Supplementary Information

Integrative genome-scale metabolic analysis of *Vibrio vulnificus* for drug targeting and discovery

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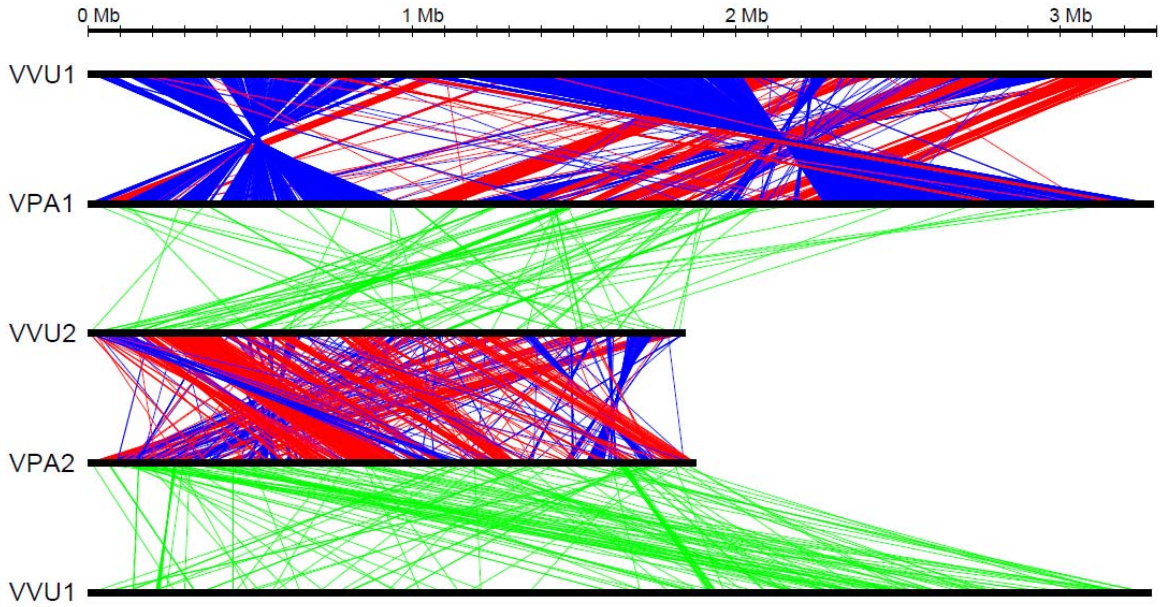
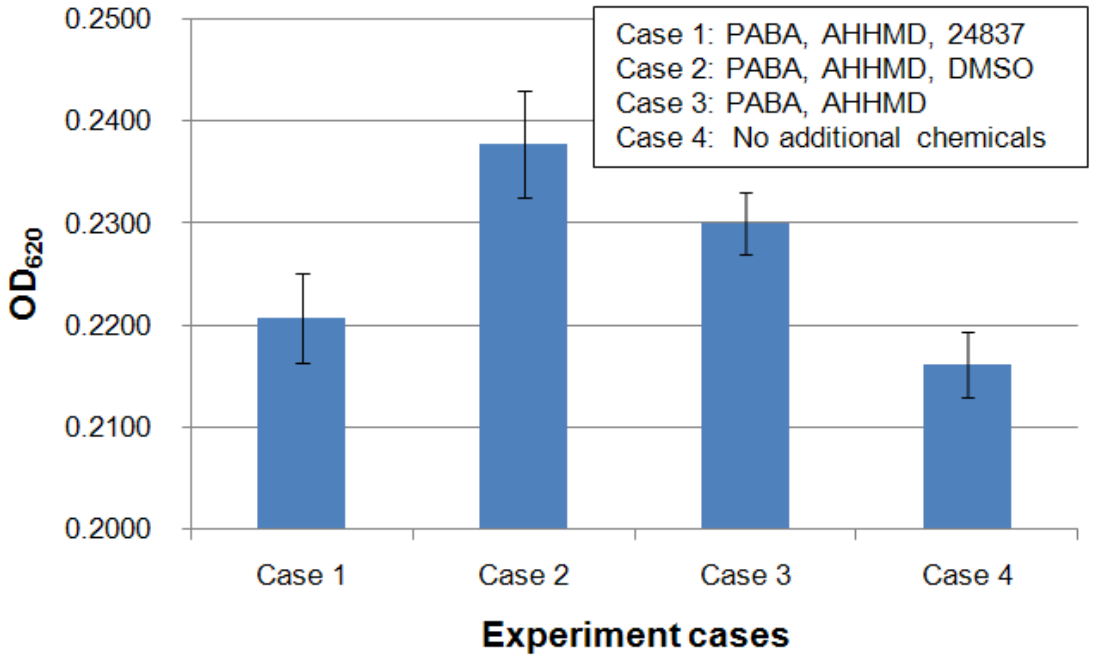
Supplementary Figure 1. Relative positions of conserved genes in *Vibrio vulnificus* and *Vibrio parahaemolyticus* genomes. The relative positions of orthologs identified by BLAST bidirectional best hits (E-value < 10^{-5}) between CMCP6 and its closest species, *V. parahaemolyticus*, were visualized by plotting lines interconnecting the pairs of corresponding genes. As shown in the plot below, gene order is more conserved throughout the large chromosome than in the small one. This suggests that genomic rearrangement of chromosome 2 is one of the marked characteristics of *Vibrionaceae* that plays a key role in shaping their genome. Red and blue lines connect genes encoded on the same and opposite strand, respectively. Gene pairs existing on the other chromosome were connected with green lines regardless of their directional orientations. Abbreviations are: VVU1, *V. vulnificus* chromosome 1; VPA 1, *V. parahaemolyticus* chromosome 1; VVU2, *V. vulnificus* chromosome 2; VPA 2, *V. parahaemolyticus* chromosome 2.



