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PROBIOTICS IN PREVENTION OF BREAST CANCER

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INTRODUCTION

Live microbial feed supplements added to foods in order to beneficially affect the consumers are known as probiotics (Fuller, 1989). Lactic acid bacteria (LAB) are the most common probiotic microorganisms used to exert a given biological function in the host. Several studies have reported the beneficial effects of the consumption of LAB or LAB-fermented products on intestinal health (Gibson *et al*., 2003). LAB have been shown to exert effects on the immune system of consumers and to increase the resistance to neoplasia and infections (Kato, 2000). It is known that oral administration of certain strains of bacteria can not only increase the local immune response in the intestine, but can also increase the systemic immune response such as macrophage function and immunoglobulin concentration in serum (Perdigo´ n *et al*., 1999, 2001) as well as the immune responses in other mucosal areas such as bronchus and mammary glands (de Moreno de LeBlanc *et al*., 2005a). These properties, among others, have led to an increase in the consumption of fermented dairy products (i.e. yoghurt and other fermented milks) containing viable LAB throughout the world. In addition to LAB, fermented milks possess other non-bacterial components produced during fermentation that can contribute to immunomodulating properties. Many beneficial effects have been attributed to bioactive peptides derived from fermented milk, including opiate, antimicrobial, antihypertensive, antithrombotic, immunomodu-latory and antitumoural activities (Matar *et al*., 2003; Shah, 2000; Clare and Swaisgood,

2000). Milk fermented by Lactobacillus (L.) helveticus R389, a microorganism with high protease and peptidase activity, exerted an antimutagenic effect while its proteolytic-deficient strain did not (Matar *et al*., 1997). In a similar way,milk fermented with L. helveticus R389 increased the number of IgA+ cells in the small intestine as well as in the bronchus of mice, but fermented milk obtained with the mutant strain did not show the same in vivo results (Matar *et al*., 2001). Also, peptidic fractions liberated during milk fermentation with L. helveticus R389 stimulated the immune system and inhibited the growth of an immunodependent fibrosarcoma in a mouse model (LeBlanc *et al*., 2002). This same fraction showed an induction of the humoral immune response against E. coli O157:H7 when mice were infected with this pathogenic bacterium (LeBlanc *et al*., 2004). It is known that breast cancer is one of the most common cancers in women and many dietary factors are related with this disease either positively or negatively. Diets rich in cultured dairy products may inhibit growth of many types of cancers such as colon cancer (the most investigated to date). There are only a few reports relating probiotics and breast cancer prevention. Biffi *et al*. (1997) studied the direct effect of milk fermented by five bacterial species (Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium animalis, Lactobacillus acidophilus, and Lactobacillus paracasei) on the growth of a breast cancer cell line and reported that the antiproliferative effect was not related to the presence of bacteria in the fermented milk. This study suggested the potential of LAB to produce compounds with antiproliferative activity useful in the prevention of solid tumours such as breast cancer during milk fermentation. Previous studies in our laboratory have demonstrated that milk fermented by L. helveticus R389 was able to delay tumour growth in an experimental breast cancer model using BALB/c mice (de Moreno de LeBlanc *et al*., 2005b). This effect was related to the induction of cellular apoptosis and to the capacity of this fermented milk to modulate the relationship between immune and endocrine systems (decreasing interleukin-6 (IL-6) levels) which are very important in an oestrogen dependent tumour.

Probiotics have been given credit for numer-ous health-promoting effects; one of which is their anticarcinogenic properties (Kato, 2000). Their connection with the prevention of animal and human cancers has been extensively reviewed (Rolfe, 2000; Hughes and Rowland, 2003). The effects of probiotics on intestinal dis-orders have been the most extensively studied because they can beneficially affect the intesti-nal microbiota, which are involved in many of these disorders. The increase of immune cell activity in the prevention of cancer by lactic acid bacteria (LAB) consumption has also been described (Kato *et al*., 2000; Hayashi and Ohwaki, 1989). For these reasons, the effects of probiotic or fermented products containing LAB are extensively studied for colon cancer. Probiotics such as lactobacilli and bifidobacteria in fermented or culture-containing dairy foods such as yogurt

may play a role in reducing the risk of colon cancer (Fernandes and Shahan, 1990; Brady *et al*., 2000; Wollowski *et al*., 2001).

