

Mini-Review on Functional Characteristics of Viili and Manufacturing Process

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Abstract

Viili (viilia) is ropey and sticky traditional fermented milk that is produced in Scandinavia. In particular, it is popular in Finland. Viili is marked by mesophilic lactic acid bacteria (LAB), fungi and yeasts. The LAB identified in viili including, the acid-producing strains *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, together with the aroma-producing strains *Lactobacillus lactis* subsp. *Lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*. In addition, most traditional viili cultures also contain *Geotrichum candidum* fungi and several yeast strains including *Kluyveromyces marxianus*. These microorganisms are being used to produce exopolysaccharides which gives a ropey, gelatinous consistency and a pleasantly mild taste to viili. In addition to its ability to provide a thick body, viili has many of the functional activities including antioxidant, immunomodulatory, ACE Inhibitory and antitumor activity that make it useful in the treatment of cancer, inflammation, diabetes, genotoxicity, accelerated ageing, hypertension and heart diseases.

Keywords: Viili; Fermented dairy; Exopolysaccharide

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Introduction

Viili (viilia) is ropey and sticky traditional fermented milk that originated in Scandinavia. In particular, it is popular in Finland [1]. The word viili is from Swedish language and describes mesophilic fermented milk that is known to have various functional benefits and the potential to improve human health [2,3].

Viili has a pleasant sharp taste and a good diacetyl aroma linked to a tough texture and can be cut easily with a spoon. Diacetyl is a major flavor compound in viili. The domestic fermented milk is typically consumed in meals, especially at breakfast, and is also used as a snack food among kids. This product is consumed either in plain form or sweetened with jam and raisins, or with fruit or cereals. A traditional way of eating viili is by mixing it with cinnamon and sugar [4]. The fat content in the product may vary from 2 to almost 12%, depending on classification (such as low fat, full fat). Viili was historically made on farms in large wooden buckets, and at a later stage, families also started to make it at home. The industrial manufacture of viili began in Finland in the 1950s. Today, this product has grown to be a vital fermented milk product in Finland. Annual consumption stands at more than 4.5 kg/capita [4]. The most traditional viili culture consists of mesophilic lactic acid bacteria (LAB) forming a culture

in the body of the milk together with a surface-growing yeast-like fungus [4-8], thus all viili cultures also contain yeasts. The effect of yeasts on the viili is not clear, however, it is believed that yeasts in viili may provide the product's unique flavor and induce the LAB to produce more exopolysaccharides (EPS) [7]. In this mini-review, we will shed a light on the manufacturing process of viili, including the identification and characteristics of microorganisms involved during culture. Moreover, biosynthesis of EPS and functional characteristics of viili are also discussed.

Identification of microorganisms in viili starter cultures

In the traditional manufacturing of viili, the propagation of the microflora was done by adding a small quantity of a previously prepared product. Today, the production of viili has developed to industrial-scale manufacturing; nevertheless, traditional starter cultures containing mixed populations of LAB are being utilized

for this purpose. Four LAB strains, namely, the acid-producing strains *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, together with the aroma-producing strains *Lactobacillus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*, were found. Wang et al. [7] identified two LAB strains, *L. lactis* subsp. *cremoris* and *Leu. mesenteroides* subsp. *mesenteroides*, in Taiwanese viili culture. On the other hand, Chen et al. [1] identified a different microbial profile in a viili culture, which included *Lb. plantarum*, *Streptococcus thermophilus*, *Lb. paracasei*, and *Bacillus cereus*. The strains other than *L. lactis* and *Leu. Mesenteroides* found in viili products, entered the culture from the local environment. Along with using the fungi (*Geotrichum candidum*), there are *Kluyveromyces marxianus*, *Saccharomyces unisporus*, and *Pichiafermentans* which has been identified in viili starter cultures at levels of 58%, 11%, and 31%, respectively. Viili yeasts produce carbon dioxide and alcohol, thus, contributing to the typical yeast flavor.

A steady and constant starter culture, which is necessary for manufacturing a quality fermented beverage, can be difficult to sustain due to the complex microbiological composition of viili culture. Therefore, knowledge of microbiological profiles of viili cultures is crucial to safety characteristics and quality control of viili products. Additionally, their probiotic effects need to be evaluated to determine their potential health benefits. Numerous studies have identified a variety of microorganisms in viili starter cultures using selective growth medium, morphological characterization, biochemical characterization, and molecular methods [1,4,7,9].

