Journal of Pharmacy and Pharmacology 7 (2019) 177-186
doi: 10.17265/2328-2150/2019.04.004

Wild-Type and Genetically Improved Strains of Dairy Origin Probiotic as Potential Treatments for Intestinal Mucositis

Flávia Figueira Aburjaile[1,2], Gwénaël Jan[3], Yves Le Loir[3], Vasco Azevedo[1] and Rodrigo Dias de Oliveira Carvalho[1]

1. Molecular and Cellular Laboratory, General Biology Department, Institute of Biological Sciences, Federal University of Minas Gerais, CEP 31270-901, Belo Horizonte, MG, Brazil

2. Laboratory of Genetics and Plant Biotechnology, Genetics Department, CCB, Federal University of Pernambuco, Avenida Prof. Moraes Rego, s/n., CEP 50.732-970, Recife, PE, Brazil

3. Laboratoire Science & Technologie du Lait & de l'Oeuf (STLO), Institut National de la Recherche Agronomique (INRA), Agrocampus Ouest, CP 35000, Rennes, France

Abstract: The use of probiotic bacteria derived from fermented foods has been explored by the scientific community as alternative strategies for the treatment of several diseases, mainly regarding intestinal dysfunction. One of the most relevant inflammatory diseases affecting the alimentary tract, for which no current intervention is entirely successful, is mucositis. In this review article, we summarize the most recent proof-of-concept studies dealing with the therapeutic use of dairy origin probiotics, for the treatment of gastrointestinal mucositis. Furthermore, we discuss several approaches for the improvement of the classical therapeutic rationale, such as supplementation with prebiotics and genetic engineering along with the respective translational issues, which may be crucial for the successful transposition of these therapeutic strategies for clinical use.

Key words: Gastrointestinal tract, chemotherapy, inflammation, probiotics.

1. Introduction

The inflammatory condition of the Gastrointestinal Tract (GIT) associated with the use of radiotherapy or chemotherapy drugs is known as mucositis. Chemical compounds such as doxorubicin, methotrexate, irinotecan and 5-fluorouracil (5-FU) are commonly used in oncology practice and widely prescribed in the treatment of several types of malignancies including gastrointestinal cancer [1]. However, these drugs also target non-malignant cells forming the mucosal epithelial tissue, which undergo rapid proliferation and replacement. For example, the analogous pyrimidine drug, 5-FU, causes cytotoxic effects through competitive inhibition of the enzyme thymidylate synthase, preventing methylation of uracil to thymine and therefore inhibiting (Deoxyribonucleic Acid) DNA replication.

Furthermore, the insertion of 5-FU in (Ribonucleic Acid) RNA molecules may also compromise several cellular processes depending on proteins, enzymes, coenzymes and ribosomes neosynthesis. Studies estimate that 60-100% of the patients submitted to chemotherapy will develop gastrointestinal mucositis symptoms such as vomiting, abdominal pain, diarrhea and weight loss [2, 3]. In addition to a reduced quality of life, the occurrence of more aggravating clinical states, characterized by sepsis, may threaten the patient's life [4].

Corresponding author: Rodrigo Dias de Oliveira Carvalho, Ph.D., research fields: bacteria, mucositis, probiotics. Email: rodrigodoc2@gmail.com.

In the pathogenesis of mucositis, reactive oxygen species (ROS) are formed as a consequence of radiotherapy/chemotherapy in normal enterocytes. They trigger pro-apoptotic and pro-inflammatory responses. Intense apoptosis of the intestinal crypt cells results in the disturbance of the epithelial barrier function and in the exposition of the mucosa to opportunistic commensal microbes, which may, in turn, induce ulcerative inflammation [2].

In fact, the scientific community has intensively debated the involvement of the intestinal microbiota in the development of mucositis in recent years. Previous studies have demonstrated that the microbiota composition was altered by 5-FU or irinotecan treatment [5–8]. Indeed, 5-FU injection caused a decrease in *Clostridium* spp., *Lactobacillus* spp. and *Streptococcus* spp., commensals that are more prevalent in the GIT of healthy individuals. It also increased sulfate-reducing bacteria such as *Desulfovibrio* spp. and opportunistic *Enterobacteriaceae* [5, 9]. Certain species of opportunistic bacteria may colonize essential niches, thus favoring the pathogenesis process [10]. For instance, the increased intestinal permeability caused by the medicaments has been associated with a bloom of commensals bacteria presenting pathogenic traits, such as *Enterococcus faecalis*.

Currently, no intervention is entirely successful in the prevention of mucositis [2]. Usually, treatment is based on local anesthetics, analgesics and/or antibiotics. The use of local anesthetics to control the symptoms of the mucositis is questioned, as their effect is short, affects the taste and reduces the flow of saliva. This, in turn, decreases food intake [11]. Broad-spectrum antibiotics (i.e., tobramycin and amphotericin B) have been associated with side effects such as abdominal pain, altered taste and abnormal dental pigmentation. Furthermore, these antibiotics may also lead to GI dysbiosis altering microbiota composition. Thus, the prolonged use of antibiotics is not indicated [7].

Several research groups have been seeking effective treatments of intestinal mucositis. As dysbiosis is involved in mucositis pathogenesis, an alternative rationale has been proposed. It relies on the administration of microbes (see Fig. 1) able to induce an anti-inflammatory response and to occupy key niches of the GIT [12, 13].

