Supplementary Information for "Culturing marine bacteria from the genus Pseudoalteromonas on a cotton scaffold activates silent biosynthetic pathways and alters secondary metabolite production"

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AntiSmash 4.0 Analysis of Bacterial Genomes

Table S1. Type of secondary metabolite biosynthetic gene clusters detected in *Pseudoalteromonas* sp. genome sequence data by antiSMASH 4.0 analysis [1].

Type of natural product pathway	*P. luteoviolacea* 2ta16 (137 scaffolds)	*P. piscicida* JCM 20779 (2 scaffolds)	*P. rubra* DSM 6842 (194 scaffolds)*
RiPPs	2	3	2
Non-ribosomal peptide (NRP)	3	2	3
Polyketide (PK)			1
Hybrid (NRP/PK)	4	4 (including alterochromide pathway)	1
Lantipeptide	1		2
Homo serine lactone	1		1
Indole	1 (violacein pathway)		
Aryl polyene cluster	1		
Other			1

* Prodiginine pathway from *P. rubra* was not detected through antiSMASH 4.0

Scanning Electron Microscopy

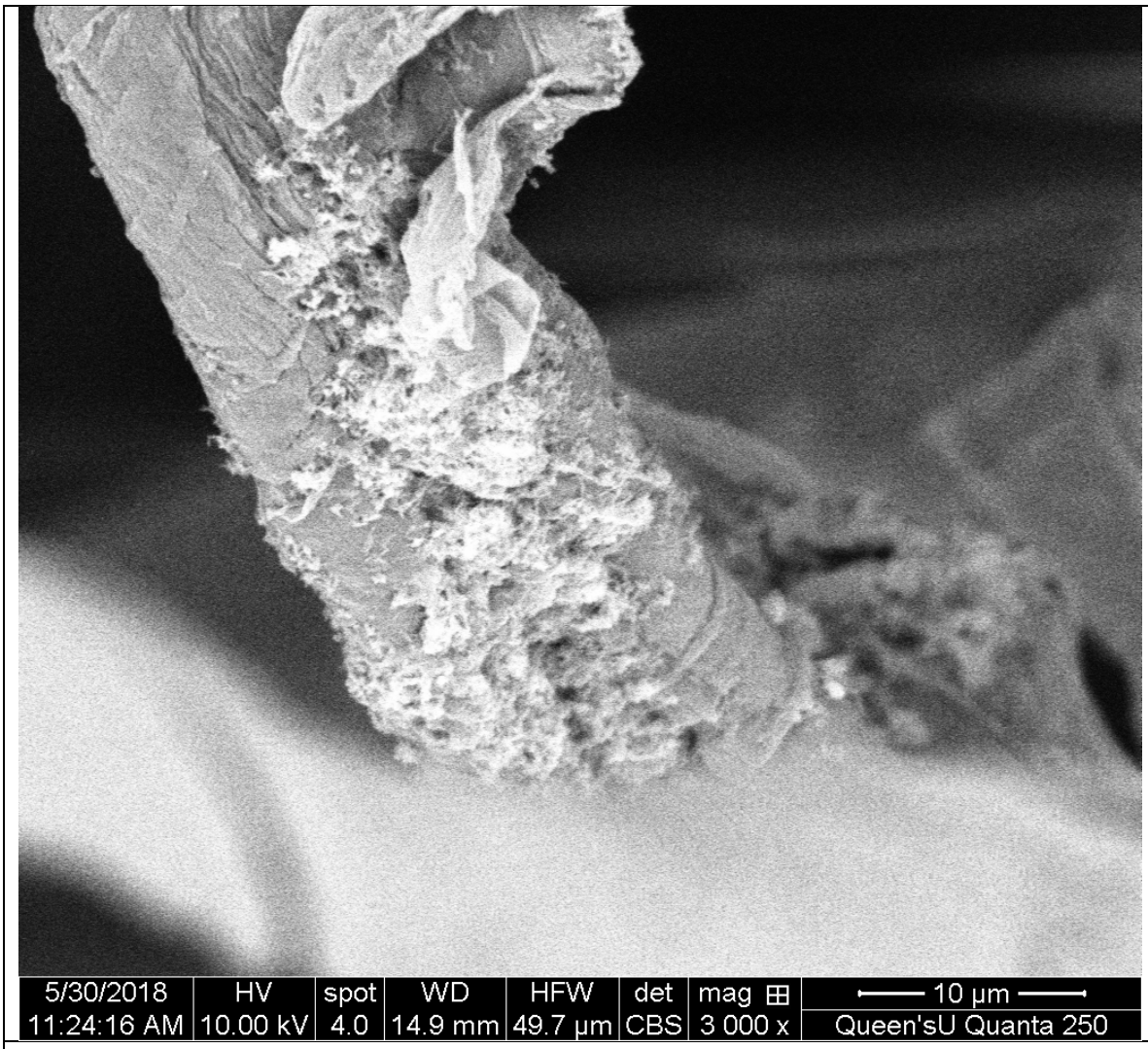

Figure S1 Scanning Electron Micrograph of cotton fibres with *P. luteoviolacea* 2ta16 biofilm adhering to its surface.

