Lactobacillus reuteri's role in the prevention of colorectal cancer: a review of literature

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Abstract

Background: Probiotics have many health benefits, which include decreasing intestinal inflammation and down-regulating metabolites and inflammatory mediators that can lead to the progression of tumors. *Lactobacillus reuteri (L. reuteri)* is a crucial bacterium that colonizes many mammals. Through analysis and evaluation of peer-reviewed articles relevant to the topic of interest, the findings in this review suggest that *L. reuteri* can slow tumor progression.

Hypothesis: *Lactobacillus reuteri* facilitates the prevention of colorectal cancer through inhibition of tumor progression.

Methodology: A database search through MEDLINE with exclusion criteria narrowed down material to the most relevant searches. The similarities that existed between various articles are highlighted in summary findings to help strengthen the argument for *L. reuteri*.

Conclusion: The findings of this review support the hypothesis that states, "*Lactobacillus reuteri* facilitates probiotics in the prevention of colorectal cancer through inhibition of tumor progression." *L. reuteri* was able to prevent colorectal cancer in animal models through the inhibition of tumor progression. Findings showed that this bacterium was critical in the inhibition of tumor progression, the reduction in inflammation, and thus the prevention of tumor progression into carcinogenesis or further metastasis.

Background

Colorectal cancer (CRC) is the third leading cause of cancer deaths worldwide, with 1.65 million new cases and almost 835 000 deaths in 2015.[1, 2] Every year, approximately 8% of cancer deaths are a result of colorectal cancer, with the highest incidence rates being in Australia, New Zealand, Europe, and North America.[2,3] The rise in the cases of CRC is due to sporadic environmental factors and genetic risk factors, which lead to random mutations in DNA; however, as advancements in screening techniques for CRC have continued to improve, mortality rates from CRC have progressively declined.

CRC affects both the colon and the rectum and has malignancy potential, which, if not caught early, can lead to metastasis. The survival rate of CRC is dependent on the stage at the time of diagnosis, with a 5-year survival rate of 90% for local metastasis, 70% for regional metastasis, and 10% for those with distant metastasis.4 CRC has multiple risk factors, which include diets high in processed meat or low in fiber, as well as chronic inflammatory diseases such as ulcerative colitis and Crohn's disease. Non-modifiable risk factors include age and genetics; however, most of the risk factors for CRC, such as lifestyle, diet , alcohol use, and exercise, are modifiable.[4] Routine medical tests like fecal occult blood test, colonoscopy, and sigmoidoscopy can help with early detection of CRC. Furthermore, due to the high incidence and mortality rates of CRC, further research and studies on promising cures or prevention methods are of great essence.

CRC Tumor Genesis

Fearon and Vogelstein, describe the developmental process of CRC as one that follows several concise and consecutive steps.[5] Cells that contain β-catenin end up developing into tumors even though initial mutations and growth from epithelial or stem cells occur at random. The adenomatous polyposis coli (APC) gene has 15 exons with a molecular weight of over 300 kDa. The protein formed by the APC gene is responsible for inhibiting the activation of β-catenin and keeping it contained just within the cytoplasm. Once the APC gene is mutated, it can no longer act as a tumor suppressor.[6]

Studies show that the loss of function of the APC gene leads to activation of β-catenin and its eventual translocation to the nucleus, causing subsequent KRAS activation that finally leads to the development of adenomas.[7] Other studies suggest that just β-catenin itself and mutations in glycogen synthase kinase-β are responsible for a subset of some CRC cases.[8] Others also suggest that *H. pylori*, mainly accountable for gastric tumors, may also have a role in some cases of CRC. The proinflammatory signaling tumor necrosis factor-α (TNF-α) that is produced by gastric tumors induces the translocation of β-catenin to the nucleus even without the mutation of the APC gene.[9]

Probiotics

There has been a growing focus on the use of probiotics in treating many gastrointestinal diseases, including cancer.[10] Some

studies link intestinal microbiota and lack of dietary fiber intake to the rise in number of CRC cases.[2] These studies have suggested that microbial imbalance in the colon leads to increased growth of bacteria, some of which are carcinogenic.[11] Because of their ability to restore microbial balance in the gastrointestinal tract, probiotics show a promising future role in the prevention and control of CRC. Evidence shows that there is an inverse correlation between the amount of healthy colon flora and the degree of the mucosal immune response.[10] It is because of these findings that Kechagia in 2013 suggested that the administration of live probiotics can help restore healthy intestinal flora, improve intestinal health, enhance immune response, and prevent cancer.

