Cloning and expression of *Eimeria necatrix* microneme5 gene in *Escherichia coli*

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Abstract:

BACKGROUND: Coccidiosis caused by *Eimeria necatrix* has the most economic impact on poultry production. Microneme proteins in *Eimeria necatrix* are thought to be critical ligands determining host cell specificity at the time of invasion. **OBJECTIVES:** Isolation and purification of *Eimeria necatrix* oocysts from Khuzestan province of Iran was performed. A cDNA encoding microneme 5 (EnMIC5) protein was cloned and expressed as recombinant protein before the evaluation of its immunogenicity by Western blotting. **METHODS:** A primer pair was designed based on the published nucleotide sequence of *Eimeria necatrix* LZ strain micronem5 gene. A Partial cDNA sequence fragment of 758 bp coding for microneme 5 protein (EnMIC5) was amplified by semi- Nested RT-PCR. PCR products were cloned and expressed in a Maltose Binding protein (MBP) containing expression vector (pMAL-c2x) in *Escherichia coli*. The cDNA which is encoded for 252 amino acids shows high degree of conservation. It contains the adhesive plasma pre-kallikrein and seven hydrophilic motifs. **RESULTS:** The results of SDS-PAGE revealed that the recombinant protein with a molecular weight of 70 kDa was over-expressed after induction with IPTG. Western blotting results revealed that the expressed recombinant protein was reacted with sera of the chicks infected with *Eimeria necatrix*. It was suggested that this protein should have a good immunogenicity and can be used for further studies. **CONCLUSIONS:** In conclusion, the high degree of sequence homology indicates that this protein is immunogenic and might be an interesting vaccine target, and deserves further investigation.

Introduction

Coccidiosis is caused by protozoan parasite which belongs to phylum of Apicomplexa and genus Eimeria. It occurs internationally and has the greatest economic impact on poultry production. The annual worldwide cost is estimated at about $800 million (Williams, 1998). For many years, prophylactic use of anticoccidial feed additives has been the main meth-

od of controlling coccidiosis in the broiler industry. However, development of anticoccidial resistance, cost of new drug development, and regular modification of drug use laws have led to the increasing suitability of using vaccines to cope with coccidiosis (Blake et al., 2006; Jenkins, 2001; Williams, 2006). Since the cost of preparing live oocyst vaccines is relatively high, some efforts have been made to search for different ways by increasing the knowledge of parasite biology, host response and defining parasite antigens that have potential use in vaccines (Jenkins, 2001).

During the last ten years, researchers have made considerable attempts to develop recombinant vaccines. However, none of them were in commercial use. They have mostly tried to emphasize on the complexity of the avian host-coccidia relationship. A major obstacle to overcome in the development of a recombinant vaccine is the lack of cross-species immune protection. The cross-protective nature of the immunity shows that the response is unlike that induced by natural infection. This has motivated researchers to concentrate on molecular cloning of genes encoding protecting antigens in order to come out with vaccines protecting against several species. In recent years, particular consideration has been allocated in different *Eimeria* species organelles associated with the attack of the host cells by motile extra cellular stages of Eimeria located inside the apical complex (Dubremetz, 1998; Striepen et al., 2001; Tomley et al., 2001). The most essential reason hindering the development of a suitable vaccine that has been mentioned lately (Jenkins, 1998; Vermeulen, 1998), is the discovery of protective antigens. To date, several potential coccidial antigens have been described. Some of these antigens are surface proteins or internal antigens related to organelles such as micronemes (Tomley et al., 1991; Tomley et al., 1996), rhoptries (Tomley, 1994), and retractile bodies (Vermeulen et al., 1993).

Sequencing of genes for organelle proteins has shown several domains and motifs conserved among genera, especially in microneme proteins, supporting the idea that apical proteins have similar functions across the phylum (Tomley, 1997). The majority of micronemes contain multiple copies of a small number of adhesive domains, which has allowed the identification of several putative microneme proteins including these domains in the parasite databases. On the basis of this, it is likely that more proteins will be recognized to engage the micronemes in future studies (Carruthers and Tomley, 2008).

