Plant Archives Vol. 19, Supplement 2, 2019 pp. 793-798 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BIO-PRODUCTION OF LACTIC ACID FROM SALTED WHEY AND WHEY PERMEATE

Dosuky Atiat S.* ; Nasr F.N. ; Yousef Eman T.A.* and Barakat Olfat S.****
*Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.
**Agricultural Microbiology Department, Faculty of Agriculture, Cairo University, Egypt

Abstract

Bio-production of lactic acid using microorganisms is promising natural processing, particularly, lactic acid production from industrial wastes such as voluble by products of dairy industries. Factors affecting lactic acid production yield were studied during fermentation process of whey permeate, salted cheese whey and their mixtures using different strains of lactic acid bacteria (LAB). Optimum conditions for production of lactic acid were mixture of salted whey and whey permeate (1:1), contained 5% sugar, 3% salt and 0.5% calcium carbonate during static state fermentation at 37°C to give 27-38 g/l with efficiency ranged between 60- 80%. *L. casei* and *L .rhamnosus* B-445 were the most efficient strains.
Keywords: Lactic acid bacteria, whey permeate, batch fermentation.

Introduction

Lactic acid (2-hydroxypropanoic acid), CH_3CHOH COOH, can be produced by either microbial fermentation or chemical synthesis There are two optical isomers of lactic acid: L(+)-lactic acid and D(–)-lactic acid. L(+) Lactic acid is classified as GRAS (generally recognized as safe) for use as a food additive by the US FDA (Food and Drug Administration), while D(–)-lactic acid is at times harmful to human metabolism and can result in acidosis as well as decalcification (Vidra *et al*., 2017).

Lactic acid is used in food, beverages, personal care products, pharmaceutical and in the production of biodegradable poly lactic acid (PLA), a well-known bioplastic material (Sodergard and Stolt, 2002). The worldwide production of lactic acid is nearly 300–400 kilo tons per year and is projected to be increased rapidly (Nattrass and Higson, 2011). According to the report of Global View Research (California, USA), the global market of lactic acid was estimated to be 714.2 kilo tons in 2013 and is expected to reach 1,960.1 kilo tons by 2020 (Grand View Research, 2014, Rodrigues *et al*., 2017).

Microorganisms that are capable of producing lactic acid are of three groups: Bacteria, fungi and yeast (Battula *et al*., 2018). Most of the lactic acid production can be occurred industrially by using of lactic acid producing bacteria in an aerobic fermentation process (Cui *et al*., 2011). Although fungal fermentation has a slight advantage over bacterial fermentation as filamentous fungi need only a simple medium to produce lactic acid, using glucose aerobically, it requires vigorous aeration (Rodrigues *et al*., 2017).

Whey is by product of cheese production after precipitation of casein, was viewed as one of the major disposal problems of dairy industry, since 9 L of whey is produced from 1 kg cheese with high carbohydrate, protein and lipid contents, because of its biochemical potential as nutrient supplementation. This high level of waste disposal represents a substantial loss of voluble resource, so, cheese has been used as a substrate for production of organic acids (i.e. lactic acid), ethanol, single cell protein, etc (Ryan and Walsh, 2016). The permeate stream after whey protein recovery is mainly composed of lactose and salts, the lactose sugar fraction in cheese whey permeate can be used for producing lactic acid, ethyl alcohol etc. whey permeate is readily a valuable and low cost substrate for production of lactic acid (Mollea *et al*., 2013).

The aim of this study production of lactic acid from whey, whey permeate, mixtures of whey and whey permeate using different strains of lactic acid bacteria i.e. *L. bulgaricus*, *L. lactis* subsp., *L. casei*, *L. reuteri* B-14171, *L .rhamnosus* B-445, *L. acidophilus 791N*, *L. casei 761N.Lactis, Lactococcus lactis diacetilactis, Ent. faceium,* and *Streptococcus*.

