Genome wide analysis of *in vivo Vibrio vulnificus* gene expression by employing a peritoneal cavity infection model

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Introduction

Vibrio vulnificus is a halophilic estuarine bacterium that causes fatal septicemia and necrotizing wound infections, and preferentially affects individuals having heavy alcohol drinking habits, underlying hepatic diseases and other immunocompromised conditions (Jones and Oliver, 2009). This opportunistic pathogen should experience dramatic environmental changes during host infection process. Virulence and survival factors required for *in vivo* growth and survival of *V. vulnificus* are expected to be expressed at right place and time as reported in other pathogens (Liu *et al*., 2009; de Vries *et al*., 2010). We previously reported that a number of virulence or virulence-associated genes are induced *in vivo* or after contact with host cells (Kim *et al*., 2003; Lee *et al*., 2007; Kim *et al*., 2008). Unraveling the genes preferentially expressed *in vivo* will enable better understanding of *V. vulnificus* pathogenicity and more intelligent screening for efficacious vaccine/therapeutic targets.

Materials and Methods

An optimized high-density oligonucleotide DNA chip of the genome project strain *V. vulnificus* CMCP6 was used to analyze the transcriptome of *V. vulnificus* infecting rat peritoneal cavity. A semipermeable tubing containing *V. vulnificus* was surgically implanted in the rat peritoneal cavity and bacterial RNA was isolated at appropriate time intervals (Figure 1). Bacteria grown for 4 hrs were harvested and the total RNA was extracted using the RNeasy minikit (Qiagen). Labeled cDNA was applied and hybridized to a *V. vulnificus* oligonucleotide DNA chip. Array images were acquired and normalized. An expression threshold was defined by the median of the $\log_2$ signal intensity of the control.

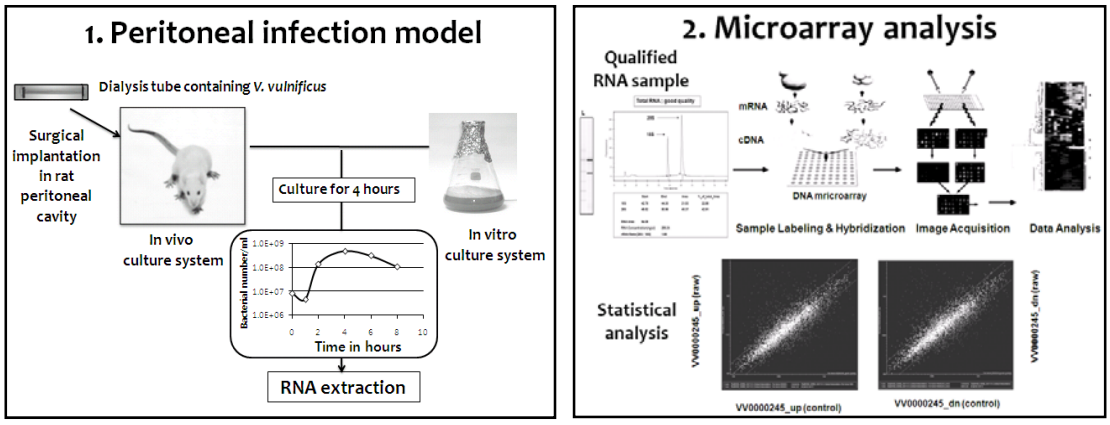

Figure 1: An optimized high-density oligonucleotide DNA chip of the genome project strain *V. vulnificus* CMCP6 was used to analyze the transcriptome of *V. vulnificus* infecting peritoneal cavity.

Results and discussion

More than 150 genes were identified as being preferentially expressed *in vivo*. Many of the up-regulated genes are involved in metabolism of inorganic ion transport, carbohydrate transport, amino acid transport, energy production/conversion, and intracellular trafficking/secretion/ vesicular transport. Many of the down-regulated genes are involved in cellular processing and signaling of signal transduction mechanisms and cell wall/membrane/envelope biogenesis, and in metabolism of energy production and conversion, carbohydrate transport, amino acid transport (Figure 2).

