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4 **Bacterial Tannases: Classification and biochemical properties**

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Abstract Tannin acyl hydrolases, also known as tannases, are a group of enzymes critical for the transformation of tannins. The study of these enzymes, which initially evolved in different organisms to detoxify and/or use these plant metabolites, has nowadays become relevant in microbial enzymology research due to their relevant role in food tannin transformation.

Microorganisms, particularly bacteria, are major sources of tannase. Cloning and heterologous expression of bacterial tannase genes and structural studies have been performed in the last few years. However, a systematic compilation of the information related to all recombinant tannases, their classification and characteristics is missing. In this review, we explore the diversity of heterologously produced bacterial tannases, describing their substrate specificity and biochemical characterization. Moreover, a new classification based on sequence similarity analysis is proposed. Finally, putative tannases have been identified *in silico* for each group of tannases taking advantage of the use of the “tannase” distinctive features previously proposed.

Keywords Tannase • Feruloyl esterase • Esterase • Hydrolysable tannins • Gallic acid

Introduction

Tannins are secondary metabolites that represent the fourth most abundant plant constituent, after cellulose, hemicellulose, and lignin (Lekha and Lonsane, 1997). They are widely distributed in the plant kingdom and found in nearly every part of the plant, such as roots, wood, fruits, seeds, and leaves (Serrano et al 2009). Structurally, this group of phenolic compounds could be divided into hydrolysable and condensed tannins. Hydrolysable tannins are comprised of a polyol carbohydrate core (usually D-glucose) esterified to phenolic acids such as gallic acid or ellagic acid, forming gallotannins and ellagitannins, respectively (Mingshu et al. 2006). In gallotannins, gallic acid or its polymerized derivatives can be esterified to polyol, catechin, triterpenoid, or flavonoid units to form hydrolysable tannins such as tannic acid, one of the most abundant reserve materials of plants.

Tannins are generally considered recalcitrant to biodegradation. However, despite their toxic effects on various organisms, some microorganisms have evolved to use gallotannins as carbon and energy sources for growth by the action of a family of enzymes, the tannin acyl hydrolases, commonly known as “tannases” (Yao et al. 2014a). Tannase, the most studied enzyme in tannin biodegradation, catalyses the hydrolysis of ester and depside bonds in varied substrates like gallotannins, gallic acid esters, epigallocatechin gallate, and epicatechin gallate leading to the release of gallic acid and glucose. The amino acid sequence analysis of bacterial, yeast, and fungal tannases showed that although the enzymes display rather divergent sequences, they share a common pentapeptide active-site motif of Gly-X-Ser-X-Gly, a typical feature for serine hydrolases (Ren et al. 2013).

The majority of industrial enzymes are classified as “hydrolases”, and “tannases” belong to this class. Since their discovery more than a century ago, tannases have been used in the food, feed, beverage, pharmaceutical and chemical industries. Among the commercial applications of tannases, the most relevant are the production of gallic acid, instant tea, and coffee-flavored refreshing drinks. Moreover, these enzymes are used for the clarification of beer and fruits juices, the improvement of flavor of grape wine, and the manufacture of animal feeds (Jana et al. 2014).

Tannase production can be achieved from microbial, animal and vegetal sources, but microorganisms are commonly used for its commercial production. Microorganisms are the main source of industrial enzymes due to their biochemical diversity and their amenability to genetic modifications. To date, commercially available tannases are mainly produced by the fermentation of fungi, especially the *Aspergillus* species, from which only crude enzymes are readily produced (Aguilar et al. 2007). Recombinant expression appears to be an appealing alternative to produce pure enzymes, however, the attempt to clone fungal tannase genes has proven difficult and inefficient, probably due to the complexity of fungal enzymes (Aguilar et al. 2007, Hatamoto et al. 1996, Zhong et al. 2004). Therefore, bacterial tannases might constitute an adequate alternative to fungal enzymes. Research on bacterial tannases has been primarily focused on the screening of new tannase-producing bacterial strains with the desired properties. The selected tannase-producer strains are often employed either directly or using their crude cellular extracts, without enzyme isolation. Surprisingly, little knowledge is available regarding the bacterial enzyme at the molecular level, as only few tannase genes have been cloned and expressed in recombinant form. This lack of knowledge could be one of the critical factors limiting the large-scale application of bacterial tannases.

Up to now, most reviews on tannases have focused on tannin hydrolysis, biodegradation, screening and identification, as well as their production, regulation, immobilization, and upstream and downstream processing with the strongest emphasis on industrial applications and patentability (Aguilar et al. 2001, Belmares et al. 2004, Aguilar et al. 2007, Natarajan et al. 2008, Rodríguez-Durán et al. 2011, Belur and Mugeraya, 2011, Prasad et al. 2012, Chávez-González et al. 2012, Beniwal et al. 2013, Chandrasekaran and Beena 2013, Aguilar-Zárate et al. 2014, Jana et al. 2014, Zhang et al. 2015). However, little is known about tannases at the molecular level and very few tannase genes have been cloned and tested for recombinant expression. The present review is an attempt to focus current knowledge of bacterial proteins that have been recombinantly produced and whose tannase activity has been biochemically confirmed.

Tannase-producing bacteria

The initial description of bacterial strains possessing tannase activity was reported by Deschamps et al. (1980) showing microorganisms capable of utilizing tannic acid as the sole carbon source. Since then, several reports have been published on bacterial tannase production and about 60 new tannase positive bacteria have been isolated (Online resource Table S1). Among tannase-producing isolates, tannase proteins have only been identified and characterized in a few of these species. Tannase-producing bacteria have been isolated from a wide variety of sources such as soil, wastewater, compost, forest litter, feces, beverages, pickle, etc. Initially, the first requisite for the isolation of novel strains is the existence of an efficient isolation technique. The available literature on bacterial tannases indicates that most authors isolated and identified the microorganisms using and enrichment liquid medium, followed by selection in solid media. The method of choice for

113 screening and the isolation of tannase producing bacteria varied from author to author.

114 Generally, all these methods are limited to cultivable microbes, although researchers have
115 recently identified bacterial tannase genes from uncultivable microorganisms using
116 metagenomics approaches (Yao et al. 2011).

117 Although the trend nowadays is to identify organism with the highest tannase
118 activities, debate has emerged due to the fact that assay protocols are both substrate and
119 wavelength specific and may not be appropriate to make any activity comparison between
120 tannases when applying different techniques. Different methods have been used to
121 evaluate the activity of tannases. Initial reports used the growth on media containing
122 tannins as an indicator of tannase activity (Online resource Table S1). Another commonly
123 used activity assay is based on the measurement of residual substrate (tannic acid) instead
124 of product quantification after enzymatic reaction. However, the method of choice should
125 determine the product of tannase reaction (gallic acid). In order to accomplish this, and
126 obtain a reliable quantification of tannase activity, the most prevalent method is the
127 colorimetric determination of gallic acid using a rhodanine assay (Inoue and Hagerman
128 (1988). Rhodanine reacts with the vicinal hydroxyl groups of gallic acid to produce a red
129 complex with a maximum absorbance at 520 nm. The red color appears only in the
130 presence of free gallic acid being absent when other gallic acid esters or phenolics are
131 present in the reaction. Therefore, tannase activity is measured by determining the quantity
132 of gallic acid formed after hydrolysis on tannins (Rodríguez et al. 2008). When the activity
133 of tannase against other potential substrates is evaluated, a more sensitive method should
134 be applied, such as HPLC-DAD. In this case, the identification of tannase products should
135 be carried out by comparing retention times and spectral data of each peak to the standards
136 obtained from commercial suppliers.

The search for new tannase proteins with the desired properties, such as optimal pH or activity, has been the focus of studies on tannases since their discovery. Recombinant DNA methodology has allowed the improvement of native proteins, therefore this methodology will be useful for the improvement of bacterial tannases once their encoding gene has been identified.

Recombinant proteins possessing tannase activity

Despite numerous bacteria have been described to possess tannase activity by the use of different techniques, only during the last years the protein sequences of some of them have been reported. The knowledge of the protein sequence allowed the recombinant production of these proteins in a heterologous host. The association of a pure protein with tannase activity on different substrates lead us to focus this review only on these proteins whose tannase activity has been unequivocally demonstrated.

Currently 12 bacterial proteins possessing tannase activity have been recombinantly produced and biochemically characterized. Among these, we find proteins from a diverse range of bacterial species, from the phyla Firmicutes (*Lactobacillus plantarum*, *Streptococcus gallolyticus* strains, among others), Actinobacteria (*Atopobium parvulum*, *Streptomyces sviveus*), and Fusobacteria (*Fusobacterium nucleatum*). In addition, a tannase protein from a cotton-field metagenomic library, Tan 410, has also been hyperproduced and characterized.

The initial classification of these proteins could be made based on the biochemical activity exhibited by the pure protein. The enzyme could only be able to hydrolyze esters bond on hydroxybenzoic acids (“tannase”) or show esterase activity on both hydroxycinnamic and hydroxybenzoic acids (“feruloyl esterase / tannase”). Up to now, ten

out 12 of these proteins exhibited only tannase activity, while two of them presented in addition, feruloyl esterase activity.

Tannases

A previous *in silico* study compared the primary structures (amino acid sequences) of proteins annotated as “tannase” in the NCBI database, 149 from bacteria and 36 from fungi. The multiple sequence alignment of these tannase protein sequences showed conserved regions along the polypeptides (Banerjee et al. 2012). Similarly, a multiple sequence alignment was performed in this work only for the 10 proteins in which tannase activity has been biochemically demonstrated. This alignment clearly revealed the existence of two different tannase subtypes based on their primary structure (Figure 1A). The first subgroup (subtype A) includes proteins from *Atopobium parvulum* DSM 20469^T (TanAAp) (Jiménez et al. 2014a), *Lactobacillus plantarum* ATCC 14917^T (TanALp) (Jiménez et al. 2014b), *Staphylococcus lugdunensis* MTCC 3614 (TanA) (Noguchi et al. 2007, Chaitanyakumar and Anbalagan 2016), and *Streptococcus gallolyticus* UCN34 (TanASg) (Jiménez et al. 2014d). The second subgroup (subtype B) is integrated by proteins from *Fusobacterium nucleatum* F0401 (TanBFn) (Tomás-Cortázar et al 2018), *Lactobacillus plantarum* (TanLpl or TanBLp) (Iwamoto et al. 2008, Curiel et al. 2009), *Lactobacillus pentosus* 21A-3 (TanLpe) (Ueda et al. 2014), *Lactobacillus paraplantarum* NOS 120 (TanLpa) (Ueda et al. 2014), *Streptococcus gallolyticus* UCN34 (TanSg1) (Jiménez et al. 2014c), and *Streptomyces sviveus* ATCC 29083 (Ss-Tan) (Wu et al. 2015). The existence of two tannase subtypes, subgroups or subfamilies was previously proposed (Iwamoto et al., 2008, Ueda et al. 2014, Jiménez et al. 2014b, 2014c, 2014d, Tomás-Cortázar et al. 2018). The comparative analysis made on lactobacilli tannases (TanLpl,

TanLpa, and TanLpe) revealed that these tannases represented a novel subfamily as they had a restricted diversity, forming a distinct cluster from other previously known tannases (TanA, TanASg, and TanAAp) (Ueda et al. 2014). Some of the newly described tannases were named as A or B attending their inclusion on these subgroups, i. e. TanAAp (Jiménez et al. 2014a), TanALp (Jiménez et al. 2014b), TanASg (Jiménez et al. 2014d), and TanBFnp (Tómas-Cortázar et al. 218).

The existence of two tannase subtypes revealed by protein sequence alignment is also supported by the identity degree exhibited among them (Figure 1B). As showed in Fig. 1B, all the proteins included in the same tannase subtype exhibited at least 34% identity among them. The level of identity among proteins included in the A subtype ranged from 38% (TanAAp vs. TanASg or TanALp) to 47% (TanASg vs. TanA). Proteins included in B subtype showed identities ranging from 34% (TanSg1 vs. Ss-Tan or TanLpl/TanBLp) to 88% (TanLpl/TanBLp vs. TanLpa). Of note, proteins belonging to different subtypes are also 27-33% identical, indicating that both subtypes are related and shared high similarity. Taking into account these two tannase subtypes, in this revision some tannase names were modified attending this criterion. Therefore, tannases were renamed as TanASl from *Staphylococcus lugdunensis* (formerly TanA), TanBLpe from *L. pentosus* (formerly TanLpe), TanBSg from *Streptococcus gallolyticus* (TanSg1), TanBLpa from *L. paraplantarum* (TanLpa), and TanBSs from *Streptomyces sviveus* ATCC 29083 (Ss-Tan).

The protein alignment showed in Figure 1A also reveals the existence of amino acid residues or motifs conserved among all the proteins included in a specific subtype, being these residues absent in the proteins belonging to the other tannase subtype (Figure 2). Figure 2 shows the amino acid alignment of two tannases that are representative of the two subtypes, the enzymes from *Lactobacillus plantarum* ATCC 14917^T (TanALp and

TanBLp). Amino acid residues conserved in all subtype A tannases are labelled on the TanALp sequence. Similarly, the amino acids conserved in all the tannase proteins from subtype B are labelled in TanBLp. Of note, it could be observed that there are amino acids conserved between TanALp and TanBLp which are not coloured as they are not conserved in all the tannases from the same subtype (Online resource Fig. 1 and 2). Importantly, this alignment allows the identification of residues conserved in all the bacterial tannases characterized so far. These residues are identified inside the box. There are 47 amino acid residues conserved in all functional bacterial tannases described so far. These amino acid signatures could be predictive of a protein having tannase activity. In addition to amino acid residues conserved among all bacterial tannases, the alignment on Figure 2 also allows the identification of residues only conserved in a specific tannase subtype while absent in any tannase from the other subtype (Figure 2).

As the TanBLp tridimensional structure have been solved, the alignment showed in Figure 1 also allows to decipher if the residues important for activity are conserved on these proteins (Ren et al. 2013). All the recombinant tannases, except TanAAp, possess the conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases (Figure 1). In TanAAp the active serine (Ser-136, in TanBLp) is substituted by an Ala residue. The catalytic triad identified in the TanBLp structure (Ser-163, Asp-419, and His-451) is only conserved in the subtype B of tannases. In A subtype proteins, Asp-419 is substituted by a Gln residue, with the exception of TanAAp with an Asn residue located in this position. The Asp to Gln amino acid variation has been previously reported, leading to the authors to suggest that the conserved residue Asp-421 may fulfil the role of Asp-419 as the acidic residue in the catalytic triad (Ren et al. 2013). The residues making contact with the three hydroxyl groups of gallic acid (Asp-421, Lys-343, and Glu-357, in TanBLp) are also conserved in all the B subtype tannases, while they are not fully conserved in tannases

belonging to A protein subtype. All the A proteins possess the Asp-421 residue, but Lys-343 and Glu-357 are not conserved in TanASl (Figure 1). Currently the relevance of these amino acid changes remains unknown. However, the absence of an aspartic acid residue on the catalytic triad of subtype A tannases is the most remarkable difference among both tannase subtypes.

The presence of several amino acid changes on residues important for activity in TanAAp led us to compare the amino acid sequence of TanAAp with sequences from other *Atopobium* species, such as *Atopobium minutum* (accession N2BXH5), *Atopobium rima*e (accession B9CMT1) or *Atopobium* sp. oral taxon 199 (accession S3A7X6) (data not shown). In all of these protein sequences the catalytic Ser-163 is conserved. Therefore, the Ser to Ala amino acid substitution on TanAAp might be a sequence mistake. However, the substitution of Lys-343 by a Leu residue is present in all the *Atopobium* sequences analysed. These amino acid changes in *Atopobium parvulum* tannase sequence might explain the very low specific activity showed by pure TanAAp protein (Jiménez et al. 2014d).

Subtype A tannases (absence of catalytic Asp)

Protein alignment showed that, in addition to sequence similarity and the absence of an Asp residue on the catalytic triad (as determined on TanBLp), proteins included in subtype A also shared several characteristics. They are all proteins around 600 amino acid long and 65 kDa of molecular size, ranging from the 596 amino acids of TanASg (63.6 kDa) to the 626 amino acids on TanALp (67.4 kDa) (Table 1). More interestingly, all tannases from subtype A contain a typical Gram-positive signal peptide. A search for conserved domains in the subtype A tannases performed in the National Center for Biotechnology internet site

rendered no putative conserved domains in any tannase with the exception of TanAAp. In this tannase, two putative conserved domains were found, Aes (acetyl esterase/lipase) and Abhydrolase_3 (alpha/beta hydrolase fold) (Table 1).

In order to find additional bacterial proteins putatively included in the A tannase subtype, the FASTA program was used as a tool to provide sequence similarity searching against protein databases. From the search, only proteins from public collection bacteria were retrieved in order to avoid mistakes due to a species misidentification of a specific strain. This search allowed retrieving, apart from lactic acid bacteria, proteins from *Actinomyces naeslundii* ATCC 12104, *Corynebacterium vitaeruminis* DSM 20294, *Methanobrevibacter millerae* SM9 (Kelly et al. 2016), *Methanolobus tindarius* DSM 2278, and *Slackia heliotrinireducens* ATCC 29202 (Table 1). These bacteria belong to the phyla *Firmicutes*, *Actinobacteria*, and *Archaea*. As other members of the A tannase subtype, these proteins are around 600 amino acids long, most of them contain a signal peptide and no putative conserved domains (Table 2). It should be mentioned that, apart from *Methanobrevibacter millerae* (Kelly et al. 2016), these bacterial species have never been associated to tannase production.

When these protein sequences were aligned along characterized subtype A tannases we observed that the amino acid residues that were conserved among characterized A subtype tannases, are also widely conserved in all the proteins retrieved from the databases. This residue conservation might support the presence of tannase activity of these proteins. More importantly, the alignment confirms that the absence of an aspartic acid residue in the catalytic triad is a general feature of subtype A tannases (Online resource Figure S1).

- TanAS1

Tannase activity has been functionally demonstrated on four proteins from subtype A. Tannase from *Staphylococcus lugdunensis* (TanASl, formerly TanA) was first recombinantly produced in 2007 (Noguchi et al. 2007), but until 2016 it was not biochemically characterized (Chaitanyakumar and Anbalagan 2016). The enzyme TanASl was produced heterologous as a 6x His-tagged protein having a specific activity of around 700 U/mg (Table 2). Pure TanASl enzyme exhibited an optimal temperature in the range of 40 °C. At this temperature, the effect of pH was measured. The highest activity for the pure enzyme was found at pH 7.0. As for the effect of additives, only SDS among the detergents assayed increased TanASl activity by 21%. On the other hand, the presence of EDTA reduced TanASl activity by 15%. Out of several metal ions tested, Na²⁺ increased the enzyme activity by 10% at 1 mM concentration. According to its classification as a member of the serine hydrolase group, β-mercaptoethanol completely abolished tannase activity. Non-polar solvents such as hexane, toluene and benzene significantly increased the activity of TanASl at a 20% concentration. However, polar solvents such as methanol, DMSO, isoamyl alcohol and butanol inhibited enzyme activity. Immobilization of TanASl tannase with calcium alginate beads shows protection of the enzyme under a wide range of pH and temperatures (Chaitanyakumar and Anbalagan 2016). TanASl activity has only been tested using the colorimetric rhodanine assay specific for gallic acid liberation from methyl gallate while no other substrates were assayed.

- TanALp

In 2014, the tannase TanALp from *Lactobacillus plantarum* was identified (Jiménez et al. 2014b). The *tanALp* (HPREF0531_11477) gene lacking the 22-amino acid

peptide signal sequence was expressed in *E. coli* as a recombinant protein containing a 6x His-tag at its N-termini. The protein was scarcely purified from the affinity purification resin (2.71 mg/L) (Table 2). Biochemical characterization of TanALp was determined by the rhodanine assay by using methyl gallate as the substrate. TanALp displays optimal activity in terms of temperature between 20 to 30 °C and an optimal pH around 6. The specific activity of TanALp was 39 U/mg, only a 9.6% of the specific activity showed by TanBLp (Jiménez et al. 2014a). In relation to the effect of additives on TanALp activity, while Ca²⁺ greatly increased it Hg²⁺ completely inhibited tannase activity. This activity was also 60% reduced by the presence of EDTA. Similarly to other serine hydrolases, tannase activity was abolished by β-mercaptoethanol (90%). Activity was also increased by the presence of Triton-X-100 (50%) and DMSO (30%). As for its substrate range, from the esters assayed, only esters derived from gallic and protocatechuic acids were hydrolysed. Cinnamic acids without hydroxyl groups and with substituents other than –H and –OH at position 2 were not metabolized by TanALp. Regarding the aliphatic alcohol constituent of the ester bond, a lauryl substituent could not be effectively hydrolysed by TanALp (Jiménez et al. 2014b).

- TanAAp

The tannase from *Atopobium parvulum* DSM 290468^T (TanAAp) was also biochemically characterized on 2014 (Jiménez et al 2014a) (Table 2). The *tanAAp* (*apar_1020*) gene was cloned incorporating the DNA sequence into a vector encoding 6-His to create a His-tagged fusion enzyme for further purification step. Around 12 mg of purified protein was routinely obtained from 1-liter cultures (Table 2). Using methyl gallate as substrate, the specific activity of TanAAp purified enzyme was 3.5 U/mg, 116

times lower than that of TanBLp (Jiménez et al. 2014a) with an optimal activity at pH 6 and 55 °C. According to its thermal stability profile, TanAAp exhibited high thermal stability under prolonged incubation up to 45 °C. Despite having a very low specific activity, TanAAp is thermostable, being able to resist thermal unfolding in the absence of its substrate. Tannase activity was not increased by any of the additives assayed. However, its activity was greatly inhibited by β -mercaptoethanol and Hg^{2+} and Zn^{2+} metal ions. In order to know the substrate specificity of TanAAp, several gallate and protocatechuate esters were assayed. None of the esters assayed were significantly hydrolysed, probably due to its low specific activity.

- TanASg

Streptococcus gallolyticus is a common tannin-degrader inhabitant of the herbivores gut on which two different tannase enzymes have been described. TanASg tannase was biochemically characterized first (Jiménez et al. 2014d). The *tanASg* (GALLO_0933) gene was cloned into a vector introducing a 6x His N-terminal affinity tag on the protein. The rhodanine assay was used to determine the specific activity of TanASg. Using methyl gallate as substrate, the specific activity of TanASg was 256 U/mg (Table 2). The biochemical characterization of TanASg revealed an optimal pH around 6, being also highly active at pH 7-8. Maximal activity occurred at 37 °C, although the enzyme also retained very high activities at higher temperatures. Non-ionic detergents showed different effect on TanASg activity. Whereas Triton-X-100 did not affect activity, Tween-80 greatly increased activity. Among metal ions, Ca^{2+} and K^{+} increased the activity while Hg^{2+} induced a severe inhibition. The ions Zn^{2+} and Mg^{2+} , and β -mercaptoethanol partially inhibited tannase activity. The TanASg protein was incubated in the presence of

different esters of gallic acid to ascertain the tannase substrate range. As expected for a tannase enzyme, TanASg was able to hydrolyze esters from gallic acid. However, the enzyme was unable to hydrolyze esters containing an alcohol substituent as long as lauryl. As a general rule of the bacterial tannases described so far, a protocatechuate ester (ethyl protocatechuate) was also hydrolysed by subtype A tannases. TanASg is also able to hydrolyze natural hydrolysable tannins, such as tannic acid and epigallocatechin gallate.

The biochemical characterization of all recombinant subtype A tannases revealed that they showed different and specific properties. The proteins found in the databases similar to subtype A and included in Table 1 (and in Online resource Fig S1) could constitute a new source of tannase proteins that might possess specific and desired biochemical properties.

Subtype B tannases (with catalytic Asp)

Similarly to subtype A enzymes, the proteins included in the B subtype group based on their protein alignment, generally shared common characteristics and possessed all the amino acid residues important for activity (active pentapeptide, catalytic triad, residues making contact with gallic acid, etc). As an example, they are proteins shorter than those in the subtype A group, ranging from 470 to 570 amino acid residues long, with a molecular size that ranges from 50 to 60 kDa (Table 2). Generally, they do not possess a signal peptide, except the recently described TanBFn (Tomás-Cortázar et al. 2018). Contrarily to most of the subtype A tannases, on subtype B proteins the putative Aes (acetyl esterase/lipase, COG0657) conserved domain was detected when a search was carried out in the NCBI site.

