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Effect of newly isolated *Lactobacillus ingluviei* ADK10, from chicken intestinal tract on acetaminophen induced oxidative stress in Wistar rats

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The total antioxidative activity of *L. ingluviei* ADK10 isolated from chicken intestine intact cells and cell free culture supernatant (CFCS) was 54- 67.95%. The ability to scavenge a,a-Diphenyl-b-Picrylhydrazyl free radical ranged from 71 and 64% in intact cells and CFCS respectively. Total reducing activity of bacteria was equivalent to 290 µM/L of cysteine. Reducing glutathione activity was equivalent to 93.95µg/mL. Oral administration of the strain at a dose of 10^9 cfu/kg body weight to acetaminophen induced oxidative stress in rats increased catalase, glutathione and superoxide dismutase activity in the blood, liver and kidney and lowered malondialdehyde level. The results indicate that *L. ingluviei* ADK10 has potential free radical scavenging activity for the treatment of oxidative stress related disease.

Keywords: Acetaminophen, Free radical scavenging activity, *Lactobacillus ingluviei* ADK10, Oxidative stress

Acetaminophen remains one of the most effective, chemotherapeutic analgesic-antipyretic agents belonging to the para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs)[1]. An acute overdose of acetaminophen (paracetamol, *N*-acetyl *p*- aminophenol; APAP) may result into potentially fatal hepatic and renal necrosis in humans and experimental animals[2]. At higher doses, acetaminophen produces a centrilobular hepatic necrosis that can be fatal. Acetaminophen poisoning accounts for approximately one-half of all cases of acute liver failure in the United States and Great Britain today[3,4]. The initial step of its toxicity is the formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P-450 which at therapeutic doses is removed by conjugation with glutathione sulfhydryl (GSH). High doses of acetaminophen result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury and oxidative stress[5,6]. Acetaminophen-induced oxidative stress could also be due to hepatic-derived acetaminophen metabolites, particularly GSH conjugates[7]. However, despite recognition of acetaminophen oxidative stress and concerted scientific efforts directed towords developing therapeutic agents to defend against acetaminophen oxidative stress, conventional chemotherapeutic options available to either treat or prevent its progress, are still restricted. In absence of reliable and effective modern antioxidative drugs for the disease treatment, concerted efforts are presently channeled toward exploring complementary or alternative chemotherapy in the disease treatment and/or anticipation. Among lactic acid bacteria (LAB), *Lactobacillus* has attracted a lot of attention for their potential probiotic effects in human health. *Lactobacillus* comprises of 100 recognized species, particularly those belonging to beneficial and non-pathogenic genera. The origin of LAB may come from mammals and birds intestines or from naturally fermented foods. LAB along with *Lactobacillus* have some probiotic functions, such as antagonistic reaction against pathogenic bacteria, adjusting the balance of intestinal flora, reducing blood cholesterol, inhibiting and reducing the risk of tumors and cancer, stimulating the immune system, stimulation of Vitamine C production and enhancement of digestion etc[8,9]. Oxidative damage has been found to play a major role in arthrosclerosis, cancer, arthritis and kidney disease. Generation of harmful free radicals and reactive oxygen species

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(ROS) may cause endogenous metabolic process. These free radicals are responsible for oxidative damage and finally cell death. Some lactobacilli have been shown to possess antioxidative activity, and are able to decrease the risk of accumulation of ROS during the ingestion of food. *Lactobacillus acidophilus*, *Bifidobacterium longum*, *L. fermentum* and *L. sake*[10] have antioxidative activity, and were able to decrease the risk of accumulation of ROS. The antioxidative activity expressed by some *Lactobacillus* strains used as food components and probiotics may have a substantial impact on human welfare. The objective of this study is to investigate the antioxidative effect of newly isolated *Lactobacillus ingluviei* ADK10, from the intestinal tract of chicken in acetaminophen induced oxidative stress.

