Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2016.169.177

Research Article
In vivo Evaluation of Safety and Probiotic Traits of Isolated *Enterococcus feacium* Strain KT712

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Abstract

Background: Enterococci represent a significant part of the microbial population in karish cheese, a traditional cheese produced in Egypt. They are Lactic Acid Bacteria (LAB) that are important in environmental, food and clinical microbiology. **Objective:** *In vitro* and *in vivo* evaluation of safety and probiotic traits of isolated enterococci strain targeted for functional foods preparations. **Materials and Methods:** Thirty three enterococci strains isolated from karish cheese were screened. The strain *Enterococcus feacium* (KT712) was selected according to preferable phenotypic and technological characterization then identified using 16S rRNA approach. *In vitro* probiotic traits were evaluated. The strain was inoculated as single culture to obtain cultured fermented milk used to feed Albino rats for *in vivo* evaluation. **Results:** The strain showed probiotic characteristics through its acid and bile salt tolerance *in vitro*, while, *in vivo* functional probiotic traits were announced in hypolipidemic effect, immunity and growth stimulation. Hematology analysis, oxidative stress parameters, carcinoembryonic antigen (CEA) levels and histological examination indicated safety of tested *Enterococcus* strain. **Conclusion:** Researchers can suggest employment of the tested *Enterococcus strain* as a probiotic in food or feed functional preparations innovations but more investigations such as; the presence of transferable antibiotic resistance genes can be recommended to guarantee safe use of the ingested strain. The sequence data of the isolated probiotic LAB strain, *Enterococcus feacium* (KT712) in this study has been deposited to the GenBank data library under the accession number KX214763.

Key words: Enterococci, probiotic, *in vitro*, *in vivo*, hypolipidemic

Received: June 16, 2016 Accepted: August 05, 2016 Published: September 15, 2016

Citation: M.G.M. Allam, A.M.G. Darwish, E.H.E. Ayad, E.S. Shokery, R.E. Mashaly and S.M. Darwish, 2016. *In vivo* evaluation of safety and probiotic traits of isolated *Enterococcus feacium* strain KT712. Res. J. Microbiol., 11: 169-177.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Enterococci bacteria play an important beneficial role in the production of various traditional fermented food products. In present years, the role of LAB in health and functionality of human have been well emphasized, mainly because of their ability to growth in low pH and to produce antimicrobial agents[1].

Human nutrition has already been using probiotics especially in dairy products. Enterococci are natural flora of fermented foods and they contribute to the bouquet and particular flavour of different kinds of regional cheeses. For that, the enterococci were suggested for use as a starter in the production of different types of cheeses such as cebreiro, feta and mozzarella. They are recognized as nosocomial pathogens from this consideration, it is important to determine safety before using enterococci for probiotic preparations. On the basis, the proper selection of enterococci is possible and can be used as ideal probiotics[2,3]. Animal nutrition is another area for successful application of probiotics and the *Enterococococcus* spp., seems to be the most utilized as additive microorganisms. Several probiotics have received temporary approval in European Union but their modes of action which lead to beneficial effects are only partly known[4].

Positive effects was reported earlier for *Enterococcus* spp., such as ability to inhibit the growth of food-borne pathogens, stimulating weight gain, hypocholesterolaemic and reduction of LDL-cholesterol, enhancing gut microbiota, immune modulatory by increasing lymphocytes and bacteriocin-producing[5–9].

The objective of this study was; the *in vivo* assessment of safety and probiotic aspects of selected probiotic *Enterococcus faecium* (KT712) strain isolated from traditional karish cheese in order to introduce a new probiotic strain helps in food or feed functional preparations innovation. The strain was selected and genetically identified on basis of favorable technological characteristics and *in vitro* potential probiotic aspects.

MATERIALS AND METHODS

Sampling: Enterococci Lactic Acid Bacteria (LAB) was isolated from edible source, traditional karish cheese and made of defatted raw cow's milk following primitive method of manufacture as the fermentation occurs as a result of the wild flora present in the raw milk and the surrounding environment, therefore, the taste the texture and the flora of the final cheese vary too much. Fifteen samples of artisanal Egyptian karish cheese were collected from different local markets from Alexandria, Egypt and kept refrigerated.

