ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Screening and Optimization of Lactic Acid Production from *Lactobacillus* Strains by Using Agro Waste Residues.

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ABSTRACT

Lactic acid is an organic acid produced by Lactobacillus strains and being used in many industries. The present study is aimed to elucidate the ability of lactic acid production with different *Lactobacillus* strains using various agro waste substrates. These Lactobacillus were isolated from various dairy products. Out of 30 isolates, 15 isolates were identified as Lactobacillus strains by morphological and physiological characterization. These strains were subjected to Solid state fermentation and Submerged fermentation by utilizing the peels of Apple, Cassava, Mosambi, Orange and Pineapple. SSF with Mosambi peel showed a higher yield of lactic acid from Lactobacillus sp LAB 23.Optimum conditions such as temperature, pH, nitrogen sources, mineral salts, inoculum concentration and fermentation time were evaluated for SSF and SmF. In SSF, the optimum culture conditions for the maximum lactic acid production were temperature 37°C, pH 6.5, 2.5% yeast extract as a nitrogen sources, 8% Calicum carbonate, 0.3% magnesium sulphate as mineral salts, 4 % inoculums concentration, substrate concentration 8g, 72 h for fermentation. In SmF, the optimum culture conditions for maximum lactic acid production were temperature 40°C, pH7.5, 3.5% yeast extract as a nitrogen sources, 9% Calicum carbonate, 0.4% magnesium sulphate as mineral salts, 8% inoculums concentration, 10 mL substrate concentration and fermentation time 96 h. The present study suggests that the Mosambi peel is an appropriate agro waste substrates for high yield of lactic acid under optimized conditions. Thus, the Mosambi peel could be used as a good substrate in various industries.
Keywords: Lactobacillus, agro waste residues, SSF, SmF, lactic acid.

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INTRODUCTION

Lactic acid (2 –hydroxypropionic, $CH_3CHCOOH$) is an organic acid. A review suggests that 85% of lactic acid in USA was used in food and food related application, biodegradable plastics(Zhan *et al* .,2007). Lactic acid is considered as GRAS (generally recognised as safe). Lactic acid can be existed naturally in 2 optical isomer L (+) lactic acid and D(-) lactic acid. L(+) lactic acid used in food and pharmaceutical industries whereas D(-) lactic acid is harmful to humans (young *et al* .,2006). Lactic acid can be produced by chemical synthesis and fermentation. In fermentation condition, a pure form of lactic acid can be produced but in chemical synthesis it is a racemic mixture.Lactic acid can be produced by bacteria and fungi. *Rhizopus* fungi is able to produce lactic acid by aerobic condition with high production rate but more incubation time is needed. Lactic acid bacteria are the important organisms for the lactic acid production. It is mostly present in plants, meat products and dairy products (Jatindra nath mohanty *et al*., 2015.)These bacteria have high growth rate with high product yield and specificity (Antonija trontel *et al.,* 2010.)

Lactic acid bacteria can be categorized into two groups, homofermentative and heterofermentative. Homofermentative lactic acid bacteria convert sugars into lactic acid, while heterofermentative convert the sugars into lactic acid and ethanol. *Lactobacillus* sp were the important genera in lactic acid bacteria and it is Gram positive, non-motile, non-spore forming, non-pigmented and catalase negative organisms and capable of producing lactic acid using various carbohydrates. In every year, food and agriculture industries release large amount of solid waste. Removal of this solid waste is a difficult task and it is responsible for environmental pollution Sailaja *et al*., 2015. Solid waste can be peels of vegetables, fruits, fibres and dairy wastes and it is able to produce enzymes, organic acids and bio products. Agro waste residue is more important because of low cost, non food nature, easy availability. Renewable agro waste substrates such as cassava, wheat bran, barley, rice bran, corn stover, bagasse, sunflower stalks, cotton stalks, woods, peels of fruits and vegetables are also able to produce lactic acid (Basa janakiram naveena *et al*., 2004 and Muhammad Idrees *et al*., 2013.)

SSF and SmF are the two common conventional approaches for the production of biotechnology products. SSF offers many advantages over SmF such as high volume productivity, low energy requirement, less moisture, high product yield, without aeration and easy downstream processing (Subramaniyam and Vimala 2012).Either in SSF or SmF the maximum production of lactic acid is influenced by microbial strains and optimization parameters such as temperature, pH, nitrogen sources, mineral salts, inoculums concentration, substrate concentration and fermentation time (Pyde acharya nagarjun *et al.,* 2005, Coelho *et al.,*2011, Antara guha *et al*., 2013, Bercem Eldeleklioglu *et al.,* 2013,Nisa saelee *et al.,* 2014,Sweta patel and Samir Parkith 2016.)The present study is aimed to screen and optimize the various *Lactobacillus* strains for the production of lactic acid by using agro waste residues as a substrate for SmF and SSF.

MATERIALS AND METHODS

Organisms

Lactobacillus strains were isolated from dairy products obtained from Coimbatore, Tamil Nadu. The strains were isolated by serial dilution method and it is identified by morphological and physiological characterization. Selected *Lactobacillus* strains were maintained on MRS agar at 37°C anaerobic condition for the subsequent use.

