

Vaccination with recombinant actin from scab mites and evaluation of its protective efficacy against *Psoroptes cuniculi* infection

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SUMMARY

The mite *Psoroptes cuniculi* is globally widespread and has a serious impact on commercial rabbit breeding. Current treatment methods are based on chemotherapy. Because of the disadvantages of these methods, alternative measures are required, and vaccination is one of the most promising strategies. Here, we cloned and expressed the recombinant *P. cuniculi* actin gene (*rPc-act*). Antiserum levels against *rPc-act* in rabbits were used to locate actin distribution in mite sections. Challenge trials were carried out to evaluate the immunity protection of *rPc-act* in rabbits, with antibody levels determined by ELISA. Sequence analysis of this gene fragment showed 89.26% and 84.91% identity to *Sarcoptes scabiei* and *Mayetiola destructor* sequences, respectively. Immunohistochemistry showed *rPc-act* to locate widely throughout the mites, especially in feet and muscle tissues. Recombinant *P. cuniculi* actin with *QuliA* adjuvant was used to immunize six rabbits. Each animal was challenge-infested with 25–50 adult mites. Although IgE levels showed no significant difference to controls, IgG levels were significantly higher, and clinical development showed no significantly different severity of lesions in vaccinated rabbits than in the controls. This study showed that *rPc-act* is a muscular isotype actin and has no clinical protective efficacy against *P. cuniculi*.

Keywords actin, immunolocalization, *Psoroptes cuniculi*, vaccine

INTRODUCTION

Ear mange is a contagious disease in rabbits caused by the mite *P. cuniculi*, a worldwide obligate ectoparasite (1). The disease is characterized by intense pruritus and serious exudations. China provides more than 70%, 90% and 95% of global rabbit meat, fur and leather, respectively (2). In China, the infection has been reported in 23 provinces (3). The infection rate of *P. cuniculi* in rabbits in China has been reported to range from 7.98% to 15.7% (4, 5). This makes *P. cuniculi* one of the most important parasites of commercial rabbit breeding in China. Therapy and control of this disease are currently based on a range of acaricides, but the resistance is emerging (6). Therefore, novel control methods are required, and vaccination represents one of the most promising candidates (7).

Actin is a component of muscle and nonmuscle cell structures and exists in all eukaryotic cells. It participates in many important cellular processes, such as muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signalling and the establishment and maintenance of cell junctions and cell shape (8, 9). Actin sequences are highly conserved among species (10). Genes encoding actin are classified into muscle or nonmuscle actin, and the latter is often used as an internal control for determination of gene expression, as it is constitutively expressed in all cells (9, 11). The actin genes from several species closely related to *P. cuniculi* have been identified, including hard ticks (11), soft ticks (12) and *Sarcoptes scabiei* mites (Accession number: EU624346, data unpublished). In 2001 (13), mice vaccinated with recombinant actin were challenged to evaluate the proteins potential as a vaccine candidate against animal schistosomiasis caused by *Schistosoma japonicum*. This study showed a significant protective effect and suggested actin as a vaccine candidate.

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Immunization with recombinant actin from *Trypanosoma evansi* was reported to induce protective immunity against *T. evansi*, *T. equiperdum* and *T. b. brucei* infection (14). It was suggested that recombinant actin may induce protection against several different trypanosome species. Together with other cytoskeletal proteins of trypanosomes, it is a potential candidate for the development of vaccines.

In this study, we determined whether immunization with recombinant *P. cuniculi* actin has a protective effect against infestation with the scab mite, *P. cuniculi*. And we report the success of cloning, expression and immunolocalization of the actin gene of *P. cuniculi*, and evaluate its potential as a vaccine candidate.

MATERIALS AND METHODS

Mites and animals

P. cuniculi mites were maintained on rabbits at the Laboratory Animal Center of Sichuan Agricultural University, China. After harvesting, mites were frozen immediately at -80°C prior to RNA extraction. Mites used in the RNA extraction included adults and nymphs of both sex. The others for the challenge trials were only adults including both genders. And the mites were unfed before the experiments.