There are many different mechanisms by which fermented products containing viable LAB may lower the risk of colon cancer:

1. The reduction of procarcinogenic substances such as mutagenic compounds commonly found in the western meat-rich diet, that can be bound to the intestinal and lactic acid bacteria *in vitro*, has been correlated with the reduction in the mutagenicity observed after exposure to the bacterial strains (Orrhage *et al*., 2002; Morotomi and Mutai, 1986). It is possible that the LAB supplementation can influence the uptake and excretion of mutagens by simply binding to them in the intestine.

2. Probiotics can also indirectly act by reducing the level of certain enzymes such as b-glucuronidase, azoreductase nitroreductase, among others, which convert procarcinogens to carcinogens in the intestine (Fernandes and Shahan, 1990; Goldin *et al*., 1980; Goldin and Gorbach, 1984). The consumption of *Lactobacillus* (*L.*) *acidophilus* in experimental animal models reduced the activity of fecal enzymes such as b-glucuronidase, azoreductase and nitroreductase (Goldin *et al*., 1980; Goldin and Gorbach, 1976). The products of these enzymes are known to be mutagenic and carcinogenic and their activities have been well correlated with the number of certain bacteria in the intestine. Goldin and Gorbach also studied the effect of feeding two *L. acidophilus* strains on the activity of these bacterial enzymes in 21 healthy volunteers (Goldin and Gorbach, 1984). The continuous consumption of these bacteria was necessary to maintain the effect; a reversal of the effect was observed within 10–30 days of stopping *Lactobacillus* feeding.

3. The production of short-chain fatty acids, such as butyrate and propionate, is another mechanism by which probiotics may help in the treatment for colorectal cancer (Geier *et al*., 2006).

4. It has also been suggested that LAB or compounds produced by these microorganisms may directly interact with tumor cells in culture and inhibit their growth, supporting the idea that they can directly produce anti-tumorigenic or anti-mutagenic compounds (Reddy *et al*., 1983).

5. The modulation of the host immune system is one of the effects attributed to the LAB or fermented products that contain them (Perdigon *et al*., 2001). This effect was studied against many pathologies and included different types of cancers.

Unfortunately, little epidemiologic evi-dence exists that relates probiotics or probiotic-containing fermented foods and cancer incidences. A few case-controlled studies have been con-ducted to evaluate the effects of yogurt or fer-mented milks on some cancer rates. Lê Monique *et al*. (1986) reported a study in France where an inverse relationship between frequency of yogurt consumption and risk of breast cancer was found. Peters *et al*. (1992) found that yogurt consumption can be a protective factor in a case-controlled study of colon cancer incidences in Los Angeles. Another case-controlled study of breast cancer in the Netherlands suggested that fermented dairy products could be pro-tective (Van't Veer *et al*., 1989). One human trial showed that the recurrence rate for superficial bladder cancer was lower for subjects receiving freeze-dried *L. casei* Shirota than a placebo (Aso and Akazan, 1992). Additional similar studies are important to clarify the role that probiotic products play in cancer rates.

Effect of fermented milk administration in a breast cancer model

Over the past decade, considerable advances have been made towards an understanding of the molecular factors involved in breast cancer development, but for women in most western countries, breast cancer still remains a major cause of death. There are genetic and envi-ronmental factors that increase the chances of breast cancer and the most common breast cancer types are estrogen-dependent. Some of the causing factors, such as the diet, can be controlled, whereas other cannot (Divisi *et al*., 2006; Matar *et al*., 2003). It is known that women with high fat content diets have a higher risk of developing breast cancer; an example of this is that the oriental diet has traditionally been related with a low risk of this type of cancer in these populations.

In addition to containing LAB, fermented milks can possess non-bacterial components produced during fermentation that may con-tribute to their immunogenicity and to proper-ties such as their anti-tumor activities. For this reason, cultured dairy products were proposed to inhibit the growth of many types of can-cers, including breast tumors. Matar *et al*. (2003) reported different roles and functions of biolog-ically active peptides released from fermented milks. For example, peptidic fractions released during milk fermentation with *L. helveticus* R389, a strain with high proteolytic activity, stimulated the immune system and inhibited the growth of an immune-dependent fibrosar-coma in a mice model (LeBlanc *et al*., 2002).

The influence of the immune cells on breast can-cer was reported using different models (Reome *et al*., 2004; Zhang *et al*., 2002). A substantial proportion (up to 50%) of breast tumors is comprised of cells from the immune system that infiltrate the tumors (Reed and Purohit, 1997). These cells produce differ-ent biological messengers such as cytokines, which are implicated in an antitumor response.

