Original Article

Optimized codons of polar tube protein 1 gene of *Encephalitozoon cuniculi* to enhance protein expression in *Escherichia coli*

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Abstract

Encephalitozoon cuniculi (*E. cuniculi*) is an intracellular zoonotic parasite that is recognized as a common parasite of rabbits. Infected rabbits show neurological and renal disorder. The polar tube protein 1 of the spore is revealed to be antigenicity. The present study aimed to produce a recombinant polar tube protein 1 (rPTP1) for which the codon usage of a native *ptp1* gene was optimized and synthesized for protein production in *Escherichia coli* (*E. coli*). Both a native *ptp1* gene amplified by PCR technique and an optimized *ptp1* gene were ligated into a pQE30 expression vector and transformed into an *E. coli* strain M15. The rPTP1 production was induced by 1M isopropyl-β-D-thiogalactoside. A protein band with a molecular weight of about 45 kDa was found in the extracted protein of both *E. coli* transformed with optimized *ptp1* and native *ptp1* plasmids. SDS-PAGE and western blot analysis using an anti-histidine antibody revealed a notably higher protein yield of the *E. coli* containing an optimized *ptp1* plasmid than that of the *E. coli* containing a native *ptp1* plasmid. Biological function of the recombinant protein was evaluated by western blotting using *E. cuniculi*-positive and negative rabbit sera. Result showed that the biological function of the optimized rPTP1 protein expressed from *E. coli* could differentiate between *E. cuniculi*-negative and positive rabbit sera. Therefore, rPTP1 can be used for the development of a diagnostic test for *E. cuniculi* infection in the future.

Keywords: *Encephalitozoon cuniculi*, *E. coli*, polar tube protein 1, rabbit, recombinant protein

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Introduction

Encephalitozoon cuniculi (*E. cuniculi*) is a member of Phylum Microsporidia and is an obligate intracellular parasite causing encephalitozoonosis in rabbits and many hosts including humans (Canning and Lom, 1986; Kunzel and Joachim, 2010; Mathis et al., 2005; Wasson and Peper, 2000; Weiss, 2001). The microsporidia is related to fungi based on phylogenetic analysis (Weiss, 2001). High percentages of seropositivity have been reported worldwide in rabbits which are the main host of the organism (Kunzel and Joachim, 2010). The seroprevalence rate of pet rabbits submitted at the Kasetsart University Teaching Hospital at Bangkhen in Thailand during October 2009 to January 2010 was 34.5% (Polpruksa et al., 2011).

The multiplied mature spores in infected host cells cause cell rupture which induces inflammation in predilection sites (i.e. brain, kidney and eye) (Kunzel and Joachim, 2010). Vestibular disease, chronic interstitial nephritis, phacoclastic uveitis, lens rupture, uveitis and cataracts may appear after infection although neurological signs including head tilt, ataxia and circling are typical symptoms of the disease in infected rabbits (Harcourt-Brown and Holloway, 2003; Jeklova et al., 2010; Kunzel et al., 2008; Kunzel and Joachim, 2010). The nature of infected rabbits is regularly found to be subclinical disease. The mode of disease transmission is ingestion or inhalation of food and water contaminated with the spores (Cox et al., 1979; Shadduck and Pakes, 1971). Transplacental transmission is described in rabbits (Baneux and Pognan, 2003).

