SUPPLEMENTAL MATERIAL

Whidbey et al., http://www.jem.org/cgi/content/full/jem.20122753/DC1

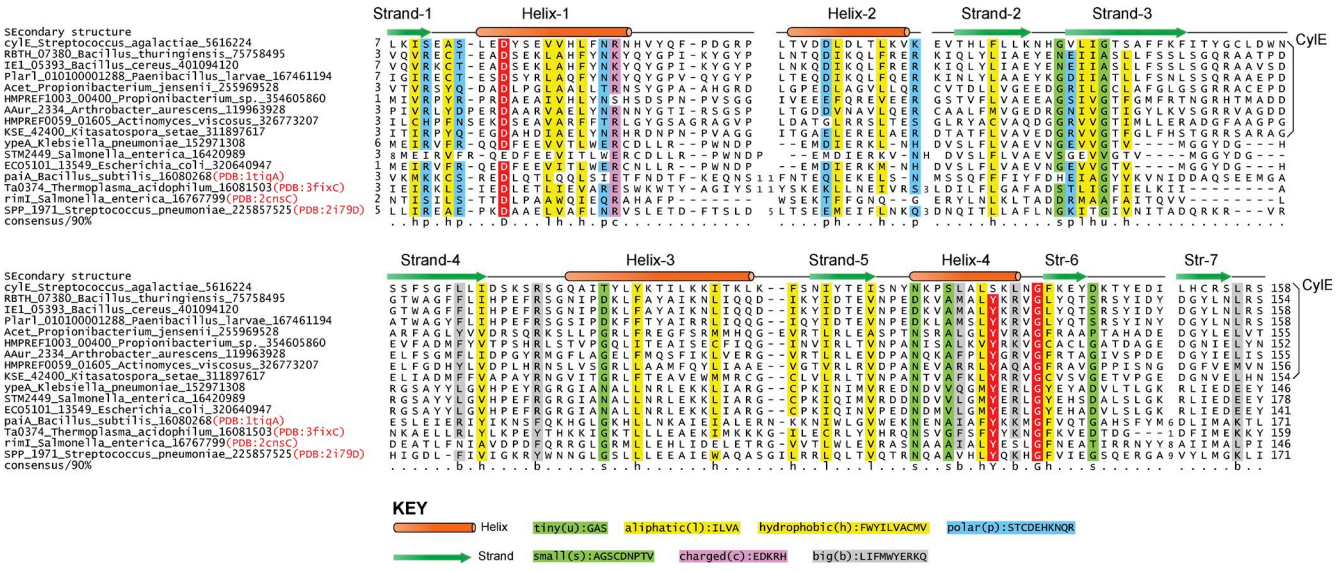


Figure S1. Alignment of CylE to known N-acyltransferases. Iterative sequence searches of GBS or *Streptococcus agalactiae* CylE using PSI-BLAST identified several homologues within the N acyltransferase superfamily and these sequences were aligned using Kalign. Secondary structure predictions show similar progression of secondary structure elements as known N-acyltransferases, further supporting the conclusion that CylE is an N-acyltransferase. Bracket denotes CylE homologues in organisms that also have genes of the Cyl operon.

