Establishing a Platform of scale-up expression of recombinant protein antigens by 30 L bioreactor

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(1)Introduction

Subunit vaccine has become the focus of the new generation of vaccine development project. To address the bottleneck of industrial production there is a need to establish a system for high density fermentation of E. coli for mass production of genetically engineered proteins. This technology can be applied to the production of vaccine antigen recombinant protein. The advantages include high volume yield, high growth rate, high product concentration and low raw material costs, shortened time frame of production of the recombinant protein. In this project, we chose swine streptococcus (*Streptococcus suis; S. suis*) recombinant protein as a model because it is a zoonotic infectious disease, according to the Animal Health Research Institute, Executive Yuan in 2008. In Taiwan's pig farms, S. suis accounted for up to 80% of bacterial respiratory pathogens. Based on the capsular surface polysaccharides (cps), *S. suis* has as many as 33 serotypes. Infect pigs show symptoms of arthritis, meningitis, sepsis, and sudden death can occur, causing economic losses. Taiwan currently lacks S. suis vaccine, the many serotypes also present a problem for vaccine development. For epidemic prevention, there is an urgent need for the development of an effective vaccine to prevent this disease.

(2)Design Concept

Industrial enzymes and medical protein usually choose *E. coli* as a recombinant protein production system since the main physiological and metabolic mechanisms of *E. coli* have been studied quite thoroughly, and the use of biotechnology is also more mature. The major bottleneck for the development of commercial recombinant subunit vaccine is the mass production technology. Most traditional production of bacteria-derived recombinant proteins is mainly based on "shaking culture" of 10 L flasks, which has limited culture space, nutrient consumption, accumulation of metabolites and unable to supply the gas, thus not suitable for long-term culture and mass production. Therefore, we need to develop efficient production platform to reduce cost.

(3)Technical Development

We cultured *E. coli* in a 30-liter pilot bioreactor and used fed-batch mode to improve production efficiency. The relationship of acetate and oxygen throughout the fermentation process were be explored. The ingredients in medium and bacterial growth conditions were tested, thus increasing the recombinant protein rSao yield. This can be developed for the production of low-cost vaccine antigen of industrialization technology platform. In addition, we selected different segments of recombinant protein antigen, made different combinations of subunit vaccines, immunized animals and carried out a series of immunoassay and effectiveness assessment tests.

(4)Technological Competitiveness

Recent research indicated that a new surface protein designated Sao (surface antigen one) reacts with 28 serotypes of sera, making Sao a good potential antigen for developing *S. suis* cross-protective vaccines. In this study, the Sao recombinant protein (rSao) has been successfully mass-produced with the pilot 30-liter bioreactor. The recombinant protein can be recognized by *S. suis* convalescent-phase sera during *S. suis* infections with Western blot. In mouse model, the immune rSao full-length group, when compared with the other groups, can effectively enhance the antibody titers ($p<0.01$), and provide resistance to mice when challenged with serotype 1, thus confirming that rSao provides cross-protection. These results showed that rSao has the potential for the development of a subunit vaccine. In addition to the patents, publish articles and technical transfer to the animal vaccine industry, this commercialization of research will benefit livestock breeders.

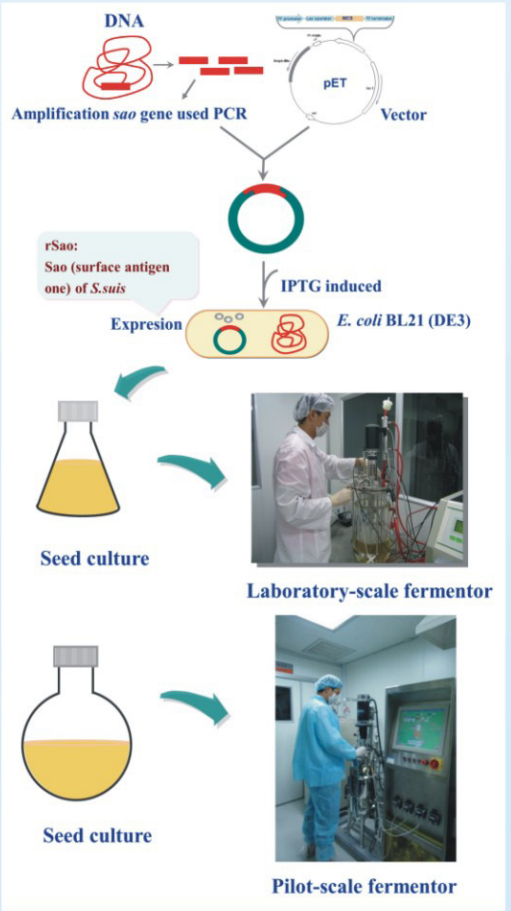

Fig. 1. From cloning the bacterial antigen to scale-up the manufactory platform of recombinant protein.

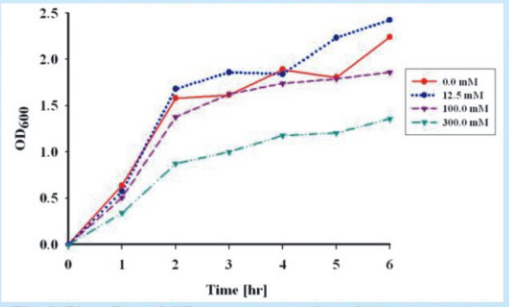

Fig. 2. The effect of different concentration of nitrogen sources in fed batch culture.

