Current Trends in Biotechnology and Pharmacy
Vol. 15 (6) 108 - 113, 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2021.6.19

Insilico Analysis of Mucin- Binding Proteins in Lactic Acid Bacteria

Shubhi Singh and Smriti Gaur*

Dept of Biotechnology, Jaypee Institute of Information Technology, Noida, India
*Corresponding author: smriti.gaur@jiit.ac.in

Abstract

Regular use of certain drugs and other antibiotics has led to the development of many side effects and drug resistance in the humans. But researchers have already started to find out various other alternatives to combat the negative effects. Among these, probiotics are being used up as an alternate and are consumed to great extent by humans in day to day life. *Lactobacillus* is the most common bacterial strain that is commercially used in various probiotic products. These LABs must bind to the human epithelial gut in order to perform their activity. For binding, mucin binding protein plays a major role by providing the attachment sites to the bacteria. In this paper we have analyzed the mucin- binding proteins in *Lactobacillus plantarum* using computational tools. *L plantarum* was chosen due to its ability to show strong binding towards epithelial surface. The FASTA sequences were extracted from UniProtkb database and were used for further comparison based on different parameters. Physicochemical analysis was done by ProtPARAM software, followed by secondary structure prediction (CFSSP software) and tertiary structure prediction (CASTp software). The MUBs motifs were also compared with the motifs of gut pathogens using TOMTOM tool. Moreover, the particular motifs responsible during adhesion were being searched in MUBs using Motif Finder. These motifs showed some similarity with the binding motifs of pathogens present in human gut. Physicochemical analysis showed that these mucin binding proteins are thermostable. GRAVY value indicates that these are soluble proteins which are hydrophilic in nature. These proteins do not have any effect of cellular proteases and hence are able to survive in the small intestine. These chosen strains have conserved sequences determined by their secondary structure analysis using the CFSSP server. Moreover, it was found that these mucin binding proteins has some of the motifs having the same sequences as present in that of pathogens. Hence, these are able to show the competitive adherence against the pathogens by blocking the binding sites for pathogens and resulting in their elimination. The study conducted showed the presence of MUBs in *Lactobacillus plantarum* and also same motifs were found in pathogen which proved the competitive adherence of *L. plantarum* against pathogens hence providing some light to the host- microbes interactions for other future studies too.

Keywords Mucin Binding Proteins, LAB, Insilico analysis, Probiotics, Pathogenic Bacteria

Introduction

Probiotics are defined as the live microorganisms which impart some health benefits to the consumer when consumed in appropriate amounts. The healthy microbes are known to reside in human intestinal gut and help the individual in numerous ways. There are several health benefits of these probiotics which are reported in the literature [1]. The food which contains probiotics in it is known as the functional foods. These functional foods share a great place in the modern market [2]. With the expansion of the modern market and with increasing awareness among people regarding the health benefits of these probiotics, there is more research work being conducted upon the usage of these organisms into several varieties of food products [3]. The incorporation of these living organisms is not an easy task and requires a great use of techniques. The stability of these organisms is a big issue while delivering the probiotics into any food item. Probiotics generally belong to the species *Bifidobacteria* and *Lactobacillus* [4]. They can also be naturally found inside the human intestinal gut. There are basic characteristics of probiotics like- the organism must of intestinal origin, should be able to survive under acidic conditions, must remain active and viable in intestine of humans, and should be able to remain alive for longer durations of time [5].

Fermented food had always been a potent source of many probiotic bacteria. Lactic acid bacteria are one of the bacteria with probiotic characteristics, majorly found in fermented food items [6-7]. LAB is able to produce many health benefits [8]. They are known to behave as the major components of functional foods. Also they can be used in other areas. They can be used as the starter cultures for both dairy and non dairy products in order to carry out fermentation as well as the hydrolysis process [9]. These can also be used as antimicrobial agents who can result in elimination of pathogenic species [10]. As we know, these organisms are able to produce the lactic acid in large amount and are able to increase the pH of the food items, hence the pathogenic bacteria mostly does not survive in this unfavorable environment [11]. They are also sometimes used as low caloric sweeteners in variety of food products. As during hydrolysis, they results in secretion of various peptides, which later on are added as a sweetening agent. Besides this, the other uses lies in the area of medicine, feed industry and chemical industry like antimicrobial, antitumor etc [12].

