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BACTERIAL TANNASES: PRODUCTION, PROPERTIES AND APPLICATIONS

TANASAS BACTERIANAS: PRODUCCIÓN, PROPIEDADES Y APLICACIONES

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Abstract

Tannins are polyphenolic compounds present in plants where they play an important role to prevent the attack of viruses, bacteria and fungi. Despite the fact that polyphenols inhibit the microbial growth, adaptation process has allowed developing mechanisms to transform them. One mechanism is the production of tannase, which has been obtained mostly from fungi. In recent years, some tannase producer bacteria have been isolated from different sources, mainly from animals and human intestine and feces as well as from fermented food and fruit wastes. Obtaining high titers of bacterial tannase depends mainly on the culture medium composition, the bacterial strain and the process optimization of culture conditions. This paper presents an overview of the recent investigations regarding the production, the physicochemical and molecular characteristics, the applications and the potential uses of bacterial tannases.

Keywords: gallotannins, bacterial tannase, tannins biodegradation, optimization, tannase gene.

Resumen

Los taninos son compuestos polifenólicos presentes en las plantas, en las cuales desempeñan un papel importante al evitar el ataque de virus, bacterias y hongos. A pesar de que los polifenoles inhiben el crecimiento microbiano, algunos microorganismos han desarrollado mecanismos para hidrolizarlos, uno de estos mecanismos es la producción de la enzima tanasa, la cual se ha obtenido mayoritariamente de hongos. Por otro lado, en los últimos años se han aislado bacterias productoras de tanasa de diversas fuentes, principalmente del intestino y heces de animales y humanos, así como de alimentos fermentados y de los desechos de algunos frutos. La obtención de altos títulos de tanasas bacterianas dependerá principalmente de la composición del medio de cultivo, de la cepa bacteriana y de una efectiva optimización de las condiciones de cultivo. Este documento presenta una revisión de las investigaciones recientes realizadas en torno a la producción, a las características fisicoquímicas y moleculares, a las aplicaciones y usos potenciales de las tanasas bacterianas.

Palabras clave: galotaninos, tanasa bacteriana, biodegradación de taninos, optimización, gen tanasa.

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1 Introduction

Tannins are polyphenolic compounds with varying molecular weight. They are found mainly in plants playing the role of defense against viruses, bacteria and fungi. Tannins have the ability to precipitate protein solutions, hence they are nutritionally undesirable. Tannins are considered a plant secondary metabolic product due to they have not an important role in the metabolism. Some microorganisms have developed the capability to use tannins as carbon source. Modification of tannins, degradation, dissociation of complex tannins, inactivation by high affinity bonds, membrane modification and chelation of metal ions, are microbial mechanisms to get over inhibition growth (Smith *et al.*, 2005).

Tannase or tannin acyl-hydrolase (E.C. 3.1.1.20) catalyzes the hydrolysis of ester bonds present in gallotannins, complex tannins and gallic acid esters. Tannic acid hydrolysis by tannase results in the releasing of glucose, gallic acid and some galloyl esters. Tannase has applications in food and beverages processes; however, the application is limited because of the little knowledge about its properties, optimal expression and scale up (Aguilar *et al.*, 2007). The major application of tannase resides in the production of acorn liquor, instantaneous tea and production of gallic acid (Belmares *et al.*, 2004).

The main sources for production of industrial enzymes are microorganisms, due to its biochemical diversity, easy culture and their docility to be genetically modified (Treviño *et al.*, 2007). Majority tannase producer microorganisms are fungi. Industrially, the problem with fungi resides in their slow tannins degradation and difficult to manipulate genetically. Few bacterial species have been reported as tannase producer such as *Bacillus* sp., *Corinebacterium* sp., *Lactobacillus* sp. and *Serratia* sp. (Mondal *et al.*, 2001; Rodríguez *et al.*, 2008; Milva *et al.*, 2010). The use of bacteria as tannase producers has received little attention.

Osawa *et al.* (2000) were the first in isolate lactobacilli strains from human gut and fermented foods. These microorganisms showed capability to hydrolyze tannins. Lactic acid bacteria (LAB) develop an important role in food tannin degradation. LAB have the ability to hydrolyze tannins and diminish their absorption to intestine cells (Ayed *et al.*, 2002).

