Investigation on antigenotoxic properties of the probiotic *Lactobacillus rhamnosus* IMC 501® by gas chromatography-mass spectrometry

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INTRODUCTION

Food contaminants entering the body through the oral route are directly exposed to the action of gut microflora. Normal healthy intestinal microflora contains many strains of lactic acid bacteria (LAB), some of which have been isolated, ascribed health benefits, and termed probiotic strains [1]. Probiotic bacteria are used as ingredient in several foods. The protective effect of LAB against food mutagens such as heterocyclic amines, *N*-nitroso compounds, and aflatoxins has been reported [2,3]. For this reason antigenotoxicity and antimutagenicity begin to be considered in characterizing the functional properties of probiotic bacteria.

The aim of the present work was to set-up a gas chromatography-mass spectrometry analysis able to point out the mechanisms involved in the LAB inhibition of 4-nitroquinoline-1-oxide (4-NQO), a direct-acting agent which produces strand scission and formation of charge-transfer adducts on DNA. Specifically, the 4-NQO antigenotoxicity of a probiotic strain (*Lactobacillus rhamnosus* IMC 501®) revealed by short-term biological assays (SOS-Chromotest and Comet assay [4]), was evaluated by GC/MS analysis.

MATERIALS AND METHODS

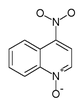
BACTERIAL STRAIN

-*Lactobacillus rhamnosus* IMC501® isolated from human faecal samples [5,6] and characterized as probiotic[7]
-Growth overnight in L-broth at 37°C
-Cells harvesting by centrifugation (6000 *g* for 15 min)

MUTAGEN

-4-nitroquinoline-1-oxide (4-NQO)

-Addedd at a final 0.1 mM concentration



GENOTOXiN-CELL COINCUBATION

-Coincubation under agitation at 37°C for 15, 30 and 135 min

-Supernatant recovery by centrifugation and filtration

CHEMICAL ANALYSIS

-Supernatant extraction by dichloromethane

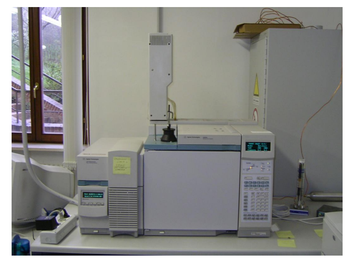
-GC-MS analysis on a gas chromatography (Agilent technologies 6890N) coupled to a mass spectometer (Agilent Technologies 5973N)

MICROBIOLOGICAL ANALYSIS

-Assessment of *Lactobacillus rhamnosus* IMC 501® viability by plate count (MRS agar, Oxoid)

OPERATING CONDITIONS

- HP5ms Agilent Techonologies column(30m - 0.25mm I.D. - 0.1um film)
- helium as carrier gas (flow rate 1 ml/min)
- 2.0 microliters of the sample was injected in the splitless mode at 250°C (purge time 2.0 min)
- Oven temperature program: initial temperature 70°C held for 3 min, increased to 320°C at 30°C/min and held at 320°C for 10 min
- data obtained by collecting full-scan mass spectra within scan range of 29- 350 amu
- NIST 08 mass spectra library for compounds identification

