Biological Forum – An International Journal **7**(2): 549-553(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Comparison study on effect of different methods on DNA extraction of *Methanobrevibacter smithii*

Shaghayegh Baradaran Ghavami*, Abbas Akhavan Sephay**, Hamid Asadzadeh Aghdaei, Taher Nejad Sattari**** and Mohammad Reza Zali********

**Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
***Department of Microbiology, Faculty of Biological Science,*
Islamic Azad University, North- Tehran Branch, Islamic Azad University, Tehran, Iran.
****Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*****Associate Professor Department of Microbiology,*
Science and Research Branch, Islamic Azad University, Tehran, Iran
******Professor Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of medical Sciences, Tehran, Iran*

(Corresponding author: Hamid Asadzadeh Aghdaei)
(Received 22 June, 2015, Accepted 13 September, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The aim of the current study was to investigate and compare effect of 3 different methods on extraction of *Methanobrevibacter smithii* DNA in human feces. Fecal samples were obtained form 21person don't use antibiotic during four week ago and randomly divided into 3 experimental group (n=7) and stored at -70°C until DNA extraction. In the first method microbial DNA was extract using boiling technique. In the second technique, microbial DNA was obtained using repeated bead-beating (RBB). In the third method, microbial DNA was extract using QIAamp DNA Stool Mini Kit (Qiagen, Germany). All DNA extractions were done using real-Time PCR. According to the data, commercial detecting kit was not useful method to detect *M. smithii* DNA (P>0.05). Also, there was no significant difference among boiling and RBB method on isolation of DNA of *M. smithii* from the feces (P>0.05) but both techniques were useful than kit (P<0.05). These results suggest, based on advantage to boiling method *e.g.* easy to use and cheaper than RBB, this method can use as an alternative technique compared to RBB for isolation of Archaea DNA.

Keywords: Boiling, Repeated bead-beating, Kit, DNA Extraction, Archaea

INTRODUCTION

Archaea are ancient and extremophiles microorganism which can find in various environment such as oceans, sewage, oil wells and soils (Dridi, Raoult *et al.* 2011). Archaea that survive in extreme condition were believed to be the most primitive on earth (Dridi, Raoult *et al.* 2011). In the past it was assumed that the Archaea and Bacteria were both in the same domain. However, detection of 16srRNA sequence illustrated that Archaea is a separate independent domain (Chaban and Hill 2012) because of Archaea and bacteria have similar characteristic including size and shape (Eckburg, Lepp *et al.* 2003, Dridi, Raoult *et al.* 2011). But most of features are more closely related to eukaryotes such as genetic and metabolic pathways. Also, Archaea possess unique flagellins and ether-linked lipids, lack murine in their cell walls thus, archaeal membranes are rigid and resistant in strict conditions. As important roles of Archaea are biochemical reaction and biodegradation of organic compounds.

Archaea that have adapted to extreme environment but they recently detected in the human and animal digestive tracts (Miller, Wolin *et al.* 1982, Zhang, Banaszak *et al.* 2009) and have syntrophy with commensal bacteria. Methanogens are the importance of the Archaea to human and animalgut. Methanogenic Archaea belonging to the Euryarchaeota phylum (Buriánková, Brablcová *et al.* 2013). Methanogens use hydrogen to produce methane. In other words, methanogens can convert CO_2 and H_2 or formate to CH_4 (Miyamoto 1997, Buriánková, Brablcová *et al.* 2013). Methanogens have highly divers and over 50 species (Tabatabaei, Rahim *et al.* 2010) including *Methanobrevibacter ruminatum* in the rumen of ruminant species e.g. cattle and sheep.

Methanobrevibacter smithii (*M. smithii*) isolated mostly in human gut and vagina (Belay, Mukhopadhyay *et al.* 1990, Aminov 2013), *M. stadtmaniae* found often in the gut (Miller and Wolin 1985) and *M. oralisin* human Oral cavity(Ferrari, Brusa *et al.* 1994).

It has been hypothesized that methanogen Archaea may play an important role in different diseases such as inflammatory bowel disease, irritable bowel syndrome, Colorectal cancer, diverticulosis, as well as obesity(Macfarlane and Gibson 1997, Scanlan, Shanahan *et al.* 2008, Horz and Conrads 2010, Mathur, Kim *et al.* 2013). Previously, it is showed lactulose breath testing significantly higher between IBS patients with constipation phenotype (Pimentel, Mayer *et al.* 2003); (Kim, Deepinder *et al.* 2012). In contrast to IBS the prevalence of methane positivity was very low among patients with Crohn's disease or Ulcerative colitis (Chatterjee, Park *et al.* 2007, Pimentel, Gunsalus *et al.* 2012).

