

Lactobacillus: A Probiotic Modulator of Gastrointestinal Motility

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Richard is a third year student in the Bachelor of Health Sciences (Honours) Program and a recipient of the Bachelor of Health Sciences Summer Research Scholarship. He spent his past summer researching the physiological effects that Lactobacillus reuteri evoke on murine gastrointestinal motility. The central focus of his research is to characterize these effects to better understand the communication between beneficial gut microorganisms and the host nervous system.

Within the gastrointestinal (GI) tract lives a highly complex community of bacteria from over 1800 genera.^[1,2] Many of these bacteria interact with each other and many are also classified as commensals, which can produce positive effects on the host organism.^[1,2] Some common examples include strains of lactic acid bacteria that aid in the absorption of lactose for humans.^[1,2] These beneficial species are often referred collectively to as “probiotics.” A vast majority of these probiotics are vital to human health. For instance, *Escherichia coli* found in the lower intestine produces Vitamin K₂ that is essential for blood coagulation in humans. But more importantly, ongoing research has demonstrated that an imbalance of these commensal species in the GI tract, known medically as “dysbiosis”, is associated with various GI diseases, gut inflammation and many disorders that elicit visceral pain.^[3] Therefore, further research on probiotics and the characterization of their physiological effects are a clinically relevant endeavor.

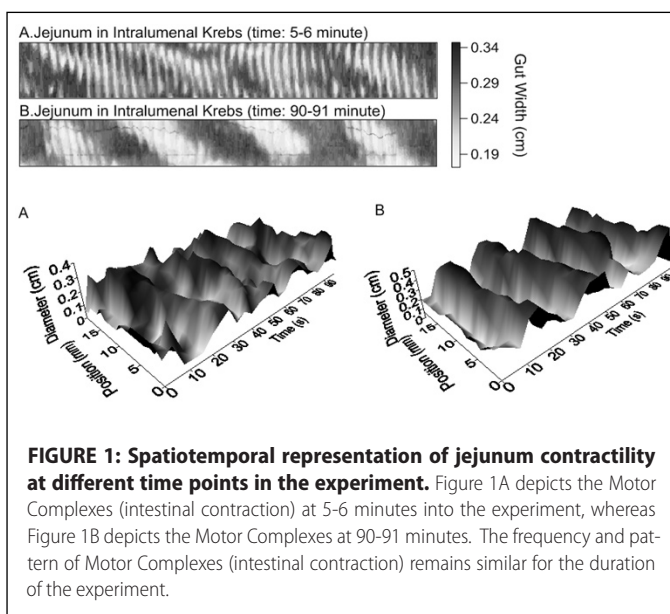
Due to the potential to extend the clinical benefits of probiotics, recent research has focused on the brain-gut axis (framework of how the interaction of intestinal organs and brain modulates organ behaviors) to explore how intestinal probiotic species communicate to the central nervous system.^[4,5,6] Little is known about the method by which bacteria communicate with the host nervous system, but preliminary evidence shows that probiotics may modulate the sensory neuron excitability (by reducing hyperpolarization) in the myenteric plexus, a network of nerves that innervates the intestines. This strongly suggests the possibility that these bacteria may signal to the central nervous system via a potent neuromodulator.^[7] To further understand signaling between probiotics and the GI tract, our laboratory utilizes the common gut bacteria *L. reuteri*. Many lactobacillus strains, including *L. reuteri*, release gamma-aminobutyric acid (GABA)—a neurotransmitter normally produced by the human body known to be able to influence intestinal motility, anxiety and depression.^[8,9] In addition to releasing GABA, *L. reuteri* also releases additional molecules that could potentially act as neuromodulators like carbohydrates, peptides or other neurotransmitters.^[8,9]

Thus, with *L. reuteri* as the focus of this research, this project seeks to characterize the probiotic's effects on intestinal motility. We believe that this approach may help to elucidate some of the unknowns underlying the communication process between the enteric nervous system and probiotics. Any discoveries from these

experiments pave the way for the development of probiotics as a cheap, effective strategy to help treat various GI diseases.

METHODS

The experimental setup comprises of an *ex vivo* organ bath stimulation in conjunction with real-time pressure and video recordings of an intestinal segment isolated from mice. The concept of “*ex vivo*” refers to the conducting of experiments on tissues in heavily controlled artificial conditions outside of the organism's body to better maintain the environmental variables.^[10] This is important because it reduces the possibility for confounding and facilitates the elucidation of true cause-and-effect relationships. To this end, an organ bath setup achieves this because the tissue is submersed in a buffer solution, which maintains constant pH, osmolarity and electrolyte content. These conditions enable the tissue to carry out its natural activities as the researchers administer different chemicals to stimulate the tissue. In this case, the ‘chemical’ is actually the probiotic *L. reuteri*, which will be applied intraluminally (to the inner surfaces of the intestine) at various concentrations. Intraluminal pressure recording and video imaging will then be performed to analyse the tissue's state of intestinal motility.