Materials and Methods

Raw Materials

Salted whey containing 4% salt and 5% sugar was obtained from Dairy Dep., Fac. of Agri., Cairo Univ., Giza, Egypt. Whey permeate containing lactose 5% was obtained from Dairy Industry Unit, Animal Production Research Institute, ARC, Ministry of Agriculture, Giza, Egypt. Whey or whey permeate were clarified through protein precipitation by heating at 85°C for 20 min then cooled to room temperature and filtered though filter paper Whatman No. 1. and sterilized at 121°C for 15 min. (Gupte and Nair, 2010).

Microorganism

Ten bacterial strains i.e *L. bulgaricus*, *L. lactis* subsp. *lactis, L. casei*, *L. reuteri* B-14171, *L .rhamnosus* B-445, *L. acidophilus 791N*, *L. casei 761N. Lactococcus lactis diacetilactis*, *Ent. faceium,* and *Streptococcus* sp. were obtained from Department of Agricultural Microbiology; Faculty of Agriculture; Cairo University to examine their potential to produce lactic acid. The strains were stored at -20°C by mixing the fresh sub – cultures with 20% glycerol (Samelis *et al*., 1994). All strains before tested were sub – cultured twice at appropriate temperature in sterile reconstitute skimmed milk.

Batch fermentation in conical flasks

Preparation of starter culture : The bacterial strains were grown in 50 mL of MRS medium for *lactobacilli* and M17 medium for *lactococci* in 250 mL Erlenmeyer flask and incubated at 37°C for 24 h under static conditions.

Fermentation media : Treated raw materials (whey permeate, whey, mixtures of whey permeate and whey) were supplemented with nutrients (SN) i.e. manganese sulphate (20 mg/L), yeast extract (0.75%, w/v) and calcium carbonate (0.5 % or 1.5%, w/v). The media were sterilized at 121°C for 15 min. All fermentation media were prepared in 250 ml conical flasks, each containing 100 ml of examined fermentation media and inoculated with 4% inoculum size of an active culture (24 h). the flasks were incubated at 37°C for 36 h. lactic acid production and consumed sugar were measured after 36 h (Miller *et al* 1959). Different set of batches were carried out to study the suitable condition of lactic acid production as following.

(a) Effect of aeration

The effect of aeration on lactic acid production was studied using whey permeate containing 5% sugar using all the examined strains or whey permeate containing 4% sugar by five strains (*L. bulgaricus*, *L. reuteri* B-14171, *L .rhamnosus* B-445, *Ent. faceium,* and *Streptococcus*) at static and agitation at 120 rpm using a rotary shaker Model SI-100, inoculum size 4% and $CaCO_3$ 1.5%. Batches were performed using all the examined strains at 37°C for 36 h in a flask with 100 ml working volume.

(b) Effect of sugar concentration

Effect of lactose concentration on lactic acid production was investigated using whey permeate by all the examined bacterial strains as a source of sugar. Dilution of whey permeate was performed with distillated water to give five different concentration of lactose 1, 2, 3, 4 and 5%, $CaCO_3$ 1.5%.

Effect of NaCl concentration

Whey permeate was mixed with salted whey to give different concentrations of salt (NaCl%) as the following; whey permeate : salted whey; 3:1 (1%), 1:1 (2%), 1:3 (3%) and 0:1 (4%) NaCl. These different mixtures were used as fermentation culture media for production of lactic acid. $CaCO_3$ 0.5% and Initial sugar concentration: 5%.

(a) Effect of $CaCO_3$ concentration

Two $CaCO_3$ concentration (0.5 or 1.5%) were added to medium contained whey permeate and salted whey (1:1) to study effect of addition of calcium carbonate on lactic acid production by strains, *L.casei* and *L .rhamnosus* B-445.

(b) Effect of sugar type and concentration

Four different sugar (glucose, lactose, sucrose, fructose) were added to mixed medium contained whey permeate and salted whey (1:1) to increase the medium sugar concentration to 10 or 15 % sugar and study its effect on lactic acid production.

