

# Synergy between *Lactobacillus paracasei* and Its Bacterial Products to Counteract Stress-Induced Gut Permeability and Sensitivity Increase in Rats<sup>1,2</sup>

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## Abstract

Stressful events result in the alteration of gut permeability and sensitivity. *Lactobacillus paracasei* NCC2461 (Lpa) therapy prevents antibiotic-induced visceral hyperalgesia in mice. This study aimed at evaluating the influence of 3 probiotic strains: *Bifidobacterium lactis* NCC362, *Lactobacillus johnsonii* NCC533, and Lpa on stress-mediated alterations of colorectal hyperalgesia, on gut paracellular permeability and whether bacteria and/or bacterial products present in the spent culture medium (SCM) were involved in the antinociceptive properties of the effective strain. Rat pups were separated from their mothers 3 h/d during postnatal d 2–14. At wk 13, gut paracellular permeability was determined as a percentage of urinary excreted <sup>51</sup>Cr-EDTA and visceral sensitivity to colorectal distension (CRD), assessed by abdominal muscle electromyography. Visceral sensitivity was also analyzed in adults rats subjected to partial restraint stress (PRS, 2 h restriction of body movements). Rats received either the probiotics resuspended in SCM or fresh growth medium as control for 2 wk. Maternal deprivation significantly increased colonic sensitivity in response to CRD and enhanced gut paracellular permeability compared with control rats. Only Lpa treatment significantly improved stress-induced visceral pain and restored normal gut permeability. Similarly, among the 3 probiotics tested, only Lpa prevented PRS-mediated visceral hyperalgesia. Both bacteria and bacterial products present in Lpa SCM were required for the antinociceptive properties against PRS. This study illustrates strain-specific effects and suggests a synergistic interplay between *L. paracasei* bacteria and bacterial products generated during fermentation and growth that confers the ability to suppress PRS-induced hypersensitivity in rats. J. Nutr. 137: 1901–1907, 2007.

## Introduction

Several studies suggest the implication of the gut microbiota in the genesis of irritable bowel syndrome (IBS)<sup>5</sup> symptoms (1,2). Abdominal pain or discomfort is often reported with the use of oral antibiotic treatment and, on the other hand, treatment with antibiotics was found to reduce symptoms in subsets of IBS patients (3,4). Moreover, both retrospective and prospective studies have recently documented the onset of IBS following confirmed bacterial gastroenteritis (5–7). The enteric microbiota may be implicated, as suggested by the presence of bacterial over-

growth in some IBS patients and the improvement of their symptoms by eradication of the overgrowth (8,9). Despite these observations, our ever-increasing understanding of gut microbiota-mucosa interactions supports the important role of inflammation and/or immune processes in the genesis of muscle dysfunction and gut hypersensitivity in IBS (10).

Several studies have already suggested that probiotics, either live or attenuated bacteria, or bacterial products may have beneficial effects in IBS patients (11,12). These can offer antibacterial and antiviral effects and could thereby prevent or modify the course of postinfective IBS (13). O'Mahony et al. (14) showed that treatment with *Bifidobacterium infantis* alleviates IBS symptoms, this symptomatic response being associated with normalization of the anti- and proinflammatory cytokine ratio.

IBS is associated with abdominal pain that may result from hypersensitivity of the colon to mechanical stimuli (15). Indeed, a lower threshold of discomfort or pain was observed in IBS patients. Stressful life events are important to the triggering of IBS symptoms, and both acute and chronic stress paradigms were developed in animals to mimic changes in visceral sensitivity

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<sup>5</sup> Abbreviations used: Bla, *Bifidobacterium lactis* NCC362; cfu, colony forming units; CRD, colorectal distension; IBS, irritable bowel syndrome; Ljo, *Lactobacillus johnsonii* NCC533; Lpa, *Lactobacillus paracasei* NCC2461; MD, maternal deprivation; MRS, Man-Rogosa-Sharpe culture medium; PRS, partial restraint stress; SCM, spent culture medium; TJ, tight junction.

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seen in IBS patients. In rats, both acute restraint and passive avoidance stress were associated with hypersensitivity to rectal distension linked to central peripheral release of corticotrophin releasing factor (CRF), these effects being blocked by a CRF-R1 receptor antagonist (16,17). Similarly, neonatal stress induced by maternal deprivation in 3-mo old rats, also triggered a long-term hypersensitivity to colorectal distension (18,19). Both restraint and maternal-deprivation stress may activate immune reactions within the gut resulting from alterations in gut paracellular permeability (20–22). Recently, acute stress-induced hypersensitivity to distension was found to be linked to alteration of colonic paracellular permeability (23).

