Evaluation of fresh water lactic acid bacteria for production of optically pure L-(+)-lactic acid

Waill A. Elkhateeb[a], Amira M. Hamdan[b], Takeshi Zendo[c], Naoki Ishibashi[c], Ghoson M. Daba[a], Yukihiro Tashiro[d], Kenji Sonomoto[c]

[a]Department of Chemistry of Natural and Microbial Products, National Research Centre, Dokki, Giza, [b]Department of Oceanography, Faculty of Science, Alexandria University, Alexandria, Egypt, [c]Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, Japan, [d]Laboratory of Soil and Environmental Microbiology, Systems Bioengineering Division, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, Japan

Correspondence to Waill A. Elkhateeb, PhD, Department of Chemistry of Natural and Microbial Products, National Research Centre, Dokki, Giza 12622, Egypt.
Tel: +20 101 324 1936; fax: +202 267-1322;
e-mail: waillahmed@yahoo.com

Received: 3 March 2022
Revised: 21 March 2022
Accepted: 23 March 2022
Published: 21 July 2022

Egyptian Pharmaceutical Journal 2022, 21:233–241

Background and objective
Lactic acid bacteria (LAB) are generous producers of many industrially important products. Of these products, optically pure lactic acid is of great value as it is essential for production of highly crystalline poly-lactic acid, which is the most widely used biodegradable synthetic polymer. Hence, this study aimed to screen for thermotolerant LAB from a new source, which is fresh water samples collected from the coast of the Nile River, Egypt, and then evaluate their ability to produce optically pure L-lactic acid.
Materials and methods
LAB strains were isolated at 50°C and evaluated for producing optically pure L-lactic acid using high-performance liquid chromatography and BF-5. Effects of medium containing different sugar sources, incubation temperature, and initial pH of the medium on the purity and productivity of L-lactic acid were also studied.
Results and discussion
All obtained isolates were capable of producing optically pure L-lactic acid on different sugar sources. Changing the incubation temperature to 30°C positively affected both productivity and optical purity, which reached 5.0 g/l of 100% optically pure L-lactic acid. On the contrary, pH of the medium was confirmed to be also one of the major factors affecting productivity and optical purity of obtained L-lactic acid. For our isolates, pH 7.0 was the optimum one for the production process. The four promising producers of 100% optically pure L-lactic acid were molecularly identified as *Lactiplantibacillus* sp.
Conclusion
This is the first study describing the evaluation of the ability of fresh water LAB isolated from the Nile River to produce optically pure L-lactic acid.

Keywords:
fresh water, lactic acid bacteria, optically pure L-lactic acid, thermophilic

Egypt Pharmaceut J 21:233–241

1687-4315

Introduction

Lactic acid bacteria (LAB) is an important biotechnological tool, as they produce many important products such as lactic acid, which is involved in food and dairy products industries (fermentation, preservation, flavoring, as an acidifier, and pH regulator); skin care and cosmetics (moisturizer, emulsifier, anti-acne, anti-aging, humectants, and skin lightening agent); pharmaceuticals and medical fields (dialysis, drug delivery controlling system, surgical sutures, and intravascular solutions); synthesis of polymers; biodegradable packaging; poly-lactic acid (PLA); leather tanning; and wool dyeing [1-3]. Biotechnological applications of sustainable chemistry to obtain ecofriendly, cost-competitive, and economically important products without using or generating any hazardous substances have become a mainstream practice in both academic and industrial fields. One of the promising examples of such applications is the microbial production of optically pure lactic acid, which is essential for production of highly crystalline PLA. PLA is currently one of the most widely used biodegradable synthetic polymer [4,5]. It has variable industrial applications owing to its high chemical and heat resistances as well as its increased physical strength [6-8]. Moreover, PLA applications extend to medical fields in tissue engineering and drug delivery [9]. Microbial production of highly optically pure lactic acid generates only one of its isomers, whereas a racemic mixture of lactic acid is obtained when synthetic methods are used [10,11]. Of different microorganisms employed for the production of optically pure lactic acid, LAB came first in terms of

safety, efficiency, and potency. Many literature studies have described the use of LAB members for such purpose, with promising high production yields [12-15]. LAB originating from different sources have been reported for their ability to produce optically pure lactic acid. However, reports describing the ability of LAB isolated from fresh water to produce optically pure lactic acid are relatively few. Hence, this study aimed to target fresh water from the River Nile, Cairo, Egypt, as a source of aquatic LAB and then evaluate their ability to produce optically pure lactic acid. Furthermore, culture conditions in terms of carbon source to be utilized, temperature, and pH for optimum optically pure lactic acid production were studied. Moreover, promising producers were molecularly identified. Targeting sources that have not been studied before can contribute in identifying promising potent isolates capable of producing optically pure lactic acid.