In order to retrieve proteins similar to subtype B tannases a search was performed on protein databases. The search revealed the existence of a larger number of putative proteins than in the case of subtype A. Similarly, only proteins from public collection of bacteria were selected (Table 3). Proteins from bacteria belonging to the phyla Firmicutes (*Hungatella hathewayi* DSM 13479, *Mitsuokella multocida* DSM 20544, and several lactic acid bacteria belonging to the *Lactobacillus* and *Oenococcus* genera), Proteobacteria (*Actinobacillus succinogenes* ATCC 55618/130Z, *Aggregatibacter segnis* ATCC 33393, *Asticcacaulis excentricus* ATCC 15261, *Campylobacter concisus* ATCC 51562, among others), Actinobacteria (*Gordonia amarae* NBRC 15530, *Mycolicibacterium vaccae* ATCC 25954, and *Streptomyces avermitilis* ATCC 31267), Fusobacteria (*Leptotrichia buccalis* ATCC 14201), and Bacteroidetes (*Capnocytophaga granulosa* ATCC 51502, *Flavobacterium rivuli* DSM 21788, and *Runella slithyformis* ATCC 29530) were found (Table 3). As showed in Table 3 these proteins are generally shorter than subtype A proteins (ranging from 427 to 589 amino acid residues) and, at minimum, the putative conserved domain Aes (COG0657) was present on them. However, the absence of signal peptides among the subtype B proteins currently characterized, was not a general feature of these proteins. Most of the similar proteins found in the databases (16 versus 12 proteins) possess a clear signal peptide, similar to the one recently characterized TanBFnp tannase (Tomás-Cortázar et al. 2018).

A multiple alignment of these proteins revealed that most of the amino acid residues conserved on the characterized B subtype tannases, were also conserved in similar proteins found in the databases supporting the existence of two tannase subtypes. Interestingly, in contrast with subtype A all proteins have an aspartic acid residue in the catalytic triad (Online resource Figure S2). Six bacterial tannases belonging to the B subtype have been recombinantly produced and biochemically characterized.

- TanBLp

Lactobacillus plantarum and *Streptococcus gallolyticus* strains contain tannase enzymes from both subtypes. All the *S. gallolyticus* strains produce both tannase proteins (TanASg and TanBSg) (Jiménez et al. 2014d), whereas TanALp is present only in some *L. plantarum* strains (Jiménez et al. 2014b). Tannase TanBLp (formerly TanLp1) from *Lactobacillus plantarum* ATCC 14917^T was expressed in *E. coli* and purified by ammonium sulphate precipitation (Iwamoto et al. 2008). The production yield was 2.97 mg/L of tannase with a specific activity of 84.3 U/mg (Table 2). The maximum activity of TanBLp was found to be at an approximately pH of 8.0 and a temperature of 40 °C. The enzyme retained more than 50% of its activity between 25 and 45 °C but it dropped markedly above 45 °C. TanBLp showed a 0.62 mM K_m value. Later, the same protein was overproduced using a different expression vector, which produced the recombinant protein attached to an amino-terminal His-tag that allows convenient purification of the native protein directly from crude cell extracts (Curiel et al. 2009). The one-step protocol used produced 17 mg of recombinant protein with a specific activity of 408 U/mg (Jiménez et al. 2014a) (Table 2). The sequence of TanBLp tannase contained a nucleotide change producing a conservative amino acid substitution at position 321 (Val-321 to Ala). This newly prepared TanBLp tannase presented an optimal pH of around 7, being also highly active at pH 6-8. This pH value is similar to the one reported for the previously purified TanBLp (pH 8.0) (Iwamoto et al. 2008). Although the dissimilar optimum pH may result from the described amino acid substitution, the effect of experimental factors such as the different buffer systems or the standard assay used for the determination cannot be discarded. The optimal temperature was found to be 40 °C, in agreement with previous

results (Iwamoto et al. 2008). The effect of additives on the activity of the new TanBLp preparation revealed that enzymatic activity was increased by K^+ and Ca^{2+} , while not significantly affected or partially inhibited by EDTA, Mg^{2+} , Zn^{2+} , Triton-X-100, Tween 80, and DMSO, and greatly inhibited by Hg^{2+} and β -mercaptoethanol. From the 18 different simple phenolic acid esters assayed only esters derived from gallic acid and those derived from protocatechuic acid were hydrolysed (Curiel et al. 2009). It seems that there are not significant esteric hindrances in TanBLp regarding the aliphatic alcohol constituent of the ester bond, as both ethyl and lauryl substituents could be effectively hydrolysed by *L. plantarum* TanBLp tannase. Tannic acid, gallo catechin gallate, and epigallocatechin gallate were also metabolysed by TanBLp tannase. This enzyme was stabilized by multipoint covalent immobilization on highly activated glyoxyl agarose (Curiel et al. 2010).

- TanBLp, TanBLpa, and TanBLpe

Ueda et al (2014) identified tannases in other lactobacilli species, such as *Lactobacillus paraplantarum* (TanBLpa, formerly TanLpa) and *L. pentosus* (TanBLpe, formerly TanLpe) and compared them with TanBLp (TanLpl) from *L. plantarum*, also performing structural and enzymological characterization. The comparative analysis of these proteins revealed that the lactobacilli tannases represented a novel subfamily of tannases as they formed a distinct phylogenetic cluster among some of the previously known tannases (TanASl, TanASg, and TanAp). The tannases from the three lactobacilli species were recombinantly produced in *Bacillus subtilis* as C-terminal His-tag fusion proteins with N-terminal secretion signal peptide (Ueda et al. 2014). Enzymatic properties of these lactobacilli tannases were investigated using methyl gallate as substrate (Table 2).

TanBLp and TanBLpa showed maximum activities at pH 8.5 and at 40 °C, whereas those of TanBLpe were optimal at pH 8.0 and at 35 °C. Although enzymes from *L. plantarum* and *L. paraplantarum* sustained more than 80% of their enzymatic activities at a pH range of 8.0-10.0, the enzyme from *L. pentosus* drastically lost its activity above pH 9.0. The tannase activities of the purified recombinant enzymes were not significantly affected (albeit partially inhibited) by PMSF, EDTA, and Mn^{2+} , Ca^{2+} , and Mg^{2+} ions. The presence of Zn^{2+} reduced TanBLp and TanBLpe activity, by 46% and 25%, respectively. The effect of the Hg^{2+} ion was not studied. All three recombinant tannases were greatly inhibited in the presence of 1 mM $FeSO_4$. The enzymes were active against methyl gallate, and catechin galloyl esters (epicatechin gallate, epigallocatechin gallate, catechin gallate, and gallocatechin gallate) (Table 2). The different kinetic properties among these tannases suggest that small differences in the amino acid sequences of tannase can exert some drastic effects. TanBLpa had markedly higher catalytic activity than TanBLp and TanBLpe against some galloyl esters of green tea catechins (Ueda et al. 2014).

- TanBSg

The gene in the *locus* GALLO_1609 from *Streptococcus gallolyticus* UCN34 was cloned and expressed in *E. coli* (Jiménez et al. 2014c). The recombinant strain produced a His-tagged TanBSg (formerly TanSg1) tannase protein purified by affinity chromatography. Twelve milligrams of purified protein were routinely obtained from one liter cultures (Table 2). Tannase activity was assayed by a rhodanine assay in order to determine free gallic acid. Using methyl gallate as substrate, the specific activity of TanBSg-purified enzyme was 577 U/mg. The optimum temperature for TanBSg was 45 °C, while 60 and 90% of the maximal activity was shown at 4 and 65 °C, respectively.

Regarding stability, the enzyme surprisingly showed about 50 and 25% of the maximal activity after 6 and 18 h of incubation at 45 °C, respectively. In terms of pH influence, the enzyme was active between pH 4 and 9, with an optimal pH around 7. Of the various metal ions and additives tested, the activity of TanBSg was increased in the presence of Ca^{2+} , DMSO and Tween-80, being strongly inhibited by Zn^{2+} , Hg^{2+} and β -mercaptoethanol. The substrate specificity of TanBSg analysed by HPLC revealed that, from the phenolic esters assayed, TanBSg hydrolysed only esters from protocatechuic acid and gallic acid. It is noteworthy to mention that only esters with a short aliphatic alcohol were effectively hydrolysed. Methyl and ethyl gallate were efficiently hydrolysed, whereas propyl gallate was only minimally hydrolysed, and lauryl gallate was not hydrolysed by TanBSg. Tannic acid was also fully hydrolysed by TanBsg.

- TanBSs

In 2015, the first subtype B tannase from an actinobacteria was characterized (Wu et al. 2015). TanBSs (formerly Ss-Tan) was chemically synthesized, and cloned into a C-terminal His-tagged vector for expression in *E. coli*. More than 80 mg purified protein were obtained from one liter cultures (Table 2). The rhodanine assay was employed to measure tannase activity. Generally, methyl gallate was the substrate used in the assay for the measurement of esterase activity. Digallic acid and tannin acid were also used to measure depsidase activity. Kinetic parameters of TanBSs using methyl gallate, digallic acid, and tannic acid as substrates were determined and compared to the activity of TanBLp. TanBSs showed higher substrate affinity and catalytic efficiency for depside bonds than ester bonds. The TanBSs depsidase activity was 6-10 times higher than that of TanBLp. In contrast, TanBLp acts more efficiently on ester bonds than depside bonds

showing an esterase activity ten times higher than TanBSs. The analysis of the thermal stability of TanBSs indicated that is much more stable than TanBLp at higher temperatures (Wu et al. 2015). Both enzymes are clearly different in substrate preference. TanBSs displayed maximum activity around pH 8.0, being highly active between pH 6 and 9 with more than 50% activity retained. TanBSs showed an optimal activity at 50 °C and was generally active between 20 and 70 °C, retaining more than 50% of the activity (Wu et al. 2015).

- TanBFnp

The *Fusobacterium* tannase (TanBFnp) contain a predicted signal peptide, which is not a common characteristic among subtype B tannases. In order to avoid solubility issues, the gene was cloned without the signal peptide into an expression vector producing a polypeptide fusion proteins (Tomás-Cortázar et al. 2018). The rhodanine colorimetric assay using methyl gallate as substrate was used to assess tannase activity. The specific activity of TanBFnp using methyl gallate as a substrate was one of the highest described to date in bacteria (699 U/mg), being equivalent to a TanBSI (716 U/mg), and 76.5% higher than TanBLp, the reference used in the same experiment (395.9 U/mg) (Table 2). The optimum pH for TanBFnp was 7.0, and 55 °C the optimal temperature for activity. TanBFnp retained around 80% of the maximal activity at all temperatures tested between 22 and 55 °C. In terms of TanBFnp thermostability, pre-incubation of the enzyme between 22 and 45 °C did not substantially affect its activity, maintaining methyl gallate hydrolysis rates above 60% of the maximum activity. The addition of Hg²⁺ and β-mercaptoethanol abolished the activity of the enzyme whereas the rest of the compounds tested increased the hydrolyzation of the substrate to different extents. This effect was especially significant

for Triton-X-100 (242%), EDTA (206%), and KCl (191%). In order to know the substrate specificity of TanBFnp, the purified enzyme was incubated with different esters of phenolic acids and the reaction products were analysed by HPLC. TanBFnp hydrolysed several simple esters of gallic acid regardless of the length of their aliphatic alcohol. Among this group of compounds, methyl gallate, ethyl gallate, propyl gallate and lauryl gallate were transformed into gallic acid by TanBFnp. Other substrates, including polyphenolic esters of gallic acid such as tannic acid, gallocatechin gallate and epigallocatechin gallate were also hydrolysed. In addition, esters of protocatechuic acid were also degraded by the enzyme (Table 2) (Tomás-Cortázar et al. 2018).

Feruloyl esterases / Tannases

Feruloyl esterases, also known as ferulic acid esterases, cinnamic acid esterases, or cinnamoyl esterases, are enzymes involved in the release of hydroxycinnamic acids, such as ferulic, *p*-coumaric, sinapic acids, from plant cell walls (Benoit et al. 2008). On the basis of their model substrate specificity they could be classified into four subclasses, type A, B, C, and D, in relation to their ability to hydrolyze the four model hydroxycinnamic acid methyl esters (methyl ferulate, methyl *p*-coumarate, methyl caffeate, and methyl sinapinate) (Crepin et al. 2004; Benoit et al. 2008).

There are some feruloyl esterases which are also able to hydrolyze esters bonds on hydroxibenzoic acids, such as gallic and protocatechuic acids. Currently, two enzymes have been described exhibiting esterase activity on both types of phenolic acids, Tan410 from a soil metagenomics library (Yao et al. 2011, 2013) and Est_1092 from *Lactobacillus plantarum* (Esteban-Torres et al. 2015). However, both proteins are only 15% identical on

their amino acid sequences (data not shown). A search for conserved domains revealed that both proteins harbor different conserved alpha/beta hydrolases domains.

“CS-D-HC” feruloyl esterase/tannase

The α/β -hydrolases superfamily did not show any disulphide bond near the triad. The nonconserved disulphides are often associated with variable structural features that are recruited resulting in the differentiation or specialization of protein function and possess structural and/or functional importance. Tan410 feruloyl esterase belongs to the pfam07519 family of the alpha/beta hydrolases and its catalytic domain contains a disulphide bond. Certain members of the pfam07519 family contain the CS-D-HC motif (Suzuki et al. 2014). The extent of conservation of the CS-D-HC motif and its functional relevance needs to be verified through functional characterization of distant homologs in pfam07519. No similar structural features to the CS-D-HC motif have been reported for enzymes having a catalytic triad apart from FAE B from *Aspergillus oryzae* (AoFaeB), which belongs to the fungal tannase family (Suzuki et al. 2014). The catalytic triad of AoFaeB comprises the residues Ser-203, Asp-417, and His-457, with the serine and histidine residues directly connected by a disulphide bond between neighbouring cysteines. This structural feature, the “CS-D-HC motif”, is unprecedented in serine hydrolases. A mutational analysis indicated that the novel structural motif plays essential roles in the function of the enzyme (Suzuki et al. 2014). The catalytic domain of Tan410 contains a disulphide bond. In the feruloyl esterase/tannase Tan410 isolated from a metagenomic library, the equivalent catalytic triad is Ser-191, Asp-399, and His-434, and similar to AoFaeB, the serine and histidine could be directly connected by a disulphide bond of the cysteine residues, Cys-190 and Cys-435 (Online resource Fig. S3).

In order to find proteins similar to Tan410 in databases, a search was performed and only proteins from bacteria collection were retrieved. The most similar proteins (34-43%) were proteins from the phyla Acidobacteria (*Granulicella mallensis* ATCC BAA-1857 and *Granulicella tundricola* ATCC BAA-1859) or Proteobacteria (*Sphingomonas sanxanigenens* DSM 19645 and *Acetobacter pasteurianus* NBRC 3283) (Online resource Figure S3). These proteins are around 500 amino acid residues long and 55 kDa (Table 4). Although Tan410 contains a signal peptide, this is not a general characteristic of this group of proteins. The search for conserved domains revealed the presence in all of these proteins of the Abhydrolase pfam07519 conserved domain, typical of some fungal tannases and feruloyl esterases. The alignment of these sequences revealed that several amino acid residues and motifs are conserved among them. The pentapeptide active site GX SXG, typical of serine hydrolases, the three catalytic residues (Ser-191, Asp-399, and His-434 in Tan410), as well as the structural motif CS-D-HC are conserved in all five proteins. The protein conservation exhibited by these proteins could indicate feruloyl esterase and tannase activity on them.

Tannase activity, as well as feruloyl esterase activity, has been functionally demonstrated in Tan410. The protein was recombinantly produced in *E. coli* as an N-terminal His-tag fusion protein and purified by affinity chromatography (Yao et al. 2011). It is a monomeric protein of 55 kDa. Tannase activity was measured by the rhodanine assay using propyl gallate as substrate. Tan410 showed its highest tannase activity at pH 6.4 and 30 °C (Table 2). The enzyme was stable below 40 °C and did not show obvious loss of activity after a prolonged incubation time at this temperature. However, its activity was drastically reduced over 40 °C. The activity of Tan410 was increased by Ca^{2+} , Cd^{2+} , and Mg^{2+} , and reduced by the presence of β -mercaptoethanol, Tween 80 and PMSF. Hg^{2+} completely inhibited the activity of Tan410. The biochemical properties of Tan410 for

esterase activity were different, as its optimum temperature and pH were 35 °C and 7.0, respectively, and its activity was enhanced by the addition of Mn^{2+} , Mg^{2+} , NH_4^+ , and Ni^{2+} . Tan410 totally hydrolysed tannic acid, epicatechin gallate, epigallocatechin gallate, ethyl ferulate, and chlorogenic acid. Methyl gallate and rosmarinic acid were partially hydrolysed, 46 and 88%, respectively. Besides these esters, methyl caffeate, and methyl *p*-coumarate were also hydrolysed (Yao et al. 2013). When Tan410 was immobilized on calcium alginate beads its optimum pH shifted towards neutrality compared with the free enzyme. Similarly, its optimum temperature also shifted to 45 °C after immobilization (Yao et al. 2014b). The immobilized Tan410 had no loss of activity after 10 cycles, and retained more than 90% of its original activity after storage for 30 days. After immobilization, the enzyme activity was only slight affected by Hg^{2+} , which completely inhibited the activity of the free enzymes.

Non-“CS-D-HC” feruloyl esterase/tannase

Not all the feruloyl esterases having tannase activity contain the CS-D-HC motif. In 2015, Esteban-Torres et al. (Esteban-Torres et al. 2015) described an esterase, Est_1092, from *L. plantarum* able to hydrolyze esters from hydroxycinnamic and hydroxybenzoic acids. Four conserved domains were found in Est_1092 (Table 5). Similarly to subtype B tannases, this type of feruloyl esterase/tannase harbours the Aes domain (COG0657) of acetyl esterase/lipase. As in the “CS-D-HC” feruloyl esterase/tannases, the alpha/beta hydrolase fold is also present in Est_1092 but with the Abhydrolase_3 domain (pfam07859) instead of the Abhydrolase (pfam07519). In Est_1092 two more domains are present, an Esterase_lipase domain, which includes esterases and lipases acting on carboxylic esters, and finally, the PRK10162 domain of acetyl esterases (Table 5).

A database search for proteins similar to Est_1092 revealed the existence of similar proteins in numerous species of the genera *Lactobacillus* (Table 5 shows 10 of these *Lactobacillus* species), *Enterococcus* (*E. cecorum* DSM 20682, *E. columbae* DSM 7374, etc), as well as *Streptococcus equinus* ATCC 700338, *Weissella paramesenteroides* ATCC 33313, *Coriobacterium glomerans* ATCC 49209, and *Parascardovia denticolans* DSM 10105, among others. These proteins exhibit a high identity degree among them, ranging from 59 to 79% (Online resource Figure S4). These bacterial species are Gram positive and belong to the Firmicutes phylum. They are around 300 amino acid residues long, with 30 kDa of molecular mass, and do not contain a signal peptide.

Est_1092 was recombinantly overproduced and biochemically characterized. The specific activity of Est_1092 on methyl gallate hydrolysis was compared to the activity reported for the two tannase enzymes (TanALp and TanBLp) previously described on *L. plantarum* (Esteban-Torres et al. 2015) Using the rhodanine assay, Est_1092 showed a specific activity of 25 U/mg, which was similar to the activity of TanALp (39 U/mg) but significantly lower than the specific activity observed for TanBLp (404 U/mg) (Table 2). For the biochemical characterization of Est_1092 only esterase activity was analysed by a spectrophotometric assay using *p*-nitrophenyl butyrate as the substrate (Esteban-Torres et al. 2015). The optimal pH for Est_1092 esterase activity was found to be 5.0, although more than 80% of the maximal activity was observed at pH 6.5. Regarding the optimal temperature, Est_1092 exhibited maximal activity at 30 °C, although the protein retained 90% of its maximal activity at temperatures ranging from 5 to 30 °C. Est_1092 also showed high thermostability, since it retained up to 70% of its activity after incubation for 20 h at 37 °C or 40% of its activity after incubation for 20 h at 45 to 65 °C.

The four model substrates for feruloyl esterases were efficiently hydrolysed by Est_1092 (methyl ferulate, methyl caffeate, methyl *p*-coumarate, and methyl sinapinate)

(Table 2). Est_1092 also hydrolysed the two model hydroxybenzoic esters substrates hydrolysed by bacterial tannases, gallate and protocatechuate esters. Unexpectedly, and in contrast to bacterial tannases, all the benzoic esters assayed were hydrolysed by Est_1092. These esters included benzoic esters (methyl benzoate, ethyl benzoate, and vinyl benzoate), hydroxybenzoic esters (methyl 4-hydroxybenzoate, and ethyl 4-hydroxybenzoate), vanillic ester (methyl vanillate), and dihydroxybenzoic esters (methyl 2,4-dihydroxybenzoate, methyl 2,5-dihydroxybenzoate, and ethyl 3,5-dihydroxybenzoate). Est_1092 is an esterase active on a broad range of esters from phenolic acids (Esteban-Torres et al. 2015) (Table 2).

In addition to the bacterial enzymes recombinantly produced with their tannase activity biochemically demonstrated, there are reports on bacterial proteins purified and partially characterized at the molecular level lacking a characterization of their biochemical activity. This is the case of a purified protein from *Enterobacter* sp. (Sharma and John 2011). Tannase activity has been demonstrated on the purified protein using the rhodanine assay, but it has not been recombinantly produced, as only a fragment of its corresponding gene is known. A database search for sequences similar to this gene fragment, the A0A0AYA379 uncharacterized protein from *Enterobacter cancerogenus* could be found. This is a 595 amino acids long, 78% identical to the *Enterobacter* sp tannase (accession CQ268323) in the 308 amino acid overlapping region. When the *E. cancerogenus* protein was analysed against the known bacterial tannases, it showed the highest identity degree (29%) to Tan410. A protein alignment of both proteins revealed that *E. cancerosus* protein possessed the “CS-D-HC” motif of some feruloyl esterase/tannase proteins. However, this protein was not included in this review due to the absence of a recombinantly produced protein and tannase activity confirmation.

Conclusions and future perspectives

Tannases form a large family of enzymes with rather divergent amino acid sequences (Banerjee et al., 2012), implying broad differences in their molecular structures and enzymatic properties. The complexity of tannin structures requires a wide number of enzymes adapted to act on diversified substrates. The distinct substrate specificities of tannases may probably result from the differences in their molecular structures. Such a finding may be of great interest to guide the search for new tannases with novel enzymatic characteristics. Given the wide application of tannases in different industries, different types of tannases that can work at a wide range of physical parameters such as temperature, pH, ionic strength, presence of detergents and organic solvents need to be identified. In general terms, for optimal activity feruloyl esterases/tannases show a preference for low temperatures (30 °C) and more acidic pH (5-6) than tannases. Not significant differences on biochemical properties are observed among tannase subtype A and B. With the exception of TanBLp, most of the tannases exhibited a high optimal temperature, being around 40 °C. Subtype A tannases exhibit a more acidic optimal pH (6-7) than subtype B (7-8). Most of tannases were inhibited by Hg^{2+} and β -mercaptoethanol, and are activated by Ca^{2+} . Only some subtype B tannases (TanBLp and TanBFnp) are able to hydrolyze gallate esters having a long aliphatic chain, such as lauryl gallate. Despsidase activity has only been tested on TanBSs. The kinetic parameters for most of the recombinant bacterial tannases remains unknown.

The soil is one of the main reservoirs of the microbial diversity on the planet, providing a vast resource for discovering biocatalysts. However, more than 99% of the microorganisms in the soil cannot be cultured, limiting the discovery of novel biocatalysts.

To overcome this limitation, metagenomics can be an alternative to study unculturable microorganisms. Moreover, besides the numerous reports describing the isolation of tannase-producing bacteria, most of the bacterial species identified in this review harboring potential tannases, do not appear among the species previously isolated. Therefore, in addition to the isolation of tannase encoding genes from metagenomics libraries, the synthetic construction of putative tannase genes included in the databases, will offer new alternatives for the selection of specific tannase proteins.