The diagnosis of encephalitozoonosis in rabbits is based on the detection of antigen using histopathological examinations (Ghosh and Weiss, 2009; Leipig et al., 2013) or polymerase chain reaction (PCR) (Csokai et al., 2009) and presence of an antibody using carbon immunoassay (CIA), indirect immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) or a western immunoblot assay (Boot et al., 2000; Garcia, 2002; Kunzel and Joachim, 2010; Zierdt et al., 1993). Serological detection of antibodies against spores of the organism in the early stages of infection is proposed as the most sensitive diagnostic method (Csokai et al., 2009). Seroconversion after infection can be detected before intracellular organisms, histopathological lesions and organisms in urine. A serological method, especially ELISA, is appropriate for disease monitoring and screening in many samples. The coating antigen for antibody detection is usually the whole spore. The spore is multiplied in a cell culture and purified; the process is rather expensive, laborious and time-consuming (Akerstedt, 2002; Furuya et al., 2008; Keohane et al., 1996). Currently, antigens for ELISA development are often prepared from recombinant protein expression in *E. coli* because it is a simple and rapid process and produces economical and high number of antigens (Hannig and Makrides, 1998). Polar tube protein 1 (PTP1) is a component of the polar tube, which is a structure in the spore and a concern for the invasion into a host cell (Delbac et al., 2001; Delbac et al., 1998; Weiss, 2001; Weiss et al., 2014). PTP1 is used for the development of serological techniques due to the protein contained in the immunological epitopes (Furuya et al., 2008; Moretto et al., 2010; van Gool et al., 1997; Weiss et al., 2014; Xu and Weiss, 2005; Zierdt et al., 1993). PTP1 is a proline-rich protein (Delbac et al., 1998) which is a rare codon (CCC, CCU and CCA) for heterologous protein expression in an *E. coli* system. Codon bias and rare codon are obstacles for the effectiveness of heterologous protein production from a gene of a eukaryotic cell in *E. coli* (Kane, 1995; Lavner and Kotlar, 2005; Rosano and Ceccarelli, 2009; Rosano and Ceccarelli, 2014). Codons including AGG/AGA, CUA, AUA, CGA or CCC are reduced, both in quality and quantity of heterologous protein expression (Kane, 1995). Codon optimization can improve a heterologous protein expression in *E. coli* (Burgess-Brown et al., 2008). The purpose of this study were to produce a recombinant polar tube protein 1 (rPTP1) of *E. cuniculi* from optimized codon in *E. coli* and to evaluate the biological functions that react with rabbit antibodies.

Materials and Methods

Sample collection and nested PCR technique: Dead rabbits suspected of encephalitozoonosis with neurological disorders such as head tilt or hind limb paresis were submitted to the Kamphaeng Saen Veterinary Diagnostic Unit (KVDU), Faculty of Veterinary Medicine at Kasetsart University. Tissue samples were collected including brains and kidneys for DNA extraction. Approximately 30 milligrams of tissue sample was ground into 100 µl of 1XPBS buffer in a 1.5 ml microcentrifuge tube for DNA extraction using a Wizard® Genomic DNA Purification System (Promega, USA) according to the manufacturer's instructions. The DNA was eluted with a 40 µl elution buffer and *E. cuniculi* infection was detected by nested PCR using specific primers based on a 16S small subunit ribosomal RNA gene which was proposed in previous reports (Csokai et al., 2009). Both first and second rounds of the nested PCR were amplified using DreamTaq™ DNA polymerase (Thermo Scientific, USA). Reaction components were prepared according to the manufacturer's recommendations. The reaction conditions of nucleic acid amplification were performed according to the original report by a thermocycler (Swift™ Maxi Thermal Cycler; ESCO, Singapore). The PCR products were analyzed by electrophoresis on a 1.5% agarose gel and visualized under UV illumination. A positive sample was used for native *ptp1* gene cloning. Codon Adaptation Index (CAI), average GC content and percentage distribution of codons were analyzed by GenScript's OptimumGene™ codon optimization tool (http://www.genscript.com/cgi-bin/tools/rare_codon_analysis).

Primer design and native PTP gene cloning: Specific primers for *ptp1* gene amplification were designed based on an accession number of AJ005666 for the native *ptp1* gene cloning. The forward primer was *ptp*-F 5'-GGAACCATAGCAAACTGTGC-3' and the reverse primer was *ptp*-R 5'-CCTCCATGGCATACTGCGT-3'. A 25 µl total volume of PCR was composed of 1X PCR buffer, 200 µM dNTPs, 2 mM $MgCl_2$, 2.5 units of DreamTaq™ DNA

Polymerase (Thermo Scientific™, USA), 25 pmol of each primer and 2 µl of DNA positive sample by nested PCR. The reaction conditions were performed with one cycle of pre-denaturation at 94°C for 3 min, followed by thirty-five cycles at 94°C for 45 sec, 55°C for 30 sec and 72°C for 1 min, and one cycle of post-elongation at 72°C for 5 min. The PCR products were visualized by a UV illuminator after electrophoresis using 1.5% agarose gel. The 915 bp of PCR product from the native *ptp1* gene was purified by FavorPrep™ GEL/PCR Purification Kit (Favorgen® Biotech Corporation, Taiwan) according to the manufacturer's protocols. The purified PCR product was ligated into a T&A cloning vector (RBC Bioscience Crop., Taiwan) and transformed into a competent *E. coli* strain JM109 by heat shock technique. Bacterial colonies that carried the native *ptp1* inserted plasmids were confirmed by PCR technique and nucleotide sequencing. Nucleotide sequence analysis was performed by BioEdit program version 7.1.3.0.

ptp1 gene optimization: A nucleotide sequence of a *ptp1* gene was optimized for suitable expression in an *E. coli* system (Figure 1). An optimized *ptp1* inserted plasmid was provided by Life Technologies Holding Pte Ltd (Life Technologies, Singapore). Recognition sites of the restriction enzymes *Bgl*II and *Hind*III were added to extend at 5′ and 3′ at the end of the optimized *ptp1* gene, respectively.

