Molecular Characterization of *Bifidobacteria* and *Lactobacillus* and Their Role in Modulating Immune System and Alleviating Ulcerative Colitis in Mice

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**Abstract:** Two probiotic bacteria (*Bifidobacteria* and *Lactobacillus*) were used in this study to evaluate their potentialities to modulate the immune system and to ameliorate ulcerative colitis (UC) in mice. Molecular genetic fingerprinting was employed to identify the two genera under study. 16S rRNA gene analysis was performed and subsequently the sequence analysis of the fragment resulted has been carried out. Cyclosporine was used to suppress the immune system while acetic acid was used to induce UC. The data obtained revealed that dosing the immune-compromised mice with *Bifidobacteria* or *Lactobacillus* resulted in a significant increase in the levels of IgE and IgM. On the other hand, the histopathological examination of the colons with UC revealed the cellular changes occurred due the treatment with acetic acid and the two probiotics were able to decrease these cellular abnormalities.

**Key words:** Probiotics • Ulcerative Colitis • Immune System • Histopathology

**INTRODUCTION**

Currently, there is a growing interest in the consumption of probiotic foods due to their reported health benefits. In developed countries, probiotics have been extensively studied and this has led to the production of a variety of probiotic foods especially with dairy milk. The term probiotics was introduced by Lilly and Stillwell in 1965 to describe growth-promoting factors produced by microorganisms [1]. Probiotics are naturally occurring beneficial organisms that aid in digestion and inhibit disease-causing bacteria in the intestine. Due to the beneficial impact of microorganisms used as probiotics; during the last decades progressive attention has been focused on the biological and molecular characterization and improvement of such [2]. Some of the positive effects of probiotics are: growth promotion of farm animals, protection of host from intestinal infections, alleviation of lactose intolerance, relief of constipation, anticarcinogenic effect, anticholesterol effects, nutrient synthesis and bioavailability, prevention of genital and urinary tract infections and immunostimulatory effects [3].

*Bifidobacteria* has a significant effect on the inflammation disease and in reducing the relapse of inflammatory diseases. It has no effect on the goblet cells but it decreases the inflammatory cells and also helps in raising the immune system efficiency and has a remarkable effect on IgM. *Lactobacillus* and *Bifidobacteria* species are the microorganisms which are most commonly used as human probiotics [1]. For ulcerative colitis, several probiotic formulations have been found effective as adjuvant therapy, both in inducing and maintaining remission [4]. The isolation of *Bifidobacteria* species from feces has assumed a considerable importance, as a consequence of interest in the potential health-promoting properties of this genus [5]. Lactobacilli are ubiquitous in nature and in humans they play a very significant role in the general health maintenance of the host. Identification of lactobacilli has previously been based on culture-dependent methods and recently molecular techniques involving gene sequencing are now the ‘gold standard’ [6].

Ulcerative colitis, together with Crohn’s disease, is a part of the spectrum of inflammatory bowel diseases (IBDs). It is a chronic inflammatory condition with unknown etiology and only a partially understood pathogenesis. Starting from the rectum, the disease may affect the mucosa of the large bowel to varying lengths. A typical clinical course of ulcerative colitis consists of rectal bleeding and diarrhea; in severe cases, however, a systemic inflammatory reaction also becomes apparent [7]. The aim of the present study was to determine the anti-inflammatory activity of the two probiotics...
Lactobacillus and Bifidobacteria to reduce inflammations in the intestinal mucosa of mice with ulcerative colitis (UC) and also to evaluate the potentialities of these bacteria to modulate the immune system of immune-compromised mice.

MATERIALS AND METHODS

Probiotic Bacteria: Both Bifidobacteria and Lactobacillus were used in this study. These bacteria were isolated from fermented milk products from Juhayna Company for food industry, 6 of Oct. city, Egypt.

Experimental Animals: Twenty one mice were used in this study as animal models. Mice (average weight 140 g) were housed in flexible plastic isolators, controlled lighting (12 hr light, 12 hr dark) and handled according to the established procedures. Mice of both sexes were used in this study. Mice were divided into 7 groups (3 mice each); Group 1: served as control in this experiment. Group 2: was treated with acetic acid (2ml/day of 2% Acetic acid/140g by intracolonic injection for three days) as an inducer of ulcerative colitis. Group 3: was treated with acetic acid and after induction of UC, they were treated with Lactobacillus (added to sterilized diet as 1ml/140g for 3 days). Group 4: was treated with acetic acid and after induction of UC, they were treated with Bifidobacteria (added to sterilized diet as 1ml/140g for 3 days). Group 5: was treated with cyclosporine (1.12ml/140g for 3 days, blood sample was taken every 24 hr) to suppress the immune system. Group 6: was treated with cyclosporine and after reducing immune system, they were treated with Lactobacillus (1ml/140g for 3 days). Group 7: was treated with cyclosporine and after reducing immune system, they were treated with Bifidobacteria. Cyclosporine (trade name: Neoral Sandimmun, 25mg/capsule). Each capsule of the drug was melted in 10 ml olive oil and given orally to mice.

Media for Growing Probiotics: Luria Bertani (LB) media were used to grow the probiotic bacteria. The composition of media was (for 1000 ml): NaCl 5g, Tryptone 10g and yeast extract 10g. Bifidobacteria and Lactobacillus were cultured in LB media at 37°C for 18hr.

Immunoglobulin Assays: Blood samples were taken every 24 hr and serum was separated and kept frozen until being subjected to ELISA assays. Indirect ELISA was used to determine the levels of both IgE and IgM as indicators on the immune system. The assays were performed according to the manufacturer procedures (Sigma Aldrich, Germany).

