

# EUROPEAN PATENT APPLICATION

Patent № EP 3 172 973 A1

Date of Patent: 31.05.2017

## PROBIOTIC FORMULA, PROCESS OF ITS PREPARATION AND USE

The invention relates to a probiotic formula involving cooperation of strains of probiotic microorganisms, isolated as it is, from a traditional dairy product in Rhodope mountains, to a process of preparation of the probiotic formula and its use as a dietary food for people with impaired liver function and structure, for controlling the symptoms of food allergy and for recovering the normal vaginal lactobacillaceae flora of women. More precisely, the probiotic cooperation of strains microorganisms includes strain *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR1, registered in CCM under N° 8591/ 17.02.2015; strain *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR2, registered in CCM under N° 8592/ 17.02.2015; strain *Streptococcus thermophilus* DMR3, registered in CCM under N° 8591/ 17.02.2015 and strain *Streptococcus thermophilus* DMR4, registered in CCM under N° 8592/ 17.02.2015. In a ratio-of 1:0.005 this probiotic cooperation is used for fermentation of a standardized and pasteurized cow's milk or goat's milk for preparation of the probiotic formula, which after lyophilization and storage for 24 months at a room temperature up to 24°C, contains alive cells of the probiotic cooperation, in concentration from 1.1x10<sup>9</sup> cfu/g to 1.6x10<sup>9</sup> cfu/g.

### Claims

1. Process of preparation of a probiotic formula from cow's or goat's milk by fermentation and lyophilization, **characterized in that** a standardized and pasteurized cow's milk with 3.6% fat or sterilized pasteurized goat's milk with 3.2% fat is cultured by a probiotic cooperation of microorganism strains, including strain *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR1, registered in CCM under N° 8591/17.02.2015, strain *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR2, registered in CCM under N° 8592/ 17.02.2015, strain *Streptococcus thermophilus* DMR3, registered in CCM under N° 8591/ 17.02.2015 and strain *Streptococcus thermophilus* DMR4, registered in CCM under N° 8592/ 17.02.2015 in a ratio 1:0.005 in order to obtain a biomass which is added to standardized and pasteurized milk in a ratio 1: 0.001 and is left to ferment for 18 hours at a temperature of 43°C- 45°C, the resultant liquid starter culture is added to 17% water solution of dry cow's or fresh goat's milk in a ratio 3:1 together with a cryoprotectant bee honey and the obtained mixture is homogenized at a temperature of 41°C - 44°C, then it is fermented for 3 hours and then is cooled down to 24°C- 25°C, then is lyophilized and is stored at a temperature of 24°C.

2. Process of preparation of a probiotic formula according to Claim 1, **characterized in that** the strains *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR1, registered in CCM under N° 8591/ 17.02.2015, *Lactobacillus delbrueckii* subsp. *bulgaricus* DMR2, registered in CCM under N° 8592/ 17.02.2015, *Streptococcus thermophilus* DMR3, registered in CCM under N° 8591/ 17.02.2015 and *Streptococcus thermophilus*

DMR4, registered in CCM under N° 8592/ 17.02.2015 are included in the probiotic cooperation in a ratio 1:1:0.5:0.5.

**3.** Process of preparation of a probiotic formula according to Claim 1, **characterized in that** the cryoprotectant bee's honey is previously heat treated.

**4.** Process of preparation of a probiotic formula according to Claims 1-3, **characterized in that** the cryoprotectant is put in the 17% water solution of dry cow's or goat's milk to which the liquid starter culture is added in a ratio 1:50.

**5.** Process of preparation of a probiotic formula according Claims 1-4, **characterized in that** the liquid starter culture is obtained also by rehydration of the lyophilized product from Claim 1 by water in a ratio water: dry product 1:0.5.

**6.** Probiotic formula, prepared according to Claims 1-5, **characterized in that** after storage for 24 months at a room temperature 24°C, it contains live cells from the probiotic cooperation in quantity from  $1,1 \times 10^9$  cfu/g to  $1,6 \times 10^9$ cfu/g.

**7.** Probiotic formula according to Claim 6, **characterized in that** after storage for 24 months at a room temperature 24°C, it contains live cells of strain *Streptococcus thermophilus DMR3*, registered in CCM under N°8591 and strain *Streptococcus thermophilus DMR4*, registered in CCM under N° 8592 in quantity from  $4,1 \times 10^8$  cfu/g to  $1,1 \times 10^9$  cfu/g, as well as live cells of strain *Lactobacillus delbrueckii ssp. Bulgaricus DMRI*, registered in CCM under N° 8591 and strain *Lactobacillus delbrueckii ssp. Bulgaricus DMR2*, registered in CCM under N° 8592 in quantity from  $5,1 \times 10^8$  cfu/g to  $8,4 \times 10^8$  cfu/g.

**8.** Use of a probiotic formula according to Claims 6 and 7 for production of a dietary food for nutritional therapy in addition to the basic treatment for restoration of the functions of the liver cells in patients with alcoholic disease.

**9.** Use of a probiotic formula according to Claims 6 and 7 for production of a food supplement for a nutritional therapy in addition to the basic treatment for restoration of the functions of the liver cells in patients with alcoholic disease.

**10.** Use of the probiotic formula according to Claim 9, **characterized in that** the food supplement for the nutritional therapy for restoration of the functions of the liver cells in patients with alcoholic disease, includes the probiotic formula from cow's milk, prepared according to Claims 1-5 and silymarin, dextrose monohydrate and magnesium stearate in a ratio 15:10:1:1.

**11.** Use of a probiotic formula according to Claims 6 and 7 for production of dietary food for nutritional therapy in addition to the basic treatment for restoration of the liver structure in patients with liver cirrhosis.

**12.** Use of a probiotic formula according to Claims 6 and 7 for production of a food supplement for a nutritional therapy in addition to the basic treatment of patients with food allergies.

**13.** Use of the probiotic formula according to Claim 12, **characterized in that** the food supplement for a nutritional therapy of patients with food allergies includes the probiotic formula from goat's milk according to Claims 6-7 and

**14.** Use of a probiotic formula according Claims 6 and 7 for the production of a medical device for restoration of the normal vaginal lactobacillus flora of women.