A Thesis for the Degree of Doctor of Philosophy

Effects of Cyclic di-GMP and Biofilm Development on Transcriptome Profile of *Vibrio vulnificus*

Cyclic di-GMP 증가와 바이오필름 형성 단계에 따른 식중독 패혈증비브리오균의 전사체 분석

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Abstract

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Biofilm formation is regulated by many factors including cyclic diguanylic acid (c-di-GMP) and calcium. Overexpression of diguanylate cyclase increased intracellular c-di-GMP level and promoted biofilm formation and rugose colony development of *Vibrio vulnificus*. Microarray analysis revealed that intracellular c-di-GMP influences the expression of over 5% of the *V. vulnificus* genome. Among the c-di-GMP regulon, the genes from several functional categories were identified to affect biofilm formation. From these combined results, it was confirmed that c-di-GMP regulates diverse cellular processes associated with the adherence to surfaces and the biofilm formation.

V. vulnificus exhibited distinct stages of biofilm development, including initial attachment, maturation, and dispersion. Transcriptomic comparison between cells in biofilm and planktonic cells were performed using RNA sequencing technique. The sequencing data revealed that, comparing to the transcriptome in planktonic

cells, the expression of 10% of the *V. vulnificus* genome was influenced in biofilm cells regardless of the stages of biofilm development. The expression levels of the two gene clusters, *brp* locus and the loci encoding calcium-binding protein and type 1 secretion system components, were changed dynamically with the development stages. Against my expectation, biofilm dispersion factor, *vvpE*, showed highly increased expression in the transition from the mature stage to the dispersion stage in biofilm development.

As the locus encoding calcium-binding protein shows notable expression levels when biofilm forms, it is inevitable to identify the relation between calcium and biofilm formation of *V. vulnifius*. In this study, a putative calcium binding protein, CabA, induced under high c-di-GMP level was identified from *V. vulnificus*. The coding regions of *cabA* and its adjacent gene cluster *cabBC* encoding type 1 secretion system (T1SS) components are located between a polysaccharide gene cluster, *brp*, and a gene encoding putative regulator of *brp*, BrpT. Reverse transcription-PCR results indicated that *cabA* is co-transcribed with *cabB* and *cabC*. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) and isothermal titration calorimetry (ITC) analyses demonstrated that CabA binds to calcium. To examine the role of CabA under high and low levels of c-di-GMP, we constructed the modified *V. vulnificus* strain in which the expression of *dcpA*

encoding diguanylate cyclase is under the arabinose-inducible promoter. When high levels of c-di-GMP were present, a considerable decrease in the biofilm formation of the mutant was observed with increasing calcium concentrations which was not shown under low levels of c-di-GMP. Confocal laser scanning microscope examination of flow-cell-grown biofilms revealed that the wild type forms well-structured and mushroom-shaped biofilms, whereas the *cabA* mutant makes unstructured and loose biofilms. c-di-GMP-induced colony rugosity of *V. vulnificus* was also found to be mediated by CabA. Western blot analysis demonstrated that CabA was secreted into biofilm matrix by the T1SS. The exogenously added CabA restored the biofilm formation activity and colony rugosity of the *cabA* mutant only in the calcium-containing media. It was additionally confirmed that the expression of *cabABC* operon was activated by BrpT. Consequently, the combined results indicated that CabA, a c-di-GMP-regulated calcium binding protein, is the major protein component of *V. vulnificus* biofilm matrix and plays crucial roles in biofilm formation.

Keywords : *Vibrio vulnificus*, biofilm, c-di-GMP, transcriptome analysis, RNA sequencing, calcium-binding protein, biofilm matrix protein

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Chapter 1.

Introduction

1-1. *Vibrio vulnificus*

1-1-1. Disease and pathogenesis of *V. vulnificus*

Vibrio vulnificus belongs to *Vibrio* genus in *Vibrionaceae*, and is Gram-negative, curved rod-shaped, halophilic, and motile with a single polar flagellum. *V. vulnificus* is found in estuarine waters and frequently contaminates oysters and other seafood (Dalsgaard *et al*., 1996; Hoi *et al*., 1998; Myatt and Davis, 1989). Consumption of seafood containing *V. vulnificus* can result in a severe, fulminant systemic infection. Characteristics of this disease include fever, chills, nausea, hypotensive septic shock, and the formation of secondary lesions on the extremities of patients (Chuang *et al*., 1992; Hlady and Klontz, 1996; Klontz *et al*., 1988; Oliver, 2006; Strom and Paranjpye, 2000). These symptoms are typically observed within 36 hours to 10 days. The mortality from septicemia is very high (>50%) and death can occur within one to two days after the first signs of illness (Gulig *et al.*, 2005; Jones & Oliver, 2009; Linkous & Oliver, 1999; Strom & Paranjpye, 2000). Like systemic disease, wound infections progress rapidly to cellulitis, ecchymoses and bullae which can progress to necrotizing fasciitis at the site of infection. Infection with *V. vulnificus* also causes a severe tissue damage and fulmination, indicating that pathogenicity of this bacterium is a multifactorial and complex phenomenon that involves many genes. Unique aspects of the infections caused by *V. vulnificus* are that most cases occur in persons over the age of 50, and, based on data compiled by the Food and Drug Administration of the USA, of 249 cases that occurred in the country over the last 10 years, 85% of the individuals who were developed into endotoxic shock from *V. vulnificus* infection were males

(Merkel *et al.*, 2001). Although the role of gender in *V. vulnificus*-induced endotoxic shock and death is not clearly understood, it has been suggested that the estrogen appears to be protective material after exposure to endotoxin. Thus, the decreased mortality from *V. vulnificus* endotoxic shock in women is supposed due to their higher estrogen levels (Merkel *et al.*, 2001).

1-1-2. Virulence factors of of *V. vulnificus*

Capsular polysaccharide (CPS) and Lipopolysaccharide (LPS)

In *V. vulnificus*, CPS is the most important virulence factor. *V. vulnificus* uses its CPS to avoid phagocytosis by host defense cells (Linkous and Oliver, 1999; Strom and Paranjpye, 2000). The presence of a capsule is involved in colonies opacity, with encapsulated strains being opaque and unencapsulated strains being translucent (Wright *et al.*, 1999). Unencapsulated mutants exhibited attenuated mortality in mouse models (Simpson *et al.*, 1987).

One of the major characteristics of *V. vulnificus* disease is endotoxic shock as the over-production of tumor necrosis factor (TNF) in response to LPS. Injections into rats of LPS extracted from *V. vulnificus* resulted in a dramatic decrease in heart rate and arterial pressure, followed by death (McPherson *et al.*, 1991). An inhibitor of nitric oxide synthase (N-monomethyl-L-arginine), LDL (low density lipoprotein) cholesterol, and estrogen were shown to mitigate the effects of LPS (Park *et al.*,

2007; Merkel *et al*., 2001). Injection of LDL prior to LPS exposure resulted in increase mouse survival and delay death in fatal cases (Park *et al*., 2007).

Hemolysin, metalloprotease, and RTX toxin

Secreted proteases, including Hemolysin (VvhA) and Metalloprotease (VvpE) have been examined and proposed to have a role in *V. vulnificus* virulence (Baffone *et al*., 2001; Miyoshi *et al*. 1993). Injection of purified samples of these proteins into animals reproduces some of the pathology of *V.* vulnificus infection. However, mutant strains that do not produce one or even both of these proteases are not significantly attenuated in animal models of infection (Kreger and Lockwood, 1981; Gray and Kreger, 1985; Jeong *et al*., 2000; Fan *et al*., 2001). The infection of mice with a double mutant for both *vvhA* and *vvpE* revealed no attenuation by the intraperitoneal (IP) route (Fan *et al*., 2001). The double mutant strain retained some cytotoxic activity in cell culture, suggesting the presence of another cytotoxin (Fan *et al*., 2001). However, these subsequent studies indicated no apparent role for these exoproteases.

RTX toxins are composed of repeated structural subunits which form pores in cellular membranes and are found in a broad range of gram-negative bacteria. *V. vulnificus* has RtxA1, which shows high homology to the RtxA sequence of *V. cholera*. The *rtxA1* mutant showed decreased cellular damage compared to the

parent strains and an inability to lyse cells or to disrupt either cell monolayers or tight junctions (Kim *et al*. 2008; Lee *et al*, 2007; Liu *et al*, 2007). It has been suggested that *V. vulnificus* RtxA1 results in cellular changes including rearrangement of cytoskeletal structure, bleb formation, and aggregation of actin leading to cellular necrosis and allow *V. vulnificus* to invade the bloodstream by crossing the intestinal epithelium (Kim *et al*. 2008).

The role of RtxA1 in relationship to *vvhA* and *vvpE* was investigated. The *vvpE vvhA* double mutant remained highly cytotoxic, however, an *rtxA1* vvpE double mutant showed a decrease in cytotoxicity similar to that of the *rtxA1* single mutant (Kim *et al*. 2008). Interestingly, a *vvhA rtxA1* double mutant and a *vvpE vvhA rtxA1* triple mutant were both devoid of cytotoxic effects. These results indicated that only mutation in *rtxA1* influences cell cytotoxicity (Kim *et al*. 2008).

Motility

Motility of pathogenic bacteria is also important in the process of infection as it allows adhesion and colonization to host cells (Ottemann & Miller, 1997). Therefore, motility can be proposed to be another virulence determinant in *V. vulnificus*. The mutation of FlgC, a flagella basal body rod protein, resulted in a significant decrease in motility, adhesion, and virulence *in vitro* and *in vivo* (Kim & Rhee, 2003). Flagellum-deficient *flgE* mutants of *V. vulnificus* also showed a

significant decrease in virulence in mice compared with that of the wild type (Lee *et al.*, 2004). In addition to attenuation of virulence in mice, the *flgE* mutation decreased the ability of *V. vulnificus* to form biofilms *in vitro* (Lee *et al.*, 2004).

1-2. Biofilm

Bacteria have adopted mechanisms for establishing microbial communities known as biofilms, which are embedded in a matrix of extracellular polymeric substances including polysaccharides, proteins, and nucleic acids (for a recent review, Flemming and Wingender, 2010). Biofilm formation contributes to a microorganism's survival in an environmental niche, and affects the likelihood of contact with a host. This surface-attached state is thought to be the primary means of persistence of bacteria in the environment, providing protection from a variety of stresses (Hall-Stoodley & Stoodley, 2005; Karatan & Watnick, 2009). Therefore, the biology of bacterial biofilms has become a major focus of microbial research

1-2-1. Biofilm matrix

The bacterial cells account for less than 10% of the dry mass of biofilms, whereas the matrix can account for over 90%. Biofilm matrix protects bacteria against various kind of stresses including desiccation, oxidizing or charged biocides, some anti biotics, ultraviolet radiation, protozoan grazers and host immune defences (Flemming and Wingender, 2010).

Exopolysaccharides (EPS) are a major component of biofilm matrix. In many bacteria, EPS is essential for biofilm formation, and mutants that cannot synthesize EPS are unable to form mature biofilms (Watnick and Kolter, 1999; Danese *et al.*,

2000). Bacteria can produce diverse types of EPS, for example, *Pseudomonas aeruginosa* produces at least three distinct EPS that contribute to biofilm development and architecture: alginate, Pel, and Psl (Ryder *et al*., 2007) . The biofilm matrix can contain considerable amounts of extracellular proteins including enzymes and structural proteins. Biopolymer-degrading enzymes allow bacteria to break down biopolymers and utilize them as carbon and energy sources (Wingender *et al*., 1999). In addition, some enzymes can degrade structural matrix component, and, in turn, promote biofilm dispersion (Sauer *et al*., 2004). The structural proteins in the biofilm matrix, such as the carbohydrate-binding proteins are involved in the formation and stabilization of the network consisting of polysaccharide matrix. Extracellular DNA (eDNA) also contributes to the formation of the biofilm matrix. It has been reported that eDNA comprise a large proportion especially in waste-water biofilms (Frølund *et al*., 1996).

1-2-2. Development stages of biofilm

Biofilm development of bacteria can be divided into several distinct stages. Firstly, planktonic bacterial cells attach to abiotic or biotic surfaces. This irreversible attachment is reinforced by adhesins or cellular appendages such as pili and fimbriae (Rosan and Lamont, 2000). In the second stage of biofilm development, attached cells multiply on the surfaces and produces large amount of matrix components to develop matured biofilm structure. The biofilm matrix provides

structural scaffold with the bacteria and contributes to antimicrobial resistance by acting as a diffusion barrier or binding directly to antimicrobial agents (Mah and O'Toole, 2001). The final stage of biofilm development is the detachment of cells from the biofilm colony and their dispersal into the environment. Like other stages of biofilm development, dispersal is a complex process that involves numerous environmental signals, signal transduction pathways, and effectors (Karatan and Watnick, 2009). This stage facilitates the dissemination of pathogens in new environments and dispersal of infections within a single host (Kaplan, 2010)

1-2-3. Cyclic di-GMP

A bacterial global second messenger, 3',5'-cyclic diguanylic acid (c-di-GMP), is known to be a key molecular factor in biofilm formation, adhesion, motility, virulence, and persistence of bacteria (for recent reviews, Boyd and O'Toole, 2012; Krasteva *et al*., 2012). c-di-GMP is synthesized and degraded by diguanylate cyclases (DGCs) containing the GGDEF domain and c-di-GMP-specific phosphodiesterases (PDEs) containing the EAL or HD-GYP domain, respectively. c-di-GMP receptors or effectors are c-di-GMP binding proteins or RNA. c-di-GMP binds to their receptors or effectors and give an effect by altering the structure and output function of the effector (Hengge, 2009). Low concentrations of c-di-GMP are associated with cells that move by using flagellar motors or retracting pili. In contrast, increasing concentrations of c-di-GMP promote the expression of

adhesive matrix components and result in multicellular behavior and biofilm formation (Jenal and Malone, 2006).

1-3. Objective of this study

V. vulnificus makes biofilms on various biotic surfaces, such as the eel body surfaces, crab shells, and oyster shells (Marco-Noales *et al*., 2001; Guo *et al*., 2010 and 2011). The mechanism of biofilm formation of *V. vulnificus* has been partially understood. Diguanylate cyclase protein A (DcpA) has been identified to regulate EPS production, biofilm formation, and rugose colony development in *V. vulnificus* (Nakhamchik *et al.,* 2008). It has been reported that *brp* gene cluster, which is involved in EPS synthesis, is crucial in biofilm formation and its expression is regulated by a global regulator, NtrC, which is required for mature biofilm development (Kim *et al*., 2009). Another study has demonstrated that the expression of *brp* gene cluster is regulated by c-di-GMP levels and also that BrpT is a putative regulator of *brp* gene cluster (Guo *et al*., 2010). However, the global gene expression profiling in response to the biofilm formation or increased level of c-di-GMP is understudied. In this study, I conducted whole-genome microarray comparing the global gene expression level under the conditions of high and low concentration of intracellular c-di-GMP. RNA sequencing was also performed to compare the transcriptome of *V. vulnificus* in different stages of biofilm development. Based on these transcriptome analyses, this study intended to identify

and characterize a gene which is regulated by c-di-GMP and essential for biofilm formation of *V. vulnificus*.

Chapter 2.

Transcriptome Analysis of *V. vulnificus* in Response to Increased Cyclic di-GMP Level

2-1. Introduction

Many bacteria live within multicellular structures known as biofilms, which are embedded in a matrix of extracellular polymeric substances including polysaccharides, proteins, and nucleic acids (for a recent review, Flemming and Wingender, 2010). Biofilm formation facilitates bacteria to survive against various unpredictable stresses including starvation, temperature change, antimicrobial agents challenge, and host defense systems (Costerton *et al*., 1987; Johnson *et al*., 2008). Therefore, the transmission and persistence of pathogenic bacteria are enhanced by forming biofilms (Huq *et al*., 2008). Previous studies have reported that a pathogenic marine bacterium, *V. vulnificus*, makes biofilms on various biotic surfaces, such as the eel body surfaces, crab shells, and oyster shells (Marco-Noales *et al*., 2001; Guo *et al*., 2010 and 2011). Furthermore, there are several lines of evidence supporting the idea that the biofilm formation is required for persistence and pathogenesis of *V. vulnificus* in its hosts. (Grau *et al*., 2005; Paranjpye *et al*., 2007; Kim *et al*., 2013)

A bacterial global second messenger, 3',5'-cyclic diguanylic acid (c-di-GMP), is known to be a key molecular factor in biofilm formation, adhesion, motility, virulence, and persistence of bacteria (for recent reviews, Boyd and O'Toole, 2012;

Krasteva *et al*., 2012). c-di-GMP is synthesized by diguanylate cyclases (DGCs) and degraded by c-di-GMP-specific phosphodiesterases (PDEs). DGCs and PDEs are ubiquitous in bacteria and c-di-GMP networks consisting of these enzymes have been identified in many bacteria (Boyd and O'Toole, 2012). *V. vulnificus* DcpA (diguanylate cyclase protein A) has been identified to influence exopolysaccharide (EPS) production, biofilm formation, and rugose colony development (Nakhamchik *et al*., 2008). In addition, c-di-GMP-regulated EPS locus (brp) and its putative regulator BrpT have been reported to be essential for biofilm formation (Guo *et al*., 2010). In this study, we presented that c-di-GMP influenced biofilm development and colony morphotype of *V. vulnificus*. Furthermore, a whole-genome microarray analysis revealed that more than 5% of *V. vulnificus* genes were differentially expressed depending on the levels of intracellular c-di-GMP.

2-2. Materials and Methods

2-2-1. Strains, plasmids, and culture media.

The bacterial strains and plasmids used in this study are listed in Table 2-1. Unless noted otherwise, *V. vulnificus* strains were grown in Luria-Bertani medium supplemented with 2.0% (wt/vol) NaCl (LBS) at 30℃. The *Vibrio fisheri* minimal medium containing 32.6 mM glycerol (VFMG) was used for biofilm formation (Kim *et al*., 2013) and AB medium (Greenberg *et al*., 1979) was used for transciptome analysis. When required, antibiotics were added to the media. All the media components were purchased from Difco (Detroit, MI), and the chemicals were purchased from Sigma (St. Louis, MO).

2-2-2. Manipulation of intracellular level of c-di-GMP

To manipulate intracellular level of c-di-GMP, the *dcpA* coding region encoding diguanylate cyclase (Nakhamchik *et al*., 2008) was amplified by PCR using *V. vulnificus* genomic DNA and a pair of primers dcpA_F and dcpA_R (Table 2-2). The amplified dcpA coding region was cloned into a broad host-range vector pJH0311 (Goo *et al*., 2006) under an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible promoter to result in pJN1002 (Table 2-1). The plasmid pJN1002 was delivered into *V. vulnificus* by conjugation (Kim *et al*., 2013), and intracellular c-

di-GMP levels of *V. vulnificus* strains carrying pJN1002 were measured using LC-MS as previously described (Irie *et al*., 2012).

2-2-3. Biofilm formation

Biofilm formation activity of the *V. vulnificus* strains in static or shaking culture was assessed as previously described (Russo *et al*, 2006; Kim *et al*., 2013). Static biofilms were formed by standing the polystyrene microtiterplates (Nunc, Roskilde, Denmark) containing 200 μl culture with VFMG for 12 h at 30^{o}C, stained with 1% (wt/vol) crystal violet (CV) solution for 15 min at room temperature, and then quantitated by measuring the amount of CV eluted from the biofilms as an absorbance at 570 nm (A_{570}) (Kim *et al*., 2009). For the shaking condition, 5ml cultures with VFMG were grown for 12 h at 30^{o}C with shaking at 220 rpm. Biofilm rings at the air–liquid interface of the shaking cultures were photographed using a digital camera (Canon POWERSHOT SX220 HS, Japan).

2-2-4. Colony morphology assay.

For the analysis of colony morphology, 2 μl of cultures grown to A_{600} 0.8 was spotted onto agar plates supplemented with 100 μg/ml ampicillin and 1 mM IPTG. The colonies grown at 30 ℃ for 24 h were photographed as described above.

2-2-5. Transcriptome analysis.

For transcriptome analysis, *V. vulnificus* strains containing either pJN1002 or pJH0311 were grown to A_{600} 0.7 with AB medium (300 mM NaCl, 50 mM $MgSO_4$, 0.2% vitamin-free casamino acids, 10 mM potassium phosphate, 1 mM L-arginine, pH 7.5; Greenberg *et al*., 1979) in the presence of 0.1 mM IPTG and total cellular RNAs were isolated. Aminoallyl-cDNA was synthesized using an Amino Allyl cDNA Labeling Kit (Ambion, Austin, TX) and were respectively labeled with Cy5 or Cy3 (Amersham Pharmacia, Uppsala, Sweden). Equal amounts of the labeled cDNA were combined to hybridize the *V. vulnificus* Whole-Genome Twin-Chip (Kim *et al*., 2013) at 42^{o}C for 16 h and the arrays were washed, dried, scanned, and analyzed by GenePix Pro 3.0 software (Axon Instruments, Union City, CA). The ORF spots that showed 2-fold or greater difference in expression with a *P*-value of $\leq$ 0.05 were considered to be regulated by c-di-GMP. Each sample was run in biological triplicate, of which 2 technical replicates were performed.

2-2-6. quantitative real-time PCR (qRT PCR)

For qRT-PCR, cDNA was synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Real-time PCR amplification of the cDNA was performed by using the Chromo 4 real-time PCR detection system (Bio-Rad) with a pair of specific primers listed in Table S2 as described previously (Kim *et al*., 2013).

2-2-7. Generation of the c-di-GMP regulon mutants

The coding regions of some of the c-di-GMP regulon on chromosome were amplified and inactivated *in vitro* by deletion of the coding region of each gene using the PCR-mediated linker-scanning mutation method as described previously (Kim *et al.*, 2012). Brifely, for construction of VV2_1570 mutant (*brpT* mutant), pairs of primers VV21570_F1_F and VV21570_F1_R (for amplification of the 5' amplicon) or VV21570_F2_F and VV21570_F2_R (for amplification of the 3' amplicon) were designed and used as listed in Table 2-2. A 405-bp deleted VV2_1570 (*brpT*) was amplified by PCR using the mixture of both amplicons as the template and VV21570_F1_F and VV21570_F2_R as primers (Table 2-2). In order to inactivate VV2_1570 (*brpT*), a 1.2-kb *nptI* DNA conferring resistance to kanamycin (Oka *et al*., 1981) was also inserted into a unique BamHI site present within the Δ*brpT* to result in Δ*brpT*::*nptI*. The resulting Δ*brpT*::*nptI* was ligated with SpeI-SphI-digested pDM4 (Milton *et al.*, 1996) to form pJN0903 (Table 2-1). The *E. coli* S17-1 λ *pir*, *tra* containing pJN0903 was used as a conjugal donor to *V. vulnificus* CMCP6 to result in the VV2_1570 mutant (JN091) (Table 2-1). Similar experimental procedures were adopted for amplification of other cancidate genes *in vitro*, except for used primers and conjugal donors (Table 2-2).

2-2-8. Microarray data accession number

All primary microarray data were deposited into the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/projects/geo/) under accession number GSE51459.

Table 2-1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics[a]	Reference or source
Bacterial strains		
V. vulnificus		
CMCP6	Wild type *V. vulnificus*, virulent	Laboratory collection
JN101	CMCP6 with Δ VV1_1829	This study
JN102	CMCP6 with Δ VV2_0264	This study
WL131	CMCP6 with Δ VV1_0525	This study
JN091	CMCP6 with Δ VV2_1570 (*brpT*)	This study
JN094	CMCP6 with Δ VV2_1582	This study
JN103	CMCP6 with Δ VV1_2716	This study
JN104	CMCP6 with Δ VV1_2715	This study
E. coli		
DH5α	*supE44* Δ*lacU169* (ϕ80*lacZ* ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*; plasmid replication	Laboratory collection
S 17-1λ*pir*	λ-*pir* lysogen; *thi pro hsdR hsdM*$^{+}$ *recA* RP4-2 Tc::Mu-Km::Tn7;Tp^{r} Sm^{r}; host for π-requiring plasmids; conjugal donor	Simon *et al*. (1983)
Plasmids		
pGEM-T Easy	PCR product cloning vector; Ap^{r}	Promega
pDM4	suicide vector; *ori*R6K; Cm^{r}	Milton *et al.* (1996)
pJH0311	0.3-kb NruI fragment containing multi-cloning site of pUC19 cloned into pCOS5; Ap^{r}, Cm^{r}	Goo *et al*. (2006)
pJN1002	pJH0311 with *dcpA*	This study
pJN1006	pDM4 with ΔVV1_1829; Cm^{r}	This study
pJN1008	pDM4 with ΔVV2_0246; Cm^{r}	This study
pWL1302	pDM4 with ΔVV1_0525; Cm^{r}	This study
pJN0903	pDM4 with ΔVV2_1570 (*brpT)*; Cm^{r}	This study
pJN0907	pDM4 with ΔVV2_1582; Cm^{r}	This study
pJN1004	pDM4 with ΔVV1_2716; Cm^{r}	This study

pJN1104	pDM4 with ΔVV1_2715; Cm^r	This study

[a]Ap^r, ampicillin resistant; Km^r, kanamycin resistant; Cm^r, chloramphenicol resistant; Sm^r, streptomycin resistant.

Table 2-2. Oligonucleotides used in this study.

Oligonucleotide[a]	Sequence (5' → 3')[b]	Use
dcpA_F	GAGCTCTCATCCCATCCTAAACTTTGT	Amplification of the *dcpA* coding region
dcpA_R	CCCGGGTGGGTTTTCTGTTG	
qRT11829_F	AATGCCTTATGCTGACGGTTGTTC	qRT-PCR
qRT11829_R	GAGATTCGCTCTAAAGTGCTTGCC	
qRT12716_F	ACGAATCCATCAATCCAGCAGAGG	
qRT12716_R	CGACGAACCATCCCAAATCAACTG	
qRT12976_F	CAAGAAGACGAGTCAACCAATCCC	
qRT12976_R	TTTCCGAAGATCAAATGGCAGAGC	
qRT13061_F	GGCGGAAGCGGTGACGAC	
qRT13061_R	GCAAATGGTTGGTGAGAACAAACG	
qRT20196_F	ACCTGCGTAAAGTGAACCAAGAAC	
qRT20196_R	TGGTGTGGGTGGCGAATACTC	
qRT20264_F	GAAGTAAGGCATCTGCGTGAGTTG	
qRT20264_R	TTAAAGTGGGCGAAAGCGAAGC	
qRT20559_F	CCAAGACCTGCTGAAGAAGTGAAG	
qRT20559_R	AATGGAGTCAACGCTTGTTTACCG	
qRT21570_F	TCAACAAAGCCTTCAGAGTGAGAATC	
qRT21570_R	GAGATCAACAAGCAGAATACTTTCAGC	
qRT21571_F	TTGGTTGCTGGCTCTGGTGAC	
qRT21571_R	ACTGTCTATACGCACTGTGTCCTC	
qRT21572_F	GCCATTGCCCAGACCCAGAG	
qRT21572_R	CCGATAATACCAACCGCACAACC	
qRT21573_F	TTGGCGGTGGTATTGGCTACTG	
qRT21573_R	TGTTGAATTGCCTGGCGTTGAC	
qRT21582_F	CGGCAAACGGAGTATCAAAGACG	
qRT21582_R	ACCAGTTCTCAGACACCACAGC	
VV11829_F1_F	GATCGCGACTTCCGGTGATTAC	Construction of
VV11829_F1_R	GGTGGATCCAACATTCATATCACCAT	

VV11829_F2_F	GTTGGATCCACCCAAGGAAGAGG	VV1_1829 mutant
VV11829_F2_R	CTCATTCCAAATGTCACGGCG	
VV20264_F1_F	AGTGACATAGCCGCGATTTGGT	Construction of VV2_0264 mutant
VV20264_F1_R	GAAGGATCCATGCCTGGCAGT	
VV20264_F2_F	CATGGATCCTTCGGGTTGGATA	
VV20264_F2_R	GCCAGTGTATTTATTCCTGCATTGTC	
VV10525_F1_F	TCAGCGCCGATGCTGATAACC	Construction of VV1_0525 mutant
VV10525_F1_R	AGGGATCCCGATAGTAAACGCAGC	
VV10525_F2_F	CGGGATCCCTCATCATGGCTAAGG	
VV10525_F2_R	GTCGGGTGATGACGAAGCCATG	
VV21570_F1_F	TGTATTTGGCGTTCTTTGACTGGAA	Construction of VV2_1570 (*brpT*) mutant
VV21570_F1_R	TATCGGATCCATCCAGTTTCAGCCA	
VV21570_F2_F	GGATGGATCCGATAAGGCAGGTG	
VV21579_F2_R	CACCTTGGCCACCGATCAAA	
VV21582_F1_F	GCGGGTTTTACGCCTATCGTGG	Construction of VV2_1582 mutant
VV21582_F1_R	CATCGGATCCATGTGCACATCTCGT	
VV21582_F2_F	ACATGGATCCGATGCAGCTCATTG	
VV21582_F2_R	GCAACTGTTGTTGATATTTCGCGAGGC	
VV12716_F1_F	ACTAGTGTTAAAAAATTGACGATTTGC	Construction of VV1_2716 mutant
VV12716_F1_R	TGCGGATCCAAGGGAAAGAATT	
VV12716_F2_F	CTTGGATCCGCACGTAAAGGTTACT	
VV12716_F2_R	GCCCCTTACGTGAAAAACTACTTCTT	
VV12715_F1_F	ATAGCTCTGCCGCGCTGATG	Construction of VV1_2715 mutant
VV12715_F1_R	GTAACGGGCTCCGTTGGAGG	
VV12715_F2_F	CCAAATGACTTGGCTGATTGCC	
VV12715_F2_R	AGCGCTTGCAGAATCAACGG	

[a] The oligonucleotides were designed using the *V. vulnificus* CMCP6 genome sequence (GenBank accession numbers AE016795 and AE016796).

2-3. Results

2-3-1. Intracellular c-di-GMP concentrations of *V. vulnificus* strains.

In order to verify whether the overexpression of *dcpA* increase c-di-GMP levels, intracellular c-di-GMP levels of *V. vulnificus* strains were measured using LC-MS as previously described (Irie *et al*., 2012). The result revealed that the c-di-GMP level in the *V. vulnificus* containing pJN1002 was about 3-fold greater than that in the *V. vulnificus* containing the control vector, pJH0311 (Fig. 2-1).

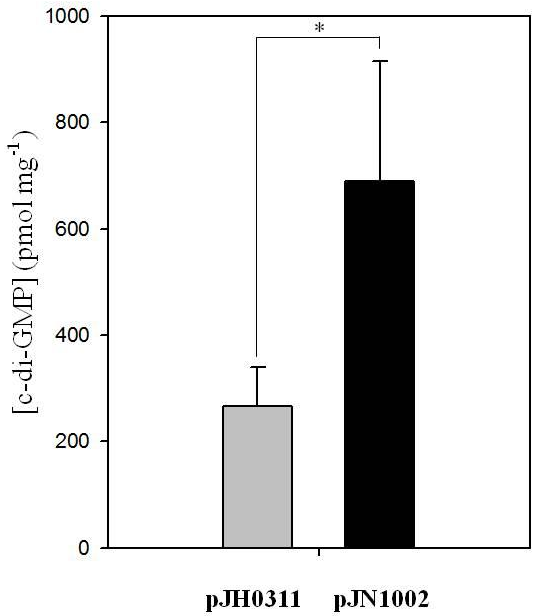

Fig. 2-1. Intracellular c-di-GMP concentrations of *V. vulnificus* strains. The wild type *V. vulnificus* containing pJH0311 or pJN1002 were grown in VFMG supplemented with 0.1mM IPTG. Intracellular concentrations of c-di-GMP were measured by LC-MS and normalized to mg of total protein. Error bars represent the SEM. ∗, $P < 0.05$ relative to the wild type containing pJH0311.

2-3-2. The effects of c-di-GMP on biofilm and rugose colony formation of *V. vulnificus*.

Biofilm formation abilities of the *V. vulnificus* strains were assessed in static and shaking cultures. When induced by 1 mM IPTG, biofilm formation activity of the *V. vulnificus* containing pJN1002 was 1.6-fold higher than that of the *V. vulnificus* containing pJH0311 (negative control) in static condition (Fig. 2-2A). Moreover, in the shaking condition, the *V. vulnificus* containing pJN1002 formed a thick biofilm, while the *V. vulnificus* containing pJH0311 did not produced biofilm at the air-liquid interface (Fig. 2-2B). These results indicated that increased c-di-GMP level in cells promoted biofilm formation of *V. vulnificus* especially at the air-liquid interface.

The *V. vulnificus* containing pJN0311 exhibited smooth colony morphology, on the other hand, the *V. vulnificus* containing pJN1002 produced rugose colonies (Fig. 2-2C). This indicated that the elevated intracellular c-di-GMP level increased colony rugosity of the pathogen.

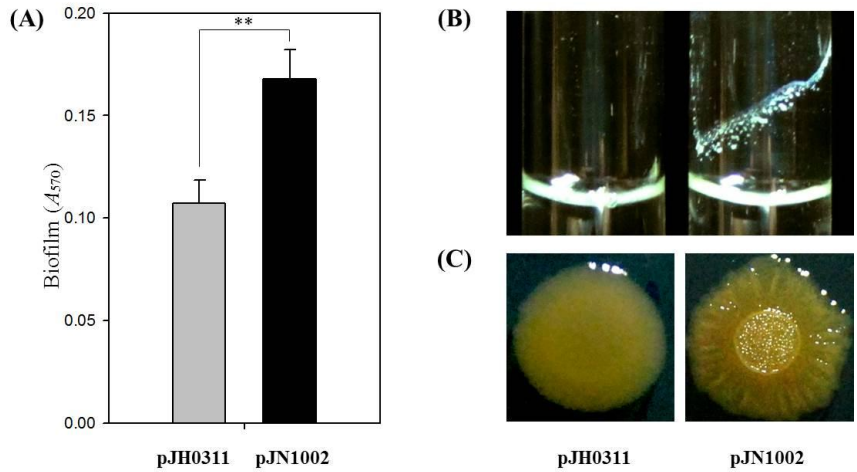

Fig. 2-2. The effect of intracellular c-di-GMP levels on biofilm formation and rugose colony development of *V. vulnificus*. (A and B) Biofilms of the wild type *V. vulnificus* containing pJH0311 or pJN1002 were grown in VFMG supplemented with 0.1mM IPTG under static (microtiterplate wells, A) and shaking (test tubes, B) culture conditions. Static biofilms were quantitated using CV staining (A). (C) The *V. vulnificus* strains were grown onto LBS agar plates containing 100 μg/ml of ampicillin., then the plates were incubated at 30℃ for 24 h. Each colony cluster showing the mean rugosity from at least three independent experiments was photographed. **, $P < 0.005$ relative to the wild type containing pJH0311.

2-3-3. Identification of the c-di-GMP regulon.

Transcripts from the *V. vulnificus* containing pJN1002 were compared to those from the pathogen containing pJH0311. The microarray analysis predicted 233 genes potentially regulated by c-di-GMP, 109 of which were up-regulated and 124 of which were down-regulated. The predicted genes of the c-di-GMP regulon are distributed throughout the two chromosomes of *V. vulnificus*. A complete list of names or locus tags of the 233 genes is shown in Table 2-3. Although a substantial portion of the predicted genes are of hypothetical or unknown functions, 55.8 % of those belong to functional categories (Fig. 3). Among these, ten categories, including amino acid transport and metabolism, carbohydrate transport and metabolism, cell wall/membrane biogenesis, defense mechanisms, energy production and conversion, inorganic-ion transport and metabolism, posttranslational modification/protein turnover/chaperones, signal transduction mechanisms, transcription, and translation contain at least 5 genes (Fig. 2-3).

Table 2-3. Genes and their products of the c-di-GMP regulon identified using the *V. vulnificus* whole genome microarray.

Locus tag[a]	M value[b]	Gene product
Amino acid transport and metabolism		
VV1_0209	-2.671	Cysteine synthase A
VV1_1001	-2.218	Amino acid ABC transporter, periplasmic amino acid binding portion
VV1_2149	-1.381	Choline dehydrogenase and related flavoprotein
VV1_1002	-1.136	Amino acid ABC transporter, permease protein
VV1_1087	1.132	Peptide ABC transporter, permease protein
VV2_1548	1.137	Protease II
VV1_1467	1.162	Arginine deiminase
VV1_0726	3.250	Sulfate adenylate transferase, subunit 2
Carbohydrate transport and metabolism		
VV2_1353	-1.255	PTS system, fructose-specific IIABC component
VV1_2312	-1.234	Hypothetical protein
VV2_1590	-1.006	Permease of the drugmetabolite transporter (DMT) superfamily
VV2_0957	1.008	Putative permease
VV2_1018	1.026	N-acetylglucosamine-6-phosphate deacetylase
VV2_0586	1.430	Putative permease
VV1_2010	2.563	Probable transmembrane water channel aquaporin Z
VV2_1626	3.108	Predicted membrane protein
Cell cycle control, mitosis and meiosis		
VV1_1157	-1.482	Cell division protein FtsX
Cell motility		
VV2_0477	-1.905	Methyl-accepting chemotaxis protein
VV2_1523	-1.613	Methyl-accepting chemotaxis protein
VV1_2868	-1.105	Methyl-accepting chemotaxis protein
Cell wall/membrane/envelope biogenesis		
VV2_0814	-4.219	Putative lipoprotein
VV2_0578	-3.404	Outer membrane protein N, non-specific porin
VV1_2309	-2.000	Putative glycosyltransferase protein
VV1_2305	-1.911	Putative exopolysaccharide biosynthesis protein
VV1_2688	-1.639	Outer membrane efflux protein

VV1_2125	-1.502	Putative outer membrane lipoprotein
VV1_2308	-1.431	Putative glycosyltransferase protein
VV1_2310	-1.322	Putative glycosyltransferase protein
VV1_2304	-1.255	Putative polysaccharide export-related protein
VV1_2302	-1.149	Putative capsular polysaccharide biosynthesis glycosyltransferase
VV1_1867	-1.032	Predicted membrane-associated Zn-dependent protease
VV1_0582	-1.020	UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate-D-alanyl-D-alanyl ligase
VV2_0478	-1.012	Alanine racemase 2
VV1_2716	1.024	Agglutination protein
VV1_1428	1.226	UDP--glucose-1-phosphate uridylyltransferase
VV2_0807	1.361	OmpA-like transmembrane domain protien
VV2_1681	1.805	D-alanyl-D-alanine carboxypeptidase
VV1_2114	1.848	Porin-like protein H precursor OmpH
VV1_2799	2.163	Outer membrane phospholipase A
VV2_0196	2.672	Opacity protein and related surface antigen
VV2_0559	3.564	Outer membrane protein, OmpA family
VV2_1579	5.523	Putative exopolysaccharide biosynthesis protein
VV2_1580	6.064	Putative polysaccharide export-related protein
VV2_1578	6.126	Putative lipopolysaccharide biosynthesis protein
VV2_1582	6.624	Undecaprenyl-phosphate glucose phosphotransferase, BrpA
VV2_1576	7.291	Glycosyltransferase
Coenzyme transport and metabolism		
VV1_0727	-2.644	Uroporphyrinogen-III methylase
VV1_1841	-2.089	Putative p-aminobenzoyl-glutamate transporter
VV1_3169	-1.531	O-succinylbenzoate-CoA synthase
VV2_1563	-1.400	vWA, von Willebrand factor (vWF) type A domain containing protein
Defense mechanisms		
VV2_0784	-1.527	Multidrug resistance efflux pump
VV2_0170	-1.388	Multidrug resistance efflux pump
VV1_2689	-1.136	Multidrug resistance efflux pump
VV2_1306	2.332	Na+-driven multidrug efflux pump
VV2_1573	3.164	HlyD family secretion protein
DNA replication, recombination, and repair		

VV1_1533	-1.523	Endonuclease I
VV1_3055	-1.026	DNA polymerase elongation subunit (family B)
VV1_0420	1.166	Exodeoxyribonuclease VII, large subunit
Energy production and conversion		
VV1_1879	-2.523	Nitrogen regulatory protein PII
VV1_2147	-1.639	NAD-dependent aldehyde dehydrogenase
VV1_2895	-1.245	Trimethylamine-N-oxide reductase
VV2_0230	-1.388	Cytochrome B561
VV2_1077	-1.105	N-ethylmaleimide reductase
VV1_1787	1.158	Glycerol kinase
VV1_2591	1.172	Formate dehydrogenase, iron-sulfur subunit
Function Unknown		
VV2_0331	-3.591	Conserved hypothetical protein
VV2_0044	-3.012	Putative chitinase
VV1_2846	-2.565	Predicted membrane protein
VV1_1837	-2.204	Conserved hypothetical protein
VV1_1545	-2.000	Conserved hypothetical protein
VV1_1144	-1.989	Conserved hypothetical protein
VV2_1409	-1.878	Predicted membrane protein
VV1_0410	-1.806	Uncharacterized membrane-associated protein
VV2_1275	-1.262	Uncharacterized membrane-associated protein
VV2_1243	1.026	Predicted membrane protein
VV1_2809	1.172	Conserved hypothetical protein
VV1_2015	1.214	Conserved hypothetical protein
VV2_0347	1.322	Conserved hypothetical protein
VV1_0634	2.115	Conserved hypothetical protein
VV2_1137	3.010	Conserved hypothetical protein
General function prediction only		
VV2_1566	-1.868	MoxR-related protein
VV2_0803	-1.801	Uncharacterized protein containing a von Willebrand factor type A (vWA) domain
VV1_2268	-1.685	Putative ATP-binding protein
VV1_2632	-1.626	ABC transporter with duplicated ATP-binding protein
VV2_1565	-1.617	Conserved hypothetical protein

VV2_1083	-1.322	Predicted hydrolase of the HAD superfamily
VV1_0192	-1.238	Predicted permease
VV1_1323	-1.238	ABC transporter, ATP-binding protein
VV1_1907	-1.184	Putative Zn-dependent protease contains TPR repeats
VV2_0875	-1.123	Putative epimerase, PhzCPhzF homolog
VV1_2869	-1.083	Na+-dependent transporter, SNF family
VV1_2857	-1.080	Predicted Fe-S protein
VV2_1360	-1.050	Putative formate dehydrogenase, alpha subunit
VV1_1543	-1.009	Lysine efflux permease
VV2_0107	1.009	Predicted pyrophosphatase, MazG
VV1_2976	1.081	Putative ABC transporter, permease protein
VV1_2193	1.101	Predicted acetyltransferase
VV1_1794	1.102	Predicted hydrolase of alkaline phosphatase superfamily
VV1_1473	1.116	Putative Zn-dependent protease with chaperone function
VV2_1472	1.207	AnkB protein
VV1_0863	1.382	Predicted amidophosphoribosyltransferase ComFC
VV2_1675	1.771	Putative homoserine kinase type II
VV2_1575	3.827	Putative membrane protein involved in the export of O-antigen and teichoic acid
VV2_1572	3.836	Proteaselipase ABC transporter, ATPase and permease protein
Hypothetical protein		
VV2_0982	-4.059	Hypothetical protein
VV1_2376	-3.252	Conserved hypothetical protein
VV2_0783	-3.198	Hypothetical protein
VV1_2856	-2.358	Hypothetical protein
VV1_2286	-2.146	Hypothetical protein
VV2_0813	-1.873	Hypothetical protein
VV1_1835	-1.852	Hypothetical protein
VV1_2727	-1.831	Hypothetical protein
VV2_1642	-1.826	Hypothetical protein
VV2_0990	-1.781	Hypothetical protein
VV2_0649	-1.776	Putative TraG protein
VV1_3250	-1.595	Hypothetical protien
VV1_2307	-1.582	Hypothetical protein

VV2_0284	-1.578	Hypothetical protein
VV1_2303	-1.548	Hypothetical protein
VV2_1359	-1.510	Hypothetical protein
VV2_1562	-1.478	TPR repeat and von Willebrand factor (vWF) type A domain containing protein
VV1_2311	-1.462	Hypothetical protein
VV2_0766	-1.373	Hypothetical protein
VV2_0404	-1.351	Hemolysincytolysin VvhA
VV2_1561	-1.347	Hypothetical protein
VV2_1312	-1.311	Hypothetical protein
VV2_0403	-1.265	Cytolysin secretion protein VvhB
VV1_2998	-1.228	Hypothetical protein
VV2_0537	-1.194	Hypothetical protein
VV1_1853	-1.162	Hypothetical protein
VV1_2105	-1.114	Hypothetical protein
VV1_3072	-1.038	Hypothetical protein
VV2_0332	-1.023	Hypothetical protein
VV1_0139	-1.014	Hypothetical protein
VV1_2971	1.004	Hypothetical protein
VV1_3034	1.021	Cytochrome c-type protein NrfB precursor
VV2_1399	1.041	Hypothetical protein
VV2_0304	1.062	Hypothetical protein
VV1_2329	1.064	Hypothetical protein
VV2_1402	1.079	Putative integral membrane protein
VV1_3114	1.087	V10 pilin-like protein
VV1_3224	1.091	Hypothetical protein
VV1_2388	1.100	Hypothetical protein
VV2_1420	1.113	Hypothetical protein
VV1_0734	1.130	Hypothetical protein
VV1_2555	1.158	Hypothetical protein
VV1_2054	1.162	Hypothetical protein
VV2_0781	1.181	Hypothetical protein
VV1_2192	1.286	Hypothetical protein
VV1_2362	1.303	Conserved hypothetical protein

VV2_1176	1.310	Hypothetical protein
VV2_0538	1.373	Hypothetical protein
VV2_1389	1.407	Hypothetical protien
VV2_1682	1.408	Leucine-rich repeats (LRRs), ribonuclease inhibitor (RI)-like subfamily
VV2_1551	1.420	Hypothetical protein
VV2_1552	1.570	Hypothetical protein
VV2_0988	1.575	Hypothetical protein
VV2_1627	2.225	O-antigen polymerase
VV2_0374	2.456	Hypothetical protien
VV2_1307	2.489	Hypothetical protein
VV1_2313	2.650	Hypothetical prtoein
VV1_0653	2.956	Hypothetical protein
VV2_1574	3.373	Hypothetical protein
VV2_1043	3.459	Hypothetical protein
VV2_1694	4.254	Hypothetical protein
VV2_1581	5.383	Hypothetical protein
VV2_1571	6.114	Putative calcium-binding protein
VV2_1577	7.654	Hypothetical protein
VV1_2971	1.004	Hypothetical protein
VV1_3034	1.021	Cytochrome c-type protein NrfB precursor
VV2_1399	1.041	Hypothetical protein
VV2_0304	1.062	Hypothetical protein
VV1_2329	1.064	Hypothetical protein
VV2_1402	1.079	Putative integral membrane protein
VV1_3114	1.087	V10 pilin-like protein
VV1_3224	1.091	Hypothetical protein
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VV2_1420	1.113	Hypothetical protein
VV1_0734	1.130	Hypothetical protein
VV1_2555	1.158	Hypothetical protein
VV1_2054	1.162	Hypothetical protein
VV2_0781	1.181	Hypothetical protein
VV1_2192	1.286	Hypothetical protein

VV1_2362	1.303	Conserved hypothetical protein
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VV2_0538	1.373	Hypothetical protein
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VV1_0653	2.956	Hypothetical protein
VV2_1574	3.373	Hypothetical protein
VV2_1043	3.459	Hypothetical protein
VV2_1694	4.254	Hypothetical protein
VV2_1581	5.383	Hypothetical protein
VV2_1571	6.114	Putative calcium-binding protein
VV2_1577	7.654	Hypothetical protein
Inorganic ion transport and metabolism		
VV1_2201	-1.340	Integral membrane protein TerC family
VV2_1549	1.083	Putative TonB system receptor
VV2_0392	1.197	Nitrate ABC transporter, permease protein
VV2_0843	1.327	Vulnibactin outer membrane receptor precursor VuuA
VV1_0723	2.150	Adenylylsulfate kinase
VV1_0724	3.348	Putative sodiumsulfate symporter
VV1_0725	3.759	Sulfate adenylate transferase subunit 1
Intracellular trafficking and secretion		
VV1_2170	-1.065	TolB protein
VV1_2330	1.150	Putative Flp pilus assembly protein CpaB
Lipid transport and metabolism		
VV1_1971	-1.194	Long-chain fatty acid transport protein

VV1_1976	-1.127	Fatty oxidation complex, alpha subunit
VV1_2871	-1.056	Cardiolipin synthase
Nucleotide transport and metabolism		
VV2_0712	1.038	GMP reductase
Posttranslational modification, protein turnover, chaperones		
VV1_0603	-2.680	Protease DO
VV2_0008	-1.932	Hypothetical protein
VV1_2858	-1.837	Heat shock protein HslJ
VV1_0189	-1.347	Heat shock protein 90 HtpG
VV1_0201	-1.155	Thioredoxin domain-containing protein
VV1_0529	-1.086	Thiol:disulfide interchange protein DsbC
VV1_2152	-1.059	Conserved hypothetical protein
VV2_1550	1.339	Putative regulatory P domain of the subtilisin-like proprotein convertase and other protease
VV1_1033	3.237	ComM-related protein
Secondary metabolites biosynthesis, transport, and catabolism		
VV1_0685	-1.171	ABC-type transporter involved in resistance to organic solvents, ATP-binding protein
VV2_0989	1.180	Alkyl sulfatase and related hydrolase
VV1_3061	5.197	Hypothetical protein
Signal transduction mechanisms		
VV2_1512	-2.977	Sensor histidine kinaseresponse regulator
VV2_0992	-2.608	Sensor histidine kinase
VV2_1510	-2.388	Putative response regulator
VV2_1511	-2.279	Putative response regulator
VV1_3201	-2.204	Putative response regulator
VV2_1509	-2.059	GGDEF family protein
VV1_2259	-1.826	Hypothetical protein
VV2_0991	-1.806	Response regulator, OmpR family
VV2_0138	-1.635	Response regulator VieA
VV2_0137	-1.523	Sensory box sensor histidine kinaseresponse regulator VieS
VV2_0464	-1.344	Putative sensor histidine kinase
VV2_1123	-1.241	Signal transduction histidine kinase regulating C4-dicarboxylate transport system

VV2_0205	-1.139	EAL domain protein
VV1_0502	-1.095	Putative regulator of cell autolysis LytS
VV2_0264	1.468	Predicted signal transduction protein containing a membrane domain, an EAL and a GGDEF domain
VV2_0778	1.627	Sensor histidine kinase
VV1_1829	1.757	Response regulator containing a CheY-like receiver domain and a GGDEF domain
VV1_0525	2.848	Sigma-54 dependent transcriptional regulator VpsR
VV2_1570	3.507	Response regulator containing a CheY-like receiver domain and an HTH DNA-binding domain, LuxR family
VV2_1569	3.976	DNA-binding HTH domain-containing protein
Transcription		
VV1_2136	-1.949	Conserved hypothetical protein
VV2_0882	-1.474	Putative transcriptional regulator
VV1_0451	1.057	Transcriptional regulator, LysR family
VV1_1358	1.189	Transcriptional regulator, LacI family
VV2_0779	1.306	Response regulator, OmpR family
VV2_0857	1.427	Hypothetical protein
VV2_0776	1.526	Transcriptional regulator containing a DNA-binding HTH domain and an aminotransferase domain (MocR family)
VV2_1674	1.546	Transcriptional regulator, LysR family
VV2_0519	1.696	Cold shock protein CSD
VV1_2692	1.715	DNA-binding HTH domain-containing protein
VV2_0503	3.858	Cold shock protein CspA
Translation		
VV1_2630	-1.569	Ribosome modulation factor
VV1_0763	1.008	30S ribosomal protein S10
VV1_0511	1.016	Ribosomal protein S20
VV1_0753	1.019	Ribosomal protein S17
VV1_0762	1.217	50S ribosomal protein L3

[a] Functional categories, locus tag numbers, and annotation of gene products are based on the database of the *V. vulnificus* CMCP6 genome, which was retrieved from GenBank (accession numbers AE016795 and AE016796). Functional categories in boldface are shown above the first gene in each category.

