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Original Article

Engineered plasmid DNA vaccine for *Staphylococcus aureus*

M.Muruganandam
Department Biotechnology, St. Michael College of Engg. & Tech., Kalayarkoil- 630551
Sivagangai District, Tamil Nadu, India.
E.mail: vaccine_2006@yahoo.com
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Abstract

Plasmid DNA has wide variety of applications in vaccine research. Here it is modified and used as vaccines. First plasmid DNA was isolated from *Staphylococcus aureus* and engineered by various restriction enzymes. In this work, 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, BamH-I, and Hae- III. Then digested plasmid DNA was used as vaccines. In the second experiment, isolated plasmid DNA was double digested by these enzymes and used as vaccines. Albino rats were used as test animal in all the treatments. In the first experiment, maximum immune response was observed in Pst-I and Hae-III digested treatment. In the second experiment, maximum immune response was observed in EcoR-I + Hind-III and Hind-III + BamH-I digested treatments. So it is concluded that, double digested treatments are highly suitable for develop plasmid DNA vaccine for *Staphylococcus aureus*

Key words: *Staphylococcus aureus*, DNA vaccine.

Introduction

Staphylococcus aureus cause disease when they get inside the host, because they can't penetrate the skin. So they are associated with wounds, cuts, needle pricks, etc. Once inside the host, they stick to host tissues and produce toxins. It can also cause wide variety of infections that are described as pyogenic (pus - forming) include impetigo, boils, wounds abscesses and pneumonia. (Rogery Stainer *et al*., 1987). It is also a major cause of hospital acquired infection of surgical wounds and infections associated with individually medical devices.

Staphylococcal food poisoning is the name of the condition caused by the enterotoxins which some strains of *S.aureus* produce. The onset of symptoms in Staphylococcal food poisoning is usually rapid and in many causes acute, depending on individual susceptibility to the toxin, the amount of the contaminated food eaten, the amount of toxin in food ingested, and general health of the victim. The most common symptoms are nausea, vomiting, and retching, abdominal gramping and demonstrate all the symptoms associated with the illness. (FDA/CFSAN)

Staphylococcus aureus has the remarkable and unfortunate features that it can become readily resistance to antibiotics. Indeed, it has acquired resistance to almost all antibiotics so far, resulting in an increase in incidence of acute hospital – acquired infections. Extensive studies have focused on how *S.aureus* acquired resistance to antibiotics, and genome sequencing analysis confirmed the existence of many resistance genes acquired by horizontal transfer from other species. In addition *S.aureus* can cope with antibiotic stresses in an adaptive manner through regulation of the expression of many genes (Yoshikaz Taraka *et al* .,2007)

Due to antibiotic resistant problem, pathogen control and prevention is very difficult. But there is another way to control the infection by development of therapeutic vaccines for immunotherapy. DNA vaccine development is one of the novel powerful methods. It has several advantages. In addition, DNA vaccines are a greater interest among researchers around the world (Whalen, 1996). In the present study the plasmid DNA was isolated from *S.aureus* and digested by single enzymes and also double digested, all these treatments are tested in albino rat. The best treatments are recommended for new DNA vaccine development against *Staphylococcus aureus*.

Materials and Methods

The bacterial pathogen *Staphylococcus aureus* was collected from the patient's samples from local hospital and confirm through regular biochemical and microbial tests. The plasmid DNA was isolated by alkaline lysis method (medox kit). The plasmid DNA was digested by different restriction enzymes and used as vaccine.

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First treatment is undigested plasmid DNA another five treatments were single digested plasmid DNA which are digested by five different enzymes. (Table :1). Then remaining treatment were double digested using combination of two enzymes. The digested plasmids DNA were provided by intramuscular injection. After one week same dose was given as booster dose. Then after two weeks, blood samples were collected for analysis.

Table 1: Various restriction enzymes and their restriction sites

S.No	Name	Source	Recognition Sequence
1	EcoR-I	*E. coli* RY 13	5' G↓ AATTC 3'
2	Bsh-I (Hae-III)	*Bacillus sphaericus*	5' GG↓CC 3'
3	Pst-I	An *E.coli* strain that carries the cloned Pst-I gene from *Providencia stuartii*	5'CTGCAG 3'
4	BamH-I	*Bacillus amyloliquefaciens.H*	5'G↓GATCC 3'
5	Hind-III	*Haemophilus influenzae Rd*	5'…..A↓AGC TT… 3' 3'….TTGA↑A ….5'

Result and Discussion

Staphylococcus aureus is a major cause of hospital and community acquired infections. It causes serious and fatal diseases. Still there is no proper vaccine for these infections. (Yoshikaz Taraka *et al* .,2007). The research is going on the whole cell killed vaccine is commonly used in many diseases. The next step is preparation of mutant strain whole cell killed vaccine. Muruganandam and Veerayee kanna (2010) is stated that the 6 minutes UV treated mutant strain is best for preparing killed vaccine. If increase the UV treatment more than 6 minutes most of the potential of virulence factors may decreased. Because, the maximum leucocyte level, hemoglobin and antibody levels are observed in 6 minutes treatments compare to other treatments. (Muruganandam and Veerayee kanna, (2010)). Muruganandam, (2007) proved that the plasmid DNA alone act as good vaccine for *Aeromonas hydrophila* infection. It induces maximum immune response.

