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Mitigating the Inhibitory Effect of Tannins on β-Glucosidase Activity Using Tannase from *Lactiplantibacillus plantarum*

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ABSTRACT

Tannins are a diverse group of plant-derived polyphenols with a hallmark property to bind to various biomolecules. Their binding with enzymes in specific ways leads to the loss of their enzymatic potential. A similar phenomenon is postulated in the case of tannins from fruits inhibiting the β-glucosidase activity, causing lesser hydrolysis of glycosidically bound volatiles in fruit juices. We first demonstrated that tannins, viz. tannic acid and epigallocatechin gallate, significantly inhibited β-glucosidase activity. Next, the cell-free supernatant (CFS) of *Lactiplantibacillus plantarum*, which is known to have tannase activity, was found to de-repress β-glucosidase inhibition caused by tannins. Our results indicate that tannase, along with β-glucosidase, can be a useful strategy for hydrolysing glycosidic phytochemicals for the release of bioactive chemicals.

1. Introduction

Tannins are one of the most abundant classes of secondary metabolites present in a broad range of fruits, vegetables, legumes, cereals, and nuts (Bule *et al.* 2020). Tannins serve as defence molecules in plants by binding to extracellular proteins and enzymes produced by invading microbes, thereby limiting the microbial colonisation. The enzyme inhibition by tannins also poses problems in food processing. For example, red wine tannins have been shown to inhibit salivary β-glucosidases, reducing the hydrolysis of glycosidically-bound volatiles (GBV) and thus aroma release (Genovese *et al.* 2009). Similarly, in our previous study, we reported the release of a lower number of volatiles from β-glucosidase-treated mango juice than those released from the β-glucosidase-treated purified fraction of GBV (Godse *et al.* 2023). We further attributed this observation to the inhibitory effect of tannins, which are known to be present in mango fruits (Marcela *et al.* 2017; Arampath & Dekker 2019). However, despite these observations, direct evidence of the inhibition of β-glucosidases by well-defined tannins is not substantial and has been limited to plant extracts instead of purified tannins (Juntheikki & Julkunen-Tiitto 2000).

Given the potential for tannins to modulate β-glucosidase activity, it is also plausible that enzymatic degradation of the inhibitory tannins can lead to restoration of the β-glucosidase activity. Tannases or tannin acyl hydrolases (EC 3.1.1.20) are enzymes that hydrolyze the ester bond in hydrolyzable tannins to release gallic acid and glucose (Chávez-González *et al.* 2012). Due to their ability to degrade tannins, tannases are useful in the food and beverage industry as a clarifying agent for instant tea, juices, and beers (Yao *et al.* 2014). Therefore, exploring the potential of tannases

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to degrade inhibitory tannins and recover β-glucosidase activity holds both theoretical and practical interest.

In this study, we aimed to assess the effect of tannins on the almond β-glucosidase activity and the potential of the cell-free supernatant (CFS) of *Lactiplantibacillus plantarum* JGR2, previously shown to exhibit tannase activity (Surve *et al.* 2022), to degrade tannins and restore β-glucosidase activity.

2. Materials and Methods

A standard 200 µL assay for β-glucosidase activity contained 100 µg of X-glucoside and 10 µg of almond β-glucosidase in 50 mM phosphate buffer (pH 7) incubated at 37°C for one hour. The product formation was assessed spectrophotometrically at 630 nm. To assess the effect of tannic acid and epigallocatechin gallate (EGCG) on the β-glucosidase activity, the standard reaction was carried out in the presence of 0.01-0.2% tannins.

L. plantarum JGR2 was cultivated as detailed earlier (Surve *et al.* 2022), and the cell-free supernatant (CFS) was obtained by centrifugation at 2,000 g for 10 min at 25°C. The total protein in the CFS was quantified using Pierce BCA assay kit by Thermo Fisher Scientific (Massachusetts, USA) with bovine serum albumin as a standard and used for enzyme assays.

To determine the activity of β-glucosidase in the CFS, varying amounts of CFS (10-40 µg protein content) were added in a 200 µL reaction containing 100 µg of X-glucoside as the substrate and incubated at 37°C for one hour, followed by measurement of OD_{630}.

