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Antiviral Activity of Hen Egg-White Lysozyme on Polio Virus.

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Abstract

The antimicrobial significance of lysozyme is not yet fully established but previous observations of its properties show enormous antiviral activity. The objective of the study is to test the antiviral effects of hen egg-white lysozyme (HEWL) on the three strains of poliovirus. Lyophilized commercial HEWL was dissolved in Eagle's Minimum Essential Medium (MEM) at a concentration of 11.6 mg/L and used for the different dilutions of log 10⁻¹ to 10⁻⁶ for the three Poliovirus in-house reference strains Sabin 1 01/528, Sabin 2 01/530 and Sabin 3 01/532 and incubated for 30 minutes and the other for 1 hour at 36°C respectively (virus dilutions in lysozyme). Two hundred microliter of each virus dilution was added onto a L20B cell monolayer in microtiter plates seeded 48 hrs earlier and incubated at 36°C and observed daily under inverted microscope for cytopathic effects. The study revealed marked absence of the cytopathic effect was exhibited in some microwells when compared with the controls. In all the tests, no CPE was observed on the first day when compared with the controls. The higher the virus dilutions, the reduced CPE showing higher antiviral effects on lesser number of virus where the concentration of the lysozyme is constant. In conclusion Lysozyme has antiviral effects on Poliovirus and from the colour and morphology of the cells on fifth day of observation, there is an indication that lysozyme is less toxic than most antiviral compounds or agents.

Keywords: Antiviral, Lysozyme, Poliovirus.

Introduction

Lysozymes (EC 3.2.1.17) are hydrolytic enzymes. It is a globular protein characterized by a molecular weight of approximately 14.4 Kilo Dalton. It is found as a single polypeptide chain consisting of 129 amino acids, in which lysine is the N – end amino acid and leucine the C – end (Cegielska-Radziejewska *et al.*, 2008). Lysozyme was first discovered by Fleming (1922) in the nasal mucus and subsequently purified from various plant, animal, microbial (bacteria, virus and fungi) materials (Parisien *et al.*, 2007; Xue, 2004; Masschalck and Michiels, 2003).

However, the amino acid sequence of known lysozymes reveals that aspartic acid is not consistently present in the active site of lysozyme molecules (Kuroki *et al.*, 1999). In contrast, substitution of glutamic acid has resulted in a complete inactivation of the enzyme (Kuroki *et al.*, 1999) confirming the critical role of this amino acid in enzymatic activity of lysozyme regardless of its origin or the class to which it belongs.

However, the chicken egg white remains the richest source of the enzyme with a concentration ranging from 3400 and 5840 mg/L (Sauter and Montoure, 1972; Wilcox and Cole, 1957). Due to the wide variability in

origin, and structural, antigenic, chemical and enzymatic properties of lysozyme molecules, they have been classified in different classes/types. The most studied and the best known is the conventional or chicken-type (i.e., c-lysozyme) with the lysozyme derived from the egg white of domestic chicken (*Gallus gallus*) as the prototype (Kuroki *et al.*, 1999). Although, c-lysozyme is typically found in the egg white of birds, it was also purified from various tissues and secretions of mammals including milk, saliva, tears, urine, respiratory and cervical secretions (Parisien *et al.*, 2007; Masschalck and Michiels 2003; Moroni and Cuccuri, 2001). Other types of lysozyme are also known; and they include the g-type derived from the egg white of domestic goose (*Anser anser*), h-type lysozyme from plant, i-type from invertebrates, b-type from bacteria (*Bacillus*) and v-type from viruses (Bachali *et al.*, 2004; Xue 2004 and Heinz *et al.*, 1992). Despite the variability in the amino acid composition and sequence of lysozyme molecules, amino acids of the catalytic centre of the active site are well conserved to keep the hydrolytic function of the enzyme (Prager and Wilson, 1974).

Non-hydrolytic antimicrobial peptides have been shown to possess more than one mode of action, and to inhibit DNA expression and synthesis pathways such as those producing cell-wall components after translocation, into the cytoplasm of sensitive microorganisms (Jenssen *et al.*, 2006; Brogden, 2005; Wu, 1999).

