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Nutrition and Cancer
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t775653687

Probiotic *Lactobacillus Acidophilus* and *L. Casei* Mix Sensitize Colorectal Tumoral Cells to 5-Fluorouracil-Induced Apoptosis

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Online publication date: 31 March 2010

To cite this Article Baldwin, Cindy , Millette, Mathieu , Oth, Daniel , Ruiz, Marcia T. , Luquet, François-Marie andLacroix, Monique(2010) 'Probiotic *Lactobacillus Acidophilus* and *L. Casei* Mix Sensitize Colorectal Tumoral Cells to 5-Fluorouracil-Induced Apoptosis', Nutrition and Cancer, 62: 3, 371 — 378

To link to this Article: DOI: 10.1080/01635580903407197
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Nutrition and Cancer, 62(3), 371–378
Copyright © 2010, Taylor & Francis Group, LLC
ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635580903407197

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Probiotic *Lactobacillus Acidophilus* and *L. Casei* Mix Sensitize Colorectal Tumoral Cells to 5-Fluorouracil-Induced Apoptosis

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To assess the potential of *Lactobacillus acidophilus* and *Lactobacillus casei* strains to increase the apoptosis of a colorectal cancer cell line in the presence of 5-fluorouracil (5-FU), LS513 colorectal cancer cells were treated for 48 h with increasing concentrations of these lactic acid bacteria (LAB) in the presence of 100 μg/ml of 5-FU. In the presence of 10^8 CFU/ml of live LAB, the apoptotic efficacy of the 5-FU increased by 40%, and the phenomenon was dose dependent. Moreover, irradiation-inactivated LAB caused the same level of induction, whereas microwave-inactivated LAB reduced the apoptotic capacity of the 5-FU. When cells were treated with a combination of live LAB and 5-FU, a faster activation of caspase-3 protein was observed, and the p21 protein seems to be downregulated. These results suggest that live *L. acidophilus* and *L. casei* are able to increase the apoptosis-induction capacity of 5-FU. The mechanisms of action are still not elucidated, and more research is needed to understand them. This is the first set of experiments demonstrating that some strains of LAB can enhance the apoptosis-induction capacity of the 5-FU. Based on these results, it is possible to speculate that LAB or probiotics could be used as an adjuvant treatment during anticancer chemotherapy.

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†Daniel Oth is now a retired professor.
Submitted 11 May 2009; accepted in final form 3 August 2009.
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INTRODUCTION

Cancer is a major public health problem worldwide. In North America, colorectal cancer (CRC) is the second most frequent cause of cancer-related deaths after lung cancer (1,2). It was estimated that in 2008, in the United States, 148,810 cases would be diagnosed with this cancer and that 49,960 persons would die from it (2), whereas 21,500 new cases would be diagnosed and 8,900 persons would die from this disease in Canada (1).

In the mid 1990s, the treatment of metastatic CRC was based on 5-fluorouracil (5-FU) alone; at that moment, the response rate (RR) was about 20%, with a progression-free survival (PFS) of 4 mo and a median survival time of 12 mo. Today, combination of 5-FU with novel molecules have improved the RR in the first-line treatment of advanced CRC to 40–50%, the PFS is approximately 8 mo, and the median survival time is 15 to 20 mo (3,4). These novel compounds include irinotecan, capecitabine, oxaliplatin, cetuximab, bevacizumab, and panitumumab, which have been recently approved by the U.S. Food and Drug Administration (FDA) for the treatment of CRC (5). Despite many advances, the prognosis of metastatic CRC remains poor due to the resistance of cancer cells to conventional chemotherapeutic drugs; therefore the search for adjuvant or alternative therapies is of utmost importance.

Dietary factors and colonic microbiota seem to play an important role in colorectal carcinogenesis, making the study of the potential protective role of probiotics of high interest. It is well known that some inhabitants of the indigenous colonic microbiota are able to produce metabolites from the degradation of