Isolation of enterococci and species identification: The samples were enriched in skim milk RSM (12.5%) inoculated in selective M17 medium for cocci isolation[10] and purified through streak plate method. Purified strains were stored and registered in Faculty of Agriculture Saba Basha, Alexandria University Culture Collection (FABA).

Fifty two cocci shaped Gram-positive and catalase-negative isolates were identified to the genus level using phenotypic (CO_2 production, growth at 45 and 10°C, growth in of 6.5% NaCl, in pH 9.6 and in SF medium) and biochemical (carbohydrate fermentation) characterization[11]. Selected strain characterization was confirmed using genotypic 16S rRNA approach.

Technological characterization of selected strains

Flavour production: Flavour production was carried out on the 33 enterococci strains according to Ayad *et al.*[12]. The intensity of flavour was scored on a scale from (1-4), 1: Slightly, 2: Moderate, 3: Strong and 4: Very strong and the overall grade of 10. Based on flavour production results, 17 enterococci strains were selected for further testing.

Acid production: The cultures were considered as fast-, medium- or slow-acidifying when a Δ pH of 0.4 U was achieved after 3, 3-6 and >6 h, respectively[13].

Autolytic activity: The rate of autolysis was determined according to Thiboutot *et al.*[14]. The autolytic activity was determined as the percentage decrease in the absorbance (OD_{650}) at different time intervals.

Proteolytic activity: The ability of strains to hydrolyze milk protein was examined as any halo of proteolysis around and underneath the growth that indicate proteolytic activity according to Ayad[15].

Antagonistic effect: Antagonistic effect was determined by agar well-diffusion assay against enteropathogenic *E. coli* obtained from the culture collection of NIZO (Food Research, Ede, the Netherlands) with measuring the diameter of the inhibition zones as described by Ayad *et al.*[16].

Exopolysaccharide (EPS) production: Inoculated loop method was used to assess slime formation according to Knoshaug *et al.*[17] strains were considered positively slime producer if the length of slime was above 1.5 mm.

Molecular identification of selected LAB isolates: After isolation of chromosomal DNA from selected LAB isolates

according to Maniatis *et al.*[18], the universal primers used for PCR, PCR products were purified prior to sequencing by using PCR clean up promega kit. The DNA sequences of PCR products were determined by using an AB 373 DNA sequence (Applied Biosystem, Mubarak city for scientific research). Basic Local Alignment Search Tool (BLAST) algorithm was used to search for homologous sequences in GenBank. Software Bioedit was used to align the query with other sequences in the GenBank then phylogenetic tree of the bacterial isolates was drawn. The sequence of 16S rRNA was deposited to the GenBank database under the accession number KX214763.

***In vitro* assessment of probiotic traits of *Enterococcus faecium* (KT712):** Acid tolerance was tested on pH (2.0, 3.0 and 4.0±0.1) with HCl while bile salt resistance was tested using MRS broth containing 0.2, 0.3 and 0.4% (w/v) bile salts (Biolife, Italy) measuring optical density at 650 nm ($O.D_{650}$) as described by Baccigalupi *et al.*[19]. Culture's growth in standard MRS broth (pH 6.6) was used as a blank.

***In vivo* evaluation of selected *Enterococcus faecium* (KT712)**

Preparation of milk culture for animals diet: The strain *E. faecium* (KT712) was selected for *in vivo* evaluation since it showed preferred technological attributes and *in vitro* probiotic traits. The selected strain was inoculated for obtaining 10^6-10^7 CFU mL^{-1} in milk culture, incubated at 40°C followed by cooling at 4°C.

