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Original Article

Cocktail Plasmid DNA Vaccine for Common Food Borne Disease

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Abstract

Our previous study shows that, plasmid DNA vaccines are one of the best vaccines. So in this attempt, try to develop a common plasmid DNA vaccine for *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. In this experiment, four treatments were tested and one control treatment was also used. In the first treatment, plasmid DNA was collected from *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. These plasmid DNA was mixed well and deliver through intramuscular injection. In the second treatment, all the plasmid DNA were isolated and digested by BamH-I enzyme and mixed well then these digested plasmid DNA was used as vaccine. In the third treatment, all the plasmid DNA were isolated from the pathogens and digested by Pst-I enzyme and these also mixed well and used as vaccine. In the fourth treatment, all plasmids were double digested by BamH-I and Pst-I enzymes and used as vaccines. The maximum immune response was observed in double digested treatment compared to other treatments. So, it is concluded that it is best for develop a cocktail vaccine for all these disease.

Keywords: Cocktail plasmid DNA vaccine, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*.

Introduction

Many bacterial pathogens act as food borne pathogenic organisms. Some pathogens produce toxins, it leads to food contamination. Some of these toxins are heat stable in some cases. The important food borne organisms are *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, etc. these bacteria are mainly spread through contaminated food and water. The contaminated drinking water mainly spread various fatal diseases. The *Staphylococcus aureus* produce toxins in food.

These toxins are heat resistant and if it is also enter through wounds, cuts, etc, lead to many fatal problems. These diseases commonly called Staphylococcal disease. But already our lab work reported mutant plasmid DNA vaccine for *Staphylococcus aureus* (Muruganandam, 2010b). The *Salmonella typhi* mainly cause typhoid. It spreads through contaminated water. In our previous work reported the advanced plasmid DNA vaccine for *Salmonella typhi* (Muruganandam, 2010a). The *Escherichia coli* mainly case bloody diarrhea. It also mainly spread through contamined drinking water. Previously our lab work, reported, the advance DNA vaccine for *E.coli* (Muruganandam, 2010c). In the present work, plasmid DNA was isolated from three different pathogen and prepares various vaccine treatment and test in albino rat. Finally, we will find out suitable DNA vaccine preparation for develop cocktail plasmid DNA vaccine for common food borne diseases.

Materials and Method

Pathogens such as *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* were collected from the patient's samples at local hospital. Done all the biochemical and biological test for confirmation. Then prepared three separate broth and individually inoculated. After 24 hours, plasmid DNA was separately isolated by alkaline lysis method. In the first treatment, all the plasmid DNA were mixed and used as vaccine. In the second treatment, all the plasmid DNA were digested by BamH-I restriction enzymes. (Medox Company). Then it was used as vaccine.

In the third treatment, plasmid DNA were isolated individually and digested by Pst-I restriction enzyme (medox company) and these are mixed used as vaccine. In the fourth treatment, plasmid DNA was isolated from all the pathogen and double digested using BamH-I + Pst-I enzymes and were mixed well and used as vaccine. One control treatment was used. Albino rats were used as test animals in all the treatment. All the vaccines were delivered through intramuscular injections. After delivered the test vaccines, two weeks later, blood samples were

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collected for analysis. The restriction enzyme recognition sites are shown in Table: 1

Table- 1: Recognition sites of restriction enzymes

Restriction enzymes	source	Recognition site
BamH-I	*Bacillus* mybolique facies-H	5'G↓GATCC 3'
Pst-I	An *E.coli* strain that carries the clonal Pst-I gene from *Providencia styartili*	5' CT GCAG 3'

Results

In this attempt, maximum immune response was observed in double digested plasmid DNA treatment, compared to other treatments (Fig.1-5). The second maximum immune response was observed in undigested plasmid DNA treatment. The lesser response observed in control treatment compared to two restriction enzymes Pst-I digested plasmid DNA treatment is gives better results than BamH-I digested treatment.

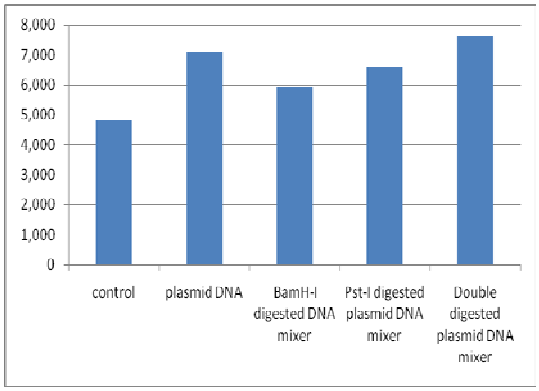

Fig. 1: Various plasmid DNA vaccine influence on WBC Count (cells/cubic mm) of Albino rat

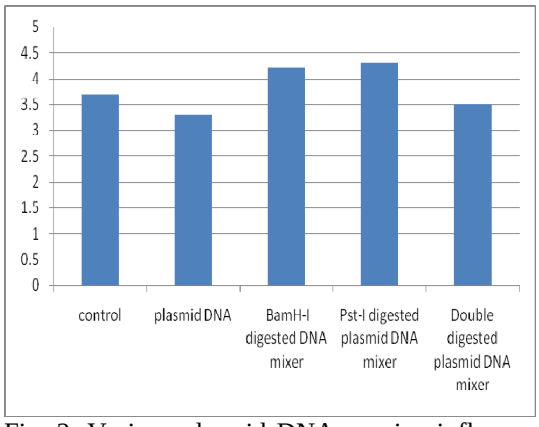

Fig. 2: Various plasmid DNA vaccine influence on RBC Count (millions) of Albino rat

