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A mini-review: valuable allies for human health: probiotic strains of *Limosilactobacillus reuteri*

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Abstract. The World Health Organization defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". One of the most well studied probiotics is *Limosilactobacillus reuteri*, a Gram-positive, rod-shaped bacterium that colonizes the mucosal surfaces of mammals and birds and is considered autochthonous to the human microbiome. Genetic analyses have confirmed that this microorganism has co-evolved with its host, a prerequisite for the development of a mutualistic relation. On the one hand, *L. reuteri* contributes to the health of the host by releasing antimicrobial compounds, such as reuterin, and numerous active metabolites that can cross the epithelial barrier and reach different targets. Secondly, it can directly prevent the mucosal colonization by pathogenic bacteria helping in the prevention and restoration of dysbiosis due to the capability of forming biofilm. The characterization of numerous effector molecules produced by *L. reuteri* has provided a broad understanding of the mechanisms by which it not only displays antimicrobial and immunomodulatory activities within the gastrointestinal tract but can also influence the correct balance of distal locations of the body. This mini-review carries out a brief overview relating to the most well-known properties of *L. reuteri* highlighting the main biological processes involved.

Keywords: *Limosilactobacillus reuteri*, probiotic, postbiotic, immunomodulation.

INTRODUCTION

Named after Gerhard Reuter, the German microbiologist who conducted pioneering studies on the subject, *Lactobacillus reuteri* has recently been reclassified as *Limosilactobacillus reuteri* (Zheng et al., 2020). This Gram-positive bacterium is currently one of the widely-used probiotics, in which an increased number of studies support its ability to elicit health benefits to the host (Mu et al., 2018). As confirmed by Oh et al., *L. reuteri* colonizes the Gastrointestinal Tract (GIT) of different vertebrates and can be considered autochthonous to the human gut. Their phylogenetic analysis speculates the

fact that, between this microorganism and the host, there is a symbiotic relationship developed from a long-term evolutionary process (Oh et al., 2009; Walter et al., 2011).

As a member of the gut microbiota, *L. reuteri* strains are involved in a complex interplay within the host and can exert different antimicrobial, immunostimulatory and anti-inflammatory activities (Abuqwider et al., 2022). In addition to lactic acid and different low-molecular mass compounds, *L. reuteri* produces a small aldehyde, known as reuterin, which is effective in inhibiting numerous bacterial pathogens (Castellani et al., 2021). At the same time, *L. reuteri* produces biofilm that provides a successful colonization of host-tissues, thus limiting the adhesion of pathogens (Grande et al., 2017). The purpose of this mini-review is to provide an overview of the most well-known properties of *L. reuteri* describing its potential role in health and disease.

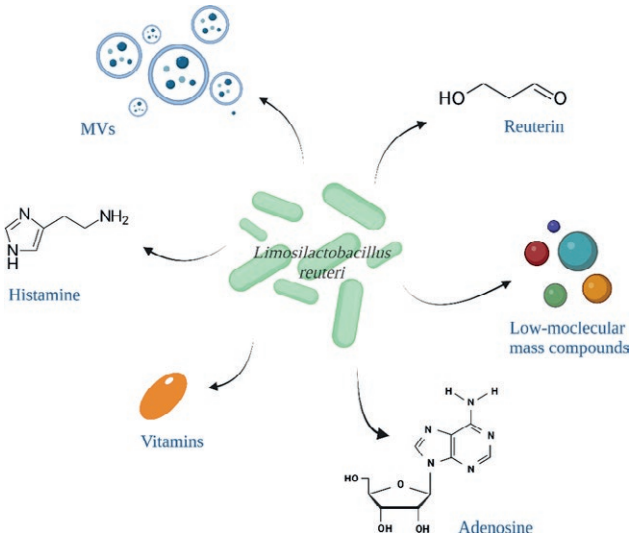

Figure 1. Graphical summary of *Limosilactobacillus reuteri* bioactive compounds. Created with BioRender.com.

L. REUTERI BIOACTIVE COMPOUNDS

According to the definition provided by the International Scientific Association of Probiotics and Prebiotics, the term "postbiotics" defines a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen et al., 2021). In line with this definition, several metabolites produced by *L. reuteri* can be mentioned for their bioactivities and they could be part of preparations with postbiotic properties (Figure 1).