Animals and conditions: Ten male Albino rats aged 4 weeks obtained from (Faculty of Medicine, Alexandria University, Egypt) were acclimatized by feeding on commercial chow (ATMIDA; protein 18.5%, fat 2.8% and fiber 11.2%) for 1 week before starting the experiment at room temperature (25±2). Animals were divided into two groups, 5 rats each. The control group fed the commercial chow diet and drank pasteurized milk. The other group fed the commercial chow diet and drank fermented milk inoculated with selected strain *E. faecium* (KT712), daily for 5 weeks. The institutional guidelines for care and use of animals have been followed and at the end of the experiment and under the approval of ethical committee, the rats were sacrificed after overnight fasting under light diethyl ether anesthesia. Blood samples were collected from abdominal aorta in heparin added tubes for blood analysis and in plain tubes for serum analysis.

Evaluation of probiotic traits

Growth performances and general health: Body weights were recorded regularly. Occurrence of diarrhea and vomiting was monitored daily. Final weights were recorded, then after necropsy, the rat's organs, liver, kidneys, brain and spleen were dissected out carefully, checked and weighed.

Plasma lipid analysis: Total cholesterol, triglyceride, plasma high density lipoprotein (HDL), plasma low density lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and total lipid.

Safety evaluation

Complete Blood Count (CBC): The CBC was used as a broad screening checking for any disorders. Hematology parameters were determined using cell dyne 6000 SL hemotology analyzer battery, USA. All blood tests were analyzed at Mabarret El-Asafra Laboratories, Alexandria, Egypt.

Antioxidative enzymes: Concentrations of TBARS in RBC and superoxide dismutase (SOD) was determined using Enzyme-linked immunosorbent assay (ELISA).

Carcinoembryonic antigen (CEA): The CEA (Hitach 7600, Japan) was used by (Applied biosystem, Mubarak city for scientific research).

Histology examination: Tissue samples of liver were embedded in paraffin wax. Six sections (4 μm thick) from each organ were cut using (Microtome mod-1130/B10 cut REICHERT-Jung, Germany) and stained by hematoxylin and eosin (H and E stain) according to Gordon *et al.*[20]. The slides prepared with synthetic resin (Entellan-Merck) were observed under light microscopy up to 400X.

Statistical analysis: Statistical analysis was performed using analytical software SPSS® 13.0 (Statistical Package for the Social Sciences) (2005). Mann-Whitney U test was used to compare between the test group and the control group regarding growth, plasma lipid profile, biochemical and hematology parameters. Differences were considered significant at $p<0.05$. Standard deviation was calculated for (N) replicates according to each experiment (All experiments held in triplicates, except for *in vivo* related analysis was for 5 rats of each group).

RESULTS

Identification of enterococci isolates: Fifty two coccci isolates gained from 15 karish cheese samples were phenotypically identified. *Enterococcus* spp., represented (63.46%) of total isolated cocci strains in karish cheese samples. Based on flavor formation, 15 enterococci isolates were selected for further

examination. Carbohydrate fermentation profile of selected enterococci isolates approved the phenotypic characterization and highlighted the limited *Enterococcus* spp., biodiversity in tested cheese samples in the two species; *Enterococcus faecium* (60%) and *Enterococcus faecalis* (40%).

Technological characterization: Table 1 illustrates technological characteristics of the 15 selected enterococci isolates. More than 53% of selected strains scored slow acidification rate, fast rate was recorded in only one strain, *Enterococcus faecalis* (KT713) and medium acidification rate was noticed in 6 *enterococcus* strains; 4 *E. faecium* and 2 *E. faecalis*. Only two *Enterococcus faecium* strains were autolytic, KT712 and KM742 isolates. The autolytic activity of the rest of enterococci isolates ranged between 2.4% (KT717) and 11.5% (KT624). None of the selected strains showed proteolytic activity. The culture supernatant of only two strains *Enterococcus faecalis* (KT724) and *Enterococcus faecium* (KM742) showed antagonistic activity against pathogenic *E. coli* with diameter 1 cm which may nominate these strains to be used as bio-preservatives. The results showed that none of selected strains produced EPS.

