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ORIGINAL ARTICLE

Black Tea Phenolics Enhance Aerobic Growth of *Lactobacillus Delbrueckii* subsp. *lactis* under Oxidative Conditions

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Abstract

Black tea is rich in phenolic compounds with known antioxidant properties, yet their protective effects on lactic acid bacteria under oxidative conditions are not fully understood. In this study, the antioxidant action of phenolic compounds present in black tea infusion (BTI) was evaluated in relation to the aerobic growth of *Lactobacillus delbrueckii* subsp. *lactis*. Aqueous BTI was prepared from black tea leaves and characterised for total phenolic content (TPC) and radical scavenging activity. A BTI concentration of 40% (v/v) was selected for bioassays to exclude bactericidal or nutritive effects. The 40% (v/v) BTI exhibited a total phenolic content of 0.328 mg mL^{-1} gallic acid equivalents and a DPPH radical scavenging activity of 3.099 mg mL^{-1} ascorbic acid equivalents. Under anaerobic conditions, BTI did not significantly influence the bacterial growth. In contrast, under aerobic conditions, colony-forming unit counts were significantly higher in BTI-incorporated medium compared with BTI-free control. These findings indicated that black tea phenolics enhance the aerobic growth of *L. delbrueckii* subsp. *lactis*, most likely by mitigating oxidative stress through extracellular antioxidant mechanisms. The study highlights the potential of black tea phenolics to improve the aerobic tolerance of lactic acid bacteria. These results highlight the potential application of black tea phenolics in improving the aerobic tolerance of lactic acid bacteria in food and fermentation systems.

Keywords*: Aerobic growth, black tea infusion, lactic acid bacteria, Lactobacillus delbrueckii subsp. lactis, oxidative stress, phenolic antioxidants*

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1. Introduction

Black tea, one of the most widely consumed beverages globally, is known for its robust, strong flavour, dark colour, and higher caffeine content, distinguishing it from green or white teas. Black tea is produced from the plucked leaves of *Camellia sinensis* (L.) *Kuntze* (tea), through partial drying, withering, and mechanical disruption, processes that facilitate the oxidation of leaf polyphenols by endogenous enzymes. Such oxidation, promoted by mechanical disruption of leaves, does not occur in green or white teas as the leaves are kept unbroken. The oxidative enzymes involved in black tea processing are mainly polyphenol oxidase (PPO) and peroxidase. These enzymes oxidize ortho-diphenolic catechin monomers in tea leaves into quinones, which subsequently undergo non-enzymatic condensation and polymerisation reactions to form black tea polyphenols, mainly theaflavins and thearubigins. Although enzymatic oxidation reduces the concentration of ortho-diphenols, which are known for their strong antioxidant properties, it does not eliminate the overall antioxidant capacity of black tea. This is because of theaflavins and thearubigins possess substantial antioxidant activity and exhibit improved stability in aqueous systems, as demonstrated by a variety of chemical and biological assays (Leung et al. 2001; Cabrera et al. 2006; Sang et al. 2011; Khan & Mukhtar, 2019).

Oxidative stress is a major constraint for the growth of lactic acid bacteria (LAB) under aerobic conditions due to their limited endogenous antioxidant defence mechanisms. Therefore, these bacteria grow optimally in low-oxygen environments. Reactive oxygen species (ROS), particularly superoxide anions ($O_2\bullet^-$) and hydroxyl radicals (•OH), generated by the oxidation of biomolecules by molecular oxygen, can readily oxidise membranes, proteins, and nucleic acids, causing extensive damage that leads to cell death. Increasing attention has been directed toward the ability of phenolic compounds to counter oxidative stress in microorganisms, as plant-derived phenolics have been shown to scavenge reactive oxygen species and modulate redox balance in biological systems (Kiokias et al. 2020; Yan et al. 2020). Similar protective effects of these compounds have also been reported in microbial systems (Feng et al. 2020). Tea polyphenols have been reported to enhance the survival rate of LAB under oxidative conditions. Due to the high molecular weight and polarity of black tea polyphenols, including theaflavins and thearubigins, antioxidant protection reported in LAB has been attributed in previous studies primarily to extracellular mechanisms, with no direct evidence for intracellular uptake (Zhao & Shah, 2014; Li et al. 2019). Similarly, Xu et al. (2016) reported protective effects during concurrent exposure.

