Construction of a Plasmid Vector and Cloning of Major Outer Membrane Protein-1 (*Omp-1)*

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ABSTRACT

Chlamydia trachomatis genital infection is a prevalent bacterial sexually transmitted disease. *C. trachomatis* infects the genital and ocular epithetlium. Genital infection may progress to serious reproductive conditions, such as pelvic inflammatory disease, infertility, and ectopic pregnancy. Vaccinology strategies are attempting to produce an effective vaccine that would confer immunity against genital chlamydial infection. A major challenge of vaccine development is the effectiveness of the delivery systems that would present the antigen(s) to the immune system and achieve lasting immunity. To overcome this problem, we chose to develop of a vaccine scheme that utilizes a commensal bacteria as a live delivery vehicle of chlamydia antigens to the immune system. *Lactobacilli* are of the normal flora of the human genital and urinary tracts. Previous results from our laboratory suggest that *Lactobacillus* can be used as an effective vehicle to deliver chlamydia or antigens of other human and animal pathogens. We hypothesize that a vaccine utilizing *lactobacilli* as a live delivery vehicle will produce significant quantities of chlamydia antigen and possess adjuvant effects to induce mucosal, humoral and cell-mediated immune responses. In our laboratory, we have developed the recombinant plasmid using plasmid pGK12 and a multiple cloning site obtained from plasmid pVAX. By gel electrophoresis and DNA sequencing, we have confirmed the construct and verified its orientation. These promising results premise the development of a prophylactic vaccine against chlamydial genital infection. The expression of plasmid pGKOMP1 in *Lactobacillus* constitutes a recombinant vaccine with the potential to produce an efficacious vaccine against *C. trachomatis* genital infection. Our vaccine scheme can be used for vaccinology efforts towards other infectious diseases. Supported by NIH grants GM08247 and A141231.

Figure 1: Restriction Site Map of Plasmid pGK12

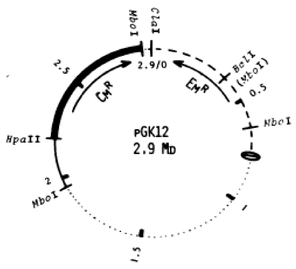

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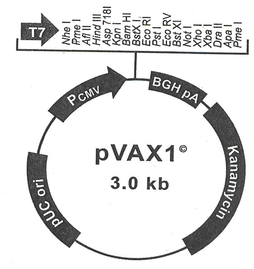
Figure 2: pVAX Vector System



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INTRODUCTION

Chlamydia genital infection caused by *Chlamydia trachomatis* is the most commonly reported bacterial causing sexually transmitted disease worldwide. The complications resulting from this sexually transmitted disease include serious reproductive conditions, such as pelvic inflammatory disease, infertility, and ectopic pregnancy. Additionally, pregnant women may experience prematurity and morbidity of the fetus; or they may infect their child during delivery causing early infant pneumonia and neonatal and ocular conjunctivitis. Although chlamydial infections can be treated with antibiotics, most are asymptomatic. Furthermore, unless therapy is administered in a timely manner, the adverse outcomes can result. Therefore, developing a vaccine is an ideal way to effectively protect against this disease. A promising preventive strategy is the development of a vaccine that utilizes a *Lactobacillus* species as a live delivery vehicle of chlamydia antigens to the immune system. *Lactobacillus* is a part of the normal flora of the human gut and genitourinary tract and they are considered to be safe organisms for human consumption. *Lactobacilli* have an adjuvant effect by enhancing antigen specific immune responses when administered in combination with antigen. The use of *Lactobacillus* as an adjuvant may produce large quantities of chlamydia antigens, and furthermore induce substantial immunity. We hypothesize that recombinant *Lactobacilli* that perpetually express chlamydia antigens will effectively provide lasting protective immunity against chlamydial challenge. Therefore, we propose to develop a system that can be used to regenerate transformed *Lactobacillus vaginalis* expressing recombinant pGK12 plasmid carrying OMP1, a gene of *Chlamydia trachomatis.* OMPI encodes the major outer membrane protein (MOMP). MOMP is an immunogenic surface protein that may play a role in the attachment of *Chlamydia trachomatis* to host cells. Previous studies have shown that MOMP can induce strong humoral and cell mediated immune responses in humans and animal models. Plasmid pGK12 (Fig. 1) found in *E. coli* is an expression vector containing chloramphenicol and erythromycin resistance genes, and plasmid pGK12 contains restriction sites which denote its ability for reconstruction and subsequent anchoring of OMP1. Plasmid pVAX is a vector system desigmed to use in DNA vaccine development. The formation of the plasmid pGKVAX results from the ligation between the Cla I and Bcl I digested ends of the linearized plasmid vector, pGK12 and the PCR amplified pVAX multiple cloning site. The ability to construct plasmid pGKVAX and to transform it into DH5α *E.coli* suggest our achievement of the objective of developing an effective vehicle to deliver chlamydia or antigens of other human and animal pathogens to the genital mucosa. This study is significant because next OMPI can then be cloned into pGKVAX whereby producing a recombinant plasmid that may be used to the transformation of *Lactobacillus vaginalis*, an effective delivery vehicle that can present OMPI chlamydial antigen to the immune system. This genetic system is a hallmark because we aim to improve chlamydial antigen delivery utilizing *Lactobacillus vaginalis* as a live carrier. This investigation will proceed to the transformation of *Lactobacillus vaginalis* with pGKOMPI. Additionally, we will analyze the ability of *Lactobacillus vaginalis* to express pGKOMPI and its ability to act as an antigen delivery vehicle.

