GENOMIC CLONING OF *FASCIOLA HEPATICA* EXCRETORY - SECRETORY ANTIGENIC GENES IN *E.COLI*

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ABSTRACT

A complementary DNA (cDNA) library was constructed from the mRNA of adult worms of *Fasciola hepatica* in the expression vector λgt11, the size of the library contained approximately 2.4 x 10^5 recombinants and the recombinant efficiency was 85%. The library was directedly screened with a rabbit antisera raised against a excretory-secretory (ES) antigen of *Fasciola hepatica*. Two positive clones of strong signal were selected from a group of 200 positive clones, named λFH3 and λFH7. The length of external fragments of two recombinants were 0.85Kb (λFH3) and 1.15Kb (λFH7). The *E.coli* 1089 host was infected by recombinants λFH3 and λFH7 respectively, through SDS - PAGE (polyacrylamide gel electrophoresis) after induced by isopropyl-D-thiogalactopyranoside (IPTG) (final concentration 5mM), the results indicated that the molecular weight of fusion protein expressed (β-galactosidase fusion protein) of recombinant λFH3 was 138KD, and that of λFH7 was 156KD, the rate of fusion proteins expression was approximately 100mg/liter. Western - blot showed that two hybrid proteins corresponded to 26.2KD protein band ((FH3) and 38.8KD protein band (λFH7) of *Fasciola hepatica* ES antigens.

KEYWORDS

Genomic cloning, *Fasciola hepatica*, ES antigen, cDNA library, immunoscreening, expression

INTRODUCTION

Fascioliasis is a serious parasitic disease over the world. *Fasciola hepatica* are a widely spread parasite in many kinds of economic herbivores such as sheep, yak, cattle, horse, deer, camel, … etc., and hinder the development of the productive force of livestock husbandry. Moreover, the economic losses caused by fascioliasis can be very huge. So it is very important to develop an effective vaccine against fascioliasis. Irving (1982) and Haroun (1986) reported that ES antigens of *Fasciola hepatica* could induce effective protection against infection. As it is impractical to obtain enough ES antigens for a commercial vaccine against these worms, an alternative approach is to use recombinant DNA methods to generate a cheap and plentiful supply of antigens. We report here the cloning and expression in *Escherichia coli* of the gene encoding ES antigen as fusion proteins with *E.coli* β-galactosidase. Vaccination of rats with these fusion proteins gave significant, although not complete, immunity against challenge infection with *F. hepatica* metacercaria. Commercial development of a vaccine is being pursued.

MATERIALS AND METHODS

Parasites, antigens preparations and antisera production. Adult *Fasciola hepatica* were harvested from the liver of yaks, washed in 0.01M phosphate-buffered saline (PBS) for three times, and cultured in liquid media RPMI 1640 for 24 hours at 37°C. ES antigens were prepared from media through centrifuging, filtrating, and dialyzing. ES antigens were mixed and emulsified with the same volume of Freund's completed adjavents (Sigma) and used to inoculate New Zealand rabbits by the standard procedure. Antisera titres were determined by ELISA (George, 1988).

Construction of cDNA library. The poly(A)$^+$RNA was separated from the total RNA of *F.hepatica* by oligo(dT) cellulose columns and was converted into cDNA(Sambrook, 1989). The cDNAs were ligated to synthetic EcoR I linkers after methylation of internal EcoR I sites. The linker ligated cDNAs were digested with EcoR I and separated from the linkers by Sephacryl S-400 chromatography, then ligated to EcoR I-digested alkaline-phosphatase-treated λgt11 vector DNA and packaged in vitro with commercial packaging reactions (BRL). Packaged phage were plated on *Escherichia coli* host strain Y1090.

Immunoscreening of cDNA library. The cDNA library was immunoscreened as phage plaques on *E.coli* host strain Y1090 at a density of 2000~4000 plaques per 85mm plates. After inducing fusion protein expression with IPTG at 42°C, the colonies were screened with 1:100 pre-absorbed rabbit antisera and anti-rabbit IgG coupled to HRP (1:200). After the primary screening (approximately 25,000 plaques), 200 colonies gave positive signals. Then they were picked up and rescreened.

