Expression of the *Pediococcus acidilactici ldh* L gene in *Propionibacterium freudenreichii* var. *shermanii* LAG16424T

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ABSTRACT

As a cloning vector for transformation in Propionibacterium sp., pPK705 shuttle vector was experimented, where it consists of the indigenous plasmid of Propionibacterium acidipropionici E214 (pRG01), plasmid pUC18 and the hygromycin resistance gene (hyg B). A 1.2 Kb BamHI-EcoRI DNA fragment encoding ldh L gene of Pediococcus acidilactici DG302 was cut from plasmid pGID150 and further subcloned in pPK705. Transformation and expression of the ldh L gene of pediococci in NAD$^+$-independent LDH Propionibacterium freudenreichii var. shermanii LAG16424T was confirmed. The absolute L (+) LDH enzyme activity was found to be (25.67 to 42.79 IU/ml) for Propionibacterium transformants cell free extract. Twenty Propionibacterium transformants were further inoculated in skimmed milk, where curdling was detected after 48 h with one of the prominent transformants. After 144 h, all transformants coagulated skimmed milk as compared to parental bacterial strain. Maximum % of lactic acid produced by Propionibacterium transformants was 0.79 % as lactic acid after 96 h. Further proof for the expression of NAD$^+$-dependent L (+) LDH through the transformed ldh L gene was confirmed by polyacrylamide gel electrophoresis.

Keywords: gene cloning, gene expression, ldh L gene, pediococcus, propionibacterium.

INTRODUCTION

The use of recombinant microbial strain in the industry is almost prohibited, however a relaxation of the use of such genetically modified strains by some laboratories is taken place. This can be accomplished by cloning systems which are composed of acceptable marker genes which would allow the detection of transformants. To be completely acceptable, a "food grade" vector should ideally be entirely composed of DNA from the target organism, or closely related non-pathogenic strains, and possess a readily selectable marker gene such as nisin (Froseth & McKay, 1991). Similar efforts in that respect were reported by Biet *et al.* (1999), Cotter *et al.* (2003), as well as Xia *et al.* (2005). Kiatpapan *and* Murooka (2001) constructed a shuttle vector that included regions of plasmid pRG01 of *Propionibacterium acidipropionici* (*P. acidipropionici*) E214 (Rehberger & Glatz, 1990) and pUC18 containing the *ori* of *Escherichia coli* (*E. coli*). The above vector was used successfully for the transformation of *Propionibacterium* even the vector still including the *hyg* B as a selectable marker. Latter gene hinders the use of pPK705 as a "food grade" vector. Thus, it is necessary to exclude the pUC18 of *E. coli* under the replacement of *hyg* B with *pct* A gene of *P. thoenii* 419 (Faye *et al.*, 2000) as a marker gene before using the pPK705 as "food grade" vector.

The aim of the present work is to study a possible expression of the *Pediococcus acidilactici ldh* L gene in the NAD$^+$-independent L (+) LDH *Propionibacterium freudenreichii* var. *shermanii* (*P. freudenreichii* var. *shermanii*) LAG16424T. The next step is to modify pPK705 to be a "food grade" transformation vector in lactic acid bacteria for applied aspects.

MATERIALS and METHODS

Bacterial strains and plasmids

Vector pPK705 was provided in *E. coli* DH5α as a gift from Dr. Murooka, Department of Biotechnology, Graduate School of Engineering, Osaka University, Yamada-Oka, Suita, Osaka 565-0871, Japan. *E. coli* T61 harboring pGID150 lactate dehydrogenase gene (*ldh* L gene) of *Pediococcus acidilactici* DG302 was obtained from Dr. Ferain, Laboratoire de Génétique Moléculaire, Unité de Génétique, Univer-

site Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium. *E. coli* DH5α (wild type) was a gift from Dr. Hesham El-Sayed Mostafa, Nucleic Acid Research Department, Institute of Genetic Engineering and Biotechnology, Mubarak City for Scientific Research, Alexandria, Egypt. *P. freudenriechii* var. *shermanii* LAG16424T was provided by BCCM[TM], Wetenschapsstraat 8, rue de la Science, B-1000 Brussels, Belgium.

