

Enhancement the Fermentation of Lactose – Whey Using Recombinants Between *Lactobacillus* And *Tetragenococcus*

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Abstract: In this study adapted isolates of *Lactobacillus*, as well as, *Lactobacillus* -*Tetragenococcus* transconjugants were used in bioconverting lactose whey via lactic acid to overcome the disposal problems of whey resulted from cheese industry. Conjugation was used in this study as a mechanism of DNA transfer to release a variety of *lactobacillus* recombinants. Eight antibiotics were used for genetic marking *lactobacillus* strains to be used in mating experiments as a selectable markers for isolating transconjugants. The results revealed that L14, L34 and L19 strains were adapted from 4 % up to 7 % NaCl, whereas strain L44 adapted from 5% up to 7.5 % NaCl, in addition, L56 adapted from 7 % up to 10 % NaCl. This indicated that 10% NaCl was a top level at which *Lactobacillus* strains could tolerated. After 30 days the isolates of L34 were stable, whereas the isolates of L14 and L19 strains were unstable because they could not tolerate the higher concentration of NaCl reached, their tolerance was reduced from 7% to 6 %, as well as L56 was reached to 9% than 10% NaCl, but L44 was reached to 6.5 % than 7.5 % NaCl. Treatment of *Tetragenococcus halophilus* with different levels of temperature has been shown to result in the elimination of chloramphenicol resistance genes at 35C°, as well as, chloramphenicol, erythromycin, K₂Cr₂O₇ and high salt tolerance at 40C°. Transconjugants resulted from the mating between; T14 x L14, T14 x L56 (except Tr28), T14 x L34, T19 x L36 and T14 x L44 (except Tr44) appeared significant increase in growth percentage. At 7.5% NaCl the trasconjugants resulted from mating between; T14 x L14, T14 x L56 and T14 x L44 appeared significant increase in growth percentage over the mid parents. However, L43 isolates (A1, A2) and L19 isolate (A1) gave biomass yield and conversion efficiency better than their parental strains. Although, transconjugants from the mating between; T14 with L56(I) except for Tr3, Tr5; T14 X L44 (I) except Tr16 and Tr20, as well as, the trasconjugants resulted from mating between T14 XL14 appeared significant increase in lactic acid production. Transconjugant isolates; Tr8,Tr14,Tr17,Tr18,Tr19,Tr20,Tr22,Tr24,Tr25 and Tr40 appeared significant increase in lactic acid yield over the mid parents. Moreover, transconjugant isolates Tr8,Tr13,Tr14,Tr18,Tr22,Tr24,Tr25 and Tr40 appeared significant increase in lactic acid conversion efficiency in relation to the mid parents. On the other hand, the following transconjugants; Tr8, Tr14, Tr18, Tr22, Tr24, Tr25 and Tr40 appeared significant increase over their mid parents in both lactic acid yield and conversion efficiency.

Key words: Bioconverting, biomass yield, conversion efficiency, *Lactobacillus*, lactose whey, *Tetragenococcus*, transconjugants, Lactic acid.

INTRODUCTION

The dairy industry represents a major and important part of the food industry and contributes significant liquid waste, whose disposal requires a large amount of capital investment (Gonzalez-Siso, 1996). Its disposal, as waste, poses serious pollution problems for the surrounding environment because of its high biological oxygen demand. Different possibilities of whey utilization have been evaluated to solve its disposal problem. However, a major portion of the world's cheese whey production is not treated and is discarded as effluent (Tyagi *et al*, 1991). To overcome this problem, a better alternative is subjecting the whey to processes through which the value-added products can be manufactured, which may contribute wholly or partially to the costs. Because whey and whey permeates contain significant quantities of lactose, these could be used as substrates for fermentations, which is an interesting way to upgrade these effluents (Panesar *et al* 2007). In the past, whey had been considered as waste. The addition of salt during biochemical action causes more whey to be

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separated off. Due to this, excess salt is present in this whey. Reduction in the generation of salty whey would reduce the disposal cost. Whey should be recovered and reused if possible. This will have less effect on the environmental and social aspects (Venkatraman and Muralidharan, 2004). Lactic acid is one of the earliest known fermentation products from microbial metabolism. Its use is more in anti-inflammatory drugs, pharmacology, textile, and tanning industries and also as a green solvent (Naveena *et al* 2005). Lactic acid production is being studied with increased interest due to its wide application in the synthesis of biodegradable biocompatible plastics and coatings (Gross and Kalra 2002). Considering the global market trends, agro-based chemicals hold a promising future and the demand for lactic acid is expected to shoot up to around 200,000 MT by the end of the year 2011 (Ramesh, 2001). Lactic acid bacteria are used for the preservation of food and feed raw materials like milk, meat, and vegetables or other plant materials. During technological processes, lactic acid bacteria involved in food technology, is exposed to several stress conditions such as the presence of salt. Certain strains of lactic acid bacteria, in particular, strains from the genus *Lactobacillus*, have been attributed to probiotic activities in humans and animals (Kalliomaki *et al*, 2001). *Tetragenococcus halophilus* extreme salt tolerance (18% NaCl), which distinguishes from other LAB, *Tetragenococci* generally require the range of 5% NaCl for growth (Garvie *et al*, 1986). *Tetragenococcus* species are important in lactic fermentation (not as lactobacillus) of high-salt-containing food, e.g., soy sauce; (Satomi *et al* 1997). These may encode important traits like resistance to phages or antibiotics, lactose catabolism, and production of proteolytic enzymes or bacteriocins (Ruiz-Barba *et al*, 1991). The application of genetic technologies to characterize and potentially manipulate lactobacilli remains highly significant on both economic and medical grounds. Genetic characterization and manipulation of the Lactobacilli await development of effective gene transfer strategies and construction of suitable vehicles for insertional mutagenesis and stable integration of genes (Scheirlinck *et al.*, 1989). The capacity for conjugal transfer is an important characteristic for plasmids. Self-transmissible conjugative plasmids have the ability to form effective cell-to-cell contact, while modifiable plasmids are only able to prepare their DNA for transfer. Mobilization involves the action of a specific DNA-protein structure called the relaxosome to produce single-stranded cleavage at the nicking site (*nic*) within the origin of transfer (*oriT*) of the plasmid. To date, there is very little information on conjugation in Lactobacilli. (Lanka and Wilkins. 1995). The present study aimed to overcome the disposal problem of whey resulted from cheese industry via inducing adapted isolates of *Lactobacillus*, as well as, *Lactobacillus-Tetragenococcus* transconjugants higher efficient in bioconverting lactose whey to lactic acid.

MATERIALS AND METHODS

I -Materials:

1- Microbial Strains:

Lactobacillus and *Tetragenococcus* strains were used in this study to induced recombinants between both. These recombinants were used as a genetic raw material for selected higher efficient isolates in bioconverting lactose whey to lactic acid. Their sources and relevant genotypes are listed in Table1.

2-Growth Media and Culture Condition:

a-MRS Medium 182:

This medium was used as complete medium of *Lactobacillus* strains according to Ronald (2006).

b- Lactobacilli MRS Broth 493 (Defect 0881):

This medium was used as complete medium for *Lactobacillus* and *Tetragenococcus* strains according to Downes and Ito (2001). PH was adjusted to 6.2-6.6.

C- Whey Fermentation Medium:

Whey clarification was carried through protein precipitation induced by heating the whey at 121°C for 5 min. Precipitated proteins were removed by cellulose acetate membranes, pH of whey medium was adjusted from 5.9 to 7.0 and then sterilized at 121°C for 20 min. The fermentation medium prepared was used for the production of lactic acid using *Lactobacillus* according to Panesar *et al* (2007).

3-Genetic Marking:

Eight antibiotics were used in this study for genetically marking the different bacterial strains as shown in Table 2.

Table 1. Bacterial strains used in this study .