In this manuscript, we describe the isolation and purification of *Eimeria necatrix* oocysts from Khuzestan province of Iran. A cDNA encoding microneme5 (EnMIC5) protein was cloned and expressed, and also the immunogenicity of the recombinant protein was evaluated by Western blotting.

Matherials and Methods

Construction of the recombinant plasmid pMALc2x-EnMIC5: *E. necatrix* were collected from cercal contents of the slaughtered chickens. Identification of *E. necatrix* was carried out by morphologic and morphometric criteria and also by PCR (Su et al., 2003). Then, single speculated oocysts were inoculated orally to ten 3-week-old coccidia-free chickens and 7 days later, pure oocysts were recovered from the ceca using either enzymatic or chemical treatments (Shirley, 1995; Tomley, 1997). Total RNA was extracted from the speculated oocysts using acid-phenol extraction procedure based on the protocol explained by Chomczynski and Sacchi (1987) (Johnston, 1998). The cDNA synthesis was performed using oligo (dT) as primer. To amplify the gene encoding the microneme protein 5 from *Eimeria necatrix*, forward Mic5-F (5'-CGCGGATCCGCACTGCAGGAAATGGGAAG) and reverse Mic5-R (5'- CGCAAGCTTTTC-

CCAGAAGCCAAGGTGAA) primers were designed based on a single EST (accession number EU335049) of *Eimeria necatrix*. The PCR products of 758 bp was electrophoresed on 1% agarose gel and stained with ethidium bromide.

PCR products were excised from agarose gel and purified using an extraction kit (CinnaGen, Iran). Following digestion of the purified PCR products by restriction enzymes BamHI (GGATCC) and HindIII (AAGCTT), the fragment was cloned into the Maltose Binding protein (MBP) containing expression vector (pMAL-c2x) (NEW ENGLAND Biolabs, UK). The ligation reaction was used to transform *E. coli* TG1 strain using standard heat shock method explained by Chung and Miller. Recombinant colonies were selected on plates containing 100 µg/ml ampicillin.

Identification of EnMIC5 cDNA: By using Gene JET plasmid miniprep kit (Fermentas, Lithuania), plasmid DNA was purified and then digested with BamHI and HindIII before running on agarose gel. The selected positive clones were sequenced from both ends using a dideoxy termination method in applied bio systems 373 DNA sequences (Gen Fanavaran, Iran).

Expression of the recombinant protein in *E. coli*: For protein expression, recombinant vector was transformed into DH5 *E. coli* bacteria. An overnight culture of recombinant bacteria was diluted 1/100 in fresh LB medium and incubated at 37 °C until the OD600 of the culture reached to 0.4-0.6. Then, expression of the recombinant protein was induced by addition of 0.3 mM isopropyl thiogalactopyranoside (IPTG) and further incubation was done for a further 2 h, at which time the cells were recovered by centrifugation. Two samples collected before and after adding IPTG were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Chicken serum samples: Blood samples were taken from chickens 3 weeks after oral inoculation with single sporulated oocysts and stored at -20°C until tested by Western blotting.

Western blotting: Total proteins of bacteria transformed with pMAL-c2x plasmid containing the EnMIC5 gene were resolved by 10% SDS-PAGE gel electrophoresis and then transferred to nitrocellulose membrane by semi-dry blotting method. Non-specific binding sites were blocked by incubation for 1 h in 5% w/v skim milk in PBS before the nitrocellulose membrane was incubated for 2h with 1:50 and 1:100 dilution of the chicken serums inoculated with *Eimeria necatrix* oocysts. After washing the membranes with PBS including 0.05% Tween 20 (PBST), they were probed for 1 h with anti-chicken IgG conjugated to horseradish peroxidase, diluted 1:3000 in PBST containing 1% skim milk. Finally, the reaction was developed by a chloronaphthol-H2O2 mixture as the chromogen-substrate solution.