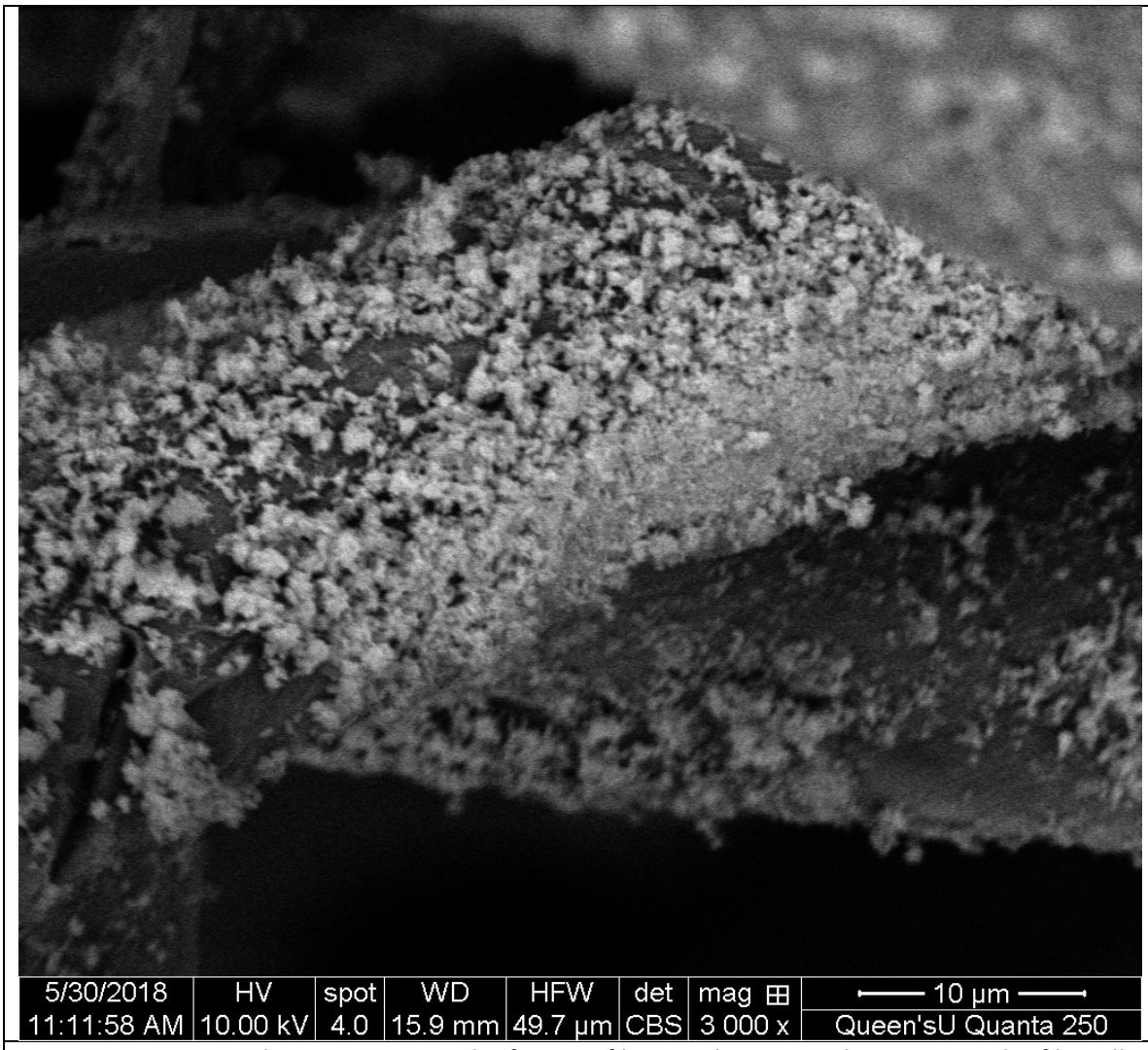

Figure S2: Scanning Electron Micrograph of cotton fibres with *P. piscicida* JCM 20779 biofilm adhering to its surface.

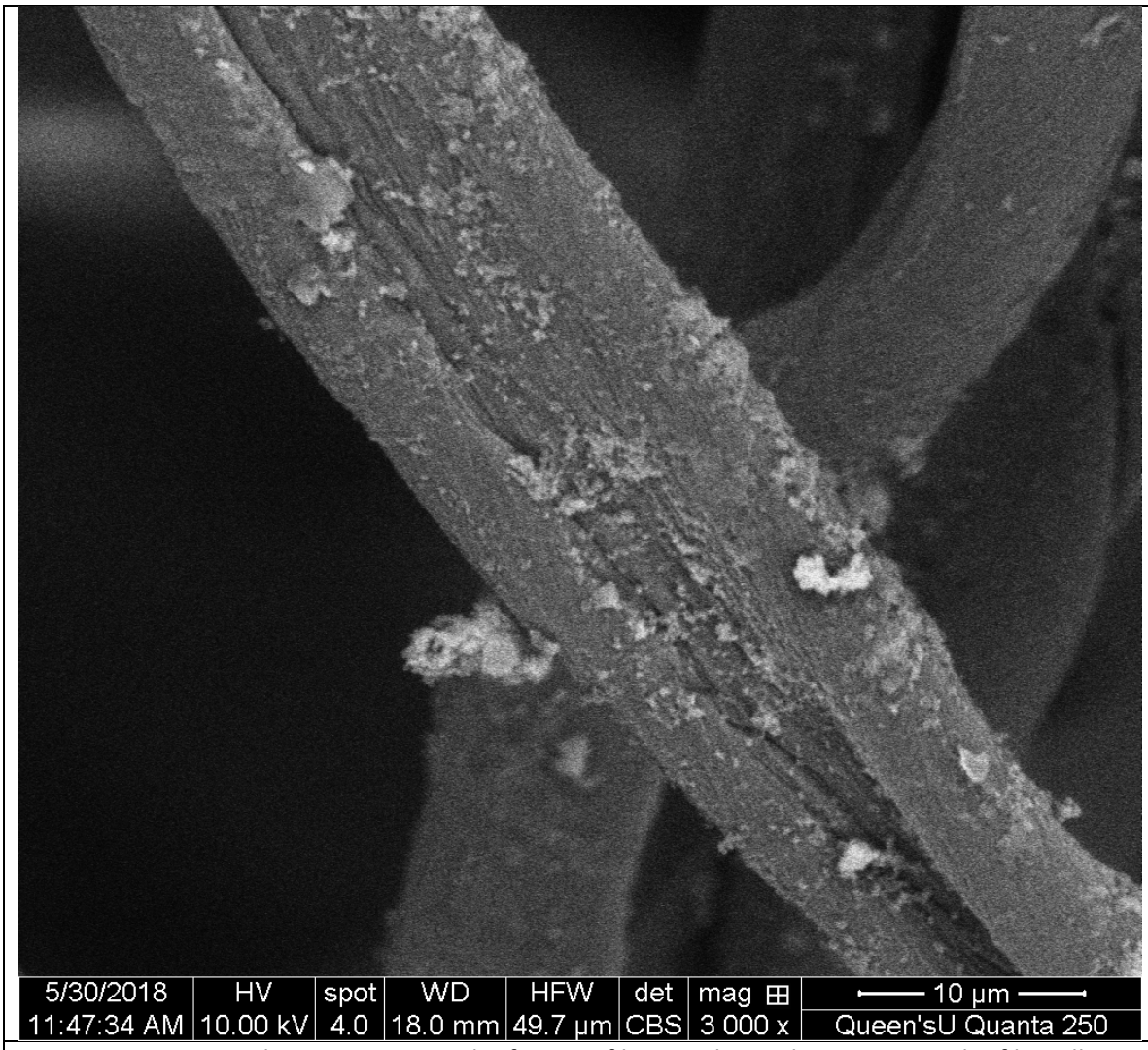

Figure S3: Scanning Electron Micrograph of cotton fibres with *P. rubra* DSM-6842 biofilm adhering to its surface. Microcolonies are visible as large clusters of cells, individual cells are also seen adhering to the surface of the cotton fibres.

***P. luteoviolacea* 2ta16**

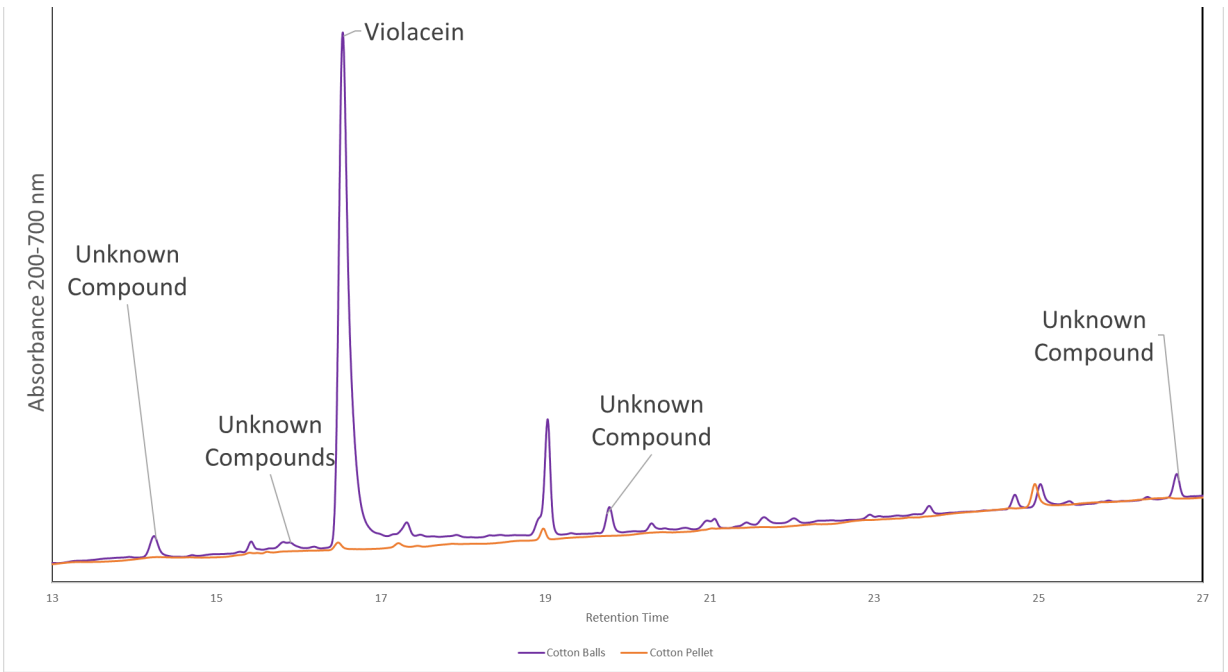

Figure S4: UPLC-PDA trace of methanol extracts of cotton balls from cotton containing culture, as well as methanol extract of cell pellets from non-cotton containing cultures of *P. luteoviolacea* 2ta16.