Strains	Genotype	Source or reference	Designation
<i>Lactobacillus delbruekii</i> (var) <i>lactis</i> 1441	Wild-type	Department of Applied Chemistry and Microbiology Division of Microbiology, University of Helsinki, Finland.	L14
<i>Lactobacillus casei sub sp</i> <i>casei</i> DSM5622	Wild-type	International culture collection obtained from DSMZ GmbH, Braunschweig, Germany.	L56
<i>Lactobacillus casei</i> NRRL-B1922	Wild-type	United states Department of Agriculture, Research Education and Economics, Agricultural Research Service USDA	L19
<i>Lactobacillus casei</i> NBIMCC3485	Wild-type	National Bank for Industrial Microorganism and cell cultures NBIMCC, Bulgaria.	L34
<i>Lactobacillus acidophilus</i> NRRL-B4495	Wild-type	United states Department of Agriculture, Research Education and Economics, Agricultural Research Service USDA	L44
<i>Tetragenococcus halophilus</i> NRRL-B14187	Wild-type	United States Department of Agriculture, Research Education and Economics, Agricultural Research Service USDA	T14

Table 2: Antibiotics and their abbreviations

Antibiotics	Designation	Concentration ($\mu\text{g/ml}$)
Chloramphenicol	<i>Cm</i>	30
Streptomycin	<i>Str</i>	2500
Cephalexin	<i>Cp</i>	250
Neomycin sulphate	<i>Nm</i>	50
Ampicillin	<i>Ap</i>	50
Erythromycin	<i>Erie</i>	40
Pencillin	<i>Pn</i>	50
Rifamycin	<i>Rf</i>	50

4. Reagents and Apparatus:

- Sulfuric acid, reagent grade 95.5% specific gravity 1.84. Phenol, 80% by weight, prepared by adding 20 grams of glass-distilled water to 80 grams of redistilled reagent grade phenol. This reagent was used in lactose determination (Michel *et al*, 1956).
- Phenol phthalin reagent: It was prepared by dissolving 1g of the solid phenolphthalein in 110 ml of ethyl alcohol (95 % per cent v/v), adding N/10 NaOH until one drop gives a faint pink coloration, and then making up to 200 ml with distilled water. It was used as an indicator with lactic acid titrated. (Edgar 1963).

II - Methodes

1-Genetic Markers Test:

Antibiotic susceptibility tests of *Tetragenococcus halophytes* and *Lactobacillus* strains were performed using disk diffusion method for the appearing of zones according to Nevien *et al.*, (2007).

2-Tolerance of *Lactobacillus* Strains and Their Transconjugants to Different NaCl Concentrations.:

Pure cultures of *Lactobacillus* strains and their transconjugants were grown in MRS amended with NaCl concentrations (1, 2, 3, 4, 6, 7 and 8 %) for *Lactobacillus* strains, as well as, 0, 2.5, 5, 7.5 and 10 for transconjugants. Plates and broth media were incubated at 37C° for maximum 4 days.

3- Adaptation to NaCl:

Cells from a log phase culture of *Lactobacillus* strains were spread on the surface of MRS agar plates and then incubated at 37C°. Each strain was tested for the final NaCl tolerated level. Saline tolerance was induced via adapting strains to different NaCl concentrations using 0.5 % intervals to enhancement the switch on of saline tolerance genes. Adapted isolates were retested for their tolerance to NaCl at the final concentration reached, this was done after 30, 60 and 180 days of isolation.

4-Plasmids Curing:

Cells from a log phase culture of strain T-14187 (Chloramphenicol, Erythromycin and K₂Cr₂O₇ resistant) were grown on MRS broth and incubated at (28, 37 and 40C°) for 5 days in 3 tubes. The growth evenly spread on the surface of MRS agar plates. Single colonies appeared were picked up and grown at 28 C° on MRS agar slants. Then the colonies were checked for the markers resistance pattern, as well as, to 18 % NaCl. Because the inability of the tested strains to grow on selective medium indicating that it was plasmid free cells. However, resistance indicating that the cells were plasmid-harboring according to Nigel *et al.* (1983).

5-Conjugation Experiments:

Matings were conducted using a nutrient agar broth (pH 6.8) containing selectable markers as shown in Table 2. The plates were incubated for 4 days at least until the transconjugants were appeared on selective media. The appropriate time for appearing transconjugants was differed from one mating to another. Samples from the mixture mating were taken from each conjugation tube every two days and plated on the appropriate media searching on the suitable time of genetic transfer needed to construct transconjugants. In conjugation experiments donors and recipients were mixed in a 1: 2 ratio and incubated for the appropriate time. Mating cell mixtures were resuspended in nutrient broth (pH 6.8) and plated on MRS containing selectable markers for isolating transconjugants. Such broad host range transferable plasmids play an important role in the spread of genetic markers resistance. Hybridization experiments used in this study were carried out according to Tun-Garrido *et al* (2003). Hybridization technique was repeated several time through four months until reached to appropriate time required for genetic transfer and appearing transconjugants on the selective media.

6-Analytical Methods:

A-reducing Sugar Assays:

Lactose concentration in cheese whey was measured using the method of reducing sugars, as well as, the absorbance was measured at 490 nm. according to Michel *et al* (1956). However, lactose concentrations in different samples treated with tested strains were assayed using the following equation;

$$x = \frac{y - a}{b}$$

Where; y = Optical density at 490 nm, x = Concentration of Lactose, b = Regression = 0.743, a: means the absorbance at 490 nm when the concentration of lactose equal zero = 0.12, r: means correlation = 0.983

b -Growth Characteristics:

Growth was assayed via measuring the turbidity at optical density 600 nm using a spectrophotometer (Spekol 11).

Conversion Efficiencies:

The efficiency of growth was measured according to Tango and Ghaly (1999), who are stated that during lactose fermentation, the cell yield (YX/S) was found to be 0.08 g cell / g lactose. A small proportion of lactose is also used for the cell maintenance and release of energy.

C- Lactic Acid and pH Measurement:

pH was measured directly using pH meter (PH890) according to Kobayashi *et al* (2004). However, lactic acid was measure by titration method against sodium hydroxide solution using phenol phthalein, as an indicator. One molecular weight of sodium hydroxide therefore neutralizes one molecular weight of lactic acid. The end point (it is generally agreed to adopt 1 ml as the quantity of indicator to be used per 10 ml of milk or whey) when a dilute solution of a strong acid is titrated with carbonate-free alkali, using phenolphthalein as indicator, a point is ultimately reached at which the addition of one drop of the alkali causes the solution to change from colourless to pink. The method described was referred to, Santo *et al* (2005).

Product Formation and Respiration:

The stoichiometric lactic acid yield (YP/S) was estimated to be 0.95 g lactic acid / g lactose. Energy release and production of lactic acid was referred to the previous equations (Ghaly *et al.*2003).

Statistical Analysis:

The data were subjected to the analysis of variance of randomized completely design according to Seducer and Cochran (1955). All experiments conducted in this study were done in three replicates.

RESULTS AND DISCUSSION

Genetic Markers Test:

Six bacterial strains used in this study were genetically marked using eight antibiotics. The results showed in Table (4) appeared that penicillin, ampicillin and cephalixin were much more effective to inhibit the growth of all bacterial strains than the other antibiotics used herein. In addition, streptomycin appeared the same trend with all strains except for, L14. This was agree with Brock (1964), who found that streptomycin inhibited plague formation when certain bacteriophages were plated on streptomycin-resistance host cells. All other antibiotics, except for penicillin, ampicillin and cephalixin revealed differences in their action against different bacterial strains. Results clearly show that L14 strain was resistant to Str, Nm, Ery and Rf. Whereas, L56 was resistant to Cm, Nm, and Rf. In addition, L19, L44 and T14 were resistant to Ery, however, L56 and L34 were sensitive to Ery. There results in agreed with those reported by Chin *et al* (2005), who found that all *Lactobacillus* strains exhibited varying degrees of resistance to chloramphenicol and erythromycin. Growth inhibition appeared in this study was not accompanied by cell death and could be readily reversed by removing the antibiotic (Fernandez and Anton 1987).The results indicated that Cm, Str and Ery revealed differences in their action against six bacterial strains used in this study. As noted in previous studies by Cresti *et al.* (2002), who found that there was a correlation between the antibiotic resistance phenotype and the genotype for each isolate. This study indicated that there are genetic variations between bacterial strains due to resistance and sensitivity to different antibiotics. These markers are sufficient to be used in intergenic or intragenic transfer. Also, this study has shown that *Lactobacillus* and *Tetragenococcus* strains contains variations against the same antibiotic. The results indicated that screening the isolates for their resistance profiles to a battery of eight antibiotics representing different results related to sensitivity and resistance, depending upon their mode of action and the genotype of each strain. Furthermore, the analysis of bacterial strains indicates that although plasmids were present in some of the strains but did not harbor antibiotic resistance genes (Chakrabarty *et al.*,1990). The location (chromosomal or extra chromosomal) of drug resistance genes was confirmed by plasmid curing strategies. Swenson *et al.* (1990) reported that resistant trait to various antibiotics were located in a high copy number of transferable plasmid DNAs and the plasmids of these strains can be used as cloning vector for gram-positive bacteria in recombinant DNA technology. According to these results, in some strains the resistance to some antibiotics may be under the control of plasmid DNAs; however, the resistance to some other antibiotics may be encoded by chromosomal genes, and different plasmids caused resistance to different antibiotics (Aslim and Beeyalti 2004).

