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Study the effect of local wide and mutation probiotic bacteria on decreasing cholesterol in produced fermented yoghurt

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Abstract:

Local Lactic acid bacteria stain which isolated from cow milk was used. The bacteria was identified after a series of screening operation, biochemical tests and carbohydrates fermentation as well as using Api_{50} technique as a confirmation tests for detecting the bacteria under consideration. Bactria were exposed to waves of ultraviolet radiation of frequency 254 nm for 10, 20, 30, 40, 50, 70 Second to make a physical mutation for bacteria. Chemical composition of fresh milk of cow was determined as well as determination of cholesterol which was 9.12 milligrams cholesterol /100 ml, yoghurt was manufacture from wide type bacteria. The cholesterol rate was 3.8 mg/100 ml with reduction rate 58.3% the rate of mutated bacteria which used produce yoghurt and for all mutation periods was 3.1 mg cholesterol /100 ml for 20 sec. with reduction rates 66% followed mutation period 30, 40, 10, 50, 70 second which was 3.2, 3.4, 3.5, 3.6, 3.6 mg cholesterol /100 ml respectively with reduction rate for cholesterol 64.9, 62.7, 61.62, 60.52, 60.52% respectively

Keywords: *lactobacillus casei*, probiotic bacteria, UV light mutation, decreasing Cholesterol, Yoghurt

INTRODUCTION

Lactobacillus has been used for several centuries in various food fermentation processes, with the majority of fermented products containing microorganisms as *Bifidobacterium* bacteria or strains of *Lactobacillus* bacteria and related species, Lactobacillus casei contains a variety of species, such as *Lactobacillus casei* and *Lactobacillus paracasei*. The classification of *Lactobacillus casei* has been subject to significant changes in recent years, causing some confusion (Collins *et al*., 1989; Dicks *et al*., 1996).

The *lactobacillus casei* bacteria is considered one of the most common species in the industrial level especially in dairy industry, since It is involved in the manufacture of cheddar cheese and has a role in the manufacture of pickles such as the fermented green olives produced in Sicily (Banks and Williams, 2004; Randazzo *et al*., 2004) According to the World Health Organization, the use of *lactobacillus casei* in the food industry as a health support has not had any adverse effects on the health of the consumer, these bacteria are considered as an effective strains in the mitigation of bacterial diseases that causing infectious diseases and intestinal (FAO/WHO, 2002).

This is evident through the use of some drinks manufactured from *lactobacillus* casei bacteria, which has been instrumental in inhibiting the growth of Helicobacter pylori bacteria (Cats *et al*., 2003). The study also showed that the treatment of children with acute diarrhea (caused by a viral infection) significantly decreased diarrhea, and many studies indicated that the use of strains of *Lactobacillus* bacteria is an effective and safe treatment of acute and infectious diarrhea (Van Niel *et al*., 2002). The Granito and Álvarez, (2006) noted that the use of *lactobacillus* bacteria in the In the natural fermentation of beans gave positive results in the reduction of a high proportion of compounds that cause flatulence when digestion. While it is confirmed (Ridwan *et al*., 2008) that adding strains of *lactobacillus casei* bacteria will reduce cytokines causing inflammation and increase the inhibition of the growth of bacteria in the small intestine as well as to dislodge from the walls of the intestine. It has the potential to boost the immune system and cholesterol-lowering antioxidants as commonly used in fermented beverages made from milk (Sujono *et al*., 2016), As well as yogurt which can reduce cholesterol by 52.17 mg / 100g (Yuniastuti, 2004).

