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*Original Research Article*

# Investigation of the Prebiotic Properties of the Yam Bean (*Pachyrhizus erosus* (L.) Urban) Tuber Extract

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ABSTRACT

Yam bean tubers have been found to possess various kinds of fructans including inulin and fructooligosaccharide, which are well-known prebiotics. However, the prebiotic effects of yam bean tuber have not been established. This study was aimed to investigate the prebiotic properties of the yam bean tuber extract. The sample was prepared by extracting the edible tubers of yam bean with 85% ethanol. The prebiotic effects of the yam bean tuber extract (1, 2, 3%) were evaluated by monitoring bacterial growth and acid production of the probiotics, *Lactobacillus plantarum* and *Lactobacillus acidophilus*. The results showed that yam bean tuber extract produced significant concentration-dependent increases in the growth and acid-producing activity of *L. plantarum* ($p$<0.05). The specific growth rate and generation time of *L. plantarum* was significantly modified by the yam bean tuber extract at the highest concentration tested (3%) ($p$<0.05). The extract also caused a significant pH decrease in the *L. acidophilus* incubation media ($p$<0.05). However, the growth kinetics, specific growth rate and generation time of *L. acidophilus* did not change significantly. The extract did not substantially influence the growth kinetics, specific growth rate, and generation time of *Escherichia coli*, the pathogenic bacteria. The effects of the yam bean tuber extract on the growth and acid production of bacteria were similar to those of the commercial inulin at the same concentrations tested. These results indicate the prebiotic properties of the yam bean tuber extract in certain probiotics, specifically *L. plantarum*.

***Keyword*:** Yam bean, *Lactobacillus plantarum*, *Pachyrhizus erosus*, Prebiotics, Probiotics.

## Introduction

Mexican yam bean or Jicama (*Pachyrhizus erosus* (L.) Urban), Fabaceae family is widely cultivated in several tropical countries in Central America, Western Africa and Southeast Asia. Its edible tuberous root is generally consumed as fresh fruit in Thailand especially in the Northeast of the country. The plant of yam bean can be cultivated simply even in the relative drought area with a growth cycle of approximately 6-8 months.[1] It was reported that the tuber of yam bean contained a high level of water with substantial amounts of various types of carbohydrate, including starch, sucrose and reducing sugar.[2] Additionally, the amount of crude fiber in yam bean tuber was considerably higher than those in potato and sweet potato for approximately 14 and 4 times, respectively.[2] Due to its nutritive value, easy-cultivable and low-priced characteristics, the yam bean tuber provides an alternative source of food to combat malnutrition in developing countries.[3] In addition to its nutritional benefits, the tuberous root of the yam bean was shown to possess inulin, a polysaccharide of fructose polymer linked by $\beta$ (2→1) linkage with a glucose residue at its end. It was demonstrated that the yields of inulin derived from the yam bean tubers varied regarding the preparation methods with the inulin yields of 11.97%–12.15%, 11.21%–11.38% and 9.9% obtained from the ultrasonic-assisted extraction (UAE), the microwave-assisted extraction (MAE) and the conventional hot water extraction methods, respectively.[4]

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Inulin is one of the well-known prebiotics, which defines as "selectively fermented ingredients that result in specific changes in the composition and/or activity of gastrointestinal microbiota, thus conferring benefits upon host health".[5] The health benefits of prebiotics have been documented cumulatively including improvement of gastrointestinal function, increase in mineral absorption, modulation of energy metabolism and satiety as well as enhancement of host defense mechanism and modulation of the immune system.[6] Inulin is indigestible by the human digestive enzyme but can be fermented by probiotic bacteria residing in the gastrointestinal tract, especially *Bifidobacterium* and *Lactobacillus* species. The bacterial metabolites derived from prebiotic fermentation, principally short-chain fatty acids i.e. acetate, propionate and butyrate, have been shown to produce both local and systemic benefits to the host, such as antimicrobial, antioxidant and immunomodulating activities.[7] According to its virtues in both nutritional and functional aspects, yam bean tubers serve as a potential nutraceutical, which can be feasibly available for all, particularly underprivileged people in developing countries.[8] This study was aimed to investigate the prebiotic properties of the yam bean tuber extract. The prebiotic effect of the yam bean tuber extract, indicated by bacterial growth and acid production of two species of probiotics, *Lactobacillus plantarum* and *L. acidophilus*, was examined in this study.