Lactobacillus reuteri (L. reuteri) is specifically examined amongst the various other probiotics because of its ability to limit the degree of inflammation in patients with ulcerative colitis (especially among children), a known risk factor for the development of CRC.[12] It is also one of the probiotics that have been of great interest to researchers.[13] *L. reuteri* is known to colonize the gastrointestinal tracts, urinary tracts, skin, and mammary glands of many mammals.[13] It has antimicrobial activity with an ability to inhibit the aggregation and growth of other microbes.[13] Some particular strains of this bacterium have reduced the production of pro-inflammatory cytokines like TNF-α, while also promoting T cell development.[13]

Probiotics modulate immune system response, induce apoptosis of cancer cells, decrease the degree of inflammation caused by chronic inflammatory diseases, and reduce a patient's chances of developing CRC.[14,15] The overall objective of this study is to demonstrate the benefits of specifically administering *L. reuteri* to help prevent the development or progression of CRC.

Methodology

The authors had free access to MEDLINE through PubMed, and searched the database using the following key terms: "colorectal cancer" and "*Lactobacillus reuteri*." The search included these terms with and without quotation tags and together with publication types. Google Scholar was also used with the terms "*Lactobacillus reuteri* and colorectal cancer," "Probiotics and colon cancer," and "Colon cancer probiotic prevention." Articles were narrowed down by selecting those that were most recently published. Abstracts of articles were then examined by the authors and filtered based on whether or not they were in English, were available in full text, and had open-access. The methodologies (cohort, randomized control trials, or systematic reviews) used in the studies were also taken into account.

Inclusion and exclusion criteria

The publication year was refined to the custom range of 2010 – 2018. Articles that were older than ten years and were not in English were excluded. In search criteria, keywords like "*Lactobacillus reuteri*," "probiotics," and "colorectal cancer" were used in combination or alone to help find relevant articles for this review. The study populations included mammalian cell lines, mice, and rats. The information was gathered and tabulated by author name, year of publication, study design, study population, outcome, and results. The quality of articles was analyzed based on their "impact factor," which is a measure of importance or rank of the journal calculated by the average number of times its articles are cited in other peer-reviewed research papers. The summary of the research articles used for this review is shown in the evidence table below.

Table 1. Evidence Table

Study details	Population and setting	Method of allocation	Outcome and methods of analysis	Results
Authors: Blasingame, Camara A.; Billups, Leonard H.; Graham, omas; Henry, JaNell; Carter, Brianna; readgill, David W.; and Alexander, A. Deloris **Year**: 2016 **Citation**: Blasingame, Camara A.; Billups, Leonard H.; Graham, omas; Henry, JaNell; Carter, Brianna; readgill, David W.; and Alexander, A. Deloris (2016) "Modulation of Colorectal Cancer by the Probiotic Organism Lactobacillus Reuteri," Professional Agricultural Workers Journal: Vol. 3: No. 2, 3. **Country of study**: United States **Aim of the study:** To determine if *L. reuteri* could protect mice from carcinogen-induced colorectal cancer **Study design**: Random trial	**University of North Carolina, Chapel Hill, Texas A&M University** **Eligible populations:** Mice were randomly assigned to controls, pre-treatment or post-treatment groups. Mice were examined after a 26- week latency period. **Excluded population:** Human subjects	Mice were randomly assigned to different groups. Confounding factors, such as different strains of mice, were eliminated. Bacterial identities were verified by gram staining. The gender of mice was also taken into account, as females had a higher tumor burden. Age did not play a factor. The mice were assigned randomly into the pre-treatment, control or post-treatment groups. The mice in the groups were injected with 10mg/kg of AOM and treated with L. reuteri; given only AOM; only the bacterium or given neither. At the end of the 26- week latency period mice were examined. 34 mice were treated; 18 males, 12 females ended up included in the study.	The bacterium *L. reuteri* was unable to prevent tumor formation in animals and this was an objective finding **Method of analysis:** -Tumor number - Mortality -Tumor penetrance -Tumor multiplicity The P-value of 0.05 was considered significant	Mice treated with bacterium alone did not show an increase in tumor formation. This was in comparison to animals that were in other treatment groups. The study determined that the bacterium did not alter morbidity in animals treated with AOM and this was quantified through tumor number, mortality, and penetrance. **Limitations:** There was a significant effect on gender with females having a higher tumor burden. There were also fewer females in the experiment, which could have skewed the results.

Authors: Chien-Chang Chen, Wei-Chuan Lin, Man-Shan Kong, Hai Ning Shi, W. Allan Walker, Chun-Yen Lin, Ching-Tai Huang, Yung-Chang Lin, Shih-Ming Jung and Tzou-Yien Lin

Year: 2012

Citation: Chen, C.C., Lin, W.C., Kong, M.S., Shi, H.N., Walker, W.A., Lin, C.Y., Huang, C.T., Lin, Y.C., Jung, S.M., Lin, T.Y. Oral inoculation of probiotics Lactobacillus acidophilus NCFM suppresses tumour growth both in segmental orthotopic colon cancer and extra-intestinal tissue. Br J Nutr. 2012;107:1623–1634.