MATERIALS AND METHODS

Isolation of Plasmid pGK12 from *Escherichia coli* GM1829

E. coli cultures supplied by the laboratory of Dr. Todd Klaenhammer at North Carolina State University, Food Science Department were prepared in chloramphenicol supplemented Luria-Bertani (LB) medium and incubated overnight. Plasmid pGK12 was isolated from the *E. coli* cultures by following the QIAprep Spin Miniprep Kit using a Microcentrifuge (QIAGEN Inc., Valencia, CA). Final DNA concentration was confirmed using the GeneQuant Pro (Amersham Biosciences, Pittsburgh, PA), and gel electrophoresis confirmed the plasmid size.

Plasmid pGK12 Processing

Plasmid pGK12 DNA isolated by QIAprep Spin Miniprep Kit using a Microcentrifuge (QIAGEN Inc., Valencia, CA). The purified plasmid was digested with restriction enzymes, and gel electrophoresis was performed noting the restriction enzymes making only one cut such as Cla I. Plasmid pGK12 was double digested with Cla I and Bcl I. Gel electrophoresis was performed to confirm its digestion. 4.0 kb is the approximate size of the plasmid after the double digestion.

pVAX Vector System Processing

The isolation of the multiple cloning site from pVAX was performed by PCR amplification. The PCR procedure was performed using specific primers that were made for the 5' and 3' ends which ensures that the whole multiple cloning site is inserted. The primers used were specific for Cla I, the forward primer and Bcl I, the reverse primer (Integrated DNA Technology, Coralville, IA). Gel electrophoresis was performed to confirm PCR amplification. The amplified product was purified using the QIAquick PCR Purification procedure (QIAGEN Inc., Valencia, CA).

Ligation

The ligation of the multiple cloning site into the linearized vector, pGK12, was performed using the LigaFast Rapid DNA Ligation System (Promega, San Luis Obispo, CA). The ligated pGK12 and pVAX multiple cloning site product, is termed pGKVAX. To confirm the ligation pGK12 and pVAX multiple cloining site, DNA sequencing was performed.

Transformation of DH5α *E. coli* with Plasmid Construct pGKVAX and Selection of Transformants on Chloramphenicol Supplemented Media

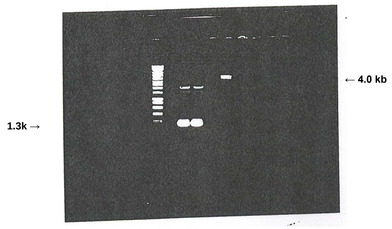
Competent *E. coli* strain DH5α cells were transformed with pGKVAX by heat shock method and propagated on LB broth agar plates supplemented with chloramphenicol. Colonies were isolated and cultures were prepared. Plasmid pGKVAX was isolated from the transformed DH5α cells by the QIAprep Spin Miniprep Kit using a Microcentrifuge (QIAGEN Inc., Valencia, CA. Restriction enzymes, Hpa II, Pst I, and Cla I were used to digest the pGKVAX construct to identify possible sites for OMP1 insertion.

