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The Penetrability of Selected Bacteria on The Raw Bovine Meat

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ABSTRACT

The aim of this experiment is to confirm the isolation of *Salmonella typhimurium* and determine the penetration ability of *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Proteus mirabilis* isolated from raw bovine meat in Kota Bharu. The identification of *Salmonella typhimurium* was done using PCR technique. The penetration experiment was conducted on meat without fat and meat with fat under at temperature and 4-8°C. After several hours, different layer the meats were sliced, homogenized with 10 ml of peptone water, and a loop full of the homogenates was streaked on agar plates to determine the penetration ability of the bacteria. *Salmonella typhimurium* and *Proteus mirabilis* was able to penetrate deep into the meat without fat at room temperature. *Staphylococcus aureus* was able to penetrate in the first hour only. In lower temperature, all bacteria were unable to penetrate except for *Proteus mirabilis.* The penetration of bacteria was concern regarding to the storage of the meat. Raw meat should be hygienically handle and properly stored to ensure the freshness and public health safety.

Keywords: **Penetrability, Bacterial invasion, Meat, Hygein, PCR**

1. Introduction

The increasing local demand for fresh meat leads to a longer production line in meat industry. The animals need to be slaughtered in higher quantity and the meat has to be stored longer in order to stock the meat for supply to the consumers. Wide ranges of bacteria are unable to grow in cold storage area, though they survive in such temperature. However, the numbers of viable cells decrease in low temperature environment (Doyle, 2002 and Temelli, *et al.*, 2011). The bacterium stays on the surface of the meat during the logarithmic phase of growth and after reaching their maximum cell density; extracellular proteases enzyme are secreted and apparently break down the connective tissue between the muscle fibers, allowing the bacteria to penetrate the meat (Gill and Penny, 1977). The surface bacteria that were not removed during washing process are prone to penetrate the meat being assisted by the washing water (Anderson, *et al.*, 1991).

The objectives of this experiment are to confirm the *Salmonella typhimurium* using PCR, and to determine the penetration ability of *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Proteus mirabilis* isolate from raw bovine meat in Kelantan

(Muhammad et al., 2012) and to determine whether fat and low temperature affects the penetration process.

2. Materials and method

2.1 Bacterial culture

The bacteria used in this experiment were *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Proteus mirabilis* that were isolated from fresh raw meat surface sold at local meat shops in Kota Bharu, Kelantan (Muhammad et al., 2011). The bacteria were maintained on nutrient agar and grown in nutrient broth (OXOID products). Motility was observed using microscopic observation and proteolysis activity was determined by hydrolysis of red blood cell in 7% sheep blood agar (Gill and Penny, 1977). The bacterial culture was diluted six times (10^6) before applied onto the meat.

2.2 DNA Extraction from *Salmonella* Isolates for PCR Test

DNA was extracted from the two isolates using Qiagen®, DNeasy Blood and Tissue DNA extraction kit. The extraction was conducted per the procedures recommended by the manufacturer.

2.3 PCR Detection of Salmonella Species

The PCR amplification was conducted by using Salmonella-specific primers, S139 (invA Forward, GTGAAATTATCGCCACGTTCGGGCAA) and S141 (*inv*A Reverse, TCATCGCACCGTCAAAGGAACC) which targets the 284 bp segment gene (Rahn *et al*., 1992). The PCR reaction was prepared in 50 µL reaction comprised of 25 µL of PCR Master Mix (Fermentas®), 1 µL of 100 µM of each primer, 30 µL of deionised distilled water and 3 µL of DNA template. The PCR was run at 94^{o}C for 2 min for initial denaturation, denaturation at 94^{o}C, 45 s; annealed at 55^{o}C for 1 min and extension at 72^{o}C for 2 min in 30 cycles. Further extension at 72^{o}C for 3 min and held at 4^{o}C.

