

Different varieties of banana flour on the survival of *Lactobacillus pentosus* and *L. plantarum*

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Research Article

Keywords: bananas flour, *Lactobacillus*, circular economy

Posted Date: June 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9607935/v1>

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Additional Declarations: No competing interests reported.

Abstract

Lactobacillus pentosus 12250 and *L. plantarum* 12251 were cultured in a liquid medium containing powdered unripe or ripe Pei-Chiao, Formosana, or Tai-Chiao No. 5 cultivar bananas to investigate the effect of banana flour supplementation on bacterial growth. At 24 h, the log counts of bacteria cultured in media supplemented with unripe or ripe Pei-Chiao, Formosana, or Tai-Chiao No. 5 bananas flour were only slightly less than the log count of the control group. However, at 48 h, the log counts of bacteria cultured in media supplemented with banana flour were higher than that in the control group. Statistical analyses showed that supplementation of the culture medium with banana flour did not negatively impact the growth of *L. pentosus* 12250 or *L. plantarum* 12251. In fact, *L. plantarum* 12251 actually benefited from supplementation with ripe banana flour. All six types of banana flour have shown similar effects on the growth of *L. pentosus* 12250. These results underscore the potential of banana flour to positively impact the growth of lactic acid bacterial growth and suggest the value of incorporating the use of banana flour into the diversified banana utilization industry to facilitate achieving the goal of a 'zero-waste' food circular economy.

1. Introduction

The banana (*Musa paradisiaca*) is a tall, annual, herbaceous plant in the genus *Musa* L. of the family Musaceae. Bananas are a widely cultivated crop worldwide, with more than 1,000 varieties produced and consumed, varying in color, flavor and chemical composition (Mostafa, 2021). Global annual banana production yields over 140 million metric tons, second only to wheat, rice, and corn, making bananas the most produced fruit crop worldwide (FAOSTAT, 2022). Bananas are a good source of natural starch and have unique properties and characteristics (Da Mota, 2000). Banana starch has been shown to be unhydrolyzable by α -amylase and glucoamylase, so banana pulp can be used as a substitute for carbohydrates, fats, eggs, or wheat and as a flavor enhancer (Biernacka et al., 2020; Horie et al., 2020; Mostafa, 2021). Unripe bananas are also used as probiotic biomass and are a source of dietary fiber due to their resistant starch content (Garcia-Valle et al., 2019). In addition, bananas are effective in controlling blood glucose, maintaining weight, lowering cholesterol, and preventing colorectal cancer (Da Silva et al., 2014; Fu et al., 2021; Mostafa, 2021). Finally, bananas are rich in natural antioxidants; in addition to their antioxidant potency (Vu et al., 2018; Qamar & Shaikh, 2018), bananas have independent antibacterial properties (Jalani et al., 2014; Jahan et al., 2018; Al-Mqbali & Hossain, 2019; Fahim et al., 2019; Mostafa, 2021).

Taiwan's banana production is approximately 350,000 metric tons annually. Sometimes, there is an oversupply of bananas due to large-scale planting. Bananas are mainly eaten fresh in Taiwan; but are also partially processed and made into banana wine, dried bananas, or crisps. Bananas are also considered to be a commodity that spans primary agriculture, secondary processing industry, and tertiary service industry. Although bananas are an important fruit in Taiwan, value-adding processing and utilization of bananas is of great significance to the industry, as the production capacity of agricultural products may be affected by climate factors, resulting in short-term imbalances between production and

sales. Circular economies and sustainable development goals are issues that have attracted much attention in recent years. The goal of a circular economy is to achieve optimal utilization of natural resources, i.e., to prevent and reduce waste, consistent with the Zero Hunger theme of the 17 Sustainable Development Goals (SDGs) adopted by the United Nations. In the context of the food industry, a 'zero-waste' food circular economy aims to minimize food waste at all stages of production and consumption, from farm to table, and to ensure that all parts of the food, including by-products and surplus, are utilized effectively and sustainably. Promoting a circular economy in the production system fosters the achievement of the SDG. Adding new economic value to raw materials, residual materials, and waste, or recycling high-value materials can increase their economic value, reduce their environmental harm and stress, and ensure sustainable development of natural resources.

