ADAPTIVE NATURE OF WATERBORNE MICROBIAL PATHOGENS IN COLD TEMPERATURES

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INTRODUCTION

Changes in the environmental physico-chemical parameters may have profound effects on the physiology of the microorganisms leading them to adaptation to the altered environment for their survival (Colwell et al. 1977; Miller et al. 1984; Singleton et al.; 1982). The adaptation to the changing environment in these microorganisms could be displayed through the change in the cell morphologies, cellular protein syntheses and overall growth patterns (Colwell and Huq 1994). In many occasions these microbial cells could enter into dormant state by compromising their culturability on microbiological media (Colwell and Huq 1994; Oliver 1993). To date, suboptimal growth temperatures or nutrient deprivation have been identified as the two most important environmental factors that trigger cellular processes in these microorganism to enter into dormant state (Colwell and Huq 1994; Oliver 1993). Some of the microbial pathogens such as *Escherichia coli* (Xu et al. 1982), *Legionella pneumophila* (Hussong et al.1987), *Salmonella enteritidis* (Roszak et al. 1984) and *Vibrio vulnificus* (Oliver 1993) have been shown to enter into dormant state and remain non-culturable on various microbiological media. Also, it has been reported that a waterborne microbial pathogen, *V. cholerae*, disappears from their water habitat during the winter months and are detected during summer months (Adikari 1975; Uchiyama 1998; Ravel et al. 1995). *V. cholerae* is a Gram negative bacterium with distinct "comma" shaped appearance and this pathogen is known to cause cholera in humans primarily through the consumption of contaminated drinking or potable waters. This pathogen is endemic to many of the developing countries including South America and Asia and causes cholera outbreaks often in an epidemic form (Kaysner and Hill 1994). In the United States, although the number of cases of cholera has been extremely low during the past decade; however, the Gulf of Mexico is considered to be the reservoir for this pathogen (Colwell and Huq 1994; Kaysner and Hill 1994). Therefore, the Federal Government guideline requires routine monitoring seafood and shellfish for the presence of this pathogen before shipped to the restaurants for human consumption (Bej et al. 1996, 1997). In this report, we have investigated the effects of the suboptimal growth temperatures in this waterborne microbial pathogen, *Vibrio cholerae*, by studying their viability on a microbiological medium and changes in their cellular morphologies.

MATERIALS AND METHODS

Bacterial Strain and Growth Media

V. cholerae O1 Inaba biotype El Torr ATCC 154 was maintained on LB agar [10 g bacto tryptone, 5 g yeast extract, 10 g NaCl, 14 g bacto agar (Difco) per L] (Miller, 1972) or grown in a liquid AKI medium [15 g peptone, 5 g NaCl, 4 g yeast extract, 30 mL $NaHCO_3$, pH 7.2 per L] (Atlas 1993).

Growth and Tolerance at Cold Temperatures

An overnight culture (1 mL) of *V. cholerae* was inoculated to 300 mL of AKI medium and grown on a shaker incubator set at 35°C. When the culture reached the exponential growth phase (OD_{450nm}= 0.2), 0.1 mL aliquot was 10-fold serially diluted in AKI medium and plated onto LB agar to determine the viable plate count. Three aliquots, each containing 50 mL, were then separated into 250 mL flasks,and immediately placed on shaker incubators set at 35°C, 15°C, 5°C. At various time intervals ranging between 1 h of initial growth and 191 h, the cell density and viable plate counts of each culture was taken. Viable plate counts were obtained on LB agar grown overnight at 35°C. Also, a viable plate count was determined on LB agar for the cultures maintained at 5°C until no culturable cells were noticed.

Microscopical Examination of Cell Morphologies

At various time intervals, aliquots (0.1 mL) from each culture at 35°C, 15°C, and 5°C were centrifuged at 10,500 xg, resuspended in 0.1 mL of M9 minimal medium (Miller 1972), treated with acridine orange fluorescent dye (0.01 mL) (Daley and Hobbie 1975), and examined under a Leitz Diaplane fluorescent microscope at 1000x magnification for morphological changes.

RESULTS AND DISCUSSION

Growth and Tolerance at Cold Temperatures

The viable plate counts of the *V. cholerae* culture grown at 15°C exhibited 2-log increase in cell counts during the first 28 h of incubation followed by only a 1-log decrease during the next 158 h of incubation suggesting a steady maintenance of the cell counts at this temperature throughout the experimentation (Figure 1). For the culture incubated at 35°C, a 1.5-log rapid increase in the total viable plate counts was noticed during the first 28 h, followed by a gradual decline of cell counts of approximately 2-log during the next 158 h of incubation (Figure 1). Although both cultures exhibited similar growth and survival patterns at two different temperatures, an approximately 2-log higher cell counts of the culture maintained at 15°C as compared to the culture incubated at 35°C was noticed. This result suggests that *V. cholerae* exhibits a relatively slower cellular metabolism resulting in longer generation time when maintained at a suboptimal growth temperature of 15°C.

