Systematic and Applied Microbiology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Comparison of DNA extraction methods for human gut microbial community profiling

Mi Young Lim[a,*,1], Eun-Ji Song[a,b,1], Sang Ho Kim[c], Jangwon Lee[c], Young-Do Nam[a,b,*]

[a] Research Group of Gut Microbiome, Division of Nutrition and Metabolism Research, Korea Food Research Institute, Jeollabuk-do 55365, Republic of Korea
[b] Department of Food Biotechnology, Korea University of Science and Technology, Daejeon 34113, Republic of Korea
[c] IVD Business Unit, SK Telecom, Seoul 04539, Republic of Korea

ARTICLE INFO

Article history:
Received 4 September 2017
Received in revised form
13 November 2017
Accepted 13 November 2017

Keywords:
Human fecal sample
Gut microbiota
DNA extraction
Bead beating
16S rRNA gene sequencing

ABSTRACT

The human gut harbors a vast range of microbes that have significant impact on health and disease. Therefore, gut microbiome profiling holds promise for use in early diagnosis and precision medicine development. Accurate profiling of the highly complex gut microbiome requires DNA extraction methods that provide sufficient coverage of the original community as well as adequate quality and quantity. We tested nine different DNA extraction methods using three commercial kits (TianLong Stool DNA/RNA Extraction Kit (TS), QIAamp DNA Stool Mini Kit (QS), and QIAamp PowerFecal DNA Kit (QP)) with or without additional bead-beating step using manual or automated methods and compared them in terms of DNA extraction ability from human fecal sample. All methods produced DNA in sufficient concentration and quality for use in sequencing, and the samples were clustered according to the DNA extraction method. Inclusion of bead-beating step especially resulted in higher degrees of microbial diversity and had the greatest effect on gut microbiome composition. Among the samples subjected to bead-beating method, TS kit samples were more similar to QP kit samples than QS kit samples. Our results emphasize the importance of mechanical disruption step for a more comprehensive profiling of the human gut microbiome.

Introduction

The human gastrointestinal tract harbors a diverse microbial community that performs important functions in health and disease. Advances in high-throughput sequencing technology have enabled researchers to discover shifts in gut microbial composition and function that are significantly associated with various disorders or diseases, such as obesity [24], type 2 diabetes [19], and inflammatory bowel diseases [15]. In addition, population-level metagenomics analysis has been performed in order to understand better the normal gut microbiome and its associations with various exogenous and intrinsic host factors [6,30]. Recent studies have suggested that microbiome profiles can be used not only for early diagnosis and prognosis of disease, but also for personalized treatment selection in daily medical practice [20].

The accuracy of microbiome data depends on how well the DNA is extracted from the gut bacteria, so that it accurately reflects the composition of the actual gut microbial community. The MetaHIT project, which is based on metagenomic shotgun sequencing, estimated that each person carries at least 160 species at any one time and that the entire European cohort harbors a total of 1000–1150 bacterial species [18]. To profile this complex human gut microbial community using 16S rRNA gene sequencing or metagenomic shotgun sequencing, it is critical to extract efficiently the DNA of all bacterial species in the community.

Human microbiome researchers have employed various DNA extraction methods [6,8,13,30], although the International Human Microbiome Standards project (IHMS; http://www.microbiome-standards.org) has suggested standard operating procedures for DNA extraction from human fecal samples [22]. This is necessary because numerous commercial fecal microbiome DNA extraction kits are available and more are continually being developed. Commercial DNA extraction kits differ in their lysis methods (e.g. mechanical, chemical, enzymatic, and heat) and specific DNA

* Corresponding authors at: Research Group of Gut Microbiome, Division of Nutrition and Metabolism Research, Korea Food Research Institute, Jeollabuk-do 55365, Republic of Korea.
E-mail addresses: mylim@kfri.re.kr (M.Y. Lim), youngdo98@kfri.re.kr (Y.-D. Nam).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.syapm.2017.11.008

isolation methods (e.g. magnetic beads and spin column-based methods). In addition, diverse platforms compatible with the commercial kits for automated DNA extraction are commonly used.

Studies of DNA extraction methods for human microbiome research indicate that the use of different DNA extraction kits can generate different microbial profiles for the same fecal sample, although the variation between different DNA extraction methods was shown in most cases to be no larger than the inter-individual variation [9,26]. One of the major factors in the observed differences was whether or not the kit protocol included mechanical disruption by bead beating in the cell lysis step [1,11,28]. Bead beating improves bacterial DNA recovery by effectively lysing not only Gram-negative but also Gram-positive bacteria, which have a thick cell wall [5,21]. Modifications to the original extraction kit protocols, such as addition of bead beating, are thus not uncommon [3,25]. Since no clear consensus regarding the most effective DNA extraction method has been reached, and DNA extraction techniques are constantly advancing, it is important to compare newly developed DNA extraction methods with established procedures.

Therefore, this study compared a new DNA extraction kit, the TianLong Stool DNA/RNA Extraction Kit (TS), with two widely used kits, the QIAamp DNA Stool Mini Kit (QS), and QIAamp PowerFecal DNA Kit (QP), formerly known as the "MO BIO's PowerFecal DNA Isolation Kit." A human fecal sample collected in OMNIgene-GUT tubes, which temporarily preserved stabilized DNA at room temperature, was used to evaluate the DNA extraction methods. Since, unlike the QP kit, the TS and QS kits do not include a mechanical pre-treatment step, the latter two kits were also compared with or without an additional bead-beating step. In addition, since the TS kit was originally developed to be used with an automated instrument, the manual and automated methods recommended for the TS and QS kits were compared.

