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Research Article

Production of peptide antibiotic bacteriocin using banana peel media

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Abstract
Many strains of lactic bacteria produce antimicrobial peptides of bacteriocins that are antibiotics used against pathogenic strains. The present work aimed to use a banana peels medium in the fermentation process to replace the commercial MRS medium for decreasing the cost of bacteriocins LAB production. Based on the result, banana peel was a cost-effective and viable alternative carbon source for the production and development of bacteriocin-producing *Lactobacilli*. The growth of lactobacilli in commercial MRS medium and Banana Peel medium showed no differences, therefore banana peel waste can be used to produce *Lactobacilli* bacteriocins. *Lactobacillus* strains grew exceptionally well at 37 C and pH 6.0.

Keywords: Bacteriocins, Lactic acid bacteria, MRS.

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Introduction

Bacteriocins are small cationic membrane-active compounds that induce cell death in target cells by forming pores and disrupting membrane potentials. They are proteinaceous toxins, ribosomally synthesized and extracellularly released as small peptides or bioactive proteins. Bacteria produce it to inhibit the growth of bacterial strain(s) which is similar or closely related (Beshkova & Frengova 2012; Martinez et al. 2013). Gratia was the first who discover bacteriocins in 1925 (Beshkova & Frengova 2012). Bacteriocins are peptides that already have an inhibitory action on Gram-positive and Gram-negative bacteria, but particular immune mechanisms protect the producing bacteria from their own bacteriocin (And & Hoover 2003; Cotter et al. 2012). Although bacteriocins can be produced from microorganisms by fermentation, there is still a need to reduce the cost of production by various methods (Makkar et al. 2011).

Bacteriocins can destroy the target bacteria by disrupting the integrity of the membrane. They have a lower chance of inducing resistance because their fragments do not interfere with the target cells and could be considered a possible solution to the growing problem of antibiotic resistance microbial (Cotter et al. 2012; Yang et al. 2014; Ahmad et al. 2017). Bacteriocins used in biopreservation systems can fulfill consumer demand for natural preservatives and are also considered an additional protection measure for minimally processed goods that rely solely on cooling as a preservation medium (da Costa et al. 2019). Consumers are more concerned about their daily dietary consumption. Allergies and carcinogenic effects are associated with preservatives influence, contributing to the preference for fresh foods manufactured with little or even no synthetic additives (Balciunas et al. 2013).

Commercial medium, which is called de-man rogosa and sharp (MRS) is being used for producing the bacteriocin from Lactic Acid Bacillus (LAB) species (de Mann & Sharp 1960). MRS components are high-cost and raise the total cost of LAB production. Waste materials that are locally available for

Table 1. Influence of different temperatures on the growth of *Lactobacillus* species in banana peel medium.

Temperature	*Lactobacillus sporogens* (OD 600nm)	*Lactobaccillus acidophilus* (OD 600nm)
28˚C	0.15	0.25
30˚C	0.83	0.49
37˚C	1.23	1.15
40˚C	0.74	0.84
45˚C	0.34	0.54
50˚C	0.30	0.21

Table 2. Effect of different pH on growth of *Lactobacillus* species in Banana Peel Medium.

pH	*Latobacillus sporegens* (OD at 600nm)	*Lactobacillus acidophilus* (OD at 600nm)
4.5	0.12	0.31
5.0	0.31	0.70
5.5	0.96	1.10
6.0	1.40	1.30
6.5	1.20	0.93
7.0	0.83	0.50
7.5	0.50	0.41
8.0	0.23	0.10

alternative carbon sources to reduce the production cost are used to produce bacteriocins (Fasakin et al. 2018). The study aimed to utilize banana peels medium in fermentation to replace MRS medium for lowering the cost of bacteriocins LAB production.

Materials and Methods

The peel of the banana protects the fruit, contributes at least 30% to the total weight of the banana, and contains essential quantities of phosphorous and nitrogen (Khawas & Deka 2016). It also supplies Mg and K (Hill 2020). Banana peel also contains higher phytochemical ingredients than flesh (pulp) (Sulaiman et al. 2011). The peel which has ripened has approximately 30% free sugars, 13.8% soluble sugars, up to 8% crude protein (CP), and 4.8% total phenolics (Wadhwa & Bakshi 2015). For LAB processing, commercial medium like de Man, Rogosa or Corn Steep Liquor has a high cost. For lowering production costs exploring locally available sources for *Lactobacilli* culture media from different agro-industrial wastes may be an alternative. Therefore, the banana peel could be used as a source of nutrition for *Lactobacilli* strains as a potential bacteriocin producer (Singh 2009). Materials used are *Lactobacillus* culture, nutrient agar, MRS broth, and banana peel medium. The strains of *L. sporogenes* and *L. acidophilus* were obtained. The strains were cultured in sterile de Mann Rogosa Sharpe (MRS) broth in 24 h incubation at 37°C (Song et al. 2015). The culture was maintained on MRS agar slants and stored at 4°C, and every month sub-cultured. Stock cultures were stored at 20°C in 40% glycerol (glycerol represents carriers to support microorganisms that are already cryo-preservative) (Eid et al. 2016).