dietary substrates (6). Lactic acid bacteria (LAB) have been widely used in various fermented food products around the world for many centuries. The preservative role and health benefits of fermented milk (7), kefir (8), and yogurt (9) are now recognized. Some LAB are also considered probiotics, which are defined as live microorganisms that when administered in adequate amounts, confer a health benefit on the host (10). The consumption of probiotics can result in a theoretical prevention of CRC by stimulating the immune system (11); reducing the incidence of microbial infection (reviewed in (12)); binding toxic compounds (13); reducing the activity of carcinogenic fecal enzymes such as azoreductase, β-glucuronidase, and nitroreductase (reviewed in (14)); and reducing the incidence of precancerous lesions as proven in animal models (6). A clinical trial demonstrated that the daily administration of a *Lactobacillus acidophilus* (*L. acidophilus*) and *Lactobacillus casei* (*L. casei*) probiotic fermented milk was safe and effective in the prevention of antibiotic-associated diarrhea in hospitalized patients (15). This study also demonstrated the potential of this probiotic milk to reduced *Clostridium difficile*-associated diarrhea incidence. Millette et al. (16) established the antimicrobial capacity of this fermented milk against various bacterial pathogens and suggested that the production of organic acids and bacteriocin-like inhibitory substances was involved in the inhibitory activity. Moreover, Millette et al. (17) demonstrated that these lactobacilli were acid and bile resistant. This research also showed that the administration of these probiotics to healthy mice modulates the intestinal microbiota.

Based on this precedent, the research on the potential use of probiotic bacteria as an adjuvant therapy for the treatment of cancer is highly justified. The aim of this project was to demonstrate the capacity of a *L. acidophilus* and *L. casei* probiotic strains mix to increase the apoptosis of a colorectal cell line in presence of 5-FU.

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MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

L. acidophilus CL1285 and *L. casei* LBC80R proprietary mix (LAB mix) is deposited in Institut Pasteur (Paris, France) and was graciously provided by Bio-K + International, Inc. (Laval, Québec, Canada). LAB mix were propagated in Lactobacilli MRS broth (MRS; Difco Laboratories, Detroit, MI) at 37°C for 18 h. LAB mix was stored at –80°C in their media containing 10% (wt/vol) glycerol (Laboratoires MAT, Montréal, Québec, Canada). Before each experiment, the bacterial content of one vial was thawed, transferred in 9 ml of MRS, and activated by two consecutive incubations of 18 h at 37°C. Thereafter, bacteria were washed twice in 10 ml sterile phosphate-buffered saline (PBS) after centrifugation at 4°C for 10 min at 2,000 *g*. A final resuspension was done in 9 ml of supplemented RPMI-1640 without antibiotics (Gibco, Burlington, Ontario, Canada).

Bacterial Enumeration and Inactivation

The bacterial concentration was determined as follows. Serial dilutions of the bacteria were done in sterile peptone (0.1% wt/vol; Difco Laboratories, Detroit, MI), and appropriate dilutions were pour plated in MRS agar and incubated at 37°C for 48 h under anaerobic atmosphere. Colony-forming units (CFU) were enumerated using a Darkfield Quebec Colony Counter (American Optical, Scientific Instrument Division, Keene, OH). Two kinds of bacterial inactivation were assayed in this study. LAB mix was heated using a 2450 MHz microwave oven for 40 s (700 W, Turntable microwave oven, General Electric, The Brick, Laval, Québec, Canada) or treated with γ irradiation at a dose of 9 kGy using a UC15-A irradiator (MDS-Nordion, Laval, Québec, Canada) having a dose rate of 23 kGy/h for the radiation inactivation. This irradiator was certified by the National Institute of Standards and Technology (Gettysburg, PA), and the dose rate was established using a correction for decay of source. After the treatments, the bacterial mortality was verified by plating on MRS agar.

Cell Culture

The colorectal carcinoma cell line CRL-2134 isolated from a primary tumor biopsy, also designated LS513, was obtained from the American Type Culture Collection (Rockville, MD) and cultured in RPMI-1640 medium supplemented with 2 mM of l-glutamine (Gibco), HEPES buffer (1% vol/vol; Gibco), gentamicin, penicillin, and FBS (10% vol/vol). The carcinoma cells were cultured at 37°C under an atmosphere containing 5% CO_2 and 95% humidity. The medium was replaced every 3 days. When confluence was reached, the cells were detached using preheated trypsin (0.25%)-EDTA (0.2 g/l) solution (Gibco) and then counted in a Bürker hemacytometer chamber (Fisher Scientific, Ottawa, Ontario, Canada).

