A potential role of *Lactobacillus acidophilus* LA1 and its exopolysaccharides on cancer cells in male albino mice

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(Received 13 February 2015; accepted 8 May 2015)

The aim of the present study was to evaluate the potential role of *Lactobacillus acidophilus* (LA1), isolated from faecal samples of breast-fed Egyptian infants (15–90 days), and its exopolysaccharides (EPS), with regard to antitumour activity *in vivo* against Ehrlich ascites carcinoma (EAC) cells. *L. acidophilus* demonstrated antioxidant properties by suppression of malondialdehyde and nitric oxide serum levels. Also, the EPS showed a powerful antitumour effect based on the obtained results from liver function tests, such as aspartate aminotransferase (AST), alanine transaminase (ALT) and albumin concentration than positive control and toward the normal values, when compared with a positive control. The kidney function of the treated and non-treated groups was not affected and there were no significant differences between the negative control and the treated groups. There was a marked suppression of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) enzymes in groups treated with LA1 and its EPS.

**Keywords:** *L. acidophilus*; EPS; enzymes; Ehrlich ascites carcinoma (EAC)

**Introduction**

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, leading to a progressive increase in the number of dividing cells.[1–3] Cancer is one of the most prevalent groups of disorders among the population in many countries worldwide.[4] Most chemotherapies are cytotoxic and cause immunotoxicity, which affects the tumour development and aggravates the patient’s recovery.[5] The discovery and identification of new antitumour drugs [6] with low side effects on the immune system has become an essential goal in many studies of immunopharmacology.[7–10] Exopolysaccharides (EPS) from probiotic bacteria have health-promoting effects, including antitumour effect and cholera toxin neutralization.[11] The ability of *Lactobacilli* to reduce the risk of cancers has been suggested based on their ability to modify gut microflora to decrease β-glucoronidase.[12,13] Studies indicated that recurrences of urinary bladder cancers appear to decrease by internal intake of probiotics like *Lactobacillus casei* Shirota, but this finding needs confirmation.[14,15] Lactic acid bacteria (LAB) and *Bifidobacterium* have generally low activities of enzymes involved in carcinogen production.[16] Several experimental animal studies demonstrated a protective effect of prebiotics, such as oligofructose, probiotics such as some *Lactobacillus* and *Bifidobacterium* strains, or their combination on the establishment, growth and metastasis of transplantable and chemically induced tumours.[17–19] Kalui et al. [20] assessed that the production of EPS in amounts ranging from 298.53 to 431 mg/L were produced by *Lactobacillus plantarum* and *Lactobacillus rhamnosus* isolate strains from a Kenyan traditional spontaneously fermented maize porridge. *Lactobacillus acidophilus* is a major species of LAB, a diverse group of economically important microorganisms, used in various food and agricultural fermentation processes.[11,21] Ghan et al. [22] reported that *L. acidophilus* and its EPS had antitumour activity *in vivo* and *in vitro*. EPS, one of the primary metabolic products of LAB, has different health benefits and has recently received an increasing attention.[11] There is an increasing awareness of the relationship between diet composition, the gastrointestinal tract bacteria and the risk of colorectal cancer.[23] A four-year study of 398 subjects showed that *L. casei* Shirota decreased the recurrence of a typical colonic polyps.[24] Liu et al. [25] reported that probiotics can improve the integrity of gut mucosal barrier. They can benefit the faecal microbiota and decrease the infectious complications in patients with colorectal cancer undergoing colorectomy.[26] The aim of the present study was to...
investigate the antitumour activity of *Lactobacillus acidophilus* (LA1) bacteria and its EPS as natural agents against Ehrlich ascites carcinoma (EAC) cells *in vivo* and to measure the activity of haematological profiles, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

**Materials and methods**

**Bacterial strains**

Fresh faecal samples were obtained from a healthy breast-fed child (15 days of age). The samples were collected early in the morning and transported to the laboratory. Then the samples were stored at 4 °C ± 1 °C. Phenotypic identification was carried out according to Bergey’s Manual of Systematic Bacteriology.[27] Further confirmation was done according to MacFaddin.[28] The culture was preserved in reconstituted skim milk in Eppendorf tubes and stored at −80 °C with glycerol (20%, v/v). Prior to use, the strain was sub-cultured (1%, v/v) twice in Lactobacilli de Man, Rogosa and Sharpe (MRS) broth and adjusted to 1 × 10⁷ colony forming unit/mL (CFU/mL). *Escherichia coli* ATCC 10536 and *L. casei* ATCC334 strains were cultivated in Nutrient broth (Lab M, IDG, UK) and incubated at 37 °C for 24 h, then preserved in reconstituted skimmed milk in Eppendorf tube. The isolated *L. acidophilus* strain is further referred to in the text as LA1.