The present review shows relevant points related to the bacterial tannase. It analyzes information about substrates for tannase production, the tannase-producing bacteria, tannin degradation pathways and

physicochemical characteristics of bacterial tannase, with the goal to contribute to the knowledge for potential applications of bacterial tannase in food and pharmaceutical industry.

2 Tannins structure

Tannins are hydro soluble and high molecular weight polyphenolic compounds. Tannins have the ability to precipitate macromolecules (such as proteins, cellulose, starch, etc.) and minerals by forming strong complexes. Tannins are the second most important group of natural phenolic compounds after lignin (Aguilar *et al.*, 2007).

Tannins are widely distributed in different vascular plant structures. They are considered as secondary metabolic products due to them do not participate directly in biosynthesis, biodegradation or any transformation of energy process. Swain (1977) informed that these compounds play vital roles in plants defense against fungi, bacteria or virus diseases, and protect them from herbivores. Actually, tannins are classified into four major groups. The most accepted classification is (Fig. 1): gallotannins, ellagitannins, complex tannins and condensed tannins (Khanbabaei *et al.*, 2001).

Gallotannins are the simplest hydrolysable tannins. They are formed by galloyl or di-galloyl units esterified with a core of glucose or a polyvalent alcohol (such as glucitol, shikimic acid, quinic acid, etc.). Tannic acid or pentagalloyl-glucose is an example of such compounds. A characteristic of gallotannins is their easy hydrolysis by heat, acid or alkali conditions, and for tannase. Gallotannins are found in plants such as oak galls where tannic acid is obtained (Hagerman, 2002).

Ellagitannins are found forming blocks of ellagic acid. Ellagitannins are esters of hexahydroxy diphenic acid (HHDP) joined to a glucose molecule. HHDP group in aqueous solution is transformed spontaneously to ellagic acid, hence the name of ellagitannins. Ellagitannins are formed from gallotannins by the oxidative coupling of two galloyl groups. Biodegradation pathway of ellagitannins is not clear yet (Khanbabaei *et al.*, 2001). Ellagitannins are obtained mainly from trees such oak (*Quercus* sp.) and chesnut (*Castanea dentata*), shrubs such as the pomegranate (*Punica granatum*) and fruits as red raspberry. Ellagitannins are capable to reduce congenital defects, promote cicatrization, reduce

