EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF A DIALYSED CRANBERRY EXTRACT AGAINST SELECTED ISOLATES OF *STAPHYLOCOCCUS AUREUS*

by

Gwendolyn Jill Leusink

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science

in

The Faculty of Graduate Studies

(Food Science)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

October 2009

ABSTRACT

This research examined the antimicrobial activities of a dialysed commercial cranberry extract (CE90), by determining the minimum inhibitory concentration against *Staphylococcus aureus* and the effect of CE90 on morphology, adhesion to fibronectin, biofilm formation and removal and salt tolerance of *Staph. aureus*. The minimum inhibitory concentration was 16 mg CE90 ml^{-1} for 10 of the 24 *Staph. aureus* isolates and 32 mg CE90 ml^{-1} for the remaining 14 isolates. Phase contrast microscopy of treated *Staph. aureus* revealed a dose-dependent bacterial clumping effect, with higher CE90 concentrations resulting in larger clumps. There were no significant differences between CE90 concentrations (0, 2, 4, 8 and 16 mg CE90 ml^{-1}) in *Staph. aureus* binding to fibronectin, for a parental strain and two mutant strains deficient in either fibronectin-binding proteins A and B or clumping factor A, with the exception of reduced adhesion for the *fnbA*$^{-}$*/fnbB*$^{-}$ mutant at 4 mg CE90 ml^{-1}. Biofilm formation by four isolates of *Staph. aureus* (PC1, SA 113, 8325-4, 2076) incubated with CE90 generally decreased as CE90 concentration increased (0 – 16 mg CE90 ml^{-1}). The amount of pre-formed biofilm removed by CE90 for *Staph. aureus* PC1 was not significantly different from the control. *Staph. aureus* SA 113 and 2076 showed a greater ($P < 0.05$) level of residual biofilm after exposure to CE90 (SA 113, 2 – 16 mg CE90 ml^{-1}; 2076, 4 – 16 mg CE90 ml^{-1}) than the negative control. There was more ($P < 0.05$) residual biofilm in the 2 mg CE90 ml^{-1} treatment versus the 16 mg CE90 ml^{-1} treatment for *Staph. aureus* 8325-4. Biofilm index at 8 and 24 h showed a significant interaction between isolates and CE90 concentrations ($P < 0.05$), and biofilm index of *Staph. aureus* PC1 and SA 113 showed a significant interaction between time intervals and CE90 concentrations ($P < 0.05$). There was a

reduction in colony forming units with increasing CE90 concentration on both tryptic soy agar and TSA plus 7.5% NaCl (TSAS), with a greater reduction on TSAS, though this difference between media was significant in only four of the 16 isolate-treatment combinations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
AFM	Atomic force microscopy
BSA	Bovine serum albumin
CLSI	Clinical and Laboratory Standards Institute
ClfA	Clumping factor A
clfA	Clumping factor A gene
ClfB	Clumping factor B
clfB	Clumping factor B gene
cfu	Colony forming units
CE90	Cranberry extract 90
DIN	Deutsche Industrie Norm-Medizinische Mikrobiologie
ELISA	Enzyme-linked immunosorbent assay
E. coli	*Escherichia coli*
ECM	Extracellular matrix
FnbpA	Fibronectin-binding protein A
fnbA	Fibronectin-binding protein A gene
FnbpB	Fibronectin-binding protein B
fnbB	Fibronectin-binding protein B gene
HBSS	Hanks' balanced salt solution
H. pylori	*Helicobacter pylori*
h	Hours
MRSA	Methicillin-resistant *Staph. aureus*

MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
µl	Microlitres
MIC	Minimum inhibitory concentration
min	Minutes
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
NCCLS	National Committee for Clinical Laboratory Standards
NDM	Non-dialysable material
PBS	Phosphate buffered saline
P. gingivalis	*Porphyromonas gingivalis*
PAC	Proanthocyanidin
Staph. aureus	*Staphylococcus aureus*
S. sobrinus	*Streptococcus sobrinus*
TSA	Tryptic soy agar
TSAS	Tryptic soy agar plus salt (7.5% NaCl)
TSB	Tryptic soy broth
UTI	Urinary tract infection
vanA	Vancomycin A gene
VanA	Vancomycin A plasmid
VRE	Vancomycin resistant enterococci

ACKNOWLEDGEMENTS

I would like to express my everlasting gratitude to my UBC supervisor, Dr. Brent Skura, for his outstanding example of excellence in academia and scientific research, his unending patience and the willingness to share his vast knowledge and experience, which have provided such a valuable contribution to my research and scientific training. His integrity, encouragement, cheerful attitude and genuine concern for the professional and personal well-being of his students have greatly enriched my life during these past few years. Thank you.

I would also like to extend sincere thanks to my project supervisor, Dr. Moussa Diarra, who provided me with the opportunity to perform this research in the microbiology laboratory at Agriculture and Agri-Food Canada; without him, this project would not have been possible. His scientific expertise, guidance and willingness to perform the SAS statistical analysis are greatly appreciated.

I am also indebted to the other members of my committee, namely Dr. David Kitts and Dr. Eduardo Jovel. I am extremely grateful to Dr. Kitts for his patient willingness to provide valuable advice throughout the duration of my time here at UBC, and for the ability to condense his extensive knowledge of statistical principles and procedures into comprehensible explanations. Heartfelt thanks goes to Dr. Jovel for his willingness to be involved in this project by serving on my committee, and the valuable suggestions he has provided therein.

This study was funded through Agriculture and Agri-Food Canada and the Cranberry Institute. I greatly acknowledge both organizations for their support.

Numerous people have contributed to this project through their professional expertise or technical assistance, which is genuinely appreciated. In particular I would like to thank Heidi Rempel, Val Skura and Pedro Aloise, the microbiology laboratory supervisor at Agriculture Canada and the senior technicians of the UBC Food, Nutrition and Health building, respectively, for their efficient organization and laboratory management skills, which have greatly facilitated my work. Sincere thanks go to Garnet Martens and Kevin Hodgson, the research manager and light microscopy technician, respectively, for providing access to equipment in the UBC BioImaging Facility, and for their extremely helpful assistance with microscopy. I thank Doug Klaiber of Decas Botanical Synergies for his generous donations of cranberry extract. Sheila Fitzpatrick and the librarians at UBC are gratefully acknowledged for sharing extensive knowledge of cranberries and assisting in the location of scientific articles and other educational resources, respectively. I thank Jane Zhao and Cynthia Lopez for providing valuable technical assistance. Sincere thanks go to Terry Rigby and the computer technicians at UBC for fixing my computer and network problems, and to Andrew Larkin for his assistance with statistics and excel and for lending me his computer when mine finally died its last death. I thank Monica Purnama for helping me learn how to program SAS.

I would also like to thank those who have contributed to my professional development through their teaching and training, which has increased my interest in science and fascination with biology. My high school and undergraduate biology teachers and professors and my graduate food science professors have provided the education upon which my scientific research career is based. Todd Kabaluk and Bob Vernon provided invaluable scientific training in their entomology research laboratories at Agriculture Canada, and Michael Lyon expanded my scientific experience to include clinical trials in his research laboratory at the Canadian Centre for Functional Medicine.

Genuine thanks go to all my friends who have encouraged and inspired me along the way. Thank you for being there for me in daily life and when I needed you most. Your shared laughter, conversations and companionship fill my life with joy. Though it would be impossible to mention everyone who comes to mind with those words, I'd particularly like to thank Fatou Diarrassouba, a constant companion during our time at Agriculture Canada and here at UBC; Carrie Vanderwal, a lifelong friend; and Michelle Contant, my favourite hiking buddy.

My greatest debt of gratitude is to my family, for their unending support, encouragement and love. Thank you, Mom, for showing me how to live out my beliefs in daily life, and for always believing in me and my ability to overcome all the obstacles I've faced. Thank you, Dad, for working so hard to provide us with the opportunity to do whatever we set our minds to, and for setting an example of achieving excellence with humility. Janna, Dan, Mark, Steph, Laura, Zach and Bev, thank you for being not only my family but also my best friends.

DEDICATION

To my parents, Henry and Helen Leusink. With loving thanks for inspiring me to always do my best, and for your daily prayers that sustain me along the way.

1. INTRODUCTION

Staph. aureus is an important human and animal pathogen (Smeltzer and Gillaspy, 2000; Ben Zakour *et al*., 2008) that produces several virulence factors including adhesins and the ability to form biofilm. This bacterium is also one of the most common causes of bovine mastitis, a disease that causes important economic losses in milk production (Vasudevan *et al*., 2003; Bannerman *et al*., 2004). The widespread use of antibiotics has led to a dramatic increase in antibiotic resistant *Staph. aureus* strains (Diarra *et al*., 2002; Tacconelli, 2009). Therefore, we have an urgent need for alternative methods of controlling *Staph. aureus* infections.

The ability of *Staph. aureus* to colonise epithelial cells is associated with surface extracellular matrix (ECM) binding proteins called adhesins (Navarre and Schneewind, 1999; Yarwood *et al*., 2004). In order to establish an infection, bacteria must first adhere to host tissues. Fibronectin-binding proteins, a type of bacterial adhesin, are required for the internalization of *Staph. aureus* by epithelial cells (Dziewanowska *et al.*, 1999). Thus, fibronectin-binding proteins may be potential targets for protection against *Staph. aureus* infection. Biofilms are adherent bacterial communities enveloped in an ECM; these bacteria may be 1,000 to 1,500 times more resistant to antibiotics than planktonic bacteria (Ceri *et al*., 1999; Wu *et al*., 2003). The ability to form biofilm is another vital virulence factor for *Staph. aureus* (Fox *et al*., 2005). The persistence of biofilm-associated bacteria can result in chronic cases of disease. In addition, the formation of adherent bacterial biofilms is the most important factor in the pathogenesis of biomaterial-associated infections (Stewart and Costerton, 2001; Mack *et al*., 2004).

Administration of antibiotics may eliminate planktonic bacteria and therefore the symptoms of infection, but symptoms often recur after removal of treatment due to residual biofilm-associated bacteria (Stewart and Costerton, 2001). Bacteria that are able to survive within a biofilm despite exposure to antibiotics may develop antibiotic resistance (Eydelnant and Tufenkji, 2008). Therefore, alternative methods of controlling *Staph. aureus* infections should focus on inhibiting bacterial adhesion and biofilm formation.

Nogueira *et al.* (2003) demonstrated that cranberry juice concentrates possess intrinsic antimicrobial properties against bacterial pathogens. Cranberry products can reduce the incidence of urinary tract infections (Howell, 2002; Gormley, 2003) and inhibit bacterial adhesion (Burger *et al*., 2002; Turner *et al*., 2005; Johnson-White *et al*., 2006; Eydelnant and Tufenkji, 2008). Because there is currently no evidence within the literature to suggest that the adhesion inhibitors present in cranberries are bactericidal, the selection of strains that are resistant to these adhesion inhibitors is unlikely to occur. In addition to its anti-adhesion potential, cranberry has been shown to reduce biofilm formation (Reid *et al.*, 2001; Yamanaka *et al*., 2004; Steinberg *et al.*, 2004, 2005; Duarte *et al.*, 2006; Koo *et al.*, 2006; Labrecque *et al*., 2006; Yamanaka *et al*., 2007), which may be crucial to the efficacy of *Staph. aureus* control. The cranberry anti-adhesion and biofilm inhibition agent(s) may lead to new therapeutic strategies that are urgently needed because of the world-wide increase in antibiotic resistant bacteria (Sharon and Ofek, 2002).

The purpose of the present work was to determine the effect of a freeze-dried high-molecular-weight non-dialysable extract of a spray-dried cranberry juice concentrate powder (CE90) on fibronectin binding, biofilm formation and removal, morphology and salt tolerance of selected *Staph. aureus* isolates.

Experiments were designed to test the following hypotheses:

1. CE90 (4 – 16 mg ml^{-1}) will have no effect on the clumping behaviour of *Staph. aureus* PC1, SA 113, 8325-4, 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$, 8325-4 *clfA*$^{-}$, 2076, SHY97-4320, ABB03-2487, ATCC 6538 and ATCC 29213.
2. CE90 (4 – 16 mg ml^{-1}) will have no effect on the fibronectin binding ability of *Staph. aureus* 8325-4 and two mutant strains, 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$ and 8325-4 *clfA*$^{-}$.
3. CE90 (2 – 16 mg ml^{-1}) will have no effect on the extent of biofilm formed by *Staph. aureus* PC1, SA 113, 8325-4 and 2076.
4. CE90 (2 – 16 mg ml^{-1}) will have no effect on the extent of removal of biofilm produced by *Staph. aureus* PC1, SA 113, 8325-4 and 2076.
5. CE90 (2 – 16 mg ml^{-1}) will have no effect on the biofilm index of *Staph. aureus* PC1, SA 113, 8325-4 and 2076.
6. CE90 (4 – 16 mg ml^{-1}) will have no effect on the salt tolerance of *Staph. aureus* PC1, SA 113, 8325-4 and 2076.

2. LITERATURE REVIEW

2.1 *Staph. aureus*

2.1.1 Physical and chemical characteristics

Staph. aureus is a Gram positive, facultatively anaerobic coccus that tends to grow in pairs, short chains, or grape-like clusters. This organism is catalase positive, enabling it to convert hydrogen peroxide to oxygen and water (Varnam and Evans, 1991; Tortora *et al*., 2001; Jay, 2005; Doyle and Beuchat, 2007). *Staph. aureus* produces carotenoid pigments that likely provide protection from phagocytic killing and the antimicrobial effects of sunlight through their antioxidant properties (Tortora *et al*., 2001; Liu *et al*., 2005). These pigments result in characteristic golden yellow colonies when the bacterium is grown on agar. Bacterial cells are approximately 0.5 - 1 μm in diameter, are non-motile, and do not form capsules or spores. Fermentation of glucose by *Staph. aureus* produces primarily lactic acid. This bacterium is coagulase positive and oxidase negative (Varnam and Evans, 1991; Tortora *et al*., 2001; Jay, 2005; Doyle and Beuchat, 2007). Coagulase is able to clot fibrin in blood; the resulting clots of fibrin may protect *Staph. aureus* from phagocytosis and other host defenses (Tortora *et al*., 2001). *Staph. aureus* is sensitive to lysostaphin and resistant to lysozyme (Varnam and Evans, 1991).

2.1.2 *Staph. aureus* growth

A wide range of environmental conditions permit growth of *Staph. aureus*: a) temperature 6.7 – 47.8^{o}C (optimum 37^{o}C); b) pH 4.0 – 9.8 (optimum 6 - 7); c) a_w 0.83 - > 0.99 (optimum > 0.99). *Staph. aureus* is very salt tolerant, growing well at NaCl

concentrations between 0 and 10%; some strains can grow in NaCl concentrations up to 20% (Varnam and Evans, 1991; Jay, 2005).

2.1.3 Virulence factors

2.1.3.1 *Staph. aureus* toxins

Staph. aureus produces a number of toxins, including alpha-toxin, which forms pores in cell membranes resulting in cell lysis. Alpha-toxin possesses hemolytic activity along with several other *Staph. aureus* hemolysins (Bhakdi and Tranum-Jensen, 1991; Lowy, 1998; Giese *et al*., 2009).

Exfoliative toxins cause staphylococcal scalded skin syndrome, which reduces cell adhesion within the epidermis, resulting in blisters and the sloughing off of skin. This syndrome occurs most frequently in children and infants (Lowy, 1998; Tortora *et al*., 2001; Castellano *et al*., 2008).

Toxic shock syndrome toxin causes a cytokine storm through release of a large amount of cytokines from immune T cells and macrophages. This results in symptoms such as fever and low blood pressure. Toxic shock syndrome may progress to coma, multiple organ failure and even death (Lowy, 1998; Seishima *et al*., 2009).

Consumption of enterotoxins present in food results in gastroenteritis (Varnam and Evans, 1991; Jay, 2005). Enterotoxins are far more heat resistant than *Staph. aureus* cells, and are unlikely to be inactivated by cooking and commercially applied heat

treatments (Jay, 2005; Doyle and Beuchat, 2007). The fact that enterotoxins are able to exert their effects even in the absence of viable *Staph. aureus* cells means that food can still cause gastroenteritis even after proper cooking or pasteurization, if the food had a high level of *Staph. aureus* before heat treatment. Re-contamination after heat treatment and improper refrigeration allowing the production of enterotoxin can also result in illness. The minimum amount of enterotoxin needed to produce symptoms of gastroenteritis in humans is approximately 1 ng/g of food, or a total of 20 ng (Jay, 2005).

Conditions favouring enterotoxin production are more limited than for *Staph. aureus* growth: a) temperature 10 – 46^{o}C (optimum 40 – 45^{o}C); b) a_w 0.86 - > 0.99 (optimum > 0.99); c) NaCl 0 – 10% (Varnam and Evans, 1991; Jay, 2005). The pH range in which enterotoxins are produced varies widely according to the type of toxin, oxygen availability and acidulant. In aerobic conditions, the minimum pH for production of most enterotoxins is approximately 5, whereas in anaerobic conditions, the minimum pH is approximately 6. However enterotoxin A was produced at pH 4.5 when hydrochloric acid was used as the acidulant, and pH 5.0 when lactic acid was used (Jay, 2005). Interactions between temperature, pH, a_w, NaCl concentration, acidulant and oxygen availability mean that the range in which each of these factors permits *Staph. aureus* growth and enterotoxin production can be restricted when the other factors are not ideal (Jay, 2005). This is the basic concept behind hurdle technology, which combines multiple inhibitory factors for food preservation (Leistner, 2000; Doyle and Beuchat, 2007).

2.1.3.2 Adhesins

Staph. aureus possesses several other important virulence factors, such as adhesins and the ability to form a biofilm. Adhesins are surface proteins embedded in bacterial cell walls, which enable bacterial attachment to a surface thus creating the opportunity for colonization and infection. Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (Beenken *et al*., 2004) are an important class of adhesins. Adhesins such as fibronectin-binding proteins A and B (FnbpA, FnbpB), collagen-binding protein and the fibrinogen-binding proteins clumping factors A and B (ClfA and ClfB) are MSCRAMMs (Gotz, 2002) and play a primary role in the initial attachment of bacteria to cells and biomaterials (Patti *et al*., 1994; Gotz, 2002; Beenken *et al*., 2004; Yarwood *et al*., 2004).

2.1.3.3 Biofilm production

After *Staph. aureus* have become established on a cell surface they may begin to produce a biofilm, which is a complex bacterial community contained within a polysaccharide matrix. The production of multiple cell layers requires polysaccharide adhesins that promote adhesion between bacterial cells (Gotz, 2002). The polysaccharide intercellular adhesin, a component of the ECM, seems to be the primary adhesin responsible for this phenomenon (Heilmann *et al*., 1996; Yarwood *et al*., 2004). Adhesion between bacteria allows the formation of densely packed bacterial clusters, with channels running through for delivery of water and nutrients and to facilitate the removal of waste (Annous *et al*., 2009). Biofilms confer bacterial protection against host defenses (Shiau and Wu, 1998; Stewart, 2002), physical and chemical stresses (Annous *et al*., 2009) and antibiotics, by

hindering delivery of antimicrobial agents (Stewart, 2002) and as a result of the intrinsically antibiotic resistant phenotype that is characteristic of biofilm-associated bacteria (Ceri *et al*., 1999; Beenken *et al*., 2004). Since bacteria within a biofilm typically have a slower growth rate than planktonic bacteria, this reduces the impact of antibiotics that target cell wall synthesis; in addition, the decreased oxidative metabolism of biofilm-associated bacteria limits their uptake of aminoglycosides (Lewis, 2001; Stewart and Costerton, 2001; Stewart, 2002). The frequency of genetic transfer within biofilms is typically higher than between planktonic bacteria (Annous *et al*., 2009), facilitating the spread of antibiotic resistance genes (Licht *et al*., 1999; Maeda *et al*., 2006). The ensuing tendency of biofilm-associated infections to resist antibiotics often leads to chronic disease, which may necessitate surgery to remove the source of the biofilm. This applies particularly to cases of indwelling biomedical devices such as prosthetic heart valves, central venous catheters, urinary catheters and prosthetic joints (Gander, 1996; Cirioni *et al*., 2003).

The genetic regulation of biofilm formation is complex and not yet fully elucidated (Lim *et al*., 2004). A cell to cell signalling system known as quorum sensing, which allows bacteria to communicate with each other, is involved in the formation of biofilm. Bacteria produce molecules that, when the bacterial cell density and therefore the signalling molecule concentration are high enough, bind to receptors that either activate or repress the transcription of certain genes. This stimulates or represses biofilm formation and allows bacteria to behave as an integrated community, producing a unified response to environmental stress (Annous *et al*., 2009).

2.1.4 *Staph. aureus* infections in livestock and poultry

The pathogenicity of *Staph. aureus* has serious consequences for human and animal health (Trivier and Courcol, 1996; Bayles *et al*., 1998; Smeltzer and Gillaspy, 2000; Zadoks *et al*., 2000; Fitzgerald *et al*., 2001a, b; Diarra *et al*., 2002; Hazariwala *et al*., 2002; Oliveira *et al*., 2002; Sifri *et al*., 2003; White *et al*., 2003; Ben Zakour *et al*., 2008). *Staph. aureus* is one of the main pathogens responsible for bovine mastitis (Forsman *et al*., 1997; Bayles *et al*., 1998; De Oliveira *et al*., 2000; Gentilini *et al*., 2000; Hebert *et al*., 2000; Zadoks *et al*., 2000; Fitzgerald *et al*., 2001a; Vasudevan *et al*., 2003; Bannerman *et al*., 2004), which results in a loss of approximately $2 billion per year according to the United States National Mastitis Council (Harmon, 1996). Bacterial chondronecrosis, associated mainly with *Staph. aureus*, is a common poultry disease (Nairn, 1973; Riddell, 1980; Daum *et al*., 1990; Cook, 2000; McNamee and Smyth, 2000; Smeltzer and Gillaspy, 2000; White *et al*., 2003) and was the primary cause of lameness in broiler chicken flocks (Thorp and Waddington, 1997; McNamee *et al*., 1998). Bacterial chondronecrosis is also known as osteomyelitis, bacterial chondronecrosis with osteomyelitis or femoral head necrosis. Additional poultry diseases caused by *Staph. aureus* include joint infections such as arthritis (Nairn, 1973; Riddell, 1980; Daum *et al*., 1990; Smeltzer and Gillaspy, 2000; Jensen and Miller, 2001; Hazariwala *et al*., 2002) and synovitis (Nairn, 1973; Riddell, 1980; Jensen and Miller, 2001; White *et al*., 2003); omphalitis, also known as yolk sac infection (Nairn, 1973; Cervantes *et al*., 1988; Jensen and Miller, 2001; Hazariwala *et al*., 2002; White *et al*., 2003; Cortes *et al*., 2004); blood infections such as bacteremia (Daum *et al*., 1990) and

septicemia (McCullagh *et al*., 1998; Jensen and Miller, 2001; Hazariwala *et al*., 2002); wound infections (Cervantes *et al*., 1988); and skin infections such as dermatitis (Cervantes *et al*., 1988; McCullagh *et al*., 1998; Jensen and Miller, 2001; Hazariwala *et al*., 2002; White *et al*., 2003), cellulitis (White *et al*., 2003) and abscesses (McCullagh *et al*., 1998; Jensen and Miller, 2001).

2.2 Antibiotic resistance

2.2.1 *Staph. aureus* antibiotic resistance

The use of antibiotics for clinical and veterinary medicine, as well as for incorporation into animal feed to control sub-clinical infections and promote animal growth, may contribute to the emergence of bacterial antibiotic resistance by creating a selective pressure favourable for resistant strains. The drastic increase in antibiotic resistant *Staph. aureus* strains has been attributed to extensive use of antibiotics (Rolinson, 1994; Gruson *et al*., 2000; Hiramatsu, 2001; Graffunder and Venezia, 2002).

Staph. aureus tends to develop resistance to antibiotics very quickly, which necessitates the continuous development of new drugs. Methicillin was the main antibiotic that was developed in response to penicillin resistant *Staph. aureus* strains that emerged in the 1940's (Revazishvili *et al*., 2006). However *Staph. aureus* soon became resistant to methicillin in addition to other antibiotics such as erythromycin, tetracycline, rifampicin, aminoglycosides and quinolones (Lilly and Lowbury, 1978; Aubry-Damon *et al*., 1998; Ito *et al*., 2003; Revazishvili *et al*., 2006). Vancomycin and teicoplanin are glycopeptides that are used as last resort antibiotics to treat methicillin-resistant *Staph.*

aureus (MRSA). *Staph. aureus* are beginning to show resistance to these drugs as well (Revazishvili *et al*., 2006; Sader *et al*., 2009; Wohlleben *et al*., 2009). The rise in multi-drug resistant *Staph. aureus* strains poses a serious concern for public health.

Staph. aureus develops resistance to antibiotics by either the mutation of a gene on its chromosome, or by acquiring an antibiotic resistance gene from another bacterium through conjugation, transduction or transformation (Ito *et al*., 2003). There is concern that MRSA may obtain the vancomycin resistance gene from enterococci.

2.2.2 Antibiotic use and development of antibiotic resistance

The *vanA* gene confers resistance to vancomycin and teicoplanin, resulting in vancomycin resistant enterococci (VRE). There is a positive correlation between the use of avoparcin as a growth promoter in farm animals and an increased prevalence of VRE in the feces of these animals (Klare *et al*., 1995; Aarestrup, 1995; Aarestrup *et al*., 1996; McDonald *et al*., 1997; van den Bogaard and Stobberingh, 1999). Avoparcin promotes the development of cross-resistance in bacteria to medically important glycopeptides such as vancomycin and teicoplanin (Bates, 1997; Endtz *et al*., 1997). The same trend was observed in the commensal flora of healthy humans and pets in the Netherlands (McDonald *et al*., 1997; van den Bogaard *et al*., 1997), Belgium (Devriese *et al*., 1996) and Denmark (Aarestrup *et al*., 1996), even with very limited use of vancomycin in hospitals throughout Europe. This suggests that using antibiotics as growth promoters may increase the prevalence of antibiotic resistant bacteria that can impact human health as well as agriculture. Additionally, administration of teicoplanin, vancomycin or

streptomycin to healthy human volunteers or to an experimental mouse model significantly increased VRE colonization of subjects' intestinal tracts (Van der Auwera *et al*., 1996; Whitman *et al*., 1996). Cephalosporins, another important contributor to VRE, also select for resistance to beta-lactams such as penicillins and cephalosporins (Ballow and Schentag, 1992; Jones, 1992; Yates, 1999). Furthermore, use of the fluoroquinolone enrofloxacin as a growth promoter in poultry was linked to quinolone-resistant *Campylobacter* spp. infections of humans (Endtz *et al*., 1991); that phenomenon supports the hypothesis that agricultural use of antibiotics has important implications for human health. Neuhauser *et al*. (2003) found that gram-negative aerobic bacteria, isolated from intensive care unit patients in hospitals across the USA between 1994 and 2000, had increased frequency of resistance to antibiotics, particularly to ciprofloxacin, over the study period. This may be linked to a concurrent national increase in the use of fluoroquinolones.

2.2.3 Reduction of antibiotic resistance following restriction of antibiotic use

Studies have shown that restricting antibiotic use may effectively decrease antibiotic resistance. After avoparcin was banned in Europe in 1997, numerous studies reported a reduction in the prevalence of VRE in the fecal matter of farm animals (Bager *et al*., 1999; Klare *et al*., 1999; Pantosti *et al*., 1999; van den Bogaard *et al*., 2000) and healthy humans (Klare *et al*., 1999; van den Bogaard *et al*., 2000). Similarly, decreased prevalence of dalfopristin/quinupristin-resistant enterococci was observed in farm animals and humans during a time of limited virginiamycin availability (van den Bogaard *et al*., 2000). Studies also show that restricting cephalosporin use may lead to a decrease

in cephalosporin resistance (Ballow and Schentag, 1992; Rice *et al*., 1996; Rahal *et al*., 1998).

2.2.4 Multidrug-resistant bacteria

The main concern regarding VRE is their potential to transfer the *vanA* gene to more pathogenic bacteria (Bates, 1997). The VanA plasmid carries resistance genes not only for vancomycin and teicoplanin, but also for tetracycline, streptomycin and erythromycin. Therefore, a variety of growth promoters, such as tylosin, which shows cross-resistance to erythromycin, may non-specifically select for the *vanA* gene (Bates, 1997). Another implication is that transmission of the VanA plasmid increases resistance to additional antibiotics besides vancomycin. *E. coli*, which is part of the normal flora of humans and many animals, can easily exchange DNA with other members of Enterobacteriaceae and may act as a reservoir of antibiotic resistance genes for these bacteria (Diarrassouba *et al*., 2007; Diarra *et al*., 2007; Costa *et al*., 2008). *Staph. aureus, Listeria monocytogenes* and several *Streptococcus* spp. have also been shown to develop vancomycin resistance through obtaining the *vanA* gene from *Enterococcus faecium* or *Enterococcus faecalis* (Leclercq *et al*., 1989; Noble *et al*., 1992, respectively). If this transfer would occur in MRSA, the resulting methicillin- and vancomycin-resistant *Staph. aureus* could cause infections that would be nearly untreatable (Bates, 1997).