**EPS extraction**

Bacterial isolates were screened for EPS-producing ability using 5% (w/v) glucose as a carbon source on nutrient agar medium. The culture plates were incubated for 48 h at 30 °C ± 1 °C. The EPS were isolated and purified according to Cerning et al.[29] EPS were lyophilized and stored at −20 °C for further analyses. EPS identification was done using high-pressure liquid chromatography (HPLC).

**Tumour cells**

The initial inoculums of EAC cells were purchased from the National Cancer Institute, Cairo University, Egypt. The EAC cells were propagated in the National Organization of Drug Control and Research (NODCAR) laboratories by weekly intraperitoneal injections of 0.2 mL of 1:5 saline solution of freshly drawn ascitic fluid (0.2 × 10⁸ EAC cells) from a donor mouse bearing 6–8-days-old ascitic tumour into three mice in order to ensure that the ascitic fluid will continue to propagate and then can be drawn from mouse/mice that survive. Transplantation was carried out using sterile disposable syringes under aseptic conditions. The tumour growth was rapid enough to kill the mice within 18–20 days, due to the accumulation of ascitic fluid. The tumour rarely showed distal metastasis or spontaneous regression.

**In vivo assessment of antitumour activity**

Adult male Swiss albino mice with an average body weight of 20–25 g were used as experimental animals throughout the study. The mice were housed in specially designed cages and maintained in a thermostatically controlled room during the experimental period. They were fed an ordinary pellet diet and given up tap water ad libitum in NODCAR. These mice were divided into six groups and each group contained five animals.

**Animal groups**

G.1: Healthy mice, negative control.
G.2: Healthy mice, injected subcutaneous in thigh with 1 × 10⁶ tumour cells/mouse (positive control).
G.3: Healthy mice, orally administered a daily dose of 500 µg/mL of LA1 (1 × 10⁷ CFU) for 14 days. The animals that had subcutaneous injections with 1 × 10⁶ tumour cells/mouse were administered orally LA1 (1 × 10⁷ CFU) three doses weekly for two weeks.
G.4: Healthy mice, injected intraperitoneally with a daily dose of 500 µg/mL with 1 mg/mL concentration of EPS extracted from LA1. The animals that were subcutaneously injected with 1 × 10⁶ tumour cells/mouse were subjected to injections with EPS three times weekly for two weeks.
G.5: Healthy mice, orally administered a daily dose of 500 µg/mL of *L. casei* (1 × 10⁷ CFU) for 14 days. The animals that were subcutaneously injected with 1 × 10⁶ tumour cells/mouse were administered orally *L. casei* (1 × 10⁷ CFU) three doses weekly for two weeks.
G.6: Healthy mice, orally administered a daily dose of 250 µg/mL of *E. coli* (1 × 10⁶ CFU/mL) for 14 days. Then, some animals that were injected subcutaneously with 1 × 10⁶ tumour cells were administered orally three doses of *E. coli* (1 × 10⁶ CFU/mL) weekly for two weeks.

**Biochemical analysis**

Collection of the blood and tumours from all groups was performed at the end of the experimental period. Fasted mice were anaesthetized by diethyl ether inhalation. Mice were sacrificed using a sharp razor blade. Blood was collected and the serum was obtained by centrifugation for 10 min at 4000 rpm. These samples were stored at
−20 °C for determination of serum ALT and AST activity,[1,30] plasma ALP and liver function tests. The results were expressed in U/L. The liver albumin content was measured according to the method described by Lowry et al. [31] by using bovine serum albumin as a standard. Liver and plasma LDH activity, as indicator of necrotic cell death, was determined using a kinetic method.[32] The liver malondialdehyde (MDA) concentrations, lipid peroxidase and serum nitric oxide (NO) level were determined according to Namiduru et al.[33] Serum creatinine was determined as described by Paroni et al.[34] Creatinine concentration was measured in mg/dL.

