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Biosynthesis, Production and Application Of Kefiran In Food Industry: A Review

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Kefiran is a water soluble exopolysaccharides produced by lactic acid bacteria and mostly synthesized during bacterial growth. Although the information regarding the biosynthesis of exopolysaccharides produced by lactic acid bacteria is still insufficient, nonetheless, the mechanism suggested for capsular polysaccharides and exopolysaccharides from gram-negative bacteria probable can also be accepted for gram-positive kefiran producer as they come from the same *Lactobacilli* sp. The production of kefiran by *Lactobacilli* sp. is significantly affected by the medium formulation where different types of nutrient (carbon, nitrogen and phosphorous) gave different results towards the ability of the cells to produced kefiran. Moreover, the pH of medium and incubation temperature also give great impact on kerafin production. The used of kefiran has received intense attention recently in food industries besides pharmaceutical industries due to its ability to provide the desired rheological properties for the dairy products. It can be used as gelling agent, water binding agent, food packaging, thickener as well as improve self-supporting gels in food industries. The present review discusses the literature on biosynthesis, production and applications of kefiran in food industry.

Keywords: Kefiran, Biosynthesis, Production, Application, Food Industry

INTRODUCTION

Microbial cells have been widely used as sustainable biofactory for the production of polysaccharides. Nowadays, different polysaccharides such as xanthan (El Enshasy et al. 2011; Elsayed et al. 2016) pullulan (Low et al. 2019; Dailin et al. 2019), alginate (Then et al. 2012), gellan (Banik et al. 2007) and different types of mushroom biopolymers (El Enshasy et al. 2010; Maftoun et al. 2013; Masri et al. 2017; Selvamani et al. 2018; Abd Alsaheb et al. 2020) are produced industrially and used extensively in food industries. Among different attractive multifunctional biopolymers, kefiran gain many attentions during the recent years because of its many health benefits (Dailin et al. 2020). Kefir is a sour, refreshing, mildly alcoholic, acidic and self-carbonated traditional fermented milk beverage which is believed to be found initially from the northern Caucasian mountains of Russia (Tratnik et al. 2006; Angelidis et al. 2020). It is the product of a mixed alcoholic and lactic fermentation and identified by a creamy texture, nutritional composition and distinctive volatile profile (Prado et al. 2015, Gao & Li, 2016; Angelidis et al. 2020). All the features (flavor and taste) are influenced by the symbiotic interactions between acetic acid bacteria, lactic acid bacteria and yeast as well as the metabolic products for instance bioactive peptides, carbon dioxide, vitamins (K, C, B1, B2, B5 and B12), acetic acids, lactic acids, some nutraceutical compounds, essential amino acids, exopolysaccharides (kefiran), other volatile components, minerals, bacteriocins, acroin, ethanol, folic acid and acetaldehyde (Farnworth, 2005; Arslan, 2015; Prado et al. 2015; Bourrie et al. 2016; Atalar, 2019). Commercially manufactured kefir and other fermented milk products have become popular around the world since it confers beneficial health effects related to its both prebiotic and probiotic content (Farnworth, 2005; Schneedorf, 2012). Kefir can be produced by using kefir grains (traditional method) or by using natural starter cultures from kefir (backslopping method) (Garofalo et al. 2020). Kefir grain, a consortium of exopolysaccharides and many microorganisms (Prado et al. 2015; Plessas et al. 2016) which is used as main component as healthy fermented milk drink with potential nutraceutical activities based on its high content of different types of probiotic bacteria and unique type of biopolymer. Different studies showed that kefir grains bioflora composed of wide range of microorganisms mainly bacteria and yeast in symbiotic relationship. However, about 90% of microbiota of grain composed of bacteria belongs to genus *Acetobacter* and lactic acid bacteria (LAB) such as *Lactobacillus, Lactococcus, Streptococcus*, and *Leuconostoc* (Chen et al. 2008; Kesmen and Kacmaz, 2011). These bacteria co-exist in symbiotic relation with different types of yeasts from species of *Yarrowia, Pichia, Zygosaccharomyces*, *Candida, Kluyveromyces*, *Torulaspora* and *Saccharomyces* species (Latorre-Garcia et al. 2007; Marsh et al. 2013). However, *Lactobacillus kefiranofaciens* and *Lactobacillus kefiri* were found to be the major bacterial populations in all kefir grains (Leite et al. 2012). Some of these microorganisms act as probiotics and give health benefits after consumption (Kechagia et al. 2013). The biosynthesis of bacterial exopolysaccharides is complex especially heteropolysaccharides compared to homopolysaccharides because of the combination of intra and extracellular process. This biosynthesis has several intracellular steps and only polymerization of repeating units is extracellular. Many factors including chemical and environmental factors contributed towards the production process of kefiran. Due to kefiran unique characteristics, it has many applications in food industries. The present review aims to summarize studies on kefiran with an emphasis on biosynthesis, production and applications in food industry.

KEFIR MILK

Currently, commercially produced kefir and other fermented milk products have become popular around the world due to its health benefits effect, sensory properties and status of natural probiotic (Nielsen et al., 2014; Garofalo, 2020). Kefir is a fermented milk beverage which is found initially from the northern Caucasian Mountains of Russia (Lopitz-Otsoa et al., 2006; Altay et al., 2013) and is extensively consumed in North and South America, Europe, Caucasus Mountains of Russia as well as Asia (Plessas et al. 2016). It is acidic, viscous, slightly carbonated and containing small amount of alcohol (Farnworth, 2005; Lopitz-Otsoa et al. 2006; Altay et al. 2013). The word kefir is come from the word “keif” of Turkish which mean pleasure or good feeling. Kefir is also known as *kefīrs*, *keefir*, *kephir*, *kewra*, *talai*, *mudu kekiya*, *milkkefir* and *búlgaros* (Gaware et al. 2011; Arslan, 2015). According to Farnworth (2005), it is unclear for the origins of kefirs which come from a single original starter culture. Kefir is different from the fermented milk yogurt where it is the product of fermentation of milk carried using only kefir grains or with mother cultures prepared from the grains.

