monocytogenes* ST121 (34). The antimicrobial activity of reuterin is at least in part attributed to the spontaneous

conversion of 3-HPA to acrolein, a cytotoxic electrophile (35). The addition of lactic acid was found to synergistically increase this bacteriolytic effect (34). In addition to producing reuterin, *L. reuteri* is known to produce lactic acid, acetic acid, ethanol, reutericin, and reutericyclin, which also have proven antimicrobial and bacteriocin effects (36,37).

2.4 Activity Against *Helicobacter pylori*

Helicobacter pylori is a gram-negative, microaerophilic, spiral (helical) bacterium commonly found in the stomach. It was estimated that over 50% of the world's population will have *H. pylori* in their upper gastrointestinal tracts at some point throughout their lives, being more common in developing countries (38). Due to this exceedingly high prevalence of *H. pylori* epidemiologically speaking, researchers have investigated if *H. pylori* can also function as a commensal member of the stomach/ duodenum flora. One study found that non-pathogenic strains of *H. pylori* improve stomach acid secretion and satiety signalling (39), another found *H. pylori* to regulate the colonization of other stomach flora (40). However, for those colonized with pathogenic strains of *H. pylori*, the term infection not colonization is more appropriate.

Pathogenic *H. pylori* infection can result in chronic gastritis, peptic ulcers, and malignancy. There are multiple pathophysiological mechanisms involved in this process, these include adaption to acidity, mucus degradation, host immune response, and cytotoxin-associated gene A driven malignancy (41).

L. reuteri has demonstrated promising anti-helicobacter activity. Three RCTs of adult patients infected with *H. pylori*, encompassing 73 patients, determined that *L. reuteri* DSM 17648 alone significantly reduced *H. pylori* load in patients and helped alleviate mild symptoms of dyspepsia with a favorable safety profile (42–44). Additionally, when combined with a PPI *L. reuteri* DSMZ 17648 has been shown to have similar efficacy to standard triple therapy (pantoprazole, clarithromycin and amoxicillin or metronidazole) in the eradication of *H. pylori*, 65.22% and 73.91% respectively (45). Another RCT showed that when *L. reuteri* was given during seven day triple therapy vs. just triple therapy, eradication rate increased from 66% to 88% (46). One meta-analysis published in 2020 (47) encompassing 40 studies and 5792 patients concluded that *L. reuteri* probiotic supplementation in addition to triple or quadruple therapy improved the eradication rate by approximately 10% relative to the control groups. Additionally, they found a

44% reduction rate in the incidence of adverse reactions. Similarly, a 2019 meta-analysis (48) examining 11 RCTs and 724 patients found that adjuvant *L. reuteri* supplementation increased eradication rate by an average of 16% compared to controls only receiving triple or quadruple therapy. They also found adjuvant *L. reuteri* supplementation reduced the incidence of taste disorders by 64%. Table 1 summarizes the effectiveness of *L. reuteri* supplementation in the treatment of *H. pylori* infection as sole therapy and as adjuvant therapy up to the year 2021.

Table 1. Clinical Efficacy of *L. reuteri* Strains as Sole Therapy and as Adjuvant Therapy Against *H. pylori* Infection

Strain	Treatment	Subjects	Result	Reference
ATCC 55730	10 days <i>L. reuteri</i>	Children (Symptomatic)	Improvement of GI symptoms	(50)
DSM 17938	20 days triple therapy + <i>L. reuteri</i>	Adults (Symptomatic)	93.3 % eradication rate	(51)
DSM 17648	14 days <i>L. reuteri</i>	Adults (Symptomatic)	Significant decrease of pathogen load in stomach	(42)
SD2112	4 weeks <i>L. reuteri</i>	Adults (Symptomatic)	Significant decrease of urea breath test and pathogen density	(52)
DSM 17938	8 weeks <i>L. reuteri</i> + pantoprazole twice daily	Adults (Symptomatic)	13.6% eradication rate	(53)
DSM 17938, ATCC PTA 6475	28 days <i>L. reuteri</i> then 7 days <i>L. reuteri</i> + triple therapy	Adults (Symptomatic)	13% eradication rate with <i>L. reuteri</i> alone. 75% eradication rate with <i>L. reuteri</i> + triple therapy	(54)

DSMZ17648	14 days <i>L. reuteri</i>	Adults (asymptomatic colonization)	Reduced <i>H. pylori</i> load in asymptomatic carriers	(43)
DSM 17648	4 weeks <i>L. reuteri</i> +triple therapy	Adults (Symptomatic)	82.69% eradication rate	(55)
DSM 17648	4 weeks <i>L. reuteri</i> + triple therapy	Adults (Symptomatic)	86.2% eradication rate, Improved GI symptoms	(56)
DSM 17648	28 days <i>L. reuteri</i>	Adults (Symptomatic)	22.5% reduction in <i>H. pylori</i> load measured by urea breath test	(44)
DSMZ17648	28 days <i>L. reuteri</i> +/- eradication therapy (omeprazole + amoxicillin + metronidazole + bismuth for 10 days)	Adults (Symptomatic)	50% eradication rate with <i>L. reuteri</i> , 60% eradication rate with <i>L. reuteri</i> + eradication therapy	(57)
DSMZ17648	8 weeks <i>L. reuteri</i> + Pantoprazole	Adults (Symptomatic)	65.22% eradication rate	(45)
DSMZ17648,	14 days <i>L. reuteri</i> + liquorice and calcium carbonate	Adults (Symptomatic)	54.3% eradication rate	(58)

There are multiple proposed mechanisms by which probiotics decreases *H. pylori* bacterial load (Figure 2). Firstly, probiotics have been shown to improve GI barrier function, specifically by upregulating tight junctions, promoting mucus secretion, and increasing growth factors (59). The GI epithelium and associated mucus are integral parts of the innate defence against pathogenic bacteria entering through the GI tract. Second, probiotics reduce the host inflammatory response caused by *H. pylori* infection (60). The sustained expression of pro-inflammatory factors, such as IL-8, TNF- α , by *H. pylori* can lead to a chronic inflammatory response which is a key pathophysiological step in the manifestation of *H. pylori* gastritis. Probiotics have been shown to

mitigate this inflammatory response by inhibiting pro-inflammatory pathways such as NF- κ B. Third, as previously mentioned, *L. reuteri* is known to produce a wide range of antimicrobial substances, such as lactic acid, acetic acid, ethanol, reutericin, and reutericyclin, which have been shown to be effective in inhibiting a wide range of pathogenic organisms (33). This likely suppresses *H. pylori* through direct toxicity, as well as, indirectly with changes in local pH. Furthermore, *L. reuteri* has been shown to interfere with *H. pylori* gastric colonization (61). This is achieved by competitive binding to adhesion receptors of *H. pylori*, specifically two glycolipids found on gastric epithelium called gangliotetraosylceramide and sulfatide. Without pathogen adhesion, it is far less likely to manifest a clinical picture of disease. Similarly, *L. reuteri* DSM17648 has shown the unique ability to effectively chelate *H. pylori* (44). This is achieved by specifically binding to *H. pylori* surface proteins to form *reuteri/pylori* co-aggregates. The aggregated *H. pylori* are not capable of adhering to gastric mucosa and have significantly reduced motility. As a result, *H. pylori* co-aggregates are subject to peristaltic contractions and are enterically excreted. The *L. reuteri* DSM17648 strain is currently available on the market for reducing the load of *H. pylori* in the stomach.

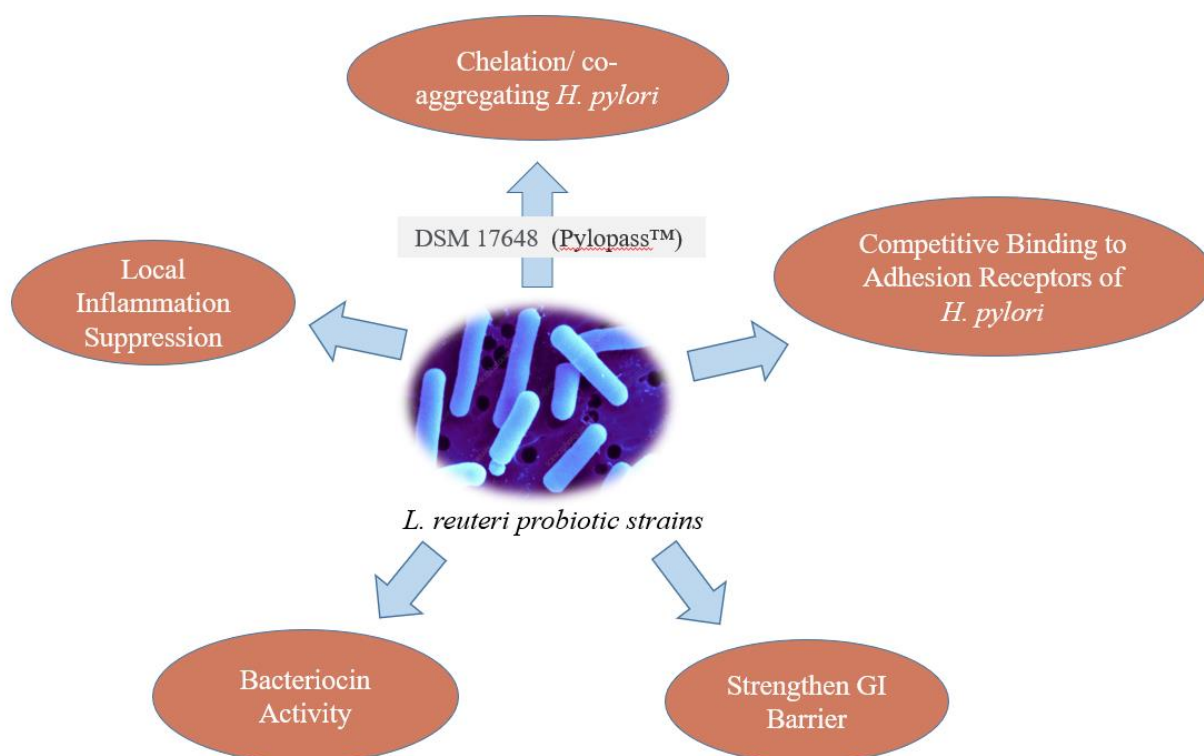


Figure 2. Schematic Diagram of *L. reuteri* Probiotic Mechanisms of Action Against *H. pylori*