Molecular identification via 16S rRNA approach: Depending on technological characterization the strain; *Enterococcus faecium* (KT712) was selected for molecular identification via 16S rRNA approach (for its medium acidification effect and autolytic activity) which confirmed the phenotypic and biochemical identification. The results of Basic Local Alignment Search Tool (BLAST) analysis derived from aligning the 16S rRNA partial sequences was used to draw phylogeny trees (Fig. 1). Multiple alignments were carried out to confirm the results of BLAST analysis and then the sequence data of the isolated probiotic strain was deposited to the GenBank data library under the accession number KX214763.

The following is the partial nucleotide sequence of 16S rRNA gene of KT712 strain (*Enterococcus faecium*) (accession number in GenBank KX214763, bases <1..>548)

AGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGCATT
TCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAG
TCTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTT
TCACATCAGACTTAAGAAACCGCCTGCGCTCGCTTTACGCCCA
ATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTG
CTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAA
GGGATGAACAGTTACTCTCATCCTTGTTCTTCTCTAACAACAGA
GTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCG
GTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCC
CGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCA
CCCTCTCAGGTCGGCTATGCATCGTGGCCTTGGTGAGCCGTTA
CCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAGCGA
CACCCGAAAGCGCCTTTCAAATCAAAA.

***In vitro* assessment:** *In vitro* assessment of the strain *Enterococcus faecium* (KT712) showed its tolerance to acid and bile salt as in Fig. 2a and b.

***In vivo* assessment:** All animals were healthy and no noticeable abnormal behavior, changes in activity or decline in hair luster were observed after 5 weeks of feeding the *Enterococcus faecium* KT712.

Probiotic traits evaluation: Figure 3 shows growth performances of the two tested groups; rats fed cultured milk with *Enterococcus faecium* KT712 gained more body weight

Table 1: Technological characteristics of selected enterococci strains

		Antimicrobial activity						
Strain code	*Enterococcius* spp.	(+, -)	Diameter (cm)	Proteolysis (+, -)	Acid producing ability	Autolytic activity (%)	EPS	Flavor (smell and taste) Description (intensity)[a]
KT622	*Enterococcus faecium*	-	-	-	Midium	9.09	-	Cheese like (3), ester (3), acidic (1)
KT624	*Enterococcus faecium*	-	-	-	Midium	11.5	-	Karish like (2), acid (1)
KT711	*Enterococcus faecium*	-	-	-	Slow	5	-	Karish-like (4), ester (1), cooked (2)
KT712	*Enterococcus faecium*	-	-	-	Midium	39.1	-	Karish-like (4), ester (2), fruity (2)
KT713	*Enterococcus faecalis*	-	-	-	Fast	4	-	Fruity (3), citrus (2), ester (2), sour (2)
KT714	*Enterococcus faecium*	-	-	-	Midium	10.11	-	Acid (3), ester (2), yoghurt-like (4), sharp (1)
KT716	*Enterococcus faecalis*	-	-	-	Midium	4.5	-	Karish-like (3), ester (1), cooked (2)
KT717	*Enterococcus faecium*	-	-	-	Slow	2.4	-	Karish-like (3), ester (3), sweet (2)
KT724	*Enterococcus faecalis*	+	1.00	-	Midium	10	-	Fruity (4), ester (4), youghurt-like (4)
KT725	*Enterococcus faecium*	-	-	-	Slow	4	-	Fruity (1), ester (2), sweet (3)
KT742	*Enterococcus faecalis*	-	-	-	Slow	2.6	-	Fruity (3), sweet (2), rayb-like (2)
KT743	*Enterococcus faecalis*	-	-	-	Slow	9.09	-	Karish-like (3), ester (1), cooked (2)
KT745	*Enterococcus faecalis*	-	-	-	Slow	9.09	-	Fruity (2), citrus (2), sweet (2), ester (3)
KM711	*Enterococcus faecium*	-	-	-	Slow	3.58	-	Ester (4), fruity (3), karish-like (2)
KM742	*Enterococcus faecium*	+	1.00	-	Slow	44	-	Citrus (3), ester (3)

