Food and Nutrition Sciences, **2013, 4, 27-39**
Published Online November 2013 (http://www.scirp.org/journal/fns)
http://dx.doi.org/10.4236/fns.2013.411A005

Scientific Research

The Role of Lactic Acid Bacteria in the Pathophysiology and Treatment of Irritable Bowel Syndrome (IBS)

Julia König, Ignacio Rangel, Robert J. Brummer

School of Health and Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden.
Email: julia.konig@oru.se

Received August 30th, 2013; revised September 30th, 2013; accepted October 7th, 2013

ABSTRACT

Irritable bowel syndrome (IBS) is a multifactorial chronic disorder characterized by various abdominal complaints and a worldwide prevalence of 10% - 20%. Although its etiology and pathophysiology are complex and still not completely understood, aberrations along the microbe-gut-brain axis are known to play a central role. IBS is characterized by interrelated alterations in intestinal barrier function, gut microbe composition, immune activation, afferent sensory signaling and brain activity. Pharmaceutical treatment is generally ineffective and, hence, most therapeutic strategies are based on non-drug approaches. A promising option is the administration of probiotics, in which lactic acid bacteria strains are considered specifically beneficial. This review aims to provide a concise, although comprehensive, overview of the role of lactic acid bacteria in the pathophysiology and treatment of IBS.

Keywords: Irritable Bowel Syndrome (IBS); Probiotics; Lactic Acid Bacteria; Gut-Brain Axis

1. Introduction

Irritable bowel syndrome (IBS) has a worldwide prevalence of 10% - 20%. It strongly affects the patients' quality of life and causes substantial economic costs due to the need for medical consultation and work absenteeism [1,2]. Symptoms vary between patients and include abdominal pain or discomfort, constipation and/or diarrhea, bloating, flatulence, fecal urgency, a sense of incomplete evacuation and relief of pain or discomfort upon defecation [3].

IBS is a multifactorial disease, and both etiology and pathophysiology are complex and still not completely understood. It is, however, well accepted that a dysregulation of the microbe-gut-brain axis plays a profound role. Associated pathophysiologic aberrations include visceral hypersensitivity, abnormal gut motility, and autonomic nervous system dysfunction [4]. Furthermore, there is a growing evidence that an aberrant function of the immune system is part of the pathogenesis of IBS. Mild immune activation has been found both locally in the gut as well as systemically [5], and mucosal biopsies from IBS patients are characterized by an increased quantity of various immune-associated cells, including mast cells [6-8]. Own preliminary data applying immune fingerprinting of intraepithelial and lamina propria lymphocytes isolated from mucosal biopsies, show that patients suffering from IBS after an episode of infectious gastroenteritis (so called post-infectious IBS) display an altered composition of immune cells compared to healthy controls. In agreement with the hypothesis that an altered bidirectional gut-brain interaction has a central role in IBS, psychological and environmental factors like anxiety, depression and significant negative life events are believed to contribute to IBS symptom development [9]. Pharmaceutical treatment, apart from anti-depressive drugs like selective serotonin reuptake inhibitors (SSRI), is generally ineffective and, hence, most therapeutic strategies are directed at improving gut-brain interaction by improving life style (diet, physical activity, stress management, etc.) and the intestinal ecosystem (especially probiotics, see below) as well as by cognitive behavioral therapy in selected cases.

A growing body of evidence points to the presence of an altered intestinal microbiota composition in IBS [10, 11]. Especially post-infectious IBS seems to be causally linked to aberrations in the gut ecosystem [12]. IBS symptoms can be improved by treatments targeting the intestinal microbial ecosystem, such as antibiotics, probiotics (living organisms which upon ingestion have

beneficial health effects) and prebiotics (food compounds that are selectively fermented by desirable intestinal microbiota) [13-15]. Among the bacterial groups composing the gut microbiota, lactic acid bacteria have gained most attention as potentially beneficial microbial strains in probiotics.

This review aims to provide a concise, although comprehensive, overview of the role of lactic acid bacteria in the pathophysiology and treatment of IBS, based on the paradigm that aberrant microbe-gut-brain interactions play a pivotal role in IBS.

2. Aberrant Ecosystem in IBS—Focus on Lactic Acid Bacteria

Numerous studies have investigated the gut microbiota composition in IBS and found a deregulated ecosystem that differs from healthy controls [10]. Here, we will put special emphasis on aberrations regarding lactic acid bacterial strains (**Table 1**).

Already earlier studies using culture-based techniques described abnormal numbers of *Lactobacillus* and *Bifidobacterium* in IBS patients. Balsari *et al*. detected decreased amounts of both species in fecal samples of IBS patients compared to controls [16], while another study found lower numbers of fecal *Bifidobacterium*, without any differences in *Lactobacillus* and *Enterococcus* spp. [17]. Tana *et al*. found a higher amount of *Lactobacillus* in IBS [18]. A study investigating fecal samples of diarrhea-predominant IBS (IBS-D) patients detected a tendency of lower amounts of *Lactobacillus* spp. using culture-based methods ($p = 0.08$) [19]. Additional qPCR analysis, however, revealed contrary results. In this case, levels of *Lactobacillus* spp. were significantly higher in IBS-D than in controls.

Even though culture-based techniques are suitable for accurate species identification, results must be interpreted with caution and are not representative, as only a fraction of the bacteria in the intestine is cultivable. Malinen *et al*. were the first to apply a culture-independent, specifically designed qPCR assay covering approximately 300 bacterial species for the analysis of fecal microbiota in IBS [20]. The assay targeted *Bifidobacterium*, *Lactobacillus* and *Enterococcus* spp., amongst others. When dividing the IBS patients according to symptoms, they found that lower amounts of *Lactobacillus* spp. were present in fecal samples of IBS-D compared to constipation-predominant patients (IBS-C). Furthermore, in combined samples from all IBS subtypes collected at three time points during a 6-month period, lower amounts of *B. catenulatum* were found compared to healthy controls. In this comparison, no difference could be detected in *Lactobacillus* and *Enterococcus* spp. or in strains such as *B. adolescentis*, *B. bifidum*, and *B. longum*. The same research group was also the first to apply high-throughput 16S rRNA (small subunit ribosomal RNA) gene cloning and sequencing [21]. Pooled bacterial genomic DNA samples were separated according to their guanine cytosine content to be able to identify also less abundant species. In one of the three selected fractions, *Lactobacillus* spp. were absent in all IBS samples, whereas in another fraction, IBS-D patients had significantly lower amounts of *Bifidobacterium* spp. Furthermore, qPCR analysis of the individual samples combining all subtypes suggested lower levels of *B. catenulatum* in IBS ($p = 0.09$). Rajilic-Stojanovic *et al*. analyzed the microbial composition of fecal samples in 62 IBS patients and 46 controls, respectively, using a high-throughput phylogenic microarray (HITChip) that enables the unbiased detection of over one thousand phylotypes [22]. One of the notable differences between IBS and control samples was a significantly decreased level of *Bifidobacterium* spp. in IBS patients (all subtypes combined). Here, the most significant differences were ascribed to *B. gallicum* and *B. pseudocatenulatum*. In addition, higher amounts of *Streptococcus intermedius* et rel., another species comprising lactic acid strains, were detected in patients with IBS and especially in those with alternating episodes of diarrhea and constipation (mixed type IBS). *Lactobacillus* and *Enterococcus* spp. did not differ significantly between IBS patients and healthy controls. The authors correlated IBS symptom scores with the abundance of specific phylogenetic groups and found a negative association of pain scores with *Bifidobacterium* spp. and a positive association with *L. gasseri* et rel. The association of specific bacteria with specific IBS symptoms is a promising tool to provide insight into factors contributing to IBS. However, it needs to be taken into account that identical symptoms are not necessarily related to the same pathophysiology in IBS. Jeffery *et al*. applied pyrosequencing of 16S rRNA to analyze the fecal microbiota in IBS [23]. In this study, the IBS patients clustered into three different groups based on their microbiota composition. The so-called "normal-like IBS group" consisting of 15 of the 37 included patients was indistinguishable from the healthy controls, whereas the two other groups clustered very differently from the healthy controls. In these, an increase in *B. adolescentis* in IBS was detected, but the number of other *Bifidobacterium* species was unchanged. Applying 16S rRNA high-throughput sequencing, Carroll *et al*. detected *Enterococcus* and unspecified *Lactobacillaceae* species in the fecal samples of IBS-D patients which were below detection limit in healthy controls [24]. Own preliminary HITChip data revealed a higher level of several *Lactobacillus* strains (*L. gasseri* et rel., *L. plantarum* et rel., *L. salivarius* et rel.) in fecal samples of IBS patients compared to healthy controls, whereas no differences in *bifidobacterium* spp. were detected.

Most studies published so far have focused on investi-

Table 1. Alterations in the intestinal microbiota in IBS—Focus on lactic acid bacteria.