Supplementary Figure 2. Results of enzyme assay with the structural analog 24837. Each experimental case contains reaction buffer, cell extract and pyrophosphate in common, but also have additional specific chemicals. See Supplementary Methods for details. Abbreviations are: AHHMD, 2-Amino-7,8-dihydro-4-hydroxy-6-(diphosphooxymethyl)pteridine; PABA, 4-Aminobenzoate; DMSO, dimethyl sulfoxide.



Supplementary Table I. Generic genome features of representative species of *Vibrionaceae* family. Abbreviations are: VVU, *Vibrio vulnificus* CMCP6 (this study); VPA, *Vibrio parahaemolyticus* RIMD 2210633; VCH, *Vibrio cholerae* N16961; VFI, *Vibrio fischeri* ES114; VHA, *Vibrio harveyi* ATCC BAA-1116; VSP, *Vibrio splendidus* LGP32.

	Chromosome 1					
	VVU	**VPA**	**VCH**	**VFI**	**VHA**	**VSP**
Size (bp)	3,281,866	3,288,558	2,961,149	2,897,536	3,765,351	3,299,302
%G+C	46.45	45.4	47.7	39.0	45.6	44.0
No. of CDSs	2,896	3,080	2,742	2,586	3,546	2,946
Avg. CDS size (bp)	970	927	948	974	906	966
% coding	85.6	86.8	87.8	86.9	85.3	86.3
No. of rRNA operons	8	10	8	11	9	7
No. of tRNAs	98	112	94	107	105	97
	Chromosome 2					
	VVU	**VPA**	**VCH**	**VFI**	**VHA**	**VSP**
Size (bp)	1,844,830	1,877,212	1,072,315	1,330,333	2,204,018	1,675,519
% G+C	47.12	45.4	46.9	37.0	45.3	43.6
No. of CDSs	1,537	1,752	1,093	1,175	2,374	1,485
Avg. CDS size (bp)	1,044	933	831	991	804	969
% coding	87.0	87.0	84.7	87.5	86.6	85.9
No. of rRNA operons	1	1	0	1	1	1
No. of tRNAs	13	14	4	11	16	17
	Plasmid					
	VVU	**VPA**	**VCH**	**VFI**	**VHA**	**VSP**
Size (bp)				45,849	89,008	
%G+C				38.4	43.8	
No. of CDSs				57	120	

Supplementary Table II. 17 largest clusters (≥ 10 members) found in six completely sequenced Vibrios. Product names were adopted from the *V. cholerae* annotation data (KEGG). Abbreviations are: VVU, *V. vulnificus* CMCP6 (this study); VPA, *V. parahaemolyticus* RIMD 2210633; VCH, *V. cholerae* N16961; VFI, *V. fischeri* ES114; VHA, *V. harveyi* ATCC BAA-1116; VSP, *V. splendidus* LGP32.

Cluster 1 (31 genes): flagellin
- VVU: VV1_0215, VV1_0214, VV1_1923, VV1_1924, VV1_1926
- VPA: VP0788, VP0790, VP2259, VP2261, VP2258
- VCH: VC2188, VC2144, VC2143, VC2187, VC2142
- VFI: VF_1862, VF_1866, VF_1864, VF_1865, VF_2079, VF_1863
- VHA: VIBHAR_01300, VIBHAR_01301, VIBHAR_03173, VIBHAR_03174, VIBHAR_03171
- VSP: VS_0813, VS_2293, VS_0812, VS_0814, VS_2294

Cluster 2 (18 genes): cold-shock protein
- VVU: VV1_2757, VV2_0503, VV2_0519
- VPA: VP1889, VPA1289, VPA0552
- VCH: VCA0166, VCA0933, VCA0184
- VFI: VF_2561, VF_A1094, VF_A0595
- VHA: VIBHAR_06418, VIBHAR_00522, VIBHAR_05396
- VSP: VS_II0326, VS_0175, VS_II0495

Cluster 3 (13 genes): transketolase (EC 2.2.1.1)
- VVU: VV1_1537, VV2_0553
- VPA: VP2605, VPA1181
- VCH: VC0473, VCA0624
- VFI: VF_0440, VF_A0686
- VHA: VIBHAR_03567, VIBHAR_06257
- VSP: VS_2654, VS_II0463, VS_0168

Cluster 4 (13 genes): DNA-binding protein HU-beta
- VVU: VV1_0019, VV1_1224
- VPA: VP2911, VP0920
- VCH: VC0273, VC1919
- VFI: VF_0799, VF_2397
- VHA: VIBHAR_00213, VIBHAR_01420
- VSP: VS_2954, VS_2201, VS_1735

Cluster 5 (12 genes): PTS-system, fructose-specific II-like component
- VVU: VV2_1353, VV2_1356
- VPA: VPA1420, VPA0297
- VCH: VC1822, VC1821
- VFI: VF_A0713, VF_A0370
- VHA: VIBHAR_05188, VIBHAR_06592
- VSP: VS_II1316, VS_II1323