To assess the possible effect of CFS on derepression of β-glucosidase activity, 0.01% of tannic acid and varying amounts of CFS (10-40 µg with an interval of 10 µg) were added to the standard reaction with X-glucoside as the substrate. Parallelly, appropriate controls, viz., those without tannic acid and CFS, were also set up. The progress of the reactions was monitored as above. Similar reactions were set up with esculin as the substrate, and the product formation was analyzed by TLC as mentioned earlier (Godse *et al.* 2025). All the assays were performed in triplicate.

3. Results

Tannic acid (0.01%) inhibited β-glucosidase activity by more than 80%. Further slight reduction in the enzyme activity was observed with increasing concentration of tannic acid. A similar inhibitory effect of EGCG on β-glucosidase activity was observed (Figure 1). Since both tannins showed a similar extent of inhibition at the lowest concentration (0.01%), only one of them was used for further assays.

Varying amounts of CFS were assessed for derepression of the β-glucosidase activity. While tannic acid reduced the enzyme activity to about 30%, provision of CFS containing tannase led to the derepression of the enzyme activity (Figure 2). Specifically, even at the lowest amount of CFS used (containing total 10 µg protein), the β-glucosidase activity increased by more than two-fold, reaching about 80% of the activity found without tannic acid and CFS. Continuous increase in the activity was observed with increasing amount of

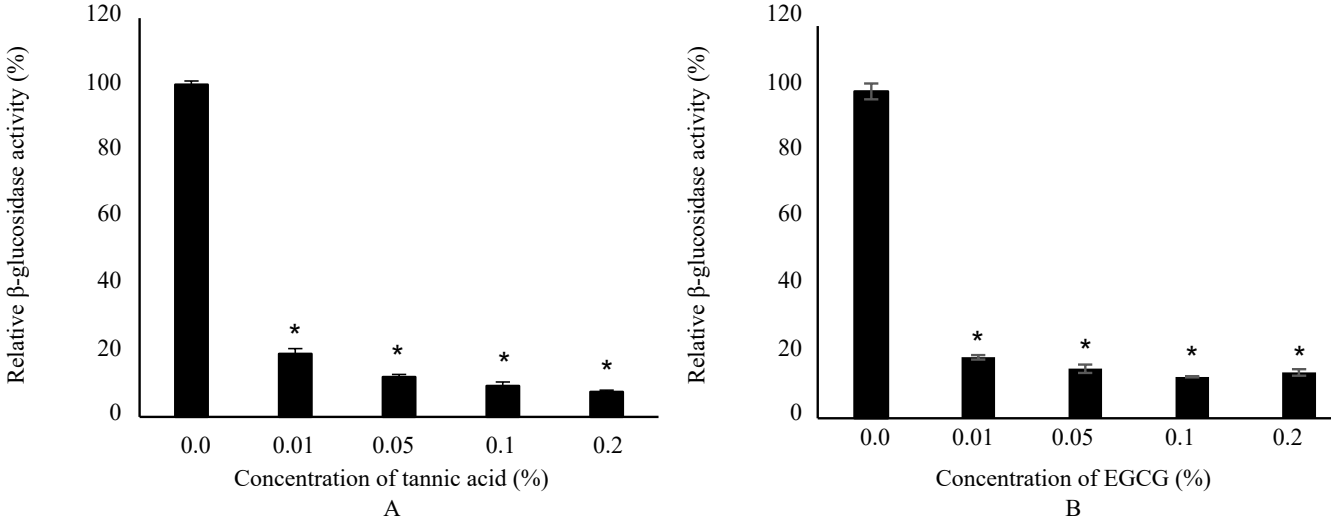


Figure 1. Effect of tannins on the β-glucosidase activity with X-glucoside as a substrate. (A) Tannic acid and (B) epigallocatechin gallate. Asterisks denote values that are significantly different than the control without these tannins (*, $p \leq 0.001$) (One-way ANOVA with Tukey's post hoc test)

