INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-8, Issue-4, Oct-Dec-2017 Coden IJABFP-CAS-USA Copyrights@2017

Received: 14th July 2017 *Revised: 17th Sep- 2017* *Accepted: 19th Sep-2017*

DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt *Research article*

EVALUATION OF BANANA PEEL WASTE AS GROWTH MEDIUM FOR PROBIOTIC LACTOBACILLUS SPECIES

Nafeesa Farees[1,2], Dejene Deresh Abateneh[1], Mathewos Geneto[1], N.V.Naidu[2]

[1]Department of Biomedical Sciences, Mizan Tepi University SSNPR, Mizan, Ethiopia .
[2]Global Institute of Biotechnology, Himayath Nagar, Hyderabad, 500029, T.S. India

ABSTRACT: In the present study the ability of probiotics strains to grow in media using Banana Peel Waste as alternative carbon source was investigated. Banana Peel Waste was used as fermentation media on which two strains of probiotics, *Lactobacillus* sp. were grown. *Lactobacilli* sp. were propagated in De Mann Rogosa (MRS) broth for three successive times prior to carrying out the fermentation in Banana Peel Waste medium substrate using cheapest and simplest technique namely shake flask fermentation and incubated at 37 °C for 24 h. The effects of different temperatures and initial pH on viability of probiotics used were determined spectrophotometrically at 600 nm wavelength. The results showed that bacterial growth in Banana Peel Waste culture medium obtained at 37^{O}C and pH 6 was comparable to that of MRS medium. It can be concluded that Banana Peel Waste can be utilized to produce culture medium for cultivation of probiotic bacteria as a substitute to MRS medium which is expensive for cultivating probiotics. This will not only result in cost reduction in production of Probiotics from cheap, inexpensive agro-waste material, but also helps in solving the environmental pollution problem caused by the extensive disposal of nutrient rich Banana Peels into nature.

Key words: Agro-waste, Lactobacilli, Banana Peels, Probiotics

*Corresponding author: Prof. N.V.Naidu, Global Institute of Biotechnology, Himayath Nagar, Hyderabad, 500029, T.S. India, E-mail naidu111_2000@yahoo.com

INTRODUCTION

The term Probiotics is used to represent the group of microorganism which forms the microbiota of human intestine and work for better digestion, infection control and health improvement (Hill et al., 2014).

In recent years increasing interest in Probiotics is being shown from Public and Research Organizations, Government agencies, Pharmaceutical and Food Companies (Sanders, 2008). Attempts are been made to utilize the lignocellulosic agro and industrial waste as alternative sources of carbon for production of probiotics by microorganisms. This would help not only in providing cheap and abundant source of raw material for the production of probiotics, but also help in solving the problem of environmental pollution by the organic waste (Anwar et al., 2014). At present a commercial medium called D-Mann Rogosa is being used for production of probiotics. The components of MRS medium are expensive and increase the cost of production of probiotics (De Man and Sharp, 1960). In the present study locally available waste material like Banana Peels are used as alternative sources of carbon to reduce cost of production. Hence the purpose of this study is to assess the utility of Banana Peels as fermentation media and substitute MRS media for reducing the cost of production of Probiotics.

Banana peels

India is the largest producer of bananas. It produces more bananas in a year than the rest of the world produces for export. Most of the bananas grown in India are for the domestic market. Even though more than half of the bananas produced in India are Cavendish types, the country is host to a wide diversity of bananas (Grossman, 1993).