Incubation of Tumorigenic Cells in the Presence of LAB Mix

Cells were seeded in 6-wells plate (Sarstedt, Montréal, Québec, Canada) at 3×10^5 cancer cells/well and incubated as described above, in the presence of 10^6 to 10^9 CFU/ml of active or inactive LAB and 5-FU for 48 h. A concentration of 100 μg/ml of 5-FU was determined in preliminary tests as the concentration allowing 50% of cell apoptosis and was used along the experiments. The cancer cells present in the supernatant were harvested by centrifugation at 500 *g* for 5 min. The cells that adhered to the wells were rinsed with PBS and detached by the addition of 200 μl of trypsin-EDTA for 3 min at 37°C. A volume of 1 ml of supplemented RPMI-1640 (without antibiotics) was added to inactivate the trypsin and centrifuged at 500 g for 5 min. The cell-containing pellets (from the supernatant and the adhered cells) were mixed together and washed twice in PBS containing 0.25% EDTA to avoid clumping.

Apoptosis Measurement

The apoptosis evaluation was done by flow cytometry in presence of propidium iodide (according to (18)). Briefly, 500 μl of a solution containing sodium citrate (0.1% wt/vol; Sigma, St-Louis, MO), triton X-100 (0.1% wt/vol; Sigma), 50 μg/ml of RNase A (Sigma), and 20 μg/ml of propidium iodide (Sigma)

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was used to resuspend the cell pellet. The samples were placed at 4°C for 15 min, and then the percentage of apoptotic cells was determined by flow cytometry (Coulter Epics XL-MCL, Beckman Coulter Canada, Inc., Mississauga, Ontario, Canada).

Immunoblotting

A total number of 6×10^5 tumorigenic cells were seeded in 6-well plates and allowed to adhere overnight. A concentration of 100 μg/ml of 5-FU and 1×10^8 of live or microwave-inactivated *L. acidophilus* and *L. casei* mix was added to the wells, and the cells were incubated as described above. After 0, 3, 6, 12, 24, 36, and 48 h, cells were washed with ice-cold PBS and scraped. The cells mixture was centrifuged at 500 g for 5 min and suspended in 50 μl of lysis buffer composed of 50 mM Tris-hydrochloride (HCl), pH 7.5, 150 mM sodium chloride, 1% Nonidet P-40 (Roche Diagnostics, Laval, Québec, Canada) and one Complete pellet (protease inhibitors cocktail; Roche Diagnostics). The cell-containing pellet was placed on ice for 30 min. Then the solution was transferred into a 1.5-ml tube and centrifuged at 10,000 g for 10 min in order to eliminate the cell debris. The supernatant was mixed with an equal volume of sample buffer [100 mM Tris-HCl (pH 6.8), 2% SDS, 2% β-mercaptoethanol, 20% glycerol, and 1 mg bromophenol blue]. From each sample, 30 μl of cell lysate were loaded onto 12% SDS-PAGE and submitted to electrophoresis at 200 V for 45 min. The proteins were then blotted onto nitrocellulose Hybond ECL membrane (GE Healthcare, Baie d'Urfé, Québec, Canada) at 100 V for 1 h. The blots were blocked with PBS buffer containing 5% skim milk powder and 0.1% Tween-80 [PBS Tween (PBST)] for 60 min at room temperature. Then, the membrane was washed once for 15 min and twice for 5 min in PBST and incubated for 60 min in presence of one of the primary antibodies. Anti-p53 and anti-p21 from mice or anticaspase-3 from rabbit (BD Pharmingen, Mississauga, Ontario, Canada) were placed in primary antibody buffer (PBST with 5% skim milk powder). Then, the blots were washed once for 15 min and twice for 5 min in PBST and incubated for 60 min at room temperature in the presence of 3/10,000 HRP-conjugated mice antiigG (Sigma) in blocking buffer. After a final wash (once for 15 min and twice for 5 min in PBST without skim milk), blots were incubated in ECL solution (GE Healthcare) as described by the manufacturer's instructions, and then chemiluminescence was detected using a AFP imaging mini-med/90 X-ray film processor (AFP Imaging Corp., Elmsford, NY) on a hyperfilm ECL (GE Healthcare).