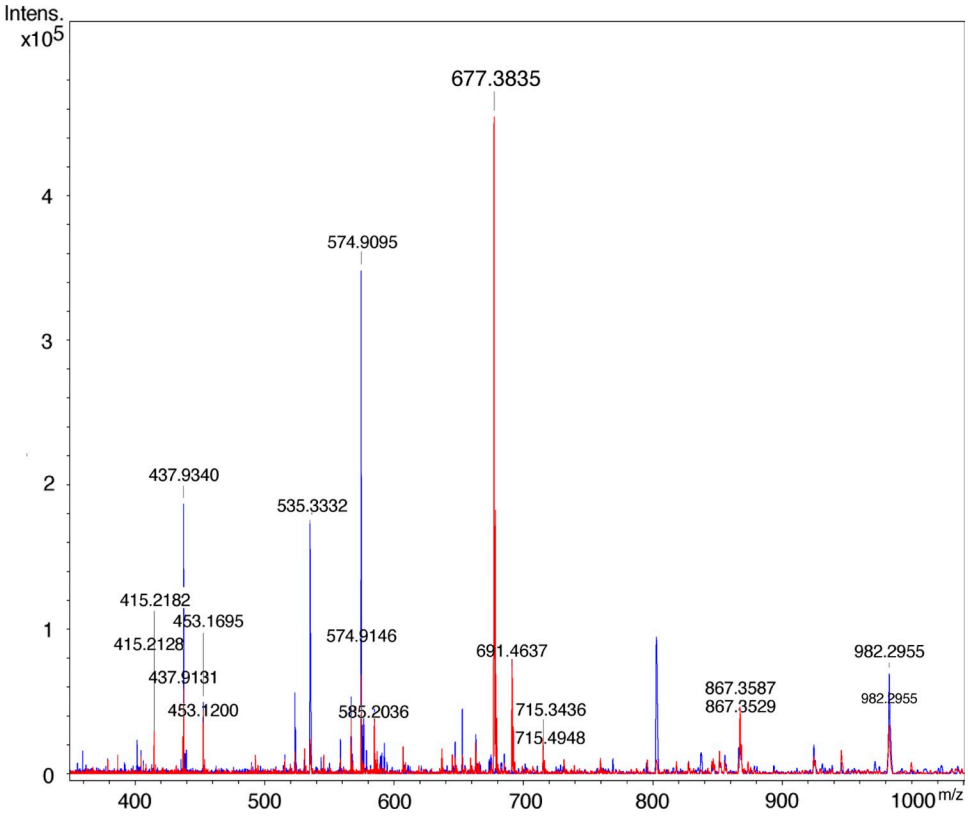
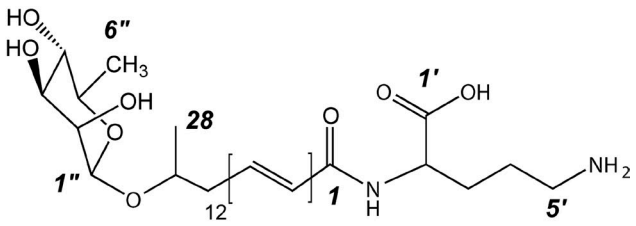


Figure S2. Mass spectrometry confirms that the purified pigment with hemolytic activity is the GBS pigment previously identified as granadaene. Purified pigment from wild-type GBS dissolved in DMSO with 0.1% TFA was analyzed by Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and the spectrum is shown in red. The peak at 677.3835 *m/z* corresponds to the full length pigment and the peak at 691.4637 *m/z* corresponds to a methylated derivative. To control for possible contaminants, extraction was also performed on the nonhemolytic and nonpigmented sample from the Δ*cylE* strain (spectrum overlaid in blue). A comparison between the MS spectra revealed that the 677.3835 *m/z* and 691.4637 *m/z* are unique to the pigmented sample. Data shown are representative spectra from experiments performed on three independent pigment preparations and controls.

A

Position	^{1}H (ppm)	$^1J_{H,H}$ (Hz)	Position	^{1}H (ppm)	$^1J_{H,H}$ (Hz)
2	6.12 (*br. s*)		5'	2.80 (*br. s*)	
3-23	6.0-7.4		NH	8.36 (*d*)	8.1
24	6.15 (*m*)		NH_2	7.68 (*br. s*)	
25	5.77 (*dd*)	14.9, 7.5	1"	4.66 (*s*)	
26	2.29 (*m*)		2"	3.54 (*m*)	
27	3.72 (*m*)		3"	3.41 (*dd*)	9.4, 3.1
28	1.07 (*d*)	6.2	4"	3.19 (*t*)	9.2
2'	4.31 (*m*)		5"	3.45 (*dd*)	9.4, 6.4
3'	1.59 (*m*)		6"	1.12 (*d*)	6.2
4'	1.51 (*m*)				