Subcloning of ptp1 gene into expression vector: Both the *E. coli* strain JM109 carrying the native *ptp1* inserted plasmids and the optimized *ptp1* inserted plasmids were cultured in LB broth containing 100 µg/ml of ampicillin overnight. The plasmids were extracted using a FavorPrep™ Plasmid Extraction Mini Kit (Favorgen® Biotech Corporation, Taiwan).

Each native *ptp1* and the optimized *ptp1* inserted plasmid digested with *Bgl*II and *Hind*III restriction enzymes (FastDigest™Thermo Scientific™, USA) were ligated into pQE30 (Qiagen Inc., USA), which was digested with *Bam*HI and *Hind*III restriction enzymes (FastDigest™ Thermo Scientific™, USA). Each ligation mixture was transformed into a competent *E. coli* strain M15 by heat shock technique. The inserted gene in each bacterial colony was checked by PCR using *ptp*-F and *ptp*-R primers.

Protein expression in E. coli: A single colony of each bacteria contained a native *ptp1* inserted plasmid, an optimized *ptp1* inserted plasmid and a non-inserted plasmid which was grown for a starter culture in LB broth containing 100 µg/ml of ampicillin and 25 µg/ml of kanamycin overnight at 200 rpm at 37°C. The starter cultures were subcultured at a concentration of 1:100 in LB broth containing 100 µg/ml of ampicillin and 25 µg/ml of kanamycin. They were shaken until the optical density (OD_{600}) reached 0.6. Recombinant proteins were induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM for 4 hours. Cell pellets and supernatant were separated by centrifugation at 10,000 rpm for 5 min. A 2X SDS-PAGE loading buffer with dithiothreitol (DTT) was added to the proteins of both parts, which were then detected by 10% SDS-PAGE and coomassie brilliant blue staining.

Western blot analysis and biological function evaluation: The proteins from pellet fraction were run in SDS-polyacrylamide gel and transferred to a nitrocellulose membrane using a Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell (BioRad, USA) according to the manufacturer's instructions. The nitrocellulose membrane was blocked with 5% skim milk in 1XPBS-T at 37°C for 1 hour. A 1:1000 dilution of Anti-His antibody (GE-Healthcare, UK) in 1X-PBS-T contained 1% skim milk which was used for detection of 6X Histidine-tag protein on a recombinant protein incubated at 37°C for 1 hour. The membrane was washed with 1X PBS-T for 5 min, 3 times. Adding a secondary antibody, Goat anti-Mouse IgG-HRP (KPL, USA) was diluted to 1:1000 with 1% skim milk in 1X-PBS-T and incubated at 37°C for 1 hour.

E. cuniculi-positive and negative rabbit sera were tested by an *Encephalitozoon cuniculi* ELISA kit (Medicago, Sweden) and were used to evaluate biological functions of the recombinant proteins. The nitrocellulose membrane bound proteins from SDS-polyacrylamide gel was blocked with 5% skim milk in 1XPBS-T at 37°C for 1 hour. Each positive and negative rabbit serum was diluted to 1:1000 with 1% skim milk in 1X-PBS-T and incubated at 37°C for 1 hour. The membrane was washed with 1X PBS-T for 5 min, 3 times, and a dilution of 1:1000 goat anti-rabbit IgG-HRP (KPL, USA) in 1% skim milk in PBS-T incubated at 37°C for 1 hour was added. The membranes were washed with 1X PBS-T for 5 min, 3 times, and the color of the TMB membrane substrate (KPL, USA) was developed. The reaction was stopped by soaking the membrane with PBS-T.

Results

The *ptp1* gene was amplified using the PCR technique from a native *ptp1* gene of *E. cuniculi* from infected rabbit tissue and was synthesized according to an optimized *ptp1* gene to improve the suitable codon usage for protein production from *E. coli*. A different nucleotide sequence (915 bp) excluding signal peptide between native *ptp1* and optimized *ptp1* genes were compared in Figure 1. The amino acid component of native *ptp1* (305 amino acid residues) was found to be composed of 36.07% of rare codons, which became 4.59% after codon optimization (Table 1). The majority of rare codons of the native *ptp1* were found in codon usage of proline (thirty-nine residues) and glycine (thirty residues), but two isoleucine (ATA) and six proline (CCC) residues were rare codons that were mentioned to be the cause of a protein expression problem in *E. coli*.

