# Food waste management - a cheap source of lactic acid produced by *Lactobacillus* sp.

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**Abstract**. The most common and important chemical compound used in pharmaceutical, cosmetic, chemical and food industry is lactic acid. There have been various attempts made to produce lactic acid efficiently from inexpensive raw materials. The main objective of present study was to produce lactic acid from cheap food waste such as potato peels, orange peels and mango peels as substrate. A total of 35 isolates were screened for *Lactobacillus* spp. On the basis of temperature and pH optimization, 4 bacterial isolates (5SA, 21SA, 22SA, 32SA) were selected for further study and fermentation. The highest lactic acid production, 12.23 g $L^{-1}$ was obtained for mango peels where as for orange peels it was 11.98 g $L^{-1}$ for 21SA isolate. Isolates 5SA and 22SA produced 13.08 g $L^{-1}$ and 12.54 g $L^{-1}$, respectively, lactic acid for potato peels at the 3rd of fermentation. For mixed peel waste, 32SA isolate was able to produce maximum 11.56 g $L^{-1}$ lactic acid after fermentation at the end of 3rd day. Thus it shown that lactic acid can be efficiently produced from food wastes at a cheaper cost and high rate.
**Key Words**: lactic acid, food waste, *Lactobacillus*, pollution, food peels.

**Introduction**. The increase in population, urbanization and development of a large industries, leads to increase in pollution (Prabhakar 2001). Environmental pollution by waste generated from economic activities such as chemical, petrochemical, agricultural and food industries are common problems faced by the world. Food waste is a global phenomenon that impacts the environment and society in four ways: the energy used to produce food, the food disposed of by retailers, the food wasted in the household and the energy used to dispose of food waste (Moon & Woodroof 1986; Kroyer 1991).

Food waste is a bigger problem in developed countries than in developing countries (Table 1). It had been calculated that 19 percent of the available, edible food supply in the U.S. is wasted by consumers, costing the average American household $936 a year (Buzby & Hyman 2012). Food waste directly affects environmental agencies and municipalities because in landfills food waste is a primary cause of methane gas emissions (a very potent greenhouse gas) and the methanogens which cause the mobilization of other pollutants in landfills, resulting in an increase in both air pollutants and leachate. Food waste is a cause of nitrogen oxide emissions, which is also a greenhouse gas, as well as a cause of smog and respiratory illness (Gunders 2012).

Table 1

Solid waste generation across the world (Naska et al 2001)

| *Countries* | *Solid waste generation per day (kg person$^{-1}$ day$^{-1}$)* |
|---|---|
| Highly developed countries | 1.2 to 1.8 |
| Industrial countries | 0.7 to 1.2 |
| Medium income countries | 0.5 to 0.7 |
| Low income countries | 0.3 to 0.6 |

Recycling food-waste for producing products, such as organic soil amendment (OSA) and bioenergy, has received a great level of interest recently. Organic waste treatment processes such as composting processes (i.e., windrow composting, vermicomposting, and powered composting) (VermiCo 2013; Purkayastha 2012; Munnoli et al 2010) and anaerobic digestion processes (Shin et al 2010; Quiroga et al 2014; Dai et al 2013; Bernstad et al 2013; Rounsefell et al 2013) are promising technologies.

Microorganisms such as *Flavobacterium, Pseudomonas, Micrococcus, Aeromonas, Xanthomonas,* and *Vibrio* are found to degrade the food waste compounds. The microbes have the potential to break and degrade the waste toxins eg: *Deinococcus radiodurans* bacterium may be used to manage the nuclear waste. During the treatment of sewage,

bacteria convert the nitrogenous compounds to carbon dioxide and nitrogen (Litchfield 1987; Kannaiyan & Lily 1999).

There is a potential for food processing waste such as fruits and vegetables waste to be used as raw material, or can be used for the conversion into useful and higher value added products (Mudaliyar & Kulkarni 2011). About 30% of the fruits are turned into waste during the canning operation. These wastes contain high content of carbohydrate that can be utilized for the production of organic acid. Based on the physio-chemical properties of the food waste can be potentially used as carbon sources for production of lactic acid by microbial systems (Holten 1971; Kroyer 1991).