Naïve female New Zealand rabbits of 1–2 kg body weight age between 2 to 3 months were obtained from the Laboratory Animal Center, Sichuan Agricultural University, China. The experimental protocols and animal care were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Gene cloning and analysis

Total RNA of mites was extracted using an RNA isolation kit (Watson, Parsippany, NJ, USA), and the cDNA was synthesized using a cDNA synthesis kit (Fermentas, Shenzhen, Guangzhou, China) according to the respective manual instructions. The *actin* gene of *P. cuniculi* was amplified from cDNA using primers designed according to published actin sequences of *S. scabiei* var. *Hominis* [forward primer: 5'-TAAGCAATGTGTGACGAC-3'; reverse primer: 5'-TTAAAAGCATTCTGTGTA-3']. The PCR temperature was 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 1 min. Samples were incubated for a further 10 min at 72°C then held at 4°C . The amplified fragments were separated by agarose gel electrophoresis and purified with a QIAquick Gel Extraction Kit (Qiagen Inc., Dusseldorf, Germany) according to the manufacturers instructions. The purified PCR products were subcloned into a pMD 19-T vector (TaKaRa Bio CO. Ltd., Dalian, Liaoning, China) and

sequenced by Invitrogen (Beijing, China). The plasmid was transferred into *E. coli* strain DH5 α (Fermentas).

Expression and purification of recombinant actin

The primers with restriction sites were as follow, forward primer: 5'-CGGGATCCATGTGTGACGACGAAGTA-3'; reverse primer: 5'-CCCAAGCTTTTAAAAGCATTTTCTGTGTA-3'. The DNA fragment was subcloned into the *Bam*HI and *Hind*III restriction sites of the pET-32a(+) vector (Novagen, Irene, Centurion, South Africa) using T4 DNA ligase under standard conditions. It was then transformed into *E. coli* DH5 α cells. Prior to being inserted into transformed *E. coli* BL21 (DE3) (Novagen) for expression, the construction was verified by sequencing of the insert from both junctions. Bacteria were transformed overnight and were transferred into a LB medium supplemented with ampicillin (50 $\mu\text{g}/\text{mL}$) and were grown in a shaking incubator at 37°C . Then, isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM. Growth was continued for 4 h after induction. The recombinant actin was purified as described by Chen (15).

Detection of recombinant actin: SDS-PAGE and western blot

Before protein samples were detected by SDS-PAGE, they were adjusted to the composition of the electrophoresis sample buffer and boiled for 5 min. The SDS-PAGE was performed as described by Sambrook and Russell (16). The gels were stained with Coomassie blue and subsequently transferred to a PVDF membrane (Millipore, Shanghai, China) for 1 h with an electrophoretic transfer cell (Bio-Rad, Shanghai, China). The blotting membrane was blocked with TBST (40 mM Tris-HCl, 0.5 M NaCl, 0.1% Tween-20, pH 7.4) containing 5% skimmed milk at 4°C for 2 h. Then, the membrane was incubated with rabbit antiserum (diluted 1 : 200 (v/v) with 1% skimmed milk-TBST) overnight at room temperature. Prior to incubation with 1 : 1000 horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (AMRESCO) for 2 h, the membrane was washed with TBST three times. After incubation, detection was performed using diaminobenzidine (DAB) substrate (Sigma, St. Louis, MO, USA) for 3–5 min to visualize proteins.

