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Expression of Transformed Aheat Tolerate Gene in *Lactobacillus gasseri*

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Abstract: Two strains *streptococcus thermophillus* and *Lactobacillus gasseri* were used in this study and examined for their antibiotic resistance patterns and plasmid carriage *S. thermophillus* strain has a plasmid containing the highly thermostable gene (tolerate up to 42°C) and chloramphincol resistance gene (Cm^+) which used as a selective marker was used as a donor. This plasmid is able to integrate in *L. gassari* genome. *L. gasseri* strain sensitive of chloramphincol (Cm^-) and growing at 37°C was used as a recipient. Transformation technique was used to transfer heat tolerant and chloramphincol resistance (Cm^+) plasmid from *S. thermophillus* to *L. gasseri* strain. Seven *L. gasseri* tranformants were isolated and examined the heat stability at different temperature degrees from 37°C to 42°C. Protein fingerprinting and statistical analysis were done of the obtained data to determine the differences between wild type and transformant strains.

Keywords:

INTRODUCTION

Lactobacillus gasseri and related lactobacilli represent an important group of lactic acid bacteria which are commonly found association with the human gastrointestinal tract and are delivered as health promoting bacteria in food and probiotics[3]. Recent research efforts focused on expanding the use of probiotic lactobacilli to include biotechnology application such as enzymes and vaccine delivery system[15].

Streptococcus thermophillus is a gram positive bacterium used in manufacture yogurt and specially cheeses and strain improvement through genetic manipulation has considerable potential[1].

To apply recombinant DNA technology to *S. thermophilus,* a suitable cloning vector is required. An ideal plasmid for use in the development of an *S. thermophillus* cloning vector should be stably maintained in *S. thermophilus,* be small, and present in high copy number also, contain unique restriction enzyme sites for insertion the selectable markers and for cloning purposes[5]. The plasmids discovered in different strains have large spectra; some of these carry genes responsible for a verity of function such as thermo resistance, sugar metabolism and antibiotics[18]. In 2000, Kykova reported that 21 *Lactobacillus* strains in normal microflora resistant to antibiotics.

The main objective in this study is to construct heat stable strain(s) of *L. gasseri* con grow up to 42°C might be used in industry for high quality production using Transformation technique.

MATERIALS AND METHODS

Bacterial Strains, Plasmid and Growth Conditions:

The bacterial strains and plasmid which used in this work are listed in table (1). *S. thermophillus* strain was grown on 42°C in Elliker broth (Difco Laboractories, Detroit, M1) supplemented with 1% beef extract (Difco) and 1.4% B- glycerophosphate[9]. *L. gasseri* strain was grown on 37°C in MRS. Plasmid DNA was isolated and purified from. *S. thermophillus* as described by O'sullivan and klaen hammer[13] followed by a continuous CsCl gradient.

Genetic Marker Test: Antibiotic susceptibility tests of *Streptococcus thermophillus* and *Lactobacillus gasseri* were performed using disk diffusion method following NCCLS standards. Active both cultures were swabbed onto plates of M17 agar medium and allowed to dry. Antibiotic disks were placed on the agar and the plates were incubated for 24h on 37°C & 42°C

Antibiotic disks including Tetracycline (Tc) 30mg, Chloramphincol (Cm) 30 mg, Erythromycin (Ery) 15 mg, Ampicillin (Am) 20 mg, Gnetamycin (Gnt) 10 mg and Penicillin G (Pn G) 10 U were used in this study (oxide).

Isolation and Purification of Plasmid: Plasmid isolation was done using the windscreen method of Rodriguez and Tait[14].

Bacterial Transformation: *L. gasseri* transformed by CaCl2 transformation methods as described by Rodriguez and Tait[14].

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Table 1: Relevant characteristics of the bacterial strains and plasmid

Strains and Plasmid	Relevant characteristics	Source
Stains:		
Lactobacillus gasseri	Growing on 37°C, Cm^- and G^- (*)	NRC
Streptococcus thermophillus	Growing on 42°C, Cm^+ and G^+ (**)	NRC
Plasmid:	1-Containing the highly thermostable gene (tolerate up to 42°C) and chloramphincol resistance gene (Cm^+)	Isolated from *S. thermophillus*, this study
NiHSP	2-Able to integrate in *L. gassari* genome.	

