Production and characterization of polyclonal antibodies specific to salmonella spp.

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1 Abstract

Anti - *Salmonella* polyclonal antiserum were produced y mouse immunization with an equal mixture of the 10 isolates of *Salmonella spp.* After the fourth immunization antisera from 5 mice were collected. All antisera at 1:5000 dilution showed different degree of immunoreactivity to mutiple bands in western blot against antigen from 5 isolates of *Salmonella spp.* The sera from the fourth mouse (PAb 4) demonstrated the strongest immunoreactivity and recognized unique and common bands of the antigen among various serovars of *Salmonella.* The reactivity of PAb against different serovars of *Salmonella* and other bacteria was investigated using Dot-ELISA. Two PAbs (PAb 4 and PAb 5) reacted only against *Salmonella spp.* Three PAbs (PAb 1-3) presented crossed reaction with *S.amsterdam* , *S.virchow07* , *S.typhimurium* , *S.panama* , *S.enteritidis enterica*, *S.hada581109* , *S.weltender* , *S.winton* , *S.enteritidis* , *S.stanley* , *E.coli* , *S.aureus* , *V.alginolyticus DMST* , *V.parahaemolyticus* , *V.ffluvialis* , *V.cholerae24* , *V. cholerae25* , *V. cholerae135*, *V. cholerae136* , *V. cholerae136* . The fourth mouse will be use as the spleen donor in the hybridoma production. Three PAbs constitute convenient immunological tools that can e used for simple ,rapid and simultaneous direct detecion of *Salmonella spp.* in food sample without the bacterial isolation or biochemical characterization.

2 Introduction

The genus *Salmonella spp.* , within the family Enterobacteriaceae, is composed of facultative anaerobic, oxidase-negative, catalase-positive, Gram-negative, rod-shaped bacteria; the rods are typically 0.7–1.5 x 2–5 μm in size, although long filaments may be formed. Most strains are motile and ferment glucose with production of both acid and gas. That is a major food-borne pathogen, causing acute gastroenteritis in humans.

Methods for detecting and isolating *Salmonella spp*. from foods involve preenrichment of foods in nonselective media, enrichment in selective enrichment media, and plating onto selective/differential plating agars. Individual colonies are then subjected to biochemical screening and biochemical/serological confirmation. These five steps are utilized by all three reference methods mentioned above, but different media are used for each of the steps.

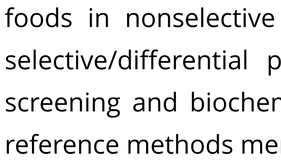
The production of polyclonal antibodies (PAbs) and development of immunoassays for the rapid and simple detection of *Salmonella spp*. such as dot blot and western blot have been interesting. Immunoassays based-on a polyclonal or monoclonal antibody can be highly sensitive, simple, specific and rapid method for the identification of salmonella contaminated in food products.

Objective of the current study were produce PAbs using the *Salmonella spp.* as antigen , characterize the PAbs for future develop the MAbs and application to detect contaminated food.