Polymerase chain reaction (PCR) was used as a suitable tool for the characterization of Archaea strains in different studies. The success in PCR depends on the quantity and quality of the extracted DNA. Various sample preparation methods have been developed for PCR assays. Many strategies currently used for the extraction of DNA from bacterial cells, such as enzymatic, chemical or thermal lysis, mechanical disruption of the cell wall by beads, sonication and go third (Ahmed *et al.* 2014). It is reported, boiling method, is the most time and cost effectiveness method, achieved the thickest bands in the PCR following grinder, crushing, freeze-thaw and glass beads (Sharbatkhori *et al.* 2009). Our aim was to find a quick, easy to perform and cost effective DNA extraction method for application in any lab that commercial DNA extraction kits are not easily available. To the best of our knowledge, until now, there is no report on detection of *M. smithii* in human gut. According to the important role on human life, our hypothesis was to isolation of *M. smithii* of Iranian.

MATERIALS AND METHODS

A. Fecal samples

Fecal samples were obtained form 21person don't use antibiotic during four week ago and randomly divided into 3 experimental group (n = 7) and stored at -70°C until DNA extraction for *M. smithii.* Research Committee and informed, written consent was obtained from each subject samples.

B. Pre-treatment of fecal sample by boiling DNA extraction protocol

Three different methods were used for the DNA extraction and all methods employed mechanical cell disruption. The First method was based on the more DNA release of Archaea by boiling as pre-treatment protocol. Samples were thawed on ice and homogenized then 250 µg of stool was weighted in to a 2 mL screw-cap tube and mixed with 1mL of lysis buffer used in the RBB-step was 4% (W/V) sodium dodecyl sulphate (SDS), 50mM Tris-HCL, 500mM NaCl and 50mM EDTA. The samples vortex continuously for 1mL and boiling for 15 min, 1mLlysis Inhibitor E1 buffer kite added and centrifuged at 4°C for 5 min at full following at full speed and supernatant was collected.

C. Pre-treatment of fecal sample by repeated bead-beating (RBB) DNA extraction protocol

The second method was RBB method. The RBB method has been done previously and they could extract Archaea DNA with high efficiency. Briefly, fecal samples were melted on ice and 250µg of feces was weighted in to 2mL screw-cap tube and added 1mL of lysis buffer (SDS, Salt and EDTA) was described completely above. Four glass-beads (3.0mm) and 0.5 of silica beada and homogenized with two rounds, duration time of homogenized was 3 min in both RBB rounds.

D. Pre-treatment of fecal sample by Qiagen kit

Latest method of DNA Archaea extraction was done using QIAamp DNA Stool Mini Kit (Qiagen, Germany). DNA was extracted by the QIAamp DNA Stool Mini Kit with standard reagent and protocols provided by manufacturer (Qiagen, Germany). Briefly, 250µg of stool was weighted and was mixed 1mL Inhibit EX buffer lysis, centrifuged for 1 min,10 Ml of proteinase K added and kept for 15 min in 70°C. The DNA extraction method was following the kit manufacturer instructions for (QIAamp DNA Stool Mini Kit; Qiagen, Germany). All DNA extractions were done using real-Time PCR.

E. Measurement of DNA concentration

DNA yield was measured with a spectrophotometer. The DNA extractions were stored at -20°C until use.

F. Quantitative PCR

Quantitative PCR analysis of *M. smithii* rpoB gene was performed using ABI7500 Real time PCR system (Applied Biosystems Foster city, CA, USA) in a 96-well plate by using SYBER Green chemistry(SYBR Green PCR Master mix, TaKaRa, Japan). We selected specific primers for *Mbb. smithii* quantification rpoB F (AAGGGATTTGCACCCAACAC) and rpoB R (GACCACAGTTAGGACCCTCTGG) and optimized amplification protocol were designed (Dridi, B., *et al* 2009, Kim, G., *et al* 2012).

Then, the synthesized gene has cloned in PUC57 plasmid (Generay company, South Korea). Ten fold dilution series were made and used to create standard curves in quantitative PCR runs.

G. Statistical and data analysis

Data were presented by frequency (percent), and mean ± standard deviation was used for continuous data. Data were analyzed by Chi-square, and if needed Exact Fisher's Test using SPSS ver21, P 0.05 was set as significance level. Real time analysis was performed by SDS software version 1.3 (Applied Biosystems-USA). Comparison of Bacterial performed using Mann-whitney U Test.