[b] The M value represents the log2 ratio of DNA hybridization intensity of each gene in the *V. vulnificus* containing pJN1002 versus the *V. vulnificus* containing pJH0311. The values shown are the mean from three independent experiments. The locus tags and genes with $M \geq 1$ or $M \leq -1$ (expression ratios of ≥ 2, $P \leq 0.05$) were considered as the genes regulated by c-di-GMP.

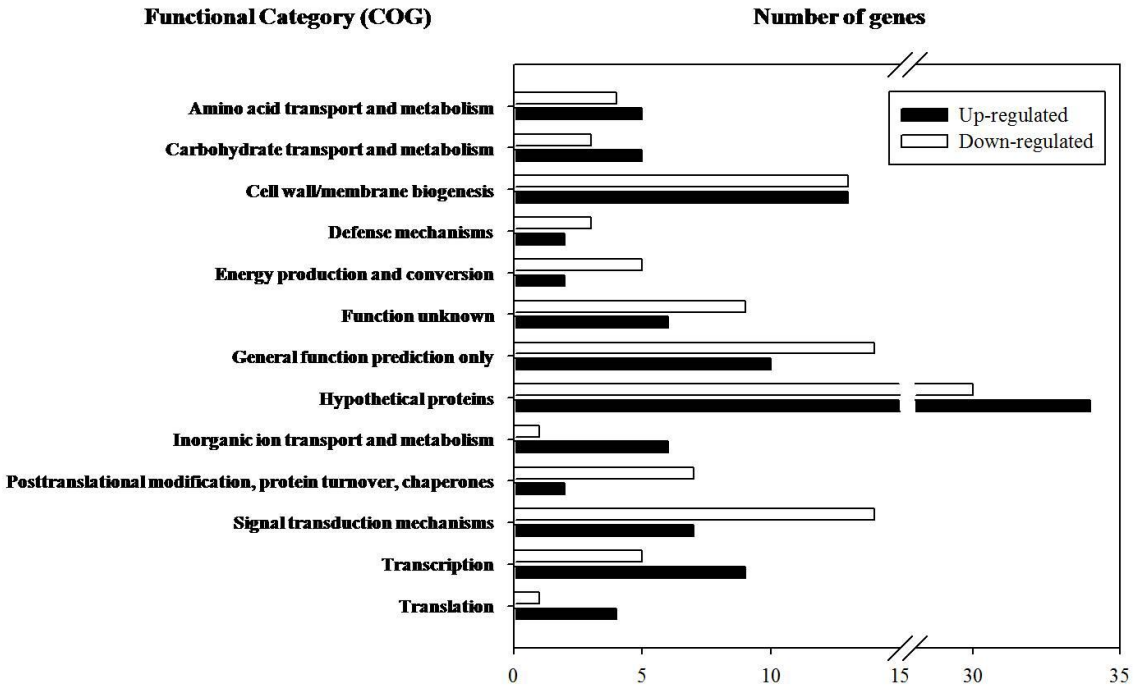


Fig. 2-3. Number of genes regulated by high intracellular c-di-GMP levels. Genes with expression ratios of ≥2 on the basis of microarray analysis results were considered to be regulated by c-di-GMP. Functional categories (COG) corresponding to at least 5 genes are presented and are based on the database for the *V. vulnificus* CMCP6 genome, which was retrieved from GenBank (accession numbers AE016795 and AE016796). Closed and open bars represent the genes up-regulated and down-regulated by c-di-GMP, respectively.

2-3-4. Verification of the genes up-regulated in response to increased c-di-GMP level.

Among the genes up-regulated by increased c-di-GMP, 11 genes encoding a variety of proteins, most likely involved in biofilm formation, were selected and their expression was experimentally verified using qRT-PCR. qRT-PCR results revealed that all genes chosen for verification were up-regulated in response to increased c-di-GMP level.

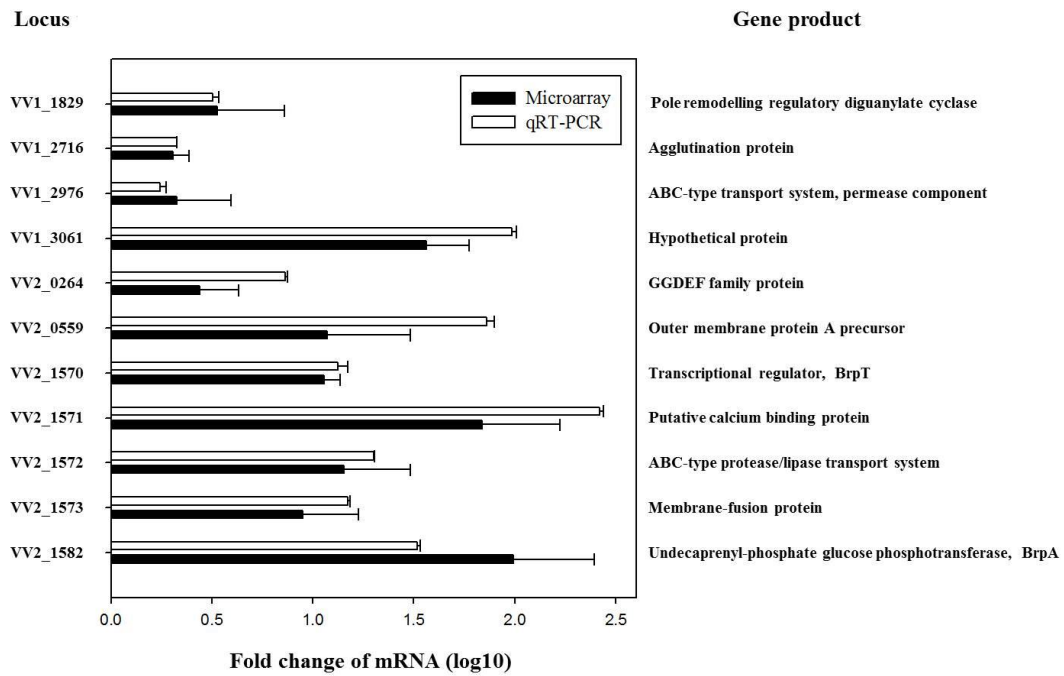


Fig. 2-4. Verification of the newly predicted genes as part of the c-di-GMP regulon which affect biofilm formation of *V. vulnificus*. Twelve genes from the pool of c-di-GMP-regulated gene members newly predicted on the basis of microarray analysis results were analyzed by qRT-PCR. Each column represents the mRNA expression level in the wild type *V. vulnificus* containing pJN1002 relative to that in the wild type containing pJH0311. Averages and SEM were calculated from the results of at least three independent experiments. Locus tags are based on the database of the *V. vulnificus* CMCP6 genome and the products of the 12 genes are listed on the right.

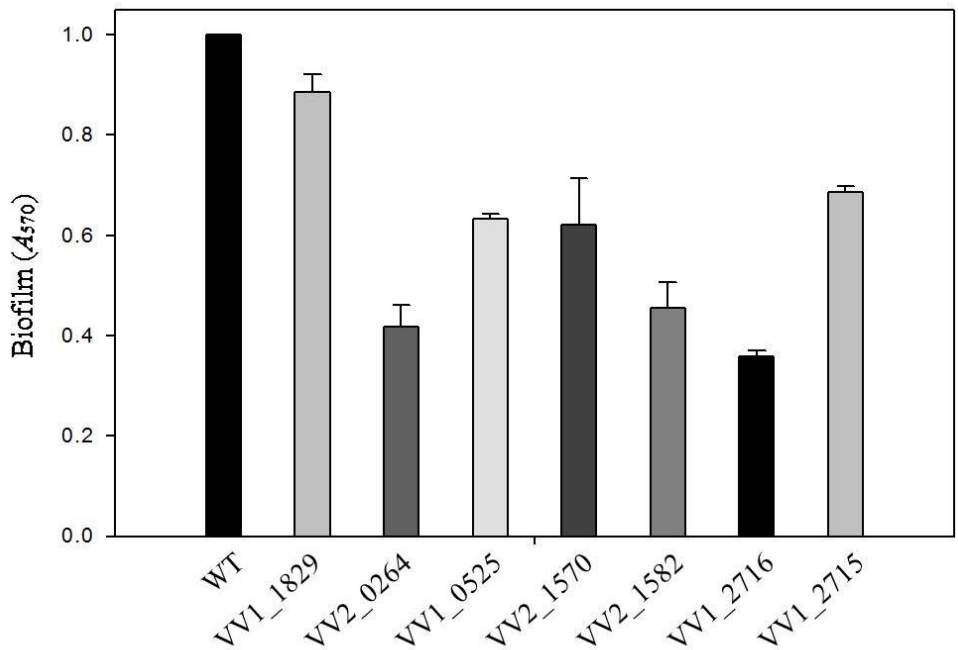


Fig. 2-5. Static biofilm formation of wild type and some mutants of c-di-GMP regulon. Selected mutants are expected to affect biofilm formation of *V. vulnificus.* Biofilm assays were performed with crystal violet for qauntifying biofilms. Biofilms were grown in VFMG for 24 h and formed biofilms were quantified by measuring the OD_{570}.

2-3-5. The effects of mutation of c-di-GMP regulons to biofilm formation of *V. vulnificus*.

In this study, it was dicoverved that some genes which are the c-di-GMP regulon are also related to biofilm formation via both microarry experiments with high/low c-di-GMP level conditions or biofilm/planktonic cells. As microarray data showed common genes, it is possible to predict that the genes may influence biofilm formation. Although there are some differences in effect of genes on biofilm formation, it is clear that the genes definitely have positive influnces on biofilm formation of *V. vulnificus*.

2-4. Discussion

A bacterial second messenger, 3',5' -Cyclic diguanylic acid (c-di-GMP), is known to be important for biofilm formation in many bacteria. Overexpression of diguanylate cyclase protein increased intracellular c-di-GMP levels and promoted biofilm formation and rugose colony development of *V. vulnificus*. Microarray analysis revealed that c-di-GMP influences the expression of over 5% of the *V. vulnificus* genome. Among the c-di-GMP regulon, the genes from several functional categories were identified to affect biofilm formation. The predicted or known functions of the genes verified to be up-regulated in response to increased c-di-GMP level and potentially associated in biofilm formation were involved in the exopolysaccharide (EPS) synthesis and c-di-GMP production, cell wall/membrane biogenesis, type 1 secretion, and adherence.

V. vulnificus brp gene cluster and *brpT* are homologues of *V. cholerae vps*-II gene cluster and *vpsT*, respectively, which are responsible for VPS (*Vibrio* polysaccharide) production. It has been reported that *brp* gene cluster and *brpT* are essential for biofilm formation and their expressions are regulated by c-di-GMP (Guo *et al*., 2010). Consistent with this, *brpT* and the first gene of *brp* gene cluster, *brpA*, appeared to be up-regulated by c-di-GMP (Fig. 4). Additionally, expression

of two genes encoding DGC-family proteins (VV1_1829 and VV2_0264) increased in response to elevated c-di-GMP concentrations. This could be explained as a consequence of a positive feedback loop of c-di-GMP production. These results indicated that c-di-GMP promotes the synthesis of EPS as well as of itself.

VV2_0559, encoding an OpmA-family protein precursor was identified as a member of the c-di-GMP regulon. Generally, OmpA-family proteins can function as adhesins and invasins in many bacteria (Smith *et al*., 2007). Furthermore, it has been demonstrated that the deletion of *ompA* reduced biofilm mass by 80% in *E. coli* (Barrios *et al*., 2006). Additionally, VV1_2976, encoding an ABC transporter permease which is a component of the oligopeptide transport system, was up-regulated in response to increased c-di-GMP concentration. It has been well known that bacteria uptakes exogenous peptides or muropeptides using the oligopeptide transport system, and, in turn, breaks down the peptides using cellular peptidases to make the amino acids available and recycle cell wall components (Detmers *et al*., 2001; Monnet, 2003). Interestingly, a previous report has suggested that EPS production and biofilm formation of *V. vulnificus* are induced under nitrogen-poor conditions (Kim *et al*., 2009). One possible explanation for these findings is that, under nitrogen-depleted conditions, *V. vulnificus* makes biofilms as a survival

strategy while trying to uptake oligopeptides as nitrogen sources. Therefore, c-di-GMP regulates the syntheses of different types of membrane proteins required for adhesion and uptake of nutrition to potentially enhance biofilm formation and survival of *V. vulnificus*.

Genes (VV1_2716, VV2_1572, and VV2_1573) encoding components of type 1 secretion systems (T1SS), were also up-regulated by increased c-di-GMP. VV1_2716 encodes a homologue of *Shewanella oneidensis* agglutination protein, AggA, which is essential for pellicle formation (Liang *et al*., 2010). VV2_1572 and VV2_1573 encode homologues of *Vibrio parahaemolyticus* MfpB and MfpC, which have been known to be regulated by c-di-GMP and required for pellicle formation (Enos-Berlage *et al*., 2005; Ferreira *et al*., 2011). Furthermore, VV2_1571 encoding a putative effector protein of the T1SS composed of the proteins coded by VV2_1572 and VV2_1573 was also predicted to belong to the c-di-GMP regulon. VV2_1571 encodes a putative calcium-binding protein which harbors GD-rich nonapeptide RTX repeat at the N-terminal domain (data not shown). In addition, another RTX protein-encoding gene, VV1_3061, was also predicted to be regulated by c-di-GMP. These RTX proteins may function as adhesins like other biofilm-associated proteins (BAPs) and adhesins which are secreted by T1SS in many bacteria (Satchell, 2011). These results suggested that c-

di-GMP promote adherence of *V. vulnificus* to surfaces by regulating T1SS and T1SS effector proteins.

Taken together, all these results suggest that c-di-GMP regulates numerous genes involved in many cellular processes including the c-di-GMP production, syntheses of EPS and membrane proteins, and protein secretion, potentially contributing to surface adherence, biofilm formation, and survival of *V. vulnificus*.

Chapter 3.

The Analysis of *V. vulnificus* Transcriptome in Planktonic and Biofilm cells at the Different Stages of Biofilm Development Using RNA Sequencing.

3-1. Introduction

Like the growth phases of planktonic or free-living bacteria, development of biofilm can be divided into several stages. Generally, they consist of initial attachment, microcolony formation, maturation, and dispersion (Kaplan, 2010). Previous study has reported that transcriptomes of developing and confluent *P. aeruginosa* biofilm cells were related to those of planktonic cells at the exponential and stationary phase, respectively (Waite *et al*., 2005). Another study has found some stage-specific *V. cholera* genes required for each biofilm development stage including *cheY*-3, *bap1,* and *leuO. cheY-3* is important in monolayer formation, on the other hand, *bap1,* and *leuO* are required for biofilm accumulation (Moorthy and Watnick, 2005).

Among the factors which influence biofilm formation of *V. vulnificus,* some genes have been known to contribute the biofilm formation at each development stage. Previous studies have revealed that EPS clusters, which are required for biofilm formation in *V. vulnificus*, are induced at the early stage of growth (Kim *et al*., 2009; Guo *et al*., 2010). Recently, a quorum sensing regulator, SmcR, has been found to be essential for biofilm detachment by inducing putative biofilm-dispersion factors. Furthermore, one of the SmcR regulon, *vvpE*, was proven to encode an elastolytic protease degrading *V. vulnificus* biofilm in the same study (Kim *et al*., 2013). CPS

also have been identified as a quorum sensing-regulated factor which determine biofilm size by restricting overgrowth of mature *V. vulnificus* biofilm (Lee *et al*., 2013).

In this study, the transcriptome analysis of biofilm and planktonic cells from different development stage were conducted using RNA sequencing (RNA-seq). Cells were harvested at the time points, which may represent the stage-specific characteristics, and their transcriptomes were analyzed using next-generation sequencing (NGS) technologies. The RNA-seq results revealed that the numerous portion of the *V. vulnificus* genome were differentially regulated in different biofilm cells from each development stage, as well as in biofilm cells compared to planktonic cells.

3-2. Materials and Methods

3-2-1. Culture conditions and biofilm formation.

V. vulnificus MO6-24/O was grown in Luria-Bertani (LB) medium supplemented with 2.0% (wt/vol) NaCl (LBS) at 30℃ to prepare an overnight culture. VFMG was used for biofilm formation (Cao *et al*., 2012). An aliquot of cultures (480 μl) grown to an A_{600} of 0.8 with LBS broth was used to inoculate 8 ml of VFMG in a 6-well culture plate (SPL, Seoul, South Korea). Biofilms were formed by incubating these cultures at 30℃ and biofilm and planktonic cells were sampled at the three selected time points (1.5, 6, and 10 h). Planktonic cells from the wells were collected by pipetting and harvested by centrifugation at 5,000 × g for 7 min at 4^{o}C and washed with phosphate-buffered saline (PBS; pH 7.4). Biofilm cells were collected with a cell scraper (SPL) and washed with PBS. The washed pellets were treated with RNAprotect Bacteria Reagent (Qiagen, Valencia, CA). To confirm the stages of biofilm development, biofilms were quantitated using crystal violet staining at every hour as described previously with slight modifications (Kim *et al*., 2013). Biofilms were formed by standing the 6-well culture plates (SPL) containing 8 ml VFMG culture with the same method as used for Once the planktonic cells were removed, the biofilm cells on the wall were washed with PBS, and then stained with 1% (wt/vol) crystal violet (CV) solution for 15 min at room

temperature. Biofilms were quantitated by measuring the amount of CV eluted from the biofilms as an absorbance at 570 nm (A_{570}) (Kim *et al*., 2009).

3-2-2 RNA extraction, sequencing, and data analysis.

Total cellular RNA was isolated from the cells harvested at each time point using an miRNeasy kit (Qiagen). Contaminated DNAs were digested by TURBO DNase (Ambion, Austin, TX) and then RNAs were cleaned up using RNeasy MinElute Cleanup kit (Qiagen). The quality of total RNAs was verified using Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano reagents (Agilent Technologies, Waldbronn, Germany). Ribosomal RNAs were removed from total RNA by using Ribo-Zero rRNA Removal kit (Epicentre, Madison, WI). cDNA libraries were generated using TruSeq Stranded mRNA sample prep kit (Illumina, San Diego, CA) following manufacturer's instruction. The quality of cDNA libraries were evaluated as described above, except that Agilent DNA 1000 Reagents (Agilent) was used. Strand-specific and paired-ended 100 nucleotide reads from each cDNA library were obtained using Hiseq 2500 (Illumina Inc, San Diego, CA). The reads were mapped to the reference genome sequence of *V. vulnificus* MO6-24/O (GenBank accession numbers CP002469 and CP002470, www.ncbi.nlm.nih.gov). The diagrams for visualization of sequence reads mapped to the *V. vulnificus* MO6-24/O chromosome were generated using CLRNASeq software (Chunlab, Seoul,

Korea). The expression level of genes was determined by counting the number of reads per kilobase per million mapped reads (RPKM), and Quantile normalization was conducted on the RPKM values. After normalization, fold changes of RPKM values and their significance (P value < 0.05) were assigned and the mRNAs with 2 or greater fold change with P values < 0.05 were considered to be differentially expressed. All data analyses were performed using CLC Genomics Workbench 6.5 (CLC Bio, Aarhus, Denmark). All samples were prepared in biological duplicates .

3-3. Results and Discussion

3-3-1. Development stage of *V. vulnificus* biofilms.

V. vulnificus MO6-24/O formed static biofilms in the 6-well culture plates with apparent development stages (Fig. 1). The amount of biofilms reached the peak point at 6 h after incubation and dramatically reduced from the peak point. Biofilm and planktonic cells were harvested at the three time points (2, 6, and 10 h)which markedly represent the growth stages of biofilms including initial attachment, maturation, and dispersion.

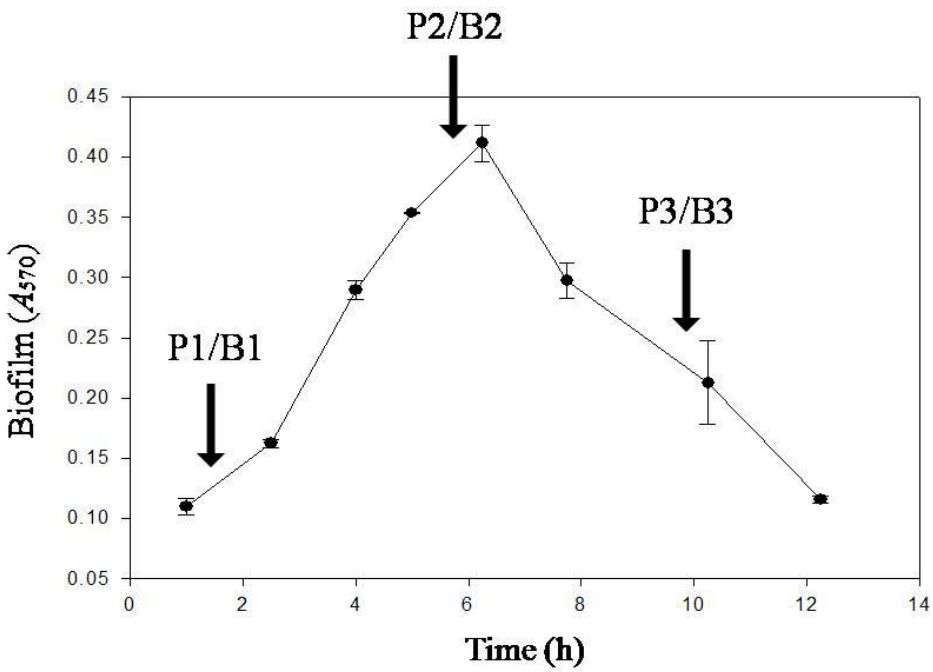


Fig. 3-1. Biofilm formation of *V. vulnificus* MO6-24/O. The wild-type biofilms were grown on the 6-well culture plate wells containing VFMG and quantitated using CV staining . The arrows indicate the time points for collecting biofilm and planktonic cells. Error bars represent the SEM. P1/B1, P2/B2, P3/B3, planktonic and biofilm cells from initial stage (1), mature stage (2), and dispersion stage (3) of biofilm development.

3-3-2. RNA-seq analysis of the transcriptome of biofilm and planktonic cells from the different growth phases.

Paired-end **s**equencing of 12 libraries generated over 35,000,000 reads for each sample and over 89% of the reads were mapped to the *V. vulnificus* MO6-24/O genome (Table 1). Volcano plots showed that the number of genes are differentially regulated with significance (*P* value < 0.05) (Fig. 2). Interestingly, The relatively low differences between the transcriptome of the samples, B2 and B3, were observed. This indicated that the biofilm cells from the mature and dispersion stages have similarity in their transcript levels. The similarity of these two transcriptome was confirmed also in PCA analysis (Fig. 3). The PCA plots of B2 and B3 samples were grouped together and hardly separated. Transcriptomes from biofilm and planktonic cells were proven to have significant differences in their expression levels. Total 461 genes were differentially expressed in the biofilm cells compared to plantonic cells at the initial stage of biofilms. Likewise, 609 and 553 genes showed differences in expression in the biofilm cells compared to planktonic cells at the mature and dispersion stages, respectively. These results indicated that 10% or more genes of *V. vulnificus* MO6-24/O were differentially expressed between biofilm and planktonic cells regardless of the development stages.

Comparison of the transcriptomes of biofilm cells from different development stages revealed that 758 genes were up- or down-regulated in biofilm cells at the mature stages compared to those at the initial stage. On the other hand, only 36 genes were differentially expressed in biofilm cells at the dispersion stage compared to those at the mature stage. These results indicated that extensive change in the expression patterns of transcriptome result in biofilm maturation, while biofilm dispersion is involved in very limited alterations of gene expression.

Fig. 3-2. Transcriptome comparisons of the RNA-seq samples. Volcano-plots of genes differentially expressed between biofilm and planktonic cells or between different stages of biofilm development were generated. Numbers on the X- and Y-axis represent the fold change($\log_2$) and *P* value ($\log_{10}$).

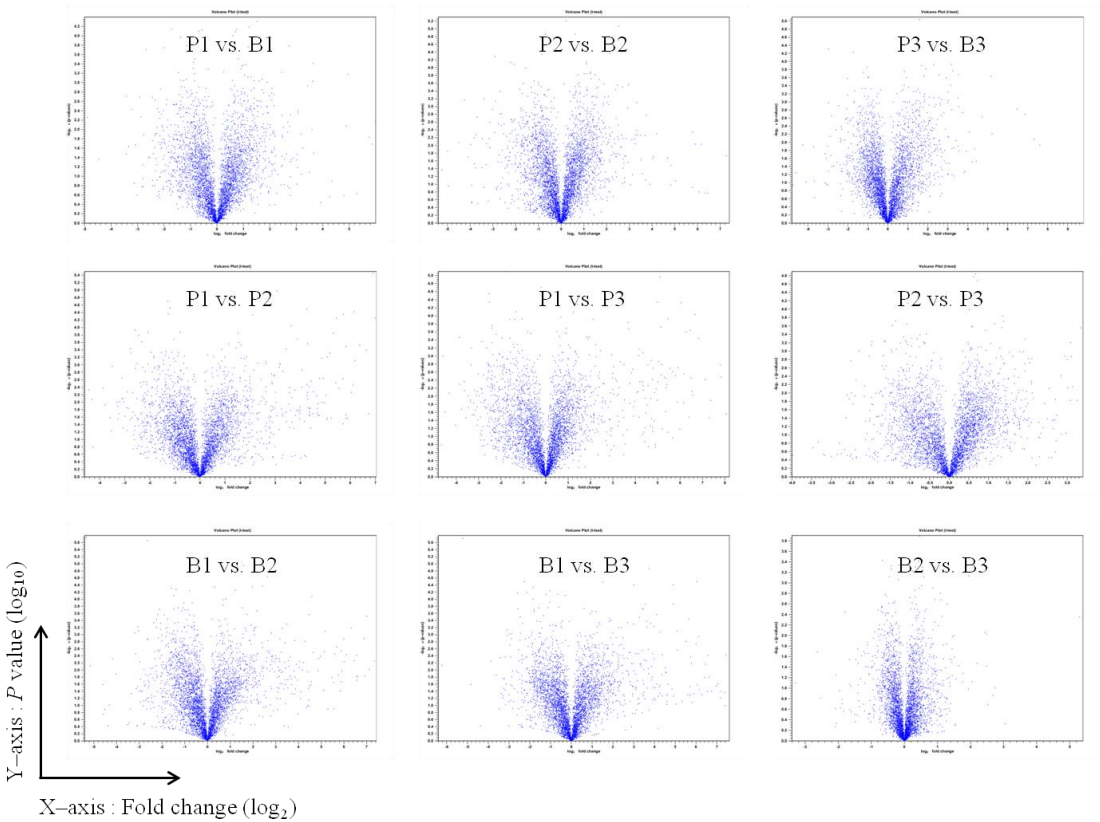
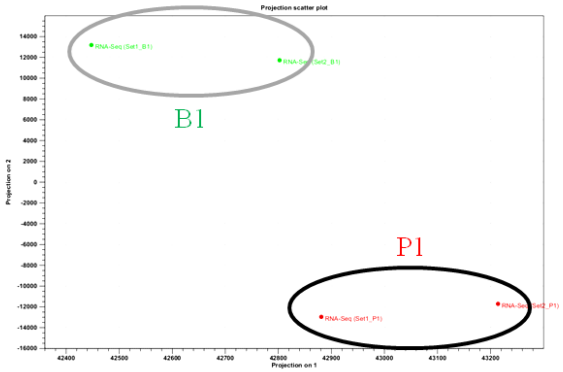
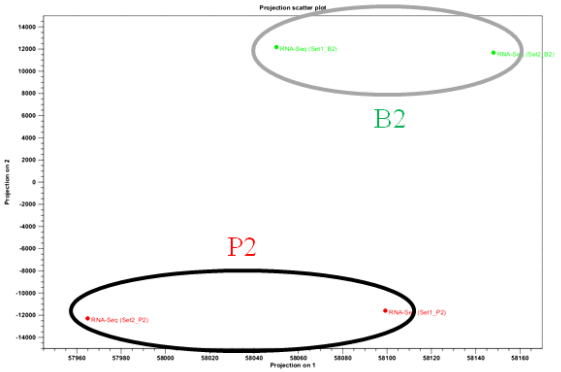

P1 vs. B1
P2 vs. B2
P3 vs. B3
P1 vs. P2
P1 vs. P3
P2 vs. P3
B1 vs. B2
B1 vs. B3
B2 vs. B3
Y–axis : P value (log10)
X–axis : Fold change (log2)

Fig. 3-3. Principal component analysis (PCA) of the RNA-seq samples. PCA analysis was performed for all samples using the gene expression values from the RNA-seq analysis. Samples from planktonic and biofilm cells at the different development stages were plotted in two dimensional plots across the first two principal components. Samples for each conditions were denoted by a different color. (A) Green dots, RNAs from biofilm cells; red dots, RNAs from planktonic cells. (B) Red dots, RNAs from cells at the initial stage; green dots, RNAs from cells at the mature stage; blue dots, RNAs from cells at the dispersion stage.

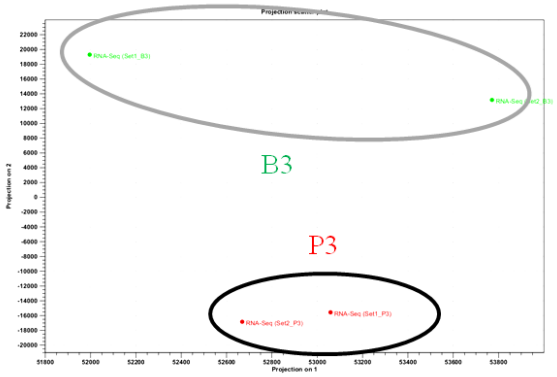
(A) Planktonic cells vs. Biofilm cells



P1 vs. B1

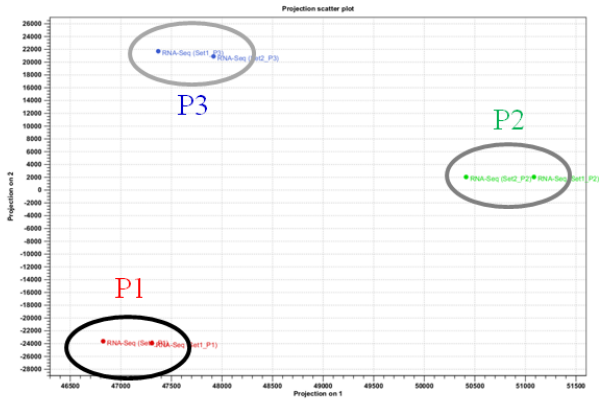


P2 vs. B2

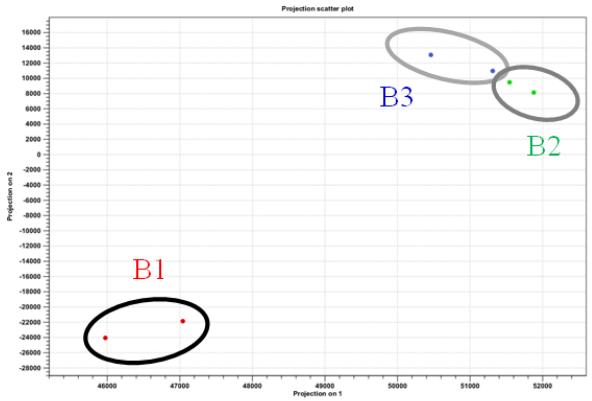


P3 vs. B3

(B) Planktonic or biofilm cells from different development stages



P1 vs. P2 vs. P3



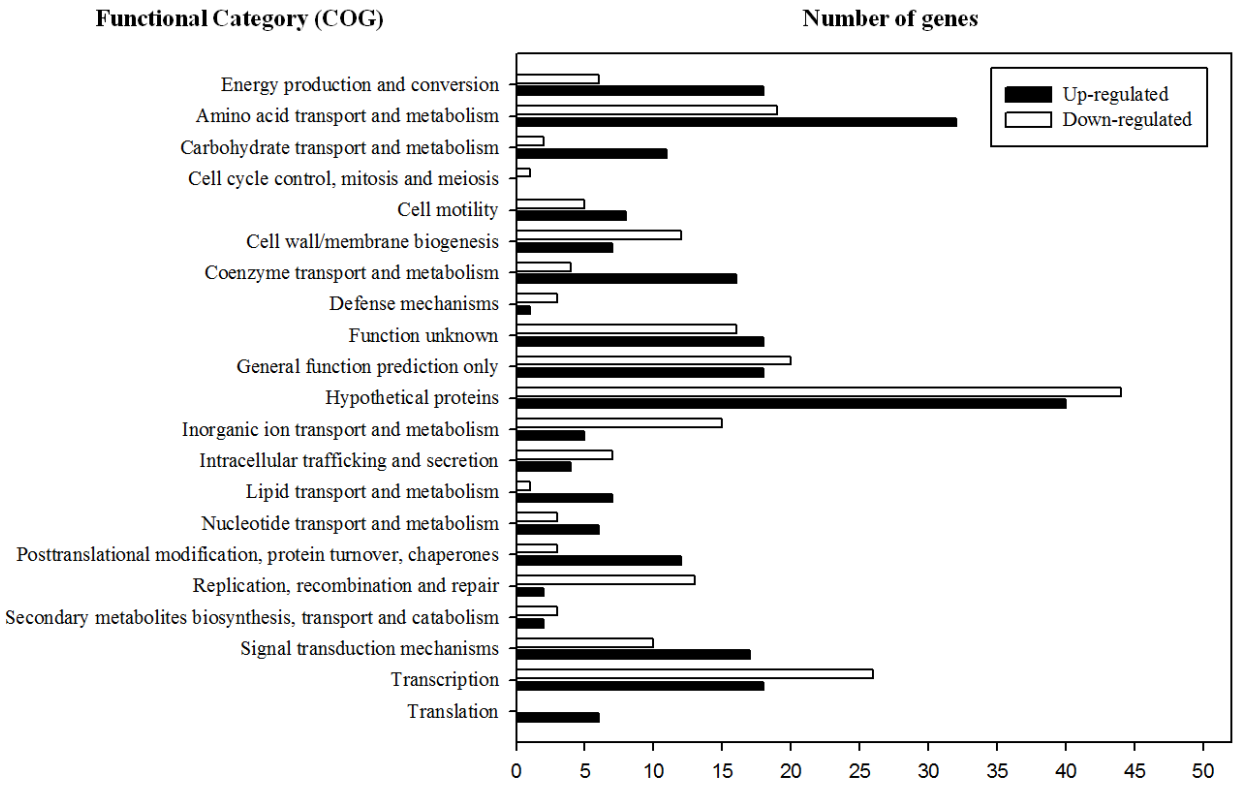
B1 vs. B2 vs. B3

Table 3-1. Analysis of RNA-seq data mapped to the *V. vulnificus* MO6-24/O genome.

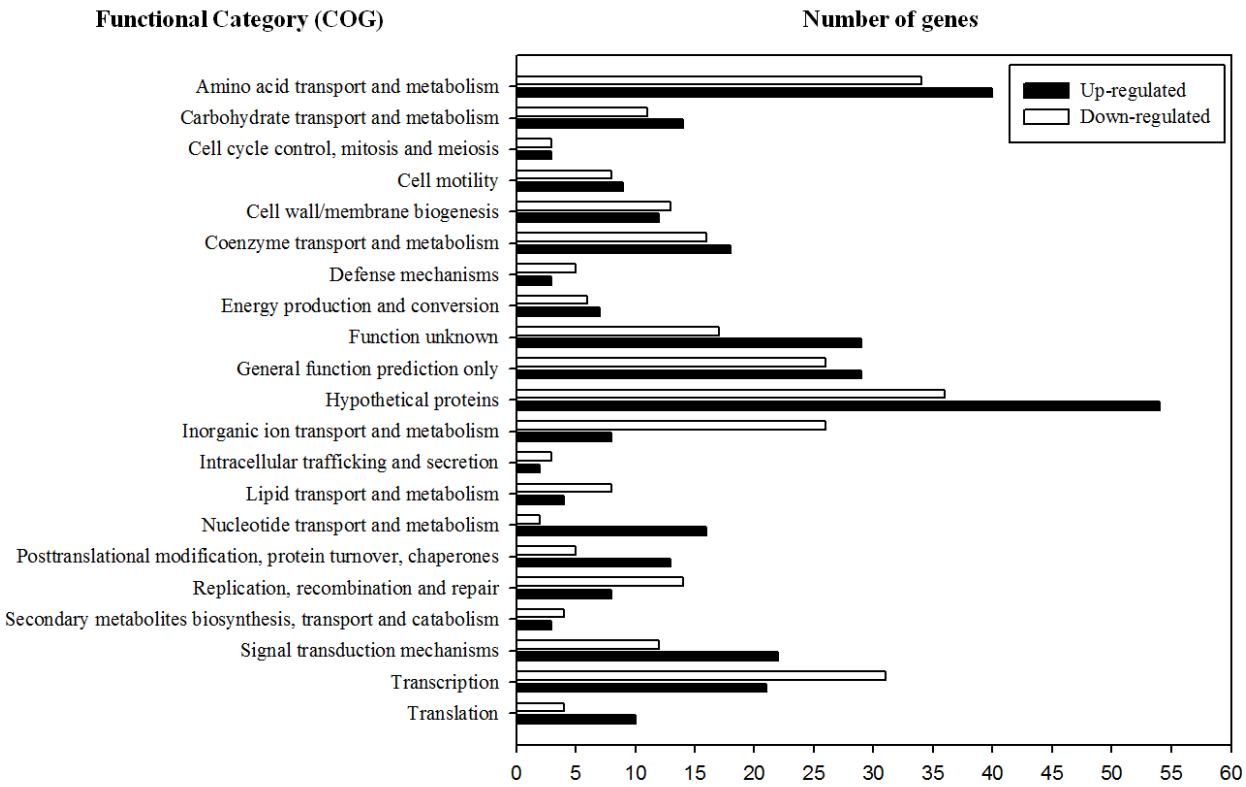
Sample	Duplicate number	Total read	rRNA read	mRNA read	Intergenic read	Unmapped read	Mapped reads(%)
P1	1	42,545,880	2,402	13,258,780	24,712,585	4,572,113	89.254
	2	35,452,904	69,818	20,354,110	12,091,141	2,937,835	91.713
B1	1	44,681,846	2,492	16,191,978	28,227,976	259,400	99.419
	2	33,611,532	7,604	22,217,616	11,049,886	336,426	98.999
P2	1	43,238,018	920	14,735,512	27,685,628	815,958	98.113
	2	30,982,836	24,614	19,416,297	11,033,885	508,040	98.360
B2	1	50,769,194	720	17,031,432	33,591,450	145,592	99.713
	2	35,928,358	10,290	23,003,871	12,812,972	101,225	99.718
P3	1	46,207,380	832	13,527,716	30,400,204	2,278,628	95.069
	2	36,170,806	20,336	17,455,165	15,525,136	3,170,169	91.236
B3	1	49,598,346	800	17,263,614	32,166,731	167,201	99.663
	2	37,438,540	8,048	23,108,882	14,211,954	109,656	99.707

Fig. 3-4. Number of genes differentially expressed in biofilm cells compared to planktonic cells at the different stages of biofilm development. Genes with expression ratios of ≥2 on the basis of the RNA sequencing results were considered to be differentially expressed in biofilm cells. Functional categories (COG) are based on the database for the *V. vulnificus* MO6-24/O genome, which was retrieved from GenBank (accession numbers CP002469 and CP002470). Closed and open bars represent the genes up-regulated and down-regulated in biofilm cells, respectively.

