Contributions of protein microenvironment in tannase industrial applicability: An in-silico comparative study of pathogenic and non-pathogenic bacterial tannase

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ABSTRACT
Tannase is an inducible industrially important enzyme, produced by several microorganisms. A large number of bacteria have reported as tannase producers; however, some of them are pathogenic in nature. Therefore, it is quite uncertain whether the application of these tannase enzymes from such pathogenic bacteria is suitable for industries and human welfare. Till date, there is no clear evidence regarding which group of bacteria (non-pathogenic or pathogenic) is better suited for their application in the edge of industries with particular reference to the food industry. The present study is following the findings of the above queries. In this study, a large number of tannase protein sequences have been retrieved from the databases, including both non-pathogenic and pathogenic bacterial species. Physiochemical and evolutionary properties of those sequences have been evaluated. Results have shown that non-pathogenic bacterial tannase possesses a high number of acidic and basic amino acid residues as compared to their pathogenic counterparts. The acidic and basic amino acid residues of tannase provide unique microenvironment to it. In the other hand, the numbers of disorder forming residues are higher in tannase sequences of pathogenic bacteria. The study of tannase microenvironment leads in the formation of salt bridges, which finally favoring the stability and proper functioning of tannase. This is the first report of such observation on tannase enzyme using in silico approach. Study of the microenvironment concept will be helpful in protein engineering.

1. Introduction
Tannase (tannin acyl hydrolase, EC 3.1.1.20) is an extracellular inducible enzyme that catalyzes the hydrolysis of ester and depside bonds of several substrates such as gallotannins, epigallocatechin-3-gallate, esters of gallic acid, and epicatechin gallate and produces glucose and gallic acid as by-products (Mohapatra et al., 2005; Natarajan and Rajendran, 2012; Jana et al., 2014). The enzyme produced from microbial sources has immense applications in various industries due to its stability (Natarajan and Rajendran, 2012; Jana et al., 2014). Tannase is produced in the presence of tannic acid by different tannase producers including a large number of filamentous fungi such as Aspergillus sp. (Sharma et al., 2007), Penicillium sp. (Batra and Saxena, 2005), a massive number of bacteria (Mondal and Pati, 2000; Mohapatra et al., 2009) and few yeasts such as Aureobasidium sp. (Zhang et al., 2019), Sporidiobolus sp. (Kanpiengjai et al., 2020) and others. There are several reports in the literature on the bacterial origin of tannase. Lewis and Starkey (1969) first reported that Achromobacter sp. able to utilize gallotannin as the energy source for its growth. Deschamps et al. (1980) isolated several bacterial strains that can use tannic acid as the sole carbon source. Mondal and Pati (2000) reported on extracellular tannase production by newly isolated Bacillus licheniformis KBR6. Jana et al. (2013) have characterized thermostable tannase from Bacillus subtilis PAB2. High tannase activity has reported in Lactobacillus Plantarum, a lactic acid bacterium (Jimenez et al., 2014; Matsuda et al., 2016). Characterization of tannase activity in cell-free
extracts of *Lactobacillus plantarum* CECT 748T was performed by Rodriguez et al. (2008). Raghuvanshi et al. (2011) reported about highest tannase producer *Bacillus sphaericus* with potential gallic acid synthesis ability. Ren et al. (2013) first reported the crystal structure of a tannase from the bacterium *L. plantarum*. Comparative study of tannase from three closely related lactobacillus species such as *L. plantarum*, *L. paraplantarum*, and *L. pentosus* was conducted by Ueda et al. (2014). Kapiengjaia et al. (2019) have isolated a new alkaline tannase from *Lactobacillus pentosus*. Jiménez et al. (2014) have reported the results of cloning and expression of the gene encoding TanSg1 tannase in *E. coli* from *Streptococcus galolyticus* UCN34.

Tannase belongs to the enzyme class ‘hydrolase’ and is one of the essential industrial enzymes (Jana et al., 2013). It has immense potential applications in several industrial sectors, including food, beverages, leather, chemical, pharmaceutical, and dye-making (Cavalcanti et al., 2020). It is extensively used for industrial effluent treatment and is also involved in the synthesis of gallic acid, instant tea (Hae-Soo et al., 2020; Urban et al., 2020; Thiyonila et al., 2020), acorn wine and coffee-flavoured soft drinks (Kar and Banerjee, 2000; Mohapatra et al., 2006; Jana et al., 2013). The enzyme also acts as a clarifier in the production of beer, fruit juices and to treat wastewater contaminated with polyphenolic compounds (Mohapatra et al., 2006; Sharma et al., 2007). It also involved in ripening of fruits (Jana et al., 2014) and wine production.

(Mohapatra et al., 2005; Patil et al., 2011). It is also reported to suppress antibiotic drug - trimethoprim and in the production of pyrogallol photographic developer, pharmaceuticals for the synthesis of an antifolic is a phenolic compound used in dye making, leather industry, as a

by hyperglycemia (Kuppan et al., 2010).

activation of proinflammatory and prooxidant gene expression induced by hyperglycemia (Ruppan et al., 2010).

Pyrogallol produced from gallic acid also has several industrial importances, like in coloring of hair, staining of fur, leathers and also in the preparation of anti-tumor and anti-cancer drugs (Jana et al., 2013).

The present study is concerned with the in Silico comparison of both non-pathogenic and pathogenic bacterial tannase and to answer about their safe application at industrial level.

2. Material and methods

2.1. Dataset

A detailed analysis of the sequence and structure of bacterial tannase was performed. A total 309 bacterial tannase sequences were retrieved from the UNIPROT database (UniProt: a hub for protein information, 2015). These sequences were divided in two groups-pathogenic and non-pathogenic bacteria tannase. These pathogenic bacteria were again divided into 4 subgroups - animal pathogens, human pathogen, plant pathogen, and both animal and plant pathogen based on their previous pathogenic reports (Hildebrand, 1971; Centurion-Lara et al., 1997; Podschun and Ullmann, 1998; Pauleta et al., 2001; Qin et al., 2011; Schröttner et al., 2014; Drancourt et al., 1997; Yadav et al., 2018; Kikuchi et al., 2020). Structures of non-pathogenic bacteria (*Lactobacillus plantarum*) were retrieved from the RCSB protein database (PDB) (Berman et al., 2000). Due to the lack of structure of pathogenic bacterial tannase, a model structure was created.

(Lieutaud et al., 2016) called disorder forming residues. Mean relative abundance (MRA) of sequences were calculated from the mean value of nonpathogenic bacterial tannase relative to pathogenic bacterial tannase to compare the data. Evolutionary properties of the sequences were calculated by the help of APBEST (Gupta et al., 2017). The phylogenetic tree was constructed by FigTree (Rambaut, 2014).

2.2. Multiple sequence alignment (MSA)

Multiple sequence alignment (MSA) was performed for all sequences using CLUSTAL Omega (Sievers et al., 2011) and the result was represented by JALVIEW (Clamp et al., 2004).