Lactic acid is the most widely used multifunctional organic acid, of which 85% is used in food and food related applications (Ronald 1946; Zhou et al 1999; Sreenath et al 2001; Naveena et al 2004). L-Lactic acid is found in many foods and beverages produced naturally by lactic acid bacteria such as *Lactobacillus* sp., *Lactococcus* sp. Lactic acid is found in virtually all tissues, physiological fluids and excretions. It is also found in human blood (1.4 µmol $mL^{-1}$), in sperm (4.1 µmol $mL^{-1}$), in sweat (4.0–40.0 µmol $mL^{-1}$), in the cerebrospinal fluid (1.6 µmol $mL^{-1}$) and in urine (0.3 µmol $mL^{-1}$) (Deraz et al 2011). Most important application of lactic acid is its use for the manufacture of biodegradable and biocompatible polylactate polymers (Khalaf 2001). Microbial sources include many bacteria such as *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Clostridium* and *Weissella* have been reported to produce lactic acid (Bogaert & Coscach 2000). The biological production of lactic acid is generated primarily through bacterial fermentation of simple sugars (Laufenberg et al 2003). Bacterial strains, such as *Lactobacillus* sp. and *Lactococcus* sp., have been used in many industrial processes because of their high growth rates and product yields (Gonzalez et al 2000). Present research having aim to elaborate the possibilty of lactic acid production from food wastes at a cheaper cost and high rate.

**Material and Method**. For the isolation of *Lactobacillus* sp*.,* soil sample was collected from the garden and field side of Bhojia Institute of Life Sciences, Budh (Baddi), H. P., India (November 2014 to July 2015). Serial dilution plate method was used and followed by spreading and streaking methods for pure culture isolation on the MRS (de Man, Rogosa and Sharpe) agar plates for the isolation of *Lactobacillus* sp. colonies at 32±2$^{o}$C for 24 hours. Identification of bacterial isolates were carried out by biochemical reactions as described in Bergey's Manual of Determinative Bacteriology (Holt 1994).

A total 35 isolates of *Lactobacillus* sp. were biochemically characterized for Gram staining, motility, starch hydrolysis, $H_2S$ production, triple sugar iron, citrate utilization, oxidation reaction, casein hydrolysis, 3-ketolactose production, urease production, catalase test, lipolysis activity, nitrate reduction, malonate utilization, indole production, pigment production, HCN production and gelatin liquefaction.

Further, isolates were optimized for pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 respectively at 32±2$^{o}$C) and temperature (25$^{o}$C, 30$^{o}$C, 35$^{o}$C, 40$^{o}$C respectively at pH 6.5 for 24 hrs ). After proper incubation absorbance was recorded at 600 nm. On the basis of maximum absorbance, isolates 5SA, 21SA, 22SA and 32SA were selected for further study.

***Lactic acid production from food waste by Lactobacillus sp.***

*Raw material processing.* A total of 3 different food wastes i.e. peels of potato, mango and orange were used for the screening of the amount of sugar content by preparing their hydrolysate and used for the production of lactic acid by *Lactobacillus* sp*.* isolates.

***Preparation of hydrolysate***

*Steam explosion.* Fourty (40) g of each food waste substrate was steam-exploded in autoclave at 121°C for 20 min. Sterilized distilled water was added to the wet pre-treated material to make up the volume 1 L and boiled at 80oC for 30 min. Later, the hydrolysate was recovered by filtration with Whattman filter paper no. 1.

*Acid hydrolysis*. Acid post hydrolysis of hydrolysate was carried out by autoclaving at 121°C with concentration of 1% HCl v/v for 30 min.

*pH adjustment.* pH of hydrolysate from acid post hydrolysis was adjusted to 6.5.

***Fermentation (batch culture)***

*Microbial strain.* The pure culture of isolated *Lactobacillus* sp*.* was grown on MRS agar media.

*Inoculum preparation:*

- *Lactobacillus* sp. grown on MRS agar media, individually, was sub cultured in liquid synthetic MRS medium and then 10 mL was inoculated in different test hydrolysate separately.
- The culture media containing hydrolysate were prepared in conical flasks by adding components of synthetic media (0.034 g $FeSO_4$, 1.0 g sodium acetate, 1.23 g $MgSO_4 \cdot 7H_2O$, 0.034 g $MnSO_4 \cdot H_2O$, 0.65 g $K_2HPO_4 \cdot 3H_2O$, 0.5 g $KH_2PO_4$, 4.0 g yeast extract) in 100 mL of each hydrolysate.
- Above media was kept for incubation at 35$^oC$ on rotary shaker at 120 rpm for 3 days.

