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THE PURIFICATION OF HELICOBACTER PYLORI UREASE β SUBUNIT PROTEIN AND DNA FOR VACCINE PRODUCTION

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THE PURIFICATION OF *HELICOBACTER PYLORI* UREASE β SUBUNIT PROTEIN AND DNA FOR VACCINE PRODUCTION

A Thesis

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Abstract

The purpose of this research was to start development of a vaccine against *Helicobacter pylori.* In our study, the focus was on the urease β subunit (*ure*B) of *Helicobacter pylori.* Urease is crucial for the bacterium's colonization of the gastrointestinal tract. Highly purified *ure*B DNA was isolated so that immunogenicity of the DNA could be tested. In addition, a pQE9 or pYES2NT plasmid containing *ure*B DNA was transformed into *Escherichia coli* BL21(DE3) cells or *Saccharomyces cerevisiae* strain 1 cells. Induction of the transformed cells produced a large amount of his-tagged ureB protein, which was purified using a nickel affinity column. The ureB protein produced in *E. coli* was used in mouse studies, while that produced in yeast was used in ELISAs. Once an adequate amount of protein and plasmid DNA were purified, they were injected into mice in various combinations. Mice injected with protein, or DNA and proteinhad immunogenic reactions to ureB. However, mice injected with only DNA did not mount an immune response.. Thus, results of my study demonstrate that ureB protein has more potential to be developed into a vaccine for production against *H. pylori* than ureB DNA.

Acknowledgements

A tremendous amount of appreciation goes to Dr. Rodolfo Bégué for letting me work on this project in his laboratory during the spring semester of 2006. Most of all, I want to thank him for being a great teacher who was always supportive and patient.

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Thank you to Dr. Sue Bartlett for her encouragement, kindness, and patience. I am so thankful for her because she took on the challenge to be my committee director on a project that I performed in New Orleans. I deeply respect her and have a great deal of gratitude for her.

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List of Figures

Introduction

Helicobacter pylori is a gram negative, spiral, microaerophilic, flagellated bacterium (Del Giudice et al., 2001). Although *Helicobacter pylori* has been known for many years to be present in the stomach, only recently has the role of the bacterium in disease been demonstrated. As reviewed by Ernst et al. (2006), Barry Marshall and Robin Warren suggested *H. pylori* was the cause of gastroduodenal disease. Dr. Marshall went so far as to intentionally infect himself with *Helicobacter pylori* in order to recover the bacterium from his stomach lining after the onset of the infection (Ernst et al., 2006).

As reviewed by Lee et al., *Helicobacter pylori* causes over 50% of gastroduodenal infections in developed countries and, more astoundingly, greater than 90% of such infections in people in developing countries. A short term infection could cause chronic gastritis or peptic ulcer disease, and a long term infection could lead to gastric adenocarcinoma or gastric B cell lymphoma (Lee et al., 1995). Although the length of time of an infection is critical, the severity of the resulting disease depends on an individual's immunity system (Futagami et al., 1998). In view of the fact that most developing countries have a larger ratio of people acquiring the bacterium in childhood, they are the ones with higher chances of forming gastric cancer (Imrie et al., 2001).

Antibiotics are reasonably effective in treating patients with symptomatic *Helicobacter pylori* infection. However, the increased use of antibiotics for patients with alleged *Helicobacter pylori* infections has led to antibiotic-resistant strains. In addition, antibiotic treatments are highly expensive and limited in number, making it impossible for everyone to even have the chance of being treated (Michetti, 1997).

Michetti and others suggest that alternative strategies are crucial for an inexpensive approach to stop future colonization of the bacterium or remove residing bacteria in order to establish a global control over *H. pylori* infections (Michetti, 1997; Lee et al., 1995). One alternative would be development of a vaccine that could be administered to an individual once and prevent infection for a lifetime. As a result, much work has focused on the genome and proteome of *Helicobacter pylori*.

Helicobacter pylori contains several proteins that help it survive while it is infecting the host. These virulence factors include urease, the vacuolating cytotoxin (VacA), the cytotoxin-associated antigen (CagA), the neutrophil-activating protein (NAP), and others (Del Giudice et al., 2001). These antigens are crucial players in the bacterium's ability to survive in the acidic environment of the gastrointestinal tract. Flagella is important for the bacterium's motility. BabA and HpaA are important for adherence to epithelial cells in the stomach. Urease and catalase are important enzymes crucial for colonization. NAP, CagA, and VacA are toxins produced in order to induce inflammatory reactions (Svennerholm, 2003). Any one of these virulence factors could be the target for a vaccine. Some researchers even suggest more than one antigen interfering with the pathogenic infection should be combined. However, these antigens should not cause any inflammatory response in the host since this would worsen the progression of gastritis (Corthesy et al., 2005; Svennerholm, 2003).

Ernst et al. (2006) and Del Giudice et al. (2001) identified urease as a major focus for vaccine development since it makes up between 5%-10% of the total cellular protein and its enzymatic activity is crucial for the bacterium's survival in an acidic environment. In fact, urease allows *Helicobacter pylori* to resist the low pH of the stomach since it

mconverts urea into ammonia and CO_2 and thus provides a buffer that allows the bacterium to entrench further into the mucosal lining of the stomach or intestines. In addition, a urease-negative mutant strain of *H. pylori* is incapable of colonizing gnotobiotic piglets (Eaton et al., 1991).

