BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Veterinary Science Research
https://ojs.bilpublishing.com/index.php/vsr

ARTICLE

A Simple Procedure for Extraction of Surface Protein of *Salmonella Serotypes* and *Escherichia coli* Strains Isolated from Poultry and Pigs

Tran Thi Quynh Lan* **Doan Thi Da Linh** **Le Ho Truc Phuong**

Department of Veterinary Biosciences, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Article history
Received: 30 October 2019
Accepted: 18 November 2019
Published Online: 30 November 2019

Keywords:
Salmonella serotypes
E.coli strains
Extraction
Protein complexes extract
SDS-PAGE

ABSTRACT

Salmonella and *E.coli* possess different surface protein structures that can induce protective immune responses. Identification of these proteins capacitates development of diverse applications in prevention and diagnosis that contribute to effectively control disease-causing enterobacteria pathogens such as *Salmonella* and *E.coli*. A simple procedure for obtaining protein complexes of *Salmonella* serotypes and *E.coli* is performed in this study. A sonication process with heat treatment of whole bacteria induced the release of protein complexes. Concentration of the protein extract was quantified using protein quantification Kits-Rapid, and protein complex profile was obtained by SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) and silver staining. The concentrations of protein ranged from 29.45 to 45.35 µg/mL in the *Salmonella* protein extracts, and from 25.35 to 36.72 µg/mL in the *E.coli* protein extracts. Six major groups of proteins from *E. coli* (YfiO, NipB, OmpF, YfgL, Talc, YaeT) and four major groups of proteins from *Salmonella* (Flagellin, OmpA, Porin, SEF21) were preliminarily determined by a simple procedure of extraction based on the molecular weight.

1. Introduction

Salmonella and *E.coli* are common bacteria in intestinal tract that is responsible for a variety of intestinal disorders in swine and poultry [1]. The expression of *E.coli* and *Salmonella* virulence genes causes gastrointestinal diseases in animals as well as intoxications in humans using the meat contamination bacteria [2]. In order to reduce the intensive intestinal colonisation of pathogenic *Salmonella* and *E.coli* in commercial farms, various strategies have been applied, such as antibiotics supplementation in the diet or supplementation of additives that inhibit bacterial adhesion to the intestinal epithelium, competitive exclusion by non-pathogenic bacteria, genetic selection of animal strains, and development of vaccines [3].

Enterobacteriaceae possesses different surface structures (proteins or antigens) that can induce protective immune responses [4]. The utilization of an optimized method for surface protein extraction should greatly enable applications for the therapy and diagnosis including the production of ELISA kit and subunit vaccine as well as other proteomic research. Nowadays, the protein extraction from the bacteria surface was done by many

**Corresponding Author:*
Tran Thi Quynh Lan,
Department of Veterinary Biosciences,Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam;
Email: lan.tranthiquynh@hcmuaf.edu.vn

DOI: https://doi.org/10.30564/vsr.v1i2.1357

methods such as the cell surface protein isolation kit, the use of a rotating cylinder probe [5] or a combination of detergent and phase separation using Triton X-114 (TX-114) [6]. However, most of these methods have limitations due to augmentation of performance duration, the expense as well as special materials in laboratory.

The aim of this study is to determine a simple extraction procedure for obtaining the surface protein complexes of *Salmonella* serotypes and *E.coli* strains. This is the first step to identify immune-reactive proteins in the next approach. Therefore, identification of protein components obtaining from extraction was carried out by examining the molecular weight of proteins separated in SDS-PAGE bands profile.

2. Materials and Methods

2.1 Bacterial Strains

The selection of bacterial strains was the first step in the protein extraction process. A total of 17 *Salmonella* serotypes and *E.coli* strains isolated from poultry and pigs from previous study were carried out in this study (Table 1). *Salmonella* Typhimurium ATCC® 14028™ was used as control. All of bacterial strains were stored at -70°C in glycerol 25 % [7].