Conjugative transposons, DNA transfer elements that have characteristics similar to plasmids, transposons and phages, contribute to the spread of antibiotic resistance genes (Salyers *et al*., 1995). The frequency that conjugative transposons are transferred by is

increased 10- to 1000-fold by pre-exposure to tetracycline (Valentine *et al*., 1988; Torres *et al*., 1991; Stevens *et al*., 1993). Therefore, the use of tetracycline as a growth promoter in livestock feed may select for antibiotic resistant bacterial strains and also enhance the transmission of DNA that contains resistance genes. Some of these transmissible elements have a wide range of bacterial hosts and therefore, can be spread among many different types of bacteria (Bertram *et al*., 1991). Similar to the VanA plasmid, conjugative transposons carrying resistance genes to tetracycline may also possess genes encoding resistance to a variety of antibiotics such as aminoglycosides, macrolides, lincosamides, streptogramins and chloramphenicol (Courvalin and Carlier, 1986; Ayoubi *et al*., 1991). This may lead to increased selective pressure for these conjugative transposons. Additionally, it means that the spread of these transmissible elements may result in increased resistance to not only tetracycline but to other clinically significant antibiotics as well (Speer *et al*., 1992).

2.3 Cranberry

2.3.1 Health benefits and antimicrobial activity

Cranberries have been associated with a wide array of health benefits such as maintaining cardiovascular health (Wilson *et al*., 1998; Porter *et al*., 2001; Reed *et al*., 2001; Reed 2002; Yan *et al*., 2002; Ruel *et al*., 2005; Vattem *et al*., 2005a; Ruel *et al*., 2006; Neto, 2007a) and protecting against cancer (Bomser *et al*., 1996; Guthrie, 2000; Sun *et al*., 2002; Ferguson *et al*., 2004; Vattem *et al*., 2005a; Neto, 2007a; Kresty *et al*., 2008; Neto *et al*., 2008; Liu *et al*., 2009; Singh *et al*., 2009). Perhaps the most well-known health benefit of cranberries is their ability to reduce the incidence of urinary tract infections

(UTIs). In addition to their effect on urinary tract health, cranberries possess antimicrobial activity against a variety of bacteria (Puupponen-Pimia *et al*., 2001; Vattem *et al*., 2004a,b; Weiss *et al*., 2004; Lin *et al*., 2004; Lin *et al*., 2005; Puupponen-Pimia *et al*., 2005; Vattem *et al*., 2005b; Nohynek *et al*., 2006; Apostolidis *et al*., 2008; Magarinos *et al*., 2008; Wu *et al*., 2009). Daily use of a cranberry NDM-containing mouthwash over a period of six weeks significantly reduced the level of salivary mutans streptococci as well as total bacterial counts, compared to a placebo mouthwash. However, the NDM-containing mouthwash had no effect on the plaque or gingival indices (Weiss *et al*., 2004). Cranberries were used for food and medicine among North American First Nation societies (Henig and Leahy, 2000; Carson and Riley, 2003; Lynch, 2004; Raz *et al*., 2004; Nowack, 2007). The fact that cranberries have a long history of use in preventing wound infections and treating sepsis suggests that there is minimal potential for the development of bacterial resistance against cranberry.

2.3.2 Ability of cranberries and cranberry extracts to reduce the incidence of UTIs

Cranberries have long been used for the prevention of UTIs, and there is increasing scientific evidence to support this use (Bodel *et al*., 1959; Moen, 1962; Sternlieb, 1963; Papas *et al*., 1968; Bate-Smith, 1973; Gibson *et al*., 1991; Rogers, 1991; Avorn *et al*., 1994; Haverkorn and Mandigers, 1994; Walker *et al*., 1997; Dignam *et al*., 1998; Henig and Leahy, 2000; Kontiokari *et al*., 2001; Howell, 2002; Stothers, 2002; Griffiths, 2003; Jepson *et al*., 2004; Raz *et al*., 2004; Bailey *et al*., 2007; Jepson and Craig, 2007; Nowack, 2007; Hess *et al*., 2008). Avorn *et al*. (1994) conducted a randomized, double-blind, placebo-controlled trial to determine the effect of cranberry juice on bacteriuria

and pyuria in elderly women. They found that the group that drank cranberry juice had fewer cases of bacteriuria with pyuria than the control group that drank a placebo drink without cranberry. Furthermore, subjects in the group that consumed cranberry were able to clear these infections within a month more often than subjects in the placebo group.

It was previously thought that the ability of cranberry to help reduce the incidence of UTIs was either due to acidification of the urine (Bodel *et al*., 1959; Kinney and Blount, 1979) or to hippuric acid, a strong antibacterial agent and metabolite of quinic and benzoic acids found in cranberries (Howell, 2002). However Kahn *et al*. (1967) found that at least 1500 ml of cranberry juice should be consumed each day in order to achieve the urinary pH and hippuric acid concentration necessary for this effect. Considering that cranberry juice exerts an antibacterial effect at much lower doses, there must be another mechanism of action (Raz *et al*., 2004).

2.3.3 Bacterial anti-adhesion mechanism of cranberries and cranberry extracts

The ability of cranberry to prevent bacterial adhesion to cell surfaces is now regarded as the likely mechanism of preventing UTIs (Sobota, 1984; Schmidt and Sobota, 1988; Zafriri *et al*., 1989; Ofek *et al*., 1991; Howell *et al*., 1998; Howell and Foxman, 2002; Raz *et al*., 2004; Howell *et al*., 2005; McMurdo *et al*., 2005; Di Martino *et al*., 2006; Liu *et al*., 2006). In order to cause a UTI, bacteria, typically uropathogenic P-fimbriated *E. coli* (Nowack, 2007), must first adhere to uroepithelial cells (Howell *et al*., 2005) by attachment of their fimbrial adhesins to carbohydrate receptors on the cell surface (Beachey, 1981; Foo *et al*., 2000a,b; Johnson *et al*., 2003). Cranberry proanthocyanidins

(PACs) have unique A-type linkages (Foo *et al*., 2000a,b; Howell *et al*., 2005) (Appendix A) and possess bacterial anti-adhesion ability (Howell *et al*., 1998; Foo *et al*., 2000a; Howell *et al*., 2005); these A-type PACs are thought to be the active ingredient in cranberry that helps prevent UTIs. Cranberry juice and A-type PACs isolated from cranberry have been shown to prevent adhesion of P-fimbriated uropathogenic *E. coli* to *in vitro* bladder epithelial cells (Gupta *et al*., 2007), uroepithelial cells (Sobota, 1984; Schmidt and Sobota, 1988; Zafriri *et al*., 1989; Ofek *et al*., 1991; Ofek *et al*., 1996; Howell *et al*., 1998; Foo *et al*., 2000a,b; Howell *et al*., 2005; Turner *et al*., 2005; Di Martino *et al*., 2006; Liu *et al*., 2006), biomaterials commonly used to make urinary catheters (Eydelnant and Tufenkji, 2008) and cell surfaces with receptor sequences similar to those found on uroepithelial cells (Foo *et al*., 2000a,b). Cranberry PACs may also affect colonic bacteria (Zafriri *et al*., 1989), inhibiting their ability to bind to the uroepithelium if they were to travel up the urinary tract (Howell, 2002).

In addition to the effect of cranberry on the urinary tract, a high-molecular-weight component of cranberry juice inhibited the adhesion of *H. pylori* to human mucus, erythrocytes and cultured gastric epithelial cells (Burger *et al*., 2002), and suppressed *H. pylori* infection of humans (Zhang *et al*., 2005; Gotteland *et al*., 2008). Cranberry juice and its non-dialysable component inhibited adhesion of oral bacteria such as *Streptococcus sobrinus* to saliva-coated hydroxyapatite beads (Steinberg *et al*., 2004; Weiss *et al*., 2004; Yamanaka *et al*., 2004).

Additionally, consumption of cranberry products has been shown to induce bacterial anti-adhesion activity of urine in both mice and humans (Sobota, 1984; Howell *et al*., 2001; Howell and Foxman, 2002; Greenberg *et al*., 2005; Howell *et al*., 2005; Di Martino *et al*., 2006), peaking between four and six h after consumption, and remaining for at least eight h (Howell *et al*., 2005). This suggests that bioactive PACs were absorbed and likely remained functional throughout metabolism. The detection of PACs in plasma of rats after they were fed grape seed extract supports the hypothesis that PACs are absorbed after ingestion (Prasain *et al*., 2009).

Conversely, B-type PACs isolated from cranberry (Foo *et al*., 2000a), apple juice, purple grape juice, green tea and dark chocolate (Howell *et al*., 2005) did not prevent adherence of P-fimbriated *E. coli* to cell surfaces with uroepithelial cell-like receptors, with the exception of purple grape juice, which showed very slight activity at a much higher concentration. None of these food products containing B-type PACs resulted in urinary anti-adhesion activity after consumption (Howell *et al*., 2005). The ability of cranberry juice to reduce non-specific adhesion of several foodborne pathogens to borosilicate glass microscope slides was not reproduced with white cranberry juice (Johnson-White *et al*., 2006); however, the level of A-type versus B-type PACs in the white cranberry juice was not reported in that study.

Fructose has also been shown to have anti-adhesion activity, specifically towards *E. coli* with type 1 fimbrial adhesins (Zafriri *et al*., 1989; Ofek *et al*., 1996). However, there have been no clinical studies supporting the use of fructose in maintaining urinary tract

health (Howell, 2002; Gupta *et al*., 2007). This may be due to the fact that fructose does not display anti-adhesive activity against P-fimbriae, which most *E. coli* isolates causing pyelonephritis possess (Vaisanen-Rhen *et al*., 1984; Arthur *et al*., 1989; Johnson, 1991; Stapleton, 2005; Di Martino *et al*., 2006; Lane and Mobley, 2007).

2.3.4 Effect of cranberries on bacterial biofilm formation

Cranberry compounds inhibited biofilm formation in uropathogens, the causative agents of urinary tract infections (Reid *et al.*, 2001), and oral pathogens, present in dental biofilm with a primary role in causing dental caries (Steinberg *et al.*, 2004; Yamanaka *et al*., 2004; Steinberg *et al.*, 2005; Duarte *et al.*, 2006; Koo *et al.*, 2006; Labrecque *et al*., 2006; Yamanaka *et al*., 2007). Cranberry juice and its non-dialysable component, as well as other PACs and flavonols derived from cranberry, significantly inhibited the activity of enzymes involved in oral biofilm formation (Steinberg *et al.*, 2004; Duarte *et al.*, 2006; Koo *et al.*, 2006). Furthermore, cranberry non-dialysable material (NDM) promoted desorption of *S. sobrinus* from biofilm (Steinberg *et al*., 2005). Biofilm inhibition may be related to inhibition of extracellular polysaccharide synthesis (Steinberg *et al*., 2004), as well as to reduction of hydrophobicity (Yamanaka *et al*., 2004).

2.3.5 Cranberry PAC structure

Cranberry PACs are composed primarily of epicatechin subunits of 4-10 degrees of polymerization, with at least one A-type interflavan linkage but often multiple A-type linkages, at each degree of polymerization (Foo *et al*., 2000b; Howell *et al*., 2005).

Cranberry PAC trimers had greater anti-adhesion activity than dimers, suggesting that a higher molecular mass may result in greater activity (Foo *et al*., 2000a). However, whether or not larger oligomers remain intact and are effective *in vivo* is not yet known (Howell, 2002).

2.4 Literature review pertaining to methodology

2.4.1 Minimum Inhibitory Concentration (MIC)

Antibiotic susceptibility testing may be performed by several different methods, including agar dilution, E-test, disk diffusion, broth macrodilution and microdilution. The agar dilution method (Baker *et al*., 1991; Wust and Hardegger, 1992; de Castillo *et al*., 1998; Kirkpatrick *et al*., 1998; Simpson *et al*., 1998; Kelly *et al*., 1999; Reynolds *et al*., 2003) consists of finding the minimum inhibitory concentrations (MICs) of antibiotic against bacteria by dipping a sterile replicator into broth suspensions of the test strains, and touching the replicator to sterile agar plates containing doubling dilutions of antibiotic. Plates are incubated, and the MIC is determined as the lowest concentration of antibiotic that completely inhibits bacterial growth. The E-test (Baker *et al*., 1991; Wust and Hardegger, 1992; Kelly *et al*., 1999) determines MIC values by dipping a cotton swab into bacterial suspensions and streaking to cover the entire surface of an agar plate. After the surface has dried, the E-test strip is placed onto the agar surface and incubated. The MIC is recorded as the point where the zone of inhibition intersects the MIC scale on the strip. Disk diffusion (Baker *et al*., 1991; de Castillo *et al*., 1998; Kelly *et al*., 1999) involves using a cotton swab to cover the entire surface of an agar plate after the swab has been dipped in bacterial inoculum. When the surface of the agar has dried, antibiotic

disks are aseptically placed on the agar plate followed by incubation under prescribed conditions. The diameters of the zones of inhibition are measured to the nearest millimetre. Broth macrodilution (Kirkpatrick *et al*., 1998) and microdilution (Baker *et al*., 1991; Kelly *et al*., 1999; Reynolds *et al*., 2003) determine MIC values by inoculating sterile broth containing doubling dilutions of antibiotic with bacterial suspensions followed by incubation under prescribed conditions. Broth macrodilution uses test tubes, while broth microdilution uses microwell plates. There is generally very good agreement between results obtained by agar dilution, E-test, disk diffusion, broth macrodilution and microdilution (de Castillo *et al*., 1998; Kirkpatrick *et al*., 1998; Kelly *et al*., 1999; Reynolds *et al*., 2003).

Agar dilution testing in North America follows the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards (NCCLS)) methods, while the CLSI guidelines or those recommended by the British Society for Antimicrobial Chemotherapy are used in Europe (Simpson *et al*., 1998; Louie and Cockerill, 2001). These methods are similar, with the choice of test media typically being the only difference between them. However laboratories in France and Germany follow testing guidelines recommended by the Societe Francais de Microbiologie and the Deutsche Industrie Norm-Medizinische Mikrobiologie (DIN), respectively (Simpson *et al*., 1998). A comparison of antibiotic susceptibilities of *E. coli* strains analysed by the NCCLS and DIN guidelines showed significant differences between the methods (Simpson *et al*., 1998). This was attributed to methodological variation as well as to a

difference in breakpoints categorizing strains as susceptible, intermediate or resistant, with NCCLS permitting higher MICs than DIN for a particular category.

2.4.2 Bacterial morphology

Many different types of microscopes are available to visualize bacterial morphology, including bright field (Nandy *et al*., 2007), dark field (Merrell *et al*., 2002), phase contrast (Kahl *et al*., 2000), differential interference contrast (Kahl *et al*., 2000) and fluorescence microscopes (Kahl *et al*., 2000; Leevy *et al*., 2006; Nandy *et al*., 2007). In addition, transmission electron microscopes (Shimoda *et al*., 1995; Kahl *et al*., 2000; Bobin-Dubreux *et al*., 2001; Diarra *et al*., 2002) and scanning electron microscopes (Kahl *et al*., 2003) have been used to provide greatly magnified images with excellent resolution.

2.4.3 ECM binding

The ability of bacteria to bind to ECM proteins may be measured by their adherence to protein-coated wells of a microplate (Dziewanowska *et al*., 1999). Wells are coated with protein solution or bovine serum albumin (BSA) to estimate nonspecific binding and rinsed. Residual binding sites are blocked with heat-denatured BSA in Hanks' balanced salt solution (HBSS). Bacterial inoculum is added and allowed to incubate, and the wells are rinsed to remove nonadherent bacteria. Adherent bacteria are detached with trypsin and quantified by plating serial dilutions.

Bauer and Spinola (1999) developed an enzyme-linked immunosorbent assay (ELISA)-based method to assess bacterial binding to ECM proteins. Similar to the microplate method described above, wells are coated with ECM proteins, BSA or fetuin to estimate nonspecific binding, rinsed, and residual binding sites are blocked. Bacteria are added to wells and incubated, and the wells are rinsed to remove unbound bacteria. Adherent bacteria are detected by incubating with rabbit antiserum against the bacterial cells, followed by horseradish peroxidase-conjugated goat anti-rabbit secondary antibody. ELISA plates are incubated with horseradish peroxidase substrate, and the absorbance at 450 nm is measured for each well.

Alternatively, the ECM-binding ability of bacteria may be measured by their ability to bind to protein-coated latex beads. ECM protein is adsorbed onto latex beads and used in a particle agglutination assay, where the beads are mixed with an equal volume of bacterial inoculum on a glass slide and examined for agglutination (Naidu *et al*., 1988; Styriak *et al*., 1999, 2002).

2.4.4 Biofilm formation and removal

The extent of biofilm formation or removal may be measured by a number of different methods, such as the test tube method, the Congo red agar plate test, microscopy and the microwell plate test (Arciola *et al*., 2002; Stepanovic *et al*., 2007). The test tube method involves inoculating broth in a test tube with an isolated colony followed by incubation at an appropriate temperature. The inoculum is removed, tubes are rinsed and dried, and culture tubes stained with an appropriate dye such as trypan blue, safranin or crystal

violet. Excess stain is removed; tubes are rinsed and dried upside down. Biofilm production is considered positive when a visible film remains on the test tube walls (Christensen *et al*., 1985; Mathur *et al*., 2006). Alternatively, crystal violet that has stained adherent biofilm may be extracted with a solvent such as dimethylsulphoxide, and the absorbance measured by spectrophotometry at 585 nm (Henoun Loukili *et al*., 2004).

Congo red agar is used to measure biofilm formation by the appearance of colonies. Bacteria are streaked onto congo red agar and plates are incubated. Biofilm producing bacteria form black or very dark colonies, generally with a dry crystalline consistency, whereas biofilm negative bacteria form pink or red colonies (Arciola *et al*., 2002; Mathur *et al*., 2006).

Fluorescence microscopy and confocal laser scanning microscopy provide high-resolution images of biofilm, and can serve as a measure of biofilm formation or removal (Hyde *et al*., 1997; Marion-Ferey *et al*., 2003). In fluorescence microscopy, biofilms are stained with a dye and examined at a wavelength appropriate for the selected dye. The extent of biofilm formation is detected visually, through use of a digital camera or a photomultiplier. Image analysis software such as Scion Image can be used to determine the amount of surface area that is covered by biofilm (Marion-Ferey *et al*., 2003). Biofilm samples may also be examined by confocal laser scanning microscopy, which provides three dimensional images and a measurement of the biofilm thickness.

The microwell plate assay is the most common method used for the analysis of biofilm formation (Stepanovic *et al*., 2007). In this method, bacterial broth suspensions with or without treatments are incubated in a 96-well microplate to form a biofilm. The inoculum is removed, wells are rinsed, and adherent organisms may or may not be fixed with a solution such as Bouin fixative or 2% sodium acetate. Biofilm is stained with crystal violet. At this point either the biofilm can be measured in the original wells (Christensen *et al*., 1985; Vasudevan *et al*., 2003; Kaplan *et al*., 2004; Mathur *et al*., 2006), or the crystal violet can be extracted and transferred to a new microwell plate for a more homogenous sample (Narisawa *et al*., 2005; Yoshida *et al*., 2009). If the biofilm is left in the original wells, the crystal violet solution is removed and the wells are rinsed to remove unbound crystal violet and the biofilm is dried at room temperature. Biofilm absorbance is measured at 570 nm with a microplate reader. Alternately, the crystal violet solution is removed and the wells are rinsed. The remaining crystal violet that has stained the biofilm is then extracted with 70 - 95% ethanol and transferred to a new microwell plate and the absorbance is measured at 590 nm with a microplate reader.

The microwell plate assay may also be used to examine the ability of a particular treatment to remove pre-existing biofilm (Pitts *et al*., 2003; Kaplan *et al*., 2004). The method is essentially the same as when examining biofilm formation, except that treatments are applied after bacteria have formed biofilm, rather than being incubated along with the bacterial suspension. After removing the treatment solutions and rinsing wells, biofilm is stained with crystal violet and measured as described above.

2.4.5 Biofilm enumeration

Viable plate counts and direct counts are common assays used to enumerate the bacteria present in a biofilm. For viable plate counts, a bacterial suspension is incubated in a tube or microplate to form a biofilm, after which the inoculum is removed. Tubes or wells are then rinsed to remove non-adherent bacteria. At this point several different methods may be used to quantify bacteria. Biofilm-associated bacteria may be resuspended by adding sterile solution to tubes or wells and sonicating (Kadouri and O'Toole, 2005; Tre-Hardy *et al*., 2008). Alternatively, biofilm can be removed from wells by scraping with a sterile spatula or cell scraper and vigorously vortexing in sterile saline or phosphate buffered saline (PBS) to disperse cells (Di Bonaventura *et al*., 2004; Kaplan *et al*., 2004). Serial dilutions of resuspended biofilm bacteria are enumerated by plating onto agar.

Biofilm-associated bacteria may also be enumerated through direct counts (Huang and Stewart, 1999; Ahmad *et al*., 2008). A biofilm is allowed to form as described above, wells are rinsed to remove planktonic bacteria, and the biofilm is stained with acridine orange. Bacteria are examined with a fluorescence microscope and visually counted.

2.4.6 Salt tolerance

Staph. aureus has a particularly high tolerance to salt, and uninjured cells remain viable in the presence of NaCl (Chapman, 1945). However bacterial injury results in a loss of salt tolerance (Hurst *et al*., 1973; Wyber *et al*., 1994; Carson *et al*., 2002). The degree of injury caused by a treatment is determined by the ability of treated *Staph. aureus* to produce colonies on salt-containing media, and compared with its viability on regular

media. The concentration of salt used in the assay is typically about 7.5% (Hurst *et al*., 1973), as usually only staphylococci are able to grow on media containing 7.5% NaCl (Chapman, 1945). Media such as nutrient agar or trypticase soy agar are generally used for either spot plating or pour plating (Hurst *et al*., 1973; Tomlinson and Palombo, 2005). However Bruins *et al*. (2007) used mannitol broth with NaCl concentrations ranging from 1.5 to 12.5%, from which they determined the maximal non-inhibiting concentrations and the MICs.

3. MATERIALS AND METHODS

3.1 Cranberry extract

Cranberry extract (spray-dried cranberry juice concentrate powder standardized to a minimum of 90% cranberry fruit solids and 35% organic acids, containing 0.6 – 0.95% PACs) was kindly provided by Decas Botanical Synergies (Wareham, MA, USA) (Appendix B). A high-molecular-weight NDM was prepared from this cranberry extract by the method of Zafriri *et al*., 1989. Briefly, cranberry extract was dialysed at 4^{o}C for three days against six changes of water that had been treated through the Barnstead nanopure water system, using Spectrum Spectra/Por 7 dialysis bags (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) with a 15,000-molecular-weight cut-off. The NDM was freeze-dried to a powder, and the resulting extract (CE90) was stored at 4^{o}C in the dark. For each experiment, CE90 was reconstituted in nanopure water the same day of the experiment. Fresh CE90 stock solutions (128 mg ml^{-1}) were filter sterilised with a 0.2 µm nylon syringe filter (Fisher Scientific, Ottawa, ON, Canada) and diluted to the desired concentration (2, 4, 8, 16 mg ml^{-1}) in media. CE90 concentrations were chosen based on the MIC of CE90 for the tested *Staph. aureus* isolates, to analyse the bioactivity of inhibitory and sub-inhibitory concentrations of CE90 against *Staph. aureus*.

3.2 *Staph. aureus* isolates

Twenty-four *Staph. aureus* isolates were used in the *in vitro* evaluation of CE90. These included bovine and poultry isolates and *Staph. aureus* PC1, SA 113, 8325-4, 8325-4 *fnbA*$^{-}$*/fnbB*$^{-}$, 8325-4 *clfA*$^{-}$ and 2076 from the collection at Agriculture and Agri-Food

Canada (Agassiz, BC, Canada), as well as *Staph. aureus* ATCC 25923 and 29213 from the American Type Culture Collection that were used as reference strains. *Staph. aureus* SA 113 and 2076 were chosen as the biofilm positive and negative isolates, respectively, based on the literature (Cucarella *et al*., 2001) and a preliminary study that confirmed a high level of biofilm production by *Staph. aureus* SA 113 and a low level of biofilm production by *Staph. aureus* 2076 (Appendix C). *Staph. aureus* 8325-4, 8325-4 *fnbA*$^-$ */fnbB*$^-$ and 8325-4 *clfA*$^-$ were chosen to test the effect of CE90 on fibronectin binding ability in a strain that possesses fibronectin binding proteins as well as clumping factor A, which allows *Staph. aureus* to bind to fibrinogen, and the two mutant strains that each possess one of these adhesins but not the other. All 24 *Staph. aureus* isolates from the collection at Agriculture and Agri-Food Canada were used in order to evaluate the MIC of CE90 against a range of different *Staph. aureus* isolates. All culture media were from Becton, Dickinson and Company (Sparks, MD, USA). *Staph. aureus* from frozen stock at -80°C in tryptic soy broth (TSB) containing 25% glycerol were streaked onto TSA plates or TSB and then incubated for 18-24 h at 37°C. For most experiments, the isolates were sub-cultured in TSB, on agar plates (TSA), Mueller Hinton agar plates (MHA) or in Mueller Hinton broth (MHB) for an additional 18-24 h.

3.3 Determination of MIC

The MIC of CE90 against 24 *Staph. aureus* isolates was determined by the agar dilution method according to the NCCLS (1999). A 48-pin replicator was sterilized by dipping in 95% ethanol and flaming with a bunsen burner and then cooled by touching the surface of a sterile TSA plate. The sterility of the TSA plate and the replicator pins was

confirmed by incubating the TSA plate for 24 h at 37°C. The replicator was dipped into a 96-well plate containing 100 µl well^{-1} of *Staph. aureus* (1.5 x 10^{8} cfu ml^{-1} in PBS) (one isolate per well). MHA plates containing doubling dilutions of CE90 ranging from 64 mg CE90 ml^{-1} – 1 mg CE90 ml^{-1} were inoculated with approximately 300 colony forming units (cfu) spot^{-1} by touching the replicator to the surface of the agar. Plates were incubated at 37°C, and the MIC of CE90 against each isolate was determined at 24 h. The MIC was defined as the lowest concentration of CE90 that completely inhibited *Staph. aureus* after 24 h incubation at 37°C.

3.4 *Staph. aureus* morphology

The effect of CE90 on *Staph. aureus* morphology was examined by exposing 10 *Staph. aureus* isolates to 0, 4, 8 or 16 mg CE90 ml^{-1} in TSB, TSB + 7.5% NaCl, or TSB + 7.5% EDTA at 37°C. Isolates used were PC1, SA 113, 8325-4, 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$, 8325-4 *clfA*$^{-}$, 2076, SHY97-4320, ABB03-2487, ATCC 6538 and ATCC 29213. Ten µl samples of an isolate suspended in each treatment (CE90 in TSB with or without NaCl or EDTA) were placed on glass slides and covered with a coverslip. The edges of coverslips were sealed onto the glass slides with fingernail polish to reduce the evaporation of samples. Samples of *Staph. aureus* in this CE90-TSB solution were observed at time 0 and after 5, 10, 15, 20 and 60 min incubation at 37°C without shaking. Glass slides were examined by phase contrast microscopy (National 163 PH, Microscope World, Carlsbad, CA, USA) at 400x magnification and digitally photographed.

3.5 Attachment to ECM protein (fibronectin)

Staph. aureus adherence to fibronectin was evaluated by the method described by Dziewanowska *et al*., 1999 using three *Staph. aureus* strains (8325-4 parental strain and two mutant strains, 8325-4 *fnbA*$^-$/*fnbB*$^-$ deficient in FnbpA and FnbpB, and 8325-4 *clfA*$^-$ deficient in ClfA). Briefly, wells in a 96-well microtiter plate (Costar Inc., Corning, NY, USA) were coated with 100 µl of fibronectin solution from bovine plasma (lyophilized powder, cell culture tested, Sigma, Oakville, Ontario, Canada, 50 µg ml^{-1} in HBSS (10 x, liquid, cell culture tested, Sigma) with bivalent ions (0.35 g L^{-1} sodium bicarbonate)) and incubated overnight at room temperature. The wells were then rinsed three times with HBSS with bivalent ions, and residual binding sites were blocked with 100 µl of heat-denatured 0.1% BSA (powder, cell culture tested, Sigma) in HBSS (without bivalent ions) for 60 min at room temperature. The wells were rinsed with TSB containing 0.25% glucose with 0.1% heat-denatured (90^{o}C water bath, 10 min) BSA. *Staph. aureus* were incubated with 0, 4, 8 or 16 mg CE90 ml^{-1} TSB for 2 h at 37^{o}C on an orbital platform shaker at 190 rpm. Samples were centrifuged at 12,850 x *g* for 12 min at 20^{o}C. The *Staph. aureus* pellets were resuspended in PBS and centrifuged at the same settings two more times. Pellets were resuspended in TSB containing 0.25% glucose with 0.1% heat-denatured BSA. Then 100 µl of *Staph. aureus* cell suspension (5×10^5 cfu ml^{-1} in this TSB-glucose-BSA solution) were added to each well. The plates were incubated for 1 h at room temperature and rinsed four times with PBS to remove non-adherent *Staph. aureus*. Adherent *Staph. aureus* were detached from the wells by incubating with 0.25% porcine trypsin in an HBSS with phenol red solution containing 0.02% EDTA (Sigma) for five min at 37^{o}C. Detached *Staph. aureus* were serial diluted in PBS to the 10^{-6}

dilution, and 20 µl were drop plated onto TSA. Plates were incubated at 37°C for 18 – 24 h, and colonies were counted. The results were recorded as the protein adherence index = (cfu ml^{-1} recovered / cfu ml^{-1} added)*100.

3.6 Biofilm formation

The effect of CE90 on biofilm formation in *Staph. aureus* PC1, SA 113, 8325-4 and 2076 was evaluated according to the modified method of Vasudevan *et al.* (2003). TSB containing 0.25% glucose and 0, 2, 4, 8 or 16 mg CE90 ml^{-1} (195 µl) was added to sterile 96-well flat bottom polystyrene tissue culture plates along with 5 µl of inoculum (approximately 1.5 x 10^8 cfu ml^{-1}) and incubated at 37°C for 18 – 24 h. The wells were rinsed three times with sterile PBS to remove planktonic cells, dried at room temperature and stained with 1% (w/v) crystal violet (microscopy grade, Sigma) (200 µl $well^{-1}$) for 15 min at room temperature. After removing the crystal violet and rinsing the wells with distilled water, the plate was thoroughly dried upside down at room temperature, and the absorbance was measured at 570 nm with a microplate reader. *Staph. aureus* SA 113 and *Staph. aureus* 2076 served as the biofilm positive and negative control isolates, respectively.

