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Lactobacilluscaseias Prebiotics

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Abstract: ***The Lactobacillus casei (LCG) group, composed of the closely related Lactobacillus casei, Lactobacillus paracasei, and Lactobacillus rhamnosus are some of the most studied and used probiotic species of lactobacilli. The group is always as interested as probiotics, and their use is widespread in the industry. Many studies have focused on recent years in their application for health promotion in the treatment or prevention of a number of diseases and disorders. LCG has the potential to be used in the prevention and treatment of diseases associated with gut microbiota disorders. This group has been extensively studied in terms of stress responses, which are essential for survival and therefore act as probiotics. Recent and progressive advances in microbiome science are empowering new research limitations for probiotics and prebiotics. The types of novels, methods, and applications currently being researched have the potential to change the scientific understanding and healthy eating and health care of these interventions. The expansion of the related fields of microbiome-directed interventions, as well as the changing global environment for use in all spheres of government, policy, regulations, and consumers, marks a period of great change. The recent increase in interest in probiotics among physicians has many causes, especially concerns about it limits the current armamentarium of pharmacy agents. Although probiotics have been widely used in dietary supplements and diets to maintain health, science and clinical studies are recognizing the potency of other probiotics has been in therapeutic activity. In this review we review the latest, health-related research on LCG and its benefits.***

Keywords: Microbiota, LCG, Psychobiotic, Heterofermentative

1. Introduction

There are more than 200 species of Lactobacillus, the largest and most diverse type of Lactobacillus within the lactic acid bacteria (LAB). Lactobacillus spp. they are part of the microbiota of humans and animals where they collect gastrointestinal tract (GIT) and urogenital tract. They are also found in a variety of food products from fruits and vegetables to a range of naturally fermented products (Minervini & Calasso, 2022). Lactobacillus spp. used and taught extensively as early fermentation cultures and as probiotics. Their long history of use in fermented products led to their being recognized as GRAS (commonly recognized as safe) by the US Food and Drug Authority (FDA) (Qin et al., 2021).

Lactobacillus species are probiotics ("good" bacteria) commonly found in digestive tract and urine. They can be used for diarrhea and "intestinal health". “Good” bacteria like Lactobacillus can help the body break down food, absorb nutrients, and fight off “bad” organisms that can cause disease. Lactobacillus is sometimes added to yoghurt foods and is also found in food additives (Mörschbächer & Granada, 2022).

Lactobacillus is commonly used for diarrhea, which includes diarrhea and diarrhea in people taking antibiotics. Some people use lactobacillus for digestive problems, irritable bowel syndrome (IBS), colic in infants, and many other conditions involving the stomach and intestines (B. Yang et al., 2019). But there is no solid scientific evidence supporting the use of this term. There is also no good evidence to support the use of COVID-19 lactobacillus (B. Yang et al., 2021).

The Lactobacillus casei (LCG) group, composed mainly of Lactobacillus casei, Lactobacillus paracasei, and Lactobacillus rhamnosus species, are among the most widely studied species due to their commercial, industrial and health potential. Commercially, they are used to ferment dairy products, often producing improved flavor and texture foods (Farzad Rahmati, 2017). They have also been found to produce many bioactive metabolites that can provide digestive benefits when consumed. Therefore, many types of LCG are considered probiotics. One member, L. rhamnosus GG (LGG), is probably one of the most studied bacterial species in terms of health applications (Karami et al., 2017).

Awareness about certain type of bacteria has been providing benefits to the human body from many years ago. This has led to the term probiotics, which are defined as living microorganisms that when controlled in sufficient quantities, give the host health benefits. The introduction of live germs into humans primarily involves food or a form of oral supplement (Jeong et al., 2022). In most countries, when making standard and non-infectious applications, the product does not need to be registered as a drug. This has allowed food companies and small and medium enterprises to enter the rapidly growing market for probiotics, where genetics are a fraction of those in the pharmaceutical industry. The perceived failure to protect the intellectual property surrounding probiotic applications, has so far maintained a major pharmacy in the production of probiotic drugs (Bull et al., 2013a).

The overall effect is that probiotics have been scientifically and clinically prescribed, have been thought of as dietary and supplemental applications. In addition, they are microbial species with a history of safe use, which is also due to their inclusion in various foods (J Nowroozi, M Mirzaii, 2004).

