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Bacterial Plasmid DNA enhances the efficacy of Vaccine and their advantages in Vaccine Development

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Abstract

Objectives: Many Bacterial components used as immunogen during vaccine development. Plasmid DNA is one among them. It is a short sequence DNA. It always carries one or more genes responsible for useful characteristics. If use DNA in vaccine development; it has more practical value and less expensive. It has resistance to temperature extremes, Storage and transport is also easy. DNA vaccines provide long term immunity.

Methods: The plasmid DNA induces humoral antibodies and cell mediated immunity against bacterial pathogens. In our studies proved that naked plasmid DNA and mutant pathogens plasmid DNA acted as good immunogens. The single and double enzyme digested plasmid DNA also produces good immune responses in many cases. The mixer of plasmid DNA of pathogenic bacteria also produces good immunity which is help to prepare. This is help to prepare mixer vaccines.

Results: The mixer vaccines give protection against more than one diseases and plasmid DNA companied with antigenic proteins or with other subunit components gives enhanced immunity. It is very much useful to produce good vaccines with least cost with short duration. This is very much suitable to developing countries and it is easy to prepare their own indigenous vaccines against various pathogenic infectious diseases.

Keywords: plasmid DNA, Vaccine development, Infectious Diseases

Introduction

Bacteria cause many infectious diseases to humans and animals. They create mortality and morbitality to the host organism. Vaccination is one of the best methods to prevention and control the infections of pathogenic bacteria. Many bacterial components have antigenic properties which are suitable for vaccine development. The main components of vaccine complex such as Antigens, adjuvants, preservatives, stabilizers, etc. The adjuvants are boosup the efficacy of vaccine. The preservatives help to avoid bacterial and fungal contamination in the vaccine. The stabilizers help to maintain the conditions of vaccine. The plasmid DNA is one of the important antigenic components of bacteria which are highly useful for vaccine development (Muruganandam, 2012, Muruganandam, 2013).

Plasmid DNA

Plasmid DNA is an extra chromosomal genetic element. It is present in cytoplasm. They vary in size from a few to several hundred kilo bases in length. The chromosome and plasmids together constitute the bacterial genome. Plasmids are small, circular and autonomously replicating double standard DNA molecules. Plasmids are transferable to other cells. They have great use in recombinant DNA technology (Muruganandam, 2013). A cell may contains1-100 plasmids. There are different types of plasmids based on their function. The important types are f plasmids, v plasmids, degradative plasmids, colplasmids; etc. The f plasmids have ability to transfer chromosomal genes to other cells. They can also transfer themselves to the cells. The R plasmid has the gene for resistance to one or more antibiotics. If the virulence plasmid is present inside the bacterium it turns that bacterium into pathogen which is an agent of diseases. The degenerative plasmids help to the host bacterium to digest compounds. The col-plasmid has the ability to synthesize a toxin called colicins (Muruganandam, 2017 & 2018b).

Components

Plasmid DNA has various general characteristics which are help to extent different way to existence. Plasmid DNA consists of different components that include, Antibiotic resistance genes, multiple cloning sites, primer binding sites, etc. The antibiotic resistance genes is one of the main components of plasmids. These genes play important role in drug resistance. Through conjugation process, plasmid DNA is capable of transferring antibiotic resistance properties to one species to another species of bacteria. A promoter region is another component of plasmid DNA which involved in transcriptional machinery. Another

component is primer binding site. It is a small sequence of DNA on a single strand that is typically used for DNA sequencing. In plasmid multiple cloning site is a short sequence DNA consisting of a few sites for cleavage by restriction enzymes. As such they allows for easy insertion of DNA through ligation or restriction enzyme digestion. Plasmids have one or few origins of replication, which are specific location for replication begins. Plasmids are used in genetic engineering to amplify or produce many copies of certain genes. Some plasmids help to resists various antibiotics. They make toxins to kill other bacteria. Some help resist environmental factors. They are also use unusual chemical compounds as nutrients. In pathogenic bacteria plasmid acts as antigens during infection. So, it is suitable to use in vaccine development. In cloning, a plasmid is a type of vector and uses in gene transfer to the cells of superior organisms to improve their resistance to diseases, growth rates and any other required traits. It is used to produce proteins and antibiotics at large scale. Plasmid are used to transfer genes into human cells as part as gene therapy. Plasmid cannot produce diseases. If it is combined with antigenic protein it will be induces immunity. So, it is highly suitable for vaccine development. These vaccines induces to produces more antibodies, it will also useful in plasma therapy for viral infection (Muruganandam, 2012, 2013 & 2017).

