## Diketopiperazines synthesis gene in *Shewanella baltica* and roles of diketopiperazines and resveratrol in quorum sensing

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\* Corresponding author: Lifang Feng E-mail: <u>fenglifang@mail.zjgsu.edu.cn</u> Tel: +(86)571-28008964 Table S1 IDs and primer sequences of genes for PCR

Gene ID	forward primer sequence (5'-3')	Note	
gyrB_f	CGTATCGGCAGCAGAAGTTATCAT	Amplify <i>gyrB</i> gene	
gyrB_r	GAAGAAGAAGGTCAACAGCAAGGT		
16s_f	GCCCCCTGGACAAAGACTGAC	endogenous control in	
l6s_r	GACATCGTTTACGGCGTGGACTA	qPCR	
3b1325_qpcr_f2456	GCGAACAGTACCAAGTACAGC	determine gene	
sb1325_qpcr_r2668	GGATCACAAACCCTTGCTGAG	expression level by	
sb1370_qpcr_f1751	CCCTGCTAACCCAGAAATCCATG	qPCR	
sb1370_qpcr_r1940	CACAGGAGAATCTGCTGGCTC		
sb1325_up_f470	GATGATGCAGGACAACTTGC	KO for <i>sb1325</i>	
sb1325_up_r1566	AAGCAGCTCCAGCCTACACACATCTAGTT		
	CACCTAAAGAGTCG		
sb1325_down_f3485	CTAAGGAGGATATTCATATGCACGTTAAC		
	GCGCAATATAGCG		
sb1325_down_r4442	CGGTGTGGCATCTTGATAATCAAC		
sb1325_nest_f473	GATGCAGGACAACTTGCGGTAC		
sb1325_nest_r4436	GGCATCTTGATAATCAACCCCTAG		
sb1370_up_f289	CACTCTTGTGCTTGGCGAAGG	KO for <i>sb1370</i>	
sb1370_up_r1313	AAGCAGCTCCAGCCTACACAGGATTGCT		
	TGCTCCTATGTTGGC		
sb1370_down_f2309	CTAAGGAGGATATTCATATGCCCAGCCCT		
	TTAAATACCCAGC		
sb1370_down_r3279	CTAGAAACTGGCTCATCCAGTAAG		
sb1370_nest_f293	CTTGTGCTTGGCGAAGGTACTG		
sb1370_nest_r3272	CTGGCTCATCCAGTAAGTATCC		
sb1370_Re_r2330	AAGCAGCTCCAGCCTACACAGCTGGGTA	Rescue for KO of <i>sb1370</i>	
	TTTAAAGGGCTGGG		
Cm_f	TGTGTAGGCTGGAGCTGCTT	amplify <i>Cm</i> gene	
Cm_r	CATATGAATATCCTCCTTAG	cassette	
sb1325_test_f411	CTGGTTATCACTACCGTTTACCTC	KO test	
sb1325_test_r4499	GTTAGTGCGGCTGCCATCACTG		
sb1370_test_f262	GGATTCATCGTCATCATCGACGC		
sb1370 test r3308	GCTCAGGATCTGGCTTGTTG		

Code	Functional Categories	Gene No.
А	RNA processing and modification	1
В	Chromatin structure and dynamics	2
С	Energy production and conversion	263
D	Cell cycle control, cell division, chromosome partitioning	35
Е	Amino acid transport and metabolism	321
F	Nucleotide transport and metabolism	76
G	Carbohydrate transport and metabolism	162
Н	Coenzyme transport and metabolism	172
I	Lipid transport and metabolism	118
J	Translation, ribosomal structure and biogenesis	199
K	Transcription	295
L	Replication, recombination and repair	184
Μ	Cell wall/membrane/envelope biogenesis	207
Ν	Cell motility	142
0	Posttranslational modification, protein turnover, chaperones	177
Р	Inorganic ion transport and metabolism	226
Q	Secondary metabolites biosynthesis, transport and catabolism	96
R	General function prediction only	467
S	Function unknown	323
Т	Signal transduction mechanisms	292
U	Intracellular trafficking, secretion, and vesicular transport	115
V	Defense mechanisms	79