However, no clear explanation has so far been provided for the sensitivity of viruses to lysozyme. Cisani *et al.* (1984) suggested that the inhibition of herpes virus would rather be due to interference with antiviral activity because of the basic nature of lysozyme, than to the hydrolytic activity. Moreover, in vitro inhibition of human immunodeficiency virus (HIV) by lysozyme was attributed to the hydrolysis of viral polysaccharides and RNA transcripts, or genomic RNA (Lee-Huang *et al.*, 1999). Therefore, lysozyme would not always act by its hydrolytic activity, but would inhibit the growth of some micro-organisms either by

permeabilizing the plasma membrane and/or acting on intracellular components by virtue of its cationic hydrophobic nature, as has been described for a variety of antimicrobial peptides (Jenssen *et al.*, 2006; Reddy *et al.*, 2004).

The antimicrobial activity of lysozyme has extensively been demonstrated in vitro or in physiological fluids and secretions including milk, blood serum, saliva, and urine (Lee-Huang *et al.*, 1999; Sexton *et al.*, 1996; Martinez and Carroll, 1980). Although, lysozyme has been shown to have antimicrobial activities towards bacteria, fungi, protozoan and viruses (Lee-Huang *et al.*, 2005; Wang *et al.*, 2005; Reddy *et al.*, 2004), it is essentially known for its antibacterial activity and has been used, on this basis, in food preservation.

Polio virus is a non-enveloped, single-stranded RNA virus that causes poliomyelitis which is an acute infectious systemic viral disease affecting human in widely varying severity from a non-specific illness to almost irreversible paralysis or death. Polio virus has three serotypes 1, 2 and 3 (Oderinde *et al.*, 2011).

Polio virus is very stable in acidic environments, including the stomach. They are susceptible to chlorine-containing cleansers but resistant to inactivation by standard disinfectants (e.g., alcohol, detergents) and can persist for days at room temperature. The aim of this study was to test the antiviral effects of HEWL on Poliovirus.

Materials and Methods

Virus. Vaccine in-house reference strains obtained from of polio virus Sabin 1 01/528, Sabin 2 01/530 and Sabin 3 01/532.

Cells. L20B Mouse cells is an epithelial genetically engineered cell expressing the human poliovirus receptor. It is specific for poliovirus isolation as recommended by World Health Organization (WHO, 2004). Cells were grown in Eagle's minimum essential medium (MEM) containing 100 units of penicillin and 100mg of streptomycin/ml and supplemented with 10% or 2% foetal calf serum (FCS). The

cells were seeded 48 hours earlier into 96 wells microtiter plates (flat bottom) with growth medium (Eagle's MEM supplemented with 10% FCS). Add 100 μ l each of L20B cells from a cell suspension containing 2×10^5 cells /ml to all wells. Cover the plate with non-toxic sealer and incubate at 36°C for 48 hours to form a monolayer.

Lysozyme. Hen egg-white crystalline powder (Roche Diagnostics, USA).

Virus Dilutions. Dilution tubes with screw caps were labelled 10^{-1} – 10^{-6} per virus strain in duplicates, 1.8 ml of Lysozyme dissolved in Maintenance medium (MM) at concentration of 11.6 mg/L were dispensed into the tubes. 0.2 ml of the isolate is picked into the first tube 10^{-1} using a pipettor with sterile Aerosol Resistant Tips (ARTs). Ryan et al. (2010), used the same concentration for maximal bacterial removal and treatment of insect samples and even before DNA extraction. Cover the tube and vortex gently. Take another pipette tip, transfer 0.2 ml from the first tube to the second tube, and discard pipette tip. Cover the tube and vortex

gently. Repeat dilution steps, transferring 0.2 ml at each time and always changing pipette tip between dilutions, up until tube 6. Incubate for each strain for 30mins and 1 hour at 36°C and add 200 μ l of virus dilutions to 96 well microtiter plates (Separate for the 30mins and 1-hour incubation time) from wells 1 to 10 in rows A to H – that is, 20 wells per dilution from 10^{-6} to 10^{-6} . Add 200 μ l of lysozyme dissolved in maintenance medium to wells A11 to H12 in rows for the cell controls. Label the edge of the microtiter plate appropriately, indicating the dilution e.g. 10^{-1} , 10^{-2} to 10^{-3} as applied in this case. Cover the plate with non-toxic sealer and incubate at 36°C. Examine for development of CPE, using an inverted microscope, and record daily readings for 5–7 days. For a valid test, the cell control should have a complete monolayer of healthy cells.

