# Supplementary Information

# The regulator of type IV pili synthesis, PilR, from *Lysobacter* controls antifungal antibiotic production via a c-di-GMP pathway

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**Running Head**: Regulation of antibiotic production in *Lysobacter* via PilR

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## Table S1 Identified two-component systems in *Lysobacter enzymogenes*

| ***L. enzymogenes* strain OH11** | | | | ***L. enzymogenes* strain C3[b]** | | | | |
|---|---|---|---|---|---|---|---|---|
| **TCS locus[a]** | **Protein ID** | **Class** | **Family[c]** | **Protein ID** | **Genebank ID (GI)** | **E-value** | **Ident(%)** | **Protein definition** |
| *1 | Le0041 | RR | NarL | ALN55927.1 | 951301690 | 2e-144 | 99 | Two-component system response regulator, LuxR family |
| 2 | Le0371 | RR | NarL | ALN59922.1 | 951305685 | 6e-88 | 61 | Transcriptional regulator, LuxR family |
| | Le0372 | HK | Classic | ALN59923.1 | 951305686 | 2e-72 | 42 | Sensor histidine kinase |
| *3 | Le0440 | HK | Hybrid | ALN55523.1 | 951301286 | 0.0 | 99 | Sensory box histidine kinase |
| *4 | Le0445 | RR | NarL | ALN55518.1 | 951301281 | 7e-160 | 100 | Transcriptional regulator, LuxR family |
| *5 | Le0537 | RR | NarL | ALN55363.1 | 951301126 | 2e-141 | 89 | LuxR family regulatory protein |
| 6 | Le0597 | HK | Classic | ALN57914.1 | 951303677 | 5e-28 | 30 | Histidine kinase |
| | Le0598 | RR | OmpR | ALN57913.1 | 951303676 | 2e-48 | 38 | Transcriptional regulatory protein, C terminal-DNA-binding response regulator |
| *7 | Le0674 | HK | Hybrid | ALN60709.1 | 951306472 | 0.0 | 99 | Sensor histidine kinase |
| 8 | Le0735 | HK | Classic | ALN60651.1 | 951306414 | 0.0 | 98 | Sensory box histidine kinase |
| | Le0736 | RR | CheY | ALN60650.1 | 951306413 | 4e-107 | 100 | Response regulator receiver protein |
| 9 | Le0752 | RR | OmpR | ALN60588.1 | 951306351 | 2e-161 | 99 | Response regulator receiver domain |
| | Le0754 | HK | Classic | ALN60589.1 | 951306352 | 0.0 | 94 | Sensor histidine kinase |
| 10 | Le0759 | HK | Classic | ALN60608.1 | 951306371 | 0.0 | 94 | Two-component system sensor protein |
| | Le0760 | RR | NarL | ALN60609.1 | 951306372 | 1e-148 | 99 | Transcriptional regulator, LuxR family |
| 11 | Le0871 | HK | Unorthodox | ALN60506.1 | 951306269 | 0.0 | 96 | Sensory box histidine kinase |
| | Le0872 | RR | VieA | ALN60505.1 | 951306268 | 0.0 | 96 | Response regulator protein |
| *12 | Le0906 | RR | PrrA | ALN60469.1 | 951306232 | 4e-141 | 99 | Photosynthetic apparatus regulatory protein RegA |
| 13 | Le0916 | RR | NarL | ALN60459.1 | 951306222 | 3e-146 | 98 | Response regulator receiver domain |
| | Le0918 | HK | Classic | ALN60456.1 | 951306219 | 0.0 | 95 | His Kinase A (phosphoacceptor) domain |
| 14 | Le0978 | HK | Classic | ALN60390.1 | 951306153 | 0.0 | 99 | Nitrogen regulation protein NtrB |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Le0979 | RR | NtrC | ALN60389.1 | 951306152 | 0.0 | 99 | Nitrogen regulation protein NR(I) |
| *15 | Le1110 | RR | FrzZ | ALN60254.1 | 951306017 | 0.0 | 100 | Response regulator protein |
| 16 | Le1119 | HK | Classic | ALN60244.1 | 951306007 | 0.0 | 98 | Sensor histidine kinase |
| | Le1120 | RR | OmpR | ALN60243.1 | 951306006 | 7e-165 | 99 | Response regulator receiver domain protein |
| 17 | Le1128 | HK | Classic | ALN60231.1 | 951305994 | 0.0 | 98 | Sensor kinase RpeA |
