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Production of lactic acid from whey permeate using lactic acid bacteria isolated from cheese

Waiel F. Sayed[1], Wesam M. Salem[1*], Zeinab A. Sayed[1], Abdelmoneim K. Abdalla[2]

[1] Department of Botany and Microbiology, Faculty of Science, South Valley University, 83523 Qena, Egypt.
[2] Food Science Department, College of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

The uses of whey permeate for lactic acid production is a dual-purpose process by producing lactic acid and decreasing the environmental pollution problem caused by dumping the lactose- rich dairy by-product. This study aimed to investigate lactic acid production from whey permeates using lactic acid bacterial isolates. Five isolates from cheese samples were identified as *lactobacillus casei MT682513, Enterococcus camelliae MT682510, Enterococcus faecalis MT682509, Enterococcus lactis MT682511, and Wissella paramesenteroides MT682512* using 16S rRNA. Small scale batch fermentations of permeate were conducted under uncontrolled pH conditions. pH, temperature, carbon, and nitrogen sources as well as fermentation time on the conversion rate of lactose to lactic acid were monitored. It was found that *Lactobacillus casei* exhibited the highest percentage of lactic acid production without any supplementations. The increasing percentage was 107% using glucose ($100 \, \mathrm{gl^{-1}}$) and 44.2 % using yeast extract ($10 \, \mathrm{gl^{-1}}$) as carbon and nitrogen sources, respectively. The optimum lactic acid production was between 30°C and 37°C within a pH value of 6. The highest production of lactic acid under the optimized conditions resulted after 14h of fermentation. This work facilitates further studies of *Lactobacillus casei* over the optimized conditions of whey parameters on the other industrially important for lactic acid production and applications.

Keywords: Batch fermentation; Lactic acid bacteria; *Lactobacillus casei*; Permeate; 16S rRNA.

Introduction

Permeate is a high- lactose dairy byproduct produced through the removal of proteins and fat from milk or whey by ultrafiltration (Kaur et al., 2020). The total sum of manufactured global whey is valued to be around 180- 190 million tons per year and only partial of this derivative (50 %) is used for nutrition or feedstuff manufacture (Ibarruri and Hernández, 2019). Nearly 30% of annual global cheese whey production remains underutilized, ending up as waste or animal feed (Affertsholt, 2007). Currently, due to their high nutritional value, proteins are separated from whey by ultrafiltration process, and the resultant products being designated whey permeate. Owing to the low solid content (4% DM), permeate has been recognized as a waste product despite its nutritional potential (Marwaha and Kennedy, 1988). As an organic waste, permeate can work as a good raw material to produce lactic acid (Alonso et al., 2010). The availability of carbohydrate sources (lactose) in permeates and other nutrients suitable for the growth of microorganisms make permeate one of the possible substrates to produce diverse bio-products including lactic acid (Panesar et al., 2007). Thus, the production of lactic acid through lactic acid bacteria could be a substitute handling path for permeate lactose utilization. The fact that a huge amount of milk permeate is now produced as a byproduct of UF treating count to the clearance problem (El-Batawy et al., 2018). Consequently, finding new approaches to use permeate is vital. The present practices monitored by the dairy productiveness for treatment permeate include setting of it as excess, land scattering, selling dry permeate powder, and combination into animal nourish (Kushwaha et al., 2011). Fermentation of whey or milk permeates to produce alcohols, methane, organic acids, microbial biomass protein, and other products could be a beneficial approach (Guimarães et al., 2010). Lactic acid is becoming more significant owing to its utilization in several applications including pharmaceutical, cosmetics, food, and chemical sectors (Gao et al., 2011). In the food sector, it is used to improve food microbial quality and flavor (Hofvendahl and Hahn-Hägerdal, 2000; Wisselink et al., 2002). It is recognized as harmless material by the American Food and Drug Administration (Wee et al., 2006; Reddy et al., 2008). Furthermore, the utilization of lactic acid in the formation of PLA (polylactic acid) is gaining more consideration in the world, due to the opportunity of replacing petrochemical plastic products with green biodegradable PLA plastics (Rasal et al., 2010). Currently, most of the used lactic acid is derived from biological sources and its production cost could be much more reasonable if the used feedstock is an organic waste (Abdel-Rahman et al., 2013; Tan et al., 2017). Most lactic acid production worldwide (about 90%) is made by lactic acid bacterial fermentation and the rest is produced synthetically (Hofvendahl and Hahn-Hägerdal, 2000). Microbial fermentation has the advantage of generating an optically pure product via selecting the applicable isolate of lactic acid bacteria, while artificial manufacture always yields a racemic combination of lactic acid. In addition, optically pure lactic acid is con-

verted to a high crystal polymer appropriate for integrity film construction and is predictable to be valuable in the fabrication of fluid crystal as well (Amass et al., 1998). Permeate removal represents a critical problem from both an economical and environmental perspective. Whereas the use of permeate as a fermentation medium could be beneficial for both the environment and the sustainable economy (Jelen, 2003; Panesar et al., 2007). Microbial fermentation could be enhanced in a sequential batch operation by supplementing the media with glycerol, acetic acid, and increasing concentrations of yeast extract (Sayed et al., 2016; 2017). Therefore, this study aimed to determine the optimal conditions for lactic acid production from whey permeate using selected isolates of lactic acid bacteria in batch fermentation.

Materials and methods

Sampling and enumeration of total lactic acid-bacteria

Three types of cheese samples (Edam, Kareish, and Domiati) were collected from different regions in Qena city, Egypt, in August 2019. For the isolation of lactic acid bacteria, the initial dilutions were made up in sterile physiological saline (0.85% w/v, NaCl) for each cheese sample. Then 0.1 ml of the appropriate dilution was streaked on the specific agar medium to enumerate the total lactic acid bacteria as a colony-forming unit (CFU). Lactobacilli were isolated on MRS agar Oxoid® (De Man et al., 1960) while Lactococci and Streptococci were isolated on M17 agar Oxoid® (Terzaghi and Sandine, 1975). The selected colonies were repeatedly

streaked to obtain pure cultures that were maintained in a sterilized 70% glycerol at -20°C for further use.

