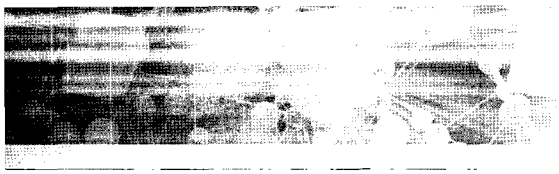

S7-1

Functional Genomics of Vibrio vulnificus: regulation of toxigenesis

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Microbial pathogenicity is a complex phenomenon in which expression of numerous virulence factors is frequently controlled by a common regulatory system. Recently, quorum sensing has been also implicated as an important global regulator controlling the expression of numerous virulence factors in bacterial pathogens. In the present study, DNA targets of the SmcR, a *Vibrio vulnificus* LuxR homologue, were selected from a random pool of DNA fragments by using a modification of SELEX procedure consisting of *in vitro* DNA-SmcR interaction, purification of SmcR-DNA complexes, and PCR amplification. The amplified DNA fragments enriched by a repetition of the cycle of the procedure were cloned and analyzed separately by EMSA to verify the specific binding of SmcR to the DNA. The sequences of the fragments were determined and revealed about 46 genes as candidates for SmcR-regulated genes. The sequences for binding of SmcR to the fragments were determined by DNase I protection assays. Alignment of the sequences revealed a 28-bp consensus SmcR-binding sequence, 5'-TWNTTATTGATWWRWTWNTNANTANNWW-3' (R: G or A; W: A or T; N: any nucleotide), as an inverted repeat. The activities of vvpEPS promoter in which the SmcR-binding sites were systemically mutated were compared and demonstrated the nucleotides that are essential for the maximum expression of the PS in vivo. Moreover, we have used MSCAN, prediction algorithm of transcription factor binding sites (TFBS), for predicting of SmcR binding sites in the whole genome sequences of V. vulnificus and identified other 55 genes to the SmcR regulon. The SmcR regulon include genes involved in nucleotide biosynthesis, protein folding, amino acid biosynthesis, stress responses, and iron transport,indicating that that SmcR is a novel global regulator controlling numerous genes contributing to pathogenesis as well as survival of V. vulnificus.