

ORIGINAL RESEARCH ARTICLE

## Lactic Acid Production Using Lactic Acid Bacteria under Optimized Conditions

A.Sheeladevi\* and N.Ramanathan

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002, India

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### ABSTRACT

Lactic acid is an organic acid 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 poly lactic acid. Lactic acid bacteria have the property of producing lactic acid from various fermentable carbohydrates. This process is called microbial fermentation. The genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are important members of lactic acid bacteria. Two kinds of lactic acid bacteria are recognized as Homofermentative and Heterofermentative. The homofermentative lactic acid bacteria belong to the genera *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were used for this study. These Lactic acid bacteria were isolated from various sources such as milk, curd, whey, fermented idly dough and pickles. Effect of carbon sources, temperature, pH and inoculum levels on the growth of lactic acid bacteria were investigated. *Lactobacillus delbrueckii* was found to produce 135 g<sup>l</sup> of lactic acid from 150 g<sup>l</sup> of glucose followed by *Lactobacillus plantarum* (120 g<sup>l</sup>) and *Lactobacillus casei* (112.5 g<sup>l</sup>). Maximum glucose conversion to lactic acid was observed at process conditions of pH 5.5, temperature 37°C, 10% inoculum level and fermentation period of 72 hours.

**Key words:** Lactic acid, Lactic acid bacteria, Glucose and Homofermentative.

### 1. INTRODUCTION

Lactic acid (2-hydroxypropanoic acid) is an invaluable chemical, it was first discovered by the Swedish chemist Scheele in 1780, who isolated the lactic acid from sour milk. It was first produced commercially by Charles E. Avery at Littleton, Massachusetts, USA in 1881. Lactic acid can be produced by either microbial fermentation or chemical synthesis, a great deal of interest has recently become focused on the microbial fermentation, because the chemical synthesis of lactic acid is associated with several serious problems, including environmental issues and the depletion of petrochemical resources [1]. Lactic acid is classified as GRAS (generally recognized as safe) for use as a food additive by the US FDA (Food and Drug Administration) and it has been utilized in a broad range of applications in the food, beverage, cosmetic, medical and pharmaceutical industries [2]. Major use of lactic acid (accounts to 85% of demand) is still in food and food related applications.

Lactic acid is widely used in almost every segment of the food industry, where it serves in a wide range of functions, such as flavouring, pH

regulation, improved microbial quality and mineral fortification. Moreover, lactic acid is used commercially in the processed meat, hams, fish and poultry industries, to provide products with an increased shelf life, enhanced flavour, and better control of food-borne pathogens. Due to the mild acidic taste of lactic acid, it is also used as an acidulant in salads and dressings, baked goods, pickled vegetables, and beverages.

Lactic acid is used in confectionery, not only for flavour, but also to bring the pH of the cooked mix to the correct point for setting. The advantages of adding lactic acid in confectionery include its low inversion rate, ease of handling, and ability to produce clear candies. Another potential application of lactic acid in the food industry is the mineral fortification of food products. Lactic acid plays a vital role in the chemical industry, where it is used as a precursor for the syntheses of ethyl lactate, propylene oxide, propylene glycol, acrylic acid, 2, 3-pentanedione and dilactide. Another very promising lactic acid application is the production of environmentally friendly “green” solvents (lactate esters). They

can replace traditional solvents made from petrochemical feedstocks<sup>[3]</sup>.

Lactic acid bacteria have the property of producing lactic acid from sugars by a process called fermentation. Lactic acid bacteria genera include *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Vagococcus*, *Oenococcus* and *Weissella*. Lactic acid bacteria (LAB) can be classified into two groups: homofermentative and heterofermentative. The homofermentative LAB are *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus casei*, *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus faecalis*, *Streptococcus thermophilus* and *Pediococcus cerevisiae*. The heterofermentative LAB are *Leuconostoc mesenteroides*, *Lactobacillus cremoris*, *Lactobacillus brevis* and *Lactobacillus fermentum*.