Materials and methods

Isolation of lactic acid bacteria and culture conditions

Six fresh water samples (water and sediments) were collected in July 2015 from the coast of the River Nile in Cairo, Egypt, as described by Abdel-Rahman *et al.* [13]. The pH values of samples were recorded, and their appropriate dilutions were cultivated on de Man, Rogosa, and Sharpe (MRS, Oxoid, UK) with 15 g of agar media supplemented with 0.5% $CaCO_3$ [13,16], and incubated anaerobically at 50°C for 3–5 days. Colonies growing on the plates and forming a clear zone by acid formation were selected, purified, gram stained, and investigated for their catalase reaction by placing a drop of 3% hydrogen-peroxide solution on the cells. Immediate formation of bubbles indicates presence of catalase in the cells. The safety of isolates was tested in terms of hemolytic activity by plating freshly growing bacterial cells onto Columbia agar supplemented with 5% (v/v) sheep blood. Then, the plate was aerobically incubated at 37°C for 24–48 h. The hemolytic reaction was recorded by the presence of a clear zone of hydrolysis around the growing colonies (β-hemolysis), a partial hydrolysis and greening zone (α-hemolysis), or no hemolysis [17]. Colonies showing halo rings around them, gram positive, non-spore formation, catalase negative activity, and no hemolytic activity were chosen for further investigation. Isolates were stored at −80°C on MRS broth medium with 30% glycerol and propagated in MRS medium at 30°C for 18 h before use.

Screening for lactic acid-producing lactic acid bacteria

Owing to the fact that cellobiose and xylose are abundant sugars in the nature, we investigated also the ability of isolates to use cellobiose and xylose as economic carbon sources when compared with glucose. Hence, obtained bacterial isolates were investigated for their ability to produce optically pure L-lactic acid and D-lactic acid as described previously [12,18]. In brief, isolates were inoculated in Erlenmeyer flasks containing 50 ml of modified MRS broth medium (mMRS), where different carbon sources were added separately (D-glucose, mMRSG, 20 g/l; D-cellobiose, mMRSC, 10 g/l; or D-xylose, mMRSX, 10 g/l), with medium pH adjusted to 7.0. After inoculation, flasks were incubated at 50°C under anaerobic conditions for 72 h. Aliquots from each sample were centrifuged at 6000*g* for 10 min at 4°C. After that, supernatant was filtered using a membrane filter (Dismic–13HP, 0.45 μm; Advantec, Tokyo, Japan) and finally injected in the high-performance liquid chromatography system (US HPLC–1210; Jasco, Tokyo, Japan) equipped with a SUGAR SH-1011 column (Shodex, Tokyo, Japan) to analyze fermentation products (lactic acid, acetate, ethanol, and residual sugars). Analyses were performed at column temperature, 50°C, and using 3 mM $HClO_4$ as the mobile phase at a flow rate of 1.0 ml/min and an injection volume of 20 μl. Concentrations of residual sugars and fermentation products were calculated using calibration curves obtained using standard solutions. The optical purity of lactic acid was measured using a BF-5 biosensor (Oji Scientific Instruments, Hyogo, Japan) according to the manufacturer's protocol. The yield of lactic acid (g lactic acid/g consumed sugar) was calculated. L-lactic acid optical purity (%) was measured using the following formula:

$$\text{L}-\text{lactic acid optical purity}(\%) = \frac{\text{L}-\text{LA concentration}-\text{D}-\text{LA concentration}}{\text{L}-\text{LA CONCENTRATION}+\text{D}-\text{LA concentration}} \times 100.$$

Optimization of culturing conditions for optically pure L-lactic acid production

Culturing conditions in terms of temperature and pH values were investigated as factors that affect optically pure L-lactic acid production. Promising isolates were investigated for production of optically pure L-lactic acid at 30°C and then at different pH values (3.5, 5.0, and 7.0).

Physiological characterization of promising optically pure L-lactic acid producers

Physiological characteristics of the isolates were determined using the API 50 CHL test kit (bioMérieux, Marcy I'Etoile, France) as described by the manufacturer. The obtained pattern was compared

with those of reference strains described by Manero and Blanch [19].

Molecular identification of promising optically pure L-lactic acid producers and construction of phylogenetic tree

Partial 16 S rRNA gene region of promising isolates corresponding to positions 8–1510 of *Escherichia coli* 16 S rRNA gene was analyzed using the universal primers 8UA (5′-AGAGTTTGATCCTGGCT CAG-3′) and 1510r (5′-GGTTACCTTGTTAC GACTT-3′) [20]. Total genomic DNA was extracted from cells treated with lysozyme (Seikagaku, Tokyo, Japan) using the Mag Extractor Kit (Toyobo, Osaka, Japan) according to the manufacturer's protocol. Obtained genomic DNA was used as a PCR template. PCR was performed using Taq DNA polymerase (Promega, Madison, Wisconsin, USA) under the following conditions: denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 90 s. The amplified products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). DNA sequencing was carried out by FASMAC (Kanagawa, Japan). Similarity search was performed in the GenBank database using the BLAST algorithm. On the contrary, molecular phylogenetic tree was constructed on the basis of the obtained 16 S rRNA genes with strains from the genus *Lactiplantibacillus* as recorded by Zheng *et al.* [21]. Phylogenetic analysis was conducted by MEGA 6.0 software [22], using the maximum likelihood method.

Results

Isolation and physiological characterization of bacterial isolates

The pH values were recorded for the six fresh water samples as shown in Table 1. Purification of colonies obtained from plating these six samples resulted in obtaining 27 bacterial isolates. Of them, only nine bacterial isolates showed clear zones on MRS agar medium supplemented with $CaCO_3$ and were assumed to be acid producers. As shown in Table 1, five isolates were obtained from sample no. 6, whereas three isolates were obtained from sample 1, and an isolate was obtained from sample 3. Remaining samples produced no isolates, showing clear zones around colonies, hence were not selected for further investigation. All of the nine isolates with clear zones showed no catalase activity, were positively Gram stained, and showed no hemolytic activity. Therefore, these nine isolates were presumptively identified as LAB and were selected for further investigations.

