Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.48.007638

Bacterial and Microbial Ghosts Preparation

Amro Abd Al Fattah Amara*

Department of Protein Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Universities and Research Centre District, New Borg El-Arab, Egypt

***Corresponding author:** Amro Abd Al Fattah Amara, Department of Protein Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Universities and Research Centre District, New Borg El-Arab, Egypt

ARTICLE INFO

Received: January 20, 2023

Published: January 31, 2023

Citation: Amro Abd Al Fattah Amara. Bacterial and Microbial Ghosts Preparation. Biomed J Sci & Tech Res 48(2)-2023. BJSTR. MS.ID.007638.

ABSTRACT

Microbial or cell evacuation is the science of evacuating microbes and other cells. There are mainly two common protocols for evacuating bacterial cells or microbial cells. The bacteriophage E lysis gene-based protocol and the Sponge-Like-Protocol. From their names the first protocol uses the bacteriophage E lysis gene to produce the E lysis protein inside the Gram-negative bacteria causing pore in the cell. The second protocol uses subcritical concentrations of some selected active chemical compounds to evacuate any kind of cells turning them to empty cells. It is able to evacuate prokaryotic, eukaryotic and Archie cells. Viruses are also evacuated and, in some applications, deactivated using the H2O2 bio-critical concentration. The evacuation process is able to remove the cytoplasm of the evacuated cells. In case of virus evacuation, it can degrade their genetic material keep it unable to infect cells or replicate inside it. Also, it keeps the cells, or the virus envelop 3D structure in correct forms. It keeps the surface antigens safe and able to activate the immune system. The absence of the cytoplasm and its contents is important for many applications. They include microbial cell ghost preparation, RNA and DNA removal or isolation, drug delivery, disinfectants, and the like. There is a misunderstanding for the concept of the Sponge-Like-Protocol. There is a mix between the minimum inhibition concentration and the minimum growth concentration. This review will revise some of the tactics used for cell evocation. It will describe the Sponge-Like Protocol in more detail. Additionally, it concerns with summarizing the main concept of the Sponge-Like Protocol aiming to a successful cell/microbe/virus ghost's preparation with correct surface antigens.

Abbreviations: BEC: Buccal Epithelial Cells; BG: Bacterial Ghosts; FT: Fowl Typhoid; GMT: Geometric Mean Titer; HCJE: Human Conjunctival Epithelial; MBP: Maltose Binding Protein; MGC: Minimum Growth Concentration; MIC: Minimum Inhibitory Consideration; OM: Outer Membrane; RITARD: Reversible Intestinal Tie Adult Rabbit Diarrhea; SDS: Sodium Dodecyl Sulfate; SG: Salmonella Gallinarum; SL: Sponge-Like; SLRP: Sponge-Like Reduced Protocol; TCP: Toxin-Coregulated Pili; VAG: Vibrio Anguillarum Ghosts; VCG: Vibrio Cholerae Ghosts; WCV: Whole-Cell Vibrio's

Introduction

The first sign about evacuating cells has been given to the ancient Egyptian. They evacuate the body of the dead person (mummy) from its organs. Then the whole body has been evacuated from its water content. Mummification is the process of embalming or artificially preserving lifeless bodies. Embalmers took delicate care to preserve it! The embalming skill hit the highest point during the New Kingdom era between 1738 and 1102 BC. The ancient Egyptians are known for their success in embalming the dead (AA Amara [1]). The mummies can be found in many museums around the entire world. The process varied from age to age in Egypt, but it always involving:

1) Drying the body from any humidity;

2) Preserving the body in a dry place.

3) Removing the internal organs;

4) Treating the body with resin;

5) Wrapping it in linen Bandages;

6) Finally, the body is isolated from the air by putting it in fixed closed containers.

DOI: 10.26717/BJSTR.2023.48.007638

It left dry, isolated from the light in an air-inaccessible chamber (Mummy, Encyclopedia Britannica 2013) (AA Amara [1]). Archaeologists have found evidence of a high degree of embalming skill in the burial chambers of the prehistoric Paraca Indians of Peru. The Guanches, aborigines of the Canary Islands, used methods much like those of the Egyptians, removing the internal organs and filling the cavity with salt and vegetable powders. The Jívaro tribes of Ecuador and Peru took, the additional precaution of ensuring the immortality of their chiefs is by roasting their embalmed bodies over very low fires. In Tibet some bodies are still embalmed according to an ancient formula: the corpse is put in a large box and packed in salt for about three months, after which it is in mummified condition. It is clear that the old civilization knew how to dry anything using the salt and by other practices (AA Amara [2]). It is Hooke who coined the word cell; in a drawing of the microscopic structure of cork, he showed walls surrounding empty spaces and refers to these structures as cells. He described similar structures in the tissue of other trees and plants and discerned that in some tissues the cells were filled with a liquid while in others they were empty (AA Amara [2]). Microbes are part of the nature, they dead in an ecological environment could somehow evacuate them from their cytoplasmic content. Ghosts are daily produced in our bodies (inside or outside). They are produced in the lung, in the stomach, in the surface of our skin, etc. In addition, they play roles in immunization. Natural ghosting phenomena in our bodies is playing certain roles in immunization.

Bacteriophage E lysis Gene for Bacterial Ghost Preparation

Phages are classified in a number of virus families, including Inoviridae and Microviridae. Phages are consisting of a core of genetic material (nucleic acid) surrounded by a protein capsid. The nucleic acid may be either DNA or RNA and maybe double stranded or single-stranded. The amino acids content of the E lysis gene sequence and for its nucleotides content as blow. From the day of the cloning of the bacteriophage E lysis gene, E protein has been expressed in different Gram-negative hosts and become a subject for improvement. The expression of cloned PhiX174 lysis E gene in Gram-negative bacteria results in lysis of the bacteria by formation of an E-specific transmembrane tunnel structure built through the cell envelope complex. Ghosts have a sealed periplasmic space and the export of proteins into this space vastly extends the capacity of ghosts or recombinant ghosts to function as carriers of foreign antigens. One important feature of the BGs that it contains at least one pore. That, of course, will enable loading drugs, DNA, plasmids, etc. to the cell. The E lysis gene during its activity introduces a hole in the bacterial envelope, which bursts the bacterial cells and the new bacteriophage progeny come out. The discovery of the heat sensitive promoter and regulator adds additional values to the process. The heat sensitive promoter and regulator enable better control for the process where, either the targeted bacteria are cultivated to its desire biomass using large flasks, bottles or fermentors then their temperature increased to be equal to the promoter activating temperature.

The increase in the temperature leads to the activation of the heat sensitive promoter. That causes the E lysis protein to be expressed and suitable amount of the lysis enzyme are produced which able to make a hole in the bacterial cells. E lysis protein exerts its lytic function by fusion of the inner and outer cell membranes (IM (inner-membrane) and OM (outer membrane)), forming a specific transmembrane tunnel structure. Through such a tunnel or poor, the bacterial cytoplasmic content is passing out. Such process enables the production of the BGs but there is a need for degrading the residue of the DNA, plasmid, etc., which still existed, or in better words still contaminated the opened bacterial cells. As the main weak point, such a process has been implemented to Gram-negative bacteria. Moreover, the produced bacterial cells might still contain residue, which effect on the quality of the produced cells and their expected applications.