3.7 Biofilm removal

The effect of CE90 on removal of *Staph. aureus* PC1, SA 113, 8325-4 and 2076 biofilms was evaluated according to the modified method of Vasudevan *et al.* (2003). TSB containing 0.25% glucose (195 µl) was added to sterile 96-well flat bottom polystyrene tissue culture plates along with 5 µl of inoculum (approximately 1.5 x 10^8 cfu ml^{-1}) and

incubated at 37°C for 18 – 24 h. Inoculum was then removed by pipetting and wells were rinsed twice with sterile PBS to remove planktonic cells. Sterile PBS containing CE90 (0, 2, 4, 8 or 16 mg ml^{-1}) were added to each well (200 µl) and left at ambient temperature for another 24 h. The CE90-PBS solutions were removed by pipetting, and wells were rinsed three times with sterile PBS. Wells were dried at room temperature and stained with 1% crystal violet (200 µl $well^{-1}$) for 15 min at room temperature. After removing the crystal violet and rinsing the wells with distilled water three times, the plate was thoroughly dried upside down at room temperature, and the absorbance was measured at 570 nm with a microplate reader. *Staph. aureus* SA 113 and *Staph. aureus* 2076 served as the biofilm positive and negative control isolates, respectively.

3.8 Biofilm index

The amount of *Staph. aureus* PC1, SA 113, 8325-4 and 2076 forming a biofilm versus remaining in a planktonic state during exposure to CE90 over a 24 h period was determined as follows. Sterile 24-well flat bottom polystyrene tissue culture plates (Cellstar TC-Plate with lid, DNase, RNase free) (Greiner Bio-One, Monroe, North Carolina, USA) containing 1.95 ml of TSB with 0.25% glucose and 0, 2, 4, 8 or 16 mg CE90 ml^{-1} were inoculated with 500 µl of *Staph. aureus* broth suspension (approximately 3.0×10^7 cfu ml^{-1}). Tissue culture plates were incubated at 37°C for 2, 4, 6, 8 or 24 h. One 24-well plate was used for each incubation time, with each plate containing every CE90 concentration and the control. At each time interval, the specified tissue culture plate was removed from the incubator, and supernatant was removed from each well and stored at 4°C until the preparation of planktonic *Staph. aureus* serial dilutions (within the

hour). Wells were rinsed three times with sterile PBS to remove unattached *Staph. aureus*. Biofilm was removed by swabbing each well with a dry, sterile cotton swab. Each swab was transferred to 1 ml of 0.85% sterile saline where it was swirled and shaken vigorously to suspend biofilm-associated *Staph. aureus*. Cotton swabs were discarded and *Staph. aureus* suspensions in the tubes were vortexed for one min. Samples of supernatant containing planktonic *Staph. aureus* were removed from the fridge, and planktonic and biofilm *Staph. aureus* were each serial diluted in sterile saline to the 10^{-5} dilution and drop plated (10 µl) onto TSA. Plates were incubated at 37^{o}C for 18 – 24 h, and planktonic and biofilm-associated *Staph. aureus* were enumerated as total planktonic cfu per well or total biofilm cfu per well. The biofilm index was calculated as: Biofilm index = (total cfu biofilm *Staph. aureus* / (total cfu biofilm *Staph. aureus* + total cfu planktonic *Staph. aureus*))*100.

3.9 Salt tolerance assay

The modified method of Tomlinson and Palombo (2005) was used to evaluate the effect of CE90 in terms of the proportion of *Staph. aureus* killed versus injured. Four *Staph. aureus* isolates (PC1, SA 113, 8325-4 and 2076) were used in the assay. This was evaluated by examining the ability of treated *Staph. aureus* to grow on TSA supplemented with 7.5% NaCl. Briefly, *Staph. aureus* (3.0×10^{7} cfu ml^{-1} final) in 96-well polystyrene tissue culture plates were treated for two h with 0, 4, 8 or 16 mg CE90 ml^{-1} in TSB (222 µl final volume) at 37^{o}C with shaking (175 rpm). Samples were centrifuged in 1.5 ml polypropylene microcentrifuge tubes (VWR, Edmonton, Alberta, Canada) with an Eppendorf 5810R centrifuge (VWR) for 10 min at 25,200 x *g* at room

temperature and resuspended in PBS twice to remove CE90. Samples were serial diluted in PBS to the 10^{-6} dilution, drop plated (10 µl) onto TSA and on TSA supplemented with 7.5% NaCl (TSAS) and incubated for 24 h at 37^{o}C. For each *Staph. aureus* isolate and CE90 concentration, growth on either normal (TSA) or salt-containing media (TSAS) was calculated as a percentage of that isolate's growth on TSA without CE90. Percent growth on TSA corresponds to the proportion of live *Staph. aureus*, while percent growth on TSAS corresponds to the proportion of uninjured *Staph. aureus* for each CE90 concentration. The number of *Staph. aureus* presumed to be killed is the difference between the TSA count at 0 mg CE90 ml^{-1} and other concentrations of CE90. The proportion of injured *Staph. aureus* was calculated as the difference between counts on TSA and TSAS for each concentration of CE90.

% surviving = (cfu ml^{-1} on TSA / cfu ml^{-1} on TSA of isolate at 0 mg ml^{-1})*100

% killed = 100 – % surviving

% healthy = (cfu ml^{-1} on TSAS / cfu ml^{-1} on TSA of isolate at 0mg ml^{-1})*100

% injured = % surviving - % healthy

3.10 Statistical analyses

All data on bacterial numbers were log transformed before statistical analysis. For each experiment, the effects of different concentrations of CE90 were assessed on two different days in duplicate (biofilm formation and removal in quadruplicate) and were analysed using the General Linear Model procedure of SAS (Statistical Analysis System Institute Inc., 2000). Biofilm index data was analysed using the repeated measurements

option. Least significant difference was used to separate the means whenever the ANOVA F value was significant. The 0.05 *P*-value was used to declare significance.

4. RESULTS & DISCUSSION

4.1 Determination of MIC

Ten of the 24 *Staph. aureus* isolates were completely inhibited by 16 mg CE90 ml^{-1} after 24 h incubation. The MIC of CE90 against the remaining 14 *Staph. aureus* isolates after 24 h was 32 mg CE90 ml^{-1} (Table 1).

The MIC values found in the current study were lower than the MIC of dried cranberry juice reported by Valentova *et al*. (2007) (179-357 mg ml^{-1}), indicating that CE90 has a greater ability to inhibit *Staph. aureus* growth than the dried cranberry juice used in their study. However, other studies have reported biological activities of cranberry components against bacteria at much lower concentrations. Wu *et al*. (2008) found that 5 µl ml^{-1} of cranberry concentrate caused morphological damage to *Staph. aureus*, *E. coli*, *L. monocytogenes* and *S. typhimurium*, including breakage of cell walls and membranes. The wide range of cranberry concentrations capable of inhibiting bacterial growth may be due to differences in the source and variety of cranberries used between studies, variations in the susceptibility to cranberry compounds between different bacteria, as well as differences in experimental methods used to measure growth inhibition.

4.2 *Staph. aureus* morphology

An examination of *Staph. aureus* morphology after treatment with CE90 permitted visualization of the effect on *Staph. aureus*. *Staph. aureus* treated with CE90 (4 – 16 mg ml^{-1}) appeared as relatively large, dose-dependent clumps of *Staph. aureus* for all 10 test isolates (PC1, SA 113, 8325-4, 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$, 8325-4 *clfA*$^{-}$, 2076, SHY97-4320,

ABB03-2487, ATCC 6538 and ATCC 29213) at each time interval tested (0, 5, 10, 15, 20 and 60 min exposure to CE90) (Figure 1a,b,c,d). The same phenomenon was found with *Staph. aureus* incubated in CE90-TSB solutions containing either 7.5% NaCl or 7.5% EDTA. There were no observable differences in *Staph. aureus* clumping in response to CE90 with the presence or absence of NaCl or EDTA. No clumping was found in the 0 mg ml^{-1} control for any isolate.

Staph. aureus clumping in the presence and absence of either NaCl or EDTA suggests that the observed clumping effect is not due to electrostatic interactions or to positive cations. The lack of clumping in the 0 mg ml^{-1} control suggests that the clumping was not due to autoaggregation of *Staph. aureus*.

To the author's knowledge, the dose-dependent clumping observed upon exposure of *Staph. aureus* to CE90 has not been previously reported for CE90 or other cranberry components. However there have been several studies that report a morphological change of bacteria after treatment with cranberry products. Matsushima *et al*. (2008) found that *H. pylori* treated with cranberry extract changed from a spiral-shaped bacterium into a coccoid form, while Ahuja *et al*. (1998) showed that P-fimbriated *E. coli* grown on cranberry agar had an elongated morphology compared to the control. Wu *et al*. (2008) found that *S. aureus*, *E. coli*, *S. typhimurium* and *L. monocytogenes* treated with cranberry concentrate experienced breakage of cell walls and membranes, with lysate and cytoplasmic material surrounding bacterial cells.

The clumping effect may help to explain the ability of cranberry to reduce the incidence of UTIs. Large clumps of causative bacteria such as *E. coli* would have greater mass and therefore momentum when travelling along the urinary tract, and therefore likely would not attach to uroepithelial cells as easily as single bacteria in the absence of cranberry.

The tendency of *Staph. aureus* to agglutinate to form increasingly larger and therefore fewer clumps with increasing concentrations of CE90 has important implications for the interpretation of the results in the present study. Experiments using colony counts to determine the effect of CE90 on *Staph. aureus* may give misleading results, if one fails to account for the fact that a clump of bacteria will give rise to only a single colony, which is indistinguishable from a colony formed from a single cell. Since the clumping effect was not discovered until after the other experiments had already been performed, it was impossible to determine, after the fact, the extent to which clumping was responsible for a reduction in colony counts, versus the extent to which CE90 was having a real effect. The implications for the interpretation of results for each experiment will be discussed in more detail below.

4.3 Attachment to ECM protein (fibronectin)

Considering the role of fibronectin-binding proteins in bacterial adhesion to cultured mammalian cells, airway epithelium, endothelium, biomaterials and intravascular catheters (Menzies, 2003), and the findings that cranberry can reduce the incidence of UTIs (Howell, 2002; Gormley, 2003) and inhibit bacterial binding to cultured uroepithelial (Turner *et al*., 2005) and gastric cells, erythrocytes and immobilized human

mucus (Burger *et al*., 2002), I examined the impact of CE90 on *Staph. aureus* adhesion to fibronectin-coated plates, using a strain deficient in FnbpA and FnbpB, a strain deficient in ClfA, and the parental strain (8325-4). CE90 (4 – 16 mg ml^{-1}) did not show any significant effect ($P > 0.05$) on fibronectin binding in these strains of *Staph. aureus*, with the exception of the fibronectin-binding protein double-mutant strain *fnbA*$^{-}$/*fnbB*$^{-}$, which showed less fibronectin binding in the 4 mg CE90 ml^{-1} concentration than either the untreated control or the higher CE90 concentrations (8 – 16 mg ml^{-1}) ($P < 0.05$) (Figure 2). The lack of any significant effect on *Staph. aureus* binding to fibronectin seems to support the conclusion that the anti-adhesion activity of CE90 was not due to interference with fibronectin binding. This would make sense if the anti-adhesion activity was due to *Staph. aureus* clumping rather than any effect on *Staph. aureus* fibronectin adhesins, in particular. However, the fact that CE90 causes *Staph. aureus* to clump complicates the interpretation of these results. Rather than having no effect, it is possible that CE90 inhibited *Staph. aureus* from binding to fibronectin, but that the few *Staph. aureus* that were able to bind were associated with a large clump of *Staph. aureus*. Since *Staph. aureus* that had bound to fibronectin were detached with a 0.25% trypsin-EDTA solution before enumeration, trypsin may also break the cell to cell adhesions within the clumps, if trypsin would digest *Staph. aureus* proteins that interact either with CE90 or with other *Staph. aureus* proteins that promote cell to cell adhesion, such as extracellular adherence protein or intercellular adhesin. Therefore, when serial dilutions and colony counts were made, the number of colonies formed would be much greater than the number of *Staph. aureus* that had actually bound to fibronectin. In this case, the ability of CE90 to inhibit *Staph. aureus* from binding to fibronectin would be masked by the clumping effect. It is

impossible to determine from this study whether the lack of significant differences was due to CE90 having no effect on fibronectin binding, or to inhibition of fibronectin binding that was masked by the clumping effect. Furthermore, CE90 could inhibit *Staph. aureus* binding to fibronectin as a consequence of clumping, in the sense that clumping would cause many of the *Staph. aureus* to be physically held apart from the fibronectin. Even if the number of *Staph. aureus* that had bound to fibronectin could somehow be determined, this still would not reveal whether the *Staph. aureus* within the clumps would have been able to bind to fibronectin, if they had been able to come in contact with it. Further research should be done to clarify the effect of CE90 on fibronectin binding, apart from the clumping effect. There were no significant differences in fibronectin binding between strains ($P > 0.05$) (Figure 2). It is unclear why the *fnbA*$^{-}$*/fnbB*$^{-}$ strain has as much fibronectin binding as the parental strain. Further research should be done to clarify the reasons for this finding.

Several studies show that cranberry products have an effect on bacterial adhesion. Labrecque *et al.* (2006) found that 50 μg ml^{-1} of cranberry NDM inhibited *Porphyromonas gingivalis* adhesion to surfaces coated with collagen, fibrinogen or human serum. Koo *et al.* (2006) reported that treating saliva- or glucan-coated hydroxyapatite with cranberry juice resulted in a 40 – 85% reduction in adherence of *Streptococcus mutans* compared to the control. There was no pH effect reported in that study. Yet when *S. mutans* rather than hydroxyapatite was treated with cranberry juice, bacteria retained their ability to attach to these untreated surfaces. This finding suggests that cranberry compounds may have an anti-adhesive mechanism apart from directly

affecting bacterial adhesins. Eydelnant and Tufenkji (2008) found that there was less attachment of uropathogenic bacteria to biomaterials that urinary catheters are made from when either the bacteria or biomaterials were coated with high-molecular-weight PACs derived from cranberry. They found similar results for non-biological latex microspheres substituted for bacteria, which suggests a non-biospecific anti-adhesion mechanism of cranberry PACs. This supports the suggestion that the anti-adhesion activity of CE90 may be due to physical clumping of *Staph. aureus*. The results of Eydelnant and Tufenkji (2008) did not correlate with theoretical predictions of adhesion based on the sum of the predominant physicochemical forces between the bacteria and a solid surface. However, a model accounting for steric hindrance provided theoretical predictions of adhesion that agreed with experimental results. Therefore the anti-adhesion activity of cranberry PACs may be at least partially explained by steric interactions.

Furthermore, several studies support the hypothesis that cranberry compounds exert an effect on bacterial fimbriae as the mechanism of inhibiting bacterial adhesion. Liu *et al.* (2006) demonstrated that P-fimbriated *E. coli* exposed to cranberry juice adjusted to pH 7 have shorter fimbriae and higher densities of biopolymer around the bacterial cell than the control, as measured by atomic force microscopy (AFM). They postulated this was due to cranberry juice inducing a conformational change in the fimbriae, causing them to go from an extended to a compressed state. These changes were completely reversible when the cranberry juice was replaced with water. Increasing concentrations of cranberry juice were correlated with lower adhesion forces between the P-fimbriated *E. coli* and the AFM silicon nitride tip. Liu *et al.* (2006) hypothesized that the cranberry

compounds responsible for inhibiting bacterial adhesion were hydrophilic PACs, so their binding to the hydrophobic fimbriae decreased adhesion between the fimbriae and the non-polar AFM tip. This hypothesis was supported by Yamanaka *et al*. (2004), who found that cranberry juice reduced the adhesion and biofilm formation, as well as the cell surface hydrophobicity of oral streptococci, presumably by binding to or masking hydrophobic cell surface proteins. The effects of cranberry on bacterial fimbriae length, biopolymer density and adhesion found by Liu *et al*. (2006) were not seen with non-fimbriated bacteria. The mass of surface biomolecules in their study was constant between treated and control bacteria, suggesting that cranberry juice does not cause removal of fimbriae, and that a loss of fimbriae is not required for inhibiting bacterial adhesion. Conversely, Ahuja *et al.* (1998) found that treatment with cranberry juice resulted in the loss of *E. coli* P- and Type 1 fimbriae, as well as decreased adhesion. This apparent discrepancy may be due to the fact that the study conducted by Liu *et al.* (2006) did not allow sufficient time for bacterial growth to occur, whereas Ahuja *et al.* (1998) used four subsequent 48 h incubations. A plausible explanation could be that cranberry does not cause fimbriae removal in mature bacteria, but may affect growing bacteria by down-regulating genes involved in fimbriae expression. Lin *et al*. (manuscript in preparation; as cited in Johnson *et al*., 2008) performed gene expression analysis on *E. coli* grown in 10% cranberry juice. They found that flagellar basal body rod and motor proteins were down regulated compared to the control. In support of these genotypic results, scanning electron microscope images showed fewer visible P-fimbriae for *E. coli* grown in media containing cranberry PACs than *E. coli* grown in media without cranberry components (Johnson *et al*., 2008).

The fact that the results of the present study did not corroborate those of Liu *et al*. (2006) and Ahuja *et al*. (1998), who demonstrated the effect of cranberry on bacterial fimbriae, may be due to the choice of *Staph. aureus* as the test bacteria, rather than *E. coli* possessing P- or Type 1 fimbriae. Perhaps the effect that cranberry has on these fimbriae was not apparent in the current study because *Staph. aureus* has different adhesins that may not be affected in the same way.

Taken together, these results suggest that the anti-adhesive activity of cranberry components is likely due to several different mechanisms of action. Cranberry compounds may affect fimbrial structure and therefore, adhesive ability; alternatively, cranberry components may result in the loss of bacterial fimbriae or may prevent their expression. Bacterial strain or length of exposure may determine the predominant mechanism. The finding that cranberry PACs inhibit the adhesion of latex microspheres to biomaterials indicates an additional non-biological mechanism of action, perhaps related to steric hindrance. Certain bacterial strains may not be affected by the anti-adhesion activity of cranberry compounds, possibly due to different adhesins that are not affected in the same way. Although CE90 did not demonstate a significant effect on *Staph. aureus* adhesion to fibronectin, it may have shown an effect on *Staph. aureus* adhesion had this been examined with different ECM proteins or surfaces. Further research should be done to analyse the effect of CE90 on *Staph. aureus* adhesion and the initial attachment stage of biofilm formation.

4.4 Biofilm formation, removal & index

The ability of biofilm-associated bacteria to resist antibiotic treatment and contribute to persistence of infection, and the results of previous research indicating that cranberry can inhibit biofilm formation, made the potential for CE90 to inhibit biofilm formation or remove pre-existing biofilm an important question to address.

4.4.1 Biofilm formation

There was a significant interaction between isolate and CE90 concentration ($P < 0.05$) (Figure 3), indicating that the CE90 concentrations had a different effect on the biofilm formation of different isolates. *Staph. aureus* PC 1 and 8325-4 produced similar amounts of biofilm in the 0 mg CE90 ml^{-1} control (OD 0.813 and 0.727, respectively) (Figure 3), and both these isolates produced more biofilm with low levels of CE90 (2 mg ml^{-1}) compared to the control ($P > 0.05$) and higher levels of CE90 (8 and 16 mg ml^{-1}) ($P < 0.05$) (Figure 3). Biofilm formation by *Staph. aureus* SA 113 was not stimulated by any concentration of CE90 compared to the control, and this isolate had the largest decrease in biofilm formation with CE90 treatment compared to the control (OD 2.21 difference between 0 and 16 mg CE90 ml^{-1}) (Figure 3). The varying effects of CE90 treatments on the biofilm formation of different isolates may be due to potentially different genotypic or phenotypic characteristics of the isolates.

CE90 treatments had a significant effect on *Staph. aureus* biofilm formation, showing a general trend of less biofilm formation at higher concentrations of CE90, which is supported by the finding that cranberry compounds inhibit biofilm production by

uropathogens such as *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Reid *et al.*, 2001) and oral pathogens such as streptococci (Yamanaka *et al*., 2004; Steinberg *et al.*, 2004, 2005; Duarte *et al.*, 2006; Koo *et al.*, 2006), *P. gingivalis* (Labrecque *et al*., 2006) and synergistic *P. gingivalis* and *Fusobacterium nucleatum* (Yamanaka *et al*., 2007), as well as the activity of enzymes involved in biofilm formation (Steinberg *et al.*, 2004; Duarte *et al.*, 2006; Koo *et al.*, 2006). The clumping effect may contribute to the inhibition of biofilm formation, as the large, seemingly unorganized clumps of *Staph. aureus* may be less capable of forming biofilm because biofilms are complex, organized structures of bacteria, which would be difficult to develop with large bacterial clumps randomly packed together.

Staph. aureus PC 1 and SA 113 formed more biofilm in the presence of CE90 than *Staph. aureus* 8325-4 and 2076 ($P < 0.05$) (Figure 3). The greater amount of biofilm formed by *Staph. aureus* SA 113 than *Staph. aureus* 2076 is in accordance with my expectations, as these isolates were used as the biofilm positive and negative control isolates, respectively. Further research should be performed to analyse the effect of CE90 on additional *Staph. aureus* isolates, as the varying effects of CE90 on these four isolates show that isolates react differently to the presence of CE90.

4.4.2 Biofilm removal

There was a significant interaction between isolate and CE90 concentration ($P < 0.05$) (Figure 4), indicating that CE90 concentrations had specific effects on the biofilm removal of different *Staph. aureus* isolates. The most noticeable difference in the effect

of CE90 between different isolates is that *Staph. aureus* SA 113 showed greater levels of residual biofilm after exposure to CE90 (2, 4, 8 and 16 mg ml^{-1}) compared to the control ($P < 0.05$) (Figure 4). The varying effects of CE90 concentrations on the biofilm removal of different isolates may be due to either genotypic or phenotypic differences between the isolates.

CE90 concentrations had a significant effect on *Staph. aureus* biofilm removal, with the exception of PC1. However, the only statistically significant differences between the untreated control biofilms and those exposed to CE90 were that the latter had greater levels of residual biofilm than the untreated controls for some isolates of *Staph. aureus* ($P < 0.05$) (Figure 4). These results show that CE90 was not effective in removing pre-existing biofilm. Steinberg *et al.* (2005) found that cranberry NDM (2 mg ml^{-1}) promoted desorption of *S. sobrinus* from biofilm; however this difference from the results of the current study may be due to the difference in bacterial species, cranberry extract, experimental methods or method of analysis. Labrecque *et al.* (2006) reported that *P. gingivalis* biofilm was not desorbed by treatment with cranberry NDM, though they used a much lower concentration (0.25 mg ml^{-1}) and a different bacterial species. The finding that exposure to CE90 results in greater levels of residual biofilm for certain isolates (SA 113) compared to the control suggests that further research should be done to determine if this is a common phenomenon, since this could have negative implications for the use of CE90 in many applications.

There was a significant difference in the amount of biofilm remaining after exposure to CE90 between each isolate (SA 113 > 8325-4 > PC1 > 2076) ($P < 0.05$) (Figure 4). The greater amount of residual biofilm in *Staph. aureus* SA 113 compared to *Staph. aureus* 2076 supports the biofilm formation results and the choice of these isolates as the biofilm positive and negative control isolates.

4.4.3 Biofilm index

There was a significant interaction between bacterial isolate and CE90 concentration at 8 and 24 h ($P < 0.05$) (Table 2), indicating that CE90 concentration had specific effects on the biofilm index of different *Staph. aureus* isolates at these time intervals. At 8 h, *Staph. aureus* PC1 and SA 113 showed a higher biofilm index at 2, 4 and 8 mg ml^{-1} compared to 0 and 16 mg ml^{-1}, whereas *Staph. aureus* 8325-4 showed no significant difference in biofilm index between CE90 concentrations at this time interval (Figure 5d). At 24 h, the different effects of CE90 among *Staph. aureus* isolates were more pronounced. For *Staph. aureus* PC1, all the CE90 concentrations showed a lower biofilm index than the 0 mg ml^{-1} control, and the 8 mg ml^{-1} treatment resulted in a lower biofilm index than the 16 mg ml^{-1} treatment ($P < 0.05$) (Figure 5e). For *Staph. aureus* SA 113, the 2, 8 and 16 mg ml^{-1} treatments resulted in a lower biofilm index than the 0 mg ml^{-1} control, the 8 mg ml^{-1} resulted in a lower biofilm index than the 4 mg ml^{-1} treatment, and the 16 mg ml^{-1} treatment showed a lower biofilm index than all the CE90 concentrations ($P < 0.05$) (Figure 5e). For *Staph. aureus* 8325-4, all the CE90 concentrations had a decreased biofilm index compared to the control, and the 8 mg ml^{-1} treatment resulted in a higher biofilm index compared to the 4 and 16 mg ml^{-1} treatments ($P < 0.05$) (Figure 5e).

The finding that CE90 concentration had a different effect between *Staph. aureus* as time progressed may be a result of the *Staph. aureus* isolates becoming more established over time. As bacteria mature, isolates may become more differentiated from each other regarding their gene expression and cell surface proteins, which may result in the tested CE90 concentrations having a wider range of effects than with less mature bacterial populations.

Staph. aureus PC1 and SA 113 also showed a significant interaction between time and concentration of CE90 ($P < 0.05$) (Table 3), indicating that the concentration of CE90 had specific effects on these bacteria at different time intervals. These isolates tended to have a higher biofilm index at 2, 4 and 8 mg ml^{-1} than at 0 and 16 mg ml^{-1} from 2 h to 8 h (Figure 5a, b, c, d). However, by 24 h, all of the CE90 concentrations resulted in a lower biofilm index than the 0 mg ml^{-1} control for both isolates ($P < 0.05$; SA 113 at 4 mg ml^{-1}, $P > 0.05$) (Figure 5e).

The finding that low to medium concentrations of CE90 resulted in an initial increase in biofilm index does not support the claim that cranberry products inhibit biofilm formation (Reid *et al.*, 2001; Yamanaka *et al*., 2004; Steinberg *et al.*, 2004, 2005; Duarte *et al.*, 2006; Koo *et al.*, 2006; Labrecque *et al*., 2006; Yamanaka *et al*., 2007). However, the finding that by 24 h each concentration of CE90 decreased the biofilm index compared to the 0 mg ml^{-1} control does support this claim as well as the optical density measurements that showed inhibition of biofilm formation at higher concentrations of

CE90 over 24 h. The finding that the concentration of CE90 had a different effect on *Staph. aureus* PC1 and SA 113 biofilm index at different time intervals may be related to the above explanation that bacteria express different genes and produce different proteins as they mature. This may result in CE90 having a different effect on biofilm index over time.

Biofilm index values were similar between isolates at each time interval, with slightly higher values for *Staph. aureus* SA 113 at 6 and 24 h (Figure 5c,e) ($P < 0.05$). This may be explained by the naturally high levels of biofilm produced by this isolate.

Staph. aureus SA 113 at 24 h showed the greatest reduction in biofilm index with increasing CE90 concentration, from 65% at 0 mg CE90 ml^{-1} to 44% at 16 mg CE90 ml^{-1} (Figure 5e) ($P < 0.05$). This 20% reduction in biofilm index is significant, considering the high levels of biofilm produced by *Staph. aureus* SA 113.

4.5 Salt tolerance assay

The question of whether CE90 has a bactericidal effect on *Staph. aureus* or simply injures these bacteria was important to address, for a number of reasons. First of all, if CE90 is able to prevent infections by inhibiting *Staph. aureus* adherence or otherwise injuring *Staph. aureus* without actually killing the bacteria, the development of *Staph. aureus* resistance to CE90 would be less likely than if CE90 has a bactericidal effect. In the latter case there would be a selective pressure for *Staph. aureus* that could survive in the presence of CE90, and resistance may develop as it has for antibiotics. However, if

the effect of CE90 on *Staph. aureus* is not bactericidal this selective pressure would not exist and therefore resistance to CE90 would be unlikely.

Another reason for addressing this question is that bacteria present in foods often suffer sublethal injuries (Hernandez *et al*., 1993), which prevent them from growing in selective media used to detect bacteria in foods. However, these injured bacteria may eventually recover under appropriate environmental conditions (Hernandez *et al*., 1993), such as the food being left at room temperature to thaw. The resulting potential for high levels of undetected food-associated bacteria to cause foodborne illness poses a serious public health risk (Hernandez *et al*., 1993). It would be beneficial to know whether CE90 has a similar effect of injuring *Staph. aureus* that may recover at a later stage to cause illness.