2. The Dairy Origin Probiotics Therapeutic Rationale for Treating Intestinal Disorders

Although the benefits of some fermented foods in health were studied many years ago, by Metchnikoff

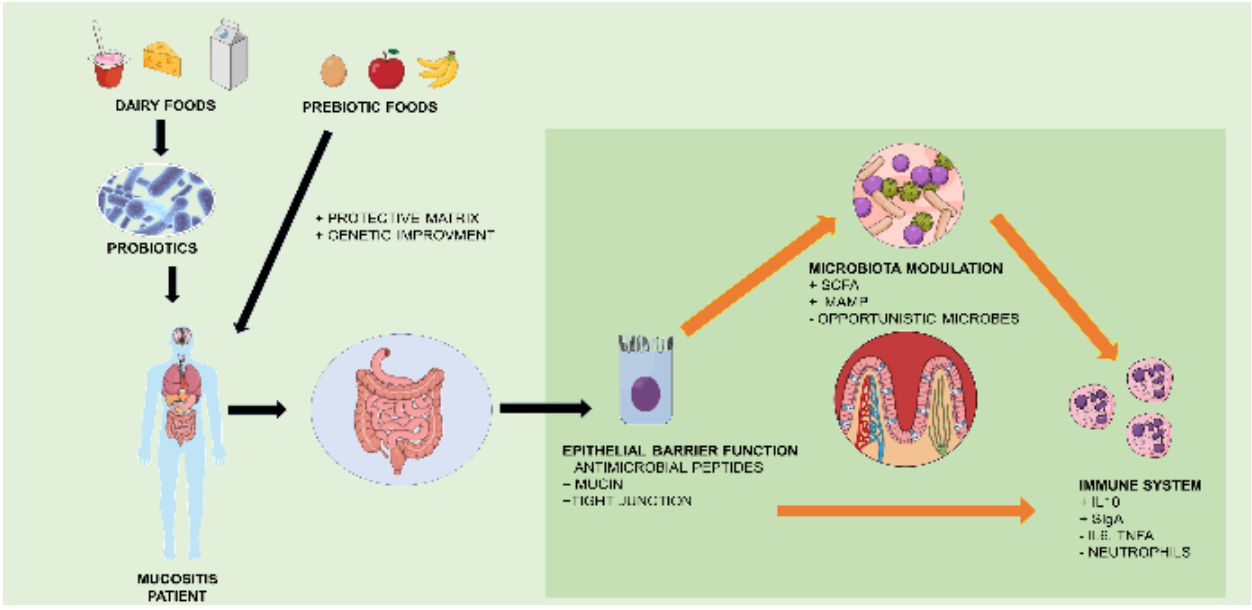

Fig. 1 Probiotics therapeutic rationale (Figure created in the Mind the Graph platform: www.mindthegraph.com).

studies at the beginning of the twentieth century, the term probiotic originated in 1965, when Lilly and Stillwell associated the survival of certain microorganisms with the beneficial effect of dairy origin probiotics administration [14]. In 2001, the World Health Organization conceptualized that the probiotics as "live microorganisms administered in adequate amounts that confer a beneficial health effect on the host" [15]. Currently, this broad concept has been revisited by the scientific community because several commercialized formulations have not shown to confer benefits to health in adequate controlled studies [16].

Many probiotics have been described in the literature, such as Gram-positive bacteria (e.g., *Lactobacillus* spp. and *Bifidobacterium* spp.) and yeasts (e.g., *Saccharomyces* spp.) [17]. Regarding Gram-negative probiotic strains, the *E. coli* Nissle 1917 has revealed a benefic potential in human studies [18]. These microorganisms have been extensively explored as they can transiently colonize the human GIT. More recently, some Archaea commensal species have also been pointed out as candidates for promoting gut health [19]. However, an increasing number of studies have shown strain-specific beneficial properties of allochthonous dairy species such as *Lactococcus lactis* and *Propionibacterium freudenreichii*. Moreover, the majority of dairy origin probiotic strains granted the Generally Regarded as Safe (GRAS) status by the Food and Drug Administration Agency (FDA) and promote the improvement of human health [20].

A limiting factor for the use of probiotics relies on the strains capacity to tolerate different environmental stresses during storage and transition through the GIT [21, 22].

For a probiotic to perform its function, it is necessary to maintain its metabolic activity and its survival within the GIT. The molecular characterization of essential genes implicated in stress adaptive responses has been of extreme importance for screening potential probiotic strains [23].

2.1 Mechanism of Interaction between Dairy Origin Probiotics and the Host

Dairy strains of bacteria can modulate several essential factors involved in intestinal homeostasis of the host during mucositis [12]. Some bacterial species, such as *L. paracasei*, *L. johnsonii*, *L. fermentum*, *L. plantarum*, *L. rhamnosus* and *P. freudenreichii* are currently considered because of their ability to promote homeostasis of intestinal microbiota [24, 25].

Selected strains of probiotics can promote beneficial effects when used in the treatment and prevention of the intestinal radiotherapy/chemotherapy induced inflammation. This includes (I) modulation of immune signaling molecules involved in mucositis pathogenesis, (II) maintenance of mucus barrier and of intestinal permeability, (III) competitive exclusion of opportunistic pathogenic bacteria and (IV) prevention of enterocytes apoptosis and oxidative damage [13, 26].

The modulation of the intestinal microbiota opens new avenues in the context of gastrointestinal pathologies. Several studies also seek a better understanding of these microbial communities and their modulation effects on the immune system [28]. Dairy probiotics, like propionibacteria, may exert effects on the microbiota of the host, either by inhibiting undesirable microorganisms and/or by increasing the proliferation of regulatory commensals as *Bifidobacterium* spp. [27]. The restoration of *Lactobacillus* spp. population also seems to be relevant in the context of 5-FU induced mucositis. Indeed, Florez and colleagues [28] suggest that lactobacilli are more susceptible to 5-FU effects than other intestinal bacteria.

