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Viili as Fermented Food in Health and Disease Prevention: A Review Study

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ABSTRACT

Viili is a unique fermented milk product from Nordic countries, particularly popular in Finland. Viili contains a symbiotic cluster of viable abundant probiotics, including lacto bacteria, fungus and yeasts, has a pleasant sharp taste and good diacetyl aroma linked to a stringy texture and can be consumed easily with spoon because of its semi-solid structure. The slime-forming *Lactococcus lactis* subsp. *cremoris* in viili produce phosphate-containing hetero polysaccharides, one of the main characteristics that distinguish viili from other fermented milk products. The symbiotic characteristics of viili and its Exopolysaccharides (EPS) provide various benefits from promoting intestinal probiotics interaction to anti oxidative, anti-inflammation, immunomodulation and antitumor activities. In addition, the tripeptides Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP) from the fermentation inhibit Angiotensin-Converting Enzyme, which reduces the blood pressure and the incidents of cardiovascular diseases. All studies show that multi microbes fermented viili not only provides probiotics, also a large number of other beneficial factors to Nordic population, and possibly to other regions of population as well.

Key words: Viili, Fermentation, Exopolysaccharides, Probiotics, *Lactococcus lactis*, Fungus, Yeasts, Antioxidants, Immunomodulation and Anti-carcinogenic activity.

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INTRODUCTION

Viili is a unique fermented thick milk product without curds from Nordic countries, but it is believed to be originally from Sweden, however, now found in Finland, where it is largely considered as a national treasure. This domestic fermented milk is typically consumed at breakfast and is also a popular snack food among children and elders in Finland. The product is consumed alone or with cereals, muesli or fruit. A traditional way of eating viili is by mixing it with cinnamon and sugar (Leporanta, 2003). Its unique stickiness is due to phosphate- containing EPS produced by the slime-forming *Lactococcus lactis* subsp. *cremoris*. Viili EPS has been claimed to exhibit several health benefits including antioxidant, anti-inflammatory, anti-carcinogenic, anti-aging and immunomodulatory activities (Kitazawa et al., 1991, 1992, 1993). Viili was historically made on farms in large wooden buckets, and with cooking utensils at home. The industrial manufacture of viili began in Finland in the 1950s. Today, this product constitutes an important fermented milk product in Finland. Annual consumption stands at more than 4.5 kg/capita (Leporanta, 2003) even though the average consumption is actually declined because of wider range of milk products in market (Alatossava et al., 2013).

It is believed that the term viili originated from the Swedish word “fil” (Leporanta, 2003). However, the tradition and skill of making viili has been passed from generation to generation for many centuries. Viili is mainly consumed in Finland, Sweden, Denmark, and, in less extent, in Norway. Nowadays, there are different viili starter providers, most of them from U.S. Since viili is always a starter, and extremely easy to be cultured at home, there are quite a large number of viili lovers worldwide. In the last several years we have studied macrophage RAW 264.7 cells to observe phagocytosis, the release of NO, and the gene expression of iNOS, IL-6

Figure 1. Commercial viili, A: viili made from whole milk, B: Structure and consistency of semi-solid viili, with the permission of Valio Limited.

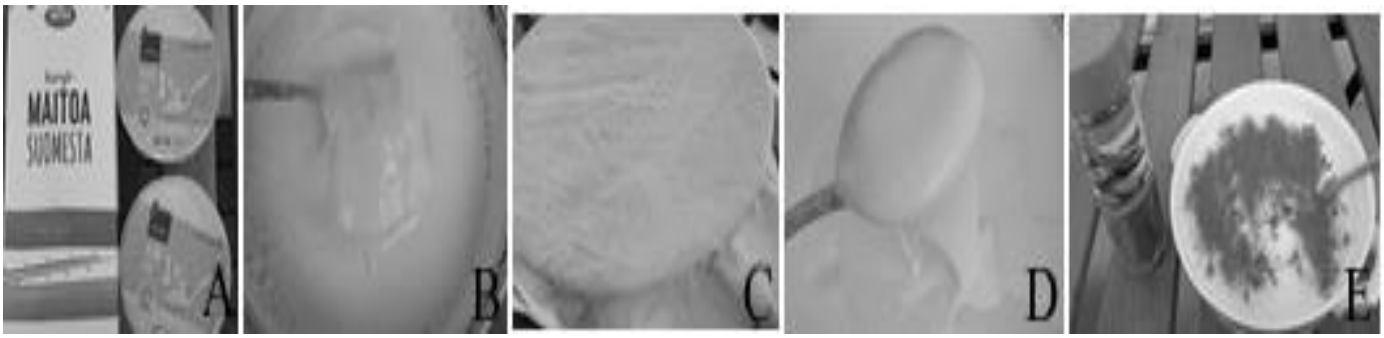

Figure 2. Viili production at home. A: starter of viili and the milk. B: Approximately 10 to 20% inoculation of viili to pasteurized milk (V/V). C: Fermentation at Room temperature for 16 to 24 h, the container need to be covered with 2 to 3 layers of textile and stand at 20 to 30°C. D: An ready viili should contain ropy polysaccharides, should also form a velvet surface layer if whole milk was applied fermented viili. E: One of the way to comsume viili with sugar and cinnamon (Leporanta, 2003).

and IL-1β as well as the morphology change at presence of viili exopolysaccharides (VEPS). We also studied VEPS influence on tumor antigen MAGEA10 and inflammatory COX-2 genes expression in cancer cells to investigate the potential possible roles of VEPS in human immunity. To understand the complex multiple mechanisms of viili in the daily health and diseases prevention, we further review our work and analyze different approaches for possible immune therapy.

