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Deoxyribonuclease Activity from Helical Bacteria

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Dedication:

To my father and mother...

To my husband, brother, and family...

To my son Ibrahim...

To my doctors...

Areej Sami Altaweel

Declaration

I certify that this thesis submitted for the degree of Master in Medical Laboratory Sciences/ Microbiology and Immunology track, is the result of my own research, except where otherwise acknowledge, and that this study has not been submitted for a higher degree to any other university or institution.

Signed

Areej Sami Altaweel

Date: 16.01.2013

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Abstract

The activity of nuclease enzyme in helical bacteria such as *Campylobacter jejuni*, *Helicobacter pylori*, and *Alphaproteobacteria* "QUBC 70" has been detected. This activity interferes with DNA preparation, isolation, storage, and DNA-based reactions including PCR and sequencing. These bacteria are fastidious; slow growing, Gram negative helical bacteria. The high rate of infections with *H. pylori* (50% worldwide) and 85% among Palestinians (and most likely the same rate with *Campylobacter jejuni*), dictates that a reliable molecular method of detection of these bacteria from stool, water, food, or other samples must be established. DNA based detection has been hampered by such putative nucleases. This work focused on expanding our understanding of the characteristics and properties of these nucleases and to explore the conditions for having stable DNA preparation from these bacteria without interference of nucleases.

Bacterial cultures from stool were used to collect bacteria for lysis. Lysates were prepared by different methods; SDS lysis, SDS and boiling, sonication, lysozyme and water. SDS-lysis was selected as the method of choice. Water saturated with Ammonium sulfate (AS) was used to fractionate proteins from cleared lysate.

This work illustrates the presence of DNase activity in bacterial lysate prepared by lysing bacterial cells in the presence of SDS followed by boiling, indicating the putative nuclease to be SDS-heat stable. When lysis was performed with lysozyme or sonication without SDS, the putative nuclease appeared to be reduced probably due to the proteases and/or due to nuclease association with the cell envelope. The addition of saturated AS (AS; 0.6 1 v/v at 25˚C) to *C. jejuni* lysate precipitated the nuclease. Ammonium sulfate was not efficient in salting-out the nuclease activity when applied at <0.6 volumes of *Campylobacter* lysate. When applied to a different bacterial lysate (*Alphaproteobacteria* QUBC 70), AS precipitated the nuclease activity at ~300%. The DNase activity was assayed by mixing exogenous λ-DNA (lambda bacteriophage) with target preparation and incubation at 36˚C or 45˚C for different times up to 48 hours.

It can be concluded that repeatedly, the DNase activity was found in cell extracts of both *C. jejuni* and "QUBC 70". Ammonium sulfate DNase precipitation profile for *C. jejuni* was different and distinguishable from that of the *Alphaproteobacteria* QUBC 70.

Campylobacter nuclease was active at 36 ^{o}C and 45˚C but poorly at 50˚C in the presence of > 0.01% SDS.

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List of abbreviations

2-ME	2-Mercaptoehanol
ATCC	American type culture
C. jejuni	*Campylobacter jejuni*
DNase	Deoxyribonuclease
dsDNA	Double-stranded DNA
E. coli	*Escherichia coli*
H. felis	*Helicobacter felis*
PCR	Polymerase chain reaction
Prk	Protease K
QUSB 70	Quds universal 70
S. aureus	*Staphylococcus aureus*
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TAE	Tris acetate EDTA
TBO	Toludine blue O
TE	Tris buffer
V/V	Volume/volume
VBNC	Viable but non culturable

Chapter I

Introduction

1.1 Helical Bacteria

Bacteria such as *Helicobacter pylori* and *Campylobacter jejuni* are medically important pathogens that require monitoring and continuous assessment of their presence in the environment, in healthy, carriers, and sick individuals and animals. In general, they can inhabit various niches in the environment. *Helicobacter pylori* inhabit areas of the stomach and duodenum. It causes a chronic low level inflammation of the stomach lining, gastritis, and is strongly linked to the development of duodenal ulcers, gastric ulcers, and has been classified as Class I carcinogen (Bargouthi, 2009). *Helicobacter pylori* was recognized as Campylobacter-like organism until it was given the current species status. Since then, tens of Helicobacter species have been discovered and are known to be strongly host specific; *H. felis* is the species found in most cats while *H. suis* is found in swine species. Few *Helicobacter* spp. have been reported to cause human diseases especially *Helicobacter hepaticus* (Bargouthi, 2009).

Common features among these species include shape (spiral or helical) and that they inhabit the intestines of the target host. In the laboratory, they are slow growers; require low oxygen tension (1-5%) and serum, blood, or a substitute (Bargouthi, 2009). Another important character that is controversial; is the ability of these species and others

(including *Legionella*, *Vibrio*, *Salmonella*, *Escherichia*, and *Shigella* spp., other spiral bacteria that show coccoid forms include *Desulfovibrio*, *Campylobacter*, *Aquaspirillum*, *Oceanospirillum*, and *Spirillum*) to morphologically transform to a coccoid form also known as viable but non-culturable (VBNC) form. Coccoid and VBNC forms may be critical stages that allow undetectable transfer and survival of the bacterium, this view is supported by the ubiquitous distribution of such bacteria. Resuscitation of 30-day old (VBNC) *Campylobacter jejuni* through an embryonated hen-egg was reported to allow recovery of the bacterium from a large proportion of the inoculated eggs**.** Other investigators view these forms as dead bacteria (Bargouthi, 2009).

Detection of *Helicobacter*, *Campylobacter*, and other fastidious pathogens and environmental bacteria becomes a serious problem due to the culture-evading forms and the difficulty of extracting stable DNA from them. The original observation is that crude DNA extracted from cultured *Helicobacter* or *Campylobacter* is highly unstable unlike those of *Escherichia coli* and *Bacillus* spp. (Barghouthi, 2011). Taken together, the following factors; VBNC forms, low infectious doses, slow growth, unstable DNA in crude cell lysate, and the vague issue of DNA extraction from coccoid forms, the detection and identification of such bacteria may be inefficient and subject to failure (Bargouthi, 2009; Nogva et al., 2000).

1.2. Literature Review

1.2.1 Nucleases

Under well contained laboratory conditions, DNA instability is essentially due to the presence of free metals and/or dexoribonucleases since the double helix is highly stable. Watson and Crick description of the double helix of the DNA molecule opened the doors to a new area in biological understanding and research. In the late 1960s, scientists Stuart Linn and Werner Arber isolated two types of enzymes responsible for phage growth restriction in *Escherichia coli* bacteria. One of these enzymes added a methyl group to the DNA, generating methylated DNA, while the other cleaved unmethylated DNA at a wide variety of locations (*dam* and *dcm*) along the length of the molecule. In 1968 Smith, Wilcox, and Kelley, working at Johns Hopkins University, isolated and characterized the first restriction nuclease whose function depends on a specific DNA nucleotide sequence.