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

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

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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.

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LIST OF CONTENTS

| | Page |
|---|-------------|
| ABSTRACT | i |
| LIST OF PRESENTATIONS | iii |
| ACKNOWLEDGEMENTS | iv |
| LIST OF CONTENTS | v |
| LIST OF FIGURES | ix |
| LIST OF TABLES | xii |
| | |
| CHAPTER 1 INTRODUCTION | 1 |
| | |
| CHAPTER 2 <i>E. SAKAZAKII</i> ASSOCIATION WITH FATAL OUTBREAKS AMONG NEONATES - A DESCRIPTION OF THE BACTERIUM AND BIOFILM FORMATION ABILITY, INFANT FORMULA AND FEEDING TUBE AND CURRENT CONCERNS REGARDING THIS ISSUE – A REVIEW OF THE LITERATURE | 3 |
| | |
| 2.0 Introduction | 4 |
| 2.1 <i>Enterobacter sakazakii</i> (<i>E. sakazakii</i>) | 4 |
| 2.1.1 Genotype and phenotype | 6 |
| 2.1.2 Sources - clinical, food and environment | 8 |
| 2.1.3 Characteristics of <i>E. sakazakii</i> - capsulated or non-capsulated | 10 |
| 2.1.4 Survival and growth characteristics | 10 |
| 2.1.5 Virulence factors and pathogenicity | 11 |
| 2.1.6 Disease associated with <i>E. sakazakii</i> | 11 |
| 2.1.7 Hazard identification | 13 |
| 2.2 Infant formula | 14 |

| | | |
|------------------|--|-----------|
| 2.2.1 | Composition | 14 |
| 2.2.2 | Sterility of infant formula milk powder | 15 |
| 2.3 | Infant feeding tube | 17 |
| 2.3.1 | Feeding tubes | 19 |
| 2.3.2 | Methods of feeding / delivery | 19 |
| 2.3.3 | Material of the tubes - polyvinyl chloride, polyurethane and silicone | 22 |
| 2.3.4 | Importance of microbial colonisation of infant feeding tubes | 24 |
| 2.4 | Conclusions | 25 |
| | | |
| CHAPTER 3 | MATERIALS AND METHODS | 27 |
| | | |
| 3.1 | Source of isolates | 28 |
| 3.2 | Maintenance of <i>E. sakazakii</i> culture | 29 |
| 3.3 | Culture preparation | 30 |
| 3.4 | Screening method for strain purity | 30 |
| 3.4.1 | TSA and skim milk plate count agar | 30 |
| 3.4.2 | Gram staining | 30 |
| 3.4.3 | API 20E | 31 |
| 3.4.4 | Capsule staining procedure | 31 |
| 3.5 | Microtitre plate assay | 31 |
| 3.6 | Enumeration of <i>E. sakazakii</i> , using impedance detection with the BacTrac® 4000 | 32 |
| 3.6.1 | Operation of the BacTrac® 4000 | 32 |
| 3.6.2 | Cleaning procedure | 32 |
| 3.6.3 | Handling error | 33 |
| 3.6.4 | Calibration curve of BacTrac® 4000 | 33 |
| 3.6.5 | Plate counts | 33 |
| 3.7 | Biofilm development | 34 |
| 3.7.1 | Tube preparation | 34 |
| 3.7.2 | Inoculum preparation | 34 |
| 3.7.3 | Inoculation media - 10% reconstituted IFM | 34 |
| 3.7.4 | Incubation | 35 |

| | | |
|------------------|---|-----------|
| 3.8 | Detection methods | 35 |
| 3.8.1 | Epifluorescence microscopy | 35 |
| 3.8.2 | Capacitance detection | 36 |
| 3.8.3 | Scanning Electron Microscopy (SEM) | 36 |
| 3.9 | Accuracy and reproducibility | 36 |
| 3.10 | Data analysis | 36 |
| | | |
| CHAPTER 4 | IDENTIFICATION AND ATTACHMENT OF <i>ENTEROBACTER SAKAZAKII</i> - PRELIMINARY RESULTS | 37 |
| | | |
| 4.1 | Introduction - biofilms | 38 |
| 4.2 | Results and discussions | 41 |
| 4.2.1 | Isolation and identification of <i>E. sakazakii</i> | 41 |
| 4.2.2 | 10% reconstituted Infant Formula Milk (IFM) | 46 |
| 4.2.3 | Microtitre plate assay | 48 |
| 4.3 | Conclusions | 56 |
| | | |
| CHAPTER 5 | BIOFILM FORMATION BY <i>ENTEROBACTER SAKAZAKII</i> ON INFANT FEEDING TUBES | 57 |
| | | |
| 5.1 | Introduction | 58 |
| 5.2 | Procedures | 60 |
| 5.3 | Results and discussions | 62 |
| 5.3.1 | Cleaning protocols | 63 |
| 5.3.2 | Handling errors | 63 |
| 5.3.3 | Calibration curves from impedance microbiology | 64 |
| 5.3.3.1 | Reproducibility of BacTrac® 4000 measurements | 66 |
| 5.3.4 | Biofilm enumerations | 69 |

| | | |
|--|---|-----|
| 5.3.4.1 Dairy strain | 69 | |
| 5.3.4.2 Clinical strain | 76 | |
| 5.3.4.3 Environment strain | 83 | |
| 5.3.5 General discussion of the results | 90 | |
| 5.3.6 SEM images of the tubing surface (control) | 92 | |
| 5.3.7 SEM images of F8 biofilm on PVC, polyurethane and silicone after different time of incubation | 94 | |
| 5.4 Conclusions | 100 | |
| | | |
| CHAPTER 6 | FINAL DISCUSSION AND CONCLUSIONS | 101 |
| | | |
| APPENDICES | | 106 |
| | | |
| BIBLIOGRAPHY | | 113 |

