

# Complexing of Green Tea Catechins with Food Constituents and Degradation of the Complexes by *Lactobacillus plantarum*

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Complexing of green tea catechins with food constituents and their hydrolysis by tannase-producing *Lactobacillus plantarum* strains, were investigated. Our observations indicated that 1) epigallocatechin gallate (EGCg) and other catechin galloyl esters bound with food ingredients (i.e., proteins) to form a complex that is likely to be unabsorbable through the intestinal wall, whereas most catechins not esterified with gallic acid (GA) remain in free form, not complexing with food ingredients; 2) tannase activity of *L. plantarum* is strain dependent, possibly grouped into those with high tannase activity hydrolyzing EGCg to epigallocatechin and GA and those with the low activity; and 3) *L. plantarum* strains with high tannase activity are capable of hydrolyzing not only intact EGCg but also EGCg and other catechin galloyl esters complexed with dietary proteins to free non-galloyl ester catechins and GA. The evidence suggests that *L. plantarum* with high tannase activity, if it colonizes the human intestine, would release free non-galloyl-ester catechins and GA that are readily absorbed through the human intestinal epithelia from the complexes, thereby ensuring maximum delivery of the bioactive polyphenols of green tea to the host.

**Key words:** epigallocatechin gallate, epigallocatechin, gallic acid, tannase, *Lactobacillus plantarum*

## INTRODUCTION

Catechins contained in green tea, black tea, and wine are known to have strong antioxidative and other potentially useful bioactivities [1]. Accumulating evidence indicates that these activities have notable effects against so-called lifestyle-related diseases, including the suppression of carcinogenesis through the “detoxification” of various oxygen-free radicals generated in the body [2], prevention of arterosclerosis by inhibiting the oxidation of blood cholesterol [3] and improving endothelial function [4], and regulation of blood sugar concentration by affecting enzymatic activities related to glycogenesis [5]. Major green tea catechins include epigallocatechin gallate (EGCg) and epicatechin gallate (ECg), which are esterified with gallic acid (GA), such as hydrolysable tannins, and epigallocatechin (EGC) and epicatechin (EC), in which EGCg is the most abundant [6]. Like other plant

polyphenols, catechins are known to bind to various proteins (e.g., albumin, casein) to form macromolecular complexes in vitro [7, 8]. Furthermore, Carbonaro et al. [9] demonstrated through animal experiments that catechins bound with proteins in the intestinal lumen, which may limit their absorption from the intestine. Several human studies [10, 11] indicate that green tea catechins of the galloyl-ester form, EGCg and ECg, were absorbed through the intestines markedly less than those of the non-galloyl ester form, EGC and EC. The evidence suggests that the galloyl ester catechins in green tea are more readily complexed with proteinous components of foods than non-ester catechins to form stable macromolecules, thereby making them difficult to absorb through the host intestinal wall.

Over the past decade, we have isolated a range of *Lactobacillus* species, including *Lactobacillus plantarum*, *L. pentosus* and *L. paraplantarum*, capable of degrading tannin-protein complexes from human feces and fermented vegetables that produce tannase (tannin acyl hydrolase [galloyl ester hydrolyzing enzyme]) [12, 13]. Furthermore, we found that the optimal pH and

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temperature for the activity of a tannase homologue cloned from a strain of *L. plantarum* were around pH7 and 40°C, respectively [14]. Since pH within the human intestinal lumen ranges from slightly alkaline or acidic (i.e., 6.0-7.5) and the mean total gastrointestinal transit time of ingested materials is approximately 30 hr in normal human subjects [15], the macromolecules of galloyl-ester catechins complexed with dietary proteins may be degraded by tannase produced by lactobacilli colonizing the human intestine, thereby yielding smaller molecules of non-galloyl-ester catechins that are absorbable through the human intestinal wall. In order to evaluate this possibility, we herein describe: 1) complexing of green tea catechins with food constituents, 2) hydrolysis of green tea catechin complex with food constituents by tannase-producing lactobacillus strains and finally 3) degradation of green tea catechin complex with food constituents by *L. plantarum* strains in vitro.

## MATERIALS AND METHODS

### Materials

A standard laboratory rodent diet (MF; Oriental Yeast, Osaka, Japan) consisting of 54.4% carbohydrate, 23.6% protein, and 5.3% fat (% total energy) was pulverized and dissolved in 50 mM phosphate buffer (pH 6.8) containing 1% ascorbic acid (Wako Chemical Industries, Osaka, Japan) at final concentrations of 4%, 10% and 20% (wt/vol) with a shaking incubator (200 rpm) at room temperature for 30 min to prepare a double strength stock solution of rodent diet. Pure digestive enzymes including pepsin, porcine pancreatic lipase, trypsin and chymotrypsin were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Pure (-)-epigallocatechin gallate (EGCg), (-)-epigallocatechin (EGC) and 3,4,5-trihydroxybenzoic acid (gallic acid [GA]) obtained from Wako Pure Chemical Industries, Ltd. were each dissolved in 50 mM phosphate buffer (pH 6.8) containing 1% ascorbic acid (Wako) at a final concentration of 0.5 mM or 1 mM as the respective stock solutions. In addition to EGCg, EGC and GA, pure catechin galloyl esters including (-)-gallocatechin gallate (GCg), (-)-epicatechin gallate (ECg) and (-)-catechin gallate (Cg) and pure non-galloyl ester catechins including (-)-gallocatechin (GC), (+)-catechin (C) and (-)-epicatechin (EC) were also obtained from Wako or Funakoshi Co., Ltd. (Tokyo, Japan) and used as standard markers for subsequent HPLC analyses. The chemical structures of the catechins and their galloyl esters as described above are presented in Figure 1 for reference.