Materials and Methods

Isolation and Identification of Lactobacillus strains—Samples of chicken intestine were collected from local markets in Midnapore town (22.4212° N, 87.3248° E), West Bengal, India. Samples (10^6 dilution) with saline solution (0.85% NaCl) were plated onto de Man-Rogosa-Sharpe (MRS) agar, and incubated at 37 °C under anaerobic condition for 24-48 h. Based on the phenotypic characteristics (colony characteristics, gram staining, cell shape, and catalase production), isolates of lactobacilli were collected. On the basis of highest acid and bile tolerance *Lactobacillus* ADK10 was selected. To identify the isolated *Lactobacilus* sp., at the level of species, 16S-rRNA sequencing was preformed[11,12] before storing in MRS containing 20% glycerol at -20 °C for further studies. The bacterial sequence was submitted in the GenBank. Identified *Lactobacillus* ADK10 was stored in laboratory and cultured in MRS broth under aerobic condition at 4 °C.

Total antioxidative activity of bacteria—The measurement of antioxidative activity of the *Lactobacillus* was performed by the thiobarbituric acid (TBA) method via the measurement of lipid peroxidation[13] based on the monitoring of inhibition of linoleic acid and rat plasma peroxidation by cell-free extracts of bacteria. Linoleic acid was chosen as the unsaturated fatty acid[14]. Linoleic acid (20 mL) emulsion was made up of 0.1 mL linoleic acid, 0.2 mL Tween-20, and 19.7 mL deionized water. Phosphate buffer (15 mL) solution (0.02 *M*, *p*H 7.4), 1 mL linoleic acid or rat plasma emulsion, 0.2 mL $FeSO_4$ (0.01%), 0.2 mL H_2O_2 (0.56 m*M*), and 0.4 mL intracellular extract were mixed and incubated at 37 °C. Intracellular extract was replaced by deionized water in the control samples. After 12 h of incubation, 2 mL reaction solution was mixed with 0.2 mL trichloroacetic acid (TCA; 4%), 2 mL TBA (0.8%), and 0.2 of butylated hydroxytoluene (BHT; 0.4%). This mixture were incubated at 100 °C for 30 min and allowed to cool. Chloroform (2 mL) was then added for extraction. The extract was obtained and then absorbance was measured at 532 nm. The percentage of inhibition of linoleic acid peroxidation and rat plasma peroxidation were defined as:

$$\text{Inhibition (\%)} = \frac{(1 - \text{A532}(\text{experimental sample})}{\text{A532}(\text{control})} \times 100$$

Reduced glutathione status—To eliminate the proteins from the sample, 10% solution of metaphosphoric acid (Sigma, USA) was added to the equal volume of sample and mixed vigorously. This mixture was allowed to stand at room temperature for 5 min and centrifuged at 3000 *g* for 5 min. The supernatant was carefully collected and stored at -20 °C, if the assay was not performed immediately. The change in optical density was measured after 10 min at 412 nm on a spectrophotometer. The glutathione content was quantitated on the basis of a standard curve generated with known amounts of glutathione[15].

Quantitative determination of a,a-diphenyl-b-picrylhydrazyl (DPPH) radical—The DPPH radical scavenging activity was measured as per Shimada *et al*.[16] with a minor modification. Intact cells (0.8 mL), 1 mL CFCS and 1 mL freshly prepared DPPH solution (0.2 mM in methanol) were mixed separately and allowed to react for 30 min. Blank samples contained either PBS or deionized water. The scavenged DPPH was then monitored by measuring the decrease in absorbance at 517 nm. The scavenging ability was defined as follows:

(A517 (blank) - A517 (sample)/A517 (blank) ×100%

Qualitative 2, 2, diphenyl-1-picrylhydrazyl (DPPH) assay on TLC—TLC was used to separate ether extract of cell free culture supernatant (CFCS). The plates were dried in the hot air oven. To detect antioxidant activity, chromatograms were sprayed with 0.2%, 2, 2, diphenyl-2-picryl-hydrazyl (DPPH) in methanol, as an indicator[17]. The presence of

antioxidant compounds was detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

Reducing power assay—The reducing power of the *Lactobacillus* strain was determined as per Oyaizu[18]. *Lactobacillus* sample (100 µL of 10 mg/mL) were mixed with an equal volume of 0.2 M phosphate buffer, *p*H 6.6, and 1% potassium ferricyanide (w/v). The mixture was incubated at 50 °C for 20 min, during which time ferricyanide was reduced to ferrocyanide. Trichloroacetic acid (10% w/v) (0.5 mL) was added to the mixture and then centrifuged at 3,000 *g* for 10 min. The upper layer of the solution was mixed with deionized water and 0.1% $FeCl_3$ (w/v) at a ratio of 1:1:1 (v/v/v) and the absorbance at 700 nm was measured to determine the amount of ferrocyanide (Prussian blue) formed. Increased absorbance of the reaction mixture indicated increased reducing power of the sample and the reducing activity of cysteine was used as a standard.

