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EVALUATION OF WHEY WATER AS GROWTH MEDIUM FOR LACTOBACILLUS SPECIES

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ABSTRACT: An attempt is made in this study to use Whey water as a cheap and economical source of carbon for the growth and production of Probiotics *Lactobacillus sp*. Two strains of lactobacilli sp. Namely *Lactobacillus sporogenes* and *Lactobacillus acodophillus* was propagated in de-Man Rogosa and Sharpe medium (MRS) for three successive times prior to carrying out the fermentation in Whey water substrate using flask fermentation technique with incubation at 37^0C for 24 h. Further the effects of initial pH and different temperatures on viability and growth of lactobacilli were determined colorimetrically at 600 nm wavelength. The results showed the best growth in Whey water culture medium was obtained at pH 6.0 at optimum temperature of 37^0C. The growth of lactobacilli sp. in Whey water medium was comparable to that of MRS medium. It can therefore we concluded that Whey water from cheese production industries can be used as a cost effective and cheap substitute for the growth of Probiotic lactobacilli in the place of MRS medium which is expensive .At the same time the present study also helps in providing a solution to the disposal of huge quantities of whey as a byproduct of dairy industry contributing to environmental pollution**.**

Key words: Dairy and Agroindustrial waste, Lactobacilli, Whey water, Probiotics.

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INTRODUCTION

Probiotics are a group of microorganisms forming gastrointestinal biota with putative health benefits. Probiotics are believed to play an important role in regulating intestinal function and digestive health benefits. Probiotics are normally consumed from fermented milk products, such as yoghurt in several countries. As per the World Health Organization's report, probiotics are defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (WHO, 2012).

There is an interest in recent years to utilize, the locally available Agroindustrial and Dairy waste materials, with rich nutrients as alternative source of carbon and other minerals etc for the production of Probiotics .This will not only result in reduction of the cost of production of Probiotics but also help in providing a solution to the disposal of huge quantities of such byproducts of dairy industry contributing to environmental pollution (Nasim et al., 2004: Soccol et al., 2010).

Whey, the greenish translucent liquid obtained from milk after precipitation of casein, has been viewed as one of the major disposal problems of the dairy industry, because of the high volumes produced and having a high biochemical oxygen demand (Marwaha and Kennedy, 988 ; Mawson,1994).

As a general rule, about nine litres of whey is obtained for every kilogram of cheese produced. Thus, the volume of whey to be processed, originating from just one typical large scale cheese making operation can exceed 1 x 106 L/day. A dairy farm processing 100 t of milk per day produces approximately the same amount of organic products in its effluent, as would a town with 55000 residents (Sienkiewicz,T and Riedel,C.-L ,1990) .

The availability of carbohydrate reservoir of lactose in whey and presence of other essential nutrients for the growth of microorganisms makes the whey one of the potential substrates for the production of different bio-products through biotechnological means (Hofvendahl, K and Hagerdal, 2000).

About 108 tonnes of whey water is produced every year and it is estimated that for 1 Kg of Cheese produced, 9 litres of whey water is obtained as an effluent. When this Whey water is disposed to the environment without pretreatment, it pollutes the water bodies by increasing the biological oxygen demand in the range of 38,000 to 46,000 ppm as opposed to 200 ppm in case of sewage. (Marwaha and Kennedy, 1988; Mawson, 1994) .About 1, 50,000 tons of cottage cheese and 2 million tons of whey (with about 1, 30,000 tons of milk nutrients) are produced annually in India. (Dernirel, B, O. Yeniguri and T.T. Onay, 2005).

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 Whey water -waste as a nutritional source for cultivation of lactobacilli strains, which are potential probiotics.

MATERIALS AND METHODS

Microorganism

The strains of *Lactobacillus sporogenes* and *Lactobacillus 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

Whey Water

250 ml of milk was heated to boiling temperature and lemon juice was added to obtain the whey. The mixture was filtered with the cheese cloth and the filtrate whey water was collected. The filtrate was centrifuged to separate residual precipitate. Then it was autoclaved and inoculated with enriched de Man Rogosa and Sharpe broth of LAB (Lactic Acid Bacillus).

Preparation of Inoculums

The culture was aseptically inoculated into a 250 ml flask which contain 100 ml of 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 whey medium. The fermentation flasks was maintained in an incubator.

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 600 nm wavelength.

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.