Actin immunolocalization

Antiserum raised against rPc-act in rabbits using standard methods (17) was used to locate rPc-act in mite sections. Mites were fixed in 1% molten agarose and embedded in paraffin wax. Then, 5- μm sections were cut off from the wax cubes and dried on Superfrost slides. Sections were

dewaxed in xylene and rehydrated. Afterwards, sections were treated to remove endogenous peroxidase and blocked in 25% normal goat serum in TBS (1X) for 30 min and then incubated overnight at 4°C with serum against rPc-act in rabbits diluted 1 : 1000 in TBS. Following washing and incubation in HRP-conjugated goat anti-rabbit IgG (AMRESCO) (1 : 200 dilution in TBS) (AMRESCO) for 1 h at room temperature, the sections were washed. Detection of the goat antibody conjugate was carried out using DAB substrate. Finally, sections were washed in water, counterstained with haematoxylin, rinsed in water, dehydrated and mounted in a resin-based mountant step-by-step. Serums from naïve rabbits group were regarded as negative controls.

Challenge trials and sampling

Eighteen *P. cuniculi*-naïve rabbits weighing 1–2 kg each were randomly divided into three groups: six were immunized with rPc-act, six with control 0.9% saline buffer and six with control QuilA (0.01 g/mL, International Laboratory, South San Francisco, CA, USA) adjuvant. Each animal was injected subcutaneously with either 170 µL (500 µg) rPc-act with 10 µL QuilA and 320 µL normal saline buffer (NS), with 10 µL QuilA and 490 µL NS, or with 500 µL NS only, respectively. The vaccination schedule contained four injections. Before the third injection, an interval of seven days took place between vaccinations. Afterwards, the interval was extended to 14 days before the last injection. Two weeks later, the left ear of each rabbit was challenged with 25–50 vigorous adult *P. cuniculi* mites (18) and wrapped with gauze for 48 h. Weight and lesion areas of each rabbit were recorded once per week since the start of infestation. The lesion areas were measured by otoscope and flexible ruler. The lesion areas and the proportions in the whole left ear were calculated. The scores of the lesion areas were classified to three levels. (1) Represented small lesions only identified by otoscope, (2) represented lesion area within 10% of the auricle, and (3) represented lesion area above 10% of the auricle. Blood samples were obtained from the ear arteries, and serum was isolated and stored at –20°C.

ELISA analysis

Recombinant *P. cuniculi* actin-specific IgG and total IgE antibody levels in vaccinated rabbits were evaluated by an enzyme-linked immunosorbent assay (ELISA). Individual wells of 96-well microtiter plates (Invitrogen) were coated with 100 µL purified rPc-act at 4°C overnight in carbonate buffer (0.06 M, pH 9.6). The plates were washed with PBS containing 0.05% Tween-20 (PBST) four times, then

blocked with BSA (AMRESCO) in PBS and incubated at 37°C for 1 h. Before 100 µL serum (1 : 200) was added to each well and incubated at 37°C for 1 h, the wells were washed with PBST four times. Then, the wells were washed again with PBST and incubated with HRP-conjugated goat anti-rabbit IgG (1 : 5000) (AMRESCO) at 37°C for 1 h. Colour reaction was developed by adding 100 µL TMB substrate (45 mM dibasic sodium phosphate, 0.22 mM citric acid, 0.42 mM 3,3',5,5' tetramethylbenzidine and 2×10^{-2} H₂O₂) (Sigma) per well, and the reaction was stopped 15 min later by adding 100 µL of 0.5 M H₂SO₄ to each well. The plates were read at 460 nm in an ELISA reader (Thermo Labsystems, Shanghai, China). Total IgE was measured by rabbit IgE ELISA kit (GDB, San Diego, CA, USA) as instructed by the manufacturer.

Statistical analysis

NCBI BLAST and DNAMAN were used to analyse the sequences and ran alignments. The structure prediction was performed by ExPASy (http://us.expasy.org/tools/pi_tool.html). The prediction of transmembrane was carried out by TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>).

Statistical analysis was performed with ANOVA and least squares difference (LSD) using SPSS version 13.0 (Shanghai, China) for Windows. $P < 0.05$ was considered as significantly different.

