© 2017 IJSRST | Volume 3 | Issue 9 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Green Approach towards Environment and Chemical Science

Evaluation of Biological Activities of Chick Egg White on Water Pollutant Bacteria

Swapnil Kamble*, Pratiksha Dake
Department of Botany, Nowrosjee Wadiacollege, Pune, Maharashtra, India

ABSTRACT

Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels. It has been suggested that water pollution is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily.That is why, inhibition of this pollution using natural and easily available resources is the intrinsic aim of the current study. Industrial methods have been developed for its economic recovery from egg whites, and the deproteinized egg whites have been approved for food use in Europe and recently in the United States. Therefore, the current study aimed at evaluation antimicrobial activity of Chick egg white and partial purified lysozyme. The experimental designs in the current work has been carried out with several aspects of antimicrobial activity of egg white and partially purified lysozyme. Interestingly, lysozyme showed articulate inhibition activity on gram positive bacteria. Results relevant to egg white are assorted with the earlier literature of not getting inhibition zone on gram negative bacteria. At the same time, the same concept should also be applied at the industrial level to amplify the research being carried out. The future prospects of the present study would be dragged to advanced aspects in molecular evidences with respect to the genes involved in bacteria which are being inhibited by the egg white.

Keywords : Water Pollution, Egg White, Lysozyme, Antibacterial Activity.

I. INTRODUCTION

Water pollution refers to the presence of components that decrease the quality of fresh or marine water (Gale 2009). Pollution of water is the presence of some foreign organic, inorganic, biological, radiological or physical substances in the water. These substances contaminate water by degrading its quality which may cause health hazard or decrease the utility of water. Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels. It has been suggested that water pollution is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. An estimated 580 people in India die of water pollution related illness every day. About 90 percent of the water in the cities of China is polluted. As of 2007, half a billion Chinese had no access to safe drinking water (Kahn, Joseph; Yardley, Jim 2009). In addition to the acute problems of water pollution in developing countries, developed countries also continue to struggle with pollution problems. For example, in the most recent national report on waterquality in the United States, 44 percent of assessed stream miles, 64 percent of assessed lake acres, and 30 percent of assessed bays and estuarine square miles were classified as polluted. The head of China's national development agency said in 2007 that one quarter the length of China's seven main rivers were so poisoned the water harmed the skin (Wachman, Richard, 2009). That is why, inhibition of this pollution using natural and easily available resources is the intrinsic aim of the current study.

Egg white mainly consists of water (88%) and protein (11%), with the remainder consisting of carbohydrates, ash, and trace amounts of lipids (1%). Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%) are considered as the main proteins and avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), ovoflavoprotein (0.8%), ovoglycoprotein (1.0%), and ovoinhibitor (1.5%) are the minor proteins found in egg white (Kovacs-Nolan et al., 2005). Egg white, therefore has been considered as the antimicrobial agent in the current study. Lysozyme is one of the most thoroughly investigated of all proteins and was the very first protein subjected to X-ray structural analysis. As implied by its name, it is an enzyme, one found in other animal tissues and secretions

as well. It cleaves specific bonds in specific polysaccharides, ones that constitute the cell walls of many bacteria. Lysozyme thus has antibacterial activity, and provides an embryo with a measure of protection against infection during its developmental phases (Klaus Roth). Antimicrobial mechanisms of lysozyme, is the degradation of the glycosidic (1-4) ß-linkage between the N-acetylglucosamine and the N-acetylmuramic acid of the peptidoglycan layer in the bacterial cell walls (muramidase activity). The peptidoglycan is a strong, woven mesh that maintains the cell's shape and allows the diffusion of solutes and other type of molecules via large openings in the mesh. The cell wall determines the shape of the cell and protects cells from osmotic lysis. Without a wall or when it is attacked by lysozyme or other antimicrobial agents, the cell would swell and burst. Bacteria that are susceptible to the enzymatic action of lysozyme are not lysed when they are in an osmotically balanced medium. Cell death occurs by the lytic action of lysozyme on peptidoglycan only when in low-osmotic-strength media, or when the rate of the synthesis and polymerization process for new peptidoglycan formation is slower than the lysozyme catalyzed degradation (N. Guyotet, al. 2013). Such a lysozyme mechanism is unfortunately, limited to certain Gram-positive bacteria. The peptidoglycan of some Gram-positive bacteria is indeed resistant to hydrolysis by lysozyme because of chemical modifications (N. Guyotet, al. 2013).Adham M. Abdou (2013) suggested that the name lysozyme was originally used to describe an enzyme which had lytic action against bacterial cells. Lysozyme is one of the oldest egg components to be utilized commercially after it was discovered by Alexander Fleming in 1922. L. Hughey and E. A. Johnson (1987) concluded that lysozyme is an important component in the prevention of bacterial growth in foods of animal origin such as hen eggs and milk . The enzyme may also have applications as a preservative in foods that do not naturally possess it. It is attractive as a food preservative because it is specific for bacterial cell walls and harmless to humans. Industrial methods have been developed for its economic recovery from egg whites, and the deproteinized egg whites have been approved for food use in Europe and recently in the United States.

