Lactobacillus plantarum BGPKM22 AND *lactobacillus brevis* BGZLS10-17 BLOCK LPS-INDUCED NUCLEAR TRANSLOCATION OF NF-κB TRANSCRIPTION FACTOR IN HUMAN BRONCHIAL EPITHELIAL CELLS

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Lactobacillus spp. is a lactic acid bacteria (LAB) naturally present in fresh milk and dairy products. Moreover, LAB present normal microbiota of healthy human lungs. However, chronic lung diseases, e.g., COPD (chronic inflammatory lung disease), asthma, bronchiectasis, etc., are featured by diminished microbiota of the lungs (1). Also, chronic lung diseases are characterized by persistent inflammation that is not fully controlled with available anti-inflammatory therapy (2). As LAB are well-known for their beneficial effects on human health, we hypothesized that autochthonous LAB strains, isolated from artisanal dairy products collected from specific ecological localities across Western Balkan Countries, could have alleviating effects on inflammation in chronic lung diseases.

The aim of this research was to test the ability of natural LAB isolates to inhibit the activity of proinflammatory transcription factor NF-κB in human bronchial epithelial cells.

Human bronchial epithelial cells, BEAS-2B, were pre-treated with natural LAB isolates, using 1:10 ratio, and subsequently treated with lipopolysaccharides (LPS). The cells were fixed and subjected to immunostaining with an anti-NF-κB antibody. Detection of NF-κB nuclear translocation was performed by confocal microscopy (Leica SP8). The quantification of the nuclear signals was done using the LAS X software.

The signal intensity of nuclear NF-κB in BEAS-2B cells treated with *Lactobacillus brevis* BGZLS10-17 and LPS was significantly lower than in the control samples, BEAS-2B cell treated with LPS, (14.6±1.2 versus 19.0±1.9; p=0.026). Also, the signal intensity of nuclear NF-κB in cells treated with *Lb. plantarum* BGPKM22 and LPS was significantly lower than in controls (13.0±2.4 versus 22.5±3.4; p=0.017).

Results of our research showed that exposure of human bronchial epithelial cells to LAB isolates, *Lb. brevis* BGZLS10-17 and *Lb. plantarum* BGPKM22, block LPS-induced nuclear translocation of NF-κB. The proinflammatory transcription factor NF-κB is activated in chronic lung diseases and implicated in amplification of inflammatory response in the lungs. Also, NF-κB plays an important role in acute exacerbations of chronic lung diseases that are associated with significant morbidity and mortality (2). Hence, autochthonous isolates *Lb. brevis* BGZLS10-17, from 10 days old Zlatar cow cheese, and *Lb. plantarum* BGPKM22, from cow sour milk from Pirot, that reduce the activity of NF-κB *in vitro* may be employed for alleviation of signs of chronic lung diseases.

References

1. Ubags NDJ, Marsland BJ. Mechanistic insight into the function of the microbiome in lung diseases. Eur Respir J. 2017:50: 1602467.
2. Barnes PJ. Kinases as Novel Therapeutic Targets in Asthma and Chronic Obstructive Pulmonary Disease. Pharmacol Rev. 2016;68(3):788-815.

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Lactobacillus plantarum BGPKM22 I *Lactobacillus brevis* BGZLS10-17 SPREČAVAJU LPS-INDUKOVANU TRANSLOKACIJU TRANSKRIPCIONOG FAKTORA NF-κB U JEDRA HUMANIH BRONHIJALNIH EPITELIJALNIH ĆELIJA

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Lactobacillus spp. je jedna od bakterija mlečne kiseline (BMK), prirodno prisutna u svežem mleku i mlečnim proizvodima. BMK predstavljaju normalnu mikrobiotu pluća kod zdravih ljudi. S druge strane, hronične plućne bolesti, npr. HOBP (hronična opstruktivna bolest pluća), astma, bronhiektazije, i slično karakteriše smanjenje plućne mikrobiote (1). Takođe, ono što karakteriše hronične plućne bolesti je stalna inflamacija koja se ne može u potpunosti kontrolisati antiinflamatornom terapijom (2). Pošto su BMK poznate po svom povoljnom uticaju na zdravlje ljudi, pretpostavili smo da autohtoni sojevi BMK, izolovani iz tradicionalnih domaćih sireva sakupljenih sa posebnih ekoloških područja u zemljama Zapadnog Balkana, mogu da ublaže inflamaciju u hroničnim plućnim bolestima. Cilj ovog istraživanja je bilo ispitivanje sposobnost prirodnih izolata BMK da inhibiraju aktivnost proinflamatornog transkripcionog faktora NF-κB u humanim bronhijalnim epitelijalnim ćelijama.

Humane bronhijalne epitelijalne ćelije, BEAS-2B, su bile pretretirane prirodnim izolatima BMK, u odnosu 1:10, a zatim tretirane lipopolisaharidom (LPS). Ćelije su fiksirane i imunohemijski obojene anti-NF-κB antitelom. Translokacija NF-κB u jedro je detektovana konfokalnim mikroskopom (Leica SP8). Signal u jedrima je kvantifikovan primenom programa LAS X.

Intenzitet signala jedarnog NF-κB u BEAS-2B ćelijama tretiranim *Lactobacillus brevis* BGZLS10-17 sojem i LPS je bio značajno manji nego u kontrolnom uzorku, odnosno BEAS-2B LPS tretiranim ćelijama (14,6±1,2 *vs.* 19,0±1,9; p=0,026). Takođe, intenzitet signala jedarnog NF-κB u bronhijalnim ćelijama tretiranim *Lb. plantarum* BGPKM22 sojem i LPS je bio značajno niži u poređenju sa kontrolom (13,0±2,4 *vs.* 22,5±3,4; p=0,017).

Rezultati našeg istraživanja su pokazali da izlaganje ćelija humanog bronhijalnog epitela izolatima BMK, *Lb. brevis* BGZLS10-17 i *Lb. plantarum* BGPKM22, sprečava LPS-indukovanu jedarnu translokaciju NF-κB. Proinflamatorni transkripcioni faktor NF-κB je aktiviran u hroničnim plućnim bolestima i uključen u amplifikaciju inflamatornog odgovora u plućima. Takođe, NF-κB ima važnu ulogu u akutnim pogoršanjima hroničnih bolesti pluća koja su povezana sa značajnim morbiditetom i mortalitetom (2). Stoga autohtoni izolati *Lb. brevis* BGZLS10-17, iz 10 dana starog kravljeg sira sa Zlatara, i *Lb. plantarum* BGPKM22, iz kiselog mleka iz Pirota, koji redukuju aktivnost NF-κB *in vitro,* mogu biti upotrebljeni za ublažavanje znakova hroničnih plućnih bolesti.

Literatura

1. Ubags NDJ, Marsland BJ. Mechanistic insight into the function of the microbiome in lung diseases. Eur Respir J. 2017; 50:1602467.
2. Barnes PJ. Kinases as Novel Therapeutic Targets in Asthma and Chronic Obstructive Pulmonary Disease. Pharmacol Rev. 2016;68(3):788-815.

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