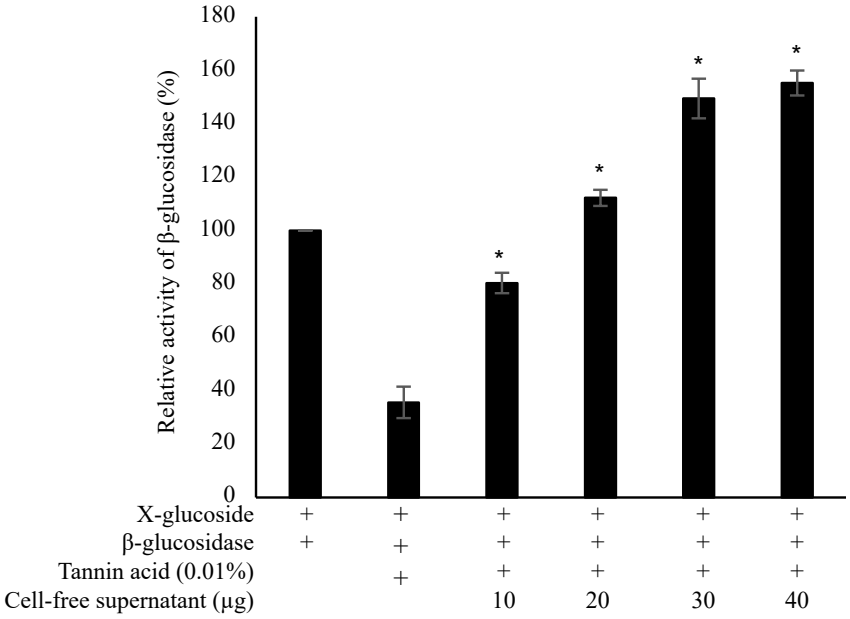


Figure 2. Assessing the derepression of β-glucosidase activity on X-glucoside by the cell-free supernatant of *L. plantarum* JGR2 as the tannase source. Asterisks denote values that are significantly different than the control (*, p≤0.001) (One-way ANOVA with Tukey's post hoc test)

CFS, with maximum activity reaching about 160% of the positive control in the presence of 40 µg of CFS (Figure 2).

The β-glucosidase activity of the CFS was examined to negate the possibility of its contribution to the observed derepression of tannic-acid-induced inhibition of almond β-glucosidase. CFS was not found to have any β-glucosidase activity (Figure 3).

In the reactions with esculin as the substrate, β-glucosidase completely degraded esculin. At the same time, the addition of tannic acid to the assay led to inhibition of β-glucosidase activity as reflected in the residual substrate spot (Figure 4). On the other hand, the addition of *L. plantarum* CFS to the reaction containing tannic acid showed no substrate spot (Figure 4), suggesting derepression of the β-glucosidase activity.

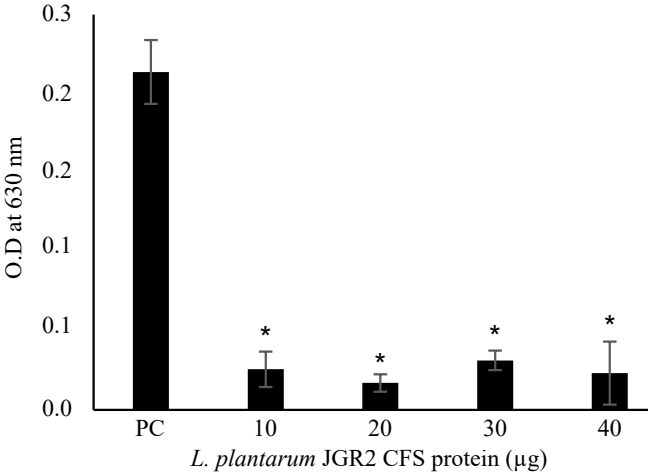
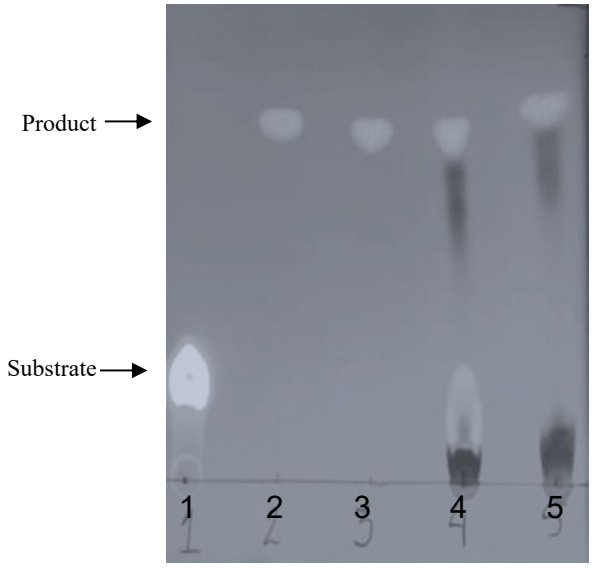