RESULTS

Gene cloning and sequence analysis

A DNA fragment (Accession number: JQ936991) of 1131 nucleotides length was amplified by RT-PCR from the total RNA of mites. Phylogenetic analysis showed the gene encoding rPc-act to cluster with *S. scabiei* actin into one branch separate from tick sequences. The rPc-act gene showed high degrees of homology to *S. scabiei* and *Mayetiola destructor* sequences, which were 89.26% and 84.91% identical, respectively (Figure 1). Sequence analysis revealed that it encodes a 376 amino acid protein of 41.4 kDa and pI = 6.09 without signal peptide. It was analysed as an outer membrane protein. This protein showed 99.2%, 90.7%, 91.2%, 91.8% and 91.0% amino acid identity to actin from *S. scabiei*, *Liposcelis bostrychophila*, *Aedes aegypti*, *Oikopleura longicauda* and *M. destructor* (Figure 2), respectively.

Expression and purification of recombinant actin

Recombinant actin of *P. cuniculi* was expressed in a pET-32a vector. The protein was found in the supernatant, and

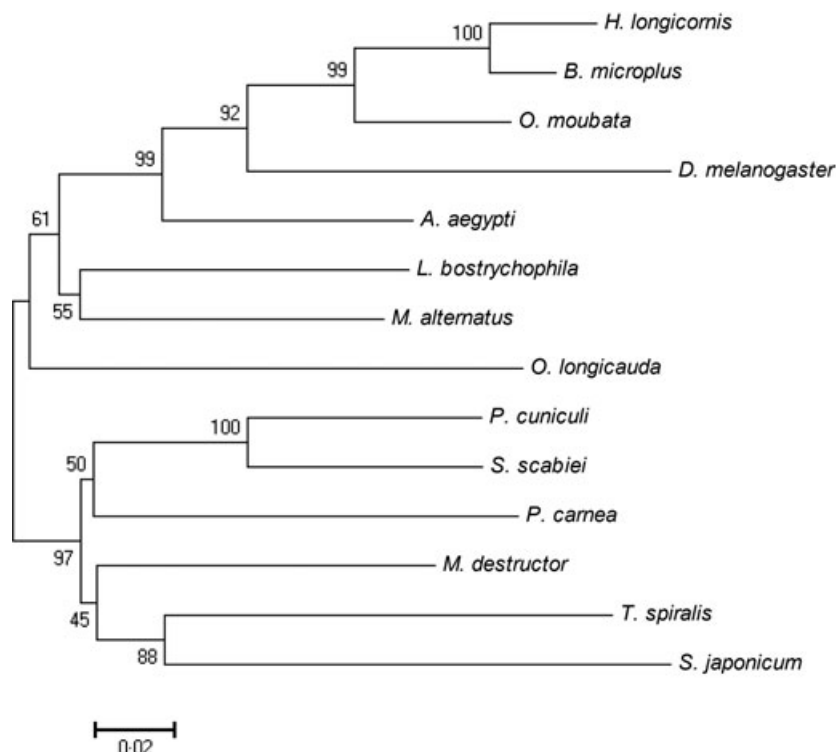


Figure 1 Phylogenetic tree of actin genes constructed by the neighbour-joining method with Clustal X program. Numbers at the branch indicate the bootstrap values with 1000 replicates. The sequences used were *Psoroptes cuniculi* (JQ936991), *Oikopleura longicauda* (AB034208.1), *Haemaphysalis longicornis* (AY254898.1), *Sarcoptes scabiei* (EU624346.1), *Drosophila melanogaster* (K00670.1), *Liposcelis bostrychophila* (FJ447483.2), *Aedes aegypti* (AY289764.1), *Ornithodoros moubata* (AB208021.1), *Boophilus microplus* (AY255624.1), *Mayetiola destructor* (AF017427.1), *Podocoryne carnea* (X69059.1), *Monochamus alternatus* (EU100951.1), *Trichinella spiralis* (XM_003373527.1) and *Schistosoma japonicum* (AF223400.1).

its mass was estimated by SDS-PAGE as approximately 62 kDa (Figure 3), which included 20 kDa His-Tags. The peak of expression was observed 4 h after induction by IPTG.