| | Le1130 | RR | OmpR | ALN60230.1 | 951305993 | 9e-171 | 100 | DNA-binding response regulator |
| 18 | Le1234 | RR | OmpR | ALN60121.1 | 951305884 | 2e-163 | 99 | Transcriptional regulatory protein, C terminal - DNA-binding response regulator |
| | Le1235 | HK | Classic | ALN60120.1 | 951305883 | 0.0 | 98 | Sensor histidine kinase |
| 19 | Le1262 | HK | Classic | ALN60094.1 | 951305857 | 0.0 | 96 | Sensor histidine kinase |
| | Le1263 | RR | OmpR | ALN60093.1 | 951305856 | 2e-156 | 97 | Transcriptional regulatory protein, C terminal - transcriptional regulator |
| 20 | Le1422 | HK | Classic | ALN59923.1 | 951305686 | 0.0 | 98 | Sensor histidine kinase |
| | Le1423 | RR | NarL | ALN59922.1 | 951305685 | 2e-157 | 100 | Transcriptional regulator, LuxR family |
| 21 | Le1445 | HK | Classic | ALN59895.1 | 951305658 | 0.0 | 98 | Two-component system sensor protein |
| | Le1446 | RR | LytTR | ALN59894.1 | 951305657 | 2e-173 | 99 | Alginate biosynthesis regulatory protein |
| 22 | Le1610 | RR | OmpR | ALN59664.1 | 951305427 | 5e-157 | 99 | DNA-binding response regulator |
| | Le1611 | HK | Classic | ALN59663.1 | 951305426 | 0.0 | 98 | Sensor histidine kinase |
| 23 | Le1647 | RR | OmpR | ALN59623.1 | 951305386 | 2e-163 | 100 | DNA-binding response regulator |
| | Le1648 | HK | Classic | ALN59622.1 | 951305385 | 0.0 | 98 | Sensor histidine kinase |
| 24 | Le1804 (PilR) | RR | NtrC | ALN59455.1 | 951305218 | 0.0 | 99 | Type IV pilus expression regulatory protein |
| | Le1805 (PilS) | HK | Classic | ALN59454.1 | 951305217 | 0.0 | 99 | Type IV pilus sensor protein |
| *25 | Le1909 | RR | CheY | ALN59304.1 | 951305067 | 2e-88 | 100 | Type IV pilus assembly protein |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *26 | Le1910 | RR | CheY | ALN59303.1 | 951305066 | 3e-67 | 98 | Type IV pilus assembly protein |
| *27 | Le1914 | HK | CheA | ALN59300.1 | 951305063 | 0.0 | 99 | Hpt domain protein |
| 28 | Le1919 | HK | Classic | ALN59294.1 | 951305057 | 0.0 | 81 | Signal transduction histidine kinase |
| | Le1921 | RR | CheY | ALN59293.1 | 951305056 | 6e-89 | 99 | Response regulator receiver domain |
| 29 | Le1936 | RR | OmpR | ALN59277.1 | 951305040 | 5e-168 | 100 | Transcriptional regulatory protein, C terminal DNA-binding response regulator v |
| | Le1937 | HK | Classic | ALN59276.1 | 951305039 | 0.0 | 99 | Histidine kinase |
| 30 | Le2134 | RR | NarL | ALN59088.1 | 951304851 | 4e-165 | 99 | Transcriptional regulator, LuxR/UhpA family |
| | Le2135 | HK | Hybrid | ALN59087.1 | 951304850 | 0.0 | 97 | Sensory box histidine kinase |
| 31 | Le2296 | RR | NarL | ALN58894.1 | 951304657 | 3e-155 | 99 | Two component transcriptional regulator, LuxR family |
| | Le2297 | HK | Classic | ALN58893.1 | 951304656 | 1e-163 | 97 | Histidine kinase |
| 32 | Le2332 | HK | Classic | ALN58862.1 | 951304625 | 0.0 | 100 | Histidine kinase |
| | Le2333 | RR | NarL | ALN58861.1 | 951304624 | 5e-145 | 100 | Transcriptional regulator, LuxR family |
| 33 | Le2666 | RR | OmpR | ALN58543.1 | 951304306 | 8e-175 | 99 | KDP operon transcriptional regulatory protein KdpE |
| | Le2667 | HK | Classic | ALN58542.1 | 951304305 | 0.0 | 99 | Two-component system sensor kinase KdpD |
| 34 | Le2947 | HK | Classic | ALN57550.1 | 951303313 | 0.0 | 99 | Histidine kinase |