Additionally, *L. reuteri* treated individuals have shown a marked decrease in antibiotic-associated adverse effects. One meta-analysis concluded that *L. reuteri* supplementation during triple therapy decreased antibiotic-associated side effects by 20–30% compared to controls only receiving triple therapy (62). Antibiotic-associated adverse effects encompassed diarrhoea, abdominal pain, nausea, taste disturbances, vomiting, and constipation.

2.5 Effect on Accelerated Wound Healing

The four classical stages of wound healing are haemostasis, inflammation, proliferation, and remodelling. Direct topical application of probiotics and their products in skin wounds has been shown to promote wound healing (63–65). This local effect primarily relies on the antibiotic properties of probiotics and their interaction with local inflammatory cells and proliferating epidermis. Considering this, researchers examined if orally consumed probiotics residing in the gut could also exert wound healing properties in distant tissue such as skin.

In one mouse study (66), researchers evaluated the healing properties of orally consumed live *L. reuteri* ATCC-PTA-6475. A standardized 2.0-millimeter full thickness dorsal skin excision was made, and then the wound was microscopically examined at three, six and twelve days after excision. Mice orally receiving *L. reuteri* exhibited complete epidermal closure in half the time required for matched control animals. When examining wound area, female mice consuming *L. reuteri* experienced a more rapid increase in healing compared to male mice. In the *L. reuteri* group, further examination of the wound bed showed accelerated maturation of granulation tissue, accelerated collagen deposition and significantly increased numbers of regulatory T lymphocytes (Tregs). Tregs are a specialized subpopulation of T cells that down-regulate host inflammatory responses and have been shown to prevent excessive inflammation surrounding tissue wounds (67). To further determine the significance of this increase in Tregs, researchers transferred purified Treg cells originating from *L. reuteri*-supplemented donor mice into mice genetically lacking T and B lymphocytes. The transferred Treg cells were sufficient to recapitulate the rapid cutaneous wound healing in the hosts.

Additionally, significantly increased plasma levels of oxytocin, known to be required in the normal mammalian wound healing processes (68–70), were demonstrated in *L. reuteri* dietary

supplemented mice. Intriguingly, *L. reuteri* supplemented mice who were subject to vagotomy did not show elevated plasma oxytocin nor accelerated wound healing. Therefore, the oxytocin-driven acceleration of wound healing is vagus nerve dependent. Researchers concluded that *L. reuteri*'s presence in the GI tract influences the immune system and oxytocin levels to exert the systemic effect of accelerated skin wound healing.

Another similar study in mice (71) evaluated the wound healing effects of topical *L. reuteri* extracts. All mice were subjected to a full thickness wound 1mm x 2mm in the area located on the mesial gingiva of the first maxillary molar. Then mice were randomly distributed into two groups: local injection of 0.9% sodium chloride (control group) or local injection of *L. reuteri* extracts. The goal was to determine whether bacterial extracts could regulate the functions of gingiva MSCs (GMSCs) and thus promote wound healing. The authors discovered that local injection of *L. reuteri* extracts increased the proliferation of gingival MSCs, as well as, their capacities of migration, expression of stem cell markers and osteogenic differentiation. Accelerated wound healing was evident histologically and macroscopically. Immunohistochemistry staining indicated that *L. reuteri* extracts promoted GMSCs migration via PI3K/AKT/ β -catenin/TGF β 1 pathway, thus accelerating wound healing.

2.6 Influence on Integumentary Health

It is well established that skin and mucosal surfaces of mammals are colonized by millions of bacteria (72). Mechanistically, microbes and their metabolites interact with immunological (73), metabolic (74), and neuroendocrine pathways (75) that modify stress-related responses in the skin through a gut-brain-skin axis (76). Mouse and human studies suggest that supplementation with probiotic bacteria has many beneficial effects on the integumentary system (63,77–80).

A study in mice investigated the integumentary effects of *L. reuteri* ATCC 6475 yogurt supplementation (81). After 24 weeks, mice showed a significant increase in dermal thickness when compared to controls. This was histologically confirmed to be attributed to additional collagen and subcutaneous fat deposition. Furthermore, probiotic-fed mice expressed an average of 12 hair follicles to every control group rat's hair follicle (a 1,200% increase), thus increasing fur density. When examining the hair cycle stages of probiotic-fed mice, a dramatic anagenic shift was found. Controls demonstrated 36% anagen phase while probiotic group showed 70% anagen

phase hair follicle cycle distribution. Additionally, when measuring the pH of the skin, oral cavity, rectum, and vagina, all mucocutaneous surfaces were slightly more acidic particularly in female mice.

These microscopic changes resulted in visibly thicker and shinier fur. Researchers measured hair luster by reflectometry and found a 100% increase in light reflectivity. The experiment was repeated comparing *L. reuteri* yogurt vs *L. reuteri* drinking water vs controls in order to rule out any confounding factors present in the yogurt (82). The results demonstrated increases in dermal thickness, folliculogenesis, sebocytogenesis, anagenic follicular shift, and fur luster in both the yogurt and drinking water groups when compared to controls. Therefore, these integumentary effects observed were attributed to *L. reuteri* dietary supplementation. The health conveying phenotype demonstrated in *L. reuteri* supplemented mice has been coined “glow of health.”

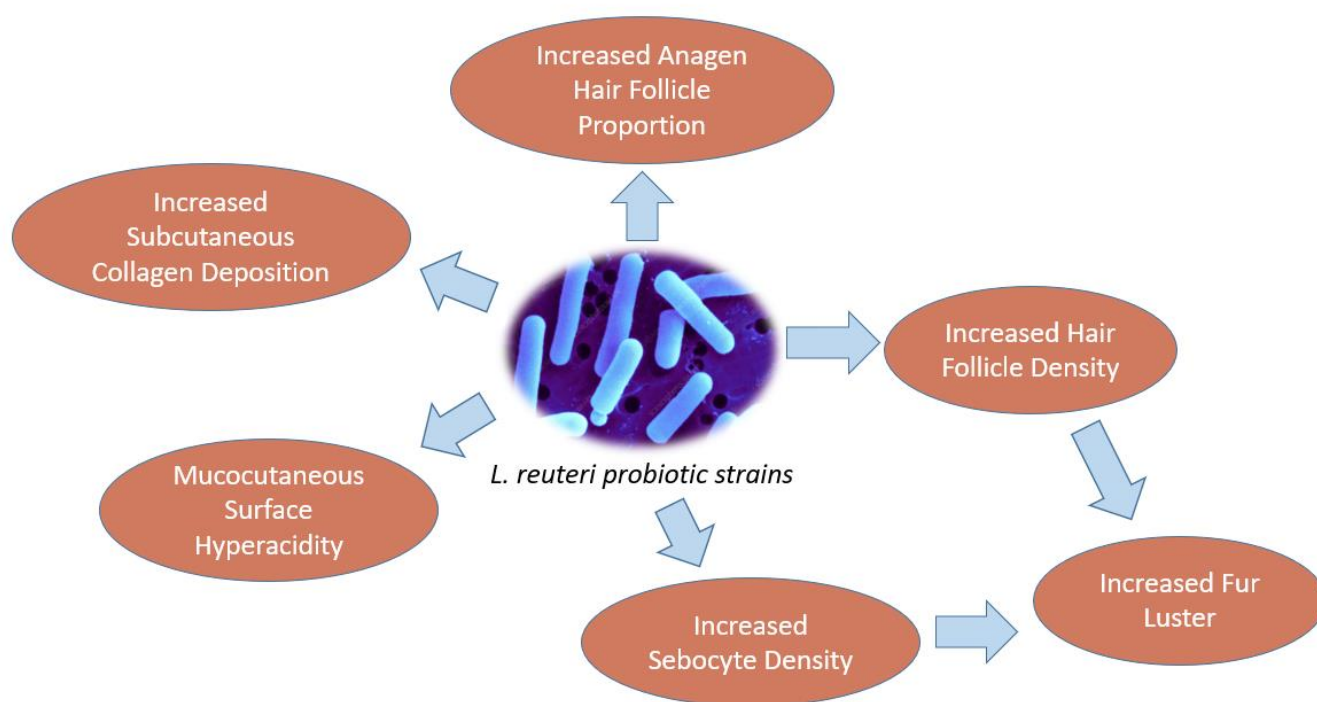


Figure 3. *L. reuteri* Effect on Integumentary Health

2.7 Testicular Health

One mouse study found that oral ingestion of *L. reuteri* ATCC 6475 over one year increased testicular weight nearly 40% versus control groups (83). Histological examination revealed that this increase in total testicular weight was due to increased seminiferous tubule cross sectional area and total germ cell volume. Likewise, Leydig cell density and individual Leydig cell volume was significantly increased. Given that the Leydig cell is the main cellular source of testosterone, researchers measured the serum testosterone after 5 months of *L. reuteri* supplementation. A 500% increase in serum testosterone was found. No analogous studies have been conducted in humans. Researchers observed the same testicular-increasing effects of *L. reuteri* when they administered an IL-17 antibody. This suggests that *L. reuteri*'s effects are mediated through increasing IL-10, thus decreasing IL-17 levels.