Major research moves towards rDNA-based vaccine development, Foreg: *Salmonella typhimurium* engineered to express antigens of vibrio cholera. So, in this presentation, another alternative way i.e.) direct plasmid DNA based vaccines development is focused. Plasmid DNA induces immunity so it is useful to vaccine development.

Isolation method

For isolation of plasmid DNA in lab, commonly follow alkaline lysis method. In this method, bacterial cells are harvested by centrifugation from bacterial broth culture. Then isolation procedure will be started. First resuspention of the pellet in isotonic solution ethylene diamine tetra acetate which is prevents nuclease activity. Alkaline lysis process involves cell lysis by using sodium dodecylsulfate to disintegrate the lipid structure on the cell membrane; dissolved proteins are precipitated by using a solution of acidic potassium acetate. A mixture of phenol and chloroform is used for purification of the plasmid DNA. This step removes protein content. In the precipitation step, first add ethanol for precipitation, by using centrifugation. Then wash with 70% ethanol to remove salt content. After that centrifuge 12,000rpm for 30 minutes to sediment plasmid DNA then. Dissolve in TE buffer solution and store for further use (Muruganandam, 2012, 2013).

Vaccine Development

Whole Plasmid DNA Vaccine

Plasmid DNA based different types of vaccines are discussed here one by one. In this study was done in bacterial pathogen *Aeromonas hydrophila* it is mainly present in drinking and ground water of various countries. In it mainly gives problem to immuno suppressive patient and normal host. *Aeromonas hydrophila*, water borne pathogen causing bacteraemia, cellulites to human has not got a main stay treatment till date. Now there is no commercial vaccine. The aim of this work is to develop novel vaccine. In this study two experiments were carried out. In this first experiment lives attenuated vaccine with various. Adjuvants such as vitamin A, C and E were tested. In this second experiment six various types of vaccines were tested such as killed vaccines live attenuated vaccines, toxic vaccine DNA vaccines, plasmid DNA vaccines and protein vaccine and the results shows the maximum humoral and cell mediated response was observed in protein and plasmid DNA vaccines. In this study, vitamin A acts as best adjuvant (Merina Sara Mathew *et al.*, 2008, Muruganandam, 2018).

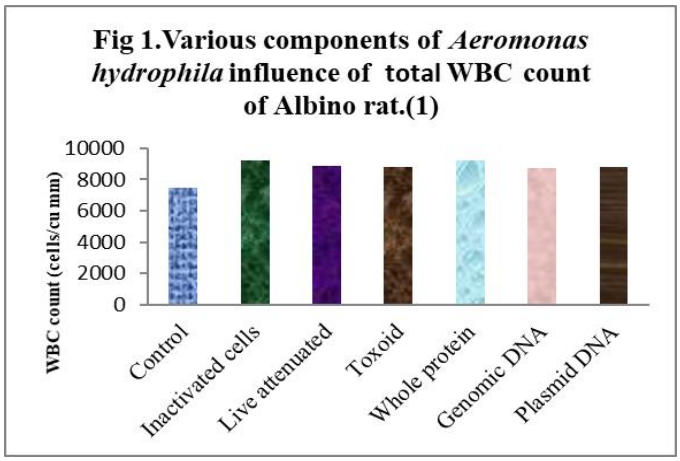


Fig 1.Various components of *Aeromonas hydrophila* influence of total WBC count of Albino rat.(1)

