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Isolation of *L. Acidophilus* and Formation of Probiotic Coconut Water

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ABSTRACT

Due to the high mineral content, coconut water is becoming a more popular beverage and sports beverage in tropical regions. Consumers would receive a unique probiotic beverage made from probiotic fermentation of coconut water, providing both hydrations plus probiotic advantages. The focus of this research was to see how well the probiotic bacteria grew, survived, and fermented in coconut water. In coconut water, Lactobacillus acidophilus thrived. Addition of biotin and vitamin c increase the importance of the drink because biotin is good for healthy hairs and nails, while vitamin c is good for glowing skin. The fermentation results in this study show that bacterial growth increases during and after fermentation, which is good for maintaining the drink's probiotic nature. Mineral content did not change significantly. These findings imply that fermenting coconut water into a probiotic beverage is feasible, particularly for sports nutrition, as it provides both electrolytes and probiotics. In this research different types of techniques are used for the isolation of desire bacteria to make a functional beverage.

Keywords: *Lactobacillus acidophilus*, probiotics, vitamin c, biotin, fermentation.

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INTRODUCTION

Coconut water has a unique chemical makeup of carbohydrates, minerals, vitamins, enzymes, amino acids, and phytohormones that play distinct physiological roles in the human system [1]. Coconut water is used as a refreshing and hydrating beverage because of its high mineral content, which includes salt, potassium, magnesium, and calcium, which helps replenish electrolytes lost via perspiration [2]. When administered in sufficient proportions, probiotics are living bacteria that provide a health benefit to the host. Probiotic bacteria are increasingly being used in food products due to the health benefits they provide, which include assisting digestion, modifying the immune system, suppressing infections, and perhaps lowering cancer risk [3]. The goal of this study was to see how well probiotic bacteria grew, survived, and fermented in coconut water.

MATERIAL AND METHODS

Bacterial sample preparation and their cultivation

The curd (dairy product) without any preservatives obtained from local market in Vadodara Gujarat. First the 1ml curd was serially diluted up to 10^{-10} by following the serial dilution technique [6] into the test tubes then 5 perti plates of standard size (100mm x 15mm) were prepared with Man, Rogosa Sharpe (MRS) agar composition in table 1 which is used to cultivate *lactobacilli* [7]. Then the 1 ml of 10^{-8} serially diluted sample were added into the MRS agar plate with spread plate technique and incubate at 37° c for 72 hrs. Then kept in the incubator at 37° c until use.

Conformation test for lactobacillus strain

Using gram stain, biochemical assays, and an automated approach for quick identification of bacteria, the isolated colony grown on MRS agar plates was identified. Bergey's manual of determinative bacteriology was used to make the identification. MRS agar slant was used to keep the culture and it was kept at 4° C.

One loop full of bacteria was mixed in a sterile vial containing porous beads preserved in glycerol as a cryo-preservation and serving as transporters for microorganisms and stored at -20° C for long-term storage.

1. **Gram staining test**
   The isolated bacteria were examined under a compound microscope at a magnification of 100x with oil emersion using the gram staining procedure described by Collins and colleagues **[9]**.
2. **Motility test**
   The hanging-drop wet method **[10]** and Carigie's approach were used **[11]**. To check the bacteria's movement, the slide was examined using a light microscope at 40x magnification. Carigie's technique, on the other hand, used the stabbing method to inoculate bacteria into the middle of a tube containing motility media. The medium was incubated for 48 hours at two distinct temperatures: 25 °C and 37 °C. Observing the spreading growth in the incubated semisolid agar revealed the bacteria's movement.
3. **catalase test**
   A single isolated colony was placed on a glass slide and a drop of 3 percent hydrogen peroxide was applied to it to perform this test. The development of oxygen bubbles indicated that the bacteria had responded positively or negatively to the catalase test **[12]**.
4. **carbohydrate fermentation test**
   The medium for this test was phenol red broth base medium. Various sugar substrates were employed, including arabinose, sucrose, maltose, lactose, sorbitol, and glucose. Each sugar substrate was added to 100 ml of medium at a concentration of 0.1 g (0.1 % w/v). Each tube received 5 mL of the respective mixture. The Durham tube was put into the glucose test tube for gas detection. All of the tubes were sterilized at 121°C for 15 minutes. An isolated colony of the bacteria under research was introduced into the tubes. Changes in the colour of the media indicated that the bacteria were reacting positively **[13].**