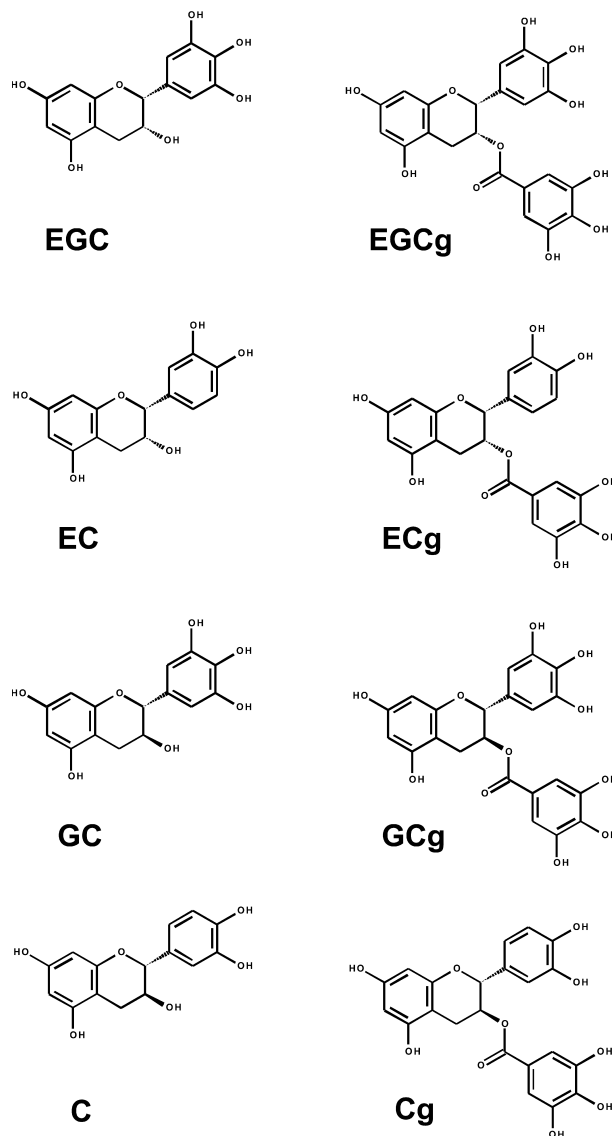


Fig. 1. Chemical structures of major green tea catechins and their galloyl esters. EGC, epigallocatechin; EGCg, epigallocatechin gallate; EC, epicatechin; ECg, epicatechin gallate; GC, gallocatechin; GCg, gallocatechin gallate; C, catechin; Cg, catechin gallate

A commercial PET bottled green tea beverage (Kao Corporation, Tokyo, Japan) was purchased from a local grocery store in Japan. Green tea was mixed with an equal volume of 100 mM phosphate buffer (pH 6.8 [PBS]) containing 2% ascorbic acid (Wako), which is referred to as “green tea solution” in the subsequent analyses.

Meanwhile, 15 *L. plantarum* strains, including those isolated from dietary fermented vegetables that were

commercially available in Japan, were incubated in MRS broth (Oxoid Ltd., Basingstoke, United Kingdom) aerobically at 37°C for 24hr. All strains were positive for tannase activity, judging from a qualitative tannase assay [16] using methyl gallate (Wako) as a substrate. All strains possessed an *L. plantarum*-specific tannase gene as determined by PCR assay, the procedure for which is described elsewhere [14]. After incubation, the bacterial cultures, collected by centrifugation at  $2,150 \times g$  for 10 min, were washed well with PBS (pH 7.0) and then suspended, with OD<sub>660</sub> values of 1.0 (ca. 8.7 log colony forming units [cfu]/ml), in 50 mM phosphate buffer (pH 6.8) containing 1% ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to prepare a “bacterial suspension” for subsequent analyses.