Country of study: China

Aim of the study: To determine if tumor growth could be suppressed in colon cancer

Study design: Randomized control trial

National Science Council, Taiwan

Eligible populations: Female mice, 4 – 6 weeks old. Using colon carcinoma cell line CT- 26

Excluded population: Human subjects

Mice were initiated into the study when they were 6 weeks old with a 20g body weight. Female mice were randomized into four groups. Mice were inoculated daily for 14 consecutive days before cancer cell implantation. After this, they were inoculated at 1 x 109 colony forming units/ mouse weekly for 3 weeks.
Primary tumors were initiated by injecting CT-26 cells suspended in serum-free medium and tumor growth was assessed every 3 days.

Statistical analysis: means and standard errors of the mean using one- way analysis of variance. Tumor volume was analyzed with two- way ANOVA
The P-value of 0.05 was considered significant.
Objective findings.
Methods of analysis: Tumour size was measured every 3 days
H&E staining was used to histologically analyze dorsal-lateral flank tumors.
PCR was utilized to determine the expression of CXCR4 mRNA, which helped quantify the amount of mRNA from the chemokine receptor type, CXCR4.

Oral administration of La (lactobacillus) reduced tumor growth and the extent of tissues that were affected.
The findings of the study concluded that there was an increase in cell death and a down-regulation of mRNA and inflammatory molecules such as MHC class 1.

Limitations: The study did not analyze different strains of La, which could limit their suggestions.

Authors: Chunxu Gao, Bhanu Priya Ganesh, Zhongcheng Shi, Rajesh Rasik Shah, Robert Fultz, Angela Major, Susan Venable, Monica Lugo, Kathleen Hoch, Xiaowei Chen, Anthony Haag, Timothy C. Wang, James Versalovic

Year: 2017

Citation: Chunxu Gao, Bhanu Priya Ganesh, Zhongcheng Shi, Rajesh Rasik Shah, Robert Fultz, Angela Major, Susan Venable, Monica Lugo, Kathleen Hoch, Xiaowei Chen, Anthony Haag, Timothy C. Wang, James Versalovic. Gut Microbe–Mediated Suppression of Inflammation-Associated Colon Carcinogenesis by Luminal Histamine Production. The American Journal of Pathology. Volume 187, Issue 10, Pages 2323-2336 (October 2017).

Country of study: United States

Aim of the study: To determine if histamine plays an important role in the suppression of inflammation-associated colon cancer.

Study design: Preclinical studies

Texas Children's Hospital

Eligible populations: Four mouse pups – two males and two female – were obtained from rederivation.

Excluded population: Human subjects

PROgeneV2 database was used and the PROgeneV2 search included 2113 patient samples in 15 data sets.
Each mouse received 5 x 109CFU of bacteria in either 0.2 mL of MRS media or MRS media only as a control. The frequency of administration was once per day for 7 days before AOM injection, followed by administration once per 3 days for 15 weeks. At 12 weeks of age, mice in the positive control group received a single dose of AOM.

Statistical analysis: GradPad Prism software version 5. Data was presented as means +/- SD. T-test and one-way analysis of variance were used and other data were presented as box and whisker plots.
Objective findings.
Methods of analysis: At 15 weeks of AOM injection, mice were euthanized, and the GIT was carefully removed and analyzed. Their femur was used for the collection of bone marrow. This was done through flow cytometry. Subsequently, a PET scan was performed 15 weeks after AOM injection. Mice deficient in histamine were given the bacterium and analyzed for the total DNA and RNA from the distal colon.

The administration of *L. reuteri* increased the expression of hdc, a gene responsible for histamine production.

The hdc gene produces histamine, which is important for the bacterium to reduce tumors when compared to the control.

The imaging revealed that there was restriction of growth for *L. reuteri*. The histamine created by the bacterium slows gene expression and decreases inflammation.

Cytokines in plasma can be reduced by *L. reuteri*.

Limitations: Histamine is only one of many microbial metabolites that can have a profound effect on human anatomy.

Authors: Melinda Anne Engevik, Berkley Luk, Anne Hall, Bhanu Priya Ganesh, and James Versalovic

Year: 2016

Citation: Engevik, Melinda, et al., "Lactobacillus reuteri decreases adherent tumor mucins and enhances chemotherapeutic susceptibilities via ERK/JNK pathways." The FASEB Journal 1. Suppl 30 (2016). Web. 22 Nov. 2017.

Country of study: United States

Aim of the study: To determine if *L. reuteri* decreases adherent tumor mucins and enhances chemotherapeutic susceptibilities via ERK/JNK pathways.

Study design: Experimental

Faculty of Medicine and Health, University of East Anglia

Eligible populations: Adenocarcinoma cell line HT-29-MTX-E12 incubated with CM from probiotic human gut microbes.

Excluded population: Human and mice subjects

The adenocarcinoma cell line HT-29-MTX-E12 was used, which expressed both adherent and secreted mucins.

Cancer-driven mucus was used in combination with chemotherapeutic agents.
Mucus was analyzed with reverse transcriptase PCR. Fluorescence was used to distinguish between the different mucins.
Resazurin was used for fluorescence to determine cancer cell viability.