In all experiments yield and efficiency were calculated as follows:

$$\text{Efficiency (\%)} = \frac{\text{Initial lactose conc. – residual lactose conc.}}{\text{Initial lactose conc.}} \text{ X 100}$$

Lactic acid yield was expressed as follows:

$$\text{Lactic acid yield (\%)} = \frac{\text{Lactic acid production}}{\text{Lactose utilized}} \text{ X 100}$$

Analytical Methods

Reducing sugar was determined using 3,5-dinitrosalicylic acid (DNS) method according to Miller *et al* (1959) as g/l. Lactose was determined according to the methods described by Sánchez-Manzanares *et al*., (1993). Lactic acid was measured by means of HPLC (column: zorbax ODS: 4.6 x 250 mm at 210 nm) as g/l (Lin *et al*., 2011). Lactate concentration was spectrophotometrically determined using lactate kit (lactate- Liquizyme, Schiffgraben, Hannover, Germany) at 546 nm (Panesar *et al*., 2010).

Statistical Analysis

All experiments were applied in triplicates arranged in a completely randomized design. The significance of the main effects was determined by analysis of variance (ANOVA). The significance of variance treatments was evaluated by Duncan's multiple range test (P ・ 0.05). All analysis were made using a software package "Costat", a product of Cohort Software Inc., Berkeley, California. All data were recorded as means of three replications.

Result and Discussion

Effect of Aeration

Data of lactic acid production due to lactose concentration was recorded in table (1). The results showed that, static state more suitable for lactic acid production than aeration with all strains tested. The highest lactic acid and its yield were 27 and 23.9g/l and 65 % and 59% with *L. rhamnosus* B-445 and *E. faceium* in static state. While, in aeration, the lactic acid and yield reached to 21.4 and 21.3g/l for lactic acid and 64% for its yield with *L. reuteri* and *L. rhamnosus* B-445.

Better five bacterial strains in lactic acid production (*L. bulgaricus*, *E. faceium, L. reuteri*, *L. rhamnosus* B-445 and *Streptococcus*) were chosen to complete experiments of fermentation in whey permeate contained 4% lactose under static and agitation states (Table 2).

The whey contains 40 g/l lactose was used for lactic acid production where the maximum lactic acid production were obtained at static where the highest lactic acid production of 20.2 g/l and yield of 69 % were obtained by using *Streptococcus* sp following lactic acid production of 18.91g/l and yield of 61% by *L .rhamnosus* B-445 while at shaking on 120 rpm, the highest lactic acid production of 19.1 g/l and yield of 76 % were obtained by using *Streptococcus* sp following lactic acid production of 17.65 g/l and yield of 70 % by *L. reuteri* (Table, 2).

In general, in all tested strains, production of lactic acid was higher under static state (anaerobic) than shaking aeration conditions (aerobic). Physiologically, pyruvic acid of glycolysis in bacteria enter fermentation pathways under anaerobic conditions in absence of oxygen (Moat *et al*., 2002). Also Panesar *et al*. (2010) experimentally reported that, static fermentation was better than agitation using orbital shaker for production of lactic acid from whey using *L. casei*.

(1) Effect of sugar concentration.

Whey permeate was diluted with distilled water to give five different concentrations of lactose sugar (Table 3). Results indicated that, increasing in lactose concentration (1 to 5%) caused increasing of lactic acid production ranged between 1.65 to 27 g/l with all tested strains. The results presented in Table (3), showed that, the optimum lactose concentration for lactic acid production and its yield was 5%. All the five strains tested were given yield ranged between 59 to 66%. No significant difference was found between the five strains tested. Approximately the yield was ranged between 63 to 66% except one strain (*E. faceium*) was given 59%. *Streptococcus* sp give the highest production of lactic acid; 6.9, 9.6, 13.3 and 20.2 g/l in 1, 2, 3 and 4% lactose whey permeate with yields; 86, 51, 49 and 69%, respectively. Except in 5% lactose, *L. rhamnosus* B-445 give the highest production 27 g/l with yield 65%.

Efficiency of lactic acid production (Fig. 1) was higher in 2% lactose with most of tested strains. *Streptococcus sp* recorded highest efficiency with 1, 2, 3 and 4% lactose but *L. rhamnosus* B-445 give the highest efficiency only with 5% lactose.