2.3. Physicochemical and evolutionary properties

Result of MSA was divided into two categories-block and non-block of sequences. Block of the sequence was prepared by BLOCK by using BLOCK MAKER (Henikoff et al., 1995). In BLOCK format all homologous positions are fixed. Each position may contain various kinds of residues. Both non block and block sequences were analysed by PHYSICO2 (Banerjee et al., 2015) for the calculation of physicochemical properties. It gives more than 20 types of residue level analysis and 46 window dependent properties form a protein FASTA file. Physicochemical parameters like amino acid compositions, GRAY (grand average of hydropathy), pl, aliphatic index, disordered forming residues and ordered forming residues etc were calculated by the help of PHYSICO2. Based on the strength of order formation C, F, Y and W are taken as strong order forming residues. On the other hand, disorder regions of proteins were found to have higher content of R, E, G, Q, S, K, P and G. Most disorder residues based on their abundance in these proteins are R, E, S and P.
| Pathogenicity          | Organism           | ACC No. | DFR | OFR | GRAVY | pI    | Aliphatic index (Ali Ind) |
|-----------------------|--------------------|---------|-----|-----|-------|-------|--------------------------|
| animal infections     | Afipla broomeae    | A0A2MRZG7 | 23.8 | 11.2 | -0.07 | 9.18  | 58.62                   |
| Alteromonas sp.       | A0A3D0T3E3        | 17.5    | 15  | 0.19 | 5.62  | 53.62 |
| human infections      | Alteromonas sp.   | A0A0N7KYJ3 | 17.5 | 8.8  | -0.29 | 4.76  | 102.38                  |
|                       | Klebsiella sp.    | A0A263JYJ0 | 13.8 | 11.2 | -0.21 | 6.58  | 84.12                   |
|                       | Alteromonas sp.   | A0A3D0T3E3 | 17.5 | 12.5 | 0.27  | 6.42  | 69.62                   |
|                       | Serratia sp.      | A0A2V1HI92 | 18.8 | 8.8  | -0.33 | 4.66  | 85.38                   |
|                       | Burkholderia pyrrhocina | A0A3D3FKB1 | 17.5 | 12.5 | 0.27  | 6.44  | 80.25                   |
|                       | Treponema sp.     | A0A353SAZ4 | 18.8 | 8.8  | -0.33 | 4.66  | 85.38                   |
|                       | Olsenella sp.     | A0A367NI18 | 22.5 | 11.2 | -0.14 | 6.86  | 78.12                   |
|                       | Prolixibacteraceae bacteria | A0A3D1L1M7 | 18.8 | 8.8  | -0.07 | 4.66  | 89.00                   |
|                       | Treponema sp.     | A0A3D3F0K1 | 17.5 | 12.5 | 0.27  | 6.44  | 80.25                   |
|                       | Comamonadaceae bacteria | A0A257MF43 | 16.2 | 8.8  | -0.09 | 5.11  | 94.00                   |
|                       | Paracoccus pantotrophus | A0A495PRV1 | 21.2 | 7.5  | -0.24 | 5.57  | 106.25                 |
|                       | Actinomadura pelletieri | A0A495QG59 | 25   | 12.05 | -0.08 | 10.0  | 79.12                   |
|                       | Burkholderia insecticola | R4WG68 | 20.00 | 11.20 | -0.3  | 6.01  | 96.25                   |
| plant pathogen        | Acidovorax delafieldii | A0A165LJ6 | 15.00 | 10   | 0.1   | 5.05  | 84.38                   |
|                       | Burkholderiales bacteria | A0A257CD03 | 23.8 | 12.5 | -0.19 | 5.58  | 85.25                   |
|                       | Acinetobacter baumannii | A0A257CJ44 | 17.5 | 10   | 0.21  | 4.99  | 90.25                   |
|                       | Acinetobacter baumannii | A0A257LE10 | 18.8 | 10   | 0.19  | 5.69  | 90.38                   |
|                       | Acinetobacter baumannii | A0A257ISY7 | 17.5 | 11.2 | 0.15  | 6.7   | 90.25                   |
|                       | Acinetobacter baumannii | A0A257M4N4 | 18.8 | 11.2 | 0.19  | 5.87  | 70.88                   |
|                       | Acinetobacter baumannii | A0A257Q1J1 | 17.5 | 8.8  | -0.13 | 7.52  | 93.88                   |
|                       | Acinetobacter baumannii | A0A257S17 | 12.5 | 11.2 | -0.15 | 7.42  | 79.38                   |
|                       | Acinetobacter baumannii | A0A257T166 | 18.8 | 10   | 0.13  | 8.47  | 82.88                   |
|                       | Acidovorax delafieldii | A0A25699K8 | 15   | 12.5 | -0.2  | 6.43  | 80.62                   |
|                       | Acidovorax delafieldii | A0A25685EL6 | 15   | 11.2 | -0.06 | 5.77  | 80.75                   |
|                       | Acidovorax delafieldii | A0A25685I3 | 15   | 11.2 | -0.29 | 6.7   | 79.38                   |
|                       | Acidovorax delafieldii | A0A25698W22 | 16.2 | 12.5 | -0.18 | 5.09  | 72.12                   |
|                       | Acidovorax delafieldii | A0A25698W88 | 16.2 | 12.5 | -0.18 | 5.09  | 72.12                   |
|                       | Acidovorax delafieldii | A0A25698W97 | 16.2 | 12.5 | -0.18 | 5.09  | 72.12                   |
|                       | Acidovorax delafieldii | A0A25698Y64 | 16.2 | 11.2 | -0.15 | 5.79  | 86.75                   |
|                       | Acidovorax delafieldii | A0A25698Y70 | 13.8 | 11.2 | -0.32 | 6.41  | 78.12                   |
|                       | Acidovorax delafieldii | A0A25698Y74 | 13.8 | 11.2 | -0.27 | 6.7   | 78.12                   |
|                       | Acidovorax delafieldii | A0A25698W88 | 17.5 | 11.2 | -0.2  | 7.42  | 63.37                   |
|                       | Acidovorax delafieldii | A0A25698X300 | 16.2 | 12.5 | -0.2  | 6.43  | 81.88                   |
|                       | Acidovorax delafieldii | A0A256X9000 | 15   | 11.2 | 0.3   | 6.7   | 78.12                   |
|                       | Acidovorax delafieldii | A0A256HDP5 | 15   | 12.5 | -0.2  | 6.43  | 79.38                   |
|                       | Acidovorax delafieldii | A0A256HJS5 | 15   | 11.2 | 0.3   | 6.7   | 78.12                   |
|                       | Acidovorax delafieldii | A0A256HJS1 | 17.5 | 11.2 | -0.04 | 5.81  | 77                  |
|                       | Acidovorax delafieldii | A0A256HDF3 | 15   | 11.2 | 0.3   | 6.7   | 78.12                   |
|                       | Acidovorax delafieldii | A0A256HDF4 | 15   | 11.2 | 0.06  | 5.77  | 80.75                   |
|                       | Acidovorax delafieldii | A0A256HDF9 | 15   | 12.5 | -0.2  | 6.43  | 80.62                   |
|                       | Acidovorax delafieldii | A0A3282HL3 | 18.8 | 10   | -0.13 | 9.02  | 85.5                    |
|                       | Acidovorax delafieldii | A0A328ILM9 | 13.8 | 11.2 | -0.29 | 6.86  | 72                  |
|                       | Acidovorax delafieldii | A0A328ILM05 | 13.8 | 11.2 | -0.18 | 7.42  | 74.37                   |
|                       | Acidovorax delafieldii | A0A328ILM20 | 16.2 | 10   | -0.04 | 5.81  | 78.25                   |
|                       | Acidovorax delafieldii | A0A328ILM19 | 16.2 | 10   | -0.04 | 5.81  | 78.25                   |
|                       | Acidovorax delafieldii | A0A328ILM5  | 15   | 11.2 | 0.3   | 6.7   | 78.12                   |
|                       | Acidovorax delafieldii | A0A328ILV01 | 15   | 12.5 | 0.2   | 6.43  | 79.38                   |
|                       | Acidovorax delafieldii | A0A495GX1  | 15   | 11.2 | -0.28 | 6.69  | 78.12                   |
|                       | Acidovorax delafieldii | A0A495HSM5 | 16.2 | 10   | -0.04 | 5.81  | 78.25                   |