Reference	Subject populations	Sample	Method	Outcome
Balsari *et al*., 1982 [16]	IBS (n = 20), HC (n = 20)	Feces	Culture	↓*Lactobacillus* and *Bifidobacterium* spp.
Si *et al*., 2004 [17]	IBS (n = 25), HC (n = 25)	Feces	Culture	↓*Bifidobacterium* spp.
Malinen *et al*., 2005 [20]	IBS (n = 27), HC (n = 22) IBS-D (n = 12), IBS-C (n = 9), IBS-A (n = 6)	Feces	qPCR	IBS-D vs. IBS-C: ↓*Lactobacillus* spp. IBS vs. HC: ↓*B. catenulatum*
Kassinen *et al*., 2007 [21]	IBS (n = 24), HC (n = 23) IBS-D (n = 10), IBS-C (n = 8), IBS-A (n = 6)	Feces	16S rRNA sequencing after GC profiling	IBS vs. HC: ↓*Lactobacillus* spp. IBS-D vs. IBS-C&HC: *Bifidobacterium* spp.
			qPCR	IBS vs. HC: ↓*B. catenulatum* (p = 0.09)
Kerckhoffs *et al*., 2009 [30]	IBS (n = 41), HC (n = 26)	Feces, duodenal mucosa	FISH (only FS)	↓*Bifidobacterium* spp. (Feces)
			qPCR	↓*B. catenulatum* (Feces + mucosa)
Tana *et al*., 2010 [18]	IBS (n = 26), HC (n = 26)	Feces	Culture	↑*Lactobacillus* spp.
			qPCR	no changes in *Bifidobacterium* spp.
Carroll *et al*., 2010 [19]	IBS-D (n = 10), HC (n = 10)	Feces, sigmoidal mucosa	Culture	↓*Lactobacillus* spp. (p = 0.08) (Feces)
			qPCR	↑*Lactobacillus* spp. (Feces) No alterations in mucosa-associated microbiota
Rajilic-Stojanovic *et al*., 2011 [22]	IBS (n = 62), HC (n = 42)	Feces	Phylogenetic microarray (HITChip)	↓*Bifidobacterium* spp. ↓*B. gallicum* and *B. pseudocatenulatum* ↓*Streptococcus intermedius* et rel.
Jeffery *et al*., 2012 [23]	IBS (n = 37), HC (n = 20)	Feces	16S rRNA pyrosequencing	IBS subgroups 1&2: ↑*B. adolescentis*
Carroll *et al*., 2012 [24]	IBS-D (n = 23), HC (n = 23)	Feces	16S rRNA sequencing	↑*Enterococcus* and *Lactobacillaceae* spp.
Parkes *et al*., 2012 [29]	IBS (n = 47), HC (n = 26) IBS-D (n = 27), IBS-C (n = 20)	Rectal mucosa	FISH	IBS-C vs. IBS-D&HC: ↑*Bifidobacterium* spp.

n—number of randomized subjects. FISH—fluorescent in situ hybridization, HC—healthy controls; HITChip—human intestinal tract chip; IBS-A—alternating mixed type IBS; IBS-C—constipation-predominant IBS; IBS-D—diarrhea-predominant IBS; qPCR—quantitative PCR.

gating fecal microbiota, and not many results can be found on mucosa-associated bacteria, even though it is known that their compositions differ significantly [25-27]. In general, IBS patients seem to have a higher number of mucosa-associated bacteria than healthy controls [28,29]. Kerckhoffs *et al*. examined fecal and duodenal mucosa brush samples in IBS patients using qPCR [30]. They detected significantly lower *B. catenulatum* levels in IBS patients (combined and in subtypes), while no difference could be found in the numbers of *B. adolescentis*, *B. bifidum*, and *B. longum*. These results applied to both fecal and mucosal samples. The only difference between the two sample types was a lower percentage of *B. bifidum* of the total bifidobacterial counts in the fecal samples in both IBS and healthy controls. The authors further investigated fecal samples using FISH analysis and detected lower numbers of bifidobacteria in IBS compared to healthy controls. An additional study investigating IBS-D patients and respective healthy controls did not detect any differences in *Lactobacillus* or *Bifidobacterium* spp. in mucosal samples obtained from unprepared sigmoidoscopies using both culture-based and qPCR analyses [19]. Own preliminary HITChip data also did not reveal any significant differences in sigmoidal mucosa lactic acid bacteria between IBS patients and healthy controls. Parkes *et al*. applied FISH to investigate the presence of selected bacterial groups in the mucosa of IBS patients' rectal biopsies from a prepared bowel [29]. When analyzing all IBS samples as one group, no differences in the numbers of bifidobacteria and lactobacillus-enterococci were detected. Analysis of subgroups, however, showed that higher numbers of bifidobacteria were present in the IBS-C samples compared to IBS-D and control samples. In addition, the maximum number of stools per day negatively correlated with the number of mucosa-associated bifidobacteria and lactobacilli-enterococci.

In conclusion, the presented studies show rather inconsistent results regarding the role of lactic acid bacteria as part of a deregulated gut ecosystem. This can partly be explained by the heterogeneous character of the IBS pathophysiology, which is characterized by a large inter-individual variation of aberrations along the microbe-gut-brain axis. Furthermore, classifications of patients according to symptoms varied between studies, and often a small number of patients were included. Importantly, studies applied various different methods and techniques for sampling and especially for microbiota analysis, which often are subject to selection biases [11]. In addition, most of the applied analyses only investigated bacteria at the species level instead of performing deeper analyses that would reveal differences between strains. Moreover, when analyzing the intestinal microbiota, it is always difficult to account for exogenous fac-

tors, and especially diet has shown to have a strong influence on the microbiota composition [31].

Further studies analyzing the microbiota composition of fecal and mucosal biopsies on a strain-specific level are essential to elucidate the role of lactic acid bacteria in IBS.

3. Clinical Intervention Studies

Lactic acid bacteria administered as probiotic compounds promise to be a new therapeutic option for the treatment of IBS, and numerous studies testing the effect of a wide selection of different probiotic strains, the majority of them lactic acid bacteria, have been published [11]. Several meta-analyses concluded that probiotics in general improve IBS symptoms [32-34]. As meta-analyses combining the results of studies using different probiotic strains carry the risk of masking the success, or failure, of a specific strain, the authors agreed that it needs to be further investigated which strains and which doses are most effective.

Clarke *et al*. gave a comprehensive overview of the various intervention trials that specifically investigated lactic acid bacteria in IBS [35]. Of the 42 evaluated studies, 34 reported beneficial effects in at least one of the endpoints or symptoms. However, a lack of standardized endpoint measurements hampered comparisons within studies. In addition, the quality of the studies varied widely and often included small patient numbers. The authors highlighted the importance of considering strain-specific effects. While some lactic acid bacteria strains were able to improve abdominal pain in IBS patients, others primarily affected bloating and flatulence or stool frequency. Even within one strain, the influence of delivery vehicle and dose-dependency needs to be considered.

Not all studies distinguished between the various IBS subtypes such as diarrhea or constipation-predominant IBS, discounting the fact that most strains are probably more effective in treating one kind than the other. As mentioned before, it also needs to be considered that IBS symptoms not necessarily predict the underlying pathophysiology. Hence, it would be ideal to administer lactic acid bacteria that specifically target the respective pathophysiologic mechanism instead of applying a treatment based on symptoms. An additional factor to be taken into account is that clinical trials are often conducted in a hospital setting, which may give rise to an inclusion bias in comparison to subjects suffering from IBS in the general population. These groups may differ in the proportion of the various pathophysiologic mechanisms contributing to IBS symptoms.

However, even considering these biases, most of the higher-quality clinical trials reported beneficial effects of lactic acid bacteria on IBS symptoms. So far, only one study reported symptom deterioration using *L. plantarum* MF1298 [36]. *B. infantis* 35624 is one of the strains that demonstrated IBS symptom improvement in more than one controlled clinical trial with a substantial number of patients. Administration of this strain significantly reduced composite and individual scores for abdominal pain/discomfort, bloating/distension and bowel movement difficulty during an 8-week treatment, compared to administration of placebo and of *L. salivarius* UCC4331. Furthermore, it was able to normalize aberrant IL-10/IL-12 ratios in peripheral blood samples of these IBS patients [37]. In a large, multicenter trial that included 362 female IBS patients in a primary care setting, *B. infantis* 35624 improved abdominal pain, the composite score, individual scores for bloating, bowel dysfunction, incomplete evacuation, straining, and the passage of gas after a 4-week study period [38].

In addition, the so-called 'Finnish combination' consisting of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii ssp. shermanii* JS and *B. breve* Bb99 or *B. animalis ssp. lactis* Bb12, respectively, yielded noteworthy positive results. In a 6-month trial including 103 patients, its administration lead to a 42% reduction in total symptom scores compared to a 6% reduction with placebo administration [39]. In a second clinical trial with 86 IBS patients, treatment with this multispecies probiotic during a 5-month period led to a 37% mean reduction in IBS score compared to a 9% reduction in the placebo group [13].

Only few probiotic intervention studies have looked deeper into the underlying mechanisms and evaluated for instance the impact of the tested lactic acid bacteria on the microbiota composition in IBS. Kajander *et al*. investigated the effect of the multispecies "Finnish combination" (see above) on the fecal microbiota composition of IBS patients using strain- and species-specific qPCR assays. They did not detect any changes, apart from an increase in *Bifidobacterium* spp. in the placebo and a decrease in the treatment group [40]. In addition, no differences in the presence of short-chain fatty acids and bacterial enzymes in fecal samples were found. They concluded that other mechanisms apart from an increased colonization with the administered bacteria must have been responsible for the beneficial effects on IBS symptoms, probably involving a direct interaction with the intestinal epithelium. Another explanation could be a more dominant effect of some lactic acid bacteria in the small bowel rather than in the colon, as some strains have been shown to provoke a direct metabolic or immunologic effect in the small bowel [41-43]. Furthermore, the applied techniques were probably not sufficient to detect all underlying microbial changes. In a subsequent study, the same group applied a similar qPCR assay with a broader target of phylotypes to evaluate the effect of the same probiotic combination on the fecal microbiota

in 42 IBS patients. They reported that a phylotype with 94% similarity to *Ruminococcus torques* was decreased and *Clostridium thermosuccino-genes* 85% was increased in the probiotic compared to the placebo group [44].

