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Protective Effect of *Lactobacillus casei* on DMH-Induced Colon Carcinogenesis in Mice

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Abstract The administration of probiotics is a promising approach to reduce the prevalence of colon cancer, a multifactorial disease, with hereditary factors, as well as environmental lifestyle-related risk factors. Biogenic polyamines, putrescine, spermidine, and spermine are small cationic molecules with great roles in cell proliferation and differentiation as well as regulation of gene expression. Ornithine decarboxylase is the first rate-limiting enzyme for polyamine synthesis, and upregulation of ornithine decarboxylase activity and polyamine metabolism has been associated with abnormal cell proliferation. This paper is focused on studying the protective role of *Lactobacillus casei* ATCC 393 in a chemically induced mouse model of colon carcinogenesis, directing our attention on aberrant crypt foci as preneoplastic markers, and on polyamine metabolism as a possible key player in carcinogenesis. BALB/c mice were administered 1,2-dimethylhydrazine dihydrochloride (DMH) to induce colon cancer (20 mg/kg body weight, subcutaneous, twice a week for 24 weeks). *L. casei* ATCC 393 was given orally (10^6 CFU, twice a week), 2 weeks before DMH administration. Hematoxylin and eosin staining, high-performance liquid chromatography, and Western blotting were used to evaluate aberrant crypt foci, urinary polyamines, and ornithine decarboxylase expression in the colon. The experimental data showed that the preventive administration of *L. casei* ATCC 393 may delay the onset of cancer as it significantly reduced the number of DMH-induced aberrant crypt foci, the levels of putrescine, and the expression of ornithine decarboxylase. Hence, this probiotic strain has a prospective role in protection against colon carcinogenesis, and its antimutagenic activity may be associated with the maintenance of polyamine metabolism.

Keywords *Lactobacillus casei* · Colon carcinogenesis · 1,2-Dimethylhydrazine · Putrescine · Ornithine decarboxylase · Aberrant crypt foci

Introduction

Worldwide, colon cancer (CC) is the third most commonly diagnosed cancer and one of the most common causes of cancer-related death in western societies. CC is a multi-step process, multifactorial disease, with hereditary, diet, and lifestyle risk factors [1–3]. A number of therapies are available to CC patients; however, they present relatively low success rate, low survival rate, and high level of recurrence [4]. Aberrant crypt foci (ACF) are the earliest precancerous lesions that appear during colon carcinogenesis and a relevant biomarker of the disease [5]; however, CC is often diagnosed in its later stage, when clinical symptoms become apparent and the success rates fall dramatically. Hence, there is an urgent need for fully efficient therapeutic schemes and prevention therapies of this disease.

Probiotics are health promoters with great prospective applications in CC remediation as a prophylactic food additive or as adjuvant to conventional therapies [3, 6]. The potential roles and mechanisms of action of probiotics, especially for CC prevention and treatment, have received considerable attention and have been extensively reviewed [3, 6–13].

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Updated experimental and clinical evidence of the health benefit and therapeutic effectiveness of different species of the genus *Lactobacillus* are available [14, 15]; in particular, *Lactobacillus casei* is a nonpathogenic probiotic strain commercially available as a health food supplement in several countries. *L. casei* Shirota has been largely studied in clinical trials and experimental models and has demonstrated beneficial effects on the gastro intestinal tract health and the immune system [16].

L. casei ATCC 393 strain is a key microorganism present in fermented dairy product and food. It belongs to the generally recognized as safe (GRAS) list and might be applicable as potent antitumor agent. It has been shown that this strain exerts antiproliferative effects on several cancer cell lines, including the human (HT-29) and mouse (CT26) colon carcinoma cell lines [17, 18], and that this effect is related with apoptotic cell death in colon cancer cells [18]. Furthermore, it was shown that the oral administration of live *L. casei* 393 inhibits growth of colon carcinoma in BALB/c mice inoculated subcutaneously with CT26 colon cancer cells. In addition, it has also been reported that this specific strain is able to adhere to CT26 and HT29 cells [18] to survive passage through the digestive system of Wistar rats and reach the colon to which it transiently adheres [18, 19]. This transient adhesion is important to influence macrobiotic balance and achieve physiological effects of the probiotic. However, there is too little experimental evidence of the effect of this strain in vivo, especially in chemically induced murine model of sporadic CC.

Among the several biochemical alterations in cancer cells, one of the most consistent is the change in intracellular polyamines (PA) content [20–24]. These molecules are small short-chain aliphatic amines ubiquitously present and with great functions in many cellular processes, such as cell growth, cell proliferation, and apoptosis. PA levels are rigorously controlled by complex regulatory mechanisms of synthesis, catabolism, and transport which warranty PA homeostasis in healthy cells; however, PA and their metabolizing enzymes are also tightly linked to neoplastic proliferation. PA biosynthesis is controlled by ornithine decarboxylase (ODC), the first rate-limiting enzyme of PA biosynthetic pathway. ODC converts ornithine to putrescine which is the precursor of the higher PA spermidine and spermine. ODC expression is upregulated in most hyperproliferative cells, thus rapidly growing cancerous tissues usually contain large amounts of PA. The central enzyme in the catabolism of PA is the spermidine-spermine-N-acetyl transferase (SSAT) which catalyzes the acetylation of SPD and SPM to mono or diacetylated derivatives. Acetylated PA are in turn substrate for the polyamine oxidase and degraded to lower PA, recycled within the cell, or exported to blood and urine where they have been proposed as diagnostic markers in initial stages of carcinogenesis [25, 26]. The cellular levels of SSAT are low in normal cells but rapidly rise when intracellular PA levels increase, thus the catabolic pathway helps in controlling PA homeostasis. As a consequence of the correlation between cancer progression and PA content, these molecules are target in carcinogenesis research and have implications in cancer prevention and treatment [27].