Several probiotics improve the intestinal barrier by reducing the alteration of gut paracellular permeability (24,25) suggesting that, through such a mechanism, they can improve stress-induced hypersensitivity to distension. Two species from the *Lactobacilli* and *Bifidobacteria* genera most commonly used as probiotics were previously tested in a mouse model of postinfective IBS (26). This article describes the results obtained with 3 of these Nestle probiotics in 2 rat models of psychological stress, mimicking other clinical features of IBS. Consequently, the aim of our work was: 1) to evaluate a possible protective influence of *Bifidobacterium lactis* NCC362 (Bla), *Lactobacillus johnsonii* NCC533 (Ljo), and *Lactobacillus paracasei* NCC2461 (Lpa) in a model of chronic stress of neonatal maternal deprivation-induced visceral hyperalgesia associated with alterations of colonic paracellular permeability; 2) to confirm these previous results in the acute stress model of partial restraint-induced visceral hyperalgesia, and 3) to investigate whether bacteria and/or bacterial products of the antinociceptive positive strain are required to get the expected protective effect.

## Materials and Methods

### Bacterial strains and culture conditions

*L. johnsonii* NCC533, *L. paracasei* NCC2461 (CNCM I-3446), and *B. lactis* NCC362 were obtained from the Nestle Culture Collection and grown under anaerobic conditions in Man-Rogosa-Sharpe (MRS, BioMérieux) broth (bifidobacteria with 0.5% cysteine). After 48 h at 37°C, the number of bacteria was estimated by measuring the optical density at 600 nm ( $1 \text{ OD}_{600} = 10^{14}$  bacteria/L). Bacterial cells were pelleted by centrifugation at  $5000 \times g$  for 15 min at 4°C and further resuspended at a concentration of  $10^{15}$ /L in their spent culture medium (SCM), namely, Ljo, Lpa, and Bla, respectively. Aliquots of 1 mL were kept frozen until use. To further analyze the effect of *L. paracasei* after centrifugation, the bacterial pellet was washed twice in fresh MRS and finally resuspended at a concentration of  $10^{15}$ /L in fresh MRS (washed Lpa). The SCM was filtered through  $0.2 \mu\text{m}$  to obtain bacteria-free SCM (Lpa-free SCM). Measurement of the amount of DNA present in filtered SCM from Ljo, Lpa, and Bla was performed using the Picogreen Quantitation kit (Molecular Probes, Juro AG) according to manufacturers' instructions.

### Experimental design

**Maternal deprivation stress and probiotic treatments.** Wistar rats were purchased from Janvier SA (Le Genest). For the protocol of maternal deprivation (MD), male and female rats (250–275 g) were used. One week after arrival in the animal facility, rats were mated by placing 2 males and 6 virgin females in the same cage for 2 wk. Pregnant females were then individually housed in standard cages and allowed free access to water and standard pellets (UAR pellets containing crude protein, 16.1%; crude fat, 3.1%; crude ash, 5.1%; and crude fiber, 3.9%) (27). Following delivery (d 1), litters were culled to 10 pups. MD was performed daily according to a previously validated methodology (27) from postnatal d 2 to d 14 for 3 consecutive hours (from 0900 to 1200), during which time pups were removed from the home cage and kept in

temperature-controlled cages at  $28 \pm 1^\circ\text{C}$ . Control pups were left in their home cage. From d 15 to d 22, all (deprived and control) pups were maintained with their dam. Weaning was performed on d 22, siblings were sex matched, and males were selected and housed by groups of 10 in the same cage. Mothers, as well as the young rats after weaning, were allowed free access to water and standard pellets (UAR pellets). At 11 wk of age, 4 groups of 10 deprived and 4 groups of control rats received a daily oral dose of 1 mL of MRS as control, or 1 mL of Ljo, Bla, or Lpa, for 2 wk. At wk 13, gut paracellular permeability and fecal microbiota composition were determined at d 89. Visceral perception was analyzed at d 91 by colorectal distension (CRD) and electromyography as described below. Microbiota composition was determined from small intestine wall and content after rats were killed, as described under *Microbiota analysis*. (See Figure 1.)