Effects of probiotic treatment on the mucosa-adherent bacteria have not been reported in IBS patients yet.

4. Putative Mechanisms of How Lactic Acid Bacteria Affect the Gut Ecosystem in IBS

Even though lactic acid bacteria seem to be effective in improving IBS symptomatology, the mechanisms behind their beneficial effects are still not completely understood. Here, we will provide an overview of putative mechanisms. It needs to be highlighted that all mechanisms described below are highly interrelated, and many specifically cluster around improving intestinal barrier function.

4.1. Microbe-Microbe Interaction

4.1.1. Competitive Action

Initially, it was hypothesized that the beneficial effects of the administered probiotic bacteria were associated with their ability to adhere and colonize to the intestinal mucosa. By this means, they were thought to act as antagonists against pathogenic species by replacing existing pathogens or by inhibiting their adherence [45]. There is, however, limited evidence of strains that can actually adhere to the mucosal tissue, and a persistent colonization after the intake has been stopped is very rare. Nevertheless, a competitive action of probiotics, mainly lactic acid bacteria, on pathogens has been demonstrated for the treatment of *Helicobacter pylori* infection in humans. After administration of probiotics, most studies showed a decrease in *H. pylori* colonization and consequently improvement of *H. pylori*-induced gastritis [46].

4.1.2. pH-Lowering Effect

An additional antimicrobial mechanism of lactic acid bacteria is their ability to lower the pH by producing lactic acid during fermentation processes [47,48]. Once this organic acid has passed the cell membrane of a pathogen, the acidity of the molecule needs to be overcome by driving out the excess protons in order to maintain the intracellular pH. This implies a high requirement of energy to sustain the activity of the ATPase in charge of the process, resulting in an inhibitory effect against respective pathogens [49].

4.1.3. Bacteriocins

Lactic acid bacteria secrete a variety of different antimicrobial substances, so-called bacteriocins [50]. Bacteriocins produced by *L. acidophilus* and *L. casei* inhibited the growth and viability of *Cronobacter sakazakii*, a pathogen that can cause bacteremia, meningitis, and necrotizing enterocolitis in infants [51]. In another study, substances secreted by a *L. plantarum* strain showed inhibitory effects on the growth, biofilm formation, and invasion and adhesion ability of *Salmonella enterica* serovar Enteriditis [52]. Gassericin A, a bacteriocin produced by *L. gasseri*, is thought to cause cell death via a pore-formation mechanism as a result of the dimerization and location of this bacteriocin on the cell membrane of gram-positive pathogens [49].

4.2. Effect on Mucus Composition

Mucin is the first barrier protecting the gastrointestinal mucosa from opportunistic pathogens [53]. There is limited information about the alterations of the mucus layer in IBS, however, changes in the expression of genes associated with the production of mucins in the colon of IBS patients have been reported [54]. In addition, an overproduction of mucus has been detected in colonic biopsies of IBS patients [55]. Lactic acid bacteria are known to regulate the expression of mucin genes such as MUC2 and MUC3 [56,57]. Results from animal studies are, however, mostly contradictory. For instance, supplementation with the multistrain probiotic product VSL#3, which contains lactic acid bacteria strains, did not affect the expression levels of MUC1, MUC2, MUC3 and MUC4 in a mouse model of colitis [58] or the expression of MUC5ac in a rat model of gastric ulcer [59]. On the contrary, administration of VSL#3 to healthy Wistar rats resulted in the upregulation of MUC2, MUC3 as well as MUC31 gene levels [60]. These examples strongly indicate that in particular human clinical trials are mandatory to clearly determine the effect of these bacteria on mucus layer production, quality and integrity.

4.3. Immunomodulatory Effect

Increased immune activation with signs of low-grade inflammation is frequently observed in subgroups of IBS patients, like those with the diarrhea-predominant or post-infectious subtype [5]. Accordingly, lactic acid bacteria are known to exert immunomodulatory effects [61]. For instance, we could show that *Lactobacillus plantarum* WCFS1 affected NFκB pathways correlating with immune tolerance in healthy subjects [41,42], and activated TLR2 signaling [62]. Toll-like receptors (TLR) are members of the family of pattern-recognition receptors (PRR) and are a fundamental part of the inherent immune system, where they are in charge of recognizing and discriminating microbial infections. Changes in intestinal microbiota in IBS are consistent with altered TLR expression in colonic biopsies as well as TLR-related cytokine responses in peripheral blood of IBS patients [63,

64]. Polymorphisms in the TLR9 gene have been associated with a higher risk of post-infectious IBS [65].

Various lactic acid strains are known to act via the expression of TLRs [66]. Recent studies demonstrated that different *Lactobacillus* species could inhibit the provoked production of cytokines such as IL-8 via TLR9 in Caco-2 cells and via TLR4 in T24 urothelial cells, respectively [67,68]. In another study it was found that stimulation of peripheral blood mononuclear cells (PBMC) from healthy volunteers with *L. rhamnosus*, *L. casei* and a *B. breve* strain was TLR9 dependent [69]. In addition, the effect of *B. breve* on production of proinflammatory cytokines was the result of TLR2 signaling activation, an effect that was not observed when PBMCs were stimulated with lactobacilli strains. We also demonstrated a strain-specificity of human cellular pathway modulation within the species *L. plantarum* [43].

4.4. Effect on Epithelial Barrier Function

As stated earlier, the intestinal barrier plays a central role in the pathophysiologic concept of IBS integrating the intestinal ecosystem, immune activation, mucosal integrity, afferent sensory signaling and brain activity. Deregulation of immune responses and deterioration of the intestinal barrier function are associated processes, and may provoke sustained immune activation, mucosal inflammation and increased afferent sensory signaling leading to abdominal complaints [70]. A disturbed intestinal barrier function coincides with changes in mucosal integrity and tight junction function. The functional consequence of this can be increased mucosal permeability. A subset of diarrhea-predominant IBS patients showed increased intestinal permeability correlating with severity of symptoms [71]. Acute stress may disturb intestinal barrier function, and corticotropin releasing factor (CRF) and post-stress intestinal mast cell activation play a central mechanistic role in this. Hence, maintenance of tight junction function plays an important role in the resilience of intestinal barrier function.

Several studies have demonstrated protective effects of lactic acid bacteria on intestinal epithelial cell integrity *in vitro* or in experimental animal studies, as shown by improved transepithelial resistance and relocalization of tight junction proteins, amongst others [72-74]. Evidence on the potential effect of lactobacilli regarding the regulation of the intestinal barrier function in humans was provided by a study of Karczewski *et al*. [62]. The administration of *L. plantarum* WCFS1 via a feeding catheter led to an upregulation of the epithelial tight junction proteins ZO-1 (zonula occludens-1) and occludin in the duodenum. In addition, lactic acid bacteria might have a protective effect on the mucosal integrity through the regulation of mucin proteins, or through TLR signaling, as outlined above. Apart from their role in immunoregulatory processes, TLRs are involved in epithelial cell proliferation, IgA production, and maintenance of tight junctions, all of which are highly relevant for a well-functioning epithelial barrier [75].

4.5. Effect on Oxidative Stress

Reactive oxygen species (ROS) are mediators of both the innate and adaptive immune regulatory function and play a role in the interaction between the intestinal ecosystem, the immune system and intestinal barrier function. Mast cell activation in IBS, resulting in release of e.g. histamine and the activation of proteases, may well lead to increased levels of ROS and thus oxidative stress. Scavenging of ROS may protect the intestinal barrier in cases of increased oxidative stress such as metabolic stress and mast cell activation.

Although clear evidence of a beneficial effect of lactic acid bacteria on oxidative stress is lacking in the human setting, a number of experimental animal studies have shown anti-oxidative properties. *L. rhamnosus* GG reduced markers of alcohol-induced intestinal and liver oxidative stress as well as improved parameters of intestinal barrier function in a rat model of alcoholic steatohepatitis [76]. *L. paracasei* F19 significantly reduced the harmful effects of ischemia/reperfusion in a rat model associated with reduction of the ischemia/ reperfusion induced alteration in the intestinal microbiota [77]. A multistrain mix of lactic acid bacteria was shown to be able to beneficially affect oxidative networking and effectively reduce doxorubicin-induced oxidative stress in rats [78]. These anti-oxidative properties are very strain specific and not clearly associated with lactic acid production. In a mice model of *Giardia* parasitic infection, *L. rhamnosus* GG was not only able to increase levels of antioxidants in the intestine but also nearly restored normal mucosal morphology [79].