Preparation of various agro waste residues

Agro waste residues such as Apple peel, Cassava peel, Mosambi peel, Orange peel and Pineapple peel were obtained from the local market, Coimbatore, Tamil Nadu, India. They were washed under running tap water, sliced into pieces, spread on the trays, shade dried and ground into fine powder. The residues were stored in polyethylene jar at room temperature for further study.

Steam explosion for Submerged fermentation (Pumiput *et al.,*2008)

Each agro waste residues of forty gram was steam exploded for 1 h and water was added to the wet pre treated material which was boiled at 80 °C for 30 min followed by filtration with cheese cloth. Later the hydrolysate is used for SmF and optimization studies.

Screening of Lactobacillus strain and Agro waste residues for lactic acid production by SSF

In SSF, each agro waste residues of 10g was taken in 250mL Erlenmeyer flasks moistened with 10 mL of sterile distilled water in the ratio 1:1 W/V and sterilized. Then, the flasks were inoculated with *Lactobacillus* sp; the content were mixed and incubated. For lactic acid extraction the distilled water was added to the solid medium and the mixture was shaken in a rotary shaker (100rpm) for 1 hr. The extract was squeezed through a Whattman filter paper and clarified by centrifugation at 10,000 × g for 20 min. The crude lactic acid was used for the estimation of lactic acid.

Screening of Lactobacillus strain and Agro waste residues for lactic acid production under SmF

The hydrolysate medium was inoculated with *Lactobacillus sp* and the content were mixed and incubated. The extract was squeezed through a Whattman filter paper and clarified by centrifugation at 10,000 × g for 20 min. The crude lactic acid is used for the estimation of lactic acid.

Optimization of cultural condition for maximum lactic acid yield

Optimization of cultural parameters for SSF and SmF to achieve maximum lactic acid production was studied at different temperature 28,30,35,37,40,45°C, pH4-8, nitrogen sources peptone, yeast extract, triammonium citrate, meat extract,mineral salts such as magnesium sulphate, manganese sulphate, dipotassium hydrogen sulphate, calcium carbonate, various concentration were also studied for nitrogen and mineral salts. Inoculum concentration 2,4,6,8,10 mL, substrate concentration 2,4,6,8,10 and fermentation time 24,48,96,120 h were studied.

Estimation of lactic acid

The amount of lactic acid in the fermentation broth was titrated..25 mL of crude lactic acid into 100mLflaks one mL of phenolphthalein indicator was added into flasks. It was titrated against 0.1M NaoH for the appearance of pink colour and calculated using the following formula.

$$\frac{\text{Volume of NaoH} \times \text{Molecular weight of glucose} \times \text{Molarity} \times 100}{1000 \times \text{Amount of sample taken}}$$

Statistical analysis

The data were expressed as Mean $\pm$ SD from triplicate determination.

RESULTS

To screening the agro waste residues for the lactic acid production for SSF and SmF were done. Hence the present study is focused to utilize the agro waste residue for the production of lactic acid which can be used for many industries

Organisms screening

Out of 30 isolates, 15 isolates were identified as *Lactobacillus sp* based on their morphology and biochemical characterization. Identified *Lactobacillus sp* were named in the Table 1

Table 1: Sources for the isolation of *Lactobacillus* sp and isolates name

Dairy samples for the isolation of Lactobacillus sp	Isolates name
Cow milk	LAB2, LAB21, LAB23
Curd	LAB4, LAB14, LAB19, LAB26
Youghrt	LAB5
Cheese	LAB11, LAB17, LAB25, LAB29
Buffalo milk	LAB13, LAB30
Goat milk	LAB18

Screening of lactic acid production under SSF and SmF

Different agro waste residues such as Apple peel, Cassava peel, Mosambi peel, Orange peel and Pineapple peel were supplemented for the screening of maximum production of lactic acid under SSF and SmF in the Fig (1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9)

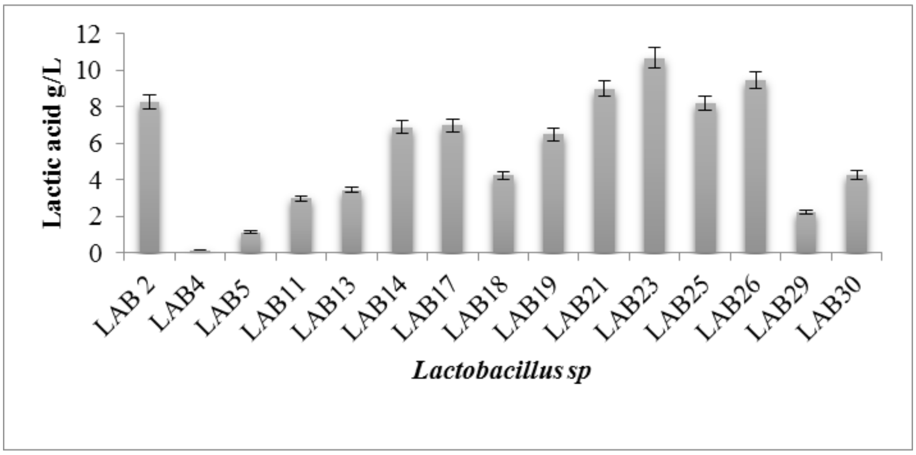

Fig 1.1Screening of lactic acid production using Apple peel using Lactobacillus sp by SSF

