Lactobacillus plantarum TENSIA® DSM 21380 – an antimicrobial and antihypertensive probiotic

The Harmony® cheese comprising *L. plantarum* TENSIA® helps to maintain heart health through lowering (high-normal) blood pressure

Western lifestyle and nutritional patterns on one hand and longevity / aging of the population associated with age-related health conditions on the other hand have created a situation, where chronic illnesses and health problems represent the most serious threat to public health. Rising healthcare costs and treatment of diseases is becoming critically exhausting the resources and being a burden to society even in highly developed countries.

All over the world, cardiovascular disease (CVD) remains one of the leading causes of death (Nichols *et al.* 2012). The prevalence of death due to elevated blood pressure is higher for coronary events than for stroke. Besides, it has been demonstrated that cardiovascular morbidity and mortality are related to both systolic and diastolic blood pressures. Overall, the prevalence of hypertension appears to be around 30–45% of the general population, with a steep increase with ageing (Mancia*et al.* 2013). However, long term exposure to elevated blood pressure in younger subjects may lead to a high and partly irreversible risk situation in middle age, with potential shortening of their otherwise longer life expectancy.

Elevated blood pressure can compromise the normal function of arteries and has a negative effect on vitality of organs. The elevation of blood pressure affects especially heart, brain and kidneys leading to increased risk of heart attack, stroke, and kidney failure. It has been shown that individuals at systolic blood pressure levels between 130 and 139 mmHg or diastolic levels of between 85 and 89 mmHg have a 1.5 to 2.5 times greater risk of experiencing a heart attack, a stroke, or heart failure in 10 years than those whose blood pressure level is below 120/80 mmHg (Vasan *et al.* 2001).

Thus, innovative science-based preventive solutions have become essential. Synergy between researchers and food producing companies is able to generate innovative health-supporting products that help to reduce disease risks.

Bio-Competence Centre of Healthy Dairy Products LLC (BioCC) owns *Lactobacillus plantarum* strain TENSIA®, a multipotent microbe of a healthy child origin with the ability to produce compounds possessing direct or indirect blood pressure lowering effect, antimicrobial activity against food borne pathogens and expressing moderate anitoxidative activity.

Functional properties of *Lactobacillus plantarum* TENSIA®:

- Production of ACE inhibitory compounds, bioactive peptides and other compounds possessing direct or indirect blood pressure lowering effect
- Antimicrobial activity e.g. listeria, salmonella, shigella
- Production of antimicrobial compounds and harboring plantaricin – encoding genes
- In food reduces the risk of food born infections by suppressing potentially pathogenic bacteria
- Supplying the human body with *Lactobacillus plantarum* strain TENSIA®, which enhances the body's natural resistance to enteropahtogens
- Increases the amount of useful lactobacilli in the gut, which ensures breakdown of the nutrients into more easily absorbable compounds
- Production of conjugated linolenic acid (CLA)
- Production of polyamines (putrescine, spermidine)
- Production of nitric oxide (NO)

In order to employ the effects of the strain for the benefit of human health, a biotechnology solution for incorporating this lactobacillus into Edam-type cheese was developed due to the cooperation between BioCC and E-piim production Ldt (Estonia). As a result, a new functional food product, Harmony® cheese comprising *L. plantarum* TENSIA® in the amount of ca 5×10^{7} viable cells / 1g was created.

Our research proves that regular, at least for 8 week consumption of 50g/day probiotic *Lactobacillus plantarum* TENSIA® comprising Harmony® cheese brand helps to maintain the cardio-vascular system / heart health through reduction of blood pressure.

The consumption of Harmony® cheese comprising *Lactobacillus plantarum* TENSIA® belongs to a versatile and balanced diet that along with physical activity is necessary for a healthy lifestyle.

Health effects of dairy products comprising *L. plantarum* TENSIA® have been tested on over 700 persons, in cheese on over 600 persons.

Several clinical trials (mainly in Estonia but also an independent trial in Russia) with volunteers consuming the Harmony® cheese comprising *L. plantarum*TENSIA® have provided positive effects on increasing the level of *L. plantarum* TENSIA® in the human organism, stabilizing the intestinal lactobiota and most importantly, helping to reduce blood pressure. Main findings are presented shortly below.