Animal study

Selection of animals and care—Healthy, adult, male albino Wistar strain rats (18) weighing 100±15 g (supplied by Ghosh Animal, Animal Foods and Animal Cages Supplier, Kolkata 54) were used. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed six per cage in a temperature-controlled room (22 ± 2 ºC) with 12-12 h dark-light cycle at 50±10 % RH. They were provided with standard food (pellet diet) and water *ad libitum*. The principle of laboratory animal care of National Institute of Health USA guideline was followed throughout the duration of experiment[19] and the Institute Ethics committee approved the experimental protocol.

Grouping of animals and experimental procedure—Animals were randomized and divided into three groups (NC, U, and UP) of six animals each. Group NC served as untreated control and was injected with distilled water (5 mL/kg body weight daily for 7 days). Groups UP and U animals were treated with 500 mg/kg body weight of the acetaminophen by intraperitoneal injection for 7 days inducing oxidative stress.

Formulation preparation—Food balls were arranged by the casein-based diet with *Lactobacillus* ADK10, sterile 10% honey and milk mixture[20]. The formulation was stored in a –70 °C freezer in aseptic conditions. While no microbial additives were given to rats of NC and U groups, UP group was administetived with 1×10^9 cfu of *L. Ingluviei* ADK10 for 7 days. After 7 days all experimental animals from all groups were sacrificed and blood, liver and kidney were collected.

Antioxidant enzymes

Catalase (CAT)—For the evaluation of CAT activity of blood, liver and kidneys were measured spectrophotometrically according to Beers *et al*[21].

Superoxide dismutase (SOD)—The SOD activity of blood, liver and kidneys were estimated by measuring the percentage of inhibition of the pyragallol autooxidation by SOD[22].

Estimation of Glutathione level—Blood, liver and kidney levels of acid soluble thiols, mainly glutathione, were determined calorimetrically at 412 nm[23]. The concentration of glutathione is expressed as mol/ g blood and tissue.

Estimation of lipid peroxidation from the levels of malondialdehyde (MDA)—The MDA in sample was calculated by using the extinction coefficient of 1.56×10^5 M/cm and expressed in the unit of nM/mg of tissue or nM/mL of plasma[24].

Statistical Analysis—All experiments were performed thrice independently and each assay was performed in triplicate. Results are expressed as mean ± SD. The level of significance was analyzed by ANOVA ($P < 0.05$).

Results

Twenty four strains of *Lactobacillus* sp. were isolated from GI tract of chicken. Results of the primary screening probiotic properties found that 3 out of 21 isolates could be classified as acid and bile tolerant strains with other essential probiotic characteristics. Among these, strain *Lactobacillus* ADK10 exhibited the highest bile and acid resistance. After using species specific PCR, this strain was identified as *Lactobacillus ingluviei*ADK10 (Fig. 1) with the accession No. JQ395039.

Total antioxidative activity of bacteria—*Lactobacillus* strain ADK 10 was tested for its antioxidative capacity in both intact cells and intracellular cell-free extracts in the present study. Both intact cells and cell-free extract of ADK 10 possess the inhibited linoleic acid and rat plasma peroxidation (Table 1). The scavenging DPPH rate of intact cells was higher than that of cell-free extracts.