4.6. Neurogenic Action

An increasing number of studies substantiate a crosstalk between the gut ecosystem and the central nervous system, and it has become evident that even behavior can be affected by the intestinal microbiota [80,81]. This has been nicely demonstrated by a study of Bercik *et al*. in which the gut microbiota of mice belonging to the timid and less explorative strain BALB/c was exchanged with the microbiota of highly explorative NIH Swiss mice. This resulted in a more explorative behavior of the BALB/c mice, similar to that of the donor mice [82]. Accordingly, specific lactic acid bacteria administered as probiotics have been shown to exert neurogenic effects. *B. infantis* 35624 reversed behavioral deficits in a rat maternal separation model and restored noradrenaline

levels in the brain [83]. An effect of lactic acid bacteria on brain function and behavior has also been demonstrated for *B. longum* NC3001, which normalized anxiety-like behavior and hippocampal brain derived neurotrophic factor (BDNF) levels in a mouse model of chronic gastrointestinal inflammation [84]. Administration of *L. rhamnosus* (JB-1) reduced anxiety and depression related behavior in mice by modulating GABA receptor expression in the brain [85]. Only a very limited number of studies have looked at a neurogenic effect of lactic acid bacteria in humans. The strains *L. helveticus* R0052 and *B. longum* R0175 showed beneficial effects on psychological distress and cortisol levels in healthy human volunteers in addition to an anxiolytic-like effect in rats [86]. A fermented milk product containing *B. animalis* subsp *Lactis*, *S. thermophiles*, *L. bulgaricus*, and *L. lactis* subsp *Lactis* modulated brain activity in healthy women. Its ingestion reduced task-related brain responses and altered resting-state networks, thus successfully demonstrating an effect on gut-brain communication in humans [87]. Consuming a milk drink containing *L. casei* Shirota elevated mood in subjects with initially poor mood [88]. The same strain led to a decrease in anxiety symptoms in patients with chronic fatigue syndrome, however, an association with enhanced bowel function and/or reduced abdominal complaints was not assessed [89].

A number of *in vitro* and animal studies suggest that the administration of specific lactic acid bacteria might be beneficial for the treatment of visceral hypersensitivity and abdominal pain in IBS. *L. acidophilus* NCFM modulated the perception of visceral pain in rodents with a morphine-like effect and induced the expression of cannabinoid receptors, while *L. acidophilus* NCFM as well as *L. salivarius* Ls-33 induced the expression of opioid receptors in human HT-29 epithelial cells *in vitro* [90]. Also *B. infantis* 35624 was able to reduce visceral pain in rats [91].

4.7. Interaction with Metabolic Networking

The intestinal microbiota in healthy adults forms a complex ecosystem that is composed of more than 1000 microbial species [92,93]. These organisms live in a close symbiotic relationship with the host as well as each other, which is based on metabolic interaction and networking. Modulation of this system by adding for instance lactic acid bacteria may lead to a chain of interrelated actions within the metabolic networking. Especially the so-called crossfeeding results in highly complex interactions. Crossfeeding denotes the nutritional interdependence between two or several species, which utilize products provided by other species for their own metabolism. For instance, the administration of lactic acid bacteria might affect the growth of bacteria utilizing lactate as their substrate, such as *Anaerostipes caccae* and *Eubacterium hallii*, which convert lactate into the beneficial short-chain fatty acid butyrate [94]. Butyrate is an important energy source for epithelial cells and has protecting effects on colonic mucosal function including inhibition of inflammation and carcinogenesis [95-97]. Our own preliminary data showed that IBS patients have a lower proportion of butyrate-producing microbiota both in fecal and unprepared mucosal samples compared to healthy controls. The administration of butyrate via enemas resulted in a substantial decrease of visceral perception in healthy volunteers [98,99], and could reduce the frequency of abdominal complaints in IBS patients [100]. Consequently, lactic acid bacteria might also contribute to improvement of IBS symptoms by promoting the growth of butyrate-producing bacteria. The increased production of butyrate in turn can affect other bacteria that utilize butyrate as a substrate. Moreover, butyrate is not the only short-chain fatty acid produced from lactate, and other compounds such as propionate also play a role in this complex metabolic networking.

The metabolic effects of lactic acid bacteria are strain-specific, as it was demonstrated by the different amounts of butyrate produced by various *B. animalis* strains [101]. In addition, a host-specific effect needs to be considered. Even though all adults share a common microbial core, each person has its own subject-specific intestinal microbiota composition [93], which is also strongly influenced by the individual diet [31,102]. Hence, it seems plausible that the gut ecosystem reacts subject-specific to the administration of lactic acid bacteria.

5. Future Applications of Lactic Acid Bacteria in the Treatment of IBS

With regard to a future application of lactic acid bacteria in the treatment of IBS, it still needs to be investigated if their efficacy is higher if administered as monospecies or in a multispecies mixture. As several pathophysiologic mechanisms are involved in IBS, and in addition, patients show different aberrations along the microbe-gut-brain axis, a combination of several lactic acid bacteria could provide a more comprehensive treatment covering various needs. In a multispecies mixture, one strain could deliver a beneficial neurogenic effect, while another strain could improve intestinal barrier function. A multispecies probiotic could also be more effective in the various segments of the intestine. Furthermore, it was shown, by applying an *in vitro* human intestinal mucus model, that individual strains may strongly enhance each other's adherence if combined with other strains, with some combinations being more effective than others [103]. However, besides a potential synergistic effect, lactic acid bacteria could also exert antagonistic effects against each other if administered in combination.

An additional consideration in the case of using lactic acid bacteria as a treatment for IBS, might be a combined administration with a specific prebiotic substance. Prebiotics are food compounds that are selectively fermented in the intestine by specific desirable bacteria, mostly bifidobacteria or lactobacilli. They confer favorable health effects on the host by stimulating the metabolism and growth of these bacteria [104]. Prebiotics and probiotics administered in combination are denoted as synbiotics. The addition of the prebiotic might enhance the viability and activity of the administered lactic acid bacteria and also of resident bacteria, resulting in a synergistic effect. So far, there is only one placebo-controlled trial evaluating the effect of synbiotics on IBS symptoms. It included 68 IBS patients and reported improvement of abdominal pain and bowel habits using a novel synbiotic known as SCM-III. Its uptake successfully increased numbers of lactobacilli, eubacteria and bifidobacteria [105]. Further beneficial effects have been described in several open-label studies. Those results, however, need to be assessed with caution, as the placebo response in IBS is high [106].

Lactic acid bacteria will probably play a central role in the probiotic treatment of IBS. One of the clear advantages of probiotics over conventional pharmacological medication is their favorable adverse effect profile, which enables chronic administration and preventive treatment.

REFERENCES

[1] H. B. El-Serag, K. Olden and D. Bjorkman, "Health-Related Quality of Life among Persons with Irritable Bowel Syndrome: A Systematic Review," *Alimentary Pharmacology & Therapeutics*, Vol. 16, No. 6, 2002, pp. 1171-1185. http://dx.doi.org/10.1046/j.1365-2036.2002.01290.x

[2] R. S. Sandler, J. E. Everhart, M. Donowitz, E. Adams, K. Cronin, C. Goodman, E. Gemmen, S. Shah, A. Avdic and R. Rubin, "The Burden of Selected Digestive Diseases in the United States," *Gastroenterology*, Vol. 122, No. 5, 2002, pp. 1500-1511. http://dx.doi.org/10.1053/gast.2002.32978

[3] G. F. Longstreth, W. G. Thompson, W. D. Chey, L. A. Houghton, F. Mearin and R. C. Spiller, "Functional Bowel Disorders," *Gastroenterology*, Vol. 130, No. 5, 2006, pp. 1480-1491. http://dx.doi.org/10.1053/j.gastro.2005.11.061

[4] T. Karantanos, T. Markoutsaki, M. Gazouli, N. P. Anagnou and D. G. Karamanolis, "Current Insights into the Pathophysiology of Irritable Bowel Syndrome," *Gut Pathogens*, Vol. 2, No. 3, 2010, pp. 3-4749-2-3.

[5] G. Barbara, C. Cremon, G. Carini, L. Bellacosa, L. Zecchi, R. De Giorgio, R. Corinaldesi and V. Stanghellini, "The Immune System in Irritable Bowel Syndrome," *Journal of Neurogastroenterology and Motility*, Vol. 17, No. 4, 2011, pp. 349-359. http://dx.doi.org/10.5056/jnm.2011.17.4.349

[6] S. C. Bischoff, "Physiological and Pathophysiological Functions of Intestinal Mast Cells," *Seminars in Immunopathology*, Vol. 31, No. 2, 2009, pp. 185-205. http://dx.doi.org/10.1007/s00281-009-0165-4

[7] V. S. Chadwick, W. Chen, D. Shu, B. Paulus, P. Bethwaite, A. Tie and I. Wilson, "Activation of the Mucosal Immune System in Irritable Bowel Syndrome," *Gastroenterology*, Vol. 122, No. 7, 2002, pp. 1778-1783. http://dx.doi.org/10.1053/gast.2002.33579

[8] C. Cremon, L. Gargano, A. M. Morselli-Labate, D. Santini, R. F. Cogliandro, R. De Giorgio, V. Stanghellini, R. Corinaldesi and G. Barbara, "Mucosal Immune Activation in Irritable Bowel Syndrome: Gender-Dependence and Association with Digestive Symptoms," *The American Journal of Gastroenterology*, Vol. 104, No. 2, 2009, pp. 392-400. http://dx.doi.org/10.1038/ajg.2008.94

[9] J. Fichna and M. A. Storr, "Brain-Gut Interactions in IBS," *Frontiers in Pharmacology*, Vol. 3, 2012, p. 127. http://dx.doi.org/10.3389/fphar.2012.00127

[10] A. Salonen, W. M. de Vos and A. Palva, "Gastrointestinal Microbiota in Irritable Bowel Syndrome: Present State and Perspectives," *Microbiology*, Vol. 156, No. 11, 2010, pp. 3205-3215. http://dx.doi.org/10.1099/mic.0.043257-0

[11] M. Simren, G. Barbara, H. J. Flint, B. M. Spiegel, R. C. Spiller, S. Vanner, E. F. Verdu, P. J. Whorwell, E. G. Zoetendal and Rome Foundation Committee, "Intestinal Microbiota in Functional Bowel Disorders: A Rome Foundation Report," *Gut*, Vol. 62, No. 1, 2013, pp. 159-176. http://dx.doi.org/10.1136/gutjnl-2012-302167