Isolation and identification of lactic acid bacterial isolates

Representative lactic acid bacterial isolates were examined according to their colony morphology, catalase, and Gram reactions. The seemingly bacteria were characterized as Gram +ve and catalase -ve cocci and/ or bacilli (Garvie, 1984). According to the above tests, the accepted bacterial isolates were subjected to further tests. Tests for biochemical activities were performed for cultures grown at 30°C for 48 hours. The identification procedure was carried out based on the criteria in the Bergey's Manual of Determinative Bacteriology (Vos et al., 2011).

Selection of isolates for lactic acid fermentation

Cultures of lactic acid bacterial isolates were prepared from the stock by two sequential transfers in 10 ml permeate sterilized at 121°C for 20 min. 100µl of each isolate culture was inoculated in 10 ml permeate followed by incubation at 30°C , concentration of lactic acid was detected in the beginning of fermentation process by HPLC. The lactic acid concentrations were monitored. Production of lactic acid was determined by titration methods (Sarantinopoulos et al., 2001) and isolates with the highest production level were selected for further fermentations.

16S rRNA gene sequences analysis of the selected isolates

DNA was extracted from the selected isolates that were grown in MRS and M17 at 30°C using a revised cetyltrimethylammonium bromide (CTAB) method (Jones, 1953). The purified DNA template was diluted with sterilized bi-distilled H_2O to 100 ng μl^{-1} for 16S rRNA gene amplification. The 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1495R (5'-CTACGGCTACCTTGTTACGA-3') Metabion® (Germany) primers were used for amplification of the partial 16S rRNA gene (Liu et al., 2012). The PCR mix (50 μl) contained 2 μl DNA templates (100 ng μl^{-1}), 5 μl 10 × buffer (Mg^{2+}), 4 μl dNTP (10 mmol l^{-1}), 1.5 μl primer FA-27F (10 pmol μl^{-1}), 1.5 μl primer RA- 1495R (10 pmol μl^{-1}), 0.5 μl Taq DNA polymerase (5 U μl^{-1}) and 35.5 μl tri- distilled water. The thermal cycling program consisted of an initial denaturation step at 94°C for 5 min and 30 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 2 min with a final extension at 72°C for 10 min and 4°C for preservation. PCR amplification was carried out on an automatic thermal cycler (PTC-200, MJ Research®, USA). The 16S rRNA gene sequences of all isolates were obtained by Macrogen Inc. laboratory, Seoul, South Korea. MEGA version 6.0 software (http://www.mega software. net) was used to create phylogenetic trees by the neighbor-joining (NJ) method (Saitou and Nei, 1987; wang et al., 2016).

Fermentation conditions for lactic acid production

Fresh permeate was obtained from a soft cheese factory, Qena city, Egypt, and was utilized as a base medium for lactic acid fermentation after sterilization at 121°C for 20 min. Batch fermentations were prepared by inoculating 100μl of the selected culture in Erlenmeyer flasks containing 200 ml of permeate followed by incubation at 30°C. Fermentations were carried out in triplicates in a temperature-controlled flask shaker (Gerhardt®, Germany) operated at 150 rpm. Lactic acid concentrations, pH values, optical density, and cell mass were monitored throughout the fermentation time.

Determination of lactic acid concentration

Lactic acid concentrations were determined by a high-performance liquid chromatography (HPLC) system and confirmed only for the isolates with the highest lactic acid production. The instrument was equipped with a UV detector at 210 nm (Agilent Technologies® G4212-90013, USA) (Oh et al., 2005). A RezexTM ROA (300 x 7.8 mm, Phenomenex® USA) column was eluted with 50 mM phosphate solution in pH 2.8 as mobile phase at a flow rate of 0.4 ml min^{-1}, injection volume 25 μl with an isocratic elution at a retention time (RT) of 8.11. The column temperature was maintained at 60°C. The system was controlled by the "Turbochrom Navigator" software.

Effect of different factors on the production of lactic acid

The effect of different parameters, such as carbon and nitrogen sources, pH, temperature, and fermentation time, were investigated for the enhanced lactic acid produc-

tion from permeate medium by the used isolates. The fermentation medium was used without any supplementations as a control medium and, to examine the effect of different sources of nutrients on lactic acid production, other supplements were added. The fermentation medium was supplemented with 100 g l^{-1} of different carbon sources (glucose, fructose, sucrose, starch, and glycerol). Nitrogen sources were added with 10g l^{-1} (yeast, peptone, and beef extract). The effect of pH on lactic acid production was evaluated by adjusting the fermentation medium at pH values of 4, 5, 6, 7, and 8 using 5 mM HCl or NaOH. The optimum temperature was determined by carrying out fermentation at different temperatures (20, 27, 30, 37, 42, 45°C and 47°C) for 24 hours. The permeate medium was inoculated with the selected cultures and fermentation was performed for 12, 24, 48, and 72 hours at 30°C to evaluate the optimum fermentation time.

Statistical analysis

For each experiment three replicates were carried out. The variability degree of results was expressed in the form of means ± standard deviation (SD). SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Deviations were calculated as the standard error of (mean ± SD). A significance level of 0.05 was applied for every analysis. Data series were tested for normality with the Kolmogorov–Smirnov test and homogeneity of variance with the Levene's test. A significance level of 0.05 was applied for every analysis. It was done to compare control and other treated groups. Differences in mean (carbon sources, nitrogen sources, pH, temperature, and fermentation time) were tested using a one- way analysis of variance (ANOVA) with Tukey's honestly significant difference HSD test as post hoc analysis. The difference was considered statistically significant when P< 0.05.

