ABSTRACT

Studies on the Acid Shock Response in Vibrio Cholerae.

Exposure to acidic pH is a common stress encountered by many bacteria during their life cycle in various niches. For enteric bacteria facing acid stress is part of their pathogenic and commensal lifestyles. Many microbes acquire an increased ability to withstand the lethal effects of acids, if they are pre-adapted to a sub-lethal acidic pH. This phenomenon has been termed as "Acid Tolerance Response" (ATR). Studies regarding ATR in some bacteria primarily *Escherichia coli* and *Salmonella typhimurium* have shown that ATR is accompanied by the induction of a subset of proteins collectively known as Acid Shock Proteins (ASPs), which probably provide defense against lethal acidic conditions.

V. cholerae, the causative agent of the disease cholera, has a dual existence as it can exist both as a free-living organism in brackish waters and also in the human gut. It infects humans through the fecal oral route. It is exposed to a variety stressful conditions like different pH, temperature upshift, nutrient starvation and many more in its journey from the environment to it's human host. Though some studies have been carried out on the stress response of *V. cholerae*, not a great deal is known about how *V. cholerae* is able to overcome these stresses. This thesis presents results of a study, which was aimed to characterize the acid shock response in *V. cholerae*.

It was found that *V. cholerae* could mount a strong ATR against both mineral and organic acid. For HCl, a prior incubation at pH 5.0 (sub-lethal pH) for protected it against an acid shock at pH 4.0. For the organic acids the lethal pH were found to be 5.7 and 5.0 respectively. It also showed an

ATR against VFA cocktail (which mimics the acid conditions within the intestine) and bile. ATR mounted by *V. cholerae*, be it by mineral acid or by organic acid could protect it against both mineral acid (HCl) and organic acids. Besides, HCl-ATR was found to cross protect *V. cholerae* against the deleterious effects of heat and oxidative stresses. The data obtained also revealed that acid adaptation at pH 5.0 helped *V. cholerae* to retain it's motility, which it otherwise lost as a result of acid shock.

ATR in *V. cholerae* was found to be sensitive to the protein synthesis inhibitor drug Chloramphenicol, demonstrating the requirement of *de novo* protein synthesis in the process. 2-dimensional protein gel analysis revealed enhanced or *de novo* synthesis of 46 proteins during adaptation with mineral acid (HCl) at pH 5.0 for 90 min. These included well-known heat shock proteins (HSPs), DnaK, GroEL and DnaJ as identified by immunobloting. A strong induction of a 16 kDa protein was seen, which is also observed in the heat shocked *V. cholerae*. In case of the VFA cocktail ATR, only four major differences between control and the adapted cells were observed, whereas adaptation in presence of bile did not reveal any difference at the protein level, pointing to different underlying mechanisms of induction of ATR in response to different agents.

Exposure of bacteria to acid stress has been shown to lead to changes in the profiles of cellular lipids, cellular lipopolysacharides (LPS) and outer membrane proteins. *V. cholerae* during adaptation at pH 5.0 (HCl) did not show any noticeable change in the profiles of the above mentioned cellular components. ASPs induced during acid adaptation unlike in the case of heat shock response in *V. cholerae* did not show any cross reactivity with human immune sera.

Though the HSPs like DnaK, GroEL, DnaJ were induced prominently as the result of acid adaptation, their enhanced synthesis alone was not sufficient to protect *V. cholerae* against acid shock, induction of heat shock could not cross protect *V. cholerae* against acid shock. Role of the global regulators ToxR and ToxS, which are involved in regulating the expression of a number of genes in *V. cholerae* ATR was investigated using null mutants of *toxR* and *toxS* in response to environmental signals. It was found that while *toxR* and *toxS* null mutants of *V. cholerae* were able to mount HCl-ATR they were unable to do so for the organic acids, suggesting that the pathways of induction of inorganic and organic ATRs are distinct in this organism. It was also shown that *toxR* and *toxS* have no role to play in acid induced loss of motility in *V. cholerae.*

Expression pattern of the *tcpA* gene coding for the major pilin subunit essential for intestinal colonization and *rpoS* gene coding for stationary phase sigma factor, which is known to play a major role in the induction of ATR in *E. coli* and *S. typhimurium* was examined in acid adapted and control. The results obtained showed an induction in *tcpA* gene expression suggesting that perhaps *V. cholerae*, which can successfully reach the intestine after negotiating the acid barriers at different places become better equipped to colonize the intestine they otherwise would have been, had they been exposed to the acidic conditions of the intestine directly. No change in the expression was observed in the case of *rpoS* gene, indicating that unlike in some other organisms RpoS plays no role in the acid tolerance response of *V. cholerae.*