JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10187–10198 10187
DOI:10.1021/jf902003a

Evaluation of Agrowastes as Immobilizers for Probiotics in Soy Milk

Sue-Siang Teh, Rosma Ahmad, Wan-Nadiah Wan-Abdullah, and Min-Tze Liong*

School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

The objective of this study was to evaluate agricultural wastes as immobilizers for probiotics in liquid foods, such as soy milk. Probiotic strains were initially evaluated for acid and bile tolerance and the ability to produce α-galactosidase. Rinds of durian, mangosteen, and jackfruit were dried, ground, and sterilized prior to immobilization of selected strains (*Lactobacillus acidophilus* FTDC 1331, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, and *Lactobacillus bulgaricus* FTCC 0411). Immobilized cells were inoculated into soy milk, and growth properties were evaluated over 168 h at 37 °C. Soy milk containing free cells without agrowastes was used as the control. Immobilized probiotics showed increased growth, greater reduction of stachyose, sucrose, and glucose, higher production of lactic and acetic acids, and lower pH in soy milk compared to the control. The results illustrated that agrowastes could be used for the immobilization of probiotics with enhanced growth, utilization of substrates, and production of organic acids.

KEYWORDS: Soy milk; probiotic; agrowastes; immobilization; durian; cempedak; mangosteen

INTRODUCTION

Probiotics are microorganisms that possess various benefits to the host upon consumption and have been reported to eliminate the growth of pathogens, alleviate lactose tolerance, decrease serum cholesterol levels, and reduce the risks of cancer (*1*).

Agrowastes can be defined as wastes generated from animals and plants agriculturally such as leaves, roots, hulls, manures, and plant fibers. Although agrowastes are not classified as hazardous wastes, they are produced abundantly from crops each year, leading to environmental and economical issues. For example, 880 million tons of cereals are produced worldwide annually, of which 550 million tons are wheat straw, whereas approximately 700,000 tons of okara are produced annually from the production of tofu in Japan (*2*). More than 6 million hectares of land in Malaysia is utilized for major crops such as oil palm, rubber, paddy, coconut, and cocoa, yet only 24.5% of the total agricultural biomass is used for energy consumption and the rest is left as wastes. There are approximately 17000 ha of land in Malaysia that are utilized for the cultivation of fruit, producing approximately 0.25 million tonnes of fruits (*3*). However, only 20% of the whole fruit is edible, whereas the skin, core, base, and rind are normally discarded as wastes. Due to increased economical and environmental concerns, agrowastes are used as bedding for animals and livestock feeding or added into soil as green fertilizer. Panthapulakkal and Sain have previously documented other uses of agrowastes such as soil conditioners or fertilizers, biofuels, thermoplastics, activated charcoal, and components of other composite materials (*4*). Fruit agrowastes are often rich in dietary fiber and sugars and could be used as substrates for microorganisms. Wastes from the pineapple fruit have been used as fermentation broth for the cultivation of *Saccharomyces cerevisiae* and *Candida utilis* (*3*), whereas sago waste and palm oil sludge have been used for the cultivation of *Myceliophthora thermophila* and *Trichoderma harzianum*, respectively, in submerged fermentation (*5*). However, limited information is available on the use of agrowastes in solid state fermentation, whereas their potential as immobilizers for probiotics has not been evaluated. To our knowledge, this is the first study to evaluate such a property.

The viability of probiotics should exceed 10^7 CFU/g of product to exhibit therapeutic effects in the host and maintained at a minimum level of 10^6 CFU/g to be recognized as a probiotic food. Stresses to organisms begin in the stomach, in the presence of acids and with pH between 1.5 and 3.0, and in the upper intestines that contain bile (*6*). The time from entrance to release from the stomach has been estimated to be approximately 90 min, with further digestive processes requiring longer residence time (*7*). Additionally, it has been found that free cells of probiotics without protection in food matrices such as yogurt do not have a long shelf life containing the required level of total viable counts (*8*). Thus, many methods have been developed to enhance the viability of probiotics, such as microencapsulation (*9*), stress adaptation, mutation, alginate coating (*10*), and lyophilization. However, some of these techniques, such as microencapsulation, are less suitable as they involve packing of cells in sealed capsules, which may hinder their release in specific sites in the lower intestines (*11*), whereas lyophilization reduces cell counts during harsh temperature changes. Immobilization has been utilized and was found to increase the growth on cells, enhance their storage stability, and prolong the shelf life of

*Corresponding author (telephone +6046532114; fax +6046573678; e-mail mintze.liong@usm.my).

© 2009 American Chemical Society
Published on Web 10/12/2009

products (*12*). Cell immobilization includes biomass entrapment within matrices of carriers such as alginate, agar, and polyacrylates (*13*) and attachment or adsorption to preformed carrier (*14*). Tuli et al. have previously evaluated solid phases for cell immobilization and found that immobilization was effective for the entrapment of *Lactobacillus casei* in the production of lactic acid (*15*). Kosseva et al. used calcium pectate gel and chemically modified chitosan beads as supports for the immobilization of *L. casei* in the fermentation of Chardonnay wine (*16*). Alginate was also evaluated as a matrix for *L. casei* in the production of lactic acid (*17*).

However, there has been no attempt to utilize agrowastes as a source of solid support for the immobilization of probiotic. Additionally, the survival and viability of immobilized probiotics on agrowastes remain unknown. Thus, in this study, we aimed to use agrowastes as immobilizers for probiotics in a food product, such as soy milk. Soy milk contains α-galactosyl sugars such as stachyose and raffinose, which could be utilized by strains of probiotics possessing α-galactosidase activity. Hence, probiotic strains were screened, and strains with higher α-galactosidase activity were selected to be incorporated into soy milk. Additionally, we also evaluated the growth properties of immobilized probiotics in soy milk.

MATERIALS AND METHODS

Bacterial Cultures. Strains of *Lactobacillus bulgaricus* FTCC 0411, *L. casei* FTCC 0442, *Lactobacillus fermentum* FTCC 0013, *Lactobacillus acidophilus* ATCC 4962, *L. acidophilus* FTDC 2333, *L. bulgaricus* FTDC 1311, *Lactobacillus* spp. FTDC 1211, *L. acidophilus* FTDC 1733, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2133, *L. bulgaricus* FTDC 1611, *L. acidophilus* FTDC 1331, *L. bulgaricus* FTDC 1511, *Bifidobacterium bifidium* FTDC 2142, and *B. bifidium* FTCC 0012 were obtained from the Culture Collection Centre of School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia. Each strain was propagated three times in sterile de Mann, Rogosa, Sharpe (MRS) broth (Himedia, Mumbai, India) supplemented with 0.05% (w/v) filter-sterilized (0.20 μm) L-cysteine·HCl (Bioshop, Burlington, Canada) solution and incubated at 37 °C for 20 h prior to each analysis. Stock cultures were stored at −20 °C in 40% (v/v) sterile glycerol.

Acid Tolerance of Probiotic Strains. All probiotic strains were subcultured three times in sterile MRS broth using 10% (v/v) inoculum and incubated at 37 °C for 20 h. Simulated gastric juices were prepared by suspending pepsin (Sigma-Aldrich, St. Louis, MO) in 0.5% (w/v) sterile NaCl (Sigma-Aldrich) to a final concentration of 3 g/L. The pH of simulated gastric juices was adjusted to 2.0, 3.0, and 4.0 with 0.1 M HCl. Probiotic culture (0.2 mL) was pipetted into a sterile Eppendorf tube, followed by centrifugation at 17530*g* and 4 °C for 5 min. The supernatant was discarded, and the pellet was washed twice with 0.5% (w/v) NaCl; 0.3 mL of 0.5% (w/v) NaCl and 1 mL of prepared simulated gastric juice (pH 2.0, 3.0, and 4.0) were added into the pellet, vortexed for 10 s, and incubated at 37 °C. To determine the acid tolerance of each probiotic strain, 0.1 mL aliquots were sampled after 0, 30, 60, 90, and 180 min for the determination of total viable counts with sterile MRS agar supplemented with 0.15% (w/v) L-cysteine·HCl. The experiments were repeated twice.

Bile Tolerance of Probiotic Strains. Washed probiotic strain suspensions were prepared as described above. Three types of bile salts solutions (cholic acid, taurocholic acid, and glycocholic acid; Sigma-Aldrich) were prepared at 3% (w/v) each. Pancreatin (Sigma-Aldrich) solution was prepared at 1 g/L. One milliliter of bile salts solution and 1 mL of pancreatin were added into 10 mL of 0.5% (w/v) sterile sodium chloride (pH 8.0) to simulate small intestinal juices. Bacterial growth was measured once every hour for 7 h at OD_{620}. The obtained absorbance values were plotted against incubation time. Bile tolerance of each strain was determined as the time required for an increase in absorbance value of 0.3 unit. The experiments were replicated twice.

α-Galactosidase Assay. Crude enzyme extract was prepared according to the method of Ng et al. with some modification (*18*). Each probiotic strain (10% v/v) was activated via three successive propagations in 10 mL of sterile MRS broth supplemented with 5% (w/v) L-cysteine·HCl at 37 °C for 20 h. The probiotic cells were harvested at time 0 and 20 h by centrifugation at 4000*g* for 10 min at 4 °C. The supernatant was discarded, and the pellet was washed twice with cold 50 mM sodium phosphate buffer (pH 5.5). Ten milliliters of the same cold buffer was added into the pellet. The pellet was cooled in an ice bath for 15 min and sonicated (ELMA, Singen, Germany; 50 kHz, 300 W) for 15 min. The cooling and sonication steps were repeated twice. The cell debris was separated via centrifugation at 10000*g* for 10 min at 4 °C. The supernatant was used as a crude enzyme extract. Crude enzyme extracts were assayed for α-galactosidase activity according to the method of Ewe et al. with some modifications (*19*). Crude enzyme extract (1.5 mL) and 3 mL of 5 mM pNPG (*p*-nitrophenyl-α-D-galactopyranoside; Sigma-Aldrich) were added into a sterile centrifuge tube and incubated at 37 °C for 30 min. The reaction was stopped by the addition of 3 mL of cold 0.2 M Na_2CO_3 into the mixture. The amount of *p*-nitrophenol released was measured spectrophotometrically at 420 nm. A series of known concentrations of *p*-nitrophenol (Sigma-Aldrich) was prepared to produce a standard calibration curve. One unit of enzyme activity is defined as the amount of enzyme that released 1 μmol of *p*-nitrophenol from pNPG per milliliter per minute under assay conditions. The specific activity was expressed as units (U) of α-galactosidase activity per milligram of protein. The Bradford assay was used to determine protein concentration in crude enzymes extracts (*20*). The strains with highest α-galactosidase activity were selected to be used for the evaluation of soy milk fermentation. Subsequent soy milk analyses were carried out at time 0, 12, 18, 24, 48, 72, and 168 h.

Preparation of Agrowastes Immobilizers. Agricultural wastes were obtained from local orchards (Penang, Malaysia). They include durian (*Durio zibethinus*), cempedak (*Artocarpus champeden*), and mangosteen (*Garcinia mangostana*). The rind portions were cut into smaller pieces, oven-dried at 70 °C for 20 h, milled with an ultracentrifugal mill (Retsch ZM 100; F-Kurt Retsch GmbH & Co., Haan, Germany), and sieved through a no. 80 test sieve (Retsch) using a vibrator sieve shaker (Retsch AS 200). The resultant powder was vacuum-packed and stored at −20 °C until further use. They are used as solid supports for the immobilization of probiotics. The average particle size was 150 μm.

