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Research Article

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Microbial Utilization of Dairy Waste for Lactic acid Production by Immobilized Bacterial Isolates on Sodium Alginate Beads

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ABSTRACT

Lactic acid has long been used in the food, chemical, textile, pharmaceutical and other industries. Ninety percentage of the worldwide production of lactic acid is by microbial fermentation. Recently, there is an increasing interest in the production of lactic acid, since it is a potential substrate for polylactic acid that is biocompatible and can be used for medical purposes. Whey, which is a by-product of dairy industry, contains approximately 5 % (w/v) lactose. Since whey has a high BOD content, it possesses serious environmental problems. Whey lactose is a good substrate for lactic acid bacteria and can be used for lactic acid fermentations. The lactic acid bacteria (LAB) were isolated from various sources such as curd, idle batter, pickle and yoghurt and identified based on their morphological and biochemical characteristics. Four potential isolates were screened out for the production of lactic acid. Immobilized cells of LAB isolated from pickle was found to produce of maximum amount of Lactic acid (109.0g/L) followed by LAB isolated from yoghurt (101.0 g/L), curd (88.0 g/L) and idle dough (80.0 g/L). This study is focused on the production of lactic acid from whey by LAB immobilized in sodium alginate beads. Lactose conversion to lactic acid was compared with immobilized and free cells. The immobilezed cells showed potential to perform beneficially in the lactic acid fermentation industries, coupled with removal of water pollutant, dairy whey.

Key words: *Fermentation, Fermented Foods, Immobilization, Lactic acid, Lactic Acid Bacteria.*

INTRODUCTION

Lactic acid has a long history of uses for fermentation and preservation of human food stuffs[1]. Lactic acid can be produced by either microbial fermentation or chemical synthesis[2, 3]. Lactic acid is now considered to be one of the most useful chemicals, used in food industry as a preservative, acidulant and flavouring agent, in the textile and pharmaceutical industries, and in the chemical industry as a raw material for the production of lactate ester, propylene glycol, 2,3-pentanedione, propanoic acid, acrylic acid, acetaldehyde, and dilactide[4,5]. Lactic acid is produced through chemical synthesis and microbial biosynthesis. The microbiological approach is preferred because it allows the obtaining of the biologically active *L(+)* form of the acid. The main biological agents applied in the production of lactic acid are the bacteria from the genus *Lactobacillus*, which can be employed as free or immobilized cells[6,7,8]. The use of immobilized lactic acid bacteria for biosynthesis of lactic acid has significant economic advantages to the application of free cells. Regardless of this fact, limited research was dedicated to this method so far. The strains proposed in the reference literature for production of lactic acid with immobilized cells belong to the species *Lactobacillus delbrueckii* sub sp.*bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus* and *Lactobacillus casei*[6,7,9]. The widely used choice of substrates for lactic acid fermentation is refined sugars, which are inexpensive. Lactic acid can also be produced from abundantly available cheaper substrate, such as whey. Whey is a by-product of cheese production. The production of dairy products in large quantities leads to enormous quantities of whey as a byproduct in the dairy industries, which represents 85-95% of the milk volume and retains 55% of milk nutrients.

Among the most abundant of these nutrients are lactose, soluble proteins, lipids and mineral salts[10,11]. 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 substrate for the production of different bio-products through biotechnological means[12]. Microbial fermentation has the advantage that by choosing a strain of LAB producing only one of the isomers, an optically pure product can be obtained, whereas synthetic production always results in a racemic mixture of lactic acid. The production of optically pure lactic acid is essential for the polymer synthesis in which lactic acid is used[4,5,12]. Moreover, L (+) lactic acid is used by human metabolism due to the presence of L-lactate dehydrogenase and is preferred in foods as preservative as well as emulsifier[10,12]. Presently, starch or sugar containing substances are used for the production of lactic acid. Whey is the attractive raw material for lactic acid production for its low cost, low levels of contaminants, fast fermentation rate, high lactic acid yield, little or no by-product formation, ability to be fermented with little or no pretreatment, year-round availability and reduction of waste products.

The aim of this study is to investigate the potential of immobiized lactic acid bacteria isolated from fermented foods to produce lactic acid using whey as a substrate in optimized conditions.

MATERIALS AND METHODS

Materials

Fermented food samples for the isolation of LAB were collected from market and whey sample was collected from dairy industry, Bangalore, Karnataka, India.

Isolation of Lactic acid bacteria & culture conditions

The lactic acid bacteria were isolated from curd, idle batter, pickle and yoghurt by using the MRS (De Man, Rogosa and Sharpe) agar medium [13]. Well grown lactic acid bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on MRS agar plates and stored at 4^0C.

Morphological and Biochemical tests

Based on the growth performance in MRS agar, lactic acid strains were selected. The isolates were identified following morphological and biochemical characterization according to Bergey's Manual of Systematic bacteriology.