In the randomized double blinded placebo controlled (DBPC) cross-over study (ISRCTN15061552) with 3-week treatment periods with 83 reasonably healthy adults of both sexes aged 18-65 years treatments were as follows: 50g/day of Harmony® cheese comprising *L. plantarum* TENSIA® (daily dose 10^{10}CFU) as *verum*and 50 g /daily regular cheese without probiotic additive as control (Mikelsaar *et al.*, 2012).

It was found that the impact of consumption of the Harmony® cheese comprising *L. plantarum* TENSIA® was associated with the baseline levels of blood pressure. Significant decrease of BP was found in persons with high normal values (systolic blood pressure (SBP): ≥130…139.5 mmHg/ diastolic blood pressure (DBP): 85…89 mmHg) during the 3-week consumption of 50g of the Harmony® cheese comprising *L. plantarum* TENSIA® ($p = 0.003$ and $p = 0.004$, mean: -4.0mmHg and -2.3mmHg respectively). The respective values in control group were as follows: ($p = 0.174$ and $p = 0.198$, -2.5mmHg and -0.3mmHg respectively). Changes in DBP of the *verum* group differed significantly ($p= 0.047$) from those of the placebo in subjects with elevated BP i.e. SBP ≥130…139.5 mmHg or/and DBP ≥85–89 mmHg according to the classification of European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) (Mancia *et al.*, 2007, 2013).

The effect of 8-week consumption of *L. plantarum* TENSIA® with Harmony® cheese on blood pressure but also on other clinical markers was evaluated during DBPC parallel-designed two-armed study in 118 subjects (aged 20…65 yrs) with baseline systolic blood pressure values defined by ESC/ESH Guidelines (Mancia *et al.*, 2007; 2013) as normal or high normal i.e. SBP between ≥120…139.5mmHg, but uncomplicated by diabetes or previous cardiovascular events and without anti-hypertensive drug treatment (Hütt *et al.*, 2014). The daily dose of *L. plantarum* TENSIA® administered with the Harmony® cheese was 2.5 $x10^9$ CFU.

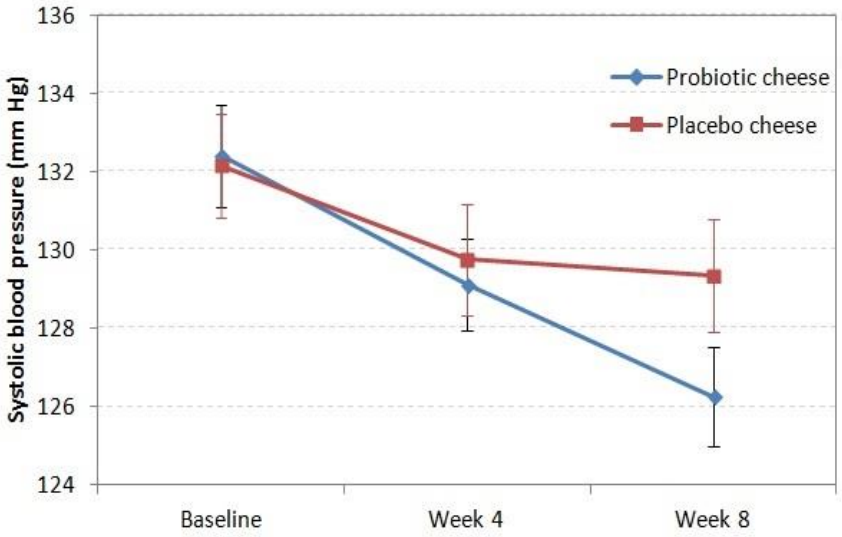
Continuous consumption of *L. plantarum* TENSIA® with the Harmony® cheese resulted in significantly different reduction ($p=0.007$) in SBP accompanied by the significantly different reduction ($p= 0.026$) in DBP in *verum* group (Harmony® cheese group) as compared to the control (regular Edam-type cheese).

Steady significant reduction of both SBP and DBP throughout the study occurred within the *verum* group ($p= 0.006$ in week 4 *vs* start and $p=0.005$ in week 4 *vs* week 8 for SBP; respective p values for DBP being 0.033 and <0.001). On the other hand, the significant effect of placebo consumption was limited to four weeks ($p= 0.023$ in week 4 *vs* start and $p=0.864$ in week 4 *vs* week 8 for SBP; respective p values for DBP being 0.011 and 0.763).

