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PROBIOTICS WITH TANNASE ACTIVITY

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Introduction. Vegetable food is the main source of tannins - biologically active phytonutrients. Tannins present in various plants used as foodstuffs and feedstuffs. Tannins, on the one hand good for health because of their chemopreventive activity against carcinogenesis and mutagenesis, on the other hand - they may be involved in cancer formation, or antinutritive hepatotoxic activity. Tannins are known as antinutrients - they reduce the efficiency of the conversion by the body absorption of nutrients into new substances. The properties of tannins are directly dependent on their molecular weight.

Although tannins are toxic to a variety of organisms, some microorganisms are resistant to the action of tannins and have the ability to degrade them to oligomeric tannins and other useful derivatives, such as pyrogallol or gallic acid. Tannase - tannin acyl hydrolase (EC 3.1.1.20), catalyzes the hydrolysis of the galloyl ester bond of tannins. Tannase belongs to the superfamily of esterases. Despite the extensive interest and long history of the study of tannase, there is surprisingly little knowledge about the enzyme at the molecular level, which has become one of the critical factors that limit the large-scale application of tannase. Only bacterial tannase analyzed at the genetic level. Only biochemistry and structure of *Lactobacillus plantarum* tannase, most often occurring during the fermentation of plant materials with a high content of tannins, were characterized.

The microflora of the gastrointestinal tract (GIT) of a person has a profound influence on the transformation of food into metabolites that can affect human health [3-5]. Thus, the high specific activity of bacteria, particularly tannin-metabolising activity can be regarded as one of the criteria for the selection of probiotic strains for further use in the pharmaceutical industry.

The aim of the study. Do a review of modern literary sources in order to analyze data on the possibilities using tannin-metabolising activity of probiotic microorganisms for creation probiotics with new therapeutic properties.

Main results. Tannins are naturally occurring polyphenolic compounds with varying molecular weights that occur naturally in the plant kingdom. These phenolic compounds differ from others by having the ability to precipitate proteins from solutions. In the plant kingdom, these tannins are found in leaves, bark, and wood. Tannins are considered to be plants' secondary metabolic products because they play no direct role in plant metabolism. After lignin, tannins are the second most abundant group of plant phenolics. One of the major characteristics of tannins is their ability to form strong complexes with protein and other macromolecules such as starch, cellulose, and minerals. They possess a certain range of biological properties that provide defense mechanisms of plants against pests and diseases caused by bacteria and viruses. Tannins inhibit the growth of a number of microorganisms and are resistant to microbial action [3, 4]. It is widely accepted that tannins are divided into

four major groups: gallotannins, ellagitannins, condensed tannins, and complex tannins.

From the ancient time tannin rich plant extracts (tea) have been used as traditional medicine in China and Japan, to prevent diarrhea, diuretics, inflammation, septic, hemorrhage and cancer. Tannin phenolics are also efficient chelators for metal ions, that's why they can be used in the treatment of poisoning caused by heavy metals. Tannin and flavonoid compounds are found to be most effective inhibitors of HIV-1.

Increased amounts of tannic acid in soil have several detrimental effects like plant vegetation, cropping systems and low production yield. Side by side, tannin also causes various nutritional and processing problems like protein indigestibility, inhibition of enzymatic reactions and essential microbial processes such as those necessary for beer brewing. Thus high concentrations of tannin depress voluntary feed intake, digestive efficiency and animal productivity. In addition, dietary tannins have been implicated in the development of some forms of cancer.

However, reception of adequate amounts of the correct type tannin is beneficial to human health due to their effects on metabolic enzymes, immunomodulation, and other functions [1-5].

Many products of anaerobic decomposition of tannins produced in the gastrointestinal tract, may also form compounds with useful effects for human health, such as propionic or phenylacetic acids [4, 5]. These compounds have anti-inflammatory effect when absorbed in the gastrointestinal tract, and have a wide range antimicrobial effect by inhibiting the development of pathogenic microorganisms.

Tannins have a range of effects on various organisms - from toxic effects on animals to growth inhibition of microorganisms. Some microbes are, however, resistant to tannins and have developed various mechanisms and pathways for tannin degradation in their natural habitat.

Microbial degradation of condensed tannins is, however, less documented than that of gallotannins in both aerobic and anaerobic environments.

Despite antimicrobial properties of tannins, many fungi, bacteria and yeasts are stable enough to tannins and able to grow and develop in their presence. The mechanisms by which microorganisms are resistant include modification, degradation, dissociation tannin-substrate complexes, inactivation of tannins by the binding capacity, etc. [1].

Fungi, yeasts and some aerobic bacteria are usually best suited for the degradation of tannins but also anaerobic degradation occurs, e.g., in the gastrointestinal tract. Each group of microorganisms has the specific properties of tannins in the process of decomposition. Thus, yeast, showing activity against gallotannins and unable to degrade tannins macromolecular compound. Bacteria have the ability to degrade gallotannins and ellagotannis. Fungi, may decompose tannins all types [1].

It is known that tannins metabolism involves a number of enzymes, but is the main enzyme tannase. In nature, this enzyme can be of animal, plant and microbial origin. Most importantly, the tannase is of microbial origin [3].

The tannase structure and properties depend of: producer, cultivation conditions, etc. [3].

Since tannase has practical significance, especially in the pharmaceutical industry, an important issue is its safety in relation to the human body, as well as the status of tannase producer as GRAS microorganisms group (Generally Recognized as Safe). It is known a limited number of issues about tannase safety, however, recent studies have demonstrated the safety of tannase produced by bacteria of the genus *Lactobacillus*. The microorganisms in the human gastrointestinal tract have a profound influence on the transformation of food into metabolites which can impact human health.