Results

Enumeration of total lactic acid bacteria

The average count of total lactic acid bacteria of the 3 types of cheese samples collected from Qena city, Egypt are presented in Table 1. The bacterial viable counts of these samples ranged from (0.8 to 6.7 ×10^7 CFU/mL). The average count of lactic acid bacteria isolated from the Kareish cheese samples (K) was 6.4×10^7 CFU/mL, which was higher than that of the Domiati cheese samples (D) (3×10^7 CFU/mL). The isolated bacteria from the Edam cheese samples (ED) showed a lower average count (8×10^6 CFU/mL).

Table 1: lactic acid bacterial (LAB) count in the examined cheese samples

Cheese sample types	No. of samples	Sample numbers	Count (Log$_{10}$ CFU/mL)	
			Average (mean±SD)	Range
Edam	4	ED1~ ED4	$8.0\times10^6 \pm 0.01$	$8.0\times10^6 \sim 9.0\times10^6$

| Domiati | 8 | D1~ D8 | $3.0\times10^7 \pm 0.01$ | $2.5\times10^7 \sim 3.0\times10^7$ |
| Kareish | 8 | K1~ K8 | $6.4\times10^7 \pm 0.06$ | $61\times10^6 \sim 6.7\times10^7$ |

Isolation and identification of lactic acid bacteria.

Based on growth characteristics of bacterial isolates in whey permeate the isolates that showed the highest growth rate was identified by 16S rRNA gene sequences, phylogenetic analysis, and were utilized for further fermentations. To accurately check the uniqueness of these isolates at a species level, the sequence of the 16S rRNA gene (around 1,400 bp) was determined and examined with the NCBI BLAST program (http://www.ncbi.nlm.nih.gov) for their closest relatives/ reference strains with a homology of over or equal to 99%. These isolates were identified as *lactobacillus casei* MT682513, *Enterococcus camelliae* MT682510, *Enterococcus faecalis* MT682509, *Enterococcus lactis* MT682511, and *Wissella paramesenteroides* MT682512. Phylogenetic tree analysis was performed to reveal the relationship between the representative isolates and the known reference strains (Figure 1, see also supplementary file S1).

Production of lactic acid

In general, lactic acid production and bacterial cell mass were increased over time for the five tested isolates during whey permeate fermentation (without supplementations). While the pH values were ranged from 4 to 5.79 under the same previous conditions of permeate fermentation (Table 2). Among the five isolates, the highest lactic acid production (44.87 mg ml^{-1}) and bacterial cell mass (0.17g) were recorded after 14 hrs incubation of permeate fermentation (without supplementations) by *Lactobacillus casei* (Table 2; see also Figure 2A for HPLC analysis as a confirmation). Followed by *Enterococcus lactis* (41.15 mg ml^{-1}; cell mass 0.16g) (Table 2; see also Figure 2B for HPLC analysis as a confirmation); *Enterococcus faecalis* (39.00 mg ml^{-1}; cell mass 0.15g); *Enterococcus camelliae* (35.94 mgml^{-1}; cell mass 0.15g) and finally, *Wissella paramesenteroides* recorded the lowest lactic acid production as (33.15 mg ml^{-1}; cell mass 0.12g). (Table 2).

Effect of carbon sources on lactic acid production.

The lactic acid concentration was intensely increased reaching 92.023 mg ml^{-1} after 14 hrs of incubation by *Lactobacillus casei* utilizing glucose as carbon source compared to 42.09 mg ml^{-1} for permeate without any supplementations. When fructose was utilized as carbon source lactic acid concentration was approximately two folds of that produced form permeate alone (72.13 mg ml^{-1}). With the addition of sucrose and starch to the permeate, the maximum concentrations of lactic acid were decreased to 61.023 and 50.44 respectively after 14 hrs of fermentation. These results represent an increase of 45% and 20% compared to permeate without additional carbon sources. The utilization of glycerol did not result in a significant increase in lactic acid production by *Lactobacillus casei* (Figure 3). For *Enterococcus faecalis*, the addition of glycerol to the fermentation

medium stimulated the production of lactic acid compared to permeate without any supplements. Lactic acid production by *Enterococcus faecalis* from permeate only was 42.00 mg ml^{-1}. But when glycerol was added, the value increased to 50.96 mg ml^{-1} (Figure 3).
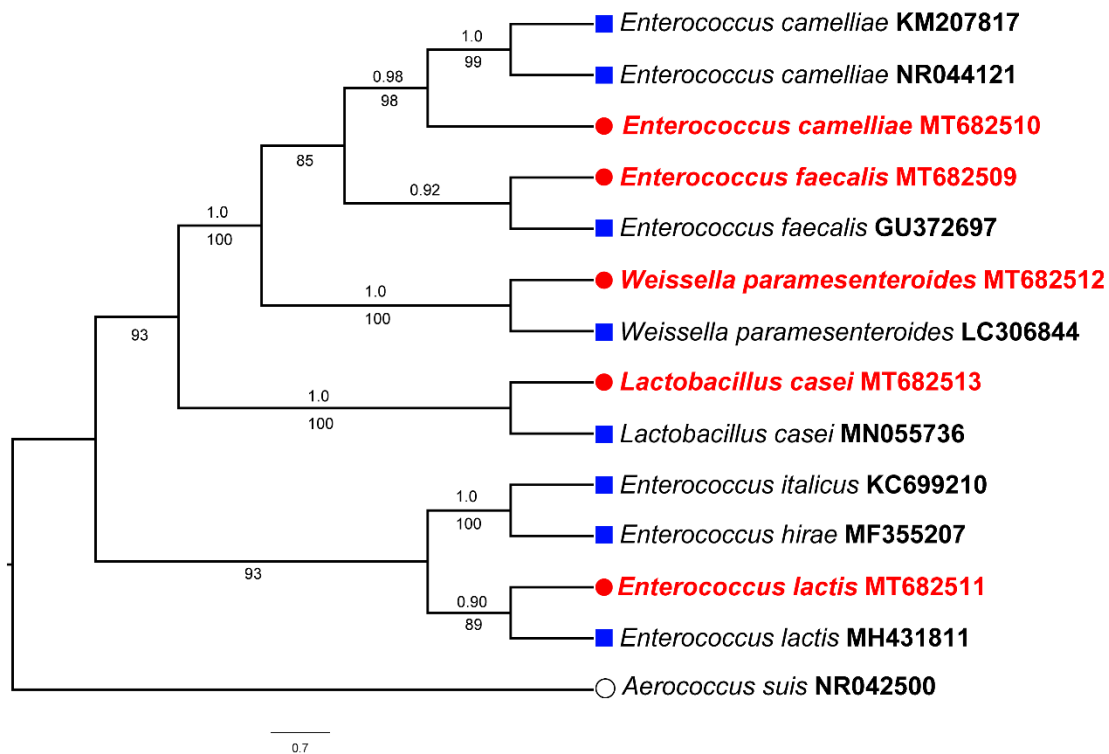

Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA sequences. Strict consensus cladogram of 90 Most Parsimonious (MP) trees found using the 16S ribosomal RNA sequence. Shown are the phylogenetic relationships among the isolated bacterial strains. The newly generated sequences are preceded by a red circle. The GenBank sequences are preceded by blue squares. The GenBank accession number appears after the species name. Values above the branches indicate Bayesian posterior probabilities (≥ 0.90), and the maximum parsimony bootstrap support values ($\geq 70\%$) are given below the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The outgroup used for tree construction preceded by an empty circle. Tree length= 685; Consistency index (CI) = 0.9489; Homoplasy index (HI) = 0.0602; Retention index (RI) = 0.9839; Rescaled consistency index (RC) = 0.9336.

Table 2: Lactic acid production by LAB isolates from whey permeates during the fermentation process over time.

Isolates	Fermentation criteria	Time (h)			
		2	**6**	**10**	**14**
Lactobacillus casei	**LA**[a]	4.96 ± 0.10	28.18 ± 0.19	**44.25** ± 0.09	**44.87** ± 0.06
	pH	5.79	4.49	4.00	4.00
	OD$_{600}$[b]	0.48 ± 0.02	2.53 ± 0.03	3.15 ± 0.04	3.15 ± 0.02
	Cell mass (g)	0.04 ± 0.00	0.08 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Enterococcus lactis	**LA**[a]	4.84 ± 0.07	25.76 ± 0.04	**41.10** ± 0.09	**41.15** ± 0.01
	pH	5.74	4.55	4.15	4.15
	OD$_{600}$[b]	0.37 ± 0.00	2.47 ± 0.03	3.12 ± 0.01	3.12 ± 0.05
	Cell mass (g)	0.04 ± 0.01	0.08 ± 0.00	0.16 ± 0.01	0.16 ± 0.01
Enterococcus faecalis	**LA**[a]	4.66 ± 0.15	25.04 ± 0.02	**38.34** ± 0.06	**39.00** ± 0.01
	pH	5.12	4.53	4.44	4.21
	OD$_{600}$[b]	0.28 ± 0.01	1.86 ± 0.06	2.84 ± 0.01	2.82 ± 0.03
	Cell mass (g)	0.03 ± 0.04	0.08 ± 0.00	0.14 ± 0.00	0.15 ± 0.01
Enterococcus camelliae	**LA**[a]	4.46 ± 0.16	24.28 ± 0.04	**35.94** ± 0.02	**35.94** ± 0.08
	pH	5.72	4.65	4.49	4.49
	OD$_{600}$[b]	0.27 ± 0.06	1.98 ±	2.40 ± 0.00	2.49 ±
	Cell mass (g)	0.03 ± 0.00	0.09 ± 0.00	0.15 ± 0.01	0.15 ± 0.00
Wissella paramesenteroides	**LA**[a]	4.42 ± 0.01	22.18 ± 0.02	**33.15** ± 0.11	**33.15** ± 0.04
	pH	5.79	4.96	4.52	4.52
	OD$_{600}$[b]	0.22 ± 0.01	1.70 ± 0.04	1.91 ± 0.00	1.91 ± 0.01
	Cell mass (g)	0.03 ± 0.00	0.07 ± 0.01	0.11 ± 0.02	0.12 ± 0.00

