Cloning and Expression of Vacuolating Cytotoxin A (*VacA*) Antigenic Protein in *Nicotiana benthamiana* Leaves a Potential Source of the Vaccine against *Helicobacter pylori*

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ABSTRACT

Background: The applications of transgenic plants in the healthcare system are immense. They offer an alternative strategy for the fabrication of antigenic determinants of medically important pathogens. **Objectives:** Cloning and transient expression of the vacuolating cytotoxin A (*vacA*) gene of *Helicobacter pylori* in *Nicotiana benthamiana* is undertaken in the present study. **Methods:** The *vacA* gene of *H. pylori* was amplified. The *vacA* and pBI121 vectors were digested with BamHI and SacI and the *vacA* gene was cloned in pBI121 by T4 ligation. The vacA-pBI121 construct was transformed into *Escherichia coli* DH5α and the transformants were confirmed by isolation and sequencing of vacA-pBI121. Further, the vacA-pBI121 was transformed into *Agrobacterium tumefaciens* EHA105 by electroporation. The transformants were used for agroinfection of *N. benthamiana* by agroinjection technique and the transgenic plant was screened for *vacA* gene expression by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). **Results:** The *vacA* gene amplification was confirmed by observing an intense DNA band in agarose electrophoresis. Sequencing of *vacA* gene of *E. coli* DH5α transformants indicated a gene size of about 2877bp which revealed 99.82% sequence similarity with online available *H. pylori vacA* gene sequence. The *A. tumefaciens* EHA105 transformants were confirmed by amplification of the *vacA* gene. The screening of transgenic leaves of *N. benthamiana* for *vacA* gene expression by SDS-PAGE showed *VacA* protein with a molecular weight of 105kDa. **Conclusion:** A novel transgenic plant expressing *VacA* protein was developed as a source of eco-friendly-based synthesis of antigenic determinants for various medical applications.

Key words: Transgenic plant, Cloning *vacA* gene, VacA determinant, Transient expression, *Nicotiana* sp., Peptic ulcer.

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INTRODUCTION

India was known to have a good collection of medicinal plants, which have been used for many years to extract drugs and perfumery products. Over many decades of efforts, plants with favorable characteristics have been produced by time-consuming conventional breeding methods.[1] To overcome this drawback genetic engineering was evolved as an alternative technology. Genetic engineering can be used to produce plants resistant against extreme conditions of temperature, salinity, drought, and also to produce monoclonal antibodies and vaccines.[2,3]

Transgenic plants also serve as a substitute resource for the production and supply of vaccines called edible vaccines.[4] In 1990, for the first time the "edible vaccine" term was coined which are nothing but, edible GM crops that contain added "immunity" against specific diseases.[5] These vaccines are being developed against several diseases like Hepatitis B and C, foot and mouth disease, cholera, and measles.[4] The transgenic plant is also used as well-established recombinant technology in the production of biopharmaceuticals like therapeutic protein, vaccine, and antigenic determinants. It is an environmentally friendly, robust, low cost, and easily scalable. Transgenic plants can be developed using a simple and low-cost agroinjection technique used for transfer for the gene of interest in plants through *A. tumefaciens* infection.[6] Agroinjection is more efficient, provides rapid scalability, easy genetic modifications, and low cost.[7] Moreover, the yield of protein is *Nicotiana* species can

be enhanced by adapting transient expression foreign protein. The transient expression has several advantages like a high yield of antibody production in less time compared to stable expression of the protein.[8]

Dwarf tobacco plant *Nicotiana benthamiana* from the Solanaceae family is a native Australian species and is an allotetraploid with 19 chromosomes in its genome.[9] In-plant virology, *N. benthamiana* is the frequently used host as most of the plant viruses can infect the plant successfully. Genes coding for beta-glucuronidase and green fluorescent protein have been successfully transferred and expressed in *N. benthamiana*.[10] *N. benthamiana* has been used for the production of many antibodies against hepatitis viruses, Influenza, Ebola virus, and coronavirus.[11]