V. cholerae culture that was transferred from 35°C to 5°C showed a decline of 3-log in the first 48 h followed by a steady decline of an additional 3-log during the next 138 h (Figure 1). This result suggests that incubation at a suboptimal growth temperature of 5°C of a culture of *V. cholerae* did not trigger adaptive advantage to grow and divide as evidenced from the culture exposed to 15°C. Therefore, *V. cholerae* seems to tolerate and continue to grow at an suboptimal temperature of 15°C; however, at a much colder temperature of 5°C, the viable plate counts declines for this pathogen and become non-culturable.

Morphology and Direct Viable Count

Microscopic examination of *V. cholerae* culture maintained at 35°C for 120 h exhibited relatively short but characteristic "comma" morphology. Cells from the culture maintained at 15°C exhibited elongated morphology with distinct "comma" configuration (Figure 2). The elongated morphology of the cells from the culture kept at 15°C was possibly due to the reduced cellular metabolic activities which resulted in the extended generation time as noticed in the viable plate count data described above. Such elongated cellular morphology following incubation at suboptimal growth temperatures between 10°C-15°C has been reported for other microbial pathogens such as *V.vulnificus* (Bryan et al. 1999) and *S. typhimurium* (Jeffreys et al. 1998; Horton et al. 1999).

V. cholerae cells from the culture incubated at 5°C directly from 35°C exhibited round coccoid morphology. Such change in the morphology was also reported in *V. vulnificus* (Bryan et al. 1999; Oliver 1993) when exposed to cold temperatures. This result suggests that the coccoid morphology could be a manifestation of the cells to remain in dormant state at 5°C (Bryan et al. 1999).

CONCLUSION

V. cholerae cultures failed to demonstrate continued growth as an adaptive response when transferred to a suboptimal growth temperature of 5°C. Rapid drop in the viable plate counts of *V. cholerae* cultures exposed to 5°C resulted in the transformation into a non-culturable dormant state by this pathogen with distinct coccoid morphologies. Further characterization of the cellular protein profiles when *V. cholerae* cultures exposed to various cold temperatures will help to understand the cold temperature-induced cellular factor that triggers the cells to enter into a non-culturable dormant state in this pathogen.

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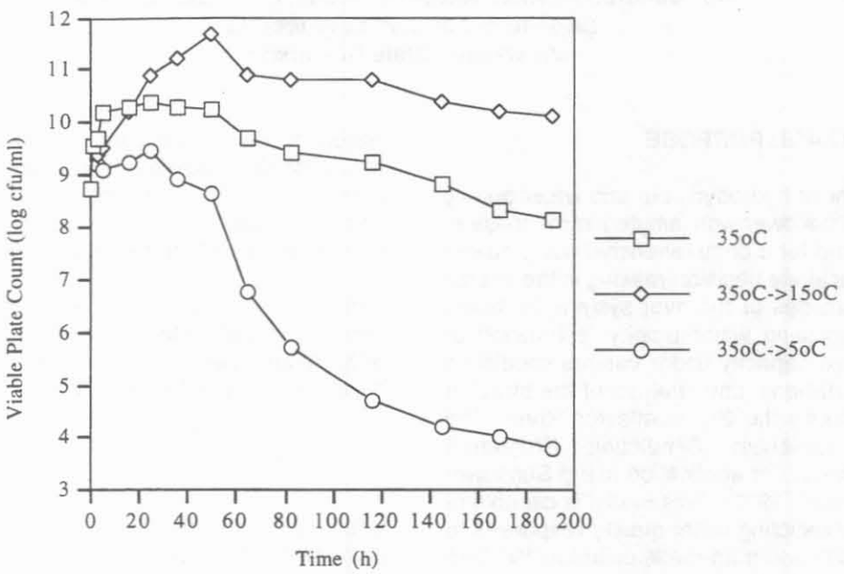

Figure 1. Viable plate counts of *Vibrio cholerae* O1 cultures grown at various temperatures between 0 and 191 h. (□), *V. cholerae* culture was maintained at 35°C; (◊), culture transferred from 35°C to 15°C and maintained at this temperature; (O), culture transferred from 35°C to 5°C and maintained at this temperature. Each data point is an average of three identical plate counts.

Figure 2. Microscopic examination of *Vibrio cholerae* O1 cultures showing changes in the morphologies when exposed to various temperatures. Panels (a), *V. cholerae* cultures was maintained at 35°C; (b), culture was grown at 35°C and then maintained at 15°C for 160 h; (c), cultures was first grown at 35°C and then transferred at 5°C for 160 h. All microscopic examinations were performed at a 1000x magnification.