Molecular biology and genetic engineering have opened up the areas of many new applications of gene cloning and recombinant DNA technology in tannase research. Recombinant DNA techniques offer new opportunities for construction of genetically modified microbial strains with selected enzyme machinery. The data exposed on this review on recombinant bacterial tannases, suggests that besides genetic differences, the variation in host cells and culture media may partly account for the different productivities observed. This review demonstrated that bacterial tannases can be produced efficiently by recombinant techniques and their productivity could be greatly improved by optimizing expression and purification parameters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

735

736 **Ethical statement** This article does not contain any studies with human participants or
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740 **References**

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Figure legends

Fig. 1. Comparison of protein sequences from bacterial tannases active only in hydroxybenzoic esters. (A) Alignment of recombinantly produced functional tannases from *Lactobacillus plantarum* (TanALp, accession C2FI97, and TanLpl, accession AB379685), *Lactobacillus pentosus* (TanLpe, BAN10247), *Lactobacillus paraplantarum* (TanLpa, BAN10246), *Streptococcus gallolyticus* (TanASg, accession GALLO_0933, and TanSg1, accession W_012962282), *Atopobium parvulum* (TanAAp, accession C8W7K9), *Staphylococcus lugdunensis* (TanA, BAF03594.1), *Fusobacterium nucleatum* subsp. *polymorphum* (TanBFnp, ALQ42581.1), and *Streptomyces sviveus* (Ss-Tan, ADY53581.2). Multiple alignments were performed using the Clustal Omega program after retrieval of sequences from BLAST homology searches. Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. Putative signal peptides are highlighted in yellow. Residues conserved in all the subtype A tannases are highlighted in green, whereas residues conserved in all subtype B tannases are in blue. The conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases is showed in a box. In a black-lined box are also shown the amino acids from the catalytic triad, Ser-163, in the pentapeptide motif, which is conserved in all bacterial tannases, except in TanAAp, and His-451 conserved in all tannases, and Asp-419 which is present only in subtype B tannases (numbering is referring to TanLp1 tannase). The absence of Asp-419 in all the subtype A tannases is shown with a red-lined box. (B) Percentage of identity among bacterial tannases resulting from Clustal Omega comparison.

Fig. 2. Alignment of *L. plantarum* proteins as representative of A and B tannase subtypes.

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905 Dashes indicate gaps introduced to maximize similarities. Amino acid residues conserved
906 in all tannases identified so far are shown in a black-lined box, amino acids conserved in
907 all subtype A tannases are highlighted in green, and in blue in amino acids conserved in
908 subtype B tannases. Black circles show amino acid residues which are only conserved in
909 subtype A tannases and are not present in any of the subtype B tannases. Black diamonds
910 indicate amino acids which are only conserved in subtype B tannases and are not present
911 in any subtype A tannases. In a red-lined box the Asp-419 from the catalytic triad only
912 conserved in subtype B tannases is shown.

Table 1. Functional bacterial tannase proteins from A subtype (absence of catalytic Asp), and putative tannases belonging to this subtype

Protein	Accession	Strain	Phylum ^a	Gram	Residues (aa)	MW (kDa)	Signal peptide	Asp-419	Conserved domains
TanAAp	C8W7K9	<i>Atopobium parvulum</i> DSM 20469 ^T	Ac	+	607	66.1	Yes	No	Aes (COG0657) Abhydrolase_3 (pfam07859)
TanALp	C2FI97	<i>Lactobacillus plantarum</i> ATCC 14917 ^T	Fi	+	626	67.4	Yes	No	No
TanASl	BAF03594.1	<i>Staphylococcus lugdunensis</i> MTCC 3614	Fi	+	613	67.9	Yes	No	No
TanASg	GALLO_0933	<i>Streptococcus gallolyticus</i> UCN34	Fi	+	596	63.6	Yes	No	No
ANA	J3F2Y7	<i>Actinomyces naeslundii</i> ATCC 12104	Ac	+	615	63.7	Yes	No	Aes (COG0657)
ARI	B9CMT1	<i>Atopobium rimae</i> ATCC 49626	Ac	+	659	70.9	Yes	No	Aes (COG0657)
CVI	W5Y437	<i>Corynebacterium vitaeruminis</i> DSM 20294	Ac	+	562	59.3	No	No	No
LGA	A0A249NJ78	<i>Lactobacillus gasseri</i> DSM 14869	Fi	+	638	68.8	Yes	No	No
LPC	A0A0R1TM22	<i>Lactobacillus paracollinoides</i> DSM 15502	Fi	+	626	67.3	Yes	No	No
LPE	G0M044	<i>Lactobacillus pentosus</i> IG1	Fi	+	627	67.4	Yes	No	No
LNA	A0A0R1K9A6	<i>Lactobacillus namurensis</i> DSM 19117	Fi	+	638	69.7	Yes	No	Aes (COG0657) Esterase_lipase (cd00312)
LZY	A0A0R1MZ09	<i>Lactobacillus zymae</i> DSM 19395	Fi	+	601	65.7	Non	No	No
MMI	ALT68817.1	<i>Methanobrevibacter millerae</i> SM9	Ar	+	605	66.6	Non	No	No
MTI	W9DZK2	<i>Methanolobus tindarius</i> DSM 2278	Ar	-	663	72.8	Yes	No	No
SHE	C7N116	<i>Slackia heliotrinireducens</i> ATCC 29202	Ac	+	640	66.4	Yes	No	No

^a Phylum, Actinobacteria (Ac), Archaea (Ar), Firmicutes (Fi)

Table 2. Biochemical properties of recombinant bacterial tannases

Protein	Bacterial strain	aa ^a (kDa)	SP ^b	Conserved domains	Yield (mg/L)	Specific activity (U/mg)	T ^c	pH	Substrates ^d	Activators	Inhibitors	Reference
TANNASE												
Subtype A (absence of catalytic Asp)												
TanAAP	<i>Atopobium parvulum</i> DSM 20469 ^T	607 (66)	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)	12	3.5	55	6.0	None by HPLC Methyl gallate by rhodanine		Hg ²⁺ , Zn ²⁺ βME	Jiménez et al. 2014a
TanALp	<i>Lactobacillus plantarum</i> ATCC 14917 ^T (CECT 748 ^T)	626 (67)	Yes	No	3	39	20-30	6.0	Gallate and protocatechuate esters (non lauryl gallate), tannic acid and EGCG	Ca ²⁺ Triton-X-100, DMSO	Hg ²⁺ EDTA, βME	Jiménez et al. 2014b
TanASl	<i>Staphylococcus</i> <i>lugdunensis</i> MTCC 3614	613 (67)	Yes	No	ND	577*	40	7.0	Non tested by HPLC Methyl gallate by rhodanine	Na ²⁺ SDS, nonpolar solvents	EDTA, βME, polar solvents	Chaitanyakumar and Anbalagan 2016
TanASg	<i>Streptococcus gallolyticus</i> UCN34	596 (63)	Yes	No	ND	256	37	6.0	Gallate and protocatechuate esters (non lauryl gallate), tannic acid and EGCG	Ca ²⁺ , K ⁺ Tween-80	Hg ²⁺ , Zn ²⁺ , Mg ²⁺ βME	Jiménez et al. 2014d
Subtype B (with catalytic Asp)												
TanBFnp	<i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>	520 (57)	Yes	Aes (COG0657)	ND	716	55	7.0	Gallate and protocatechuate esters (non lauryl gallate), tannic acid, GCG, and EGCG	K ⁺ , Mg ²⁺ Triton-X-100, EDTA	Hg ²⁺ βME	Tomás-Cortázar et al. 2018
TanBLpa	<i>Lactobacillus</i> <i>paraplantarum</i> NOS 120	469 (50)	No	Aes (COG0657)	ND	ND	40	8.5	Gallate esters, and ECG, EGCG, CG, and GCG		FeSO ₄	Ueda et al. 2014
TanBLpe	<i>Lactobacillus pentosus</i> 21A-3	470 (51)	No	Aes (COG0657)	ND	ND	35	8.0	Gallate esters, and ECG, EGCG, CG, and GCG		Zn ²⁺ , FeSO ₄	Ueda et al. 2014
TanBLp	<i>Lactobacillus plantarum</i> ATCC 14917 ^T	469 (50)	No	Aes (COG0657)	3	84*	40	8.0	ND	ND	ND	Iwamoto et al. 2008
					17	408	40	7.0	Gallate and protocatechuate esters, tannic acid, GCG, and EGCG	Ca ²⁺ , K ⁺	Hg ²⁺ , Zn ²⁺ , Mg ²⁺ βME, DMSO, Triton-X-100	Curiel et al. 2010
					90 ND	ND ND	30 40	8.0 8.5	ND Gallate esters, and ECG, EGCG, CG, and GCG	ND Ca ²⁺	ND Zn ²⁺ , FeSO ₄	Wu et al. 2013 Ueda et al. 2014
TanBSg	<i>Streptococcus gallolyticus</i> UCN34	470 (52)	No	Aes (COG0657)	12	577	45	7.0	Gallate (ethyl and methyl) and protocatechuate esters, and tannic acid, GCG, EGCG	Ca ²⁺ DMSO, Tween-80	Hg ²⁺ , Zn ²⁺ βME	Jiménez et al. 2014c
TanBSs	<i>Streptomyces sviveus</i> ATCC 29083	570 (60)	No	Aes (COG0657)	80	ND	50	8.0	Gallate esters and depsidase activity	ND	ND	Wu et al. 2015
FERULOYL ESTERASE / TANNASE “CS-D-HC” motif												
Tan410	Uncultured bacterium	520 (54)	Yes	Abhydrolase (pfam07519) CS-D-HC motif	ND	ND	30	6.4	Tannic acid, methyl gallate, ECG, and EGCG. Chlorogenic acid, ethyl ferulate, methyl caffeate, and methyl <i>p</i> -coumarate	Ca ²⁺ , Cd ²⁺ , Mg ²⁺	Hg ²⁺ βME, Tween-80	Yao et al. 2011 Yao et al. 2013
Non-“CS-D-HC” motif												
Est_1092	<i>Lactobacillus plantarum</i> JDM1	295	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162	ND	25	30 ^c	5.0 ^c	Hydroxycinnamate esters and benzoate and hydroxybenzoate esters	ND	ND	Esteban-Torres et al. 2015

^a amino acid, kDa; ^b signal peptide; ^c temperature; ^d catechin gallate (CG), gallocatechin gallate (GCG), epicatechin gallate (ECG), epigallocatechin gallate (EGCG); ^e determined by esterase activity, ND not determined

Table 3. Functional bacterial tannase proteins from B subtype (with catalytic Asp), and putative proteins belonging to this subtype

Protein	Accession	Strain	Phylum ^a	Gram	Residues (aa)	MW (kDa)	Signal peptide	Asp-419	Conserved domains
TanBFnp	ALQ42581.1	<i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>	Fu	-	520	57.8	Yes	Yes	Aes (COG0657)
TanBLpa	BAN10246	<i>Lactobacillus paraplantarum</i> NOS 120	Fi	+	469	50.7	No	Yes	Aes (COG0657)
TanBLpe	BAN10247	<i>Lactobacillus pentosus</i> 21A-3	Fi	+	470	51.1	No	Yes	Aes (COG0657)
TanBLp	AB379685	<i>Lactobacillus plantarum</i> ATCC 14917 ^T	Fi	+	469	50.6	No	Yes	Aes (COG0657)
TanBSg	W_012962282	<i>Streptococcus gallolyticus</i> UCN34	Fi	+	470	52.9	No	Yes	Aes (COG0657)
TanBSs	EDY53581.2	<i>Streptomyces sviceus</i> ATCC 29083	Ac	+	570	60.2	No	Yes	Aes (COG0657)
ASU	A6VQU7	<i>Actinobacillus succinogenes</i> ATCC 55618	P	-	510	55.8	Yes	Yes	Aes (COG0657)
ASE	E6KXG9	<i>Aggregatibacter segnis</i> ATCC 33393	P	-	513	56.1	Yes	Yes	Abhydrolase_3 (pfam07859)
AEX	E8RUH5	<i>Asticcacaulis excentricus</i> ATCC 15261	P	-	523	55.4	Yes	Yes	Aes (COG0657)
CCO	U2F494	<i>Campylobacter concisus</i> ATCC 51562	P	-	478	52.5	No	Yes	Aes (COG0657)
CGR	S2VLN4	<i>Capnocytophaga granulosa</i> ATCC 51502	B	-	534	58.5	No	Yes	Aes (COG0657)
CHO	C8NA23	<i>Cardiobacterium hominis</i> ATCC 15826	P	-	485	51.8	Yes	Yes	ThiJ (COG0693)
ECO	C0DUD8	<i>Eikenella corrodens</i> ATCC 23834	P	-	496	53.6	Yes	Yes	GATase1_Hsp31_like (cd03141)
FRI	A0A0A2MDE7	<i>Flavobacterium rivuli</i> DSM 21788	B	-	518	56.4	No	Yes	Aes (COG0657)
GAM	G7GR49	<i>Gordonia amarae</i> NBRC 15530	Ac	+	572	59.5	Yes	Yes	Aes (COG0657)
HHa	D3AJB8	<i>Hungatella hathewayi</i> DSM 13479	Fi	+	506	56.2	No	Yes	Aes (COG0657)
HHT	D3AMC3	<i>Hungatella hathewayi</i> DSM 13479	Fi	+	454	50.8	No	Yes	Abhydrolase_3 (pfam07859)
HGR	F3KTR5	<i>Hylemonella gracilis</i> ATCC 19624	P	-	575	60.5	Yes	Yes	Peptidase_s15 (pfam02129)
KOR	C4GHB9	<i>Kingella oralis</i> ATCC 51147	P	-	427	45.8	No	Yes	Aes (COG0657)
LAL	A0A2K9HQ50	<i>Lactobacillus alimentarius</i> DSM 20249	Fi	+	427	46.9	No	Yes	Abhydrolase_3 (pfam07859)
LFA	A0A0R2NRK2	<i>Lactobacillus fabifermentans</i> DSM 21115	Fi	+	466	50.7	No	Yes	Esterase_lipase (cd00312)
LFF	A0A0R2NFY2	<i>Lactobacillus fabifermentans</i> DSM 21115	Fi	+	562	59.4	Yes	Yes	Aes (COG0657)
LGA	A0A0R1V835	<i>Lactobacillus gastricus</i> DSM 16045	Fi	+	460	51.1	No	Yes	Peptidase_S15 (pfam02129)
LPB	A0A0R1H2G9	<i>Lactobacillus parabrevis</i> ATCC 53295	Fi	+	471	51.3	No	Yes	Aes (COG0657)
LSE	A0A246EIV6	<i>Lactobacillus senmaizukei</i> DSM 21775	Fi	+	466	50.7	No	Yes	No
LVE	A0A0R1SD40	<i>Lactobacillus versmoldensis</i> DSM 14857	Fi	+	547	59.2	Yes	Yes	Aes (COG0657)
LBU	C7NCM7	<i>Leptotrichia buccalis</i> ATCC 14201	Fu	-	537	59.7	Yes	Yes	Aes (COG0657)

Table 3 (cont.)

Protein	Accession	Strain	Phylum ^a	Gram	Residues (aa)	MW (kDa)	Signal peptide	Asp-419	Conserved domains
MMU	C9KQE8	<i>Mitsuokella multacida</i> DSM 20544	Fi	-	589	62.6	Yes	Yes	Aes (COG0657) Peptidase_S9 (pfam00326)
MCU	A0A1N7G800	<i>Moraxella cuniculi</i> DSM 21768	P	-	503	55.2	Yes	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)
MPO	A0A1T0CUS9	<i>Moraxella porci</i> DSM 25326	P	-	498	53.9	No	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)
MVA	K0UPD3	<i>Mycolicibacterium vaccae</i> ATCC 25954	Ac	+	523	53.8	No	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)
NBA	F2BGE0	<i>Neisseria bacilliformis</i> ATCC BAA-1200	P	-	481	51.2	Yes	Yes	Aes (COG0657)
NMU	D2ZY62	<i>Neisseria mucosa</i> ATCC 25996	P	-	500	53.9	Yes	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)
OOE	Q04F19	<i>Oenococcus oeni</i> ATCC BAA-331	Fi	+	425	47.7	No	Yes	Aes (COG0657)
RTH	S9R622	<i>Rubellimicrobium thermophilum</i> DSM 16684	P	-	479	49.2	Yes	Yes	No
RSL	F8EDM8	<i>Runella slithyformis</i> ATCC 29530/DSM 19594	B	-	482	52.0	Yes	Yes	Aes (COG0657) Abhydrolase_3 (pfam07859)
SNA	A0A059KHH4	<i>Sphaerotilus natans subsp. natans</i> DSM 6575	P	-	511	54.8	Yes	Yes	Aes (COG0657)
SAV	Q82MV9	<i>Streptomyces avermitilis</i> ATCC 31267	Ac	+	566	59.9	No	Yes	Aes (COG0657)

^a Phylum, Actinobacteria (Ac), Bacteroidetes (B), Firmicutes (Fi), Proteobacteria (P)

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Table 4. Functional Tan410 feruloyl esterase/tannase, and putative proteins belonging to this tannase group

Protein	Accession	Strain	Phylum ^a	Gram	Residues (aa)	MW (kDa)	Signal peptide	Conserved domains
Tan410	E7D7J5	Uncultured bacterium			520	54.8	Yes	Abhydrolase (pfam07519) CS-D-HC motif
APA	C7JI56	<i>Acetobacter pasteurianus</i> NBRC 3283	P	-	549	59.5	No	Abhydrolase (pfam07519) CS-D-HC motif
GMA	G8NXE6	<i>Granulicella mallensis</i> ATCC BAA-1857	Aci	-	498	55.0	No	Abhydrolase (pfam07519) CS-D-HC motif
GTU	E8WXF9	<i>Granulicella tundricola</i> ATCC BAA-1859	Aci	-	509	55.1	Yes	Abhydrolase (pfam07519) CS-D-HC motif
SSA	W0AHR6	<i>Sphingomonas sanxanigenens</i> DSM 19645	P	-	507	53.9	Yes	Abhydrolase (pfam07519) CS-D-HC motif

^a Phylum, Acidobacteria (Aci), Proteobacteria (P)

Table 5. Functional Est_1092 feruloyl esterase/tannase, and putative proteins belonging to this tannase group

Protein	Accession	Strain	Phylum	Gram	Residues (aa)	MW (kDa)	Signal peptide	Conserved domains
Est_1092	ACT61979.1	<i>Lactobacillus plantarum</i> JDM1	Fi	+	295	33.5	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LAL	A0A2K9HRX0	<i>Lactobacillus alimentarius</i> DSM 20249	Fi	+	293	33.8	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LFA	A0A0R1IRP4	<i>Lactobacillus farciminis</i> DSM 20184	Fi	+	294	33.7	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LGA	A0A0R1VI90	<i>Lactobacillus gastricus</i> DSM 16045	Fi	+	297	33.9	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LGS	A0A249NLB4	<i>Lactobacillus gasseri</i> DSM 14869	Fi	+	305	34.9	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LKI	A0A0R1VGX3	<i>Lactobacillus kitasatonis</i> DSM 16761	Fi	+	297	33.9	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LMA	J1F5K6	<i>Lactobacillus mali</i> DSM 20444	Fi	+	296	33.7	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LPA	A0A0R1HXG6	<i>Lactobacillus paralimentarius</i> DSM 13961	Fi	+	286	32.8	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162

Table 5 (cont.)

Protein	Accession	Strain	<i>Phylum</i>	Gram	Residues (aa)	MW (kDa)	Signal peptide	Conserved domains
LPE	G0M1G3	<i>Lactobacillus pentosus</i> IG1	Fi	+	296	34.6	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162 /NonD (TIGR00976)
LRU	E7FSS6	<i>Lactobacillus ruminis</i> ATCC 25644	Fi	+	304	34.6	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) PRK10162
LVI	A0A0R2CA25	<i>Lactobacillus vini</i> DSM 20605	Fi	+	296	33.7	No	Aes (COG0657) Abhydrolase_3 (pfam07859) Esterase_lipase (cd00312) Esterase_phb (TIGR01840) PRK10162

^a*Phylum*, Firmicutes (Fi)

Figure 1

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TanAAp	-----MAFTRKEFL-SLSALGVAGTAACTPQART	29
TanASg	-----MPRKKWFFTSSAVLLCSAMLLTACSSSSNS	30
TanA	-----MKKTFISLLSATVILSGCGVGEHQ	24
TanALp	-----MQFRK-IVPLMSGLLVMSVGLAACGHSETK	29
TanSg1	-----	0
TanBSs	MTLDCWQFCPHSGYVRRRQQIPPRERTVKHRAVRRRSVV--LGIGATAGAMAAGGLALNA	58
TanBFnp	-----MRKFKF---LMLFCLFG-----SMLFAA	20
TanLpe	-----	0
TanLpl	-----	0
TanLpa	-----	0
..		
TanAAp	SDNTN-----QPASNVDLEEFKSLKLDMTQNSY-----DEDNDCCYYQL	67
TanASg	STSSSS--QNTTASTSSLSSGEVSTTLDKVDNSKQI-----NADDNVYYQI	76
TanA	NNNSNHDAKGVNT--SNVKIKNYNQASSALQIDNSKWKY-----DSKNNVYYQL	71
TanALp	TKHPTSTVAKVAKATKQTVTKADVKNAKKLLINQKQWHY-----NATNKVYYQV	78
TanSg1	-----MSINQWIF-----DETNNCYMSL	18
TanBSs	NAS-----TGSTSTSRDDGLVDFPDARWKLTTTVDTDQTEHEVYTHFWK	103
TanBFnp	--Q-----KTTKTVKNEYDLKFNPDKYVSKE-----TEINGQIKIRAYE	58
TanLpe	-----MTDTLIFDADWLTPEQ-----IQLDGKTINYLAHA	30
TanLpl	-----MSNRLIFDADWLVEPQ-----VQVAGQAIQYYAAR	30
TanLpa	-----MSNRLIFDADWLVEPQ-----VQVAGQAIQYYAAR	30
..		
TanAAp	GIQVCTKPASKSVNTLSVFVFGKVFSGKKNG-STVECEVSEKAVVGSFTARTAPIVMPIN	126
TanASg	GISAAANPTDAEQQTLSIFVPGDYMTATDNGNGTYTCEINTSATVGNYSSETAPIVMPIN	136
TanA	NISVSNPQAKNVEKLGIIYVPAAYFKGKKNHNGTYTFTVNDAKKNGYSARTAPIVYVFN	131
TanALp	GVKGTGKTTSTYESMGIFIAKVVNAKASGQKTYTITFNNKAKVKGYTAKTAPIVMPFN	138
TanSg1	KNVYCAQFKDSELEALHIFVPAVYMTADGTIDR---DAVVTNKNGTIYTSQTVPIIFYND	75
TanBSs	AVTVVSKFVDATYQSLIVSAPVEIDG-----TAVDASNAPILLANS	144
TanBFnp	NIVYVKNFIDKDYQNMNIYIPEEYFNNS-----SIGSYNSSNAPIFFPNT	103
TanLpe	DIQYVQHPPAA-AIQRLNVFVPAAYENG---TVNGYTRDTAPIFMPNT	74
TanLpl	NIQYVQHPPAA-AIQVLNVFVPAAYLHGS-----SVNGYQRATAPILMPNT	74
TanLpa	NIQYVQHPPAA-AIQVLNVFVPAAYLHGS-----SVNGYQRATAPILMPNT	74
	* . : : *	..
TanAAp	TATLFPQSAPTSTY-----AYEGL	144
TanASg	TPGYSAMSALTEY-----TS-DA	153
TanA	TPGYAEQSAPTSTY-----RYSNI	149
TanALp	TPGYAAQTAPTGY-----DS-SA	155
TanSg1	IGGYAECQPAMVT-----PRN	91
TanBSs	VGGYMPSSVTDATGVGAGGMGGGGGAPGGSPTGTAAPSASASANANANTNATGGATSSNQ	204
TanBFnp	VGGYMPGKADIVG-LGRDGK-----ANSI	126
TanLpe	VGGYFPGPVDDPTRTQWPNN-----ATTI	98
TanLpl	VGGYLPGPADDPQRVTWPTN-----AGTI	98
TanLpa	VGGYLPGPADDPQRVTWPTN-----AGTI	98
..		
TanAAp	APYLEAGFVYVYAGFRGRSAGYD--STTGSDELYAGGSWPVAVDFKAAIRYLRYNNELLP	202
TanASg	TDYTSQGMIIYVSAGLRGRDS-----GAFSGVTDAAKAAIRYLRYNQGNIS	197
TanA	SKYMKAGFIYVEAGLRGRSMSGNNSNASTKSYETGSGWGVTDLKAAIRYFRNDSSLP	209
TanALp	NKYTKAGFIYVAAGCRGLSQSDK-----SNGSSWGVTDLKAAVRLRLNRSRIA	205
TanSg1	QRYLEDGYVLVSVGARGRQSQN-----GIGKAPAGLVDLKAAVRWLKHHNDIP	140
TanBSs	LLALAAGYVVIEPGARGRTLKNS-----SGEYGTAPAAIVDLKAAVRYIKSNKGRIP	257
TanBFnp	TYALSKGYVVAAPGARGRTLTDN-----KGNYTGKAPAAIVDLKAAVRYLYLNDEVMP	179
TanLpe	KQALKRGYVVVSAGLRGHTTVNE-----SGQRVGQAPAFIVDMKAAVRYVKYNQERLP	151
TanLpl	QQALKRGYVVVAAGIRGRTTVDK-----SGQRVGQAPAFIVDMKAAIRYVKYNQGRLP	151
TanLpa	QQALKRGYVVVAAGIRGRTTVDK-----SGQRVGQAPAFIVDMKAAIRYVKYNQGRLP	151
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Figure 2