Table S2 Functional category in COG of S. baltica SB-19

GenelD	RNA-Seq	qPCR
sb3101	10.606	7.863
sb3099	8.97716	7.527
sb1327	8.40107	8.753
sb1325	8.09412	7.322
sb3103	7.75368	6.457
sb1720	6.28173	5.478
sb2760	5.67806	4.658
sb2506	4.53189	4.862
sb3835	4.21485	5.833
sb0224	3.88691	3.675
sb2181	3.3524	3.951
sb1005	2.3014	2.162
sb2505	1.44095	0.784
sb0579	-1.64697	-1.086
sb2523	-3.32798	-2.376
sb0974	-3.4259	-1.292
sb3824	-4.15724	-3.544
sb0974	-4.22165	-3.044
sb2395	-5.04157	-4.659
sb0064	-5.30656	-4.927
sb2603	-5.43974	-6.073
sb0065	-6.54014	-4.925

Table S3 Log-transformed expression ratios from RNA-Seq and qPCR

Table S4 IDs and annotation of differentially genes with expression levels more than 2-fold

Gene ID	Gene description	Log <sub>2</sub> (fold change)	<i>p</i> -value
sb2190	Cro/Cl family transcriptional regulator	inf	5.00E-05
sb0224	transcriptional regulator, MerR family	3.88691	0.0002
sb2181	integrase	3.3524	0.00055
sb0974	purine nucleoside phosphorylase	-4.22165	0.0005
sb2395	carbon starvation protein CstA	-5.04157	0.00015
sb2603	cold-shock protein	-5.43974	0.0001

in log phase cells

Table S5 IDs and annotation of differentially genes with expression levels more than 2-fold

Gene ID	Gene description	Log <sub>2</sub> (fold	<i>p</i> -value
		change)	
sb3101	TonB-denpendent receptor	10.606	5.00E-05
sb3099	pyridoxamine 5'-phosphate oxidase	8.97716	5.00E-05
sb1327	L-lysine 6-monooxygenase (NADPH)	8.40107	0.00055
sb1325	lucA/lucC family protein	8.09412	5.00E-05
sb3100	heme utilization cystosolic carrier protein HutX	8.02429	5.00E-05
sb2448	phage-shock protein	7.92005	5.00E-05
sb1324	TonB-dependent receptor	7.88322	5.00E-05
sb3103	flagellar motor protein MotA	7.75368	0.00015
sb2446	envelope stress response membrane protein PspC	7.75221	5.00E-05
sb3357	hypothetical protein	6.98078	5.00E-05
sb2332	TonB-denpendent receptor	6.9628	0.0002
sb0440	iron ABC transporter substrate-binding protein	6.9576	5.00E-05
sb3105	ABC transporter substrate-binding protein	6.95556	5.00E-05
sb3834	ligand-gated channel protein	6.64529	5.00E-05
sb1011	DUF2884 family protein	6.42248	5.00E-05
sb1720	chemotaxis protein CheY	6.28173	5.00E-05
sb3190	iron-regulated protein A precursor	6.17928	5.00E-05
sb1503	RND transporter MFP subunit	6.04505	0.0001
sb1406	4-hydroxyphenylpyruvate dioxygenase	5.99485	5.00E-05
sb3107	hemin ABC transporter ATP-binding protein	5.9145	5.00E-05
sb0687	elongation factor GreAB	5.88868	5.00E-05
sb1606	TonB-denpendent receptor	5.7347	5.00E-05
sb3106	iron ABC transporter permease	5.72566	5.00E-05
sb2760	isocitrate lyase	5.67806	5.00E-05
sb3191	hypothetical protein	5.43339	0.0001
sb0484	DUF3103 family protein	5.40777	5.00E-05
sb1419	membrane protein	5.39578	5.00E-05
sb4008	TonB-denpendent receptor	5.25152	0.0002
sb2839	siderophore transporter component 1	5.21576	5.00E-05
sb1763	cation diffusion facilitator family transporter	5.08142	0.00065
sb3360	TonB-denpendent receptor	5.01282	5.00E-05
sb3515	TonB-denpendent receptor	4.82074	0.0003
sb2468	YIP1 family protein	4.80005	5.00E-05
sb3189	c-type cytochrome	4.7314	5.00E-05
sb2506	phosphoglucomutase	4.53189	0.00045
sb0803	nickel transporter	4.44726	5.00E-05
sb2761	malate synthase	4.41859	5.00E-05
sb2679	TonB-denpendent receptor	4.32537	0.0002

