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Antitumor Activity of *Lactobacillus plantarum* Cytoplasm on Teratocarcinoma-Bearing Mice

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Abstract Potential antitumor activity of *Lactobacillus plantarum* cytoplasm was examined using F9 teratocarcinoma-bearing BALB/c mice. The cytoplasmic fraction of *L. plantarum* was separated by sonication followed by ultracentrifugation. The fraction at a dose of 100 or 200 mg/kg/day was orally administered for 7 consecutive days before or after tumor inoculation to 16 mice. As a control, heat-killed whole cell was used at a dose of 100 mg/kg/day. Upon oral administrations of both the cytoplasm and heat-killed whole cell, when performed after and before tumor inoculation, the survival of F9-bearing mice prolonged more effectively. Administration of the cytoplasm after tumor inoculation extended the average survival days by 30 and 40% at daily dosages of 100 and 200 mg/kg/day, respectively. This result suggests that the cytoplasmic fraction of *L. plantarum* has strong antitumor activity against mouse F9 teratocarcinoma *in vivo*.

Key words: Antitumor, cytoplasm, *Lactobacillus plantarum*, teratocarcinoma

During the past decade, the importance of lactic acid bacteria (LAB) in the maintenance of gut health has increasingly been recognized. Accordingly, the possible health-related benefits associated with consumption of LAB as a dietary supplement are well-documented [4, 5, 8, 13]. In particular, the potentiality of dietary LAB to prevent chronic diseases such as cancer is very promising [1, 11, 16]. The anticancer activity of LAB has been well demonstrated by culture, cell, or some cellular components.

Fermented milk products of *L. bulgaricus*, *L. acidophilus*, or *Streptococcus thermophilus* have been shown to have strong chemopreventive activity towards carcinogenesis both in rats and in hamsters [2, 21]. Cultures or cells of LAB such as *L. casei* [24], *L. rhamnosus* [7], *L. acidophilus* [6, 15], and *Bifidobacterium longum* [12, 18, 22] were found to inhibit the growth of both implantable and chemically-induced tumors in rodents. LAB preparations including heat-killed whole cells, cell walls, and peptidoglycans [3, 20], and other cellular fractions such as polysaccharides [17] and glycoproteins [14], also appeared to possess the antitumor activity. Accordingly, most studies of antitumor activity of LAB have been focused on cultures, whole cells, or cell wall fractions of LAB. Recently, several investigations reported that cytoplasmic fractions of LAB stimulate the immune systems of hosts [10, 23]. However, to the best of our knowledge, study of antitumor activity of LAB has never been carried out with cytoplasmic fractions. Therefore, in this study, the anticancer activity of the *L. plantarum* cytoplasmic fraction was investigated using a teratocarcinoma-bearing mice model.

Six-week-old male BALB/c mice, obtained from DAIHAN Biolinc Inc. (Chungbuk, Korea), were housed in plastic cages in an air-conditioned room, and given food and water *ad libitum*. F9 cells were maintained *in vitro* in DMEM medium (GibcoBRL, Grand Island, NY, U.S.A.) containing 10% fetal bovine serum (FBS, GibcoBRL, Grand Island, NY, U.S.A.). Before inoculation into mice, tumor cells were washed with phosphate buffered saline (PBS), then suspended in sterile saline, and the concentration was appropriately adjusted for injection. The protocol of cytoplasm fractionation was adopted from Hosonos [10] as follows; *L. plantarum* was cultured in MRS broth (Difco Laboratories, Detroit, MI, U.S.A.) at 37°C for 18 h. After cultivation, the cells were harvested by centrifuging at 4°C, washed three times with distilled water, and lyophilized for storage. The lyophilized cells were resuspended in distilled water to 10 mg/ml concentration, then disrupted ultrasonically in ice for 30 min. After centrifuging the suspension at