Table S1. Described bacteria possessing tannase activity

Bacterial species	Strain	Isolation	Tannase detection method	Reference
<i>Achromobacter sp.</i>		Loamy sandy soil from a hardwood forest	Growth on media containing natural plant tannins	Lewis and Starkey 1969
<i>Atopobium parvulum</i>	DSM 20469		Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Jiménez et al. 2014a
<i>Azotobacter sp.</i>	SSBB1		Residual tannic acid content after enzymatic reaction and HPLC and LC-MS analysis of the degradation products	Gauri et al. 2012
<i>Bacillus cereus</i>	KBR9		Residual tannic acid content after enzymatic reaction	Mondal et al. 2001
<i>Bacillus gottheilii</i>	M2S2	Tannery effluent soil sample	Gallic acid spectrophotometric detection after enzymatic reaction	Selvaraj and Murty 2017a
<i>Bacillus gottheilii</i>	M2S2	Tannery effluent soil sample	Gallic acid spectrophotometric detection after enzymatic reaction	Selvaraj and Murty 2017b
<i>Bacillus licheniformis</i>	KBR 6	Lateritic soil	Paper chromatography analysis of tannic acid degradation	Mondal and Pati 2000
<i>Bacillus licheniformis</i>	KBR6	Forest soli	Residual tannic acid content after enzymatic reaction	Mondal et al. 2000
<i>Bacillus licheniformis</i>	KRB6		Residual tannic acid content after enzymatic reaction	Mohapatra et al. 2009
<i>Bacillus massiliensis</i>		Rotting grape must	Gallic acid colorimetric determination by rhodanine assay	Prasanna et al. 2012
<i>Bacillus polymyxa</i>	Q47	Decaying bark	TLC analysis of chesnut tannin fermentation broth	Deschamp et al. 1983
<i>Bacillus pumilus</i>	M26	Forest litter	TLC analysis of chesnut tannin fermentation broth	Deschamp et al. 1983
<i>Bacillus sphaericus</i>		Soil	Tannin-protein complex degraatation on culture media and visual reading method	Raghuwanshi et al. 2011
<i>Bacillus subtilis</i>	PAB2		Gallic acid colorimetric determination at 254.6 and 293.8 nm	Jana et al. 2013
<i>Bacillus subtilis</i>	PAB2		Gallic acid colorimetric determination at 254.6 and 293.8 nm	Jana et al. 2013
<i>Citrobacter sp.</i>		Tannery effluent	Tannic acid utilization as a sole carbon source and visual reading method	Wilson et al. 2009
<i>Citrobacter freundii</i>	TB3	Tannery effluent	Tannic acid utilization as a sole carbon source and TLC analysis of degradation products.	Kumar et al. 1999
<i>Citrobacter freundii</i>	NCTC 9750		Gallic acid spectrophotometric detection	Murugan et al. 2008
<i>Corynebacterium</i>	Q40/QM40	Decaying bark	TLC analysis of chesnut tannin fermentation broth	Deschamp et al. 1983
			HPLC analysis of tara tannin fermentation broth	Deschamps and Lebeault 1984
<i>Enterobacter sp.</i>	KPJ03		Gallic acid colorimetric determination by rhodanine assay	Sharma and John 2011
<i>Enterobacter sp.</i>	Ruminal fecal matter		Gallic acid colorimetric determination by rhodanine assay and HPLC analysis of tannic acid degradation products	Sharma et al. 2017
<i>Enterobacter asburæ</i>	LR01	Rohu gut	Residual tannic acid content after enzymatic reaction	Mandal and Ghosh 2013
<i>Enterobacter cloacæ</i>	MTCC 9125	Compost sample	Gallic acid colorimetric determination by rhodanine assay	Beniwal et al. 2013
<i>Enterobacter ludwigii</i>	GRT-1	Goat rumen	Tannin-protein complex degradation. Gallic acid detection by visual reading and colorimetric rhodanine assay. TLC detection of tannic acid metabolites	Singh et al. 2012
<i>Enterococcus faecalis</i>		Goat feces	Tannic acid utilization as a sole carbon source and HPLC and TLC detection of degradation products	Goel et al. 2011
<i>Fusobacterium nucleatum subsp. polymorphum</i>			Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Tomás-Cortázar et al. 2018

Bacterial species	Strain	Isolation	Tannase detection method	Reference
<i>Gluconoacetobacter hansenii</i>	UAC09	Contaminated grape wine	Spectrophotometric detection at 310 nm of residual tannic acid content after enzymatic reaction	Usha Rani and Anu Appaiah 2012
<i>Klebsiella sp.</i>	C2A	Olive mil waste	Residual tannic acid content after enzymatic reaction. Visual Reading method and HPLC detection of tannic acid degradation products	Pepi et al. 2013
<i>Klebsiella pneumoniae</i>	Q52	Decaying bark	TLC analysis of chesnut tannin fermentation broth	Deschamp et al. 1983
<i>Klebsiella pneumoniae</i>	TM20, TM21, TM24, QM26, QM29		HPLC analysis of tara tannin fermentation broth	Deschamps and Lebeault 1984
<i>Klebsiella pneumoniae</i>	MTCC 7162	Tannery effluent	Tannic acid utilization as a sole carbon source and gallic acid colorimetric determination by rhodanine assay	Sivashanmugan and Jayaraman 2011
<i>Klebsiella pneumoniae</i>	KP715242	Thermostable	Gallic acid spectrophotometric detection	Kumar et al. 2015
<i>Klebsiella pneumoniae</i>	KP715242		Residual tannic acid content after enzymatic reaction	Kumar et al. 2016
<i>Klebsiella pneumoniae</i>	PLP G-17 SC	Goat rumen	Gallic acid colorimetric determination by rhodanine assay and HPLC analysis of tannic acid degradation products	Sharma et al. 2017
<i>Klebsiella variicola</i>	PLP G-17 LC	Goat rumen	Gallic acid colorimetric determination by rhodanine assay and HPLC analysis of tannic acid degradation products	Sharma et al. 2017
	PLP S-18			
<i>Lactobacillus spp.</i>			Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus sp.</i>	ASR-S1	Sheep excreta	Gallic acid colorimetric determination by rhodanine assay	Sabu et al. 2006
<i>Lactobacillus acidophilus</i>	KB96	Human feces	Gallic acid spectrophotometric detection at 440 nm	Nishitani et al. 2004b
	KB249			
<i>Lactobacillus apodemi</i>	ASB1	Wild mouse feces	Tannin-protein complex degratation	Osawa et al. 2006
<i>Lactobacillus brevis</i>	AScc-4300		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus buchneri</i>	CSCC 5400		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus casei</i>	CSCC-2600		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus fermentum</i>	CSCC-5217		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus helveticus</i>	ASCC-905		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus hilgardii</i>	CSCC-5489		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Lactobacillus pentosus</i>	ATCC 8041	Silage	Tannin-protein complex degratation on selective media and visual reading method	Osawa et al. 2000
	CNRZ 1544	Fermented olives		
	CNRZ 1561			
<i>Lactobacillus pentosus</i>		Human feces and fermented foods	Gallic acid spectrophotometric detection at 450 nm	Nishitani et al. 2004a
<i>Lactobacillus pentosus</i>		Human feces	Tannin-protein complex degratation on selective media	Noguchi et al. 2007
<i>Lactobacillus pentosus</i>		Vegetable pickled with rice bran	Tannin-protein complex degratation and gallic acid spectrophotometric detection at 520 nm	Iwamoto et al. 2008
<i>Lactobacillus pentosus</i>	LT7	kimchi	HPLC and TLC pyrogallol detection from tannic acid	Kwon et al. 2008
<i>Lactobacillus pentosus</i>	21A3		HPLC analysis of degradation products	Ueda et al. 2014

Bacterial species	Strain	Isolation	Tannase detection method	Reference
<i>Lactobacillus paraplantarum</i>	KOG 1	Pickled rice bran	Tannin-protein complex degravation on selective media and visual reading method	Osawa et al. 2000
	KOG 3	Korean cabbage kimchi		
	KOG 15	Pickled cuccumber and radish		
	KOG16	Beer contaminant		
	KOG 20	Human feces		
	KOG 25			
	ATCC 700211			
	61D			
<i>Lactobacillus paraplantarum</i>		Human feces and fermented foods	Gallic acid spectrophotometric detection at 450 nm	Nishitani et al. 2004a
<i>Lactobacillus paraplantarum</i>			Tannin-protein complex degravation and gallic acid spectrophotometric detection at 520 nm	Iwamoto et al. 2008
<i>Lactobacillus paraplantarum</i>	NOS120		HPLC analysis of degradation products	Ueda et al. 2014
<i>Lactobacillus plantarum</i>	KLH 1, etc	Human feces	Tannin-protein complex degravation on selective media and visual reading method	Osawa et al. 2000
	KOG 1, etc.	Turnip pickled with rice bran		
	ATCC	Cucumber, radish, eggplant, and		
	14917	vegetables pickled		
	ATCC 8014	Cheese		
	CNRZ 1228			
<i>Lactobacillus plantarum</i>		Stored olive wastes	Residual tannic acid content after enzymatic reaction	Ayed and Hamdi 2002
<i>Lactobacillus plantarum</i>		Human feces and fermented foods	Gallic acid spectrophotometric detection at 450 nm	Nishitani et al. 2004a
<i>Lactobacillus plantarum</i>		Grape must and wine	Gallic acid spectrophotometric detection at 450 nm	Vaquero et al. 2004
<i>Lactobacillus plantarum</i>			Gallic acid spectrophotometric detection at 440 nm	Matthweus et al.2006
<i>Lactobacillus plantarum</i>		Human feces	Tannin-protein complex degravation on selective media	Noguchi et al. 2007
<i>Lactobacillus plantarum</i>	ATCC	Pickled cabbage	Tannin-protein complex degravation and gallic acid spectrophotometric detection at 520 nm	Iwamoto et al. 2008
	14917T			
<i>Lactobacillus plantarum</i>	CECT 748	Pickled cabbage		Rodríguez et al. 2008
<i>Lactobacillus plantarum</i>		kimchi	HPLC and TLC pyrogallol detection from tannic acid	Kwon et al. 2008
<i>Lactobacillus plantarum</i>	MTCC 1407		Spectrophotometric detection at 310 nm of tannic acid degradation	Natarajan and Rajendran 2009
<i>Lactobacillus plantarum</i>	CECT 748	Pickled cabbage	Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Curiel et al. 2009
<i>Lactobacillus plantarum</i>	MTCC 1407		Spectrophotometric detection of tannic acid degradation	Natarajan and Rajendran 2012
<i>Lactobacillus plantarum</i>	ATCC	Pickled cabbage	Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Jiménez et al. 2014b
	14917T			
<i>Lactobacillus plantarum</i>	ATCC		HPLC analysis of degradation products	Ueda et al. 2014
	14917T			
<i>Lactobacillus plantarum</i>	CIR1	Plum fruit juice	Gallic acid colorimetric determination by rhodanine assay	Aguilar-Zárate et al. 2014
<i>Lactobacillus plantarum</i>	H78	Rice, barley, and pickles		Matsuda et al. 2016

Bacterial species	Strain	Isolation	Tannase detection method	Reference
<i>Lonepinella koalarum</i>	ACM 3666	Feces of koala	Tannin-protein complex degratation on selective media	Osawa et al. 1995
	ACM 3667			
	ACM 3496			
	ACM 3894			
	ACM 3900			
	ACM 3895			
	ACM 3896			
<i>Methanobrevibacter millerae</i>	SM9	Sheep rumen	Analysis of the complete genome sequence	Kelly et al. 2016
<i>Microbacterium terregens</i>	DT5	Tea processing factory	Gallic acid colorimetric determination by rhodanine assay	Belur et al. 2010
<i>Oenococcus oeni</i>			Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Pantoea sp.</i>	2AT2	Oilve mil waste	Growth on media containing tannic acid as carbon source and HPLC analysis of degradation products	Pepi et al. 2010
<i>Pantoea agglomerans</i>	UQM 1615	Fresh cecal Wall of koala	Tannin-protein complex degratation on selective media	Osawa 1992
<i>(formerly Enterobacter agglomerans)</i>	UQM 3666			
	UQM 3667			
<i>Pantoea agglomerans</i>	T71	Soil simple from an orchard	Growth on media containing tannic acid as the main carbon source and HPLC determination of pyrogallol production	Zeida et al. 1998
<i>Pediococcus spp</i>			Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Pediococcus acidilactici</i>	NOS122	Korean cabbage kimchi	Gallic acid spectrophotometric detection at 450 nm	Nishitani et al. 2004b
<i>Pediococcus acidilactici</i>	CSCC-2302		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Pediococcus pentosaceus</i>	NOS141	Korean cabbage kimchi	Gallic acid spectrophotometric detection at 450 nm	Nishitani et al. 2004b
<i>Pediococcus pentosaceus</i>	CSCC-2304		Gallic acid spectrophotometric detection at 440 nm	Matthews et al. 2006
<i>Providencia rettgeri</i>	DT6	Tea processing factory	Gallic acid colorimetric determination by rhodanine assay	Belur et al. 2010a
<i>Rhodococcus</i>	NCIM 2891		Residual tannic acid content after enzymatic reaction	Nadaf and Ghosh,2011
<i>Pseudomonas aeruginosa</i>	IIIB 8914	Tannery soil	Residual tannic acid content after enzymatic reaction	Selwal et al. 2010
<i>Pseudomonas citronellolis</i>		Tannery soil	Tannic acid utilization as a sole carbon source and visual reading method	Chowdhury et al. 2004
<i>Selenomonas ruminantium</i>	ES3	Rumen fluid from sheep (ES3 and ES4)	Gallic acid colorimetric determination by rhodanine assay	Odenyo et al. 2001
	ES4			
	EG4.2	from goat (EG4.2 and EG19)		
	EG19	from duiker EAT2		
	EAT2			
<i>Serratia sp.</i>	2AT1	Olive mil waste	Growth on media containing tannic acid as carbon source and HPLC determination of degradation products	Pepi et al. 2010
	2AT3			
<i>Serratia ficaria</i>	DTC	Tea processing factory	Gallic acid colorimetric determination by rhodanine assay	Belur et al. 2010a
<i>Serratia ficaria</i>	DTC	Tea processing factory	Gallic acid colorimetric determination by rhodanine assay	Belur et al. 2010b
<i>Serratia marcescens</i>	DT1	Tea processing factory	Gallic acid colorimetric determination by rhodanine assay	Belur et al. 2010a
<i>Serratia marcescens</i>		Human septicemia	DNS glucose determination from tannic acid after enzymatic acivity	Nsayef Muslim et al. 2017

Bacterial species	Strain	Isolation	Tannase detection method	Reference
<i>Staphylococcus lugdunensis</i>	MTCC 3614	Human feces	Tannin-protein complex degravation on selective media	Noguchi et al. 2007
<i>Staphylococcus lugdunensis</i>			PCR detection of the TanA _{SI} gene	Noguchi et al. 2010
<i>Staphylococcus lugdunensis</i>			Gallic acid colorimetric determination by rhodanine assay	Chaitanyakumar and Anbalagan 2016
<i>Staphylococcus lugdunensis</i>	UQM 3546	Human colon carcinoma	Tannin-protein complex degravation on selective media	Noguchi et al. 2018
<i>Streptococcus bovis</i>		Animal feces	Tannin-protein complex degravation on selective media	Osawa and Sly 1992
<i>Streptococcus bovis</i>		Koala feces	Tannin-protein complex degravation on selective media	Osawa 1992
	UQM 3611			
<i>Streptococcus caprinus</i>	ACM 3969	Rumen from feral goats	Tannic acid-protein complex degradation	Brooker et al. 1994
<i>Streptococcus gallolyticus</i>	UCN34	Human feces	Tannin-protein complex degravation on selective media	Noguchi et al. 2007
<i>Streptococcus gallolyticus</i>		Koala feces	Tannin-protein complex degravation and gallic acid spectrophotometric detection at 520 nm	Iwamoto et al. 2008
<i>Streptococcus gallolyticus</i>		Human endocarditis	Analysis of the complete genome sequence	Rusniok et al. 2010
<i>Streptococcus gallolyticus</i>	UCN34	Human endocarditis	Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Jiménez et al. 2014c
<i>Streptococcus gallolyticus</i>	UCN34	Human endocarditis	Gallic acid colorimetric determination by rhodanine assay and HPLC detection of degradation products	Jiménez et al. 2014d
<i>Streptomyces sp.</i>	AL1L	Leaves of Ailanthus excelsa Roxb	Gallic acid spectrophotometric detection at 550 nm	Roy et al. 2018
<i>Streptomyces sviveus</i>			Gallic acid colorimetric determination by rhodanine assay	Wu et al. 2015
<i>Unkown</i>		Cotton filed	Gallic acid colorimetric determination by rhodanine assay	Yao et al. 2011
<i>(metagenomic library)</i>				
<i>Unkown</i>		Cotton soil	Gallic acid colorimetric determination by rhodanine assay	Yao et al. 2013
<i>(metagenomic library)</i>				
<i>Unkown</i>		Cotton soli	Gallic acid colorimetric determination by rhodanine assay	Yao et al. 2014
<i>(metagenomic library)</i>				

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Figure S1

1	TanAap	-----MAFTRKEFLSLS---ALGV--AGTLAACTPQARTS-----DNTN	34
2	ARI	-----MDFTRKQFLALA---AAGATSVGALCACSPQQKKEG-----DSSK	37
3	TanASg	-----MPRKKWFFTS---SAVLLCSAMLLTACSSSSNSSTSSSSSQNT----TAS	43
4	ANA	---MPEGTMMNLPRRTILTALPA-TA---GILGVGACSAGG-----T-----AS---QA	39
5	CVI	-----	0
6	MMI	-----MDKKYIILMI-VAIVIIAAA--VSVYTHHT-----SER	30
7	SHE	-----MTKTTCTRRNFLSMLAIGAAGVAGSAALAACAQDQDTTASGT-----ASGSATA	49
8	MTI	-----MKLLKRILVLMMLL-----AVILPVCSGSTA-----EEQ	29
9	TanASl	-----MKKTFISLL--SA-----TVILSGCGVGEHQNNNSNH-DAKGVN--TS	38
10	LPC	-----MRPKRVLTIL--GCAS-AVALVLTACGSTKKSST-----ATKAPVTTTS	41
11	LNA	MINEIKGVSMTMIRHKAIPVI--SGLLILLVTT-TACARTASH--TKKQTTQQKVTKATK	55
12	LGA	-----MKVKKRVILL--GGMVLASLTLLTACQASGTDSTSSSTNTSKIVAKATK	47
13	LZY	-----M--SGLLIISVGLG-ACQATT-KTKHKTSTAAQVAKATK	36
14	TanALp	-----MQFRKIVPLM--SGLLVMSVGLA-ACGHSET-KTKHPTSTVAKVAKATK	45
15	LPE	-----MQFKKLAPLM--GGLLVMSVGLA-ACGQTKT-KTKQTTSTATKVAKATK	45
16	TanAap	QPASNVDL EEFSKSLKDMTQWSTDEDNDQCYQGLGIQCTKPASKSVNTLSVFVPGKYFSG	94
17	ARI	NAPASVDLEEFSKSLTLDMGAWSYDKDNVYQGLGLQYCMKPASKTYNSLSIYVPGKYFTG	97
18	TanASg	TSSLSGSEVSTTLDKVDNSKWQYNADDNVYQIGISAAANPTDAEQQTLSIFVPGDYMTA	103
19	ANA	SATASSQAPTDTKLVNLKGAWRYDAKGDVYQGLGLRVATPQASDYETLGIYVPGAYLTG	99
20	CVI	-----MDGKGWSYDETNNAYYILGLTYCSKPQATDYETLSLIVPGASVDA	45
21	MMI	KNTLALDDKQIANITVMNNWSYDSANDIYQNLNLSYCNRAKAPEYETLSIYVPGKYFDG	90
22	SHE	AVDVEPVELANTALAI DTAAWNYDADNDVYQIGVATCAAPQAADYESMGI FVPGAYMDA	109
23	MTI	AELISNYDQDNEALTFDNSAWNYDEDNDVYQINVVYCSSPETTDYESMGIYVPGAYMDA	89
24	TanASl	NVKIKNYNQASSALQIDNSKWYDSKNNVYQNLNISYVSNPQAKNVEKLG IYVPAAYFKG	98
25	LPC	QNVTKSDVSNAKKLLISTKKWRYNAKNKVYQVGLKYGTKTT-STYESMGIYVPASYVTA	100
26	LNA	LSVTKRDVSNAKKLLLKTSKWRYNAKHQVYQVGVRYGKTTSTKYESMGI FIPAQYVTA	115
27	LGA	YQVNSDISNAKKLLINSKWHYNAKNKVYQVGVRYGKTK-SSYESMGIYIPAAVYVNA	106
28	LZY	QTVTKADV TNAKKLLINHKWRYNATNKVYQVGVRIYGTKTTSSTYESMGI FIPAKYVKA	96
29	TanALp	QTVTKADVKNAKKLLINQKQWRYNATNKVYQVGVRYGTKTTSSTYESMGI FIPAKYVNA	105
30	LPE	QTVTTADVKNAKKLLINQKKWRYNAKNKVYQVGVRYGTKTSSSTYESMGI FIPAKYVTA	105
31	TanAap	KKNG-SYEECEVSEKAVVGSFTARTAPIVMPINTATLFPQSAPTSYAYEGLAPYLEAGFV	153
32	ARI	EKNSNKTYTCKINTKAVVGSFTPTAPIVAMPINTGTLSPQACPTESY EGLSTYLETGCI	157
33	TanASg	TDNGNGTYTCEINTSATVGNYTSEAPIVIPINTPGYSAMSALTEYTS-DATDYTSQEMI	162
34	ANA	KDNGNGTYTATVNASGANGDFTAATAPIVFFVNTPGYSAQKPPTESYDTIKAYMEAGFI	159
35	CVI	TDNGDGTYSLKPK-----QTATAVVMELNTPGYAAQAPMTEYSWDTVSEYIEAGLV	96
36	MMI	EKNTNGTYNCTINEKNKVANYSAKDAPVIPINTPGYSAQTAPTSYNPQEVKNYTDSGII	150
37	SHE	TDNGDGTFTCTVNDGNEVNGFTGTAPIVMPINTAGYSAQAAPTESYSGLDYLEAGLI	169
38	MTI	TENDGTFTCTVNTDNEINGYTAETAPIVFFVNTAGYSAQAAPTSYSSISSYLEEFGV	149
39	TanASl	KKNHNGTYTVTVNDAKKVNGYSARTAPIVYFPVNTPGYAEQSAPTSYRYSNISKYMKAGFI	158
40	LPC	KSNGDKTYTLTLNTKNKVKGYTAKTAPIVMPVNPAGYSAQAAPTGYDS-STAQFTKAGFI	159
41	LNA	TKSGSKTYHLKVNSKRKVNGYSAKTAPIVMPINTPGYAAQAAPTGYDQ-SSAKYTKAGFI	174
42	LGA	KKSGTNTYKISINTNKKVNGYTASTAPIVMPINTPGYSAQAAPTGYES-GSAKFTKAGFI	165
43	LZY	KTSGHQTYTITFNKAKVKGYTAKTAPIVMPVNTPGYAAQAAPTGYDS-SANKYTKAGFI	155
44	TanALp	KASGQKTYTITFNKAKVKGYTAKTAPIVMPVNTPGYAAQTAPTGYDS-SANKYTKAGFI	164
45	LPE	KASGQKTYTITFNKAKVKGYTAKTAPIVMPVNTPGYAAQTAPTSYDS-NANKYTKAGFI	164
46	TanAap	YVYAGFRGRSAGYDS--TTGSDELYAGGSEWPAVDFKAAIRYLRYNNELLPCNTSKIFVF	211
47	ARI	YVYAGFRGRSAGYDS--ASGSDDLYAGGSEWPAVDFKAAIRYLRYNAASLPCDTTKVVFAY	215
48	TanASg	YVSAGLRGRDSDG-----PSGVTDAKAAIRYLRYNQGNISGNTDSIFVF	206
49	ANA	YVHAGLRGKDSNSQ-----TYSGNAPWGVTDLKAAVRYRRYNAALIPGDAAKVYVF	210
50	CVI	YAHAGMRGRDSMSD-----SYVGNAPWGVTDLKAAVFIRLNAEFIPADTSKVYTF	147
51	MMI	YVYPGCRGRDNGD-----NFTGGAPWGVTDLKASIMYLKFNKDIIPGDTEKIFTF	200
52	SHE	YVYAGCRGRDNGD-----TFAGGAPWGVTDLKAIVRTLRNGGNIPGDKDRIFTF	219
53	MTI	YVYAGMRGRDNGYDEN----DDLIIYSGGAPWGVTDLKAIRYYRFNQELLPGSTDLMFTE	205
54	TanASl	YVEAGLRGRSMGMGNSSNASTKSYETGSEWGVTDLKAIRYYRFNDSSLPGNSSKIYTF	218
55	LPC	YVEAGFRGLNNTD-----KSNGSSEWGVTDLKAIVRTLHLNANRLAGDTSRIFTF	209
56	LNA	YVLACGCHLNQTD-----KTNGSAPWGVTDLKAIVRTLRLNQSLAGNTNRIFTF	224
57	LGA	YVLACGRGLNQTD-----SSNGSSEWGVTDLKAIVRTLRLNENRIAGNTNRIFTF	215
58	LZY	YVAACGRGLNQSD-----KSNGSSEWGVTDLKAIVRTLRLNRQNIAGNTNRIFTF	205
59	TanALp	YVAACGRGLSQSD-----KSNGSSEWGVTDLKAIVRTLRLNRSRIAGNTNRVFTF	214
60	LPE	YVAACGRGLNQTD-----KSNGSSEWGVTDLKAIVRTLRLNSKRIAGDNTNRVFTF	214
61		*. * : * . * : : * : : : : *	
62			
63			
64			
65			

