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

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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 (CH_4) 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 H_2/CH_4 lactulose breath test (LBT) in our institution showing a CH_4 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 CH_4 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 CH_4 production (< 5 ppm at LBT) was observed in 11 patients, while, we did not observe any significant decrease of H_2 production (from 13.2 ± 8.8 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 CH_4 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[1]. Constipation can be either primary or idiopathic, or secondary to systemic disorders, medications, organic pathologies, obstructing colonic lesions, anorectal dysfunction that impede or obstruct flow and others[2].

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[3].

Recent studies have shown a role of methane (CH_4) production from gut microbiota, mainly *Methanobrevibacter smithii,* in constipated patients that reduce the oro-cecal transit time[4,5].

Interestingly, intestinal CH_4, produced by converting hydrogen (H_2) 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[6].

The determination of gut microflora gas production (CH_4 and/or H_2) is performed usually by lactulose breath test (LBT). This is a non-invasive test able to determine H_2/CH_4 production from gut bacteria along the gastrointestinal (GI) tract after the ingestion of 10 g of lactulose. CH_4 breath test positivity is variably defined; based on the most common classification, patients may be

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Table I. Rome III criteria for chronic constipation.

A	Stained during at least 25% of defecations
B	Lumpy or hard stools in at least 25% of defecations
C	Sensation of incomplete evacuation for at least 25% of defecations
D	Sensation of anorectal obstruction/blockage for at least 25% of defecations
E	Manual manoeuvres to facilitate at least 25% of defecations
F	Fewer than 3 evacuations per week

divided into CH_4-producers and non-producers, based on detection of a CH_4 production higher than 5 ppm at any time during the test[7].

Only a fraction of patients with constipation have successful treatment with laxatives requiring, however, a long-term therapy[3]; new and more effective therapies are thus needed to treat this condition.

Several and recent studies assessed the beneficial role of some probiotics in organic and functional GI disorders characterized by dysbiosis, such as inflammatory bowel disease, traveller diarrhoea, and small intestine bacterial overgrowth (SIBO)[8-10]. Since dysbiosis is a frequent finding in patients with chronic constipation, the use of specific bacterial strains may be a therapeutic option[11]. This may be the case of bifidobacteria and lactobacilli, which are able to produce lactic and acetic acid, thus lowering of colonic pH and improving intestinal motility. Moreover, those probiotics may accelerate intestinal transit by decreasing the proliferation of methanogens in patients with functional constipation[12].

Several studies performed in children with functional chronic constipation showed a positive effect of *Lactobacillus reuteri* (*L. reuteri*), endogenous Lactobacillus species in the human GI tract, in increasing bowel movements[13]. A double-blinded randomized controlled trial recently published by our group on the effect of *L. reuteri* (DSM 17938) supplementation in adult patients affected by chronic functional constipation for 4 weeks, reported an increasing of bowel movements and a reduced stool consistency in all treated patients[14].

The specific mechanism underlining this phenomenon is not well understood. We have hypothesized that *L. reuteri* may exert a beneficial effect by reducing the gut methanogenic flora then increasing bowel movements. We have therefore designed a study aimed at testing our hypothesis.

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)[15], 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 local 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 H_2 and CH_4 with a breath tracker quintron gas chromatograph (Quintron Instrument Company, Milwaukee, WI, USA).

Exclusion criteria for a positive SIBO were an increase in H_2 > 20 ppm by 90 min or 180 min[16].

Two validated criteria used in previous studies to define CH_4 excretion were considered for comparison with the value we employed in our investigation: any detection of CH_4 > 5 ppm and baseline methane value ≥ 3 ppm. Areas Under the Curve (AUCs) of H2 and CH_4 were assessed with the trapezoidal rule and methane producer patients were defined by an AUC_{CH4} ≥ 1 200 ppm*4h, equal to a mean CH_4 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 CH_4/H_2 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 H_2 and CH_4) 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 H_2 production ± SD	13.2 ± 8.8
Mean CH_4 production ± SD	20.8 ± 11.5

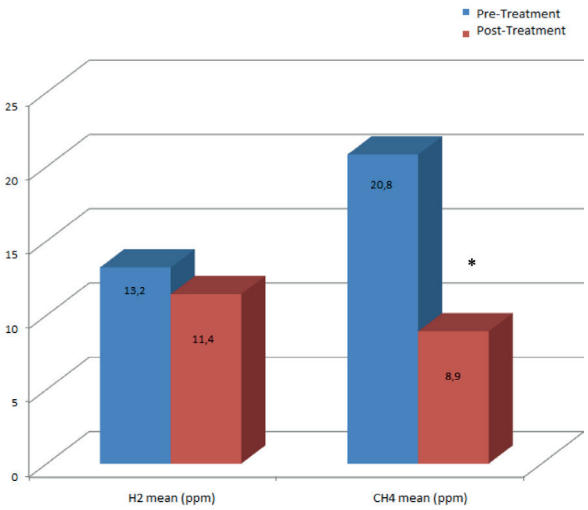

Figure 1. Mean H_2 and CH_4 production during LBT at enrolment and 4 weeks after the administration of *L. reuteri* twice a day. * $p < 0.0001$

Effect of L. reuteri on CH_4/H_2 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 CH_4 production (< 5 ppm at LBT) was observed in 11 patients.