The biological production of lactic acid via microbial fermentation has been studied extensively by a many research groups<sup>[4]</sup>. While the homofermentative LAB convert glucose almost exclusively into lactic acid, the heterofermentative LAB catabolize glucose into ethanol and CO<sub>2</sub> as well as lactic acid. The homofermentative LAB usually metabolize glucose via the Embden-Meyerhof pathway (*i.e.* glycolysis). Since glycolysis results only in lactic acid as a major end-product of glucose metabolism, two lactic acid molecules are produced from each molecule of glucose with a yield of more than 0.90 g/g. Only the homofermentative LAB are used for the commercial production of lactic acid<sup>[5]</sup>. The present investigation was carried out, to study the potential of Lactic acid bacteria produce Lactic acid using glucose as substrate in optimized conditions.

## 2. MATERIALS AND METHODS

### 2.1. Microorganisms

The Lactic acid bacteria was isolated from milk, curd, whey, fermented idly dough and pickles by using the MRS<sup>[6]</sup> Agar medium. Well grown Lactic acid bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on MRS agar slants and stored at 4°C. Identification of the Lactic acid bacterial isolates was carried out by the routine bacteriological methods *i.e.*, By the colony morphology, preliminary tests like Gram staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests.

## 2.2. Growth studies of Lactic acid bacteria Isolates

### 2.2.1. Preparation of standard inoculum

All the five lactic acid bacteria isolates *i.e.*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were grown in MRS broth at the temperature of 37°C for 24 hours. Then the broth was centrifuged at 3000 rpm for 10 min to harvest the cells and the pellets washed three times with 0.1 M phosphate buffer (pH 6.0). Finally, the cells were resuspended in the same buffer to a cell concentration of 1 x 10<sup>7</sup>/ml by measuring the absorbance at 480 nm and used as standardized inoculum.

### 2.2.2. Fermentation Parameters

For lactic acid production, the following fermentation parameters *i.e.*, Growth rate of Lactic acid bacteria Isolates in different carbon sources at different concentrations, in different temperature, in different pH level, in different Inoculum levels were tested for five LAB isolates *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae*. The cell density of the LAB were measured by optical density (OD) at 480 nm in a Double-beam UV-VIS spectrophotometer.

### 2.2.3. Effect of different fermentation periods on Lactic acid production

The MRS broth was prepared and 10 % of the standard inoculum of LAB isolates (*Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae*) was inoculated separately. All the five LAB isolates were grown for different fermentation periods *viz.*, 36, 48, 72 and 96 hours respectively. At the end of the fermentation period, lactic acid produced (g l<sup>-1</sup>) was determined using standard protocols.

## 2.3. Lactic acid production

### 2.3.1. Preparation of starter culture for Lactic acid production

Among the five LAB isolates, the three LAB culture *viz.*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, and *Lactobacillus casei* were selected based on the growth studies. The pure culture of *Lactobacillus* sp. were inoculated into MRS broth with the pH of 5.5 and incubated at 37°C for 24 hours. The MRS broth was prepared with the glucose concentration of 15%. Then 10% of the standard starter cultures of LAB (*Lactobacillus delbrueckii*, *Lactobacillus plantarum*, and *Lactobacillus casei*) were

inoculated and incubated in a temperature controlled shaker at 37°C, the fermentation process was carried out up to 72 hours for lactic acid production.

### 2.3.2. Estimation of Lactic acid

The amount of lactic acid in fermentation broth was determined by transferring 25 ml of culture broth of LAB isolates into 100 ml flask. One ml of phenolphthalein indicator (0.5% in 5% alcohol) was added into the flask. This was titrated with 0.25 M NaOH for the appearance of pink colour. The titratable acidity was calculated as lactic acid % W/V [7]. Each millilitre of 1 N NaOH is equivalent to 90.08 mg of lactic acid. The titratable acidity was then calculated.

## 3. RESULTS AND DISCUSSION

Lactic acid has a long history of uses for fermentation and preservation of human food stuffs. It can be produced by either microbial fermentation or chemical synthesis. Due to environmental concerns and the limited nature of petrochemical feedstocks, lactic acid can be commercially produced by microbial fermentation. Recently lactic acid consumption has increased considerably because of its role as a monomer in the production of biodegradable Poly lactic acid (PLA), which is well-known as a sustainable bioplastic material. However, the global consumption of lactic acid is expected to increase rapidly in the near future.