Preparation of coconut water

Fresh coconut water was obtained from green coloured mature coconut imported from Andaman and Nicobar Islands. In the early stage of coconut water total soluble solid was 6.60% and the pH was 6.76. First the coconut water 350 ml was prefiltered in the 500ml of sterile conical flask with the help of funnel and filtered with 0.45-m polyether sulfone filter membrane. After the filtration of coconut water loop full colony of *Lactobacillus acidophilus* bacteria were added in the flask and mixed well.

Fermentation of coconut water with probiotic strain

The coconut water with probiotic strain was placed in a conical flask for fermentation in a shaker incubator for 7 days under observation. The vitamin C tablet of 500 mg and biotin tablet of 10000 mcg were added in the flask in a powdered form before fermentation. The MCW (mature coconut water) was fermented for 7 days and samples were taken every 24 hours to check for properties that would allow them to be considered functional beverages, such as pH, antibacterial activity, acidity, antioxidant activity, amino acids, elements, GABA, vitamin B12, and viable LAB count. In addition, the level of vitamin C and biotin was evaluated to ensure that they would not affect the growth of microorganisms; and live LAB was counted during the first week of storage at room temperature (25^{o} C) and then every week for the next four weeks at 4^{o} C. A spectrophotometer **[14]** was utilised to determine the growth conformation by subtracting the OD (optical density).

Results

Result of gram staining test

A light microscope was used to examine the bacteria that had been isolated. The bacteria were clearly rod-shaped coccobacilli, gram-positive, that could be found single or in chains. The isolated bacteria were identified as lactobacilli based on the gram staining results**.**

Result of motility test

The isolated bacteria were found to be nonmotile using the hanging-drop wet method. Carigie's method, on the other hand, revealed that the bacteria developed only along the stab line in the media. As a result, these techniques confirmed that the bacteria in question was nonmotile. The nonmotile nature of *L. acidophilus* is a distinguishing feature.

Result of catalase test

Because of its simplicity, the catalase test is one of the most useful diagnostic procedures for identifying bacteria. No bubble was noticed during the catalase test, showing that the isolated bacterium is catalase negative and incapable of mediating the breakdown of H_2O_2 to create O_2. *Lactobacillus acidophilus* is known to be catalase negative.

Carbohydrate test

The basic goal of the carbohydrate fermentation test is to see if bacteria can ferment various types of carbohydrates. The phenol red broth base medium was utilised as an indication to distinguish the bacteria based on their carbohydrate usage characteristics. The isolated bacteria could ferment maltose, lactose, sucrose, and glucose, but not sorbitol or arabinose, as shown in **Table 2.** The glucose introduced using the Durham tube produced no bubbles, indicating that no gas production was connected with the growth. As a result, the acquired results were consistent with *L. acidophilus* strain characteristics.

Result of fermentation process

Changes in pH, cell population, total acidity in coconut water broth by *Lactobacillus acidophilus* are present in figure 4. MCW fermentation resulted in a considerable rise in cell density after 48 hours of incubation, throughout the fermentation, the bacteria multiplied at a rapid rate. After two days of fermentation, the pH dropped dramatically from 6.78 to 3.6. the bacterial growth was largely stable up to 1.41×108 CFU/ml or slightly decreased after 26 days at 4 °C, and reached significantly high viable cell counts of 5.04x107 CFU/ml on day 28. Similarly, over the 26 days of storage at 4° C, the pH stayed largely steady. After 2 days of fermentation, while ^{o}Brix level decreased slightly from 6.60 % to 6.38 %. Sugars found in coconut water were fructose, glucose, and sucrose. The overall sugar composition of the coconut water did not alter much before and after fermentation. Except for sucrose and fructose, the sugar level of the coconut water inoculated with the cultures fell dramatically after 2 days of fermentation. More glucose was eaten by *L. acidophilus*. The consumption of sucrose and fructose was higher than that of glucose. Despite this, the coconut water fermented by microorganisms contained large levels of residual sugars, allowing sweetness to be retained. According to the **[15]** probiotic bacteria *bacillus coagulans* consuming the sugar from coconut water up to 3.15% and providing less viscous product and having less sugar retain. The lactobacillus consumes less sugar up to 2.6 % they only use the glucose due to which the sweetness of the product is retains. The viable cell count, ph ^{o} Brix, and sugar concentration is given below in **(fig 1)** for better understanding.