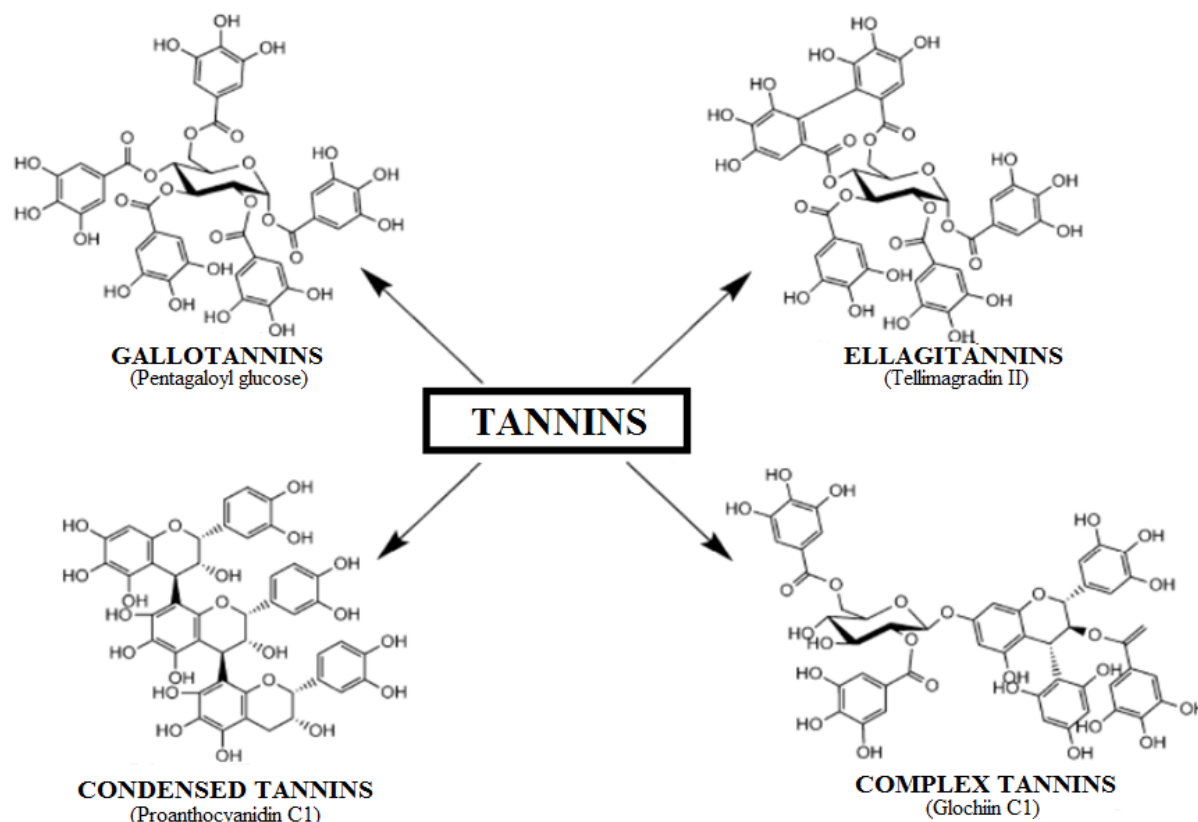


Fig. 1. Tannins classification (Khanbabaee and Van Ree, 2001).

the possibilities of suffering heart attacks, stop proliferation of viruses and prevent the appearance of cancer cells (Robledo *et al.*, 2008).

Condensed tannins are oligomeric and polymeric proanthocyanidins that are conformed by unites of flavan-3-ol (catechin) or flavan-3-4-ol linked to each other by C-C bonds. Catechin and epicatechin are the main monomérica unites of condensed tannins which to be condensed can build polymers. Polymeric chains could be formed by two or more than 50 unites. Condensed tannins cannot be used as substrate for tannase neither easily hydrolyzed by other methods. Major constituents of these compounds are cyanidin and delphinidin that are the responsible for the astringent flavor in fruits and wine (Belmares *et al.*, 2004; Ramirez-Coronel *et al.*, 2004; Aguilar *et al.*, 2007).

Complex tannins structure are formed by several units of gallotannins or ellagitannin and a catechin or epicatechin (Khanbabaee *et al.*, 2001). Complex tannins are formed probably due to reactions catalyzed in presence of light, heat or oxygen. A classical

example is catechin-gallate with hydrolysable and condensed bonds (Aguilera-Carbó *et al.*, 2008).

3 Bacterial tannin-biodegradation pathways

Enzymatic biodegradation of tannins is the most efficient way to hydrolyze high molecules to smaller one with important biological activities (Chavez-Gonzalez *et al.*, 2011). The ability to degrade tannins is different between yeast, fungi and bacteria. Yeast hydrolyzes easily gallotannins but not high molecular weight compounds such as ellagitannins or complex tannins. Fungi efficiently degrade hydrolysable and condensed tannins (Belmares-Cerda *et al.*, 2003) while bacteria only hydrolyze gallotannins and ellagitannins (Bhat *et al.*, 1998).

Enzymes involved in tannin hydrolysis are tannase and gallic acid decarboxylase. Tannase has been the most studied enzyme. Tannase hydrolyzes the ester and depsidic bonds of gallotannins, ellagitannins, and