Table 1 pH values and the isolates' numbers of fresh water samples collected from the River Nile coast, Cairo, Egypt

Fresh water samples	pH	Number of isolates	Number of isolates with clear zones	Isolates numbers to be investigated
1	7.30	6	3	2, 3, 6
2	7.49	1	0	–
3	7.35	5	1	8
4	7.63	4	0	–
5	7.92	2	0	–
6	7.16	9	5	9, 10, 11, 17, 19

Investigation of the ability of bacterial isolates to produce L-lactic acid

Using different carbon sources

The nine isolates obtained (isolates nos. 2, 3, 6, 8, 9, 10, 11, 17, and 19) were investigated for their ability to produce lactic acid after cultivation on different carbon sources. Moreover, other produced byproducts (acetic acid, ethanol, and residual sugars) were measured. As shown in Table 2, all obtained isolates were capable of producing acids with variable degrees. Isolates 11 (11G) and 19 (19G) cultivated on glucose (20 g/l) were the highest producers of L-lactic acid by producing 7.12 g/l followed by isolates 2 (2G), 10 (10G), and 17 (17G) cultivated on glucose that achieved 6.34, 6.48, and 6.02 g/l, respectively. On the contrary, isolate 19 (19X) cultivated on xylose (10 g/l), isolate 11 (11X) cultivated on xylose, isolate 11 (11C) cultivated on cellobiose (10 g/l), and isolate 2 (2X) cultivated on xylose were the highest producers of optically pure L-lactic, recording 93.1, 91.7, 89.7, and 86.8%, respectively. It should be noted that acetic acid was produced by six isolates on cultivation on xylose (3X, 8X, 9X, 10X, 11X, and 17X).

Effect of changing incubation temperature on production of L-lactic acid

Isolates were investigated for L-lactic acid production after changing incubation temperature to 30°C. As shown in Table 3, changing incubation temperature had a positive effect on both L-lactic acid production and its optical purity. Many isolates were capable of producing L-lactic acid with optical purity, which reached 100% (10C30, 11C30, 17C30, 17×30, and 19×30). However, among these isolates, isolate 11 cultivated on cellobiose (11C30) achieved the highest production of 100% optically pure L-lactic acid by producing 5.0 g/l, followed by isolate 10 cultivated on cellobiose (10C30, 3.6 g/l), and then isolate 17 cultivated on cellobiose (17C30, 3.4 g/l) and isolate 19 cultivated on xylose (19×30, 3.2 g/l). Hence, isolates 10C30, 11C30, 17C30, and 19×30 were chosen for further investigation.

Table 2 Ability of fresh water lactic acid bacteria isolates to produce L-lactic acid using D-glucose, 20 g/l; D-cellobiose, 10 g/l; or D-xylose, 10 g/l at 50°C

Isolates designation	Lactic acid (g/l)	Acetic acid (g/l)	Consumed sugar (g/l)	YLA/TS (g/g)	L-lactic acid (g/l)	OP (%)
2G	9.48	–	14.85	0.63	6.34	33.7
2C	5.21	–	7.88	0.66	4.48	71.9
2X	1.67	–	4.09	0.4	1.56	86.8
3G	11.03	–	14.32	0.77	5.62	1.9
3C	3.05	–	6.31	0.48	2.78	82.2
3X	2.27	0.10	4.16	0.54	1.76	55.0
6G	9.9	–	14.53	0.68	4.66	–5.8
6C	4.36	–	8.69	0.50	2.30	5.5
6X	1.59	–	4.09	0.38	1.34	68.5
8G	10.61	–	14.63	0.72	4.30	–18.9
8C	3.25	–	6.65	0.48	2.70	66.1
8X	2.48	0.10	4.44	0.55	1.48	19.3
9G	11.31	–	14.61	0.77	5.34	–5.5
9C	4.16	–	7.39	0.56	3.76	80.7
9X	2.17	0.45	5.62	0.38	1.86	71.4
10G	10.21	–	13.73	0.74	6.48	26.9
10C	3.64	–	6.95	0.52	3.08	69.2
10X	2.87	0.20	4.69	0.61	2.22	54.7
11G	10.56	–	14.70	0.71	7.12	34.8
11C	4.09	–	6.77	0.60	3.88	89.7
11X	2.67	0.54	5.95	0.44	2.56	91.7
17G	9.49	–	14.16	0.67	6.02	26.8
17C	5.43	–	7.95	0.68	4.68	72.3
17X	2.67	0.30	4.96	0.53	2.36	76.7
19G	10.39	–	14.42	0.72	7.12	37.0
19C	5.20	–	8.54	0.60	4.30	65.3
19X	1.47	–	4.07	0.36	1.42	93.1

X, C, and G, xylose, cellobiose, and glucose added as carbon source to the medium, respectively.
YLA/TS, yield of lactic acid to total consumed sugar.
OP, optical purity of L-lactic acid. Cultivation was carried out at 50°C, pH 7.0 for 24 h.
Experiments were carried out in triplicates, and the average data are presented.

