Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 1987-1990

Received: 09-01-2018
Accepted: 10-02-2018

Sudam Chandra Sahoo
Division of Veterinary Public Health, IVRI, Bareilly, Uttar Pradesh, India

Sarada Prasanna Sahoo
Livestock Officer, Department of Animal Husbandry, Dairying & Fisheries, Government of India New Delhi, India

Rashmi Ranjan Mishra
Department of Veterinary Clinical Medicine Ethics and Jurisprudence, College of Veterinary Science and Animal Husbandry, O.U.A.T, Odisha, India

Madhu R
Division of Veterinary Biochemistry, IVRI, Bareilly, Uttar Pradesh, India

Satsarthak Tripathy Department of Veterinary Preventive Medicine and Epidemiology, College of Veterinary Science and Animal Husbandry, O.U.A.T, Odisha, India

Correspondence
Sudam Chandra Sahoo Division of Veterinary Public Health, IVRI, Bareilly, Uttar Pradesh, India

Insilico analysis of Listeriolysin-O with its natural inhibitors

Sudam Chandra Sahoo, Sarada Prasanna Sahoo, Rashmi Ranjan Mishra, Madhu R and Satsarthak Tripathy

Abstract
Present study was conducted to understand the molecular interaction of listeriolysin-O with its natural inhibitors such as Fisetin and Lutein. The crystal structure of protein listeriolysin-O was revealed and molecular docking study was conducted by AutoDock tool. The result was analysed in terms of binding energy and position of binding. Further the interacting of amino acid residues was analysed. The energy obtained from docking study of Lutein was -6.05 whereas for fisetin was -4.75. It was identified that, both inhibitors possessed the strong interaction with listeriolysin-O. Hence lutein and fisetin may represent promising therapeutic agent for treatment of Listeriosis.

Keywords: listeriosis, listeriolysin-O (LLO), fisetin, lutein, docking energy

1. Introduction
Listeriosis is an important food borne zoonotic disease of public health significance caused by pathogenic strains of *Listeria* spp. particularly *Listeria monocytogenes* [1] and *Listeria ivanovii* [2]. It is an infectious and fatal disease of animals, birds, fish, crustaceans and humans where septicaemia and encephalitis are predominantly observed [3, 4, 5, 6, 7]. In humans, the young ones, especially new-borns, elderly persons, pregnant women, immunocompromised and immature individuals are at higher risk of acquiring listeriosis [8, 9, 10]. In India, *L. monocytogenes* is one of the major etiological agents in the causation of abortions and premature births in human [11]. For diagnoses of Listeriosis, molecular techniques are extremely accurate, sensitive and specific. Now-a-days Polymerase chain reaction (PCR) is the widely used molecular tool for identification of *Listeria* at genomic level [12]. The most common method of treatment is prescription of antibiotics.

Listeriolysin O **(**LLO) is one of the most important virulence factor produced *by L. monocytogenes* essential for promoting the intracellular growth of bacteria. LLO encoded by cholesterol-dependent cytolysin toxin encoding gene (*hly*A) present only in virulent strains of the species [11]. LLO is a (58-kD) secreted protein that can be easily detected with the use of blood agar or haemolysis assays. It contained 529 amino acids and showed strong regional homologies to Streptolysin and pneumolysin-O [14]. LLO has been proposed to be a promising target for the development of anti-listeriolisis drugs. Screening of inhibitors of LLO would facilitate the process of development of anti-LLO drugs. There are many natural proteins which inhibit the virulent action of LLO. Lutein, a natural small molecule existing widely in fruits and vegetable is demonstrated to be an effective inhibitor of LLO that works by blocking its oligomerization during invasion, thus showing significant bacteriostastic effect [15]. Fisetin, another natural flavonoid, is a potent antagonist of LLO mediated haemolysis. Fisetin inihibits *L.monocytogenes* infections in both tissue culture and animal infection models [16]. Molecular modelling and mutational analysis revealed that, fisetin directly engages the Loop2 and Loop3 of LLO which leads to block the cholesterol binding ability and reduces its oligomerization, thereby inhibiting its haemolytic activity [16]. Unlike traditional antibiotic treatment lutein does not kill the bacteria rather inhibit its virulence factor. Hence it shows potential to serve as complimentary constituent to the existing therapy of *L.monocytogenes* infections [15]. Due to selective targeting to the virulence factor, these lutein and fisetin cause less drug resistance as compared to the traditional antibiotic therapy. Further these compounds put less selective pressure on bacteria unlike antibiotic which minimise the probability mutation in bacteria [15]. In this regard, the present study was conducted to understand the detail molecular interaction of listeriolysin-O with its selective inhibitors.