(A) Initial stage (B1/P1)



(B) Mature stage (B2/P2)



(C) Dispersion stage (B3/P3)

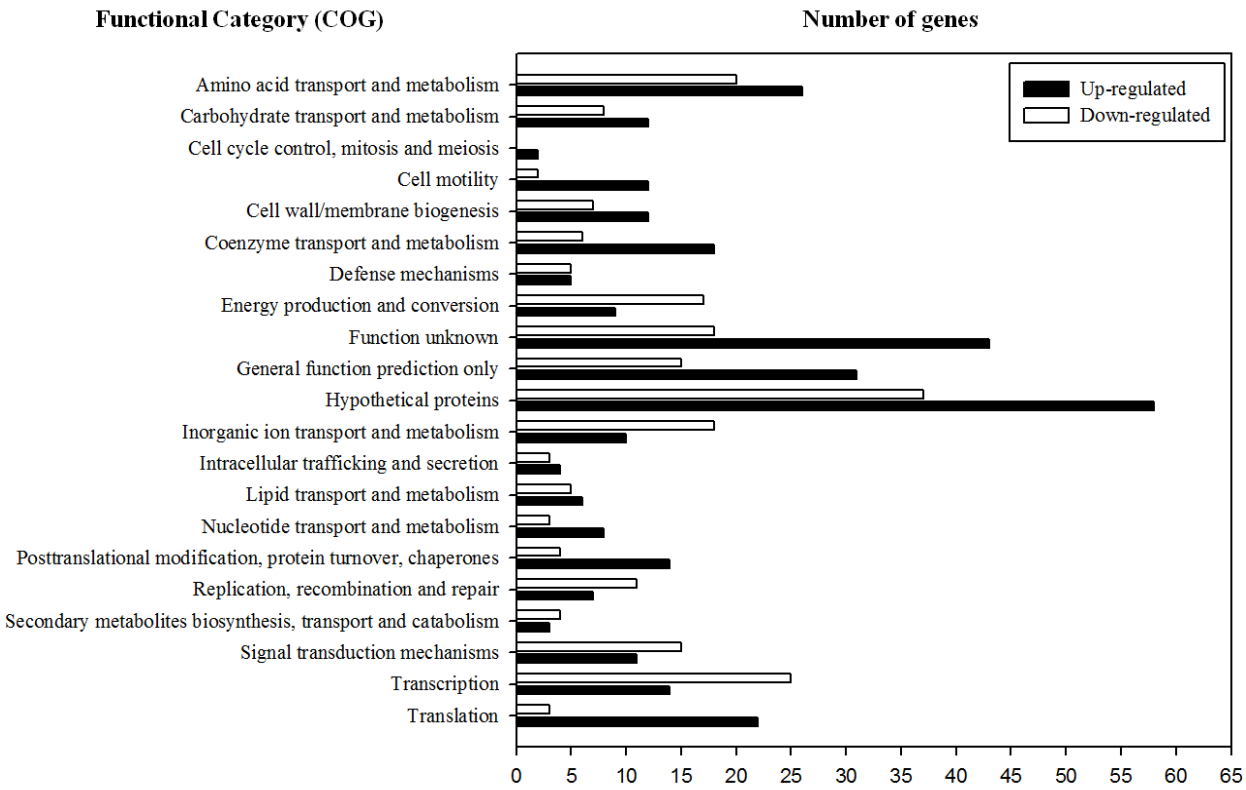
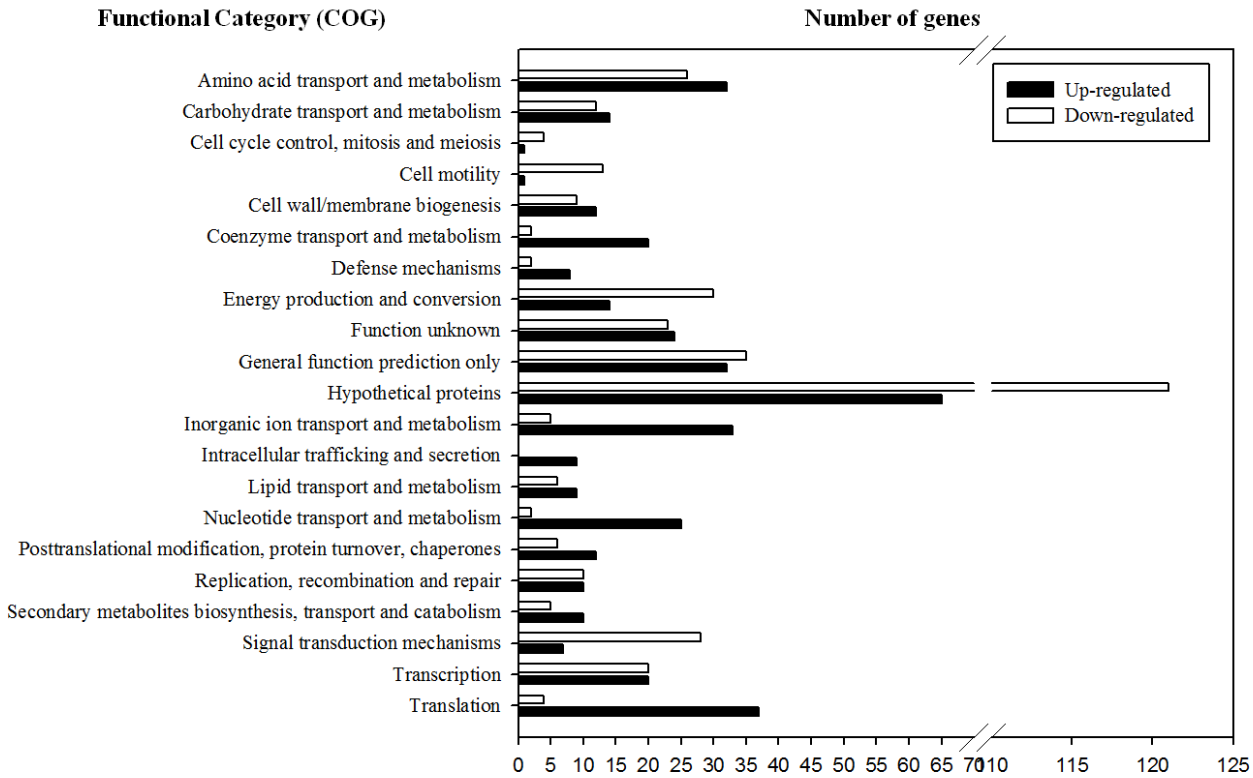


Fig. 3-5. Number of genes differentially expressed in biofilm cells from the different stages of biofilm development. Genes with expression ratios of ≥2 on the basis of the RNA sequencing results were considered to be differentially expressed in biofilm cells. Functional categories (COG) are based on the database for the *V. vulnificus* MO6-24/O genome, which was retrieved from GenBank (accession numbers CP002469 and CP002470). Closed and open bars represent the genes up-regulated and down-regulated in the B2 sample compared to the B1 sample (A) and in the B3 sample compared to the B2 sample (B).

(A) Biofilm cells from the initial stage and mature stage (B2/B1)



(B) Biofilm cells from the mature stage and dispersion stage (B3/B2)

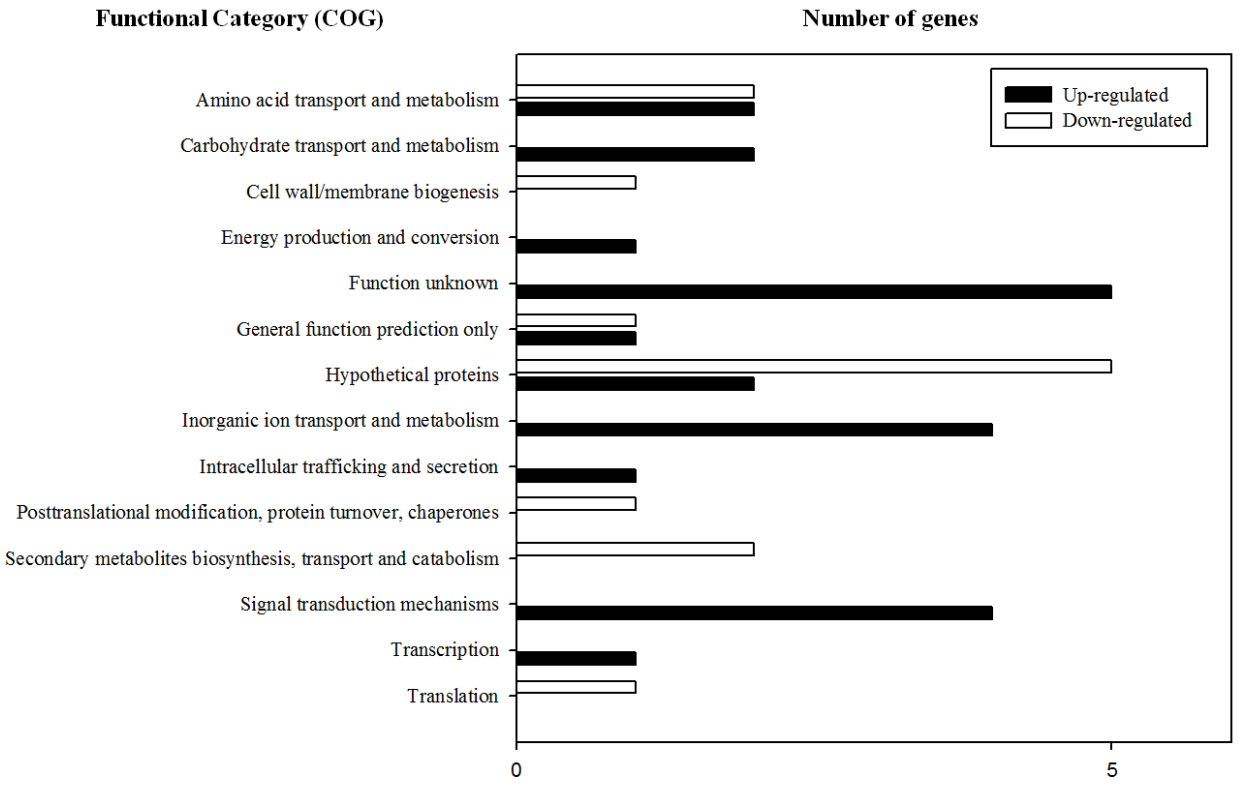


Table 3-2. Genes differentially expressed in biofilm cells compared to planktonic cells at the initial stage of biofilm development (B1/P1).

Locus tag[a]	Gene product	Fold change
Up-regulated genes (248 genes)		
VVMO6_01837	membrane protein	63.59241
VVMO6_04423	Ornithine decarboxylase (EC 4.1.1.17) / Arginine decarboxylase (EC 4.1.1.19)	58.41435
VVMO6_03235	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	54.28936
VVMO6_03010	Glutathione synthetase (EC 6.3.2.3)	31.08412
VVMO6_03011	Uncharacterized protein conserved in bacteria	29.61556
VVMO6_01639	Putative lipoprotein	12.61272
VVMO6_03009	hypothetical protein	12.41698
VVMO6_02535	hypothetical protein	12.32338
VVMO6_01693	Tricarboxylate transport protein TctC	12.08157
VVMO6_02370	Predicted deacylase	10.74558
VVMO6_02324	hypothetical protein	9.508646
VVMO6_04167	Predicted L-lactate dehydrogenase2C Fe-S oxidoreductase subunit YkgE	9.05841
VVMO6_00583	Outer membrane protein OmpU	8.391864
VVMO6_03427	Aspartate aminotransferase (AspB-4) (EC 2.6.1.1)	8.351252
VVMO6_04185	Arginine ABC transporter2C periplasmic arginine-binding protein ArtI	8.195906
VVMO6_02482	Ribosome hibernation protein YfiA	8.018222
VVMO6_04225	hypothetical protein	7.426379
VVMO6_04168	Predicted L-lactate dehydrogenase2C Iron-sulfur cluster-binding subunit YkgF	7.035131
VVMO6_03475	Phage shock protein E	6.85698
VVMO6_04237	Aldehyde dehydrogenase (EC 1.2.1.3)	6.703812
VVMO6_02180	Formyltetrahydrofolate deformylase (EC 3.5.1.10)	6.614142
VVMO6_03882	Glycerol-3-phosphate transporter	6.414611
VVMO6_03619	Methylglyoxal synthase (EC 4.2.3.3)	6.379637
VVMO6_04166	L-lactate permease	5.888304
VVMO6_03027	Uncharacterized protein conserved in bacteria	5.688501
VVMO6_02371	DNA-directed RNA polymerase2C beta' subunit/160 kD subunit	5.619089
VVMO6_04184	Arginine ABC transporter2C ATP-binding protein ArtP	5.528383
VVMO6_01765	Phosphoserine aminotransferase (EC 2.6.1.52)	5.504358
VVMO6_01725	5'-nucleotidase (EC 3.1.3.5); 2'2C3'-cyclic-nucleotide 2'-phosphodiesterase (EC 3.1.4.16); Putative UDP-sugar hydrolase (EC 3.6.1.45)	5.499722
VVMO6_02120	DNA-binding protein HU-beta	5.469879
VVMO6_03689	Predicted transcriptional regulator	4.599736
VVMO6_03883	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	4.506946
VVMO6_01680	Putative hydrolase of the HAD superfamily	4.502742

VVMO6_01605	COG19562C GAF domain-containing protein	4.497195
VVMO6_03122	hypothetical protein	4.481343
VVMO6_01928	Mlc2C transcriptional repressor of MalT (the transcriptional activator of maltose regulon) and manXYZ operon	4.443824
VVMO6_03922	Type III effector HopPmaJ	4.395157
VVMO6_02216	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	4.385787
VVMO6_04187	Arginine ABC transporter2C permease protein ArtM	4.319181
VVMO6_01122	Aspartate aminotransferase (EC 2.6.1.1)	4.297339
VVMO6_01274	putative SpoOM-related protein	4.229046
VVMO6_04513	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	4.209314
VVMO6_03984	Cold shock protein CspE	4.204294
VVMO6_01236	Agmatinase (EC 3.5.3.11)	4.186668
VVMO6_02325	hypothetical protein	4.160804
VVMO6_03358	Acyl-CoA hydrolase (EC 3.1.2.20)	4.081094
VVMO6_03388	Universal stress protein family 8	4.047764
VVMO6_04429	FOG: TPR repeat protein2C SEL1 subfamily	4.04073
VVMO6_03995	dCMP deaminase (EC 3.5.4.12)	4.011038
VVMO6_02645	Probable transcriptional activator for leuABCD operon	3.956494
VVMO6_00199	Aspartate ammonia-lyase (EC 4.3.1.1)	3.949851
VVMO6_03417	OsmC/Ohr family protein	3.946365
VVMO6_04169	Predicted L-lactate dehydrogenase2C hypothetical protein subunit YkgG	3.88664
VVMO6_01656	hypothetical protein	3.822172
VVMO6_01657	Putative threonine efflux protein	3.79902
VVMO6_02669	Malate dehydrogenase (EC 1.1.1.37)	3.765265
VVMO6_00673	ribosomal protein S6 glutaminyl transferase related protein	3.764048
VVMO6_03361	hypothetical protein	3.743177
VVMO6_02632	TRAP transporter solute receptor2C unknown substrate 6	3.668519
VVMO6_03424	Phosphoenolpyruvate-protein kinase	3.658603
VVMO6_02078	Uncharacterized protein YeaC	3.636402
VVMO6_03067	Phosphoserine phosphatase (EC 3.1.3.3)	3.604525
VVMO6_02215	PTS system2C N-acetylglucosamine-specific IIB component (EC 2.7.1.69) / PTS system2C N-acetylglucosamine-specific IIC component (EC 2.7.1.69)	3.596063
VVMO6_03937	Adenosine deaminase (EC 3.5.4.4)	3.572831
VVMO6_03696	membrane protein	3.532091
VVMO6_00425	Glutaminase (EC 3.5.1.2)	3.521721
VVMO6_01990	hypothetical protein	3.480213
VVMO6_04325	Phospholipid-binding protein	3.451785
VVMO6_04379	C4-dicarboxylate transport transcriptional regulatory protein	3.436642
VVMO6_02217	N-acetylglucosamine-6P-responsive transcriptional repressor NagC2C ROK family	3.433502
VVMO6_02111	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-	3.39737

	carboxylic-acid synthase (EC 2.2.1.9)	
VVMO6_01162	hypothetical protein	3.36911
VVMO6_04327	hypothetical protein	3.36844
VVMO6_02109	Naphthoate synthase (EC 4.1.3.36)	3.3576
VVMO6_01264	hypothetical protein	3.349097
VVMO6_02896	Porphobilinogen synthase (EC 4.2.1.24)	3.326224
VVMO6_00880	Cyn operon transcriptional activator	3.27959
VVMO6_03813	Aspartate aminotransferase (EC 2.6.1.1)	3.275992
VVMO6_03517	Chemotaxis protein CheV (EC 2.7.3.-)	3.232843
VVMO6_01711	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	3.209864
VVMO6_01643	6-phosphogluconate dehydrogenase2C decarboxylating (EC 1.1.1.44)	3.207358
VVMO6_04354	Ferredoxin--NADP(+) reductase (EC 1.18.1.2)	3.20494
VVMO6_02963	hypothetical protein	3.175441
VVMO6_00940	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.144796
VVMO6_00520	LSU ribosomal protein L19p	3.082702
VVMO6_00096	Homolog of E. coli HemX protein	3.076276
VVMO6_02501	FKBP-type peptidyl-prolyl cis-trans isomerase slpA (EC 5.2.1.8)	3.074075
VVMO6_00663	UDP-glucose 4-epimerase (EC 5.1.3.2)	3.06468
VVMO6_01836	hypothetical protein	3.032091
VVMO6_02618	General secretion pathway protein A / General secretion pathway protein B	3.02474
VVMO6_00503	Aspartokinase (EC 2.7.2.4)	3.018473
VVMO6_00690	N-acetylglutamate synthase (EC 2.3.1.1)	2.980586
VVMO6_01392	hypothetical protein	2.934908
VVMO6_03374	Transcriptional regulator LuxT	2.933537
VVMO6_03477	hypothetical protein	2.910787
VVMO6_01773	hypothetical protein	2.910771
VVMO6_02703	Inorganic pyrophosphatase (EC 3.6.1.1)	2.902176
VVMO6_03516	hypothetical protein	2.888747
VVMO6_01163	HDIG domain protein	2.883955
VVMO6_03300	Protease-related protein	2.881512
VVMO6_02287	YbaK family protein	2.871764
VVMO6_03425	Methyl-accepting chemotaxis protein	2.871698
VVMO6_01644	6-phosphogluconolactonase (EC 3.1.1.31)2C eukaryotic type	2.862301
VVMO6_02362	Gamma-glutamyl phosphate reductase (EC 1.2.1.41)	2.854727
VVMO6_01140	Fumarate hydratase class I2C aerobic (EC 4.2.1.2)	2.854134
VVMO6_00558	Ferric iron ABC transporter2C iron-binding protein	2.853573
VVMO6_00097	Uroporphyrinogen-III synthase (EC 4.2.1.75)	2.842013
VVMO6_04236	Acetyltransferase	2.841402
VVMO6_02880	hypothetical protein	2.839212
VVMO6_01846	DNA-binding protein inhibitor Id-2-related protein	2.815345
VVMO6_01476	ROK family protein	2.802164
VVMO6_02705	hypothetical protein	2.790263

VVMO6_02007	hypothetical protein	2.787331
VVMO6_01242	hypothetical protein	2.786159
VVMO6_03109	Maltose operon transcriptional repressor MalR2C LacI family	2.743254
VVMO6_02306	FIG002095: hypothetical protein	2.736127
VVMO6_02110	2-succinyl-6-hydroxy-22C4-cyclohexadiene-1-carboxylate synthase (EC 4.2.99.20)	2.708742
VVMO6_00262	Cyclic AMP receptor protein	2.697158
VVMO6_02199	Succinate dehydrogenase cytochrome b-556 subunit	2.680207
VVMO6_04380	Signal transduction histidine kinase	2.674955
VVMO6_01817	Cystathionine beta-lyase (EC 4.4.1.8)	2.667075
VVMO6_03443	L-serine dehydratase (EC 4.3.1.17)	2.663452
VVMO6_03048	Copper metallochaperone2C bacterial analog of Cox17 protein	2.661645
VVMO6_00272	Isoaspartyl aminopeptidase (EC 3.4.19.5) @ Asp-X dipeptidase	2.660847
VVMO6_02198	Succinate dehydrogenase hydrophobic membrane anchor protein	2.654819
VVMO6_02500	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2)	2.648346
VVMO6_00743	UPF0325 protein yaeH	2.647976
VVMO6_04402	hypothetical protein	2.643919
VVMO6_04247	Predicted dehydrogenase	2.637256
VVMO6_02566	Dihydrodipicolinate reductase (EC 1.3.1.26)	2.632549
VVMO6_03084	DNA-binding response regulator	2.61795
VVMO6_02240	Putative stomatin/prohibitin-family membrane protease subunit YbbK	2.611528
VVMO6_02681	Uncharacterized ABC transporter2C ATP-binding protein YrbF	2.586503
VVMO6_01301	Glyoxalase family protein	2.570262
VVMO6_01598	hypothetical protein	2.569905
VVMO6_03872	Anthranilate phosphoribosyltransferase like (EC 2.4.2.18)	2.551507
VVMO6_00185	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	2.548283
VVMO6_04353	Permease of the drug/metabolite transporter (DMT) superfamily	2.539917
VVMO6_01469	Chemotaxis protein CheC -- inhibitor of MCP methylation	2.535761
VVMO6_01637	hypothetical protein	2.532224
VVMO6_00477	Pyridoxine 5'-phosphate synthase (EC 2.6.99.2)	2.526897
VVMO6_02071	Signal transduction histidine kinase	2.525526
VVMO6_00420	N-acetylglucosamine regulated methyl-accepting chemotaxis protein	2.524823
VVMO6_00095	Homolog of E. coli HemY protein	2.51214
VVMO6_01470	diguanylate cyclase (GGDEF domain) with PAS/PAC sensor	2.506579
VVMO6_01005	Positive regulator of competence TfoX	2.500259
VVMO6_03771	DamX-related protein	2.495177
VVMO6_02006	YciL protein	2.495157

VVMO6_00419	Methyl-accepting chemotaxis protein	2.491054
VVMO6_00519	tRNA (Guanine37-N1) -methyltransferase (EC 2.1.1.31)	2.469993
VVMO6_00405	Cytosol aminopeptidase PepA (EC 3.4.11.1)	2.468382
VVMO6_03770	Protein acetyltransferase	2.455596
VVMO6_02047	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)	2.455461
VVMO6_02363	Glutamate 5-kinase (EC 2.7.2.11)	2.453038
VVMO6_03062	Transcriptional regulator2C AsnC family	2.451995
VVMO6_00790	Arsenate reductase (EC 1.20.4.1)	2.431843
VVMO6_01277	Translation initiation factor 3	2.431821
VVMO6_00465	YgfY COG2938	2.429719
VVMO6_02447	Preprotein translocase subunit YajC (TC 3.A.5.1.1)	2.419406
VVMO6_00300	Transcriptional (co)regulator CytR	2.417815
VVMO6_03662	Hypothetical protein2C restriction endonuclease-like VRR-NUC domain	2.41742
VVMO6_03049	Cytochrome oxidase biogenesis protein Sco1/SenC/PrrC2C putative copper metallochaperone	2.402022
VVMO6_01777	Glutaredoxin 1	2.392698
VVMO6_03478	hypothetical protein	2.391612
VVMO6_03677	ABC-type multidrug transport system2C ATPase and permease component	2.39146
VVMO6_04085	GMP reductase (EC 1.7.1.7)	2.391361
VVMO6_03721	Methyl-accepting chemotaxis protein	2.386774
VVMO6_00276	FKBP-type peptidyl-prolyl cis-trans isomerase FkpA precursor (EC 5.2.1.8)	2.386736
VVMO6_00098	Porphobilinogen deaminase (EC 2.5.1.61)	2.385589
VVMO6_03532	Transcriptional regulator	2.384634
VVMO6_00671	hypothetical protein	2.38382
VVMO6_02445	Protein-export membrane protein SecF (TC 3.A.5.1.1)	2.373154
VVMO6_03812	FIG008443: hypothetical protein	2.371661
VVMO6_04049	hypothetical protein	2.367988
VVMO6_00312	N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38)	2.367019
VVMO6_03764	Allophanate hydrolase 2 subunit 1 (EC 3.5.1.54)	2.357161
VVMO6_03711	Iron-containing alcohol dehydrogenase	2.354302
VVMO6_03020	Na+/H+ antiporter NhaD type	2.351099
VVMO6_04253	FOG: TPR repeat protein	2.349838
VVMO6_01881	Probable protease htpX homolog (EC 3.4.24.-)	2.347391
VVMO6_02374	Phosphoribosylformylglycinamidine synthase2C synthetase subunit (EC 6.3.5.3) / Phosphoribosylformylglycinamidine synthase2C glutamine amidotransferase subunit (EC 6.3.5.3)	2.343801
VVMO6_02112	Menaquinone-specific isochorismate synthase (EC 5.4.4.2)	2.334263
VVMO6_02901	Twin-arginine translocation protein TatA	2.325619
VVMO6_00446	hypothetical protein	2.317656
VVMO6_00989	Cold shock protein CspD	2.311297
VVMO6_03479	hypothetical protein	2.310615
VVMO6_02369	ABC-type multidrug transport system2C ATPase and	2.310497

	permease component	
VVMO6_00046	Coproporphyrinogen III oxidase2C aerobic (EC 1.3.3.3)	2.304889
VVMO6_03600	Signal transduction histidine kinase regulating citrate/malate metabolism	2.301863
VVMO6_00282	SSU ribosomal protein S7p (S5e)	2.300644
VVMO6_03726	hypothetical protein	2.296211
VVMO6_02723	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases	2.282172
VVMO6_03725	FOG: EAL domain protein	2.270762
VVMO6_00488	Enolase (EC 4.2.1.11)	2.263524
VVMO6_00070	DNA-directed RNA polymerase specialized sigma subunit	2.26212
VVMO6_04156	Putative magnesium transporter MgtE	2.25346
VVMO6_00047	Shikimate 5-dehydrogenase I alpha (EC 1.1.1.25)	2.249638
VVMO6_00175	DNA-binding protein HU-alpha	2.248758
VVMO6_00196	Transcriptional regulator2C LuxR family	2.247662
VVMO6_01671	Methyl-accepting chemotaxis protein	2.242175
VVMO6_01175	Na(+)-linked D-alanine glycine permease	2.2278
VVMO6_04479	hypothetical protein	2.224733
VVMO6_02451	Putative acetoin utilization protein AcuB	2.224215
VVMO6_00764	cytochrome c554	2.215902
VVMO6_02161	FIG138576: 3-oxoacyl-[ACP] synthase (EC 2.3.1.41)	2.214888
VVMO6_01853	Peptidyl-prolyl cis-trans isomerase ppiB (EC 5.2.1.8)	2.214488
VVMO6_01835	Alpha-12C6-galactosidase2C putative	2.209553
VVMO6_01224	Predicted redox protein	2.204249
VVMO6_03215	12C4-alpha-glucan branching enzyme (EC 2.4.1.18)	2.192264
VVMO6_02710	Protein ytfJ precursor	2.191094
VVMO6_02197	Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1)	2.188756
VVMO6_03753	DnaJ-related protein	2.173033
VVMO6_00478	Holo-[acyl-carrier protein] synthase (EC 2.7.8.7)	2.16166
VVMO6_01000	Glucose-1-phosphate adenylyltransferase (EC 2.7.7.27)	2.158305
VVMO6_03007	Glucosamine-6-phosphate deaminase (EC 3.5.99.6)	2.155252
VVMO6_00727	COG1720: Uncharacterized conserved protein	2.152329
VVMO6_01603	Tail-specific protease precursor (EC 3.4.21.102)	2.137739
VVMO6_02200	Citrate synthase (si) (EC 2.3.3.1)	2.13475
VVMO6_01105	Periplasmic thiol:disulfide oxidoreductase DsbB2C required for DsbA reoxidation	2.133592
VVMO6_02746	SSU ribosomal protein S17p (S11e)	2.133205
VVMO6_04254	Transcriptional regulator2C LysR family	2.13019
VVMO6_01543	Membrane alanine aminopeptidase N (EC 3.4.11.2)	2.125574
VVMO6_01532	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.60)	2.124008
VVMO6_03416	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11) / Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)	2.120168
VVMO6_04368	D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)	2.120098
VVMO6_00570	(GlcNAc)2 ABC transporter2C periplasmic substrate-	2.11967

	binding protein	
VVMO6_03765	Lactam utilization protein LamB	2.112661
VVMO6_04478	hypothetical protein	2.110691
VVMO6_02283	hypothetical protein	2.08492
VVMO6_01927	Chemotaxis protein CheV (EC 2.7.3.-)	2.072815
VVMO6_02337	GGDEF domain family protein	2.072061
VVMO6_02320	Serine hydroxymethyltransferase (EC 2.1.2.1)	2.064353
VVMO6_00731	Carbamoylphosphate synthase large subunit	2.061432
VVMO6_02160	3-oxoacyl-[ACP] reductase (EC 1.1.1.100)	2.058164
VVMO6_00733	FIG003671: Metal-dependent hydrolase	2.056293
VVMO6_01738	Succinylglutamate desuccinylase (EC 3.5.1.96)	2.05549
VVMO6_00069	hypothetical protein	2.051706
VVMO6_01037	18K peptidoglycan-associated outer membrane lipoprotein; Peptidoglycan-associated lipoprotein precursor; Outer membrane protein P6; OmpA/MotB precursor	2.051164
VVMO6_01524	hypothetical protein	2.049621
VVMO6_00789	Trp repressor-binding protein	2.047888
VVMO6_04256	Transcriptional regulators2C LysR family	2.047449
VVMO6_03817	Metal-dependent hydrolase	2.042689
VVMO6_02822	Methyl-accepting chemotaxis protein	2.029744
VVMO6_02701	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (EC 6.3.2.-)	2.026574
VVMO6_00422	FIG001341: Probable Fe(2+)-trafficking protein YggX	2.01449
VVMO6_01778	hypothetical protein	2.008311
VVMO6_01647	Sensor kinase CitA2C DpiB (EC 2.7.3.-)	2.005368
VVMO6_00049	carbonic anhydrase2C family 3	2.004792

Down-regulated genes (213 genes)

VVMO6_01047	hypothetical protein	-22.0059
VVMO6_03003	hypothetical protein	-10.7317
VVMO6_01713	hypothetical protein	-10.7304
VVMO6_03970	Inner membrane protein YrbG2C predicted calcium/sodium:proton antiporter	-10.1528
VVMO6_03004	Sodium/glutamate symporter	-9.392
VVMO6_02519	hypothetical protein	-7.57554
VVMO6_02509	Sodium-dependent phosphate transporter	-7.34405
VVMO6_02637	Transcriptional regulator of glmS gene2C DeoR family	-6.82077
VVMO6_03519	Histone acetyltransferase HPA2	-6.38528
VVMO6_01116	High-affinity choline uptake protein BetT	-5.96701
VVMO6_00033	COG1272: Predicted membrane protein hemolysin III homolog	-5.8407
VVMO6_02838	TonB system biopolymer transport component; Chromosome segregation ATPase	-5.43516
VVMO6_00034	Potassium uptake protein TrkH	-5.37784
VVMO6_01263	hypothetical protein	-5.29462
VVMO6_03452	HTH-type transcriptional regulator BetI	-5.09706
VVMO6_02636	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (EC 2.6.1.16)	-5.06406

VVMO6_00316	hypothetical protein	-5.03836
VVMO6_02366	Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22)	-4.9723
VVMO6_03242	Permease of the drug/metabolite transporter (DMT) superfamily	-4.9524
VVMO6_00141	O-methyltransferase-related protein	-4.82182
VVMO6_02982	hypothetical protein	-4.80923
VVMO6_01171	hypothetical protein	-4.73711
VVMO6_02839	MotA/TolQ/ExbB proton channel family protein	-4.70767
VVMO6_00908	hypothetical protein	-4.61757
VVMO6_02840	Ferric siderophore transport system2C biopolymer transport protein ExbB	-4.57966
VVMO6_04340	hypothetical protein	-4.50381
VVMO6_02254	hypothetical protein	-4.45041
VVMO6_01634	Sugar transferase SypR involved in lipopolysaccharide synthesis	-4.42104
VVMO6_02841	Biopolymer transport protein ExbD/TolR	-4.3967
VVMO6_02837	TonB-dependent receptor; Outer membrane receptor for ferrienterochelin and colicins	-4.31519
VVMO6_02983	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)	-4.30769
VVMO6_00140	SOS-response repressor and protease LexA (EC 3.4.21.88)	-4.28947
VVMO6_00094	NAD(FAD)-utilizing dehydrogenases	-4.2655
VVMO6_02842	Ferric siderophore transport system2C periplasmic binding protein TonB	-4.20992
VVMO6_00607	Transcriptional regulator2C MarR family	-4.18671
VVMO6_02466	Phosphate transport system permease protein PstC (TC 3.A.1.7.1)	-4.16892
VVMO6_01115	Transcriptional regulator2C MarR family	-4.09804
VVMO6_02424	GTP-binding protein EngA	-4.08566
VVMO6_03971	Transcriptional regulator2C TetR family	-4.07667
VVMO6_03969	hypothetical protein	-4.06466
VVMO6_03453	Betaine aldehyde dehydrogenase (EC 1.2.1.8)	-4.01631
VVMO6_01118	DNA polymerase III epsilon subunit (EC 2.7.7.7)	-3.9813
VVMO6_01119	Predicted signal-transduction protein containing cAMP-binding and CBS domains	-3.96174
VVMO6_03472	Alcohol dehydrogenase (EC 1.1.1.1)	-3.9352
VVMO6_01863	Histidinol dehydrogenase (EC 1.1.1.23)	-3.88003
VVMO6_02843	TPR domain protein2C putative component of TonB system	-3.77902
VVMO6_01864	ATP phosphoribosyltransferase (EC 2.4.2.17)	-3.73536
VVMO6_01712	Catalase (EC 1.11.1.6) / Peroxidase (EC 1.11.1.7)	-3.7145
VVMO6_00962	FIG004684: SpoVR-like protein	-3.67419
VVMO6_03325	Phosphate ABC transporter2C periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)	-3.61476
VVMO6_01674	Agglutination protein	-3.5729
VVMO6_01862	Histidinol-phosphate aminotransferase (EC 2.6.1.9)	-3.57246
VVMO6_01160	hypothetical protein	-3.56355
VVMO6_03529	hypothetical protein	-3.5352

VVMO6_02639	Transcriptional regulator LuxZ	-3.49622
VVMO6_02717	Adenylylsulfate kinase (EC 2.7.1.25)	-3.43269
VVMO6_03012	Transcriptional regulator2C LysR family	-3.39457
VVMO6_01996	Anthranilate synthase2C aminase component (EC 4.1.3.27)	-3.34372
VVMO6_02253	hypothetical protein	-3.3429
VVMO6_02935	LysR-family transcriptional regulator VC0068	-3.32143
VVMO6_04339	hypothetical protein	-3.30616
VVMO6_01997	Anthranilate synthase2C amidotransferase component (EC 4.1.3.27)	-3.26507
VVMO6_04334	ABC-type branched-chain amino acid transport system2C periplasmic component	-3.26363
VVMO6_01861	Histidinol-phosphatase (EC 3.1.3.15) / Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19)	-3.24992
VVMO6_00963	FIG002076: hypothetical protein	-3.24637
VVMO6_02367	Xanthine/uracil/thiamine/ascorbate permease family protein	-3.18497
VVMO6_00907	hypothetical protein	-3.16712
VVMO6_01896	ATPase involved in DNA repair	-3.14634
VVMO6_00785	Uracil phosphoribosyltransferase (EC 2.4.2.9)	-3.11961
VVMO6_00317	Hydrogen peroxide-inducible genes activator	-3.10609
VVMO6_01662	Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) / Sirohydrochlorin ferrochelatase (EC 4.99.1.4)	-3.10484
VVMO6_02713	ABC transporter ATP-binding protein YvcR	-3.10417
VVMO6_04336	Histone acetyltransferase HPA2	-3.08435
VVMO6_01998	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)	-3.07923
VVMO6_04335	FOG: GGDEF domain	-3.07874
VVMO6_02481	hypothetical protein	-3.07813
VVMO6_01758	hypothetical protein	-3.02699
VVMO6_00394	Arginine deiminase (EC 3.5.3.6)	-3.0235
VVMO6_01165	ABC transporter2C periplasmic substrate-binding protein-related protein	-3.01345
VVMO6_03594	hypothetical protein	-3.00813
VVMO6_01999	Indole-3-glycerol phosphate synthase (EC 4.1.1.48) / Phosphoribosylanthranilate isomerase (EC 5.3.1.24)	-3.00516
VVMO6_00127	Methyltransferase (EC 2.1.1.-)	-2.99812
VVMO6_01860	Imidazole glycerol phosphate synthase amidotransferase subunit (EC 2.4.2.-)	-2.96771
VVMO6_01200	hypothetical protein	-2.95041
VVMO6_00211	Uncharacterized low-complexity protein	-2.93861
VVMO6_04392	FOG: GGDEF domain	-2.91495
VVMO6_00906	2-methylthioadenine synthetase	-2.90959
VVMO6_02714	ABC-type antimicrobial peptide transport system2C permease component	-2.88519
VVMO6_00628	hypothetical protein	-2.87953
VVMO6_02682	Inner membrane protein YrbG2C predicted calcium/sodium:proton antiporter	-2.87279
VVMO6_04086	hypothetical protein	-2.85246
VVMO6_00212	membrane protein	-2.85228
VVMO6_02592	Stringent starvation protein A	-2.84839

VVMO6_02623	Acyl-phosphate:glycerol-3-phosphate O-acyltransferase PlsY	-2.84615
VVMO6_00750	Undecaprenyl pyrophosphate synthetase (EC 2.5.1.31)	-2.84163
VVMO6_01661	Ferredoxin-type protein NapG (periplasmic nitrate reductase)	-2.82233
VVMO6_02190	Zinc ABC transporter2C ATP-binding protein ZnuC	-2.80716
VVMO6_00099	Adenylate cyclase (EC 4.6.1.1)	-2.7958
VVMO6_02074	Protein ydjA	-2.78276
VVMO6_02213	Ferric uptake regulation protein FUR	-2.78132
VVMO6_00593	COG0779: clustered with transcription termination protein NusA	-2.76275
VVMO6_03197	Transcriptional regulator2C GntR family	-2.75262
VVMO6_00360	Single-stranded DNA-binding protein	-2.75001
VVMO6_00718	DNA polymerase IV (EC 2.7.7.7)	-2.74962
VVMO6_00918	hypothetical protein	-2.7458
VVMO6_00406	FIG000988: Predicted permease	-2.7451
VVMO6_01106	Na+/H+ antiporter NhaB	-2.73341
VVMO6_00650	Flp pilus assembly protein TadB	-2.70885
VVMO6_00128	Signal recognition particle receptor protein FtsY (alpha subunit) (TC 3.A.5.1.1)	-2.69751
VVMO6_03400	CbbY family protein	-2.67426
VVMO6_03029	hypothetical protein	-2.66071
VVMO6_01528	hypothetical protein	-2.65171
VVMO6_01859	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase (EC 5.3.1.16)	-2.64992
VVMO6_04312	Carbon-nitrogen hydrolase	-2.6331
VVMO6_00649	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF2C TadA subfamily	-2.62951
VVMO6_00234	Ribosome small subunit-stimulated GTPase EngC	-2.61352
VVMO6_01675	FIGfam020323	-2.61229
VVMO6_04338	probable exported protein YPO3233	-2.60001
VVMO6_01515	Fumarate and nitrate reduction regulatory protein	-2.59795
VVMO6_04529	Sialic acid utilization regulator2C RpiR family	-2.58404
VVMO6_02831	hypothetical protein	-2.58088
VVMO6_02915	hypothetical protein	-2.57661
VVMO6_03640	ABC-type phosphate transport system2C periplasmic component	-2.56415
VVMO6_02591	Stringent starvation protein B	-2.55946
VVMO6_00647	Similar to TadZ/CpaE2C associated with Flp pilus assembly	-2.55674
VVMO6_03615	hypothetical protein	-2.55464
VVMO6_03684	hypothetical protein	-2.5461
VVMO6_00651	Type II/IV secretion system protein TadC2C associated with Flp pilus assembly	-2.54351
VVMO6_00964	Serine protein kinase (prkA protein)2C P-loop containing	-2.54315
VVMO6_00292	Predicted transcriptional regulator	-2.53198
VVMO6_02926	ATP-dependent DNA helicase UvrD/PcrA	-2.51413
VVMO6_04089	hypothetical protein	-2.4939

VVMO6_00481	GGDEF domain protein	-2.49172
VVMO6_03631	outer membrane protein2C MtrB	-2.47715
VVMO6_04090	Chemotaxis protein CheC -- inhibitor of MCP methylation	-2.47642
VVMO6_02106	hypothetical protein	-2.4739
VVMO6_02377	Transcriptional regulator2C LysR family2C in formaldehyde detoxification operon	-2.46979
VVMO6_00961	GGDEF family protein	-2.42928
VVMO6_01636	hypothetical protein	-2.42121
VVMO6_03528	Exonuclease SbcD	-2.41793
VVMO6_02936	Xaa-Pro aminopeptidase (EC 3.4.11.9)	-2.41777
VVMO6_02934	Multidrug resistance transporter2C Bcr/CflA family	-2.4056
VVMO6_00589	Dihydropteroate synthase (EC 2.5.1.15)	-2.3978
VVMO6_01992	hypothetical protein	-2.39503
VVMO6_00093	Universal stress protein B	-2.39251
VVMO6_02598	ATPase2C AFG1 family	-2.38709
VVMO6_01473	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-2.38644
VVMO6_00115	Transcriptional regulator2C ArsR family	-2.37148
VVMO6_01801	hypothetical protein	-2.3675
VVMO6_02570	FIG006972: hypothetical protein	-2.36562
VVMO6_01987	FOG: EAL domain	-2.35061
VVMO6_01514	Universal stress protein E	-2.336
VVMO6_04018	Outer membrane protein A precursor	-2.33051
VVMO6_01984	Excinuclease ABC subunit C	-2.31328
VVMO6_00645	Von Willebrand factor type A domain protein2C associated with Flp pilus assembly	-2.30787
VVMO6_02066	Potassium uptake protein2C integral membrane component2C KtrA	-2.30647
VVMO6_00648	Type II/IV secretion system ATPase TadZ/CpaE2C associated with Flp pilus assembly	-2.30227
VVMO6_01222	hypothetical protein	-2.29421
VVMO6_01107	Transcriptional regulator for fatty acid degradation FadR2C GntR family	-2.2814
VVMO6_00845	ABC transporter involved in cytochrome c biogenesis2C CcmB subunit	-2.275
VVMO6_02458	hypothetical protein	-2.27267
VVMO6_01660	Polyferredoxin NapH (periplasmic nitrate reductase)	-2.27183
VVMO6_02436	Chaperone protein HscB	-2.25944
VVMO6_02490	Chorismate mutase I (EC 5.4.99.5) / Cyclohexadienyl dehydrogenase (EC 1.3.1.12)(EC 1.3.1.43)	-2.25232
VVMO6_02696	Thiamin ABC transporter2C ATPase component	-2.2501
VVMO6_00480	hypothetical protein	-2.24481
VVMO6_02056	hypothetical protein	-2.24223
VVMO6_03886	MSHA pilin protein MshA BUT NOT	-2.2356
VVMO6_00015	Putative amino acid ABC transporter2C permease protein	-2.23225
VVMO6_00362	MSHA biogenesis protein MshI	-2.22336
VVMO6_00129	Cell division transporter2C ATP-binding protein FtsE (TC 3.A.5.1.1)	-2.22164

VVMO6_00771	Ribonuclease HI (EC 3.1.26.4)	-2.20446
VVMO6_02763	UDP-glucose dehydrogenase (EC 1.1.1.22)	-2.20237
VVMO6_03089	Transcriptional regulator CdgA	-2.19897
VVMO6_02026	Electron transport complex protein RnfA	-2.19889
VVMO6_00441	Extracellular deoxyribonuclease Dns (EC 3.1.21.-)	-2.19282
VVMO6_00882	hypothetical protein	-2.18797
VVMO6_00407	FIG000906: Predicted Permease	-2.17558
VVMO6_02807	UDP-N-acetylglucosamine 42C6-dehydratase (EC 4.2.1.-)	-2.17516
VVMO6_04121	Transcriptional regulator2C AsnC family	-2.17338
VVMO6_00915	hypothetical protein	-2.17089
VVMO6_02295	Outer membrane lipoprotein LolB precursor	-2.16926
VVMO6_00253	Phosphoglycolate phosphatase (EC 3.1.3.18)	-2.14552
VVMO6_00905	Phosphatidylinositol kinase and protein kinase of the PI-3 kinase family	-2.14422
VVMO6_02365	hypothetical protein	-2.14219
VVMO6_01986	DNA polymerase II (EC 2.7.7.7)	-2.13328
VVMO6_00075	Gluconate utilization system Gnt-I transcriptional repressor	-2.13326
VVMO6_00092	Ferritin-like protein 2	-2.128
VVMO6_03423	Transcriptional regulator2C AraC family	-2.12034
VVMO6_02629	RNA polymerase sigma factor RpoD	-2.10494
VVMO6_03719	Response regulator	-2.1039
VVMO6_00357	Excinuclease ABC subunit A	-2.09826
VVMO6_01894	SAM-dependent methyltransferases	-2.08418
VVMO6_00694	HesA/MoeB/ThiF family protein related to EC-YgdL	-2.07954
VVMO6_03159	Phosphoserine phosphatase	-2.07707
VVMO6_00720	Methyl-accepting chemotaxis protein	-2.074
VVMO6_00772	DNA polymerase III epsilon subunit (EC 2.7.7.7)	-2.0697
VVMO6_00245	Putative inner membrane protein YjeT (clustered with HflC)	-2.06809
VVMO6_02272	Flagellar protein FlgP	-2.05878
VVMO6_01866	hypothetical protein	-2.05872
VVMO6_00644	hypothetical protein	-2.05025
VVMO6_02806	Bacillosamine/Legionaminic acid biosynthesis aminotransferase PglE; 4-keto-6-deoxy-N-Acetyl-D-hexosaminyl-(Lipid carrier) aminotransferase	-2.04511
VVMO6_00291	hypothetical protein	-2.04412
VVMO6_03670	Transcriptional regulator2C LysR family	-2.03337
VVMO6_02471	Phosphate regulon transcriptional regulatory protein PhoB (SphR)	-2.0295
VVMO6_00911	Type I restriction-modification system2C DNA-methyltransferase subunit M (EC 2.1.1.72)	-2.02197
VVMO6_02328	Trehalose operon transcriptional repressor	-2.02094
VVMO6_02508	Transcriptional activator NhaR	-2.02037
VVMO6_04519	ABC transporter2C transmembrane region:ABC transporter:Peptidase C392C bacteriocin processing	-2.01433
VVMO6_00970	hypothetical protein	-2.01011
VVMO6_03806	Molybdenum transport ATP-binding protein ModC (TC	-2.00416

	3.A.1.8.1)	
VVMO6_03488	Serine transporter	-2.00308
VVMO6_02538	Endonuclease IV (EC 3.1.21.2)	-2.00049

[a] Locus tags are based on the database for the *V. vulnificus* MO6-24/O genome, which was retrieved from GenBank (accession number CP002469 and CP002470).

Table 3-3. Genes differentially expressed in biofilm cells compared to planktonic cells at the in mature stage of biofilm development (B2/P2).