Western blot analysis

Recombinant Pc-act was transferred onto nitrocellulose membranes and probed with sera from rabbit infected with *P. cuniculi* or naïve animals. IgG from rabbits previously infected with the parasites reacted with the recombinant protein, whereas it did not react with the negative control (Figure 4).

Actin immunolocalization

Immunolocalization was carried out to visualize anatomical distribution of rPc-act in the sections of adult *P. cuniculi* (Figure 5). Immunostaining was found widespread through the sections, and rPc-act was localized specifically in the chelicerae and in body muscle tissue.

Vaccination trials

Mites could be found in the left auricle of each rabbit 24 h after infestation. Through four weeks of observation, the lesion area of the auricles was recorded (Table 1). A visible inflammatory response could only be identified by otoscope in all three groups 1 week p.i. In the rPc-act group, crust was only detected in two vaccinated rabbits during the first week after challenge. However, until the end of the trial, most animals (4/6) were infested with *P. cuniculi*, and one of the infested rabbits was detected with more evident clinical development. In the QuilA and NS control groups, more rabbits were infested compared to the rPc-act group. However, no significant difference in the severity of lesion areas could be identified between the vaccination group and the two control groups. Live mites were re-isolated from each infested rabbit, and no obvious decrease was found. Body weight of the animals remained unchanged during the challenge trial (data not shown).

Serological responses were determined by ELISA, and Figure 6 shows the specific IgG antibody responses. The

A.aegypti	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
D.melanogaster	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
H.longicornis	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
L.bostrychophila	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
M.destructor	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
O.longicauda	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
P.cuniculi	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
R.microplus	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
S.scabiei	MCDDEVALVINGSGMCKAGFAGDDA.PRAVFPISVGRPHGQVAVGMQKDSVYGEAQ
A.aegypti	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
D.melanogaster	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
H.longicornis	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
L.bostrychophila	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
M.destructor	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
O.longicauda	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
P.cuniculi	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
R.microplus	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
S.scabiei	SRGILTLKPYIEHGIITNMDEKINHHFTFNELRVAPEHPVILLTEALPKNRKN
A.aegypti	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
D.melanogaster	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
H.longicornis	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
L.bostrychophila	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
M.destructor	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
O.longicauda	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
P.cuniculi	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
R.microplus	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
S.scabiei	TOIMETFNTPAMVYALQAVLSVAGRTTGVILDSGDSVSHTPVIEGYALPHAIIRLD
A.aegypti	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
D.melanogaster	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
H.longicornis	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
L.bostrychophila	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
M.destructor	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
O.longicauda	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
P.cuniculi	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
R.microplus	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
S.scabiei	LAGRLTDYIMKILTERGYSFTTAREIYRDIREKICVYALDFEOMATAAASSTLEKS
A.aegypti	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
D.melanogaster	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
H.longicornis	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
L.bostrychophila	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
M.destructor	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
O.longicauda	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
P.cuniculi	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
R.microplus	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
S.scabiei	YELPDGAVITIGNERFCPEALPQSPFIAMEAGIHETTYSINMKCDIDIRKOLYANTVL
A.aegypti	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
D.melanogaster	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
H.longicornis	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
L.bostrychophila	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
M.destructor	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
O.longicauda	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
P.cuniculi	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
R.microplus	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
S.scabiei	SGGTTMYPGIADMOKEITLAPSTIKIILAPPRKYSWIGGSIILASTFOQWWSK
A.aegypti	EYDSESGSIVHRCF
D.melanogaster	EYDSESGSIVHRCF
H.longicornis	EYDSESGSIVHRCF
L.bostrychophila	EYDSESGSIVHRCF
M.destructor	EYDSESGSIVHRCF
O.longicauda	EYDSESGSIVHRCF
P.cuniculi	EYDSESGSIVHRCF
R.microplus	EYDSESGSIVHRCF
S.scabiei	EYDSESGSIVHRCF