2. Materials and Methods

2.1 Protein sequence and structure retrieval

The target protein Listeriolysin -O primary sequence was retrieved from UniProt with the accession ID as P13128. The sequence was from 39 to 526 amino acids long. Protein crystal structure was retrieved from the Protein Data Bank (PDB) with the PDB ID 4CDB. It was reported as x-ray diffraction methods and the resolution was 2.15 Å.

2.2 Ligand preparation

Various ligands suitable for this target protein (Listeriolysin–O) were searched from different literatures. The selected ligands which also take part to inhibit the natural protein were Fisetin and Lutein. These were retrieved from the PubChem database with a compound ID of 5281614 and 5281243 respectively.

2.3 Molecular Docking

To know the binding site and binding position, molecular docking was performed with the help of AutoDock tool. During protein preparation, the polar hydrogen and suitable collman charges were added to the protein 3D structure (Fig-1). The torsion angle and the torsion tree were taken for consideration during ligand preparation. Grid box was set on the whole surface of the protein in order to perform a blind docking (Figure-2).

3. Results and Discussion

The crystal structure of protein listeriolysin-O was reported (Fig-1) as x- ray diffraction method and the resolution were 2.15 Å and 0.246 as R-free value [15]. Analysis of crystal structure reveals that the structure consists of polypeptide chain A having sequence length 488 residues [17].

In order to find out the molecular mechanism of protein listeriolysin-O with the selected two ligands, molecular docking study was conducted by AutoDock tool. The result was analysed in terms of binding energy/docked energy and position of binding. Best binding position of Listeriolysin-O with Lutein (Fig-3) and Listeriolysin-O with fisetin (Fig-5) was revealed. The energy was obtained from docking study of Lutein was -6.05. It also indicates the best rank within the total surface of target protein for the ligand binding. Similarly, for fisetin, the best binding pose was generated. The docking energy between target protein listeriolysin-O and fisetin was evaluated as -4.75. As the Autodock tool indicate more effective binding as more negative score, hence it was identified that, both inhibitors were possessing the strong interaction with listeriolysin. Further the interacting amino acid residues were analysed as they play significant role in binding mechanism. These amino acid residues between Listeriolysin-O and Lutein within 4Å were GLU139, SER135, HIS79, TRY78, VAL77, VAL75, ALA286, TYR70AND SAP69(Fig-4).Similarly for fisetin PRO502, LEU503, ARG475, TYR444, ASN473, VAL438, TYR98 and TYR440 (Fig-6) residues are playing important role in binding of protein with in its suitable best pose on the surface of listeriolysin-O. The results obtained in accordance with the finding of Wang *et al.*, 2015 [16].

Antibiotics are used rampantly in most of the bacterial infection. More than 200 antibiotics have been utilised for treatment of both human and animals. Now-a-days, the negative side of this rampant uses of antibiotics draws attention of researcher. The gram positive bacteria *Listeria monocytogenes* has not been escaped from this global problem of antibiotic resistance. According to recent reports, Drug resistance *Listeria monocytogenes* have been identified in many times [18, 19, 20]. Therefore it is the essential to identify new method of treatment of bacterial infection without inducing selective pressure unlike antibiotics. In this regard, Lutein and fisetin were identified as potent inhibitor of listeriolysin-O [15, 16]. Hence the study described about the molecular mechanism of target protein Listeriolysin-O with its two selective inhibitors which may lead a step towards the clear understanding of their interactions in a more deeply manner. The interactions of inhibitor with listeriolysin-O were supported by the finding of Loose *et al.*, 2015; Wang *et al* 2015; Liu *et al*., 2017 [15, 16, 21]. According to study fisetin protects against *L. monocytogenes* infection by reducing the production of listeriolysin-O also it reduces *L. monocytogenes*-mediated cell death but not toxin-mediate cell death. Which indicate significant decreases in the haemolytic activity without hampering the growth of bacteria [21].Whereas Lutein inhibits intracellular growth of *L. monocytogenes* but also block *L. monocytogenes* escape from phagocytic vacuole which inhibits cell to cell migration [15]. This present study explained the effectiveness of natural compound in inhibiting the activity of LLO which create a new way for future use of fisetin and lutein as highly potent LLO-specific therapeutic drugs.

