UNIVERSITI TEKNOLOGI MARA

THE INDUCTION OF ACID TOLERANCE IN *Vibrio cholerae* – FACTORS INFLUENCING AND CROSS-PROTECTION RESPONSES

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Master of Science

January 2007

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Date 15 JANUARY 2007

Abstract

The factors influencing the ability of *Vibrio cholerae* AC-V99 El Tor O1 Ogawa to tolerate acid killing after a pre-exposure to sub-lethal acid pH were studied. The effect of pre-exposure to sub-lethal acid pH on tolerance to other stresses (cross-protection) including heat, osmotic and oxidative stresses was also investigated. The involvement of *de novo* proteins synthesis and effect on cell morphology were also determined. Acid sensitivity test showed that the sublethal pHs for this strain ranged from pH 5.0 to pH 6.0 and at pH 4.5 and below the cells are completely killed. The ability of this bacterium to induce acid tolerance was tested by pre-exposing early exponential cells in LB + 3% NaCl at pH 5.0 for one hour before challenging them to pHs 3.5, 3.0 and 2.5 for a further one hour. Viable counts were determined by plating dilutions of the samples on LB + 3% agar (pH 7.0) at various intervals of time. Cells that were pre-exposed to pH 5.0 survived significantly better ($P \leq 0.05$) at these lethal pHs compared to those that were pre-exposed to pH 7.0. This response is partly dependent on *de novo* protein synthesis as treatment with chloramphenicol, a protein synthesis inhibitor during pre-exposure did not completely eliminate the response. Microscopic examination showed that acid induced cells were found to be elongated and segmented probably due to arrested cell division. The induction of acid tolerance response was found to be affected by growth phase, pH, temperature, pre-exposure time and initial cell density. Early exponential phase cells pre-exposed to pH 5.0 were found to have highest survival rate after one hour of challenge at pH 3.5 followed by stationary phase, late exponential phase and mid-exponential phase cells under the same conditions. A pre-exposure at pH 5.0 for three hours gave maximal protection against acid killing compared to one, two and three hours of pre-exposure time. The induction of acid tolerance was higher when cells were pre-exposed at sub lethal pH 5.0 than at sub-lethal pHs 5.5 and 6.0. Pre-exposure to pH 5.0 at 30°C gave better protection to acid killing than pre-exposure to same pH at 37°C. Cells pre-exposed at pH 5.0 at an initial density of $OD_{600} \sim 0.1$ to give maximum protection upon challenge to pH 3.5 compare to those pre-exposed at an initial density of $OD_{600} \sim 0.025$ and $OD_{600} \sim 0.05$. Pre-exposure to sublethal acid pH was also found to confer resistance to osmotic and oxidative stress but not to heat stress.

Keywords: *Vibrio cholerae*, acid adaptation, acid stress, acid tolerance response (ATR), low pH adaptation, cross-protection responses, morphological changes

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CHAPTER 1

INTRODUCTION

Vibrio cholerae is a Gram-negative enteric pathogen that is responsible for the endemic and epidemic secretory diarrhoeal disease known as cholera. This disease is endemic in South East Asia, parts of Africa and much of Central and South America (Faruque et al., 1998).

This bacterium lives within the aquatic reservoirs and enters its human host via the oral route through ingestion of contaminated food and water. As *V cholerae* enters the human body, it immediately encounters a variety of stressful stimuli, such as elevated temperature, oxygen radicals, extremes of pH, degradative enzymes, and deprivation of certain nutrients, which it has to combat successfully before it can reach the intestinal epithelium and proliferate there (Alvarez et al., 2003). Those that survived will subsequently encounter volatile fatty acids, bile, low oxygen and normal flora in the intestine and specific immune defenses provided by lymphoid tissues of the gastrointestinal tract (Smith, 2003).

Studies in the last decade have showed that one of the ways in which enteric bacteria can survive low pH stress in their hosts is, if they had prior exposure to mild acid stress. This phenomenon has been well documented in a number of gastrointestinal or food-borne pathogenic bacteria such as *Escherichia coli* (Castanie-Cornet et al. 1999; Conner & Kotrola, 1995), *Salmonella typhimurium* (Foster, 1995, 1993, 1992, 1991), *Aeromonas hydrophila* (Karem et al., 1994), *Vibrio parahaemolyticus* (Wong et al. 1998), *Helicobacter pylori* (Mooney et al., 1990), *Listeria monocytogenes* (Kroll & Patchett, 1992), and *Enterococcus faecalis*