Determination of the Expression of the Fas Receptor and Fas Ligand

About 5×10^5 LS513 cells/well were used as described before. An incubation of 24 h was done in the presence of 100 μg/ml of 5-FU alone or in combination with increasing concentrations of live or microwave-inactivated LAB. After incubation, cells were detached and washed in RPMI-1640 as described above. The cell pellets were placed on ice, and 50 μl of flow cytometry buffer (PBS, 1% BSA, 0.02% sodium azide, and 0.25% EDTA) was added. Then, a volume of 20 μl of the Fas receptor or Fas ligand antibodies (BD Pharmingen) were added to the cell suspension, kept for 30 min on ice, and washed twice with 4 ml of flow cytometry buffer. A volume of 0.25 μl of streptavidin-phycoerythrin (BD Pharmingen) was then added to the suspension, placed on ice for 30 min, and washed twice. Finally, 250 μl of a solution of 2% paraformaldehyde in PBS and 250 μl of flow cytometry buffer were used to resuspend the cell pellets. The samples were placed at 4°C until the Fas/Fas ligand concentrations were determined by flow cytometry.

Statistical Analysis

To calculate the percentages of apoptotic cells in the presence of 5-FU and LAB, the experiments were repeated 3 times. Analysis of variance (ANOVA) and Duncan's multiple-range test was done using the SPSS statistics program (version 17.0) to determine significant differences in percentages of apoptotic cells between the control (untreated cells) and the LS513 cells treated with 5-FU and/or LAB. All significant differences were determined at a P value ≤ 0.05.

RESULTS

Results presented in Fig. 1 show that a concentration of 100 μg/ml of 5-FU is needed to obtain approximately 50% of apoptotic cells. This concentration was further used for the other experiments in order to assess the apoptosis changes of LS513 cells when they were treated with a combination of 5-FU and live or inactivated LAB. The apoptosis values obtained by cytometry were the percentage of cells (out of 10,000) showing a sub-G1 pattern characteristic of apoptotic DNA. Figure 1 also shows a dose-dependent response curve, indicating a correlation between the quantity of chemotherapeutic agent and the percentage of cells undergoing apoptosis.

The results presented in Fig. 2 show that the treatment of LS513 cells with 100 μg/ml 5-FU in the presence of various

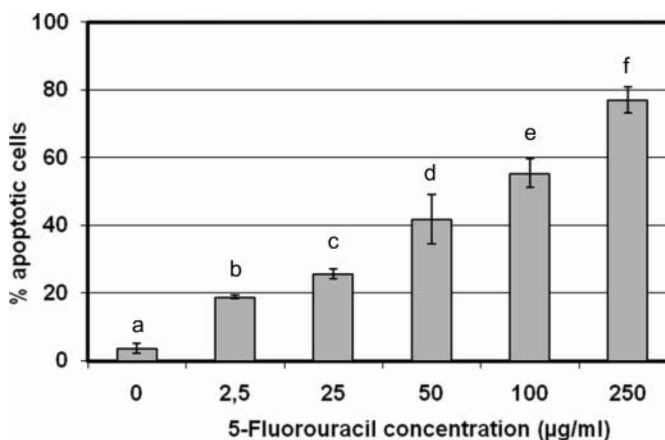

FIG. 1. Effect of 5-fluorouracil (5-FU) on LS513 cell apoptosis induction. LS513 cells were treated for 48 h with increasing concentrations of 5-FU (0–250 μg/ml). a-f: Results with a different letters correspond to a significant difference of apoptosis as compared to the other groups ($P \leq 0.05$).