H. pylori bacterium is a helical-shaped, motile, microaerophilic, Gram-negative in cell wall nature was discovered by Robin Warren in 1979. Approximately, 70 to 90% population of age of fewer than 10 years in developing nations are a carrier of infection caused by the *H. pylori* bacterium.[12] Also the *H. pylori* infection is associated with various disorders of including gastritis, 10–15% of peptic ulcer disease,1–3% of gastric cancer, and 0.1% of mucosa-associated lymphoid tissue lymphoma.[13] In *H. pylori*, the cytotoxin-associated gene A and vacA are the main antigenic determinants and the pathogen also possess several

adhesions to facilitate gastric mucosal adhesions and vaccine design against *H. pylori* is an challenging task.[14,15]

MATERIALS AND METHODS

Amplification of *vacA* gene and preparation of *vacA*-pBI121 construct

The genomic DNA of *H. pylori* ATCC 26695 (the United States of America, procured through Genei) was used as the template for the amplification of *vacA* gene (~2877bp) using specific forward primer "atatcaggatccATGAATAAAACCCCAGATAAACCCGA" and reverse primer "tatcgaGAGCTCttaAGCGTTAGCCCAAACATTGGTAG" (Invitrogen). Polymerase chain reaction (PCR) reaction mixture of 25µl containing 50ng genomic DNA of *H. pylori*, Taq DNA polymerase of 1 unit, 0.2mM each dNTPs, PCR buffer of 1X, each *vacA* primer at 10pM and 3mM $MgCl_2$ was amplified in a gradient PCR. The amplification conditions were set for 35 cycles with denaturation carried out at 94°C for 1 min, annealing of DNA at 55°C for 1min followed by extension step for 10min at 72°C. The PCR product was electrophoresed at 100volts on 0.1% agarose gel in 1X TAE buffer. The gel was stained with 1µg/ml ethidium bromide and the DNA bands were observed. Furthermore, the DNA fragments from agarose gel were purified using Genei Gel extraction Kit.

Restriction digestion of *vacA* fragment and pBI121

The gene and pBI121 (contributed by Dr. Manmohan M. ICAR, Indian Institute of Horticulture Research) were restriction digested separately. Digestion of pBI121 was carried out in a reaction mixture consisting of 163.9µl double distilled (DD) water, 20µl 10X assay buffer D, 16µl plasmid DNA and *BamHI* and *SacI* of 2µl each. The *vacA* digestion was carried in the same reaction mixture except for the volume of DD water and PCR product added were 120µl and 60µl respectively. Both vials were incubated at 37°C for 2h. The digested products were separated on 1% agarose gel followed by this, both fragments were recovered from gel using Genei Gel extraction kit.

Ligation of vacA fragment into pBI121

The *vacA* fragment was ligated into the pBI121 vector using a ligation reaction mixture. The ligation was carried out in a vial containing 11µl of DD water, 2µl 10X ligase buffer, 1µl T4 DNA ligase, 5µl *BamHI/SacI* digested pBI121 vector and 6µl of *BamHI/SacI* digested vacA gene fragment. The binary vector pBI121 was maintained as the experimental control. Both vials were incubated at 16°C overnight and the ligation was confirmed by electrophoresis of the ligation mixture on the agarose gel.