Media and growth conditions

All mentioned *E. coli* strains were cultivated at 37°C in Luria Bertani Medium (Rehberger & Glatz, 1990) containing the appropriate antibiotic. *P. freudenriechii* var. *shermanii* LAG16424T was grown at 30°C in tomato juice medium (ATCC). *P. freudenriechii* var. *shermanii* LAG16424T transformants were grown at 30°C in tomato juice medium containing 250μg hygromycin B /ml.

Isolation of plasmid DNA

Plasmid DNA was isolated using alkaline lysis method (Jore, *et al.* 2001). After the bacteria was grown in the appropriate broth medium, cells were collected by centrifugation at 138000 Xg for 10 min at 4°C, washed with 200 μl of solution I (25% sucrose -50 mM Tris – HCl, pH 8) and resuspended in 250 μl of solution II (25% sucrose -50 mM Tris – HCl, 50 mM NaCl and 5 mM EDTA, pH 8) containing 10 mg of lysozyme [activity ~ 20000 IU/mg of egg albumin, Amersham Biosciences (Voulhagmenis Avenue 16, Glyfada, Greece)] per ml. After 30 min of incubation at 37°C, cells were lysed by adding 500μl of solution III (0.2 N NaOH and 1% sodium dodecyl sulphate) and left on ice for 2 to 5 min, solution IV 400 μl containing 3 M sodium acetate (pH 4.8) was added, and the mixture was left on ice for 5 min followed by centrifugation at 138000Xg for 15 min at 4°C. The supernatants were transferred to new eppendorf tubes, where 200μl of solution V (phenol: chloroform: iso-amyl alcohol ratio 1:1:0.04, 0.1% hydroxyquinoline and 0.2% β-mercaptoethnol) were added and mixed well. Centrifugation at 138000 Xg for 2 to 3 min at 4°C was carried out. The upper aquatic layer was transferred to fresh eppendorf tubes. Later step of extraction was repeated to ensure the removal of protein residues. One ml of solution VI containing isopropanol (99 %) was added. The eppendorf tubes were placed on dry ice/ ethanol bath at -70°C for 10 min and finally were centrifuged at 138000 Xg for 10 min at 4°C. The plasmid DNA was dissolved in 30 μl of solution VII [10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)].

Agarose gel electrophoresis

Agarose gel electrophoresis was carried out according to (Sambrook, *et al.*, 1989). Agarose (Amersham Biosciences) 1.5% was dissolved in TBE buffer (0.0089M Tris, 0.089M Boric acid and 0.002M EDTA dissolved in 1 liter distilled water). The slurry was boiled until the agarose dissolved totally. The solution was cooled to 50°C and ethidium bromide (from a stock solution of 10 mg/ml in distilled water, stored at 4°C in a light-proof bottle, Amersham Biosciences) was added to a final concentration of 0.5 μg/ml. The warm agarose solution was poured into the sealed tray after the comb was clamped near one end of the tray. After the gel was completely hardened at room temperature, the comb was removed carefully and the gel was mounted in the electrophoresis tank. The electrophoresis buffer was added to cover the gel. Samples were mixed with loading buffer (0.25% bromophenol blue and 4% sucrose) and loaded into the wells of the submerged gel beside the *Eco*RI – *Hind*III digested λ DNA marker (Amersham Biosciences, Sweden). Electrophoresis was run at 75 volts until the bromophenol blue dye reaches 1 cm before the end of the tray.

Plasmid DNA purification

Purification was performed using NAP-5 column (Amersham Pharmacia Biotech) by removing the upper cap of the column and the preservative (Kathon[®]) was poured, whereas, Kathon[®] is provided by manufacturer to preserve the column gel from spoilage. After removing the bottom column cap, preservative residues are removed by successive passage of solution VIII (0.1M sodium phosphate buffer, pH 7.5) through the column for 3 times.

Measurement of plasmid DNA

According to Sambrook, *et al.* (1989), the plasmid DNA concentration was calculated from the following equation: DNA concentration (μg/μl)= ($O.D._{260\ nm}$ × B × 50) /1000, where B is the dilution inversion factor.

Digestion, dephosphorylation and ligation of recombinant plasmids

Digestion, dephosphorylation and ligation were carried out according to Johnson and Kaiser (1993). For digestion, the following ingredients were transferred into an eppendorf tube in

the following order: plasmid solution (0.2-1.0µg plasmid /20µl of solution VII), 2µl of the appropriate 10X digestion buffer (Promega, USA) and one unit of each appropriate restriction enzyme (Promega, USA) was added. The tubes were mixed by tapping after which incubation at 37°C was carried for 2 hr. The reaction was terminated by incubation in a water bath at 65°C for 10 min.