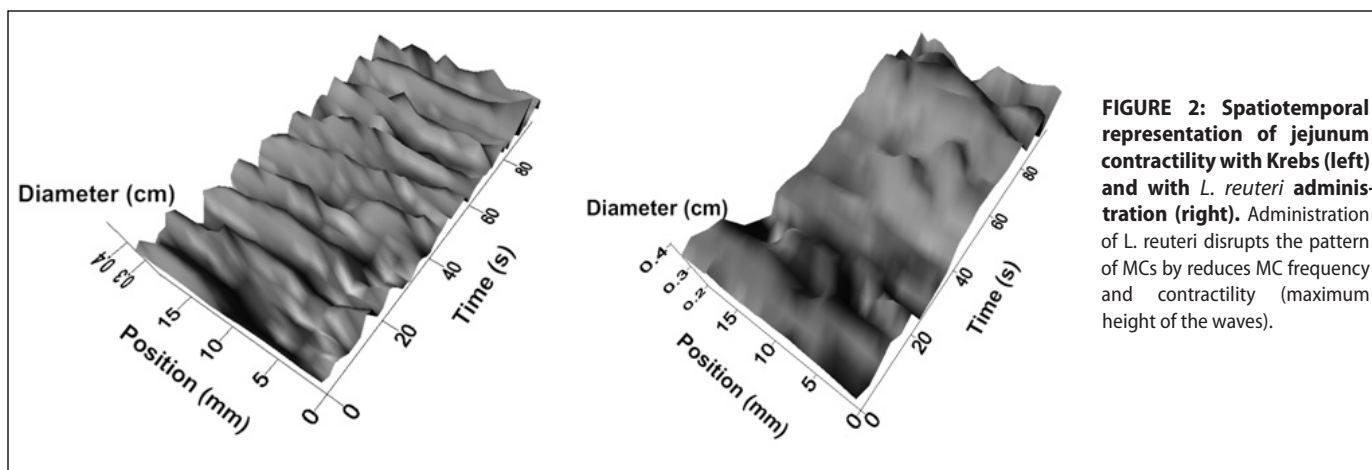


FIGURE 2: Spatiotemporal representation of jejunal contractility with Krebs (left) and with *L. reuteri* administration (right). Administration of *L. reuteri* disrupts the pattern of MCs by reduces MC frequency and contractility (maximum height of the waves).

RESULTS

***L. reuteri* reduces intraluminal pressure:** During peristalsis, the intestine carries out alternating events of distension and contraction in order to facilitate the digestion and passage of food through the intestine. These regular contractions are known as motor complexes (MCs).^[10,13] Experimentally, MCs can be identified through two approaches. The first being the rise in intraluminal pressure when a contraction is initiated (think of squeezing a tube that contains water), and the second being the decrease in diameter when a contraction occurs. Therefore, since MCs are neural-dependent events,^[10,13] investigating their changes in response to *L. reuteri* helps us to understand how probiotics influence GI motility.

Following the intraluminal application of *L. reuteri*, the average intraluminal pressure of the intestinal MCs demonstrated robust decreases in amplitudes in a concentration-dependent manner.^[10]

This modulating effect on the pressure amplitudes was also accompanied by a decrease in MC frequency. These two findings reflect an overall diminished contractility of the intestine evoked by intraluminal *L. reuteri* administration, resulting in weakened and infrequent MCs.^[10]

