PlpE Epitopes of Pasteurella multocida Fusion Protein as Novel Subunit Vaccine Candidates
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Saied Mostaan, Abbas Ghasemzadeh, Parastoo Ehsani, Soroush Sardari, Mohammad Ali Shokrgozar, et al.. PlpE Epitopes of Pasteurella multocida Fusion Protein as Novel Subunit Vaccine Candidates. Advanced Biomedical Research, 2020, 9 (1), pp.43. 10.4103/abr.abr_245_19:10.4103/abr.abr_245_19. pasteur-03134189

HAL Id: pasteur-03134189
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PlpE Epitopes of Pasteurella multocida Fusion Protein as Novel Subunit Vaccine Candidates

Abstract

**Background:** Pasteurella multocida is the causative agent of many diseases. Antimicrobial treatment disadvantages highlight the need to find other possible ways such as prophylaxis to manage infections. Current vaccines against this agent include inactivated bacteria, live-attenuated bacteria, and nonpathogenic bacteria, which have disadvantages such as lack of immunogenicity, reactogenicity, or reversion to virulence wild bacteria. Using bioinformatical approaches, potentially immunogenic and protective epitopes identified and merged to design the best epitope fusion form in case of immunogenicity as a vaccine candidate. **Materials and Methods:** In this study, the fusion protein (PlpE1 + 2 + 3) and full PlpE genes (PlpE-Total) were cloned in pET28a in BL21 (DE3) firstly and later in pBAD/gIII A and expressed in Top10 Escherichia coli. Overlap polymerase chain reaction (PCR) using different primers for 5’ and 3’ end of each segment produced fusion segment 1 + 2 and (1 + 2) +3 fragments and was used for cloning. **Results:** Cloning of both PlpE1 + 2 + 3 and PlpE-Total into the pET28a vector and their transform into the BL21 (DE3) E. coli host was successful, as the presence of the cassettes was proved by digestion and colony PCR, however, their expression faced some challenges independent of expression inducer (isopropyl β-d-thiogalactopyranoside) concentration. **Conclusion:** Changing the vector to pBAD/gIII A and consequently changing the host to Top10 E. coli have resulted in sufficient expression, which shows that Top10 E. coli may be a good substitute for such cases. Furthermore, it is concluded that adding 8M urea results in sufficient purification, which hypothesizes that denature purification is better for such cases than native one. Purified proteins headed for further analysis as vaccine candidates.

**Keywords:** Fusion PlpE, Pasteurella Multocida, vaccine candidate

Introduction

*Pasteurella multocida* is a Gram-negative, nonmotile, nonspore-forming, and facultative aerobic/anaerobic coccobacillus that is the etiological agent of a wide range of animal diseases such as pneumonia in cattle and sheep, atrophic rhinitis in swine, hemorrhagic septicemia in buffalo and cattle, and fowl cholera in chicken.[1] P. multocida-related infections also suffer economic incomes due to its damage to sheep, goats, rabbits, poultry, and other livestock industries. besides high mortality rates, *P. multocida* inhibit gain weight of poultry and causes a shortage in production which suffer farm holders lifestyle. This bacterium is responsible for 30% of total cattle deaths around the world and losses of one billion dollars annually in this industry in North America alone.[3] Besides these findings, it is responsible for considerable economic losses of pigs, poultry, and cattle. It has also been isolated both from healthy animals as a member of the oropharyngeal flora of calves and does not cause a serious disease but, in stress conditions, it is one of the bacterial pathogens associated with disease.[4] *P. multocida* strains are classified into five capsular serogroups (A, B, D, E, and F) and 16 somatic lipopolysaccharide serotypes numbered from 1 to 16.[5]

Vaccination of livestock plays a vital role in improving the health and welfare of livestock and preventing animal to human transmission, which is one of the main goals of public health strategies. The present vaccine candidates against this agent consist of live-attenuated vaccines, which have several disadvantages such as induction of short-term or ineffective

How to cite this article: Mostaan S, Ghasemzadeh A, Ehsani P, Sardari S, Shokrgozar MA, Abolhassani M, et al. PlpE epitopes of Pasteurella multocida fusion protein as novel subunit vaccine candidates. Adv Biomed Res 2020;9:43.

