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RESEARCH ARTICLE

Sustainable Low-Cost Agro-Waste media as Alternatives for the Cultivation of Lactic Acid Bacteria

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Abstract Lactic Acid Bacteria (LAB) are widely used in the food industry, particularly in fermented products like yogurt, cheese, fermented meats and vegetables. However, the reliance on commercial culture media such as MRS remains costly, and limited studies have explored integrated agro-waste-based formulations as sustainable alternatives for LAB cultivation. This study examines the possibility of using agricultural waste (corn cob waste, banana peels, and mature coconut water), as plant-based alternatives in the proliferation of LAB. Wastes medium were used to grow two LAB strains, *Lacticaseibacillus paracasei* BUM6 and *Lactobacillus casei* Shirota, and the growth were compared to that observed in commercial MRS broth and agar. The findings indicated that enrichment of corn cob media with mature coconut water promoted the growth of LAB as compared to the control MRS media. Banana peel media on the other hand had some or no bacterial growth ($p < 0.05$) probably due to some antimicrobial elements and absence of available nutrients. The formulated corn-cob based media with mature coconut water had potential in offering a feasible and cost-effective alternative. The research focuses on the utilization of agricultural waste into culture media of microbial strains that can be used in resourceful biotechnology incorporating halal requirements and the original concepts of a circular economy.

Keywords: Lactic acid bacteria, agricultural waste, corn cob, banana peel, mature coconut water.

Introduction

Lactic acid bacteria (LAB) are widely employed as starter cultures in dairy products such as milk, yoghurt, and cheese, and are also applied in the commercial processing of meats, alcoholic beverages, and vegetables. Beyond their role as starter cultures, LAB plays a central role in food fermentation, which remains one of the most established methods of food preservation [1]. Given their extensive applications, efficient cultivation of LAB requires suitable growth media that can support their nutritional demands. Microbiology relies heavily on the development of culture media as a growth medium to accommodate their nutritional needs. For example, MRS media contains nutrients that are helpful to *Lactobacillus* species by providing an ideal pH and energy sources.

Malaysia produces huge quantities of agricultural wastes in various crops industries, and it significantly contributes to the overall biomass production in the country. The country has the potential to valorize its waste with an estimated 168 million tons of biomass resources estimated which can be utilized such as palm oil residues, rice husks, coconut shells, sugarcane bagasse, urban waste, and forestry by-products [2]. Agricultural waste alone manufactures more than 103 million tons annually, showing a giant prospect of sustainable resource recovery and usage [3]. These wastes hold high nutritional and bioactive substances and are therefore potential sources to be redesigned into microbial culture media or other biotechnological usages. Corn cobs, banana peels and mature coconut water are some of the many agricultural wastes abundant in Malaysia that has demonstrated potential constituents that could be used as growth media by microbes.

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One of the wastes from agriculture industries that is abundance in Malaysia is corn cob. Corn is a staple food for billions around the world, supplying necessary calories and nutrients. It is a key source of food products such as cornmeal, tortillas, and breakfast cereal [4]. Due to its high lignocellulosic composition, corn cob represents a potential carbon-rich substrate that can be valorized for microbial cultivation. Meanwhile, bananas are a popular fruit crop in the tropical and subtropical regions. Banana consumption reaches 12 kg/person, making it the world's biggest food crop after rice, wheat, and corn [5]. Given the importance of bananas as a crop worldwide, plantations create tons of waste after each harvest season and during processing to yield banana pulp. Banana peel waste contains carbohydrate and minerals, making it a relevant candidate for evaluation as a low-cost culture medium component. On the other hand, mature coconut water, carbohydrates from mature coconuts, is different from fresh, green coconut water in several ways. As the coconut matures, the water inside gets less sweet, more earthy, and may appear cloudy, as well as discarded. Despite this, mature coconut water retains soluble sugars and micronutrients which may support microbial growth.

While many agricultural wastes are wasted or left in fields as organic matter, they offer overlooked potential for a variety of uses. So far, there's only one study found on utilising corn cob as an alternative to culture agar, which was used for the growth of *Escherichia coli* and *Staphylococcus aureus* [6]. Therefore, the present work aimed to look into the use of corn cobs, banana peel and mature coconut water as an alternative media for LAB.