Effect of changing medium pH on L-lactic acid production

Isolates that were capable of producing high optically pure L-lactic acid on the cheap carbon sources (xylose and cellobiose) were selected to investigate the effect of changing initial pH on their ability to produce optically pure L-lactic acid. Therefore, isolates 10C30, 11C30, 17C30, and 19×30 were evaluated for this purpose. As shown in Table 4, pH values have a significant influence on L-lactic acid production as well as its optical purity %. Cultivation of the four selected isolates at 30°C, pH, 3.5, for 24 h revealed that cultivation at pH 3.5 had a negative effect on optically pure L-lactic acid production by all isolates. Similarly, the optical purity of L-lactic acid produced by all isolates was undesirably affected. Highest recorded optical purity at this pH was 73.8% and was achieved by isolate 19 cultivated on xylose (19×30). All isolates remained unable to produce 100% optically pure L-lactic acid at pH 5.0 except for isolate 11C30, which produced it in considerable concentration (3.78 g/l) in comparison with other tested isolates. On the contrary, pH 7.0 was the optimum pH for production of optically pure L-lactic acid, as all isolates retained their ability to produce it. Isolate 11 cultivated on cellobiose (11C30) remained the highest producer of 100% optically pure lactic acid (5.0 g/l).

Molecular identification of bacterial isolates

Primary identification of isolates using API 50 CHL suggested that all of them belonged to the genus *Lactiplantibacillus*. Additionally, analyzing the partial 16 S rRNA gene regions of the promising four isolates (10, 11, 17, and 19) obtained from fresh water sample no. 6 revealed that all isolates had high similarity to *Lactiplantibacillus* sp. KLDS 1.0702 and *Lactiplantibacillus* sp. KLDS 1.0704 sequences, recording 98.41, 97.21, 97.79, and 97.01%, respectively. Sequences of these isolates were deposited in the international gene bank under the names and the accession numbers, *Lactiplantibacillus* sp. WA10 (OL986008), *Lactiplantibacillus* sp. WA11 (OL986012), *Lactiplantibacillus* sp. WA17

Table 3 Ability of fresh water lactic acid bacteria isolates to produce L-lactic acid using D-glucose, 20 g/l; D-cellobiose, 10 g/l; or D-xylose, 10 g/l at 30°C

Isolates designation	Lactic acid (g/l)	Acetic acid (g/l)	Consumed sugar (g/l)	YLA/TS (g/g)	L-lactic acid (g/l)	OP (%)
2G30	10.49	–	15.45	0.67	5.88	12
2C30	5.28	–	8.64	0.61	4.44	68
2×30	3.12	0.58	7.14	0.43	2.76	77
3G30	10.75	–	14.12	0.76	6.64	23.5
3C30	4.98	1.88	6.45	0.77	3.50	40.5
3×30	11.24	–	13.71	0.81	5.66	0.71
6G30	8.80	–	14.84	0.59	4.74	7.7
6C30	3.92	–	6.88	0.56	3.28	67
6×30	2.49	–	5.44	0.45	2.48	99.1
8G30	11.39	–	13.20	0.60	6.32	10.9
8C30	6.46	1.49	8.81	0.73	3.80	17.6
8×30	9.57	–	17.26	0.55	6.56	37
9G30	11.31	–	14.61	0.77	5.16	–8.7
9C30	5.39	–	9.33	0.57	5.38	99.6
9×30	2.55	0.30	5.85	0.43	2.42	89.8
10G30	6.57	–	20.00	0.32	4.80	46
10C30	3.60	–	8.71	0.41	3.60	100
10×30	2.53	0.30	4.96	0.51	2.48	96
11G30	9.35	–	15.38	0.60	5.38	15
11C30	5.00	–	8.92	0.56	5.00	100
11×30	3.02	0.63	7.14	0.42	3.00	98.6
17G30	6.02	–	11.00	0.54	3.18	5.6
17C30	3.40	–	8.47	0.40	3.40	100
17×30	1.80	–	4.71	0.38	1.80	100
19G30	8.42	–	14.65	0.57	7.14	69
19C30	3.54	–	6.59	0.53	3.12	76
19×30	3.20	0.20	4.40	0.72	3.20	100

X, C, and G, xylose, cellobiose, and glucose, respectively.
YLA/TS, the yield of lactic acid to total consumed sugar.
OP, optical purity of L-lactic acid. Cultivation was carried out at 30°C, pH 7.0 for 24 h.
Experiments were carried out in triplicates, and the average data are presented.

Table 4 Effect of different medium pH on production of L-lactic acid by selected isolates at 30°C

Isolate designation	pH 3.5 Lactic acid (g/l)	Consumed sugar (g/l)	L-lactic acid (g/l)	OP %	pH 5.0 Lactic acid (g/l)	Consumed sugar (g/l)	L-lactic acid (g/l)	OP %	pH 7.0 Lactic acid (g/l)	Consumed sugar (g/l)	L-lactic acid (g/l)	OP %
10C30	1.79	5.09	1.47	64.2	3.93	10.00	3.56	81.1	3.6	8.71	3.6	100
11C30	2.32	6.66	1.86	60.3	3.78	7.94	3.78	100	5.0	8.92	5.0	100
17C30	4.33	16.89	2.40	10.8	11.01	15.82	6.96	26.4	3.4	8.47	3.4	100
19×30	1.91	6.16	1.66	73.8	2.36	6.51	2.26	91.5	3.2	4.40	3.2	100

