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Determining antioxidant activities of lactobacilli by cellular antioxidant assay in mammal cells

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ABSTRACT

Among the beneficial effects of *Lactobacillus* spp., the common mechanism underlying health protection is believed to be related to antioxidant activity. The antioxidant activities of cell-free suspension (CFS) and strain suspension (SS) of 13 *Lactobacillus* strains derived from 4 species (*L. rhamnosus*, *L. acidophilus*, *L. casei*, and *L. fermenti*) were determined. The correlation of CFSs and SSs in different cell lines, as well as the correlation among the three cell models (RAW264.7, Caco-2, and EA.hy926 cell lines), were compared. All CFSs and SSs of lactobacilli showed that they significantly inhibited the production of intracellular reactive oxygen species without creating obvious cytotoxic effects in the three mammal cell lines. These results suggested that RAW264.7, Caco-2, and EA.hy926 cell lines could be used as cell models to assay the cellular antioxidant activity of CFS and SS of lactobacilli. Thus, lactobacilli and by-products are a potential source of natural antioxidants and to reduce oxidative stress.

1. Introduction

One factor that underlies the pathology of many diseases, including diabetes, cancer, liver disease and several degenerative chronic diseases, is an increase in oxidative stress (Bjelakovic et al., 2011; Firuzi, Miri, Tavakkoli, & Saso, 2011; Hallwell & Gutteridget, 1984). Both eucaryotic and aerobic procaryotic organisms have developed inherent antioxidant mechanisms and repair systems to scavenge or neutralise reactive oxygen species (ROS) (Kullisaar et al., 2002); however, insufficient antioxidant capacity exists to prevent ROS-mediated injury when abnormally high levels of ROS are generated. Therefore, attention has been paid to antioxidant therapy, with a potentially important alternative treatment to reduce the oxidative damage (Lykkesfeldt & Svendsen, 2007).

Given the potential toxic effect of synthetic antioxidants, considerable focus has been placed on the antioxidant capacity of natural products (Miremadi, Ayyash, Sherkat, & Stojanovska, 2014), with particular interest on *Lactobacillus*

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strains (Lin & Yen, 1999). *Lactobacillus* spp. are important members of the healthy human microbiota, which can modify the gut microbiota and may be beneficial for the treatment and the prevention of inflammatory bowel disease (Mikel'saar, Tjuri, Valjaots, & Lencner, 1984; Saez-Lara, Gomez-Llorente, Plaza-Diaz, & Gil, 2015). In particular, besides the long history of consumption of lactic acid bacteria, *Lactobacillus* spp. have been reported to have a range of health-promoting features (Choi, Lee, & Paik, 2015; Fernandes & Shahani, 1989; Petrova, Lievens, Malik, Imholz, & Lebeer, 2015; Waters, Mauch, Coffey, Arendt, & Zannini, 2015). Numerous studies have demonstrated that *Lactobacillus* strains can decrease oxidative stress and accumulation of ROS, which help to prevent diseases (Forsyth et al., 2009; Hathout et al., 2011; Lee, Hwang, Heo, Lee, & Park, 2005). *Lactobacillus* strains could degrade the superoxide anion and hydrogen peroxide (Ahotupa, Saxelin, & Korpela, 1996; Xing, Wang, Gu, et al., 2015). Bing, Kinouchi, Kataoka, Kuwahara, and Ohnishi (1998) suggested that the culture supernatant of *Lactobacillus acidophilus* contains anti-ulcer and anti-oxidative metabolites. Wang et al. (2012) reported that the *Lactobacillus rhamnosus* GG culture supernatant ameliorated acute alcohol-induced intestinal permeability and liver injury. Therefore, increasing the consumption of beneficial *Lactobacillus* strains, which can be added as biologically active compounds for transforming a "functional foods," could be an elegant way to limit the number and incidence of disorders and to recover from dysbiosis (Miremadi et al., 2014; Persichetti, De Michele, Codini, & Traina, 2014).