Cluster 6 (12 genes): inosine kinase (EC 2.7.1.73)
- VVU: VV1_2924, VV2_0218
- VPA: VP1132, VPA0835
- VCH: VC1129, VCA0801
- VFI: VF_0911, VF_A0696
- VHA: VIBHAR_01826, VIBHAR_05942
- VSP: VS_2030, VS_II0808

Cluster 7 (12 genes): elongation factor EF-Tu (EC 3.6.5.3)
- VVU: VV1_1203, VV1_1339
- VPA: VP2770, VP2930
- VCH: VC0321, VC0362
- VFI: VF_0233, VF_2423
- VHA: VIBHAR_00054, VIBHAR_00233
- VSP: VS_2972, VS_2834

Cluster 8 (12 genes): spermidine/putrescine transport system substrate-binding protein
VVU: VV1_2600, VV1_2601
VPA: VP1526, VP1525
VCH: VC1425, VC1424
VFI: VF_1319, VF_1318
VHA: VIBHAR_02285, VIBHAR_02286
VSP: VS_1478, VS_1477

Cluster 9 (12 genes): peptide/nickel transport system ATP-binding protein
VVU: VV1_1670, VV2_1294, VV2_1178
VPA: VP2482, VPA0004
VCH: VC0617
VFI: VF_2142, VF_A0200
VHA: VIBHAR_03426, VIBHAR_07043
VSP: VS_2509, VS_II1528

Cluster 10 (12 genes): bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
VVU: VV1_0461, VV2_1559
VPA: VP0576, VPA1458
VCH: VC0726, VCA0073
VFI: VF_1983, VF_1613
VHA: VIBHAR_01033, VIBHAR_05140
VSP: VS_0587, VS_II0208

Cluster 11 (12 genes): purine-nucleoside phosphorylase (EC 2.4.2.1)
VVU: VV1_1728, VV2_1540
VPA: VP2433, VPA1475
VCH: VC2347, VCA0053
VFI: VF_0507, VF_A0402
VHA: VIBHAR_03374, VIBHAR_05118
VSP: VS_2460, VS_II0197

Cluster 12 (12 genes): nitrogen regulatory protein P-II
VVU: VV1_1658, VV1_1879
VPA: VP2299, VP2493
VCH: VC0606, VC2239
VFI: VF_1942, VF_2153
VHA: VIBHAR_03218, VIBHAR_03437
VSP: VS_2333, VS_2520

Cluster 13 (11 genes): glycine hydroxymethyltransferase (EC 2.1.2.1)
VVU: VV2_0188, VV1_0286
VPA: VPA0803, VP0715
VCH: VCA0278, VC0941
VFI: VF_0695
VHA: VIBHAR_05971, VIBHAR_01208
VSP: VS_II0818, VS_2373

Cluster 14 (11 genes): D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)
VVU: VV1_0281, VV2_1681
VPA: VP0719, VPA1296
VCH: VC0947, VCA0270
VFI: VF_0745
VHA: VIBHAR_01214, VIBHAR_06426
VSP: VS_II0550, VS_0710

Cluster 15 (11 genes): UDP-glucose 4-epimerase (EC 5.1.3.2)
VVU: VV1_1770, VV2_1095, VV1_1342
VPA: VP2400, VPA0879
VCH: VCA0774
VFI: VF_A0352, VF_0201
VHA: VIBHAR_05793, VIBHAR_03331
VSP: VS_II0752

Cluster 16 (11 genes): general L-amino acid transport system ATP-binding protein (EC 3.6.3.-)

VVU: VV1_2706
VPA: VP1623, VPA0822
VCH: VC1359, VCA1037
VFI: VF_1535, VF_A0393
VHA: VIBHAR_02364, VIBHAR_05950
VSP: VS_1400, VS_II0534

Cluster 17 (10 genes): hypothetical protein

VVU: VV1_1828, VV2_0944
VPA: VP2344, VPA0324
VCH: VC2288, VC1322
VFI: VF_0733, VF_1434
VHA: VIBHAR_03268
VSP: VS_0700

Supplementary Table V. Biomass composition of *V. vulnificus* CMCP6.

Supplementary Table V-A. Macromolecular composition. Macromolecular composition of *V. vulnificus* was assumed to be the same as that of *Escherichia coli* based on the fact that they are both gram-negative, and possess similar size of DNA molecules (Neidhardt & Umbarger, 1996).

Component	Composition (g/gDCW)	Comments
Protein	0.550	-
DNA	0.031	-
RNA	0.205	-
Phospholipid	0.091	-
Cofactors and vitamins	0.030	-
Lipopolysaccharide	0.034	-
Peptidoglycan	0.025	-
Glycogen	0.025	-
Ash	0.009	Other unknown compounds were assumed to be ash (not included in this model).
Sum	1.000	

Supplementary Table V-B. Amino acid composition. The amino acid composition was determined by the Waters HPLC systems (Water Corporation, Milford, MA). Absorbance at 254 nm was measured. See Supplementary Methods for details.

Amino acids	mmol/g protein
Alanine	1.521
Arginine	0.384
Asparagine	0.438
Aspartate	0.438
Cysteine	0.108
Glutamine	0.645
Glutamate	0.645
Glycine	1.002
Histidine	0.196
Isoleucine	0.479
Leucine	0.673
lysine	0.373
Methionine	0.126
Phenylalanine	0.283
Proline	0.392
Serine	0.407
Threonine	0.500
Tryptophan	0.109
Tyrosine	0.115
Valine	0.712

Supplementary Table V-C. DNA composition. The DNA composition was determined from the genomic sequence of *V. vulnificus*. GC content of *V. vulnificus* is set to 46.79% (= (46.45 + 47.12)/2).

