# ANTIMICROBIAL POLYLACTIC ACID FILMS AGAINST SOME IMPORTANT FOOD-BORNE PATHOGENS, *LISTERIA MONOCYTOGENES, SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* O157:H7

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Abstract-The Active Packaging (AP) concept is an emerging trend in the food packaging industry. Antimicrobial (AM) active packaging can be made by incorporating suitable AM agents into suitable food packages. This paper presents a study on the suitability of environmentally friendly active packaging films to be produced by coating process. In particular, biodegradable commercial polylactic acid (PLA) product was used as an environmentally friendly polymer matrix. Natural antimicrobial, Lauric Arginate and polylysine at various concentrations were coated on each polymeric matrix. The antibacterial activity of these films was evaluated against *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 by qualitative agar diffusion assay, quantitative determination by using JIS Z 2801:2000 method. Inhibition of *L. Monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 by the AM films were also clearly observed by agar diffusion assay. During the quantitative antibacterial evaluation, the *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 viable counts decreased by more than 5.13, 4.80 and 5.30 log cfu respectively, compared to the control. Further investigation will be done in food products to prove whether the AM film can enhance safety of the product when it is used as a packaging material. Greater emphasis on safety features associated with the addition of antimicrobial agents offers promise as the new development in bio-based active packaging field.

***Index Terms*—antimicrobial packaging, food safety, polylactic acid films, food-borne pathogens, *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O157:H7**

## I. INTRODUCTION

Processed meat manufacturers select packaging for two major reasons: preservation of product quality (appearance, flavor, odor and texture) and inhibition of microbial growth. Product quality attributes are paramount to consumer acceptance. The inhibition of microbial growth becomes highly concerned with increased sales of sliced and diced and shredded cooked products. Consumers are also demanding consumer-friendly packaging (Lopez *et al*, 2004). Meat is rich nutrient matrixes that provide good sources for growing of food-borne pathogens. The application of natural antimicrobial agents such as lauric arginate and polylysine to packaging can create an environment inside the package that may prevent the growth of common foodborne pathogens on meat products surface such as *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 and, hence, lead to control the outgrowth of those food borne pathogens during the shelf life of ready-to-eat meat products.

In response to the current consumer demand and market trends, the area of active packaging is becoming increasingly significant (Vermeiren L *et al*, 1999). Active packaging (AP) performs some desired role other than providing an inert barrier between the product and external conditions (Yam, 2005). The concept of this project was to develop a biodegradable polylactic acid (PLA) antimicrobial film that would minimize risk of illness or death from food-borne pathogens. An important requirement was the need to incorporate some natural antimicrobial agents in the packaging that can control the outgrowth of *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 on meat products during the shelf life of ready-to-eat meat products. The aim of this study was to evaluate antimicrobial PLA film for its suitability to be used as antimicrobial food packaging by varying certain type of natural antimicrobial agents.

## II. MATERIALS AND METHODS

### A. Antimicrobial polylactic acid film preparation

Coated plastic films were prepared by applying antimicrobial solution in suitable composition onto PLA films by spraying method. Antimicrobial agents were prepared at various concentrations in order to investigate the best effective condition.

### B. Preparation of bacteria cells

*Listeria monocytogenes* (ATCC 19115), *Salmonella typhimurium* (DMST 0562) and *Escherichia coli* O157:H7 (DMST 12743) were grown separately in 25 ml tryptic soy broth (TSB, Difco) at 37 ºC, 200 rpm for 24 hr. Each culture will be centrifuged (7500 *x* g *at* 4 °C for 10 min) and the resulting pellet will be washed twice and re-suspended in 25 ml 0.1% (w/v) peptone water solution. This method consistently produced a suspension containing approximately $10^9$ colony forming units (cfu) ml, which will enumerated by serial dilution in 0.1% (w/v) peptone water and spread-plating on trypic soy agar (TSA, Difco).

### C. Antibacterial activity evaluation of the antibacterial-containing coated films

For the qualitative evaluation of the antibacterial activity by agar diffusion assay, samples of the coated films (20×20 $mm^2$) were placed onto Tryptic Soy agar (TSA, Oxoid) plates seeded with $10^6$ cfu from overnight cultures of *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7. The plates were incubated at 37 °C for 24 h and the antagonistic activities were quantified by a clear zone of inhibition in the indicator lawn around and in contact with the coated plastic films. Coated plastic films without antimicrobial substance were tested as negative controls.

