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HEAT KILLED PROBIOTIC LACTOBACILLUS PLANTARUM INDUCES NF KAPPA B FACTOR COMPARED WITH LIVE BACTERIA AND LIPOPOLYSACCHARIDES ON HEK – 293 CELL LINES

PRATHIBA SANDEEP* AND P. SARAVANA KUMARI[1]

[*]Research and Development centre, Bharathiar University, Coimbatore, India.
[1]Department of microbiology, Sree Narayana guru college, Coimbatore, India.

ABSTRACT

Probiotics are live microorganisms which have shown beneficial effects on human health.[1] Conditions susceptible to treatment with probiotics include traveler's, antibiotic-induced, and childhood diarrhea. Recently, several controlled clinical studies have also proven a role for probiotic therapy in different states of inflammatory bowel diseases.The aim of our study was to compare Heat killed probiotic bacterial strain of Lactobacillus plantarum with live bacteria with respect to innate defense mechanisms in the HEK-293 cells. Here we report *L.plantarum* tested to induce the NF -κB activation or expression in HEK 293 cell lines in a time - and dose-dependent manner. Activation of NF -κB through Heat killed bacteria was confirmed by fold change in HEK-293 cell lines. Luciferase gene reporter analyses assay experiments demonstrated that NF -κB activation is seen in HEK-293 cell lines through Heat killed cells of L.plantarum. This report demonstrates that Heat killed probiotic bacteria, L.plantarum can stimulate the immune cells by induction of NF –κB.

KEYWORDS: **Renilla luciferase enzyme, Probiotics, HEK-293 Cell lines, *L.plantarum.***

PRATHIBA SANDEEP
Research and Development centre, Bharathiar University, Coimbatore, India

INTRODUCTION

Probiotics are live microorganisms which have shown beneficial effects on human health.[1] Conditions susceptible to treatment with probiotics include traveler's, antibiotic-induced, and childhood diarrhea. Recently, several controlled clinical studies have also proven a role for probiotic therapy in different states of inflammatory bowel diseases [2, 3]. The convincing outcome of these clinical trials using probiotic bacteria has encouraged us to further explore the mode of action of these bacteria. Several modes of probiotic action have been considered. Bacterial interference with intestinal pathogens is a well-established mode of action[4, 5]. May be mediated by bacteriocins [6, 7, 8]. Specific antimicrobial substances that antagonize intestinal pathogens. However, probiotics also appear to directly affect mucosal immune function through modulation of immunoglobulin A (IgA) synthesis, mucus formation, or alterations of the pro-versus anti-inflammatory balance of local cytokines 9.Recent findings raise the possibility that microbe-host cell signaling might be a mode of action by which probiotic bacteria could stabilize intestinal microbiology and effectively prevent colonization by enteric pathogens[10]. Prompted by studies on defensins in colonic mucosa [11, 12]. We hypothesized that probiotics may act through an induction of these endogenous antibiotics. Probiotic bacteria that interact with the host epithelium to resolve inflammation. Probiotics have been defined as live microbial food supplements that beneficially affect the host by improving its intestinal microbial balance[13]. The most widely used probiotics in humans are bifidobacteria and lactobacilli. Lactic acid bacteria (LAB) are safe microorganisms that improve disturbances in the indigenous microflora, ameliorate the development of microflora[14]. Have anti-diabetic and anti-hyperlipidemia effects[15, 16]. Inhibit carcinogenesis, have anticolitic effects[17]. And induce nonspecific activation of the host's immune system[18]. Nevertheless, the anticolitic mechanism of LABs has not been thoroughly examined. Therefore, this study was conducted to demonstrate the probable therapeutic effect of probiotics in patients with UC, and to evaluate their effect on the inflammatory mediators and nuclear factor (NF)-κB activation in these patients. Intestinal epithelial cells (IEC) are the first point of contact for bacteria within the gut lumen, and they interact with the gut immune system. Consequently, IEC have a pivotal function in bacteria-host communication. Bacterial signatures generally activate signaling cascades that can trigger proinflammatory gene transcription through specific receptors (e.g., Toll-like receptors) expressed on apical and/or basolateral surface of epithelial cells. This mechanism is largely controlled by the transcriptional factor NF-*κ*B. NF-*κ*B is a dimeric DNA binding protein whose major form is represented by the association of p65 and p50 proteins. In steady state, NF-*κ*B is locked in the cytoplasm by an inhibitory protein of the I*κ*B family. Upon receptor activation, I*κ*B is phosphorylated by the I*κ*B kinase complex (IKK) before undergoing degradation by the proteasome. Then, free NF-*κ*B translocate to the nucleus to turn on a large number of genes involved in proinflammatory processes at the site of infection or tissue damage[19].