Identification of recombinants. Positive recombinant phages were incubated to lysate on *E.coli* Y1090, the phage DNA was extracted. The insert fragments were cut down with E*coR*I, separated in 0.7% agarose gel, and recovered by low melting agarose. Then they were subcloned to pUC18 (E*coR*I site), transformed into *E.coli* DH5α, and spread in X-gal plate. Recombinant plasmids were extracted and their physical maps were analysed using restriction enzymes (B*amH*I, P*st*I, H*ind*III, S*ma*I, X*ba*I, and S*al*I).

Expression of recombinant fusion proteins. *E.coli* Y1089 was infected by recombinant phages λFH3 and λFH7 respectively, and cultured in LB media containing ampicillin at final concentration of 80 g/ml and shaken at 30°C for 12-15 hours until the cell density reached $O.D._{600}$=1.5, then transfered culture to 45°C for 15 min, add IPTG at final concentration of 5mM to induce. After incubated for 2 hrs, pelleted at 4000rpm for 10 min, the cells were frozen and disrupted, and were analysed by PAGE, in 10% gels containing SDS (Laemmli, 1970).

Western blotting.Recombinant hydrid proteins were fractionated in 10% SDS slab gel and transferred onto nitrocelluse filter (probind, Pharmacia AB.) by using the Semidryt Transblot Appateurs. The nitrocelluse filter was stained with anti-ES antiserum, developed with Biotin-Anti-rabbit IgG (Sigma), Avidin-HRP (Sigma) and 4-chloro-1-Naiphenal, by the procedure of Sambrook (1989).

RESULTS AND DISCUSSION

Antigens preparations and antisera production. ES antigens were separated by SDS-PAGE gel, gels were stained with 0.01% Coomassie blue R-250. It was shown that ES antigens mainly contained five protein bands, their molecular weight were 38.8KD, 28KD, 26.2KD, 23.4KD, 18.7KD (1KD = 1, 000 dalton). Among them the 38.8KD and 26.2KD bands show comparatively high rate, then these two bands were recovered from gel and antisera against these two proteins and total ES antigens were prepared.

cDNA library. The cDNA library of *F.hepatica* constructed in this paper contained approximately 2.4 x 10^5 recombinant phages and the recombinant efficiency was determined by growth on IPTG-X-gal (5-bromo-4-chloro-3-indolyl-D-galactoside) plates was 85%.

Immunological screening of recombinants. After inducing hybrid protein expression at 42°C and screening with pre-absorbed rabbit antisera, 200 colonies gave positive signals. Positive colonies on master plate were identified, hand-picked and rescreened with ES antisera and protein band antisera (38.8KD, 26.2KD), two strong positive colonies were selected, named λFH3 and λFH7.

Identification of recombinants. The insert fragment in λFH3 was 0.85Kb, that in λFH7 was 1.15Kb. Recombinant plasmids pFH3 and pFH7, corresponding to recombinant phages λFH3 and λFH7, was digested by restriction enzymes, the results showed that the restriction map of insert fragment in λFH3 was different to that in λFH7. So two different ES antigenic genes had been obtained.

Nature of expressed hybrid proteins. Induced bacterial extracts were examined by SDS-PAGE. The fusion proteins (β-galactosidase hybrid proteins) expressed by recombinants had molecular weight of 138KD (λFH3) and 156KD (λFH7), of which the *F.hepatica* components were 22KD (λFH3) and 40KD (λFH7) (The molecular mass of β-galactosidase expressed in recombinant λgt11 was 116KD). By Western blotting and screening with either 26.2KD band or 38.8KD band antisera, the results showed that the fusion protein expressed by λFH3 was corresponding to 26.2KD protein band of ES, that expressed by λFH7 was corresponding to 38.8KD protein band of ES.

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