2.8 Effects on Immune Function

Multiple studies have shown that *L. reuteri* induces anti-inflammatory Treg cells (84–86). It is believed that *L. reuteri*'s ability to promote Tregs is a key contributor to the myriad of beneficial effects of *L. reuteri* demonstrated in both diseased and non-diseased conditions. The Treg-inducing property of *L. reuteri* is strain-dependent. However, the immunomodulatory effects of *L. reuteri* are not solely mediated by Treg induction, as *L. reuteri* suppressed Th1/Th2 proinflammatory responses in Treg-deficient mice (87). Also, specific *L. reuteri* strains can reduce the production of many pro-inflammatory cytokines. For instance, *L. reuteri* GMNL-263 can reduce serum MCP-1, TNF α , and IL-6 levels in mice fed with high fat diet (88). In fact, the immunomodulatory effects of *L. reuteri* appear to rely on its metabolites. One mouse study found that the *L. reuteri* BM36301 culture supernatant alone reduced TNF α production from human myeloid THP-1 cells (89).

Interestingly, when *L. reuteri* catabolizes tryptophan, the metabolite, indole-3-aldehyde, acts as an agonistic ligand for the aryl hydrocarbon receptor (AhR). The AhR is expressed on cells of the innate immune system, specifically macrophages, dendritic cells, NK cells, B lymphocytes and certain subtypes of T cells as Th17 and Treg cells (90). One mouse study found that activating the AhR upregulates IL-22 production from innate lymphoid cells (ILCs) (91). This upregulation in IL-22 was shown to induce immune tolerance to known probiotic species, while also increasing

colonization resistance to pathobionts such as *Candida albicans*. Therefore, it is postulated that this form of probiotic metabolite – host immune system interaction has evolved as a mechanism to ensure selective commensalism of the host.

Secretory IgA is an antibody component of the innate immune system, specifically, produced by mucus membranes of gastrointestinal, respiratory, and urogenital tracts (92). One mouse study found that dietary supplementation of *L. reuteri* increased free secretory IgA levels (93). However, this secretory IgA upregulation was not evident in vitamin A-deficient rats, suggesting a vitamin A-dependent mechanism. It is currently unclear how exactly *L. reuteri* induces B cells to upregulate IgA production.

One randomized control study followed 262 employees at a company in Sweden for 80 days (94). The goal was to measure the amount of total sick-leave caused by respiratory or gastrointestinal infections, between the probiotic supplementing and control groups. The authors found the probiotic group *L. reuteri* ATCC 55730 decreased the number of reported sick days from 26.4% (controls) to 10.6% (probiotic) over the course of the study. This clinically demonstrated *L. reuteri*'s immune system augmenting effect.

2.9 Oral Health Benefits

After the gut, the oral cavity has the second most diverse microbiota community consisting of over 700 species of bacteria (95). Oral dysbiosis is a risk factor in the development of caries, periodontal disease, halitosis, and cardiovascular disease (96–98). A major etiological factor in the development of caries is the dysbiotic proliferation of a bacteria called *Streptococcus mutans* (99). This bacteria thrives by fermenting dietary sugars, leading to a reduction in pH levels that leads to tooth demineralization (100).

One RCT examining the oral health effects of *L. reuteri* ATCC 55730 in children found the prevalence of caries was significantly lower in the probiotic group (0.67 ± 1.61) than controls (1.53 ± 2.64) (101). Additionally, the probiotic group had a lower incidence and severity of gingivitis compared to controls. Similarly, an *in vitro* study assessed the effect of *L. reuteri* ATCC 55730 on *S. mutans* (102). An inverse relationship was found between the relative abundance of *L. reuteri*

and *S. mutans*, suggesting that the presence of *L. reuteri* has an inhibitory effect on *S. mutans* colonization.

Another RCT examined the effect of *L. reuteri* ATCC PTA 5289 & DSM 17938 dual strain supplementation on residual pocket depth in periodontitis patients (103). After twenty-four weeks, the overall probing pocket depth in the probiotic lozenges group (2.64 ± 0.33 mm) was significantly lower compared to the control lozenges (2.92 ± 0.42 mm). This difference was even more pronounced in moderate (4-6 mm) and deep (≥ 7 mm) pockets. A similar study with chronic periodontitis patients found that after twelve weeks of oral *L. reuteri* ATCC PTA 5289 & DSM 17938 dual strain supplementation, pocket depth was significantly reduced. Researchers also reported a reduction in the population density of *Porphyromonas gingivalis*, a bacteria implicated with periodontal disease (104).

2.10 Influence on Bone Health

Estrogen deficiency is a major risk factor for osteoporosis that is associated with bone inflammation and resorption (105). Mouse studies have shown dietary supplementation of *L. reuteri* ATCC PTA 6475 significantly suppresses bone loss associated with estrogen deficiency in ovariectomized mice (106). Osteoclast bone resorption markers and activators (Trap5 and RANKL), as well as, osteoclastogenesis are significantly decreased in *L. reuteri*-supplemented mice.

An analogous RCT conducted in postmenopausal women (107) assessed tibial bone mineral density in *L. reuteri* group vs placebo, in both pre-existing osteoporosis patients and healthy patients after twelve months of treatment. In the osteoporosis comparison, the *L. reuteri* group had a 1,02% mean average reduction in bone loss ($-0,83\%$ vs $-1,85\%$). Similarly in the non-osteoporosis comparison, the *L. reuteri* group showed a 0,93% mean average reduction ($-0,93\%$ vs $-1,86\%$). Hence, both *L. reuteri* and placebo group experienced a reduction in bone mineral density, however this loss of density was significantly less in the *L. reuteri* group in both osteoporosis patients and non-osteoporosis patients. Researchers concluded that *L. reuteri* 6475 should be further explored as a novel approach to prevent age-associated bone loss and osteoporosis.

2.11 Vitamin Synthesis

The human genome is not capable of synthesizing vitamins required for its cellular physiology (108). Therefore, thirteen vitamins have been termed essential because they must be exogenously sourced. It is common knowledge that many of the essential vitamins can be attained through a variety of dietary sources, however some vitamins are also actively produced by gut microorganisms as metabolites. This can be achieved symbiotically by having select vitamin producing bacteria colonize the host GI tract, or entirely exogenously by consuming fermented foods. Thus, fermented foods have been called bifunctional foods since they already contain the liberation of microbial metabolites (bioactive effect) (109).

Currently, *L. reuteri* strains CRL1098, JCM1112, DSM 20016, and ZJ03 have demonstrated the ability to produce vitamin B12 (cobalamin) (110–112). Genome analysis of *L. reuteri* has identified a cluster of cobalamin biosynthesis genes, specifically coding for the CobA, CbiJ, and CbiK enzymes (110). It is now understood that *L. reuteri* requires cobalamin in the biosynthesis of reuterin. Specifically, the conversion of 3-hydroxypropionaldehyde to reuterin is catalyzed by an enzyme called 3-hydroxypropionaldehyde reductase, which requires cobalamin as a cofactor to function properly. Therefore, all strains of *L. reuteri* which produce reuterin also produce cobalamin. In one mouse study, the administration of *L. reuteri* CRL1098 together with a diet lacking vitamin B12 was shown to ameliorate pathologies in B12-deficient pregnant female mice and their offspring (113). In addition to B12, *L. reuteri* strains 6475 and JCM1112 have been found to synthesize vitamin B9 (folate) (111,114).

2.12 Microbiota Modulation

Numerous metabolic, autoimmune, endocrine, neuropsychiatric, and oncological conditions have been associated with perturbances in the microbiota (115). This heterogeneous group of microbiota imbalances have collectively been labelled dysbiosis. Hallmarks of dysbiosis include alteration in microbial composition (bacterial, archaea, fungi), metabolome, anatomical microbe localization, reduced diversity, opportunistic shift, and pathogen colonization (116–119). Studies have demonstrated disease specific gut microbiota signatures on the metagenomic, meta-transcriptomic and metabolomic levels of various chronic diseases such as inflammation bowel disease, colorectal cancer, obesity, type 2 diabetes, atherosclerosis, and allergy (120–123).