Nucleotide	mol/mol DNA	MW, g/mol	mmol/g DNA
dAMP	0.266	313.2	0.861
dGMP	0.234	329.2	0.757
dCMP	0.234	289.2	0.757
dTMP	0.266	304.2	0.861

Supplementary Table V-D. RNA composition. It was assumed that mRNA makes up 5% and rRNA 80% of the total RNA. The rest was assumed to be tRNA (Brown, 1999).

Nucleotide	mol/mol RNA			MW, g/mol	mol/mol RNA	mmol/g RNA
	5% mRNA	80% rRNA	15% tRNA			
AMP	0.266	0.260	0.194	329.2	0.250	0.773
GMP	0.234	0.308	0.319	345.2	0.306	0.946
CMP	0.234	0.217	0.265	305.2	0.225	0.696
UMP	0.266	0.214	0.223	306.2	0.218	0.674

Supplementary Table V-E. Phospholipid composition. The composition of phospholipids was taken from (Oliver & Colwell, 1973).

Component	mmol/g phospholipids
Lysophosphatidylethanolamine	0.155
Phosphatidylethanolamine	0.908
Phosphatidylglycerol	0.268

Supplementary Table V-F Composition of fatty acids in phospholipids. The composition of fatty acids in phospholipids was determined by gas chromatographic analysis of fatty acid methyl esters (GC-FAME). See Supplementary Methods for details.

Fatty acid	g/g total fatty acids	MW, g/mol	mmol/g total fatty acids	mol/mol total fatty acids
12:0	0.041	200.320	0.205	0.052
14:0	0.164	228.370	0.721	0.182
15:0	0.048	242.400	0.199	0.050
16:0	0.394	256.420	1.544	0.390
16:1	0.241	254.400	0.952	0.240
18:0	0.011	284.480	0.040	0.010
18:1	0.084	282.460	0.300	0.076

Supplementary Table V-G. Cofactors and vitamins incorporated in the biomass. Cofactors and vitamins are assumed to be the same ratio (w/w).

Molecule	MW, g/mol	g/g cofactors and vitamins	mmol/g cofactors and small molecules
Coenzyme A	767.535	0.1	0.130
Flavin adenine dinucleotide	785.550	0.1	0.127
Flavin mononucleotide	456.344	0.1	0.219
Menaquinone	308.414	0.1	0.324
NAD	664.433	0.1	0.151
NADP	744.413	0.1	0.134
Pyridoxine	169.178	0.1	0.591
Tetrahydrofolate	445.430	0.1	0.225
Thiamin	265.356	0.1	0.377
Ubiquinone	318.407	0.1	0.314

Supplementary Table V-H. Lipopolysaccharide (LPS) composition. Lipopolysaccharide composition of *V. vulnificus* was taken from (Iguchi et al, 1989).

Component	Molar ratio	MW, g/mol	mmol/g LPS
UDPglucose	8.9	566.302	1.149
beta-D-Fructose 6-phosphate	0.7	260.136	0.090
ADP-L-glycero-D-manno-heptose	3.0	619.368	0.387
UDP-N-acetyl-D-glucosamine	1.1	607.354	0.142

Supplementary Table VI. Topological parameters of VvuMBEL943 and metabolic networks of other organisms. The topology of VvuMBEL943 was analyzed using Cytoscape (Cline et al, 2007; Shannon et al, 2003) with the plugin NetworkAnalyzer (Assenov et al, 2008), and compared with metabolic networks of other organisms previously reported (Assenov et al, 2008; Ma & Zeng, 2003a; Shannon et al, 2003). The average path length (APL) and the network diameter (ND) were of particular interest as they show distinct values among different domains of organisms (Assenov et al, 2008; Ma & Zeng, 2003a). APL represents the expected distance between two connected nodes, while ND refers to the maximum length of the shortest paths between two nodes (Assenov et al, 2008). APL and ND of VvuMBEL943 were calculated to be 7.311 and 28, respectively, which are consistent with those of bacteria on average, 7.22 and 20.6 (Ma & Zeng, 2003a). These values are distinct from those of eukaryotes and archaea (Ma & Zeng, 2003a); the average APL and ND for eukaryotes are 9.57 and 33.1, respectively, while those of archaea are 8.50 and 23.4. The APL and ND values of VvuMBEL943 were more similar to those of *V. cholerae* (7.64 and 26) than those of *E. coli* (8.2 and 23) (Ma & Zeng, 2003a).

Topological parameters	*V. vulnificus*	*E. coli*	*V. cholerae*	Bacteria	Eukaryotes	Archaea
Average path length	7.311	8.2	7.64	7.22	9.57	8.50
Network diameter	28	23	26	20.6	33.1	23.4
Clustering coefficient	0.066	-	-	-	-	-
Connected components	33	-	-	-	-	-
Network radius	1	-	-	-	-	-
Shortest paths	341,403	-	-	-	-	-
Avg. number of neighbors	3.268	-	-	-	-	-
Number of nodes	828	-	-	-	-	-
Number of edges	2,077	-	-	-	-	-
Network density	0.005	-	-	-	-	-
Isolated nodes	0	-	-	-	-	-
Number of self-loops	0	-	-	-	-	-
Multi-edge node pairs	446	-	-	-	-	-

Supplementary Table VII. Initial set of 193 essential metabolites predicted under the arbitrary complex medium. Full name of each essential metabolite is available in Supplementary Table IV.