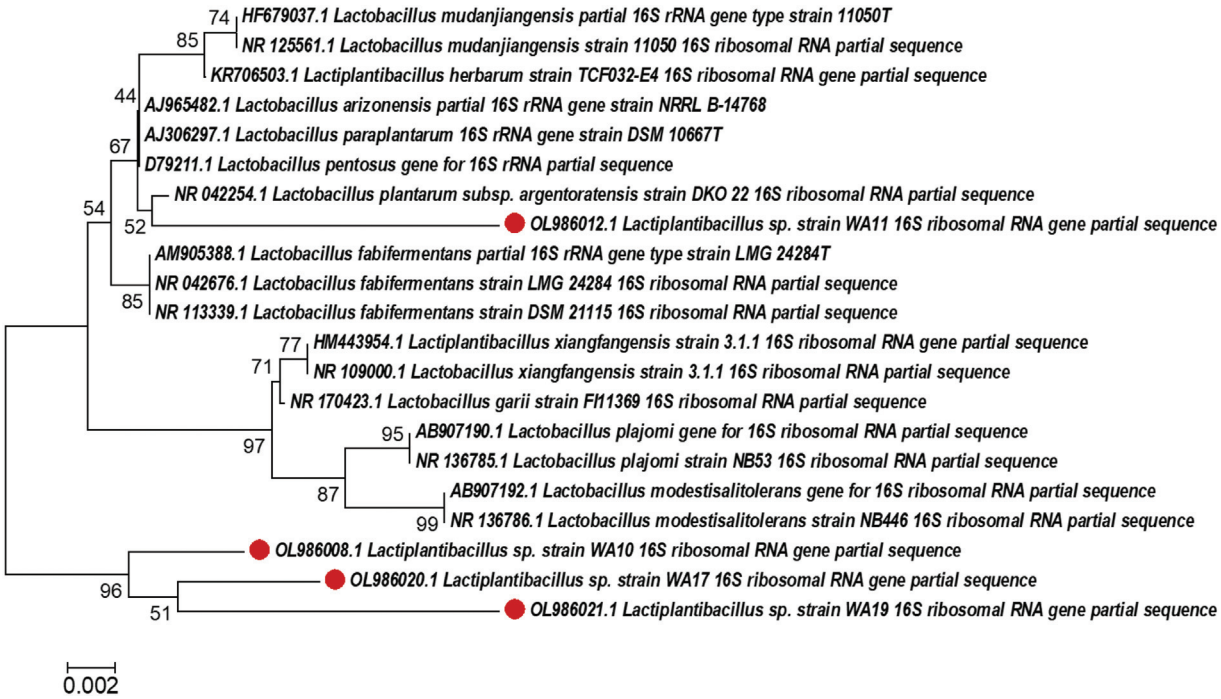
X and C, xylose and cellobiose, respectively.
OP, optical purity of L-lactic acid. Cultivation was carried out at 30°C, pH 3.5, 5.0, and 7.0 for 24 h.
Experiments were carried out in triplicates, and the average data are presented.

(OL986020), and *Lactiplantibacillus* sp. WA19 (OL986021). On the contrary, phylogenetic analysis was conducted by MEGA6 software using the maximum likelihood method (Fig. 1).

Discussion

LAB are famous producers of different industrially important products [23]. Of these products, lactic acid is attracting extra attention owing to its applications in different fields. However, applications of lactic acid in biodegradable polymers have grown significantly, owing to the increased awareness among consumers of the effects of using harmful nondegradable plastic, besides the increased consumption of sustainable products, as well as use of biodegradable plastic packaging in food-related applications. Owing to its involvement in many industries, the global lactic acid market size reached 1.1 billion USD in 2020 and is estimated to reach 2.1

Figure 1



Molecular phylogenetic tree on the basis of 16 S rRNA gene for *Lactiplantibacillus* strains WA10, WA11, WA17, and WA19 (marked with red closed circles) with strains in the genus *Lactiplantibacillus* as recorded by Zheng *et al.* [21]. Phylogenetic analysis was conducted by MEGA6 software [22] using the maximum likelihood method. Accession number was indicated in parentheses adjacent to each strain. The tree was drawn to scale with branch lengths measured in the number of substitutions per site. Bootstrap values above or equal to 50% based on 500 resampled data sets were indicated at nodes.

billion USD by 2025. On the contrary, global PLA market size reached 786 million USD in 2020 and is expected to reach 1756 USD million by 2025 [24]. Additionally, it was observed that the early COVID-19 effect have also increased the use of packaged products owing to the elevated restrictions on public activities, and the bulk purchase of different packaged products. In 2010, the manufacturing cost of lactic acid production at an industrial scale has been estimated to be around 0.55$/kg [25]. Lactic acid is generally produced by either microbial fermentation or chemical synthesis, which results in two optical isomers of lactic acid, that is, L(+)-lactic acid and D(–)-lactic acid, or a racemic mixture of both DL-lactic acid [26]. It should be noted that high optically pure lactic acid has much more value than the racemic form and has wider applications. Generally, chemical synthesis results in obtaining racemic DL-lactic acid, which cannot be used for PLA production, as it requires either pure L(+)- or D(–)-lactic acid for polymerization to a high crystalline PLA [27]. On the contrary, microbial fermentation can produce optically pure lactic acid, and the obtained product depends on many factors such as the bacterial producing strain, composition of the medium, and culturing conditions. Hence, we screened in this study for LAB capable of producing optically pure L-lactic acid and tried to study the effect of carbon source, temperature, and pH value on the productivity and optical purity of the obtained lactic acid.