Histopathological Examinations: One mouse of each group was slaughtered and dissected. A biopsy of colon tissue was removed to examine the UC features. Samples were kept frozen until being processed and prepared for the electron microscopy. Transmission EM was used to clarify the features of UC.

16S rRNA Analysis: Genomic DNA of the two probiotic bacteria under study was extracted according to Sambrook et al. [8]. The extracted DNA was subjected to PCR to generate the pattern specific to 16S rRNA. The PCR mix was as follows: 2 µl (about 40-60 ng) of the genomic DNA, 1.5 µl forward primer, 1.5 µl reverse primer, 12.5 µl master mix and 7.5 µl of D. H2O (the final volume was 25 µl). The PCR profile was: 94°C for 45 seconds, 58°C for 1 minute and 72°C for 2 minutes. A final extension step at 72°C for 10 minutes was applied. The number of cycles was 35. The oligonucleotides primers used in this study were purchased from LabTechnology (Promega Corp.). Primers PAF [5' AGA GTT TGA TCC TGG CTC AG 3'] position 8-27 (using the Escherichia coli numbering system) and 536R [5' GTA TTA CCG CGG CTG CTG 3'] position 519- 536 were used to amplify the 5' region of the 16S rRNA gene [9].

RFLP Analysis: The amplified product (16S rRNA fragment) was subjected to Restriction Fragment Length Polymorphism (RFLP) analysis by using three different restriction enzymes PstI (recognition site 5'-CTGCAG-3'), HindIII (recognition site 5'-AAGCTT-3') and BamHI (recognition site 5'-GGATCC-3') [10] (Fermintas life science). The reaction mix was as follows: 17µl D.H2O, 5µl buffer, 2µl enzyme and 5 µl DNA (PCR product). The mix was then incubated for 20 minutes at 37°C and the reaction was terminated by raising the temperature to 80°C for 10 min. The product was then separated on 1.2% agarose and then visualized on UV-transilluminator after being stained with ethidium bromide.

RESULTS AND DISCUSSION

16S rRNA Analysis: Phylogenetic analysis of rRNA genes, amplified by PCR, has been used as a rapid and efficient strategy to investigate the biodiversity of intestinal bacteria and revealed many novel species [11]. Although the diversity of the gut microbiota has been investigated extensively by anaerobic culture techniques, it is receiving renewed interest due to the development and application of molecular techniques, especially those based on the 16S and 23S rRNA genes [12, 13].
A 460-bp fragment could be obtained as a result of amplification of the 16S rRNA gene in both Lactobacillus and Bifidobacteria using the primers designed by Yeng et al.[9] (Figure 1). The data showed an increase in the levels of both IgE and IgM which gave 2 IU/ml and 28.2 mg/dl. On the other hand, when treating the mice with Lactobacillus after being treated with cyclosporine, the mice serum levels of both IgE and IgM were elevated as it showed 1.7 IU/ml and 26.7 mg/dl, respectively (Figures 2-5).

Several studies have demonstrated that Lactobacillus is able to boost the immunity of the host by producing the strong colonies in the intestinal tract, so that pathogenic bacteria are not able to create any destruction in the host body [14]. Meanwhile, probiotics are the viable microorganisms, which upon digestion exert...
Fig. 6: H and E stained section of specimen of the Control group

Fig. 7: H and E stained section of specimen of the UC-diseased group

Fig. 8: H and E stained section of specimen after treatment with Bifidobacteria or Lactobacillus

Fig. 9: H and E stained section of specimen after treatment with Bifidobacteria or Lactobacillus

Histopathological Examinations and Ulcerative Colitis:
Ulcerative colitis (UC) has been associated with a defective colonic mucus layer and reduced number of sugar residues [17]. The human gastrointestinal (GI) tract consists of different habitats, in which the entire colon is occupied by mostly obligatory anaerobic bacteria [18]. Data obtained indicated that the mice treated with intracolonic application of acetic acid [19] have developed health-promoting effects on host [15]. Our data indicated that probiotics were able to boost the immune system of mice used as host. This effect may be either direct by altering the resident microbiota or indirect by enhancing the gut barrier function and hence reducing the ability of commensal bacteria to activate the immune system as probiotics have been shown in many experimental models to stimulate regulatory T cells [16].

As a fact, a key requirement for probiotic strains is that they should not carry transmissible antibiotic resistance genes [20] and this was the case for the strains used in the present study. The amelioration observed in the UC may be partially due to the action of probiotics given to the mice as it may serve as a protector against the pathogens in the intestine and meanwhile results in vital benefits for developing healthy gastrointestinal function [21]. In the same context, it is widely believed that Bifidobacteria have an anti-infectious action and also the majority of the Lactobacillus strains produce bacteriocins and this explain the importance of these bacteria for the gastrointestinal tract [22, 23]. Improving the immune...
system through the positive influence on the intestinal flora and having anti-colon cancer effects is also a mechanism by which probiotics could promote UC [2]. Probiotics have also been used to treat other intestinal disease like Crohn’s Disease as researchers have made attempts on CD patients to control the severity of this disease by the administration of probiotics [24].

**RFLP Analysis:** The PCR product of 16S rRNA was subjected to three restriction enzymes. Under the applied conditions and the given concentrations, no pattern could be obtained and this may be due to lack of specific recognition site in *Bifidobacteria* and *Lactobacillus* 16S rRNA gene.

*Bifidobacteria* and *lactobacillus* are very promising probiotic bacteria to treat UC and to elevate the immune system parameters as (IgE and IgM) in mice. According to the obtained data, we recommend applying *Bifidobacteria* and *Lactobacillus* as food supplement to patients who are suffering from immune deficiency.

**REFERENCES**