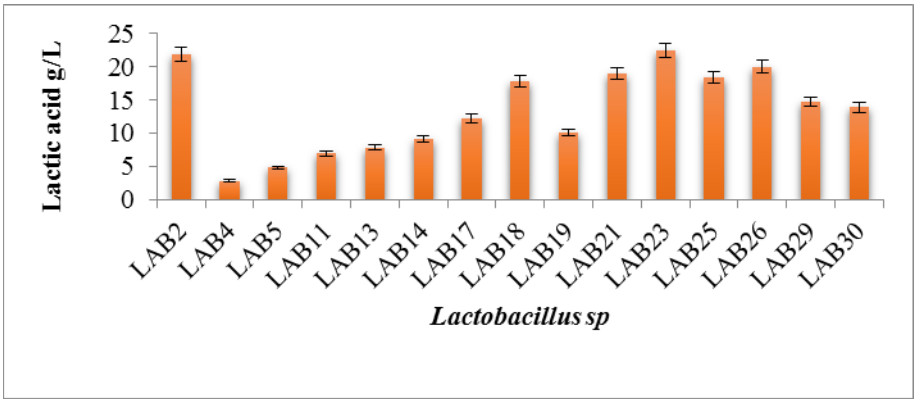

Fig 1.2 Screening of lactic acid production using Cassava peel using *Lactobacillus sp* by SSF

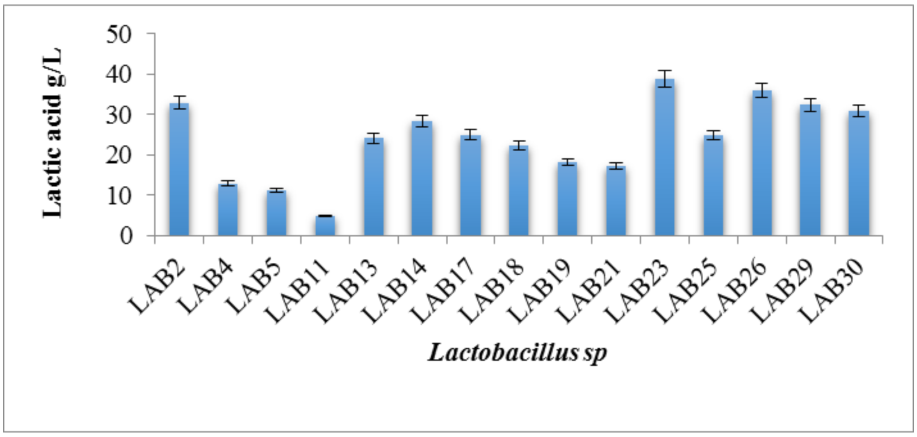

Fig 1.3 Screening of lactic acid production using Mosambi peel from *Lactobacillus sp* by SSF

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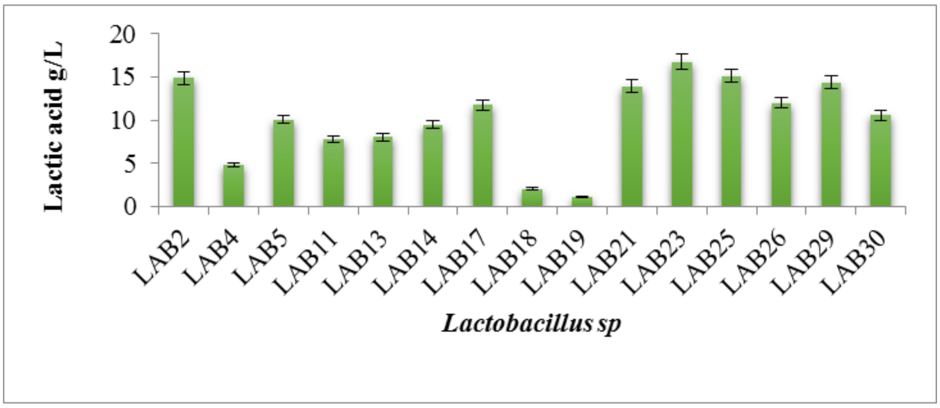

Fig 1.4 Screening of lactic acid production using Orange peel from *Lactobacillus sp* by SSF

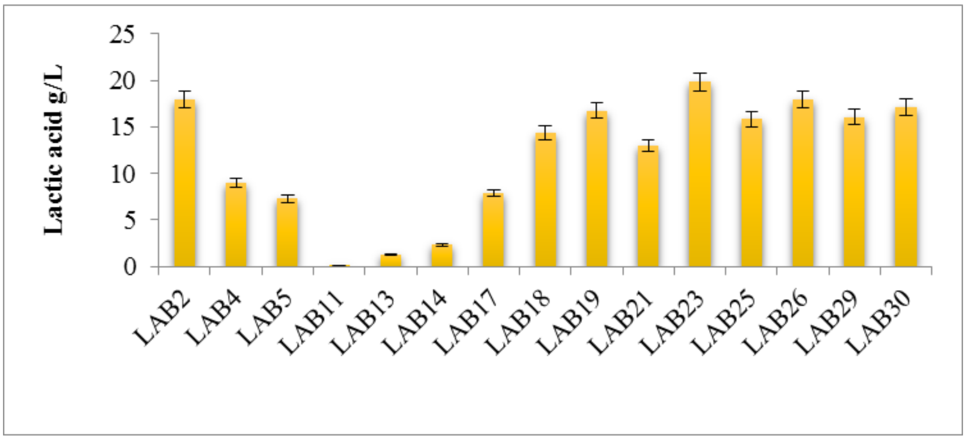

Fig 1.5 Screening of lactic acid production using Pineapple peel from *Lactobacillus sp* by SSF