To perform its function, any probiotic must be able to colonize itself on the human gut epithelia. The epithelial surface of the human gut is known to made up of the mucus secreting cells or epithelial cells [13]. These cells have been greatly known for their secretion of mucin. These mucins are high molecular weight proteins playing a major role in adhesion of bacteria [13]. The bacteria have the special proteins present on their surface which results in its binding with the mucins. These special glycosylated proteins are called as mucin binding proteins. There are some special receptor interactions and

hence the bacteria gets adhere to the epithelial surface with strong affinity [14]. When attached, these probiotic bacteria are known to show the competitive adherence to the gut pathogens and hence results in their elimination by several other ways [15]. The result of this is the gut pathogens do not find any free surface to bind and hence the acidic environment created by the LAB will result in their elimination [16]. Here in our study we analyzed the mucin binding proteins in LAB with the help of computational tools. The *Lactobacillus* strains were found having the highest number of mucin binding proteins in it. The *L. plantarum* was further chosen as the base for this study. The FASTA sequences were extracted from UniProt kb database and were used for further comparison based on different parameters. The MUBs motifs were also compared with the motifs of gut pathogens. This study focused on showing the relation between probiotics and pathogens on the basis of competitive adherence.

Materials and Methods:

Sequence Retrieval

UniprotKb is the platform for collection of accurate information on proteins. This server was used for searching the strains of *Lactic acid bacteria* having the mucin binding proteins [17]. Different strains of *Lactobacillus* with different number of mucin binding proteins were retrieved. Total 11 strains were available showing the presence of Mubs. Out of these, single strain was chosen for further in silico studies on the basis of strong affinity towards human gut epithelium and presence of maximum number of Mubs.

Physicochemical Properties

For analyzing the Physicochemical Properties of retrieved sequences, ProtPARAM software was used, from the Expasy website [18]. The properties included were Amino acid composition of the mucin binding proteins, molecular weight of the protein, Theoretical pI or isoelectric point- this is critical parameter to analyze the function of the protein. Instability indexes which states the stability of proteins, its functions and also the interactive patterns of protein with other molecules [19]. Aliphatic index is defined as the volume of whole protein captured by the aliphatic side chains present in the protein. This parameter gives an idea about the thermo stability of the protein [20]. GRAVY or Grand Average Hydropathy is defined as sum of all hydropathy values of all the amino acids divided by number of residues in a sequence [21].

Secondary Structure Prediction:

The secondary structures of chosen mucin binding protein sequence were analyzed using the CFSSP server [22]. This Chou and Fasman Secondary Structure Prediction tool usually gives an idea about the presence of sheets, helices and turns in the protein [23]. The secondary structures of proteins will lead to the formation of tertiary structures and hence will determine the function of a protein.

Tertiary Structure Prediction

Tertiary structure is defined as the three dimensional structure of the protein. It involves the joining of all the functional parts of proteins together which helps the protein to become an active functional element. The tool which was used to predict the tertiary structure was CASTp- Computed Atlas of Surface Topography of Proteins web server [24 which helps to locate and measure gaps in interior of proteins and pockets which are present on the surface of proteins [25].

Motif Search

Motifs are the super secondary structures which are present in the proteins. These can be a part of a domain and generally exists in combinations of β- sheets, α- helix and turns. For searching the active motifs present in the protein having the mucin binding regions, the online tool – Motif Search was used (www.genome.jp/tools/motif/) [26].

Motif Comparison

TOMTOM is an online comparison tool, which compares the query motifs against the list of the existing motifs which are present in its database. During our search, the FASTA sequence of one chosen motif from each protein was inserted in the search box to compare the results [27].

Results and Discussions:

Sequence Retrieval

After searching the strains of *Lactic acid bacteria* having the mucin binding proteins, this resulted in 11 different strains of *Lactobacillus* with different number of mucin binding proteins present in them. Out of these, initially 2 strains were chosen having highest number of mucin binding proteins (mubs), namely, *Lactobacillus fermentum* and *Lactobacillus plantarum*. Further reported data was searched and it was found that *Lactobacillus fermentum* shows either no or very less attachment to the cell surface whereas the other strain shows the strong attachment [28]. Hence, we preceded our work with *Lactobacillus plantarum.* Further full length protein sequences were downloaded for the in silico study. Total 4 sequences were retrieved for different *Lactobacillus plantarum* species which are mentioned below in table 1. Moreover, several reported studies also show the analysis of the mucin binding proteins in LAB, and their ability to show association with intestinal proteins during adhesion through docking [29].