For dephosphorylation, the following ingredients in the following order were added directly to the digested plasmid: 10µl of calf intestinal alkaline phosphatase buffer (10X), 0.01U of calf intestinal alkaline phosphatase (CIAP) and the volume was made to 100 µl with nuclease-free-water (Amersham Biosciences, Sweden) followed by incubation at 37°C for 30min. Another 0.01U of CIAP were added to the reaction mixture followed by incubation for additional 30 min at 37°C To stop the reaction, 2µl of 0.5M of EDTA (BDH, UK, pH 8.0) were added and the tubes were heated in a water bath for 20 minutes at 65°C. The calf intestinal alkaline phosphatase was removed by phenol: chloroform extraction for protein removal, as mentioned in plasmid isolation section, followed by isopropanol treatment to precipitate DNA.

For ligation, the following ingredients were added in an eppendorf tube: 0.1µg plasmid, 0.017µg DNA insert, 1 unit of T_4 DNA ligase (USB, USA), 1.0µl of T_4 DNA ligase (10X) buffer and DNase-free-water (Promega, USA) to a final volume of 10µl. Ligation reaction was performed at 4°C for overnight.

Bacterial transformation

Transformation of competent cells was carried out as mentioned below (Perball, 1989). Frozen bacterial pellet was resuspended in 100µl of ice-cold 100mM magnesium chloride. The mixture was kept on ice for 15min followed by centrifugation at 10300 Xg for 5 min at 4°C. The supernatant was poured off and cell pellet was placed on ice. The pellet was resuspended very gently in 10 µl of ice-cold 50 mM calcium chloride. The mixture was placed on ice where the overnight cells were ready to be transformed. Ten ng (in 0.5- 10µl) of plasmid DNA were transferred to 100µl of competent cells and the tube was swirled very gently just to mix and left on ice for 30min. The competent cells were heat-shocked by placing the tube in a warm bath at 42°C for 1.5min. One ml of the appropriate broth was added to the cells followed by incubation at the appropriate temperature for 18hr to cure transformants that were detected by plating dilutions of the broth on the appropriate agar medium containing the appropriate bacterial antibiotic.

Total protein determination

Total protein was measured spectrophotometrically at 280 nm and calculated from the following equation according to Vassault (1983):

$$\text{Total protein (mg/ml)} = (10 \times A_{280nm} \times 100) / 8.85$$

LDH activity

Bacterial cells were collected by centrifugation at 1380000 Xg for 10 min at 4°C and the pellet was washed twice with 0.01M of potassium sodium phosphate buffer (pH 7.0) and re-centrifuged at 41050 Xg for 20 min at 4°C. The pellet was ground for 20 min in a mortar using glass beads (0.1mm) and resuspended in 0.5 ml of 0.01 M potassium sodium phosphate buffer (pH 7.0). The mixture was centrifuged at 138000 Xg for 1 hr at 4°C. The pellet was discarded and the NAD^+-dependent and independent LDH activity in the crude extract was measured according to Vassault, (1983) and Molinari and Lara (1960), respectively. One unit of NAD^+-dependent LDH activity was defined as the amount of enzyme catalyzing the oxidation of 1 µmole NADH (Amersham Biosciences, Sweden) per min under the assay conditions. Enzyme activity and specific enzyme activity were calculated as follows:

$$\text{Enzyme activity (IU/ml)} = [(\Delta A_{340nm}/\text{minute}) \times 3.1 \times 101 \times 51 / (6.22 \times 0.1)]$$

$$\text{Specific enzyme activity (EU/mg protein)} = \text{Enzyme activity / Protein content}$$

Whereas, one unit of NAD^+-independent LDH activity was defined as the amount of enzyme catalyzing the oxidation of 1.0µM of 2, 6-dichlorophenolindophenol (Amersham Biosciences, Sweden) per min under the assay conditions. Enzyme activity and specific enzyme activity were calculated as follows:

$$\text{Enzyme activity (EU/ ml)} = (\Delta A_{600nm}/\text{ minute}) / 20.6$$

$$\text{Specific Activity (EU/ mg protein)} = \text{Enzyme activity} / A_{280nm}$$

Lactic acid determination

Overnight cultured broth (0.2ml) was transferred to an eppendorf tube. The tube was centrifuged at 41050 Xg at 4°C for 5 min. The supernatant was transferred to a cleaned dried fresh test tube and 0.2 ml of distilled water was added to dilute the supernatant (1:1). Concen-

trated pure sulphuric acid (AR, BDH) (2.0ml) were added gently, but quickly, to the mixture. The mixture was mixed gently followed by adding 1.0 to 2.0 droplets of 1.0% (w/v) guaiacol (Oxoid, England) dissolved in ethanol. The sample is positive if a red ring to the top of the test tube was seen immediately after the addition of guaiacol (Rauscher, *et al.*, 1972).