Table 3:Mating between *Lactobacillus* strains and *Tetragenococcus halophilus* NRRL-B14187 that having the opposite genetic markers.

No. of mating	Mating
1 L.14XT.14	St ⁺ Ery ⁻ Co ⁻ X St ⁻ Ery ⁺ Co ⁺
2 L.56 X T.14	Ery ⁻ Cr ⁻ Co ⁻ X Ery ⁺ Cr ⁺ Co ⁺
3 L.34 X T.14	Ery ⁻ Cr ⁻ Co ⁻ X Ery ⁺ Cr ⁺ Co ⁺
4 L.19 X T.14	Cu ⁻ Cr ⁻ Co ⁻ X Cu ⁺ Cr ⁺ Co ⁺
5 L.44 X T.14	Cr ⁻ Co ⁻ X Cr ⁺ Co ⁺

Table 4: Genetic variations in sex bacterial strains tested against eight antibiotics .

Bacterial strains	Antibiotics							
	Cm	Str	Pn	Nm	Ap	Ery	CP	Rf
L.14	-	+	-	+	-	-	-	+
L.56	+	-	-	+	-	-	-	+
L.34	+	-	-	+	-	-	-	+
L.19	+	-	-	+	-	+	-	+
L.44	+	-	-	+	-	+	-	+
T.14	+	-	-	+	-	+	-	+

++, - = Resistance and sensitive, respectively.

Tolerance of *Lactobacillus* Strains to Different NaCl Concentrations:

Sodium chloride is important ingredient of cheese which exerts a major influence on its composition, micro flora, ripening, texture, flavor and quality (Salem and abide 1997). As shown from the results summarized in Table (5) all *Lactobacillus* strains were tolerate NaCl until 4%, however two strains L56 and L44 could tolerate until 5 % NaCl. In addition, only one strain L56 reached to 7% NaCl.

The results obtained here are in agreement with those reported by Santo *et al* (2005), who found that *Lactobacillus sakei* tolerated 6% NaCl. In addition, sharaf *et al* (2007), found that *Lactobacillus casei subsp casei* tolerated 6% NaCl. This agreed with Leroy and Luc (1999), who reported that homofermentative lactic

acid bacteria are more resistant to sodium chloride than heterofermentative lactic acid bacteria. Although, the *Lactobacillus* strains tolerated to high NaCl concentrations was not enough to solve the fermentation of salty whey problems and production of sterile Domiati cheese which traditionally made from milk salted with 5 – 15 % NaCl. This was very significant problems in the field of dairy production and cheese industry. Thus, the present study was contributed to solve these problems via induced recombinations in *Lactobacillus* strains carrying effective genes related to tolerate higher concentration of NaCl via bacterial mating technique. Methods in improving microbial strains have relied upon either mutagenesis followed by selection to improve properties, or manipulation of specific gene known to play an important role in the desired phenotype. Other reports have described the successful use of protoplast fusion and gene transfer via conjugation to combine metabolic capabilities of two different organisms, as was done in this investigation. Consequently, the increase of NaCl concentrations above a certain value within this range will render the culture bacteriostatic and indicated that the bacterium was tolerant, but not yet resistant. Hop resistance is therefore defined as the stage when the growth rate is virtually unaffected by the hop concentration in this definite range (Jürgen, 2008). To delineate hop resistance mechanisms and study their respective role in adaptation, cells from *Lactobacillus* strains were adapted to increasing concentrations of NaCl (Simpson and Fernandez 1992).

Table 5: Tolerance of *Lactobacillus* strains to different NaCl concentrations .

Strains	NaCl Concentrations %							
	1	2	3	4	5	6	7	8
L.14	+	+	+	+	-	-	-	-
L.56	+	+	+	+	+	+	+	-
L.34	+	+	+	+	-	-	-	-
L.19	+	+	+	+	-	-	-	-
L.44	+	+	+	+	+	-	-	-

+ , - Growth and suppress growth, respectively.

Adaptation for Salt Tolerance:

The microbial response towards osmotic stress is an important issue in industry. Inhibition of microbial growth by high osmolarity is in many cases a desired effect, e.g. in the food industry where osmotically active agents, such as salt and sugars, are used to suppress spoilage - related and pathogenic microbes. (O'Byrne and Booth 2002). In other cases, growth inhibition is an unwanted side-effect, for instance in food-products in which food-grade microorganisms fulfill a positive role and in fermentations where alkaline salts are added for pH control. For many applications the long term adaptation towards high osmolarity is more relevant than the acute, short term adaptation, e.g. in processes in which the osmolarity of the environment increases gradually due to drying or pH control. In contrast, most studies on the prokaryotic adaptation towards osmotic stress focuss on the effect of osmotic upshock, i.e. a sudden increase in osmolarity of the environment (Csonka. 1989). As shown from the results presented in Table (6) the adaptation of *Lactobacillus* strains taken eight steps, each step takes the same time with increasing 0.5% of NaCl, all strains tested started from the last level of NaCl, which reached in the previous test (Table 6). The results obtained clearly appeared that L14, L34 and L19 adapted from 4% up to 7 % NaCl, whereas strain L44 adapted from 5% up to 7.5 % NaCl. In addition, L56 adapted from 7 % up to 10 % NaCl. This indicated that 10 % NaCl was a top level at which *Lactobacillus* strains could tolerated. The results obtained here are in agreement with those reported by Tanaka *et al* (2004), who found that high-pressure adaptation was examined using a moderately halophilic bacterium (*Micrococcus roseus*) isolated from open seawater and capable of growing in 15 % w/v NaCl (optimum NaCl concentration 3 % w/v). It was cultured in 1, 3, 5, 10 and 15% NaCl, the survival ratio proportionally increased at increased NaCl concentration. As noted in previous studies (Woojin *et al* 2001), *L. acidophilus* is capable of displaying adaptive response to stress. The adaptive response to one stress was also shown to provide cross-protection against different stresses tested. Pieterse (2006) studied the effect of continuous exposure to NaCl on gene expression in *Lactobacillus* strains, and found that no increased expression was observed for genes that had previously been implicated to play a role in the acute response to hyperosmotic stress. Jürgen (2008) reported that hop adaptation appears as a multifactorial process, which results in changes in metabolism, protein profile, membrane and cell wall composition and intracellular manganese levels. These structural defense mechanisms imply an altered membrane composition which accounts for maintaining the membrane integrity even at high NaCl concentrations and protects from acid and oxidative stress. As non-growing bacteria are hard to investigate and adaptation and thus the transition from hop tolerance to resistance is a dynamic property, an alternative definition, which is analytically accessible, has to be used. Accordingly, in this experience hop tolerance is defined as state, where the growth rate of the bacterium is dependent on hop concentration in the growth medium within a definite range (Jürgen, 2008).

Stability Testing of Adaptive Isolates:

Isolates of *Lactobacillus* strains resulted from adaptation to high NaCl concentrations was tested after 30, 60 and 180 days to the last concentration adapted. The results were summarized in Table 7. It was appeared that after 30 days the isolates of L34 were stable, whereas the isolates of L14 and L19 strains were unstable because they are not reached to their tolerance arrived before, it was reached to 6% from 7% NaCl, as well as L56 was reached to 9% from 10% NaCl, but L44 was reached to 6.5 % from 7.5 % NaCl. When L14 and L56 were re-test for gene stability after 180 days at the final concentration of NaCl reached, their growth are suppressed. These results appeared that L34, L19 and L44 were succeeded in growth with reduced efficiency. The results obtained here are in agreement with those reported by Pieterse (2006), who found that the absolute levels of AMP, ADP and ATP were reduced at high NaCl concentrations which implicates a less preferable energetic status. This decrease in absolute levels of adenine nucleotides corresponds with the lower expression of genes involved in purine biosynthesis. This leading to limitation of the nucleotide levels which may serve a role in counteracting the negative effects of high osmolarity on the intracellular water contents. Stability of salt tolerance may be due to the stability of genes related to salt tolerance which turn to switch on via adaptation.

Table 6: Adaptation of *lactobacillus* strains to different grades of NaCl concentrations.