#### HPLC analysis

Prior to HPLC analysis, all samples were filtered through a 10-kDa cut-off centrifugal filter unit (Amicon Ultra-0.5; Millipore Corp., Bedford, USA) by centrifugation for 15 min at 14,000g in order to collect fractions containing free EGCg, EGC, and GA. The filtrates were then applied to a Shim-pack XR-ODS (3.0 mm i.d.  $\times$  50 mm, 2.2  $\mu$ m particle size; Shimadzu Corporation, Kyoto, Japan) using a Prominence Ultra Fast Liquid Chromatography system (Shimadzu). Chromatograms were processed with an LC solution program (Shimadzu). The separation solvents were 0.1% phosphoric acid (A) and 0.1% phosphoric acid / acetonitrile 50:50 v/v (B). Separation was carried out at 37°C, according to the following program: 01 min, isocratic of 5% B; 1–5 min, linear gradient of 5–20% B; 5–9 min, linear gradient of 20–40% B; 9–10 min, linear gradient of 40–100% B; 1011 min, isocratic of 100% B; and 11–15 min, isocratic of 5% B. The flow rate and injection volume were 1.0 mL/min and 2  $\mu$ L, respectively. Catechins and GA in eluates were detected by monitoring absorbance at 230 nm and quantified by the means of the calibration curves obtained with respective authentic standard markers of catechins and GA.

#### Food constituents complexing assay

For EGCg, EGC and GA, one milliliter of the reaction mixtures consisted of 0.5 mM each of EGCg, EGC, or GA, 2, 5, and 10% wt/vol rodent diet, 1% wt/vol ascorbic acid, with or without addition of 2% wt/vol pepsin, porcine pancreatic lipase, trypsin, or chymotrypsin (Wako) in order to simulate human digesta. When green tea catechins were used, the mixture consisted of 50 mM phosphate buffer (pH 6.8), 50% vol/vol green tea solution, 2, 5 and 10% wt/vol rodent diet and 1% wt/vol

ascorbic acid, in which the final concentration of commercial green tea was 25% vol/vol. The mixtures were incubated at 37°C for 3 min, 7 min, 15 min, 30 min, and 45 min, respectively. After incubation, they were subjected to HPLC analysis as described above. Each assay was performed in triplicate.

#### Assay for green tea catechin hydrolysis by tannase-producing *L. plantarum* strains

One milliliter of each bacterial suspension was transferred to an Eppendorf tube and centrifuged at 14,000 g for 5 min. After centrifugation, the supernatant was removed, leaving the bacterial cell pellets on the bottom of the tube. One milliliter of the mixture consisting of 50 mM phosphate buffer (pH 6.8), 0.475 mM EGCg, EGC or GA and 1% wt/vol ascorbic acid was then added to the tube. The bacterial cell pellet was then resuspended thoroughly with a vortex test-tube mixer for 30 sec. The prepared mixtures were then incubated at 37°C for 10hr. After incubation, the mixtures were subjected to HPLC analysis as described above. The same reaction without EGCg, EGC and GA was carried out as a control. Each assay was performed in triplicate.

As shown later, the EGCg hydrolyzing activity of the lactobacilli was strain dependent, with some of the strains having high activity and others having low activity. Strain *L. plantarum* 22A-4, which had high hydrolyzing activity, and strain *L. plantarum* 20A-2, which had low activity were used for the subsequent assay. One milliliter of the bacterial suspension of *L. plantarum* 22A-4 or 20A-2 was transferred to an Eppendorf tube and centrifuged at 14,000 g for 5 min. After centrifugation, the supernatant was removed, leaving the bacterial cell pellets on the bottom of the tube. Five hundred  $\mu$ l of PBS containing 1% ascorbic acid (Wako) was then added to the tube, and the bacterial cell pellet was resuspended thoroughly by a vortex test-tube mixer for 30 sec. Five hundred microliters of green tea solution was then added to the tube and mixed by a vortex test-tube mixer for 30 sec. The prepared mixtures were incubated at 37°C in a water bath for 10hr. After incubation, the mixtures were subjected to HPLC analysis as described above. The green tea mixture without the bacterial cells was manipulated as a control. Each assay was performed in triplicate.

#### Assay for hydrolysis of EGCg complexed with food constituents by tannase-producing *L. plantarum* strains

*L. plantarum* 22A-4 with high hydrolyzing activity and *L. plantarum* 20A-2 with low activity were used for

the subsequent assay. First, a 1-ml bacterial suspension of *L. plantarum* 22A-4 or 20A-2 was transferred to 7 Eppendorf tubes and centrifuged at 14,000 g for 5 min. After centrifugation, supernatants were removed, leaving the bacterial cell pellets on the bottom of the tubes. To each respective pellet, one milliliter of the phosphate buffer (pH 6.8) containing 10% wt/vol rodent diet, 0.5 mM EGCg, and 1% wt/vol ascorbic acid, which were preincubated at 37°C for 15 min, was added. The bacterial cell pellet was then immediately suspended thoroughly in the mixtures with a vortex test-tube mixer for 30 sec. Immediately after mixing, one of the mixtures was subjected to HPLC analysis, and the rest of the tubes containing the mixtures were incubated at 37°C in a water bath for 1h, 2, 4, 6, 8 and 10 hr. After each incubation, the mixtures were subjected to HPLC analysis as described above. Each assay was performed in triplicate.