Locus tag	Gene product	Fold change
Up-regulated genes (325 genes)		
VVMO6_04423	Ornithine decarboxylase (EC 4.1.1.17) / Arginine decarboxylase (EC 4.1.1.19)	59.83597
VVMO6_03235	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	54.82087
VVMO6_04215	hypothetical protein sometimes fused to ribosomal protein S6 glutaminyl transferase	18.36133
VVMO6_01237	Biosynthetic arginine decarboxylase (EC 4.1.1.19)	16.95476
VVMO6_01236	Agmatinase (EC 3.5.3.11)	16.32894
VVMO6_03011	Uncharacterized protein conserved in bacteria	13.4486
VVMO6_00896	hypothetical protein	12.98818
VVMO6_01161	SM-20-related protein	11.29442
VVMO6_04214	Ribosomal protein S6 glutaminyl transferase	10.10421
VVMO6_02370	Predicted deacylase	10.09453
VVMO6_02113	Aspartate/tyrosine/aromatic aminotransferase	9.680725
VVMO6_02453	Malate synthase (EC 2.3.3.9)	9.577385
VVMO6_03829	Permease of the major facilitator superfamily	9.324282
VVMO6_04237	Aldehyde dehydrogenase (EC 1.2.1.3)	8.642919
VVMO6_01932	Sodium-dependent transporter	7.787406
VVMO6_03830	Transcriptional regulator2C AraC family	7.733907
VVMO6_03711	Iron-containing alcohol dehydrogenase	7.640804
VVMO6_01837	membrane protein	6.615645
VVMO6_02180	Formyltetrahydrofolate deformylase (EC 3.5.1.10)	6.54282
VVMO6_01680	Putative hydrolase of the HAD superfamily	6.532906
VVMO6_00673	ribosomal protein S6 glutaminyl transferase related protein	6.302234
VVMO6_02497	Carbon starvation protein A	6.096218
VVMO6_03831	Na+-driven multidrug efflux pump	6.016737
VVMO6_03663	Oxalate/formate antiporter	5.783748
VVMO6_02371	DNA-directed RNA polymerase2C beta' subunit/160 kD subunit	5.779647
VVMO6_02216	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	5.708137
VVMO6_04175	membrane protein	5.654738
VVMO6_01639	Putative lipoprotein	5.61847
VVMO6_02498	Autolysin sensor kinase (EC 2.7.3.-)	5.568705
VVMO6_02535	hypothetical protein	5.365445
VVMO6_00054	Ketol-acid reductoisomerase (EC 1.1.1.86)	5.351589
VVMO6_01678	Queuosine Biosynthesis QueC ATPase	5.338363
VVMO6_01656	hypothetical protein	5.306974
VVMO6_01657	Putative threonine efflux protein	5.289646
VVMO6_01765	Phosphoserine aminotransferase (EC 2.6.1.52)	5.284997
VVMO6_01487	ABC transporter ATP-binding protein uup	5.145478

VVMO6_01679	Queuosine Biosynthesis QueE Radical SAM	5.044
VVMO6_03752	Isopenicillin N synthase	5.022817
VVMO6_02352	Octaprenyl-diphosphate synthase (EC 2.5.1.-) / Dimethylallyltransferase (EC 2.5.1.1) / Geranyltranstransferase (farnesyldiphosphate synthase) (EC 2.5.1.10) / Geranylgeranyl pyrophosphate synthetase (EC 2.5.1.29)	4.884204
VVMO6_01990	hypothetical protein	4.759074
VVMO6_00897	Lactoylglutathione lyase (EC 4.4.1.5)	4.713346
VVMO6_02533	Sodium/alanine symporter	4.622777
VVMO6_00424	tRNA (guanine46-N7-)-methyltransferase (EC 2.1.1.33)	4.479341
VVMO6_03259	L-threonine 3-dehydrogenase (EC 1.1.1.103)	4.406803
VVMO6_00880	Cyn operon transcriptional activator	4.403016
VVMO6_00671	hypothetical protein	4.370178
VVMO6_01508	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)	4.340804
VVMO6_03027	Uncharacterized protein conserved in bacteria	4.333002
VVMO6_03517	Chemotaxis protein CheV (EC 2.7.3.-)	4.266102
VVMO6_00173	hypothetical protein	4.236466
VVMO6_03118	Membrane fusion component of tripartite multidrug resistance system	4.211482
VVMO6_02217	N-acetylglucosamine-6P-responsive transcriptional repressor NagC2C ROK family	4.077632
VVMO6_04527	tRNA and rRNA cytosine-C5-methylase	4.04369
VVMO6_04513	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	3.932534
VVMO6_02499	FIG001014_Response regulator of the LytR/AlgR family	3.891791
VVMO6_01989	Translation elongation factor P-related protein	3.844152
VVMO6_00699	Outer membrane protein OmpK	3.814993
VVMO6_01122	Aspartate aminotransferase (EC 2.6.1.1)	3.769878
VVMO6_04064	Putative transporter	3.721197
VVMO6_02452	Isocitrate lyase (EC 4.1.3.1)	3.671297
VVMO6_01509	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	3.669281
VVMO6_03067	Phosphoserine phosphatase (EC 3.1.3.3)	3.647777
VVMO6_00425	Glutaminase (EC 3.5.1.2)	3.645903
VVMO6_03008	hypothetical protein	3.645405
VVMO6_03173	membrane protein	3.622147
VVMO6_04236	Acetyltransferase	3.582729
VVMO6_01455	hypothetical protein	3.559911
VVMO6_01142	Conserved protein YcjX with nucleoside triphosphate hydrolase domain	3.492596
VVMO6_02650	2-isopropylmalate synthase (EC 2.3.3.13)	3.468723
VVMO6_03361	hypothetical protein	3.437655
VVMO6_00862	Phosphohistidine phosphatase SixA	3.417142
VVMO6_01507	Spermidine Putrescine ABC transporter permease component potC (TC_3.A.1.11.1)	3.401281
VVMO6_00583	Outer membrane protein OmpU	3.367368

VVMO6_00663	UDP-glucose 4-epimerase (EC 5.1.3.2)	3.350932
VVMO6_00267	Glutathione-regulated potassium-efflux system ATP-binding protein	3.34765
VVMO6_02353	1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7)	3.345492
VVMO6_00207	6-phosphofructokinase (EC 2.7.1.11)	3.332186
VVMO6_01907	Iron-regulated protein A precursor	3.331549
VVMO6_04225	hypothetical protein	3.329379
VVMO6_03499	Predicted deacylase	3.326676
VVMO6_03985	YaeQ protein	3.311931
VVMO6_00392	Aspartate carbamoyltransferase (EC 2.1.3.2)	3.298952
VVMO6_02034	hypothetical protein	3.280492
VVMO6_04065	Transcriptional regulator2C MarR family	3.263951
VVMO6_01721	Fumarylacetoacetase (EC 3.7.1.2)	3.25899
VVMO6_01844	Formate efflux transporter (TC 2.A.44 family)	3.253815
VVMO6_01506	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	3.235188
VVMO6_04017	2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I alpha (EC 2.5.1.54)	3.216051
VVMO6_01124	hypothetical protein	3.214682
VVMO6_04231	Peptide deformylase (EC 3.5.1.88)	3.211576
VVMO6_03995	dCMP deaminase (EC 3.5.4.12)	3.210416
VVMO6_03813	Aspartate aminotransferase (EC 2.6.1.1)	3.195223
VVMO6_02349	Flagellar motor rotation protein MotB	3.188963
VVMO6_02645	Probable transcriptional activator for leuABCD operon	3.185415
VVMO6_03119	Permease of the drug/metabolite transporter (DMT) superfamily	3.175205
VVMO6_03475	Phage shock protein E	3.166529
VVMO6_03689	Predicted transcriptional regulator	3.145049
VVMO6_02004	Intracellular septation protein IspA	3.087154
VVMO6_04156	Putative magnesium transporter MgtE	3.083936
VVMO6_03633	ATP-dependent RNA helicase DbpA	3.077837
VVMO6_00199	Aspartate ammonia-lyase (EC 4.3.1.1)	3.048169
VVMO6_00973	54K polar flagellar sheath protein A	3.043391
VVMO6_03705	hypothetical protein	3.039745
VVMO6_02896	Porphobilinogen synthase (EC 4.2.1.24)	3.027949
VVMO6_01872	Adenylosuccinate lyase (EC 4.3.2.2)	3.024231
VVMO6_03619	Methylglyoxal synthase (EC 4.2.3.3)	3.009567
VVMO6_04396	DNA polymerase III alpha subunit (EC 2.7.7.7)	3.007212
VVMO6_02081	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)	3.006687
VVMO6_03209	Transcriptional regulatory protein RstA	2.999787
VVMO6_01605	COG19562C GAF domain-containing protein	2.999519
VVMO6_01456	hypothetical protein	2.979308
VVMO6_03986	hypothetical protein	2.9562
VVMO6_04402	hypothetical protein	2.952395
VVMO6_03492	Methyl-accepting chemotaxis protein	2.950776
VVMO6_01802	hypothetical protein	2.949831

VVMO6_01272	Imidazolonepropionase (EC 3.5.2.7)	2.933532
VVMO6_02071	Signal transduction histidine kinase	2.921967
VVMO6_03007	Glucosamine-6-phosphate deaminase (EC 3.5.99.6)	2.919702
VVMO6_04068	ATP-dependent RNA helicase RhlE	2.917761
VVMO6_03662	Hypothetical protein2C restriction endonuclease-like VRR-NUC domain	2.892186
VVMO6_01644	6-phosphogluconolactonase (EC 3.1.1.31)2C eukaryotic type	2.888512
VVMO6_00478	Holo-[acyl-carrier protein] synthase (EC 2.7.8.7)	2.861391
VVMO6_03811	hypothetical protein	2.847636
VVMO6_04428	exopolysaccharide synthesis protein ExoD-related protein	2.846162
VVMO6_04066	Lactoylglutathione lyase	2.845007
VVMO6_00786	Uracil permease	2.842715
VVMO6_00213	Lysine 22C3-aminomutase (EC 5.4.3.2)	2.836548
VVMO6_02350	Flagellar motor rotation protein MotA	2.834519
VVMO6_03374	Transcriptional regulator LuxT	2.833586
VVMO6_01982	Diaminobutyrate-pyruvate transaminase & L-22C4-diaminobutyrate decarboxylase	2.807711
VVMO6_04320	Membrane fusion protein of RND family multidrug efflux pump	2.79558
VVMO6_02618	General secretion pathway protein A / General secretion pathway protein B	2.785357
VVMO6_04328	Redox-sensitive transcriptional activator SoxR	2.784627
VVMO6_03294	Small-conductance mechanosensitive channel	2.775818
VVMO6_01488	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.767643
VVMO6_02325	hypothetical protein	2.760559
VVMO6_03677	ABC-type multidrug transport system2C ATPase and permease component	2.750901
VVMO6_01386	hypothetical protein	2.748606
VVMO6_04247	Predicted dehydrogenase	2.741675
VVMO6_03937	Adenosine deaminase (EC 3.5.4.4)	2.736929
VVMO6_00546	C4-type zinc finger protein2C DksA/TraR family	2.73219
VVMO6_00156	Pantothenate kinase (EC 2.7.1.33)	2.717204
VVMO6_03516	hypothetical protein	2.717029
VVMO6_01779	TrkA2C Potassium channel-family protein	2.716512
VVMO6_00352	Na+/H+ antiporter2C putative	2.715256
VVMO6_03753	DnaJ-related protein	2.709407
VVMO6_01928	Mlc2C transcriptional repressor of MalT (the transcriptional activator of maltose regulon) and manXYZ operon	2.703391
VVMO6_04539	Uncharacterized protein conserved in bacteria	2.697857
VVMO6_02501	FKBP-type peptidyl-prolyl cis-trans isomerase slpA (EC 5.2.1.8)	2.694313
VVMO6_00097	Uroporphyrinogen-III synthase (EC 4.2.1.75)	2.693874
VVMO6_03872	Anthranilate phosphoribosyltransferase like (EC 2.4.2.18)	2.685972
VVMO6_02062	DNA-cytosine methyltransferase (EC 2.1.1.37)	2.672885

VVMO6_02500	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2)	2.670058
VVMO6_04479	hypothetical protein	2.658723
VVMO6_00405	Cytosol aminopeptidase PepA (EC 3.4.11.1)	2.656967
VVMO6_03171	Ribosyl nicotinamide transporter2C PnuC-like	2.653139
VVMO6_02277	hypothetical protein	2.646842
VVMO6_03364	Dihydroorotase (EC 3.5.2.3)	2.646773
VVMO6_04047	GGDEF family protein	2.641147
VVMO6_03172	N-Ribosylnicotinamide phosphorylase (EC 2.4.2.1)	2.636193
VVMO6_03358	Acyl-CoA hydrolase (EC 3.1.2.20)	2.633552
VVMO6_00951	hypothetical protein	2.633149
VVMO6_01274	putative SpoOM-related protein	2.621013
VVMO6_02890	Protein of unknown function DUF414	2.619537
VVMO6_00477	Pyridoxine 5'-phosphate synthase (EC 2.6.99.2)	2.614249
VVMO6_02362	Gamma-glutamyl phosphate reductase (EC 1.2.1.41)	2.611735
VVMO6_01143	Membrane protein YcjF	2.609942
VVMO6_01796	Signal transduction histidine kinase	2.602519
VVMO6_00902	Uncharacterized iron-regulated protein	2.59532
VVMO6_00725	hypothetical protein	2.583501
VVMO6_03478	hypothetical protein	2.580905
VVMO6_03817	Metal-dependent hydrolase	2.578559
VVMO6_00952	Lipoprotein releasing system transmembrane protein LolC	2.574435
VVMO6_01711	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	2.573738
VVMO6_02644	Long-chain-fatty-acid--CoA ligase (EC 6.2.1.3)	2.567741
VVMO6_01645	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)	2.565391
VVMO6_04512	Transcriptional regulator2C LuxR family	2.561748
VVMO6_02701	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (EC 6.3.2.-)	2.560775
VVMO6_03922	Type III effector HopPmaJ	2.560687
VVMO6_02047	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)	2.551821
VVMO6_04447	Uncharacterized protein conserved in bacteria	2.550218
VVMO6_02723	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases	2.532025
VVMO6_04037	Outer membrane protein N2C non-specific porin	2.521307
VVMO6_00338	Glucose-6-phosphate isomerase (EC 5.3.1.9)	2.515313
VVMO6_02320	Serine hydroxymethyltransferase (EC 2.1.2.1)	2.504754
VVMO6_02451	Putative acetoin utilization protein AcuB	2.501693
VVMO6_03417	OsmC/Ohr family protein	2.495374
VVMO6_02986	hypothetical protein	2.494697
VVMO6_03109	Maltose operon transcriptional repressor MalR2C LacI family	2.493979
VVMO6_00079	4-Hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16)	2.493371

	/ 2-dehydro-3-deoxyphosphogluconate aldolase (EC 4.1.2.14)	
VVMO6_04085	GMP reductase (EC 1.7.1.7)	2.484508
VVMO6_04045	Multidrug resistance protein D	2.481433
VVMO6_00723	hypothetical protein	2.471716
VVMO6_02112	Menaquinone-specific isochorismate synthase (EC 5.4.4.2)	2.465129
VVMO6_00832	Flagellar biosynthesis protein FlhA	2.461944
VVMO6_02375	Transglycosylase2C Slt family	2.455841
VVMO6_02542	Aspartokinase (EC 2.7.2.4) / Homoserine dehydrogenase (EC 1.1.1.3)	2.45133
VVMO6_00781	Phosphoheptose isomerase 1 (EC 5.3.1.-)	2.441905
VVMO6_01513	tRNA(Cytosine32)-2-thiocytidine synthetase	2.438163
VVMO6_04403	Hypothetical protein in aerobactin uptake cluster	2.436168
VVMO6_03479	hypothetical protein	2.427477
VVMO6_00111	Cof protein2C HD superfamily hydrolase	2.423075
VVMO6_00378	Rod shape-determining protein MreB	2.412323
VVMO6_02306	FIG002095: hypothetical protein	2.411235
VVMO6_02698	Thiamin ABC transporter2C substrate-binding component	2.411153
VVMO6_00098	Porphobilinogen deaminase (EC 2.5.1.61)	2.410269
VVMO6_01301	Glyoxalase family protein	2.409176
VVMO6_01152	Predicted signal transduction protein	2.399548
VVMO6_00727	COG1720: Uncharacterized conserved protein	2.397451
VVMO6_01846	DNA-binding protein inhibitor Id-2-related protein	2.39156
VVMO6_03474	hypothetical protein	2.384199
VVMO6_00387	RNA polymerase associated protein RapA (EC 3.6.1.-)	2.382697
VVMO6_02455	Alkyl hydroperoxide reductase subunit C-like protein	2.370278
VVMO6_03334	hypothetical protein PA3071	2.366844
VVMO6_01672	LSU m5C1962 methyltransferase RlmI	2.365144
VVMO6_00782	Predicted glutamine amidotransferase	2.355012
VVMO6_02889	Periplasmic/membrane protein associated with DUF414	2.340271
VVMO6_03064	Pyrrolidone-carboxylate peptidase (EC 3.4.19.3)	2.332641
VVMO6_01023	Aspartyl-tRNA synthetase (EC 6.1.1.12)	2.328296
VVMO6_00872	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)	2.326619
VVMO6_03060	Ribosomal protein S12p Asp88 (E. coli) methylthiotransferase	2.314083
VVMO6_04071	Acetyltransferase	2.311614
VVMO6_00940	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.305581
VVMO6_01770	Regulator of competence-specific genes	2.302245
VVMO6_03765	Lactam utilization protein LamB	2.30146
VVMO6_00096	Homolog of E. coli HemX protein	2.297615
VVMO6_03675	Predicted amidohydrolase	2.297141
VVMO6_03174	Predicted N-ribosylNicotinamide CRP-like	2.296887

	regulator	
VVMO6_03017	membrane protein	2.29683
VVMO6_02852	Transcription elongation factor GreB	2.289159
VVMO6_03083	Signal transduction histidine kinase	2.28543
VVMO6_01258	Phosphoribosylglycinamide formyltransferase 2 (EC 2.1.2.-)	2.285177
VVMO6_03726	hypothetical protein	2.279185
VVMO6_00272	Isoaspartyl aminopeptidase (EC 3.4.19.5) @ Asp-X dipeptidase	2.278605
VVMO6_03084	DNA-binding response regulator	2.27807
VVMO6_01887	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase (EC 2.6.1.62)	2.270582
VVMO6_02849	Xanthine permease	2.267018
VVMO6_01720	Maleylacetoacetate isomerase (EC 5.2.1.2) @ Glutathione S-transferase2C zeta (EC 2.5.1.18)	2.262477
VVMO6_00724	hypothetical protein	2.259441
VVMO6_01768	Response regulator	2.250752
VVMO6_01817	Cystathionine beta-lyase (EC 4.4.1.8)	2.242789
VVMO6_02181	Protein yecM	2.242647
VVMO6_03226	hypothetical protein	2.237073
VVMO6_01643	6-phosphogluconate dehydrogenase2C decarboxylating (EC 1.1.1.44)	2.233954
VVMO6_00110	Protein of unknown function DUF55	2.23206
VVMO6_03333	hypothetical protein	2.225807
VVMO6_04192	ABC-type amino acid transport/signal transduction systems	2.224837
VVMO6_02287	YbaK family protein	2.222183
VVMO6_00102	Diaminopimelate decarboxylase (EC 4.1.1.20)	2.217001
VVMO6_02374	Phosphoribosylformylglycinamidine synthase2C synthetase subunit (EC 6.3.5.3) / Phosphoribosylformylglycinamidine synthase2C glutamine amidotransferase subunit (EC 6.3.5.3)	2.212742
VVMO6_00190	Probable 3-phenylpropionic acid transporter	2.203726
VVMO6_00262	Cyclic AMP receptor protein	2.201716
VVMO6_02351	Exodeoxyribonuclease VII small subunit (EC 3.1.11.6)	2.19865
VVMO6_00101	putative lipoprotein L	2.198155
VVMO6_00941	Bis(5'-nucleosyl)-tetraphosphatase (asymmetrical) (EC 3.6.1.17)	2.19155
VVMO6_02369	ABC-type multidrug transport system2C ATPase and permease component	2.19008
VVMO6_03812	FIG008443: hypothetical protein	2.190049
VVMO6_01853	Peptidyl-prolyl cis-trans isomerase ppiB (EC 5.2.1.8)	2.183539
VVMO6_02224	Adenylate kinase (EC 2.7.4.3)	2.182694
VVMO6_02614	Adenylate cyclase (EC 4.6.1.1)	2.180804
VVMO6_04478	hypothetical protein	2.179501
VVMO6_02851	Two-component system response regulator OmpR	2.179132
VVMO6_01524	hypothetical protein	2.178492

VVMO6_03503	Low-specificity L-threonine aldolase (EC 4.1.2.5)	2.174873
VVMO6_03921	hypothetical protein	2.173465
VVMO6_01777	Glutaredoxin 1	2.171639
VVMO6_02363	Glutamate 5-kinase (EC 2.7.2.11)	2.171113
VVMO6_00404	DNA polymerase III chi subunit (EC 2.7.7.7)	2.163466
VVMO6_00166	Regulator of sigma D	2.16262
VVMO6_00390	Endoribonuclease L-PSP	2.160734
VVMO6_04102	Sialic acid utilization regulator2C RpiR family	2.160568
VVMO6_01988	hypothetical protein	2.147933
VVMO6_03398	hypothetical protein	2.146753
VVMO6_03208	Sensory histidine kinase in two-component regulatory system with RstA	2.140925
VVMO6_01681	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.137361
VVMO6_03622	hypothetical protein	2.136241
VVMO6_03521	Aerotaxis sensor receptor protein	2.133441
VVMO6_03339	Transcriptional regulator VpsT	2.12289
VVMO6_02616	Probable low-affinity inorganic phosphate transporter	2.11853
VVMO6_04350	ATPase involved in DNA repair	2.117283
VVMO6_03028	Pirin-related protein	2.116923
VVMO6_00250	Cytosine deaminase	2.110035
VVMO6_00227	Protein export cytoplasm chaperone protein (SecB2C maintains protein to be exported in unfolded state)	2.10101
VVMO6_04457	Uxu operon transcriptional regulator	2.095653
VVMO6_03418	Acetyltransferase	2.088775
VVMO6_00626	Lipoate-protein ligase A	2.086427
VVMO6_03645	Acetate kinase (EC 2.7.2.1)	2.085269
VVMO6_00419	Methyl-accepting chemotaxis protein	2.081789
VVMO6_02523	Peptide chain release factor 2; programmed frameshift-containing	2.079594
VVMO6_01640	hypothetical protein	2.078008
VVMO6_03477	hypothetical protein	2.076588
VVMO6_00622	Deoxyribose-phosphate aldolase (EC 4.1.2.4)	2.07573
VVMO6_02020	Molybdenum cofactor biosynthesis protein MoaA	2.075451
VVMO6_02285	hypothetical protein	2.074147
VVMO6_01769	hypothetical protein	2.073553
VVMO6_02301	tRNA-i(6)A37 methylthiotransferase	2.069836
VVMO6_00950	Transcription-repair coupling factor	2.068272
VVMO6_02510	hypothetical protein	2.062278
VVMO6_01264	hypothetical protein	2.061872
VVMO6_01902	FIG001587: exported protein	2.059309
VVMO6_01717	Potential queD like	2.054359
VVMO6_03664	NADH:ubiquinone oxidoreductase subunit 2	2.053147
VVMO6_03362	hypothetical protein	2.053128
VVMO6_02554	Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	2.053052
VVMO6_01273	Histidine utilization repressor	2.045912

VVMO6_02988	ABC-type amino acid transport/signal transduction system	2.041385
VVMO6_00145	tRNA (Uracil54-C5-)-methyltransferase (EC 2.1.1.35)	2.040719
VVMO6_02243	N-acetylglucosamine-regulated outer membrane porin	2.037746
VVMO6_01022	COG1801: Uncharacterized conserved protein	2.037381
VVMO6_04503	hypothetical protein	2.03427
VVMO6_00674	GNAT family acetyltransferase VC2332	2.027587
VVMO6_00337	Uncharacterized conserved protein	2.024727
VVMO6_00693	Membrane-bound lytic murein transglycosylase A precursor (EC 3.2.1.-)	2.023529
VVMO6_03532	Transcriptional regulator	2.021787
VVMO6_00418	Uncharacterized flavoprotein	2.019219
VVMO6_03760	ABC transporter ATP-binding protein YvcR	2.018642
VVMO6_03250	hypothetical protein	2.016131
VVMO6_02215	PTS system2C N-acetylglucosamine-specific IIB component (EC 2.7.1.69) / PTS system2C N-acetylglucosamine-specific IIC component (EC 2.7.1.69)	2.014101
VVMO6_01849	Nucleoside-diphosphate-sugar epimerase	2.012244
VVMO6_01133	L-serine dehydratase (EC 4.3.1.17)	2.01009
VVMO6_02123	ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)	2.008408
VVMO6_00535	Quorum-sensing regulator of virulence HapR	2.004925
VVMO6_01302	hypothetical protein	2.004845

Down-regulated genes (284 genes)

VVMO6_01047	hypothetical protein	-37.7982
VVMO6_03325	Phosphate ABC transporter2C periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)	-31.858
VVMO6_03004	Sodium/glutamate symporter	-22.6738
VVMO6_02509	Sodium-dependent phosphate transporter	-18.9609
VVMO6_01116	High-affinity choline uptake protein BetT	-16.9622
VVMO6_03003	hypothetical protein	-14.0959
VVMO6_02637	Transcriptional regulator of glmS gene2C DeoR family	-14.0464
VVMO6_02466	Phosphate transport system permease protein PstC (TC 3.A.1.7.1)	-13.2681
VVMO6_03970	Inner membrane protein YrbG2C predicted calcium/sodium:proton antiporter	-12.8643
VVMO6_00930	Transcriptional activator of cad operon	-11.0746
VVMO6_04146	Amino acid transporter	-9.79109
VVMO6_02391	Proton/glutamate symport protein @ Sodium/glutamate symport protein	-8.93455
VVMO6_03519	Histone acetyltransferase HPA2	-8.81006
VVMO6_03451	TPR repeat containing protein	-8.12167
VVMO6_03640	ABC-type phosphate transport system2C periplasmic component	-7.47234
VVMO6_03688	hypothetical protein	-6.86387

VVMO6_00346	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)	-6.79259
VVMO6_03806	Molybdenum transport ATP-binding protein ModC (TC 3.A.1.8.1)	-6.66213
VVMO6_01115	Transcriptional regulator2C MarR family	-6.4527
VVMO6_00033	COG1272: Predicted membrane protein hemolysin III homolog	-6.42867
VVMO6_02636	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (EC 2.6.1.16)	-6.18825
VVMO6_02519	hypothetical protein	-6.17762
VVMO6_03805	Permease of the drug/metabolite transporter (DMT) superfamily	-6.11629
VVMO6_03328	Phosphate transport ATP-binding protein PstB (TC 3.A.1.7.1)	-6.01609
VVMO6_00345	Sulfite reductase [NADPH] flavoprotein alpha-component (EC 1.8.1.2)	-5.79519
VVMO6_00140	SOS-response repressor and protease LexA (EC 3.4.21.88)	-5.68693
VVMO6_03835	C4-dicarboxylate like transporter	-5.3037
VVMO6_03969	hypothetical protein	-5.24273
VVMO6_04340	hypothetical protein	-5.15611
VVMO6_02491	2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I alpha (EC 2.5.1.54)	-5.12678
VVMO6_02517	hypothetical protein	-5.12657
VVMO6_02471	Phosphate regulon transcriptional regulatory protein PhoB (SphR)	-5.06041
VVMO6_01995	Trp operon leader peptide	-4.99125
VVMO6_03971	Transcriptional regulator2C TetR family	-4.8702
VVMO6_00347	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)	-4.82245
VVMO6_02935	LysR-family transcriptional regulator VC0068	-4.76689
VVMO6_02721	Uroporphyrinogen-III methyltransferase (EC 2.1.1.107)	-4.75242
VVMO6_02332	Methionine ABC transporter substrate-binding protein	-4.72167
VVMO6_01997	Anthranilate synthase2C amidotransferase component (EC 4.1.3.27)	-4.60939
VVMO6_01996	Anthranilate synthase2C aminase component (EC 4.1.3.27)	-4.58348
VVMO6_00316	hypothetical protein	-4.50833
VVMO6_00116	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)	-4.45454
VVMO6_01167	Tyrosine-specific transport protein	-4.43797
VVMO6_00681	hypothetical protein	-4.32968
VVMO6_00317	Hydrogen peroxide-inducible genes activator	-4.2991
VVMO6_02718	Sulfate permease2C Trk-type	-4.25055
VVMO6_02763	UDP-glucose dehydrogenase (EC 1.1.1.22)	-4.16887
VVMO6_00739	hypothetical protein	-4.10427
VVMO6_00501	Regulatory protein RecX	-4.09209
VVMO6_01758	hypothetical protein	-4.08352
VVMO6_02972	Amino acid ABC transporter2C periplasmic	-4.01449

	amino acid-binding portion	
VVMO6_01835	Alpha-12C6-galactosidase2C putative	-3.98276
VVMO6_02331	Methionine ABC transporter permease protein	-3.97806
VVMO6_04122	Permease of the drug/metabolite transporter (DMT) superfamily	-3.9743
VVMO6_01634	Sugar transferase SypR involved in lipopolysaccharide synthesis	-3.97296
VVMO6_01263	hypothetical protein	-3.88449
VVMO6_02490	Chorismate mutase I (EC 5.4.99.5) / Cyclohexadienyl dehydrogenase (EC 1.3.1.12)(EC 1.3.1.43)	-3.88306
VVMO6_01118	DNA polymerase III epsilon subunit (EC 2.7.7.7)	-3.8709
VVMO6_04108	N-acetylmannosamine kinase (EC 2.7.1.60)	-3.86341
VVMO6_00718	DNA polymerase IV (EC 2.7.7.7)	-3.77016
VVMO6_02895	DNA polymerase I (EC 2.7.7.7)	-3.76265
VVMO6_02635	Mannitol operon repressor	-3.72273
VVMO6_00034	Potassium uptake protein TrkH	-3.71613
VVMO6_00684	Tellurite resistance protein	-3.65758
VVMO6_02247	Cysteine synthase (EC 2.5.1.47)	-3.6241
VVMO6_00738	Acetyltransferase	-3.57193
VVMO6_02190	Zinc ABC transporter2C ATP-binding protein ZnuC	-3.51982
VVMO6_02714	ABC-type antimicrobial peptide transport system2C permease component	-3.50226
VVMO6_01661	Ferredoxin-type protein NapG (periplasmic nitrate reductase)	-3.4947
VVMO6_01662	Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) / Sirohydrochlorin ferrochelatase (EC 4.99.1.4)	-3.44401
VVMO6_04206	Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	-3.4353
VVMO6_01713	hypothetical protein	-3.40538
VVMO6_02773	hypothetical protein	-3.39794
VVMO6_02973	Amino acid ABC transporter2C permease protein	-3.39584
VVMO6_02012	Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1)	-3.38912
VVMO6_01998	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)	-3.38508
VVMO6_04109	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	-3.36499
VVMO6_02465	Phosphate transport system permease protein PstA (TC 3.A.1.7.1)	-3.36083
VVMO6_01119	Predicted signal-transduction protein containing cAMP-binding and CBS domains	-3.34057
VVMO6_04111	ABC-type uncharacterized transport system2C periplasmic component	-3.33933
VVMO6_01660	Polyferredoxin NapH (periplasmic nitrate reductase)	-3.32865
VVMO6_00607	Transcriptional regulator2C MarR family	-3.31811
VVMO6_00715	Thiamin biosynthesis lipoprotein ApbE	-3.30979
VVMO6_02974	ABC-type polar amino acid transport system2C	-3.27808

	ATPase component	
VVMO6_01545	hypothetical protein	-3.2761
VVMO6_03012	Transcriptional regulator2C LysR family	-3.25993
VVMO6_02013	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)	-3.24195
VVMO6_02968	DNA recombination and repair protein RecF	-3.23205
VVMO6_02024	Excinuclease ABC subunit B	-3.20679
VVMO6_00678	Glycerol kinase (EC 2.7.1.30)	-3.20564
VVMO6_00357	Excinuclease ABC subunit A	-3.19879
VVMO6_00117	Predicted protein-tyrosine phosphatase	-3.18433
VVMO6_02717	Adenylylsulfate kinase (EC 2.7.1.25)	-3.17098
VVMO6_01262	Outer membrane protein	-3.16394
VVMO6_00480	hypothetical protein	-3.15878
VVMO6_00740	hypothetical protein	-3.13486
VVMO6_04207	Isochorismate pyruvate-lyase of siderophore biosynthesis	-3.11833
VVMO6_01911	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)	-3.1013
VVMO6_00315	Argininosuccinate lyase (EC 4.3.2.1) / N-acetylglutamate synthase (EC 2.3.1.1)	-3.08799
VVMO6_03701	Fructose repressor FruR2C LacI family	-3.07443
VVMO6_00679	Glycerol uptake facilitator protein	-3.06521
VVMO6_03407	hypothetical protein	-3.05269
VVMO6_02719	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)	-3.0429
VVMO6_04339	hypothetical protein	-3.03756
VVMO6_01168	Lactoylglutathione lyase	-3.03099
VVMO6_02715	FIG003461: hypothetical protein	-3.02662
VVMO6_02010	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)	-3.00973
VVMO6_01547	hypothetical protein	-3.00193
VVMO6_01999	Indole-3-glycerol phosphate synthase (EC 4.1.1.48) / Phosphoribosylanthranilate isomerase (EC 5.3.1.24)	-2.99456
VVMO6_00500	RecA protein	-2.99097
VVMO6_00128	Signal recognition particle receptor protein FtsY (alpha subunit) (TC 3.A.5.1.1)	-2.9888
VVMO6_01856	GTG start codon	-2.98026
VVMO6_01712	Catalase (EC 1.11.1.6) / Peroxidase (EC 1.11.1.7)	-2.95969
VVMO6_02926	ATP-dependent DNA helicase UvrD/PcrA	-2.93607
VVMO6_01514	Universal stress protein E	-2.93144
VVMO6_00344	Thymidylate kinase	-2.92363
VVMO6_04197	Phosphopantetheinyl transferase component of siderophore synthetase (EC 2.7.8.-)	-2.90816
VVMO6_03684	hypothetical protein	-2.90664
VVMO6_02865	General secretion pathway protein J	-2.90488
VVMO6_02940	Sulfur carrier protein adenylyltransferase ThiF	-2.89572
VVMO6_00911	Type I restriction-modification system2C DNA-methyltransferase subunit M (EC 2.1.1.72)	-2.86539
VVMO6_02941	Thiamin-phosphate pyrophosphorylase (EC	-2.84934

	2.5.1.3)	
VVMO6_01544	hypothetical protein	-2.82425
VVMO6_00479	hypothetical protein	-2.8198
VVMO6_01169	hypothetical protein	-2.7966
VVMO6_00682	DNA damage-inducible gene in SOS regulon2C dependent on cyclic AMP and H-NS	-2.78801
VVMO6_02508	Transcriptional activator NhaR	-2.78044
VVMO6_02191	Zinc ABC transporter2C inner membrane permease protein ZnuB	-2.77658
VVMO6_00845	ABC transporter involved in cytochrome c biogenesis2C CcmB subunit	-2.77159
VVMO6_00683	Transcriptional regulator2C LysR family	-2.76841
VVMO6_02011	Oligopeptide transport ATP-binding protein OppD (TC 3.A.1.5.1)	-2.75861
VVMO6_04198	Non-ribosomal peptide synthetase modules2C siderophore biosynthesis	-2.75133
VVMO6_00146	Outer membrane vitamin B12 receptor BtuB	-2.74471
VVMO6_02969	DNA polymerase III beta subunit (EC 2.7.7.7)	-2.74368
VVMO6_02422	GGDEF domain protein	-2.72515
VVMO6_03224	Membrane-associated phospholipid phosphatase	-2.71857
VVMO6_02772	hypothetical protein	-2.71798
VVMO6_04408	Aerobactin siderophore receptor IutA	-2.71124
VVMO6_02423	Predicted metal-dependent hydrolase	-2.6905
VVMO6_00969	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-2.68592
VVMO6_04507	Proline/sodium symporter PutP (TC 2.A.21.2.1) @ Propionate/sodium symporter	-2.66714
VVMO6_00127	Methyltransferase (EC 2.1.1.-)	-2.65076
VVMO6_02366	Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22)	-2.63876
VVMO6_01635	hypothetical protein	-2.62886
VVMO6_02866	General secretion pathway protein I	-2.62137
VVMO6_00844	ABC transporter involved in cytochrome c biogenesis2C ATPase component CcmA	-2.61344
VVMO6_00970	hypothetical protein	-2.61058
VVMO6_00118	Permease of the major facilitator superfamily	-2.60532
VVMO6_01170	Non-specific DNA-binding protein Dps / Iron-binding ferritin-like antioxidant protein / Ferroxidase (EC 1.16.3.1)	-2.58372
VVMO6_02867	General secretion pathway protein H	-2.56338
VVMO6_02771	glycosyl transferase2C group 1	-2.56263
VVMO6_02938	Thiazole biosynthesis protein ThiG	-2.53593
VVMO6_04205	Vulnibactin utilization protein VuuB	-2.51687
VVMO6_00608	Multi antimicrobial extrusion protein (Na(+)/drug antiporter)2C MATE family of MDR efflux pumps	-2.50235
VVMO6_00314	Argininosuccinate synthase (EC 6.3.4.5)	-2.48839
VVMO6_00998	hypothetical protein	-2.48484
VVMO6_02470	Phosphate regulon sensor protein PhoR (SphS) (EC 2.7.13.3)	-2.47373

VVMO6_02417	Chitinase (EC 3.2.1.14)	-2.47238
VVMO6_02774	hypothetical protein	-2.4679
VVMO6_01784	hypothetical protein	-2.45661
VVMO6_01825	Psp operon transcriptional activator	-2.45082
VVMO6_02298	Peptidyl-tRNA hydrolase (EC 3.1.1.29)	-2.45014
VVMO6_02864	General secretion pathway protein K	-2.44481
VVMO6_00120	hypothetical protein	-2.43159
VVMO6_03834	Methyl-accepting chemotaxis protein	-2.43135
VVMO6_02623	Acyl-phosphate:glycerol-3-phosphate O-acyltransferase PlsY	-2.40862
VVMO6_00193	Acetate permease ActP (cation/acetate symporter)	-2.40453
VVMO6_03730	Transcriptional regulator2C LysR family	-2.39731
VVMO6_03734	Integral membrane protein TerC	-2.39418
VVMO6_02521	Transcriptional regulator VpsR	-2.39123
VVMO6_01828	Peptide transport system permease protein sapC (TC 3.A.1.5.5)	-2.38866
VVMO6_00194	hypothetical protein	-2.38337
VVMO6_04361	ATP-dependent RNA helicase VC1407	-2.38309
VVMO6_01896	ATPase involved in DNA repair	-2.38232
VVMO6_02367	Xanthine/uracil/thiamine/ascorbate permease family protein	-2.37461
VVMO6_00589	Dihydropteroate synthase (EC 2.5.1.15)	-2.35131
VVMO6_02329	D-glycero-D-manno-heptose 12C7-bisphosphate phosphatase (EC 3.1.1.-)	-2.34975
VVMO6_00752	1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267)	-2.34735
VVMO6_01862	Histidinol-phosphate aminotransferase (EC 2.6.1.9)	-2.34594
VVMO6_01334	hypothetical protein	-2.34519
VVMO6_00750	Undecaprenyl pyrophosphate synthetase (EC 2.5.1.31)	-2.33484
VVMO6_02914	3-polyprenyl-4-hydroxybenzoate carboxy-lyase (EC 4.1.1.-)	-2.32688
VVMO6_02570	FIG006972: hypothetical protein	-2.3248
VVMO6_00129	Cell division transporter2C ATP-binding protein FtsE (TC 3.A.5.1.1)	-2.32303
VVMO6_03804	Transcriptional regulator2C MarR family	-2.32291
VVMO6_00751	Phosphatidate cytidylyltransferase (EC 2.7.7.41)	-2.31604
VVMO6_02424	GTP-binding protein EngA	-2.31234
VVMO6_02831	hypothetical protein	-2.30729
VVMO6_03854	Methylase of chemotaxis methyl-accepting protein	-2.29481
VVMO6_01912	Oligopeptide transport ATP-binding protein OppD (TC 3.A.1.5.1)	-2.29423
VVMO6_01860	Imidazole glycerol phosphate synthase amidotransferase subunit (EC 2.4.2.-)	-2.29252
VVMO6_01484	Signal transduction histidine kinase	-2.29221
VVMO6_02824	Transcriptional regulator SlmA2C TetR family	-2.27235
VVMO6_02634	Mannitol-1-phosphate 5-dehydrogenase (EC 1.1.1.17)	-2.27138

VVMO6_00841	CheW domain protein	-2.27119
VVMO6_00785	Uracil phosphoribosyltransferase (EC 2.4.2.9)	-2.25985
VVMO6_01521	Cytochrome c oxidase subunit CcoQ (EC 1.9.3.1)	-2.25847
VVMO6_02937	Thiazole biosynthesis protein ThiH	-2.25765
VVMO6_03472	Alcohol dehydrogenase (EC 1.1.1.1)	-2.25711
VVMO6_01861	Histidinol-phosphatase (EC 3.1.3.15) / Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19)	-2.25326
VVMO6_02641	Sensory box/GGDEF family protein	-2.24838
VVMO6_01473	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-2.2468
VVMO6_00360	Single-stranded DNA-binding protein	-2.24492
VVMO6_00369	MSHA biogenesis protein MshG	-2.23804
VVMO6_03719	Response regulator	-2.23287
VVMO6_04021	Hypotehtical protein in Cytochrome oxidase biogenesis cluster	-2.23221
VVMO6_02478	COG1496: Uncharacterized conserved protein	-2.22898
VVMO6_03197	Transcriptional regulator2C GntR family	-2.2283
VVMO6_02592	Stringent starvation protein A	-2.22777
VVMO6_02299	GTP-binding and nucleic acid-binding protein YchF	-2.22646
VVMO6_02767	COG0438: Glycosyltransferase	-2.22272
VVMO6_02598	ATPase2C AFG1 family	-2.21851
VVMO6_02365	hypothetical protein	-2.21583
VVMO6_01106	Na+/H+ antiporter NhaB	-2.21187
VVMO6_02894	GTP-binding protein EngB	-2.20822
VVMO6_03859	ATP-dependent RNA helicase VVA0939	-2.20496
VVMO6_02105	membrane protein	-2.20316
VVMO6_01759	CBSS-345074.3.peg.1627: Cysteine desulfurase (EC 2.8.1.7)	-2.20179
VVMO6_04006	Molybdopterin biosynthesis protein MoeA	-2.18718
VVMO6_02539	Pyruvate formate-lyase (EC 2.3.1.54)	-2.18671
VVMO6_00313	Acetylglutamate kinase (EC 2.7.2.8)	-2.18549
VVMO6_00304	Methionine repressor MetJ	-2.18524
VVMO6_00211	Uncharacterized low-complexity protein	-2.176
VVMO6_02014	Oligopeptide ABC transporter2C periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)	-2.174
VVMO6_03836	TPR domain protein2C putative component of TonB system	-2.17004
VVMO6_02805	hypothetical protein	-2.16703
VVMO6_00234	Ribosome small subunit-stimulated GTPase EngC	-2.16362
VVMO6_00136	Chorismate--pyruvate lyase (EC 4.1.3.40)	-2.16282
VVMO6_00542	3-methyl-2-oxobutanoate hydroxymethyltransferase (EC 2.1.2.11)	-2.16189
VVMO6_03228	hypothetical protein	-2.15882
VVMO6_02869	General secretion pathway protein F	-2.15619
VVMO6_02328	Trehalose operon transcriptional repressor	-2.15537

VVMO6_04195	Putative phosphatase	-2.15431
VVMO6_01184	Putative glycosyltransferase protein	-2.1383
VVMO6_02712	Predicted zinc-binding protein	-2.13791
VVMO6_01863	Histidinol dehydrogenase (EC 1.1.1.23)	-2.13446
VVMO6_02825	Lipid A biosynthesis lauroyl acyltransferase (EC 2.3.1.-)	-2.12584
VVMO6_03288	Toxin secretion ATP-binding protein	-2.12528
VVMO6_00760	Ribonuclease HII (EC 3.1.26.4)	-2.1235
VVMO6_00251	3'-to-5' exoribonuclease RNase R	-2.12047
VVMO6_02811	Diacylglycerol kinase (EC 2.7.1.107)	-2.11222
VVMO6_01687	PrpF protein involved in 2-methylcitrate cycle	-2.10858
VVMO6_00298	ATP-dependent protease HslV (EC 3.4.25.-)	-2.10679
VVMO6_02684	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase (EC 3.1.3.45)	-2.10368
VVMO6_01158	LysR-family transcriptional regulator VCA0830	-2.0992
VVMO6_00561	GlcNAc phosphomutase (EC 5.4.2.3)	-2.08951
VVMO6_00394	Arginine deiminase (EC 3.5.3.6)	-2.0844
VVMO6_01944	3-oxoacyl-[acyl-carrier-protein] synthase2C KASIII (EC 2.3.1.41)	-2.08397
VVMO6_03135	Galactose/methyl galactoside ABC transport system2C D-galactose-binding periplasmic protein MglB (TC 3.A.1.2.3)	-2.08338
VVMO6_02806	Bacillosamine/Legionaminic acid biosynthesis aminotransferase PglE; 4-keto-6-deoxy-N-Acetyl-D-hexosaminyl-(Lipid carrier) aminotransferase	-2.08281
VVMO6_00092	Ferritin-like protein 2	-2.08028
VVMO6_00130	Cell division protein FtsX	-2.07936
VVMO6_04007	Molybdopterin biosynthesis protein MoeB	-2.07859
VVMO6_00978	hypothetical protein	-2.07819
VVMO6_00481	GGDEF domain protein	-2.07796
VVMO6_00401	Inner membrane protein YjgN	-2.07662
VVMO6_00137	4-hydroxybenzoate polyprenyltransferase (EC 2.5.1.-)	-2.07323
VVMO6_01505	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	-2.06858
VVMO6_00370	MSHA biogenesis protein MshF	-2.06733
VVMO6_03518	Fatty acid desaturase (EC 1.14.19.1); Delta-9 fatty acid desaturase (EC 1.14.19.1)	-2.0671
VVMO6_00602	hypothetical protein	-2.06175
VVMO6_00004	Chromosome (plasmid) partitioning protein ParB / Stage 0 sporulation protein J	-2.06068
VVMO6_01755	SanA protein	-2.05121
VVMO6_03133	Galactose/methyl galactoside ABC transport system2C permease protein MglC (TC 3.A.1.2.3)	-2.04995
VVMO6_02670	Arginine pathway regulatory protein ArgR2C repressor of arg regulon	-2.04856
VVMO6_01261	hypothetical protein	-2.04679
VVMO6_04509	Proline dehydrogenase (EC 1.5.99.8) (Proline oxidase) / Delta-1-pyrroline-5-carboxylate	-2.04294

	dehydrogenase (EC 1.5.1.12)	
VVMO6_03423	Transcriptional regulator2C AraC family	-2.04217
VVMO6_03979	Mannose-6-phosphate isomerase (EC 5.3.1.8)	-2.03987
VVMO6_04023	hypothetical protein	-2.03961
VVMO6_00984	ATP-dependent helicase DinG/Rad3	-2.0391
VVMO6_03885	TldE/PmbA protein2C part of proposed TldE/TldD proteolytic complex (PMID 12029038)	-2.03618
VVMO6_03421	Transcriptional regulator2C LysR family	-2.03568
VVMO6_02479	Ribosomal large subunit pseudouridine synthase D (EC 4.2.1.70)	-2.03557
VVMO6_02591	Stringent starvation protein B	-2.03547
VVMO6_01884	Biotin synthesis protein bioC	-2.03445
VVMO6_03853	Signal transduction histidine kinase	-2.02782
VVMO6_00367	MSHA biogenesis protein MshN	-2.02177
VVMO6_02807	UDP-N-acetylglucosamine 42C6-dehydratase (EC 4.2.1.-)	-2.01851
VVMO6_03594	hypothetical protein	-2.01726
VVMO6_01943	Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39)	-2.00503
VVMO6_01836	hypothetical protein	-2.00429
VVMO6_01686	Propionate--CoA ligase (EC 6.2.1.17)	-2.00322
VVMO6_01101	Maltodextrin glucosidase (EC 3.2.1.20)	-2.00226

Table 3-4. Genes differentially expressed in biofilm cells compared to planktonic cells at the dispersion stage of biofilm development (B3/P3).