**Measuring the tumour volume**

Tumour volume was measured as described previously.[35] Briefly, the tumour volume (V) (mm³) was calculated by measuring the length (L), width (W) and height (H) of the tumour. It was assumed that the tumours were approximately hemiellipsoids, whose volume is lit L-W-H. There were daily measurements of the tumours and the mice weight.

**Statistical analysis**

The obtained data were subjected to analysis of variance and the means were compared using the ‘Least Significant Differences (LSD)’ test at the 0.05 level.

**Results and discussion**

**The effect of L. casei, L. acidophilus and LA1 EPS on the volume of solid tumour**

In our previous study, we obtained promising results with L. acidophilus bacteria and showed its antioxidant and antitumour activity.[22] Here, we further expanded our experiments by including new strains, such as L. casei, L. acidophilus and L. acidophilus EPS.

*In vivo* studies for the cytotoxicity of *Lactobacillus* and its EPS have been done on solid tumour-bearing mice. As shown in Figure 1, L. casei, L. acidophilus and L. acidophilus EPS caused significant reduction in the tumour’s volume, when compared with that of the positive control group and the group treated with *E. coli*. The reduction of the tumour’s volume (1.02 ± 0.022 mm³, 1.21 ± 0.019 mm³ and 1.29 ± 0.0141 mm³) was observed when solid tumour-bearing mice were treated with LA1 EPS, *L. acidophilus* and *L. casei*, respectively. The EPS from LA1 showed a more powerful reduction effect on solid tumour volume than *L. acidophilus* itself, while *E. coli* caused propagation of the solid tumour volume when compared with the control mice.[36] This suggested that probiotics may be used as adjuvants in anticancer chemotherapy. Daniluk [37] reported that the anticancer activity through induction of apoptosis of cancer cells seems to be a promising approach for use of some probiotic strains as support therapy or disease prevention.[22] Probiotics also have a role in prevention of colon cancer.[38]

**Enzymatic assay**

**Effect of probiotic/EPS treatment on serum ALT and AST concentrations in mice bearing solid tumour**

The results presented in Figures 2 and 3 show the effect of *L. casei*, LA1 and LA1 EPS in mice bearing solid tumour on ALT and AST levels compared with the *E. coli*-inoculated mice, positive and negative controls. The results revealed that the concentrations of 500 μg/mL from *L. casei*, *L. acidophilus* and LA1 EPS had a marked effect

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![Figure 1](image1.png)  
**Figure 1.** Effect of tested bacteria and EPS on the volume of solid tumour (mm³) after 20 days of injection.

![Figure 2](image2.png)  
**Figure 2.** Serum ALT concentrations in mice, bearing solid tumour treated with bacteria and EPS.

![Figure 3](image3.png)  
**Figure 3.** Effect of bacteria and EPS on serum AST in mice bearing solid tumour.
on both enzymes’ activity levels, in which the mean ± standard deviation (SD) of different samples belonging to normal mice, mice bearing solid tumour and other treated with LA1 and L. casei was significant, \( p < 0.05 \). However, the application of EPS had a marked reduction on ALT and AST, which was highly significant, \( p < 0.05 \). In the case with E. coli (250 \( \mu \)g/mL), the results revealed that the enzymes’ activity levels became elevated when compared with the positive controls, which was significant, \( p < 0.05 \). This meant that LA1 EPS, L. acidophilus and L. casei were effective and had the ability to reduce ALT and AST toward the normal values. Some authors reported that the activities of ALT, AST and ALP enzymes are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity.[39,40] LA1 EPS, L. acidophilus and L. casei administered to mice provoked a marked suppression in serum AST, ALT and ALP activities, indicating a recovery from hepatocellular damage potentiated by EAC.[41,42] The elevation of the serum levels of those enzymes could potentially be attributed to their release from the cytoplasm into the blood circulation,[43] indicating a necrosis and inflammatory reactions.[44–46]