#### *Hydrolysis assay of green tea catechins complexed with food constituents*

Prior to the assay, the green tea solution was mixed with an equal volume of 50 mM phosphate buffer (pH 6.8) containing 1% ascorbic acid (Wako). The mixture was subjected to HPLC analysis in order to qualify and quantify the catechin contents in the mixture. *L. plantarum* 22A-4 with high hydrolyzing activity and *L. plantarum* 20A-2 with low activity were used for the subsequent assay. First, a 1-ml bacterial suspension of LP22A-4 or 20A-2 was transferred to 7 Eppendorf tubes and centrifuged at 14,000 g for 5 min. After centrifugation, the supernatants were removed, leaving the bacterial cell pellets on the bottom of the tubes. To the respective pellet, one milliliter of the phosphate buffer (pH 6.8) containing 10% wt/vol rodent diet, 50% vol/vol green tea solution and 1% wt/vol ascorbic acid, which were preincubated at 37°C for 15 min, was added. The bacterial cell pellets were then resuspended thoroughly in the mixtures with a vortex test-tube mixer for 30 sec. Immediately after mixing, one of the mixtures was subjected to HPLC analysis, and the rest of the tubes containing the mixtures were incubated at 37°C in a water bath for 10 hr. After incubation, the mixtures were subjected to HPLC analysis as described above. Each assay was performed in triplicate.

## RESULTS

*Complexing of catechins with food constituents.* A marked proportion of pure EGCg was complexed with the food constituents within 3 min of incubation (Fig. 2a). The extent of complexing was dependent on the

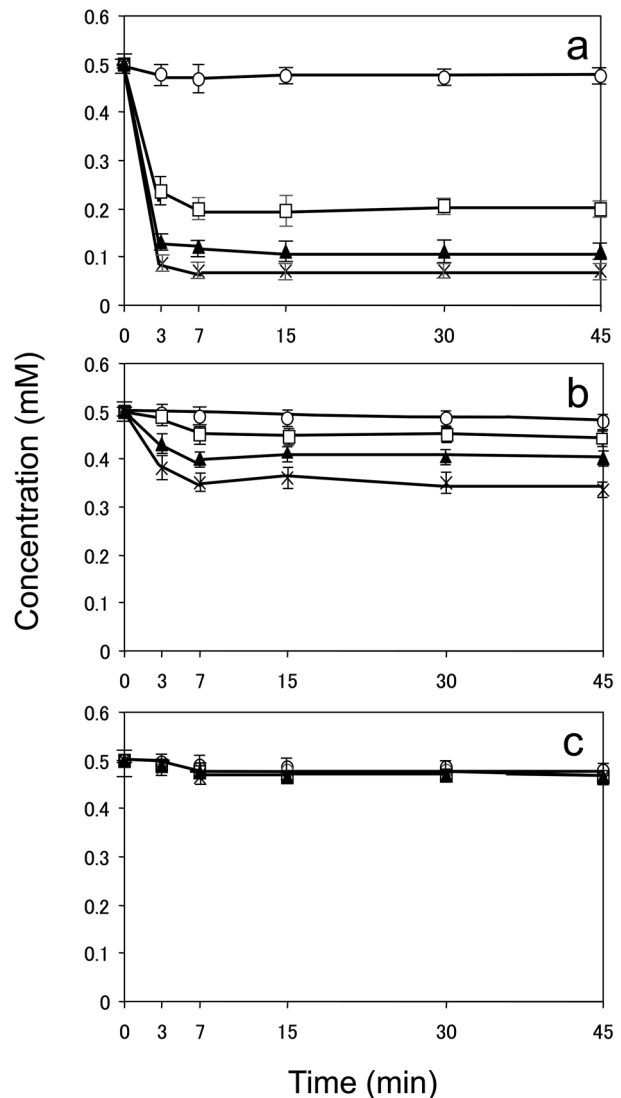


Fig. 2. Concentrations of free EGCg (a), EGC (b) and GA (c) in the mixture containing 0.5 mM of pure (-)-epigallocatechin gallate, (-)-epigallocatechin, or gallic acid and 0% (○), 2% (□), 5% (▲) and 10% (×) of a standard laboratory rodent diet (wt/vol) detected by HPLC during 45 min of incubation at 37°C.

concentration of laboratory rodent diet in the mixture, in which less than 50%, 30% and 20% of EGCg were detected as the free form in the 2%, 5% and 10% diet mixtures, respectively. Similar diet concentration-dependent complexing was observed for pure EGC (Fig. 2b), but the extent of complexing was more moderate, with approximately 70% of EGC detected as the free form even in the 10% diet mixture. The concentration of pure GA remained intact regardless of the concentration of the diet in the mixtures throughout incubation (Fig.

