Effect of *Lactobacillus reuteri* (DSM 17938) on methane production in patients affected by functional constipation: a retrospective study

V. OJETTI¹, C. PETRUZZIELLO¹, A. MIGNECO¹, M. GNARRA², A. GASBARRINI¹, F. FRANCESCHI¹

¹Department of Medical Sciences, Division of Gastroenterology, Catholic University of the Sacred Heart, Rome, Italy
²Dermatology Unit, Columbia University Medical Center, NYC, NY, USA

**Abstract.** – **OBJECTIVE:** Constipation is a common symptom affecting up to 30% of the Western population and is strongly associated with the presence of intestinal methanogens, which may directly inhibit motor activity. Two recent studies performed on adult and children affected by chronic constipation showed that the supplementation with *L. reuteri* significantly improved bowel movements. Whether its action is related to a decreasing of methane (CH₄) production has never been tested. We have therefore designed a study aimed at testing this hypothesis.

**PATIENTS AND METHODS:** Data of 20 adults (12 females, mean age 36.2 ± 13.7) affected by functional constipation, treated with the probiotic *L. reuteri* (DSM 17938) for 4 weeks who performed a H₂/CH₄ lactulose breath test (LBT) in our institution showing a CH₄ production higher than 5 ppm were retrospectively analyzed from March to June 2015. Data recorded in their stool diary, reporting the frequency of defecations and stool consistency were also analysed, as well as the result of the LBT performed at the end of the treatment with *L. reuteri*.

**RESULTS:** Four weeks of *L. reuteri* administration was associated with a significant decrease of mean CH₄ production determined by LBT (from 20.8 ± 15 to 8.9 ± 8.6; *p* < 0.0001 CI 95%) and of AUC value (from 5101.5 ± 3571.13 to 2128.4 ± 2110.8; *p* < 0.0001 CI 95%). Moreover, a total disappearance of CH₄ production (< 5 ppm at LBT) was observed in 11 patients, while, we did not observe any significant decrease of H₂ production (from 13.2 ± 6.8 to 11.4 ± 7.3, CI 95%, n.s.).

**CONCLUSIONS:** This study highlights for the first time the beneficial effect of *Lactobacillus reuteri* (DSM 17938) on chronic constipation, via a significant decrease of CH₄ production.

**Key Words:** Constipation, Methanogenic flora, Lactulose breath test, Probiotics, *Lactobacillus reuteri*.

**Introduction**

Constipation is defined by Rome III criteria (Table I) as less than three defecations in a week, often with hard stools, excessive straining, and/or a sense of incomplete evacuations. It affects up to 30% of the western population, with a higher prevalence in women and the elderly. Constipation can be either primary or idiopathic, or secondary to systemic disorders, medications, organic pathologies, obstructing colonic lesions, ano-rectal dysfunction that impede or obstruct flow and others.

Primary constipation is the most common kind; patients often have a long history of medical examinations and negative diagnostic tests and with a considerable reduced quality of life.

Constipation is induced by a slow transit time with a hypo motility of neuromuscular apparatus.

Recent studies have shown a role of methane (CH₄) production from gut microbiota, mainly *Methanobrevibacter smithii*, in constipated patients that reduce the oro-cecal transit time.

Interestingly, intestinal CH₄, produced by converting hydrogen (H₂) produced by other bacteria, can slow intestinal transit, directly inhibiting gut motility. However, whether those alterations are either a cause or a consequence of constipations is still debated.

The determination of gut microflora gas production (CH₄ and/or H₂) is performed usually by lactulose breath test (LBT). This is a non-invasive test able to determine H₂/CH₄ production from gut bacteria along the gastrointestinal (GI) tract after the ingestion of 10 g of lactulose. CH₄ breath test positivity is variably defined; based on the most common classification, patients may be
**Table I.** Rome III criteria for chronic constipation.

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<tr>
<td>A</td>
<td>Stained during at least 25% of defecations</td>
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<tr>
<td>B</td>
<td>Lumpy or hard stools in at least 25% of defecations</td>
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<td>C</td>
<td>Sensation of incomplete evacuation for at least 25% of defecations</td>
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<tr>
<td>D</td>
<td>Sensation of anorectal obstruction/blockage for at least 25% of defecations</td>
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<td>E</td>
<td>Manual manoeuvres to facilitate at least 25% of defecations</td>
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<tr>
<td>F</td>
<td>Fewer than 3 evacuations per week</td>
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L. reuteri in methane producer constipated patients

Patients and Methods

A retrospective study was conducted in 20 adult patients (12F, 8M; mean age 36.2 ± 13.7) with functional constipation who performed a H\(_2\)/CH\(_4\) LBT in the Gastroenterology Unit of the Catholic University of Rome, positive for CH\(_4\) production (> 5 ppm) in the absence of SIBO.