in stationary phase cells

sb1407	homogentisate 1,2-dioxygenase	4.27634	0.00065
sb3835	LysR family transcriptional regulator	4.21485	0.0001
sb1799	TonB-denpendent receptor	4.1722	0.00025
sb3461	hypothetical protein	4.15593	0.00025
sb0802	hypothetical protein	4.14182	0.0001
sb4259	hypothetical protein	3.96664	0.0001
sb2484	gluconate transporter	3.95684	0.00025
sb3755	TonB-denpendent receptor	3.93265	0.00045
sb2302	biotin synthase	3.84469	5.00E-05
sb2642	hypothetical protein	3.7756	0.00045
sb1384	exonuclease	3.36075	0.0006
sb2485	glycerate kinase	3.23507	0.0005
sb4017	MerR family transcriptional regulator	2.97726	0.0005
sb4029	DNA-binding protein	-3.26577	0.0004
sb2523	beta-aspartyl peptidase	-3.32798	0.00055
sb3258	RNA helicase	-3.373	0.00055
sb0974	purine nucleoside phosphorylase	-3.4259	0.00025
sb3875	hypothetical protein	-3.58541	0.00055
sb3586	histidine kinase	-3.68046	0.00025
sb4133	protoporphyrinogen oxidase	-3.73387	0.00035
sb0158	hemerythrin	-3.96804	0.00055
sb3500	thiosulfate reductase	-4.03588	0.00025
sb0638	cytochrome C biosynthesis protein	-4.10152	0.0001
sb3824	formate dehydrogenase	-4.15724	0.00015
sb2115	quinone-reactive Ni/Fe-hydrogenase small chain	-4.20461	5.00E-05
sb2581	membrane protein	-4.24104	0.00045
sb2603	cold-shock protein	-4.28337	0.00065
sb0609	radical SAM protein	-4.29668	0.00045
sb2478	cystathionine beta-synthase	-4.30298	0.0005
sb3825	formate dehydrogenase subunit gamma	-4.44914	5.00E-05
sb3187	hypothetical protein	-4.48254	0.0001
sb4224	membrane protein	-4.53705	0.00025
sb0063	porin	-4.73681	5.00E-05
sb4217	formate dehydrogenase, alpha subunit	-4.88881	5.00E-05
sb0633	fumarate reductase	-4.92351	0.00025
sb3423	hypothetical protein	-4.94677	0.0002
sb0064	putrescine transporter	-5.30656	5.00E-05
sb1225	short-chain dehydrogenase	-5.33312	5.00E-05
sb4218	formate dehydrogenase-N subunit beta	-5.58097	0.00015
sb3005	transcriptional regulator	-5.75377	5.00E-05
sb0423	hypothetical protein	-6.26541	5.00E-05
sb0065	ornithine decarboxylase	-6.54014	5.00E-05

Query of CDPSs	E value	Identity	Subject of Gene ID <sup>a</sup>	Reference
AlbC	0.022	22%	sb1747	10
Rv2275	1.6	30%	sb0868	10
YvmC_Blic	2.8	25%	sb1843	10
YvmC_Bsub	1.5	30%	sb1331	10
YvmC_Bthu	1.5	30%	sb3303	10
pSHaeC06	0.049	26%	sb1302	10
Plu0297	1.7	36%	sb0036	10
JK0923	1.8	30%	sb2950	10
Nvec-CDPS2	0.58	25%	sb0386	10
DmtB1	0.048	34%	sb1370	29

Table S6 Local BLAST alignment of CDPSs with genomic database of SB-19 strain

<sup>a</sup> The table lists only one gene with the highest E value.