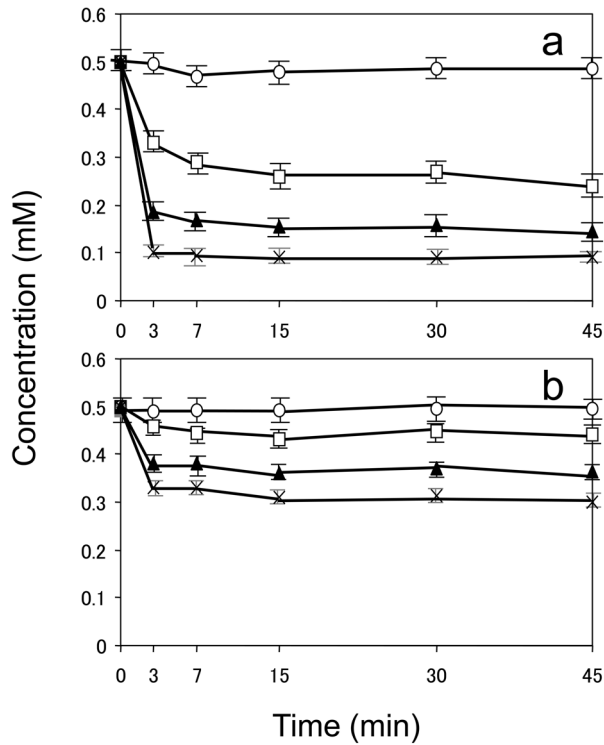


Fig. 3. Concentrations of free EGCg (a) and EGC (b) in the mixture containing quarter-strength commercial PET bottled green tea beverage and 0% (○), 2% (□), 5% (▲) and 10% (X) of a standard laboratory rodent diet (wt/vol) detected by HPLC during 45 min of incubation at 37°C.

2c). It should be noted that coincubation with either pepsin, porcine pancreatic lipase, trypsin or chymotrypsin did not affect complexing and the results were comparable to those without the digestive enzymes (data not shown).

Figures 3a and 3b show the transition of the concentrations of EGCg and EGC detected as the free form in the green tea-rodent diet mixture during incubation, respectively. Approximately 20% EGCg was detected as the free form, while approx. 70% EGC was detected after 7 min incubation with the diet mixture, and the ratio remained constant throughout the incubation. Figures 4a and 4b show a comparison of HPLC chromatograms between the commercial green tea mixed equally with PBS only and green tea mixed equally with 20% of the diet suspension after 45 min incubation at 37°C. Peaks of not only EGCg but also other galloyl-ester catechins (i.e., GCg, ECg, Cg) were diminished markedly after incubation with the food suspension, whereas more than half the contents of EGC and other non-galloyl ester catechins (i.e., GC, EC, C) remained as the free form.

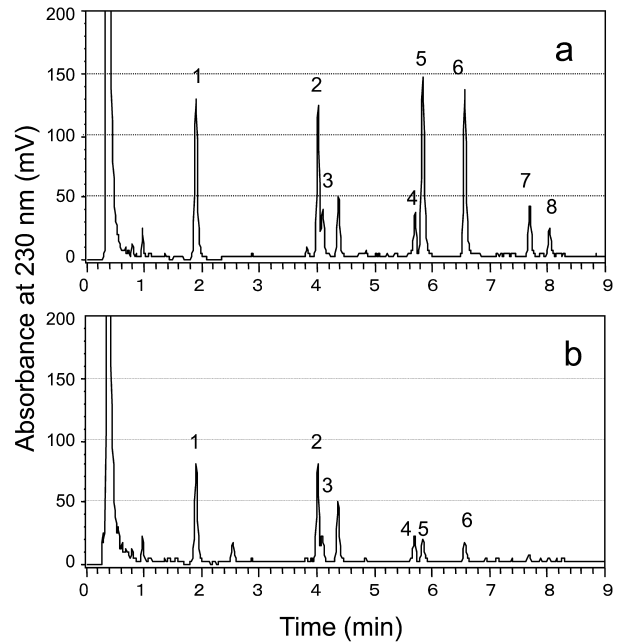


Fig. 4. HPLC chromatograms of the mixture containing half-strength commercial green tea mixed equally with PBS only (a) and with the solution of 20% (wt/vol) standard laboratory rodent diet (b) after 45 min of incubation at 37°C. Peaks: 1, GC; 2, EGC; 3, C; 4, EC; 5, EGCg; 6, GCg; 7, ECg; and 8, Cg.

*Hydrolysis of catechin galloyl esters by tannase-producing L. plantarum strains.* Of the 14 *L. plantarum* strains tested in the present study, 8 strains hydrolyzed more than 80% of pure EGCg added in the test medium to EGC and GA after 10 hr of incubation. The rest of the strains showed marginal hydrolyzing activity (Fig. 5). It should be noted that EGC and GA added to the test media were not degraded by any of the strains tested after 10 hr of incubation (data not shown). Strain 20A-2, which had low hydrolyzing activity, showed limited hydrolyzing activity on catechin galloyl esters in the green tea mixture, in which the chromatographic peak pattern (Fig. 6b) was almost comparable to that of the green tea mixture without addition of the bacteria (Fig. 6a). By contrast, strain 22A-4, which showed high EGCg hydrolyzing activity, hydrolyzed not only EGCg but also other catechin galloyl esters (i.e., GCg, ECg, Cg) in the green tea mixture, yielding non-galloyl-ester catechins (i.e., GC, EC, C) and GA after 10 hr of incubation at 37°C (Fig. 6c). These results indicated that the tannase of *L. plantarum* can hydrolyze not only EGCg but also other catechin galloyl esters to non-galloyl esters in green tea.

