Protein Profiles of *Escherichia coli* Inactivation Results with Gamma Irradiation on Doses 600-800 Gy

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Protein Profiles of *Escherichia coli* Inactivation Results with Gamma Irradiation on Doses 600-800 Gy

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Abstract. Escherichia coli is one of the main bacteria of the coliform type which can cause mastitis in dairy cows. Efforts to prevent mastitis are by the vaccination program. In this study, the E. coli (S1) bacterial cell strains tested were the result of isolation from milk from dairy cows infected with mastitis. Aims of the research were to know the protein profile of E. coli inactivation results with gamma irradiation on doses 600-800 Gy. Bacterial cells were inactivated by gamma irradiation at doses of 600, 650, 700, 750 and 800 Gy. The parameters analyzed are the viability of bacteria by TPC method, total protein by Lowry method and protein profile by electrophoresis using SDS-PAGE. The results showed that the dose to inactivate E. coli bacterial cells with gamma irradiation was 700 Gy. The total protein content of E. coli cells inactivated by gamma irradiation at doses of 0 Gy (control), 600 Gy, 650 Gy, 700 Gy, 750 Gy, 800 Gy respectively was 4.60 mg / ml; 4.77 mg / ml; 13.77 mg / ml; 9.43 mg / ml; 4.40 mg / ml; and 5.60 mg / ml. Protein bands of E. coli bacteria cells from inactivation with gamma irradiation were not different from before irradiation and protein antigens were still detected in 22kDa, 26kDa, 35kDa, and 58kDa.
Keywords: Escherichia coli, vaccine, protein, irradiation, and gamma

1. Introduction

Escherichia coli is one of the main bacteria of the coliform types that common cause of mastitis in dairy cattle [1]. The bacteria are normal in the gastrointestinal tract of human and endothermic (warm-blooded) animals, mostly not pathogenic [2]. Mastitis is an inflammation of the udder caused by microorganisms such as bacteria and fungi. The problem of mastitis can be overcome by using various kinds of antibiotics, but the method of treatment causes microorganism resistance and residues in milk [1], so it is necessary to get alternatives for preventing disease, such as development of a vaccine against pathogen [3-5].

A vaccine is a suspension of microorganism that made from killed or weakened of the microbe and is stimulate immunity or antibodies to a particular disease. Bacterial inactivation for vaccines can be done using gamma irradiation. Inactivation methods with gamma rays have effectiveness in increasing immune responses compared to conventional techniques, such as heating [6]. Irradiation methods in a vaccine will prevent replication but retain their metabolic activity, stimulate immune response and

protection againts bacteria in the host. The types of irradiation common used for developed vaccine are gamma rays because they have high penetrating and short wavelength [7,8].

Protein profiles defined as total protein levels and the number of protein bands must be seen after gamma irradiation because potential vaccines must contain antigenic proteins. Analysis of protein bands can be performed by electrophoresis using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The advantages of the SDS-PAGE method are to analyze the purity of proteins, to determine the molecular weight of proteins and the number of polypeptide chains, to detect proteolysis and to determine a monomer or oligomer protein [9].

In the previous studies, inactivation of E. coli bacterial cells with gamma irradiation at range doses from 0-1000 Gy with intervals of 100-200 Gy showed fluctuating levels of E. coli protein cells, with the highest of total protein at 600 Gy is 325 mg/mL. Protein bands of E. coli cells after irradiation determine no different profile protein with molecular weight was 15 – 220 kDa. From the above study, inactivation of E. coli cells occurred at a range of 800-1000 Gy [10]. The aim of this study is to know the protein profile of E. coli inactivation results with gamma irradiation on doses 600-800 Gy.