Preparation of SDS- PAGE gel

The preparation of the gel and the electrophoresis technique was carried out (Bio RAD using Protean II cell). Electrophoresis was performed in 12% polyacrylamide gel as described by Laemmli (1970). Bacterial cell extracts were mixed with sample loading buffer and loaded onto 12% polyacrylamide gel along with the protein marker 10–100KD (Pharmacia Biotech, Sweden).

Statistical analysis

As described by Snedecor and Cochran (1956), the statistical analysis was carried out according to "student's" *t*- distribution, hence this distribution has revolutionized the statistics of small samples. The quantity *t* is given by the equation:

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

That is, *t* is the deviation of the estimated mean from that of the population, measured in terms of $s/\sqrt{n}$ as the unit. Both x and s are calculated from a sample of observations number (n).

RESULTS AND DISCUSSION

In order to select the most appropriate bacterial host that will be used for the transformation studies concerning *ldh* L gene sequence of *Pediococcus acidilactici* DG302 into *Propionibacterium*, different bacterial host strains available in our laboratory were tested with respect to their sensitivity towards hygromycin B (selectable marker, Sigma). Among all the investigated bacterial strains, *P. freudenreichii* var. *shermanii* LAG16424T revealed sensitivity towards hygromycin B. Thus, later bacterial strain was used as host in the transformation studies concerning the application of pPK705 as a vector containing the hygromycin B resistance gene.

The purified plasmid pGID150 was cut with *Bam*HI – *Eco*RI restriction enzymes to give a 1.2 Kb DNA fragment. This fragment carries the *ldh* L gene of *Pediococcus acidilactici* DG302 (Figure 1). Similarly, the vector pPK705 was digested with the above restriction enzymes to give two DNA fragments 2.075 and 6.225 Kb. Dephosphorylation of the open 6.225 Kb plasmid was carried out, accordingly self-recircularization was prevented during ligation. The dephosphorylated pPK705 and the 1.2 Kb fragment reaction mixtures were mixed and ligation was performed as described in materials and methods. Figure (1) illustrates a scheme for the construction of the modified pPK705 (7.425 Kb) vector.

As a proof for the construction of modified pPK705 encoding a 1.2Kb *Bam*HI – *Eco*RI DNA fragment, digested and ligated products were subjected to agarose gel electrophoresis. Figure (2) shows intact and digested pGID150 and pPK705, as well as ligated DNA products. Accordingly, the molecular size of the modified plasmid was found to be 7.425 Kb which is the sum of the *ldh* L gene fragment (1.2 Kb) of *Pediococcus acidilactici* DG302 and the 6.225 Kb open plasmid resulting from the digestion of the plasmid pPK705 with *Bam*HI – *Eco*RI.

Ligation products were added to competent *P. freudenreichii* var. *shermanii* LAG16424T. Transformation was carried out on the basis of the heat shock as described by Perball (1989). The transformants were subcultured in Luria Bertani broth without the addition of the antibiotic and incubation was performed at 30°C for 16 hr in dark. This period allows transformants to express genes. Growing right transformant clones were transferred to tomato juice broth containing 250 µg hygromycin B/ ml in case of *Propionibacterium sp.* as described by Kiatpapan, *et al* (2000). The number of *Propionibacterium* transformants / µg plasmid DNA was found to be 0.9×10^4 CFU/ µg DNA. According to Kiatpapan *et al* (2000), the transformants of *Propionibacterium* with intact vector pPK705 revealed a value of 1.0×10^6 CFU/ µg DNA. Later difference in transformation efficiency may be due to the different basics of transformation procedures used in each case. Since Kiatpapan *et al* (2000) used the electrical shock which is more efficient than heat shock used in the present work.