(2) Effect of salt concentration

The more suitable medium tested (3%) followed by (2%) and (4%) salt for the best strains *L. casei* and *L. rhamnosus* B-445 were given yield ranged between 88 and 93%; 89 and 79% and 90 and 83% respectively. The highest production of lactic acid was 33.24 g/l with yield 83% by *L. rhamnosus* B-445 and 30.73 g/l with yield 90% by *L. casei* recorded in 3% salted whey. For the production of lactic acid, in 4% salt, the highest lactic acid production of 29.92 g/l and yield of 93% were obtained using *L .rhamnosus* B-445. Following lactic acid production of 28.24 g/l and yield of 88% were obtained using *L. casei*. In 2% salt, the highest lactic acid production of 30.1 g/l and yield of 89% were obtained using *L. casei*. Following *L. rhamnosus* B-445 with lactic acid production of 28.64 g/l and yield of 79%. In 1% salt, the highest lactic acid production of 10.90 g/l and yield of 32% were obtained using *L. casei*. Following *L. rhamnosus* B-445 with lactic acid production of 10.60 g/l and yield of 44%. Chikthimah *et al*. (2001) reported that, salt (3.5% NaCl) influenced growth of lactic acid bacteria. Also, salted whey can be used as a substrate for growth of other microorganisms such as yeasts for production of valuable products like carotenoid (Kanzy *et al*., 2015).

Efficiency was presented in Fig. 2. Highest efficiencies were in 2, 3, and 4% salted whey reached using *L. rhamnosus* B-445 and *L. casei*. Consequently, *L. casei* and *L. rhamnosus* B-445 were considered as the best bacterial strains for production of lactic acid using of fermentation of cheese whey, which consistent with other researchers who used *L. casei* (Büyükkileci and Harsa, 2004; Panesar *et al*., 2010) and *L. rhamnosus* (Cui *et al*., 2012) for production of lactic acid using fermentation of cheese whey and whey permeate.

(3) Effect of sugar type and concentration

The best strains in previous experiment were used for fermentation of mixture of whey and whey permeate (1:1) contained 10 and 15% sugar of glucose, lactose, sucrose or fructose (Table 5). Results reveled that, 10% sugar was better for production of lactic acid. While the best sugar were glucose and sucrose which give lactic acid production average 33.5 g/l using *L. casei* and 38.5 g/l using *L. rhamnosus* B-445 with efficiency around 75%. Other researchers deduced that, lactose, sucrose, glucose and fructose can be utilized by Lactobacillus spp as fermentable sugars for production of lactic acid (Srinivas *et al*., 1990; Vidra *et al*., 2017).

(4) Effect of $CaCO_3$ concentration

Mixtures of whey and whey permeate (1:1) contained 10% sugar and 0.5 or 1.5% calcium carbonate were fermented using *L. casei* and *L. rhamnosus* B-445 (Table 6). Results indicated that, 0.5% $CaCO_3$ recorded higher production of lactic acid ranged between 28 and 38.94 g/l with higher efficiencies ranged between 74 and 87%. Calcium carbonate was added as a buffer to reduce effect of produced acid as inhibition factor for bacterial strains. Similarly, calcium carbonate was added to neutralize the acid formed during production of lactic acid by fermentation of whey and whey permeate using LAB (Büyükkileci and Harsa, 2004; Panesar *et al*., 2010 and Cui *et al*., 2012).

Table 1: Effect of aeration on lactic acid production using whey permeate

Bacterial strains	Aeration	*LA(g/l)	** CS (g/l)	Yield %	Efficiency (%)
L. bulgaricus	static	21.2	33.6	63	67
	120 rpm	20.1	33.2	61	66
L. lactis	static	16.2	23.8	54	48
	120 rpm	12.82	23.8	68	48
E. faceium	static	23.9	40.5	59	81
	120 rpm	17.4	33.9	51	68
L. casei	static	22.1	23.7	93	47
	120 rpm	17	23.7	72	47
L. reuteri	static	22	33.6	65	67
	120 rpm	21.4	33.5	64	67
L. rhamnosus B-445	static	27	41.4	65	83
	120 rpm	21.3	33.4	64	67
Streptococcus	static	21.92	33.4	66	66
	120 rpm	20.3	42.5	48	46
L. acidophilus 791N	static	11.2	32.9	34	67
L. casei 761N	static	10.73	23	47	66

