Investigating the effect of sugarcane extract and lotus root extract on the growth of *Lactobacillus* spp.

Group 1-15

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Abstract

The beneficial properties of *Lactobacillus* spp. have been widely researched all over the world. It is also a known fact that prebiotics like xylo-oligosaccharide (XOS), galacto-oligosaccharide (GOS) and fructo-oligosaccharide (FOS) are able to effectively proliferate the growth, and hence further boost the beneficial properties of probiotics. This project recognised the issue with having low amounts of food waste being recycled and hence causing high amounts of discarded food. Thus, this project aims to mitigate this issue by recycling and harvesting the prebiotic contents present in sugarcane bagasse and lotus roots. Sugarcane bagasse is an abundant agricultural waste and has high XOS content. Lotus root is a very widely produced plant in both Southeast Asia and Australia and has high polyphenolic content, which also serves as prebiotics. The prebiotic extracts were prepared from powdered sugarcane bagasse and lotus roots separately. These extracts were mixed with *Lactobacillus gasseri, Lactobacillus plantarum* and *Lactobacillus rhamnosus* in different set-ups and grown in MRS agar. All three *Lactobacillus* spp. had higher mean absorbance and also mean colony counts in the experimental set-ups than in the control set-ups where no extracts were added. This indicates that the addition of sugarcane bagasse and lotus root extracts were able to effectively proliferate the growth of *L. gasseri, L. plantarum* and *L. rhamnosus.* Hence, they are viable prebiotics.

Introduction

According to Singapore's National Environment Agency's website, in 2019, out of the 744000 tons of food waste generated, only 136000 tons gets recycled, which is a mere 18%. Most of the food thrown away still contains beneficial contents, but these beneficial contents usually become wasted. Therefore, recycling sugarcane bagasse and lotus roots, and making use of the high prebiotic contents available in them to proliferate the growth of *Lactobacillus*

spp. is a feasible solution for the waste problem.

Lactobacillus is a genus of bacteria that is defined as Gram-positive, facultative anaerobic bacteria that mainly converts sugars into lactic acid (Makarova et al., 2006). La Fata, Weber and Mohajeri (2017) reported that *Lactobacilli* can be used as probiotics to modify the gut microbiota, which is prominent in the treatment of diseases (Azad, Sarker, Li & Yin, 2018). For example, by administering *L. acidophilus* orally in mice with dextran sodium sulfate (DSS)-induced colitis, which is an inflammatory bowel disease, *L. acidophilus* was able to reduce the impact of the DSS-induced colitis by suppressing proinflammatory cytokines such as IL-6, tumor necrosis factor-a, and IL-1b in colon tissues (Park et al., 2018). Hence, it is of interest to find methods to promote the growth of *Lactobacillus*. We decided to use *Lactobacillus gasseri, Lactobacillus plantarum* and *Lactobacillus rhamnosus* species as these *Lactobacillus* species have not been experimented on as much as species like *L. acidophilus.*

Sugarcane bagasse (*Saccharum officinarum)* has high xylan content at 21.46% (Kaur, Uppal & Sharma, 2018). Xylan hydrolysate samples were found to have increased effect on growth of all species of *Lactobacillus* as compared to standard glucose and fructooligosaccharides. (Kaur et al., 2018). Hence, it is of interest to study the effects of sugarcane extract on the growth of *Lactobacillus* spp.

Lotus (*Nelumbo nucifera* Gaertn.) is grown in large scales across Southeast Asia and Australia for commercial purposes (Wang, Yi, Sun, Lamikanra & Min, 2018). Studies show that lotus root contains high levels of polyphenolic compounds and they can be used as prebiotics to enhance the growth of beneficial gut microbes such as *Bifidobacterium* and *Lactobacillus* spp. (Thilakarathna, Langille & Rupasinghe, 2018). Hence, it is of interest to study the effects of lotus root extract on the growth of *Lactobacillus* spp.