Controls. There were two class of controls; i. wells with the diluted MM with lysozyme and the cells (no virus); ii. Wells with viruses diluted in MM and cells (no lysozyme).

Results



Fig. 1a.

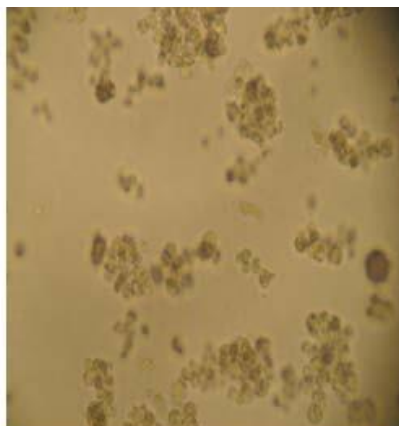


Fig. 1b.

Fig. 1a and **1b** are the pictures of a healthy monolayer of L20B cell and with typical Poliovirus cytopathic effects formed in control wells as observed under the inverted light microscope.

Thirty Minutes Incubation of Virus Dilutions in Lysozyme

Table 1A: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 1 (P1) on L20B.

Virus Dilution/ Days	Log 10⁻¹	Log 10⁻²	Log 10⁻³	Log 10⁻⁴	Log 10⁻⁵	Log 10⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	++	++	-	-
Day 3	+++	+++	+++	+++	-	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

Table 1B: Control Wells Observation for Virus + Cell for P1, for Day 1 and 2.

Days	Virus Dilutions					
	Log 10⁻¹	Log 10⁻²	Log 10⁻³	Log 10⁻⁴	Log 10⁻⁵	Log 10⁻⁶
Day 1	++++	++++	++++	++++	++	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + -- represents 25% cytopathic effect (CPE)
- ++ -- represents 50% cytopathic effect (CPE)
- +++ -- represents 75% cytopathic effect (CPE)
- ++++ -- represents 100% cytopathic effect (CPE)

Table 1A shows the sensitivity of poliovirus type 1 to lysozyme on the day 1 with no cytopathic effect on any dilution, this effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 4 in comparison to the control (cell + virus) on Table 1B where the cytopathic effects were 100% on day 2.



Table 2: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 2 (P2) on L20B.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	++	++	-	-
Day 3	+++	+++	+++	+++	-	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

Table 2B: Control Wells Observation for Virus + Cell for P2 for Day 1 and 2.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	++++	++++	++++	++++	+++	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + --- represents 25% cytopathic effect (CPE)
- ++ --- represents 50% cytopathic effect (CPE)
- +++ --- represents 75% cytopathic effect (CPE)
- ++++ --- represents 100% cytopathic effect (CPE)

Table 2A shows the sensitivity of poliovirus type 2 to lysozyme on the day 1 with no cytopathic effect on any dilution, this effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 4 in comparison to the control (cell + virus) on table 2B where the cytopathic effects were 100% on day 2. This is same observation for poliovirus type 1.

Table 3A: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 3 (P3) on L20B.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	++	++	-	-
Day 3	+++	+++	+++	+++	++	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

TABLE 3B: Control Wells Observation for Virus + Cell for P3 for Day 1 and 2.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	++++	++++	++++	++++	+	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + --- represents 25% cytopathic effect (CPE)
- ++ --- represents 50% cytopathic effect (CPE)
- +++ --- represents 75% cytopathic effect (CPE)
- ++++ --- represents 100% cytopathic effect (CPE)

Table 3A shows the sensitivity of poliovirus type 3 to lysozyme on the day 1 with no cytopathic effect on any dilution, this effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 3 at dilution of log 10⁻⁵ (50% CPE) in comparison to the control (cell + virus) on table 3B where the cytopathic effects were 100% on day 2.