Bacteria that have sustained damage to their cell membranes may become more permeable and are unable to maintain an appropriate osmotic pressure or to exclude toxic materials (Carson *et al*., 2002). The resulting loss of salt tolerance is suggestive of membrane damage in sublethally injured *Staph. aureus* (Iandolo and Ordal, 1966; Carson *et al*., 2002). There was a dose-dependent decrease in *Staph. aureus* viability on both TSA and TSAS. Isolate PC1 formed fewer colonies on TSA at the higher CE90 concentrations (8 and 16 mg ml^{-1}) than in the absence of CE90 (control) (Figure 6a) ($P < 0.05$). There was also a significant reduction in colonies formed by PC1 on TSAS at all CE90 concentrations (4, 8 and 16 mg ml^{-1}) compared to the control ($P < 0.05$), as well as at 16 mg ml^{-1} compared to 4 mg ml^{-1} ($P < 0.05$) (Figure 6a). *Staph. aureus* SA 113 on TSA and TSAS, and *Staph. aureus* 8325-4 on TSA, formed fewer colonies at 16 mg ml^{-1}

than at 4 and 8 mg ml^{-1} ($P < 0.05$), and fewer colonies at 4 and 8 mg ml^{-1} than the control ($P < 0.05$) (Figure 6b,c). *Staph. aureus* 8325-4 showed a decrease in viability on TSAS at 16 mg ml^{-1} compared to 0 and 4 mg ml^{-1} ($P < 0.05$), and at 8 mg ml^{-1} compared to 0 mg ml^{-1} ($P < 0.05$) (Figure 6c). There was a significant reduction in colonies formed by isolate 2076 on TSA and TSAS at 16 mg ml^{-1} compared to 0 and 4 mg ml^{-1} ($P < 0.05$), and at 4 and 8 mg ml^{-1} compared to 0 mg ml^{-1} ($P < 0.05$) (Figure 6d). For a particular concentration, *Staph. aureus* counts were lower on TSAS than on TSA, with the exception of the controls without CE90, and *Staph. aureus* 8325-4 at the 4 mg CE90 ml^{-1} treatment (Figure 6a,b,c,d). However this difference between media was significant only for PC1 at 4 and 16 mg ml^{-1}, 8325-4 at 8 mg ml^{-1}, and 2076 at 16 mg ml^{-1} CE90 ($P < 0.05$) (Figure 6a,c and d, respectively). There were no significant interactions between isolate and CE90 concentration ($P > 0.05$).

Based on the assumption that reduced viability on TSA and TSAS corresponds to *Staph. aureus* death and injury, these results suggest that CE90 not only injures *Staph. aureus* but also has a bactericidal effect. In addition, the ability of CE90 to increase *Staph. aureus* sensitivity to NaCl, which is used as a food preservative, suggests that CE90 may have the potential to be used as an additional barrier in hurdle technology to minimize foodborne illness and food spoilage.

However, the interpretation of these results as an indication of *Staph. aureus* death and injury may need to be re-examined in light of the effect of CE90 on *Staph. aureus* morphology. The dose-dependent *Staph. aureus* clumping effect observed after

treatment with CE90 means that even if CE90 neither injures nor kills *Staph. aureus*, we would still expect to see a dose-dependent reduction in cfu on TSA and TSAS, since each clump of bacteria gives rise to only one colony. In that case the reduced cfu on TSA and TSAS should be interpreted not as an indication of *Staph. aureus* death or injury, but merely as the result of *Staph. aureus* clumping in response to treatment with CE90. The fact that most samples of the biofilm index experiment did not show a significant reduction in either planktonic- or biofilm-associated *Staph. aureus* viability on TSA after treatment with CE90 over 24 h (Appendix D.1 – D.8) suggests that CE90 does not have any significant bactericidal effect on *Staph. aureus*. Yet the difference in *Staph. aureus* counts between TSA and TSAS (Figure 6a,b,c,d) suggests that there is a certain amount of injury taking place, and therefore the clumping effect is not solely responsible for the reduced counts. It may be likely that the reduction in colony forming units is partially explained by *Staph. aureus* clumping, but that CE90 injures and possibly kills *Staph. aureus*, as well. Further studies are required to separate this confounding variable in order to elucidate the effect of CE90 on *Staph. aureus* health and survival. To the author's knowledge there are no previously published studies examining the effect of cranberry or cranberry extracts on the salt tolerance of *Staph. aureus*.

5. CONCLUSION

The concentration of CE90 for practical applications should be carefully selected, as certain isolates may actually produce more biofilm in response to low concentrations of CE90, or they may produce biofilm that is more resistant to removal by rinsing after exposure to CE90 than in the absence of CE90. In addition, the observed variations between isolates in the effect of CE90 means that we can't generalize among all isolates of *Staph. aureus*, as some isolates react differently to CE90 than others.

Staph. aureus PC1, SA 113, 8325-4 and 2076 showed decreased salt tolerance with increasing CE90 concentration. This increase in bacterial sensitivity to salt may have potential applications to food preservation. However further research must be done to determine whether the salt tolerance results are a true reflection of *Staph. aureus* injury and killing, or whether they were a result of clumping. If salt tolerance results were due to *Staph. aureus* clumping in response to treatment with CE90, the clumps may break apart either in the food or within the host after food consumption.

The results of this study show that the clumping effect of CE90 has important implications for the microbiological analysis of cranberry products. Since bacterial counts of samples plated onto agar are a measure of the colony forming units, if large clumps of bacteria are plated, each clump would give rise to only one colony. This would make it appear as though the bacterial counts had decreased, even if the number of viable bacteria remains the same.

In summary, CE90 has the potential to cause clumping, reduce biofilm formation (8, 16 mg ml^{-1}) and decrease salt tolerance of *Staph. aureus* PC1, SA 113, 8325-4 and 2076. CE90 was not effective at removing pre-formed biofilm for these four isolates, and even resulted in higher levels of residual biofilm than the control for *Staph. aureus* SA 113 and 2076. CE90 did not affect *Staph. aureus* 8325-4, 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$ or 8325-4 *clfA*$^{-}$ fibronectin binding, except for 8325-4 *fnbA*$^{-}$/*fnbB*$^{-}$ at 4 mg ml^{-1}, which showed less fibronectin binding than the control.

6. RECOMMENDATIONS FOR FURTHER RESEARCH

A-type PACs are also found in cinnamon, curry, peanuts, plums and avocados (Gu *et al*., 2003). Additional research should be done to determine whether these alternative sources of A-type PACs also have anti-adhesion activity (Howell *et al*., 2005). The level of A-type versus B-type PACs present in white cranberries should be analysed, and further research could be done to confirm or contradict the findings of Johnson-White *et al*. (2006), which claimed that white cranberry juice did not possess anti-adhesion activity. Different sources of cranberry compounds and various methods of extraction and preparation, which would result in different compositions and purities of extracts, should be evaluated.

The effect of CE90 on the normal flora of humans and animals, and other beneficial bacteria such as probiotics, should be studied. Similarly, the effect of CE90 on multi-drug resistant bacteria such as MRSA and VRE should be examined.

The extent to which different concentrations of CE90 result in *Staph. aureus* clumping could be quantified and statistically analysed. The viability of clumps could be confirmed by staining *Staph. aureus* that had been exposed to CE90 with Live/Dead staining and examining the clumps with a microscope. Additionally, further studies could determine whether clumps remain stable over time and in different environments, or whether they tend to break apart.

Further studies could examine the effect of CE90 on *Staph. aureus* adhesion to additional ECM proteins besides fibronectin, such as fibrinogen and collagen, and to epithelial cells.

The observation among the biofilm formation, removal and biofilm index experiments in the present study that low to medium concentrations of CE90 result in higher levels of biofilm with specific isolates requires further study since enhanced biofilm formation and diminished biofilm removal may have negative consequences. Studies should be performed to examine the effect of CE90 on quorum sensing and the genetic regulation of biofilm formation. Different methods of quantifying the biofilm index could be tested to ensure the most accurate enumeration of planktonic and biofilm bacteria. Using a sterile spatula or cell scraper to remove biofilm might result in a more accurate measurement, as bacteria may be washed away from these instruments into saline more easily than from a cotton swab. Alternatively, saline could be added to the biofilm coated wells, and biofilm-associated bacteria could be suspended by sonication or glass beads.

The effect of CE90 on the survival and health of *Staph. aureus* could be clarified by repeating the experiment and examining samples under a microscope to determine the extent of clumping. This information, along with an assessment of viability using Live/Dead staining, could assist in estimating whether the reduced colony counts are primarily due to *Staph. aureus* injury and killing, or are simply a result of bacterial clumping. Alternatively, an attempt could be made to break apart the clumps before plating onto TSA and TSAS, perhaps by adding trypsin or sonicating the samples.

Preliminary experiments would need to be performed to determine the effectiveness of trypsin or sonication in breaking apart the clumps without negatively affecting viability.

The *in vitro* nature of these experiments means that the results cannot be extrapolated *in vivo* to provide an indication of the effect of CE90 in a human or animal model. Results are limited to situations where CE90 has direct contact with bacteria, since the complex environment of a living system and the effects of digestion and absorption on the bioactivity of CE90 is unknown. Testing the effect of CE90 on bacteria incubated with cell lines, and eventually on bacteria within animal models or human subjects would provide an indication of the effect of CE90 *in vivo*. For example the ability of CE90 to reduce the risk of UTIs may be a valuable research question to address. It may be of interest to determine whether CE90 can control sub-clinical infections and help promote growth and feed efficiency in farm animals. If successful, this may reduce the reliance on feed supplemented with antibiotics by providing the agricultural industry with a more natural alternative that produces minimal, if any, selective pressure for resistance (Howell and Foxman, 2002; Eydelnant and Tufenkji, 2008; McMurdo *et al*., 2009).

Studies should be done to verify the belief that bacteria do not develop resistance to cranberry compounds. The sites of action, effective dose, and structure-activity relationships of CE90 are also important research questions to address.

7. TABLES

Table 1: MICs of CE90 in mg ml^{-1} against 24 different isolates of *Staph. aureus*

Isolate	MIC (mg CE90 ml^{-1})	Origin	Reference
SHY97-3906	32	1*	Diarra, 2002
SHY97-3923-3	32	1*	Diarra, 2002
SHY97-4085	32	2*	
SHY97-4242	32	2*	
SHY97-4320	32	1*	Diarra, 2002
SHY97-4343	16	1*	Diarra, 2002
ATCC 25923	16	3	Diarra, 2002
PC1	16	4	Diarra, 2002
NCTC 9789	32	4	Diarra, 2002
2076	32	4	Diarra, 2002
22260	32	4	Diarra, 2002
ST79/741	32	4	Diarra, 2002
3804 (pII)	32	4	Diarra, 2002
RN9	32	4	Diarra, 2002
FAR 8	32	4	Diarra, 2002
FAR 10	16	4	Diarra, 2002
3906 PGR	32	2	
SA 113	16	5	
8325-4	16	6	
8325-4 *fnbA*$^-$/*fnbB*$^-$	16	6	
8325-4 *clfA*$^-$	16	6	
SHY99-730-2	32	2*	
MRSA	16	7	
ATCC 29213	16	3	Diarra, 2002

1 = Laboratoire Provincial de Pathologie Animale of St-Hyacinthe (QC, Canada)
2 = Dr Diarra (Agassiz, BC, Canada)
3 = American Type Culture Collection (ATCC) (Manassas, VA, USA)
4 = Vanderbilt University School of Medicine (Nashville, TN, USA) (beta-lactam antibiotic-resistant)
5 = Pharmacia
6 = Dr Foster (Ontario, Canada)
7 = Serge Messier (University of Montreal) (St-Hyacinthe, QC, Canada)
* = clinical bovine mastitis

Table 2: Interactions between CE90 concentrations and isolate (*Staph. aureus* PC1, SA 113, 8325-4 and 2076) at different time intervals[1.]

Time	Concentration*Isolate
2 h	$P > 0.05$
4 h	$P > 0.05$
6 h	$P > 0.05$
8 h	$P < 0.05$
24 h	$P < 0.05$

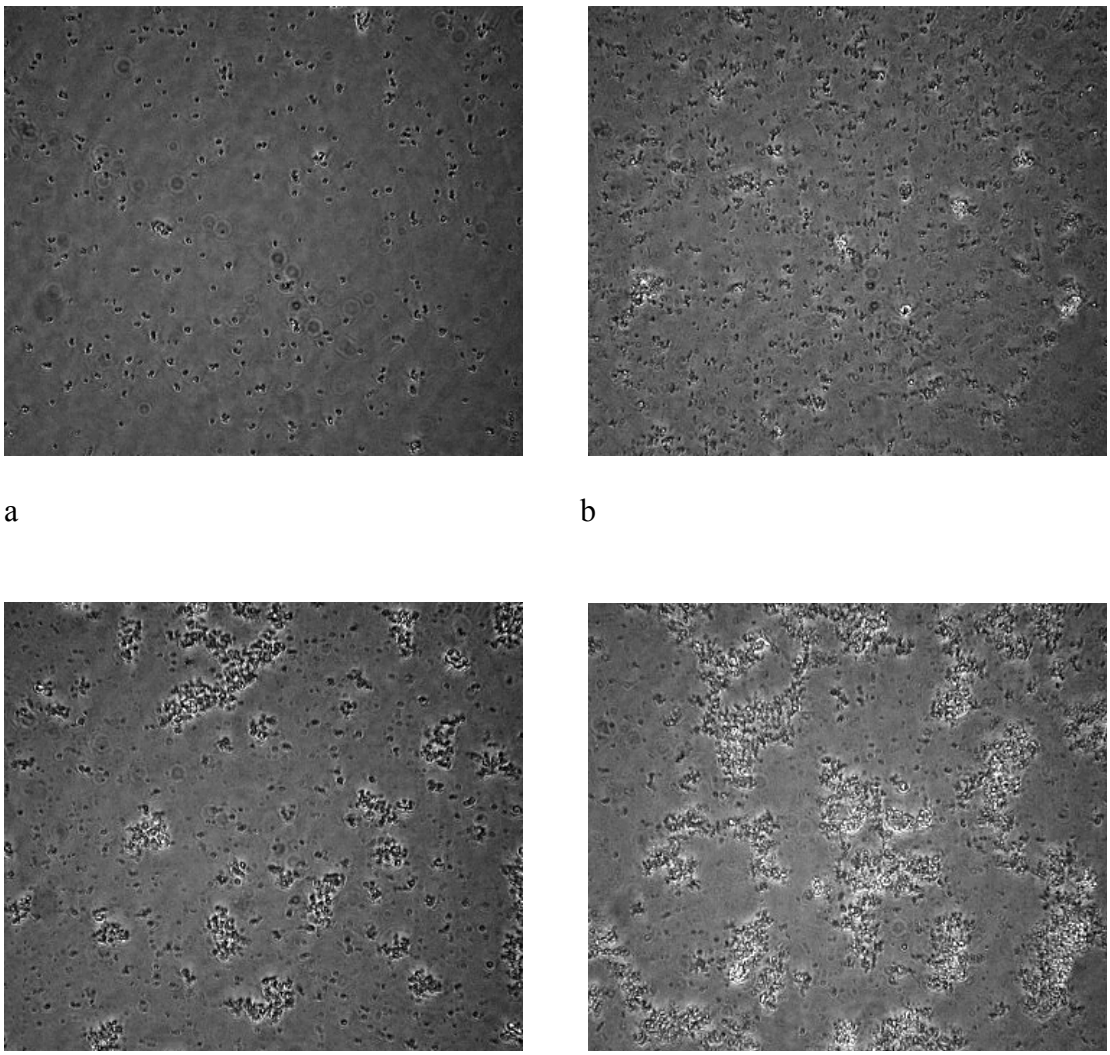
[1.] Analysis is a two-way ANOVA of the biofilm index from two separate experiments performed in duplicate.

Table 3: Interactions between CE90 concentrations and time for different *Staph. aureus* isolates[1.]

Isolate	Concentration*Time
PC1	$P < 0.05$
SA 113	$P < 0.05$
8325-4	$P > 0.05$

[1.] Analysis is a two-way ANOVA of the biofilm index from two separate experiments performed in duplicate.

8. FIGURES



a b

c d

Figure 1: Effect of CE90 on *Staph. aureus* PC1 morphology viewed through a phase contrast microscope: formation of *Staph. aureus* clumps (magnification: 400x). Image processing steps used: red channel selected; unsharp mask (3x) (Adobe Photoshop 5.5) (Russ, J. C. 2007).

a: 0 mg CE90 ml^{-1}, 5 min exposure to CE90
b: 4 mg CE90 ml^{-1}, 5 min exposure to CE90
c: 8 mg CE90 ml^{-1}, 5 min exposure to CE90
d: 16 mg CE90 ml^{-1}, 5 min exposure to CE90

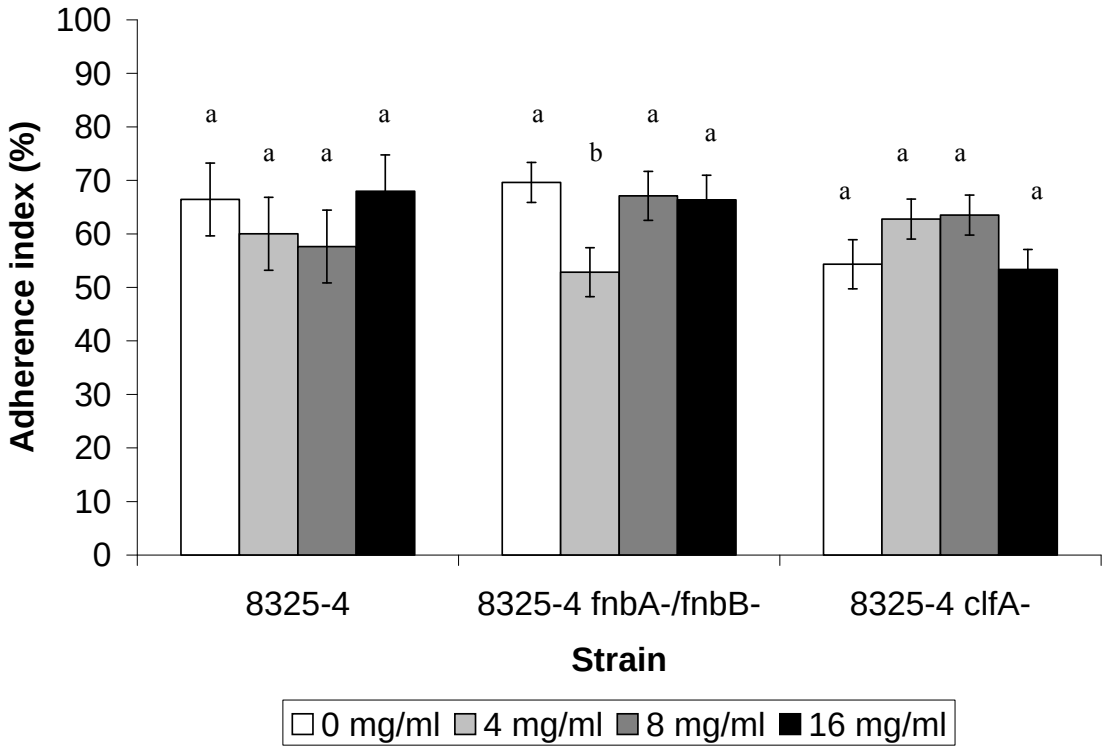


Figure 2: Effect of CE90 on *Staph. aureus* binding to fibronectin. Treatments within each strain with different letters are significantly different ($P < 0.05$). There were no significant differences between strains ($P > 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

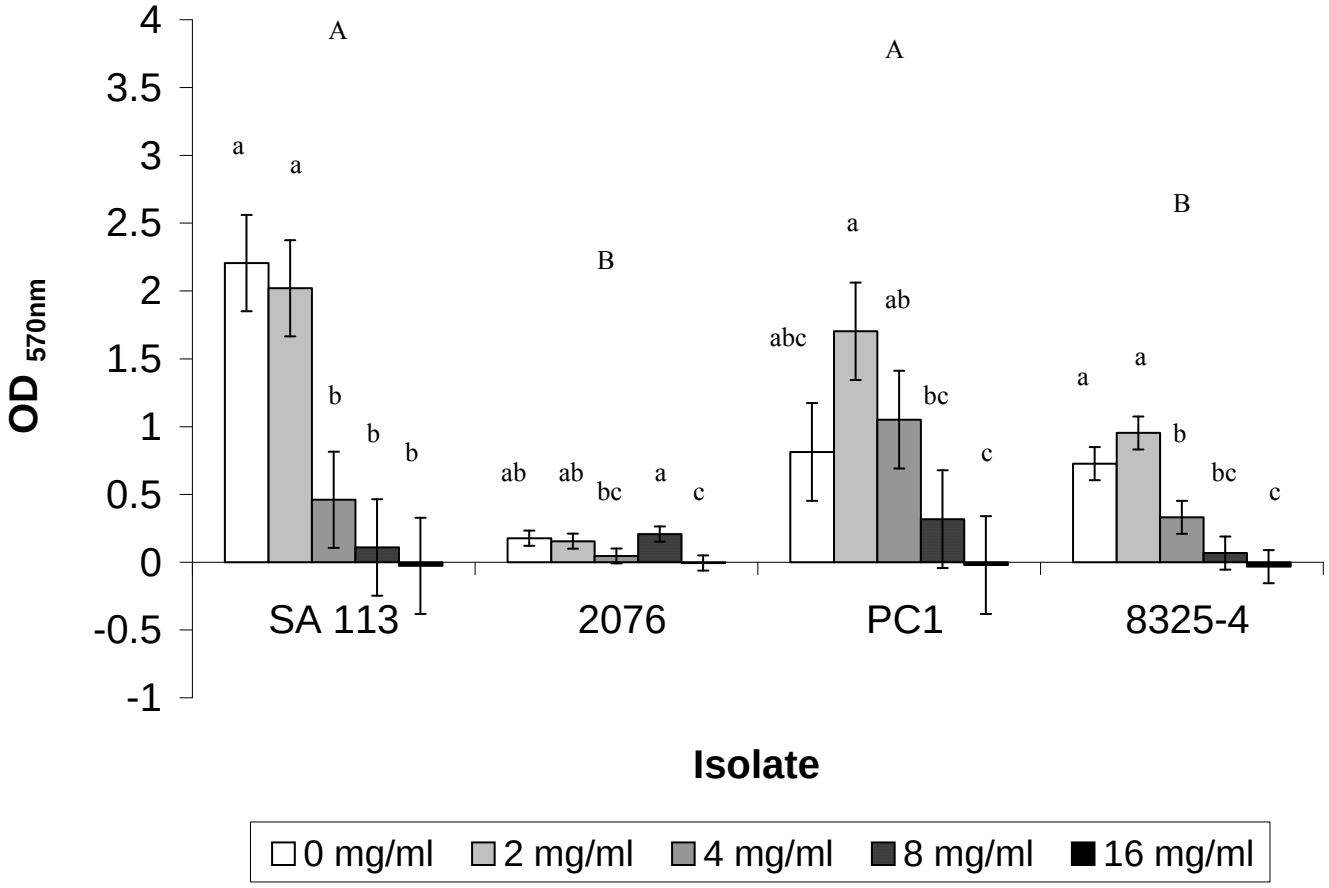


Figure 3: Effect of CE90 on *Staph. aureus* biofilm formation. Treatments within each isolate with different letters (a, b, c) are significantly different ($P < 0.05$). Isolates with different letters (A, B) are significantly different ($P < 0.05$). Values represent the least squares mean and standard error of optical density measurements at 570nm from two separate experiments performed in quadruplicate. There was a significant interaction between *Staph. aureus* isolate and CE90 treatment ($P < 0.05$).

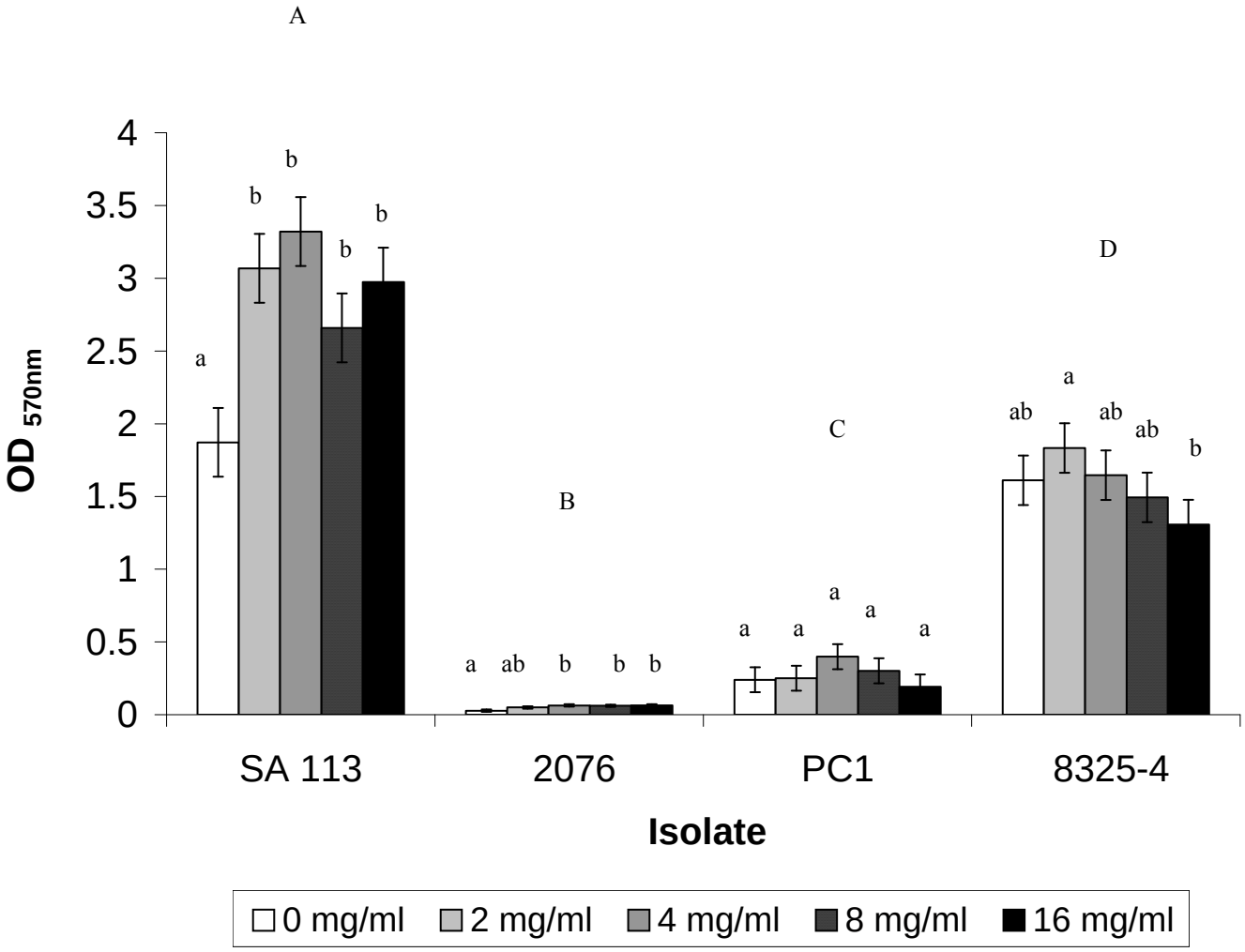


Figure 4: Effect of CE90 on removal of biofilm formed by *Staph. aureus*. Treatments within each isolate with different letters (a, b) are significantly different ($P < 0.05$). Isolates with different letters (A, B, C, D) are significantly different ($P < 0.05$). Values represent the least squares mean and standard error of optical density measurements at 570nm from two separate experiments performed in quadruplicate. There was a significant interaction between *Staph. aureus* isolate and CE90 treatment ($P < 0.05$).

