Therapy of irritable bowel syndrome in Vietnamese residents with probiotic enterococci

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For citation: Ermolenko El, Kotyleva MP, Tsapieva AN, Karaseva AB, Bui Thi Lan Anh, Suvorov AN Therapy of irritable bowel syndrome in residents of Vietnam with probiotic enterococci. Experimental and Clinical Gastroenterology. 2021;196(12): 35-43. DOI: 10.31146/1682-8658-ecg-196-12-35-43

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Summary

This paper compares the composition of intestinal microbiota of healthy volunteers and patients with irritable bowel syndrome (IBS) of Hanoi before and after treatment with probiotic sourdough based on the bacteriococcus *faecium* L3 (L3) strain, successfully used for dysbiosis correction and IBS treatment in Russia.

IBS patients (IBS group) had a higher a-diversity than the control group (Nealthey) and IBS patients after probiotic therapy (IBS+L3 group). Phylogenetic analysis of the studied samples reflected the division of the studied communities into 2 clusters, one of which grouped samples predominantly taken in IBS before therapy. Principal components method (PCA) showed that most samples from the IBS group are localized on the graph in a separate sector distant from the adjacent samples IBS+L3 and Nealthy. The study of the microbiome of Vietnamese IBS patients after probiotic administration at the level of types (phyla) revealed a tendency

to an increase in *Firmicutes* representation. Introduction of L3 led to an increase in the population of *Lachnospiraceae*, such as butyrate-producing *Roseburia* and acetate-producing *Blautia*. At the same time, the percentage of staphylococci and bacteria belonging to the family *Enterobacteriaceae* decreased. In addition to the positive changes in the gastrointestinal tract function revealed by the clinical d a t a , the metagenomic analysis of 16S rRNA genes showed the specificity of the gut microbiota of Hanoi IBS patients compared to healthy volunteers and the same patients after probiotic treatment.

The positive changes in the gut microbiome of IBS patients in Vietnam after treatment w i t h L3-based probiotic sourdough do not differ in principle from the effects described earlier by the authors when using this probiotic in Russia, which creates the conditions for expanding its use in the Asian region.

Keywords: microbiota, irritable bowel syndrome, Enterococcus faecium L3, probiotics

Conflict of Interest. The authors declare no conflict of interest.

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https://doi.org/10.31146/1682-8658-ecg-196-12-35-43

Irritable bowel syndrome therapy in Vietnam with probiotic enterococci

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For citation: Ermolenko E. I., Kotyleva M. P. Tsapieva A. N., Karaseva A. B., Bui Thi Lan An, Suvorov A. N. Chlrritable bowel syndrome therapy in Vietnam with probiotic enterococci. *Experimental and Clinical Gastroenterology*. 2021;196(12): 35-43. (In Russ.) DOI: 10.31146/1682-8658-ecg-196-12-35-43

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Summary

In this work, we compared composition of the intestinal microbiota of healthy volunteers and patients with irritable bowel syndrome (IBS) in Hanoi residents before and after therapy with a probiotic starter culture based on the bacteriocinogenic stra *Enterococcus faecium* L3 (L3), which is successfully used to correct dysbiosis and treat IBS in Russia.

In IBS patients (IBS group) α -diversity was higher than in the control group (Healthy) and in IBS patients after probiotic therapy (IBS + L3 group). Phylogenetic analysis of the studied samples reflected the division of the studied communities into 2 clusters, one of which grouped samples mainly taken for IBS before therapy. Principal component analysis (PCoA) showed that most of the samples from the IBS group are located on the graph in a separate sector, far from the adjacent IBS + L3 and Nältra samples. A study of the microbiome of Vietnamese patients with IBS after taking probiotic at the level of types (phyls) revealed a tendency towards an increase in the representation of *Firmicutes*. The introduction of L3 led to an increase in the population of members of the family Lachnospiraceae, such as butyrate-producing *Roseburia* and acetate-producing *Blautia*. At the same time, the percentage of staphylococci and bacteria belonging to the *Enterobacteriaceae* family was decreased. In addition to positive changes in the functioning of the gastrointestinal tract, revealed on the basis of clinical data, the features of the intestinal microbiota of IBS residents of Hanoi compared with healthy volunteers and in the same patients after probiotic therapy were revealed by metagenomic analysis of 16S rRNA genes. Positive shifts in the intestinal microbiome of IBS patients in Vietnam after therapy with a probiotic starter culture based on L3 do not fundamentally differ from the previously described effects noted by the authors when using this probiotic in Russia, which creates the preconditions for expanding the possibility of its use in the Asian region.