The Egyptian River Nile was chosen as a source for bacterial isolation, which resulted in obtaining 27 bacterial isolates. Of them, only nine strains were presumptively identified as LAB. Fresh water is rarely targeted as a source of LAB isolation, as majority of literature studies have screened for LAB from fresh water creatures, not from water and sediment samples, as we have done in the current work. Moreover, isolation was conducted at 50°C to obtain heat-tolerant isolates, as an extra trait to facilitate their application in industrial fields. Many studies have nominated Egyptian sources as a repository for promising microorganisms [28-31].

Investigating the ability of the nine isolates to produce lactic acid at 50°C as an incubation temperature was conducted using different sugars such as glucose besides cellobiose and xylose which are abundant sugars in the nature and considered as economic carbon sources when compared with glucose. The nine isolates showed variable ability to produce L-

lactic acid. Majority of isolates cultivated on glucose as carbon source were capable of producing L-lactic acid, whereas isolates 2, 11, and 19 showed the highest ability to produce L-lactic acid on xylose and cellobiose (Table 2). Xylose is ranked second in the list of the most abundant sugars in the nature. It exists in hemicellulose of woods and also in agricultural wastes [14]. This made it an economic substrate that is also not used as food supply as mentioned before. On the contrary, cellobiose is the main product resulting from enzymatic hydrolysis of the abundant cellulose. β-glucosidases are the enzymes working on cellobiose and convert it into two glucose molecules. Generally, ability of LAB to consume sugars other than glucose is highly favorable. However, its presence results in a phenomenon called carbon catabolite repression where the microorganism prefers consuming glucose (as a rapidly metabolized carbon source) over nonpreferred carbon sources such as xylose, which inhibits expression of some genes and enzymes related to the catabolism of those nonpreferred carbon sources [32,33]. It should be noted that some isolates were also capable of producing highly pure D-lactic acid (Table 1). So far, few reports have described the ability of wild-type stains such as *Lactobacillus delbrueckii* and *Lactobacillus coryniformis* subsp. *torquens* [34,35] to produce D-lactic acid. On the contrary, several studies have reported production of D-lactic acid by metabolically engineered *Lactobacillus plantarum* [36-38]. The influence of incubation temperature on productivity and optical purities of L-lactic acid was studied by cultivating the nine isolates on the three different sugar sources but at 30°C as an incubation temperature. It was noticed that the optical purity of produced lactic acid was enhanced after incubation at 30°C, as four isolates (10, 11, 17, and 19) were able to produce 100% optical pure L-lactic acid when cultivated on cellobiose or xylose (Table 3). Moreover, isolate 11 cultivated on cellobiose showed the highest productivity (5.0 g/l) among the tested isolates. Hence, isolates 10C30, 11C30, 17C30, and 19×30 were chosen for investigating the effect of initial pH values of the medium on their L-lactic acid production and optical purity.

pH 3.5 had a negative effect on both productivity and optical purity of produced L-lactic acid. On the contrary, pH 7.0 was the optimum pH for production of optically pure L-lactic acid, as all isolates were capable of producing 100% optically pure L-lactic acid, and isolate 11 cultivated on cellobiose (11C30) was also the highest producer of 100% optically pure lactic acid (5.0 g/l). Homofermentative LAB are favored in large-scale applications as they possess aldolase enzymes capable of producing lactic acid as their primary end product. This is not the case for heterofermentative LAB that produce lactic acid besides other byproducts [10]. Our four isolates can be considered as homofermentative LAB, especially when cultivated on glucose and cellobiose. This was not the case when using xylose as a sugar source, except for isolate 17, which produced lactic acid as its primary end product even during cultivation on xylose. Molecular identification of the four isolates revealed that all belong to the genus *Lactiplantibacillus*.

The genus *Lactiplantibacillus* (previously known as *Lactobacillus*) is reported to be a producer of both L-lactic acid and D-lactic acid because it has stereospecific NAD-dependent lactate dehydrogenases [39]. Moreover, it was reported that D-lactic acid production is connected to the biosynthesis of cell wall through incorporation of it as the last residue of the muramyl-pentadepsipeptide peptidoglycan precursor [40]. Hence, different approaches have been applied to improve L-lactic acid production from *Lactobacillus* species such as metabolic engineering, genome shuffling, using mixed microbial cultures, or optimizing physiological and fermentation conditions [41-44]. Many studies have described the effect of controlling physiological factors and culturing conditions on improving L-lactic acid and eliminating D-lactic acid produced by different *Lactobacillus* species. Using glucose controller to regulate glucose concentration in culture broth as well as in fed-batch culture has improved productivity to 170 g/l L-lactic acid by *Lactobacillus rhamnosus* LA-04-1 [45]. Modifying the nitrogen source used during the fermentation process has improved L-lactic acid by *L. plantarum* As.1.3 [45]. Tian *et al.* [46] have reported the positive influence of adding vitamin C on L-lactic acid production and cell growth in *Lactobacillus thermophilus* A69. The results obtained in the current study confirmed the influence of some culturing conditions on production of 100% optically pure L-lactic acid from the four different *Lactiplantibacillus* strains isolated from the River Nile in Egypt.

Conclusion

Nine thermal tolerant bacterial strains were isolated from fresh water samples collected from the coast of the Nile River in Egypt. Physiological studies revealed that these isolates are promising producers of 100% optically pure L-lactic acid. This the first report describing isolation of thermal tolerant, 100%

optically pure L-lactic acid producers from fresh water samples in general and from the River Nile in particular. Further studies are required to optimize cultivation and production conditions to increase production yield of 100% optically pure L-lactic acid from these promising isolates.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Tarraran L, Mazzoli R. Alternative strategies for lignocellulose fermentation through lactic acid bacteria: the state of the art and perspectives. FEMS Microbiol Lett 2018; 365:fny126.

