Protective effects of lactic acid bacteria against genotoxicity in mice

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The possible protective effects of lactic acid bacteria (LAB) against *in vivo* genotoxicity were assessed in mice. Culture broth containing *Lactobacillus casei* was orally administered to mice either 1 h before intake of the genotoxins, cyclophosphamide (CPH), procarbazine (PCB) and urethane (URE) or together with PCB. In addition, an experiment was carried out in which heat-inactivated *L. casei* was orally administered to mice 1 h before PCB. Control animals received cell-free culture broth. Genotoxic effects were assessed in the mouse bone-marrow micronucleus test. The results suggest that pretreatment of mice with *L. casei* 1 h before the administration of genotoxins can lead to a moderate reduction ($P < 0.05$) in the genotoxicity of PCB and URE, but not CPH. However, co-administration of PCB with *L. casei* did not show any significant effect on genotoxicity. Similarly, no significant change in genotoxicity was observed after administration of heat-inactivated LAB. From this investigation, there is limited evidence for the protective effect of LAB against *in vivo* genotoxicity.

EVIDENCE obtained from laboratory investigations and epidemiological surveys indicate that our commonly consumed food contains certain ingredients which can possibly play an important role in reducing the incidence of cancer[1]. Many of the compounds with antigenotoxic and anticarcinogenic properties are known to occur naturally in vegetables, fruits, nuts, cereals, spices and beverages like tea and coffee[2–7]. Hence intake of chemopreventive food components has been suggested as an effective strategy for minimizing the possible deleterious effects resulting from human exposure to genotoxic and/or carcinogenic agents in our environment[8–10].

Currently there is considerable interest in the potential health benefits, including antigenotoxic and anticarcinogenic effects associated with the consumption of yoghurt and other fermented milk products. Lactic acid producing bacteria (LAB), particularly lactobacilli and bifidobacteria are considered as the most probable agents responsible for these effects[11]. From *in vitro* studies, there are many reports on the antigenotoxic effects of LAB against a variety of food-borne carcinogens[12–16]. In addition, a few reports have appeared on the *in vivo* protective effects of LAB. *Lactobacillus acidophilus* feeding has reduced the incidence of 1,2-dimethylhydrazine-induced colon tumours in rats[17]. Similarly, administration of *Bifidobacterium longum* to rats decreased the incidence of colon and liver tumours induced by 2-amino-3-methylimidazo [4,5-f] quinoline[18]. Protection against *in vivo* genotoxicity has been observed after co-administration of LAB with busulfan[19]. Furthermore, LAB administration has exerted protective effects against DNA damage induced by carcinogens in gastric and colonic mucosae of rats[20,21]. In contrast, a recent report shows that administration of LAB to mice does not lead to major changes in absorption and distribution of carcinogens or their genotoxic activity in the liver[11].

In view of the above findings on the potential beneficial effects of LAB and the consumption of LAB-containing fermented milk products by a large section of the human population, it would be of interest if more information is obtained on the possible *in vivo* antigenotoxic effects of LAB against other genotoxins. Hence, the present work was undertaken with the main aim of evaluating the *in vivo* antigenotoxic effects of LAB against the well known genotoxins, cyclophosphamide (CPH), procarbazine (PCB) and urethane (URE). For this purpose, genotoxic effects were assessed in the mouse bone-marrow micronucleus test after oral administration of LAB, either before or together with a genotoxin.

Experiments were carried out with 12-week-old male Swiss albino mice weighing 24–28 g, which were bred and maintained in the university animal house at 25 ± 2°C on standard mouse diet and water *ad lib*. Pure culture of *Lactobacillus casei* (obtained from Institute of Microbial Technology, Chandigarh) was grown in MRS broth (Himedia, India) for 16 h at 37°C with constant shaking at 225 rpm and the final pH was adjusted to 6.5 ± 0.2. In the first experiment LAB culture containing 10^7 cells/ml was administered to mice by gavage (10 ml/kg body weight) 1 h before the genotoxins. The control animals received cell-free culture broth. The genotoxins CPH (Sigma, USA), PCB (Hoffmann La Roche, Switzerland) and URE (Fluka, Switzerland) were dissolved in distilled water and administered by gavage (5 ml/kg body weight) 1 h after the animals received LAB. This was followed by another experiment in which PCB was dissolved either in the LAB culture or cell-free culture broth and administered by gavage. Additional work was carried out to evaluate the effect of 'heat-inactivated' LAB culture, which was administered 1 h before the animals received PCB (25 mg/kg body weight). After 27 h treatment, genotoxic effects were assessed in the bone-marrow micronucleus test as described by Schmid[22].