**Partial-restraint stress and probiotic treatments.** Female rats, 13 wk of age (225–250 g), were housed individually in a temperature-controlled room ( $21 \pm 1^\circ\text{C}$ ). Stress effects were studied using a unique, 2-h session of the wrap partial restraint stress (PRS) model (28). Rats were lightly anaesthetized with ethyl ether, and their fore shoulders, upper forelimbs, and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not to prevent, body movements. Rats were then placed in their home cages for 2 h. Sham-stress rats (sham-PRS), considered as controls, were anaesthetized but not wrapped. They were allowed to move freely in their cages. Two weeks before PRS (at 11 wk of age), 4 groups of 10 rats received an oral dose of 1 mL/d of Ljo, Bla, Lpa, or MRS. To evaluate the role of the bacterial cell compared with the SCM, 4 additional groups of 10 rats received, for 2 wk before PRS, a mL/d oral gavage of MRS, Lpa, washed-Lpa, or Lpa-free SCM. Microbiota composition was determined from fecal samples at d 13 before the PRS session as described under *Microbiota analysis*. At the end of treatments (d 15) visceral perception was assessed by CRD and electromyography (see below) 60 min before (basal condition) and 30 min after the PRS or sham stress sessions. Microbiota composition was analyzed from small intestinal wall and content after PRS and after killing. (See Figure 1.)

**Gut permeability measurements.** In both humans and in animal models  $^{51}\text{Cr}$ -ethylenediamine tetra-acetic acid ( $^{51}\text{Cr}$ -EDTA), as well as sugars lactulose and mannitol, are commonly used to evaluate gut permeability. However, the dual sugar probe is metabolized into the large intestine and only allows measurement of small bowel permeability (29). Consequently, the assessment of total gut paracellular permeability to large molecules was performed using  $^{51}\text{Cr}$ -EDTA (Perkin Elmer Life Science) in adult, 13-wk-old deprived and control rats (30).  $^{51}\text{Cr}$ -EDTA (25.9 kBq) was diluted in 0.5 mL of saline and administered by gavage. Rats were then placed in metabolic cages and feces and urine were collected for 24 h. Total radioactivity found in urine was measured with a gamma counter (Cobra II; Packard). Permeability to  $^{51}\text{Cr}$ -EDTA was expressed as a percentage of the total radioactivity administered.

**Colorectal distension.** To measure abdominal contractions as the index of pain, rats were equipped with 3 groups of 3 NiCr wire electrodes implanted into the abdominal external oblique muscle at 12 wk of age and 1 wk after the start of probiotic treatments (31). Briefly, the myoelectrical activity was recorded on an electromyograph (EMG). EMG recordings began at least 5 d after surgery. Rats were placed in polypropylene tunnels. A balloon consisting of an arterial embolectomy catheter (Fogarty, Edwards Laboratories) was introduced into the colorectum at 1 cm from the anus and fixed at the base of the tail. The balloon was progressively inflated by steps of 0.4 mL, from 0 to 1.2 mL, each step of inflation lasting 5 min.

**Microbiota analysis.** Microbiota were assessed in the luminal content and wall of the small intestine and colon, as well as in fecal samples. Samples were processed within 30 min of collection and kept frozen at  $-80^\circ\text{C}$  until analysis for the endogenous population of lactobacilli, bifidobacteria, enterobacteria, enterococci, and *Clostridium perfringens*. Bacteria were detected on selective or half-selective medium, as already reported (32). Microbial concentrations were expressed as  $\log_{10}$  colony