Therefore, the current study aimed at evaluation antimicrobial activity of Chick egg white and partial purified lysozyme.

II. MATERIALS AND METHODS

Sample preparation:

Water sample

Polluted water sample were collected from Mula-Mutha River, Pune (Maharashtra, India).

Egg white

Eggs were obtained from the shop near NowrosjeeWadia College, Pune.

Eggs were hand broken and the egg white was manually separated from the whole egg (N.Guyotet al.2013)

Isolation of microorganism shows antimicrobial activity.

Nutrient agar medium were prepared, autoclaved and poured in the petri dish and allowed to settle. 150 µl of polluted water sample were poured on petri plates and spread by using spreader. 5mm diameter well was made by using borer in the middle of petri plates. 200µl of egg white were poured in the well and sealed with parafilm properly. The petri plates were leaved at room temperature for 2 hours to allow the diffusion of egg white. The plates were incubated at 37^0C temperature for 24 hours. After incubation the colony which shows antimicrobial activity was isolated and maintained in nutrient agar slant. Above Procedure were carried out for 2 to 3 times to obtain pure culture of bacteria (Seham Abdel‐Shafi et al. 2016).

Partial purification of lysozyme.

For the partial purification of lysozyme, egg whites, carefully separated from the egg yolks, were diluted 3- or 3.3-fold with 0.05 M NaCl solution. To precipitate the egg white proteins other than lysozyme, the pH of this mixture was set to 4.0 by carefully adding several drops of 1N acetic acid and it was diluted with an equal volume of 40% ethanol. After 6 hours incubation at room temperature in the presence of ethanol, the mixtures were centrifuged at 15,000 x g for 15 min at 4 °C; then the precipitates were discarded. The supernatant were collected and stored in refrigerator (SeyhunGEMiLi et al. 2007).

Antimicrobial activity of egg white and partial purified lysozyme

Nutrient agar medium were prepared, autoclaved and poured in the petri dish and allowed to settle. 150 µl of pure bacterial culture were poured on petri plates and spread by using spreader. 5mm diameter well was made in middle of petri plate. Partially purified lysozyme and egg white were poured in well. Petri plates were sealed with parafilm properly and leaved at room temperature for 2 hours to allow the diffusion of egg white. The plates were incubated at 37^0C temperature for 24 hours. The radius of zone of inhibition was measured in mm (Seham Abdel‐ Shafi et al. 2016).

III. RESULT AND DISCUSSION

Isolation of microorganism shows antimicrobial activity

In the present study, on nutrient agar petri plate 2 to 3 types of colonies were observed, among them 8 mm zone of inhibition was formed by inhibition of one microorganism. The zone was not clear because there was growth of bacteria which was not inhibited by the egg white. SompongThammasirirak et al., (2008) concluded that the egg white was not active against the Gram-positive bacteria. The bacteria which showed the zone of inhibition, was isolated and separately tested against egg white to confirm the isolation of purecolonies. Isolated bacteria was gram positive. 12mm zone of inhibition of isolated bacteria against egg white was observed. The bacteria was identifies by Gram's staining. The bacteria were Gram-positive. The bacteria were rod shaped and violet in color. The positively charged crystal violet pass through the cell wall and cell membrane and binds to negatively charged components inside the cell. Addition of negatively charged iodine (in the mordant) binds to the positively charged dye and forms a large Crystal violet (hexamethyl-para-rosaniline chloride) interacts with aqueous KI-I2 via a simple anion exchange to produce a chemical precipitate. The small chloride anion is replaced by the bulkier iodide, and the complex thus formed becomes insoluble in water. During decolorization, alcohol dissolves the lipid present in the outer membrane of Gram negative bacteria and it leaches the dye-iodine complex out of the cell. A thin layer of peptidoglycan does not offer much resistance either. The dye-iodine complexes are washed from the Gram negative cell along with the outer membrane. Hence Gram negative cells readily get decolorized. On the other hand Gram positive cells become dehydrated from the ethanol treatment, closing the pores as the cell wall shrinks during dehydration. The dyeiodine complex gets trapped inside the thick peptidoglycan layer and does not get decolorized (ShridharRao, 2014).