## Materials and Methods

*Preparation of the yam bean tuber extract*

The tubers of yam bean (*Pachyrhizus erosus* (L.) Urban) were obtained from Borabue district, Maha Sarakham, Thailand, in January 2019. Plant materials were authenticated by Asst. Prof. Wanida Caichompoo, a botanist, and the specimens were archived at the Faculty of Pharmacy, Mahasarakham University, with voucher numbers of MSU.PH-LEG-P01. The tubers were cleaned thoroughly with tap water and cut into small pieces of 2×2 cm using a knife. The

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samples were freeze-dried in the freeze dryer (-103 to -80$^{o}$C) for 24 h and subsequently extracted with 85% ethanol. The conditions were a solvent of 14L per 140 g dried yam bean. After the incubation at 50°C for 1 h, the extract solution was collected by centrifugation at 4,000 × g at 4°C for 15 minutes. The solvent was removed by rotary evaporation and subsequently by freeze dryer (-103 to -80$^{o}$C) for 24 h.[9] The % yield of the yam bean tuber extract obtained was 26.56% (by dry weight). The obtained sample was kept at -20$^{o}$C until use.

*Carbohydrate content assay*

The total carbohydrate content of the yam bean tuber extract (0.5 mg/mL) was determined using the phenol-sulfuric acid method.[10] Briefly, 100 µL of the yam bean tuber extract or sucrose (standard), 100 µL of deionized water and 200 µL of 6.5% phenol were added. Subsequently, 1,000 µL of 85% concentrated sulfuric acid ($H_2SO_4$) was included and mixed completely. The plate was kept at room temperature (25$^{o}$C) for 30 min and the absorbance was measured at the wavelength of 490 nm by using an absorbance microplate reader (BMG LABTECH, Germany). The total carbohydrate content was calculated from the sucrose standard curve.

The dinitrosalicylic acid colorimetric method was conducted to determine the amount of reducing sugar in the yam bean tuber extract. The reducing sugar assay was performed according to the method of Ali *et al*. (2006) with slight modification.[11] 200 µL of the yam bean tuber extract (0.5 mg/mL) or glucose (standard) was mixed with 100 µL of 3,5-dinitrosalicylic acid (DNS) color reagent (96 mM DNS and 5.31 M sodium potassium tartrate in 2 M NaOH) and then boiled at 85°C for 15 min. The absorbance of the mixture was measured at the wavelength of 540 nm by using an absorbance microplate reader. The concentration of reducing sugar was calculated from the glucose standard curve. The percent of reducing sugar in the total carbohydrate content of the extract was calculated. The percent of non-reducing sugar content in the total carbohydrate content was subsequently calculated by 100 - % of reducing sugar content. The experiments were performed in triplicate.

*Effects of the yam bean tuber extract on growth and acid production of Lactobacillus plantarum, L. acidophilus and Escherichia coli*

Two strains of probiotics, *L. plantarum* (TISTR1465) and *L. acidophilus* (TISTR2365) were used to investigate the prebiotic effects of the yam bean tuber extract in this study. The probiotic strains were obtained from the Thailand Institute of Scientific and Technological Research Institute culture collection, Ministry of Science, and Technology, Thailand. *Escherichia coli* O157:H7 (DMST12743) was obtained from the Department of Medical Sciences Thailand (DMST), Ministry of Public Health, Thailand. The *Lactobacillus* strains were cultivated anaerobically at 37°C in de man Rogosa Sharpe (MRS) broth as described in Li *et al.*[12] *E. coli* was cultivated aerobically at 37°C in tryptic soy broth. The bacteria were cultured to reach their stationary phase after which they were collected by centrifugation at 2,500 × *g* at 4°C for 15 minutes and washed twice with phosphate-buffered saline (PBS). The density of the organism suspensions was adjusted to equal that of the 0.5 McFarland standard by adding basal media (the same as MRS media but without glucose). The yam bean tuber extract (1, 2, and 3% w/v), the commercial inulin (1, 2, and 3% w/v), and glucose (2% w/v) in basal media were prepared in 250-mL bottles. The amount of 1.5 g, 3.0 g, or 4.5 g of sample was added to 150-mL basal media to make 1, 2, or 3% w/v sample preparation. The bacteria suspensions (1% v/v) were subsequently added to these sample preparations. Before incubation, the samples were aliquoted to 15-mL test tubes for ease of testing at each time point. Glucose (2% w/v) was served as the positive control, and the MRS broth formula without carbohydrate source (glucose-free media or basal media) was used as the negative control. The *Lactobacillus* strains were incubated anaerobically at 37°C. *E. coli* was incubated aerobically at 37°C. The growth of bacteria was monitored at 0, 2, 4, 6, 8, 10, 12, 22, and 24 h for *L. plantarum* and *E. coli*. For *L. acidophilus*, the growth was monitored at 0, 12, 24, 28, 32, 36, 48, 56, and 60 h by measuring the incubation media's optical density (OD) at 620 nm. The pH of the culture media was used as an indicator of the acid-producing capability of the bacteria. The pH was determined by using pH meter (Mettler-Toledo).