Cell Immobilization. Agrowaste powders 2% (w/v) were added into universal bottles containing 10 mL of MRS broth. The mixtures were autoclaved at 121 °C for 15 min. All probiotic strains were subcultured three times in sterile MRS broth using 10% (v/v) inoculum and incubated at 37 °C for 20 h prior to use. The final subcultures were then centrifuged at 1233*g* for 15 min. The pellets were washed twice with peptone (2% w/v). The probiotic pellets were transferred aseptically into the MRS broth containing (2% w/v) agrowaste powder, which acted as immobilizers. The cultures were fermented at 37 °C for 20 h in the presence of the immobilizers.

Preparation of Soy Milk. Dried soybeans (*Glycine max*) were purchased from TESCO (Penang, Malaysia), soaked overnight to promote swelling, and blended with distilled water at a ratio of 1:6 (w/v). The blended mixture was filtered with muslin cloth, and the resultant soy milk was pasteurized at 95 °C for 15 min.

Innoculation of Immobilized Probiotic into Soy Milk. MRS broth containing fermented probiotic cultures and agrowaste immobilizers was centrifuged at 1233*g* for 15 min. The supernatants were discarded, and the biocatalyzed probiotic pellets were washed twice with sterile peptone (2% w/v; Sigma-Aldrich). The biocatalyzed probiotic pellets were introduced aseptically into 10 mL of sterile soy milk and fermented at 37 °C for 20 h. Fermented soy milk without agrowastes was used as the control.

Scanning Electron Microscopy (SEM). The immobilization of probiotics on agrowaste rind powder was observed by SEM. Sterilized fruit rind powder in soy milk without probiotics was observed for comparison. Probiotic-fermented soy milk with immobilizers was centrifuged at 1233*g* for 15 min. The supernatant were discarded and the pellet was resuspended with McDowell Trump fixative (Sigma-Aldrich) prepared in 0.1 M phosphate buffer (Sigma) (pH 7.2) for 2 h. The resuspended samples were centrifuged, and the supernatant was discarded. Then, the pellet was resuspended in 1% (w/v) osmium tetroxide (Sigma-Aldrich) prepared in the phosphate buffer for 1 h. The sample was washed twice with distilled water and dehydrated in a sequence using ethanol (50%, 10 min), ethanol (75%, 10 min), ethanol (100%, 10 min), and hexamethyldisilazane (HMDS; 10 min) (Sigma). The HMDS was

decanted from the tube, and the residue was air-dried at 25 °C. The dried cells are then mounted onto a SEM specimen stub, coated with gold in a sputter coater (Polaran model SC515) for 5 min and examined with a scanning electron microscope (Leo Supra 50 VP Field Emission, Oberkochen, Germany).

Microbial Analysis. Viability tests of probiotic strains in fermented soy milk were carried out using the pour-plate method. Prior to pour-plate, glass beads (0.25 mm) were added into the fermented medium and homogenized (Ika, Staufen, Germany) to release the probiotic cells from agrowastes. A 10-fold serial dilution was carried out with sterilized peptone water (2% w/v). MRS agar was added with sterile L-cysteine·HCl (0.05% w/v) and incubated at 37 °C anaerobically with gas-generating kits (Merck, Darmstadt, Germany).

Chemical Analyses. Sugars in fermented soy milk were quantified via high-performance liquid chromatography (HPLC). Soy milk samples were filtered (125 mm diameter × 100 circles, Whatman, Maidstone, U.K.) prior to treatment with cation powder (Fluka, Steinheim, Germany) and anion powder (Merck). The filtrate was subsequently filtered through a Sep-Pak C18 cartridge (Waters, Milford, MA) prior to filtration through a 0.20 μm filter (Sartorius, Goettingen, Germany). The HPLC system consisted of a Sugar-Pak 6.5 × 300 mm column (Waters), a HPLC pump (Waters), and a refractive index detector (Waters). The temperature of the column was maintained at 90 °C. Ethylenediaminetetraacetic acid calcium disodium salt (Ca-EDTA) (Fluka) (4 mg/L) was used as the mobile phase with a flow rate of 0.5 mL/min and a pressure of 400 psi. Known concentrations of stachyose, raffinose, sucrose, glucose, and fructose (Sigma) were used as standards for sugar analysis.

The concentration of organic acids in soy milk samples was also determined using HPLC. Soy milk samples (1.5 mL) were treated with 0.1 mL of nitric acid (15.8 N) (Sigma-Aldrich) followed by the addition of 0.1 mL of sulfuric acid (0.1 N) (Sigma-Aldrich) to precipitate residual proteins. The aliquots were filtered through a 0.20 μm filter (Sartorius). The HPLC system consisted of a Rezex ROA-organic acid H 300 × 7.80 mm column (Phenomenex, Torrance, CA), a HPLC pump (Waters) and a 2487 Dual λ Absorbance Detector (Waters). Sulfuric acid (0.001 N) was used as the mobile phase with a flow rate of 0.5 mL/min and a pressure of 500 psi. Lactic and acetic acids were detected at 214 nm.

The pH of the soy milk samples was determined using a pH-meter with a glass electrode (Delta 320, Shanghai, China).

Statistical Analysis. Data analysis was performed using SPSS Inc. software (version 15.0) (Chicago, IL). A repeated measure ANOVA was used for time-based analyses. One-way ANOVA was used to evaluate the significant differences between sample means, with significance level at $\alpha = 0.05$. Mean comparisons were assessed by Tukey's test. All data presented were mean values of duplicates, obtained from two separate runs, unless stated otherwise.

RESULTS

Acid Tolerance. All probiotic strains showed tolerance to pH 2.0, 3.0, and 4.0 for 3 h despite variations in viability (**Table 1**). Most probiotic strains showed higher reduction in growth in simulated gastric juice at pH 2.0 compared to pH 3.0 or 4.0. *L. acidophilus* FTDC 2333, an acid-sensitive probiotic strain, showed great reduction (46.24%) of viability at pH 2.0 after 180 min of simulated gastric transit. *L. acidophilus* ATCC 4962 and *L. bulgaricus* FTCC 0411 were also acid-sensitive strains, with growths reduced by 3 log at 180 min during simulated gastric transit at pH 4.0, whereas *L. acidophilus* FTDC 2133, *L. casei* FTCC 0442, and *L. acidophilus* FTDC 1331 were more acid-tolerant, with a reduction of only 1 log under the same conditions. *L. bulgaricus* FTDC 1511 was the most acid-tolerant probiotic strain, with the least reduction in growth under all pH values studied, pH 2.0 (6.52%), pH 3.0 (5.55%), and pH 4.0 (4.97%).

Bile Tolerance. All probiotic strains were evaluated for their bile tolerance using simulated small intestinal juice containing three different bile acids, such as cholic acid, taurocholic acid, and glycocholic acid (**Table 2**). The pH of the media was monitored due to the addition of bile acids. There was no difference in the initial pH of all media ($P < 0.05$). Our results showed that media containing cholic acid had lower pH upon fermentation compared to the other media studied. *B. bifidium* FTCC 0012 and *B. bifidium* FTDC 2142 were the most bile-tolerant strains in the presence of cholic acid, but not for media supplemented with taurocholic and glycocholic acids. *L. acidophilus* FTDC 1331, *Lactobacillus* FTDC 1211, and *L. acidophilus* FTDC 2333 also showed good growth in media supplemented with cholic acid. However, *L. bulgaricus* FTCC 0411 was the most sensitive to cholic acid. All probiotic strains showed higher tolerance toward cholic acid compared to taurocholic and glycocholic acids, whereas most strains showed better tolerance toward glycocholic acid compared to taurocholic acid. *L. bulgaricus* FTDC 1511, *B. bifidium* FTCC 0012, *Lactobacillus* FTDC 1211, *L. bulgaricus* FTDC 1611, *L. bulgaricus* FTDC 2133, *L. acidophilus* FTDC 2333, and *L. acidophilus* FTDC 1331 showed the highest tolerance toward taurocholic acid, whereas *B. bifidium* FTCC 0012, *L. bulgaricus* FTDC 1511, *L. acidophilus* FTDC 1311, and *L. acidophilus* FTDC 2333 showed the highest tolerance toward glycocholic acid. *L. casei* FTCC 0442 was the most bile-sensitive strain in the presence of taurocholic and glycocholic acids.

α-Galactosidase Activity. The α-galactosidase activity of all probiotic strains was determined over 20 h (**Table 3**). Strains with the highest specific α-galactosidase activity will be selected for incorporation into soy milk for subsequent growth analyses. Our results showed that the α-galactosidase activity was strain-dependent. *L. acidophilus* FTDC 1331 exhibited the highest specific α-galactosidase activity (20.95 U/mg) at 20 h, followed by *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, and *L. bulgaricus* FTCC 0411. Thus, these strains were chosen for further evaluation in soy milk.

Scanning Electron Microscopy. Our SEM photos showed that the probiotic cells attached well on agrowaste powder, which acted as immobilizers (**Figure 1**). This indicated that agrowastes could be used for probiotic cell immobilization.

Growth of Probiotics. The growth of immobilized *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1331, *L. bulgaricus* FTCC 0411, *L. acidophilus* FTDC 2631, and *L. acidophilus* FTDC 1733 in soy milk was evaluated over 168 h at 37 °C (**Table 4**). Free cells of probiotics (without immobilization) in soy milk were used as the control. In general, all probiotic strains showed increasing growth over 168 h. In the control medium, the growths of all probiotic strains increased from 0 to 48 h but reduced after 72 h. However, in soy milk containing immobilized cells on agrowastes, the growth of all probiotic strains increased from 0 to 72 h and began to decrease only after 168 h. In general, our results indicated that probiotic strains survived longer upon immobilization on agrowaste powder compared to the control. Among all of the probiotic strains studied, *L. acidophilus* FTDC 1733 showed the highest viability in all agrowastes over 168 h, followed by *L. acidophilus* FTDC 2631.

Analysis of Sugars. All agrowastes contained various amounts of sugars, which contributed to various concentrations of sugars in soy milk prior to fermentation. Thus, to better illustrate the utilization of sugars, data are presented as milligram per milliliter reduction, instead of the concentration of individual sugars.