Banana varieties available for consumption in Taiwan can be categorized into Cavendish (AAA) varieties and *Musa* section varieties. Cavendish bananas are commercially cultivated and are represented chiefly by the Pei-Chiao variety, which has been modified to include the Formosana, Tai-Chiao No. 5, and other AAA genome group varieties common in Taiwan (Ministry of Agriculture (Taiwan), 2024). A recent analysis of the starch content and resistant starch (RS) content in unripe and ripe Taiwanese bananas of the Pei-Chiao, Formosana, and Tai-Chiao No. 5 varieties by Li et al. (2020) showed that total starch content was 42.05–59.05 g/100 g dry base in unripe bananas and 23.16–30.70 g/100 g dry base in ripe bananas, straight-chain starch content was 27.07–37.17 g/100 g dry base in unripe bananas and 14.46–19.30 g/100 g dry base in ripe bananas, and resistant starch content was 27.07–37.17 g/100 g dry base in unripe bananas and 14.46–19.30 g/100 g dry base in ripe bananas. The highest total starch, straight-chain starch, and resistant starch content was found in Pei-Chiao bananas whether ripe or unripe, followed by Formosana and Tai-Chiao No. 5. Another study showed that banana peel contains high antioxidant capacity, the total phenolic content of Tai-Chiao No. 5 banana peel was approximately 872.7 mg GAE/100 g DM, and its ferrous iron chelating capacity, ferric ion reducing power, and free radical scavenging capacity were all higher in the peel than in the pulp (Chueh et al., 2019).

In recent years, bananas are being considered as a potentially important functional food ingredient because they are rich in resistant starch type 2. The resistant starch is considered a prebiotic that promotes the growth of probiotics. Powdered banana (also named banana flour) has been used as a fermentation substrate for *Lactobacillus acidophilus* and *L. casei* (Massounga Bora et al., 2019). Three banana varieties from Thailand, such as Saba (*Musa acuminata* × *balbisiana* (ABB Group)), Pisang Awak (*Musa sapientum* Linn), and Silver bluggoe (*Musa* (ABB group)) have also been found to be useful as prebiotic food ingredients (Powthong et al., 2020); the green banana pulp of Brazil has shown prebiotic potential against *Lactobacillus acidophilus* LA-5™, *Bifidobacterium* Bb-12™, *Streptococcus thermophilus* and *L. delbrueckii* spp. Bulgaricus without affecting the physicochemical and sensorial properties of yogurts (Costa et al., 2017). However, there is little studies on whether the Pei-Chiao, Formosana, and Tai-Chiao No. 5 banana cultivars can be used as substrates for the growth of lactic acid bacteria. Since *L. plantarum* and *L. pentosus* have been used in the production of fermented products and have been studied for their excellent health benefits, which include improving the gut microbiome, reducing LDL cholesterol, and inhibiting the growth of pathogenic bacteria (Behera et al., 2018; Lee et al., 2019; Ho et al.,

2019; Ma et al., 2022; Yilmaz et al., 2022). In the present study, *L. pentosus* 12250 and *L. plantarum* 12251 were cultured in liquid media containing powdered unripe or ripe Pei-Chiao (PC), Formosana (FS), or Tai-Chiao No. 5 (TC5) banana cultivars to investigate the effect of banana flour supplementation on their growth, and to determine whether banana flour is suitable for culture of lactic acid bacteria.

2. Material and Methods

2.1. Preparation of banana flour

Unripe (banana with full green peel, stage 1 of ripening) and ripe (banana with full yellow, stage 6 of ripening) bananas were obtained from the local market, Pingtung (Taiwan). After cleaning and brushing the bananas, their ends were removed. Whole fruits were cut into slices within thickness of 0.5 cm, and then immediately rinsed in 0.3% (w/v) citric acid solution. A DKN 612 oven procured from Yamoto Company (Tokyo, Japan) and maintained at 50°C was employed to dry the slices for 12 h. Subsequently, the slices were ground into a powder using a laboratory grinder; for the derived powder, filtration was executed using a 50-mesh sieve (270 µm) procured from Retsch (Haan, Germany) to obtain a homogenous mixture, placed into sealed bottles and kept at room temperature until used for further analysis.