Keywords: microbiota, irritable bowel syndrome, Enterococcus faecium L3, probiotics

Conflict of interest. Authors declare no conflict of interest.

1. Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder in which recurrent abdominal pain is associated with defecation or changes in the rhythm of defecation. Typically, there is impaired bowel emptying (constipation, diarrhea, or alternation) and intestinal bloating or distention for more than three months [1,2].

The prevalence of IBS depends on the geographic region, epidemiological situation, and diagnostic criteria and is found in 10-45% of

of the world's population [3, 4, 5]. In the countries of the Asian region, this functional bowel disorder has also become widespread. For example, the incidence of IBS in Beijing, Hong Kong, and Taiwan is 0.82%, 6.6%, and 22.1%, respectively [6]. It cannot be excluded that this is due to the peculiarities of the gut microbiota in different regions. For example, Chinese residents are characterized by a decrease in the intestinal content of bifidobacteria, lactobacilli, Prevotella, and bacteroidetes, while the population of Escherichia spp, pseudomonads and fecal bacteria [7]. Previously, we found that the intestinal microbiota of people living in large metropolitan areas of Vietnam (Hanoi) and Russia (Saint Petersburg) are characterized by lower content of lactobacilli and higher representation of opportunistic enterobacteria and staphylococci. Enterococcus *hirae* and *E. faecium* were predominantly isolated from the feces of Vietnamese [8].

A comparative study of microbiota of healthy and IBS patients in Russia was carried out earlier by means of 16S rRNA gene metagenome analysis. The following features of microbiome of IBS patients were revealed: 1) increased representation of Actinobacteria, Firmicutes, Streptococcaceae Lachnospiraceae (Streptococcus), (Dorea spp.), Veillonellaceae (Dialister), Proteobacteria (Enterobacteriaceae and Desulfovibrionaceae families), Bifidobacterium spp. 2) decrease of Bacteroidetes phylum representation, including representatives of families Prevotellacea (Prevotella spp.), Bacteroidaceae (Bacteroides spp.), Firmicutes bacteria belonging to families Clostridiaceae and Ruminococcaceae (Faecalibacterium spp.) [9].

Probiotics are recommended for IBS therapy [1, 2, 10, 11], but objective evaluation of the effectiveness of probiotic bifidobacteria, lactobacilli, escherichia, probiotic strains complex in IBS therapy was performed mainly in European countries and the USA, and conflicting clinical results related to the specific action of probiotic agents were obtained [12, 13]. It should be emphasized that the composition of microbiota in clinical studies has not always been considered.

As a result of the comprehensive clinical and laboratory The use of *Enterococcus faecium* L3 probiotic strain in the treatment and correction of dysbiotic conditions in patients with IBS in RF has been proved to be highly effective [1, 14].

The purpose of this study was to identify changes in the gut microbiota and evaluate the effectiveness of probiotic enterococci on the functional state of the gastrointestinal tract in the treatment of IBS in Hanoi.

2. MATERIALS AND METHODS

2.1. Patient Characteristics

Residents of Hanoi, 11 men aged 23-51 (31.23 ± 7.33) with IBS were studied. Healthy volunteers of similar age and gender groups were included in the control group.

To rule out the presence of infectious pathology when forming groups of fecal samples

2.2. Probiotic strain Eptegococcus faecium L3

Eptegosoccus faecium L3 - a probiotic strain isolated from fermented milk products, used as functional food products "Bio-bio", "Laminolact", "Bakfir" (LLC.