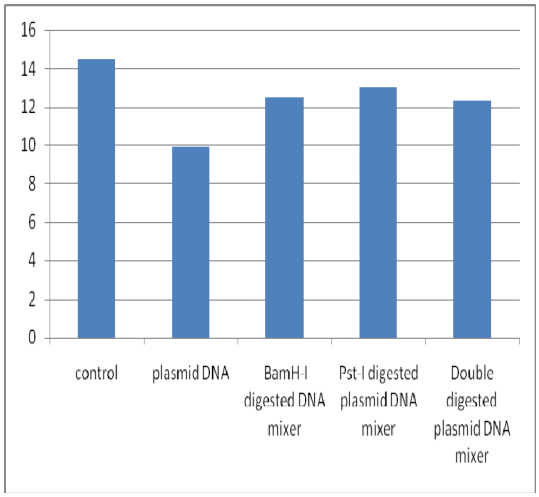

Fig. 3: Various plasmid DNA vaccine influence on Hb (gm %) of Albino rat

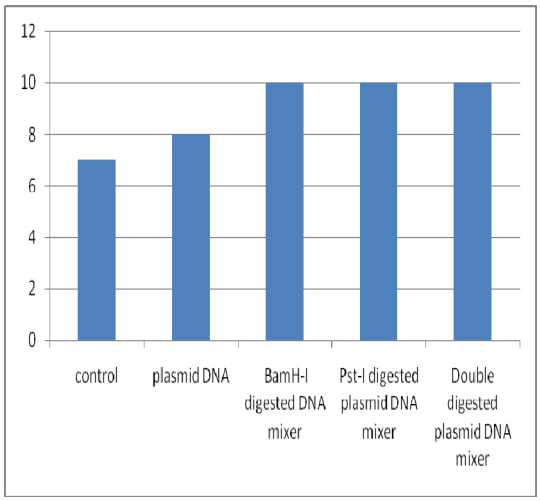

Fig. 4: Various plasmid DNA vaccine influence on antibody levels of Albino rat

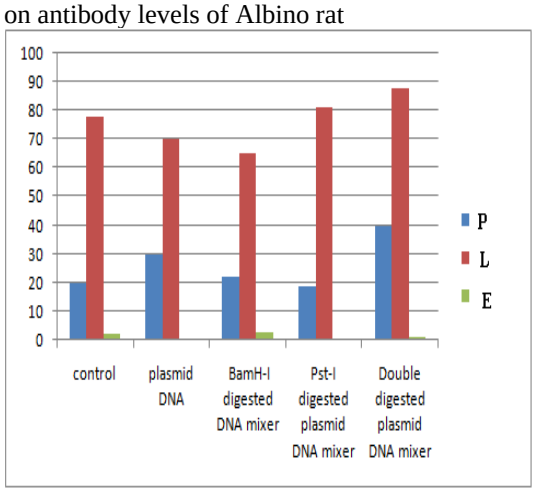

Fig. 5: Various plasmid DNA vaccine influence on WBC differential count (gm %) of Albino rat

The RBC count was more or less same level in undigested plasmid DNA treatment and double digested plasmid DNA treatments. The Pst-I and BamH-I digested plasmid DNA have similar RBC counts. Higher level of polymorph and lymphocytes were observed in double digested plasmid DNA treatments, compare to other treatments. The antibody levels were higher in single and double digested treatments. The control treatment has lesser antibody levels compared to other treatments. In this attempt, maximum immune response was observed in double digested treatment. So it is highly suitable for cocktail plasmid DNA vaccine preparations.

Discussion

Our previous studies proved that plasmid DNA vaccine is best compared to other traditional vaccines (Muruganandam, 2007). In our lab various plasmid DNA vaccines developed for various bacterial pathogens. For Staphylococcal disease, mutant strain plasmid DNA vaccine was developed (Muruganandam, 2010b). Then for *Salmonella typhi* single digested plasmid DNA vaccine and double digested plasmid DNA vaccines were developed (Muruganandam, 2010a) and for *E.coli* also single and double digested plasmid DNA vaccines were developed (Muruganandam, 2010c). So now we try to develop a cocktail plasmid vaccine for three common food borne pathogens such as *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*.

In this study plasmid DNA was isolated from each pathogen. Then the isolated plasmid DNA was single digested, double digested and undigested treatments were prepared. Then the mixed of plasmid DNA, mixer of single digested plasmid DNA and mixer of double digested plasmid DNA were prepared and used as vaccine. In our lab previously, developed cocktail genomic DNA vaccine for these diseases (Muruganandam, 2010d). But compared to these vaccines, cocktail plasmid DNA vaccine give better results. In this study, the maximum immune response was observed in double digested plasmid DNA mixer treatment, compared to single digested DNA treatment and undigested plasmid DNA treatments. So it is concluded that the double digested plasmid DNA vaccine mixer was highly suitable for develop a cocktail plasmid DNA vaccine preparation compared to other treatments.

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