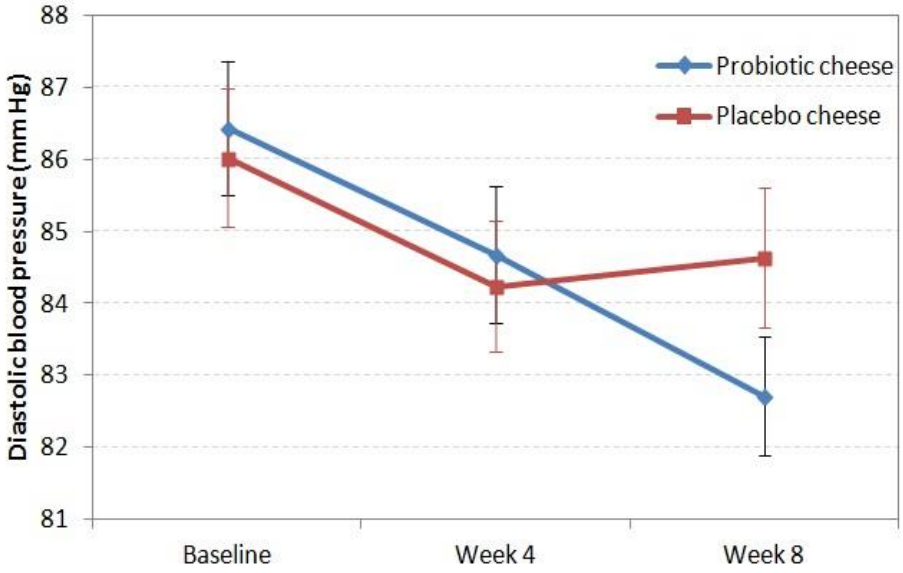
Continuous consumption of the Harmony® cheese with *L. plantarum* TENSIA® over longer period (8 weeks) resulted in significantly different reduction (p=0.007) in SBP accompanied by the significantly different reduction (p= 0.026) in DBP in *verum* group as compared to the control (Figure 1)

Figure 1. Course of change in average systolic blood pressure (a) and diastolic blood pressure (b) in *verum* and placebo groups during 4 and 8 week administration of 50g of the probiotic cheese Südamejuust Harmony™ comprising *L. plantarum* TENSIA® or placebo cheese, respectively. Reduction of SBP *verum* vs placebo after 8-week administration p=0.007.

a)



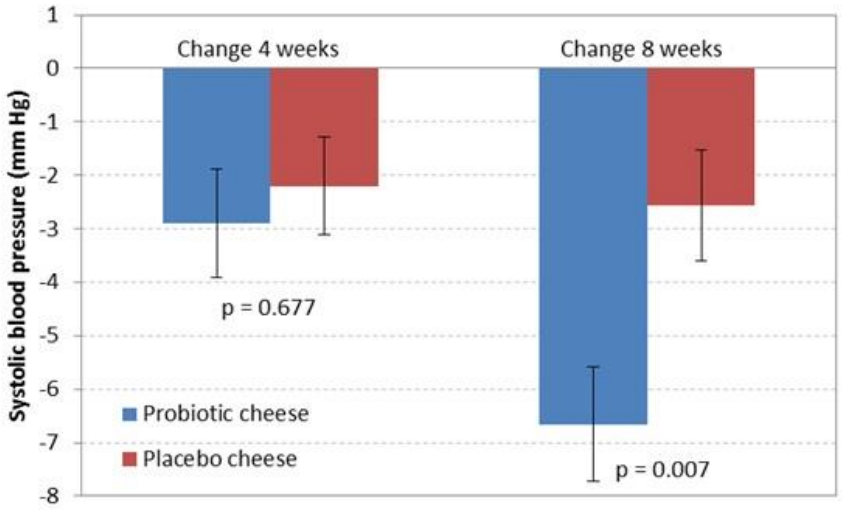
b)