Kefir has been a home-made product for centuries and it is also manufactured industrially in various countries across the globe (Angelidis et al. 2020). Generally, kefir is made from cow’s milk, nonetheless, there have been some studies on different milk types from plant based milk and other animal based milks. Plant based milks for example peanut milk (Bensmira & Jiang, 2015) and soy milk (Botelho et al., 2014) whilst animal based milks include ewe milk (Yilmaz-Ersan et al. 2018) and buffalo milk (Gul et al. 2018) were also evaluated in the production of kefir like beverages.

Kefir contains minerals, amino acids, enzymes, tryptophan, calcium, phosphorus, magnesium, and many vitamins such as B2, B12, K, A and D (Gaware et al. 2011). Due to its high nutritional value and content of natural probiotics, kefir has conferred many health benefits. Since the early eighteen centuries, it is believed that kefir have healing ability (Lopitz-Otsoa et al. 2006; Erdogan et al. 2019). Besides, kefir also possesses beneficial effects such as fat deposition reduction (Gao et al. 2019), antimutagenic (Guzel-Seydim et al. 2011), antibacterial, hypocholesterolemic, antioxidant (Slattery et al. 2019), immunoregulatory (Hong et al. 2009), antidiabetic, antiallergic (Hong et al. 2010), anti-inflammatory (Diniz et al. 2003) and antitumoral (Gao et al. 2013). Several compounds produced during microbial fermentation for example peptides may have bioactive properties (Savastano et al. 2020). The bioactive peptides deriving from kefir milk protein have revealed health promoting characteristics recently by several studies (Ebner et al. 2015; Dallas et al. 2016; Amorim et al., 2019; Izquierdo-González et al. 2019). During the fermentation process, lactose is transformed into lactic acid mainly by lactic acid bacteria resulting in a pH drop and thus helps in preservation of the milk (Rattray and O'Connell., 2011). People who are sensitive to lactose can safely consume the kefir drink where lactose in milk is decreased by 75% after the fermentation (Yilmaz et al. 2006).

Traditionally, kefir is made by inoculation of pasteurized, cooled milk with natural starter cultures prepared from kefir grains or with kefir grains, yeasts (*Kluyveromyces*, *Saccharomyces* and *Candida*) (around 83-90%), acetic and lactic acid bacteria (*Acetobacter*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Lactococcus*) (approximate 10-17%) embedded with complex sugars and casein in a polysaccharides matrix (Prado et al. 2015; Baschali et al. 2017; Elsayed et al. 2017; Savastano et al. 2020). The final characteristics of kefir for example functional properties, sensorial, microbiological, physicochemical and structural are influenced by the production conditions, types of milk and starter culture (Atalar, 2019). A milder and sweeter kefir taste can be obtained by having a shorter fermentation time. Sour taste kefir obtained with longer fermentation process.

KEFIR GRAINS

Kefir grains is a mix of microorganisms coexist in symbiotic association which held together by a polysaccharide called kefiran, a type of water soluble polysaccharides which contain equal amount of galactose and glucose and is predominantly produced by yeasts and lactic acid bacteria present in the kefir grains. (Lopitz-Otsoa et al. 2006; Garofalo et al. 2015; Prado et al. 2015; Dailin et al. 2015; Dailin et al. 2016; Elsayed et al. 2017). Kefir grains are small, hard, gelatinous, irregular, lobed-shape, yellowish-white granules varying in diameter between 3 and 35 mm, with the appearance of miniature cauliflowers (Arslan, 2015). The structure of the grains suggests that grains arise from curling of flat sheet-like structures with subsequent folding and refolding, the grain size growing with the increase of carbohydrate/microflora increase (Nielsen et al. 2014). Kefir grains have the biochemical compositions include magnesium, vitamins K and B, fat, phosphorous, tryptophan, proteins (5.6% free amino acids, 6% soluble and 27% insoluble), calcium and mucopolysaccharides (Rosa et al. 2017). Historically, kefir grains were considered as a gift from Allah to the Muslim people of the Northern Caucasian Mountains (Lopitz-Otsoa et al. 2006).

Traditionally, kefir grains are used as starter culture to manufacture fermented milk beverages (Lopitz-Otsoa et al. 2006). At artisanal level, kefir grains are added to milk at different ratios (typically from 1% to 20% w/v) and are left to ferment at 20 – 25 ℃ for 18 – 24 h (Leite et al. 2013). During fermentation, the grains' biomass increase and eventually break down into new, smaller grains and release viable cells into the milk (Prado et al. 2015). Aromatic compound, ethyl alcohol, lactic acid and CO_2 released during the fermentation process create distinctive sensorial properties of kefir. At the end of the process, kefir grains are recovered by separating the grains from kefir using sieving and used in another fermentation process (Otles & Cagindi, 2003, Leite et al. 2013).

Kefir grains contain wide range of bacteria and yeast from different species and live in symbiotically relationship. However, the interactions between both of the bacteria and yeast are still unclear and further study is needed. The microbiota in kefir grains may differ relying on the milk types used in fermentation (coconut milk, buffalo milk, soy milk, sheep milk, bovine milk, camel milk, rice milk and goat milk), time and temperature in fermentation process as well as the ratio of kefir grains to milk (Altay et al. 2013; Bourrie et al. 2016). Due to the different microbial consortium exist in the kefir grains, thus, varying kefir milk products with different sensorial, physico-chemical, microbiological and nutritional properties may be attained (Bengoa et al. 2018). Isolation of microorganisms from kefir microflora has been carried out and the isolated microbes included lactic acid bacteria, yeasts, streptococci and acetic acid bacteria (Simova et al. 2002; Garofalo et al. 2015). The microorganism population in kefir and kefir grains is showed in Table 1 as reported by many authors.