Essential metabolites
2AG3PE, 2PCDPMDE, 3A2OP, 3DDAH7P, 3PSME, 4PPNCYS, 4PPNTE, 4PPNTO, A6RP, A6RP5P, A6RP5P2, ACACP, ACCOA, ACP, ADCHOR, ADP, ADPDGDMHEP, ADPG, ADPHEP, AGL3P, AHHMP, AHTD, AKG, ALA, ALAALA, AMP, ARG, ASN, ASP, ASPSA, ATP, bALA, C120ACP, C140ACP, C150ACP, C160ACP, C161ACP, C180ACP, C181ACP, CAV, CDPDG, CDPMDE, CHOR, CO2, COA, CTP, CYS, D6RP5P, D8RL, DALA, DAPIM, DATP, DB4P, DCTP, DGDMH17BP, DGDMH1P, DGDMH7P, DGLU, DGTP, DHAP, DHDP, DHF, DHN, DHP, DHPANT, DHPT, DHSK, DMK, DMPP, DNA, DPCOA, DQT, DTDP, DTMP, DTTP, DX5P, E4P, ER4P, F6P, FAD, FMN, FPP, FUM, G1P, G3P, G6P, GDP, GGPP, GL3P, GLN, GLU, GLY, GLYCOGEN, GMP, GPP, GTP, HEPPP, HIS, HMB4PP, HPPP, IASP, ICHOR, ILE, IPP, LEU, LPS, LYS, MALACP, MALCOA, MDAPIM, MDE4P, MDECPP, MET, METTHF, MK, MKH2, MTHF, NAAD, NACN, NAD, NADH, NADP, NADPH, NH3, OBUT, OHB, OIVAL, OPP, OSB, OSBCOA, OTHIO, P5P, PA, PABA, PANT, PE, PEP, PEPTIDO, PG, PGP, PHE, PHOSPHOLIPID, PHT, PI, PNTO, PPAACP, PPACOA, PPEPTIDO, PPPP, PRO, PROTEIN, PRPP, PS, PYR, PYRDX, QA, R5P, RIBFLAV, RL5P, RNA, RTHIO, S7P, SAM, SAOPIM, SDAPIM, SER, SHCHC, SME, SME3P, SUCCOA, TDHDP, THF, THR, TRP, TYR, UDCP, UDCPP, UDP, UDPG, UDPMNLADGMD, UDPMNLADGMDDADA, UDPNAG, UDPNAGEP, UDPNAM, UDPNAMA, UDPNAMAG, UMP, UPPMNGNLADGMDDADA, UPPMNGNLADGNMDG5DADA, UPPMNGNLADGNMDDADA, UPPMNLADGMDDADA, UTP, VAL

Supplementary Table VIII. List of 140 essential reactions predicted from reaction essentiality analysis under the arbitrary complex medium. Information of each reaction is available in Supplementary Table III. See Supplementary Methods for details.

Reaction number of essential enzymes
R006, R040, R041, R089, R090, R120, R121, R149, R157, R197, R198, R199, R200, R204, R206, R207, R209, R210, R213, R214, R218, R219, R220, R221, R222, R223, R224, R225, R226, R227, R228, R229, R230, R231, R232, R233, R234, R236, R237, R238, R239, R240, R254, R255, R259, R260, R262, R263, R265, R266, R324, R342, R343, R350, R351, R353, R413, R451, R453, R456, R457, R500, R503, R504, R505, R506, R568, R569, R570, R572, R573, R574, R575, R628, R644, R645, R646, R647, R648, R649, R650, R651, R652, R653, R654, R655, R656, R657, R658, R659, R660, R668, R669, R670, R671, R672, R673, R674, R675, R676, R679, R680, R681, R683, R684, R695, R697, R698, R703, R704, R709, R713, R714, R715, R716, R719, R720, R721, R722, R732, R733, R734, R736, R737, R740, R783, R793, R794, R795, R796, R797, R798, R801, R802, R803, R804, R805, R806, R807, R838

Supplementary Table IX. Six sets of synthetic lethal reactions, each caused by essential metabolites. All sets of reactions affected by each essential metabolite were investigated under the arbitrary complex medium to see if true synthetic lethals exist. The synthetic lethal reactions refer to those that are not essential when alone, but become essential when other relevant reaction(s) are inactivated simultaneously. In this regard, metabolite essentiality analysis itself conveys, although limited to those around the essential metabolites, the concept of synthetic lethality because all the reactions connected with the removed metabolite are also removed from the cellular system. As a result of this analysis, 6 sets of reactions around 6 essential metabolites were predicted to be complete synthetic lethals, in which none of the reactions are individually essential. Five sets of reactions were caused by essential metabolites involved in the metabolism of cofactors and vitamins, including dihydrofolate (DHF), pyridoxine 5'-phosphate (P5P), 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine (AHHMP), 4-aminobenzoate (PABA) and 2,3-dihydrodipicolinate (DHDP), while the other set was related to the reduced thioredoxin (RTHIO). Reaction information is available in Supplementary Table III, respectively.