Figure 2 Comparison of rPc-act with the amino acid sequences of actin of other species. Accession numbers were as follows: *Drosophila melanogaster*: NP_523625-1, *Aedes aegypti*: AAQ24506-1, *Rhipicephalus microplus*: AAP79880-1, *Liposcelis bostrychophila*: ACJ65018-2, *Haemaphysalis longicornis*: AY254898-1, *Oikopleura longicauda*: BAA86216-1, *Mayetiola destructor*: O16808-1, *Sarcoptes scabiei*: ACC85688-1.

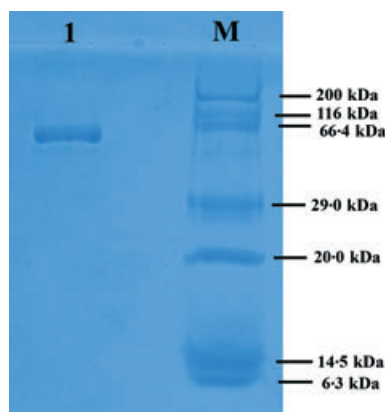


Figure 3 Coomassie-stained gel examined by SDS-PAGE analysis of the recombinant actin of *P. cuniculi*. Lane 1: purified recombinant proteins; lane M: protein marker.

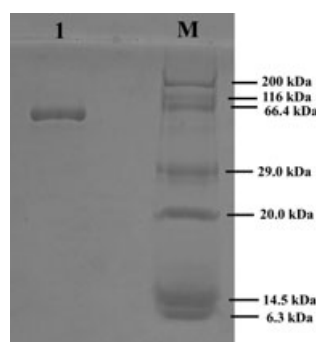


Figure 4 Western blot analysis of recombinant actin of *P. cuniculi*. Lane 1: purified recombinant proteins; lane M: protein marker; lane NC: negative control.

specific IgG rose rapidly since the first injection with recombinant actin protein in the rPc-act group. Four weeks after the vaccination, the specific IgG levels in the vaccination group started to stabilize gradually, and no significant difference could be detected since then ($P > 0.05$). Afterwards, the OD₄₅₀ reached its peak (1.96) seven days later. Although significant differences could be observed between their maxima and minima ($P < 0.01$), the OD₄₅₀ value of the specific IgG antibody still remained low and was significantly different ($P < 0.01$) at each time point in the negative controls compared to the vaccinated animals. The total IgE level is shown in Figure 7. In the rPc-act group, IgE levels remained relatively low with a few fluctuations during the whole trial. However, the IgE titres of the NS group increased after the challenge and were significantly higher ($P < 0.05$) than the levels of the vaccination group since 1 week p.i.

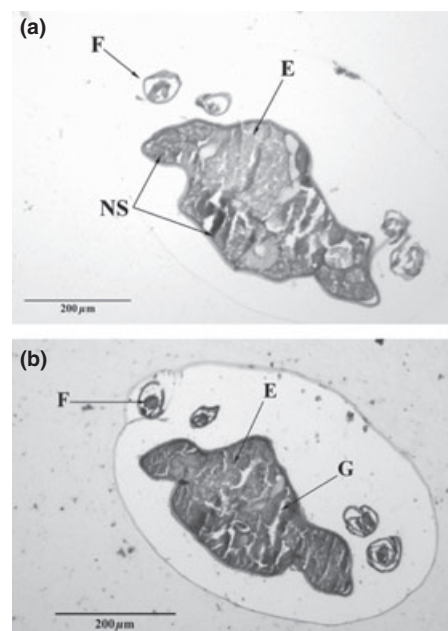


Figure 5 Immunolocalization of rPc-act in sections of adult *P. cuniculi*. (b) Incubated with rabbit anti rPc-act as primary antibody; (a) primary antibody omitted. Immunoreactivity was visualized using goat anti-rabbit HRP conjugate, followed by incubation in DAB substrate. Areas annotated with G show sections through gut; C: chelicera; E: egg; MT: muscle tissue; NS: nonspecific staining area.