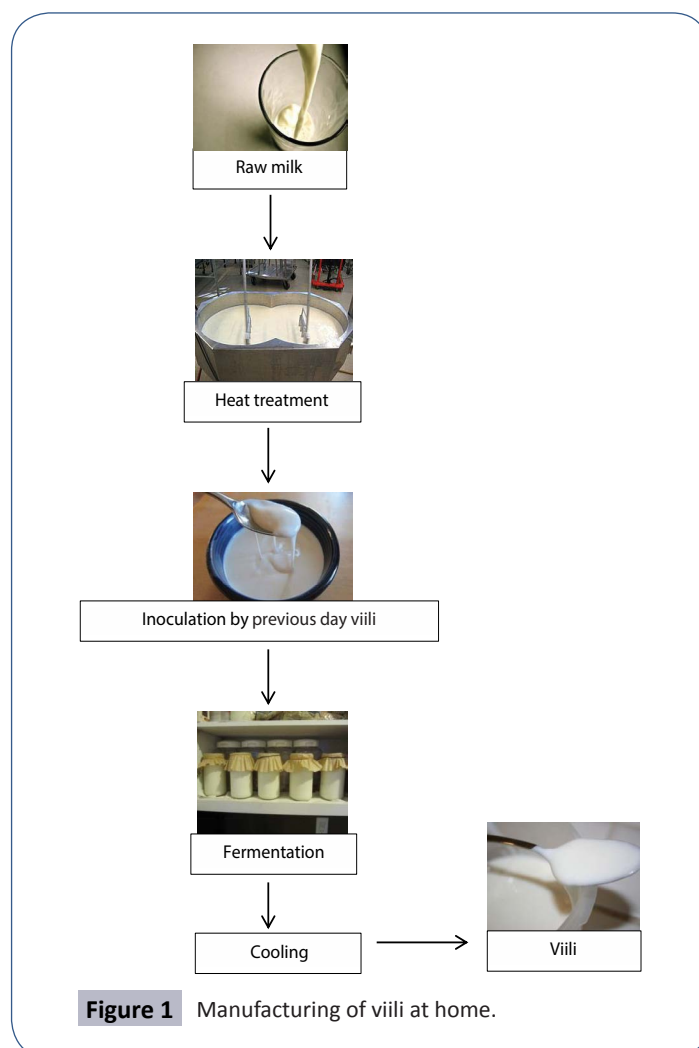
The characteristics of microorganisms of viili during culture

The fermentation of viili starters is characterized by a rapid increase in the number of LAB from an initial value of 10^6 to 10^9 CFU/g after the first 20 h of fermentation. This level then remains stable for the rest of process [7]. A study of the microbial dynamics at different fermentation stages demonstrated that *L. Lactis* subsp. *cremoris* was the leading bacterial species in the samples, followed by *Leu. mesenteroides* subsp. *mesenteroides*; moreover, there was no change in this proportion as the fermentation progressed. Uchida et al. [10] also reported that *L. lactis* subsp. *cremoris* was the dominant strain in all samples of Japanese domestic ropy fermented milk. A high population of *L. lactis* subsp. *cremoris*, which results in the production of a high level of EPS, is necessary to produce the ropiness of the fermented milk product. The viili fungus, *G. candidum*, contributes to the typical musty aroma of this produce. Lipases and proteases produced by *G. candidum* release fatty acids and peptides that are metabolized by the microbial populations, and this contributes to the development of viili's distinctive flavors and other qualities [11]. Also, *G. candidum* neutralizes the curd by catabolizing the lactic acid produced by the LAB and by releasing ammonia during the metabolism of the amino acids [12]. The viili yeast, *K. marxianus*, is able to utilize both lactose and galactose as carbon sources, which explains why this strain is the primary yeast in the viili samples. The participation of *K. marxianus* in the starter culture ensures the metabolism of lactose via alcohol