The urease gene cluster contains 9 genes that are clustered within an 8 kb region of the genome. The *ure*A and *ure*B genes encode the structural proteins of urease subunits, and the other genes encode accessory proteins (Tsuda et al., 1994). The two polypeptides making up urease are the 30,000 dalton urease α subunit and the 62,000 dalton urease β subunit (Labigne et al., 1991; Dunn et al., 1990). Michetti et al. (1994) found enzymatic activity is not necessary for protection. Thus, most researchers have focused their attention on the β subunit because it contains the catalytic site. Mouse stomachs immunized with UreB were 100% urease negative, while mouse stomachs immunized with UreA were only 50% urease negative (Ferrero et al., 1994). Furthermore, oral immunization with recombinant *H. pylori* urease B subunit eradicates a preexisting *H. felis* infection (Corthesy-Theulaz et al., 1995).

In summary, vaccines containing adjuvants will be more useful in stopping *Helicobacter pylori* infections than antibiotics and urease may be the crucial antigen for invoking an immune response to a new infection or to an old infection. Adjuvants are particularly important for inducing protection in a host. Del Giudice et al. (2001) suggest protection is caused for two reasons. Adjuvants help increase the quality and quantity of antigen specific antibodies. In addition, they increase the types of immune responses stimulated (Del Giudice et al., 2001). Adjuvants can be added to protein based vaccines or DNA based vaccines. The concept of using purified DNA in a vaccine is a rather new

approach started within the past 10-15 years. There are two possible advantages to using a DNA vaccine. One advantage is the vaccine would be easier and cheaper to make and the second advantage is the shelf life of the vaccine would be longer. However, to date there is insufficient data on the efficacy of DNA vaccines.

The goal of this research project was to carry out the initial experiments that would lead to production of a ureB vaccine. Toward this end ureB DNA was isolated and purified. In addition, methods were developed for expression of ureB protein in *E. coli* and *Saccharomyces cerevisiae*. Finally, sufficient ureB protein was purified from each organism to use in vaccines or ELISAs, respectively.

Methods and Materials

Digestion of plasmid DNA (pQE9/*ure*B) using Sal I restriction enzyme

The pQE9/*ure*B plasmid was constructed by Dr. Andres Ramgoolan and Dr. Rodolfo Bégué. For this, the 1.6 kilobase pair gene encoding the urease B subunit (*ure*B) (GenBank accession number: AF352376) was amplified by polymerase chain reaction (PCR) using *Helicobacter pylori* genomic DNA as template, and the product was cloned into the Sal I site of the pQE9 vector (Quiagen) and transformed into *E. coli* XL10 Gold cells (Invitrogen). Successful cloning of the *ure*B gene was confirmed by restriction enzyme digestion of 0.3 µg of pQE9/*ure*B with Sal I and subjecting the digested DNA to 1% agarose gel electrophoresis (showing the 1.6 kbp *ure*B segment). Sequencing (done at LSUHSC) confirmed that the 1.6 kbp insert was the *ure*B gene. For large scale preparation of plasmid DNA to be used as an antigen, *ure*B was cloned into pTopo which can express ureB under control of a cytomegalovirus promoter.

Plasmid Preparation

Small and large scale preparations of plasmid DNA were performed using the QIAprep Spin Miniprep Kit (Qiagen) or the QIAprep Megaprep Kit (Qiagen), respectively.

E. coli XL10 Gold Transformation

Next, 0.6 µg of the plasmid was used to transform *E. coli* XL10 Gold cells. After incubation with the plasmid (30 minutes at 4°C), the cells were heat shocked at 42°C for 1 minute, and then incubated at 37°C for 1 hour in LB. The cells were pelleted by centrifugation and the pellet was resuspended and plated on Luria-Bertani (LB) medium containing ampicillin for selection of transformed cells.

E. coli BL21(DE3) Transformation

A similar protocol as above was used, as recommended by the manufacturer (Novagen). Briefly, 0.3 ug plasmid was used to transform *E. coli* BL21(DE3) cells. The transformed cells were heat shocked at 42°C for 30 seconds, centrifuged to pellet the cells, resuspended and plated in LB medium containing ampicillin.

24 Hour Induction Time Course in *E.coli*

Transformed *E. coli* XL10 Gold and BL21(DE3) cells were grown overnight in 10 ml of LB media containing ampicillin (100 µg/ml) in a 37°C shaking incubator. The next day, the overnight cultures were transferred to new tubes containing 90 ml of LB plus ampicillin. After a 1 hour incubation, 200 µl of 0.5 M IPTG was added to make a final concentration of 1 mM. 2.5 mls of culture were collected before IPTG was added as well as 2, 4, 6, 8, 10, and 24 hours following the IPTG induction. 1.5 mls were collected to have their absorbance checked at 600 nm. 1 ml was centrifuged at max speed in a minicentrifuge for 10 minutes. The supernatant was decanted and the pellet was resuspended in SDS buffer and distilled water, sonicated for cell lysis, and kept at –20°C.

