

Supplementary Materials: Effect of Different Species of *Prorocentrum* Genus on the Japanese Oyster *Crassostrea gigas* Proteomic Profile

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Table S1. Summary of three-way analysis of variance (ANOVA) of *Crassostrea gigas* exposure to whole live cells of *Prorocentrum* spp.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	3	4.9	1.64	4.10	0.008135 **
Exposure time	3	921.4	307.13	765.818	<2e-16 ***
Cell concentration	4	1.6	0.41	1.025	0.396954
Species:Exposure time	9	55.3	6.14	15.319	<2e-16 ***
Species:Cell concentration	8	0.3	0.03	0.080	0.999640
Exposure time:Cell concentration	12	16.1	1.34	3.43	0.000299 ***
Species:Exposure time:Cell concentration	24	19.7	0.82	2.051	0.005696 **
Residuals	128	51.3	0.40		

Table S2. Summary of three-way analysis of variance (ANOVA) of *Crassostrea gigas* exposure to aqueous extract (AE) of *Prorocentrum* spp.

AE	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	3	10.1	3.36	7.913	0.00011 ***
Exposure time	3	339	112.99	265.856	<2e-16 ***
Concentration	2	1.4	0.7	1.656	0.19742
Species:Exposure time	9	26.2	2.91	6.85	3.05e-07 ***
Species:Concentration	4	0.9	0.22	0.512	0.72707
Exposure time:Concentration	6	4.8	0.8	1.888	0.09296
Species:Exposure time:Concentration	12	22	1.83	4.317	2.96e-05 ***
Residuals	80	34	0.42		

Table S3. Summary of three-way analysis of variance (ANOVA) of *Crassostrea gigas* exposure to organic extract (OE) of *Prorocentrum* spp.

OE	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	3	0.89	0.3	0.409	0.74718
Exposure time	3	293.7	97.9	135.034	<2e-16 ***
Concentration	2	1.39	0.69	0.958	0.388071
Species:Exposure time	9	25.24	2.8	3.869	0.000419 ***
Species:Concentration	4	0.06	0.01	0.019	0.999268
Exposure time:Concentration	6	11.5	1.92	2.644	0.021670 *
Species:Exposure time:Concentration	12	7.72	0.64	0.888	0.562656
Residuals	80	58	0.73		

Table S4. Relative expression values of regulated protein spots that showed statistical differences ($p < 0.005$) in *Crassostrea gigas* exposure to aqueous extract (AE) of *Prorocentrum* spp.

Spot ID	Control	<i>P. lima</i>	<i>P. minimum</i>	<i>P. rhathymum</i>	<i>P. lima</i>	<i>P. minimum</i>	<i>P. rhathymum</i>
	Original data				Processed data		
	Whole Cells						

1	1.138	1.166	1.120	0.856	0.028	-0.018	-0.282
5	1.212	3.966	5.003	2.739	2.754	3.792	1.528
8	0.716	1.262	1.518	1.345	0.546	0.802	0.629
11	1.149	0.808	0.314	1.178	-0.340	-0.835	0.029
13	1.117	1.130		1.586	0.013	0	0.468
14	1.433			1.247	0	0	-0.186
15	4.723	5.541	5.074	6.122	0.818	0.351	1.399
16	1.733	4.312	4.784	4.049	2.579	3.051	2.317
21	0.954	1.246	2.640	1.638	0.292	1.687	0.684
25	3.354	5.323	1.413	5.928	1.969	-1.941	2.574
29	1.938	7.362	7.053	6.715	5.424	5.115	4.777
30	1.650	2.264	2.239	3.007	0.613	0.589	1.357
31	2.134	0.332			-1.802	0	0
35	5.529	9.017	7.555	6.826	3.488	2.026	1.297
36	1.284	2.124	1.274	0.760	0.840	-0.010	-0.525
37	2.617	2.437	4.123	3.374	-0.180	1.506	0.756
38	2.027	3.594	2.547	2.791	1.567	0.520	0.764
39	3.213	5.233	2.226	2.802	2.020	-0.987	-0.411
40				1.283			1.283
41				1.409			1.409
43			1.906			1.906	
44		1.424			1.424		
45		1.238			1.238		
46		0.882			0.882		
47	1.718				0	0	0
48	1.411				0	0	0
Aqueous Extract							
21	1.719			0.423	0	0	-1.297
27	5.823	6.338	5.576	4.471	0.515	-0.246	-1.351
38	1.710	1.260		1.228	-0.451	0	-0.483
47	1.646	1.911			0.265	0	0
50		0.992	1.996		0.992	1.996	
55		0.314			0.314		
57	1.542				0	0	0
Organic Extract							
9	0.374			0.341	0	0	-0.033
17	0.624	0.682	0.650	0.816	0.059	0.027	0.193
19	0.306		1.256	0.700	0	0.950	0.394
24	4.380	0.903	1.975	2.576	-3.477	-2.405	-1.804
29	0.789			0.566	0	0	-0.222
34	0.891	0.294	0.159	0.560	-0.597	-0.732	-0.331
39	3.644	2.899	2.625	3.641	-0.745	-1.019	
40	1.222	0.613	1.079	1.857	-0.609	-0.143	0.635
50	1.115		1.400		0	0.285	0
52	1.884		1.531		0	-0.353	0
58		0.598	0.577	0.721	0.598	0.577	0.721
70			0.815			0.815	
77	4.207				0	0	0