Table1. *Lactobacillus plantarum* strains

Lactobacillus plantarum	**A0A0S2Z342**
Lactobacillus plantarum	**A0A0S2Z2N2**
Lactobacillus plantarum	**A0A0S2Z2J1**
Lactobacillus plantarum	**A0A0A8J814**

Physicochemical Analysis:

After entering the FASTA sequences in the ProtPARAM online software, the physicochemical properties of the query sequences were generated. The data showed that LAB strain 1 has highest of the molecular weight and LAB strain 4 has the lowest molecular weight. pI value was in the range of 4 for the first three strain while the last strain has pI value of 11. The instability index values were in the range of 15-69. Whereas the aliphatic index values were in the range of 73-111. LAB strain 1 has total 527 amino acids, LAB strain 2 has 462 amino acids, LAB strain 3 has 394amino acids and LAB strain 4 has only 35 amino acids. The GRAVY values of the first three LAB strains were negative while the GRAVY value of the last strain is positive (Table 2).

According to the data obtained, it can be concluded that the proteins are stable as the all the values of instability index obtained

Table2. Physicochemical properties of selected Mubs in LAB strains

LAB strain	No. of Ami-noAcids	GRAVY	pI	Mol. Weight	Instability Index	Aliphatic index
Lactobacillus plantaru-mA0A0S2Z342	527	-0.431	4.84	56.6kDa	15.43	73.17
Lactobacillus plantaru-mA0A0S2Z2N2	462	-0.439	4.71	49.7kDa	19.48	72.75
Lactobacillus plantaru-mA0A0S2Z2J1	394	-0.457	4.54	42.1kDa	22.13	71.98
Lactobacillus plantaru-mA0A0A8J814	35	0.180	11.72	3.901kDa	69.29	111.43

are below 40, except the last one whose value is above 60 which indicates that this particular protein is not stable [30]. The molecular weights of the three proteins are in the range of 40-60kDa. However, last protein shows the molecular weight of 3.901kDa. Since aliphatic indexes are the good indicator of thermostability, hence high values of these index shows that protein is thermostable in nature [31]. The pI obtained for the first three proteins is near to 4 and above and the pH of the small intestine is between 5 to 7, therefore the proteins that fall near this range would be able to survive and colonize in the small intestine to show their beneficial effects. The GRAVY data tells us about the hydrophobicity of the protein and the value below 0 i.e. a negative value indicates that the protein is hydrophilic in nature [32].

Secondary structure prediction:

The secondary structures of the selected strains were analyzed using CFFSP online server. The FASTA sequences were entered into the query box and the results were generated. It was found that the most of the part of the mucin binding protein was being covered by the β- sheets, followed by the α- helix and then the turns. All of the retrieved sequences under study were found to have almost similar secondary structure composition stating that these protein structures are highly conserved by nature and may hence play an important role in the determining the functional activity. However, the last protein showed some variation (Table 3). The crystal structure determination in of these mucin binding proteins in probiotics has put more light upon the mechanisms of how human pathogens with associate with these gut probiotics [33].

Table3. Secondary structure composition of selected Mubs in LAB

LAB Strain	Amino Acids	Helix	Sheet	Turn
Lactobacillus plantarum A0A0S2Z342	527	47.2%	78.4%	14.6%
Lactobacillus plantarum A0A0S2Z2N2	462	50.6%	78.6%	14.1%
Lactobacillus plantarum A0A0S2Z2J1	394	52.8%	71.8%	14.0%
Lactobacillus plantarum A0A0A8J814	35	34.3%	62.9%	14.3%

Tertiary Structure Analysis:

Tertiary Structure Analysis was done to calculate the volume as well as the surface area of the mucin binding proteins. The FASTA sequences of the chosen strains were used to generate the values. It was found that CASTp online tool generated the results for the first two strain of LAB

Table4. Tertiary structure composition for selected Mubs in LAB

LAB strain	Volume	Surface Area
Lactobacillus plantarum A0A0S2Z342	68.906	145.038
Lactobacillus plantarum A0A0S2Z2N2	570.283	371.627

It was found that the first strain has total volume of 68.906 and surface area of 145.038, whereas the second strain has volume of 570.283 and surface area value of 371.627. It has been reported that the sites with the largest volume and surface area are known to be regarded as the active sites. Hence, the more volume and surface are of the protein, the more tightly it will fit to the receptor through its active site, as a result it will show a great binding [34].