Results

Molecular characterization of EnMIC5: A 758 bp fragment of microneme 5 gene from *Eimeria necatrix* (EnMIC5) was amplified by PCR. The authenticity of the recombinant clones was confirmed by PCR and DNA sequencing. The cDNA fragment (EnMIC5) was cloned into pMALc2x expression vector. Positive recombinant clones after confirmation by electrophoresis and double digestion with BamHI and HindIII enzymes were sequenced by Gen Fanavaran, Iran (Fig. 1). The cloned DNA fragment represents a single Open Reading Frame (ORF) of 758 bp encoding a protein of 252 amino acids. The calculated molecular mass is 27254.60 Da with an isoelectric point of 4.86. The encoded polypeptide is predominantly hydrophilic (Fig. 2) [14] and rich in cysteine (8.33%) (Table 1). The amplified fragment includes approximately half of the entire coding sequence of the original sequence registered in the NCBI data base (accession number EU335049). The expressed region con-

Table 1. Amino acid composition of EnMIC5.

Amino acids	Number	Percentage
A	26	10.32
C	21	8.33
D	12	4.76
E	23	9.13
F	9	3.57
G	23	9.13
H	1	0.40
I	2	0.79
K	15	5.95
L	22	8.73
M	4	1.59
N	7	2.78
P	6	2.38
Q	11	4.37
R	11	4.37
S	21	8.33
T	17	6.75
V	10	3.97
W	4	1.59
Y	7	2.78

tains adhesive plasma pre-kallikrein (codons 130-201), and is rich in cysteine amino acids.

Expression of EnMIC5 as fusion protein with Maltose binding protein (MBP): A 758 bp amplified fragment of EnMIC5 cDNA was ligated into pMALc2x expression vector and the construct was transformed into *E. coli* DH5. The induced cell lysates displayed a prominent band of 70 kDa (MBP- EnMIC5 including MBP with 42.5kDa and EnMIC5 with 27.5 kDa) which was not present in the non-induced lysate cells (Fig. 4).

Immunogenicity of EnMIC5 recombinant protein: To examine the antibody reactivity to EnMIC5 fusion protein, sera from *E. necatrix* infected chicks were tested using Western blotting. Reaction of chicken serum with recombinant protein band corresponding to the expected recombinant protein in the IPTG induced bacteria cells in comparison with the non-induced bacteria cells is shown in Fig. 5. The results indicated the good immunogenicity of EnMIC5 fusion protein.

Discussion

In this study, we expressed and analyzed a partial cDNA (EnMIC5) encoding a MIC5 gene from *Eimeria necatrix*. EnMIC5 has a calculated molecular mass of 27.5 kDa. Analysis of the deduced amino acid sequence revealed the presence of a signal peptide and an apple domain. The predicted amino acid sequence of MIC5 is highly conserved and has major sequence similarity with MIC5 sequences from other diverse organisms (Murray et al., 1986).

Natural infection of any chicken Eimeria creates small or no cross protection (Rose and Mockett, 1983). However, this can be achieved by immunization of chicken with soluble parasite antigens (Murray et al., 1986) or recombinant protein (Bhogal et al., 1992; Crane et al., 1991). Micronemes are the smallest of the apical organelles, structurally and functionally preserved in all Apicomplexans (Bumstead and Tomley, 2000). Micronemal proteins are thought to be critical ligands determining host cell specificity at the time of invasion. Recent studies provide strong evidence that the transmembrane micronemal proteins of the TRAP family participate not only in attachment but also to gliding motility. Therefore, they actively contribute in the invasion course (Tomley et al., 1996; Tomley and Soldati, 2001) and play an essential role in the invasion process of apicomplexan parasites. To ensure delivery of ligands at the right time and optimal place, it is necessary that micronemes exocytose adhesins and other factors in a regulated fashion onto the parasite surface during an early phase of invasion (Carruthers and Tomley. 2008).

