Beer spoilage bacteria and hop resistance in Lactobacillus brevis

Sakamoto, Kanta

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CHAPTER 3
Electrotransformation of *Lactobacillus brevis*

Kanta Sakamoto

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SUMMARY
The conditions for electrotransformation of five *Lactobacillus brevis* strains lacking *horA* or its homologue were investigated. Two of them were successfully transformed. The highest efficiency was $2.5 \times 10^3$ transformants per µg of DNA (T/µg) for JCM1059 and $5.5 \times 10^2$ T/µg for ABBC45C, a segregant strain lacking pRH45. No transformants were obtained from the other strains.
INTRODUCTION

The horA gene was isolated from a hop resistant strain of Lb. brevis ABBC45 (Sami et al., 1997a). This gene is present on plasmid pRH45, which is amplified during subculturing of the strain in medium containing increasing concentrations of hop compounds. Most beer-spoilage lactobacilli strains harbor the horA gene or its homologue (Sami, 1997b). The deduced amino acid sequence of HorA is very homologous to that of LmrA, a lactococcal ABC-type multidrug transporter (van Veen et al., 1996) which makes HorA a potential player in the hop resistance mechanism.

Attempts to express HorA in Escherichia coli failed due to cell lysis soon after the expression of this protein (Sami, 1999). Also the introduction of a marker gene into pRH45 was unsuccessful possibly due to the instability of plasmid DNA fragments in E. coli (Sami et al., 1997a). Recently Lb. brevis ABBC45C, which spontaneously had lost pRH45, was segregated from the original strain ABBC45 by continuous culturing in the absence of hop compounds (Sami et al., 1998; Suzuki et al., 2002). Re-introduction of pRH45 in ABBC45C or the other strains lacking horA would be extremely helpful for studying the role of this protein in hop resistance.

Successful gene transformation of Lactobacillus spp. has been developed (Chassy and Flickinger, 1987; Luchansky et al., 1988; Hashiba et al., 1990; Aukrust and Blom, 1992; Bhowmik and Steele, 1993; Sasaki et al., 1993; Aukrust et al., 1995; Klein et al., 1995; Berthier et al., 1996; Serror et al., 2002) but successful transformation of Lb. brevis has not yet been reported. In this study the conditions for electrotransformation of Lb. brevis were determined. The developed transformation procedure was used for the re-introduction of pRH45 into ABBC45C (Sami et al., 1998: Chapter 3).

MATERIALS AND METHODS

Bacteria and a plasmid

Lactobacillus brevis type strain JCM1059 was obtained from JCM (Japan Collection of Microorganisms, Saitama, Japan). Lb. brevis ABBC45 and horA-lacking strains 45C, 216, 218 and 241 (Sami et al., 1997b) were from our laboratory culture collection.

The plasmid pGK13, which has both a chloramphenicol resistance gene and an erythromycin resistance gene, was a gift from Dr. J. Kok (University of Groningen, The Netherlands) and prepared from Escherichia coli DH5α.

Preparation of competent cells

The method previously described by Aukrust et al. (1995) was used with some
Cells were cultured anaerobically at 30°C in 100 ml of MRS medium supplemented with 1% (w/v) glycine. At mid-exponential phase (A_{600}= ~0.6), cells were chilled on ice for 10 min and harvested by centrifugation at 1,500 × g for 5 min, washed once with 100 ml of cold 10 mM MgCl₂ and collected by centrifugation at 1,500 × g for 5 min. Subsequently, cells were washed with 100 ml of cold SM solution (952 mM sucrose and 3.5 mM MgCl₂) and harvested by centrifugation at 5,000 × g for 10 min. After repeating this step twice, cells were suspended gently in 1 ml of cold SM solution and used for electroporation.

**Electroporation**

Competent cells (40 µl) prepared as described above were mixed with 0.2 µg of pGK13 and subjected to an electric pulse in a 0.1 cm cuvette by using a Gene Pulser and a Pulse Controller apparatus (Bio-Rad, USA). Immediately MRS-SM medium (MRS containing 0.5 M sucrose and 0.1 M MgCl₂) was added and cells were incubated for 2 h at 30°C before plating on MRS containing 15 µg/ml of chloramphenicol and 5 µg/ml of erythromycin.

**RESULTS**

Among the *Lb. brevis* strains of our bacterial collection, five (ABBC45^C, 216, 218, 241, and the type strain JCM1059) were found to lack horA or its homologue (Sami *et al.*, 1997b). In order to transform successfully pRH45 in these strains, the electroporation procedure for *Lb. brevis* was optimized.

Aukrust *et al.* (1995) reported two different methods for preparing competent cells of lactobacilli. One of them includes a SM solution to wash cells (SM method), while the other includes a polyethylene glycol solution (PEG method). Both methods were applied for *Lb. brevis* ABBC45. The SM method was found to yield 2 to 40 times higher transformation efficiencies than the PEG method (data not shown). The SM method was further optimized in this study. Some strains have extracellular polysaccharides, which can be removed by a washing step with 10 mM MgCl₂ (Aukrust *et al.*, 1992; Berthier *et al.*, 1996). To improve electroporation of competent cells MgCl₂ was therefore included. Due to the high osmolarity of the SM solution some strains did not pellet at 1,500 or 3,000 × g centrifugation for 10 min and centrifugation at 5,000 × g for 10 min was needed for successful pelleting. Parameters of the electrical pulse (capacitance, resistance and voltage) were varied. The highest transformation efficiency of 2.5 × 10³ T/µg for JCM1059 was obtained at 25 µF, 100 or 200 Ω and 2.0 kV (Fig. 1A). The transformation efficiency increased with the capacitance from 0.25 to 25 µF when the other parameters were fixed at 400 Ω and 1.5 kV (data not shown). For ABBC45^C the highest efficiency was 2.9 × 10² T/µg at 25 µF, 200 Ω and 1.5 kV (Fig. 1B). No transformants were obtained for ABBC216, 218 and 241.
DISCUSSION

In this study we demonstrated for the first time successful electrotransformation of *Lb. brevis* strains. The transformation efficiency varied strongly between the different strains. The highest transformation efficiency was in the order of $10^3$ T/µg for JCM1059 and of $10^2$ T/µg for ABBC45C. These values are comparable to those of other *Lactobacillus* spp. reported so far. The type strain of *Lb. brevis* JCM1059 has no plasmid, which makes this a very useful strain for genetic and molecular studies for *Lb. brevis*. However several attempts to transform *Lb. brevis* JCM1059 with pRH45 failed (data not shown). The main reason for this failure can be the large size of plasmid pRH45 (15 kb). Successful electroporation of pRH45 into *Lb. brevis* ABBC45C was achieved and resulted in restoration of hop resistance (Sami et al., 1998, Chapter 3).

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