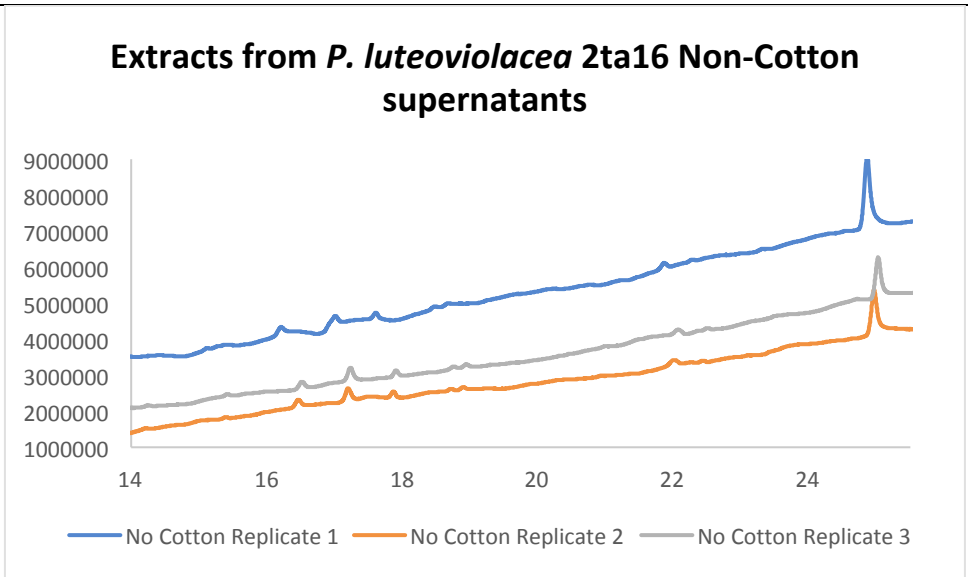

Figure S5: UPLC-PDA Trace of ethyl-acetate extracts from biological replicates of planktonic cultures of *P. luteoviolacea* 2ta16.

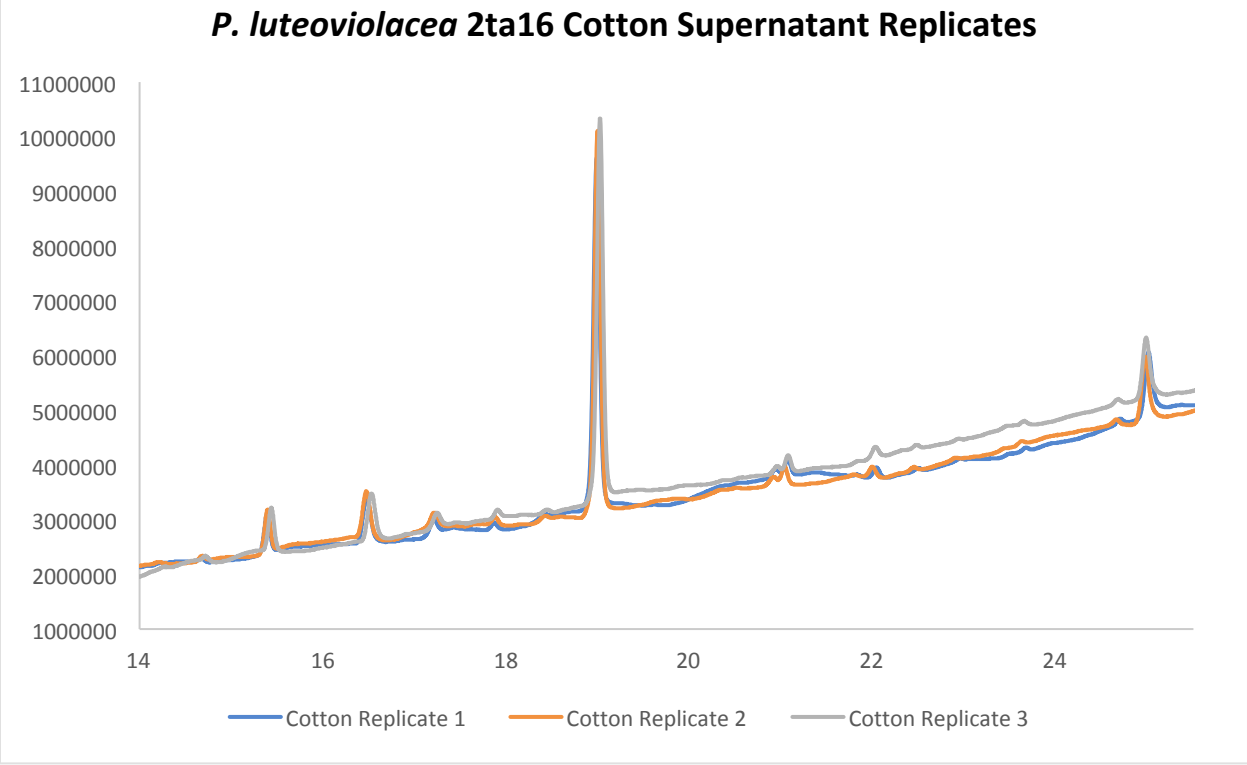

Figure S6: UPLC-PDA Trace of ethyl-acetate extracts from biological replicates of cotton containing cultures of *P. luteoviolacea* 2ta16.