Our results showed that the change of sucrose was not strain-dependent but was agrowaste-dependent (**Table 5**). In general, the highest reduction of sucrose was found in soy milk containing immobilized cells on mangosteen, followed by cempedak and durian over 168 h. Our results showed that the concentration of sucrose in the control was reduced by only 0.05−0.06 mg/mL over 168 h for control (**Table 5**), whereas those in soy milk containing immobilized cells on agrowastes were reduced by 0.09−0.21 mg/mL

Table 1. Effect of pH 2.0, 3.0, and 4.0 on Viability of 15 Strains of Probiotics

		viable count[a] (log CFU/mL) during simulated gastric transit tolerance					
strain	pH of simulated gastric juice	0 min	30 min	60 min	90 min	180 min	% reduction
Lactobacillus acidophilus ATCC 4962	2	9.51 ± 0.09 aB	9.10 ± 0.05 bB	8.56 ± 0.06 cA	7.89 ± 0.07 dA	7.51 ± 0.01 eA	2.00 ± 0.08 **34**
	3	9.43 ± 0.10 aB	9.06 ± 0.04 abB	8.52 ± 0.10 bcA	7.81 ± 0.27 cdA	7.51 ± 0.28 dA	1.93 ± 0.39 **23**
	4	10.25 ± 0.03 aA	10.04 ± 0.01 aA	8.67 ± 0.05 abA	8.10 ± 0.51 bA	7.07 ± 0.75 bA	3.18 ± 0.72 **1**
Lactobacillus acidophilus FTDC 2333	2	9.43 ± 0.03 aA	8.49 ± 0.11 bC	7.68 ± 0.02 cC	6.62 ± 0.06 dC	5.07 ± 0.29 eB	4.35 ± 0.33 **1**
	3	9.45 ± 0.03 aA	8.87 ± 0.08 bB	7.79 ± 0.04 cB	6.85 ± 0.01 dB	5.66 ± 0.14 eB	3.79 ± 0.11 **1**
	4	9.55 ± 0.01 aA	9.85 ± 0.01 aA	8.90 ± 0.03 bA	7.64 ± 0.01 cA	7.00 ± 0.19 dA	2.55 ± 0.25 **12**
Lactobacillus bulgaricus FTCC 0411	2	10.26 ± 0.02 aA	10.11 ± 0.02 bA	9.33 ± 0.07 cB	9.00 ± 0.02 dA	7.69 ± 0.02 eA	2.46 ± 0.16 **2**
	3	10.25 ± 0.02 aA	9.97 ± 0.18 aA	8.83 ± 0.07 bB	8.50 ± 0.10 bA	7.94 ± 0.02 cB	2.32 ± 0.01 **2**
	4	10.26 ± 0.01 aA	10.07 ± 0.01 aA	8.98 ± 0.02 bA	8.75 ± 0.21 bA	7.95 ± 0.02 cB	2.32 ± 0.02 **2**
Lactobacillus fermentum FTCC 0013	2	9.45 ± 0.01 aA	9.39 ± 0.01 aA	9.31 ± 0.02 bA	8.20 ± 0.01 cC	8.07 ± 0.01 cB	1.47 ± 0.04 **4**
	3	9.43 ± 0.01 aA	9.36 ± 0.03 bA	9.23 ± 0.04 cA	8.08 ± 0.01 dB	7.97 ± 0.03 eA	1.37 ± 0.01 **45**
	4	9.45 ± 0.01 aA	9.42 ± 0.01 aA	9.32 ± 0.02 bA	8.12 ± 0.01 cA	8.01 ± 0.03 dAB	1.44 ± 0.04 **3**
Lactobacillus bulgaricus FTDC 1311	2	10.45 ± 0.02 aA	10.27 ± 0.01 bB	10.20 ± 0.01 cB	9.10 ± 0.01 dA	8.99 ± 0.01 eA	1.47 ± 0.01 **4**
	3	10.47 ± 0.01 aA	10.34 ± 0.01 bA	10.22 ± 0.01 cA	9.11 ± 0.01 dA	9.01 ± 0.02 eA	1.45 ± 0.01 **45**
	4	10.44 ± 0.02 aA	10.27 ± 0.01 bB	10.19 ± 0.01 bB	9.02 ± 0.03 cB	8.96 ± 0.02 cA	1.49 ± 0.01 **3**
Lactobacillus FTDC 1211	2	10.40 ± 0.01 aA	10.34 ± 0.01 bA	10.27 ± 0.01 cA	8.80 ± 0.01 dB	8.73 ± 0.01 eB	1.67 ± 0.01 **3**
	3	10.36 ± 0.01 aA	9.99 ± 0.05 bB	9.95 ± 0.01 bC	8.83 ± 0.01 cB	8.75 ± 0.02 cB	1.61 ± 0.04 **34**
	4	10.36 ± 0.01 aA	10.05 ± 0.02 abB	9.99 ± 0.01 abB	8.96 ± 0.01 abA	8.87 ± 0.02 bA	1.48 ± 0.01 **3**
Lactobacillus acidophilus FTDC 1733	2	10.00 ± 0.04 aA	9.87 ± 0.02 bA	9.64 ± 0.03 cA	9.67 ± 0.02 cA	8.76 ± 0.01 dC	1.25 ± 0.04 **45**
	3	9.86 ± 0.03 aA	9.83 ± 0.02 aA	9.74 ± 0.02 bA	9.63 ± 0.04 cA	8.81 ± 0.01 dB	1.06 ± 0.01 **56**
	4	9.90 ± 0.05 aA	9.79 ± 0.02 abA	9.77 ± 0.04 abA	9.69 ± 0.01 bA	8.85 ± 0.01 cA	1.05 ± 0.04 **34**
Lactobacillus acidophilus FTDC 2631	2	10.38 ± 0.01 aA	10.11 ± 0.01 bA	9.75 ± 0.02 cA	9.63 ± 0.04 dAB	8.93 ± 0.01 eAB	1.45 ± 0.01 **4**
	3	10.37 ± 0.01 aA	10.06 ± 0.01 bB	9.53 ± 0.07 cB	9.37 ± 0.10 cB	8.89 ± 0.01 dB	1.47 ± 0.00 **45**
	4	10.39 ± 0.01 aA	10.12 ± 0.01 bA	9.81 ± 0.03 cA	9.69 ± 0.04 cA	8.96 ± 0.01 dA	1.43 ± 0.03 **3**
Lactobacillus acidophilus FTDC 2133	2	9.75 ± 0.02 aB	9.62 ± 0.07 aA	9.46 ± 0.02 bB	9.09 ± 0.01 cB	9.01 ± 0.03 cB	0.74 ± 0.01 **6**
	3	9.84 ± 0.02 aB	9.69 ± 0.05 bA	9.64 ± 0.04 bAB	9.14 ± 0.01 cAB	9.07 ± 0.01 cAB	0.77 ± 0.01 **67**
	4	9.99 ± 0.04 aA	9.81 ± 0.03 bA	9.68 ± 0.06 bA	9.16 ± 0.01 cA	9.12 ± 0.01 cA	0.87 ± 0.03 **34**
Lactobacillus bulgaricus FTDC 1611	2	10.23 ± 0.02 aA	9.99 ± 0.01 bC	9.88 ± 0.01 cA	8.92 ± 0.01 dB	8.81 ± 0.01 eC	1.42 ± 0.01 **4**
	3	10.24 ± 0.01 aA	10.10 ± 0.01 bB	9.96 ± 0.04 cA	8.99 ± 0.01 dAB	8.89 ± 0.01 eB	1.36 ± 0.01 **45**
	4	10.28 ± 0.01 aA	10.14 ± 0.01 bA	9.98 ± 0.02 cA	9.01 ± 0.02 dA	8.91 ± 0.01 eA	1.36 ± 0.01 **3**
Bifidobacterium spp. 12	2	10.44 ± 0.01 aA	10.27 ± 0.01 bB	10.02 ± 0.01 cC	9.01 ± 0.02 dB	8.88 ± 0.01 eB	1.56 ± 0.02 **4**
	3	10.44 ± 0.01 aA	10.34 ± 0.01 bA	10.09 ± 0.01 cB	9.06 ± 0.01 dB	8.94 ± 0.01 eB	1.50 ± 0.01 **4**
	4	10.45 ± 0.01 aA	10.36 ± 0.01 bA	10.16 ± 0.01 cA	9.13 ± 0.01 dA	8.98 ± 0.01 eA	1.48 ± 0.01 **3**
Bifidobacterium FTDC 2142	2	10.38 ± 0.01 aA	10.30 ± 0.01 aA	10.10 ± 0.02 bB	9.07 ± 0.01 cA	8.80 ± 0.07 dA	1.58 ± 0.07 **4**
	3	10.41 ± 0.02 aA	10.31 ± 0.02 bA	10.14 ± 0.01 cAB	9.09 ± 0.01 dA	8.89 ± 0.02 eA	1.52 ± 0.01 **34**
	4	10.43 ± 0.01 aA	10.35 ± 0.02 aA	10.18 ± 0.02 bA	9.13 ± 0.02 cA	9.00 ± 0.06 cA	1.42 ± 0.06 **3**
Lactobacillus casei FTCC 0442	2	10.46 ± 0.01 aA	10.31 ± 0.01 bB	10.14 ± 0.01 cC	9.81 ± 0.02 dB	9.44 ± 0.07 eB	1.01 ± 0.04 **56**
	3	10.46 ± 0.01 aA	10.33 ± 0.01 bAB	10.20 ± 0.01 cB	9.88 ± 0.04 dAB	9.60 ± 0.03 eAB	0.86 ± 0.04 **67**
	4	10.48 ± 0.01 aA	10.36 ± 0.01 bA	10.25 ± 0.01 cA	9.95 ± 0.03 dA	9.71 ± 0.02 eA	0.77 ± 0.01 **34**
Lactobacillus acidophilus FTDC 1331	2	10.43 ± 0.01 aA	10.07 ± 0.03 bA	10.04 ± 0.01 cA	9.79 ± 0.04 dA	9.58 ± 0.07 eA	0.62 ± 0.01 **6**
	3	10.44 ± 0.01 aA	10.17 ± 0.02 bA	10.11 ± 0.01 cA	10.03 ± 0.01 dA	9.82 ± 0.02 eA	0.53 ± 0.03 **7**
	4	10.44 ± 0.01 aA	10.23 ± 0.01 bB	10.14 ± 0.02 bB	10.06 ± 0.02 cB	9.91 ± 0.03 dB	0.85 ± 0.06 **34**
Lactobacillus bulgaricus FTDC 1511	2	10.42 ± 0.01 aA	10.27 ± 0.01 bB	10.00 ± 0.01 cB	9.88 ± 0.02 dB	9.74 ± 0.03 eB	0.68 ± 0.01 **6**
	3	10.44 ± 0.01 aA	10.34 ± 0.02 bA	10.15 ± 0.01 cA	9.99 ± 0.01 dA	9.86 ± 0.02 eA	0.58 ± 0.01 **7**
	4	10.46 ± 0.01 aA	10.38 ± 0.02 bA	10.16 ± 0.01 cA	10.04 ± 0.01 dA	9.94 ± 0.02 eA	0.52 ± 0.01 **4**

[a] Results are expressed as mean ± standard error of means; each data point is the average of measurement from two independently replicated experiments, $n = 2$. Means in the same column followed by lower case letters are significantly different ($P < 0.05$). Means in the same row followed by upper case letters are significantly different ($P < 0.05$). Means of percentage reduction (%) between strains within the same pH followed by boldface numbers are significantly different ($P < 0.05$).