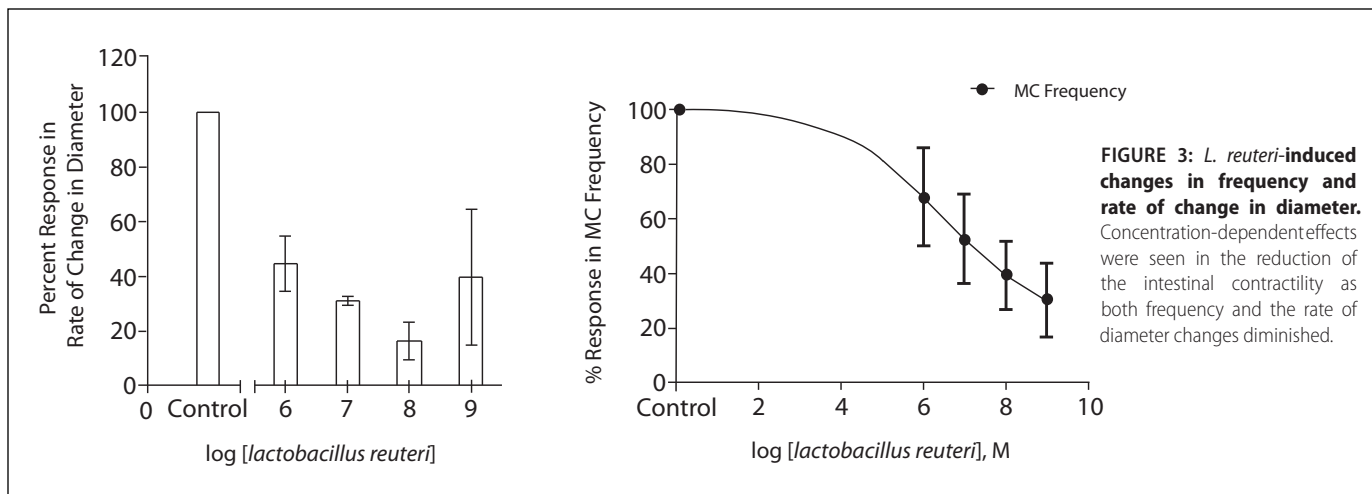
Intestinal imaging shows that *L. reuteri* reduces the frequency and contractility of MCs: Despite these findings, one drawback to this technique is that the pressure is only recorded at one location along the intestine; thus, the pressure probe may easily pick up noises or sporadic contractile events that are not truly MCs. To account for this possibility, imaging was also conducted because it captures the changes in diameter of all positions along the intestine. This gives a better indication of which contractions are true MCs (which are represented by the light bands in Figure 1). Initial control recordings without *L. reuteri* were performed to determine the natural pattern of intestinal contraction (Figure 1), and it was observed that this natural pattern was consistent over the duration of the experiment. In agreement with the intraluminal pressure measurements, the imaging results reflect a similar relaxing effect of *L. reuteri* on intestinal motility as evidenced by the reduced frequency and contractility of MCs upon administration of the probiotic (Figure 2).

In addition to providing information about MC frequency, the imaging technique provides another useful measure to characterize the patterns of motility: rate of change in diameter. This measure looks at how fast the intestinal diameter at a specific location on the intestine reaches its minimum as it undergoes a propagating MC. Interestingly, remarkable decreases were observed in rate of the change of diameter following *L. reuteri* application, suggesting an altered mode of distension/contraction in the intestinal peristalsis.

The two pieces of information yielded by the imaging technique—the MC frequency and rate of change of diameter—are represented graphically in Figure 3. Dose-response relationships were observed for both measures, and this further strengthens the evidence for *L. reuteri* as the causative agent. Therefore, the imaging results reinforce those from the pressure analysis by demonstrating that *L. reuteri* weakens the contractility of the intestine through weakened, infrequent, and slower MCs.

CONCLUSION

Based on the present findings, *L. reuteri* can serve as a potent modulator of GI motility. The observed decreases in MC frequency, contractility and rate of change in diameter indicate that *L. reuteri* administration induces a moderation of peristalsis, which needs to be further characterized. These decreased effects were also dose-dependent in nature. Dose-dependent relationships are characteristic of ligand-receptor interactions, and as such, this suggests that probiotic to nervous system interactions occur at a molecular receptor level.^[10] Consequently, this further extends our laboratory focus into *L. reuteri* conditioned media (the culture medium containing the microorganism's secretion of proteins, cytokines, neurotransmitters and other chemicals), as it is plausible that the ligand responsible for these events may be secreted by the bacterium. These findings contribute to the scientific state of the art on multiple levels. They provide pioneering evidence of the possible therapeutic benefits of utilizing *L. reuteri* as drug substitutes or supplements to therapeutically affect patient intestinal motility. This is especially important given the present findings pertaining to *L. reuteri*'s relaxant effects on the gut, which may point to *L. reuteri* as a potential treatment to diseases involving hypermotility.



ity of the gut, such as ulcerative colitis, irritable bowel syndrome and inflammatory bowel disease.^[11] These results further mount to the existing benefits of providing “living drugs” (probiotics) as therapy. This is a compelling approach since probiotic delivery removes the potential side-effects that drug-users would incur; they are easier and cheaper to culture than drug pharmaceuticals, and from a patient’s standpoint, they can be readily supplemented into patients’ diet (i.e. probiotic yogurts).^[12] Hence, the rigorous investigation of probiotic effects and their mechanisms of action are paramount to the eventual advancements in treating human digestive diseases. However, before we progress onto human appli-

cations, more detailed research on the nature of the neuromodulator and its human applicability is required before this work can lead to practical clinical trials for public usage. Regardless, the findings presented herein contribute to an emerging body of literature concerning the influence of *L. reuteri* on intestinal motility and sets an important stage for the development of probiotics as feasible alternatives to treat GI diseases.

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Reviewed by Dr. Wolfgang Kunze, Ph.D.

Dr Wolfgang Kunze is an Associate Professor of Psychiatry at McMaster University. He obtained his PhD in Melbourne University, Melbourne, Australia. He is a biophysicist with a strong interest in electrophysiology, neuronal sensory processing and enteric nervous system function. Dr Kunze has supervised Richard in his laboratory for the past 2 years and wholeheartedly supports his projects.

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