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Simplifying the White Egg Lysozyme Protocol for Microbial Ghosts Preparation

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Abstract

Recently a protocol for preparing cell ghosts from the extremothermophilic gram-positive *Bacillus stearothermophilus* using the lysozyme was proposed. *B. stearothermophilus* which is extremothermophilic and spore former Bacillus strain was selected to be a model due to their exceptional properties. lysozyme are existed in all types of birds' eggs and could be considered as perfect selected natural enzymes can be found everywhere and can be used by any one at any time. In this study the protocol is adjusted and introduced in simpler form and steps as an instructions to the one who are going to vaccine his animals against unknown infection existed around. One should be aware with the basic steps. Each communal disease could be studied as case by case study, and for each a specific protocol can be installed. Such protocol is simple for the microbiologist but it need more precautions for those who did not have the basic microbiological knowledge. However, separate protocol for each microbe could be evaluated by the experts and can be introduced to the farmers, grazer, as well as to the human in emergence cases. This study even could do a critical shift in the protection against diseases. I recommended for each government to establish this protocol for the endemic diseases in different areas.

Keywords: *Bacillus stearothermophilus;* Microbial ghosts; Emergency protocol; Birds' egg; Lysozyme.

Introduction

Weibull (1956) is succeeded to prepare *Bacillus megaterium* as protoplast (Weibull 1956). Its protoplast was given the name "ghosts". The phage ghosts and bacterial ghosts were known in the middle of the 19th century because of the development of the molecular biology and the microscopy [19- 21, 24-26, 27, 28, 31,35]. Red Blood Cells (RBCs) ghosts were also prepared [12-14, 17, and 18]. Other forms of different cells have the ghosts feature were described [15, 16, 22, 30, 33, 34].

The concept of bacterial lysis by the bacteriophage *E* lysis protein leads to establish a protocol for gram negative ghost cells preparation. A new protocol proves success of all types of microbes (till now) was recently introduced. It has been given the name Sponge-Like Protocol (SLP), which is derived from using the critical chemical concentration of some chemical compounds and some physical and biological parameters [4-6, 29] The SLP protocol found acceptance and proved to be efficient [8-10].

The prepared bacterial cells using the SLP protocol maintain correct cell structure and surface antigen [11]. Using the bio-critical concentration of a single compound such as H_2O_2 is enabling the evacuation of the Newcastle virus from their RNA constituents [29].

Lysozyme is an enzyme able to lysis most of the bacterial strains and it was used in the original SLP protocol [9, 23, 37, 38].

In recent study a harsh extermothermophilic spore former, protease and keratinase producer *B. stearothermophilus* strain was prepared as ghost cells using the minimum activity concentration of the hen white egg lysozyme [7]. Two protocols are summarized to enable better use for the lysozyme either in direct or emergence cases.

Protocol number one

Preparing Cell Ghosts from *B. Stearothermophilus* (Or from any Other Microbes)

- The microbial strain

Any pure bacterial strain can be used after preparing a suitable biomass under aseptic conditions [2, 3, 8-10, 7].

Native eggs white preparation

Native egg white can be gained from any of the bird eggs (in nature) or can be obtained from the local market.

- Eggs white preparation

A clean and healthy eggs were collected. Their surface are wet with ethanol by immersing them in the container containing 95% ethanol for 10 seconds (Amara 2016) or by any available disinfectant. The egg then transferred to clean and sterile Petri dish (without its cover) and then flamed. The ethanol (on the surface) enables surface flaming and sterilization without denaturing the egg's contents. The eggs then were taken under

aseptic conditions and broken in another sterile Petri dish. The egg white part was separated carefully without damaging its yolk. The white part then collected in sterile falcon tube and put in -80°C for at least one day or until their usage [7].

- Purified eggs white stock

Stock solution represent 15.99 mg/ml from the purified eggs white lysozyme is prepared in sterile distilled water. The solution then subjected to filtration using bacterial filter to get rid from any contaminant might contaminate the original purified lysozyme.

Protein Standard curve

Protein standard curve was generated by dissolving 19 mg of Bovine Serum albumin (BiowestTM) in 1.5 ml of distilled water. Then 10, 20, 30, 40 50, 75 and 100 µl of the stock were taken and transferred to sterile Eppendorf tube. Then each was completed to 1 ml using distilled water. The solution in each Eppendorf then mixed well. The absorbance of each sample then determined spectrophotometrically at 280 nm using quartz cuvette.

The obtained data after calculating the concentration of each sample against the obtained absorbance under the experimental condition were plotted.