Taxonomic History

LCG is composed of three genotypically and phenotypically related species facultatively heterofermentative, L. casei, L. paracasei, and L. rhamnosus. The taxonomy of these species has been extensively investigated in the past, as many species within the group are often used as original cultures and probiotics (Skerman et al., 1980). As LCG species are highly commercially viable, their relationships have become the subject of much research. It is important to emphasize that differentiation is important to ensure proper literature review and to avoid confusion between the related types of probiotic in the diet and health-related products. However, the taxonomic history of LCG has been both controversial and challenging (Bull et al., 2013b).

Lactobacillus casei was first proposed as a novelty in 1971, but this description of the characters was first questioned in 1996 (Salvetti et al., 2012). It was proposed that the type L. casei ssp. casei ATCC 393 and L. rhamnosus ATCC 15820 should be repositioned as L. zeae described earlier, due to the high similarity with each other and the diversity of the entire LCG collection. The name L. paracasei was rejected, and L. casei ATCC 334 was proposed as a new type of case (Candra et al., 2021). Further research has continued to question the phylogenetic relationships between animal species using highly refined methods such as comparant sequence analysis of recA and 23S-5S rRNA intergenic spacer regions. In 2002, it was suggested that the brand nominate L. casei ssp. casei ATCC 393 should actually be replaced as L. zeae and that L. paracasei strains should be rearranged below L. casei. In 2008, type L. casei was confirmed to be the first version of ATCC 393, and the designated version of ATCC 334 was rejected as it represented a separate tax, L. paracasei (Sanders et al., 2018).

L. Casei and Health Related Research

LCG contains many species with proven probiotic activity. Probiotics are defined as living microorganisms that, when used in sufficient quantities, provide the host with health benefits. Bacteria with related health benefits have been researched since that daily consumption of fermented milk products had a beneficial effect on human health (Kechagia et al., 2013). This research has been growing steadily over the past 100 years, with significant improvements in linking health benefits to probiotic use. The activity of these species has improved beyond the use of these bacteria in fermented dairy products, or even boiled foods in general, to a wide range of technological and medical purposes. As ways to improve their health skills are being addressed, potential applications of these types are being developed in the field of nutrition, biotechnology and medicine (Lew et al., 2019). This provides evidence for a future novelty of non-probiotic diets and therapeutic potential based on specific pathogens and diseases. Some of the promising LCG applications in these fields will be discussed below. The ability of a number of these species to help maintain a healthy microbiota may provide non-invasive treatments for a range of problems. They can be grown prophylactically or with treatment for many diseases related to gut microbiota disorders (Nagpal et al., 2012).

There is great potential in the areas of active novel nutrition and medicine found in LCG. However, it should be noted that although many of these strains have been shown to have an effect on in vitro or in vivo mouse models, the basic methods require further research on any future use as microbial therapies (Ranjha et al., 2021).

LCG-related health benefits have been reported for a variety of health conditions, from atopic dermatitis to cancer. The mechanisms by which these viruses directly or indirectly have a beneficial effect on human health are not yet fully understood and require further research (Kirstin Hendrickson, 2022). Possible mechanisms include the production of antibacterial agents such as bacteriocins, enhancing the epithelial barrier by attachment, competition for pathogenic binding sites, or immune modification (Claesson et al., 2007).

Allergic Diseases

Childhood asthma rates are rising which has been linked to the concept of hygiene. Essentially, this suggests that children get fewer infections at an early age due to better hygiene, in addition to antibiotic exposure, congenital malformations, and dietary changes (Asher et al., 2006). These factors influence the development of the gut microbiota and its renewal of the immune system in childhood (Huang et al., 2022). However, with the evolution of gut microbiota, probiotics have been shown to reduce the risk of developing allergic reactions such as atopic dermatitis (Van Der Heijden et al., 2008). For example, the risk of developing allergies has been shown to be reduced when you are 5 years old with a colony of premature health with LCG in children of both parents who are allergic to certain substances (Yavuz et al., 2016). The use of probiotics such as LGG has been investigated for its potential protective effects in children at high risk of developing allergic reactions such as asthma and atopic dermatitis (Slattery et al., 2019). However, this study was recently repeated and found to be ineffective in preventing this allergy in 2 years (Hilty et al., 2010). Recent meta-analyzes show that there are confusing results in this area (L. Li et al., 2020). Although it is important to note that this analysis includes studies that use a wide variety of species and varieties, it was concluded that the total data show benefits in probiotic use, although this depends on many factors other than the specific type used in the study (Björkstén et al., 2001).