Initial sugar concentration: 5% $CaCO_3$ 1.5% inoculum size: 4% *LA: lactic acid (g/l)
**CS: consumed sugar (g/l) LSD 0.05 = 0.391 for bacterial strains LSD 0.05 = 0.184 for aeration

Table 2 : Effect of aeration on lactic acid production using diluted whey permeate

Bacterial strains	Aeration	*LA (g/l)	** CS (g/l)	Yield %	Efficiency (%)
L. bulgaricus	static	15.9	26.4	58	66
	120 rpm	15.2	26.4	60	66
E. faceium	static	17.65	26.8	64	67
	120 rpm	16.82	27	70	67
L. reuteri	static	18.72	28.2	63	71
	120 rpm	17.65	26.8	70	67
L. rhamnosus B-445	static	18.91	27.8	61	70
	120 rpm	17.2	26.8	66	68
Streptococcus	static	20.2	27.8	69	70
	120 rpm	19.1	26.6	76	67

Initial sugar concentration: 4% $CaCO_3$ 1.5% *LA: lactic acid (g/l)
** CS: consumed sugar (g/l) LSD 0.05 = 0.429 for bacterial strains LSD 0.05 = 0.271 for aeration

Table 3: Effect of dilution of whey permeate (sugar concentration) on lactic acid production

Bacterial strains	Initial sugar concentration														
	1%			2%			3%			4%			5%		
	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %
L. bulgaricus	2.3	5.7	40	7.5	13.05	57	8.15	19.5	42	15.9	26.4	58	21.2	33.6	63
E. faceium	4.41	6.6	67	6.6	15.9	42	9.22	19.8	47	17.6	27.8	61	23.9	40.5	59
L. reuteri	1.65	3.9	42	5.44	14.85	37	7.47	19.65	38	18.72	28.2	63	22	33.6	65
L. rhamnosus B-445	3.9	4.2	93	7.42	15.3	48	10.2	19.65	52	18.91	26.8	64	27	41.4	65
Streptococcus	6.9	8	86	9.6	19	51	13.3	27	49	20.2	27.8	69	21.92	33.4	66

$CaCO_3$ 1.5% *LA: lactic acid (g/l) ** CS: consumed sugar (g/l)
LSD 0.05 = 0.237 for bacterial strains and sugar concentration

Table 4 : Effect of salt concentration on production of lactic acid using different LAB strains

Bacterial strains	Salt concentration (%)											
	1			2			3			4		
	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %	*LA (g/l)	** CS (g/l)	Yield %
L. bulgaricus	10.40	24.2	43	10.91	24.1	45	5.55	14.2	39	11.02	22.3	49
Lactococcus lactis diacetilactis	10.55	23.9	44	10.84	24.3	45	11.45	24.2	47	11.09	22	50
L. lactis	5.95	14	43	6.3	24.1	26	10.5	14.4	73	5.71	12.1	47
E. faceium	9.40	23.9	39	10.79	24.5	44	9.25	24.5	38	10.95	22	50
L. casei	10.90	34.4	32	30.1	33.9	89	30.73	34.2	90	28.24	32.2	88
L. reuteri	10.20	23.9	43	7.78	24.4	32	5.5	13.8	40	10.66	22.4	48
L. rhamnosus B-445	10.60	24.1	44	28.64	36.2	79	33.24	39.9	83	29.92	32.1	93
L. acidophilus 791N	9.30	24	39	9.04	24.4	37	9.8	23.8	41	9.60	22.3	43
L. casei 761N	7.10	16.2	44	6.18	24.6	25	5.4	16.2	33	5.94	12.2	49

$CaCO_3$ 0.5% Initial sugar concentration: 5% *LA: lactic acid (g/l) ** CS: consumed sugar (g/l)
LSD 0.05 = 0.228 for bacterial strains LSD 0.05 = 0.152 for salt concentration