Lactobacillus kefiranofaciens

L. kefiranofaciens was isolated from kefir grains (Kandler and Kunath, 1983; Fujisawa et al. 1988; Santos et al. 2003; Chen and Chen, 2013). It was found to be gram-positive, non-motile, non-spore-forming rods that are generally having size in the range of 0.6-0.8 × 3.0-15.0 μm with tendency to form chains of shorts rods or long filaments where it often containing polyphosphate granules that is usually terminal (Logan and De vos, 2009). *L. kefiranofaciens* belongs to the *Therrnobacterium* group and facultative anaerobic producing lactic acid homofermentatively (Fujisawa et al. 1988) and produce almost the same amount of lactic acid from lactose (Cheirsilp et al.2003).

BIOSYNTHESIS OF KEFIRAN

The biosynthesis of most exopolysaccharides are quite similar to the process by which the bacterial cell wall polymer, peptidoglycan and lipopolysaccharide are formed (Kumar et al. 2007). Polysaccharides produced via cell wall, intercellular and exocellular polysaccharides (Mathur and Mathur, 2006). Kefiran is most likely synthesized during bacterial growth (Cheirsilp et al. 2001). Kefiran is released into the media, reaching 218 mg L^{-1} and 247 mg L^{-1} in fermented milk and whey, respectively (Rimada and Abraham, 2006). It is known that the nature and composition of EPS as capsular or slime material are influenced by several factors such as medium composition, biosynthetic pathways, growth phase, and rate of microbial growth (De Vuyst and Degeest, 1999).

It is important to terminate the fermentation process at a necessary time when the nutrients in the culture medium almost consumed. This can be done by studying the growth kinetic of microbial. This step will help to prevent the degradation of produced polysaccharides by polysaccharases enzymes. The degradation process of microbial polysaccharides occurred either by polysaccharide hydrolases (polysaccharases) or by polysaccharide lyases (Sutherland, 1999). Polysaccharaces enzymes convert polysaccharides into energy as a result of nutrient exhaustion. There is still lack of information regarding the biosynthesis of exopolysaccharides produced by lactic acid bacteria. It is, however, probable that the mechanism proposed for exopolysaccharides and capsular polysaccharides from gram-negative bacteria can also be accepted for gram-positive kefiran producer since they come from the same *Lactobacilli* sp. The heteropolysaccharides biosynthetic pathway are generally divided into four parts: the first one involves with sugar transport into the cytoplasm; the second is synthesis of sugar-1-phosphates and; the third region activation of and coupling of sugars; and the fourth is transport and polymerization process (Laws et al.2001).

Several enzymes are involved during biosynthesis and secretion of heteropolysaccharides. Numerous types of carbohydrates from the surrounding medium are able to be transported to the cytoplasm through bacterial phosphoenolpyruvate (PEP)-sugar phosphotransferase sytem (PTS) which also function in carbohydrate catabolic repression (Deutscher et al., 2006). Glucose-6-phosphate is a key intermediate linking the anabolic pathways of EPS production and the catabolic pathways of sugar degradation. It is the flux of carbon bifurcates between the formation of fructose-6-phosphate toward the products of glycolysis, biomass and ATP formation and toward the biosynthesis of sugar nucleotides which is the precursors of EPSs. Phosphoglutcomutase is an enzyme involved in the conversion of glucose-6-phosphate to glucose-1-phosphate.It plays an important role in the divergence of flux between these catabolic and anabolic pathways (Degeest and De Vuyst, 2000). The sugar nucleotides required for the development of majority exopolysaccharides structures are UDP-glucose, UDP-galactose and dTDP-rhamnose (Laws et al. 2001). Glucose-1-phosphate serves as a branch point for the formation of the sugar nucleotides UDP-glucose and dTDP-glucose via the action of UDP-glucose pyrophosphorylase and dTDP-glucose pyrophosphorylase, respectively. The mechanism of polymerization of the repeating unit in lactic acid bacteria and its subsequent export from the cell is still remain unclear. Figure 1 shows the generalized diagram of lactic acid bacteria for glycolysis and the conversion of lactose and galactose to EPS.