Table 1. Bacterial sources of tannase

Microorganismo	Referencia
<i>Achromobacter</i> sp	Lewis and Starkey, (1969)
<i>Bacillus plumilus</i>	Deschamps <i>et al.</i> (1983)
<i>Bacillus polymixa</i>	Deschamps <i>et al.</i> (1983)
<i>Corinebacterium</i> sp.	Deschamps <i>et al.</i> (1983)
<i>Klebsiella pneumoniae</i>	Deschamps <i>et al.</i> (1983)
<i>Pseudomonas solanaceum</i>	Deschamps <i>et al.</i> (1983)
<i>Citrobacter freundii</i>	Kumar <i>et al.</i> (1999)
<i>Lactobacillus plantarum</i>	Osawa <i>et al.</i> (2000)
<i>Lactobacillus paraplantarum</i>	Osawa <i>et al.</i> (2000)
<i>Lactobacillus pentosus</i>	Osawa <i>et al.</i> (2000)
<i>Bacillus licheniformis</i>	Mondal <i>et al.</i> (2000)
<i>Bacillus cereus</i>	Mondal <i>et al.</i> (2001)
<i>Lactobacillus plantarum</i>	Ayed and Hamdi (2002)
<i>Selenomonas ruminantium</i>	Belmares <i>et al.</i> (2004)
<i>Citrobacter freundii</i>	Belmares <i>et al.</i> (2004)
<i>Lactobacillus paraplantarum</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus acidophilus</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus pentosus</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus animalis</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus murinus</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus faecalis</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus acidilactici</i>	Nishitani <i>et al.</i> (2004)
<i>Lactobacillus pentosaceus</i>	Nishitani <i>et al.</i> (2004)
<i>Enterococcus faecalis</i>	Goel <i>et al.</i> (2005)
<i>Lactobacillus</i> sp. ASR-S1	Sabu <i>et al.</i> (2006)
<i>Pediococcus pentosaceus</i>	Guzmán-López, <i>et al.</i> (2009)
<i>Lactobacillus buchneri</i>	Guzmán-López, <i>et al.</i> (2009)
<i>Lactobacillus hilgardii</i>	Guzmán-López, <i>et al.</i> (2009)
<i>Weissella confusa</i>	Guzmán-López, <i>et al.</i> (2009)
<i>Bacillus thuringiensis</i>	BN2 Belur <i>et al.</i> (2009)
<i>Lactobacillus plantarum</i>	Curiel <i>et al.</i> (2009)
<i>Pseudomonas aeruginosa</i>	Selwal <i>et al.</i> (2010)
<i>Serratia ficaria</i>	Belur <i>et al.</i> (2010)
<i>Serratia marcescens</i>	Belur <i>et al.</i> (2010)
<i>Microbacterium terregens</i>	Belur <i>et al.</i> (2010)
<i>Providencia rettgeri</i>	Belur <i>et al.</i> (2010)
<i>Bacillus sphaericus</i>	Raghuwanshi <i>et al.</i> (2011)
<i>Lactobacillus plantarum</i>	Kannan <i>et al.</i> (2011)
<i>Bacillus massiliensis</i>	Belur <i>et al.</i> (2012)

complex tannins but do not affect the C-C bonds. Hence, tannase cannot hydrolyze condensed tannins (Haslam and Stangroom, 1966).

Monomers of gallic acid can be used as substrate after breakdown of simple aliphatic acids (Fig. 2). Gallic acid is converted to pyrogallol by the enzyme gallate decarboxylase. Anaerobic decomposition of gallic acid and hydrolysable

tannins occurs by different mechanisms. The first step is the decarboxylation of gallic acid to form pyrogallol, which is converted to phloroglucinol by pyrogallol-phloroglucinol isomerase and to dihydrophloroglucinol by phloroglucinol reductase. Then, dihydrophloroglucinol is converted to 3-hydroxy-5-oxohexanoate (HOHN) by diphloroglucinol hydrolase.

