Lactic Acid Production from Kitchen Waste with a Newly Characterized Strain of *Lactobacillus plantarum*

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To enhance the fermentative production of lactic acid (LA) from kitchen waste, a strain of high-yield lactic acid bacterium, designated as TD46, was characterized and its fermentation profiles were investigated. The strain TD46 could produce 91.34 g l^{-1} of LA from 100 g l^{-1} of glucose in 96 h of fermentation at pH 5.5~6.0 and 30 °C. On the basis of its fermentation ability to 49 carbohydrates and additional physio-biochemical tests, the strain TD46 was tentatively identified as *Lactobacillus plantarum*. The strain TD46 produced 28.85 g l^{-1} of LA from non-autoclaved kitchen waste in 48 h of fermentation at pH 5.5~6.0, which was 75.1 % higher than that of the spontaneous fermentation without inoculum (control). Meanwhile, 0.39 g g^{-1} of LA yield and 0.60 g l^{-1} h^{-1} of average productivity were reached, respectively. This study shows that enhancement of LA production from kitchen waste can be realized by using the high-yield strain TD46.

Key words
Kitchen waste, lactic acid, *Lactobacillus plantarum*, fermentation

Introduction

Biological solid wastes, such as kitchen waste discharged from households and restaurants and refuse from the food industry, account for a considerable proportion of municipal solid garbage in China. For example, over 1 300 tons of kitchen waste was generated per day in Shanghai, and over 1 000 tons in Beijing.[1] When the waste is left alone, it easily putrefies, resulting in groundwater contamination and offensive odour generation. On account of its high water content, the incineration process is not suitable to dispose this kind of waste.[2] It is difficult to combust without auxiliary fuel, and incineration facilities can be damaged by temperature fluctuations. So far, the recovery of energy by methane fermentation and the production of organic fertilizer by composting have been used as recycling methods for kitchen waste.[3] *Shirai*[4] developed a new system for reducing and recycling kitchen waste. In this system, discharged waste from families and restaurants is transported to a production factory for lactic acid (LA). After the waste is sterilized and inoculated with lactic acid bacteria (LAB), LA can be produced. The remainder obtained after separating LA is utilized as high quality fertilizer.

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LA is a valuable industrial chemical, used 1) as an acidulant, flavor and preservative in the food, pharmaceutical, leather, and textile industries; 2) for the production of base chemicals; 3) for polymerization to biodegradable poly LA (PLA).[5–7] LA exists as two optical isomers, D- and L-LA. For the production of PLA, either L- or D-LA or a racemic mixture can be used. The properties of PLA vary with isomer composition; it can, therefore, be used for different applications. Pure L- and D-polymers are crystalline and more stable than amorphous, racemic polymers. PLA is considered to be one of the most promising biodegradable polymers, which might play an increasing role in solving a worldwide environmental problem. That is, PLA could be a substitute for non-biodegradable plastics, if more economical raw materials, processes and suitable microorganisms could be developed.[8]

Recently, renewable resources including cellulose, hemicellulose, and starch used as the substrates for fermentative production of LA have been studied in detail.[8–10] Fermentative LA production from these renewable resources usually comprises hydrolysis of substrate to sugars followed by fermentation of sugars to LA using lactic acid bacteria (LAB). The fastidious LAB have complex nutritional requirements due to their limited ability to synthesize B-vitamins and amino acids.[11] As a result, the addition of various concentrations of nutri-

ents in the form of e.g. yeast extract, peptone or corn steep liquor is crucial to facilitate LAB proliferation and to increase LA yield from cellulose or starch. *Norton* et al.[12] stated that the use of these nutrient supplements in large quantities is very expensive and can reach as high as 32 % of the total LA production cost. From the viewpoint of reducing costs, therefore, it is more advantageous for LA production with organic wastes containing carbohydrates and other nutrients enough for LAB proliferation.

Kitchen waste is characterized by a high organic content, as it contains soluble sugars, starch, lipids, proteins, cellulose, and other compounds that are readily biodegradable, and generally few compounds that inhibit bacteria.[2] Therefore, it is the fine substrate for LA production by LAB. Fermentative production of LA with kitchen waste, not only can eliminate its pollution problems, but is also conducive to reducing production cost of LA. *Sakai* et al.[13] and *Wang* et al.[14] investigated LA production from kitchen waste, and confirmed that LA could be stably accumulated by controlling suitable fermentation conditions (temperature, oxygen, pH etc.). To our knowledge, however, the application of high-yield LAB on enhancement of LA fermentation of kitchen waste has not been particularly researched.

