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NF-κB in Anti-Inflammatory Activity of Probiotics: An Update

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Abstract: The NF-κB (nuclear factor κappa B) belongs to a family of transcription factors that regulate a number of key cellular pathways and control many physiological processes, including the immune and inflammatory responses. The beneficial effect of probiotics is presently recognized in both the intestinal and extra-intestinal development. The growing literature on the subject suggests an important role of specific probiotic strains, mixtures of strains, and secreted products, all of which have a role in either the preventive or therapeutic treatment of certain human disorders. During the last two decades, a number of studies have shown that probiotic strains and their extracellular products can join the immune system and activate receptors in different key pro-inflammatory pathways such as the NF-κB route, either by increasing or suppressing the signaling pathways. This brief review does not include an exhaustive bibliography of the subject, but focuses instead on an update to the latest research of the past three years on the role of the NF-κB signaling pathway in the anti-inflammatory effect of probiotics.

Keywords: NF-κB, probiotic, signaling, inflammation, probiotic-host interaction, probiotic anti-inflammatory activity.

INTRODUCTION

The NF-κB family of transcription factors is a signaling pathway that controls the expression of several genes. These genes control key physiological processes such as cell growth, survival and cell adhesion, the inflammatory and immune responses, and the responses of oxidative stress and apoptosis [1]. Moreover, many human diseases such as cancers and inflammatory diseases are related to the deregulation of the NF-κB signaling pathway [1]. Therefore, regulation of NF-κB can be used as a target to either treat or ameliorate symptoms in several human diseases. Lactic acid bacteria (LAB) are a heterogeneous group of microorganisms present in many foods. Some specific LAB strains are beneficial to the host either human or animal, and are known as probiotics. In addition, a diverse group of functional microorganisms, including probiotics, or potentially viable microorganisms that benefit the host, are included in the intestinal microbiota. The use of probiotics, as either potentially preventive or therapeutic agents has been studied in several diseases, including inflammatory disorders [2]. Increasing evidence has accumulated over the past fifteen years demonstrating that probiotics interact with the host through the modulation of key signaling pathways, including NF-κB that either enhances or suppresses the cellular responses derived there from [2]. The aim of this minireview is to summarize and discuss the latest research on the modulation by probiotics of the signaling pathway NF-κB in inflammatory conditions.

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THE NF-κB PATHWAY

The route of NF-κB in mammals is a multi-component complex signaling pathway. This signaling pathway includes positive and negative regulatory elements that activate a group of transcription factors known as NF-κB. Five structurally related members, RelA / p65, RelB, c-Rel and synthesized as precursor proteins, p50 (p105 / NFKB1) and p52 (p100 / NFKB2), are the NF-κB family of transcription factors [3].

Under non-stimulating conditions, NF-κB is present in the cytoplasm in an inactive form that interacts with any of several the IκB inhibitory proteins (IκBα, IκBβ, IκBε, NF-κB p105, p100 NF-κB) [3]. IκB degradation is controlled by IκB kinase complex (IKK), which consists of the catalytic subunit (IKK / β) and non-catalytic subunits (for example, NF-κB essential modulator or NEMO). A number of physical and chemical stimuli both, endogenous and exogenous, activate IKK, resulting in the phosphorylation, ubiquitination and degradation of the IκB proteins by the proteasome [1].

Among the many ways under which NF-κB is activated, the most common are the classical or canonical, and the non-canonical or alternative pathways (Fig. **1**) that differ in the mechanisms that induce the activation of the IKK complex [1]. In the canonical pathway the IKK / IKKβ heterodimer (or IKKβ homodimer), in a complex with NEMO, phosphorylates IκB, with the release of NF-κB dimers p50- or p-65. In the non-canonical pathway the NF-κB-inducing kinase (NIK) phosphorylates the IKK homodimer, leading to the induction of the p100-RelB complex. Once NF-κB is released from IκB, it migrates to the nucleus, where it binds and either activates or enhances specific gene transcription promoters. Various combinations of NF-κB heterodimers