Brain Function

'Psychobiotic' is a bacterium that when used in sufficient amounts have good mental health benefits. LCG members are involved in a number of important courses in this area (Dr. Willem de Vos, 2008). In another study focusing on the brain-gut-axis type L. rhamnosus JB1 highlighted both the ability of bacteria to positively affect brain function and the importance of the vagus nerve in dual communication between the intestinal tract and the brain (De Angelis et al., 2014). Administration of JB1 directly affected the expression of GABA receptors in the brain which led to a reduction in anxiety and depressive behavior in mice. The study included healthy animals in different study areas with the same effect (Di Cerbo et al., 2016).

In a recent study, the preparation of the LGG tablet with Bifidobacteriumlactis Bb12 as a complementary therapy in hospitalized patients with dementia was effective in lowering hospital admission rates (Salami, 2021). Although it is important to note that this study had a number of limitations; the direct probiotic effect on the microbiome and CNS inflammation was not measured, and the medication taken by the staff was specific to the patient. It highlights the possibilities of microbial treatment for mental disorders ensuring more investment and learning (Kim et al., 2020).

The combination of the L combination. rhamnosus R0011 and L. helveticus R0052 and highlights the ability of bacteria to influence behavior in a mouse model. Studies have shown that mutations in the gut microbiota can have a positive effect on the behavior of certain animals, in this case memory disturbance caused by stress. The normal formation of gut microbiota by absorbing this probiotic compound has helped to prevent abnormal behavior caused by infection with Citrobacterrodentium (Leclercq et al., 2017).

Obesity

Obesity is a complex syndrome that has many adverse effects that trigger onset. These include diet, physical activity and genetic factors, but can also be influenced by the formation of gut microbiota. Exploitation of microbiota through the administration of probiotics is an area that can provide interventions to prevent and treat obesity (López-Moreno et al., 2020). In a recent study, probiotic L. rhamnosus CGMCC1.3724 was given to obese men and women 24 weeks. Men did not show significant differences in weight loss, but female participants in the probiotic group had significant weight loss (Mazloom et al., 2019). This was found to be associated with a decrease in blood leptin concentration. Obesity is associated with changes in the gut microbiota and body limits. Boiled milk containing L. The mouse-fed casei CRL 431 has been able to have a positive effect on microbiota and other obesity-related biological symptoms (Oh et al., 2015).

In the murine figure of the fat L. caseiShirota was tested for its ability to improve weight management compared to Orlistat, a drug used to treat obesity. The results of this study showed promise in the use of probiotic species as an alternative to weight management (Rosing et al., 2017). The impact of exposure to probiotics on weight gain before birth has also been investigated. L. rhamnosus given to pregnant women has been found to alter childhood obesity during the first 6 months of life (Chung et al., 2016).

Cancer

Cancer is a global problem with great efforts being devoted to preventing and / or treating the disease. Cancer is an abnormal growth of cells beyond their normal limits that can invade the connective tissue and / or spread to other organs. Probiotics have been investigated for their potential as complementary therapies and for the treatment of cancer cells (Z. P. Li et al., 2021).

Type L. casei previously mentioned type ATCC 393 was investigated in a screening model for colon cancer using both murine cell lines and human colon carcinoma. The in vivo model showed a approximately 80% reduction in tumor volume in live L-fed mice. casei 13 days (Spangler et al., 2021). Bacteria attach to cancer cells and reduce the activity of cancer cells and cause apoptotic cell death. Although the mechanisms are not fully understood, this provides promising evidence for the use of L. casei in the treatment of cancer (Chee et al., 2020). Types of L. casei have also been investigated for prophylactic treatment in the latest stage of colorectal cancer (CRC). The main cause of death is CRC tumor metastasis in other organs. A non-cellular supernatant from L. casei and L. rhamnosus has been found to reduce the ability of the metastatic tumor cell line to invade in vitro (Tukenmez et al., 2019).

