Expression of Transformed Aheat Tolerate Gene in Lactobacillus gasseri

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Abstract: Two strains streptococcus thermophilus and Lactobacillus gasseri were used in this study and examined for their antibiotic resistance patterns and plasmid carriage. S. thermophilus strain has a plasmid containing the highly thermostable gene (tolerate up to 42°C) and chloramphicol resistance gene (Cm+) which used as a selective marker was used as a donor. This plasmid is able to integrate in L. gasseri genome. L. gasseri strain sensitive of chloramphicol (Cm+) and growing at 37°C was used as a recipient. Transformation technique was used to transfer heat tolerant and chloramphicol resistance (Cm+) plasmid from S. thermophilus to L. gasseri strain. Seven L. gasseri transformants 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[14].

Streptococcus thermophilus is a gram positive bacterium used in manufacture yogurt and specially cheeses and strain improvement through genetic manipulation has considerable potential[10].

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. thermophilus 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[6]. 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[11]. 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. thermophilus strain was grown on 42°C in Elliker broth (Difco Laboractories, Detroit, M1) supplemented with 1% beef extract (Difco) and 1.4% B- glycerophosphate. L. gasseri strain was grown on 37°C in MRS. Plasmid DNA was isolated and purified from S. thermophilus as described by O'sullivan and klaen hammer[11] followed by a continuous CsCl gradient.

Genetic Marker Test: Antibiotic susceptibility tests of Streptococcus thermophilus 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, Chloramphicol (Cm) 30 mg, Erythromycin (Ery) 15 mg, Ampicillin (Am) 20 mg, Gnetamyacin (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

<table>
<thead>
<tr>
<th>Strains and Plasmid</th>
<th>Relevant characteristics</th>
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<tr>
<td>Stains:</td>
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<tr>
<td><em>Lactobacillus gasseri</em></td>
<td>Growing on 37°C, Cm and G&quot;*&quot;</td>
<td>NRC</td>
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<tr>
<td><em>Streptococcus thermophilus</em></td>
<td>Growing on 42°C, Cm and G&quot;**&quot;</td>
<td>NRC</td>
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<td>Plasmid:</td>
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<td>NiHSP</td>
<td>1-Containing the highly thermostable gene (tolerate up to 42°C) and chloramphicol resistance gene (Cm&quot;)</td>
<td>Isolated from <em>S. thermophilus</em>, this study</td>
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<td></td>
<td>2-Able to integrate in <em>L. gasseri</em> genome.</td>
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The isolated plasmid NiHSP from *S. thermophilus* was as a donor, while *L. gasseri* strain was used as a recipient. Transformants were selected in MRS broth containing chloramphicol (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. gasseri* and *S. thermophilus* were tested for their sensitivity against five antibiotics (tetracycline, chloramphicol, 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 chloramphicol. Their was different, *S. thermophilus* 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 thermophillic 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[39]. 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[3].

**Transformation of *L. gasseri*:** The gene which responsible of thermophilic 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. thermophilus* as a donor to *L. gasseri* as a recipient were 0.6, 0.3 and 0.2% for the total viable cell count when it grow at 10, 20 and 30 mg of chloramphicol, respectively. Successful transformation can be found by growing the obtained transformants on media supplemented with chloramphicol as a selective media and incubated these colonies on 37°C and 42°C. Four transformants (T1, T2, T3, T4) which able to grow on supplemented medium with chloramphicol at 42°C were isolated (Table 3).
Law et al. [4] described an integration strategy of *lactobacillus* that utilizes Pwvol delivered vectors from which the repA gene has been removed. These "Ori" integration vectors in a desired host strain are to transformation (PMPT). Originally described for *Bacillus subtilis* [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, PNHKO1, from Thermous sp., they found 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 chloramphinol 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* T1, T2, T3 and T4 and their two parents; *L. gasseri* control-1 & *S. thermophilus* 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. gasseri* T1 (Lan 2) to 15 bands for the two transformants; *L. gasseri* T1 (lan 3) and transformant *L. gasseri* T4 (Lan 4) each. As below, the higher total number of bands was for the two transformants; T1 (lan 3) and T4 (Lan 4) were 15 bands each, followed by *L. gasseri* (lan 5) was 14 bands, transformant *L. gasseri* T1 (lan 1) 13 bands, control-2 *S. thermophilus* (Lan 6) 11 bands and transformant *L. gasseri* T4 (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. thermophilus* 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**