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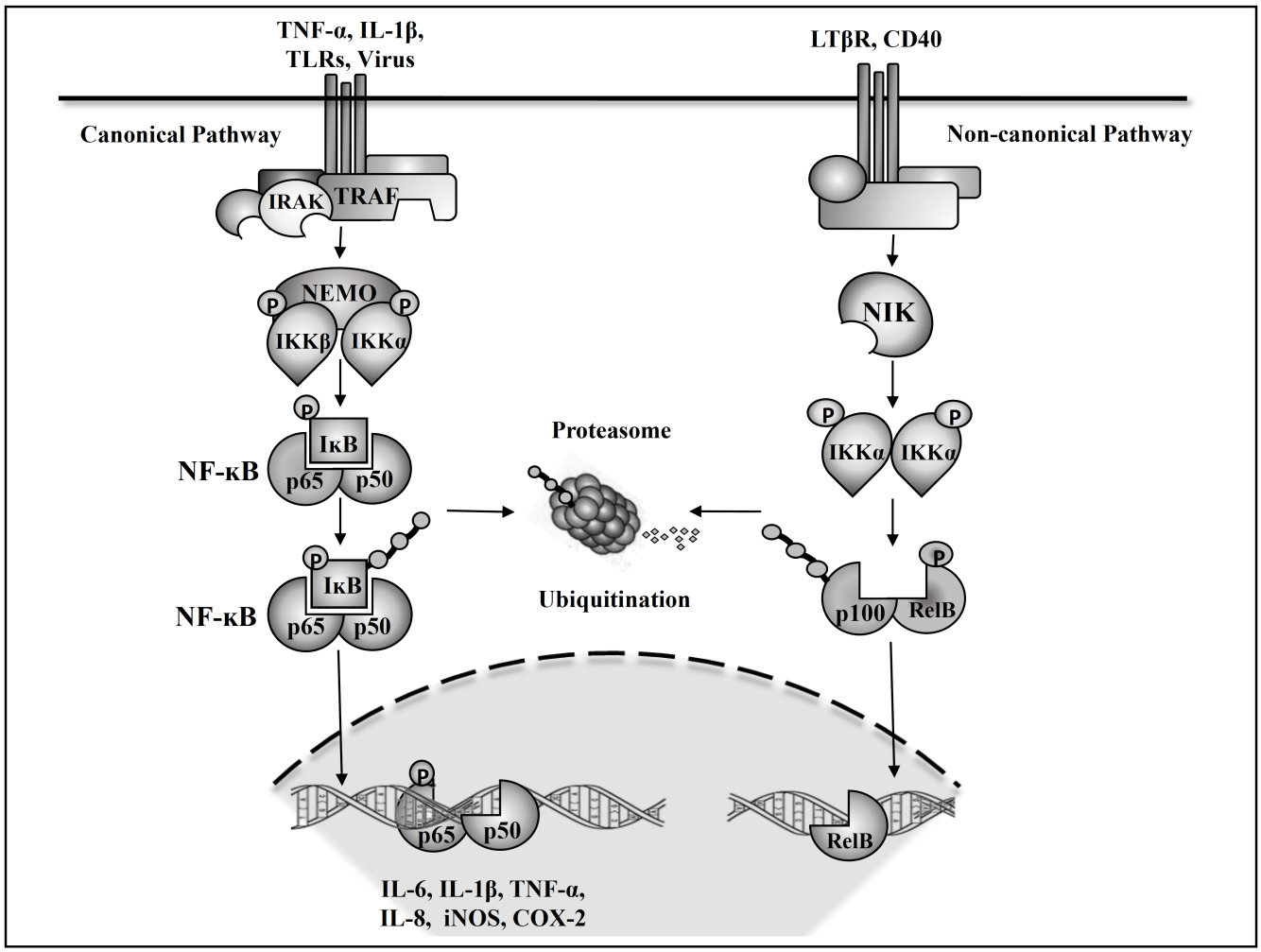

Fig. (1). NF-κB signaling pathways. Different extracellular effectors can initiate the canonical and non-canonical NF-κB pathways. Induction stimuli trigger IKK activation leading to phosphorylation, ubiquitination, and degradation of IκB proteins in the canonical pathway or p100 in the non-canonical pathway. Released NF-κB dimers translocate to the nucleus, where they bind to specific DNA sequences, promoting the transcription of target genes.

and structurally associated homodimers are present in mammalian cells, and exhibit different affinities for the target DNA binding sites, and protein-protein interactions in DNA. Thus, different transcriptional responses in different cell types or under different stimuli are generated [1, 3].

NF-κB IN INFLAMMATORY DISEASES

Since the discovery of NF-κB by Sen and Baltimore in 1986 [4], this signaling pathway has been one of the most studied by researchers worldwide, since it is a vital intracellular regulator that connects to several environmental cues with the activation of many cellular genes. Since deregulation of this signaling pathway is involved in many human disorders [3], the search for agents which regulate the signaling pathway NF-κB is continuously ongoing.

Several human diseases and chronic inflammatory disorders associated with activated NF-κB, including those induced by microorganisms and viruses [1]. When the human body interacts with some microorganisms or is exposed to irritating molecules, an immune response is activated in order to fight infection and to prevent tissue damage. The expected result of inflammation is the protection of the spread of infection, followed by resolution-tissue restoration to normal function [5]. Tissue mononuclear phagocytes, which are cells that activate during the early innate immune response, recognize pathogens and tissue damage molecules through pattern recognition receptors (PRRs). PRRs have the specific capacity to recognize directly or indirectly essential structures for the survival of microbes, known as microbe associated molecular patterns (MAMPs). MAMPs are widely conserved and distributed among organisms, and include microbial nucleic acids, cell wall lipopolysaccharide (LPS) and proteoglycans, and cell wall components of fungi such as alpha -mannan and β-glucan. These molecular patterns also include danger/damage associated molecular patterns (DAMPs) released by injured cells, including nucleic acids, uric acid, adenosine triphosphate (ATP) and β amyloid molecules [6]. When the inflammatory response is either excessive or lower than normal, inflammation can progress from acute to chronic, settling for long periods. Chronic inflammation involves tissue infiltration by lymphocytes, dendritic cells and macrophages, to produce large amounts of various reactive compounds, including cytokines, chemokines,