3 Method

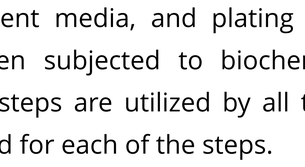
1. Antigen preparation

- Heat kill
- Formalin fix



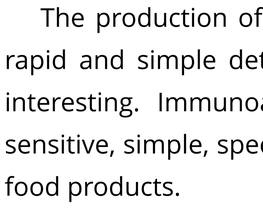
2. Mouse immunizaton

- Intraperitoneally



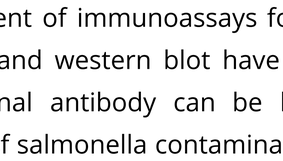
3. Mouse blood collection

- Orbital sinus



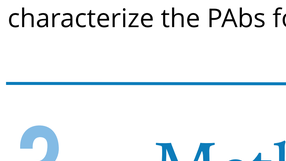
4. Specific testing

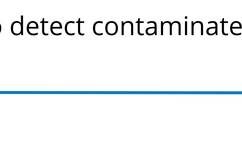
- Western blot
- Dot blot



5. Screening of *Salmonella*

-XLD
- Hektoen enteric agar





6. Detection of *Salmonella*

- Future plan

7. Development of monoclonal antibody production

4 Result

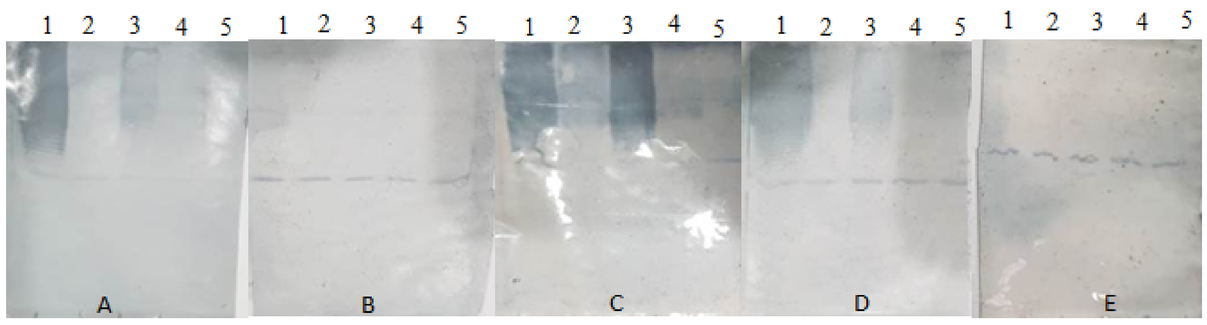


Fig.1 Western blot analysis showed specificity of PAb (A:PAb1 , B:PAb2 , C:PAb3 , D:PAb4 , E:PAb5) lane(1);S.Amsterdam , lane(2);*S.virchow07* , lane(3);*S.typhimurium* , lane(4);*S.panama* , lane(5);*S.enteritidis enterica*

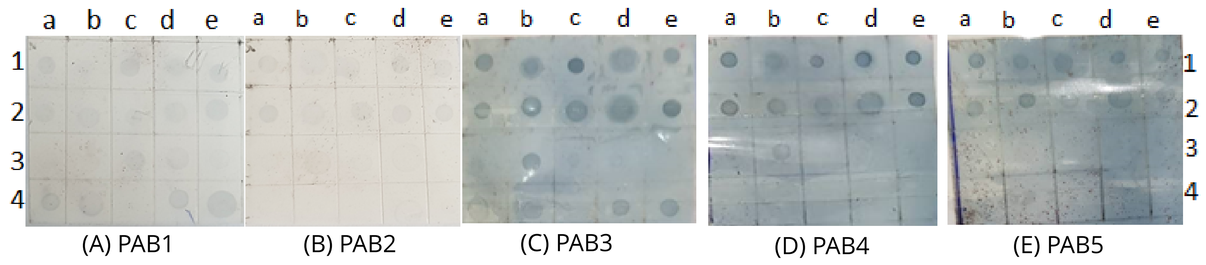


Fig.2 Cross-reactivity of the PAbs as determined by dot blotting. Heat killed *Salmonella spp.* and other bacteria were spotted onto each square of nitro cellulose membrane and treated with PAbs from each group (A) PAb1 , (B) PAb2 , (C) PAb3 , (D) PAb4 and (E) PAb5. The bacteria on the membrane were as follows : Row 1. (a) *S.amsterdam* , (b) *S.virchow07* , (c) *S.typhimurium* , (d) *S.panama* , (e) *S.enteritidis enterica*. Row 2. (a) *S.hada581109* , (b) *S.weltender* , (c) *S.winton* , (d) *S.enteritidis* , (e) *S.stanley*. Row 3. (a) *E.coli* , (b) *S.aureus* , (c) *V.alginolyticus DMST* , (d) *V.parahaemolyticus* , (e) *V.ffluvialis*. Row 4. (a) *V.cholerae24* , (b) *V. cholerae25* , (c) *V. cholerae135* , (d) *V. cholerae136* , (e) *V. cholerae136*

5 Discussion

Western blot of 5 strain of salmonella using PAb1 , PAb2 , PAb3 , PAb4 , PAb5 respectively is shown in Fig.1. PAb have a specific clear band protein. PAb1 , PAb3 and PAb4 have specificity with lipopolysaccharide. Moreover, all three PAbs selectively bound to the LPS present in the upper region of the gel. This indicates that the binding sites were in the long-chain O antigen region bearing the O-acetyl group. However the result has very weak result because of error for several reasons such as sample was more dilution , poor retention protein bound to membrane weakly , primary and/or secondary antibody were inactive or overly diluted. However the solution of errors was done by loading the larger amount of protein onto the gel or increasing concentration of proteins , determination the antibody activity by performing a serial dilution using dot blot and increase antibody concentration as necessary.

Dot blot results show the cross-reactivity of PAb1 , PAb3 and PAb4 in Fig.2. PAb1 crossed reaction with *V.alginolyticus DMST* , *V.parahaemolyticus* , *V.cholerae24* , *V.cholerae25* , *V.cholera136* and *V.cholerae137.* PAb3 crossed reaction with *E.coli* , *S.aureus* , *V.cholerae24* , *V.cholerae25* , *V.cholera136* and *V.cholerae137*. And PAb4 crossed reaction with *E.coli* and *S.aureus.* The cross-reactive is a reaction between an antigen and an antibody, which have react with similar protein like target antigen but different type of antigen. Expected they had some epitopes that were similar to *Salmonella*, which caused the cross reaction with the antibody against *Salmonella spp*. were chosen in the experiment because these bacteria are commonly found in many kinds of food and drink and specimens from patients, so it was necessary to solve this problem by using selective enrichment broth to enrich and select the *Salmonella*, but to be able to inhibit the other bacteria before detection.

6 Reference

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