Essential metabolites	No. of total consuming reactions	Consuming reactions
AHHMP	2	R735/R739
DHDP	2	R501/R502
DHF	4	R741/R742/R743/R744
P5P	2	R686/R687
PABA	2	R738/R739
RTHIO	11	R193/R322/R326/R329/R330/R347/R362/R363/R364/R449/R463

Supplementary Table X. Comparison of essential metabolites in *V. vulnificus* with those of two Gram negative bacteria, *E. coli* and *Helicobacter pylori*. Full name of each essential metabolite is available in Supplementary Table IV.

Categories	List of essential metabolites
Common essential metabolites in *V. vulnificus* and *E. coli* (119 metabolites)	3DDAH7P, 3PSME, 4PPNCYS, 4PPNTE, 4PPNTO, A6RP, A6RP5P, A6RP5P2, ACCOA, ACP, ADCHOR, ADPDGDMHEP, ADPG, ADPHEP, AHTD, ALA, ALAALA, AMP, ARG, ASN, ASP, ATP, C120ACP, C161ACP, C181ACP, CDPDG, CHOR, CO2, COA, CTP, CYS, D6RP5P, D8RL, DALA, DATP, DB4P, DCTP, DGDMH17BP, DGDMH1P, DGDMH7P, DGLU, DGTP, DHF, DHP, DHPT, DHSK, DPCOA, DQT, DTDP, DTMP, DTTP, E4P, FAD, FMN, G1P, G3P, GDP, GL3P, GLN, GLU, GLY, GLYCOGEN, GMP, GTP, HIS, ILE, LEU, LPS, LYS, MALACP, MALCOA, MET, METTHF, MTHF, NAD, NADH, NADP, NADPH, OTHIO, PA, PABA, PE, PEP, PEPTIDO, PG, PGP, PHE, PI, PNTO, PRO, PS, RIBFLAV, RL5P, RTHIO, S7P, SER, SME, SME3P, SUCCOA, THF, THR, TRP, TYR, UDCP, UDCPP, UDP, UDPG, UDPMNLADGMD, UDPMNLADGMDDADA, UDPNAG, UDPNAGEP, UDPNAM, UDPNAMA, UDPNAMAG, UMP, UPPMNGNLADGMDDADA, UPPMNLADGMDDADA, UTP, VAL
Common essential metabolites in *V. vulnificus* and *H. pylori* (115 metabolites)	3DDAH7P, 3PSME, ACCOA, ACP, ADCHOR, ADP, ADPDGDMHEP, ADPHEP, AHHMP, AHTD, AKG, ALA, ALAALA, AMP, ARG, ASN, ASP, ASPSA, ATP, C180ACP, C181ACP, CDPDG, CHOR, COA, CTP, CYS, DALA, DATP, DCTP, DGDMH17BP, DGDMH1P, DGDMH7P, DGLU, DGTP, DHAP, DHDP, DHF, DHP, DHPT, DHSK, DQT, DTDP, DTMP, DTTP, E4P, F6P, FAD, G3P, GDP, GL3P, GLN, GLU, GLY, GMP, GTP, HIS, ILE, LEU, LPS, LYS, MALACP, MALCOA, MET, METTHF, MK, MTHF, NAD, NADH, NADP, NADPH, NH3, OTHIO, PA, PABA, PE, PEP, PEPTIDO, PG, PGP, PHE, PI, PRO, PRPP, PS, PYR, R5P, RL5P, RTHIO, S7P, SAM, SER, SME, SME3P, SUCCOA, TDHDP, THF, THR, TRP, TYR, UDCP, UDCPP, UDP, UDPG, UDPMNLADGMD, UDPMNLADGMDDADA, UDPNAG, UDPNAGEP, UDPNAM, UDPNAMA, UDPNAMAG, UMP, UPPMNGNLADGMDDADA, UPPMNLADGMDDADA, UTP, VAL
Essential metabolites of *V. vulnificus* commonly predicted in both *E. coli* and *H. pylori* (98 metabolites)	3PSME, ACCOA, ACP, ADCHOR, ADPDGDMHEP, ADPHEP, AHTD, ALA, ALAALA, AMP, ARG, ASN, ASP, ATP, C181ACP, CDPDG, CHOR, COA, CTP, CYS, DALA, DATP, DCTP, DGDMH17BP, DGDMH1P, DGDMH7P, DGLU, DGTP, DHF, DHP, DHPT, DHSK, DQT, DTDP, DTMP, DTTP, E4P, FAD, GDP, GL3P, GLN, GLU, GLY, GMP, GTP, HIS, ILE, LEU, LPS, LYS, MALACP, MALCOA, MET, METTHF, MTHF, NAD, NADH, NADP, NADPH, OTHIO, PA, PABA, PE, PEP, PEPTIDO, PG, PGP, PHE, PI, PRO, PS, RL5P, RTHIO, S7P, SER, SME, SME3P, SUCCOA, THF, THR, TRP, TYR, UDCP, UDCPP, UDP, UDPG, UDPMNLADGMD, UDPMNLADGMDDADA, UDPNAG, UDPNAGEP, UDPNAM, UDPNAMA, UDPNAMAG, UMP, UPPMNGNLADGMDDADA, UPPMNLADGMDDADA, UTP, VAL
Essential metabolites of *V. vulnificus* predicted in *E. coli*, but not *H. pylori* (21 metabolites)	3DDAH7P, 4PPNCYS, 4PPNTE, 4PPNTO, A6RP, A6RP5P, A6RP5P2, ADPG, C120ACP, C161ACP, CO2, D6RP5P, D8RL, DB4P, DPCOA, FMN, G1P, G3P, GLYCOGEN, PNTO, RIBFLAV
Essential metabolites of *V. vulnificus* predicted in *H. pylori*, but not *E. coli* (17 metabolites)	3DDAH7P, ADP, AHHMP, AKG, ASPSA, C180ACP, DHAP, DHDP, F6P, G3P, MK, NH3, PRPP, PYR, R5P, SAM, TDHDP

Supplementary Table XI. Primers used for the mutant construction.