Protein Expression (small scale) in *E. coli*

The procedure recommended in the Qiagen manual was followed in order to express the recombinant protein (rUreB) contained in the construct pQE9/*ure*B. Stock bacteria of *E. coli* BL21(DE3) cells were grown overnight in three ml of LB media containing ampicillin (100 µg/ml) in a 37°C shaking incubator. The next day, 200 µl of the overnight culture was transferred to a new tube containing two ml of LB plus

ampicillin. After a 1 hour incubation, IPTG (0.5 M isopropylthio-B-D-galactoside)(Invitrogen) was added to a final concentration of 1 mM. Since the *ure*B gene is under the control of the lac promoter, transcription is activated in the presence of galactose residues. After three hours of incubation, 1 ml of culture was taken from the tube and centrifuged. The supernatant was decanted and the pellet was resuspended in sodium dodecyl sulfate (SDS) buffer (950 µl of Laemmli Sample Buffer and 50 µl of 2 B-mercapto-ethanol)(Biorad), sonicated for cell lysis, and kept at –20°C.

Protein Expression in *E. coli* (large scale)

The procedure recommended in the Qiagen manual was followed in order to express the recombinant protein in large amounts. On the first day, *E. coli* BL21(DE3) cells containing the pQE9/*ure*B plasmid were grown in 20 ml of LB containing 100 µg/ml ampicillin. On the second day, the first day's growth was transferred into 500 ml of LB containing 100 µg/ml ampicillin. IPTG (1 mM final concentration) was added to induce protein expression. The cells were harvested by centrifugation and were resuspended in urea buffer (100 mM NaH_2PO_4, 10 mM Tris-Cl, 8 M urea, pH 8.0) to lyse the cells. The cells were pelleted by centrifugation and were resuspended in 0.1 mM EGTA/0.25% Tween to remove the protein from the cell pellet.

Protein Expression (large scale) in Yeast

Saccharomyces cerevisiae strain 1 cells (INVSc1; Invitrogen) were transformed with the pYES2NT/*ure*B plasmid constructed by Dr. Rodolfo Bégué. In this construct, expression of *ure*B is under control of the gal 1 promoter. To produce yeast-derived rUreB (y-UreB), the INVSc1/pYES2NT/ *ure*B stock cells were suspended in yeast growth medium and plated onto synthetically derived medium minus uracil (SD-Ura)

and incubated at 30°C for several days. A single colony was picked from the plate and regrown in 25 ml SD-Ura 2% glucose at 30°C in a shaking incubator overnight. The culture was then poured into 75 ml SD-Ura 2% glucose media and incubated again in a 30°C shaking incubator overnight. The next day, cells were pelleted by centrifugation, resuspended in10 ml water, and added to 500 mls of SD-Ura plus 2% galactose for induction of protein expression. After overnight growth, the cells were harvested by centrifugation and were resuspended in breaking buffer (water, 0.1 M sodium phosphate pH 7.4, 100% glycerol, 500 mM EDTA, 100 mM PMSF). Glass beads were added to the solution to mechanically lyse the cells (cycles of vortexing and ice incubation, one minute each, six times). The cells debris was then pelleted by centrifugation and resuspended in EGTA/0.25% Tween to release the protein.

Protein Purification

A nickel column (Ni-NTA Superflow Columns, Quiagen) was installed in order to purify the protein. Since the pQE9 vector or the pYES2NT vector adds six histidines (His-6) to the *ure*B or rUreB protein, the nickel residues bind the His-6 of the recombinant protein when the lysate is applied. The column was then washed with urea buffer at decreasing pH's (8.0, 6.3, 5.9, and 4.5). In order to determine the fractions with the most protein, absorbance at 280 nm (A_{280}) was measured. We determined that the bacterial protein eluted off of the column at pHs 5.9 and 4.5 and the yeast protein eluted off the column at pH 6.3. The protein content was analyzed by SDS- polyacrylamide gel electrophoresis (PAGE), protein staining with Coomassie Blue, BCA, and Western blot (detailed below).

Western Blot

In order to check for the expression of recombinant *ure*B protein, a western blot was performed. After the recombinant bacteria were lysed in reducing SDS buffer (950 µl of Laemmli Sample Buffer and 50 µl of 2 ß-mercaptoethanol (Biorad), the proteins were separated by SDS-PAGE through a 10% gel (40 minutes at 200 V) and transferred onto a nitrocellulose membrane (100 V for 90 minutes). The gel was rocked for an hour in Blocking Buffer (100 ml PBS, 0.1% Tween, 3 g powdered milk). The primary antibody, 1:4,000 dilution of Rabbit-$\alpha(His)_6$ in blocking buffer, was added next. After washing with PBS, the secondary antibody, 1:4,000 dilution of Goat α Rabbit IgG conjugated to horseradish peroxidase in blocking buffer, was added for detection of rUreB. Chemiluminescent substrate solution (Pierce) was sprinkled over the nitrocellulose membrane and chemiluminescence was detected using a BioRad VersaDoc Transilluminator.

Concentrating and Exchanging Purified Protein's Urea Buffer to PBS

Vivaspin-20 (50,000 Molecular Weight cutoff) concentrators were used to exchange the urea buffers to PBS and to concentrate the proteins produced in *Saccharomyces cerevisiae* and *Escherichia coli*.

Results and Discussion

Isolation of Purified *ure*B DNA

A plasmid purification was performed on a large scale to confirm the cloning of ureB into Topo 10. The agarose gel confirmed that the digestions worked and that the *ure*B DNA is the correct size. There was no digestion in lane C, therefore there would only be one band. The Bstx1 digestion in lane B was correct because it produced a 1.6 kbp band, and the BamHI digestion in lane A was correct because it produced a 1 kbp band. There was a final yield of 1,767 µg of purified *ure*B DNA.

