LWT - Food Science and Technology 58 (2014) 633–638

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Biotransformation and resulting biological properties of green tea polyphenols produced by probiotic bacteria

Ana M. López de Lacey, Efrén Pérez-Santín, M. Elvira López-Caballero*, Pilar Montero

*Institute of Food Science, Technology and Nutrition, ICTAN (CSIC) (formerly Institute of Refrigeration), C/José Antonio Nováis, 10, 28040 Madrid, Spain*

ARTICLE INFO

*Article history:*
Received 7 June 2012
Received in revised form
21 March 2014
Accepted 28 March 2014
Available online 5 April 2014

*Keywords:*
Polyphenols
Probiotic bacteria
Survival
Antioxidant properties
ACE inhibition

ABSTRACT

This study evaluates the antioxidant properties and ACE inhibition capacities of eight individual flavonoids before and after incubation with *Bifidobacterium animalis* ssp. *lactis* LAFTI@B94®. Changes in flavonoid content and the survival of the probiotics, incubated in individual flavonoid solutions as the only source of carbon, were also determined. The glycosylated flavonoids permitted a higher survival rate of the probiotic bacteria with survival percentages above 50% at 48 h of incubation. The incubation of *B. animalis* B94 with individual catechins would supposedly produce an increase in all the biological activities tested (ABTS, FRAP and ACE inhibition), but the increments were minor or imperceptible in the flavonol solutions. Epigallocatechin, catechin and epicatechin solutions registered the most important changes in ABTS, FRAP and ACE inhibition capacity, in some cases even doubling their biological activities after incubation with the bacteria. These results are encouraging as the incorporation of *B. animalis* B94 in various polyphenol-rich foods could be a way of improving the antioxidant properties of the foods. In addition, the polyphenol-rich food could act as a matrix for probiotic strains.



## 1. Introduction

Flavanols, particularly those of the catechin and catechin-gallate ester family, together with the flavonols quercetin, kaempferol and their glycosides, are the major polyphenols in green teas (Rice-Evans, Miller, & Paganga, 1996). The biological activity of green teas has been associated principally with this group of polyphenol compounds (Higdon & Frei, 2003). Numerous *in vitro* assays have demonstrated the well-known strong antioxidant properties of catechins (Majchrzak, Mitter, & Elmadfa, 2004). However, the beneficial effects of polyphenols depend on their bioavailability (Walle, 2004).

Probiotic bacteria, live cells with a wide variety of positive health effects, are principally members of the *Bifidobacterium* and *Lactobacillus* family, although some strains of *Bacillus*, *Pediococcus* and yeast have also been found to be suitable candidates (Soccol et al., 2010). These microorganisms contribute to intestinal microbial balance and play a role in maintaining health (Soccol et al., 2010). The recent interest in adding probiotics to some foods rich in phenolic compounds (Marotti, Bonetti, Biavati, Catizone, & Dinelli, 2007; Otieno, Ashton, & Shah, 2006) is because certain enzymatic activities such as deglycosylation, ring-fission, dehydroxylation, demethylation or decarboxylation, etc. (Aura, 2008) are present in some probiotics. These enzymatic activities can, in some cases, convert polyphenols into more bioavailable or bioactive forms than the original phenolic compounds (Donkor & Shah, 2008; Tsangalis, Ashton, McGill, & Shah, 2002; Uskova, Kravchenko, Avrenjeva, & Tutelyan, 2010). The fermentation of flavonol glycosides with some probiotic microorganisms with glucosidase activity, such as $\beta$-glucosidase, $\beta$-galactosidase or $\alpha$-rhamnosidase, could improve their bioavailability. This bioavailability is increased since the bacteria transform the flavonol glycoside into its aglycone which is more absorbable (Izumi et al., 2000). Moreover, the deglycosylation of flavonol glycosides such as rutin or quercetin rutinoside to quercetin, improves their antioxidant capacity (Rice-Evans et al., 1996).