	TanAAp	GFAAGGGLSAVLGTSGDSSLYMPYIKAVGAATHSEKGEQLSDSIYGSASWCPATSYDLAD	271
	ARI	GFSSGGGLSAVLGTSGDSPLYSPYLDAGI GAATHDTQGTSLSDAIYGSASWCPSTSFQVAD	275
1	TanASg	GMSGGGAQSAIIGSSGDSSLYDDYITEIGAVE-----GVSDSVAGVMAWCPITNLDTAN	260
2	ANA	GHSGGGAQSAVAGASGDSGMFAPYIALGAATTDSSGKALSDAVAAMCWCPITSLGSAN	270
3	CVI	GHSGGGAQSAVMGASGSDSLYTPYLETIGAAMTGANGEKLSDAIAGAMCWCPITSLDFAN	207
4	MMI	GHSGGGAQSAIVGSSGDNELYESYLEAIGAMTDQNGYVISSSVYSGMCWCPITNLDTAD	260
5	SHE	GHSGGGAQSAIIGCATGNAEGYTPYLETIGAALQDDEGNDISDAIMGAMCWCPITNLDTAN	279
6	MTI	GHSGGGAQSCLTGATGDSVLYDYDYESIGAAYIDEDGNYISDVICGAMCWCPITSLDTAN	265
7	TanASl	GHSGGGAQSAIAGASGDSKLYYKYLEQIGAAMTDKNGKYISDKIDGAMAWCPITSLDQAD	278
8	LPC	GMSGGGAESAIVGATGNSRGYTKYIKAI GAPMATASGKHTRDNVDGAMAWCPITSLDTAN	269
9	LNA	GHSGGGAQSSLMGTTGDSKQYLYKYLRAIGAPLATTSGQATSDAIMGAMAWCPVTSLODTAN	284
10	LGA	GHSGGGAQSAIVGSSGNSKAYIKYIKAI GAPLATTSGKATSDATEGAMSWCPITSLDTAN	274
11	LZY	GHSGGGAQSAIMGATGDSKKYTKYIKAI GAPLATTGNGATSDAVAGAMAWCPITSLDTAN	265
12	TanALp	GHSGGGAQSAIMGATGDSKKYTKYIKAI GAPLATTGKSTSDAVAGAMAWCPITSLDTAN	274
13	LPE	GHSGGGAQSAIMGATGDSKKYTKYIKAI GAPMATTSGKSTSDAVAGAMAWCPITSLDTAN	274
14		* :.*. *. : :.* : ** : ** . * .*** * . . * :	
15	TanAAp	AAEWSAGQYADATDSRAEGVWTPQLSQDLGAYISFVNNDMLDSNDSKVSLEDTNSGV	331
16	ARI	AAEWSAGQYADG-DTRASGTWTHALSSDLASAYGTWVNSMDFLSDGNKLELDQTNSEI	334
17	TanASg	EAYEWNMGSTRSD-LSDE---EQTISDGLATAFAKYINKLGLQDEDDGNKLTLLKKSDDGI	315
18	ANA	AAEWNMGQFASS-DTRADGTWTRAYSQDLAAAFPAYLNALRLTDSKGKPLTLESSSQGT	329
19	CVI	LAYEQMGQFATT-GTRADGTWTKAYSNDLAKAFGDYLNGLGLED-GGKRMSLEQSGDGY	265
20	MMI	EGYEWYMGQYT-----RSNDTWYELSKDLAMKYGEYINKLGLKDPGKTLTLLDKSNDGI	315
21	SHE	EAYEWNMGQFASS-GTRAEGTFTAQLSQDMAEAYADYINVGLLRDADGNELTLEDGEGEI	338
22	MTI	EAYEWNMGQYSSS-GTRADDTWTSAMSDELAEAYASYINGLGLMDENGEVLTLEESDGV	324
23	TanASl	AAEYWMGQYGNB-GNRKKNSEFKQLSTDASSYASYLNKLNKNG-NTTSLTKSKNGQ	336
24	LPC	EAYEWNMGQYSNS-GTRKSGTWTKALSNDMAKNFAQYINKIGLKDKAGKTLTLLKKSCTGI	328
25	LNA	EAYEWNMGQYATT-GTRKSGTWTKALSNDLATRYAKYLNQLKLNKNTDGTTLKLNNSGSGI	343
26	LGA	EAYEWNMGQYSTS-RTRASGTFTKALSNDMAKKYAEYINKLGLKDSKNQKTLTLLKSSSGI	334
27	LZY	EAYEWNMGQYSNS-GTRKQGTWTKALSNDMATSAYQYINKLGLKDGASQTTLTLLKKSANGI	324
28	TanALp	EAYEWNMGQYSNS-GTRKQGTWTKALSNDMATSAYQYINKLGLKDANGKTLTLLKKSSTGI	333
29	LPE	EAYEWNMGQYSNS-GTRKQGTWTKALSNDMATSAYQYINKLGLKADAGKTLTLLKKSSTNGI	333
30		.*** * . * : * : : : . : * . *	
31	TanAAp	VTAGSYADLVNELKTSASNFIFKDNAPFYTFTFPQRLEEPTFPQDPNPLATVR-----	382
32	ARI	VTMGSYMEAIQAELETSAANFARETSFPYTATPQRFEDPLFPQDPNPLAKSR-----	385
33	TanASg	VQAGSYINYLKSVIEDSLNTFLANTTFFPYDASSSSQGGGLGG-----	356
34	ANA	VLAGSYYDHLVAVIQKSLNDFLAATTFFPYTPSSTEMAGMEP-GGGGGAPSG-----TP	381
35	CVI	FLAGSYYDKIVAVVEKSLNDFLSVTTFFPYTPSNTTMAGMSV-GVGGTGAAAQSGSSSGAS	324
36	MMI	VTSGSYDYDQISVIEESLNNFLADTTFFPYTPSNDMKSMGGSPLG--NAPTGEM---PT--	368
37	SHE	KTQGSYDYDVVAEVERSLNNFLADTEFFPYTPSNAFNADMGAAGGGSGAPSGEM---PGEA	395
38	MTI	VTSGTYDYDQLSVIEGSLNNFLSDTTFFPYTESAGTTMADGG-FGGSGAPSGEM---PD--	378
39	TanASl	VTEGSYAKYLKKEIEDSATEFLNNTTFFPYKQNSTEQAGMGN-----GG	379
40	LPC	VTSGSYERYLKNEIQTSLNNFLKDTKFFPYKASNSMTAGMNISGNTSGKGTG-----AA	381
41	LNA	VTSGTYAKYLKQEVQSLNNFLKDTTFFPYKATMTSGPS--GAAQSGSQ-----KM	392
42	LGA	VTSGTYAEYLKAEVDQSLNNFLKDTKFFPYTATSMNGPS--GAAQSGS-----K	381
43	LZY	VTSGSYATYLLKKEVEQSLNNFLKDTTFFPYKATSNEGPS--SAATQ---TSG-----KK	372
44	TanALp	VTSGTYATYLLKKEVEQSLNNFLKDTTFFPYKATSNEGPS--GAASQT-LTSG-----KM	383
45	LPE	VTSGSYATYLLKKEVEQSLNNFLKDTTFFPYKATSNEGPS--GAAAQTGTTTG-----KM	384
46		* : * . : . * . * .*** .	
47	TanAAp	-GTDNAA-----	388
48	ARI	-GVEAPSTVLQTDATKSA---DTQSQQGQLQDQGQSQDQS-----	421
49	TanASg	--GDMPTGEAPTDLGTTDD-----TTSIEDVDD-----	382
50	ANA	PSG-----GGGTPSKG-----	392
51	CVI	PSDAAGAGAAGA-----SGAMPSGG-----	344
52	MMI	--GEAPAGDVP-----NGELPAGNIQNNG-----	390
53	SHE	PSGEAPSGEAP-----SGEAPSGG-----	414
54	MTI	--GEMPSGDFP-----GETTSDSSIQSTDSTDVVSASFDSIINFLNSLFGND	425
55	TanASl	PSGGKPSGKM-----GS-----	391
56	LPC	PSGTPKPSGKAPSGAKS-----TSGTPKPSGNMP-----	408
57	LNA	PSGQKPSGKK-----PSGQSSTGKPA-----	413
58	LGA	PSGQKPSGAPNNNMKNGNNSKPSGKKPSGSMP-----	413
59	LZY	PSGSKPTGAT-----KSGSKPSGSAP-----	393
60	TanALp	PSGSKPSGTA-----KSGSKPSGSAP-----	404
61	LPE	PSGSKPSGTA-----KSGSQPSGGAP-----	405

59
60
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[illegible]

TanAAp	A----	607
ARI	A----	659
TanASg	DNS-	596
ANA	S----	615
CVI	-----	562
1 MMI	-----	605
2 SHE	-----	640
3 MTI	Q----	663
4 TanASl	K----	613
5 LPC	-----	626
6 LNA	ATAD	638
7 LGA	N----	638
8 LZY	-----	611
8 TanALp	-----	626
9 LPE	-----	627

Fig. S1. Comparison of amino acid sequences of functional subtype A tannases and similar putative tannases included in the databases. Multiple alignments were done using the program ClustalOmega after retrieval of sequences from BLAST homology searches. The functional subtype A tannases are from *Atopobium parvulum* DSM 20469^T (TanAAp, accession C8W7K9), *Lactobacillus plantarum* ATCC 14197^T (TanALp, accession C2FI97), *Staphylococcus lugdunensis* MTCC 3614 (TanASl, accession BAF03594.1), and *Streptococcus gallolyticus* UCN34 (TanASg, accession GALLO_0933). Putative subtype A tannases are from *Actinomyces naeslundii* ATCC 12104 (ANA, accession J3F2Y7), *Atopobium rimae* ATCC 49626 (ARI, accession B9CMT1), *Corynebacterium vitaeruminis* DSM 20294 (CVI, accession W5Y437), *Lactobacillus gasseri* DSM 14869 (LGA, accession A0A249NJ78), *Lactobacillus paracollinoides* DSM 15502 (LPC, accession A0A0R1TM22), *Lactobacillus pentosus* IG1 (LPE, accession G0M044), *Lactobacillus namurensis* DSM 19117 (LNA, accession A0A0RIK9A6), *Lactobacillus zymae* DSM 19395 (LZY, accession A0A0R1MZ09), *Methanobrevibacter millerae* SM9 (MMI, accession ALT68817.1), *Methanobolus tindarius* DSM 2278 (MTI, accession W9DZK2), and *Slackia heliotrinireducens* ATCC 29202 (SHE, accession C7N116). Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. Putative signal peptides are highlighted in yellow. Residues conserved in all the functional subtype A tannases described so far are highlighted in green. The conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases is showed in a black-lined box. In a box are also showed the amino acids from the catalytic triad, Ser-163, in the pentapeptide motif, is conserved in all bacterial tannases, except in TanAAp, and His-451 which is conserved in all tannases. However, the residue Asp-419, also marked in a red-lined box, from the catalytic triad is absent in all subtype A tannases (numbering is referring to TanBLp tannase).

Figure S2

1				
2				
3	RTH	-----MTIGSTMRLMLIGGLT	17	
4	GAM	-----MP-----SE---ITRRAALAGFGALVL-A	20	
5	MVA	-----MKKAAMAD---AQ---MTRRALLAGMGYTAG-A	26	
6	AEX	-----MIDRRRLMQATLAAG-I	16	
7	TanBSs	MTLDCWQFCPHSGYVRRRQQI-----PPRERTVKH---RA---VRRRSVVLGIGATAG-A	48	
8	SAV	----MLTISR RVFYVRKRKISTERGREWERSMKH---RA---MKRRTVVMGVGAAAG-V	48	
9	TanBSg	-----	0	
10	HHA	-----	0	
11	OOE	-----	0	
12	LGA	-----	0	
13	LSE	-----	0	
14	LAL	-----	0	
15	LPB	-----	0	
16	LFA	-----	0	
17	TanBLpe	-----	0	
18	TanBLp	-----	0	
19	TanBLpa	-----	0	
20	LFF	-----MRLKKVVAVSLCAVSL-I	17	
21	LVE	-----MFLAV-I	6	
22	CCO	-----MKGFRVAI-----	8	
23	MMU	-----MGISMN-----LQQYVLTALAVSALTM	22	
24	HHT	-----	0	
25	ASU	-----MKLLKISMALFSAVF-F	16	
26	ASE	-----MQAKTWLASLVGLAV-S	16	
27	TanBFnp	-----MRKFKFLMLFCLFGSML--	17	
28	FRI	-----MKNKI-NYLLYTVV--	13	
29	RSL	-----MF--IRQKFPANLLFAVG--	16	
30	MPO	-----	0	
31	HGR	-----MIYANRRQFTLALMAASLAT	20	
32	SNA	-----MMI-----PRRTFTMSLLAAS---	16	
33	CGR	-----MSGKTLSPFTNEEEEAVKLTKVVPYSLEDKLKEH	34	
34	LBU	-----MNN-----KIKLMIISLMVSSMVI	19	
35	MCU	-----MKKPIYLCAMT--MAL	14	
36	KOR	-----	0	
37	CHO	-----MKKTAIL-----	7	
38	NMU	-----MNT-----LNKTLLS--VS--VAL	15	
39	ECO	-----MMK-----FKPALLAGILA--LAC	17	
40	NBA	-----MP-----FKPFALAALTA--LAT	16	
41				
42				
43				
44	RTH	-----TAAGTVRAE	30	GVFD
45	GAM	AVAACSDDDSD-----SGSGSSSA-----	50	SPKSSGTGTAT
46	MVA	LAMA-S-----CAGS-----	44	APREPDPTA
47	AEX	F-----SGT-----	29	ALRAACAET
48	TanBSs	MAAG-GLALNA-----NAS-----	70	TGSTSTSRD
49	SAV	VAAG-GFALSA-----QASGT-----	72	SGSKSSDSS
50	TanBSg	-----	0	
51	HHA	-----MK---QDYKEYEEKIKMN	15	
52	OOE	-----	0	
53	LGA	-----	0	
54	LSE	-----MQ	2	
55	LAL	-----	0	
56	LPB	-----MAMT	4	
57	LFA	-----M	1	
58	TanBLpe	-----MT	2	
59	TanBLp	-----MS	2	
60	TanBLpa	-----MS	2	
61	LFF	GLAACASNNSASTTKKSAS-----TSSSNTKILSNVTYQHAVKYYRGTTYSGKA	67	
62	LVE	VLTCSSNKSSSSSSK-----TIKAGDLSSTSYKKNASYRRGGNAYKGKK	51	
63	CCO	-----LGVCLAGACFG	19	
64	MMU	-----PWAPGVSQAAAADASAQAATAKTAADVSSATAVKADTAAKSDEAEAKAKQVK	74	
65	HHT	-----MS	2	
66	ASU	G-----NVF-A-----LPPS-----QNPLGDKPFANKAADG	41	

1	ASE	G-----MAMAN-----PPQG-----NDPLADAPYFNKTDSG	42
2	TanBFnp	-----FAAQKTTTKTVKNE	30
3	FRI	-----LLYSAFTFCQEKSV	27
4	RSL	-----ALFA-NTVCG-QSQ	28
5	MPO	-----MTH-----I-----NENPIN-ISVIDGKL-----PSITATQSKTA	29
6	HGR	TARAA-----ESA-P-----QGTNTSE	37
7	SNA	LAGAC-----SSTPMTPTPSPA	34
8	CGR	GAIDKAGLWQDKVSDNRVIT-----GQN-P-----QSAKSVGEAILKELQK	76
9	LBU	GANSN--KKVNTNVSASQKDIQN--NGKDSEKK-PQMEGPMKKTIVITEDMIVNKTANG	73
10	MCU	SLAAC-----VN--AGNQTPSN-AADMDKR-----QRSELTRLPAP	47
11	KOR	-----	0
12	CHO	--AAM-----S-----LANLAQA	18
13	NMU	GLNAC-----A-----NVQTAKT	28
14	ECO	GSGAA-----K-----TIEVSQG	30
15	NBA	-----HAQA	20
16			
17	RTH	ATLRIDP-DAFVTITVTI-----DGKPLTLRRYEA-VVGRPVAMATEQPARRMGPGGSP	83
18	GAM	GDLVFDP-ASFTEKTATISAADGSSKKVTYRFYGPITYVTKPVDE-----	94
19	MVA	GKLAFDP-AVFTQKTATVA-TAAGDKQVYHFFGPITYVANPVDA-----	87
20	AEX	SRLVFNA-DDFTELKKTIT-TAKGERVVYRFYKAIPYVSEPIDV-----	72
21	TanBSs	DGLVFDP-DAWRKLTITVTDTQGTTEHVYHFWKAVTYVSKPVDA-----	114
22	SAV	DALVFDK-DAFTVLTTIT-TDGGDKEVKYHFKAITVYVAKPVDE-----	115
23	TanBSg	-----MSINQ-WI-----FDETNNCYMSLKNVYCAQPKDS-----	29
24	HHA	DNLKFDA-ERGETRTYEA-----AGKKITCRAYLGIVYCERPAD-----	53
25	OOE	-----	0
26	LGA	-MLKIRV-DQYQT--KA-----LLNGFLYREYRDLTYVERPVS-----	34
27	LSE	APLRFDE-STYEKATTTV-----AGEAIAYRSFKQIDYVSRPVD-----	40
28	LAL	-----	0
29	LPB	TPLRFDE-TRYQSAQVTI-----DGQVLRYRAFLGLDYVTMPVD-----	42
30	LFA	TQLEFDT-AQFQPQLVTL-----DGQTVRYQAACDIPYVAQPV-----	39
31	TanBLpe	DTLIFDA-DWLTPEQIQL-----DGKTINYLAAHDIQYVQHPAA-----	40
32	TanBLp	NRLIFDA-DWLVPQVQV-----AGQAIQYYAARNIQYVQHPVA-----	40
33	TanBLpa	NRLIFDA-DWLVPQVQV-----AGQAIQYYAARNIQYVQHPVA-----	40
34	LFF	KSLKLDT-KKYTTKTITV-----NKKKIKVRCYTNLTIVSKPVST-----	106
35	LVE	VSLNFKN-AKYTVRTLTS-----GNKKIKYRAYENITYVSKPVDK-----	90
36	CCO	GELKFDE-NKFELKSVQV-----GERTLKFRAYEGIVYVAKPVS-----	57
37	MMU	YSLKFNA-KNYTKETLQL-----DGKDVAFRAYRNIVYVSKPASV-----	113
38	HHT	ISLKFHE-ENYTIREISI-----LDKHIKYRAFENIVYAGKPVDR-----	41
39	ASU	YNLEFND-KQYTVETGEV-----DGKTVKYRAFEKIVYVKNPVEP-----	80
40	ASE	YDLKFSP-KTYRTLEAKV-----NGETVKFRAFEKIVYVQNPVEP-----	81
41	TanBFnp	YDLNFPN-DKYVSKETEI-----NGQIKYRAYENIVYVKNPIDK-----	69
42	FRI	YDLNFPN-ENYKMQLTL-----EGKSEKVRAYENIVYVANPVDT-----	66
43	RSL	GALRFDP-SKFTLQSQEL-----NGKTVNVRAYEKIVYVANPVDT-----	67
44	MPO	KPLQFDV-NNYKTLDVTV-----GDQTIKVRAYENIVYVANPVDS-----	68
45	HGR	SSLDFNP-HRYTRQDFVL-----KGLAVDVRAEGLPTVRRALEP-----	76
46	SNA	PPPPFDPKRGYQRREVS-----GGRTVVVRAWEGLAYVSRPVEP-----	74
47	CGR	YPLRFDA-TKYTTQQVTQ-----GTQTLTVRAYEGIVYVANPVEE-----	115
48	LBU	YNLNFVNKNYTTKTATV-----NGKTVTYRAYENIVYVAKPADI-----	113
49	MCU	ADLDFAK-QSGSVQQFEL-----EGQTVRYRAFENIVYVLPVDT-----	86
50	KOR	-----MYVLKPADT-----	9
51	CHO	HSLDFAA-DKYTAKTAEV-----NGATIAYRAYEGIPYVSKPVEP-----	57
52	NMU	YDLDFSK-QKYTEQSIEV-----NGQAVKFRAYENVYVVRNPVDT-----	67
53	ECO	YGLDFRT-QTYTRQSTVV-----NGQTVAYRAYENIVYVANPVDS-----	69
54	NBA	ADLDFSK-QPYTAQTAEV-----NGQTVQYRAFENIPYVKNPVEA-----	59
55			
56	RTH	SGAETQTLEDPLTYQKMAIFVPEGASD-----TAAMILNVSNG	121
57	GAM	-----TYQCLTVSVPTAIDG--KKVD-----ASTAFIVFANSVG	126
58	MVA	-----DYQSLVISVPTAIDG--RVVD-----ATGAPIVFANSVG	119
59	AEX	-----AYQSLNISVPVPSIDG--KRVD-----SSGSPIILFSNSVG	104
60	TanBSs	-----TYQSLIVSAPVEIDG--TAVD-----ASNAPILLANSVG	146
61	SAV	-----KYQSLNVSVPVEIDG--TAVD-----ATNAPILFANSVG	147
62	TanBSg	-----ELEALHIFVPAYVMTADGTIDRDAVVTNKNGTIYTSQTVPIIFYNDIG	77

