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Research Article

Antioxidant Effect of *Lactobacillus acidophilus* as a Probiotic at Different Time Intervals

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Abstract

Background: Probiotics are survival microorganisms that, when administered in sufficient amounts, confer health benefits to the host and can be used in an antioxidative role.
Objectives: The antioxidative effect of whole cells and intracellular cell-free extracts of the lactic acid bacteria *Lactobacillus acidophilus* (PTCC 1643) as a probiotic at three different time intervals was investigated.
Materials and Methods: Antioxidant biomarkers, such as total antioxidant power (TAP), measured with the FRAP (ferric-reducing ability of plasma) method, were evaluated at 24, 48, and 72 hours.
Results: The results showed that extracts and bacteria of *L. acidophilus* were able to significantly increase TAP after 24 and 72 hours.
Conclusions: The results showed that the effect of *L. acidophilus* is time-dependent.

Keywords: Antioxidants, Probiotics, *Lactobacillus acidophilus*

1. Background

Probiotics are microbially derived factors that stimulate the growth of other organisms. Currently, probiotics are selected from the strains most favorable for the most intestinal bacteria, which belong to the yeast genera and to *Bifidobacterium* and *Lactobacillus* (1, 2), which are the most commonly used. The major target of the probiotic *Lactobacillus* is the human gastrointestinal tract. The benefits of probiotics, due to their antimicrobial and antioxidative properties, are predicted to increase the popularity of their use in humans (3). Various authors have reported the defense in opposition to the capability to reduce the risk for gathering of reactive oxygen species (ROS) and oxidative toxic stress (4, 5). The antioxidant properties of probiotics could be due to metal ion chelation, enzyme inhibition, reduction of ascorbate autoxidation, and ROS scavenging (6). The causes of this decline have been suggested to be increased oxidative stress and disorders in energy metabolism, which might participate in important functions (7, 8). Oxidative stress arises when there is a marked imbalance between the production and the elimination of ROS. It has been shown that exposure of living systems to various chemicals results in the urgent formation of free radicals that last for a matter of milliseconds and lead to oxidative damage to biomolecules, such as DNA, proteins, and lipids (9). Also, in acute and chronic oxidative stress, the existence of extreme amounts of free radicals may lead to several unrecoverable effects, such as fibrosis, necrosis, atrophy, vascular damage, and DNA breakage (10). In the ROS theory of disease, therefore, it is necessary to develop and use effective and powerful antioxidants in order to protect the human body from free radicals and to retard the progress of several chronic diseases (11, 12).

2. Objectives

This study aimed to examine the antioxidant effects of *L. acidophilus* as a probiotic at different time intervals in extracts and bacterial samples, in an in vitro study.

3. Materials and Methods

3.1. L. acidophilus and Growth Conditions

L. acidophilus (PTCC 1643) samples were obtained from our frozen stock collection in the medical microbiology laboratory. *L. acidophilus* was plated onto lactobacilli MRS agar (Difco), and the plate was incubated at 37°C for 24 hours in an anaerobic chamber (BBL GasPak anaerobic system). *L. acidophilus* was re-cultured three times in MRS broth for activation prior to experimental use.

3.2. Preparation of Whole Cells and Intracellular Cell-Free Extracts

L. acidophilus PTCC 1643 was collected via centrifugation for 10 minutes at 4400 g after 24 hours of incubation at 37°C. For the preparation of whole cells, the bacteria were washed with phosphate-buffered saline (PBS) three times, then resuspended in PBS. For the preparation of cell-free extracts, the pellets were rapidly washed twice with deionized water, then resuspended in deionized water, followed by sonication disruption. Sonication was performed on ice five times at intervals of 1 minute. Then, the cell debris was removed by centrifugation at 7800 g for 10 minutes, and the supernatant was the resultant cell-free extract. The total cell numbers were adjusted to 10^9 CFU/mL for the preparation of whole cells and cell-free extracts (13).