Keystone taxa are highly connected microbial taxa that individually or in a guild exert a considerable influence on microbiota structure and functioning irrespective of their abundance (124). *L. reuteri* has shown significant evidence to be considered a keystone taxa, as it is able to influence the diversity, composition, and metabolic function of gut microbiota in mice, piglets, and humans.

One study assessed oral administration of *L. reuteri* DSM17938 to mice, which were induced to have gut microbial dysbiosis due to FOXP3 gene mutation. FOXP3 gene mutation impairs functioning of Treg cells, manifestations of this include multiorgan inflammation, which in humans is called “immune dysregulation, polyendocrinopathy, enteropathy with X-linked inheritance” (or IPEX syndrome) (125). The results demonstrated that *L. reuteri* was able to prolong the lifespan of these mice and reduce multi-organ inflammation while remodelling the gut microbiota (126). The disease-ameliorating effect of *L. reuteri* was attributed to the remodelled gut microbiota, notably increased phylum Firmicutes and the genera *Lactobacillus* and *Oscillospira*. The study also demonstrated that *L. reuteri* supplementation resulted in inosine production (postbiotic effect). Through adenosine A2A receptor engagement, inosine can reduce Th1/Th2 cell responses and their associated pro-inflammatory cytokines. These results suggest that the *L. reuteri* – gut microbiota – inosine – adenosine A2A receptor axis could serve as a potential therapeutic method for Treg-deficient disorders. Likewise, in mice studies *L. reuteri* C10-2-1 has been shown to increase microbiota alpha diversity (93,106). Alpha diversity is a term used to describe the diversity, in terms of taxa number, within a single sample. High alpha diversity, i.e. high number of different taxa, with regard to the gut microbiome, is emerging as an important indicator of health (127–130).

A study in piglets examining the microbiota modulating effects of *L. reuteri* ZLR003 supplementation found increased alpha diversity when compared to the antibiotic-treated and control groups. Specifically noteworthy, the *L. reuteri* group showed significant microbiota shift in the jejunum with regard to asserting Proteobacteria as the dominant phylum (131). In another piglet study, the authors found that three weeks of *L. reuteri* TMW1.656 fermented fed supplementation increased alpha diversity, especially in the Firmicutes phyla, while reducing the abundance of *Enterobacteriaceae*, when compared to controls (132). Researchers concluded that

the microbiota modulating effect of this strain was due to its ability to produce the bacteriocin reutericyclin.

2.13 Effects of *L. reuteri* on Infant Health

There are many reports demonstrating beneficial effects of *L. reuteri* on different aspects of infant health, from infant colic, acute infectious gastroenteritis to general microbiota modulation leading to prevention of atopic phenotypes.

L. reuteri is an effective treatment against infant colic. One meta-analysis encompassing four double-blind RCTs involving 345 infants with colic (174 probiotic and 171 placebo) found that *L. reuteri* DSM17938 supplementation reduced crying and/or fussing time by 25% after 21 days when compared to controls (133). Similarly, the probiotic group was almost twice as likely as the control group to completely resolve colic at all time points. This significant treatment effect was only found in breast fed infants and was absent in formula fed infants. Therefore, it is speculated that a prebiotic nutrient in human breast milk, likely human milk oligosaccharide (HMOs), provides the microbial substrate to enable microbial symbiotic effects (134).

L. reuteri has been shown to significantly reduce symptom duration of acute infectious diarrhoea in children. One RCT of children (6-36 months of age) hospitalized for watery diarrhoea studied the effect of *L. reuteri* DSM 17938 on symptom duration. On day two and three of treatment, watery diarrhoea persisted in 82% and 74% of the placebo and 55% and 45% of the *L. reuteri* recipients respectively (135). Hence, the probiotic group saw expedited infectious recovery. An analogous study also found that application of *L. reuteri* DSM 17938 reduced the duration of acute diarrhoea in hospitalized children. The prevalence of diarrhoea-free children, *L. reuteri* vs controls, after 24 hours was 50% versus 5% and after 72 hours 69% versus 11% (136). One systematic review concluded *L. reuteri* DSM 17938 oral administration reduces the duration of diarrhoea and increases the likelihood of cure. In preventive settings, *L. reuteri* has the potential to reduce the risk of community-acquired diarrhoea in otherwise healthy children (137).

Additionally, there are reports showing differences in infants' microbiota depending on the type of birth. Compared to vaginally delivered infants, Cesarean (C)-section delivered infants display a dysbiotic GI microbiota, specifically microbiota with less probiotic *Lactobacillus* and

Bifidobacterium species, and a higher relative abundance of *Enterococcus*, *Enterobacter*, and *Clostridium* species (138–140).

One study found that supplementing C-section babies with *L. reuteri* DSM 17938 from 2 weeks to 4 months of age shifted the gut microbiota composition toward the distribution pattern found in vaginally delivered infants (138). Also noteworthy, as long as the mother's GI tract is colonized with *L. reuteri*, *L. reuteri* will be present in her breast milk (10). This gastrointestinal to mammary duct translocation was shown to occur due to dendritic cells sampling bacteria directly from the gut lumen by creating openings between enterocyte tight junctions (141). Once internalized by dendritic cells (immune sequestration) bacteria may spread to other locations via the bloodstream to arrive at mucosal-associated lymphoid tissue system (141). Growing evidence suggests earlier infancy lactobacilli colonization may protect the infant from developing atopic allergy (142). Also, *L. reuteri* supplementation in children has been shown to ameliorate atopic dermatitis phenotype (143). Thus, breast milk is considered a natural synbiotic, since it contains both probiotics, as well as prebiotic fiber (HMOs) to bestow the engraftment of a eubiotic infant microbiome.

2.14 Benefits in Neuropsychiatric and Neurodevelopmental Disorders

The evidence supporting the role of gut microbiota in the pathogenesis and/or progression of many neurodevelopmental, neuropsychiatric, and neurological conditions is mounting (144). Consequently, restoring a microbiota balance with probiotics is dawning as a novel preventive and therapeutic approach for managing such disorders (145,146). Clinical trials investigating the treatment of neuropsychiatric disorders with chronic exogenous oxytocin administration have raised several issues regarding CNS absorption efficacy and safety, especially in children and adolescents (147,148). It is believed that chronic exogenous oxytocin may lead to desensitization of the endogenous oxytocinergic system (148).

Currently, *L. reuteri* is the only probiotic known to upregulate endogenous oxytocin production (66). This has led researchers to propose *L. reuteri* as a psychobiotic. Psychobiotics are probiotics that confer mental health benefits to the host when consumed in a particular quantity through the interaction with commensal gut bacteria (149). It is hypothesized that administering psychobiotics, such as *L. reuteri*, would not only elevate endogenous oxytocin levels, but rather mitigate the adverse effects driven by exogenous oxytocin administration. This is because stimulating

endogenous oxytocin production also stimulates complex homeostatic pathway involving interrelated gut, immune, endocrine, and brain functions (150). The use of *L. reuteri* supplementation has yet to be studied in human neuropsychiatric conditions.

Exposure to maternal obesity in utero is positively associated with neurodevelopmental disorders, such as autism spectrum disorder (ASD) in children (151). One mouse study demonstrated that a maternal high fat, low fiber diet induced social deficits in the offspring (152). Shotgun genomic sequencing revealed significantly different microbiota composition when compared to mice fed a regular diet. The most striking difference was the relative abundance of *L. reuteri*, which was nine-fold less abundant in the maternal high fat diet (MHFD) offspring group compared to controls. Remarkably, after four weeks of live *L. reuteri* consumption MHFD offspring significantly improved sociability and preference for social novelty (152). This suggests that *L. reuteri* may also offer benefit to human neurodevelopmental disorders.

3. Conclusion

L. reuteri has been shown to be a safe and effective probiotic for attenuating both GI diseases and diseases in remote tissues, as well as, augmenting human host physiology. These various probiotic mediated effects are strain dependant. Current research suggests that *L. reuteri* and its metabolites promote human health through direct and indirect mechanisms. Specific *L. reuteri* strains have demonstrated a myriad of probiotic effects including the ability to kill a range of enteropathogenic organisms, accelerate wound healing, increase skin thickness, increase hair follicle density and the prevalence of anagen stage hair follicles, increase serum testosterone, increase serum oxytocin, increase male germ cell volume, upregulate Tregs, increase secretory IgA, reduce the incidence of dental caries, improve periodontitis treatment, reduce age related bone loss, synthesize B12, beneficially modulate GI microbiota, reduce the duration of infectious gastroenteritis, ameliorate infant colic, and improve ASD in mice (Figure 1).