One Hour Incubation of Virus Dilutions with Lysozyme

Table 4A: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 1 (P1) on L20B.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	+	-	-	-
Day 3	+++	+++	++	++	-	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

Table 4B: Control Wells Observation for Virus + Cell for P1, On Day 1 and 2.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	++++	++++	++++	++++	++	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + --- represents 25% cytopathic effect (CPE)
- ++ --- represents 50% cytopathic effect (CPE)
- +++ --- represents 75% cytopathic effect (CPE)
- ++++ --- represents 100% cytopathic effect (CPE)

Table 4A shows the sensitivity of poliovirus type 1 to lysozyme on the day 1 with no cytopathic effect on any dilution, this effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 3. On day 2, dilutions of 10⁻¹ to 10⁻³ have CPE of 25 and 50% while CPE is absent from 10⁻⁴ to 10⁻⁶ in comparison to the control (cell + virus) on Table 4B where the cytopathic effects was 100% on day 2. Higher sensitivity is shown by the virus to lysozyme.

Table 5A: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 2 (P2) on L20B.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	+	-	-	-
Day 3	+++	+++	++	++	-	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

Table 5B: Control Wells Observation for Virus + Cell for P2 for Day 1 and 2.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	++++	++++	++++	++++	+	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + --- represents 25% cytopathic effect (CPE)
- ++ --- represents 50% cytopathic effect (CPE)
- +++ --- represents 75% cytopathic effect (CPE)
- ++++ --- represents 100% cytopathic effect (CPE)

Table 5A shows the sensitivity of poliovirus type 2 to lysozyme on the day 1 with no cytopathic effect on any dilution, this effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 3. On day 2, dilutions of 10⁻¹ to 10⁻³ have CPE of 25 and 50% while CPE is absent from 10⁻⁴ to 10⁻⁶ in comparison to the control (cell + virus) on Table 5B where the cytopathic effects was 100% on day 2. Higher sensitivity is shown by the virus to lysozyme.



Table 6A: Representation of the Sensitivity to Lysozyme and Cytopathic Effect (CPE) formed by Reference Polio Virus Strain 3 (P3) on L20B.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	-	-	-	-	-	-
Day 2	++	++	+	-	-	-
Day 3	+++	+++	++	++	-	-
Day 4	++++	++++	++++	++++	+++	+++
Day 5	++++	++++	++++	++++	++++	++++
Cell + lysozyme	-	-	-	-	-	-

Table 6B: Control Wells Observation for Virus + Cell for P3 for Day 1 and 2.

Days	Virus Dilution					
	Log 10 ⁻¹	Log 10 ⁻²	Log 10 ⁻³	Log 10 ⁻⁴	Log 10 ⁻⁵	Log 10 ⁻⁶
Day 1	++++	++++	++++	++++	++	-
Day 2	++++	++++	++++	++++	++++	++++

Key:

- -- represents no cytopathic effect (CPE)
- + --- represents 25% cytopathic effect (CPE)
- ++ --- represents 50% cytopathic effect (CPE)
- +++ --- represents 75% cytopathic effect (CPE)
- ++++ --- represents 100% cytopathic effect (CPE)

Table 6A shows the sensitivity of poliovirus type 3 to lysozyme on the day 1 with no cytopathic effect on any dilution. This effect affected the higher virus dilutions of 10⁻⁵ and 10⁻⁶ by not causing cytopathic effects (CPE) until day 3. On day 2, dilutions of 10⁻¹ to 10⁻³ have CPE of 25 and 50% while CPE is absent from 10⁻⁴ to 10⁻⁶ in comparison to the control (cell + virus) on table 6B where the cytopathic effects was 100% on day 2. Higher sensitivity is shown by the virus to lysozyme.

Discussion

Our study demonstrated that lysozyme has antiviral activity against poliovirus. Our finding is consistent with a previous report which indicated that Modified hen egg white lysozyme (HEWL) inhibit herpes simplex virus-1 from inducing cytopathic effects (Cisani et al., 1984). Similarly, anti -HIV activity of the chicken and human lysozyme has been reported (Lee-Huang, 1999).