Several mechanisms could explain the effect of *L. casei* against colon carcinogenesis onset. In the present study, we evaluated the anticarcinogenic effect of *L. casei* 393 in a chemically induced mouse model of colon carcinogenesis and attempted to demonstrate whether such positive effect was related with PA metabolism. For this purpose, histopathological (ACF count), biochemical (PA levels), and molecular (ODC protein expression) studies were conducted.

Materials and Methods

Preparation of the Bacterial Strain and Administration to Mice

Lactobacillus casei 393 was purchased from the American Type Culture Collection (*L. casei* ATCC® 393™, USA) and grown to exponential phase in Lactobacillus Mann-Rogosa-Sharp (MRS) broth (Becton, Dickinson and Company, USA) under aerobic conditions at 37 °C [28]. Just before administration, bacteria were harvested and suspended for oral administration in 100 μL of sterile phosphate-buffered saline (PBS), at 10^6 CFU per mouse.

Animals

Inbred, female BALB/c mice were used. At the beginning of the experiment, all animals were 14 to 16 weeks old and weighed 25 ± 2 g. Animal origin, housing, and handling have been described before [29]. The experimental protocol was conducted in accordance with the experimental animal local guidelines of our institution and the government guidelines for animal studies [30].

Determination of the Lethal Dose 50 of DMH in BALB/c Mice

1,2-Dimethylhydrazine hydrochloride (DMH, CH3-NH-NH-CH3) (Sigma-Aldrich, USA) was used as a pre-carcinogen agent [31]. The lethal dose 50 (LD_{50}) assays consisted of the subcutaneous administration of mice ($n = 5$ per concentration) to increasing concentrations (50–70 mg/kg of body weight) of DMH dissolved in saline solution. Mortality was observed for 96 h. Data were recorded and analyzed using EPA Probit Software, version 1.5 [32].

Experimental Groups

The mice were randomly assigned to four experimental groups (10 mice per group). The healthy control group (HC) did not receive any treatment throughout the experimental course. A second control group (LC) received *L. casei* probiotic bacteria only, twice a week (on days 1 and 5), over the course of 31 weeks. The DMH experimental group was only treated with DMH (on day 3) for 24 weekly injections, adjusted to the sublethal dose of 20 mg/kg of body weight. The *L. casei* preventive group (LCP) was injected with DMH to induce carcinogenesis as was done for the DMH group, but also received *L. casei* bacteria for 31 weeks, starting 2 weeks before the onset of the DMH treatment.

Identification and Quantification of ACF

After 31 weeks, mice were anesthetized with a lethal dose of sodium pentobarbital (65 mg/mL), sacrificed and dissected in order to obtain a segment of the distal colon. The segment was washed with physiological saline solution and fixed in 10% buffered formalin (Caledon Laboratories Ltd., Canada) for at least 24 h and later embedded in a paraffin block (McCormick Scientific Paraplast, USA). Five-micron thick paraffin slices were cut in transverse sections and stained according to a classic hematoxylin-eosin (H&E) protocol [33]. For each colon sample, 1 cm^2 of H&E-stained colonic mucosa was scored for ACF under the light microscope. The total number of ACF is reported.

Determination of Urinary Polyamines

The recollection of urine to determine the dynamic of PA levels during the full experimental period presents the advantage that it is non-invasive and harmless for the experimental animal. Urine was collected in sterile conditions at weeks 6, 13, 16, 25, and 31 and stored at −20 °C until analysis. The sample was deproteinized with a solution containing 5% trichloroacetic acid (TCA, Merck, Germany) and 0.05 N HCl (Mallinckrodt Baker, Mexico) in a 1:2 *v/v* proportion, vortexed, and allowed to stand at room temperature for 20 min. The mix was centrifuged at 9,200×*g* for 20 min, and the supernatant was filtered using syringe-driven 0.20-μm filters (Millex-LG, Millipore Corporation, USA). For high-performance liquid chromatography (HPLC) analysis of the main free biogenic PAs, putrescine, spermidine, and spermine, the ion exchange principle was applied, and post-column derivatization using o-phthalaldehyde (Sigma-Aldrich, USA) was used. HPLC conditions have been previously described [34]. A reverse phase C18 column (150 mm × 4.6 mm, Inertsil ODS-2, with 5 μm of particle diameter, Varian GL Sciences Inc., USA) and a Galaxie Chromatography Data Acquisition System were used (version 1.9 SP2b, Varian GL Sciences Inc., USA). Each sample was analyzed in duplicate.

Western Blot Analysis of ODC Protein

ODC was quantified at week 31 at the end of the experiment. Colon tissues were homogenized and lysed in RIPA buffer and a cocktail of proteases and phosphatases inhibitors (Santa Cruz Biotechnology, USA). Then, protein extracts were quantified by the micro method of Bradford (Bio-Rad Laboratories, USA) and used for Western blot analysis. Twenty micrograms of protein were separated by SDS-PAGE and transferred onto PVDF membrane (Millipore Corporation, USA) as described previously [35]. Membranes were exposed to primary mouse monoclonal anti-ODC antibody (diluted 1:30,000) or the primary mouse anti-β-actin antibody (diluted 1:16,000) as a control. Horseradish peroxidase-labeled goat anti-mouse IgG was used as a secondary antibody (1:50,000). All antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). For chemiluminescence detection, the ECL Prime Western Blotting Detection Reagents were used (Amersham GE healthcare, UK). In addition, the X-ray films (Kodak) were scanned and proteins quantified using the 1D Image Analysis Software 3.5 package (Eastman Kodak Company, USA). Finally, ODC protein expression values were normalized to that of β-actin.