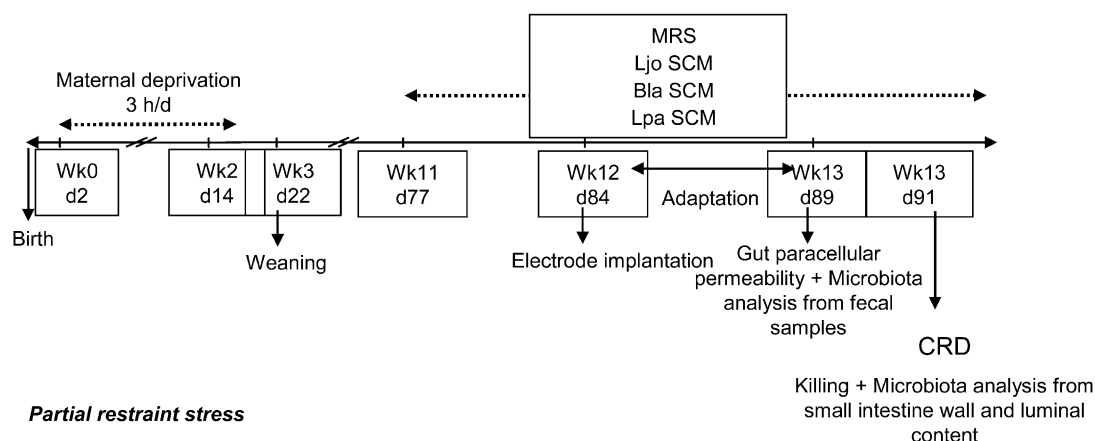
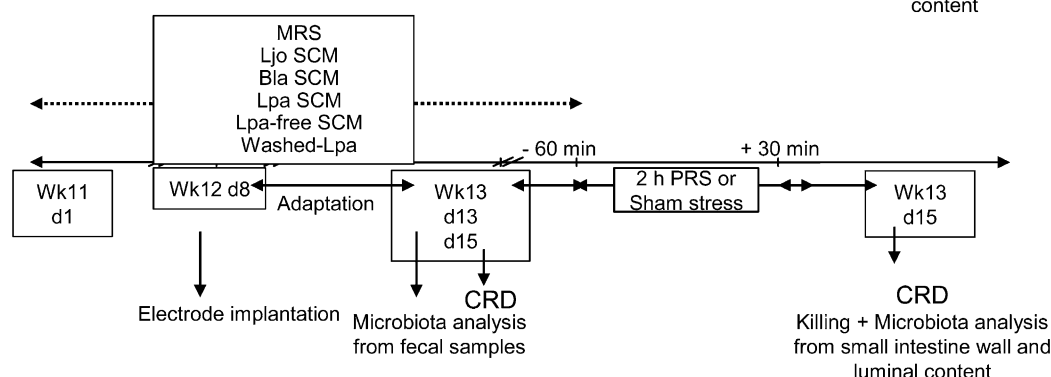
**Maternal deprivation stress****Partial restraint stress**

**FIGURE 1** Experimental design of both maternal deprivation stress and partial restraint stress procedures.

forming units (cfu)/g of the sample, with a detection limit at  $3.00 \log_{10}$  cfu/g. The presence of live probiotics was monitored by random amplification of polymorphism DNA (RAPD) fingerprint, as described previously (26), on bacteria isolated from the dominant population of the respective genera, lactobacilli, or bifidobacteria. Lactobacilli isolates were obtained from plating a  $10^6$ -fold dilution of fecal samples and/or gut contents with a limit of detection of  $7.00$ – $8.00 \log_{10}$  cfu/g. Bifidobacteria were isolated from plating a  $10^4$ -fold dilution, detection limit =  $5.00 \log_{10}$  cfu/g.

All experimental protocols described in this study were approved by the Local INRA Animal Care and Use Committee.

**Statistical analysis.** For each experimental design, i.e., MD and PRS, the number of abdominal spike bursts/5 min and gut paracellular permeability (percentage of  $^{51}\text{Cr}$ -EDTA found in the urine) were analyzed by a 2-way ANOVA (3 probiotics  $\times$  2 treatments). A post hoc Student's *t* test was calculated on this ANOVA when data followed a normal distribution, and differences were considered significant at  $P < 0.05$ . For microbiota, ANOVA at a level of 5% was performed. Fisher's least significant difference (LSD) was calculated on the ANOVA when data followed normal distribution; otherwise Kruskal-Wallis test was performed. Values were expressed as means  $\pm$  SEM.

## Results

### Maternal deprivation stress

**Probiotics and gut permeability.** In control rats receiving MRS growth medium,  $1.49 \pm 0.09\%$  of orally administered  $^{51}\text{Cr}$ -EDTA was excreted in urine over 24 h, and this value was greater in deprived rats (Table 1). Neither Bla nor Ljo treatments had any effect on the increase in gut permeability seen in deprived adult rats (Table 1). In contrast, Lpa treatment restored

gut permeability of deprived rats to levels similar to those of control rats receiving the same treatment (Table 1).

**Probiotics and visceral pain.** In 12-wk-old control rats receiving MRS as vehicle, a volume of 0.8 mL was the threshold at which CRD increased ( $P < 0.05$ ) the number of abdominal contractions compared with the predistension period. Deprived rats treated with MRS exhibited an increase ( $P < 0.05$ ) in the number of abdominal contractions for 0.8 and 1.2 mL distensions compared with control rats (Fig. 2). A chronic treatment with Bla and Ljo did not reverse the MD-induced visceral hypersensitivity in adult rats (Fig. 2B). In contrast, treatment of deprived rats with Lpa reduced ( $P < 0.05$ ) by 57 and 33% the number of abdominal contractions for the distending volumes of 0.8 and 1.2 mL, respectively (Fig. 2B). None of the 3 probiotics tested had a significant effect on visceral sensitivity in control rats (Fig. 2A).