fermentation and the formation of the product's typical yeasty flavor. In addition to *K. marxianus*, various lactose-negative yeasts seem to play very important roles in the formation of viili flavor. Simova et al. [13] reported that the product's typical yeasty flavor and aroma were absent, when ropy fermented products were produced by *K. marxianus* only. Additionally, it is worthwhile mentioning that there are remarkable differences in the cell surface properties, co-aggregation, auto-aggregation, and biofilm formation between the kefir and viili strains [14]. The LAB and yeasts in viili do not show any significant auto-aggregation and biofilm formation, both of which are necessary for forming starter grains. Grain formation begins with the self-aggregation of LAB strains to form small granules. Biofilm producing strains then begin to attach to the surface of the granules and co-aggregate with other organisms and components in the milk to form the grains [14]. The surface properties of viili microorganism explain why there is no starter grain formation in viili starter cultures.

Manufacture of viili at home

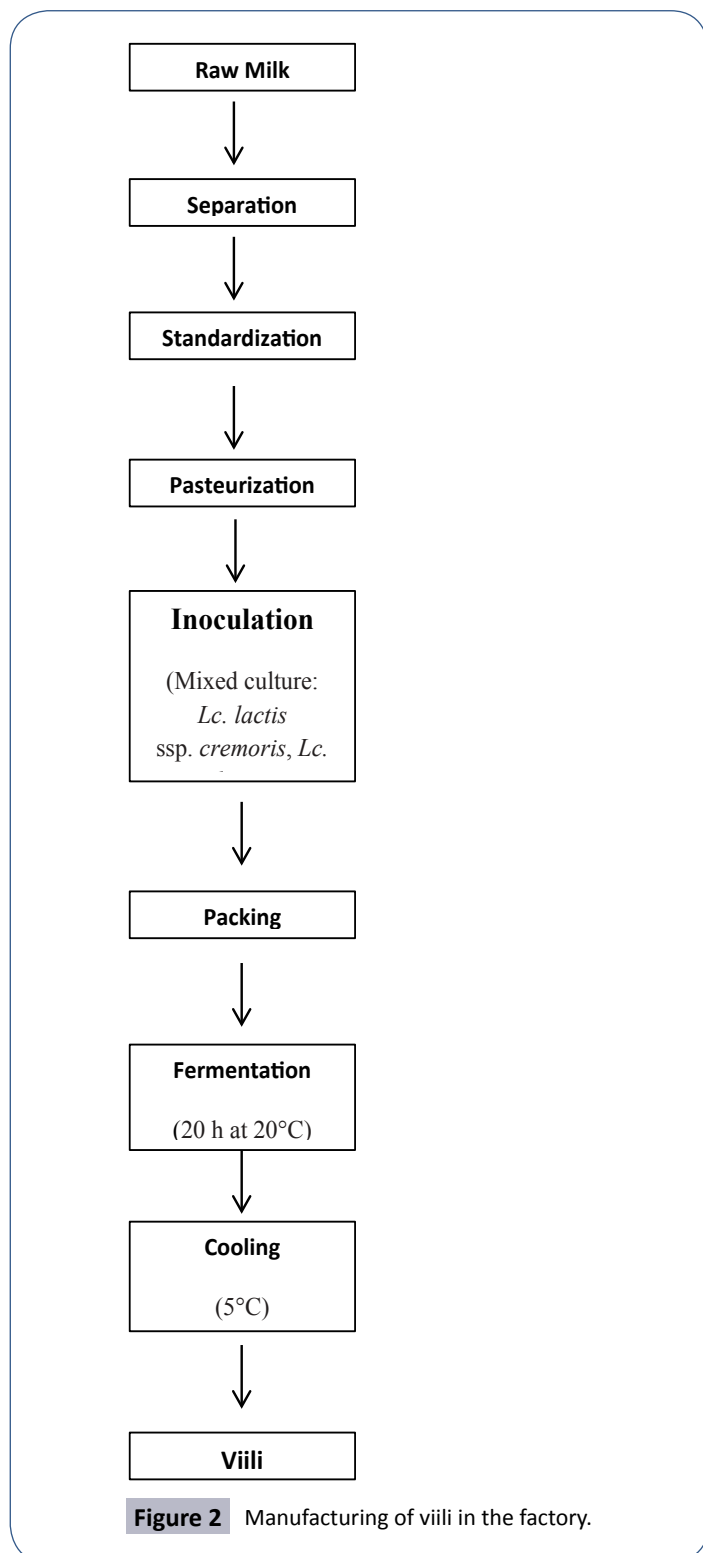
To make homemade viili (Figure 1), raw milk is pasteurized. Active starter (5%, w/v), usually from the previous day viili, is then inoculated into the milk, and the mixture is incubated at 20°C for 20 h. After fermentation, the viili is cooled to 4°C and



is ready to consume. However, the ropy nature of viili products seems to become unstable if it is produced at higher fermentation temperatures and, when there are frequent transfers of starter cultures.

