Purification of genomic DNA from *Lactobacillus* Sp using QuickPick™ SML gDNA kit

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ABSTRACT

Lactobacilli are gram positive, facultative bacteria, which convert lactose and simple sugars to lactic acid. Species belonging to the genus *Lactobacillus* are used specifically in dairy industry.

The QuickPick™ SML gDNA purification kits provide fast and simple means of purifying genomic DNA from different sample materials. The method does not require organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation.

The reagent volumes of the QuickPick™ SML gDNA purification kits can be scaled up or down for different sample amounts either with the PickPen® manual tools or with the MagRo™ 8-M robotic workstation. The size of the purified genomic DNA is typically at least 30 kbp. DNA fragments of this size denature completely during thermal cycling and can be used in downstream applications such as PCR amplifications.

INTRODUCTION

The cell wall of gram-positive bacteria contains a high amount of peptidoglycan making the cell wall more rigid than that of gram-negative bacteria. The release of nucleic acids out of the cell demands that the gram-positive bacteria are treated in harsher conditions either enzymatically or by mechanical grinding. In this study, cells of *Lactobacillus* Sp were treated with lysozyme enzyme. Lysozyme binds on bacterial surface and attacks to peptidoglycans.

An efficient lysozyme treatment for *Lactobacillus* Sp cell disruption is presented followed by the purification of genomic DNA with QuickPick™ SML gDNA kit and MagRo™ 8-M robotic workstation.

PRINCIPLE OF THE QuickPick™ SML gDNA KIT

After lysozyme treatment step the genomic DNA in the sample is released using Proteinase K and Lysis Buffer. Released DNA is bound to the Magnetic Particles in the presence of Binding Buffer. Magnetic Particles with the bound DNA are washed with the Wash Buffer 1 and 2. The DNA is then eluted from Magnetic Particles with the Elution Buffer.

MATERIALS & METHODS

About 10 million cells of *Lactobacillus* Sp were dispensed into parallel six wells of a 96-well microplate and suspended into 50 µl of Lysozyme Buffer (20 mM Tris-HCl, pH 8; 2 mM EDTA; 1.2 % Triton X-100 and 20 mg/ml Lysozyme). The plate was transferred on the deck of the MagRo™ 8-M.

During the MagRo™ 8-M process the samples in the 96-well microplate are incubated at +37°C for an hour to make the rigid cell walls susceptible to lysis (lysozyme treatment). After the incubation 50 µl of Lysis Buffer and 10 µl of Proteinase K are added. Samples are lysed at +56°C for 30 minutes on the thermal shaker. After lysis step 260 µl of Magnetic Particle/Binding Buffer mix is dispensed into lysates followed by incubation for 10 minutes at room temperature. After binding magnetic particles are collected and washed two times in Wash Buffer 1 and once in Wash Buffer 2. Magnetic Particles with bound DNA are transferred into Elution Buffer (50 µl) and incubated at room temperature for 5 minutes. Finally, the Magnetic Particles are collected from the eluates and discarded. The samples are analysed by spectrophotometer and agarose gel electrophoresis. PCR amplification is carried out using purified genomic DNA as a template.

RESULTS

Genomic DNA purification of six parallel samples from gram-positive bacteria, *Lactobacillus* Sp was carried out by MagRo™ 8-M robotic workstation by using QuickPick™ SML gDNA purification kit. Before genomic DNA purification samples were treated with lysozyme enzyme to break the cell walls.

Yields and purities of the purified genomic DNAs were measured by spectrophotometer (Table 1).

Table 1: Yields and purities of the genomic DNA from gram positive *Lactobacillus* Sp, purified with QuickPick™ SML gDNA kit and MagRo™ 8-M.

No of replicate	Purity (260 nm/280 nm)	Concentration (µg/ml)	Yield (µg)
1	1.9	19	1.9
2	1.9	23	2.3
3	1.7	23	2.3
4	1.8	17	1.7
5	1.8	24	2.4
6	1.9	24	2.4

The average yield between six replicates was 2.1 µg ± 0.3 µg. The purities of all replicates were higher than 1.7, which clearly demonstrates that the purified genomic DNA is of high quality. The integrity of the purified genomic DNA was also analyzed by agarose gel electrophoresis (Fig.1).

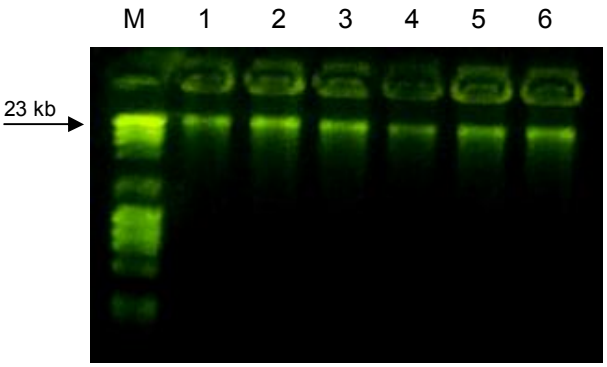

Figure 1: Agarose gel electrophoresis (1% agarose gel) analysis of the genomic DNA purified from *Lactobacillus* Sp. with QuickPick™ SML gDNA kit and MagRo™ 8-M. M = molecular size standard (λ DNA / *Hin*dIII); 1-6 = six replicates of genomic DNA eluates.

Intact high molecular weight DNA was detected from all samples. This confirms that the purified genomic DNA from gram-positive *Lactobacillus* Sp is of high-quality and can be used in downstream applications.

The genomic DNA was used as template for PCR utilizing primers specific for the 16S/23S ribosomal RNA intergenic spacer region (Dubernet et al., 2002) of *Lactobacillus* Sp (Fiq. 2). The amplified PCR products were of expected size (about 250 bp). The results demonstrate that the purity of the purified genomic DNA is sufficiently high for a sensitive PCR analysis.

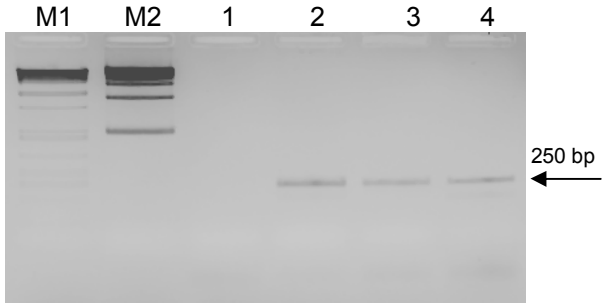

Figure 2: PCR amplification results from purified DNA, where primers specific for lactobacilli were used. M1 = molecular size standard (I PstI); M2 = molecular size standard (1 kb DNA-ladder); 1 = negative control; 2 – 3 = Amplified PCR products; 4 = positive control.

The accomplishment of the PCR amplification also confirms the integrity of the purified genomic DNA.

CONCLUSION

According to the results, the QuickPick™ SML gDNA kit, combined with lysozyme treatment, generates high-quality genomic DNA as purified from gram-positive *Lactobacillus* Sp. Purified DNA can be used directly as template for PCR amplification.

The QuickPick™ SML gDNA purification kit provides a fast and simple genomic DNA purification method from different sample materials and can be applied to both manual PickPen® tools and the automated MagRo™ 8-M robotic workstation.

REFERENCE

Dubernet, S., Desmasures, N. and Gueguen, M. (2002) A PCR-based method for identification of lactobacilli at the genus level. *FEMS Microbiology Letters* 214, 271-275.