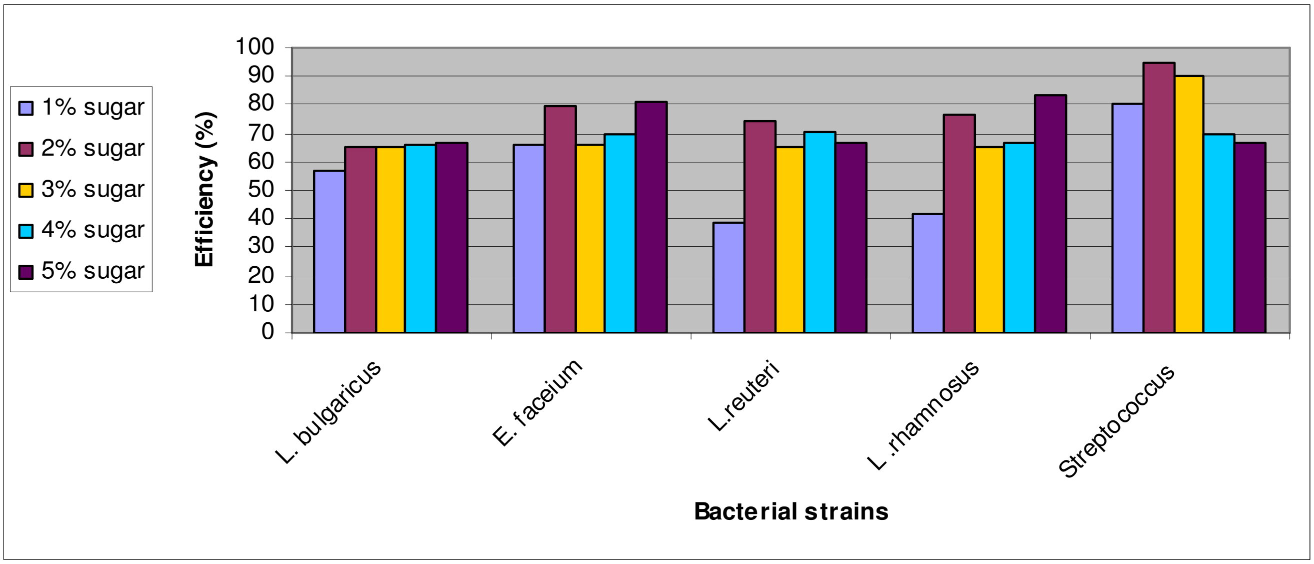

Fig.1. Efficiency of lactic acid production using LAB strains using whey permeate with different concentrations of lactose sugar.