Preparation of competent *E. coli* DH5α and transformation

The *vacA*-pBI121 construct was transformed into the competent *E. coli* DH5α (genotype F', *endA1 hsdR17* (rk–mk+) *glnV44 thi-1 recA1gyrA* (Nalr) *relA1*(*lacZY, AargF*)*U169deo RΦ* 80d*lac*(*lacZ*)M15, Genei) cells by the calcium chloride method.[16] A single colony *E. coli* DH5α grown in LB agar (Genei) was inoculated in sterile LB broth and incubated at 37°C till the culture O.D.$_{A660nm}$ reaches 0.4-0.5. The culture was chilled for 30 min and cells were centrifuged at 3000rpm for 15min at 4°C. The pellet was suspended in 30ml of an ice-cold salt buffer consist of 100mM $CaCl_2$, 70mM $MgCl_2$ and 40mM Sodium acetate of pH 5.2 to 5.5. The solution was incubated for 45 min on ice and centrifuged again. The pellet was dissolved in acid salt buffer with 20% glycerol and stored at -70°C. The solution was incubated for 30min on ice followed by the heat shock for 5min at 37°C to transfer *vac*A-pBI121 construct into competent cells. On an LB agar plate containing kanamycin (20µg/ml),

the solution was subcultured and the plate was incubated at 37°C for 12h. After incubation, the transformants containing *vacA*-PBI121 were selected.

Positive clones confirmation by purification of recombinant *vacA*-pBI121

The recombinant *vacA*-pBI121 from transformants of *E. coli* DH5α was isolated by alkaline lysis protocol.[17] The vial containing cell pellet of *E. coli* DH5α collected by centrifugation 5ml of 12h broth at 6000rpm 4°C for 5min, 200µl of pre-chilled solution I, and II were mixed, and kept for 5min on ice. After 5min, 200µl of solution III was added, mixed gently and the solution was incubated on ice for 10min. The cell lysate was centrifuged for 10min at 4°C at 8000rpm and to the supernatant, an equal volume of phenol:chloroform:iso-amyl alcohol (25:24:1:v/v) was added, mixed gently and re-centrifuged. To the aqueous phase, an equal volume of pre-chilled isopropanol was added, mixed, and re-centrifuged. Pellet collected was washed with 70% alcohol and dried for 2h. The purity of the *vacA*-pBI121 was checked on 0.8% agarose gel electrophoresis and used for positive clones confirmation by restriction digestion and DNA sequencing.

Confirmation of clones by *vacA* homology study using nBlast

The query nucleotide sequence, *vacA* gene was matched with the homologous sequences deposited in the NCBI (National Center for Biotechnology Information) database using the online available nBlast tool. The percentage sequence similarity from the online database similar to the query sequence was calculated. The phylogenetic tree was constructed using the query sequence and identical sequences from the NCBI database using ClustalW.

Plant material

N. benthamiana seeds were surface sterilization with 100% ethanol for 1min followed by 15min treatment with 50% (v/v) Clorox solution containing 3% sodium hypochlorite. Seeds were washed in sterile water and blotted in Whatman filter paper. In standard germination soil containing 10 cm^2 plastic pots, 15 seeds of *N. benthamiana* were sown and pots were maintained in the growth conditions of temperature 25°C, 70% humidity, and 15h light and 9h dark photoperiod with 74 mol/m^2/s light intensity. Followed by 8 days of sowing, seedlings were transferred to potting soil containing 10 cm plastic pots (one seedling per pot) and grown in 12h light and 12h dark photoperiod with 159 mol/m^2/s light intensity with 60% humidity at 22°C. After reaching optimal developmental stage they were used for agroinfiltration (4–5 week old).[18]

Transformation of *Agrobacterium tumefaciens* with *vacA*-pBI121 vector

To prepare competent cells, *A. tumefaciens* strain EHA105 (contributed by Dr. Manmohan M. ICAR, IIHR) was grown on an LB agar plate containing 25µg/ml rifamycin. In 5ml of LB broth containing 25µg/ml rifamycin, a single colony of *A. tumefaciens* strain EHA105 was inoculated and incubated overnight at 28°C in a shaker incubator at 200rpm. In 200ml of LB medium, 2ml of the overnight culture was inoculated and grown till it reaches OD$_{600}$ of 0.6. The broth was chilled for 10min and cells were recovered by centrifugation at 4°C for 15min at 4000rpm. The cell pellet was washed with 1M HEPES buffer (Genei) of pH 7.0 thrice.[19] The above cells were transformed with vacA-pBI121 construct by electroporation.[19] In a pre-chilled 2mm electroporation cuvette; 50µl of competent cells was mixed with 1µl of *vacA*-pBI121. The mixture was pulsed for 5micro sec at 2800V, followed by immediate inoculation in 1ml LB medium and incubation at 28°C for 2h in 200rpm