By recycling sugarcane bagasse and lotus roots to produce prebiotic extracts, this helps to reduce the amount of food waste generated. Since the production of these prebiotic extracts is inexpensive, food production companies can add these prebiotic extracts into their food at a lower cost. This increases the nutritional benefit for the consumers at a cheap price.

This project gathers more information on how the prebiotic contents present in sugarcane bagasse and lotus roots affect the growth of *L. gasseri, L. plantarum* and *L. rhamnosus* strains, which have not been widely experimented on.

Objective

The objective of this study is to investigate if sugarcane and lotus root extracts are viable prebiotics that can increase the growth of *Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus gasseri*.

Hypotheses

The hypothesis of this study is that sugarcane and lotus root extracts are viable prebiotics that will enhance the growth of *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus gasseri*.

Materials and Methods

a) Materials

Lactobacillus plantarum, Lactobacillus rhamnosus and Lactobacillus gasseri were obtained from ATCC (American Type Culture Collection). Sugarcane bagasse (*Saccharum officinarum*) was obtained from Bukit Timah Market & Food Centre. Lotus root (*Nelumbo nucifera Gaertn.*) was purchased from NTUC FairPrice Co. (Singapore). MRS broth and MRS agar from BD (Becton, Dickinson and Company) were used.

b) Methods

(i) Preparation of Prebiotic Extracts

Sugarcane bagasse and lotus root were washed and cut into small pieces. Then, they were dried in a hot air oven at 70°C for 24h. Dried samples were blended to powder form and added separately to deionised water in an extraction ratio of 1g of powder to 30ml of water. Subsequently, the suspensions were heated at 85°C for 30 min on a hot plate, then centrifuged at 8000 rpm for 10 min. Supernatant was collected and autoclaved at 10 psi for 10 min.

(ii) Growth of pre cultures of *Lactobacillus*

Lactobacillus plantarum ATCC 8014, *Lactobacillus rhamnosus* ATCC 7469 and *Lactobacillus gasseri* ATCC 19992 were inoculated separately into 10ml of MRS broth and grown for 24h at 30°C in a shaking incubator. The absorbance of each culture at 600nm was standardised at 0.8 the following day.

(iii) Testing effects of prebiotic extracts on growth of *Lactobacillus spp.*

20µl of *Lactobacillus* pre culture added to 4.98 ml of MRS broth. 5 replicates of this mixture were prepared for each type of *Lactobacillus* pre culture. These replicates would act as the control set-up. Equal volumes of double strength MRS broth were mixed separately with sugarcane and lotus root extracts. 20µl of *Lactobacillus* culture supernatants were added to 4.98 ml of the mixture. 5 replicates of this mixture were prepared for each type of prebiotic extract and *Lactobacillus* pre culture added. These replicates would act as the experimental set-up. All replicates were placed in a shaking incubator at 30°C for 24h. Serial 10-fold dilution was then carried out with normal saline to appropriate dilution factor. 0.1ml of the diluted culture was spread on MRS agar plates, and they were then incubated at 30°C for 24h. Subsequently, the number of colony forming units on each MRS agar plate was determined.

Results and Discussion

a) *Lactobacillus gasseri*

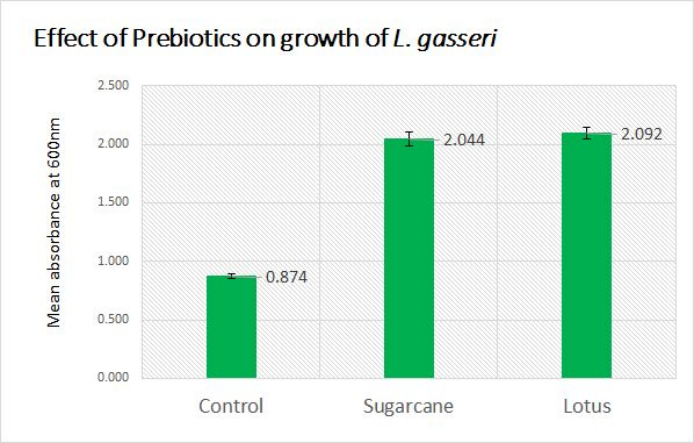

Fig. 1.1: Mean absorbance of *L. gasseri* in the control and experimental set-ups.