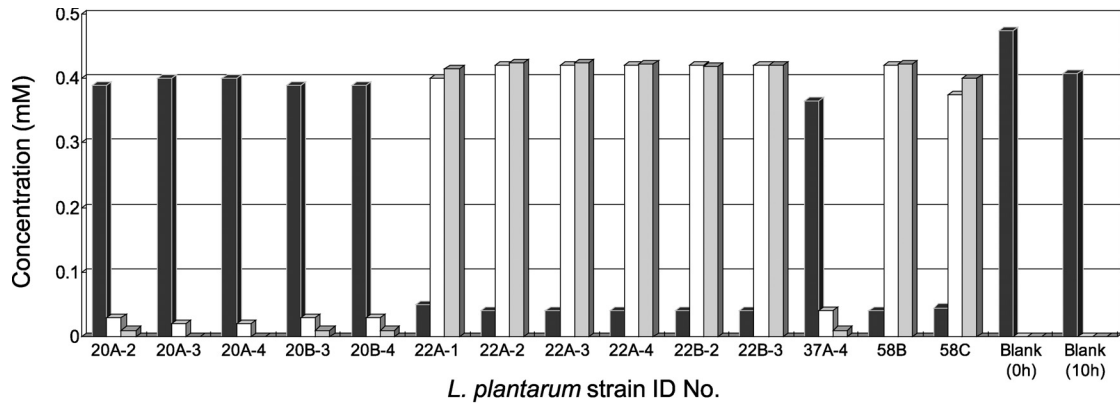


Fig. 5. Concentrations of EGCg (solid bars), EGC (white bars), and GA (striped bars) detected by HPLC in pure EGCg solutions incubated with 15 *L. plantarum* strains (ca.  $8.7 \log \text{cfu/ml}$ ) for 10 hr at  $37^\circ\text{C}$ . Concentration of pure EGCg solution without the addition of bacterial cells at the start of the assay (Blank, 0 hr) decreased slightly after 10 hr of incubation (Blank, 10 hr) due to auto-oxidation of EGCg.

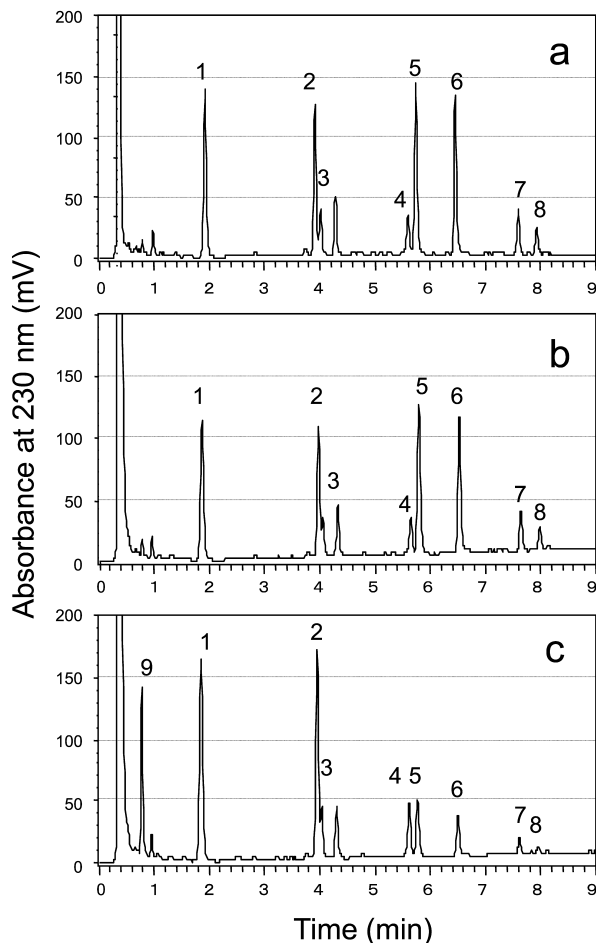


Fig. 6. HPLC chromatograms of half-strength commercial green tea mixed equally with PBS only (a), half-strength commercial green tea mixed equally with PBS containing ca.  $8.7 \log \text{cfu}$  *L. plantarum* 20A-2 (b) and half-strength commercial green tea mixed equally with PBS containing ca.  $8.7 \log \text{cfu}$  *L. plantarum* 22A-4 after 10 hr of incubation at  $37^\circ\text{C}$ . Peaks: 1, GC; 2, EGC; 3, C; 4, EC; 5, EGCg; 6, GCg; 7, ECg; 8, Cg; and 9, GA.