shaking condition. The culture was inoculated in an LB plate containing antibiotic and incubated at 28°C for 72h. Transformation of *vacA*-pBI121 in *A. tumefaciens* was confirmed by conducting colony PCR technique using *vacA* specific primers.

Agroinfiltration

Agroinfiltration of *A. tumefaciens* EHA105 containing vacA-pBI121 into *N. benthamiana* has performed by the agroinjection method.[20] In 5ml of LB medium containing kanamycin, a distinct colony of *A. tumefaciens* EHA105 was inoculated and incubated overnight at 28°C. In 25 ml LB broth, 1ml of the overnight culture was inoculated and incubated overnight at 28°C. The culture was centrifuged at 5,000rpm for 15min and the pellet was resuspended in suspension solution. The culture $O.D_{A600}$ was adjusted to 0.4. Four to five-week-old leaves of *N. benthamiana* were placed on the bench at room temperature for 2-3h (or overnight) before used for infiltration. bacterial culture was injected using a 5ml syringe simply by pressing the syringe (no needle) on the underside of the leaf by avoid cotyledons and exerting a counter-pressure with a finger on the other side. Additionally, a vacuum pressure of 80 mbar was applied for 1.5min. The plants were incubated in a growth room with 8h dark, 16h light at 25°C with 55–65% humidity until harvesting of leaves which lasted 2 to 4 days.

Screening of vacA gene expression in transgenic plant

Protein purification

The crude leaves extract of *N. benthamiana* was prepared by crushing in extraction buffer (pH 7.5) containing 1%Triton X-100 and 100 mM Tris/HCl. The extract was centrifuged for 5min at 18407rpm and to the supernatant containing VacA, 50% saturated $(NH_4)_2SO_4$ solution was added to precipitate protein which was further purified by anti-VacA specific antibody (IgG) affinity column by washing with RX buffer of pH 7.3 (KCl -10mM, NaCl-0.3mM, $MgCl_2$-0.35mM, and 0.125mM EGTA containing HEPES-1 mM). The protein precipitate was eluted with glycine-HCl-50mM, pH 1 to activate VacA. Lastly, inactivation of VacA was done at 95°C for 10 min and protein was stored at -80°C.[21]

Determination of molecular weight of the protein

The transgenic plants were screened for the expression of the *vacA* gene using the Genei SDS-PAGE kit. The VacA protein purified from leaves extract and standard marker proteins were mixed with 2X loading buffer in equal proportion. The mixtures were heated for 5 min at 95°C with sudden quenching on ice and loaded in 12.5% polyacrylamide gel and electrophoresed in 1X reservoir buffer for 2 h at 10mA. The gel was stained in 20ml of Ezee blue staining solution containing Coomassie Brilliant Blue R-250 and the protein bands were visualized and molecular weight on VacA protein was determined.

RESULTS

Amplification of *vacA* gene and preparation of *vacA*-pBI121 construct

The *vacA* gene was successfully amplified in PCR using *vacA* specific forward and reverse primers and gDNA of *H. pylori* as a template. The gel documentation results of agarose gel showed intense DNA bands (run in triplicate in lanes 1-3 as shown in Figure 1) of amplified PCR product consist of *vacA* gene. When distance traveled by amplified bands compared with distance traveled by DNA markers, the bands were observed between 2.5 and 3.0 kb of DNA markers (Figure 1). Determination of the molecular size of the amplified product on agarose gel matched with the original size of the *vacA* gene *i.e.* ~2877bp.