Statistical Analysis

Differences between groups were analyzed by ANOVA with Bonferroni post-hoc test (two tailed) for parametric data (ACF, putrescine, ODC), and Kruskal-Wallis and Mann-Whitney *U* tests for non-parametric data (spermidine), using SPSS version 17 software.

Results

Lethal Dose 50 of DMH in BALB/c Mice

The Probit analysis of mortality of female BALB/c mice after 96 h of exposure to different concentrations of DMH revealed a LD$_{50}$ of 55 mg/kg of body weight (bw). This data allowed proceeding with the carcinogenesis induction in these mouse strain using the sublethal dosage of 20 mg/kg bw/week.

Effect of *L. casei* ATCC 393 on Aberrant Crypt Foci

Figure 1 shows representative histopathological microphotographs of colonic sections and Fig. 2 presents the ACF data. A significantly lower number of ACF was found in the LCP group (23 ± 2.5 ACF) compared to the DMH group which developed abundant ACF (72 ± 6.5 ACF)
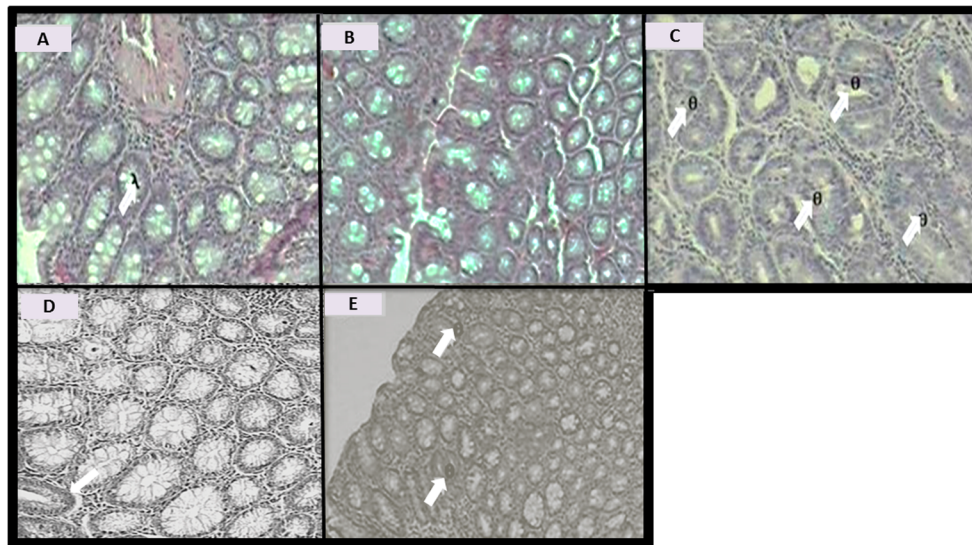
Fig. 1 Representative views of the histopathology of distal colon tissue of BALB/c mice at week 31 of experimentation. **a** Normal architecture of colonic crypts (λ) of healthy control (*HC*) mice (×40). **b** Intact architecture of *L. casei* ATCC 393 control (*LC*) mice (×40). **c** Abundant aberrant crypt foci (*ACF*, θ), which correspond to multiple hyperplasic lesions, in DMH mice (×40). **d** and **e** Combination of normal and damaged structures in colon tissue of mice from the *L. casei* ATCC 393 preventive group (*LCP*) (x40 and x10, respectively)

($p < 0.01$). Moreover, the histopathological microphotographs of the colonic sections of the DMH group showed severe inflammatory infiltrates, compared to the other groups, even though this characteristic was not scored. As expected, no ACF were found in the HC and LC groups.

Effect of *L. casei* ATCC 393 on Urinary Polyamine Levels

Figure 3a presents the putrescine data at week 6. Putrescine concentration was significantly higher in the DMH group (0.3844 nmol/μl) ($p < 0.05$) compared to the other groups. Interestingly, the LCP group maintained
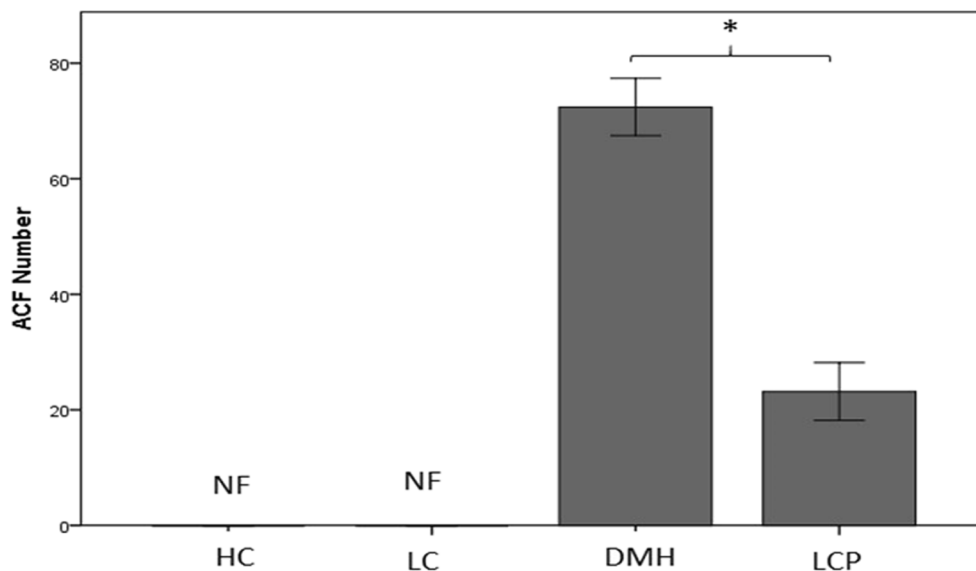

