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Research Article

POTENTIALS OF PINEAPPLE WASTE AS GROWTH MEDIUM FOR *LACTOBACILLUS* SPECIES

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ABSTRACT

Objective: The present study was conducted to prove that pineapple wastes can be treated through the biochemical reaction called fermentation by commercial probiotics, lactobacilli which is a friendly bacteria. In the present study, pineapple waste was used as sole carbon source for preparation of fermentation media on which three strains of probiotics, *Lactobacillus* sp. FTDC 8133, FTDC 3666 and FTDC 8264 were grown. In addition, the ability of probiotics strains to grow in pineapple waste was investigated.

Methods: Lactobacilli sp. were propagated in MRS broth for three successive times prior to carry out the fermentation in pineapple waste substrate using cheapest and simplest technique namely shake flask fermentation incubated at 37 °C for 24 h. The effects of different temperatures and initial pH on viability of probiotics were determined spectrophotometrically at 600 nm wavelength.

Results: The results showed that best growth was significantly obtained at 37°C and pH 6. Effect of the pineapple-wastes culture medium on the bacterial growth was comparable to that of MRS.

Conclusion: It can be concluded that pineapple wastes from these processing industries can be utilized to produce culture medium for cultivation of probiotics bacteria whereas MRS medium which is an expensive medium for cultivating probiotics. At the same time we can use the wastes to increase the economic value of the wastes obtained after fermentation. The present finding helps in probiotics production from cheap, inexpensive agrowaste material.

Keywords: Agro-waste, Lactobacilli, Pineapple, Probiotics.

INTRODUCTION

The world is facing problems of environmental pollution. To overcome those problems, there is an alternative process for using these wastes. There has been an increase in the utilization of agro wastes as an alternative source for cultivation of probiotics in recent years [1]. To make the price of probiotics more competitive, the yield should be improved through developing the rich nutrient culture medium with a low cost of production [2]. The economical production of such nutrient rich culture medium could be achieved by using locally available agro wastes that are abundant in nutrients that meet the microbial requirement [3]. A commercial medium such as de Man, Rogosa and Sharpe (MRS) is usually too costly for commercial production of probiotics [4]. Therefore, exploring locally available sources as culture media for probiotics from various agro-industrial wastes could be a better alternative to reduce the cost of production. A better fermentation process using inexpensive waste would be essential for commercial production [5].

Pineapple is the second most consumed and produced fruit after bananas, contributing to more than 20% of the world production of tropical fruits [6, 7]. More than 70% of pineapple is consumed as fresh fruit in producing countries [8]. Pineapple waste is a by-product of the pineapple processing industry and it consists of residual pulp, peels and skin. About 30% of the pineapples are turned into waste during the canning operation [9]. These wastes can cause environmental pollution problems if not utilized because its still contains high content of carbohydrates as well as high fiber and low protein contents [10, 11]. Based on the physico-chemical properties of the pineapple waste, it can be potentially used as carbon sources for production of lactic acid by microbial systems [12]. Utilization of *Lactobacillus* in probiotic production has been increasing remarkably [13]. Nutritional growth sources for lactobacilli could be substituted with agro-industrial wastes which are readily and inexpensively available [14]. Pineapple waste is an applicable source of not only carbon, but it is also a potential source of nutrition for probiotics, considering it could significantly promote high growth rate [15, 16]. Thus, to develop commercial scale of probiotic production, alternative nutritional sources shall be screened from our local agro-industrial wastes.

Hence, the purpose of this study is to investigate the applicability of pineapple agro-waste as a nutritional source of cultivation of lactobacilli strains, which are potential probiotics.

MATERIALS AND METHODS

Microorganism

Strains of lactobacilli coded as *Lactobacillus* sp. FTDC 8133, *Lactobacillus* sp. FTDC 3666 and *Lactobacillus* sp. FTDC 8264 were obtained from the culture collection of School of Industrial Technology, Universiti Sains Malaysia (Penang, Malaysia). The strains were propagated in sterile de Mann Rogosa Sharpe (MRS) broth supplemented with 0.15% w/v filter sterilized (0.45 µm) L-cysteine hydrochloride for three successive times with inoculums size (10% v/v) and incubated for 24 h at 37 °C prior to use. The culture was then maintained on MRS agar slants and stored at 4 °C and subcultured every month. The stock cultures were kept in 40% (v/v) glycerol (glycerol as cry-preservative and serves as carriers to support microorganisms) and stored at -20 °C.

Isolation and enumeration of lactobacilli

For the isolation of lactobacilli, 10 ml of bacterial culture was inoculated into 90 ml of MRS broth and incubated at 37 °C for 36-48 h (Memmert, Germany). The enumeration of viable cells was conducted by ten-fold serial dilution from fresh culture (10^{-1}-10^{-8} with MRS broth as diluents), and then 1.0 ml of each dilution was cultivated in MRS agar using pour plate method. The plates were incubated at 37 °C for 72 h [17].