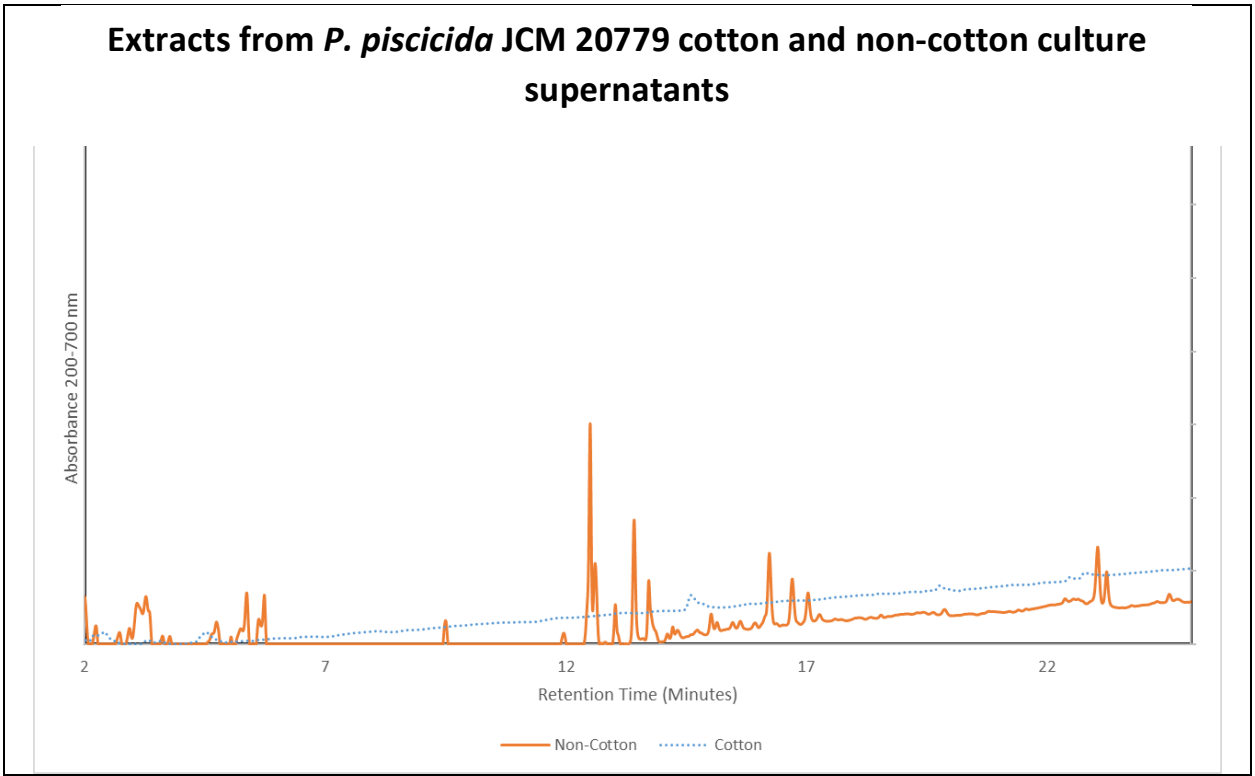

Figure S7: UPLC-PDA trace of ethyl acetate extracts of cell free supernatants from *P.piscicida* JCM 20779

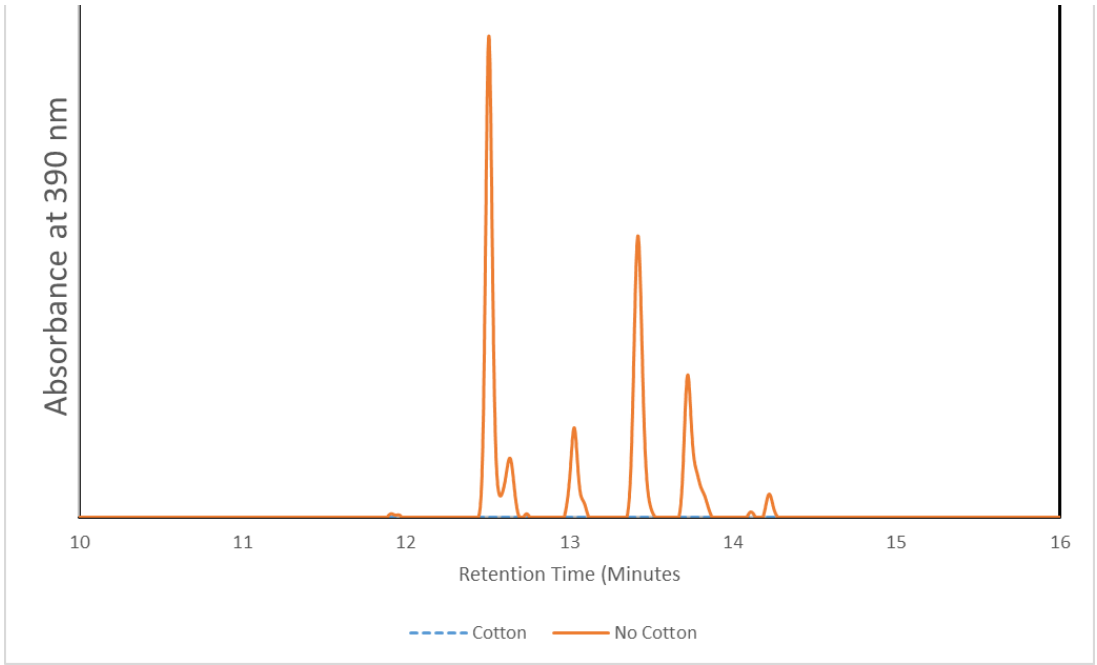

Figure S8: UPLC-PDA chromatograms of ethyl acetate extracts of cell-free supernatants from cultures of *P. piscicida* JCM 20779 grown with and without cotton. Absorbance at 390 nm is indicative of alterochromide family members.

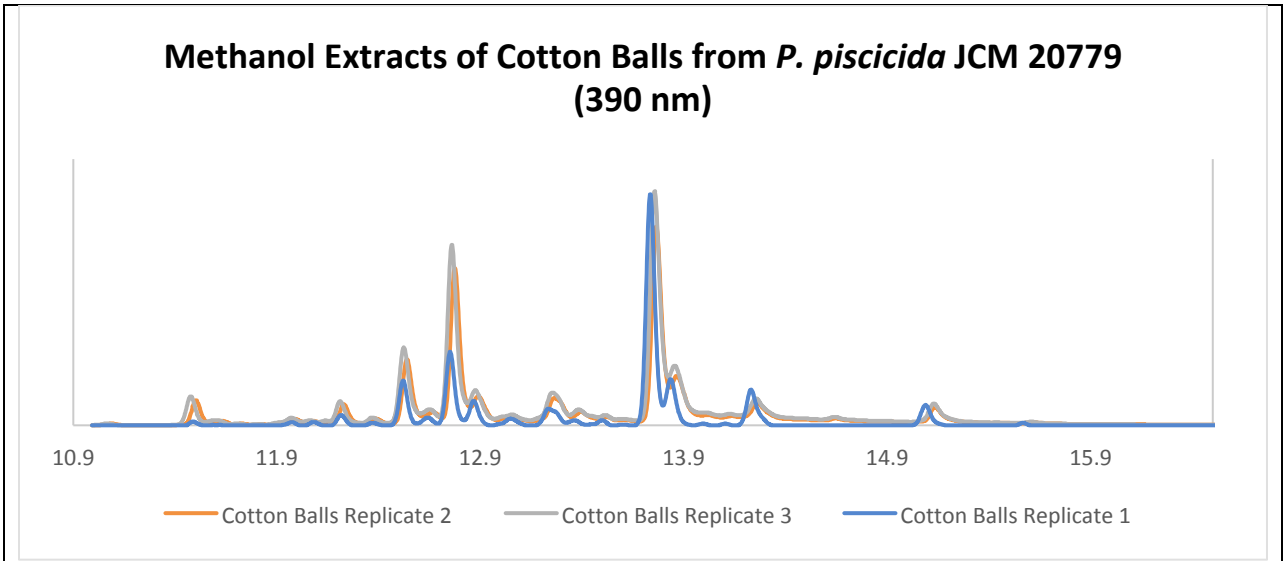

Figure S9: UPLC-PDA Trace of methanol extracts from biological replicates of cotton containing cultures of *P. piscicida* JCM 20779.