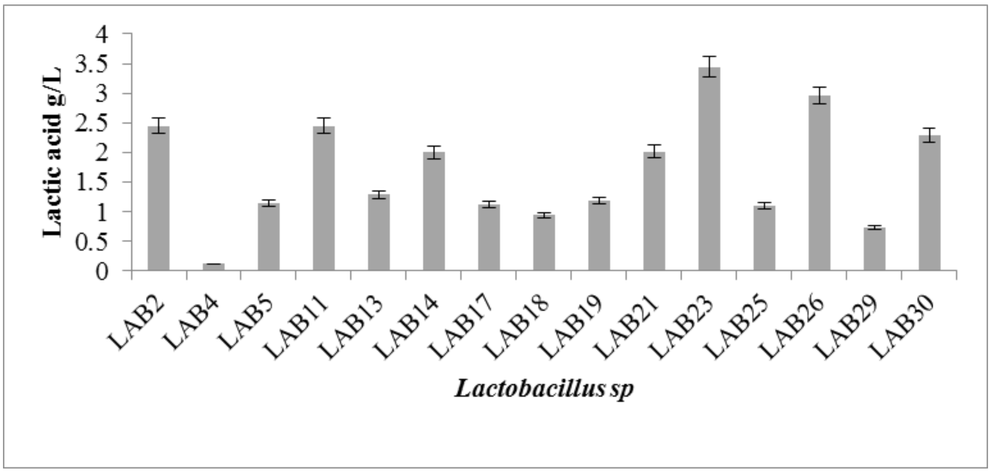

Fig 1.6 Screening of lactic acid production using Apple peel from Lactobacillus sp by SmF

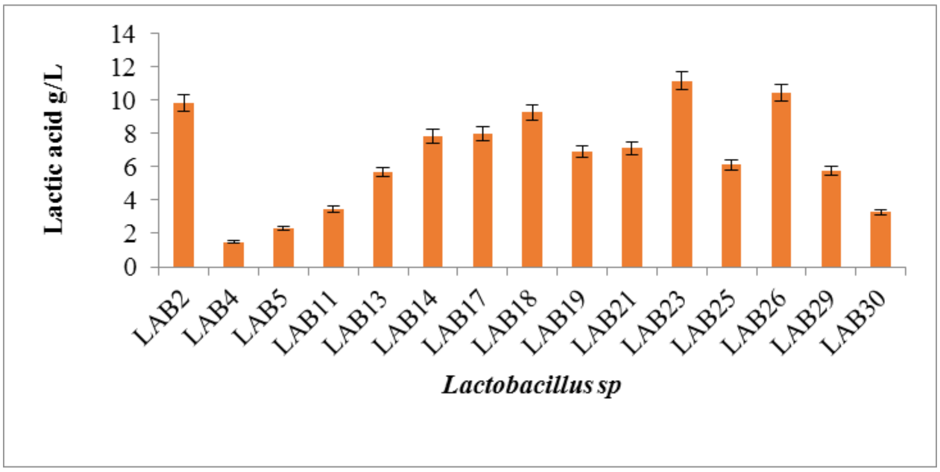

Fig 1.7 Screening of lactic acid production using Cassava peel from *Lactobacillus sp* by SmF

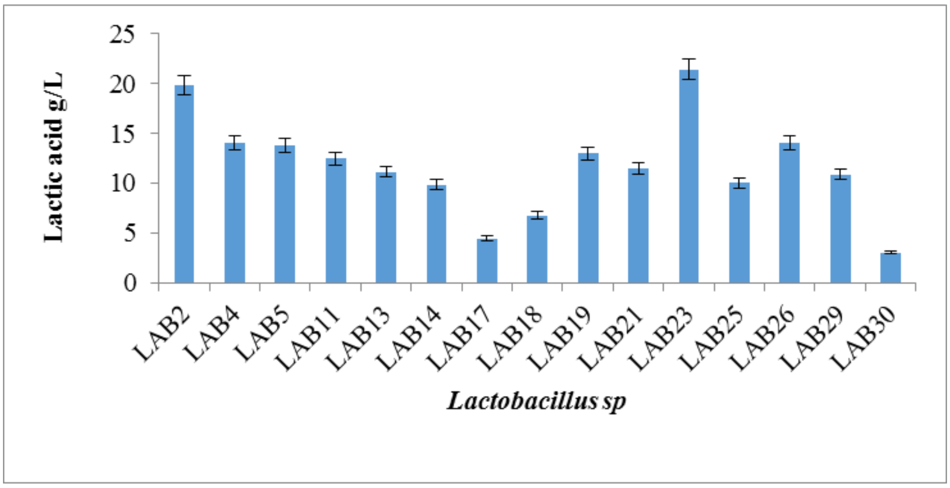

Fig 1.8 Screening of lactic acid production using Mosambi peel from *Lactobacillus sp* by SmF