Table (1) reflects the NAD^+ - dependent L (+) LDH enzyme activities of twenty *P. freudenreichii* var. *shermanii* LAG16424T transformants that ranged between 25.67 and 42.79 IU/ml cell free extracts. While, the specific enzyme activity (Units / mg protein) varied between 1.51 and 2.97 IU/mg protein.

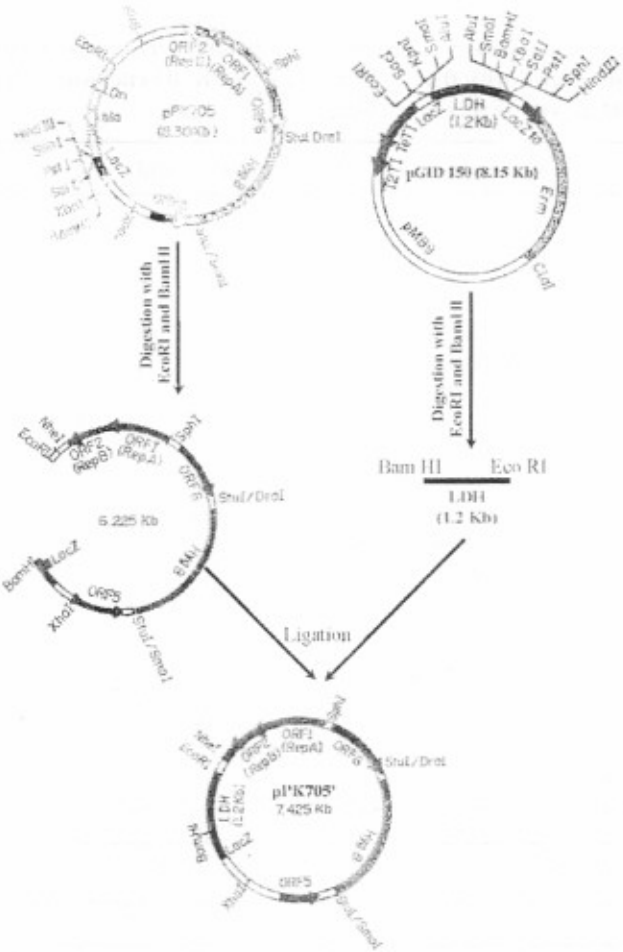

Fig. 1: Scheme for construction of modified pPK705' (7.425Kb) vector. Abbreviations: *Hyg* B, hygromycin B resistance gene; *Rep* A, gene coding for Rep A protein; *Rep* B, gene coding for Rep B protein. *bla*, β-lactamase; *lac* Z', β-galactosidase; *ori*, *col*E1 replication origin; MCS, multicloning site

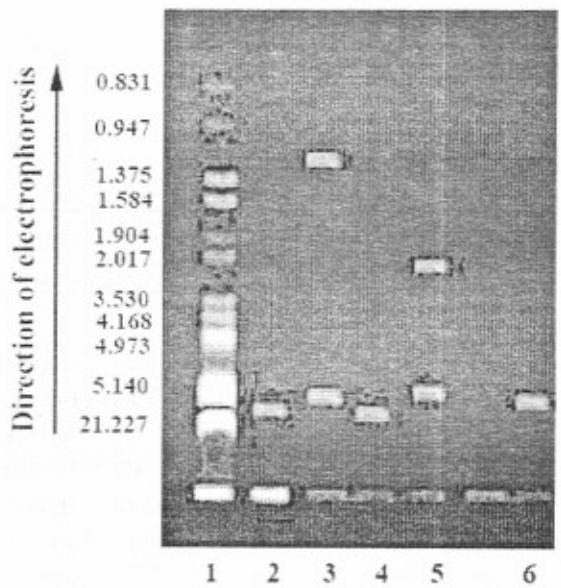

Fig. 2: Agarose gel electrophoretic pattern of intact pGID150 and pPK705 plasmids as well as their digested and legated products

Lane 1, λ *Eco*RI – *Hind*III DNA marker; Lane 2, Intact plasmid pGID150; Lane 3, plasmid pGID150 digested with *EcoR*I- *BamH*I; Lane 4, Intact plasmid pPK705; Lane 5, plasmid pPK705 digested with *EcoR*I- *BamH*I; Lane 6, modified pPK705' (7.425 Kb) plasmid

Table 1: NAD^+- dependent lactate dehydrogenase of *Propionibacterium freudenreichii* var. *shermanii* LAG16424T transformed with the modified vector pPK705 enclosing *ldh* L gene of *Pediococcus acidilactici* DG302 as well as t-Test calculations.