At the same time, we did not observe any significant decrease of H_2 production (from 13.2±8.8 to 11.4 ± 7.3, CI 95%, n.s.).

The reduction of mean CH_4 and H_2 production for each patient were summarized in Figure 3.

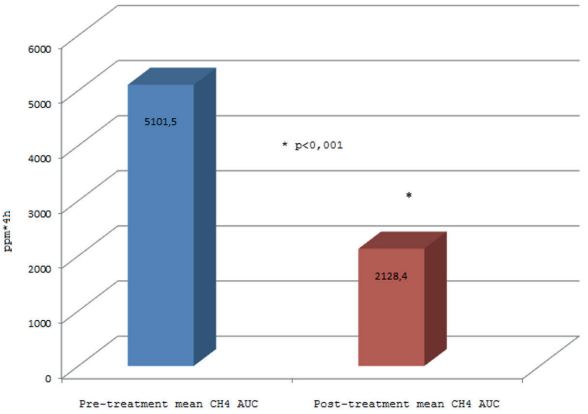

Figure 2. Significantly decrease of CH_4 AUC at enrolment and 4 weeks after the administration of L. reuteri twice a day.

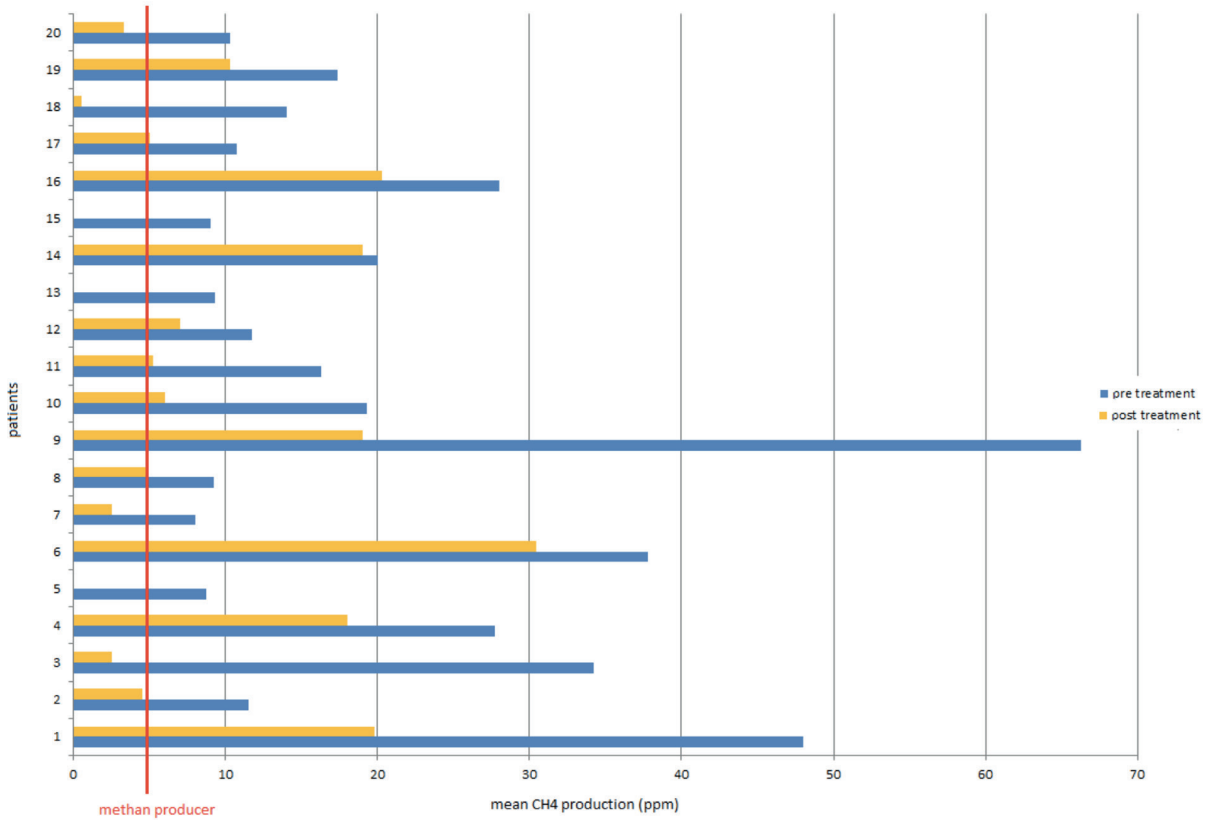

Figure 3. Reduction of CH_4 and H_2 mean production for each patient after treatment with *L. reuteri*.