Although some studies have been published dealing with flavonoid biotransformations produced by *Bifidobacterium* or *Lactobacillus* sp. (Ávila et al., 2009; Donkor & Shah, 2008; Marotti et al., 2007), there are not many published reports on the effect of probiotic bacteria on flavonol antioxidant activity.

Catechin fermentation with bacteria is even less studied than flavonols. The work done by Macedo, Battestin, Ribeiro, and Macedo (2011) demonstrated that green tea polyphenols treated with *Paecilomyces variotti* tannase exhibited a greatly increased antioxidant capacity *in vitro*. However, no additional studies have

* Corresponding author. Tel.: +34 91 5492300; fax: +34 91 5493627.
*E-mail address:* elvira.lopez@ictan.csic.es (M.E. López-Caballero).

http://dx.doi.org/10.1016/j.lwt.2014.03.040
0023-6438/

been found dealing with the biological activities of catechin metabolites produced by a specific strain, since research is focused principally on catechin metabolism by faecal species (Aura et al., 2008; Meselhy, Nakamura, & Hattor, 1997).

Thus, the objectives of this study were to investigate the survival of *Bifidobacterium animalis* ssp. *Lactis LAFTI@B94* in different polyphenols solutions and explore the changes in the composition of polyphenols incubated with this probiotic. The antioxidant properties and ACE activity of the metabolites derived from polyphenol solutions in the presence of probiotic bacteria were also studied.

## 2. Materials and methods

### 2.1. Chemicals

Standard polyphenol compounds: (−)-epigallocatechin (CAS: 970-74-1), (+)-catequin (CAS:154-23-4), (−)-epicatequin (CAS: 490-46-0), (−)-epicatechin-3-gallate (CAS: 1257-08-5), quercetin-3-*O*-galactoside (CAS: 482-36-0), quercetin-3-*O*-glucoside (CAS: 482-35-9), kaempferol-3-*O*-rutinoside (CAS: 17650-84-9), kaempferol-3-*O*-glucoside (CAS: 480-10-4) were purchased from Extrasynthese (Genay, Cedex, France).

Reagents for enzyme activity assay: *ρ*-nitrophenyl-*β*-D-glucopyranoside, *ρ*-nitrophenyl-*β*-D-galactopyranoside, *ρ*-nitrophenyl-*α*-D-ramnopyranoside, *ρ*-nitrophenol were provided by Sigma–Aldrich (Stenheim, Germany).

HPLC–MS: HPLC grade methanol, acetonitrile and formic acid (VWR international, Inc., Barcelona, Spain). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratory Bedford, MA).

Antioxidant activity; FRAP: (2,4,6-tripyridyl-*s*-triazine), $FeCl_3$ and $FeSO_4$ (Sigma–Aldrich, St. Louis, Mo., USA). ABTS: [2,2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid)], potassium persulphate, Vitamin C (Sigma–Aldrich, St. Louis, Mo., USA).

Reagents for ACE-inhibition: HHL (Hippuryl-L-Histidyl-L-Leucine), ACE (CAS: 9015-82-1), potassium phosphate buffer, containing 300 mMol/L NaCl, pH 8.3 (Sigma–Aldrich, St. Louis, Mo., USA).

Culture media: MRS (de Man, Rogosa and Shape, Oxoid, Basingstoke, UK).

### 2.2. Bacterial strain and growth conditions

*B. animalis* ssp. *Lactis LAFTI@B94* (*B. animalis* B94) was selected as a commercial probiotic strain. This strain was purchased lyophilized in DSM (DSM Food Specialties Ltd., Sydney, Australia) and kept at −20 °C until use.

For experiments, one portion of lyophilized strain was dissolved in 0.05 g/100 mL cysteine MRS broth. Then one aliquot of this solution was streaked on 0.05 g/100 mL cysteine MRS agar plates, which were incubated at 37 °C for 24 h under anaerobic conditions. After the incubation, one colony of *B. animalis* B94 was transferred into broth medium and was grown at 37 °C under anaerobic conditions for 24 h.

