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BACTERIAL ATTACHMENT TO MEAT SURFACES



A thesis presented in partial fulfilment of the requirements for the
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Valarmathi Narendran

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ABSTRACT

The aim of this study was to optimise the hygienic efficiency of slaughter and dressing operations. Three strategic approaches, namely reducing and removing or killing the bacteria attached to meat surfaces, were considered. The second option of removal was selected for development, as current technology inevitably results in bacterial contamination, while killing bacteria on meat surfaces requires drastic treatments that may adversely affect quality parameters.

The initial attachment mechanism between bacteria and the carcass surface (reversible attachment) was studied using the collagen film model system. Bacterial attachment to the collagen model was compared with attachment to cut beef muscle and uncut beef muscle using viable count procedure. Scanning electron microscopy and direct microscopic count procedure using an epifluorescence microscope was also developed using both collagen films mounted on microscope slides and collagen coated microscope slides. The collagen film viable count system was the method selected to model bacterial attachment to meat because of ease and consistency of quantification.

There was no positive correlation between attachment and many bacterial cell surface factors such as charge, hydrophobicity, protein and polysaccharide surface molecules. Different eluents were used to identify the principal component interfering with single attachment mechanisms on electrostatic interaction and hydrophobic interaction chromatographic columns and on collagen film. Three components interfering with the isolated attachment mechanisms were identified. They were Tween, sodium chloride (NaCl) and mannose. Further column studies indicated that cell surface proteins play a more important role in cell surface negative charge and hydrophobicity than do surface polysaccharides.

A wash solution was formulated using the components Tween, NaCl and mannose to reverse what were believed to be the major attachment mechanisms. Further trials

with Tween, NaCl and mannose and increasing their concentrations and the application of increased vigorous rinsing also proved ineffective for washing the cells from meat surfaces. This result also supports the hypothesis that bacterial attachment to meat surface is very complex and multifactorial. Elution studies using 10 % Tri sodium orthophosphate pH 12.0 killed the cells rather than removing them and further work will be directed towards the killing.

FRONTISPIECE



Scanning electron micrograph of *E. coli* E6 attaching to cut beef muscle surface. The cells are in clumps and appear to be preferentially colonising the muscle fibres. Magnification = 7000 x.

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LIST OF FLOWCHARTS

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ABBREVIATIONS

APC	-	Aerobic plate count
Ab-DEFT	-	Antibody- direct epifluorescent filter technique
BSA	-	Bovine serum albumin
CF	-	Collagen Film
CCS	-	Collagen coated slide
CPC	-	Cetyl pyridinium chloride
DF	-	Diluent fluid
DEFT	-	Direct epifluorescent filter technique
EIC	-	Electrostatic Interaction chromatography
ETEC	-	Enterotoxigenic <i>Escherichia coli</i>
FCU	-	Femoris culna ulnaris
FISH	-	Fluorescent in situ hybridisation
GMP	-	Good manufacturing practices
HIC	-	Hydrophobic Interaction chromatography
HCGR	-	High concentration gentle rinse
HCVR	-	High concentration vigorous rinse
LCGR	-	Low concentration gentle rinse
LCVR	-	Low concentration vigorous rinse
LPS	-	Lipo polysaccharides
MRGR	-	Multiple rinse gentle rinse

MRVR	-	Multiple rinse vigorous rinse
MIRINZ	-	Meat Industrial Research Institute
MATH	-	Microbial adhesion to hydrocarbons
NaCl	-	Sodium chloride (salt)
PW	-	Prewetting
PB	-	Phosphate buffer
PBS	-	Phosphate buffered saline
REA	-	Restriction endonuclease analysis
SEM	-	Scanning electron microscopy
SRGR	-	Single rinse gentle rinse
SRVR	-	Single rinse gentle rinse
STD-DF	-	Standard diluent fluid
TSM	-	Tween, Salt (NaCl) and Mannose
TEM	-	Transmission Electron Microscopy
TVC	-	Total Viable Count
TSP	-	Trisodium ortho phosphate

DEFINITIONS

ADHESION: Adhesion is a physicochemical process of interaction between molecules that are situated in the outermost layer of the inert surface and of the microorganisms, and molecules of the surrounding fluid. This term is used when the process is permanent and irreversible.

ATTACHMENT: Attachment is defined as a physicochemical process of interaction between molecules that are situated in the outermost layer of the inert surface and of the microorganisms, and molecules of the surrounding fluid. This term is used when the process is temporary and reversible.

CARCASS: is the body of any slaughtered animal or bird often, but not always, after bleeding and dressing.

CUT BEEF MUSCLE: Meat which has only muscle and is free from subcutaneous fat and fascia.

EVISCERATION: is the removal of the viscera from a carcass.

FRESH MEAT: is meat that has not been treated in any way other than refrigeration, with or without preservative packaging to maintain its fitness for human consumption.

INITIAL MICROFLORA: is the association of microorganisms present on an eviscerated carcass after skin removal (if appropriate) but prior to washing, grading, chilling and further processing.

MEAT: is the edible part (musculature and edible offal) of an animal or bird slaughtered for human consumption.

MICROBIAL CONTAMINATION: refers to microorganisms directly or indirectly transferred onto a carcass or edible offal, hence contaminating microflora means those microorganisms present as a consequence of such transmission.

RIGOR: The postmortem changes that occur during conversion of muscle to meat resulting in stiffening of a body after death.

SPOILAGE: describes changes that render meat objectionable to consumers: hence spoilage microflora describes an association of microorganisms whose development on meat renders that meat objectionable to consumers.

SPOILAGE POTENTIAL: is a measure of the propensity of microorganisms to render meat objectionable to consumers through the production of offensive metabolic by products.

SLAUGHTER: is the killing of an animal or bird for human consumption generally but not necessarily performed within premises (abattoir) that are approved and registered for that purpose.

UNCUT BEEF MUSCLE: Meat which is completely surrounded by fascia which is a membranous connective tissue covering.