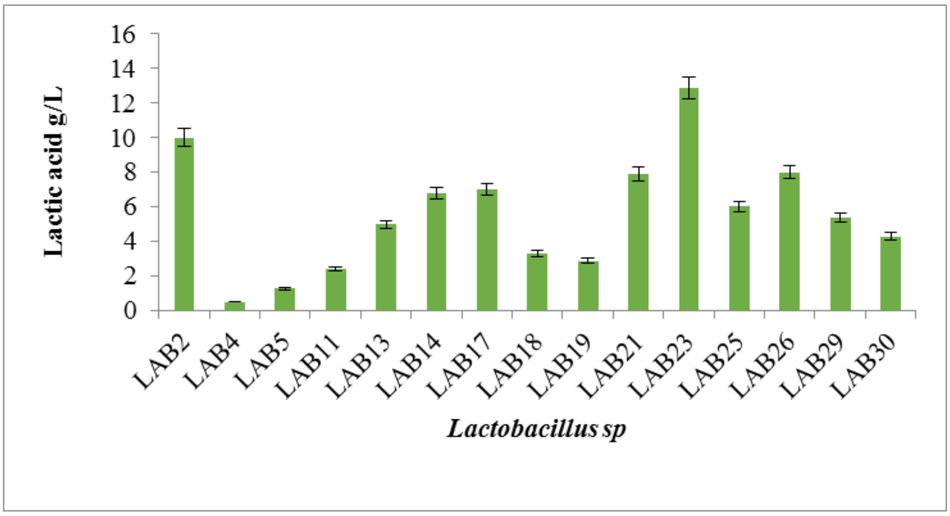

Fig 1.9 Screening of lactic acid production using Orange peel from *Lactobacillus sp* by SmF

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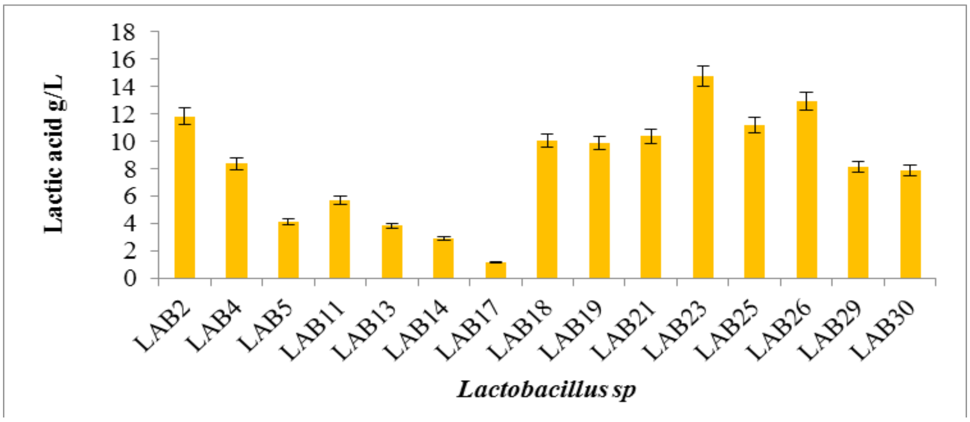

Fig 1.10 Screening of lactic acid production using Pineapple peel from Lactobacillus sp by SmF

Different agro waste substrates such as Apple peel, Cassava peel, Mosambi peel, Orange peel and Pineapple peel were used as a substrate for SSF and SmF. It has been showed that isolated *Lactobacillus sp* was able to produce lactic acid by using these substrates. Among used substrates Mosambi peel served as the best substrate for the production of lactic acid using *Lactobacillus sp* (LAB 23) from SSF.

Effect of temperature

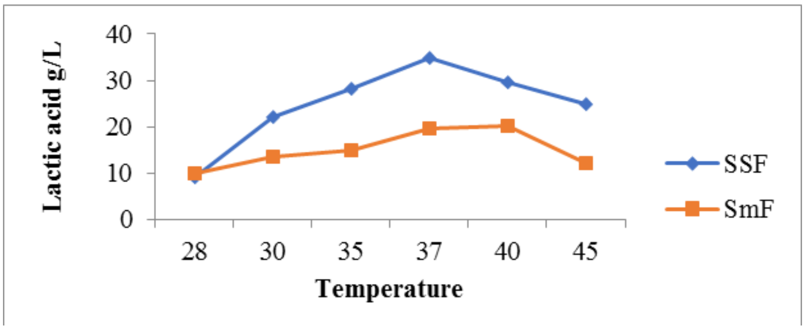

Fig 2: Effect of temperature on lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel

The effect of different temperature on lactic acid production was evaluated. The highest yield of lactic acid was achieved at 37°C while in SmF the maximum production of lactic acid were at 35 °C (Fig 2).