growth factors, reactive oxygen and nitrogen species that can cause tissue damage with lasting responses in time [7]. Furthermore, several families of virus activate the NF-κB pathway in a number of ways to promote the process of viral replication or prevent virus-induced apoptosis. For example, the promoter-proximal (enhancer) region of HIV-1 contains NF-κB binding sites controlling HIV-1 inducible gene expression [6].

Toll-like (TLR) and other inflammatory signaling pathways including the PRR, NLRs group (receptor-like nucleotide oligomerization domain) receptors and mediated by C -type lectin receptors have signal transduction pathways through the NF-κB [8]. Furthermore, an abnormal expression of NF-κB is present in inflammatory diseases. This is in addition to the activation of pro-inflammatory cytokines, cell proliferation, the expression of anti-apoptotic genes, and increased angiogenesis through vascular endothelial growth factor, all of which are induced by NF-κB [8]. For example, in the pathogenesis of asthma there is a continuous expression of genes containing the κB site within the promoter, indicating a role of NF-κB in the initiation and maintenance of processes such as allergic inflammation. Pro-inflammatory cytokines, chemokines, and adhesion molecules and inflammatory enzymes are encoded by these genes [9].

NF-κB is also involved in the initiation and perpetuation of rheumatoid arthritis (RA), with proliferation of synovial fibroblast-like cells and activation of bone resorption by osteoclasts [10]. Studies of patients with Crohn's disease, a chronic inflammatory bowel disorder, showed an increase in NF-κB in colon biopsies and mononuclear cells in the lamina propria [11]. Many other human inflammatory disorders and diseases are associated with activation of NF-κB, including chronic obstructive pulmonary disease (COPD) [12]; acid injury lung [13] induced inflammatory bowel disease (IBD) [14]; peritoneal [15] endometriosis; Behcet's disease [16]; psoriasis [17]; periodontitis [18]; lupus erythematosus [19] and the antiphospholipid syndrome [20]. Moreover, activation of NF-κB participates not only in the viral replication process during some types of viral infection, but it also acts by inducing a host protective inflammatory response [21]. For more detail regarding NF-κB and diseases / disorders, see [22]. In short, external factors such as microorganisms, virus and pro-inflammation irritant molecules act through NF-κB signaling. Further, NF-κB dysfunction is also involved in autoimmune and inflammatory disorders.

PROBIOTICS

The World Health Organization (FAO / WHO) and the Food and Agriculture Organization of the United Nations defines probiotics as "live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host" [23]. The beneficial effects of probiotics have been studied both *in vitro* and *in vivo*, which include modulatory immune responses by regulating the cytokine profile, increased antibody secretion, reduced inflammation and increased intestinal epithelial cell function [2, 24]. Probiotics are indigenous and exogenous bacterial species in particular of the intestinal genus *Lactobacillus* and *Bifidobacterium*, which interact with dendritic cells (DC) and intestinal epithelial cells (IEC) through PRRs. PRRs identify bacterial surface molecules, the MAMPs, such as LPS, flagellin, lipoteichoic acid (LTA) and peptidoglycan [25]. Sometimes, the cell-bacteria interaction includes cooperation with co-receptors present on groups of multi-receptors present in lipid rafts, which are specific cell membrane microdomains [26]. The MAMPs-PRRs interaction triggers cellular signaling pathways such as the mitogen-activated protein kinase (MAPK) and NF-κB. These signaling pathways are initiated at the surface and transmitted to the core, resulting in a specific cellular response that includes cytokine expression, mucin secretion by IEC and Ag presentation by DC [24, 25].

Surprisingly, while the traditional definition assumes that the probiotic bacteria have to be alive to exercise the health-promoting effects [2], a substantial body of scientific evidence has shown that some of the mechanisms of action of probiotic LAB are produced by some of their own cell surface and extracellular associated compounds. In fact the administration of metabolites either derived or isolated from bacteria, such as exopolysaccharides, proteins / peptides or LTA may be sufficient to promote the desired beneficial effects and may represent a safer alternative inflammatory disorders [24, 27 - 30]. Thus, both live bacteria as well as associated soluble and secreted factors can have a probiotic effect.