Motif Search:

The FASTA sequence for the target proteins was entered in the in the search box and later the lists for the active motifs present in the proteins were generated. It was reported that *Lactobacillus plantarum* bacteria contain the motifs which are leucine rich in nature. These leucine rich motifs are important for protein- protein interactions, hence making possible the binding of MucBP with mucins [35]. The generated results for the available motifs present in mucin binding proteins are shown below:

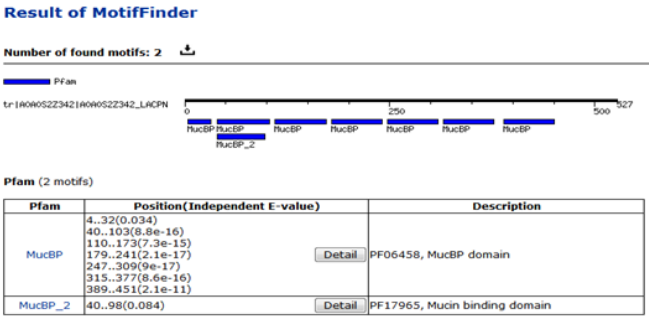

Figure1. Motifs present in L plantarum (A0A0S2Z342)

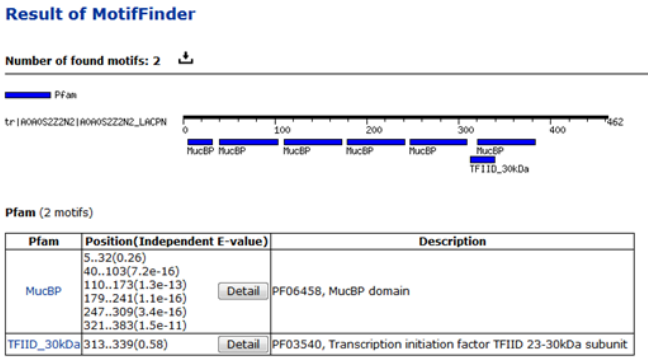

Figure2. Motifs present in L plantarum (A0A0S2Z2N2)

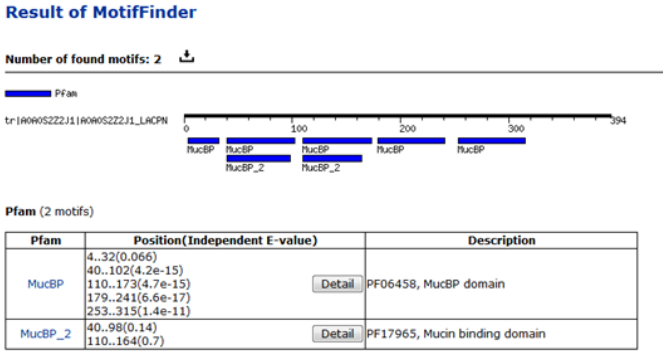

Figure3. Motifs present in L plantarum (A0A0S2Z2J1)

The above results showed the presence of different mucin binding motifs present in the protein. The first strain shows that this protein has the 2 different motifs and first type of motif is present at seven different positions, which are specified in the result data only. Likewise, the second protein also shows the presence of two motifs- MucBP and TGIID_30kDa. The MucBP is present at 6 different positions, which is implied by different amino acid positions. The third protein also has two active motifs –MucBP (present at 5 different positions) and MucBP_2. Whereas the last protein which is highly unstable do not have any active mucin binding motif, according to the results, as compared to others. Other studies were also conducted to study the complete domains of these mucin binding proteins. It is known that the domains like flagelin domain in association with pfam00669 and 700 are present. In addition to these, there are several other important peptides too which are present in these mucin proteins. The complete study on mucin binding proteins encoding genes was performed by Buntin and others in 2017 [36].