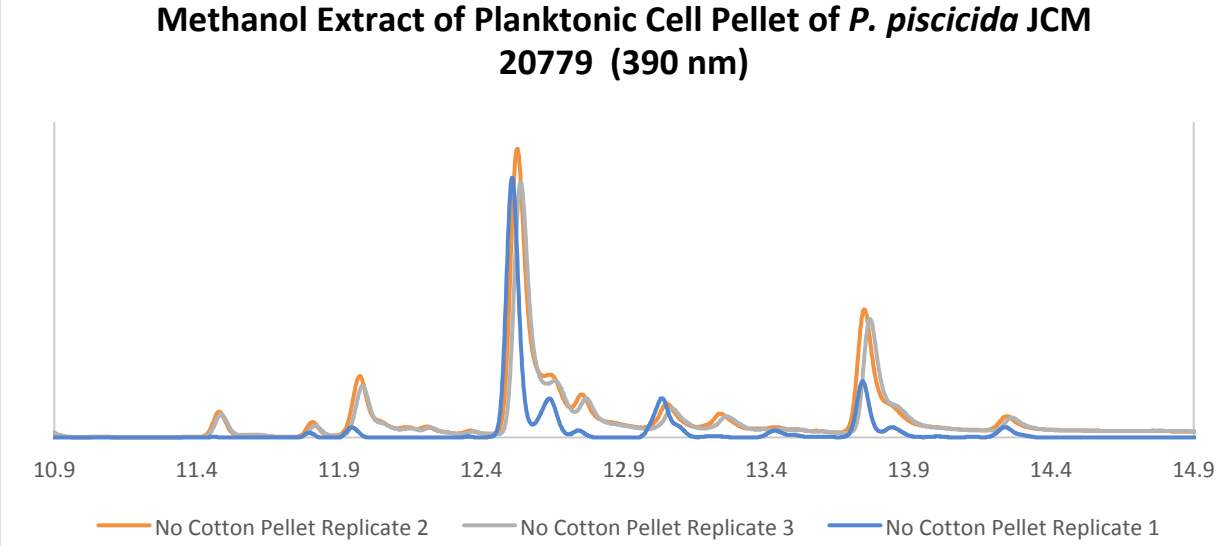

Figure S10: UPLC-PDA Trace of methanol extracts from biological replicates of planktonic cultures of *P. piscicida* JCM 20779.

***P.rubra* DSM-6842**

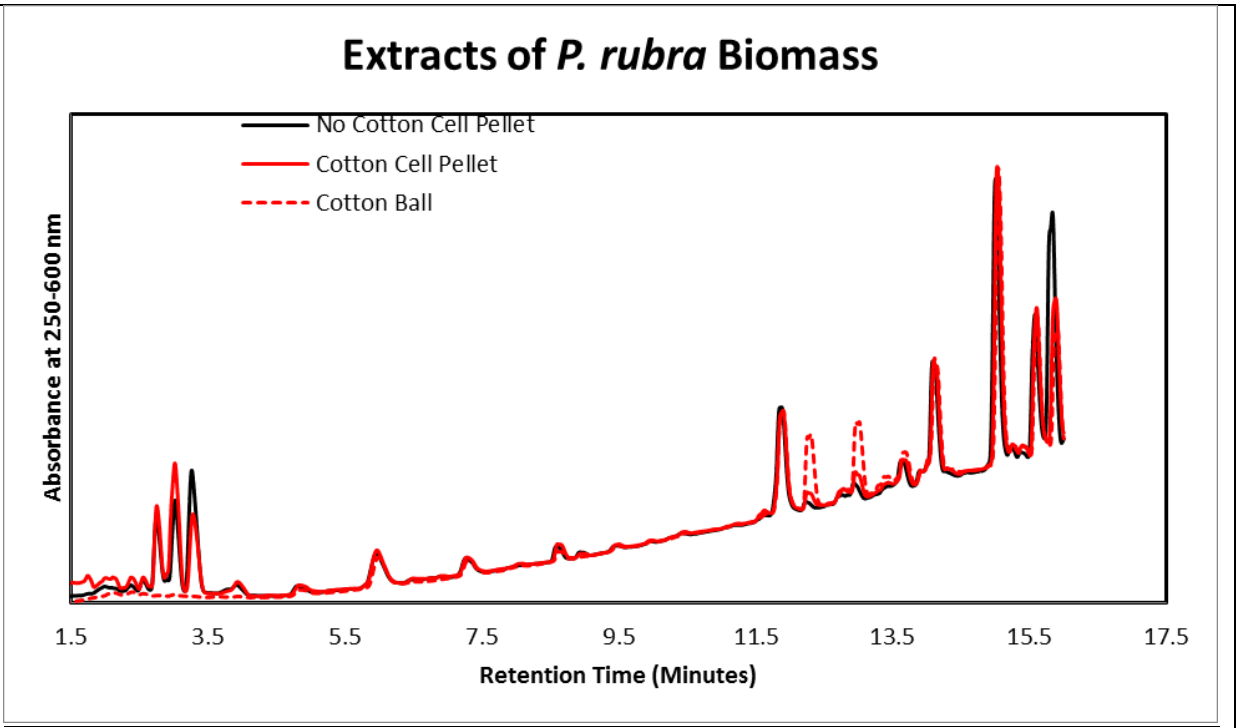

Figure S11: Averaged UPLC-PDA chromatograms of methanol extracts of *P. rubra* DSM-6842 biomass from cultures containing cotton scaffold and cultures lacking cotton scaffold.

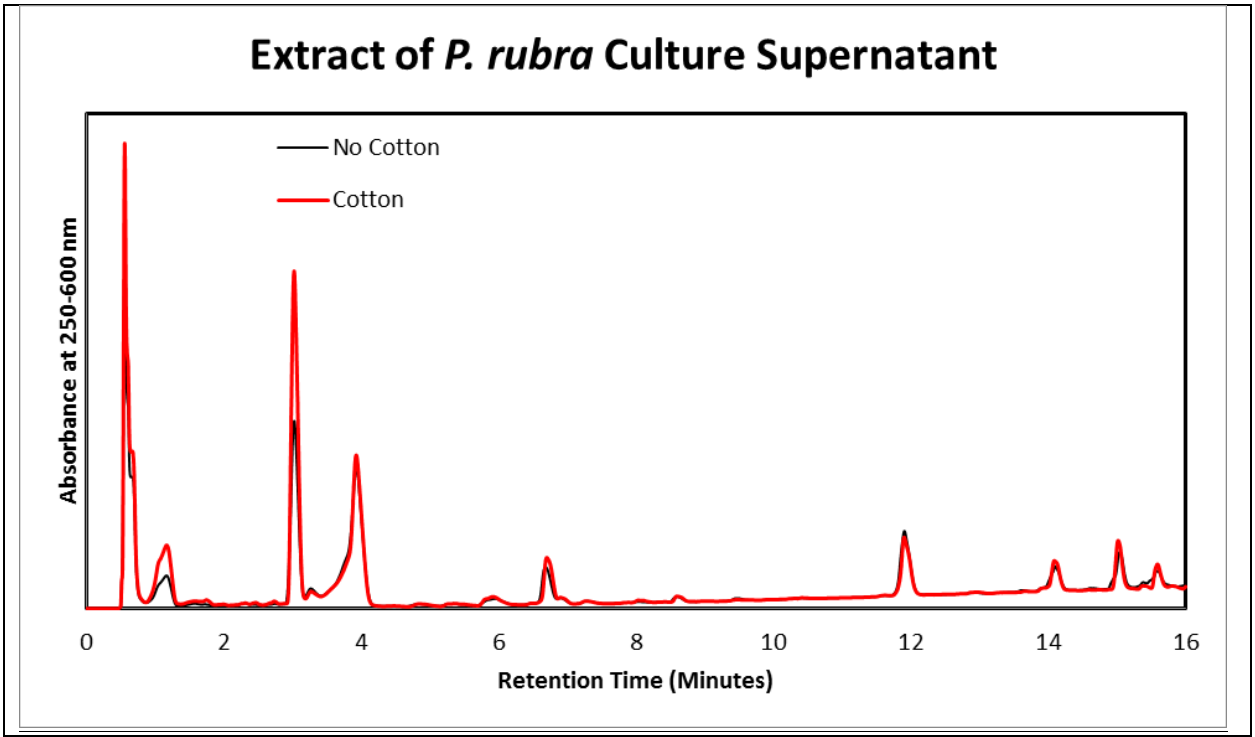

Figure S12: Averaged UPLC-PDA chromatograms of ethyl acetate extracts of cell-free supernatants from cultures of *P. rubra* DSM-6842 grown with and without cotton scaffolds.