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| Pathogenicity          | Organism                             | ACC No. | DFR  | OFR  | GRAVY | pI   | Ali | Ind  |
|------------------------|--------------------------------------|---------|------|------|-------|------|-----|------|
| plants and animals     | Pseudomonas sp.                       |         |      |      |       |      |     |      |
|                        |                                      | A0A348R1P3 | 18.8 | 11.2 | -0.14 | 4.84 | 80  | 80.5 |
|                        |                                      | A0A348SKP4 | 21.2 | 11.2 | -0.21 | 8.49 | 90.25 |      |
|                        |                                      | A0A350ABK1 | 26.2 | 11.2 | 0.08  | 5.76 | 69.62 |      |
|                        |                                      | A0A350ABR3 | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A350GWW1 | 26.2 | 10   | 0.07  | 7.63 | 82  |      |
|                        |                                      | A0A350JWB4 | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A352G1I3 | 22.5 | 11.2 | 0.06  | 7.46 | 69.62 |      |
|                        |                                      | A0A352HVP1 | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A355LIN0 | 18.8 | 11.2 | -0.09 | 5.17 | 75.62 |      |
|                        |                                      | A0A356N026 | 18.8 | 10   | -0.21 | 7.59 | 90.25 |      |
|                        |                                      | A0A357P7D1 | 25    | 11.2 | 0.07  | 5.76 | 69.62 |      |
|                        |                                      | A0A358FT95 | 27    | 11.3 | 0.07  | 5.54 | 69.67 |      |
|                        |                                      | A0A3885FW3 | 18.8 | 11.2 | -0.09 | 5.17 | 75.62 |      |
|                        |                                      | A0A388JBL2 | 26.2 | 11.2 | 0.08  | 5.76 | 69.62 |      |
|                        |                                      | A0A389B824 | 26.2 | 10   | 0.11  | 6.87 | 82  |      |
|                        |                                      | A0A389TB99 | 26.4 | 10.1 | 0.12  | 6.88 | 80  |      |
|                        |                                      | A0A389TL63 | 26.2 | 11.2 | 0.08  | 5.76 | 69.62 |      |
|                        |                                      | A0A3C0P4C6 | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A3C1IRP7 | 26.2 | 11.2 | 0.08  | 5.76 | 69.62 |      |
|                        |                                      | A0A3C1RML0 | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A3D0K9E4 | 22.5 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A3D2CS8 | 26.2 | 11.2 | 0.02  | 5.75 | 68.38 |      |
|                        |                                      | A0A3D2M686 | 17.5 | 11.2 | -0.12 | 4.84 | 75.62 |      |
|                        |                                      | A0A3D2YC8F | 21.2 | 11.2 | -0.22 | 8.5  | 90.25 |      |
|                        |                                      | A0A3D2YC1U | 26.2 | 11.2 | 0.08  | 5.76 | 69.62 |      |
|                        |                                      | A0A3D5QXO | 26.2 | 10   | 0.07  | 7.63 | 82  |      |
| Average                | Pseudomonas sp.                       |         |      |      |       |      |     |      |
| nonpathogenic          | Acidobacteria bacterium               |         |      |      |       |      |     |      |
|                        |                                      | A0A1Q6JAT5 | 15.7 | 12.9 | -0.26 | 5.08 | 69.86 |      |
|                        |                                      | A0A1Q6JAT7 | 15.7 | 12.9 | -0.22 | 5.08 | 67  |      |
|                        |                                      | A0A1Q7CDH1 | 15.7 | 12.9 | 0.3   | 4.72 | 69.86 |      |
|                        |                                      | A0A1Q7FN8 | 19.9 | 12.9 | -0.16 | 6.44 | 79.57 |      |
|                        |                                      | A0A1Q7GJ8 | 15.3 | 11.4 | -0.04 | 5.64 | 71.14 |      |
|                        |                                      | A0A1Q7GR3 | 17    | 12.9 | -0.23 | 4.66 | 86.57 |      |
|                        |                                      | A0A1Q7NE8 | 20.7 | 12.9 | -0.18 | 5.16 | 83.71 |      |
|                        |                                      | A0A1Q7RT7 | 19.9 | 12.9 | -0.16 | 6.44 | 79.57 |      |
|                        |                                      | A0A1Q8BB2 | 18.4 | 12.9 | -0.2  | 7.42 | 79.57 |      |
|                        |                                      | A0A3D4FST8 | 15.7 | 11.4 | -0.47 | 6.69 | 94.86 |      |
|                        |                                      | A0A3D4FV4 | 20.7 | 11.4 | 0.04  | 6.45 | 68.29 |      |
|                        |                                      | A0A3D4FVS1 | 15.7 | 12.9 | -0.37 | 6.85 | 72.71 |      |
| Average                | Acidobacteria bacterium               |         |      |      |       |      |     |      |
| nonpathogenic          | Algoriphagus antarcticus             |         |      |      |       |      |     |      |
|                        |                                      | A0A3E0DS9 | 11.4 | 17.1 | -0.51 | 6.13 | 91.86 |      |
|                        |                                      | A0A3G9HC6 | 12.9 | 11.4 | -0.25 | 5.51 | 89.29 |      |
| Average                | Algoriphagus antarcticus             |         |      |      |       |      |     |      |