Table 1: Microorganism population in kefir and kefir grains

Microorganism	References
Lactobacilli	
Lactobacillus acidophilus *Lactobacillus brevis* *Lactobacillus casei subsp. pseudoplantarum* *Lactobacillus casei subsp. tolerans* *Lactobacillus confuses* *Lactobacillus crispatus* *Lactobacillus fermentum* *Lactobacillus kefis*	(Yuksekdag et al., 2004; Kesmen and Kacmaz 2011; Tas et al., 2012; Garofalo et al., 2015) (Marshall, 1987; Simova et al., 2002; Tas et al., 2012; Yuksekdag et al., 2004; Garofalo et al., 2015) (Angulo et al., 1993) (Angulo et al., 1993) (Yaman, 2004) (Tas et al., 2012) (Angulo et al., 1993) (Pintado et al., 2003; Garofalo et al., 2015)
Lactobacillus kefirgranum	(Takizawa et al., 1994; Tas et al., 2012; Garofalo et al., 2015)
Lactobacillus parakefir	(Takizawa et al., 1994; Garofalo et al., 2015)
Lactobacillus plantarum	(Yuksekdag et al., 2004; Gao et al., 2012; Garofalo et al., 2015)
Lactobacillus gasseri	(Angulo et al., 1993; Garofalo et al., 2015)
Lactobacillus helveticus	(Simova et al., 2002; Jianzhong et al., 2009; Tas et al., 2012)
Lactobacillus kefiranofaciens *Lactobacillus kefiri*	(Takizawa et al., 1994; Santos et al., 2003; Tas et al., 2012; Garofalo et al., 2015) (Takizawa et al., 1994; Gao et al., 2012; Kesmen and Kacmaz, 2011; Garofalo et al., 2015)
Lactobacillus paracasei	(Santos et al., 2003; Garofalo et al., 2015)
Lactobacillus rhamnosus	(Angulo et al., 1993; Santos et al., 2003)
Lactobacillus thermophiles	(Tas et al., 2012)
Lactobacillus viridescens *Lactococcus lactis subsp. cremoris* *Lactococcus lactis subsp. Lactis*	(Angulo et al., 1993) (Yuksekdag et al., 2004) (Garrote et al., 2001; Simova et al., 2002; Yuksekdag et al., 2004)
Lactobacillus helveticus *Lactobacillus delbrueckii*	(Garofalo et al., 2015)
Yeast	
Brettanomyces anomalus	(Garrote et al., 2001)
Candida friedricchii	(Garrote et al., 2001)
Candida holmii	(Garrote et al., 2001; Witthuhn et al., 2004)
Candida inconspicua *Candida kefir*	(Simova et al., 2002) (Marshall, 1987; Garofalo et al., 2015)
Candida kefyr	(Witthuhn et al., 2004)
Candida lipolytica	(Witthuhn et al., 2004)
Candida maris	(Simova et al., 2002)
Candida valida	(Garrote et al., 1997)
Issatchenkia occidentalis	(Diosma et al., 2013)
Kazachstania exigua	(Garofalo et al., 2015)
Kazachatania unispora	(Magalhães et al., 2010; Garofalo et al., 2015)
Kluyveromyces lactis	(Latorre-García et al., 2007; Garofalo et al., 2015)
Kluyveromyces marxianus *Pichia ferments*	(Kesmen and Kacmaz, 2011; Gao et al., 2012; Diosma et al., 2013; Garofalo et al., 2015) (Angulo et al., 1993; Garrote et al., 1997; Garofalo et al., 2015)
Pichia guilliermondii	(Gao et al., 2012)
Pichia kudriavzevii	(Gao et al., 2012)
Saccharomyces cerevesiae	(Marshall,1987; Gao et al., 2012; Diosma et al., 2013; Garofalo et al., 2015)
Saccharomyces exiguss	(Latorre-García et al., 2007)
Saccharomyces lactis	(Pintado et al., 2003)
Saccharomyces unisporus	(Latorre-García et al., 2007; Diosma et al., 2013)
Torulaspora delbrueckii	(Garofalo et al., 2015)
Other Bacteria	
Acetobacter sp. *Bacillus sp.* *Dysgonomonas*	(Garrote et al., 2001; Gao et al., 2012) (Angulo et al., 1993; Gao et al., 2012) (Gao et al., 2013)
Escherichia coli	(Angulo et al., 1993; Chen et al., 2008)
Micrococuccus sp. *Pelomonas* *Shewanella*	(Angulo et al., 1993) (Gao et al., 2013) (Gao et al., 2013)
Streptococcus thermophiles	(Simova et al., 2002; Yuksekdag et al., 2004; Kesmen et al., 2011)

Weissella	(Gao et al., 2013)