**Determination of ALP serum concentrations in mice bearing solid tumour treated with bacteria and EPS**

The effects of L. casei, LA1 and LA1 EPS on ALP in comparison with that of E. coli and positive and negative controls in different mice sera are shown in Figure 4. The results revealed that there was a marked decrease in the ALP serum levels in each group treated with LA1 EPS (85.6 ± 4.41 U/L), LA1 (95.6 ± 4.41 U/L) and L. casei (105.6 ± 4.41 U/L), in comparison with the positive control, whereas there was a marked increase in the group treated with E. coli (139.6 ± 4.93 U/L). L. casei, LA1 and its EPS had a highly significant effect on the activity levels of ALP, \( p < 0.05 \). In the case with E. coli, the results revealed that, the enzymes’ activity levels became elevated, when compared with the positive control. This meant that LA1 EPS, L. acidophilus and L. casei were effective and had the ability to reduce ALP toward the normal values.

**Determination of serum LDH enzyme concentrations in mice bearing solid tumour treated with bacteria and EPS**

LDH can be used as an indicator for cellular damage and cytotoxicity of toxic agents. In fact, elevation of LDH indicates a switch from respiration to anaerobic glycolysis.[47] In the present study, LDH activity was increased in the serum of control mice and mice treated with E. coli, whereas it was decreased in groups treated with probiotics and EPS. The change in LDH activity resulted from overproduction of superoxide anions and hydroxyl radicals. This causes oxidative damage to the cell membrane [1,48] and increase in the membrane permeability.[39] The effects of L. casei, L. acidophilus and LA1 EPS on the activity of LDH enzyme, which is responsible for tissue necrosis, compared with E. coli, negative and positive control in different mice serum, are shown in Figure 5. The results revealed that the treatment with administration of L. casei, L. acidophilus and LA1 EPS had a marked effect on the enzymes’ activity levels. The application of LA1 EPS had a marked reduction on the activity of LDH enzyme, which was highly significant, \( p < 0.01 \). In the case of E. coli, when compared with the positive control, the results revealed that the enzymes’ activity levels became elevated. This meant that L. acidophilus EPS, L. acidophilus and L. casei were effective and had an ability to reduce the LDH activity toward the normal values.
Table 1. Effect of bacteria and EPS on serum MDA, nitric oxide and albumin concentrations in mice bearing solid tumour.

| Groups                                      | MDA (mol/L) | Nitric oxide (μmol/L) | Albumin (g/dL) |
|---------------------------------------------|-------------|-----------------------|----------------|
| Negative control                            | 15.5 ± 1.51 | 32.6 ± 2.31           | 2.31 ± 0.25    |
| Positive control                            | 29 ± 1.98** | 52.8 ± 4.10**         | 2.37 ± 0.24**  |
| Mice, bearing solid tumour, treated with EPS| 20.5 ± 1.75*** | 40 ± 1.37***        | 1.98 ± 0.11*** |
| Mice, bearing solid tumour, treated with L. acidophilus bacteria | 23.4 ± 1.00* | 41.9 ± 2.25*        | 2.1 ± 0.11**  |
| Mice, bearing solid tumour, treated with L. casei bacteria | 21.4 ± 1.05** | 41.06 ± 2.51**       | 2.3 ± 0.11***  |
| Mice, bearing solid tumour, treated with E. coli bacteria | 31.4 ± 2.47*** | 56.36 ± 1.04***      | 2.6 ± 0.07***  |

Note: ""highly significant; ""moderate; "low.

Different studies suggest elevated ALP and LDH levels as predicting factors in neoplastic disorders.[49] Our study revealed that both ALP and LDH enzymes had significantly elevated activities in the positive control and in E. coli-treated groups, when compared with LA1 EPS-, L. acidophilus- and L. casei-treated groups. The bacteria-and EPS-treated groups suppressed the enzymes' activities and had mildly elevated values when compared with the negative control. The LDH increase probably results from more use of glucose in metastatic tissues and high conversion rate of glucose into lactate.[50]

Serum creatinine concentrations in mice bearing solid tumour treated with bacteria and EPS

The results, presented in Figure 6, show that the creatinine level remained the same in all treated groups, when compared with the negative control and the difference remained insignificant even in mice with transplanted cancer cells. This was an indication that the kidney function was not affected in this experiment.