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| Pathogenicity Organism | ACC No.  | DFR  | OFR  | GRAVY | pI  | Ali Ind |
|------------------------|---------|------|------|-------|-----|--------|
| *Bacillus megaterium*  | A0A2A8XE9 | 18.6 | 12.9 | -0.09 | 6.71 | 80.86  |
|                        | A0A2AW381 | 18.6 | 12.9 | -0.09 | 6.71 | 80.86  |
|                        | A0A2AW3A4 | 17.1 | 12.9 | -0.07 | 10.1 | 71.14  |
|                        | A0A2AY08  | 17.1 | 12.9 | -0.09 | 10.1 | 69.71  |
|                        | A0A2AY620  | 18.6 | 14.3 | -0.12 | 6.44 | 80.86  |
|                        | A0A588Q4C2 | 17.1 | 12.9 | -0.13 | 7.56 | 80.86  |
|                        | A0A588QEF4 | 17.1 | 12.9 | -0.07 | 10.1 | 71.14  |
| *Bradyrhizobium huanghuaiense* | A0A562B4X4 | 17.1 | 10   | -0.17 | 6.69 | 82.29  |
|                        | A0A562B517 | 15.7 | 18.6 | -0.31 | 9.54 | 82.29  |
|                        | A0A562B989 | 15.7 | 12.9 | -0.44 | 4.41 | 86.43  |
| *Bradyrhizobium lablabi* | A0A2J9V55S | 18.4 | 10   | -0.19 | 5.87 | 100.43 |
|                        | A0A2J9VC52 | 14.3 | 12.9 | -0.45 | 4.19 | 96.29  |
|                        | A0A2J9VGU1 | 18.6 | 12.9 | -0.01 | 5.68 | 78.29  |
|                        | A0A2J9VHC6 | 12.9 | 12.9 | -0.57 | 8.35 | 106    |
| *Brevundimonas diminuta* | A0A246KNG1 | 18.9 | 11.4 | -0.07 | 8.5  | 83.57  |
| *Brevundimonas* sp. | A0A2S8WC05 | 12.9 | 11.4 | -0.23 | 8.49 | 76.86  |
| *Bryobacterales bacteria* | A0A3D1B235 | 14.3 | 14.3 | -0.38 | 5.53 | 82.57  |
| *Collimonas pratensis* | A0A127PZK5 | 12.9 | 17.1 | -0.36 | 6.41 | 88     |
|                        | A0A127QSE8 | 11.4 | 17.1 | -0.35 | 6.41 | 85.29  |
| *Cyanobacteria bacteria* | A0A350X46 | 15   | 14.3 | -0.31 | 7.4  | 92.14  |
| *Dyadobacter jiangsuensis* | A0A2P8GI72 | 14.3 | 11.4 | -0.19 | 7.26 | 75.43  |
| *Edaphobacter aggregans* | A0A3R9PWC6 | 14.3 | 17.1 | -0.4  | 7.42 | 81     |
| *Edaphobacter dinghuensis* | A0A495B1U9 | 15.7 | 12.9 | -0.38 | 7.42 | 96.14  |
| *Edaphobacter modestus* | A0A4J7Y7T20 | 10  | 14.3 | -0.4  | 6.42 | 80.86  |
|                        | A0A4J7YX34 | 12.9 | 11.4 | -0.42 | 8.54 | 74     |
| *Geodermatophilus tzadiensis* | A0A2T0TQP9 | 12.9 | 17.1 | -0.35 | 4.93 | 80.86  |
| *Granulicella* sp. | A0A3R8QIC9 | 11.4 | 12.9 | -0.42 | 8.43 | 74     |
| *Herbaspirillum seropedicae* | A0A4R8NQC9 | 17.1 | 12.9 | -0.13 | 4.49 | 75.57  |
| *Hydrogenophaga* sp. | A0A350SR60 | 11.4 | 12.9 | -0.32 | 9.06 | 82.43  |
|                        | A0A350SZD1 | 14.3 | 15.7 | -0.46 | 8.43 | 87.86  |
| *Hylemonella gracilis* | F3KUL5    | 17.1 | 12.9 | -0.34 | 5.72 | 82.29  |
| *Janthinobacterium* sp. | A0A2Q7TPQ9 | 12.9 | 12.9 | -0.05 | 4.35 | 72.71  |
| *Kribbella* sp. | A0A318PH4 | 18.6 | 14.3 | -0.4  | 4.65 | 76.71  |
| *Komagatanabacter xylinus* | A0A2D3HEP9 | 17.1 | 14.3 | -0.42 | 4.65 | 76.71  |
| *Lactobacillus acidophilus* | A0A2Q7W28 | 10  | 12.9 | -0.53 | 4.32 | 106    |
| *Lactobacillus alimentarius* | A0A4R1WPV4 | 14.3 | 12.9 | -0.21 | 4.64 | 89.14  |
| *Lactobacillus brevis* | A0A4R1WQ69 | 18.6 | 14.3 | -10.3 | 8.43 | 87.86  |
| *Lactobacillus curieae* | A0A4R2C1W1 | 15  | 15.7 | -0.17 | 10.2 | 75.43  |
|                        | A0A4V2F7E5 | 15  | 17.1 | -0.26 | 9.48 | 83.71  |
| *Kutzneria buriramensis* | A0A3E0HZ0 | 8.6  | 11.4 | -0.17 | 4.88 | 79.57  |
| *Lactobacillus acidophilus* | A0A4R8F3E0 | 15  | 11.4 | -0.07 | 9.74 | 57.43  |
| *Lactobacillus alimentarius* | A0A4R9HIQ50 | 15.7 | 14.3 | -0.31 | 8.99 | 75.29  |
| *Lactobacillus brevis* | A0A388ETC4 | 17.1 | 17.1 | -0.34 | 9.82 | 87.71  |
| *Lactobacillus casei* | A0A0R2BA86 | 11.4 | 11.4 | -0.21 | 10.2 | 58.86  |
| *Lactobacillus casei* | A0A161XSA7 | 11.4 | 11.4 | -0.21 | 10.2 | 58.86  |
| *Lactobacillus curvatus* | A0A4R2NKR2 | 11.4 | 14.3 | -0.39 | 9.27 | 68.43  |
| *Lactobacillus curvatus* | W6T5V7    | 11.4 | 14.3 | -0.4  | 9.27 | 72.57  |
| *Lactobacillus curvatus* | A0A1V3Y4K2 | 15  | 11.4 | -0.07 | 9.74 | 74     |
| *Lactobacillus curvatus* | A0A0R1V835 | 18.6 | 15.7 | -0.28 | 6.42 | 81.14  |
| *Lactobacillus curvatus* | A0A199QAR2 | 18.6 | 14.3 | -0.3  | 8.63 | 75.29  |
| *Lactobacillus curvatus* | A0A4G84F30 | 15  | 11.4 | -0.07 | 9.74 | 57.43  |
| *Lactobacillus curvatus* | A0A0R1K9A6 | 17.1 | 11.4 | -0.14 | 10.7 | 65.86  |
| *Lactobacillus curvatus* | A0A0R1H2G9 | 17.1 | 15.7 | -0.36 | 8.67 | 83.57  |
| *Lactobacillus curvatus* | A0A4R1S2M2 | 11.4 | 11.4 | -0.23 | 10.2 | 67.14  |
| *Lactobacillus curvatus* | A0A4R1S2M1 | 15  | 11.4 | -0.11 | 9.55 | 57.43  |
| *Lactobacillus curvatus* | A0A4R1S2M1 | 15  | 11.4 | -0.11 | 9.55 | 57.43  |
| Pathogenicity | Organism                          | ACC No. | DFR | OFR | GRAVY | pI  | Ali | Ind |
|---------------|----------------------------------|---------|-----|-----|-------|-----|-----|-----|
|               | Lactobacillus paucivorans         | A0A0R2LXK0 | 17.1 | 15.7 | -0.36 | 9.92 | 80.71|
|               | Lactobacillus pentosus           | A0A2K9HZY9 | 12.9 | 15.7 | -0.23 | 4.42 | 75.14|
|               |                                  | A0A2K9J2I6 | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | A0A2P1JP46 | 15.7 | 14.3 | -0.18 | 4.5  | 71   |
|               |                                  | A0A2P1JP47 | 12.9 | 15.7 | -0.24 | 4.72 | 71   |
|               |                                  | A0A2P1JP53 | 11.4 | 15.7 | -0.32 | 4.33 | 78   |
|               |                                  | A0A2P1JP57 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP59 | 11.4 | 15.7 | -0.3  | 4.71 | 72.43|
|               |                                  | A0A2P1JP60 | 14.3 | 14.3 | -0.19 | 4.42 | 72.43|
|               |                                  | A0A2P1JP62 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP63 | 11.4 | 15.7 | -0.26 | 4.42 | 72.43|
|               |                                  | A0A2P1JP64 | 11.4 | 15.7 | -0.3  | 4.71 | 73.86|
|               |                                  | A0A2P1JP65 | 14.3 | 12.9 | -0.16 | 4.42 | 72.43|
|               |                                  | A0A2P1JP66 | 14.3 | 14.3 | -0.19 | 4.42 | 72.43|
|               |                                  | A0A2P1JP67 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP68 | 11.4 | 15.7 | -0.3  | 4.71 | 72.43|
|               |                                  | A0A2P1JP70 | 14.3 | 17.1 | -0.35 | 4.33 | 79.43|
|               |                                  | A0A2P1JP71 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP73 | 11.4 | 15.7 | -0.3  | 4.71 | 72.43|
|               |                                  | A0A2P1JP74 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP75 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP76 | 14.3 | 14.3 | -0.21 | 4.42 | 75.14|
|               |                                  | A0A2P1JP77 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP78 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP79 | 15.7 | 12.9 | -0.15 | 4.42 | 71   |
|               |                                  | A0A2P1JP80 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP81 | 12.9 | 18.6 | -0.3  | 6.45 | 73.86|
|               |                                  | A0A2P1JP82 | 14.3 | 14.3 | -0.19 | 4.42 | 72.43|
|               |                                  | A0A2P1JP84 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP85 | 15.7 | 14.3 | -0.19 | 4.42 | 72.43|
|               |                                  | A0A2P1JP87 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP89 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP90 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2P1JP91 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A2S9YNQ5 | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | A0A2S9YVG1 | 14.3 | 14.3 | -0.18 | 4.42 | 68.29|
|               |                                  | A0A2S9YVVX | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | A0A2S9YVVX8 | 14.3 | 14.3 | -0.23 | 4.72 | 72.43|
|               |                                  | A0A2S9YVM2 | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | A0A2S9W5G2 | 15.7 | 14.3 | -0.2  | 4.5  | 73.71|
|               |                                  | A0A3M6KL4 | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | A0A3M6LU9 | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | A0A4S1G2K2 | 12.9 | 15.7 | -0.25 | 5.62 | 71   |
|               |                                  | A0A4S1G5S5 | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | F6IR14     | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | F6IR18     | 11.4 | 15.7 | -0.3  | 4.71 | 72.43|
|               |                                  | G6M044     | 15.7 | 12.9 | -0.22 | 10.4 | 58.86|
|               |                                  | G6M5S6     | 14.3 | 14.3 | -0.19 | 4.42 | 72.43|
|               |                                  | H5KY14     | 14.3 | 14.3 | -0.23 | 4.72 | 71   |
|               |                                  | M5AP49     | 15.7 | 14.3 | -0.2  | 4.5  | 73.71|
|               |                                  | T2HN93     | 12.9 | 15.7 | -0.25 | 4.72 | 72.43|
|               |                                  | T2HN96     | 15.7 | 14.3 | -0.1  | 4.18 | 71   |
|               |                                  | T2HNG4     | 15.7 | 14.3 | -0.15 | 4.31 | 73.71|