Transformat	Protein (mg/ml)	Activity (U/ml)	Specific activity (U/mg)
1G	12.655	25.672	2.029
2G	11.525	34.230	2.970
3G	22.712	34.230	1.507
4G	12.881	34.230	2.657
5G	17.062	25.672	1.505
6G	18.644	34.230	1.836
7G	11.638	25.672	2.206
8G	15.819	34.230	2.164
9G	16.949	34.230	2.020
10G	14.915	34.230	2.295
11G	20.339	34.230	1.683
12G	13.446	25.672	1.909
13G	14.915	25.672	1.721
14G	19.774	34.230	1.731
15G	20.000	34.230	1.711
16G	18.757	42.787	2.281
17G	22.599	34.230	1.515
18G	19.435	34.230	1.761
19G	16.045	34.230	2.133
20G	17.740	34.230	1.930
The parent	28.249	0.000	0.000

G refers to transformants with *ldh* L modified pPK705.

t-Test		
	X_1	X_2
Mean	0	1.978166
Variance	0	0.145908
Observations	20	20
Pooled Variance		0.072954
df		38
t Calc.		23.16124058
t Tabu. (0.05 Conf. limit)		2.093284625
t Tabu. (0.01 Conf. limit)		2.681382451
X_1 = NAD(+)Dependent LDH Enzyme Activity before transformation		
X_2 = NAD(+)Dependent LDH Enzyme Activity after transformation		

The transformation of *P. freudenreichii* var. *shermanii* LAG16424T with *ldh* L gene of *Pediococcus acidilactici* DG302 was further confirmed using *t*-test as a statistical parameter. All transformants exhibited highly significant NAD^+ -dependent L (+) LDH enzyme activities than parental *Propionibacterium* strain used in the study. This can be observed from calculated *t* value that was found to be 23.161, which is much greater than tabulated *t* that revealed values of 2.093 and 2.861 at 0.05 and 0.01 limits of confidence and 19 degrees of freedom. The results describing the NAD^+-independent LDH activity indicated no change of that enzyme system in *Propionibacterium* transformants.

The inclusion of *ldh* L gene in *Propionibacterium* genome resulted in NAD^+-dependent L (+) LDH expression and consequently the production of lactic acid. Thus, clotting of milk was detected as shown in Table (2). Milk clotting was detected after 48 hr, where only one transformant out of 20 investigated showed

Table 2: The influence of *Propionibacterium freudenreichii* var. *shermanii* LAG16424T transformed with *ldh* L gene of *Pediococcus acidilactici* DG302 on clotting skimmed milk

Transformat	Curdling time (hr.)					
	24	48	72	96	120	144
1G	–	–	–	–	–	×
2G	–	–	×	×	×	×
3G	–	–	×	×	×	×
4G	–	×	×	×	×	×
5G	–	–	×	×	×	×
6G	–	–	–	–	–	×
7G	–	–	×	×	×	×
8G	–	–	–	–	–	×
9G	–	–	×	×	×	×
10G	–	–	×	×	×	×
11G	–	–	×	×	×	×
12G	–	–	×	×	×	×
13G	–	–	–	–	–	×
14G	–	–	–	–	–	×
15G	–	–	–	–	–	×
16G	–	–	×	×	×	×
17G	–	–	×	×	×	×
18G	–	–	–	–	–	×
19G	–	–	–	–	–	×
20G	–	–	–	–	–	×
The parent	–	–	–	–	–	–

G refers to transformants with *ldh* L modified pPK705.

clotting of skimmed milk, whereas, all transformants coagulated skimmed milk after 144hr. This may be explained as the transformant that could coagulate skimmed milk after 48 hr may have many copies of the *ldh* L gene which is encoded in the modified pPK705 vector. On the other hand, parental *P. freudenreichii* var. *shermanii* LAG16424T did not indicate any capacity to coagulate skimmed milk even after 144hr.