A number of the genes that encode micronemal proteins including EnMIC-2 (Qin et al., 2005), EnMIC-5 (Cai et al., 2008; Masaeli et al., 2011), EtMIC-2 (Bashar et al., 2003), EtMIC 5 (Brown et al., 2000) and TgMIC4 (Soldati et al., 2001) have already been cloned and characterized. In all cases, high homology

between the sequence of target gene and the other related genes was shown. Identification and characterization of these genes revealed the existence of a distinct type of adhesive motif called an "apple domain" (Fig. 3). This domain has strong similarity to the adhesive plasma pre-kallikrein. In Apple/PAN domains which have three conserved disulphide bridges that are essential for their tertiary structure, similarity at the amino acid level between domains is generally low. This may contribute to their highly diverse and particular ligand binding properties (Carruthers and Tomley. 2008). The existence of several different types of micronemes (such as: MIC1, MIC2) as adhesion in Apicomplexans, shows the diversity of strategies used by the parasite to create host-parasite interactions. This diversity may contain the wide range of host cell type specificity.

Western blot analysis revealed that the expressed recombinant protein was reacted with sera of chickens against *Eimeria necatrix* infection demonstrating ability of this protein to produce specific antibody response. These results are in accordance with a study performed by Cai Xue-peng et al., 2008. Xie Ming-quan et al immunized chickens with recombinant EnMic-2 in *Salmonella typhymurium* and challenged the chickens with *Eimeria necatrix* sporulated oocysts 3 weeks PI, resulting in reducing the oocyst shedding.

In conclusion, the strong sequence conservation indicates that the recombinant protein coded by this gene is immunogenic and might be a potential vaccine target and deserves further investigation.

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References

1. Bashar, A.E., Jian-ping, C., Ming-quan, X., Guo-qing, L., Zong-hua, Q., Hui-xian, W., Xing-yu, P., Weng-kang, W. (2003) Characterization of Microneme-2 (EtMlC-2) gene of *Eimeia tenella* Guangdong strain. Int J Poult Sci. 2: 133-138.
2. Bhogal, B., Miller, S., Anderson, G.A., Jessee, A.C., Strousberg, E.J., Mc Candliss, S., Nagle, R.J., Strousberg, R. (1992) Potential of a recombinant antigen as a prophylactic vaccine for day-old broiler chickens against Eimeria acervuline and *E. tenella* infections. Vet Immunol Immunopathol. 31: 323-335.
3. Blake, D.P., Shirley, M.W., Smith, A.L. (2006) Genetic identification of antigens protective against coccidia. Parasit Immunol. 28: 305-314.
4. Brown, P.J., Billington, K.J., Bumstead, J.M., Clark, J.D., Tomley, F.M. (2000) A microneme protein from *Eimeria tenella* with homology to the Apple domains of coagulation factor XI and plasma pre-kallikrein. J Molecul Biochem Parasitol. 107: 91-102.
5. Bumstead, J., Tomley, F.M. (2000) Induction of secretion and surface capping of microneme proteins in *Eimeria tenella*. J Molecul Biochem Parasitol. 110: 311-321.
6. Cai, X.P., Liu, H., Jia, W., Guo, A., Zhang, Sh., Yan, H., Yue, Ch. (2008) Recombinant expression of microneme protein 5 gene of *Eimeria necatrix* LZ strain in *E. coli* and antigenicity of recombinant protein. Act Vet Zootechnic Sini. 39: 1575 -1580.
7. Carruthers, V.B., Tomley, F.M. (2008) Receptor-ligand interaction and invasion: Microneme proteins in Apicomplexans. Subcell Biochem. 47: 33-45.
8. Chomczynski, P., Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analitic Biochem. 162: 156-159.
9. Crane, M.S.J., Goggin, B., Kirj, K.E., Chakoaboity, P.R. (1991) Cross protection against four species of chicken coccidian with a single