- Determination of the minimum inhibition activity (MIA) and MGA of the native and purified enzyme

Standard serial dilution experiment was conducted to determine the MIA and the MGA of both of the native eggs white against the bacterial cells under investigation [7,9]. Five sterile test tubes were used. The tubes each contain sterile 4 ml of LB medium or any suitable medium. One ml from the white egg was added to the first tube. Then the first tube mixed well. After that serial dilution is conducted by transferring one ml from the first tube to the second one following by mixing their contents well then one ml is transferred to the third tube and mixed well and so on till the last tube. From the last tube one ml is discharged.

After that 20 µl of previously cultivated broth culture (LB) Which containing the bacterial cells under investigation was transferred to each test tube. The sample kept for two days at room temperature.

- MIA/MGA and MIC/MGC validation using viable cell test

Due to that lysozyme is protein in nature, it shows some turbidity upon their mixing with the used medium. For that an additional step was added to validate the data obtained from the serial dilution experiment. 10 µl of each test tube was transferred to the surface of NA or any suitable medium. The plates were incubated at 37°C (or any other suitable temperature) for overnight and the data is evaluated based on the existence or the absence of bacterial growth. In case of the presence of viable cells the experiment is repeated with higher amount of the lysozyme.

- Protein determination

10 µl of each test tube and from each stock was taken and completed to 1 ml water. The mixture absorbance (of each) was determined spectrophotometrically using the quartz cuvette at 280 nm. The amount of the lysozyme activity in each sample was calculated using the portion standard curve.

- DNA determination

10 µl of each sample was taken and completed to 1 ml and the mixture absorbance was determined spectrophotometrically using the quartz cuvette at 260 nm. The amount of the DNA is calculated based on the following formula [32, 36].

[An extension E260 = 1 corresponds to 50 µg dsDNA/ml]

- Samples from the prepared cells were taken and evaluated using light microscope for the quality of the cells.

- The lysozyme activity and the exposure time are adjusted if there is excess of the damaged cells (at 37°C).

- The prepared ghost free from any viable cells are preserved in suitable containers aiming to be used as a vaccine.

Note: Only microbiologist are able to adjust the preparation conditions safely.

Protocol Number Two

For Preparing Cell Ghosts from Contaminated Sample(S)

- Shack the sample which is collected perfectly from the infection site proposed to contain bacterial cells in sterile water or saline to be sure that the bacterial cells are located in the suspension. It can be used as it is, or one can add suitable selective medium to increase the microbial biomass

- Remove any course materials either by decantation or by tissue filtration. In some cases (specially in fastidious microbes) it is recommended to use the solutions where the original samples are obtained from.

- Collect eggs, and disinfect their surface in solution contains disinfectant (if it is evaluable). Or just wash their surface with water carefully.

- Break each egg cover carefully and collect the egg white without contaminating it with the egg yolk. Do that one by one. The egg white then transferred to clean and sterile container.

- According to Amara 2016, 23.41 mg/ml is enough to turn most microbes to ghosts after overnight incubation. However, increasing the dosage is recommended.

- If microscope is existed so it is recommended to observe the quality of the prepared ghosts. For the expert, one could know if the bacterial cells still existed or they are already lysed from their turbidity. And it is recommended to be sure that the microbes under investigation are completely dead by searching for the existence of any viable cells. In emergence cases excess of the white eggs can be used till complete death of any viable cells.

- If the microbes still viable, the treatment with eggs lysozyme should be repeated.

- For each microbe in a particular area a separate protocol should be optimized by experts. The increase in the turbidity is an indicator about the of microbial activity. Full instruction

should be written.

Discussion

This study proposed two protocols based on the results obtained from using the lysozyme in the hen egg white during preparing *B. stearothermophilus* as ghost cells [7]. Lysozyme is the favorite enzyme in the DNA preparation protocols from various bacterial cells [36]. It is successfully able to lysis most of the bacterial cell walls. It is existed naturally in the saliva and in the egg white and lysis every day millions of microbes. It is wise to use such natural phenomenon for our best. Amara et al. [8], succeeded to establish a protocol for preparing bacterial ghosts from *E. coli* using the MIC and the MGC of some chemical compounds [9]. And later the same concept was used by using the lysozyme (Amara 2016). This study re-introduces the idea of using the lysozyme and of course any other enzyme could degrade the bacterial cell walls or other genetic materials(using Dnases or RNases) in a form of protocol.