#### Hydrolysis assay of catechin galloyl esters complexed with food constituents

Approximately 10% of the EGCg initially added was detected as the free form after 15 min incubation in the 10% food suspension immediately before viable cells (ca.  $5.0 \times 10^8 \text{CFU/ml}$ ) of strain 22A-4 with high tannase activity and strain 20A-2 with low activity were added to the media. In the medium containing strain 22A-4, measurable amounts of EGC and GA were detected after 4 hr of incubation, and the amounts increased with time; after 10 hr of incubation, approximately 0.2 mM of EGC and GA in the free form was detected, indicating that approximately 40% of the EGCg complexed with proteins in the test medium was hydrolyzed to EGC and GA as the free form (Fig. 7a). However, little EGCg complexed with proteins in the test medium was hydrolyzed in the media containing 20A-2 during incubation (Fig. 7b). Likewise, the concentrations of EGCg and other galloyl catechins of green tea declined markedly on the HPLC graph after 15 min of incubation in 10% food suspension immediately before viable cells of *Lp* 22A-4 with high tannase activity were suspended in the media (data not shown). After 10 hr of incubation, the concentrations of EGC, EC and GA detected by HPLC (Fig. 8c) were approximately 5- to 10-fold higher than those detected in the media incubated with or without 20A-2 (Fig. 8b and Fig. 8a). The above observations provide evidence supporting that *L. plantarum* strains with high tannase activity are capable of hydrolyzing not only intact EGCg but also EGCg and other galloyl-ester catechins complexed with dietary proteins to free EGC and GA.

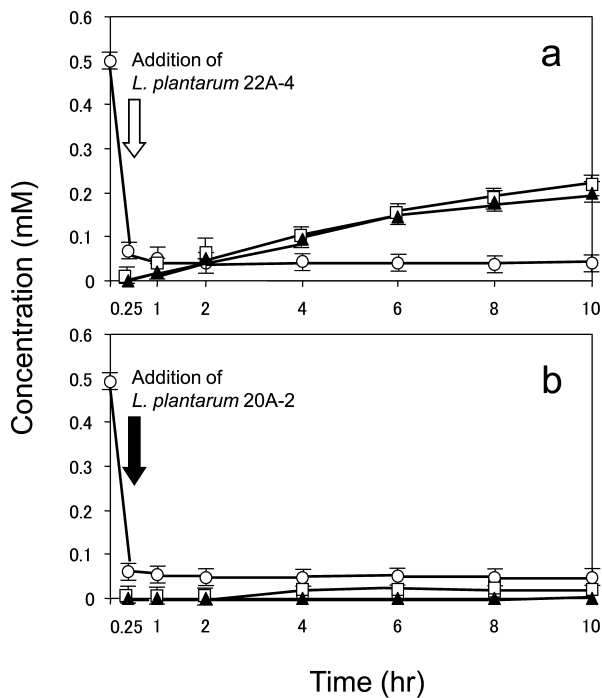


Fig. 7. Concentration of free EGCg (○) in the mixture containing 10% of a standard laboratory rodent diet (wt/vol) and concentrations of EGC (▲) and GA (□) detected by HPLC after addition of ca.  $8.7 \log$  cfu *L. plantarum* 22A-4 (a) or *L. plantarum* 20A-2 (b) during 10 hr of incubation at 37°C.

## DISCUSSION

Catechins complexing with food constituents have been demonstrated in several in vitro experiments reported elsewhere [7, 8], in which catechins formed macromolecular complexes with proteins (e.g., bovine serum albumin, casein), with EGCg forming the most stable complexes. Since substances with a molecular weight of >10 kDa cannot cross normal gastrointestinal mucosa [17] and free catechins including EGCg and EGC were detected in the 10-kDa cut-off filtrate of the mixtures in the present study, a large proportion of green tea catechin galloyl esters drunk by a human host would complex with dietary proteins or proteinous substances secreted from the alimentary tract (e.g., salivary proteins and mucins) to form macromolecules over 10 kDa, thereby not being absorbed through the intestinal wall, whereas most EGC remains uncomplexed and is readily absorbed through the alimentary epithelia. This might account for the markedly high area under the blood concentration time curve for EGC and EC as compared with EGCg and ECg in human volunteers consuming green tea, as observed by Henning et al. [10].