MATERIALS AND METHODS

Bacterial preparation

The Gram positive *Lactobacillus plantarum* culture was procured from ATTC cultures(ATCC- 8014) The bacteria were grown in Deman Rogosa Sharpe (MRS) broth for 24 hours 37 ℃. The whole cells were obtained by centrifugation at 6000* rpm for 10 min which was washed twice with sterilized phosphate buffer at pH 7.2. The heat killed *Lactobacillus plantarum* was prepared by killing them by increasing heat in the water at 100 ℃ for 15 min, the mixture was mixed with 20% Heat killed Lactobacillus plantarum and 80% dextrin and used for further studies.

Cell Culture and Reagents

Human Embryonic kidney cell line (ATCC - CRL-1573) was grown in DMEM-Dulbaccos modified Agar medium with L-glutamine and 4.5 g L glucose, supplemented with fetal bovine serum, 100 units of penicillin G and 0.1 mg of streptomycin sulfate in a humidified atmosphere of a 5% CO_2 at a 37 ℃ [20].

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Analysis of NF-κB Activation

Hek-293 reporter cells were seeded at 100,000 cells per well, into 96-wells plates and cells were stimulated with 10% (vol/vol) of Heat killed Lactobacillus bacterial cells in comparison with Live cells after 8 hours of growth, keeping LPS as control. Cells were then incubated for 24 hours, later cells were harvested using 250micro liter of passive lysis buffer (promega) per well. Renilla Luciferase activity were analyzed with lusiferase repoter assay, promoter activity was normalized to the activity of the Renilla luciferase control.[21]

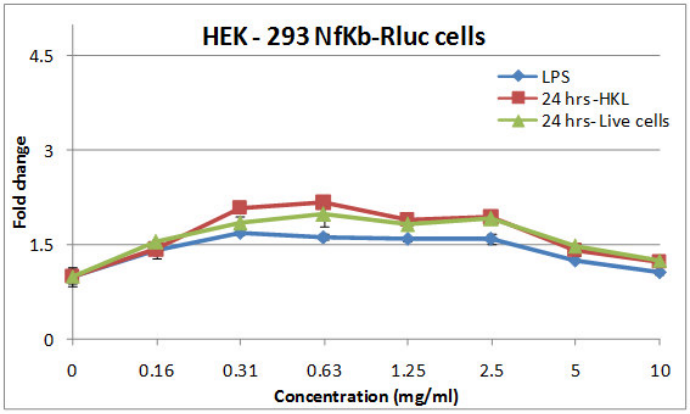
RESULTS

Analysis of NF-κB Activation

The HEK-293NF kappa B reporter cells were incubated with live Lactobacillus plantarum, heat killed lactobacillus plantarum and lipopolysaccharides as control with different concentrations, later the harvested cells were taken for luciferase assay. The present report clearly indicates that the heat killed probiotic strain induces NF kappa b activation in HEK – 293 NF kappa b Reporter cell lines when compared with LPS and live bacterial cells.[22].

Table 1
Luciferase assay results

Concentration mg/ml	LPS – 24 hours	Live cells-24 hours	HKL-24 hours
0	1	1	1
0.16	1.40787878	1.536101522	1.423757172
0.31	1.68729384	1.843132404	2.090099647
0.63	1.627427862	1.976325131	2.161094001
1.25	1.59201401	1.817529484	1.903483377
2.5	1.594234644	1.90742428	1.932760115
5	1.248433932	1.467707297	1.413297772
10	1.061288767	1.24644057	1.220351946



DISCUSSION

In this study we have investigated that there is an effect of heat killed probiotic - *Lactobacillus plantarum* on HEK 293 reporter cells in comparison with live L*actobacillus plantarum* and lipopolysaccharides. The HEK 293 cells were incubated for 24 hours on different concentrations of lipopolysaccharides, live probiotics and heat killed *L. Plantarum.* The effect is clearly proven through NF kappa B indication on cells by Heat killed probiotics. LPS induces NF kappa B indication which is taken as control along with live probiotics on different concentrations which was incubated for 24 hours and assay results clearly indicates that heat killed probiotics induces immune system by activation of NF kappa B factor.[22.]

CONCLUSION

With respect to the above study it has been concluded that Heat killed probiotic L.plantarum induces NF kappa B factor in HEK 293 cell lines, in comparison with live probiotic cells and Lipopolysaccharides.

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To my sweet family and my Guide.

CONFLICT OF INTEREST

I declare there is no conflict of interest in this study.

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