Previous studies by Karunasiri et al. (2020) and Gunawardane et al. (2025) demonstrated that overnight pre-treatment of some microorganisms, including some LAB, with aqueous solutions of phenolics extracted from coconut milk gave antioxidant capacity to those organisms, as shown by enhanced growth and survival under subsequent aerobic conditions compared with untreated controls. This carryover protection could be explained by the relatively small molecular size of

coconut milk phenolics that may have allowed their intracellular uptake. As intracellular accumulation by LAB has not been demonstrated with black tea phenolics, pre-treatment with BTI was not expected to confer sustained antioxidant protection during subsequent aerobic growth. The present study tested the hypothesis that black tea phenolics, when present during aerobic exposure of *Lactobacillus delbrueckii* subsp. *lactis* can enhance its aerobic growth, possibly by mitigating oxidative stress through extracellular scavenging of ROS and/or modulation of the redox environment of the culture medium.

2. Materials and Methods

2.1. Preparation of Black Tea Infusion (BTI)

Market samples of loose black tea leaves of the BOPF (Broken Orange Pekoe Fannings) grade were obtained from four commercially available brands (50 g each). Equal amounts from each brand were combined to prepare a composite sample. This approach was used to minimise brand-specific variation and obtain a representative sample of commercially available black tea. The grade BOPF was selected due to its relatively fine particle size, which enhances the extraction efficiency of water-soluble phenolic compounds, and its widespread use in commercial tea products, thereby improving the reproducibility and practical relevance of the study.

Two-step sequential aqueous extraction was done. The first extraction was done with a sample of 6 g of black tea kept in 300 mL of distilled water at 30°C for 30 min. Thereafter, leaves were gently squeezed in a cheesecloth to extract the compounds out. The squeezed tea leaves were then kept in 300 mL of distilled water at 75 °C for 30 min. The two extracts were mixed and filtered using a plastic strainer three times, followed by a final filtration through a filter paper (90 mm F1/KA4, Smith filters, England) to remove particulate matter. Then the filtrate was centrifuged at 5000 g for 15 min, the supernatant was filter-sterilised using a bacteriological filter (0.45 µm), and stored in the dark at 4°C. This aqueous extract of black tea, approximately 550 mL after filtration, was labelled as the black tea infusion (BTI), and was used for subsequent chemical and microbiological analyses.

2.2. Determination of the Appropriate BTI Concentration for Bioassays

To identify a concentration of BTI suitable for bioassays, a dilution series of BTI (0–100%, v/v) was prepared in MRS (De Man, Rogosa, and Sharpe agar) broth and inoculated with the test organism *L. delbrueckii* subsp*. lactis*. Cultures were incubated overnight under anaerobic conditions. Following incubation, bacterial growth was assessed by colony-forming units (CFU) enumeration on MRS agar. The BTI concentration of 40% was selected for subsequent experiments, as it exhibited neither bactericidal effects nor growth enhancement relative to the BTI-free control. This concentration ensured that any observed effect under aerobic conditions could be attributed to antioxidant protection rather than nutritional or inhibitory effects.

2.3. Determination of Total Phenolic Content (TPC) of BTI

Determination of TPC of 40% BTI was carried out according to the method described previously (Lamuela-Raventós, 2018; Karunasiri et al. 2020), with minor modifications. Two microlitres of aqueous BTI was diluted with 100 µL of deionized water to bring the concentration within the level of absorbance. Next, 10 µL of Folin-Ciocalteu reagent was added to the diluted sample, followed by 40 µL of Sodium carbonate (25% w/v) to provide the alkaline conditions required for the assay. The mixture was then further diluted with 100 µL of deionized water and incubated at room temperature for 30 min to allow complete reaction of phenolic compounds with the Folin-Ciocalteu reagent, resulting in the formation of a blue-coloured complex. The absorbance at 765 nm was measured using a UV-visible spectrophotometer. A control sample prepared in the same manner, but using distilled water in place of BTI, was used as the blank. A standard curve was prepared using gallic acid solutions of known concentrations ranging from 1 to 7 mg mL^{-1}. The total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) (mg mL^{-1}) for the 40% (v/v) BTI test concentration. All measurements were performed in triplicate.

2.4. Determination of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity of BTI

A fresh 50 µmolL^{-1} of DPPH solution was prepared by dissolving 0.0986 mg of DPPH in 5 mL of methanol. This concentration was selected to produce an initial absorbance within the optimal range, approximately 0.8–1.2 at 517 nm, ensuring sufficient sensitivity for detecting changes in absorbance due to antioxidant activity. In a microplate, 100 µL of 40% BTI was added, followed by 100 µL of DPPH solution. 100 µL of Ascorbic acid 100 µmol L^{-1} in methanol) mixed with 100 µL of DPPH solution was used as the positive control, while 100 µL of methanol mixed with 100 µL DPPH solution served as the negative control. A volume of 200 µL of methanol alone was used as the blank. All reactions were carried out in a final volume of 200 µL. The mixtures were incubated in the dark at room temperature for 30 min to give sufficient time for the reaction between DPPH and the antioxidants in BTI. After incubation, the absorbance was measured at 517 nm using a spectrophotometer, corresponding to the maximum absorbance of DPPH. The results were expressed as mg mL^{-1} ascorbic acid equivalents (AAE) of the test concentration of 40% (v/v) BTI. All assays were conducted in triplicate.