Given the existence of a common mucosal immune system, a fermented product that enters the organisms by the oral route can exert its influence on the immune cells associated not only with the gastrointestinal tract but also with other mucosal sites and associated glands, such as mammary glands. In this way, it was reported that the oral administration of milk fermented with the proteolytic strain *L. helveticus* R389 increased the number of IgA secreting cells in the small intes-tine as well as in the bronchus of mice. However, fermented milk obtained with the proteolytic deficient mutant strain did not show the same results (LeBlanc et al., 2002). Considering these previous results, the effect of milk fermented by *L. helveticus* R389 or its proteolytic deficient variant, *L. hel-veticus* L89 was assayed on a murine hormone-dependent breast cancer model (using the ATCC tumoral cell line 4T1 injected in the upper right mammary gland), studying the systemic and local immune responses in the mammary glands and tumors.

Mice were fed with milk fermented by *L. hel-veticus* R389 (P+) or L89 (P-), for 7 days prior to the tumor cell injection. After that, they again received the fermented milk in a cyclical basis. The results obtained showed that the adminis-tration of both fermented milks either delayed or stopped tumor development (De Moreno *et al*., 2005) mmune mechanisms were studied, and these two groups compared with the tumor control, different cytokines were analyzed in serum, in the mammary gland tissues and in the immune cells isolated from the tumor. The study of cytokine-positive cells in mammary glands furthered the understanding of the local cell response after mice were fed with fermented milk as well as after tumor injection. In the tumor isolated cells the analysis was performed because the role of tumor-infiltrating immune cells in antitumor immunity, as well as their potential for cancer immunotherapy, has been extensively reported (Ferrarini *et al*., 2002; Bingle *et al*., 2002).

It was observed that in serum TNFá increased significantly in the basal sample from mice receiving milk fermented by *L. helveticus* R389 or L89 (Table 1). This increase prior to tumor induction could be related to the decrease of tumor growth in both experimental groups. The P (+) group maintained the TNFáconcentration at close to the basal level throughout the trial, demonstrating a regulation of this cytokine, whereas the P(-) group showed increased TNFá in the last sample (similar to the control group, Table 1). In the tumor control group, TNFá levels increased in the function of the time, as did the tumor volume (DeMoreno *et al*., 2005). In the cells isolated from the tumor, TNFá increased in both groups fed with fermented milk where the tumor growth was delayed, leading to an induction of the cytokine production by fermented milk, which may play a biological role in the induc-tion of cellular apoptosis (DeMoreno *et al*., 2005) (Table 1).

IL-6 was studied in this model because this cytokine is implicated in estrogen synthesis (Purohit *et al*., 2002), a hormone that the tumor needs to

Table: 1. Effect of probiotics on tumor volume and cyokines in blood serum (Adapted from Bioactive Foods in Promoting Health: Probiotics and Prebiotics, 2010, Elsevier Inc).

Experimental Control	*Time(days)*	*Tumor Volume*	*Blood Serum*		
			TNFα	*IL-6*	*IL-10*
Tumor Control	Basal	ND	42.1 ± 2.1^{e}	15.0 ± 2.0^{e,f}	121.2 ± 35.1^{f}
	12	0.02 ± 0.01^{e}	207.1 ± 24.5^{f,g}	12.1 ± 1.1^{e}	409.1 ± 113.2^{g}
	18	0.12 ± 0.07^{f}	226.6 ± 39.4^{f,g}	40.4 ±6.0^{h}	943.5 ± 87.3^{h}
	27	0.21 ± 0.12^{f}	522.7 ± 71.8^{i}	93.2 ± 4.1^{i}	50.4 ± 15.1^{e}
*L. helveticus*R389 (P+)	Basal	ND	207.7 ± 43.6^{g,h}	17.1 ± 2.1^{f}	552.2 ± 69.2^{g}
	12	0.01 ± 0.01^{e}	176.8 ± 2.1^{f}	20.1 ± 2.2^{g}	974.5 ± 48.5^{h}
	18	0.03 ± 0.03^{e}	248.4 ± 11.1^{h}	14.0 ± 4.1^{e,f}	3338.9 ± 689.2^{j}
	27	0.04 ± 0.02^{e}	283.5 ± 34.74	15.2 ± 2.1^{e,f}	4796.3 ± 859.5^{j}
*L. helveticus*R89 (P-)	Basal	ND	256.1 ± 51.5^{g}	22.0 ± 2.1^{g}	1441.2 ± 69.2^{i}
	12	0.01 ± 0.01^{e}	242.8 ± 9.9^{h}	13.1 ± 2.0^{e,f}	1361.3 ± 155.1^{i}
	18	0.03 ± 0.02^{e}	233.6 ± 18.2^{h}	17.1 ± 2.2^{f}	836.2 ± 131.2^{h}
	27	0.05 ± 0.03^{e}	144.8 ± 49.5^{f,g}	33.2 ± 4.1^{h}	1556.7 ± 169.1^{i}