The major banana growing states are in the northeastern and southern parts of the country. Tamil Nadu has the largest area followed by Maharashtra and Karnataka. Tamil Nadu also ranks first in production, followed by Maharashtra, but the highest productivity is recorded in Maharashtra, followed by Tamil Nadu and Madhya Pradesh. Maharashtra's high productivity is the result of growing in monocultures high-yielding Cavendish clones, coupled with the adoption of improved technologies such as high density planting and the use of tissue-culture planting material. Banana peel constitutes about 30 percent of fresh banana by weight. Ripe banana peels contain up to 8 percent Crude Protein (CP) and, 13.8 percent soluble sugars and 4.8 percent total phenolics (Wadhwa and Bakshi, 2013). Banana peels are rich in trace elements, the ripened peel has approximately 30 percent free sugars. Green plantain peels contain 40 percent starch. A commercial medium such as de Man, Rogosa and Sharpe (MRS) or Corn Steep Liquor is usually too costly for commercial production of probiotics. Therefore, exploring locally available sources as culture media for probiotics from various agro-industrial wastes could be a better alternative for reducing the cost of production. Hence, the purpose of this study was to investigate the applicability of Banana Peel as a nutritional source for cultivation of *Lactobacilli* strains, which are potential probiotics (Ansari et al., 2016).

MATERIALS AND METHODS

MATERIALS:

Lactobacillus culture, Nutrient agar, MRS broth, Banana peel

METHODS

Microorganism

The strains of *L. sporogenes* and *L. acidophilus* were obtained as probiotics from the local market in Hyderabad. The strains were propagated in sterile de Mann Rogosa Sharpe (MRS) broth and incubated for 24 h at 37°C prior to use. The cultures was then maintained on MRS agar slants and stored at 4°C and subcultured every month. The stock cultures were kept in 40 % glycerol (glycerol as cryo-preservative and serves as carriers to support microorganisms) and stored at -20°C.

CULTURE MEDIUM

Banana Peel Medium

Ripe banana peels were collected, chopped in to small pieces and made in to the paste.20 grams of banana peel paste was mixed with 100 ml of distilled water. Then the mixture was autoclaved and inoculated with MRS broth of Lactobacillus culture (Gilliland and Speck, 1977).

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℃ for 24 h.

Fermentation conditions

The submerged fermentations were carried out in 250 ml Erlenmeyer flasks containing 100 ml of banana peel medium. Fermentation flasks were maintained in an incubator (Taskin and Erdal, 2011).

Effect of initial pH on 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 600nm wavelength (Todoroy and Dicks, 2005)**.**

Effect of temperature on growth of lactobacilli strains

The effect of different temperatures on fermentation were carried out at various temperatures of 30°C, 37°C, 40°C, 45°C,50°C and at optimum pH 6.0 for 36 h. The initial pH of fermentation medium was 6± 0.5.Growth of lactobacilli strains was determined spectrophotometrically at 600 nm wavelength (Ghatani et al., 2017).

RESULTS AND DISCUSSION

Growth of probiotics lactobacilli strains in Banana Peel medium

De Man and colleagues (1960) have reported that the MRS medium is the most common source for cultivation of *Lactobacillus* sp because it provides suitable growth factors and nutrition.

However, when applied at industrial level of processing it has proved to be expensive and has a negative impact on the economics of probiotic production. In this study, we evaluated Banana Peel medium as a potentially low-cost media for *Lactobacillus* production. Banana Peel waste is available in huge quantities as an agro waste material and can be potentially used as carbon source for cultivation and fermentation of probiotics.

The experiments were carried out in shake flask fermentation using *L. sporogenes* and *L. acidophillus* strains. The results showed that *Lactobacillus sp.* tested utilize Banana Peel waste as growth medium and the growth of these species was comparable to that of MRS medium.

Since Probiotics production is a fermentation process, several extracellular factors influence the production. Among thease factors the initial pH and temperature have been found to have crucial effects on the growth and viability of the bacteria (Shah, 2000).

Effect of Initial pH

The study on the effect of initial PH of Banana peel waste medium was carried out in 250 ml Erlenmeyer flask with working volume of 100 ml at 37°C using whey water. The pH of the medium was adjusted using 0.1 N HCL and 0.2M NaOH. The range of initial pH was 4.0,4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5.The results of the effect of pH on bacterial growth are shown in figures 1 and figure 2 on *L. sporogenes* and *L. acidophilus* respectively