**TABLE 1** Effect of Bla, Ljo, and Lpa treatments on the increase of gut paracellular permeability in rats induced by neonatal maternal deprivation stress<sup>1</sup>

| Probiotics | Control                 | Deprived             |
|------------|-------------------------|----------------------|
|            | $\%^{51}\text{Cr EDTA}$ |                      |
| MRS        | $1.49 \pm 0.09$         | $2.00 \pm 0.09^{*a}$ |
| Bla        | $1.63 \pm 0.08$         | $2.16 \pm 0.28^{*a}$ |
| Ljo        | $1.31 \pm 0.16$         | $2.19 \pm 0.22^{*a}$ |
| Lpa        | $1.71 \pm 0.09$         | $1.66 \pm 0.14^b$    |

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 8$ . Means in a column with superscripts without a common letter differ,  $P < 0.05$ . \*Different from the corresponding control group,  $P < 0.05$ .

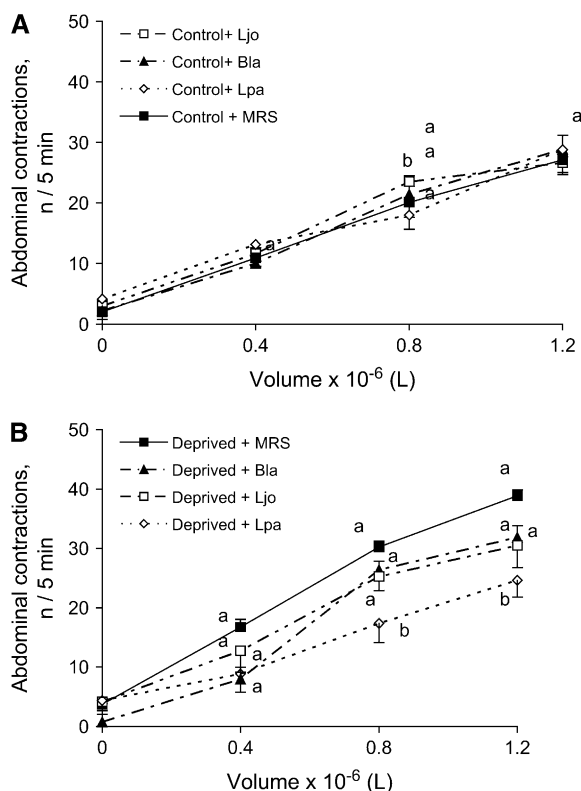


**FIGURE 2** Stress-induced visceral sensitivity in control (A) and maternally deprived (B) rats that were or were not treated with Bla, Ljo, Lpa, or MRS. Values are means  $\pm$  SEM,  $n = 9$ . At each volume of CRD, labeled means without a common letter differ,  $P < 0.05$ .

### Partial restraint stress

**Probiotics and visceral pain.** We tested the effects of the 3 probiotics on visceral sensitivity using the PRS model in adult rats. Under basal conditions in rats treated with MRS, CRD was associated with a significant increase in the frequency of abdominal contractions observed at volumes of 0.8 and 1.2 mL and no significant increase for the lowest volume of 0.4 mL. None of the probiotics in their SCM affected these basal abdominal responses to CRD (Fig. 3A). A 2-h PRS session is known to enhance the abdominal responses for 0.8 and 1.2 mL and also to trigger a response for the lowest volume of distension, i.e., 0.4 mL. Rats receiving MRS showed a similar response (Fig. 3A). Treatment with Bla, Ljo, and Lpa attenuated the increase of abdominal contractions induced by PRS for the lowest volume of distension, 0.4 mL (reduction of 55, 74, and 76%, respectively, compared with MRS-treated rats) but only Lpa ( $P < 0.05$ ) reduced by 47 and 36%, respectively, the poststress abdominal contractions for the highest volumes of distension were 0.8 and 1.2 mL (Fig. 3B).