Received: 13 November 2019
Revised: 08 January 2020
Accepted: 11 March 2020
Published: 28 August 2020

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Access this article online
Website: www.advbiores.net
DOI: 10.4103/abr.abr_245_19
Quick Response Code:

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SOEing PCR to construct a fusion form \((1 + 2)\) without a linker due to separated epitopes. Then, the latter DNA fragment was mixed with fragment 3 and fused using SOEing PCR program. The final product of overlap PCR is segment \(1 + 2 + 3\) as a fused form called \(\text{PlpE}1 + 2 + 3\).

For segments 1, 2, and 3, initial denaturation was at 94°C for 5 min, and denaturation and annealing and extension were at 94°C for 45 s, 55°C for 45 s, and 72°C for 30 s, respectively, and repeated for 30 cycles. The final extension was performed at 72°C for 7 min. For segments \(1 + 2\) and \((1 + 2) + 3\), PCR program is as above but needs pre-PCR stage. Primers designed considering overlap segments and restriction sites for \(NcoI\) and \(XhoI\). CCATGG sequence is a restriction site for \(NcoI\) and CTTCGAG sequence is restriction sites for \(XhoI\).

To merge segment 1 to segment 2 and also segment 1+2 to segment 3, a extra cycle called pre-PCR performed as 94°C for 3 minutes followed by 40°C for 5 minutes and finally 72°C for 1 min. Then, it was paused to add the related primers followed by PCR cycle, as discussed above.

### Cloning

The mixture was incubated at a temperature appropriate for that restriction enzyme (37°C) for 3–5 h. PCR products of \(\text{PlpE}-\text{Total}, \text{PlpE}1 + 2 + 3, \text{and pET}-28a(+)\) and \(\text{pBAD/gIII}\) A vectors were digested using \(NcoI\) and \(XhoI\) restriction enzymes. Then, the ligation of digested PCR fragments and vectors was done.

Briefly, 10 μL of 2X ligase buffer, 50 ng of vectors, 100 ng of PCR product, and 5 Weiss units of T4 DNA ligase were mixed and \(\text{H}_2\text{O}\) was added to total volume of 20 μL. Ligation was carried out as overnight incubation at 4°C. When the vectors were used, vector and insert DNA were mixed in 1:3 molar ratio, 5 Weiss units of T4 DNA Ligase (Fisher Scientific, USA) and 2 μL of 10X ligase buffer were added, and the mixture was incubated 16 h at 4°C.

Competent BL21 (DE3) (Novagen, Germany) and Top10 (Novagen, Germany) \(E.\text{coli}\) cells were prepared. Briefly, a single fresh \(E.\text{coli}\) colony was inoculated in LB medium until the OD600 reached 0.5–0.6 to obtain a middle of logarithmic phase culture. Then, the culture was incubated on ice and centrifuged at 3500 rpm for 5 min. The supernatant was decanted and the pellet was resuspended in ice-cold buffer. The cells were spun down at 3500 rpm for 5 min at 4°C. Finally, the supernatant was decanted and the pellet was resuspended gently. 100 μL of aliquots was stored at −80°C.

For transformation, briefly, 100 μL aliquot of competent \(E.\text{coli}\) was mixed gently with gel-extracted, cleaned up ligation products. The mixture was incubated on ice and heat shocked at 42°C and then incubated on ice. Luria-Bertani (LB) was added to the mixture and incubated at 37°C for 80 min by gentle agitation (100 rpm). The cells were centrifuged...
and resuspended in 100 µL of LB. Transformed cells were plated on selective medium containing appropriate antibiotic (100 µg/mL ampicillin or 30 µg/mL kanamycin).