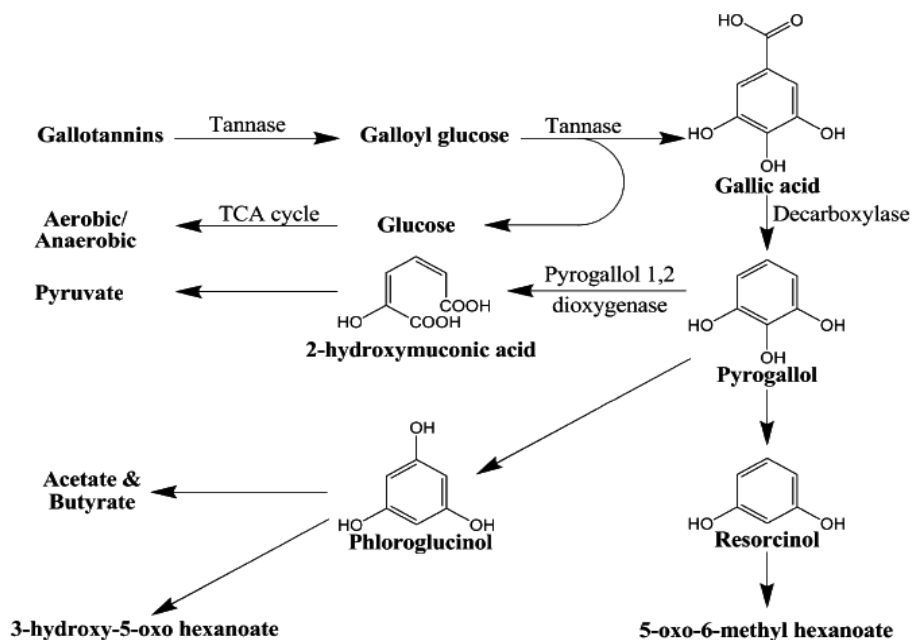


Fig. 2. Gallotannins biodegradation pathway (Mingshu *et al.*, 2006).

The HOHN is degraded by different pathways. In anaerobic conditions, HOHN is converted to 3,5-docosaheptaenoate or triacetate by the enzyme HOHN dehydrogenase and finally to three molecules of acetyl-CoA by the sequential enzymatic action of triacetyl-CoA transferase, triacetate β -ketothiolase, acetoacetyl-CoA β -ketothiolase, phosphotransacetylase, and acetate kinase (Brune and Schink, 1992). HOHN can be converted to butyrate and acetate in rumen system. HOHN-CoA is derived from the enzymatic action of HOHN-CoA transferase and is converted to acetate and butyrate by the rumen bacteria by sequential action of β -hydroxybutyryl-CoA dehydrogenase, butyryl-CoA dehydrogenase, acetyl-CoA acetyltransferase, enoyl Co-A hydratase, phosphate acetyl transferase and acetate kinase (Brune and Schink, 1992; Nelson *et al.*, 1995).

4 Tannase producer bacteria

Available literature on bacterial tannase indicates that most of the authors isolated and identified the microorganisms using an enrichment liquid medium, followed by selection in solid media.

First reported bacterium capable of hydrolyzing gallotannins as sole energy source was *Achromobacter* sp. (Lewis *et al.*, 1969). Deschamps *et al.* (1983), isolated bacterial strains able to hydrolyze tannic

acid and to produce tannase. In recent years interest on bacterial tannase has increase and new strains have been isolated from different sources. Strain of *Lactobacillus plantarum* isolated from olive mill waste was the first to be tested in tannase production (Ayed and Hamdi, 2002).

Lactic acid bacteria (LAB) have been isolated from specific habitats including food, plants, meat, human and animal feces (Pelinescu *et al.*, 2009). Cavin *et al.* (1993), isolated strains of *Lactobacillus* and *Pediococcus*, and demonstrated their ability to transform ferulic acid and *p*-cumaric acid to volatile phenols. Osawa *et al.* (2000), described tannins hydrolysis by *Lactobacillus* strains isolated from human feces and fermented foods. Also, Gaime-Perraud *et al.* (2000), demonstrated that polyphenolic content is decreased in coffee cherry husk by LAB.

LAB play an important role in food-tannin biodegradation. LAB possess the ability to hydrolyze tannins when they are into the intestine and to decrease their absorption to cells. Some LAB are used as inoculum in silages or as probiotic in ruminants (Weinberg *et al.*, 2004) due to their capability to grow, tolerate, and hydrolyze phenolic compounds. Vaquero *et al.* (2004), isolated from wine and grape wastes some tannase-producer LAB. The finding of tannin hydrolysis by LAB has sparked their use as tannase producer.

There exist some reports on bacterial tannase production using high-tannin content plant extracts. Deschamps *et al.* (1983), reported high tannase activity (0.5 U/ml) by *Klebsiella* using chestnut bark extracts as sole carbon source. Mohapatra *et al.* (2006), showed tannase activity upper than 0.6 U/mL by *Bacillus subtilis* KBR6 and using crude extracts of *Anacardium occidentale*. These works are an interesting way to produce high tannase activity by using cheaper tannin sources.

The specific case of bacterial tannase production, many reports have indicated that the enzyme is completely extracellular. In recent years interesting studies done by Belur *et al.* (2010) and Belur *et al.* (2012) reported cell associated tannase by *Serratia ficaria* and *Bacillus massiliensis*. Cell-associated tannase is probably related to microorganism growth and reaches the peak in late stationary phase (Belur *et al.*, 2010). Until now there are not works related to intracellular bacterial tannase.