**Lpa bacteria/SCM and visceral pain.** The PRS model was further used to determine the component of Lpa responsible for the prevention of visceral hyperalgesia. As described above, under basal conditions, CRD significantly increased abdominal contractions from a threshold volume of 0.8 mL. A similar nociceptive response profile was observed in rats pretreated with all Lpa preparations compared with MRS-treated rats (Fig. 4A). PRS increased ( $P < 0.05$ ) the number of abdominal contractions in response to all CRD volumes in the MRS control group (Fig. 4B). As previously described, treatment with Lpa ( $P < 0.05$ )

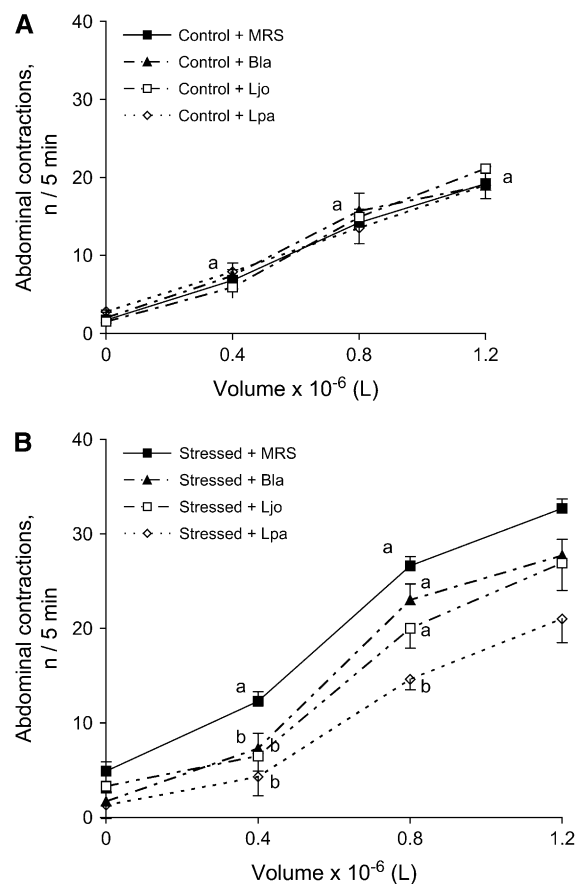


**FIGURE 3** Stress-induced visceral sensitivity in control (A) and partial restraint stressed (B) rats that were or were not treated with Bla, Ljo, Lpa, or MRS. Values are means  $\pm$  SEM,  $n = 8$ . At each volume of CRD, labeled means without a common letter differ,  $P < 0.05$ .

reduced by 75, 47, and 41% the increase in the number of abdominal contractions induced by PRS for the distension volumes of 0.4, 0.8, and 1.2 mL, respectively. In contrast, neither the Lpa-free SCM devoid of live bacteria nor the washed Lpa treatment showed any antinociceptive properties on PRS-induced visceral hyperalgesia (Fig. 4B).

### Maternal deprivation and partial restraint stresses

**Probiotics and gut microbiota.** Maternal deprivation had only a few effects on gut microbiota including: 1) an increase of the Enterococci in the unstressed rats receiving a probiotic [Bla ( $0.6.0 \pm 0.3$  cfu/g), Ljo ( $6.6 \pm 0.3$  cfu/g), or Lpa ( $6.5 \pm 0.3$  cfu/g)] compared with MRS ( $5.0 \pm 0.4$  cfu/g), thus suggesting a non-specific probiotic effect; 2) an increase of Lactobacilli in the colon tissue of the stressed rats receiving Bla ( $7.3 \pm 0.3$  cfu/g) compared with the stressed groups control ( $6.0 \pm 0.3$  cfu/g).

Restraint stress also resulted in minor effects on the gut microbiota: 1) fecal Enterobacteria were decreased in the groups treated with Lpa and Bla, after the stress session ( $5.2 \pm 1.1$  vs.  $4.1 \pm 1.1$  and  $5.1 \pm 0.9$  vs.  $4.2 \pm 0.8$  cfu/g, respectively); 2) the Enterococci were significantly higher in the colonic content of rats receiving Lpa ( $5.1 \pm 0.6$  cfu/g) than in controls ( $4.1 \pm 1.0$  cfu/g) suggesting a strain-dependent probiotic effect. In all rats, total Bifidobacteria, including Bla, was always under the limit of detection. Fingerprinting analysis indicated that Ljo or Lpa were present in amounts  $>7.00$  log<sub>10</sub> cfu/g in 20% of the rats treated with the respective probiotic (data not shown).