Manufacture of viili in the factory

For the industrial production of viili (Figure 2), the milk is separated and standardized to a fat content in the range of 1.0%–



3.5%. Usually, viili has been made from raw milk. Consequently, a layer of cream forms on its surface. After standardization, milk is heated to a high temperature (83°C for 20–25 min), tempered to the incubation temperature of 20°C, and inoculated with 4% starter culture consisting of *L. lactis* subsp. *lactis*, a diacetylactis culture, *Leuc. mesenteroides* subsp. *cremoris*, and *Geotrichum candidum* or a mixed culture of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, and *G. candidum*. Next, the mixture is packed in a cup and removed to a storage area for ripening, where the viili fermentation takes place for 20 h at 20°C (final acidity of 0.9%). The purpose of incubation in consumer cups is to allow the fat to rise to the surface during incubation where the *Geotrichum* mold will grow and contribute to the typical musty aroma. Furthermore, complex carbohydrates formed by the organisms employed give the product a heavy and ropy characteristic. The viili is cooled to below 6°C after fermentation. The mold stops growing after the oxygen is completely consumed. Most of the CO₂ formed dissolves in the product, leading to a slight underpressure in the package. The shelf life of viili is around 3 weeks. There is a wide range of different types of viili in the market, including products with dissimilar fat contents, ones with reduced lactose and flavored varieties. Viili is also produced commercially from homogenized milk without mold growing on the surface and from milk other than cow's, but traditional viili is still the most popular in the Finnish region [4,15].

Slime properties of viili

The characteristic ropy consistency of viili is caused by the slime forming variants of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* found in the starter culture [16]. Both strains are able to produce slime as capsule or exocellular EPS. Scanning electron micrographs of milk gels prepared using a ropy strain, *L. Lactis* subsp. *cremoris*, showed that EPS was present in the form of a network that attached the bacterial cells to the protein matrix. A thick network of slime also attached the casein micelle-clusters to each other producing casein conglomerates, which seems to be the origin of the characteristic consistency of viili [17].

Sugar components and composition of the EPS

The composition of the EPS excreted by *L. lactis* subsp. *cremoris* consists of 3–47% protein and 29–85% carbohydrate. The sugar components are predominantly galactose, glucose, and often rhamnose [18], with repeating units of “→4-β-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl—(1→,” and groups of α-l-rhamnopyranosyl and α-d-galactopyranosyl-1-p attached to each side of galactopyranosyl [16,18,19]. The composition and sugar components of EPS are strain and medium dependent. The EPSs secreted by *L. lactis* subsp. *cremoris* SBT 0495, ARH53, ARH74, ARH 84, ARH 87, and B30 are composed of repeating units of galactose, glucose, and rhamnose with a phosphodiester structural element [18,20]. In contrast, the EPS produced by *L. lactis* subsp. *cremoris* H414 is a homopolymer consisting of galactose with a branched-pentasaccharide repeating unit [21].

Marshall et al. [22] reported that *L. lactis* subsp. *cremoris* strain LC33 was able to generate two different EPSs. One contains glucose, galactose, rhamnose, glucosamine, and phosphate. The other was composed of galactose, glucose, and glucosamine with branched terminal galactose moieties. The slime material obtained from *L. lactis* subsp. *cremoris* SBT0495 supernatant in whey permeate medium consisted of 42% carbohydrate and 21% protein with large amount of mannans present in the cultured medium [20]. Furthermore, *L. lactis* produced more EPS on glucose than on fructose as the sugar substrate, although the transcription level of the EPS gene cluster was independent of the sugar source [23].