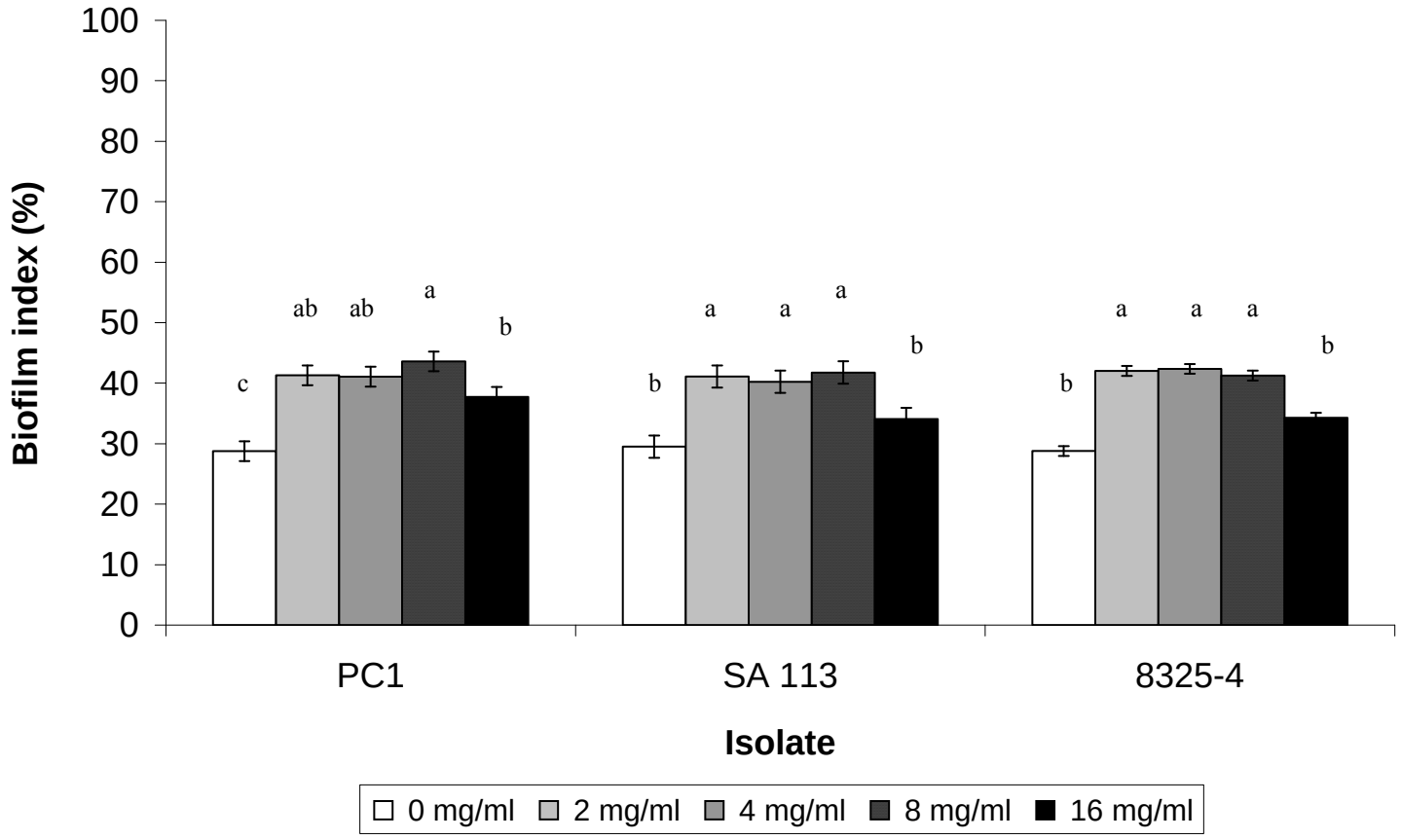


Figure 5a: Effect of CE90 on biofilm index of *Staph. aureus* after 2 h. Biofilm index = (cfu ml^{-1} biofilm *Staph. aureus* / (cfu ml^{-1} biofilm *Staph. aureus* + cfu ml^{-1} planktonic *Staph. aureus*))*100. Different letters (a, b, c) represent significant differences of treatments within each isolate ($P < 0.05$). There were no significant differences between isolates ($P > 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

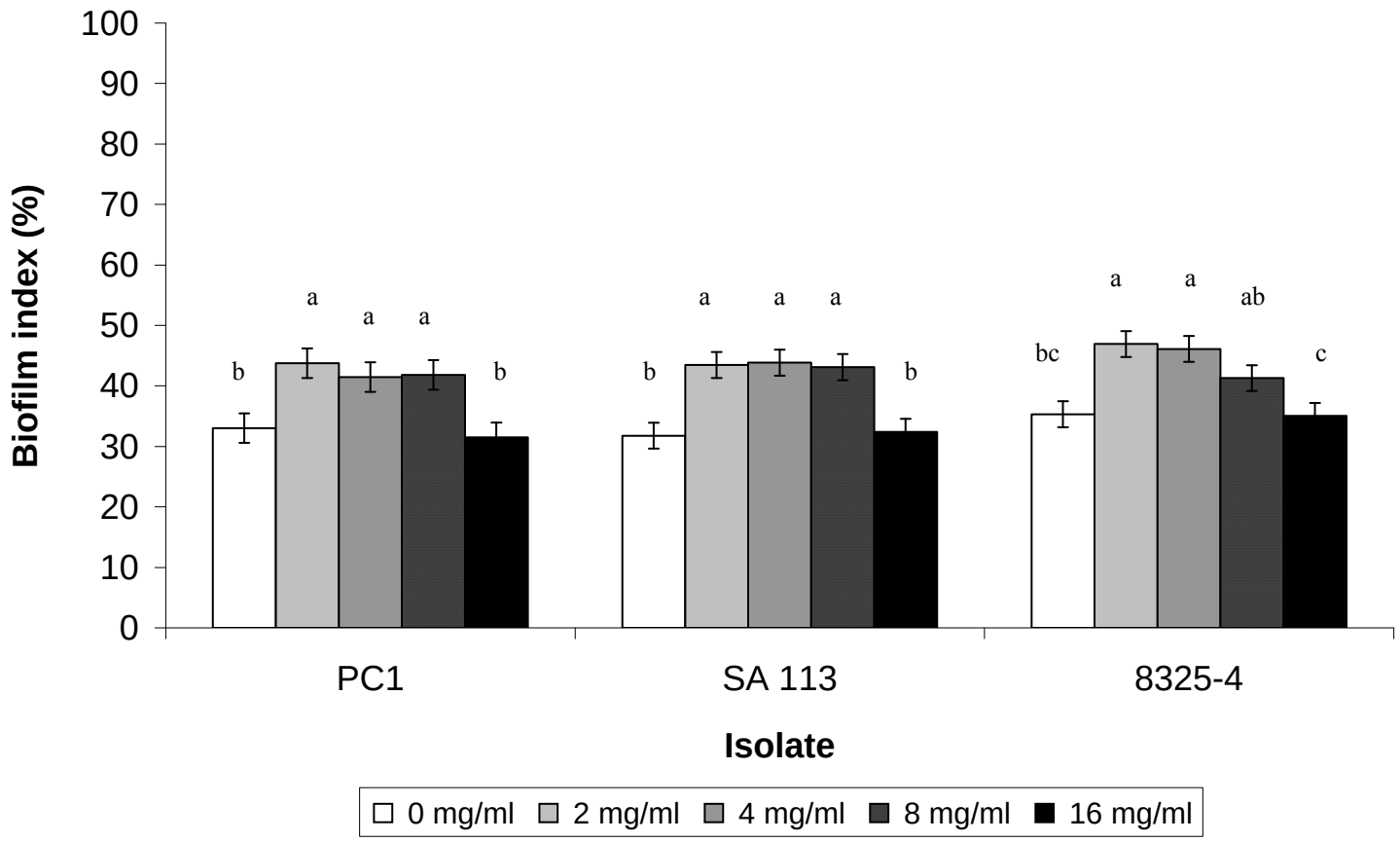


Figure 5b: Effect of CE90 on biofilm index of *Staph. aureus* after 4 h. Biofilm index = (cfu ml^{-1} biofilm *Staph. aureus* / (cfu ml^{-1} biofilm *Staph. aureus* + cfu ml^{-1} planktonic *Staph. aureus*))*100. Different letters (a, b, c) represent significant differences of treatments within each isolate ($P < 0.05$). There were no significant differences between isolates ($P > 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

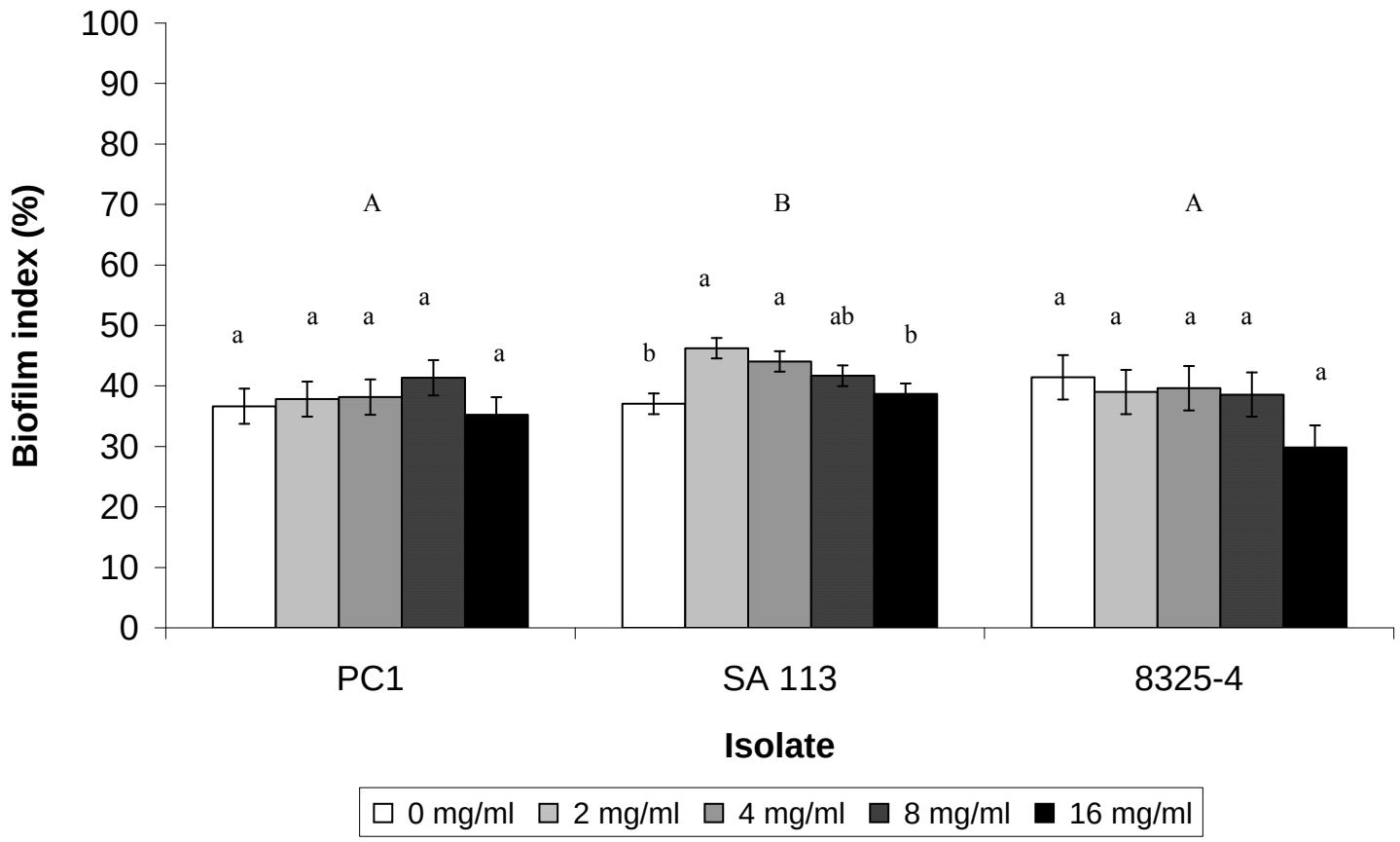


Figure 5c: Effect of CE90 on biofilm index of *Staph. aureus* after 6 h. Biofilm index = (cfu ml^{-1} biofilm *Staph. aureus* / (cfu ml^{-1} biofilm *Staph. aureus* + cfu ml^{-1} planktonic *Staph. aureus*))*100. Different letters (a, b) represent significant differences of treatments within each isolate ($P < 0.05$). Different letters (A, B) represent significant differences between isolates ($P < 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

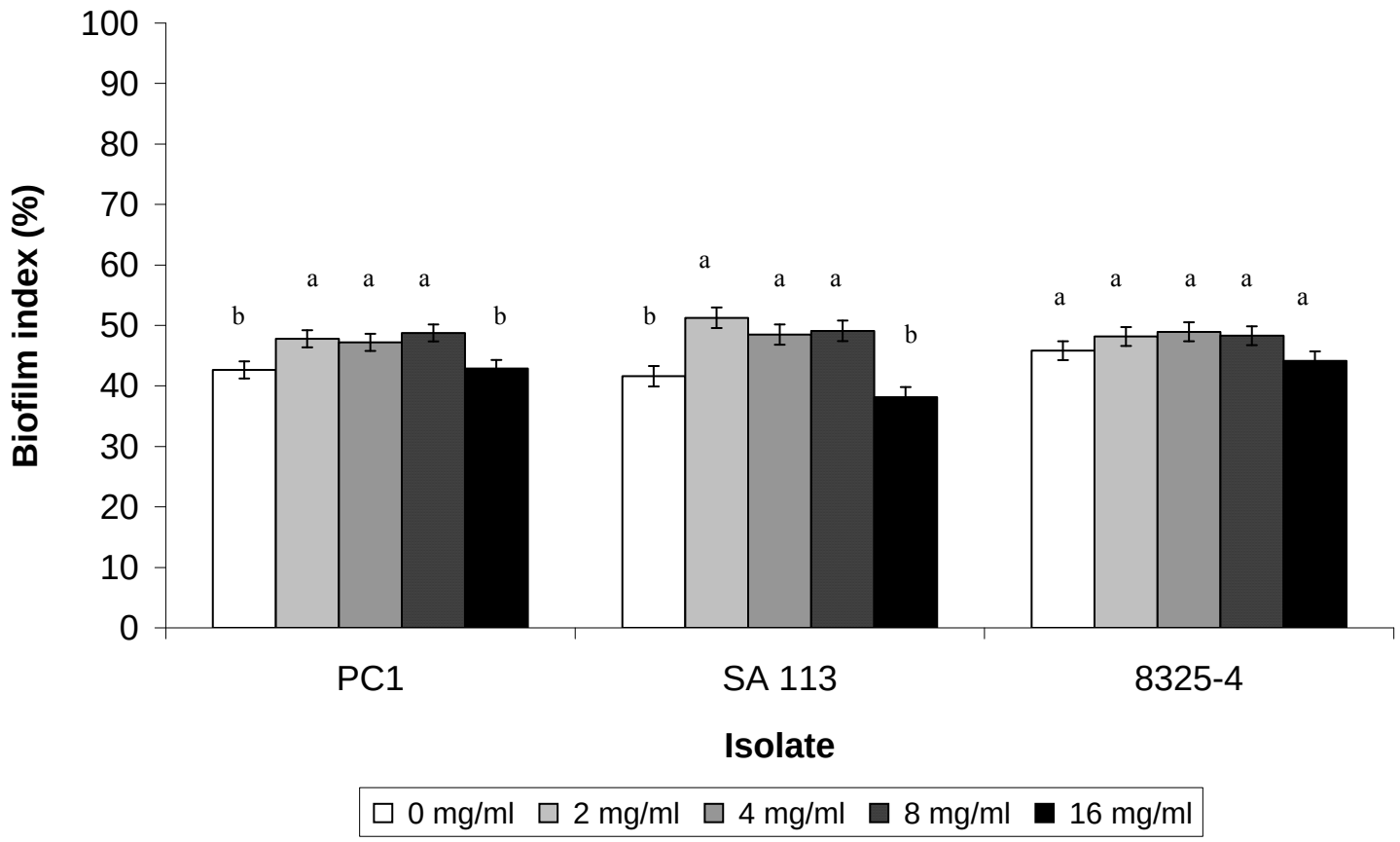


Figure 5d: Effect of CE90 on biofilm index of *Staph. aureus* after 8 h. Biofilm index = (cfu ml^{-1} biofilm *Staph. aureus* / (cfu ml^{-1} biofilm *Staph. aureus* + cfu ml^{-1} planktonic *Staph. aureus*))*100. Different letters (a, b) represent significant differences of treatments within each isolate ($P < 0.05$). There were no significant differences between isolates ($P > 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

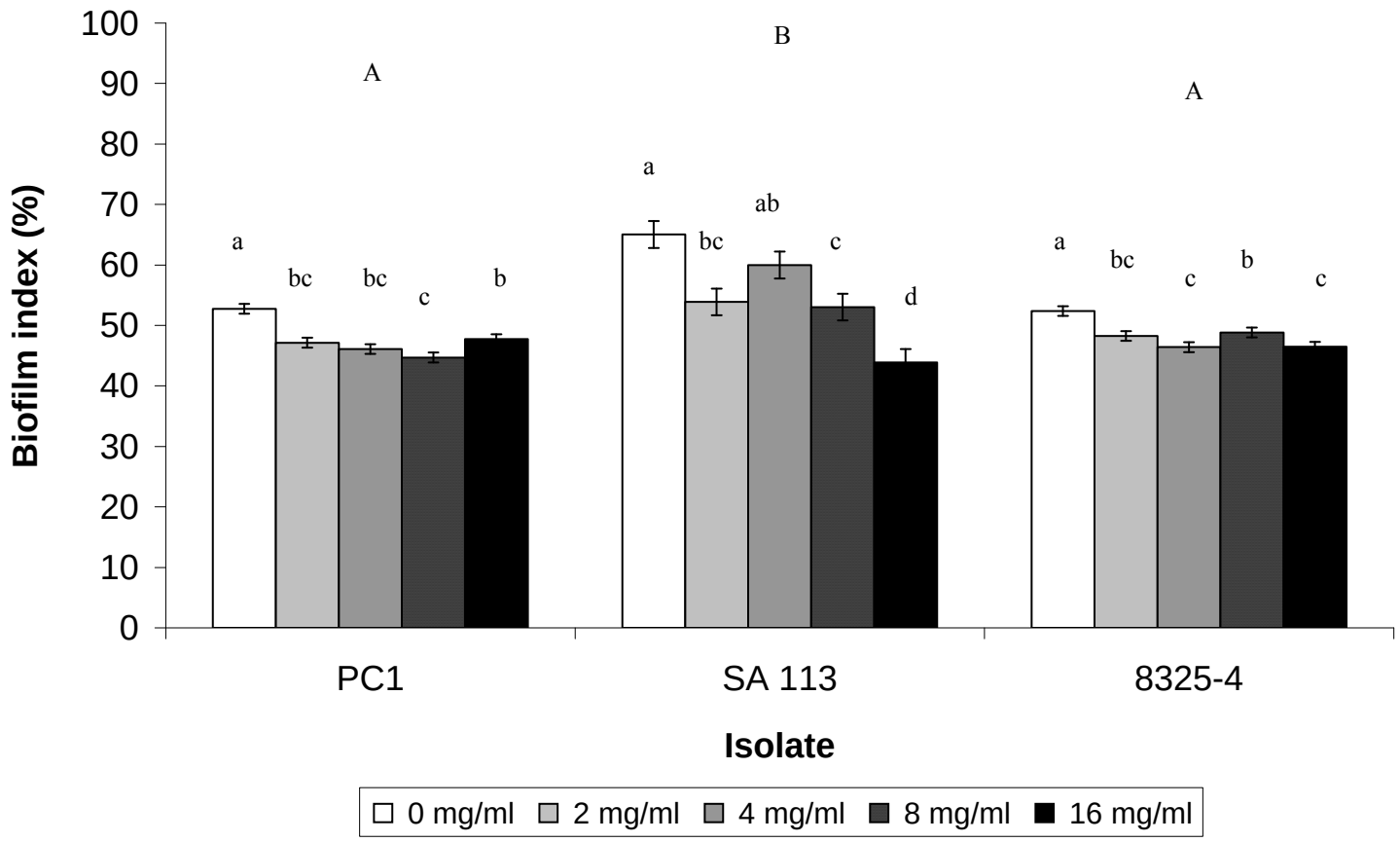


Figure 5e: Effect of CE90 on biofilm index of *Staph. aureus* after 24 h. Biofilm index = (cfu ml^{-1} biofilm *Staph. aureus* / (cfu ml^{-1} biofilm *Staph. aureus* + cfu ml^{-1} planktonic *Staph. aureus*))*100. Different letters (a, b, c, d) represent significant differences of treatments within each isolate ($P < 0.05$). Different letters (A, B) represent significant differences between isolates ($P < 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

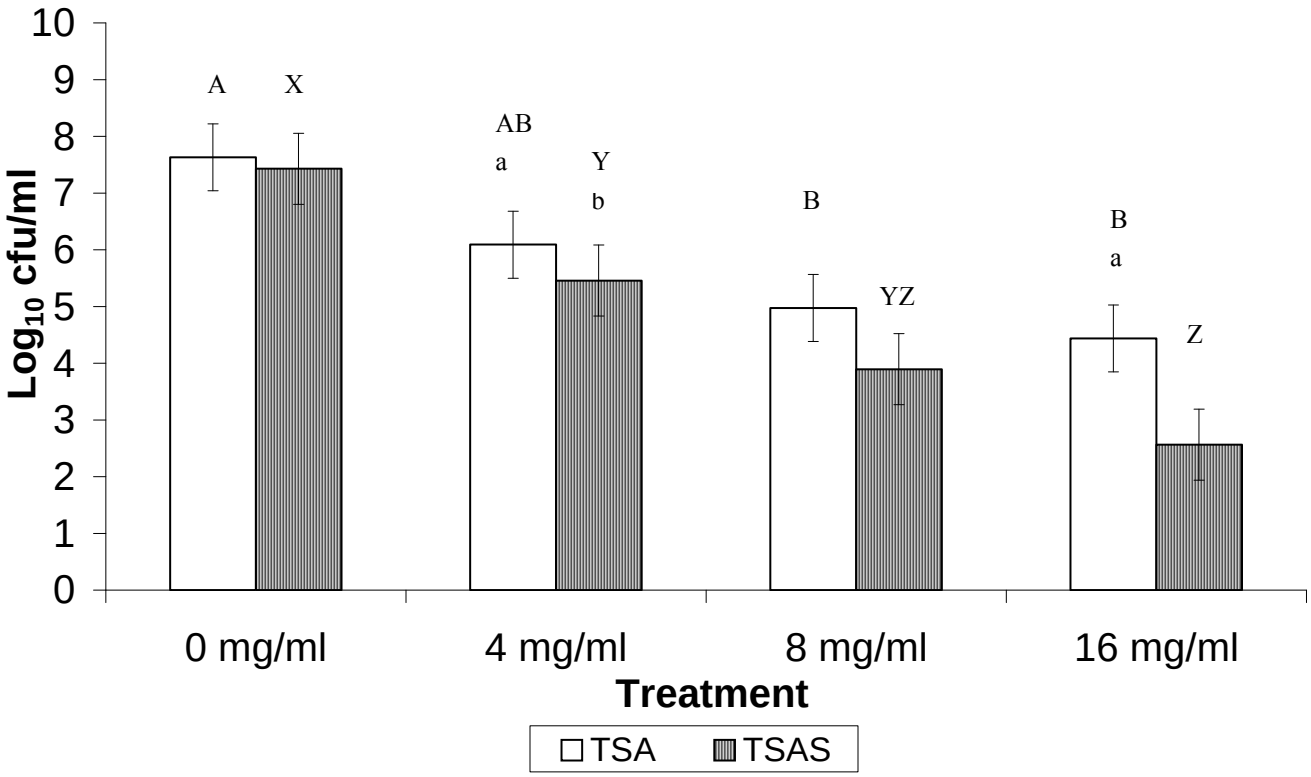


Figure 6a: Effect of CE90 on *Staph. aureus* PC1 salt tolerance. Different letters within each media (A, B = TSA; X, Y, Z = TSAS) represent significant differences between treatments ($P < 0.05$). Different letters within each treatment (a, b) represent significant differences between media ($P < 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

% surviving = (cfu ml^{-1} on TSA / cfu ml^{-1} on TSA of isolate at 0 mg ml^{-1})*100
% killed = 100 – % surviving
% healthy = (cfu ml^{-1} on TSAS / cfu ml^{-1} on TSA of isolate at 0mg ml^{-1})*100
% injured = % surviving - % healthy

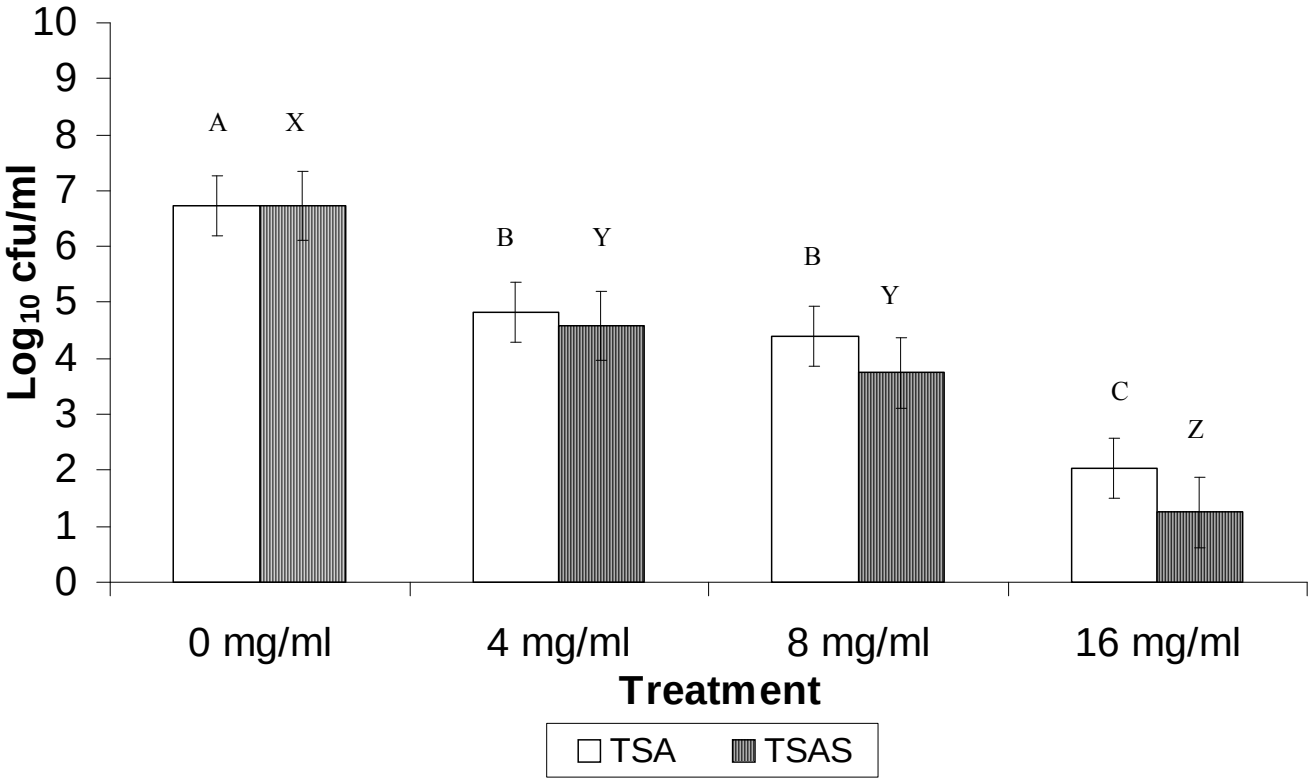


Figure 6b: Effect of CE90 on *Staph. aureus* SA 113 salt tolerance. Different letters within each media (A, B, C = TSA; X, Y, Z = TSAS) represent significant differences between treatments ($P < 0.05$). There were no significant differences between media ($P > 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

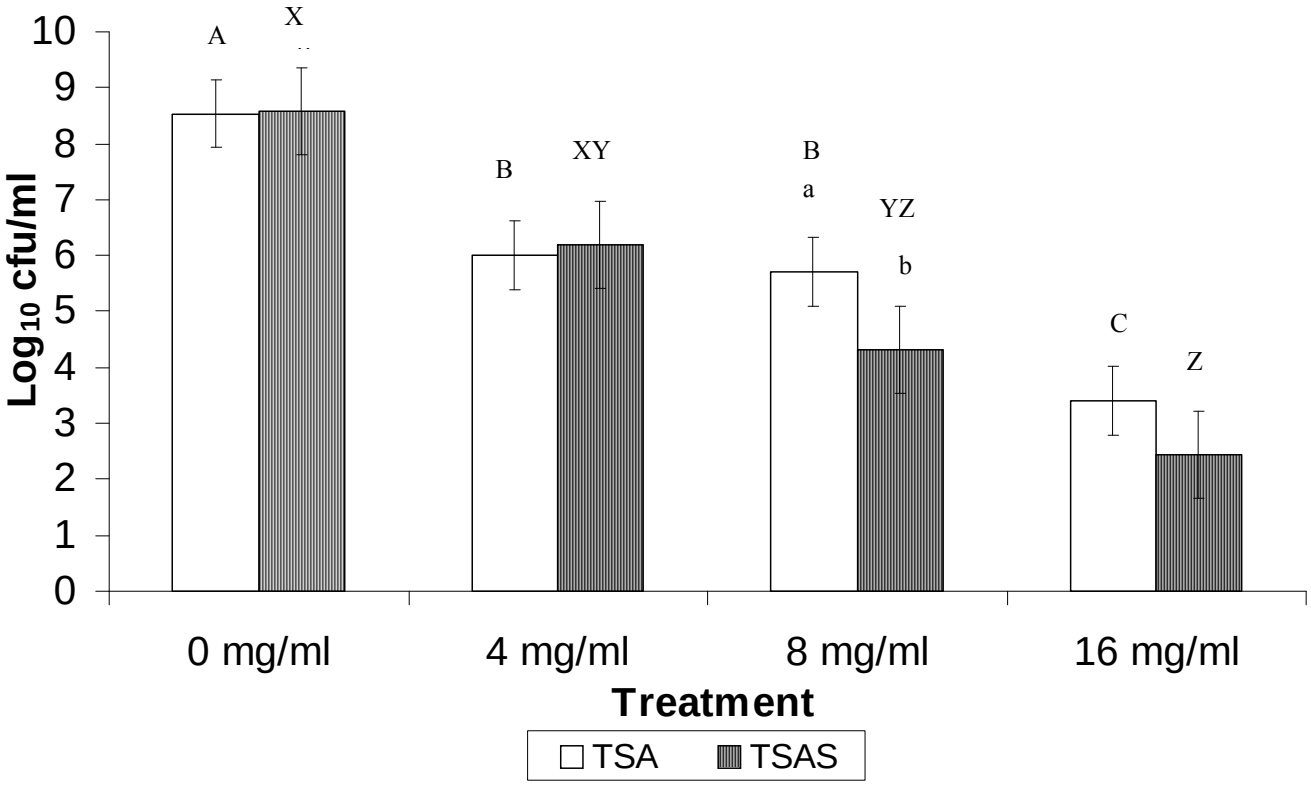


Figure 6c: Effect of CE90 on *Staph. aureus* 8325-4 salt tolerance. Different letters within each media (A, B, C = TSA; X, Y, Z = TSAS) represent significant differences between treatments ($P < 0.05$). Different letters within each treatment (a, b) represent significant differences between media ($P < 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

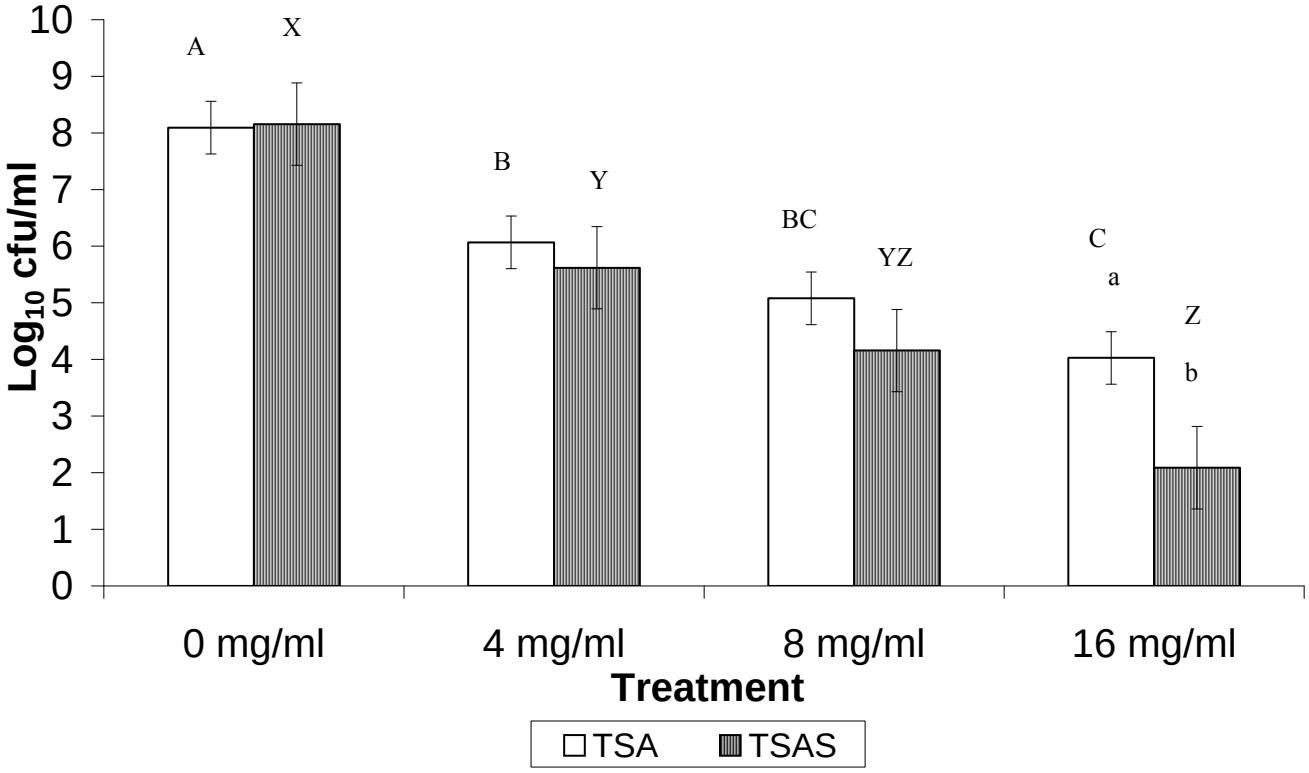


Figure 6d: Effect of CE90 on *Staph. aureus* 2076 salt tolerance. Different letters within each media (A, B, C = TSA; X, Y, Z = TSAS) represent significant differences between treatments ($P < 0.05$). Different letters within each treatment (a, b) represent significant differences between media ($P < 0.05$). Error bars represent the standard error of two repeats performed in duplicate.