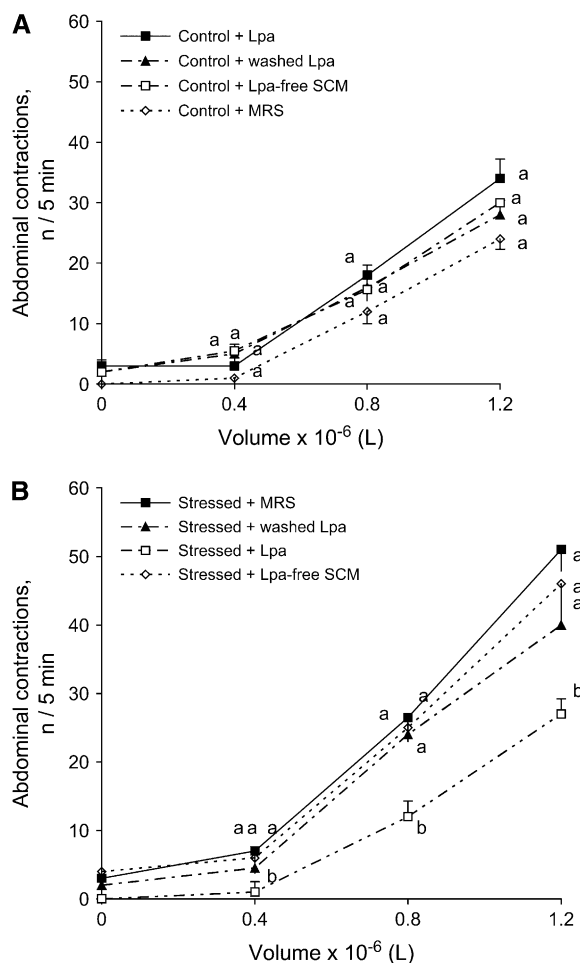


**FIGURE 4** Stress-induced visceral sensitivity in control (A) and partial restraint stressed (B) rats that were or were not treated with Lpa, washed Lpa, Lpa-free SCM, or MRS. Values are means  $\pm$  SEM,  $n = 9$ . At each volume of CRD, labeled means without a common letter differ,  $P < 0.05$ .

## Discussion

Stressful life events are important to trigger IBS symptoms. Acute and chronic stress paradigms have been developed in animals to mimic changes in visceral sensitivity seen in IBS patients. Accumulated data support a potential role of probiotics for alleviating IBS symptoms and suggest that the effects are strain-dependent (11,12,33–35). We clearly show here that 3 probiotic strains have different effects on gut sensitivity and permeability in 2 rat models of psychological stress. Although chronic treatments with Bla and Ljo were unable to reduce MD stress-induced alterations in gut permeability and visceral hypersensitivity, a 2-wk treatment with Lpa reversed visceral hypersensitivity to CRD and completely restored gut paracellular permeability. In addition, using the PRS model, we also observed that Lpa was the most effective probiotic in the prevention of restraint stress-mediated induction of visceral hyperalgesia and demonstrated that this effect involves a synergistic interplay between the Lpa bacteria and the bacterial products generated during the fermentation process.

Several mechanisms by which certain probiotic strains may mediate their different potential therapeutic effects in IBS have been proposed (36). Recent interest has been directed to the potential role of intestinal microbiota in the pathogenesis of IBS

(36–42). However, it is not clear whether altered microbiota has a role in the development of this syndrome or whether it is a consequence of gut dysfunction. Probiotics can restore the intestinal microbiota balance. Our results do not permit the inference as to whether reequilibration of endogenous gut microbiota composition and/or function was part of the probiotic effect observed. The psychological stresses applied only showed a minor impact on the part of the rat microbiota that we investigated. Nor can we associate the effect to the survival of the different probiotics, as their recovery from feces or gut contents of the treated rats was very low. In the case of Ljo or Lpa, this can be attributed to the high rate of colonization by the Lactobacilli of the rat microbiota. This is not unexpected, as it is quite unlikely that exogenous Lactobacilli displace the highly concentrated endogenous Lactobacillus population. The lack of Bla detection may be explained by the fact that Bifidobacteria are not normal commensals of the rat. Indeed, in rodents, the balance of lactic acid bacteria is toward Lactobacilli (43). Although Lpa or Ljo were not present in concentrations over the detection limit in 80% of the rats, only Lpa was able to restore gut dysfunctions altered by both MD and PRS, suggesting that the biological effect was not directly related to the presence of the specific probiotic in the dominant population. Interestingly, Lpa-mediated attenuation of visceral hypersensitivity induced by antibiotic treatment in mice was not accompanied by a normalization of the dominant intestinal microbial species (44).

Intestinal permeability is increased in postinfectious (PI)-IBS and other diarrheic patients (45,6). Stressful life is also a decisive factor to trigger IBS. In rats, PRS as well as MD increase the response to CRD (16,17,22). Changes in colonic paracellular permeability resulting from epithelial cell cytoskeleton contraction are responsible for visceral hyperalgesia induced by stress through myosin light chain kinase activation (23). However, the mechanism by which probiotics may reduce such epithelial barrier dysfunction, and subsequently visceral pain, remains to be elucidated.