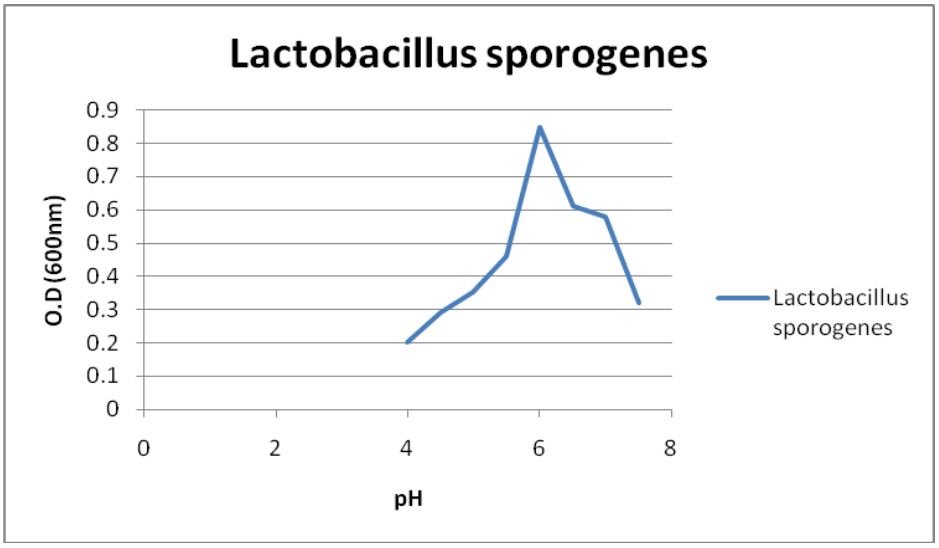

Fig-1. Effect of pH on *Lactobacillus sporogenes* at 37°C in Banana Peel medium

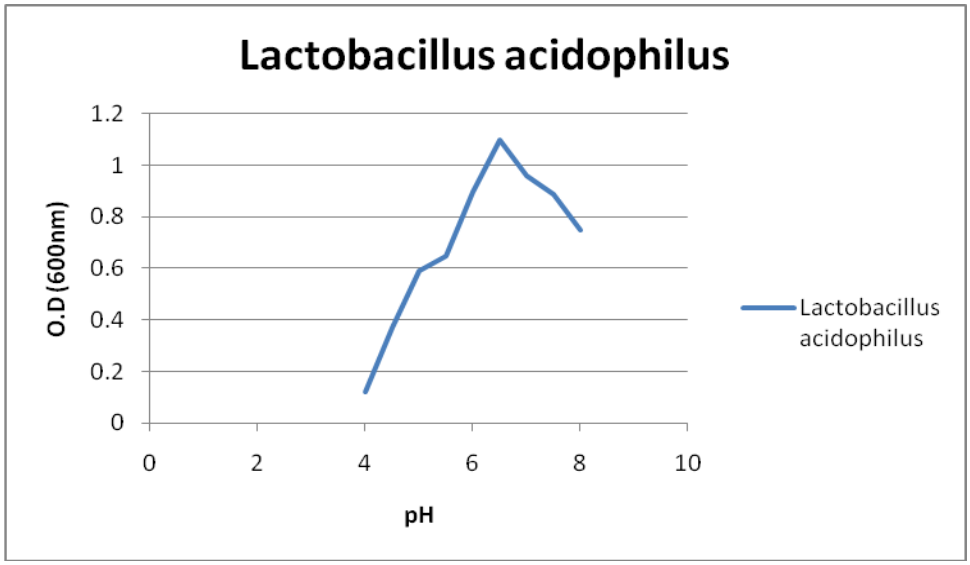

Fig-2. Effect of pH on *L. acidophilus* at 37°C in Banana Peel medium

The exponential growth rate at initial pH 6.0 was the fastest compared to 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 for the whey water fermentation using *L. sporogenes* and *L. acidophilus* species is 6.0.

Effect of Temperature

Temperature is a crucial factor for the growth of microorganisms. Most organisms need an optimum temperature or a characteristic range of temperature in which they grow. Any attempt on the improvement of the production of Probiotic lactobacilli involves determination of optimum pH and temperature. In this study the two species of lactobacilli showed optimum growth in Banana Peel medium at 370C in figures 3 and figure 4. When the temperature of the medium was increased from 37^0C to 60^0C the lactobacilli species showed considerable decrease in growth. The present results are consistent with the observations of others who found optimum growth of lactobacilli between 37^0C and 37^0C.