MODULATION OF SIGNALING *VIA* NF-κB BY PROBIOTICS

Despite the fact that the potential of probiotics efficacy in a variety of diseases has been well demonstrated, their mechanisms of action are not yet fully known. The host-probiotic cell interaction involves the modulation of key signaling pathways to enhance or suppress the activation of downstream signaling pathways [31, 32]. Several different stimuli induce immune responses *via* the NF-κB signaling pathway (Fig. **2**). As described above, under non-stimulating conditions, NF-κB is in its inactive form in the cytoplasm, bound to the inhibitor molecule, IκB. Under stimulated conditions, the different steps of the signaling pathway, namely IκB phosphorylation by IKK, ubiquination, and degradation by the proteasome complex, translocation to the nucleus and transcription of the target gene, are activated and may be targeted by a probiotic to either enhance or suppress the cellular response. (Fig. **2** and Table 1). Each probiotic strain can modify this route in different ways inducing a different response [2]. Lebeer *et al*. [24] have suggested that the final outcome of the probiotic-induced host cell response will depend on the combination and concentration of both the various MAMPs that can interact with PRRs and their associated co-receptors. In addition, two important factors that determine the responsiveness of the host cells are the accessibility of PRRs for MAMPs (i.e., the subcellular distribution, compartmentalization and the levels of expression of PRRs in different tissues) and direct or indirect negative regulators, derived from PRR signaling [25].

While more studies on the anti-inflammatory effect of probiotics have been conducted in intestinal tissues [2], several studies have suggested that the potential effects of these bacteria are not limited to the intestine. These studies have shown that the immune system of other tissues as the