The isolated plasmid NiHSP from *S. thermophillus* was as a donor, while *L. gasseri* strain was used as a recipient. Transformants were selected in MRS broth containing chloramphincol (cm) and grown to mid log phase at 37°C and 42°C to allow the plasmid to propagate in the cell.

SDS-PAGE Protein Fingerprint: Total protein extractions, protein banding patterns and SDS Polyacrylamide gel electrophoresis was performed according to Sheri *et al.*[17].

RESULTS AND DISCUSSIONS

Genetic Marker: Genetic marker is an important tool for tracing genetic characters through genetic experiments therefore the two strains *L. gassari* and *S. thermophillus* were tested for their sensitivity against five antibiotics (tetracycline, chloramphincol, erythromycin, ampicillin, gentamicin and penicillinG. The antibiotic patterns (resistance and sensitive) are presented in Table (2).

Data in Table (2) revealed that the results clear of the two strains had a same behavior against all the antibiotics except for chloramphincol. Their was different, *S. thermophillus* was resistant (Cm^+) but it was sensitive (Cm^-) for the another strain.

Lykova concluded that the use of stable antibiotic resistant strains of normal microflora was favorable as an addition to antibiotic therapy. Industrially important thermophilli starters for milk fermentation have been reported to carry plasmid DNA. Some plasmids are important for industrial applications

Some researchers have reported that the resistant trait for strains to various antibiotics were located in a high copy number and transferable plasmid DNAs and the plasmids of these strains can be used as cloning vector for Gram-positive bacteria in recombinant DNA technology because[19]. 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 antibiotics may be coded by chromosomal genes, and different plasmids caused resistance to different antibiotics[1].

Transformation of *L. gasseri*: The gene which responsible of thermophelic trait carrying on the plasmids. It contains genes responsible for thermophilic resistance is maintained by the use of antibiotic resistant selection[16].

The transformation efficiencies of NiHSP from *S. themophillus* as a donor to *L. gasseri* as a recipient

Table 2: The antibiotic Susceptibility tests of *Streptococcus thermophillus* and *Lactobacillus gasseri.*

Antibiotics	*S. thermophillus*	*L. gassari*
Amp	++	++
Pn G	-	-
Ery	++	+
Gnt	-	-
Tc	-	-
Cm	++	-
	++	-

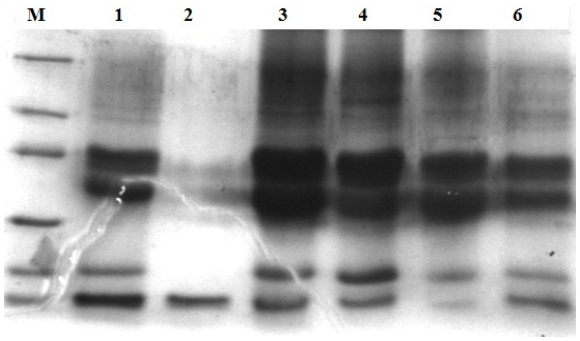

Fig. 1: The SDS - PAGE total Protein Pattern for transformant strains for L. gasseri; T1 (lan 1) , T2 (lan 2), T3 (lan 3), T4 (lan 4), L. gasseri (lan 5), S. thermophillus (lan 6) and (lan M) the marker with molecular weight (14 to 83 KDs).

were 0.6, 0.3 and 0.2% for the total viable cell count when it grow at 10, 20 and 30 mg of chloramphincol, respectively. Successful transformation can be found by growing the obtained transformants on media supplemented with chloramphincol as a selective media and incubated these colonies on 37°C and 42°C. Four transformants (T_1, T_2, T_3 and T_4) which able to grow on supplemented medium with chloramphincol at 42°C were isolated (Table 3).