[12] R. Spiller and C. Lam, "An Update on Post-Infectious Irritable Bowel Syndrome: Role of Genetics, Immune Activation, Serotonin and Altered Microbiome," *Journal of Neurogastroenterology and Motility*, Vol. 18, No. 3, 2012, pp. 258-268. http://dx.doi.org/10.5056/jnm.2012.18.3.258

[13] K. Kajander, E. Myllyluoma, M. Rajilic-Stojanovic, S. Kyronpalo, M. Rasmussen, S. Jarvenpaa, E. G. Zoetendal, W. M. de Vos, H. Vapaatalo and R. Korpela, "Clinical Trial: Multispecies Probiotic Supplementation Alleviates the Symptoms of Irritable Bowel Syndrome and Stabilizes Intestinal Microbiota," *Alimentary Pharmacology & Therapeutics*, Vol. 27, No. 1, 2008, pp. 48-57. http://dx.doi.org/10.1111/j.1365-2036.2007.03542.x

[14] R. Spiller, "Review Article: Probiotics and Prebiotics in Irritable Bowel Syndrome," *Alimentary Pharmacology & Therapeutics*, Vol. 28, No. 4, 2008, pp. 385-396. http://dx.doi.org/10.1111/j.1365-2036.2008.03750.x

[15] A. H. Sachdev and M. Pimentel, "Antibiotics for Irritable Bowel Syndrome: Rationale and Current Evidence," *Current Gastroenterology Reports*, Vol. 14, No. 5, 2012, pp. 439-445. http://dx.doi.org/10.1007/s11894-012-0284-2

[16] A. Balsari, A. Ceccarelli, F. Dubini, E. Fesce and G. Poli, "The Fecal Microbial Population in the Irritable Bowel Syndrome," *Microbiologica*, Vol. 5, No. 3, 1982, pp. 185-194.

[17] J. M. Si, Y. C. Yu, Y. J. Fan and S. J. Chen, "Intestinal Microecology and Quality of Life in Irritable Bowel Syndrome Patients," *World Journal of Gastroenterology*, Vol.

10, No. 12, 2004, pp. 1802-1805.

[18] C. Tana, Y. Umesaki, A. Imaoka, T. Handa, M. Kanazawa and S. Fukudo, "Altered Profiles of Intestinal Microbiota and Organic Acids May Be the Origin of Symptoms in Irritable Bowel Syndrome," *Neurogastroenterology and Motility*, Vol. 22, No. 5, 2010, pp. 512-9,e114-e115.

[19] I. M. Carroll, Y. H. Chang, J. Park, R. B. Sartor and Y. Ringel, "Luminal and Mucosal-Associated Intestinal Microbiota in Patients with Diarrhea-Predominant Irritable Bowel Syndrome," *Gut Pathogens*, Vol. 2, No. 1, 2010, pp. 19-4749-2-19.

[20] E. Malinen, T. Rinttila, K. Kajander, J. Matto, A. Kassinen, L. Krogius, M. Saarela, R. Korpela and A. Palva, "Analysis of the Fecal Microbiota of Irritable Bowel Syndrome Patients and Healthy Controls with Real-Time PCR," *The American Journal of Gastroenterology*, Vol. 100, No. 2, 2005, pp. 373-382. http://dx.doi.org/10.1111/j.1572-0241.2005.40312.x

[21] A. Kassinen, L. Krogius-Kurikka, H. Makivuokko, T. Rinttila, L. Paulin, J. Corander, E. Malinen, J. Apajalahti and A. Palva, "The Fecal Microbiota of Irritable Bowel Syndrome Patients Differs Significantly from That of Healthy Subjects," *Gastroenterology*, Vol. 133, No. 1, 2007, pp. 24-33. http://dx.doi.org/10.1053/j.gastro.2007.04.005

[22] M. Rajilic-Stojanovic, E. Biagi, H. G. Heilig, K. Kajander, R. A. Kekkonen, S. Tims and W. M. de Vos, "Global and Deep Molecular Analysis of Microbiota Signatures in Fecal Samples from Patients with Irritable Bowel Syndrome," *Gastroenterology*, Vol. 141, No. 5, 2011, pp. 1792-1801. http://dx.doi.org/10.1053/j.gastro.2011.07.043

[23] I. B. Jeffery, P. W. O'Toole, L. Ohman, M. J. Claesson, J. Deane, E. M. Quigley and M. Simren, "An Irritable Bowel Syndrome Subtype Defined by Species-Specific Alterations in Faecal Microbiota," *Gut*, Vol. 61, No. 7, 2012, pp. 997-1006. http://dx.doi.org/10.1136/gutjnl-2011-301501

[24] I. M. Carroll, T. Ringel-Kulka, J. P. Siddle and Y. Ringel, "Alterations in Composition and Diversity of the Intestinal Microbiota in Patients with Diarrhea-Predominant Irritable Bowel Syndrome," *Neurogastroenterology and Motility*, Vol. 24, No. 6, 2012, pp. 521-530,e248.

[25] E. G. Zoetendal, A. von Wright, T. Vilpponen-Salmela, K. Ben-Amor, A. D. Akkermans and W. M. de Vos, "Mucosa-Associated Bacteria in the Human Gastrointestinal Tract Are Uniformly Distributed along the Colon and Differ from the Community Recovered from Feces," *Applied and Environmental Microbiology*, Vol. 68, No. 7, 2002, pp. 3401-3407. http://dx.doi.org/10.1128/AEM.68.7.3401-3407.2002

[26] P. B. Eckburg, E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson and D. A. Relman, "Diversity of the Human Intestinal Microbial Flora," *Science*, Vol. 308, No. 5728, 2005, pp. 1635-1638. http://dx.doi.org/10.1126/science.1110591

[27] I. M. Carroll, T. Ringel-Kulka, T. O. Keku, Y. H. Chang, C. D. Packey, R. B. Sartor and Y. Ringel, "Molecular Analysis of the Luminal- And Mucosal-Associated Intestinal Microbiota in Diarrhea-Predominant Irritable Bowel Syndrome," *American Journal of Physiology*, *Gastrointestinal and Liver Physiology*, Vol. 301, 2011, pp. G799-G807. http://dx.doi.org/10.1152/ajpgi.00154.2011

[28] A. Swidsinski, J. Weber, V. Loening-Baucke, L. P. Hale and H. Lochs, "Spatial Organization and Composition of the Mucosal Flora in Patients with Inflammatory Bowel Disease," *Journal of Clinical Microbiology*, Vol. 43, No. 7, 2005, pp. 3380-3389. http://dx.doi.org/10.1128/JCM.43.7.3380-3389.2005

[29] G. C. Parkes, N. B. Rayment, B. N. Hudspith, L. Petrovska, M. C. Lomer, J. Brostoff, K. Whelan and J. D. Sanderson, "Distinct Microbial Populations Exist in the Mucosa-Associated Microbiota of Sub-Groups of Irritable Bowel Syndrome," *Neurogastroenterology and Motility*, Vol. 24, No. 1, 2012, pp. 31-39. http://dx.doi.org/10.1111/j.1365-2982.2011.01803.x

[30] A. P. Kerckhoffs, M. Samsom, M. E. van der Rest, J. de Vogel, J. Knol, K. Ben-Amor and L. M. Akkermans, "Lower Bifidobacteria Counts in Both Duodenal Mucosa-Associated and Fecal Microbiota in Irritable Bowel Syndrome Patients," *World Journal of Gastroenterology*, Vol. 15, No. 23, 2009, pp. 2887-2892. http://dx.doi.org/10.3748/wjg.15.2887

[31] G. D. Wu, J. Chen, C. Hoffmann, K. Bittinger, Y. Y. Chen, S. A. Keilbaugh, M. Bewtra, D. Knights, W. A. Walters, R. Knight, R. Sinha, E. Gilroy, K. Gupta, R. Baldassano, L. Nessel, H. Li, F. D. Bushman and J. D. Lewis, "Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes," *Science*, Vol. 334, No. 6052, 2011, pp. 105-108. http://dx.doi.org/10.1126/science.1208344

[32] L. V. McFarland and S. Dublin, "Meta-Analysis of Probiotics for the Treatment of Irritable Bowel Syndrome," *World Journal of Gastroenterology*, Vol. 14, No. 17, 2008, pp. 2650-2661. http://dx.doi.org/10.3748/wjg.14.2650

[33] S. Nikfar, R. Rahimi, F. Rahimi, S. Derakhshani and M. Abdollahi, "Efficacy of Probiotics in Irritable Bowel Syndrome: A Meta-Analysis of Randomized, Controlled Trials," *Diseases of the Colon and Rectum*, Vol. 51, No. 12, 2008, pp. 1775-1780. http://dx.doi.org/10.1007/s10350-008-9335-z

[34] N. Hoveyda, C. Heneghan, K. R. Mahtani, R. Perera, N. Roberts and P. Glasziou, "A Systematic Review and Meta-Analysis: Probiotics in the Treatment of Irritable Bowel Syndrome," *BMC Gastroenterology*, Vol. 9, No. 15, 2009, pp. 15-230X-9-15.