RESULTS

Comparison effect of different DNA extraction methods on Archaea load isolated from human feces is presented in figure 1. According to the data, commercial detecting kit was not able to detect Archaea DNA human feces (P>0.05). Also, there was no significant difference among boiling and RBB method on isolation of DNA of *M. smithii* from the feces (P>0.05) but both techniques were useful than kit (P<0.05).

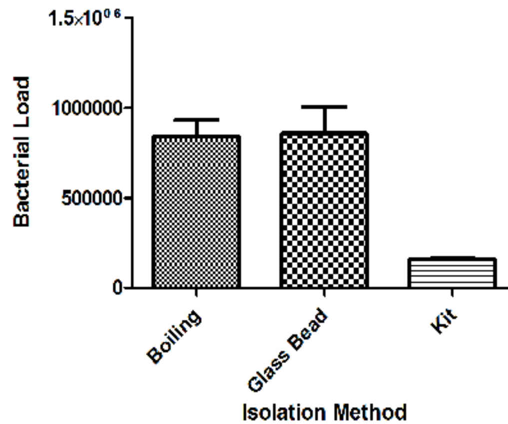

Fig. 1. Comparison effect of different DNA extraction methods on Archaea load isolated from human feces (n = 7). Kit: Qiagen kit.

Table 2: DNA OD (260/280 nm) and bacterial load of Archaea using three different method.

Methods	Pre-treatment	DNA OD 260/280 nm
Boling	yes	$8.40*10^6$ gene copies per reaction
RBB	yes	$8.58*10^6$ gene copies per reaction
Qiagen kit	No	$1.58*10^6$ gene copies per reaction

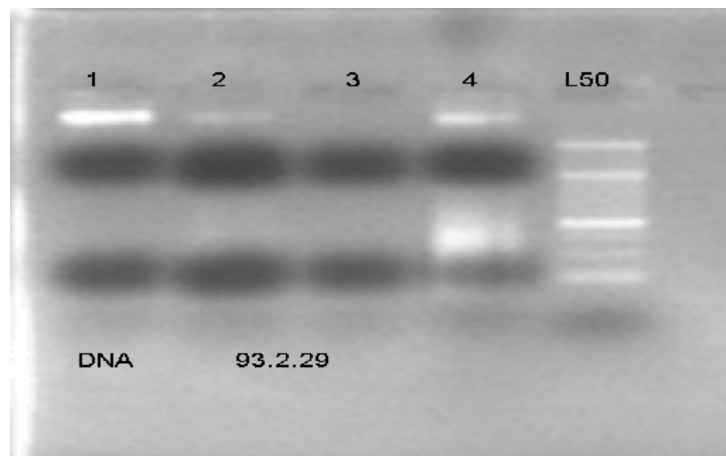

Fig. 2. Result of real-Time PCR of Archaea DNA. 1: boiling, 2 RBB, 3:negative control, 4:Qiagen kit.

DISCUSSION

In this study we used three different methods, to perform DNA extractions of Archaea from fecal samples. As seen from the results boiling and glass bead had parallel and better results than commercial detecting kit. However, in comparison among boiling and glass bead techniques, the second one is much more expensive, time consuming and needs laboratory skill. PCR was used as a suitable tool for the characterization of Archaea strains in different studies. The success in PCR depends on the quantity and quality of the extracted DNA. Various sample preparation methods have been developed for PCR assays. It is reported, boiling method, is the most time and cost effectiveness method, achieved the thickest bands in the PCR following grinder, crushing, freeze-thaw and glass beads (Sharbatkhori *et al.* 2009).

Many strategies currently used for the extraction of DNA from bacterial cells, such as enzymatic, chemical or thermal lysis, mechanical disruption of the cell wall by beads, sonication and go third (Ahmed *et al.* 2014). In most of molecular studies of Archaea, commercial DNA extraction kits, such as High Pure PCR Preparation Kit (Roche, Germany) (Maillard *et al.* 2007), QIAamp DNA mini Kit (Qiagen, Germany), Wizard Genomic DNA Purification Kit (Bart *et al.* 2006), Q-bio Gene Kit (USA) have been used for extracting DNA. Commercial kits have a proper application when a large numbers of samples containing low numbers of organisms are processed. Although the application of kits is quick, and easy for obtaining nucleic acids, they can be expensive and not readily available in some countries.

