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Review Article ***Open Access***

KEFIRAN - A THERAPEUTIC BIOFILM: A REVIEW

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Kefir, a unique alcoholic fermented milk beverage made using kefir grains is originated in Caucasus Mountains. Kefir grains consist of heterogeneous microflora embedded in a polysaccharide matrix called kefiran which is considered as Biofilm which has many food applications due to its therapeutic value. Kefiran exhibits significant properties that have inspired the scientists to unravel the secrets of it. Factors like medium, incubation condition, nitrogen source and so on will affect the production of kefiran. Kefiran has prebiotic nature stimulating the growth of probiotics in the gastro-intestinal tract of human beings. It extends certain therapeutic benefits major being, balancing of the microbiota in intestine, Treating Lactose Intolerance, Anti-cholesterolemic property, Anti-carcinogenic property.

Keywords: Kefiran, Biofilm, Exopolysaccharide, Prebiotic

INTRODUCTION

India ranks first in milk production in the world accounting 155.5 million tonnes 2015-16 (http://www.nddb.org/information/stats/milkprodindia). Milk turns sour and will be exploited due to increase in microbial load when kept at room temperature. To minimize milk spoilage, preservation methods such as Heat treatments and Fermentation are adopted. Milk is also utilized to manufacture variety of milk products to increase its shelf life and to preserve the nutrients (Fox *et al.,* 2000). Fermentation is a chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts produced by microorganisms such as yeasts, bacteria (Kosikowski and Mistry, 1999).

However, over time, it has soon become apparent that many fermented foods had longer storage lives and improved nutritional values, making this form of food processing a popular technique (Farnworth, 2004). Fermented milk products are classified based on the starter culture used by Tamime and Robinson (1988). Kefir is an alcoholic fermented milk beverage produced by the action of lactic acid bacteria and yeasts which exist in symbiotic association in kefir grains. The name kefir has been derived from a Turkish word "keif" which means good feeling. It is originated in the Caucasus Mountains which is located between Russia and Georgia (Hertzler and Clancy, 2003). Research shows that kefir contains a special lipid Sphingomyelin, which enhances body cells to secrete 14 times more interferon-beta (Osada *et al.,* 1994) a glycoprotein produced by body cells to help the body fight against viral infection.

Kefiran an exopolysaccharide is produced by *Lactobacillus kefiranofaciens* (Wang *et al*., 2010) found in "kefir grains" and is composed of protein, polysaccharide, and complex symbiotic microbial mixture (Jianzhong *et al*., 2009). Kefiran consist similar ratios of glucose and galactose. Exo Polysaccharides produced by lactic acid bacteria are considered as GRAS, i.e., generally recognized as safe.

Origin of Kefir

"The grains were a gift from Allah (God) provided over 1,000 years ago"

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Table 1: History

Scientist	Year	Contribution
Mohammed	570 BC	Original grains gifted by 'Angel Gabriel'
Genghis Khan	1200 AD	Mare milk was preserved by transferring kefir grains in goat skin bag
Irina Sakharova and Blandov	1908	Commercial production of Kefir in Moscow Dairy started
La Rivire *et al.*	1967	Discovered exopolysaccharide of kefir grains by naming it as kefiran
Toba *et al.*	1983	Discovered the polysaccharide producing organism and named as *Lb. kefiranofaciens*
Gibson and Roberfroid	1995	Exopolysaccharides produced by lactic acid bacteria possess prebiotic nature

The origin of Kefir is considered to be in the northern slopes of Caucasian mountains. A well-known legend states that the Prophet Mohammed gave Kefir grains to the Orthodox people living in the Caucasian Mountains in Eastern Europe. He also taught them how to use the grains and made them promise that they would keep the existence of the Kefir grains secret; otherwise the grains would lose their strength and healing power (Koroleva, 1988). Their existence was kept as secret for a long period and it was only in 1908 that "Dairy Moscow" owned by a man called Blandov started to produce Kefir. Immigration of the Eastern people also contributed to popularizing Kefir across the world (Garrote *et al.*, 2001).

KEFIR GRAINS

Kefir grains are a mass of complex symbiotic microbial mixture which includes species of yeasts, Lactic Acid Bacteria (LAB), imbedded together by matrix of protein and polysaccharide. The microorganisms present in the kefir grains ferment the milk, and the grains can be recovered at the end of the fermentation process. Kefir grains when observed using Scanning Electron Microscopy (SEM) methods indicated the yeast colonization on the surface and middle part of the kefir grain and a higher concentration of bacteria found inside the grains (Guzel-Seydim *et al.*, 2005). The matrix of kefir grains consist a specific water soluble polysaccharide, which hasn't isolated from any other substrates, and hence called original kefiran.