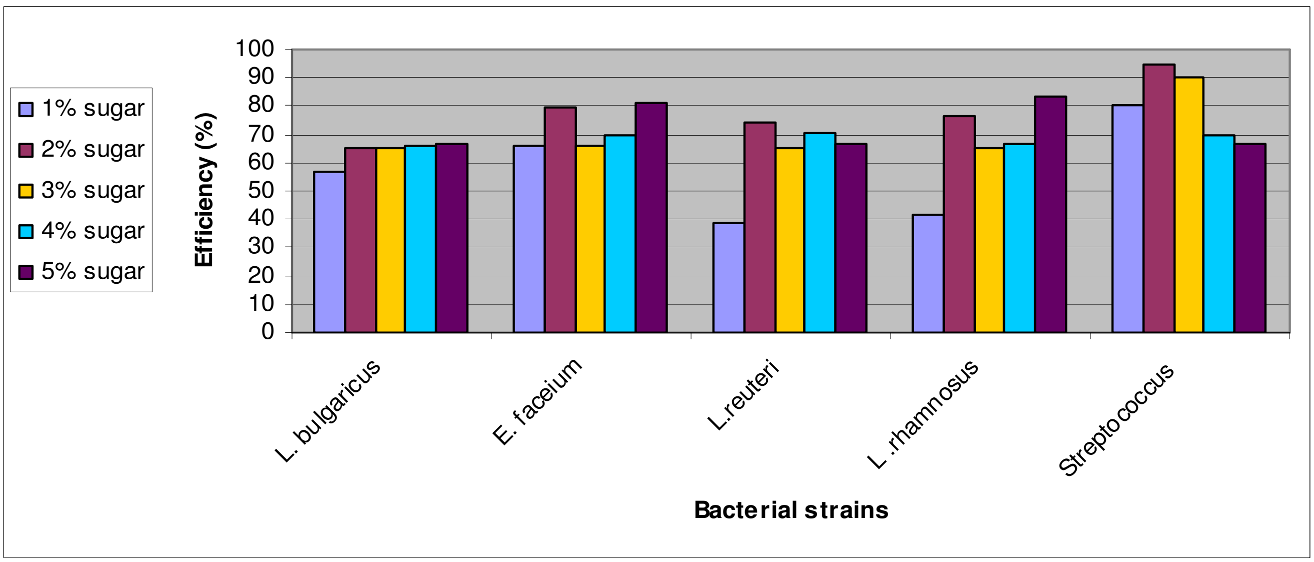

Fig. 2 : Efficiency of lactic acid production using LAB strains using mixture of whey permeate and salted whey with different concentrations of salt (NaCl).

Table 5: Effect of the type and concentration of sugar on lactic acid production using whey: whey permeate in ratio of 1:1

Bacterial strains	Sugar type	Initial sugar concentration							
		10%				15%			
		*LA (g/l)	** CS (g/l)	Yield %	Efficiency (%)	*LA (g/l)	** CS (g/l)	Yield %	Efficiency (%)
L. casei	Glucose	33.61	74.7	45	75	32.62	114.5	28	76
L. rhamnosus B-445		38.94	75.2	52	75	36.25	114.7	32	76
L. casei	Lactose	36.18	74.3	49	74	32.83	114.9	29	77
L. rhamnosus B-445		36.00	74.8	48	75	35.82	114.7	31	76
L. casei	Sucrose	33.32	73.9	45	74	32.80	114.5	29	76
L. rhamnosus B-445		38.11	74.4	51	74	37.25	114.2	33	76
L. casei	Fructose	28.00	84.0	33	84	ND	ND	ND	ND
L. rhamnosus B-445		29.00	86.9	33	87	ND	ND	ND	ND

$CaCO_3$ 0.5% *LA: lactic acid (g/l) ** CS: consumed sugar (g/l) LSD 0.05 = 0.094 for bacterial strains and sugar concentration
LSD 0.05 = 0.133 for sugar type

Table 6 : Effect of $CaCO_3$ concentration on lactic acid production using whey: whey permeate in ratio of 1:1

Bacterial strains	Sugar type	$CaCO_3$ **concentration**							
		0.5%				**1.5%**			
		***LA (g/l)**	**** CS (g/l)**	**Yield %**	**Efficiency (%)**	***LA (g/l)**	**** CS (g/l)**	**Yield %**	**Efficiency (%)**
L. casei	Glucose	33.61	74.7	45	75	22.6	63.8	35	64
L. rhamnosus B-445		38.94	75.2	52	75	22.3	64.5	35	65
L. casei	Lactose	36.18	74.3	49	74	20.7	64.4	32	64
L. rhamnosus B-445		36.00	74.8	48	75	20.4	64.5	32	65
L. casei	Sucrose	33.32	73.9	45	74	22.3	63.9	35	64
L. rhamnosus B-445		38.11	74.4	51	74	20.5	63.8	32	64
L. casei	Fructose	28.00	84.0	33	84	21.7	64.2	34	64
L. rhamnosus B-445		29.00	86.9	33	87	20.6	63.9	32	64

Initial sugar concentration: 10% *LA: lactic acid (g/l) ** CS: consumed sugar (g/l)
LSD 0.05 = 0.131 for bacterial strains and sugar concentration LSD 0.05 = 0.185 for sugar type

Conclusion

Fermentation process can be efficiently used for production of lactic acid from huge dairy industries wastes; whey permeate and salted cheese whey using lactic acid producing bacterial strains *Streptococcus sp., L. casei* and *L. rhamnosus* B-445 were efficient and promising bacterial strains for production of lactic acid under stress such as salt or sugar contained whey or whey permeate.

References

Batulla, S.K.; Nikhilesh, G.S.S.; Tarun, B.; Narayana, S.K.V. and Gopinadh, R. (2018). Industrial production of lactic acid and its applications. International Journal of Biotech Research. 1: 42-54.