Loss of intestinal epithelial barrier function is an aggravating factor for mucositis patients, as it may lead to increased epithelial permeability, and thus to translocation of intestinal bacteria to the systemic circulation [29]. Therefore, stimulation of expression of epithelial wall factors is another way in which dairy

origin probiotics interact with the host cells, providing defensive mechanisms against harmful invasive bacteria, which can worsen the prognostic of mucositis. Several studies demonstrated that bacterial cells could activate the expression of defensins by Paneth cells, mucins by goblet cells or proteins that constitute tight junctions of intestinal epithelial cells such as claudins and occludins [30-32].

Immunomodulation is an essential property of probiotic bacteria. It allows the induction and regulation of immune responses, modulation of inflammatory diseases and control of undesirable microorganisms [26, 33]. In mucositis, the inhibition of the NF-kB activation is one of the most important effects that bacterial probiotics may exert on host cells. As intestinal mucositis initial stage consists of acute innate immune responses, hampering of the NF-kB pathway limits downstream induction of pro-inflammatory cytokines [34, 35]. For instance, *L. rhamnosus* inhibits TNF-alpha-induced secretion of IL-8 [36].

Moreover, *L. rhamnosus* GG supernatant limits 5-FU induced apoptosis in IEC-6 rat intestinal epithelial cells, by reducing the expression of caspase 3 and 7 [37]. As the epithelial barrier is disrupted by the primary effects of mucositis, such as apoptosis, some studies suggest that translocated bacterial products could serve as sensitizing antigens to create secondary adaptive immune responses against the commensal microbiota colonizing the mucosa [29]. In this context, some dairy origin lactobacilli have also been described to stimulate the production of anti-inflammatory cytokines, like IL-10, involved in immunological tolerance to intestinal microbes antigens [12, 25].

2.2 Use of Dairy Origin Probiotic Strains to Contain Mucositis

Dairy origin probiotic strains gave encouraging results in animal models of intestinal mucositis (see Table 1). *L. fermentum* BR11, in a mice model of 5-FU induced mucositis, reduced myeloperoxidase enzyme activity and improved jejunal inflammation [40]. Moreover, experiments in rats exposed to irinotecan revealed the therapeutic effect of an association of several *Lactobacillus* spp. Strains's consumption reduced diarrhea and inhibited apoptosis of small bowel mucosal crypts [38].

Similarly, live and dead cells of the probiotic *S. thermophilus* TH4, as well as its culture supernatant content, prevent 5-FU induced damages in rodents [39].

Table 1 Beneficial microbes in the treatment of intestinal mucositis.

Probiotic effect in intestinal mucositis	Probiotics/synbiotic	References
Prevented the degeneration of goblet cells, weight loss and mucosal damage	*Lactobacillus casei* (BL23) and *Propionibacterium freudenreichii* (138)	[41]
Decrease mucosal damage and intestinal permeability caused by mucositis; Increased butyrate concentration	Synbiotic (Simbioflora®): *Lactobacillus paracasei* (LPC-31), *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (NCFM) and *Bifidobacterium lactis* (HN019) + fructooligosaccharide	[47]
Reduced the severity of diarrhea and intestinal mucositis in colorectal cancer-bearing mice. Decreased TNF-a and IL-6 expression. Intestinal microbiota composition of Firmicutes and Bacteroidetes was restored	*Lactobacillus casei* variety *rhamnosus* (Lcr35)	[34]
Villous architecture improvement and stimulation of Paneth cells activity	*Lactococcus lactis* (NZ9000) + Pancreatitis-Associated Protein (PAP)	[12]
Reduction of ileum histological damage and mitigation of myeloperoxidase activity	*Lactococcus lactis* (NZ9000)	[12]
Jejunal villi architecture preservation and suppression of TNF-α, IL-1β and IL-6 mRNA expression	*Lactobacillus casei* variety *rhamnosus* (Lcr35, Antibiophilus®); *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (LaBi, Infloran®)	[44]
Reduced severity of mucositis and prevent weight loss	*Lactococcus lactis* (AG013) + Trefoil Factor I (TFF-1)	[49]
Reduced myeloperoxidase enzyme activity and improved jejunal inflammation	*Lactobacillus fermentum* (BR11)	[48]

Functional dairy beverages, fermented by lactobacilli and streptococci strains, were shown to strengthen barrier function in methotrexate-treated rats [40].

The administration of *L. casei* and *P. freudenreichii* strains, used to make yogurt and ripening of emmental cheese respectively has been proved to prevent goblet cells degeneration and avoid mucosal damage [41].

In another study, it investigated the impact of the probiotic mixture DM#1, containing *B. breve* DM8310, *L. acidophilus* DM8302, *L. casei* DM8121, and *S. thermophilus* DM8309, on the intestinal microbiota of rats, in the context of 5-FU induced mucositis. The consumption of DM#1 ameliorated the dysbiosis state caused by 5-FU, possibly by increasing the proportion of potential anti-inflammatory commensal as *Clostridium* clusters III and XIVa and *Lactobacillus* [42, 43]. Another study confirmed the efficacy of oral administration of the of probiotics *L. casei* variety *rhamnosus* or *L. acidophilus*, associated with *B. bifidum*. It evidenced an improvement of 5-FU induced intestinal mucositis in mice [44].

These results suggest that probiotic-based strategies are potentially suitable for the treatment and prevention of mucositis. However, the choice of an adequate probiotic preparation is essential. It should take into consideration the patient-specific clinical conditions and his intestinal microbiota composition, as these parameters may directly determine the success of mucositis treatment. Therefore, it is important to evaluate the limitations of each specific lineage. In this context, further preclinical and clinical studies are necessary [13, 26, 44].