Figure 3. Determining the β-glucosidase activity of the *L. plantarum* JGR2 cell-free supernatant. Y-axis indicates the extent of β-glucosidase activity in terms of OD_{630}. PC indicates reaction containing 10 µg of almond β-glucosidase. Asterisks denote values that are significantly different than the control (*, p≤0.001) (One-way ANOVA with Tukey's post hoc test)

4. Discussion

Tannins inhibit various enzymes in food processing, including β-glucosidase. We aimed at addressing these issues using the CFS of *L. plantarum*, which possesses tannase activity (Surve *et al.* 2022). The experiments were devised using one of the most commonly used enzymes (almond β-glucosidase), a chromogenic substrate (X-glucoside) that yields a blue coloured product which does not overlap with the yellow colour of tannic acid, unlike p-nitrophenyl-β-glucopyranoside, and the tannin concentrations based on their concentrations in mango fruits (Verghese *et al.* 2017; Anoman Jean-Claude *et al.* 2022). The results with tannic acid and EGCG confirmed that tannins inhibit β-glucosidase activity. Furthermore, both tannins showed a similar extent of inhibition of β-glucosidase. An earlier study on the inhibition of



	1	2	3	4	5
Esculin (substrate)	+	-	+	+	+
Esculetin (product)	-	+	-	-	-
β-glucosidase	-	-	+	+	+
Tannin acid (0.01%)	-	-	-	+	+
Cell-free supernatant	-	-	-	-	+

Figure 4. Assessing the derepression of β-glucosidase activity on esculin by the cell-free supernatant of *L. plantarum* JGR2 as the tannase source

collagenase by tannic acid and EGCG also reported that comparable amounts of both these tannins showed a similar extent of inhibition (Jackson *et al.* 2010).

L. plantarum CFS was indeed found to relieve the inhibition of β-glucosidase caused by tannic acid. This derepression is highly likely because of the extracellular tannase secreted by *L. plantarum* (Surve *et al.* 2022). We also confirmed that *L. plantarum* CFS does not have the β-glucosidase, which is in agreement with a previous study (de Oliveira Galdino *et al.* 2023). Next, we wanted to know if the inhibition of β-glucosidase by tannic acid and its derepression by tannase activity of CFS is also observed with a natural plant substrate of β-glucosidase. For this purpose, we used esculin, a coumarin glucoside found in plants, which is a commonly used natural substrate for assessing the activity of new β-glucosidases (Godse *et al.* 2025). These results confirmed that the tannase activity of *L. plantarum* CFS can remove the inhibitory effect of tannic acid, restoring the activity of almond β-glucosidase towards natural substrates as well.

The potential of β-glucosidase to hydrolyze glycosidic phytochemicals, thus releasing bioactive compounds, is well-established (Godse *et al.* 2021). However, its application in the food industry has been limited, possibly because of inhibition by tannins present in the plant matrices. Our findings demonstrate that the CFS of *L. plantarum*, which contains tannase activity, can effectively reduce the inhibitory effects of tannins on β-glucosidase. This approach aligns with the growing demand for natural and sustainable methods in food processing.

Conflict of Interest

The authors declare no competing interests.

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