Further research strengthening clinical evidence of effectiveness for various conditions is needed to be able to use this bacterial species in the validated clinical therapeutic protocols. Additionally, to unravel the mechanisms behind the observed probiotic effects more studies evaluating transcriptome and metabolome are needed. Understanding the metabolome could be pivotal in the application of precision medicine. Dietary interventions together with edible bacterial cocktails

may be used to activate latent host genetic programs, originating from host-microbiota coevolution, as well as to treat disease and optimise health.

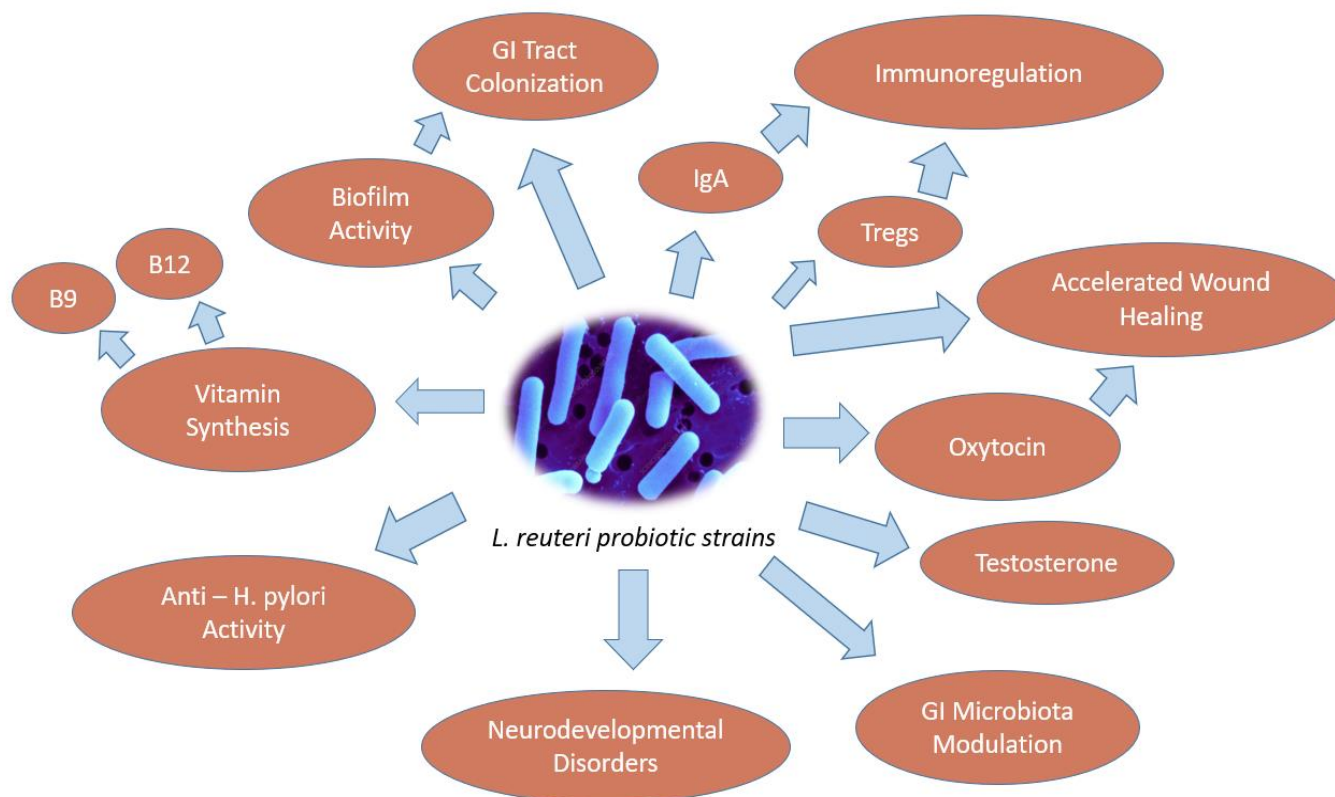


Figure 4. Probiotic Properties of *L. reuteri*

4. References

1. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014 Aug;11(8):506–14.
2. Montalban-Arques A, De Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM, et al. Selective Manipulation of the Gut Microbiota Improves Immune Status in Vertebrates. *Front Immunol*. 2015;6:512.
3. Wolf BW, Wheeler KB, Ataya DG, Garleb KA. Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the human immunodeficiency virus. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc*. 1998 Dec;36(12):1085–94.
4. Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K. Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol*. 2004 Feb;70(2):1176–81.
5. Weizman Z, Alsheikh A. Safety and tolerance of a probiotic formula in early infancy comparing two probiotic agents: a pilot study. *J Am Coll Nutr*. 2006 Oct;25(5):415–9.
6. Mangalat N, Liu Y, Fatheree NY, Ferris MJ, Van Arsdall MR, Chen Z, et al. Safety and tolerability of *Lactobacillus reuteri* DSM 17938 and effects on biomarkers in healthy adults: results from a randomized masked trial. *PloS One*. 2012;7(9):e43910.
7. Hoy-Schulz YE, Jannat K, Roberts T, Zaidi SH, Unicomb L, Luby S, et al. Safety and acceptability of *Lactobacillus reuteri* DSM 17938 and *Bifidobacterium longum* subspecies *infantis* 35624 in Bangladeshi infants: a phase I randomized clinical trial. *BMC Complement Altern Med*. 2016 Feb 2;16:44.
8. Sinkiewicz G. *Lactobacillus reuteri* in health and disease. 2010 [cited 2023 Feb 11]; Available from: <http://urn.kb.se/resolve?urn=urn:nbn:se:mau:diva-7368>
9. Mu Q, Tavella VJ, Luo XM. Role of *Lactobacillus reuteri* in Human Health and Diseases. *Front Microbiol* [Internet]. 2018 [cited 2023 Mar 17];9. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2018.00757>
10. Sinkiewicz G, Ljunggren L. Occurrence of *Lactobacillus reuteri* in human breast milk. *Microb Ecol Health Dis*. 2008 Jan 1;20(3):122–6.
11. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinction in the gut microbiota compounds over generations. *Nature*. 2016 Jan 14;529(7585):212–5.

12. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* 2016 Aug;14(8):e1002533.
13. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010 Mar 4;464(7285):59–65.
14. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, et al. Postbiotics: An evolving term within the functional foods field. *Trends Food Sci Technol.* 2018 May 1;75:105–14.
15. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell.* 2015 Apr 9;161(2):264–76.
16. Salas-Jara MJ, Ilabaca A, Vega M, García A. Biofilm Forming *Lactobacillus*: New Challenges for the Development of Probiotics. *Microorganisms.* 2016 Sep 20;4(3):35.
17. Krumbeck JA, Marsteller NL, Frese SA, Peterson DA, Ramer-Tait AE, Hutkins RW, et al. Characterization of the ecological role of genes mediating acid resistance in *Lactobacillus reuteri* during colonization of the gastrointestinal tract. *Environ Microbiol.* 2016;18(7):2172–84.
18. Berger A. Scientists discover how helicobacter survives gastric acid. *BMJ.* 2000 Jan 29;320(7230):268.
19. Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C. A review of probiotic supplementation in healthy adults: helpful or hype? *Eur J Clin Nutr.* 2019 Jan;73(1):24–37.
20. Li XJ, Yue LY, Guan XF, Qiao SY. The adhesion of putative probiotic lactobacilli to cultured epithelial cells and porcine intestinal mucus. *J Appl Microbiol.* 2008 Apr;104(4):1082–91.
21. Roos S, Jonsson H. A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. The GenBank accession number for the sequence reported in this paper is AF120104. *Microbiology.* 2002;148(2):433–42.
22. Frese SA, MacKenzie DA, Peterson DA, Schmaltz R, Fangman T, Zhou Y, et al. Molecular Characterization of Host-Specific Biofilm Formation in a Vertebrate Gut Symbiont. *PLoS Genet.* 2013 Dec 26;9(12):e1004057.
23. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev.* 2002 Apr;15(2):167–93.
24. Wasfi R, Abd El-Rahman OA, Zafer MM, Ashour HM. Probiotic *Lactobacillus* sp. inhibit growth, biofilm formation and gene expression of caries-inducing *Streptococcus mutans*. *J Cell Mol Med.* 2018 Mar;22(3):1972–83.