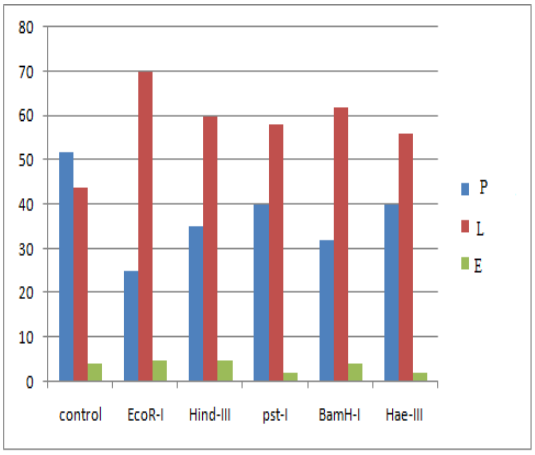

Fig. 1: Various enzyme digested plasmid DNA vaccine influence on WBC Differential count (%) of Albino rat.

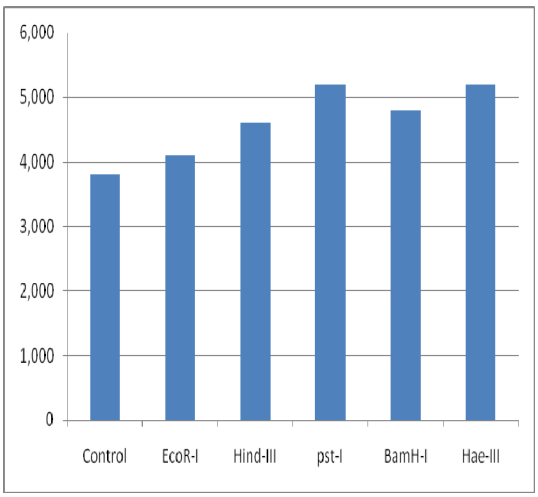

Fig. 2: Various enzyme digested plasmid DNA vaccine influence on WBC count (Cells/cubic mm) of Albino rat.

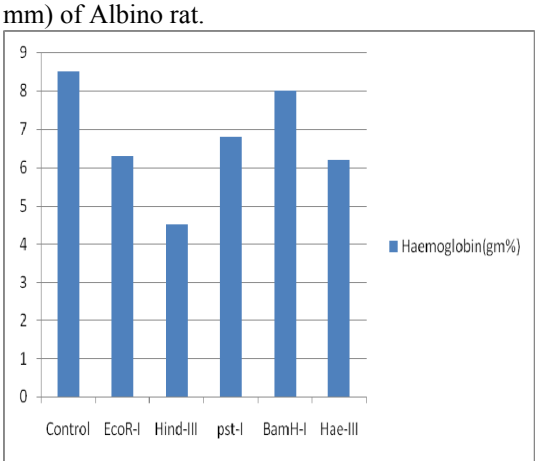

Fig 3: Various enzyme digested plasmid DNA vaccine influence on Hemoglobin (gm %) of Albino rat.

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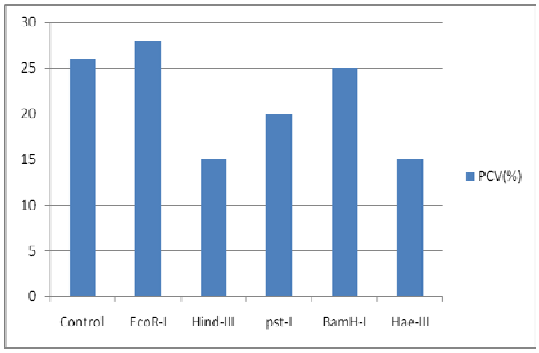


Fig. 4: Various enzyme digested plasmid DNA vaccine influence on PCV (%) of Albino rat.