Applications and biosynthesis of EPS

EPS synthesized by LAB is playing a major role in viili product and other fermented dairy products including cheese, drinking yoghurt, yoghurt, and fermented cream. EPS is increasing the viscosity of a final product and binding hydration water and interacting with other milk constituents, including proteins and micelles, to strengthen the rigidity of the casein network, thereby, improving the texture of final product. As a consequence, EPS can decrease syneresis and improve product stability. Furthermore, it has been conveyed that EPS has beneficial effects on gut health [24].

Recent studies have demonstrated that the ropy phenotype of *L. Lactis* subsp. *cremoris* is associated with an 18.5-30 MDa plasmid [23,25]. Several enzymes under positive or negative control are involved in polysaccharide synthesis and excretion. Therefore, the plasmid may code for certain proteins that are involved in slime production [26]. The metabolic pathways that are involved in the biosynthesis of the EPS molecules from the milk sugar lactose are illustrated in **Figure 3**. The biosynthesis can be roughly divided into three steps: the intracellular formation of EPS precursors, the sugar nucleotides, and the formation of a repeating unit on a lipid carrier that is located at the cytoplasmic membrane [23,27]. The repeating unit of EPS produced by most of *L. lactis* subsp. *cremoris* strains is composed of glucose, galactose, rhamnose, and phosphate [18,20]. The sugar nucleotides UDP-glucose, UDPgalactose, and dTDP-rhamnose are the donors of the monomers used for the biosynthesis of the pentasaccharide unit. The last step of EPS formation involves the transportation of the repeating units across the membrane to the outer layer of the bacteria and polymerization of several hundred to several thousand repeating units to form the final EPS [23,27]. The enzymes involved in EPS formation by *L. lactis* subsp. *cremoris* include the enzymes responsible for the original metabolism of the carbohydrate, enzymes leading to sugar nucleotide synthesis and interconversion, glycosyl transferases that form the repeating unit attached to the glycosyl carrier lipid, and translocases and polymerases that form the polymer. The genes encoding the enzymes involved in the biosynthesis of EPS by *L. lactis* subsp. *cremoris* are borne by an EPS plasmid. The gene products EpsD, EpsE, EpsF, and EpsG are glycosyltransferases and are required for the synthesis of the EPS backbone [28,29]. The presence of the EPS genes on a plasmid has been suggested to be the cause of EPS expression instability at higher temperatures and when there are frequent transfers of starter culture [30,31].

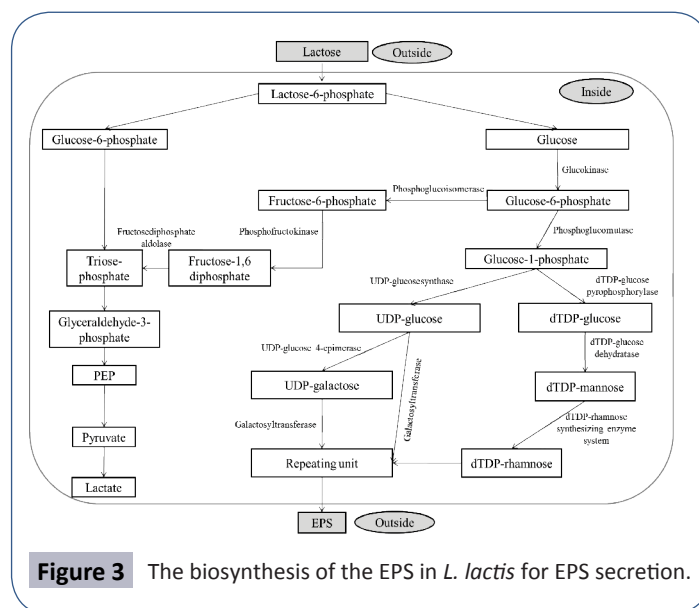


Figure 3 The biosynthesis of the EPS in *L. lactis* for EPS secretion.

Functional characteristics of viili

Viili and its isolated bacteria have been reported to possess several health benefits including anti-oxidation, ACE inhibitory, anti-tumor, and immunomodulatory activity.