LIST OF FIGURES

| Figure | | Page |
|---------------|---|-------------|
| 2.1 | Scanning electron micrograph of <i>E. sakazakii</i> cells that attached to polyurethane infant feeding tube after 12 hours incubation in 10% reconstituted infant formula | 5 |
| 2.2 | Diagram of standard feeding tube made from polyurethane | 18 |
| 4.1 | The image represents the growth of <i>E. sakazakii</i> on tryptic soy agar (TSA) after 48 hours of incubation at 25°C | 41 |
| 4.2 | The image represents the growth of <i>E. sakazakii</i> on skim milk plate count agar after 48 hours of incubation at 25°C | 42 |
| 4.3 | The images showed the capsule staining of F8 strain | 43 |
| 4.4 | The results from heat treatment of the 10% reconstituted IFM | 47 |
| 4.5 | Microtitre plate assay | 50 |
| 4.6 | Biofilm score by dairy isolate (F1) | 53 |
| 4.7 | Biofilm score by clinical isolate (F8) | 54 |
| 4.8 | Biofilm score by environmental isolate (A6) | 55 |

| | | |
|------|--|----|
| 5.1 | Calibration curve of F1, F8 and A6 | 65 |
| 5.2 | Regression calibrations of F8 strain | 66 |
| 5.3 | Reproducibility of BacTrac® 4000 | 67 |
| 5.4 | F1 planktonic cells of PVC tube | 69 |
| 5.5 | F1 biofilm cells of PVC tube | 70 |
| 5.6 | F1 planktonic cells of polyurethane tube | 71 |
| 5.7 | F1 biofilm cells of polyurethane tube | 72 |
| 5.8 | F1 planktonic cells of silicone tube | 73 |
| 5.9 | F1 biofilm cells of silicone tube | 74 |
| 5.10 | The numbers of F1 cells forming biofilm on three types of infant feeding tubes tube at three time intervals - 4, 12 and 24 h | 75 |
| 5.11 | F8 planktonic cells of PVC tube | 76 |
| 5.12 | F8 biofilm cells of PVC tube | 77 |
| 5.13 | F8 planktonic cells of polyurethane tube | 78 |
| 5.14 | F8 biofilm cells of polyurethane tube | 79 |
| 5.15 | F8 planktonic cells of silicone tube | 80 |
| 5.16 | F8 biofilm cells of silicone tube | 81 |

| | | |
|------|---|----|
| 5.17 | The numbers of F8 cells forming biofilm on three types of infant feeding tubes tube at three time intervals - 4, 12 and 24 h | 82 |
| 5.18 | A6 planktonic cells of PVC tube | 83 |
| 5.19 | A6 biofilm cells of PVC tube | 84 |
| 5.20 | A6 planktonic cells of polyurethane tube | 85 |
| 5.21 | A6 biofilm cells of polyurethane tube | 86 |
| 5.22 | A6 planktonic cells of silicone tube | 87 |
| 5.23 | A6 biofilm cells of silicone tube | 88 |
| 5.24 | The numbers of A6 cells forming biofilm on three types of infant feeding tubes tube at three time intervals - 4, 12 and 24 h | 89 |
| 5.25 | Scanning electron micrograph of PVC, polyurethane and silicone infant feeding tube surfaces that acted as control | 93 |
| 5.26 | Scanning electron micrograph of the attachment of strain F8 on PVC, polyurethane and silicone tubing after 4 h of incubation | 95 |
| 5.27 | Scanning electron micrograph of the biofilm formation by F8 strain on PVC, polyurethane and silicone tubing at 12 h of incubation | 97 |
| 5.28 | Scanning electron micrograph of the biofilm formation by F8 strain on PVC, polyurethane and silicone tubing at 24 h of incubation | 99 |

LIST OF TABLES

| Table | | Page |
|--------------|--|-------------|
| 2.1 | Environmental samples tested for the presence of <i>E. sakazakii</i> | 7 |
| 2.2 | Biochemical differentiation of opportunistic <i>Enterobacter</i> species | 9 |
| 2.3 | Methods of feeding / delivery | 20 |
| 2.4 | Materials of feeding tubes | 22 |
| 3.1 | List of isolates from Fonterra and their origin | 28 |
| 3.2 | List of isolates from AsureQuality and their origin | 29 |
| 3.3 | List of isolates from AUT and their origin | 29 |
| 4.1 | List of isolates from Fonterra and their purity result | 44 |
| 4.2 | List of isolates from AsureQuality and their purity result | 45 |
| 4.3 | List of isolates from AUT and their identification result | 45 |
| 4.4 | The score based on biofilm formation | 51 |
| 4.5 | Summary of the biofilm score by <i>E. sakazakii</i> | 52 |
| 5.1 | List of repetition of biofilm experimental runs | 62 |