Numerous studies have explored techniques for how probiotic species can be used to help reduce tumor. L. casei in combination with dietary fiber due to its ability to reduce plant recurrence in colorectal cancer, with earlier studies showing promising results. LGG has also been investigated as a synbiotic containing oligofructose and Bifidobacteriumlactis Bb12 (Ilhan et al., 2019). This promising study showed that synbiotic was able to reduce the uncontrolled growth of intestinal cells. This is thought to be due to the presence of a sybiotic that improves mucosal formation, reduces exposure to epithelial cells to cytotoxic and genotoxic agents, and decreases the growth of colon cells (Ilhan et al., 2019).

Remedies for cancer patients can place great strain on the immune system and can cause serious side effects, such as diarrhea, which can be fatal in a person with a weakened immune system. The proven probiotics reduce the risk of radiation-induced diarrhea during cancer treatment (Wang et al., 2022). LGG has also been considered as a complementary therapeutic agent for cancer treatment to reduce the incidence and severity of diarrhea. It was shown that additional treatment with LGG pills caused diarrhea less than 3/4 grade, with less abdominal discomfort reported (X. Yang et al., 2018).

Diarrhea

According to the WHO, diarrhea is the second leading cause of death among children under 5, and it remains a major problem associated with antibiotics. Clostridium antibiotic-related infections are the leading cause of diarrhea in high-income countries in all ages (Ruiz-Palacios et al., 2006). LCG variants have been associated with the development of symptoms and / or the duration of diarrhea in many studies, but in particular LGG is a promising type in this regard. The ability of many types of L. casei prevention of antibiotic-related diarrhea is associated with its ability to maintain diversity of the human gut microbiome during antibiotic treatment. This may be due to the direct adhesion of stress to epithelial cells. LGG get attaches to the mucosal cells of the body through the SpaCBApilus. SpaCadhesin is available for all lengths of pilus in large quantities (Wilkins et al., 2017). This adhesin allows for both long-term and close adhesion of bacteria and resources for long-term strain accumulation. Vancomycin-resistant enterococci, like Enterococcus faecium also bind and bind to intestinal-based intestines with the same high sequence as the LGG SpaCBApilus. LGG can be used prophylactically or as a direct competitor in the treatment of enterococcal infections (Guandalini, 2011).

Stress

Like probiotics the LCG must withstand a great deal of stress during processing and remain active as it passes through its working environment, the GIT. These pressures include but are not limited to bile salts, oxidative stress, cold stress, osmotic stress, acid stress and long-term storage (Takada et al., 2016). Resistance to these pressures in the LCG depends on the problem, with some having high resistance to multiple pressures while others provide little resistance. LCG uses many different strategies to survive these pressures, including cell membrane modification, metabolic pathways, and control of chaperone proteins (Hosseini Nezhad et al., 2015a).

Due to the natural LCG nature of the heterofermentative LCG, hexose sugar is almost fermented into lactic acid. LCG has a natural tolerance to acid stress for this reason. Probiotic bacteria also have to deal with an acidic environment during bowel movement. Tolerance to acid pressure is very important in the probiotic bacteria industry. In acidic environments, acids can slowly disperse into cells where they can rapidly degrade into protons (Takada et al., 2017). This lowers the intracellular pH (pHi) and affects the transmembrane pH gradient and the proton motive power. Low pH also causes damage to proteins and DNA. Methods used to combat acid stress in L. Casei include Arginine deiminase, F0F1 proton pump method, cell membrane modification and repair of damaged DNA and proteins. The use of amino acids also plays a physiological role in regulating pH (Wu et al., 2012b).

Bacteria also adapt to natural changes (pressures) by modifying their membranes. L. Casei Zhang-tolerant acid can increase the expression of the protein MurA and MurG, enzymes essential for the synthesis of peptidoglycan. It has been shown that bacteria alter the fatty acid structure of the cytoplasmic membrane in response to acid conditions but different effects have also been reported. These conflicting results may be due to the use of different types or different test conditions (Hathout et al., 2011). In response to DNA damage, there is an overproduction of DNA repair proteins involved in repairing base extraction, nucleotide remodeling, differentiation, and homologous reunification. Proteins involved in normal stress response (DnaK, DnaJ and Hspl) and chaperone proteins (GroEL, GrpE) are also over-reported. These proteins are involved in preventing improper protein wrapping and repairing damaged proteins at the expense of ATP (Alcántara et al., 2011). Most cell reactions to acid stress depend on energy and therefore require ATP. The activity levels of the glucose-phosphotransferase system (PTS) are significantly lower in stress-containing cells, which were higher than those in sensitive cells. This means that more glucose goes into glycolysis and more energy (ATP) for the cell to respond to acid stress (Puya Yazdi, 2021).

