

### PROBIOTICS DECREASE LPS INDUCED INFLAMMATORY RESPONSE IN HUMAN BRONCHIAL CELLS – A POTENTIAL THERAPEUTICS IN CHRONIC INFLAMMATORY LUNG DISEASES

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#### Objective:

The alteration of lung microbiota in healthy and diseased lungs implicates that its composition can modulate inflammatory response of the lungs. We suppose that health-benefit of probiotics could be effective in treatment of chronic inflammatory lung conditions. However, the effects of lactic-acid bacteria (LAB), that feature the microbiota of healthy human lungs, on bronchial cells has been insufficiently investigated.

The main objective of this research is to explore the immunomodulatory abilities of natural LAB isolates in human bronchial cells as potential therapeutics for chronic inflammatory lung diseases.

#### Methods:

The ability of 21 LAB isolates to reduce the expression and secretion of pro-inflammatory mediators IL-1, IL-6, IL-8, TNF and MCP-1 is tested in LPS induced BEAS-2B cells using RT-PCR and ELISA approaches, respectively. The cytotoxicity is measured by LDH assay.

#### Results:

Five LAB strains showed a decrease of the expression of at least one pro-inflammatory cytokine and did not increase the secretion of measured cytokines. The strains *Lactobacillus brevis* BGZLS10-17, *Lb. plantarum* PKM22 and GO7-29, significantly decreased LPS induced IL8, MCP and TNF ( $p < 0,01$ ) while *Lb. rhamnosus* BGHi22 and *Streptococcus thermophilus* BGKMJ1-36 significantly attenuated the induction of IL6 and TNF ( $p < 0,05$ ). The strains with anti-inflammatory abilities showed no cytotoxicity.

#### Conclusions:

Available anti-inflammatory pharmacological treatments, are not effective in suppression of lung inflammation and there is an urgent need for the development of novel approaches. LAB isolates with anti-inflammatory abilities, identified in our research, may be employed in manipulation of the lung microbiota that can alleviate the symptoms in chronic inflammatory lung diseases.

### DARK CHOCOLATE AS A PROMISING CARRIER FOR PROBIOTIC STRAINS

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#### Objective:

Chocolate is one of the most attractive food products among the majority of population. Furthermore, probiotic-containing chocolates have been proposed as new functional food candidates. Our study aimed to evaluate flavanol-rich dark chocolate as a carrier for probiotic bacteria (*Bifidobacterium animalis* subsp. *lactis* BB-12 and *Akkermansia muciniphila* DSM 22959), through measurement of cell viability during aerobic storage at 20°C.

#### Methods:

Upon cultivation, pellets from probiotic strains (*B. animalis* subsp. *lactis* BB-12 and *A. muciniphila* DSM 22959) were incorporated, at 10% (w/w) inoculum, in melted 70% cocoa dark chocolate at 37°C, followed by tempering at 34°C during 10 minutes. Next, chocolates were molded and cooled at 11°C, for 2h. Lastly, produced chocolates were stored aerobically at 20°C. Viability of probiotic strains was assessed after 6 and 12 days by plating colony-forming units in appropriate media.

#### Results:

Incorporation of *B. animalis* subsp. *lactis* BB-12 into dark chocolate showed a high viability level (ranging between 8 to 9 log CFU/g), even after 12 days of aerobic storage at 20°C. In contrast, *A. muciniphila* DSM 22959 incorporated into dark chocolate displayed a high viability reduction at similar storage conditions.

#### Conclusions:

Our findings indicate that dark chocolate constitutes a promising carrier for delivery of probiotic *B. animalis* subsp. *lactis* BB-12. However, incorporation of *A. muciniphila* DSM 22959 in dark chocolate still requires further improvements.