Büyükkileci, A.O. and Harsa, S. (2004). Batch production of L(+) lactic acid from whey by *Lactobacillus casei* (NRRL B-441). J Chem Technol Biotechnol 79: 1036–1040.

Chikthimah, N.; Anantheswaran, R.C.; Roberts, R.F.; Mills, E.W. and Knabel, S.J. (2001). Influence of sodium chloride on growth of lactic acid bacteria and subsequent destruction of *Escherichia coli* 0157: H7 during processing of Lebanon bologna. J. Food Protection 64(8): 1145- 1150.

Cui, F.; Li, Y. and Wan, C. (2011). Lactic acid production from corn stover using mixed cultures of *Lactobacillus rhamnosus* and *Lactobacillus brevis*. Bioresource Technology,102:1831e6.

Cui, F.; Wan, C.; Li, Y. and Liu, Z. (2012). Co-production of lactic acid and *Lactobacillus rhamnosus* cells from whey permeate with nutrient supliments. 5: 1278- 1286.

Gupte, A. and Nair, J. (2010). β- Galactosidase production and ethanol fermentation from whey using *Kluveromyces marxinus* NCIM 3551. J. Sci. Ind. Res., 69:855-869.

Kanzy, H.M.; Nasr, N.F.; El- Shazly, H.A.M. and Barakat, O.S. (2015). Optimization of carotenoids production by yeast strains of *Rhodotorula* using salted cheese whey. Int. J. Curr. Microbial. Appl. Sci., 4(1): 456-469.

Lin, J.T.; Liu, S.C.; Shen, Y.C. and Yang, D.J. (2011). Comparison of various preparation methods for determination of organic acids in fruit vinegars with a simple ion- exclusion liquid chromatography. Food Anal. Methods, 4:531-539.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426–428.

Moat, A.G.; Foster, J.W. and Spector, M.P. (2002). Microbial physiology. Fourth Edition. Wiley-Liss, New York. Pp 417.

Mollea, C.; Marmo, L. and Bosco, F. (2013). Valorisation of Cheese Whey, a By-Product from the Dairy Industry. In: Muzzalupo I (ed) Food Industry. InTech. doi:10:5772-53159.

Nattrass, L. and Higson, A. (2011). NNFCC Renewable Chemicals Fact Sheet: Lactic Acid, United Kingdom. York, UK: NNFCC.

Panesar, P.S.; Kennedy, J.F.; Knill, C.J. and Kosseva, M.R. (2010). Production of L(+) lactic acid using *Lactobacillus casei* from whey. Brazilian Archives of Biology and Technology, 53: 219–226.

Ryan, M.P. and Walsh, G. (2016). The biotechnological potential of whey. Rev Environ Sci Biotechnol. 15:479–498.

Rodrigues, C.; Vandenberghe, L.P.S.; Woiciechowski, A.L.; Oliveira, J.de Letti, L.A.J. and Soccol, C.R. (2017). Production and Application of Lactic Acid.

Samlis, J.; Maurogenakis, F. and Metaxopoulas, J. (1994). Characterization of lactic acid bacteria isolated from semi- hard goat's cheese. J. Dairy Res., 58: 137-145.

Sánchez-Manzanares, J.A.; FernándezVillacañas, M.R.; Marín-Iniesta, F. and Laencina, J. (1993). Determination of lactose by an enzymatic method. Food chem., 46(4): 425-427.

Sodergard, A. and Stolt, M. (2002). Properties of lactic acid based polymers and their correlation with composition. Progress in Polymer Science, 27: 1123–1163.

Srinivas, D.; Mital, B.K. and Garg, S.K. (1990). Utilization of sugars by *Lactobacillus acidophilus* strains. International Journal of Food Microbiology. 10(1): 51-57.

Vidra, A.; Tóth, A.J. and Németh, A. (2017). Lactic acid production from cane molasses. Liquid Waste Recovery, 2: 13–16

Yadav A.K.; Chaudhari A.B. and Kothari R.M. (2011). Bioconversion of renewable resources into lactic acid: an industrial view. Critical Reviews In Biotechnology.