Materials and Methods

Preparation of Bacteria Culture

L. casei Shirota was isolated from a probiotics drink (Yakult) while *L. paracasei* BUM6 was originally isolated from budu, a traditional Malaysian fermented food [7]. To prepare the overnight culture of *L. casei* Shirota and *L. paracasei* BUM6, the cultures were placed into a test tube containing MRS broth, respectively, and incubated for 18 to 20 h. Cultures were checked for turbidity as the sign of bacterial growth.

Preparation of Alternative Media

Fresh corn cob and banana peel waste were collected from discarded materials. For corn cobs, any residual kernels were removed and rinsed thoroughly under running water to clean any dirt or debris on the cobs. The cobs were then chopped into smaller pieces to increase the surface area for efficient nutrient extraction. Similarity, banana peels were washed under running water and cut into small pieces. This will ensure that the extracts are free from contaminants. Both wastes were dried at 45°C for 24 h to get rid of moisture before it was placed into two different pots. Approximately 500ml of water was added to cover the materials in each pot. The materials were subjected to aqueous extraction by decoction for 40 min to extract necessary nutrients such as sugars, minerals and organic compounds into the water [5].

The decoctions were filtered using a muslin cloth and were separated into two portions. The first portion was set aside for the preparation of broth media, whilst the second portion was added with agar powder to produce solid mediums (Table 1). Mature coconut water was also added in both media as additional nutrients source. The pH was adjusted as necessary by adding 0.2M NaOH until the pH is approximately 6.5 – 6.8 since most LAB grow the best in slightly acidic pH.

Table 1. Formulation for alternative broth and solid media

Media	Ingredients	Quantity
Corn cob broth media	Corn cob decoction	200 ml
	Mature coconut water	200 ml
Corn cob solid media	Corn cob decoction	200 ml
	Mature coconut water	400 ml
	Agar powder	4 g
Banana peel broth media	Banana peel decoction	200 ml
	Mature coconut water	400 ml
Banana peel solid media	Banana peel decoction	200 ml
	Mature coconut water	400 ml
	Agar powder	6 g

The prepared mixture was poured into flasks and covered with aluminum foil. All media were autoclaved for 30 min at 121°C (15 psi). This process is important to ensure that any unwanted contaminants are eliminated, making the media ready for microbial cultivation. Once sterilized, the broth was left to cool down while the solid media were transferred into plates and allowed to solidify. All media were stored in sterile environment if not used immediately.

Cultivation and Quantification of LAB Growth

The overnight-grown LAB cultures in MRS broth were adjusted to an optical density (OD) of 1.0 (10^8 CFU/mL) at wavelength 600 nm to ensure uniform cell density. Then, a loopful of each culture was inoculated into 5 mL of corn cob broth media and banana peel broth media, respectively. MRS broth was served as control. These broths were incubated at 37°C before the OD was measured again after 24 h incubation. Each corresponding uninoculated broth was used as the blank control. Therefore, the OD readings were interpreted as relative growth comparisons within each medium. The experiment was performed in triplicates and repeated thrice to ensure the reliability and reproducibility of the results and allowed for statistical analysis of the bacterial growth patterns across different media formulations.

Simultaneously, a loopful of *L. casei* Shirota and *L. paracasei* BUM6 was streaked onto the corn cob solid media and banana peel solid media using the four-quadrant method, respectively. Each culture was also streaked onto the MRS agar to serve as control. The agar plates were incubated in 37°C for 48 h to allow bacterial growth. Observations were performed after 48 h, during which the morphology of the colonies were examined and recorded.

Statistical Analysis

The results obtained were expressed as mean ± standard deviation (mean ± SD) and were analysed using SPSS (Statistical Package for the Social Sciences) version 26.0. One-Way ANOVA statistical test was carried out to measure if the growth in each medium yields a significant different compared to the control media and was indicated by a probability value of less than 0.05 ($p < 0.05$).

Results and Discussion

Comparative Analysis of Alternative and Commercial Media

Culture media serve as the fundamental platform for microbial growth, supplying carbon, nitrogen, and other micronutrients that are essential for cellular metabolism and biomass production. The reliance on commercially available media, however, poses significant economic challenges, particularly for large-scale applications in food, pharmaceutical, and agricultural biotechnology. In response, increasing attention has been given to the use of agro-industrial by-products, which are not only inexpensive and readily available but also rich in fermentable substrates such as cellulose, hemicellulose, proteins, and simple sugars [8 – 10]. The utilization of these agricultural wastes as alternative culture media therefore provides a dual advantage, reducing production costs while simultaneously addressing environmental concerns related to waste accumulation.