[35] G. Clarke, J. F. Cryan, T. G. Dinan and E. M. Quigley, "Review Article: Probiotics for the Treatment of Irritable Bowel Syndrome—Focus on Lactic Acid Bacteria," *Alimentary Pharmacology & Therapeutics*, Vol. 35, No. 4, 2012, pp. 403-413. http://dx.doi.org/10.1111/j.1365-2036.2011.04965.x

[36] S. C. Ligaarden, L. Axelsson, K. Naterstad, S. Lydersen and P. G. Farup, "A Candidate Probiotic with Unfavourable Effects in Subjects with Irritable Bowel Syndrome: A Randomised Controlled Trial," *BMC Gastroenterology*, Vol. 10, No. 16, 2010.

[37] L. O'Mahony, J. McCarthy, P. Kelly, G. Hurley, F. Luo, K. Chen, G. C. O'Sullivan, B. Kiely, J. K. Collins, F. Shanahan and E. M. Quigley, "Lactobacillus and Bifidobacterium in Irritable Bowel Syndrome: Symptom Responses and Relationship to Cytokine Profiles," *Gastroenterology*, Vol. 128, No. 3, 2005, pp. 541-551. http://dx.doi.org/10.1053/j.gastro.2004.11.050

[38] P. J. Whorwell, L. Altringer, J. Morel, Y. Bond, D. Charbonneau, L. O'Mahony, B. Kiely, F. Shanahan and E. M. Quigley, "Efficacy of an Encapsulated Probiotic Bifidobacterium Infantis 35624 in Women with Irritable Bowel Syndrome," *The American Journal of Gastroenterology*, Vol. 101, No. 7, 2006, pp. 1581-1590. http://dx.doi.org/10.1111/j.1572-0241.2006.00734.x

[39] K. Kajander, K. Hatakka, T. Poussa, M. Farkkila and R. Korpela, "A Probiotic Mixture Alleviates Symptoms in Irritable Bowel Syndrome Patients: A Controlled 6-Month Intervention," *Alimentary Pharmacology & Therapeutics*, Vol. 22, No. 5, 2005, pp. 387-394. http://dx.doi.org/10.1111/j.1365-2036.2005.02579.x

[40] K. Kajander, L. Krogius-Kurikka, T. Rinttila, H. Karjalainen, A. Palva and R. Korpela, "Effects of Multispecies Probiotic Supplementation on Intestinal Microbiota in Irritable Bowel Syndrome," *Alimentary Pharmacology & Therapeutics*, Vol. 26, No. 3, 2007, pp. 463-473. http://dx.doi.org/10.1111/j.1365-2036.2007.03391.x

[41] F. J. Troost, P. van Baarlen, P. Lindsey, A. Kodde, W. M. de Vos, M. Kleerebezem and R. J. Brummer, "Identification of the Transcriptional Response of Human Intestinal Mucosa to Lactobacillus Plantarum WCFS1 *in Vivo*," *BMC Genomics*, Vol. 9, No. 374, 2008.

[42] P. van Baarlen, F. J. Troost, S. van Hemert, C. van der Meer, W. M. de Vos, P. J. de Groot, G. J. Hooiveld, R. J. Brummer and M. Kleerebezem, "Differential NF-KappaB Pathways Induction by Lactobacillus Plantarum in the Duodenum of Healthy Humans Correlating with Immune Tolerance," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 106, No. 7, 2009, pp. 2371-2376. http://dx.doi.org/10.1073/pnas.0809919106

[43] P. van Baarlen, F. Troost, C. van der Meer, G. Hooiveld, M. Boekschoten, R. J. Brummer and M. Kleerebezem, "Human Mucosal *in Vivo* Transcriptome Responses to Three Lactobacilli Indicate How Probiotics may Modulate Human Cellular Pathways," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 108, Suppl. 1, 2011, pp. 4562-4569. http://dx.doi.org/10.1073/pnas.1000079107

[44] A. Lyra, L. Krogius-Kurikka, J. Nikkila, E. Malinen, K. Kajander, K. Kurikka, R. Korpela and A. Palva, "Effect of a Multispecies Probiotic Supplement on Quantity of Irritable bowel Syndrome-Related Intestinal Microbial Phylotypes," *BMC Gastroenterology*, Vol. 10, No. 110, 2010.

[45] A. L. Servin, "Antagonistic Activities of Lactobacilli and Bifidobacteria against Microbial Pathogens," *FEMS Microbiology Reviews*, Vol. 28, No. 4, 2004, pp. 405-440. http://dx.doi.org/10.1016/j.femsre.2004.01.003

[46] A. Patel, N. Shah and J. B. Prajapati, "Clinical Appliance of Probiotics in the Treatment of *Helicobacter pylori* Infection—A Brief Review," *Journal of Microbiology, Immunology, and Infection*, 2013, in press. http://dx.doi.org/10.1016/j.jmii.2013.03.010

[47] S. Tejero-Sarinena, J. Barlow, A. Costabile, G. R. Gibson and I. Rowland, "*In Vitro* Evaluation of the Antimicrobial Activity of a Range of Probiotics against Pathogens: Evidence for the Effects of Organic Acids," *Anaerobe*, Vol. 18, No. 5, 2012, pp. 530-538. http://dx.doi.org/10.1016/j.anaerobe.2012.08.004

[48] P. Hutt, J. Shchepetova, K. Loivukene, T. Kullisaar and M. Mikelsaar, "Antagonistic Activity of Probiotic Lactobacilli and Bifidobacteria against Entero- and Uropatho0 gens," *Journal of Applied Microbiology*, Vol. 100, No. 6, 2006, pp. 1324-1332. http://dx.doi.org/10.1111/j.1365-2672.2006.02857.x

[49] K. Selle and T. R. Klaenhammer, "Genomic and Phenotypic Evidence for Probiotic Influences of Lactobacillus Gasseri on Human Health," *FEMS Microbiology Reviews*, Vol. 37, No. 6, 2013, pp. 915-935.

[50] P. Kanmani, R. Satish Kumar, N. Yuvaraj, K. A. Paari, V. Pattukumar and V. Arul, "Probiotics and Its Functionally Valuable Products—A Review," *Critical Reviews in Food Science and Nutrition*, Vol. 53, No. 6, 2013, pp. 641-658. http://dx.doi.org/10.1080/10408398.2011.553752

[51] S. S. Awaisheh, A. A. Al-Nabulsi, T. M. Osaili, S. Ibrahim and R. Holley, "Inhibition of *Cronobacter sakazakii* by Heat Labile Bacteriocins Produced by Probiotic LAB Isolated from Healthy Infants," *Journal of Food Sciences*, Vol. 78, No. 9, 2013, pp. M1416-M1420. http://dx.doi.org/10.1111/1750-3841.12209

[52] J. K. Das, D. Mishra, P. Ray, P. Tripathy, T. K. Beuria, N. Singh and M. Suar, "*In Vitro* Evaluation of Anti-Infective Activity of a *Lactobacillus plantarum* Strain against *Salmonella enterica* Serovar Enteritidis," *Gut Pathogens*, Vol. 5, No. 1, 2013, p. 11. http://dx.doi.org/10.1186/1757-4749-5-11

[53] M. A. McGuckin, S. K. Linden, P. Sutton and T. H. Florin, "Mucin Dynamics and Enteric Pathogens," *Nature Reviews Microbioly*, Vol. 9, No. 4, 2011, pp. 265-278.

[54] C. Martinez, A. Gonzalez-Castro, M. Vicario and J. Santos, "Cellular and Molecular Basis of Intestinal Barrier Dysfunction in the Irritable Bowel Syndrome," *Gut and Liver*, Vol. 6, No. 3, 2012, pp. 305-315. http://dx.doi.org/10.5009/gnl.2012.6.3.305

[55] A. E. Dorofeyev, E. A. Kiriyan, I. V. Vasilenko, O. A. Rassokhina and A. F. Elin, "Clinical, Endoscopical and Morphological Efficacy of Mesalazine in Patients with Irritable Bowel Syndrome," *Clinical and Experimental Gastroenteroly*, Vol. 4, 2011, pp. 141-153.

[56] D. R. Mack, S. Michail, S. Wei, L. McDougall and M. A. Hollingsworth, "Probiotics Inhibit Enteropathogenic *E. coli* Adherence *in Vitro* by Inducing Intestinal Mucin Gene Expression," *American Journal of Physiology*, Vol. 276, No. 4, 1999, pp. G941-G950.

[57] A. M. O'Hara and F. Shanahan, "Mechanisms of Action of Probiotics in Intestinal Diseases," *Scientific World Journal*, Vol. 7, 2007, pp. 31-46. http://dx.doi.org/10.1100/tsw.2007.26

[58] E. Gaudier, C. Michel, J. P. Segain, C. Cherbut and C. Hoebler, "The VSL# 3 Probiotic Mixture Modifies Mi-

croflora but Does not Heal Chronic Dextran-Sodium Sulfate-Induced Colitis or Reinforce the Mucus Barrier in Mice," *Journal of Nutrition*, Vol. 135, No. 12, 2005, pp. 2753-2761.

[59] P. Dharmani, C. De Simone and K. Chadee, "The Probiotic Mixture VSL#3 Accelerates Gastric Ulcer Healing by Stimulating Vascular Endothelial Growth Factor," *PLoS ONE*, Vol. 8, No. 3, 2013, Article ID: e58671. http://dx.doi.org/10.1371/journal.pone.0058671

[60] C. Caballero-Franco, K. Keller, C. De Simone and K. Chadee, "The VSL#3 Probiotic Formula Induces Mucin Gene Expression and Secretion in Colonic Epithelial Cells," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, Vol. 292, No. 1, 2007, pp. G315-G322. http://dx.doi.org/10.1152/ajpgi.00265.2006

[61] J. M. Wells, "Immunomodulatory Mechanisms of Lactobacilli," *Microbial Cell Factories*, Vol. 10, Suppl. 1, 2011, p. S17.