Restriction digestion of *vacA* fragment and pBI121and T_4 ligation

The plasmid PBI121 and an amplified *vacA* gene were digested with *BamHI* and *SacI*. Both the *vacA* fragment and the linearized PBI121 vector were recovered from agarose gel and ligated by T_4 process. The agarose electrophoresis of the ligated sample indicated a single intense DNA (Lanes 2 to 6 in Figure 2). On contrary, the control sample containing only pBI121 (lane 1 in Figure 2) indicated a single band.

Preparation of competent *E. coli* DH5α and transformation

The ligated sample *i.e.,* a *vacA*-pBI121 construct was transformed into the competent *E. coli* DH5α cells which were grown on LB agar medium
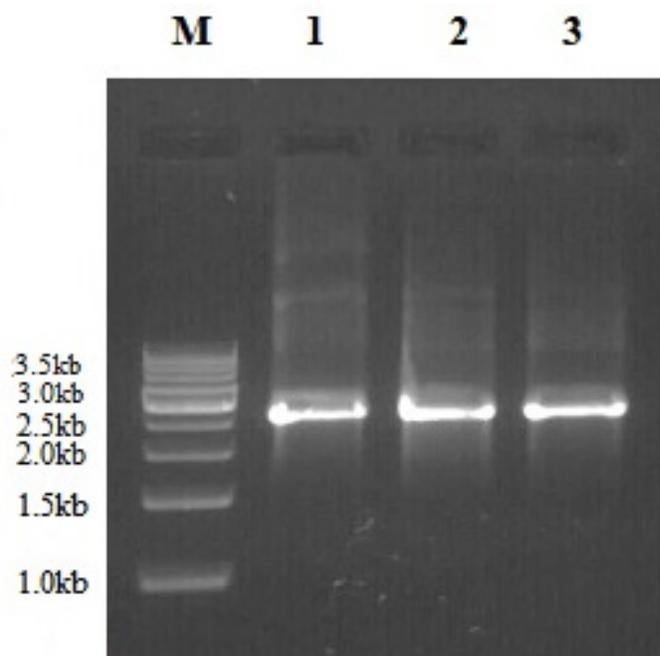
Figure 1: Agarose gel indicating an amplified product consists of the vacA gene.
Lane M: DNA markers, Lane 1-3: bands of amplified products consist of vacA gene.
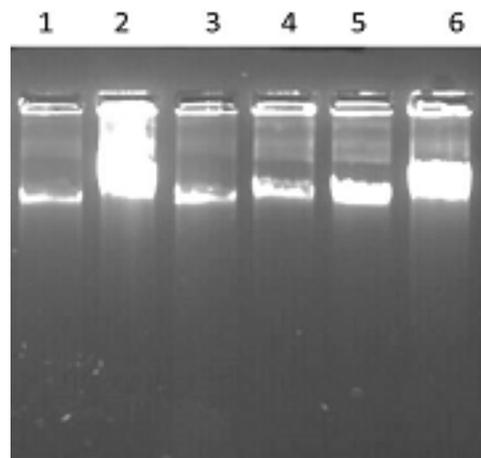
Figure 2: Ligation of vacA gene in pBI121 vector.
Lane 1: PBI121 plasmid DNA, Lane 2-6: vacA-pBI121 plasmid DNA.

containing kanamycin. After incubation, *E. coli* DH5α transformants formed colonies which confirmed the successful transformation of a *vacA*-pBI121 construct. These positive clones were further confirmed by isolation, restriction digestion, and sequencing of a *vacA*-pBI121 construct.