Figures 1–3 show the incidence of micronucleated polychromatic erythrocytes (MnPCEs) which indicate the genotoxic effect. From Figure 1, it is evident that pretreatment with LAB can lead to a moderate reduction

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in the genotoxicity of PCB and URE ($P < 0.05$), but not CPH. Intake of LAB together with PCB did not show any significant difference in the incidence of MnPCEs when compared to that with cell-free culture broth (Figure 2). This observation is contrary to a report on a reduction in genotoxicity after the co-administration of LAB with busulfan[19]. Furthermore, there is no significant reduction in the genotoxicity of PCB after pretreatment with heat-inactivated LAB (Figure 3).

Our present investigation has yielded limited evidence for a moderate antigenotoxic effect against PCB and URE when LAB-containing broth is administered to mice by gavage 1 h before the genotoxins. Inactivation of genotoxins and carcinogens has been proposed as one possible mechanism by which LAB exert protective effects[11]. From *in vitro* studies, several reports have appeared on the adsorption of genotoxins and carcinogens by LAB[15,23–26], depending on the genotoxin, bacterial strain and pH conditions[24,27]. Since the binding also occurs with dead cells, it appears to be a physical phenomenon[25]. From the point of *in vivo* effects, the binding of genotoxins/carcinogens with LAB could lead, in theory, to a decrease in bioavailability of the ingested genotoxins in the gut, thereby reducing the absorption into the blood and the subsequent interaction with other tissues in the body[11]. However, because of dependence on pH conditions, the binding process may not occur or may be reversed in the gut by unfavourable conditions of pH and other factors like nutritional and physiological state of the animals[11].

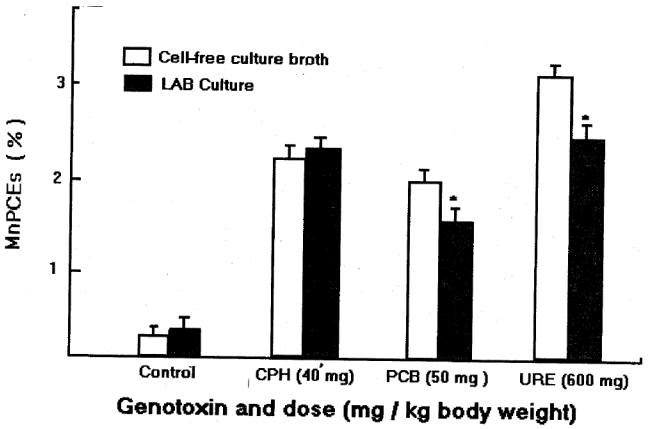

Figure 1. Induction of micronucleated polychromatic erythrocytes (MnPCEs) by cyclophosphamide (CPH), procarbazine (PCB) and urethane (URE) in bone marrow cells of mice which received either cell-free culture broth or LAB-containing culture broth 1 h before the genotoxins. All values are means ± SEm from 4 mice. 2500 PCEs were scored per animal. *Significantly different from the control (cell-free culture broth) at $P < 0.05$.

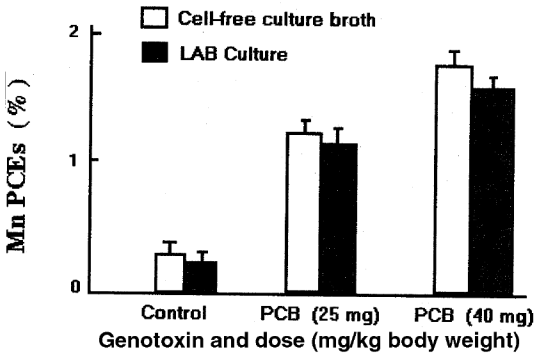

Figure 2. Induction of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow cells of mice after co-administration of procarbazine (PCB) with either cell-free culture broth or LAB culture. All values are means ± SEm from 4 mice. 2500 PCEs were scored per animal.