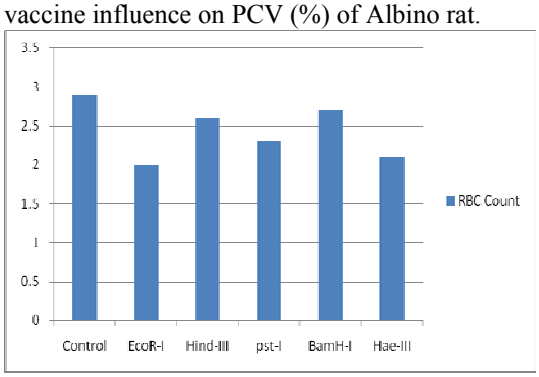


Fig. 5: Various enzyme digested plasmid DNA vaccine influence on RBC Counts (millions) of Albino rat.

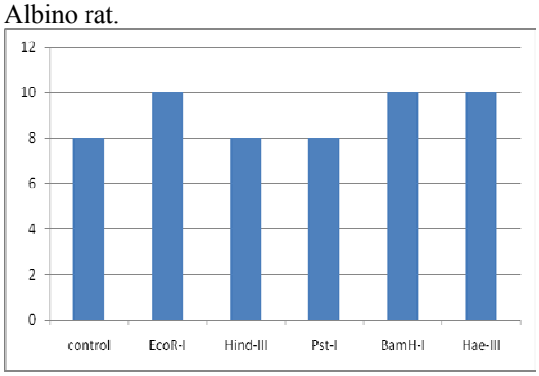


Fig. 6: Various enzyme digested plasmid DNA vaccine influence on antibody level of Albino rat.

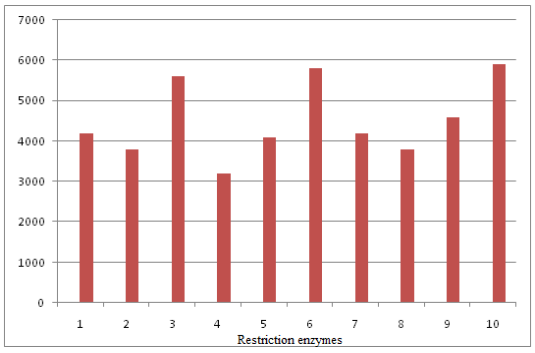


Fig. 7: Various enzymes double digested plasmid DNA vaccine influence on WBC Counts (Cells/ ccmm) of Albino rat.

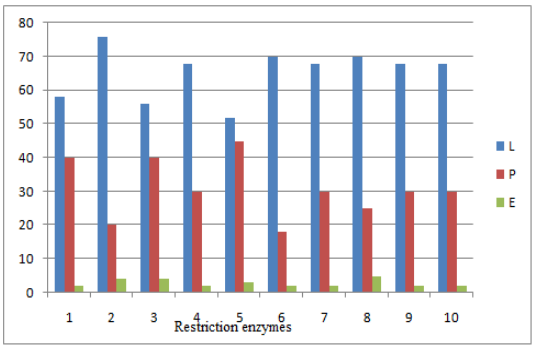


Fig. 8: Various enzymes double digested plasmid DNA vaccine influence on WBC Differential Counts (%) of Albino rat.

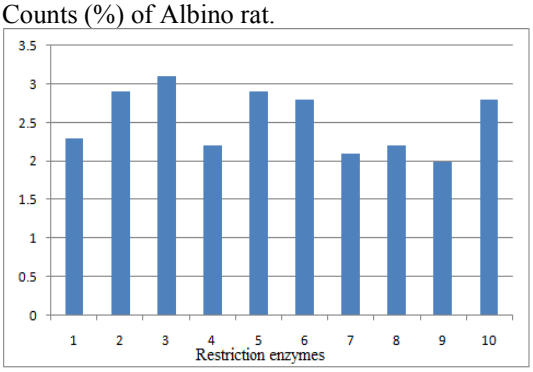


Fig 9: Various enzymes double digested plasmid DNA vaccine influence on RBC Counts (Millions) of Albino rat.

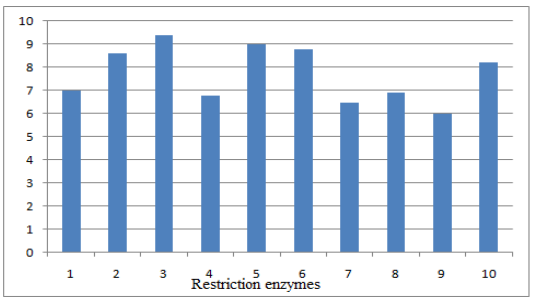


Fig.10: Various enzymes double digested plasmid DNA vaccine influence on Hb (gm %) of Albino rat.