Mice fed with the milk fermented by *L. helveticus* R389 (strain with high proteolytic activity, P+) or by *L. helveticus* L89 (proteolytic deficient mutant strain, P-) were compared with the tumor control (without specific feeding).

aTumor growth rate is expressed as the volume (cm3) of the tumor (cm3). bFor blood serum, the concentration for each cytokine was evaluated by ELISA. Results are expressed as the mean concentration of each cytokine (pg/ml) ± standard deviation. cFor mammary gland tissues, cytokine-positive cells were analyzed using indirect immunofluorescence. Results are expressed as means ± SD of cytokine-positive cells counted in 10 fields of vision at 1000x of magnification. dFor cells isolated from tumor, cytokine-positive cells were analyzed by immunoperoxidase technique and results are expressed as means ± SD of cytokine-positive cells each 100 counted cells (cells/100). e,f,g,h,i,j Means in each column without a common letter differ significantly (p <0.05). ND = Not determined.

Table 2: Effect of probiotics on tumor volume and cyokines profile in mammary glands ***(Adapted from Bioactive Foods in Promoting Health: Probiotics and Prebiotics, 2010, Elsevier Inc).***

Experimental Control	*Time(days)*	*Tumor Volume*	*Mammary gland tissues*		
			TNFα	*IL-6*	*IL-10*
Tumor Control	Basal	ND	$9 \pm 3^{e,f}$	$9 \pm 3^{e,f,g}$	$6 \pm 3^{e,f}$
	12	0.02 ± 0.01^{e}	$9 \pm 4^{e,f,g}$	15 ± 4^{f}	$11 \pm 3^{f,g}$
	18	0.12 ± 0.07^{f}	21 ± 6^{h}	$13 \pm 6^{e,f,g}$	$14 \pm 4^{g,h}$
	27	0.21 ± 0.12^{f}	21 ± 6^{h}	28 ± 8^{h}	$16 \pm 3^{g,h}$
*L. helveticus*R389 (P+)	Basal	ND	$7 \pm 2^{e,f}$	6 ± 2^{e}	$7 \pm 2^{e,h}$
	12	0.01 ± 0.01^{e}	$13 \pm 4^{f,g,h}$	$11 \pm 3^{e,f,g}$	$15 \pm 4^{g,h}$
	18	0.03 ± 0.03^{e}	$12 \pm 4^{f,g,h}$	$13 \pm 4^{f,g}$	25.0 ± 5^{i}
	27	0.04 ± 0.02^{e}	$13 \pm 2^{f,g,h}$	14 ± 4^{f}	21 ± 5^{i}
*L. helveticus*R89 (P-)	Basal	ND	5 ± 1^{e}	$7 \pm 2^{e,g}$	6 ± 1^{e}
	12	0.01 ± 0.01^{e}	18 ± 4^{h}	15 ± 5^{f}	$14 \pm 4^{g,h}$
	18	0.03 ± 0.02^{e}	$16 \pm 3^{g,h}$	17 ± 2^{f}	17 ± 2^{h}
	27	0.05 ± 0.03^{e}	$15 \pm 2^{g,h}$	12 ± 3^{f}	$12 \pm 3^{f,g,h}$

Mice fed with the milk fermented by *L. helveticus* R389 (strain with high proteolytic activity, P+) or by *L. helveticus* L89 (proteolytic deficient mutant strain, P-) were compared with the tumor control (without specific feeding).

aTumor growth rate is expressed as the volume (cm3) of the tumor (cm3). bFor blood serum, the concentration for each cytokine was evaluated by ELISA. Results are expressed as the mean concentration of each cytokine (pg/ml) ± standard deviation. cFor mammary gland tissues, cytokine-positive cells were analyzed using indirect immunofluorescence. Results are expressed as means ± SD of cytokine-positive cells counted in 10 fields of vision at 1000x of magnification. dFor cells isolated from tumor, cytokine-positive cells were analyzed by immunoperoxidase technique and results are expressed as means ± SD of cytokine-positive cells each 100 counted cells (cells/100). e,f,g,h,i,j Means in each column without a common letter differ significantly ($p < 0.05$). ND = Not determined.

grow. It is also a pro-angiogenic factor (Urban *et al*., 1986), supporting the growth of new blood vessels that are also essen-tial for tumor growth.The tumor control group showed elevated levels of IL-6 in the serum and that IL-6+ cells increased in mammary gland tissues and in the immune cells infiltrating the tumor for this group. However, the P(+) and P(-) groups did not show increased levels of this cytokine in serum during the study, suggesting that this IL-6 decrease could be involved as one of the mech-anisms for tumor growth delay. In the mam-mary glands, IL-6 secreting cell numbers were constant and similar in all groups until 18 days after tumor injection (Table 2).

