Probiotic DNA Extraction of *Bifidobacterium lactis* and *Lactobacillus fermentum* via High Osmotic Dual- Bead Beating Technique

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Abstract

DNA extraction of probiotic *Bifidobacterium lactis*, and *Lactobacillus fermentum* were subjected to a novel dual-sized bead extraction approach. Compartmentalizing the bead-beating chamber increased mechanical compression forces on gram-positive bacteria to overcome the high-strength peptidoglycan cell wall. 100-Micron glass powder ensures effective capture and compression of bacteria in solution with additional 3mm glass beads aiding in powder compaction during bead-beating.

This technique is further enhanced by increasing osmotic pressure through 100mM sodium phosphate and thermal heating of the sample, which yielded greater results over commercially available kits. This novel technique is universally applicable to both gram-positive and gram-negative bacteria for a reliable, and economical DNA extraction by avoiding the necessary use of costly enzymatic reagents.

Application of this technique was successful in producing adequate amounts of double-stranded DNA from *Bifidobacterium lactis* and *Lactobacillus fermentum* for downstream molecular assays.

Introduction

DNA extraction from gram-positive bacteria has proven to be difficult due to varying densities of the peptidoglycan cell wall in different bacteria. Studies into efficient DNA extractions for *Lactobacillus casei* from Alimolaei et al [1], show an industrial-wide difficulty in searching for an efficient method of gram-positive DNA extraction. A quick, economical, efficient and reproducible DNA extraction method is essential to modern molecular assays. Current commercial microbial DNA extraction methods require enzymatic lysis which do not always produce reliable and consistent results as was previously encountered with a lysozyme based extraction method. Adequate amounts of extracted DNA template was extremely difficult to obtain for required identification of probiotic raw materials. Our approach, is to provide a reliable DNA extraction method using a three-stage approach using dual-sized beads to aid in microbial capture and compression. The method utilizes high osmotic pressure and mechanical stress on the cell wall which increases the overall chances of cellular fracture.

This proposed method avoids costly enzymes, lengthy incubation, and limited shelf-life for extracting DNA from microbial samples.

Materials and Methods

Materials: Microbiologics *Bifidobacterium lactis* and *Lactobacillus fermentum*. Hardy Diagnostics Lactobacilli MRS Agar, Anaerobe Systems Bifido Agar, 100mM Sodium Phosphate with EDTA, Tween-20 pH 7.4, TE Buffer w/ 10mM Tris, 1mM EDTA, pH 8.0 DNase/RNase Free, Sterile PCR Grade Water, Invitrogen Qubit Buffer, Invitrogen Qubit dsDNA HS Reagent 200x, MasterPure Gram Positive DNA Purification Kit

Equipment: 35°C Incubator, Invitrogen Qubit 3 Fluorometer, Anaerobic GasPak/Chamber, Eppendorf Centrifuge, MiniG 1600 Homoginizer, Sigma-Aldrich 100 Micron Acid-washed Glass beads/powder, Pyrex 3mm Solid Glass beads, Sarstdet gasketed tubes, Qubit 3 Fluorometer, Amicon 0.22µm GV Durapore Centrifuge Filter, Amicon Ultra 0.5mL Ultracel 30K Spin Column, Amicon Ultra Capture Tubes

Probiotic reference organisms were grown under anaerobic conditions at 35°C for 2 days. A 1000µl 2.0 McFarland suspension was made for each organism to standardize the approximate cfu/ml comparison. Cultures were then centrifuged to pellet the bacteria, and liquid suspension reduced to 400µl volume. Both organisms were transferred to Sarstedt tubes containing 100 micron glass bead powder and 3mm glass beads.

Samples were subjected to incubation at 100°C for 5 minutes. Sarstedt tubes were then immediately placed in the homogenizer and allowed to bead-beat for 1 minute at 1500 rpm. Tubes were briefly spun down to accumulate glass powder and beads at the bottom of the tube. A lysate volume of 400µl were then transferred to 0.22GV Durapore centrifuge column to filter out larger cellular components. Filtrates were then transferred to Amicon Ultracel 30K centrifugal filters and centrifuged until fully filtered. Amicon filters were transferred to new collection tubes for DNA purification. Purification of the Amicon Ultracel filters consisted of 3 washes of the spin column with TE buffer. A 400µl aliquot was transferred to the Ultracel filter, in which 100µl of filtrate was saved before a final water wash with 400µl of PCR grade water. Ultracels filters were inverted into collection tubes and centrifuged for DNA extract capture.

