Isolation of *Campylobacter* from water and its fitness in an aquatic biofilm

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any other university for a degree.

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Date: 1 FEBRUARY 2002

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Summary

Campylobacter is the leading cause of gastroenteritis globally and has been isolated from several water sources. The organism is reportedly capable of integrating into biofilms and can thus become a major problem for water suppliers, since many water distribution systems can harbor biofilms. There is currently no information available regarding the prevalence of *Campylobacter* in South African water sources. It would thus be important to investigate the occurrence of the organism in our water sources and also to determine whether it is capable of surviving in a biofilm.

Two approaches were used for the isolation of *Campylobacter* from water. The first was based on a filtration method that involved the movement of the organism through a 0.6µm membrane filter onto a non-selective agar. This method was modified from the

one described by Steele and McDermott (1984) and subsequently used for analysis of environmental waters during two sampling rounds (March and June 2000). Although high bacterial counts were obtained, subsequent identification of the isolates obtained indicated that none of these were *Campylobacter*, but rather a wide range of Gram positive and Gram negative bacteria.

Due to the poor performance of the filter approach, the use of selective enrichment in Bolton broth, prior to selective culturing, was investigated. In this approach, different volumes of the various water sources were filtered, the filter was then placed in the enrichment broth and incubated for 24 h where after it was streaked onto a selective and non-selective agar. Three *C. jejuni* isolates were obtained with this enrichment approach. All other isolates were identified as *Arcobacter butzleri*. There was no correlation between the presence of fecal coliforms and *Campylobacter*.

The fitness of *Campylobacter* in a biofilm was investigated by introducing *Campylobacter* into an established *Methylobacterium* biofilm grown on PVC coupons in sterilized tap water. The *Campylobacter* cells were monitored using a fluorescently labelled rDNA probe at various time intervals. The *Campylobacter* cells could be detected in the biofilm after 24 h, but its numbers decreased rapidly and no cells could be detected on the coupons after 48 h.

Since the prevalence of *Campylobacter* in South African waters appears to be very low, there may not be a need for the routine monitoring thereof. However, a rather high

incidence of the closely related *Arcobacter* species was found which may warrant further investigation.

Isolering van *Campylobacter* uit water en hul oorlewing in 'n akwatiese biofilm

deur

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Opsomming

Campylobacter is die hoof oorsaak van gastrointeridis wêreldwyd en is vanuit verskeie waterbronne geïsoleer. Die organisme is in staat om in biofilms te integreer en kan dus 'n groot probleem vir waterverskaffers wees aangesien baie waterverspreidingsisteme biofilms mag hê. Huidiglik is daar baie min bekend oor die voorkoms van *Campylobacter* in Suid-Afrikaanse watersisteme. Dit is dan juis belangrik om die voorkoms van die organisme in ons waterbronne te bepaal en ook hul vermoë om binne biofilms te oorleef.

Twee benaderings is gevolg om *Campylobacter* vanuit water te isoleer. Die eerste was gebaseer op 'n filtrasie-metode waar die beweging van die organisme deur 'n 0.6µm membraan na 'n nie-selektiewe agar medium ondersoek is. Hierdie metode is 'n modifikasie van die een beskryf deur Steele & McDermott (1984) en is gevolglik gebruik

vir die analise van omgewingswatermonsters tydens twee periodes (Maart en Junie 2000). Alhoewel hoë bakteriese tellings verkry is, het verdere identifikasie van die isolate geen *Campylobacter* opgelewer nie, maar wel 'n wye verskeidenheid Gram positiewe en Gram negatiewe organisms.

As gevolg van die swak isoleringsvermoë van die filtrasie-metode, is die gebruik van selektiewe verryking in Bolton medium, voor selektiewe kweking, ondersoek. Tydens hierdie benadering is verskillende volumes vanaf verskeie waterbronne gefiltreer. Die filter is dan gevolglik in verrykingsmedium geplaas, 24 h geïnkubeer en onderskeidelik op selektiewe en nie-selektiewe agarmedium uitgeplaat. Drie *C. jejuni* isolate is deur middel van hierdie verrykingsmetode verkry. Die res van die isolate is geidentifiseer as *Arcobacter butzleri*. Geen korrelasie tussen die teenwoordigheid van fekale koliforme en *Campylobacter* is gevind nie.