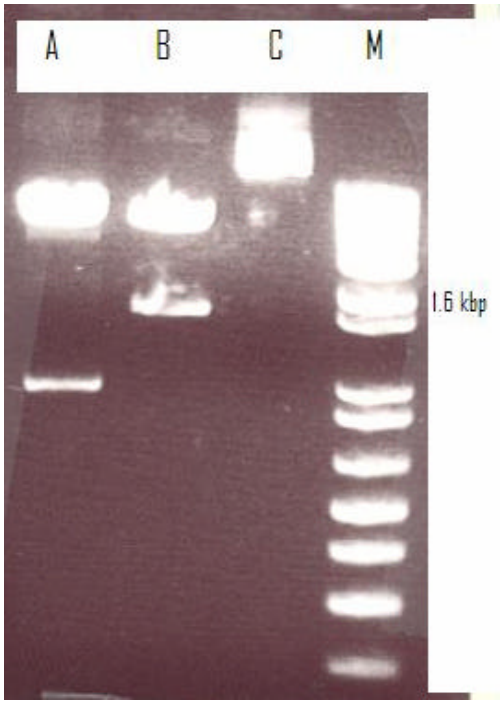


Figure 1. Agarose gel of plasmid DNA digestions. Lane A: BamHI digestion; lane B: Bstx1 digestion; lane C:plasmid pQE9/*ure*B not digested; lane M: 1 kb Plus DNA ladder marker.

Selecting Host Cells for ureB Expression

Since the stock of *E. coli* XL10Gold cells transformed with pQE9/ureB was no longer expressing ureB protein, nine colonies of newly transformed *E.coli* XL10Gold and BL21(DE3) cells were induced to check for expression. The Western blots in Figure 2 confirmed that the 64 kDa ureB protein was expressed. Lane C in Picture A and lane D in Picture B represent the best cultures for the *E. coli* XL10 Gold and BL21(DE3) cells.

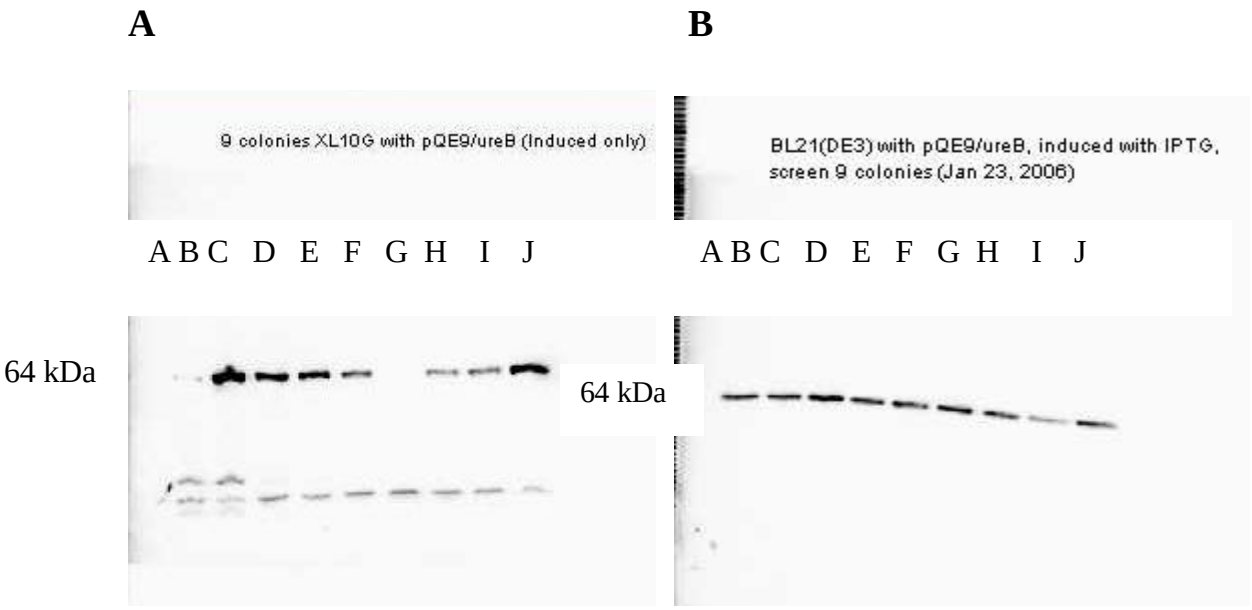


Figure 2. Western blots comparing the expression of rUreB in eighteen cultures of (A) *E. coli* XL10 Gold cells and (B) *E. coli* BL21(DE3) cells transformed with pQE9/ureB. The proteins were separated on a 10% SDS-PAGE gel and transferred to nitrocellulose. The primary antibody was a 1:4000 dilution of Rabbit-$\alpha(His)_6$ in blocking buffer and the secondary antibody was a 1:4000 dilution of Goat α Rabbit IgG conjugated to horseradish peroxidase in blocking buffer. The recombinant protein is about 64 kDa.

Time Course

As shown in Figure 2, Lane C in Picture A and lane D in Picture B represent the best isolates of *E. coli* XL10 Gold and BL21(DE3) cells, respectively. These two isolates were induced with IPTG separately in 250 ml of LB media. Samples were taken at specified times after induction to check for expression of the 64 kDa ureB protein. The best culture was lane C in picture B, which represents a four hour induction period. Note that there was no induction in the XL10 Gold cells.