Results from our present study showed that the changes of glucose were also dependent on the type of agrowastes (**Table 6**). In general, soy milk containing immobilized cells on mangosteen showed the highest reduction of glucose, where a reduction

Table 2. Bile Tolerance of Probiotic Strains in Different Bile Media[a]

	growth media								
	MRS broth + 0.1% cholic acid			MRS broth + 0.1% taurocholic acid			MRS broth + 0.1% glycocholic acid		
		pH			pH			pH	
strain	time (h)	T1	T2	time (h)	T1	T2	time (h)	T1	T2
Bifidobacterium 12	4.87 ± 0.03 bA	6.25	4.78	6.29 ± 0.21 abC	6.88	5.78	5.64 ± 0.09 aB	6.35	5.01
L. casei FTCC 0442	5.99 ± 0.01 deA	6.20	4.62	14.01 ± 0.60 gB	6.78	5.62	12.7 ± 0.99 gB	6.44	5.13
Bifidobacterium FTDC 2142	3.96 ± 0.06 aA	6.21	4.65	10.64 ± 0.91 defB	6.55	5.22	9.16 ± 0.47 eB	6.20	5.12
L. bulgaricus FTDC 1511	5.99 ± 0.10 deA	6.12	4.33	6.09 ± 0.10 aA	6.53	5.23	6.27 ± 0.44 abA	6.08	5.07
L. acidophilus FTDC 1331	5.32 ± 0.11 cA	6.14	4.28	8.56 ± 0.57 cB	6.81	5.45	5.63 ± 0.03 aA	6.15	4.98
L. acidophilus ATCC 4962	6.19 ± 0.11 defA	6.24	4.20	11.59 ± 0.19 fB	6.77	5.16	10.99 ± 0.17 fB	6.23	4.94
L. fermentum FTCC 0013	6.15 ± 0.21 defA	6.13	4.30	9.05 ± 0.01 cdB	6.55	5.28	8.87 ± 0.24 eB	6.14	5.10
Lactobacillus FTDC 1211	5.95 ± 0.05 dA	6.05	4.11	6.67 ± 0.49 abA	6.14	5.34	6.85 ± 0.20 abA	6.07	5.02
L. acidophilus FTDC 2631	6.47 ± 0.03 fA	6.08	4.02	9.32 ± 0.12 cdC	6.25	5.18	6.87 ± 0.10 abB	6.09	5.11
L. acidophilus FTDC 1733	6.44 ± 0.21 fA	6.16	4.05	11.04 ± 0.23 efB	6.35	5.35	6.75 ± 0.10 abA	6.17	5.14
L. bulgaricus FTCC 0411	8.07 ± 0.09 gA	6.21	4.02	9.43 ± 0.77 cdeA	6.46	5.20	8.59 ± 0.02 cdeA	6.13	5.03
L. bulgaricus FTDC 1311	7.98 ± 0.06 gA	6.11	4.27	10.62 ± 0.14 defB	6.61	5.16	8.65 ± 0.56 deA	6.24	5.05
L. acidophilus FTDC 2333	5.97 ± 0.03 dA	6.02	4.08	8.57 ± 0.26 cB	6.23	5.14	5.98 ± 0.23 abA	6.26	5.16
L. acidophilus FTDC 2133	6.37 ± 0.04 defA	6.06	4.12	7.94 ± 0.03 bcC	6.15	5.23	7.17 ± 0.06 bcB	6.11	5.17
L. bulgaricus FTDC 1611	6.41 ± 0.16 efA	6.22	4.13	7.81 ± 0.27 bcB	6.45	5.21	7.28 ± 0.01 bcdB	6.12	5.03

[a] Results are expressed as mean ± standard error of means; $n = 2$. Means in the same column followed by different lower case letters are significantly different ($P < 0.05$). Means in the same row followed by different lower case letters are significantly different ($P < 0.05$).

Table 3. α-Galactosidase Activity of Selected Probiotic Strains at 37 °C for 20 h[a]

	α-galactosidase activity		
strain	total activity (U/mL)	total protein (mg/mL)	specific activity (U/mg of protein)
L. acidophilus FTDC 2631	0.33 ± 0.01 B	0.02 ± 0.01 FG	14.96 ± 0.06 B
Bifidobacterium 12	0.02 ± 0.01 IJ	0.02 ± 0.01 EFG	0.88 ± 0.01 GH
L. acidophilus ATCC 4962	0.01 ± 0.00 JK	0.02 ± 0.01 G	0.56 ± 0.01 H
L. bulgaricus FTDC 1611	0.02 ± 0.01 JK	0.03 ± 0.01 B	0.56 ± 0.06 H
L. acidophilus FTDC 2133	0.04 ± 0.01 G	0.03 ± 0.01 BC	1.41 ± 0.01 FG
L. bulgaricus FTDC 1511	0.11 ± 0.01 E	0.03 ± 0.01 CDE	4.06 ± 0.13 E
L. acidophilus FTDC 1733	0.18 ± 0.01 C	0.03 ± 0.01 BCD	6.43 ± 0.21 D
L. acidophilus FTDC 2333	0.19 ± 0.01 C	0.02 ± 0.01 EFG	8.04 ± 0.50 C
L. bulgaricus FTDC 1311	0.04 ± 0.01 GH	0.02 ± 0.01 FG	1.60 ± 0.06 F
Lactobacillus spp. FTDC 1211	0.01 ± 0.01 K	0.03 ± 0.01 B	0.32 ± 0.08 H
L. fermentum FTCC 0013	0.03 ± 0.01 HI	0.04 ± 0.01 A	0.71 ± 0.01 GH
L. acidophilus FTDC 1331	0.62 ± 0.01 A	0.03 ± 0.01 BC	20.95 ± 0.21 A
L. casei FTCC 0442	0.08 ± 0.01 F	0.02 ± 0.01 G	3.89 ± 0.30 E
Bifidobacterium FTDC 2142	0.02 ± 0.01 IJ	0.04 ± 0.01 A	0.48 ± 0.05 H
L. bulgaricus FTCC 0411	0.15 ± 0.01 D	0.03 ± 0.01 DEF	5.73 ± 0.05 D

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs: $n = 2$. Means in the same column followed by different upper case letters are significantly different ($P < 0.05$). α-Galactosidase activity from cell-free extracts of selected probiotic strains grown in MRS broth supplemented with 0.5% (w/v) L-cysteine·HCl.

of > 0.18 mg/mL was observed over 168 h, followed by cempedak and durian ($P < 0.05$). In the control, the concentration of glucose was reduced by only 0.04−0.07 mg/mL over 168 h.

Most probiotic strains in the control utilized approximately 0.07 mg/mL of fructose over 12 h and > 0.18 mg/mL over 168 h (**Table 7**). Soy milk containing immobilized cells on agrowastes showed a higher reduction of fructose over 168 h compared to the control, where soy milk containing immobilized cells on mangosteen showed the highest reduction of fructose (0.69−1.04 mg/mL), followed by cempedak (0.54−0.72 mg/mL) and durian (0.47−0.77 mg/mL) ($P < 0.05$).

There was a higher reduction of stachyose in soy milk containing immobilized cells on agrowastes compared to the control (**Table 8**). In general, the highest reduction of stachyose was found in soy milk supplemented with immobilized cells on cempedak (1.89−2.51 mg/mL), followed by durian and mangosteen. Among all strains studied, *L. acidophilus* FTDC 1331 showed the highest utilization of stachyose, whereas *L. acidophilus* FTDC 2631 showed the lowest utilization ($P < 0.05$). However, the utilization of raffinose was not significantly affected by the supplementation of immobilized cells on agrowastes in soy milk (**Table 9**).

Organic Acids. The production of organic acids by probiotic strains increased significantly ($P < 0.05$) over time. Our results showed that the concentration of lactic acid in soy milk was higher than that of acetic acid in all media studied. After 12 h, most strains in soy milk supplemented with immobilized cells on agrowastes showed higher concentration of lactic acid than the control (**Table 10**). In general, the highest production of lactic acid occurred in soy milk with added immobilized cells on cempedak and mangosteen powder ($P < 0.05$). Among the strains studied, *L. acidophilus* FTDC 1733 produced the highest concentration of lactic acid, whereas *L. acidophilus* FTDC 2631 produced the least ($P < 0.05$).

Our results show that the concentration of acetic acid in the control was higher than in soy milk containing immobilized cells on agrowastes after 12 h of fermentation (**Table 11**). However, after 72 h, the production of acetic acid in soy milk containing immobilized cells on agrowastes was higher than the control ($P < 0.05$). The production of acetic acid was higher in

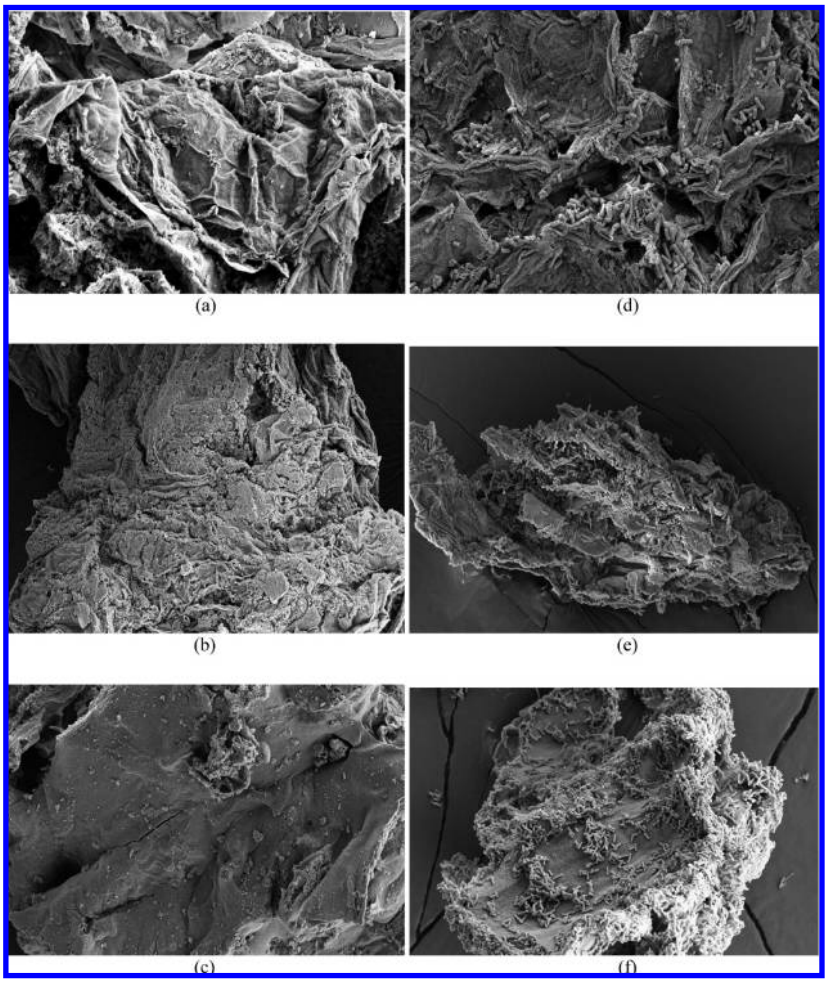

Figure 1. Scanning electron micrographs of agrowastes powder without probiotic (**a**) durian, (**b**) cempedak, or (**c**) mangosteen; immobilized cells of *L. acidophilus* FTDC 1331 on (**d**) durian rind powder, (**e**) cempedak rind powder, or (**f**) mangosteen rind powder.

soy milk supplemented with immobilized cells on cempedak compared to the other agrowastes and the control ($P < 0.05$). Among all of the strains studied, *L. acidophilus* FTDC 2631 produced the highest acetic acid, whereas *L. bulgaricus* FTCC 0411 produced the least ($P < 0.05$).

pH. The pH in the control was higher than that in soy milk supplemented with immobilized cells on agrowastes at 0 h; the control had pH >6.0, whereas the agrowaste-supplemented soy milk had pH >5.0 (**Table 12**). The pH of all fermentation media decreased over time. The pH in all fermentation media after 168 h was above 4.0. The control showed higher reduction of pH than soy milk containing immobilized cells on agrowastes over 168 h. The reduction of pH in the control for all strains ranged from 29.6 to 31.2% over 168 h, whereas soy milk containing immobilized cells on durian ranged from 18 to 19%, cempedak ranged from 20 to 24%, and mangosteen ranged from 16.4 to 22.4%.