[62] J. Karczewski, F. J. Troost, I. Konings, J. Dekker, M. Kleerebezem, R. J. Brummer and J. M. Wells, "Regulation of Human Epithelial Tight Junction Proteins by *Lactobacillus plantarum in Vivo* and Protective Effects on the Epithelial Barrier," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, Vol. 298, No. 6, 2010, pp. G851-G859. http://dx.doi.org/10.1152/ajpgi.00327.2009

[63] E. K. Brint, J. MacSharry, A. Fanning, F. Shanahan and E. M. Quigley, "Differential Expression of Toll-Like Receptors in Patients with Irritable Bowel Syndrome," *The American Journal of Gastroenterology*, Vol. 106, No. 2, 2011, pp. 329-336.

[64] S. Ishihara, Y. Tada, N. Fukuba, A. Oka, R. Kusunoki, Y. Mishima, N. Oshima, I. Moriyama, T. Yuki, K. Kawashima and Y. Kinoshita, "Pathogenesis of Irritable Bowel Syndrome—Review Regarding Associated Infection and Immune Activation," *Digestion*, Vol. 87, No. 3, 2013, pp. 204-211. http://dx.doi.org/10.1159/000350054

[65] A. C. Villani, M. Lemire, M. Thabane, A. Belisle, G. Geneau, A. X. Garg, W. F. Clark, P. Moayyedi, S. M. Collins, D. Franchimont and J. K. Marshall, "Genetic Risk Factors for Post-Infectious Irritable Bowel Syndrome Following a Waterborne Outbreak of Gastroenteritis," *Gastroenterology*, Vol. 138, No. 4, 2010, pp. 1502-1513. http://dx.doi.org/10.1053/j.gastro.2009.12.049

[66] C. Gomez-Llorente, S. Munoz and A. Gil, "Role of Toll-Like Receptors in the Development of Immunotolerance Mediated by Probiotics," *The Proceedings of the Nutrition Society*, Vol. 69, No. 3, 2010, pp. 381-389. http://dx.doi.org/10.1017/S0029665110001527

[67] Y. Hiramatsu, T. Satho, K. Irie, S. Shiimura, T. Okuno, T. Sharmin, S. Uyeda, Y. Fukumitsu, Y. Nakashima, F. Miake and N. Kashige, "Differences in TLR9-Dependent Inhibitory Effects of H_2O_2-Induced IL-8 Secretion and NF-Kappa B/I Kappa B-Alpha System Activation by Genomic DNA from Five *Lactobacillus* Species," *Microbes and Infection*, Vol. 15, No. 2, 2013, pp. 96-104. http://dx.doi.org/10.1016/j.micinf.2012.11.003

[68] M. Karlsson, N. Scherbak, G. Reid and J. Jass, "*Lactobacillus rhamnosus* GR-1 Enhances NF-kappaB Activation in *Escherichia coli*-Stimulated Urinary Bladder Cells Through TLR4," *BMC Microbiology*, Vol. 12, No. 15, 2012.

[69] T. S. Plantinga, W. W. van Maren, J. van Bergenhenegouwen, M. Hameetman, S. Nierkens, C. Jacobs, D. J. de Jong, L. A. Joosten, B. van't Land, J. Garssen, G. J. Adema and M. G. Netea, "Differential Toll-Like Receptor Recognition and Induction of Cytokine Profile by *BifidoBacterium breve* and *Lactobacillus* Strains of Probiotics," *Clinical and Vaccine Immunology*, Vol. 18, No. 4, 2011, pp. 621-628. http://dx.doi.org/10.1128/CVI.00498-10

[70] J. D. Wood, "Neuropathophysiology of Functional Gastrointestinal Disorders," *World Journal of Gastroenterology*, Vol. 13, No. 9, 2007, pp. 1313-1332.

[71] Q. Zhou, B. Zhang and G. N. Verne, "Intestinal Membrane Permeability and Hypersensitivity in the Irritable Bowel Syndrome," *Pain*, Vol. 146, No. 1, 2009, pp. 41-46. http://dx.doi.org/10.1016/j.pain.2009.06.017

[72] R. Mennigen, K. Nolte, E. Rijcken, M. Utech, B. Loeffler, N. Senninger and M. Bruewer, "Probiotic Mixture VSL#3 Protects the Epithelial Barrier by Maintaining Tight Junction Protein Expression and Preventing Apoptosis in a Murine Model of Colitis," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, Vol. 296, No. 5, 2009, pp. G1140-1149. http://dx.doi.org/10.1152/ajpgi.90534.2008

[73] E. Miyauchi, J. O'Callaghan, L. F. Butto, G. Hurley, S. Melgar, S. Tanabe, F. Shanahan, K. Nally and P. W. O'Toole, "Mechanism of Protection of Transepithelial Barrier Function by *Lactobacillus salivarius*: Strain Dependence and Attenuation by Bacteriocin Production," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, Vol. 303, No. 9, 2012, pp. G1029-G1041. http://dx.doi.org/10.1152/ajpgi.00003.2012

[74] Y. K. Zhou, H. L. Qin, M. Zhang, T. Y. Shen, H. Q. Chen, Y. L. Ma, Z. X. Chu, P. Zhang and Z. H. Liu, "Effects of *Lactobacillus plantarum* on Gut Barrier Function in Experimental Obstructive Jaundice," *World Journal of Gastroenterology*, Vol. 18, No. 30, 2012, pp. 3977-3991. http://dx.doi.org/10.3748/wjg.v18.i30.3977

[75] M. T. Abreu, "Toll-Like Receptor Signalling in the Intestinal Epithelium: How Bacterial Recognition Shapes Intestinal Function," *Nature Reviews Immunology*, Vol. 10, No. 2, 2010, pp. 131-144.

[76] C. B. Forsyth, A. Farhadi, S. M. Jakate, Y. Tang, M. Shaikh and A. Keshavarzian, "Lactobacillus GG Treatment Ameliorates Alcohol-Induced Intestinal Oxidative Stress, Gut Leakiness, and Liver Injury in a Rat Model of Alcoholic Steatohepatitis," *Alcohol*, Vol. 43, No. 2, 2009, pp. 163-172. http://dx.doi.org/10.1016/j.alcohol.2008.12.009

[77] G. Nardone, D. Compare, E. Liguori, V. Di Mauro, A. Rocco, M. Barone, A. Napoli, D. Lapi, M. R. Iovene and A. Colantuoni, "Protective Effects of *Lactobacillus paracasei* F19 in a Rat Model of Oxidative and Metabolic Hepatic Injury," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, Vol. 299, No. 3, 2010, pp. G669-G676. http://dx.doi.org/10.1152/ajpgi.00188.2010

[78] A. Amaretti, M. di Nunzio, A. Pompei, S. Raimondi, M.

Rossi and A. Bordoni, "Antioxidant Properties of Potentially Probiotic Bacteria: *In Vitro* and *in Vivo* Activities," *Applied Microbiology and Biotechnology*, Vol. 97, No. 2, 2013, pp. 809-817. http://dx.doi.org/10.1007/s00253-012-4241-7

[79] N. Goyal, P. Rishi and G. Shukla, "*Lactobacillus rhamnosus* GG Antagonizes Giardia Intestinalis Induced Oxidative Stress and Intestinal Disaccharidases: An Experimental study," *World Journal of Microbiology & Biotechnology*, Vol. 29, No. 6, 2013, pp. 1049-1057. http://dx.doi.org/10.1007/s11274-013-1268-6

[80] J. F. Cryan and T. G. Dinan, "Mind-Altering Microorganisms: The Impact of the Gut Microbiota on Brain and Behaviour," *Nature Reviews*. *Neuroscience*, Vol. 13, No. 10, 2012, pp. 701-712.

[81] J. A. Bravo, M. Julio-Pieper, P. Forsythe, W. Kunze, T. G. Dinan, J. Bienenstock and J. F. Cryan, "Communication between Gastrointestinal Bacteria and the Nervous System," *Current Opinion in Pharmacology*, Vol. 12, No. 6, 2012, pp. 667-672. http://dx.doi.org/10.1016/j.coph.2012.09.010

[82] P. Bercik, E. Denou, J. Collins, W. Jackson, J. Lu, J. Jury, Y. Deng, P. Blennerhassett, J. Macri, K. D. McCoy, E. F. Verdu and S. M. Collins, "The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice," *Gastroenterology*, Vol. 141, No. 2, 2011, pp. 599-609.