B

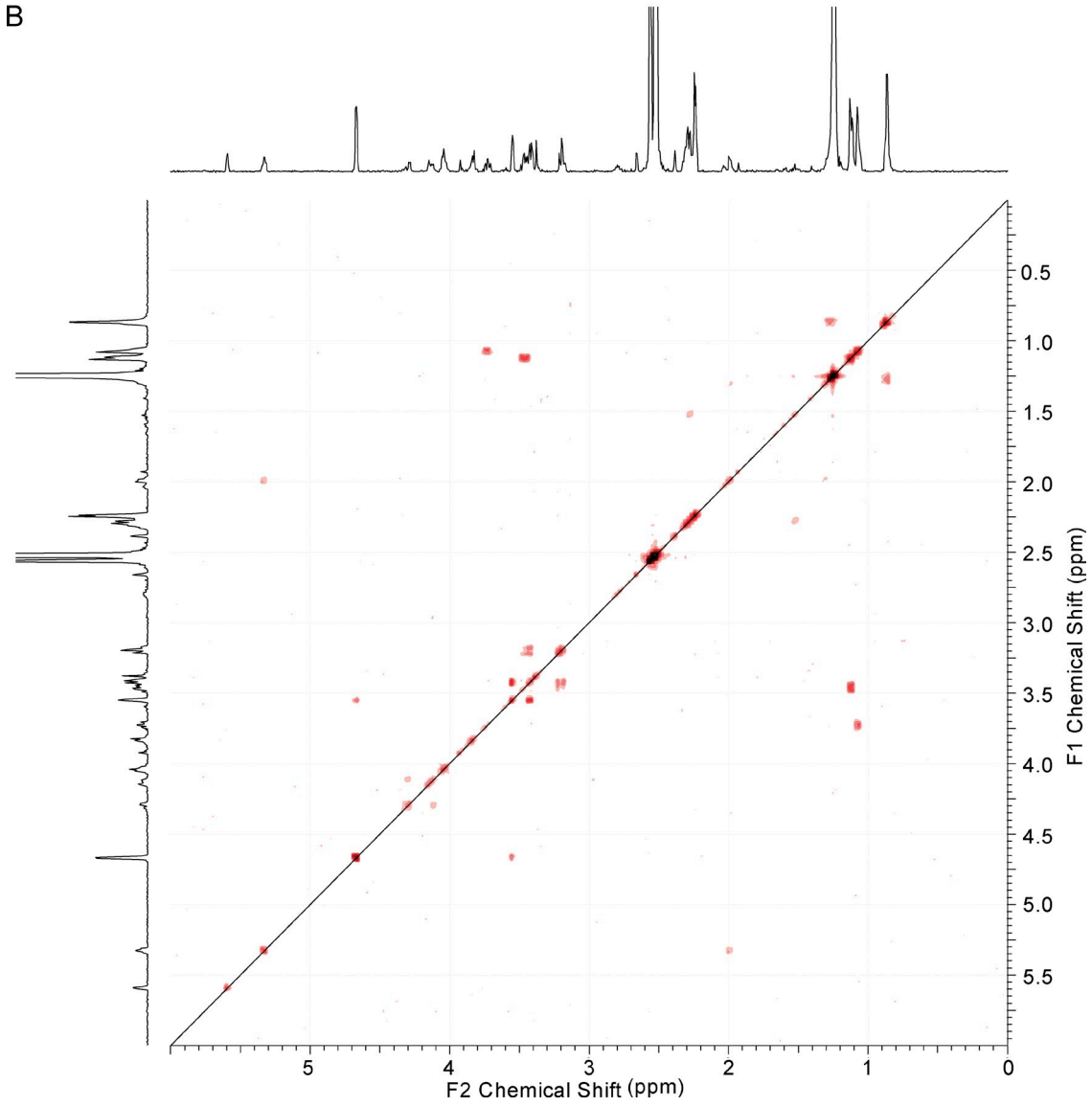


Figure S3. ^{1}H chemical shifts and ^{1}H–^{1}H COSY NMR of the purified GBS pigment. The pigment from WT GBS or control extract from Δ*cylE* were dissolved in DMSO-d_6: 0.1% *d*-TFA and analyzed by ^{1}H NMR and COSY at 298K on a Bruker AV-500 instrument. NMR was performed on two independent pigment preparations and controls. (A) ^{1}H shift assignments are listed. The broad peak from 6.4 to 6.8 ppm corresponds to the olefinic hydrogens present in the polyene; peaks at 7.68, 4.31, 1.62, 1.51, and 2.8 ppm correspond to hydrogens present in ornithine, whereas peaks at 4.66, 3.54, 3.41, 3.19, 3.46, and 1.12 ppm correspond to hydrogens on the rhamnose. Pigment structure is shown on the right. (B) The C27 hydrogen shift (3.73 ppm) corresponds to the ether linkage between rhamnose and C27. Likewise, the C2' hydrogen shift (4.31 ppm) suggests that ornithine is linked to the polyene by an amide bond at the α carbon (C2'). Collectively, these results indicate that the hemolytic molecule purified from GBS is the ornithine rhamnolipid previously described as granadaene (Rosa-Fraile et al., 2006; Vanberg et al., 2007).