DNA concentration was measured on the Invitrogen Qubit 3 Fluorometer using the dsDNA High Sensitivity kit.

The MasterPure gram-positive extraction and purification kit was used to compare DNA recovery. Manufacturer protocol was followed using a standardized 2.0 McFarland solution. DNA extract was immediately frozen prior to quantification.

Results

DNA extraction methods were analyzed via Invitrogen Qubit 3 Fluorometer using high sensitivity dsDNA assay. Dual-bead extraction showed increased DNA yield over commercially lysozyme-based kits across both organisms and at ambient and elevated temperatures. Dual-bead heated extraction on Lactobacillus fermentum resulted in over-saturation of the Qubit 3 Fluorometer requiring extracts for L.fermentum to be diluted to a 1:10 ratio for DNA concentration analysis.

DNA extraction via bead-beating and spin column filtration resulted in PCR ready extracts in approximately 2 hours versus overnight incubation of lysozyme-based kits for adequate DNA products.

Probiotic organism	Commerically Available Kits	
	Prepman Ultra Gram Negative Sample Prep Reagent	Lucigen MasterPure Gram Positive Kit
Bifidobacterium lactis	0.0128 ng / µL	1.95 ng / µL
Lactobacillus fermentum	0.324 ng / µL	11 ng / µL
Probiotic organism	**Dual-Bead Extraction Technique**	
	22°C Dual Bead-Beating Technique	**100°C Dual Bead-Beating Technique**
Bifidobacterium lactis	0.632 ng / µL	6.1 ng / µL
Lactobacillus fermentum	11.2 ng / µL	460.4 ng / µL

Table 1: Tabular data of DNA concentrations by extraction method.

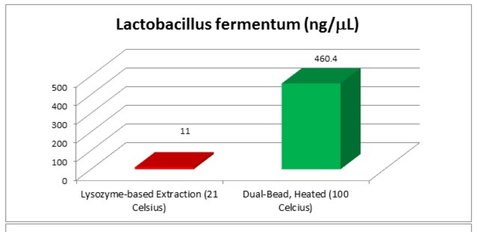

Figure 1: Relative DNA concentrations released into solution via dual-bead-beating protocol and commercially available kits.

Discussion

By using the compartmenalized dual-bead technique, the combining effects of osmotic, thermal, and mechanical methods on gram-positive bacteria, increased amounts of genomic DNA were observed via Qubit data. Employing this new dual-bead technique, we have successfully analyzed that the DNA yields were greatly improved over the lysozyme-based cellular lysis for these probiotic organisms. This may be attributed to in part by the low ratio of enzyme reagent to probiotic bacteria and also to the diminishing lysozyme enzyme as it is incorporated into the gram-positive cell wall during the lengthy incubation stage.

Additionally, increased efficiency in lysis was due in part to heating the probiotic bacterium with the dual-glass bead mixture in sodium phosphate. Increased DNA released into solution was observed when compared to the ambient bead-beating sample preparation. A significantly greater output of DNA from *L.fermentum* can be attributed to the weaker cell wall when compared to *B.lactis*.

Economical savings can be seen in avoiding the need to store enzymatic lysis kits with a limited shelf life.

Conclusion

The unique combination of high osmotic pressure, heat, and mechanical disruption can yield higher concentrations of liberated DNA for virtually the same amount of bacterial concentration than from the tested commercial kits. Centrifugal spin column purification aided in concentrating and purification of the desired DNA extract.

The unique combination of 3mm glass-beads and 100 Micron bead powder benefit mechanical extraction by assuring microbial compartmentalization and assuring mechanical compression during the lysing procedure. By adopting this relatively economical method for DNA extraction of probiotics *B.lactis*, and *L. fermentum*, greater yields of DNA are possible by avoiding the use of costly enzymes. Additionally, the dual-bead extraction technique is experimentally easier to conduct than the commercial multistage enzymatic kits. The osmotic pressure, thermal, and mechanical sheering of the glass beads and powder offers endless cycles of cellular lysing capabilities.

References

[1] Mojtaba Alimolaei and Mehdi Golchin. "An Efficient DNA Extraction Method for Lactobacillus casei, a Difficult-to-Lyse Bacterium". In: *International Journal of Enteric Pathogens* 4.1 (Mar. 2016).