Authors have recently isolated a strain of high-yield LAB, designated as TD 46, from anaerobically fermented kitchen waste. In this paper, we report its characterization and application on enhancement of LA production from kitchen waste.

Materials and methods

Microorganisms

The strain TD46 was isolated from kitchen waste. The procedures of isolation were based on the method of *Wang* et al.[15] *Lactobacillus plantarum* AS1.555 provided kindly by Department of Food Engineering, Changchun University of Agriculture and Animal Science, China, was used as a reference strain. Both of them were stored at –20 °C in Man Rogosa Sharpe (MRS)[16] broth containing ρ = 20 % of glycerol and were subcultured every 6 months.

Microorganism identification was based on the following examinations: (1) Gram stain, (2) microscopic examination of morphology, mobility and spore-forming, (3) absence of catalase, (4) homolactic or heterolactic character, determined by the capability of the strain to produce CO_2 in MRS broth by "hot-loop" test, [17] (5) arginine deamination, (6) H_2S generation, (7) growth at 15 °C and 45 °C, (8) carbohydrate fermentation profiles using API 50 CH strips and API 50 CHL medium according to manufacturer's instructions (API system, Bio-Merieux, France). Evaluation of results and identification of the strain TD46 were carried out according to Bergey's Manual[18] and APILAB Plus program (Bio-Merieux).

Glucose fermentation by the strain TD 46

Anaerobic batch fermentations were conducted in duplicate in 300 ml of serum vials with 100 ml of pre-reduced medium at 30 °C. Inoculation at ρ = 5 % was performed with an 18 h pre-culture.

The fermentation medium contained (per liter of distilled water): 20 g glucose, 10 g peptone, 5 g yeast extract, 2 g K_2HPO_4, 2 g triammonium citrate, 0.58 g $MgSO_4 \cdot 7H_2O$, 0.2 g $MnSO_4 \cdot H_2O$, pH 6.2. The pH was not controlled during the fermentation. For the fermentation with pH control, the same medium as above-mentioned was used apart from an increase in glucose mass concentration to 100 g l^{-1}. The pH was controlled at 5.5~6.0 by addition of w = 50 % of $CaCO_3$ slurry.

Disposal of kitchen waste

The kitchen waste, used in the following experiments, was collected from the canteen of Harbin Institute of Technology, China. Its basic characteristics are shown in Table 1. The waste was smashed by disposer, stored at –20 °C.

Table 1 – *Characteristics of kitchen waste used in the experiment*

Total solid w/%	Dissolved solid w/%	Suspended solid w/%	Total sugar w_{dm}/%	Crude protein w_{dm}/%	Crude lipid w_{dm}/%	Crude fiber w_{dm}/%
17.22	7.58	9.64	62.68	15.56	18.06	2.26

Fermentation of kitchen waste for LA production

100 g of smashed kitchen waste mixed with 150 ml of water were added to a 500 ml of fermentor installed with gas inflow and effluent ports without feeding any other nutritional substrates. Two types of fermentation modes were employed, respectively, i.e. the fermentation using autoclaved kitchen waste (condition of autoclave: at 121 °C for 30 min) and an open fermentation mode using non-autoclaved waste as substrate. Both of them were carried out with 15 % (v/w) of inoculum size of the strain TD46 at 30 °C. The pH was maintained at 5.5~6.0 by addition of w = 50 % of $CaCO_3$ slurry, unless other stated. During the sampling, N_2 gas was sparged in the substrate.

Analytical methods

The samples were collected at regular intervals. For fermentations of glucose, the biomass concentration was determined by measurement of optical density at 650 nm and related to the dry mass (DM) obtained at 105 °C for 24 h, after two centrifugation (n = 4000 min^{-1} for 15 min) and washing cycles. The rest of the analytical determinations were carried out in the supernatant. Glucose mass concentration was measured by the 3, 5 – dinitrosalicylic acid method.[19] For kitchen waste fermentation, the total sugar mass concentration was measured by the phenol sulfuric method, using glucose as the standard.[20] Each sample was filtered through 0.45 μm-pore membrane after being centrifuged at n = 4000 min^{-1} for 15 min. The filtrate was subjected to analyses of LA. Viable LAB counts in kitchen waste were determined by the method of double MRS agar layer at 30 °C for 72 h.[21] The LA mass concentration was measured by Dionex 2010i (USA) ion chromatograph equipped with a HPICE-AS1 column (Dionex Corporation, USA) operating at 25 °C with a conductivity detector. The eluent was 1 m mol l^{-1} octanesulphur with a flow rate of 0.9 ml min^{-1}, and the regeneration solution was TBA(OH) (tetrabutyl ammonium hydroxide).