In this study, the concentration of the lysozyme, the quantity or virus dilutions, and the contact time of the lysozyme is a factor, as this is remarkable between the 30 mins and 1-hour contact time with the constant concentration of the lysozyme of 11.6 mg/L. The 1-hour contact time show much more sensitivity of the virus to the lysozyme thereby reducing CPE formation and delay in earlier days. However, there will be

need for further studies that will analyse the increasing concentrations of lysozyme with increasing contact time.

However, no clear explanation has so far been provided for the sensitivity of viruses to lysozyme. However, Cisani *et al.* (1984) suggested that the inhibition of herpes virus would rather be due to interference with antiviral activity because of the basic nature of lysozyme, than to the hydrolytic activity. In addition, in-vitro inhibition of human immunodeficiency virus (HIV) by lysozyme was attributed to the hydrolysis of viral polysaccharides and RNA transcripts, or hydrolytic activity, but would inhibit the growth of some micro-organisms either by permeabilizing the plasma membrane and or acting on intracellular components by virtue of its cationic hydrophobic nature, as has been described for a variety of antimicrobial peptides (Jensen *et al.*, 2006; Reddy *et al.*, 2004; Epan and Vogel, 1999). In conjecture, further research is needed for a clearer understanding of the mechanism of action by which lysozyme acts on microorganisms that are sensitive to it, in general, eukaryotic and non-cellular microorganisms, in particular.

Lysozyme has found usage in medicine, pharmaceuticals, agriculture and biotechnology. However, more avenues are yet to be exploited. In this study, poliovirus was sensitive to HEWL but the concentration, contact time and temperature is a critical factor. The incubation / contact time of virus and HEWL of 1- hour showed more sensitivity than 30 mins.

The constant concentration of 11.6 mg/L used in this study was replicated from Ryan *et al.* (2010) in usage for bacterial and nucleic acid decontamination. In conjecture, increased concentration of HEWL will result in increased sensitivity of the poliovirus as well.

In addition, the colour of the medium and the morphology of the L20B cell line from the fifth to seventh day shows that HEWL is less toxic than most antimicrobial substances or agents.

Conclusion

This study has shown that lysozyme (HEWL) has antiviral effects on poliovirus and less toxic than most antimicrobials.

Recommendation

It is recommended that further researches on antimicrobial potentials of lysozyme be carried out for better exploitation.

Conflict of Interest.

There is no any conflict of Interest.

References

- Bachali, S., Bally, X., Jolles, J., Jolles, P., Deutsch, J.S. (2004). The lysozyme of the starfish *Asterias rubens*. A paradigmatic type1 lysozyme. *European Journal of Biochemistry*; **271**: 237-242.
- Brogden, K.A. (2005). Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nature Reviews of Microbiology*; **3**: 238-250.
- Cegielska-Kadziejewska, R., Lesnierowski, G., Kijowski, J. (2008). Properties and application of egg white lysozyme and its modified preparations-A review. *Polish Journal of Food and Nutrition Sciences*; **58** (1): 5-10.
- Cisani, G., Varaldo, P.E., Ingianni, A., Pompei R., Satta, G. (1984). Inhibition of herpes simplex virus-induced cytopathic effect by modified hen egg-white lysozymes. *Current Microbiology*; **10**: 35-40.
- Epan, R.M., Vogel, H.J. (1999). Diversity of antimicrobial peptides and their mechanisms of action. *Biochimica et Biophysica Acta (BBA)*; **1462** (1-2): 11-28.
- Fleming, A. (1922). On a remarkable bacteriolytic element found in tissues and secretions. *Proceedings of the Royal Society of London Series B*; **93**: 306-317.
- Heinz, D.W., Baase, W.A., Matthews, B.W. (1992). Folding and function of a T4