Gene E Codes for 91 Amino Acids

Gene E: The amino acids content of the E lysis gene sequence of the Enterobacteria phage ϕX174 and for its nucleotides content were well sequenced as blow. One can obtain uncut phage DNA from the market and make cloning for the E lysis gene. Alternatively, one can isolate the phage and made DNA isolation then clone the E lysis gene.

E lysis Gene Nucleotides Sequence:

Atggtacgctggactttgtgggataccctcgctttcctgctcctgttgagtttattgctgccgtc attgcttattatgttcatcccgtcaacattcaaacggcctgtctcatcatggaaggcgctgaatttac ggaaaacattattaatggcgtcgagcgtccagttaaagccgctgaattgttcgcgcttaccttgcgt gtacgcgcaggaaacactgacgttcttgctgacgcagaagaaaacgtgcgtcaaaaattgcgtgc agaaggagtga

Amino Acids Sequence:

MVRWTLWDTLAFLLLLSLLLPSLLIMFIPSTFKRPVSSWKALNLRKT LLMASSVQLKPLNCSRLPCVYAQETLTFLLTQKKTCVKNCVQKE

ϕX174 Lysis E Gene and their Action in Gram-Negative Bacteria

The expression of the cloned ϕX174 lysis E gene in Gram-negative bacteria particularly in E coli results in lysis of bacteria by forming an E specific transmembrane tunnel structure built through the cell envelope complex (Eko, et al. [3-6]). The E lysis gene during its activity introduces a hole in the bacterial envelope, which bursts the bacterial cells and the new bacteriophage progeny come out. E lysis protein exerts its lytic function by fusion of the inner and outer cell membranes (IM and OM), forming a specific transmembrane tunnel structure. Through such tunnel or poor, the bacterial cytoplasmic content is passing out. The discovery of the heat sensitive promoter and regulator add additional values to the process. The heat sensitive promoter and regulator enable better control for the process where, either the targeted bacteria are cultivated to its desire biomass using large flasks, bottles or fermentors then their temperature increased to be equal to the promoter activating temperature. That increase leads to activating the heat sensitive promoter. That, cause the E lysis

protein to be expressed and suitable amount of the lysis enzyme are produced which enable pores in the bacterial cell wall. Through such pore(s), the cytoplasmic contents are come out.

Examples About Miscellaneous Successful Stories and the Used Tactics

Antigen-presenting cells have the capacity to stimulate specific T cells after the internalization and processing of Actinobacillus ghosts, as demonstrated by a strong specific T-cell response generated against the ghost antigens (Felnerova, et al. [7]). Pigs aerogenously immunized with genetically inactivated (ghosts) or irradiated Actinobacillus pleuropneumoniae are protected against a homologous aerosol challenge despite differing in pulmonary cellular and antibody responses (Katinger, et al. [8]). Pigs immunized with Actinobacillus pleuropneumoniae ghosts have been found to be protected against clinical disease in both vaccination groups, whereas colonization of the lungs with A. pleuropneumoniae has been prevented in ghost-vaccinated pigs (Huter, et al. [9]). The A. hydrophila ghosts (AHG) suggests that the bacterial ghost vaccine has higher potential to induce protective adaptive immunity than normal vaccine (Chu, et al. [10]). A method of fractionation of ghosts has been devised to identify the chemical components of the cytoplasmic membrane. The method consists of dialyzing the ghosts against distilled water, and then dissolving the ghosts in dilute alkali. The ghosts have been fractionated into four fractions by use of differential centrifugation (Yamaguchi, et al. [11]). The combination vaccine regimen comprising rVCG expressing chlamydial MOMP (*Chlamydia trachomatis*) and HSV-2 glycoprotein D (Herpes simplex virus type 2 (HSV-2)) delivered by recombinant Vibrio cholerae ghosts (rVCG) elicited adequate immune effectors that simultaneously protected against the individual pathogens (Macmillan, et al. [12]).

An efficacious vaccine is needed to control the morbidity and burden of rising healthcare costs associated with genital *Chlamydia trachomatis* infection. rVCG expressing chlamydial proteins may constitute a suitable subunit vaccine for inducing an efficient mucosal T cell response that protects against *C. trachomatis* infection. rVCG offer a significant technical advantage as a chlamydial vaccine (Eko, et al. [13]). The lack of genetic material in the oral EHEC bacterial ghost vaccine abolished any hazard of horizontal gene transfer of resistance genes or pathogenic islands to resident gut flora. Intragastric immunization of mice with EHEC ghosts without the addition of any adjuvant induced cellular and humoral immunity. It is possible to develop an efficacious single dose oral EHEC bacterial ghost vaccine (Mayr, et al. [14]). Immunization with *Escherichia coli* (EPEC) E2348/69 ghosts can elicit protective immune responses in BALB/c mice. EPEC ghosts represent a promising new approach for vaccination against EPEC infection (Liu, et al. [15]). Rod-shaped «ghosts» that are free of murein have been isolated from E. coli. The shape of these «ghosts» is maintained by a unit membrane soluble in sodium dodecyl sulfate (Henning, et al. [16]). The efficiency of internalization of BGs produced from probiotic *Escherichia coli* Nissle 1917 (EcN) by human conjunctival epithelial (HCjE) cell line, the EcN BGs cytotoxicity for HCjE cells, and *in vivo* uptake of EcN BGs by conjunctival guinea pig epithelial cells. The uptake of EcN BGs has been detected in the conjunctival cells after *in vivo* administration of EcN BGs into the eye of the guinea pig.

The findings that EcN BGs are nontoxic and effectively internalized *in vitro* by human and *in vivo* by guinea pig conjunctival cells comprise an important contribution to the future use of BGs as a system for conjunctival delivery of drugs and vaccines, either to treat or prevent ocular surface diseases (Stein, et al. [17]). BGs derived from E. coli NK9373 strongly induced the release of the pro-inflammatory cytokines IL-6 and IL-8 (Abtin, et al. [18]). BGs derived from wt E. coli NK9373 strongly induced the release of the pro-inflammatory cytokines IL-6 and IL-8 (Abtin, et al. [18]). Eight melanoma cell lines have been investigated for their capacity to bind and phagocyte BG derived from E. coli NM522 and *Mannheimia haemolytica* A23. High capability to bind BG has been observed in almost all of the analyzed cell lines, furthermore, cells have been able to independently take up BG of the used bacterial species. Further, transfection efficiency of BG loaded with DNA *in vitro* has been measured. The Bowes cells exhibited a high expression level of GFP and the incubation of cells with plasmid loaded BG led up to 82 % transfection efficiency (Kudela, et al. [19]). The introduced common brushtail possum (Trichosurus vulpecula) is a major pest in New Zealand and immunocontraceptive vaccines are being developed for biocontrol of possum populations, with BGs (BGs) being evaluated as a means of oral delivery. Recombinant BGs expressing possum zona pellucida 3 protein (ZP3) as an L' membrane anchored protein (ZP3-L') or as an S-layer SbsA-fusion protein (MBP-SbsA-ZP3) have been produced by the expression of the cloned bacteriophage phiX174 lysis gene E in E. coli NM522. (Walcher, et al. [20]).