2.2. Bacterial strains

L. pentosus 12250, *L. plantarum* 12251 (Bioresource Collection and Research Center, Hsinchu, Taiwan), and *Escherichia coli* strain ATCC 25922 were used for this study. The specific test strains of *Lactobacilli* were selected because they are already established as probiotics and are used in dairy products or they have potential probiotic properties. The *E. coli* strain was chosen to represent the enteric portion of the commensal biota. The *Lactobacillus* cultures were maintained at -80 °C in MRS Broth (BD Difco, USA) containing 15% (w/v) glycerol and *E. coli* cultures were maintained at -80 °C in Tryptic Soy Broth (TSB; BD Difco, USA) containing 15% (w/v) glycerol.

2.3. Growth of *L. pentosus* and *L. plantarum*

According to the method described by Huebner et al. (2007), with certain modifications. The amount of different banana flour added to the MRS broth was extrapolated so that its resistant starch content would be equivalent to 1% glucose. The experimental media (30 ml) was sterilized at 121°C for 15 min before inoculation. Colonies isolated from MRS agar plates were pre-cultured twice in MRS liquid broth at 37°C, first for 24h and then for 18 h, to ensure that all the cells were harvested from the early stationary phase. The assay was performed by adding *L. pentosus* 12250 or *L. plantarum* 12251 to separate flasks containing MRS Broth with 1% (w/v) glucose or different banana flour. In all cases, the initial microbial concentration was approximately at 10² CFU/mL. These media were incubated in micro-aerophilic conditions for 24 h and 48 h. After incubation, samples were enumerated on MRS agar. Each assay was replicated three times.

2.4. Growth of *E. coli*

According to the method described by Huebner et al. (2007), with certain modifications. For the *E. coli* strain, a single colony from Tryptic Soy Agar (TSA; BD Difco, USA) plate was transferred into 10mL TSB and pre-cultured twice to ensure that all the cells were fresh. The quantity of various banana flours incorporated into the M9 Minimal Medium broth (BD Difco, USA) was calculated to ensure their resistant starch content was equivalent to 1% glucose. Before inoculation, the experimental media (30 ml) was sterilized at 121°C for 15 minutes. The assay was performed by adding *E. coli* ATCC 25922. The assay was conducted by introducing *E. coli* ATCC 25922 into the media. The initial microbial concentration was approximately 10^2 CFU/mL in all cases. The media were incubated at 37°C for 0, 24, and 48 hours. After incubation, samples were enumerated on TSA using a plate count procedure. Each assay was performed in triplicate.

2.5. Prebiotic index

The prebiotic index was calculated according to Palframan et al. (2003); it is the ratio of probiotic growth in the prebiotic to probiotic growth in a control carbohydrate (glucose was used as standard in this study). A prebiotic index higher than 1 means that the sample positively affects the probiotic growth. If the prebiotic index is near 1, it indicates a low effectiveness of the evaluated sample. The prebiotic index was calculated according to the following equation.

Prebiotic index = CFU of probiotics in prebiotic/ CFU of probiotics in control

2.6. Statistical analysis

The experimental data were calculated using Microsoft Office Excel 2021 (Microsoft Corporation, Redmond, WA, USA). The results are shown as Mean $\pm$ Standard Deviation. The data were analyzed with analysis of variance using Statistical Analysis System software (SAS Institute, Cary, NC, USA). Duncan's multiple range test was used to establish the multiple comparisons of the mean values; differences between means were considered significant if $p < 0.05$.

3. Results and discussion

In the present study, unripe or ripe Pei-Chiao (PC), Formosana (FS), or Tai-Chiao No. 5 (TC5) banana flour was added to MRS broth to investigate its effect on the growth of *L. pentosus* 12250 and *L. plantarum* 12251. The amount of banana flour added to MRS broth was extrapolated so that its resistant starch content would be equivalent to 1% glucose (the resistant starch content of bananas used in this study was as described in a previous study (Li et al., 2020)). Therefore, MRS broth supplemented with 1% (w/v) glucose served as the control group, and MRS broth supplemented with banana flour from unripe Pei-Chiao (UPC), ripe Pei-Chiao (PRC), unripe Formosana (UFS), ripe Formosana (RFS), unripe Tai-Chiao No. 5 (UTC5), or ripe Tai-Chiao No. 5 (RTC5) were the experimental groups. The results of *L. pentosus* 12250 and *L. plantarum* 12251 growth are presented in Table 1. The prebiotic index obtained with the different banana flour is shown in Table 2.