Values ± SD (n=3), [a]: Lactic acid concentration (mg/ml), [b]: Optical density of the fermentation medium.
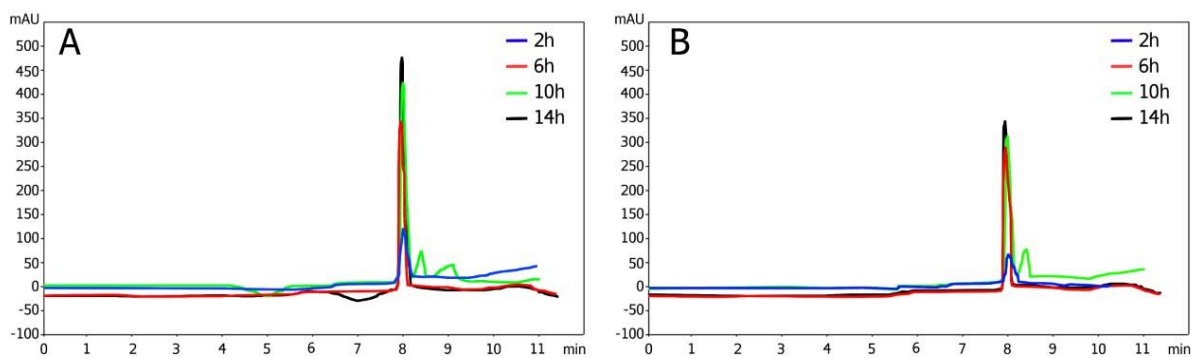
Figure 2: Chromatogram of lactic acid concentration by the fermentation of whey permeate using (A): *Lactobacillus casei* and (B): *Enterococcus lactis* at 30℃.
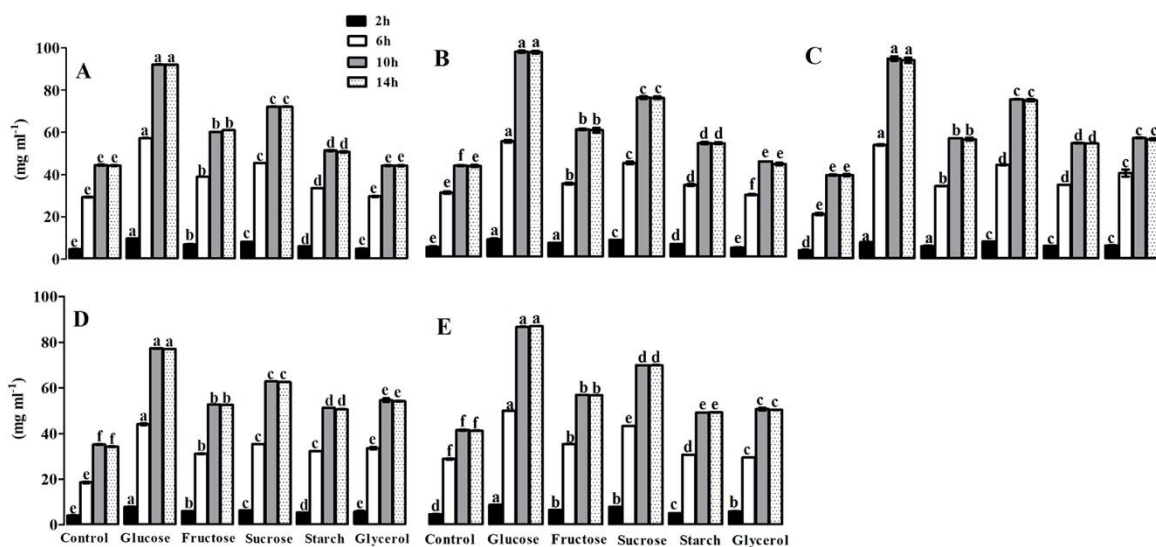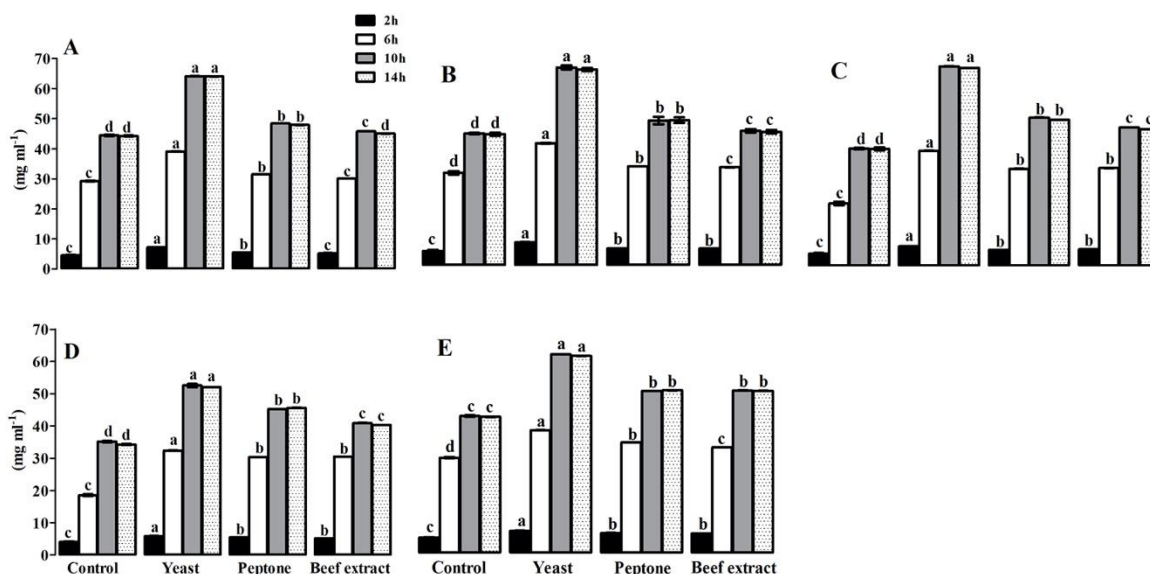

Figure 3: Effects of different carbon sources on the lactic acid production during whey permeate fermentation. (A): *Lactobacillus casei*, (B): *Enterococcus lactis*, (C): *Enterococcus faecalis* (D): *Enterococcus camelliae* and (E): *Weissella paramesenteroids*.

Effect of nitrogen source on lactic acid production.