Omp-I Processing

Omp-I was amplified by polyermase chain reaction using primers specific for restriction sites, Bcl1 and Not1, located within the pGKVAX MCS.

RESULTS

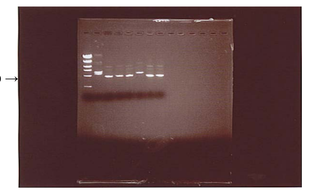
Figure 3: pGK12 and pVAX Processing



Lanes 2 & 3 Amplified pVax MCS
Lane 4 pGK12 digested w/ Cla I & Bcl I

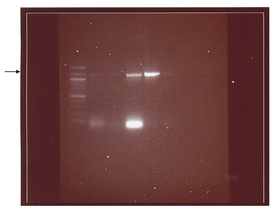
Results: The multiple cloning site from pVAX was isolated by PCR amplification using primers specific for Cla I and Bcl I. pGK12 was digested with Cla I and Bcl I.

Figure 4: pGKVAX isolated from DH5α Clones



Results: Lanes 3, 4, 5, 7, and 8 denote positive clones because they are 4.0 kb.

Figure 8: Omp-I PCR Product

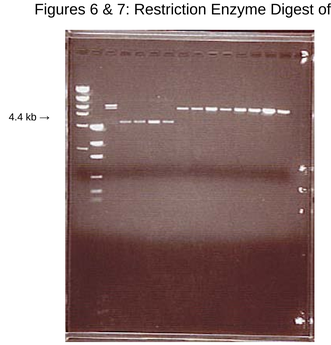


Results: Omp-I (Lanes 4 & 5) was amplified using primers specific for Bcl 1 and Not 1.

Figure 5: Insertion of pVAX MCS.

Results: **DNA sequencing confirmed the ligation of the multiple cloning site from pVAX and digested pGK12.**

Figures 6 & 7: Restriction Enzyme Digest of pGKVAX



Results: Pst I and Cal I (Lanes 8-16) linearize pGKVAX and Hpa II (Lanes 4-7) removes a segment of the construct.

Results: Bcl 1 (Lane 2) linearizes pGKVAX and Kpn1 (Lanes 3-6) removes a segment of the construct.

SUMMARY

- **This study provides evidence of the construction of recombinant plasmid pGKVAX.**
- **Recombinant plasmid pGKVAX can be used for the ligation of Omp-I.through Pst 1, Bcl 1, and Not 1 restriction sites located within the MCS**
- **A vaccine utilizing recombinant plasmid pGKOmp-I may possibly induce a strong cell-mediated and humoral immune response in a mouse model, protecting against *Chlamydia trachomatis* challenge.**
- **This vaccine may be applicable to other animals and humans, and the techniques used for its development may also be used for the development of other vaccines.**

FUTURE STUDIES

Ligation of pGKVAX and Omp-I

Analysis of Omp-I expression

Transformation of *Lactobacillus vaginalis* with pGKOMP1

Evaluation of Immune Response in a murine model

ACKNOWLEDGEMENTS

Dr. Godwin Ananaba, Mr. Eno Ekong, Dr. Godwin Ifere, Dr. Tesfaye Belay, Dr. Francis O. Eko, and Dr. Joseph Igietseme

MBRS-SCORE and the Department of Biological Sciences, Clark Atlanta University

Department of Microbiology/Biochemistry/Immunology, Morehouse School of Medicine

Dr. Todd Klaenhammer and Ms. Evelyn Durmaz, Food Science Department, North Carolina State University for *Escherichia coli* GM1829

PHS grants: GM08247, RR03062, and A141231

MBRS RISE Program-NIH-NIGMS Grant #5R25GM060414