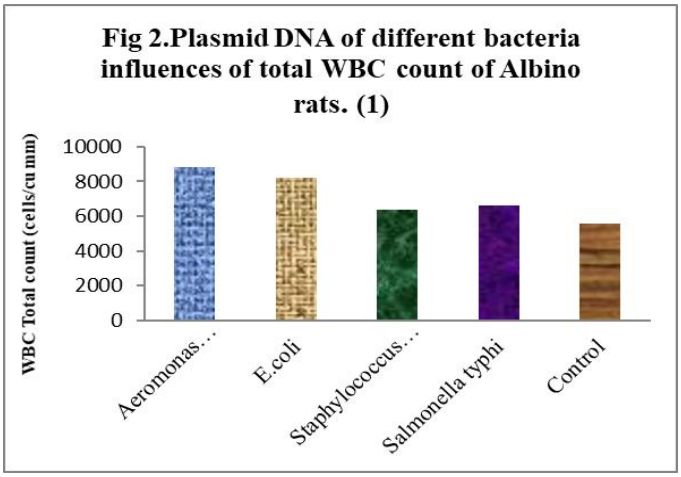


Fig 2.Plasmid DNA of different bacteria influences of total WBC count of Albino rats. (1)

Mutant Strains' plasmid DNA Vaccine

Staphylococcus aureus cause a variety of disease in human either through toxin production or invasion. The most common cause of food poisoning is staph toxin, this bacterium grows in improperly stored food the cooking process kill them but the toxins are heat resistant and it also infect wounds. In this experiment, the pathogen *Staphylococcus aureus* was collected from patients in hospital for confirmation of *S. aureus* all routine micro biological and Biochemical tests were done. During these experiments five sets of spread plate culture were maintained and four sets are exposed to UV radiation with different time intervals (0, 2, 4, 6 and 8minutes). First sets were maintained as a control after 24 hours the mutant strains were observed. Then it was isolated and put in to subculture. The mutant strains plasmid DNA was isolated by alkaline, lyses method and these was used as vaccine. In this experiment albino rats was used as test animal. The same level vitamin C (200mg) was provided as adjuvants. After fifteen days inactive pathogens were injected to all treatment including control treatment. After that blood samples of the entire albino rat were taken for analysis. The maximum, immune response was observed in 6th minute treatment strain. In all the treatment, inactivated whole cell pathogens were tested, and also the similar result was observed. So it is concluded that this 6 minute treatment strain's plasmid DNA and inactivated whole cell is suitable for vaccine development of *S.aureus* vaccine (Muruganandam and Verrayee Kanna, 2010, Muruganandam and Kochet George Ray, 2009, Muruganandam, 2018b).

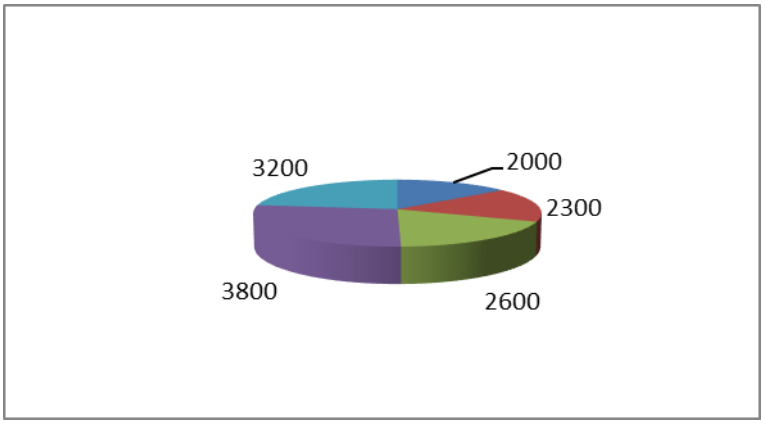


Fig.3. Mutant Strains' plasmid DNA Vaccines effect on WBC (cells/cumm) counts of Albino rats. 1. Control (2000 cells) 2. Two-minute U.V. treatment, (2,300 cells) 3. Four-minute U.V. treatment (2,600 cells), 4. Six minutes U.V. treatment (3,800 cells) 5. Eight-minute U.V. treatment (3,200 cells)**.**