Partial purification of lysozyme

The partial purification of lysozyme by ethanol precipitation was applied by SeyhunGemili et al. (2007). Lysozyme was partially purified from egg white and confirmed by testing against microorganisms. 14mm zone of inhibition was observed. During the incubation period, the fluctuations also occurred in the protein contents and changes in both activity and protein content were similar up to the sixth hour of incubation in the presence of 40% ethanol. Thus, it seems that these fluctuations are due to the change in lysozyme and other proteins' solubility in the extract during incubation. The further increase to 8 h of incubation at the 40% ethanol concentration precipitated particularly non-lysozyme proteins and this increased the specific enzyme activity considerably (SeyhunGemili et al. 2007).

Antibacterial activities of egg white and partially purified lysozyme

Antibacterial activities were carried out against Gram-positive bacteria.12mm zone of inhibition of egg white and 14 mm zone of inhibition of partial purified lysozyme was observed. S.Gomathi et al.(2015) tested Quail egg white against Micrococcus Luteus. They observed 12mm zone of inhibition of Purified Quail egg white Lysozyme and 18mm zone of inhibition of standard lysozyme. Lysozyme is a hydrolase that cleaves the glycosidic bond between N-acetylemuramic and N-acetylglucosamineheteropolymer of the peptidoglycan, the components of the bacterial cell wall (SompongThammasirirak et al., 2008). These enzymes are strongly active against Gram-positive bacteria bus inactive against Gram-negatve bacteria (SompongThammasirirak et al., 2008).EdySusanto et al (2014) concluded that antimicrobial activity of lysozyme can be converted into active against gram-negative bacteria through genetic hydrophobic peptide C terminal

to lysozyme. Bakteriolitic lysozyme activity against gram-negative bacteria through the destruction of the function of the phosphate groups of phospholipids with lipopolysaccharide in the outer V. membrane of gramnegative bacteria. The research results prove that egg white lysozyme thermal modification can increase the antibacterial spectrum mainly on gram-negative bacteria *E.coli*.RenataCegielska-Radziejewska et al,(2003) concluded that, investigations indicated a possibility to extend the range of lysozyme activity using thermal and chemical-thermal modification. It was observed that lysozyme concentration in the solution subjected to thermal modification, the pH value of the solution, the temperature and time of modification had a significant effect on the content of the forming polymers. The time of oxidation influenced also the amounts of polymers in the case of the chemical-thermal modification. The investigation indicated also a possibility to extend the range of lysozyme activity to include Gram-negative bacteria, i.e. Escherichia coli. Modification of lysozyme by the membrane technique also broadened the spectrum of enzyme antibacterial action especially against Pseudomonas fluorescens and Proteus mirabilis bacteria. It may be stated that increased antibacterial activity against Gram–negative bacteria is not connected with a decrease in the activity of modified lysozyme preparations against Gram-positive bacteria. Studies showed that the applied lysozyme preparations showed a varying activity, depending on the type of bacteria. *C. glutamicum, X. oryzae, S. flexneri, and S. cerevisiae,* including *E. coli*, were influenced by the lysosome treatment, except for S. albus. In the case of S. albus, the antimicrobial effect was lower than the other species. This suggests that the lysosomal activity against some strains forming bacteria.