recombinant antigen. Infect Immun. 59: 1271-1277.
10. Dubremetz, J.F. (1998) Apical organelles and host-cell invasion by Apicomplexa. Int J Parasitol. 28: 1007- 1013.
11. Jenkins, M.C. (1998) Progress on developing a recombinant coccidiosis vaccine. Int J Parasitol. 28: 1111-1119.
12. Jenkins, M.C. (2001) Advances and prospects for subunit vaccines against protozoa of veterinary importance. Vet Parasitol. 101: 291-310.
13. Johnston, D.A., Bratty, J., Fernando, M.A. (1998) A fast and inexpensive method for isolation of high-quality RNA from eimerian oocysts. Parasitol Res. 84: 549-551.
14. Kyte, J., Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. J Molecul Biol. 157: 105-32.
15. Masaeli, Sh., Mayahi, M., Jolodar, A., Hamidinejat, H., Seyfi Abad Shapouri, M.R. (2011) Partial sequence determination of a cDNA encoding microneme 5 protein of *Eimeria necatrix* isolated in Khuzestan province, Iran. Iranian Journal of Veterinary Science Technology. 3: 39-48.
16. Murray, P.K., Bhogal, B.S., Crane, M.St J. (1986) Eimeria tenella - in vivo immunization studies with sporozoite antigen. In: Research in Avian Coccidiosis. Mc Dougald, L.R., Joyner, L.P., Long, P.L. (eds.). University of Georgia Press. Athens, Georgia, USA. p. 564-573.
17. Qin, Z.H., Xie, M.Q., Cai, J.P., Elhag, A.B., Peng, X.Y., Wei, W.K., Wu, H.X. (2005) Expressing microneme-2 gene of *Eimeria necatrix* in *Escherichia coli*. Chin J Vet Sci. 25: 22-25.
18. Rose, M.E., Mockett, A.P.A. (1983) Antibodies to coccidia: detection by the enzyme-linked immunosorbent assay (ELISA). Parasite Immunol. 5: 479-489.
19. Shirley, M.W. (1995) *Eimeria* species and strains of chickens. In: Biotechnology Guidelines on Techniques in Coccidiosis Research. European Commission DGXII, Luxembourg. p. 1- 24.
20. Soldati, D., Brecht, S., Carruthers, V.B., Ferguson, D.J.P., Giddings, O.K., Wang, G., Jäkle, U., Harper, J.M., Sibley, L.D. (2001) The toxoplasma micronemal protein MIC4 is an adhesin composed of six conserved apple domains. J Biol Chem. 276: 4119-27.
21. Striepen, B., Soldati, D., Garcio-Reguet, N., Dubremetz, J.F., Roos, D. (2001) Targeting of soluble proteins to the rhoptries and micronemes in *Toxoplasma gondii*. Molecul Biochem Parasitol. 113: 45- 53.
22. Su, Y.C., Fei, A.C., Tsai, F.M. (2003) Differential diagnosis of five avian *Eimeria* species by polymerase chain reaction using primers derived from the internal transcribed spacer 1 (ITS-1) sequence. Vet Parasitol. 117: 221-227.
23. Tomley, F.M. (1994) Characterization of rhoptry proteins of *Eimeria tenella* sporozoites: antigenic diversity of rhoptry epitopes within species of the genus Eimeria and among three asexual generations of a single species, E. tenella. Infect Immunol. 62: 4656-4658.
24. Tomley, F.M. (1997) Techniques for isolation and characterization of apical organelles from *Eimeria tenella* sporozoites. Methods: A companion to Methods in enzymology. 13: 171-176.
25. Tomley, F.M., Clark, L.E., Kawazoe, U., Dijkema, R., Kok, J.J. (1991) Sequence of gene encoding an immunodominant microneme protein of *E. tenella*. Molecul Biochem Parasitol. 49: 277-288.
26. Tomley, F.M., Billington, K.J., Bumstead, J.M., Clarka, J.D., Monaghanb, P. (1996) Molecular cloning and characterization of a novel acidic microneme protein (EtMIC-2) from the apicomplexan protozoan parasite, *Eimeria tenella*. Molecul Biochem Parasitol. 79: 195- 206.
27. Tomley, F.M., Billington, K.J., Bumstead, J.M., Clark, J.D., Monaghan, P. (2001) EtMIC-4: a microneme protein from *Eimeria tenella* that contains tandem arrays of epidermal growth factor-like repeats and thrombospondin type-1 repeats. Int J Parasitol. 31: 1303-1310.
28. Tomley, F.M., Soldati, D.S. (2001) Mix and

match modules: structure and function of microneme protein in apicomplexan parasites. Parasitol Today. 31: 1303- 1310.