Further proof for the implication of *ldh* L gene of *Pediococcus acidilactici* DG302 into the whole genome of *P. freudenreichii* var. *shermanii* LAG16424T was confirmed by subjecting the cell free extract of transformed cells (the new clones) to electrophoretic mobility by SDS-PAGE technique. Figure (3) shows the protein pattern of different bacterial clones. As the L (+) LDH enzyme protein has a molecular weight of about 140 KDa and it is a tetramer, each subunit has a molecular weight of about 34.5 KDa (Garmyn, *et al.* 1995). All bacterial clones expressing L (+) LDH enzyme activity showed a protein band that has a molecular weight of about 34.5 KDa as compared to the protein marker (Pharmacia Biotech, Sweden) as shown in lane 1. *E. coli* DH5α crude extract (lane 2) did not contain this specific band.

From the present work, it is clear that there is a possibility of transforming propionibacteria with a shuttle vector which consists of any indigenous plasmid and a selectable marker gene.

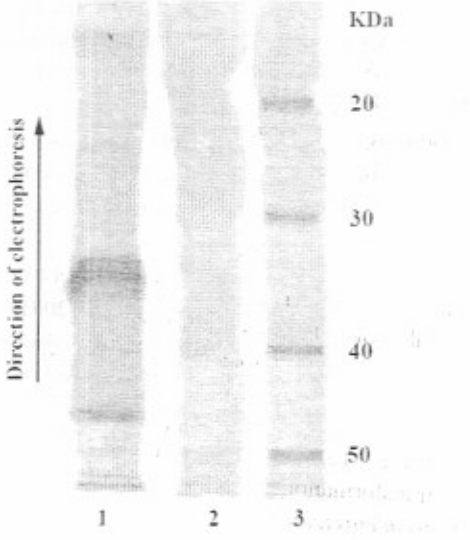

Fig. 3: SDS-PAGE pattern of lactate dehydrogenase enzyme of *P. freudenreichii* var. *shermanii* LAG16424T transformed with ldh L gene of *P. acidilactici* DG302 (lane 1), *E. coli* DH5α (harboring the native plasmid pPK705; lane 2), and lane 3 represents molecular weight marker 20 - 50 KDa

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التعبير الجيني لجين L *ldh* الخاص ببكتيريا بيديوكوكاس أسيديلاكتيسي في بكتيريا حامض البروبيونيك

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من المعروف أن بكتيريا حامض البروبيونيك تقوم بتحويل حامض اللاكتيك إلى حامض البروبيونيك المسئول عن إظهار الطعم والنكهة في أصناف الجبن السويسري بالإضافة إلى ثاني أكسيد الكربون؛ وهو المسئول عن تكوين العيون كبيرة الحجم في تلك المجموعة من الأجبان. إلا أن تلك البكتيريا ليس لها مقدرة على إنتاج حامض اللاكتيك، ومن ثم تتكون بادئات تلك الأصناف من الأجبان من بكتيريا حامض البروبيونيك وتلك المسئولة عن تحويل اللاكتوز إلى حامض اللاكتيك ألا وهي بكتيريا حامض اللاكتيك التابعة للجنس لاكتوباسيللاس. و في محاولة من جانبنا للبدء في استنباط سلالات معدلة وراثيا آمنة للاستخدام الصناعي، فقد بدأ العمل أولا في التعرف على مقدرة بكتيريا حامض البروبيونيك (التي تمتلك فقط إنزيم لاكتات ديهيدروجينيز الذي لا يعتمد على مركب نيكوتيناميد أدنين داينيكليوتيد كمجموعة مرافقة (NAD^+- independent LDH) في التعبير عن إنزيم لاكتات ديهيدروجينيز (الذي يعتمد على مركب نيكوتيناميد أدنين داينيكليوتيد كمجموعة مرافقة (NAD^+- dependent LDH)، وقد استخدم بلازميد pPK705 المتضمن لجزء من التركيب الجيني لبكتيريا حامض البروبيونيك كناقل لجين L *ldh* الخاص ببكتيريا بيديوكوكاس أسيديلاكتيسي. هذا وقد تم الحصول على نتيجة إيجابية في هذا الشأن أسفرت عن مقدرة السلالات المعدلة وراثيا من بكتيريا *Propionibacterium freudenreichii* var. *shermanii* LAG16424T على إنتاج حامض اللاكتيك ذي المقدرة على تجبين اللبن. وننوه إلى أنه لاستخدام تلك السلالات في الصناعة فإنه يلزم إزالة الأجزاء الداخلة في تركيب البلازميد pUC18 والتي مصدرها بكتيريا إشيريشيا كولاي *Escherichia coli*.