All organic causes of constipation, use of oral laxatives, antibiotics, prebiotics or probiotics in the last month were considered as exclusion criteria.

We have then considered patients treated with L. reuteri (DSM 17938) at a dose of 10\(^8\) colony-forming units in 1 capsule (Reuflor, Italchimici; Pomezia, Italy), 30 minutes after eating twice per day for 4 weeks. We analyzed their stool diary recording the frequency of defecations and stool consistency (defined as hard, normal or watery Bristol stool scale)\(^1^5\), the occurrence of adverse effects causing discomfort and/or causing interruption of the normal daily activity and missing doses. We have also considered all clinical evaluations conducted during follow-up visits performed at the outpatient clinic from the enrolment to 4 weeks after the starting of the therapy, as well as the results of H\(_2\)/CH\(_4\) LBT to evaluate gas production performed at the end of the treatment.

The primary outcome was the modification of CH\(_4\) and H\(_2\) production after the treatment, while secondary outcome was the measure of stool frequency per week and stool consistency.

The study was conducted in accordance with the Declaration of Helsinki and was approved by our Ethical Committee (No. 1149/2016). A control group of patients affected by functional constipation and with a H\(_2\)/CH\(_4\) LBT positive for CH\(_4\) production not treated with L. reuteri was not included in this study as disposed by our Ethical Committee, who considered unethical not to treat those patients. None of the patients or authors received any honorary or economic benefits for the participation in this study.

**H\(_2\)/CH\(_4\), Lactulose Breath Test**

LBT was performed in the morning after a carbohydrate-restricted dinner and fasting for at least 12 h the day before the test to minimize basal H\(_2\) excretion. Physical exercise, smoking, and food were not allowed for 30 min before and during the test. Before the test, patients performed a mouth wash with 20 ml of a ch-
lorhexidine 0.05% solution. End-alveolar breath samples were collected immediately before the ingestion of lactulose 10 g in 200 ml solution and were taken every 15 min for 4 h with the two-bag system, consisting of a mouthpiece, a T-valve and two collapsible bags; the first one collects alveolar air. Samples were analyzed immediately for H₂ and CH₄ with a breath tracker quintron gas chromatograph (Quintron Instrument Company, Milwaukee, WI, USA).

Exclusion criteria for a positive SIBO were an increase in H₂ > 20 ppm by 90 min or 180 min. Two validated criteria used in previous studies to define CH₄ excretion were considered for comparison with the value we employed in our investigation: any detection of CH₄ > 5 ppm and baseline methane value ≥ 3 ppm. Areas Under the Curve (AUCs) of H₂ and CH₄ were assessed with the trapezoidal rule and methane producer patients were defined by an AUC₁₈₄ ≥ 1 200 ppm*4h, equal to a mean CH₄ production of 5 ppm.

**Statistical Analysis**

All data were collected in a database and statistically analyzed with SPSS software version 8.0 (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using the Student’s t-test and independent samples Mann-Whitney U test with 95% confidence intervals at a significance level of < 0.05. Variables concerning CH₄/H₂ excretion and clinical score were expressed as mean values ± SD.

**Results**

The demographic characteristic of the 20 evaluated patients (age, gender, constipation characteristics and mean level of H₂ and CH₄) are shown in Table II.

None of the patients recorded severe “adverse events”; only one patient reported a herpes simplex I infection, which did not determine any interruption of the study.

**Table II.** Demographic character of the 20 patients.

| Gender, n M/F | 7/13 |
| Mean age ± SD | 36.2 ± 13.7 |
| Bowel movements ± SD | 4.1 ± 1.1 |
| Mean H₂ production ± SD | 13.2 ± 8.8 |
| Mean CH₄ production ± SD | 20.8 ± 11.5 |

**Figure 1.** Mean H₂ and CH₄ production during LBT at enrolment and 4 weeks after the administration of L. reuteri twice a day. *p < 0.0001

**Effect of L. reuteri on CH₄/H₂ Production**

Four weeks of L. reuteri administration was associated with a significant decrease of mean CH₄ production determined by LBT (from 20.8 ± 15 to 8.9 ± 8.6; p < 0.0001 CI 95%) and of AUC value (from 5101.5 ± 3571.13 to 2128.4 ± 2110.8; p < 0.0001 CI 95%) (Figure 2).

