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# *Lactobacillus gasseri* SF1183 protects the intestinal epithelium and prevents colitis symptoms *in vivo*

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ABSTRACT

*Lactobacillus gasseri* SF1183 belongs to a subpopulation of bacteria tightly associated to the human ileal epithelium. Cells of SF1183 survive and grow in simulated intestinal and gastric conditions, have a strong antimicrobial activity against Gram-positives and Gram-negatives and secrete molecule(s) sensed by human intestinal cells. We report that the oral administration of SF1183 cells had a protective effect in a murine model of DSS (Dextran-Sulfate-Sodium)-induced colitis. The analysis of the intestinal microbial composition indicated that several bacterial genera were differently represented in the intestine of DSS-treated animals. An overall similar alteration was observed in the microbiota of DSS-treated animals that received SF1183, suggesting that the beneficial role of the probiotic was not played through a reshuffling of the intestinal flora. Based on our *in vivo* data we propose the SF1183 strain of *L. gasseri* as a new anti-inflammatory probiotic, potentially useful as a therapeutic agent for the treatment of IBDs.

## 1. Introduction

Inflammatory bowel diseases (IBDs) such as Ulcerative Colitis (UC) and Crohn's Disease (CD) are chronic inflammatory disorders of the gastrointestinal tract due to an abnormal immune response to commensal bacteria (Santos Rocha et al., 2014; Xavier & Podolsky, 2007). IBD patients often present damages of the mucosal barrier and are characterized by gut dysbiosis, a general decrease of the gut microbial complexity with respect to healthy controls (Manichanh et al., 2012). A series of evidence indicates that the inflammatory status is induced by the interaction of luminal bacteria with the mucosal immune cells, which over-respond producing high levels of inflammatory cytokines (TNF-α, IFN-γ), then responsible of the increased intestinal permeability. This, in turn, allows luminal antigens to cross the epithelial barrier and stimulate the immune cells underneath the epithelium, causing the exacerbation of colitis symptoms (Coskun, 2014). The characterization of the intestinal microflora of UC patients allowed to correlate the presence or absence of specific bacteria with the pathogenesis. In a study by Sokol et al. (2008), *Fecalibacterium prausnazii* was shown to be present in low proportion in ileal biopsies of patients with recurrent CD disease. A different study focused on the analysis of the *Lactobacillus* population and showed that, in UC patients, some species of the genus were less represented than in healthy controls, highlighting the importance of the relative proportion of bacteria belonging to the same genus (Cui et al., 2016).

In order to balance the altered microbiota population, probiotic bacteria, mainly belonging to the *Lactobacillus* and *Bifidobacterium* genera, have been often proposed as therapeutic agents for the treatment of inflammatory disorders (Fedorak et al., 2015; Santos Rocha et al., 2014). Some strains have been reported to exert immune-regulatory activity and directly influence cytokine expression while others have demonstrated effects on the intestinal permeability (Rao & Samak, 2013), protecting the gut barrier integrity (Karczewski et al., 2010; Laval et al., 2015) and reducing the inflammation (Ewaachuk et al., 2008; Madsen et al., 2001; Yan et al., 2007). However, the use of probiotics for the treatment of IBDs is not always recommended. Being

*Abbreviations:* DSS, dextran sulfate sodium; DGGE, denaturing gradient gel electrophoresis; UC, ulcerative colitis; IBD, inflammatory bowel disease; CD, Crohn's Disease; MPO, myeloperoxidase; TNFα, tumor necrosis factor α; TJ, tight junction; HTAB, hexadecyltrimethylammonium bromide; PCA, Principal Component Analysis; OTUs, Operational Taxonomic Units

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IBDs due to an abnormal and aggressive immune response to the resident microflora, a bacterial treatment can also be detrimental and cause the exacerbation of symptoms (Cui et al., 2016; Mileti, Matteoli, Iliev, & Rescigno, 2009). Therefore, a deep knowledge of the effects of a probiotic strain in a reliable *in vivo* model is an essential preliminary step to propose a probiotic as a therapeutic agent for IBDs treatment.

The murine model of DSS (Dextran Sulfate Sodium)-induced colitis has been widely used to study IBDs and, in particular, UC disorders (Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014). Indeed, DSS induces in mice a severe colon inflammation with symptoms such as mucosal damages at intestinal level, disruption of intestinal barrier integrity and a general inflammatory status that resemble these typically observed in UC patients (Chassaing et al., 2014). Here we used DSS-murine model to assess the *in vivo* effects of strain SF1183 of *L. gasseri,* a species previously suggested as directly involved in protecting the intestine from inflammatory diseases (Cui et al., 2016). The SF1183 strain has been isolated from intestinal biopsies of healthy human volunteers and belongs to a subpopulation of bacteria found to be tightly bound to the epithelial cells underlying the mucosal surface (Fakhry et al., 2009). SF1183 has potential probiotic properties being able to survive and grow in simulated intestinal and gastric conditions and to produce antimicrobials active against Gram-positive and Gram-negative bacteria (Fakhry et al., 2009). In addition, *in vitro* studies with human colon cancer (HCT116) cells evidenced that SF1183 produces and secretes molecule(s) able to be sensed by the eukaryotic cells and to interfere with their survival and proliferation (Di Luccia et al., 2013). We now report an *in vivo* study using a murine model of experimentally-induced colitis to assess the potential of SF1183 in protecting mice from the development of the inflammation.

## 2. Materials and methods

### 2.1. Bacterial strains, growth conditions

*L. gasseri* SF1183 (Fakhry et al., 2009) was grown anaerobically in MRS broth (Difco, Detroit, MI) for 24 h at 37 °C. Cell were collected, washed two times with PBS and suspended in PBS containing 20% glycerol. Cells were aliquoted ($2x10^9$ cells/aliquot) and stored at −80 °C. For the *in vivo* experiment, cells were quickly defrosted and immediately given to mice.