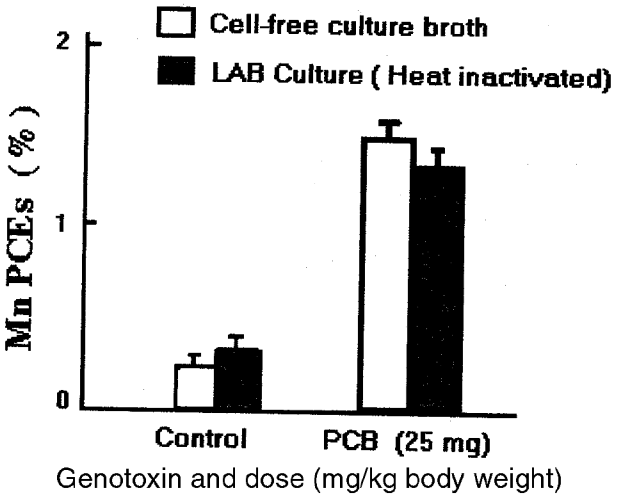

Figure 3. Induction of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow cells of mice which received either cell-free culture broth or heat-inactivated LAB culture 1 h before the genotoxin. All values are mean ± SEm from 4 mice. 2500 PCEs were scored per animal.

In conclusion, the findings from our present study have furnished limited evidence for the *in vivo* antigenotoxic effects of LAB. Further studies are needed to identify the favourable conditions under which LAB can exert protective effects against *in vivo* genotoxicity. In India, such studies are important in view of the widespread consumption of fermented milk products and 'idly' which is known to be a source of LAB with antimutagenic activity[28].

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Fluoride concentration in river waters of south Asia

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Fluoride levels in various types of environmental samples show wide variations from a low of 1.2 μg/m^3 in the air samples over Delhi to a very high value of over 18,000 μg/l in a hot spring in the Western Ghats region, due to which the surface water samples in the mountain streams generally show higher F levels. Large rivers with large run-off show higher levels of fluoride and hence greater fluoride flux to the oceans. Higher fluoride exposures due to enhanced application of rock phosphates adversely affect the health of our aquatic environment, in addition to decreasing the per capita availability of safe drinking water.

WATER availability is a critical factor in socio-economic development, limiting progress in many areas such as south Asia and other arid and semi-arid zones. In most parts of the world, the finite supply of freshwater is put to heavy use[1]. Industrial wastes, sewage and agricultural run-off can overload rivers and lakes with chemicals, wastes and nutrients, and poison water supplies. At present, the annual freshwater consumption is around 4000 km^3 throughout the world with India's consumption being just 10% of this value[2,3]. But the quantity of freshwater demand does not reflect the problems associated with water quality parameters such as hardness, fluoride, bacterial count and toxic metal content. In India, the arsenic-related problem in drinking water is already well known[4–6]. An estimated 62 million people, including 6 million children suffer from fluorosis because of consuming fluoride-contaminated (> 1000 ppb) water[7].

Fluoride is ubiquitous in the environment and is always present in plants, soils and phosphatic fertilizers[8]. Various rock types contain fluoride at different levels: basalt, 360 μg/g; granites, 810 μg/g; limestone, 220 μg/g; sandstone and greywacke, 180 μg/g; shale, 800 μg/g; oceanic sediments, 730 μg/g; and soils, 285 μg/g (ref. 9). The F concentration in the upper continental crust is 611 ppm (ref. 10). It is an essential constituent in minerals such as fluorite, apatite, cryolite, and topaz[11]. Whereas minerals such as biotite, muscovite and hornblende may contain large per cent of F (ref. 12) and therefore, would seem to be the main source of F in surface waters. It appears, therefore, that the F content of surface water is largely dependent on the mineralogical composition of the inorganic fraction in surface soils and sediments. Apatite may perhaps exchange some of its hydroxyl ions for fluoride following reaction of the type:

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^{-1} = Ca_{10}(PO_4)_6F_2 + 2OH^{-1} \quad (1)$$

$$K = a^2OH^{-1}/a^2F^{-1} = 10^{6.6} \quad (2)$$

i.e. the process converts the hydroxyl apatite of bones and calcium phosphate into fluorapatite, where K is equilibrium constant and a is activity[13]. With increasing use of fertilizers[14] containing fluoride, the fluoride content of surface water also increases. Approximately 20 to 400 g F per hectare is annually leached from soils, about the same amount that is added to the soil from the atmosphere, but fertilizing adds another 5 to 30 kg F per hectare annually[15]. This fluoride accumulates in the soils. The main part of fluoride in rainwater may originate in sea aerosols: K_2SiF_6 (hieratite) and Na_2SiF_6 (malladrite), where tiny droplets of foam are caught up by the wind[16] and may be carried far from the ocean to continental areas. The F content of various continental precipitations shows a range of 4–89 ppb and in the vicinity of cities and industrial areas, an average of 290 ppb can be found[17]. The order of magnitude of the normal fluoride content in the air is < 0.01–0.4 μg/m^3 and in industrial areas up to 5–111 μg/m^3 from chemical

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