Culture medium

The substrate used to carry out the fermentation process was pineapple waste obtained from the local market in Penang, Malaysia. The reference culture medium used in the study was De Man-Rogosa–Sharpe (MRS) medium as described by De Man and colleagues [18].

Preparation of liquid pineapple waste

The pineapple waste was boiled for 15 min at 121°C resulting in the flocculation of particulates and these settled rapidly upon cooling to room temperature. Then, the particulate was separated by centrifugation for 10 min at 5000 rpm. The clear

supernatant was filtered using Whatman filter paper under vacuum and was stored at –18 °C.

Preparation of inoculum

The culture was aseptically inoculated into a 250 ml flask which contains 100 ml MRS medium. The flask was incubated at 37 °C for 24 h.

Fermentation conditions

The submerged fermentations were carried out in 250 ml Erlenmeyer flasks containing 100 ml of pineapple waste or MRS. The fermentation flasks were maintained in an incubator.

Effect of temperature on the growth of lactobacilli strains

The effect of different temperatures on fermentation were carried out at various temperatures of 28 °C, 30 °C, 37 °C, 40 °C, 45 °C and 50 °C for 36 h. The initial pH of the fermentation medium was 6.0 ± 0.5. Growths of lactobacilli strains were determined spectrophotometrically at 600 nm wavelength.

Effect of initial pH on the growth of lactobacilli strains

The effect of initial pH was studied by conducting fermentation at various initial pH of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with 0.1 N HCl and 0.1 M NaOH. The flasks were incubated at 37 °C. Growth of lactobacilli strains was determined spectrophotometrically at 600 nm wavelength.

RESULTS AND DISCUSSION

Growth of probiotics lactobacilli strains in pineapple waste medium

A previous study by De Man and colleagues [18] indicated that MRS medium is the most common source for *Lactobacillus* sp cultivation because it provides suitable growth factors and nutrition. However, its high cost has a negative economic impact when applied in industrial-scale processes. In this study, we evaluated potentially low-cost media for *Lactobacillus* production using pineapple agro wastes. It therefore can potentially be used as a carbon source for cultivation and fermentation of probiotics. The experiments were carried out in shake flask fermentation using *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264). The result showed that *Lactobacillus* utilizes pineapple waste and grows as a growth medium. There is a potential for food processing waste such as pineapple waste to be used as raw material, or for conversion into useful and higher value-added products. The pineapple waste can also be used as food or feed after biological treatment.

Commercial probiotics production, being a fermentation end-product, will be influenced by a number of criteria. The purpose will always be the highest amount of probiotics production at the lowest cost. Although, cellular growth factors have been found to be essential for bacterial growth, however, extracellular factors such as pH and temperature have been proven to be the critical requirement for bacterial growth [19].

Effect of Temperature

Temperature is an important parameter to control growth of lactobacilli [20]. Most species have a characteristic range of optimum temperature in which they can grow, but they do not grow at the same rate over the whole temperature range. Furthermore, improvement in the production of *Lactobacillus* usually involves optimization of physical properties such as pH and temperature [21].

The optimum growth for the different strains of lactobacilli in pineapple medium was at 37 °C as shown Fig. 1. There was no significant difference ($p > 0.05$) in the growth of the different strains. On this condition, lactic acid bacteria grew well than compared to the other temperatures. The maximum population density was reduced by an order of magnitude when the temperature was increased from 37 °C to 45 °C. These findings are consistent with the results of previous research [22]. Bâati et al. [24] reported that *Lactobacillus* sp is a thermophilic bacterium, which can generally survive at higher temperature up to 45 °C. Other researchers also reported that the optimum growth of most *Lactobacillus* sp between 35 and 38 °C [19, 24].

Fig. 2 shows the comparison of *Lactobacillus sp* FTDC 8133, FTDC 3666 and FTDC 8264 growth at optimum temperature 37 °C between pineapple waste-medium and the commercial MRS medium. The result indicated that no significant difference ($P>0.05$) was found between two media.

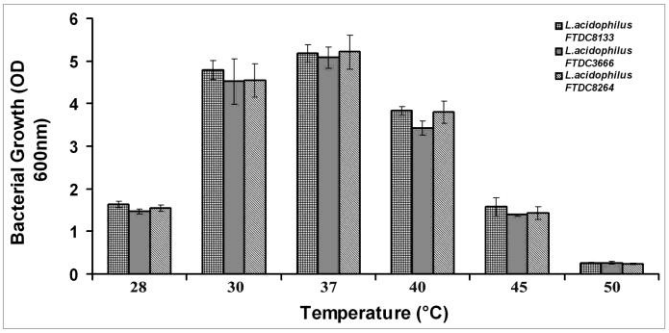

Fig. 1: Effect of different temperature on growth of *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264).