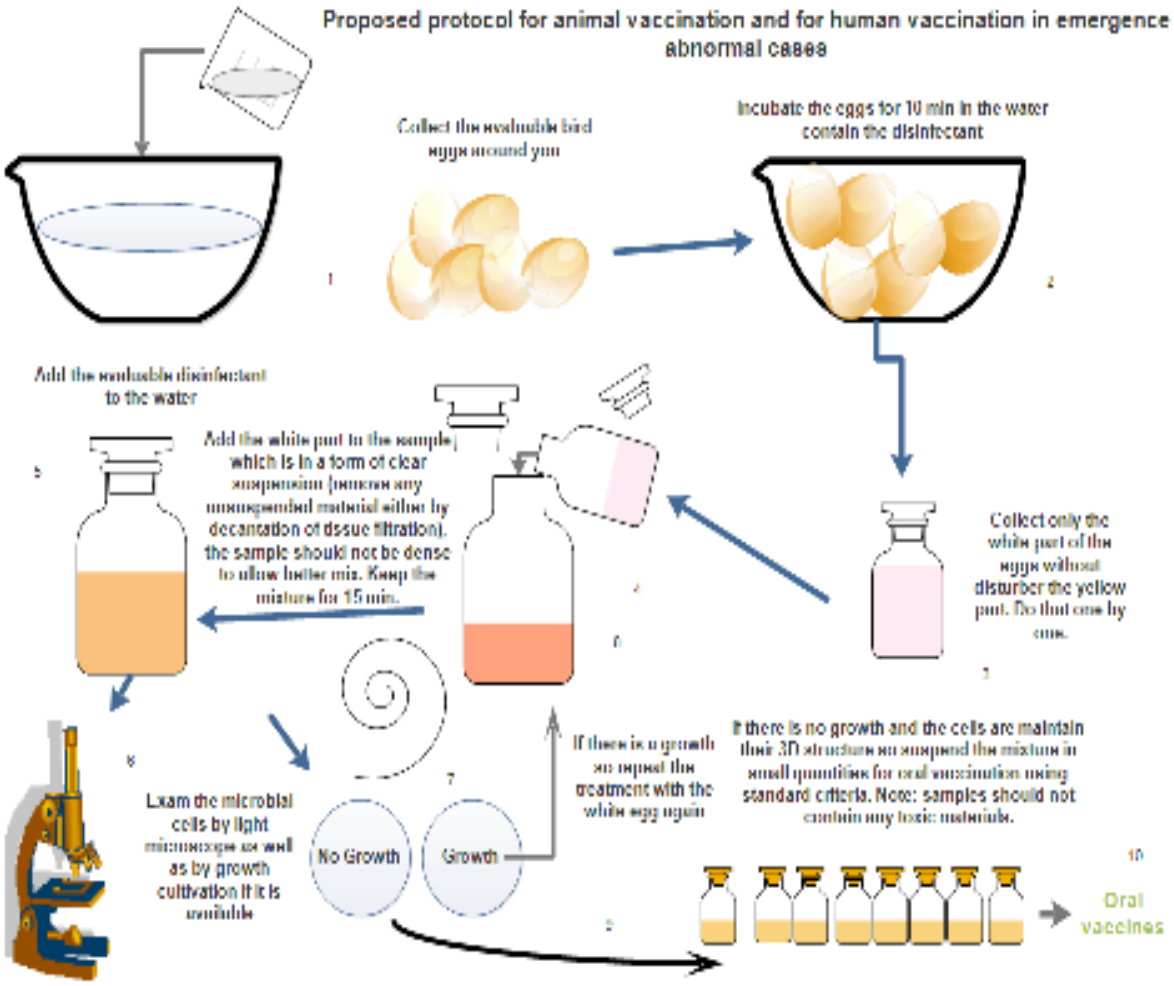

Figure 1: 1) Prepare the disinfectant solution and the water surface washed eggs; 2) Incubate for proper time (10 min) in disinfectant; 3) Collect the white eggs in a clean container; 4) Mix the white eggs with a clear filtrate contain the proposed pathogens, 5) Incubate for the propitiate time based on the cell density, the microbes MAC and MGC; 6) Test using light microscope; 7) Test for cell viability; 8) If there are viable cells repeat the treatment process and if the lysozyme lysis the cell completely dilute the concentration of the used lysozyme or shorten the exposure time; 9) If there is no viable cells so distribute the sample in quantities for oral vaccines. Note: be sure that the sample did not contain toxic materials other than the microbes and the used amount should be standardized by expert. In emergence case the protocol is flexible and the responsibility should be taken based on each case by the responsible person in the field.