DISCUSSION

Our results showed that most strains survived the acidic and bile conditions similar to those of the human gastrointestinal tract. Most strains showed good tolerance toward acids, even at low pH conditions, with growth exceeding 7 log CFU/mL over 180 min. A greater reduction in growth was observed at pH 2.0 than at pH 4.0. This may be attributed to the higher concentration of H^+ ion at pH 2.0. It has been found that proton motive force across the cell membrane can cause membrane damage and injury, leading to the loss of viability under conditions of severe acidification (*21*). All probiotic strains showed better growth in the presence of cholic acid compared to the other bile acids studied. It was previously found that conjugated bile has higher inhibition effect on the growth of lactic acid bacteria, due to greater solubility and detergent activity than their deconjugated counterpart (*5*). Our results also showed that most strains exhibited better tolerance toward glycocholic acid than toward taurocholic acid. It has been found that glycine-conjugated bile salts are more toxic than taurine-conjugated bile salts. Probiotics have the ability to deconjugate bile as a protective mechanism against the toxicity raised (*22*). Thus, such a protective mechanism was expressed more in the presence of glycocholic acid, leading to better growth as compared to taurocholic acid.

Past studies have illustrated that strains of probiotics could proliferate well in soy milk (*23*, *24*), most probably due to their ability to utilize soy α-galactosyl oligosaccharides such as raffinose and stachyose. Thus, strains of probiotics were subsequently screened for α-galactosidase activity. The sucrose moiety in raffinose and stachyose is linked by α-1,6 bonds to one raffinose and two stachyose units of galactose. α-Galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) catalyzes the cleavage of terminal α-1,6-linked galactosyl residues, yielding glucose, galactose, and fructose (*25*). Therefore, a high α-galactosidase activity exhibited by probiotic strains would indicate better proliferation in soy-based medium. *L. acidophilus* FTDC 1331, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, and *L. bulgaricus* FTCC 0411 exhibited

Table 4. Total Viable Count of Probiotic Strains in Selected Media at 37 °C for 168 h

		total viable count[a] (CFU/mL)							
strain	medium[b]	0 h	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 2333	control	8.88 ± 0.02	9.10 ± 0.01	9.41 ± 0.03	9.70 ± 0.07	9.92 ± 0.04	8.91 ± 0.03	8.49 ± 0.05	T: <0.0001
	D	8.91 ± 0.06	9.13 ± 0.04	9.33 ± 0.02	9.78 ± 0.02	9.95 ± 0.01	9.89 ± 0.01	9.78 ± 0.04	T × S: <0.0001
	C	8.90 ± 0.02	9.26 ± 0.01	9.42 ± 0.03	9.90 ± 0.04	10.18 ± 0.03	10.38 ± 0.02	9.86 ± 0.05	T × m: <0.0001
	M	8.83 ± 0.05	9.04 ± 0.06	9.33 ± 0.01	9.87 ± 0.06	10.12 ± 0.01	10.35 ± 0.02	9.88 ± 0.02	T × S × m: <0.0001
									S: <0.0001
L. acidophilus FTDC 1331	control	8.95 ± 0.01	9.17 ± 0.01	9.42 ± 0.02	9.76 ± 0.05	10.07 ± 0.03	8.98 ± 0.04	8.66 ± 0.03	m: <0.0001
	D	8.82 ± 0.04	9.13 ± 0.03	9.25 ± 0.01	9.72 ± 0.01	10.17 ± 0.01	10.23 ± 0.01	9.82 ± 0.04	S × m: <0.0001
	C	8.81 ± 0.02	9.15 ± 0.02	9.31 ± 0.05	9.80 ± 0.05	10.11 ± 0.01	10.42 ± 0.02	9.74 ± 0.04	
	M	8.88 ± 0.02	9.18 ± 0.01	9.26 ± 0.01	9.78 ± 0.04	10.07 ± 0.03	10.40 ± 0.02	9.86 ± 0.05	
L. bulgaricus FTCC 0411	control	8.91 ± 0.02	9.15 ± 0.03	9.37 ± 0.02	9.84 ± 0.03	10.11 ± 0.04	9.06 ± 0.01	8.60 ± 0.09	
	D	8.85 ± 0.06	9.05 ± 0.02	9.29 ± 0.04	9.84 ± 0.04	10.11 ± 0.03	10.30 ± 0.04	9.90 ± 0.06	
	C	8.83 ± 0.03	9.17 ± 0.02	9.31 ± 0.04	9.85 ± 0.03	10.13 ± 0.02	10.27 ± 0.01	9.76 ± 0.05	
	M	8.86 ± 0.04	9.15 ± 0.03	9.27 ± 0.01	9.84 ± 0.06	10.14 ± 0.01	10.28 ± 0.01	9.84 ± 0.01	
L. acidophilus FTDC 2631	control	8.95 ± 0.02	9.34 ± 0.02	9.76 ± 0.04	10.10 ± 0.01	10.28 ± 0.01	9.01 ± 0.04	8.72 ± 0.08	
	D	8.94 ± 0.04	9.33 ± 0.01	10.01 ± 0.04	10.21 ± 0.02	10.42 ± 0.01	10.09 ± 0.02	9.77 ± 0.08	
	C	8.92 ± 0.03	9.33 ± 0.01	9.99 ± 0.04	10.20 ± 0.02	10.34 ± 0.01	10.25 ± 0.01	9.87 ± 0.01	
	M	8.94 ± 0.03	9.37 ± 0.01	9.85 ± 0.03	10.13 ± 0.01	10.35 ± 0.02	10.23 ± 0.01	9.95 ± 0.01	
L. acidophilus FTDC 1733	control	8.89 ± 0.04	9.18 ± 0.02	9.28 ± 0.01	9.89 ± 0.01	10.13 ± 0.01	9.01 ± 0.05	8.69 ± 0.09	
	D	8.97 ± 0.04	9.38 ± 0.02	10.07 ± 0.03	10.17 ± 0.01	10.40 ± 0.01	10.25 ± 0.01	9.84 ± 0.03	
	C	8.90 ± 0.03	9.14 ± 0.01	9.26 ± 0.01	9.79 ± 0.06	10.15 ± 0.01	10.37 ± 0.01	9.85 ± 0.02	
	M	8.88 ± 0.03	9.11 ± 0.04	9.27 ± 0.01	9.71 ± 0.08	10.28 ± 0.01	10.30 ± 0.01	9.90 ± 0.03	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

Table 5. Reduction of Sucrose at 37 °C for 168 h

		sucrose[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 1331	control	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	T: <0.0001
	D	0.01 ± 0.01	0.03 ± 0.02	0.05 ± 0.03	0.08 ± 0.02	0.11 ± 0.02	0.14 ± 0.02	T × S: 0.476
	C	0.03 ± 0.01	0.05 ± 0.01	0.08 ± 0.03	0.10 ± 0.04	0.10 ± 0.03	0.11 ± 0.04	T × m: <0.05
	M	0.08 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.15 ± 0.03	0.17 ± 0.03	0.18 ± 0.05	T × S × m: 0.644
								S: 0.497
L. bulgaricus FTCC 0411	control	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	m: <0.0001
	D	0.02 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.09 ± 0.03	S × m: 0.936
	C	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.10 ± 0.03	0.12 ± 0.05	
	M	0.04 ± 0.01	0.10 ± 0.05	0.12 ± 0.05	0.14 ± 0.07	0.16 ± 0.08	0.19 ± 0.10	
L. acidophilus FTDC 1733	control	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	
	D	0.02 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.07 ± 0.03	0.08 ± 0.02	0.09 ± 0.02	
	C	0.03 ± 0.01	0.04 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	
	M	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	
L. acidophilus FTDC 2631	control	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	
	D	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.10 ± 0.03	0.12 ± 0.02	0.13 ± 0.03	
	C	0.03 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.08 ± 0.04	0.09 ± 0.04	0.11 ± 0.05	
	M	0.11 ± 0.04	0.13 ± 0.04	0.14 ± 0.04	0.17 ± 0.05	0.19 ± 0.06	0.21 ± 0.08	
L. acidophilus FTDC 2333	control	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	
	D	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	
	C	0.03 ± 0.03	0.06 ± 0.05	0.08 ± 0.06	0.09 ± 0.06	0.10 ± 0.06	0.11 ± 0.07	
	M	0.06 ± 0.03	0.08 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.14 ± 0.04	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

higher α-galactosidase activity and were thus selected to be incorporated into soy milk for further evaluation.

Probiotic strains bound well onto agrowastes as shown in our SEM micrographs. Hence, agrowastes could play a vital role as solid supports for the immobilization of probiotics in food product. The presence of food and food ingredients has been reported to improve the viability of microorganism during gastric transit (*26*). Cereals have been evaluated as a new solid-phase support for the delivery of microorganisms because of their high content of essential vitamins, minerals, and fiber that led to enhanced microbial growth (*27*). In our present study, probiotics survived longer and growth increased upon immobilization on

Table 6. Reduction of Glucose at 37 °C for 168 h

		glucose[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 1331	control	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	T: <0.0001
	D	0.02 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	T × S: 0.246
	C	0.08 ± 0.06	0.10 ± 0.07	0.12 ± 0.07	0.13 ± 0.06	0.13 ± 0.06	0.14 ± 0.06	T × m: <0.05
	M	0.14 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	T × S × m: 0.742
								S: 0.784
L. bulgaricus FTCC 0411	control	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	m: <0.0001
	D	0.02 ± 0.01	0.07 ± 0.03	0.08 ± 0.04	0.10 ± 0.05	0.12 ± 0.05	0.13 ± 0.06	S × m: 0.594
	C	0.04 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	
	M	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	
L. acidophilus FTDC 1733	control	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	
	D	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.04	0.10 ± 0.03	0.12 ± 0.04	
	C	0.06 ± 0.03	0.08 ± 0.04	0.10 ± 0.04	0.11 ± 0.05	0.12 ± 0.06	0.12 ± 0.06	
	M	0.13 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	
L. acidophilus FTDC 2631	control	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	
	D	0.03 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	
	C	0.11 ± 0.03	0.14 ± 0.02	0.16 ± 0.02	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	
	M	0.13 ± 0.02	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	
L. acidophilus FTDC 2333	control	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	
	D	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	
	C	0.09 ± 0.09	0.11 ± 0.08	0.11 ± 0.08	0.12 ± 0.07	0.13 ± 0.08	0.14 ± 0.08	
	M	0.15 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