Fig. 2 Effect of *L. casei* ATCC 393 on the formation of aberrant crypt foci (number of ACF) in the distal colon tissue of BALB/c mice treated with DMH and control groups, at week 31 of experimentation. Healthy control (*HC*), *L. casei* ATCC 393 control (*LC*), DMH (*DMH*), and *L. casei* ATCC 393 preventive (*LCP*) groups are shown. Data are presented as means ± SE. One-way ANOVA and Bonferroni post hoc tests were applied to the experimental data. **Indicates statistical significance ($p < 0.01$) between experimental groups. *NF* indicates that ACF were not found. For each colon sample, 1 cm² of H&E-stained colonic mucosa was scored for ACF under a light microscope

putrescine levels (0.1784 nmol/µl) similar at the healthy control group (0.1758 nmol/µl). No statistical differences were found between the HC, LCP, and LC group (0.1658 nmol/µl).

Remarkably similar results were found at week 31 (Fig. 3b) as the LCP mice presented significantly lower levels of putrescine (0.1291 nmol/µl) than the DMH group (0.2893 nmol/µl) ($p < 0.05$) and maintained no statistical differences in the level of this PA when compared to the HC (0.1397 nmol/µl), and LC (0.1314 nmol/µl) groups.

The dynamic of putrescine concentration in urine of BALB/c mice over the full course of the study is presented in Fig. 3c. Interestingly, putrescine levels transiently decreased in the DMH group at weeks 13, 16, and 25, compared to weeks 6 and 31, reaching similar values to the HC, LC, and LCP groups.

The comparison of urinary spermidine levels (pmol/µl) between the different groups at varying time is presented in Table 1. The dynamics of spermidine and putrescine in urine of DMH mice followed similar patterns. Spermidine concentration significantly increased ($p < 0.05$) at weeks 6 and 31 in the DMH group (40.57 ± 32.2 and 25.1 ± 2.0, respectively) compared with the HC group (5.49 ± 5.48 and 7.83 ± 4.35, respectively) and transiently decreased in the DMH group at week 16 (11.9 ± 1.15) reaching, at this sampling date, similar values to the other groups (HC, LC, and LCP). The spermidine levels decreased in the LCP group at weeks 6 and 31 (12.60 ± 8.5 and 14.03 ± 3.3, respectively) compared to the DMH group, even though the differences did not reach significance.

Urinary spermine was not detected in any of our experimental conditions (data not shown).
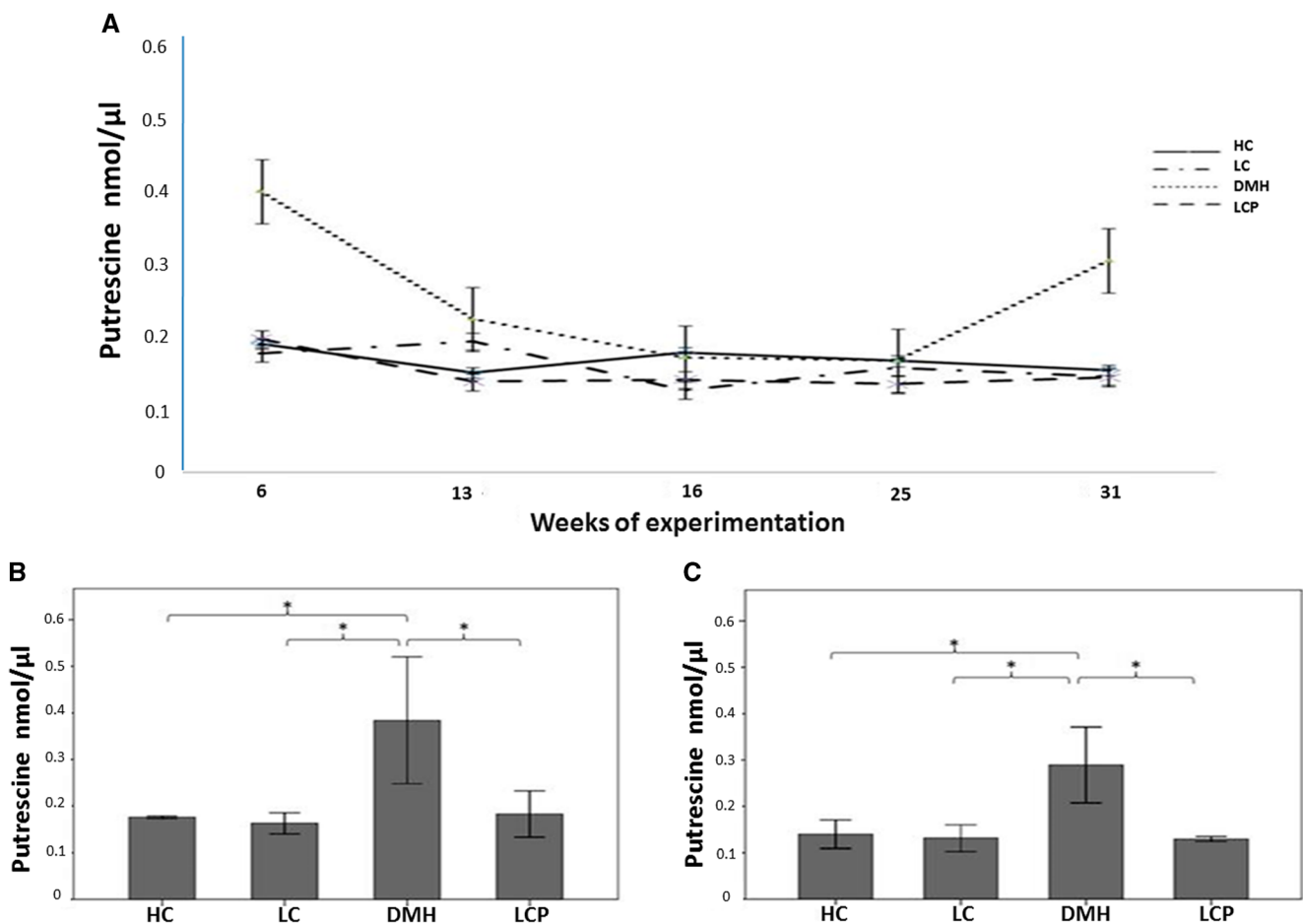