The percentage distribution of codons in computed codon quality groups showed that the value of 100 was the highest usage frequency to give amino acid the desired expression (Figure 2). Seven percent of the codons in the native gene had a value less than 30 (Figure 2, A1) while the majority of codon percentage distribution of optimized *ptp1* was located between 91-100 (Figure 2, A2). The distribution of codon usage frequency along the length of a *ptp1* gene expressed in

E. coli is shown in Figure 2B. The native *ptp1* had a 0.59 CAI value and 55.47 of the average GC content while the optimized *ptp1* had a 0.85 CAI value and 57.11 of the average GC content.

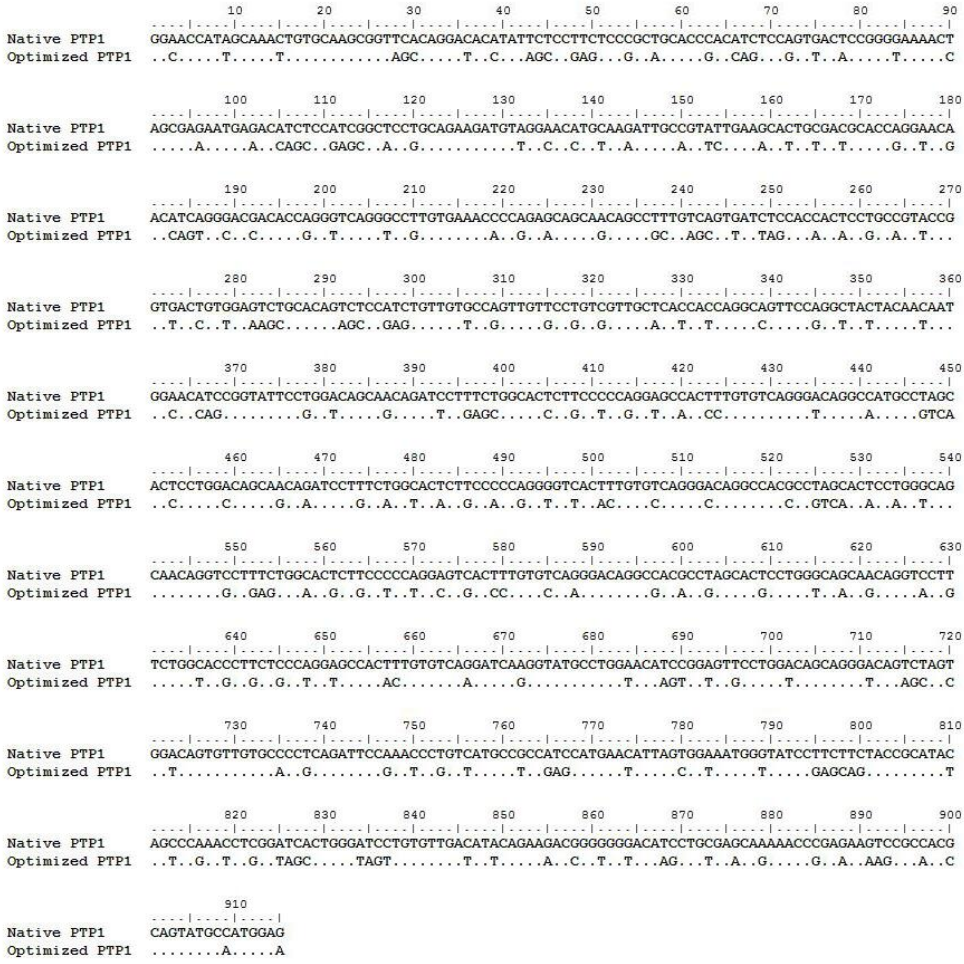

Figure 1 The nucleotide alignment between native (upper row) and optimized (lower row) *ptp1* nucleotide sequences were analyzed by ClustalW in the BioEdit program version 7.1.3.0. Dots represent identical sequences with a native *ptp1* sequence.