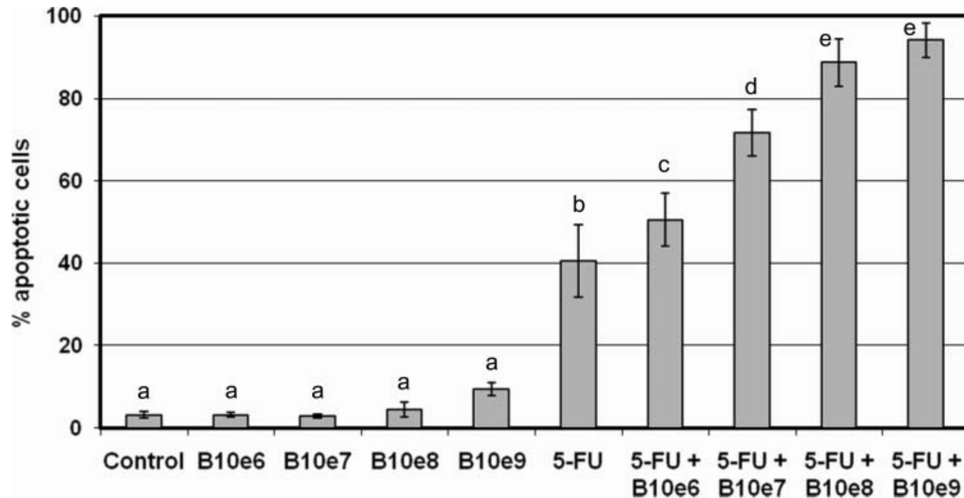

FIG. 2. Effect of live (B) *Lactobacillus acidophilus* and *Lactobacillus casei* mix in the presence of 5-fluorouracil (5-FU) on LS513 cell apoptosis induction. LS513 cells were treated for 48 h with increasing concentrations of lactic acid bacteria in presence of 100 μg/ml of 5-FU.a-e: Results with a different letters correspond to a significant difference of apoptosis as compared to the other groups ($P \leq 0.05$).

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concentrations (>10^6CFU/ml) of live *L. acidophilus* and *L. casei* mix increased significantly ($P \leq 0.05$) the percentage of apoptotic cells. A concentration of 10^8 active LAB doubled the level of cell apoptosis as compared to the cells treated with the 5-FU alone. However, in absence of 5-FU, the presence of live bacteria did not have any influence on the apoptosis of the tumorigenic cell line. In order to verify if the LAB viability was important in this effect, the bacteria were killed by γ irradiation or by a microwave treatment. The results presented in Fig. 3 demonstrate that the irradiation-inactivated LAB in combination with 5-FU had similar apoptotic effects on the LS513 cells as the live LAB mix with 5-FU. The results presented in Fig. 4 show that at concentrations $\geq 10^7$ CFU/ml, microwaved LAB reduced the apoptosis-stimulating effect of the 5-FU as compared to the live or irradiated LAB.

In order to confirm the apoptosis-enhancing effect of the LAB obtained by flow cytometry, the caspase 3 expression was verified by immunoblotting. An antibody raised against the inactive proenzyme form of the molecule was used in this study. When the protein undergoes proteolytic processing during the caspase cascade, it is cleaved in 2 smaller subunits. As a result, it is possible to observe three bands: one of 32 kDa corresponding to procaspase 3 and two other bands that correspond to its cleavage products of 17 kDa and 11 kDa, respectively. Figure 5 shows the expression of the procaspase 3 in the LS513 cells treated with 5-FU alone or in combination with 10^8 active

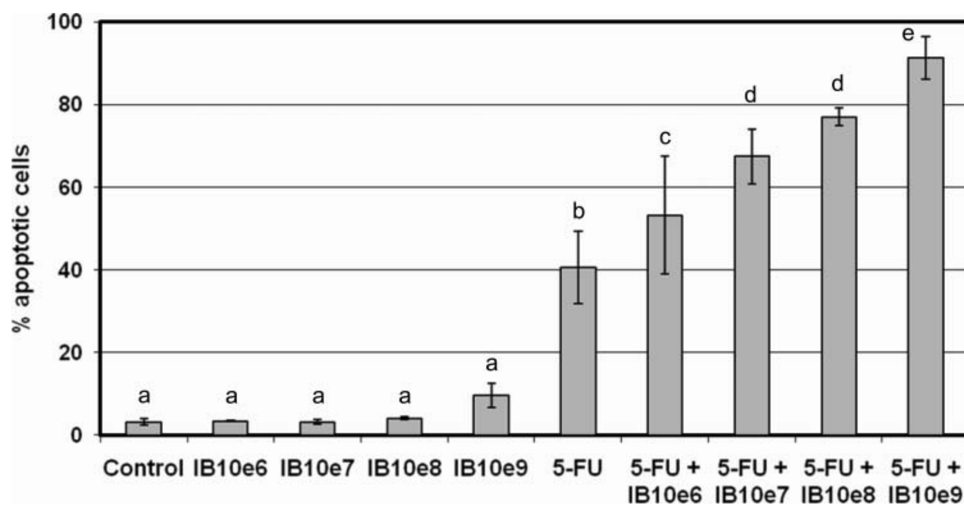

FIG. 3. Effect of irradiation-inactivated (IB) *Lactobacillus acidophilus* and *Lactobacillus casei* mix in the presence of 5-fluorouracil (5-FU) on LS513 cell apoptosis induction. LS513 cells were treated for 48 h with increasing concentrations of lactic acid bacteria in presence of 100 μg/ml of 5-FU. a-e: Results with a different letters correspond to a significant difference of apoptosis as compared to the other groups ($P \leq 0.05$).