(continued on next page)
Table 1 (continued)

| Pathogenicity Organism       | ACC No. | DFR   | OFR   | GRAVY | pI      | Ali Ind |
|------------------------------|---------|-------|-------|-------|---------|---------|
| Lactobacillus plantarum     | A0A0F7GJK2 | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A0G9GPE8  | 15.7  | 12.9  | -0.23 | 10.4    | 58.86   |
|                             | A0A199QIQ2  | 14.3  | 12.9  | -0.19 | 6.48    | 79.43   |
|                             | A0A1E3KV81  | 15.7  | 12.9  | -0.29 | 6.86    | 75.29   |
|                             | A0A1S0RU34  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A1W6NR26  | 15.7  | 12.9  | -0.22 | 10.4    | 58.86   |
|                             | A0A387DIN7  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A3Q9OYE5  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A39SKSF6  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A39SSF33  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A39SKSF4  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A495835   | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | A0A5F0YDB8  | 15.7  | 12.9  | -0.25 | 9.27    | 75.29   |
| Lactobacillus senmaizukei   | T2HN98    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T2HNH1    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T2HNZ3    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T2HNZ8    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T2HPES    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T2HQZ7    | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
|                             | T5JWNI    | 12.9  | 15.7  | -0.25 | 4.72    | 71      |
| Lactobacillus sp.           | A0A8R2D670 | 15.7  | 15.7  | -0.57 | 9.3     | 87.86   |
|                             | A0A3R8IJD4  | 18.6  | 12.9  | -0.12 | 9.27    | 83.57   |
| Lactobacillus spicheri      | A0A199QIQ2  | 14.3  | 12.9  | -0.3  | 8.63    | 75.29   |
| Lactobacillus suartsaii     | A0A4Q0VH3  | 17.1  | 11.4  | -0.25 | 10.2    | 72.86   |
| Lactobacillus sSYMAe        | A0A9R1MZ09 | 14.3  | 12.9  | -0.23 | 10.4    | 60.29   |
| Marinobacterium mangrovicola| A0A4R1G7Z5  | 15.7  | 8.6   | -0.25 | 5.74    | 93.57   |
|                             | A0A4R1G8V6  | 14.3  | 11.4  | -0.29 | 5.66    | 86.43   |
|                             | A0A4R1G8H8  | 11.4  | 11.4  | -0.39 | 4.8     | 104.57  |
| Massilia flava              | A0A862PHV0  | 14.3  | 10    | -0.15 | 5.74    | 78.29   |
|                             | A0A862Q3C7  | 10    | 17.1  | -0.49 | 6.69    | 75.29   |
|                             | A0A9R1JE6V1 | 14.3  | 14.3  | -0.41 | 6.41    | 78      |
|                             | A0A9R1JRC9  | 14.3  | 8.6   | -0.29 | 4.94    | 112.86  |
|                             | A0A9R1JHD9  | 12.9  | 12.9  | -0.32 | 5.66    | 76.86   |
| Nostoc sp.                  | A0A318ARQ9  | 17.1  | 12.9  | -0.26 | 6.86    | 94.71   |
|                             | A0A318BBV5  | 20.4  | 15.7  | -0.2  | 9.17    | 68.57   |
|                             | A0A318DSP5  | 17.1  | 12.9  | -0.19 | 6.43    | 86.57   |
|                             | A0A318BPC8  | 14.3  | 14.3  | -0.25 | 7.25    | 71.57   |
|                             | A0A318B76  | 18.6  | 11.4  | 0.04  | 7.52    | 61.57   |
| Novosphingibium taibuenense | A0A562JN73  | 15.7  | 12.9  | -0.24 | 6.16    | 86.57   |
|                             | A0A562JN15  | 19.9  | 14.3  | -0.11 | 6.44    | 75.29   |
| Paraburkholderia sp         | A0A495SPL1  | 18.4  | 11.4  | -0.3  | 4.17    | 78.14   |
|                             | A0A495G1V4  | 14.3  | 10    | -0.21 | 5.74    | 86.57   |
|                             | A0A495G633  | 10    | 14.3  | -0.29 | 4.56    | 82.43   |
| MSPH034                  | A0A495S8F9  | 18.4  | 14.3  | -0.12 | 4.15    | 68.43   |
| MSPH034                  | A0A495T4J4  | 17.1  | 15.7  | -0.33 | 4.94    | 83.86   |
| MSPH034                  | A0A495T4W0  | 18.6  | 12.9  | -0.04 | 6.16    | 80.86   |
| MSPH034                  | A0A495T7R8  | 15.7  | 15.7  | -0.34 | 7.42    | 76.86   |
| MSPH034                  | A0A495TQA4  | 15.7  | 15.7  | -0.11 | 8.42    | 67      |

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of the network salt bridge). NUM is a new concept to calculate those energies of network salt bridges. The identification of salt bridges microenvironment residues and their energies were calculated by an in house automated method (Mitra et al., 2019). PDB2PQR v1.9.0 (Dolinsky et al., 2004) was used to generate the partial atomic charge (Q) and radius (R) with a force field, CHARMM22 (Buck et al., 2006). The Poisson-Boltzmann calculations were performed to determine atomic potentials with the help of APBS (Jurrus et al., 2018). The concept of the microenvironment is new in this field (Nayek et al., 2015).

3. Results and discussion

3.1. Physiochemical and evolutionary analysis

Physiochemical properties of all the bacterial sequences were individually evaluated. Some differences have been identified between pathogenic and non-pathogenic bacterial tannase sequences (Table 1). The number of disorder forming residue (S + E + P + R) and order forming residue (C + F + Y + W) are critical properties for a protein (Lieutaud et al., 2016). GRAVY indicates the hydrophobic and hydrophilic nature of the protein (Kaur and Pati, 2018).