Moreover, a total disappearance of CH₄ production (< 5 ppm at LBT) was observed in 11 patients.

At the same time, we did not observe any significant decrease of H₂ production (from 13.2 ± 8.8 to 11.4 ± 7.3, CI 95%, n.s.).

The reduction of mean CH₄ and H₂ production for each patient were summarized in Figure 3.
Effect of L. reuteri on Bowel Movements

At the end of the therapy, the reduction of CH\textsubscript{4} production was associated with an increase of bowel movements per week, as described in Figure 4. Interestingly, patients experienced a significant increase in the frequency of bowel movements per week at the end of the therapy (from 4.1 ± 1.2 to 6.4 ± 0.7 CI 95%; \( p < 0.001 \)), as reported in Figure 5. Therefore, the mean overall increase in bowel movements per week was 2.3 ± 1.14.

Effect of L. reuteri on Stool Consistency

At baseline, the stool consistency was reported to be abnormally hard (type 1-2-3 BSS) in 13 patients (65%) and normal (type 4-5 BSS) in the remaining 7 subjects (35%). At the end of the therapy, only two patients reported an improvement of stool consistency.

Discussion

Our study highlights the beneficial effect of L. reuteri (DSM 17938), in the treatment of chronic constipation by decreasing CH\textsubscript{4} production in the gut.

We showed that the administration of L. reuteri (DSM 17938) twice a day for 4 weeks in patients affected by idiopathic chronic constipation significantly reduces CH\textsubscript{4} production as assessed by LBT, with a total disappearance in 55% of the subjects.

It is well known that several gasses, such as CO\textsubscript{2}, H\textsubscript{2} and CH\textsubscript{4}, are produced through enteric fermentation, while the exact composition has an interindividual diversity. Some of them, including CH\textsubscript{4} and H\textsubscript{2}, are excreted in the flatus and in the breath, giving then the opportunity to indirectly measure their production using breath testing\textsuperscript{17}. On this subject, different studies\textsuperscript{18,19} have demonstrated the correlation between CH\textsubscript{4} production and irritable bowel syndrome (IBS), delayed oro-cecal transit time and constipation. Hwang et al\textsuperscript{20} in 2010 showed that CH\textsubscript{4} production during LBT may highly predict IBS-C. Similarly, Attaluri et al\textsuperscript{21} analyzed a cohort of chronic constipation patients, reporting a delayed oro-cecal transit time and CH\textsubscript{4} production in about 50% of the subjects.
Another study demonstrated an improvement of IBS-C related symptoms concomitant to the reduction of CH₄ production as assessed by LBT²², while Savarino et al²³, demonstrated that methane production is significantly associated with constipation, and that the mean CH₄ excretion seemed to increase with the reduction of bowel movements. There is also preliminary evidence that some antibiotics, particularly gentamycin, neomycin or rifaximin, improve clinical outcome of IBS-C patients, by altering gut microflora and, therefore, reducing CH₄ production²⁴,²⁵. Interestingly, a recent study²⁶ showed that CH₄ production is not only associated to constipation and IBS-C, but also to diverticulitis and colon cancer.

Despite the results of all those studies, whether intestinal motility is the cause or the consequence of CH₄ production is still undemonstrated²⁷. Our hypothesis is that *L. reuteri* (DSM 17938) may inhibit gut microbiota gram-positive bacteria more than negative, shifting gut microflora towards dominant H₂ consuming microorganisms. The possibility to reduce CH₄ methane production with the administration of *L. reuteri* (DSM 17938) could then represent the basis for new and more effective therapies even for other GI disorders linked to CH₄ production.

**Figure 4.** Methane AUC reduction and bowel movements/week increase for each patient at the end of therapy.

**Figure 5.** Significant increase of bowel movements 4 weeks after the administration of *L. reuteri* twice a day. *p < 0.001.
Further double-blind randomized trials are then needed in order to better elucidate the mechanism underlining constipation and CH₄ production.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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