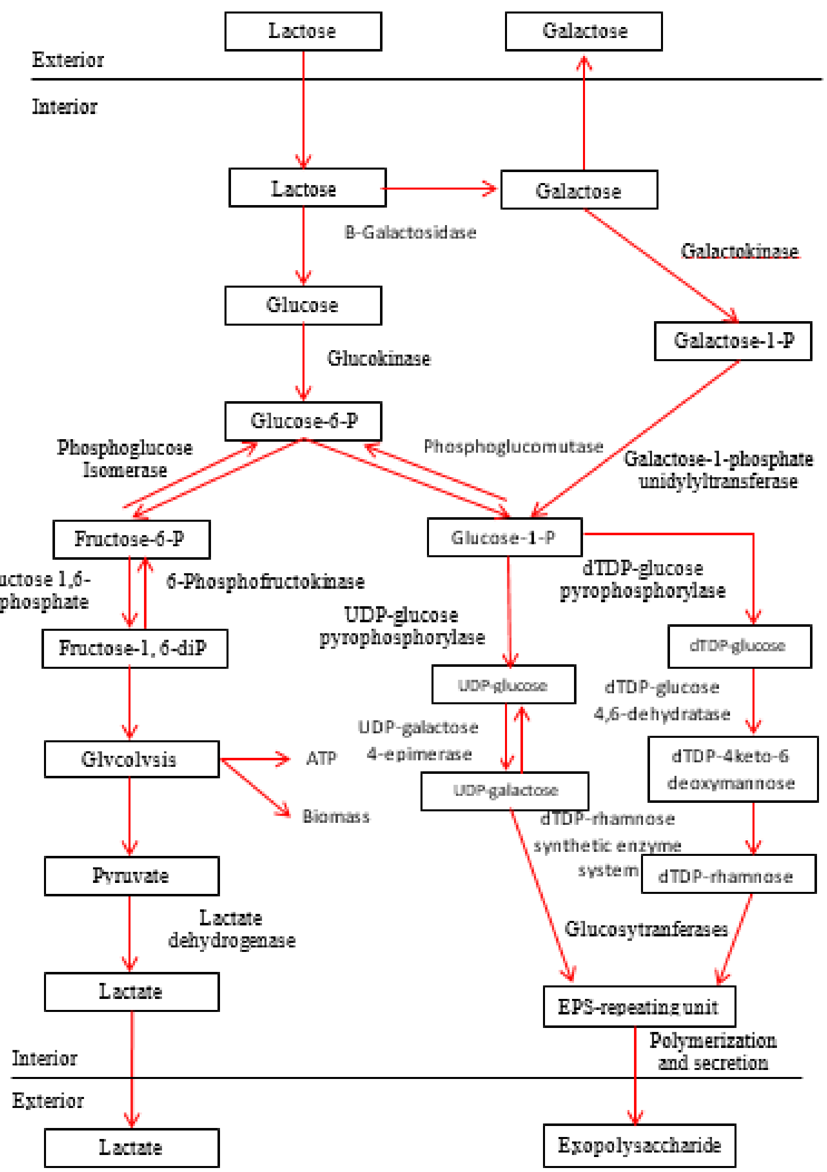

Figure 1: Generalized diagram of lactic acid bacteria for glycolysis and the conversion of lactose and galactose to EPS with some modifications (Welman and Maddox, 2003)

FACTORS EFFECTING THE CELL GROWTH AND KEFIRAN PRODUCTION

There are several factors that influence cell growth and kefiran production including medium formulation and cultivation conditions. Medium formulation for example different carbon sources, nitrogen sources and phosphate has great impact on microbial growth as well as polysaccharides production. Thus, the types and concentration of each nutrient must be determined and optimized in shake flask level prior to large-scale production. Other than medium formulation, cultivation conditions for instance pH of medium, incubation temperature and aeration rate also play a notable role in biomass and kefiran production.

Medium formulation

Different medium formulation has been established by previous researchers as to produced kefiran. These formulations which include different types of nutrients gave different results towards the capability of the cells to produced kefiran. As demonstrated in Table 2, different cultivation media have been used for cell cultivation and kefiran production.

Carbon sources

Production of polysaccharides is strongly influenced by the type, concentration and source the of carbon sources. Carbohydrates are the major sources of cellular energy and carbon in all living organisms (Shuhong et al. 2014). The type of carbon source utilized is dependent on the species of microorganism where different species of organism may require different types of carbon source for living. Various sources or carbon source can be found abundantly on the earth such as lactose, glucose, sucrose, maltose, fructose, galactose, molasses and starch. The types and compositions of carbohydrates gave significant effect on the production of polysaccharides and the characteristic (Wang and Bi, 2008; Wu et al., 2014; Shuhong et al. 2014). The possible effect of each different individual carbon sources varied is because of the different impact of cellular catabolic repression on the cellular secondary metabolism (Maeda et al. 2003). However, commercial carbon sources such as glucose and sucrose readily available in the market are quite expensive. Therefore, several investigators have described the utilization for cheaper carbon sources for production of EPS. It is mainly the easily available agricultural waste such as potato residues (Bilanovic et al. 2011), whey lactose (Cheirsilp et al. 2011), molasses (Bakhtiyari et al. 2014) and vegetable waste (Donato et al. 2014).

Several studies have been focused on the utilization of several carbon sources for kefiran production. The types and amount of carbon source used can affect polysaccharides production significantly. According to Yokoi and Watanabe (1992), lactose was found to be the best chemically defined carbon sources compared to glucose and sucrose. At the concentration of 10%, lactose gave the highest yield with a total kefiran of 66% difference for glucose and 46% difference for sucrose. Similarly, lactose was also found to be the best carbon source for kefiran production followed by sucrose, fructose and glucose (Zajsek and Gorsek, 2011). It was also reported that whey lactose was used at optimal concentration of 4% to achieve maximal kefiran production (Cheirsilp and Radchabut, 2011). Another research reported by Taniguchi et al. (2001) used different types of carbon sources of glucose, galactose, lactose, sucrose, or a mixed sugar of glucose and galactose. The results showed that lactose gave the highest yield for kefiran production compared to others carbon sources. Similar result was also reported by Yokoi and Watanabe (1992).

In contrast, Wang and Bi (2008) reported maltose was better than lactose for kefiran production. Eight different types of carbon sources were tested in their study including glucose, lactose, maltose, sucrose, fructose, galactose and soluble starch. This result shows that besides lactose, maltose can be also used for kefiran production. On the other hand, sago starch was utilized for kefiran production (Yeesang et al., 2008). Sago starch can be converted into glucose as carbon source and yielded 0.85 g L^{-1} kefiran. Yeesang et al. (2008) used different sago starch concentrations of 2, 3, 4 and 5% w/v. The results showed that 4% sago starch gave the highest yield of kefiran where yield decrease when sago starch was used beyond this concentration. It is also worthy to note that rice starch hydrolysate was used as a source of carbon source (Maeda et al. 2003). Medium contained 10% of rice starch hydrolysate was also found to be the best carbon source followed by 10% of glucose and 10% of lactose.

Nitrogen sources

Nitrogen is essential nutrient for the cell growth and biosynthesis of polysaccharides. Basically, complex nitrogen source such as yeast extract was used as a source for vitamins and other growth factors like amino acids (Cheirsilp and Radchabut, 2011). The importance role of nitrogen source has been well showed in many studies related to polysaccharides production such as kefiran (Cheirsilp and Radchabut, 2011), levan (Silbir et al. 2014), pullulan (Sugumaran et al. 2014) and gellan gum (Zhang et al. 2015). Nitrogen sources can be divided into organic and inorganic nitrogen sources. Organic nitrogen sources include yeast extract, soybean meal, meat extract, corn steep liquor, rice protein hydrolysate and casein hydrolysates. Inorganic nitrogen sources include ammonium salts such as triammonium citrate, ammonium nitrate, ammonium phosphate, ammonium lactate, ammonium acetate, ammonium hydrochloride and ammonium sulfate. The nitrogen source competition between *L. kefiranofaciens* and yeast might occur for growth in the mixed culture system.