- lysozyme containing 10 consecutive alanines illustrate the redundancy of information in an amino acid sequence. *Proceedings of National Academy of Science (USA)*; **89**:3751-3755.
- Jenssen, H., Hamill, P., Hancock, R.E.W. (2006). Peptide antimicrobial agents. *Clinical Microbiology Reviews*; **19** (3): 491-511.
- Kuroki, R., Weaver, L.H., Matthews B.W. (1999). Structural basis of the conversion of T4 lysozyme into a transglycosidase by reengineering the active site. *Proceedings of National Academy of Science (USA)*; **96**:8949-8954.
- Lee-Huang, S., Huang, P.L., Sun, Y., Kung, H.F., Blithe, D.L., Chen, H.C. (1999). Lysozyme and RNases as Anti-HIV components in beta core preparations of human chorionic gonadotropin. *Proceedings of the National Academy of Sciences (USA)*; **96** (6): 2678-2682.
- Lee-Huang, S., Malorov, V., Huang, P.L., Ng, A., Hee, C.L., Chang, Y.T., Kallenbach, N., Chen, H.C. (2005). Structural and functional modelling of human lysozyme reveals a unique nonapeptide, HL9, with anti-HIV activity. *Biochemistry*; **44**: 4648-4655.
- Maroni, P., Cuccuri, C (2001). Relationship between mammary gland infections and some milk immune parameters in Sardinian breed ewes. *Small Ruminants Research*; **41**:1-7.
- Martinez, R.G., Carroll, S.P. (1980). Sequential metabolic expressions of the lethal process in human serum-treated *Escherichia coli*: Role of lysozyme. *Infection and Immunity*; **28**:735-745.
- Masschalck, B., Michiels, C.W. (2003). Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. *Critical Reviews in Microbiology*; **29**(3): 191-214.
- Oderinde, B.S., Baba, M.M., Rimamnyang, C.M., Muhammad, T., Ogunmola, O.O. (2011). Comparison of the potency of circulating vaccine derived poliovirus type 2 with sabin like type 2 by micro titration. *Australian Journal of Basic and Applied Sciences*; **5** (11):1352-1357.
- Parisien, A., Allain, B., Zhang, J., Mandeville, R., Lan, C.Q. (2007). Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. *Journal of Applied Microbiology*; **104**: 1-13.
- Prager, E.M., Wilson, A.C. (1974). Widespread distribution of lysozyme in egg white of birds. *Journal of Biological Chemistry*; **249**: 7295-7297.
- Reddy, K.V.R., Yedery R.D., Aranha, C. (2004). Antimicrobial peptides: premises and promises: Review 2004. *International Journal of Antimicrobial Agents*; **24**: 536-547.
- Ryan, T.J., Rob, K., Andrew, P.M. (2010). Bacterial communities of disease vectors sampled across time, space and species. *International Society for Microbial Ecology Journal*; **4**: 223-231.
- Sauter, E.A., Montoure, J.E. (1972). The relationship of lysozyme content of egg white to volume and stability of foams. *Journal of Food Science*; **37**(6):918.
- Sexton, C., Buss, D.H., Powell, B., O'Connor, M., Rainer, R., Woodruff, R., Cruz, J., Pettenati, M., Rao, P.N., Case, L.D. (1996). Usefulness and limitations of serum and urine lysozyme levels in the classification of acute myeloid leukemia: An analysis of 208 cases. *Leukemia Research*; **20**: 462-472.
- Wang, S., Ng, T.B., Chen, T., Lin, D., Wu, J., Rao, P., Ye, X. (2005). First report of a novel plant lysozyme with both antifungal and antibacterial activities. *Biochemistry Biophysics, Research Communication*; **327**: 820-827.



Wilcox, F.H., Cole R.K. (1957). The inheritance of differences in the lysozyme level of Hen's egg white. *Genetics*; **42**: 264-272.

World Health Organization (2004). Polio Laboratory Diagnosis Manual:49.

Wu, T., Samaranayake, L.P., Leung, W.K., Sullivan, P.A. (1999). Inhibition of growth and secreted aspartyl proteinase production in *Candida albicans* by

lysozyme. *Journal of Medical Microbiology*; **48**: 721-730.

Xue, Q.G., Schey, K.L., Volety, A.K., Chu, F.L., La Peyre, J.F. (2004). Purification and characterization of lysozyme from plasma of the eastern oyster (*Crassostrea virginica*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*; **139**: 11-25.

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