	HHA	-----PVQKLNIFVPEVYDR-GGMIG-----GYTKLTAPIFVFPNTVG	89
1	OOE	-----MNVFVPLGYK-NEIIN-----DYGLNTAPIFMPNTVG	32
2	LGA	-----EIQQNLNFI PQAYYQ-QESIN-----GYTAATAPIFVFPNTVG	70
3	LSE	-----PIQRLNIFVPEAYFN-GGQVN-----GYTRVSAPIFMPNTIG	76
4	LAL	-----MNI FVPLAYYH-QGTIN-----GYTAKTAPIFMPNTVG	32
5	LPB	-----AIQQNLNVFVPEPYFN-NGQVN-----GYTRVTAPIFMPNTVG	78
6	LFA	-----DIQQNLNIFVPAAYFE-GGTLN-----GYTAATAPIFMPNTVG	75
7	TanBLpe	-----AIQRLNVFVPAAYEN-GGTVN-----GYTRDTAPIFMPNTVG	76
8	TanBLp	-----AIQVLNVFVPAAYLH-GSSVN-----GYQRATAPIILMPNTVG	76
9	TanBLpa	-----AIQVLNVFVPAAYLH-GSSVN-----GYQRATAPIILMPNTVG	76
10	LFF	-----KYQTMNIYVPEKYFH-NGKIN-----GYTKTAPIFMPNNVG	142
11	LVE	-----KYQTMNIYIPEAYFK-NKKIN-----GYTKNSAPVFFPNSVG	126
12	CCO	-----GYEALNFYVPEDKFG-----DQKGAIFMPNAIG	85
13	MMU	-----AAESMSIFIPEAYFQKGGTVN-----GYTKETAPIFLPNGVG	150
14	HHT	-----EHQSLSIFVPEWYFE-EAD-G-----KEQLKKVPVFFPNTVG	76
15	ASU	-----DYQTINFYVPEDYFA-DKEIN-----GFNAQTAPIFLPNSIG	116
16	ASE	-----DYQTLNIYVPEAYFN-NGKIN-----GFNAKSAPIFLPNAV	117
17	TanBFnp	-----DYQNMNIYIPEEYFN-NSSIG-----SYNSSNAPIFFPNTVG	105
18	FRI	-----SYQKLNIIYIPEEYFS-GKSIN-----GYTGANAPIFFPNEIG	102
19	RSL	-----TQQVLNIYIPEAYFN-QQSIN-----GYTAQTAPIFFPNNRV	103
20	MPO	-----TYQSMNIYIPESYFE-NQSIN-----GYTASTAPIFMPNAV	104
21	HGR	-----SYQTLNLIYIPDAYFK-GGTVG-----RYNAQTAPIFLPNQVG	112
22	SNA	-----AWQVMNLIYIPEAYFQ-GGQID-----GRTARTAPIFLPNSIG	110
23	CGR	-----QYQQLNIIYIPEAYFN-GETIN-----GFNAQTAPIFLPNGIG	151
24	LBU	-----AYQTINIYIPEEYFN-NKTVG-----KYNARTAPIFFPNTVG	149
25	MCU	-----RYQFMNIYIPEAYFQ-DESID-----DFTAETAPIFLPNQVG	122
26	KOR	-----RYQTLNVYIPEAYFS-GGSID-----GYTAQTAPIFFPNNIG	45
27	CHO	-----EYQQINIYIPEAYFN-GGSIN-----GYSATAPIFLPNQIG	93
28	NMU	-----RYEIIINIYVPEAYYN-GGEID-----GFTAETAPIFLPNQIG	103
29	ECO	-----AYQTINIYIPEAYFN-GGSIN-----GYTAQTAPIFLPNQIG	105
30	NBA	-----DYQTINIYIPEAYFN-GGSVN-----GYTAATAPIVFLPNRIG	95
		: . * : . . *	

31	RTH	GWFASELRP-GIEDGG-----	136
32	GAM	GYMPSSVAEATAVGGGQ-----MVGMGTQGSSSSSSASGSSAP	164
33	MVA	GYLPASVKDVTGVGEAG-----RRGPPPPG-----	144
34	AEX	GYMPSSVADATGIGGAP-----MRGLPPTGMQAPPQGV-----	137
35	TanBSs	GYMPSSVTDATGVGAGG-----MGGGGGA-PGGSPGTGT-----	178
36	SAV	GYMPSSVADATGVGGAA-----MTGMPGGGASASPSAS-----	180
37	TanBSg	GYAECQPAMVTPRN-----	91
38	HHA	GYLPGPAEEPGLNIRT-----	105
39	OOE	GYFPGPRTFPGNKKIL-----	48
40	LGA	DYLPGPADFPGNQWP-----	86
41	LSE	GYRPGPSDFPENVARY-----	92
42	LAL	GYMPGPRDFPGNAAFP-----	48
43	LPB	DYLPGPDRDFPGNQESF-----	94
44	LFA	GYMPGPRDYPGNQQQP-----	91
45	TanBLpe	GYFPGPVDDPTRTQWP-----	92
46	TanBLp	GYLPGPADDPQRVWTP-----	92
47	TanBLpa	GYLPGPADDPQRVWTP-----	92
48	LFF	GYMSGTAGTLTGSSSG---A--A-----PSGSTGKMS	170
49	LVE	GYMPGEATTIGKSNSST---M--T-----MPKST-KSS	153
50	CCO	GYMSAKPQKPEIQNEK-----	101
51	MMU	GYMPGEIKEPSANDRM-----	166
52	HHT	GYMPGLPEPPGPNRSG-----	92
53	ASU	GYMPAMAATAAQKGIG-----	132
54	ASE	GYMPAKAATYDAKGFG-----	133
55	TanBFnp	GYMPGKADIVGLGRDG-----	121
56	FRI	GYMPAHPATASPSKKGK---FPSHMGDIPAGTKPPEGEKL-----PLGGLGSMP	148
57	RSL	GYMPALPATFKASPOR-----GP--	121
58	MPO	GYMPAKPMTVGVGRDG-----	120
59	HGR	GYMPAKPGTLAVRVEGPPGGMGMEMGGGGGGPQGPGRGGMGGGGGGMRMPPGGMGGE	172
60	SNA	GYMPAKPGTPEGNGP-----	126
61	CGR	GYMPAKPLSLTGGNKD-----	167
62	LBU	GYMPGAAGIPGNGRDG-----	165
63	MCU	GYMPATAGRPELDQRS-----	138

	KOR	GYMPATAGQPENDQRA-----	61
1	CHO	GYMPAKPGVPGERRHG-----	109
2	NMU	GYMPAEPGKPALEGKR-----	119
3	ECO	GYMPAKAGVPGKGGFG-----	121
4	NBA	GYMPAKAGVPGKGGHG-----	111
5		..	
6			
7			
8	RTH	-----SY--VSDSDSKVGAALAAGY	155
9	GAM	TSSAASSSSAAASSFAAPSSSAAQSSTASGTEVESGGNAMLNSMGKMVSIPEIALAEGF	224
10	MVA	-----LPGGEVTPGGNAMVDARGDLVNLAAALAVAAGY	176
11	AEX	-----APTGIGG-EVQ-SGGNAMIAGGKRVSNAELALAAGL	171
12	TanBSs	-----AA--PSASAASNANANTNATGGATSSNQLLALAAGY	212
13	SAV	-----ASAGTGAGEVQSGGNGQVNAGGEMVNNGKLALAAGY	216
14	TanBSg	-----QRYLEDGY	99
15	HHA	-----GMPNSIFAALHGC	119
16	OOE	-----YNSSTMVKALSRGY	62
17	LGA	-----SRSKTIEAALQRGY	100
18	LSE	-----GQGATMLAALKQGY	106
19	LAL	-----NNSTTIVKAIEHGY	62
20	LPB	-----SRGATILQALMRGY	108
21	LFA	-----TNSKTLIQALRRGY	105
22	TanBLpe	-----NNATTIKQALKRGY	106
23	TanBLp	-----TNAGTIQQALKRGY	106
24	TanBLpa	-----TNAGTIQQALKRGY	106
25	LFF	GT-----KPSGKAPSGSGSSTSLNANGGASTTSASQKAISEGY	208
26	LVE	SS-----TPAA--PGAGGTGTNATGGAPSGANSAPKALKEGY	189
27	CCO	-----PNATLEALLRGY	113
28	MMU	-----SGGANASLVALSRGY	181
29	HHT	-----EPNASFYAILNGY	105
30	ASU	-----GRTSTILTALSKGY	146
31	ASE	-----SGDKSNAILTALSKGY	149
32	TanBFnp	-----KANSPTYALSKGY	134
33	FRI	MG-----MM-----GPNRPSAVLTALSKGY	168
34	RSL	-----MP-----GGNQGSSVLAALSKGY	139
35	MPO	-----KPNVAQALHRGL	133
36	HGR	GT-----RPGGGGARGAGGPGAGPNAGPSAGPSAIMQALARGF	210
37	SNA	-----PPAPGQTPLPASIAVALTRGL	147
38	CGR	-----TNNSLIMALSKGF	180
39	LBU	-----QPDASLIALSNGY	178
40	MCU	-----GSPNAMMVALSKGY	152
41	KOR	-----G-----GKPNAIMLALSKGY	76
42	CHO	-----GQP-----GADAMQTALAKGY	125
43	NMU	-----GEPEDGQKSPNAALTALSKGY	140
44	ECO	-----PS--KEGQVDAMQAALARGY	139
45	NBA	-----QS--KG--ADAMQTALARGL	127
46		: *	
47			
48	RTH	VYVDICSRGRGILA-----ADG-SLPCKAPAAVVDKAAIRFLRLND--	196
49	GAM	VVVEPGARGRTLVS-----DSG-DYYGTAPAVIVDLKAAVRYVRFNE--	265
50	MVA	VAVEPGCRGRTLVT-----ADG-VCYGTAPAAVVDLKAAVRYLRSNA--	217
51	AEX	TVVEPGARGRTLTD-----AAG-TYYGVAPAAIVDLKAAVKYVRFNK--	212
52	TanBSs	VVIEPGARGRTLKN-----SSG-EYYGTAPAAIVDLKAAVRYIKSNK--	253
53	SAV	VVVEPGARGRTLTD-----SSG-TYYGTAPAAIVDLKAAVRYVRFNK--	257
54	TanBSg	VLVSVGARGRQSQN-----GICKAPAGLVDLKA AVRWLKHH--	136
55	HHA	VVVSQVGRGRTSGMKSTEFFEGGKVDDTGQDNG-KMVGKAPALIVDMKAVIRYIRHNR--	176
56	OOE	VVVSAGLRGRTLTN-----EKG-EFIGKAPAFIIDMKAAIRYIKFNK--	103
57	LGA	VVASPGLRGRTQQN-----ESG-DYICKAPAFIVDLKAAIRFLRHNA--	141
58	LSE	VVVSAGARGWTSRD-----SNN-EFTGKAPAFIVDLKAAVRYVITYNA--	147
59	LAL	VVISAGLRGRSLTD-----ETK-RFIGKAPAFIVDMKAAIRYVRHNQ--	103
60	LPB	VVVSAGLRGRTSKT-----AQG-EYSCKAPAFIIDMKAAIRYVKFNA--	149
61	LFA	VVVSAGLRGWSQQN-----AAG-DFICKAPAFIVDMKAAIRYVKANQ--	146
62	TanBLpe	VVVSAGLRGHTTVN-----ESG-QRVGQAPAFIVDMKAAVRYVKYNQ--	147
63	TanBLp	VVVAAGIRGRTTVD-----KSG-QRVGQAPAFIVDMKAAIRYVKYNQ--	147
64	TanBLpa	VVVAAGIRGRTTVD-----KSG-QRVGQAPAFIVDMKAAIRYVKYNQ--	147
65	LFF	IVAAPGARGRDAKQ-----NG--KYTGKAPAGIVDLKAAVRYLKYNS--	248
66	LVE	IVAAPGARGRTTKQ-----KS--NYTGKAPAAIVDLKAAVRYLKLNS--	229

1	CCO	VVASVGARGRTLK-----DGEKFIKAPAAI	VDLKA	AVRYLK	FND--	153
2	MMU	VVAAPAIRGRTTVG-----DDGKTYVGKAPALI	VDYKA	AVRYL	RNRH-	224
3	HHT	VVVSPGARGRGLKD-----EKG-AFIGNAPACI	VDLKA	AI	RYLKHN	146
4	ASU	VVASVGARGRTLK-----KTG-AYTGKAPAVI	VDLKA	AVRYL	HANN-	186
5	ASE	VVASVGARGRTLE-----KDG-KYTGKAPAAI	IDLKA	AVRYL	LHFN	189
6	TanBfnp	VVAAPGARGRTLTD-----NKG-NYTGKAPAAI	VDLKA	AVRYL	LYLND-	175
7	FRI	VVASAGARGRTT-----KTGKAPAVI	VDLKA	AI	RYLKFN	203
8	RSL	VVASAGARGRIS-----PSGKAPAGI	VDLKA	AVRYL	KAND--	174
9	MPO	IVASAGARGRSLKN-----EQG-QYYGKAPAGI	VDLKA	AVRYL	KAND--	174
10	HGR	IVAAPGARGRTLRA-----EDG-SWTGKAPAAI	VDLKA	AVRWLR	RHNALA	253
11	SNA	VVASPGARGRTLQA-----ADG-TWTGKAPAAI	IDLKA	AVRFLR	RHHD--	188
12	CGR	VVASPGARGRTS-----ATGKAPAVI	VDLKA	AVRYL	KYND--	215
13	LBU	VVASPGARGRTLE-----KDG-KYTGKAPAVI	DLKA	AVRYL	RYND--	218
14	MCU	VVASAGARGRTE-----STGRAPAAI	VDLKA	AVRYL	KAND--	187
15	KOR	VVASAGARGRTE-----ANGHAPAAI	VDLKA	AVRYL	KAND--	111
16	CHO	IVASPGARGRTS-----PTGKAPAAI	YDLKA	AVRYL	RHND--	160
17	NMU	VVASPGARGRTE-----PTGKAPAAI	VDLKA	AVRYL	KAND--	175
18	ECO	VVASPGARGRTA-----ATGKAPAAI	VDLKA	AVRYL	RFND--	174
19	NBA	VVASPGARGRTS-----STGKAPAAI	VDLKA	AVRYL	KAND--	162

20	RTH	--DLLPGSAERIVIT	GTSGGGGLSAAVAASGN	SPD-YLPYLAEI	GAAGIAADGSSTLADD	253
21	GAM	--GRIPGRTDRIVST	GTSAGGAVSALLGASG	SDSL-YSSLEAEI	GAA-----DASDA	314
22	MVA	--ATMPGDTDRIVST	GTSAGGALSALLGASG	DSPL-YAGYLDL	GAA-----DAGDA	266
23	AEX	--GRIPGDTDRIVSS	GTSAGGALSALLGASG	DSPL-FAPYLEAI	GAA-----DASDA	261
24	TanBSs	--GRIPGDVERIVSA	GTSAGGALSLLGASG	DSPL-YDRLLKEI	GAA-----DASDA	302
25	SAV	--GRIPGNTARIVSS	GTSAGGALSALLGASG	DSPL-YDSYLLKDL	GAA-----EASDA	306
26	TanBSg	--NDIPGDIEKIIISV	GTSAGGAMSSLLGSTG	NRAE-YSFLLEEI	GAEL-----DQRDD	186
27	HHA	--GLIPGNTERIIITS	GTSAGGALSALAGATG	NSRD-YVPYLKEI	GAA-----DERDD	225
28	OOE	--DNFPGDVNKIIITN	GTSAGGAISALIGASG	NNVF-FEKELDEI	GAA-----DSSDN	152
29	LGA	--DHLPGNYERIIITN	GTSAGGATSALAGVSG	NHPF-FQPLLDEL	GAA-----KESDA	190
30	LSE	--GWLPGDPNHIVVS	GTGAGGAAAALLGASG	EADF-FETPLQEL	GAA-----PGTDA	196
31	LAL	--QLIPGNVEQIIITN	GTSAGGATSATVGTSG	NAQF-FEKYLQEL	GAA-----NESDA	152
32	LPB	--GWLPGNPARIIVTN	GSAGGAIAALAGASG	NADF-FETALQDL	GAA-----PATDD	198
33	LFA	--GRFPGNTDRIIITN	GTSAGGATSALVGASG	NAKF-FEPALAKL	GAA-----QATDD	195
34	TanBLpe	--ERLPGNVKRIIITN	GTSAGGATSALMGASG	NAKF-FEGALKTL	GAA-----PATDD	196
35	TanBLp	--GRLPGDANRIIITN	GTSAGGATSALAGASG	NSAY-FEPALTAL	GAA-----PATDD	196
36	TanBLpa	--GRLPGDANRIIITN	GTSAGGATSALAGASG	NSAY-FEPALTAL	GAA-----PATDD	196
37	LFF	--RSLPGNTNRIISL	GTSAGGALSALLGSTG	NSKA-YAPYLKAI	GAA-----NTSDN	297
38	LVE	--KKIPGNQNKIIISL	GTSAGGAMSALLGASG	NSKD-YAPYLKAI	GAA-----DTSBN	278
39	CCO	--KFMPGDANKIIISN	GTSAGGAMSALLGTS	ANAKE-YEPYLKEL	GAA-----KADDQ	202
40	MMU	--RLPAGNTDKIIISN	GTSAGGALSALLGATG	NSKE-YAAELKAI	GAA-----NERDD	273
41	HHT	--DQIPGVDVKIIISN	GTSAGGALSALLGTTG	NHPD-YEPYLEEI	GAA-----EETDD	195
42	ASU	--AKMPGDANKIIISN	GTSAGGAMSALLAASG	DSND-YAEYLDL	GAA-----NASDA	235
43	ASE	--EAMPGDANKIIISN	GTSAGGALSALLGASG	DSMD-YVEYLLKQV	GAA-----EASDV	238
44	TanBFnp	--EVMPGDANKIIISN	GTSAGGALSALLGATG	NSSD-YEPYLKEI	GAA-----ETRDD	224
45	FRI	--SRMAGDANKIVSN	GTSAGGAISALLGATG	NNTD-YDPYLLKAL	GAA-----NATDD	252
46	RSL	--AAIPGDANKIIISN	GTSAGGAMSALLGATG	NHPD-YEPLLKSL	GAA-----DATDH	223
47	MPO	--SLMAGDAGKIIIN	GTSAGGAMTALLGATG	DSAD-YEPYLDAI	GAA-----KASDS	223
48	HGR	NGGRLPGNTERIVSN	GTSAGGALSALLGASG	NHAD-FERELQAL	GAA-----DARDD	304
49	SNA	--AVMPGNTARIISN	GTSAGGALSALLGASG	NSPD-HEEALRAL	GAA-----PARDD	237
50	CGR	--KEIPGDANKIIISN	GTSAGGASSALLGASG	DQAA-YEPYLKEL	GAA-----PATDA	264
51	LBU	--NKMPGRADRIIISN	GTSAGGGVSALLGATG	NNTD-YEPYLKEI	GAL-----NARDD	267
52	MCU	--QKMGDANKIIISN	GTSAGGALSALLGTSG	NAPE-FEPYLLQQL	GAA-----TADDT	236
53	KOR	--QAMAGDANKIIISN	GTSAGGALSALLGTSG	GSKD-YEPYLRQL	GAA-----RANDT	160
54	CHO	--AVMPGDAEKIIISN	GTSAGGALSILLGASIT	GDGDRKLFVEIM	GAA-----DARDD	210
55	NMU	--KAMPGDAEKIIISN	GTSAGGAMSALLGATAD	QKD-YENHLKAL	GAA-----EGSDK	224
56	ECO	--AVMAGDAEKIIISN	GTSAGGALSILLGASG	NQAD-YEPYLLQQL	GAA-----PAKDH	223
57	NBA	--RAMPGDARKIIISN	GTSAGGALSILLGASAN	QAD-YEGSLKAL	GAA-----EADDS	211

58	RTH	VLAVIAYCPITDLGHADLAYEWLYQGIRSAANTAGS-----	289
59	GAM	VYATGSWCPITDLEHADAAEFCWGANPYT-----	344
60	MVA	IYATGAWCPITDLEHADGAYEWNWGANRLS-----	296

	AEX	IFAVGSWCPIADLEHADMAYEWNWGANPLS-----	291
1	TanBSs	IFAVGAWCPITDLEHADGAYEWNWGTNTLA-----	332
2	SAV	IFASGDWCPITDLEHADMAYEWNWGANPLS-----	336
3	TanBSg	IFAAQCFCPIITNLEHADMAYEWMFQAKKIYTFNSRV-----	222
4	HHA	VFAANCYCPITNLEHADSAYEWFQGGIRDYCRTRHK---K-----	262
5	OOE	IFAVSAYCPIHNLNADAAAYEWFQFNGINDWHTQKID---I-----	189
6	LGA	IYAASCYCPITNLEHADLAYEWFQFNGITTYHNNQMD---F-----	227
7	LSE	VFAVATYCPITNLEHADDAAYEWFQFHGISDWTQRSRK---L-----	233
8	LAL	IYAASCYCPITNLEHADAAAYEWFNNITDWNRVQMVTPPH-----	192
9	LPB	IFAVSAYCPIHNLHADAAAYEWFQFNGLTEWHQHQHCT---V-----	235
10	LFA	IFAVSAYCPIHNLHADMAAYEWFQFNGINDWHRYKMD---F-----	232
11	TanBLpe	IFAVSAYCPIITNLEHADMAAYEWFQFNGINDWNRSQPVAGSM-----	236
12	TanBLp	IFAVSAYCPIHNLHADMAAYEWFQFNGINDWHRYQPVAGTT-----	236
13	TanBLpa	IFAVSAYCPIHNLHADMAAYEWFQFNGINDWHRYQPVAGTA-----	236
14	LFF	IYTAVAFCPITNLDHADSAAYEWFQFNGINSISGGGTPG-----	334
15	LVE	IFASMDFCPITNLDNADSAAYEWFQFNGINSYQGMAMPG-----	315
16	CCO	IYAVSAYCPITNLEHEDAEAYEWMFGYLDKFESIDFASLDA-----	242
17	MMU	IFASMDYCPITNLDHADMAAYEWMFNGVNEAHQSSAQMSPLLPLKSGSMPGTIKAGGQLVP-----	333
18	HHT	VYAASCYCPITNLEHADMAAYEWFQFNGDDYYWGE-----	229
19	ASU	IFAASVYCPITDLEHADMAAYEWFQFNGLNQYERMDMSEL-NAE-----	276
20	ASE	IFAVSAYCPIITNLEHADSAAYEWFQFNGLNDRYRMDMSRL-NAQ-----	279
21	TanBFnp	IFAVSAYCPIITNLEHADAAAYEWMYNGVNSYSRMEFTRNTSAQ-----	266
22	FRI	IFAVSAYCPIITNLDNADMAAYEWFQFNGINSTAQRKFPG-----	289
23	RSL	IFAVSAYCPIITNLEHADMAAYEWFQFNGLNKIHAFFKFRG-----	257
24	MPO	VFAVSSYCPITNLEHADMAAYEWFQFNGDVVDYKKISATM-----	260
25	HGR	IYAVSAYCPIITNLDHADGAYEWFQFNGHGLTEYRNVAVSM-----	341
26	SNA	IFAVSAYCPIITNLEHADAAAYEWFQFNGHGLTEYRNVAVSM-----	274
27	CGR	IFAVSAYCPIITNLEHADKAYEWFQFNGHGLTEYRNVAVSM-----	301
28	LBU	IYAVSAYCPIITNLDNADMAAYEWFQFNGHGLTEYRNVAVSM-----	304
29	MCU	IFAVSAYCPIITNLEHADMAAYEWFQFNGHGLTEYRNVAVSM-----	273
30	KOR	IFAVSAYCPIITNLEHADMAAYEWFQFNGHGLTEYRNVAVSM-----	197
31	CHO	IYAVSAYCPIITNLEHADMAAYEWFQFNGHGLTEYRNVAVSM-----	247
32	NMU	VFAVSSYCPITNLDHADMAAYEWFQFNGHGLTEYRNVAVSM-----	261
33	ECO	IFAVSAYCPIITNLEHADMAAYEWFQFNGHGLTEYRNVAVSM-----	260
34	NBA	IFAVSAYCPIITNLEHADMAAYEWFQFNGHGLTEYRNVAVSM-----	248
35		: : : ** : * : . ** :	
36	RTH	-----EWSEAAARASAEALAAAYPAYLEGLGLTSADG	320
37	GAM	-----S--SIDTTVTKELAAQFAEYQDKLKLEGRNG	373
38	MVA	-----TGGQVDREVSAALGAQFAEYKLAEEIHGLND	327
39	AEX	-----TGNTVDQNLSQLKNGFATYQSSLLKLGGLG	322
40	TanBSs	-----TGKQVDQAVSKELQSQFAEYQASLKLRLNG	363
41	SAV	-----TGTLVDQSVSKELSTAFADYQASLRLRA-KG	366
42	TanBSg	-----RPQIINKRQQLLSQSLAAEFPEYVNSLHLDDES--	254
43	HHA	-----TEH----GIVRIQEDGTMTDRQMEISGKLQKLFAYLNSLKLKDENG	305
44	OOE	-----KNG----KAEFSPNGQLNELEKQLSSKLKNQFIEYLNSLRLRNPDG	232
45	LGA	-----KTD----PPRAIAVDGELTNEQQKLSKELKQGFRAFYFNQLNLHDDQQG	270
46	LSE	-----VDE----QPQITVQSSHLSGEEQRWSSERQQAFAIYINGLKLRRDDQ	276
47	LAL	-----ETT----PPVFKINGELTAEKDNKSKILKDEFVKYLNGLSLIDANG	235
48	LPB	-----VNN----QVKRTSSSGELTVSEQRLSSELKSAFVPYLNHLELKDHDAG	278
49	LFA	-----SGA----QPKFTPWAGTLTAAQQAQSAVLKAQFATYLNLSLQLTDTAG	275
50	TanBLpe	-----KNG----RPKFESITGTLSASDQSLADLKRQFSDYLNGLQLHDANG	279
51	TanBLp	-----KNG----RPKFEPVSGQLTVEEQALSIALKAQFSTYLNQLKLKTASDG	279
52	TanBLpa	-----VNG----RPKFEPVSGVLDSEAQALSATLKRQFRDYLNHLGLKATDG	279
53	LFF	-----STNTATTLTTKQKKASQQLKADEFVTVNGLNLKNGS-	370
54	LVE	-----QTAETSTLTSQAQKISNDLKKEFPSPYVNSLNLKDDNG	352
55	CCO	-----ASFNDRSK--KPKTITGELNATQKELSRLEKSKFPAYLNSLNLKDAKG	288
56	MMU	LGPGPVVQGDGGMVTDPRANAPTETSAGTEMTQEQRVASDQLKALFPDYVNRGLKDRQG	393
57	HHT	-----KSGVMSEEQKKLSAMEKLLFPYVNSLNLKLVGDSG	263
58	ASU	-----SFNNRSKPMK--KTEGTLSPAQVAVSNELKAQFPAYLNSLNLKDEKG	321
59	ASE	-----SFNDRSQAAA-KATIEGTLTAAEQMSDQLKAEFPYVNSLNLKLEDEKG	326
60	TanBFnp	-----EYNDR--SLT-RSTVQGNLTDEIKISNKLKTLFPFYVNSLNLKLTDDAG	311
61	FRI	-----M-----N-TTAGNTALTADQLKISSELKKSFTAYVNNLQLTGSRG	328
62	RSL	-----REGTLDAEALKVSDDELKKAFTYVNGLNKLKDDAG	291
63	MPO	-----LDY--NVK-RELTAGQLTDSEQQIAQALKQDFPNYINSKLKLTGHDG	303
64	HGR	-----LDF--RVE-RTETVARLDAEQQLSTELRAEFYAYVNGLNKLKLRGAQG	384