Taniguchi et al. (2001) studied the effect of yeast extract addition as nitrogen source for the production of kefiran. From the results obtained, it has been shown that in medium free of yeast extract, poor growth was observed and kefiran was hardly produced. The addition of 5 g L^{-1} yeast extract gave a significant increase in the concentration of kefiran compared to 2.5 g L^{-1} yeast extract culture. Similar studies conducted by Yokoi and Watanabe (1992) and Cheirsilp and Radchabut (2011) investigated the effect of different nitrogen sources such as trypton, yeast extract, meat extract, and triammonium citrate for the production of kefiran. It was found that increasing the nitrogen concentration increased the yield of kefiran and cell growth. Yeast extract was found to be the best nitrogen source for kefiran production in other studies as well (Yokoi and Watanabe, 1992; Wang and Bi, 2008; Cheirsilp and Radchabut, 2011).

Phosphate

A salt of phosphoric acid known as phosphate is an inorganic chemical that were used usually in the production medium to enhance cell growth and product formation (Dhivya et al., 2014). Only few studies reported so far regarding the effect of phosphate on *L. kefiranofaciens* growth and kefiran production. It was reported that an optimal concentration of phosphate at 0.25 g/L is used for production of kefiran (Dailin et al., 2015; Dailin et al., 2016). Other researchers have reported the effect of phosphate for other types of polysaccharide producing cells. For levan producing strain it was found that addition of phosphate in cultivation medium increased both the cell growth and polysaccharide production (Sarilmiser et al., 2015; Abou-Taleb et al., 2014).

A similar study showed also that phosphate plays a crucial role in cell growth and production of polysaccharides by *Bacillus licheniformis* KS-17 strain (Song et al. 2014). Different phosphate sources were examined such as dipotassium, monopotassium, disodium, and monosodium phosphates. It was found that dipotassium phosphate greatly enhanced polysaccharides production while potassium phosphate significantly enhanced biomass. Furthermore, Gunter and Ovodov (2005) shows that cell growth and production of polysaccharides were limited by the absence of phosphate in the medium. For xanthan production, studies show that xanthan production was highly effected by the phosphate concentration. Higher phosphate concentration more than 50 mM inhibited xanthan production (Souw and Demain, 1979). It was also reported that relatively low phosphate concentrations of about 0.1 g L^{-1} was needed for optimal production of curdlan (Kim et al. 2000). Further addition of phosphate concentration beyond this concentration showed significant in reduction of curdlan production.

Cultivation Conditions

Besides medium formulations, cultivation conditions which involve production of xanthan is an important factor affecting kefiran production. Some of the important parameters are pH of medium, incubation temperature and aeration rates.

pH of medium

PH of culture medium plays an important role for the microbial growth and product yield in submerged cultures. pH is a measure of the concentration of hydrogen ions in a particular solution. The more the ions of hydrogens present in a solution, the lower the pH value of that solution bringing an acidic environment and vice versa. Different pH results in different polysaccharides production yield and microbial growth. In general, the optimal pH medium for cell growth is about the range from 2.0 to 4.0 but the optimal medium pH for exopolysaccharides formation is with the range from 5.0 to 7.0 (Shu and Lung, 2004). Initial pH of culture medium potentially affects function of cell membrane, morphology and structure of cell, salts solubility, substrates ionic state, various nutrients uptake and also synthesis of product (Fang and Zhong, 2002). In addition, the effect of pH on cell growth and polysaccharides production is different depending on the types of microorganism, operational conditions and medium composition (Shu and Lung, 2004).

Several researches have investigated the effect of pH on microbial growth and kefiran production. Yokoi and Watanabe (1992) tested three different pH conditions that were 4.5, 5.0, 5.5 and 6.0, and pH of culture broth was controlled throughout the process. The results show that the highest yield of kefiran was obtained at pH 5.0. Similar approach used by Taniguchi et al. (2001) where different pH of 4.5, 5.5 and 6.5 were studied to obtained the maximum production yield of kefiran. The highest amount of kefiran obtained was at the initial pH of 5.5. This result was concurrent with the findings of Yeesang et al. (2008) and Cheirsilp and Radchabut (2011) where the highest kefiran production was also obtained at the pH of 5.5.

Another research pointed out that the cell growth of lactic acid bacteria is optimum at the pH of 5.5 (Yokota et al. 1995). Ghasemlou et al*.* (2012) reported that pH 5.7 was the best for kefiran production using response surface methodology. According to Cheirsilp et al. (2001), no growth of *L. kefiranofaciens* was observed at pH of 7.0 and at pH 4.0. Initial pH was controlled along the process to gain optimum production and to avoid product inhibition and drop in pH value caused by the production of lactic acid (Yokoi and Watanabe, 1992).

Incubation temperature

Temperature is important factor effecting on the microbial growth, yield and polysaccharides formation (Shu et al. 2007). According to their temperature optima, organisms can be classified into three groups that are psychrophiles (T_{opt}<20°C), mesophiles (T_{opt} range between 20 to 30°C) and thermophiles (T_{opt}>50°C) (Shuler and Kargi, 2002). The production of exopolysaccharides is often to be greater on lower temperature (Cerning, 1995). However, the biosynthesis of polysaccharides is inhibited if the temperature is decreased by 10°C from it optimal temperature (Sutherland, 2001). When temperature increased toward optimal growth temperature, the growth rate approximately doubles for each 10°C increase in temperature (Shuler and Kargi, 2002). The growth rate decreases and thermal death may occur above the optimal temperature range (Shuler and Kargi, 2002).