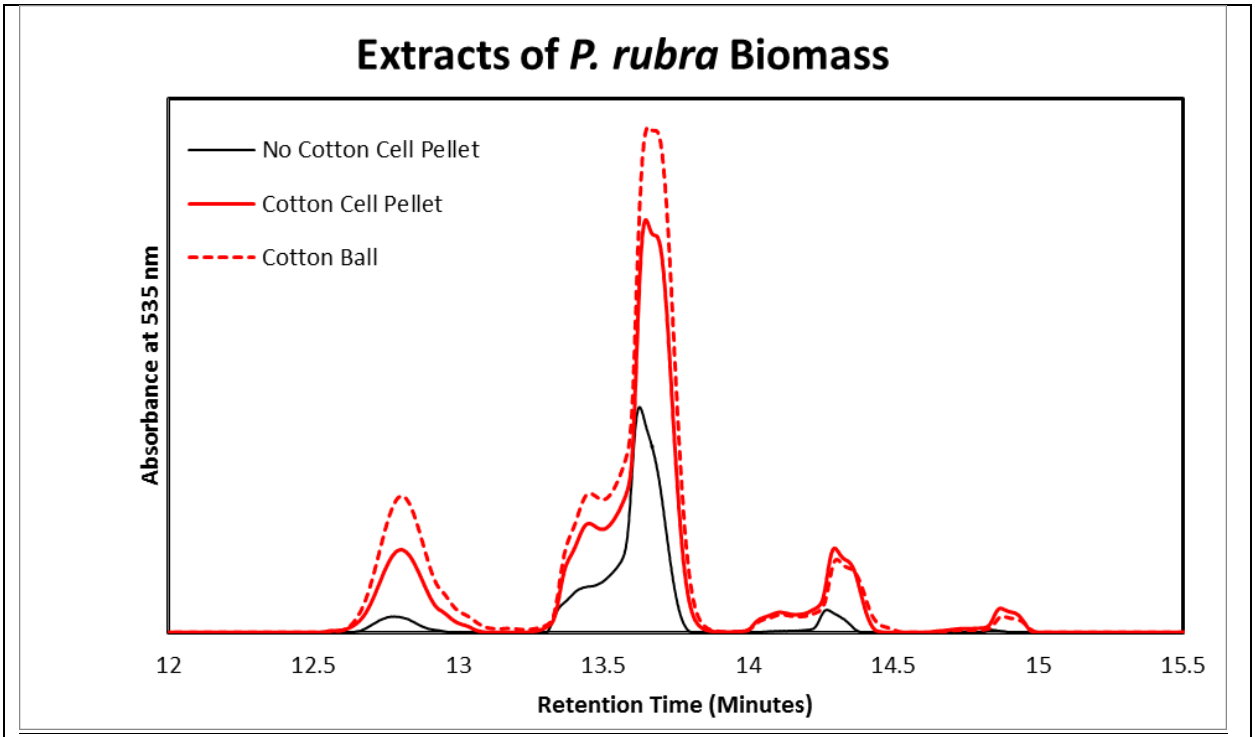

Figure S13: Averaged UPLC-PDA chromatograms of extracts from the biomass of cultures of *P. rubra* DSM-6842 grown with and without cotton scaffolds. Compounds identified are Cycloprodigiosin/4''-(n-butyl)prodigiosin (Co-eluted at 12.8 minutes, Prodigiosin (RT = 13.69 minutes), and 4''-(n-hexyl)prodigiosin (RT = 14.35), and 4''-(n-heptyl)prodigiosin (RT = 14.87)

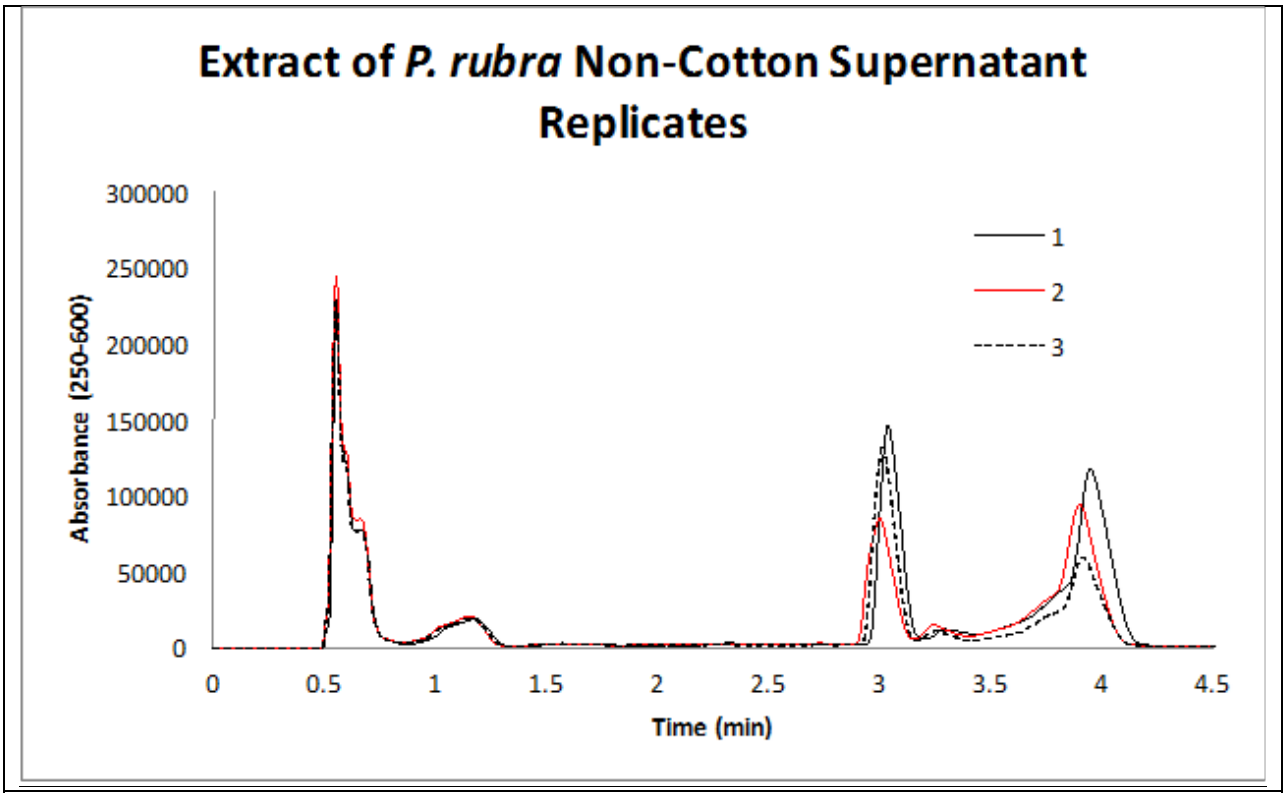

Figure S14: UPLC-PDA Traces of ethyl acetate extracts from biological replicates of planktonic cultures of *P. rubra* DSM-6842.