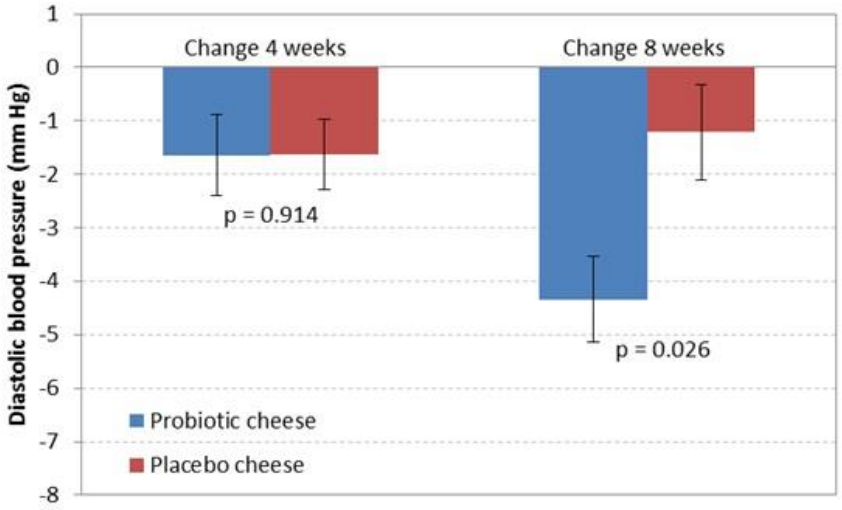
The average reduction of SBP during 8 weeks of administration of *L. plantarum* TENSIA® with Harmony® cheese was 6.66 mmHg and 4.34 mmHg for DBP. The corresponding figures for placebo were respectively 2.56 mmHg and 1.21 mmHg (Figure 2)

Figure 2. Average change with standard error in systolic blood pressure (a) and in diastolic blood pressure (b) in *verum* and placebo groups during 4 and 8 week administration of 50g of the probiotic cheese Südamejuust Harmony™ comprising *L. plantarum* TENSIA® or placebo cheese, respectively.

a)



b)



It has been shown that the reduction of blood pressure for 5mm/Hg reduced the prevalence of cardiovascular diseases and stroke for 20-40%. Even a 2 -3mmHg lower usual SBP would involve about 8-10% lower stroke mortality and about 5-7% lower mortality from other vascular causes in and the reduction of SBP for 10 mmHg lower usual or 5 mmHg lower usual DBP would, in the long term, be associated with about 40% lower risk of stroke death and about 30% lower risk of death from vascular causes (Appel *et al.*, 2006; Chobanian *et al.*, 2003, Vasan *et al.*, 2001).

Administration for 8 weeks of *L. plantarum* TENSIA® with the Harmony® cheese improved also some atherosclerosis markers. A significant reduction in LDL/HDL ratio occurred within *verum* group both during short term i.e. 4-week (p=0.0397) as well as log-term i.e. 8-week (p=0.0155) consumption period.

More importantly, continuous consumption of the Harmony® cheese with *L. plantarum* TENSIA® resulted in significantly different reduction (p=0.0028) in oxidized-LDL (ox-LDL), oxidative stress index (OSI) (p= 0.0004), total peroxide count (TPX) (p=0.0039) in *verum* group as compared to the control. The improvement of oxidative stress markers due to administration of *L. plantarum* TENSIA® with the Harmony® cheese could explainable with the antioxidative activity of the strain.

Reactive oxygen species (ROS) are products of normal cellular metabolism and derive from various sources in different cellular compartments. Oxidative stress resultant from imbalance between ROS generation and antioxidant defence mechanisms is important in pathogenesis of cardiovascular diseases, such as hypertension, heart failure, atherosclerosis, diabetes, and cardiac hypertrophy. Thus, the consumption of the Harmony® cheese with *L. plantarum* TENSIA® could also help to improve the antioxidant defense of human body and through this contribute to CV health.

A DBPC study carried out in cooperation between E-piim production Ltd (Estonia) and the Institute of Nutrition under the Russian Academy of Medical Sciences in Moscow (Russia) evaluated the clinical efficacy of a hypocaloric diet supplemented with Harmony® cheese comprising *L. plantarum* TENSIA® in Russian adult patients with obesity and hypertension with particular accompanying diseases under standard treatment.

The study resulted in several beneficial shifts of health markers in subjects randomly selected from consecutive patients admitted to the Clinic of the Institute of Nutrition. First, body mass index (BMI) was significantly reduced (p=0.031) in the Harmony® cheese group versus the control cheese group. The changes in BMI were closely associated with the water content of the body (r=0.570, p=0.0007) when adjusted for sex and age (Sharafedtinov *et al.*, 2013). In patients simultaneously treated with BP-lowering drugs, similar reductions of BP were observed in both groups. A positive association was detected between *L. plantarum*TENSIA® colonization and the extent of change of morning diastolic BP (r=0.617, p=0.0248) and a trend toward lower values of morning systolic BP (r=-0.527, p=0.0640) at the end of the study after adjusting for BMI, age, and sex. In both the probiotic and control groups, the reduction of total cholesterol and low-density lipoprotein was observed after the consumption of 50 g of cheese containing 26% fat for 3 weeks. Significant reduction of the plasma triglyceride level was detected only in the Harmony® cheese group.