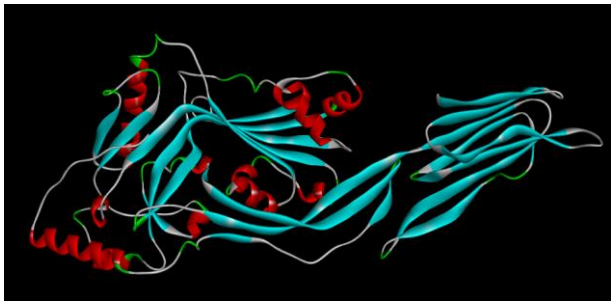

Fig 1: 3D crystal structure of target protein Listeriolysin O

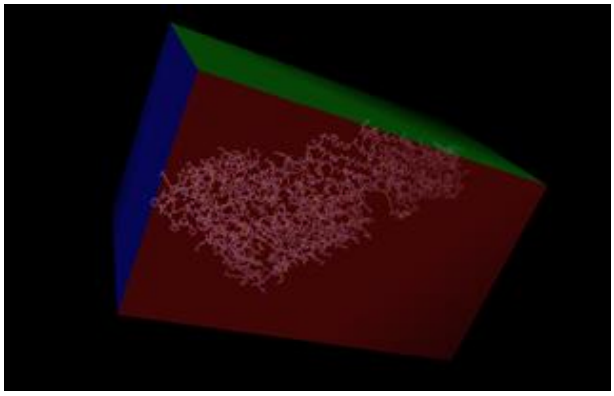

Fig 2: Grid box occupying the whole protein during docking

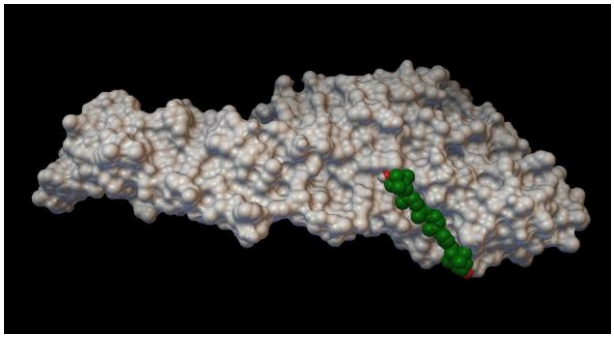

Fig 3: Best binding position of Listeriolysin-O with Lutein

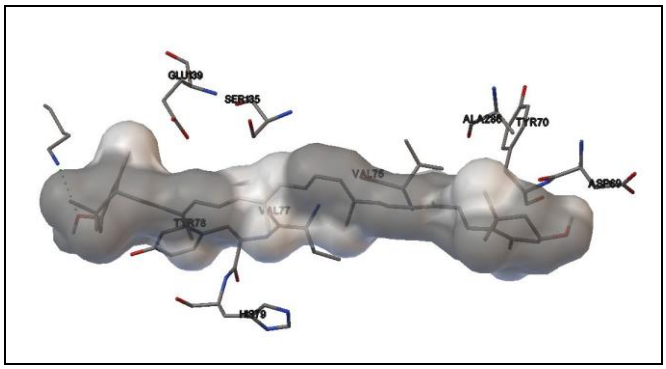

Fig 4: Interacting amino acid residues between Listeriolysin-O with Lutein

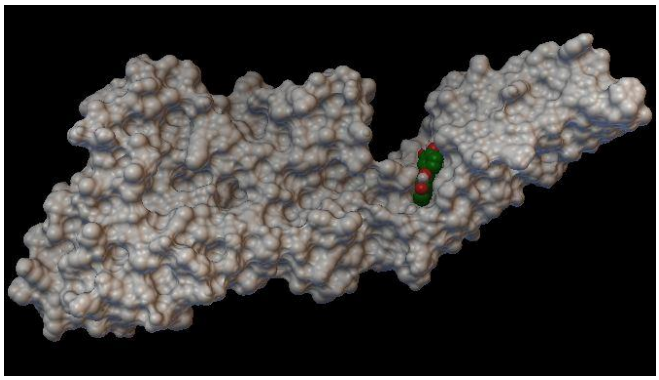

Fig 5: Best binding position of Listeriolysin-O with fisetin

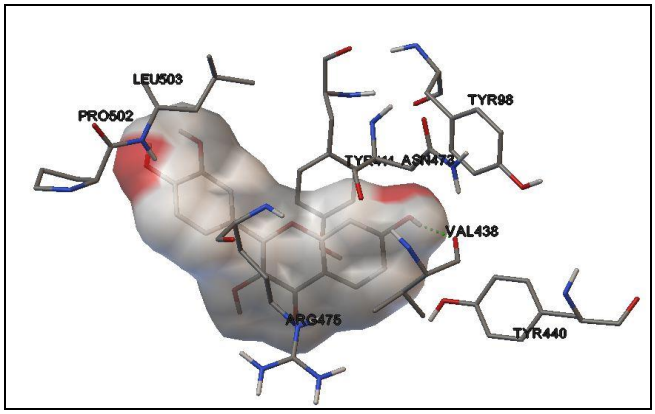

Fig 6: Interacting amino acid residues between Listeriolysin-O with fisetin

4. Conclusion

Both fisetin and lutein have antilisterial effects by inhibiting the target protein LLO. These compounds have shown specific binding position to listeriolysin-O. Further their binding energy with listeriolysin was scored negatively. Lutein has more negative binding energy as compare to fisetin, which indicates lutein bind more strongly to listeriolysin than fisetin. Hence lutein and fisetin may represent and novel therapeutic agent for treatment of Listeriosis. In this context, consumption of selected fruits and vegetables that contain large quantity of lutein and fisetin or adding those supplements may directly play a role in preventing listeria infection in susceptible animal and human population. However, extensive molecular studies necessary before administrating these compounds in clinical setting.