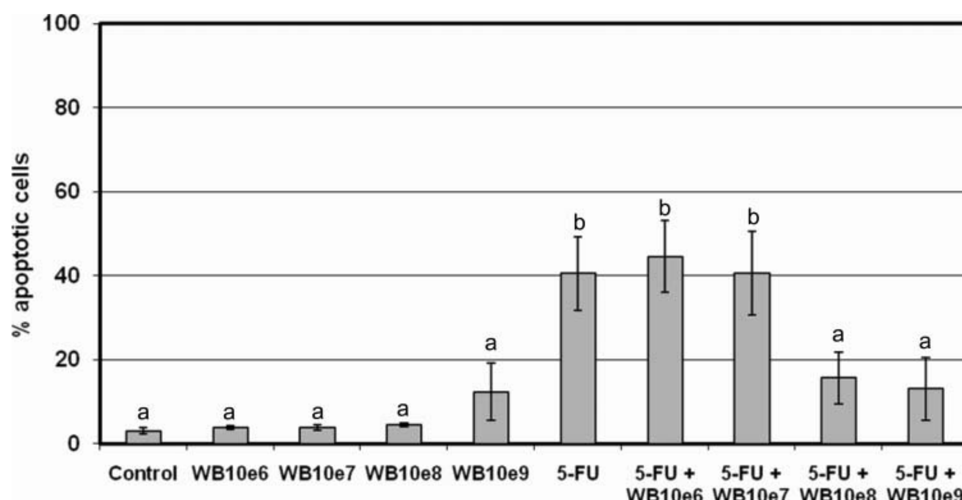

FIG. 4. Effect of microwave-inactivated (WB) *Lactobacillus acidophilus* and *Lactobacillus casei* mix in the presence of 5-fluorouracil (5-FU) on LS513 cell apoptosis induction. LS513 cells were treated for 48 h with increasing concentrations of lactic acid bacteria in presence of 100 μg/ml of 5-FU. a–b: Results with a different letters correspond to a significant difference of apoptosis as compared to the other groups ($P \leq 0.05$).

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or microwave-inactivated LAB. These results demonstrate that 5-FU in presence of live LAB accelerates the proteolytic processing of the inactive form to the active form of the caspase 3 as observed by a faster decline of the 32 kDa band after 24 h of incubation. Moreover, the expression of the procaspase 3 in cells treated with a combination of 5-FU and microwave-killed LAB is the same as observed in cells treated with 5-FU alone.

The results in Fig. 6 show the expression pattern of the p21 protein in the LS513 cells in presence of 5-FU or combination of 5-FU and live or microwave-inactivated bacteria visualized by Western blot. The expression of the p21 increased during time until 36 h and decreased after 48 h when cells were treated with 5-FU alone or in combination with microwave-killed lactobacilli. When the cells were treated with 5-FU in combination with live LAB, the increase in the p21 expression was lower and shorter. The induction was observed up to 24 h, followed by a reduction of the band intensity after 36 and 48 h of incubation.

In addition, results obtained by immunoblotting showed an increase in the expression of the Fas receptor, and the p53 protein, when the LS513 cells were treated with 5-FU alone. However, when 5-FU was associated with live or inactivated LAB, no enhancing effect was observed (results not shown).

DISCUSSION

CRC is the second most frequent cause of cancer-related death in the United States (5). Many epidemiological and clinical studies have demonstrated that lifestyle, including the frequency of physical activity and especially the diet, can reduce the incidence of CRC (19,20). CRC research is currently focused to reduce chemotherapy resistance by tumoral cells with a combination of treatments [chemotherapy, radiotherapy, immunity adjuvants; as reviewed by (21)]. As an example, many clinicians now accept 5-FU/leucovorin/oxaliplatin as the best treatment regimen for patients with resected colon cancer with lymph node metastases, and there are many other clinical trials that have been done to study various combinations of chemotherapeutic agents after surgery to prolong the disease-free survival period (21).