Expression and purification

For the production of His-tagged fusion proteins, recombinant BL21 (DE3) E. coli were grown in Luria Broth containing isopropyl β-d-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1, 0.4, and 1 mM for the induction of gene expression under the control of phage T7 promoter (Kothari et al., 2006), and kanamycin was added to a final concentration of 30 µg/mL as a selection marker.

Recombinant E. coli Top10 were grown in Luria Broth containing serial dilution (0.2%, 0.02%, 0.002%, 0.0002%, and 0.00002%) of L-Arabinose (Merck, Germany) for the induction of gene expression. Ampicillin to a final concentration of 100 µg/mL was a selection marker.

Induced cultures were centrifuged and the pellets were resuspended in binding buffer (Ayalew et al., 2008) containing 8M urea. The cells were freeze-thawed 20 times, for 20 s, 10 s interval and then sonicated 20 times on ice, 20 s interval and the lysates were centrifuged and supernatants were saved. Ni-NTA (Qiagen) columns were used for the purification of fusion proteins on the bases of nickel affinity of histidine-tagged proteins. The purity of eluates was monitored on glycine-sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Western blotting is used to confirm the target proteins.

Results

Amplification of PlpE-Total and construction of the chimeric gene

Sequence evaluation

The sequencing result of amplified PlpE confirmed that P. multocida strain X-73 lipoprotein E (PlpE) gene (Genbank: EF219452.1) has been used.

Overlap PCR using different primers for 5’ and 3’ end of each segment produced fusion segment 1 + 2 and (1 + 2) +3 fragments and was used for cloning, as shown in figure 1. The final sequence of overlap fragments was 640 bp but not 680 bp.

pET-28a(+) and pBAD/gIII A vectors including gene of interest were transformed into the prepared BL21 (DE3) and Top10 competent E. coli, respectively. PCR performed to select 5 colonies which uptaken genes of interests, digestion (Ncol and Xhol) and colony.

Expression and purification

For gene expression in E. coli, the gene constructs and recombinant plasmids were introduced to the BL21 (DE3) and Top10 E. coli, and recombination was verified by restriction enzyme digestion, colony PCR, and sequencing. For the production of His-tagged fusion proteins, recombinant BL21 (DE3) E. coli were grown in Luria Broth containing IPTG to a final concentration of 0.5 mM for the induction of gene expression under the control of phage T7 promoter (Kothari et al., 2006) as supplier recommendation. Recombinant Top10 E. coli were grown in LB containing 0.002 mM L-arabinose which was the best concentration for both PlpE-Total and PlpEI + 2 + 3. Cloning into pET28a vector in BL21 (DE3) E. coli was successful but not its expression. The presence of the cassettes in the pBAD/gIII A vector in Top10 E. coli was confirmed and also its expression for both PlpE-Total and PlpEI + 2 + 3 had an adequate amount of proteins as shown in figure 2.

Discussion

Our goal was to identify polytope regions of PlpE protein common to all serotypes and construct a polytope, which offers the possibility of a vaccine that will induce protection against all P. multocida serotypes.

In our previous work, based on bioinformatics studies, three different conserved segments of PlpE fulfilled the designed criteria. Briefly, the selection of epitope region via IEDB tools, possessing high antigenicity via
immunomedicine group, is including different serotypes’ coverage via homology modeling, amelioration of half-life via ProtParam, and antibody accessibility. Conserved region 1 comprising amino acids (aa) 23–104 of PlpE, conserved region 2 from aa 135 to 202, and conserved region 3 from aa 262 to 321 are hypothesized as the most promising segments of PlpE to confer protection against *P. multocida*. The candidate fusion protein for cloning and expression was fragment 1 + fragment 2 + fragment 3.

In this study, the construction of both expression cassettes via pET28a vector within BL21 (DE3) *E. coli* was successful, however, the expression resulted no protein after evaluating 20 colonies on glycine-SDS-acrylamide gel despite successful cloning. Surprisingly, changing the vector to pBAD/gIII A within Top10 *E. coli* has resulted in sufficient expression, which hypothesizes that Top10 *E. coli* may be a good substitute for CodonPlus BL21 (DE3) *E. coli*.