Yokoi and Watanabe (1992) reported in their studies that highest kefiran production obtained at 30°C. Similar results were also reported that the optimal temperature for kefiran production is 30°C (Taniguchi et al. 2001; Yeesang et al*.* 2008; Zajšek and Gorsek, 2011). According to Yeesang et al. (2008), for temperature higher than 32°C, reducing sugars concentration is at higher level during fermentation and inhibits the cell growth. Lower cell growth brought into less reducing sugars consumed but in increased conversion of starch to reducing sugars. However, a much lower temperature of 24°C was optimum for maximal kefiran production reported (Ghasemlou et al., 2012).

Aeration rate

Aeration rate is one of the vital parameter for all aerobic processes and influencing the successful progress of fermentation process. Aeration could be advantageous to the performance and growth of microorganisms by enhancing the mass transfer characteristics with respect to products/by-products, oxygen and substrate (Roukas & Mantzouridou, 2001; Mantzouridou et al. 2002; Kim et al. 2003). It exhibits a remarkable effect on the polysaccharides production such as pullulan (Roukas & Mantzouridou, 2001), xanthan (Borges et al. 2008), kefiran (Cheirsilp, 2003), curdlan (Lee et al. 1999) and gellan (Giavasis et al. 2006). Aeration determines the oxygenation of the fermentation process and ensure the better mixing in fermentation medium particularly where the agitation speed is low (Mantzouridou et al. 2002; Kim et al. 2003), therefore, helping maintain a concentration gradient between the exterior and interior of the cell and result in high biomass production (Roukas & Mantzouridou, 2001).

The concentration of critical O_2 for most of the bacteria is between the ranges of 5% to 10% of the saturated dissolved oxygen (DO) concentration. According to Cheirsilp and the colleagues (2003), the spurge of oxygen at control DO of 5% shortened the fermentation time and also kefiran production. Other than that, in an environment without aeration, there is no different in the amount of broth kefiran and biomass (cells) produced in pure culture and mixed culture of *L. kefiranofaciens* and *S. cerevisiae*.

Besides dissolved oxygen, the dissolved carbon dioxide (DCO_2) concentration may have a huge impact on the performance of the growth of microorganism. High concentration of DCO_2 may cause toxicity to the cells; however, certain amount of DCO_2 is required for proper metabolic function. Similar to the oxygen supplied for fermentation process, the DCO_2 concentration are controlled by altering the CO_2 content in the air supply as well as agitation speed (Shuler & Kargi, 2002). There have been relatively few reports on the effect of CO_2 concentration on kefiran production. Based on Taniguchi et al. (2001), none of the cell growth and kefiran produced in the cultivation by aeration with N_2 alone at 0.3 $vv^{-1}m^{-1}$. A slight increase in the amount of kefiran was observed when a mixed gas of nitrogen and carbon dioxide at a ratio of 9:1 as compare to none aerated culture. The ratio of N_2 to CO_2 (9:1) was better than 1:1 and this indicates that the important of introducing a small quantity of carbon dioxide for microbial growth and kefiran production. Moreover, it was also found that the gas containing CO_2 led to the change in lactose uptake and metabolism. Another research performed by Cheirsilp et al. (2003) demonstrates opposite results of stress from CO_2 and O_2 where it did not enhance kefiran production.

Table 2: Media for kefiran production in submerged culture

Strain	Medium Composition (g L^{-1})	Reference
Lactobacillus kefiranofaciens ATCC 43761	rice starch hydrolysate, 100; rice protein hydrolysate, 3.5; glucose, 100; polypeptone, 15; yeast extract, 10; Tween 80, 1; K_2HPO_4, 2; sodium acetate, 5; triammonium citrate, 2; $MgSO_4.7H_2O$, 0.2;$MnSO_4.5H_2O$, 0.05; *pH 5.0; T, 33°C*	Maeda et al., 2005
Lactobacillus kefiranofaciens ATCC 43761	maltose, 100; yeast extract, 10; tryptone, 20; meat extract, 20; K_2HPO_4 2; triammonium citrate, 4; sodium acetate, 5; Tween 80, 1; $MnSO_4·4H_2O$, 0.28; $MgSO_4.7H_2O$, 0.58; $CaCl_2·2H_2O$, 0.74; *pH 5.5;T, 25°C*	Wang and Bi., 2008
Lactobacillus kefiranofaciens ATCC 43761	sago starch, 40; yeast extract, 10; tryptone, 20; meat extract, 20; K_2HPO_4, 2; triammonium citrate, 4; sodium acetate, 5; Tween 80, 1; $MnSO_4.4H_2O$, 0.28; $MgSO_4.7H_2O$, 0.58; $CaCl_2.2H_2O$, 0.74; *pH 5.5; T, 30°C*	Yeesang et al., 2008
Lactobacillus kefiranofaciens ATCC 43761	whey lactose, 40; Yeast extract, 40; tryptone, 20; meat extract, 20; K_2HPO_4, 2;Triammonium citrate, 4; sodium acetate, 5; Tween 80, 1, $MnSO_4.4H_2O$, 0.28; $MgSO_4.7H_2O$, 0.58; $CaCl_2/2H_2O$, 0.74; *pH 5.5; T, 30°C*	Cheirsilp and Radchabut, 2011
Lactobacillus kefiranofaciens ATCC 43761	lactose monohydrate, 10; yeast extract, 10; tryptone, 20; meat extract, 20; K_2HPO_4, 2; triammonium citrate, 4; sodium acetate, 5; Tween 80, 1; $MnSO_4.4H_2O$, 0.28; $MgSO_4.7H_2O$, 0.58; $CaCl_2.2H_2O$, 0.74; *pH 5.0; T, 30°C*	Cheirsilp et al., 2007
Lactobacillus kefiranofaciens K_1	deprotenized whey, 1000 ml; white table wine, 70 ml; glucose, 10; galactose, 10; Tween 80, 1; *pH 5.5; T, 30°C*	Mukai et al., 1990
Lactobacillus sp. KPB16-7B	lactose monohydrate, 20; trypton, 10; yeast extract, 5; meat extract, 10; K_2HPO_4, 2; triammonium citrate, 2; Tween 80, 1; sodium acetate, 5; $MnSO_4.4H_20$, 0.28; $MgSO_4.7H_20$, 0.58; *pH 6.5; T, 30°C*	Yokoi and Watanabe, 1992
Lactobacillus sp. LM 17	lactose monohydrate, 20; trypton, 10; yeast extract, 5; meat extract, 10; K_2HPO_4, 2; triammonium citrate, 2; Tween 80, 1; sodium acetate, 5; $MnSO_4.4H_20$, 0.4; $MgSO_4.7H_20$, 0.7; $CaCl_2$, 7 mM; *pH 6.5; T, 30°C*	Micheli et al., 1999
Lactobacillus kefiri ATCC 35411	protease peptone, 10; beef extract, 10; yeast extract, 5; dextrose, 20; sorbitan monooleate,1; ammonium citrate, 2; sodium acetate, 5; $MnSO_4.4H_20$, 0.05; Na_2HPO_4, 2; *pH 6.5; T, 34°C*	Kandler and Kunath, 1983