Table 1. *Salmonella* serotypes and *E.coli* strains

Bacterial strains	Fecal samples	Quantity	Identification code
Salmonella serotypes*			
*S.*Typhimurium 14028™	control	1	ATCC
S. Typhimurium	pig	2	ST1, S5
S. Paratyphi A	pig	1	ST5
S. Senftenberg	pig	1	ST7
S. Saintpaul	pig	1	SR6
S. Montevideo	pig	1	S6
S. Anatum	pig	1	S7
S. Paratyphi B	pig	1	S8
S. Enteritidis	chicken	2	S9, S10
E.coli strains **	chicken	1	E3
	chicken	1	E4
	chicken	1	E5
	chicken	1	E6
	chicken	1	E7
	chicken	1	E8

Note: (*) Provided by Laboratory of Department of Veterinary Biosciences, FMVS, NLU-HCMC [7, 8] , and (**) Laboratory of Department of Veterinary Public Health, FMVS, NLU-HCMC

2.2 Antigenic (Protein) Extract Complexes of Bacterial Strains

The production of surface protein complexes was carried out from the whole bacterial cells. Briefly, a colony isolated on blood agar (Nam Khoa Co.Ltd, HCMC,VN) was incubated in 100 mL Brain Heart Infusion media (BHI) (HiMEdia Laboratories Pvt. Ltd) (pH 7.4) overnight at 37°C with shaking of 150 rpm (until OD_{600} = 0.65) in shaker inoculator machine (Hanbaek Scientificco, HB-201SF, Korea). The bacterial suspension was centrifuged at 2400 x g for 20 minutes at room temperature by centrifuge machine (Hanil Science Industrial, FLETA 5, Korea) and then the pellet was suspended in 5 mL of Phosphate Buffer Saline (PBS) (pH 7.4). The suspension of bacterial cells was sonicated 20 times x 10 seconds on ice (Hwashin technology, powersonic 410, Korea), and then was centrifuged (11000g; 10 min.; 4°C) by cool centrifuge machine (Hanil Science Industrial, centrifuge Smart-R17, Korea). The supernatant was dialyzed and stored at -70°C.

Protein concentrations were determined by Quantification Kit-Rapid (51254, Sigma-Aldrich) (High Sensitivity Assay for Microplate reader) with bovine serum albumin (BSA) as standard.

2.3 SDS-PAGE Procedure

Briefly, samples (25-75 µg of total protein extract) were resolved by one-dimensional sulphate polyacrylamide gel electrophoresis (SDS-PAGE, Amersham, Buckinghamshire, UK). Protein samples were solubilised in buffer (0.5M Tris, pH 6.8, 2% sodium dodecyl sulphate (SDS), 10% glycerol, 5% mercaptoethanol) at 100°C for 5 min, and then loaded into 10% Bis-Acrylamide (Fisher scientific, FairLawn, New jersey, USA) separating gels. The gels were run at room temperature in buffer (25mM Tris, 0.2M glycine, 0.1% SDS) at 40V/3h and then 50V for sufficient time when the dye front reached the bottom of the gel casing. They were stained with silver and the apparent molecular masses of the proteins in the antigenic extracts were determined by PD Quest software (Bio-Rad) and then, comparing their electrophoretic mobility with that of the molecular mass markers (Precision Plus ProteinTM Standards, Bio-Rad).

Results of the procedure and protein identification of bacterial strains were demonstrated in OD_{595} values, protein concentrations (µg/mL of complex extract) and SDS-PAGE bands profiles. In term of procedure parameters, the main points focused on optic density of bacteria culture and sonication of bacteria suspension.

3. Results and Discussion

3.1 Procedure Parameters

Optic density (OD): Optic density of bacteria culture was measured at achieve the stationary phase in standard curves of bacteria growth. At that phase, the number of bacteria was high and stable so the concentration of bacterial protein would be at higher quantity.

 DOI: https://doi.org/10.30564/vsr.v1i2.1357

According to the previous procedure of growth conditions [9], the OD_{600} results of bacterial culture suspensions is shown in Table 2.