25. Jones SE, Versalovic J. Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiol.* 2009 Feb 11;9:35.
26. Olson JK, Rager TM, Navarro JB, Mashburn-Warren L, Goodman SD, Besner GE. Harvesting the benefits of biofilms: A novel probiotic delivery system for the prevention of necrotizing enterocolitis. *J Pediatr Surg.* 2016 Jun;51(6):936–41.
27. Navarro JB, Mashburn-Warren L, Bakaletz LO, Bailey MT, Goodman SD. Enhanced Probiotic Potential of *Lactobacillus reuteri* When Delivered as a Biofilm on Dextranomer Microspheres That Contain Beneficial Cargo. *Front Microbiol.* 2017 Mar 27;8:489.
28. Ragan MV, Wala SJ, Goodman SD, Bailey MT, Besner GE. Next-Generation Probiotic Therapy to Protect the Intestines From Injury. *Front Cell Infect Microbiol* [Internet]. 2022 [cited 2023 Feb 28];12. Available from: <https://www.frontiersin.org/articles/10.3389/fcimb.2022.863949>
29. McMillan A, Dell M, Zellar MP, Cribby S, Martz S, Hong E, et al. Disruption of urogenital biofilms by lactobacilli. *Colloids Surf B Biointerfaces.* 2011 Aug 1;86(1):58–64.
30. Siedler S, Balti R, Neves AR. Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Curr Opin Biotechnol.* 2019 Apr 1;56:138–46.
31. PubChem. 3-Hydroxypropanal [Internet]. [cited 2023 May 5]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/75049>
32. Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrob Agents Chemother.* 1988 Dec;32(12):1854–8.
33. Cleusix V, Lacroix C, Vollenweider S, Duboux M, Le Blay G. Inhibitory activity spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. *BMC Microbiol.* 2007 Nov 12;7:101.
34. El-Ziney MG, van den Tempel T, Debevere J, Jakobsen M. Application of reuterin produced by *Lactobacillus reuteri* 12002 for meat decontamination and preservation. *J Food Prot.* 1999 Mar;62(3):257–61.
35. Engels C, Schwab C, Zhang J, Stevens MJA, Bieri C, Ebert MO, et al. Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. *Sci Rep.* 2016 Nov 7;6:36246.
36. Kabuki T, Saito T, Kawai Y, Uemura J, Itoh T. Production, purification and characterization of reuterin 6, a bacteriocin with lytic activity produced by *Lactobacillus reuteri* LA6. *Int J Food Microbiol.* 1997 Feb;34(2):145–56.
37. Gänzle MG, Höltzel A, Walter J, Jung G, Hammes WP. Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Appl Environ Microbiol.* 2000 Oct;66(10):4325–33.

38. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology*. 2017 Aug;153(2):420–9.
39. Ackerman J. The ultimate social network. *Sci Am*. 2012 Jun;306(6):36–43.
40. Gravina AG, Zagari RM, De Musis C, Romano L, Loguercio C, Romano M. *Helicobacter pylori* and extragastric diseases: A review. *World J Gastroenterol*. 2018 Aug 7;24(29):3204–21.
41. Alfarouk KO, Bashir AHH, Aljarbou AN, Ramadan AM, Muddathir AK, AlHoufie STS, et al. The Possible Role of *Helicobacter pylori* in Gastric Cancer and Its Management. *Front Oncol*. 2019;9:75.
42. Holz C, Busjahn A, Mehling H, Arya S, Boettner M, Habibi H, et al. Significant Reduction in *Helicobacter pylori* Load in Humans with Non-viable *Lactobacillus reuteri* DSM17648: A Pilot Study. *Probiotics Antimicrob Proteins*. 2015;7(2):91–100.
43. Mehling H, Busjahn A. Non-Viable *Lactobacillus reuteri* DSMZ 17648 (Pylopass™) as a New Approach to *Helicobacter pylori* Control in Humans. *Nutrients*. 2013 Aug 2;5(8):3062–73.
44. Buckley M, Lacey S, Doolan A, Goodbody E, Seamans K. The effect of *Lactobacillus reuteri* supplementation in *Helicobacter pylori* infection: a placebo-controlled, single-blind study. *BMC Nutr*. 2018;4:48.
45. Muresan IAP, Pop LL, Dumitrascu DL. *Lactobacillus reuteri* versus triple therapy for the eradication of *Helicobacter pylori* in functional dyspepsia. *Med Pharm Rep*. 2019 Oct;92(4):352–5.
46. Efrati C, Nicolini G, Cannaviello C, O'Sed NP, Valabrega S. *Helicobacter pylori* eradication: Sequential therapy and *Lactobacillus reuteri* supplementation. *World J Gastroenterol WJG*. 2012 Nov 21;18(43):6250–4.
47. Zhang M, Zhang C, Zhao J, Zhang H, Zhai Q, Chen W. Meta-analysis of the efficacy of probiotic-supplemented therapy on the eradication of *H. pylori* and incidence of therapy-associated side effects. *Microb Pathog*. 2020 Oct;147:104403.
48. Yu M, Zhang R, Ni P, Chen S, Duan G. Efficacy of *Lactobacillus*-supplemented triple therapy for *H. pylori* eradication: A meta-analysis of randomized controlled trials. *PloS One*. 2019;14(10):e0223309.
49. Lü M, Yu S, Deng J, Yan Q, Yang C, Xia G, et al. Efficacy of Probiotic Supplementation Therapy for *Helicobacter pylori* Eradication: A Meta-Analysis of Randomized Controlled Trials. *PloS One*. 2016;11(10):e0163743.
50. Lionetti E, Miniello VL, Castellaneta SP, Magistá AM, de Canio A, Maurogiovanni G, et al. *Lactobacillus reuteri* therapy to reduce side-effects during anti-*Helicobacter pylori*

- treatment in children: a randomized placebo controlled trial. *Aliment Pharmacol Ther.* 2006 Nov 15;24(10):1461–8.
51. Dore MP, Soro S, Rocchi C, Loria MF, Bibbò S, Pes GM. Inclusion of *Lactobacillus Reuteri* in the Treatment of *Helicobacter pylori* in Sardinian Patients. *Medicine (Baltimore)*. 2016 Apr 18;95(15):e3411.
 52. Imase K, Tanaka A, Tokunaga K, Sugano H, Ishida H, Takahashi S. *Lactobacillus reuteri* tablets suppress *Helicobacter pylori* infection--a double-blind randomised placebo-controlled cross-over clinical study. *Kansenshogaku Zasshi*. 2007 Jul;81(4):387–93.
 53. Dore MP, Cuccu M, Pes GM, Manca A, Graham DY. *Lactobacillus reuteri* in the treatment of *Helicobacter pylori* infection. *Intern Emerg Med*. 2014 Sep;9(6):649–54.
 54. Francavilla R, Polimeno L, Demichina A, Maurogiovanni G, Principi B, Scaccianoce G, et al. *Lactobacillus reuteri* Strain Combination In *Helicobacter pylori* Infection: A Randomized, Double-Blind, Placebo-Controlled Study. *J Clin Gastroenterol*. 2014 May;48(5):407–13.
 55. Ismail NI, Ali RAR, Wong Z, Nawawi KNM, Kok WH, Mahmood NRKN, et al. IDDF2022-ABS-0107 The effect of *lactobacillus reuteri* probiotic as an adjunct treatment for *helicobacter pylori* infection in adults. *Gut*. 2022 Sep 1;71(Suppl 2):A9–10.
 56. Yang C, Liang L, Lv P, Liu L, Wang S, Wang Z, et al. Effects of non-viable *Lactobacillus reuteri* combining with 14-day standard triple therapy on *Helicobacter pylori* eradication: A randomized double-blind placebo-controlled trial. *Helicobacter*. 2021 Dec;26(6):e12856.
 57. Kornienko EA, Parolova NI, Ivanov SV, Polev DS, Zykin PA, Kondratenko YD, et al. Gastric microbiota and probiotics opportunities in *helicobacter pylori* eradication in children. *Gastroenterol Hepatol Open Access*. 2020 Jan 16;11(1):13–23.
 58. Mihai L, Mihai BM, Dranga M, Prelipcean CC. LACTOBACILLUS REUTERI – AN ALTERNATIVE IN THE FIRST-LINE OF HELICOBACTER PYLORI ERADICATION. 2019;67.
 59. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. *Nat Med*. 2019 May;25(5):716–29.
 60. Ji J, Yang H. Using Probiotics as Supplementation for *Helicobacter pylori* Antibiotic Therapy. *Int J Mol Sci*. 2020 Jan;21(3):1136.
 61. Mukai T, Asasaka T, Sato E, Mori K, Matsumoto M, Ohori H. Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*. *FEMS Immunol Med Microbiol*. 2002 Jan 14;32(2):105–10.
 62. Shi X, Zhang J, Mo L, Shi J, Qin M, Huang X. Efficacy and safety of probiotics in eradicating *Helicobacter pylori*: A network meta-analysis. *Medicine (Baltimore)*. 2019 Apr;98(15):e15180.