DISCUSSION

In this study, we demonstrate the successful cloning and expression of recombinant *P. cuniculi* actin in *E. coli*. The amino acid sequence of this putative actin showed a high degree of homology with actin from *S. scabiei*, *Haemaphysalis longicornis*, *Rhipicephalus appendiculatus* (11), *A. aegypti* (19), *Ornithodoros moubata*, *Schistosoma*, *T. evansi* and *Drosophila melanogaster* (20). The amino acid sequence of rPc-act showed high similarity to muscle actin genes in these species. Moreover, because of the distribution of the recombinant actin, which is mostly localized in the musculature, it can be classified as an isotype of muscle actin (18). Mounting evidence suggests that actin is involved in multiple processes during the life cycle of parasites and has critical importance in motility (19, 21) and endocytosis (14). Our immunolocalization results indicate that rPc-act is mainly expressed in muscle tissue and in the feet, which are both critical for feeding and motility. Therefore, some negative effects should be expected in these survival activities, while the mite is exposed to actin antibodies.

Actin has been investigated as a vaccine candidate in a number of parasitic species. An earlier study (13) showed

Table 1 Clinical lesion area development of rabbits with experimental aural infestations with 25–50 *P. cuniculi* mites

Group (n = 6)	Number of rabbits with clinical manifestation (severity of lesion area)			
	1 week p.i.	2 weeks p.i.	3 weeks p.i.	4 weeks p.i.
rPc-act	2 ^a	2 ^a , 1 ^b	2 ^a , 1 ^b	3 ^a , 1 ^c
QuilA	4 ^a	1 ^a , 4 ^b	1 ^a , 4 ^b	1 ^a , 3 ^b , 1 ^c
NS	4 ^a	1 ^a , 3 ^b	3 ^b , 1 ^c	3 ^b , 1 ^c

^aSmall lesions only identified by otoscope; ^blesion area within 10% of the auricle; ^clesion area above 10% of the auricle.

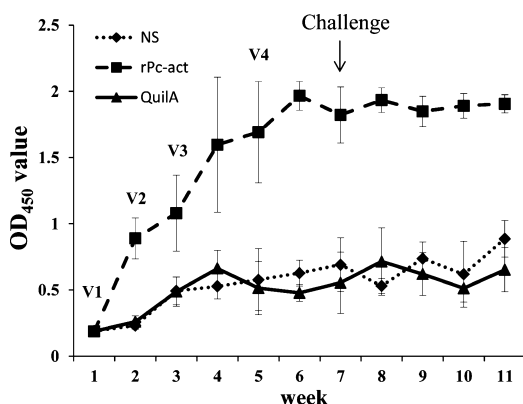


Figure 6 Serological development determined by ELISA for specific IgG against rPc-act in rabbits before and after the experimental infestation with 25–50 live *P. cuniculi* mites. OD value determined as absorbance at 450 nm. V1: first vaccination; V2: second vaccination; V3: third vaccination; V4: fourth vaccination.

that in vaccinated mice, the number of *Schistosoma japonicum* parasites and eggs per gram of liver tissue were significantly lower than in the control animals. It was demonstrated that mice vaccinated with recombinant actin from *T. evansi* elicited protective immunity against infection with three species of *Trypanosoma* (14). These results suggest that rPc-act might be used to induce immunity protection against *P. cuniculi*. Our ELISA experiment demonstrates the elicitation of IgG and low-titre IgE isotypes. Similar to other reports (17, 22), specific IgG antibody response against *P. cuniculi* was detected. Although there may be some positive correlation between lesion area reduction and IgG level increase, the differences between vaccinated group and the controls are not statistically significant, suggesting that rPc-act possesses no clinical protective efficacy against *P. cuniculi*.