Reverse transcriptase PCR revealed several spp. from lactobacillus that were able to alter mucins. This bacterium was able to release storages mucins. Furthermore, there was decreased viability of cells when pretreated with the bacterium. Tumors were also decreased as a result.

Reviews

Lactobacillus reuteri's role in the prevention of colorectal cancer: a review of literature

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Authors: Gao, C., Major, A., Rendon, D., Lugo, M., Jackson, V., Shi, Z., Mori-Akiyama, Y., Versalovic, J.

Year: 2015

Citation: Gao, C., Major, A., Rendon, D., Lugo, M., Jackson, V., Shi, Z., Mori-Akiyama, Y., Versalovic, J. 2015. Histamine H2 receptor-mediated suppression of intestinal inflammation by probiotic Lactobacillus reuteri. MBio. 2015;6:e01358-15.

Country of study: United States

Aim of the study: To determine if histamine could suppress intestinal inflammation with the administration of probiotics.

Study design: Experimental

Department of Pathology, Texas Children's Hospital, Houston, Texas, USA

Eligible populations: Female mice (45 days old).

Excluded population: Human subjects

All *L. reuteri* mice were prepared freshly before administration. Each mouse received diluted water with an equal volume of absolute ethanol. Adult female mice were fed *L. reuteri*, daily by orogastric gavage following acclimatization and at least 5 days prior to TNBS instillation.

Histidine was converted to histamine and this resulted in the suppression of inflammation. The subjects were analyzed based on weight loss, colon injury and inflammatory mediators such as serum amyloid protein (SAA). PET scan was used to better analyze the data.
Statistics: T-test or analysis of variance. Other data were presented as box-and-whisker plots showing the median, 10th and 90th percentile. Objective findings.

The bacterium suppressed inflammation in the colon. PET scan was able to demonstrate that a specific strain of *L. reuter*, 6475 was able to suppress inflammation. The bacterium increased the expression of the histamine gene. In addition, administration affected cytokines, which are inflammatory mediators generated in the inflammatory process.

Limitations: There is limited information that exists regarding mechanisms of probiotic- mediated immunomodulation in vivo.

Authors: Kahouli I, Malhotra M, Tomaro-Duchesneau C, Saha S, Marinescu D, LS Rodes, MA Aloui-Jamali and S Prakash

Year: 2015

Citation: Kahouli I, Malhotra M, Tomaro-Duchesneau C, Saha S, Marinescu D, et al. (2015) Screening and In-Vitro Analysis of Lactobacillus reuteri Strains for Short Chain Fatty Acids Production, Stability and Therapeutic Potentials in Colorectal Cancer. J Bioequiv Availab 7:039-050. DOI: 10.4172/jbb.1000212

Country of study: Canada

Aim of the study: *L. reuteri* analysis of different strains for short-chain fatty acid production and the possible therapeutic benefits.

Study design: Research article

Faculty of Medicine, McGill University

Eligible populations: Human epithelial CRC adenocarcinoma cell line Caco – 2.

Excluded population: Human and mice subjects

Cells were maintained in medium and supplemented with 20% fetal bovine serum. All cells were left to attach for 24-48 h in 96 well plates.

Specific strains of the bacterium showed the ability to stunt growth of tumors. Short-chain fatty acids produced by the bacterium were viewed while the bacteria grew. The expansion of colon cancer cells with the administration of probiotics was evaluated using an assay.
Statistics: SCFAs were calculated using linear regression equations from corresponding standard curves. Data were presented as means +/- standard errors and correlations were determined using the Pearson correlation method. Analysis of variance with Tukey's comparison test and student's T-test.

Objective findings.

The bacterium produced lactic acid and the production of SCFAs was strain-dependent. These fatty acids may actually be the reason this probiotic has inhibitory effects.

Authors: Liu, Y., Fatheree, N.Y., Mangalat, N., Rhoads, J.M.

Year: 2010

Citation: Liu, Y., Fatheree, N.Y., Mangalat, N., Rhoads, J.M. 2010. Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation. Am J Physiol Gastrointest Liver Physiol. 2010;299:G1087–G1096.

Country of study: United States

Aim of the study: To determine if *L. reuteri* strains were able to suppress intestinal inflammation.

Study design: Experimental

University of Texas (UT) Medical School at Houston, TX

Eligible populations: Human derived L. reuteri and Sprague-Dawley rat pups.

Excluded population: Human subjects

Strains DSM17938, ATCC PTA4659, ATCC PTA 5289, and ATCC PTA 6475. Newborn pups were separated from their mothers and fed for 3 days with: 1) special rodent formula, 2) rodent formula containing L. reuteri, 3) formula containing LPS from E. coli or 4) LPS in combination with one of the four designated L. reuteri strains

L. reuteri strains were able to control inflammation that was induced by the endotoxin LPS.
The study was analyzed first through tissue harvest, with the terminal ileum being excised and stained. The colon was thoroughly examined.