Fast: Δ pH of 0.4 U was achieved after 3 h, Medium: in 3-6 h, Slow: >6 h, [a]Intensity on scale from 1-4, 1: Slightly, 2: Moderate, 3: Strong and 4: Very strong, data of autolytic activity percentage based on the mean of triplicates

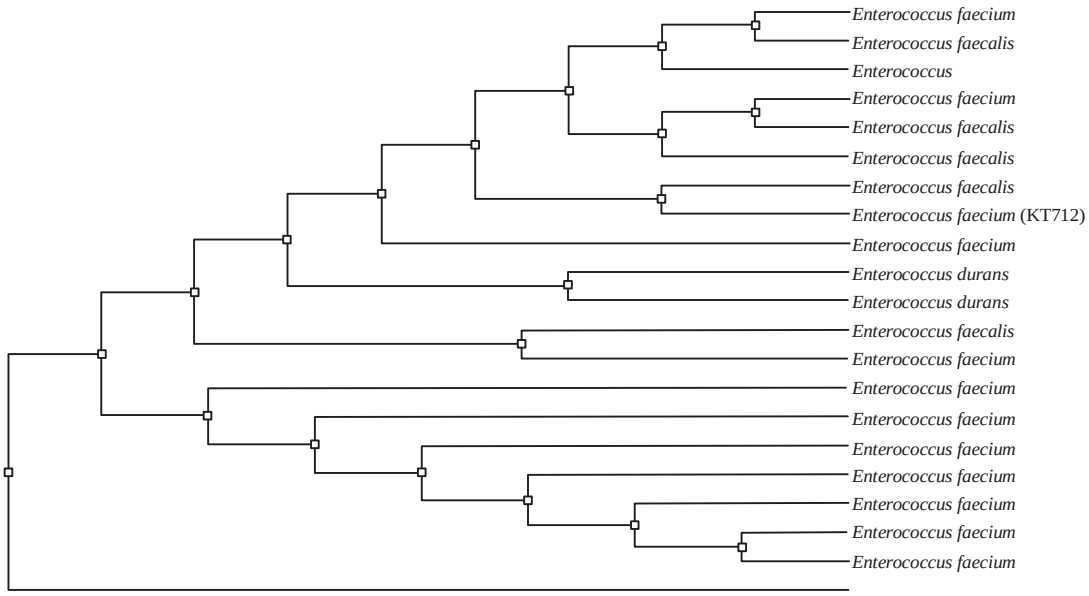

Fig. 1: Phylogenic tree of partial sequences of *Enterococcus faecium* (KT712) strain [Constructed using a BioEdit version 5.0.6.]

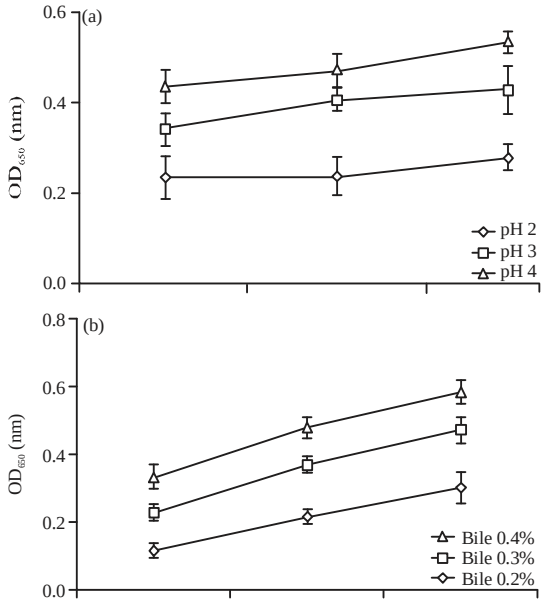

Fig. 2(a-b): (a) Acid tolerance of the strain *Enterococcus faecium* (KT712) and (b) Bile salt tolerance of the strain *Enterococcus faecium* (KT712), data are the Mean of triplicates±SD