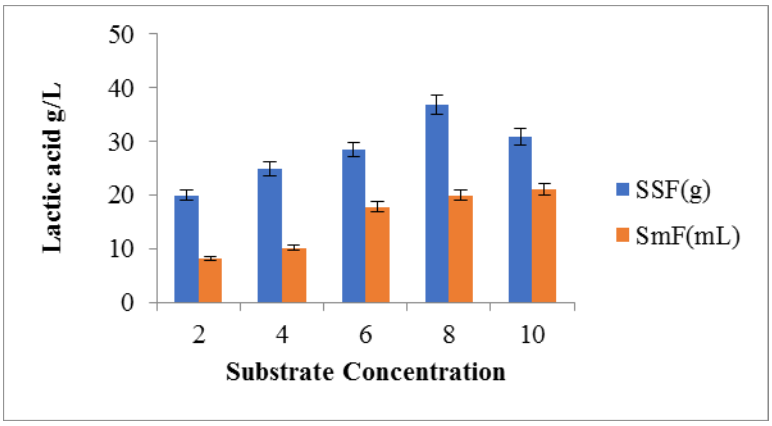

Fig 3: Effect of substrate concentration on the lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel

The different concentration of Mosambi peel for lactic acid production were also studied for the maximum production on substrate concentration in SSF at 8g but SmF 10 mL substrate concentration produce maximum lactic acid production(Fig. 3).

Fermentation time plays a vital role in the production of lactic acid. The medium flasks were incubated at different hours.

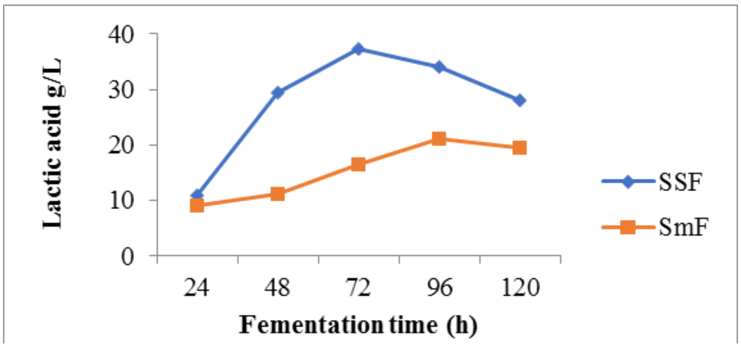

Fig 4: Effect of fermentation time on lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel

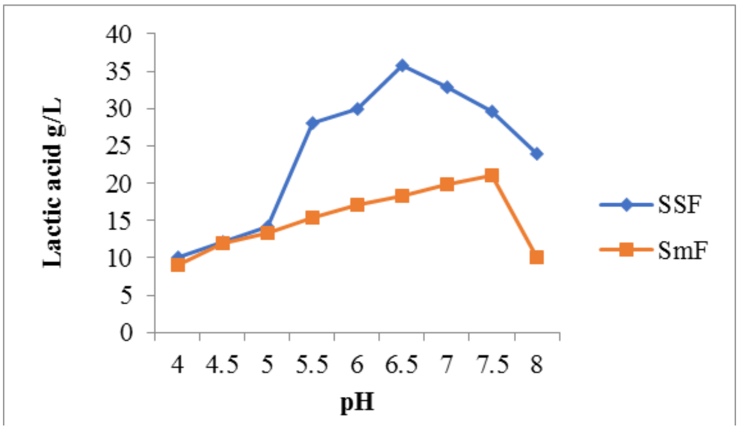

Fig 5: Effect of pH on lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel

The maximum production of lactic acid occurred in 72 h in SSF but in SmF 96 h shows maximum production (Fig 4).To study the effect of pH for lactic acid production in different pH level such as 4 to 8studied. The higher yield of lactic acid occurred in 6.5 pH in SSF and it is reduced to pH7.5 in SmF. (Fig 5).

In the present study inoculums concentration was also play a vital role. The maximum production of lactic acid was found in SSF at 4% inoculums concentration. In SmF 8% inoculums concentration shows the production of lactic acid (Fig 6).

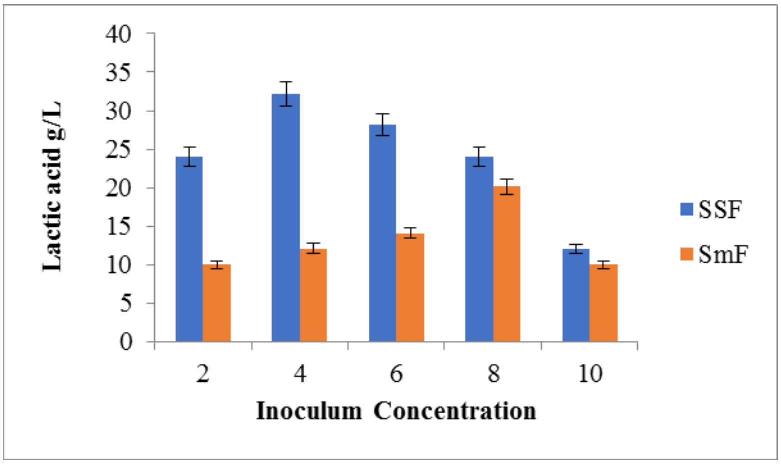

Fig 6: Effect of Inoculum concentration on lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel.