VIILI PREPARATION

For the commercial industrial production of viili (Figure 1) the milk is separated and standardized to a fat content in the range of 1.0 to 3.5%. Traditionally, viili has been made from non-homogenized milk, which is the reason why a thicker layer of cream forms on its surface. After standardization, milk is pasteurized and cooled to 20°C. A mixed culture of *L. lactis* sub sp. *cremoris*, *L. lactis* sub sp. *lactis*, and *G. candidum* is then inoculated and mixed. The mixture is then packed in a cup and to an incubator for ripening, where viili fermentation is conducted for 20 h at 20°C. After fermentation, viili is cooled (6°C) throughout the transportation and sale shelf. Viili has a stringy/ropy texture at optimal temperature of 28°C. The shelf life of viili is around 3 weeks. There are a wide range of different types of viili on the market, including products with different fat contents, with reduced lactose and flavoured varieties. Viili is also made commercially from homogenized milk without mould growing on the surface and from milk other than cow's milk, but traditional viili is still the most popular in the Finnish region (Leporanta, 2003). However, the consumption of viili has been variable and versatile by multiple food cultures in Figure 2. Commercial viili carries living microorganisms, that make it easy for anyone to reproduce anywhere, at home, farm, or industry scale without infiltration. This procedure is not possible with almost all other yogurt, because they do not carry living microorganisms or they are protected by a patent.

VIILI AS A SOURCE OF PROBIOTICS

Probiotics are microorganisms that are believed to provide health benefits when consumed. Introduction of the concept is generally attributed to the Nobel Prize Élie

Metchnikoff, who in 1907 suggested that the dependence of the intestinal microorganisms on the food makes it possible to adopt measures to modify the intestinal microbiota and to replace the harmful microorganisms by the useful ones. In addition, probiotics secrete a large number of vitamins, or effective peptides. However, the profile of probiotics for each individual was actually established in a quite early age (Bäckhed et al., 2015). Normally probiotics in viili are bacteria, fungus and yeasts that provide positive health benefits, especially for digestive system. Viili presents high population of symbiotic bacteria, yeasts and moulds that act synergically. Viili contains several lactic acid bacteria (LAB) including *L. lactis* sub sp. *cremoris, L. lactis* sub sp. *Lactis* biovar. *diacetylactis*, and *Leuconostoc mesenteries* subsp. *cremoris*. Among them, the slime-forming *L. lactis* subsp. *cremoris* produces a characteristic phosphate-containing heteropolysacharide or EPS. These LAB strains are also responsible for lactic acid fermentation, as well as citrate-based aroma formation. In addition, traditional viili cultures also contain yeast strains such as *Kluveromycesmarxianus* and *Pichia fermentans*. Because milk mainly consists of casein protein, or peptides, so the role of yeasts in viili fermentation is minimum, but in the end of fermentation, or as the lactose or glucose increase, small amount of ethanol can be produced by yeast, this is particularly the case for overdue viili, which can be smelled by olfactory system. However, the effect of the yeasts on the viili as symbiosis is not clear, it is probably that CO_2 by the fermentation of yeast may act as growth factors for synergistic effects beside the roles of stripping H_2S (Butzke and Park, 2011), this is particularly the case for yogurt production.

In addition, it is also believed that yeasts in viili may provide the product's unique flavour and induce the LAB to produce more EPS (Wang et al., 2008). However, yeast is frequently excluded in today's viili industry to keep alcohol free (personal communication with Kalle Leporanta, 2016).

Viili contains at least one kind of fungus: *Geotrichum candidum.* White filamentous yeast- like mould, which forms a velvet-like surface at the end of viili fermentation. *G. candidum*is a pathogen of many plants, but it is well known for its role as a probiotic microorganism present in viili and cheese fermentation processes. It can grow at temperatures ranging from 5 to 38°C, with an optimal growth at around 25°C, and at a large pH interval from 3 to 11, with an optimal pH value at 5.0 to 5.5. Besides the long lag phase, the generation time of *G. candidum* is 66 min in liquid culture at 30°C, being one of the shortest among eukaryotes, with final counts lower than 10^6 thallus forming units (tfu)/g (Boutrou and Gueguen, 2005; Hudevoca et al., 2009). *G. candidum* creates a velvet-like creamy surface on viili and it is also involved in the ripening process of various cheeses. Typically during the manufacture of viili, the fermentation takes place in the package and lasts about 18 to 20 h at 18 to 20°C (Roginski, 2002).

COMPOSITION AND BASIC STRUCTURE OF EPS OF VIILI

The bacteria strains used for viili manufacturing produce EPS which give viili a ropey, gelatinous consistency and a pleasantly mild taste resulting from lactic acid fermentation (Fondén et al., 2006). *L. lactis* subsp. *Cremoris* in viili produces phosphate-containing hetero polysaccharides, named viilian. The composition of the EPS secreted by *L. lactis*s subsp. *Cremoris* consists of 3 to 47% protein and 29 to 85% carbohydrates (Macura and Twonsley, 1984; Nakajima et al., 1990). Viili EPS has a molecular weight of about 2000 kDa and it is mainly composed of D-glucose, D-galactose, L-rhamnose, and phosphate, with a repeating unit of "→4-β- Glcp-(1→4)-β-D-Galp (1→4)-β-D-Glcp-(1→", and groups of α-L-Rhap and α -D-Galp-1-p attached to each side of Galp (Nakajima et al., 1990; 1992, Higashimura et al., 2000; Sletmoen et al., 2003). The composition and sugar components of EPS are sub-strains 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 (Nakajima et al., 1990; Yang et al., 1999). In contrast, the EPS produced by *L. lactis* subsp. *Cremoris* H414 is a homopolymer consisting of galactose with a branched-pentasaccharide repeating unit (Gruter et al., 1992). Marshall et al. (1995) reported that *L. lactis* subsp. *Cremoris* strain LC33 was able to generate two different EPSs. One of them 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 (Yang et al., 1999). Additionally, *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 (Looijesteijn et al., 1999).