Reuterin is a water-soluble mixture of different forms of 3-hydroxypropionaldehyde synthesized only in the presence of glycerol. It shows antimicrobial activity against Gram-positive and Gram-negative bacteria maintaining this effect in a wide range of pH (Cleusix et al., 2007). Then, Thomas and colleagues characterized the function of *L. reuteri* 6475-derived histamine. This compound is the result of the L-histidine metabolism and exerts anti-inflammatory activity via tumor necrosis factor suppression (Thomas et al., 2012). Similarly, several authors reported that *L. reuteri* can produce adenosine which can also reduce inflammation by interacting with T-cell receptors (Pang et al., 2022; Liu et al., 2023). Moreover, some strains, such as *L. reuteri* CRL1098 and *L. reuteri* JCM1112, can produce vitamins including vitamin B12 and vitamin B9 (Mu et al., 2018).

However, it is reasonable to assume that all the health promoting properties could be related to a synergy between the compounds released by this bacterium. For instance, Maccelli et al. found that the Cell Free Supernatant (CFS) of *L. reuteri* DSM 17938 displayed antimicrobial and antibiofilm activities versus different pathogens, although it was almost impossible to attribute these functions to a single compound. In fact, the metabolomic analysis revealed the complexity of the CFS composition making its characterization a challenging issue (Maccelli et al., 2020; Vitale et al., 2023). Furthermore, these studies focused on *L. reuteri*-derived Membrane Vesicles (MVs) which can be produced in the planktonic and biofilm phenotypes. As reviewed by Krzyżek et al., the MVs secreted by *L. reuteri*, as well as other probiotics strains, are likely one of the effectors of the probiotic activity in maintaining physiological homeostasis and ameliorate disease conditions (Krzyżek et al., 2023).

HEALTH PROMOTING PROPERTIES OF *L. REUTERI*

It is widely known that a balanced gut microbiota promotes the health of the host and that probiotics play a key role in maintaining tissue homeostasis. Given their resistance to low pH and bile salts, multiple *L. reuteri* strains have the potential to colonize the GIT; the adherence to epithelial cells is guaranteed by the expression of mucus-binding proteins and the production of exopolysaccharides which result in the biofilm formation (Mu et al., 2018). *L. reuteri* competes for nutrients and space with other microorganisms, thus limiting their growth. As mentioned above, it can release antimicrobial compounds that directly kill pathogens. This probiotic is also known for enhancing the function of the intestinal epi-

thelial barrier by reversing altered transepithelial electrical resistance and increasing the expression of tight junction proteins (Gao et al., 2022). It is interesting to note that in 2022 Lee and colleagues demonstrated that *L. reuteri* DS0384 accelerated the maturation of fetal intestine in stem cell-derived models as well as *in vivo* models. The results obtained using different strains gave way to the conclusion that the effect is strain-dependent rather than species-dependent (Lee et al., 2022).

The colonization of the GIT mucosa and the immunomodulatory properties attribute *L. reuteri* as having key function that may be effective for the management of inflammatory bowel disease. Numerous studies reported that the treatment with *L. reuteri* decreased the levels of pro-inflammatory markers, in both *in vitro* and *in vivo* models, by regulating Treg and dendritic cells (Abuqwider et al., 2022). At the same time, Liu and colleagues demonstrated that four *L. reuteri* strains can differentially modulate the release of cytokines and chemokines from cultured intestinal cells and rat intestine resulting in both immunosuppression and immunostimulation (Liu et al., 2010).

As extensively studied, the cross-talk between probiotics and immune system does not only impact the gastrointestinal tissue, but correlates with the homeostasis of different areas of the body. Fang et al. documented the efficacy of *L. reuteri* treatment in alleviating atopic dermatitis in mice (Fang et al., 2022), while, in 2023 Lu et al. investigated whether maternal *L. reuteri* supplementation could restore detrimental neurological alterations in offspring. Given that maternal inflammatory states can induce Blood-Brain Barrier (BBB) dysfunction and neurodevelopment deficits in children, they employed rodent models of maternal immune activation and demonstrated that *L. reuteri* treatment during lactation rescued the BBB deficits in offspring improving their spatial learning later in life. Even though the detailed mechanisms are still unknown, it is plausible that these effects are mediated by metabolites and neurotransmitters systemically released by *L. reuteri* (Lu et al., 2023). Thus, as documented for other probiotics, *L. reuteri* participates in the balance of the gut-brain axis (Abuqwider et al., 2022).

CONCLUSION

The existing body of literature concerning *L. reuteri* allowed its characterization in terms of sites of colonization, capability of forming biofilm and release of bioactive compounds. Therefore, all these findings highlighted the numerous health promoting properties related to this probiotic. This mini-review summarizes some of the most well studied *L. reuteri* strains that are involved in health and disease conditions and widely described are some of the mechanisms related to their properties. In conclusion, studies in this area of research are constantly evolving with the purpose of continuous learning relating to this probiotic.

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