2 Lübeck M, Lübeck PS. Application of lactic acid bacteria in green biorefineries. FEMS Microbiol Lett 2019; 366:fnz024.

3 Ahmad A, Banat F, Taher H. A review on the lactic acid fermentation from low-cost renewable materials: recent developments and challenges. Environ Technol Innov 2020; 101138.

4 Auras RA, Lim LT, Selke SE, Tsuji H. *Poly (lactic acid): synthesis, structures, properties, processing, and applications*. Michigan city: John Wiley & Sons; 2011.

5 Roy S, Rhim JW. Preparation of bioactive functional poly (lactic acid)/ curcumin composite film for food packaging application. Int J Biol Macromol 2020; 162:1780–1789.

6 Lunt J. Large-scale production, properties and commercial applications of polylactic acid polymers. Polym Degr Stab 1998; 59:145–152.

7 Chen Y, Geever LM, Killion JA, Lyons JG, Higginbotham CL, Devine DM. Review of multifarious applications of poly (lactic acid). Polym Plast Technol Eng 2016; 55:1057–1075.

8 Di Lorenzo ML, Androsch R. *Industrial applications of poly (lactic acid)*. Cham: Springer; 2018.

9 Liu S, Qin S, He M, Zhou D, Qin Q, Wang H. Current applications of poly (lactic acid) composites in tissue engineering and drug delivery. Compos Part B Eng 2020; 199:108238.

10 Abdel-Rahman MA, Tashiro Y, Sonomoto K. Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits. J Biotechnol 2011; 156:286–301.

11 Huang S, Xue Y, Yu B, Wang L, Zhou C, Ma Y. A review of the recent developments in the bioproduction of polylactic acid and its precursors optically pure lactic acids. Molecules 2021; 26:6446.

12 Shibata K, Flores DM, Kobayashi G, Sonomoto K. Direct l-lactic acid fermentation with sago starch by a novel amylolytic lactic acid bacterium, *Enterococcus faecium*. Enzyme Microb Technol 2007; 41:149–155.

13 Abdel-Rahman MA, Tashiro Y, Zendo T, Shibata K, Sonomoto K. Isolation and characterisation of lactic acid bacterium for effective fermentation of cellobiose into optically pure homo l-(+)-lactic acid. Appl Microbiol Biotechnol 2011; 89:1039–1049.

14 Abdel-Rahman MA, Tashiro Y, Zendo T, Sakai K, Sonomoto K. *Enterococcus faecium* QU 50: a novel thermophilic lactic acid bacterium for high-yield l-lactic acid production from xylose. FEMS Microbiol Lett 2015; 362:1–7.

15 Tan J, Abdel-Rahman MA, Sonomoto K. Biorefinery-based lactic acid fermentation: microbial production of pure monomer product. Synthesis 2017; 27–66.

16 de Man JC, Rogosa M, Sharpe M. A medium for the cultivation of lactobacilli. J Appl Bacteriol 1960; 23:130–135.

17 Lombardi A, Gatti M, Rizzotti L, Torriani S, Andrighetto C, Giraffa G. Characterization of *Streptococcus macedonicus* strains isolated from artisanal Italian raw milk cheeses. Int Dairy J 2004; 14:967–976.

18 Elkhateeb WA, Hamdan AM, Zendo T, Tashiro Y, Sonomoto K. Innovative green chemical production by novel Egyptian aquatic bacterial isolates. J Fac Agr 2018; 63:331–337.

19 Manero A, Blanch AR. Identification of *Enterococcus* spp. with a biochemical key. Appl Environ Microbiol 1999; 65:5173–5176.

20 Sawa N, Zendo T, Kiyofuji J, Fujita K, Himeno K, Nakayama J, Sonomoto K. Identification and characterization of lactocyclicin Q, a novel cyclic bacteriocin produced by *Lactococcus* sp. strain QU 12. Appl Environ Microbiol 2009; 75:1552–1558.

21 Zheng J, Wittouck S, Salvetti E, Franz CM, Harris HM, Mattarelli P, Lebeer S. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus Beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol 2020; 70:2782–2858.

22 Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013; 30:2725–2729.

23 Daba GM, El-Dien AN, Saleh SA, Elkhateeb WA, Awad G, Nomiyama T, Yamashiro K, Zendo T. Evaluation of *Enterococcus* strains newly isolated from Egyptian sources for bacteriocin production and probiotic potential. Biocatal Agr Biotechnol 2021; 35:102058.

24 Market and Market. Market report, 2021. Available at: https://www.marketsandmarkets.com/Market-Reports/polylacticacid-387.html?gclid=Cj0KCQiAk4aOBhCTARIsAFWFP9FHCgtwVBUZn_FBMkeDTeHhT3LHlHo1jMhZc_Iuc_NWQgUP0nl_b30aApsWEALw_wcB [Accessed February 27, 2022].

25 Madhavan NK, Nair NR, John RP. An overview of the recent developments in polylactide (PLA) research. Bioresour Technol 2010; 101:8493–8501.