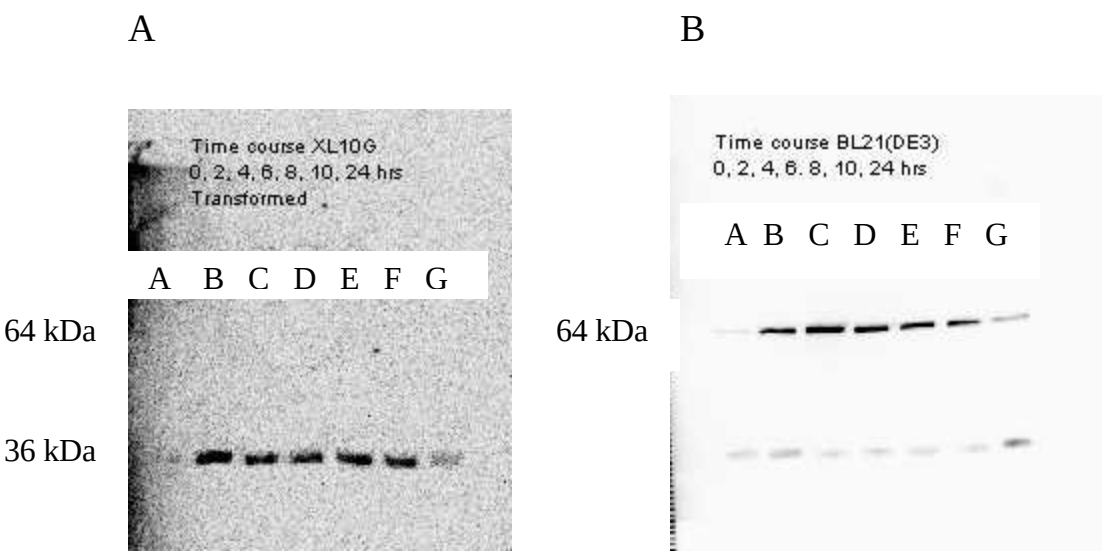


Figure 3. Western blots of (A) *E. coli* XL10 Gold cells and (B) *E. coli* BL21(DE3) competent cells transformed with pQE9/ureB. (A- Enhanced picture of the induced samples) Lane A: 0 hours; lane B: 2 hours; lane C: 4 hours; lane D: 6 hours; lane E: 8 hours; lane F: 10 hours; lane G: 24 hours. (B) Lane A: 0 hours; lane B: 2 hours; lane C, 4 hours; lane D, 6 hours; lane E: 8 hours; lane F: 10 hours; lane G: 24 hours. Method as shown in figure 1.

First Induction

A large scale expression of *E. coli* BL21(DE3) cells transformed with pQE9/ureB previously shown in Figure 2 as the best culture was regrown. The Western blot in

Figure 4 shows that protein has only been produced in the uninduced sample. Therefore, the process of finding competent cells producing the ureB protein started again.

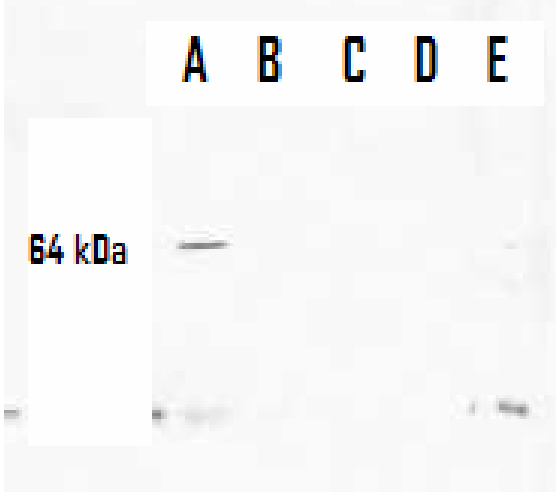


Figure 4. Western blot of *E. coli* BL21(DE3) competent cells transformed with pQE9/ureB. Lane M: Invitrogen See Blue Plus 2 Pre-Stained Standard marker; lane A: uninduced sample; lane B: induced sample; lane C: supernatant; lane D: clear lysate; lane E: pellet. Method as shown in figure 1.

Small Scale Expression of *ure*B in *E. coli*

In order to obtain optimal expression of ureB, expression in *E.coli* XL10 Gold and BL21(DE3) cells was compared again. The Western blot in Figure 5 confirmed that *E. coli* BL21(DE3) cells were the better choice since there was more rUreB protein produced by the sample in lane C at the 64 kDa band. The optimized procedure consists of *E.coli* BL21(DE3) cells induced for 4 hours, unsonicated, and run on a 10% polyacrylamide gel.

Figure 5. Western blot of comparing expression of rUreB in *E. coli* XL10 Gold and BL21(DE3) cells. Lanes A through D: *E. coli* BL21(DE3) cells transformed with pQE9/ureB; lanes E through H: XL10 Gold *E. coli* cells transformed with pQE9/ureB. Method as shown in figure 1.

Large Scale Expression of ureB in *E. coli*

The Western blot in Figure 3 shows the 64 kDa band typical of the ureB protein is present in the pellet. In previous work, the *ure*B protein was present in the lysate, but our bacterial system produced a different result.