Fig. 3 Effect of *L. casei* ATCC 393 on putrescine urinary levels. **a** Putrescine urinary levels at week 6. **b** Putrescine urinary levels at week 31. **c** Time-course evaluation of putrescine (nmol/µl) in the urine of BALB/c mice. Data (nmol/µl) are presented as means ± SE ($n = 5$).

One-way ANOVA and Bonferroni post hoc test were applied to the experimental data. *Indicates statistically significant differences ($p < 0.05$) between experimental groups

Effect of *L. casei* ATCC 393 on ODC Expression

The expression of ODC in colon was evaluated by Western blotting at the end of the experiment (week 31). Results indicate that the expression of ODC was significantly lower in the LCP group (1.861 ± 0.557) ($p < 0.05$) than in the DMH group (3.076 ± 0.208) which coincides with the dynamic of urinary putrescine in this same week. No statistical differences were observed in the expression of ODC between the HC (1.602 ± 0.344), LC (2.070 ± 0.311), and LCP groups (Fig. 4).

Discussion

In view of the essential role of food in colon carcinogenesis, the prospect of dietary bacteria to delay its onset is a matter of great interest. Recent in vitro studies of *L. casei* 393 have focused on its antiproliferative and pro-apoptotic effects on several cancer cell lines [17, 18, 36–38] and on colonic tumor size reduction [18]. The present research used a DMH-induced mouse model for the study of the protective effect of *L. casei* 393 against carcinogenesis and focused attention on PA metabolism as a possible key player and on ACF as preneoplastic markers.

In the liver of mice, DMH is metabolized to azoxymethane and then to methylazoxymethanol, which leads to methyl carbonium ion, believed to be the ultimate carcinogen which binds stem cells DNA in the epithelium of the distal colon of murine models [39]. ACF are precursor lesions of colon cancer both in rodents and in humans. These preneoplastic lesions may progress to early adenoma, advanced adenoma and, finally, malignant neoplastic injuries [5, 31, 39]. In later stages adenomas can appear in the rectal area as observed in our laboratory in a preliminary experiment that lasted 40 weeks.

ACF counts have been used as biomarkers of carcinogenesis in animal studies aimed at the identification of colon carcinogens and putative chemopreventive or anticancer agents. In this context, we showed here for the first time that the preventive ingestion of *L. casei* 393 was efficient in decreasing the number of ACF in BALB/c mice at week 31 of experimentation even in the continuous presence of DMH and, hence, significantly delay the initial stages of colon carcinogenesis. This experimental data complements previous findings reporting that other probiotic strains have beneficial effects, reducing precancerous lesions against chemically induced models of carcinogenesis [23, 40, 41].

Several mechanisms by which probiotics may act in a positive fashion against neoplastic transformation have been described. In particular, the transient adherence of *L. casei* 393 to the colonic epithelial cells may preserve the epithelial barrier and thereby reduce the binding and contact time of epithelial cells with the carcinogen and its metabolites; otherwise, the probiotic may bind to the mutagen, favor its early degradation, avoid its entrance to the cell, DNA damage and succeeding mutations [19, 42–44]. Moreover, as described for other probiotics, *L. casei* may increase the production of short-chain fatty acids such as butyrate, found to play an important role in decreasing proliferation and inducing apoptosis of damaged cells [3, 44, 45].

Some of the abovementioned mechanisms may also involve PA synthetic and catabolic pathways. Mutations of genes involved in the regulation of cell cycle identified in CC epithelial cells, include the deactivation of the tumor suppressor gene *Apc* (*adenomatous polyposis coli*) and the mutational activation in the oncogen *K-ras* [46]. Several notable genetic mutations in *Apc* and *K-Ras* have been described within the colonic crypts of CC patients [5]. *Apc* and *K-Ras* gen products are important factors for cell cycle, involved in cancer progression and growth through PA metabolism. The inhibition of *Apc* is related with an increase in ODC activity and PA biosynthesis, meanwhile the activation of *K-Ras* downregulates the expression of SSAT [47–50].