ntragastric immunization of mice with E. coli O157:H7 ghosts without the addition of any adjuvant induced cellular and humoral immunity (Mayr, et al. [14]). BGs prepared from E. coli O26:B6 induce dose-dependent antibody responses against bacterial cells, or their corresponding lipopolysaccharides (LPS) show successful immunization in rabbits after applying a standard immunization protocol (Mader, et al. [21]). An EPEC reference strain E2348/69 (serotype O127:H6) has been transformed with the lysis plasmids to produce EPEC Ghosts. Vaccination trials showed that mice immunized with EGE or EGES have been significantly protected against subsequent challenges with the wildtype virulent parent strain, EPEC E2348/69 (42/50 and 45/50 survival, respectively); in contrast, none of the control mice survived. Immunization with EPEC Ghosts can elicit protective immune responses in BALB/c mice (Liu, et al. [22]). ETG have been generated via coexpression of the SNA gene and lysis gene E under the control of each lambda promoter (DJ Lee, et al. [10]). Most important, ETG-immunized mice have been significantly protected against *E. tarda* challenges (87. 7% survival) compared to 73. 3 and 33. 3% survival in the FKC-immunized and PBS-treated control, respectively, suggesting that an ETG oral vaccine

DOI: 10.26717/BJSTR.2023.48.007638

could confer protection against infection in a mouse model of disease (Wang, et al. 2009). Tilapia immunized with *E. tarda* ghosts (ETG) and formalin killed *E. tarda* (FKC) vaccines showed significantly higher serum agglutination titers than control fish (Kwon, et al. [23]).

Flavobacterium columnare is a bacterial pathogen causing high mortality rates for many freshwater fish species. These results showed that FCG could confer immune protection against *F. columnare* infection. As a non-living whole-cell envelope preparation, FCG may provide an ideal alternative to pathogen-based vaccines against columnaris in aquaculture (Zhu, et al. [24]). The S-layer gene sbsA from Bacillus stearothermophilus PV72 has been used for the construction of fusion proteins. Fusion of maltose binding protein (MBP) to the N-terminus of SbsA allowed the expression of the S-layer in the periplasm of *Escherichia coli*. The OM protein (Omp) 26 of NTHi has been inserted into the N-terminal and C-terminal regions of SbsA. Recombinant E. coli cell envelopes (ghosts) have been produced by the expression of SbsA/Omp26 fusion proteins prior to gene E-mediated lysis. Intraperitoneal immunization with these recombinant BGs induced an Omp26-specific antibody response in BALB/c mice (Riedmann, et al. [25]). BG vaccine generated from the *Haemophilus parasuis* serovar five reference strain Nagasaki has been prepared and used to inoculate piglets. The results showed that the piglets inoculated with the BG vaccine developed higher antibody activity and higher gamma interferon and interleukin four levels than those vaccinated with IB or those in the PC group after primary and secondary exposure to the antigens and challenge. CD4(+) T lymphocyte levels have been observed to increase following secondary immunization.

Higher CD4(+) T lymphocyte levels and both CD4(+) major histocompatibility complex class II-restricted Th1-type and Th2-type immune responses in the BG group are relevant for protection (Hu, et al. [26]). Prophylactic oral vaccination experiments using these *H. pylori* ghosts in the BALB/c mouse model showed a significant reduction of the bacterial load in the ghost group, as measured by a quantitative bacterial reisolation procedure (Panthel, et al. [27]). Generation of *H. pylori* ghosts as vaccine candidates and highlight the need to concentrate on alternative animal models and the use of fully virulent *H. pylori* type I strains for vaccination. An effective vaccine strategy against *H. pylori* has the potential to significantly improve population health worldwide (Hoffelner, et al. 2008). Coadministration of ghosts with Cholera toxins as mucosal adjuvant resulted in a complete protection of 10 of 10 and 8 of 8 mice against *H. pylori* challenge, with three animals showing a sterile immunity (Panthel, et al. [27]). BGs from *Mannheimia haemolytica* have been used for site-specific delivery of doxorubicin (DOX) to human colorectal adenocarcinoma cells (Caco-2). The *in vitro* release profile of DOX-ghosts demonstrated that the loaded drug has been non-covalently associated with the BGs and that the drug delivery vehicles themselves represent a slow-release system. Adherence studies showed that the M. haemolytica ghosts are more efficiently targeted the Caco-2 cells than E. coli ghosts, and released the loaded DOX within the cells (Paukner, et al. [28]).

Mass implementation of DNA vaccines is hindered by the requirement of high plasmid dosages and poor immunogenicity. The capacity of *Mannheimia haemolytica* ghosts as a delivery system for DNA vaccines. *In vitro* studies showed that BGs loaded with a plasmid carrying the green fluorescent protein-encoding gene (pEGFP-N1) are efficiently taken up by APC, thereby leading to high transfection rates (52-60%). BGs not only target the DNA vaccine construct to APC, but also provide a strong danger signal, acting as natural adjuvants, thereby promoting efficient maturation and activation of dendritic cells (Ebensen, et al. 2004). Eight melanoma cell lines have been investigated for their capacity to bind and phagocyte BG derived from *Escherichia coli* NM522 and *Mannheimia haemolytica* A23. High capability to bind BG has been observed in almost all of the analyzed cell lines, furthermore, cells have been independently able to take up BG of the used bacterial species. Further, transfection efficiency of BG loaded with DNA *in vitro* has been misused. The Bowes cells exhibited a high expression level of GFP and the incubation of cells with plasmid loaded BG led up to 82% transfection efficiency (Kudela et al. [19]). The translocation of energy-rich phosphate in Mycobacterium phlei protoplast ghosts by a soluble protein fraction (SH Lee, Kalra, et al. [29]). Ethambutol and rifampin resulted in more drastic alterations in the bacterial morphology than have been seen with any of the drugs used alone, leading to the removal of the bacterial outer layer, homogenization of cytoplasm, complete cell lysis, and formation of ghosts (Rastogi, et al. [30]).

Viridin B, a bacteriocin produced by Streptococcus mitis strain 42885, has been shown previously to exert a bactericidal effect against a Neisseria sicca strain but only a bacteriostatic effect against a coagulase-negative staphylococcus (Dajani, et al. [31]). Pasteurella ghosts have been used for immunization of rabbits and mice. Rabbits immunized subcutaneously with either *P. multocida* or *P. haemolytica* ghosts developed antibodies reacting with the immunizing strain, as well as with other Pasteurella strains. The number of proteins in whole-cell protein extracts recognized by the sera is constantly increased during the observation period of 51 days. In addition, dose-dependent protection against homologous challenge has been observed in mice immunized with *P. multocida*-ghosts (Marchart, et al. [32]). Protective immunization of cattle against homologous challenge has been induced by adjuvanted P. hemolytic ghosts. The level of protection has been similar to a commercially available vaccine (Marchart, et al. [32]). The binding of OM ghosts derived from Pseudomonas aeruginosa strain 492c to human buccal epithelial cells (BECs) has been examined. These sugar inhibition data demonstrated a difference in the binding of OM ghosts to trypsinized and untrypsinized BECs and possibly reveal the nature of the receptor(s), free of possible bacterial metabolic effects (Doig, et al. [33]). Synovial fibroblasts have been infected with *Yersinia enterocolitica* or *Salmonella enterica* serovar Enteritidis and analyzed by electron microscopy and fluorescence in situ hybridization.