RESULTS AND DISCUSSION

Growth of probiotics lactobacilli strains in whey medium

De Man and colleagues (1960) have reported that the MRS medium indicated that 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 Whey water as a potentially low-cost media for *Lactobacillus* production. Whey water, which is available in huge quantities as a waste byproduct of Cheese industry can be potentially used as carbon source for cultivation and fermentation of probiotics. The experiments were carried out in shake flask fermentation using *Lactobacillus sporogenes* and *Lactobacillus acidophillus* strains. The results showed that *Lactobacillus* tested utilise whey water 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 (Brandt M, Hammes W, Ganzle M. 2004).

Effect of Initial pH

The study on the effect of initial PH of Whey water 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 0.2 M NaOH. The range of initial pH was 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.The results of the effect of pH on bacterial growth are shown in figures 1 and figure 2 on *Lactobacillus sporogenes* and *Lactobacillus acidophilus* respectively.

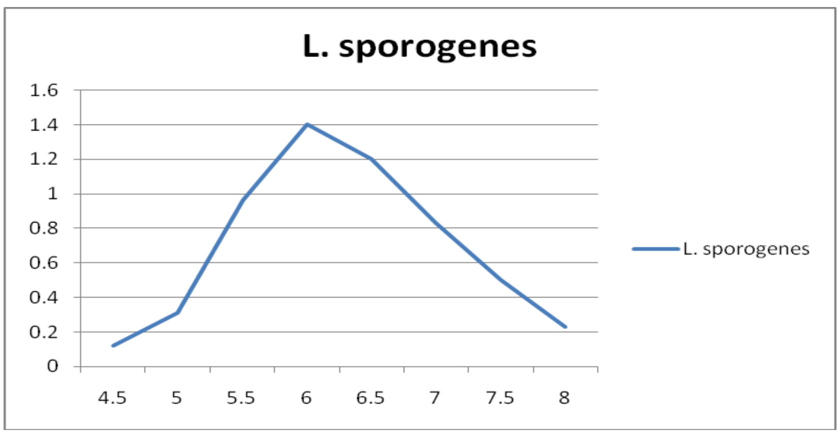

Fig-1. Effect of pH on *Lactobacillus sporogenes* at 37°C in whey medium

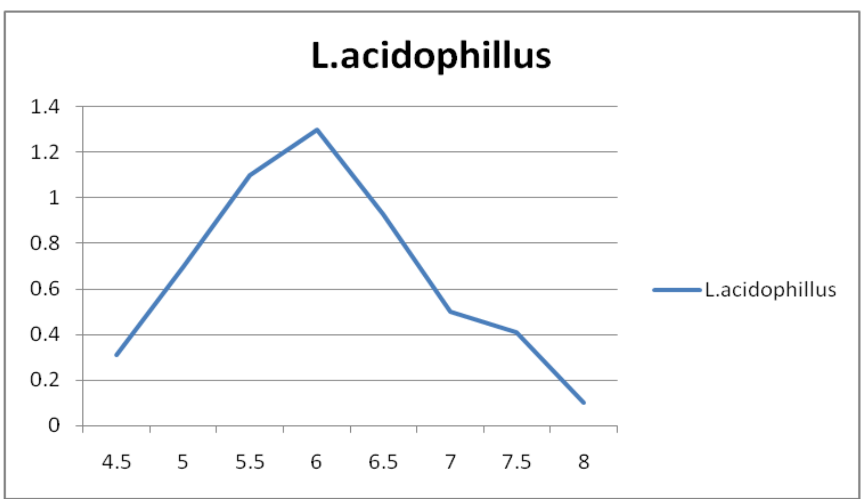

Fig-2. Effect of pH on *Lactobacillus acidophilus* at 37°C in whey medium

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 *Lactobacillus sporogenes* and *Lactobacillus acidophilus* species is 6.0.

Effect of Temperature

Temperature is an another crucial factor for the growth of microorganisms (Brandt M, Hammes W, Ganzle M. 2004). Most organisms need an optimum temperature or a characteristic range of temperature in which they grow. Any attempt on the improvement of the productin\of Probiotic lactobacilli involves determination of optimum pH and temperature. In this study the two species of lactobacilli showed optimum growth in whey medium at 37^0C in figures 3 and figure 4. When the temperature of the medium was increased from 37^0C to 37^0C 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 (Diep and Nes 2002, Baati et al 2004).