Table 1 Amino acid component of PTP1 between native and optimized *ptp1* expressed in *E. coli*

Amino Acid	Rare codons[a]	Native PTP1	Optimized PTP1	Total amino acid
Alanine	-	-	-	21
Cysteine	TGT/TGC	9/3	7/0	12
Aspartic acid	-	-	-	5
Glutamic acid	-	-	-	9
Phenylalanine	-	-	-	0
Glycine	GGG/GGA	10/20	0/1	38
Histidine	-	-	-	3
Isoleucine	ATA*	2	0	9
Lysine	-	-	-	6
Leucine	CTA*/CTC	0/2	0/0	17
Methionine	-	-	-	5
Asparagine	-	-	-	8
Proline	CCC*/CCA/CCT	6/15/18	0/0/4	42
Glutamine	-	-	-	34
Arginine	AGG*/AGA*/CGA*/CGG	-	-	0
Serine	AGT/TCA/TCC/UCG	2/5/7/1	0/1/0/0	35
Threonine	ACA	10	1	34
Valine	-	-	-	21
Tryptophan	-	-	-	0
Tyrosine	-	-	-	6
		110	14	305

[a]Codons used by *E. coli* at a frequency of <1% (Kane, 1995)

*Rare codons reported as the cause of reduction in protein expression in *E. coli* (Kane, 1995)

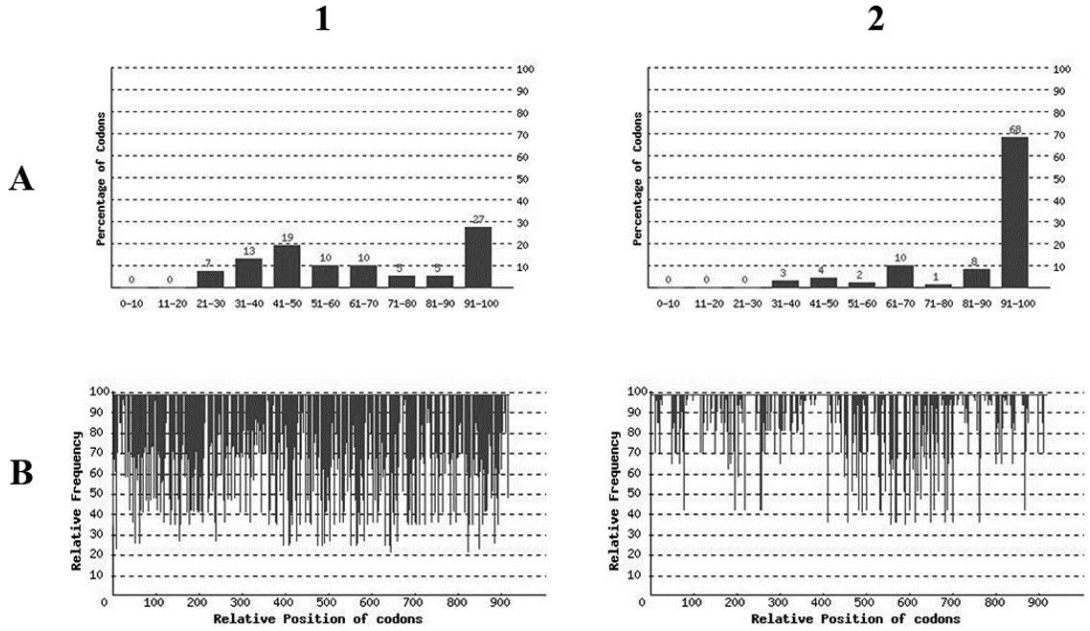

Figure 2 The histograms show the percentage distribution of codons in computed codon quality groups (A) and the distribution of codon usage frequency along the length of a *ptp1* gene expressed in *E. coli* (B). The histograms of the native and optimized *ptp1* genes are the first (1) and second (2) columns, respectively.

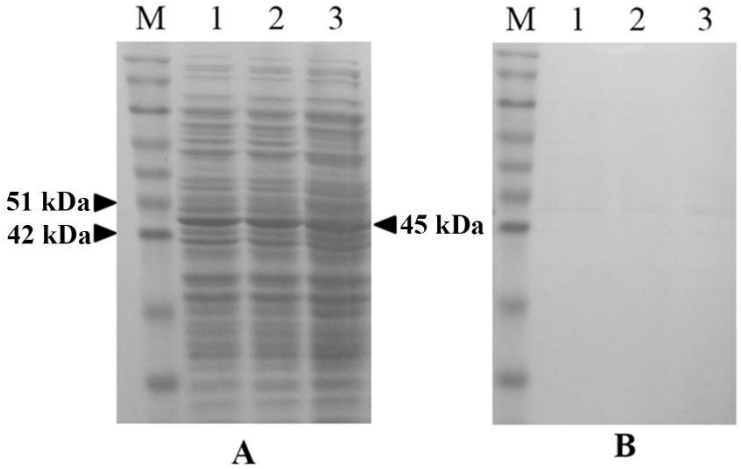

Figure 3 SDS-PAGE analysis shows the expressed *ptp1* from *E. coli*. The samples included pellet fraction (A) and supernatant fraction (B) which were collected and evaluated by SDS-PAGE. M represents a Chromatein prestained protein ladder (Vivantis, USA). Lane 1 is control pQE30 plasmid, Lane 2 is native *ptp1* inserted plasmid, and Lane 3 is optimized *ptp1* inserted plasmid.