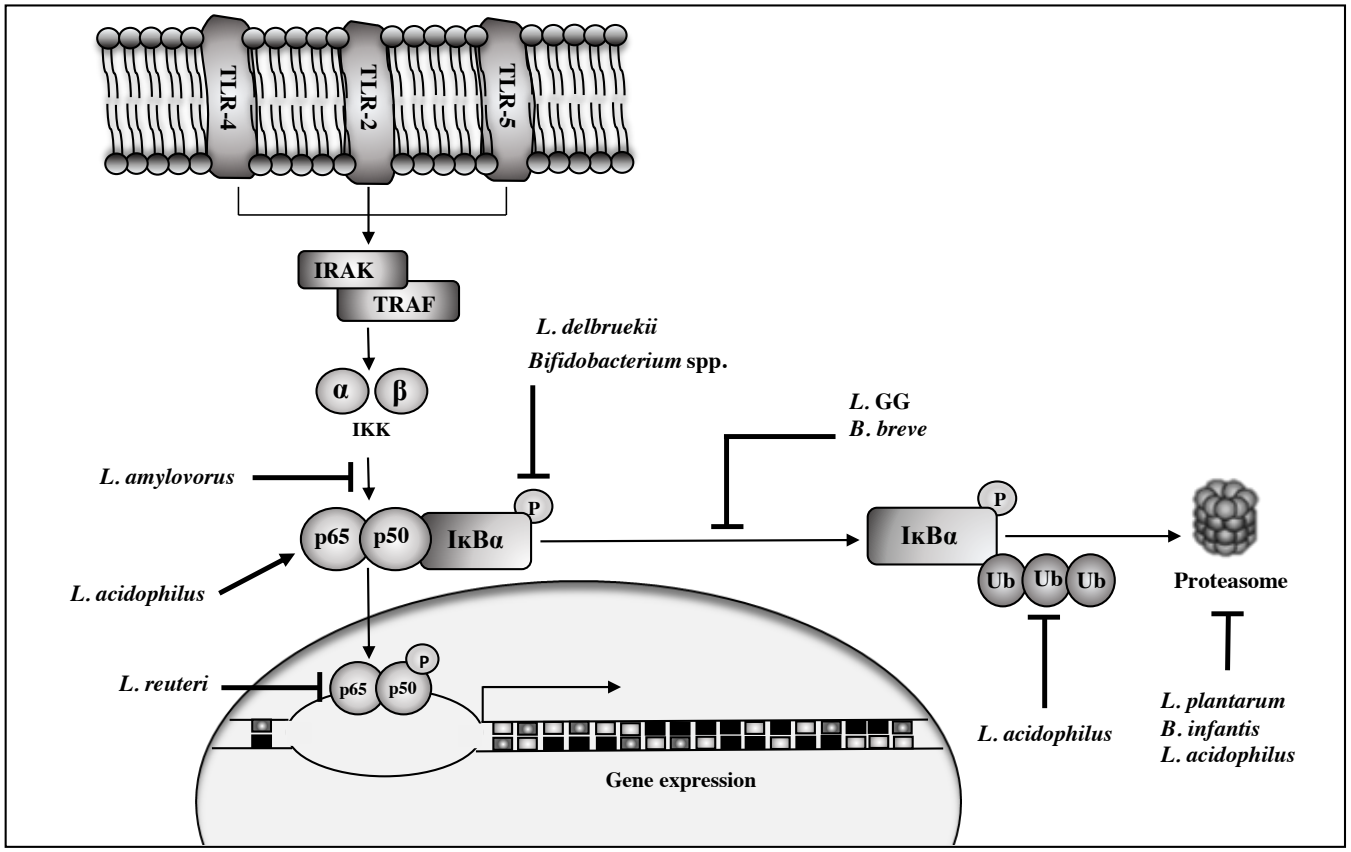

Fig. (2). Probiotics modulate NF-κB signaling pathways. Probiotics interact with the host by modulating key signaling pathways, to enhance or suppress activation of downstream signaling pathways. Several probiotics prevent NF-κB activation by inhibiting IκBα phosphorylation, ubiquitination, proteasomal degradation, or NF-κB translocation into the nucleus (⊣). Other probiotics increase NF-κB activation through enhanced NF-κB p65 phosphorylation (→). IRAK: Interleukin receptor-associated kinase; TRAF: TNF receptor associated factor; TLR: Toll-like receptor.

urogenital mucosa can be modulated by these bacteria [33]. The probiotic *Lactobacillus rhamnosus* GR-1, isolated from a female urethra, activated NF-κB in *Escherichia coli*-stimulated T24 bladder cells by increasing levels of TLR4. This NF-κB increased levels of TNF-alpha, whereas IL-6 and CXCL8 levels were reduced. TLR4 modulation is one of the mechanisms used by probiotic to fight pathogens [33, 34]. Interestingly, *L. rhamnosus* GR-1 and *Lactobacillus reuteri* RC, both probiotic strains used in commercial products to maintain vaginal health, suppressed *Candida albicans*-induced Iκκα, TLR2, TLR6, IL-8, and TNF-α, in the vaginal epithelial cell line VK2. These lactobacilli induce the expression of IL-1α and IL-1β mRNA that is not suppressed by an inhibitor of NF-κB, suggesting that their mechanism of action may induce an alternative inflammatory signal transduction pathway such as the MAPK/Ap-1 [35]. These two results, both activation and inhibition of NF-κB by *L. rhamnosus* GR-1 could be explained by different PRR levels in the host cells, and their different effectors *E. coli* and *C. albicans* [24].

Platelet activating factor (PAF), a potent bioactive phospholipid responsible for causing intestinal injury, is implicated in the pathogenesis of IBD and necrotizing enterocolitis (NEC) [36, 37, 38]. The severity of the disease correlates with high levels of PAF in tissues and / or serum of patients with Crohn´s disease, ulcerative colitis, and NEC [39]. PAF-induced inflammation is reduced by *Lactobacillus acidophilus* ATCC 4357-conditioned media, by blocking IκB phosphorylation in human IECs. Moreover, increased ubiquitination in response to PAF is substantially attenuated by *L. acidophilus*-conditioned medium [36]. Another strain, *L. acidophilus* LA5®, has been tested for anti-inflammatory effects in MKN45 gastric cells incubated with *Helicobacter pylori*. Pre-treatment with *L. acidophilus* increased cytoplasmic IκB that decreased the levels of nuclear NF-κB, and significantly reduced the Smad7 pathway. Further, TNF- and IL-8 levels also decreased, indicating that this strain could neutralize *H. pylori*-induced gastric inflammation through the NF-κB pathway [40]. A study by Jiang *et al.* [41] investigated the effects of a different L. *acidophilus* strain in a model of unstimulated intestinal epithelial cells. Their results showed an increased phosphorylation of p65 NF-κB by *L. acidophilus* NFCM. Moreover, the NF-κB inhibitor pyrrolidine dithiocarbamate [PDTC], significantly reduced the secretion of IL-1α, IL-1β, CCL2 and CCL20 secretion by intestinal epithelial cell lines treated with *L. acidophilus* NCFM. Thus, the NF-κB signaling pathway was involved in the production of cytokines and chemokines modulated by the NFCM strain. Similar results were described by Klaenhammer and O'Flaherty [42]. Their work showed an increase in the expression of target genes *via* NF-κB in human intestinal cells treated with *L. acidophilus*

Table 1. Probiotic Modulation of NF-κB Signaling Pathway in Different Models.