2.3 Prebiotics Supplementation

Prebiotics are nondigestible dietary fibers or substances that increase the growth and/or activity of beneficial bacteria that colonize the GIT [45]. Some studies have shown the improvement of the probiotic effect of micro-organisms by their combination with prebiotics, a term defined as synbiotics [46]. *L. paracasei* jointly administered with Fructo-oligosaccharides (FOS) has been reported to regulate the balance of the intestinal microbiota of weaning piglets by significantly augmenting the population of *Lactobacillus* spp., *Bifidobacterium* spp., and also reducing *Clostridium* sp. and *Enterobacterium* sp. Interestingly, recent work revealed that a symbiotic preparation containing several strains of lactobacilli species plus FOS was able to control mucosal inflammation and increase butyrate concentration in mice experimental mucositis induced by 5-FU [47].

However, negative results were also obtained with FOS where authors suggest *L. fermentum* BR11 in combination with FOS does not confer any therapeutic benefit for the alleviation of 5-FU mucositis in rats [48]. In this context, further investigation is required to elucidate whether the synergistic anti-inflammatory effects of FOS are strain specific. Another potential candidate for the control mucositis is carboxy-methylated pachyman (CMP), a modified polysaccharide isolated from *Poria cocos* [8]. A study demonstrated CMP restored the intestinal microbiota in 5-FU treated tumor-bearing mice. As this compound supports the growth of lactobacilli species, and short chain fatty acids producing bacteria, we suggest it might have potential to improve the probiotic effects of dairy origin bacterial groups such as *Lactobacillus* and *Propionibacterium*.

2.4 Use of Genetically Improved Dairy Origin Probiotic Strains for the Treatment of Mucositis

Most of the studies involving genetic engineering of dairy origin probiotics are based on the *L. lactis*. It is indeed the most characterized lactic acid bacterium, with a large number of genetic tools available [12]. Several works using recombinant strains of *L. lactis*, producing anti-inflammatory molecules gave promising results in the context of inflammatory bowel diseases in animal models [50]. Therefore, other studies sought similar protective effect in the context of mucositis, using recombinant *L. lactis*.

Carvalho and colleagues tested *L. lactis* secreting a

human antimicrobial peptide, known as Pancreatitis-Associated Protein (PAP). In this study, PAP secreted by *L. lactis* inhibited pathogenic E. faecalis, *in vitro*. It furthermore preserved villous architecture and increased Paneth cells activity in mice inflamed with 5-FU [12].

Rottiers and colleagues [49] investigated the effects of another *L. lactis* strain secreting human trefoil factor I (TFF-1), a peptide presenting proliferative function in epithelial tissues. Authors suggested that TFF-1 delivered by *L. lactis* prevented mucositis in hamsters submitted to chemotherapy. Further, by using a biological confinement strategy, a safe recombinant TFF1-secreting strain, AG013, was designed and tested in a Phase 1b clinical trial. In this trial, the genetically modified *L. lactis* administrated to mucositis patients was safer and more effective than the placebo [51].

3. Translational Perspectives for Applying Dairy Probiotics and Genetically Modified Organism (GMO) Strains in Clinical Treatment of Mucositis

For probiotics to be used in humans rigorous tests are required, which aim to guarantee the quality and safety of these microorganisms before their possible applications in the treatment of clinical diseases [52, 53].

3.1 Biological Confinement Strategies and Food-Grade Expression Systems

Biological confinement strategies are required for the use of recombinant strains of dairy origin probiotics in humans. In 2003, Steidler and colleagues developed a refined ecological confinement system for *L. lactis*. In this system, the essential gene coding for thymidylate synthase (*thyA*) located in *L. lactis* chromosome was inactivated [54]. As the *L. lactis* strain becomes auxotrophic (i.e., only able to grow in the presence of thymine), the strain cannot survive out of the human intestine. This avoids its dissemination to the external environment.

Regarding heterologous protein expression systems available for dairy origin probiotics, most of them present safety bottlenecks such as the need for chemical compounds to control the expression of proteins, making them inadequate for use in humans [55]. In this context, several works are being conducted to design sophisticated food-grade expression systems. Benbouziane and colleagues [56] constructed the stress-inducible controlled expression system (SICE). The stresses, naturally found in the digestive tract, are acidic pH and bile salts. This eliminates the need for synthetic compounds to induce heterologous expression. However, further studies are required to replace antibiotic resistance markers, as they could be transferred to commensal species of the human microbiota [56].

3.2 Protection Matrices

A protective matrix, based on encapsulation technology, protects probiotic cells while transiting through the GIT. Biopolymers encapsulation may protect dairy origin probiotics against biotic and abiotic stresses, including during the drying process used in formulations [57, 58]. Alginate, and others non-toxic polymers such as β-D-mannuronic and αL-guluronic acids, are commonly used. Alginate-based encapsulation increased protection for *L. lactis*, *L. casei* and *B. longum* in different stress conditions [30, 33, 59].

Milk proteins have also appeared as an innovative methodology to provide encapsulation to probiotic bacteria for increasing viability during passage in the GIT and through the spray-drying process [57, 60, 61]. For example, whey proteins-encapsulated *L. paracasei* subsp. *paracasei* E6 demonstrated increased survival compared to the same bacteria without encapsulation when subjected to gastric juice [62]. The emergence of functional foods for improving the survival of therapeutic dairy origin probiotics, such as wild-type and genetically improved strains, is a promising research area. Depending on the food matrix

biochemical, it may protect bacteria towards digestive stresses, whereas the fermented dairy products confer a high amount of essential nutrient for consumer [63, 64]. In this context, cheese matrices have been explored for probiotic strains of *Lactobacillus* spp. and of *P. freudenreichii*. In a recent study, *B. bifidum* BB-12 and *L. acidophilus* LA-5 strains in white-brined cheese showed increased viability, compared to the strains that were not protected [65].