5 Bacterial tannase production: optimization process

Due to the immense potential of bacterial tannase application is necessary the development of processes to optimize the enzyme production. First bacterial tannase optimization work included the effect of glucose and tannic acid concentration, and also the pH effect, reaching a maximal tannase activity of 6 U/mL by *Lactobacillus plantarum* (Ayed and Hamdi, 2002). From this work many studies include an optimization step for physical culture conditions and media.

Conventional methods for optimization of medium and fermentation conditions imply vary one parameter at the time keeping constant the others. Authors have reported the use of this methodology for tannase production (Selwal *et al.*, 2010; Beniwal *et al.*, 2010). However, the process is time consuming and expensive. It also does not take into account the combined interactions between various physicochemical parameters (Rao *et al.*, 2008).

Taguchi design of experiments (DOE) is an orthogonal array (OA) that consists in the study of any system given by a set of independent variables (factors) over a specific region of interest (levels) (Taguchi, 1986). This focus allows identifying the influence of individual factors, and also establishing the relationship between variables and operative conditions, also experimental data ANOVA gives a statistic relationship of system production. Das

Mohapatra *et al.* (2009), applied this methodology to optimize the production process of tannase by *Bacillus licheniformis* KBR6 testing six factors (pH, temperature, tannic acid, phosphate, nitrogen and magnesium). Taguchi methodology allowed them to increase tannase production 2.58-fold under optimized conditions.

Response Surface Methodology (RSM) is an efficient tool for optimization of different parameters. It is an experimental design upper than conventional methods. RSM is used to determine the influence of factors over the response and to optimize these variables in order to achieve maximum yield under the best possible economic conditions (Naidu *et al.*, 2008). In bacterial tannase production, Raghuwanshi *et al.* (2011) reported the higher tannase activity by *Bacillus sphaericus* employing RSM. They increased the enzyme activity 9.26-fold compared with unoptimized conditions.

Literature review suggest that optimal conditions for tannase production can vary considerably depending on the microorganism, cultural conditions, type of fermentation, and experimental process.

6 Molecular and physicochemical characteristics

The most studied topic on tannase is the related to its physicochemical characteristics. Fungal and bacterial tannases have been characterized and purified. Important differences corresponding to microorganisms and culture conditions have been found in both tannases (Aguilar *et al.*, 2001).

An important characteristic of bacterial tannases is their tolerance to temperatures between 30-50 °C (Kumar *et al.*, 1999; Sabu *et al.*, 2006; Raghuwanshi *et al.*, 2011). However important activities reaching 50% of effectiveness have been observed in the range of 25 to 80 °C (Iwamoto *et al.*, 2008; Raghuwanshi *et al.*, 2011). Optimal pH for bacterial tannases has resulted to be between 3-7 (Mondal *et al.*, 2001). Raghuwanshi *et al.* (2011), reported that *Bacillus sphaericus* tannase reach the maximal activity at pH 8. Tannase obtained from *Lactobacillus plantarum* showed a retention activity of 88% at pH 9 (Iwamoto *et al.*, 2008).

Molecular characteristics of bacterial tannases have been studied. Noguchi *et al.* (2007) were the first in cloned the tannase gene *tanA* from *Staphylococcus ludgunensis* that encode to a protein with 613 amino acid residues.