(5)R&D Result

We have successfully scaled-up expression of the target protein from a small amount to the mass production of the pilot plant level fermentor technology (Fig. 1), improved medium composition and culture conditions (Fig. 2), and established the parameters of fermentation condition (Fig. 3) in order to improve the yield of the recombinant protein. On the other hand, screening and prediction of the antigenicity site of antigen fragments was done with computer softwares (Fig. 4) We cloned and expressed the proteins using genetic engineering techniques,

including Sao full-length, N-terminus and C-terminus (Fig. 5). After that, proteins were combined with adjuvant as subunit vaccines: (A) rSao-full-length (B) rSao-N-terminus, (C) rSao-C-terminus, (D) empty vector, (E) control group, to verify immunogenicity in mice model. Mice were intramuscularly (0.2 mL) immunized, and boosted with the same vaccine 2 weeks after the primary immunization. All mice were challenged intraperitoneally (i.p.) with *S. suis* serotype 1 strain (P1) 2 weeks after the secondary immunization. Daily observation showed that rSao full length group survival rate was 80%, the C terminal group 40%, the N-terminal group, empty vector, and the control group were all 0% (Fig. 6). Analysis of IgG antibody titers, rSao full length and the C-terminal group had significant difference ($p < 0.05$) compared with the other three groups (Fig. 7). In summary, this study has successfully produced biologically active rSao with 30-liter bioreactor and established mass production technology platform. This research has resulted in technology transfer, patent applications and new drug registration procedures. These results could be applied in commercial-scaled manufacture of animal vaccines, recombinant protein production processes, reaching the goal of consistency and reducing production costs. These results should assist local animal vaccine industry upgrade their technology and enter the international market.

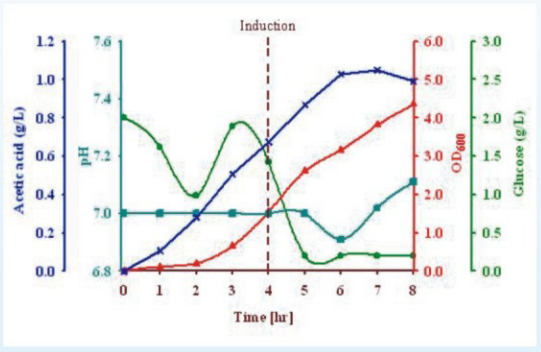

Fig. 3. The parameters of fermentation process during the cell growth. The arrow indicates the time of induction.

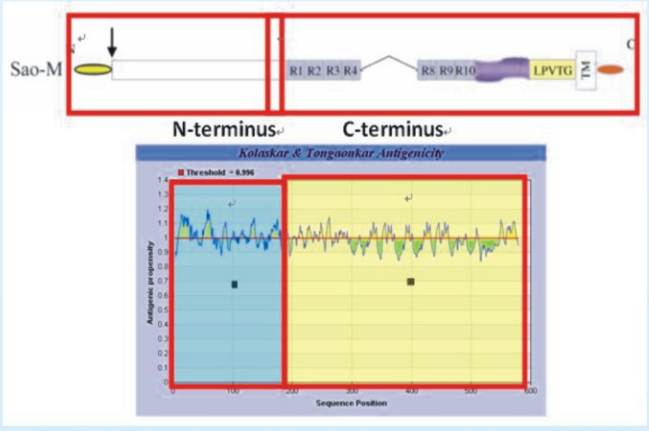

Fig. 4. Prediction the antigenic site of the N- and C-terminus of Sao with IEDB (Immunoepitope database).

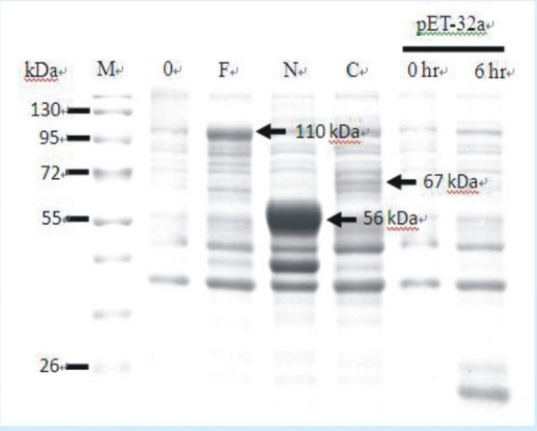

Fig. 5. Analysis the molecular weight of recombinant protein (rSao) with SDS-PAGE. Full length (110 KDa), N-terminus (58 KDa), C- terminus

Group	Dead	Alive	Survival rate
Full-length	1	4	80 %
N-terminus	5	0	0 %
C-terminus	3	2	40 %
E. coli (vector)	5	0	0 %
Control	5	0	0 %

Fig. 6. The survival rate of immunized mice after challenge. (A)rSao full-length, (B)r Sao-N terminus, (C)Sao-C terminus, (D) vector only (E) control. All mice were challenged intraperitoneally (i.p.) with S. suis serotype 1 strain (P1) 2 weeks after the secondary immunization.

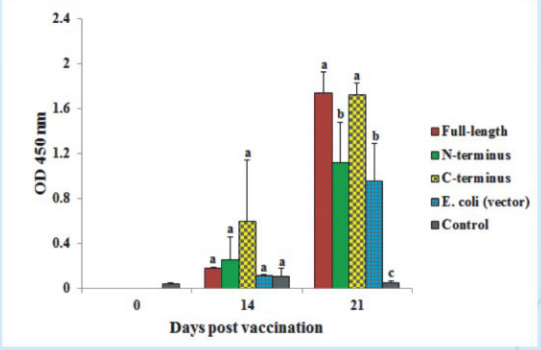

Fig. 7. Antibody responses of total IgG in mice. Mice were i.m. immunized with Full-length, N-terminus, C-terminus, vector only and saline (unvaccinated control) respectively, and boosted with the same vaccine 2 weeks after the primary immunization.

Acknowledges

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