The addition of yeast extract as a nitrogen source to permeate increased lactic acid production by *Lactobacillus casei* to 64.14 mg ml^{-1} after 10 hrs of fermentation (repre-senting 44.2% increase compared to con-trol). The concentrations of LA were 48.44 mg ml^{-1} and 45.84 mg ml^{-1} when peptone and beef extract was added to the fermen-tation medium with 9% and 3% increase compared to control (Figure 4).
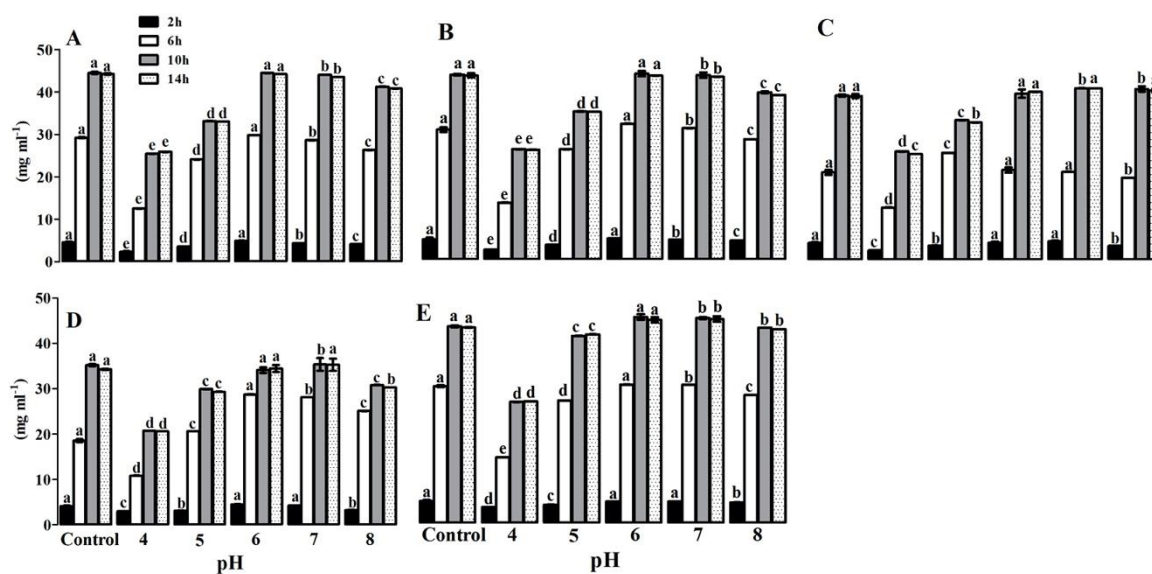

Figure 4: Effects of different nitrogen sources on the lactic acid production during whey permeate fermentation. (A): *Lactobacillus casei*, (B): *Enterococcus lactis*, (C): *Enterococcus faecalis* (D): *Entero-coccus camelliae,* and (E): *Weissella paramesenteroids*.
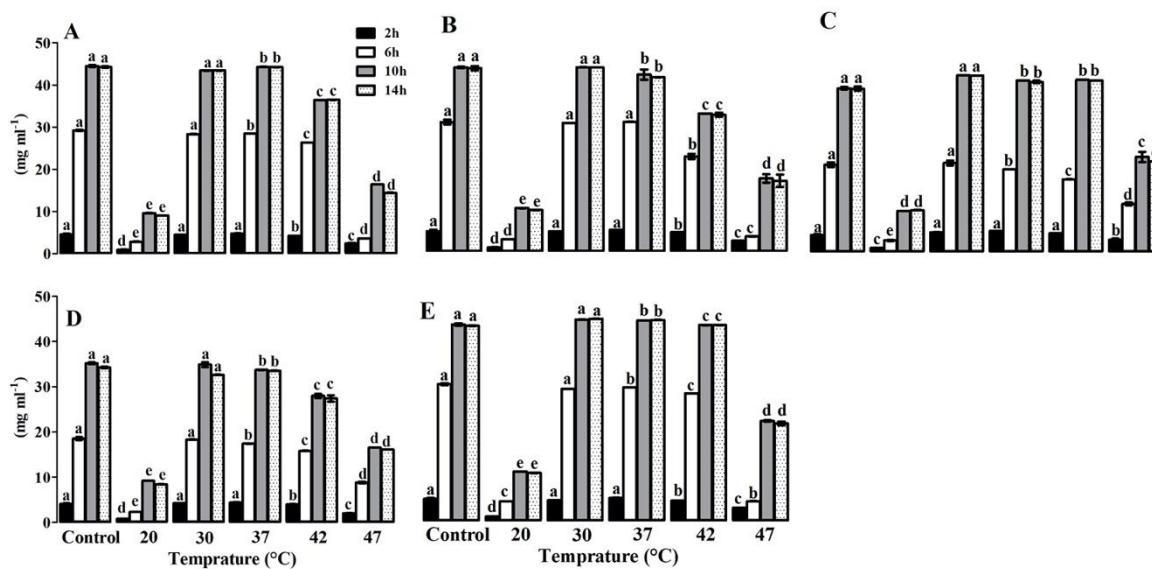
Effect of pH on lactic acid production.

The maximum lactic acid production was recorded around neutrality at pH 6 and pH 7. The lowest value was observed at pH 4, while at pH 5 and pH 8; lactic acid produc-tion was low (Figure 5).

Effect of fermentation temperature on lactic acid production.

The maximum lactic acid concentrations were recorded between (30 °C and 37°C) for all the tested isolates. Other tested tem-peratures yielded lower concentrations of lactic acid. A significant decrease in lactic acid yield was observed at 20 °C and 47°C (Figure 6).