5. Conflict of Interest

There is no conflict of interest for this Article.

6. References

1. Farber JM, Peterkin PI. Listeria monocytogenes,A food borne pathogens. Microbiological review. 1991; 55(3):476-511
2. Day JB, Basavanna U. Magnetic bead Immuno-detection of Listeria monocytogenes and Listeria ivanovi from infant formula and leafy green vegetables using the Bio-Plex suspension array system. Food microbiology. 2015; 46:564-72.
3. Low JC, Donachie W. A review of Listeria monocytogenes and listeriosis. The Veterinary Journal. 1997; 153(1):9-29.
4. George LW. Listeriosis. In: Smith BP, editor. Large animal internal medicine. St Louis (MO): Mosby; 2002, 946-949.
5. Wesley IV. Listeriosis in animals. In: Ryser ET, Marth EH, editors. Listeria, listeriosis and food safety. 3rd ed. Boca Raton (FL): CRC Press, 2007, 55-84.
6. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, *et al* Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and environmental microbiology. 2008; 74(17):5402-5407
7. OIE. Listeria monocytogenes. Chapter 2.9.7. Manual of diagnostic tests and vaccines for terrestrial animals. 2014, 1-18.
8. Martinez G. Rapid Detection of Listeria spp. from Environmental Samples Using a New Immunomagnetic Assay. In 2013 Annual Meeting. 2013, 28-31.
9. Poulsen KP, Czuprynski CJ. Pathogenesis of listeriosis during pregnancy. Animal health research reviews. 2013; 14(1):30-39.
10. Sappenfield E, Jamieson DJ, Kourtis AP. Pregnancy and susceptibility to infectious diseases. Infectious diseases in obstetrics and gynecology, 2013.
11. Soni DK, Singh DV, Dubey SK. Pregnancy-associated human listeriosis: Virulence and genotypic analysis of Listeria monocytogenes from clinical samples. Journal of Microbiology. 2015; 53(9):653-660.
12. Barbuddhe SB, Malik SVS. Recent advances in the molecular diagnosis of listeriosis. In: ICAR sponsored winter school on "Molecular diagnostic techniques for zoonotic and foodborne infections", Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, 2009, 191-199.
13. Portnoy DA, Chakraborty T, Goebel W, Cossart P. Molecular determinants of Listeria monocytogenes pathogenesis. Infection Immunuty. 1992, 1263-1267
14. Leimeister-Wächter M, Chakraborty TRINAD. Detection of listeriolysin, the thiol-dependent hemolysin in Listeria monocytogenes, Listeria ivanovii, and Listeria seeligeri. Infection and immunity. 1989; 57(8):2350-2357.
15. Liu B, Teng Z, Wang J, Lu G, Deng X, Li L. Inhibition of listeriolysin O oligomerization by lutein prevents Listeria monocytogenes infection. Fitoterapia. 2017; 116:45-50.
16. Wang j, Qiu J, Tan Zhang, Wang Y, Zhou H, Liu X *et al*. Fisetin inhibits Listeria monocytogenes virulence by interfering with the oligomerization of listeriolysin O. The Journal of infectious diseases. 2014; 211(9):1376-1387.
17. Köster S, Van Pee K, Hudel M, Leustik M, Rhinow D, Kühlbrandt W *et al*. Crystal structure of listeriolysin O reveals molecular details of oligomerization and pore

formation. Nature communications. 2014; (5):36-90.
18. Sharma S, Sharma V, Dahiya DK, Khan A, Mathur M, Sharma A. Prevalence, virulence potential, and antibiotic susceptibility profile of Listeria monocytogenes isolated from bovine raw milk samples obtained from Rajasthan, India. Foodborne pathogens and disease. 2017; 14(3):132-140.
19. Gowda TK, Van Damme I. Occurrence and Antibiotic Susceptibility of Listeria Species and Staphylococcus aureus in Cattle Slaughterhouses of Kerala, South India. Foodborne pathogens and disease. 2017; 14(10):573-579.
20. Yadav MM, Roy A, Bhanderi B. Multiple Antibiotic Resistance among Listeria Strains, Including Listeria monocytogenes Isolated from Animals of Gujarat State, India. International. Journal. Of Current. Microbiology. Appiled. Science. 2018; 7(1):1493-1501.
21. Loose M, Pillich H, Chakraborty T. Fisetin protects against Listeria monocytogenes infection by reducing the production of listeriolysin O. The journal of infectious diseases. 2015; 213(4):684-685.