Locus tag	Gene product	Fold change
Up-regulated genes (327 genes)		
VVMO6_03011	Uncharacterized protein conserved in bacteria	159.3517
VVMO6_03235	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	115.9761
VVMO6_03010	Glutathione synthetase (EC 6.3.2.3)	37.20995
VVMO6_03829	Permease of the major facilitator superfamily	35.73892
VVMO6_03178	hypothetical protein	21.46585
VVMO6_03177	Permease of the drug/metabolite transporter (DMT) superfamily	18.50944
VVMO6_04166	L-lactate permease	16.93369
VVMO6_03179	Argininosuccinate synthase (EC 6.3.4.5)	15.17244
VVMO6_03831	Na+-driven multidrug efflux pump	13.39747
VVMO6_02113	Aspartate/tyrosine/aromatic aminotransferase	11.8641
VVMO6_04175	membrane protein	11.7237
VVMO6_03009	hypothetical protein	11.53616
VVMO6_03350	Capsular polysaccharide synthesis enzyme CpsB	11.39095
VVMO6_01237	Biosynthetic arginine decarboxylase (EC 4.1.1.19)	10.72432
VVMO6_02497	Carbon starvation protein A	9.849923
VVMO6_03663	Oxalate/formate antiporter	9.485464
VVMO6_01487	ABC transporter ATP-binding protein uup	9.254084
VVMO6_02324	hypothetical protein	9.079899
VVMO6_03633	ATP-dependent RNA helicase DbpA	9.055341
VVMO6_01639	Putative lipoprotein	8.863016
VVMO6_03204	Inner membrane protein YccF	8.385007
VVMO6_03711	Iron-containing alcohol dehydrogenase	8.270394
VVMO6_02498	Autolysin sensor kinase (EC 2.7.3.-)	8.122717
VVMO6_04242	Glutathione S-transferase (EC 2.5.1.18)	8.115744
VVMO6_01844	Formate efflux transporter (TC 2.A.44 family)	8.035486
VVMO6_01989	Translation elongation factor P-related protein	7.992846
VVMO6_03117	hypothetical protein	7.605219
VVMO6_01679	Queuosine Biosynthesis QueE Radical SAM	7.570653
VVMO6_01932	Sodium-dependent transporter	7.391944
VVMO6_03424	Phosphoenolpyruvate-protein kinase	7.194045
VVMO6_04421	Transcriptional regulator2C AraC family	7.138581
VVMO6_03810	hypothetical protein	7.003601
VVMO6_03118	Membrane fusion component of tripartite multidrug resistance system	6.607258
VVMO6_04068	ATP-dependent RNA helicase RhlE	6.579908
VVMO6_02370	Predicted deacylase	6.467314
VVMO6_02372	Pressure-regulated ORF-like protein	6.405693
VVMO6_01680	Putative hydrolase of the HAD superfamily	6.39449
VVMO6_02378	S-(hydroxymethyl)glutathione dehydrogenase	6.28823

	(EC 1.1.1.284)	
VVMO6_03349	Capsular polysaccharide synthesis enzyme CpsC2C polysaccharide export	6.25697
VVMO6_03704	FIG015136: hypothetical protein	6.247788
VVMO6_03830	Transcriptional regulator2C AraC family	6.144357
VVMO6_03425	Methyl-accepting chemotaxis protein	6.129474
VVMO6_04064	Putative transporter	6.080168
VVMO6_00673	ribosomal protein S6 glutaminyl transferase related protein	5.961157
VVMO6_01236	Agmatinase (EC 3.5.3.11)	5.955026
VVMO6_02533	Sodium/alanine symporter	5.935603
VVMO6_01678	Queuosine Biosynthesis QueC ATPase	5.832886
VVMO6_03832	hypothetical protein	5.696434
VVMO6_04168	Predicted L-lactate dehydrogenase2C Iron-sulfur cluster-binding subunit YkgF	5.612778
VVMO6_04231	Peptide deformylase (EC 3.5.1.88)	5.520599
VVMO6_04320	Membrane fusion protein of RND family multidrug efflux pump	5.48471
VVMO6_01657	Putative threonine efflux protein	5.465732
VVMO6_01513	tRNA(Cytosine32)-2-thiocytidine synthetase	5.374817
VVMO6_04428	exopolysaccharide synthesis protein ExoD-related protein	5.313727
VVMO6_03387	Peptidyl-prolyl cis-trans isomerase ppiC (EC 5.2.1.8)	5.217052
VVMO6_02645	Probable transcriptional activator for leuABCD operon	5.202747
VVMO6_02371	DNA-directed RNA polymerase2C beta' subunit/160 kD subunit	5.102591
VVMO6_03364	Dihydroorotase (EC 3.5.2.3)	5.076335
VVMO6_01142	Conserved protein YcjX with nucleoside triphosphate hydrolase domain	5.054499
VVMO6_04527	tRNA and rRNA cytosine-C5-methylase	5.039186
VVMO6_01990	hypothetical protein	5.031178
VVMO6_01645	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)	5.022737
VVMO6_03811	hypothetical protein	5.009707
VVMO6_04196	hypothetical protein	4.917532
VVMO6_03242	Permease of the drug/metabolite transporter (DMT) superfamily	4.869646
VVMO6_00425	Glutaminase (EC 3.5.1.2)	4.71511
VVMO6_03173	membrane protein	4.668843
VVMO6_01455	hypothetical protein	4.668578
VVMO6_02442	Inositol-1-monophosphatase (EC 3.1.3.25)	4.638317
VVMO6_01796	Signal transduction histidine kinase	4.61009
VVMO6_03985	YaeQ protein	4.59652
VVMO6_03340	STRUCTURAL ELEMENTS; Cell Exterior; surface polysaccharides/antigens	4.557374
VVMO6_03122	hypothetical protein	4.526121
VVMO6_03644	Cold-shock DEAD-box protein A	4.521091
VVMO6_02644	Long-chain-fatty-acid--CoA ligase (EC 6.2.1.3)	4.38775

VVMO6_00546	C4-type zinc finger protein2C DksA/TraR family	4.380273
VVMO6_03484	Signal recognition particle GTPase	4.337993
VVMO6_00205	P pilus assembly/Cpx signaling pathway2C periplasmic inhibitor/zinc- resistance associated protein	4.336909
VVMO6_04432	hypothetical protein	4.289143
VVMO6_03664	NADH:ubiquinone oxidoreductase subunit 2	4.273202
VVMO6_00173	hypothetical protein	4.25162
VVMO6_04503	hypothetical protein	4.231661
VVMO6_01972	3-demethylubiquinone-9 3-methyltransferase (EC 2.1.1.64)	4.217334
VVMO6_03398	hypothetical protein	4.217051
VVMO6_01143	Membrane protein YcjF	4.194968
VVMO6_00895	conserved protein of unknown function; putative YcgN protein	4.180943
VVMO6_04396	DNA polymerase III alpha subunit (EC 2.7.7.7)	4.116306
VVMO6_03417	OsmC/Ohr family protein	4.114249
VVMO6_04071	Acetyltransferase	4.108567
VVMO6_02352	Octaprenyl-diphosphate synthase (EC 2.5.1.-) / Dimethylallyltransferase (EC 2.5.1.1) / Geranyltranstransferase (farnesyldiphosphate synthase) (EC 2.5.1.10) / Geranylgeranyl pyrophosphate synthetase (EC 2.5.1.29)	4.10413
VVMO6_00190	Probable 3-phenylpropionic acid transporter	4.087834
VVMO6_00725	hypothetical protein	4.082732
VVMO6_01656	hypothetical protein	4.082442
VVMO6_00902	Uncharacterized iron-regulated protein	4.06873
VVMO6_03027	Uncharacterized protein conserved in bacteria	4.000274
VVMO6_00267	Glutathione-regulated potassium-efflux system ATP-binding protein	3.970055
VVMO6_02180	Formyltetrahydrofolate deformylase (EC 3.5.1.10)	3.928337
VVMO6_00424	tRNA (guanine46-N7-)-methyltransferase (EC 2.1.1.33)	3.919609
VVMO6_03791	hypothetical protein	3.848596
VVMO6_01644	6-phosphogluconolactonase (EC 3.1.1.31)2C eukaryotic type	3.838702
VVMO6_01787	AttE component of AttEFGH ABC transport system	3.827806
VVMO6_04539	Uncharacterized protein conserved in bacteria	3.725918
VVMO6_03226	hypothetical protein	3.698419
VVMO6_01371	hypothetical protein	3.688962
VVMO6_01509	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	3.677867
VVMO6_01765	Phosphoserine aminotransferase (EC 2.6.1.52)	3.657343
VVMO6_00302	LSU ribosomal protein L31p	3.63379
VVMO6_04065	Transcriptional regulator2C MarR family	3.619672
VVMO6_04513	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	3.610261
VVMO6_03499	Predicted deacylase	3.604634
VVMO6_00786	Uracil permease	3.593368

VVMO6_01105	Periplasmic thiol:disulfide oxidoreductase DsbB2C required for DsbA reoxidation	3.581622
VVMO6_02351	Exodeoxyribonuclease VII small subunit (EC 3.1.11.6)	3.559186
VVMO6_00583	Outer membrane protein OmpU	3.531074
VVMO6_03982	Uncharacterized conserved protein	3.525463
VVMO6_04044	hypothetical protein	3.505314
VVMO6_00867	Putative transporting ATPase	3.500205
VVMO6_03986	hypothetical protein	3.492123
VVMO6_01731	Glutaredoxin	3.466154
VVMO6_03833	hypothetical protein	3.427233
VVMO6_01508	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)	3.395745
VVMO6_03207	Diacylglycerol kinase (EC 2.7.1.107)	3.383102
VVMO6_01795	Two-component system response regulator QseB	3.377698
VVMO6_03862	Predicted SAM-dependent methyltransferase	3.360257
VVMO6_00111	Cof protein2C HD superfamily hydrolase	3.352489
VVMO6_04319	Putative multidrug resistance protein	3.349872
VVMO6_03064	Pyrrolidone-carboxylate peptidase (EC 3.4.19.3)	3.349236
VVMO6_02535	hypothetical protein	3.252844
VVMO6_00973	54K polar flagellar sheath protein A	3.252159
VVMO6_00213	Lysine 22C3-aminomutase (EC 5.4.3.2)	3.248236
VVMO6_02286	Ribosomal large subunit pseudouridine synthase F (EC 4.2.1.70)	3.228136
VVMO6_01256	hypothetical protein	3.222881
VVMO6_03342	Membrane-fusion protein	3.214926
VVMO6_03341	ABC-type protease/lipase transport system2C ATPase and permease component	3.212293
VVMO6_03369	AraC-type DNA-binding domain-containing protein	3.195666
VVMO6_02616	Probable low-affinity inorganic phosphate transporter	3.195312
VVMO6_03752	Isopenicillin N synthase	3.192834
VVMO6_00897	Lactoylglutathione lyase (EC 4.4.1.5)	3.188287
VVMO6_02890	Protein of unknown function DUF414	3.173951
VVMO6_00156	Pantothenate kinase (EC 2.7.1.33)	3.168709
VVMO6_02325	hypothetical protein	3.153468
VVMO6_00184	Biotin carboxylase of acetyl-CoA carboxylase (EC 6.3.4.14)	3.149806
VVMO6_03186	hypothetical protein	3.144366
VVMO6_01076	hypothetical protein	3.118472
VVMO6_01609	Ribosomal RNA small subunit methyltransferase F (EC 2.1.1.-)	3.07813
VVMO6_00868	hypothetical protein	3.063252
VVMO6_03067	Phosphoserine phosphatase (EC 3.1.3.3)	3.062126
VVMO6_03119	Permease of the drug/metabolite transporter (DMT) superfamily	3.057395
VVMO6_03339	Transcriptional regulator VpsT	3.050638
VVMO6_04350	ATPase involved in DNA repair	3.049916

VVMO6_02407	YrdC/Sua5 family protein2C required for threonylcarbamoyladenosine (t(6)A) formation in tRNA	3.039594
VVMO6_01907	Iron-regulated protein A precursor	3.033866
VVMO6_01794	membrane protein	3.028513
VVMO6_03143	NAD(FAD)-utilizing dehydrogenase2C sll0175 homolog	3.026813
VVMO6_03923	Predicted signal transduction protein	3.021873
VVMO6_00185	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	3.016898
VVMO6_00951	hypothetical protein	3.0136
VVMO6_02615	Phosphate transport regulator (distant homolog of PhoU)	3.002778
VVMO6_00894	proteinase inhibitor2C putative	3.001866
VVMO6_01274	putative SpoOM-related protein	2.999576
VVMO6_02891	Putative methyltransferase associated with DUF414	2.994158
VVMO6_00613	FIG002208: Acetyltransferase (EC 2.3.1.-)	2.991969
VVMO6_03141	RNA methyltransferase2C TrmH family	2.981211
VVMO6_01822	Phage shock protein C	2.976305
VVMO6_03160	hypothetical protein	2.96384
VVMO6_03863	FOG: GGDEF domain	2.955543
VVMO6_03689	Predicted transcriptional regulator	2.94811
VVMO6_04402	hypothetical protein	2.943864
VVMO6_01254	Nicotinamidase (EC 3.5.1.19)	2.935206
VVMO6_02455	Alkyl hydroperoxide reductase subunit C-like protein	2.925712
VVMO6_02349	Flagellar motor rotation protein MotB	2.917689
VVMO6_01475	Putative cytoplasmic protein	2.917555
VVMO6_01456	hypothetical protein	2.913047
VVMO6_03142	4Fe-4S ferredoxin2C iron-sulfur binding	2.91121
VVMO6_02285	hypothetical protein	2.907611
VVMO6_00392	Aspartate carbamoyltransferase (EC 2.1.3.2)	2.899297
VVMO6_00808	Flagellin protein FlaD	2.896611
VVMO6_01122	Aspartate aminotransferase (EC 2.6.1.1)	2.876069
VVMO6_01301	Glyoxalase family protein	2.8751
VVMO6_01104	Protein export cytoplasm protein SecA ATPase RNA helicase (TC 3.A.5.1.1)	2.874376
VVMO6_00862	Phosphohistidine phosphatase SixA	2.871743
VVMO6_00148	Glutamate racemase (EC 5.1.1.3)	2.862862
VVMO6_00818	Flagellar M-ring protein FliF	2.861875
VVMO6_00871	Erythronate-4-phosphate dehydrogenase (EC 1.1.1.290)	2.855709
VVMO6_02181	Protein yecM	2.848493
VVMO6_01982	Diaminobutyrate-pyruvate transaminase & L-22C4-diaminobutyrate decarboxylase	2.841833
VVMO6_02650	2-isopropylmalate synthase (EC 2.3.3.13)	2.831603
VVMO6_00896	hypothetical protein	2.828414
VVMO6_02896	Porphobilinogen synthase (EC 4.2.1.24)	2.799493

VVMO6_03060	Ribosomal protein S12p Asp88 (E. coli) methylthiotransferase	2.776897
VVMO6_02068	Putative sugar isomerase involved in processing of exogenous sialic acid	2.774143
VVMO6_01539	Dihydroorotate dehydrogenase (EC 1.3.3.1)	2.770308
VVMO6_02889	Periplasmic/membrane protein associated with DUF414	2.766502
VVMO6_00181	DNA-binding protein Fis	2.76538
VVMO6_01532	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.60)	2.759373
VVMO6_04479	hypothetical protein	2.753941
VVMO6_02112	Menaquinone-specific isochorismate synthase (EC 5.4.4.2)	2.744106
VVMO6_00387	RNA polymerase associated protein RapA (EC 3.6.1.-)	2.738989
VVMO6_02819	LSU ribosomal protein L33p	2.726926
VVMO6_01507	Spermidine Putrescine ABC transporter permease component potC (TC_3.A.1.11.1)	2.697813
VVMO6_01672	LSU m5C1962 methyltransferase RlmI	2.694922
VVMO6_01506	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	2.69218
VVMO6_02473	Putative protease	2.690666
VVMO6_04041	Putative transporter2C DME family	2.686426
VVMO6_00872	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)	2.685139
VVMO6_04236	Acetyltransferase	2.683803
VVMO6_04035	Organic hydroperoxide resistance transcriptional regulator	2.683493
VVMO6_00274	Protein slyX	2.671716
VVMO6_01302	hypothetical protein	2.668677
VVMO6_02004	Intracellular septation protein IspA	2.660916
VVMO6_00257	Para-aminobenzoate synthase2C amidotransferase component (EC 2.6.1.85)	2.653368
VVMO6_03071	Succinate-semialdehyde dehydrogenase [NADP+] (EC 1.2.1.16)	2.646192
VVMO6_02350	Flagellar motor rotation protein MotA	2.639746
VVMO6_03361	hypothetical protein	2.632257
VVMO6_03063	hypothetical protein	2.630402
VVMO6_00145	tRNA (Uracil54-C5-)-methyltransferase (EC 2.1.1.35)	2.581302
VVMO6_01479	Molybdopterin-guanine dinucleotide biosynthesis protein MobA	2.560431
VVMO6_02430	Ribosomal RNA large subunit methyltransferase N (EC 2.1.1.-)	2.549341
VVMO6_02301	tRNA-i(6)A37 methylthiotransferase	2.519653
VVMO6_00861	hypothetical protein	2.512767
VVMO6_03922	Type III effector HopPmaJ	2.51111
VVMO6_02353	1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7)	2.50597
VVMO6_03995	dCMP deaminase (EC 3.5.4.12)	2.503465
VVMO6_02003	Mu-like prophage protein gp16	2.502707

VVMO6_03393	membrane protein	2.498716
VVMO6_00186	3-dehydroquinate dehydratase II (EC 4.2.1.10)	2.490579
VVMO6_04169	Predicted L-lactate dehydrogenase2C hypothetical protein subunit YkgG	2.474431
VVMO6_03872	Anthranilate phosphoribosyltransferase like (EC 2.4.2.18)	2.45724
VVMO6_04441	hypothetical protein	2.428092
VVMO6_03294	Small-conductance mechanosensitive channel	2.426676
VVMO6_01823	Phage shock protein B	2.416465
VVMO6_00832	Flagellar biosynthesis protein FlhA	2.415514
VVMO6_03085	hypothetical protein	2.413407
VVMO6_00568	(GlcNAc)2 ABC transporter2C permease component 2	2.408065
VVMO6_00272	Isoaspartyl aminopeptidase (EC 3.4.19.5) @ Asp-X dipeptidase	2.405748
VVMO6_02626	SSU ribosomal protein S21p	2.404709
VVMO6_04512	Transcriptional regulator2C LuxR family	2.399412
VVMO6_00833	Flagellar biosynthesis protein FlhF	2.396282
VVMO6_00182	tRNA dihydrouridine synthase B (EC 1.-.-.-)	2.395292
VVMO6_04457	Uxu operon transcriptional regulator	2.392806
VVMO6_01837	membrane protein	2.383683
VVMO6_03724	ATP-dependent RNA helicase VCA0768	2.382842
VVMO6_01461	hypothetical protein	2.379367
VVMO6_02277	hypothetical protein	2.372794
VVMO6_01988	hypothetical protein	2.363913
VVMO6_04045	Multidrug resistance protein D	2.362378
VVMO6_02698	Thiamin ABC transporter2C substrate-binding component	2.360552
VVMO6_02449	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)	2.358618
VVMO6_01882	hypothetical protein	2.355522
VVMO6_00671	hypothetical protein	2.34935
VVMO6_00528	Quinolinate phosphoribosyltransferase [decarboxylating] (EC 2.4.2.19)	2.342207
VVMO6_03300	Protease-related protein	2.340141
VVMO6_03473	Putative heat shock protein YegD	2.335258
VVMO6_01683	Membrane-associated phospholipid phosphatase	2.33341
VVMO6_02316	Proposed lipoate regulatory protein YbeD	2.328023
VVMO6_04478	hypothetical protein	2.314648
VVMO6_01812	Methyltransferase (EC 2.1.1.-)	2.313243
VVMO6_03921	hypothetical protein	2.310683
VVMO6_00819	Flagellar motor switch protein FliG	2.307693
VVMO6_02291	FIG002082: Protein sirB2	2.301057
VVMO6_01778	hypothetical protein	2.298661
VVMO6_01130	Queuosine biosynthesis QueD2C PTPS-I	2.294296
VVMO6_03751	Predicted membrane protein	2.285155
VVMO6_02280	Lead2C cadmium2C zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)	2.279673

VVMO6_04151	Putative response regulator	2.279034
VVMO6_02618	General secretion pathway protein A / General secretion pathway protein B	2.277957
VVMO6_02039	Glutaredoxin-related protein	2.273946
VVMO6_00207	6-phosphofructokinase (EC 2.7.1.11)	2.269682
VVMO6_03426	Lipoate-protein ligase A	2.26897
VVMO6_01242	hypothetical protein	2.268661
VVMO6_02854	Putative ATP-dependent Lon protease	2.262762
VVMO6_02632	TRAP transporter solute receptor2C unknown substrate 6	2.261311
VVMO6_02627	Transamidase GatB domain protein	2.254331
VVMO6_03217	Periplasmic thiol:disulfide interchange protein DsbA	2.232628
VVMO6_02357	62C7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.9)	2.228086
VVMO6_04129	hypothetical protein	2.221839
VVMO6_02975	LSU ribosomal protein L34p	2.216028
VVMO6_00477	Pyridoxine 5'-phosphate synthase (EC 2.6.99.2)	2.212618
VVMO6_02506	SSU ribosomal protein S20p	2.209725
VVMO6_04506	hypothetical protein	2.201203
VVMO6_03017	membrane protein	2.19861
VVMO6_04066	Lactoylglutathione lyase	2.193597
VVMO6_02258	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)	2.188725
VVMO6_00807	Flagellin protein FlaF	2.18617
VVMO6_01087	hypothetical protein	2.183318
VVMO6_02356	Transcription termination protein NusB	2.181118
VVMO6_00408	FIG023103: Predicted transmembrane protein	2.179808
VVMO6_04156	Putative magnesium transporter MgtE	2.178696
VVMO6_03988	Protein F-related protein	2.169603
VVMO6_01605	COG19562C GAF domain-containing protein	2.16485
VVMO6_01303	hypothetical protein	2.161183
VVMO6_00398	Beta-galactosidase/beta-glucuronidase	2.159833
VVMO6_00875	Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)	2.154917
VVMO6_03619	Methylglyoxal synthase (EC 4.2.3.3)	2.147423
VVMO6_00685	Predicted hydrolase	2.147137
VVMO6_01946	LSU ribosomal protein L32p	2.143757
VVMO6_02375	Transglycosylase2C Slt family	2.142035
VVMO6_04034	2-keto-4-pentenoate hydratase (EC 4.2.1.-)	2.139464
VVMO6_04225	hypothetical protein	2.118452
VVMO6_01922	Possible protease sohB (EC 3.4.21.-)	2.115382
VVMO6_02978	Inner membrane protein translocase component YidC2C long form	2.114787
VVMO6_02755	LSU ribosomal protein L3p (L3e)	2.114154
VVMO6_01438	hypothetical protein	2.108019
VVMO6_02560	Putative inner membrane protein	2.106377
VVMO6_00352	Na+/H+ antiporter2C putative	2.104895
VVMO6_02756	SSU ribosomal protein S10p (S20e)	2.090071

VVMO6_00880	Cyn operon transcriptional activator	2.084925
VVMO6_00221	Lysyl-tRNA synthetase-related protein	2.069283
VVMO6_00723	hypothetical protein	2.064439
VVMO6_00727	COG1720: Uncharacterized conserved protein	2.063741
VVMO6_02224	Adenylate kinase (EC 2.7.4.3)	2.063141
VVMO6_03370	hypothetical protein	2.062238
VVMO6_02651	3-isopropylmalate dehydrogenase (EC 1.1.1.85)	2.059759
VVMO6_00938	Uridine phosphorylase (EC 2.4.2.3)	2.054973
VVMO6_04325	Phospholipid-binding protein	2.047504
VVMO6_02362	Gamma-glutamyl phosphate reductase (EC 1.2.1.41)	2.040831
VVMO6_02044	Putative membrane protein	2.036886
VVMO6_01531	ATP-dependent protease La (EC 3.4.21.53) Type II	2.030059
VVMO6_03620	Periplasmic protein torT precursor	2.027937
VVMO6_00236	Iron-sulfur cluster-binding protein	2.025756
VVMO6_04254	Transcriptional regulator2C LysR family	2.02402
VVMO6_00952	Lipoprotein releasing system transmembrane protein LolC	2.021961
VVMO6_00817	Flagellar hook-basal body complex protein FliE	2.008401
VVMO6_03335	MoxR-like ATPase in aerotolerance operon	2.005707
VVMO6_02448	tRNA-guanine transglycosylase (EC 2.4.2.29)	2.004859
VVMO6_01786	AttF component of AttEFGH ABC transport system / AttG component of AttEFGH ABC transport system	2.004187
VVMO6_01779	TrkA2C Potassium channel-family protein	2.003127

Down-regulated genes (226 genes)

VVMO6_01047	hypothetical protein	-19.0671
VVMO6_02186	Ferrous iron transport protein A	-13.1474
VVMO6_01116	High-affinity choline uptake protein BetT	-8.79657
VVMO6_03003	hypothetical protein	-8.43352
VVMO6_01995	Trp operon leader peptide	-8.12411
VVMO6_01115	Transcriptional regulator2C MarR family	-7.84889
VVMO6_03519	Histone acetyltransferase HPA2	-7.42895
VVMO6_02637	Transcriptional regulator of glmS gene2C DeoR family	-7.20098
VVMO6_02185	Ferrous iron transport protein B	-7.08475
VVMO6_04086	hypothetical protein	-6.95878
VVMO6_03697	PTS system2C fructose-specific IIB component (EC 2.7.1.69) / PTS system2C fructose-specific IIC component (EC 2.7.1.69)	-6.51571
VVMO6_01119	Predicted signal-transduction protein containing cAMP-binding and CBS domains	-6.03308
VVMO6_03197	Transcriptional regulator2C GntR family	-5.79025
VVMO6_03325	Phosphate ABC transporter2C periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)	-5.51423
VVMO6_02190	Zinc ABC transporter2C ATP-binding protein ZnuC	-5.24746

VVMO6_00683	Transcriptional regulator2C LysR family	-5.21546
VVMO6_01118	DNA polymerase III epsilon subunit (EC 2.7.7.7)	-5.17843
VVMO6_01835	Alpha-12C6-galactosidase2C putative	-5.06938
VVMO6_01893	Alanine dehydrogenase (EC 1.4.1.1)	-5.01097
VVMO6_00140	SOS-response repressor and protease LexA (EC 3.4.21.88)	-4.95027
VVMO6_03134	Galactose/methyl galactoside ABC transport system2C ATP-binding protein MglA (EC 3.6.3.17)	-4.58094
VVMO6_01662	Siroheme synthase / Precorrin-2 oxidase (EC 1.3.1.76) / Sirohydrochlorin ferrochelatase (EC 4.99.1.4)	-4.50493
VVMO6_01825	Psp operon transcriptional activator	-4.25621
VVMO6_01996	Anthranilate synthase2C aminase component (EC 4.1.3.27)	-4.22698
VVMO6_01204	hypothetical protein	-4.16883
VVMO6_02048	Na+/H+ antiporter NhaC	-4.14926
VVMO6_01263	hypothetical protein	-4.13349
VVMO6_00858	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	-4.12648
VVMO6_04312	Carbon-nitrogen hydrolase	-4.09017
VVMO6_03825	hypothetical protein	-4.03215
VVMO6_00033	COG1272: Predicted membrane protein hemolysin III homolog	-4.02746
VVMO6_02184	Ferrous iron transport protein C	-3.96916
VVMO6_02056	hypothetical protein	-3.91864
VVMO6_04207	Isochorismate pyruvate-lyase of siderophore biosynthesis	-3.7662
VVMO6_03029	hypothetical protein	-3.76129
VVMO6_00092	Ferritin-like protein 2	-3.75048
VVMO6_01167	Tyrosine-specific transport protein	-3.6397
VVMO6_02074	Protein ydjA	-3.61997
VVMO6_03012	Transcriptional regulator2C LysR family	-3.56414
VVMO6_00738	Acetyltransferase	-3.48661
VVMO6_01834	Predicted manganese transporter2C 11 TMS	-3.44828
VVMO6_01125	hypothetical protein	-3.40809
VVMO6_00316	hypothetical protein	-3.37554
VVMO6_02935	LysR-family transcriptional regulator VC0068	-3.37511
VVMO6_02509	Sodium-dependent phosphate transporter	-3.31983
VVMO6_02636	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (EC 2.6.1.16)	-3.31153
VVMO6_03423	Transcriptional regulator2C AraC family	-3.28742
VVMO6_03934	Signal transduction histidine kinase	-3.28477
VVMO6_01758	hypothetical protein	-3.25104
VVMO6_00679	Glycerol uptake facilitator protein	-3.23239
VVMO6_01756	Di-and tricarboxylate transporter	-3.22952
VVMO6_02466	Phosphate transport system permease protein PstC (TC 3.A.1.7.1)	-3.22067
VVMO6_03466	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3)	-3.21232

VVMO6_03826	hypothetical protein	-3.1797
VVMO6_01997	Anthranilate synthase2C amidotransferase component (EC 4.1.3.27)	-3.12608
VVMO6_01189	hypothetical protein	-3.12554
VVMO6_00845	ABC transporter involved in cytochrome c biogenesis2C CcmB subunit	-3.06531
VVMO6_01097	hypothetical protein	-2.99688
VVMO6_03450	hypothetical protein	-2.91227
VVMO6_00930	Transcriptional activator of cad operon	-2.90643
VVMO6_03701	Fructose repressor FruR2C LacI family	-2.87465
VVMO6_01914	Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1)	-2.87211
VVMO6_04206	Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	-2.85515
VVMO6_02970	Chromosomal replication initiator protein DnaA	-2.85335
VVMO6_00479	hypothetical protein	-2.83615
VVMO6_01158	LysR-family transcriptional regulator VCA0830	-2.8252
VVMO6_00640	ABC-type uncharacterized transport system2C permease component	-2.82232
VVMO6_01896	ATPase involved in DNA repair	-2.82176
VVMO6_02640	hypothetical protein	-2.80609
VVMO6_03853	Signal transduction histidine kinase	-2.80018
VVMO6_03615	hypothetical protein	-2.76615
VVMO6_04006	Molybdopterin biosynthesis protein MoeA	-2.75773
VVMO6_00602	hypothetical protein	-2.74914
VVMO6_00881	Response regulator	-2.74774
VVMO6_02024	Excinuclease ABC subunit B	-2.73298
VVMO6_03400	CbbY family protein	-2.72487
VVMO6_03946	Alanine racemase (EC 5.1.1.1)	-2.71047
VVMO6_04121	Transcriptional regulator2C AsnC family	-2.70566
VVMO6_00133	Thiosulfate sulfurtransferase GlpE (EC 2.8.1.1)	-2.70531
VVMO6_00357	Excinuclease ABC subunit A	-2.69325
VVMO6_00034	Potassium uptake protein TrkH	-2.68929
VVMO6_00201	tRNA (cytosine34-2'-O-)-methyltransferase (EC 2.1.1.-)	-2.67345
VVMO6_01755	SanA protein	-2.67188
VVMO6_03885	TldE/PmbA protein2C part of proposed TldE/TldD proteolytic complex (PMID 12029038)	-2.64886
VVMO6_03884	TldD protein2C part of proposed TldE/TldD proteolytic complex (PMID 12029038)	-2.64607
VVMO6_02519	hypothetical protein	-2.64158
VVMO6_04007	Molybdopterin biosynthesis protein MoeB	-2.62853
VVMO6_00646	hypothetical protein	-2.62674
VVMO6_03804	Transcriptional regulator2C MarR family	-2.62051
VVMO6_03628	Cytochrome c553	-2.61949
VVMO6_02727	FKBP-type peptidyl-prolyl cis-trans isomerase fklB (EC 5.2.1.8)	-2.61936
VVMO6_02623	Acyl-phosphate:glycerol-3-phosphate O-	-2.61067

	acyltransferase PlsY	
VVMO6_00188	DNA polymerase III epsilon subunit (EC 2.7.7.7)	-2.58065
VVMO6_00093	Universal stress protein B	-2.57832
VVMO6_00127	Methyltransferase (EC 2.1.1.-)	-2.57596
VVMO6_02366	Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22)	-2.57081
VVMO6_02722	2'2C3'-cyclic-nucleotide 2'-phosphodiesterase (EC 3.1.4.16)	-2.56658
VVMO6_00234	Ribosome small subunit-stimulated GTPase EngC	-2.5611
VVMO6_01089	GGDEF family protein	-2.55889
VVMO6_01262	Outer membrane protein	-2.5507
VVMO6_04340	hypothetical protein	-2.5238
VVMO6_02670	Arginine pathway regulatory protein ArgR2C repressor of arg regulon	-2.51964
VVMO6_02013	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)	-2.51472
VVMO6_00645	Von Willebrand factor type A domain protein2C associated with Flp pilus assembly	-2.49185
VVMO6_00996	5-Enolpyruvylshikimate-3-phosphate synthase (EC 2.5.1.19)	-2.48221
VVMO6_00607	Transcriptional regulator2C MarR family	-2.46166
VVMO6_00310	Phosphoenolpyruvate carboxylase (EC 4.1.1.31)	-2.45901
VVMO6_01515	Fumarate and nitrate reduction regulatory protein	-2.45879
VVMO6_01107	Transcriptional regulator for fatty acid degradation FadR2C GntR family	-2.4535
VVMO6_01828	Peptide transport system permease protein sapC (TC 3.A.1.5.5)	-2.45073
VVMO6_03527	Response regulator	-2.44377
VVMO6_00142	Soluble pyridine nucleotide transhydrogenase (EC 1.6.1.1)	-2.4344
VVMO6_03631	outer membrane protein2C MtrB	-2.42664
VVMO6_00766	hypothetical protein	-2.42587
VVMO6_02188	hypothetical protein	-2.42486
VVMO6_01913	ABC-type dipeptide transport system2C periplasmic component	-2.41945
VVMO6_00134	GlpG protein (membrane protein of glp regulon)	-2.41193
VVMO6_00947	NADH dehydrogenase (EC 1.6.99.3)	-2.40896
VVMO6_03471	membrane protein	-2.39264
VVMO6_00345	Sulfite reductase [NADPH] flavoprotein alpha-component (EC 1.8.1.2)	-2.37836
VVMO6_00202	Manganese superoxide dismutase (EC 1.15.1.1)	-2.37115
VVMO6_00889	hypothetical protein	-2.36736
VVMO6_00647	Similar to TadZ/CpaE2C associated with Flp pilus assembly	-2.36324
VVMO6_02926	ATP-dependent DNA helicase UvrD/PcrA	-2.36121
VVMO6_02684	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase (EC 3.1.3.45)	-2.34717
VVMO6_02365	hypothetical protein	-2.34629
VVMO6_00085	Transcriptional regulator AsnC	-2.34627
VVMO6_00141	O-methyltransferase-related protein	-2.33831

VVMO6_00107	Sensory box/GGDEF family protein	-2.33646
VVMO6_01168	Lactoylglutathione lyase	-2.33289
VVMO6_02014	Oligopeptide ABC transporter2C periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)	-2.32657
VVMO6_00346	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)	-2.32582
VVMO6_03421	Transcriptional regulator2C LysR family	-2.32163
VVMO6_01815	Putative sodium-dependent transporter	-2.30284
VVMO6_02328	Trehalose operon transcriptional repressor	-2.30184
VVMO6_00340	Zinc uptake regulation protein ZUR	-2.29364
VVMO6_04162	ABC transporter2C ATP-binding protein	-2.29298
VVMO6_03963	Methylglutaconyl-CoA hydratase (EC 4.2.1.18)	-2.29238
VVMO6_02807	UDP-N-acetylglucosamine 42C6-dehydratase (EC 4.2.1.-)	-2.28545
VVMO6_01483	Sigma-54 dependent response regulator	-2.27971
VVMO6_03913	Uncharacterized protein ImpJ/VasE	-2.27958
VVMO6_03202	Endonuclease I	-2.27816
VVMO6_01661	Ferredoxin-type protein NapG (periplasmic nitrate reductase)	-2.2706
VVMO6_00750	Undecaprenyl pyrophosphate synthetase (EC 2.5.1.31)	-2.27021
VVMO6_03245	putative histidinol phosphatase and related hydrolases of the PHP family	-2.26992
VVMO6_02380	Aminobenzoyl-glutamate transport protein	-2.26906
VVMO6_02831	hypothetical protein	-2.26852
VVMO6_02875	Phosphoenolpyruvate carboxykinase [ATP] (EC 4.1.1.49)	-2.26367
VVMO6_00771	Ribonuclease HI (EC 3.1.26.4)	-2.25973
VVMO6_00480	hypothetical protein	-2.25776
VVMO6_04339	hypothetical protein	-2.25368
VVMO6_03640	ABC-type phosphate transport system2C periplasmic component	-2.25198
VVMO6_03145	Transcriptional regulator2C AraC family	-2.24661
VVMO6_02089	hypothetical protein	-2.24071
VVMO6_02895	DNA polymerase I (EC 2.7.7.7)	-2.23701
VVMO6_00998	hypothetical protein	-2.23352
VVMO6_00765	Nitrogen regulatory protein P-II	-2.22842
VVMO6_02213	Ferric uptake regulation protein FUR	-2.22533
VVMO6_00211	Uncharacterized low-complexity protein	-2.22345
VVMO6_03470	PQQ-dependent oxidoreductase2C gdhB family	-2.21788
VVMO6_00632	nonspecific acid phosphatase precursor	-2.21452
VVMO6_03718	hypothetical protein	-2.20786
VVMO6_00751	Phosphatidate cytidylyltransferase (EC 2.7.7.41)	-2.20774
VVMO6_00990	ATP-dependent Clp protease adaptor protein ClpS	-2.20725
VVMO6_03962	Methylcrotonyl-CoA carboxylase carboxyl transferase subunit (EC 6.4.1.4)	-2.19851
VVMO6_04341	hypothetical protein	-2.19806
VVMO6_00263	Phosphoribulokinase (EC 2.7.1.19) homolog2C	-2.19606

	function unknown	
VVMO6_02823	Phosphopantothenoylcysteine decarboxylase (EC 4.1.1.36) / Phosphopantothenoylcysteine synthetase (EC 6.3.2.5)	-2.19563
VVMO6_03685	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P protein) (EC 1.4.4.2)	-2.19327
VVMO6_03627	Putative cytochrome c-type protein	-2.19188
VVMO6_01484	Signal transduction histidine kinase	-2.18313
VVMO6_01934	PTS system2C glucose-specific IIB component (EC 2.7.1.69) / PTS system2C glucose-specific IIC component (EC 2.7.1.69)	-2.18104
VVMO6_01270	Urocanate hydratase (EC 4.2.1.49)	-2.18075
VVMO6_02137	DNA-binding response regulator GltR	-2.17845
VVMO6_00911	Type I restriction-modification system2C DNA-methyltransferase subunit M (EC 2.1.1.72)	-2.1773
VVMO6_04338	probable exported protein YPO3233	-2.17462
VVMO6_02478	COG1496: Uncharacterized conserved protein	-2.17239
VVMO6_04516	ABC-type protease exporter2C membrane fusion protein (MFP) family component PrtE/AprE	-2.17076
VVMO6_03943	Electron transfer flavoprotein-ubiquinone oxidoreductase (EC 1.5.5.1)	-2.15988
VVMO6_01094	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14)	-2.14668
VVMO6_02508	Transcriptional activator NhaR	-2.1386
VVMO6_03730	Transcriptional regulator2C LysR family	-2.13411
VVMO6_04187	Arginine ABC transporter2C permease protein ArtM	-2.12785
VVMO6_03638	GGDEF family protein	-2.12221
VVMO6_02643	Acetolactate synthase large subunit (EC 2.2.1.6)	-2.11453
VVMO6_00785	Uracil phosphoribosyltransferase (EC 2.4.2.9)	-2.11395
VVMO6_01827	Peptide transport system permease protein sapB (TC 3.A.1.5.5)	-2.10403
VVMO6_01763	Transport ATP-binding protein CydC	-2.10363
VVMO6_02683	Arabinose 5-phosphate isomerase (EC 5.3.1.13)	-2.10112
VVMO6_02105	membrane protein	-2.09939
VVMO6_04190	FOG: EAL domain protein	-2.09828
VVMO6_04008	hypothetical protein	-2.09698
VVMO6_01762	Transport ATP-binding protein CydD	-2.08453
VVMO6_02269	Flagellar basal-body P-ring formation protein FlgA	-2.0818
VVMO6_00260	Succinylglutamic semialdehyde dehydrogenase (EC 1.2.1.71)	-2.07706
VVMO6_03501	Aldehyde dehydrogenase (EC 1.2.1.3); Probable coniferyl aldehyde dehydrogenase (EC 1.2.1.68)	-2.07266
VVMO6_01245	Transcriptional regulator2C TetR family	-2.07242
VVMO6_03356	Maltose/maltodextrin ABC transporter2C permease protein MalG	-2.0716
VVMO6_00631	Small-conductance mechanosensitive channel	-2.07111
VVMO6_03795	Signal transduction histidine kinase	-2.0703
VVMO6_03667	Multidrug resistance protein A	-2.06828

VVMO6_01249	GGDEF family protein	-2.06579
VVMO6_03137	Beta-galactosidase (EC 3.2.1.23)	-2.06346
VVMO6_00359	Transcriptional regulator2C LuxR family	-2.06153
VVMO6_00918	hypothetical protein	-2.05883
VVMO6_02695	FIG138315: Putative alpha helix protein	-2.04982
VVMO6_00514	Hemolysins and related proteins containing CBS domains	-2.04708
VVMO6_02205	Endoribonuclease L-PSP	-2.04634
VVMO6_02969	DNA polymerase III beta subunit (EC 2.7.7.7)	-2.04244
VVMO6_02128	Transglutaminase-like enzyme	-2.04234
VVMO6_00649	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF2C TadA subfamily	-2.04124
VVMO6_00651	Type II/IV secretion system protein TadC2C associated with Flp pilus assembly	-2.04103
VVMO6_02806	Bacillosamine/Legionaminic acid biosynthesis aminotransferase PglE; 4-keto-6-deoxy-N-Acetyl-D-hexosaminyl-(Lipid carrier) aminotransferase	-2.04043
VVMO6_00769	Hydroxyacylglutathione hydrolase (EC 3.1.2.6)	-2.03715
VVMO6_02642	Acetolactate synthase small subunit (EC 2.2.1.6)	-2.0366
VVMO6_00279	tRNA 5-methylaminomethyl-2-thiouridine synthase TusC	-2.03396
VVMO6_01542	hypothetical protein	-2.033
VVMO6_01030	Cytochrome d ubiquinol oxidase subunit II (EC 1.10.3.-)	-2.03165
VVMO6_03932	Sigma cross-reacting protein 27A	-2.02728
VVMO6_04161	hypothetical protein	-2.02645
VVMO6_00922	HipB protein	-2.02448
VVMO6_00015	Putative amino acid ABC transporter2C permease protein	-2.02395
VVMO6_00543	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3)	-2.02352
VVMO6_03026	hypothetical protein	-2.02062
VVMO6_03691	Secreted trypsin-like serine protease	-2.01672
VVMO6_02458	hypothetical protein	-2.00515

Table 3-5. Genes differentially expressed in biofilm cells from the mature stage compared to those from the initial stage of biofilm development (B2/B1).