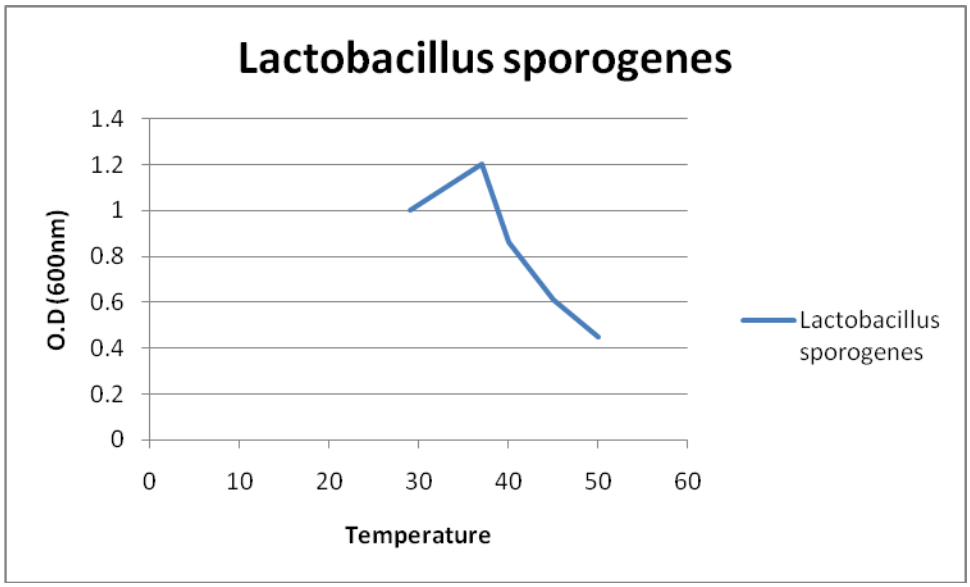

Fig -3 .Effect of temperature on growth on *L. sporogenes* at pH 6 in Banana peel medium

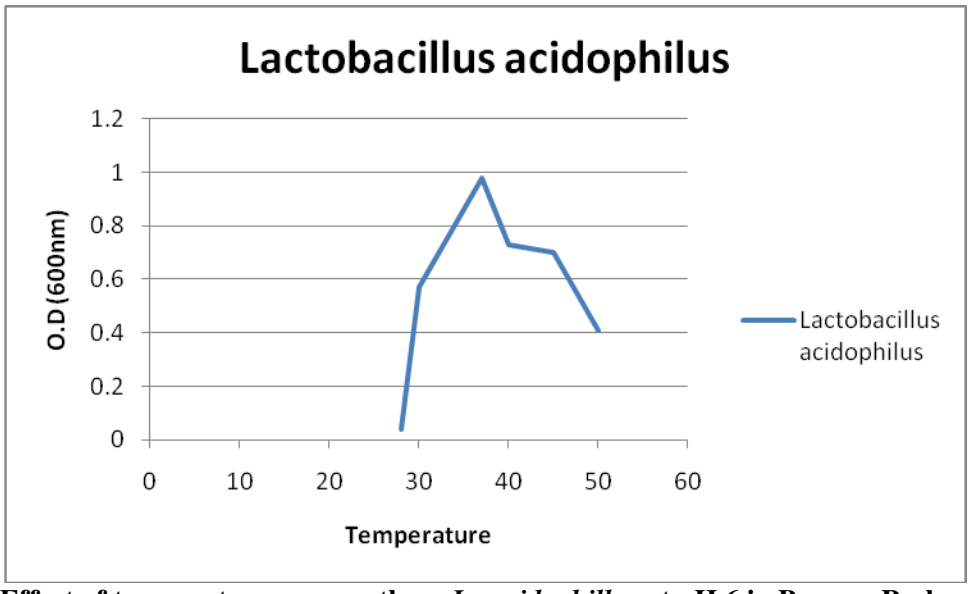

Fig-4 Effect of temperature on growth on *L. acidophillus* at pH 6 in Banana Peel medium

Table 1 shows the comparison of growth of lactobacilli at optimum conditions pH 6.0 and 37°C temperature for the two tested lactobacilli species of *L. sporogenes* and *L. acidophilus* in MRS and banana medium respectively.