In order to have therapeutic benefits, probiotic goods must include a significant number of active and viable probiotic bacteria (10^6–10^7 CFU/g or ml of product). **Fig 2** shows the result of fermentation, increasing in turbidity is detected by spectrophotometer through taking OD (optical density)

DISCUSSION

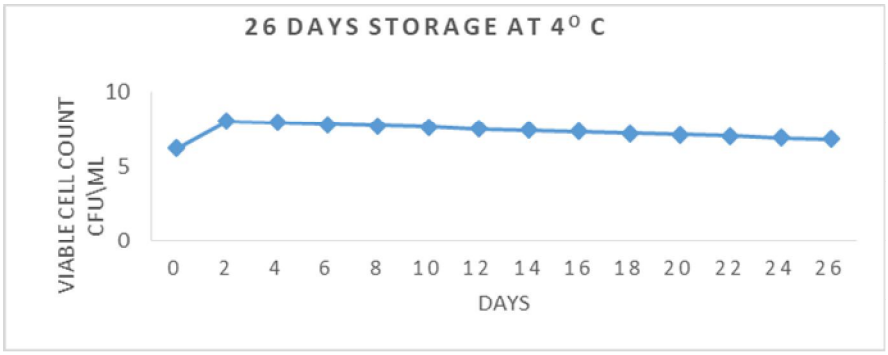
Probiotic fermentation of coconut water was investigated in this work, with a focus on the viability and fermentation performance of *Lactobacillus acidophilus*. Only a few investigations on the fermentation of coconut water have been carried out till now. The ability to produce exopolysaccharide or docosahexaenoic acid using coconut water **[16]**, as well as the growth and survival of L. bulgaricus and Streptococcus thermophilus in a coconut water-based medium, are just a few examples. Similarly, after refrigerated storage, the growth and survival of L. plantarum in a fermented oatmeal–coconut water matrix at various levels. Microbial growth in coconut water has been discovered in all of these experiments. To ensure that the coconut water was the only raw material that governed the development and metabolism of the probiotic bacteria in this investigation, this research employed it as the sole fermentation medium, without any additives, in comparison to those used in prior studies **[17].**

Table 1 Composition of MRS media [8]

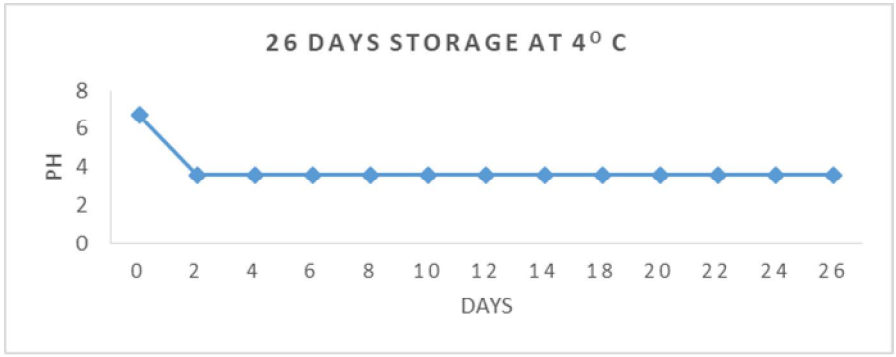
composition	Concentration (g/l)
agar	12g/l
diammonium hydrogen citrate	2g/l
Dipotassium hydrogen phosphate	2g/l
D (+)-glucose	20g/l
Magnesium sulfate	0.1g/l
Manganous sulphate	0.05g/l
Meat extract	5g/l
Sodium acetate	5g/l
Universal peptone	10g/l
Yeast extract	5g/l