29. Vermeulen, A.N. (1998) Progress in recombinant vaccine development against coccidiosis. A review and prospects into the next millenium. Int J Parasitol. 28: 1121-1130.
30. Vermeulen, A.N., Kok, J.J., van den Boogart, P., Dijkema, R., Claessens, J.A.J. (1993) Eimeria refractile body proteins contain 2 potentially functional characteristice-transhydrogenase and carbohydrate transport. FEMS Microbiol Lett. 110: 223-230.
31. Williams, R.B. (1998) Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int J Parasitol. 28: 1089-1098.
32. Williams, R.B. (2006) Relative virulences of a drug-resistant and a drug-sensitive strain of *Eimeria acervulina*, a coccidium of chickens. Vet Parasitol. 15: 15-23.
33. Xie, Ming-quan., Qin, Zong-hua., Cai, Ji-an-ping., Bashar, A .E., Peng, Xin-yu., Wei, Wen-kang., Wu, Hui-xian. (2005) Immunogenic characterization of the recombinant attenuated *Salmonella typhymurium* expressing Mic2 gene of *Eimeria necatrix*. Acta Veterinaria et Zootechnica Sinica. 36: 705-710.

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کلونینگ و بیان ژن میکرونم ۵ اِیمریا نکاتریکس در باکتری *E. coli*

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چکیده

زمینــه مطالعــه: اِیمریا نکاتریکس عامل کوکسـیدیوز حداکثر تأثیر اقتصـادی را بر روی تولید پرندگان دارد. به نظر می‌رسـد پروتئین‌های میکرونم نقش مهمی در حرکت انگل و تهاجم به سلول‌های میزبان دارد. **هدف:** جداسازی و خالص‌سازی اووسیست اِیمریا نکاتریکس از استان خوزستان-ایران بود. یک cDNA کد کننده پروتئین میکرونم ۵ (EnMIC۵) کلون و پس از بیان کردن این پروتئین نوترکیب، خاصیت ایمنی‌زایی آن توسط وسترن بلوتینگ مورد آزمایش قرار گرفت. **روش کار:** یک جفت پرایمر بر اساس توالی نوکلئوتیدی موجود برای ژن میکرونم ۵ اِیمریا نکاتریکس سویه LZ طراحی و به وسیله آن توالی بخشی از cDNA کد کننده این پروتئین به میزان ۷۵۸ bp با استفاده از روش Semi- Nested RT-PCR تکثیر گردید سپس قطعه بدست آمده به درون وکتور pMAL-c۲x حاوی پروتئین باند شونده به مالتوز کلون و در باکتری *E. coli* سویه TG۱ بیان گردید. قطعه تکثیر شده، یک open reading frame ۲۵۲ اسیدآمینه ای داشت که به میزان قابل توجهی حاوی مناطق حفاظت شده شامل پری کالکرئین پلاسمایی چسبنده و هفت جزء آبدوست بود. **نتایج:** در SDS-PAGE پروتئین الحاقی با وزن مولکولی در حدود ۷۰ کیلو دالتون پس از القاء توسط IPTG بیان شد. آزمایش وسترن بلات نشان داد که پروتئین نوترکیب با سرم بدست آمده از جوجه‌های آلوده به اِیمریا نکاتریکس واکنش می‌دهد. **نتیجه گیری نهایی:** یافته‌ها نشان داد پروتئین بدست آمده قدرت ایمنی‌زایی نسبتاً خوبی داشته و می‌تواند در آینده مورد مطالعه بیشتری قرار گیرد.

واژه‌های کلیدی: اِیمریا نکاتریکس، بیان ژن، ایمنی‌زایی، میکرونم

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