Statistics: Experimental results were expressed as means +/- SE and one-way analysis of variance was used.

A P value of <0.05 was considered statistically significant.

Objective findings.

There are strains of the bacterium that impaired LPS and initiated Il-8 production. Some strains specifically reduced LPS induction in rats. Specific strains affected Th1 and Th2, and helper T cells.

Lactobacillus reuteri's role in the prevention of colorectal cancer: a review of literature

Authors: Liu, Yuying, Fatheree, Nicole Y., Mangalat, Nisha and Rhoads, March Jon **Year**: 2010 **Citation**: Thomas, Carissa M. et al. "Histamine Derived from Probiotic Lactobacillus ReuteriSuppresses TNF via Modulation of PKA and ERK Signaling." Ed. Markus M. Heimesaat. PLoS ONE 7.2 (2012): e31951. PMC. Web. 22 Nov. 2017. **Country of study**: United States **Aim of the study**: To determine how histamine derived from probiotic *L. reuteri* suppresses inflammatory mediators. **Study design**: Experimental	**Department of Pediatrics of the University of Texas Health Science Center at Houston Medical School** **Eligible populations:** Compounds with TNF inhibitory activity were isolated from L. reuteri cell pellets treated and acidified from L. reuteri strain 6475. **Excluded population:** Human and mice subjects	Cell pellets were separated based on relative hydrophobicity. The fractions were tested for the retention of TNF-inhibitory compounds by activating human monocytoid cells.	Histamine was produced by *L. reuteri*. **Method of analysis:** TNF - ELISA Quantification of histamine by ELISA Quantification of cAMP by ELISA **Statistics:** Mean values with a P-value of <0.05 was considered statistically significant. Objective findings.	*L. reuteri* produced histamine and this inhibited TNF production. Histamine increased secondary messenger cAMP signaling through H2 receptors.
Authors: Verma, A., Shukla, G **Year**: 2013 **Citation**: Verma, A., Shukla, G. Probiotics Lactobacillus rhamnosus GG, Lactobacillus acidophilus suppresses DMH-induced pro-carcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. Nutr Cancer. 2013;65:84–91. **Country of study**: India **Aim of the study**: To determine the protective potential of different probiotics against chemical-induced CRC. **Study design**: Experimental	**Department of Microbiology, Panjab University** **Eligible populations:** Sprague Dawley rats **Excluded population:** Human subjects	Animals belonging to different probiotic groups were fed orally with 1 x 10⁹ lactobacilli daily for 1 week and then injected weekly with DMH to induce cancer.	Lactobacillus GG and lactobacillus + DMH-treated animals had the most reduced ACF counts. This was analyzed through body weight, ACF count and pathological studies. Statistics: Results were expressed as mean +/- SD. The intergroup variation was assessed by a 1-way analysis of variance and a P-value of <0.05 was considered statistically significant. Objective findings.	The increase in the rats' body weights when treated with only probiotics was more than when rats were treated with probiotics and DMH. The rats which were treated with the probiotic alone and DMH, showed a significant decrease in ACF counts.
Authors: Zhong, Li, Xufei Zhang, and Mihai Covasa **Year**: 2014 **Citation**: Zhong, Li, Xufei Zhang, and Mihai Covasa. "Emerging Roles of Lactic Acid Bacteria in Protection against Colorectal Cancer." World Journal of Gastroenterology : WJG 20.24 (2014): 7878–7886. PMC. Web. 22 Nov. 2017 **Country of study**: USA **Aim of the study**: To determine the emerging roles in the protection of CRC. **Study design**: Literature review	**Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, CA** **Eligible populations:** N/A **Excluded population:** N/A	N/A	N/A	**Results:** LTA related protection against pro-inflammation during tumor development.

Results

A 2012 cohort study by Chen was examined in which mice were orally pretreated with a Lactobacilli strain. This study sought to determine if probiotics can prevent CRC. They did the research using *L. acidophilus*, a strain of lactobacillus, to find out if it can increase cell death and halt cancer growth.[14] The researchers used female mice aged 4 to 6 weeks old that were then divided into four groups. One group of BALB/c mice were inoculated with a colon cancer cell line CT-26 only; the next BALB/c group was pretreated with 1 x 10⁸ CFU and L. acidophilus for 14 days, before the administration of CT-26; the third BALB/c group was pretreated with 1 x 10⁸ CFU and E. Coli for 14 days, before administration of CT-26; finally, the fourth BALB/c group was not pretreated.[14]

Chen's findings demonstrated that there was a 36% reduction in tumor volume in the mice that received the bacterium three weeks post-CT-26 inoculation, and a 42% reduction in tumor volume 24 days post-CT-26 inoculation. The researcher then used an analysis of variance to assess the findings. H&E staining of dorsal-lateral flank tumors suggested that oral administration of lactobacilli enhanced apoptosis of tumor cells due to the down-regulation of MHC class molecules, hence decreasing the extent of metastasis.[14] Chen concluded based on these findings that probiotics play a pivotal role in modern healthcare and hold a promising future in treatment, prevention, and management of CRC.