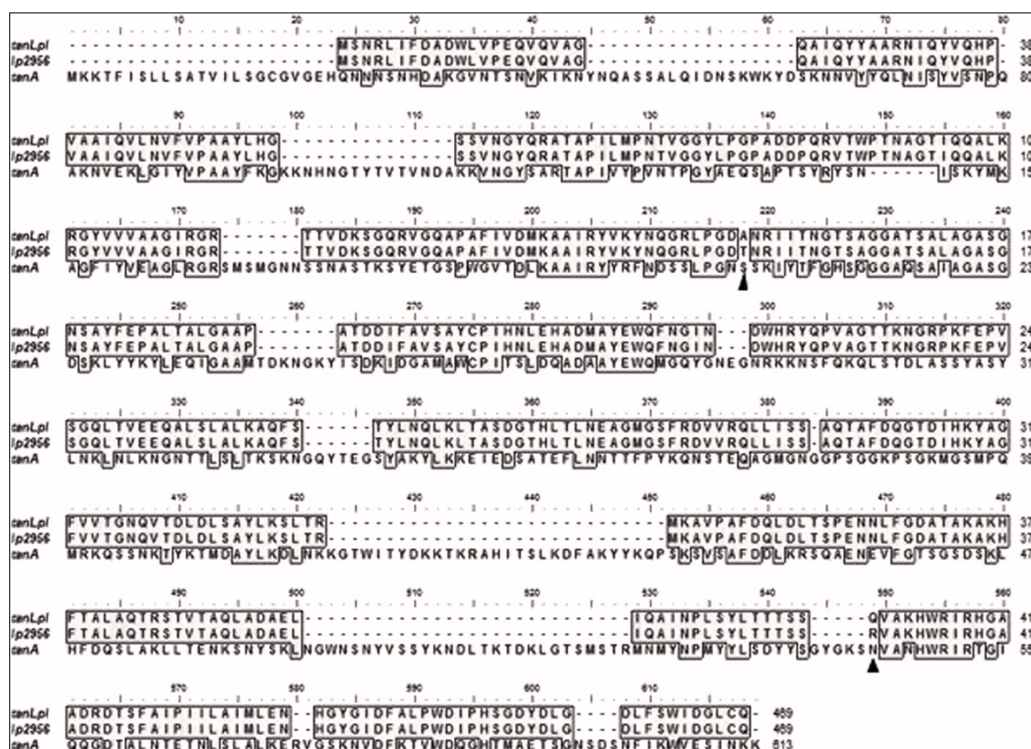


Fig. 3. Comparison of amino acid sequences of *tanLpl* and *lp2956* genes obtained from *L. plantarum* WCFS1 and *TanA* gene from *S. ludgunensis* (Iwamoto et al., 2008).

Later, Iwamoto et al. (2008) searched for a similar sequence to *tanA* gene in *Lactobacillus plantarum* WCFS1 complete genome. They found a DNA fragment called *lp2956*, from a hypothetical protein, with 46.7% of similarity to *tanA*. Then, they cloned on *E. coli* the responsible gene for tannase activity obtained from *L. plantarum* ATCC 14917 (CECT 748). The new gene was called *tanLpl* and encoded for a residue of 469 amino acids with estimated molecular weight of 50,747 Da. The amino acid sequence analysis revealed an identity of 99.6% between genes *tanLpl* and *lp2956* (Fig. 3). Those amino acid differences showed some changes in catalytic conditions of the enzyme modifying its optimal pH and temperature (Iwamoto et al., 2008).

Curiel et al. (2009) described some changes in *tanLpl* gene in relation to previous works. Changes were over positions 801 (C to T) and 962 (T to C) in the sequence of *L. plantarum* ATCC 14917. The changes caused the substitution of amino acids in the position 321 (Val-321 to Ala) in the corresponding protein despite it was the same strain of *L. plantarum* studied previously. Evolution could be the reason of those changes.

The amino acid sequence (623 amino acid residues) of *tanA* gene is longer than *tanLpl* (469 amino acids). However, both genes possess similarities in their sequences but showed no significant similarities to other tannases deposited in the databases, including those from bacteria or from fungi. For example, low *K_m* values of *L. plantarum* tannase compared to *A. oryzae* tannase, suggest that the binding site between bacterial enzyme and substrate assumes a different conformation than fungal tannase. Hence, probably the tannase from *L. plantarum* can act as a monomer while it is well known that *A. oryzae* tannase consist in two units linked by a disulfide bond (Hatamoto et al., 1996). Therefore, *tanLpl* from *L. plantarum* and *tanA* from *S. ludgunensis* may have different catalytic residues than bacterial and fungal tannases, and are thus classified into a novel family of tannases (Rodriguez et al., 2011).

7 Applications

Use of tannase is centered in food industry, leather and pharmaceutical. Actually, main applications of tannase are elaboration of instantaneous tea, acorn

wine and gallic acid production. Also, tannase is used as clarifying agent in juice and coffee flavored beverages (Aguilar *et al.*, 2001; Belmares *et al.*, 2004).