*Media and fermentation conditions.* To screen for lactic acid production from food waste hydrolysate, 5% of the inoculum was added separately to 250 mL of fermentation medium and incubated at 35$^oC$ temperature at 120 rpm for 6 days. The fermentation medium was similar to the inoculum medium for each test hydrolysate. The substrate consumption and also product (lactic acid) formation was examined on the basis of titration.

***Lactic acid production and estimation***. For estimation of lactic acid 50 mL of fermented broth was taken in centrifuge tubes and centrifuged at 10000 rpm for 5 minutes to pellet out the bacterial growth for each hydrolysate. Then the supernatant was transferred to a beaker and the solution was heated to 80$^oC$. pH was adjusted to 7. Broth was filtered by using Whatmann filter paper no. 42, filtrate was discarded. The precipitate in the filter paper was dissolved in a conical flask by using minimal volume of 0.1 N HCl. After dissolution of the precipitate, the solution was diluted using distilled water for titration against 0.1 N NaOH using phenolphthalein as indicator. The end point of titration was recorded. Lactic acid production was estimated by following formula:

$$(S \times X) \times 1.8\ gL^{-1}$$

Where: S = strength of NaOH;
X = volume of tyrant.

## Results

***Isolation of Lactobacillus sp***. A total of 35 colonies of *Lactobacillus* sp. were isolated from the soil sample on MRS agar media and characterized on the basic of staining techniques and biochemical tests. These isolates were named from 1SA to 35SA for further experiments. All 35 isolates were gram positive non motile, rods of varying size, endospore negative. Isolates shown positive reaction in triple sugar iron, urease, casein, methyl red, malonate utilization and starch hydrolysis while catalase, citrate, nitrate reduction, indole production, Voges proskauer test, hydrogen sulphide production, gelatin liquefaction, O/F and HCN were found to be negative.

Isolated bacterial strains showed the complete hydrolysis of maltose, dextrose, lactose, mannose, galactose, fructose, xylose and raffinose as a sole carbon source.

***Optimization of growth conditions***. Isolated bacterial strains were optimized for growth at different pH ranging from 6 to 9 at 32±2$^oC$ and temperature (25$^oC$, 30$^oC$, 35$^oC$, 40$^oC$ respectively at pH 6.5) over a period of 24 hours. The absorbance was recorded at 600 nm. All isolates shown maximum absorbance at pH 6.5 (Table 2). Temperature based optimizatoion shown that isolates 5SA, 21SA, 22SA and 32SA shown maximum absorbance at temperature 35$^oC$ as shown in Table 3.

Table 2

Data of pH optimization of bacterial isolates

| *Ph* | *Absorbance at 600 nm* | | | |
|---|---|---|---|---|
| | *5SA* | *21SA* | *22SA* | *32SA* |
| 6.0 | 0.310 | 0.211 | 0.330 | 0.221 |
| 6.5 | 0.665 | 0.451 | 0.610 | 0.510 |
| 7.0 | 0.418 | 0.410 | 0.512 | 0.425 |
| 7.5 | 0.417 | 0.321 | 0.406 | 0.398 |
| 8.0 | 0.225 | 0.301 | 0.200 | 0.312 |
| 8.5 | 0.111 | 0.118 | 0.115 | 0.198 |
| 9.0 | 0.100 | 0.090 | 0.100 | 0.118 |

Table 3

Data of temperature optimization of bacterial isolates

| *Temperature* | *Absorbance at 600 nm* | | | |
|---|---|---|---|---|
| | *5SA* | *21SA* | *22SA* | *32SA* |
| 25°C | 0.04 | 0.09 | 0.02 | 0.10 |
| 30°C | 0.08 | 0.15 | 0.10 | 0.21 |
| 35°C | 0.41 | 0.38 | 0.45 | 0.55 |
| 40°C | 0.12 | 0.11 | 0.09 | 0.14 |

***Lactic acid production from different food substrates***. Results of lactic acid produced from peels of potato, mango, orange and mixture (peels of potato + mango + orange) on different food substrates are shown in Figures 1-4 for all four isolates.