Motif Comparison

Once the active motifs were searched in the selected proteins, these were then compared with other motifs using an online comparison tool. TOMTOM was used as an online comparison tool and results which were generated are shown below:

Figure4. Compared motif of L plantarum (A0A0S2Z342)

Figure5. Compared motif of L plantarum (A0A0S2Z2N2)

Figure6. Compared motif of L plantarum (A0A0S2Z2J1)

The query sequence was taken from the motif finder tool where motif sequence which was present in the mucin binding proteins of the specific LAB bacteria. These small FASTA sequences were entered into the query box and generated the above mentioned results. These results explains that the motifs which were present

in our target proteins has the similar sequences present in the other bacterial species like *S. pyogenes, P. aeruginosa, V. cholera, L. monocytogenes, P.putida, E.coli* etc. Hence, it can be concluded that if probiotics enriched with *Lactobacillus plantarum* strain is taken with normal diet, can help in elimination of these above mentioned pathogenic bacteria species [37]. Since *Lactobacillus plantarum* has the mucin binding proteins, hence these have positive effects on showing the competitive adhesion against the pathogenic bacteria [38]. Once probiotics bind to the gut surface in the host, these blocks the binding site of the pathogenic bacteria and hence conveying the advantage to the host by reducing chances of occurrence of many diseases [39-40].

Conclusion

Mucin Binding Proteins are usually found in all the strains of LAB. These proteins play a vital role in adhesion and help the bacteria to attach itself to the inner membrane of human intestine. *Lactobacillus plantarum* had the maximum number of mucin binding proteins and hence was chosen further for the study. Physicochemical analysis showed that these mucin binding proteins are thermostable. GRAVY value indicates that these are soluble proteins which are hydrophilic in nature. These proteins do not have any effect of cellular proteases and hence are able to survive in the small intestine. These four strains have conserved sequences determined by their secondary structure analysis using the CFSSP server. These forms the motifs which are formed by the combinations of the secondary structures and these were found by using the online tool –Motif Search. These motifs were further analyzed and were found in pathogenic bacterial strains. It was found that these mucin binding proteins has some of the motifs having the same sequences as present in that of pathogens. Hence, are able to show the competitive adherence against the pathogens by blocking the binding sites for pathogens and resulting in their elimination. These types of studies will further clarify the microbes and hosts interactions and will lead to more research in this area. With more insights on these mechanisms will lead to development of more novel concepts and will result as a great help in order to prevent and treat gastrointestinal illness.

Consent for Publication Not Applicable.

Conflict of Interest The authors declare no conflict of interest, financial or otherwise

Acknowledgement We thank Dr. Anukriti Verma for her support in proofreading of the manuscript.

References

[1] Abatenh, E., Gizaw, B., Tsegay, Z., Tefera, G. and Aynalem, E., (2018). Health benefits of probiotics. *J Bacteriol Infec Dis*, *2*(1).

[2] Chakrabarti, S., Guha, S. and Majumder, K., (2018). Food-derived bioactive peptides in human health: Challenges and opportunities. *Nutrients*, *10*(11), p.1738.

[3] Terpou, A., Papadaki, A., Lappa, I.K., Kachrimanidou, V., Bosnea, L.A. and Kopsahelis, N., (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, *11*(7), p.1591.

[4] Lebeer, Sarah, Jos Vanderleyden, and Sigrid CJ De Keersmaecker (2008), "Genes and molecules of lactobacilli supporting probiotic action." *Microbiology and Molecular Biology Reviews* 72, no. 4: 728-764.

[5] Gotcheva, Velitchka, Eli Hristozova, Tsonka Hristozova, Mingruo Guo, Zlatka Roshkova, and Angel Angelov, (2002) "Assessment of potential probiotic properties of lactic acid bacteria and yeast strains." *Food Biotechnology* 16, no. 3: 211-225.

[6] Choi, A.R., Patra, J.K., Kim, W.J. and Kang, S.S., (2018). Antagonistic activities and probiotic potential of lactic acid bacteria derived from a plant-based fermented food. *Frontiers in microbiology*, *9*, p.1963.

[7] anlier, N., Gökcen, B.B. and Sezgin, A.C., (2019). Health benefits of fermented foods. *Critical reviews in food science and nutrition*, *59*(3), pp.506-527.