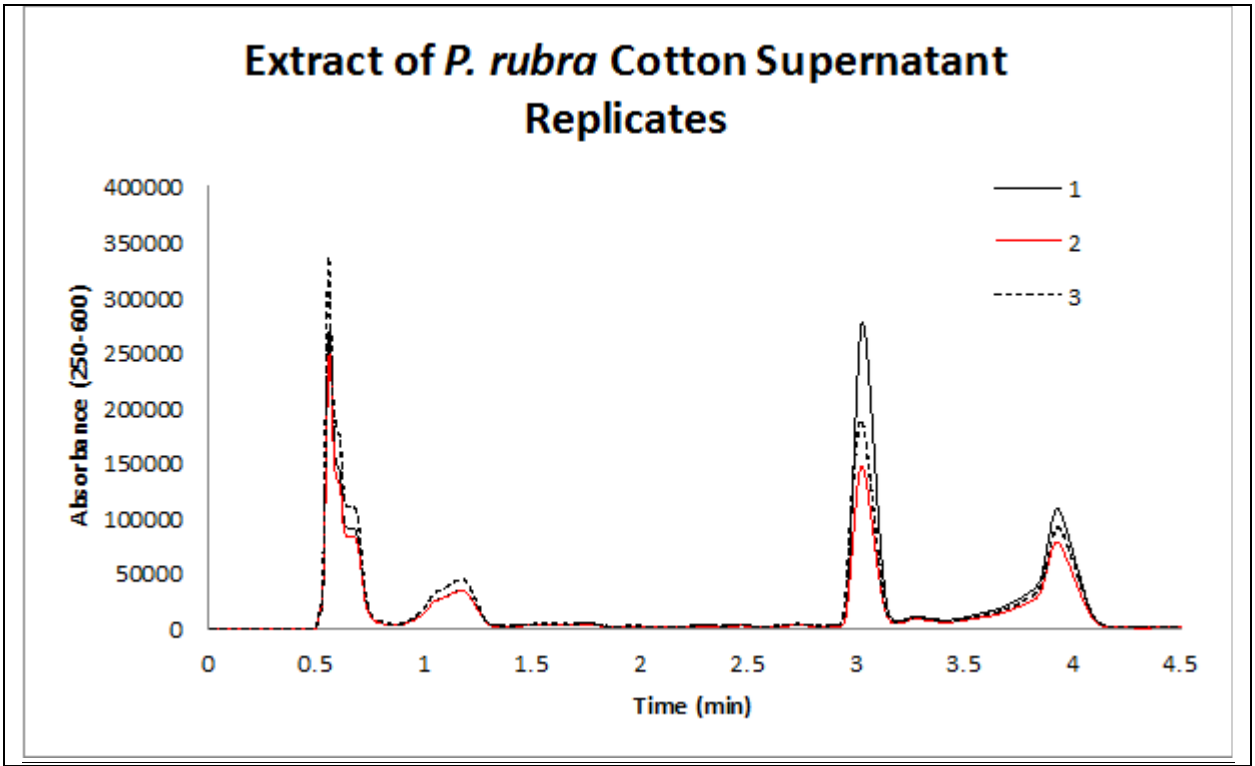

Figure S15: UPLC-PDA Traces of ethyl acetate extracts from biological replicates of cotton containing cultures of *P. rubra* DSM-6842.

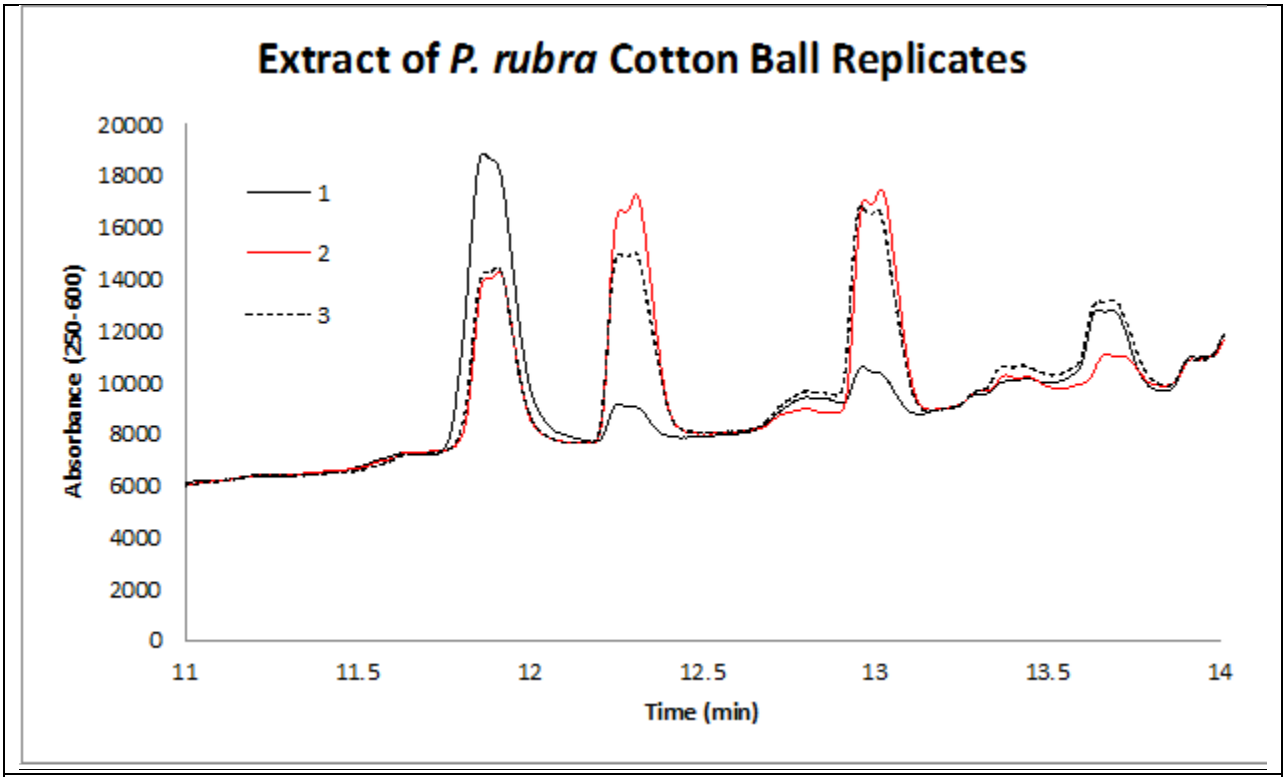

Figure S16: UPLC-PDA Traces of methanol extracts from biological replicates of cotton containing cultures of *P. rubra* DSM-6842.

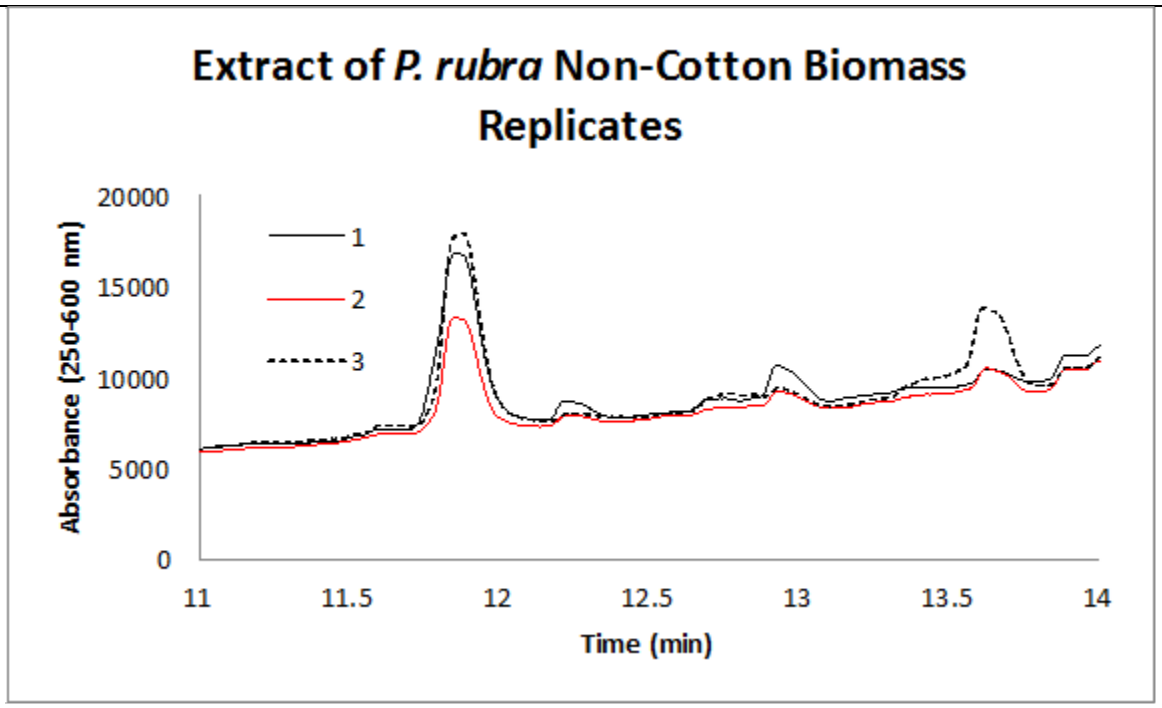

Figure S17: UPLC-PDA Trace of methanol extracts from biological replicates of planktonic cultures of *P. rubra* DSM-6842.