There are some comparative studies of different DNA extraction methods for organisms, such as fungi, bacteria, viruses and protozoa (Klerks *et al.* 2006). Our aim was to find a quick, easy to perform and cost effective DNA extraction method for application in any lab that commercial DNA extraction kits are not easily available. The major advantage of the "glass beads method" is that there is no need for reusable equipment, and so is a low risk for accidental contamination of DNA among samples. However, the expense of this technique is too much. Other disadvantages of this method are difficulties in its handlings and safety hazards in use (Sharbatkhori *et al.* 2009).

The boiling procedure developed by Reischl *et al.* involves thermal lysis and has been used for total DNA extraction from *S. aureus* (Reischl *et al.,* 2000). However, the cell wall of Gram-positive bacteria, which contains a peptidoglycan layer, is more robust compared to that of Gram-negative bacteria. Although the boiling method is more rapid, simple, and effective than standard methods for bacterial DNA isolation (Queipo-Ortuño *et al.,* 2008), its yields are too low for subsequent analytical procedures (Ahmed *et al.* 2014). Moreover, the thickness of bands can be for more amplification of the additional band that more or less observed in all methods. It seems that employing boiling method by using less amount of extracted DNA in PCR reaction or decreasing PCR cycle numbers will attain a high quality of PCR bands. The "boiling method" took just 10 min, without any additional effort. This technique needs minimal equipment, performed at 95°C boiling water, and is available in almost all laboratories. Although glass beads methods also did not yielded DNA smear, like crushing method, but the PCR bands quality in crushing is much better than glass beads in all samples (Sharbatkhori *et al.* 2009).

Methanogens Archaea not only regulate levels of atmospheric H_2 but also help balance levels of H_2 in human gut(Pimentel, Gunsalus *et al.* 2012, Buriánková, Brablcová *et al.* 2013). Methane is as important microbiota byproduct and has considerable effect on the gut. So Archaea as strict anaerobic members of human microbiota that finding most significant position in different studies nowadays. Methanogen archaea have symbiotis and syntrophic relationships with commensal and pathogens bacteria in intestine (Dridi, Raoult *et al.* 2011, Pimentel, Gunsalus *et al.* 2012). Also, have key role in fermentation to produce short-chain fatty acids (SCFAs) such as acetate, ropionate and butyrate. SCFAs provide daily energy in host and can result in host adiposity (McNeil 1984, Mathur, Kim *et al.* 2013).

In conclusion, in PCR gels of all methods were seen some advantages and disadvantages but considering with different aspects of suitability for a DNA extraction method such as PCR band quality, time consuming, cost effectiveness, labor using and simplicity, boiling method was the most suitable methods considering their quality, simplicity, quickness and low cost for the DNA extraction of *M. smithii.*

REFERENCE

Aboushelib M, De Jager N, Pallav P, Feilzer AJ, (2005). Microtensile bond strength of different components of core veneered all-ceramic restorations. *Dent Mater.* **21**: 984-91.

Ahmed, O.B., Asghar, A.H., Elhassan, M.M. (2014). Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. *African J Microbiol Res* **8**(6): 598-602.

Aminov, R. I. (2013). "Role of archaea in human disease." Frontiers in cellular and infection microbiology 3.

Bart, J., Morariu, S., Knapp, J., Ilie, M., Pitulescu, M., Anghel, A., Cosoroaba, I., Piarroux, R. (2006). Genetic typing of *Echinococcus granulosus* in Romania. *Parasitol Res* **98**:130-37.

Belay, N., B. Mukhopadhyay, E. C. De Macario, R. Galask and L. Daniels (1990). "Methanogenic bacteria in human vaginal samples. *J clin microbiol.* **28**(7): 1666-1668.

Buriánková, I., L. Brablcová, V. Mach, P. Dvorák, P. P. Chaudhary and M. Rulík (2013). "Identification of methanogenic archaea in the Hyporheic Sediment of Sitka Stream." *PloS one* **8**(11): e80804.

Chaban, B. and J. E. Hill (2012). A 'universal'type II chaperonin PCR detection system for the investigation of Archaea in complex microbial communities. *The ISME journal* **6**(2): 430-439.

Chatterjee, S., S. Park, K. Low, Y. Kong and M. Pimentel (2007). The degree of breath methane production in IBS correlates with the severity of constipation. *The Am J Gastroenterol* **102**(4): 837-841.

Dridi, B., D. Raoult and M. Drancourt (2011). Archaea as emerging organisms in complex human microbiomes." *Anaerobe* **17**(2): 56-63.

Dridi, B., et al., (2009). High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. *PLoS One,* **4**(9): 7063.