After fermentation, the probiotic LCG will deal with cold stress in the form of a refrigerator. Cold storage survival is necessary for the probiotic bacteria to be refrigerated to reach their final destination in the human GIT. Cold is a physical stress that affects the physico-chemical properties within cells. It does this by influencing membrane fluidity, distribution levels and interactions of macromolecules such as proteins, DNA and RNA (Zotta et al., 2017). Cold shock proteins (CSPs) are produced by bacteria in response to growth at temperatures below ideal conditions. CspA and CspB in L. casei has a similar sequence of C-terminal EIIA proteins of the glucose-PTS system. It has been suggested that Hpr phosphorylates these cold proteins to activate them in response to cold shock (Strandwitz, 2018). L. casei-capable Hpr ser-45 were more sensitive to freezing / soluble glucose compared to the growth of other sugars. This suggests that CSPs play a key role in metabolism at low temperatures. Many cold-blooded proteins (CIPs) are expressed in cold shock to maintain the fluid of the cell membrane by increasing the amount of short and / or incomplete fatty acids and maintaining DNA formation by reducing excessive negative stress (Hill et al., 2018).

Bile acids (sometimes called salts) are sent to the intestines of mammals to aid digestion. They also provide another barrier to bacteria as they have antimicrobial activity. Bile is known to affect the formation of membranes, DNA, RNA and protein binding using a proteomic method of testing the tolerance of bile in the L. casei also elevated RMlC membrane modification protein, cell wall synthesis protein NagA and NagB, molecular chaperone protein ClpP and protein. involved in central metabolism was the key to bile tolerance (Reale et al., 2015). Bile salt hydrolase (BSH) is a protein produced by bacteria found in the intestinal tract that can eliminate bile salts and have a more positive effect on lowering serum cholesterol levels and reducing the risk of obesity and arthrosclerosis in men. BSH activity found in L. casei but you are not always there. There is conflicting evidence as to whether BSH increases bile tolerance to many studies that find a link while many others fail to find a link between BSH activity and bile tolerance (Hosseini Nezhad et al., 2015b).

Oxidative stress means the production of Reactive Oxygen Species (ROS) and cell accumulation. ROS comprises superoxide (O2 -), hydrogen peroxide (H2O2), and hydroxyl radical (OH). ROS can lead to the depletion of proteins, lipids, and nucleotides, which contribute to the binding of cell growth and cell death. Another LAB, which includes L. casei, can use oxygen in aerobic conditions to produce energy using a process that includes pyruvate oxidase, NADH oxidase, NADH peroxidase and acetone kinase, which leads to the formation of ROS (Wu et al., 2012a). Catalase is an enzyme that can degrade H2O2 and is therefore an important factor in oxidative stress. The presence of oxygen shows the increase in activity of L. casei. High Mn content in L. casei can act as an active scavenger of O2, thus compensating for the depletion of superoxide dismutase, whereas currently there are no known enzymes to reduce hydroxyl radicals (Amira et al., 2019).

High osmolality is often associated with LCG in ripe foods. Digestive parasites and viruses are often more sensitive to high osmotic conditions compared to LAB, so NaCl is often added to aid with native LAB or initiation during the fermentation process (Gul & Atalar, 2018). The salt pressure leads to a change in the cell wall alignment to L. casei, which increases its ability to make biofilms and bind cation. Elevated salts cause L. casei to be easily found in antimicrobial peptides that regulate cell wall formation (X. Li et al., 2021).

Immunity

L. casei promotes the recovery of immunosuppression that is being caused by chemotherapeutic agents in animal model mice, by activating the natural killer (NK) cells, cytotoxic T cells and macrophages. These are all white blood cells that recognize and eliminate tumor cells and infected cells (Guandalini, 2011).