Locus tag[a]	Gene product	Fold change
Up-regulated genes (395 genes)		
VVMO6_04209	Aryl carrier domain	178.5529
VVMO6_01834	Predicted manganese transporter2C 11 TMS	138.293
VVMO6_00202	Manganese superoxide dismutase (EC 1.15.1.1)	129.4809
VVMO6_03381	hypothetical protein	118.2936
VVMO6_04207	Isochorismate pyruvate-lyase of siderophore biosynthesis	95.94346
VVMO6_04205	Vulnibactin utilization protein VuuB	95.54847
VVMO6_04206	Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	95.12045
VVMO6_03379	Ferric siderophore transport system2C biopolymer transport protein ExbB	94.40746
VVMO6_04210	Catechol siderophore ABC transporter2C substrate-binding protein	87.59587
VVMO6_03380	Ferric siderophore transport system2C periplasmic binding protein TonB	79.89452
VVMO6_03378	Biopolymer transport protein ExbD1	68.93925
VVMO6_04161	hypothetical protein	63.82343
VVMO6_04162	ABC transporter2C ATP-binding protein	60.24116
VVMO6_03382	Radical SAM family protein HutW2C similar to coproporphyrinogen III oxidase2C oxygen-independent2C associated with heme uptake	59.68158
VVMO6_03280	hypothetical protein	59.09393
VVMO6_04202	Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	57.61611
VVMO6_04201	22C3-dihydro-22C3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28)	51.34356
VVMO6_03377	Periplasmic hemin-binding protein	48.96587
VVMO6_04203	22C3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58)	46.5518
VVMO6_04198	Non-ribosomal peptide synthetase modules2C siderophore biosynthesis	44.32741
VVMO6_03383	Putative heme iron utilization protein	36.05261
VVMO6_03375	ABC-type hemin transport system2C ATPase component	35.31463
VVMO6_04200	2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I alpha (EC 2.5.1.54)	34.38988
VVMO6_04197	Phosphopantetheinyl transferase component of siderophore synthetase (EC 2.7.8.-)	31.4367
VVMO6_03613	hypothetical protein	29.58768
VVMO6_03384	Pyridoxamine 5'-phosphate oxidase-related putative heme iron utilization protein	23.95413
VVMO6_04367	Vibriolysin2C extracellular zinc protease (EC 3.4.24.25) @ Pseudolysin2C extracellular zinc protease (EC 3.4.24.26)	23.72009
VVMO6_00937	Lead2C cadmium2C zinc and mercury transporting	23.3074

	ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)	
VVMO6_04113	hypothetical protein	22.61556
VVMO6_03348	Capsular polysaccharide synthesis enzyme CpsD2C exopolysaccharide synthesis	21.65766
VVMO6_04112	Polyhydroxyalkanoic acid synthase	21.06585
VVMO6_03606	hypothetical protein	19.52398
VVMO6_03768	TonB-dependent heme and hemoglobin receptor HutA ; TonB-dependent hemin 2C ferrichrome receptor	18.54939
VVMO6_00201	tRNA (cytosine34-2'-O-)-methyltransferase (EC 2.1.1.-)	18.17819
VVMO6_04211	Ferric vulnibactin receptor VuuA	17.14122
VVMO6_03144	Ferrichrome-iron receptor	16.35504
VVMO6_03607	Ferric vibriobactin2C enterobactin transport system2C permease protein VctD (TC 3.A.1.14.6)	15.36533
VVMO6_00896	hypothetical protein	15.08277
VVMO6_04403	Hypothetical protein in aerobactin uptake cluster	14.19951
VVMO6_04242	Glutathione S-transferase (EC 2.5.1.18)	14.17552
VVMO6_04114	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	13.02642
VVMO6_02284	hypothetical protein	12.98061
VVMO6_01907	Iron-regulated protein A precursor	12.50636
VVMO6_02937	Thiazole biosynthesis protein ThiH	12.34558
VVMO6_03663	Oxalate/formate antiporter	11.77121
VVMO6_00902	Uncharacterized iron-regulated protein	11.58639
VVMO6_01887	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase (EC 2.6.1.62)	11.29301
VVMO6_02938	Thiazole biosynthesis protein ThiG	10.80422
VVMO6_03841	TonB system biopolymer transport component; Chromosome segregation ATPase	10.62614
VVMO6_03840	MotA/TolQ/ExbB proton channel family protein	10.47702
VVMO6_04115	Acetoacetyl-CoA reductase (EC 1.1.1.36)	10.21942
VVMO6_03839	Ferric siderophore transport system2C biopolymer transport protein ExbB	9.843962
VVMO6_03218	hypothetical protein	9.822333
VVMO6_00189	Predicted signal-transduction protein	9.749871
VVMO6_01906	Probable thiol oxidoreductase with 2 cytochrome c heme-binding sites	9.453637
VVMO6_03838	Biopolymer transport protein ExbD/TolR	9.401937
VVMO6_04422	hypothetical protein	9.134589
VVMO6_00141	O-methyltransferase-related protein	9.110394
VVMO6_02224	Adenylate kinase (EC 2.7.4.3)	9.079357
VVMO6_02497	Carbon starvation protein A	9.034735
VVMO6_03837	Ferric siderophore transport system2C periplasmic binding protein TonB	8.943285
VVMO6_03320	Hypothetical protein in cluster with HutR2C VCA0066 homolog	8.937768
VVMO6_02034	hypothetical protein	8.851765
VVMO6_03608	Ferric vibriobactin2C enterobactin transport system2C	8.807967

	permease protein VctG (TC 3.A.1.14.6)	
VVMO6_03279	Uncharacterized conserved protein	8.646415
VVMO6_02940	Sulfur carrier protein adenylyltransferase ThiF	8.564266
VVMO6_03347	Glycosyltransferase	8.414876
VVMO6_04406	Ferric aerobactin ABC transporter2C permease component	8.40742
VVMO6_01886	Biotin synthase (EC 2.8.1.6)	8.187888
VVMO6_04068	ATP-dependent RNA helicase RhlE	7.974994
VVMO6_03836	TPR domain protein2C putative component of TonB system	7.888543
VVMO6_03190	RNA methyltransferase2C TrmA family	7.728189
VVMO6_00897	Lactoylglutathione lyase (EC 4.4.1.5)	7.710512
VVMO6_03633	ATP-dependent RNA helicase DbpA	7.238693
VVMO6_03340	STRUCTURAL ELEMENTS; Cell Exterior; surface polysaccharides/antigens	7.216679
VVMO6_01885	8-amino-7-oxononanoate synthase (EC 2.3.1.47)	7.196224
VVMO6_04214	Ribosomal protein S6 glutaminyl transferase	7.13594
VVMO6_01982	Diaminobutyrate-pyruvate transaminase & L-22C4-diaminobutyrate decarboxylase	7.111128
VVMO6_00178	IMP cyclohydrolase (EC 3.5.4.10) / Phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3)	7.035733
VVMO6_04421	Transcriptional regulator2C AraC family	7.027274
VVMO6_01208	hypothetical protein	7.007758
VVMO6_01679	Queuosine Biosynthesis QueE Radical SAM	6.836612
VVMO6_02697	Thiamin ABC transporter2C transmembrane component	6.786882
VVMO6_03829	Permease of the major facilitator superfamily	6.652643
VVMO6_00285	Bacterioferritin-associated ferredoxin	6.538926
VVMO6_00531	Transcriptional repressor for pyruvate dehydrogenase complex	6.511037
VVMO6_00532	Pyruvate dehydrogenase E1 component (EC 1.2.4.1)	6.320744
VVMO6_03056	Glycogen phosphorylase (EC 2.4.1.1)	6.217521
VVMO6_02442	Inositol-1-monophosphatase (EC 3.1.3.25)	6.125731
VVMO6_04215	hypothetical protein sometimes fused to ribosomal protein S6 glutaminyl transferase	6.122349
VVMO6_03117	hypothetical protein	6.116009
VVMO6_03864	hypothetical protein	5.982357
VVMO6_01216	hypothetical protein	5.85846
VVMO6_02374	Phosphoribosylformylglycinamidine synthase2C synthetase subunit (EC 6.3.5.3) / Phosphoribosylformylglycinamidine synthase2C glutamine amidotransferase subunit (EC 6.3.5.3)	5.550383
VVMO6_01905	Iron-regulated protein A precursor	5.516492
VVMO6_01487	ABC transporter ATP-binding protein uup	5.423302
VVMO6_00533	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex (EC 2.3.1.12)	5.36003
VVMO6_03052	hypothetical protein	5.350971
VVMO6_02453	Malate synthase (EC 2.3.3.9)	5.339938
VVMO6_02498	Autolysin sensor kinase (EC 2.7.3.-)	5.322104

VVMO6_02616	Probable low-affinity inorganic phosphate transporter	5.264659
VVMO6_01513	tRNA(Cytosine32)-2-thiocytidine synthetase	5.249572
VVMO6_03321	Hypothetical protein in cluster with HutR2C VCA0067 homolog	5.24143
VVMO6_00302	LSU ribosomal protein L31p	5.008364
VVMO6_03830	Transcriptional regulator2C AraC family	4.98644
VVMO6_04404	Ferric aerobactin ABC transporter2C ATPase component	4.897994
VVMO6_00187	Acetyl-coenzyme A synthetase (EC 6.2.1.1)	4.866031
VVMO6_03831	Na+-driven multidrug efflux pump	4.860953
VVMO6_04064	Putative transporter	4.751849
VVMO6_02983	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)	4.685841
VVMO6_01904	putative exported protein	4.633719
VVMO6_03145	Transcriptional regulator2C AraC family	4.585269
VVMO6_02849	Xanthine permease	4.551891
VVMO6_03609	Ferric vibriobactin2C enterobactin transport system2C ATP-binding protein (TC 3.A.1.14.6)	4.534788
VVMO6_00181	DNA-binding protein Fis	4.494479
VVMO6_01980	Carboxynorspermidine decarboxylase2C putative (EC 4.1.1.-)	4.47313
VVMO6_04321	hypothetical protein	4.470392
VVMO6_01883	Dethiobiotin synthetase (EC 6.3.3.3)	4.418373
VVMO6_00215	hypothetical protein	4.395398
VVMO6_01884	Biotin synthesis protein bioC	4.381615
VVMO6_02528	Branched-chain amino acid transport system carrier protein	4.375352
VVMO6_00938	Uridine phosphorylase (EC 2.4.2.3)	4.373575
VVMO6_04065	Transcriptional regulator2C MarR family	4.330906
VVMO6_01731	Glutaredoxin	4.30916
VVMO6_02698	Thiamin ABC transporter2C substrate-binding component	4.284609
VVMO6_01678	Queuosine Biosynthesis QueC ATPase	4.272049
VVMO6_03614	Hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34)	4.270704
VVMO6_02944	Multicopper oxidase	4.228821
VVMO6_03716	Inosine-guanosine kinase (EC 2.7.1.73)	4.201755
VVMO6_00177	Phosphoribosylamine--glycine ligase (EC 6.3.4.13)	4.200993
VVMO6_00044	Phosphoribosylaminoimidazole carboxylase catalytic subunit (EC 4.1.1.21)	4.193773
VVMO6_03711	Iron-containing alcohol dehydrogenase	4.190907
VVMO6_02372	Pressure-regulated ORF-like protein	4.16033
VVMO6_00894	proteinase inhibitor2C putative	4.158779
VVMO6_02882	GTP-binding protein TypA/BipA	4.152191
VVMO6_04405	Ferric aerobactin ABC transporter2C periplasmic substrate binding protein	4.099356
VVMO6_00182	tRNA dihydrouridine synthase B (EC 1.-.-.-)	4.084002
VVMO6_01258	Phosphoribosylglycinamide formyltransferase 2 (EC 2.1.2.-)	4.07195
VVMO6_01981	Carboxynorspermidine dehydrogenase2C putative (EC 1.1.1.-)	4.063475

VVMO6_01237	Biosynthetic arginine decarboxylase (EC 4.1.1.19)	4.047094
VVMO6_00139	Glycerol-3-phosphate acyltransferase (EC 2.3.1.15)	4.030818
VVMO6_04212	Amide synthase component of siderophore synthetase	4.013658
VVMO6_04231	Peptide deformylase (EC 3.5.1.88)	3.937603
VVMO6_02430	Ribosomal RNA large subunit methyltransferase N (EC 2.1.1.-)	3.936135
VVMO6_00783	Phosphoribosylglycinamide formyltransferase (EC 2.1.2.2)	3.93349
VVMO6_01844	Formate efflux transporter (TC 2.A.44 family)	3.920993
VVMO6_03146	Hypothetical protein in aerobactin uptake cluster	3.908617
VVMO6_02379	S-formylglutathione hydrolase (EC 3.1.2.12)	3.897941
VVMO6_01638	Glyoxalase family protein	3.864908
VVMO6_02533	Sodium/alanine symporter	3.859237
VVMO6_02407	YrdC/Sua5 family protein2C required for threonylcarbamoyladenosine (t(6)A) formation in tRNA	3.833576
VVMO6_01209	Pilus assembly protein CpaE-like protein	3.771858
VVMO6_02488	hypothetical protein	3.762588
VVMO6_03057	4-alpha-glucanotransferase (amylomaltase) (EC 2.4.1.25)	3.762455
VVMO6_00695	Cysteine desulfurase CsdA-CsdE2C sulfur acceptor protein CsdE	3.69074
VVMO6_04044	hypothetical protein	3.687392
VVMO6_02452	Isocitrate lyase (EC 4.1.3.1)	3.677935
VVMO6_00094	NAD(FAD)-utilizing dehydrogenases	3.671834
VVMO6_00694	HesA/MoeB/ThiF family protein related to EC-YgdL	3.657471
VVMO6_01750	Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6)	3.644951
VVMO6_02945	Multicopper oxidase	3.64201
VVMO6_01813	Pseudouridylate synthase2C 23S RNA-specific	3.631384
VVMO6_03127	Pseudouridylate synthase2C 23S RNA-specific	3.592653
VVMO6_01812	Methyltransferase (EC 2.1.1.-)	3.591902
VVMO6_03118	Membrane fusion component of tripartite multidrug resistance system	3.578844
VVMO6_01872	Adenylosuccinate lyase (EC 4.3.2.2)	3.540821
VVMO6_00992	Translation initiation factor 1	3.49327
VVMO6_01672	LSU m5C1962 methyltransferase RlmI	3.478391
VVMO6_03142	4Fe-4S ferredoxin2C iron-sulfur binding	3.447116
VVMO6_03832	hypothetical protein	3.446379
VVMO6_01202	hypothetical protein	3.426405
VVMO6_03339	Transcriptional regulator VpsT	3.416964
VVMO6_02418	GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2)	3.400988
VVMO6_00786	Uracil permease	3.377501
VVMO6_01272	Imidazolonepropionase (EC 3.5.2.7)	3.357317
VVMO6_00043	Phosphoribosylaminoimidazole carboxylase ATPase subunit (EC 4.1.1.21)	3.356318
VVMO6_02984	Peptide ABC transporter2C ATP-binding protein	3.345188
VVMO6_03704	FIG015136: hypothetical protein	3.332993
VVMO6_00056	RND multidrug efflux transporter; Acriflavin	3.325151

	resistance protein	
VVMO6_01751	hypothetical protein	3.297745
VVMO6_03664	NADH:ubiquinone oxidoreductase subunit 2	3.293135
VVMO6_04341	hypothetical protein	3.290258
VVMO6_03644	Cold-shock DEAD-box protein A	3.282635
VVMO6_01952	Sulfate permease	3.272507
VVMO6_02627	Transamidase GatB domain protein	3.266519
VVMO6_00286	Bacterioferritin	3.265117
VVMO6_01236	Agmatinase (EC 3.5.3.11)	3.264604
VVMO6_00879	Amidophosphoribosyltransferase (EC 2.4.2.14)	3.254544
VVMO6_02626	SSU ribosomal protein S21p	3.21966
VVMO6_01029	Cytochrome d ubiquinol oxidase subunit I (EC 1.10.3.-)	3.215043
VVMO6_03499	Predicted deacylase	3.201656
VVMO6_02615	Phosphate transport regulator (distant homolog of PhoU)	3.191591
VVMO6_02414	D-amino acid dehydrogenase small subunit (EC 1.4.99.1)	3.178161
VVMO6_01087	hypothetical protein	3.165536
VVMO6_02531	ATP-dependent RNA helicase SrmB	3.15202
VVMO6_02297	Ribose-phosphate pyrophosphokinase (EC 2.7.6.1)	3.150741
VVMO6_02419	Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205)	3.144778
VVMO6_00596	Ribosome-binding factor A	3.136489
VVMO6_02780	predicted protein	3.106422
VVMO6_00784	Phosphoribosylformylglycinamidine cyclo-ligase (EC 6.3.3.1)	3.09744
VVMO6_02286	Ribosomal large subunit pseudouridine synthase F (EC 4.2.1.70)	3.080918
VVMO6_04361	ATP-dependent RNA helicase VC1407	3.061472
VVMO6_02296	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (EC 2.7.1.148)	3.056215
VVMO6_03395	Predicted arginine uptake transporter	3.03726
VVMO6_01833	Manganese-dependent inorganic pyrophosphatase (EC 3.6.1.1)	3.024111
VVMO6_04527	tRNA and rRNA cytosine-C5-methylase	3.016985
VVMO6_01899	hypothetical protein	3.011342
VVMO6_03278	hypothetical protein	3.00714
VVMO6_00057	Probable Co/Zn/Cd efflux system membrane fusion protein	3.000014
VVMO6_02013	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)	2.99597
VVMO6_02011	Oligopeptide transport ATP-binding protein OppD (TC 3.A.1.5.1)	2.968724
VVMO6_02420	Exodeoxyribonuclease VII large subunit (EC 3.1.11.6)	2.953135
VVMO6_03053	hypothetical protein	2.944678
VVMO6_03493	Glutamate decarboxylase	2.923583
VVMO6_02182	Arginyl-tRNA synthetase (EC 6.1.1.19)	2.906415
VVMO6_03833	hypothetical protein	2.903816
VVMO6_00597	tRNA pseudouridine synthase B (EC 4.2.1.70)	2.901006

VVMO6_02473	Putative protease	2.895103
VVMO6_02348	Thiamine biosynthesis protein thiI	2.894968
VVMO6_02506	SSU ribosomal protein S20p	2.873894
VVMO6_02476	hypothetical protein	2.825943
VVMO6_02317	Octanoate-[acyl-carrier-protein]-protein-N-octanoyltransferase	2.800429
VVMO6_03143	NAD(FAD)-utilizing dehydrogenase2C sll0175 homolog	2.798552
VVMO6_04455	ATP-dependent RNA helicase VCA0990	2.796166
VVMO6_00878	Bacteriocin production protein	2.778548
VVMO6_00424	tRNA (guanine46-N7-)-methyltransferase (EC 2.1.1.33)	2.777291
VVMO6_01786	AttF component of AttEFGH ABC transport system / AttG component of AttEFGH ABC transport system	2.774282
VVMO6_01508	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)	2.771696
VVMO6_02499	FIG001014_Response regulator of the LytR/AlgR family	2.771178
VVMO6_03200	hypothetical protein	2.769365
VVMO6_02010	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)	2.762024
VVMO6_00387	RNA polymerase associated protein RapA (EC 3.6.1.-)	2.756289
VVMO6_02638	Pyruvate kinase (EC 2.7.1.40)	2.756204
VVMO6_00593	COG0779: clustered with transcription termination protein NusA	2.744637
VVMO6_02650	2-isopropylmalate synthase (EC 2.3.3.13)	2.739053
VVMO6_01190	GGDEF family protein	2.718377
VVMO6_03862	Predicted SAM-dependent methyltransferase	2.717172
VVMO6_04357	Transcriptional regulator2C LysR family	2.701165
VVMO6_04432	hypothetical protein	2.696272
VVMO6_02416	hypothetical protein	2.691131
VVMO6_01269	Histidine ammonia-lyase (EC 4.3.1.3)	2.69034
VVMO6_01506	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	2.68336
VVMO6_04091	hypothetical protein	2.682599
VVMO6_02665	COG0536: GTP-binding protein Obg	2.682111
VVMO6_00558	Ferric iron ABC transporter2C iron-binding protein	2.678418
VVMO6_02827	Ribonuclease PH (EC 2.7.7.56)	2.666727
VVMO6_00274	Protein slyX	2.658347
VVMO6_03152	Putative PTS system2C nitrogen regulatory IIA component	2.657364
VVMO6_04075	Regulator of nucleoside diphosphate kinase	2.656687
VVMO6_00157	Translation elongation factor Tu	2.645205
VVMO6_00145	tRNA (Uracil54-C5-)-methyltransferase (EC 2.1.1.35)	2.636985
VVMO6_01645	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)	2.635726
VVMO6_02819	LSU ribosomal protein L33p	2.624186
VVMO6_03876	Acetyltransferase	2.619599
VVMO6_00594	Transcription termination protein NusA	2.618438
VVMO6_01946	LSU ribosomal protein L32p	2.618375

VVMO6_00585	Transcription elongation factor GreA	2.610512
VVMO6_03173	membrane protein	2.607521
VVMO6_00898	hypothetical protein	2.607449
VVMO6_03065	Spindolin-related protein	2.604739
VVMO6_03172	N-Ribosylnicotinamide phosphorylase (EC 2.4.2.1)	2.599242
VVMO6_04045	Multidrug resistance protein D	2.58566
VVMO6_03863	FOG: GGDEF domain	2.581225
VVMO6_02881	Predicted ATPase	2.578465
VVMO6_00607	Transcriptional regulator2C MarR family	2.54746
VVMO6_01507	Spermidine Putrescine ABC transporter permease component potC (TC_3.A.1.11.1)	2.534147
VVMO6_02012	Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1)	2.524185
VVMO6_00190	Probable 3-phenylpropionic acid transporter	2.523046
VVMO6_00183	Ribosomal protein L11 methyltransferase (EC 2.1.1.-)	2.520571
VVMO6_02316	Proposed lipoate regulatory protein YbeD	2.517019
VVMO6_00283	Translation elongation factor G	2.504934
VVMO6_01787	AttE component of AttEFGH ABC transport system	2.504514
VVMO6_03473	Putative heat shock protein YegD	2.498571
VVMO6_03153	PTS system2C fructose-specific IIB component (EC 2.7.1.69)	2.496391
VVMO6_03974	hypothetical protein	2.488867
VVMO6_02005	Acyl-CoA thioesterase YciA2C involved in membrane biogenesis	2.480838
VVMO6_02844	Guanylate kinase (EC 2.7.4.8)	2.477701
VVMO6_03950	RTX toxin transporter	2.474154
VVMO6_00861	hypothetical protein	2.473477
VVMO6_01870	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase (EC 2.1.1.61)	2.467346
VVMO6_01127	hypothetical protein	2.463627
VVMO6_02777	hypothetical protein	2.460978
VVMO6_03119	Permease of the drug/metabolite transporter (DMT) superfamily	2.457387
VVMO6_02037	Histidine permease YuiF	2.437538
VVMO6_02378	S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)	2.435122
VVMO6_03407	hypothetical protein	2.433873
VVMO6_01195	DnaK-related protein	2.415895
VVMO6_01031	hypothetical protein	2.410274
VVMO6_00267	Glutathione-regulated potassium-efflux system ATP-binding protein	2.404131
VVMO6_00257	Para-aminobenzoate synthase2C amidotransferase component (EC 2.6.1.85)	2.401073
VVMO6_00473	Signal peptidase I (EC 3.4.21.89)	2.398345
VVMO6_03645	Acetate kinase (EC 2.7.2.1)	2.390724
VVMO6_00684	Tellurite resistance protein	2.389435
VVMO6_02318	Lipoate synthase	2.381122
VVMO6_00874	tRNA pseudouridine synthase A (EC 4.2.1.70)	2.367821
VVMO6_03051	membrane protein	2.363722

VVMO6_02367	Xanthine/uracil/thiamine/ascorbate permease family protein	2.356906
VVMO6_01644	6-phosphogluconolactonase (EC 3.1.1.31)2C eukaryotic type	2.354872
VVMO6_04376	Hypothetical protein YgaF	2.352685
VVMO6_02891	Putative methyltransferase associated with DUF414	2.35108
VVMO6_02436	Chaperone protein HscB	2.349995
VVMO6_01509	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	2.345399
VVMO6_02936	Xaa-Pro aminopeptidase (EC 3.4.11.9)	2.341641
VVMO6_02560	Putative inner membrane protein	2.340352
VVMO6_00545	glutamyl-Q-tRNA synthetase	2.326738
VVMO6_02976	Ribonuclease P protein component (EC 3.1.26.5)	2.326195
VVMO6_02356	Transcription termination protein NusB	2.325342
VVMO6_03951	RTX toxin transporter	2.316707
VVMO6_03066	hypothetical protein	2.307786
VVMO6_03474	hypothetical protein	2.307783
VVMO6_02625	YgjD/Kae1/Qri7 family2C required for threonylcarbamoyladenosine (t(6)A) formation in tRNA	2.306853
VVMO6_02449	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)	2.306351
VVMO6_01196	hypothetical protein	2.302872
VVMO6_03333	hypothetical protein	2.293464
VVMO6_02355	Thiamine-monophosphate kinase (EC 2.7.4.16)	2.291393
VVMO6_01477	hypothetical protein	2.288103
VVMO6_03258	2-amino-3-ketobutyrate coenzyme A ligase (EC 2.3.1.29)	2.284767
VVMO6_01505	ABC transporter2C periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)	2.273743
VVMO6_02666	LSU ribosomal protein L27p	2.265871
VVMO6_03025	Chitinase (EC 3.2.1.14)	2.257071
VVMO6_00559	Ferric iron ABC transporter2C permease protein	2.255742
VVMO6_02522	Lysyl-tRNA synthetase (class II) (EC 6.1.1.6)	2.255217
VVMO6_02654	Uncharacterized protein DUF547	2.255112
VVMO6_03985	YaeQ protein	2.25414
VVMO6_03369	AraC-type DNA-binding domain-containing protein	2.254079
VVMO6_02639	Transcriptional regulator LuxZ	2.248393
VVMO6_01475	Putative cytoplasmic protein	2.246411
VVMO6_02782	Polysaccharide export lipoprotein Wza	2.236725
VVMO6_02920	Putative preQ0 transporter	2.23059
VVMO6_02285	hypothetical protein	2.227202
VVMO6_04507	Proline/sodium symporter PutP (TC 2.A.21.2.1) @ Propionate/sodium symporter	2.220892
VVMO6_00517	SSU ribosomal protein S16p	2.219294
VVMO6_02014	Oligopeptide ABC transporter2C periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)	2.201945
VVMO6_02221	Di-/tripeptide transporter	2.201747
VVMO6_04071	Acetyltransferase	2.200153

VVMO6_02975	LSU ribosomal protein L34p	2.196283
VVMO6_03353	hypothetical protein	2.189757
VVMO6_02294	Glutamyl-tRNA reductase (EC 1.2.1.70)	2.18366
VVMO6_02890	Protein of unknown function DUF414	2.181583
VVMO6_00014	N-acetylglucosamine-1-phosphate uridyltransferase (EC 2.7.7.23) / Glucosamine-1-phosphate N-acetyltransferase (EC 2.3.1.157)	2.180888
VVMO6_02895	DNA polymerase I (EC 2.7.7.7)	2.180113
VVMO6_00099	Adenylate cyclase (EC 4.6.1.1)	2.17809
VVMO6_02472	DNA recombination-dependent growth factor C	2.176838
VVMO6_00162	LSU ribosomal protein L10p (P0)	2.172526
VVMO6_04448	transcriptional regulator2C LysR family	2.169537
VVMO6_01103	tRNA-dihydrouridine synthase C (EC 1.-.-.-)	2.162811
VVMO6_03715	Formate--tetrahydrofolate ligase (EC 6.3.4.3)	2.160431
VVMO6_03189	NADPH-flavin oxidoreductase	2.154836
VVMO6_03643	Exoribonuclease II (EC 3.1.13.1)	2.151933
VVMO6_02648	hypothetical protein	2.150719
VVMO6_00899	Alkaline phosphatase (EC 3.1.3.1)	2.145049
VVMO6_00246	Adenylosuccinate synthetase (EC 6.3.4.4)	2.140867
VVMO6_01921	Cytidylate kinase (EC 2.7.4.14)	2.140324
VVMO6_02769	Probable dTDP-4-dehydrorhamnose reductase (EC 1.1.1.133)	2.137768
VVMO6_03141	RNA methyltransferase2C TrmH family	2.1371
VVMO6_01273	Histidine utilization repressor	2.13232
VVMO6_03064	Pyrrolidone-carboxylate peptidase (EC 3.4.19.3)	2.131829
VVMO6_02039	Glutaredoxin-related protein	2.129403
VVMO6_00472	Translation elongation factor LepA	2.126481
VVMO6_02916	Transcription termination factor Rho	2.122986
VVMO6_00198	C4-dicarboxylate transporter DcuA	2.122495
VVMO6_00173	hypothetical protein	2.113473
VVMO6_02898	hypothetical protein	2.102026
VVMO6_00398	Beta-galactosidase/beta-glucuronidase	2.098623
VVMO6_00352	Na+/H+ antiporter2C putative	2.098386
VVMO6_02455	Alkyl hydroperoxide reductase subunit C-like protein	2.095216
VVMO6_01130	Queuosine biosynthesis QueD2C PTPS-I	2.088468
VVMO6_02768	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)	2.086178
VVMO6_02549	hypothetical protein	2.073627
VVMO6_03293	Carbonic anhydrase (EC 4.2.1.1)	2.07156
VVMO6_01681	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.071193
VVMO6_02765	Lipid carrier : UDP-N-acetylgalactosaminyltransferase (EC 2.4.1.-)	2.063743
VVMO6_02301	tRNA-i(6)A37 methylthiotransferase	2.06354
VVMO6_02889	Periplasmic/membrane protein associated with DUF414	2.057538
VVMO6_01735	hypothetical protein	2.056382
VVMO6_03364	Dihydroorotase (EC 3.5.2.3)	2.052308
VVMO6_02922	ATP-dependent DNA helicase RecQ	2.049963

VVMO6_02899	Twin-arginine translocation protein TatC	2.047847
VVMO6_03406	hypothetical protein	2.044247
VVMO6_00158	Preprotein translocase subunit SecE (TC 3.A.5.1.1)	2.043966
VVMO6_01785	AttH component of AttEFGH ABC transport system	2.043147
VVMO6_02539	Pyruvate formate-lyase (EC 2.3.1.54)	2.039682
VVMO6_00058	Transcriptional regulator2C TetR family	2.038634
VVMO6_02921	Aminopeptidase N	2.037703
VVMO6_03674	hypothetical protein	2.036682
VVMO6_01030	Cytochrome d ubiquinol oxidase subunit II (EC 1.10.3.-)	2.032323
VVMO6_04358	hypothetical protein	2.02679
VVMO6_01830	Peptide transport system ATP-binding protein sapF (TC 3.A.1.5.5)	2.025987
VVMO6_01680	Putative hydrolase of the HAD superfamily	2.024056
VVMO6_01827	Peptide transport system permease protein sapB (TC 3.A.1.5.5)	2.023397
VVMO6_01791	MFS family multidrug transport protein2C bicyclomycin resistance protein	2.017457
VVMO6_02276	Glutamyl-tRNA synthetase (EC 6.1.1.17)	2.016828
VVMO6_01004	COG0398: uncharacterized membrane protein	2.014733
VVMO6_00869	5-methylaminomethyl-2-thiouridine-forming enzyme mnmC	2.013648
VVMO6_03259	L-threonine 3-dehydrogenase (EC 1.1.1.103)	2.00488

Down-regulated genes (363 genes)

VVMO6_01175	Na(+)-linked D-alanine glycine permease	-40.2423
VVMO6_01837	membrane protein	-35.5537
VVMO6_02986	hypothetical protein	-24.6065
VVMO6_02491	2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I alpha (EC 2.5.1.54)	-17.8349
VVMO6_00969	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-16.184
VVMO6_01664	Amino acid ABC transporter2C permease protein	-10.0975
VVMO6_01663	Glutamate Aspartate periplasmic binding protein precursor GltI (TC 3.A.1.3.4)	-9.2183
VVMO6_03134	Galactose/methyl galactoside ABC transport system2C ATP-binding protein MglA (EC 3.6.3.17)	-8.91131
VVMO6_01255	Integration host factor alpha subunit	-8.73616
VVMO6_01239	hypothetical protein	-8.36021
VVMO6_02490	Chorismate mutase I (EC 5.4.99.5) / Cyclohexadienyl dehydrogenase (EC 1.3.1.12)(EC 1.3.1.43)	-8.34776
VVMO6_03135	Galactose/methyl galactoside ABC transport system2C D-galactose-binding periplasmic protein MglB (TC 3.A.1.2.3)	-8.20891
VVMO6_01995	Trp operon leader peptide	-7.95262
VVMO6_03882	Glycerol-3-phosphate transporter	-7.8721
VVMO6_02482	Ribosome hibernation protein YfiA	-7.71292
VVMO6_01639	Putative lipoprotein	-7.58766
VVMO6_01665	Amino acid ABC transporter2C permease protein	-7.29391
VVMO6_03464	Anaerobic glycerol-3-phosphate dehydrogenase	-6.90434

	subunit A (EC 1.1.5.3)	
VVMO6_03295	hypothetical protein	-6.8324
VVMO6_01309	DNA-damage-inducible protein J	-6.67474
VVMO6_03741	Long-chain fatty acid transport protein	-6.52764
VVMO6_04354	Ferredoxin--NADP(+) reductase (EC 1.18.1.2)	-6.25656
VVMO6_02120	DNA-binding protein HU-beta	-6.22609
VVMO6_01160	hypothetical protein	-6.15412
VVMO6_03465	Anaerobic glycerol-3-phosphate dehydrogenase subunit B (EC 1.1.5.3)	-6.10792
VVMO6_03466	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3)	-6.05224
VVMO6_00690	N-acetylglutamate synthase (EC 2.3.1.1)	-5.99499
VVMO6_03883	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	-5.94712
VVMO6_00312	N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38)	-5.62466
VVMO6_03740	putative lipase	-5.59781
VVMO6_00637	Transcriptional regulator2C AraC family	-5.28365
VVMO6_00679	Glycerol uptake facilitator protein	-5.18304
VVMO6_00291	hypothetical protein	-5.00995
VVMO6_03133	Galactose/methyl galactoside ABC transport system2C permease protein MglC (TC 3.A.1.2.3)	-5.00566
VVMO6_04374	Response regulator	-4.94029
VVMO6_02715	FIG003461: hypothetical protein	-4.81878
VVMO6_03390	Methyl-accepting chemotaxis protein	-4.72795
VVMO6_04559	Anti-anti-sigma regulatory factor	-4.72524
VVMO6_03411	GGDEF family protein	-4.71476
VVMO6_04325	Phospholipid-binding protein	-4.65328
VVMO6_00636	Long-chain-fatty-acid--CoA ligase (EC 6.2.1.3)	-4.59975
VVMO6_04096	Periplasmic nitrate reductase precursor (EC 1.7.99.4)	-4.59017
VVMO6_04160	Putative protease La homolog (EC 3.4.21.-)	-4.56691
VVMO6_04558	Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY	-4.53137
VVMO6_01527	hypothetical protein	-4.4942
VVMO6_03625	Chromosome segregation ATPase	-4.41936
VVMO6_04094	Ferredoxin-type protein NapF (periplasmic nitrate reductase)	-4.27814
VVMO6_00583	Outer membrane protein OmpU	-4.24776
VVMO6_03358	Acyl-CoA hydrolase (EC 3.1.2.20)	-4.16561
VVMO6_03030	GGDEF family protein	-4.16348
VVMO6_03627	Putative cytochrome c-type protein	-4.14741
VVMO6_01928	Mlc2C transcriptional repressor of MalT (the transcriptional activator of maltose regulon) and manXYZ operon	-4.11151
VVMO6_00883	hypothetical protein	-4.10645
VVMO6_01529	hypothetical protein	-4.10263
VVMO6_04237	Aldehyde dehydrogenase (EC 1.2.1.3)	-4.06601
VVMO6_03427	Aspartate aminotransferase (AspB-4) (EC 2.6.1.1)	-4.06563
VVMO6_00462	hypothetical protein	-4.05611

VVMO6_02275	Hypothetical protein2C specific for Vibrio	-4.05369
VVMO6_02338	Decarboxylase family protein	-4.01591
VVMO6_00678	Glycerol kinase (EC 2.7.1.30)	-4.01042
VVMO6_02709	hypothetical protein	-3.9484
VVMO6_01705	Putative response regulator	-3.93633
VVMO6_01358	hypothetical protein	-3.93254
VVMO6_04027	Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	-3.8798
VVMO6_01530	putative dehydrogenase	-3.87496
VVMO6_01264	hypothetical protein	-3.84177
VVMO6_03300	Protease-related protein	-3.7296
VVMO6_03475	Phage shock protein E	-3.72783
VVMO6_04095	Periplasmic nitrate reductase component NapD	-3.69786
VVMO6_04353	Permease of the drug/metabolite transporter (DMT) superfamily	-3.68071
VVMO6_01111	TRAP dicarboxylate transporter2C DctQ subunit2C unknown substrate 6	-3.60518
VVMO6_03216	hypothetical protein	-3.59853
VVMO6_03410	Alkylated DNA repair protein	-3.56803
VVMO6_02535	hypothetical protein	-3.56582
VVMO6_02632	TRAP transporter solute receptor2C unknown substrate 6	-3.56182
VVMO6_03263	DNA-damage-inducible protein J	-3.55971
VVMO6_01836	hypothetical protein	-3.55325
VVMO6_03413	Predicted transcriptional regulator	-3.54793
VVMO6_02324	hypothetical protein	-3.51334
VVMO6_00663	UDP-glucose 4-epimerase (EC 5.1.3.2)	-3.50428
VVMO6_03202	Endonuclease I	-3.47439
VVMO6_03773	hypothetical protein	-3.45133
VVMO6_02198	Succinate dehydrogenase hydrophobic membrane anchor protein	-3.43759
VVMO6_01422	Predicted acetyltransferase	-3.43576
VVMO6_01249	GGDEF family protein	-3.43307
VVMO6_00935	GGDEF family protein	-3.42465
VVMO6_00315	Argininosuccinate lyase (EC 4.3.2.1) / N-acetylglutamate synthase (EC 2.3.1.1)	-3.41413
VVMO6_02907	hypothetical protein	-3.40115
VVMO6_03522	RsbR2C positive regulator of sigma-B	-3.39657
VVMO6_03960	Predicted transcriptional regulator LiuR of leucine degradation pathway2C MerR family	-3.39454
VVMO6_01703	Predicted permease	-3.35914
VVMO6_00073	Chromosome segregation ATPase	-3.34618
VVMO6_01420	hypothetical protein	-3.33489
VVMO6_04322	hypothetical protein	-3.28858
VVMO6_01965	NrfC protein	-3.27671
VVMO6_01407	hypothetical protein	-3.2714
VVMO6_00258	Acetylornithine aminotransferase (EC 2.6.1.11) / N-succinyl-L2CL-diaminopimelate aminotransferase (EC 2.6.1.17) / Succinylornithine transaminase (EC 2.6.1.81)	-3.26662

VVMO6_02915	hypothetical protein	-3.2352
VVMO6_00496	Lipoprotein NlpD	-3.22962
VVMO6_01408	hypothetical protein	-3.22787
VVMO6_03942	Electron transfer flavoprotein2C beta subunit	-3.21265
VVMO6_03626	hypothetical protein	-3.1773
VVMO6_03505	Putative regulator protein	-3.17224
VVMO6_03774	Translation initiation factor SUI1-related protein	-3.16846
VVMO6_04177	Zn-dependent hydrolase	-3.16269
VVMO6_00881	Response regulator	-3.15694
VVMO6_02197	Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1)	-3.15369
VVMO6_02196	Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1)	-3.11447
VVMO6_04098	Cytochrome c-type protein NapC	-3.0986
VVMO6_01438	hypothetical protein	-3.09549
VVMO6_03264	YafQ toxin protein	-3.0896
VVMO6_00666	Aldose 1-epimerase (EC 5.1.3.3)	-3.08091
VVMO6_03521	Aerotaxis sensor receptor protein	-3.07907
VVMO6_01136	Guanylate cyclase-related protein	-3.07398
VVMO6_01319	hypothetical protein	-3.06685
VVMO6_00665	Galactokinase (EC 2.7.1.6)	-3.05677
VVMO6_01448	hypothetical protein	-3.04862
VVMO6_01409	Glyoxalase family protein	-3.0448
VVMO6_03445	Exopolyphosphatase-related protein	-3.04137
VVMO6_00664	Galactose-1-phosphate uridylyltransferase (EC 2.7.7.10)	-3.01536
VVMO6_00638	Flp pilus assembly protein	-3.01328
VVMO6_00634	putative transcriptional regulator2C LysR family	-3.01251
VVMO6_03047	Probable GTPase related to EngC	-3.00658
VVMO6_01328	hypothetical protein	-3.00307
VVMO6_01367	DinB family protein	-2.99514
VVMO6_00443	Putative cytochrome c oxidase2C subunit I	-2.9873
VVMO6_01335	Prevent host death protein2C Phd antitoxin	-2.97382
VVMO6_01421	hypothetical protein	-2.97081
VVMO6_01340	hypothetical protein	-2.95503
VVMO6_00764	cytochrome c554	-2.94445
VVMO6_02517	hypothetical protein	-2.93435
VVMO6_01412	hypothetical protein	-2.93405
VVMO6_03799	hypothetical protein	-2.89729
VVMO6_03507	Uncharacterized protein conserved in bacteria	-2.89352
VVMO6_02710	Protein ytfJ precursor	-2.88854
VVMO6_04238	Sigma-54 dependent transcriptional regulator	-2.88319
VVMO6_01336	Death on curing protein2C Doc toxin	-2.87444
VVMO6_01347	Prevent host death protein2C Phd antitoxin	-2.8684
VVMO6_00497	RNA polymerase sigma factor RpoS	-2.86039
VVMO6_02199	Succinate dehydrogenase cytochrome b-556 subunit	-2.85509
VVMO6_03992	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-2.83413

VVMO6_01411	hypothetical protein	-2.82431
VVMO6_03612	hypothetical protein	-2.81774
VVMO6_03471	membrane protein	-2.81387
VVMO6_01930	Predicted esterase of the alpha-beta hydrolase superfamily	-2.80832
VVMO6_01369	hypothetical protein	-2.795
VVMO6_01341	unknown	-2.79044
VVMO6_03981	Extracellular solute-binding protein2C family 3/GGDEF domain protein	-2.78517
VVMO6_04392	FOG: GGDEF domain	-2.77416
VVMO6_01446	hypothetical protein	-2.77401
VVMO6_04026	Cytochrome c oxidase polypeptide I (EC 1.9.3.1)	-2.77147
VVMO6_01410	hypothetical protein	-2.77085
VVMO6_02217	N-acetylglucosamine-6P-responsive transcriptional repressor NagC2C ROK family	-2.77034
VVMO6_03297	hypothetical protein	-2.76781
VVMO6_00940	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	-2.75799
VVMO6_00946	YcfP protein: probably an esterase that is part of a salvage cluster	-2.7473
VVMO6_01405	hypothetical protein	-2.73915
VVMO6_04326	Transcriptional regulator2C AraC family	-2.72341
VVMO6_01048	COG1132	-2.72255
VVMO6_02179	hypothetical protein	-2.72127
VVMO6_01447	hypothetical protein	-2.69719
VVMO6_01316	hypothetical protein	-2.68784
VVMO6_04165	Predicted Lactate-responsive regulator2C LysR family	-2.68545
VVMO6_03001	Methyl-accepting chemotaxis protein	-2.68432
VVMO6_01364	Putative acetyltransferase	-2.67477
VVMO6_00860	Protease III precursor (EC 3.4.24.55)	-2.67047
VVMO6_03688	hypothetical protein	-2.66919
VVMO6_01623	Sigma-54 dependent transcriptional regulator SypG	-2.65784
VVMO6_01339	hypothetical protein	-2.65654
VVMO6_01167	Tyrosine-specific transport protein	-2.656
VVMO6_03853	Signal transduction histidine kinase	-2.65471
VVMO6_01312	hypothetical protein	-2.65387
VVMO6_01599	hypothetical protein	-2.64433
VVMO6_01399	hypothetical protein	-2.64056
VVMO6_02467	Polyphosphate kinase (EC 2.7.4.1)	-2.63913
VVMO6_02345	hypothetical protein	-2.63451
VVMO6_01647	Sensor kinase CitA2C DpiB (EC 2.7.3.-)	-2.63349
VVMO6_01397	Phenazine biosynthesis protein PhzF like	-2.63222
VVMO6_01646	Transcriptional regulatory protein CitB2C DpiA	-2.62855
VVMO6_03443	L-serine dehydratase (EC 4.3.1.17)	-2.62089
VVMO6_00635	hypothetical protein	-2.61547
VVMO6_03299	Histone acetyltransferase HPA2	-2.60297
VVMO6_01415	hypothetical protein	-2.60235
VVMO6_00676	Aerobic glycerol-3-phosphate dehydrogenase (EC	-2.60189

	1.1.5.3)	
VVMO6_01414	FIG032766: hypothetical protein	-2.59815
VVMO6_01468	Fructosamine kinase family protein2C At3g61080 homolog	-2.59764
VVMO6_03721	Methyl-accepting chemotaxis protein	-2.5937
VVMO6_01413	FIG001353: Acetyltransferase	-2.59207
VVMO6_01598	hypothetical protein	-2.58668
VVMO6_02987	Uncharacterized domain COG3236 / GTP cyclohydrolase II (EC 3.5.4.25)	-2.58621
VVMO6_01444	hypothetical protein	-2.58513
VVMO6_02337	GGDEF domain family protein	-2.58447
VVMO6_00990	ATP-dependent Clp protease adaptor protein ClpS	-2.58294
VVMO6_01449	hypothetical protein	-2.58141
VVMO6_01156	hypothetical protein	-2.57376
VVMO6_01435	conserved hypothetical protein	-2.57028
VVMO6_03120	FIG032766: hypothetical protein	-2.56424
VVMO6_04279	Transcriptional regulators2C LysR family	-2.55922
VVMO6_03516	hypothetical protein	-2.55821
VVMO6_03771	DamX-related protein	-2.5548
VVMO6_03798	hypothetical protein	-2.54478
VVMO6_03070	FOG: EAL domain protein	-2.5404
VVMO6_03391	hypothetical protein	-2.53644
VVMO6_00113	Homoserine/homoserine lactone efflux protein	-2.52396
VVMO6_02089	hypothetical protein	-2.52288
VVMO6_01523	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1)	-2.5197
VVMO6_00420	N-acetylglucosamine regulated methyl-accepting chemotaxis protein	-2.51685
VVMO6_04253	FOG: TPR repeat protein	-2.51204
VVMO6_00479	hypothetical protein	-2.51138
VVMO6_01392	hypothetical protein	-2.49931
VVMO6_01311	hypothetical protein	-2.49493
VVMO6_01361	hypothetical protein	-2.48992
VVMO6_00166	Regulator of sigma D	-2.48862
VVMO6_03890	Succinyl-CoA synthetase2C alpha subunit	-2.48141
VVMO6_01637	hypothetical protein	-2.47893
VVMO6_03961	Isovaleryl-CoA dehydrogenase (EC 1.3.99.10)	-2.47581
VVMO6_01313	Lactoylglutathione lyase	-2.46917
VVMO6_01366	hypothetical protein	-2.46832
VVMO6_03641	hypothetical protein	-2.46537
VVMO6_01330	Phosphohydrolase (MutT/nudix family protein)	-2.46469
VVMO6_00936	Inosine monophosphate dehydrogenase-related protein	-2.46242
VVMO6_03934	Signal transduction histidine kinase	-2.45568
VVMO6_03732	Predicted ATP-dependent endonuclease of the OLD family	-2.45395
VVMO6_02714	ABC-type antimicrobial peptide transport system2C permease component	-2.45363
VVMO6_01431	histone acetyltransferase HPA2	-2.44288