Specific growth rate (µ) was calculated for each bacterial strain during its exponential growth phase by the equation: $\mu = (\ln X - \ln X_0) / (t - t_0)$, where X and $X_0$ were absorbance measured at time t and $t_0$, respectively. The generation time (*tg*) was calculated for each culture from the corresponding value of µ by the equation: $tg = \ln 2/\mu$ .[13]

*Statistical analysis*

The data were expressed as mean ± SD. The statistical analysis was performed by using a one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. The difference between data is considered statistically significant when a *p*-value is less than 0.05.

## Results and Discussion

*Carbohydrate content assay*

The carbohydrate content in the yam bean tuber extract is shown in Table 1. The yam bean tuber extract contained significantly high amount of carbohydrate at 85.88 ± 2.70% of the extract. The main proportion of carbohydrate in the extract was found as reducing sugar (79.24 ± 2.35%), whereas the percentage of non-reducing sugar was 20.76 ±2.35 %. The amounts of reducing sugar and non-reducing sugar in the yam bean tuber extract were 68.05 ± 2.02% and 5.64 ± 2.02%, respectively. Inulin and its fructooligosaccharides derivatives, which are well-known prebiotics, can be classified as non-reducing sugars. It can be roughly estimated that the inulin content in the yam bean tuber extract was at approximately 5%. This was relatively lower than those reported earlier by Sarkar *et al*. (2021) in which the percent (%) yield of inulin obtained from the yam bean tubers by using the conventional hot water extraction was at about 10%.[4] However, various factors may influence the inulin content in the yam bean tubers including plant variety, planting condition, harvest time, nutritional status as well as the extraction methods. The percent (%) yield of inulin obtained from the tubers of yam bean is relatively lower than those achieved from tuberous roots of Jerusalem artichoke (*Helianthus tuberosus* L.) or chicory (*Cynara scolymnus* L.) reported earlier.[14–16] Nonetheless, it provides an alternative source of inulin especially in the drought area where prolonged shortages in the water supply.

*Effects of the yam bean tuber extract on growth and acid production of L. plantarum and L. acidophilus*

The yam bean tuber extract produced concentration-dependent changes in the growth and/or acid production of the probiotics tested. The growth and acid-producing activity of *L. plantarum* were significantly enhanced when the yam bean tuber extract (1, 2 and 3%) were included in the bacterial culture ($p < 0.05$) (Figure 1a and 1b). The growth-promoting effect of the extract (2 and 3%) was detected as early as 12 h. The pH declines of the incubation media, which indicates acid production of *L. plantarum* was also observed early at 10 h incubation. The incubation media pHs in the presence of the yam bean tuber extract at every concentration tested were also significantly lower than those of the negative control at the incubation times of 10, 12, 22 and 24 h ($p<0.05$) (Figure 1b). The changes of pH in the incubation media were consistent with the *L. plantarum* growth as indicated by the optical density absorbance. The specific growth rate and generation time of *L. plantarum* were substantially increased and decreased, respectively with the yam bean tuber extract (3%) at 48-h incubation (Table 2). These results thus indicate the prebiotic effect of the yam bean tuber extract on *L. plantarum.*

The growth of *L. acidophilus* was increased in the presence of the yam bean tuber extract at every concentration tested (1, 2, 3%) (Figure 2a). However, its growth stimulating effect did not reach a statistical significance when compared with the negative control. The yam bean tuber extract at every concentration tested significantly decreased the media pH of *L. acidophilus* at the incubation periods of 60 h ($p < 0.05$) (Figure 2b). The yam bean tuber extract at the concentration of 3% produced an increase in specific growth rate (µ) and a decrease in generation time (tg) of *L. acidophilus* at 48-h incubation (Table 2).