"Avena, Russia). The strain was deposited in GenBank (No. SUB167269, genome size 2,629,318 base pairs, contains 2,717 genes) and in the All-Russian Research Institute of Agricultural Microbiology, ND-79,

2.3. Research design

The patients with IBS for 10 days received the soy milk-based sourdough *of Epteococcus faecium* L3 ("Supro", Belgium). The sourdough was administered twice a day, 50 ml of the product containing lg 8.5 colony-forming units (CFU/ml) of entero-cocci in 1 ml of the product for 10 days. While taking the sourdough, patients filled out a "gastro-enterological diary" designed to monitor the presence of dyspeptic symptoms. Assessment of the course of IBS during probiotic therapy included the following parameters: stool consistency according to Bristol scale, frequency of defecation,

2.4. Microbiota study The study of fecal samples was performed using 16S rRNA gene sequencing on the Illumina platform (*MiSeq* sequencer, USA).

patients were analyzed for the presence of Rota viruses, adenoviruses noroviruses, *Entamoeba histolytica*, and *Giardia lamblia* using immunochromatographic rapid tests (Vegal,

"Biotech, Russia) for direct detection of antigens of the listed pathogens in feces.

Russian patent No. 2220199. This strain is vancomycin-sensitive, lacks hemolysins and other pathogenicity factors, and has five bacteriocin genes (lactobin, enterocins A and B, entXa and entXb), due to which it displays antagonistic activity against opportunistic and pathogenic microorganisms [15].

bloating, rumbling, abdominal pain and heaviness, heartburn, nausea, vomiting, and decreased appetite.

Before the start of sourdough administration and immediately after the end of the course of administration, fecal samples were collected to assess the state of the intestinal microbiome during therapy. Before analyzing the microbiota composition, fecal samples were stored at "80°C.

Patient groups and corresponding fecal samples were designated as follows: healthy volunteers -"Healthy"; patients with IBS (Irritable bowel syndrome) before and after pro-biotic therapy - "IBS" and "IBS+L3," respectively.

DNA from feces was isolated using the Express-DNA-Bio DNA extraction kit (AlkorBio, Russia). For microbiome sequencing

Table 1. Primers used in the study	Primers for V3-V4 region of 16S rRNA		Size amplicon (p.n.)
,	Direct primer (341)	tcgtcggcagcagcgtcagatgtgtataagagacacagcctacgggnggcwgcag	161
	Reverse primer (785)	gtctcgtgtgctcgtaggtgtgtataagagagacaggactachvgggtatctaatcc	404

DnA libraries were prepared using the Illumina Nextera sample preparation kit with DnA primers corresponding to the V3 to V4 sites of the 16S rRNA gene. The nucleotide sequence of the primers is shown in Table 1. Illumina MiSeq was used for library sequencing. Sequencing was performed a t the St Petersburg State University Biobank Resource Center. Fastqc Operational Taxonomic Units (OTUs) generation (http://www.bioinformatics.babraham.ac.uk/ projects/ fastqc) was used to assess the quality of the raw reads. CDHIT-OTU-Miseq was used to search for OTUs. CD-HIT-OTU-Miseq allows extraction of OTUs from paired-end reading operations without pairwise sequence combining by comparing the clustering results for reads R1 and R2. CD-HIT-OTU-Miseq used only high-quality reading regions for clustering. Clustering was performed using the following parameters - lengths of high-quality reading regions

R1 and R2 200 and 180 bp, respectively, 97% similarity reads for the clustering cutoff and 0.00001 for the abundance cutoff. OTUs were annotated using the Greengenes database version 13.5 [16]. OTUs present in less than 5% of samples were excluded from the analysis when filtered as noise. The R-package phyloseq [17] was used to plot phylum counts. The R prcomp function was used for principal component analysis (PCA). Vectors for PCA corresponded to OTU content filtered for noise and normalized for relative content. The noise filtering threshold was increased for PCA to exclude OTUs present in less than 25% of the samples. This was done to increase the percentage of variance explained by the first and second principal components and to obtain a more representative 2D image. The composition and biodiversity of the gut microbiota were assessed at the phyla (type), family, and genus levels.