Materials and methods

Fecal sample collection

Fecal samples were obtained from participants in the Korean gut microbiome project. This study was approved by the Institutional Review Board of Daegu Korean Medicine Hospital, which is affiliated with Daegu Haany University (IRB No: DHUMC-D-16005-PRO-03). Informed consent had been obtained from the participants. A fecal sample from one of the participants was collected in four OMNIgene-GUT tubes (DNA Genotek, Ontario, Canada) and homogenized for 30 s by vigorous shaking. These samples were preserved in the OMNIgene-GUT stabilizing buffer and stored at room temperature for 1 week. The samples were pooled, and the pooled sample was used for all the DNA extraction methods.

DNA extraction

Three DNA extraction kits were used in this study: the TianLong Stool DNA/RNA Extraction Kit (TS; Xi'an TianLong Science and Technology Co., Ltd., Xi'an, China), QIAamp DNA Stool mini kit (QS; Qiagen, Hilden, Germany), and QIAamp PowerFecal DNA Isolation kit (QP; Qiagen, Hilden, Germany). For the TS and QS kits, DNA extraction was performed with or without an additional bead-beating step using manual or automated methods. For the QP kit, DNA extraction was performed by one method according to the manufacturer's instructions. Therefore, nine different methods were employed in the present study – one method for the QP kit and four methods for each of the TS and QS kits. For each method, three aliquots of the fecal sample were purified, resulting in a total of 27 DNA samples for high-throughput sequencing (Supplementary Fig. S1). The concentration and quality of the extracted nucleic acids were measured spectrophotometrically using a NanoDrop™ ND-1000 spectrophotometer (NanoDrop Technologies Inc., DE, USA) and the integrity of genomic DNA was evaluated by visualizing the extracted DNA in a 1% (w/v) agarose gel through electrophoresis. The extracted DNA samples were stored at −20 °C until library preparation and sequencing.

TianLong stool DNA/RNA extraction kit

For the methods including a bead-beating step, 250 μL of the fecal samples were transferred to a 2 mL tube containing 0.3 g of sterile 0.1 mm zirconia beads (BioSpec, OK, USA) and 600 μL of pretreatment solution 1. The samples were mixed by vortexing, and subsequently heated at 95 °C for 20 min. The samples were homogenized twice by bead beating with the MO BIO vortex adapter at maximum speed for 1 min each with a 5 min rest on ice in between, and then centrifuged. A 500 μL aliquot of supernatant was transferred to a new tube containing 200 μL of pretreatment solution 2, heated at 95 °C for 5 min, and centrifuged. A 500 μL aliquot of the supernatant was used in the subsequent purification steps. For the methods without a bead-beating step, 250 μL of the fecal samples were transferred to a 2 mL tube containing 600 μL of pretreatment solution 1, and then processed as described above with the exception of the beat-beating step.

For the manual extraction method, a 500 μL aliquot of supernatant was mixed with 500 μL lysis buffer, 30 μL of magnetic beads, and 10 μL proteinase K (included in the TS kit) by vortexing for 5 min. The mixture was placed on a magnetic station for 90 s, and the supernatant was removed. The magnetic beads were washed sequentially with 750 μL of W1 buffer, W2 buffer, and W3 buffer. Finally, DNA was eluted from the beads by incubation with 200 μL elution buffer. For the automated method, a 500 μL aliquot of supernatant was transferred into a well containing 500 μL lysis buffer in a purification plate, and 10 μL proteinase K were added to the wells. The sample mixture was processed using the NP968 Nucleic Acid Extraction System (Xi'an TianLong Science and Technology Co., Ltd., Xi'an, China) with a stool DNA extraction method program. The final elution volume was set to 200 μL.

QIAamp DNA stool mini kit

For the methods including a bead-beating step, 250 μL of the fecal samples were transferred to a 2 mL tube containing 0.3 g sterile 0.1 mm zirconia beads (BioSpec, OK, USA) and 1.2 mL ASL lysis buffer. The samples were mixed by vortexing, and subsequently heated at 95 °C for 15 min. The samples were subjected twice to bead beating using the Qiagen TissueLyser II at a frequency of 30 Hz for 1 min each with a 5 min rest on ice in between, and then centrifuged. A 1.2 mL aliquot of the supernatant was transferred to a new tube, vortexed with an InhibitEX tablet, and centrifuged. The supernatant was transferred to a new sample tube and used in the subsequent purification steps. For the methods without a bead-beating step, 250 μL of fecal samples were transferred to a 2 mL tube containing 1.2 mL ASL lysis buffer and then processed according to the manufacturer's instructions.

For the manual extraction method, the remaining steps were performed as per the manufacturer's instructions. For the automated method, the remaining steps were carried out using a QIAcube system (Qiagen, Hilden, Germany). In both cases, DNA was eluted in 200 μL AE buffer.

QIAamp PowerFecal DNA kit

A 250 μL aliquot of the fecal sample was transferred to a Dry Bead Tube provided in the kit, and subsequent steps were performed according to the manufacturer's instructions. The bead-beating step was performed with the MO BIO vortex adapter. DNA was eluted in 100 μL C6 elution buffer solution.

Please cite this article in press as: M.Y. Lim, et al., Comparison of DNA extraction methods for human gut microbial community profiling, Syst. Appl. Microbiol. (2017), https://doi.org/10.1016/j.syapm.2017.11.008