Note: The processed data were obtained by subtracting the expression value of each spot with respect to the control value.

Table S5. Peptide sequences of protein spots expressed differently and selected for identification by mass spectrometry.

[illegible]

TALAPSTMKMQKEITALAPSTMKVAPEEHPVLLTEAPLNPKVAPEEHPVLLTEAPLNPKSYELPDGQVITI
 GNERSYELPDGQVITIGNERVAPEEHPVLLTEAPLNPKLCYVALDFEQEMGTAASSSSLEKMQKEITALAP
 STMKVAPEEHPVLLTEAPLNPKVAPEEHPVLLTEAPLNPKDLYANTVLSGGSTMYPGIADRDLYANTVL
 SGGSTMYPGIADRSYELPDGQVITIGNERMQKEITALAPSTMKMQKEITALAPSTMKMQKEITALAPSTM
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 YANTVLSGGSTMYPGIADREITALAPSTMKIKVAPEEHPVLLTEAPLNPKVAPEEHPVLLTEAPLNPKM
 QKEITALAPSTMKSYELPDGQVITIGNERLDLAGRDLTDYLMKLDLAGRDLTDYLMKSYELPDGQVITIGN
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 SFTTTAEREIVREITALAPSTMKMQKEITALAPSTMK

17 SLALQINDEQLKSLALQINDEQLKSLALQINDEQLKQLTDAHNTFNLFDK

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 GNERVAPEEHPVLLTEAPLNPKSYELPDGQVITIGNERSYELPDGQVITIGNERVAPEEHPVLLTEAPLNPK
 VAPEEHPVLLTEAPLNPKVAPEEHPVLLTEAPLNPKSYELPDGQVITIGNERLNDQIGEYEGEATLRLN
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 AVEEDLTFRIGGLEDEVSKQRNVIDELSKEKLASEKECAELRSGSLDFFEVYNVLQKIFQSLDVKNEKVG
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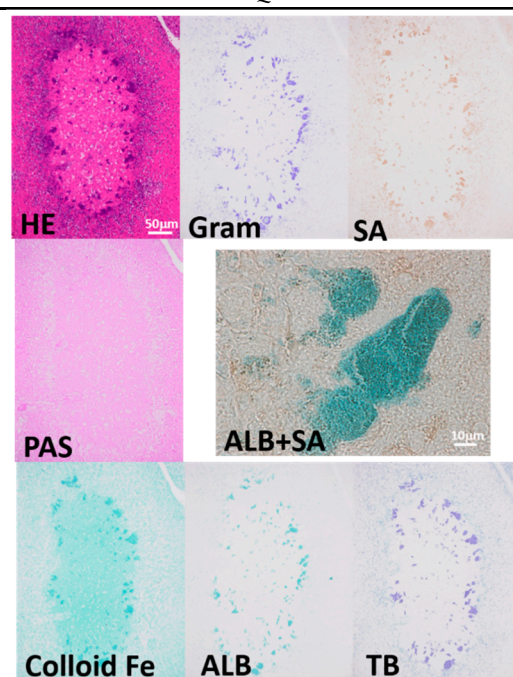


Figure S1. A large infected necrotic lesion in the liver of a mouse 10 days after OJ-1 injection. After injection of OJ-1 $\times 1$ solution, some mice survived and their general condition was fine 10 days after bacterial injection; however, large extracellular necrotic lesions were sometimes developed in the liver. The lesion was examined for biofilm formation. The result was similar to the lesion 24 h after injection (Figure 1); many MRSA colonies developed around the necrotic focus, and they were accompanied with acidic polysaccharides detected by Colloidal Fe, ALB, and TB staining, but were negative for neutral polysaccharides (PAS). The double staining of ALB + SA showed a biofilm matrix containing acidic mucopolysaccharides.