Dietary Factors

Prebiotics are indigestible substances that exert beneficial physiological effects on the host by selectively stimulating the beneficial growth or activity of a limited number of native bacteria. Ancient people ate many prebiotic sources (such as plant roots) and used fermentation as a means of preserving and cooking food (Terpou A., et al 2019). To provide probiotic or prebiotic products or to develop new foods to intentionally and specifically alter the gut microbiota to reduce the incidence of chronic diseases, prebiotics or fermentation A maternal diet supplemented with food and plant roots may be required (Hori T, Matsuda K., et al, 2020). Alters milk levels of lactobacilli and other species. Also, such diets can be tested during weaning to see if they alter the gut microbiome or susceptibility to asthma or allergies. It is impossible to know exactly what the Paleolithic diet was and whether it was beneficial to the health of babies. However, while diet and microbes influence fundamental aspects of immunity and development, how to acquire bacteria and which ones need to be supplemented to achieve good health in the short and long term. Little is known. Over time, new commensal products (i. e., mixtures of probiotics and prebiotics) will be developed that, given the existence of such microbiota, may contribute to the acquisition of a healthy gut microbiota (RavinderNagpal, Ashwani Kumar., et al, 2012).

Infections

L. casei stimulated the acquisition of immune defenses caused by chemotherapeutic agents in mice, activating natural killer cells (NK), cytotoxic T cells and macrophages. All of these white blood cells detect and destroy tumor cells and infected cells (Castro-González et al., 2019). Respiratory and Intestinal Infections Although some studies have found no evidence that eating L. casei protects respiratory symptoms, many others found that L. casei was beneficial in both respiratory and intestinal diseases (Kalliomäki et al., 2001). L. casei significantly reduced the incidence and duration of respiratory tract infections (URTIs) in medically healthy office workers (Arshad et al., 2010). Similarly, for healthy shift workers, L. casei reduced the incidence of tuberculosis of the stomach and common respiratory infections (CID), increased the time to the onset of CID, and reduced the total number of CIDs in a small group of smokers. With the advent of CID, the duration of the temperature was lower and an increase in leukocyte, neutrophil, and natural killer cells (NK) and activity (Antoun et al., 2020).

L. casei also reduced the incidence of common infectious diseases (CID) in children, reduced the duration of CID, and significantly reduced respiratory infections (URTI) in adults such as rhinopharyngitis in adults (Nieto-Martinez et al., 2021). For men and women athletes who engage in winter-based exercise activities, L. casei reduced the proportion of subjects who experienced 1 week or more with symptoms of high respiratory infections (URTI) and reduced the number of episodes of URTI. The administration of probiotic L. casei in combination with albendazole reduces Giardia infection and improved recovery in mice (Salminen et al., 2004).

2. Conclusion and Future Perspective

Current technological advances and methodologies offer exciting research opportunities for probiotics and prebiotics and applications. New tools that allow real-time lessons for humans and tracking the microbe as it integrates into existing microbiota, as well as programs that can measure health standards, will further this field. A study of what viruses are present, their interaction with the host and the impact of environmental factors (e. g., drugs, nutrients) will be the standard for future physical examinations.

Sample novel systems will determine how a used probiotic or prebiotic interacts with the host at various levels, including the immune system, metabolism and all components of the microbiome. Ultimately, an integrated approach will support your specific type of medication to establish a dose-response relationship for treatment, but even more so try to direct what goes into our system, how it is processed, and which probiotics or prebiotics deliver the best desired results. The mechanical details in the active atoms will pave the way for emerging ideas, such as postbiotics.

LCG contains type L. rhamnosus, L. casei, and L. paracasei; these have been well researched because of their effectiveness in the food, biopharmaceutical and medical industries. Despite this, the group has a long taxonomic history of distinguishing and discriminating animal species from each other. The taxonomic debate surrounding the LCG is likely to continue to be interesting and challenging as new adherence to new diagnostic approaches is developed.

Stress resistance is associated with LCG through physiological and metabolic modification; although these phenotypes are resistant to some form. LCG must be able to survive many stressful situations when used in the industry, including oxidative stress, osmotic stress, cold pressure, acid stress and long-term storage. In addition to their need to tolerate these conditions in food processing, the living species must be able to survive when it passes through GIT if it is intended for health improvement purposes.

LCG health-promoting skills have been documented in numerous studies that highlight the real potential for their use in the treatment, or prevention, of various diseases. Going forward, it is important for scientists to define the basic mechanisms involved in being able to use these types or components of their bacteria as a novel treatment or prophylactic intervention.

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