This result can be explained by the relationship between this cytokine and estrogen synthesis in the mam-mary gland; estrogens being essential in pro-moting the proper growth of this tumor cell line. These cytokine-positive cells increased even more after 18 days in the tumor control group, and remained unmodified in both P(+) and P(-) groups (DeMoreno *et al*., 2005). In the isolated cells, all mice fed with fermented milk showed decreases in the number of IL-6+ cells compared to the tumor control group; P(+) being the group which pre-sented the lowest number of cells positive for this cytokine (Table 2).

IL-10 was the cytokine that showed differ-ences between both groups where the tumor growth was delayed. Serum IL-10 levels were significantly increased only in the P(+) group in relation to the tumor control group. Mice fed *L. helveticus* R389 showed increased IL-10 secreting cell numbers in the mammary glands throughout the time of the entire study. These were significantly higher numbers compared to the tumor control on days 18 and 22 after tumor injection. This outcome did not seem to occur in the P (-) group, since IL-10 secreting cell num-bers were not higher than those from the tumor control group (Table 3). Similar increases were reported for the immune cells isolated from the tumor where the mice receiving the milk fermented with the proteolytic strain showed the highest increases for IL-10 (DeMoreno *et al*., 2005).

The study of the cytokines showed that the consumption of both fermented milks dimin-ished IL-6, a cytokine that the tumor needs for growth, and this decrease could be related with the delay of the tumor growth observed in both mice fed with the proteolytic and the proteolytic deficient strain of *L. helveticus*. The increase of IL-10 in mice fed with milk fermented by *L. hel-veticus* R389 could explain the important differ-ence between both fermented milks, attributed principally to the components released into the milk after the fermentation with the proteo-lytic strain where the regulation of the immune response was observed in the three levels stud-ied (serum, mammary glands and tumor).

Table 3: Effect of probiotics on tumor volume and cyokines in breast tumor ***(Adapted from Bioactive Foods in Promoting Health: Probiotics and Prebiotics, 2010, Elsevier Inc)***

Experimental Control	*Time(days)*	*Tumor Volume*	*Tumor infiltrating cells*		
			TNFα	*IL-6*	*IL-10*
Tumor Control	Basal	ND	ND	ND	ND
	12	0.02 ± 0.01^{e}	ND	ND	ND
	18	0.12 ± 0.07^{f}	24 ± 2^{g}	29 ± 2^{g}	18.0 ± 2^{g}
	27	0.21 ± 0.12^{f}	13 ± 1^{g}	23 ± 4^{g}	8.0 ± 2^{e}
L. helveticusR389 (P+)	Basal	ND	ND	ND	ND
	12	0.01 ± 0.01^{e}	ND	ND	ND
	18	0.03 ± 0.03^{e}	$22 \pm 7^{f,g}$	4 ± 2^{e}	$14 \pm 3^{f,g}$
	27	0.04 ± 0.02^{e}	13 ± 1^{e}	4 ± 1^{e}	13 ± 2^{f}
L. helveticusR89 (P-)	Basal	ND	ND	ND	ND
	12	0.01 ± 0.01^{e}	ND	ND	ND
	18	0.03 ± 0.02^{e}	12.0 ± 2^{e}	4 ± 1^{e}	$13 \pm 6^{e,f,g}$
	27	0.05 ± 0.03^{e}	$11.0 \pm 4^{e,f}$	13 ± 4^{f}	$9 \pm 3^{e,f}$

Mice fed with the milk fermented by *L. helveticus* R389 (strain with high proteolytic activity, P+) or by *L. helveticus* L89 (proteolytic deficient mutant strain, P-) were compared with the tumor control (without specific feeding).

aTumor growth rate is expressed as the volume (cm3) of the tumor (cm3). bFor blood serum, the concentration for each cytokine was evaluated by ELISA. Results are expressed as the mean concentration of each cytokine (pg/ml) ± standard deviation. cFor mammary gland tissues, cytokine-positive cells were analyzed using indirect immunofluorescence. Results are expressed as means ± SD of cytokine-positive cells counted in 10 fields of vision at 1000x of magnification. dFor cells isolated from tumor, cytokine-positive cells were analyzed by immunoperoxidase technique and results are expressed as means ± SD of cytokine-positive cells each 100 counted cells (cells/100). e,f,g,h,i,j Means in each column without a common letter differ significantly ($p < 0.05$). ND = Not determined.