63. Guéniche A, Bastien P, Ovigne JM, Kermici M, Courchay G, Chevalier V, et al. Bifidobacterium longum lysate, a new ingredient for reactive skin. *Exp Dermatol*. 2010 Aug;19(8):e1-8.
64. Huseini HF, Rahimzadeh G, Fazeli MR, Mehrazma M, Salehi M. Evaluation of wound healing activities of kefir products. *Burns J Int Soc Burn Inj*. 2012 Aug;38(5):719–23.
65. Peral MC, Huaman Martinez MA, Valdez JC. Bacteriotherapy with *Lactobacillus plantarum* in burns. *Int Wound J*. 2009;6(1):73–81.
66. Poutahidis T, Kearney SM, Levkovich T, Qi P, Varian BJ, Lakritz JR, et al. Microbial Symbionts Accelerate Wound Healing via the Neuropeptide Hormone Oxytocin. *PLoS ONE*. 2013 Oct 30;8(10):e78898.
67. Boothby IC, Cohen JN, Rosenblum MD. Regulatory T cells in Skin Injury: At the Crossroads of Tolerance and Tissue Repair. *Sci Immunol*. 2020 May 1;5(47):eaaz9631.
68. Macciò A, Madeddu C, Chessa P, Panzone F, Lissoni P, Mantovani G. Oxytocin both increases proliferative response of peripheral blood lymphomonocytes to phytohemagglutinin and reverses immunosuppressive estrogen activity. *Vivo Athens Greece*. 2010;24(2):157–63.
69. Johnson HM, Torres BA. Regulation of lymphokine production by arginine vasopressin and oxytocin: modulation of lymphocyte function by neurohypophyseal hormones. *J Immunol Baltim Md 1950*. 1985 Aug;135(2 Suppl):773s–5s.
70. Barnard A, Layton D, Hince M, Sakkal S, Bernard C, Chidgey A, et al. Impact of the neuroendocrine system on thymus and bone marrow function. *Neuroimmunomodulation*. 2008;15(1):7–18.
71. Han N, Jia L, Su Y, Du J, Guo L, Luo Z, et al. *Lactobacillus reuteri* extracts promoted wound healing via PI3K/AKT/ β -catenin/TGF β 1 pathway. *Stem Cell Res Ther*. 2019 Aug 7;10:243.
72. Gordon JI. Honor thy gut symbionts redux. *Science*. 2012 Jun 8;336(6086):1251–3.
73. Chow J, Mazmanian SK. Getting the bugs out of the immune system: do bacterial microbiota “fix” intestinal T cell responses? *Cell Host Microbe*. 2009 Jan 22;5(1):8–12.
74. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell*. 2012 Mar 16;148(6):1258–70.
75. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A*. 2011 Sep 20;108(38):16050–5.
76. Arck P, Handjiski B, Hagen E, Pincus M, Bruenahl C, Bienenstock J, et al. Is there a “gut-brain-skin axis”? *Exp Dermatol*. 2010 May;19(5):401–5.

77. Chapat L, Chemin K, Dubois B, Bourdet-Sicard R, Kaiserlian D. *Lactobacillus casei* reduces CD8+ T cell-mediated skin inflammation. *Eur J Immunol*. 2004 Sep;34(9):2520–8.
78. Floch MH, Walker WA, Madsen K, Sanders ME, Macfarlane GT, Flint HJ, et al. Recommendations for probiotic use-2011 update. *J Clin Gastroenterol*. 2011 Nov;45 Suppl:S168-171.
79. Guéniche A, Benyacoub J, Buetler TM, Smola H, Blum S. Supplementation with oral probiotic bacteria maintains cutaneous immune homeostasis after UV exposure. *Eur J Dermatol EJD*. 2006;16(5):511–7.
80. Krutmann J. Pre- and probiotics for human skin. *J Dermatol Sci*. 2009 Apr;54(1):1–5.
81. Levkovich T, Poutahidis T, Smillie C, Varian BJ, Ibrahim YM, Lakritz JR, et al. Probiotic Bacteria Induce a ‘Glow of Health.’ *PLoS ONE*. 2013 Jan 16;8(1):e53867.
82. Erdman S, Poutahidis T. Probiotic ‘glow of health’: it’s more than skin deep. *Benef Microbes*. 2014 Jun 1;5(2):109–19.
83. Poutahidis T, Springer A, Levkovich T, Qi P, Varian BJ, Lakritz JR, et al. Probiotic Microbes Sustain Youthful Serum Testosterone Levels and Testicular Size in Aging Mice. *PLoS ONE*. 2014 Jan 2;9(1):e84877.
84. Karimi K, Inman MD, Bienenstock J, Forsythe P. *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med*. 2009 Feb 1;179(3):186–93.
85. Lorea Baroja M, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clin Exp Immunol*. 2007 Sep 1;149(3):470–9.
86. Liu Y, Fatheree NY, Dingle BM, Tran DQ, Rhoads JM. *Lactobacillus reuteri* DSM 17938 changes the frequency of Foxp3+ regulatory T cells in the intestine and mesenteric lymph node in experimental necrotizing enterocolitis. *PloS One*. 2013;8(2):e56547.
87. He B, Hoang TK, Wang T, Ferris M, Taylor CM, Tian X, et al. Resetting microbiota by *Lactobacillus reuteri* inhibits T reg deficiency–induced autoimmunity via adenosine A2A receptors. *J Exp Med*. 2017 Jan;214(1):107–23.
88. Hsieh FC, Lan CCE, Huang TY, Chen KW, Chai CY, Chen WT, et al. Heat-killed and live *Lactobacillus reuteri* GMNL-263 exhibit similar effects on improving metabolic functions in high-fat diet-induced obese rats. *Food Funct*. 2016 May 18;7(5):2374–88.
89. Lee J, Yang W, Hostetler A, Schultz N, Suckow MA, Stewart KL, et al. Characterization of the anti-inflammatory *Lactobacillus reuteri* BM36301 and its probiotic benefits on aged mice. *BMC Microbiol*. 2016 Apr 19;16:69.
90. Ambrosio LF, Insfran C, Volpini X, Acosta Rodriguez E, Serra HM, Quintana FJ, et al. Role of Aryl Hydrocarbon Receptor (AhR) in the Regulation of Immunity and

- Immunopathology During *Trypanosoma cruzi* Infection. *Front Immunol* [Internet]. 2019 [cited 2023 Apr 28];10. Available from: <https://www.frontiersin.org/articles/10.3389/fimmu.2019.00631>
91. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 2013 Aug 22;39(2):372–85.
 92. Corthésy B. Multi-Faceted Functions of Secretory IgA at Mucosal Surfaces. *Front Immunol*. 2013 Jul 12;4:185.
 93. Wang P, Li Y, Xiao H, Shi Y, Le GW, Sun J. Isolation of *Lactobacillus reuteri* from Peyer's patches and their effects on sIgA production and gut microbiota diversity. *Mol Nutr Food Res*. 2016 Sep;60(9):2020–30.
 94. Tubelius P, Stan V, Zachrisson A. Increasing work-place healthiness with the probiotic *Lactobacillus reuteri*: a randomised, double-blind placebo-controlled study. *Environ Health Glob Access Sci Source*. 2005 Nov 7;4:25.
 95. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol JOMFP*. 2019;23(1):122–8.
 96. Nath SG, Raveendran R. Microbial dysbiosis in periodontitis. *J Indian Soc Periodontol*. 2013;17(4):543–5.
 97. Li Y, Zhu M, Liu Y, Luo B, Cui J, Huang L, et al. The oral microbiota and cardiometabolic health: A comprehensive review and emerging insights. *Front Immunol*. 2022 Nov 18;13:1010368.
 98. Zhang Y, Zhu C, Cao G, Zhan J, Feng X, Chen X. Dynamic Alterations of Oral Microbiota Related to Halitosis in Preschool Children. *Front Cell Infect Microbiol* [Internet]. 2021 [cited 2023 May 1];11. Available from: <https://www.frontiersin.org/articles/10.3389/fcimb.2021.599467>
 99. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev*. 1986 Dec;50(4):353–80.
 100. FRCP DMJ MD. The influence of oral health on the risk of cardiovascular disease [Internet]. *Observatoire de la prévention*. 2022 [cited 2023 Apr 29]. Available from: <https://observatoireprevention.org/en/2022/03/27/the-influence-of-oral-health-on-the-risk-of-cardiovascular-disease/>
 101. Stensson M, Koch G, Coric S, Abrahamsson TR, Jenmalm MC, Birkhed D, et al. Oral administration of *Lactobacillus reuteri* during the first year of life reduces caries prevalence in the primary dentition at 9 years of age. *Caries Res*. 2014;48(2):111–7.
 102. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol*. 2004 Sep 1;95(2):219–23.