2.5. Statistical analysis

Statistical analysis was performed using Statistica 8.0 software package. (StatSoft, USA). Differences between the groups were tested using Wilcoxon W-criterion, differences at $p \le 0.05$ were considered significant.

3. RESULTS

3.1. Clinical effect

The analysis of the "gastroenterology diaries" of the patients showed that the stools of 6 subjects during the probiotic therapy were characterized as normal and optimal in terms of transit time of the food lump in the GI tract (stool type 3 and 4 according to the Bristol scale). The stools of three subjects changed from liquid stools characteristic of diarrhea (stool types 6 and 5 on the Bristol scale) at the beginning of therapy to normal stool consistency and shape after therapy (Fig. 1).

3.2. Examination of the gut microbiome

The structure of the gut microbiome was analyzed using 16S rRNA gene metagenomic analysis, comparing the representation of individual gut microbiota taxa of healthy volunteers and IBS patients before and after treatment with E. faecium L3-based probiotic starter. To assess the α -diversity of the microbiomes of the studied groups, we used indices of diversity saturation taking into account the relative numbers of community components and their numbers. No significant differences were found in a d i v e r s i t v parameters estimated using the observed species richness and Shannon index in fecal samples from the groups

The frequency of defecation in all the subjects (from 1 to 6 times per day) both before and at the end of therapy did not change significantly. However, three patients showed fluctuations in stool frequency while taking the starter (Fig. 2).

Monitoring of clinical manifestations of IBS during therapy, such as bloating, rumbling, abdominal pain and heaviness, heartburn, nausea, vomiting, appetite disorders, showed that the above symptoms were not detected at the end of therapy. Cases of nausea, vomiting, and appetite disorders were not recorded during the entire observation period.

Healthy and IBS+L3. At the same time, adiversity in the IBS group was markedly higher compared to that in the Healthy and IBS+L3 groups (Fig. 3).

The cluster analysis of the studied samples reflected the division of the studied communities into two clusters. IBS samples were predominantly grouped in one of the clusters. Two IBS+L3 samples were also included in this cluster. The second cluster grouped samples of all Healthy Vietnamese, IBS+L3 samples, and only two IBS samples (Fig. 4).

Visualization of the results of the analysis using the Principal Component Method, PCA (Fig. 5) showed that most IBS samples form

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Figure 1.

Dynamics of changes in the Bristol stool scale in three IBS





•IBS

oIBS+L3

6

Figure 5.

Analysis of differences in the structure of microbial communities using the PCA method.

Figure 6. Representation of Firmicutes in the gut microbiome of healthy Vietnamese and IBS patients before and after probiotic therapy.

a separate group distant from the IBS+L3 and Healthy samples grouped together.

7

6

The use of E. faecium L3 for the therapy of IBS resulted in changes in the gut microbiota not only at the level of community meta-parameters, such as phylogenetic diversity, but also at the level of individual taxa.

A study of the microbiome of Vietnamese IBS patients after probiotic administration at the phyla (type) level revealed an increasing trend in the representation of Firmicutes and Bacteroidetes (Fig. 6 and Fig. 7, respectively).

Statistical analysis at the family level showed a decrease in the proportion of Enterobacteriaceae and an increase in Lachnospiraceae (Fig. 8 and Fig. 9, respectively).

Administration of E. faecium L3 led to a decrease in the content of bacteria of the genus Staphylococcus (Fig. 10), the representation of which was minimal in the group of healthy patients. In addition, administration of the probiotic led to a significant increase in the population of Roseburia sp. (Fig. 11) and a tendency to an increase in the genus Blautia (Fig. 12), apparently compensatory, since the representation of these bacteria was low in healthy patients. There was also a tendency for Lactobacillus spp. (Fig. 13) to decrease in the Vietnamese microbiome after therapy to the level typical of healthy volunteers. At the same time, the population of lactobacilli in the IBS + L3 and Healthy groups was significantly lower compared to the IBS group.