Mechanisms of action

Blood pressure regulation in human body is quite complicated. At least three mechanisms have been described. The blood pressure lowering mechanisms of *Lactobacillus*

plantarum TENSIA® is complex, connected with the absorption of several blood pressure-lowering property having metabolites produced by the strain

In virto studies indicate, that the strain *Lactobacillus plantarum* TENSIA® expresses proteolytic properties *in vitro* (in milk and cheese) incl. ACE inhibitory activity and is able to produce ACE inhibitory activity-having bioactive di-peptides (Ile-Leu and Phe-Leu) and acetylcholine into the cheese matrix during cheese ripening and shelf-life.

Additionally, TENSIA® is able to produce nitric mono-oxide (NO, well-known vaso-dilatator) and to use nitrates as a substrate for NO production.

The moderate anitoxidative activity of the strain also contributes to the improvement of other cardio-vascular risk factors (e.g. excessive oxidative stress).

L.plantarum TENSIA® tolerates well technological processing. This property makes the strain usable as probiotic adjunct starter in functional food.

L. plantarum strain TENSIA® was elaborated into a probiotic according to the regulations of FAO /WHO (2002). *'*

Safety

Lactobacilli incl. Species *Lactobacillus plantarum* have been consumed for centuries in form of fermented foods and are generally recognised as safe (FAO/WHO, 2002). The bacterial species *L. plantarum* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach (EFSA, 2007 and 2012).

At the same time, a heterofermentative lactic acid bacterium *Lactobacillus plantarum* as species is a normal component of intestinal microbiota in humans. The human origin of the strain *Lactobacillus plantarum* TENSIA® is a prerequisite for its harmless status and suitability for oral application.

Safety of the Harmony® cheese and the strain *L. plantarum* TENSIA® have been studied thoroughly. Antibiotic resistance profile and absence of virulence factors of the strain was confirmed by laboratory tests. In a mice toxicity model during 30 days of feeding no translocation of intestinal bacteria into bloodstream and no pathological shifts of organs were found (Songisepp *et al.*, 2012).

Safety of the consumption of the Harmony® cheese comprising *L. plantarum* TENSIA® has been proved in two age groups of general healthy population: adults (age 20…60 y) and also on more vulnerable population group: aged people (> 65 y). In human intervention trials with healthy adult volunteers and aged people no adverse gastro-intestinal effects were seen and no increased inflammatory indices were found (Songisepp *et al.*, 2012).

The fat content of the Harmony® cheese is 26% (fat in dry matter 45-47%). In human intervention studies the daily dose of 50 g of cheese was administered to healthy adults (ISRCTN38739209) and elderly (ISRCTN45791894). During these trials and even by consuming twice the dose of cheese (i.e.100g/d) by healthy adults in trial ISRCTN42449576, the impact of fat on host plasma lipids by consuming three weeks the Harmony® cheese was considered negligible. No negative impact on levels of total cholesterol or cholesterol-fractions (HDL-, LDL-cholesterol, triglycerides) was assessed. No adverse effects in inflammation markers of blood (WBC count and IgG, IgM and IgE levels) were detected (Songisepp *et al.,* 2012).

Consumption of probiotics (live microbes) can cause unpleasant gastrointestinal symptoms (abdominal pain, flatulence, bloating). In vulnerable population groups also some adverse side effects (endocarditis, sepsis) have been reported (Boyle *et al.,* 2006). A relatively high amount (2×10^{10}CFU per serving for 3-weeks) of the probiotic strain *L. plantarum* TENSIA® was consumed with cheese by adult volunteers (study ISRCTN38739209). No adverse side effects (flatulence, bloating, abdominal pain, stool frequency) were detected. Consumption of cheese in double dose (i.e. 100g/daily, 2.5 $x10^{10}$CFU per serving of the probiotic for 3-weeks) in study ISRCTN42449576 caused abdominal pain, flatulence and/or bloating in single cases. As the probiotic daily dose was similar in both studies with adult volunteers, such indigestion could be caused by the consumption of cheese in excess, not by the probiotic strain (Songisepp *et al.,* 2012). Also the consumption of 100 g/d of cheese for 3 weeks caused hard stools from the second week of the trial (Songisepp *et al.,* 2012). Cheese is rich in saturated fats and proteins and lacks fibre and therefore the excessive consumption of cheese could result in constipation.