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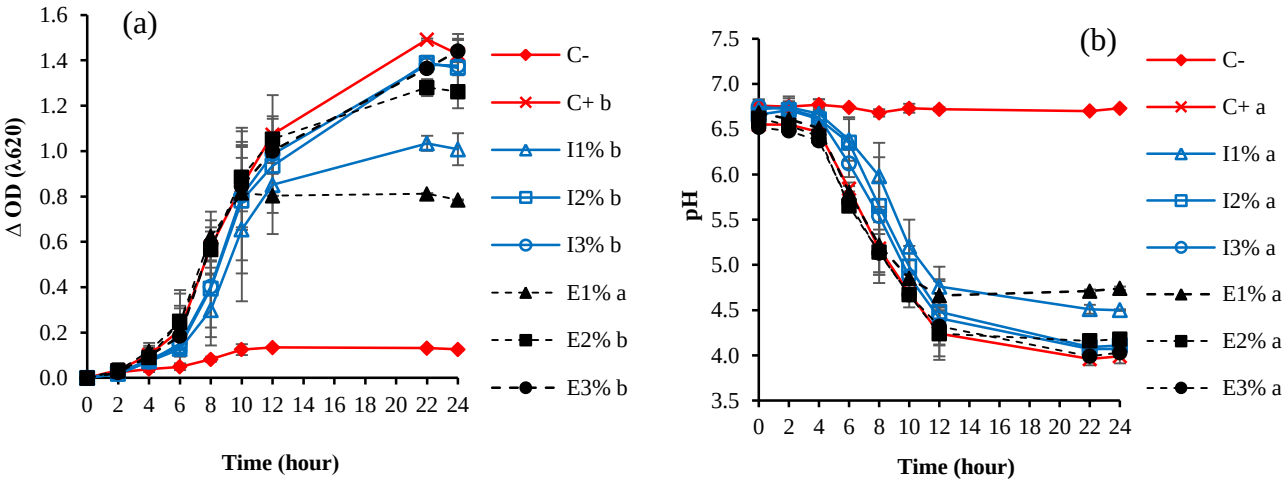
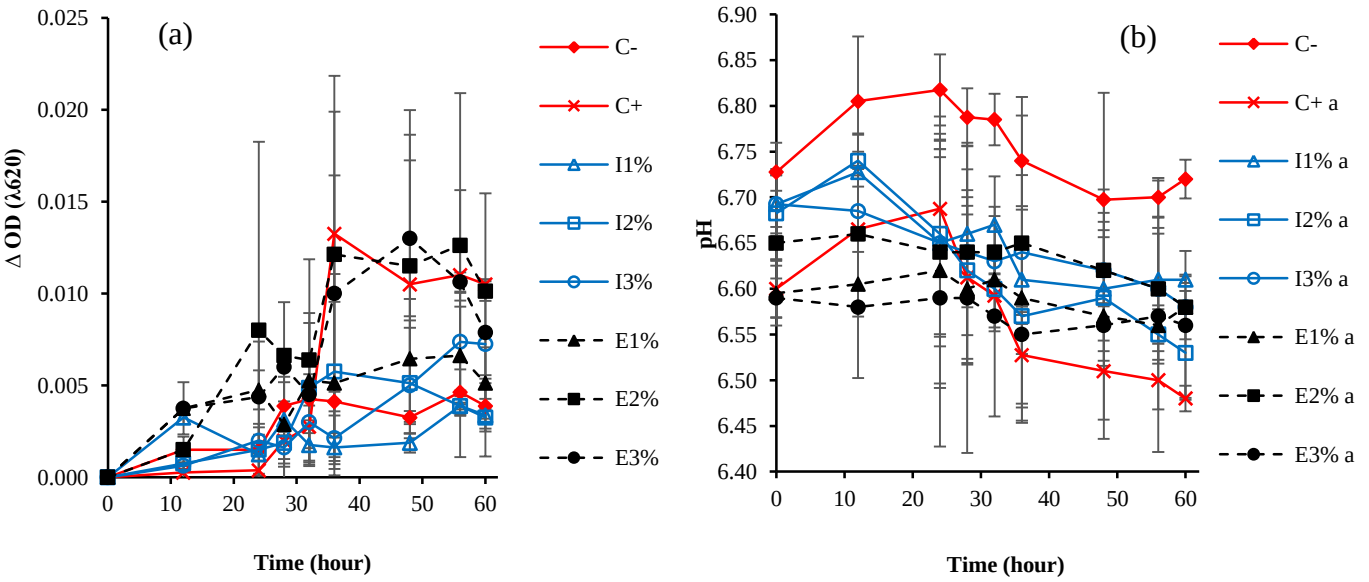



[a] $p < 0.05$ when compared with negative control (MRS without glucose) at t = 22 and 24
b $p < 0.05$ when compared with negative control (MRS without glucose) at t = 12, 22 and 24

[a] $p < 0.05$ when compared with negative control (MRS without glucose) at t = 10, 12, 22 and 24

**Figure 1:** Growth kinetics of *L. plantarum* **(a)** and pH changes in basal media **(b)** supplemented with the yam bean tuber extract (E), inulin (I), positive control (glucose; C+) and negative control (C-) (n = 3).