IV. CONCLUSION

The present work has been focused on the antimicrobial activity of egg white and the lysozyme as it would channelize the indirect control of water pollution. The study has been more emphasized upon the lysosomal antimicrobial activity which has been articulately proved via experimental designs. Further, the study concludes that egg white being an easy source for purified lysozyme should be worked upon at laboratory level in various aspects. This is because very meager work has been carried out on egg white as an antimicrobial agent. At the same time, the same concept should also be applied at the industrial level to amplify the research being carried out. The future prospects of the present study would be dragged to advanced aspects in molecular evidences with respect to the genes involved in bacteria which are being inhibited by the egg white. The identification of wide range of inhibiting bacteria by lysozyme is also to be carried out. The specificity of inhibition activity on gram positive bacteria is also an intrinsic issue to be concerned which has to be investigated further.

FIGURES:

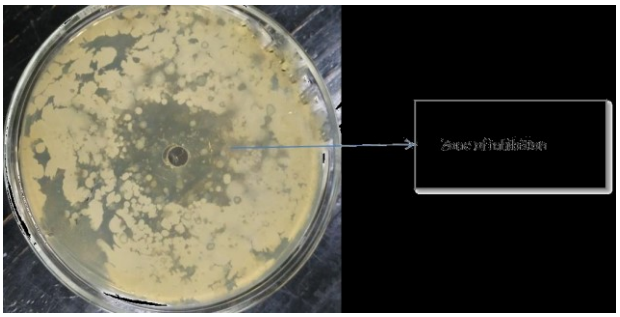

Figure 1. Zone of inhibition of water sample

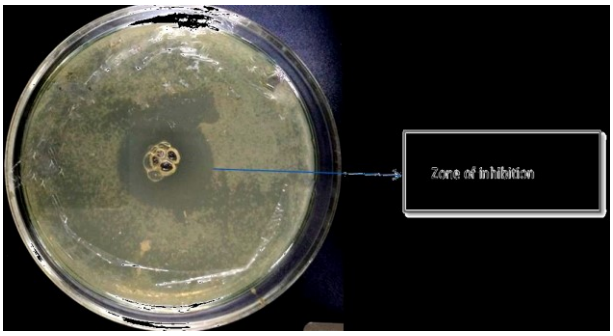

Figure 2. Zone of inhibition of isolated bacteria

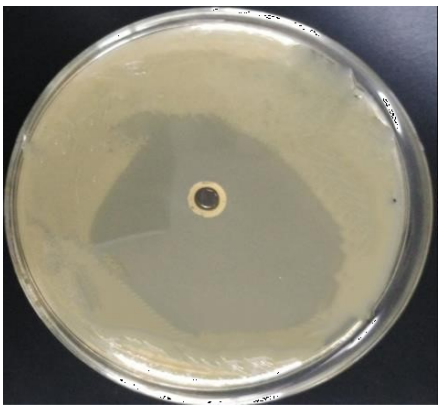

Figure 3. Lysozyme zone of inhibition.

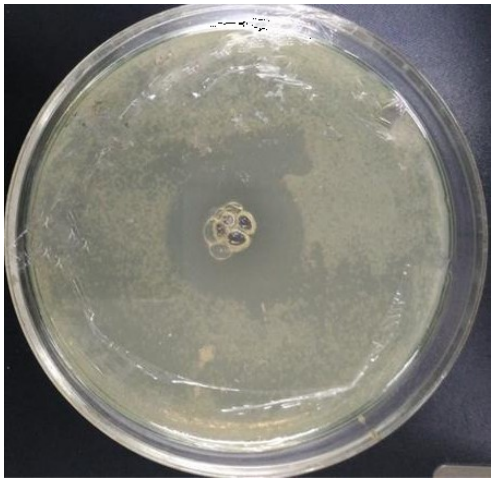

Figure 4. Egg white zone of inhibition.

V. REFERENCES

[1]. Aleksandra Zambrowicz, Marta Pokora, Ewelina Eckert, MarekSzoltysik, Anna Dabrowska, JozefaChrzanowska, TadeuszTrziszka Antioxidant and antimicrobial activity of lecithin free egg yolk protein preparation hydrolysates obtained with digestive enzymes. Functional Foods in Health Disease, 2(12):487-500, (2012).

[2]. Carrillo W., Tubon J and Vilcacundo R. Isolation of hen egg white lysosome by cation exchange chromatography , analysis of it digestibility and evaluation of the inhibition lipid peroxidation in the zebrafish model. Asian journal of pharmaceutical and clinical researchvol 9, (2016).