Positive clones confirmation by purification of recombinant *vacA*-pBI121

The positive clones of *E. coli* DH5α were selected by isolating the *vacA*-PBI121 construct from them to confirm the cloning of the *vacA* gene. The *vacA*-PBI121 construct was digested with the restriction enzymes *BamHI* and *SacI* and separated on agarose gel indicated two bands (lanes 2 and 4 in Figure 3) which correspond to *vacA* gene and *pBI121* plasmid DNA respectively. The relative migration of the second band in lanes 2 and 4 was slightly before 3 kb, this is because *vacA* gene size is approximately 2877bp. Lane 1 and 3 show the DNA band of plasmid pBI121 used as control. The result indicated cloning of *vacA* gene in pBI121 binary vector and its transformation into the *E. coli* DH5α.

Confirmation of *vacA* cloning by homology studies using Blast

The cloning of *vacA* in pBI121 was further confirmed by sequencing the *vacA* gene. The sequence analysis using the nBlast web interface available at the NCBI indicated 99.82% similarity and homologous to the sequence of *H. pylori* strain (Figure 4). The phylogenetic tree was constructed using identical sequences (Figure 5).

Plant material

The seedling of *N. benthamiana* shifted to 10 cm^2 plastic pots containing potting soil from germination soil revealed the optimal growth stage of plants after 3 weeks of transfer. At this stage no flower buds were visible and few leaves were well developed.
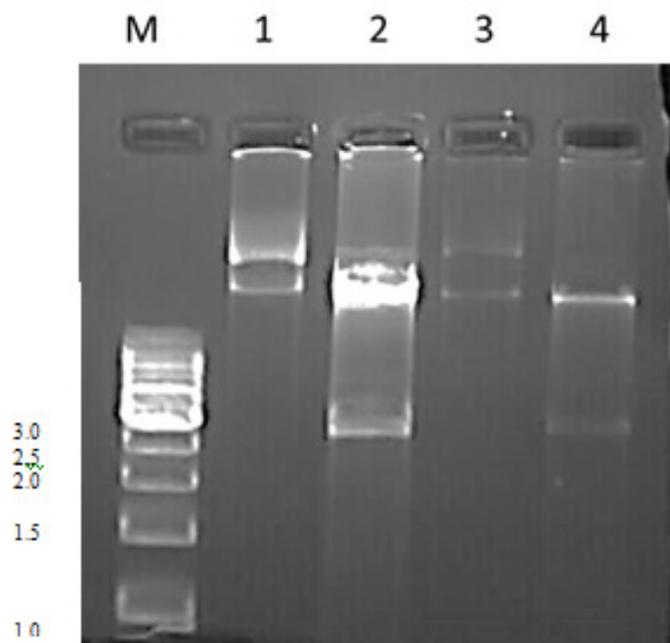
Agrobacterium tumefaciens transformation with *vacA*-PBI121 construct

The expression cassette *vacA*-pBI121 with added *BamHI* and *SacI* restriction sites was transformed into the competent cells of *A. tumefaciens* strain EHA105. The transformants of *A. tumefaciens* strain EHA105 confirmed by colony PCR technique revealed a successful amplification of the *vacA* gene when vacA gene-specific primers are used for amplification.

Agroinfiltration

Among the agroinfiltration techniques, Agro-injection techniques offer direct injection of inoculum into the plant enhancing rapid infection. Successful infiltration of *A. tumefaciens* strain EHA105 containing *vacA*-pBI121 expression cassette was often noticed as a spreading "wetting" area in the leaf. The successful infiltration was further confirmed by studying the expression of the *vacA* gene product in transgenic *N. benthamiana* (Figure 6).