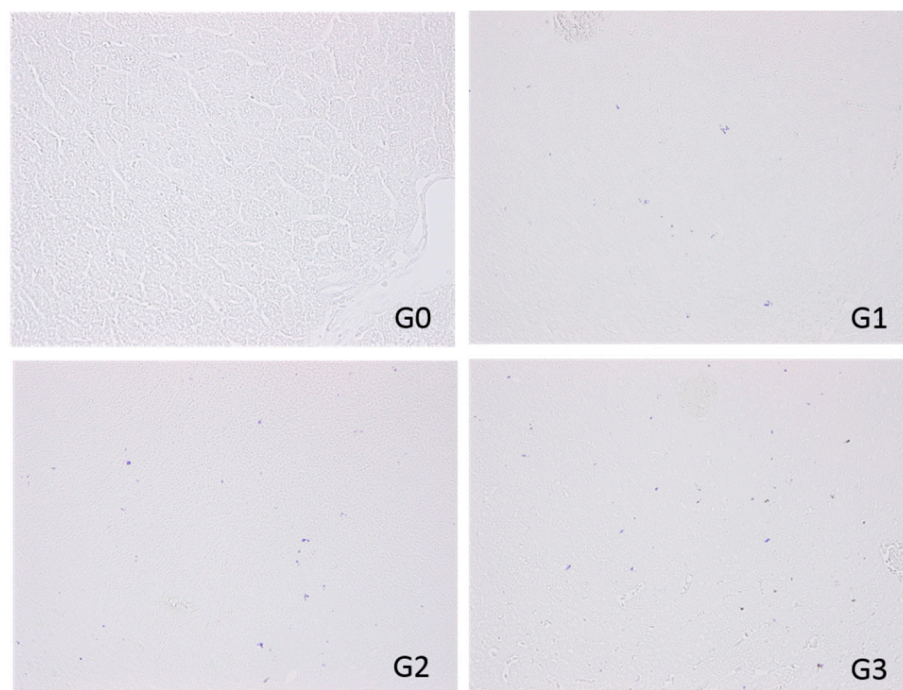


Figure S2. Reference picture for grading of gram-positive MRSA in the liver. Each liver picture was evaluated using a set of reference pictures for the grading of gram-positive MRSA.