Total estimation of lactose content from whey

The whey collected from dairy was titrated with Fehling's solution 1 and Fehling's solution 2 for the estimation of lactose. The whey added with nutrients was used as medium to which free cells and immobilized cells are inoculated and incubated at 37^0C for 24 hours. The filtrate after incubation was used for titration with Fehling's solution 1 and Fehling's solution 2 for the estimation of lactose.

Fed batch fermentation

Preparation of starter culture

The inoculum of four different LAB were prepared in growth medium (MRS)[14]. MRS growth medium composition (g/l): peptone (10.0), yeast extract (5.0), meat extract (10.0), glucose (20.0), sodium acetate (5.0), ammonium citrate (2.0), K_2HPO_4 (5.0), $Na_2HPO_4.2H_2O$ (2.0), $MgSO_4.2H_2O$ (0.1) and $MNSO_4.4H_2O$ (0.05). Incubation temperature was 37 ± 1^0C for 18hours. Initial pH of the medium was adjusted to 6.7.

Immobilization of cells - Entrapment in alginate

All the potential lactic acid bacterial isolates LABC, LABI, LABP and LABY isolated from curd, idli batter, pickle and yoghurt respectively were harvested from MRS broth during early stationary phase routinely by centrifugation at 5000 rpm for 10 min. The supernatant was removed and the pellet was mixed with 40 ml of 2% (w/v) sodium alginate that was prepared previously by dissolving 0.8 g of sodium alginate in 40 ml of distilled water. The bead-forming solution was dropped into 2% (w/v) $CaCl_2$ solution with mild stirring maintained for 30 min at room temperature in order to cure. The resulting beads which had 2 mm diameters were washed twice with normal saline and used as the biocatalyst[15].

Production of lactic acid

Whey was supplemented with yeast extract (0.75%, w/v), manganese sulphate (20 mg/L), and calcium carbonate (1.5%, w/v)[14]. The whey medium was sterilized at 121°C for 15 min. The free cells & immobilized cells of LABC, LABI, LABP and LABY were inoculated on whey medium and incubated at 200rpm & 37 $\pm$ 1^0C for 48 hours.

Determination of Lactic acid from free cells

After completion of fermentation the fermented broth was centrifuged at 10,000 rpm for 10 min to pellet out the bacterial growth and the supernatant was used for estimation of lactic acid by titrimetric method[16].

Determination of Lactic acid from Immobilized cells

After completion of fermentation the fermented broth was filtered, the filterate was used for estimation of lactic acid by titrimetric method.

For the determination of the acid-forming ability of the lactic acid bacteria, the titratable acidity (expressed as Toerner degree, 1°T) was determined by titration of $10cm^3$ sample with 0.1N NaOH to a pink endpoint using phenolphthalein as an indicator (1°T = 0.009 g lactic acid)[16].

RESULTS AND DISCUSSION

Isolation and Identification of Lactic acid bacteria

The four lactic acid bacteria isolated from curd, idle batter, pickle and yoghurt were tentatively identified as *Lactobacillus* sp based on their morphological and biochemical characteristics (Table 1).

Table 1: Morphological and biochemical characteristics

Characteristics	Result			
	LAB C	**LAB I**	**LAB P**	**LAB Y**
Gram reaction	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Rod	Rod	Rod	Rod
Motility	Non Motile	Non Motile	Non Motile	Non Motile
Endospore	Non Spore Forming	Non Spore Forming	Non Spore Forming	Non Spore Forming
Catalase	-	-	-	-
Oxidase	-	-	-	-
Rhamnose	-	-	-	-
Dextrose	-	+	+	+
Galactose	+	+	-	-
Xylose	+	+	-	-
Lactose	+	+	+	+
Sucrose	+	+	+	+
Esculin	-	-	-	-
Mannitol	-	-	+	-

(+ Positive; - Negative)

Isolates were subjected to test on their ability to produce lactic acid from both lactose (MRS) medium and on the special whey medium (pH 5.5). The percentage of lactic acid produced from the respective medium (Table 2) clearly indicates that the isolates show differential ability and the medium plays a pivotal role in accumulation of the desired end product. Though whey is a non-defined medium, the isolates produced lactic acid on par with the lactose medium with an average of 0.55% less in comparison to defined lactose medium.

The cultures were tested for their ability to produce the lactic acid as a free cell and also as an immobilized cell. LAB C and LAB I isolates from curd and idli batter samples showed higher percentage of end product in comparison to LAB P and LAB Y, with LAB C producing more lactic acid in defined lactose medium and LAB I in whey medium respectively (Table 2).