EPS CHARACTERIZATION AND FUNCTION

There are different enzymes involved in EPS formation by *L. lactis* subsp. *Cremoris* suchasthose responsible for carbohydrate metabolism, enzymes leading to sugar nucleotide synthesis and interconversion,

glycosyltransferases 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 are placed in an EPS plasmid in *Lc. lactis* subsp. *cremoris*. The gene products *EpsD*, *EpsE*, *EpsF*, and *EpsG*areglycosyl transferases and are required for the synthesis of the EPS backbone (Van Kranenburg et al., 1997, 1999). 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 batch inoculations of starter culture (Vedamuthu and Neville, 1986; Cerning et al., 1992). It has been shown that the slime-forming capacity is stable when the bacteria are grown at17°C but is lost when they are grown at >30°C(Forsén et al.,1973).

HEATH EFFECTS OF VIILI

Besides its high nutritional value, viili exhibits positive benefits for human health. Viili contains high populations of different probiotic bacteria (10^8/mL), antihypertensive effects as well as anti-inflammatory, antioxidant and anti-carcinogenic activities associated to the presence of viili. Hence, viili can be considered a traditional natural functional food. It has been shown that viili EPS help the interaction with intestine mucusa (Ruas-Madiedo et al., 2006), but it acts in symbiosis with other microorganism in viili, multiple metabolites in the same life chain, which make it safe to consume. The precise mechanism is unknown, but the risk of developing an infection due to "pathogen" of *G. candidum* in connection with its technological use and consumption of dairy products has been virtually nil because fewer than 100 cases reported only between year 1842 to 2006 (Pottier et al., 2007).

ANTIOXIDATIVE EFFECTS OF VIILI

Many dietary compounds are known to have health benefits owing to their antioxidative and anti-inflammatory properties. In addition to the antioxidant diets, an intracellular Nrf2/ARE antioxidant pathway has also been established. That is, there is possible an association of intracellular anti-ROS's activation via Nrf2/ARE with the hypoxia, inflammation as well as the promotion of cell migration and invasion in cellular micro environment, and extracellular antioxidant's interaction may remain cell health by balancing the oxidative state (Luo et al., 2011). Viili and its isolated bacteria have been reported to possess several health benefits including anti-oxidative effects (Wang et al., 2014). Endogenous metabolic processes and exogenous chemicals in the human body or in a food system are able to produce, in some circumstances, highly reactive oxygen species that are able to oxidize biomolecules, resulting in tissue damage and cell death. This may lead to inflammation, diabetes, genotoxicity, cancer, and accelerated aging (Biswas et al., 2010).

In a recent study that showed the antioxidant capacity of ursolic acid (UA) increased with concentration from 0 to 100 µg/ml in a dose dependent manner, while antioxidant power of viili EPS (VEPS) was milder, but clear synergic interaction was observed from 0 to 50 µg/ml of both compounds. Significant anti-proliferation of HepG2 cells with UA and viili EPS at concentration of 200 µg/ml by MTT assay was observed, however, the cells started sene scences at 12.5 µg/ml, which indicated multiple molecular modulation mechanisms rather than an induction of apoptosis or necrosis. All these tests have shown a compatibility of herbal UA and foodborne viili EPS, which means they can be either processed together for health products or functional food because viili EPS is an antioxidant synergic with ursolic acid (Liu et al., 2012). In addition to viili EPS, viili bacteria have been demonstrated to contain α,α-diphenyl-β-picylhydrazl, which has a free radical-scavenging effect and Fe^{2+}-chelating ability (Chiang et al., 2011). The anti-oxidative nature of viili and its products may help the human body to reduce oxidative damage. Since milk proteins are precursors of many different biologically active compounds, some peptides with free radical-scavenging activities have been identified in fermented dairy. Viili's proteolytic abilities that aid the digestion of the milk protein into peptides and free amino acids were characterized in early 1990s (Tan and Konings, 1990; Alting et al., 1995). The change in antioxidant activity noted in low-fat cheeses made with viili is probably also associated with the viable populations of LAB plus the anti-oxidative peptides.