Serum MDA, nitric oxide and albumin concentrations in mice bearing solid tumour treated with bacteria and EPS

As shown in Table 1, there was a marked decrease in MDA serum level in all groups treated with LA1 EPS (20.5 ± 1.75 mol/L), L. casei (21.4 ± 1.05 mol/L) and LA1 (23.4 ± 1.00 mol/L) toward the normal value (15.5 ± 1.51 mol/L) in comparison with the positive control group. The group treated with E. coli (31.4 ± 2.47 mol/L) had an increased MDA serum level, when compared with the positive control. A marked decrease in NO[51] serum level was observed in all groups treated with LA1 EPS (40 ± 1.37 μmol/L), L. casei (41.06 ± 2.51 μmol/L) and LA1 (41.9 ± 2.25 μmol/L) toward the normal value (32.6 ± 2.31 μmol/L), in comparison with the positive control. In contrast, the group treated with E. coli (56.36 ± 1.04 μmol/L) showed elevated NO serum levels, when compared with the positive control. Albumin serum levels are also shown in Table 1. There was a marked decrease in the albumin serum level in groups treated with L. acidophilus EPS (1.98 ± 0.11 g/dL), L. acidophilus (2.1 ± 0.11 g/dL) and L. casei (2.3 ± 0.11 g/dL) toward the normal value (2.31 ± 0.25 g/dL), in comparison with the positive control.

Table 1 shows that the mice treated with LA1 EPS had a highly significant effect in reducing serum NO level, when compared with the mice treated with L. acidophilus and L. casei. L. casei bacteria had better antioxidant properties than L. acidophilus. Oxidative damage primarily occurs through the production of reactive oxygen species, including hydroxyl radicals and hydrogen peroxide that are generated during the reaction with biological molecules. This may eventually lead to damage of membranes and other tissues.[52–54] Lipid peroxidation and the oxidative protein damages, provoked by free radicals' attack on biological structures, have been demonstrated to play a significant role in several pathological events.[55,56] The chemopreventive effects of L. casei, LA1 and LA1 EPS on albumin concentrations, in comparison with E. coli in different mice sera, are presented in Table 1. The results revealed that L. casei, LA1 and its EPS had a marked effect on albumin levels. The difference in mean ± SDs of samples belonging to normal mice, mice bearing solid tumour and mice treated with LA1 and L. casei was significant, p < 0.05. The highest effect of albumin level reduction was obtained with LA1 EPS, which was highly significant, p < 0.05. In the case of E. coli, the result revealed that the albumin level was elevated above the values of the positive control. On the other hand, L. casei, L. acidophilus and LA1 EPS were effective and had the ability to reduce MDA and NO levels toward the normal values.

In the case with E. coli, the results revealed that the tested strains caused significant elevation in the serum liver proteins, in comparison with the positive control. Different Lactobacillus strains produce different concentrations of lactic acid, which depends on the strain and ecological diversity. The antioxidant activity of the cultures was also characterized.[57]

Conclusions

The EPS, produced by LA1, showed a powerful effect, based on the obtained results from the liver function tests, such as AST, ALT and albumin concentration, in
comparison with \textit{L. acidophilus} itself. \textit{E. coli}-treated groups showed a marked elevation in liver function tests (AST, ALT) and albumin concentrations, with an elevation in MDA and NO levels. There was a significant relationship between negative control and groups treated with probiotics and EPS after implantation with EAC, $p < 0.05$. The kidney function in treated and non-treated groups remained unaffected and there was no significant relation between negative control and the other treated groups. There was a marked suppression of LDH and ALP enzymes in groups treated with probiotics and EPS, with significant relationship, $p < 0.05$. The albumin concentrations, LDH and ALP enzymes levels became elevated in positive control groups and in groups treated with \textit{E. coli}. Based on our present observations, we propose that the studied LA1 bacteria and their EPS may serve as an alternative for prolonged therapeutic option against cancer, without harmful side effects.

Acknowledgements
The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project no. RGP-VPP-202.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This study was funded by Deanship of Scientific Research at King Saud University through the research group [project number RGP-VPP-202].

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