Results and discussion

Production of LA from glucose by the strain TD 46

To investigate the fermentation performance of the strain TD46, cultivation was firstly conducted in the medium containing glucose under the conditions of with and without pH control, respectively.

Fig. 1 shows its growth kinetics, pH variation, and LA accumulation in the fermentation medium containing 20 g l^{-1} of glucose without pH control, compared with *L. plantarum* AS1.555. As shown in Fig. 1, the strain TD 46 produced 14.05 g l^{-1} of LA with γ_{DM} = 2.80 g l^{-1} of biomass accumulated in 48 h of fermentation at 30 °C, which were 42.6 % and 97.2 % higher than those of *L. plantarum* AS1.555, respectively. Meanwhile, the strain TD46 resulted in a lower pH (3.22) than the strain AS1.555 (3.51) did, which was consistent with the different amounts of LA produced by them. This suggests that the strain TD46 is of higher acid tolerance than the strain AS1.555.

When pH was maintained at 5.5~6.0, 91.34 g l^{-1} of LA was produced from 100 g l^{-1} of glucose by the strain TD46, 21.9 % higher than that of the strain AS1.555 in 96 h of fermentation at 30 °C (Fig. 2). Meanwhile, 98.9 % of glucose was utilized by the strain TD46 (Fig. 3), and LA yield (grams LA per gram glucose utilized) was 0.92.

According to the results illustrated by Figs. 1, 2 and 3, it can be concluded that the ability to converse glucose to LA for the strain TD46 is better

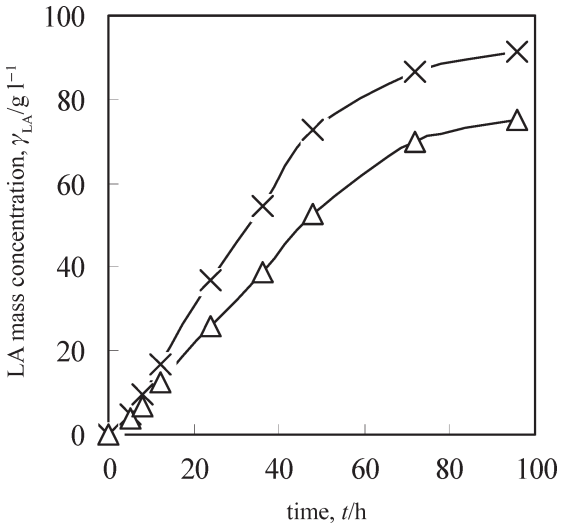

Fig. 2 – *LA production by the strain TD46 from 100 g l^{-1} of glucose at 30 °C and pH 5.5~6.0 compared with L. plantarum AS1.555. (×) TD46, (△) AS1.555*

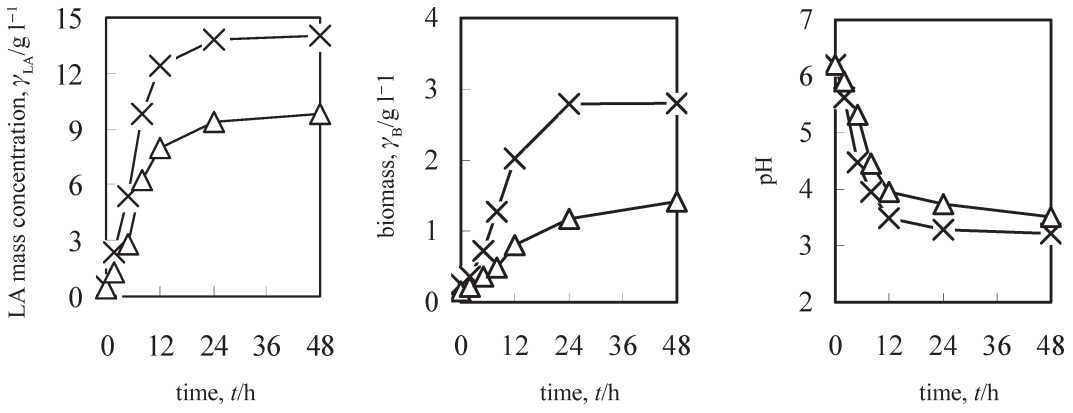

Fig. 1 – *Culture kinetics of the strain TD46 on 20 g l^{-1} of glucose at 30 °C without pH control compared with L. plantarum AS1.555. (×) TD46, (△) AS1.555*