	SNA	-----LDY--QMQ--RREVAGTLTADQIALADALKASFAYLNTLDLRDAAG	317		
1	CGR	-----LDY--NVQ--RTYKTGFTTPEQTKVSADLKKDFPAYLNSLKLKDSKG	344		
2	LBU	-----LDY--NVE--RKYTEGTLTDDEISRNDLKKMFAYLNSLKLKGKNG	347		
3	MCU	-----VDY--RIE--RKLINGVQTSEQIKLSDELKQAFIPYINSLNLYNKQG	316		
4	KOR	-----LDY--KVE--RKLVKGTQTPAQIQLSGSLKALFPAYVNSLKLKNAQG	240		
5	CHO	-----LDY--KVE--RKLVKGTLDAAEQRVSDCLKAQFPAYVNSLGLKNAQG	290		
6	NMU	-----LDY--RVK--RELVAGTLTDDEKKLSDLLKPLYPAYLNSLNLKSPEG	304		
7	ECO	-----IDY--HVE--RKLVNGTLTNEQIQISGSLKAQFPAYVNSLRLRDLQG	303		
7	NBA	-----LDY--KVE--RKLVKGTLNEAEQSLSGRLKPLFAPYINSLKLKDAQG	291		
8		:	:	*	:
9					
10					
11	RTH	SPLTATTMKSIAIDELTRE--IERHIAAGGTVPARGETF--SLTLPGPGG--ERQIEQPNDWL	377		
12	GAM	FGAL----NAGNYHDYLLREYLIP-SATTYLSG-----LS--TADRQTYLKENSFI	417		
13	MVA	FGVL----NAANYPDYLLHESLQP-AATTYLTN-----LT--EHDRAGYLAQNPF	371		
14	AEX	FGPL----TSANYS DYLLKTYLQP-AATQYLAK-----LS--SEDRAAYLTRNPHI	366		
15	TanBSs	FGAL----TARNYDEYLVKQYLEP-SATTYLAA-----LS--DSDRETYLAANTFI	407		
16	SAV	FGAL----TARNLDDYLLR TYLQP-SATTY LKG-----LS--ETERSTYLAANTFI	410		
17	TanBSg	--LTADGRGG-NFYQG IILN-QLSL-SLNKFLAKHAQTNDE----KEBLARELD PQGLWC	304		
18	HH A	NSLNL DENGEGSFLEYIKG-EILK-SAGRELAFHETANHLSGLSAPGS AV---NTFPAL	359		
19	OOE	DYLT LNKIGEGNFDLTKK-FLIK-SAQTEIE-----KGNEL---NNPNFL	273		
20	LGA	KELTIDQDGKGSFLKLIQA-LIMQ-SAQQVLN-----EGQTL---SAEAGL	311		
21	LSE	HDLTLNLNGTGSFREAIVA-DLLS-AAQAAGV-----AGQAL---GNSAGV	317		
22	LAL	NRLSLNADGTGSFLEYVKD-FIEQ-SAQKALD-----SGQKI---SADTGV	276		
23	LPB	NPLQLQADGSGSFRRFIDD-YLTV-SAQ RALS-----TGNDV---TAFPGV	319		
24	LFA	AQLTL DADGNGSFRELVRQ-TVMA-SAQ TALD-----QGVDI---HKYAGF	316		
25	TanBLpe	QALTVPSPDGTGTGFQNYLVQ-LLTD-SAQ AALD-----KGVDI---HKYAGF	320		
26	TanBLp	THLTLNEAGMGSFRDVRQ-LLIS-SAQ TAFD-----QGTDI---HKYAGF	320		
27	TanBLpa	TTLTVTAA GTGPFREVVRH-LLMA-SAQ TALD-----QGTDI---HKYAGF	320		
28	LFF	TKLQLNSDGTGSFATLVEK-EIMN-SAQ TALD-----NGTTIT--TKKYPWL	413		
29	LVE	KTLKMNKDGSGSFNNLIKK-TVEN-SANTA I K-----NGKNIT--EKKYPWL	395		
30	CCO	HALSLDENGEGNFKEYINA-LISR-AFTATKS-----SDKNIT--LTPKFI	329		
31	MMU	HTLMLNEDGTGSFADYIKS-IYAA-SAQ SALT-----AGEDL---SSLDWL	434		
32	HHT	RQLTLKEDGNGSFKEYIAE-YVLK-SADRAAE-----CGTDL---EALTWL	304		
33	ASU	NALSLEQDGSGSFKDFIKF-YLIE-SANKAIT-----QGADL---SQIGWL	362		
34	ASE	NALTLDGQNGSFKDYMN-VIVR-SADKARK-----SGVTF---EDKPVW	367		
35	TanBFnp	NSLTL DKNNGSFKTYLSI-IIRN-SANKALK-----EGKDISQF---KKAF	353		
36	FRI	EKLTLDKEKGNGAFKDYVKK-CVIA-SAQ KELD-----KGTDL---AKHAWL	369		
37	RSL	KKLELNAQEGGSFKEYYKS-FLAA-SAQ KALT-----AGVDI---AKYDFL	332		
38	MPO	KLLTL DKGNGSFKDEVLY-YLNK-SVNTAHK-----QGVDL---SSYTFL	344		
39	HGR	QPLSLQADGTGTLRDEIQA-LLMA-SAQ GALA-----AGVNV---GQRDWV	425		
40	SNA	RPLRLDAGGHGSFREHVKS-LVIA-SAQ QALD-----AGADL---SSRRWL	358		
41	CGR	EQLTLNSKGGSFKE LKQ-TIIA-AAEKAQR-----EGTDL---SKYSFL	385		
42	LBU	KLLTL DKNNGSFKEQIKQ-YYID-SANVALK-----KGTDL---SEFEFL	388		
43	MCU	VKLTL DADGNGSFKRHIEG-LLMQ-SAQ EAIN-----RGEDL---SDQTLW	357		
44	KOR	TPMTLDANGNGTFKTEMEA-MLLQ-SAQ KALD-----NGKDL---SDQTLW	281		
45	CHO	EPLALNADGSGSF RDYVAA-FVAA-AANAELQ-----KGADPAVLQAENPWL	335		
46	NMU	KPLTLDAQNGSFKNHIAG-LLAQ-SAQ IQLD-----AGKDL---SDRAWL	345		
47	ECO	RALRLDAQNGSFKNYVVS-FLRR-SAQ SQLD-----AGKDL---SNRNWL	344		
48	NBA	RVLTL DKNNGSFKNYVAS-FLAK-SAQ QQLD-----AGKNL---SDRRWL	332		
49		:			
49	RTH	TVEGGKV---TDLDLDAFLRFVAAITPLKPVPAFDR TANSNGNPGVDGENTLFGGPDVAYA	434		
50	GAM	TYSSGKA----SFTWDGFT--DHVGT RKKPAPAFDAF DLS-----LDTNLNF GHGTEKAR	466		
51	MVA	TWTAGEA----TFDWAGFV--GHVGT RRTAPAFDAF DLS-----TAENNLFGAGTV AAR	420		
52	AEX	RWKANQA----RFGWTD FL--SHVGARKKGLPAFDAF DLS-----SGENNLFG RDKTKAR	415		
53	TanBSs	TWKNGKA----TFTWADFL--THVGARKKTTPAFDAF DLS-----TGENNLFGAGTTQNR	456		
54	SAV	TWDGTVA----TFTWADFL--THVGARKKDTPAFDAF DLS-----AGENNEFGTGT TQAR	459		
55	TanBSg	HFENGQA---TVFDL DAYV--VNYMGRKKDCPAFDSLDYQ----TPETEVFGNRDKNHR	354		
56	HH A	KILGERV---VGLDWKAY---MKEITRMKPAFADATDLK----SPENEEFGSMDMEAR	408		
57	OOE	DIRNGKV---VSLKWKAY---LRYITRMKKVPAFDDLG L N-----NPENDLFGDIKIKAK	322		
58	LGA	TVVDQQV---VGIDWPQY---LAYIGRKKAVPAFDDLT LA-----SAENDLFGTRQIPQQ	360		
59	LSE	TTKDGH I---VAIDWRY---TAALGRQKGLPAFDALDLS-----HPENQLFGNRLMPAR	366		
60	LAL	HVDSGVV---NGLDWDKY---LQFITRMKGVPAFDD LKMQ-----NPEPNLFGDGKTKAS	325		
61	LPB	KVNNGRV---ASLDWSAY---LRGITRMKRVSAFDALDLS-----SPASIFGSRLIAAR	368		

	LFA	EVNAGRV---TDLDLTAY---FVSITRMKAVPAFDELDLS-----SPETKLFQDAQTPAK	365
1	TanBLpe	TVTNGRV---TGLDLKAY---LTSLTRMKAVPAFDQLDLG-----SLENRLFQDATVMNR	369
2	TanBLp	VVTGNQV---TDLDSAY---LKSLTRMKAVPAFDQLDLT-----SPENNLFQDATAKAK	369
3	TanBLpa	TVTDOGQV---TELDLTAY---LKSLTRMKAVPAFDQLDLT-----SPENNLFQDALVLGK	369
4	LFF	TIKNGQV---TAVNLTKY---FKSVGRSKATPAFDADFIS-----NAENIEFGDSTTNAK	462
5	LVE	TIKNGKA---TDVNLSKY---FKAVGRMKAAPAFDGVDLS-----NAENQEMGTATVNKK	444
6	CCO	TLDTQGCSLGYTFKLEDF---IASLKRAKEVVAFDGFGLF-----NPENDLFQDGTKPAK	381
7	MMU	TIENGLV---KVDLDLKY---AAWATRLKAAPAFDKFDLS-----SGENDEFGTSDNTPR	483
8	HHT	TMENGRP---VGLDWDSE---IKYRTRMKTPAFDDVHLA-----TPENELFGSSDTERR	353
9	ASU	KFNRNKI---TDLDFTGF---VHSKKRMKLPFAFDADFDS-----SAENNEFGDSNINNKK	411
10	ASE	KLNKEGV---SDIDWEGY---IHSEKRMKSPAFDALNLS-----SGENNLFGTESVNNQ	416
11	TanBFnp	TIENNKV---VAVDLVDY---THIGDRMKSPAFDSDLAS-----SGENNLFGDKSDNK	402
12	FRI	TIKGGKV---TDIDFESY---IKYMTRMKTPAFDALDLS-----AGENQLFGTDKIDKQ	418
13	RSL	QIKDGKV---LSVDFDGY---MTYLERMKTPAFDALDNT-----SPENQLFGSATVDKR	381
14	MPO	AQKKSAAE-PYYIADYSEFL--KAYTGRSKTPAFDALDLS-----AGENNLFGDGATDNR	396
15	HGR	RVKDGQV---SALDFAAH---VRGMGRMKGLPAFDGLELE-----TGENQLFGNAQTDKR	474
16	SNA	RIASGRV---VDLDFDAY---GLAATRMKLPFAFDGVALD-----TGENQLFGSSRLDKR	407
17	CGR	TLKNGKV---TTIDWDGY---ITYMERHKSPPAFDGLDLS-----IGENQLFGDATTDKK	434
18	LBU	TIKNGKV---VDLDYDKY---IVYMGRQKTPGAFDNVDLS-----TGENNEFGDETNNK	437
19	MCU	TIITNGRV---VAADFAAY---AKFVGRQKTPAFDGIDLS-----TAENNLFGDQQVAKK	406
20	KOR	TIQNGKA---IAADFAAY---AQFVGRQKTPAFDGVDLS-----TGENQLFGDARTDKK	330
21	CHO	TIDGKRV---KNVDFAAAY---AKAMGRQKTPAFDALDLS-----SGENQLFGDEHQDTR	384
22	NMU	TVRNGKV---VSVDFDAY---AKAAGRQKTPAFDGVDLS-----AGENQLFGNNTVDKR	394
23	ECO	TIRNGKV---SAVDFAAY---ARATGRQKTPAFDALDLS-----SGENQLFGTATTNNQ	393
24	NBA	TVKNGKV---SAVDFDKY---AAAAGRQKTPAFDAVDLS-----AGENQLFGSATQDTR	381
25		. * ** *	:
26	RTH	NFTPFQGNHNEVA-----GDGSGPDD---T-GMDWDAWTA----TA	467
27	GAM	HITEYSQKRDTT-----GLKGKALPDDTAKRTNQMNPMYHIA-A-KNP	507
28	MVA	HFTGYSAARNTD-----G--AVPLAAGIPELCLDMNPMYHLS-R-AEA	459
29	AEX	HFTAFSQTHDTG-----K--TAIDADI PALLDLMNPMFFIG-K-PNV	453
30	TanBSs	HFTAYGAKNDTT-----GLTSKRVASDIPEKLHLMNPMHHLVEK-VNG	498
31	SAV	HFTLYSLRHEKG-----G--SARLSDSLPTKLDLMNPMYHLVKE-QNP	499
32	TanBSg	HFSENVAKHIEKLPALS----DY----QKAFQVDLAEEDLILARKLLNPMFTLQSDLEEK	406
33	HHA	HFSEFSMEYTET-----EAEMAPEQ---LIRMLNPTKYIGKA----	442
34	OOE	HFTETGQKYESEK-----TAKKADPE---LVTAINPLNYLNNQ-SNS	359
35	LGA	HFTDFGQLNSQV-----KASQADPA---LVAAVNPIHYLTET--SG	396
36	LSE	HFTFEFAQSHTLE-----AASRADPD---LVAAINPMTYLKTT--QS	402
37	LAL	HFTKFSQSNSQV-----KSQADSD---LIKAINPLTYIKSA--DG	361
38	LPB	HFTTFQAQESAV-----PAQLADTS---LVAAINPLTYLMAH--QS	404
39	LFA	HFTTFASEHSTV-----PAKLASYE---LVTNVNPFVYLLHD--QS	401
40	TanBLpe	HFTSFSQEHTTA-----TAQLADPE---LVEAINPLTYLTGK-QSA	406
41	TanBLp	HFTALAQTRSTV-----TAQLADAE---LIQAINPLSYLTTT--SS	405
42	TanBLpa	HFTALSQARSTV-----TAQLADDD---LVKAVNPISYLLTQ--QA	405
43	LFF	HFTNFSMQRNTA-----KQAT-QADSS---IVKLMNPMAYIGSD--NA	499
44	LVE	HFTDYSQKNNTA-----KNKTAKASDN---IKMMNPMYISNN--NA	482
45	CCO	HFTKFAKERSWG-----EMADAG---VIKMMNAMNYTKNK----	413
46	MMU	HFTGFALTRDKK-----MGATMATWD---DIRRMNPLYFIGQK--GV	520
47	HHT	HFTSFSLKHSASV-----SG-KMADEV---QIRQMNPMNYIQDP--IA	389
48	ASU	HFTVYSLKRSTV-----NG-KLADSE---TIKLLNAMNYTNNR----	445
49	ASE	HFTDYSMQHSSE-----KG-KMADKH---VIQLMNAMNYVDHG----	450
50	TanBFnp	HFTKFSFDINNKAIEYFSSGKFNDKNNKISVPKMADKN---IKMMNPMYIIDNN----	455
51	FRI	HFTAFSANN--A-----QVKSTADAA---IKIMNPMKYIGLP--NT	453
52	RSL	HFTFPFALHYSNP-----NLSSMAEAQ---QIKMMNPMNYIGAK--GA	418
53	MPO	HFTAFGVKHKSTA-----TDAKMADAQ---TVKLMNPMAYLDDK--TA	433
54	HGR	HFTAFSARHSRS-----EAGAFVADPL---TVRQMNAMHYATDS--SA	512
55	SNA	HFTAFSMQHSRV-----VGAQQADER---TVRMMNPMHYIGRS--AA	444
56	CGR	HFTPYALKNSVV-----ES-QMADAN---IVKLMNPMFSIGNK--NA	470
57	LBU	HFTYMLKHSTV-----NGT-MADKK---IKMMNPMNYLSNT----	471
58	MCU	HFTDFSMANSTV-----LGASKADSR---TVYMMNPMNFQSK----	440
59	KOR	HFTAFSLKNSTV-----AGSLKADAH---TVKLMNAMNYTSP----	364
60	CHO	HFTAYSAANSASV-----KGAGSVGRA---AVKTMNPLYYISEK----	419
61	NMU	HFTAFSMQHNTA-----ANAEIADEE---TVKIMNPLNYIGKP--GA	431
62	ECO	HFTAFSYQHRAN-----AQATLADPQ---TVKLMNPMHYISRH----	428
63	NBA	HFTFEFAQNSTV-----KGAGRADAQ---TVKMMNPMHYIAKT----	416
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3	RTH	GEGSNWPPRSGW-----	479
4	GAM	GRSKQWWIRLGTKDS\$DTS\$LTISTNLTASLVMLGDKVDHLMYWDQGHGANDDADDFLAWVR	567
5	MVA	AVAPHWWIRVGTSDTDTSLSVSANLAAAAAAGASVSHRMYWDEGHGANPDAAEFITWID	519
6	AEX	STAQHWWIRVGTKDS\$DTS\$LTIVGNLAAALSANKRKVNSAMYWDGGHGANEDADAFVKWVS	513
7	TanBSs	RRSKHWWIRLGTNDTDTSHVISANLAAAAKGLGDEVNHLYYWDQGHGANTDPGDFITWIA	558
8	SAV	HRSKHWWIRVGTKDS\$DTS\$LSVVGNLAAASLEQLGDDVDLTYWDQGHGANTDAADFITWIA	559
9	TanBSg	QVASHYRICLGAKDADTSFAISYLLALALKKRGIDVHYELIWGMGHADADYNEEFSQWVD	466
10	HHA	DTAKHWRIRHGSFDRDTSFAIPVILALLKKNKGYDVFLLPWGLPHSGDYDLQELFTWID	502
11	OOE	LIAKHWRIRHGAADRDT\$FAVPIILATTLQNMNLDIDFFMPWAKPHSGDYDLIDLFDWID	419
12	LGA	QTAHYWRIRHGASDRDTAFSIPILILALFLQDQGGKQVDFAYPWGIGHGDDYDLVALFDWID	456
13	LSE	QVAPHWWIRHGAADAETAFAPKLLATRLANQGYDVNFAYRWATKHIGDYDLPDLFNWVN	462
14	LAL	QTAKYWRIRHGASDRDTSFAIPVILATLLRNAGFDVDFAMPWATPHSGDYDLRDLFTWID	421
15	LPB	QIAQHWRIRHGAADRDTAFAPVILATNLQNHGLDVDFALPWRPHSGDYDLSELFDWID	464
16	LFA	TRAKHWRVRHGAADRDT\$FAIPLIILVTLQNRGLDVDFTLPWDTPHSGDYDLGELFTWID	461
17	TanBLpe	KMAQHWRIRHGAADRDT\$FAIPIILATVQLNQGREVDFALPWDI PHSGDYDLGELFAWID	466
18	TanBLp	QVAKHWRIRHGAADRDT\$FAIPIILAIMLENHGYGIDFALPWDI PHSGDYDLGDLFSWID	465
19	TanBLpa	QVAKHWRIRHGAADRDT\$FAIPIILATMLKNPGYDVFALPWDI PHSGDYDLGELFAWID	465
20	LFF	TLAKHFWIRYGEKDANTS\$VAVPTLLSQKLKNAGADVNYHVQWNTGHAGDYDLNAMFKWLN	559
21	LVE	DTADHWYIRYGEKDANTS\$IAVPTIFQKLKNENKDVNYHLEWNKGHAGDYNLNMFTWMN	542
22	CCO	EAAKFYRIRQGTNDTDLALAVPAMLLSLKNAGKEVDFEAVWGQGHGDDYDLDELFAWIK	473
23	MMU	TVAPHWRIRHGAKDRDTCLAI\$PAILATLQNNGYDVFSSPWGKGHAGDYDLTELFDWAD	580
24	HHT	KKAKYFRIRHGAADRDTSLAISAILTAGLRNEGLLVYHLPWGVPHAGDYDLEELFNWIK	449
25	ASU	KAARHWRIRAGSADYDT\$SHAISAILTVKLRMSGKNVDYAI\$PWAI PHSGDYDLDELFWQMD	505
26	ASE	K-TAYWRIRAGTS\$DRDTS\$HAISAILAIKLRMAGKQVDYETPWGVPHSGDYDLDELFWQMD	509
27	TanBFnp	TSTKYWRIRHGAIDKDTSLAIPAILALKLKNSGKVVDFASPWGQGHGDDYDLEELFNWID	515
28	FRI	NTSKYWRIRHGSKDS\$DTGLAVSVILATYLENKGFNVDFALPWDKPHSGDYDLEELFAWAD	513
29	RSL	KTAAYWRVRHGA\$KDKDTGFAIPVLLAAALKNKGFAIDFALPWDRPHSGDYDLEELFNWMD	478
30	MPO	NTALHWRIRHGA\$KDS\$DTGFAIPVILATKLKNQGGKNVDFAMPWDKGGHGGDYDLDELFAWVD	493
31	HGR	RPAPHWRIRHGTMDSDTSFAVPTLLAALRERQLQVDLALPWDRPHSGDYDLDELFTWIE	572
32	SNA	TVAPHWRIRHGTMDRDTSLAVPVMLGTLLAQHGIDVDLALPWDRPHSGDYDLDELFAWID	504
33	CGR	HLAKYWRIRHGA\$KDS\$TSAAILSLILATLQNNHY\$PVDYALPWDPKPHSGDYDLGELFTWAE	530
34	LBU	--KVYWRIRHGAIDKDTSLAIPAILAIKLQNLGKNVDFASPWATPHSGDYDLDELFRWMD	530
35	MCU	--TKYYRIRHGENDRDTSLAISTLMALKLDKENKVDFALPWGRGHSGDYDLQELFDWAK	498
36	KOR	--TQHYRIRVGENDRDTSLAIGLLLSLKLKNSGKNVDYALPWGVPHSGDYDLDELFAWMD	422
37	CHO	TVPQHWRIRVGT\$KDRDT\$SHAIAAILAAKLQNSGKNVDMAMPWGVPHSGDYDLDELFAWMD	479
38	NMU	NLPQNWRIRVGTNDRDTSLAVSAILAAKLQNNGYTVDYALPW\$DVGHGGDYDLDELFAWMD	491
39	ECO	TTPQHWRIRVGTADSDTSLAIAAILTAKLQNSGKRVD\$FALPW\$DVPHSGDYDLDELFWMD	488
40	NBA	-PTQHWRIRVGTADSDTSLAVSAILAAKLQNSGK\$VDYALPW\$DVPHSGDYDLDELFAWVD	475
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44	RTH	-----	479
45	GAM	ELGSK-----	572
46	MVA	RISR-----	523
47	AEX	RLNKDTRMRG--	523
48	TanBSs	EVTGYRKAGRTK	570
49	SAV	KVTGYKK-----	566
50	TanBSg	AIVH-----	470
51	HHA	TICR-----	506
52	OOE	SLVKEK-----	425
53	LGA	QICQ-----	460
54	LSE	QCCR-----	466
55	LAL	KICAKN-----	427
56	LPB	NLCQTAT-----	471
57	LFA	QLCQP-----	466
58	TanBLpe	GLCQ-----	470
59	TanBLp	GLCQ-----	469
60	TanBLpa	HLCQ-----	469
61	LFF	SKM-----	562
62	LVE	KIAKK-----	547
63	CCO	RVVEK-----	478
64	MMU	GLCKASTKK---	589
65	HHT	TVCKK-----	454
66	ASU	GISKK-----	510