Probiotic	Experimental Model	Observed effect	Reference
NF-κB Pathway Activation			
Bifidobacterium bifidum PRL2010	Caco-2 cells	Increases of NF-κB activation	[49]
Lactobacillus acidophilus NCFM	Caco-2 cells	Increases NF-κB p65 phosphorylation	[41, 42]
Lactobacillus casei ATCC 11578	NIH-3T3 cells	Induces NF-κB activation	[48]
Lactobacillus rhamnosus GR-1	T24 cells	Increases NF-κB activation	[33, 34]
Lactobacillus jensenii	End1/E6E7 epithelial cells	Induces NF-κB activation	[59]
NF-κB Pathway Inhibition			
Bifidobacterium strains	HT-29 cells	Inhibit IκB phosphorylation	[43]
Bifidobacterium breve DSMZ 20213 and LGG ATCC 53103	IMIS	Inhibit NF-κB p50/p65 subunits	[47]
Bidobacterium infantis ATCC	Rat ileum lysate	Stabilizes the NF- κB/Iκκα complex	[44]
Lactobacillus acidophilus	CMT93 cells	Inhibits NF-κB activation	[60]
Lactobacillus acidophilus LA5®	MKN45 cells	Decreases nuclear NF-κB	[40]
Lactobacillus acidophilus TCCC 11036	HT-29 cells	Decreases NF-κB transcriptional activity	[46]
Lactobacillus acidophilus ATCC 4357	NCM460 cells	Atenuates IKKγ (NEMO) ubiquitination	[36]
Lactobacillus amylovorus DSM 16698T	Pig intestinal explants and Caco-2 cells	Supress P-IKKα, P-IκBα	[54]
Lactobacillus brevis G-101	Mice peritoneal Θ	Inhibits NF-κB activation	[68]
Lactobacillus casei	KATO3 cells	Decreases NF-κB and IκB expression	[61]
Lactobacillus casei	Mice peripheral blood	Decreases NF-κB levels	[62]
Lactobacillus delbruekii	HT-29 cells	Reduces IκB phosphorylation	[45]
Lactobacillus jensenii TL2937	Porcine intestinal epithelial cells	Inhibit NF-κB activation	[53]
Lactobacillus helveticus HY7801	Mice vaginal tissues	Decreases NF-κB p- p65 levels	[58]
Lactobacillus plantarum WCFS1	HEK-293 cells	Decreases NF-κB activation	[63, 64]
Lactobacillus plantarum HY7712	Mice perithoneal Θ	Decreases NF-κB p- p65 levels	[57]
Lactobacillus plantarum ATCC14917, *Lactobacillus acidophilus* ATCC 53544 and *Bifidobacterium infantis* ATCC 15697	Rat intestinal explants	Preserve IκBα expression	[52]
Lactobacillus plantarum MTCC 2621	Rat liver	Reduces NF-κB	[65]
Lactobacillus plantarum HY7712	RAW 264.7cells	Inhibits NF-κB activation	[56]
Lactobacillus rhamnosus LGG	Co-culture model	Modulate NF-κB p65 translocation and p-p65 activation	[66]
L. rhamnosus GR-1® and *L. reuteri* RC-14®	VK2 cells	Decrease Iκκα and Iκκβ expression	[35]
Lactobacillus reuteri CRL 1098	RAW 264.7 cells	Reduces p65 NF-κB translocation	[55]
Lactobacillus reuteri ATCC PTA 4659 and DSM 17938	Rat Intestinal explants	Inhibit IκB phosphorylation	[50]
L.actobacillus salivarius B101, *Lactobacillus rhamnosus* B103 and *Lactobacillus plantarum* XB7	AGS cells	Suppress NF-κB activation	[67]
VSL#3	Rat colon homogenates	Decreases NF-κB expression	[51]

NFCM. These studies collectively demonstrated the effect of different strains belonging to the same species as had previously been described by Thomas and Versalovic [2]. The studies further supported the idea that only studies of challenge where each bacterial strain tested in each experimental model will provide the appropriate conclusion as to the strain-specific response to their anti-inflammatory properties.

Many other studies with IECs described the inhibitory effect of probiotics on the NF-κB signaling pathway. Conditioned media obtained from various strains of *Bifidobacterium*, for example, inhibit in a dose and time-dependent manner IL-8-induced expression of TNF-α in human colon cells stimulated with LPS from *E. coli* 055:B5. Conversely, this medium also decreased the expression of NF-κB target genes, suggesting that bifidobacteria may

actually produce anti-inflammatory effect(s) at least in part, through the inhibition of IκBα phosphorylation [43]. Another study showed that *Bifidobacterium infantis* ATCC 15697 (BICM)-conditioned medium protected against *Cronobacter sakazakii*-induced intestinal inflammation in newborn mice [44]. Pretreatment with BICM restored reduced levels of IκB in the infected ileum. In addition, nuclear translocation of NF-κB p65 was observed in *in vitro* assays using H4 cells exposed to *C. sakazakii*. This effect was prevented in cells pretreated with BICM. Another study showed that dairy *Lactobacillus delbrueckii* strains diminished IκB phosphorylation in TNF-α treated HT-29 cells, suggesting a potential use of these strains in the treatment of chronic inflammation disorders like IBD [45]. Using the same human HT-29 experimental model, Chen *et al.* [46] demonstrated that live *L. acidophilus* 11036 TCCC negatively regulated gene expression of inducible nitric oxide synthase (iNOS) and prostaglandin endoperoxide synthase-2 (PTGS-2) associated with the inflammatory response. Moreover, as expected, *L. acidophilus* decreased NF-κB transcriptional activity, because there are binding sites for NF-κB in the iNOS, PTGS-2 and IL-8 promoters. Another study explored the effects of *Bifidobacterium breve* (DSMZ 20213) and *L. rhamnosus* GG (LGG) (ATCC 53103), as representative of six commensal probiotics, on IL-17 and IL-23 expression, two cytokines that play an important role in IBD. *B. breve* and LGG significantly inhibited basal and LPS-induced IL-17 secretion in a 3D co-culture model composed of human intestinal HT-29/B6 or T84 cells and PBMCs. In parallel, NF-κB nuclear translocation diminished due to a decrease in IRAK1 and IκBα, with the subsequent reduction of cytokine gene expression both *in vitro* and *in vivo*. This study described a novel regulatory mechanism by which commensal probiotics inhibit the NF-κB-mediated transcriptional activation of inflammatory genes [47].