### 2.2. Animals and treatment

Male C57BL/6 mice (Charles River, Italy), of 8 weeks of age were used as a model of DSS-induced colitis as previously reported (Melgar, Karlsson, & Michaelsson, 2005). Mice were singularly caged in a temperature-controlled room (23 ± 1 °C) with a 12-h light/dark cycle (6.30am – 6.30 pm). Treatment, housing, and euthanasia of animals met the guidelines set by the Italian Health Ministry. All experimental procedures were approved by the "Comitato Etico-Scientifico per la Sperimentazione Animale" of the Federico II University of Naples (Italy).

Mice were divided in 2 groups that were fed with a standard diet and daily administered with PBS (n = 12) or $2 \times 10^9$ cells of SF1183 (n = 6). After 7 days, animals treated with SF1183 (n = 6) and half of the animals treated with PBS (n = 6) were treated with 2.5% DSS directly added in drinking water (day 0). The other half of animals that received only PBS served as control. Body weight, food and water intake were assessed daily. At day +5 all the animals were sacrificed and the whole colon was collected.

Macroscopic score was assessed at sacrifice by an unbiased observer as described by Kim, Shajib, Manocha, and Khan (2012). Scores were defined as follows: rectal bleeding: 0 (none), 1 (red), 2 (dark red), 3 (gross bleeding); rectal prolapse: 0 (none), 1 (signs of prolapse), 2 (clear prolapsed), 3 (extensive prolapsed); diarrhea: 0 (normal), 1 (soft), 2 (very soft), 3 (diarrhea); colonic bleeding: 0 (normal), 1 (red), 2 (dark red), 3 (black).

### 2.3. Measurement of colonic inflammation

The myeloperoxidase (MPO) activity has been assessed in colonic samples as reported by Kim et al. (2012). Briefly, tissue samples (50 mg) were homogenized in 1 ml of hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0) and centrifuged at 13,400*g* for 6 min at 4 °C. MPO activity was measured spectrophotometrically: 10 µl of supernatant were combined with 200 µl of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml 0-dianisidine hydrochloride and 1.25% hydrogen peroxide. The change in absorbance at 450 nm was measured and one unit of MPO activity was defined as the amount that degraded 1 µmol of peroxide per minute at 25 °C.

The TNF-α concentration in protein extracts from colon was determined using a mouse specific enzyme linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instruction. Briefly, the wells of a microtiter plate were coated with 100 µl of mouse anti-mouse TNF-α (4 µg/ml) in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, pH 7.4), and incubated overnight at room temperature. The antibody excess was then removed by washing with Wash Buffer (containing 0.05% (v/v) Tween 20 in PBS, pH 7.4), and the remaining sites on the plate were blocked with reagent diluent (PBS containing 1% BSA) (1h, room temperature). After extensive washing, 100 µl of samples (1:2–1:10 dilution in reagent diluent) were added to the wells and incubated for 2 h at room temperature. After further washing, the wells were incubated with biotinylated goat anti-rat TNF-α (225 ng/ml in reagent diluent) followed by treatment with Streptavidin-HRP (1:200 dilution; 1 h, room temperature). Peroxidase-catalysed colour development from o-Phenylenediamine was measured at 492 nm. Colonic content of cytokines TNF-alpha, IL-10 and IL-12 was measured by using ELISA kits according to manifacturer instructions (R&D Systems, Minneapolis, MN. USA for TNF-alpha and ThermoFisher Scientific, Whaltman, MA, USA for IL-10 and IL-12).

The data, obtained on 6 animals for each experimental group, were analysed using one-way ANOVA followed by Tukey post-test and presented as mean ± SEM. Probability values less than 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

### 2.4. Histological analysis

Distal colonic samples (n = 4 for each experimental group) were fixed in Bouin solution for 48 h and embedded in paraffin. Six serial transversal sections of 6 µm for each sample were stained with Hematoxylin and Eosin (H&E) to study the histology of the tissue. The images were analysed and acquired at light microscope by a Kontron Electronic Imaging System KS300 (Zeiss, Germany).

H&E-stained colonic serial sections were analysed to evaluate the tissue damages. Histological scoring was based on 3 parameters as reported in Laroui et al. (2012) considering the morphology of epithelium and the crypt damages (from 0 to 5 as maximum score), infiltration of inflammatory cells in mucosa and submucosa (from 0 to 3 as maximum score) and ulcerations (from 0 to 3 as maximum score). Values were added to give a maximal histological score of 11 (Laroui et al., 2012**)**. The obtained data, were analysed by GraphPad Prism 5 and elaborated by one-way ANOVA with post-test Tukey to compare multiple treatments and presented as mean ± standard deviation (S.D.).

### 2.5. Immuno-histochemical analysis

For the detection of occludin we used the immuno-histochemical staining of the avidin–biotin–peroxidase complex technique. Serial sections were exposed to polyclonal anti-occludin antibodies

(ThermoFisher Scientific, Whaltman, MA, USA) at a working dilution of 1:100 at 4 °C overnight. Visualization was carried out using the Vectastain Elite ABC kit (Vector Labs, Inc., Burlingame, CA, USA) and revealed by 3 mg of 3.3′- diaminobenzidine-tetrahydrochloride (Sigma, St. Louis, MO, USA) dissolved in 10 ml of a phosphate buffered saline solution and 150 µl of 3% $H_2O_2$. Antibody specificity was assessed by omitting the primary antiserum.

### 2.6. PCR-DGGE analysis

Total genomic DNA was extracted from 200 mg of faecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions.