The two proposed protocols were summarized and proposed for the specialist basically the microbiologist and the governments (Figure 1) to encourage them to apply such protocol (of course under control) in the areas where there are a need for excessive vaccinations in conditions where vaccines are not available. I am personally recommended reevaluating these protocols and standardize them for the proposed used microbes. The precautions which are well known by microbiologist should be followed. In fact ancient Egyptians and till now were using it to grow the Turkey. Young Turkey usually not survive in the Egyptian environment if not feed just after their hatching on a mixture of egg, cheese and the *Allium porrum*, a traditional folk mixture.

I recommended that local microbiologist can optimize this protocol for each local pathogen and summarizing their finding in steps. Good awareness with the microbiology aspects is recommended. It might be that this protocol can find acceptance and save a lot of our economic losses.

References

1. Amara, A. A. Opportunistic pathogens and their biofilm Food for thought. Science against microbial pathogens: communicating current research and technological advances. Microbiology Book Series 3, 2011.813-25.
2. Amara, A. A. Back to natural fiber: wool color influences its sensitivity to enzymatic treatment. Scientific World Journal. 2012; 4.35 6239. doi:10.1100/2012/356239
3. Amara, A. A. Back to natural fiber: wool color influences its sensitivity to enzymatic treatment. The Scientific World Journal.2012;4. doi:10.1100/2012/356239
4. Amara, A. A. Bacterial and Yeast Ghosts: *E. coli* and Saccharomyces cerevisiae preparation as drug delivery model ISIJ Biochemistry 4, 11-22.2015.
5. Amara, A. AKostenlos viral ghosts, bacterial ghosts' microbial ghosts and more. Schuling Verlag – Germany.2015.
6. Amara, A. A. *Saccharomyces cerevisia*e Ghosts Using the Sponge-Like Re-Reduced Protocol SOJ Biochem.2014; 4(1-4).doi:10.15226 /2376-4589/2/1/00107
7. Amara, A. A. "The critical activity for the cell wall degrading enzymes: Could the use of the lysozyme for Microbial Ghosts preparation establish emergence oral vaccination protocol?" International Science and Investigation journal.2016; 5(2):351-369.
8. Amara, A. A., Salem-Bekhit, M. M., and Alanazi, F. K. Preparation of bacterial ghosts for *E. coli* JM109 using sponge-like reduced protocol. Asian J Biol Sci.2013;6(8):363-369.doi:10.3923/ajbs.2013.363.369
9. Amara, A. A., Salem-Bekhit, M. M., and Alanazi, F. K. (2013b): Sponge-like: a new protocol for preparing bacterial ghosts. The Scientific World Journal.2013;7.doi:10.1155/2013/545741'
10. Amro, A. A., Neama, A. J., Hussein, A., Hashish, E. A., and Sheweita, S. A. Evaluation the Surface Antigen of the *Salmonella typhimurium* ATCC 14028 Ghosts Prepared by "SLRP". The Scientific World Journal.2014;6. doi:10.1155/2014/840863
11. Amro, A. A., Salem-Bekhit, M. M., and Alanazi, F. K. Plackett–Burman randomization method for bacterial ghosts preparation form *E. coli* JM109. Saudi Pharmaceutical Journal.2014;22(3):273-279. doi:10.1016/j.jsps.2013.06.002
12. Ansorge, I., Benting, J., Bhakdi, S., and Lingelbach, K. Protein sorting in Plasmodium falciparum-infected red blood cells permeabilized with the pore-forming protein streptolysin O. Biochem J.1996;315(1):307-314. Doi:10.1042/bj3150307
13. Arienti, G., Carlini, E., Laureti, S., Brunetti, P., and Santeusanio, F. Red blood cell ghosts are affected by adrenoleucodystrophy. Eur J Clin Invest.1996; 26(10):917-922.doi:10.1111/j.1365-2362.1996.tb02138.x
14. Basch, P. F. Development and behavior of cultured *Schistosoma mansoni* fed on human erythrocyte ghosts. Am J Trop Med Hyg.1984;33(5):911-917.doi:10.4269/ajtmh.1984.33.911
15. Bello, I. O., Qannam, A., Al-Zahrani, A., and AlDosari, A. Peripheral dentinogenic ghost cell tumor: report of a case and literature review. Int J Surg Pathol.2012;20(5):494-499. doi:10.1177%2F1066896911429299
16. Bonucci, E., Silvestrini, G., and di Grezia, R. Histochemical properties of the "crystal ghosts" of calcifying epiphyseal cartilage. Connect Tissue Res.1989; 22(1-4):43-50.discussion 53-61. doi:10.3109/03008208909114119
17. Boogaard, C., and Dixon, G. H. Red cell ghost-mediated microinjection of RNA into HeLa cells. I. A comparison of two techniques for the entrapment and microinjection of tRNA and mRNA. Exp Cell Res.1983;143(1):175-190.doi:10.1016/0014-4827(83)90119-2
18. Bowman, M. H., Ottolenghi, A. C., and Mengel, C. E. Effects of phospholipase C on human erythrocytes. J Membr Biol.1971; 4:156-164.
19. Buller, C. S. Phospholipase activity in bacteriophage-infected Escherichia. II. Activation of phospholipase by T4 ghost infection. J Virol.1975; 15(5):1141-1147.
20. Childs, J. D. Superinfection exclusion by incomplete genomes of bacteriophage T4. J Virol.1973; 11(1):1-8.
21. Cornett, J. B. Spackle and immunity functions of bacteriophage T4. J Virol.1974; 13(2):312-321.
22. Devi, K. R., Shyamasundari, K., and Rao, K. H. Histology and histochemistry of the intermoult integument in the ghost crab Ocypoda platytarsis (Milne-Edwards) (Crustacea: Brachyura). Arch Ital Anat Embriol.1991; 96(2):121-133.
23. Duckworth, D. H. Role of lysozyme in the biological activity of bacteriophage ghosts. J Virol.1969;3:92-94.
24. Duckworth, D. H. Inhibition of T4 bacteriophage multiplication by superinfecting ghosts and the development of tolerance after bacteriophage infection. J Virol.1971;7(1):8-14.1
25. Duckworth, D. H., and Bessman, M. J. Assay for the Killing Properties of T2 Bacteriophage and Their "Ghosts". J Bacteriol.1965; 90(8):724-728.
26. Duckworth, D. H., and Winkler, H. H. Metabolism of T4 bacteriophage ghost-infected cells. II. Do ghosts cause a generalized permeability change? J Virol.1972; 9(6):917-922.
27. Dyson, R. D. A procedure for the extraction of DNA and protein ghosts from bacteriophage lambda. Biochem Biophys Res Commun.1966;22(1-4):106-11.doi:10.1016/0006-291X(66)90610-3
28. Dyson, R. D., and van Holde, K. E. An investigation of bacteriophage lambda, its protein ghosts and subunits. Virology.1967;33(4):559-66.

