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BIOFILM FORMATION OF
ENTEROBACTER SAKAZAKII **ON THREE**
DIFFERENT MATERIALS OF INFANT
FEEDING TUBE

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2009

**BIOFILM FORMATION OF
ENTEROBACTER SAKAZAKII ON THREE DIFFERENT MATERIALS
OF INFANT FEEDING TUBE**

A THESIS PRESENTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENT S
FOR THE DEGREE OF MASTER OF TECHNOLOGY IN FOOD MICROBIOLOGY
AT MASSEY UNIVERSITY, PALMERSTON NORTH, NEW ZEALAND

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2009

IN LOVING MEMORIES OF MY BELOVED DAD

MD ZAIN BIN ABDULLAH

(1955 - 2003)

YOU ARE ALWAYS IN MY HEART

ABSTRACT

The aim of this study was to observe biofilm formation by *Enterobacter sakazakii* (*E. Sakazakii*) from different clinical, dairy and environmental origins on three infant feeding tubes made of different materials. Infant formula milk was selected as the medium for *E. sakazakii* growth.

Seventeen isolates from different origins were retrieved and tested for purity, using a plating method and biochemical tests to eliminate the non *E. sakazakii* strains from this study. A method to rapidly and accurately detect viable cells of *E. sakazakii* on infant feeding tube surfaces using of the BacTrac® 4000 microbiological growth analyser was developed. The sources of errors such as from cleaning, operation and handling procedures were assessed prior to experimental runs.

The strength of biofilm formation by different isolates of *E. sakazakii* on plastic surfaces was scrutinised using a microtiter plate assay. The results from the microtitre plate assay were based on the absorbance at 550 nm of crystal violet stained films and showed that all the clinical isolates were able to attach and form strong biofilms on the plate. Some environmental isolates formed strong or weak biofilms and some did not produce biofilm at all. However, dairy isolates formed both strong and weak biofilms in the microtitre plate when incubated in 10% reconstituted infant formula milk.

The further studies were to quantify biofilm formation by three isolates of different origin on three different materials of infant feeding tubes using a batch system. Tubing pieces were incubated with infant formula milk inoculated with *E. sakazakii* cells at approximately $8 \log \text{CFU mL}^{-1}$ and the biofilm formation was assessed at three time intervals: 4, 12 and 24 hours. Biofilm formation on the

tubing by clinical isolates was also observed using epifluorescence microscopy and the scanning electron microscope.

E. sakazakii from clinical, dairy and environmental isolates were able to form biofilm on three different materials of infant feeding tubes. The results showed that the initial attachment at 4 h on silicone tubing was low compared with the other two tubes. The scanning electron micrographs showed the surface characteristics of each tubing and the biofilm formation by *E. sakazakii* clinical isolates after 4, 12 and 24 hours. Silicone tubing appeared to be the best choice for premature babies that need feeding using feeding tubes, as it was slow to become colonised compared with the PVC and polyurethane tubing.

LIST OF PRESENTATIONS

This work has been presented in part in the following presentations at scientific conferences:

1. Zain, S. N. M, Flint, S. H., Brooks, J. D. (2008). Assessment of *Enterobacter sakazakii* growth on three types of infant feeding tubes. *14th World Congress of Food Science and Technology*, Shanghai, China, October 2008.
2. Zain, S. N. M, Flint, S. H., Brooks, J. D. (2008). Differences in attachment of *Enterobacter sakazakii* in reconstituted infant formula with respect to origin. *New Zealand Microbiological Society conference*, Christchurch, November 2008.

ACKNOWLEDGEMENTS

I am indebted to my main supervisor, Associate Professor Dr. Steve Flint, Director (Food Science and Technology), Institute of Food, Nutrition and Human Health, Massey University, who provided much information and encouragement, who was always keen to meet, discuss and question the project and also inspired me to be a dedicated researcher.

I am also thankful to my co-supervisor, Professor John Brooks from Auckland University of Technology, Auckland, who guided me since I started my postgraduate diploma in Food Technology at Massey University. He also provided his expert guidance in the realm of microbiology, especially biofilms, and promptly and thoroughly reviewed my scripts.

I gratefully acknowledge the Massey University Institute of Food Nutrition and Human Health for providing an excellent laboratory and the support technicians Ann-Marie Jackson, Jon Palmer and Judy Collins.

I am indebted to my sponsor Universiti Teknologi Mara and the Malaysia government for funding my study at Massey University for two and half years. I have learnt much knowledge and I believe that I can contribute something to the University when I return to start lecturing in future years.

Special thanks to Doug Hopcroft and Dmitry Sokolov from Manawatu Microscopy Imaging centre who provided expert assistance in scanning electron microscopy and confocal laser microscopy and image capture.

This work could not have succeeded without the support of my family; my mother, Norhayati Hamid, brother and sister that always believed in me that I am capable to be independent in New Zealand. Last but not least, to my beloved husband, Mohamed Ridzuan who was always there during my ups and downs.

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