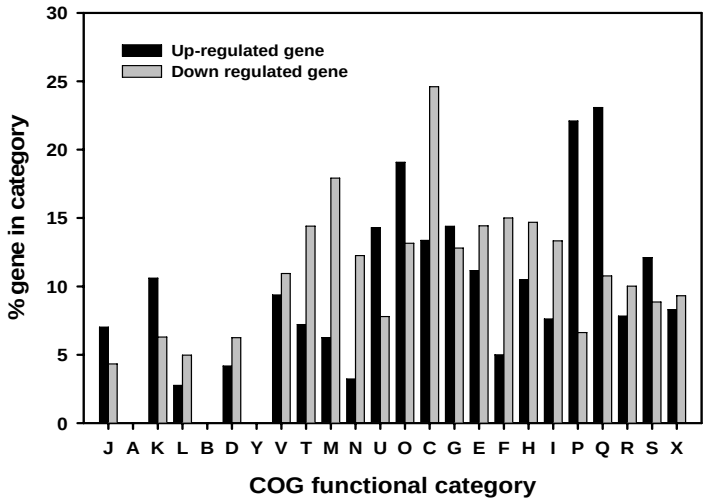

Figure 2: Functional attributions of the *in vivo* up- and down-regulated genes of *V. vulnificus* by COG category. The "% gene in category" refers to the percentage of *in vivo* induced genes out of total number of the indicated functional category genes.

Of particular interest was the up-regulation of various types of iron and iron-assimilation genes such as catechol/hydroxamate siderophore, heme, and free iron acquisition system (Table 1). Especially, it has been shown that the vulnibactin-mediated iron uptake system was the most highly induced gene class among different iron acquisition systems. Expression of an Flp pilus operon was significantly increased during the *in vivo* growth. Additionally, genes encoding a fructose phosphotransferase (PTS) system and several hypothetical genes appeared to be highly up-regulated.

Table 1: Interesting *in vivo* up-regulated iron assimilation gene clusters

Locus	Description	Fold increase
VV11660-VV11662	Iron(III) ABC transporter system	4-9.6
VV20110-VV20112		4.6-5.6
VV20276	Heme receptor, HupA	12.2
VV20360-VV20364	TonB system	6-7.8
VV20829-VV20844	Vulnibactin-related gene clusters	7.4-15.4
VV21010-VV21012	Hydroxamate-dependent iron ABC transporter	4.2-9.2
VV21609-VV21614	Hemin ABC transporter and TonB system	7.6-12.8
VV21615-VV21617	Putative heme iron utilization protein	10.6-13.6

Expression of an alcohol dehydrogenase and fumarate reductase/formate dehydrogenase-related genes were significantly decreased during the *in vivo* growth. Moreover genes encoding a tungstate ABC transport system and putative capsular polysaccharide biosynthesis appeared to be highly down-regulated (Table 2).

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Table 2: Interesting *in vivo* down-regulated gene clusters

Locus	Description	Fold decrease
VV11266-VV11269	Fumarate reductase	4.8-6.2
VV12302-VV12305	Putative capsular polysaccharide biosynthesis genes	4.2-5
VV12574-VV12576	Tungstate ABC transporter system	4.2-5
VV12588-VV12592	Formate dehydrogenase system	4-8.8
VV12617-VV12619	Cytochrome c oxidase, subunits	4.4-4.8
VV20010-VV20012	Anaerobic glycerol-3-phosphate dehydrogenase	4.8-6.2
VV20019	Alcohol dehydrogenase, class IV	8.6

Conclusions

The microarray-based *in vivo* gene expression analysis demonstrated that the iron assimilation systems play the most essential role for *in vivo* survival and growth. Several interesting genes that are supposed to be important for the host-parasite interactions appeared to be induced in the rat peritoneal cavity infection model. Of note was that metabolic pathways of *V. vulnificus* seemed to be robustly reprogrammed *in vivo* after infection.

Acknowledgements

This work was supported by Grant No. RTI 05-01-01 from the Regional Technology Innovation Program of the Ministry of Knowledge Economy (MKE)

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