Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

"Isolation and Identification of *Pseudomonas* species Associated With Rapid Deterioration of Freshly Slaughtered Chilled Beef Meat"

A thesis
presented by

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Abstract:

(**Key words**: beef meat- *Pseudomonas aeruginosa* - *Pseudomonas fluorescens*)

Microbial contamination of the freshly slaughtered meat contributes significantly to meat deterioration or spoilage. In the present work, the prevalence of *Pseudomonas spp* in raw beef meat samples was investigated. A total of 320 raw beef meat samples were randomly collected from retail outlets and abattoir in Cairo - Egypt during the periods from June 2013 to January 2014. The samples were collected, labeled and transported with minimum of delay in an ice box (4-5ºC) to the laboratory. Bacteriological and molecular examination of the collected samples revealed the recovery of 46 isolates of *Pseudomonas* with a rate of 14.37%. Two species belonging to genus *Pseudomonas* were recovered and identified, *P. fluorescens* and *P. aeruginosa*, which were isolated at a rate of 8.75 and 5.6%, respectively. Studying the effect of organic acids treatment of beef meat samples on removal of Pseudomonas contaminants revealed that using acetic acid or citric acid at concentration of 2% for 2 min induced significant reduction of *P. aeruginosa* and *P. fluorescens* to uncountable levels (less than 30 CFU/cm^2).

Dedication

No words can express my gratitude to my whole family who used every means of their power for helping and supporting me.

Dedicated to:

My father

, my mother

, my wife

and my children

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1. Introduction

Meat is recognized as one of the most perishable foods, which might be attributed, among other reasons, to its contamination with microorganisms that grow to unacceptable levels. Many groups of organisms potentially contribute to meat spoilage under appropriate conditions **(Ercolini *et al*., 2006).**

The chemical composition of meat favours microbial growth to unacceptable levels, which contributes significantly to meat deterioration or spoilage. Presence of large numbers of microorganisms in raw meat induces changes that render it unfit for human consumption **(Fung, 2010).**

Living plants and animals have structural and chemical defenses that prevent microbial colonization, but once they are dead or in a dormant state, these defense systems deteriorate and become less effective. Many different microbes may potentially be able to use the nutrients in a food but some species have a competitive advantage under certain conditions. Food processors should note that certain spoilage organisms may not grow on particular foods because some nutrient is missing. If the food product is reformulated, then a new ingredient may allow growth of a previously unimportant microbe. Different food categories present different challenges for inhibition of spoilage organisms **(Pitt and Hocking , 1997)**.

Spoilage of meat occurs if the meat is untreated in a matter of hours and this result in the meat becoming unappetizing, poisonous or infectious. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria, which are borne by the animal itself, by the people handling the meat, by their implements and the environment in which the animals are kept. Meat can be kept edible for a much longer time though not indefinitely, if proper hygiene is observed during production, processing, and if

appropriate food safety, food preservation and food storage procedures are applied (**Lawrie, 2006)**.

The most frequent bacteria to occur on fresh meat are bacteria of the genera *Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, Micrococcus*, lactic acid bacteria and various genera of the family *Enterobacteriaceae* (**Pennacchia *et al*., 2011)**. The survival and growth of these microbes is influenced, to a great extent, by the composition of the atmosphere surrounding the meat.

Meat can be spoiled quickly under aerobic conditions. This is caused by the rapid growth of Pseudomonades. Psychrotrophic species such as *Pseudomonas fragi, P. lundensis, P. putida and P. fluorescens* can be isolated from unpacked meat showing signs of spoilage. *P. fluorescens* occurs more frequently on fresh meat, though during longer periods of storage *P. fragi* becomes dominant **(Bruckner *et al*., 2012)**. Higher concentrations of CO_2 (10%) inhibit the growth of both *P. fluorescens and P. fragi* on red meat. *P. fragi* plays a significant role in meat spoilage; meat is even considered the ecological niche for this species. *P. fragi* represents the dominant species among the *Pseudomonades* regardless of the packaging of the meat. All other species occur primarily on unpacked meat, i.e. under aerobic conditions.

P. putida accounting for the greater proportion on fresh pork and poultry meat under aerobic conditions (around 90% of *Pseudomonas spp*.); with *P. fluorescens* occuring less frequently. The population of *Pseudomonades* attained values of 9-10 $\log_{10}$ CFU/g^{-1} after several days depending on the storage temperature **(Bruckner *et al*., 2012)**.

P. fragi and P. putida are the principal species in minced beef. A population of pseudomonades of 10^7 – 10^8 CFU/g^{-1} causes slime to form on

meat and a bad smell to appear. Both of these deviations appear, however, when pseudomonades exhaust the glucose and lactic acid in the meat and begin to metabolize nitrogenous compounds, particularly amino acids **(Doulgeraki and Nychas, 2013)**.

The aim of the present work was to determine the microbial agents associated with meat spoilage, however, after examination of the first 50 samples it was found that *Pseudomonas* spp. were the most prevalent microbial agents. Therefore, the plan of the work was modified to elucidate the role of *Pseudomonas* spp. as one of the important **specific spoilage organism** (SSO) of chilled beef meat during aerobic storage. It was planned also to explore the effect of some organic acids in decontamination of meat from *Pseudomonas* spp.

To achieve this aim the following procedures were done:

- Isolation and Identification of *Pseudomonas* species associated with rapid deterioration of freshly slaughtered chilled beef meat.
- Bacteriological and Molecular identification of *Pseudomonas* isolates recovered from freshly slaughtered chilled beef meat for 8 hrs.
- Experimental evaluation of the antimicrobial effect of some organic acids on *Pseudomonas* spp.