Gene	Primer used for insertional mutation		Primer used for deletion mutation	
	Name	Sequence (restriction enzyme)[a]	Name	Sequence (restriction enzyme and overlapped region)[b]
VV10323	323-1	GAGCTCGTAAACAATCAGTTCGTCCAC (*Sac*I)	323-3	GAGCTCGTAATCCAAGACGCTGGCAAC (*Sac*I)
	323-2	CTCGAGGAGCCTTGTTCACACTATG (*Xho*I)	323-4	**CGGTTCACCATG**TGATATGTTCACAGGAATTATC
			323-5	**GAACATATCA**CATGGTGAACCGTTACCTTGCG
			323-6	TCTAGACCTTCCGCTTGTAATGCGCTGG (*Xba*I)
VV11644	1644-1	TCTAGAGGGAGGGTTAAATTTGGTGC (*Xba*I)	1644-3	TCTAGAGATCTGTTCACGAAGGGCTG (*Xba*I)
	1644-2	GAGCTCTAAAAACGCTGCCAAAATCG (*Sac*I)	1644-4	**TCATGATTACT**TAGCCAAATCCTGGTAAGGAAAAAT
			1644-5	**GGATTTGGCTA**AGTAATCATGATTTGGCTTTGC
			1644-6	GAGCTCACGTGATTTACTGCTGGGAC (*Sac*I)
VV11691	1691-1	GAGCTCGAAGTCTCATTGGAAGAAGA (*Sac*I)	1691-3	GAGCTCGTGCTGATTTAGCCAACCTA (*Sac*I)
	1691-2	TCTAGAACCATCGATTTACGAGACAT (Xbal)	1691-4	**CTTTTTTACTCA**CATCACAACAGGGAATGAAAG
			1691-5	**CTGTTGTGATG**TGAGTAAAAAAGAATATAAGAAGG
			1691-6	TCTAGAACCTTGTCCAGCAAAGCGCA (*Xba*I)
VV11175	1175-1	GAGCTCACGACTGCCTGAAGTGAACT (*Sac*I)	1175-3	GAGCTCAAGAATGAGTTACCATCGGTAAG (*Sac*I)
	1175-2	CTCGAGTGTCTTGGTACTTTGTGGTG (*Xho*I)	1175-4	**CCCATCGTTTT**CACTCTGATAAACCTACTTAGA
			1175-5	**TTATCAGAGTG**AAAACGATGGGGTTTAACGCTATA
			1175-6	TCTAGACAATTTCCCAGGCATTACTCACCC (*Xba*I)
VV10580	580-1	GAGCTCACTCGATTTGTTGGAGCAAG (*Sac*I)	580-3	GAGCTCACCTTTGGTAGGGACCATC (*Sac*I)
	580-2	TCTAGATCTTCCATCCGCTCAAAACG (*Xba*I)	580-4	**TAATGAAATATG**TAGCGCAACCTCTAACGCGTATTT
			580-5	**GGTTGCGCTA**CATATTTCATTACACCGTAAAAAG
			580-6	TCTAGAGGCCAACGAAAATGAAGCCTGTCA (*Xba*I)
VV10567	567-1	GAGCTCGGTTAAAGCAGCACATCACA (*Sac*I)	567-3	GAGCTCCAATTCGCTGTCATCTTTCG (*Sac*I)
	567-2	CTCGAGGGTAATTTCTACACGCTCAC (*Xho*I)	567-4	**GAAAAATGCAATG**TAAATAAATAATTATGCGCCAGCC
			567-5	**TAATTATTTATTTA**CATTGCATTTTTCTCCGTGTTTCTG
			567-5	TCTAGACATTAATACCGCGGTTGAAGTCTAC (*Xba*I)
VV11866	1866-1	GAGCTCCACTTTGCTATGTCGCTAC (*Sac*I)	1866-3	CTCGAGCCCGATTTTGGTGAAGAAAG (*Xho*I)
	1866-2	CTCGAGGTTCAAAATCAGGTTGTAGG (*Xho*I)	1866-4	**TCTCTAACAAC**CATAACTGTTGACCGAATAAAG
			1866-5	**CAACAGTTATG**GTTGTTAGAGAGCGTGCACAATG
			1866-6	TCTAGACCCACATCACCAGTTATCAGCTTC (*Xba*I)
VV11568	1568-1	CTCGAGGGCATTACCATTCATTTACG (*Xho*I)	11568-3	CTCGAGAGCGTAGCGGCCAGAAGAAA (*Xho*I)
	1568-2	CTCGAGTGTTCAGCTCGTAAATCTCA (*Xho*I)	1568-4	**CAATAGCCATTTA**ACTCATTTTTCCATACTCCGTGTTC
			1568-5	**GAAAAATGAGT**TAAATGGCTATTGTTGGACTGGGTAC
			1568-6	TCTAGAGGCTGTCTGCTGCGGTATAG (*Xba*I)

[a] The restriction enzyme site is underlined.

[b] The overlapping PCR sequence is bolded.