4. Conclusion

The therapeutic use of dairy origin probiotics strains has been explored in animal models of intestinal mucositis. Furthermore, considerable progress is being achieved for the improvement of probiotic effects by association with other beneficial strains, supplementation with prebiotics and genetic engineering to produce recombinant anti-inflammatory molecules. As shown in this mini-review, biological confinement strategies along with food grade expression systems and protective matrices formulations may provide safety, improvement of therapeutic effects and enhanced survival. Therefore, we emphasize the importance of such translational approaches for the successful use of dairy origin probiotics, recombinant or wild-type, in clinical therapy against mucositis in the future.

Acknowledgments

All the authors acknowledge the Federal University of Minas Gerais, the Federal University of Pernambuco and the funding agencies CNPq, CAPES and FAPEMIG. N. Jan, English teacher, is thanked for constant correction of English. G. J. thanks Institut National de la Recherche Agronomique (INRA) for support and P. Lardon for fruitful discussions. The authors declare no conflict of interest.

References

[1] Koohi-Hosseinabadi, O., Andisheh-Tadbir, A., Bahadori, P., Sepehrimanesh, M., Mardani, M., and Tanideh, N. 2015. "Comparison of the Therapeutic Effects of the Dietary and Topical Forms of Zizyphus Jujuba Extract on Oral Mucositis Induced by 5-Fluorouracil: A Golden Hamster Model." *Journal of Clinical and Experimental Dentistry* 7: 304–9.

[2] Cinausero, M., Aprile, G., Ermacora, P., Basile, D., Vitale, M. G., Fanotto, V., Parisi, G., Calvetti, L., and Sonis, S.T. 2017. "New Frontiers in the Pathobiology and Treatment of Cancer Regimen-Related Mucosal Injury." *Frontiers in Pharmacology* 8 (354): 1-16.

[3] Soares, P. M. G., Mota, J. M. S. C., Souza, E. P., Justino, P. F. C., Franco, A. X., Cunha, F. Q., Ribeiro, R. A., and Souza, M. H. L. P. 2013. "Inflammatory Intestinal Damage Induced by 5-Fluorouracil Requires IL-4." *Cytokine* 61: 46–9.

[4] Antunes, H. S., Herchenhorn, D., Small, I. A., Araújo, C. M. M., Viégas, C. M. P., de Assis Ramos, G., Dias, F. L., and Ferreira, C. G. 2017. "Long-Term Survival of a Randomized Phase III Trial of Head and Neck Cancer Patients Receiving Concurrent Chemoradiation Therapy with or without Low-Level Laser Therapy (LLLT) to Prevent Oral Mucositis." *Oral Oncology* 71: 11–5.

[5] Stringer, A. M., and Logan, R. M. 2015. "The Role of Oral Flora in the Development of Chemotherapy-Induced Oral Mucositis." *Journal of Oral Pathology & Medicine* 44: 81–7.

[6] von Bültzingslöwen, I., Brennan, M. T., Spijkervet, F. K. L., Logan, R., Stringer, A., Raber-Durlacher, J. E., and Keefe, D. 2006. "Growth Factors and Cytokines in the Prevention and Treatment of Oral and Gastrointestinal Mucositis." Supportive Care in Cancer: *Official Journal of the Multinational Association of Supportive Care in Cancer* 14: 519–27.

[7] Touchefeu, Y., Montassier, E., Nieman, K., Gastinne, T., Potel, G., Bruley des Varannes, S., Le Vacon, F., and de La Cochetière, M. F. 2014. "The Role of the Gut Microbiota in Chemotherapy—or Radiation-Induced Gastrointestinal Mucositis—Current Evidence and Potential Clinical Applications." *Alimentary Pharmacology & Therapeutics* 40: 409–21.

[8] Wang, C., Yang, S., Gao, L., Wang, L., and Cao, L. 2018. "Carboxymethyl Pachyman (CMP) Reduces Intestinal Mucositis and Regulates the Intestinal Microflora in 5-Fluorouracil-Treated CT26 Tumour-Bearing Mice." *Food & Function* 9: 2695–704.

[9] Lin, X. B., Farhangfar, A., Valcheva, R., Sawyer, M. B., Dieleman, L., Schieber, A., Gänzle, M. G., and Baracos, V. 2014. "The Role of Intestinal Microbiota in Development of Irinotecan Toxicity and in Toxicity Reduction through Dietary Fibres in Rats." *PloS One* 9 (1): 1–11, e83644.

[10] van Vliet, M. J., Harmsen, H. J. M., de Bont, E. S. J. M., and Tissing, W. J. E. 2010. "The Role of Intestinal Microbiota in the Development and Severity of

Chemotherapy-Induced Mucositis." *PLoS Pathogens* 6 (5): 1–7, e1000879.

[11] Herbers, A. H. E., van der Velden, W. J. F. M., de Haan, A. F. J., Donnelly, J. P., and Blijlevens, N. M. A. 2014. "Impact of Palifermin on Intestinal Mucositis of HSCT Recipients after BEAM." *Bone Marrow Transplantation* 49: 8–10.

[12] Carvalho, R. D., Breyner, N., Menezes-Garcia, Z., Rodrigues, N. M., Lemos, L., Maioli, T. U., da Gloria Souza, D., Carmona, D., de Faria, A. M. C., Langella, P., Chatel, J.-M., Bermúdez-Humarán, L. G., Figueiredo, H. C. P., Azevedo, V., and de Azevedo, M. S. 2017. "Secretion of Biologically Active Pancreatitis-Associated Protein I (PAP) by Genetically Modified Dairy *Lactococcus lactis* NZ9000 in the Prevention of Intestinal Mucositis." *Microbial Cell Factories* 16: 27.

[13] Cereda, E., Caraccia, M., and Caccialanza, R. 2018. "Probiotics and Mucositis." *Current Opinion in Clinical Nutrition and Metabolic Care* 21 (5): 399-404.