Strains	Adaptation steps														
	Step 1			Step 2			Step 3			Step 4			Step 5		
	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)
L.14	4	+	48	4.5	+	72	5	+	144	5.5	+	192	6	+	192
L.56	7	+	48	7.5	+	72	8	+	144	8.5	+	192	9	+	192
L.34	4	+	48	4.5	+	72	5	+	144	5.5	+	192	6	+	192
L.19	4	+	48	4.5	+	72	5	+	144	5.5	+	192	6	+	192
L.44	5	+	48	5.5	+	72	6	+	144	6.5	+	192	7	+	192

Table 6. Continued

Strains	Adaptation steps											
	Step 6			Step 7			Step 8			Step 9		
	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)	NaCl %	Growth	Time (h)
L.14	6.5	+	240	7	+	240	7.5	-	288	7.25	-	288
L.56	9.5	+	240	10	+	240	10	-	288	10.25	-	288
L.34	6.5	+	240	7	+	240	7.5	-	288	7.25	-	288
L.19	6.5	+	240	7	+	240	4.5	-	288	7.25	-	288
L.44	7.5	+	240	8	-	240	7.75	-	288			

+, - = Growth and suppress growth, respectively .Time(h) = Time of incubation.

Table 7: Genetic stability of NaCl tolerance in *Lactobacillus* strains .

Strains and adapted isolates	Final NaCl %	30 day						60 day		180 day at new final concentrations	
		At final concentration		New concentration tolerated %		At final concentration		New concentration tolerated %			
L14	4	+	4	+	4	+	4	+			
A1	7	-	6	+	6	+	6	-			
A2	7	-	6	+	6	+	6	-			
L.56	7	+	7	+	7	+	7	+			
A1	10	-	9	+	9	+	9	-			
A2	10	-	9	+	9	+	9	-			
L.34	4	+	4	+	4	+	4	+			
A1	7	+	7	+	7	+	7	+			
A2	7	+	7	+	7	+	7	+			
L19	4	+	4	+	4	+	4	+			
A1	7	-	6	+	6	+	6	+			
A2	7	-	6	+	6	+	6	+			
L44	5	+	5	+	5	+	5	+			
A1	7.5	-	6.5	+	6.5	+	6.5	+			
A2	7.5	-	6.5	+	6.5	+	6.5	+			

+, - = Growth and growth suppressed , respectively .

Plasmid Curing Test:

As shown from the results presented in Table 8, treatment of *Tetragenococcus halophilus* with different levels of temperature has been shown to result in the elimination of chloramphenicol resistance genes at 35C°, as well as, chloramphenicol, erythromycin, K₂Cr₂O₇ and high salt tolerance at 40C°. This may be due to the elimination of their genes from plasmid DNA. This indicated that plasmid DNA of these genes was cured at 40C°. In addition, the frequencies of plasmid bearing cells were higher at 28C° than that of plasmid free cells at 40C°. The results indicated that treatment of *Tetragenococcus* with low levels of temperature (40 C°) has been shown to result in higher frequency of plasmid free cells for all markers tested. It seems reasonable to

assume that under these conditions where plasmid - borne loci enhanced the survival probability or growth rates of their host cells, these extra - chromosomal elements would become established and bacteria carrying them would maintain high frequencies in the populations. Some strains may contain as many as ten plasmids (Stanisich,1988). The alteration in ability to utilize various substrates after curing of plasmids suggested that the plasmids may encoded genes (Baldani *et al*, 1992).The results obtained in this study exhibited varying degrees of resistance to chloramphenicol, erythromycin, and. $K_2Cr_2O_7$.

This are in agreement with those reported by Chin *et al* (2005), who found that all *Lactobacillus* strains exhibited varying degrees of resistance to chloramphenicol and erythromycin. According to these results, some strains used in this study appeared resistance to some antibiotics may be under the control of plasmid DNAs; however, the resistance to some antibiotics may be encoded by chromosomal genes. This are important in testing plasmid transfer in filter mating experiment between strains having the opposite genetic markers.

Table 8: Effect of heat shock on plasmide curing of T .14 grown in MRS broth for 5 days.

Treatments	28C°	35C°	40C°
00	+	+	+
Chloramphenicol	+	-	-
Erythromycin	+	+	-
$K_2Cr_2O_7$	+	+	-
18 % NaCl	+	+	-

+, - = Means resistant and sensitive to heavy metals and antibiotics , respectively .

Evaluation of Transconjugants for Salinity Tolerances in MRS Media:

Experiments described the growth behavior for all bacterial strains and their transconjugants under four levels of NaCl concentrations (0, 2.5, 5, and 7.5) add to MRS media are shown in Table 9. The results appeared that at zero level of NaCl, all bacterial transconjugants induced low levels of pH, as an indicator of higher amounts of lactic acid production in relation to their mid parents. However, many of bacterial transconjugants appeared significant increase in growth percentage except some others like that resulted from the mating between T14 X L44. At 2.5 % NaCl, transconjugants resulted from all crosses revealed significant increase in pH value, except for, three transconjugants; Tr16, Tr19 and Tr20. Whereas, the following transconjugants; Tr2, Tr3, Tr16, Tr19, Tr20, Tr22, Tr26, Tr27, Tr29, Tr41, Tr42, Tr44 and Tr45 failed to appear any significant increase in growth and growth percentage at the second level (2.5%) of NaCl.

Table 9: Testing transconjugants for salinity tolerances

Strains and Trans.	o.o % NaCl										2.5 % NaCl		5% NaCl		7.5% NaCl	
	pH	Turbidity		pH	Turbidity		pH	Turbidity		pH	Turbidity					
		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %				
T .14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100				
L.56	2.89	2.584	100	3.12	1.891	100	3.69	1.236	100	6.2	0	100				
MP	4.34	1.616	100	4.19	1.312	100										
Tr1	3.00	2.730	168.0	3.15	1.630	119.6	6.20	0	0	6.2	0	0				
Tr2	3.05	2.350	145.4	3.36	1.075	75.50	6.20	0	0	6.2	0	0				
Tr3	3.00	2.590	160.4	3.22	1.090	80.00	6.20	0	0	6.2	0	0				
Tr4	2.93	2.640	163.4	3.08	1.640	120.4	6.20	0	0	6.2	0	0				
Tr5	3.0	2.380	147.2	3.60	1.500	110.1	6.20	0	0	6.2	0	0				
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100				
L34	3.41	1.343	100	3.70	1.173	100	6.20	0	0	6.2	0	0				
MP	4.64	0.995	100	4.48	0.953	100										
Tr6	3.86	1.410	141.8	3.90	1.078	107.5	6.20	0	0	6.2	0	0				
Tr7	3.91	1.450	145.8	3.98	1.091	108.8	6.20	0	0	6.2	0	0				
Tr8	3.86	1.480	148.8	3.95	1.076	107.3	6.20	0	0	6.2	0	0				
Tr9	3.74	1.440	144.8	3.88	1.106	110.3	6.20	0	0	6.2	0	0				
Tr10	3.88	1.410	141.8	3.93	1.126	112.3	6.20	0	0	6.2	0	0				
T14	5.86	0.674	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100				
L19	3.22	1.762	100	3.55	1.004	100	6.20	0	0	6.20	0	0				
MP	4.54	1.205	100	4.41	0.868	100										
Tr11	3.24	1.550	128.7	3.53	1.249	128.7	6.20	0	0	6.20	0	0				
Tr12	3.22	1.540	127.8	3.51	1.266	127.8	6.20	0	0	6.20	0	0				
Tr13	3.24	1.430	118.7	3.45	1.275	118.7	6.20	0	0	6.20	0	0				
Tr14	3.25	1.730	144.2	3.55	1.585	114.2	6.20	0	0	6.20	0	0				
Tr15	3.23	1.710	143.9	3.54	1.425	143.9	6.20	0	0	6.20	0	0				