ANGIOTENSIN I-CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY

ACE (EC 3.4.15.1) is known to be associated with hypertension and congestive heart failure. The enzyme converts angiotensin I into angiotensin II, the former being an inert peptide and the latter being a pressor agent. The enzyme is also responsible for the breakdown of bradykinin, which is a dilatory peptide. The enzyme is thus an obvious drug target for the treatment of certain cardiovascular diseases, including hypertension. LAB is known to produce inhibitors of the enzyme during fermentation. Viili containing *L. lactis* subsp. *Cremoris* strain has been demonstrated to have a strong inhibitory effect on ACE activity (Chiang et al., 2011). The proteolytic activity of the starters and the rate of proteolysis seem to play an important role in the inhibitory activity of these dairy products. The proteolytic system of *L. lactis* has been studied, and it consists of a cell

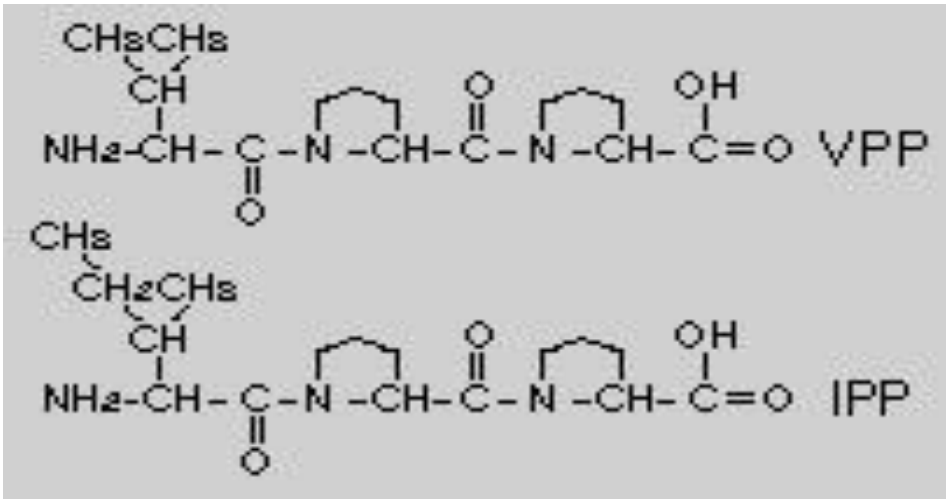

Figure 3. Tripeptides from viili and many other fermented milk products (Turpeinen et al., 2009).

wall bound proteinase and several intracellular peptidases (Tan and Konings, 1990; Alting et al., 1995). Since the high proteolytic activity, viili, as fermented milk product demonstrates a better ACE inhibitory effects comparing to other fermented milk products. Research has mainly focused on IPP + VPP from viili, two lacto tripeptides that can inhibit the ACE *in vitro*. In both Finnish and Japanese subjects with (mild) hypertension, IPP and VPP in Figure 3 showed their inhibition on ACE and reduced diastolic blood pressure (DBP) and systolic blood pressure (SBP) by 7 to 14 mmHg, which is a statistically significant reduction (Hata et al., 1996; Pins and Keena, 2006). Moreover, many studies have reported that the types and concentrations of bioactive peptides are able to significantly affect the functional properties of the dairy product (Ong and Shah, 2008a, b).

IMMUNOMODULATORY EFFECTS

Viili and its LAB have been demonstrated to have immune regulatory effects *in vitro* and *in vivo*, including anti-allergic effects and anti-colitis effects (Huang et al., 2010). 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 beneficial and improve the Th1/Th2 balance. Oral feeding of *Lc*. *lactis* subsp. *Cremoris* TL1 contained in viili 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 intestinal physiology have been demonstrated. This strain is able to ameliorate dextran sulfate sodium (DSS)-induced colitis as exemplified by a significant attenuation of the bleeding score and a reduction in colon shortening. Histological analysis also showed regeneration and epithelial restitution in the colon among the animals included in the *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 effect may lead to inhibition of neutrophil accumulation in the mucosal region of the DSS-colitis mice. It is widely known that the toll-like receptor (TLR) family plays an important role in host defense through recognizing bacterial pathogen-associated molecular patterns that engages anti-inflammatory processes, that is why colitis caused uncomfortability can be minimized because the inflammation is kept to a minimum. The immune regulatory effects of viili probiotic LAB has been investigated in a swine study, showing an intestinal immune regulation by probiotic LAB mediated by the TLR in the gut, which is similar to TLR signalling transduction pathways through the molecular mechanisms of the ligands of lipopolysaccharides (LPS) of *Escherichia coli* in human (Tohno et al., 2007, 2008). Recently such TLR family protein was defined as RP105/MD1 complex that is involved in the immunoregulatory effect of EPS from *Lactobacillus plantarum* N14 (Murofushi et al., 2015). By studying the effects of VEPS in RAW 264.7 macrophages the phagocytosis was observed by scanning electronic microscopy (SEM), the titer of NO, IL-6, and IL-1β was increased by ELISA, the gene expression of IL-6 and IL-1β was enhanced by RT-PCR, the gene and protein expression of inducible nitric oxide synthase (iNOS) were also increased by RT-PCR and Western blotting, which suggest the potential possible roles of VEPS in human immunity (Figure 4). The cytokines' induction indicates a