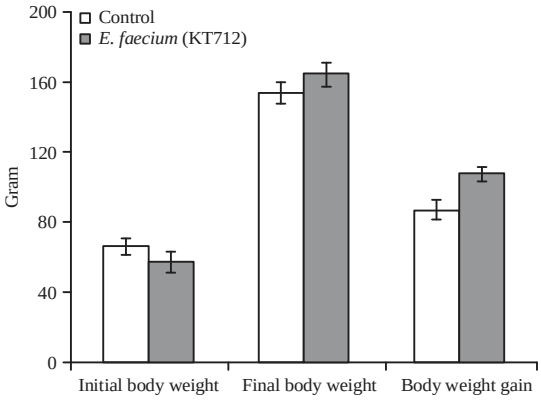

Fig. 3: Growth performances of the tested groups, data are the Mean±SD of 5 rats per group

at the end of the experiment than the control group. While, the relative organs' weights, liver, spleen, kidneys and brain weight (g organ/100 g b.wt.) were comparable to control.

Figure 4a and b show plasma lipid profile and atherogenic indices of the two groups of rats. Although, results showed significantly higher concentration in total lipids, triglycerides and VLDL-Ch in *E. faecium* (KT712) group but this was accompanied with remarkable suppression in Low Density Lipoprpteins (LDL) (up to 37.5%) that led to a suppression in atherogenic indices (up to 38.8%) comparing to control group.

Safety assessment: The hematology parameters results showed in Table 2 revealed two controversial results concerning the rat's group fed fermented milk with *Enterococcus faecium* (KT712). Firstly, the significant increase of WBCs counts of the experimental group. Secondly, the significant decrease in platelets count.

Table 2 presents biochemical parameters of rats group fed fermented milk with *E. faecium* (KT712). The results clearly

showed that the strain didn't cause the production of carcinoembryonic antigen protein (CEA). The *E. faecium* (KT712) strain fed group showed slight decrease in superoxide dismutase (SOD) and increase in thiobarbituric acid reactive substance (TBARS).

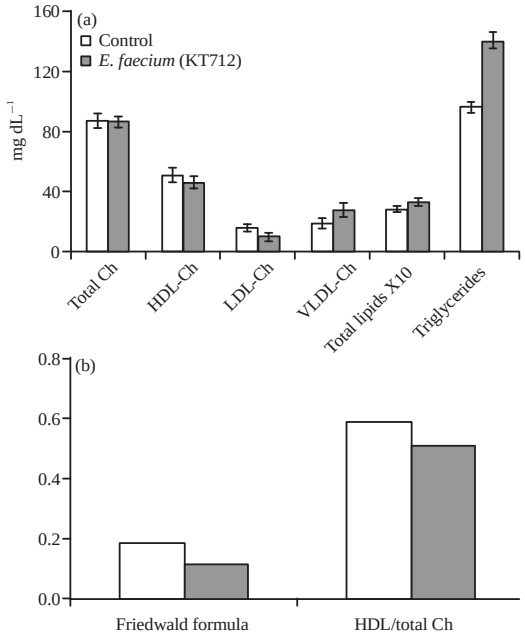

Fig. 4(a-b): (a) Plasma lipid profile and (b) Atherogenic indices, Ch: Cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, Friedwald formula: LDL-Ch/HDL-Ch, data are the mean of 5 rats per group ±SD, except for atherogenic indices that calculated as a ratio using mean values

Figure 5 illustrates the light micrograph of a rat liver, the histological examination of rat's levers revealed normal

Table 2: Hematology and biochemical parameters of treated rats

Parameters	Control	*Enterococcus faecium* (KT712)
Hematology parameters		
WBCs×(10)^3	8.90±1.28^a	15.90±2.11^c
RBCs×(10)^6	6.63±1.08^a	6.77±1.48^a
Hb (%)	84.00±3.08^a	85.00±2.92^a
Ht (%)	41.00±2.92^a	40.00±3.16^a
Platelets×(10)^3	773.00±4.18^a	552.00±3.39^b
RDW	19.50±2.83^a	17.90±2.28^b
MCV	61.70±1.93^a	59.40±2.70^b
MCH	18.10±2.65^a	18.30±1.88^a
MCHC	29.60±2.47^a	30.30±2.85^a
Leucocytes		
Poly	15.00±1.58^a	16.00±1.87^a
Mono	7.00±1.87^a	5.00±1.41^b
Lymph	74.00±3.39^a	77.00±2.65^a
Eosino	3.00±1.22	1.00±0.70
Baso	0	0
Band	1	1
Biochemical parameters		
SOD (μM mg^{-1})	53.00±2.83^a	49.00±2.92^a
TBARS (μM mg^{-1})	23.00±2.12^a	28.00±2.24^a
CEA (mg dL^{-1})	<0.2	<0.2