[a] $p < 0.05$ when compared with negative control (MRS without glucose) at t = 60

**Figure 2:** Growth kinetics of *L. acidophilus* (a) and pH changes in basal media (b) supplemented with the yam bean tuber extract (E), inulin (I), positive control (glucose; C+) and negative control (C-) (n = 3).

**Table 1:** Carbohydrate content assay of the yam bean tuber extract (0.5 mg/mL)

| | **Total carbohydrate (mean ± SD)** | **Reducing sugar (mean ± SD)** | **Non-reducing sugar (mean ± SD)** |
|---|---|---|---|
| Amount in the extract (mg/mL) | 0.43 ± 0.01 (n = 3) | 0.34 ± 0.01 (n = 3) | 0.03 ± 0.01 (n = 3) |
| Percentage in the extract (%) | 85.88 ± 2.70 (n = 4) | 68.05 ± 2.02 (n = 3) | 5.64 ± 2.02 (n = 3) |
| Percentage in total carbohydrate (%) | - | 79.24 ± 2.35 (n = 3) | 20.76 ± 2.35 (n = 3) |

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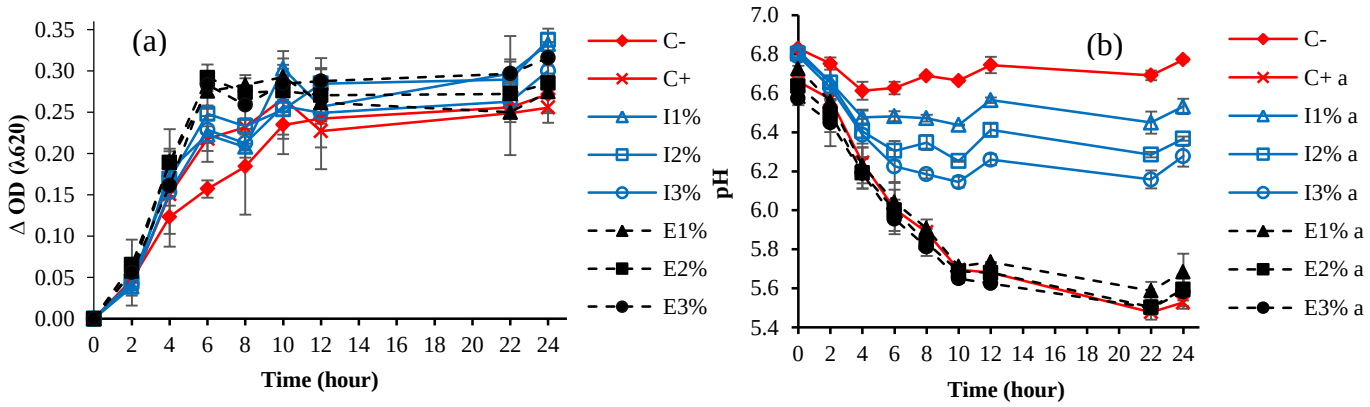
However, these were not statistically different from the negative control. The growth and acid-producing activity of *L. acidophilus* in the presence of the yam bean tuber extract were similar to those of inulin at the same concentration tested. The specific growth rate and generation time of *L. plantarum* It has been reported that different species of lactobacilli selectively utilized different sources of carbohydrate.[17] The divergence in energy source selectivity depends on the capability of bacterial enzyme required for carbohydrate digestion, such as extracellular glycosidases, and transport systems in each probiotic species.[7] Additionally, the bacteria must be able to metabolise the available carbohydrate and also tolerate the changes of surrounding conditions, especially pH, which occur during substrate metabolism. From our results, *L. plantarum* was likely to use both the yam bean tuber extract and commercial inulin as energy sources more efficiently than *L. acidophilus*. The prebiotic activity of the yam bean tuber extract on other lactobacilli species or other probiotics such as bifidobacteria has not been clearly investigated. Further experiments regarding the difference in probiotic sensitivity toward the yam bean tuber extract should be performed.