Engineered Plasmid DNA vaccine

Nowadays plasmid DNA has wide variety of applications in vaccine research. Here it is modified and used as vaccine. First, plasmid was isolated from *Staphylococcus aureus* and digested by various restricted enzymes. In this study two experiments were carried out. In the first experiment, plasmid DNA was isolated and digested individually by five restriction enzymes, such as EcoR-I, Hind-III, PST-I, Bam H-I and Hae-III. then digested plasmid DNA was used as vaccine. In the second Experiment isolated Plasmid DNA was double digested by these enzymes and used as vaccine. Albino rats were used as test animal, In the first experiment, maximum immune response was observed in pst-I and Hae-III enzyme digested treatment. In the second experiment, maximum immune response was observed in EcoR-I+Hind-III and Hind-III+BamH-I digested treatments. So, these are suitable to develop plasmid DNA based vaccine for *staphylococcus aureus* (Muruganandam, 2012, Muruganandam, 2013 & 2018).

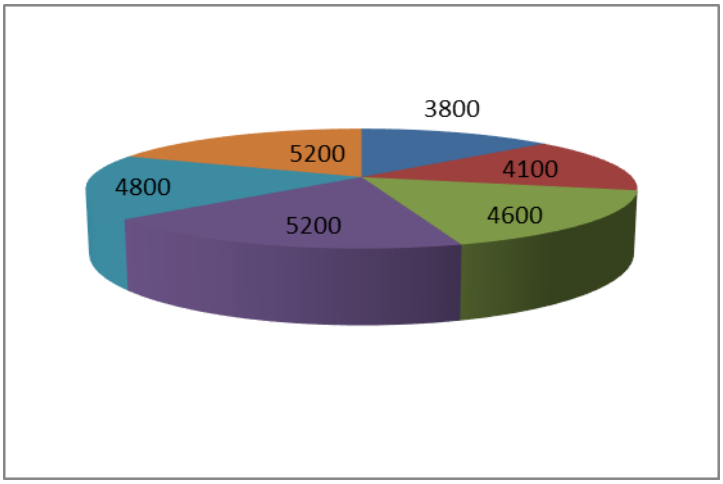
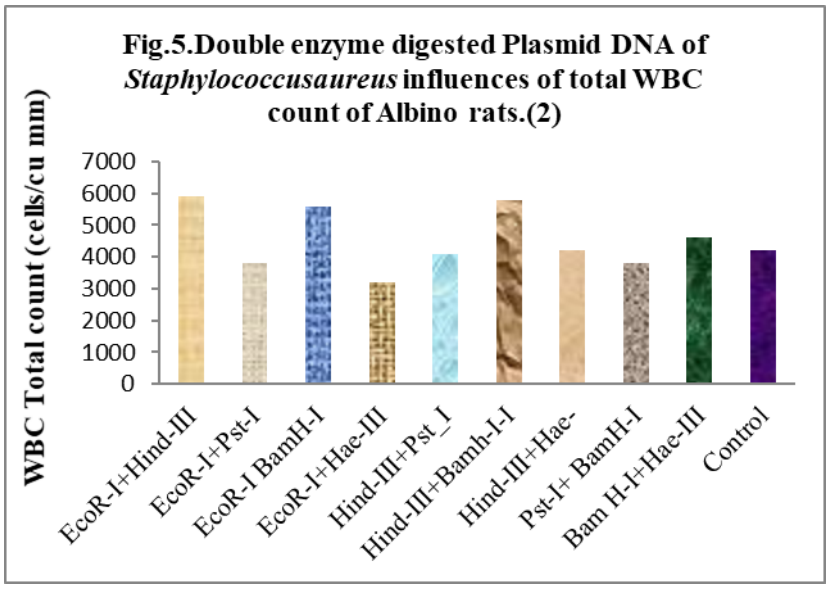