Table 2: - Biochemical test results

test	observation
Glucose(gas)	No gas formation (Durham tube with no bubble)
Sorbitol	No Fermentation
Glucose (acid)	Fermentation (acid production)
Maltose	Fermentation (acid production)
Sucrose	Fermentation (acid production)
Lactose	Fermentation (acid production)
Arabinose	No fermentation



(a)



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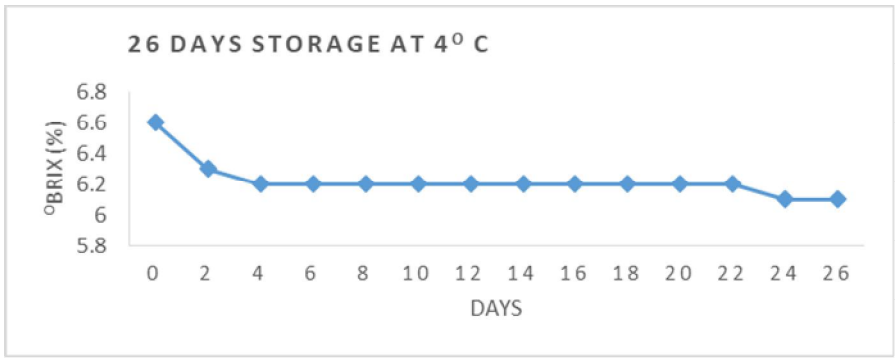
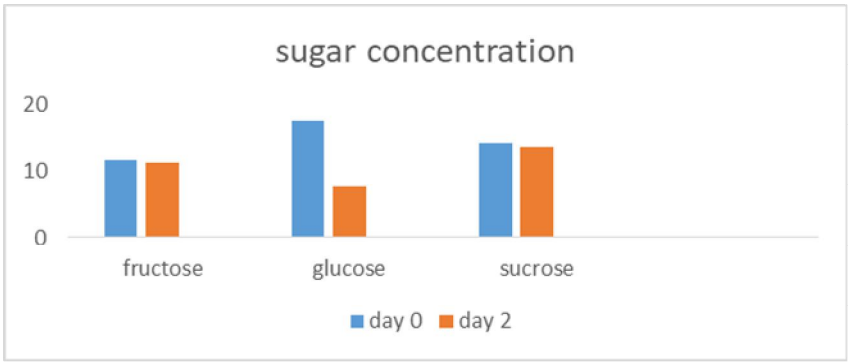


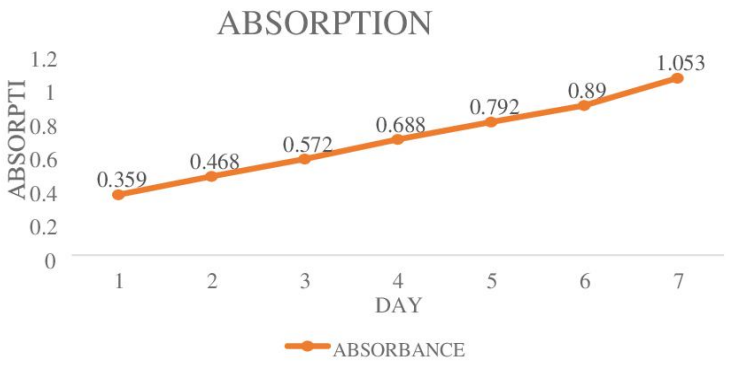
(c)



(d)

Fig 4 (a) viable cell count of coconut water containing *Lactobacillus acidophilus*
(b) ph graph of coconut water containing lactobacillus acidophillus
(c) ^{0}Brix % of dissolved sugar content in product
(d) 3 types of sugar and their concentration in the product

Fig: 2: Turbidity test of the final product



CONCLUSION

Lactobacillus acidophilus, a strain with possible probiotic properties, was found to be effective in the production of a fermented coconut water beverage. It was discovered that the fermented beverage includes vital minerals, vitamin B12, and antioxidants. After a 24-hour fermentation with *L. acidophilus*, even after 28 days of storage at 4°C, the *L. acidophilus* levels in fermented broth were consistent with the daily recommended probiotic dose. The inclusion of vitamin C and biotin had no effect on *L. acidophilus* growth, which is a positive indicator for a functional drink. Overall, the results demonstrated that the prospective probiotic strain contributed significant value to simple coconut water, resulting in a low-cost novel functional fermented beverage with a variety of health advantages.