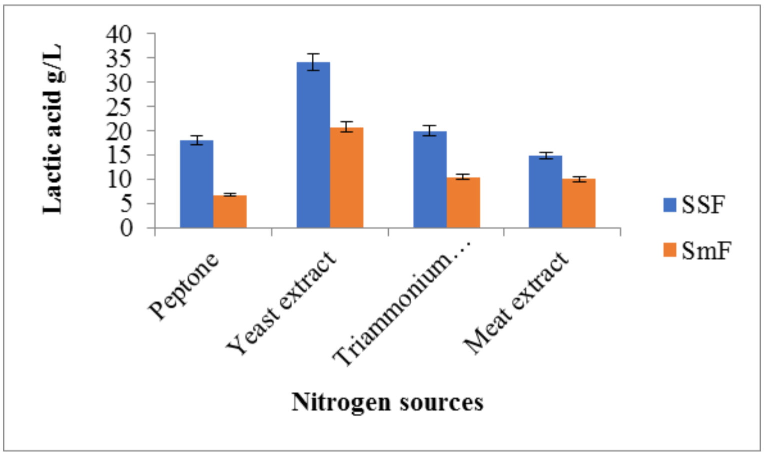

Fig 7: Effect of inoculum concentration on lactic acid production by Lactobacillus sp (LAB23) using Mosambi peel

The *Lactobacillus sp* requires substrate with high nitrogen content. In the present study four different nitrogen sources like peptone, yeast extract, tri-ammonium citrate, meat extract were taken to enhance the production of lactic acid. In SSF and SmF the yeast extract showed maximum production at a concentration of 2.5% when compared to other nitrogen sources and in SmF, the nitrogen concentration was found at 3.5% in yeast extract (Fig. 7 and 8).

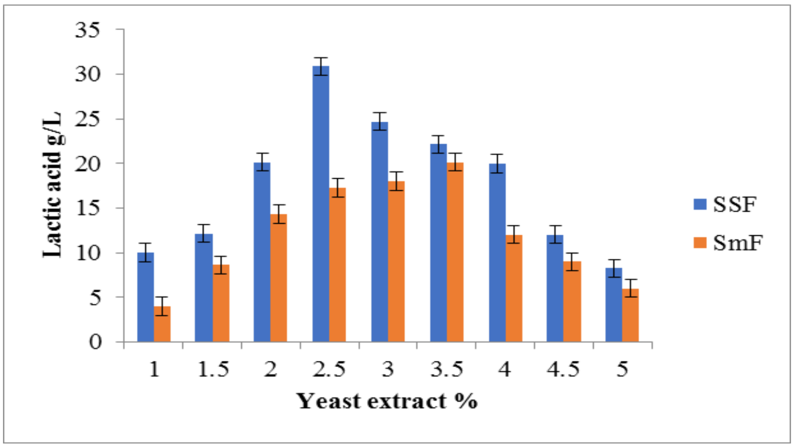

Fig 8: Effect of nitrogen concentration on lactic acid production by *Lactobacillus sp* (LAB23) using Mosambi peel

Mineral salts play a vital role in fermentation. Different mineral salts such as magnesium sulphate, di-potassium hydrogen phosphate, sodium acetate, manganese sulphate, calcium carbonate.

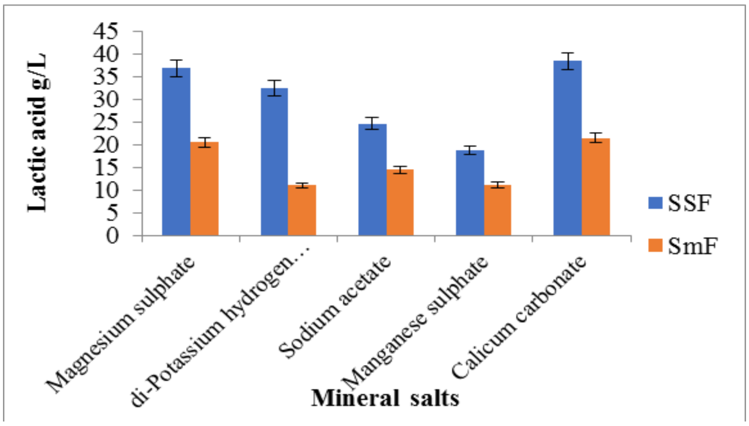

Fig 9: Effect of mineral salts on lactic acid production by Lactobacillus sp (LAB23) using Mosambi peel.

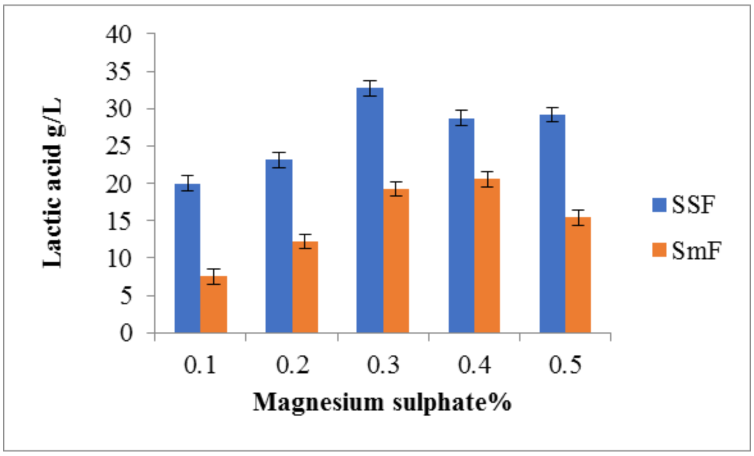

Fig 10: Effect of mineral salts on lactic acid production by Lactobacillus sp (LAB23) using Mosambi peel.