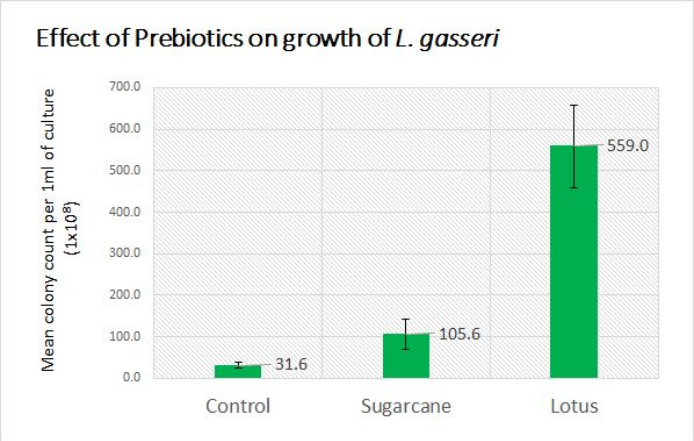

Fig. 1.2: Mean colony count of *L. gasseri* per 1ml of culture in the control and experimental set-ups ($1x10^8$).

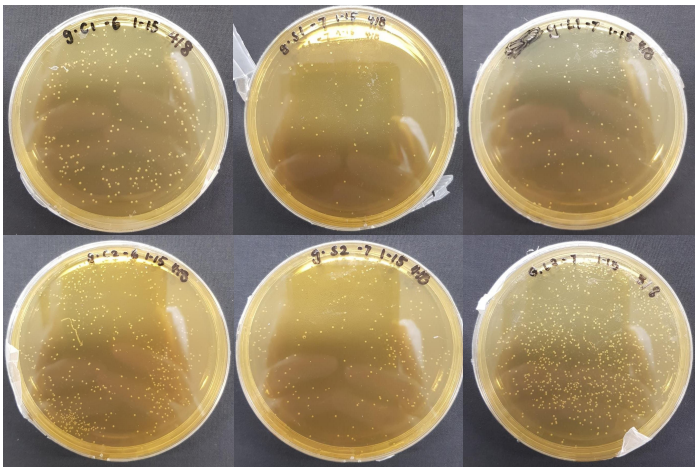

Fig 1.3: *L. gasseri* colony count in Control and Experimental Set-up replicates 1 & 2

From Fig 1.1, the mean absorbance of *L. gasseri* in the control set-ups was 0.874, compared to 2.044 and 2.092 in the sugarcane and lotus roots set-ups, respectively. The results showed that the mean absorbance of *L. plantarum* in the experimental set-ups were higher than in the control set-ups. From Fig 1.2, the mean colony count of *L. gasseri* in the control set-ups was 31.6 x 10^8, compared to 105.6 x 10^8 and 559.0 x 10^8 in the sugarcane and lotus roots set-ups, respectively. From Fig 1.3, as serial dilution for the control set-ups were carried out to a dilution factor of $1x10^{-6}$, compared to $1x10^{-7}$ for the experimental set-ups, visual comparison is inaccurate. However, when the colony counts in the experimental agar plates are multiplied by another 10, the counts would be higher than that in the control set-up. The results thus showed that there was a higher mean colony count of *L. gasseri* in the experimental set-ups compared to the control set-ups.