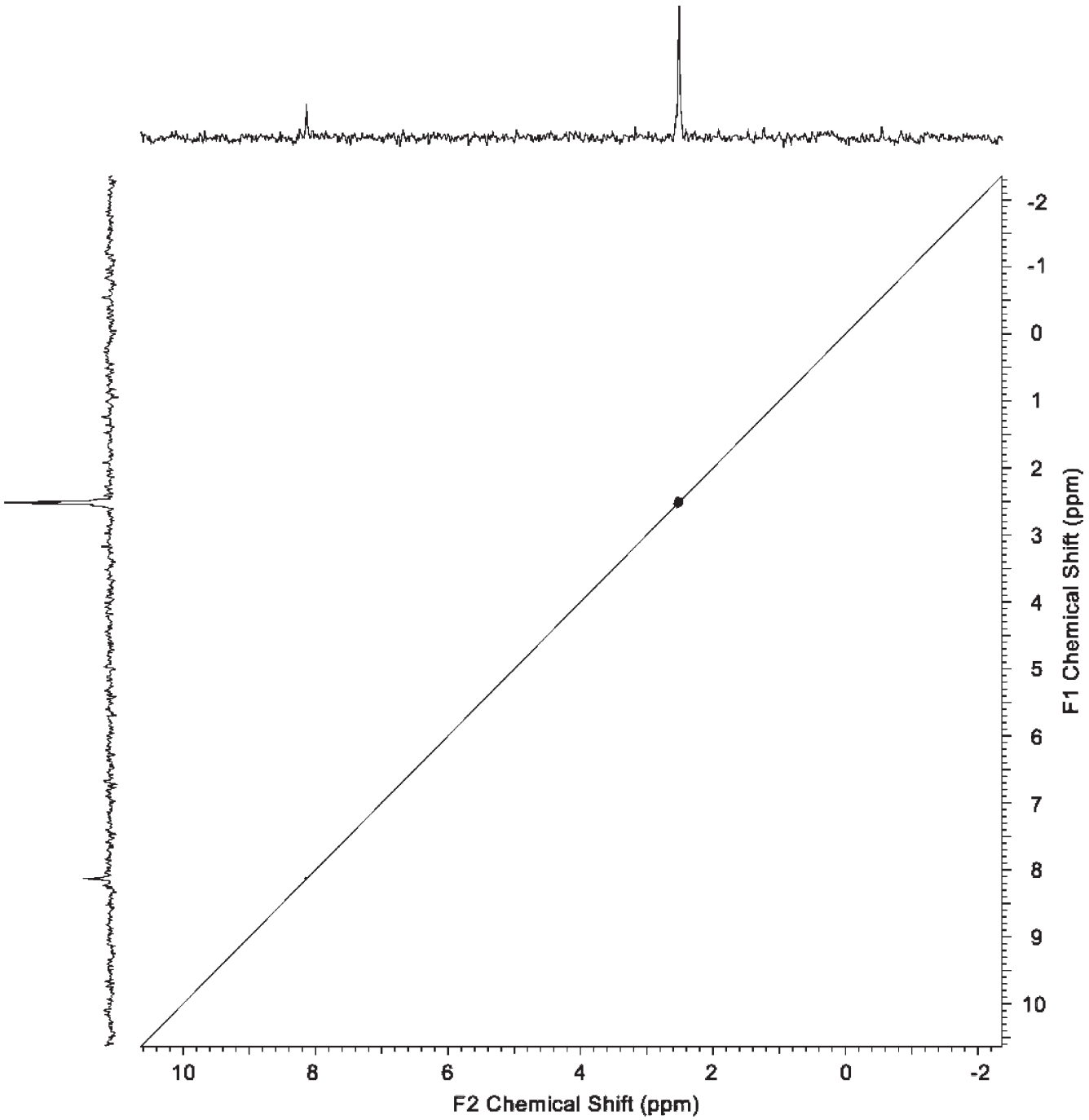


Figure S4. COSY analysis of the Δ*cylE* extract only shows peaks corresponding to residual DMSO (2.52 ppm) and TFA (8.13 ppm).

Table S1. Primers used in this study (listed 5′-3)

Name	Sequence
CylEF	TAGCGAATTCAGGAGGATGAAAGATGATAATAAATTAAAG
CylER	TAGCGAATTCATGAAAGATGATAATAAATTAAAG
CylABEF	AAAAAA GAATTCAGGAGGATATACATATGGAAATTAAACTCAAAAATATTGG
CylABER	TTTTTTGGATCCCTGTTCTCAATGTATTATAAATAGTATTC
dCylopupF	AAAAAATCTAGAAAGCTGGACCGTGCC
dCylopupR	CACTGTTCCTTGCATATTATCACCTTCAAC
dcylkanF	GTTGAAGGTGATAATATGCAAGGAACAGTG
dcylkanR	GGAACAACATGTTGCGAGTTGCGGATGTACTTCAG
dCylopdnF	CTGAAGTACATCCGCAACTCGCAACATGTTGTTCC
dCylopdnR	TTTTTTCTCGAGCAGTTTCACTTTTGACAACC
*qRTcylE*L	GGAAGTTACCCGATTGAGCA
*qRTcylE*R	TGCCAGGAGGAGAATAGGAA
*rps*LF	TGCCCTTCGTAAATTTGCTC
*rps*LR	AACGTACCCCTGGAAGGTCT