Figure 5: Effects of medium pH on the lactic acid production during whey permeate fermentation. (A): *Lactobacillus casei*, (B): *Enterococcus lactis*, (C): *Enterococcus faecalis*. (D): *Enterococcus camelliae* and (E): *Weissella paramesenteroids*.
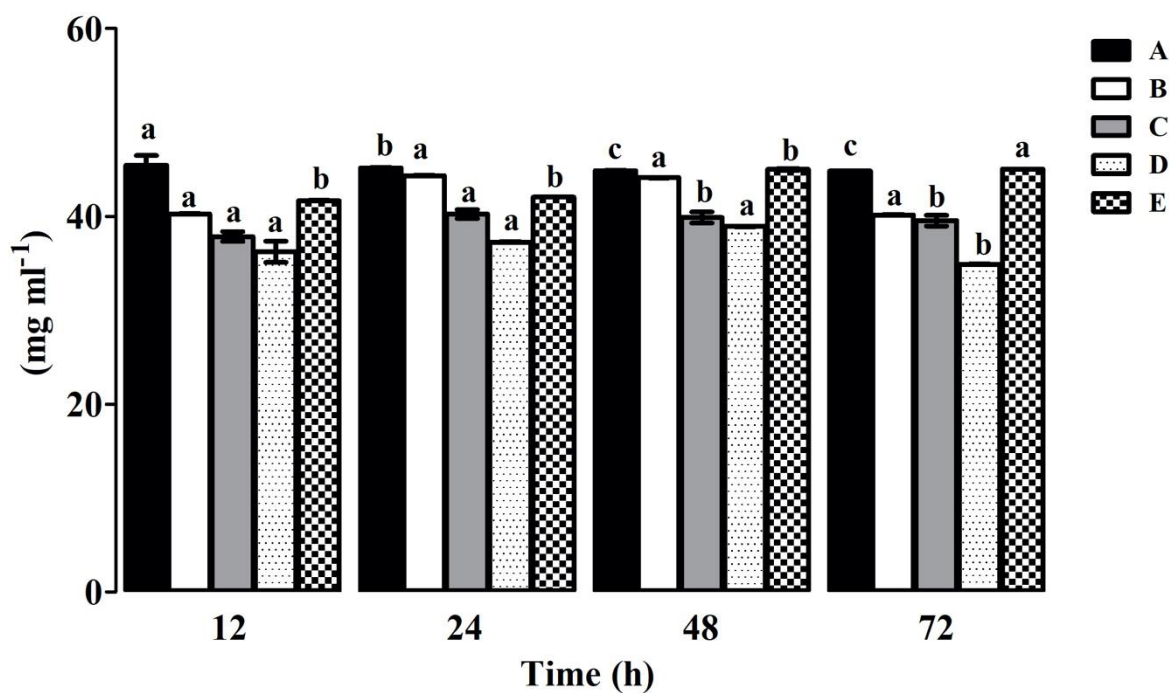

Figure 6: Effects of fermentation temperature on the lactic acid production from whey permeate. (A): *Lactobacillus casei*, (B): *Enterococcus lactis*, (C): *Enterococcus faecalis* (D): *Enterococcus camelliae* and (E): *Weissella paramesenteroids*.

Effect of fermentation time.

The results obtained are presented in (Figure 7). A gradual increase in lactic acid production for all the tested isolates was observed up to 24 h. A maximum lactic acid production of 45.15 mg ml^{-1} was detected after 24 h of incubation by *Lactobacillus casei* followed by *Enterococcus lactis* with (LA) level of 44.34 mg ml^{-1}. A fermentation time of 24 h was considered optimal for maximum lactose conversion to lactic acid (Figure 8).

Figure 7: Effects of fermentation time on the production of lactic acid from whey permeate. (A): *Lactobacillus casei*, (B): *Enterococcus lactis*, (C): *Enterococcus faecalis* (D): *Enterococcus camelliae,* and (E): *Weissella paramesenteroids*.
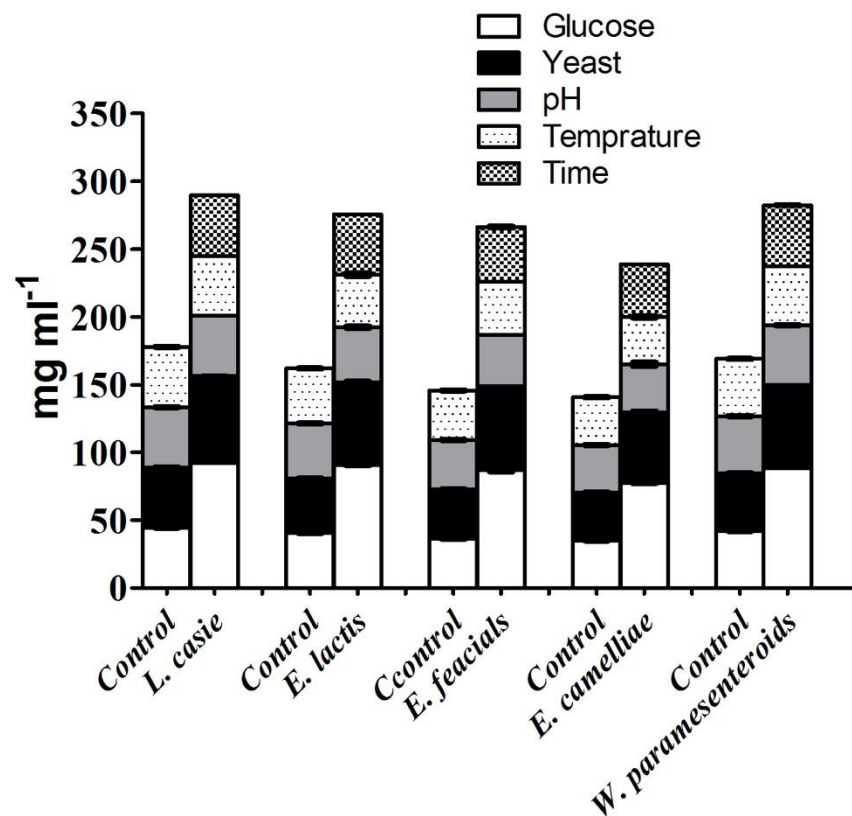
Figure 8: The optimized process parameters influencing lactic acid production during whey permeate fermentation. Shown are the highest lactic acid production (mg ml^{-1}) by utilizing additional sources of carbohydrate such as glucose (100g l^{-1}) and supplementing the permeate medium with yeast extract (10g l^{-1}) as nitrogen sources. Adjusting the permeate medium at pH 6 and fermentation temperature between 30°C and 37°C, over a fermentation time of 24 h compared to control bacteria.