[83] L. Desbonnet, L. Garrett, G. Clarke, B. Kiely, J. F. Cryan and T. G. Dinan, "Effects of the Probiotic *Bifidobacterium infantis* in the Maternal Separation Model of Depression," *Neuroscience*, Vol. 170, No. 4, 2010, pp. 1179-1188. http://dx.doi.org/10.1016/j.neuroscience.2010.08.005

[84] P. Bercik, A. J. Park, D. Sinclair, A. Khoshdel, J. Lu, X. Huang, Y. Deng, P. A. Blennerhassett, M. Fahnestock, D. Moine, B. Berger, J. D. Huizinga, W. Kunze, P. G. McLean, G. E. Bergonzelli, S. M. Collins and E. F. Verdu, "The Anxiolytic Effect of *Bifidobacterium longum* NCC3001 Involves Vagal Pathways for Gut-Brain Communication," *Neurogastroenterology and Motility*, Vol. 23, No. 12, 2011, pp. 1132-1139. http://dx.doi.org/10.1111/j.1365-2982.2011.01796.x

[85] J. A. Bravo, P. Forsythe, M. V. Chew, E. Escaravage, H. M. Savignac, T. G. Dinan, J. Bienenstock and J. F. Cryan, "Ingestion of *Lactobacillus* Strain Regulates Emotional Behavior and Central GABA Receptor Expression in a Mouse via the Vagus Nerve," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 108, No. 38, 2011, pp. 16050-16055. http://dx.doi.org/10.1073/pnas.1102999108

[86] M. Messaoudi, R. Lalonde, N. Violle, H. Javelot, D. Desor, A. Nejdi, J. F. Bisson, C. Rougeot, M. Pichelin, M. Cazaubiel and J. M. Cazaubiel, "Assessment of Psychotropic-Like Properties of a Probiotic Formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in Rats and Human Subjects," *The British Journal of Nutrition*, Vol. 105, No. 5, 2011, pp. 755-764. http://dx.doi.org/10.1017/S0007114510004319

[87] K. Tillisch, J. Labus, L. Kilpatrick, Z. Jiang, J. Stains, B. Ebrat, D. Guyonnet, S. Legrain-Raspaud, B. Trotin, B. Naliboff and E. A. Mayer, "Consumption of Fermented Milk Product with Probiotic Modulates Brain Activity," *Gastroenterology*, Vol. 144, No. 7, 2013, pp. 1394-1401.

[88] D. Benton, C. Williams and A. Brown, "Impact of Consuming a Milk Drink Containing a Probiotic on Mood and Cognition," *European Journal of Clinical Nutrition*, Vol. 61, No. 3, 2007, pp. 355-361. http://dx.doi.org/10.1038/sj.ejcn.1602546

[89] A. V. Rao, A. C. Bested, T. M. Beaulne, M. A. Katzman, C. Iorio, J. M. Berardi and A. C. Logan, "A Randomized, Double-Blind, Placebo-Controlled Pilot Study of a Probiotic in Emotional Symptoms of Chronic Fatigue Syndrome," *Gut Pathogens*, Vol. 1, No. 1, 2009, p. 6.

[90] C. Rousseaux, X. Thuru, A. Gelot, N. Barnich, C. Neut, L. Dubuquoy, C. Dubuquoy, E. Merour, K. Geboes, M. Chamaillard, A. Ouwehand, G. Leyer, D. Carcano, J. F. Colombel, D. Ardid and P. Desreumaux, "*Lactobacillus acidophilus* Modulates Intestinal Pain and Induces Opioid and Cannabinoid Receptors," *Nature Medicine*, Vol. 13, No. 1, 2007, pp. 35-37.

[91] D. P. McKernan, P. Fitzgerald, T. G. Dinan and J. F. Cryan, "The Probiotic *Bifidobacterium infantis* 35624 Displays Visceral Antinociceptive Effects in the Rat," *Neurogastroenterology and Motility*, Vol. 22, No. 9, 2010, pp. 1029-1035.

[92] E. G. Zoetendal, M. Rajilic-Stojanovic and W. M. de Vos, "High-Throughput Diversity and Functionality Analysis of the Gastrointestinal tract Microbiota," *Gut*, Vol. 57, No. 11, 2008, pp. 1605-1615. http://dx.doi.org/10.1136/gut.2007.133603

[93] J. Qin, R. Li, J. Raes, M. Arumugam, K. S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez, T. Yamada, D. R. Mende, J. Li, J. Xu, S. Li, D. Li, J. Cao, B. Wang, H. Liang, H. Zheng, Y. Xie, J. Tap, P. Lepage, M. Bertalan, J. M. Batto, T. Hansen, D. Le Paslier, A. Linneberg, H. B. Nielsen, E. Pelletier, P. Renault, T. Sicheritz-Ponten, K. Turner, H. Zhu, C. Yu, S. Li, M. Jian, Y. Zhou, Y. Li, X. Zhang, S. Li, N. Qin, H. Yang, J. Wang, S. Brunak, J. Dore, F. Guarner, K. Kristiansen, O. Pedersen, J. Parkhill, J. Weissenbach, P. Bork, S. D. Ehrlich and J. Wang, "A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing," *Nature*, Vol. 464, No. 7285, 2010, pp. 59-65. http://dx.doi.org/10.1038/nature08821

[94] S. H. Duncan, P. Louis and H. J. Flint, "Lactate-utilizing Bacteria, Isolated from Human Feces, that Produce Butyrate as a Major Fermentation Product," *Applied and Environmental Microbiology*, Vol. 70, No. 10, 2004, pp. 5810-5817. http://dx.doi.org/10.1128/AEM.70.10.5810-5817.2004

[95] H. M. Hamer, D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost and R. J. Brummer, "Review Article: The Role of Butyrate on Colonic Function," *Alimentary Pharmacology & Therapeutics*, Vol. 27, No. 2, 2008, pp. 104-119. http://dx.doi.org/10.1111/j.1365-2036.2007.03562.x

[96] H. M. Hamer, D. M. Jonkers, A. Bast, S. A. Vanhoutvin, M. A. Fischer, A. Kodde, F. J. Troost, K. Venema and R. J. Brummer, "Butyrate Modulates Oxidative Stress in the Colonic Mucosa of Healthy Humans," *Clinical Nutrition*, Vol. 28, No. 1, 2009, pp. 88-93.

http://dx.doi.org/10.1016/j.clnu.2008.11.002

[97] H. M. Hamer, D. M. Jonkers, S. A. Vanhoutvin, F. J. Troost, G. Rijkers, A. de Bruine, A. Bast, K. Venema and R. J. Brummer, "Effect of Butyrate Enemas on Inflammation and Antioxidant Status in the Colonic Mucosa of Patients with Ulcerative Colitis in Remission," *Clinical Nutrition*, Vol. 29, No. 6, 2010, pp. 738-744. http://dx.doi.org/10.1016/j.clnu.2010.04.002

[98] S. A. Vanhoutvin, F. J. Troost, T. O. Kilkens, P. J. Lindsey, H. M. Hamer, D. M. Jonkers, K. Venema and R. J. Brummer, "The Effects of Butyrate Enemas on Visceral Perception in Healthy Volunteers," *Neurogastroenterology and Motility*, Vol. 21, No. 9, 2009, pp. 952-976. http://dx.doi.org/10.1111/j.1365-2982.2009.01324.x

[99] S. A. Vanhoutvin, F. J. Troost, H. M. Hamer, P. J. Lindsey, G. H. Koek, D. M. Jonkers, A. Kodde, K. Venema and R. J. Brummer, "Butyrate-Induced Transcriptional Changes in Human Colonic Mucosa," *PloS ONE*, Vol. 4, No. 8, 2009, Article ID: e6759. http://dx.doi.org/10.1371/journal.pone.0006759

[100] T. Banasiewicz, L. Krokowicz, Z. Stojcev, B. F. Kaczmarek, E. Kaczmarek, J. Maik, R. Marciniak, P. Krokowicz, J. Walkowiak and M. Drews, "Microencapsulated Sodium Butyrate Reduces the Frequency of Abdominal Pain in Patients with Irritable Bowel Syndrome," *Colorectal Disease*, Vol. 15, No. 2, 2013, pp. 204-209. http://dx.doi.org/10.1111/j.1463-1318.2012.03152.x

[101] S. Arboleya, N. Salazar, G. Solis, N. Fernandez, A. M. Hernandez-Barranco, I. Cuesta, M. Gueimonde and C. G. de los Reyes-Gavilan, "Assessment of Intestinal Microbiota Modulation Ability of *Bifidobacterium* Strains in *In vitro* Fecal Batch Cultures from Preterm Neonates," *Anaerobe*, Vol. 19, 2013, pp. 9-16. http://dx.doi.org/10.1016/j.anaerobe.2012.11.001

[102] K. P. Scott, S. W. Gratz, P. O. Sheridan, H. J. Flint and S. H. Duncan, "The Influence of Diet on the Gut Microbiota," *Pharmacological Research*, Vol. 69, No. 1, 2013, pp. 52-60. http://dx.doi.org/10.1016/j.phrs.2012.10.020

[103] M. C. Collado, J. Meriluoto and S. Salminen, "Development of New Probiotics by Strain Combinations: Is It Possible to Improve the Adhesion to Intestinal Mucus?" *Journal of Dairy Science*, Vol. 90, No. 6, 2007, pp. 2710-2716. http://dx.doi.org/10.3168/jds.2006-456

[104] M. Roberfroid, "Prebiotics: The Concept Revisited," *The Journal of Nutrition*, Vol. 137, No. 3, 2007, pp. 830S-837S.

[105] J. Tsuchiya, R. Barreto, R. Okura, S. Kawakita, E. Fesce and F. Marotta, "Single-Blind Follow-Up Study on the Effectiveness of a Symbiotic Preparation in Irritable Bowel Syndrome," *Chinese Journal of Digestive Diseases*, Vol. 5, No. 4, 2004, pp. 169-174. http://dx.doi.org/10.1111/j.1443-9573.2004.00176.x

[106] E. M. Quigley, "Prebiotics and Probiotics: Their Role in the Management of Gastrointestinal Disorders in Adults," *Nutrition in Clinical Practice*: *Official publication of the American Society for Parenteral and Enteral Nutrition*, Vol. 27, No. 2, 2012, pp. 195-200.