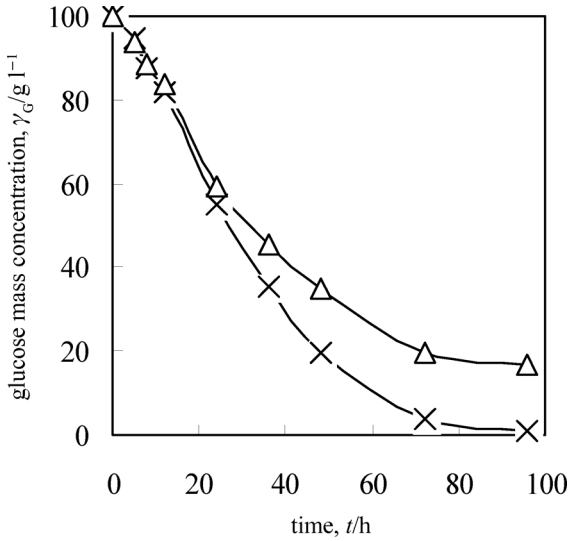

Fig. 3 – *Utilization of 100 g l^{-1} of glucose by the strain TD46 at 30 °C and pH 5.5~6.0 compared with L. plantarum AS1.555. (×) TD46, (△) AS1.555*

than *L. plantarum* AS1.555. This might attribute to its high acid tolerance and high conversion rate for LA.

Identification of the strain TD 46

Morphological, physiological and biochemical characteristics of the strain TD46 are presented in Table 2, which was regarded as belonging to the genus *Lactobacillus* based on the taxonomic criteria of *Kandler & Weiss*[18] and *Axelsson*.[22] The ability of the strain TD46 to utilize 49 different carbohydrates was studied using API 50 system as described in the section of "Materials and methods". The results were recorded after 24 h and 48 h of incubation and interpreted using the APILAB Plus computer-aided identification program. A percentage correct identification value (% Id)[23] was obtained for the strain TD46. The results obtained by identification program show that the strain TD46 possesses 99.9 % (% Id) of similarity with *L. plantarum*, and hence it is identified as a strain of *L. plantarum*.

Table 2 – *Characteristics of the strains TD 46 and L. plantarum AS 1.555*

Strain	TD 46	AS 1.555
Homolactic	+	+
Gram stain	+	+
Catalase	–	–
Cell shape	Short rod	Short rod
Spore	–	–
Mobility	–	–
H_2S production	–	–
Deamination of arginine	–	–
Growth at 15 °C	+	+
Growth at 45 °C	–	–

+ positive, – negative

L. plantarum is common in nature and can be found in many fermentation products.[24–26] It has been shown to be the dominant organism at the end of most vegetable fermentations.[22] Because LA fermentation is a product inhibited process, the high acid tolerance indicates a great potential for the use of *L. plantarum* in LA fermentation for industrial applications.[27]

Although, both, the strain TD46 and reference strain belong to the same species, they exhibit different capacity for LA production and growth kinetics on glucose as represented in Figs. 1 and 2. This may be due to the difference of their fermentation character. In the present study, the strain TD46 resulted in better LA production and exhibited higher biomass accumulated as well as higher tolerance to low pH than *L. plantarum* AS1.555. Therefore, the strain TD46 may be promising to be used to produce LA.

Effect of fermentation mode on LA production from kitchen waste

To simplify LA fermentation process of kitchen waste, an open fermentation mode (without any sterilization) was carried out in contrast to the fermentation with autoclaved waste as substrate. Fig. 4 shows LA production kinetics of two different fermentation modes described above with inoculation

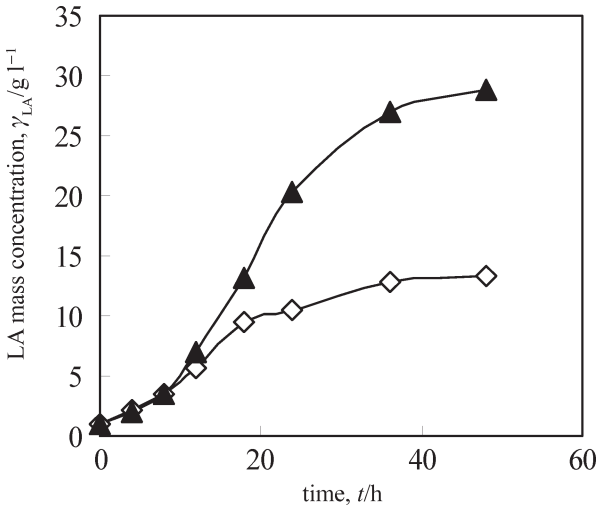

Fig. 4 – *Comparison in LA production between two types of fermentation modes. (▲) non-autoclaved waste as substrate, (◇) autoclaved waste as substrate*