Table 1

The total viable counts (log cfu/ml) of *L. pentosus* 12250 and *L. plantarum* 12251 in cultural media with different sample

Bacterial strains	Sample	Cultural time	
		24 h	48 h
<i>L. pentosus</i> 12250	Glucose	8.95 ± 0.35 ^a	8.60 ± 0.36 ^a
	RPC	8.71 ± 0.34 ^a	8.75 ± 0.37 ^a
	RFS	8.66 ± 0.28 ^a	8.82 ± 0.33 ^a
	RTC5	8.78 ± 0.25 ^a	8.92 ± 0.21 ^a
	UPC	8.78 ± 0.42 ^a	8.91 ± 0.13 ^a
	UFS	8.64 ± 0.40 ^a	8.98 ± 0.05 ^a
	UTC5	8.72 ± 0.12 ^a	8.89 ± 0.10 ^a
<i>L. plantarum</i> 12251	Glucose	9.06 ± 0.15 ^b	8.66 ± 0.06 ^b
	RPC	9.06 ± 0.07 ^b	8.96 ± 0.03 ^c
	RFS	8.98 ± 0.17 ^{bc}	9.04 ± 0.09 ^c
	RTC5	8.89 ± 0.25 ^{bc}	9.00 ± 0.04 ^c
	UPC	8.91 ± 0.06 ^{bc}	8.96 ± 0.27 ^c
	UFS	8.77 ± 0.04 ^c	8.85 ± 0.22 ^c
	UTC5	8.77 ± 0.10 ^c	8.84 ± 0.14 ^c
1. UPC, unripe Pei Chiao; RPC, ripe Pei Chiao; UF, unripe Formosana; RFS, ripe Formosana; UTC, unripe Tai-Chiao No. 5; RTC, ripe Tai-Chiao No. 5.			
2. Values are Mean ± SD.			
3. Different lowercase letters indicate significant differences between groups (* $p < 0.05$), while same lowercase letters do not show significant differences.			

Table 2
Prebiotic index of different varieties of banana flour

Bacterial strains	Sample	Prebiotic Index	
		24 h	48 h
<i>L. pentosus</i> 12250	RPC	0.973	1.017
	RFS	0.968	1.025
	RTC5	0.981	1.037
	UPC	0.981	1.036
	UFS	0.965	1.044
	UTC5	0.974	1.033
<i>L. plantarum</i> 12251	RPC	1.000	1.034
	RFS	0.991	1.044
	RTC5	0.981	1.039
	UPC	0.983	1.034
	UFS	0.968	1.021
	UTC5	0.968	1.020
1. UPC, unripe Pei Chiao; RPC, ripe Pei Chiao; UF, unripe Formosana; RFS, ripe Formosana; UTC, unripe Tai-Chiao No. 5; RTC, ripe Tai-Chiao No. 5.			

The respective log counts of *L. pentosus* 12250 in MRS culture medium supplemented with glucose, banana flour from UPC, UFS, and UTC5 were 8.95, 8.78, 8.64, and 8.72 after 24 h of incubation, and 8.60, 8.91, 8.98, and 8.89 after 48 h of incubation. For MRS culture medium supplemented with glucose, banana flour from RPC, RFS, and RTC5, the respective log counts were 8.95, 8.71, 8.66, and 8.78 after 24 h of incubation and 8.60, 8.75, 8.82, and 8.92 after 48 h of incubation. When comparing *L. pentosus* 12250 cultured for 24 h with glucose or ripe banana flour as the substrate, although the bacterial log counts in the medium supplemented with banana flour were slightly lower than those of the control group, the difference was only 0.17–0.31 log CFU/mL. However, at 48 h, the log counts of the bacteria cultured in the media supplemented with either unripe or ripe banana flours were higher than those of the control group.