Figure 6. Western blot of *E. coli* BL21(DE3) competent cells transformed with pQE9/ureB. Lane M: Invitrogen See Blue Plus 2 Pre-Stained Standard marker; lane A: uninduced sample; lane B: induced sample; lane C: supernatant; lane D: cleared lysate ; lane E: pellet. Method as shown in figure 1.

Confirmation that rUreB was removed from the pellet (small scale expression)

The rUreB protein was extracted from the pellet of the BL21(DE3) *E.coli* cells using 0.25% Tween 20 and 0.1 mM EGTA. The Western blot in Figure 7 confirms that ureB protein was extracted from the pellet and was present in one wash.

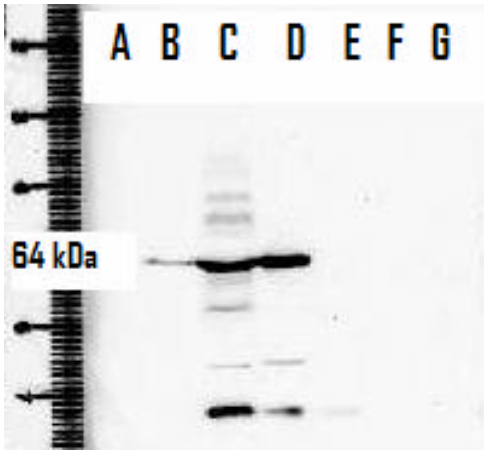


Figure 7. Western blot of *E. coli* BL21(DE3) competent cells transformed with pQE9/ureB. Lane A: Invitrogen See Blue Plus 2 Pre-Stained Standard marker; lane B: clear lysate; lane C: pellet; lane D: wash 1; lane E: wash 2; lane F: wash3; lane G: wash 4. Method as shown in figure 1.

Confirmation that rUreB was removed from the pellet (large scale expression)

Since the rUreB protein was successfully extracted from the pellet of the BL21(DE3) *E.coli* cells using 0.25% Tween 20 and 0.1 mM EGTA in the smaller expression test, the same procedure was performed in the larger expression test. The Western blot in Figure 8 confirms that ureB protein was extracted from the pellet and was present in three washes.

Figure 8. Western blot of *E. coli* BL21(DE3) competent cells transformed with pQE9/ureB. Lane M: Invitrogen See Blue Plus 2 Pre-Stained Standard marker; lane A: pellet; lane B: wash 1; lane C: wash 2; lane D: wash 3; lane E: pellet resuspended in SDS. Method as shown in figure 1.

Confirmation that ureB elutes from nickel columns at low pH

The filtered washes of protein collected using 0.25% Tween 20 and 0.1 mM EGTA were run over a nickel column because the pQE9 vector adds a $(His)_6$ tag on the C terminal of the expressed protein. The SDS-PAGE gel and the western blot in Figure 9 confirm that the protein was eluted most efficiently by buffer D (pH 5.9) and buffer E (pH 4.5). The SDS-PAGE gel was run to check for any contaminants within the sample and the western blot was performed to check for our specific 64 kDa recombinant protein.