Growth % = Means growth percentage , Trans. = Means transconjugants

Table 9 . Continued

Strains and Trans.	o.o % NaCl			2.5 % NaCl			5% NaCl			7.5% NaCl		
	pH	Turbidity		pH	Turbidity		pH	Turbidity		pH	Turbidity	
		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L44	3.14	2.230	100	3.48	1.551	100	3.98	0.955	100	6.20	0	0
MP	4.50	1.439	100	4.37	1.142	100						
Tr16	3.68	1.690	117.4	4.39	0.693	58.10	6.20	0	0	6.20	0	0
Tr17	3.93	1.900	137.3	3.12	1.320	110.7	6.20	0	0	6.20	0	0
Tr18	3.43	2.490	206.7	3.58	1.970	165.3	6.20	0	0	6.20	0	0
Tr19	3.34	1.820	151.1	4.20	0.785	65.80	6.20	0	0	6.20	0	0
Tr20	3.53	0.606	50.02	4.63	0.529	44.40	6.20	0	0	6.20	0	0
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L14	3.36	2.432	100	3.77	1.426	100	0	0	0	0	0	0
MP	4.61	1.539	100	4.52	1.079	100	2.58	0.396	50.00	2.50	0.451	50.00
Tr21	3.08	2.146	139.5	3.70	1.503	133.1	3.87	0.996	125.7	4.59	0.796	88.30
Tr22	3.52	1.521	98.80	3.40	0.831	73.50	4.02	0.775	97.80	4.50	0.696	77.20
Tr23	3.59	1.623	105.5	3.73	0.831	97.40	3.92	0.942	118.9	5.02	0.605	67.10
Tr24	3.48	1.507	97.90	3.88	1.078	95.40	4.23	0.865	109.2	4.6	0.684	75.90
Tr25	3.73	1.573	102.2	3.87	1.152	102.0	4.02	0.976	123.2	4.78	0.748	0.830
T .14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L.56	2.89	2.584	100	3.12	1.891	100	3.69	1.236	100	0	0	0
MP	4.39	1.616	100	4.19	1.312	100	4.42	1.014	100	2.50	0.451	50.00
Tr26	3.05	2.180	134.9	3.2	1.187	87.10	3.55	1.092	101.2	3.88	0.741	82.20
Tr27	3.14	1.620	100.2	3.19	1.297	95.20	3.43	1.095	101.5	3.68	0.814	90.30
Tr28	2.86	2.650	163.9	3.05	1.542	113.2	3.54	0.825	76.40	4.42	0.688	76.30
Tr29	2.98	2.430	150.3	3.21	1.298	95.30	3.44	1.260	116.8	3.76	0.917	101.7
Tr30	2.99	2.570	159.06	3.11	1.460	107.2	3.51	1.107	102.6	4.61	0.703	78.00

Growth % = Means growth percentage , Trans. = Means transconjugants

Table 9. Continued

Strains and Trans.	o.o % NaCl			2.5 % NaCl			5% NaCl			7.5% NaCl		
	pH	Turbidity		pH	Turbidity		pH	Turbidity		pH	Turbidity	
		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %		OD at 600 nm	Growth %
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L34	3.41	1.343	100	3.70	1.173	100	0	0	0	6.20	0.00	0.00
MP	4.64	.995	100	4.48	0.952	100	2.58	0.396	50.00			
Tr31	3.90	1.409	141.8	3.98	1.007	100.4	4.14	0.896	113.1	6.20	0.00	0.00
Tr32	3.91	1.414	142.3	4.03	1.033	103.0	4.08	0.908	114.6	6.20	0.00	0.00
Tr33	3.95	1.373	138.2	4.03	1.018	101.5	4.34	0.803	101.3	6.20	0.00	0.00
Tr34	3.80	1.303	131.1	3.86	1.051	104.8	3.93	0.906	114.3	6.20	0.00	0.00
Tr35	3.95	1.078	108.5	4.07	0.895	89.21	4.35	0.703	88.70	6.20	0.00	0.00
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L19	3.22	1.762	100	3.55	1.004	100	0	0	0	6.20	0.00	0.00
MP	4.54	1.205	100	4.41	0.868	100	2.58	0.396	50.00			
Tr36	3.49	1.573	131.0	3.55	1.229	133.8	3.72	0.911	115.0	6.20	0.00	0.00
Tr37	3.58	1.583	131.4	3.67	1.261	137.3	3.74	0.687	86.70	6.20	0.00	0.00
Tr38	3.52	1.558	129.0	3.62	1.283	139.7	3.83	0.756	95.50	6.20	0.00	0.00
Tr39	3.51	1.556	129.3	3.65	1.259	137.2	3.69	0.654	87.60	6.20	0.00	0.00
Tr40	3.52	1.595	132.3	3.67	1.242	135.3	3.71	0.916	115.6	6.20	0.00	0.00
T14	5.86	0.647	100	5.26	0.732	100	5.15	0.792	100	5.00	0.901	100
L44	3.14	2.230	100	3.48	1.551	100	3.98	0.955	100	0	0.00	0.00
MP	4.50	1.439	100	4.37	1.142	100	4.57	0.874	100	2.50	0.451	50.00
Tr41	3.17	1.267	88.05	3.49	0.840	70.50	3.76	0.796	84.80	4.08	0.715	79.30
Tr42	3.35	1.580	109.7	3.26	1.085	91.00	3.60	1.060	112.9	3.98	0.775	86.00
Tr43	2.95	2.248	156.2	3.40	1.822	153.0	3.59	1.087	115.8	3.93	0.713	79.10
Tr44	3.41	1.503	104.4	3.22	0.966	80.90	3.49	0.801	85.30	4.25	0.605	67.10
Tr45	3.22	1.563	119.2	3.67	1.043	87.50	3.82	0.920	98.00	3.92	0.827	91.70
F.test	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D												
5%	0.06	0.12	3.70	0.05	0.009	1.42	0.03	0.003	0.46	0.019	0.34	0.17
1%	0.08	0.16	4.99	0.07	0.012	1.89	0.04	0.004	0.62	0.025	0.45	0.23

**=p<0.01.

This are in harmony with Michael *et al.* (1998), who found that the increasing in ionic strength was inhibit the growth of lactobacilli to a much greater degree than it inhibits the growth of yeasts, whereas, Verluyten *et al.* (2004) found that salt affects the growth and bacteriocin production of lactic acid bacteria, as well as, the growth of *L. curvatus* LTH 1174, was not affected at a low concentration of NaCl (2%, wt/vol). At 5 % NaCl, transconjugants resulted from the mating between T14 x L56 and T14 x L44 exhibited significant reduction in pH value in relation to the parental strains. However, transconjugants resulted from the mating between; T14 x L14, T14 x L56 (except for Tr28), T14 x L34, T19 x L36 and T14 x L44 (except for Tr44) appeared significant increase in growth percentage. The present results are in accordance with Shockey and Borger (1991), who found that greater amounts of salt were required to inhibit the initial growth rates of the lactic acid bacteria compared with *C. buryicum*, although even at 1200 mM salt some growth was observed for all lactic acid bacteria. Tolerance of lactic acid bacteria to high concentrations of salt is well known. Growth in 6.5% NaCl is one criterion for identification of certain species of *Streptococcus* (Buchanan *et al.* 1974). Lactic acid bacteria are an integral part of the milk and cheese processing industry, where NaCl concentrations can reach from 6 to 9 % (El-Gendy *et al.* 1983). Taken together, at 7.5 % NaCl, the transconjugants resulted from the mating between; T14 x L14, T14 x L56 and T14 x L44 appeared significant increase in growth percentage over the mid parents. However, all transconjugants did not appeared significant increase in pH value and growth percentage, except for, Tr27, Tr29 and Tr45. This agreed with Verluyten *et al.* (2004), who found that the inhibition increased linearly was evident with higher salt concentrations. The strong negative effect of high salt concentrations on the growth of lactic acid bacteria has been reported previously, however, low concentrations of salt (1 to 2 %, wt/vol) can sometimes enhance bacterial growth (Uguen *et al.* 1999). Michael *et al.* (1998) found that the growth of lactobacilli is inhibited by 4 % NaCl.

Evaluation of Adapted Isolates for Salty Cheese Whey Utilization:

Utilization of raw lactose by different *Lactobacillus* strains and it's adapted isolates was compared in small-scale batch fermentation using 25 ml of salty cheese whey as the only carbon source. The ability of *Lactobacillus* strains and their adapted isolates to utilize raw lactose as the only carbon source in small – scale fermentation was shown in Table 10. *Lactobacillus* strains and their adapted isolates appeared significant differences for all traits tested including utilizing lactose, amounts of biomass yield and conversion efficiency. The data clearly showed that L43 isolates (A1, A2) and L19 isolate (A1) gave biomass yield and conversion efficiency better than their parental strains. On the other hand, all isolates appeared significant decrease in growth percentage than their parental strains. This was agree with Steil *et al* (2003), who found that decreased expression of genes involved in lipoteichoic acid biosynthesis has also been observed for *Bacillus subtilis* subjected to long term salt stress in response to salt stress. As well as, Pieterse (2006) found that, the total amount of NADP (H) in *L. plantarum* was actually lower in the NaCl - stressed cells. It should be noted that the absolute NAD (H) levels and the NAD + NADH ratio also showed a significant reduction under continuous NaCl stress, which may have a negative effect on the metabolic potential of the cells.