Bacterial 16S rDNA fragments were amplified using nested PCR. For the primary amplification PCR was conducted with the primers 27F and 1492R (Chong et al., 2009). The PCR mixtures contained 5 µl of extracted nucleic acids, 0.5 µM of each primer, 250 µM of each type of deoxyribonucleotide triphosphate, 10 µl of 5X GoTaq® Flexi Buffer, 1.25 U GoTaq® DNA Polymerase, 1.5 mM $MgCl_2$ (Promega Corporation, Madison, WI, USA) and sterile MilliQ water for a final volume of 50 µl. PCR amplification was performed using the following program: 95 °C for 2 min, 20 cycles of denaturation at 92 °C for 45 s, annealing at 50 °C for 2 min, and extension at 72 °C for 1 min and 45 s, and a single final extension at 72 °C for 5 min.

For the secondary amplification, the primer pair 341F-GC (with 40 bp GC-clamp) and 907R (Chong et al., 2009) was used to amplify the hypervariable regions V3, V4 and V5. The reaction mixtures consisted of 2 µl of template, 0.5 µM of each primer, 400 µM of each deoxyribonucleotide triphosphate, 10 µl of 5X GoTaq® Flexi Buffer, 1.25 U GoTaq® DNA Polymerase, 1.5 mM $MgCl_2$ (Promega Corporation, Madison, WI, USA) and sterile MilliQ water for a final volume of 50 µl.

Touchdown PCR was performed with an initial denaturation step of 94 °C for 5 min, 10 touchdown cycles of 94 °C for 1 min, 65 °C (-1°C per cycle) for 1 min and 72 °C for 3 min, 5 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, and a final elongation step of 72 °C for 4 min. The presence of PCR products was confirmed by analyzing 5 µl of product on 1.5% agarose gels and staining with ethidium bromide.

DGGE was performed with the BIO-RAD DCode™ Universal Mutation Detection System.

PCR samples were loaded onto 7% (wt/vol) polyacrylamide gels in 1X TAE (40 mM Tris base, 20 mM acetic acid, glacial, 1 mM EDTA [pH 8.0]). The 7% (wt/vol) polyacrylamide gels (acrylamide/Bis solution, 37.5:1; BIO-RAD Laboratories, Inc., Hercules, CA, USA) were made with denaturing gradients ranging from 35% to 60% (where the 100% denaturant contains 7 M of urea and 40% deionised formamide). The electrophoresis was performed at 60 °C for 16 h at 70 V. After electrophoresis, the gels were soaked for 15 min in 250 ml of 1X TAE running buffer and 0.5 µg/ml ethidium bromide, rinsed for 15 min in 250 ml of 1X TAE running buffer and photographed.

DGGE patterns were analysed using the FPQuest Software Version 5.1 (BIO-RAD Laboratories, Inc., Hercules, CA, USA) to generate a band-matching table. All bands are divided into classes of common bands and for each pattern a particular band class can have two states: present or absent (binary matrix). The binary matrix obtained was exported to perform the Principal Component Analysis (PCA) with the PAST software (Hammer, Harper, & Ryan, 2001).

### 2.7. Microbiota identification by 16S rRNA sequencing

Partial 16S rRNA gene sequences were amplified from extracted DNA using primer pair Probio_Uni and Probio_Rev, which target the V3 region of the 16S rRNA gene sequence (Milani et al., 2013). 16S rRNA gene amplification and amplicon checks were carried out as previously described (Milani et al., 2013). 16S rRNA gene sequencing was performed using a MiSeq (Illumina) at the DNA sequencing facility of GenProbio srl (www.genprobio.com) according to the protocol previously reported (Milani et al., 2013).

Following sequencing and demultiplexing, the obtained reads of each sample were filtered to remove low quality and polyclonal sequences. All quality-approved, trimmed and filtered data were exported as .fastq files.

The .fastq files were processed using a script based on the QIIME software suite (Caporaso et al., 2010). Paired-end reads pairs were assembled to reconstruct the complete Probio_Uni/Probio_Rev amplicons. Quality control retained those sequences with a length between 140 and 400 bp and mean sequence quality score > 20. Sequences with homopolymers > 7 bp and mismatched primers were omitted.

In order to calculate downstream diversity measures (alpha and beta diversity indices, Unifrac analysis), 16S rRNA Operational Taxonomic Units (OTUs) were defined at ≥99% sequence homology using uclust (Edgar, 2010) and OTUs with < 10 sequences were filtered. All reads were classified to the lowest possible taxonomic rank using QIIME (Caporaso et al., 2010) and the SILVA database v. 123 clustered at 99% identity as reference dataset (Quast et al., 2013). Biodiversity of the samples (alpha-diversity) was calculated with Chao1 and Shannon indexes. Similarities between samples (beta-diversity) were calculated by weighted uniFrac (Lozupone & Knight, 2005). The range of similarities is calculated between the values 0 and 1. PCoA representations of beta-diversity were performed using QIIME (Caporaso et al., 2010).

## 3. Results and discussion

### 3.1. L. gasseri SF1183 protects mice from the DSS-induced inflammation

Eight weeks old mice were divided into two groups, one receiving a daily dose of 2 x $10^9$ cells of *L. gasseri* SF1183 suspended in PBS (n = 6) and the other receiving only PBS (n = 12). After seven days, all animals treated with SF1183 and half of the animals that received only PBS were used to induce acute ulcerative colitis by adding 2.5% DSS in the drinking water, given *ad libitum* for 5 days, as previously reported (Perse & Cerar, 2012). For the entire duration of the treatment mice of all three groups (Control, DSS and DSS/SF1183) consumed the same amount of food and water as shown in the supplemental material (Fig. S1).

To evaluate the development of colitis symptoms during the DSS treatment and the effects of the SF1183, a disease activity index (DAI) was determined considering weight loss, diarrhea and presence of occult blood in stools at the end of the treatment. A score was assigned according to the scale (1–3) as previously reported (Kim et al., 2012). As expected, no signs of disease were observed in Control animals receiving only PBS (Fig. 1A). Mice of the DSS group showed a DAI score significantly higher respect to control and also respect of mice of the DSS/SF1183 group, suggesting a partial protective effect induced by SF1183 cells (Fig. 1A).