In a comparative study, Kahouli used human epithelial colorec-

tal cancer cell lines to demonstrate that probiotics can be used as preventive agents against colorectal cancer. Specifically, Kahouli investigated the efficacy of short-chain fatty acids (SCFAs): acetate, propionate and butyrate (all of which are secreted by L. reuteri), and examined how they function to impede the growth of colorectal cancer cells. Low levels of SCFAs have been reported in populations with a high incidence of CRC. In Kahouli's study, five strains of L. reuteri were categorized based on their ability to inhibit cancer growth by synthesizing SCFAs.[15]

Cells were maintained in a medium where they were left to attach for 24 to 48 hours in 96-well plates.[15] SCFAs produced by *L. reuteri* were analyzed and then compared to the proliferation of colon cancer cells. The SCFAs produced by the bacterium were found to have inhibitory effects on inflammation. SCFAs levels were calculated using linear regression and presented as means. Interestingly, the lactic acid produced by the five strains showed that certain strains of *L. reuteri*, NCIMB 702656 and NCIMB 701359, produced more lactic acid when compared to multiple other strains.[15] It was also determined that L. reuteri did alter the metabolism of cancer cells, causing growth inhibition among them.[15]

An experiment by Blasingame was conducted to determine if the bacterium L. reuteri could offer protection to mice that were exposed to a carcinogen from developing colorectal cancer. This randomized control study used mice models that were assigned to either treated or non-treated groups. The mice were injected with 10mg/kg bodyweight of Azoxymethane (AOM), which is a carcinogenic compound known to induce colorectal cancer in mice.[16] The groups of mice were either given AOM alone or AOM and L. reuteri, *L. reuteri* alone, or neither. After a 26-week latency period, a total of 34 mice were euthanized and examined.[16]

Using analysis of variance on collected data, findings showed that while there was reduced tumor size with mice treated with L. reuteri, there was no change in mortality.[16] Of note in this study, there were increased tumor numbers in female mice compared to males. For those treated with *L. reuteri*, in addition to the reduction of tumor size, it was noted that angiogenesis in these cells was also reduced, affecting cancer tissue establishment.[16]

Research done in 2012 combined the study of metabolites and bacterial genetics to investigate TNF-α inhibitory factors produced by the bacterium *L. reuteri*.[17] The study found that histamine blocked TNF-α production by 80% when compared to the control.[17] It was found that *L. reuteri* produces the biogenic amine histamine, which was identified and quantified in TNF-α inhibitory fractions using nuclear magnetic resonance (NMR) and mass spectrometry.17 The findings showed that histamine was able to suppress TNF-α production through the activation of a histamine receptor. This study demonstrated that lactobacilli were able to convert L-histidine into an immunoregulatory signal, histamine, which in turn suppressed the production of pro-inflammatory TNF-α production.[17] While many previous studies used animal models, findings by Thomas were strengthened by the fact that the study was performed on human monoclonal cell lines.

Gao's research, concluded in 2017, investigated and analyzed results from histidine deficient mice. Histidine is a common amino acid that makes up histamine. Histidine deficient mice have a predisposition to carcinogenesis. To investigate whether histidine deficiency was linked to the increased cancer rates, Gao examined the relationship between survival rates, mortality rates, and genetic names.[18] In the study, groups were divided based on their expression of histidine, and the data was then plotted and compared among 2113 patient samples of 15 data sets. Histidine deficient mouse-tail clippings were used for DNA extractions. L. reuteri was then cultured, and cells were centrifuged with this bacterium before the introduction of AOM. Fifteen weeks later, the mice were euthanized and cells from their ilea, ceca, and colons retrieved to determine mRNA expression of histidine. The findings indicated that L. reuteri administration increased the abundance of histamine in the HDC deficient mice.[18] Elevated levels of the gene responsible for histamine production inferred a higher rate of survival in the case of CRC (P = 0.02).

Gao in 2015 investigated whether *L. reuteri* had any effects on intestinal inflammation in trinitrobenzene sulfonic acid (TNBS) induced mice models.[19] *L. reuteri* collected from the breast milk of women was used to colonize the mice. Colitis caused by TNBS produced different cytokines in the colon of mice that were then quantified based on their mRNA expression. Data was collected, measured, and then analyzed using a T-test and analysis of variance. This study further supported that histamine was shown to play an essential role in suppressing colonic inflammation. The hdc-positive strains contained human TNF-α production by myeloid cells.[19] Adjunctively, PET scan imaging was also used to offer visuals on the ability of *L. reuteri* to suppress intestinal inflammation.