Table 3: Applications of kefiran in food industry

Application	Reference
Thickeners Gelling agent Water binding agent Stabilizer Emulsifier Improve self-supporting gels	(Rimada & Abraham, 2006) (Medrano et al., 2008; Piermaria et al., 2008; Zavala et al., 2014; Moradi & Kalanpour, 2019; Moradi et al., 2019) (Piermaria et al., 2011) (Piermaria et al., 2008; Moradi & Kalanpour, 2019; Moradi et al., 2019) (Piermaria et al., 2008; Moradi & Kalanpour, 2019; Moradi et al., 2019)(Piermaria et al., 2008)
Food packaging Fat Substitute	(Ghasemlou et al., 2011; Piermaria et al., 2011; Zolfi et al., 2014b; Babaei-Ghazvini et al., 2018; Goudarzi & Shahabi-Ghahfarrokhi, 2018; Júnior et al., 2020) (Moradi & Kalanpour, 2019; Moradi et al., 2019)

Application In Food

Interest in kefiran has increased recently in food industry due to its ability to provide the desired rheological properties for the dairy products. The food industry is often looking for new attractive and healthy foods with low fat content to improve the firmness, creaminess and to add more biofunctional properties (Sarmidi and El Enshasy, 2012). Polysaccharides such as kefiran could function as thickeners (Rimada and Abraham, 2006), gelling agents (Piermaria et al. 2008; Zavala et al. 2014) and water binding agents (Piermaria et al. 2011) when added to food products**.** Kefiran is able to improve the rheological properties of chemically acidified skim milk gels, increasing their apparent viscosity, the storage and modulus of these gels (Rimada and Abraham, 2006). Hence, it can be applied as a food grade additive for fermented products. This polysaccharide also improves self-supporting gels (Piermaria et al. 2008). It can be formed as a result of cryogenic treatment from their solutions. In addition, they also found that the characteristics of kefiran cryogels are translucent, self-supporting, retain water with high content and melt at mouth temperature of 37°C.

Over the past decade, the food packaging industries depends mostly on using petroleum-based plastic materials. However, the increases of awareness of environmental issues and demand for innovative biodegradable packaging have turned this issue around. Therefore, efforts have been taken where food packaging are derived from renewable materials such as polysaccharides. Extensive studies have been taken by many researchers to apply kefiran as novel material in food packaging. Recent study shows that kefiran can produce films with good appearance and satisfactory mechanical properties (Ghasemlou et al. 2011). In this study, they found that the barrier properties of kefiran films can be improved by addition of a relatively high amount of oleic acid and low concentration of glycerol without effect on their appearance. Another study shows that kefiran able to form edible transparent films but with brittle and rigid characteristics (Piermaria et al. 2011). However, the addition of sugar improved the water vapor permeability and mechanical properties. The growth of food microbes can be prevented since kefiran films possess low water activity. In addition, the characteristic nature of kefiran as antibacterial and antifungal makes it more attractive in food packaging. Recent studies also showed the implementation of nanotechnology for the preparation of UV-protective kefiran/nano-ZnO nanocomposites, formation of kefiran nanofibers and for the production of biodegradable kefiran-whey protein isolate (WPI) nanocomposite (Esnaashari et al. 2014; Zolfi et al. 2014a; Shahabi-Ghahfarrokhi et al. 2015)

CONCLUSION

Kefiran is a unique exopolysaccharide produced by lactic acid bacteria using submerged fermentation. It possesses various advantages with its unique characteristic with strong applications in the food industries covering emulsifier, thickeners, gelling agents, fat substitute, food packaging and few others. For this, more research work need to be done to identify novel application of this material especially in the food industry. Furthermore, research on the maximal production of kefiran in large scale using optimal condition is needed to maximize the potential used of this product for wider community.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

DJD, RAM, TLT, and TH involved in data collection and writing the manuscript. SR, RW, EAE, OML, and HAE reviewed the manuscript. All authors read and approved the final version.

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