VVMO6_03764	Allophanate hydrolase 2 subunit 1 (EC 3.5.1.54)	-2.44049
VVMO6_01711	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	-2.43534
VVMO6_03306	GTPase (EC 3.6.1.-)	-2.43467
VVMO6_01332	putative acetyltransferase	-2.43109
VVMO6_00300	Transcriptional (co)regulator CytR	-2.43047
VVMO6_01179	Capsular polysaccharide synthesis enzyme CpsC2C polysaccharide export	-2.42005
VVMO6_00743	UPF0325 protein yaeH	-2.41911
VVMO6_02637	Transcriptional regulator of glmS gene2C DeoR family	-2.39982
VVMO6_01432	putative acetyltransferase	-2.39857
VVMO6_01326	hypothetical protein	-2.39448
VVMO6_00120	hypothetical protein	-2.38828
VVMO6_03761	Predicted ABC-type transport system2C permease component	-2.38788
VVMO6_01395	hypothetical protein	-2.38719
VVMO6_03703	hypothetical protein	-2.38271
VVMO6_01428	hypothetical protein	-2.38232
VVMO6_01469	Chemotaxis protein CheC -- inhibitor of MCP methylation	-2.37736
VVMO6_02640	hypothetical protein	-2.37637
VVMO6_03515	Ribose operon repressor	-2.3754
VVMO6_01365	hypothetical protein	-2.37474
VVMO6_03026	hypothetical protein	-2.37277
VVMO6_02063	HNH endonuclease	-2.3726
VVMO6_00107	Sensory box/GGDEF family protein	-2.36938
VVMO6_01434	hypothetical protein	-2.36799
VVMO6_01398	hypothetical protein	-2.36683
VVMO6_00480	hypothetical protein	-2.3631
VVMO6_01353	hypothetical protein	-2.3583
VVMO6_02370	Predicted deacylase	-2.35798
VVMO6_01325	hypothetical protein	-2.35796
VVMO6_03517	Chemotaxis protein CheV (EC 2.7.3.-)	-2.35382
VVMO6_01141	hypothetical protein	-2.34771
VVMO6_01337	hypothetical protein	-2.33273
VVMO6_03941	Electron transfer flavoprotein2C alpha subunit	-2.33264
VVMO6_01439	Plasmid stabilization element ParE2C putative	-2.32813
VVMO6_03854	Methylase of chemotaxis methyl-accepting protein	-2.32267
VVMO6_01738	Succinylglutamate desuccinylase (EC 3.5.1.96)	-2.31687
VVMO6_01169	hypothetical protein	-2.31334
VVMO6_02062	DNA-cytosine methyltransferase (EC 2.1.1.37)	-2.3127
VVMO6_01396	hypothetical protein	-2.30721
VVMO6_01288	hypothetical protein	-2.3034
VVMO6_03959	3-ketoacyl-CoA thiolase [isoleucine degradation] (EC 2.3.1.16)	-2.30079
VVMO6_01522	Cytochrome c oxidase subunit CcoO (EC 1.9.3.1)	-2.29292
VVMO6_01333	hypothetical protein	-2.29019

VVMO6_01402	hypothetical protein	-2.28992
VVMO6_00416	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein	-2.28974
VVMO6_01453	NTP pyrophosphohydrolase	-2.27884
VVMO6_02200	Citrate synthase (si) (EC 2.3.3.1)	-2.27763
VVMO6_01315	hypothetical protein	-2.26962
VVMO6_01400	hypothetical protein	-2.26239
VVMO6_03305	Membrane fusion component of tripartite multidrug resistance system	-2.26166
VVMO6_03763	Allophanate hydrolase 2 subunit 2 (EC 3.5.1.54)	-2.26149
VVMO6_01394	hypothetical protein	-2.2609
VVMO6_01375	Lactoylglutathione lyase	-2.24219
VVMO6_01528	hypothetical protein	-2.24087
VVMO6_00841	CheW domain protein	-2.23678
VVMO6_01867	DNA-binding protein H-NS	-2.23282
VVMO6_03935	Response regulator	-2.23165
VVMO6_03266	hypothetical protein	-2.23127
VVMO6_03402	Glycosyltransferase involved in cell wall biogenesis (EC 2.4.-.-)	-2.23127
VVMO6_03508	Putative PRS2 protein	-2.22748
VVMO6_02065	hypothetical protein	-2.22301
VVMO6_04379	C4-dicarboxylate transport transcriptional regulatory protein	-2.21685
VVMO6_02064	hypothetical protein	-2.21537
VVMO6_01140	Fumarate hydratase class I2C aerobic (EC 4.2.1.2)	-2.21051
VVMO6_03271	lipoprotein Blc	-2.20743
VVMO6_01135	Hypothetical nudix hydrolase YeaB	-2.20698
VVMO6_00919	hypothetical protein	-2.20651
VVMO6_02822	Methyl-accepting chemotaxis protein	-2.20148
VVMO6_01919	Integration host factor beta subunit	-2.19913
VVMO6_01667	Putative TEGT family carrier/transport protein	-2.19414
VVMO6_01521	Cytochrome c oxidase subunit CcoQ (EC 1.9.3.1)	-2.19291
VVMO6_02544	Ribonuclease E inhibitor RraA	-2.19181
VVMO6_01334	hypothetical protein	-2.19117
VVMO6_02078	Uncharacterized protein YeaC	-2.19093
VVMO6_01393	putative acetyltransferase	-2.18786
VVMO6_04088	YjeF protein2C function unknown	-2.18422
VVMO6_04023	hypothetical protein	-2.17045
VVMO6_01745	Predicted nucleoside-diphosphate-sugar epimerases	-2.16916
VVMO6_01317	hypothetical protein	-2.16861
VVMO6_01717	Potential queD like	-2.16804
VVMO6_01379	hypothetical protein	-2.16726
VVMO6_00049	carbonic anhydrase2C family 3	-2.15965
VVMO6_02594	Ubiquinol--cytochrome c reductase2C cytochrome B subunit (EC 1.10.2.2)	-2.15558
VVMO6_01442	Vco30	-2.15069
VVMO6_01357	hypothetical protein	-2.1472

VVMO6_01390	hypothetical protein	-2.14602
VVMO6_01927	Chemotaxis protein CheV (EC 2.7.3.-)	-2.1376
VVMO6_00091	Universal stress protein A	-2.13487
VVMO6_03501	Aldehyde dehydrogenase (EC 1.2.1.3); Probable coniferyl aldehyde dehydrogenase (EC 1.2.1.68)	-2.13471
VVMO6_03231	ABC-type amino acid transport/signal transduction system	-2.13392
VVMO6_01274	putative SpoOM-related protein	-2.13284
VVMO6_00092	Ferritin-like protein 2	-2.12044
VVMO6_01403	glyoxalase family protein	-2.11612
VVMO6_01121	hypothetical protein	-2.11609
VVMO6_03765	Lactam utilization protein LamB	-2.11515
VVMO6_02670	Arginine pathway regulatory protein ArgR2C repressor of arg regulon	-2.11169
VVMO6_04049	hypothetical protein	-2.11062
VVMO6_01314	hypothetical protein	-2.11025
VVMO6_01327	hypothetical protein	-2.10762
VVMO6_01426	hypothetical protein	-2.1066
VVMO6_01177	Capsular polysaccharide synthesis enzyme CpsA2C sugar transferase	-2.10627
VVMO6_01238	Predicted hydrolase	-2.10613
VVMO6_00630	Putative membrane GGDEF domain involved in signal transduction	-2.09769
VVMO6_01331	hypothetical protein	-2.09248
VVMO6_02255	Flagellin protein FlaC	-2.08636
VVMO6_02216	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	-2.08103
VVMO6_01374	hypothetical protein	-2.07913
VVMO6_03797	NAD(P) transhydrogenase alpha subunit (EC 1.6.1.2)	-2.07854
VVMO6_02236	Cell division inhibitor	-2.07325
VVMO6_01716	hypothetical protein	-2.07283
VVMO6_03802	Response regulator	-2.07127
VVMO6_01318	hypothetical protein	-2.07035
VVMO6_01321	hypothetical protein	-2.06607
VVMO6_01382	Glyoxalase family protein	-2.0608
VVMO6_00655	hypothetical protein	-2.06017
VVMO6_01997	Anthranilate synthase2C amidotransferase component (EC 4.1.3.27)	-2.0584
VVMO6_02681	Uncharacterized ABC transporter2C ATP-binding protein YrbF	-2.05744
VVMO6_02593	ubiquinol cytochrome C oxidoreductase2C cytochrome C1 subunit	-2.05714
VVMO6_02001	Tryptophan synthase alpha chain (EC 4.2.1.20)	-2.05638
VVMO6_01322	hypothetical protein	-2.05401
VVMO6_00611	C-di-GMP phosphodiesterase A-related protein	-2.05318
VVMO6_00991	ATP-dependent Clp protease ATP-binding subunit ClpA	-2.04898
VVMO6_01349	hypothetical protein	-2.04612
VVMO6_02215	PTS system2C N-acetylglucosamine-specific IIB component (EC 2.7.1.69) / PTS system2C N-	-2.04386

	acetylglucosamine-specific IIC component (EC 2.7.1.69)	
VVMO6_03020	Na+/H+ antiporter NhaD type	-2.04023
VVMO6_03720	Putative phosphatase YieH	-2.03741
VVMO6_00765	Nitrogen regulatory protein P-II	-2.03727
VVMO6_02180	Formyltetrahydrofolate deformylase (EC 3.5.1.10)	-2.03674
VVMO6_03307	GGDEF family protein	-2.0365
VVMO6_03244	2-oxoglutarate dehydrogenase complex2C dehydrogenase component	-2.03489
VVMO6_01307	hypothetical protein	-2.02844
VVMO6_01429	hypothetical protein	-2.02684
VVMO6_03726	hypothetical protein	-2.02368
VVMO6_03518	Fatty acid desaturase (EC 1.14.19.1); Delta-9 fatty acid desaturase (EC 1.14.19.1)	-2.02134
VVMO6_03527	Response regulator	-2.01663
VVMO6_01520	Cytochrome c oxidase subunit CcoP (EC 1.9.3.1)	-2.00937
VVMO6_03888	LemA protein	-2.00894
VVMO6_01881	Probable protease htpX homolog (EC 3.4.24.-)	-2.00865
VVMO6_01329	hypothetical protein	-2.00495

Table 3-6. Genes differentially expressed in biofilm cells from the dispersion stage compared to those from the mature stage of biofilm development (B3/B2).

Locus tag[a]	Gene product	Fold change
Up-regulated genes (23 genes)		
VVMO6_03242	Permease of the drug/metabolite transporter (DMT) superfamily	39.10754
VVMO6_04389	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14)	5.61235
VVMO6_03707	Phosphoglycerate transporter protein PgtP	5.349824
VVMO6_03243	Chemotactic transducer-related protein	3.857728
VVMO6_03671	hypothetical protein	3.134972
VVMO6_03710	Phosphoglycerate transport system transcriptional regulatory protein PgtA	2.863282
VVMO6_01256	hypothetical protein	2.805467
VVMO6_03711	Iron-containing alcohol dehydrogenase	2.732157
VVMO6_02184	Ferrous iron transport protein C	2.698303
VVMO6_02185	Ferrous iron transport protein B	2.696235
VVMO6_02497	Carbon starvation protein A	2.593696
VVMO6_03810	hypothetical protein	2.458858
VVMO6_02637	Transcriptional regulator of glmS gene2C DeoR family	2.432348
VVMO6_00346	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)	2.369672
VVMO6_01208	hypothetical protein	2.272506
VVMO6_00345	Sulfite reductase [NADPH] flavoprotein alpha-component (EC 1.8.1.2)	2.260354
VVMO6_00725	hypothetical protein	2.205785
VVMO6_01794	membrane protein	2.1645
VVMO6_01094	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14)	2.152205
VVMO6_03708	Phosphoglycerate transport regulatory protein PgtC	2.151158
VVMO6_01209	Pilus assembly protein CpaE-like protein	2.130861
VVMO6_04045	Multidrug resistance protein D	2.038135
VVMO6_01795	Two-component system response regulator QseB	2.002153
Down-regulated genes (13 genes)		
VVMO6_03347	Glycosyltransferase	-6.18714
VVMO6_00054	Ketol-acid reductoisomerase (EC 1.1.1.86)	-3.48075
VVMO6_03320	Hypothetical protein in cluster with HutR2C VCA0066 homolog	-2.76811
VVMO6_01127	hypothetical protein	-2.65783
VVMO6_03256	Regulatory P domain of the subtilisin-like proprotein convertase	-2.65587

VVMO6_04225	hypothetical protein	-2.45023
VVMO6_03321	Hypothetical protein in cluster with HutR2C VCA0067 homolog	-2.33907
VVMO6_00597	tRNA pseudouridine synthase B (EC 4.2.1.70)	-2.27143
VVMO6_02654	Uncharacterized protein DUF547	-2.25357
VVMO6_01272	Imidazolonepropionase (EC 3.5.2.7)	-2.16027
VVMO6_01269	Histidine ammonia-lyase (EC 4.3.1.3)	-2.14489
VVMO6_02632	TRAP transporter solute receptor2C unknown substrate 6	-2.12257
VVMO6_03752	Isopenicillin N synthase	-2.00143

3-3-3. Increase of the expression levels of biofilm-associated genes in mature biofilm cells.

In order to identify genes potentially involved in maturation of biofilm, I compared the transcriptome of mature biofilm cells (B2) with that of planktonic cells (P2) at the same development stage. Firstly, the gene encoding a GGDEF domain protein (VVMO6_04047) was up-regulated in the biofilm cells compared to the planktonic cells (Fig. 3-6A), which can result in the increase of intracellular level of c-di-GMP and the synthesis of EPS and other matrix component contributing to biofilm maturation.

The expression level of the gene encoding quorum sensing regulator, SmcR, was increased in mature biofilm cells (Fig. 3-6B). This could be the consequence of the increase of cell density in biofilm. However, the expression levels of the genes encoding proteins in the AI (auto inducer)-2 signalling pathway and the small RNAs (*qrr*) which affect the stability of the smcR transcript were not altered in biofilm cells. Therefore, the up-regulation of *smcR* in biofilm cells may not be induced by AI-2. It is still possibe that other unknown quorum sensing pathway (s) induced the increase in amount of *smcR* mRNA.

c-di-GMP-regulated *brp* gene cluster, which is homologue of *V. cholera vps*-II gene cluster, is essential for biofilm formation of *V. vulnificus* (Guo *et al.*, 2010; Nakhamchik *et al.*, 2008). The alteration of the expression level of *brp* cluster was confirmed using the RNA-seq analysis. As expected, the expression levels of *brp* gene cluster and the gene encoding a putative regulator of *brp*, BrpT, were increased in mature biofilms compared to

planktonic cells (Fig. 3-6C). The gene cluster, VVMO6_03340-03342, encoding the putative calcium-binding protein and type 1 secretion system components was also up-regulated in mature biofilm cells (Fig. 3-6D). EPS produced by the Brp proteins and the putative adhesin encoded by VVMO6_03340 possibly promotes the maturation of *V. vulnificus* biofilm.

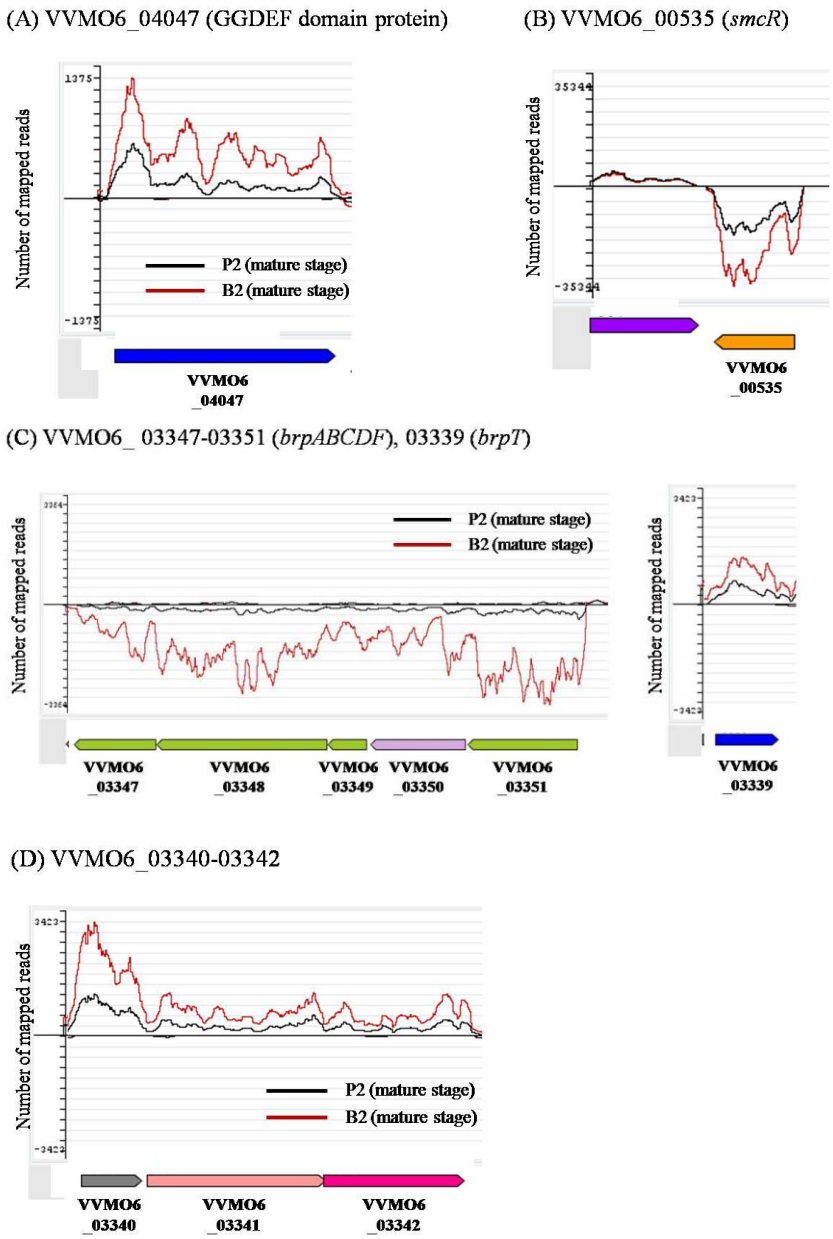


Fig. 3-6. Mapped sequence data of genes up-regulated in biofilm cells at the mature stage. Snapshots represent some RNA-seq data mapped to the *V. vulnificus* MO6-24/O genome. Black and red lines represent the mapped reads of each RNA sample from the planktonic cells and biofilm cells at the mature stage of biofilm development, respectively. Reads mapped to VVMO6_04047, VVMO6_00535, VVMO6_00347-00351, VVMO6_03339, and VVMO6_03340-03342 are shown.

3-3-4. Dynamic changes of the expression levels of biofilm-associated genes with the biofilm development stages.

The expression level of *brp* gene cluster was increased in mature biofilms and decreased in biofilm cells at the dispersion state (Fig. 3-7A). This dynamic change of the expression level of *brp* indicated that *brp* locus is required for the maturation of biofilm, however, it can be unnecessary in dispersing biofilms. The gene cluster, VVMO6_03340-03342, showed similar change in expression levels with the *brp* gene cluster (Fig. 3-7B). Surprisingly, VVMO6_04367 encoding VvpE, which is reported to affect the detachment of *V. vulnificus* biofilm, showed increased fold change in mature biofilms and the expression level was maintained to the dispersion stage (Fig. 3-7C) (Kim *et al*., 2013). One possible explanation for this inconsistency is that, when biofilms reach the maturation stage, *V. vulnificus* cells may start to detach immediately for maintaining the size and shape of biofilms.

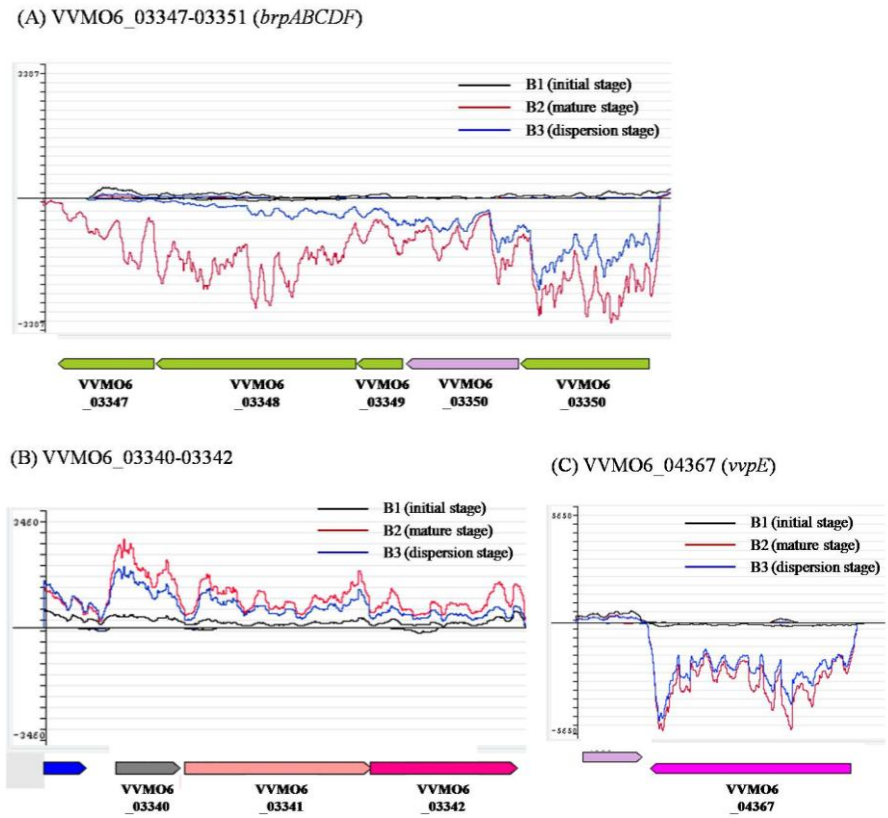


Fig. 3-7. Mapped sequence data of genes differentially expressed in biofilm cells at the different stages of biofilm development. Snapshots represent some RNA-seq data mapped to the *V. vulnificus* MO6-24/O genome. Black, red, and blue lines represent the mapped reads of each RNA sample from the initial, mature, and dispersion stage of biofilm development. Reads mapped to VVMO6_03347-03351, VVMO6_03340-03342, and VVMO6_04367 are shown.

Chapter 4.

CabA, a Cyclic-di-GMP-Regulated Calcium Binding Protein, Is an Essential Component of Biofilm Matrix of *V. vulnificus*

4-1. Introduction

In natural habitat, microorganisms do not live as pure cultures of freely suspended single cells (planktonic state) but instead grow in biofilms. Biofilm is a complex community of microorganisms that are embedded in a matrix of extracellular polymeric substance they have produced (Flemming and Wingender, 2010). By forming biofilms, microorganisms can adapt to various unpredictable stresses including starvation, temperature changes, antimicrobial agents challenge, immune host defenses and so on (Costerton *et al*., 1987; Johnson *et al*., 2008). These characteristics of biofilms are attributed to biofilm matrix accounting for over 90 % of the dry mass of biofilms. Biofilm matrix consisting of polysaccharides, proteins, nucleic acids, and lipids encases and immobilizes microbial aggregates, providing the mechanical stability of biofilms and acting as protective barriers (Flemming and Wingender, 2010).

A representative factor influencing biofilm formation is 3',5'-Cyclic diguanylic acid (c-di-GMP), a bacterial global second messenger. Recent studies have indicated that the high intracellular c-di-GMP levels induced biofilm formation, exopolysaccharide (EPS) synthesis and rugose colony development in many bacteria (for recent reviews, Boyd and O'Toole, 2012; Krasteva *et al*., 2012). c-di-GMP is synthesized by diguanylate cyclases (DGCs) containing the GGDEF domain and degraded by phosphodiesterases (PDEs) containing the EAL domain or HD-GYP domain (Galperin, 2004). Genes encoding DGCs or PDEs are highly prevalent among different bacteria (Galperin *et al.,* 2001). *Vibrio vulnificus* also has large numbers of DGCs and PDEs; 66 proteins with the GGDEF domain and 33 proteins with the EAL domain (Römling *et al.,* 2005). Among the DGCs in *V. vulnificus*, DcpA (diguanylate cyclase protein A) has been identified to regulate EPS production, biofilm formation, and rugose colony development (Nakhamchik

et al., 2008). Moreover, c-di-GMP-regulated polysaccharide locus (*brp*), and its putative regulator BrpT of *V. vulnificus* have been proved to be important for biofilm formation and stress resistance (Guo *et al*., 2010).

Calcium is known to be another important factor for biofilm formation in many bacteria. Although the inhibitory roles of calcium in biofilm formation have been reported (Arrizubieta *et al*., 2004; Boyd *et al*., 2012), many researchers have found that calcium promotes surface attachment and biofilm formation (Cruz *et al*., 2012; Kierek *et al*., 2003; Martinez-Gil *et al*., 2012). Calcium has ability to form ionic cross bridges with negatively charged bacterial polysaccharides (Chen *et al*., 2002). For example, calcium is important for the ionic cross-bridging of O-antigen polysaccharide in the biofilm matrix of *V. cholerae* (Kierek *et al*., 2003). Alginate, which is produced in mucoid *Psedomonas aeruginosa*, has been known to form gel network by binding calcium with a high affinity (Lattner *et al.*, 2003). In addition, calcium-induced multimerization of the large adhesion, LapF, has been reported to modulate biofilm formation of *Pseudomonas putida* (Martinez-Gil *et al*., 2012). These findings suggest that calcium can have positive effects on biofilm formation with different types of mechanisms. In *V. vulnificus*, increased calcium promotes switching from the virulent opaque strains to the translucent or rugose strains and also promotes biofilm formation (Garrison-Schilling *et al*., 2010).

In the present study, a c-di-GMP-induced calcium binding protein, CabA (Calcium binding protein involved in biofilm formation), was identified. *cabA* was found to be co-transcribed as an operon with *cabB* and *cabC* encoding type 1 secretion system (T1SS) components. The roles of CabA were accessed under high and low c-di-GMP levels and the results revealed that CabA is essential to biofilm formation and rugose colony development under high c-di-GMP levels. The localization study suggested that CabA was secreted by T1SS composed of CabB and CabC. Furthermore, *cabABC* expressions were activated by BrpT. Therefore, it

appeared from the results that CabA is a biofilm matrix protein and plays an essential role in biofilm formation and rugose colony development.

4-2. Materials and Methods

4-2-1. Strains, plasmids, and culture media.

The strains and plasmids used in this study are listed in Table 4-1. Unless noted otherwise, *E. coli* strains were grown in Luria-Bertani (LB) medium at 37 ℃ and *V. vulnificus* strains were grown in LB medium supplemented with 2.0 % (wt/vol) NaCl (LBS) at 30 ℃. In addition, experiments about biofilm formation of *V. vulnificus* were conducted in the VFMG (Kim *et al*., 2013). All the media components were purchased from Difco (Detroit, MI), and the chemicals were purchased from Sigma (St. Louis, MO).

4-2-2. Construction of JN111 for manipulating c-di-GMP level.

To manipulate c-di-GMP level in cells, the arabinose-inducible P_{BAD} promoter was integrated in the upstream regulatory region of *dcpA* on the *V. vulnificus* CMCP6 chromosome as described in Fig. 4-3. The conjugation and isolation of the transconjugants were conducted as previously described (Kim *et al*., 2011), and the strain that expresses *dcpA* under P_{BAD} was chosen and named JN111. Intracellular c-di-GMP level of JN111 was manipulated by adding arabinose in the growth media.Intracellular c-di-GMP levels of *V. vulnificus* strains were measured using LC-MS as previously described (Irie *et al*., 2012).

4-2-3. RNA purification and transcript analysis.

Total cellular RNA was isolated from the *V. vulnificus* strains grown to A_{600} of 0.6 with LBS using an RNeasy® mini kit (QIAGEN) (Kim *et al*., 2011). Reverse transcription was carried out with Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) using the primers which are designed to hybridize to neighboring coding regions of *cabA*, *cabB* and *cabC* as depicted in Fig. 4-1A (Table 4-2). The resulting cDNAs were amplified by PCR with the same set of

primers.

For quantitative real-time PCR (qRT-PCR), cDNA was synthesized with iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA) and real-time PCR amplification of the cDNA was performed with the specific primer pairs for each *cab* gene (Table 4-2). Relative expression levels of the *cab* transcripts were calculated by using the 16S rRNA expression level as the internal reference for normalization as described previously (Kim *et al*., 2011).

4-2-4. Purification of CabA, metal contents analysis, and isothermal titration calorimetry (ITC).

The coding region of *cabA* was amplified by a PCR using the *V. vulnificus* CMCP6 chromosomal DNA and a pair of primers, cabAexp_F and cabAexp_R (Table 4-2), and cloned into a His_6 tag expression vector, pET28a(+) (Novagen, Madison, WI) to result in pYM1202 (Table 4-1). The His-tagged CabA was then expressed in *E. coli* BL21 (DE3), and purified by affinity chromatography according to manufacturer's procedure (Qiagen, Valencia, CA). The purified CabA was dialyzed against the storage buffer (20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT and 40 % glycerol) and kept frozen until use.

The concentrations of five metal ions (Ca, Mg, Mn, Fe, and Zn) in the purified CabA were measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optima-4300 DV, PerkinElmer, Waltham, MA) as previously described (Cubadda and Raggi, 2005). For ITC analyses, CabA was dialyzed intensively against the storage buffer containing 10 mM EGTA. The resulting calcium-free CabA and $CaCl_2$ solutions were prepared using Tris buffer (50 mM Tris·HCl, pH 7.0; 300 mM NaCl). An 1.2 mM $CaCl_2$ solution in an injection syringe was titrated against 0.08 mM CabA in a reaction cell at 25 ^{o}C (VP-ITC, MicroCal Inc., Northampton, MA). The stirring speed was 270 RPM, and the thermal power was recorded every 15 sec. Raw data were processed and plotted by using the ORIGIN software package (MicroCal Inc.) (Hwang *et al*.,

2013).

4-2-5. Generation of the *cabA* and *cabBC* mutant.

The *cabA* gene was inactivated *in vitro* by deletion (530-bp of 573-bp) of the *cabA* coding region using the PCR-mediated linker-scanning mutation method as described previously (Kim *et al.*, 2012). Pairs of primers cabA_F1_F and cabA_F1_R (for amplification of the 5' amplicon) or cabA_F2_F and cabA_F2_R (for amplification of the 3' amplicon) were designed and used as listed in Table 4-2. The Δ*cabA*, a 530-bp deleted *cabA*, was amplified by PCR using the mixture of both amplicons as the template and cabA_F1_F and cabA_F2_R as primers (Table 4-2). Similar experimental procedures were adopted for amplification of the Δ*cabBC in vitro*, except that primers cabBC_F1_F, cabBC_F1_R, cabBC_F2_F and cabBC_F2_R (for 2,977-bp deleted Δ*cabBC*) were used (Table 4-2). The resulting Δ*cabA* and Δ*cabBC* were ligated with SpeI-SphI-digested pDM4 (Milton *et al.*, 1996) to form pYM1102 and pYM1203, respectively (Table 4-1). The *E. coli* S17-1 λ *pir, tra* containing either pYM1102 or pYM1203 was used as a conjugal donor to *V. vulnificus* CMCP6 to result in the *cabA* mutant (YM112), or the *cabBC* mutant (YM121), respectively.

Using *E. coli* S 17-1λ*pir, tra* containing pYM1102 or pYM1203 as conjugal donors and JN111 as recipients, *cabA* mutant (YM112D) and *cabBC* mutant (YM121D) were generated by homologous recombination, respectively. All of the conjugation and isolation of the transconjugants were conducted using the methods previously described (Kim *et al.*, 2012).

Table 4-1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristicsa	Reference or source
Bacterial strains		
V. vulnificus		
CMCP6	wild type *V. vulnificus*, virulent	Laboratory collection
JN091	CMCP6 with Δ*brpT*	This study
JN111	CMCP6 with P_{BAD}-*dcpA*	This study
YM112D	JN111 with Δ*cabA*	This study
YM121D	JN111 with Δ*cabBC*	This study
E. coli		
BL21 (DE3)	F^-*ompThsdS*$_B$ (r_B^-, m_B^-) *gal dcm* (DE3)	Laboratory collection
DH5α	*supE44* Δ*lacU169 (*ϕ80*lacZ* ΔM15*) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*; plasmid replication	Laboratory collection
S 17-1λ*pir*	λ-*pir*lysogen; *thi pro hsdRhsdM*$^+$*recA* RP4-2 Tc::Mu-Km::Tn7;Tp^rSm^r; host for π-requiring plasmids; conjugal donor	Simon *et al*. (1983)
Plasmids		
pJH0311	0.3-kb NruI fragment containing multi-cloning site of pUC19 cloned into pCOS5; Ap^r, Cm^r	Goo *et al*. (2006)
pET-28a(+)	His-tag protein expression vector; Km^r	Novagen
pYM1202	pET-28a(+) with *cabA*; Km^r	This study
pDM4	suicide vector; *ori*R6K; Cm^r	Milton *et al.* (1996)
pYM1102	pDM4 with Δ*cabA*; Cm^r	This study
pYM1203	pDM4 with Δ*cabBC*; Cm^r	This study
pBAD24	Expression vector with the P_{BAD} promoter; Ap^r	Guzman *et al.*, (1995)
pDrive	PCR product cloning vector ;Ap^r, Km^r	Qiagen
pJN1105	pDrive with P_{dcpA}-*dcpA;* Ap^r, Km^r	This study
pJN1106	pDrive with P_{dcpA}-P_{BAD}-*dcpA;* Ap^r, Km^r	This study
pJN1107	pDM4 with P_{dcpA}-P_{BAD}-*dcpA* ; Cm^r	This study
pJN0908	pBAD24 with *brpT*	This study
pBBR_lux	Broad host range vector containing *luxCDABE* operon; Cm^r	Lenz *et al*., (2004)
pYM1204	pBBR_lux with P_{cabA}	This study
pKS1101	pBAD24 with *oriT* of RP4; Ap^r	Kim *et al*. (2012)
pJK1113	pKS1101(pBAD24 with *oriT*) with *nptI*; Ap^r, Km^r	This study
pYM1109	pJK1113 with*cabA*	This study

[a]Ap^r, ampicillin resistant; Km^r, kanamycin resistant; Cm^r, chloramphenicol resistant; Sm^r, streptomycin resistant.

Table 4-2. Oligonucleotides used in this study.

Oligonucleotide[a]	Sequence(5' → 3')[b]	Use
cabAexp_F	CCATGGCTGTTTATTCTGGAACTG	Amplification of the *cabA*coding region
cabAexp_R	CTCGAGGAATTGGAACATGTCATC	
cabA_F1_F	ACTAGTGGTGGAGCGAAGAAGGAA	Construction of the *cabA* mutant
cabA_F1_R	CGGGATCCGTTCCAGAATAAACA	
cabA_F2_F	ACGGATCCCGGATGACATGTT	
cabA_F2_R	GCATGCGATAAAGAGAGGAACCAC	
cabBC_F1_F	ACTAGTGGGGCTGGCGATGATAG	Construction of the *cabBC* mutant
cabBC_F1_R	GAGGGATCCCTACCCACCTAGAAGTAG	
cabBC_F2_F	TAGGGATCCCTCAAACCGATTACAG	
cabBC_F2_R	GCATGCCTATGTGAAACGATACTTAGG	
dcpA_F1_F	ACCCCGCTTGTGAGGTGAAC	Integration of P_{BAD} in the P_{dcpA}
dcpA_F1_R	GATTCCATGGCGTTAGCCAACTGTAAT	
dcpA_F2_F	AACGCCATGGAATCATATTTAGGTAAAGGAAG	
dcpA_F2_R	GAGCCCGTTAGATCAGAGACATGTCAG	
pBAD24_F	CCATGGTGCATAATGTGCCTGTC	
pBAD24_R	CCGGGTACCATGGTGAATTCCT	
RTcabAB_F	CAGATTTTGTCGCTCATGCGG	RT-PCR
RTcabAB_R	CGGAGTATGACGAAATCACTCGGT	
RTcabBC_F	ACGGCAGAGAGTTGCGTTGG	
RTcabBC_R	TTGCCTGGCGTTGACTGCTT	
qRT21571_F	TTGGTTGCTGGCTCTGGTGAC	qRT-PCR
qRT21571_R	ACTGTCTATACGCACTGTGTCCTC	
qRT21572_F	GCCATTGCCCAGACCCAGAG	
qRT21572_R	CCGATAATACCAACCGCACAACC	
qRT21573_F	TTGGCGGTGGTATTGGCTACTG	
qRT21573_R	TGTTGAATTGCCTGGCGTTGAC	
dualbrpT_F	GCACCCATGGCAGACACAACG	*E. coli* dual plasmid system
dualbrpT_R	ATTAGCATGCCCAGGCGGTTT	
dualPcabA_F	CTGAGCTCGGAATTTATAAAACCGC	
dualPcabA_R	CAGGATCCGTTCCAGAATAAACAGC	

[a] The oligonucleotides were designed using the *V. vulnificus* CMCP6 genome sequence (GenBank accession numbers AE016795 and AE016796).
[b]Regions of oligonucleotides not complementary to corresponding genes are underlined.

4-2-6. Biofilm formation.

The micotiter plate biofilm assay was conducted as described previously. (Kim *et al*., 2013). Biofilms were quantitated by measuring the amount of CV eluted from the biofilms as an absorbance at 570 nm (A_{570}) (Kim *et al*., 2009). When required, VFMG supplemented with various amounts of $CaCl_2$, arabinose, and/or Ca-free CabA was used.

For biofilm structure analyses, biofilms were formed in flow cell chambers as described elsewhere (Borlee *et al*., 2010). Glass coverslips were attached on a polycarbonate flow cells with individual channel dimensions of 1 × 4 × 40 mm. Each flow cell was inoculated with 100 μl of the culture diluted to A_{600} of 0.1, and inverted to allow bacteria to attach to the coverslip for 1 h without flow. Then VFMG was flowed at a constant rate of 8 ml/h using a Minipuls Evolution peristaltic pump (Gilson, Villiers, France) to grow biofilm for 3 days. Biofilms were stained with LIVE/DEAD Baclight Viability Kit containing SYTO9 and *propidium iodide* (Invitrogen, Carlsbad, CA) for 15 min in the dark and photographed by confocal laser scanning microscopy (CLSM) (LSM710, Zeiss, Jena, Germany). The biofilm images were processed using Zeiss Zen software (Zeiss).

4-2-7. Colony morphology assay.

For the analysis of colony morphology, 2 μl of cultures grown to A_{600} of 0.8 with LBS broth was spotted onto LBS agar plates supplemented with 0.02% of arabinose, 200 μg/ml of ampicillin and kanamycin. When required, the calcium ions in LBS were chelated by adding of EGTA, and CabA was exogenously provided to the spotted colonies by adding Ca-free CabA.

4-2-8. Biofilm fractionation and Western blot analysis.

Biofilms were formed by gentle shaking the glass flask (Schott-Duran, Mainz,

Germany) containing 500 ml VFMG culture for 8 h at 30℃ as described above (Kim *et al*, 2013). Planktonic cells were removed by rinsing gently with phosphate-buffered saline (PBS, pH 7.4), after which the biofilms were collected with a cell scraper (SPL) and resuspended in PBS. Biofilms were disrupted by sonication (VC130 Ultrasonic Processor, Sonics & Materials, Inc., Newtown, CT) and then fractionated into cell fractions (pellets) and matrix fractions (supernatants) by centrifugation as previously described (Branda *et al*., 2006). Proteins (10 μg) from the lysates of cell fractions and concentrates of matrix fractions were resolved by SDS-PAGE (Sambrook and Russel, 2001). Western immunoblotting of CabA was performed using a rat anti-CabA antiserum (AbFrontier, Seoul, South Korea) as described previously (Lim *et al*., 2011).

4-2-9. *E. coli* dual plasmid system.

The coding region of *brpT* was amplified by PCR using primers dualbrpT_F and dualbrpT_R (Table 4-2) and cloned into the pBAD24 under an arabinose-inducible promoter (Guzman *et al*., 1995) to generate the pJN0908. The *cabA-lux* fusion reporter pYM1204 was constructed by subcloning the *cabA* upstream region (317-bp) amplified by PCR using primers dualPcabA_F and dualPcabA_F (Table 4-2) into the pBBR_lux (Lenz *et al*., 2004). *E. coli* DH5α was cotransformed with the pJN0908 and pYM1204 and The cellular luminescence of the *E. coli* was measured with an Infinite^{TM} M200 microplate reader (Tecan, Männedorf, Switzerland) and the relative luminescence unit was calculated by dividing the luminescence by the A_{600}, as described previously (Hwang *et al*., 2013).

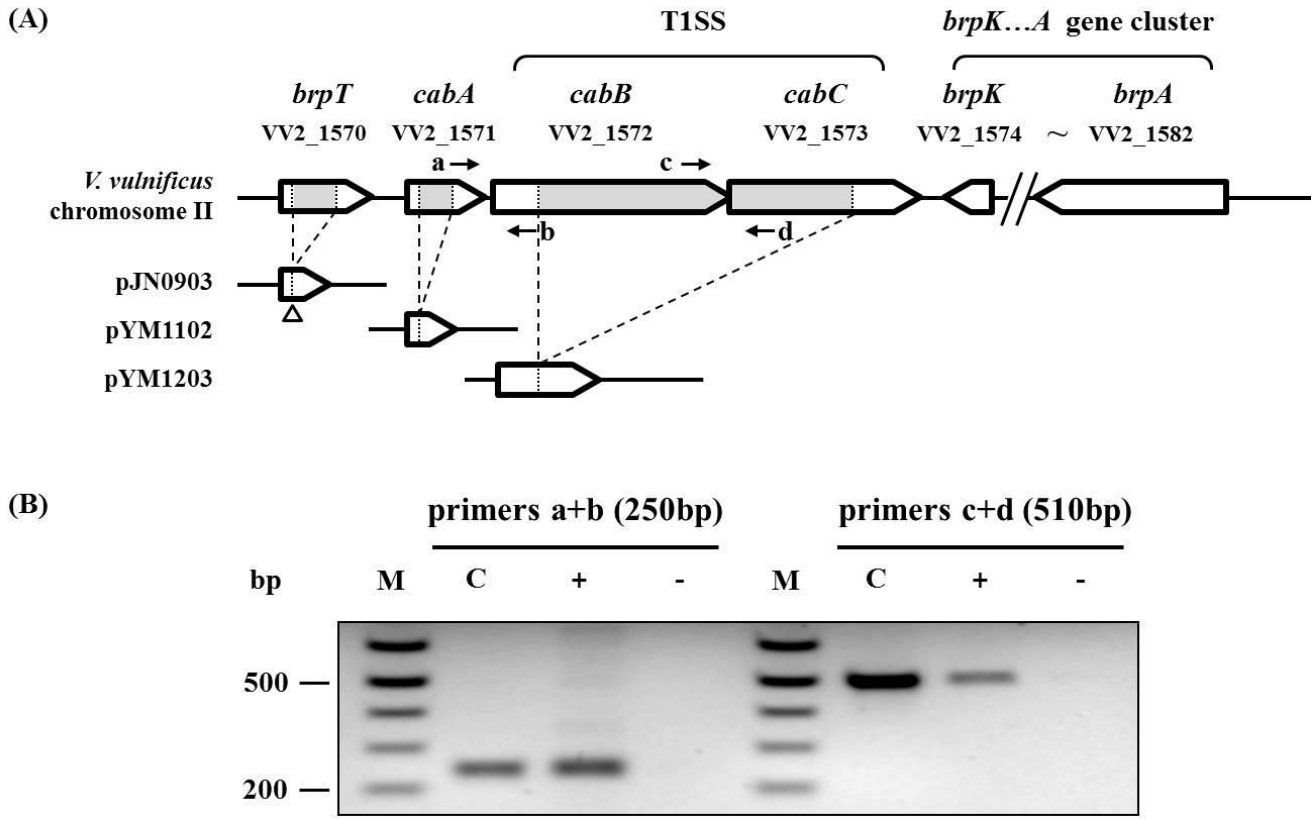
4-3. Results

4-3-1. Identification of a *cabABC* operon.

In the previous study, a whole genome microarray analysis predicted 121 genes potentially up-regulated by ci-di-GMP (Table 2-3). Among the genes, *cabA*, *cabB*, and *cabC* (VV2_1571-3) were identified and annotated to encode a putative calcium-binding protein, a protease/lipase ABC transporter, and a membrane-fusion protein, respectively (GenBank accession number AE016795 and AE016796) (Fig. 4-1A).

The presence of transcripts of the intergenic regions of *cabA*, *cabB*, ad *cabC* was examined using reverse transcription-PCR methods. The results revealed that the genes were transcribed as a transcriptional unit (Fig. 4-1B). Many coding regions that presumably participate in biofilm development were identified immediately upstream and downstream the *cabABC* operon from the *V. vulnificus* genome sequence (GenBankTM accession numbers AE016795 and AE016796). The *brp* gene cluster is downstream of the *cabABC* operon and consists of *brpK…brpA* known to participate in the production of EPS essential for biofilm matrix (Kim *et al*., 2009). In contrast, *brpT* encoding a putative regulator and a homologue of VpsT essential for expression of *V. cholerae* EPS genes is located upstream of the *cabABC* operon (Fig, 4-1A). The *brp* gene cluster consists of another transcriptional unit (data not shown).

Fig. 4-1. Genetic organization of the *cabABC* operon. (A) The physical map of the *cabABC* operon located between *brpT* and *brp* gene cluster. The open arrows represent the transcriptional directions and coding regions of the genes. The figure was derived using the nucleotide sequences of the *V. vulnificus* CMCP6 genome in the GenBank databases (NCBI). The gene identifications are shown above each coding region. The size of *brp* gene cluster is reduced as indicated. Regions cloned in the plasmids pJN0903, pYM1102, and pYM1203 used for the construction of the mutants JN091, YM112(D), and YM121(D), respectively. The regions deleted are depicted by the dashed lines. (B) Co-transcription of the *cabABC* operon was determined by RT-PCR using total RNA extracted from *V. vulnificus* CMCP6 and primers (solid arrows) hybridizing to neighboring coding regions. M, DNA marker; C, genomic DNA PCR (positive control); +, RTase; -, no RTase (negative control).