doi:10.1016/0042-6822(67)90055-4

29. El-Baky, N. A., and Amara, A. A. Newcastle disease virus (LaSota strain) as a model for virus Ghosts preparation using H2O2 bio-critical concentration. International Science and Investigation journal.2014; 3(5):38-50.
30. Fejerskov, O., and Krogh, J. The calcifying ghost cell odontogenic tumor - or the calcifying odontogenic cyst. J Oral Pathol.1972; 1(6):273-287. doi:10.1111/j.1600-0714.1972.tb01666.x
31. French, R. C., and Siminovitch, L. The action of T2 bacteriophage ghosts on Escherichia coli B. Can J Microbiol.1955; 1(9):757-764. doi:10.1139/m55-090
32. Green, M. R., and Sambrook, J. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press New York.1989.
33. Gunhan, O., Mocan, A., Can, C., Kisnisci, R., Aksu, A. Y., and Finci, R. Epithelial odontogenic ghost cell tumor: report of a peripheral solid variant and review of the literature. Ann Dent.1991; 50(2):8-11, 48.
34. Hong, S. P., Ellis, G. L., and Hartman, K. S. Calcifying odontogenic cyst. A review of ninety-two cases with reevaluation of their nature as cysts or neoplasms, the nature of ghost cells, and subclassification. Oral Surg Oral Med Oral Pathol.1991; 72(1):56-64.doi:10.1016/0030-4220(91)90190-N
35. Lubitz, W., Witte, A., Eko, F. O., Kamal, M., Jechlinger, W., Brand, E., Marchart, J, e.tal. Extended recombinant bacterial ghost system. J Biotechnol.1999; 73(2-3):261-273.doi:10.1016/S0168-1656(99)00144-3
36. Sambrook, J., and Russell David, W. Molecular cloning: a laboratory manual. Vol. 3. Cold spring harbor laboratory press.1989.
37. Varadhachary, A., and Maloney, P. C. A rapid method for reconstitution of bacterial membrane proteins. Mol Microbiol.1990;4(8):1407-1411. doi:10.1111/j.1365-2958.1990.tb00720.x
38. Weibull, C. The nature of the ghosts obtained by lysozyme lysis of Bacillus megaterium. Exp Cell Res.1956; 10(1):214-221. doi:10.1016/0014-4827 (56)90087-8