The World Health Organization (WHO) predicted that by 2030, cardiovascular disease will be a leading cause of death, affecting approximately 23.6 million people worldwide (WHO, 2009). The high cholesterol rate in 45% of heart attacks in Western Europe and 35 % Of seizures in Central and Eastern Europe from 1999-2003 (Yusuf *et al*., 2004). A high-cholesterol diet continuously accumulates harmful cholesterol in the blood vessels and cholesterol oxidation in addition to producing a set of free radicals (Towil and Pramono, 2014). People with hypercholesterolemia may avoid taking medicine and drugs while going to use dietary supplements such as probiotic and prebiotic Which are nutritional supplements that have a beneficial effect on the host by improving intestinal & microbial storage (FAO/WHO, 2001)**,** Several studies have been conducted to evaluate the probiotic and prebiotic effects on cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides**.** No definitive results were obtained and this is evident by showing some strains of probiotic species of susceptibility to lowering cholesterol while other types did not have the same effect (Ooi and Liong, 2010) While studies confirmed that even when 1% of blood cholesterol is reduced, still it can reduce the risk of coronary heart disease by 2-3% (Manson *et al*., 1992).

The aim of this research is to study the effect of *lactobacillus casei* bacteria on lowering the cholesterol levels in a fermented milk product and comparing them with the same mutated strain in the physical method at different levels.

MATERIALS AND METHODS

Isolation Source

Cow raw milk was obtained from a local farm in Basrah Governorate in southern Iraq. Milk was stored directly at 5 ° C. In preparation for the isolation of lactic acid bacteria, samples of raw milk were grown in the center of *Lactobacillus* MRS (Oxoid agar), and the dishes were incubated in anaerobic conditions with Co2 and at 37 ° C for 48 hours.

Isolation Method

The colonies were selected based on Cell morphology, Gram stain was tested, temperature was maintained at 15 °C and 45 °C, Catalase test, ammonia production of arginine, nitrate reduction, gelatin ester, sugar fermentation test (20) included (Melibiose, Melezetose, Gluconate, Escullin, Arabinose, Trehalose, Sucrose, Salicine, Raffinose, Manitol, Manose, Maltose, Lactose, Galactose, Cellibiose, Amygdaline, Fructose, Ribose, Sorbitol, Xylose).

Yogurt Preparation

Yogurt prepared by using the method mentioned by Al-Rawi (2005).

Cholesterol Determination

Prepared according to Al-Sheikh Dhahir (1999).

Determination of cholesterol rate

Determined according to Francy and Elias (1968).

Mutation of *Lactobacillus casei* bacteria by ultraviolet radiation

Lactobacillus casei bacteria been developed in 20m of LAPTg environment at 37 ° C. The cell output was subjected to 5000g centrifuge for 15 minutes and then washed twice in 20 mL of Phosphate buffer solution (BPS), 2ml of cells were transferred to a sterile Petri dish and exposed to ultraviolet (254nm) at a distance of 30 cm for 10, 20, 30, 40, 50, 70 sec. After a series of dilution, 0.1ml of stranded cells were deployed in the hard center LAPTg (Yeast extract / peptone / tryptone / tween80 / glucose) After incubating at a temperature of 37 ° C for 48 hours and observing its growth, they were stored in aluminum coated containers in total darkness (Goodarzi, 2016).

RESULTS & DISCUSSION

12 isolates of lactic acid bacteria were isolated using selective media MRS agar and MRS broth, which were subjected to screening and a series of biochemical tests. *Lactobacillus casei* bacterial was isolated by morphological grown colonies and microscopic examination, which showed that the bacteria were rod shape, with separated short chains, non spore, Gram positive , immobile, grow at 15 °C and do not grow at 45 °C, table (1) negative in the production of ammonia from arginine ,negative in catalase, negative in nitrate reduction, and unable to Liquefaction of gelatin and the results are consistent with what is mentioned by (ERDOÚRUL and ERBÜLÜR, 2006) and it has the ability to digest a wide range of sugars (20) type sugar table (2) which is due to its ability to produce the amylase enzyme.

The API_{50} test was used to confirm the reliability of the sugary fermentation agent by the bacterial isolates, and the positive effect of the test was determined by changing the color of the diabetics. It is effective in diagnosing Lactobacilli bacteria between (95.6 - 98.6) % (Liu *et al*., 2011).