Supplementary Methods

Biomass composition of *V. vulnificus* CMCP6

Biomass composition was determined based on the previously reported literature except for compositions of amino acids and fatty acids; they were experimentally determined, which constitute a major portion of the cell. Biomass composition studied includes compositions of macromolecules, amino acids, DNA, RNA, phospholipids and their constituting fatty acids, cofactors, vitamins, and lipopolysaccharides. They are presented in detail in Supplementary Table V. Firstly, overall macromolecular composition of *V. vulnificus* was assumed to be the same as that of *E. coli* based on their biological similarities (Neidhardt & Umbarger, 1996). DNA and RNA compositions were determined based on the genome sequence of *V. vulnificus* CMCP6. Compositions of phospholipids and lipopolysaccharides were determined from Oliver and Colwell (1973) and Iguchi (1989), respectively. Compositions of cofactors and vitamins were calculated with the assumption that they exist in the equal amount in a unit gram pool. To establish a reaction for the biosynthesis of proteins, amino acid composition was experimentally determined at Korea Basic Science Institute (KBSI, Daejeon, Korea). They were determined by the Waters HPLC systems (Waters, Milford, MA) that consist of two Waters 510 HPLC Pumps, Gradient Controller, 717 Automatic Sampler, 996 photodiode array detector, and Millennium 32 chromatography manager together with Waters Pico-tag column (3.9 x 300 mm, 4 μm). Absorbance at 254 nm was measured. Fatty acids composition was determined by gas chromatographic analysis (6890 GC system, Agilent Technologies, Palo Alto, CA) of fatty acid methyl esters (Peltroche-Llacsahuanga et al, 2000) at Center for Research Facilities of Chungnam National University. Both experimental data were eventually converted to mmol/g protein for amino acids and mol/mol total fatty acids for fatty acids as in Supplementary Table V.

Calculation of topological parameters

Cytoscape was used to calculate topological parameters of metabolic network, VvuMBEL943 (Supplementary Table VI) (Cline et al, 2007; Shannon et al, 2003). The metabolic model in GAMS was first converted into a Simple Interaction Format (SIF) file, and imported by Cytoscape. The Cytoscape plug-in NetworkAnalyzer was used to calculate topological parameters of VvuMBEL943 (Assenov et al, 2008; Cline et al, 2007). Shortest path distance between two connected metabolites was calculated with the plug-in ShortestPath. For

physiologically meaningful interpretation of metabolic network, currency metabolites, such as ATP, NADH etc., were removed prior to the network analysis (Ma & Zeng, 2003a; Ma & Zeng, 2003b).

***In vitro* enzyme assay**

Cells grown in the Mueller Hinton broth were harvested from mid-exponential growth phase in flasks with working volume of 100 ml. This enzyme assay followed the protocol in our previous study (Jang et al, 2007). Cells were then washed twice with 100 mM Tris-HCl (pH 7.0) containing 20 mM KCl, 5 mM MnSO4, 2 mM DTT and 0.1 mM EDTA, and stored at -70 ºC for future use. Cells were disrupted using an ultrasonic homogenizer (Vibra-Cell VCX-600, Sonics & Materials, Inc., CT). Cells were kept on ice throughout sonication. Sonication was carried out at 20 kHz for five cycles with 1 min of rest between each cycle. Each cycle consists of 3 s sonication and 3 s rest for 1 min. Cell extracts were then filtered with Microcon Ultracel YM-30 (Millipore Corp., MA) by centrifuging at 16,000*g* for 10 min at 4 ºC. Centrifugation was repeated at least four times. The supernatant was used for subsequent enzyme assay.

Enzyme assay was conducted using the cell extract from the above procedure, in order to confirm if the chemical compound 24837 inhibits its hypothetical target, dihydropteroate synthase (DHPS). DHPS releases a mole of pyrophosphate as a byproduct from reactions of 2-amino-7,8-dihydro-4-hydroxy-6-(diphosphooxymethyl)pteridine (AHHMD) and 4-aminobenzoate (PABA). Enzyme assay was designed to cleave this pyrophosphate into two moles of orthophosphates by using inorganic pyrophosphatase and measure their concentration using Malachite Green Phosphate Assay Kits (BioAssay Systems, CA), which quantifies the green complex formed from Malachite Green, molybdate and free orthophosphate. First, AHHMD was prepared by reducing 6-hydroxymethylpterin diphosphate, lithium salt (Schircks Laboratories, Switzerland) according to (Shiota et al, 1969). Then, in the control experiment, 10 uM of PABA and AHHMD were incubated with 0.25 U/ul inorganic pyrophosphatase and 20 ul of cell extract in the total reaction volume of 320 ul (Gengenbacher et al, 2008). Reaction buffer consists of 20 mM Tris-HCl, 200 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol in 200 ul at 37 ºC (Gengenbacher et al, 2008). In our study, 50 uM of the chemical compound 24837 was used in place of PABA to examine its inhibition ability. Also, DMSO, solvent of 24837, alone was also tested in place of 24837 at its volume equivalent to 50 uM PABA as DMSO appeared to affect final results as well.

Reactions were allowed to take place for 15 min at room temperature, and 10-fold diluted. Then, orthophosphates were quantified in accordance with the protocol of the Malachite Green Phosphate Assay Kits. Resultant green complexes were measured with SpectraMax M2 (Molecular Devices, CA) at $OD_{620\,nm}$. Experiments were performed in at least triplicates.

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