Figure 1

Characteristics of Kefir Grains

Kefir grains are small, hard, irregularly shaped, yellowish granules varying in diameter from 3 to 35 mm, with appearance of miniature cauliflowers. Kefir grains can ferment any fresh mammalian milk, and they can also be used to ferment soy, rice, or almond milks. These grains resemble a living ecosystem and as long they are "fed" with milk regularly, the grains can be preserved indefinitely. One of the advantages of Kefir grains is that as they grow, the grains can be subdivided and thereby used to ferment other batches of milk (Otles and Caginidi, 2003).

Table 2: Composition of Wet Kefir Grains

Contents	%
Moisture	86.3
Polysaccharide	8
Protein	4.5
Ash	1.2
Fat	0.03

Source: Garrote et al. (2001)

BIOFILM

A biofilm is produced by any group of microorganisms to which cells stick to each other. These adherent cells are frequently embedded within a self-produced polysaccharide matrix of Extracellular Polysaccharide Substance (EPS). Biofilm is an EPS, which is referred as slime but not all slimes are biofilms. Biofilms may form on living or non-living surfaces, and can tolerate high acidic, alkaline, hot, and

cool conditions. Biofilm formation involves the transport of organic and inorganic molecules and microbial cells to the surface by a subsequent adsorption to it and finally an irreversible attachment aided by the production of EPS. However, to date there has been little information available on its film characteristics (Ghasemlou *et al.*, 2010)

Biofilms are produced by some species of LAB, and a number of genes due to auto-aggregation properties of the cells, clumping of cells of the same strain due to hydrophobicity and adhesion ability has been observed in some lactobacilli assist cells which tolerate higher levels of acetic acid and alcohol (Kubota *et al.*, 2008). Cell aggregation observed to involve in the interaction of cell surface components, i.e., lipoteichoic acid, proteins, and carbohydrates, as well as soluble protein. Biofilms may be both helpful and harmful, whereas helpful biofilm such as kefiran produced by beneficial microorganisms has therapeutic benefits. Investigations of biofilms are generally focused on structural, functional as well as ecological aspects (Denkhaus *et al*., 2007).

Characteristics of Kefiran

Kefiran is a yellow coloured gel; Lactose present in the milk is used as the substrate by the microorganisms for its production it is produced in the center of the kefir grain. Kefiran is synthesized by homo-fermentative lactobacilli *species Lb. kefiranofaciens*. Encapsulated LAB, *Lb. kefiranofaciens, Lb. kefiri* and other strains can synthesize kefiran from lactose under anaerobic conditions and in the presence of alcohol (Arihara *et al.*, 1990).

Major producer of kefiran is *Lb. kefiranofaciens* followed by *Lb. casei*, *Lb. brevis* with symbiotic association of *Sacharomyces cerevisiae*. Kefiran is a water soluble branched exopolysaccharide. It consist similar ratios of D-glucose and D-galactose, and is classified as a water-soluble glucogalactan, which makes it suitable to be used as additive (Abraham *et al.*, 2010). Bacteria that produce exopolysaccharides are often found in milk or milk products but maximum production of exopolysaccharide may occur in chemical media (containing carbohydrate source, mineral salts, amino acids, vitamins and nucleic acids) at a constant pH.

The molecular structure of kefiran is a branched hepta or hexa saccharide repeating unit, which in itself is composed of a regular penta saccharide unit to which one or two sugar residues are randomly linked (Kooiman, 1968). The variety of linkage types of the molecule accounts for the poor accessibility of kefiran to enzymatic attack. This property may be important in the stability of the kefir grain, including the therapeutic activity of kefiran, due to the polysaccharide remaining chemically stable to the process of fermentation including gastric digestion.

Table 3: Composition of Kefiran (in Dry Kefir Grain)

Contents	%
Polysaccharide	65.7
Total protein	24.3
Ash	7
Fat	3

These cell-surface carbohydrates confer protective properties on their bacterial producers; hence they are normally loosely bound to cell membrane; therefore they are easily lost to their environment (Jolly *et al.*, 2002). In food products, exopolysaccharides contribute to organoleptic and stability characteristics. Kefiran dissolves slowly in cold water and fastly in hot water, and forms a viscous solution at 2% concentration.

THERAPEUTIC PROPERTIES OF KEFIRAN

- Impact on GIT (Gastrointestinal Tract)
- Reduction of lactose intolerance
- Anti-carcinogenic
- Anti-cholesterolemic

Impact on Gastrointestinal Tract

Stimulation of the immune system is one of the mechanism by which probiotic bacteria exert many of their beneficial effects. Thoreux and Schmucker (2001) fed traditional kefir to young (6 months) and old (26 months) rats and found an improved mucosal immune response in the young animals, as shown by a higher anti-Cholera Toxin (CT) IgA response compared to controls.