By the analysis of physicochemical properties (Table 1), it showed that the tannase sequences of pathogenic bacteria contain high number of disorder forming residues, which means it can cause some diseases in human or create toxicity in enzymatic reaction (Tretyachenko et al., 2017). On the other hand, tannase sequences of non-pathogenic bacteria contain high number of order forming residue. The present study reveals that the negative value of GRAVY of non-pathogenic bacterial tannase indicates that it is hydrophilic in nature. It could easily mix with any aqueous medium (Schroeder, 2017). Enzymes with less disorder forming residues and highly hydrophilic in nature are highly stable and are could be very helpful and profitable for industries (Rigoldi et al., 2018). The

Table 1 (continued)

| Pathogenicity | Organism          | ACC No. | DFR | OFR | GRAVY | pI  | Ali | Ind |
|---------------|-------------------|---------|-----|-----|-------|-----|-----|-----|
| pathogenic    | Pseudomonas       | A0A495TR06 | 12.9 | 14.3 | -0.22 | 4.62 | 90.57 |
| non-pathogenic| Pseudomonas       | A0A495TUG7 | 14.3 | 10   | -0.24 | 6.42 | 90.86 |
| pathogenic    | Pseudomonas       | A0A4R1J326 | 14.3 | 14.3 | -0.36 | 5.97 | 75.14 |
| non-pathogenic| Pseudomonas       | A0A562G7H4 | 12.9 | 12.9 | -0.2  | 4.58 | 74.14 |
| pathogenic    | Pseudomonas       | A0A1X0N509 | 18.6 | 12.9 | -0.27 | 7.4  | 93.57 |
| non-pathogenic| Pseudomonas       | A0A2T0SB96 | 17.1 | 12.9 | -0.3  | 5.46 | 72.71 |
| pathogenic    | Pseudomonas       | A0A562E413 | 19.9 | 12.9 | -0.46 | 4.51 | 93.57 |
| non-pathogenic| Roseobacter       | A0A562STT3 | 18.4 | 10   | -0.18 | 8.36 | 89.29 |
| pathogenic    | Simplicispora      | A0A2U1C866 | 18.6 | 15.7 | -0.17 | 5.09 | 79.71 |
| non-pathogenic| Ureacoccus        | A0A2T0SBY3 | 19.9 | 15.7 | -0.09 | 7.3  | 71.14 |
| pathogenic    | Variospirax       | A0A109D427 | 18.4 | 15.7 | -0.23 | 8.47 | 84   |
| non-pathogenic| Pseudomonas       | A0A125NU60 | 14.3 | 11.4 | -0.29 | 5.55 | 81   |
| pathogenic    | A0A2G6NT685       | 15.7 | 12.9 | -0.27 | 9.05 | 100.43 |
| non-pathogenic| A0A2G6SN6         | 14.3 | 20   | -0.46 | 8.99 | 85   |
| pathogenic    | A0A2G6ST73        | 12.9 | 15.7 | -0.28 | 4.88 | 86.57 |
| non-pathogenic| A0A2G6TX73        | 14.3 | 11.4 | -0.28 | 4.51 | 95   |
| pathogenic    | A0A2G6X9A91       | 15.7 | 8.6  | -0.26 | 5.08 | 96.43 |
| non-pathogenic| A0A2G6X5T3        | 14.3 | 11.4 | -0.28 | 4.51 | 95   |
| pathogenic    | A0A2G6X9A91       | 15.7 | 8.6  | -0.26 | 5.08 | 96.43 |
| non-pathogenic| A0A2G6X9A91       | 15.7 | 8.6  | -0.26 | 5.08 | 96.43 |
| Average       |                   | 14.9 | 13.6 | -0.245 | 6.73 | 76.42 |

Table 2. Evolutionary properties like maximum conserved residue (MCR), Maximum diverse residue (MDR), Dominant hetero pair (DHP), Non conserve and conserve ratio (R) and E value of non-pathogenic bacterial tannase and pathogenic bacterial tannase sequences.

| Properties       | Non pathogenic | Pathogenic |
|------------------|---------------|------------|
| MCR              | A > T > V    | A > L > V  |
| MDR              | G > A > Y    | G > A > D  |
| DHP              | AG > DV > EA | ML > SA > AG |
| R ratio          | 100.14        | 85.99      |
| E value          | 65.98         | 57.68      |
Table 3. Type of organism with their site profile name and accession number in relation to tannase.

| Type of organism | Site profile name | Acc. No. |
|------------------|-------------------|----------|
| Nonpathogenic    | Eukaryotic thiol (cysteine) proteases histidine active site | A0A2P8GI72 |
|                  | Glycoprotein hormones beta chain signature 1 | A0A350X46 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2H9V655 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2H9VIF2 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6X5T3 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A125NU60 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A190D437 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6WT85 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6X91 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6X7X3 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A4R1G7Z5 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A495T4W0 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A495G1V4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A3D4FV49 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A3D4FST8 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A4G9GPE8 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A4G9V8F4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A1W6R26 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A448F360 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A0R1TM22 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A358K1G1 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A1V3Y4K2 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A0R2BA66 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A161XSA7 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A0R1K9A6 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A0F3H8R38 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | F6RL4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | G0M044 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A494SU8 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2K9I2I6 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A127QSE8 |
| Human pathogen   | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A318IM73 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A353SAZ4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A257FZ4W4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2V1HH92 |
|                  | Serine/threonine dehydratases pyridoxal-phosphate attachment site | A0A495SPR1 |
|                  | Type-1 copper (blue) proteins signature | A0A3D4FWC6 |
|                  | Twin arginine translocation (Tat) signal profile | A0A2T0TQP9 |
|                  | Twin arginine translocation (Tat) signal profile | A0A2H9V9G1 |
|                  | Type-1 copper (blue) proteins signature | A0A3D4FWC6 |
|                  | Serine/threonine dehydratases pyridoxal-phosphate attachment site | A0A495SPR1 |
|                  | Twin arginine translocation (Tat) signal profile | A0A3D4FWC6 |
|                  | Twin arginine translocation (Tat) signal profile | A0A356GSK5 |
|                  | Twin arginine translocation (Tat) signal profile | A0A356GSK5 |
|                  | Twin arginine translocation (Tat) signal profile | A0A356GSK5 |
| plant pathogen   | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A257LMN4 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A257LS17 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6X2L6 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A257LYY7 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A514E1B9 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | G0EY7 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A168F6C6 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6EL6 |
|                  | Prokaryotic membrane lipoprotein lipid attachment site profile | A0A2G6EL6 |

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isoelectric point of non-pathogenic sequences is slightly higher than pathogenic bacterial tannase. The result of pI indicates that non-pathogenic bacterial tannase can tolerate a wide range of pH (Kiraga et al., 2007). The aliphatic index of the non-pathogenic and pathogenic bacterial tannase is almost equal. That means the thermal stability of those proteins is nearly the same (Panda and Chandra, 2012).

From the analysis of amino acid composition (Figure 1.) of two groups of bacterial tannase sequences, it is clear that high number of acidic and basic residues (except R) are present in non-pathogenic bacterial tannase. Hydrophobic amino acids showed higher abundance in pathogenic sequences. Kyte Dolittle hydrophobicity scale indicated that those non-pathogenic bacterial tannases are highly hydrophilic in nature (Figure 1b green line). It means it could easily interact with the liquid or aqueous medium which is beneficial for industrial applications (Schröder, 2017). On the other hand, those pathogenic bacterial tannase are hydrophobic in nature (Figure 1b red line).