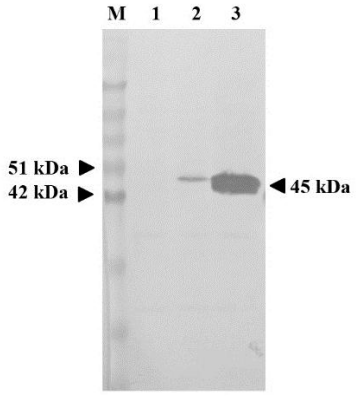

Figure 4 Western blotting with Anti-His antibody. Lane 1 is pellet fraction from *E. coli* containing control pQE30 plasmid, Lane 2 is pellet fraction from *E. coli* containing native *ptp1* inserted plasmid, and Lane 3 is pellet fraction from *E. coli* containing optimized *ptp1* inserted plasmid. M represents a Chromatein prestained protein ladder (Vivantis, USA).

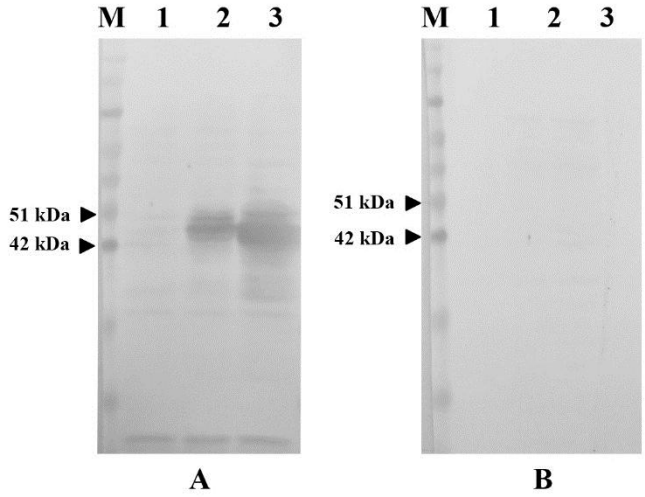

Figure 5 Evaluation of biological rPTP1 function by western blotting using rabbit antibodies. The proteins from pellet fraction reacted with *E. cuniculi*-positive (A) and negative (B) rabbit sera. M represents a Chromatein prestained protein ladder, vivantis. Lane 1 is control pQE30 plasmid, Lane 2 is native *ptp1*, and Lane 3 is optimized *ptp1*.

The pellet and supernatant of each *E. coli* contained native *ptp1* and optimized *ptp1* inserted plasmids which were collected and analyzed by SDS-PAGE. A protein band that increased after being induced by IPTG was found to be approximately 45kDa in pellet fraction from both native *ptp1* and optimized *ptp1*. The protein band of the optimized *ptp1* was thicker than the protein band in the native *ptp1* lane (Figure 3A). In SDS-PAGE, it was shown that the *ptp1* protein did not secrete in the supernatant. The indistinct *E. coli* protein bands appeared from both *E. coli*-contained plasmids with and without *ptp1* gene (Figure 3B).

The expressed proteins from pellet fraction were detected by western blotting using an Anti-His antibody. A protein band was visualized on the nitrocellulose membrane with both the native gene clone and optimized gene clone at a molecular weight of about 45 kDa and was suspected to be rPTP1 (Figure 4). The protein from the optimized gene could produce higher yield than the native gene. The biological function test of rPTP1 showed that rPTP1 would only react with the positive serum against *E. cuniculi* of rabbits (Figure 5A) while indistinct nonspecific bands appeared from the negative rabbit serum by western blotting (Figure 5B).