These results indicated that *L. gasseri* had higher growth rates in the experimental set-ups

compared to the control set-ups.

b) *Lactobacillus plantarum*

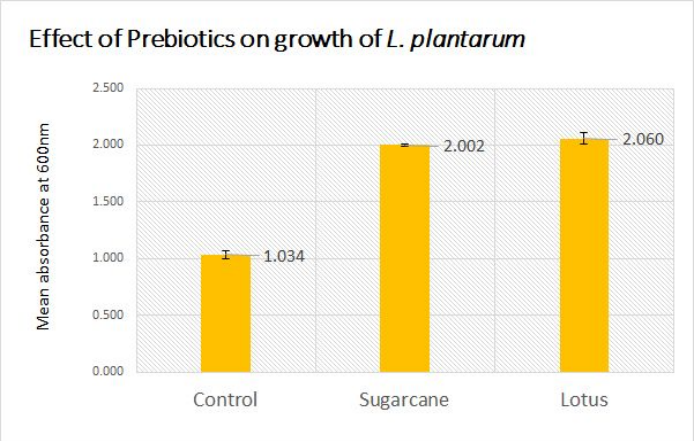

Fig. 2.1: Mean absorbance of *L. plantarum* in the control and experimental set-ups.

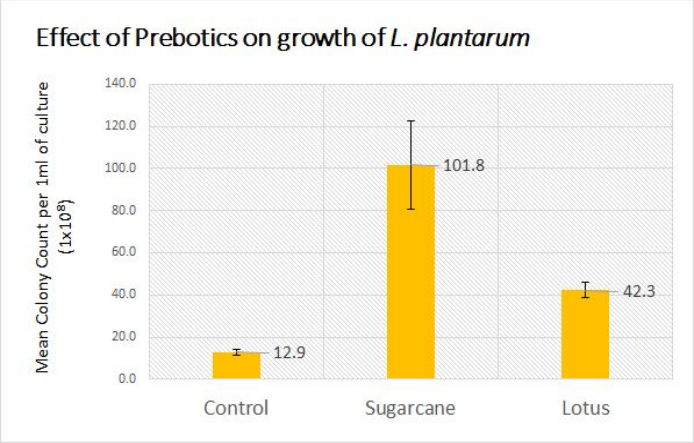

Fig. 2.2: Mean colony count of *L. plantarum* per 1ml of culture in the control and experimental set-ups ($1x10^8$).

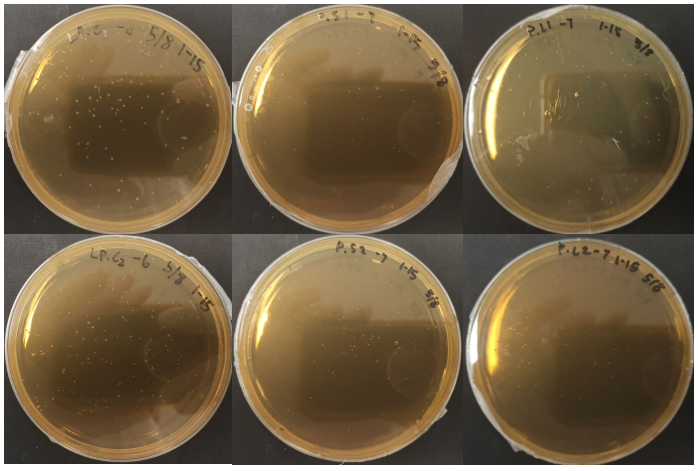

Fig 2.3: *L. plantarum* colony count in Control and Experimental Set-up replicates 1 & 2

From Fig 1.1, the mean absorbance of *L. plantarum* in the control set-ups was 1.034, compared to 2.002 and 2.060 in the sugarcane and lotus roots set-ups, respectively. The results showed that the mean absorbance of *L. plantarum* in the experimental set-ups were

higher than in the control set-ups. From Fig 2.2, the mean colony count of *L. plantarum* in the control set-ups was 12.9 x 10^8. The mean colony count of *L. gasseri* in the sugarcane and lotus root set-ups were 101.8 x 10^8 and 42.3 x 10^8 respectively. From Fig 2.3, similar to Fig 1.3, the colony counts in the experimental set-ups would be much higher than that in the control set-up if multiplied but another 10. The results thus showed that there was a higher mean colony count of *L. plantarum* in the experimental set-ups compared to the control set-ups.