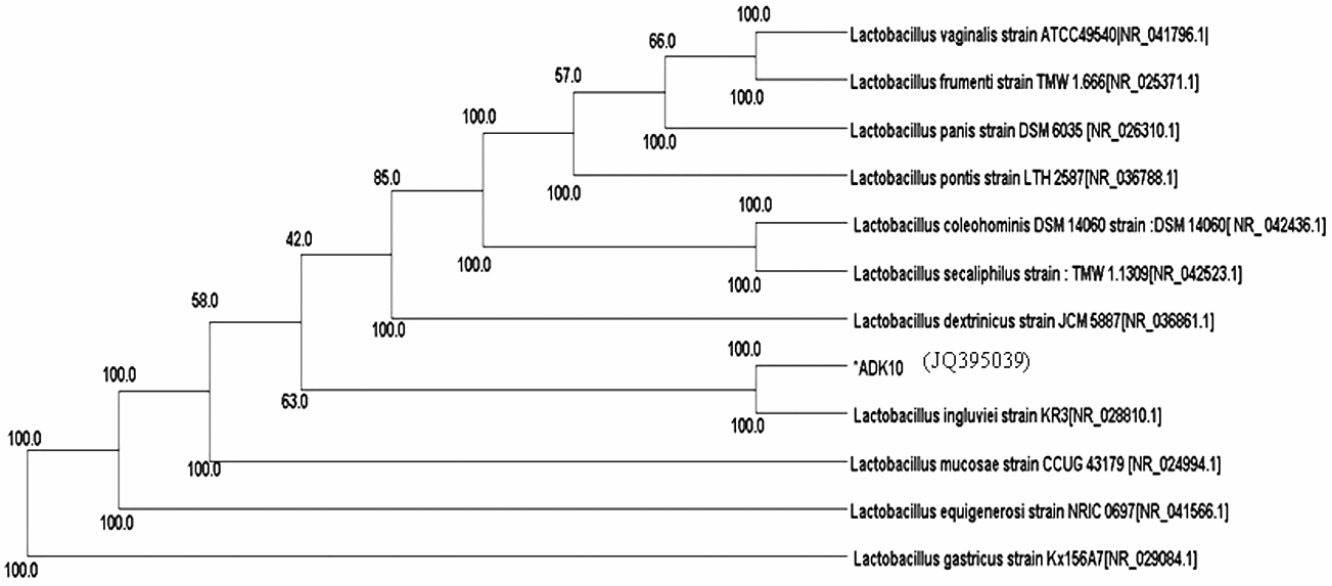

Fig. 1—Phylogenetic tree of ADK10 with its accession number

Table 1—Antioxidative activity of bacteria

Methods	The intact cells of 10^9 cells of ADK10 (%)	Cell-free extract (1 mL)
Linoleic acid peroxidation	67.95± 4.98	61.73±4.77%
Rat plasma peroxidation	64.4± 4.23	56.96±5.98%
Glutathione content	-	93.95µg/mL
DPPH free radical scavenging	71.50±6.12	63.99±6.09%

Reducing power assay—Strain exhibited strong reducing capacity. The intact cells of ADK10 showed higher reducing capacity than the cell-free extract, equivalent to 290±3.21 and 273±7.28 µM L cysteine respectively.

Activities of blood, liver and kidney catalase, SOD, glutathione and MDA—Lipid peroxidetion level (MDA level) and plasma, liver and kidney level of SOD, CAT, GSH are shown in Table 2.

Discussion

Two types of antioxidative pathways of lactic acid bacteria enzymatic and non-enzymatic defence systems have been described. Antioxidant enzymes play a critical role in defence against ROS. Such as superoxide dismutase (SOD) eliminates direct toxicity of superoxide anions and glutathione peroxidase (GPx) scavenges H_2O_2 and hydroxyl radicals[25]. However, to prevent excessive oxidative stress, cells and organisms had to develop non-enzymatic defence mechanisms, including reduction activity and the chelating capacity of metal ion, which can eliminate active oxygen[26]. Evidently, the high total antioxidative activity (TAA) of the present antioxidative strain is one of the reasons for their increased resistance to ROS. Lin and Chang[8] found that some intestinal lactic acid bacteria, inhibiting linoleic acid oxidation, revealed significant antioxidative activity (in the case of *L. acidophilus* ATCC 4356 an antioxidative capacity was 28–45%). The *Lactobacillus* strain in the present study was facultative aerobic and grew both in the anaerobic and aerobic CO_2 enriched milieu. The aerobic growth conditions are very important as one of the purposes was to establish the mechanisms by which *Lactobacillus* ADK10 possess ROS eliminating effects. If there is more oxygen in a medium, there will be more oxygen free radicals generated and the antioxidative activity of the *Lactobacillus* can be evaluated more precisely. The results of the present study confirm these findings as the data showed that *Lactobacillus* ADK10 intact cells and CFCS inhibit linoleic acid and rat plasma oxidation.