Table 2. OD_{600} of *Salmonella* serotype and *E.coli* strains

Bacterial identification code	OD_{600} average
ATCC	0.99
ST1	0.97
S5	1.06
ST5	1.06
ST7	1.03
SR6	1.01
S6	1.05
S7	1.12
S8	1.10
S9	1.00
S10	0.85
E3	0.96
E4	1.00
E5	0.95
E6	0.96
E7	1.03
E8	0.86

OD_{600} of *Salmonella* culture suspensions ranged from 0.85 to 1.12 that is higher than the standard curve, which is due to increasing of the growth of bacteria strains. That means the bacterial strains in the study possessed possibly high virulence. *Salmonella* strains isolates from pigs increased the growth in comparision to *Salmonella* Enteritidis isolates from chickens. Among all *Salmonella* isolates used in this study, the predominant genotypic virulence profile (virulotype) was characterized by the concomitant presence of *invA*, *sopB*, and *stn* in carrier strains. In contrast, two virulotypes comprising either *invA*, *sopB*, *spvC*, and *stn* or *invA* and *sopB* were identified for the *Salmonella* Typhimurium isolates. Virulotypes made up of multiple virulence genes were predominant in most *Salmonella* strains tested in the previous study, indicating that pigs might act as a reservoir for these virulent strains [7]. In contrast, the optic density was ranged at lower level from 0.86 to 1.03 from *E.coli* culture suspensions. All *E.coli* strains were isolated from clinically sick chickens; however, the virulence of these strains was still not investigated in our previous study.

Sonication: The bacteria suspension was destroyed by sonication wave at high temperature and then immediately was quickly chilled by ice at 4^0C. The sudden change in temperature causes to destroy bacterial cell wall. In our study, times of sonication were increased to 20 times at the highest frequency (40 kHz) to ensure that bacterial whole cell was completely broken.

3.2 Protein Concentration in Complexes Extract

Table 3. Quantification of *Salmonella* protein in extract

Salmonella serotypes	Bacterial identification	OD_{595}	Protein concentration (µg/mL)
Salmonella Typhimurium	ST1	0.97	37.3
	S5	1.06	41.05
Salmonella Paratyphi A	ST5	1.06	29.45
Salmonella Senftenberg	ST7	1.03	36.6
Salmonella Saintpaul	SR6	1.01	31.75
Salmonella Montevideo	S6	1.05	45.35
Salmonella Anatum	S7	1.12	35.3
Salmonella Paratyphi B	S8	1.10	33.95
Salmonella Enteritidis	S9	1.00	37.85
	S10	0.85	41.25
Salmonella Typhimurium	ATCC	1.27	76.4

Table 4. Quantification of *E.coli* protein in extract

Sample	OD_{595}	Protein concentration (µg/mL)
E3	0.96	25.35
E4	1.00	25.5
E5	0.95	32.55
E6	0.96	27.33
E7	1.02	36.72
E8	0.86	32.79

Concentrations of proteins was ranged from 29.45 µg/mL (*S.* Paratyphi A) to 45.35 µg/mL (*S.* Montevideo) in *Salmonella* serotypes (Table 3), and between 25.35-36.72 (µg/mL) in *E.coli* extracts (Table 4). The highest concentrations were approximately equivalent in *S.* Typhimurium and *S.* Enteritidis although the two serotypes were isolated from different hosts. The protein concentrations of extracts depend on many factors. The most important is the manifestation of the virulence factor, which is strains specificity. These two serotypes are dominant serotypes in many countries and also the most important cause of foodborne intoxication throughout the world, so that is a major challenge to the industry. The second factor is the quality of the extraction process in our experience. Protein concentrations of extracts were relatively linear in all bacterial samples and sufficient to use SDS-PAGE method for characterization of protein complexes.

DOI: https://doi.org/10.30564/vsr.v1i2.1357

3.3 Identification of Protein Complexes

The SDS-PAGE profile of protein extract obtained from five *Salmonella* serotypes (*S.* Montevideo, S6; *S.* Paratyphi B, S8; *S.* Senftenberg, ST7; *S.* Enteritidis, S10 and *Salmonella* Typhimurium ATCC® 14028™, *Salmonella* enterica subsp.enterica) and three *E.coli* strains (E_5, E_7, E_8).