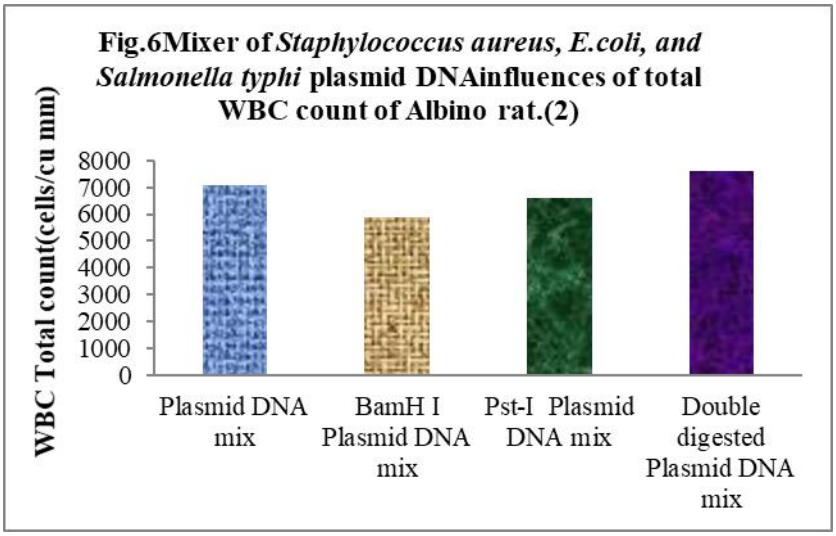
Fig.4. Single Enzyme Digested plasmid DNA vaccine influence on WBC counts (cells/cumm). 1.EcoR-I,(4,100 cells) 2.Hind-III(4,600 cells),3.Pst-I,(5,200 cells) 4.Bam H-I(4,200 cells) and 5.Hae-III (5,200 cells) 6.control (3,800 cells) digested plasmid DNA fragments.

Cocktail plasmid DNA Vaccine

Water borne diseases are caused due to intake of contaminated drinking water. The potable water is degraded due to the faecal contamination, which contain many pathogens like. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, etc. which causes fever, dysentery, vomiting, diarrheal, rapid pulse, cramps, etc. Still now there is no proper cocktail vaccine recognanised. Here is an attempt to develop a cocktail vaccine for these diseases. In this experiment, four treatments were tested and one control was maintained. In the first

treatment, the plasmid DNA was isolated from three pathogens and it was mixed then use as vaccine. In the second experiments, the plasmid DNA was isolated and all the pathogens were digested by Pst-I enzyme and in third treatment, plasmid DNA was digested by BamH-I and in fourth treatment these plasmid DNA was double digested by both enzymes used as vaccine. Albinorats were used as test animals. It was observed that double digested plasmid DNA mixture induces more immune response compared to other treatment and so it was recommended for development of cocktail vaccine for these water borne diseases (Muruganandam, 2018a).





Peptide Plasmid DNA vaccine

Plasmid DNA with extracellular protein was tested in *Staphylococcus aureus.* In this study, plasmid DNA, extracellular protein and mixed of both treatment were tested. Albino rates were used as test animals. First test vaccines were injected and after 15 days, inactivated

pathogens were injected. After one week, blood samples were collected for analysis. The maximum immune response was observed in mixer treatment compared to other treatment. Based on this study, plasmid DNA with other immunogenic protein produces good immune response.

Based on these experiments, it is concluded that, Plasmid DNA act as good antigen. The plasmid DNA from the mutant pathogen is also acts as good antigens. The single and double digested plasmid DNA acted as good antigen. The mixer of various pathogenic bacteria is also giving better immune response which are also help to prepare mixer vaccines for more than one diseases. Our lab studies prove that plasmid DNA of various pathogenic bacteria induces antibody production and it also induces cell mediated immunity. If plasmid DNA combined with antigenic proteins gives better immunity, it will be very much useful to produce good vaccines with least cost (Muruganandam, 2018b).

Potential Merits

For plasmid DNA based vaccine development, required short duration. The plasmid DNA vaccines produces long term immunity. It is stable and easy to transport. It is also thermo liable i.e.) heat resistant. It is easy to sequence. Formalin is used to inactivation of pathogen and preservation of amino acids in vaccines. Here formalin is not required for DNA vaccine. If plasmid DNA combined with suitable antigenic proteins, it will enhance the efficacy of vaccine. Now it is possible to change the sequence of antigenic protein and also sequence of plasmid DNA. So, the production is easy and also suitable to develop advanced vaccines in future. The DNA vaccine development is easy to prepare more than one infection. It is also suitable for pain free vaccination. The plasmid DNA vaccines are highly suitable for developing countries. Because they are highly effective but it is low cost production vaccine (Muruganandam, 2019).