Healthy

Figure 7: Representation of *Bacteroidetes* in the gut microbiome of healthy Vietnamese and IBS patients before and after probiotic treatment.

Figure 8. Representation of *Enterobacteriaceae* in the gut microbiome healthy Vietnamese as well as IBS patients before and after probiotic therapy.

Figure 9. Representation of the family *Lachnospiraceae* in the gut microbiome of healthy Vietnamese as well as IBS patients before and after probiotic therapy.

Figure 10. Representation of *Staphylococcus* genus in the gut microbiome of healthy Vietnamese and IBS patients before and after probiotic therapy.

Figure 11. Representation of the genus *Roseburia* in the gut microbiome of healthy Vietnamese and IBS patients before and after probiotic therapy.

Figure 12. Representation of *Blautia* genus in the intestinal microbiome of healthy Vietnamese, as well as IBS patients before and after probiotic therapy.

Figure 13. Representation of the genus *Lactobacillus* in the gut microbiome of healthy Vietnamese and IBS patients before and after probiotic therapy.



4. Discussion

This paper compares the microbiota of healthy Hanoi residents and IBS patients and evaluates the effectiveness of pro-biotic therapy for this functional gastrointestinal disorder. The probiotic strain *E. faecium* L3 [15], which has a beneficial effect on the intestinal microbiota in the correction of dysbiosis, IBS therapy [18, 19] and neuroimmune disease therapy [20, 21, 22] in Russia, was used in therapy. *E. faecium* L3 is characterized by

the ability to produce bacteriocins with an antagonistic effect on certain representatives of the pathogenic microbiocenosis, including and opportunistic Klebsiella, staphylococci, streptococci, and pseudomonads [15]. This was an additional argument in selecting a probiotic, since the population of pathogenic enterobacteriaceae is increased in Vietnamese residents suffering from IBS [23].

The indisputable expected positive effect of the use of *E. faecium* L3 in the therapy of

The effect on intestinal motility has also been shown in an experimental model [24] and in clinical applications [21, 15]. The effect on intestinal motility, as well as on the activity of digestive enzymes [25], is closely related to changes in the intestinal microbiocenosis.

Earlier it was noted that the following bacterial taxa increased in IBS in Russian residents: are Actinobacteria, Firmicutes, Proteobacteria (families Enterobacteriaceae and Desulfovibrionaceae), Gammaproteobacteria, Lachnospiraceae (Dorea spp.), Veillonellaceae (Dialister), Ruminococcus sp, Clostridium sp., Dorea sp., Bacteroidetes, Clostridium, influ-Haemophilus enza, Streptococcaceae (Streptococcus), while Prevotellaceae (Prevotella spp.), Clostridiaceae, Ruminococcaceae (Faecalibacterium spp.), Bifidobacterium spp., Bacteroides, Dorea spp. and Ruminococcus spp. decrease [26, 9].

In this work, some similar structural features of the microbiota characteristic of IBS patients of Vietnam were found in IBS patients: the representation of *Bacteroidetes* phyla was reduced. However, in contrast to the data of other authors, *Firmicutes* representation was also minimal compared to the control group.

A fundamental difference was the higher content of Firmicutes lactobacilli in Hanoi residents with IBS compared to patients after therapy and healthy people. Apparently, this is related to the peculiarities of the microbiota of the inhabitants of the Asian region identified earlier [9, 23].