Our experimental data showed that the exposure to DMH exacerbated putrescine and spermidine synthesis but the preventive administration of *L. casei* 393 was able to maintain PA

Table 1 Comparison of spermidine levels (pmol/μl) in the urine of BALB/c mice from the different groups at weeks 6, 16, and 31 of experimentation

Sampling week	HC	LC	DMH	LCP
6	5.49 ± 5.48	13.36 ± 2.35	40.57 ± 32.2*	12.60 ± 8.5
16	15.35 ± 9.47	18.33 ± 6.8	11.9 ± 1.15	9.39 ± 1.9
31	7.83 ± 4.35	16.36 ± 3.6	25.1 ± 2.0*	14.03 ± 3.3

Experimental data are given as median ± SE ($n=5$)

HC healthy control, *LC Lactobacillus casei* control, *DMH* DMH-only control group, *LCP L. casei* preventive group

*Indicates statistical differences $p < 0.05$ compared with HC group according to Mann-Whitney *U* test
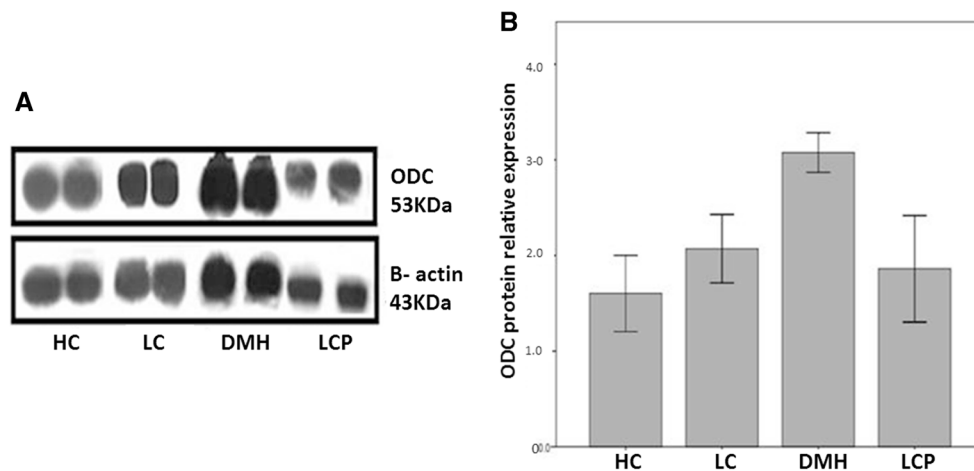
Fig. 4 Effect of *L. casei* ATCC 393 on ODC expression in distal colon tissue of BALB/c mice. **a** Representative blots of each study group. The specific bands for ODC (53 KDa) and β-actin (43 KDa) can be observed. β-actin was used as a constitutive protein. **b** Graphic representation of ODC expression normalized against β-actin. Healthy control (*HC*), *L. casei* ATCC 393 control (*LC*), DMH (*DMH*), and *L. casei* ATCC 393 preventive (*LCP*) groups are shown. One-way ANOVA and Bonferroni multiple range tests were applied to the experimental data, which are expressed as means ± SE. *Indicates statistically significant differences ($p < 0.05$) between experimental groups

metabolism at levels of the control group even in presence of DMH. These data match a previous report that the consumption of *Bifidobacterium longum* decreases ODC activity and PAs content in colon tumors [51] and provide further experimental evidence that probiotics can actually modulate PA content in colon cells. In view of the time-course evaluation of putrescine in the group of mice exposed to DMH for 24 weeks, we hypothesize that colonic epithelial cells exposed to DMH may have suffered early mutations in the *Apc* gene which lead to the upregulation of *Odc* gene expression and exacerbated synthesis of putrescine. These mutated cells would still be able to recuperate PA homeostasis through upregulation of SSAT as observed during intermediate weeks of experimentation. However, the constant exposure to the carcinogen may have induced an activating mutation in *K-Ras* and the consequent loss of the PA catabolic pathway, as indicated by the high levels of putrescine and ODC in the DMH group at the end of the experimental period. This would explain the increased number of ACF in colon of DMH-treated mice. By opposite, our results from mice that received *L. casei* in a preventive way indicate that the protective effect of *L. casei* against carcinogenesis may result, at least in part, from the prevention of mutations in *Apc* and K-*Ras*, among other important cell cycle genes, which hence allowed the maintenance of PA homeostasis and the control of cell proliferation within the colonic crypts.

Among the several mechanisms by which probiotics may act in a positive fashion against neoplastic transformation, we cannot rule out that probiotic strains, modulate the intestinal microbiota as antagonist of pathogens, participate in epigenetic changes, and also affect the innate and acquired immune systems of the host. Further studies of the role of *L. casei* 393 on BALB/c mice immune response are being undertaken.

Conclusion

In conclusion, the preventive ingestion of *L. casei* ATCC 393 could have a protective role against colon carcinogenesis, as it decreased the formation of precancerous lesions. The results of the evaluation of colonic ODC and urinary putrescine and spermidine provide us with evidence that the anticarcinogenic property of this probiotic strain involves the maintenance of PA homeostasis within colon epithelial cells, probably through antimutagenic activity.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Azcárate-Peril MA, Sikes M, Bruno-Bárcena JM (2011) The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? Am J Physiol Gastrointest Liver Physiol 301(3):G401–G424. doi:10.1152/ajpgi.00110.2011

2. Pericleous M, Mandair D, Caplin ME (2013) Diet and supplements and their impact on colorectal cancer. J Gastrointest Oncol 4:409–423. doi:10.3978/j.issn.2078-6891.2013.003