IgE levels showed no significant increase during the entire trial in the rPc-act group. The actin is not an allergen to cause allergy hypersensitivity in the animals immune response system, and IgE is an important index in the hypersensitivity. So the low IgE level should mainly attribute to the features of the protein and the relatively

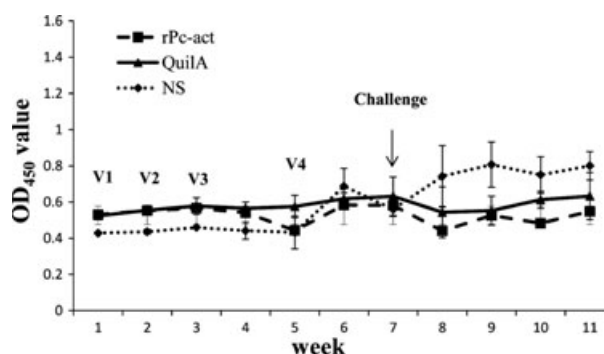


Figure 7 Serological development determined by ELISA for specific IgE against rPc-act in rabbits before and after the experimental infestation with 25–50 live *P. cuniculi* mites. OD value determined as absorbance at 450 nm. V1: first vaccination; V2: second vaccination; V3: third vaccination; V4: fourth vaccination.

short monitoring period. In addition, van den Broek *et al.* (22) reported that the mean IgE levels did not increase until six weeks p.i. These findings suggest that although IgE may be involved in host defence against *Psoroptes* mites, its detailed role in immunopathogenesis still needs to be investigated. An increase in total IgE shows in the normal saline controls but not in the other two groups. This may cause a wonder that whether the QuilA adjuvant may reduce the responsiveness to *P. cuniculi* allergens. Indeed, the IgE level in the NS controls showed a little higher than the other two after challenge. First, this is not a statistical significant difference. Second, we have not seen any published references indicated that QuilA adjuvant might depress IgE levels. At last, a colleague in our laboratory had finished her study with exact the same dose of the same adjuvant (QuilA), and the results of her study did not show IgE depression in *S. Scabiei*-infected rabbits (23). Therefore, we concluded that it was not the QuilA adjuvant that depressed IgE levels, but something else which worth more further study in the future.

However, the immune mechanisms of infestations with *Psoroptes* remain unclear. Huntley *et al.* (24) found that

both the lesion area and the numbers of mites in sheep treated with an immunosuppressive agent were higher than in the control group. Interestingly, this immunosuppressant did not have any negative effect on mites *in vitro*. Eosinophils (25) and host immunoglobulin (26, 27) have been detected in *P. ovis* and *P. cuniculi*. These findings suggest that mites can take immunocytes and immunoglobulin of their host as a food source instead of being killed by the hosts immune response. McNair (28) pointed out that mites could feed and survive on the host with the benefits of certain interactions between mite secretions and the host immune response. The different number of mites (25 to 50) did not seem to impact the severity of the infection. In previous studies (17, 22), they took the same range of number to infected rabbits or sheep, which indicates this is a reliable and mature method. It suggested that the tiny difference of the mite numbers among this range might not influence the result.

In conclusion, we show the characterization and immunolocalization of actin, which suggest that rPc-act may be critical to mite survival. However, no protection is offered against mite infection in rabbits vaccinated with the recombinant actin. This is inconsistent with previous reports describing full protection against parasites in other species. Our results still remain valuable for further investigations, which may concentrate on the development of optimal applications of allergens or immunogens as vaccines to prevent *P. cuniculi* infection.

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