

# **Anti-oxidative Activity of Probiotic *Lactobacillus rhamnosus* 0908 Towards 2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ) And 2-Amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhIP)**

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## **INTRODUCTION**

Nowadays, humans are exposed to increasingly harmful xenobiotic substances which diffuse from the contaminated environment to food. Additionally, thermal processing of food, especially of meat, gives rise to pyrolytic products, such as heterocyclic aromatic amines (HAAs). HAAs may be linked to an increased cancer incidence in such organs as the colon, breast, stomach and liver. Carcinogenic mechanisms of HAAs include the initiation of tumours through reactive oxygen species (ROS) production, resulting in the formation of oxidised DNA bases, apurinic/aprimidinic (AP) sites, or DNA strand breaks (Brown et al., 2014). ROS may participate in HAA-induced tumour development. HAAs can cause significant oxidative damage to DNA, lipids and proteins in the human body. The *Lactobacillus* bacteria are regarded as beneficial for host health, and they are most often used as probiotics. The human colon microbiota and probiotics are important factors that may play a major role in preventing colorectal cancer. One of the mechanism is antioxidative activity, which involves the detoxification of ROS (Koller et al., 2008).

## **EXPERIMENTAL METHODS**

The aim of the research was to estimate, if the probiotic strain *Lactobacillus rhamnosus* 0908 protects from the basic and oxidative DNA damage induced by IQ and PhIP, what was measured in human colon derived cells Caco-2. The strain acquired from the collection of the Institute of Fermentation Technology and Microbiology (ŁOCK 105), Lodz University of Technology, Poland and it possesses well documented probiotic properties (Cukrowska et al., 2009). Heterocyclic aromatic amines – IQ and PhIP were purchased from Toronto Research Chemicals, Canada. The protective effect of lactobacilli toward Caco-2 cells was investigated following Caco-2 preincubation with lactobacilli and treatment with IQ (252 µM) or PhIP (223 µM) in alkaline comet assay. Next, the strain was used in oxidative DNA damage measurement with Endo III and Fpg enzymes. Endo III converts oxidized pyrimidines into strand breaks, which can be detected by the comet assay. Fpg is involved in the first step of base excision repair. It removes modified bases from DNA and creates AP sites, which are then cleaved due to its AP lyase activity, producing gaps in the DNA strand. The gaps can be detected by the comet assay (Błasiak et al., 2004).

## **RESULTS AND DISCUSSION**

In the alkaline comet assay the genotoxicity of the positive controls was  $41.0\% \pm 8.4$  (IQ) and  $23.0\% \pm 0.6$  (PhIP). *Lb. rhamnosus* 0908 was very effective in protecting against IQ and PhIP genotoxicity (up to 80%) (ANOVA,  $P < 0.05$ ). In the following experiment, IQ induced oxidative DNA damage 11.2% (Endo III) and 13.8% (Fpg), while PhIP 14.9% (Endo III) and 17.8% (Fpg). *Lb. rhamnosus* 0908 reduced oxidative DNA damage from 15.1% to 4.6% (PhIP) (ANOVA,  $P < 0.05$ ). It also reduced oxidative DNA breaks induced by hydrogen peroxide (50 µM) and recognised by Fpg, from 28.5 to 6.9%. Oxidative stress is considered to play a key role in cancer development. Antioxidants can act through different pathways, preventing mutagen formation by free radical scavenging, blocking the biotransformation of promutagens into reactive metabolites (by inhibiting their metabolic activation), stimulating detoxification enzymes, and modulating (as suppressing agents) intracellular processes which are involved in DNA repair mechanisms

and tumour promotion (Vitaglione and Fogliano, 2004). While ROS generation may account for an increased risk of cancer, probiotics can inactivate ROS through enzymatic and non-enzymatic mechanisms, e.g., by peroxidase, superoxide dismutase, or scavenging by  $Mn^{2+}$  (Koller et al., 2008).

## CONCLUSIONS

The probiotic strain *Lb. rhamnosus* 0908 have been found to exhibit anti-genotoxic and anti-oxidative activity towards IQ and PhIP. It also shows anti-oxidative action by preventing oxidative DNA damage induced by hydrogen peroxide (50  $\mu M$ ). Supplementation with the probiotic bacteria may enhance the protective component of the human gut and help to reduce the genotoxicity induced by food mutagens.

## REFERENCES

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