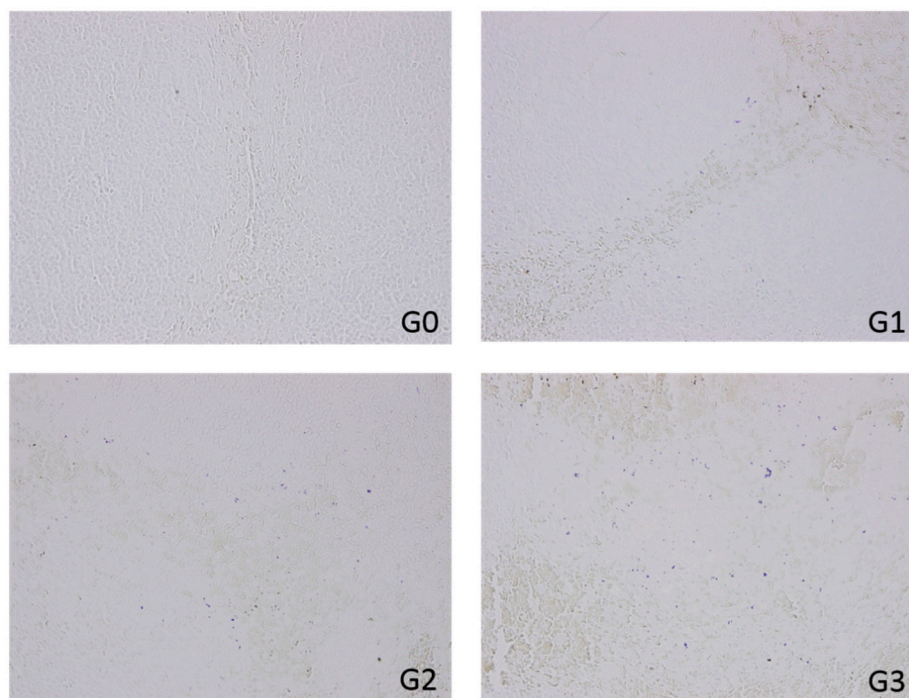


Figure S3. Reference picture for grading of gram-positive MRSA in the spleen. Each spleen picture was evaluated using a set of reference pictures for the grading of gram-positive MRSA.

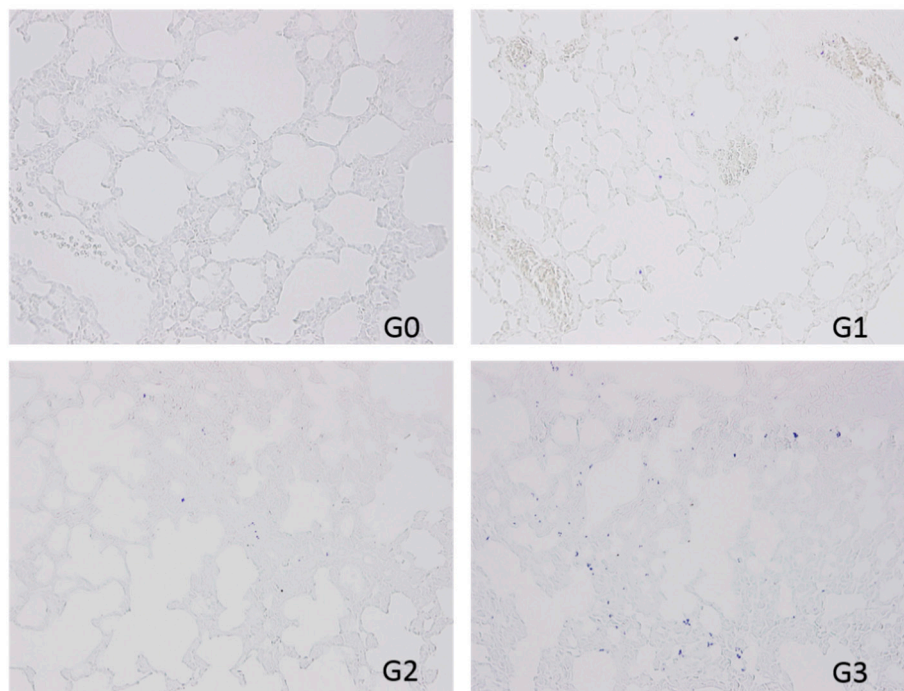


Figure S4. Reference picture for grading of gram-positive MRSA in the lung. Each lung picture was evaluated using a set of reference pictures for the grading of gram-positive MRSA.

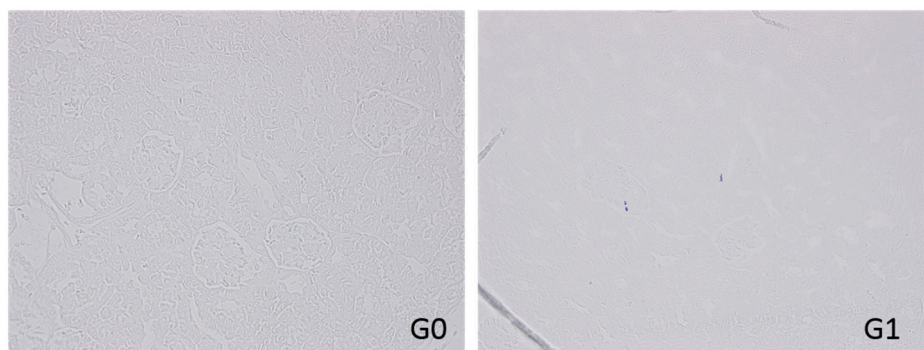


Figure S5. Reference picture for grading of gram-positive MRSA in the kidney. Each kidney picture was evaluated using a set of reference pictures for the grading of gram-positive MRSA.