Several Lactobacilli adhere to mucosal surfaces inhibiting the attachment of pathogenic bacteria and enhancing the secretion of mucins (46,47). These properties may counterbalance the stress-induced opening of tight junctions (TJ) and subsequently limit the paracellular entry of pathogens. Several pathogenic bacteria modulate intestinal permeability by altering TJ (48). Live probiotics prevent enteroinvasive *Escherichia coli*-induced alteration of the phosphorylation of TJ proteins, occludin and zonula occludens 1 (ZO-1). In addition, they prevent enteroinvasive *E. coli*-induced increase of permeability by decreasing transepithelial resistance of polarized HT29/cl.19A cell monolayers (49).

Immune changes occurring in the intestine of IBS patients are now well documented (50–52). An increased mast cell density has often been reported in the intestinal and colonic mucosa of different IBS subtypes patients (53,54). Interestingly, alleviation of IBS symptoms by the probiotic *B. infantis* 35624 is associated specifically with normalization of the ratio of proinflammatory to antiinflammatory cytokines IL-12/IL-10 (55). Moreover, a mixture of probiotics is able to decrease the production of proinflammatory cytokines, such as IFN $\gamma$  responsible for the increase of intestinal permeability related to stress (56). In rats subjected to MD stress, alterations of gut paracellular permeability are accompanied by an elevated number of colonic mast cells and increased colonic mucosal expression of IL-1 $\beta$ , IL-2, IL-4, IL-10 and IFN $\gamma$  mRNA (27). We measured the cytokine protein levels (data not shown) but were unable to detect an effect of probiotics, as MD only induced a moderate nonsignificant increase of INF- $\gamma$  and IL-1 $\beta$  mucosal levels. The participation of the

immune system in the Lpa effect has already been underlined by 2 studies in mice. In the *Trichinella spiralis* model of PI-IBS, Lpa attenuates muscle hypercontractility by influencing the immune response to infection (26). Furthermore, Lpa therapy prevents visceral hypersensitivity as well as the increase of colonic myeloperoxidase activity and of SP immunoreactivity induced by antibiotic treatment (44).

The role of CpG DNA motifs in probiotic effect has been recently explored (57) and TLR9 has been identified as its mucosal pattern recognition molecule (58,59). A study by Jijon et al. (60) illustrated that epithelial cells not only respond but also differentiate between DNA from probiotic and pathogenic bacteria. Indeed, DNA from the 8 probiotic strains found as part of VSL#3 exhibit significant differences in their individual ability to downregulate epithelial responses. CpG DNA is unlikely to be responsible for Lpa activity as the Lpa-free SCM alone did not have any effect. All SCM preparations from Ljo, Lpa, and Bla contained ~20, 10, and 40 mg DNA/L, respectively, which indicated a consumption of ~20, 10, and 40 ng DNA · d<sup>-1</sup> · rat<sup>-1</sup>. These amounts, in particular that of Lpa, are >0.1% of the amount of bacterial DNA required for exerting an immunostimulatory effect, i.e., 50 µg DNA/mouse (57). Furthermore, the CpG content of the active strain Lpa, and the inactive strain Bla are not different (Lpa: RRCpGYY: 22834, RTCpGYY: 14798 vs. Bla: RRCpGYY: 22904, RTCpGYY: 14574, where R is A/G and Y is C/T; B. Berger, unpublished data), which argues once more against a CpG motifs-mediated effect.

We showed here that both bacteria and bacterial products generated during fermentation are required to prevent PRS-mediated induction of visceral hyperalgesia. In the mouse model of *T. spiralis* infection, Lpa bacteria were not necessary to attenuate muscle hypercontractility during active therapy (26) but were required to get a long-lasting effect on gut motility (61). Although the animal species (rats vs. mice), the stress applied (infectious vs. psychological) and the outcomes (contractility and motility vs. visceral perception) differed among these studies, they all indicated that the probiotic beneficial effect was linked to interactions between live bacteria, bacterial products, and the host.

In conclusion, Lpa bacteria, together with its bacterial products, normalize intestinal paracellular permeability altered by MD. This implies a regulation of the TJ complex that may limit stress-mediated mast-cell degranulation. We hypothesize that Lpa may reduce visceral hypersensitivity response induced by MD stress linked to intraluminal bacterial products, which can activate the submucosal immune cells sensitizing sensory terminals to mechanical stimuli. However, further investigations aimed at understanding how some probiotic strains are able to regulate the TJ complex and influence intestinal paracellular permeability, are still warranted.

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