2.5. Bacterial Strains Used to Test the Antioxidant Activity of BTI

The strain of *L. delbrueckii* subsp. *lactis* (DSM 20072; DMBUK 113040) was obtained from the culture collection at the Department of Microbiology, University of Kelaniya (DMBUK), which was maintained at 37°C for the study under anaerobic conditions in MRS medium (HI Media, India).

2.6. The Aerobic Growth of the Bacterium in the Presence of BTI

Based on a preliminary experiment, a 10^{-6} dilution of an overnight anaerobic culture of *L. delbrueckii* subsp. *lactis* was selected as the plateable inoculum. Accordingly, 1 mL aliquots from the 10^{-6} dilution were aseptically transferred into 12 sterile petri dishes. Molten MRS agar supplemented with BTI to a final concentration of 40% (v/v) was poured into six plates, while standard MRS agar without BTI was poured into the remaining six plates, which served as controls. After solidification, all plates were incubated under aerobic conditions at 37°C for 24 h. Following incubation, bacterial growth was quantified by enumeration of CFU on each plate. The mean CFU counts obtained from BTI-incorporated plates were compared with those from BTI-free control plates to evaluate the effect, if any, of BTI on the aerobic growth of the bacterium.

2.7. Statistical Analysis

Statistical analysis was performed using Minitab (Version 17, Minitab LLC) and Microsoft Excel software. Differences between means were evaluated using an independent samples *t*-test. Data are presented as mean ± standard deviation (SD), and statistical significance was accepted at $p \leq 0.05$. Microbiological assays were performed with $n = 9$ biological replicates, while chemical assays (TPC and DPPH) were conducted in triplicate.

3. Results and Discussion

The total phenolic content of the 40% (v/v) BTI, determined by the Folin–Ciocalteu assay, was 0.328 $mgmL^{-1}$ gallic acid equivalents. Quantification of DPPH radical scavenging activity was performed using a calibration curve constructed with ascorbic acid standards (0–100 $\mu molL^{-1}$). The relationship between absorbance at 517 nm and ascorbic acid concentration was linear, with the regression equation $Y = -0.1206X + 1.5098$ ($R^2 = 0.998$). The antioxidant capacity of BTI was calculated by substituting the measured absorbance values into the regression equation. The DPPH radical scavenging activity of the test concentration (40% BTI) was 3.099 $mgmL^{-1}$ ascorbic acid equivalents,

Under anaerobic conditions, no significant difference in bacterial growth was observed between BTI-free and BTI-incorporated (40%) media ($p = 0.82$; $n = 9$). In contrast, under aerobic conditions, CFU counts were significantly higher in the BTI-incorporated (40%) medium compared with the BTI-free control ($p = 0.044$; $n = 9$), indicating a growth enhancement associated with the presence of BTI under aerobic conditions (Fig. 1). This aerobic-specific growth enhancement suggests a protective role of BTI under oxidative conditions.