Lactic acid bacteria can be classified into two groups: homofermentative and heterofermentative. While the homofermentative LAB convert glucose almost exclusively into lactic acid, the heterofermentative LAB catabolize glucose into ethanol and CO<sub>2</sub> as well as lactic acid [4]. Only the homofermentative LAB have the industrial importance and used as commercial production of lactic acid [5]. The homofementative Lactic acid bacteria were from the genera *Lactobacillus*, *Sreptococcus* and *Pediococcus* [8]. The experimental results of the present study in the Lactic acid production using Lactic acid bacteria are briefly discussed.

Lactic acid bacteria are identified as Gram-positive, non-spore forming rods, catalase-negative, usually non-motile, that do not reduce nitrate, indole is not formed and that utilize glucose (Bergey's manual, 1986). According to the morphological and biochemical characteristics of the five LAB isolates *viz.*, *Lactobacillus*, *Streptococcus*, *Lactobacillus*, *Lactobacillus* and *Pediococcus* respectively based on the Bergey's manual of systematic Bacteriology. The five LAB isolates isolated from milk, curd, whey, fermented

idly dough and pickles were used for the carbohydrate fermentation. Based on their carbohydrate fermentation characters LAB isolates were identified as *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae*.

Glucose was used as a substrate for D-lactic acid production using *Bacillus* (*Lactobacillus*) *laevolacticus*. The result indicated that 97% of D-lactic acid was produced from 50 kgm<sup>-3</sup> of glucose in a chemostat culture at pH 6.0 [9]. *Lactobacillus coryniformis* sub sp. *torquens* produced 39 kgm<sup>-3</sup> of D-lactic acid from 40 kgm<sup>-3</sup> of glucose at pH 6.4 [10]. In the present study, glucose was selected for lactic acid production to get maximum yield of lactic acid to reduce the cost of purification process of lactic acid and also less chance of contamination during lactic acid fermentation.

Glucose was used as a substrate for lactic acid production using *Lactococcus lactis* subsp *lactis*. The fermentation process was carried out at 32°C with 60 gl<sup>-1</sup> of glucose and a pH of 6.0. The results indicated that 54 gl<sup>-1</sup> of lactic was produced. Maximum production and glucose conversion was achieved at 15% of glucose concentration [11]. This present study revealed that the growth of five LAB isolates *viz.*, *Lactobacillus casei*, *Strptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were higher in glucose as a substrate when compared with fructose, sucrose, lactose and starch and also maximum growth was recorded in 15% of glucose concentration, when compared with 10 and 20%.

The effects of temperature, pH and medium composition on lactic acid production by *Lactobacillus casei* were investigated. The highest lactic acid productivity values were obtained at 37°C and pH 5.5. [12]. In the present study, the similar results were also obtained - the growth of five LAB isolates *viz.*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were higher in the temperature of 37°C, when compared with 28, 30 and 40°C. (Fig 1)

The effect of pH on growth and lactic acid production of *Lactobacillus helveticus* was investigated in a continuous culture using supplemented whey ultrafiltrate. Maximum lactate productivity of 5 gl<sup>-1</sup> h<sup>-1</sup> occurred at pH 5.5 [13]. In the present study revealed that the growth of five LAB isolates *viz.*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus*

*cerevisiae* were higher in the pH of 5.5, when compared with the pH of 4.5, 5.0 and 6.0.(Fig 2).

Cock *et al.* (2006)<sup>[11]</sup> have reported that lactic acid production was increased at 10% level of inoculum. The results indicated that 54 g<sup>l</sup><sup>-1</sup> of lactic acid were produced from 60 g<sup>l</sup><sup>-1</sup> of glucose using *Lactococcus lactis*. In the present study, the similar results were also obtained - the growth of five LAB isolates *viz.*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were higher in the inoculum level of 10%, when compared with 3, 5 and 12% levels of inoculum (Fig 3).