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CONFLICT OF INTEREST

The author declare that they have no conflict of interest.

REFERENCES

1. Lee, P. R., Boo, C. X., & Liu, S. Q. (2013). Fermentation of coconut water by probiotic strains *Lactobacillus acidophilus* L10 and Lactobacillus casei L26. *Annals of microbiology*, *63*(4), 14411450.

2. Giri, S. S., Sukumaran, V., Sen, S. S., & Park, S. C. (2018). Use of a potential probiotic, Lactobacillus casei L4, in the preparation of fermented coconut water beverage. *Frontiers in Microbiology*, 1976.
3. Kantachote, D., Ratanaburee, A., Hayisama-ae, W., Sukhoom, A., & Nunkaew, T. (2017). The use of potential probiotic Lactobacillus plantarum DW12 for producing a novel functional beverage from mature coconut water. *Journal of Functional Foods*, *32*, 401-408.
4. Pachori, R. R., & Kulkarni, N. S. (2017). Studies on development of probioticated coconut water. *Online International Interdisciplinary Research Journal*, *7*(spe), 9-17.
5. Prado, F. C., Lindner, J. D. D., Inaba, J., Thomaz-Soccol, V., Brar, S. K., & Soccol, C. R. (2015). Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of functional foods*, *12*, 489-497.
6. Ben-David, A., & Davidson, C. E. (2014). Estimation method for serial dilution experiments. *Journal of microbiological methods*, *107*, 214-221.
7. Renschler, M. A., Wyatt, A., Anene, N., Robinson-Hill, R., Pickerill, E. S., Fox, N. E., ... & McKillip, J. L. (2020). Using nitrous acid-modified de Man, Rogosa, and Sharpe medium to selectively isolate and culture lactic acid bacteria from dairy foods. *Journal of dairy science*, *103*(2), 1215-1222.
8. Liew, S. L., Ariff, A. B., Raha, A. R., & Ho, Y. W. (2005). Optimization of medium composition for the production of a probiotic microorganism, Lactobacillus rhamnosus, using response surface methodology. *International Journal of Food Microbiology*, *102*(2), 137-142.
9. Grange, J., & Lyne, P. (2004). *Collins and Lyne's Microbiological Methods*. Hodder Education.
10. MacFaddin, J. F. (2000). Deoxyribonuclease (DNase) and thermonuclease (Tnase) tests. *Biochemical Tests for Identification of Medical Bacteria, 3rd ed., ed. by MacFaddin, JF, Lippincott Williams and Wilkins, Philadelphia, PA*, 136-159.
11. Barrow, G. I., & Feltham, R. K. A. (2004). Motility test. *Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edition. Cambridge University Press, UK*, 26.
12. Hattori, T., Matsumoto, H., & Murakami, Y. (1987). Standardization of catalyst test methods by the committee on reference catalyst of the catalysis society of Japan. In *Studies in Surface Science and Catalysis* (Vol. 31, pp. 815-826). Elsevier.
13. Bronpenbre, J., & Schlesinger, M. J. (1918). Carbohydrate fermentation by bacteria as influenced by the composition of the medium. *Proceedings of the Society for Experimental Biology and Medicine*, *16*(3), 44-46.
14. Mielenz, K. D., & Eckerle, K. L. (1972). Spectrophotometer linearity testing using the double-aperture method. *Applied Optics*, *11*(10), 2294-2303.
15. Gangwar, A. S., Bhardwaj, A., & Sharma, V. (2018). Fermentation of tender coconut water by probiotic bacteria Bacillus coagulans. *International Journal of Food Studies*, *7*(1).
16. Shivakumar, S., & Vijayendra, S. V. N. (2006). Production of exopolysaccharides by Agrobacterium sp. CFR-24 using coconut water–a byproduct of food industry. *Letters in applied microbiology*, *42*(5), 477-482.
17. Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M., & Verduyn, C. (2007). Coconut water as a medium additive for the production of docosahexaenoic acid (C22: 6 n3) by Schizochytrium mangrovei Sk-02. *Bioresource technology*, *98*(2), 281-287.

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