For *Salmonella* serotypes analysis (Figure 1), the frequency of the bands mostly appeared from *Salmonella* Typhimurium ATCC (96%) and *S.* Senftenberg, ST7 (85%) samples. The dominant protein obtained from protein extract of *Salmonella* serotypes is indicated with apparent molecular weights of 53 kDa (flagellin). This antigen, easily exposed to antibodies, has variability (it belongs to *Salmonella* serotyping proteins) and a time-regulated expression (phase depending) that represent some drawbacks, considering its use as a vaccine antigen [9]. There are other major proteins in the extracst that appear by SDS-PAGE with apparent molecular weights of 45, 35 , 34, 28.4, 26.8, 25.2, 23.2, 22.1, and 21 kDa (SEF21). These results were also described by Ochoa-Repáraz [10]. In fact, different authors have taken advantage of this property of these components for the development of specific serodiagnostic tests, like flagellin in ELISA.

For *E.coli* strains analysis (Figure 2), major proteins in the protein complexes extract appeared with apparent molecular weights of 26kDa (YfiO), 34kDa (NipB), 37kDa (OmpF), 40kDa (YfgL), 50kDa (TolC) and 85kDa (YaeT). The results may correspond with some of the major antigenic proteins from protein complexes of the *E.coli* cell envelope described by Stenberg (2005) [11] and Lasserre (2006) [12].

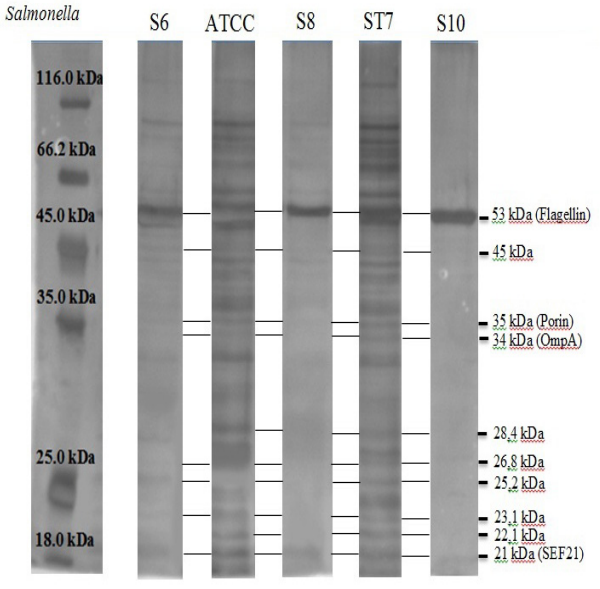

Figure 1. SDS PAGE of protein complexes extract of *Salmonella* serotypes (silver staining), and the position of some identify bands (arrows)

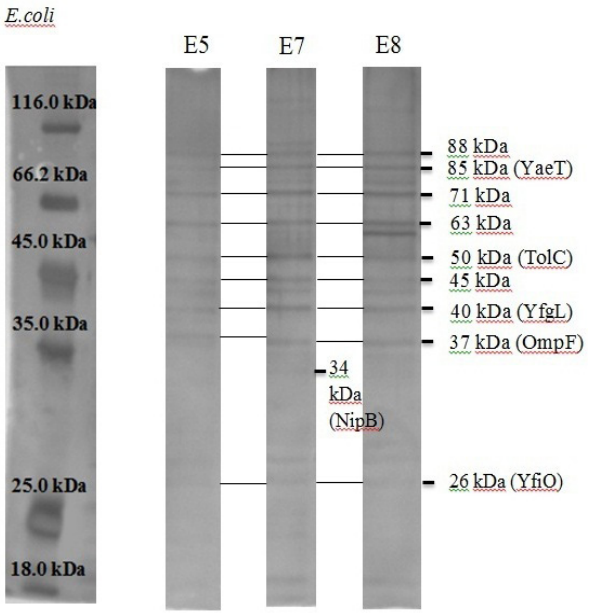

Figure 2. SDS PAGE of protein complexes extract of *E.coli* strains (silver staining), and the position of some identify bands (arrows)

4. Conclusions

Salmonella serotypes and *E.coli* strains possess antigenic protein structures that can induce protection in poultry and pigs. We obtained an antigenic protein complexes of *Salmonella* and *E.coli* from whole bacteria cells by a simple procedure. Further evaluation will be necessary to assess the immunogenicity of these antigens.