Table (1) Biochemical test for *Lactobacillus casei* isolate

Strain	Gram	15 °C	45 °C	Ammonia from arginine	Nitrate reduction	Liquefaction of gelatin	Catalase
Lactobacillus casei	+	+	-	-	-	-	-

Table (2) Results of carbohydrate fermentation tests for *Lactobacillus casei* isolate

Strain	Escullin	Melibiose	Trehalose	Ribose	Arabinose	Gluconate	Sucrose	Manitol	Raffinose	Galactose	Manose	Sorbitol	Amygdaline	Maltose	Lactose	Salicine	Fructose	Melezetose	Cellibiose	Xylose
Lactobacillus casei	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-

Table (3) Chemical composition of milk used in the manufacture of fermented milk product

Fat	Protein	Total Solid	Cholesterol mg/100 ml
3.6	3.2	12	9.12

The ability of the bacteria *Lactobacillus casei* wide type and mutation on reducing cholesterol

Noting from table (3) that The chemical composition of raw bovine milk used in the Yogurt industry contains 3.6% fat , 3.2% protein, 12% total solids, Cholesterol 9.12 mg 9.12 mg Cholesterol/100m, noting that the value of the cholesterol was less than the value mentioned by (Piironen *et al*.2002) Comparative Milk research since the cholesterol level is connected with the amount of fat (Faye *et al*., 2015).

From figure (1) observing a comparison between the ratios of cholesterol in the current study samples which reached 9.12 mg cholesterol/100m in the curd milk. The cholesterol level in the fermented milk product decreased when the *Lactobacillus casei* bacteria wide type was used to 3.8 mg cholesterol / 100 ml and a reduction in cholesterol reached 58.3%, which is higher than the percentage mentioned by (Utami *et al*., 2017) That the use of bacteria *Lactobacillus casei* led to a reduction of cholesterol by 32%, which showed (Yuniastuti, 2004) that the bacteria *Lactobacillus casei* able to reduce the total cholesterol and levels of triglycerides and LDL cholesterol levels significantly (Utami *et al*., 2017). As cleared by Kumar *et al*., (2010) that the Dietary supplementation with Lactic acid bacterium can significantly reduce total cholesterol by 23.6%. As for the mutant bacteria used in the yogurt industry, it is noted that the value of cholesterol in the fermented milk product, which was manufactured from the mutant strain at the level of 20 seconds, was reduced to 3.1 mg Cholesterol/100 ml and with cholesterol reduction of 66% followed by mutation levels 30, 40, 10, 50 and 70 sec. Levels of cholesterol were 3.2, 3.4, 3.5, 3.6, 3.6 mg cholesterol / 100 ml and cholesterol reduction of 64.9, 62.7, 61.62 and 60.52 , 60.52%, respectively figure (2). The Mutagenesis is widely used to increase the properties of microorganisms and improve their activity. However, they are rarely used against Lactic acid bacteria because of their genetic properties (Goodarzi, 2016).