Probiotic organisms inhibit pathogenic bacteria by adhering to the intestinal epithelial cells as they are capable to produce acids and bacteriocin. This aids in promoting bowel movement evacuating putrefactive bacteria, leads to a balanced intestinal microflora there by establishing a healthier digestive system. Increase in IgA levels due to more probiotic population protects intestinal epithelial cells by inhibiting colonization of enteric pathogens.

Reduction in Lactose Intolerance

Lactose intolerance is the most common food intolerances, and is estimated that it affects three quarters of the world's population as a result of decrease in intestinal lactose activity, and almost 60 to 70% of Indian population is lactose intolerant (Swaggerty *et al.*, 2002). The absorption capacity of lactose goes on decreasing as we grow up, it is least in old age. People with lactose intolerance have difficulty in digesting milk sugar present in dairy products due to insufficient amounts of gut enzyme lactase. Due to lack of this enzyme, lactose will be used by coliforms where it produces carbon dioxide which causes flatulence.

It has also been shown that fermented milk products have a slower transit time than milk, which may improve lactose digestion (Labayen *et al.*, 2001). The ability to reduce lactose concentrations and the β-galactosidase activity in kefir make it suitable for consumption by people classified as lactose intolerant (Farnworth and Mainville, 2008). It has been noticed that some kefir grains show β-galactosidase enzyme activity, which remains active when consumed, and kefir contains less lactose than milk (Farnworth, 2005).

Zheng *et al.* (2013) studied and suggest that probiotic bacteria stimulated by kefiran in kefir consumer's gut are abundant and are correlated with health improvement, so in that way it had been demonstrated that the cell-free fraction of kefir enhances the ability to digest lactose relieving symptoms (Rizk *et al.*, 2009).

Commercial kefir which consist kefiran has proved to be as effective as yoghurt in reducing expired hydrogen and flatulence in adults suffering from lactose intolerant when compared to the ingestion of milk. De Vrese *et al.* (1992) demonstrated that when pigs fed with kefir showed increase in galactose plasma concentrations, resembling improvement of intestinal lactose hydrolysis by the microbial enzyme β-galactosidase.

Anti-Tumour Property

Living organisms need Cell proliferation for growth and body development, when cell grows fast, body has the capability to manage this condition by replacing the old cells with new ones. Tumour is the condition which occurs when the dead cells accumulate and if attacked by virus causes cancer. Tumours are classified as benign-which are ovoid in shape, non-cancerous and treatable; malignant – irregular in shape, grow fast and causes cancer (Kuby, 1994).

The process of proliferation is controlled by genes of DNA of cell nucleus which is considered as procarcinogen. In case of malignancy procarcinogens become carcinogens and other procarcinogens are suppressed. So that abnormal and unwanted cells invade surrounding tissues and spread to other body parts.

Shiomi *et al.* (1982) were the first to report the antitumour effects of a water-soluble polysaccharide isolated from kefir grains. The polysaccharide kefiran has multitherapeutic activity; it reduces the size of tumours in tumour bearing mice fed on kefiran (Kubo *et al.*, 1992).

The anticarcinogenic role of kefiran can be attributed to cancer prevention and the suppression of early-stage tumors, by delaying the enzyme activities which convert pro-carcinogenic compounds to carcinogens, e.g., nitroreductase is the enzyme which converts nitrate to nitrite or by the activation of the immune system (Sarkar, 2007). Kefiran a prebiotic stimulates the growth of probiotics which digest kefiran and releases short chain fatty acids especially butyrate. This butyrate promotes the growth of normal cells and suppresses the tumour cells by promoting apotosis.

Apotosis is the condition where cell which has DNA damage or infected with virus commits suicide so as to protect the other normal cells. Kefiran promotes the growth of probiotic organisms because of which the Carcinogens will be bound to probiotic organisms like *Lactobacillus delbrueiki* ssp *bulgaricus*, *Bifidobacterium bifidum* to

Table 4: Case Studies

Scientist	Year	Research
Furukawa *et al.*	1990	Fed kefir to mice, i.e., 2 g of product/kg body weight of mice for a period of 9 days inhibited tumour.
Kubo *et al.*	1992	Fed kefir 0.1-0.5 g/kg of body weight of mice for 15 days inhibited tumour growth.
Liu *et al.*	2002	Inhibition of tumour growth in mice by apoptic cell lysis accounted by kefir consumption.
Thoreux and Schmucker	2001	Fed mouse with 1 g of kefir for 27 days which inhibited tumour.

their binding sites (phosphate, carboxyl groups) present in the cell wall and are inactivated. As cancer causing carcinogens are inactivated the chance of causing cancer will be reduced.