26 Sharma A, Singh S, Khare SK, Sharma A, Tiwari R, Nain L. Green lactic acid production using low-cost renewable sources and potential applications. In: Anuj K. Chandel and Fernando Segato. *Production of top 12 biochemicals selected by USDOE from renewable resources*. Sao Paulo: Elsevier; 2022. 345–365.

27 Singhvi M, Zendo T, Sonomoto K. Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects. Appl Microbiol Biotechnol 2018; 102:5911–5924.

28 Hawas UW, Ahmed EF, Halwany A, Atif A, Ahmed W, El-Kassem LT. Bioactive metabolites from the Egyptian Red Sea Fungi with potential anti-HCV protease effect. Chem Natl Compd 2016; 52:104–110.

29 Abou El-Kassem L, Hawas UW, El-Souda S, Ahmed EF, El-Khateeb W, Fayad W. Anti-HCV protease potential of endophytic fungi and cytotoxic activity. Biocatal Agr Biotechnol 2019; 19:101170.

30 Elkhateeb WA, Mohamed MA, Fayad W, Emam M, Nafady IM, Daba GM. Molecular identification, metabolites profiling, anti-breast cancer, anti-colorectal cancer, and antioxidant potentials of *Streptomyces zaomyceticus* AA1 isolated from a remote bat cave in Egypt. Res J Pharma Technol 2020; 13:3072–3080.

31 Daba GM, Elnahas MO, Elkhateeb WA. Beyond biopreservatives, bacteriocins biotechnological applications: history, current status, and promising potentials. Biocatal Agr Biotechnol 2021; 39:102248.

32 Mitchell WJ. Physiology of carbohydrate to solvent conversion by clostridia. Adv Microb Physiol 1997; 39:31–130.

33 Vinuselvi P, Kim MK, Lee SK, Ghim CM. Rewiring carbon catabolite repression for microbial cell factory. BMB Rep 2012; 45:59–70.

34 Yáñez R, Moldes AB, Alonso JL, Parajó JC. Production of D (–)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis* subsp. torquens. Biotechnol Lett 2003; 25:1161–1164.

35 Fukushima K, Sogo K, Miura S, Kimura Y. Production of D-lactic acid by bacterial fermentation of rice starch. Macromol Biosci 2004; 4:1021–1027.

36 Okano K, Zhang Q, Shinkawa S, Yoshida S, Tanaka T, Fukuda H, Kondo A. Efficient production of optically pure D-lactic acid from raw corn starch by using a genetically modified L-lactate dehydrogenase gene-deficient and α-amylase-secreting *Lactobacillus plantarum* strain. Appl Environ Microbiol 2009; 75:462–467.

37 Okano K, Yoshida S, Yamada R, Tanaka T, Ogino C, Fukuda H, Kondo A. Improved production of homo-D-lactic acid via xylose fermentation by introduction of xylose assimilation genes and redirection of the phosphoketolase pathway to the pentose phosphate pathway in L-lactate dehydrogenase gene-deficient *Lactobacillus plantarum*. Appl Environ Microbiol 2009; 75:7858–7861.

38 Okano K, Yoshida S, Tanaka T, Ogino C, Fukuda H, Kondo A. Homo-D-lactic acid fermentation from arabinose by redirection of the phosphoketolase pathway to the pentose phosphate pathway in L-lactate dehydrogenase gene-deficient *Lactobacillus plantarum*. Appl Environ Microbiol 2009; 75:5175–5178.

39 Hammes WP, Vogel RF. The genus lactobacillus. In: B.J.B. Wood and W.H. Holzapfel. *The genera of lactic acid bacteria*. Boston, MA: Springer; 1995. 19–54.

40 Goffin P, Deghorain M, Mainardi JL, Tytgat I, Champomier-Verges MC, Kleerebezem M, Hols P. Lactate racemization as a rescue pathway for supplying D-lactate to the cell wall biosynthesis machinery in *Lactobacillus plantarum*. J Bacteriol, 2005; 187:6750–6761.

41 Yu L, Pei X, Lei T, Wang Y, Feng Y. Genome shuffling enhanced L-lactic acid production by improving glucose tolerance of *Lactobacillus rhamnosus*. J Biotechnol 2008; 134:154–159.

42 Ge XY, Qian H, Zhang WG. Improvement of l-lactic acid production from Jerusalem artichoke tubers by mixed culture of *Aspergillus niger* and *Lactobacillus* sp. Bioresour Technol 2009; 100:1872–1874.

43 Okano K, Uematsu G, Hama S, Tanaka T, Noda H, Kondo A, Honda K. Metabolic engineering of *Lactobacillus plantarum* for direct l-lactic acid production from raw corn starch. Biotechnol J 2018; 13:1700517.

44 Liu B, Yang M, Qi B, Chen X, Su Z, Wan Y. Optimizing L-(+)-lactic acid production by thermophile *Lactobacillus plantarum* As. 1.3 using alternative nitrogen sources with response surface method. Biochem Eng J 2010; 52:212–219.

45 Li Z, Lu J, Zhao L, Xiao K, Tan T. Improvement of l-lactic acid production under glucose feedback controlled culture by *Lactobacillus rhamnosus*. Appl Biochem Biotechnol 2010; 162:1762–1767.

46 Tian X, Hu W, Chen J, Zhang W, Li W. The supplement of vitamin C facilitates l-lactic acid biosynthesis in *Lactobacillus thermophilus* A69 from sweet sorghum juice coupled with soybean hydrolysate as feedstocks. Ind Crops Prod 2020; 146:112159.