of the strain TD 46 at pH 5.5~6.0. It should be noted that LA mass concentration was much higher in the open fermentation than that in the fermentation using autoclaved substrate. The total sugar mass concentration decreased to a lower level in the open fermentation (Fig. 5), which suggests that the larger amounts of sugar was converted into LA than in the fermentation using autoclaved substrate. This explains why the open fermentation could result in the higher LA mass concentration.

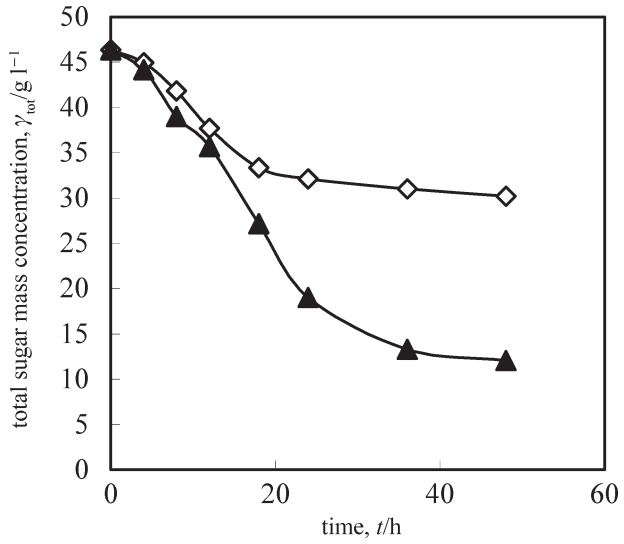

Fig. 5 – *Comparison in the changes of total sugar mass concentration between two types of fermentation modes. (▲) non-autoclaved waste as substrate, (◊) autoclaved waste as substrate*

LA fermentation of kitchen waste consists of the following stages: hydrolysis of kitchen waste by the extracellular enzymes of various bacteria; soluble sugars fermented to produce LA by LAB. Since the strain TD 46 cannot ferment polysaccharides (main carbon source in kitchen waste), including starch and cellulose to produce LA, indigenous microorganisms in the waste would exert a positive effect on the degradation of large molecules such as proteins, lipids and polysaccharides, which would be beneficial to LA production by the inoculum. The inactivation of indigenous microorganisms by autoclaving caused polysaccharides not to be degraded into fermentable sugars, thereby resulting in the decrease of LA mass concentration compared with the open fermentation. Based on this result, an open fermentation mode was adopted in the following investigations.

Enhancement of LA fermentation of kitchen waste by the strain TD 46

To enhance LA production from kitchen waste, the strain TD 46 and *L. plantarum* AS1.555 as starter cultures were investigated. The quantities for LA fermentation of various strains in 48 h of fermentation at pH 5.5~6.0 and 30 °C are illustrated in Table 3. The strain TD46 gave the highest LA mass concentration, yield, and productivity from kitchen waste, followed by *L. plantarum* AS1.555 and control. LA yield for the strain TD46 was 34.2 % and 75.1 % higher than that of *L. plantarum* AS 1.555 and control, respectively.

Table 3 – *LA production from kitchen waste by different strains in 48 h of fermentation at pH 5.5~6.0 and 30 °C*

	Mass concentration γ / g l^{-1}	Yield Y / g g^{-1}	Average productivity ρ / g l^{-1} h^{-1}
TD 46	28.85	0.39	0.60
AS 1.555	21.50	0.29	0.45
Control	16.48	0.22	0.34

Yield (g g^{-1}): grams LA per gram dry kitchen waste

Control: spontaneous fermentation of kitchen waste without any microorganisms inoculated

Since the strain TD46 was isolated from kitchen waste, it accommodated well to complex substrates in kitchen waste and could proliferate largely. During the 12~48 h of fermentation by the strain TD46, the viable number of LAB was maintained at over 10^9 CFU ml^{-1} (CFU: colony forming units), far more than the fermentation by the strain AS1.555 and the spontaneous fermentation (control) (Fig. 6). The viable number of LAB may determine the rate of conversion of substrate to LA.