Table 7. Reduction of Fructose at 37 °C for 168 h

		fructose[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 1331	control	0.08 ± 0.07	0.11 ± 0.06	0.18 ± 0.08	0.24 ± 0.11	0.27 ± 0.11	0.32 ± 0.12	T: <0.0001
	D	0.19 ± 0.04	0.26 ± 0.04	0.46 ± 0.20	0.61 ± 0.38	0.68 ± 0.35	0.77 ± 0.33	T × S: 0.387
	C	0.26 ± 0.04	0.37 ± 0.05	0.52 ± 0.03	0.62 ± 0.04	0.68 ± 0.02	0.72 ± 0.03	T × m: <0.05
	M	0.15 ± 0.08	0.49 ± 0.16	0.66 ± 0.37	0.86 ± 0.34	0.95 ± 0.38	1.03 ± 0.43	T × S × m: 0.699
								S: 0.225
L. bulgaricus FTCC 0411	control	0.07 ± 0.01	0.15 ± 0.03	0.21 ± 0.03	0.34 ± 0.08	0.37 ± 0.07	0.47 ± 0.06	m: <0.0001
	D	0.17 ± 0.06	0.26 ± 0.07	0.30 ± 0.07	0.35 ± 0.08	0.44 ± 0.14	0.60 ± 0.05	S × m: 0.306
	C	0.33 ± 0.01	0.35 ± 0.01	0.38 ± 0.01	0.48 ± 0.01	0.57 ± 0.02	0.62 ± 0.01	
	M	0.08 ± 0.05	0.51 ± 0.03	0.68 ± 0.11	0.71 ± 0.09	0.76 ± 0.14	0.82 ± 0.14	
L. acidophilus FTDC 1733	control	0.03 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.21 ± 0.06	0.27 ± 0.10	0.31 ± 0.11	
	D	0.21 ± 0.01	0.24 ± 0.01	0.27 ± 0.02	0.33 ± 0.02	0.38 ± 0.01	0.47 ± 0.03	
	C	0.20 ± 0.08	0.36 ± 0.16	0.47 ± 0.14	0.56 ± 0.12	0.62 ± 0.14	0.67 ± 0.11	
	M	0.11 ± 0.01	0.27 ± 0.00	0.53 ± 0.18	0.61 ± 0.16	0.65 ± 0.14	0.69 ± 0.10	
L. acidophilus FTDC 2631	control	0.07 ± 0.04	0.11 ± 0.08	0.23 ± 0.09	0.29 ± 0.11	0.39 ± 0.10	0.46 ± 0.10	
	D	0.18 ± 0.05	0.38 ± 0.03	0.50 ± 0.01	0.57 ± 0.02	0.62 ± 0.07	0.73 ± 0.07	
	C	0.18 ± 0.01	0.22 ± 0.01	0.25 ± 0.01	0.40 ± 0.02	0.51 ± 0.01	0.54 ± 0.02	
	M	0.33 ± 0.01	0.68 ± 0.04	0.89 ± 0.31	0.97 ± 0.38	1.00 ± 0.41	1.04 ± 0.41	
L. acidophilus FTDC 2333	control	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	
	D	0.24 ± 0.04	0.40 ± 0.02	0.50 ± 0.08	0.53 ± 0.08	0.68 ± 0.11	0.77 ± 0.09	
	C	0.27 ± 0.01	0.32 ± 0.02	0.45 ± 0.03	0.55 ± 0.02	0.60 ± 0.01	0.64 ± 0.02	
	M	0.11 ± 0.04	0.40 ± 0.02	0.53 ± 0.04	0.62 ± 0.08	0.71 ± 0.06	0.76 ± 0.03	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

agrowastes compared to the control. We postulate that the minerals and fibers of the agrowastes provided growth support for probiotic organisms.

Agrowastes often contain sugars, in both the reducing and oligosaccharides forms. Haruhito et al. previously reported that when whole cereal containing cereal fiber was mixed with distilled water and heated during the production of liquid fermentation media, soluble components such as sugars and free amino nitrogen were released (*28*). We prepared the agrowastes via addition into MRS broth and autoclaved at 121 °C for 15 min, hence releasing soluble components such as sugars to the MRS broth. Our preliminary results showed that agrowastes

Table 8. Reduction of Stachyose at 37 °C for 168 h

		stachyose[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P^c
L. acidophilus FTDC 1331	control	0.08 ± 0.05	0.10 ± 0.06	0.16 ± 0.03	0.26 ± 0.06	0.43 ± 0.01	0.55 ± 0.02	T: <0.0001
	D	0.27 ± 0.01	0.63 ± 0.21	1.71 ± 0.06	1.79 ± 0.11	2.05 ± 0.06	2.14 ± 0.02	T × S: <0.0001
	C	0.55 ± 0.11	0.94 ± 0.13	1.13 ± 0.10	1.54 ± 0.11	1.68 ± 0.13	1.89 ± 0.06	T × m: <0.0001
	M	0.59 ± 0.01	1.41 ± 0.12	1.58 ± 0.10	1.64 ± 0.11	1.82 ± 0.06	1.90 ± 0.05	T × S × m: <0.0001
								S: <0.0001
L. bulgaricus FTCC 0411	control	0.15 ± 0.05	0.18 ± 0.04	0.31 ± 0.04	0.40 ± 0.03	0.44 ± 0.03	0.55 ± 0.02	m: <0.0001
	D	0.74 ± 0.01	0.88 ± 0.01	1.25 ± 0.06	1.45 ± 0.07	1.67 ± 0.05	1.97 ± 0.19	S × m: <0.0001
	C	0.89 ± 0.04	1.09 ± 0.03	1.26 ± 0.04	1.71 ± 0.05	2.05 ± 0.02	2.39 ± 0.04	
	M	0.73 ± 0.01	1.39 ± 0.16	1.65 ± 0.06	1.79 ± 0.01	1.80 ± 0.00	2.12 ± 0.07	
L. acidophilus FTDC 1733	control	0.14 ± 0.07	0.28 ± 0.10	0.33 ± 0.12	0.37 ± 0.12	0.43 ± 0.10	0.48 ± 0.11	
	D	1.48 ± 0.01	1.52 ± 0.01	1.58 ± 0.01	1.60 ± 0.01	1.66 ± 0.04	2.02 ± 0.00	
	C	0.21 ± 0.01	0.50 ± 0.12	1.19 ± 0.01	1.66 ± 0.06	2.20 ± 0.02	2.51 ± 0.02	
	M	1.27 ± 0.01	1.51 ± 0.01	1.71 ± 0.04	1.92 ± 0.09	2.15 ± 0.08	2.33 ± 0.08	
L. acidophilus FTDC 2631	control	0.20 ± 0.01	0.38 ± 0.01	0.55 ± 0.07	0.67 ± 0.05	0.73 ± 0.07	0.79 ± 0.08	
	D	0.34 ± 0.05	1.41 ± 0.06	1.64 ± 0.05	1.82 ± 0.08	2.33 ± 0.03	2.61 ± 0.06	
	C	0.36 ± 0.19	0.77 ± 0.16	0.89 ± 0.24	1.54 ± 0.27	1.71 ± 0.16	2.05 ± 0.05	
	M	0.61 ± 0.02	0.85 ± 0.10	1.85 ± 0.06	1.89 ± 0.06	1.96 ± 0.06	2.38 ± 0.06	
L. acidophilus FTDC 2333	control	0.02 ± 0.01	0.17 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.39 ± 0.01	0.52 ± 0.01	
	D	0.97 ± 0.10	1.33 ± 0.08	1.82 ± 0.08	2.06 ± 0.21	2.30 ± 0.16	2.56 ± 0.15	
	C	0.26 ± 0.12	0.59 ± 0.06	1.17 ± 0.04	1.36 ± 0.01	1.65 ± 0.03	1.90 ± 0.11	
	M	0.16 ± 0.12	1.00 ± 0.01	1.49 ± 0.02	1.65 ± 0.05	1.99 ± 0.01	2.39 ± 0.03	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

Table 9. Reduction of Raffinose at 37 °C for 168 h

		raffinose[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P^c
L. acidophilus FTDC 1331	control	0.05 ± 0.01	0.11 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.28 ± 0.01	T: 0.296
	D	0.22 ± 0.04	0.51 ± 0.05	0.67 ± 0.09	1.01 ± 0.05	1.14 ± 0.07	1.17 ± 0.04	T × S: 0.432
	C	0.88 ± 0.20	1.00 ± 0.34	1.11 ± 0.44	1.17 ± 0.41	1.23 ± 0.38	1.30 ± 0.39	T × m: 0.420
	M	0.11 ± 0.05	0.16 ± 0.08	0.23 ± 0.10	0.30 ± 0.08	0.33 ± 0.08	0.35 ± 0.05	T × S × m: 0.482
								S: 0.429
L. bulgaricus FTCC 0411	control	0.04 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.18 ± 0.01	0.27 ± 0.01	m: 0.480
	D	0.39 ± 0.05	0.55 ± 0.06	0.74 ± 0.03	0.85 ± 0.08	1.00 ± 0.03	1.12 ± 0.01	S × m: 0.484
	C	1.11 ± 0.01	1.42 ± 0.19	1.49 ± 0.23	1.56 ± 0.20	1.63 ± 0.15	1.67 ± 0.15	
	M	0.14 ± 0.05	0.20 ± 0.01	0.25 ± 0.06	0.28 ± 0.08	0.29 ± 0.08	0.31 ± 0.09	
L. acidophilus FTDC 1733	control	0.05 ± 0.01	0.09 ± 0.02	0.15 ± 0.03	0.20 ± 0.02	0.23 ± 0.03	0.26 ± 0.03	
	D	0.67 ± 0.01	0.74 ± 0.09	0.78 ± 0.12	0.92 ± 0.14	0.95 ± 0.16	1.00 ± 0.18	
	C	0.14 ± 0.01	1.46 ± 0.22	1.60 ± 0.22	1.66 ± 0.18	1.69 ± 0.16	1.74 ± 0.16	
	M	0.07 ± 0.02	0.08 ± 0.01	0.12 ± 0.01	0.26 ± 0.11	0.30 ± 0.06	0.33 ± 0.05	
L. acidophilus FTDC 2631	control	0.03 ± 0.01	0.12 ± 0.01	0.20 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	
	D	0.68 ± 0.16	0.72 ± 0.16	0.83 ± 0.27	0.93 ± 0.26	1.00 ± 0.24	1.11 ± 0.24	
	C	0.87 ± 0.38	1.05 ± 0.43	1.13 ± 0.42	1.23 ± 0.42	1.28 ± 0.44	1.40 ± 0.40	
	M	0.10 ± 0.01	0.16 ± 0.06	0.41 ± 0.17	0.48 ± 0.22	0.50 ± 0.23	0.54 ± 0.27	
L. acidophilus FTDC 2333	control	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	
	D	1.03 ± 0.06	1.05 ± 0.06	1.16 ± 0.07	1.24 ± 0.08	1.42 ± 0.03	1.55 ± 0.04	
	C	0.50 ± 0.05	0.82 ± 0.04	0.99 ± 0.20	1.11 ± 0.18	1.18 ± 0.27	1.27 ± 0.33	
	M	0.06 ± 0.03	0.13 ± 0.02	0.27 ± 0.03	0.29 ± 0.03	0.31 ± 0.02	0.37 ± 0.03	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; m, effect of media.

containing soy milk had higher concentrations of stachyose, raffinose, sucrose, glucose, and fructose than the control (data not shown). Thus, our results were presented as percent utilization instead of the actual concentrations of individual sugars. Fung et al. stated that increased utilization of oligosaccharides and reducing sugars contributed to increased growth of *L. acidophilus* in soy whey medium (*29*). Our results showed that the reduction of oligosaccharides and monosaccharides was higher in soy milk containing immobilized cells on agrowastes than the control. This was in tandem with the increased growth of probiotic in soy milk containing immobilized cells on agrowastes.