Effect of L. reuteri on Bowel Movements

At the end of the therapy, the reduction of CH_4 production was associated with an increases 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_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_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_2, H_2 and CH_4, are produced trough enteric fermentation, while the exact composition has an interindividual diversity. Some of them, including CH_4 and H_2 are excreted in the flatus and in the breath, giving then the opportunity to indirectly measure their production using breath testing[17]. On this subject, different studies[18,19] have demonstrated the correlation between CH_4 production and irritable bowel syndrome (IBS), delayed oro-cecal transit time and constipation. Hwang et al[20] in 2010 showed that CH_4 production during LBT may highly predict IBS-C. Similarly, Attaluri et al[21] analyzed a cohort of chronic constipation patients, reporting a delayed oro cecal transit time and CH_4 production in about 50% of the subjects.

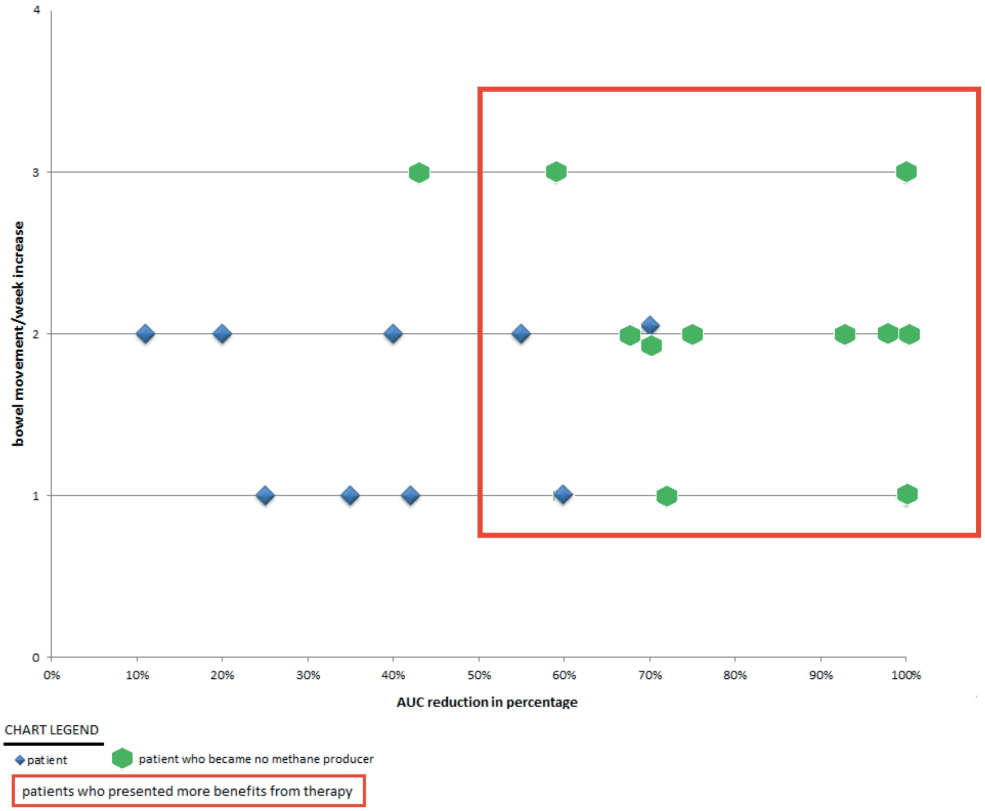

Figure 4. Methane AUC reduction and bowel movements/week increase for each patient at the end of therapy.

Another study demonstrated an improvement of IBS-C related symptoms concomitant to the reduction of CH_4 production as assessed by LBT[22], while Savarino et al[23], demonstrated that methane production is significantly associated with constipation, and that the mean CH_4 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 CH_4 production[24,25]. Interestingly, a recent study[26] showed that CH_4 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 CH_4 production is still undemonstrated[27]. 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 CH_4 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 CH_4 production.

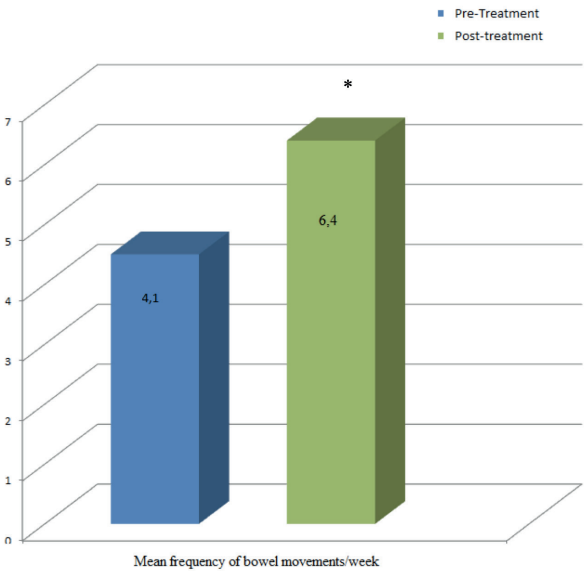

Figure 5. Significant increase of bowel movements 4 weeks after the administration of *L. reuteri* twice a day. *$p < 0.001$.

Further double-blind randomized trials are then needed in order to better elucidate the mechanism underlining constipation and CH_4 production.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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