### 2.3. Survival of *B. animalis* B94 in eight separately prepared polyphenol standard solutions

*B. animalis* B94 was grown as mentioned previously. Then the cells were harvested by centrifugation (6000 *g*, 10 min, 5 °C), washed twice with 0.9 g/100 mL NaCl solution and suspended in the same solution to an $OD_{620} \sim 0.6$. 50 μL of 0.9 g/100 mL NaCl solution was used for control purpose.

To study *B. animalis* B94 survival in polyphenol standards, 1 mL of harvested cells adjusted to $OD_{620} \sim 0.6$ were mixed with 19 mL of 0.9 g/100 mL NaCl solution. The standards were first dissolved in HPLC grade methanol before being added to the NaCl solution to a final concentration of 25 μg/mL. The same concentration of standards (25 μg/mL) was used for all the samples in order to compare the effect on probiotic survival of each compound. The final bacterial concentration was approximately $10^6$ CFU/mL. The bacterial strains were incubated at 37 °C for 72 h in anaerobic conditions.

Cell viability was assessed by determining the viable population present at 0, 24, 48 and 72 h of incubation by analysing 100 μL of each culture using the microdot method (Strahsburger, Baeza, Monasterio, & Lagos, 2005) on 0.05 g/100 mL cysteine MRS agar. All the counts were performed at least in triplicate.

### 2.4. Biotransformation of phenolic compounds

*B. animalis* B94 was used for the study of polyphenol biotransformation. The bacteria solutions ($OD_{620} \sim 0.6$) were prepared and mixed with each standard separately as explained above. The final bacterial concentration was approximately $10^6$ CFU/mL and the final concentration of each standard in this solution was 25 μg/mL. The bacterial strains were incubated at 37 °C for 72 h and in anaerobic conditions.

One aliquot of the solution was taken for the different analyses (counts, HPLC-MS, antioxidant activity and ACE inhibition) at 0, 24, 48 and 72 h of incubation at 37 °C. After the counts each aliquot was centrifuged 6000 *g*, 10 min and 5 °C and the supernatant was kept for the rest of the analyses. All the analyses were performed at least in triplicate, except for the HPLC–MS analysis which was carried out in duplicate.

### 2.5. Quantification of polyphenols by HPLC–MS

Reverse phase high performance liquid chromatography was performed to analyse phenolic compounds. The separation module consisted of an Agilent 1100 series HPLC (Agilent Technologies, Waldbroon, Germany), equipped with a $C_{18}$ size column (250 × 4.6 mm, 5 μm, $C_{18}$ Tracer Excel ODS-A column, Teknokroma) and a diode array detector (DAD). The samples were eluted with a gradient system consisting of solvent A (deionized water) and solvent B (acetonitrile) both containing 1 mL/100 mL formic acid, used as the mobile phase, with a flow rate of 1 mL/min.

The temperature of the column was maintained at 25 °C and the injection volume was 20 μL. The gradient system started at 90% solvent A and decreased to 74% A within 40 min, followed by a decreased to 35% solvent A in 10 min. The final conditions were held for an additional 5 min. The peaks of the phenolic compounds were monitored by atmospheric pressure electrospray ionization source (ESI), operated in negative ion mode, with the electrospray capillary voltage set to 3000 V, a nebulizing gas flow rate 12 L/h, and a drying temperature of 350 °C. Mass spectrometry data were acquired in the scan mode (mass range *m*/*z* 200–700) and in the sim mode (*m*/*z* 289, 305, 441, 441, 447, 457, 609).

The quantification was carried out using the external standard method. Solutions of each standard at various concentration levels of 0.62, 1.25, 2.5, 5, 10 and 20 mg/L were injected into column and the elution was performed in the same manner with the samples. Individual compound was quantified using a calibration curve of the corresponding standard compound.

### 2.6. Antioxidant activity before and after biotransformation

#### 2.6.1. FRAP

The ferric reducing/antioxidant power (FRAP) assay was carried out according to the method described by Benzie and Strain (1996) with some modification (Alemán et al., 2011). This assay measures