Screening of *vacA* gene expression in transgenic plant

Protein purification and determination of the molecular weight of the protein

The expression of the *vacA* gene in the transgenic plant of *N. benthamiana* was confirmed by purification of VacA protein from leaves extract and determining the molecular weight of the VacA protein by SDS-PAGE. The polyacrylamide gel revealed various protein bands with a different molecular weight in marker proteins and leaves extract of the transgenic plant. Molecular weight determination of unknown protein band of transgenic leaves extracts revealed 105kDa VacA protein (Figure 7). The

Description	Query Cover	Per. Ident	Accession
Helicobacter pylori strain dRdM2addM2 chromosome, complete genome	100%	96.35%	CP026515.1
Helicobacter pylori strain 26695-dR chromosome, complete genome	100%	96.35%	CP026326.1
Helicobacter pylori strain 26695-dRdM1dM2 chromosome, complete genome	100%	96.35%	CP026323.1
Helicobacter pylori strain 26695-dRdM2 chromosome, complete genome	100%	96.35%	CP026324.1
Helicobacter pylori strain dRdM1 chromosome, complete genome	100%	96.35%	CP026325.1
Helicobacter pylori strain 26695-1MET, complete genome	100%	96.35%	CP010436.1
Helicobacter pylori 26695-1CL DNA, complete genome	100%	96.35%	AP013356.1
Helicobacter pylori 26695-1CH DNA, complete genome	100%	96.35%	AP013355.1
Helicobacter pylori 26695-1 DNA, complete genome	100%	96.35%	AP013354.1
Helicobacter pylori 26695, complete genome	100%	96.35%	AE000511.1
Helicobacter pylori Rif2, complete genome	100%	96.35%	CP003906.1
Helicobacter pylori Rif1, complete genome	100%	96.35%	CP003905.1
Helicobacter pylori strain HP42K chromosome, complete genome	100%	91.86%	CP034314.1

Figure 4: nBlast result of homology search for cloned vacA gene sequence.

Figure 3: Positive clone confirmation by vacA-pBI121 digestion. M Lane-1kb ladder DNA, Lane 1 and 3- vacA-PBI121 construct, Lane 2 and 4- vac A and pBI121

Figure 5: Phylogenetic analysis of cloned *vacA* gene sequence.

results confirmed successful cloning of the *vacA* gene in *N. benthamiana* which can be used as a bioreactor for the production of the antigenic determinant of VacA proteins.

DISCUSSION

Plants are being exploited for various purposes in the field of science as a resource of medicines for various human illnesses. Traditionally, though plants are majorly used as sources of drugs, the recent innovation in recombinant technology has established an excellent platform for plant-derived biopharmaceutical compounds production like bacterial

Figure 6: Transgenic plant of *N. benthamiana* expressing VacA protein.

Figure 7: Screening of VacA protein in transgenic *N. benthamiana*. Lane 1 to 6-VacA, Lane 7- VacA Control, Lane 8-Marker proteins.

and viral antigenic proteins, antibodies, and human therapeutic proteins. Production of biopharmaceutical proteins in transgenic plants is accomplished either by stable transformation (direct) or transient transformation (indirect) of recombinant gene(s) encoded for therapeutic protein(s).[22] Meanwhile, *H. pylori* infections are found to be worldwide prevalent. The regional prevalence data of 2015 revealed that there are around 4.4 billion cases of *H.pylori* infection have been reported all over the world.[23] Moreover, the emergence of drug resistance in *H. pylori* is further worsening the situation.[24] Hence, a great need arose for early detection and prevention of *H. pylori* infection for which the present study is undertaken.

The present investigation focuses on the cloning and transient expression of the *vacA* gene in leaves of *N. benthamiana* as a source for the production of the antigenic determinant of *H. pylori* by adopting recombinant DNA technology. The virulence gene *vacA* was amplified and cloned in the pBI121 vector. The vector consists of the *vacA* gene that was transferred into *E. coli* DH5α. The *vacA*-pBI121 construct purified from *E. coli* DH5α was further transformed into competent cells of *A. tumefaciens* EHA105. The *A. tumefaciens* EHA105 containing *vacA*-PBI121 was infiltrated into the leaves of *N. benthamiana* to develop the transgenic plant. Cloning and transient expression of the *vacA* gene in the transgenic plant were confirmed by identification of *vacA* gene product *i.e.,* VacA protein of molecular weight 105kDa by conducting SDS-PAGE.

