Methanogenesis in Irritable Bowel Syndrome: A Lot of Hot Air?

Jasmohan S. Bajaj · Patrick M. Gillevet ·
Phillip B. Hylemon

Published online: 21 October 2012
© Springer Science+Business Media New York 2012

The human gut consists of several thousand species of prokaryotes including bacteria and archaea that have multiple metabolic and constitutive effects. A large proportion of these species are autochthonous, i.e., bacteria that are present among the gut mucosal niches that have evolved to survive in this ecological space while presumably providing a beneficial effect to the host [1]. Changes in this flora have been associated with inflammatory bowel disease, cirrhosis, and irritable bowel syndrome (IBS) [2–4]. In most disease states, there is a relative increase in potentially pathogenic bacteria compared to healthy hosts along with a reduction in the overall bio-diversity. A stumbling block in the characterization of the gut microbiome is the inability of the majority of species, especially anaerobic species, to be grown using standard culture methods [5]. For example, it has been suggested that less than 1 % of an environmental community can be cultured using typical growth media [6]. Initially, cloning and sequencing was performed to elucidate the community in the human gut but this was both tedious and expensive [7]. The introduction of NextGen sequencing technology

has revolutionized the field of microbial ecology and allows for the rapid in-depth analysis of many different environmental samples. To put this into perspective, the typical fluorescent sequencer will produce 96 reads per day while the NextGen Roche GS-FLX will produce 500,000 reads per day. Thus, the new technology produces 20 years worth of cloning and sequencing in an afternoon. Furthermore, multitag pyrosequencing (MTPS) has become a powerful and routine method for microbial community analysis [8–11] that takes advantage of this high-throughput technology. This methodology essentially barcodes samples that are then mixed and sequenced on one high-throughput sequencing run and then the reads are sorted into their corresponding samples based on the barcode. Specific amplification primers can be used to selectively amplify variable regions of the 16S rRNA of archaea or bacteria to enumerate these components in the gut microbiome. Therefore, the profusion of recent studies that evaluate the culture-independent methods of fecal bacterial enumeration opens an interesting chapter in our understanding of this complex system.

It has been known for decades that adult humans excrete methane and approximately one-third of all Americans produce detectable methane [12, 13]. Methane is produced in the human colon by methanogenic archaea by reducing CO_2 and using H_2 as the electron donor ($4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$). *Methanobrevibacterium smithii* is the most common methanogen in the human colon, with levels ranging from undetectable to 10^{10} /g dry weight [14]. Methanogens compete for available hydrogen gas with dissimilatory sulfate reducing bacteria [15]. Methane was initially thought to be an inert gas, however there is experimental and clinical evidence that methane is capable of reducing intestinal transit [16]. While the exact growth rate of *M. smithii* is unclear, the in vivo energetics of the

J. S. Bajaj (✉) · P. B. Hylemon
Division of Gastroenterology, Hepatology and Nutrition,
Virginia Commonwealth University and McGuire VA Medical
Center, 1201, Broad Rock Boulevard, Richmond, VA 23249,
USA
e-mail: jsbajaj@vcu.edu

P. M. Gillevet
Microbiome Analysis Center, George Mason University,
Manassas, VA, USA

P. B. Hylemon
Department of Microbiology, Virginia Commonwealth
University and McGuire VA Medical Center, Richmond, VA,
USA

methane formation reaction realistically makes this a slow-growing organism. The reduction in intestinal transit induced by methane production could potentially be a survival mechanism to allow numbers of this organism to grow in the intestine, but this is unproven. The study by Kim et al. in this issue highlights the role of quantitative PCR for the detection of *M. smithii* in a case–control study of patients with constipation-predominant IBS (C-IBS) [17]. They compared the presence of this species in methane-producing constipation-predominant IBS patients and compared them to non-methane-producing IBS patients and found that while this organism was detected in both groups, its abundance was clearly higher in those with C-IBS. Interestingly, they also demonstrated a correlation between the concentration of the *M. smithii* and its functional aspect, i.e., production of methane in the exhaled air. This study is provocative, although limited by its sample size, in that it shows a linkage between function and presence of *M. smithii* using culture-independent techniques. As prior studies have shown a significant difference between the stool and intestinal mucosal microbiome, it would have been interesting to compare the abundance of the organism on the colonic mucosa and correlate that with function [18, 19]. Also, as a significant proportion of methane is expelled as flatus, a methane breath test may underestimate methane production. The use of the primer just for *M. smithii* limits the detection of archaea species and also underestimates the role other species might be playing in overall methane production. However, this paper opens up an exciting chapter in IBS investigation which, if confirmed in larger populations, a concentration of *M. smithii* can be used as a biomarker for diagnosis as well as response to therapy.

References

1. Nava GM, Stappenbeck TS. Diversity of the autochthonous colonic microbiota. *Gut Microbes*. 2011;2:99–104.
2. Joossens M, Huys G, Cnockaert M, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut*. 2011;60:631–637.
3. Bajaj JS, Ridlon JM, Hylemon PB, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol*. 2012;302:G168–G175.
4. Malinen E, Rinttilä T, Kajander K, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol*. 2005;100:373–382.
5. Hamady M, Knight R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res*. 2009;19:1141–1152.
6. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev*. 1995;59:143–169.
7. Komanduri S, Gillevet PM, Sikaroodi M, Mutlu E, Keshavarzian A. Dysbiosis in pouchitis: evidence of unique microfloral patterns in pouch inflammation. *Clin Gastroenterol Hepatol*. 2007;5:352–360.
8. Gillevet P, Sikaroodi M, Keshavarzian A, Mutlu EA. Quantitative assessment of the human gut microbiome using multitag pyrosequencing. *Chem Biodivers*. 2010;7:1065–1075.
9. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. *Plos One*. 2008;3:1–8.
10. Wu GD, Lewis JD, Hoffmann C, et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16s sequence tags. *BMC Microbiol*. 2010;10:206–215.
11. Gillevet P. In: EPO ed. Multitag sequencing and ecogenomic analysis. BioSpherex LLC; 2006.
12. Calloway DH. Respiratory hydrogen and methane as affected by consumption of gas-forming foods. *Gastroenterology*. 1966;51:383–389.
13. Bond JH Jr, Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J Exp Med*. 1971;133:572–588.
14. Miller TL, Wolin MJ, Conway de Macario E, Macario AJ. Isolation of *Methanobrevibacter smithii* from human feces. *Appl Environ Microbiol*. 1982;43:227–232.
15. Gibson GR, Cummings JH, Macfarlane GT. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl Environ Microbiol*. 1988;54:2750–2755.
16. Attaluri A, Jackson M, Valestin J, Rao SS. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. *Am J Gastroenterol*. 2010;105:1407–1411.
17. Kang HW, Kim JM, Cha MY, Jung HC, Song IS, Kim JS. Deguelin, an Akt inhibitor, down-regulates NF- κ B signaling and induces apoptosis in colon cancer cells and inhibits tumor growth in mice. *Dig Dis Sci*. 2012;57:2873–2882.
18. Bajaj JS, Hylemon PB, Ridlon JM, et al. The colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol*.
19. Zoetendal EG, von Wright A, Vilpponen-Salmela T, Ben-Amor K, Akkermans AD, de Vos WM. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl Environ Microbiol*. 2002;68:3401–3407.