The first report on the ability of certain strains of bacteria used tannic acid as a source of carbon nutrition, appeared in the early 1980s. Since then it has been allocated more than 60 strains of bacteria - producers tannase: namely bacteria of the genera *Lactobacillus, Bacillus, Enterococcus, Pentococcus* some other [1-5] (table), however, only some of them can be used for commercial production. There are several methods for screening of bacteria producing tannase.

Table

Bacteria producers tannase

Bacteria producers	**Reference**
Bacillus plumilus	Deschamps et al. (1983)
Bacillus polymyxa	Deschamps et al. (1983)
Corynebacterium sp.	Deschamps et al. (1983)
Klebsiella pneumoniae	Deschamps et al. (1983)
Pseudomonas solanaceaum	Deschamps et al. (1983)
Citrobacter freundii	Kumar et al. (1999)
Lactobacillus plantarum	Osawa et al. (2000)
Lactobacillus paraplantarum	Osawa et al. (2000)
Lactobacillus pentosus	Osawa et al. (2000)
Bacillus lichiniformis	Mondal et al. (2000)
Bacillus cereus	Mondal et al. (2001)
Lactobacillus plantarum	Ayed and Hamdi (2002)
Lactobacillus paraplantarum	Nishitani et al. (2004)
Lactobacillus acidophilus	Nishitani et al. (2004)
Lactobacillus pentosus	Nishitani et al. (2004)
Lactobacillus animalis	Nishitani et al. (2004)
Lactobacillus murinus	Nishitani et al. (2004)
Lactobacillus faecalis	Nishitani et al. (2004)
Lactobacillus acidilactici	Nishitani et al. (2004)

Lactobacillus pentosaceaus	Nishitani et al. (2004)
Enterococcus faecalis	Goel et al. (2005)
Lactobacillus sp. ASR-S1	Sabu et al. (2006)
Pentococcus entosaceaus	Guzman-Lopez et al. (2009)
Lactobacillus buchneri	Guzman-Lopez et al. (2009)
Lactobacillus hilgardii	Guzman-Lopez et al. (2009)
Weissella confusa	Guzman-Lopez et al. (2009)
Bacillus thurangiences BN2	Belur et al. (2009)
Pseudomonas aeruginosa	Selwal et al. (2010)
Serratia ficaria	Belur et al. (2010)
Serratia marcescens	Belur et al. (2010)
Microbacterium terregens	Belur et al. (2010)
Providencia rettgeri	Belur et al. (2010)
Lactobacillus plantarum	Matoba et al. (2013)
Lactobacillus plantarum	Ren et al. (2013)
Lactobacillus plantarum	Jimenez et al. (2014)
Streptococcus gallolyticus	Jimenez et al. (2014)
Roseburia intestinalis XB6B4	de Felipe et al. (2014)
Streptococcus gallolyticus ATCC 3143	de Felipe et al. (2014)
Streptococcus gallolyticus ATCC BAA-2069	de Felipe et al. (2014)
Streptococcus gallolyticus UCN34	de Felipe et al. (2014)
Fusobacterium nucleatum subsp. vincentii	de Felipe et al. (2014)
Fusobacterium nucleatum subsp. nucleatum ATCC 25586	de Felipe et al. (2014)
Fusobacterium nucleatum subsp. animalis	de Felipe et al. (2014)
Aggregatibacter actinomycetemcomitans D7S-1	de Felipe et al. (2014)
Aggregatibacteractinomycetemcomitans D11S-1	de Felipe et al. (2014)
Aggregatibacteraphrophilus	de Felipe et al. (2014)
Aggregatibacteractinomycetemcomitans ANH9381	de Felipe et al. (2014)
Slackia heliotrinireducens	de Felipe et al. (2014)
Lactobacillus plantarum CIR1	Aguilar-Zarate et al. (2014)
Lactobacillus paraplantarum NSO120	Ueda et al. (2014)
Lactobacillus pentosus 21A-3	Ueda et al. (2014)
Lactobacillus plantarum ATCC 14917	Ueda et al. (2014)
Lactobacillus plantarum	Esteban Torres et al. (2015)

Lactobacillus plantarum DSM 15313	Ahrén et al. (2015)
Klebsiella pneumoniae	Tahmourespour et al. (2016)

According to a visual method (qualitative reaction), when treated with alkali culture liquid, followed by incubation at 23 ° C for one hour, there is a color change from green to brown at $\lambda = 440$ nm - positive reaction.

For growth and selective isolation of bacteria with tannase activity can be applied nutrient medium with the addition of tannic acid (% w / v: meat extract - 0.3; peptone - 0.5, tannic acid - 2; agar - 2). After 3-4 days of incubation is carried out painting solution 0.01 M FeCl3 for analysis gallotannins.

Both methods have been successfully used for the detection of bacteria with tannase activity. Various methods for the isolation of producers were suggested. To quantify tannase activity of producers are also known various methods, the most commonly used: titrimetric, colorimetric, UV spectrophotometry, photometry [1].

However, these methods are time consuming and require the stage of cultivation and do not allow establishing the full potential of tannase activity of the test strains. So, now particularly relevant genetic research of tannase strain-producers that gives an accurate result and allows identifying the genes responsible for tannase activity.

Conclusions. Thus, prospective study is analyzed presence of tannase activity in the well-characterized probiotic strains that can be used for the development of probiotics in the pharmaceutical industry, enriched with probiotic microorganisms with tannase activity and therefore consequently with promising - antioxidant and antitumor properties

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