Analysis of evolutionary properties of both groups of sequences (Table 2) have shown that the maximum conserve residues are almost same in both non-pathogenic and pathogenic bacterial tannases. In the case of the maximum diverse residue (MDR), it also showed almost identical residues except for the third one of pathogenic bacterial tannase. It is neutral polar in non-pathogenic bacterial tannase whereas it is acidic polar in pathogenic bacterial tannase. The R ratio, i.e. non conserve/conserve residue ratio is low in pathogenic bacterial tannase sequences. That means their divergence is strictly restricted (Bandyopadhyay et al., 2019a). E value, i.e. use of

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**Figure 2.** Phylogenetic tree showed relationship between non-pathogenic (green, pink) and pathogenic (red) bacterial tannase. A similar relation clade (blue) also found between them.
The dominant hetero pair is high in non-pathogenic bacterial tannase. That means they used their charged residues rigorously. So, the overall analysis of sequences of both groups indicates that acidic and basic residues have a principal role in non-pathogenic bacterial tannase sequences.

### 3.2. Conservation more in pathogenic bacterial tannase

The result of MSA showed that a large number of single conserve amino acid positions are present in pathogenic bacterial tannase. Conserved amino acid positions 193(G), 196(G), 231(G), 232(H), 314(Y), 317(G), 322(G), 323(R), 334(P), 362(G), 431(D), 484(C), 485(L), 643(K), 648(G), 652(D), 692(P), 693(G), 740(W), 741(E) were found in all pathogenic bacterial tannase. Most positions are prevalent by neutral non-polar amino acid Glycine. Conserved amino acids position number 196(P), 297(G), 298(Y), 432(G), 433(G), 932(H), 979(W) are found in non-pathogenic bacterial tannase. From the above result, it is found that single conserve amino acid positions are much higher in pathogenic bacterial tannase that support the R ratio value.

### 3.3. Similarity in site profile

Six types of site profile in non-pathogenic bacterial tannase and four types of site profile in pathogenic bacterial tannase have been identified (Table 3). Both types of bacterial tannase showed a common site profile which is prokaryotic membrane lipoprotein lipid attachment site profile. It was found in 36 sequences of non-pathogenic bacterial tannase and 23 sequences of pathogenic bacterial tannase. Twin arginine translocation

Table 4. Details of the Ramachandran plot of *Lactobacillus plantarum* (4J0K) and *Treponema* sp. (model).

| Properties                  | *Lactobacillus plantarum* (PDB ID-4J0K) | *Treponema* sp. (model) |
|-----------------------------|----------------------------------------|-------------------------|
| Plot area                   | residues in favored region             | 89.90%                  | 82.90 % |
|                             | residues in allowed region              | 9.20%                   | 13.40%  |
|                             | residues in generously region           | 0.40%                   | 1.50%   |
|                             | residues in disallowed region           | 0.50%                   | 2.20%   |
| Residue properties          | Max. deviation                         | 19.9                    | 7.7     |
|                             | Bad contacts                           | 0                       | 0       |
| G-factors                   | Dihedrals                              | -0.16                   | -0.43   |
|                             | Covalent                               | -0.02                   | 0.08    |
|                             | Overall                                | -0.12                   | -0.27   |
(Tat) signal profile was also found in both groups of sequences. In case of non-pathogenic bacterial tannase, 4 types of signature specially found; Glycoprotein hormones beta chain signature 1, Eukaryotic thiol (cysteine) proteases histidine active site, Type-1 copper (blue) proteins signature and Zinc-containing alcohol dehydrogenases. Pathogenic bacterial tannase also possesses some unique sites like Serine/threonine dehydratases pyridoxal-phosphate attachment site, TonB-dependent receptor proteins signature 1.

3.4. Relationship between two groups

Phylogenetic group reveals the relation between non-pathogenic and pathogenic bacterial tannase (Figure 2.). Total 4 clades have been found. All the firmicutes (green) were found to form a big clade at one side of the Phylogenetic tree. This clade contains all species of Lactobacillus. The pink clade contains all non-pathogenic proteobacteria that produce tannase. All pathogenic bacteria has formed a different clade (red) at one side of the phylogenetic tree. The most exciting clade is blue clade which contained both pathogenic and non-pathogenic proteobacteria. It indicates that they had some sequence similarity. The decoration of branching in a phylogenetic tree demonstrates how those groups of tannase producing bacteria evolved from a series of familiar forbears.

3.5. Evaluation of model

After preparation, the model was evaluated by verify3D (Eisenberg et al., 1997) and Procheck (Laskowski et al., 1993) (Figure 3). Global Model Quality Estimation (GMQE) was showed 0.60, which indicated that it got higher reliability. In case of the Ramachandran plot, the model showed 82.90% in favored regions, 13.40 % in additional allowed regions, 1.50% in generously allowed regions (Table 4). It was almost similar to the template structure. The maximum deviation of the model structure was 7.7 and no bad contacts were found. The overall G-factors are negative, which indicates that it was a good model (Maheshwari and Jain, 2019). RMSD of the model with the template was

| Table 5. No. of salt bridges (isolated and network) with desolvation (ΔΔG_{dsvl}), bridge (ΔΔG_{brd}), background (ΔΔG_{bac}), total net energy (ΔΔG_{net}) of structures of Lactobacillus plantarum and model structure of non-pathogenic bacterial tannase. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Protein name | No. of isolated salt bridges | ΔΔG_{dsvl} (Kcal/mol) | ΔΔG_{brd} (Kcal/mol) | ΔΔG_{bac} (Kcal/mol) | ΔΔG_{net} (Kcal/mol) |
| 3WA6 | 9 | 99.95 | -117.75 | -25.45 | -43.25 |
| 3WA7 | 9 | 97.57 | -114.76 | -27.13 | -44.32 |
| 4JO0 | 11 | 121.95 | -142.6 | -40.92 | -61.57 |
| 4JO0 | 11 | 121.95 | -142.6 | -40.92 | -61.57 |
| 4JO0 | 8 | 81.82 | -98.93 | -35.21 | -52.32 |
| 4JO0 | 8 | 96.13 | -104.1 | -38.43 | -46.4 |
| 4JO1 | 10 | 121.24 | -123.92 | -49.53 | -52.21 |
| 4JO1 | 10 | 124.53 | -124.16 | -49.29 | -48.92 |
| 4JO1 | 12 | 122.84 | -124.22 | -50.6 | -67.98 |
| 4JO1 | 10 | 119.36 | -125.67 | -45.37 | -61.68 |
| MODEL | 11 | 97.64 | -114.28 | -12.25 | -28.89 |
| Protein name | No. of Network salt bridges | ΔΔG_{dsvl} (Kcal/mol) | ΔΔG_{brd} (Kcal/mol) | ΔΔG_{bac} (Kcal/mol) | ΔΔG_{net} (Kcal/mol) |
| 3WA6 | 3 | 88.62 | -117.46 | -8.66 | -37.5 |
| 3WA7 | 3 | 72.79 | -99.31 | -7.41 | -33.99 |
| 4JO0 | 3 | 95.57 | -133.87 | -0.51 | -38.8 |
| 4JO0 | 3 | 67.11 | -91.91 | -1.24 | -26.04 |
| 4JO0 | 5 | 105.43 | -141.32 | -8.45 | -44.37 |
| 4JO0 | 5 | 93.13 | -130.3 | -12.14 | -49.31 |
| 4JO1 | 4 | 93.39 | -125.08 | -6.23 | -37.93 |
| 4JO1 | 5 | 103.09 | -148.2 | -12.84 | -57.96 |
| 4JO1 | 3 | 59.93 | -79.31 | -7.57 | -26.97 |
| 4JO1 | 4 | 72.39 | -101.29 | -2.54 | -31.46 |
| MODEL | 2 | 40.1 | -54.88 | -3.33 | -18.1 |
3.6. Proof of higher stability

Not only the number of salt bridges but also the energies per salt bridge were analyzed. Both types of salt bridges, i.e. isolated and network salt bridges, were analyzed. In case of isolated salt bridges, structures of non-pathogenic bacterial tannase showed higher number of salt bridges than the pathogenic model structure (Table 5). 4J0K showed highest isolated salt bridge energies. In case of network salt bridges, structures of non-pathogenic bacterial tannase showed higher number of salt bridges than pathogenic bacterial tannase (Table 5). 4J0J showed highest network salt bridge microenvironment energies. It is also found that high number of residue involvement in the microenvironment of non-pathogenic bacterial tannase gives more stability. These residues affect the stability of proteins by contributing favorable or unfavorable environment.