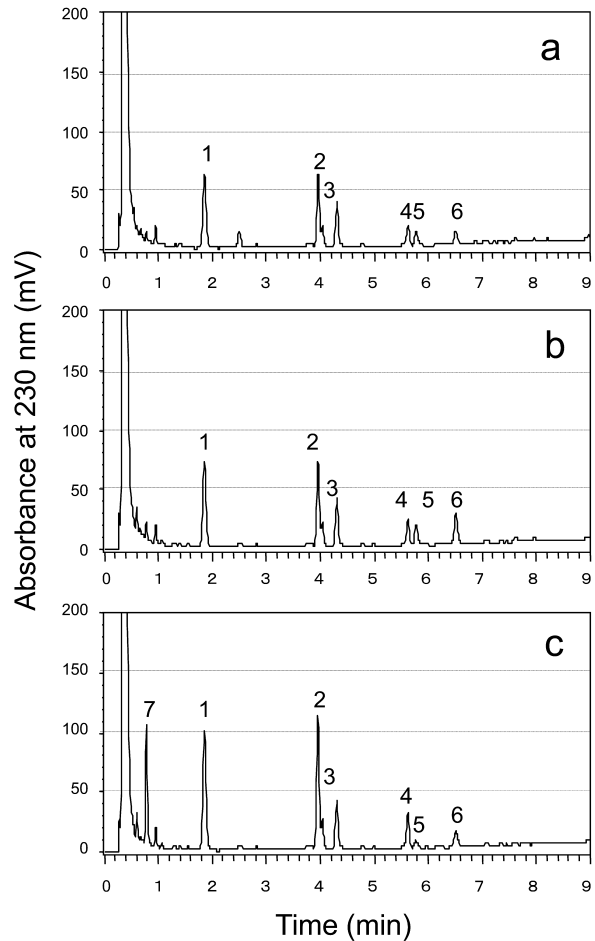


Fig. 8. HPLC chromatograms for the mixture with 50% vol/vol commercial green tea mixed and 10% wt/vol of rodent diet (a), the mixture with addition of ca.  $8.7 \log$  cfu *L. plantarum* 20A-2 (b) and the mixture with addition of ca.  $8.7 \log$  cfu *L. plantarum* 22A-4 (c) after 10 hr of incubation at 37°C. Peaks: 1, GC; 2, EGC; 3, C; 4, EC; 5, EGCg; 6, GCg; and 7, GA.

Tannases of several fungal origins (e.g., *Aspergillus oryzae*, *A. niger*) are currently being added to most commercial tea, wine and beer beverages in order to remove undesirable haze or precipitates [18] that are insoluble coacervates or condensation products composed of caffeine and tea flavonoids, including catechins [19]. This treatment leads to better color stability and organoleptic properties of green tea [20]. Although the safety of fungal tannase as a food additive has long been confirmed [21, 22], *L. plantarum* tannase may be an alternative and even safer additive, since the enzyme is produced by bacteria contained in the human diet that already colonize humans [23]. In addition, the evidence indicated that the tannase activity of *L. plantarum* is strain dependent, with strains being

grouped into those with high hydrolyzing activity and those having low activity on EGCg. Similar strain-dependent tannase activity was reported previously when methyl gallate was used as a substrate [12].

Many previous *in vitro* experiments have demonstrated that galloyl ester catechins hold stronger reactive oxygen scavenging activities than non-galloyl ester catechins; the scavenging activity of ECg is greater than that of Ec, and that of EGCg is greater than that of EGC [24–26]. For example, EGCg showed scavenging rates for  $\cdot\text{OH}$  and  $\text{O}_2\cdot$  that were by approx. 2-folds higher than those of EGC [26]. Correspondingly, higher cancer preventive activities of EGCg than EGC have been reported in several *in vitro* experiments [27–29]. EGCg showed higher growth suppressing activity against human promyelocytic leukemia cells that was by nearly 2-fold higher than that of EGC [29]. However, galloyl ester catechins have been known to have markedly poor bioavailability attributing to its low absorption as compared with non-galloyl-ester catechins. For example, Henning et al. [10] reported that EGC and EC had nearly 10 and 4-fold greater average areas under the plasma concentration–time curve than EGCg and ECg over 0–8 hr after a single consumption of green tea. More recently, Renouf et al. [30] reported in their human volunteer experiment that EGC was the major catechin, appearing rapidly in the plasma after green tea consumption, and showed an approx. 5–6 folds higher concentration than EGCg. The evidence suggests that the high absorbability of non-galloyl-ester catechins would well compensate for their relatively low bioreactivities. This in turn suggests that conversion of the galloyl ester catechins to non-galloyl ester catechins via *L. plantarum* delivers green tea's health promoting effects more efficiently to the human body, since galloyl ester catechins comprised more than 70% of total green tea catechins [6], most of which will be complexed with proteins in digesta and the complex excreted intact in feces otherwise.

Recent *in vitro* experiments [31, 32] indicated that fungal tannase-treated green tea inhibited the formation of carcinogenic and mutagenic N-nitrosamines in preserved meat due to the higher cumulative antioxidant activity of non-galloyl ester catechin contents (e.g., EGC, EC, GA) in tannase-treated green tea compared with untreated tea. Furthermore, non-galloyl ester catechins and GA were found to have marked antioxidant effects, which may exert synergistically beneficial health effects to ameliorate metabolic diseases such as diabetes mellitus [33]. In this context, the present findings lead us to propose that a greater population of

the *L. plantarum* strain with high tannase activity would degrade macromolecular complexes of catechin galloyl esters and dietary proteins to release more non-galloyl ester catechins and GA in the human host intestine. These catechins and GA would then be readily absorbed through the intestinal epithelia, thereby ensuring maximum delivery of the antioxidative properties of green tea to the host. If this is the case, *L. plantarum* with high tannase activity could be used as a novel probiotic to enhance the functionality of green tea. This probiotic application is feasible, as the consumption of a fermented oatmeal drink containing a probiotic strain of *L. plantarum* for 4 weeks achieved 8.2 log cfu/gram feces in human volunteers [34]. Further studies are in progress to evaluate such a probiotic potential of *L. plantarum* in animal experiments, which will then be developed for human volunteer experiments.

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