These results indicated that *L. plantarum* had higher growth rates in the experimental set-ups compared to the control set-ups.

c) *Lactobacillus rhamnosus*

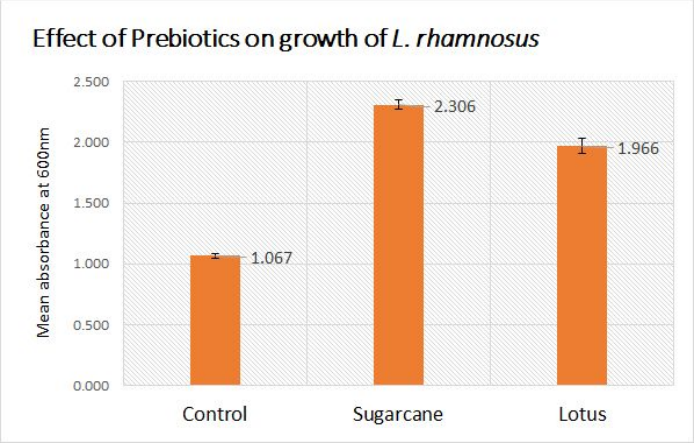

Fig. 3.1: Mean absorbance of *L. rhamnosus* in the control and experimental set-ups.

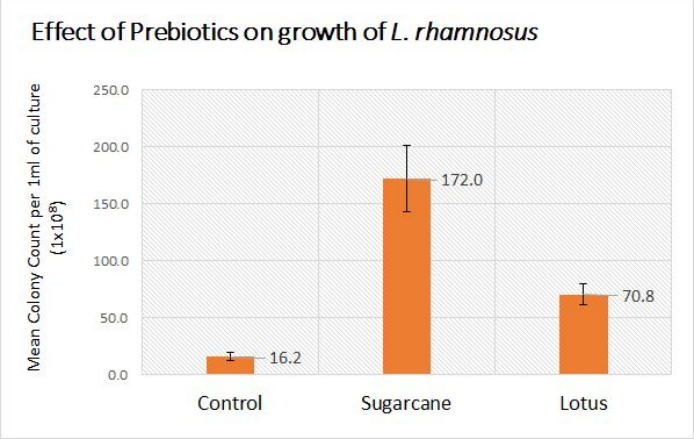

Fig. 3.2: Mean colony count of *L. rhamnosus* per 1ml of culture in the control and experimental set-ups (1x10^8).

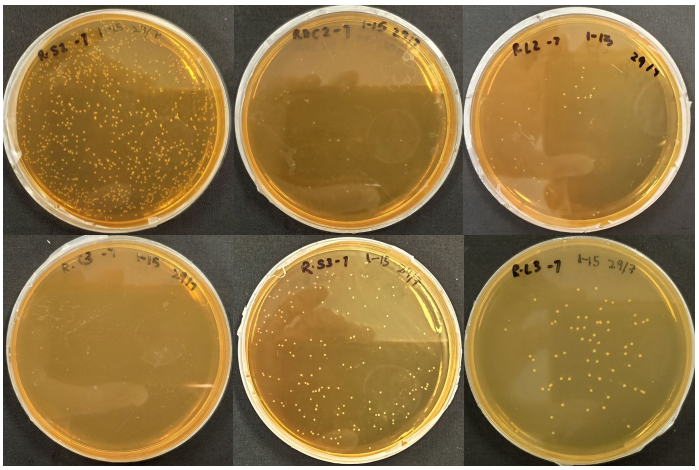

Fig 3.3: *L. rhamnosus* colony count in Control and Experimental Set-up replicates 2 & 3

From Fig 3.2, the mean absorbance of *L. rhamnosus* in the control set-ups was around 1.067, compared to 2.306 and 1.966 in the sugarcane and lotus root set-ups, respectively. The results showed that the mean absorbance of *L. rhamnosus* in the experimental set-ups were higher than in the control set-ups. From Fig 3.2, the mean colony count of *L. rhamnosus* in the control set-ups was 16.2 x 10^8, compared to 172.0 x 10^8 and 70.8 x 10^8 in the sugarcane and lotus root set-ups, respectively. From Fig 3.3, since the dilution factor was constant at 1x10^{-7}, visual comparison is accurate and higher colony counts can be seen in the experimental set-ups than in the control set-ups. The results showed that there was a higher mean colony count of *L. rhamnosus* in the experimental set-ups compared to the control set-ups.