Consuming 1.5×10^{8} log CFU with 50 g/ cheese daily for 3-weeks, one fourth of participants complained abdominal pain or bloating and half participants reported flatulence throughout the trial with elderly.

Thus, the Harmony® cheese comprising *L. plantarum* TENSIA® does not figure a direct health risk if consumed to excess for longer period (i.e. 100g per serving for 3 weeks).

In conclusion,

The consumption of Harmony® cheese comprising *L. plantarum* TENSIA® reduces both systolic and diastolic blood pressure and improves also following atherosclerosis markers: LDL/HDL, oxidized-LDL (ox-LDL) and oxidative stress index (OSI).

The consumption of the Harmony® cheese with *L. plantarum* TENSIA® could also help to improve the antioxidant defence of human body and through this contribute to cardio-vascular health.

The use of *Lactobacillus plantarum* TENSIA® as a blood pressure-lowering probiotic has been patented (Estonian Patent EE5340, European Patent EP2309870).

According to the results of clinical trials the Harmony® cheese comprising *Lactobacillus plantarum* TENSIA® helps to maintain the cardio-vascular system and heart health through reduction of blood pressure.

Thus, the Harmony® cheese as a functional food is a natural way to support human health together with physical activity and healthy diet.

The consumption of Harmony® cheese comprising *Lactobacillus plantarum* TENSIA® belongs to a versatile and balanced diet that along with physical activity is necessary for a healthy lifestyle.

E-piim produces the Harmony® cheese with TENSIA® under the license of the Bio-Competence Centre of Healthy Dairy Ltd.

References

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Patents and patent applications regarding the probiotic *Lactobacillus plantarum* Tensia DSM 21380

- European patent EP2309870, filed 12.05.2009, granted 04.05.2012, "Isolated microorganism strain *Lactobacillus plantarum* Tensia DSM 21380 as antimicrobial and antihypertensive probiotic, food product and composition comprising said microorganism and use of said microorganism for preparation of antihypertensive medicine and method for suppressing pathogens and nonstarter lactobacilli in food product", inventors E. Songisepp, M. Mikelsaar, M. Rätsep, M. Zilmer, P. Hütt, M. Utt, K. Zilmer, J.Üksti, S. Kõljalg. Patent owner: Bio-Competence Centre of Healthy Dairy Products (Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ). EP2288360 is validated in following countries:
  - United Kingdom – GP/EP2309870
  - Sweden – SE/EP2309870
  - Germany – DE/EP2309870 (60 2009 007 496.2
  - France – FR/EP2309870
  - Finland – FI/EP2309870
  - Czech Republic – CZ/EP2309870
  - Poland – PL/EP2309870
  - Hungary – HU/EP2309870
  - Lithuania – LT/EP2309870
  - Latvia – LV/EP2309870

- **Russian patent** RU2477750 filed 12.05.2009, granted 20.03.2013, Изолированный штамм микроорганизма *Lactobacillus plantarum* Tensia DSM 21380 в качестве антимикробного и антигипертензивной пробиотика, пищевого продукта и композиции, содержащей указанный микроорганизм и использование указанного микроорганизма для приготовления антигипертензивного лекарства и способу для подавления патогенов и нестартующий лактобактерии в пищевом продукте
- **Korean Patent** KR10-1587195 filed 13.12.2010, granted 14.01.2016 "Isolated microorganism strain *Lactobacillus plantarum* Tensia DSM 21380 as antimicrobial and antihypertensive probiotic, food product and composition comprising said microorganism and use of said microorganism for preparation of antihypertensive medicine and method for suppressing pathogens and nonstarter lactobacilli in food product", inventors E. Songisepp, M. Mikelsaar, M. Rätsep, M. Zilmer, P. Hütt, M. Utt, K. Zilmer, J.Üksti, S. Kõljalg. Patent owner: Bio-Competence Centre of Healthy Dairy Products (Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ)US Patent Application US2011/0177198, Patent pending