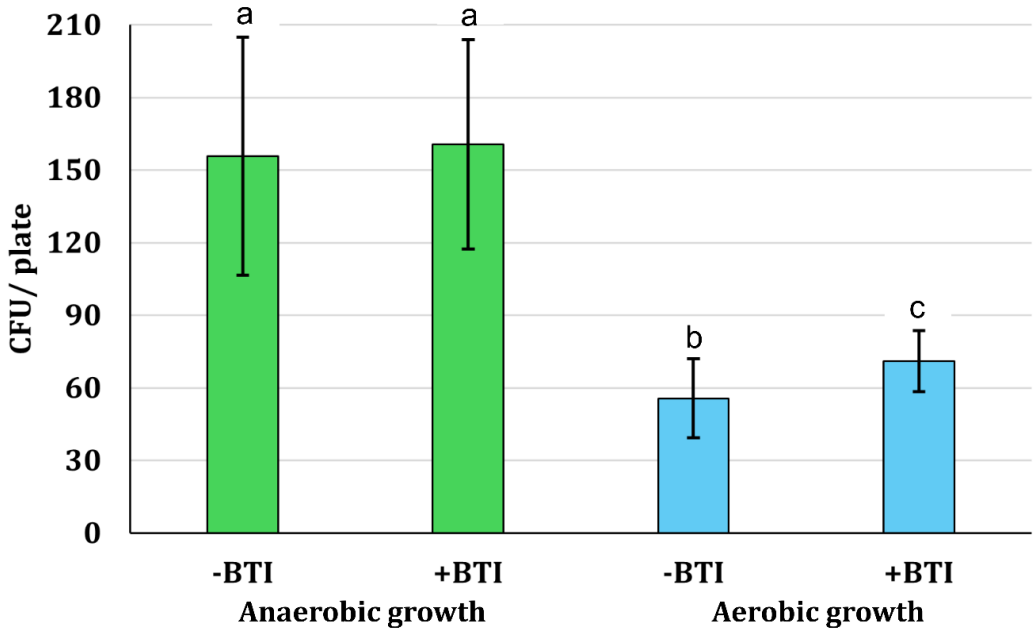

Figure 1. Effect of black tea infusion (BTI) on the growth of *Lactobacillus delbrueckii* subsp. *lactis* under aerobic and anaerobic conditions. Bacterial growth was assessed as colony-forming units (CFU) per plate. Bars represent mean CFU ± standard deviation (SD) (n = 9). Under aerobic conditions, growth in BTI-incorporated medium was significantly higher than in BTI-free medium (p = 0.044). Bars with different letters differ significantly ($p \leq 0.05$).

The 40% BTI used in the bioassays contained a substantial total phenolic content together with considerable radical scavenging activity, confirming the presence of phenolic compounds in BTI capable of mitigating oxidative stress during aerobic exposure of the organism. The total phenolic content and radical scavenging activity determined for the 40% BTI in the present study are consistent with values reported previously for black tea infusions (Leung et al. 2001; Cabrera et al. 2006; Sang et al. 2011). Theaflavins and thearubigins, the major phenolic constituents of black tea, have been demonstrated to possess strong antioxidant activity, although with different kinetics compared with green tea catechins (Leung et al. 2001; Khan & Mukhtar, 2019).

The results demonstrated that BTI confers a growth advantage to *L. delbrueckii* subsp. *lactis* under aerobic conditions, indicating a possible antioxidant protective effect. Notably, BTI did not significantly influence CFU under anaerobic conditions. Since anaerobic growth was not significantly enhanced by BTI incorporation, the observed increase in aerobic CFU is unlikely to be attributable to a general nutritional effect of the infusion at the concentration tested. Rather, the growth enhancement observed specifically under aerobic conditions suggests that BTI phenolics may have alleviated oxygen-associated oxidative stress experienced by the bacterium during aerobic growth (Fig. 1).

Zhao and Shah (2014) and Li et al. (2019) suggested that tea-derived phenolics improve the survival of lactic acid bacteria under aerobic conditions by extracellular scavenging of ROS and redox modulation of the medium rather than intracellular accumulation. The aerobic growth enhancement

observed in the present study, together with the absence of a growth-promoting effect under anaerobic conditions, supports this interpretation.

In contrast, studies using coconut milk phenolics have demonstrated sustained antioxidant protection following overnight pre-treatment, resulting in improved aerobic survival of lactic acid bacteria even after removal of the phenolic source (Karunasiri et al. 2020; Gunawardane et al. 2025). This carryover effect has been attributed to the relatively smaller molecular size and lower degree of polymerisation of coconut milk phenolics, which may facilitate intracellular uptake or stable cell-associated effects. By comparison, black tea phenolics, such as theaflavins and thearubigins, are larger and more polar oxidation products, and their intracellular uptake by lactic acid bacteria has not been demonstrated in previous studies. In the present study, no pre-treatment experiments were performed. Instead, BTI was present continuously during aerobic growth. Therefore, the observed antioxidant protection is more plausibly explained by extracellular scavenging of reactive oxygen species and modulation of the redox environment of the medium during active growth.

4. Conclusions

This study demonstrates that BTI confers a selective growth advantage to *L. delbrueckii* subsp. *lactis* under aerobic conditions, while exerting no significant effect under anaerobic conditions. The 40% (v/v) BTI used in the bioassays exhibited measurable total phenolic content and strong radical scavenging activity, confirming the presence of antioxidant phenolic compounds. The absence of significant growth enhancement under anaerobic conditions suggests that BTI, at least at the concentration tested, did not primarily function as a general nutrient supplement. The enhanced aerobic growth observed in the presence of BTI suggests a protective effect against oxygen-associated oxidative stress. Although the precise mechanism was not directly investigated in the present study, the findings are consistent with previous reports proposing extracellular antioxidant activity of black tea phenolics during microbial growth. These results highlight the potential of black tea–derived phenolics as functional antioxidant additives for improving the aerobic tolerance of lactic acid bacteria in food and fermentation systems.

Conflicts of interest: The authors have no conflicts of interest regarding this publication.

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