Table 3: Transformation efficiencies and stability of transformant strains

% of Cm^+	No of Comp.	No of transformant	Trait % of transformant	Stability ratio*	
				37°dC	42°/C
10	640	4	0.6%	98.0	95.0
20	602	2	0.3%	95.8	90.0
30	620	1	0.2%	88.0	77.0

* Stability indicates the ratio of Cm^+ colonies per total viable cell count after cultivation of 210 generation.

Law *et al.,*[8] described an integration strategy of *lactobacillus* that utilizes Pwvol delivered vectors from which the rep a gene has been removed. These "Ori" integration vectors in a desired host strain are to transformation (PMPT). Originally described for *Bacillus subtillis.*[4], describe the method to increase transformation efficiency by recombination between a transforming plasmid vector and homologues resistant plasmid in recipient cells. In 2005, Kohayshi *and* others could isolate a small multi copy cryptic plasmid, PNHKIO1, from *Thermous* sp., they fond that the efficiencies were very low and another copy numbers increased the efficiency.

The segregation stabilities of NiHSP plasmid and its derivatives in strain *L. gasseri* were measured after 10 generations of cultivation. Interestingly, if other plasmid (contain chloramphincol sensitive gene, Cm^-) was existed within the same cells the stabilities of NiHSP was improved.

Wateret *et al.,*[21] and Neu & Henrich[12] discovered the new thermo sensitive delivery vector and its use to enable Nisin-controlled gene expression in *lactobacillus gasseri* and high molecular mass surface supply repA plasmid from a second temperature.

Protein Fingerprinting: Total protein extraction and banding patterns SDS polyacrylamid gel electrophoresis[6] for four transformations; *L. gasseri* T_1, T_2, T_3 and T_4 and their two parents; *L. gasseri* control-1 & *S. thermophillus* control-2 are illustrated in Fig (1). There are observable differences in the protein banding pattern for all four transformants and there control. Some minor differences in banding patterns between the four transformants. Data from Fig (1) also revealed that the total bands number for all the four transformations and their two parents ranged from 8 bands for transformant *L. gasser* T_2 (Lan 2) to 15 bands for the two transformants; *L. gasseri* T_3 (lan 3) and transformant *L. gasseri* T_4 (Lan 4) each. As below, the higher total number of bands was for the two transformants; T_3 (lan 3) and T_4 (Lan 4) were 15 bands each, followed by *L. gasseri* (lan 5) was 14 bands, transformant *L. gasseri* T_1 (lan 1) 13 bands, control-2 *S. thermophllus* (Lan 6) 11 bands and transformant *L. gasseri* T_2 (Lan 2) 8 bands, respectively. The molecular weight of these bands ranged from 14, 4 to 100 KDs. There are common bands found in all strains. The four transformations and the parent *S. thermophillus* have one specific band at molecular weigh 92 KDs. This band of transformant strains and the parent may be referring to the occurred tolerate on 42°C and growing on chloramphincol (Cm^+). These results could be used to distinguish between the transformants and the parent *L. gasseri*. These results are in agreement with Turgeon and Moineau[20].

REFERENCES

1. Aslim, B. and Y. Beeyalti, 2004. Antibioti resistance and plasmid DNA contents of Streptococcus thermophilus strains isolated Turkish yogurts. Turk. JVet Anim, (28): 275-263.
2. Casey, M.G., J. Jimeno, 1989. Lactobacillus delbrucki, subsp. Lactis plasmids. Net.Milt Dariy,; 43: 279-286.
3. Charteris, W.P., P.M. Kelly, L. Morelli, J.k. Collins, 1998. Antibiotic susceptibility of potentially probiotic lactobacillus species, J. Food. Prot., 61: 1636-1643.
4. Cryezan, T., S. Contente, S.D. Dubnau, 1980. Molocular cloning ofheteroloyous chromosomal DNA by recombination between a plasmid vector and, helogus resitent plasmid in Bacillus Subtilis. Mol. Gan. Genet., 177: 459-467.
5. Herman, R.E, L.L. Mckay, 1985. Isolation and partial characterization of plasmid DNA from Streptococcus thermophilus Appl. Environ. Microbial, 50: 1103-1106.
6. Iruela, M., Y, Rubio, J.I. Cubero, J. Gil and Millan, 2002. Phylogeneticanalysis in the genus cicer and cultivated chickpea using RAPD and ISSr markers. Theor Appl. Genet, 104: 643-651.
7. Kobayasshi, H., A. Kuwae, H. Masedo, A. Nakamura and T. Hoshino, 2005. Isolation of low, molecular weight, multi copy plasmid, PwHK101, from thermotick and its use and expression vector for tothemophulus HB_{27}.
8. Law, J., G. Buist, A. Haandrikman, J. Kok, G. Venerma and K. Leenhouts, 1995. A system of generate chromosomal mutation in Lactococcus lactic which allows fast analysis of targeted genes. J. Bacterial, 177: 7011-7018.