It was determined that when the bacterium was suppressed, it was unable to decrease intestinal inflammation as determined by weight loss and Wallace scores for colitis, and demonstrated a significant decrease (p <0.05) in both categories.[19] A cohort study was done in 2010 by Liu which demonstrated similar findings, showing that different strains of *L. reuteri* can differentially control LPS- induced inflammation.[20] These researchers explored the effects of the bacterium on the rats. Further supporting previous studies, the *L. reuteri* used were also gathered from the breast milk of Finnish women. Newborn rats were separated from their mothers and given either a unique rodent formula, a formula containing *L. reuteri*, a formula containing LPS from *E. coli*, or LPS in combination with one of the four designated strains of the bacterium.[20] After this, the rats were euthanized, and their terminal ilea were excised and examined.

In addition,, the study by Liu et al. demonstrated and further supported the findings of Gao that multiple strains of *L. reuteri* can inhibit LPS induced IL-8 secretion.[20] The mRNA expression of IL-13 (expressed by CD4 helper T cells) was also significantly decreased in the intestines of rats that were fed with a formula containing the bacterium. The findings demonstrated that histamine was produced by *L. reuteri* strain 6475, and that histamine inhibits TNF-α production, a cytokine.20 Histamine further increased the intracellular cAMP and histamine signaling through histamine receptors that blocked the activation of downstream signal cascades that ultimately decreased TNF-α production.

The random trial completed by Engevik suggested that probiotics increased tumor cell susceptibility to 5- Fluorouracil (5- FU) treatment, a chemotherapeutic drug. The results showed a significant decrease in cancer cells.[21] Engevik also showed that after incubating a mucus-producing adenocarcinoma cell line with probiotics, those that had been pre-treated with *L. reuteri* and Bifidobacterium species were able to reduce the level of inflammation by decreasing the ability of adherent and secreted mucins expressed.

More specifically, in this particular case, *L. reuteri* ATCC 6475 was found to be capable of altering the amount of mucin (this was determined by immunofluorescence).[21]

Verma in 2013 explored the importance of lactobacilli as a prophylactic agent against colon cancer.[22] This study used Dawley rats to illustrate how one's diet can be a contributing risk factor for CRC. In the study, different probiotics were used for their protective potential against 1, 2 dimethylhydrazine dihydrochloride (DMH) induced colon carcinogenesis.[22] The animals, which were placed in groups, were fed the probiotic for one week and then were injected with DMH. Results showed a significant decrease in tumor counts in the rats that were pretreated with the bacterium.[22]

Also similar to previous studies used in this review, Verma used Sprague Dawley rats that were orally fed with 1 x 10^9 lactobacilli daily for one week and then injected with dimethylhydrazine (DMH), a carcinogen used to induce cancer.[22] For the rats pretreated with lactobacilli and DMH, a significant decrease in tumor counts was noted when compared to the DMH treated rats.[22] The findings of this research and others used in this review are summarized in table 1 above. Notwithstanding the different methods and approaches used in said studies, they demonstrate typical results supporting the hypothesis of this review.

Discussion

While examining the relevant literature, it was evident that the results strongly supported the argument that the administration of the bacterium *L. reuteri* can prevent colorectal cancer in certain cell lines and animal models by inhibiting tumor progression. This finding needs to be further explored in human trials. The only study that contradicted the findings was Blasingame, which showed that the bacterium did not significantly alter morbidity in AOM treated animals, as measured by tumor number, mortality, penetrance, or multiplicity.[16] However, the findings also showed that there was a significant effect along gender lines, with females experiencing a higher tumor burden compared to males. That said, there also was a smaller number of females used in the study, which could have skewed the results.

The other studies in this review suggest that the bacterium *L. reuteri* can decrease inflammation by inhibiting mediators such as Il-8 and TNF. Inflammation plays a crucial role in the pathogenesis of cancer. As the study by Engevik demonstrated, histamine from *L. reuteri*, specifically strain 6475, stimulated increased levels of cAMP, a well known second messenger that plays a role in signal cascades. This increase in cAMP inhibited the downstream pathway of MEK/ERK MAPK signaling via protein kinase A. Inhibition of this pathway resulted in the suppression of TNF-α production, an important cytokine secreted by macrophages and responsible for activating endothelium.[21] Furthermore, increased cAMP causes leukocyte extravasation and vascular leakage. Thus, when TNF-α is inhibited, there is a reduction in inflammation, thereby decreasing the progression of carcinogenesis responsible for CRC.

The study by Chen et al. in 2012 demonstrated that oral administration of lactobacillus reduced tumor growth and the extent of tumor invasion into local tissues. The study also showed that *L. reuteri* enhanced the apoptosis of cancer cells by down-regulating MHC class 1. Apoptosis is the phenomenon of programmed cell death, which can be activated by two pathways: either the intrinsic or the extrinsic pathway. Both pathways utilize caspases, functioning on eliminating cells. These pathways are essential in getting rid of cells that are no longer functional, thereby decreasing the risk that they could become cancerous. BAX and BAK are pro-apoptotic proteins that encourage the apoptosis of cells, whereas Bcl-2 prevents apoptosis. MHC class 1 molecule is found on the surface of most nucleated cells and presents antigens to CD8+ cytotoxic T cells, which are cells that directly kill virus-infected cells. Reduction in tumor growth and enhanced apoptosis strengthened the researchers' resolve that the administration of the bacterium can prevent CRC. However, this study showed two limitations; only female mice were used, which may introduce a selection bias into the study, and the researchers did not analyze the different strains of lactobacilli.