Discussion

The antigen production from the entire *E. cuniculi* spore requires many resources. This study provided optimized codon of a *ptp1* gene to enhance polar tube protein expression in an *E. coli* produced antigen. The PTP1 protein of microsporidian can react with antibodies of animals immunized with whole spores of *E. cuniculi* and also induce polyclonal antibodies for immunoblotting and immunofluorescence techniques (Delbac et al., 1998). Heterologous protein production in *E. coli* is preferred to develop diagnostic methods due to fast growth rate, inexpensive media and well-understood genetics (Baneyx, 1999; Gopal and Kumar, 2013; Rosano and Ceccarelli, 2009). The main obstacles for heterologous protein production in *E. coli* are rare codon and biased codon usage which causes low or no production due to insufficient tRNAs for bias codon usages (Burgess-Brown et al., 2008; Rosano and Ceccarelli, 2014). The lower one percent of codon usage in a native gene of *E. coli* is defined as a rare codon (Kane, 1995). Moreover, protein translation from a rare codon is the cause of translational errors including mistranslational amino acid substitutions, frameshift or premature translational termination (Sørensen and Mortensen, 2005). The native *ptp1* gene is more of a rare codon based on our study (Table 1). A proline-rich protein is presented in the PTP1 (Delbac et al., 1998). Proline is one of the codons that is not commonly used in *E. coli* (Sørensen and Mortensen, 2005). The majority of rare codons of a native *ptp1* are found in the codon usage of proline from our study. CAI is a value to determine a directional, synonymous codon usage bias that is correlated with the potential expression level of a gene in *E. coli* (Sharp and Li, 1987). A CAI value of more than 0.8 is proposed as a good expression. The optimized *ptp1* had a 0.85 CAI value. The average GC of both native and optimized *ptp1* was between 30-70 percent, which was appropriate for transcription and translation. The percentage distribution of codon of a value lower than 30 was an obstacle to the expression efficiency. The percentage distribution of codons in the native *ptp1* had seven percent of the codons with a value lower than 30. Thus, the result of this study indicates that *E. coli* which contains native *ptp1* inserted plasmid expresses protein less than *E. coli* containing optimized *ptp1* inserted plasmid.

The protein production in a codon bias-adjusted *E. coli* strain or DNA synthesis according to optimized codon is an alternative method to improve the heterologous protein production in *E. coli* (Burgess-Brown et al., 2008; Rosano and Ceccarelli, 2009; Rosano and Ceccarelli, 2014; Wu et al., 2004). Our result from the expressed rPTP from a nucleotide sequence of a native *ptp1* gene in an *E. coli* expression system showed a low level of protein production because the parasite is a eukaryotic cell of which amino acid sequence of a protein could affect the protein production in a prokaryotic cell. Moreover, the protein encoded from a

ptp1 gene is an acidic proline-rich protein (Delbac et al., 1998). Therefore, the *ptp1* gene sequence was synthesized with codon optimization to adjust the suitable codon usage for *E. coli.* The key finding of this study from a gene optimization viewpoint is the success in increasing the level of rPTP1 production in an *E. coli* expression system, indicating that codon bias in rPTP1 affected the protein production.

The PTP1 presence of multiple cysteine residues can form a disulfide bond (Weidner, 1976). The cysteine residues may also affect protein production because *E. coli* is not suitable for the production of proteins containing disulfide bonds (Choi et al., 2006). The composition of cysteine in rPTP1 did not block the protein production but affected the protein secretion and deviation of protein size in the SDS-PAGE analysis. The SDS-PAGE analysis showed that the suspected protein size was about 45 kDa while the predicted size of rPTP1 was about 33 kDa. The suspected protein probably had to deal with disulfide bond formation. Although DTT is added in the SDS-PAGE loading buffer before SDS-PAGE analysis to reduce disulfide bond formation, oxidation of cysteine might occur during the stacking phase of gel electrophoresis, causing the protein band to be greater than expected (Grabski and Novagen, 2001). The proline rich in protein can affect mobility due to a net charge on the protein causing slower mobility in SDS-PAGE (Bell, 2001). In this case, western blotting with histidine tag can help to confirm the interested protein.

The rPTP1 produced from *E. coli* could differentiate between *E. cuniculi*-positive and negative rabbit sera in this study. This result indicates that rPTP1 can maintain biological function although PTP1 has predicted positions of post-translation modification such as cysteine oxidation and glycosylation, which *E. coli* cannot process (Bouzahzah and Weiss, 2010; Delbac and Polonais, 2008; Xu and Weiss, 2005). The post-translation modification is associated with the invasion process into the host cell (Bouzahzah and Weiss, 2010; Xu et al., 2004). Previous reports have revealed that recombinant PTP1 of *E. hellem* expressed from *E. coli* can induce specific CD8+ T-cell immunity in immunized mice (Moretto et al., 2010) and also react with polyclonal antibodies produced from immunized rabbits with purified PTP (Keohane et al., 1998).