To evaluate the effect of DSS and SF1183 on the intestinal inflammation, the myeloperoxidase (MPO) activity and the levels of TNF-α in the colon were measured. As shown in panel B and C of Fig. 1 both parameters were significantly higher in the animals of the DSS group than in the other two groups, indicating that the DSS treatment induced an inflammation to the treated animals and that SF1183 cells had a protective effect. We also measured the plasma levels of IL12 and IL10, two cytokines with inflammatory and anti-inflammatory activities, respectively, and whose ratio is a common marker of inflammation (Foligne et al., 2007; Sokol et al., 2008). We observed a high IL12/IL10 ratio in animals of the DSS group that was partially reduced in animals pre-treated with SF1183 (Fig. 1D). Altogether data of Fig. 1 clearly indicate that 5 days of DSS treatment caused an inflammation that was reduced in animals pre-treated with a daily dose of SF1183 cells.

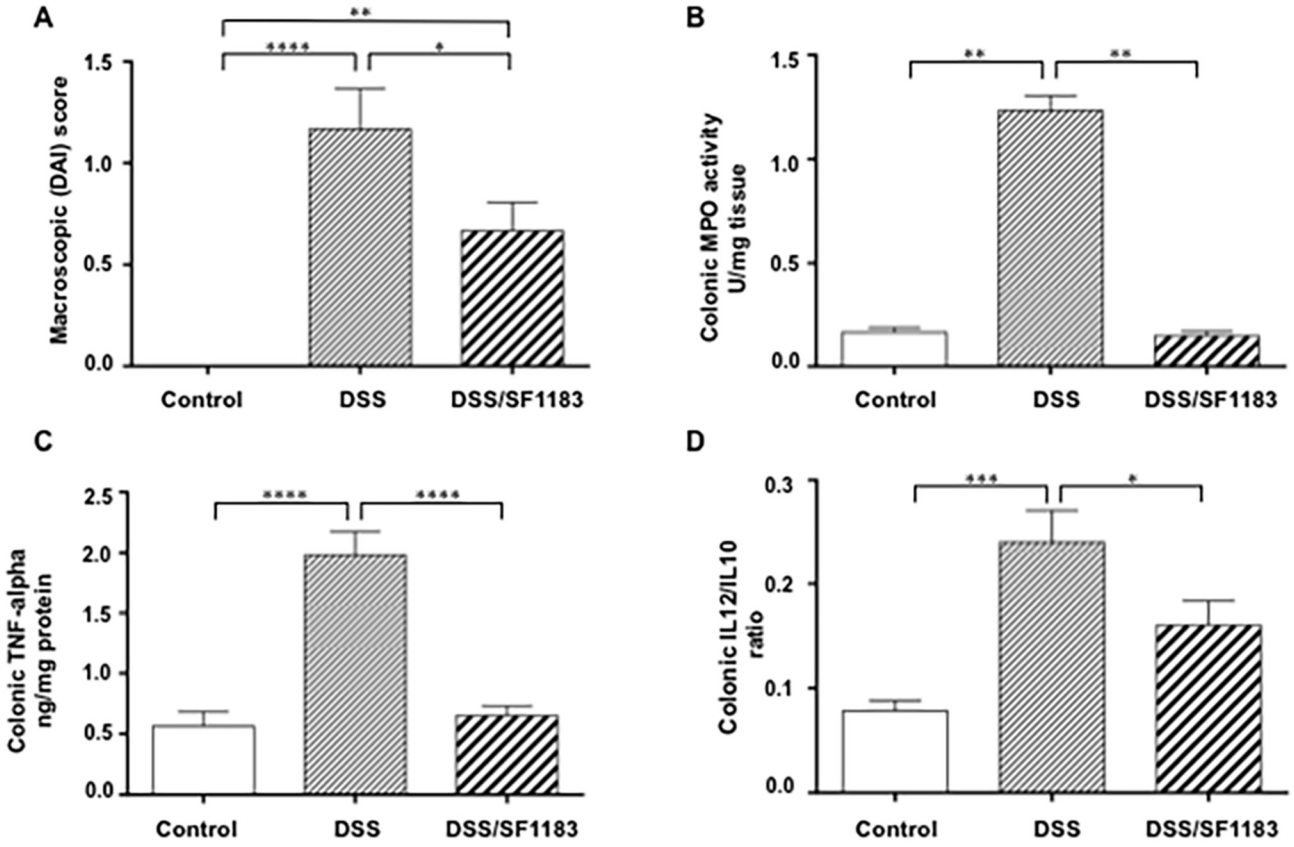


**Fig. 1.** Effects of the DSS-induced inflammation. (A) Disease activity index (DAI) evaluated at the end of the treatments. (B) Mieloperoxidase (MPO) activity, TNFα levels (C) and IL12/IL10 ratio (D) in colonic extracts. Results are the mean ± SEM of six different mice for each experimental group. $^{*}P < .05$; $^{**}p < .01$; $^{***}P < .001$; $^{****}P < .0001$.

### 3.2. *L. gasseri SF1183 protects the intestinal integrity and preserves tight junction assembly*

In order to investigate the mechanisms used by *L. gasseri* SF1183 to reduce the inflammation, the structure of the intestinal epithelium was analysed by histological and immune-histochemical analysis of colon tissues isolated from animals of the three groups. Fixed tissue samples were embedded in paraffin and transversal sections of 6 µm stained with Hematoxylin and Eosin (H&E). As shown in Fig. 2A, epithelial erosion, disappearance of crypts, mucosal and submucosal inflammation and oedema were observed in DSS-treated samples. Such tissue defects were not observed in animals pre-treated with SF1183 cells (DSS/SF1183) and the epithelium morphology resembled that observed in samples from the control group. The histology score was obtained for each sample combining the observed phenotypes, according to Laroui et al. (2012). The score value measured for animals of the DSS group was significantly higher than that of the Control group (Fig. 2B) as an indication of tissue damage. On the other hand, the score value of the DSS/SF1183 group was lower than that of the DSS group confirming that the pre-treatment with SF1183 cells reduced the mucosal damages caused by the DSS (Fig. 2B).