Table 2: Comparitive analysis of lactic acid production for free cells

S.No	Organism	Lactose medium (% of lactic acid produced)	Whey medium (% of lactic acid produced)
1	LAB C	10.8	9.8
2	LAB I	10.5	10.1
3	LAB P	6.4	6.0
4	LAB Y	7.2	6.8

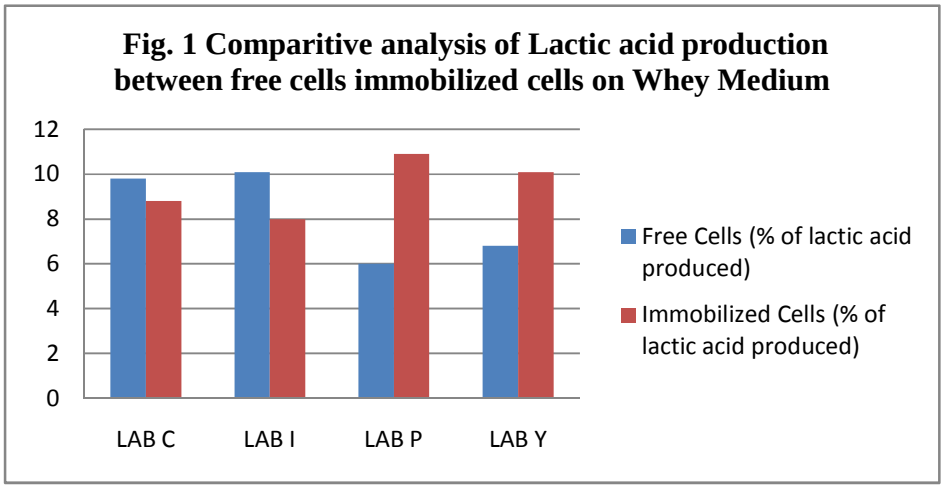
The performance of the immobilized cells was found to be far better than the free cells whereby the yield of lactic acid was comparitively higher (Table 3). All the four isolates were found to produce higher percentage of end product than as a free cellls in a fermentation media. The isolates LAB P and LAB Y outperformed the other two isolates when considered for immobilization. The effect of immobilization seems to suit LAB P and LAB Y isolates far better than LAB C and LAB I, especially LAB P isolate showed 82.98 % (11.35 g/L) higher yield contrasting to its average yield of 6.2 g/L as a free cell in a fermentation medium (Figure 1).

The results clearly show that the immobilized cells preferentially produce more end product than the free cells and the whey medium is equally suitable as a production medium for fermentation process. The whey medium is more suitable than defined lactose medium in terms of their cost and is economically more viable as an industrial process. The immobilization may be an added advantage as the yield increases dramatically and also the immobilized cells can be reused and the desired culture can be retained for subsequent fermentation process.

Table 3: Comparitive analysis of lactic acid production for immobilized cells

S. No.	Organism	Lactose medium (% of lactic acid produced)	Whey medium (% of lactic acid produced)
1	LAB C	9.7	8.8
2	LAB I	8.2	8.0
3	LAB P	11.8	10.9
4	LAB Y	10.9	10.1

According to the experiments of this study, immobilization of LAB on sodium alginate is the best approach for lactic acid production on a laboratory scale in comparison with free cells. It also has the capability of being used during repeated batch cultures for lactic acid production. Further investigations are underway to increase the strength of the sodium alginate beads. In comparison with lactose medium, the whey medium showed equally good production of lactic acid.



Whey is a major by-product of the dairy industry, and it contains lactose, protein, fat, and mineral salts. The initial concentration of the lactose in whey medium was at a average of 51 g/L and an estimation of the lactose concentration in the spent fermentation medium was found to be at an average of 29.25 g/L (Table 4) and this shows that the spent whey has to be properly utilized and there is a scope for more production of lactic acid end product. But the reason for high percentage of lactose in spent medium can be attributed to limitation in nitrogen concentration, thus it is necessary to supplement whey with an additional nitrogen source[10].

Table 4: Total estimation of lactose content from whey medium before and after fermentation

S.No	Strains	% of Lactose in Pre fermented Whey medium	% of Lactose in fermented Whey medium
1	LAB C	51.0	32.0
2	LAB I		36.0
3	LAB P		23.0
4	LAB Y		26.0

Further efforts will be made to check for the co-culturing and its effect on increase in yield of end product and scope for further research includes the optimization the whey medium for fermentation process, the role of supplements is envisaged on these aspects.

CONCLUSION

Lactic acid is widely used in the food, cosmetic, pharmaceutical, and chemical industries and has received increased attention for use as a monomer for the production of biodegradable polylactic acid. It can be produced by either biotechnological fermentation or chemical synthesis, but the former route has received considerable interest recently, due to environmental concerns and the limited nature of petrochemical feedstocks. There have been various attempts to produce lactic acid efficiently from inexpensive raw materials. Lactic acid production was compared with Immobilized and free cell using whey as medium. These studies clearly indicated that the dairy waste can be utilized for lactic acid production at its lower concentration effectively by using the identified *Lactobacillus* sp.

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