Discussion

The current research used whey permeate (lactose-rich dairy by product) as lactic acid fermentation media to be viable substrate for production with lactic acid bacteria, in line with (Saeedi et al., 2015) which indicated the use of cheap raw materials for lactic acid production, such as starch and cellulose, whey and molasses. In this sense, five lactic acid bacteria have been isolated from different cheese samples. Due to the main role played by lactic acid bacteria in fermentation processes and the

production of lactic acid, the lactic acid bacteria have a predominant microbial community in dairy yields (Table 1; 2). The findings are confirmed in Wang et al. (2016), which stated that dairy products constitute an important source of isolation and testing of lactic acid bacteria. The efficiency of lactic acid bacteria in lactic acid production has been demonstrated through Panesar et al. (2007). Strict consensus cladogram of 90 Most Parsimonious (MP) trees found using the 16S ribosomal RNA sequence. The phylogenetic relationships

among the isolated bacterial strains were shown in (Figure 1). The newly generated sequences are preceded by a red circle. The GenBank sequences are preceded by blue squares. The GenBank accession number appears after the species name. Values above the branches indicate Bayesian posterior probabilities (≥ 0.90), and the maximum parsimony bootstrap support values ($\geq 70\%$) are given below the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The out-group used for tree construction preceded by an empty circle. Tree length= 685; Consistency index (CI) = 0.9489; Homoplasy index (HI) = 0.0602; Retention index (RI) = 0.9839; Rescaled consistency index (RC) = 0.9336. Several studies proved the efficiency of lactic acid bacteria to produce lactic acid (Panesar et al., 2007; Park et al., 2010). The addition of some sugars such as glucose to permeate considerably enhanced lactic acid yield when compared with control (permeate alone; Figure 3). Hexoses are degraded through several stages to acetyl- phosphate 2- glyceraldehyde 3- phosphate. The latter is metabolized by the Embden Meyerhof- Parnas pathway to lactic and acetic acid in the ratio 2: 3. This pathway yields 2.5 moles of ATP per mole of glucose, whereas homofermentative lactic acid fermentation generates 2 moles of ATP per mole of glucose (Idler et al., 2015). The fermentation enhancing effect of yeast extract was reported earlier (Agerholm-Larsen et al., 2000). This study indicated that the addition of yeast extract not only improved the bacterial return but also compact the time necessary for the

achievement of fermentation (Figure 4). This could be due to ingredients such as amino acids, peptides, vitamins, and some organic acids including pyruvic acid and glycerol in the yeast extract (Arasaratnam et al., 1996). The fermentation enhancing effect of yeast extract could be due to its high vitamin B content (Nancib et al., 2005; Smith et al., 2014). In the current study, the maximum lactic acid production (44.47 mg ml^{-1}) was observed at pH 6 by *Lactobacillus casei* while at pH 7 there was a slight decrease in lactic acid concentration as its value was 44.07 mg ml^{-1}. In general, at higher and lower pH levels, a decrease in the Lactic acid yield was observed (Figure 5). A pH ranges from 6.0 to7.0 has been reported as optimal for lactic acid production using lactic acid bacteria. Similarly, the pH ranges from 6.0 to 6.5 have been reported as optimal for lactic acid production using *L. casei* strain (Krischke et al., 1991; Beitel et al., 2020). It is well known that the hydrogen ion concentration of the medium has a great impact on microbial growth. The pH affects several aspects of microbial cells, including the function of its enzymes and the transport of nutrients into the cell. It also affects the synthesis of metabolic enzymes responsible for the synthesis of new protoplasm with an impact on RNA and protein synthesis (Kua and Bada, 2011). Growth temperature is one of the important factors that influence the activity of cellular enzymes. Enzymes are intensely active at optimum temperature and the enzymatic reaction proceeds at the maximum rates. The optimal temperature for the growth of lactic acid bacteria varies between the genera

from 20 to 45°C at 37°C for lactic acid production using *L. casei* (Wood and Holzapfel, 2012; Beitel et al., 2020). The obtained results are in agreement with the previous data (Figure 6). From the above observations, a temperature range of 30-40°C was considered optimal to produce lactic acid from whey permeate using lactic acid bacteria. Pescuma et al. (2008) mentioned that a specific strain of *Streptococcus thermophilus* CRL 804 used up to 12% of the original lactose content and formed the maximum quantity of lactic acid at 24h. The same finding was observed for all the tested isolates. On the other hand, fermentation of whey for 48 h has been used for lactic acid production by different *lactobacilli* strains (Chiarini et al., 1992; Gandhi et al., 2000; Kumar et al., 2001). The shorter the fermentation time, the more cost-effective the fermentation process (Figure 7,8). While longer time may result in contamination accompanied by economic losses.

Conclusion:

In the current study, several factors influencing lactic acid production from permeate were investigated. The obtained results showed that during fermentation, the production of lactic acid from whey permeate could be improved by utilizing additional sources of carbohydrate such as glucose (100g l^{-1}) and supplementing the permeate medium with yeast extract (10g l^{-1}) as nitrogen sources. Also, the results of the present study indicated that adjusting the permeate medium at pH 6 and fermentation temperature between 30°C and 37°C have a great impact on the fermentation process

in terms of lactic acid yield. The above-optimized process parameters with the selection of the proper isolate such as *Lactobacillus casei* in this study could be applied and recommended in for further scaling- up studies in dairy industries. This could be more profitable for these industries as an additional income from the production of lactic acid using the permeate byproduct that is not currently beneficial for these industries as well as representing an environmental problem.

Conflicts of Interest

The authors declare no conflict of interest.

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