Intracellular bacterial replication has been followed by degradation leading to «ghosts» possessing lipopolysaccharides but not DNA. However, single bacteria survived for more than 2 weeks. Therefore, transient intra-articular infection might be the missing link between initial intestinal infection and late synovial inflammation in the pathogenesis of reactive arthritis (Meyer-Bahlburg, et al. [34,5]). The formation of the Salmonella gallinarum ghost with tunnel formation and loss of cytoplasmic contents has been observed by scanning electron microscopy and transmission electron microscopy. The safety and protective efficacy of the Salmonella gallinarum ghost vaccine has been tested in chickens that have been divided into four groups: group A (non-immunized control), group B (orally immunized), group C (subcutaneously immunized) and group D (intramuscularly immunized). The birds have been immunized at day seven of age. None of the immunized animals showed any adverse reactions such as abnormal behavior, mortality, or signs of FT such as anorexia, depression, or diarrhea ***(Chaudhari, et al. [35]). A safety enhanced Salmonella gallinarum (SG) ghost has been constructed using an antibiotic resistance gene free plasmid and evaluated its potential as fowl typhoid (FT) vaccine candidate. The antibiotic resistance free pYA3342 plasmid possesses aspartate semialdehyde dehydrogenase gene which is complimentary to the deletion of the chromosomal asd gene in the bacterial host.

The results provide a promising approach of generating SG ghosts using the antibiotic resistance free plasmid in order to prepare a non-living bacterial vaccine candidate which could be environmentally safe yet efficient to prevent FT in chickens (Jawale, et al. [35]). HIV vaccine delivery using Salmonella typhi Ty21a bacterial ghosts (BGs) prove that Ty21a BGs loaded with an HIV gp140 DNA vaccine (Ty21a BG-DNA) have been readily taken up by murine macrophage RAW264.7 cells, and gp140 has been efficiently expressed in these cells. Peripheral and intestinal mucosal anti-gp120 antibody responses in mice vaccinated with BGs-DNA vaccines have been significantly higher than those in mice immunized with naked DNA vaccine (Zhu, et al. 2012). BGs prepared from *Escherichia coli* O26:B6 and Salmonella typhimurium C5 induce dose-dependent antibody responses against bacterial cells or their corresponding lipopolysaccharides (LPS). *In vitro* systems can be used as easy predictive test systems for preparations of bacterial vaccines, particularly for BGs (Mader, et al. [21]) (Michael P Szostak, et al. [36]). Recently, BGs have been prepared using a protocol based on critical chemical concentrations. It has been given the name «sponge-like» (SL) protocol and used in its reduced form «sponge-like reduced protocol» (SLRP). While specific antibody for Salmonella is available on the market under the commercial names (of some kits) such as Febrile Antigen Kit (N.S. BIO-TEC), the described Kit has been used to investigate the validity of the SLRP.

The antigen-antibody interaction (agglutination) results of both the SLRP and the animal experiments prove that correct STGs able to immunize the rats against viable Salmonella have been obtained. STGs could be used as vaccine and as adjuvant and in the antibodies and in the diagnostic kits production. This study is an additional step for the establishment of correct BGs for immunological purposes (Amro, et al. [37]). BGs prepared from *Escherichia coli* O26:B6 and Salmonella typhimurium C5 induce dose-dependent antibody responses against bacterial cells or their corresponding lipopolysaccharides (LPS) in doses 25 ng kg-1 when administered intravenously to rabbits in a standard immunization protocol. The endotoxic activity of the bacterial preparations analyzed by a standard limulus amoebocyte lysate and 2-keto-3-deoxyoctonate assay correlated with the capacity to stimulate the release of PGE2 and TNF alpha in RAW mouse macrophage cultures and the endotoxic responses in rabbits. It can be concluded that these *in vitro* systems can be used as easy predictive test systems for preparations of bacterial vaccines, particularly for BGs (Mader, et al. [21]). The introduced common brushtail possum (Trichosurus vulpecula) is a major pest in New Zealand and immunocontraceptive vaccines are being developed for biocontrol of possum populations, with BGs being evaluated as a means of oral delivery. Recombinant BGs expressing possum zona pellucida 3 protein (ZP3) as an L' membrane anchored protein (ZP3-L') or as an S-layer SbsA-fusion protein (MBP-SbsA-ZP3) have been produced by the expression of the cloned bacteriophage phiX174 lysis gene E in E. coli NM522 (Cui, et al. [20]).

The N-terminal (amino acid residues 41-316, ZP2N) and C-terminal (amino acid residues 308-636, ZP2C) regions of possum ZP2 have been fused to maltose binding protein and expressed in the periplasmic space of *Escherichia coli* NM522 bacterial ghosts. Bacterial ghosts containing possum ZP antigens can reduce possum fertility when delivered by mucosal immunization and offer a promising delivery system for fertility control of wild possum populations (Walcher, et al. [20]). The immunogenic potential of *Vibrio cholerae* ghosts (VCG) in comparison with heat-killed whole-cell vibrios (WCV) has been evaluated after intraperitoneal immunization of adult mice. The immunogenicity of VCG in evoking serum IgG responses has been higher than that of WCV. However, the immunogenicity of the two antigen preparations has been comparable in terms of seroconversion for vibriocidal antibodies. These results demonstrate that VCG administered intraperitoneally evoke Vibrio-specific serum IgG responses as well as vibriocidal antibody activity in mice (Eko, et al. [38]). Ghosts were prepared from *Vibrio cholerae* strains of O1 or O139 serogroup after growth under culture conditions, which favor or repress the production of toxin-coregulated pili (TCP) (Eko, et al. [39]). Ghosts were prepared from V. cholerae O1 or O139 and evaluated as vaccines in the reversible intestinal tie adult rabbit diarrhea (RITARD) model. Rabbits were orally immunized with different doses of V. cholerae ghost (VCG) formulations. The vaccine formulations elicited high levels of serum vibriocidal titers against indicator strains.

The magnitude of the response has been measured as the geometric mean titer (GMT) increase for all rabbits in relation to prevaccination titers. The induction of cross protection has been evidenced by the ability of serum from VCG-immunized rabbits to mediate complement-dependent killing of both the homologous

and the heterologous strains. Immunized rabbits were protected against intraduodenal challenge 30 days after primary immunization. Protective immunity against challenge appeared to be dose-dependent and has been associated with marked inhibition of colonization. These results indicate that VCGs represent a novel approach to cholera vaccine development and constitute an effective vaccine delivery vehicle (Eko, et al. [13]). Vibrio anguillarum ghosts (VAG) were generated, for the first time, using a conjugation vector containing a ghost bacteria inducing cassette, pRK-lambdaP(R)-cI-Elysis, in which the expression of PhiX174 lysis gene E has been controlled by the P (R)/cI regulatory system of lambda phage. By scanning electron microscopy, holes ranging 80-200 nm in diameter were observed in the VAG (Kwon, et al. [40]). *Yersinia enterocolitica* O:3 has been maintained in primary cultures of human synovial cells for 6 weeks as cultivable organisms and thereafter for 2 more weeks as antigen aggregates containing specific lipopolysaccharides (LPS). Some seemingly intact bacteria were «ghosts,» bacterial rods possessing LPS but not DNA. The prolonged persistence of Yersiniae, and its antigens, in synovial cells may be the cause of the maintenance of the inflammatory host responses in the joints of patients with reactive arthritis due to Yersinia infection (Huppertz, et al. [41]).