REFERENCES

Aarestrup FM. 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb. Drug Resist. 1:255-257.

Aarestrup, F. M., P. Ahrens, M. Madsen, L. V. Pallesen, R. L. Poulsen and H. Westh. 1996. Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin. Antimicrob. Agents Chemother. 40:1938-1940.

Ahmad, S., M. Ashraf, M. Siddique, F. Mehmood, M. Arshad and H. A. Khan. 2008. Biofilm formation and drinking water quality in relation to *Escherichia coli* at commercial poultry farms. JASS. 4:77-80.

Ahuja, S., B. Kaack and J. Roberts. 1998. Loss of fimbrial adhesion with the addition of *Vaccinium macrocarpon* to the growth medium of P-fimbriated *Escherichia coli*. J. Urol. 159:559-562.

Annous, B. A., P. M. Fratamico and J. L. Smith. 2009. Quorum sensing in biofilms: why bacteria behave the way they do. J. Food Sci. 74:R24-R37.

Apostolidis, E., Y.-I. Kwon and K. Shetty. 2008. Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. Int. J. Food Microbiol. 128:317-324.

Arciola, C. R., D. Campoccia, S. Gamberini, M. Cervellati, E. Donati and L. Montanaro. 2002. Detection of slime production by means of an optimized Congo red agar plate test based on a colourimetric scale in *Staphylococcus epidermidis* clinical isolates genotyped for *ica* locus. Biomaterials. 23:4233-4239.

Arthur, M., C. E. Johnson, R. H. Rubin, R. D. Arbeit, C. Campanelli, C. Kim, S. Steinbach, M. Agarwal, R. Wilkinson and R. Goldstein. 1989. Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect. Immun. 57:303-313.

Aubry-Damon, H., C.-J. Soussy and P. Courvalin. 1998. Characterization of mutations in the *rpoB* gene that confer rifampin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 42:2590-2594.

Avorn, J., M. Monane, J. H. Gurwitz, R. J. Glynn, I. Choodnovskiy and L. A. Lipsitz. 1994. Reduction of bacteriuria and pyuria after ingestion of cranberry juice. JAMA. 271:751-754.

Ayoubi, P., A. Kilic and M. N. Vijayakumar. 1991. Tn*5253*, the Pneumococcal Ω(*cat tet*) BM6001 element, is a composite structure of two conjugative transposons, Tn*5251* and Tn*5252*. J. Bacteriol. 173:1617-1622.

Bager, F., F. M. Aarestrup, M. Madsen and H. C. Wegener. 1999. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. Microb. Drug Resist. 5:53-56.

Bailey, D. T., C. Dalton, F. J. Daugherty and M. S. Tempesta. 2007. Can a concentrated cranberry extract prevent recurrent urinary tract infections in women? A pilot study. Phytomedicine. 14:237-241.

Baker, C. N., S. A. Stocker, D. H. Culver and C. Thornsberry. 1991. Comparison of the E test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. J. Clin. Microbiol. 29:533-538.

Ballow, C. H. and J. J. Schentag. 1992. Trends in antibiotic utilization and bacterial resistance. Report of the National Nosocomial Resistance Surveillance Group. Diagn. Microbiol. Infect. Dis. 15(2 Suppl):37S-42S.

Bannerman, D. D., M. J. Paape, J.-W. Lee, X. Zhao, J. C. Hope and P. Rainard. 2004. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. Clin. Diagn. Lab. Immunol. 11:463-472.

Bate-Smith, E. C. 1973. Hemanalysis of tannins: the concept of relative astringency. Phytochemistry. 12:907-912.

Bates, J. 1997. Epidemiology of vancomycin-resistant enterococci in the community and the relevance of farm animals to human infection. J. Hosp. Infect. 37:89-101.

Bauer, M. E. and S. M. Spinola. 1999. Binding of *Haemophilus ducreyi* to extracellular matrix proteins. Infect. Immun. 67:2649-2653.

Bayles, K. W., C. A. Wesson, L. E. Liou, L. K. Fox, G. A. Bohach and W. R. Trumble. 1998. Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. Infect. Immun. 66:336-342.

Beachey, E. H. 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Inf. Dis. 143:325-345

Beenken, K. E., P. M. Dunman, F. McAleese, D. Macapagal, E. Murphy, S. J. Projan, J. S. Blevins and M. S. Smeltzer. 2004. Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol. 186:4665-4684.

Ben Zakour, N. L., C. M. Guinane and J. R. Fitzgerald. 2008. Pathogenomics of the staphylococci: insights into niche adaptation and the emergence of new virulent strains. FEMS Microbiol. Lett. 289:1-12.

Bertram, J., M. Stratz and P. Durre. 1991. Natural transfer of conjugative transposon Tn*916* between gram-positive and gram-negative bacteria. J. Bacteriol. 173:443-448.

Bhakdi, S. and J. Tranum-Jensen. 1991. Alpha-toxin of *Staphylococcus aureus*. Microbiol. Rev. 55:733-751.

Bobin-Dubreux, S., M.-E. Reverdy, C. Nervi, M. Rougier, A. Bolmstrom, F. Vandenesch and J. Etienne. 2001. Clinical isolate of vancomycin-heterointermediate *Staphylococcus aureus* susceptible to methicillin and *in vitro* selection of a vancomycin-resistant derivative. Antimicrob. Agents Chemother. 45:349-352.

Bodel, P. T., R. Cotran and E. H. Kass. 1959. Cranberry juice and the antibacterial action of hippuric acid. J. Lab. Clin. Med. 54:881-888.

Bomser, J., D. L. Madhavi, K. Singletary and M. A. Smith. 1996. *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. Planta Med. 62:212-216.

Bruins, M. J., P. Juffer, M. J. H. M. Wolfhagen and G. J. H. M. Ruijs. 2007. Salt tolerance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. J. Clin. Microbiol. 45:682-683.

Burger, O., E. Weiss, N. Sharon, M. Tabak, I. Neeman and I. Ofek. 2002. Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. Crit. Rev. Food Sci. Nutr. 42:279-284.

Carson, C. F., B. J. Mee and T. V. Riley. 2002. Mechanism of action *of Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. 46:1914-1920.

Carson, C. F. and T. V. Riley. 2003. Non-antibiotic therapies for infectious diseases. Commun. Dis. Intell. 27(Suppl):S143-S146.

Castellano, L. R., D. N. Silva-Teixeira, E. J. Antonelli, V. Rodrigues, Jr. and M. B. Cavalcanti-Cordeiro. 2008. Cytokine and nitric oxide production in an adult patient with staphylococcal scalded skin syndrome. Invest. Clin. 49:547-552. [Abstract]

Ceri, H., M. E. Olson, C. Stremick, R. R. Read, D. Morck and A. Buret. 1999. The Calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol. 37:1771-1776.

Cervantes, H. M., L. L. Munger, D. H. Ley and M. D. Ficken. 1988. Staphylococcus-induced gangrenous dermatitis in broilers. Avian Dis. 32:140-142.

Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol. 50:201–203.

Christensen, G. D., W. A. Simpson, J. J. Younger, L. M. Baddour, F. F. Barrett, D. M. Melton and E. H. Beachey. 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22:996-1006.

Cirioni, O., A. Giacometti, R. Ghiselli, G. Dell'Acqua, Y. Gov, W. Kamysz, J. Lukasiak, F. Mocchegiani, F. Orlando, G. D'Amato, N. Balaban, V. Saba and G. Scalise. 2003. Prophylactic efficacy of topical temporin A and RNAIII-inhibiting peptide in a subcutaneous rat pouch model of graft infection attributable to staphylococci with intermediate resistance to glycopeptides. Circulation. 108:767-771.

Cook, M. E. 2000. Skeletal deformities and their causes: introduction. Poult. Sci. 79:982-984.

Cortes, C. R., G. T. Isaias, C. L. Cuello, J. M. V. Flores, R. C. Anderson and C. E. Campos. 2004. Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. Rev. Latinoam. Microbiol. 46:12-16.

Costa, D., P. Poeta, Y. Saenz, L. Vinue, A. C. Coelho, M. Matos, B. Rojo-Bezares, J. Rodrigues and C. Torres. 2008. Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. Microb. Drug Resist. 14:71-77.

Courvalin, P. and C. Carlier. 1986. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. Mol. Gen. Genet. 205:291-297.

Cucarella, C., C. Solano, J. Valle, B. Amorena, I. Lasa and J. R. Penades. 2001. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. J. Bacteriol. 183:2888-2896.

Daum, R. S., W. H. Davis, K. B. Farris, R. J. Campeau, D. M. Mulvihill and S. M. Shane. 1990. A model of *Staphylococcus aureus* bacteremia, septic arthritis, and osteomyelitis in chickens. J. Orthop. Res. 8:804-813.

de Castillo, M. C., O. A. de Saab, O. M. de Nader and A. P. de Ruiz Holgado. 1998. *In vitro* comparison of disk diffusion and agar dilution antibiotic susceptibility test methods for *Neisseria gonorrhoeae*. Mem. Inst. Oswaldo Cruz. 93:517-522.

De Oliveira, A. P., J. L. Watts, S. A .Salmon and F. M. Aarestrup. 2000. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. J. Dairy Sci. 83:855-862.

Devriese, L. A., M. Ieven, H. Goossens, P. Vandamme, B. Pot, J. Hommez and F. Haesebrouck. 1996. Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob. Agents Chemother. 40:2285-2287.

Diarra, M. S., D. Petitclerc and P. Lacasse. 2002. Effect of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. J. Dairy Sci. 85:1141-1149.

Diarra, M.S., F. G. Silversides, F. Diarrassouba, J. Pritchard, L. Masson, R. Brousseau, C. Bonnet, P. Delaquis, S. Bach, B. J. Skura and E. Topp. 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and *Enterococcus* counts, and antibiotic resistance phenotypes and distribution of antibiotic resistance determinants in *Escherichia coli* isolates. Appl. Environ. Microbiol. 73:6566-6576.

Diarrassouba, F., M. S. Diarra, S. Bach, P. Delaquis, J. Pritchard, E. Topp and B. J. Skura. 2007. Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler chicken farms. J. Food Prot. 70:1316-1327.

Di Bonaventura, G., I. Spedicato, D. D'Antonio, I. Robuffo and R. Piccolomini. 2004. Biofilm formation by *Stenotrophomonas maltophilia*: modulation by quinolones, trimethoprim-sulfamethoxazole, and ceftazidime. Antimicrob. Agents Chemother. 48:151-160.

Dignam, R. R., M. Ahmed, K. G. Kelly, S. J. Denman, M. Zayon and M. Kleban. 1998. The effect of cranberry juice on urinary tract infection rates in a long-term care facility. Ann. Long-Term Care. 6:163-167.

Di Martino, P., R. Agniel, K. David, C. Templer, J. L. Gaillard, P. Denys and H. Botto. 2006. Reduction of *Escherichia coli* adherence to uroepithelial bladder cells after consumption of cranberry juice: a double-blind randomized placebo-controlled cross-over trial. World J. Urol. 24:21–27.

Doyle, M. P. and L. R. Beuchat. 2007. *Food microbiology: fundamentals and frontiers.* 3rd ed. ASM press. Washington, D. C. 1038 p.

Duarte, S., S. Gregoire, A. P. Singh, N. Vorsa, K. Schaich, W. H. Bowen and H. Koo. 2006. Inhibitory effects of cranberry polyphenols on formation and acidogenicity of *Streptococcus mutans* biofilms. FEMS Microbiol. Lett. 257:50-56.

Dziewanowska, K., J. M. Patti, C. F. Deobald, K. W. Bayles, W. R. Trumble and G. A. Bohach. 1999. Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. Infect. Immun. 67:4673-4678.

Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden and R. P. Mouton. 1991. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother. 27:199-208.

Endtz, H. P., N. Van Den Braak, A. Van Belkum, J. A. J. W. Kluytmans, J. G. M. Koeleman, L. Spanjaard, A. Voss, A. J. L. Weersink, C. M. J. E. Vandenbroucke-Grauls, A. G. M. Buiting, A. Van Duin and H. A. Verbrugh. 1997. Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in The Netherlands. J. Clin. Microbiol. 35:3026-3031.

Eydelnant, I. A. and N. Tufenkji. 2008. Cranberry derived proanthocyanidins reduce bacterial adhesion to selected biomaterials. Langmuir. 24:10273-10281.

Ferguson, P., E. Kurowska, D. J. Freeman, A. F. Chambers and D. J. Koropatnick. 2004. A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. J. Nutr. 134:1529-1535.

Fitzgerald, J. R., S. R. Monday, T. J. Foster, G. A. Bohach, P. J. Hartigan, W. J. Meaney and C. J. Smyth. 2001a. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J. Bacteriol. 183:63-70.

Fitzgerald, J. R., D. E. Sturdevant, S. M. Mackie, S. R. Gill and J. M. Musser. 2001b. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. PNAS. 98:8821-8826.

Foo, L. Y., Y. Lu, A. B. Howell and N. Vorsa. 2000a. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. J. Nat. Prod. 63:1225-1228.

Foo, L. Y., Y. Lu, A. B. Howell and N. Vorsa. 2000b. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli in vitro*. Phytochemistry. 54:173-181.

Forsman, P., A. Tilsala-Timisjarvi and T. Alatossava. 1997. Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S-23S rRNA spacer regions. Microbiology. 143:3491-3500.

Fox, L., R. Zadoks and C. Gaskins. 2005. Biofilm production by *Staphylococcus aureus* associated with intramammary infection. Vet. Microbiol. 107:295-299.

Gander, S. 1996. Bacterial biofilms: resistance to antimicrobial agents. J. Antimicrob. Chemother. 37:1047-1050.

Gentilini, E., G. Denamiel, P. Llorente, S. Godaly, M. Rebuelto and O. DeGregorio. 2000. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. J. Dairy Sci. 83:1224-1227.

Gibson, L., L. Pike and J. P. Kilboum. 1991. Clinical study: effectiveness of cranberry juice in preventing urinary tract infections in long-term care facility patients. J. Naturopath. Med. 2:45-47.

Giese, B., S. Dittmann, K. Paprotka, K. Levin, A. Weltrowski, D. Biehler, T.-T. Lam, B. Sinha and M. J. Fraunholz. 2009. Staphylococcal alpha-toxin is not sufficient to mediate escape from phagolysosomes in upper-airway epithelial cells. Infect. Immun. 77:3611-3625.

Gormley, E. A. 2003. Recurrent urinary tract infection in women: emerging concepts regarding etiology and treatment considerations. Curr. Urol. Rep. 4:399-403.

Gotteland, M., M. Andrews, M. Toledo, L. Munoz, P. Caceres, A. Anziani, E. Wittig, H. Speisky and G. Salazar. 2008. Modulation of *Helicobacter pylori* colonization with cranberry juice and *Lactobacillus johnsonii* La1 in children. Nutrition. 24:421-426.

Gotz, F. 2002. *Staphylococcus* and biofilms. Mol. Microbiol. 43:1367-1378.

Graffunder, E. M. and R. A. Venezia. 2002. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. J. Antimicrob. Chemother. 49:999-1005.

Greenberg, J. A., S. J. Newmann and A. B. Howell. 2005. Consumption of sweetened dried cranberries versus unsweetened raisins for inhibition of uropathogenic *Escherichia coli* adhesion in human urine: a pilot study. J. Altern. Complement. Med. 11:875-878.

Griffiths, P. 2003. The role of cranberry juice in the treatment of urinary tract infections. Br. J. Commun. Nurs. 557–561.

Gruson, D., G. Hilbert, F. Vargas, R. Valentino, C. Bebear, A. Allery, C. Bebear, G. Gbikpi-Benissan and J.-P. Cardinaud. 2000. Rotation and restricted use of antibiotics in a medical intensive care unit: impact on the incidence of ventilator-associated pneumonia caused by antibiotic-resistant gram-negative bacteria. Am. J. Respir. Crit. Care Med. 162:837-843.

Gu, L., M. A. Kelm, J. F. Hammerstone, G. Beecher, J. Holden, D. Haytowitz and R. L. Prior. 2003. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. J. Agric. Food Chem. 51:7513-7521.

Gupta, K., M. Y. Chou, A. Howell, C. Wobbe, R. Grady and A. E. Stapleton. 2007. Cranberry products inhibit adherence of p-fimbriated *Escherichia coli* to primary cultured bladder and vaginal epithelial cells. J. Urol. 177:2357-2360.

Guthrie, N. 2000. Effect of cranberry juice and products on human breast cancer cell growth. Exp. Biol. San Diego, CA. Abstract #531.13.

Harmon, R. J. 1996. Controlling contagious mastitis. Page 11 in Natl. Mastitis Council, Reg. Mtg. Proc., Queretero, Mexico. Natl. Mastitis Council, Inc., Madison, WI. (as referenced in: Nair, M. K. M., J. Joy, P. Vasudevan, L. Hinckley, T. A. Hoagland and K. S. Venkitanarayanan. 2005. Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. J. Dairy Sci. 88:3488-3495.)

Haverkorn, M. J. and J. Mandigers. 1994. Reduction of bacteriuria and pyuria using cranberry juice. JAMA. 272:590.

Hazariwala, A., Q. Sanders, C. R. Hudson, C. Hofacre, S. G. Thayer and J. J. Maurer. 2002. Distribution of staphylococcal enterotoxin genes among *Staphylococcus aureus* isolates from poultry and humans with invasive staphylococcal disease. Avian Dis. 46:132-136.

Hebert, A., K. Sayasith, S. Senechal, P. Dubreuil and J. Lagace. 2000. Demonstration of intracellular *Staphylococcus aureus* in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk. FEMS Microbiol. Lett. 193:57-62.

Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack and F. Gotz. 1996. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol. Microbiol. 20:1083-1091.

Henig, Y. S. and M. M. Leahy. 2000. Cranberry juice and urinary-tract health: science supports folklore. Nutrition. 16:684-687.

Henoun Loukili, N., E. Zink, S. Grandadam, M. Bientz and O. Meunier. 2004. Effectiveness of detergent-disinfecting agents on *Escherichia coli* 54127 biofilm. J. Hosp. Infect. 57:175-178.

Hernandez, F. J., J. Goyache, J. A. Orden, J. L. Blanco, A. Domenech, G. Suarez and E. Gomez-Lucia. 1993. Repair and enterotoxin synthesis by *Staphylococcus aureus* after thermal shock. Appl. Environ. Microbiol. 59:1515-1519.

Hess, M. J., P. E. Hess, M. R. Sullivan, M. Nee and S. V. Yalla. 2008. Evaluation of cranberry tablets for the prevention of urinary tract infections in spinal cord injured patients with neurogenic bladder. Spinal Cord. 46:622-626.

Hiramatsu, K. 2001. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. Lancet Infect. Dis. 1:147-155.

Howell, A. B., N. Vorsa, A. DerMarderosian and L. Y. Foo. 1998. Inhibition of adherence of P-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. N. Engl. J. Med. 339:1085-1086.

Howell, A. B., M. Leahy, E. Kurowska and N. Guthrie. 2001. *In vivo* evidence that cranberry proanthocyanidins inhibit adherence of P-fimbriated *E. coli* bacteria to uroepithelial cells. FASEB J. 15:A284.

Howell, A. B. 2002. Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit. Rev. Food Sci. Nutr. 42:273-278

Howell, A. B., J. D. Reed, C. G. Krueger, R. Winterbottom, D. G. Cunningham and M. Leahy. 2005. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. Phytochemistry. 66:2281-2291

Howell, A.B. and B. Foxman. 2002. Cranberry juice and adhesion of antibiotic-resistant uropathogens. JAMA. 287:3082-3083

Huang, C.-T. and P. S. Stewart. 1999. Reduction of polysaccharide production in *Pseudomonas aeruginosa* biofilms by bismuth dimercaprol (BisBAL) treatment. J. Antimicrob. Chemother. 44:601-605.

Hurst, A., A. Hughes, J. L. Beare-Rogers and D. L. Collins-Thompson. 1973. Physiological studies on the recovery of salt tolerance by *Staphylococcus aureus* after sublethal heating. J. Bacteriol. 116:901-907.

Hyde, F. W., M. Alberg and K. Smith. 1997. Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal. J. Ind. Microbiol. Biotechnol. 19:142-149.

Iandolo, J. J. and Z. J. Ordal. 1966. Repair of thermal injury of *Staphylococcus aureus*. J. Bacteriol. 91:134-142.

Ito, T., K. Okuma, X. X. Ma, H. Yuzawa and K. Hiramatsu. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist. Updat. 6:41-52.

Jay, J. M., M. J. Loessner and D. A. Golden. 2005. *Modern food microbiology.* 7th ed. Springer. New York. 790 p.

Jensen, E. L. and C. L. Miller. 2001. Staphylococcus infections in broiler breeders. AviaTech. 1:1-6.

Jepson, R.G., L. Mihaljevic and J. Craig. 2004. Cranberries for preventing urinary tract infections. Cochrane Database Syst. Rev: CD001321.

Jepson, R. G. and J. C. Craig. 2007. A systematic review of the evidence for cranberries and blueberries in UTI prevention. Mol. Nutr. Food Res. 51:738-745.

Johnson, B. J., B. Lin, M. A. Dinderman, R. A. Rubin, A. P. Malanoski and F. S. Ligler. 2008. Impact of cranberry on *Escherichia coli* cellular surface characteristics. Biochem. Biophys. Res. Commun. 377:992-994.

Johnson, J. R., N. Kaster, M. A. Kuskowski and G. V. Ling. 2003. Identification of urovirulence traits in *Escherichia coli* by comparison of urinary and rectal *E. coli* isolates from dogs with urinary tract infection. J. Clin. Microbiol. 41:337–345.

Johnson, J. R. 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. 4:80-128.

Johnson-White, B., L. Buquo, M. Zeinali and F. S. Ligler. 2006. Prevention of nonspecific bacterial cell adhesion in immunoassays by use of cranberry juice. Anal. Chem. 78:853-857.

Jones, R. N. 1992. The current and future impact of antimicrobial resistance among nosocomial bacterial pathogens. Diagn. Microbiol. Infect. Dis. 15(2 Suppl):3S-10S.

Kadouri, D. and G. A. O'Toole. 2005. Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl. Environ. Microbiol. 71:4044-4051.

Kahl, B. C., M. Goulian, W. Van Wamel, M. Herrmann, S. M. Simon, G. Kaplan, G. Peters and A. L. Cheung. 2000. *Staphylococcus aureus* RN6390 replicates and induces apoptosis in a pulmonary epithelial cell line. Infect. Immun. 68:5385-5392.

Kahl, B. C., G. Belling, R. Reichelt, M. Herrmann, R. A. Proctor and G. Peters. 2003. Thymidine-dependent small-colony variants of *Staphylococcus aureus* exhibit gross morphological and ultrastructural changes consistent with impaired cell separation. J. Clin. Microbiol. 41:410-413.

Kahn, D. H., V. A. Panariello, J. Saeli, J. R. Sampson and E. Schwwartz. 1967. Implications for therapy of urinary tract infection and calculi: effect of cranberry juice on urine. J. Am. Dietetic Assoc. 51:251.

Kaplan, J. B., C. Ragunath, K. Velliyagounder, D. H. Fine and N. Ramasubbu. 2004. Enzymatic detachment of *Staphylococcus epidermidis* biofilms. Antimicrob. Agents Chemother. 48:2633-2636.

Kelly, L. M., M. R. Jacobs and P. C. Appelbaum. 1999. Comparison of agar dilution, microdilution, E-test, and disk diffusion methods for testing activity of cefditoren against *Streptococcus pneumoniae*. J. Clin. Microbiol. 37:3296-3299.

Kinney, A. B. and M. Blount. 1979. Effect of cranberry juice on urinary pH. Nurs. Res. 28:287-290.

Kirkpatrick, W. R., R. K. McAtee, S. G. Revankar, A. W. Fothergill, D. I. McCarthy, M. G. Rinaldi and T. F. Patterson. 1998. Comparative evaluation of National Committee for Clinical Laboratory Standard broth macrodilution and agar dilution screening methods for testing fluconazole susceptibility of *Cryptococcus neoformans*. J. Clin. Microbiol. 36:1330-1332.

Klare, I., H. Heier, H. Claus, R. Reissbrodt and W. Witte. 1995. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. 125:165-171.

Klare, I., D. Badstubner, C. Konstabel, G. Bohme, H. Claus and W. Witte. 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb. Drug Resist. 5:45-52.

Kontiokari, T., K. Sundqvist, M. Nuutinen, T. Pokka, M. Koskela and M. Uhari. 2001. Randomized trial of cranberry-lingonberry juice and *Lactobacillus* GG drink for the prevention of urinary tract infections in women. Br. Med. J. 322:1571-1573.

Koo, H., P. Nino de Guzman, B. D. Schobel, A. V. Vacca Smith and W. H. Bowen. 2006. Influence of cranberry juice on glucan-mediated processes involved in *Streptococcus mutans* biofilm development. Caries Res. 40:20-27.

Kresty, L. A., A. B. Howell and M. Baird. 2008. Cranberry proanthocyanidins induce apoptosis and inhibit acid-induced proliferation of human esophageal adenocarcinoma cells. J. Agric. Food Chem. 56:676-680.

Labrecque, J., C. Bodet, F. Chandad and D. Grenier. 2006. Effects of a high-molecular-weight cranberry fraction on growth, biofilm formation and adherence of *Porphyromonas gingivalis*. J. Antimicrob. Chemother. 58:439-443.

Lane, M. C. and H. L. T. Mobley. 2007. Role of P-fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. Kidney Int. 72:19-25.

Leclercq, R., E. Derlot, M. Weber, J. Duval and P. Courvalin. 1989. Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. Antimicrob. Agents Chemother. 3:10-15.

Leevy, W. M., J. R. Johnson, C. Lakshmi, J. Morris, M. Marquez and B. D. Smith. 2006. Selective recognition of bacterial membranes by zinc(II)-coordination complexes. Chem. Commun. 1595-1597.

Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. 55:181-186.

Lewis, K. 2001. Riddle of biofilm resistance. Antimicrob. Agents Chemother. 45:999-1007.

Licht, T. R., B. B. Christensen, K. A. Krogfelt and S. Molin. 1999. Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. Microbiology. 145:2615-2622.

Lilly, H. A. and E. J. L. Lowbury. 1978. Antibiotic resistance of *Staphylococcus aureus* in a burns unit after stopping routine prophylaxis with erythromycin. J. Antimicrob. Chemother. 4:545-550.

Lim, Y., M. Jana, T. T. Luong and C. Y. Lee. 2004. Control of glucose- and NaCl-induced biofilm formation by *rbf* in *Staphylococcus aureus*. J. Bacteriol. 186:722-729.

Lin, Y.-T., R. G. Labbe and K. Shetty. 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. Appl. Environ. Microbiol. 70:5672-5678.

Lin, Y. T., Y. I. Kwon, R. G. Labbe and K. Shetty. 2005. Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. Appl. Environ. Microbiol. 71:8558-8564.

Liu, G. Y., A. Essex, J. T. Buchanan, V. Datta, H. M. Hoffman, J. F. Bastian, J. Fierer and V. Nizet. 2005. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J. Exp. Med. 202:209-215.