2.4 Agarose gel electrophoresis

0.4mg Agarose gel was weight and top up with 40ml TBE buffer. Heated inside a microwave oven about 1 minute and let cool for 10 minute. 2µl of Midori Green® DNA staining solution was added after the gel at ±50^{0}C. The solidified gel was placed into an electrophoresis tank filled with TBE buffer, and 5µl of PCR product which mixed with 2µl of loading dye (7µl in total) was carefully pipette into the well of the gel. The electrophoresis volt was 80V, 400mA for 60 minute. The gel was viewed using WisDoc® gel documentation equipment. 100bp DNA Ladder was used as gene ruler.

2.5 Penetration of meat by the bacteria

Blocks of meat were cut into 2cm X 3cm x 8cm dimension. The surface of the meat block was sterilized by using a hot knife. Two types of meat were used in this experiment: 1) Meat that was covered with fat and 2) Meat that was not covered with fat. Each meat block was placed in the base of polystyrene petri dish which had a thin layer of nutrient agar. Another

base of petri dish was placed on top of the first one, and a hole was made using a glass rod and sealed with parafilm. The remainder of the nutrient agar was poured until it is covered the meat block. Bacteria were inoculated on the surface area that was not burned (fat-covered and non-fat-covered) to give an initial cell density in excess of $10^6/cm^2$.

To determine the rate of penetration, a strip of meat, 1cm in cross section was removed from the agar aseptically with sterile instruments, beginning at the inoculated end for interval of 1st hour, 2nd hour, 3rd hour, 6th hour and 24th hour. Each slice was homogenized with 10 ml of peptone water, and a loop full of the homogenates was streaked on agar plates. Penetration occurred when a strip contain bacteria at a depth of at least 2 cm.

3. Result

3.1 Bacteria (*Salmonella typhimurium*) Identification

The PCR result showed amplification of a 284 bp segment Salmonella-specific gene, *inv*A confirming the findings from biochemical tests (Figure 1). The biochemical test was performed using the same isolation. The isolation producing gas and H_2S in TSI slant medium. Negative result in urease test, positive in citrate. The bacteria were highly motile and Indole negative, confirm using S.I.M media.