3. Kumar KS, Sastry N, Polaki H, Mishra V (2015) Colon cancer prevention through probiotics: an overview. J Cancer Sci Ther 7(2):081–092. doi:10.4172/1948-5956.1000329

4. Mishra J, Drummond J, Quazi SH, Karanki SS, Shaw JJ, Chen B, Kumar N (2013) Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis. Crit Rev Oncol Hematol 86(3):232–250. doi:10.1016/j.critrevonc.2012.09.014

5. Wargovich MJ, Brown VR, Morris J (2010) Aberrant crypt foci: the case for inclusion as a biomarker for colon cancer. Cancers 2(3):1705–1716. doi:10.3390/cancers2031705

6. Kahouli I, Tomaro-Duchesneau C, Prakash S (2013) Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. J Med Microbiol 62:1107–1123. doi:10.1099/jmm.0.048975-0

7. Uccello M, Malaguarnera G, Basile F, D'agata V, Malaguarnera M, Bertino G, Vacante M, Drago F, Biondi A (2012) Potential role of probiotics on colorectal cancer prevention. BMC Surg 12(Suppl 1):S35. doi:10.1186/1471-2482-12-S1-S35

8. Baffoni L, Gaggìa F, Di Gioia D, Biavati B (2012) Role of intestinal microbiota in colon cancer prevention. Ann Microbiol 62:15–30. doi:10.1007/s13213-011-0306-6

9. Shmuely H, Domniz N, Cohen D (2013) Probiotics in the prevention of colorectal cancer. Curr Colorectal Cancer Rep 9(1):31–36. doi:10.1007/s11888-012-0153-2

10. Patel S, Goyal A (2013) Evolving roles of probiotics in cancer prophylaxis and therapy. Probiotics Antimicro Prot 5(1):59–67. doi:10.1007/s12602-012-9124-9

11. Serban DE (2014) Gastrointestinal cancers: influence of gut microbiota, probiotics and prebiotics. Cancer Lett 345(2):258–270. doi:10.1016/j.canlet.2013.08.013

12. Chong ESL (2014) A potential role of probiotics in colorectal cancer prevention: review of possible mechanisms of action. World J Microbiol Biotechnol 30:351–374. doi:10.1007/s11274-013-1499-6

13. Senan S, Prajapati JB, Joshi CG (2015) Feasibility of genome-wide screening for biosafety assessment of probiotics: a case study of Lactobacillus helveticus MTCC 5463. Probiotics Antimicro Prot 7(4):249–258. doi:10.1007/s12602-015-9199-1

14. Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T (2016) Mechanisms and therapeutic effectiveness of lactobacilli. J Clin Pathol 69(3):187–203. doi:10.1136/jclinpath-2015-202976

15. Chandra P (2016) Effect of lactobacillus on biological properties: anticancer, immunomodulatory properties and improvement of bone health. J Microbiol Biotech Res 6(3):17–23

16. Ishikawa H, Akedo I, Otani T, Suzuki T, Nakamura T, Takeyama I, Ishiguro S, Miyaoka E, Sobue T, Kakizoe T (2005) Randomized trial of dietary fiber and Lactobacillus casei administration for prevention of colorectal tumors. Int J Cancer 116(5):762–767. doi:10.1002/ijc.21115

17. Choi SS, Kim Y, Han KS, You S, Oh S, Kim SH (2006) Effects of lactobacillus strains on cancer cell proliferation and oxidative stress in vitro. Lett Appl Microbiol 42(5):452–458. doi:10.1111/j.1472-765X.2006.01913.x

18. Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou E, Lamprianidou EE, Saxami G, Ypsilantis P, Lampri ES, Simopoulos C, Kotsianidis I, Galanis A, Kourkoutas Y, Dimitrellou D, Chlichlia K (2016) Lactobacillus casei exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. PLoS One 11(2):e0147960. doi:10.1371/journal.pone.0147960

19. Saxami G, Ypsilantis P, Sidira M, Simopoulos C, Kourkoutas Y, Galanis A (2012) Distinct adhesion of probiotic strain Lactobacillus casei ATCC 393 to rat intestinal mucosa. Anaerobe 18(4):417–420. doi:10.1016/j.anaerobe.2012.04.002

20. Mandal S, Mandal A, Johansson HE, Orjalo AV, Park MH (2013) Depletion of cellular polyamines, spermidine and spermine, causes a total arrest in translation and growth in mammalian cells. Proc Natl Acad Sci U S A 110(6):2169–2174. doi:10.1073/pnas.1219002110

21. Nowotarski SL, Woster PM, Casero RA Jr (2013) Polyamines and cancer: implications for chemotherapy and chemoprevention. Expert Rev Mol Med 15:e3. doi:10.1017/erm

22. Pegg AE (2016) Functions of polyamines in mammals. J Biol Chem 291(29):14904–14912. doi:10.1074/jbc.R116.731661

23. Russo F, Linsalata M, Orlando A (2014) Probiotics against neoplastic transformation of gastric mucosa: effects on cell proliferation and polyamine metabolism. World J Gastroenterol 20(37):13258–13272. doi:10.3748/wjg.v20.i37.13258

24. Kusano T, Suzuki H (2015) Polyamines: a universal molecular nexus for growth, survival, and specialized metabolism. Springer, Tokyo

25. Paik MJ, Kuon D, Cho J, Kim KR (2009) Altered urinary polyamine patterns in cancer patients under acupuncture therapy. Amino Acids 37:407–413. doi:10.1007/s00726-008-0169-8