ASE	SISK-----	513
TanBFnp	GVVK-----	520
FRI	TICK-----	518
RSL	GICK-----	482
MPO	SVAK-----	498
HGR	QRV-----	575
SNA	RVTAAPR----	511
CGR	KISR-----	534
LBU	KVVAEGR-----	537
MCU	SISAP-----	503
KOR	SVAMK-----	427
CHO	GVVKGK-----	485
NMU	QVSRSTEK----	499
ECO	KTVRQPRG----	496
NBA	KVAKGK-----	481

Fig. S2. Comparison of amino acid sequences of functional subtype B tannases and similar putative tannases included in the databases. Multiple alignments were done using the program ClustalOmega after retrieval of sequences from BLAST homology searches. The functional subtype B tannases are from *Fusobacterium nucleatum* subsp. *polymorphum* (TanBFnp, accession ALQ42581.1), *Lactobacillus paraplantarum* NOS 120 (TanBLpa, accession BAN10246), *Lactobacillus pentosus* 21A-3 (TanBLpe, accession BAN10247), *Lactobacillus plantarum* ATCC 14917T (TanBLp, accession AB379685), *Streptococcus gallolyticus* UCN34 (TanBSg, accession W_012962282), and *Streptomyces sviveus* ATCC 55618 (TanBSs, accession EDY53581.2). Putative subtype B tannases are from *Actinobacillus succinogenes* ATCC 55618 (ASU, accession A6VQU7), *Aggregatibacter segnis* ATCC 55618 (ASE, accession E6KXG9), *Asticcacaulis excentricus* ATCC 15261 (AEX, accession E8RUH5), *Campylobacter concisus* ATCC 51562 (CCO, accession U2F494), *Capnocytophaga granulosa* ATCC 51502 (CGR, accession S2VLN4), *Cardiobacterium hominis* ATCC 15826 (CHO, accession C8NA23), *Eikenella corrodens* ATCC 23834 (ECO, accession C0DUD8), *Flavobacterium rivuli* DSM 21788 (FRI, accession A0A0A2MDE7), *Gordonia amarae* NBRC 15530 (GAM, accession G7GR49), *Hungatella hathewayi* DSM 13479 (HHA, accession D3AJB8, and HHT, accession D3AMC3), *Hylemonella gracilis* ATCC 19624 (HGR, accession F3KTR5), *Kingella oralis* ATCC 51147 (KOR, accession C4GHB9), *Lactobacillus alimentarius* DSM 20249 (LAL, accession A0A2K9HQ50), *Lactobacillus fabifermentans* DSM 21115 (LFA, accession A0A0R2NRK2, and LFF, accession A0A0R2NFY2), *Lactobacillus gastricus* DSM 16045 (LGA, accession A0A0R1V835), *Lactobacillus parabrevis* ATCC 53295 (LPB, accession A0A0R1H2G9), *Lactobacillus senmaizulei* DSM 21775 (LSE, accession A0A246E1V6), *Lactobacillus versmoldensis* DSM 14857 (LVE, accession A0A0R1SD40), *Leptotrichia buccalis* ATCC 14201 (LBU, accession C7NCM7), *Mitsuokella multocida* DSM 20544 (MMU, accession C9KQE8), *Moraxella cuniculi* DSM 21768 (MCU, accession A0A1N7G800), *Moraxella porci* DSM 25326 (MPO, accession A0A1T0CUS9), *Mycolicibacterium vaccae* ATCC 25954 (MVA, accession K0UPD3), *Neisseria bacilliformis* ATCC BAA-1200 (NBA, accession F2BGE0), *Neisseria mucosa* ATCC 25996 (NMU, accession D2ZY62), *Oenococcus oeni* ATCC BAA-331 (OEO, accession Q04F19), *Rubellimicrobium thermophilum* DSM 166484 (RTH, accession S9R622), *Runella slithyformis* ATCC 29530 (RSL, accession F8EDM8), *Sphaerotilus natans* subsp. *natans* DSM 6575 (SNA, accession A0A059KHH4),

and *Streptomyces avermitilis* ATCC 31267 (SAV, accession Q82MV9). Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. Putative signal peptides are highlighted in yellow. Residues conserved in all the functional subtype A tannases described so far are highlighted in blue. The conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases is showed in a box. In a box are also showed the conserved amino acids from the catalytic triad, Ser-163, in the pentapeptide motif, Asp-419, and His-451 (numbering is referring to TanBLp tannase).

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SSA	-----MAGLIAAALAGCAALTG	17
GTU	-----MRSILIPLALLISSAAHAATCASLAA	27
APA	MAPYYCVTFSSAGFLMPYPQPLSALLTALPRRFSLCVTGATLAAIMLPHMAHATSCAELAH	60
Tan410	-----MPAKTRLS-IATALAAIGLAHSASA	32
GMA	-----MFTGRKPKYKIVLF-----LMMVLPAALYAEP CERLLQ	32
	: * *	
SSA	THAGDVKVIGATPIAPPPAWEAPN-----SRGPYRGLFVSVPFCRVEAVI	62
GTU	LSLPDPTTIHAEAV-PPGPLKPPY-----GDPIASLP---AFCRVVTGIL	67
APA	QALPDATFTTVGVV-EAGKWQPPQNQLSRIMSAPGMNVAGHATQAPNP---AFCRVAITL	116
Tan410	LQLDNGQVTAATLI-EAGKFTPTTAPGA----PPG---VGAGNYANLP---AFCRVQAIL	81
GMA	LVSPKVSITFAKIV-EAGNLT PAG-----SENTLRDLF---SFCRVAADL	73
	. . * : *	
SSA	-----EGNIGFELNLPAN-----NNGRLLGAGVGGDAGVYNYIDMSQRVAEGFATVTT	110
GTU	RPTPDSEIRFEVNLPEKEAPKPKPANNRLLDVGNGGFAGTIAYGQMASNLRKGYATAGT	127
APA	HPSADSQIRTEFVNLPLK-----GWNKGKLLGVNFGWAGSLMYYGMATGVGEGYAVAST	169
Tan410	TPTPDSDIKVEVNLPEAD-----NWNKGKYVGINGVWAGQLSLSQLADPLSRGYAAATT	134
GMA	KPTSDSDIRIEVNLPTV-----RWNGKFIAVCSGGWGCISISY GEMADALRRGYATSAT	126
	: : * * : * : *	
SSA	DSGHKAS-----QARW-MADPKARVDYEHRAVHLTTGAARKLAAFFYGRAVDRSYFTGCS	164
GTU	DTGHQA---EAEDASWAYHHPEKIKDFGYRALHLTTQRAKTILAAFYGEPQKKAYFDACS	184
APA	DTGHDSSTPEGQGGRTLGHPKELIDYAYRADHLMTITAKALIEAFYKKPASHAYWIGCS	229
Tan410	DTGHSG---NGLSADWAVGHPEKLVDFGHRADVHLMVTAKQAIEAFYGN SAEYSFWN SCS	191
GMA	DDGHTG---P---SASFIVNHPEKLIIDFAYRADHEMIVEAKTLIHAFYGRDPRYSFWNIGCS	181
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SSA	GGGRQALKEMQNYPGDYGVIIAGAPGPYMP LQ---SVRMMWFSLQQKQMPDAGLT DADWS	221
GTU	DGGRREALMEARFPEDYDGILAGAPANNWTGLLSAGVDVSH---TVIAHPEGYISSFKLR	241
APA	LGGLEGLEIAEKRYPEDYDGIVAGAPPNPPIVNFNAEQLWAGWM---SYHNPALRVSRDKFR	286
Tan410	TGGRQGLMAASRYPDYDAISAMAPANPMTDLMTQSMWASWV---NKRSADAQLDPGKLS	248
GMA	GGGREGLLQASRYPEEFDGIIAGDEANIRRN-----AWALELAVQTFKDPEAYIPAAKYP	236
	* : : * . : : * : : * *	
SSA	LYEAKATAACDAADGVVDGIIANMACRFRVETLCKPGQTGRCLTAPRLAMLKTVSPM	281
GTU	AIN TAVLNACDAQDGLKDGILLNDPRTCHFDPATLCKSGDELTCLTAPQVASLSKYAGG	301
APA	LLNTAVMNACASPVKKQGF LDEPDKCTFTPDQ LCKGNDTSSCLTANEVQAAKNTYQGP	346
Tan410	LIHQAVMNQCDALDGLDEGVIGRPDACTFDPASLVNTS----NGLTQAQADAMQATYDGV	304
GMA	MIHRAVLEACDAKDGLKDG LIEAPESONVDFKSLQCKAEDGIDCLTARQVQTAQTITSPV	296
	. . * : * : * : * *	
SSA	R-DEQGRAMDGGLLPGVTRTR-----PGP-PSPLL RAMWADGVY-NDPNW NEDGFRPTA	331
GTU	H-DSTGNPIFPGLMPGGEADWRDWITGGGPSESRYMLNFFRYMVY-DDPAN S PLAAAVDA	359
APA	ANPQTHEVIFPGPAKCS EDGFS---ADGKA--FPVALDLFKYAAF-QNPNDWTT LKWD T	400
Tan410	R-ASDGSII LPGWPRGAEMQLTALVMGPEP--FPVAMSWFKLLVHGDDSSINWLTMDWSK	361
GMA	A-TRTGQILFPRVEPCTELRWSRLAGGPQP--ADLFLDEF RYV VY-QDPNDWRTFDLER	352
	: * . : .. : * .	
SSA	DLDAANRAMPEL-RADKLAIAPFIKAGGKAILYQGWADPSTNAGPTIDYFAALARAHGGA	390
GTU	SVRTAHEKMAEPLNATNPDLAPFIAHGGKLLLYHGWNDAISPLNTIAYYQQVVAKV-GP	418
APA	DIEEATRKGVP LLHV-DDNLTPFQHGGRLLMYIGWNDC HNPE-QLAGYYQSLQHNA-GP	457
Tan410	EIAASRDY GADI NVSAETLAPFARGGKLLLSHGWNDC LIPANN TVKFY GELQTAG-A-	419
GMA	DSAKAHAI DKDIDEL-NPNLTAF AKHGKLLLYHGWNDAQVAPGSSVEFYKVELEAS-KV	410
	: : : * : * : *	

1	SSA	ARLGEAVRLFMVPGMYHCRGGCPGDAFGASGQPTWPNDPGRDMLWALIRNVERGQAPQQI	450
2	GTU	AQAEAAVKLIYMPGMGHCAGGPGPSGFGQIGLPS----PGAGAIDQLQDWNVETGKAPTST	474
3	APA	E-YQNDVKLITIPGMGHCYGGAGCDTFSKLG-----AIDNWNVSNKQAPETI	502
4	Tan410	--PQDQLRLFMAPGMGHCAGGEGPNSFDTLG-----TLDANVSTGQAPDRI	463
5	GMA	T-EQNWVRLFMAPGMAHCSGGEGPDNFDKIN-----VMEQNVVELGKTPEQI	455
6		::* *** ** ** * . * . . : ** . ::* *	
7			
8	SSA	TAAKRID-----GKDVLTFRKLCPFYQAARYQCKGAAADAGSYRC TVDPDLEKAMARRSL	504
9	GTU	IAAKYTA-----DRKIQMTFRPLCPYFQLAQYTCKGSTEDSTNFTCAAPPQ-----	519
10	APA	VASRVSN-----GKVVRTRPLCAWPKVARYDGHGNMDDASSFTCITPESQSK-----	549
11	Tan410	VATRLSSPNPNAAQLPAMTRALCAYFRVARFDGTGNPAEEGNFSC ELPDKDGF TNGN---	520
12	GMA	IAAHKAA-----GKIDRTFRPLCPYFQVARYKCVGSIDEASNFS SSSR-----	498
13		*:: ** ** ::* *:: * * : .: *	
14			
15	SSA	VGS 507	
16	GTU	--- 519	
17	APA	--- 549	
18	Tan410	--- 520	
19	GMA	--- 498	

Fig. S3. Comparison of amino acid sequences of functional Tan410, a feruloyl esterase/tannase containing the “CS-D-HC” motif, and similar putative feruloyl esterases/tannases from the same group included in the databases. The feruloyl esterases/tannases represented are from an uncultured bacterium (Tan410, accession E7D7J5), *Acetobacter pasteurianus* NBRC 3283 (APA, accession C7JI56), *Granulicella mallensis* ATCC BAA-1857 (GMA, accession G8NXE6), *Granulicella tundricola* ATCC BAA-1859 (GTU, accession E8WXF9), and *Sphingomonas sanxanigenens* DSM 19645 (SSA, W0AHR6). Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. Putative signal peptides are highlighted in yellow. Residues conserved in all the proteins are highlighted in green. The conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases is showed in a box. In a box is also showed the “CS-D-HC” motif. The “CS” is included in the conserved pentapeptide motif.

Figure S4

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8			
9	LGA	-----MVDEATKQAILDLRVAFKESDDQORDAGLPTEVPGVTRHNDLTYGADPKWH	50
10	LVI	-----MVEKRMNEILKIRSEWKKGDDQORDAGLPLEVPGVTRINNLAYGDPKWH	50
11	LMA	-----MPKQSVLDEIRQIRVDWKKGDDERDSGLPREVAGVKRINDIAYGDPKWN	50
12	LRU	MPCEIFLGGINM-TDTMEEVLEMRRTCKKSDDARDAGLPREVPGVRRIDDLSYGDPKWH	59
13	LKI	-----MVDQKVLDAVNEMRTSWKASDDKRDSGLPHDIPEVKRIDDLOYGEDPKWN	50
14	LGS	---MREGNIMKEQEILNAVKEMRISWKENDDKRDSGLPHDIPEVTRVDDLQYGEDPKWN	56
15	LFA	-----MEENITEAILQMRKQFKTQDDKRDAGLPTEIPEVTRIDNLSYGPDPKWN	49
16	Est_1092	-----MISKELSRSIIELRRTAFKKNDARDAGLPREVAGVERLDNLSYGPDPKWH	50
17	LPE	-----MTEKDLKQAIIELRRTAFKTNDDARDAGLPREVAGVERLDDLQSYGPDPKWH	50
18	LPA	-----MDFRKGAKANDDKRDEGLPTEVEGVKRVDIAAYGDPKWN	40
19	LAL	-----MMKQELIDEIMQFRKSAKNDKRDAGLPTEVKGVKRIDNLSYGPDPKWN	50
20		.: * * * * * : : * * : : * * * * * :	
21			
22	LGA	RLDIYLPDAIKGKIPVIIINIHGGGWCYGKTETYQFFGLGLAKRGFAFVNPYRLAPEVQF	110
23	LVI	LLDIYRFPQNVGTGKIPTIISIHGGGWCYGKTETYQFFGLGLAKRGFAFINPNYRLAPEVTF	110
24	LMA	LLDIYIPQNVGDGRIPVIIISIHGGGWCYGKTETYQFFGLGMAQRGFAFVNPYRLAPDVVF	110
25	LRU	LLDLYLPENVEGKIPVIIINVHGGGYCYGKTETYQFFGLNLAAGHFAFVNPYRLAPEVVF	119
26	LKI	LLDLYLPKNVEGKIPVIIINIHGGGWVYGTETYQFFGLMAKRGFVNPYKLGPEVSF	110
27	LGS	LLDLYLPKNVKGKIPVIIINIHGGGWVYGTETYQFFGLMAKRGFVNPYKLGPEVKF	116
28	LFA	LLDIYLPKNVTKPIPIIINIHGGGWCYGKTETYQFFGLNWAQRGFVNPYRLAPDVEF	109
29	Est_1092	LLDLYRPTKVIKPLPVIIINIHGGGWCYGKTETYQFFGLNWAQRGFVNPYRLAPDVTF	110
30	LPE	LLDLYRPTDARRPLPVIIINIHGGGWCYGKTETYQFFGLNWAQRGFVNPYRLAPDAVF	110
31	LPA	LLDLYLPENVEGPIPTIINIHGGGWCYGKTETYQFFGLNWAQRGFVNPYRLAPDVVF	100
32	LAL	LLDLYLPENVDKPIPTIINIHGGGWCYGKTETYQFFGLNWAQKGFVNPYRIAPDVVF	110
33		** : * * : * * : * * : * * : * * : * * : * * : * * : * * : * * : *	
34			
35	LGA	PGEMDDVNRYLHWVDEHADEYQLDRQNVFLMGDSAGQMAEQYVTILTNPYRDLFGYKL	170
36	LVI	PAELDDVDRIYHWAQHADEYNLDPENVFLVGDSDAGQMAEQYVAMLKNPSYRQKFGYQL	170
37	LMA	PEELDDVNRYIHWIDEHADEYGLDRNNVFLVGDSDAGQMAEQYVAIKNEDYRKKFGYKL	170
38	LRU	PGELDDVDRIYHWAQHADEYGLDRNNVFLVGDSDAGQMAEQYIAILTNPEYRSLFGYEP	179
39	LKI	PKELDQVNEYIYWVADHADEYNLDKNNVFLVGDSDAGQMAEQYTAILTNPEYREKFGYKL	170
40	LGS	PKELDQVNEYIHWVAHADEYNLDKNNVFLVGDSDAGQMAEQYTAILTNPKYREKFGYTL	176
41	LFA	PKELDDVNQYVHFVAQHADEYNLDKNNVFLVGDSDAGQMAEQYVTILTNPYRQFLNYEM	169
42	Est_1092	PGELDDVDRIYHWAQHADEYNLDSNNVFLVGDSDAGQMAEQYATILTNPYRSKFGYEL	170
43	LPE	PDELDDVDRIYHWAQHADEYGLDRNNVFLVGDSDAGQMAEQYAAILTNPYRDLFGYQL	170
44	LPA	PEELDDVDRIYHWAQHADEYGLDTNNVFLVGDSDAGQMAEQYTTILTNEKYRQMFNYEL	160
45	LAL	PEELDDVDRIYHWAQHADEYGLDTQNVFLVGDSDAGQMAEQYATILTNEKYRQMFNYEL	170
46		* * : * * : * * : * * : * * : * * : * * : * * : * * : * * : *	
47			
48	LGA	TQLKIRAVAFNSAAFFTLDEGMLEGAVTGXYFTDEVLNPKLHNMIDTEKYITKNFLPSFM	230
49	LVI	TNLHFRAVALNSAAVFLDPGMISGPVKGYFTDEVLLK--QRDLLATEEYIINDNFLPTFI	228
50	LMA	TDLKFRAVALNSAATFVLNPGVISGAPEAYFTDKVVKE--KLDMLDEKYIISSEFLPTYI	228
51	LRU	VDLKFRAAALNSGAFFLLDPGMIAGELDAYFPKDIVEK--DRRRISAEDYITEDFLPCYL	237
52	LKI	TDLKFRAVALNSPATFMDLKGMIAGGTLAYFPKPDVME--KNRDLLNVEKYITEDFLPTFI	229
53	LGS	TDLKFRAVALNSPATFIKDPGMIAGGTLAYFPDPEVMKSAKNQDLIDIEKYITEDFLPTFI	236
54	LFA	TDLKFRAVALNSAANFMAEPNAGVAGYFTPESVRK--YSEQLNVEKYITKDFLPTYI	227
55	Est_1092	TNLKFRAVVLNSAAAFPLDEGMVTGAVAAAYFTPSIRQ--QREQIMAENYIQKNYLPTFI	228
56	LPE	TDLHFRAVVLNSAAAFPLDEGMVTGAVAAAYFTPEAIQK--QRNQIEAERYIQANYLPTFI	228
57	LPA	TDLHFRAVALNSSANFMYDPGTVTDVVGAYFTPELLNS--KRDQINTEEYIQSNFLPAYI	218
58	LAL	TDLNFRAVALNSSANFVLESRG---VTEAYFTPEIKKN--QREKLDTEKYIQENFLPTYI	225
59		.. : * * : * * : * * : * * : * * : * * : * * : * * : * * : * * : *	
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1	LGA	TTANEDDFIRDQTMKFDGFLRAKGLSHVTKSYGDEEHPEGHVFCNPKDVLAAQQAMDEEVA	290
2	LVI	STANQDFIHDASVKFADFLETEKGIEHVFKKEYGDQEHPRPHVFLINQKDPIAAQANDDEVA	288
3	LMA	STANEDDFLKELSIKFDSFLTDLGVEHVFKKEYGDKNNPQSHVFLINQKDEIANQANDDEAD	288
4	LRU	STANEDDFIHDQTIKLDGFLTARGVEHVCRSYGDKDNPQPHVFLMNQKDETARQCNEDEVE	297
5	LKI	STANQDFIHDCAVRLDGFLLRAKGIEVIQKSWGDEKHPEPHVFLINQKDELAKDANDEEAA	289
6	LGS	STANEDDFIHDCAVRLDGFLLRAKGIEVIQKSWGDEKHPEPHVFLINQKDELAKEANDEEAE	296
7	LFA	STANQDFLRNSAFKMDGFLTAKNISHICKMYGDEKSPRGHVFLINQKDELAKKANDDEMA	287
8	Est_1092	CTATADDFLRNNAAKLDGFLTAKKVEHVFKMYGDDHPRGHVFLISQKDELAQQATDDEMR	288
9	LPE	CTATEDDFIRNNSAKLDGFLTAKGIEHVFKMYGDEKHPRGHVFLVSQKDDIAKQATDDEMA	288
10	LPA	CTANYDFIRNNSVKFDGFLTAKKVPHVFKMYGDEKNPREHVFLINQRTIAKQANDDEMA	278
11	LAL	CTAIDDFIRNSSLKFDGFLTAKNVPHICKMYGDEKNRRGHVFLIDQKDEIAKKANDDELV	285
12		** **::: : :: .** : : : : **.. . ***: .*: * * .. ::*	
13	LGA	FFKQYLV--	297
14	LVI	FFKKYLQK-	296
15	LMA	FFKKFIVE-	296
16	LRU	FFRKHMG--	304
17	LKI	FFKKHINK-	297
18	LGS	FFRKHLVKD	305
19	LFA	FLKSFLN--	294
20	Est_1092	FLQEHMA--	295
21	LPE	FLNAHLSK-	296
22	LPA	FFKKYLVK-	286
23	LAL	FFQKYLIK-	293
24		*:. .:	

Fig. S4. Comparison of amino acid sequences of functional Est_1092, a feruloyl esterase/tannase, and similar putative feruloyl esterases/tannases from the same group included in the databases. The feruloyl esterases/tannases represented are from *Lactobacillus plantarum* JDM1 (Est_1092, accession ACT61979.1), *Lactobacillus alimentarius* DSM 20249 (LAL, accession A0A2K9HRX0), *Lactobacillus farciminis* DSM 20184 (LFA, accession A0A0R11RP4), *Lactobacillus gastricus* DSM 16045 (LGA, A0A0R1V190), *Lactobacillus gasseri* DSM 14869 (LGS, accession A0A249NLB4), *Lactobacillus kitasatonis* DSM 16761 (LKI, accession A0A0R1VGX3), *Lactobacillus mali* DSM 20444 (LMA, accession JIF5K6), *Lactobacillus paraalimentarius* DSM 13961 (LPA, accession A0A0R1HXG6), *Lactobacillus pentosus* IG1 (LPE, accession G0M1G3), *Lactobacillus ruminis* ATCC 25644 (LRU, accession E7FSS6), and *Lactobacillus vini* DSM 20605 (LVI, accession A0A0R2CA25). Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. Residues conserved in all the proteins are highlighted in yellow. The conserved pentapeptide motif Gly-X-Ser-X-Gly typical of serine hydrolases is showed in a box.