WBCs: White blood cells count, RBCs: Red blood cell count, Hb: Hemoglobin, Ht: Hematocrit, RDW: Red cell distribution width, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, Poly: Polymorphonuclear, Mono: Monocytes, Lymph: Lymphocytes, Eosino: Eosinophils, Baso: Basophils, Band: Band cells, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substances, CEA: Carcinoembryonic antigen, data are the mean for 5 rats per group ±SD ($p<0.05$), ^{a,b,c}Means values in the same row marked with unlike letters are significantly different ($p<0.05$)

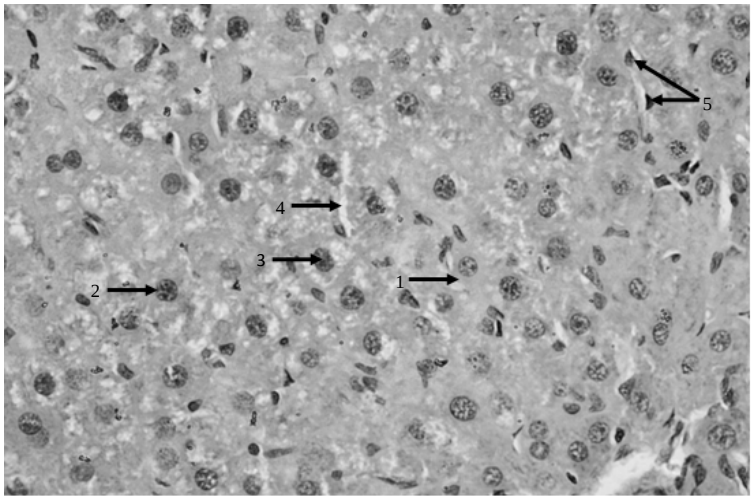

Fig. 5: Light micrograph of a rat liver (Mic. Mag. X400), H and E stain, 1: Hepatocytes, 2: Nuclei, 3: Nucleoli, 4: Blood sinusoids and 5: Kupffer cells

architecture of hepatocytes separated by blood sinusoids. Hepatocytes appeared with central active nuclei and prominent nucleoli, surrounding by vacuolated acidophilic cytoplasm. Blood sinusoids showed normal caliber and lined by flat endothelial cells and kupffer cells (macrophages). No abnormal clinical signs were noticed in the group receiving viable cells of *E. faecium* (KT712) and was similar to (control).

DISCUSSION

Enterococci are recognized as an essential part of the natural microflora of many dairy products and in some cheeses, they dominate over lactobacilli and lactococci[21]. *Enterococcus* spp., showed dominance in karish cheese samples. This result reflect their ability to withstand adverse environmental conditions during cheese-making, ripening and storage of dairy products[22]. That is a positive point since stability of probiotic culture is crucial to ensure it exerts a beneficial effect on the consumer[23]. The presence of *Enterococcus faecium* was previously reported to be relatively the higher number of enterococci in the Egyptian dairy products[13]. In addition that, the dominance of the two species, *Enterococcus faecalis* and *Enterococcus faecium* was reported earlier by Cebrian *et al*.[24].

The fact that, the isolates may adapt to their environment can be noticed in the results which was suggested before by Barbosa *et al*.[23]. Previous studies reported that, in the cheeses produced with *E. faecium* and *E. faecalis* strains, lipolysis rates were higher and flavor were improved[25] which was clear in flavor formation test. That was the main pillar nominating the 15 selected strains.