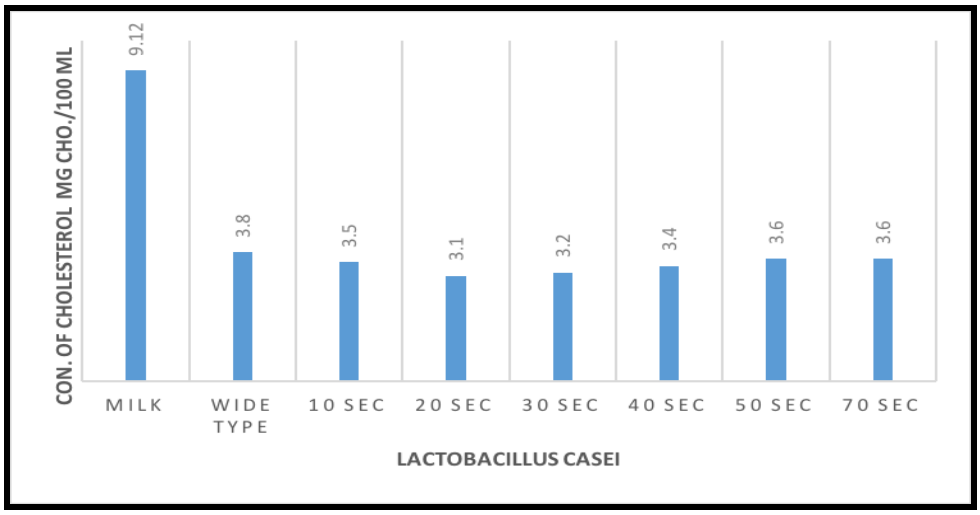

Figure (1) Total amount of mg cholesterol / 100 ml in milk and fermented milk product by *Lactobacillus casei* wide type and mutation

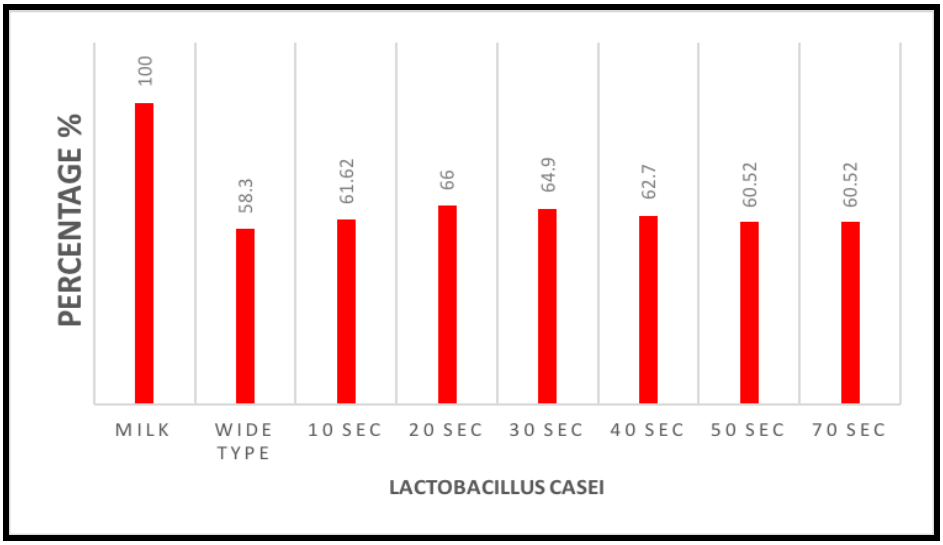

Figure (2) Percentage of cholesterol reduction in milk and fermented milk products by *Lactobacillus casei* wide type and mutation

Research is under way to improve the technological properties of starters and dairies to improve the texture, flavor and fermented milk products and to enhance the properties of micro-organisms and adaptive to lactic acid bacteria and in the pharmaceutical fields (Derkx *et al*., 2014; Klaenhammer, 1995).

Several studies have examined the effect of Probiotic in reducing the amount of cholesterol as several mechanisms have been assumed, including the enzymatic production of Bile salt hydrolase (BSH) of probiotic, which analyzes cholesterol (Lambert, 2008) and digesting the cholesterol by Probiotic (Pereira and Gibson, 2002) and deposition of cholesterol with non-dehydrated bile salts (Liong and Shah, 2006) in addition to the association of cholesterol with walls of probiotic cells (Lye *et al*., 2010) as well as the integration of cholesterol in the cellular membranes of probiotic and conversion of cholesterol into coprostanol and production of short-chain fatty acids from probiotic in the presence of prebiotics (De Preter *et al*., 2007). Another important mechanism is that cholesterol is used to create a portion of the new bile acids in a balanced manner, while the rest of the water-soluble bile acids are thrown out of the body (Ooi and Liong, 2010).

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