Synovial fibroblasts were infected with *Yersinia enterocolitica* or *Salmonella enterica* serovar Enteritidis and analyzed by electron microscopy and fluorescence in situ hybridization. Intracellular bacterial replication has been followed by degradation leading to «ghosts» possessing lipopolysaccharides but not DNA. However, single bacteria survived for more than 2 weeks. Therefore, transient intra-articular infection might be the missing link between initial intestinal infection and late synovial inflammation in the pathogenesis of reactive arthritis (Meyer-Bahlburg, et al. [34]).

Sponge-Like Protocol for BGs' Preparation

In 2013, and for the first time a protocol based on calculating the critical concentration of some chemical compounds that effect on the cell walls of the bacteria and on their genetic material has been proposed (Amro A Amara, et al. [37]). In 2013 three studies were generated on E. coli JM109 and BL21 (one published later) (Amro A Amara, et al. [42-44]). A new method for bacterial and microbial ghosts' preparation has been given the name «SL Protocol» has been published in 2013. It has been succeeded to empty *Escherichia coli* BL21 and JM109 cells from their cytoplasm content turning them to ghost cells with correct 3D structure (AA Amara, et al. [41-45]). The basic main breakthrough is the success of using the critical concentration of some cheap and available chemical compounds to introduce pores in the bacterial cell wall. The first used compounds are sodium dodecyl sulfate (SDS), NaOH, $CaCO_3$ and H_2O_2. SDS, NaOH and H_2O_2 are active compounds that influence cell macromolecules. $CaCO_3$ increases the cell wall rigidity and allows the entrance of these compounds into the cell. The minimum inhibitory consideration (MIC) and minimum growth concentration (MGC) of each of the used chemical compound were obtained from the serial dilution method against the targeted microbe. And the $CaCO_3$ used concentration has been calculated from the E. coli combatant cells preparation method. The MIC and the MGC for each compound has been calculated.

To develop the pore formation and the cytoplasm removal, the used chemicals have been applied in sufficient time and shaking has been used. In addition, successive washing steps with 0.5% NaCl and 60% Ethanol have been processed to ensure the complete evacuation of the bacterial cells 60% ethanol is able to extract many of the cytoplasmic components particularly the salts and any genomic residues. Furthermore, a mild centrifugation process has been used to gently press the cells. To do extra optimization for the process the Plackett-Burman design 12 experiments have been conducted with variable randomization to obtain the best condition for cell evacuation without the deterioration of the cell wall. The quality of the cells has been observed using a light microscope. From the beginning the used cells have been aged for seven days in broth medium to ensure good cell wall rigidity. The MIC is considered as the +1 (while it is higher in the chemical compounds concentration) and the MIC consider as the -1 (while it is lower in the chemical compounds concentration) in the design. The first design has been applied to evacuate the E. coli BL21 which harbors a plasmid caring the lysozyme gene under the chloramphenicol promoter (Amro A Amara, et al. [37]). During the first trial, the result has been promising where out of the 12 experiment only a single colony in one trial has been survived and still viable. All the cells in this experiment has been subjected to the lysis protocol by inducing the expression of the lysozyme using the chloramphenicol antibiotic.

The experiment has been repeated in another E. coli the E. coli JM109. Again the protocol shows successes without any viable colony but only with evacuated cells. An interesting point is even the two strains (BL21 and JM109) are related to the E. coli species but they show different MIC and MGC which prove the sensitivity of the protocol (AA Amara, et al. [43-46]). It can also be used to differentiate between two different but close in similarity bacterial strains. An additional handy experiment has been conducted by monitoring the released DNA and protein from the evacuated cells using the spectrophotometer at 260 and 280 nm respectively. Moreover, the agarose gel electrophoresis has been used to prove or disprove the removing of the DNA as an indicator for the removing of the other components. To ensure that all still existed DNA are tested an additional slot lysis protocol has been used to ensure the lysis of any cells that still not subjected to the evacuation process. The result has been more than excellent and agree with that obtained from the light microscope. After being sure that ghost cells have been well prepared, samples have been taken to the electron microscope for a better visualization and validation for the cells 3D structure. Special tactic has been used to ensure that the process of the investigation using the electron microscope did not change the cell 3D structure (data not shown).

The third paper has been concerned with reducing the original protocol to be more accessible for the scientists, particularly those who are not familiar with the experimental design like the Plackett-Burman (Amro A Amara, et al. [42]). In the same year the surface

 DOI: 10.26717/BJSTR.2023.48.007638

antigens of the Salmonella typhimurium ATCC 14028 ghost cells have been proved to be unaffected by the protocol (Amro, et al. [47]). Additionally, the first evacuated virus has been published (N Abd El-Baky, et al. [48]). At 2015 and the following years great international progress and acceptance have been remarked. In 2015 a full book has been published with most of the ideas and the protocols used to design the Sponge-Like Protocol (AA Amara, et al. [49]). The book gives more space for in detail description. Some are following the protocol precisely while others used one or more of its ideas such as performing microbial deactivation (Vinod, et al. [50]). Generally, the ideas described in the original protocol have been used either for preparing real evacuated microbial cells/viruses, or deactivating cells/viruses or even for strains differentiation (N Abd El-Baky, et al. [48,42,47,51,50]). The critical steps have been summarized in (Figure 1). The idea of introducing the 'cell evacology' as a separate new science has been proposed. Microbes like yeast, fungi, and mushrooms are evacuated (N Abd El-Baky, et al. [48,52,49,53]).