IL6F	GGAGACTTGCCTGGTGAAAA
IL6R	CAGGGGTGGTTATTGCATCT
IL8F	AGCTCTGTGTGAAGGTGCAG
IL8R	AATTTCTGTGTTGGCGCAGT
CXCL1F	CTCTTCCGCTCCTCTCACAG
CXCL1R	GGGGACTTCACGTTCACACT
CCL20F	GCGCAAATCCAAAACAGACT
CCL20R	CAAGTCCAGTGAGGCACAAA
IL1bF	TGGGCCTCAAAGGAAAGA
IL1bR	GGTGCTGATGTACCAGTT
GAPDHF	GAAGGTGAAGGTCGGAGTCAACG
GAPDHR	TCCTGGAAGATGGTGATGGGAT
CovRF	GCGCGGAGCTCTTGTTAAGTAAAGAATAAG
CovR R	GCGCGAGGATCCTTTATTTTTCACGAATCAC
CovS F	GCGCGCGAGCTCTATTCAAACTGTTCGCGGAAT
CovS R	GCGCGCGGATCCTTTATATTTCTTTAGTTTCTT
CovS220F	GTGCTACTAAGCGTATTGTTCGTCCGGTTAAAATTTACACGAC
CovS220R	GTCGTGTAAATTTTAACCGGACGAACAATACGCTTAGTAGCAC
CovSV343MF	CTTGGAACTTCATCAAGATGAAATGACAGATTTATCAAGCTC
CovSV343MR	GAGCTTGATAAATCTGTCATTTCATCTTGATGAAGTTCCAAG
CovR/S F	AAAAAA GGATCC GAGCTCGTTGATCAGGATTTGGTC
CovR/S R	TTTTTTGGTACCACTAAAAGAGGCAATTCTTCCAAACG