The result of the present study shows that the *Lactobacillus* ADK10 supernatant contains glutathione, which is known as an important cellular scavenger of hydroxyl radicals. However, the existence of other ROS scavenging thiol-compounds in lactobacilli is not excluded. Such understanding is supported both by data that thioglycollate, cysteine,

Table 2—2Effect of acetaminophen and *Lactobacillus* ADK10 treatment on superoxide dismutase (SOD), catalase (CAT), melondialdehyde (MDA) and glutathione (GS H) of blood (B), kidney (K), liver (L) activities in acetaminophen induced oxidative stress and uremia in rat.

[Values are mean ± SE from 6 animals each group]

Parameters	Group NC (Control)	Group U (Acetaminophen receiving control)	Group UP (*Lactobacillus* ADK10 treatment followed by acetaminophen receiving)
Superoxide dismutase (mmol/mL/min) B	4.11±0.44[a]	0.85±0.2[b]	4.43±0.55[a]
K	2.13±0.26	0.90.95±0.62[b]	2.38±0.24[a]
L	2.78±0.46[a]	7±0.16[b]	3.16±0.8[a]
Catalase (mmol/mL/min)B	8.67±0.12[a]	3.12858±0.02[b]	9.36±.01a
K	1.54±.014[a]	0.236±.05[b]	1.99±0.04[a]
L	2.38±.016[a]	0.57±.08[b]	2.37±.016[a]
Blood glutathione (µmol/ mL)	139.5±2.716[a]	73.3±4.01[b]	132.8±5.08[a]
Kidney glutathione (µmol/ g of tissue)	127.8±6.8[a]	59.81±6.33[b]	126.7±4.9[a]
Liver glutathione (µmol/ g of tissue)	122±5.56[a]	74±5.96[b]	127±1.9[a]
Blood MDA (nM/mL)	622.49±19[a]	946.45±217[b]	619.82±130[a]
Kidney MDA (nM/mg of tissue)	171.8±48.1[a]	552.2±52.5[b]	115.7±43.4[a]
Liver MDA (nM/mg of tissue)	82.51±8.51[a]	283.78±9.49[b]	124.76±31.98[a]

ANOVA followed by multiple two tail t-test and data with different superscript (a, b) in specific horizontal column differ from each other significantly ($P< 0.05$).

dithiotreitol, etc., stabilize the multiplication of *L. delbrueckii* due to their low redox potential[27].

The DPPH method measures electron-donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen to a mixture will react with and bleach DPPH. DPPH is reduced from a purple compound to a light yellow compound by electrons from oxidant compounds. Reaction of DPPH with hydroxyl groups involves a homolytic substitution of one of the phenyl rings of DPPH yielding 2- (4-hydroxyphenyl)-2-phenyl-1- picryl hydrazine as a major product while 2- (4nitrophenyl)-2phenyl-1-picrylhydrazine is also formed via a series of secondary processes. The concentration of DPPH at the end of a reaction will depend on the concentration and structure of the compound being scavenged[28]. *Lactobacillus* ADK10 had significant ability to scavenge the DPPH. The TLC-DPPH screening method indicated the presence of antioxidant compounds in *Lactobacillus* ADK10 showing the most prominent antioxidant activity (Fig. 2). Visualization of the compound with antioxidant activity enables the localization and the subsequent identification of the potential active compounds. This finding of high free radical scavenging activity of *L. ingluviei* ADK10 suggested a high efficacy of these strains as a promising antibacterial agent with potential antioxidant activity for health promotion of the host.