An immune-histochemical analysis was then performed to evaluate the assembly of the tight junctions (TJ) and the integrity of the intestinal barrier. To this aim the presence of the protein occludin, a component of the TJ (Groschwitz & Hogan, 2009; Turner, 2009), on the fixed tissues samples was analysed. A continuous brown signal along the whole epithelial surface, indicative of the presence of occludin, was observed on the epithelium of animals of the Control and DSS/SF1183 groups (Fig. 3). As previously reported (Srutkova et al., 2015), such signal was weak or totally absent on the epithelium of DSS-treated animals (Fig. 3). Although we do not know if the presence of *L. gasseri* had a stimulating effect on occludin expression and/or localization or, instead, if it avoided TJ degradation by DSS, we observed that SF1183 maintained a TJ pattern similar to that of animals of the Control group,

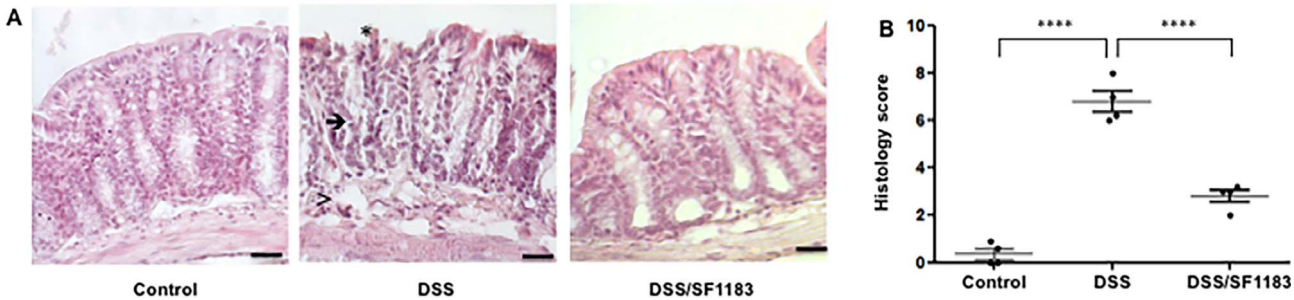


**Fig. 2.** Histology of the colonic mucosa. (A) Representative photomicrographs of colon histology in the three experimental groups. Samples were stained with Haematoxylin eosin. Epithelial erosion ($^{*}$), disappearance of crypts (→) and inflammation of mucosa and submucosa with oedema ( > ) are indicated. Scale bar: 40 µm. (B) Histology scores based on the colon morphology of the three experimental groups presented as mean ± standard deviation (S.D.); n = 4, $^{****}P < .0001$.

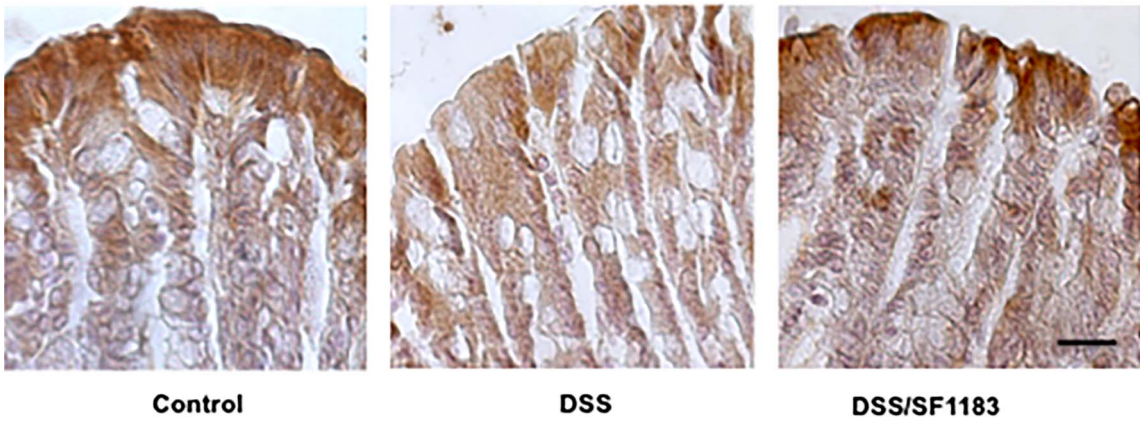


**Fig. 3.** Immuno-histochemical analysis of the colonic mucosa. Representative photomicrographs of the immuno-histochemical detection of occludin on paraffin-embedded sections of colon derived from Control, DSS and DSS/SF1183 groups. Scale bar: 20 μm;

indicating that the pre-treatment with SF1183 preserved the integrity of the epithelium.

### 3.3. Bacterial community analysis

To evaluate whether the treatment with DSS and DSS/SF1183 had an effect on the microbial composition of the gut faecal microbial communities of the various experimental groups were compared by DGGE. Total faecal DNA was extracted, PCR amplified with oligonucleotides for the V3-V4-V5 hypervariable regions of 16S rDNA and the obtained DGGE profiles were analysed by the principal component analysis (PCA). The comparison between different groups indicated that both the DSS and the DSS/SF1183 treatments caused a variation of the microbial community of the gut with respect to the untreated animals (data not shown). This preliminary indication, induced us to analyse the microbial composition of our samples in more details by sequencing the V3 region of the bacterial 16S rRNA genes on an Illumina MiSeq platform.

PCoA based on Bray-Curtis distance showed that the gut microbiota of mice of both DSS and DSS/SF1183 groups varied with respect to those of the Control group (Fig. 4), and the permutational multivariate analysis of variance (PERMANOVA) confirmed the shift of the overall structure of gut microbiota between control and treated groups ($p$ value $< .05$).