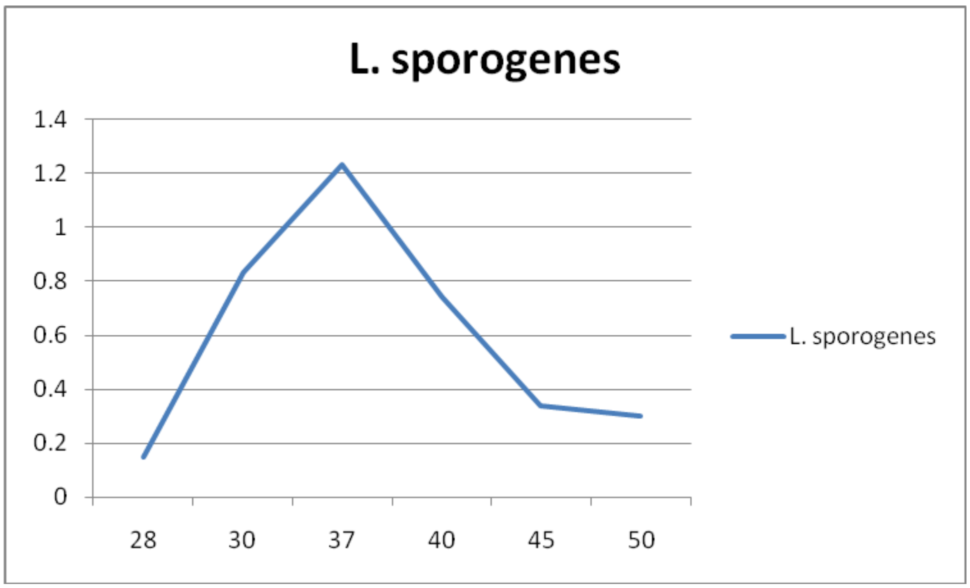

Fig 3. -Effect of temperature on growth on *Lactobacillus sporogenes* at pH 6 in whey medium

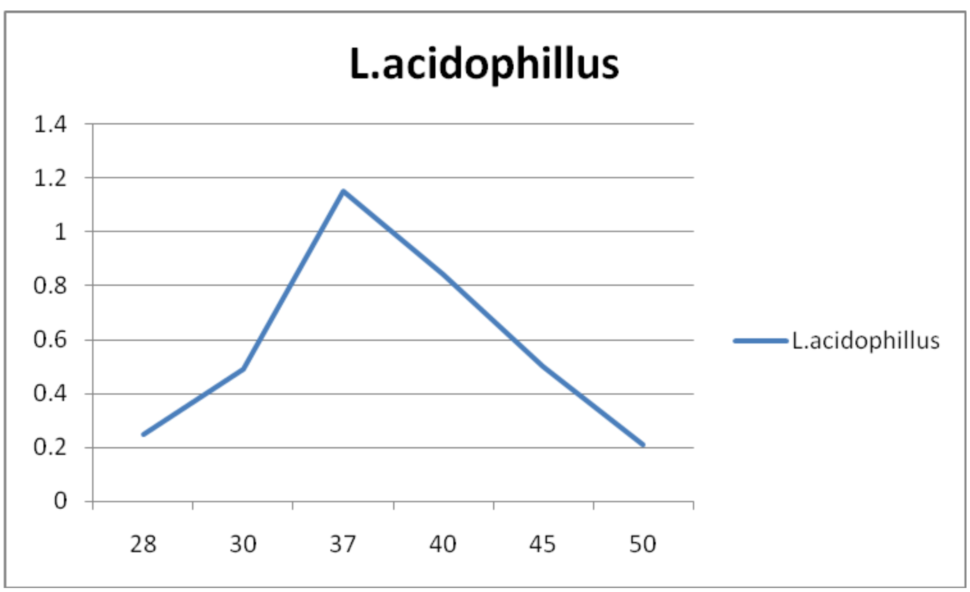

Fig-4. Effect of temperature on growth on *Lactobacillus acidophillus* at pH 6 in whey medium

Table 1 shows the comparison of growth of lactobacilli at optimum conditions pH 6.0 and 37^{o}C temperature for the two tested lactobacilli species of *Lactobacillus sporogenes* and *Lactobacillus acidophilus* in MRS and Whey medium respectively.

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

Medium	***Lactobacillus sporogenes* (OD600 nm)**	***Lactobacillus acidophillus*(OD600 nm)**
MRS medium	1.10	1.28
Whey medium	0.95	1.00

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

CONCLUSION

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

The tested both strains showed remarkable growth at 37°C and pH 6.0. It can be concluded that using whey water which is obtained as a byproduct in cheese production would be optimal both economically as well as environmentally for probiotics production.

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