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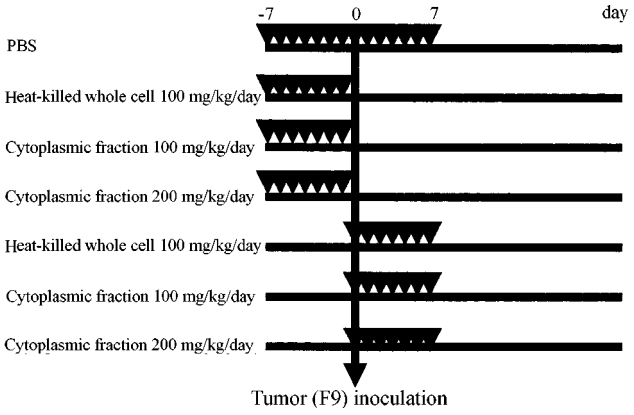

Fig. 1. Experimental schedule for oral feeding of heat-killed whole cell and cytoplasm fraction of *Lactobacillus plantarum* to mice.

800 ×g at 5°C for 30 min, the pellet was removed, and the cell walls were then sedimented from the supernatant by centrifugation at 70,000 ×g for 30 min using an ultracentrifuge. The resulting supernatant was designated as cytoplasmic fractions. The protocol of the detailed procedures is illustrated in Fig. 1. At the start of the experiment, the animals were assigned to one of seven groups, each composed of 16 animals. Mice were intraperitoneally inoculated with F9 teratocarcinoma cells (1×10^6 cells/body) on day zero. Heat-killed *L. plantarum* whole cell (100 mg/kg/day), cytoplasmic fraction (100 and 200 mg/kg/day), and PBS (control) were administered daily using a stainless steel feeding needle for 7-consecutive days before or after tumor inoculation. One day after 7-consecutive administrations, each active substance was administered orally every three days, unless otherwise stated. The animals, including the control group, were examined and weighed twice a week, and their survival rates were monitored. The significances of differences between the control group and the groups treated with *L. plantarum* preparations were analyzed by Student's *t*-test, where probability values of less than 5% were considered significant.

No significant differences in body weight changes were observed among the experimental groups (Fig. 2). The increase in body weight was attributed to the tumor mass. Figure 3 shows the results of antitumor activities of the *L. plantarum* cytoplasmic fractions, where toxicity or marked side effects were not observed. Only the cytoplasmic fraction at two concentrations (100 and 200 mg/kg/day) showed antitumor activity, when supplemented before tumor inoculation, having approximately 27% increase in average survival days compared to the control group (PBS) (Fig. 3a). In contrast to the effectiveness of the treatment with the cytoplasmic fraction, that with the heat-killed whole cell before tumor inoculation exhibited no significant activity. Figure 3b shows that both the cytoplasmic fraction and the heat-killed whole cell, when