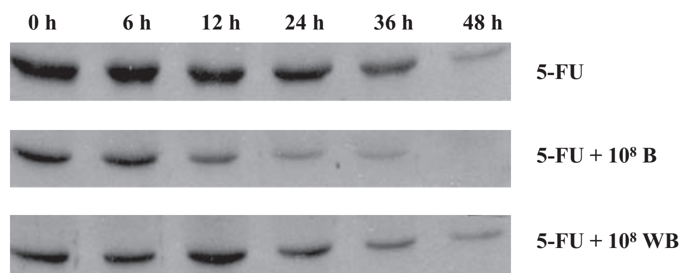

FIG. 5. Caspase 3 expression in LS513 cells treated with 100 μg/ml of 5-fluorouracil (5-FU) alone, in the presence of 10^8 live (B), or in the presence of microwave-inactivated (WB) *Lactobacillus acidophilus* and *Lactobacillus casei* mix for 48 h.

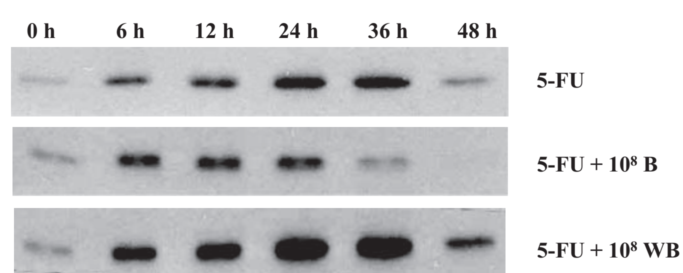

FIG. 6. Expression of the p21 protein in LS513 cells treated with 100 μg/ml of 5-fluorouracil (5-FU) alone, in presence of 10^8 live (B), or in presence of microwave-inactivated (WB) *Lactobacillus acidophilus* and *Lactobacillus casei* mix for 48 h.

In this study, we evaluated the adjuvant potential of a probiotic mix of *Lactobacillus* during treatment of LS513 cells with 5-FU using a simple in vitro model. However, this model had some limitations. First, in this type of experiment, it was not possible to verify if immune system modulation could interfere in this proapoptotic effect, although it is known that the modulation of the immune system is a strain-specific property that is widely recognized for many probiotic LAB (22,23). Furthermore, a tumoral cell line presenting a wild-type p53, a gene that is altered in a majority of human CRC cells (21), was used in this study. Further studies should be done using CRC cell lines with altered p53 (HT-29, Caco-2). Regardless of this limitation, the utilization of the LS513 cell line permits to verify the implication of the p53 on the synergistic apoptotic process observed.

Many studies have demonstrated the proapoptotic effect of the 5-FU in human cell lines (24,25). Our study confirmed this effect and showed that a concentration of 100 μg/ml produces 50% of apoptotic cells. Moreover, some components of LAB or metabolites produced during milk fermentation by LAB are known to exert antiproliferative effects on cancer cells of various types in vitro (26–28). According to Refs. 26 and 29, this is a strain-specific effect; and each effective strain gives a different rate of inhibition. In our study, treatment of the LS513 cells with high concentrations of live or inactivated LAB ($>10^8$ CFU) in presence of 5-FU resulted in a reduction of cell viability as determined by the MTT assay (results not shown). However, when the same concentrations of LAB, in absence of 5-FU, were placed in presence of the tumoral cells, no effect was observed on the percentage of the cell population undergoing apoptotic process. When the cells were treated with a combination of 100 μg/ml of 5-FU and 10^8–10^9 CFU of live or irradiation-inactivated LAB, a synergistic proapoptotic was measured (approximately 40% more cells under apoptosis as compared to 5-FU alone). On the other hand, when microwave-inactivated LAB was combined to 5-FU, the opposite effect was observed, that is, the efficacy of the 5-FU was reduced by approximately 50%. These results suggest that one or many bacterial membrane compounds could enhance the apoptotic signal of the 5-FU. The apoptotic effect is lost when the bacteria are microwaved due probably to the denaturation of membrane components during the microwave process. It can be hypothesized that teichoic acid, peptidoglycan, bacterial DNA, exopolysaccharides, or other molecules could mediate the 5-FU effect via an unknown mechanism.

In order to verify if the synergistic effect of the LAB implicated the caspases, we assessed the expression of the procaspase 3 by Western blot. This latter enzyme is cleaved, by active caspase 8 or 9, into a large and one small subunits, which then dimerize; two large and two small subunits are required for full enzymatic activity (30). Effectively, our results indicate that the caspase 3 is activated faster when cells were treated with 5-FU and 10^8 live LAB as compared to 5-FU treatment alone. The observation of the disappearance of the 32 kDa band (inactive procaspase 3) on the Western blot suggests that it is cleaved in its subunits. However, it was not possible to visualize the 17 kDa band maybe because of an insufficient protein concentration, whereas the 11 kDa band was undetectable with the BD Pharmingen rabbit antibody. This correlates with the apoptosis-enhancing effect of the combined treatment, and it confirms that cell apoptosis is caspase-3 dependent.