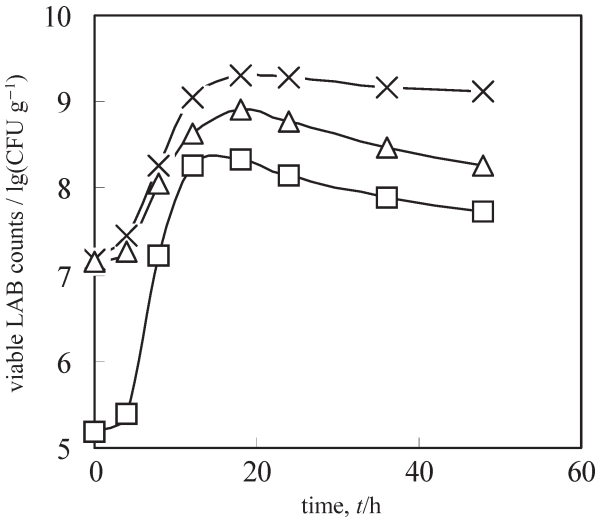

Fig. 6 – *Changes of viable LAB counts in the kitchen waste fermented by the strain TD46 compared with the spontaneous fermentation (control) and the fermentation by L. plantarum AS1.555. (×) TD46, (△) AS1.555, (□) control*

Therefore, the strain TD46 gave the higher LA yield than the strain AS1.555 and control, and has considerable potential in the fermentation of kitchen waste to LA.

Although, a certain amount of LA was accumulated when the fermentation was performed by spontaneously occurring microorganisms with LAB as key actors, the yield of LA could differ widely between different batches depending on the composition of the active microflora.[13] Thus, it would be beneficial for the enhancement of LA production to use the high-yield LAB as starter cultures.

The strain TD46, a high-yield LAB, is a new characterized strain of *L. plantarum*. Among LAB, *L. plantarum* has a relatively lower nutritional requirement, a wider spectrum of sugar assimilation, oxygen tolerance and range of growth pH.[13] Although it produces mixture of L- and D-LA,[28] D,L-LA is currently used in the chemical industry for deliming, metal etching, and for cosmetics and textile applications.[29] Even if poly-D,L-LA synthesized from optically inactive LA has lower crystallinity and tensile strength than poly-L-LA, it could be used as the controlled release agent for pesticides and fertilizer. Moreover, poly-D,L-LA could be manufactured as plastics with fine properties by copolymerization with other polymers such as polyethylene, polybutadiene, and polyethyleneglycol etc.. In order to produce optically active LA from kitchen waste, it has to be autoclaved, followed by inoculating with LAB producing L-LA or D-LA. This requires more energy for sterilization than the open fermentation, and might be not cost-effective.

There are some problems which need to be solved before putting LA production from kitchen waste to practical use, such as LA extraction problems, due to the complexity of fermentation broth and reproducibility problems caused by the variable composition of kitchen waste. However, LA production from kitchen waste is not only a simple and low cost method for LA production, but is also advantageous as a waste treatment. For example, collection and disposal of kitchen waste often is a nasty process on account of its unpleasant odor caused by putrefactive bacteria. It has been demonstrated that LA fermentation of waste can inhibit putrefactive bacteria growth, which makes it possible to realize the preservation and deodorization of waste.[14]

Conclusions

A strain of wild LAB, designated as TD46, was isolated from kitchen waste, and was tentatively identified as *L. plantarum* according to its physiological and biochemical characteristics. *L. plantarum* TD46 produced higher concentration of LA on glucose and kitchen garbage, compared with *L. plantarum* AS1.555. When pH was maintained at 5.5~6.0, the strain TD46 produced 28.85 g l^{-1} of LA with 0.39 g g^{-1} of LA yield and 0.60 g l^{-1} h^{-1} of average productivity from non-autoclaved kitchen waste in 48 h of fermentation at 30 °C.

In conclusion, the strain TD 46 was effective to enhance fermentative production of LA from kitchen waste. This recycling system is conducive to pollution abatement for kitchen waste and to reducing production cost of LA, if suitable separation process for LA is established. The potential use of our isolate for LA production will depend on the further characterization and assessment of it.

ACKNOWLEDGES

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List of symbols

n – rotation speed, min^{-1}
P – productivity, g l^{-1} d^{-1}
t – time, h
w – mass concentration, %
Y – yield, g g^{-1}
γ – mass concentration, g l^{-1}
ρ – volume fraction, %

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