2. Experimental Details

2.1. Bacterial strain and culture

E. coli S1 was isolated from milk of dairy cows which infected mastitis. The bacteria is a collection of Center for Isotope and Radiation Application (PAIR), National Nuclear Energy Agency (BATAN). One ose of E. coli SI was grown on Tryptic Soya Agar (TSA) medium at room temperature for 24 hours. These cultures were inoculated amount 3 ose into 30 ml on Tryptic Soya Broth (TSB) medium and then incubated at room temperature with 120 rpm of agitation for 24 hours. 10% of the inoculum culture was inserted into 30 ml of TSB medium and then incubated at room temperature with 120 rpm of agitation for 150 minutes (midlog phase)[11].

2.2. Inactivation of E. coli S1 with Gamma Irradiation at doses from the range 600 - 800 Gy

The inoculum culture of E. coli in the midlog phase was centrifuged at 10,000 rpm for 1 hour. Pellets were rinsed twice with 0.85% NaCl then added with 0.85% NaCl into glass vials. After that the bacteria were irradiated with gamma rays in varies dose i.e., 600, 650, 700, 750, and 800 Gy in Gamma Chamber Irradiator 4000 A with dose rate 1046 Gy /hour. The irradiated E coli bacteria were determined by plating 10-fold serial dilution of the culture on TSA medium and incubated at room temperature for 24 hours. After that the number of bacteria cells in TSA medium were counted then inactive dose of E. coli S1 culture was obtained [12].

2.3. Measurement of E. coli Protein

Standard curves for protein was determined by Bovine Serum Albumin (BSA) standard solution. BSA (40 mg/mL) solution was diluted with distillated water up to 5 mg/mL, 10 mg/mL, 20 mg/mL and 40 mg/mL. Then Lowry I and II solution was added to each standard BSA solution. The spectrophotometric absorbance of each standard was measured by a UV-VIS spectrophotometer at 700 nm[13]. The absorbance of E. coli cells was checked by interpolation using the standard curve and the protein concentration was calculated.

2.4. Sodium Deodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE) for protein analysis

Protein bands were analyzed by one-dimesion electrophoresis method of SDS-PAGE and electrophoresis was used on 10% polyacrylamide gels [14]. After electroforesis, the gel was stained with Coomassie Briliant Blue and analyzed by gel doc.

3. Results and Discussion

3.1. Effect of gamma irradiation on the viability of E. coli S1

After *E. coli* were exposed with different dose gamma irradiation, the number of bacteria were determine on TSA medium. The number of *E. coli* suspension after gamma irradiation is shown in Table 1. Inactivation bacterial cells of *E. coli* by gamma irradiation at doses of 600-800 Gy showed that increase of gamma irradiation dose impacted on decrease of the number bacteria cells. The dose for inactivate *E. coli* bacterial cells at 700 Gy and above (Figure 1).

Table 1. The number of *E. coli* bacterial suspention after gamma irradiation.

No.	Radiation dose (Gy)	The number of bacteria (cfu/mL)
1	0	1×10^{12}
2	600	3.50×10^{5}
3	650	2×10^{3}
4	700	0
5	750	0
6	800	0

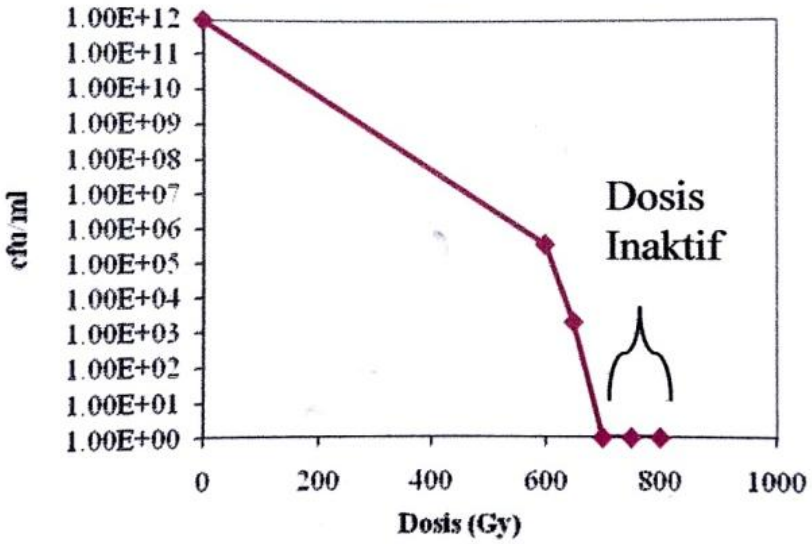

Figure 1. Relationship curve of gamma irradiation doses to the number of *E. coli* cells