Table 10. Production of Lactic Acid in Selected Media by Probiotic Strains at 37 °C for 168 h

		lactic acid[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 2333	control	9.41 ± 0.01	9.75 ± 0.01	10.04 ± 0.01	10.28 ± 0.01	10.24 ± 0.01	10.17 ± 0.01	T: <0.0001
	D	9.89 ± 0.03	10.60 ± 0.11	11.30 ± 0.09	11.70 ± 0.08	12.14 ± 0.01	11.79 ± 0.04	T × S: <0.0001
	C	8.38 ± 0.05	9.74 ± 0.02	10.18 ± 0.04	10.84 ± 0.01	11.25 ± 0.01	10.35 ± 0.05	T × M: <0.0001
	M	8.84 ± 0.04	9.31 ± 0.04	9.75 ± 0.01	10.37 ± 0.01	11.18 ± 0.08	10.80 ± 0.05	T × S × M: <0.0001
								S: <0.0001
L. acidophilus FTDC 1331	control	10.09 ± 0.01	10.24 ± 0.01	10.47 ± 0.02	11.14 ± 0.04	10.33 ± 0.01	10.19 ± 0.01	M: <0.0001
	D	8.77 ± 0.06	10.21 ± 0.04	11.21 ± 0.04	11.78 ± 0.01	12.80 ± 0.03	12.17 ± 0.06	S × M: <0.0001
	C	8.90 ± 0.01	9.57 ± 0.01	10.78 ± 0.02	12.70 ± 0.02	14.08 ± 0.01	13.80 ± 0.03	
	M	8.31 ± 0.01	10.12 ± 0.01	10.55 ± 0.04	12.84 ± 0.03	14.58 ± 0.01	13.72 ± 0.08	
L. bulgaricus FTCC 0411	control	8.59 ± 0.21	9.11 ± 0.01	9.59 ± 0.01	11.03 ± 0.01	10.83 ± 0.03	10.38 ± 0.01	
	D	7.85 ± 0.01	8.55 ± 0.04	8.96 ± 0.20	10.54 ± 1.07	14.95 ± 0.24	14.31 ± 0.02	
	C	8.10 ± 0.02	10.64 ± 0.02	12.75 ± 0.02	12.82 ± 0.01	17.62 ± 0.05	17.20 ± 0.02	
	M	8.20 ± 0.01	10.45 ± 0.02	11.76 ± 0.01	12.72 ± 0.22	18.28 ± 0.10	15.50 ± 0.25	
L. acidophilus FTDC 2631	control	10.25 ± 0.01	10.35 ± 0.01	10.46 ± 0.01	11.64 ± 0.01	11.52 ± 0.01	10.83 ± 0.01	
	D	7.48 ± 0.09	8.55 ± 0.04	11.08 ± 0.37	12.71 ± 0.06	14.95 ± 0.24	12.97 ± 0.19	
	C	9.78 ± 0.01	9.88 ± 0.05	10.64 ± 0.02	11.31 ± 0.04	12.79 ± 0.01	12.47 ± 0.05	
	M	8.70 ± 0.01	10.72 ± 0.09	11.29 ± 0.09	12.72 ± 0.09	13.29 ± 0.11	11.82 ± 0.01	
L. acidophilus FTDC 1733	control	7.37 ± 0.06	8.20 ± 0.06	8.93 ± 0.09	10.69 ± 0.17	10.42 ± 0.01	9.86 ± 0.03	
	D	10.15 ± 0.04	10.20 ± 0.01	11.27 ± 0.04	12.29 ± 0.03	12.80 ± 0.03	12.60 ± 0.04	
	C	8.38 ± 0.05	9.74 ± 0.02	10.18 ± 0.04	10.84 ± 0.01	11.25 ± 0.01	10.35 ± 0.05	
	M	8.84 ± 0.04	9.31 ± 0.04	9.75 ± 0.01	10.37 ± 0.01	11.18 ± 0.08	10.80 ± 0.05	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; M, effect of media.

Table 11. Production of Acetic Acid in Selected Media by Probiotic Strains at 37 °C for 168 h

		acetic acid[a] (mg/mL)						
strain	medium[b]	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 2333	control	5.34 ± 0.17	5.53 ± 0.21	5.81 ± 0.22	6.60 ± 0.04	6.28 ± 0.06	5.78 ± 0.04	T: <0.0001
	D	3.10 ± 0.01	3.61 ± 0.02	4.79 ± 0.04	5.47 ± 0.08	6.18 ± 0.06	5.59 ± 0.02	T × S: <0.0001
	C	3.87 ± 0.08	4.31 ± 0.05	5.40 ± 0.05	6.77 ± 0.05	7.57 ± 0.06	6.29 ± 0.03	T × M: <0.0001
	M	3.52 ± 0.09	3.71 ± 0.08	4.58 ± 0.08	6.41 ± 0.04	7.51 ± 0.08	7.15 ± 0.18	T × S × M: 0.001
								S: <0.0001
L. acidophilus FTDC 1331	control	4.37 ± 0.13	4.46 ± 0.01	4.87 ± 0.01	5.67 ± 0.06	5.47 ± 0.06	5.16 ± 0.06	M: <0.0001
	D	2.41 ± 0.06	3.07 ± 0.07	3.60 ± 0.03	4.31 ± 0.04	5.40 ± 0.07	5.22 ± 0.04	S × M: <0.0001
	C	2.01 ± 0.02	2.89 ± 0.03	3.90 ± 0.11	4.67 ± 0.06	6.17 ± 0.06	5.87 ± 0.08	
	M	2.07 ± 0.01	3.17 ± 0.08	3.80 ± 0.11	4.80 ± 0.11	6.94 ± 0.11	5.69 ± 0.10	
L. bulgaricus FTCC 0411	control	4.93 ± 0.14	5.39 ± 0.05	5.60 ± 0.04	6.80 ± 0.02	6.44 ± 0.02	6.05 ± 0.04	
	D	2.21 ± 0.08	2.93 ± 0.15	3.93 ± 0.13	4.78 ± 0.06	5.83 ± 0.16	5.49 ± 0.04	
	C	2.48 ± 0.06	3.59 ± 0.06	4.82 ± 0.08	5.67 ± 0.07	6.48 ± 0.06	6.40 ± 0.06	
	M	2.49 ± 0.09	3.66 ± 0.13	4.32 ± 0.06	5.55 ± 0.11	6.65 ± 0.09	5.95 ± 0.18	
L. acidophilus FTDC 2631	control	4.54 ± 0.01	4.68 ± 0.01	4.84 ± 0.02	5.73 ± 0.01	5.36 ± 0.12	5.15 ± 0.04	
	D	2.21 ± 0.08	2.93 ± 0.15	4.78 ± 0.06	5.49 ± 0.04	5.83 ± 0.16	5.59 ± 0.08	
	C	3.11 ± 0.02	3.61 ± 0.04	4.60 ± 0.05	5.29 ± 0.04	5.87 ± 0.06	5.60 ± 0.03	
	M	2.11 ± 0.07	3.38 ± 0.15	3.95 ± 0.10	5.47 ± 0.13	5.95 ± 0.15	4.45 ± 0.18	
L. acidophilus FTDC 1733	control	4.09 ± 0.04	4.73 ± 0.08	5.16 ± 0.13	6.18 ± 0.01	5.84 ± 0.04	5.50 ± 0.06	
	D	3.28 ± 0.04	3.69 ± 0.09	4.27 ± 0.07	5.18 ± 0.06	6.07 ± 0.07	5.79 ± 0.04	
	C	3.60 ± 0.10	4.10 ± 0.03	4.87 ± 0.06	5.70 ± 0.04	6.58 ± 0.06	5.49 ± 0.04	
	M	3.19 ± 0.14	3.95 ± 0.10	4.41 ± 0.08	4.69 ± 0.09	6.18 ± 0.13	5.71 ± 0.04	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; M, effect of media.

Probiotics also utilize oligosaccharides and produce byproducts such as lactic and acetic acids. Lactobacilli produce the majority of the fermentation metabolites as lactic acid, with a small proportion as acetic acid. Our results showed that the production of lactic and acetic acids was higher in soy milk containing immobilized cells on agrowastes than the control. The stability of cell immobilization is often confirmed by the consistency in the production of lactic acid. *L. casei* was previously found to consistently produce lactic acid in 15 successive fermentation batches during 50 days of fermentation using solid state biocatalysts, and this was accompanied by a consistent viability (*30*). We postulate that the immobilization of probiotics onto

Table 12. pH Value of Probiotic Strains in Selected Media at 37 °C for 168 h

		pH[a]							
strain	medium[b]	0 h	12 h	18 h	24 h	48 h	72 h	168 h	statistical significance of effect: P[c]
L. acidophilus FTDC 2333	control	6.31 ± 0.66	5.73 ± 0.16	5.03 ± 0.30	4.74 ± 0.19	4.59 ± 0.06	4.50 ± 0.03	4.39 ± 0.16	T: <0.0001
	D	5.40 ± 0.63	4.51 ± 0.06	4.49 ± 0.07	4.47 ± 0.08	4.44 ± 0.08	4.41 ± 0.08	4.38 ± 0.08	T × S: 1
	C	5.67 ± 0.18	5.10 ± 0.34	4.91 ± 0.37	4.73 ± 0.35	4.56 ± 0.25	4.48 ± 0.24	4.34 ± 0.30	T × M: <0.0001
	M	5.59 ± 0.03	4.72 ± 0.05	4.60 ± 0.04	4.53 ± 0.07	4.43 ± 0.17	4.39 ± 0.16	4.34 ± 0.18	T × S × M: 1
									S: 0.994
L. acidophilus FTDC 1331	control	6.31 ± 0.81	5.56 ± 0.28	4.67 ± 0.16	4.40 ± 0.28	4.36 ± 0.25	4.35 ± 0.25	4.34 ± 0.26	M: 0.057
	D	5.53 ± 0.94	5.15 ± 0.83	5.05 ± 0.75	4.89 ± 0.54	4.55 ± 0.08	4.52 ± 0.09	4.48 ± 0.07	S × M: 0.926
	C	5.61 ± 0.28	4.96 ± 0.70	4.83 ± 0.63	4.50 ± 0.21	4.44 ± 0.15	4.37 ± 0.15	4.27 ± 0.13	
	M	5.60 ± 0.16	4.67 ± 0.16	4.61 ± 0.09	4.50 ± 0.03	4.48 ± 0.01	4.45 ± 0.01	4.42 ± 0.02	
L. bulgaricus FTCC 0411	control	6.28 ± 0.52	5.34 ± 0.18	4.95 ± 0.33	4.60 ± 0.08	4.45 ± 0.11	4.43 ± 0.13	4.39 ± 0.16	
	D	5.32 ± 0.65	4.71 ± 0.10	4.59 ± 0.02	4.57 ± 0.04	4.52 ± 0.02	4.47 ± 0.03	4.32 ± 0.06	
	C	5.64 ± 0.29	5.05 ± 0.31	4.79 ± 0.11	4.69 ± 0.08	4.60 ± 0.11	4.41 ± 0.10	4.27 ± 0.15	
	M	5.51 ± 0.09	5.00 ± 0.34	4.97 ± 0.30	4.65 ± 0.13	4.58 ± 0.04	4.56 ± 0.04	4.50 ± 0.01	
L. acidophilus FTDC 2631	control	6.22 ± 0.53	5.32 ± 0.20	4.88 ± 0.28	4.65 ± 0.13	4.58 ± 0.09	4.44 ± 0.08	4.38 ± 0.10	
	D	5.27 ± 0.71	4.71 ± 0.10	4.62 ± 0.07	4.48 ± 0.09	4.43 ± 0.06	4.37 ± 0.01	4.29 ± 0.03	
	C	5.49 ± 0.39	4.86 ± 0.42	4.60 ± 0.25	4.51 ± 0.19	4.39 ± 0.19	4.32 ± 0.16	4.22 ± 0.13	
	M	5.33 ± 0.50	5.21 ± 0.37	4.92 ± 0.47	4.77 ± 0.30	4.63 ± 0.17	4.54 ± 0.13	4.46 ± 0.06	
L. acidophilus FTDC 1733	control	6.31 ± 0.64	5.27 ± 0.08	4.92 ± 0.22	4.69 ± 0.22	4.59 ± 0.09	4.50 ± 0.04	4.44 ± 0.01	
	D	5.25 ± 0.66	4.71 ± 0.18	4.56 ± 0.16	4.48 ± 0.14	4.39 ± 0.11	4.30 ± 0.06	4.28 ± 0.06	
	C	5.49 ± 0.42	4.89 ± 0.64	4.79 ± 0.57	4.60 ± 0.31	4.56 ± 0.26	4.50 ± 0.20	4.36 ± 0.27	
	M	5.37 ± 0.29	4.93 ± 0.37	4.79 ± 0.35	4.67 ± 0.25	4.58 ± 0.15	4.50 ± 0.07	4.45 ± 0.01	