Obtained results in Table 1 indicated that acidifying activity of wild LAB enterococci was rather low. In this study, it was targeted the selection of medium acidification rate strain (where Δ pH of 0.4 U was achieved after 3-5 h) to be convenient to wide range of functional food applications. The most important criteria when selecting probiotic cultures being autolysis and proteolysis characteristics of the added strain[26]. Similar observation of poor autolytic activity of some enterococci isolates was reported by Hassaine *et al*.[27]. The data suggest no relationship between the proteolytic and acidifying activities of the isolates, as also suggested by Fortina *et al*.[28]. The application of antagonistic cultures provide *in situ* production of antagonistic compounds during food fermentation which seems promising[29].

Proving its pH and bile salts resistance which is one of the bases for selecting probiotic strain that can be inhibitory in the gut[30], encouraged proceeding to *in vivo* investigations.

These results indicated that the tested strain have no adverse effects on the general health status of the rats when orally administered in milk culture for 5 weeks. Body weight gain as a result of ingested probiotics was recorded earlier by Zommara *et al*.[31] as they increase food digestion availability. Plasma lipid profile indicated a hypolipidemic effect that supported with atherogenic indeces results. Atherogenic index is more sensitive than measurement of total cholesterol as predictor of coronary heart disease and cardiovascular risk[32]. Hypolipidemic effects of lactic acid cultures have been reported by several researchers[33,34].

The safety of each particular *Enterococcus* strain should be thoroughly studied and documented, as the bacteria from this genus in spite of their beneficial activities may present a danger as pathogens associated with infections[35]. The increase in WBCs count is attributed to an immune response when accompanied with increase in lymph, as the immune system responds in a regulated fashion to microbes and eliminate them[36]. On connecting with other observations it can be summaries that, the spleen was in normal size when dissected out after necropsy, no deaths, bleeding or peculiar symptoms was recorded on the 5 rats in this group throughout the experiment or after necropsy and the histological examination of liver cells was similar to control. So, decrease in platelets point to only one explanation attributed to an immune response that caused this effect. However, both WBCs and platelets counts was within the range in male rats reported by Lang[37] where, the highest value reported for WBC was (17.90×10^3 μL^{-1}) and the lowest value for platelets count was (412×10^3 μL^{-1}).

The biochemical results clearly indicated the safety of the tested strain as it didn't cause the production of carcinoembryonic antigen protein (CEA) that widely used for measuring and monitoring the amount of this protein in the blood of healthy adults. Although, *E. faecium* (KT712) strain fed group showed slight decrease in superoxide dismutase (SOD) and increase in thiobarbituric acid reactive substance (TBARS), the histological examination of liver tissue indicated no negative effect on oxidative stress. Similar observation reported by Darwish *et al*.[38] in rats fed fermented milk with *Enterococcus faecalis.*

CONCLUSION

A survey of *Enterococcus* spp. was held for strains isolated from traditional karish cheese to select a potential LAB probiotic strain for animal study based on phenotypic and favorable technological characterization. On confirming

identification using 16S rRNA approach, the strain *E. faecium* (KT712) showed *in vitro* acid and bile salt tolerance encouraged proceeding to its *in vivo* assessment. The *in vivo* evaluation showed that this strain hold a great promise exerting potential health benefits expressed in lowering blood lipids and stimulating growth and immunity. Although the safety evaluation showed good results of hematology, biochemical and histological parameters, further investigations such as; the presence of transferable antibiotic resistance genes which comprises a theoretical risk of transfer to a less innocuous member of the gut microbial community, may be warranted to elucidate its application as promising probiotic strain in the food and feed fermentation innovative products.

ACKNOWLEDGMENT

This study is a part of the "MILAB" project (Molecular Identification of Some Industrial Important Lactic Acid Bacteria for Dairy Industries) which was financially supported by Alexandria Research Enhancement Program (ALEXREP) (2008-2010), Alexandria University, Egypt. This promising strain is registered in Faculty of Agriculture Saba Basha, Alexandria University Culture Collection (FABA).

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