Not all probiotic strains inhibit the activation of NF-κB. Some strains stimulate NF-κB leading to an increased gene expression. Saito *et al.* [48] showed that *Lactobacillus casei* ATCC 11578 cell wall extract significantly enhanced NF-κK phosphorylation and cell proliferation of NIH-3T3 fibroblast. Another study, demonstrated that *Bifidobacterium bifidum* PRL2010 increased NF-κB activation and IL-8 production in Caco-2 cells treated with IL-1β [49].

Modulation of the NF-κB signaling pathway has also been investigated in animal models of inflammation. A study by Liu *et al.* [50] demonstrated that the strains DSM 17938 and ATCC PTA 4659 of *L. reuteri* decreased inflammation in an experimental model of NEC in neonatal rat. Pretreatment of small intestinal explants from newborn rats with two *L. reuteri* strains significantly inhibited LPS-induced IκB phosphorylation. Another study by Dai *et al.* [51] investigated the effects of VSL#3, a probiotic- mixture (containing *Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *L. acidophilus*, *Lactobacillus plantarum*, *L. casei*, and *Lactobacillus bulgaricus*), on dextran sulfate sodium (DSS)-induced colitis in rats. The results showed that VSL # 3 exerts an anti-inflammatory effect by inhibiting PI3K / Akt and the NF- κB pathway. In addition, the synergistic protection of combined probiotics, *L. plantarum* ATCC 14917 (Lp), *L. acidophilus* ATCC 53544 (La) and *B. longum* sbsp. *infantis* ATCC 15697 (Bi) conditioned media (CM) against NEC like intestinal injury was investigated in rats. Lp, La/Bi, and La/Bi/Lp CM significantly decreased p-NF-κB- positive intestinal epithelial cell numbers in both control and diseased animal groups. All CM preserved IκBα expression in the diseased animal group, suggesting that the anti-inflammatory effect of these CM could be mediated through the NF-κB signaling pathway. Also, proteasome of control animals showed a higher chymotrypsin-like activity compared to Lp and La/Bi CM-treated animals, indicating that these CM inhibited proteasome activity *in vivo* [52]. The IκBα degradation inhibition may be the result of a block in ubiquitination or in the proteasome itself. Different probiotic species inhibit both of these components of the NF-κB activation pathway; however, as the protection mechanism of probiotics depends on the specific probiotic strain and particular disease model, the mechanism must be studied in each case [2]. Shimazu *et al.* [53] demonstrated that *Lactobacillus jensenii* TL2937 attenuated LPS- and enterotoxigenic *E. coli* (ETEC)-induced NF-κB and MAPK activation and expression of pro-inflammatory cytokines in a porcine intestinal epithelial cell line. In another study, *Lactobacillus amylovorus* strain DSM 16698T and its cell free supernatant completely abolished high levels of P-IKKα, P-IκBα, and P-p65 induced by ETEC in pig intestinal explants and Caco-2 cells. Moreover, *L. amylovorus* did not activate the TLR4 cascade; the P-p65 did not translocate into the nucleus after *L. amylovorus* addition, indicating its capacity to prevent or reduce the inflammatory response to ETEC in piglets [54].

The probiotic effects mediated by the NF-κB signaling pathway were additionally explored in immune cells such as macrophages. Investigating the mechanism of action of the anti-inflammatory capacity of soluble factors from *L. reuteri* CRL 1098 (Lr-SF), Griet *et al.* [55] found that Lr-SF significantly diminished pro-inflammatory mediators (NO, COX-2, and Hsp70) and pro-inflammatory cytokines (TNF-α, and IL-6) induced by LPS in RAW 264.7 cells. In addition, Lr-SF reduced NF-κB p65 subunit translocation into the nucleus of LPS-treated cells. In another study on the probiotic capacity to restore the aging-impaired immune responses, *L. plantarum* HY7712 potently induced NF-κB activation in RAW 264.7 macrophages, but inhibited LPS-stimulated NF-κB activation. Moreover, *L. plantarum* HY7712 protected against the downregulation of interferon (IFN)-γ and upregulation of IL-13 caused by γ-irradiation in mice [56]. Another study showed that *L. plantarum* HY7712 improved cyclophosphamide-induced immunosuppression in mice, inducing TNF-α expression in peritoneal macrophages *via* NF-κB activation. However, *L. plantarum* inhibited NF-κB activation due to reduction P-p65 NF-κB expression, in LPS-stimulated mice peritoneal macrophages [57].