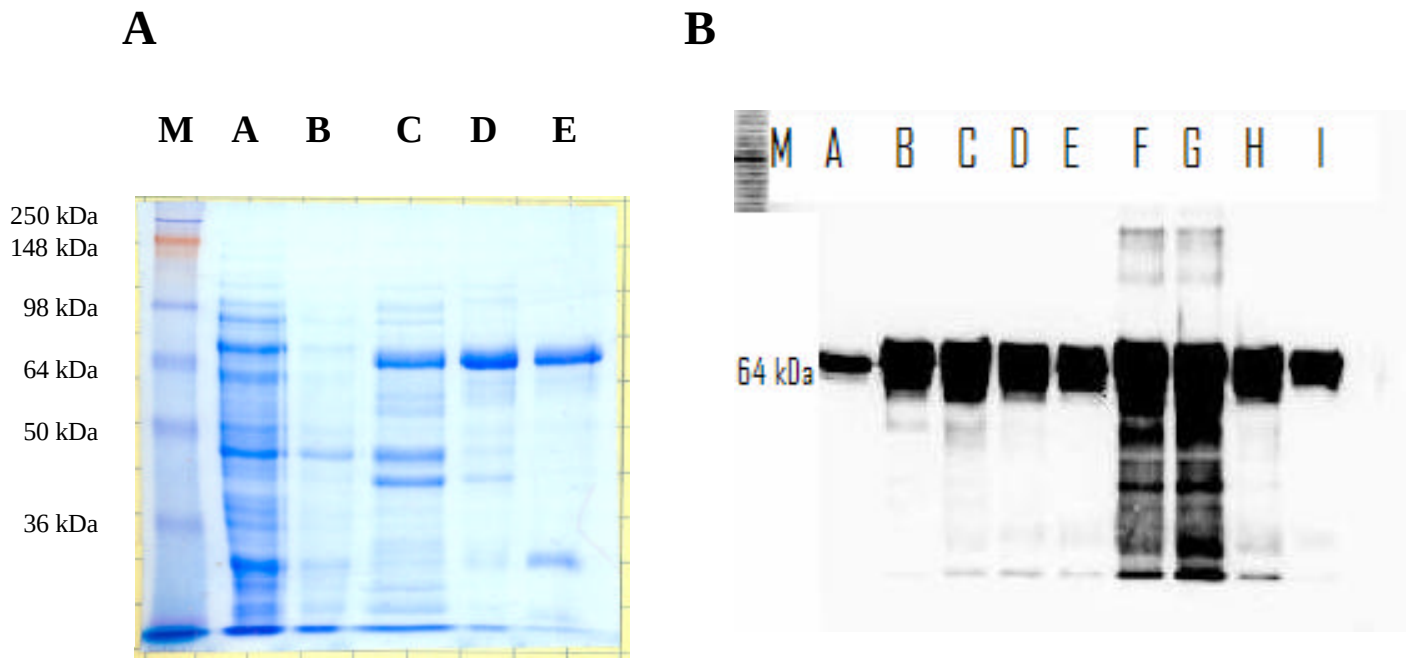


Figure 9. Polyacrylamide gel (A) and western blot (B) of eluates from the Nickel column binding made up of recombinant purified protein. (A) Lane M: Marker; lane A: flow-through; lane B: buffer B (pH 8.0); lane C: buffer C (pH 6.4); lane D: buffer D (pH 5.9); lane E: buffer E (pH 4.5). (B) Lane M: Marker; lane A: flow-through; lane B: 20 ml of buffer C-pH 6.3; lane C, first 7 ml of buffer D-pH 5.9; lane D, second 7 ml of buffer D-pH 5.9; lane E, third 6 ml of buffer D-pH 5.9; lane F, first 5 ml of buffer E-pH 4.5; lane G, second 5 ml of buffer E-pH 4.5; lane H, third 5 ml of buffer E-pH 4.5; lane I, fourth 5 ml of buffer E-pH4.5.Method as shown in Figure 1.

Concentration of purified ureB protein

All of the eluates at pH 5.9 and 4.5 were concentrated and their buffer was exchanged from the urea buffers to phosphate buffered saline (PBS). The polyacrylamide gel was run to check for the purity of the recombinant protein typically present at 64 kDa. The BCA assay kit was used to quantitate the purified protein a final yield of 13.70 mg per 500 mls cell culture was obtained.

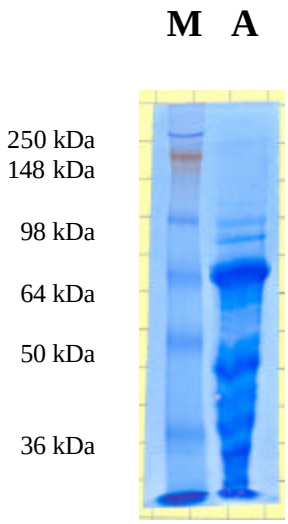


Figure 10. Polyacrylamide gel of concentrated rUreB protein. Lane M: Marker; lane A: rUreB protein.

Extraction of ureB from yeast

The rUreB protein was purified from yeast to be later used in ELISAs. Although the induction of growth was different for the yeast compared to the *Escherichia coli* cells, the purification using EGTA washes and nickel columns was the same. The Western blot in Figure 11 confirms that the protein was extracted from the pellet and was present in the washes.

Figure 11. Western blot of pYES2NT/*ure*B plasmid protein produced in yeast by transformation of Invitrogen *Saccharomyces cerevisiae* strain 1 cells (INVSc1). Lane M: Marker; lane A: lysate; lane B: lysate filtered; lane C: wash 1; lane D: wash 1 filtered; lane E: wash 2; lane F: wash 2 filtered; lane G: negative control of untransformed INVSc1. Method as shown in figure 1.

Purification of ureB expressed in yeast

The filtered washes of the transformed yeast cells were run over a nickel column and eluted by a gradient of pH buffers. The Western blot in Figure 12 confirmed that the yeast rUreB protein eluted from the column at pH 6.3 (buffer C).

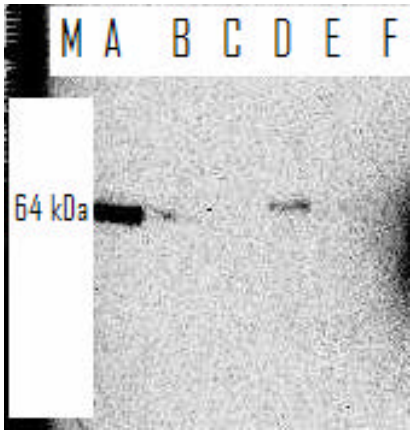


Figure 12. Western blot of INVSc1 cells transformed with pYES2NT/*ure*B plasmid. Lane M: Marker; lane A: lysate and washes; lane B: flow-through; lane C: buffer B-pH

8.0; lane D: buffer C-pH 6.3; lane E: buffer D-pH 5.9; lane F: buffer E-pH 4.5. Method as shown in figure 1.

Confirmation of Concentrated Yeast ureB Protein

The buffer C aliquot was concentrated in a Vivaspin-20 (50,000 MW cut-off) concentrator and the buffer was exchanged from urea buffer to phosphate buffered saline. A polyacrylamide gel was run to check for the purity of the 64 kDa yeast rUreB protein. The final yield of yeast rUreB was 129.4 µg per 500 ml culture.