Antioxidation activity: Endogenous metabolic processes and exogenous chemicals in the human body or in a food system are able to “in some circumstances” produce highly reactive oxygen species that are able to oxidize biomolecules, which results in tissue damage and cell death, thereby, leading to inflammation, diabetes, genotoxicity, cancer, and accelerated aging [32]. Viili made with viili bacteria have been demonstrated to contain α, α -diphenyl- β -piclylhydrazl, which has a free radical-scavenging effect and Fe^{2+} -chelating ability [2]. Viili EPS also possesses a strong antioxidant activity in the gastric intestine *in vivo* [33]. The antioxidative nature of viili and its products may help the human body to reduce oxidative damage. Several antioxidative peptides with free radical-scavenging activities have been identified in fermented dairy products [34-36]. Moreover, some studies have shown that certain *L. lactis* subsp. *cremoris* strains possess proteolytic abilities that aid the digestion of the milk protein into peptides and free amino acids [37,38]. The change in antioxidant activity noted in the low-fat cheeses made with viili is probably associated with the viable populations of LAB present in the cheese and the levels of antioxidative peptides.

Immunomodulatory activity: Viili and its LAB have been known to have immunoregulatory activities *in vitro* and *in vivo*, including antiallergic and anticolitis activities [39]. Viili and the viili bacterium *L. lactis* subsp. *cremoris* TL1 have been shown to induce the production of the helper cell type I (Th1) cytokine tumor necrosis factor- α , the proinflammatory cytokine interleukin (IL)-6, and T regulatory cell (Treg) cytokine IL-10 *in vitro*, which suggests that viili may be helpful in improving the Th1/Th2 balance. Oral feeding of the viili bacterium *L. lactis* subsp. *cremoris* TL1 has been shown to suppress total immunoglobulin IgE and ovalbumin (OVA)-specific IgE levels in the serum of OVA-

sensitized mice. Suppression of IgE production is an important target when treating allergies. Additionally, *in vivo* effects of the viili bacterium *L. lactis* subsp. *cremoris* TL1 on the regulation of dextran sulfate sodium (DSS) intestinal physiology have been demonstrated. This strain is able to ameliorate DSS-induced colitis as exemplified by a significant attenuation of the bleeding score and a reduction in colon shortening. Histological analysis also showed crypt regeneration and epithelial restitution in the colon among the animals in *L. lactis* subsp. *cremoris* TL1 treated group. These findings suggest that the viili isolated strain, *L. lactis* subsp. *cremoris* TL1, has a potential direct anti-inflammatory activity with respect to epithelial cells and that this may lead to inhibition of neutrophil accumulation in the mucosal region of the DSS-colitis mice.

ACE Inhibitory activity: ACE is known to associate with hypertension and congestive heart failure. Viili have been demonstrated to have a strong inhibitory effect on ACE activity [2]. The proteolytic activity of the starters and the rate of proteolysis both seem to play an important role in the inhibitory activity of these dairy products. The proteolytic system of *L. lactis* has been studied, which shows that the system consists of a cell wall-bound proteinase and several intracellular peptidases [37,38]. *L. Lactis* seems to possess high proteolytic activity, may produce a viili with better ACE inhibitory effect. Moreover, many studies have reported that the type and concentration of bioactive peptides present in a product are able to significantly affect the

functional properties of the dairy product [40,41]. ACE inhibitory peptides are released from α 1-casein and β -caseins and also from α -lactalbumin and β -lactoglobulin during fermentation of milk by various microbiological cultures.

Antitumor activity: Studies have identified that viili possesses antitumor activity. Kitazawa et al. [42] reported that viili and one of the starter bacteria, *L. lactis* subsp. *cremoris* KVS20, exhibited antitumor activity against the metastasis of Lewis lung carcinoma, as well as acting to reduce the growth of solid and ascetic forms of sarcoma-180 *in vivo*. The antitumor effect of viili might be due to increase in the cytotoxic activity of the macrophages stimulated by *L. lactis* subsp. *cremoris* KVS20. Liu et al. [33] observed the senescence of HepG2 cancer cells after treatment with viili EPS, which supports the idea that viili EPS might have anti-tumor activity

Conclusion

Viili is a traditional fermented milk product involving LAB, as well as a surface mould which makes the product unique in taste, aroma and appearance compared to all other Nordic fermented milks. Viili is healthy food product which has beneficial effects on gut health, in addition to treatment many diseases such as cancer, inflammation, diabetes, genotoxicity, accelerated ageing, hypertension and heart diseases. Mostly, this is due to its functional activities including antioxidant, immunomodulatory, ACE Inhibitory and antitumor activity.

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