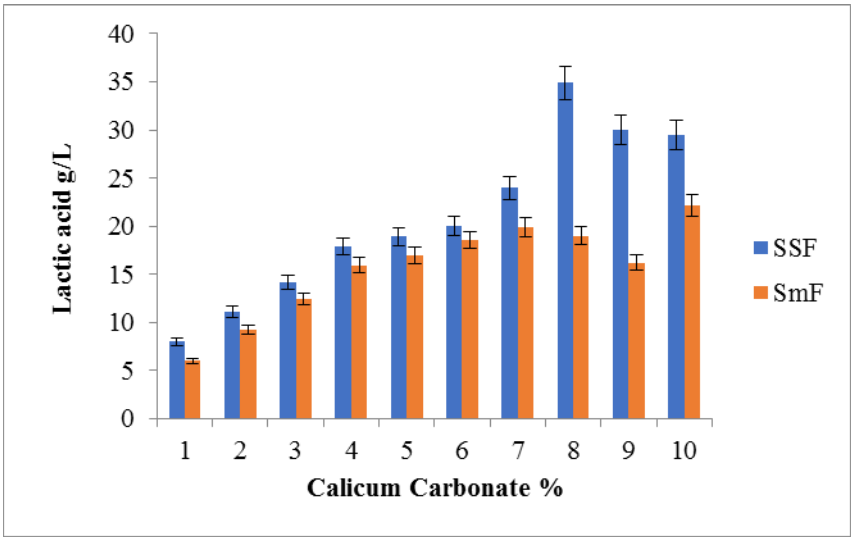

Fig 11: Effect of mineral salt concentration on lactic acid production by Lactobacillus sp (LAB23) using Mosambi peel.

Among these mineral salts highest quantity of lactic acid was produced by magnesium sulphate at a concentration of 0.3% in SSF and in SmF 0.4%. and Calicum carbonate shows maximum production at a concentration 8% in SSF and 10% in Sm F (Fig 9,10 and 11).Magnesium sulphate is added for the salt concentration and calcium carbonate is added for pH balance.

DISCUSSION

In dairy products such as milk, cheese, curd, yoghrut, and fermented milk the lactobacillus are mostly present. It is a starter culture in food industry (Valerie Coeuret *et al*.,2003). 30 strains are isolated from dairy products. According to Ankita chakarborty and Jayati Bhowal 2015 MRS agar is the selective media for the isolation of *Lactobacillus sp.* Colonies appeared as creamy white, circular and low convex in agar plates. These isolates are identified with reference to Abdi *et al.,* 2006 , Ashwani Kumar and Dinesh Kumar 2014 on the basis of morphological and biochemical characteristics. Lactobacillus strains are able to produce lactic acid using various agro waste substrates. *Lactobacillus casei* can produce L (+) lactic acid using potato starch as a substrate Palaniraj and Nagarajan 2012. Niju Narayanan 2004 who report that Lactobacillus delbreuckii, *Lactobacillus helveticus, Lactobacillus amylophylus, Lactobacillus lactis Lactobacillus pentosus*, *Lactobacillus plantarum* these organisms are able to produce lactic acid using carbohydrates. The agro waste residues are generated in the large amount every year in agricultural and food products. These agro waste residues contain cellulose, hemicelluloses, starch etc. SSF has many advantages such as high yield, low water concept etc (Krishna 2005). In the submerged fermentation mango peel produce 10.08g/L minimum lactic acid (Mridul umesh and preethi 2014) while in SSF, it produces 38.84g/L of lactic acid using Mosambi peel by *Lactobacillus* *sp*. Optimization parameters are very important for the production of lactic acid. The effect of temperature has a predominant role in lactic acid production which high in 37°C in SSF. While compared with (Sheeladevi and Ramanathan 2011 and Vethakanraj Helen Shiphrah et al., 2013) high production of lactic acid is observed in 37°C. Substrate concentration plays a vital role in fermentation process, 8g of substrate concentration shows the maximum production of lactic acid. In SSF whole substrates is used while in SmF only the hydrolysate is used and only the few nutrients are supplied for the production of lactic acid. Maximum lactic acid production was observed in 72 h in SSF but in SmF, it produced minimum production of lactic acid in 120. Optimum condition of fermentation time120h shows the maximum production of lactic acid in (Panda and Ray 2008). Various researchers studied for the effect of pH on lactic acid production. In the present study, pH 6.5 shows high yield of lactic acid however pH 6.8 is considered for the production of high yield lactic acid. The maximum production of lactic acid is observed in 4% inoculum level (Antara guha *et al.*2013). Nitrogen sources are needed for the production of lactic acid. Yeast extract shows the maximum production of lactic acid in SSF and SmF. Highest production of lactic acid is found in yeast extract (Gupta and Gandhi 1995). Mineral salts play a vital role in fermentation. In contrast Zhang and Jin 2009 reported that magnesium sulphate and calcium carbonate produce maximum lactic acid production.

CONCLUSION

In this current research work, screening of lactic acid production from the different *Lactobacillus* strains, the highest lactic acid production is found in (LAB 23) *Lactobacillus sp* by using Mosambi peel as a substrate. From the present study it was concluded that Mosmabi peel has the potential to produce high yield of lactic acid from (LAB 23) *Lactobacillus sp*. This would be ensure for the conversion of waste product into a by product and it have the potential application in various industries.

ACKNOWLEDGEMENT

The authors are sincerely grateful to the management authorities of Karpagam Univesity, Coimbatore-641 021, Tamil Nadu, India, for the constant encouragement and support.

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