[14] Lilly, D. M., and Stillwell, R. H. 1965. "Probiotics: Growth-Promoting Factors Produced by Microorganisms." *Science* 147: 747–8.

[15] Food and Agriculture Organization of the United Nations, World Health Organization. "The State of Food and Agriculture." Accessed January 15, 2006. http://www.fao.org/3/a-a0800e.pdf.

[16] Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., and Sanders, M. E. 2014. "Expert Consensus Document: The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic." *Nature Reviews Gastroenterology & Hepatology* 11: 506–14.

[17] Cousin, F. J., Mater, D. D. G., Foligné, B., and Jan, G. 2010. "Dairy Propionibacteria as Human Probiotics: A Review of Recent Evidence." *Dairy Science & Technology* 91: 1–26.

[18] Henker, J., Laass, M., Blokhin, B. M., Bolbot, Y. K., Maydannik, V. G., Elze, M., Wolff, C., and Schulze, J. 2007. "The Probiotic Escherichia Coli Strain Nissle 1917 (EcN) Stops Acute Diarrhoea in Infants and Toddlers." *European Journal of Pediatrics* 166: 311–8.

[19] Brugère, J. F., Ben Hania, W., Arnal, M. E., Ribière, C., Ballet, N., Vandeckerkove, P., Ollivier, B., and O'Toole, P. W. 2018. "Archaea: Microbial Candidates in Next-Generation Probiotics Development." *Journal of Clinical Gastroenterology* 52: 71–3.

[20] Felis, G. E., and Dellaglio, F. 2007. "Taxonomy of Lactobacilli and Bifidobacteria." *Current Issues in Intestinal Microbiology* 8: 44–61.

[21] Vinderola, G., Binetti, A., Burns, P., and Reinheimer, J. 2011. "Cell Viability and Functionality of Probiotic Bacteria in Dairy Products." *Frontiers in Microbiology* 2 (70): 1–6.

[22] Sarao, L. K., and Arora, M. 2015. "Probiotics, Prebiotics, and Microencapsulation: A Review." *Critical Reviews in Food Science and Nutrition* 57: 344–71.

[23] Papadimitriou, K., Zoumpopoulou, G., Foligné, B., Alexandraki, V., Kazou, M., Pot, B., and Tsakalidou, E. 2015. "Discovering Probiotic Microorganisms: *In Vitro*, *in Vivo*, Genetic and Omics Approaches." *Frontiers in Microbiology* 6: 58.

[24] Cousin, F. J., Louesdon, S., Maillard, M.-B., Parayre, S., Falentin, H., Deutsch, S.-M., Boudry, G., and Jan, G. 2012. "The First Dairy Product Exclusively Fermented by *Propionibacterium freudenreichii*: A New Vector to Study Probiotic Potentialities *in Vivo*." *Food Microbiology* 32: 135–46.

[25] Santos Rocha, C., Lakhdari, O., Blottière, H. M., Blugeon, S., Sokol, H., Bermudez-Humaran, L. G., Azevedo, V., Miyoshi, A., Doré, J., Langella, P., Maguin, E., and van de Guchte, M. 2012. "Anti-Inflammatory Properties of Dairy Lactobacilli." *Inflammatory Bowel Diseases* 18: 657–66.

[26] Prisciandaro, L. D., Geier, M. S., Butler, R. N., Cummins, A. G., and Howarth, G. S. 2011. "Evidence Supporting the Use of Probiotics for the Prevention and Treatment of Chemotherapy-Induced Intestinal Mucositis." *Critical Reviews in Food Science and Nutrition* 51: 239–47.

[27] Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., and Matuchansky, C. 2005. "Bifidobacteria as Probiotic Agents—Physiological Effects and Clinical Benefits." *Alimentary Pharmacology & Therapeutics* 22: 495–512.

[28] Flórez, A. B., Sierra, M., Ruas-Madiedo, P., and Mayo, B. 2016. "Susceptibility of Lactic Acid Bacteria, Bifidobacteria and Other Bacteria of Intestinal Origin to Chemotherapeutic Agents." *International Journal of Antimicrobial Agents* 48: 547–50.

[29] Bachour, P., and Sonis, S.T. 2018. "Predicting Mucositis Risk Associated with Cytotoxic Cancer Treatment Regimens: Rationale, Complexity, and Challenges." *Current Opinion in Supportive and Palliative Care* 12 (2): 198–210.

[30] Anderson, R. C., Cookson, A. L., McNabb, W. C., Park, Z., McCann, M. J., Kelly, W. J., and Roy, N. C. 2010. "*Lactobacillus plantarum* MB452 Enhances the Function of the Intestinal Barrier by Increasing the Expression Levels of Genes Involved in Tight Junction Formation." *BMC Microbiology* 10: 316.

[31] Eun, C. S., Kim, Y. S., Han, D. S., Choi, J. H., Lee, A. R., and Park, Y. K. 2011. "*Lactobacillus casei* Prevents Impaired Barrier Function in Intestinal Epithelial Cells." *APMIS: Acta Pathologica, Microbiologica, et*

Immunologica Scandinavica 119: 49–56.

[32] Robinson, K., Deng, Z., Hou, Y., and Zhang, G. 2015. "Regulation of the Intestinal Barrier Function by Host Defense Peptides." *Frontiers in Veterinary Science* 2: 57.

[33] Sathish, J. G., Sethu, S., Bielsky, M. C., de Haan, L., French, N. S., Govindappa, K., Green, J., Griffiths, C. E. M., Holgate, S., Jones, D., Kimber, I., Moggs, J., Naisbitt, D.J., Pirmohamed, M., Reichmann, G., Sims, J., Subramanyam, M., Todd, M. D., Van Der Laan, J. W., Weaver, R. J., and Park, B. K. 2013. "Challenges and Approaches for the Development of Safer Immunomodulatory Biologics." *Nature Reviews Drug Discovery* 12: 306–24.