3.2. The concentration of total protein

The results of statistical analysis showed that there were significantly different in the total protein of *E. coli* cells before and after irradiation with a significance value ($p \leq 0.05$). In the previous studies, the inactivation of *E. coli* bacterial cells by heating at a temperature of 65°C also showed a significance value ($P \leq 0.05$) which meant that there was a significantly different in total protein levels before and after heating.

Table 2. Total Protein Levels of *E. coli* Cells after Gamma Irradiation.

Dosis	Absorbans		Total protein level		
(Gy)	A	B	A	B	Mean ± SD
0	0.140	0.150	4.27	4.93	$4.60 \pm 0.47^{(a)}$
600	0.140	1.155	4.27	5.27	$4.77 \pm 0.71^{(a)}$
650	0.290	0.275	14.27	13.27	$13.77 \pm 0.71^{(c)}$
700	0.225	0.210	9.93	8.93	$9.43 \pm 0.71^{(b)}$
750	0.146	0.138	4.67	4.13	$4.40 \pm 0.38^{(a)}$
800	0.165	0.155	6.93	5.27	$5.60 \pm 0.47^{(a)}$

^{a,b,c} Different superscripts show significantly differences ($P \leq 0.05$)

3.3. Identification Molecular Weight By SDS PAGE

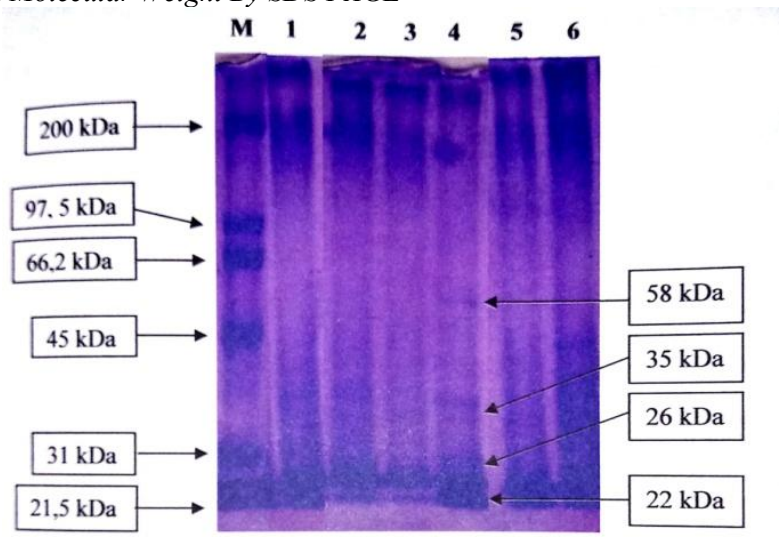

Figure 2. Protein bands of *E.coli* after inactivation by gamma irradiation in 10% polyacrilamyde gel. M : protein marker; lane 1: 0 Gy (control); lane 2: 600 Gy; lane 3: 650 Gy; lane 4: 700 Gy; lane 5 : 750 Gy; lane 6 : 800 Gy

4. Conclusions

Inactivate *E. coli* S1 with gamma irradiation at range doses of 600-800 Gy was at 700 Gy. The total protein content of E. coli cells inactivated by gamma irradiation at doses of 0 Gy (control), 600 Gy, 650 Gy, 700 Gy, 750 Gy, 800 Gy respectively was 4.60 mg/mL; 4.77 mg/mL; 13.77 mg/mL; 9.43 mg/mL; 4.40 mg/mL; and 5.60 mg/mL. The molecular weight of antigen *E. coli* S1 were detected in 22 kDa, 26 kDa, 35 kDa, and 58 kDa.

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