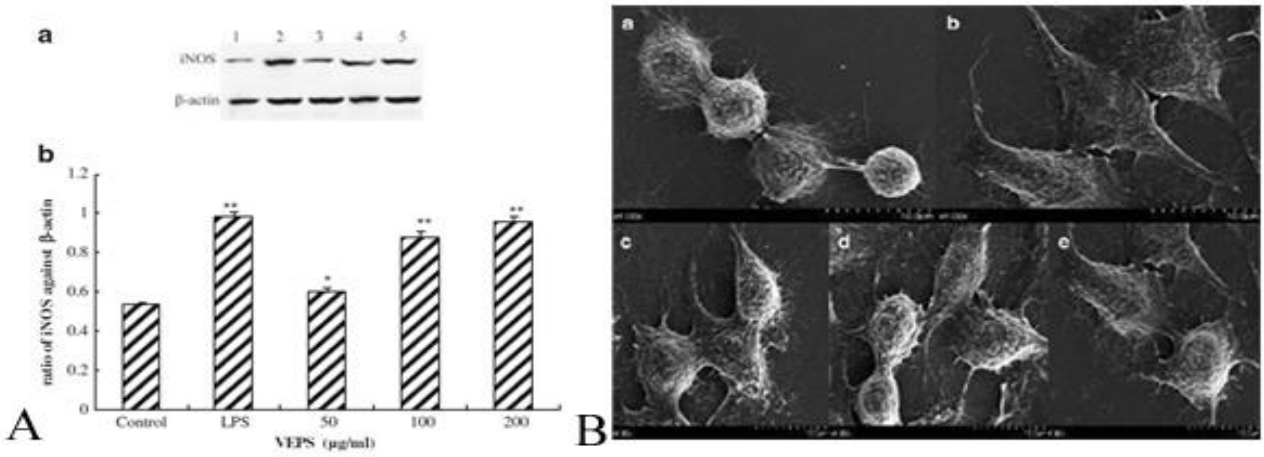

Figure 4. Viili EPS influence on macrophage RAW 264.7 cells (Wu et al., 2013)

possibility of boosting humoral immunity via T-helper cells (Wu et al., 2013). A: Protein expression of iNOS in macrophage RAW264.7 cell with different concentrations of VEPS by Western blotting. B: Cell morphology of RAW264.7 under stimulation of viili EPS and LPS by SEM. a) Control, b) 1 μg/mL of LPS, c–e) with 50, 100, and 200 μg/mL of viili EPS, respectively. Original magnification was × 4,000, the original scale of SEM represents 10 μm in each image (Wu et al., 2013).

TUMOR IMMUNOTHERAPY PROMOTED BY VIILI EPS

Immunotherapy is a kind of treatment of disease by inducing, enhancing, or suppressing an immune response through a unique matching HLA typing epitope, or nonspecific antigenic epitope. Immunotherapies designed to elicit or amplify an immune response are classified as activation immunotherapies, while those that reduce or suppress the immune response are classified as suppression immunotherapies. MAGE-A antigens belong to a cluster of cancer/testis antigens (CTA) that are expressed in tumors but not in normal tissues with the exception of testis and placenta. Among MAGE-A antigens, MAGEA10 represents an attractive target for cancer immunotherapy because its epitopes extensively elicit cytotoxic T - lymphocyte responses. However, the suppressive cyto environment for gene expression and the requirement of specific HLA-alleles presentation have frequently led to immunotherapy failure. By studying the genes expression and methylation of MAGE genes, MAGEA10 was scarcely expressed in cancer patients, but it has been recently shown that its expression can be enhanced by viili polysaccharides, which indicate a possibility of increasing epitopes presentation Figure 5.

ANTI TUMORAL IMMUNOTHERAPY

A few studies have reported the antitumor activities of viili Kitazawa et al. (1992) reported that viili and one of the starter bacteria, *L. lactis* subsp. *Cremoris* KVS20, inhibited the metastasis of Lewis lung carcinoma and reduced the growth of solid and ascetic forms of sarcoma-180 *in vivo*. The antitumor effect of viili might be due to the increase of cytotoxic activity of the macrophages stimulated by *L. lactis* subsp. *Cremoris* KVS20. Liu et al. (2012) observed the senescence of HepG2 cancer cells after treatment with viili EPS, which supports the idea that viili EPS might have anti-tumor activity (Figure 6). Moreover, villi EPS induced cancer cell apoptosis via inhibition of COX-2 gene and protein expression (Liu et al., 2014). However, comparing to other polysaccharides, such as coix polysaccharides, the anti-oxidative power of villi EPS was less competent, and the induction of antioxidation dependent apoptosis was less sensitive (Liu et al., 2014; Wang et al., 2014). On March 25, 2011, the U. S. Food and Drug Administration (USFDA) approved ipilimumab injection (Yervoy, made by Bristol-Myers Squibb Company) for the treatment of unrespectable or metastatic melanoma. Ipilimumab is a monoclonal antibody that activates the immune system by targeting CTLA-4, a protein receptor that down regulates the immune system, thus activating cytotoxic T lymphocytes (CTL), which increase the opportunities for fighting against cancer cells.

In a recent study it was shown that viili polysaccharides were able to induce cancer antigen MAGEA10's gene and protein expression, which gives a higher opportunity for immunotherapy because over expression of cancer antigen will increase the CTL opportunity to destroy the cancer cells (Wang et al., 2015). Increasing cancer antigen expression and presentation is possible to increase the opportunities of CTL mediated cytotoxicity against cancer cells (Figure 7). Cancer antigen (epitope) presented by type 1 of MHC (HLA-A) could be recognized by TCR of CTL, and eventually led to cancer cell destruction. The right inserted circle image featured

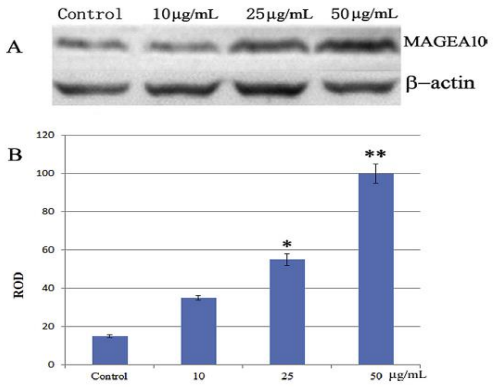

Figure 5. Enhancement of MAGEA 10 protein expression of A549 cells under influence of villi EPS by Western blotting (Wang et al., 2015).