The analysis of immune B and T cells also showed differences between the mice that received both fermented milks. Only mice fed with milk fermented by *L. helveticus* R389 increased IgA+ B cells in mammary glands after tumor injection. However, this increase was not observed when a tumor was not induced, which could mean that enhancement of IgA+ cells in mammary glands needs a stronger stimulation such as that induced by tumor cells (DeMoreno *et al*., 2005). When T cells were studied in our model, it was possible to observe changes in the balance between CD4+ and CD8+ cells in mammary glands in mice from the group fed with milk fermented by *L. helveti-cus* R389 and injected with tumor cells. CD4+ cell numbers increased, whereas CD8+ cell numbers remained unmodified. This outcome was differ-ent in the tumor control group, which maintained the balance of these cells in mammary glands towards CD8+ cells more than towards CD4+ cells (DeMoreno *et al*., 2005).

It is possible to conclude in this experimen-tal model that 7 days of cyclical feeding with milk fermented by *L. helveticus* R389 or L89 delayed tumor development and consequently decreased IL-6 secreting cells. However, milk fermented by *L. helveticus* R389 induced not only a decrease of IL-6, but also an increase of the regulatory cytokine IL-10 and cell apopto-sis in the tumor. These effects were observed when a local stimulus such as tumor cells was present.

CONCLUSIONS

There are many reports about the anticarcino-genic effect of probiotics strains and fermented product that contain them. Even when the epide-miological data and those obtained from human trails are promising, animal models are still nec-essary to elucidate the mechanisms by which they can act. At the intestinal level, a fermented product can exert an antitumoral effect by several mech-anisms. The studies carried out with the model of colon cancer inhibition through cyclic yogurt feeding demonstrated that yogurt modulates the immune system response and exerts its anti-tumor activity through its anti-inflammatory capacity with high levels of the regulatory IL-10 in the large intestine. In addition to this immu-nomodulator capacity, other mechanisms by which yogurt could exert the antitumor activ-ity observed in this model would be through the activation of the apoptosis pathways and through the yogurt bacteria interaction with the intestinal microbiota inducing decreases in the certain enzyme activities involved in the devel-opment of tumors in the intestine.

The introduction of antioxidant enzyme genes (SOD and CAT) in current probiotic strains that have natural anti-inflammatory properties, such as the ability to modulate the immune-dependent anti-inflammatory processes, could generate very potent strains that could be used for the preven-tion of inflammatory diseases or post-cancer drug treatments. The use of other

genetically modified LAB, such as the IL-10 producing strain of *Lc. lac-tis* (Steidler *et al*., 2000), could be suitable for use as treatments of intestinal diseases by delivering beneficial com-pounds to specific sites in the gastrointestinal tract where they are required.

It can also be concluded that it is possible to obtain a beneficial effect in other mucosal sites distant to the intestine with the oral administra-tion of a probiotic bacteria or fermented milk. Oral administration of probiotic bacteria also exerted antitumoral effects against a non-intestinal tumor such as fibrosarcoma. Fermented milk administration can regulate the response of the immune cells associated to the mammary glands and in the cells infiltrating the tumor in an estrogen-dependent breast tumor model. The regulation of the immune response can also exert an inhibitory influence on the estrogen synthesis, necessary to the tumor growth. The probiotic strain selection would play an important role in the mucosal acti-vation observed.

The principal cause that can be attributed to the probiotic and/or fermented product against cancer is the immune surveillance, which differs accord-ing to the site where the tumor is present. In con-trast to the results observed in the intestine, where the administration of the fermented products itself induces changes on the gut associated immune cells and in other immune cells as peritoneal mac-rophages, in the mammary glands, changes on immune cell balances were observed only when immune cells have to act against a target like tumor cells, avoiding an exacerbated immune response. This fact could be explained because at intestinal level, where there are constant stimuli on the immune system, the immune cell stimulation is maintained with the probiotic administration whereas at other sites, such as mammary glands, the immune system is maintained alert and when the target affects this site, the immune cells quickly react against the agent.

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