In this present study the optimum levels of fermentation parameters were selected for lactic acid production *viz.*, Carbon source (substrate): Glucose (15%), Temperature-37°C, pH-5.5 and the Inoculum level was 10%. Kanwar *et al.* (1995)<sup>[14]</sup> have reported that maximum lactic acid was obtained at a fermentation period of 72 hours. Hujanen *et al.*, (1996)<sup>[15]</sup> selected homofermentative strain of *Lactobacillus casei* subsp *rhamnosus* NRRL B-445 for lactic acid

production using grass extract as a nitrogen source. The result showed that 74 g<sup>l</sup><sup>-1</sup> of lactic acid was produced at 73 hours of fermentation period. In the present investigation also revealed the same result that lactic acid yield was maximum in the fermentation period of 72 hours, when compared with 36, 48 and 96 hours. All the five LAB isolates *viz.*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* were showed higher lactic acid yield at the fermentation period of 72 hours (Fig 4).

Kadam *et al.* (2006)<sup>[16]</sup> have reported the highest lactic acid concentration (135 g/l) in batch fermentation was obtained with 150 g/l of cane sugar with 90% lactic acid yield by using the strain of *Lactobacillus delbrueckii* (NCIM 2365). In the present study, the similar results were obtained. Among the three selected LAB isolates *Lactobacillus delbrueckii* showed the maximum lactic acid yield (135 g<sup>l</sup><sup>-1</sup>) from 150 g<sup>l</sup><sup>-1</sup> of glucose followed by *Lactobacillus plantarum* (120 g<sup>l</sup><sup>-1</sup>) and *Lactobacillus casei* (112.5 g<sup>l</sup><sup>-1</sup>).