[34] Chang, C. T., Ho, T. Y., Lin, H., Liang, J. A., Huang, H. C., Li, C. C., Lo, H. Y., Wu, S. L., Huang, Y.-F., and Hsiang, C. Y. 2012. "5-Fluorouracil Induced Intestinal Mucositis via Nuclear Factor-KB Activation by Transcriptomic Analysis and *in Vivo* Bioluminescence Imaging." *PloS One* 7 (3): e31808.

[35] Zhang, L., Li, N., Caicedo, R., and Neu, J. 2005. "Alive and Dead *Lactobacillus rhamnosus* GG Decrease Tumor Necrosis Factor-Alpha-Induced Interleukin-8 Production in Caco-2 Cells." *The Journal of Nutrition* 135: 1752–56.

[36] Ma, D., Forsythe, P., and Bienenstock, J. 2004. "Live *Lactobacillus rhamnosus* is Essential for the Inhibitory Effect on Tumor Necrosis Factor Alpha-Induced Interleukin-8 Expression." *Infection and Immunity* 72: 5308–14.

[37] Prisciandaro, L. D., Geier, M. S., Chua, A. E., Butler, R. N., Cummins, A. G., Sander, G. R., and Howarth, G. S. 2012. "Probiotic Factors Partially Prevent Changes to Caspases 3 and 7 Activation and Transepithelial Electrical Resistance in a Model of 5-Fluorouracil-Induced Epithelial Cell Damage." *Supportive Care in Cancer* 20: 3205–10.

[38] Bowen, J. M., Stringer, A. M., Gibson, R. J., Yeoh, A. S. J., Hannam, S., and Keefe, D. M. K. 2007. "VSL#3 Probiotic Treatment Reduces Chemotherapy-Induced Diarrhea and Weight Loss." *Cancer Biology & Therapy* 6: 1449–54.

[39] Whitford, E. J., Cummins, A. G., Butler, R. N., Prisciandaro, L. D., Fauser, J. K., Yazbeck, R., Lawrence, A., Cheah, K. Y., Wright, T. H., Lymn, K. A., and Howarth, G. S. 2009. "Effects of Streptococcus Thermophilus TH-4 on Intestinal Mucositis Induced by the Chemotherapeutic Agent, 5-Fluorouracil (5-FU)." *Cancer Biology & Therapy* 8: 505–11.

[40] Southcott, E., Tooley, K. L., Howarth, G. S., Davidson, G. P., and Butler, R. N. 2008. "Yoghurts Containing Probiotics Reduce Disruption of the Small Intestinal Barrier in Methotrexate-Treated Rats." *Digestive Diseases and Sciences* 53: 1837–41.

[41] Cordeiro, B. F., Oliveira, E. R., Silva, S. H., Assis, B. M., Acurcio, L. B., Santos, L. L., Alves, J. L., Assis, H. C., Vieira, A. T., Faria, C., Maria, A., Ferreira, E., Le Loir, Y., Jan, G., Goulart, L. R., Azevedo, V. A. D. C., Carvalho, R. D. D. O., and Carmo, F. L. R. D. 2018. "Whey Protein Isolate-Supplemented Beverage Fermented by Lactobacillus Casei BL23 and *Propionibacterium freudenreichii* 138 in the Prevention of Mucositis in Mice." *Frontiers in Microbiology* 9: 2035.

[42] Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A., and LeBlanc, J. G. 2018. "Protective Effect of the Riboflavin-Overproducing Strain *Lactobacillus plantarum* CRL 2130 on Intestinal Mucositis in Mice." *Nutrition* 54: 165–72.

[43] Tang, Y., Wu, Y., Huang, Z., Dong, W., Deng, Y., Wang, F., Li, M., and Yuan, J. 2017. "Administration of Probiotic Mixture DM#1 Ameliorated 5-Fluorouracil–Induced Intestinal Mucositis and Dysbiosis in Rats." *Nutrition* 33: 96–104.

[44] Yeung, C. Y., Chan, W. T., Jiang, C. B., Cheng, M. L., Liu, C. Y., Chang, S. W., Chiang Chiau, J. S., and Lee, H. C. 2015. "Amelioration of Chemotherapy-Induced Intestinal Mucositis by Orally Administered Probiotics in a Mouse Model." *PloS One* 10 (9): e0138746.

[45] Bindels, L. B., Delzenne, N. M., Cani, P. D., and Walter, J. 2015. "Towards a More Comprehensive Concept for Prebiotics." *Nature Reviews Gastroenterology & Hepatology* 12: 303–10.

[46] Bomba, A., Nemcová, R., Gancarc ̌ková, S., Herich, R., Guba, P., and Mudronová, D. 2002. "Improvement of the Probiotic Effect of Micro-Organisms by Their Combination with Maltodextrins, Fructo-Oligosaccharides and Polyunsaturated Fatty Acids." *British Journal of Nutrition* 88 (S1): 95–9.

[47] Trindade, L. M., Martins, V. D., Rodrigues, N. M., Souza, E. L. S., Martins, F. S., Costa, G. M. F., Almeida-Leite, C. M., Faria, A. M. C., Cardoso, V. N., Maioli, T. U., and Generoso, S. V. 2018. "Oral Administration of Simbioflora® (Synbiotic) Attenuates Intestinal Damage in a Mouse Model of 5-Fluorouracil-Induced Mucositis." *Beneficial Microbes* 9: 477–86.