The quantitative antibacterial evaluation was performed by using JIS Z 2801:2000 method. An approximately 2.5 x $10^5$ - 1.0 x $10^6$ cfu/ml cell suspension of either *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* O157:H7 were prepared in 1/500 nutrient broth. An aliquot (400 µl) is then placed onto at least 3 replicate sub-samples per species of the treated surface under test and 6 replicate sub-samples per species of the untreated surface and held in intimate contact using a sterile polylactic acid film (typically 40 x 40 mm on a test piece measuring 50 x 50 mm). The 3 replicate sub-samples of the treated material and 3 of the 6 replicate sub-samples of the untreated material were then incubated for 24 hours at 35°C at saturation humidity. After incubation, the samples were transferred to individual containers containing an aliquot (typically 10 ml) of a neutralizer validated for the biocide used in the treated material. The films were separated from the surface and the suspension remaining on the surface homogenized with the neutralizer. Three replicate sub-samples of the untreated material were also processed in this manner prior to incubation to provide base-line data. The numbers of colony forming units of either bacterium species tested within the resulting suspensions were then enumerated on nutrient agar validated using a spread plate technique

## III. RESULTS AND DISSCUSSIONS

### A. Qualitative antimicrobial activity of films

In the agar diffusion test, antimicrobial films were placed on solid agar medium containing desired target microorganisms. The plates were incubated at 37 ºC for 24 hr. Figure 1 shows the results of qualitative antibacterial activity evaluation of the PLA coating against *L. monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7. The activities are revealed by a clear zone of inhibition in contact with and around (about 4 mm) the PLA coated film. A clear zone surrounding the films indicated antimicrobial diffusion from the films and subsequent inhibited the growth of microorganisms. No activity against the indicator strain is observed for the uncoated films. Lack of growth under the film may indicate the inhibitory effect, but, appropriate control must be included to avoid false positive result.

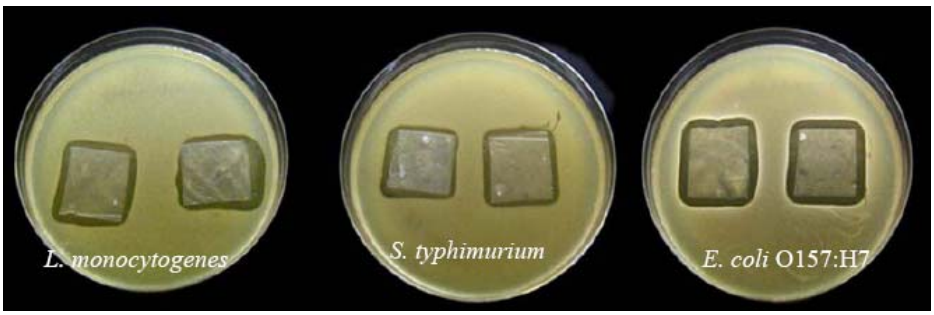


**Figure 1:** Effect of antimicrobial PLA film containing Lauric Arginate (LAE) on *L. monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7


**Figure 2:** Effect of antimicrobial PLA film containing polylysine on *L.monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7

### B. Antibacterial activity evaluation of the PLA coated film

Viable cells count in nutrient agar showed that the AM films tested had a strong antimicrobial activity compared to the control (Table 1). The PLA film coated with 10 % Lauric Arginate more than 5.13, 4.80 and 5.30-log decrease in *L. monocytogenes*, *S. typhimurium* and *E.coli* O157:H7 cells respectively. The strong antimicrobial activity was also observed in PLA film coated with polylysine.

**Table 1** Antibacterial activity of the AM films against *L.monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7

| No. | Test specimens | *Listeria monocytogenes* ATCC 19115 | | *Salmonella typhimurium* DMST 0562 | | *Escherichia colli* O157:H7 DMST 12743 | |
|---|---|---|---|---|---|---|---|
| | | Number at "24h" contact time (cfu per test piece) | Antimicrobial activity (Log reduction) | Number at "24h" contact time (cfu per test piece) | Antimicrobial activity (Log reduction) | Number at "24h" contact time (cfu per test piece) | Antimicrobial activity (Log reduction) |
| 1 | PLA | $1.4 \times 10^6$ | - | $6.4 \times 10^5$ | - | $2.0 \times 10^6$ | - |
| 2 | PLA + 10% LAE | <10 | >5.13 | <10 | >4.80 | <10 | >5.30 |
| 3 | PLA + 10% Polylysine | <10 | >5.13 | <10 | >4.80 | <10 | >5.30 |

## IV. CONCLUSION

This study describes the potential of the use of renewable packaging films as antimicrobial films in food application. The antimicrobial polylactic acid films exhibited interesting qualities in the field of bioactive packaging due to antimicrobial properties of PLA films. The qualitative antimicrobial activity of films and antibacterial activity evaluation of the PLA coated films by by using JIS Z 2801:2000 method demonstrate that polylactic acid films treatment with antimicrobial compounds are sufficient to reduce the risk of illness or death from food borne pathogens in meat products.

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