103. Laleman I, Pauwels M, Quirynen M, Teughels W. A dual-strain *Lactobacilli reuteri* probiotic improves the treatment of residual pockets: A randomized controlled clinical trial. *J Clin Periodontol*. 2020 Jan;47(1):43–53.
104. Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M, Haytac MC. Clinical and microbiological effects of *Lactobacillus reuteri* probiotics in the treatment of chronic periodontitis: a randomized placebo-controlled study. *J Clin Periodontol*. 2013 Nov;40(11):1025–35.
105. Isales CM, Seeman E. Menopause and Age-related Bone Loss. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* [Internet]. John Wiley & Sons, Ltd; 2018 [cited 2023 Feb 24]. p. 155–61. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119266594.ch21>
106. Britton RA, Irwin R, Quach D, Schaefer L, Zhang J, Lee T, et al. Probiotic *L. reuteri* Treatment Prevents Bone Loss in a Menopausal Ovariectomized Mouse Model. *J Cell Physiol*. 2014;229(11):1822–30.
107. Nilsson AG, Sundh D, Bäckhed F, Lorentzon M. *Lactobacillus reuteri* reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med*. 2018 Sep;284(3):307–17.
108. Vitamins and Minerals [Internet]. NCCIH. [cited 2023 Feb 24]. Available from: <https://www.nccih.nih.gov/health/vitamins-and-minerals>
109. Linares DM, Gómez C, Renes E, Fresno JM, Tornadijo ME, Ross RP, et al. Lactic Acid Bacteria and Bifidobacteria with Potential to Design Natural Biofunctional Health-Promoting Dairy Foods. *Front Microbiol*. 2017;8:846.
110. Taranto MP, Vera JL, Hugenholtz J, De Valdez GF, Sesma F. *Lactobacillus reuteri* CRL1098 Produces Cobalamin. *J Bacteriol*. 2003 Sep;185(18):5643–7.
111. Santos F, Wegkamp A, de Vos WM, Smid EJ, Hugenholtz J. High-Level folate production in fermented foods by the B12 producer *Lactobacillus reuteri* JCM1112. *Appl Environ Microbiol*. 2008 May;74(10):3291–4.
112. Sriramulu DD, Liang M, Hernandez-Romero D, Raux-Deery E, Lünsdorf H, Parsons JB, et al. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. *J Bacteriol*. 2008 Jul;190(13):4559–67.
113. Molina VC, Médici M, Taranto MP, Font de Valdez G. *Lactobacillus reuteri* CRL 1098 prevents side effects produced by a nutritional vitamin B deficiency. *J Appl Microbiol*. 2009 Feb;106(2):467–73.
114. Thomas CM, Saulnier DMA, Spinler JK, Hemarajata P, Gao C, Jones SE, et al. FolC2-mediated folate metabolism contributes to suppression of inflammation by probiotic *Lactobacillus reuteri*. *MicrobiologyOpen*. 2016 Oct;5(5):802–18.

115. Bulsiewicz W. Fiber fueled: the plant-based gut health program for losing weight, restoring your health, and optimizing your microbiome. New York: Avery, an imprint of Penguin Random House; 2020. 1 p.
116. Tiffany CR, Bäumlér AJ. Dysbiosis: from fiction to function. *Am J Physiol - Gastrointest Liver Physiol.* 2019 Nov 1;317(5):G602–8.
117. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 2015 Sep;33(9):496–503.
118. Wang Q, Luo Y, Ray Chaudhuri K, Reynolds R, Tan EK, Pettersson S. The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. *Brain J Neurol.* 2021 Oct 22;144(9):2571–93.
119. Takakura W, Pimentel M. Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome - An Update. *Front Psychiatry.* 2020;11:664.
120. Proffitt C, Bidkhorí G, Moyes D, Shoaie S. Disease, Drugs and Dysbiosis: Understanding Microbial Signatures in Metabolic Disease and Medical Interventions. *Microorganisms.* 2020 Sep 9;8(9):1381.
121. De Filippis F, Paparo L, Nocerino R, Della Gatta G, Carucci L, Russo R, et al. Specific gut microbiome signatures and the associated pro-inflammatory functions are linked to pediatric allergy and acquisition of immune tolerance. *Nat Commun.* 2021 Oct 13;12(1):5958.
122. Vatn S, Carstens A, Kristoffersen AB, Bergemalm D, Casén C, Moen AEF, et al. Faecal microbiota signatures of IBD and their relation to diagnosis, disease phenotype, inflammation, treatment escalation and anti-TNF response in a European Multicentre Study (IBD-Character). *Scand J Gastroenterol.* 2020 Oct;55(10):1146–56.
123. Zhao L, Cho WC, Nicolls MR. Colorectal Cancer-Associated Microbiome Patterns and Signatures. *Front Genet [Internet].* 2021 [cited 2023 May 28];12. Available from: <https://www.frontiersin.org/articles/10.3389/fgene.2021.787176>
124. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. *Nat Rev Microbiol.* 2018 Sep;16(9):567–76.
125. Liu Y, Freeborn J, Armbrister SA, Tran DQ, Rhoads JM. Treg-associated monogenic autoimmune disorders and gut microbial dysbiosis. *Pediatr Res.* 2022 Jan;91(1):35–43.
126. He B, Hoang TK, Wang T, Ferris M, Taylor CM, Tian X, et al. Resetting microbiota by *Lactobacillus reuteri* inhibits T reg deficiency-induced autoimmunity via adenosine A2A receptors. *J Exp Med.* 2017 Jan;214(1):107–23.
127. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature.* 2013 Aug 29;500(7464):541–6.

128. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*. 2006 Feb;55(2):205–11.
129. Scher JU, Ubeda C, Artacho A, Attur M, Isaac S, Reddy SM, et al. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. *Arthritis Rheumatol Hoboken NJ*. 2015 Jan;67(1):128–39.
130. Turnbaugh PJ, Hamady M, Yatsunencko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009 Jan 22;457(7228):480–4.
131. Zhang D, Ji H, Liu H, Wang S, Wang J, Wang Y. Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of *Lactobacillus* or an antibiotic. *Appl Microbiol Biotechnol*. 2016 Dec;100(23):10081–93.
132. Yang Y, Zhao X, Le MHA, Zijlstra RT, Gänzle MG. Reutericyclin producing *Lactobacillus reuteri* modulates development of fecal microbiota in weanling pigs. *Front Microbiol*. 2015 Jul 28;6:762.
133. Sung V, D'Amico F, Cabana MD, Chau K, Koren G, Savino F, et al. *Lactobacillus reuteri* to Treat Infant Colic: A Meta-analysis. *Pediatrics*. 2018 Jan 1;141(1):e20171811.
134. Wiciński M, Sawicka E, Gębalski J, Kubiak K, Malinowski B. Human Milk Oligosaccharides: Health Benefits, Potential Applications in Infant Formulas, and Pharmacology. *Nutrients* [Internet]. 2020 Jan [cited 2023 Feb 28];12(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7019891/>
135. Francavilla R, Lionetti E, Castellaneta S, Ciruzzi F, Indrio F, Masciale A, et al. Randomised clinical trial: *Lactobacillus reuteri* DSM 17938 vs. placebo in children with acute diarrhoea - a double-blind study. *Aliment Pharmacol Ther*. 2012 Aug;36(4):363–9.
136. Dinleyici EC, PROBAGE Study Group, Vandenplas Y. *Lactobacillus reuteri* DSM 17938 effectively reduces the duration of acute diarrhoea in hospitalised children. *Acta Paediatr Oslo Nor 1992*. 2014 Jul;103(7):e300-305.
137. Urbańska M, Gieruszczak-Białek D, Szajewska H. Systematic review with meta-analysis: *Lactobacillus reuteri* DSM 17938 for diarrhoeal diseases in children. *Aliment Pharmacol Ther*. 2016 May;43(10):1025–34.
138. Garcia Rodenas CL, Lepage M, Ngom-Bru C, Fotiou A, Papagaroufalis K, Berger B. Effect of Formula Containing *Lactobacillus reuteri* DSM 17938 on Fecal Microbiota of Infants Born by Cesarean-Section. *J Pediatr Gastroenterol Nutr*. 2016 Dec;63(6):681–7.
139. Nagpal R, Tsuji H, Takahashi T, Kawashima K, Nagata S, Nomoto K, et al. Sensitive Quantitative Analysis of the Meconium Bacterial Microbiota in Healthy Term Infants Born Vaginally or by Cesarean Section. *Front Microbiol*. 2016;7:1997.

140. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. *Open Biol.* 2019 Sep 11;9(9):190128.
141. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol.* 2001 Apr;2(4):361–7.
142. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet Lond Engl.* 2001 Apr 7;357(9262):1076–9.
143. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, et al. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol.* 2003 Feb;111(2):389–95.
144. Socała K, Doboszevska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. *Pharmacol Res.* 2021 Oct;172:105840.
145. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci.* 2012 Oct;13(10):701–12.
146. Foster JA, McVey Neufeld KA. Gut–brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* 2013 May 1;36(5):305–12.
147. Taylor AE, Lee H en, Buisman-Pijlman FTA. Oxytocin treatment in pediatric populations. *Front Behav Neurosci* [Internet]. 2014 [cited 2023 Mar 6];8. Available from: <https://www.frontiersin.org/articles/10.3389/fnbeh.2014.00360>
148. Lefevre A, Sirigu A. The two fold role of oxytocin in social developmental disorders: A cause and a remedy? *Neurosci Biobehav Rev.* 2016 Apr 1;63:168–76.
149. Luang-In V, Katisart T, Konsue A, Nudmamud-Thanoi S, Narbad A, Saengha W, et al. Psychobiotic Effects of Multi-Strain Probiotics Originated from Thai Fermented Foods in a Rat Model. *Food Sci Anim Resour.* 2020 Nov;40(6):1014–32.
150. Erdman SE, Poutahidis T. Microbes and Oxytocin. In: *International Review of Neurobiology* [Internet]. Elsevier; 2016 [cited 2023 May 2]. p. 91–126. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0074774216301180>
151. Connolly N, Anixt J, Manning P, Ping-I Lin D, Marsolo KA, Bowers K. Maternal metabolic risk factors for autism spectrum disorder—An analysis of electronic medical records and linked birth data. *Autism Res Off J Int Soc Autism Res.* 2016 Aug;9(8):829–37.
152. Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell.* 2016 Jun 16;165(7):1762–75.