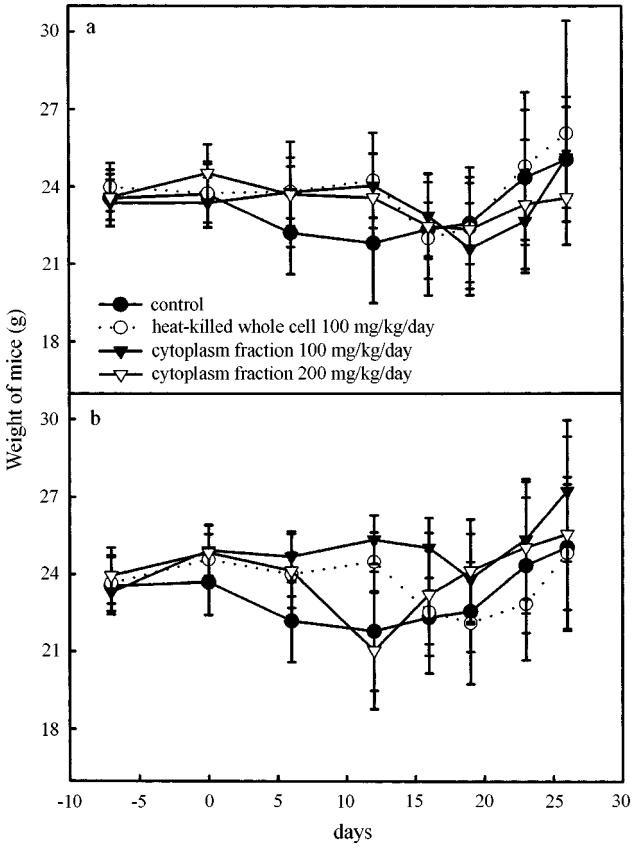

Fig. 2. Mean body weight of teratocarcinoma-bearing mice treated with *Lactobacillus plantarum* fractions.
PBS was used for the control group. (a), mice groups treated with *L. plantarum* fraction before tumor inoculation; (b), after tumor inoculation.

administered after tumor inoculation, were effective in prolonging the survival days. The cytoplasmic fraction showed antitumor activity in a dose-dependent manner. The group supplemented with the cytoplasmic fraction at 200 mg/kg/day after tumor inoculation showed the highest antitumor activity, in which the average survival days were extended by approximately 44% compared to the control group. The groups of mice supplemented with the heat-killed whole cell or the cytoplasmic fraction at 100 mg/kg/day each after tumor inoculation showed similar antitumor activities, with about 30% increase of the average survival days. All groups, except the one treated with the whole cell before tumor inoculation, showed antitumor activities. Their effectiveness were significantly different from the control group. This result suggested that the antitumor activity of heat-killed whole cell was mainly due to the cytoplasmic fraction. Although the inhibitory activity of LAB cytoplasm against tumors has not been studied yet, it has recently been reported that LAB cytoplasmic fraction enhances the capability of the host immune system by stimulating Peyer's patch and lymph node lymphocytes [10] or macrophages [23]. Therefore, it is quite possible

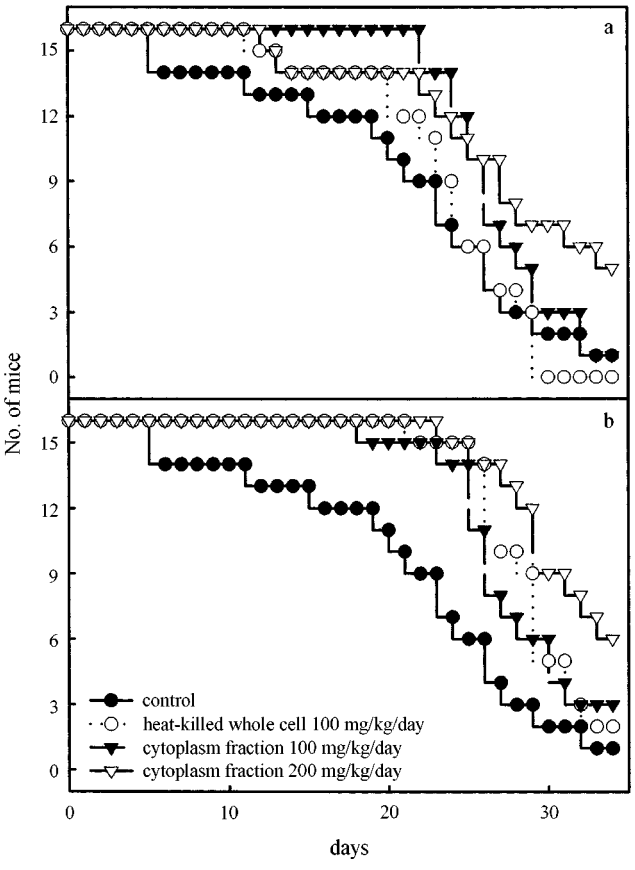

Fig. 3. Effects of oral administration of heat-killed whole cell and cytoplasmic fraction of *Lactobacillus plantarum* on the prolongation of the expected life span of Balb/c mice inoculated with F9.
PBS was used for the control group. (a), groups treated with *L. plantarum* fraction before tumor inoculation; (b), after tumor inoculation.

that the antitumor activity shown in this study was due to stimulation of the host immune system by the cytoplasm of *L. plantarum*.

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