Acknowledgments

The authors wish to thank Dr. Nguyen Ngoc Tuan and Dr. Tran Thi Dan for their help in manuscript revision. The authors would like to thank VHI organization for supplying SDS-PAGE materials. We also thank the technician group at Veterinary Laboratory Korea-Vietnam for kindly provided the space and lab technique. This work would not have been possible without funds from a scientific research project of NLU-HCMC.

References

[1] Giannella RA. *Salmonella*. In S. Baron, Medical microbiology, University of Texas Medical Branch at Galveston, Galveston, Texas, 1996, 4th.

[2] Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S and Jayaprakash NS. Veterinary Microbiology and Microbial Disease, III. 2nd edition. Willey Blackwell, USA, 2011: 263-287.

[3] Edens FW. An alternative for antibiotic use in

 DOI: https://doi.org/10.30564/vsr.v1i2.1357

poultry: probiotics, Department of Poultry Science of North Caroline State University, December 10th, 2015.
http://www.scielo.br/scielo.php?script=sci_arttext&pid=s1516-635x2003000200001#end

[4] Tizzard IR. Veterinary immunology. W.B. Saunders Co.,United States, 2004: 272-280, 7th edition.

[5] Singh K and Lahiri T. An improved protein surface sxtraction method using rotating cylinder probe. Interdisciplinary Sciences: Computational Life Sciences, 2017, 9 (1): 65-71.

[6] Padmanabhan V, Govind GA, Kamalanathan AS and Yayprakash NS. Extraction of Antigenic Membrane Proteins from *Salmonella* Using Detergent and Phase Partition Method. Res. J. Microbiol, 2018, 13 (1): 47-52.

[7] Tran TQ Lan, Marie-Lou G, Nguyen TM. Nhan, Letellier A and Quessy S. Distribution of Virulence Genes among *Salmonella* Serotypes Isolated from Pigs in Southern Vietnam. J. Food Protect., 2018, 81(9): 1459-1466.

[8] Tran TQ Lan, Nguyen TM Nhan, Nguyen T Huyen. Survey on the prevalence of *Salmonella* group and serotype isolated from pig and poultry feces. J. Vet. Sci. & Technol. 2013, (In Vietnamese) XX (3): 30-36.

[9] Tran TQ Lan, Lewandowski SL, Quessy S, Letellier A and Boulianne M. Identification of Immunoreactive Antigens of *Salmonella* Enteritidis, Processding of 8th science conference at Nong Lam University, 2011.

[10] Ochoa-Reparaz Javier, Begona Sesma, Miguel Alvarez, M. Jesus Renedo, Juan Irache, Carlos Gamazo. Humoral immune response in hens naturally infected with *Salmonella* Enteritidis against outer membrane proteins and other surface structural antigens. EDP sciences Vet. Res. 2004, 35: 291-298.

[11] Stenberg F, Chovanec P, Maslen SL, Robinson CV, Ilag LL, von Heijne G and Daley DO. Protein Complexes of the Escherichia coli Cell Envelope. J. Biol. Chem, 2005, 41: 34409-34419.

[12] Lasserre JP, Beyne E, Pyndiah S, Lapaillerie D, Claverol S and Bonneu M. A complexomic study of Escherichia coli using two-dimensional blue native/SDS polyacrylamide gel electrophoresis. Electrophoresis, 2006, 27: 3306-3321.

DOI: https://doi.org/10.30564/vsr.v1i2.1357