Several other studies demonstrated that probiotics act through the NF-κB pathway in other epithelial cells. For example, *Lactobacillus helveticus* HY7801 improved vulvovaginal candidiasis symptoms in mice, inhibiting NF-κB activation. In addition, *L. helveticus* inhibited COX-2, iNOS and P-p65 NF-κB expression in vaginal tissue of *C. albicans* infected mice [58]. Wild type (WT) *Lactobacillus jensenii* 1153 and its engineered derived strains activated NF-κB in endocervical epithelial cells; however, microorganisms did not induce significant changes in the secretion of IL-1α, IL-1β, IL-6 IL-8 and TNF-α [59].

In summary, probiotic strains of different bacterial species interact with host cells by modulating the NF-κB route, to either increase or suppress the activation of downstream signaling pathway. The net activation or inhibition effect of NF-κB will depend on several factors that include the bacterial strain, the tissue and cellular context, the interaction of a number of extracellular and intracellular-factors, and the nature of the activation signal. Only probiotic strains that prove to be effective in inhibiting NF-κB signaling could be selected for potential therapeutic application in inflammatory processes.

PERSPECTIVES

During the past decade, many scientific and pharmaceutical companies have conducted intensive research in the NF-κB field, because of its critical role in the pathogenesis of several chronic inflammatory diseases and cancer. Inhibitors of NF-κB signaling and general strategies to their use in the treatment of diseases have been extensively discussed by Gilmore and Garbati [1].These authors reported over 800 inhibitors of NF-κB signaling which include biomolecular inhibitors, natural products (and their derivatives), and synthetic chemicals [1]. The plethora of NF-κB inhibitors includes antioxidants, peptides, small RNA/DNA, microbial and viral proteins, small molecules, and engineered dominant-negative or constitutively active polypeptides [69]. Further, developed inhibitors from patents granted between 2011 and 2014 were reviewed by Arepalli *et al*. [21]. We have not found, however, any patents concerning probiotics or their extracellular compounds that specifically target the inhibition of NF-κB signaling.

Despite numerous studies on the NF-κB signaling pathway, many questions remain unanswered, both in basic science and clinical applications. For example, how does NF-κB translocate into the nucleus? Will the capacity of several natural products to inhibit NF-κB in tissue culture assays have relevance to human disease? Thus, it is almost certain that interest in identification and characterization of NF-κB inhibitors will remain an active research field. Among the challenges that remain open in the field of identification and development of inhibitors of NF-κB signaling as therapeutic agents of human disease, the identification of probiotic strains to be used in inflammatory disorders such as inflammatory bowel diseases ranks among the most promising ones.

CONCLUSION

This review summarizes new advances during the last three years on the ability of probiotics to modulate the NF-κB signaling pathway in different cell types and experimental models. Probiotic strains interact with intestinal epithelial cells, macrophages, dendritic cells, lymphocytes and bladder in different ways. Examples of the variable effects of strains belonging to same or different species on NF-κB signaling were discussed. Given the critical role that NF-κB plays in several inflammatory diseases, further research in the field of probiotics will shed new light on the mechanisms as to how new probiotic strains with anti-inflammatory effect target NF-κB pathway. Further, the use of proteomics and metabolomics approaches may help us understand the means in which specific strains modulate one or more steps of the NF-κB signaling pathway. Only with a deeper understanding of the molecular mechanisms of strain-host interactions, we will be able to put forward new approaches as to how to treat a disease with probiotics.

ABBREVIATIONS

AGS	=	Human gastric adenocarcinoma cell line
Caco-2	=	Human colonic epithelial cell line
CMT93	=	Mouse rectum carcinoma cell line
End1/E6E7	=	Human endocervical epithelial cell lines
HEK-293	=	Human embryonic kidney cell line
HT-29	=	Human colon adenocarcinoma cell line
IMIS	=	Intestinal mucosa immune system: 3D co-culture model using-HT-29/B6 or T84 cells and PBMC
IRAK1	=	Interleukin-1 receptor-associated kinase 1
MKN45	=	Human gastric epithelial cancer cell lines
NCM460	=	Human colonic epithelial cell line.
NIH/3T3	=	Mouse embryo fibroblast cell line
SMAD7	=	Mothers against decapentaplegic homolog 7
T24	=	Human bladder carcinoma cell line
TRAF	=	TNF receptor associated factor
VK2	=	Vaginal epithelial cell line

CONFLICT OF INTEREST

The authors report no conflict of interest in the preparation of this manuscript.

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