Preparation of banana peel medium: A paste was made by blending 20g of the chopped ripe banana peels into small pieces in a blender by adding 100ml of distilled water. With MRS broth containing *Lactobacillus* culture, the mixture was autoclaved and inoculated (Gilliland & Speck 1977).

Preparation of inoculum: The culture was sterilely inoculated into a 250ml flask containing 100ml MRS medium. The flask was then incubated for 24 hours at 37°C.

Fermentation conditions: Fermentations were done in 250ml Erlenmeyer flasks with 100ml of banana peel medium, and incubators were used to keep the fermentation flasks in the incubator for 48 hrs.

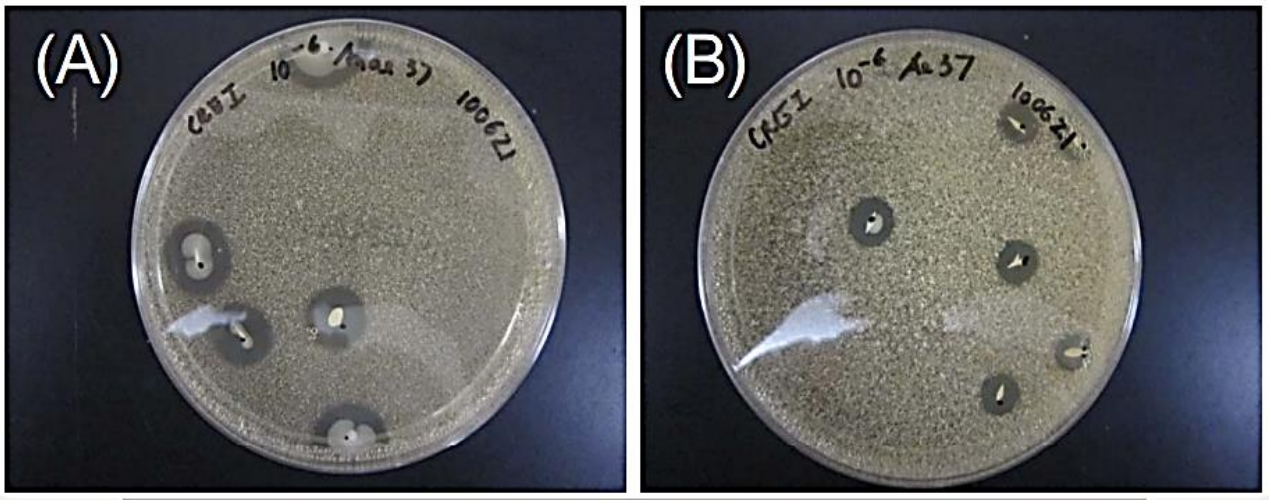

Fig.1. Inhibitory zones by bacteriocin in (A) MRS and (B) banana peel media.

Table 3. Comparison of growth of *Lactobacillus* spices at optimum temperature of 37°C and Optimum pH 6.0 between MRS and banana peel media.

Medium	*Lactobacillus sporogenes*	*Lactobacillus acidophilus*
MRS	1.11	1.26
Banana Peel	0.95*	1.09*

**P*>0.05 (Each Value is an average of 6 replicates.

The influence of initial pH on lactobacilli strains growth: Fermentation was carried out at different initial pH of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 with 0.1N HCL and 0.1M NaOH to see their influence. At 37°C, the flasks were incubated. By spectrophotometer, at 600nm wavelength, the lactobacilli strains' growth was determined.

The effect of temperature on lactobacilli strains growth: The fermentation was done at different temperatures of 30, 37, 40, 45, and 50°C and at optimum pH 6.0 for 36 h. At 6±0.5, the initial pH of the fermentation medium was kept. Using a spectrophotometer at 600nm wavelength, the *Lactobacilli* strains' growth was determined.

Results and discussion

Lactobacilli strains grow in banana peel medium and MRS medium, mostly the popular source for *Lactobacillus* sp. cultivation (de Mann & Sharp 1960); however, it is used in industrial-scale applications, but it has a high cost. Banana peel medium was used in this study to assess potentially lowering the cost of *Lactobacillus* growth media. The results showed that *Lactobacillus* utilizes banana peel medium and grows on it. Horticultural waste, such as banana peel, have the ability to be converted into usable and higher-value-added materials.

The temperature and pH effects: Temperature is an essential factor in lactobacilli formation. Most species have a distinct range of temperatures from which they will be able to grow. Hence, optimizing physical properties such as pH and temperature can increase *Lactobacillus* production. The optimum growth for various strains of *lactobacilli* in fermented product at 37°C is shown in Table 1. At pH 6.0, the various *lactobacilli* strains grew best in banana peel medium (Table 2).

The growth of all strains was not significantly different (P>0.05). The lactic acid bacteria were successful when the temperature was raised from 37 to 45°C. The overall density of the population was lowered by an order of quantity. *Lactobacillus* species is a thermophilic bacterium and this species can survive at higher temperatures up to 45°C (Adiguzel et al. 2009).

Banana peel medium proved to be a proper substitute for lactic acid bacteria cultivation. The bacteria were produced almost similar to that of the industrial MRS medium. In terms of bacterial development, there were no major differences between the MRS and banana peel media. At 37°C and pH= 6.0, both the used bacterial species developed on the banana peel medium that is a cost-effective and environmentally-friendly choice for probiotic culture.

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