All media (Figure 1) were inspected visually, and the observations are stated in Table 2. The MRS media showed clear and transparent broth as the commercial media composition is refined and standardized, with no huge undissolved particles after it is added in distilled water. On the other hand, both formulated media, corn cob and banana peel-showed slight turbidity due to their origin as natural agricultural waste products, which contain natural fibrous and particulate components. The banana peel medium was opaquer compared to the corn cob and MRS media since it contains more solid residues that were evidently visible such as starch, fiber and other insoluble products and these did not dissolve completely in preparation. These left particles add to appearances of cloudiness and lower the transparency of the medium.

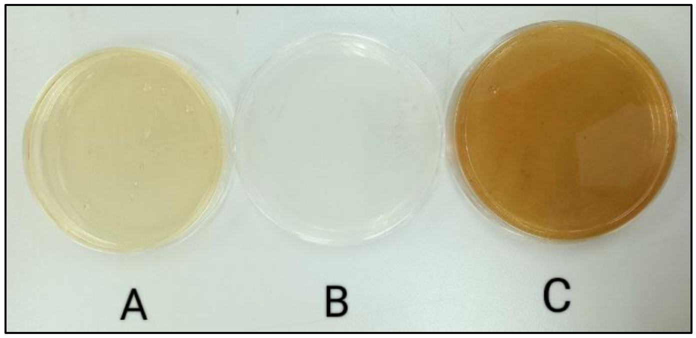


Figure 1. Visual comparison of MRS (A), corn cob (B) and banana peel (C) solid media.

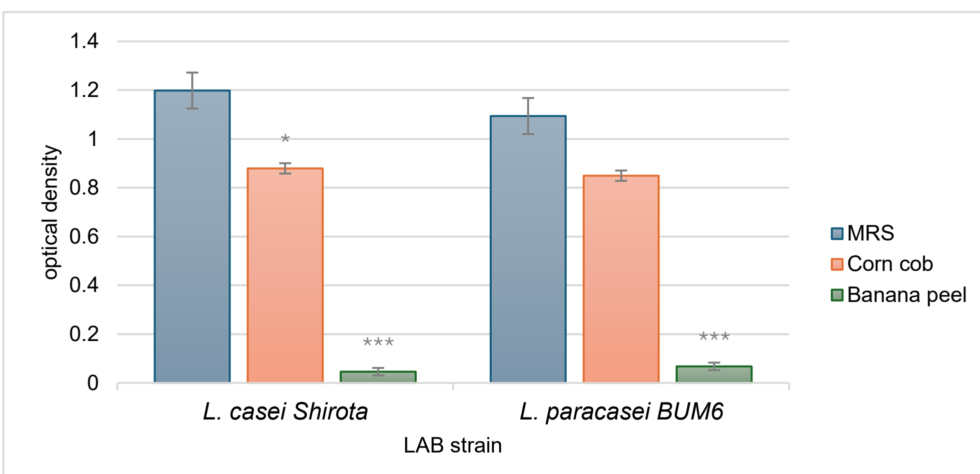
Table 2. Comparative analysis of commercialized MRS media, corn cob media and banana peel media

Characteristics	MRS broth/solid media	Corn cob broth/solid media	Banana peel broth/solid media
Mass used/100ml distilled water (g)	Agar: 6.2 Broth: 5.2	30.0	18.0
Colour	Pale yellow	Milky white	Dark brown
Turbidity	Clear	Slightly turbid	Slightly turbid
Presence of particle	No	No	Yes

Corn cobs are a well-known agricultural waste that is rich in both cellulose and hemicellulose, and this can be hydrolysed to release simple sugars such as glucose and xylose which are a major source nutrition for microbial growth [11]. Mature coconut water also contains minerals, amino acids and vitamins that occur naturally thus enriching the presence of nutrients in the media. This qualifies the mature coconut water as a supplement to increase the availability of nutrients in the media. In combination, corn cob and mature coconut water create a balanced environment that enhance microbial metabolic activities and proliferation. Banana peels are easily accessible and are found to contain carbohydrates, potassium and polysaccharides, which theoretically favoured by microorganisms [12].