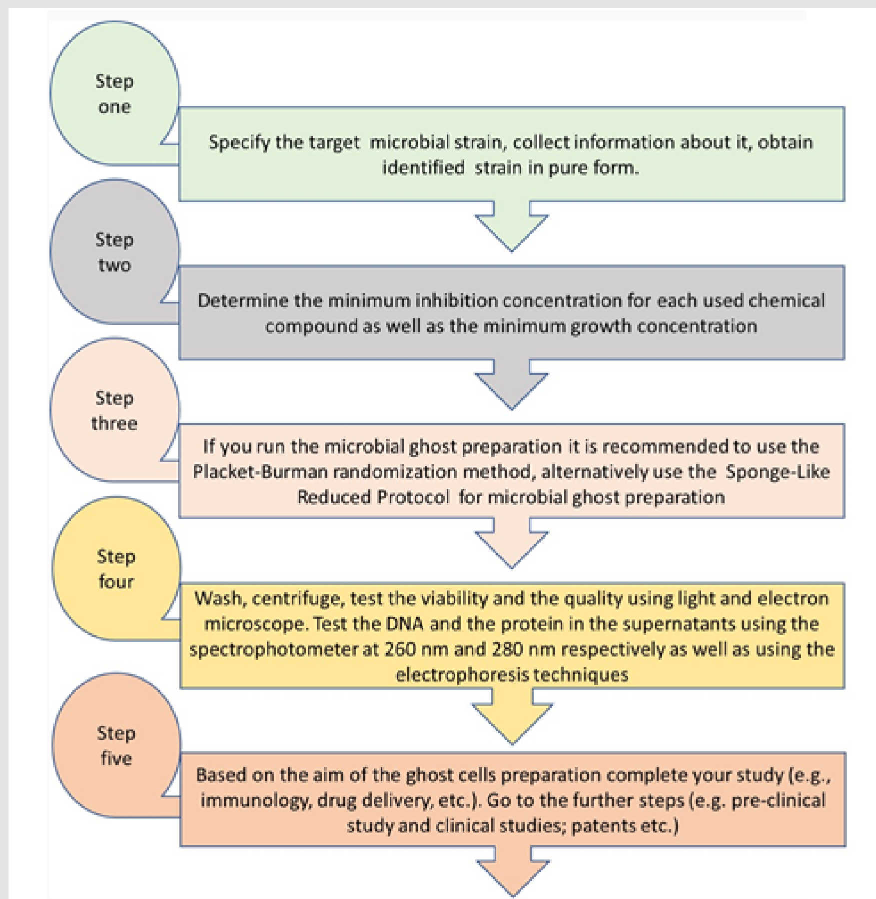

Figure 1: The Most Important Steps Concerning the SL-Protocol for Bacterial Ghosts Preparation.

None of the many investigated microbes is resisting the evacuation processes following the main protocol. The protocol is not covered by patent and is introduced to be free for anyone. Additionally, proteins that affect the cell wall have been used with the same concept; where their critical activities, which cause evacuation without degrading the cell wall, have been used. The minimum inhibition or the MGCs/activities of the used chemicals or proteins are successfully control all the investigated microbes (N Abd El-Baky, et al. [54,44]). In particular cases such as in the case of evacuating a virus where no cell wall is existed; the bio-critical concentration of H2O2 which calculated during the evacuation of the E. coli succeeded to degrade the virus genetic materials (N Abd El-Baky, et al. [48]). All such ideas have been described in nearly 64 publications which have been published from 2013 until 2023 beside a book published in 2015 (AA Amara, et al. [1]). Many students overall the world obtain their MSc or PhD from Alexandria University and other universities in another places are become familiar with such protocol and their own work are published and become available (Batah, et al. [51]). After many efforts the protocol become recognized globally and many become well familiar with its details. Besides being fast and effective, it is inexpensive. The future might show a more perfect chemical compounds or a strong modification in the protocol but the concept should be given to the original six used chemical compounds (to gentle evacuating the bacterial cells from their cytoplasmic content). The SL protocol will

DOI: 10.26717/BJSTR.2023.48.007638

be able to prepare nearly any cell as ghost. for that it is a universal protocol for cell evacuation. Bacterial, microbial, viruses' and cells' ghosts expected to stand-alone as a separate scientific filed.

The Misunderstand to the SL-Protocol

Apparently, there are some misunderstood for the concept of the SL-Protocol can be found in the literatures (data not shown). The protocol did not use the minimum inhibition concentration of the NaOH to prepare the E. coli ghosts but instead it uses the MGC which is diluted ten time. During the Plackett-Burman experiment one should use two different variables level to optimize the randomization [55]. For each variable the first protocol uses +1 which mean the MIC and the -1 which mean the MGC which both have been calculated from the serial dilation method. The MIC mean the concentration which responsible for the death of the microbes. Alternatively, the MGC is the first concentration which follow the MIC. It is of great importance to use the MGC with the chemical compounds that prove to be affect on the microbial cell wall. For more details, kindly read the first four published paper about the SL Protocol. Some authors have used higher concentrations than that used in the Sponge-Like Protocol which of course will lead to inactivation of the microbial cells and might cause evacuation but finally will not be excepted to be used in immunization purposes. However, such higher dosage could be beneficial and used in purposes where the microbial cells are used in another application (e.g. drug delivery system, nanotechnology applications, and the like). One who did not familiar with the Plackett-Burman experiment is invited to use the SL-Reduced protocol which has been tested and simplified for this purposes. By revising this protocol, one could observe that NaOH did not used in the level of the MIC but in the MGC level.

Additionally, the SL Protocol has recommended the most successful two experiments which have been actually equal to the best two one in the original protocol [56]. Furthermore, the reduced protocol has recommended two experiment represented in the following concentration for the used chemical compounds: Experiment one (NaOH -1, CaCO2 +1, H2O2 +1 and SDS +1) the second experiment used more diluted concentrations: Experiment two (NaOH -1, CaCO2 -1, H2O2 -1 and SDS +1). Only SDS has been used in the obtained MIC level. One should conduct those two experiments with the recommended used physical conditions and after that investigate the quality of the ghost cells. Again the MGC is diluted ten time and it is the concentration which show correct ghost cells with correct 3D structure and later has been used with all the experiments that used later to prepare the bacterial and the microbial ghosts. For more details, refer to the different publications using the SL Protocol and the reduced protocol for preparing microbial ghosts by our scientific group. In our scientific group the reduced protocol show success with the bacteria, but during turning yeasts, fungi and the spore of the mushrooms. NaHCO3 has been used instead of the CaCO2 from a personal experiences based on the previous work on the Spirulina platansis, where NaHCO3 eradicate all prokaryotic and eukaryotic microbes in the nature but only Spirulina platensis could be surviving. The Sponge-Like Protocol did not recommend a fixed experiment or universal one but it introduce a concept that the MIC and the MGC could introduce pores to evacuate microbes and one should change the concentration, the condition as recommended to get the points between the MIC and the MIC or one of them that satisfy the demand of their experiment.

For that, in case of the spore forming bacteria the lysozyme has been used following the same protocol to evacuate The critical activity for lysozyme (native or purified) as cell wall degrading enzymes has been calculated and the bio-critical concentration of the H2O2 that succeed to evacuate the E. coli has been used to evacuate the Newcastle virus.

Conclusion

Microbial ghosts are produced naturally and during our activities. Many tactics have been used to evacuate the microbial cells. Meanwhile there is two methods that have been designed to produce bacterial or microbial ghosts that can be used in immunization. The first on is concerned with the use of the bacteriophage E lysis gene to turn the gram negative bacteria to ghost cells upon their activation in the correct time. The other one is named the Sponge-Like protocol which is based on the use of the critical concentration of some chemical compounds or the critical activity of some enzymes to produce ghost cells with correct 3D structure and surface antigens. The effort that have been used by the researchers concerning the bacterial and the microbial ghosts not only succeeded in the production of evacuated cells that could be immunize but have been opened the get to many other applications like the drug delivery, the microbial taxonomy (based on different cell wall responses to the used chemicals), the plant pathogens control, the structure analysis and the like. The future of the microbial ghosts is promising particularly in infection control and vaccine production.