[3]. Cegielska-RadziejewskaRenata and Szablewski Tomasz Inhibition of food-borne bacteria by thermo-chemically modified egg white lysozyme. African Journal of Microbiology Research, Vol. 8(6), pp. 590-597, (2014).

[4]. Chang-Qing Ruan, Yu-Jie Chi and Rui-Dong Zhang Kinetics of Hydrolysis of Egg White Protein by Pepsin. Czech J. Food Science Vol. 28, 2010, No. 5: 355-363, (2010).

[5]. EdySusanto, DjalalRosyidi, LilikEkaRadiati and Abdul Manab Improved antibacterial spectrum of hen egg white lysozyme with thermal modified. Research and review in sciences 8(11), [437-442], (2014).

[6]. Ewelina Wesierska, YousifSaleh, Tadeusz Teziszka, WieslawKopec, MaciejSiewinski and KamilaKorzekwa Antimicrobial activity of chicken egg white cystatin. World Journal of Microbiology and Biotechnology 21: 59-64, (2005).

[7]. FlavianaBombarda de Andrade1, Jair Caetano de Oliveira2, Marjorie Takei Yoshie2, Bruno Martini Guimarães1, Rafael Braga Gonçalves3, Waleska Dias Schwarcz4 Antimicrobial Activity and Synergism of Lactoferrin and Lysozyme Against Cariogenic Microorganisms. Brazilian Dental Journal 25(2): 165-169, (2014).

[8]. KhothibulUmam Al Awwaly, Abdul Manab1, ManikEirrySawitri, Eny Sri Widyastuti1 and YunySusantiHaniyah.Hen Egg White Lysozyme Extraction Using SiO2: Effect of pH and Mineral on Antimicrobial Activity International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 5 /3pp. 432-436, (2016)

[9]. N. Guyoti, Jan, S. Rehault-Godbert, Y. Nys, M. Gautier, and F. Baron. Antibacterial activity of egg white: influence of physico-chemical conditions. Bergamo 15-19, (2013).

[10]. RenataCegielska- Radziejewska, Grzegorz Lesnierowski and JacekKijowski Properties and application of egg white lysosome and its modified preparations. polish journal of food and nutrition sciences Vol. 58, No. 1, pp. 5-10, (2008).

[11]. RenataCegielska-Radziejewska, GrzegorzLesnierowski, JacekKijowski, Tomasz Szablewski and Jan Zabielski Effects of treatment with lysozyme and its polymers on the microflora and sensory properties of chilled chicken breast muscle. Bull Vet InstPulawy 53, 455-461, (2009).

[12]. S. Gomathi, M.Vijitha, Senthilkumar Rathnasamy Affinity Separation of Lysozyme from Quail Egg (Coturnixypsilophora) and its Antimicrobial Characterization. International Journal of PharmTech Research, Vol.6, No.4, pp 1286-1291, (2014).

[13]. SeyhunGemili Partial Purification of Hen Egg White Lysozyme by Ethanol Precipitation Method and Determination of the Thermal Stability of Its Lyophilized Form. Turk J Agric For 31 125-134, (2007).

[14]. Sivaramakrishnan Rajaraman , Guruprakash Subbiahdoss and PadmanabanPatchirajan Effect of Hen Egg White on Microbial Adhesion and Biofilm Growth of Biomaterial Associated Infection Causing Pathogens International Journal

of Bio-Science and Bio-Technology Vol.6, No.2 (2014), pp.99-106, (2014).
[15]. SompongThammairirak, YuwatidaPukcothannung, SutthidechPreecharram, SakdaDaduang, RinaPatramanon, TamoFukamizo and Tomahiro Araki.Antimicrobial peptides derived from goose egg white lysosome. Comparative Biochemistry and Physiology, Prat C 151 , Pa no 84-91, (2009).
[16]. Yoon, Jihee, Jae-Min Park, Ki-Ju Kim1, Yang-Hoon Kim, and Jiho Min.Antimicrobial Activity of the Cell Organelles, Lysosomes, Isolated from Egg White. Journal of Microbiology and Biotechnology, 19(11), 1364-1368, (2009).