[48] Smith, C. L., Geier, M. S., Yazbeck, R., Torres, D. M., Butler, R.N., and Howarth, G.S. 2008. "*Lactobacillus fermentum* BR11 and Fructo-Oligosaccharide Partially Reduce Jejunal Inflammation in a Model of Intestinal Mucositis in Rats." *Nutrition and Cancer* 60: 757–67.

[49] Rottiers, P., Caluwaerts, S., Steidler, L., Vandenbroucke, K., Watkins, B., Sonis, S., and Coulie, B. 2009. "Effect of a Mouth Rinse Formulation with Human Trefoil Factor 1-Secreting *Lactococcus lactis* in Experimental Oral Mucositis in Hamsters." *Journal of Clinical Oncology* 27 (15S): e14570.

[50] Carvalho, R. D. O., Morais, K., Bastos, V.,Gomes-Santos, A. C., Luerce, T. D., Azevedo, M. S. P., Rocha, C. S., Sousa, C. S., Próperi, C., Cara, D. C., Faria, A. M. C., Bermudez-Humaran, L., Blottiere, H., Langella, P., Moreno, A., Figueiredo, H. C. P., LeBlanc, J. G., Miyoshi, A., and Azevedo, V. 2016. "Oral Administration of *Lactococcus lactis* Expressing Recombinant 15-Lipoxygenase-1 (15 LOX-1) Modulates Chemically Induced Colitis in Mice." *Medical Research Archives* 4 (7).

[51] Limaye, S. A., Haddad, R. I., Cilli, F., Sonis, S. T., Colevas, A. D., Brennan, M. T., Hu, K. S., and Murphy, B. A. 2013. "Phase 1b, Multicenter, Single Blinded, Placebo-Controlled, Sequential Dose Escalation Study to Assess the Safety and Tolerability of Topically Applied AG013 in Subjects with Locally Advanced Head and Neck Cancer Receiving Induction Chemotherapy: AG013 for Oral Mucositis." *Cancer* 119: 4268–76.

[52] Sanders, M. E., Akkermans, L. M. A., Haller, D., Hammerman, C., Heimbach, J. T., Hörmannsperger, G., and Huys, G. 2010. "Safety Assessment of Probiotics for Human Use." *Gut Microbes* 1: 164–85.

[53] Shigemori, S., and Shimosato, T. 2017. "Applications of Genetically Modified Immunobiotics with High Immunoregulatory Capacity for Treatment of Inflammatory Bowel Diseases." *Frontiers in Immunology* 8: 22.

[54] Steidler, L., Neirynck, S., Huyghebaert, N., Snoeck, V., Vermeire, A., Goddeeris, B., Cox, E., Remon, J. P., and Remaut, E. 2003. "Biological Containment of Genetically Modified *Lactococcus lactis* for Intestinal Delivery of Human Interleukin 10." *Nature Biotechnology* 21: 785–9.

[55] MacCormick, C. A., Griffin, H. G., and Gasson, M. J. 1995. "Construction of a Food-Grade Host/Vector System for Lactococcus Lactis Based on the Lactose Operon." *FEMS Microbiology Letters* 127 (1-2): 105–9.

[56] Benbouziane, B., Ribelles, P., Aubry, C., Martin, R., Kharrat, P., Riazi, A., Langella, P., and Bermúdez-Humarán, L. G. 2013. "Development of a Stress-Inducible Controlled Expression (SICE) System in *Lactococcus lactis* for the Production and Delivery of Therapeutic Molecules at Mucosal Surfaces." *Journal of Biotechnology* 168: 120–9.

[57] Guerin, J., Petit, J., Burgain, J., Borges, F., Bhandari, B., Perroud, C., Desobry, S., Scher, J., and Gaiani, C. 2017. "*Lactobacillus rhamnosus* GG Encapsulation by Spray-Drying: Milk Proteins Clotting Control to Produce Innovative Matrices." *Journal of Food Engineering* 193: 10–9.

[58] Huq, T., Khan, A., Khan, R. A., Riedl, B., and Lacroix, M. 2013. "Encapsulation of Probiotic Bacteria in Biopolymeric System." *Critical Reviews in Food Science and Nutrition* 53: 909–16.

[59] Ouwehand, A. C., Suomalainen, T., Tölkkö, S., and Salminen, S. 2002. "*In Vitro* Adhesion of Propionic Acid Bacteria to Human Intestinal Mucus." *Le Lait* 82 (1): 123-30.

[60] Wang, X., Zhao, X., Huang, D., Pan, X., Qi, Y., Yang, Y., Zhao, H., and Cheng, G. 2017. "Proteomic Analysis and Cross Species Comparison of Casein Fractions from the Milk of Dairy Animals." *Scientific Reports* 7: 43020.

[61] Guo, M., and Wang, G. 2016. "Milk Protein Polymer and Its Application in Environmentally Safe Adhesives." *Polymers* 8 (9): 324.

[62] Dias, D. R., Botrel, D. A., Fernandes, R. V. D. B., and Borges, S. V. 2017. "Encapsulation as a Tool for Bioprocessing of Functional Foods." *Current Opinion in Food Science* 13: 31–7.

[63] Aburjaile, F., Madec, M.-N., Parayre, S., Miyoshi, A., Azevedo, V., Le Loir, Y., and Falentin, H. 2015. "The Long-Term Survival of *Propionibacterium freudenreichii* in a Context of Nutrient Shortage." *Journal of Applied Microbiology* 120 (2): 432–40.

[64] Shori, A. B. 2016. "Influence of Food Matrix on the Viability of Probiotic Bacteria: A Review Based on Dairy and Non-dairy Beverages." *Food Bioscience* 13: 1–8.

[65] Özer, B., Kirmaci, H. A., Şenel, E., Atamer, M., and Hayaloğlu, A. 2009. "Improving the Viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in White-Brined Cheese by Microencapsulation." *International Dairy Journal* 19: 22–9.