Table 10: Growth of *Lactobacillus* adapted isolates grown on lactose whey at batch fermentation .

Strains and adapted isolates	NaCl%	Turbidity		Lactose consumption %	Cells		
		OD at 600nm	Growth percentage		Weight mg/ml	Biomassmg/ mg/mg lactose	Conversion efficiency %
L14	4	1.463	100	3.41	0.0924	2.710	20.96
A1	6	0.445	30.3	2.01	0.0492	2.448	19.58
A2	6	0.543	38.3	2.84	0.0484	1.737	13.90
L.56	7	1.879	100	4.18	0.1044	2.499	20.07
A1	9	0.556	29.5	1.91	0.0392	2.499	16.42
A2	9	0.615	32.6	3.36	0.0528	1.571	12.57
L.34	4	0.891	100	4.02	0.042	1.045	8.364
A1	7	0.517	57.9	2.11	0.0468	2.219	17.76
A2	7	0.526	59.0	2.21	0.0448	2.028	16.236
L19	4	0.973	100	4.07	0.0696	1.710	13.686
A1	6	0.456	46.8	2.08	0.0416	2.001	16.01
A2	6	0.543	55.8	3.07	0.0476	1.551	12.41
L44	5	1.523	100	4.13	0.0989	2.396	19.14
A1	6.5	0.499	32.7	2.43	0.0436	1.794	14.36
A2	6.5	0.475	31.1	2.25	0.0372	1.654	13.23
F. test		**	**	**	**	**	**
L.S.D5%		0.03	0.8	0.21	0.0015	0.15	1.16
1%		0.05	1.1	0.29	0.002	0.002	1.57

*, ** P 0.05, P 0.01 probability levels , respectively .

Lactic Acid Production:

The results in Table 11 revealed that all isolates used in lactic acid production via fermentation lactose whey showed that all isolates appeared significant decrease in pH values, lactose consumption and lactic acid production. On the other hand, all adapted isolates appeared significant decrease in lactic acid yield and conversion efficiency except for L34 isolates (A1, A2) and L44 isolates (A1, A2) which appeared significant increase in lactic acid yield and conversion efficiency over their parental strains. This results agreed with Jürgen (2008), who found that the production of lactate and acetate when increasing stress level (acid- and hop stress) was decreased and increased, respectively. This metabolic change was also reflected by an elevated mannitol production under acid- and hop stress conditions. Furthermore, Pieterse (2006) found that no increased expression was observed for genes that had previously been implicated to play a role in the acute response to hyperosmotic stress. However, clear trends were observed over multiple functional classes, which indicated that the long term effects of hyperosmotic stress requires more adaptations than restoration of the turgor alone. This was corresponding to previous observations by Koch *et al* (1985), who found that high salt concentrations have been suggested to inhibit D-alanine incorporation in lipoteichoic acid in growing *Staphylococcus aureus* by inhibition at the enzyme level, instead, the D-alanine is incorporated in teichoic acids.

Table 11: Converting lactose whey to a lactic acid by adapted isolates of *Lactobacillus* strains.

Strains and adapted isolates	NaCl%	pH	Lactose consumption %	Lactic acid		
				Production%	Yield %	Conversion efficiency %
L14	4	3.85	3.41	0.65	19.06	20.07
A1	6	5.04	2.01	0.37	18.40	19.36
A2	6	4.63	2.84	0.4	12.89	14.93
L.56	7	2.84	4.18	0.76	18.18	19.16
A1	9	5.03	1.91	0.36	18.80	19.84
A2	9	4.69	3.36	0.48	14.28	15.04
L.34	4	3.81	4.02	0.67	16.66	17.55
A1	7	5.90	2.11	0.44	20.85	21.97
A2	7	5.16	2.21	0.41	18.55	19.52
L19	4	3.30	4.07	0.65	15.98	16.81
A1	6	5.18	2.08	0.35	16.82	17.73
A2	6	4.58	3.07	0.47	15.33	16.33
L44	5	3.11	4.13	0.63	15.25	16.05
A1	6.5	4.75	2.43	0.45	18.51	19.50
A2	6.5	4.95	2.25	0.43	19.11	20.12
F. test		**	**	**	**	**
L.S.D5%		0.07	0.21	0.05	1.7	1.8
1%		0.1	0.29	0.06	2.3	2.4

Notes : Initial pH was 7.0 , ** = P > 0.01 probability level

Evaluation of Transconjugants in the Utilization of Salty Cheese Whey:

The results summarized in Table 12 revealed that the ability to produce lactic acid from lactose whey in batch fermentation was differed. However, transconjugants from the mating between; T14 with L56 (I) except for; Tr3, Tr5; T14 X L44 (I) except for Tr16 and Tr20, as well as, the trasconjugants resulted from mating between T14 X L14 appeared significant increase in lactic acid production. The data clearly showed that transconjugant isolates; Tr8, Tr14, Tr17, Tr18, Tr19, Tr20, Tr22, Tr24, Tr25 and Tr40 appeared significant increase in lactic acid yield over the mid parents. Moreover, transconjugant isolates Tr8, Tr13, Tr14, Tr18, Tr22, Tr24, Tr25 and Tr40 appeared significant increase in lactic acid conversion efficiency in relation to the mid parents. On the other hand, the following transconjugants; Tr8, Tr14, Tr18, Tr22, Tr24, Tr25 and Tr40 appeared significant increase over their mid parents in both lactic acid yield and conversion efficiency. This agreed with Thompson *et al* (1999), who found that - Glucanase activity was detected for all transconjugants harboring the pSA3b6:: pVA797 plasmid co - integrate, although the diameters of the zones of lichenin hydrolysis around individual colonies were varied, this could be due to differences in either the level of expression in different hosts or the ability of the bacteria to export the enzyme.

In conclusion, the disposal of whey as waste poses serious pollution problems for the environment because of its high biological oxygen demand. Efficient utilization of whey via recombinant isolates of *Lactobacillus* have been used to solve the disposal problem of whey via converting the contents of lactose to lactic acid. This because whey and whey permeates contain significant quantities of lactose which was a suitable substrates for fermentations to up grade these effluents. One attractive genetic target as conjugation used in this study is to create molecular signatures or tags on the genomes of probiotic cultures. This will be an important area for future research.

Table 12: Efficiency of *Lactobacillus* transconjugants in the conversion of lactose to lactic acid from salty whey at batch fermentation.

Strains and transconjugants	NaCl%	pH	Lactose consumption %	Lactic acid		
				Production%	Yield %	Conversion efficiency %
T .14	18	5.84	3.84	0.31	8.532	8.981
L.56	7	2.84	4.17	0.76	18.60	19.57
M.P	12.5	4.34	4.01	0.54	13.60	14.77
Tr1	4	3.45	4.18	0.72	17.28	18.18
Tr2	4	3.70	4.12	0.63	15.08	15.87
Tr3	4	3.44	4.12	0.55	14.55	15.31
Tr4	4	3.91	4.09	0.63	15.24	16.04
Tr5	4	3.21	3.61	0.56	14.96	15.74
T14	18	5. 84	3.84	0.31	8.532	8.981
L34	4	3.81	4.02	0.67	16.82	17.70
M.P	11	4.83	3.93	0.49	12.70	13.34
Tr6	4	4.41	4.06	0.45	11.76	12.37
Tr7	4	5.26	2.77	0.36	11.82	12.44
Tr8	4	5.63	2.25	0.38	16.87	17.75
Tr9	4	4.53	3.50	0.40	12.40	13.05
Tr10	4	4.59	3.43	0.44	13.55	14.26
T14	18	5. 84	3.84	0.31	8.530	8.981
L19	4	3.30	4.06	0.65	16.19	17.04
M.P	11	4.57	3.95	0.48	12.40	13.01
Tr11	4	5.50	2.38	0.37	13.38	1408
Tr12	4	4.32	3.38	0.42	14.22	14096
Tr13	4	5.43	2.45	0.38	15.79	16.62
Tr14	4	4.47	2.74	044	16.57	17.44
Tr15	4	3.23	3.36	042	13.22	13.91
T14	18	5. 84	3.84	0.31	8.532	8.981
L44	5	3.11	4.12	.63	15.55	16.36
M.P	11.5	4.48	3.98	0.47	12.00	12.67
Tr16	4	3.63	4.15	0.50	12.17	12.81
Tr17	4	3.83	3.22	0.55	16.34	17.20
Tr18	4	2.38	4.18	0.63	19.47	20.49
Tr19	4	4.21	4.04	0.59	16.51	17.37
Tr20	4	3.25	2.46	0.42	16.83	17.71
T14	18	5. 84	3.84	0.31	8.532	8.981
L14	4	3.85	3.41	0.65	18.26	19.22
M.P		4.85	3.63	0.48	13.40	14.10
Tr21	7.5	3.78	3.91	0.45	11.35	11.94