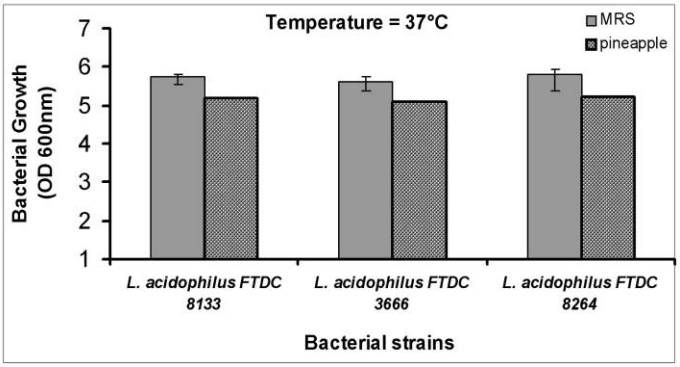

Fig. 2: Comparison of *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264) growth at the optimum temperature between pineapple waste-medium and MRS

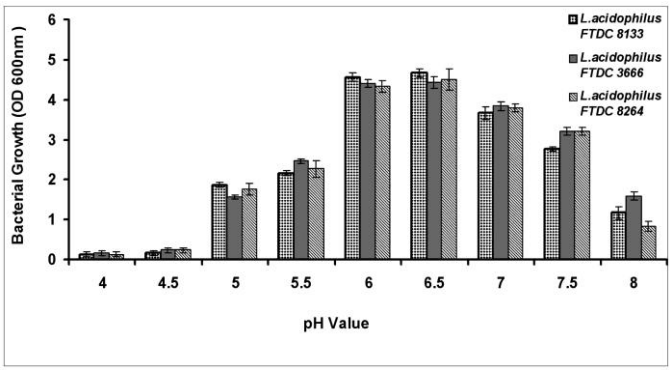

Fig. 3: Effect of different pH on growth of *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264)

Effect of Initial pH

The effect of initial pH was conducted in 250 ml Erlenmeyer flask with working volume of 100 ml at 37 °C using liquid pineapple waste. The initial pH of the fermentation medium was controlled using 0.1 N HCl and 0.2 M NaOH as pH control agent. The effect of initial pH was studied at different initial pH values (4.5, 5.5, 6.5, 7.5, and 8.0). The results of bacterial growth are shown in Fig. 3. The exponential growth rate at initial pH 6.0 was the fastest compared with the other initial pH values. In the beginning at the initial pH of 4.5 and 8.0, the bacteria exhibited a prolonged lag phase and the bacteria did not grow as well as at higher initial pH value. As the initial pH was increased above 4.5, the cell growth was increased, however, until up to a certain limit. Beyond the initial pH 6.0, its growth rate was decreased. Therefore, the optimal initial pH growth for the liquid pineapple waste fermentation using *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264) is 6.0. Goderska and colleagues [25] reported that the growth occurs at initial pH values between 5 and 7 with an optimum growth between 5.5 and 6.5. Results obtained seem to be in agreement with those obtained by Goksungur and Guvenc [26] where the optimum initial pH of 6.5 is obtained using beet molasses as a substrate.

Fig. 4 shows the comparison of bacterial growth at pH 6.0 for all *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264) between pineapple waste-medium and the commercial MRS medium. The result indicated that no significant (P>0.05) difference was observed between two media. This result supports the utilization of waste pineapple for probiotics growth is worthwhile.

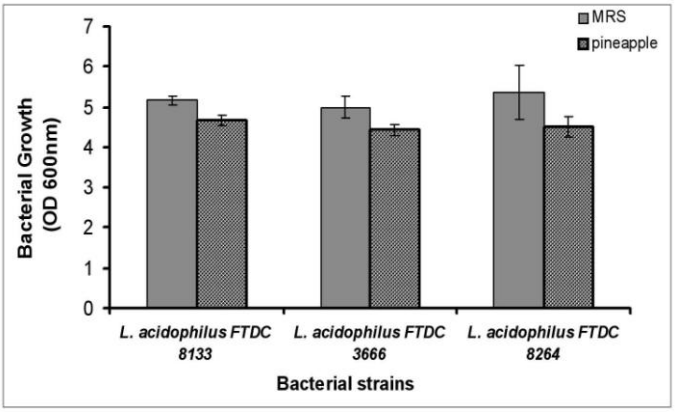

Fig. 4: Comparison of *Lactobacillus sp* (FTDC 8133, FTDC 3666, and FTDC 8264) growth at the optimum pH between pineapple waste-medium and MRS

CONCLUSION

Pineapple waste was a feasible alternative for the cultivation of lactic acid bacteria. Growth of the bacteria on pineapple waste medium was comparable to that of commercial MRS medium. The study revealed no significant differences between the pineapple waste-medium and MRS medium with respect to bacterial growth. All tested bacterial strains showed remarkable growth at 37 °C and pH 6.0. It can be concluded that using waste pineapple material for probiotic production would be optimal both economically as well as environmentally. Further study on the optimization of the pineapple waste-medium with respect to the other external factors could be carried out by comparing with the other agro-industrial wastes media.

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