(A)
T1SS
brpK…A gene cluster
brpT
cabA
cabB
cabC
brpK
brpA
VV2_1570
VV2_1571
VV2_1572
VV2_1573
VV2_1574
~
VV2_1582
a
c
b
d
V. vulnificus
chromosome II
pJN0903
pYM1102
pYM1203
(B)
primers a+b (250bp)
primers c+d (510bp)
bp
M
C
+
-
M
C
+
-
500
200

4-3-2. Calcium-binding ability of CabA.

To confirm whether the purified CabA contained calcium, concentrations of some metal ions in the CabA solution were determined using Inductively coupled plasma-Atomic emission spectrometry (ICP-AES). It was found that the level of calcium ion in the CabA solution was about 1.54 ppm while other elements including magnesium, manganese, iron and zinc were not detected from the sample (Fig. 4-2A). Because the buffers used in the purification and storage of CabA did not contain calcium ion, ICP-AES result suggested that CabA possess calcium-binding ability *in vivo.*

Isothermal titration calorimetry (ITC) experiment was performed to accurately determine the binding parameters of calcium-free CabA with calcium ion ($CaCl_2$). Calcium-free CabA was obtained by chelating calcium ions with EGTA and proved not to contain calcium ion using ICP-AES (data not shown). The ITC result revealed the binding stoichiometry of CabA with calcium ion with a dissociation constant (Kd) of 0.75 μM (Fig. 4-2B). The combined results suggested that CabA is a calcium-binding protein.

(A)

Concentration of metal ions in the CabA solution

(ppm=mg/L)

Metal ion	**Ca**	Mg	Mn	Fe	Zn
Concentration	**1.54 ± 0.00078**	ND	ND	ND	ND

(B)

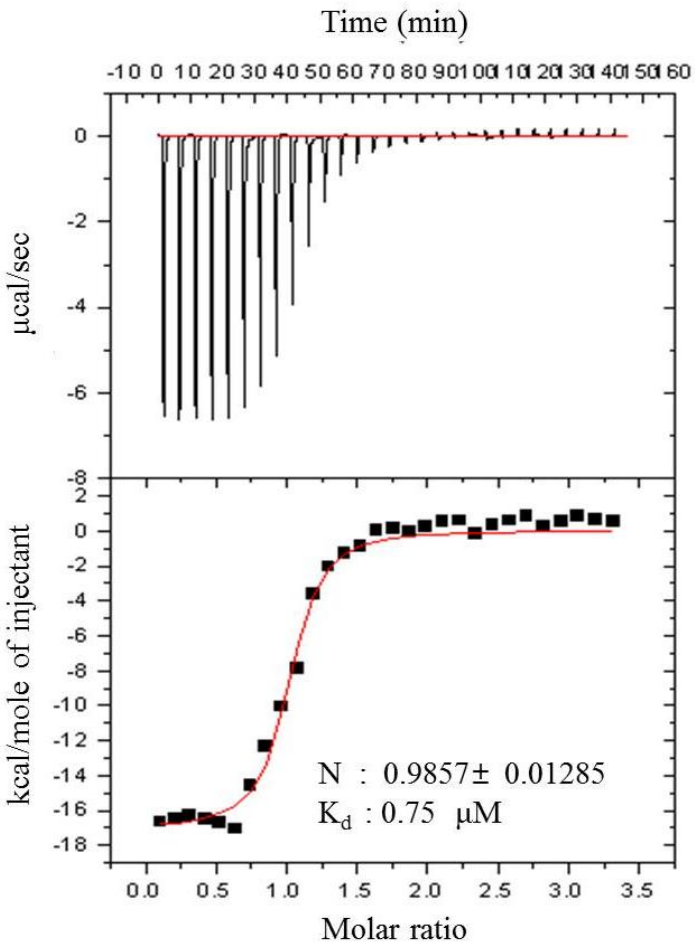
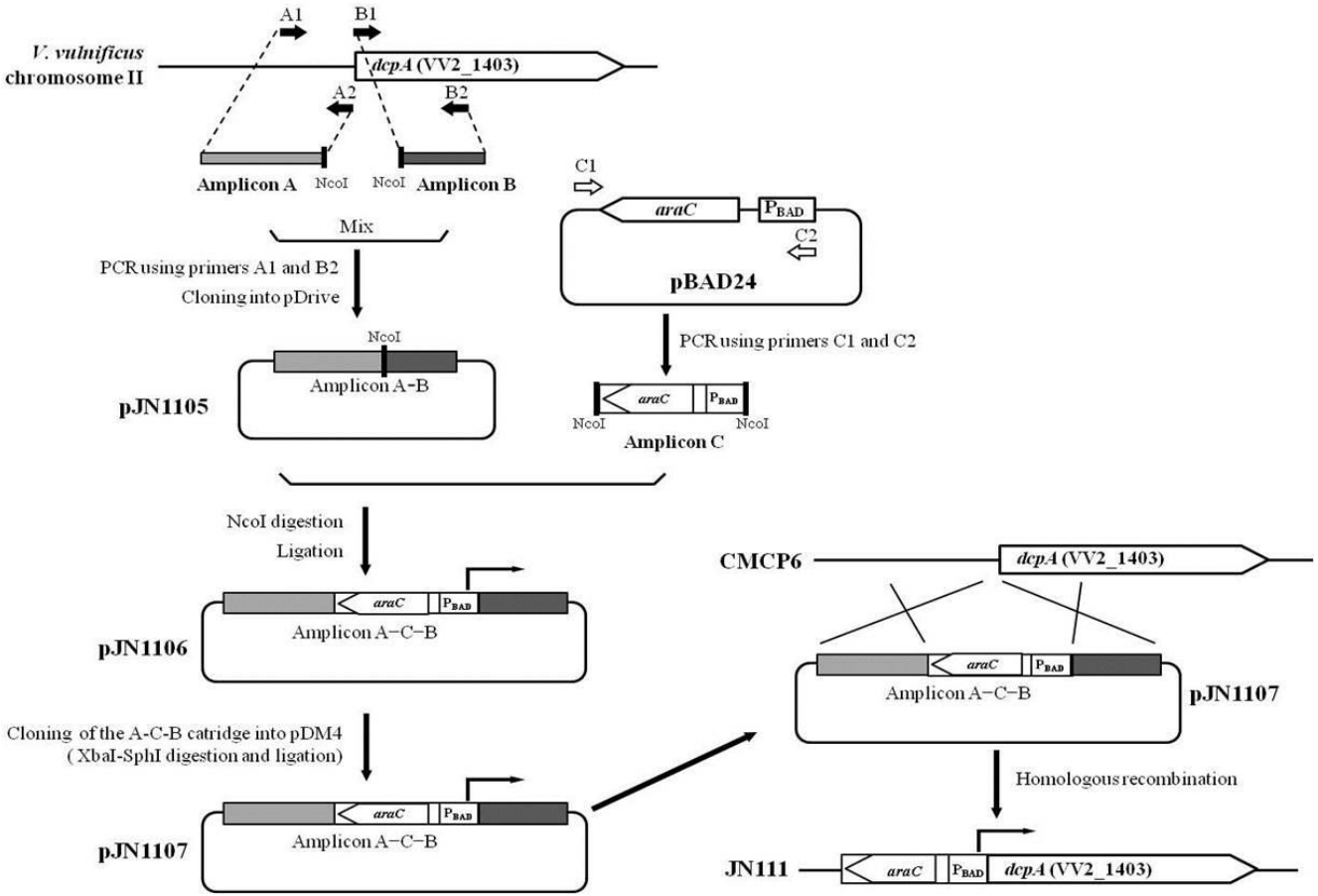


Fig. 4-2. The interaction between CabA and calcium. (A) The concentrations of metal ions in CabA were measured by ICP-AES. The values were presented as ppm (mg/L) with SEM. ND, non-detection. (B) Titration of calcium against CabA was performed using ITC. Upper panel shows raw data obtained from 30 automatic injections of $CaCl_2$ against calcium-free CabA. Low panel shows integration plot of the data calculated from the raw data. Data were fitted using the one-site binding model of the ORIGIN software package (MicroCal Inc.). N, number of binding sites; K_d, dissociation equilibrium constant.

4-3-3. Manipulation of intracellular c-di-GMP levels.

To control the intracellular level of c-di-GMP in *V. vulnificus* CMCP6, the arabinose-inducible promoter P_{BAD} was integrated in the promoter region of *dcpA* encoding diguanylate cyclase in *V. vulnificus* (Nakhamchik *et al*., 2008) (Fig. 4-3A). The intracellular c-di-GMP levels of the strain (JN111) with P_{BAD}-controlled expression of *dcpA* were determined using mass spectrometry. Relative intracellular c-di-GMP level was gradually increased as arabinose concentration in the medium was elevated (Fig. 4-3B). The *cabA* mRNA level in JN111 grown with different levels of arabinose was also determined using qRT-PCR. Consistent with the microarray results showing that *cabA* was up-regulated under high c-di-GMP condition (Table 4-3), the qRT-PCR revealed that the *cabA* mRNA level was induced by elevated intracellular c-di-GMP levels caused by increased concentrations of arabinose (Fig. 4-3C). These data indicated that JN111 was the appropriate system to examine the role of CabA under high and low c-di-GMP levels.

(A) Generation of JN111



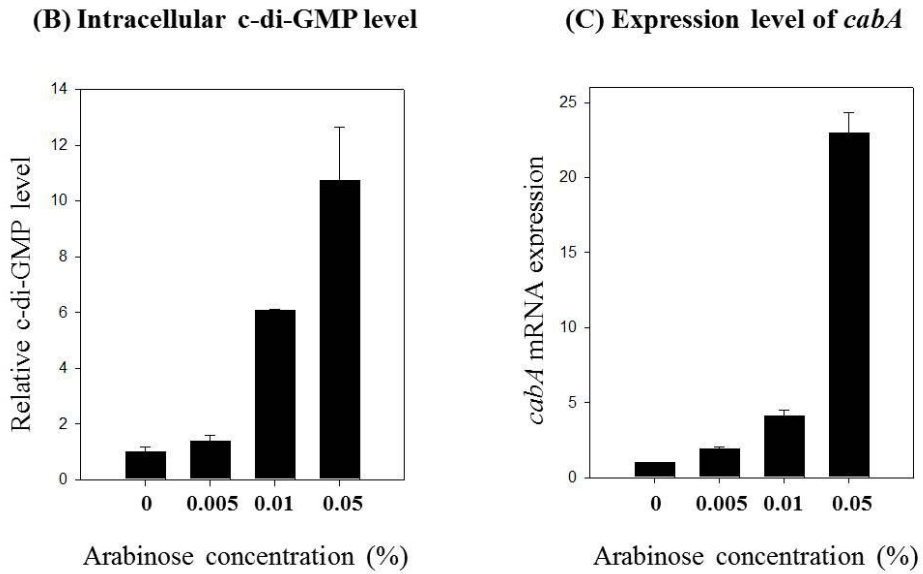


Fig. 4-3. Manipulation of the intracellular level of c-di-GMP. (A) Diagram for construction of P_{BAD}-*dcpA* fusion by insertion of the *araC*-P_{BAD} cassette in the upstream regulatory region of *dcpA* on the *V. vulnificus* CMCP6 chromosome. Solid lines, chromosomal and plasmid DNA; shaded boxes, PCR products of the upstream regulatory region (amplicon A) and 5'-terminal part of the coding region (amplicon B) of *dcpA;* open box, PCR product of the *araC*-P_{BAD} cassette (ampliconC). Shaded and open arrows, locations of the oligonucleotide primers used for PCR of amplicon A,B, and C, respectively; large Xes, genetic crossing over. Abbreviations for primers listed in Table 4-2. A1, dcpA_F1_F; A2, dcpA_F1_R; B1, dcpA_F2_F; B2, dcpA_F2_R; C1, pBAD24_F; C2, pBAD24_R. (B and C) JN111 cultures were grown with different levels of arabinose and intracellular levels of c-di-GMP (B) and expression levels of *cabA* (C) were measured. The relative c-di-GMP levels were presented as the c-di-GMP level in cells grown without arabinose as 1. Error bars represent the SEM.

4-3-4. Effect of *cabA* mutation on static biofilm formation.

To understand the role of CabA and the effect of calcium concentration in biofilm formation, static biofilm assays were conducted with JN111 and YM112D strains under low (0 % arabinose) and high (0.01 % arabinose) c-di-GMP levels with different levels of calcium. Firstly, the wild type (JN111) and mutant (YM112D) produced very low levels of biofilm in the absence of calcium regardless of the c-di-GMP levels (Fig. 4-4A and 4B). When calcium is present and c-di-GMP levels are low (0 % arabinose), there is no significant difference in biofilm-forming abilities between the wild type and *cabA* mutant (Fig. 4-4A). On the other hand, as the c-di-GMP levels were high (0.01 % arabinose), the dramatically enhanced biofilm formation with the increase of calcium concentration was observed in the wild type whereas impaired biofilm formation was shown in the *cabA* mutant. The biofilm formed by the wild type showed a 1.5-fold increase relative to that of the mutant. (Fig.4-4B).

For the complementation of the *cabA* mutant, plasmid pYM1109 was constructed by subcloning the *cabA* coding region into pJK1113 (pBAD24 with *oriT* and *nptI*, Table S1). Complementation of the *cabA* gene in YM112D with a functional *cabA* gene (pYM1109) restored the biofilm formation activity to levels comparable to those of the wild type (Fig. 4-4C). The combined results suggested that CabA is required for biofilm formation in the presence of calcium under high intracellular c-di-GMP conditions.

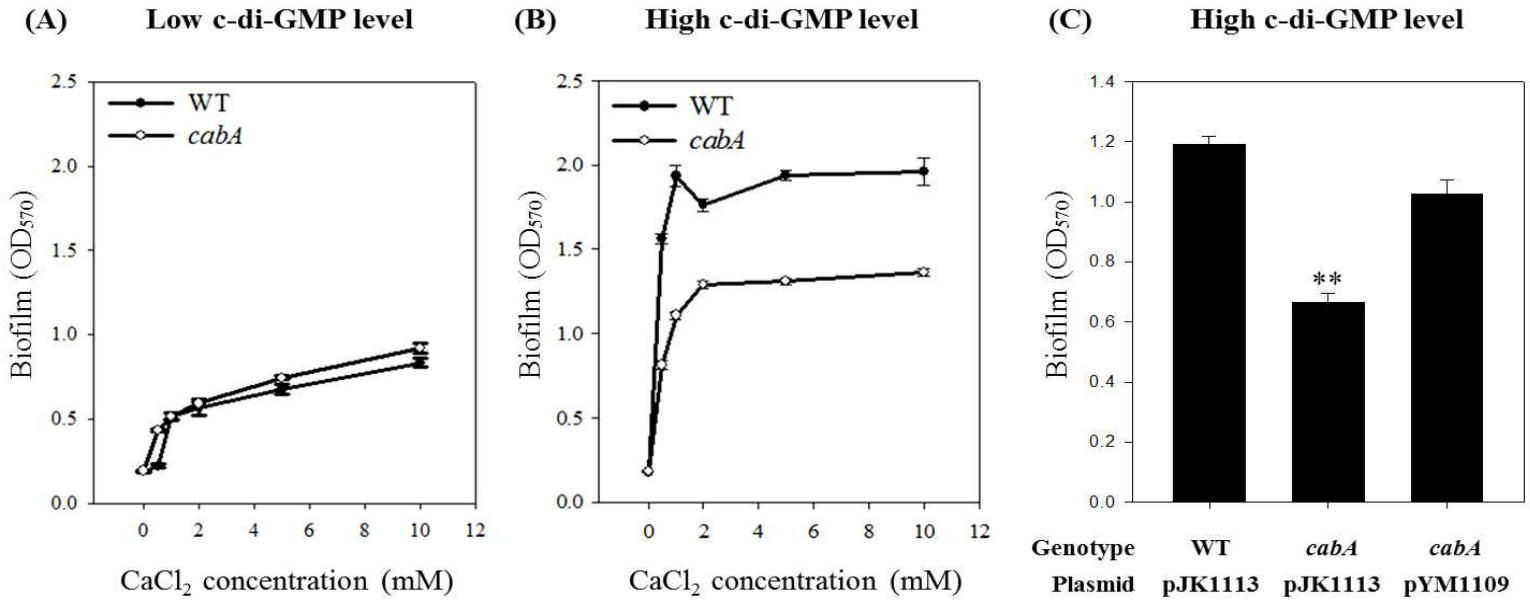


Fig. 4-4. Effects of *cabA* mutation on biofilm formation. (A and B) The wild-type and *cabA* mutant biofilms were grown on the microtiter plate wells containing VFMG supplemented with none (low c-di-GMP, A) or 0.01% (high c-di-GMP, B) of arabinose and with various levels of $CaCl_2$ for 24 h and quantitated using CV staining (Material and Method). (C) Biofilms of the strains were grown for on the microtiter plate wells containing VFMG supplemented with 0.01% of arabinose, 10 mM of $CaCl_2$, 100 μg/ml of ampicillin and kanamycin for 24 h and quantitated using CV staining. Error bars represent the SEM. **, $P < 0.05$ relative to the wild type. WT (pJK1113), wild type; *cabA* (pJK1113), *cabA* mutant; *cabA* (pYM1109), complemented strain.

4-3-5. Effect of *cabA* mutation on biofilm structure.

To determine if the *cabA* mutation effects on not only amount of biofilm but also biofilm structure, flow cell biofilms of the wild type (JN111) and mutant (YM112D) was observed using confocal laser scanning microscope (CLSM). As shown in Fig. 4-5, wild type displayed dense, uniform, and mushroom-shaped biofilms. In comparison to the wild type, markedly unstructured and loose biofilms were observed for the *cabA* mutant. In addition, it was shown that some parts of the mutant biofilms were washed out by medium flow. Consequently, it was confirmed that CabA contributed to forming and maintaining well-structured biofilms.

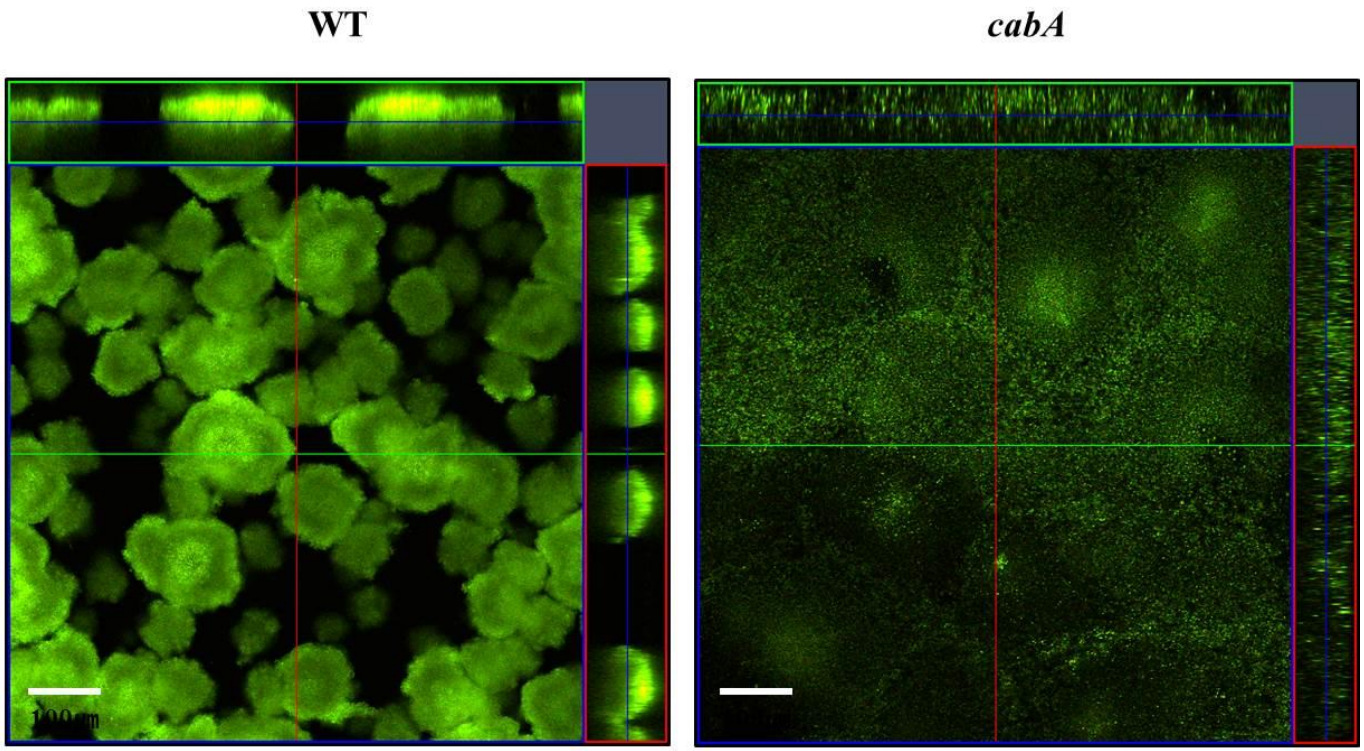


Fig. 4-5. Three-dimensional structure of the biofilms. The wild-type and *cabA* mutant biofilms were grown in the polycarbonate flow cells and were stained by LIVE/DEAD Baclight Viability Kit (Invitrogen) after 72 h of incubation. CLSM (LSM710, Zeiss) images were acquired using 10× objective. Scale bars represent 100 μm. WT, wild type; *cabA*, *cabA* mutant.

4-3-6. Effect of cabA mutation on rugose colony morphology.

Whereas the wild type (JN111) exhibited smooth colony morphology at low c-di-GMP levels (0 % arabinose), the wild type with high levels of c-di-GMP (0.01 % arabinose) formed highly wrinkled (rugose) colonies. However, *cabA* mutant (YM112D) formed smooth colonies regardless of the c-di-GMP levels. Complementation of the *cabA* mutant by introduction of pYM1109 restored colony rugosity to levels comparable to those of the wild type (Fig. 4-6). These results suggested that colony rugosity observed under high c-di-GMP levels were mediated by CabA.

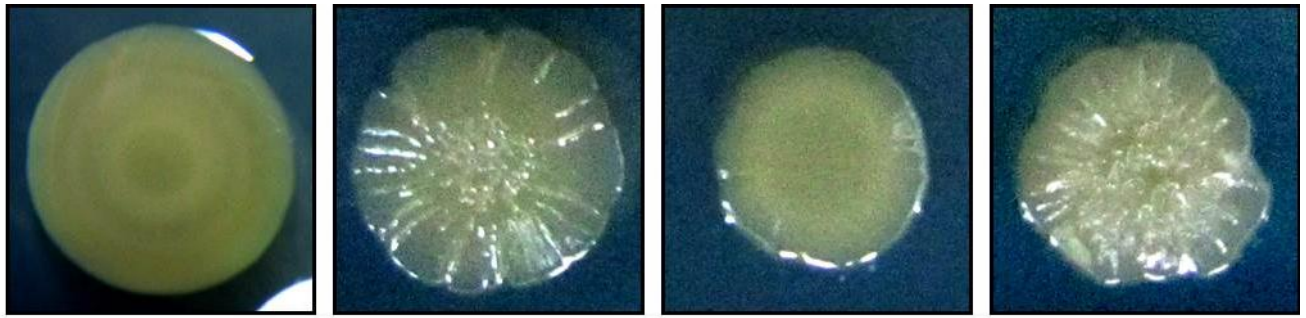


Fig. 4-6. Effects of *cabA* mutation on colony morphology. The *V. vulnificus* strains were spotted onto LBS agar plates containing either none (low c-di-GMP) or 0.01% arabinose (high c-di-GMP), then the plates were incubated at 30℃ for 24 h. Each colony showing the mean rugosity from at least three independent experiments was photographed. WT (pJK1113), wild type; *cabA* (pJK1113), *cabA* mutant; *cabA* (pYM1109), complemented strain.

4-3-7. CabA, secreted by type 1 secretion system (T1SS), exists in biofilm matrix.

It was revealed that phenotypic differences between the wild type and mutant are not attributed to the amount of EPS (data not shown). Therefore, I hypothesized that CabA could act as a structural protein and be localized to biofilm matrix. It was further hypothesized that CabA could be secreted by type 1 secretion system (T1SS) because the coding regions of *cabB* and *cabC* encoding components of T1SS were located adjacent to the coding region of *cabA*. To examine these hypotheses, Western blot analysis was performed with the wild type (JN111), *cabA* mutant (YM112D), and *cabBC* mutant (YM121D). Biofilms of the strains were fractionated through mild sonication to generate the cell fractions and the matrix fractions. To verify that the fractionation was performed without lysis of cells, the antibody against an intracellular regulator IscR, which has found to exist in the *V. Vulnificus* cytosol (data not shown), was used as a control.

CabA was not detected in medium fractions (data not shown) of the wild type, *cabA* mutant, and *cabBC* mutant, indicating that CabA was not secreted to the surrounding medium. Whereas CabA was detected in both the cell and matrix fractions of the wild type, there was no band in the matrix fraction of the *cabBC* mutant (Fig. 4-7). Thus, these data implied that CabA was secreted into biofilm matrix by T1SS composed of the proteins coded by *cabB* and *cabC*.

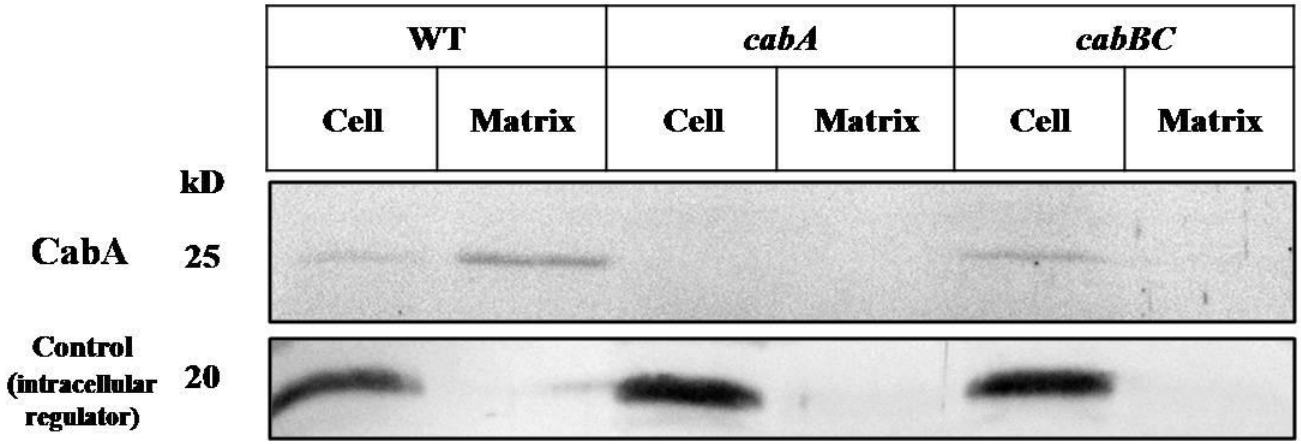
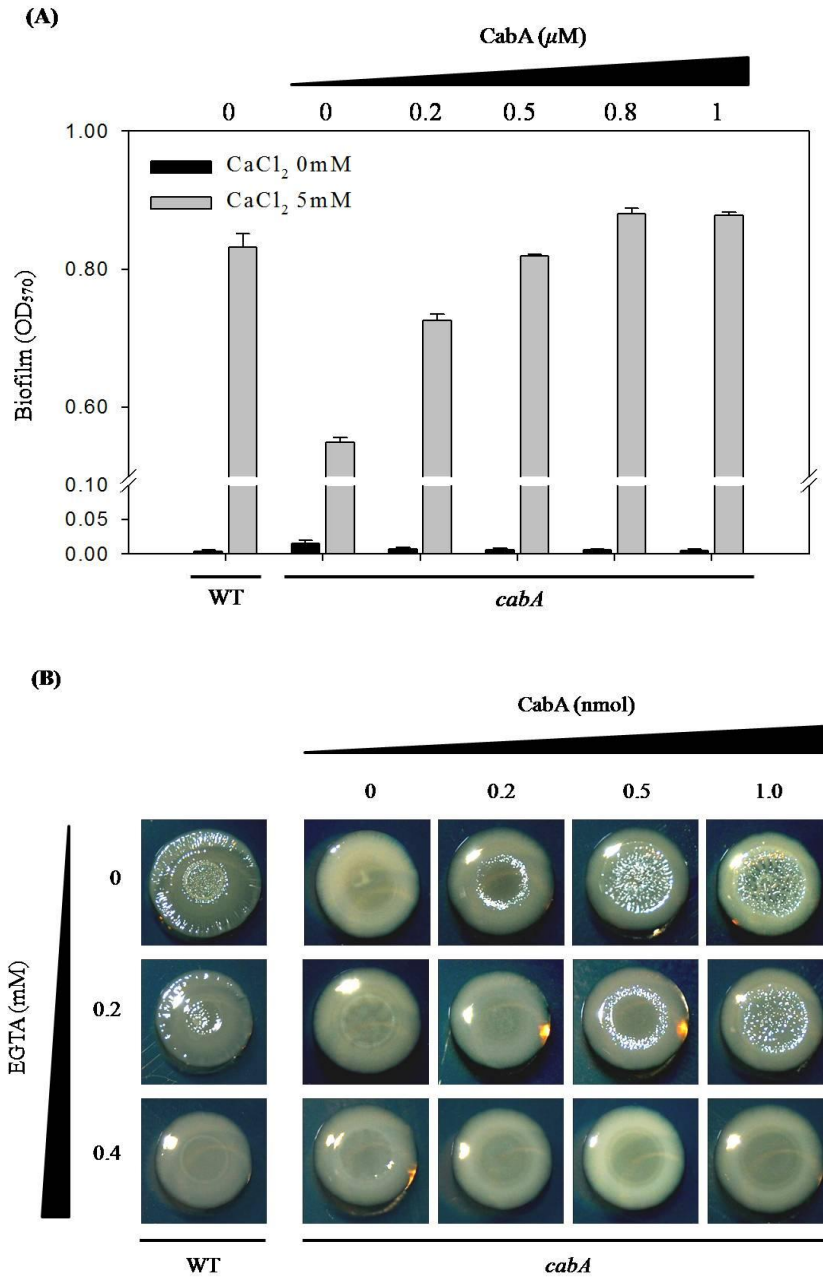


Fig. 4-7. CabA in the cell lysates and biofilm matrix fractions. Cell and matrix fractions of the wild-type (JN111), *cabA* mutant (YM112D), and *cabBC* mutant biofilms grown in the flask containing VFMG supplemented with 0.01% of arabinose were prepared. The resulting fractions, equivalent to 11 μg of total proteins, were loaded in each lane and examined for the presence of CabA protein by Western blot analyses. An intracellular regulator IscR was detected in each fraction to demonstrate that cells were not lysed during fractionation. WT, wild type; *cabA*, *cabA* mutant; *cabBC*, *cabBC* mutant; Cell, cell lysate; Matrix, matrix fraction.

4-3-8. Purified CabA restored biofilm formation activity and colony rugosity of the *cabA* mutant.

Calcium-free CabA was added exogenously to static biofilms and spotted colonies of the *cabA* mutant. The results showed that the CabA addition complemented the *cabA* mutation in terms of biofilm formation and colony rogusity in a concentration-dependent manner. The effects of the CabA addition were also dependent on the calcium concentration of the medium. When there was not available calcium in the medium, the effects of CabA addition completely disappeared (Fig. 4-8).

Fig. 4-8. Effects of CabA on biofilm and colony morphology. (A) The wild-type (JN111) and *cabA* mutant (YM112D) biofilms were grown on the microtiter plate wells containing VFMG supplemented with 0.01% arabinose and various amounts of purified CabA in the absence or presence of $CaCl_2$ for 24 h. Biofilms were quantitated by CV staining (Material and method). Error bars represent the SEM. WT, wild type; *cabA*, *cabA* mutant. (B) The colonies of the wild type (JN111) and *cabA* mutant (YM112D) were grown at 30℃ for 9 h on LBS agar plates containing 0.02 % arabinose and various levels of EGTA. Various amounts of purified CabA were added exogenously onto the colonies and then incubated for 12 hr. Each colony showing the mean rugosity from at least three independent experiments was photographed.WT, wild type; *cabA*, *cabA* mutant.


(A)
CabA (μM)
0
0
0.2
0.5
0.8
1
1.00
0.80
0.60
0.10
0.05
0.00
CaCl2 0mM
CaCl2 5mM
Biofilm (OD570)
WT
cabA
(B)
CabA (nmol)
0
0.2
0.5
1.0
EGTA (mM)
0
0.2
0.4
WT
cabA

4-3-9. Effect of BrpT on *cabABC* expression.

The effect of the *brpT* mutation on the expression of *cabABC* operon was revealed by qRT-PCR. The expression levels of *cabA*, *cabB*, and *cabC in the brpT mutant* were significantly lower than those in the wild type (Fig. 4-9A). The dependency of the *cabA* promoter activity on BrpT was investigated using *E. coli* dual plasmid system. The luciferase activity of the cells cotransformed with the plasmid (pJN0908) that express BrpT and the plasmid (pYM1204) contains *cabA* promoter and luciferase reporter increased by addition of arabinose in the medium in a concentration-dependent manner (Fig. 4-9B). The cells cotransformed with the control vector (the empty pBAD24) and pYM1204 showed no significant luciferase activity which is same in the cells cotransformed with pJN0908 and pYM1204 grown without arabinose(data not shown). These results suggested that BrpT directly activated the expression of *cabA.*

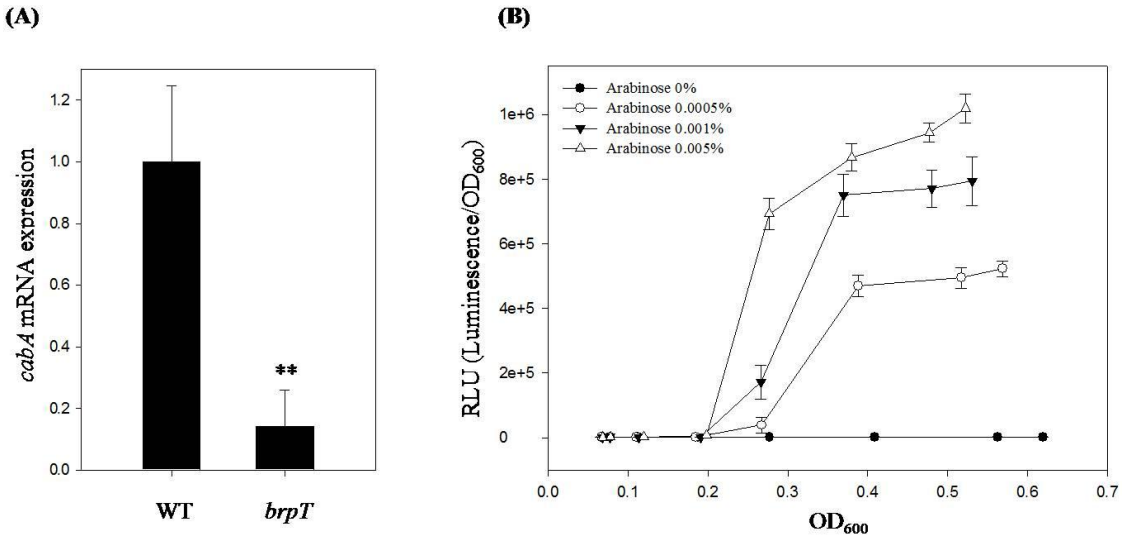


Fig. 4-9. Effect of BrpT on the expression of *cabA*. (A) The *cabA* mRNA levels were determined by qRT-PCR analyses and the *cabA* mRNA level in the wild type was presented as 1. (B) Activities of P_{cabA} were measured by using an *E. coli* dual plasmid system assay in which cells were cotransformed with pJN0908 (pBAD24::*brpT*) and pYM1204 (pBBR_lux::P_{cabA}). The luminescence of E. coli grown with different level of arabinose as indicated was measured and expressed in arbitrary RLU. Error bars represent the SEM.**, $P< 0.05$ relative to the wild type. WT , wild type; *brpT*, *brpT* mutant.

4-4. Discussion

It is well known that c-di-GMP promotes the expression of adhesive extracellular matrix components, and, in turn, regulates biofilm formation (Jenal and Malone, 2006). c-di-GMP also has been reported to promote biofilm formation and rugose colony development in *V. vulnificus* by controlling an EPS locus, *brp* (Nakhamchik *et al.*, 2008; Guo *et al*., 2010*)*. However, c-di-GMP-regulated genes have not been identified in *V. vulnificus* except brp gene cluster. The DNA microarray (Table 2-3) and the qRT-PCR analyses (Fig. 4-3B) revealed that the expression of *cabABC* operon was increased under high c-di-GMP levels. Furthermore, the *cabA* mutation caused a significant decrease in biofilm formation under high c-di-GMP levels (Fig. 4-4B). The combined results suggested that CabA is a mediator of c-di-GMP-dependent biofilm formation in *V. vulnificus*.

It is noteworthy that the effect of calcium on biofilm formation of *V. vulnificus* was much greater than those of other metal ions (data not shown). Furthermore, in the absence of calcium, the increase of c-di-GMP levels could not improve biofilm formation (Fig. 4-4A and 4B). These data implicated that calcium is an essential factor for biofilm formation and its effect is even epistatic to that of c-di-GMP. However, the mechanism how calcium promotes biofilm formation of *V. vulnificus*

has not been understood thoroughly. This study presented that CabA binds to calcium and contributes to biofilm formation and rugose colony development when calcium presents in the media (Fig. 4-4 and 4-6). Although the exact function of calcium when it binds to CabA needs additional works, calcium may have a role in multimerization or stabilization of CabA as in other calcium binding proteins, *P. putida* LapF and *P. aeruginosa* alkaline protease, reported previously elsewhere (Martinez-Gil *et al*., 2012; Zhang *et al*., 2012).

A wrinkled or rugose colony morphology is attributed to localized cell death, exopolysaccharide production, extracellular DNA release, and fibers or amyloid proteins (for a recent review, Haussler and Fuqua, 2013). *V. vulnificus* also generates morphologically and physiologically distinct rugose colonies which are regulated by the intracellular levels of c-di-GMP (Nakhamchik *et al.,* 2008). It was noted that the wild type and *cabA* mutant produced similar amount of EPS (data not shown) although they exhibited different colony morphologies (Fig. 4-6). These results are not consistent with previous studies demonstrating that rugose variants formed robust biofilms with a large amount of EPS (Anriany *et al*., 2001; Starkey *et al*., 2009; Yildiz *et al*., 2004). Our results revealed that the differences in colony morphologyof *V. vulnificus* resulted from the existence of CabA in biofilm matrix. Although the biofilm matrix proteins, RbmA, RbmC, and Bap1, have been

identified to be required for rugose colony development in *V. cholera* (Fong *et al*., 2006; Fong and Yildiz, 2007), there are not many reports about biofilm matrix proteins involved in this phenotype. The precise mechanism how CabA functions as a matrix protein to induce colony rugosity is remain unknown, nevertheless it is noteworthy that non-EPS matrix component, CabA, is required for the rugose colony development.

Another interesting feature of CabA is its localization to biofilm matrix. Biofilm matrix proteins have been studied in some bacteria. CdrA is a biofilm matrix protein which strengthens biofilm structure by binding to Psl, a major EPS component of P. aeruginosa (Borlee *et al*., 2010). RbmC and Bap1 of *V. cholerae* play crucial roles in biofilm formation and rugose colony development (Fong and Yildiz, 2007). These proteins have predicted carbohydrate binding domains (CBDs) and thus they could bind to EPS in the biofilm matrix. Although *V. vulnificus* CabA has no predicted CBDs, I cannot exclude the possibility that CabA binds to EPS. An alternative possibility involves the interaction of CabA with other matrix proteins. A structural study of CabA is underway to provide explanations about possible interactions with other matrix components.

V. vulnificus BrpT is a homologue of *V. cholera* VpsT which is a transcriptional

regulator for expression of *vps* (Vibrio polysaccharide) genes (Casper-Lindley and Yildiz, 2004). Further study about the regulation of *vps* by VpsT uncovered that VpsT activates *vps* by directly sensing c-di-GMP and that the c-di-GMP-dependent dimerization of VpsT is a key mechanism of the regulation (Krasteva *et al*., 2010). Although BrpT has been reported to regulate biofilm formation and function as a putative regulator of *brp* gene cluster in *V. vulnificus* (Guo *et al.,* 2010), its regulons were not identified experimentally. This study revealed that *V. vulnificus* BrpT activates the expression of *cabABC* operon (Fig. 4-9). Sequence analysis of *V. vulnificus* BrpT indicated that the domains required for c-di-GMP binding and dimerization are well conserved in BrpT (data not shown). It is therefore possible that BrpT activates the expression of *cabABC* by sensing c-di-GMP. If this was the case, the c-di-GMP-dependent regulation of *cabABC* expression could be explained. In summary, this study identified a secreted protein, CabA, which binds to calcium and promotes biofilm formation under high c-di-GMP condition. Our data presented here extended our understanding on the role of the calcium-binding proteinin *V. vulnificus* biofilm by demonstrating that CabA whose expression is induced by high intracellular c-di-GMP level is secreted by T1SS to the extracellular matrix, and, in turn, induces biofilm formation and rugose colony development when calcium exists in the medium. Also, I found that the expression of *cabA* and T1SS component genes, *cabB* and *cabC*, are activated by BrpT.

Although the exact mechanism how CabA functions in extracellular matrix needs additional works, the combined results suggest that CabA is one of the extracellular matrix components which play an essential role in biofilm formation and rugose colony development in *V. vulnificus.*

Chapter 5.

Conclusion

Biofilm formation abilities and colony rugosity of *V. vulnificus* increased when c-di-GMP level was elevated. A change of colony morphology from smooth to rugose was observed upon the overproduction of c-di-GMP. A whole-genome microarray screen predicted 233 genes potentially regulated by c-di-GMP, 109 of which were up-regulated and 124 of which were down-regulated. Among the c-di-GMP regulon, 12 genes were predicted to affect biofilm formation The functions of these 12 genes were classified into some categories including c-di-GMP synthesis, exopolysaccharide (EPS) synthesis, cell wall/membrane biogenesis, type 1 secretion, and adherence.

RNA-sequencing analysis revealed that 10% or more genes of *V. vulnificus* were differentially expressed between biofilm and planktonic cells regardless of the development stages. Comparison of the transcriptomes of biofilm cells from different development stages revealed that 758 genes were up- or down-regulated in biofilm cells at the mature stages compared to those at the initial stage. However, only 36 genes were differentially expressed in biofilm cells compared to those at the mature stage. These results indicated that extensive change in the expression patterns of transcriptome result in biofilm maturation, while biofilm dispersion is involved in very limited alterations of gene expression. The genes involved in the synthesis of EPS, a matrix protein, and biofilm-dispersing enzyme exhibited biofilm stage-specific expression.

V. vulnificus cabABC operon, encoding a calcium binding protein and T1SS components, is induced by high c-di-GMP levels. The disruption of *cabA* causes a significant decrease in biofilm formation and rugose colony development under the

increased intracellular level of c-di-GMP. CabA is secreted to the biofilm matrix by type 1 secretion system composed of CabB and CabC. Therefore, biofilm formation ability and colony rugosity of the *cabA* mutant can be restored by adding purified CabA exogenously. In addition, *cabA* expression is positively controlled by *V. cholera* VpsT homologue, BrpT.

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초 록

미생물의 바이오필름은 많은 인자들에 의해 조절된다고 알려져 있다. 그 중 대표적인 것이 세균의 second messenger 중 하나인 c-di-GMP이다. 세포 내의 c-di-GMP 수준이 증가하게 되면 비브리오패혈증균의 바이오필름 형성이 증가하고 콜로니의 rugosity가 증가한다. c-di-GMP에 의한 조절 유전자들을 탐색하기 위해 마이크로어레이를 수행한 결과, 비브리오패혈증균의 전체 유전체 중 5퍼센트가 넘는 유전자들이 c-di-GMP의 조절을 받는 것으로 밝혀졌다. 그 중 바이오필름에 영향을 미칠 것으로 추정되는 12개의 유전자들을 바이오필름 관련 유전자들로 동정하였고 그 유전자들의 기능은 여러 가지 범주에 걸쳐있었다. 이를 통해 c-di-GMP에 의한 바이오필름 형성 증가는 다양한 생합성 과정과 대사과정을 통해 이루어진다는 것을 알 수 있었다.

RNA sequencing을 통해 바이오필름의 각 생장단계별로 전사체의 특징을 분석하였다. 초기 부착 단계, 성숙 단계, 분해 단계에서 각각 바이오필름 세포와 부유세포를 획득하여 전사체를 분석한 결과 모든 생장 단계에서 바이오필름 세포들은 부유세포에 비해서 전체 유전체의 10퍼센트 이상이 다르게 발현된다는 것을 알 수 있었다. 각 생장단계에서의 바이오필름 세포들 간의 비교도 이루어졌다. EPS 합성에 관여하는 brp 유전

자와 c-di-GMP 농도 증가에 의해 발현이 크게 증가했던 칼슘부착단백질의 발현이 바이오필름 생장 단계와 비슷하게 증감 양상을 보였다. 바이오필름 분해에 관여하는 *vvpE* 유전자의 발현은 예상과는 다르게 성숙 단계에서부터 높은 수준으로 유지되는 것 또한 발견하였다.

앞의 두 전사체 분석 결과에서 공통적으로 발현 증가를 보였던 칼슘 부착 단백질을 coding하는 유전자 *cabA*를 동정하고 특성을 분석하였다. CabA라 명명된 이 단백질은 type 1 secretion system 구성 단백질을 coding하는 유전자들과 operon을 이루고 있다. cabA 돌연변이 균주를 만들어 그 특성을 야생종과 비교한 결과, c-di-GMP 수준이 높을 때 바이오필름 형성의 차이와 colony의 rugosity 차이를 크게 보이는 것으로 나타났다. 또한 이러한 차이는 칼슘이 배지 내에 존재 할 때만 나타났다. CabA 단백질을 type 1 secretion sytem을 통해 세포 외부의 바이오필름 matrix로 분비되어 matrix의 구성성분으로 존재하는 것으로 보인다.

주요어 : *비브리오패혈증균*, 바이오필름, c-di-GMP, 전사체 분석, RNA sequencing, 칼슘부착단백질, biofilm matrix 단백질

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