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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

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SITI NORBAIZURA BINTI MD ZAIN

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 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.

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