3.7. Effect of intrinsic microenvironment

The microenvironment plays an excellent role in increasing the stability of the protein. The interaction of microenvironment residues and its partner salt bridge is equally likely neutral or favourable or unfavourable depending on the amino acid composition of the proteins.

Table 6 showed that the microenvironment residues of both types of salt bridges contribute high energies to increase the stability of non-pathogenic bacterial tannase than pathogenic bacterial tannase. 4J0K shows highest isolated salt bridge microenvironment energies whereas 4J0J shows highest network salt bridge microenvironment energies. It is also found that high number of residue involvement in the microenvironment of non-pathogenic bacterial tannase gives more stability. Charged residues play the leading role in forming of the microenvironment of salt bridges. Those charged residue does not constitute any salt bridge. The hydrophobic residues showed lower abundance in microenvironment residues. Some microenvironment residues contribute high energies in salt bridge stability. The unfavourable residues of microenvironments provide a clue for site-directed mutagenesis and help in protein engineering (Mitra et al., 2019). By changing a single associated higher energy help the protein to stabilize in an extreme condition such as high temperature (Kumar and Nussinov 1999), high salt (Bandyopadhyay et al., 2019a) and high pH (Gallivan and Dougherty 2000). Salt bridge of protein can remain stable within an increased range of temperature (284–348K) (Belur and Mugeraya 2011). These salt bridges are surrounded by polar, charged and non-polar residues which have identified as microenvironment of salt bridges (Mitra et al., 2019). These residues affect the stability of proteins by contributing favorable or unfavorable environment.
unfavorable microenvironment residue with a favourable one, the more stable structure will be created for industrial applications.

4. Conclusion

In silico study of bacterial tannase of non-pathogenic bacteria in comparison to pathogenic bacteria showed positive MRA of acidic and basic residues which alter the sequence property and not the structure. Due to the low number of disorder forming residues but high order basic residues which alter the sequence property and not the structure.

Stabilization and application of spray-dried tannase from Aspergillus fumigatus CAS21 in the presence of different carriers. J Biotechnol 10, 1-14

Centurion-Lara, A., Arrol, T., Castillo, R., Shaffer, J.M., Castro, C., Van Voorhis, W.C., Lukehart, S.A., 1997. Conservation of the 15-kilodalton lipoprotein among Treponema pallidum subspecies and strains and other pathogenic treponemes: antigenic and genomic analyses. Infect. Immun. 65, 1440–1444. PubMed:9119485.

Clamp, M., Cuff, J., Searle, S.M., Barton, G.J., 2004. The jalview java alignment editor. Bioinformatics 20, 426–427.

Dolinsky, T.J., Nielsen, J.E., McCammon, J.A., Baker, N.A., 2004. FDBZQR: an automated pipeline for the setup of Poisson–Boltzmann electrostatics calculations. Nucleic Acids Res. 32, W665–W667.

Drancourt, M., Brouqui, P., Raoult, D., 1997. Aflpia clevelandensis antibodies and crossreactivity with Brucella spp. and Yersinia enterocolitica O: 9. Clin. Diagn. Lab. Immunol. 4, 748–752. PubMed:9384302.

Eisenberg, D., Lüthy, R., Bowie, J.U., 1997. VERIFY3D: assessment of protein models with three-dimensional profiles. Methods Enzymol. 277, 396–404.

Fiser, A., Sali, A., 2003. Modeller: generation and refinement of homology-based protein structure models. Methods Enzymol. 374, 461–491.

Gallivan, J.P., Dougherty, D.A., 2000. A computational study of cation–π interactions vs salt bridges in aquatic media: implications for protein engineering. J. Am. Chem. Soc. 122, 870–874.

Gupta, P.S.S., Nayek, A., Banerjee, S., Seth, P., Das, S., Sur, V.P., Roy, C., Bandyopadhyay, A.K., 2015. SBION2: analyses of salt bridges from multiple structure files, version 2. Bioinformatics 11, 39–42.

Gupta, P.S.S., Banerjee, S., Islam, N.R.U., Sur, V.P., Bandyopadhyay, A.K., 2017. Substitutional analysis of orthologous protein families using BLOCKS. Bioinformatics 13 (1), 1–7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5405086/

Hae-Soo, K.I.M., Do Yeon, J.E.O.N., JaiHa, H.M.A., Sahar, N.E., Ha-Nul, L.E.E., Seong-Jin, H.O.N.G., Young-Min, K.I.M., 2020. Bio-transformation of green tea infusion with tannase and its improvement on adipocyte metabolism. Enzym. Microb. Technol. 135, 109496.

Henikoff, S., Henikoff, J.G., Alford, W.I., Pietroskovski, S., 1995. Automated construction and graphical presentation of protein blocks from unaligned sequences. Gene 163, GC17-GC26.

Hildebrand, D.C., 1971. Pectate and pectin gels for differentiation of Pseudomonas sp. and other bacterial plant pathogens. Phytopathology 61.

Hult, N., Boino, A., Bulliard, V., Ceruti, L., De Castro, E., Langendijk-Genevaux, P.S., Pagni, M., Sigrist, C.J., 2006. The PROSITE database. Nucleic Acids Res. 34, D227–D230.

Islam, N.R.U., Mitra, D., Gupta, P.S.S., Banerjee, S., Mondal, B., Bandyopadhyay, A.K., 2018. AUTOMINv1.0: an automation for minimization of Protein Data Bank files and its usage. Bioinformatics 14, 525–529.

Jana, A., Maiti, C., Halder, S.K., Das, A., Pati, B.R., Mondal, K.C., Mohapatra, P.K.D., 2013. Structural characterization of thermostable, solvent tolerant, cytosafe tannase from Bacillus subtilis PAR2. Biochem. Eng. J. 77, 161–170.

Jana, A., Halder, S.K., Banerjee, A., Paul, T., Pati, B.R., Mondal, K.C., 2014. Biosynthesis, structural architecture and biotechnological potential of bacterial tannase: a molecular advancement. Bioreour. Technol. 157, 327–340.

Jimenez, N., Barcenailla, J.M., Lopez de Felipe, F., de las Rivas, B., Muiot, R., 2014. Characterization of a bacterial tannase from Streptococcus galactolyticus UCN34 suitable for tannin biodegradation. Appl. Microbiol. Biotechnol. 98, 6329–6337.

Jimenez, N., Esteban-Torres, M., Marcheiro, J.M., de las Rivas, B., Muiot, R., 2014. Tannin degradation by a novel tannase enzyme present in some Lactobacillus plantarum strains. Appl. Environ. Microbiol. 80 (10), 2991–2997.

Jurrus, E., Engel, D., Star, K., Monson, K., Brandi, J., Felberg, L.E., Brookes, D.H., Lukehart, S.A., 1997. Conservation of the 15-kilodalton lipoprotein among Treponema pallidum subspecies and strains and other pathogenic treponemes: antigenic and genomic analyses. Infect. Immun. 65, 1440–1444. PubMed:9119485.

Kiraga, J., Mackiewicz, P., Mackiewicz, D., Kowalczyk, M., Biercz, P., Polak, N., Smolarczyk, K., Dudek, M.R., Cebra, S., 2007. The relationships between the