Acknowledgment

The authors acknowledge and thanks from his hart all his family members, partners, students, researchers, and any members has by any mean help him from 2013 till now to introduce the BGs which become "microbial ghosts" to the world and to the scientific community. All those who their names are included in the research papers, reviews, letter to editors and the like concerning this protocol. And all of those who do works but their data did not publish yet and anyone by any mean support him from 2013 till nowadays. Many thanks.

Conflict of Interest

The author declares that there is no conflict of interest.

References

1. Amara AA (2015) Kostenlos viral ghosts, bacterial ghost's microbial ghosts and more. Schuling Verlag - Germany.

2. Amara AA (2015) Kostenlos Viral Ghosts, Bacterial Ghosts, Microbial Ghosts and More. Schüling Verlag Munster Germany.
3. Eko FO, Witte A, Huter V, Kuen B, Fürst-Ladani S, et al. (1999) New strategies for combination vaccines based on the extended recombinant bacterial ghost system. Vaccine 17(13-14): 1643-1649.
4. Haidinger W, Szostak MP, Jechlinger W, Lubitz W (2003) Online monitoring of *Escherichia coli* ghost production. Appl Environ Microbiol 69(1): 468-474.
5. Szostak MP, Mader H, Truppe M, Kamal M, Eko FO, et al. (1997) Bacterial ghosts as multifunctional vaccine particles. Behring Inst Mitt (98): 191-196.
6. Witte A, Wanner G, Sulzner M, Lubitz W (1992) Dynamics of PhiX174 protein E-mediated lysis of *Escherichia coli*. Arch Microbiol 157(4): 381-388.
7. Ebensen T, Paukner S, Link C, Kudela P, de Domenico C, et al. (2004) Bacterial Ghosts Are an Efficient Delivery System for DNA Vaccines. The Journal of Immunology 172(11): 6858-6865.
8. Katinger A, Lubitz W, Szostak MP, Stadler M, Klein R, et al. (1999) Pigs aerogenously immunized with genetically inactivated (ghosts) or irradiated Actinobacillus pleuropneumoniae are protected against a homologous aerosol challenge despite differing in pulmonary cellular and antibody responses. J Biotechnol 73(2-3): 251-260.
9. Huter V, Hensel A, Brand E, Lubitz W (2000) Improved protection against lung colonization by Actinobacillus pleuropneumoniae ghosts: characterization of a genetically inactivated vaccine. J Biotechnol 83(1-2): 161-172.
10. Lee DJ, Kwon SR, Zenke K, Lee EH, Nam YK, et al. (2008) Generation of safety enhanced Edwardsiella tarda ghost vaccine. Diseases of Aquatic Organisms 81: 249-254.
11. Yamaguchi T, Tamura G, Arima K (1967) Substructure of the cytoplasmic membrane of Bacillus megaterium. I. Method for the fractionation of "Ghosts". J Bacteriol 93(1): 483-489.
12. Macmillan L, Ifere GO, He Q, Igietseme JU, Kellar KL, et al. (2007) A recombinant multivalent combination vaccine protects against Chlamydia and genital herpes. FEMS Immunol Med Microbiol 49(1): 46-55.
13. Eko FO, Lubitz W, McMillan L, Ramey K, Moore TT, et al. (2003) Recombinant Vibrio cholerae ghosts as a delivery vehicle for vaccinating against *Chlamydia trachomatis*. Vaccine 21(15): 1694-1703.
14. Mayr UB, Haller C, Haidinger W, Atrasheuskaya A, Bukin E, et al. (2005) Bacterial ghosts as an oral vaccine: a single dose of *Escherichia coli* O157:H7 bacterial ghosts protects mice against lethal challenge. Infect Immun 73(8): 4810-4817.
15. Liu J, Wang WD, Liu YJ, Liu S, Zhou B, et al. (2012a) Mice vaccinated with enteropathogenic *Escherichia coli* ghosts show significant protection against lethal challenges. Lett Appl Microbiol.
16. Henning U, Rehn K, Hoehn B (1973) Cell envelope and shape of *Escherichia coli* K12. Proc Natl Acad Sci U S A 70(7): 2033-2036.
17. Stein E, Inic-Kanada A, Belij S, Montanaro J, Bintner N, et al. (2013) *In vitro* and *in vivo* uptake study of *Escherichia coli* Nissle 1917 bacterial ghosts: cell-based delivery system to target ocular surface diseases. Invest Ophthalmol Vis Sci 54(9): 6326-6333.
18. Abtin A, Kudela P, Mayr UB, Koller VJ, Mildner M, et al. (2010) *Escherichia coli* ghosts promote innate immune responses in human keratinocytes. Biochem Biophys Res Commun 400(1): 78-82.
19. Kudela P, Paukner S, Mayr UB, Cholujova D, Kohl G, et al. (2008) Effective gene transfer to melanoma cells using bacterial ghosts. Cancer Lett 262(1): 54-63.
20. Walcher P, Cui X, Arrow JA, Scobie S, Molinia FC, et al. (2008) Bacterial ghosts as a delivery system for zona pellucida-2 fertility control vaccines for brushtail possums (*Trichosurus vulpecula*). Vaccine 26(52): 6832-6838.
21. Mader HJ, Szostak MP, Hensel A, Lubitz W, Haslberger AG (1997) Endotoxicity does not limit the use of bacterial ghosts as candidate vaccines. Vaccine 15(2): 195-202.
22. Liu J, Wang WD, Liu YJ, Liu S, Zhou B, et al. (2012b) Mice vaccinated with enteropathogenic *Escherichia coli* ghosts show significant protection against lethal challenges. Letters in Applied Microbiology 54(3): 255-262.
23. Kwon SR, Nam YK, Kim SK, Kim KH (2006) Protection of tilapia (*Oreochromis mosambicus*) from edwardsiellosis by vaccination with Edwardsiella tarda ghosts. Fish & Shellfish Immunology 20(4): 621-626.
24. Zhu W, Yang G, Zhang Y, Yuan J, An L (2012) Generation of Biotechnology-Derived Flavobacterium columnare Ghosts by PhiX174 Gene E-Mediated Inactivation and the Potential as Vaccine Candidates against Infection in Grass Carp. Journal of Biomedicine and Biotechnology 2012: 1-8.
25. Riedmann EM, Kyd JM, Smith AM, Gomez-Gallego S, Jalava K, et al. (2003) Construction of recombinant S-layer proteins (rSbsA) and their expression in bacterial ghosts–a delivery system for the nontypeable Haemophilus influenzae antigen Omp26. FEMS Immunol Med Microbiol 37(2-3): 185-192.
26. Hu M, Zhang Y, Xie F, Li G, Li J, et al. (2013) Protection of piglets by a *Haemophilus parasuis* ghost vaccine against homologous challenge. Clin Vaccine Immunol 20(6): 795-802.
27. Panthel K, Jechlinger W, Matis A, Rohde M, Szostak M, et al. (2003) Generation of Helicobacter pylori ghosts by PhiX protein E-mediated inactivation and their evaluation as vaccine candidates. Infect Immun 71(1): 109-116.
28. Paukner S, Kohl G, Lubitz W (2004) Bacterial ghosts as novel advanced drug delivery systems: antiproliferative activity of loaded doxorubicin in human Caco-2 cells. Journal of Controlled Release 94(1): 63-74.
29. Lee SH, Kalra VK, Brodie AF (1974) The translocation of energy-rich phosphate in Mycobacterium phlei protoplast ghosts by a soluble protein fraction. J Biol Chem 249(24): 8048-8050.
30. Rastogi N, Labrousse V (1991) Extracellular and intracellular activities of clarithromycin used alone and in association with ethambutol and rifampin against Mycobacterium avium complex. Antimicrobial Agents and Chemotherapy 35(3): 462-470.
31. Dajani AS, Law DJ, Bollinger RO, Ecklund PS (1976) Ultrastructural and biochemical alterations effected by viridin B, a bacterocin of alpha-hemolytic streptococci. Infection and Immunity 14(3): 776-782.
32. Marchart J, Dropmann G, Lechleitner S, Schlapp T, Wanner G, et al. (2003) Pasteurella multocida- and Pasteurella hemolytic ghosts: new vaccine candidates. Vaccine 21(25-26): 3988-3997.
33. Doig P, Franklin AL, Irvin RT (1986) The binding of Pseudomonas aeruginosa outer membrane ghosts to human buccal epithelial cells. Can J Microbiol 32(2): 160-166.
34. Meyer-Bahlburg A, Brinkhoff J, Krenn V, Trebesius K, Heesemann J, et al. (2001) Infection of synovial fibroblasts in culture by *Yersinia enterocolitica* and *Salmonella enterica* serovar Enteritidis: ultrastructural investigation with respect to the pathogenesis of reactive arthritis. Infect Immun 69(12): 7915-7921.
35. Jawale CV, Chaudhari AA, Lee JH (2014) Generation of a safety enhanced Salmonella Gallinarum ghost using antibiotic resistance free plasmid and its potential as an effective inactivated vaccine candidate against fowl typhoid. Vaccine 32(9): 1093-1099.
36. Szostak MP, Hensel A, Eko FO, Klein R, Auer T, et al. (1996) Bacterial ghosts: non-living candidate vaccines. Journal of Biotechnology 44(1-3): 161-170.
37. Amro AA, Neama AJ, Hussein A, Hashish EA, Sheweita SA (2014a) Evaluation of the Surface Antigen of the Salmonella typhimurium ATCC 14028 Ghosts Prepared by "SLRP". The Scientific World Journal, p. 1-6.
38. Eko FO, Mayr UB, Attridge SR, Lubitz W (2000) Characterization and immunogenicity of Vibrio cholerae ghosts expressing toxin-coregulated pili. Journal of Biotechnology 83(1-2): 115-123.