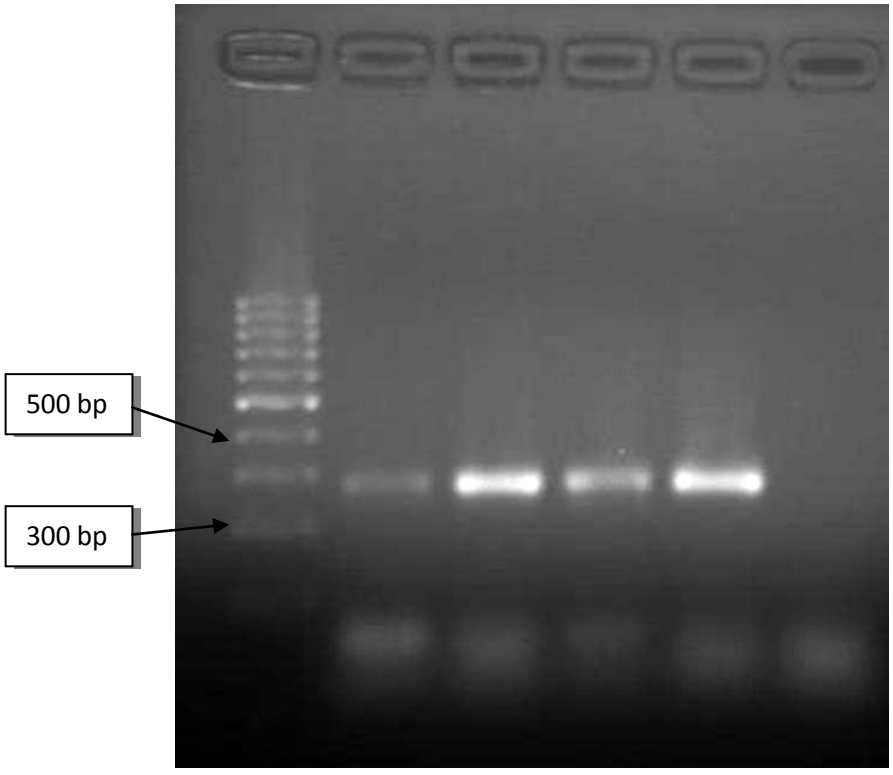

Fig.1. PCR product targeting selected gene

3.2 Meat Penetration

3.2.1 Penetration ability of different bacteria in non-fat-covered meat is shown in Table 1. *Proteus mirabilis* was the most motile and able to penetrate meat through the experiment, followed by *Salmonella typhimurium* which was found to penetrate until 3rd hour of experiment. *Staphylococcus aureus* only be able to penetrate at the first hour of the experiment. *Escherichia coli* were not able to penetrate the meat in any temperature condition.

Table 1: Penetration ability of different bacteria in on-fat-covered meat

Bacteria	Temp.	1st hour	2nd hour	3rd hour	6th hour	24th hour
Escherichia coli	25 - 28°C	-	-	-	-	-
	4- 8°C	-	-	-	-	-
Staphylococcus aureus	25 - 28°C	+	-	-	-	-
	4- 8°C	-	-	-	-	-
Salmonella typhimurium	25 - 28°C	+	+	+	-	-
	4- 8°C	+	-	-	-	-
Proteus mirabilis	25 - 28°C	+	+	+	+	+
	4- 8°C	+	-	-	-	-

3.2.2 Penetration ability of different bacteria in fat-covered meat is shown in Table 2. In fat-covered meat, only *Salmonella typhimurium* and *Proteus mirabilis* was be able to penetrate the meat, however in low temperature condition, both of them are unable to penetrate the meat. There were no penetration was observed for *Escherichia coli* and *Staphylococcus aureus*.

Table 2: Penetration ability of different bacteria in fat-covered meat

Bacteria	Temp.	1st hour	2nd hour	3rd hour	6th hour	24th hour
Escherichia coli	25 - 28°C	-	-	-	-	-
	4- 8°C	-	-	-	-	-
Staphylococcus aureus	25 - 28°C	-	-	-	-	-
	4- 8°C	-	-	-	-	-
Salmonella typhimurium	25 - 28°C	+	+	-	-	-
	4- 8°C	-	-	-	-	-
Proteus mirabilis	25 - 28°C	+	+	-	-	-
	4- 8°C	-	-	-	-	-

4. Discussion

Bacteria can penetrate into meat due to the breakdown of the connective tissue between muscles fibers by proteolytic enzymes secreted by the bacteria. *Salmonella typhimurium* and *Proteus mirabilis* was known to be highly motile (Toguchi *et al.*, 2000 and Pearson *et al.*, 2008) (Table 3). These bacteria also actively produce proteolytic enzyme that enable them to penetrate the meat. In low temperature, the bacteria will reduce its cell size, the growth rate

and the motility rate (Price and Sowers, 2004). Therefore the penetration rate in the lower temperature environment was reduced. A study using Blue Lake- dye revealed that fat-covered meat was resistant to bacterial penetration (Anderson *et al.*, 2007). The bacteria must be able to utilized lipid in order to penetrate through the fat.

Table 3: The motility and penetration ability of bacteria

Species	Motility	Penetration	
Escherichia coli	+	-	(Mittal *et al.*, 2003)
Staphylococcus aureus	+	+	(Kaito and Sekimizu, 2006)
Salmonella typhimurium	+	+	(Toguchi *et al.*, 2000)
Proteus mirabilis	+	+	(Pearson *et al.*, 2008)

5. Conclusion

In conclusion, this study showed that *Escherichia coli* grew on the surface but did not penetrate the meat. In addition more motile bacteria, *Salmonella typhimurium* and *Proteus mirabilis*, grew well on the surface and penetrate the meat. However in low temperature environment, the penetration rate of these bacteria was greatly reduced. We urge that meat seller in Kelantan to display the meat that they sell in lower temperature environment in order to reduce bacterial contamination on surface and inner part of the meat.

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