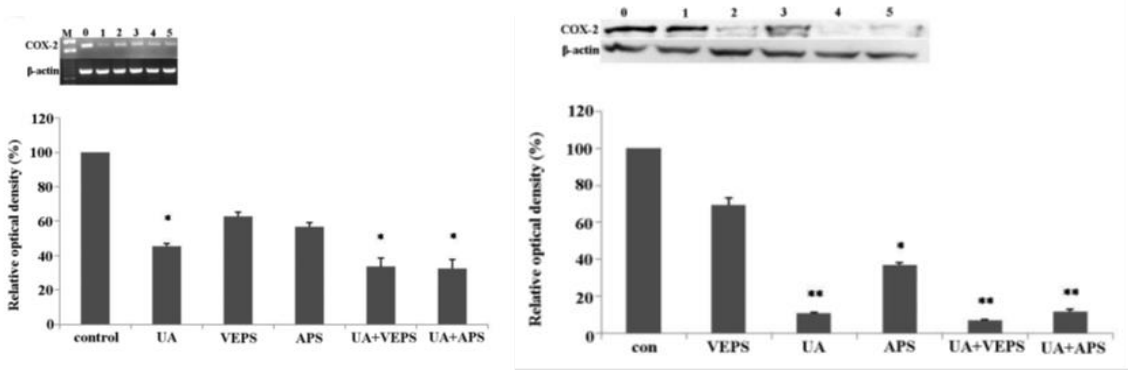

Figure 6. Inhibitory effects of different compounds on (COX)-2 expression in HepG2 cells. HepG2 cells were treated with doses of UA (5 μg/ml), villi EPS (50 μg/ml), Astragalus polysaccharides (APS) (50 μg/ml) or combined treatments for 48 h. (Left): RT-PCR analysis of COX-2 genes. Lane 0, control; lane 1, UA (5 μg/ml); lane 2, VEPS (50 μg/ml); lane 3, APS (50 μg/ml), lane 4, UA+VEPS; lane 5, UA+APS for 48 h. (Right): Quantitative analysis of protein levels with the same samples. *P<0.05, **P<0.01, compared with the control group (Liu et al., 2014).

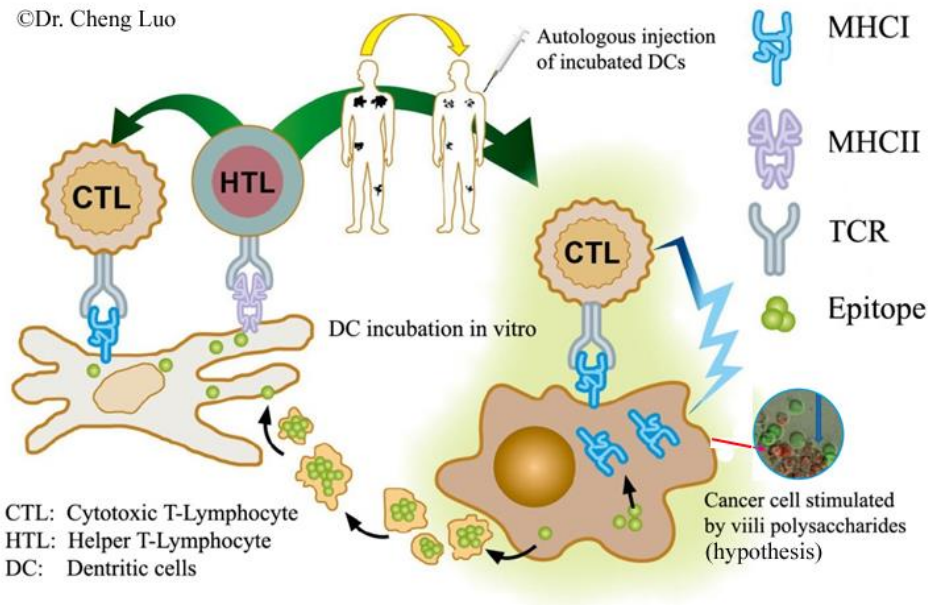

Figure 7. Autologous / non autologous immunotherapy with CTL activator (such as ipilimumab) and cancer antigen presenting promoter (such as viili polysaccharides).

the MAGEA3 pulsed cancer cells (red) were attacked by corresponding specific CTL, where non pulsed cancer cells (green) kept intact (Wang et al., 2014)

Conclusion

The functional health roles from different cultural foods, such as fermented viili, have been widely recognized. Foodborne polysaccharides VEPS possess anti-inflammation and anticancer properties, particularly by down regulating Interleukins and COX-2 expression, which may have increased internal oxidation and triggered apoptosis together with a change in internal antioxidant response elements, leading to a reduction in cell proliferation. These specific mechanisms need to be further studied.

REFERENCES

Alatossava T, Li R, Munsch-Alatossava P (2013). From “Viili” Towards “Termoviili”, a Novel Type of Fermented Milk: Characterization of Growth Conditions and Factors for a co-culture of *Lactobacillus delbrueckii* and *Geotrichumcandidum* . J. Food Process. Beverages. 1(2): 8

Alting RC, Engels WJM, Schalkwijk S, Exterkate FA (1995). Purification and characterization of cystathionineβ-lyase from *Lactococcuslactis*subsp. *Cremoris* B78 and its possible role in flavor development in cheese. Appl. Environ. Microbiol., 61:4037-42.

Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J (2015).Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell. Host Microbe.,17 (5): 690-703.

Biswas M, Haldar PK. and Ghosh AK (2010). Antioxidant and free-radicalscavenging effects of fruits of *Dregeavolubilis*.J. Natural Sci. Biol. Med., 1(1): 29-34.

Boutrou R, Guéguen M (2005). Interest in *Geotrichumcandidum*for cheese technology.Int. J. Food Microbiol., 102:1-20.

Butzke CE, Park SK (2011). Impact of fermentation rate changes on potential hydrogen sulfide concentrations in wine. J. Microbiol. Biotechnol., 21(5): 519-24.

Cerning J, Bouillanne C, Landon M, Desmazeaud M (1992). Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria.J. Dairy Sci., 75: 692-699.

Chiang ML, Chen HC, Wang SY, Hsieh YL, Chen MJ (2011). Use of Taiwanese ropy fermented milk (TRFM) and *Lactococcuslactis*subsp. *cremoris*isolated from TRFM in manufacturing of functional low-fat cheeses. J. Food Sci., 76: M504-M510.

Fondén R, Leporanta K, Svensson U (2006). Nordic/Scandinavian Fermented Milk Products, in Fermented Milks (ed A. Tamime), Blackwell Publishing Ltd, Oxford, UK. doi: 10.1002/9780470995501.ch7

Forsén R, Raunio V, Myllymaa R, Nousiainen R, and Päkkilä M (1973). Studies on the slime forming group N *Streptococcus* strains. I. Differentiation between some lactic streptococcusstrains by polyacrylamide gel electrophoresisof soluble cell proteins. Acta Universitatis Ouluensis: Scientiae Rerum Naturalium, 3: l.

Gruter M, Leeflang BR, Kuiper J, Kamerling JP, Vliegenthart JFG (1992). Structure of the exopolysaccharide produced by *Lactococcuslactis*subsp. *cremoris*H414 grown in a defined mediumor skimmed milk. Carbohydr. Res., 231: 273-291.

Hata Y, Yamamoto M, Ohni M, Nakajima K, Nakamura Y, Takano T (1996). A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. Am. J. Clin.Nutr., 64(5): 767-71.

Higashimura M, Mulder-Bosman BM, Reich R, Iwasaki T. and Robijn GW (2000). Solution properties of viilian, theexopolysaccharide from Lactococcuslactis subsp. cremoris SBT0495. Biopolymers, 54: 143-158.

Huang IN, Dai TY, Wang SY, Chen MJ (2010). Inhibitory effect of Taiwanese ropy fermented milk in an ovalbumin-induced allergy mouse model. J.Dairy Sci.,93 S(1): 807.

Hudevoca A, Valik L, Liptakova D (2009). Quantification of Geotrichumcandidum growth in co-culture with lactic acid bacteria. Czech J. Food Sci., 27: 8-27.

Kitazawa H, Toba T, Itoh T, Kumano N, Adachi S, Yamaguchi T (1991). Antitumoral activity of slime-forming, encapsulated *Lactococcuslactis*sssp. *Cremoris*isolated from Scandinavian ropy sour milk viili. Anim. Sci. Technol.,62: 277-283.

Kitazawa H, Yamaguchi T, Miura M, Saito T. and Itoh T (1993). B-cell mitogen produced by slime-forming encapsulated *Lactococcuslactis*s sp. *Cremoris* isolated from ropy sour milk, viili. J.Dairy Sci.,76: 1514-1519.

Kitazawa H, Yamaguchi T. and Itoh T (1992). B-Cell Mitogenic activity of slime products produced from slime-forming, encapsulated *Lactococcuslactis*sssp. *cremoris*. J. Dairy Sci.,75: 2946-2951.

Leporanta K (2003).Viili and Långfil-Exotic fermented products from Scandinavia. Valio Foods & Functionals. On line versión. www.valio.fi. (Access June 8, 2010).

Ling Liu, Jingkai Z, Meiling Li, Xiaohong Z, Jinlu Z, Zhenjing L, Likui W, Jihui W, Cheng L (2014). Inhibition of HepG2 cell proliferation by ursolic acid and polysaccharides via the downregulation of cyclooxygenase-2. Mol. Med. Rep.,9(6): 2505-2511.

Liu L, Wu J, Zhang J, Li Z, Wang C, Chen M, Wang Y, Sun Y, Wang L. and Luo C (2012).A compatibility assay of ursolic acid and foodborne microbial exopolysaccharides by antioxidant power and anti-proliferative properties in hepatocarcinoma cells. J. Food, Agric. Environ., 10: 111-114.

Luo C, Urgard E, Vooder T, Metspalu A (2011). The role of COX-2 and Nrf2/ARE in anti-inflammation and antioxidative stress: Aging and anti-aging. Med. Hypotheses, 77(2):174-178 .

Macura D, Townsley PM (1984). Scandinavian ropy milk-identification and characterization of endogenous ropy lactic streptococci and their extracellular excretion. J. Dairy Sci., 67: 735.

Marshall VM, Cowie EN, Moreton RS (1995). Analysisand production of two exopolysaccharides from *Lactococcuslactis* sub sp. *Cremoris* LC330. J. Dairy Res., 62: 621-628.

Murofushi Y, Villena J, Morie K, Kanmani P, Tohno M, Shimazu T, Aso H, Suda Y, Hashiguchi K, Saito T, Kitazawa H (2015). The toll-like receptor family protein RP105/MD1 complex is involved in the immunoregulatory effect of exopolysaccharides from Lactobacillus plantarum N14. Mol. Immunol., 64(1): 63-75.