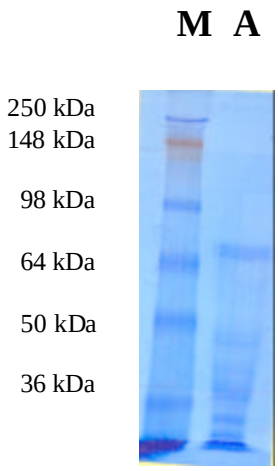


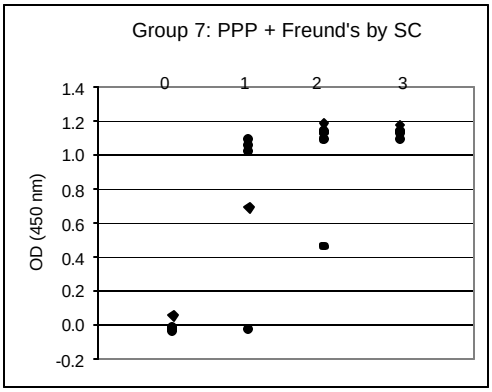
Figure 13. Polyacrylamide gel electrophoresis of concentrated Y-rUreB transformed in INVSc1 cells. Lane M: Marker; lane A: concentrated Y-rUreB.

Immunogenicity of Protein and/or DNA in Mice

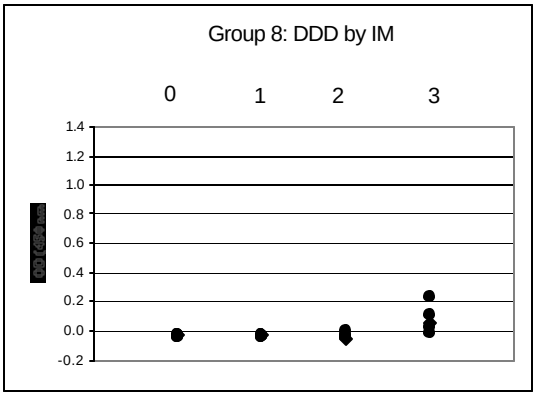
Mice were injected by Dr. Rodolfo Bégué three times with various combinations of rUreB protein and/or DNA. Serum was collected in order to test for urease B specific antibodies by an ELISA using the purified yeast rUreB protein. The following graphs confirm that the purified protein alone creates a faster and better immunogenic reaction than the DNA alone. In fact, it is not yet clear whether there is a weak immunogenic response, or no response, when DNA alone was injected. Also, the combination of

purified *ure*B DNA and protein create the same immunogenic reaction except that injecting the protein before the DNA produced a quicker response than DNA injections followed by protein injections. The reasons for the lag in an immunogenic response when DNA precedes protein as an antigen are not clear. Overall, the purified protein product worked in producing an immunogenic response in the mice studies. An adjuvant was not added to the DNA, which may be the cause its apparent failure to induce an immune response. Since DNA vaccines have only been developed recently, it is not clear what adjuvant is best to use with the DNA to induce an immunogenic response.

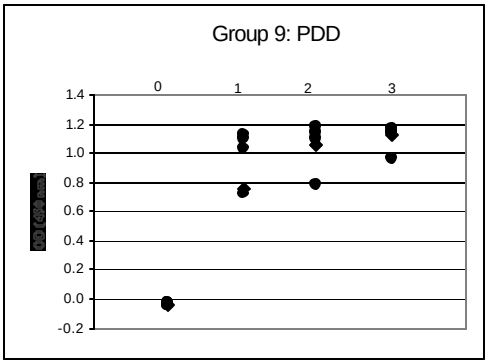
A



B



C



D

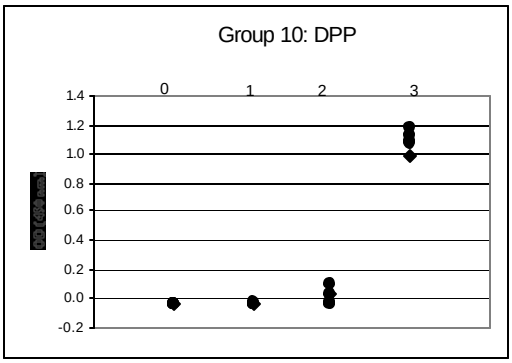


Figure 14. ELISA of four groups of BALB/c mice injected with purified *ure*B DNA and/or rUreB protein. The X axis indicates the number of injections the mice had received at the time the serum was drawn. The Y axis shows OD_{450} of the ELISA assay. Graph A showed the immunogenic response produced in five mice given three injections of purified protein. Graph B showed the immunogenic response produced in five mice given three injections of purified DNA. Graph C showed the immunogenic response produced in five mice given a protein injection followed by two DNA injections. Graph D showed the immunogenic response produced in five mice given a DNA injection followed by two protein injections.

Conclusions and Future Research

There were two goals for this project. One goal was successfully express and purify the urease β subunit protein from *Escherichia coli* and *Saccharomyces cerevisiae* expressing the his-tagged recombinant protein. The second goal was to produce enough purified recombinant protein and DNA to be tested as a vaccine for *in vivo* mice studies. This project was successful in accomplishing both of those goals. The first goal took a long time to achieve since brand new bacterial and yeast systems had to be developed. Once a method was established, it was easy to start growing larger amounts of the protein. Purification was laborious but eventually a product of enough purity and quantity was produced to be used for *in vivo* studies in mice. Even though the mice studies were not a part of this project, immunization of mice elicited a good immune response. Therefore, the quality of the products made (recombinant protein and DNA plasmid) was confirmed.

Further *in vivo* studies will be performed to check for the protection of mice against colonization by *Helicobacter pylori*. Eventually, *in vivo* studies will be performed in more complex animal systems and then humans. The long-term goal of the project is the development of a working vaccine against *Helicobacter pylori* suitable for use in humans.

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