Vaccine Delivery methods

There are many methods are used in vaccination programmes in all over the world. Here the important methods are discussed.

Oral vaccination

Oral vaccination is one of the best methods in needle free vaccination. It may replace vaccine injections. The primary problem with this type of delivery system is that the environment of the digestive system which is possible to destroy vaccines. Now vaccine capsule has been

developed as an alternative to these problems. Oral delivery is an even more attractive route of administration. Polio virus vaccine administrated orally is the greatest current examples and it is user friendly, economically feasible and clinically effective vaccines. This method is mainly used for larger population. It is a most effective method. It ensures that the vaccine reaches the individuals quickly. The eye drop administration provides good protection. It need only short duration. But it is commonly used in poultry industry (Muruganandam, 2019b).

Intranasal vaccination

Some vaccines are protecting against respiratory diseases which is to be given as drops in the nose. These vaccines generally provided faster production than those given intramuscularly or subcutaneously. Intranasal vaccines are less likely to cause allergic reactions and are more likely to provide protection. Different types of needle free injections are now developed. They are mainly used to vaccine delivery and other drug delivery functions without pain. The needle free injectors offers less pain, avoid needle stick injuries and contamination that also allows self-administration and results is no needle phobia and thus strongly preferred by the patients (Muruganandam, 2019b).

Skin Cream vaccination

In our lab experiment proves that skin cream plasmid DNA vaccine also induces immunity. In that study, the skin cream is prepared by using beeswax with coconut oil. During cream preparation, the plasmid DNA was dissolved in double distilled water and mixed well with cream. The skin cream vaccine was tested by applying on the surface of the ear's skin. The vaccine cream was applied three times with 24 hours interval. After 24 hours of vaccination, blood samples were collected for analysis. The cell mediated immunity was increased in treatment compared to control. This is the positive indication of vaccine function. In future, various skin cream vaccines will be developed; It is highly suitable for children (Muruganandam, 2019b).

Edible vaccine

Edible vaccine helps to vaccinate a big population during short duration**.** It is also pain free vaccination method. In future more and more edible vaccines will be developed for control more infectious diseases. The edible vaccines differ from traditional ones in the way, they stimulate the production of antibodies in the receiver, which is the case of edible vaccines, is

during digestion, and protein-antigen would interact with the mucous membrane of the gastrointestinal tract, activating so called mucous type of immune protection. it leads to synthesis of antibodies against virus protein. So the protein antigen is the beginning stimulator of the body to produce the antibodies necessary to have a successful vaccine (Muruganandam, 2019b).

Nanopatch technology

In nanopatch technology, Micro projection array form is the part of the push for physical targeting of vaccines to the skin. The key concept is to fabricate array of micro projections to pierce the touch scalier and rapidly deliver vaccine payloads to the epithelium. The first reports on the fabrication and testing of silicon arrays for transdermal delivery appeared in the late 1990 s. after that, many researchers discovered this technology and develop many Nano patch technology devices for vaccine delivery. Injectable vaccines are given in to the muscle (intramuscular) or under the skin subcutaneous). Some vaccines can be given either way, other must only be given one way.eg.some rabies vaccines can only be given in the muscle (Muruganandam, 2019b).

Problems to be solved

Based on this ideology, vaccines will be developed for any bacterial infectious diseases with limited lab facilities and even low budget. The plasmid DNA Sequencing, production, and isolation methods are easy. Preservatives are not required for nucleotides. So, it is practically convenient for vaccine development. This is highly suitable for developing countries. In developing countries, most of the poor people live in town and cities with poor hygienic conditions. They have infected many common infectious diseases due to thickly populations. The plasmid DNA based vaccines help to control more than one infection by mixer of plasmid fragments. It is practically convenient and possible one. If add plasmid DNA with suitable antigenic proteins, it will increase the vaccine efficacy at very high range (Muruganandam, 2018b & 2019a).

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