Nakajima H, Suzuki Y, Kaizu H and Hirota T (1992). Cholesterol lowering activity of ropy fermented milk. J. Food Sci., 57: 1327-1329.

Nakajima H, Toyoda S, Toba T, Itoh T, Mukai T, Kitazwa H, Adachi S (1990). A novel phosphophlysaccharide from slime-forming *Lactococcuslactis* sub sp. *Cremoris* SBT 0495. J. Dairy Sci., 73: 1472-1477.

Ong L, Shah NP (2008a). Influence of probiotic *Lactobacillus acidophilus* and *L. helveticus*on proteolysis, organic acid profiles, and ACE-inhibitory activity of Cheddar cheeses ripened at 4, 8, and 12°C. J. Food Sci., 73: M111-M120.

Ong L, Shah NP (2008b). Release and identification of angiotensin-converting enzyme inhibitory peptides as influenced by ripening temperatures and probiotic adjuncts in Cheddar cheeses. LWT Food Sci. Technol., 41: 1555-1566.

Pins JJ, Keenan JM (2006). Effects of whey peptides on cardiovascular disease risk factors.J. Clin. Hypertens., (Greenwich). 8(11): 775-82.

Roginski H (2002) Fermented milk: Northern Europe. Encyclopedia of Dairy Sci., 2: 1034-1041, Academic Press.

Ruas-Madiedo P, Gueimonde M, de los Reyes-Gavilán GC, Salminen S

(2006). Short communication: Effect of exopolysaccharide isolated from “viili” on the adhesion of probiotics and pathogens to intestinal mucus. J. Dairy Sci., 89: 2355-2358.
Sletmoen M, Maurstad G, Sikorski P, Paulsen BS, Stokke BT (2003). Characterization of bacterial polysaccharides: Steps towards single molecular studies. Carbohydr. Res., 338: 2459-2475.
Tan PST, Konings WN (1990). Purification and characterization of an aminopeptidase from *Lactococcuslactis* sub sp. *Cremoris* Wg2. Appl. Environ. Microbiol., 56: 526-532.
Tohno M, Shimazu T, Aso H, Kawai Y, Saito T, Kitazawa H (2007). Molecular cloning and functional characterization of porcine MyD88 essential for TLR signaling. Cell. Mol. Immunol., 4(5): 369-76
Tohno M, Shimazu T, Ueda W, Anzawa D, Aso H, Nishimura J, Kawai Y, Saito Y, Saito T, Kitazawa H (2007). Molecular cloning of porcine RP105/MD-1 involved in recognition of extracellular phosphopolysaccharides from Lactococcuslactisssp. cremoris. Mol. Immunol., 44: 2566-2577.
Tohno M, Ueda W, Azuma Y, Shimazu T, Katoh S, Wang JM, Aso H, Takada H, Kawai Y, Saito T, Kitazawa H (2008). Molecular cloning and functional characterization of porcine nucleotide-binding oligomerization domain-2 (NOD2). Mol. Immunol., 45(1): 194-203.
Van Kranenburg R, Marugg JD, Van Swam II, Willem J, DeVos WM (1997). Molecular characterization of the plasmid-encoded *eps* gene cluster essential for exopolysaccharide biosynthesis in *Lactococcuslactis*. Mol. Microbiol., 24: 387-397.
Van Kranenburg, R, Van Swam II, Marugg JD, Kleerebezem M, De Vos, WM (1999). Exopolysaccharide biosynthesis in *Lactococcuslactis* NIZO B40: Functional analysis of the glycosyltransferase genes involved in synthesis of the polysaccharide backbone. J. Bacteriol.,181: 338-340.
Vedamuthu ER, Neville JM (1986). Involvement of a plasmid in production of ropiness (mucoidness) in milk cultures by *Streprococcuscremoris* MS. Appl. Environ. Microbiol., 51: 677.
Wang L, Gao S, Jiang W, Luo C, Xu M, Bohlin L, Rosendahl M, Huang W (2014). Antioxidative Dietary Compounds Modulate Gene Expression Associated with Apoptosis, DNA Repair, Inhibition of Cell Proliferation and Migration. Int. J. Mol. Sci., 15: 16226-16245.
Wang L, XuY, Luo C, Sun J, Zhang J, Lee M-W, Bai A, Chen G, M. Frenz C.M., Huang W (2015). MAGEA10 gene expression in non small cell lung cancer and A549 cells, and the affinity of epitopes with the complex of HLA-A*0201 allel，Cell. Immunol.,297(1): 10-18.
Wang SY, Chen HC, Liu JR, Lin YC.and Chen MJ (2008). Identification of yeasts and evaluation of their distribution in Taiwanese kefir and viili starters.J. Dairy Sci., 91: 3798-3805.
Wu J, Li M, Liu L, An Q, Zhang J, Zhang J, Li M, Duan W, Liu D, Li Z, Luo C (2013). Nitric Oxide and Interleukins are Involved in Cell Proliferation of RAW264.7 Macrophages Activated by Viili Exopolysacchrides. Inflammation, 36 (4): 954-961.
Yang Z, Huttunen E, Staaf M, Widmalm G, Tenhu H (1999). Separation, purification and characterisation of extracellular polysaccharides produced by slime-forming *Lactococcuslactis*s sp. *Cremoris* strains. Int. Dairy J., 9: 631-638.