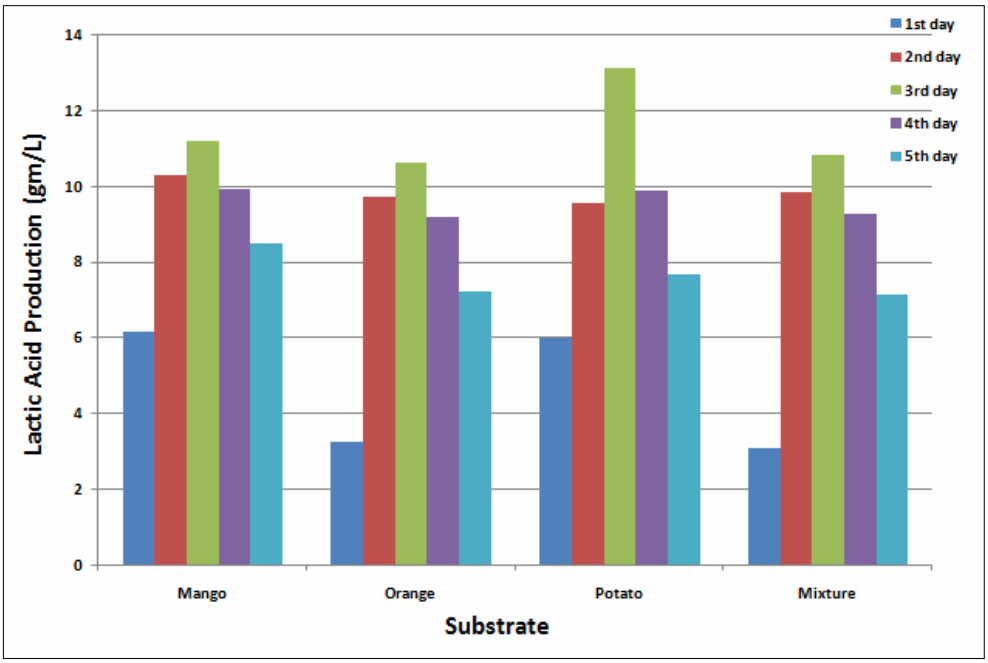


Figure 1. Lactic acid produced from different food substrates for bacterial isolate 5SA.