Table 12: Continued

Strains and transconjugants	NaCl%	pH	Lactose consumption %	Lactic acid		
				Production%	Yield %	Conversion efficiency %
Tr22	7.5	3.68	2.26	0.63	23.63	24.87
Tr23	7.5	3.62	2.78	0.50	17.36	18.27
Tr24	7.5	3.65	2.94	0.53	17.99	18.93
Tr25	7.5	3.47	4.06	0.67	17.31	18.22
T .14	18	5.84	3.84	0.31	8.532	8.983
L.56	7	2.84	4.17	0.76	18.60	19.57
M.P	12.5	4.34	4.01	0.54	13.60	14.27
Tr26	8.5	4.62	2.33	0.38	13.78	14.51
Tr27	8.5	4.81	2.31	0.41	16.66	17.53
Tr28	8.5	4.50	2.49	0.39	13.68	14.40
Tr29	8.5	4.57	2.99	0.41	12.53	13.18
Tr30	8.5	5.67	2.22	0.32	13.10	13.78
T14	18	5.84	3.84	0.31	8.534	8.983
L34	4	3.81	4.02	0.67	16.66	17.70
M.P	11	4.83	3.93	0.49	12.70	13.34
Tr31	7	4.40	3.71	0.43	11.58	12.18
Tr32	7	5.00	2.25	0.34	15.10	15.89
Tr33	7	5.56	2.92	0.39	13.35	14.05
Tr34	7	5.59	2.48	0.36	14.91	15.69
Tr35	7	5.88	2.73	0.37	13.54	14.25
T14	18	5.84	3.84	0.31	8.534	8.983
L19	4	3.30	4.06	0.65	15.98	17.04
M.P	11	4.57	3.95	0.48	12.40	13.01
Tr36	7	4.96	3.92	0.41	10.45	11.00
Tr37	7	5.03	3.12	0.39	12.49	13.14
Tr38	7	5.68	2.91	0.38	13.04	13.72
Tr39	7	4.77	3.52	0.44	12.49	13.14
Tr40	7	5.56	2.79	0.45	16.12	16.96

Table 12: Continued

T14	18	5.84	3.84	0.31	8.534	8.983
L44	5	3.11	4.12	0.63	15.27	16.36
M.P	11.5	4.48	3.98	0.47	12.00	12.67
Tr41	7.5	5.02	3.14	0.36	11.45	12.05
Tr42	7.5	4.90	3.87	0.46	11.86	12.48
Tr43	7.5	4.95	3.81	0.41	10.75	11.31
Tr44	7.5	4.85	3.11	0.48	15.42	16.23
Tr45	7.5	4.84	3.93	0.49	12.45	13.11
F.test		**	**	**	**	**
L.S.D	5%	0.04	0.24	0.04	3.7	3.95
	1%	0.05	0.32	0.06	4.9	5.24

REFERENCES

- Aslim, B. and Y. Beeyalti, 2004. Antibiotic resistance and plasmid DNA contents of *Streptococcus thermophilus* strains isolated Turkish yogurts. Turk. JVet Anim, (28): 275-263.
- Baldani, J.I., R.W. Weaver, M.F. Hynes and B.O. Eardly, 1992. Utilization of carbon substrate, electrophoretic enzyme patterns, and symbiotic performance of plasmid-cured clover rhizobia. Appl. Environ. Microbiol., 58(7): 2308–2314.
- Brock, T.D., 1964. Action of streptomycin and related antibiotics. Fed. Proc., 23: 965-975.
- Bucbanan, R.E. and N.E. Gibbons, 1974. Bergey's manual of determinative bacteriology. 7th ed. The Williams and Wilkins Co., Baltimore, MD., pp: 52–54.
- Chakrabarty, A.M., S.G. Dastidar and M. Ganguli, 1990. DNA as contaminant in antibiotics and its capacity to transfer bacteria to drug resistance. Ind. J. Exp. Biology, 28: 58-62.
- Chin.S.C., A. Norhani, W.S. Tan and Y.W. Ho, 2005. Plasmid Profiling and Curing of *Lactobacillus* strains Isolated from the Gastrointestinal Tract of Chicken. The J. Microbiol., 43(3): 251- 256.
- Cresti, S., M. Lattanzi, A. Zanchi, F. Montaguani, S. Pollini and Rossolini, 2002. Resistance determinants and cloned diversity in group a Streptococci collected during a period of increasing macrolide resistance. Antimicrobial Agen. Chemo., 46 (6): 1816-1822.
- Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev., 53(1): 121-47.
- Downes and E.D. Ito, 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C. Cited from http://www.bd.com/ds/technicalCenter/inserts/Lactose_Broth.
- El-Gendy, S.M., H. Abdel- Gali, Y. Shahin and F. Hegazi, 1983. Use of salt-tolerant lactic acid bacteria for manufacture of white pickled cheese (Domiat) ripened without whey in sealed polyethylene pouches. J. Food Prot, 46: 335.
- Edgar, R.L., 1963. A text – Book of Dairy Chemistry, Third Edition Reprinted with Revisions. London, 2.
- Fernandez, R.O. and D.N. Anton, 1987. Bacteriostatic Action of Streptomycin on Ribosomally Resistant Mutants (rpsL) of *Salmonella typhimurium*. Antimicrobial Agen. Chem., 31(10): 1627-1631.
- Garvie, E.I., P.H. Sneath, N.S. Mair, M.E. Sharpe, J.G. Holt, E.D. Williams and W.C. Baltimore, 1986. Genus *Pediococcus*. In Bergey's Manual of Systematic Bacteriol, 2: 1075 – 1079.
- Ghaly, A.E., M.S.A. Tango and M.A. Adams, 2003. Enhanced lactic acid production from cheese whey with nutrient supplement addition. Agricultural Engineering International: the CIGR J. of Sci. Res. Develop. Manuscript FP 02 009. 5: 1-20.
- Gonzalez - Siso, M.I., 1996. The biotechnological utilization of cheese whey: a review. Bioresour Technol., 57(1): 1–17.
- Gross, R.A. and B. Kalra, 2002. Biodegradable polymers for the environment. Polytechnic University, National Science Foundation, Center for Biocatalysis and Bioprocessing of Macromolecules, Six Metrotech Center, Brooklyn, NY11201, USA. Science, 97(5582): 803-807.
- Jürgen, B., 2008. Mechanisms of hop inhibition, tolerance and adaptation in *Lactobacillus brevis*. Doctoral thesis. Technische Universität München. Lehrstuhl für Technische Mikrobiologie. Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines.
- Kalliomaki, M., S. Salminen, H. Arvilommi, P. Kero, P. Koskinen and E. Isolauri, 2001. Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet, 357 (9262): 1076 – 1079.
- Kobayashi, T., M. Kajiwara, M. Wahyuni, N. Hamada - Sato, C. Imada and E. Watanabe. 2004. Effect of culture conditions on lactic acid production of *Tetragenococcus* species. J. Appl. Microbiol, 96 (6): 1215–1221.