These results indicated that *L. rhamnosus* had higher growth rates in the experimental set-ups compared to the control set-ups.

d) Overall data

Table 1: Mean Absorbance of each *Lactobacillus* spp. in different set-ups

Bacteria	Set-up	Mean Absorbance
L. gasseri	Control	0.874
	Sugarcane extract	2.044

Table 2: Mean Colony Count of each *Lactobacillus* spp. in each set-up

Bacteria	Set-up	Mean Colony Count (x10^8)
L. gasseri	Control	0.874
	Sugarcane extract	2.044

	Lotus root extract	2.092		Lotus root extract	2.092
L. plantarum	Control	1.034	*L. plantarum*	Control	1.034
	Sugarcane extract	2.002		Sugarcane extract	2.002
	Lotus root extract	2.060		Lotus root extract	2.060
L rhamnosus	Control	2.037	*L rhamnosus*	Control	2.037
	Sugarcane extract	2.306		Sugarcane extract	2.306
	Lotus root extract	1.966		Lotus root extract	1.966

Comparing Tables 1 and 2, a trend can be observed. For all *Lactobacillus* species tested, there was higher absorbance as well as higher colony counts in the sugarcane and lotus root set-ups compared to the control set-ups. This signifies that there were higher growth rates of the *Lactobacillus* spp. when sugarcane and lotus root extracts were added, respectively. This also meant that the addition of sugarcane and lotus root extracts respectively proliferated the growth of all 3 *Lactobacillus* spp.

e) Data analysis

The Mann-Whitney U test was carried out to determine if there were significant differences in the mean absorbance, and also mean colony count between the experimental and control set-ups.

Table 3: P-values of each *Lactobacillus* spp. for Absorbance data and Colony Count data

Bacteria	**Set-up**	**P-value (Absorbance)**	**P-value (Colony Count)**
L. gasseri	Sugarcane vs Control	0.022	0.016
	Lotus Root vs Control	0.037	0.025
L. plantarum	Sugarcane vs Control	0.012	0.012
	Lotus Root vs Control	0.012	0.036
L. rhamnosus	Sugarcane vs Control	0.012	0.012

	Lotus Root vs Control	0.012	0.012

As shown in Table 3, all p-values of all 3 *Lactobacillus* spp. for absorbance and colony count data were lower than 0.05. This indicates that there were significant statistical differences between the mean values in the control and experimental set-ups.

In another study, XOS extracted from sugarcane bagasse proliferated growth of *L. brevis*, *L. acidophilus* and *L. viridescens* (Gibson et al., 1995). The project made use of the xylan content extracted from the sugarcane bagasse while this study used powdered sugarcane bagasse.

In another study, Lotus root extract was reported to be able to proliferate the growth of *L. casei subsp. rhamnosus* in fermented milk. (Muadsri, 2017)

Similarly, a positive result was obtained in this study with sugarcane and lotus root extract on *L. gasseri*, *L. plantarum* and *L. rhamnosus*.

Conclusion

From the colony count done, it can be concluded that *Lactobacillus spp.* had higher growth rates in both sugarcane and lotus root set-ups compared to the control set-up. Thus, sugarcane and lotus root extracts are able to proliferate the growth of *L. gasseri, L. plantarum* and *L. rhamnosus* used in this experiment. The waste products used in this study can be recycled, and used for production of prebiotic extracts, decreasing overall food wastage in Singapore. Since it is economically feasible to prepare the prebiotic extracts, food production companies can add them into the probiotics nutritional supplements at a low cost, increasing nutritional benefits of food for consumers. Lotus root extract and sugarcane extract can be tested on other *Lactobacillus* species to determine if they are able to proliferate the growth of the other *Lactobacillus* species as well.

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