Table 1. Comparison of *Lactobacillus sp* growth at optimum pH 6.0 and optimum temperature 37°C between MRS and Banana Peel medium:

Medium	*L. acidophilus*	*L. sporogenes*
MRS	**0.96**	**1.12**
Banana Peel	**0.88**	**0.99**

The results indicate that there was no significant difference in the growth of lactobacilli between the Banana Peel medium and the commercial MRS medium ($p>.05$).

CONCLUSION

Banana Peel, an abundant byproduct of Agro waste has been investigated as an economical and feasible alternative carbon source for the cultivation and the growth of probiotic lactobacilli. It was found that there was no significant difference in the growth of lactobacilli between Banana Peel medium and commercial MRS medium. It can therefore be concluded that Banana Peel waste can be used for Probiotic lactobacilli production.

The tested strains of lactobacillus showed remarkable growth at 37°C and pH 6.0. It can be concluded that using Banana Peel which is obtained as an agro waste would be optimal both economically as well as environmentally for probiotics production.

REFERENCES

Ansari, Nadeem Fatima, Adoni Chetana, E. Maruthi Prasad, Rupali Birajdar, and N. V. Naidu. (2016). Evaluation of Whey water as growth medium for *Lactobacillus species*. IJABPT,; 8(1): 38-42.

Anwar, Zahid, Muhammad Gulfraz, and Muhammad Irshad. (2014). "Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review." Journal of radiation research and applied sciences 7, no. 2: 163-173.

De Man, J. C., deM Rogosa, and M. Elisabeth Sharpe. (1960). "A medium for the cultivation of lactobacilli." *Journal* of Applied Microbiology 23, no. 1: 130-135.

Ghatani, Kriti, and Buddhiman Tamang. (2017). "Assessment of probiotic characteristics of lactic acid bacteria isolated from fermented yak milk products of Sikkim, India: Chhurpi, Shyow, and Khachu." Food Biotechnology 31, no. 3: 210-232.

Gilliland, S. E., and M. L. Speck. (1977). "Antagonistic action of Lactobacillus acidophilus toward intestinal and foodborne pathogens in associative cultures." Journal of food protection 40, no. 12: 820-823.

Grossman, Lawrence S. (1993). "The political ecology of banana exports and local food production in St. Vincent, Eastern Caribbean." Annals of the Association of American Geographers 83, no. 2: 347-367.

Hill, Colin, Francisco Guarner, Gregor Reid, Glenn R. Gibson, Daniel J. Merenstein, Bruno Pot, Lorenzo Morelli (2014). "Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic." Nature reviews Gastroenterology & hepatology 11, no. 8: 506-514.

Sanders, Mary Ellen. (2008). "Probiotics: definition, sources, selection, and uses." Clinical Infectious Diseases 46, no. Supplement, 2: S58-S61.

Shah, N. P. (2000). "Probiotic bacteria: selective enumeration and survival in dairy foods." Journal of dairy science 83, no. 4: 894-907.

Taskin, Mesut, and Serkan Erdal. (2011)."Production of carotenoids by Rhodotorula glutinis MT5 in submerged fermentation using the extract from waste loquat kernels as substrate." Journal of the Science of Food and Agriculture 91, no. 8: 1440-1445.

Todorov, S. D., and L. M. T. Dicks. (2005). "Lactobacillus plantarum isolated from molasses produces bacteriocins active against Gram-negative bacteria." Enzyme and Microbial Technology 36, no. 2: 318-326.

Wadhwa, M., and M. P. S. Bakshi. (2013)."Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products." Rap Publication 4

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com