The species identification of representatives of the family Lacto- bacillaceae, characterized by significant species and, after reclassification, generic diversity, is of further interest: Homofermentative Lactobacillus, Holzapfelia, Amylolactobacillus, Bombilactobacillus, Companilactobacillus, Lapidilactobacillus, Agrilactobacillus, Schleiferilactobacillus, Loigolactobacillus, Lacticaseibacillus, Latilactobacillus, Dellaglioa, Liquorilactobacillus, Ligilactobacillus and heteroenzymatic Lactiplantibacillus, Furfuri lactobacillus, Paucilactobacillus, Limosilactobacillus, Fructilactobacillus, Acetilactobacillus, Apilactobacillus, Levilactobacillus, Secundilactobacillus, Lentilactobacillus species [27].

The increased α -diversity in IBS patients was also noteworthy and was more often detected in healthy individuals when compared to patients receiving antimicrobial preparations or suffering from various intestinal and extraintestinal somatic diseases and dysbiotic conditions accompanied by decreased colonization resistance of the body [28, 29]. It should be emphasized that the increased α -diversity in IBS patients tended to decrease after probiotic therapy. At the same time, the level of α -diversity after taking *E. faecium* L3 probiotic became similar to the control group (Healthy), which can be considered as a trend towards the restoration of normal microbiocenosis.

Analysis of the β -diversity (phylogenetic diversity) of the studied groups showed that the structure of the IBS microbiome differs from that of the IBS+L3 and Healthy microbiomes, while the IBS+L3 and Healthy communities appeared similar to each other, which confirms the favorable effect of therapy.

More interesting and statistically reliable data were obtained when considering the effect of probiotic L3 on individual components of gut microbiocenosis. There was an increase in members of the family Lachnospiraceae, such as butyrate-producing Roseburia and acetate-producing Blautia [30]. Bacteria of the genera Roseburia cecicola and Roseburia faecis are among the main producers of butyric acid (butyrate) in the colon. Butyric acid plays an important role in physiology; it is the main energy material for epithelial cells, maintains intestinal homeostasis, controls normal cell development and prevents the development of various intestinal diseases. It has been shown that a decrease in the number of Roseburia bacteria is associated with the development of atherosclerosis, which is presumably associated with a decrease in butyrate produced by the bacteria, which has antiinflammatory properties [31].

The role of acetate-producing bacteria of the genus *Blautia* is not completely clear; there are conflicting data on the representation of these bile-resistant bacteria in the gut microbiome in IBS, which largely depends on the dietary habits of patients [32]. However, the anti-microbial effect of acetate and its key positions in the regulation of metabolism by "chemical communication" are known [33]. [33]. Crucially, blautia can participate in the reaction of hydrogen and carbon dioxide conversion into non-gaseous acetate, thereby reducing the total gas volume in the colon [34], then involving in key metabolic processes of the body, primarily in carbohydrate metabolism [35, 32].

In general, the effect of the probiotic *E. faecium* L3 on gut microbiome in the treatment of IBS in Vietnamese has a favorable effect, bringing it closer to the group of healthy volunteers: decrease in the content of opportunistic representatives of *Staphylococcus spp.*, *Enterobacteriaceae* population, increase in the producers of ECCs such as *Roseburia* and *Blautia*.

Restoration of gut microbiocenosis is usually accompanied by normalization of peristalsis, visceral sensitivity and/or gas formation in the intestine, which mitigates the severity of IBS symptoms [30, 36]. According to several placebo-controlled studies with a large number of subjects, when taking probiotics, a reduction of some IBS symptoms was observed, in particular: abdominal pain [37, 38] and flatulence [39]. In this work, positive changes associated with a decrease in dyspeptic symptoms were also observed, primarily as a normalization of stool frequency and its character as assessed by the Bristol stool scale. No adverse effects were observed during therapy.

Conclusion

The probiotic strain *Enterococcus faecium* L3 has not previously been used in Vietnam, and this pilot study was conducted for the first time.

Although *E. faecium is* less common in the fecal microbiota of Vietnamese compared to *E. hirae, the* L3 strain did not cause adverse effects in the pilot study.

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The clinical manifestations, and the favorable effects on the microbiota noted, set the stage for expanding its use to the Asian region and other regions of the world, as has been the case with many probiotic agents originally developed for local use in Europe, the United States, and Japan.

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