Die oorlewing van *Campylobacter* binne 'n biofilm is ondersoek deur die byvoeging van *Campylobacter* in 'n gevestigde *Methylobacterium* biofilm. Hierdie biofilm is ontwikkel op PVC skyfies in gesteriliseerde kraanwater. Die teenwoordigheid van *Campylobacter* selle is deur middel van fluoreserende rDNS peilers op verskillende tydsintervalle gemonitor. *Campylobacter* selle kon na 24 h binne die biofilm aangedui word, maar getalle het baie vinnig afgeneem en geen selle kon na 48 h waargeneem word nie.

Aangesien dit wil voorkom asof lae getalle *Campylobacter* in Suid-Afrikaanse water teenwoordig is, is roetinemonitering daarvan nie nodig nie. In teenstelling hiermee, is hoë getalle van die verwante *Arcobacter* spesies gevind wat verdere ondersoek mag vereis.

Preface

The following aspects of this work have been presented as talks or posters at international and national conferences:

Conference	:	BioY2K Combined Millenium Meeting 2000, Grahamstown, South Africa, 23-28 January 2000
Poster	:	Development of a method for the detection and recovery of Campylobacters from water
Presented by	:	S. M. Diergaardt
Conference	:	Campylobacter, Helicobacter and Arcobacter. Summer Conference of the Society for Applied Microbiology, Glascow, Scotland, 11-13 July 2000
Poster	:	Detection of Campylobacters from environmental water in South Africa
Presented by	:	Dr P. A. Gouws
Conference	:	*Campylobacter* 2000, University of the Western Cape, Bellville, South Africa, August 2000
Talk	:	Adaptation of the Cape Town Protocol for the environmental isolation of Campylobacters
Presented by	:	S. M. Diergaardt

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CONTENTS

List of abbreviations

°C - degrees Celsius

µl - microliter

µm - micrometer

Anon. - anonymous

Biochem ID - biochemical identification

bp - base pairs

ca. – approximately

CAT – cefoperazone-amphotericin-teicoplanin

cfu - colony forming units

CLO - *Campylobacter*-like organisms

CO_2 - carbon dioxide

Cy - indocarbocyanine dye

d – day

DAPI - 4', 6-diamidino-2-phenylindole

dH_2O - distilled water

DMSO - dimethylsulfoxide

DNA - deoxyribonucleic acid

dNTP - deoxyribonucleic-5'-triphosphate

EDTA – ethylenediamine-tetra-acetic acid

EPS - exopolysaccharides

$FeSO_4$ - ferrous sulphate

FISH – fluorescent *in situ* hybridization

fla - flagellin

GBS – Guillain-Barré Syndrome

h- hour

HL – heat-labile

H_2O_2 - hydrogen peroxide

HS – heat-stable

H_2S - hydrogen sulphide

ISO - International Organization for Standardization

KNO_2 - potassium nitrite

KNO_3 - potassium nitrate

KZN - Kwazulu Natal

l - liter

LB - Luria Bertani broth/ agar

mCCD - modified charcoal cefoperazone deoxycholate

mCCDA - modified charcoal cefoperazone deoxycholate agar

mFC - media for fecal coliforms

min - minute

ml - milliliter

MM - molecular mass

NCBI - National Centre for Biotechnology Information

ND - not detected

NT - not tested

PBS – phosphate-buffered saline

PCR - polymerase chain reaction

PHA – passive haemagglutination

PFGE – pulse field gel electrophoresis

rDNA - ribosomal deoxyribonucleic acid

RFLP – restriction fragment length polymorphism

RNA – ribonucleic acid

s – second

SDS – sodium dodecyl sulphate

TBA - tryptose blood agar

TCA - tricarboxylic acid cycle

TEM – transmission electron microscopy

TNTC - too numerous to count

TSI - triple sugar iron agar

UHQ - ultra high quality water

UK – United Kingdom

UVB - ultraviolet B rays

VBNC – viable but non-culturable

v/v - volume per volume

w/v - weight per volume