The respective log counts of *L. plantarum* 12251 in MRS culture medium supplemented with glucose, banana flour from RPC, RFS, RTC5, UPC, UFS, and UTC5 after 24 h of incubation were 9.06, 9.06, 8.98, 8.89, 8.91, 8.77, and 8.77, and the log counts for media supplemented with glucose and the corresponding banana flour were 8.66, 8.96, 9.04, 9.00, 8.96, 8.85, and 8.84 after 48 h of incubation. Although UFS or UTC5 was added to the MRS medium, the log counts of *L. plantarum* 12251 after 24 h of incubation were lower than that of other groups. Results showed that supplementation of the culture

medium with banana flour as the culture substrate did not negatively impact the growth of *L. plantarum* 12251, and that supplementation with ripe banana flour exerted a beneficial effect. With respect to supplementation with ripe banana flour, the log counts were no significant difference at 24 h of incubation, but at 48 h, the counts of *L. plantarum* 12251 in all these groups had significant higher log counts than glucose (control group). This demonstrates that supplementation with banana flour can also provide nutrients continuously to support the growth of target specie.

A substrate with high prebiotic activity supports good growth of the probiotic bacteria, and should have cell densities comparable to that when grown on glucose (Huebner et al., 2007). All prebiotic indexes obtained in this study showed a beneficial effect of the tested banana flours. The results in this study showed that glucose supplementation can provide these two *Lactobacillus* strains with a rapid source of energy for reproduction within the first 24 h of incubation, but supplementation with banana flour provides continued nutrients up to 48 h, thereby maintaining a steady rate of bacterial count in these *Lactobacillus* strains.

The growth results for *E. coli* ATCC 25922 are presented in Table 3. Since banana flour is rich in nutrients, adding RFS, RTC5, UPC, UFS, and UTC5 banana flour to the culture medium, *E. coli* ATCC 25922 could still grow. It is worth noting that *E. coli* ATCC 25922 could not grow when RPC was added to the medium. The underlying mechanisms for this inhibition warrant further investigation.

Table 3

The total viable counts (log cfu/ml) of *E. coli* ATCC25922 in cultural media with different sample

Bacterial strains	Sample	Score	
		24 h	48 h
<i>E.coli</i> 25922	Glucose	8.73 ± 0.26 ^a	8.74 ± 0.30 ^a
	RPC	< 1 ^b	< 1 ^b
	RFS	6.19 ± 0.16 ^c	8.87 ± 0.25 ^a
	RTC5	9.34 ± 0.33 ^d	9.56 ± 0.13 ^c
	UPC	9.29 ± 0.11 ^d	9.39 ± 0.30 ^c
	UFS	9.33 ± 0.22 ^d	9.50 ± 0.04 ^c
	UTC5	9.39 ± 0.04 ^d	9.69 ± 0.01 ^c
1. UPC, unripe Pei Chiao; RPC, ripe Pei Chiao; UF, unripe Formosana; RFS, ripe Formosana; UTC, unripe Tai-Chiao No. 5; RTC, ripe Tai-Chiao No. 5.			
2. Values are Mean ± SD.			

The banana flours used in the present study can serve as culture substrates for *L. pentosus* 12250 and *L. plantarum* 12251. The growth of these experimental strains was not affected by the type of banana flour (i.e., ripe or unripe Pei-Chiao, Formosana, or Tai-Chiao No. 5). The study demonstrates that the three varieties of unripe or ripe banana flour can serve as a good substrate for culturing *L. pentosus* and *L. plantarum*.

4. Conclusion

The utilization of banana flour as a cultivation substrate represents a viable and effective strategy for the growth of lactic acid bacteria. The results demonstrate that banana flour serves as an alternative nutrient source, particularly advantageous during agricultural surpluses, thereby promoting circular bio-economies and contributing to zero-waste sustainability goals. Furthermore, substituting conventional synthetic media—such as MRS medium—with banana flour-based substrates provides a strategic pathway for carbon footprint reduction and the advancement of green biotechnology. Future investigations into diverse banana cultivars are warranted to elucidate their potential in modulating specific metabolic pathways within the gut microbiota, facilitating the development of targeted precision prebiotics.

Declarations

CRedit author Contribution.

Shu Chen Hsu: Writing-original draft preparation, methodology, conceptualization, data analysis, writing and editing; Jyun-Yu Huang: investigation, formal analysis, data analysis; Jhe-Jia Cao: investigation, data curation. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The manuscript is original, and it strictly follows all ethical procedures. No part of it is under consideration for publication in another journal. No AI tools were used to analyze and draw insights from data of the research process. The authors declare that there is no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics statement

Not applicable.

Publication statement

Not applicable.

Acknowledgements

The authors would like to acknowledge Chang Jung Christian University for providing the research facility.

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