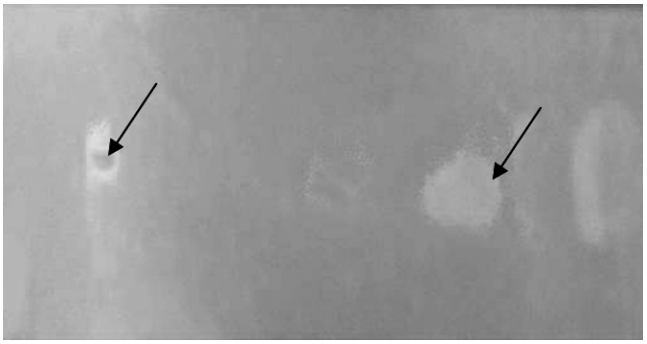

Fig. 2—Antioxidant compounds in *Lactobacillus* ADK10 showing the most prominent spots on TLC paper

In the present study, administration of nephrotoxic dose of acetaminophen to rats resulted in progress of oxidative stress harmful to hepatic and renal tissues. During kidney injury, superoxide radicals are generated at the site of damage, which modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism[29]. The present study also demonstrated that acetaminophen overdose resulted in a decrease in the SOD and CAT activities in group U,

when compared with normal control rats (group NC). It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When rats were treated with the *L. ingluviei* ADK10*,* SOD and CAT activity increase significantly when compared with induced group (P<0.05) (Group U). However, acetaminophen was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes cell damage in acetaminophen hepatotoxicity[30]. Superoxide dismutase (SOD) and catalase are antioxidant enzymes. They protect cells against oxygen free radical damage. Glutathione has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; glutathione is also a scavenger of hydroxyl radicals and singlet oxygen. Free oxygen radicals can induce lipid peroxidation in cells, and malondialdehyde (MAD) is formed during oxidative degeneration and accepted an indicator of lipid peroxidation[28]. Current evidence suggests that intracellular glutathione plays an essential role in detoxification of acetaminophen and prevention of acetaminophen-induced toxicity in liver and kidney[31]. The generation of the reactive oxygen species appears as an early event which precedes intracellular glutathione depletion and cell damage in acetaminophen hepatotoxicity[32]. Acetaminophen administration also caused a significant decrease in glutathione content. Administration of *L. ingluviei* ADK10*,* helped to uplift the glutathione depletion induced by acetaminophen. ROS can be detoxified by a number of antioxidant enzymes, glutathione contents are antioxidant components, which protect cells from oxidative damage by scavenging ROS. *Lactobacillus ingluviei* ADK10 maintaining the level of glutathione increase the level of this enzyme resulting in higher resistance of the cell toward oxidative stress and acetaminophen induced oxidative stress.

Based from results obtained of the present study, it can be concluded that *Lactobacillus ingluviei* ADK10 isolated from chicken intestine possess a number of interesting important properties that constitute the requirement for their use as high potential antioxidative agents with health-promoting effects.

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References

1 Jackson-Roberts II L & Morrow JD, Analgesic- antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout, in *Goodman and Gilman's The pharmacological basis of therapeutics*, 10th edition, edited by Hardman JG, Limbird LE and Gilman AG (McGraw-Hill Medical Publishing Division, New York) 2001, 687.

2 Nelson SD, Mechanisms of the formation and disposition of reactive metabolites that can cause acute liver injury, *Drug Metab Rev*, 27 (1995) 147.

3 Lawson JA, Farhood A, Hopper RD, Bajt ML & Jaeschke H, The hepatic inflammatory response after acetaminophen overdose: role of neutrophils, Toxicol Sci, 54 (2000) 509.

4 Ostapowicz G, Fontana RJ, Schiodt FV, *et al*. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States, *Ann Intern Med*, 137 (2002) 947.

5 Jones AF & Vale JA, Paracetamol poisoning and the kidney, *J Clin Pharm Ther*, 18 (1993) 5.

6 Hart SG, Beierschmitt WP, Wyand DS, *et al*. Acetaminophen nephrotoxicity in CD-1 mice. I. Evidence of a role for in situ activation in selective covalent binding and toxicity, *Toxicol Appl pharmacol*, 126 (1994) 267.

7 Tumper L, Monasterolo LA & Elias MM, Probenecide protects against invivo acetaminophen-induced nephrotoxicity in male wistar rats, *J Pharmacol Exp. Ther*, 24 (1998) 606.

8 Gilliland S E, Health and nutritional benefits from lactic acid bacteria, *FEMS Microbiol. Lett*, 87 (1999) 175.

9 Leroy F & Vuyst LD, Lactic acid bacteria as functional starter cultures for the food fermentation industry, *Trends Food Sci. Tech*, 5 (2004) 67.