Liu Y., M. A. Black, L. Caron and T. A. Camesano. 2006. Role of cranberry juice on molecular-scale surface characteristics and adhesion behavior of *Escherichia coli*. Biotechnol. Bioeng. 93:297–305.

Liu, M., L. Q. Lin, B. B. Song, L. F. Wang, C. P. Zhang, J. L. Zhao and J. R. Liu. 2009. Cranberry phytochemical extract inhibits SGC-7901 cell growth and human tumor xenografts in Balb/c *nu/nu* mice. J. Agric. Food Chem. 57:762-768.

Louie, M. and F. R. Cockerill. 2001. Susceptibility testing: phenotypic and genotypic tests for bacteria and mycobacteria. Infect. Dis. Clin. North Am. 15:1205-1226.

Lowy, F. D. 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. 339:520-532.

Lynch, D. M. 2004. Cranberry for prevention of urinary tract infections. Am. Fam. Physician. 70:2175-2177.

Mack, D., P. Becker, I. Chatterjee, S. Dobinsky, J. Knobloch, G. Peters, H. Rohde and M. Herrmann. 2004. Mechanisms of biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*: functional molecules, regulatory circuits, and adaptive responses. Int. J. Med. Microbiol. 294:203-212.

Maeda, S., M. Ito, T. Ando, Y. Ishimoto, Y. Fujisawa, H. Takahashi, A. Matsuda, A. Sawamura and S. Kato. 2006. Horizontal transfer of nonconjugative plasmids in a colony biofilm of *Escherichia coli*. FEMS Microbiol. Lett. 255:115-120.

Magarinos, H. L., C. Sahr, S. D. Selaive, M. E. Costa, F. E. Figuerola and O. A. Pizarro. 2008. *In vitro* inhibitory effect of cranberry (*Vaccinium macrocarpon* Ait.) juice on pathogenic microorganisms. Prikl. Biokhim. Mikrobiol. 44:333-336. [Abstract]

Marion-Ferey, K., M. Pasmore, P. Stoodley, S. Wilson, G. P. Husson and J. W. Costerton. 2003. Biofilm removal from silicone tubing: an assessment of the efficacy of dialysis machine decontamination procedures using an *in vitro* model. J. Hosp. Infect. 53:64-71.

Mathur, T., S. Singhal, S. Khan, D. J. Upadhyay, T. Fatma and A. Rattan. 2006. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian J. Med. Microbiol. 24:25-29.

Matsushima, M., T. Suzuki, A. Masui, K. Kasai, T. Kouchi, A. Takagi, T. Shirai and T. Mine. 2008. Growth inhibitory action of cranberry on *Helicobacter pylori*. J. Gastroenterol. Hepatol. 23(Suppl 2):S175-S180.

McCullagh, J. J., P. T. McNamee, J. A. Smyth and H. J. Ball. 1998. The use of pulsed field gel electrophoresis to investigate the epidemiology of *Staphylococcus aureus* infection in commercial broiler flocks. Vet. Microbiol. 63:275-281.

McDonald, L. C., M. J. Kuehnert, F. C. Tenover and W. R. Jarvis. 1997. Vancomycin-resistant enterococci outside the health-care setting: prevalence, sources, and public health implications. Emerging Infect. Dis. 3:311-317.

McMurdo, M. E. T., L. Y. Bissett, R. J. G. Price, G. Phillips and I. K. Crombie. 2005. Does ingestion of cranberry juice reduce symptomatic urinary tract infections in older people in hospital? A double-blind, placebo-controlled trial. Age Ageing. 34:256-261.

McMurdo, M. E. T., I. Argo, G. Phillips, F. Daly and P. Davey. 2009. Cranberry or trimethoprim for the prevention of recurrent urinary tract infections? A randomized controlled trial in older women. J. Antimicrob. Chemother. 63:389-395.

McNamee, P. T., J. J. McCullagh, H. J. Ball, D. Graham, S. J. McCullough, J. A. Smyth, B. H. Thorp and D. McConaghy. 1998. Study of leg weakness in two commercial broiler flocks. Vet. Rec. 143:131-135.

McNamee, P. T. and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avian Pathol. 29:253-270.

Menzies, B.E. 2003. The role of fibronectin binding proteins in the pathogenesis of *Staphylococcus aureus* infections. Curr. Opin. Infect. Dis. 16:225-229.

Merrell, D. S., S. M. Butler, F. Qadri, N. A. Dolganov, A. Alam, M. B. Cohenk, S. B. Calderwood, G. K. Schoolnik and A. Camilli. 2002. Host-induced epidemic spread of the cholera bacterium. Nature. 417:642-645.

Moen, D. V. 1962. Observations on the effectiveness of cranberry juice in urinary infections. Wis. Med. J. 61:282-283.

Naidu, A. S., M. Paulsson and T. Wadstrom. 1988. Particle agglutination assays for rapid detection of fibronectin, fibrinogen, and collagen receptors on *Staphylococcus aureus*. J. Clin. Microbiol. 26:1549-1554.

Nairn, M. E. 1973. Bacterial osteomyelitis and synovitis of the turkey. Avian Dis. 17:504-517.

Nandy, P., A. R. Thakur and S. R. Chaudhuri. 2007. Characterization of bacterial strains isolated through microbial profiling of urine samples. OnLine J. Biol. Sci. 7(1):44-51.

Narisawa, N., S. Furukawa, H. Ogihara and M. Yamasaki. 2005. Estimation of the biofilm formation of *Escherichia coli* K-12 by the cell number. J. Biosci. Bioeng. 99:78-80.

Navarre, W. W. and O. Schneewind. 1999. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Mol. Biol. Rev. 63:174-229.

NCCLS. 1999. Performance standards for antimicrobial susceptibility testing. 9th Informational Supplement (aerobic dilution). M100-S9. Wayne, PA: National Committee for Clinical Laboratory Standards.

Neto, C. C. 2007a. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. Mol. Nutr. Food Res. 51:652-664.

Neto, C. C. 2007b. Cranberry and its phytochemicals: a review of *in vitro* anticancer studies. J. Nutr. 186-193.

Neto, C. C., J. W. Amoroso and A. M. Liberty. 2008. Anticancer activities of cranberry phytochemicals: an update. Mol. Nutr. Food Res. 52(Suppl 1):S18-S27.

Neuhauser, M. M., R. A. Weinstein, R. Rydman, L. H. Danziger, G. Karam and J. P. Quinn. 2003. Antibiotic resistance among gram-negative bacilli in US intensive care units: Implications for fluoroquinolone use. JAMA. 289:885-888.

Noble, W. C., Z. Virani and R. G. Cree. 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol. Lett. 72:195-198.

Nogueira, M. C., O. A. Oyarzabal and E. D. Gombas. 2003. Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in cranberry, lemon, and lime juice concentrates. J. Food Prot. 66:1637-1641.

Nohynek, L. J., H.-L. Alakomi, M. P. Kahkonen, M. Heinonen, I. M. Helander, K.-M. Oksman-Caldentey and R. H. Puupponen-Pimia. 2006. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. Nutr. Cancer. 54:18-32.

Nowack, R. 2007. Cranberry juice – a well-characterized folk-remedy against bacterial urinary tract infection. Wiener Medizinische Wochenschrift. 157:325-330.

Ofek, I., J. Goldhar, D. Zafriri, H. Lis, R. Ada and N. Sharon. 1991. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. N. Engl. J. Med. 324:1599.

Ofek, I., J. Goldhar and N. Sharon. 1996. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol. 408:179–183.

Oliveira, D. C., A. Tomasz and H. de Lencastre. 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. Lancet Infect. Dis. 2:180-189.

Pantosti, A., M. Del Grosso, S. Tagliabue, A. Macri and A. Caprioli. 1999. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet. 354:741-742.

Papas, P. N., C. A. Brusch and G. C. Ceresia. 1968. Cranberry juice in the treatment of urinary tract infections. Southwest. Med. 47:17-20.

Patti, J. M., B. L. Allen, M. J. McGavin and M. Hook. 1994. MSCRAMM-mediated adherence of microorganisms to host tissues. Annu. Rev. Microbiol. 48:585-617.

Pitts, B., M. A. Hamilton, N. Zelver and P. S. Stewart. 2003. A microtiter-plate screening method for biofilm disinfection and removal. J. Microbiol. Methods. 54:269-276.

Porter, M. L., C. G. Krueger, D. A. Wiebe, D. G. Cunningham and J. D. Reed. 2001. Cranberry proanthocyanidins associate with low-density lipoprotein and inhibit *in vitro* Cu^{2+}-induced oxidation. J. Sci. Food Agric. 81:1306-1313.

Prasain, J. K., N. Peng, Y. Dai, R. Moore, A. Arabshahi, L. Wilson, S. Barnes, J. M. Wyss, H. Kim and R. L. Watts. 2009. Liquid chromatography tandem mass spectrometry identification of proanthocyanidins in rat plasma after oral administration of grape seed extract. Phytomedicine. 16:233-243.

Puupponen-Pimia, R., L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K. M. Oksman-Caldentey. 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 90:494-507.

Puupponen-Pimia, R., L. Nohynek, S. Hartmann-Schmidlin, M. Kahkonen, M. Heinonen, K. Maatta-Riihinen and K. M. Oksman-Caldentey. 2005. Berry phenolics selectively inhibit the growth of intestinal pathogens. J. Appl. Microbiol. 98:991-1000.

Rahal, J. J., C. Urban, D. Horn, K. Freeman, S. Segal-Maurer, J. Maurer, N. Mariano, S. Marks, J. M. Burns, D. Dominick and M. Lim. 1998. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. JAMA. 280:1233-1237.

Raz, R., B. Chazan and M. Dan. 2004. Cranberry juice and urinary tract infection. Clin. Infect. Dis. 38:1413-1419.

Reed, J. D., C. G. Krueger and M. L. Porter. 2001. Cranberry juice powder decreases low density lipoprotein cholesterol in hypercholesterolemic swine. FASEB J. 15:54.

Reed, J. 2002. Cranberry flavonoids, atherosclerosis and cardiovascular health. Crit. Rev. Food Sci. Nutr. 42(3 Suppl.):301-316.

Reid, G., J. Hsiehl, P. Potter, J. Mighton, D. Lam, D. Warren and J. Stephenson. 2001. Cranberry juice consumption may reduce biofilms on uroepithelial cells: pilot study in spinal cord injured patients. Spinal Cord. 39:26-30.

Revazishvili, T., L. Bakanidze, T. Gomelauri, E. Zhgenti, G. Chanturia, M. Kekelidze, C. Rajanna, A. Kreger and A. Sulakvelidze. 2006. Genetic background and antibiotic resistance of *Staphylococcus aureus* strains isolated in the republic of Georgia. J. Clin. Microbiol. 44:3477-3483.

Reynolds, R., J. Shackcloth, D. Felmingham and A. MacGowan. 2003. Comparison of BSAC agar dilution and NCCLS broth microdilution MIC methods for *in vitro* susceptibility testing of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*: the BSAC Respiratory Resistance Surveillance Programme. J. Antimicrob. Chemother. 52:925-930.

Rice, L. B., E. C. Eckstein, J. DeVente and D. M. Shlaes. 1996. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. Clin. Infect. Dis. 23:118-124.

Riddell, C. 1980. A survey of skeletal disorders in five turkey flocks in Saskatchewan. Can. J. Comp. Med. 44:275-279.

Rogers, J. 1991. Pass the cranberry juice. Nurs. Times. 27(87):36-37.

Rolinson, G. N. 1994. A review of the microbiology of amoxycillin/clavulanic acid over the 15 year period 1978-1993. J. Chemother. 6:283-318.

Ruel, G., S. Pomerleau, P. Couture, B. Lamarche and C. Couillard. 2005. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. Metab. Clin. Exp. 54:856-861.

Ruel, G., S. Pomerleau, P. Couture, S. Lemieux, B. Lamarche and C. Couillard. 2006. Favourable impact of low-calorie cranberry juice consumption on plasma HDL-cholesterol concentrations in men. Br. J. Nutr. 96:357-364.

Russ, J. C. 2007. *The image processing handbook.* 5th ed. Taylor & Francis Group. New York. 817 p.

Sader, H. S., R. N. Jones, K. L. Rossi and M. J. Rybak. 2009. Occurrence of vancomycin-tolerant and heterogeneous vancomycin-intermediate strains (hVISA) among *Staphylococcus aureus* causing bloodstream infections in nine USA hospitals. J. Antimicrob. Chemother. Sept 10. [Epub ahead of print]

Salyers, A. A., N. B. Shoemaker, A. M. Stevens and L.-Y. Li. 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. Microbiol. Rev. 59:579-590.

Schmidt, D. R. and A. E. Sobota. 1988. An examination of the anti-adherence activity of cranberry juice on urinary and nonurinary bacterial isolates. Microbios. 55:173-181.

Seishima, M., G. Kato, Y. Shibuya and S. Matsukawa. 2009. Cytokine profile during the clinical course of toxic shock syndrome. Clin. Exp. Dermatol. June 22. [Epub ahead of print]

Sharon, N. and I. Ofek. 2002. Fighting infectious diseases with inhibitors of microbial adhesion to host tissues. Crit. Rev. Food Sci. Nutr. 42(Suppl):267-272.

Shiau, A. L. and C. L. Wu. 1998. The inhibitory effect of *Staphylococcus epidermidis* slime on the phagocytosis of murine peritoneal macrophages is interferon-independent. Microbiol. Immunol. 42:33-40.

Shimoda, M., K. Ohki, Y. Shimamoto and O. Kohashi. 1995. Morphology of defensin-treated *Staphylococcus aureus*. Infect. Immun. 63:2886-2891.

Sifri, C. D., J. Begun, F. M. Ausubel and S. B. Calderwood. 2003. *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. Infect. Immun. 71:2208-2217.

Simpson, I., J. Durodie, S. Knott, B. Shea, J. Wilson and K. Machka. 1998. Effects of following National Committee for Clinical Laboratory Standards and Duetsche Industrie Norm-Medizinische Mikrobiologie guidelines, country of isolate origin, and site of infection on susceptibility of *Escherichia coli* to amoxicillin-clavulanate (Augmentin). J. Clin. Microbiol. 36:1361-1365.

Singh, A. P., R. K. Singh, K. K. Kim, K. S. Satvan, R. Nussbaum, M. Torres, L. Brard and N. Vorsa. 2009. Cranberry proanthocyanidins are cytotoxic to human cancer cells and sensitize platinum-resistant ovarian cancer cells to paraplatin. Phytother. Res. 23:1066-1074.

Smeltzer, M. S. and A. F. Gillaspy. 2000. Molecular pathogenesis of staphylococcal osteomyelitis. Poult. Sci. 79:1042-1049.

Sobota, A. E. 1984. Inhibition of bacterial adherence by cranberry juice: potential use for the treatment of urinary tract infections. J. Urol. 131:1013-1016.

Speer, B. S., N. B. Shoemaker and A. A. Salyers. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin. Microbiol. Rev. 5:387-399.

Stapleton, A. 2005. Novel mechanism of P-fimbriated *Escherichia coli* virulence in pyelonephritis. J. Am. Soc. Nephrol. 16:3458-3460.

Statistical Analysis System. 2000. Release 8.02. SAS Institute Inc., Cary NC.

Steinberg, D., M. Feldman, I. Ofek and E. I. Weiss. 2004. Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm. J. Antimicrob. Chemother. 54:86-89.

Steinberg, D., M. Feldman, I. Ofek and E. I. Weiss. 2005. Cranberry high molecular weight constituents promote *Streptococcus sobrinus* desorption from artificial biofilm. Int. J. Antimicrob. Agents. 25:247-251.

Stepanovic, S., D. Vukovic, V. Hola, G. Di Bonaventura, S. Djukic, I. Cirkovic and F. Ruzicka. 2007. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 115:891-899.

Sternlieb, P. 1963. Cranberry juice in renal disease. N. Engl. J. Med. 268:57.

Stevens, A. M., N. B. Shoemaker, L.-Y. Li and A. A. Salyers. 1993. Tetracycline regulation of genes on *Bacteroides* conjugative transposons. J. Bacteriol. 175:6134-6141.

Stewart, P. S. 2002. Mechanisms of antibiotic resistance in bacterial biofilms. Int. J. Med. Microbiol. 292:107-113.

Stewart, P. S. and J. W. Costerton. 2001. Antibiotic resistance of bacteria in biofilms. Lancet. 358:135-138.

Stothers, L. 2002. A randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women. Can. J. Urol. 9:1158-1162.

Styriak, I., A. Laukova, C. Fallgren and T. Wadstrom. 1999. Binding of selected extracellular matrix proteins to enterococci and *Streptococcus bovis* of animal origin. Curr. Microbiol. 39:327-335.

Styriak, I., A. Laukova and A. Ljungh. 2002. Binding of extracellular matrix molecules by staphylococci from wild herbivores. Acta Vet. Brno. 71:369-374.

Sun, J., Y.-F. Chu, X. Wu and R. H. Liu. 2002. Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem. 50:7449-7454.

Tacconelli, E. 2009. Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. Curr. Opin. Infect. Dis. 22:352-358.

Thorp, B. H. and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Tomlinson, S. and E. A. Palombo. 2005. Characterisation of antibacterial Australian medicinal plant extracts by investigation of the mechanism of action and the effect of interfering substances. J. Basic Microbiol. 45:363-370.

Torres, O. R., R. Z. Korman, S. A. Zahler and G. M. Dunny. 1991. The conjugative transposon Tn*925*: enhancement of conjugal transfer by tetracycline in *Enterococcus faecalis* and mobilization of chromosomal genes in *Bacillus subtilis* and *E. faecalis*. Mol. Gen. Genet. 225:395-400.

Tortora, G. J., B. R. Funke and C. L. Case. 2001. *Microbiology: an introduction.* 7th ed. Benjamin Cummings; Addison Wesley Longman, Inc. Toronto. 887 p.

Tre-Hardy, M., F. Vanderbist, H. Traore and M. J. Devleeschouwer. 2008. *In vitro* activity of antibiotic combinations against *Pseudomonas aeruginosa* biofilm and planktonic cultures. Int. J. Antimicrob. Agents. 31:329-336.

Trivier, D. and R. J. Courcol. 1996. Iron depletion and virulence in *Staphylococcus aureus*. FEMS Microbiol. Lett. 141:117-127.

Turner, A., S.-N. Chen, M. Joike, S. Pendland, G. Pauli and N. Farnsworth. 2005. Inhibition of uropathogenic *Escherichia coli* by cranberry juice: a new antiadherence assay. J. Agric. Food Chem. 53:8940-8947.

Vaisanen-Rhen, V., J. Elo, E. Vaisanen, A. Siitonen, I. Orskov, F. Orskov, S. B. Svenson, P. H. Makela and T. K. Korhonen. 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun. 43:149-155.

Valentine, P. J., N. B. Shoemaker and A. A. Salyers. 1988. Mobilization of *Bacteroides* plasmids by *Bacteroides* conjugal elements. J. Bacteriol. 170:1319-1324.

Valentova, K., D. Stejskal, P. Bednar, J. Vostalova, C. Cihalik, R. Vecerova, D. Koukalova, M. Kolar, R. Reichenbach, L. Sknouril, J. Ulrichova and V. Simanek. 2007. Biosafety, antioxidant status, and metabolites in urine after consumption of dried cranberry juice in healthy women: a pilot double-blind placebo-controlled trial. J. Agric. Food Chem. 55:3217-3224.

van den Bogaard, A. E., P. Mertens, N. H. London and E. E. Stobberingh. 1997. High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in The Netherlands: is the addition of antibiotics to animal feeds to blame? [letter]. J. Antimicrob. Chemother. 40: 454-456.

van den Bogaard, A. E., N. Bruinsma and E. E. Stobberingh. 2000. The effect of banning avoparcin on VRE carriage in The Netherlands. J. Antimicrob. Chemother. 46:145-153.

van den Bogaard, A. E. and E. E. Stobberingh. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs. 58:589-607.

Van der Auwera, P., N. Pensart, V. Korten, B. E. Murray and R. Leclercq. 1996. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. J. Infect. Dis. 173:1129-1136.

Varnam, A. H. and M. G. Evans. 1991. *Foodborne pathogens: an illustrated text.* Mosby-Year Book, Inc. Toronto. 557 p.

Vasudevan, P., M. Nair, T. Annamalai and K. Venkitanarayanan. 2003. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet. Microbiol. 92:179-185.

Vattem, D. A., Y.-T. Lin, R. G. Labbe and K. Shetty. 2004a. Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bioprocessing using the food grade fungus *Rhizopus oligosporus*. Process Biochem. 39:1939-1946.

Vattem, D. A., Y.-T. Lin, R. G. Labbe and K. Shetty. 2004b. Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus *Lentinus edodes* and effect on antimicrobial activity against select food borne pathogens. Innovat. Food Sci. Emerg. Technol. 5:81-91.

Vattem, D. A., R. Ghaedian and K. Shetty. 2005a. Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. Asia Pac. J. Clin. Nutr. 14:120-130.

Vattem, D. A., Y.-T. Lin, R. Ghaedian and K. Shetty. 2005b. Cranberry synergies for dietary management of *Helicobacter pylori* infections. Process Biochem. 40:1583-1592.

Walker, E. B., D. P. Barney, J. N. Mickelsen, R. J. Walton and R. A. Mickelsen. 1997. Cranberry concentrate: UTI prophylaxis. J. Fam. Pract. 45:167-168.

Weiss, E. I., A. Kozlovsky, D. Steinberg, R. Lev-Dor, R. B. N. Greenstein, M. Feldman, N. Sharon and I. Ofek. 2004. A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits *in vitro* adhesion to hydroxyapatite. FEMS Microbiol. Lett. 232:89-92.

White, D. G., S. Ayers, J. J. Maurer, S. G. Thayer and C. Hofacre. 2003. Antimicrobial susceptibilities of *Staphylococcus aureus* isolated from commercial broilers in northeastern Georgia. Avian Dis. 47:203-210.

Whitman, M. S., P. G. Pitsakis, E. DeJesus, A. J. Osborne, M. E. Levison and C. C. Johnson. 1996. Gastrointestinal tract colonization with vancomycin-resistant *Enterococcus faecium* in an animal model. Antimicrob. Agents Chemother. 40:1526-1530.

Wilson, T., J. P. Porcari and D. Harbin. 1998. Cranberry extract inhibits low density lipoprotein oxidation. Life Sci. 62, 381-386.

Wohlleben, W., E. Stegmann and R. D. Sussmuth. 2009. Complex enzymes in microbial natural product biosynthesis, Part A: Overview articles and peptides. Chapter 18: Molecular genetic approaches to analyze glycopeptide biosynthesis. Meth. Enzymol. 458:459-486.

Wu, J., C. Kusuma, J. Mond and J. Kokai-Kun. 2003. Lysostaphin disrupts *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms on artificial surfaces. Antimicrob. Agents Chemother. 47:3407-3414.

Wu, V. C. H., X. Qiu, A. Bushway and L. Harper. 2008. Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. LWT – Food Sci. Technol. 41:1834-1841.

Wu, V. C. H., X. Qiu, B. G. de los Reyes, C.-S. Lin and Y. Pan. 2009. Application of cranberry concentrate (*Vaccinium macrocarpon*) to control *Escherichia coli* 0157:H7 in ground beef and its antimicrobial mechanism related to the downregulated *slp*, *hdeA* and *cfa*. Food Microbiol. 26:32-38.

Wust, J. and U. Hardegger. 1992. Comparison of the E test and a reference agar dilution method for susceptibility testing of anaerobic bacteria. Eur. J. Clin. Microbiol. Infect. Dis. 11:1169-1173.

Wyber, J.-A., J. Andrews and P. Gilbert. 1994. Loss of salt-tolerance and transformation efficiency in *Escherichia coli* associated with sub-lethal injury by centrifugation. Lett. Appl. Microbiol. 19:312-316.

Yamanaka, A., R. Kimizuka, T. Kato and K. Okuda. 2004. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol. Immun. 19:150-154.

Yamanaka, A., T. Kouchi, K. Kasai, T. Kato, K. Ishihara and K. Okuda. 2007. Inhibitory effect of cranberry polyphenol on biofilm formation and cysteine proteases of *Porphyromonas gingivalis*. J. Periodont. Res. 42:589-592.

Yan, X., B. T. Murphy, G. B. Hammond, J. A. Vinson and C. C. Neto. 2002. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). J. Agric. Food Chem. 50:5844-5849.

Yarwood, J. M., D. J. Bartels, E. M. Volper and E. P. Greenberg. 2004. Quorum sensing in *Staphylococcus aureus* biofilms. J. Bacteriol. 186:1838-1850.

Yates, R. R. 1999. New intervention strategies for reducing antibiotic resistance. Chest. 115(Suppl 1):24S-27S.

Yoshida, S., N. Ogawa, T. Fujii and S. Tsushima. 2009. Enhanced biofilm formation and 3-chlorobenzoate degrading activity by the bacterial consortium of *Burkholderia* sp. NK8 and *Pseudomonas aeruginosa* PA01. J. Appl. Microbiol. 106:790-800.

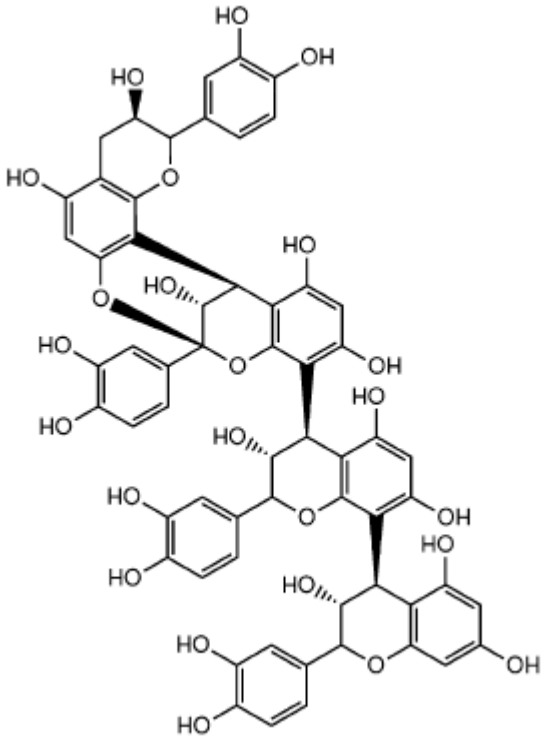
Zadoks, R., W. van Leeuwen, H. Barkema, O. Sampimon, H. Verbrugh, Y. H. Schukken and A. van Belkum. 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J. Clin. Microbiol. 38:1931-1939.

Zafriri, D., I. Ofek, R. Adar, M. Pocino and N. Sharon. 1989. Inhibitory activity of cranberry juice on adherence of Type 1 and Type P fimbriated *Escherichia coli* to eucaryotic cells. Antimicrob. Agents Chemother. 33:92-98.

Zhang, L., J. Ma, K. Pan, V. L. W. Go, J. Chen and W. You. 2005. Efficacy of cranberry juice on *Helicobacter pylori* infection: a double-blind, randomized placebo-controlled trial. Helicobacter. 10:139-145.

APPENDICES

Appendix A: Structure of a typical cranberry proanthocyanidin tetramer composed of epicatechin units with one A-type linkage and two B-type linkages. (Neto, 2007b)



Appendix B: Ingredient specifications of the undialysed cranberry extract[1.]

Ingredient specification	Typical range
Cranberry fruit solids (%)	90% minimum
Moisture	NMT 5%
pH (10% solution)	3.7 – 4.3
Titratable acidity (w/w)	8.5 – 13%
Bulk density (tapped)	0.5 +/- 0.1 g/ml
Organic acids	35% (9% quinic acid typical)
Total phenolics	2.00 – 3.80%
Total anthocyanins	0.15 – 1.00%
Total proanthocyanidins	0.95% maximum
Antioxidant (ORAC)	175 – 400 µmol Trolox/g
Antioxidant (DPPH)	55 – 70% radical inhibition
Ellagic acid	200 – 415 µg/g
Quercetin	300 – 435 µg/g
Yeast and mould	< 50/g
Standard plate count	< 500/g
E. coli	Negative/25g
Salmonella	Negative/25g
Coliform	< 10/g

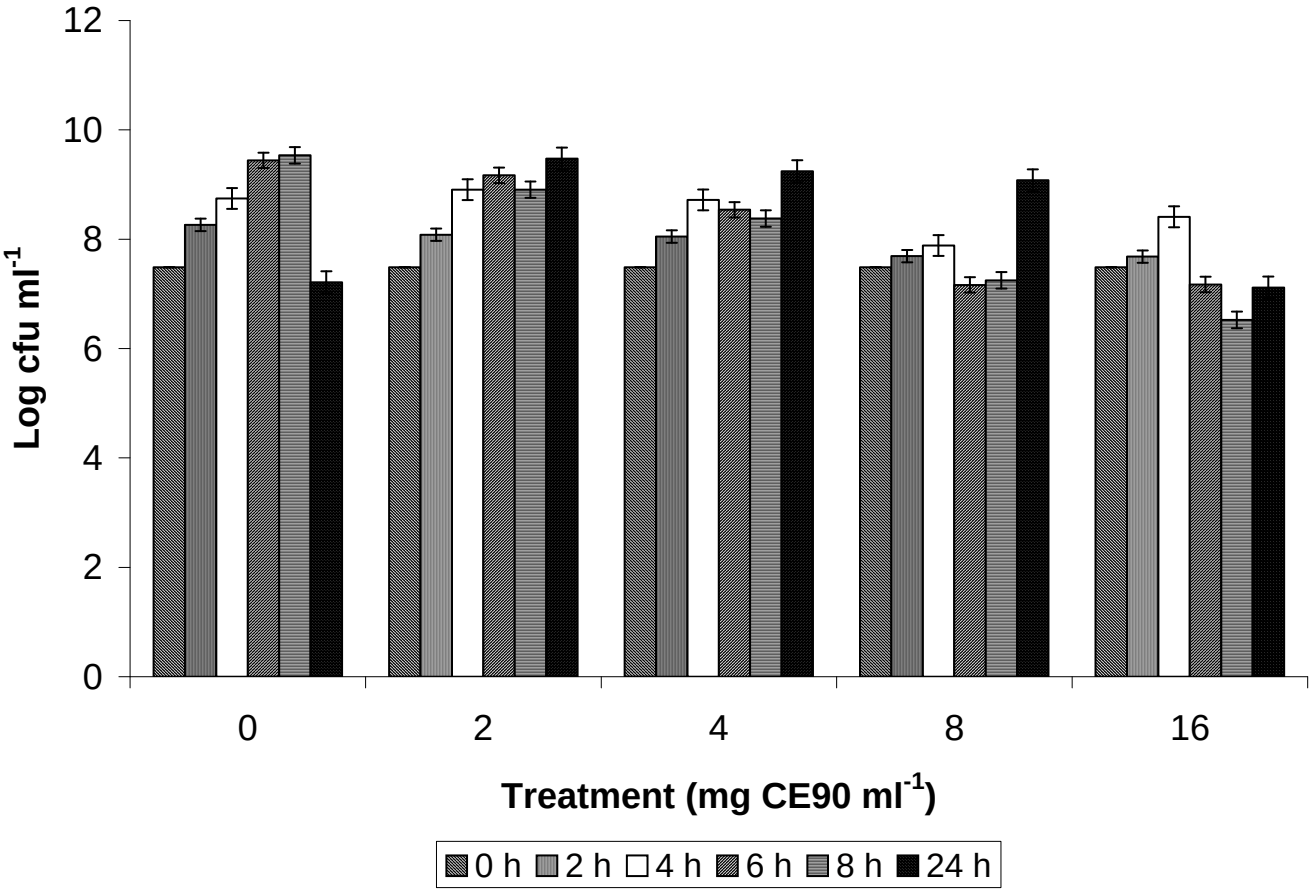
[1.] Undialysed cranberry extract = the cranberry extract provided by Decas Botanical Synergies, which was used in the preparation of a high-molecular-weight NDM (CE90) tested against *Staph. aureus*.