Evaluation of LAB Growth on Alternative Media

Each alternative media was evaluated based on LAB growth to assess their response to different formulations. Figure 2 illustrates the bacterial growth in both MRS broth and the alternative media after 24 h of incubation.



* The p-value is significant at <0.05 compared to control media
*** The p-value is significant at <0.001 compared to control media

Figure 2. Growth comparison of LAB strains in different broth media based on optical density

The growth of LAB for MRS broth is the highest compared to the others across all the trials. It is expected from MRS broth, as the control it is specialized and rich in nutrients, specifically formulated for cultivation of Lactobacilli. The growth of *L. casei* Shirota in corn cob broth is significantly lower than in MRS broth ($p<0.05$) while the growth difference seen for *L. paracasei* BUM6 was not significant. The corn cob broth proved that it could support microbial growth consistently, suggesting that corn cob waste can be utilised as potential alternative culture medium. Meanwhile, banana peel broth showed significantly low growth of LAB ($p < 0.001$) compared to MRS broth and corn cob broth. OD600 was used to estimate bacterial biomass. Although it does not differentiate viable cells and may be affected by media pigmentation, the respective uninoculated broth served as the blank control. Thus, results were interpreted as relative growth comparisons within each medium. Despite this limitation, the consistent trend observed across triplicates suggests that OD600 was sufficient to indicate relative growth differences under the experimental conditions.

The observed higher performance of MRS broth is in line with existing literature since the MRS medium has optimized nutrients uniquely placed to promote bacterial fermentation processes [13]. The similarity between the corn cob and MRS performance of the *L. paracasei* BUM6 analysis indicates that there may be strain differences in their nutritional use efficiency or adaptation. These results serve as evidence of the projections of agricultural waste media in their ability to display comparative bacterial growth performance with commercial media in optimized conditions [14]. This positive outcome for corn cob media can be attributed to the cellulose present in corn cob, which provides a valuable carbon source for microbial fermentation [5]. These findings are supported by the recent research stating that medium composition is one of the most influential factors having effects on *Lactobacillus* growth, where the choice of carbon sources becomes especially important [15]. Thus, the minimal microbial growth might suggest that there is lack in sufficient nutrients for growth or presence of inhibitory compounds that limit the proliferation of microorganism. The failure of banana peel media to serve as an alternative growth media may be attributed to its high amount of pectin and low concentration of available sugars, which limit bacterial growth and metabolic potential [16].

Colony Morphological Comparison

Figure 3 illustrates the colony morphology of *L. casei* Shirota and *L. paracasei* BUM6 on both MRS agar and corn cob agar. The overall growth comparison of both LAB is concluded in Table 3 and Table 4.

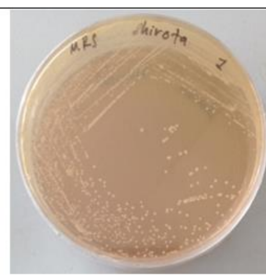
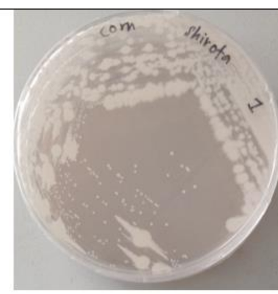
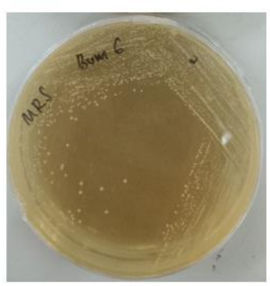
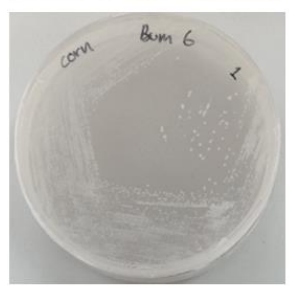
Strain	MRS	Corn cob
Shirota		
BUM6		

Figure 3. Morphological observation of *L. casei* Shirota and *L. paracasei* BUM6 on MRS agar and corn cob solid media

Table 3. Colony morphology comparison of *L. casei* Shirota grown on different solid media

Colony morphology	MRS agar	Corn cob agar	Banana peel agar
Texture	Smooth and moist	Smooth and moist	None
Margin	Entire	Entire to slightly undulate	None
Opacity	Opaque	Opaque	None
Colour	Milky white	Milky white to slightly creamy	None
Colony size	Small, 1mm to 2mm in diameter	Small with few larger colonies, 1mm to 4mm in diameter	None
Consistency	Buttery	Buttery	None
Form	Circular	Circular, some slightly irregular	None
Elevation	Low convex	Low convex	None