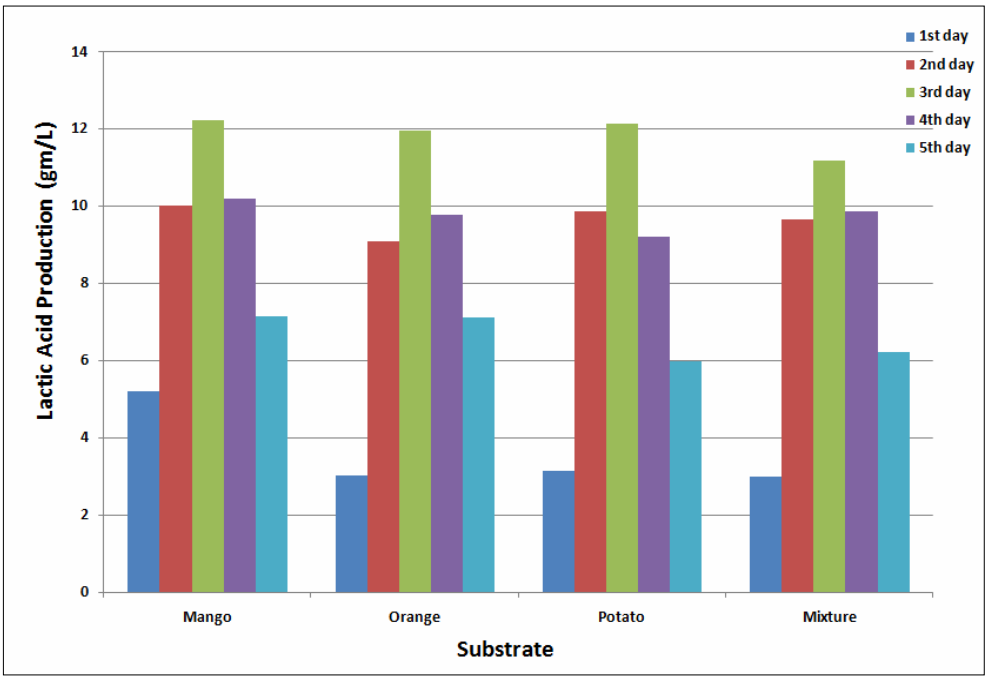


Figure 2. Lactic acid produced from different food substrates for bacterial isolate 21SA.

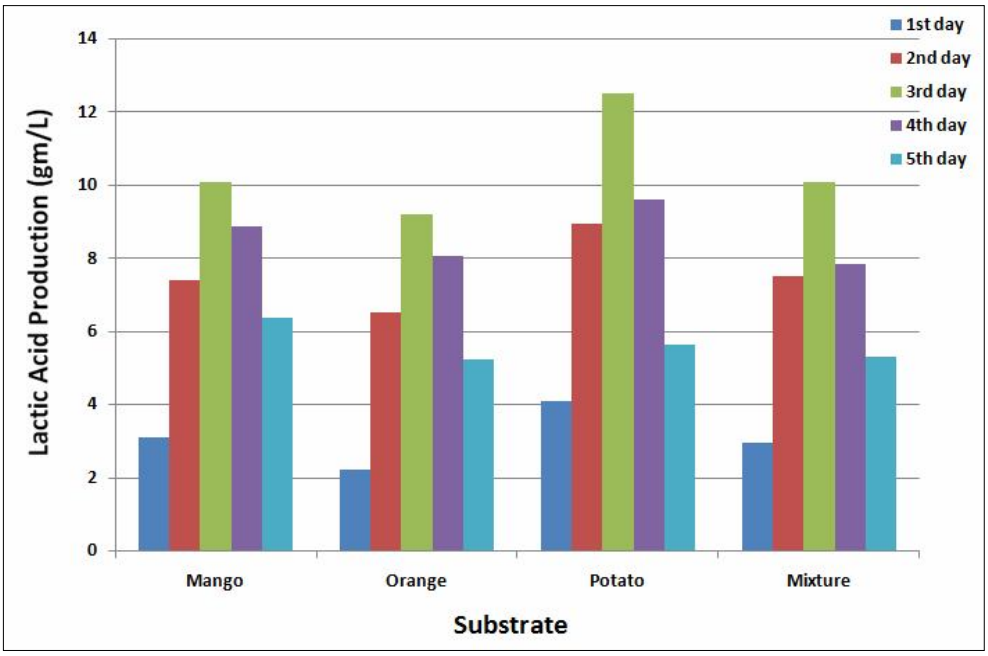


Figure 3. Lactic acid produced from different food substrates for bacterial isolate 22SA.