[a] Results are expressed as means ± standard deviation; values are means of duplicates from two separate runs; $n = 2$. [b] D, soy milk with 2% (w/v) durian rind powder; C, soy milk with 2% (w/v) cempedak rind powder; M, soy milk with 2% (w/v) mangosteen rind powder. [c] T, effect of fermentation time; S, effect of strain; M, effect of media.

agrowastes also increased the stability of growth, which subsequently led to an increased production of lactic and acetic acids.

Despite a reduced pH, the viability of immobilized probiotic cells was maintained over 168 h. We postulate that cell immobilization on agrowastes contributed to increased stability in acidic conditions. Haruhito et al. showed that *L. plantarum* immobilized onto cereal fiber was more stable in the presence of gastric juice than those cultured in the conventional MRS medium (*28*). The author suggested that higher concentrations of sugars in the malt extract coupled with the fact that cells were immobilized within the cereal fiber had synergistically contributed to the stability of *L. plantarum* under acidic conditions. We have also noted that the decrease in pH of the control was higher over 168 h compared to soy milk containing agrowastes. The pH was decreased by approximately 2 units in the control, whereas agrowaste−soy milk showed a decrease of approximately 1 unit. Fung et al. had previously suggested that free amino acids in the fermentation medium could extrude protons from the cells as part of the pH homeostasis mechanism, thus minimizing pH fluctuation in the fermentation medium and leading to enhanced bacterial survival (*29*). We believe that the thermal processing of agrowastes liberated free amino nitrogen, leading to a lower reduction of pH in soy milk containing agrowastes than the control.

In conclusion, agrowastes obtained from the rind of fruits could be a good support for the immobilization of probiotic cells. Probiotics immobilized on agrowastes maintained a viable count exceeding 10^7 CFU/mL in soy milk. Growth properties were also improved, indicating that agrowastes could be used in the production of functional foods and, at the same time, reduce the amount of agrowastes generated and reduce environmental and economical liabilities. Our present findings could also benefit the agricultural industries for sustainable approaches in waste management.

LITERATURE CITED

(1) Gomes, A. M. P.; Xavier, M. F. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technology and therapeutical properties relevant for use as probiotics. *Trends Food Sci. Technol.* **1999**, *10*, 139–157.

(2) Arvanitoyannis, I. S.; Tserkezou, P. Wheat, barley and oat waste: a comparative and critical presentation of methods and current and potential uses of treated waste. *Int. J. Food Sci. Technol.* **2007**, *43*, 694–725.

(3) Liong, M. T.; Rosma, A.; Azhar, M. E.; Afiza T. S.; Wan Nadiah, W. A. Vitamin-B contents of yeast extracts from yeasts grown on agrowastes. *Proceedings of the International Conference on Environmental Research and Technology*; Universiti Sains Malaysia: Penang, Malaysia, 2008; pp 197−201.

(4) Panthapulakkal, S.; Sain, M. Agro-residue reinforced high-density polyethylene composites: fiber characterization and analysis of composite properties. *Composites: Part A* **2007**, *38*, 1445–1454.

(5) Sabaratnam, V. *Management of agricultural waste*, **2009**; available at http://www.cebar.um.edu.my.

(6) Liong, M. T.; Shah, N. P. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.* **2005**, *88*, 55–66.

(7) Berada, N.; Lemeland, J. F.; Laroch, G.; Thouvenot, P.; Piala, M. Bifidobacterium from fermented milks: survival during gastric transit. *J. Dairy Sci.* **1991**, *74*, 409–413.

(8) Lee, Y. K.; Salminen, S. The coming age of probiotics. *Trends Food Sci. Technol.* **1995**, *6*, 241–245.

(9) Kim, S. J.; Seung, Y. C.; Sae, H. K.; Song, O. J.; Shin, S.II.; Cha, D. S.; Park, H. J. Effect of micro encapsulation on 439 viability and other characteristics in *Lactobacillus acidophilus* ATCC 440 43121. *LWT−Food Sci. Technol.* **2008**, *41*, 493–500.

(10) Mokarram, R. R.; Mortazavi, S. A.; Najafi, M. B. H.; Shahidi, F.; Khomeiri, M. The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulategastric and intestinal juice. *Food Res. Int.* **2009**, doi: 10.1016/j.foodres.2009.04.023.

(11) Shahidi, F.; Han, X. Q. Encapsulation of food ingredients. *Crit. Rev. Food Sci. Nutr.* **1993**, *33*, 201–247.

(12) Plessas, S.; Bekatorou, A.; Kanellaki, M.; Psarianos, C.; Koutinas, A. Cells immobilized in a starch−gluten−milk matrix usable for food production. *Food Chem.* **2005**, *89*, 175–179.

(13) Walsh, P. K.; Malore, O. M. Cell growth patterns in immobilization matrices. *Biotechnol. Adv.* **1995**, *13* (1), 13–43.

(14) Lacroix, C.; Grattepanche, F.; Doleyres, Y.; Bergmaier, D. Immobilized cell technology for the dairy industry. In *Cell Immobilization Biotechnology, Focus in Biotechnology*; Nevidovic, V., Willaert, R., Eds.; Springer: Heidelberg, Germany, 2009, in press.

(15) Tuli, A.; Sethi, P. K.; Khanna, P. R.; Marwaha, S. S. Lactic acid production from whey permeate by immobilized *Lactobacillus casei*. *Enzyme Microb. Technol.* **1985**, *7*, 164–168.

(16) Kosseva, M.; Beschkov, V.; Kennedy, J. F.; Lloyd, L. L. Malolactic fermentation in Chardonnay wine by immobilized *Lactobacillus casei* cells. *Process Biochem.* **1998**, *33*, 793–797.

(17) Yoo, I. K.; Seong, G. H.; Chang, H. N.; Park, J. K. Encapsulation of *Lactobacillus casei cells in* liquid-core alginate capsules for lactic acid production. *Enzyme Microb. Technol.* **1996**, *19*, 428–433.

(18) Ng, K. H.; Lye, H. S.; Easa, A. M.; Liong, M. T. Growth characteristics and bioactivity of probiotics in tofu-based medium during storage. *Ann. Microbiol.* **2008**, *58*, 477–487.

(19) Ewe, J. A.; Wan-Nadiah, W. A.; Liong, M. T. Viability and growth characteristics of *Lactobacillus* in soy milk supplemented with B-vitamins. *Int. J. Food Sci. Nutr.* **2009**, in press.

(20) Bradford, M. M. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(21) Hong, S. I.; Kim, Y. J.; Pyun, Y. R. Acid tolerance of *Lactobacillus plantarum* from kimchi. *Lebensm.−Wiss. −Technol.* **1999**, *32*, 142–148.

(22) Grill, J. P.; Perrin, S.; Schneider, F. Bile salt toxicity to some bifidobacteria strains: role of conjugated bile salt hydrolase and pH. *Can. J. Biochem.* **2000**, *46*, 878–884.

(23) Liu, C. F.; Hu, C. L.; Chiang, S. S.; Tseng, K. C.; Yu, R. C.; Pan, T. M. Beneficial preventive effects of gastric mucosal lesion for soy-skim milk fermented by lactic acid bacteria. *J. Agric. Food Chem.* **2009**, *57*, 4433–4438.

(24) Pyo, Y. H.; Song, S. M. Physicochemical and sensory characteristics of a medicinal soy yogurt containing health-benefit ingredients. *J. Agric. Food Chem.* **2009**, *57*, 170–175.

(25) Ademark, P.; Larsson, M.; Tjerneld, F.; Stålbrand, H. Multiple α-galactosidase from *Aspergillus niger*, purification, characterization and substrate specificities. *Enzyme Microb. Technol.* **2001**, *29*, 441–448.

(26) Charteris, W. P.; Kelly, P. M.; Morelli, L.; Collins, J. K. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacteria* species in the upper human gastrointestinal tract. *J. Appl. Microbiol.* **1998**, *84*, 759–768.

(27) Charalampopoulos, D.; Wang, R.; Pandiella, S. S.; Webb, C. Application of cereals and cereal components in functional foods: a review. *Int. J. Food Microbiol.* **2002**, *79*, 131–141.

(28) Haruhito, M.; Sriappareddy, T.; Severino, S. P.; Colin, W.; Hideki, F.; Akihiko, K. Effect of cereal extracts and cereal fiber on viability of *Lactobacillus plantarum* under gastrointestinal tract conditions. *Biochem. Eng. J.* **2006**, *28*, 73–78.

(29) Fung, W. Y.; Woo, Y. P.; Liong, M. T. Optimization of growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium: a response surface methodology approach. *J. Agric. Food Chem.* **2008**, *56*, 7910–7918.

(30) Kourkoutas, Y.; Xolias, V.; Kallis, M.; Bezirtzoglou, E.; Kanellaki, M. *Lactobacillus casei* cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production. *Process Biochem.* **2005**, *40*, 411–416.

Received for review June 11, 2009. Revised manuscript received September 26, 2009. Accepted September 28, 2009. This work was supported by the RU Grant (1001/PTEKIND/811020) and USM Fellowship provided by Universiti Sains Malaysia.