Anticholestrolemic Property

Several hypothesis have been proposed regarding the possible mechanism of action employed by probiotic bacteria to reduce cholesterol levels (St. Onge *et al.* 2002). Kefiran a prebiotic stimulates the growth of probiotic strains, which has the capacity to lower cholesterol levels. According to Yanping *et al.* (2009), there are 3 different ways by which bacteria can alter serum cholesterol:

- Before cholesterol can be absorbed into the body, binding of cholesterol by probiotics settled in gut with the help of kefiran takes place in the presence of bile acids and anaerobic conditions, and is absorbed into the organism's cell. Tahri *et al.* (1995) studied the hypothesis of the proposed assimilation of cholesterol by *Bifidobacterium* species. They observed the existence of an intense binding between cell surface and cholesterol, which was considered as uptake of cholesterol into the cells. This assimilation was dependent on cell growth and the presence of bile salts was shown to be a prerequisite for significant cholesterol removal.
- By the Production of free and Deconjugated bile acids - Bile is yellow green aqueous solution and is the mixture of bile acids, cholesterol, and phospholipids. It is secreted via bile duct to gall bladder and concentrated and is released into the duodenum, when food is taken for the digestion process. Bile acids (cholic acid, chenodeoxycholic acid) are synthesized through cholesterol and are conjugated with glycine and taurine in the liver.

  Conjugated bile acids act as surfactants and help in emulsification of lipids but won't be absorbed with the digested product. Bile acids in intestine are not sufficient for the digestion process so often they have to be circulated after they are used for digestion, they will be returned from small intestine to liver through portal vein this process is called enterohepatic circulation.

  In Begley *et al.* (2006) studied the mechanisms behind the deconjugation of bile acids by probiotic organisms which have active bile salt hydrolase activity. Deconjugated bile acids are less soluble and less likely to be absorbed from the intestinal lumen than conjugated bile salts, and they are precipitated at low pH, and do not participate in circulation and are excreted through feces (De Rodas *et al.*, 1996). Thus, deconjugation of bile acids in the small intestine could result in a greater excretion of bile acids from the intestinal tract, especially as free bile acids are excreted more rapidly than their conjugated forms. Increased excretion of bile acids should result in lowered serum concentrations, which in turn would decrease the amount of bile acids reaching the liver for secretion back into the intestine through enterohepatic circulation. To replace the excreted bile acids, more would have to be synthesized from cholesterol in the liver for digestion process. Thus, deconjugation of bile acids could lead to the reduction of serum cholesterol by increasing the formation of new bile acids or by reducing the absorption of cholesterol throughout the intestinal lumen. Hence, there will be reduced serum cholesterol by the intake of kefiran.
- Inhibiting the enzyme HMG-CoA reductase: The inhibition of 3HMG-Co A, which is an intermediate of mevalonate, in the synthesis of cholesterol from acetyl-Co A by fermented milk products, was the reason for the reduced level of cholesterol in the serum. Regulation of cholesterol synthesis from acetyl Co-A is done by β-hydroxy-β-methyl glutaryl Co-A reductase enzyme. Hence it is considered as the key enzyme of cholesterol biosynthesis. Inhibition of this enzyme will reduce serum cholesterol which can be achieved by Colestipol which is produced by probiotic strains stimulated by kefiran is a bile acid sequestrant used to lower blood cholesterol, specifically Low-Density Lipoprotein (LDL) (Wang *et al.*, 2009).

Table 5: Case Studies

Scientist	Year	Findings
Mann *et al.*	1974	Warriors in Africa were fed with 3L of kefir/Day for a period of 2 months showed reduction in cholesterol (LDL-upto 50%).
St. Onge *et al* .	2002	Human volunteers were fed with 500 ml of kefir/day for 4 weeks, reduced 27% serum cholesterol
Maeda *et al.*	2005	Fed rats with kefir of 50 ml/21 days and observed reduction in serum cholesterol

CONCLUSION

The microbiological and chemical composition of kefir resembles that it is a much more complex probiotic, as large number of different bacteria and yeast found in it distinguishes it from other probiotic products. During the fermentation process, the yeasts and bacteria present in kefir grains produce a variety of ingredients which give kefir its unique taste and texture. After fermentation, the finished kefir contains many ingredients that are proving to be bioactive. Many bacteria found in kefir shows proteinase activity and a large number of bioactive peptides are found in kefir. Furthermore, there is evidence that kefir consumption not only affects digestion process but also influences metabolism and immune function in humans. Kefiran, and in fact kefir grains and kefir are useful as functional food to prevent common occurring diseases.

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