Other proteins implied in the apoptotic process such as Fas, p53, and p21 were also evaluated by immunoblotting. Various researchers have demonstrated that 5-FU treated tumoral cells have a higher concentration of Fas (31,32). Moreover, Borralho et al. (33) demonstrated that 5-FU exerts its cytotoxic effects, in part, through a p53/Fas-dependent apoptotic pathway that involves Bax translocation and mitochondrial permeabilization. Our results indicate an increase in the Fas receptor when the cells were treated with 5-FU. However, when 5-FU was associated with live or inactivated LAB, no enhancing effect was observed. Other systems could induce cell apoptosis through, by example, activation of the tumor necrosis factor-related apoptosis inducing ligand (TRAIL) system including its death receptors, DR4 and DR5, as well as its decoy receptors DcR1 and DcR2 (34). We are now evaluating if treatment with live bacteria can activate the TRAIL system or increase the death receptors on the tumoral cell surface.

An increase in the p53 concentration after a treatment with 5-FU alone was also observed, which was proportional to the incubation period. However, combination of 5-FU with live bacteria did not modulate this effect. Thus, it is possible to conclude that 5-FU induced DNA damage as observed by the increase in the p53 concentration; however, this molecule does not seem to be implicated in the proapoptotic effect of the combined treatment. It is known that p53 is a transcription factor that modulates the expression of various proteins such as the p21 and Bcl-2 family such as Bax (35). The cyclin-dependent kinase inhibitor 1A, also called p21, regulates the cell cycle progression at G1-S. The expression of this protein is under the control of the p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress. Thus, when overexpression of the p21 is activated, cell growth will be inhibited. The treatment of LS513 cells with 5-FU increased the expression of p21; but unlike what was observed with p53 and Fas, the combination of 5-FU and live or microwave-inactivated LAB resulted in changes of p21 protein expression. When live LAB was used, a slight and short increase of p21 was noticed followed by a decline, whereas the presence of microwave-killed bacteria caused a strong increase of p21 over time. Given that live bacteria induced a faster apoptotic response, it is obvious that the p21 was reduced because the cells already undergo the programmed cell death and do not need p21. However, when microwave-inactivated bacteria were used, the apoptotic process was reduced, and p21 seem to be slightly increased. It thus can be suggested that p21 might be an inhibitor of the apoptotic process. This role for p21 has been previously described (36–38).

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Consumption of probiotics as a dietary supplement has been reported to show some anticancer capacity (11,39). Most studies, however, have focused on the effects of probiotics to prevent the growth of cancerous cells. In this study, we evaluated the capacity of *Lactobacillus* mix to increase the apoptosis of a colorectal cell line in presence of 5-FU. To our knowledge, no paper has been published up to now on the effect of LAB as an adjuvant to improve the 5-FU efficiency during treatment of CRC. However, Österlund et al. (40) demonstrated that the consumption of gelatine capsules twice daily, containing about 1 to 2×10^{10} of *L. rhamnosus* GG during 24 wk, as an adjuvant to cancer chemotherapy, reduced the severe (grade III and IV) episodes of diarrhea ($P = 0.027$). Some studies conducted on animal models have suggested that the reduction of the activity of potentially carcinogenic fecal enzymes (β-glucuronidase, azoreductase, nitroreductase) could result in a reduced incidence of cancer (41).

Overall, this study demonstrates a synergistic proapoptotic effect of the combination of a chemotherapeutic agent, 5-FU, and a mixture of live or irradiated *L. acidophilus* and *L. casei* probiotic strains. This apoptosis induction seems to be correlated with the activation of the caspase 3 and a faster reduction of p21 expression. The mechanisms of action leading to this effect are still not fully elucidated, and more research is needed to elucidate them.

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ACKNOWLEDGMENTS

This research was supported by the Quebec Research Council in Fishing and Agri-Food (CORPAQ) of the Quebec ministry of Agriculture, Fishery and Food (MAPAQ) and by Bio-K Plus International Inc. through their "Volet Compétitivité des Entreprises" program. The authors are grateful to Bio-K Plus International Inc. for providing the lactobacilli probiotic preparation.

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