However, although the extracellular antioxidant activity (AA) of lactobacilli has been extensively studied, information on its antioxidant properties within cells is limited. This information is significant because extracellular assays mostly rely on chemical methods that do not consider the metabolism and bioavailability of antioxidants (López-Alarcón & Denicola, 2013). Therefore, tests of the intracellular AA of lactobacilli, which address issues of uptake, distribution, and metabolism, are urgently required. The cellular antioxidant activity (CAA) assay is a newly developed approach that quantifies the antioxidant capacity of bioactive compounds in cell cultures (Wolfe & Liu, 2007). The CAA assay is improved over traditional chemical AA assays because it simulates cellular biochemical processes, including cellular uptake and metabolism. Therefore, the CAA assay should allow a better understanding of how antioxidants behave under physiological conditions (Liao et al., 2014). The CAA assay is applied to determine the AA of cell free suspension (CFS) of lactobacilli in HepG2 cell line in our previous study (Xing, Wang, Zhang, et al. 2015). The intestinal macrophages are also essential for local homeostasis, and intestinal epithelium is the first cellular barrier encountered by ingested antioxidants. However, what is absorbed in plasma might still be highly bioactive at the level of the vascular endothelium cells (Bornsek et al., 2012). Limited information is presently available about the effects of lactobacilli on the AA of different cell lines under oxidative stress. Therefore, pieces of evidence inspired us to focus our research on the protective effect of lactobacilli against AAPH-induced oxidative stress in three cell models (RAW264.7, Caco-2, and EA.hy926 cell lines).

This study aims (i) to evaluate the toxicity effect of CFS and strain suspension (SS) on three cell models (RAW264.7, Caco-2, and EA.hy926 cell lines); (ii) to apply the CAA method to detect the AAs of CFS and SS in three cell lines; (iii) to analyse the difference between CFS and SS of AA, and assay the AA difference of inter- and intra-specific by comparing the AAs of 13 *Lactobacillus* strains from four species which are used as probiotic bacteria; and (iv) to study the correlations among three cell lines to provide theoretical guidance in CAA assay.

2. Materials and methods

2.1. Chemicals

2′, 7′-dichlorofluorescin diacetate (DCFH–DA), 2, 2-azobis (2-amidinopropane) dihydrochloride solution (ABAP), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and Hanks' Balanced Salt Solution (HBSS) were purchased from Gibco Life Technologies (Grand Island, NY, USA). Dulbecco's minimum essential medium–high glucose (DMEM), RPMI 1640, foetal bovine serum (FBS), penicillin, and streptomycin were obtained from HyClone (Logan, UT, USA). DeMan, Rogosa, and Sharpe (MRS) broth were purchased from Qingdao Hopebio Company (Qingdao, China). All chemicals used in this study were of analytical grade.

2.2. Bacterial strains and culture conditions

All lactic acid bacteria (LAB) strains used in this study are *Lactobacillus* strains available for food and listed in Table 1. These strains include *L. rhamnosus GG* ATCC 53103 (LGG), which was used in all of the experiments as a reference strain (Chen et al., 2014; Sun, Hu, Le, & Shi, 2010). *L. acidophilus*, *L. fermentum*, *L. rhamnosus*, and *L. casei* are known species that display AAs (Chen et al., 2014; Lee, Hwang, Chung, Cho, & Park, 2005; Lin & Chang, 2000). These strains were maintained as frozen stocks (–80 °C) in MRS broth and supplemented with 30% (v/v) glycerol. These strains were consecutively reactivated at least thrice using 1% (v/v) inoculum in MRS broth at 37 °C for 20 h prior to use.

Table 1 – LAB used in this study.

Lactobacillus strain	Source or reference
L. rhamnosus GG ATCC 53103	Valio Ltd., Helsinki, Finland
L. rhamnosus CCFM237	CCFM-JU*
L. rhamnosus CCFM469	CCFM-JU
L. rhamnosus CCFM310	CCFM-JU
L. acidophilus CCFM137	CCFM-JU
L. acidophilus CCFM6	CCFM-JU
L. acidophilus CCFM8	CCFM-JU
L. casei 9	CCFM-JU
L. casei 5	CCFM-JU
L. casei 30	CCFM-JU
L. fermenti CCFM381	CCFM-JU
L. fermenti CCFM424	CCFM-JU
L. fermenti CCFM620	CCFM-JU

CCFM-JU*: Culture Collection of Food Microorganisms of Jiangnan University (Wuxi, China).