Fig 1: Growth rate of Lactic Acid Bacteria Isolates in different Temperature

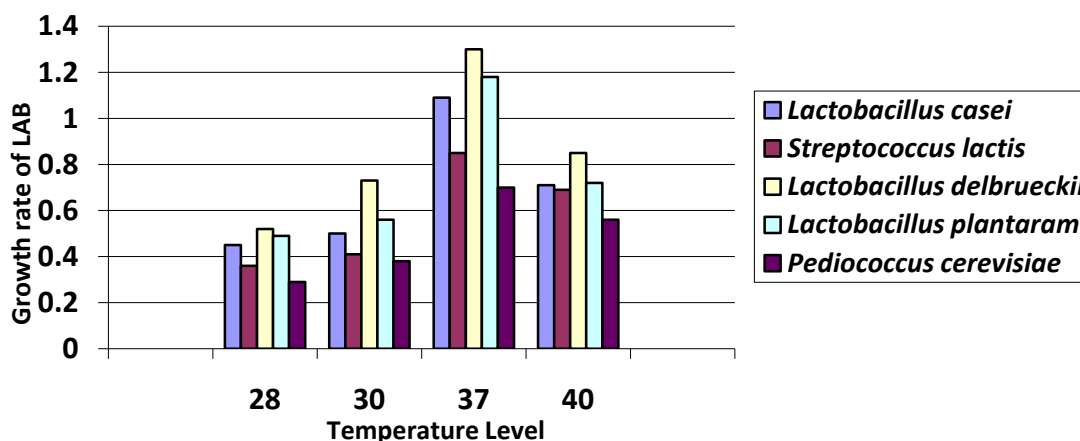


Fig 2: Growth rate of Lactic Acid Bacteria Isolates in different pH level

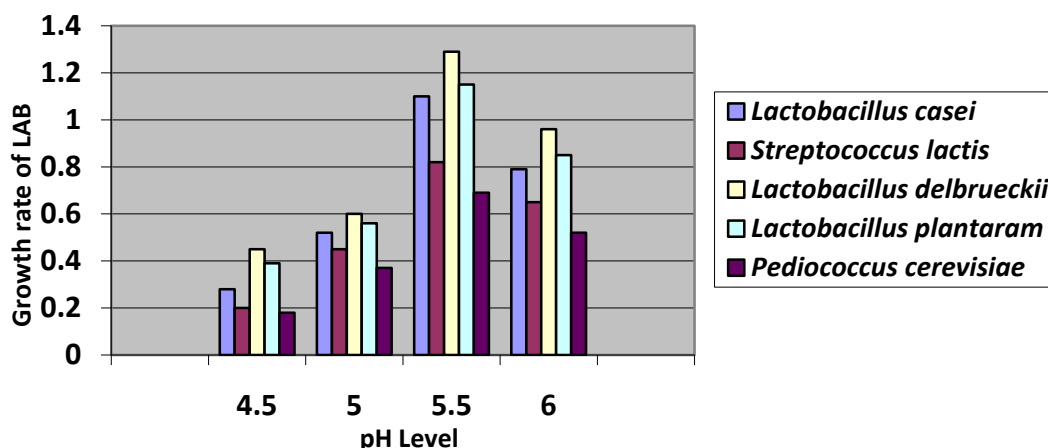


Fig 3: Growth rate of Lactic Acid Bacteria Isolates in different Inoculum levels

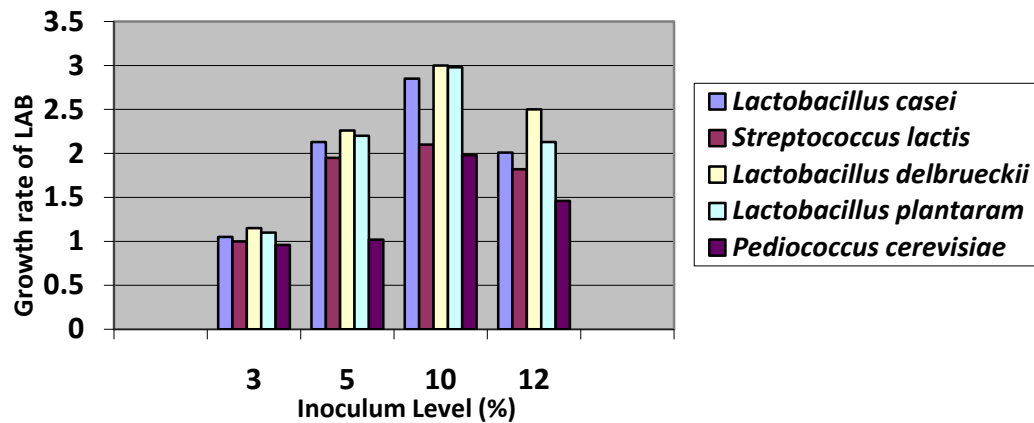
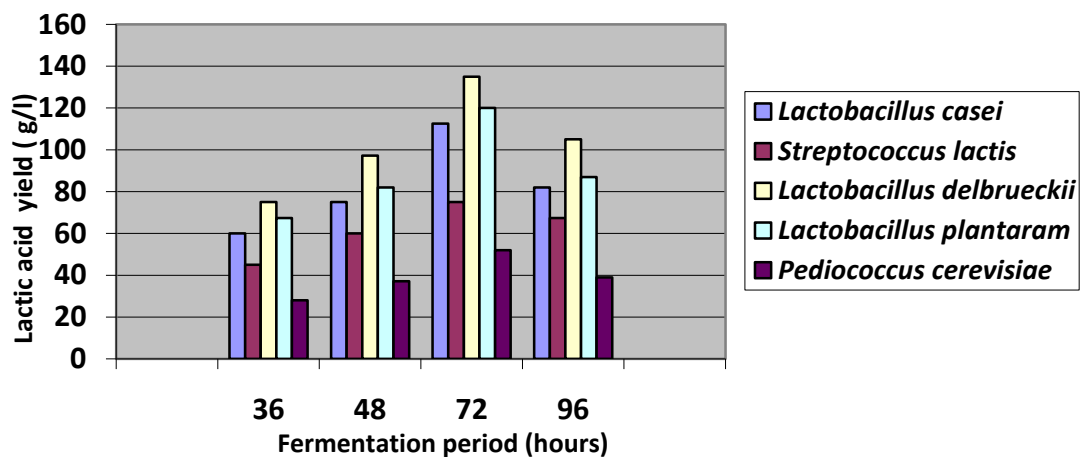


Fig 4: Effect of different fermentation periods on lactic acid production



#### 4. CONCLUSION

The present research results showed that Lactic acid bacteria have the potential to produce lactic acid in large quantity at the optimized fermentation conditions. Glucose was used as a substrate for lactic acid production because it reduces the cost of purification process and also less chance of contamination during the recovery process.

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