Gallic acid is used in pharmaceutical industry as an intermediary compound for the synthesis of trimethoprim; in chemical industry is used as substrate for the chemical or enzymatic synthesis of propyl-gallate and other antioxidant compounds with varying applications. Gallic acid is used in manufacture of semiconductor, inks and as photographic developer. Studies have demonstrated that gallic acid possesses important therapeutic properties (Abdelwahed *et al.*, 2007; Banerjee *et al.*, 2007; Yu and Li, 2008).

Use of tannase on beverages and foods helps to diminish the undesirable effects of tannins. Application of tannase on manufacturing of instantaneous tea eliminates insoluble precipitates formed when the beverage is cooled at 4 °C. Precipitates are formed by interaction of phenolic compounds and caffeine. Tannase treatment breaks the ester bonds of polyphenols avoiding its polymerization and complex with caffeine. Chemical processes for diminution of precipitates in tea can delete some aromatic compounds. However, the enzyme treatment allows obtaining a cold water soluble tea with high content of aromatic compounds and appropriate color (Aguilar *et al.*, 2001).

Reduction of bitter in fruit juices by enzymatic treatment possesses the advantage of increasing the quality of the beverages. High concentration of tannins in fruits, such as blueberry, pomegranate and raspberry, leads to the formation of sediment, color and bitter taste during the storage of its juices. In these cases is recommended an enzymatic treatment with tannase (Aguilar *et al.*, 2007). Rout and Banerjee (2006), reported a reduction of 25% of tannin content in pomegranate juice by using a treatment with tannase, while by using tannase and gelatin the tannin content diminishes in 49%.

Some varieties of sorghum possess high content of tannins and are not able for use in animal feed because of its bad nutrition effects and also for bioethanol production due to its antimicrobial effect. The treatment of sorghum with tannase or some microorganisms capable to produce the enzyme is the best way to decrease the tannin content and thus use it as a complement for animal feed (Aguilar *et al.*, 2001) and for bioethanol production (Chuck-Hernández *et al.*, 2011). Finally, tannase has been used in environmental biotechnology for the treatment of tannery effluent (Aissam *et al.*, 2005).

Table 2. Novel applications of tannase.

Applications	Reference
Identification of <i>Staphylococcus lugdunensis</i> in humans.	Noguchi <i>et al.</i> (2007)
Indicator of colon cancer.	Noguchi <i>et al.</i> (2007)
Production of derived Prunioside A esters.	Jun <i>et al.</i> (2007)
Manufacture of laundry detergent	Dykstra <i>et al.</i> (2011)
Manufacture of cosmetics	Dykstra <i>et al.</i> (2011)
Leather industry.	Dykstra <i>et al.</i> (2011)
Treatment of agroindustrial wastes for ethanol production.	Tejirian and Xu, (2011)

Novelty applications of tannase have been developed (Table 2). In recent years, ethanol production as fuel from agroindustrial wastes has gained importance. Pre-treatment of agroindustrial wastes generates phenols from lignin. It is well known that phenols inhibit the hydrolytic activity of cellulase. Tannase can be used for degradation of those phenolic compounds and thus increase the catalytic activity of cellulase (Tejirian and Xu, 2011).

Tannase gene and the enzyme activity can be employed for the identification of *Staphylococcus lugdunensis* in humans and to prevent the colon cancer (Noguchi *et al.*, 2007). Tannase has also been employed for the production of molecules with therapeutic applications, such as prunioside A with anti-inflammatory activity (Jun *et al.*, 2007). Other potential applications of tannase are found in the manufacture of laundry detergents as an additive and in the leather industry to homogenize the tannins preparation for high grade leather tannins (Dykstra *et al.*, 2011).

Final remarks

Since tannase was discovered in 1897 many researcher groups have developed studies to produce the enzyme by using microorganisms. Production of bacterial tannase is a less studied topic. The use of bacterial strains for production of tannase possesses the advantage of a short period time of fermentation and extracellular enzyme. Optimal conditions of fermentation and tannase titles are different between bacterial species. Hence, the better culture conditions should be tested for each bacterium. In view of growing demand of tannase and its potential applications in food, pharmaceutical and chemical industries, it is necessary

to isolate new bacterial strains capable to produce high titles of enzyme.

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