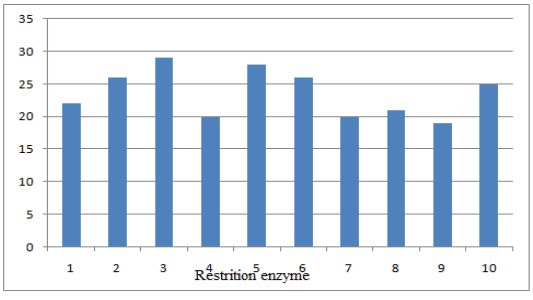
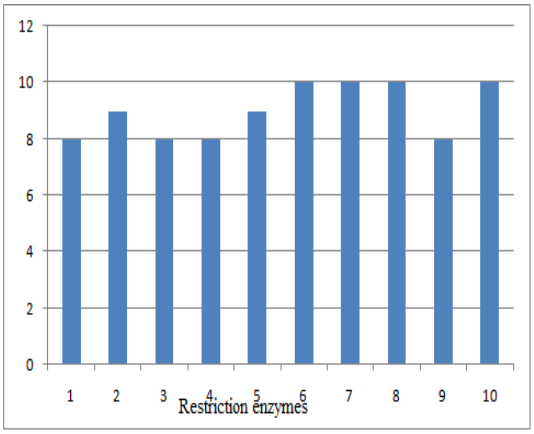


Fig.11: Various enzymes double digested plasmid DNA vaccine influence on PCV (%) of Albino rat.



Restriction Enzymes: 1 – control, 2 – EcoR-I+Pst-I, 3 - EcoR-I+BamH-I, 4 - EcoR-I+Hae-III,5 - Hind-III+Pst-I, 6 - Hind-III+BamH-I, 7 - Hind-III+Hae-III, 8 - Pst-I+ BamH-I,9 - BamH-I+Hae-III, 10-EcoR-I+Hind-III.
Fig.12: Various enzymes double digested plasmid DNA vaccine influence on Antibody level of Albino rat.

The potential of virulence was increased in mutant strain plasmid DNA vaccine (Muruganandam 2010b). The maximum antibody production and WBC counts was observed in 6 minutes UV treated mutant strains plasmid DNA treatment, which is also act as best vaccine compared to other plasmid DNA vaccines in *Staphylococcus aureus* (Muruganandam 2010b).

Next level trail is the enzyme digested plasmid DNA role in immune response was studied. The single and double digested plasmid DNA used as vaccine in the case of *Salmonella typhi*. The maximum response was observed in double digested plasmid DNA treatments(Muruganandam 2010a).

So that, here various digested plasmid DNA were used as test vaccines. In the present work, two experimental trials were conducted. In the first experiment five restriction enzymes were individually used. First plasmid was isolated and digested by these enzymes. The digested plasmid DNA was used as vaccine. The maximum immune response was observed in Pst-I digested treatment and Hae-III digested treatments. The second maximum immune response was observed in BamH-I, compared to other treatments.

In the second experiment, nine treatments and one control treatments were tested. All the treatments contain double digested plasmid DNA with various enzyme combinations. The maximum immune response was observed in EcoR-I + Hind III digested treatment. The second maximum immune response was observed in Hind-III + BamH-I digested treatments. So compare to single digestion, the double digestion DNA especially EcoR-I + Hind III and Hind-III + BamH-I treatments induce higher immune response. So it is concluded that these double digested plasmid DNA treatments are suitable for new DNA vaccine preparation.

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Reference

Muruganandam, M.2007. New DNA vaccine for *Aeromonas hydrophila*, *Aquatech*, Aug .pp:79.

Muruganandam, M.2010a. Engineered DNA vaccine for Typhoid. *J. Nat. Con*., 22(1):123-126.

Muruganandam, M.2010b. Plasmid DNA vaccine for *Staphylococcus aureus* *J. Nat. Con.*, 22(1):73-76.

Muruganandam .M and Veerayee kanna,2010. Mutant strain vaccine for *Staphylococcus aureus*

FDA/CFSAN – Bad bug book – *Staphylococcus aureus* – internet reference.

Yoshikaz Taraka, Kazuya Morikawa, Yu Ohki, Minyao, Kouhei Tsumoto, Nobushisa Watanabe, Toshiko Ohha, and Isao Tanaka, 2007. Structural and Mutationalanalysis of Drp 35 from *Staphylococcus aureus*, *J. Bio.Chem*.,282(8): 5770-5780.

Rogery Stanier, Ingraham,J.L., wheelis,M.L. and Painter,P.R.1987. General Microbiology, book. Pub. By MAC MILLAN press LTD London, International edition. pp. 689.

Whalen,R.G.1996. DNA vaccine for Emerging Infection's disease: what if? E.D., 2(3): 1-15 (web reference).