- Koch, H.U., R. Döker and W. Fischer, 1985. Maintenance of D-alanine ester substitution of lipoteichoic acid by reesterification in *Staphylococcus aureus*. J. Bacteriol., 164 (3): 1211-1217.
- Lanka, E. and B.M. Wilkins, 1995. DNA processing reactions in bacterial conjugation. Annu. Rev. Biochem., 64: 141-169.
- Leroy, F. and Luc De Vuyst, 1999. The Presence of Salt and a Curing Agent Reduces Bacteriocin Production by *Lactobacillus sakei* CTC 494, a Potential Starter Culture for Sausage Fermentation. Appl. Environ. Microbiol., 67(12): 4407-4413.
- Martin, J.K. and T.R. Klaenhammer, 2000. Genetic Modification of Intestinal Lactobacilli and Bifidobacteria. Curr. Issues Mol. Biol., 2 (2): 41-50.
- Michael, H.G., D.M. Rubin, O. Coux, I. Wefes, G. Pfeifer, Z. Cjeka, W. Baumeister, V.A. Fried and D. Finley, 1998. A Subcomplex of the Proteasome Regulatory Particle Required for Ubiquitin - Conjugate Degradation and Related to the COP9-Signalsome and eIF3. Cell, 94 (5): 615 - 623.
- Michel, D.K.A., J.K. Gilles, P.A. Hamilton, R. Fred and Smith, 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical. Chem., 28(3): 350 - 356.
- Naveena, B.J., M. Altaf, K. Bhadriah, G. Reddy, 2005. Selection of medium components by Plackett - Burman design for production of L (+) Lactic acid by *Lactobacillus amylophilus* GV6 in SS Fusing wheat bran. Bioresource Technol., 96(4): 485 - 490.
- Nevien, A., A. Sereih, A. El-Aal, S. Kh and A.G. Attallah, 2007. Expression of Transformed A heat Tolerate Gene in *Lactobacillus gasserii*. Egypt. J. Appl. Sci. Res., 3 (11): 1523-1526.
- Nigel, A., C.Y. Morrison, M.J. Hau, J.S. Trinick and B.G. Rolfe, 1983. Heat Curing of a Sym Plasmid in a Fast-Growing Rhizobium sp. That Is Able To Nodulate Legumes and the Nonlegume Parasponia sp. J. Bacteriol., 153 (1): 527-531.
- O'Byrne, C.P. and I.R. Booth, 2002. Osmoregulation and its importance to foodborne microorganisms. Int. J. Food Microbiol., 74 (3): 203-216.
- Panesar, P.S., J.F. Kennedy, C.J. Knill and M.R. Kosseva, 2007. Applicability of pectate-entrapped *Lactobacillus casei* cells for L (+) lactic acid production from whey. Biotechnological products and process engineering. Appl. Microbiol. Biotechnol., 74(1): 35-42.
- Pieterse, B., 2006. Transcriptome analysis of the lactic acid and NaCl - stress response of *Lactobacillus plantarum*. Thesis Wageningen University, Wageningen, The Netherlands - with summary in Dutch pp: 160.
- Ramesh, M.V., 2001. A wonder chemical that will help make biodegradable plastic why India needs to milk the full potential of lactic acid. India markets empowering business. Cited from Naveena *et al* 2005. Bioresource Technol., 96(4): 485-490.
- Rasool, S.A., 1992. Bacteriocins: the protein antibiotics. (Fazlee Sons, Karachi) p.15. Cited by Rasool *et al* 2003. Pak. J. Bot., 35 (2): 243-248.
- Ronald, M.A., 2006. Hand book of Microbiological Media for the Examination of Food Technology and Engineering. Cited from, <http://www.amazon.com>.
- Ruiz-Barba, J.L., J.C. Piard and R. Jimhez - Diaz, 1991. Plasmid profiles and curing of plasmids in *Lactobacillus plantarum* strains isolated from green olive fermentations. Instituto de la Grasa y sus Derivados (CSIC), UEI de Biotecnologia de Alirmentos, Sevilla, Spain and 'Station de Recherches Laitieres (INRA), Unite de Microbiologie, Domaine de Vilvert, Jouy-en-Josas, France. J. Appl. Microbiol., 71 (5): 417 - 421.
- Salem, A.S. and A.M. Abeid, 1979. Low sodium and cholesterol domiati cheese. Egyptian Dairy Sci., 25: 123 - 134.
- Santo, M.L., L. Cristiane, G.A. Fernanda, M. Daniela, H.B. Luiz, S.S.A. Ernani and D.G. Bernadette, 2005. Effect of Different Levels of Sodium Chloride and Glucose on Fermentation of Sardines (*Sardinella brasiliensis*) by *Lactobacillus sakei* 2a. Brazilian Archives of Biology and Technology, An International J., 48 (1): 45-52.
- Satomi, M., B. Kimura, M. Mizoi, T. Sato, T. Fujii, 1997. *Tetragenococcus muriaticus* sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce. Int. J. Syst. Bacteriol., 47(3): 832-836.
- Scheirlinck, T., J. Mahillon, H. Joos, P. Dhaese and F. Michiels, 1989. Integration and expression of a -amylase and endoglucanase genes in the *Lactobacillus plantarum* chromosome. Appl. Environ. Microbiol., 55: 2130-2137.
- Seduce, G.W. and W.G. Cochran, 1955. Statistical Methods, sixth edition. The Iowa state University Press, Iowa, U.S.A.
- Sharaf, O.M., N.A. Absoereh, A.B. Abd- El-Khalak and Kawther El - Shafei, 2007. Genetic improvement of *Lactobacillus casei* sub sp *casei* via protoplast fusion. J. Agric. Sci. Mansoura Univ., 32 (10): 8273 - 8286.
- Shockey, W.L. and D.C. Borger, 1991. Effect of Salt on Fermentation of Alfalfa. Treatment with Sodium Chloride, Clostridium butyricum, and Lactic Acid Bacteria'. J Dairy Sci., 74(1): 160 - 166.
- Simpson, W.J. and J.L. Fernandez, 1992. Selection of beer- spoilage lactic acid bacteria and induction of their ability to grow in beer. " Lett. Appl. Microbiol., 14(1): 13 - 16.

- Stanisich, V.A., 1988. Identification and analyses plasmids at the genetic level. *Methods Microbiol.*, 21: 11-47.
- Steil, L., T. Hoffmann, I. Budde, U. Völker and E. Bremer, 2003. Genome-wide transcriptional profiling analysis of adaptation of *Bacillus subtilis* to high salinity. *J.Bacteriol.*, 185(21): 6358-6370.
- Swenson, J.M., R.R. Facklam, C. Thornsberry, J.M. Swenson, R.R. Facklam and C. Thornsberry, 1990. Antimicrobial Susceptibility of vancomycin resistant *Leuconostoc*, *Pediococcus* and *Lactobacillus* species. *Antimicrob. Agents chemoter*, 34 (40): 543 –549.
- Tanaka, T., J. Burgess and P. Wright, 2004. High-pressure adaptation by salt stress in a moderately halophilic bacterium obtained from open seawater. *Appl. Microbiol. Biotechnol.*, 57 (1-2): 200 – 204.
- Tango, M.A. and A.E. Ghaly, 1999. Amelioration of lactic acid production from cheese whey using microaeration. *Biomass and Bioenergy J.*, 17 (2): 221-238.
- Thompson, J.K., S. Foley, K.J. McConville, C. Nicholson, M.A. Collins and R.D. Pridmore, 1999. Complete sequence of plasmid pLH1 from *Lactobacillus helveticus* ATCC15009: analysis reveals the presence of regions homologous to other native plasmids from the host strain. *Plasmid*, 42 (3): 221 – 235.
- Tun-Garrido, C., B. Patricia, G. Víctor and B. Susana, 2003. Conjugative Transfer of p42a from *Rhizobium etli* CFN42, Which Is Required for Mobilization of the Symbiotic Plasmid Is Regulated by Quorum Sensing. *J. Bacteriol.*, 185 (5): 1681-1692.
- Tyagi, R.D., D. Kluepfel and D. Couillard. 1991. Bioconversion of cheese whey to organic acids. In: Martin AM (ed) *Bioconversion of waste materials to industrial products*. Elsevier, Essex, UK, pp: 313–334. Cited from Panesar *et al* 2007. *Appl. Microbiol. Biotechnol.*, 74(1): 35 – 42.
- Uguen, P., J. Hamelin, J.P. Le Pennec and C. Blanco, 1999. Influence of osmolarity and the presence of an osmoprotectant on *Lactococcus lactis* growth and bacteriocin production. *Appl. Environ. Microbiol.*, 65(1): 291 – 293.
- Venkatraman, K. and A. Muralidharan, 2004. To eliminate the disposal of salty whey from a dairy industry into the sewer in an environmental, social and economical way – a case study from dairy farmers, Toowoomba, Queensland, Australia. *International J. Enviro. Technol. Manag*, 4(4): 365 – 374.
- Verluyten, J., M. Winy and L. De Vuyst, 2004. Sodium Chloride Reduces Production of Curvacin A, a Bacteriocin Produced by *Lactobacillus curvatus* Strain LTH 1174, Originating from Fermented Sausage. *Appl. Enviro. Microbiol.*, 70 (4): 2271 – 2278.
- Woojin, S., K.L. Perl, J.H. Park, J.E. Tandianus and N.W. Dunn, 2001. Assessment of Stress Response of the Probiotic *Lactobacillus acidophilus*. *J Current Microbiol.*, 43 (5): 346 – 350.