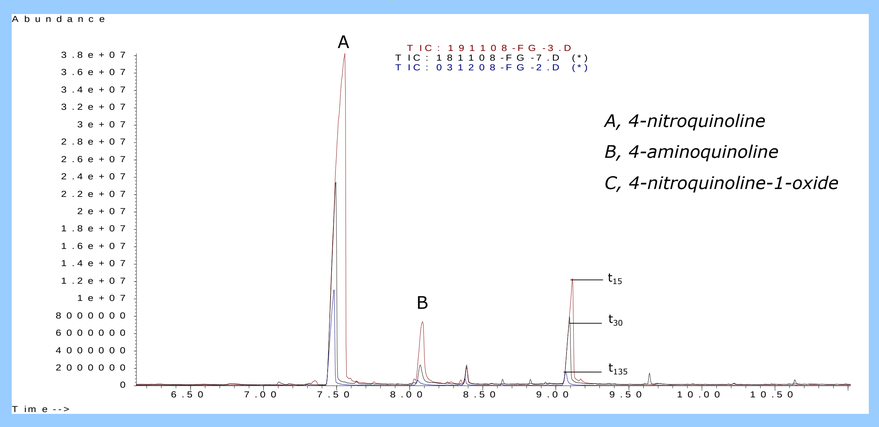


RESULTS

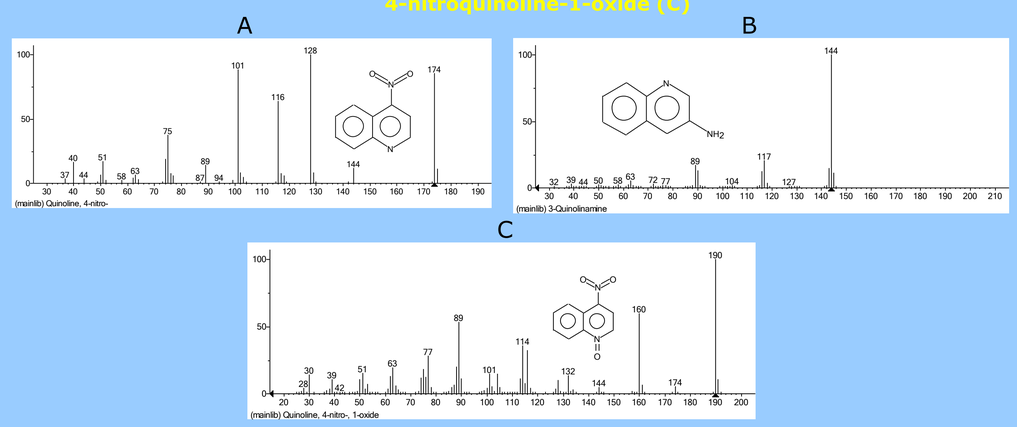
The mass spectral data from supernatants of the strain *Lactobacillus rhamnosus* IMC 501® treated with 4-NQO at different times of incubation evidence the gradual disappearance of the 4-NQO and the appearance of 4-aminoquinoline and 4-nitroquinoline at time 15 min. Also the concentration of these two products showed a gradual reduction at time 30 and 135 min.

A high survival degree (90%) of the *Lactobacillus rhamnosus* IMC 501® was found after genotoxin exposure.

Mass spectra of supernatants from *Lactobacillus rhamnosus* IMC 501® strain coincubated with 4-NQO at different time of incubation



Mass spectra of 4-nitroquinoline (A), 4-aminoquinoline (B) and 4-nitroquinoline-1-oxide (C)



CONCLUSIONS

The data presented here show that *Lactobacillus rhamnosus* IMC 501® has a potential antigenotoxic activity revealed by GC mass spectrometry when coincubated with the reference 4-NQO genotoxin. Lactic acid bacteria have been reported to have antimutagenic/anticarcinogenic properties *in vitro* and *in vivo*. Different mechanisms for this effect may be hypothesized like a physical binding of the mutagenic compounds to the bacteria, genotoxin bioconversion or conjugation.

The GC/MS protocol set-up in this study is able to reveal the inhibition of 4-NQO genotoxicity for the strain *Lactobacillus rhamnosus* IMC 501® and to detect physicochemical modifications of the genotoxic agent originated by bacterial preincubation. The inhibition of the tested genotoxin was related to the maintenance of cell viability after co-incubation. Moreover the spectra evidence the appearance of 4-amino-quinoline as bioconversion product and this compound is known to be inactive.

Results suggest that GC/MS could be a good methodology to reveal the genotoxin deactivation by probiotic bacteria and it could be applied to different genotoxic compounds which may be present in food.

In conclusion, the incorporation of *Lactobacillus rhamnosus* IMC 501® in the diet can suppress or reduce the genotoxic activity of potentially harmful compounds. Studies in humans, however, could be resulted in contradictory outcomes. So, further clinical trials to confirm these effects must be conducted.

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