[8] Barboza, N. and Usaga, J., (2020). Lactic Acid Bacteria (LAB) Applications in the Food Industry: Probiotic Foods-A Mini Review. *J Nutr Food Sci 3: 019. Henry Publishing Groups Barboza N, et al*, *3*(1), p.100019.

[9] García-Burgos, M., Moreno-Fernández, J., Alférez, M.J., Díaz-Castro, J. and López-Aliaga, I., (2020). New perspectives in fermented dairy products and their health relevance. *Journal of Functional Foods*, *72*, p.104059.

[10] Ren, D., Zhu, J., Gong, S., Liu, H. and Yu, H., (2018). Antimicrobial characteristics of lactic acid bacteria isolated from homemade fermented foods. *BioMed research international*, *2018*.

[11] Field, D., Ross, R.P. and Hill, C., (2018). Developing bacteriocins of lactic acid bacteria into next generation biopreservatives. *Current Opinion in Food Science*, *20*, pp.1-6.

[12] Chang, J-H., Y. Y. Shim, S-K. Cha, and K. M. Chee (2010) "Probiotic characteristics of lactic acid bacteria isolated from kimchi." *Journal of Applied Microbiology* 109, no. 1: 220-230.

[13] Sicard, J.F., Le Bihan, G., Vogeleer, P., Jacques, M. and Harel, J., (2017). Interactions of intestinal bacteria with components of the intestinal mucus. *Frontiers in cellular and infection microbiology*, *7*, p.387.

[14] Kameyama, A., Yamakoshi, K. and Watanabe, A., (2019). A rapid separation and characterization of mucins from mouse submandibular glands by supported molecular matrix electrophoresis. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1867*(1), pp.76-81.

[15] Lukić, Jovanka, Ivana Strahinić, Branko Jovčić, Brankica Filipić, Ljubiša Topisirović, Milan Kojić, and Jelena Begović. (2012) "Different roles for lactococcal aggregation factor and mucin binding protein in adhesion to gastrointestinal mucosa." *Applied and environmental microbiology* 78, no. 22 : 7993-8000.

[16] Khaneghah, A.M., Abhari, K., Eş, I., Soares, M.B., Oliveira,

R.B., Hosseini, H., Rezaei, M., Balthazar, C.F., Silva, R., Cruz, A.G. and Ranadheera, C.S., (2020). Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Trends in Food Science & Technology*, *95*, pp.205-218.

[17] Pramanik, Krishnendu, Priyanka Pal, Tithi Soren, Soumik Mitra, Pallab Kumar Ghosh, Anumita Sarkar, and Tushar Kanti Maiti. (2018) "In silico structural, functional and phylogenetic analysis of Klebsiella phytases." *Journal of Plant Biochemistry and Biotechnology* 27, no. 3: 362-372.

[18] Singhvi, M., Zendo, T. and Sonomoto, K.,(2018). Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects. *Applied microbiology and biotechnology*, *102*(14), pp.5911-5924.

[19] Khatoon, Nazia, Rajan Kumar Pandey, and Vijay Kumar Prajapati. (2017) "Exploring Leishmania secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach." *Scientific reports* 7, no. 1: 1-12.

[20] Faya, Ngonidzashe, David L. Penkler, and Özlem Tastan Bishop. (2015) "Human, vector and parasite Hsp90 proteins: A comparative bioinformatics analysis." *FEBS Open Bio* 5: 916-927.

[21] Verma, Anukriti, Vinay Kumar Singh, and Smriti Gaur. "Computational based functional analysis of Bacillus phytases." *Computational biology and chemistry* 60 (2016): 53-58.

[22] Begum, F., Mukherjee, D., Thagriki, D., Das, S., Tripathi, P.P., Banerjee, A.K. and Ray, U., (2020). Analyses of spike protein from first deposited sequences of SARS-CoV2 from West Bengal, India. *F1000Research*, *9*.

[23] Arun, PV Parvati Sai. (2017) "Identification of hypothetical proteins with putative arsenate reductase properties in cyanobacteria by bioinformatics approach." *Journal of Biotech Research* 8: 93.