The rarefaction curves showing the microbial richness in each sample, were obtained by using Chao1 or Shannon index. While the curve obtained with the Chao1 index did not show differences among samples, by using the Shannon index both treated groups showed a slightly reduced microbial diversity with respect to the Control group (Fig. S2). It has been previously reported that DSS-treatment caused the reduction of the gut microbial diversity in faecal samples (Berry et al., 2015; Nagalingam, Kao, & Young, 2011). However, this effect was not strong in our samples, probably as a consequence of a mild DSS treatment (Håkansson et al., 2015; Mar et al., 2014). Therefore, the DSS treatment used in our experiments (five days of 2.5% DSS supplementation) was sufficient to cause an inflammatory status (Figs. 1-3) but not strong enough to alter the overall gut microbial diversity in statistically significant way.

The analysis of the bacterial composition, reported as the average of the relative abundance of bacterial taxa at the Phylum, Family and Genus level did not show dramatic differences among the three experimental groups (Fig. 5). At the Phylum level, a general predominance of *Firmicutes* (violet) over *Bacteroidetes* (purple) was observed in all samples (Fig. 5A). In all three experimental groups the third most abundant Phylum was the *Deferribacteres* (green), although they were more represented in the gut of DSS animals (4.59%) than in animals of the other two groups (0.43% and 1.75% in Control and DSS/SF1183, respectively) (Fig. 5A). The analysis at family or genus level confirmed this trend, with the Family *Deferribacteriaceae*, green in Fig. 5B, and Genus *Mucispirillum*, light green in Fig. 5C (both members of the *Deferribacteres* Phylum) more represented in the DSS group than in the Control or DSS/SF1183 groups. However, a great variation in the relative abundance of these bacteria among different animals was observed and, consequently, the differences observed were not statistically significant.

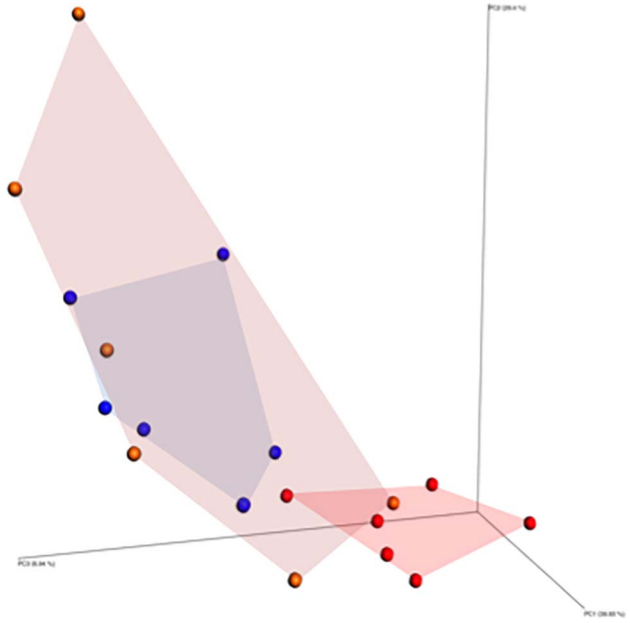

**Fig. 4.** Principal Coordinate Analysis (PCoA). Plots were generated using weighted UniFrac distance matrix. Symbols are: Control (untreated, red), DSS-treated (blue) and DSS/SF1183-treated (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To evaluate the possibility that specific bacterial groups involved in the development of inflammation could be influenced by the SF1183 treatment, the analysis at the genus level was focused on the bacterial taxa of the DSS or DSS/SF1183 groups that showed a statistically significant variation ($p < .05$) in their representation with respect to the Control group. By this approach 20 bacterial genera in the Control vs DSS comparison (Fig. S3) and 23 genera in the Control vs DSS/SF1183 comparison (Fig. S4) were identified. Most of the taxa differently represented in the Control vs DSS comparison also came up in the Control vs DSS/SF1183 comparison (Figs. S3 and S4). Since mice of the DSS/SF1183 group showed a strong reduction of the inflammation symptoms with respect to mice of the DSS group (Figs. 1–3), we inferred that