**Conclusion**

We also came to this conclusion that 8M urea within lysis buffer results in sufficient purification, which hypothesizes that denature purification is better for such cases than native one 3 times in yield despite the gIII in vector which makes the target protein secretory.

Hatfaludi *et al.*, in 2012, used 10 mM of 2ME in addition of 8M urea to insoluble protein including PlpE for purification and showed that denature purification is suitable for PlpE protein despite its expression vector or host[12] which supports our hypothesis also.

Further studies are underway to evaluate in vivo immunogenicity of these cloned and expressed subunit candidate vaccines against infection by *P. multocida*.

**Acknowledgments**

We would like to show our gratitude to the Ben Adler, Emeritus Professor, Monash University for sharing their pearls of wisdom with us during the course of this research.

**Financial support and sponsorship**

This project was honorably sponsored by the Pasteur Institute of Iran as Ph.D. dissertation project.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Peng Z, Wang X, Zhou R, Chen H, Wilson BA, Wu B. *Pasteurella multocida*: Genotypes and genomics. Microbiol Mol Biol Rev 2019;83:e00014-19.
2. Wilson BA, Ho M. *Pasteurella multocida*: From zoonosis to cellular microbiology. Clin Microbiol Rev 2013;26:631-55.
3. Dabo SM, Taylor JD and Confer AW. Characterization of *Pasteurella multocida* and bovine respiratory disease. Animal Health Research Reviews 2007;8-2:129-150.
4. Ujvári B, Weiczner R, Deim Z, Terhes G, Tóth AR, et al. Characterization of *Pasteurella multocida* strains isolated from human infections. Comp Immunol Microbiol Infect Dis 2019;63:37-43.
5. Farahani MF, Esmaeiliad M, Jabbari AR. Investigation of iron uptake and virulence gene factors (fur, tonB, cxrD, exbB, hgbA, hgbB1, hgbB2 and tbpA) among isolates of *Pasteurella multocida* from Iran. Iran J Microbiol 2019;11:191-7.
6. Okerman L, Devriese LA. Failure of oil adjuvants to enhance immunity induced in mice by an inactivated rabbit *Pasteurella multocida* vaccine. Vaccine 1987;5:315-8.
7. Dowling A, Hodgson JC, Dagleish MP, Eckersall PD, Sales J. Pathophysiological and immune cell responses in calves prior to and following lung challenge with formalin-killed *Pasteurella multocida* biotype A: 3 and protection studies involving subsequent homologous live challenge. Vet Immunol Immunopathol 2004;100:197-207.
8. Hopkins BA, Huang TH, Olson LD. Differentiating turkey postvaccination isolants of *Pasteurella multocida* using arbitrarily primed polymerase chain reaction. Avian Dis 1998;42:265-74.
9. Mariana S, Hirst R. The immunogenicity and pathogenicity of *Pasteurella multocida* isolated from poultry in Indonesia. Vet Microbiol 2000;72:27-36.
10. Ahmad FA, Rammah SS, Sheweita SA, Haroun M, El-Sayed LH. Development of immunization trials against *Pasteurella*...
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11. Hansson M, Nygren PA, Ståhl S. Design and production of recombinant subunit vaccines. Biotechnol Appl Biochem 2000;32:95-107.

12. Hatfaludi T, Al-Hasani K, Gong L, Boyce JD, Ford M, Wilkie IW, et al. Screening of 71 *P. multocida* proteins for protective efficacy in a fowl cholera infection model and characterization of the protective antigen PlpE. PLoS One 2012;7:e39973.

13. Singh AP, Singh S, Ranjan R, Gupta SK, Singh VP, Sharma B. Molecular heterogeneity of plpE gene in Indian isolates of *Pasteurella multocida* and expression of recombinant PlpE in vaccine strain of *P. multocida* serotype B: 2. J Vet Sci 2010;11:227-33.
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