*Effects of the yam bean tuber extract on growth and acid production of E. coli*

The yam bean tuber extract (3%) did not significantly increase the growth of *E. coli* at 24-h incubation. This was similar to those of inulin and glucose (Figure 3a). However, the media pH was significantly decreased in the presence of the yam bean tuber extract (1, 2, 3%) at the incubation times of 8 h onward when compared to those of the negative control (Figure 3b). Although, the yam bean tuber extract decreased the *E. coli* media pH significantly, the growth kinetics, specific growth rate and generation time of *E. coli* were not changed significantly in the presence of the yam bean tuber extract (Table 2). These effects were similar to those of the commercial inulin. Thus, the yam bean tuber extract can be potentially considered as "prebiotics" since it selectively stimulated the growth of the probiotics, *L. plantaum*, which is known to provide benefits upon host health, without causing any substantial growth-stimulating action on the growth of pathogenic bacteria, *E. coli*. However, various types of microorganisms reside simultaneously in the human gut as a microbial complex called the human gut microbiota. Bacteria in gut microbiota can compete for nutrients. However, the metabolites derived from one strain's metabolism can also be used as essential nutrients for other strains of microbes, which is known as the cross-feeding process.[18] Therefore, *in vitro* co-culture assays as well as *in vivo* experiments are necessary to confirm this suggestion.

Our results suggested that the yam bean tuber extract possessed a relatively low amount of inulin. However, both the yam bean tuber extract and inulin at the same concentration of 3% produced comparable actions on the growth and acid production of the probiotics tested in this study. According to the carbohydrate content assay, the yam bean tuber extract at the concentration of 3% provided approximately only 0.15% of inulin. It has been demonstrated that the degree of polymerization of fructans is essentially linked with the utilization of fructans by probiotics.[19] A degree of polymerization of inulin can be varied broadly in the range of 2–60.[20] It was reported that inulin with a low degree of polymerization has the higher prebiotic activities.[12] Since the yam bean tuber extract and the commercial inulin had similar effects on the growth and acid-producing activities of the probiotics tested, the degree of polymerization of fructans in the yam bean tuber extract is not likely to be dissimilar to those of the commercial inulin. Nonetheless, further experiments are required to investigate the degree of polymerization of fructans in the yam bean tuber extract. Additionally, the yam bean tuber extract may potentially contain other types of prebiotics apart from inulin-type fructans.




[a] $p < 0.05$ when compared with negative control (MRS without glucose) at t = 8, 10, 12, 22, 24

**Figure 3** Growth kinetics of *E. coli* (a) and pH changes in basal media (b) supplemented with the yam bean tuber extract (E), inulin (I), positive control (glucose; C+) and negative control (C-) (n = 3).

**Table 2:** Specific growth rate (μ) and generation time (*tg*) of *L. plantarum, L. acidophilus and E. coli* during its exponential growth phase (mean ± SD, n = 3)

| Treatment | *L. plantarum* (t = 24 hr) | | *L. acidophilus* (t = 48 hr) | | *E. coli* (t = 10 hr) | |
|---|---|---|---|---|---|---|
| | μ | *tg* | μ | *tg* | μ | *tg* |
| MRS without glucose (negative control) | 0.0073±0.0000[a] | 94.6398±1.2440[a] | 0.0027±0.0010 | 300.9327±158.2583 | 0.2092±0.0350 | 3.3774±0.5148 |
| MRS with glucose (positive control) | 0.0478±0.0016[b] | 14.5286±0.4911[b] | 0.0063±0.0049 | 88.4164±29.1042 | 0.1929±0.0156 | 3.6117±0.2952 |
| 3% yam bean tuber extract | 0.0465±0.0008[c] | 14.9094±0.2454[c] | 0.0056±0.0023 | 139.3808±53.2680 | 0.1888±0.0092 | 3.6781±0.1719 |
| 3% inulin | 0.0470±0.0017[d] | 14.7538±0.5225[d] | 0.0056±0.0026 | 146.1050±70.3188 | 0.2188±0.0111 | 3.3142±0.3356 |

[a-d] Different letter indicates statistical significance ($p < 0.05$)

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## Conclusion

The results from this study indicated that the yam bean tuber extract could enhance the growth of *L. plantarum*, one of the two probiotics tested, and did not significantly change the growth of pathogenic *E. coli* O157:H7. The tuberous roots of yam bean thus show some prebiotic potentials and can serve as an alternative source of functional foods in some geographic areas where other crop productions are limited due to water supply shortage.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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