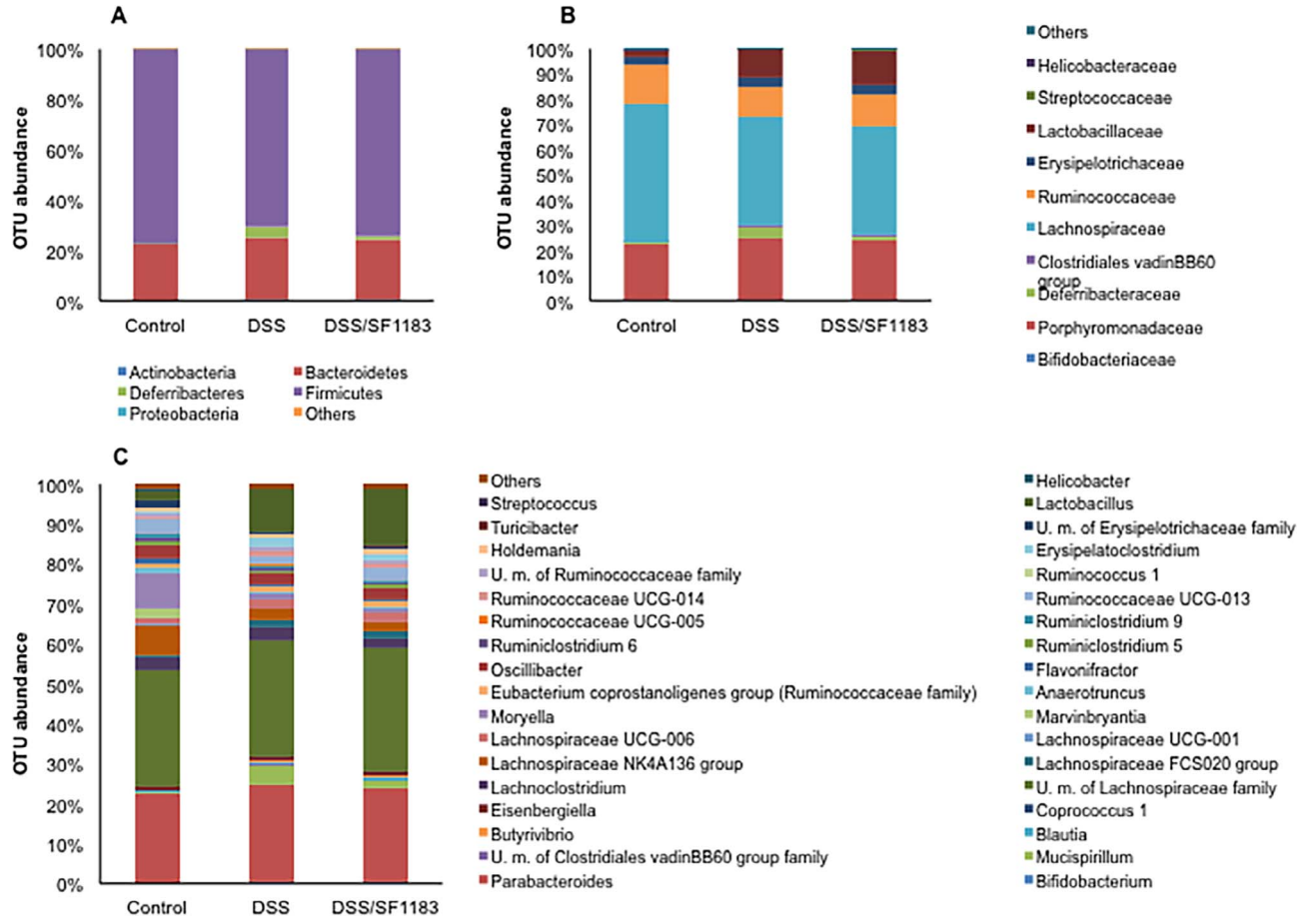


**Fig. 5.** Faecal bacterial composition. Relative Operational Taxonomic Units (OTUs) abundance at the Phylum (A), Family (B) and Genus (C) level in Control, DSS and DSS/SF1183 groups, reported as means of six mice for each group. Only Taxa represented by OTUs abundance > 0.5% have been considered for the analysis.

the microbial genera varying in both comparisons did not correlate with the development of the DSS-induced symptoms. Further analyses were then limited to the genera that showed a statistically significant variation only in the DSS vs Control comparison and not in the DSS/SF1183 vs Control comparison. By this approach members of the genus *Ruminoclostridium* 5 were found reduced only in the gut of animals of the DSS group while members of the genera *Lactobacillus, Ruminoclostridium* and *Robinsoniella* were increased only in DSS-treated animals. *Ruminiclostridium, Ruminiclostridium 5* and *Robinsoniella* are Gram-positive, anaerobic, spore formers. While it is still unclear whether *Ruminiclostridium, Ruminiclostridium 5* are distinct genera and what effects could be associated to their presence in the murine gut (Guanghong & Bailin, 2015; Yutin & Galperin, 2013), members of the *Robinsoniella* genus, commonly isolated from faeces of animals (Linnenbrink et al., 2013; Núñez-Díaz, Balebona, Alcaide, Zorrilla, & Moriñigo, 2017) and humans (Ferraris, Aires, & Butel, 2012; Gomez et al., 2011), have been associated to bacteraemia in patients with cancer and pneumonia (Jeon et al., 2012; Lim et al., 2017; Shen, Chen, Ye, Luo, & Tang, 2010) and found over-represented in NAFLD (nonalcoholic fatty liver disease) patients (Raman et al., 2013). However, the average of relative abundance of the *Ruminoclostridium 5, Ruminoclostridium* and *Robinsoniella* genera in the faecal microbial population was very low with less than 1% of *Ruminoclostridium* 5, 0.3% of *Ruminoclostridium* and 0.02% of *Robinsoniella*, in both Control and DSS groups (Fig. 6). In addition, the statistical significance of the variations between Control and DSS groups for *Ruminoclostridium 5* and *Robinsoniella* was close to the threshold of $p < .05$ (Fig. 6). For the *Lactobacillus* genus, wich includes well characterized members of the human intestinal microbiota, a strong increase was observed in both DSS (10.11%) and DSS/SF1183 (13.83%) groups with respect to the Control (2.68%) (Fig. 6). Although the variation between Control and DSS/SF1183 groups was not statistically significant ($p = .06$) (Fig. 6), it was very close to the threshold, suggesting a trend of *Lactobacillus* increase in both DSS and DSS/SF1183 groups.

In conclusion, due to the high variations observed between animals of the same experimental group, further studies involving a high number of animals per group would be needed to convincingly link these four bacterial genera to the development of the inflammation and to identify them as responsible of the protective effect of SF1183.

## 4. Conclusion

Probiotics are often proposed as therapeutic agents for the treatment of IBDs. However, such diseases are caused by an abnormal and aggressive immune response to the resident microflora and in some cases, the treatment with probiotics had detrimental effects causing the exacerbation of symptoms. This is the case of *L. rhamnosus* strain GG (LGG), one of the best characterized commercially available probiotics (Lievin-Le Moal & Servin, 2014), that resulted unable to protect mice against experimentally-induced colitis and was responsible of the exacerbation of the DSS-induced effects (Cui et al., 2016; Mileti et al., 2009). Therefore, the use of a probiotic strain for the treatment of IBDs has to be tested *in vivo* in an appropriate model.