The colony morphological characteristics of the *L. casei* Shirota varies slightly when cultivated in different culture media, with MRS agar and corn cob agar favouring bacterial growth and banana peel agar limiting growth across all parameters examined. The results on MRS agar align well with established characteristics of *L. casei* Shirota. The moist and milky white colour of the strain identified in this study is associated with the characteristics of the strain. The tiny range of colony size and the low convex elevation are typical characteristics that promote the identification of the strain as *L. casei* Shirota. Colony features on corn cob agar were strikingly similar to those on MRS agar with just clear differences in margin features (entire to slightly undulate) and a range in colony size. The consistency of the buttery texture and round shape in the two media demonstrates a consistent morphological expression of *L. casei* Shirota regardless of the culture medium. The fact that no growths were observed on the banana peel agar signifies that the banana peel agar does not provide basic nutrients that are needed by the strain.

Table 4. Colony morphology comparison of *L. paracasei* BUM6 grown on different solid media

Colony morphology	MRS agar	Corn cob agar	Banana peel agar
Texture	Smooth and moist	Smooth and moist	None
Margin	Entire	Entire to slightly undulate	None
Opacity	Opaque	Opaque	None
Colour	Milky white	Milky white to slightly creamy	None
Colony size	Small, 1mm to 2mm in diameter	Small, 1 mm to 2mm in diameter	None
Consistency	Buttery	Buttery	None
Form	Circular	Circular, some slightly irregular	None
Elevation	Low convex	Low convex	None

The morphological profiles of *L. paracasei* BUM6 are very close to the *L. casei* Shirota strain as cultivated in MRS and corn cob agar. The reproducible morphological properties between both strains seen in this study are also complimented by the comparative measure in colony morphology between *L. paracasei*, *L. casei*, and *L. rhamnosus* [17]. Both strains showed effective growth on corn cob agar. The consistency in morphology among different strains indicates that LAB has a sufficient nutritional base to grow on the corn cob agar.

The banana peel medium fails to support the growth of LAB used. It could be caused by various potential factors, including lack of readily available sources of nutrients. The main components of banana peels consist of structural polysaccharides including cellulose, hemicellulose and pectin which bacteria cannot access without first breaking them down into simple sugars. The absence of easily accessible carbon sources makes it impossible for bacteria to start metabolic processes or cell division. The combination of coconut water vitamins and minerals does not compensate for the nitrogenous compound deficiency which limits microbial growth [18]. Moreover, there is presence of antimicrobial phenolic components in banana peels, which include flavonoids, phenolic acids, tannins, and alkaloids. Banana peel extracts could contain variety of these compounds such as caffeic acid, and ferulic acid, which have antibacterial properties against certain types of bacteria by disrupting bacterial membranes and enzymes [19]. The bioactive chemicals obtained using boiling extraction techniques probably could remain in aqueous extracts, indicating that they were possibly present in the banana peel medium to inhibit microbial growth [20]. The combination of insufficient nutritional availability and nitrogen deficit, along with antimicrobial phytochemicals, gives a convincing reason for the complete absence of colony growth on agar plates.

Conclusions

From an environmental perspective, this study tackles key waste management challenges by demonstrating how agricultural residues can be changed into valuable biotechnological resources. The findings in this research indicate that the formulated corn cob-based agro-waste medium supplemented with mature coconut water demonstrates potential as a cost-effective and sustainable alternative for LAB cultivation. This highlights the feasibility of utilizing integrated agricultural waste components to develop functional microbial culture media. On the other hand, the banana peel media provided poor support for LAB growth suggesting that additional improvements are needed before it should be considered a possible low-cost option.

In the future, the pre-treatment steps of corn cob faces should be refined aiming to increase the recovering of fermentable sugars and vital nutrients. Effective pretreatment is crucial because lignocellulosic materials like corn cobs are naturally resistant to microbial breakdown due to their complex structure. This can improve the availability of sugars for microbial fermentation. It's also promising to explore mixing different agricultural wastes, which could boost the overall performance of the substrates and help tackle waste management problems at the same time. By doing this, nutritional balance of the culture medium can be enhanced to support the growth of LAB.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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