[24] Sharma, A.D., Molecular Modeling And 3d Analysis Of Water Stress Responsive Tapase Phosphatase Encoding Gene In Wheat (Triticum Aestivum).

[25] Kumar, T. Ashok (2013). "CFSSP: Chou and Fasman secondary structure prediction server." *Wide Spectrum* 1, no. 9: 15-19.

[26] Cer, R. Z., K. H. Bruce, D. E. Donohue, N. A. Temiz, U. S. Mudunuri, M. Yi, N. Volfovsky et al. (2012) "Searching for non-B DNA-forming motifs using nBMST (non-B DNA motif search tool)." *Current protocols in human genetics* 73, no. 1: 18-7.

[27] Gupta, Shobhit, John A. Stamatoyannopoulos, Timothy L. Bailey, and William Stafford Noble. (2007) "Quantifying similarity between motifs." *Genome biology* 8, no. 2: R24.

[28] Yadav, Ashok Kumar, Ashish Tyagi, Ashwani Kumar, Asha Chandola Saklani, Sunita Grover, and Virender Kumar Batish. (2015) "Adhesion of indigenous Lactobacillusplantarum to gut extracellular matrix and its physicochemical characterization." *Archives of microbiology* 197, no. 2: 155-164.

[29] Das, Jugal Kishore, Rajani Kanta Mahapatra, Shubhransu Patro, Chandan Goswami, and Mrutyunjay Suar. (2016) "Lactobacillus acidophilus binds to MUC3 component of cultured intestinal epithelial cells with highest affinity." *FEMS microbiology letters* 363, no. 8: fnw050.

[30] Idicula-Thomas, Susan, and Petety V. Balaji. (2005) "Understanding the relationship between the primary structure of proteins and their amyloidogenic propensity: clues from inclusion body formation." *Protein Engineering, Design and Selection* 18, no. 4: 175-180.

[31] Fuller, Roy. "History and development of probiotics." In *Probiotics*, pp. 1-8. Springer, Dordrecht, 1992.

[32] Panda, Subhamay, and Goutam Chandra. (2012) "Physicochemical characterization and functional analysis of some snake venom toxin proteins and related non-toxin proteins of other chordates." *Bioinformation* 8, no. 18: 891.

[33] Singh, K.S., Kumar, S., Mohanty, A.K., Grover, S. and Kaushik, J.K., 2018 Mechanistic insights into the host-microbe interaction and pathogen exclusion mediated by the Mucus-binding protein of Lactobacillus plantarum. *Scientific reports*, *8*(1), pp.1-10.

[34] Gao, Mu, and Jeffrey Skolnick. (2013) "A comprehensive survey of small-molecule binding pockets in proteins." *PLoS Comput Biol* 9, no. 10: e1003302.

[35] Volkening, Jeremy D., Kelly E. Stecker, and Michael R. Sussman. (2019) "Proteome-wide analysis of protein thermal stability in the model higher plant Arabidopsis thaliana." *Molecular & Cellular Proteomics* 18, no. 2: 308-319.

[36] Buntin, Nirunya, Willem M. de Vos, and Tipparat Hongpattarakere. (2017) "Variation of mucin adhesion, cell surface characteristics, and molecular mechanisms among Lactobacillus plantarum isolated from different habitats." *Applied microbiology and biotechnology* 101, no. 20: 7663-7674.

[37] Rossi, F. and Lathrop, A., (2019). Effects of Lactobacillus plantarum, Pediococcus acidilactici, and Pediococcus pentosaceus on the Growth of Listeria monocytogenes and Salmonella on Alfalfa Sprouts. *Journal of food protection*, *82*(3), pp.522-527.

[38] Markowiak, Paulina, and Katarzyna Śliżewska. (2017) "Effects of probiotics, prebiotics, and synbiotics on human health." *Nutrients* 9, no. 9: 1021.

[39] Tuomola, E.M., Ouwehand, A.C. and Salminen, S.J., (1999). The effect of probiotic bacteria on the adhesion of pathogens to human intestinal mucus. *FEMS Immunology & Medical Microbiology*, *26*(2), pp.137-142.

[40] Monteagudo-Mera, A., Rastall, R.A., Gibson, G.R., Charalampopoulos, D. and Chatzifragkou, A., (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Applied microbiology and biotechnology*, *103*(16), pp.6463-6472.