26. Nakayama Y, Torigoe T, Minagawa N, Yamaguchi K (2012) The clinical usefulness of urinary N^1,N^{12}-diacetylspermine (DiAcSpm) levels as a tumor marker in patients with colorectal cancer. Oncol Lett 2:970–974. doi:10.3892/ol.2012.625

27. Babbar N, Gerner EW (2011) Targeting polyamines and inflammation for cancer prevention. Recent Results Cancer Res 188:49–64. doi:10.1007/978-3-642-10858-7_4

28. Chen CC, Lin WC, Kong MS, Shi HN, Walker WA, Lin CY, Huang CT, Lin YC, Jung SM, Lin TY (2012) Oral inoculation of probiotics Lactobacillus acidophilus NCFM suppresses tumour growth both in segmental orthotopic colon cancer and extra-intestinal tissue. Br J Nutr 107:1623–1634. doi:10.1017/S0007114511004934

29. Zamora-González EO, Santerre A, Palomera-Avalos V, Morales-Villagrán A (2013) A chronic combinatory stress model that activates the HPA axis and avoids habituation in BALB/c mice. J Neurosci Meth 213:70–75. doi:10.1016/j.jneumeth.2012.10.015

30. NOM-062-ZOO-1999 (1999) Norma oficial mexicana, especificaciones técnicas para la producción, cuidado y uso de los animales de Laboratorio. http://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF. Accessed 31 October 2013

31. Rosenberg DW, Giardina C, Tanaka T (2009) Mouse models for the study of colon carcinogenesis. Carcinogenesis 30:183–196. doi:10.1093/carcin/bgn267

32. Hahn ED, Soyer R (2005) Probit and logit models: Differences in a Multivariate Realm. http://home.gwu.edu/_soyer/mv1h.pdf, accessed 20 July 2016

33. Kiernan JA (2008) Histological and histochemical methods: theory and practice. Scion Publishing, UK

34. Farkas S, Hajós G (1998) Monitoring of biologically active amines in cereals and cereal based food products by HPLC. Chromatographia 48:37–42. doi:10.1007/BF02467513

35. Chi W, Song X, Jiang C, Liu X, Li W, Wang X (2006) Lentiviral vector-mediated downregulation of ornithine decarboxylase

inhibits tumor cell growth in vitro and in vivo. Tumor Biol 27:243–251. doi:10.1159/000094843

36. Kim SN, Lee WM, Park KS, Kim JB, Han DJ, Bae J (2015) The effect of *Lactobacillus casei* extract on cervical cancer cell lines. Contemp Oncol (Pozn) 19(4):306–312. doi:10.5114/wo.2014.45292

37. Soltan Dallal MM, Mojarrad M, Salehipour Z, Atapour Mashhad H, Raoofian R, Rajabi Z (2012) Effects of probiotic *lactobacillus acidophilus* and *lactobacillus casei* on the behavior of colorectal tumor cells. Tehran Univ Med J 70(4):220–227

38. Lenoir M, Del Carmen S, Cortes-Perez NG, Lozano-Ojalvo D, Muñoz-Provencio D, Chain F, Langella P, de Moreno de LeBlanc A, LeBlanc JG, Bermúdez-Humarán LG (2016) *Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. J Gastroenterol doi. doi:10.1007/s00535-015-1158-9

39. Corpet DE, Taché S (2002) Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. Nutr Cancer 43(1):1–21. doi:10.1207/S15327914NC431_1

40. Park E, Jeon GI, Park JS, Paik HD (2007) A probiotic strain of *Bacillus polyfermenticus* reduces DMH induced precancerous lesions in F344 male rat. Biol Pharm Bull 30(3):569–574

41. Verma A, Shukla G (2013) Probiotics *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* suppresses DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. Nutr Cancer 65(1):84–91. doi:10.1080/01635581.2013.741746

42. Ohland CL, Macnaughton WK (2010) Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Gastrointest Liver Physiol 298(6):G807–G819. doi:10.1152/ajpgi.00243.2009

43. Le Leu RK, Brown IL, Hu Y, Bird AR, Jackson M, Esterman A, Young GP (2005) A synbiotic combination of resistant starch and *Bifidobacterium lactis* facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. J Nutr 135(5):996–1001

44. Raman M, Ambalam P, Kondepudi KK, Pithva S, Kothari C, Patel AT, Purama RK, Dave JM, Vyas BR (2013) Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes 4(3):181–192. doi:10.4161/gmic.23919

45. Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, Gautam SK, Singh B (2013) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev 71(1):23–34. doi:10.1111/j.1753-4887.2012.00542.x

46. McIntyre RE, Buczacki SJA, Arends MJ, Adams DJ (2015) Mouse models of colorectal cancer as preclinical models. BioEssays 37(8):909–920. doi:10.1002/bies.201500032

47. Gerner EW, Meyskens FL Jr (2004) Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 4(10):781–792

48. Babbar N, Gerner EW (2011) Targeting polyamines and inflammation for cancer prevention. Recent Res Cancer 188:49–64. doi:10.1007/978-3-642-10858-7_4

49. Soda K (2011) The mechanisms by which polyamines accelerate tumor spread. J Exp Clin Cancer Res 30:95. doi:10.1186/1756-9966-30-95

50. Ramani D, De Bandt JP, Cynober L (2014) Aliphatic polyamines in physiology and diseases. Clin Nutr 33:14–22. doi:10.1016/j.clnu.2013.09.019

51. Reddy BS (1999) Possible mechanisms by which pro- and prebiotics influence colon carcinogenesis and tumor growth. J Nutr 129(7 Suppl):1478S–1482S