10 Lin MY & Chang FY, Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356*, Digest Dis. Sci*, 45 (2000) 1617.

11 Kiss H, Kögler B, Petricevic L, Sauerzapf I, Klayraung S, Domig K, Viernstein H & Kneifel W, Vaginal *Lactobacillus* microbiota of healthy women in the late first trimester of pregnancy*, BJOG Int. J. Obst. Gyn*. 114 (2007) 1402.

12 Halder K S, Maity C, Jana A, Pati R B & Mondal C K, Chitinolytic enzymes from the newly isolated Aeromonas hydrophila SBK1 study of the mosquitocidal activity, *Bio Control* (in press) doi 10.1007/s1052601194053.

13 Decker E & Faraji H. Inhibition of lipid oxidation by carnosine*, JAOCA*, 67 (1990) 650.

14 Wanasundrara U, Amarowicz R & Shahidi F, Isolation and identification of an antioxidative component in canola meal*, J Agric Food Chem,* 420 (1994) 1285.

15 Griffith OW, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine, *Anal. Biochem*, 106 (1980) 207.

16 Shimada K, Fujikawa K, Yahara K & Nakamura T, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion*, J Agric Food Chem*, 40 (1992) 945.

17 Deby C & Margotteaux G, Relationship between essential fatty acids and tissue antioxidant levels in mice, *C R Seances Soc Biol Fil,* 165 (2003) 2675.

18 Oyaizu M, Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine*, Jpn. J. Nutr*, 44 (1986) 307.

19 Olert ED, Cross BM & McWilliam AA, Guide to care and use of experimental animals, in *Canadian Council on Animal Care*, 2nd edn edited by ED Olert and McWilliam (1993). Ottawa, pp90.

20 Ranganathan N, Patel B & Ranganathan P, *In Vitro* and in vivo assessment of intraintestinal bacteriotherapy in chronic kidney disease*, ASAIO Journal,* (2006) 70.

21 Beers RF & Sizer IW, A spectrophotometric method for measuring the breakdown of hydrogen peroxide of catalase, *J Biol Chem*, 195 (1952) 133.

22 Marklund S & Marklund G, Involvement of superoxide anion radical in autotoxidation of pyrogallol and a convenient assay of superoxide dismutase*, Eur J Biochem*, 47 (1974) 469.

23 Ellman GL, Tissue sulfhydryl groups, *Arch Biochem Biophys*, 82 (1959) 70.

24 Amanatidou A, Bennik MH, Gorris LG & Smid EJ, Superoxide dismutase plays an important role in the survival of *Lactobacillus sake* upon exposure to elevated oxygen, Arch, Microbiol, 176 (2001) 79.

25 Saide JAO & Gilliland SE, Antioxidative activity of lactobacilli measured by oxygen radical absorbance capacity, *J Dairy Sci,* 88 (2005) 1352.

26 Rees JF & Pirt SJ, The stability of lactic acid production in resting suspensions of *Lactobacillus delbrueeckii*, *J Chem Technol Biotechnol*, 29 (1979) 591.

27 Naik G H, Priyadarsini K I, Satav J G, Banavalikar M M, Sohoni D P, Biyani M K & Mohan H, Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine*, Phytochemistry*, 63 (1) (2003) 97.

28 Abdel-Zaher OA, Abdel-Rahman MM & Hafez MM, Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage, *Toxicology*, 243 (2007) 124.

29 Pande M & Flora SJS , Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats, *Toxicology*, 17 (2002) 187.

30 Neilsen F, Mikkelsen BB, Neilsen JB, Andersen HR & Grandjean P, Plasma malondialdehyde as biomarker for oxidative stress reference interval and effects of life-style factors*, Clin Chemis*, 47 (1997) 1209.

31 Newton J, Hoefle D, Gemborys M, Mugede G & Hook J, Metabolism and excretion of a glutathione conjugate of acetaminophen in the isolated rat kidney, *J Pharmacol Exp Ther*, 237 (1996) 519.

32 Manov I, Hirsh M & Ianccu TC, Acetaminophen hepatotoxicity and mechanisms of its protection by N-acetylcysteine a studyof Hep3B cells, *Exp Toxicol Pathol*, 53 (2003) 489.