39. Eko FO, Hensel A, Bunka S, Lubitz W (1994) Immunogenicity of Vibrio cholerae ghosts following intraperitoneal immunization of mice. Vaccine 12(14): 1330-1334.
40. Kwon SR, Kang YJ, Lee DJ, Lee EH, Nam YK, et al. (2009) Generation of Vibrio anguillarum ghost by coexpression of PhiX 174 lysis E gene and staphylococcal nuclease A gene. Mol Biotechnol 42(2): 154-159.
41. Huppertz HI, Heesemann J (1996) Experimental Yersinia infection of human synovial cells: persistence of live bacteria and generation of bacterial antigen deposits including “ghosts,” nucleic acid-free bacterial rods. Infect Immun 64(4): 1484-1487.
42. Amara AA, Salem-Bekhit MM, Alanazi FK (2013) Sponge-Like: A New Protocol for Preparing Bacterial Ghosts. The Scientific World Journal 2013: 1-7.
43. Amara AA, Salem-Bekh MM, Alanazi FK (2013) Preparation of Bacterial Ghosts for *E. coli* JM109 Using Sponge-like Reduced Protocol. Asian Journal of Biological Sciences 6(8): 363-369.
44. Amro AA, Salem-Bekhit MM, Alanazi FK (2014) Plackett-Burman randomization method for Bacterial Ghosts preparation form E. coli JM109. Saudi Pharmaceutical Journal 22(3): 273-279.
45. Amara AA, Salem-Bekhit MM, Alanazi FK (2013a) Preparation of bacterial ghosts for E. coli JM109 using sponge-like reduced protocol. Asian Journal of Biological Sciences 6: 363-369.
46. Amara AA, Salem-Bekhit MM, Alanazi FK (2013b) Sponge-like: a new protocol for preparing bacterial ghosts. The Scientific World Journal 2013(3): 7.
47. Amro AA, Neama AJ, Hussein A, Hashish EA, Sheweita SA (2014b) Evaluation the Surface Antigen of the Salmonella typhimurium ATCC 14028 Ghosts Prepared by â€œSLRPâ€. The Scientific World Journal 2014: 1-6.
48. Abd El-Baky N, Amara AA (2014) Newcastle disease virus (LaSota strain) as a model for virus Ghosts preparation using H_2O_2 bio-critical concentration. International Science and Investigation journal 3(5): 38-50.
49. Amara AAAF (2015) Saccharomyces cerevisiae Ghosts Using the Sponge-Like Re-Reduced Protocol. SOJ Biochemistry 2(1): 1-4.
50. Vinod N, Oh S, Kim S, Choi CW, Kim SC, et al. (2014) Chemically induced Salmonella enteritidis ghosts as a novel vaccine candidate against virulent challenge in a rat model. Vaccine 32(26): 3249-3255.
51. Batah AM, Ahmad TA (2020) The development of ghost vaccines trials. Expert Review of Vaccines 19(6): 549-562.
52. Amara AA (2019) Oyster Mushroom Spores Ghost Preparation for Medicinal, Biotechnological and Forensic Applications. Biomedical Journal of Scientific & Technical Research 24(1): 17961-17967.67
53. El-Baky NA, Abdel Rahman RA, Sharaf MM, Amara AAAF (2021) The Development of a Phytopathogenic Fungi Control Trial: Aspergillus flavus and Aspergillus niger Infection in Jojoba Tissue Culture as a Model. The Scientific World Journal 2021: 1-8.
54. Abd El-Baky N, M Sharaf M, Amer E, Reda Kholef H, Zakaria Hussain M, et al. (2018) Protein and DNA Isolation from Aspergillus Niger as well as Ghost Cells Formation. SOJ Biochemistry 4(1): 1-7.
55. Abrams A, Nielsen L, Thaemert J (1964) Rapidly synthesized ribonucleic acid in membrane ghosts from Streptococcus fecalis protoplasts. Biochimica et Biophysica Acta (BBA) - Specialized Section on Nucleic Acids and Related Subjects 80(2): 325-337.
56. Chu W, Zhuang X, Lu C (2008) [Generation of Aeromonas hydrophila ghosts and their evaluation as oral vaccine candidates in Carassius auratus gibelio]. Wei Sheng Wu Xue Bao 48(2): 202-206.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.48.007638

Amro Abd Al Fattah Amara. Biomed J Sci & Tech Res

This work is licensed under Creative *Commons* Attribution 4.0 License

Submission Link: https://biomedres.us/submit-manuscript.php

Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles

https://biomedres.us/