Table S2: Retention Times and Masses of known compounds based on dereplication using Marinlit [2]

***Pseudoalteromonas luteoviolacea* 2ta16**				
Compound	Calculated (M+H)	Observed *m/z*	Observed Adducts	Retention Time (Minutes)
Violacein	344.10	344.07	(M+Na)	16.49
Thiomarinol A	641.80	641.37	(M+Na)	19.02
***P. piscicida* JCM20779**				
Compound	Calculated (M-H)	observed *m/z*	observed adducts	Retention Time (Minutes)
Alterochromide A/A'	764.37	764.29	(M-H+CH_2O_2) 810.28	11.55/11.65
Bromoalterochromide A/A'	842.28/844.28	842.15/844.09	(M-H+CH_2O_2) 888.05/890.00	12.82/13.08
Alterochromide A''	750.36	750.56	(M-H+CH_2O_2) 796.30	10.94

Bromoalterochromide A''	828.27/830.27	828.10/830.09	(M-H+CH_2O_2) 874.15/876.03	12.28
Alterochromide B/B'	790.39	790.28	(M-H+CH_2O_2) 836.27	12.55/12.69
Bromoalterochromide B/B'	868.30/870.30	868.15/870.14	(M-H+CH_2O_2) 914.05/916.08	13.77/13.87
Alterochromide B''	776.37	776.24	(M-H+CH_2O_2) 822.22	12.00
Bromoalterochromide B''	854.28/856.28	854.03/856.24	(M-H+CH_2O_2) 900.12/902.27	13.26

***P. rubra* DSM-6842**

Compound	Predicted (M+H)	Observed *m/z*	Observed Adducts	Retention Time (Minutes)
Cycloprodigiosin	322.19	322.08	n/a	12.8
4''-(*n*-butyl)prodigiosin	310.14	310.04	n/a	12.8
Prodigiosin	324.21	324.06	(2M+2H+Cl^-) 683.29	13.69
4''-(*n*-hexyl)prodigiosin	338.22	338.09	n/a	14.35
4''-(*n*-heptyl)prodigiosin	352.24	352.16	n/a	14.87

Table S3: List of Other Secondary Metabolites Known to be Produced by *Pseudoalteromonas rubra, Pseudoalteromonas piscicida or Pseudoalteromonas luteoviolacea* but not detected in these experiments based on data from Marinlit [2]

Compound	Molecular Mass	Reference	Notes:
P. rubra			
2-(*p*-hydroxybenzyl)prodigiosin	429.24	DOI: 10.1021/np800493p	Strain not specified in this paper.
Pseudoalteromone A	320.38	DOI: 10.1007/s00253-014-5530-0	Described in strain QD1-2
alkyl-quinolinones	215.13, 243.16, 271.19	DOI: 10.3390/md14070129	Described in strain DSM-6842, as well as *P. piscicida* A1-J11
P. piscicida			
Norharman	168.199	DOI: 10.1042/BA20050176	Described in strain NJ6-3-1
Alteramide A	496.26	DOI: 10.3390/md14070129	Described in strain OT59
P. luteoviolacea			
Pentabromopseudilin	548.62	DOI: 10.1038/nchembio.1564	Described in strain I-L-33 and 2ta16

Various Bromophenol/bromopyrroles and their dimers	497.71, 599.53, 627.56, 525.74, 249.86, 327.77, 300.77, 378.68	DOI: 10.1038/nchembio.1564	Described in strain I-L-33 and 2ta16
Cyclo-(L-prolyl-L-glycine)	154.14	DOI: 10.1080/10575630008043781	Strain not Described
Cyclo-(L-phenylalanyl-4*R*-hydroxy-L-proline)	260.24	DOI: 10.1080/10575630008043781	Strain not Described
2,4-dibromo-6chlorophenol	283.82	DOI: 10.1080/10575630008043781	Strain not Described
Indolmycin	257.06	DOI: 10.1021/np100151y	Described in strain NCIMB 2035
4-hydroxy benzaldehyde	122.04	DOI: 10.3390/md14070129	Described in strain I-L-33
n-propyl-3-hydroxybenzoate	180.08	DOI: 10.3390/md14070129	Described in strain I-L-33
Thiomarinols B,C,D,E,F, G	672.80, 624.81, 654.83, 668.86, 638.79, 624.81	DOI: 10.3390/md14070129	Described in strain SANK 73390
Xenorhabdins,	338.15, 366.18, 336.13, 392.2, 420.23	DOI: 10.3390/md14070129	Described in strain SANK 73390

Table S4: Statistical analysis of metabolite profiles*

P. luteoviolacea			
Compound	Average Peak Area in planktonic culture (AU). [Standard Deviation]	Average peak area in cotton containing culture (AU). [Standard Deviation]	P Value (Paired Student's T test)
Violacein	0 (Not detected)	99068.27 [20543.4]	0.014
Thiomarinol A	0 (Not detected)	567851.50 [24149.8]	$6.0X10^{-4}$
P. piscicida			
Compound	Average ratio of Brominated analogue to non-brominated analogue in planktonic culture [Standard Deviation]	Average ratio of Brominated analogue to non-brominated analogue in cotton containing culture [Standard Deviation]	P Value (Paired Student's T test)
Bromoalterochromide A/A':Alterochromide A/A'	3.55 [0.276]	9.39 [1.415]	0.024

Bromoalterochromide B/B':Alterochromide B/B'	0.39 [0.148]	4.025 [1.159]	0.028
P. rubra			
Compound	Average Peak Area in planktonic culture (AU). [Standard Deviation]	Average peak area in cotton containing culture (AU). [Standard Deviation]	P Value (Paired Student's T test)
Unknown compound at 12.29 minutes RT	622.6 [287.8]	9155.6 [5161.7]	0.15125
Unknown compound at 12.96 minutes RT	1586.3 [273.6]	8869.6 [5836]	0.23385
Cycloprodigiosin	10695.33 [8628.3]	25597.67 [11485.98]	0.162958
Prodigiosin	87475 [53979.75]	96010.33 [32157.31]	0.831869
4''-(*n*-hexyl) prodigiosin	12503.0 [9168.827]	14142.67 [5693.175]	0.810407
4''-(*n*-heptyl) prodigiosin	3012.333 [2300.602]	3956.333 [1580.594]	0.566131

*Analyisis was performed using the Analysis ToolPak module of Microsoft Excel based on the following formula for *t* statistic:

$$t = \frac{\bar{x} - \bar{y} - \Delta_0}{\sqrt{\frac{S_1^2}{m} + \frac{S_2^2}{n}}}$$

1 Blin, K. *et al.* (2017) antiSMASH 4 . 0 — improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res.* **45**, 36–41

2 Royal Society of Chemistry (2018) , MarinLit - A database of the marine natural products literature. [Online]. Available: http://pubs.rsc.org/marinlit/. [Accessed: 15-May-2018]