Here we used C57BL/6 mice treated with DSS as a model of inflammatory intestinal disease to evaluate the *in vivo* activity of *L. gasseri* SF1183. This strain was isolated from ileal biopsies of healthy human volunteers and found to be in tight association with epithelial cells underneath the mucus layers (Fakhry et al., 2009). It is well adapted to

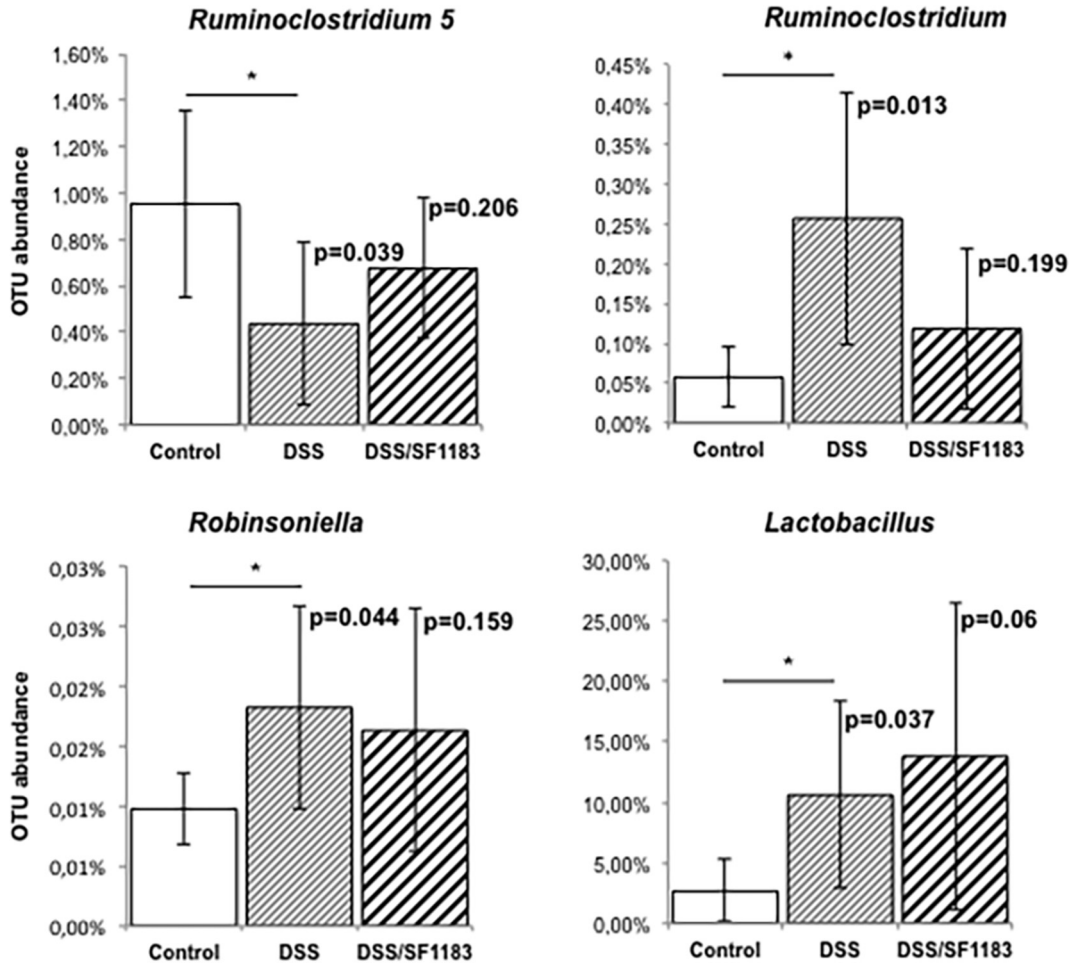


**Fig. 6.** Representativeness of four bacterial genera. The histograms show the variation of four genera in Control, DSS and DSS/SF1183 groups. Values are reported as means ± SD of six different mice ($^{*}P < .05$).

the intestinal environment being able to survive and grow in simulated intestinal and gastric conditions (Fakhry et al., 2009). In addition, SF1183 cells have strong antimicrobial activity (Fakhry et al. 2009) and have been shown to produce molecule(s) able to be sensed *in vitro* by human colon cancer (HCT116) cells interfering with their survival and growth (Di Luccia et al., 2013).

Main result of our analysis is the observation that *L. gasseri* SF1183 had a clear protective effect *in vivo* in a murine model of DSS-induced colitis avoiding the induction of an inflammatory status and maintaining the intestinal tissue integrity. This observation is supported by recent findings showing that, among the *Lactobacillus* population, the relative abundance of the *L. gasseri* species is reduced in faecal samples of UC patients (Cui et al., 2016). Our results together with literature data, then, indicate the *L. gasseri* species as important determinants of a healthy human microbiota, directly involved in the protection against the development of intestinal inflammatory diseases.

The mechanism by which *L. gasseri* SF1183 exerts its protective activity has been investigated. Our immune-histochemical analysis suggested that SF1183 beneficial effects are, at least in part, due to a protective role on the gut barrier integrity, while our rRNA 16S-sequencing analysis of DNA extracted from faecal samples denoted only a mild effect on the bacterial diversity and on the bacterial composition of the gut. Taken together our results suggest that SF1183 supplementation does not reshuffle the overall composition of the intestinal microbiota but protects mice from the DSS-induced inflammation by restoring the level of the inflammatory markers and avoiding the mucosal damages induced by the DSS treatment. Since previous *in vitro* data have shown that SF1183 secretes molecule(s) sensed by the eukaryotic epithelial cells (Di Luccia et al., 2013), we hypothesize that molecules secreted by SF1183 interact with intestinal cells protecting mice from DSS-induced damages. Although further experiments will be needed to clarify the molecular aspects of such activity, we propose *L. gasseri* SF1183 as a potential candidate for the bacteriotherapy of inflammatory intestinal disorders.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.12.049.

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