3.3. Assay for the Antioxidant Power of probiotics: the FRAP Method

Total antioxidant power (TAP) was determined with the ferric-reducing ability of plasma (FRAP) assay. This method is based on the reduction of ferric tripyridyltriazine [Fe (III)-TPTZ] complex to ferrous-tripyridyltriazine [Fe (II)-TPTZ] in the presence of antioxidants. The FRAP reagent was prepared using 10 mmol/L of TPTZ solution in 40 mmol/L of HCl plus $FeCl_3$ (20 mmol/L) and acetate buffer (0.3 mol/L; pH 3.6) at a 1:1:10 ratio. Freshly prepared FRAP reagent was warmed at 37°C for 5 minutes. The serum sample or standard (50 µL) was mixed with 1.5 mL of FRAP reagent and incubated at 37°C for 10 minutes. The absorbance of the colored Fe (II)-TPTZ was measured at 593 nm and compared to a blank. $FeSO_4$ solution at various concentrations (125, 250, 500, and 1000 µM) was used as the standard (14).

3.4. Statistical Analysis

In order to compare the three groups based on time as the quantitative variable, one-way analysis of variance (ANOVA) for symmetrical distribution with Tukey's post hoc analysis was applied. Differences between groups were considered significant when $P < 0.05$.

4. Results

4.1. TAP

A significant increase ($P < 0.05$) in TAP was observed in the extracts and bacterial samples, as recognized by the FRAP method after 24 hours of incubation. The mean ± SE values for extracts and bacterial solutions were 95.32 ± 9.5 and 119 ± 11.1 Umol/mL, respectively (Table 1).

The TAP level was significantly lower ($P < 0.05$) among the extract samples in comparison to the bacterial samples after 72 hours (Table 2). The mean ± SE values for extracts and bacterial solutions were 95.32 ± 9.5 and 119 ± 11.1 Umol/mL, respectively (Table 2). No significant difference was observed in TAP between the groups after 48 hours (Table 3).

Table 1. Total Antioxidant Power of Probiotics After 24 Hours of Incubation

Group A (After 24 Hours)	TAP, Umol/mL[a]	P Value
Extract	95.32 ± 9.5	0.04
Bacteria	119 ± 11.1	0.04

[a] Values are expressed as mean ± SD.

Table 2. Total Antioxidant Power of Probiotics After 72 Hours of Incubation

Group C (After 72 Hours)	TAP, Umol/mL[a]	P Value
Extract	91.32 ± 8.6	0.04
Bacteria	125 ± 12.9	0.04

[a] Values are expressed as mean ± SD.

Table 3. Total Antioxidant Power of Probiotics After 48 Hours of Incubation

Group B (After 48 Hours)	TAP, Umol/mL[a]	P Value
Extract	260 ± 13.6	0.65
Bacteria	295 ± 75	0.65

[a] Values are expressed as mean ± SD.

5. Discussion

In the present study, our purpose was to investigate novel activity of *L. acidophilus* as a probiotic at different time intervals (24, 48, and 72 hours) in extracts and bacterial samples in an in vitro study. Collectively, the results established that a significant increase in TAP was observed in extracts and bacterial samples after 24 and 72 hours, as shown in Tables 1 - 3. There has been increasing interest in the role and use of probiotics as a means of preventing oxidative damage in diseases due to high oxidative stress (15). ROS generation overwhelms antioxidant defenses, and ROS can interact with endogenous macromolecules and change cellular functions (16). A high level of ROS may also result in protein oxidation (PO) and lipid peroxidation (LPO). Consequently, PO and LPO levels can be used as biomarkers of ROS-induced tissue damage in various diseases (17, 18). Accumulating research has suggested that certain probiotics play various biological roles through several mechanisms, one of the most-debated of these being antioxidant activity (19). In fact, among the useful effects of probiotics in humans, protection against oxidative stress has been reported in several studies (20, 21). In this experimental study, specific strains of *L. acidophilus* showed antioxidant properties after different time intervals. Therefore, the results indicate that probiotic bacteria have antioxidative properties, calculated according to the FRAP method, which is used in many studies (22, 23). In every microbial collection, irrespective of the method used, broad dispersion of the values for antioxidative parameters was observed in different concentrations, and for TAP with the FRAP method. In this survey, the probiotic formulations were chosen from within the genera *Lactobacillus* and *Bifidobacterium*, the most commonly used probiotic bacteria. The special probiotic strains acted in concert to neutralize the oxidative stress induced in animal and human models (24). Some authors theorize that probiotics exert their protective effects against oxidative stress by restoring the gut microbiota (25). Acting in this way, antioxidant probiotic strains can be chosen and investigated as promising candidates for the prevention and control of several free radical-related disorders (26-28). Finally, *L. acidophilus* as a probiotic plays an important role in the alteration of oxidative injuries through TAP.

Footnote

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