The experimental outcome was also confirmed by the published scientific literature. The *vacA* gene consists of approximately 2877bp encoded for 959 amino acids and each amino acid's average molecular mass is 110 Daltons thereby contributing an overall molecular mass of 105490 or 105.5 Daltons VacA protein. It has been reported that, in *H. pylori,* the VacA toxin is expressed as 140kDa precursor protein. On reaching the bacterial cell surface the 40kDa C-terminal domain is excised from 140kDa which is essential for extracellular secretion. The mature 95kDa protein presents both in the extracellular domain and on the bacterial cell surface. In *H. pylori* cell supernatant, the 95kDa matured protein is recovered as 58kDa and 37kDa protein fragments.[25] Expression study of *N. benthamiana* leaves extract by the SDS-PAGE confirmed the successful cloning of *vacA* gene and its expression in the transgenic plant.

The current research is the first scientific report on the cloning and transient expression of *vacA* gene in leaves of *N. benthamiana* which has wide diagnostic and medical applications. The antigenic determinants synthesis of VacA protein by adapting present investigation plays a crucial role in medical diagnosis, vaccine, or anti-VacA-antibody production against *H. pylori*. Furthermore, the antibiotic resistance in *H. pylori* has further intensified the need for the current research for the prevention, control, and eradication of *H. pylori* infections. The production of antigenic proteins by exploiting mammalian cell culture, viral, bacterial, and yeast cells suffers several drawbacks like high cost, difficulty in the purification of pure recombinant protein, and upscaling problems. Since the plant is an easily available resource in large quantity which is essential for upscaling of biopharmaceutical proteins production, hence the expression of antigenic proteins in transgenic plants circumvents the difficulties encountered in the traditional system of therapeutic protein production. The present study is the best example for the production of VacA antigen in the transgenic plant of *N. benthamiana* that has several potential advantages like easy scalability, eco-friendly, safe, and more economical.

CONCLUSION

Ease of access to antigenic determinants of pathogenic microbes plays a significant role in successful vaccine production. The present study proposes the ecofriendly ground for the production of VacA protein as
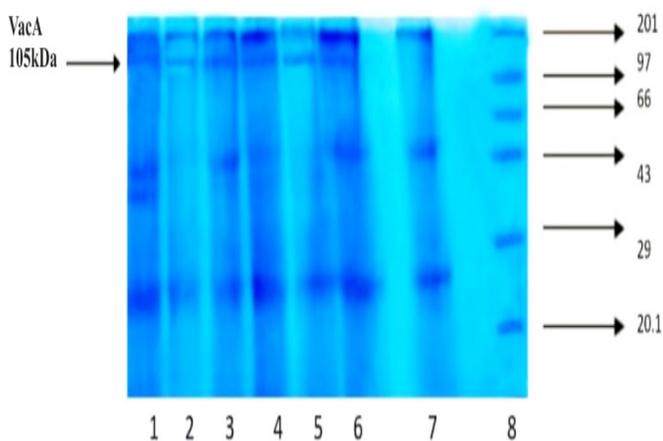
a source of antigenic protein expressed by cloning the *vacA* gene of *H. pylori* in *N. benthamiana*. Screening of *N. benthamiana* transgenic leaves for cloned *vacA* gene product by protein purification and determination of molecular weight revealed the expression of *vacA* protein of 105.5kDa. A novel transgenic plant of *N. benthamiana* expressing VacA protein was developed that unlocks huge applications in a various science discipline and play a significant role in the production of antigenic determinants used development of the novel vaccines in animals and human.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

VacA: Vacuolating cytotoxin A; **SDS-PAGE:** Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis; **LB:** Luria Bertini; **EtBr:** Ethidium bromide; **MES:** 2-(N-morpholino)ethanesulfonic acid; **HEPES:** (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; **DMSO:** DimethoxySulfoxide; **EGTA:** ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; **D.D water:** Double distilled water.

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