Eckburg, P. B., P. W. Lepp and D. A. Relman (2003). "Archaea and their potential role in human disease. *Infection and immun* **71**(2): 591-596.

Ferrari, A., T. Brusa, A. Rutili, E. Canzi and B. Biavati (1994). Isolation and characterization of *Methanobrevibacter oralis* sp. nov. *Current Microbiol* **29**(1): 7-12.

Horz, H.-P. and G. Conrads (2010). The discussion goes on: what is the role of *Euryarchaeota* in humans? Archaea 2010.

Kim, G., F. Deepinder, W. Morales, L. Hwang, S. Weitsman, C. Chang, R. Gunsalus and M. Pimentel (2012). *Methanobrevibacter smithii* is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. *Digestive diseases sci* **57**(12): 3213-3218.

Kim, G., et al., (2012). *Methanobrevibacter smithii* is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. *Digestive diseases and sciences*, **57**(12): 3213-3218.

Klerks, M.M., Bruggen, A.H.C. Van, Zijlstra, C., Donnikov, M. (2006). Comparison of methods of extracting *Salmonella enteric serovar* Enteritidis DNA from environmental substrates and quantification of organisms by using a general internal Procedural Control. *App EnvMicrob* **72**: 3879-86.

Macfarlane, G. T. and G. R. Gibson (1997). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. Gastrointestinal microbiology, Springer: 269-318.

Maillard, S., Benchikh-Elfegoun, M., Knapp, J., Bart, J., Koskei, P., Gottstein, B., Piarroux, R. (2007). Taxonomic position and geographical distribution of the common sheep G1 and camel G6 strains of *Echinococcus granulosus* in three African countries. *Parasitol Res* **100**: 495-503.

Mathur, R., G. Kim, W. Morales, J. Sung, E. Rooks, V. Pokkunuri, S. Weitsman, G. M. Barlow, C. Chang and M. Pimentel (2013). Intestinal *Methanobrevibacter smithii* but not total bacteria is related to diet-induced weight gain in rats. *Obesity* **21**(4): 748-754.

McNeil, N. (1984). The contribution of the large intestine to energy supplies in man. *Am J clin nutr* **39**(2): 338-342.

Miller, T. L., M. Wolin, E. C. de Macario and A. Macario (1982). Isolation of *Methanobrevibacter smithii* from human feces. *App envir microbiol* **43**(1): 227-232.

Miller, T. L. and M. J. Wolin (1985). *Methanosphaera stadtmaniae* gen. nov., sp. nov.: a species that forms methane by reducing methanol with hydrogen. *Arch microbial* **141**(2): 116-122.

Miyamoto, K. (1997). Renewable biological systems for alternative sustainable energy production, Food & Agriculture Org.

Pimentel, M., R. P. Gunsalus, S. S. Rao and H. Zhang (2012). Methanogens in human health and disease. *Am J Gastroenterol Supplements* **1**(1): 28-33.

Pimentel, M., A. G. Mayer, S. Park, E. J. Chow, A. Hasan and Y. Kong (2003). Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Digestive diseases and sci* **48**(1): 86-92.

Queipo-Ortuño Ml, Colmenero JDD , Macias M, Bravo MJ, Morata P (2008). Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. *Clin Vaccine Immunol* **15**(2): 293-296.

Reischl U, Linde HJ, Metz M, Leppmeier B, Lehn N (2000).Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR.J. Clin. Microbiol., **38**: 2429-2433.

Scanlan, P. D., F. Shanahan and J. R. Marchesi (2008). Human methanogen diversity and incidence in healthy and diseased colonic groups using mcrA gene analysis. *BMC microbiol* **8**(1): 79.

Sharbatkhori, M., Kia, E.B., Harandi, F.M., Jalalizand, N., Zahabiun, F., Mirhendi, H. (2009). Comparison of Five Simple Methods for DNA Extraction from *Echinococcus granulosus* Protoscoleces for PCR Amplification of Ribosomal DNA. *Iranian J Parasitol* **4**(2): 54-60.

Tabatabaei, M., R. A. Rahim, N. Abdullah, A.-D. G. Wright, Y. Shirai, K. Sakai, A. Sulaiman and M. A. Hassan (2010). Importance of the methanogenic archaea populations in anaerobic wastewater treatments. *Process Biochem* **45**(8): 1214-1225.

Zhang, H., J. E. Banaszak, P. Parameswaran, J. Alder, R. Krajmalnik-Brown and B. E. Rittmann (2009). Focused-pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester. *Water research* **43**(18): 4517-4526.