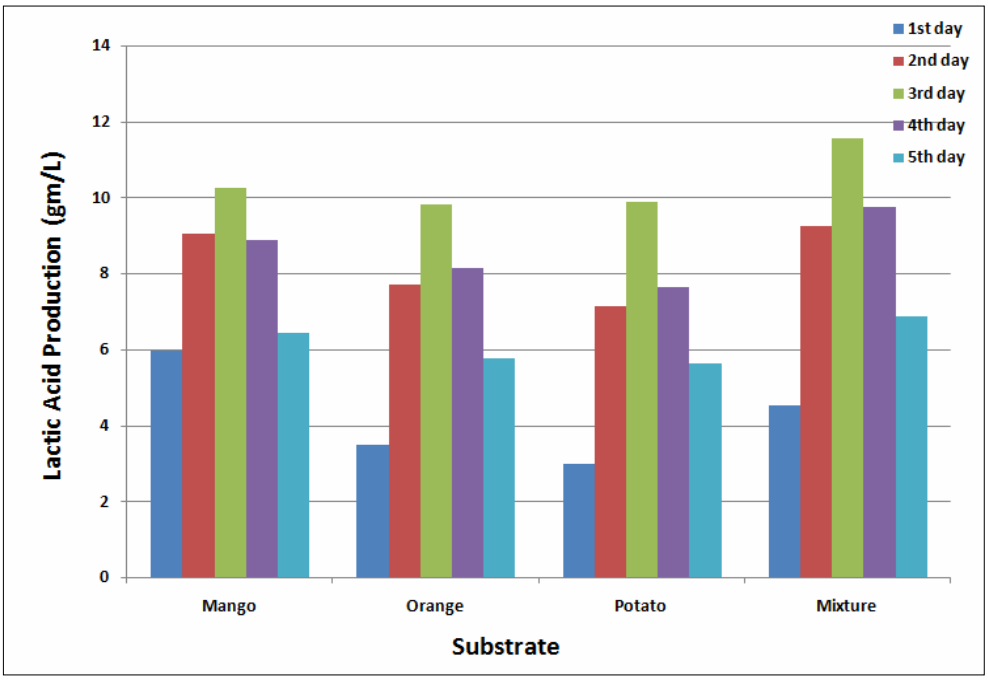


Figure 4. Lactic acid produced from different food substrates for bacterial isolate 32SA.

It observed that isolate 21SA produced maximum lactic acid from mango and orange peel waste (Figure 2) while 5SA and 22SA produced maximum lactic acid in potato waste (Figures 1 and 3, respectively) at 3$^{rd}$ day. Isolate 32SA was only able to produce highest quantity of lactic acid with mixture of all waste. All 4 isolates shown a increment in lactic acid production from day 1 to 3 but shown declined after day 3.

**Discussion**. The isolated bacterium was characterized on the basis of biochemical tests. In case of sugar hydrolysis isolated bacterium showed the complete hydrolysis of maltose, dextrose, lactose mannose and galactose after 24 hours. Isolated bacterium showed positive triple sugar iron test, urease, malonate utilization, casein hydrolysis and starch hydrolysis while catalase, citrate, nitrate reduction, indole, methyl red, Voges Proskauer, hydrogen sulphide production, gelatin liquefaction, O/F and HCN were found to be negative. Similar observations were recorded by Cheesbrough (1992) for *Lactobacillus delbruekii.*

The isolated bacterium was checked for its maximum growth at optimum pH and temperature. Jose et al (1998) and Berecka et al (2010) recorded that the isolated *Lactobacillus* sp. showed maximum growth at pH 6.5 and at temperature 35$^{o}$C and then bacterium showed minimum growth, similar results was recorded with our bacterial isolate which showed maximum growth at pH 6.5 (0.665) and temperature 35$^{o}$C (0.55) and minimum growth at pH 9.0 (0.090) and temperature 40$^{o}$C (0.09) in MRS broth, when optimization of pH was carried from 6.0 to 9.0 and temperature from 25$^{o}$C to 40$^{o}$C.

After optimization lactic acid production by *Lactobacillus* sp. from food wastes (mango, orange, potato and mixture of all these) was recorded as shown in Figures 1-4. It was observed that there were increase in lactic acid production and then rapidly decreased. Similar observation was recorded by Wee et al (2006). Maximum lactic acid was produced at 3$^{rd}$ day from mango waste (12.23 g L$^{-1}$), orange waste (11.98 gL$^{-1}$), potato waste (13.08 g L$^{-1}$) and mixture of wastes (11.56 g L$^{-1}$) then lactic acid production was decreased, when lactic acid production was carried out for 5 days. Similar observations were recorded by Umar et al (2012).

**Conclusions**. Pollution in the environment is caused due to chemical, petrochemical, agricultural and food industries. Various methods of waste treatment are carried for the proper environmental regulation. Our study concluded that food waste can be used for

the production of industrially important lactic acid through lactobacillus, that can be used in pharmaceutical, chemical and food industry

The main objective of this work was to produce lactic acid from food waste such as potato peels, orange peels, mango peels as substrate. The bacteria of genera Lactobacillus was used to produce lactic acid. The highest lactic acid production was obtained from potato, mango, orange and mixtures of all three peels samples (13.08g.$l^{-1}$, 12.23g.$l^{-1}$, 11.98g.$l^{-1}$ and 11.56g.$l^{-1}$ respectively). Thus it shows that lactic acid can be efficiently produced from food wastes at a cheaper cost and high rate.

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