(10) Belin, P.; Moutiez, M.; Lautru, S.; Seguin, J.; Pernodet, J.; Gondry, M. The nonribosomal synthesis of diketopiperazines in tRNA-dependent cyclodipeptide synthase pathways. *Nat. Prod. Rep.* **2012**, *29*, 961-979.

(29) Yao, T.; Liu, J.; Liu, Z.; Li, T.; Li, H.; Che, Q.; Zhu, T.; Li, D.; Gu, Q.; Li, W. Genome mining of cyclodipeptide synthases unravels unusual tRNA-dependent diketopiperazine-terpene biosynthetic machinery. *Nat. Commun.* **2018**, *9*, 4091.



Figure S1 Sensory changes in overall acceptability of fish fillet under treatment of resveratrol stored at 4 °C for 15 days.

The sensory experiment of fish fillet under treatment of resveratrol was carried as our previous reports.<sup>18</sup> The sensory of raw fillet samples under resveratrol treatment was evaluated by 10 experienced panelists (five males and five females, ranging from 21 to 48 years old), and their voting numbers were set at  $k, k \in (1, 10)$ . Fillet quality was divided into *m* grades, and the score of a particular grade was recorded as  $h_i, j \in (1, m)$ . Fillet attributes were divided into *n* elements, and a particular element was recorded as  $u_i, i \in (1, n)$ . The contributory weight was depended on pairwise comparison of each attributes was recorded as  $x_i$  ( $\sum x_i = 1$ ). the relation set (matrix) of *f* between two objects of  $h_i$  and  $u_i$  was calculated as follows:

$$F = \begin{bmatrix} f11/k & f12/k & \dots & f1m/k \\ f21/k & f22/k & \dots & f2m/k \\ \dots & \dots & \dots & \dots \\ fn1/k & fn2/k & \dots & fnm/k \end{bmatrix}$$

So the overall acceptability of fillet was calculated using the weight grade method as follows:

$$Z = \sum_{i=1}^{n} x_i \cdot \sum_{m=1}^{m} \frac{f_{ij}}{k} \cdot h_j$$

(18) Gao, M. S.; Feng, L. F.; Jiang, T. J.; Zhu, J. L.; Fu, L. L.; Yuan, D. X.; Li, J. R. The use of rosemary extract in combination with nisin to extend the shelf life of pompano (*Trachinotus ovatus*) fillet during chilled storage. *Food Control* **2014**, *37*, 1-8.



Figure S2 Gene expression levels with a correlation between RNA-Seq (x-axis) and qPCR (y-axis). Each Log<sub>2</sub> ratio of fold changes was compared between RNA-Seq and qPCR.



Figure S3 Comparison of *sb1370* gene between strains of SB-19 and SB-20. (a) The levels of DKPs. (b) The levels of AI-2. (c) Biofilm proportion of strains under treatment with cyclo-(L-Pro-L-Phe) (40  $\mu$ g/mL) or resveratrol (10  $\mu$ g/mL). (d) Spoilage capability of strains under treatment with cyclo-(L-Pro-L-Phe) (40  $\mu$ g/mL) or resveratrol (10  $\mu$ g/mL).



Figure S4 SB-19 strains of WT, KO, or RE under cyclo-(L-Pro-L-Phe) (40  $\mu$ g/mL) and resveratrol (10  $\mu$ g/mL) treatment.