According to Gao, histamine is one of the many microbial metabolites that can have a profound effect on humans and showed that *L. reuteri* increased hdc gene expression and histamine production in the intestines of the histidine deficient mice.[18] The hdc gene is crucial as it reduced the number and size of colonic tumors in mice that were treated with specific strains of *L. reuteri*.[18] The histamine generated by the probiotic caused suppressed cytokine gene expression, which down-regulated inflammatory mediators and therefore decreased colonic inflammation.

Gao and colleagues' study findings further strengthened Gao's argument by showing that hdc+ *L. reuteri* did indeed suppress inflammation in the colon by decreasing cytokine gene expression.[19] They also noted that H2 histamine receptors are found predominantly in the mammalian gastrointestinal tract, which further strengthens this argument. A drawback to this finding is that the mechanism behind the phenomenon displayed here is not well understood, as there is limited information on probiotic- mediated immunomodulation in-vivo.[19]

Further support of histamine production by *L. reuteri* and decreasing carcinogenesis by inhibiting TNF-α production via H2 receptor activation downstream and cAMP was demonstrated by Liu in 2010. As mentioned in the above sections, TNF-α contributes to acute inflammation through the recruitment of white blood cells. Thomas' research in 2017 complimented Liu's study findings as they also found that histamine was responsible for the decrease in inflammation in CRC cells.

The study by Gao in 2015 showed that *L. reuteri* reduced inflammation by inhibiting LPS induced Il-8 production in cultured intestinal epithelial cell lines in rats. LPS is a pathogen-associated molecular pattern (PAMP) found on gram-negative bacteria, which helps in eliciting an immune response when detected in the human body. LPS is an endotoxin comprised of a polysaccharide and Lipid-A. LPS induces multiple effects in the body, including edema, neutrophil chemotaxis, shock, and macrophage activation. Reducing LPS expression would reduce the inflammatory response in the body. Gene expression of Interleukins 1 and 6, mediators of acute inflammation, was measured in the colons of healthy mice, and suggested a significant decrease (p<0.05) after pretreatment with *L. reuteri*.[19]

Engevik's study showed how adherent mucins play a role in the pathogenesis of cancer. The study then demonstrated that Lactobacillus species were capable of altering total adherent mucin by stimulating the release of stored mucin.[21] It was found that these adherent mucins caused an increase in Sialyl-Lewis X expression. Sialyl-Lewis X is a trisaccharide antigen that serves as a ligand for

selectins. Increased numbers of these ligands lead to an increase in metastatic capacity of colon cancer cells. *L. reuteri* Engevik found that mice colonized with L. reuteri had decreased mucin levels.

Limitations

The limitations of each study have been detailed, where applicable in the results section above. For example, in Gao et al.'s 2015 study, even though it concluded that *L. reuteri* reduced the number and size of colonic tumors, it is important to note that they examined only certain strains of the bacterium and this could limit its applicability to only the specific strains that were used. As for Gao et al. 2017, the study only used four separate mouse pups, two males and two females, which is not a sufficient sample size.

For this review, considering that studies in languages other than English were excluded and that studies included in the review ought to have been published recently narrowed down the amount of data and pool of resources available to help support or disprove the hypothesis of this study. Also, the use of only free-access publications available on the MEDLINE database further reduced the pool of data that the researchers could have access to in order to strengthen the case for *L. reuteri's* use as a critical probiotic. These researchers are of the view that more research is needed to further investigate *L. reuteri* in the treatment of CRC in humans. More funding should be made available, and the body of knowledge about this promising probiotic further explored.

Conclusion

In closing, the findings of this review support the hypothesis that states, "*Lactobacillus reuteri* facilitates in the prevention of colorectal cancer through inhibition of tumor progression in animal models." Results in various studies above show that *L. reuteri* is critical in the inhibition of tumor progression through its ability to halt the progression of tumors and to decrease inflammation, which would otherwise progress to uncontrolled growth and metastasis.

This research helps expand the body of knowledge on a topic that is heavily underresearched considering the encouraging outcomes thus far. Probiotics are a promising healthcare resource. In this case, we recommend that further research be pursued to investigate *L. reuteri's* capacity to decrease susceptibility to tumors and to decrease immune response and inflammatory mediators like TNF-α in the human form of CRC. For instance, we recommend that studies in the future focus on how *L. reuteri* interferes with the binding of selectins to mucin. This finding shows a promising breakthrough in CRC management, especially when it comes to therapeutic and prophylactic treatments.[23] Probiotics could eventually play a significant role in cancer treatment, possibly as adjuvant therapies or as mono-therapies.

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