Appendix C: Biofilm formation of 24 *Staph. aureus* isolates in the absence of CE90[1.]

Isolate	OD_{570nm}
SHY97-3906	0.052
SHY97-3923-3	-0.006
SHY97-4085	0.123
SHY97-4242	0.1215
SHY97-4320	0.083
SHY97-4343	0.152
ATCC 25923	0.1205
PC1	0.262
NCTC 9789	0.0815
2076	0.004
22260	-0.0115
ST79/741	0.01
3804 (pII)	0.0945
RN9	0.276
FAR 8	0.031
FAR 10	0.0855
3906 PGR	0.1895
SA 113	0.784
8325-4	0.262
8325-4 *fnbA*$^-$/*fnbB*$^-$	0.2055
8325-4 *clfA*$^-$	0.3035
SHY99-730-2	0.2155
MRSA	0.105
ATCC 29213	0.292

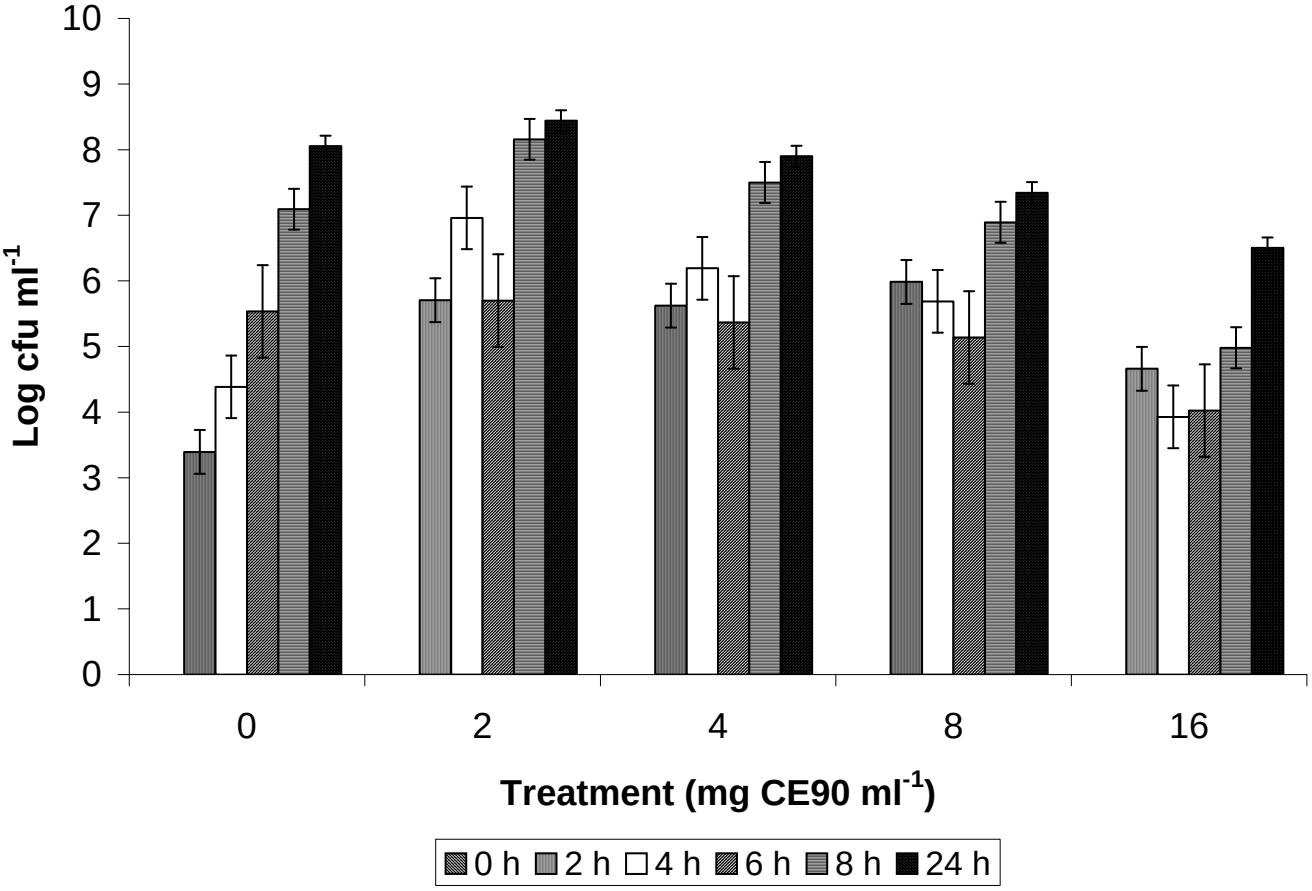
[1.] Values represent optical density measurements at 570nm, in a preliminary experiment.

Appendix D.1: Effect of CE90 on planktonic *Staph. aureus* PC1 growth[1.]



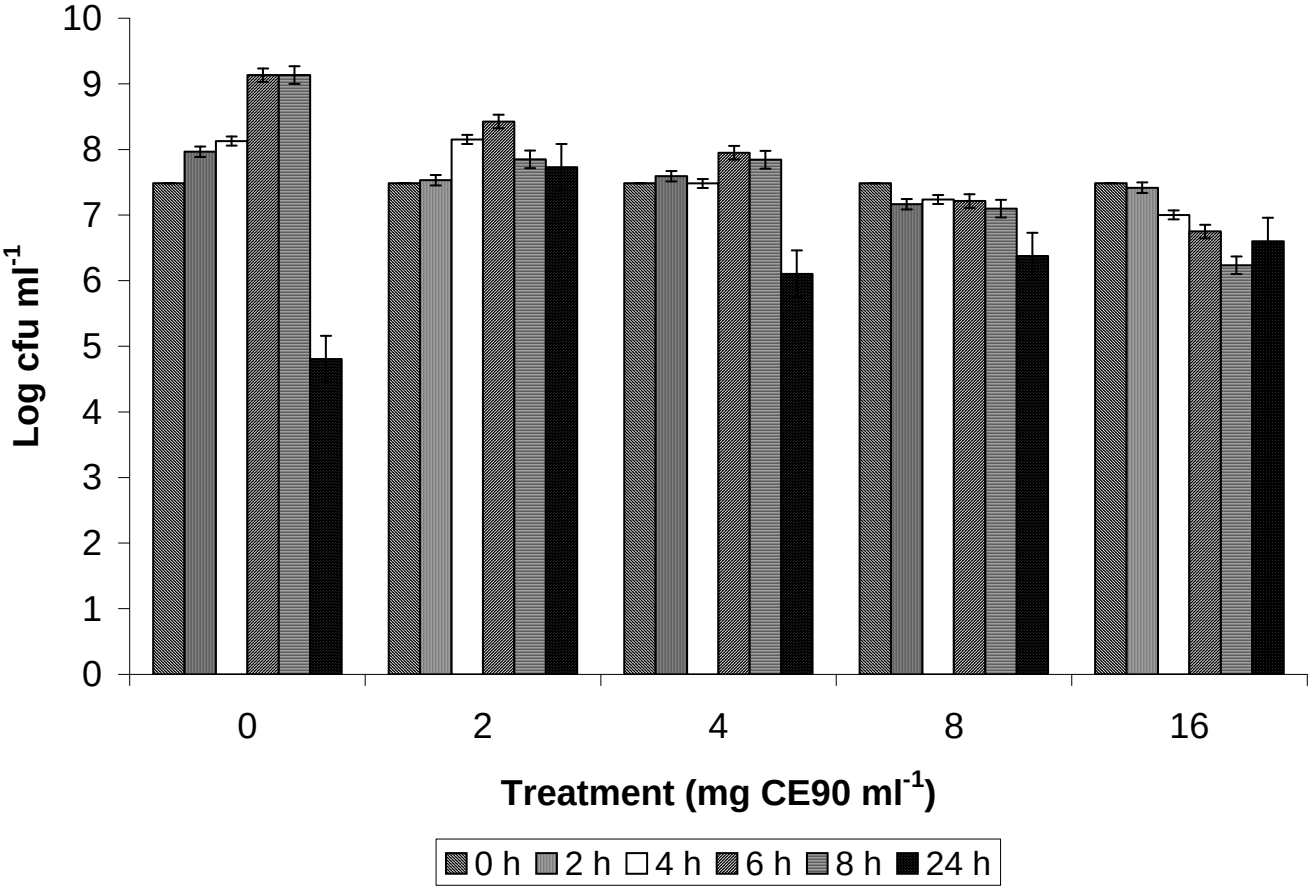
[1.] Planktonic cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.2: Effect of CE90 on *Staph. aureus* PC1 biofilm formation[1.]



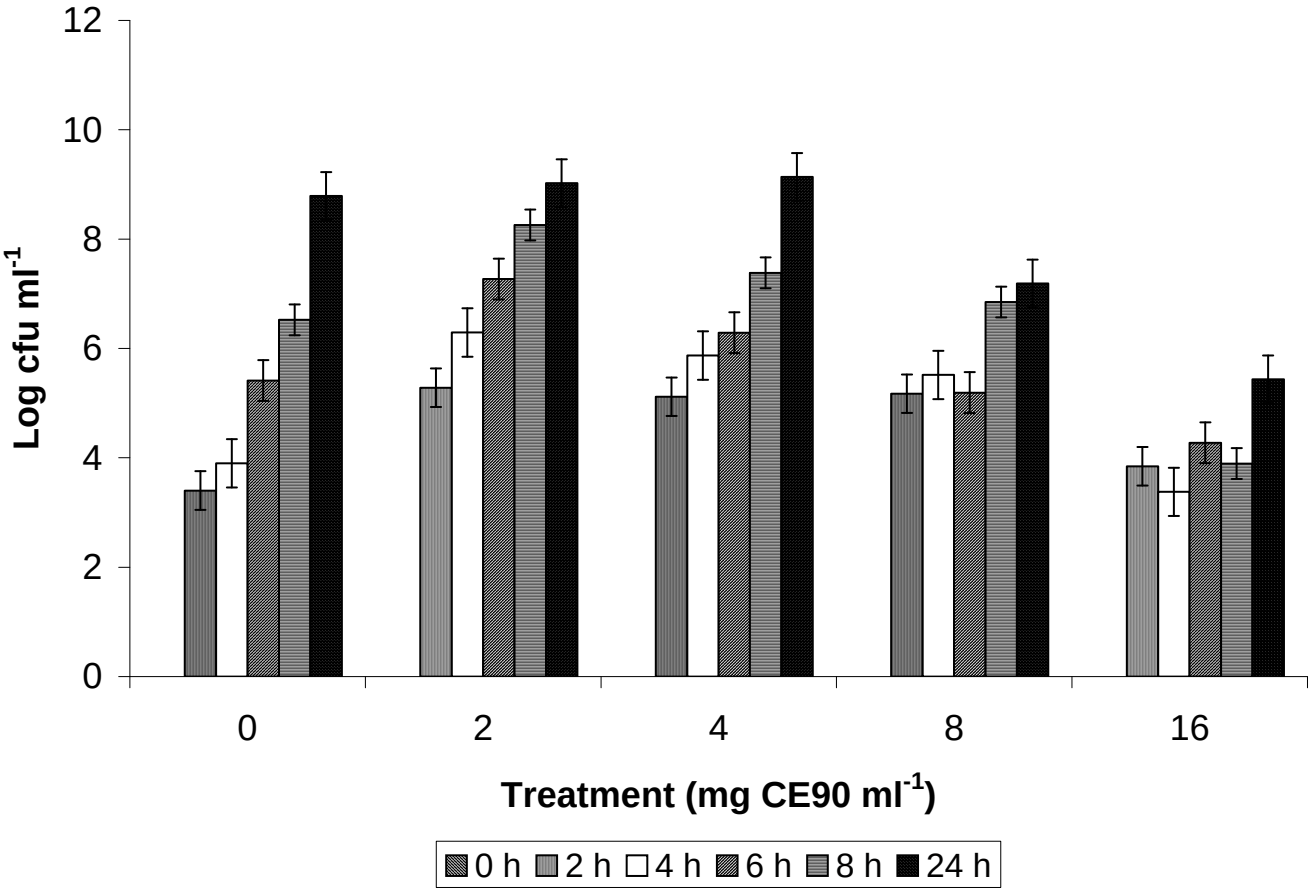
[1.] Biofilm cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.3: Effect of CE90 on planktonic *Staph. aureus* SA 113 growth[1.]



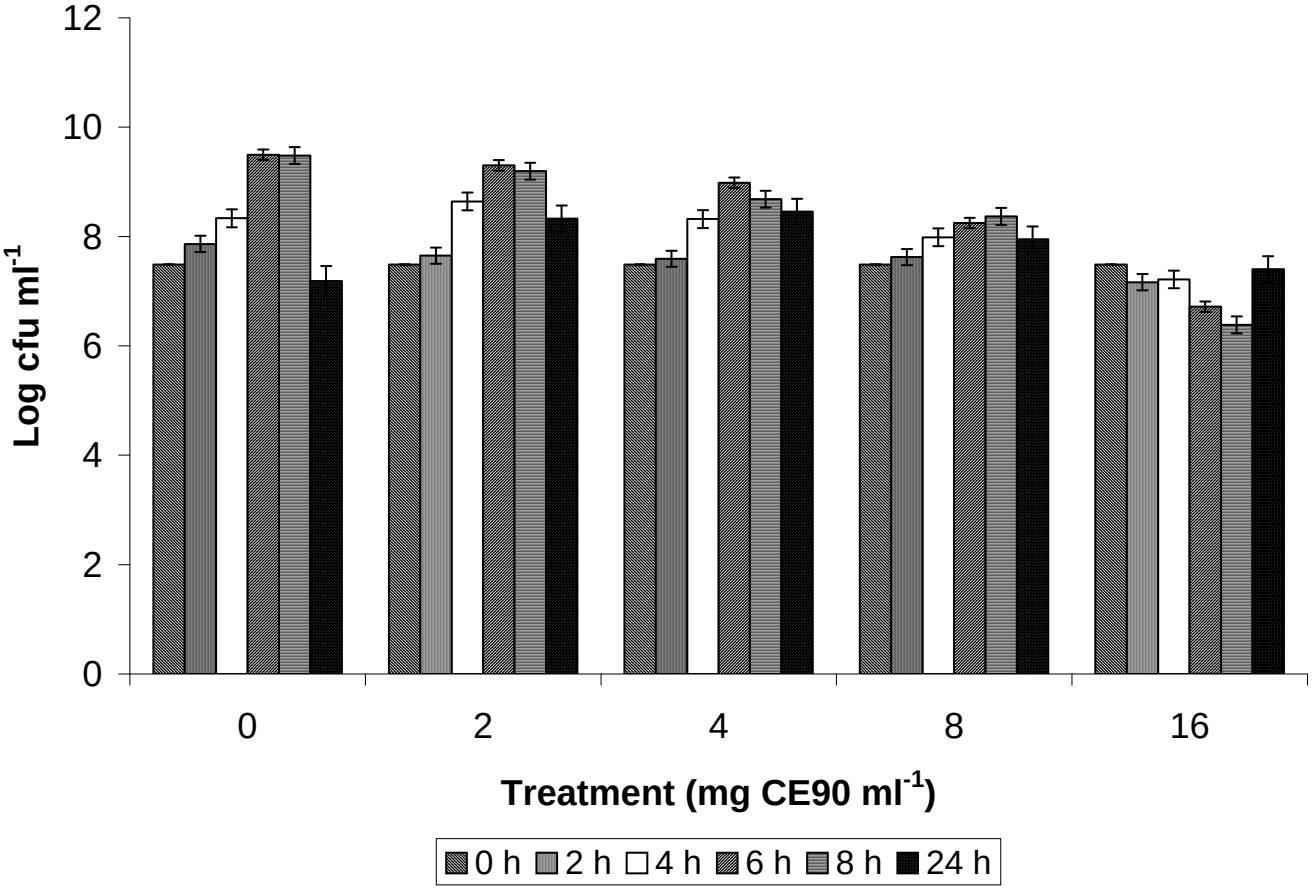
[1.] Planktonic cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.4: Effect of CE90 on *Staph. aureus* SA 113 biofilm formation[1.]



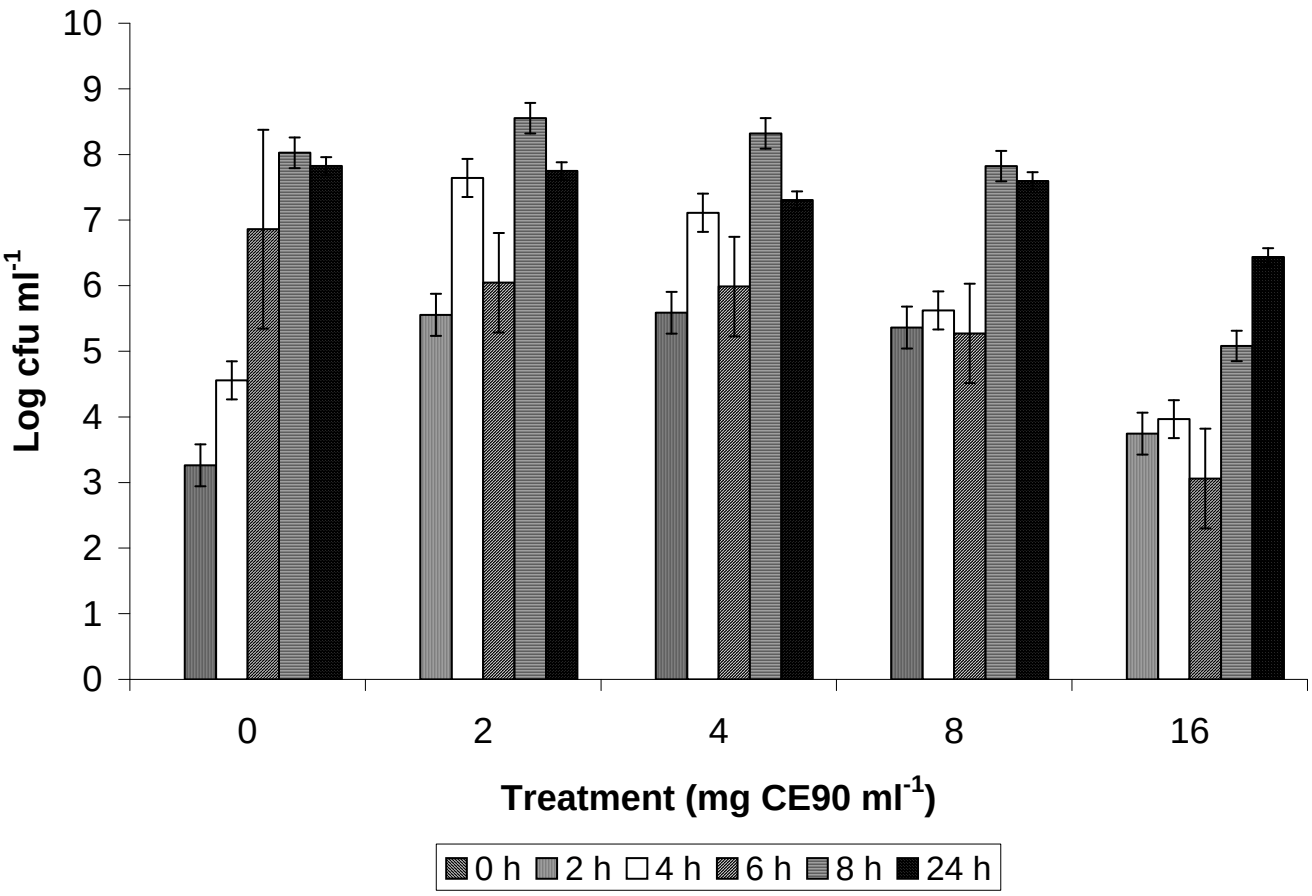
[1.] Biofilm cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.5: Effect of CE90 on planktonic *Staph. aureus* 8325-4 growth[1.]



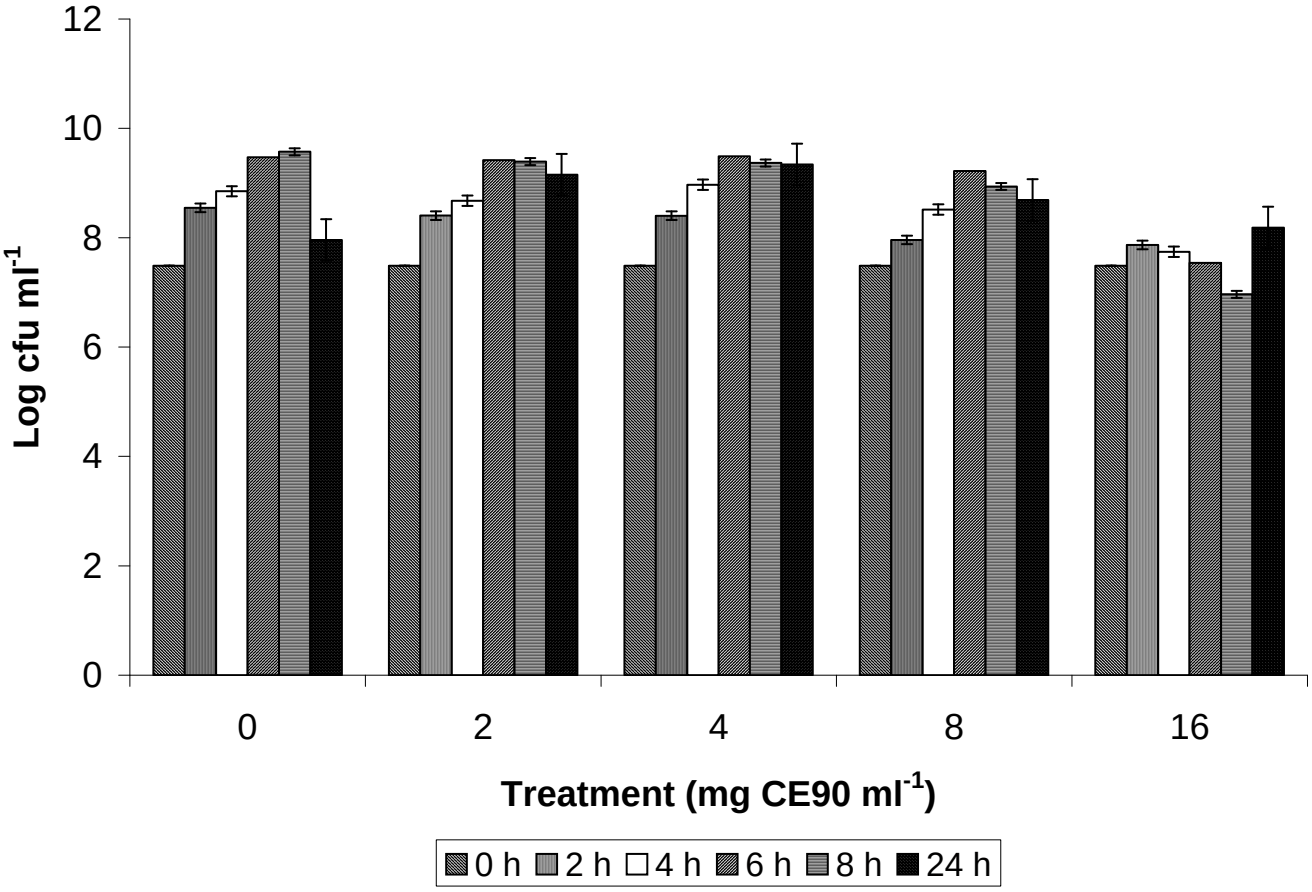
[1.] Planktonic cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.6: Effect of CE90 on *Staph. aureus* 8325-4 biofilm formation[1.]



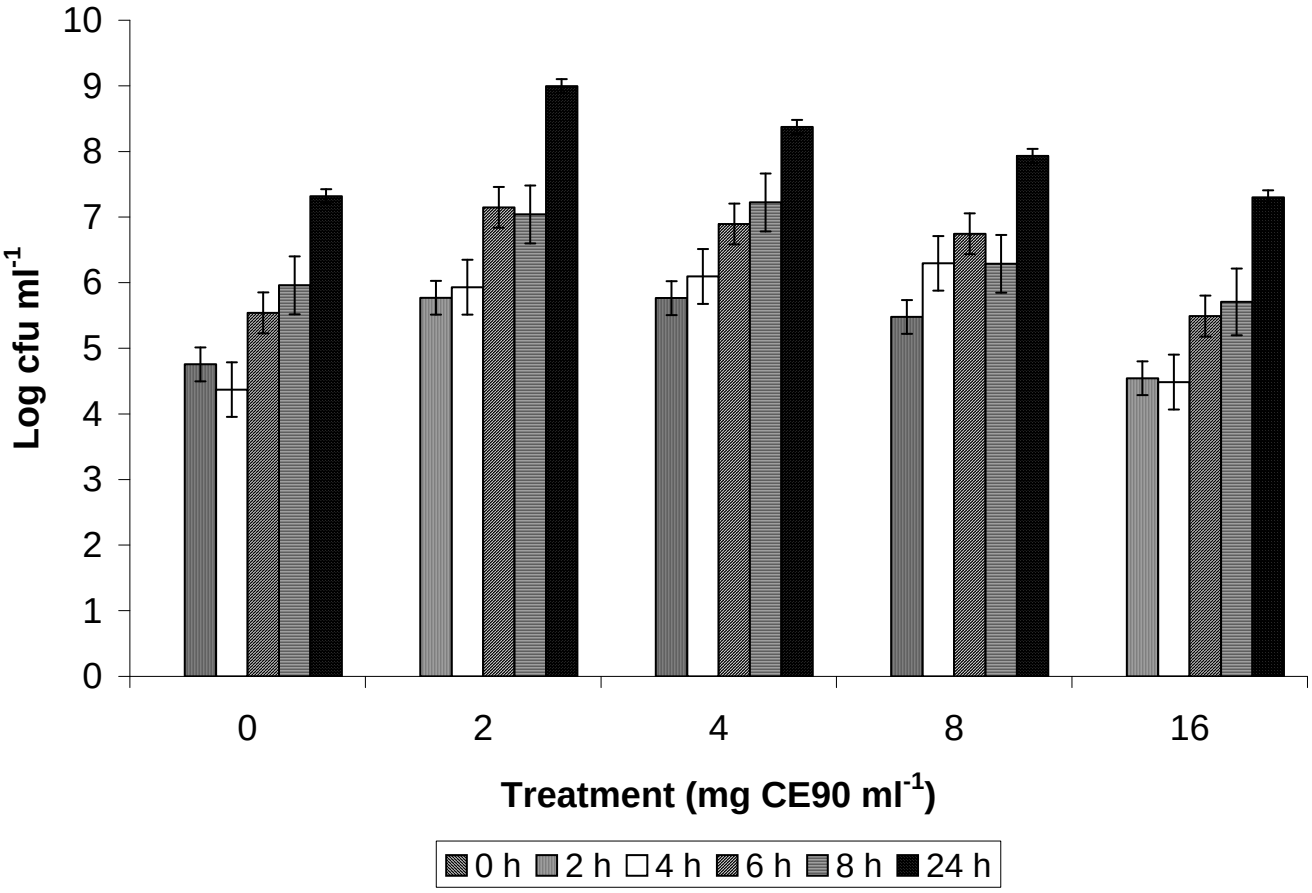
[1.] Biofilm cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.7: Effect of CE90 on planktonic *Staph. aureus* 2076 growth[1.]



[1.] Planktonic cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.

Appendix D.8: Effect of CE90 on *Staph. aureus* 2076 biofilm formation[1.]



[1.] Biofilm cfu ml^{-1} values of the biofilm index experiment over 24 h. Error bars represent the standard error of two repeats performed in duplicate.