In conclusion, codon optimization of a *ptp1* gene can improve the concentration of expression of rPTP1 protein expressed from *E. coli*. rPTP1 protein also has a biological function. Therefore, rPTP1 is suitable to perform animal immunization for polyclonal antibody production to detect organisms in samples and can also be used as an antigen for ELISA plate coating to develop an ELISA test kit.

Acknowledgements

This work was partially supported by the Center for Advanced Studies for Agriculture and Food (CASAF), Institute for Advanced Studies, Kasetsart University Under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand and the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE), Thailand.

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บทคัดย่อ

การปรับเปลี่ยนรหัสโคดอนของยีนโพลาร์ทิวป์โปรตีน 1 ของเชื้อเอ็นเซ็พฟาลิโตซูน คูนิคูไลเพื่อเพิ่มประสิทธิภาพในการผลิตโปรตีนในแบคทีเรียเอสเชอริเชีย โคไล

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เชื้อเอ็นเซ็พฟาลิโตซูน คูนิคูไลเป็นเชื้อโรคสัตว์สู่คนที่อาศัยภายในเซลล์โฮสต์และพบได้บ่อยในกระต่าย โดยกระต่ายที่ติดเชื้อจะแสดงอาการทางระบบประสาทและไต เชื้อมีการสร้างสปอร์ซึ่งมีองค์ประกอบของโพลาร์ทิวป์โปรตีน 1 ที่สามารถกระตุ้นการตอบสนองต่อภูมิคุ้มกันของโฮสต์ วัตถุประสงค์ของการศึกษานี้เพื่อผลิตรีคอมบิแนนท์โพลาร์ทิวป์โปรตีน 1 จากเชื้อแบคทีเรียเอสเชอริเชีย โคไล โดยปรับเปลี่ยนรหัสโคดอนของยีนดั้งเดิม ทั้งสายดีเอ็นเอของยีนโพลาร์ทิวป์โปรตีน 1 ดั้งเดิมที่ได้จากการเพิ่มจำนวนยีนโดยวิธีปฏิกิริยาลูกโซ่โพลีเมอเรส และสายดีเอ็นเอที่สังเคราะห์ตามยีนโพลาร์ทิวป์โปรตีน 1 ได้ถูกปรับเปลี่ยนรหัสโคดอนเพื่อเชื่อมเข้ากับเวคเตอร์ pQE30 และย้ายเข้าสู่เซลล์อีโคไลสายพันธุ์ M15 กระตุ้นการผลิตรีคอมบิแนนท์โพลาร์ทิวป์โปรตีนโดยเติม 1M isopropyl-β-D-thiogalactoside พบแบนของโปรตีนขนาด 45 กิโลดาลตันจากโปรตีนที่สกัดจากเชื้ออีโคไลที่มียีนทั้งสองแบบ โดยพบว่าเชื้ออีโคไลที่มีส่วนของยีนที่ปรับเปลี่ยนรหัสโคดอนผลิตโปรตีนได้มากกว่าเชื้ออีโคไลที่มีส่วนของยีนดั้งเดิมจากการทดสอบด้วยเทคนิค SDS-PAGE และ western blot โดยใช้แอนติบอดีต่อกรดอะมิโนฮีสติดีน 6 ตำแหน่ง ประเมินคุณสมบัติทางชีวภาพของโปรตีนโดยทดสอบกับซีรัมของกระต่ายที่ติดเชื้อและไม่ติดเชื้อเอ็นเซ็พฟาลิโตซูน คูนิคูไลด้วยวิธี western blot โดยรีคอมบิแนนท์โพลาร์ทิวป์โปรตีนที่ผลิตจากอีโคไลนั้นสามารถแยกระหว่างซีรัมของกระต่ายที่ติดเชื้อและไม่ติดเชื้อเอ็นเซ็พฟาลิโตซูน คูนิคูไลได้ ซึ่งโปรตีนที่ได้นี้จะนำไปใช้พัฒนาชุดทดสอบสำหรับการตรวจการติดเชื้อเอ็นเซ็พฟาลิโตซูน คูนิคูไลต่อไป

คำสำคัญ: เอ็นเซ็พฟาลิโตซูน คูนิคูไล อีโคไล โพลาร์ทิวป์โปรตีน 1 กระต่าย รีคอมบิแนนท์โปรตีน

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