9. Le Marrec, C., D. Van Sinderen, L. Walsh, E. Stenley, E. Vlegeles, S. Moineau, P. Heinze, G. Fitzgerald and Fayard, 1997. Two groups of bacteriophage in facting *S. thermophillus* can be distinguish on the bases of made of packaging and genetic determination for major structural proteins. Appl. Environ. Microbial., 63: 3246-3253.
10. Lykova, F.A., 2000. The antibacterial resistance of strains making up the componets of preparation Zh microbial Epidomal Immunobial, 2: 63-66.
11. Murray, P.R., E. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolkan, 1995. Manual of clinical microbiology (Antimicrobial susceptibility tests) 6th ed., ASM, Washington, DC.
12. Neu, T. and B. Henrich, 2003. New thermosensitive delivery vector and its use to enable Nisine-controlled gene expression in *Lactobacillus gassri.* Appl, Environ. Microbial., 69: 137-1382.
13. O'sullivian, D.J. and T.R. Klaenhammer, 1993. Rapid min-prep isolation of high quality plasmid DNA from *L. lactis* and *L. spp.* Environ. Microbial., 59: 2730-2733.
14. Rodriguez, R.L and R.C. Tait, 1983. Recombinant DNA Tehcniques: An introduction the Benjamin / Cummings Puplishing company, Inc, Menlo park, California, reading. London, Ansterdam, Don Mills, pp :37-51.
15. Rush, C.M., A. Mercenier and G. Pozzi, 1997. Expression of vaccine antigens in Latobacillus, P. InG- Pojji and J.M. wells, 107-144.
16. Russell, W.M. and T.R. Klaenhammer, 2001. Efficient system for directed integration into L. Acidophulus and L. Gasseri chromosomes via homologues recombination. Appl. Envirn. Microbiology, 67: 4361-4364.
17. Sheri, L.H., E.S. Micholas, T.K. Michae and B. Joanna, 2000. Comarisson of prtoen expressed by pseudomonas arygines, strain represisting initial and chronic isolted from cystic fibrosis patient: an analysis by 2-Dgel electrophoresis and capillary columen liquid chromatography tandan mass spect romory Microbiology, 146: 2495-2508.
18. Somkuti, G.A. and D.H. Steinberg, 1986. Genral method for plasmid DNA isolation from thermophilic lactic and bacteria, J. Biotech, 3: 323-332.
19. Swenson, J.M., R.R Facklam, C. Thornsberry, 1990. Antimicrobial Susceptibility of vancomycin resistant leuconostoc, pedoccus and lactobacillus species. Antimicrob. Agents chemoter, 34: 543-549.
20. Turgeon, N. and S. Moineau, 2001. Isolation and characterization of Streptococcus thermophillus plasmid closely related to the PMV148 family. Plasmid, 45: 171-183.
21. Walter, J., P. Changnaud, G.W. Tannock, D.M. hoach, F. Dal Ballo, H.F Jankinosn, W.P. Hammes, G. Hertel, 2005. A high molecular mass surface protein (LSP) and methionine sulfoxide reductase B (MrsB) contribute of the ecological performance of Lactobabcillus reuteri in murine Cut. Appl. Environ. Microbial, 71: 797-986.