| | Le2949 | RR | PrrA | ALN57551.1 | 951303314 | 6e-128 | 97 | DNA-binding response regulator |
| *35 | Le2973 | HK | Classic | ALN57572.1 | 951303335 | 0.0 | 98 | Sensory box histidine kinase |
| 36 | Le3034 | RR | RpfG | ALN57630.1 | 951303393 | 0.0 | 100 | Response regulator |
| | Le3035 | HK | Unorthodox | ALN57631.1 | 951303394 | 0.0 | 99 | Sensory/regulatory protein RpfC |
| 37 | Le3126 | RR | PrrA | ALN57720.1 | 951303483 | 6e-145 | 99 | Photosynthetic apparatus regulatory protein RegA |
| | Le3127 | HK | Classic | ALN57721.1 | 951303484 | 0.0 | 99 | Histidine kinase |
| *38 | Le3199 | RR | PleD | ALN57796.1 | 951303559 | 0.0 | 99 | WspR |
| 39 | Le3200 | RR | CheB | ALN57797.1 | 951303560 | 0.0 | 99 | Protein-glutamate methylesterase CheB |
| | Le3201 | HK | CheA | ALN57798.1 | 951303561 | 0.0 | 99 | Histidine kinase |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 40 | Le3342 | HK | Hybrid | ALN57980.1 | 951303743 | 0.0 | 90 | Sensory box histidine kinase |
| | Le3343 | RR | NarL | ALN57981.1 | 951303744 | 1e-137 | 93 | DNA-binding response regulator, LuxR family |
| *41 | Le3450 | RR | CheY | ALN58078.1 | 951303841 | 2e-91 | 99 | Type IV pilus assembly protein |
| 42 | Le3590 | RR | NarL | ALN58210.1 | 951303973 | 3e-156 | 99 | Two component transcriptional regulator, LuxR family |
| | Le3591 | HK | Hybrid | ALN58212.1 | 951303975 | 0.0 | 98 | Sensory box histidine kinase |
| *43 | Le3679 | RR | NarL | ALN58299.1 | 951304062 | 5e-152 | 100 | Two-component system response regulator, LuxR family |
| 44 | Le3696 | RR | OmpR | ALN58320.1 | 951304083 | 2e-179 | 100 | Transcriptional regulatory protein |
| | Le3697 | HK | Classic | ALN58321.1 | 951304084 | 0.0 | 98 | Signal transduction histidine kinase |
| *45 | Le3816 | RR | OmpR | ALN58438.1 | 951304201 | 0.0 | 99 | Two component transcriptional regulator, winged helix family |
| *46 | Le4011 | RR | PleD_VieA | ALN57164.1 | 951302927 | 0.0 | 99 | Sensory box-containing diguanylate cyclase/cyclic diguanylate phosphodiesterase |
| 47 | Le4033 | HK | Classic | ALN57142.1 | 951302905 | 0.0 | 98 | Sensor protein RstB |
| | Le4034 | RR | OmpR | ALN57141.1 | 951302904 | 3e-165 | 99 | DNA-binding response regulator |
| 48 | Le4041 | HK | Classic | ALN57135.1 | 951302898 | 0.0 | 97 | Putative heavy metal histidine sensor kinase |
| | Le4042 | RR | OmpR | ALN57134.1 | 951302897 | 1e-171 | 99 | Two component heavy metal response transcriptional regulator |
| *49 | Le4104 | HK | Hybrid | ALN57068.1 | 951302831 | 2e-163 | 98 | Sensor histidine kinase |
| 50 | Le4189 | RR | OmpR | ALN57006.1 | 951302769 | 2e-172 | 99 | Phosphate regulon transcriptional regulatory protein PhoB |
| | Le4190 | HK | Classic | ALN57005.1 | 951302768 | 0.0 | 99 | Phosphate regulon sensor kinase PhoR |
| 51 | Le4212 | HK | Classic | ALN56986.1 | 951302749 | 0.0 | 99 | Sensor histidine kinase |
| | Le4215 | RR | OmpR | ALN56985.1 | 951302748 | 2e-161 | 98 | DNA-binding response regulator |
| 52 | Le4259 | HK | Classic | ALN56935.1 | 951302698 | 0.0 | 99 | Histidine kinase |
| | Le4260 | RR | NarL | ALN56936.1 | 951302699 | 7e-151 | 99 | Two-component system regulatory protein |
| 53 | Le4302 | HK | Classic | ALN56897.1 | 951302660 | 0.0 | 95 | Histidine kinase |
| | Le4303 | RR | LytTR | ALN56896.1 | 951302659 | 0.0 | 96 | Response regulator receiver/LytTR DNA-binding domain protein |

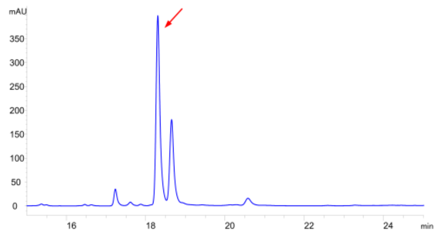
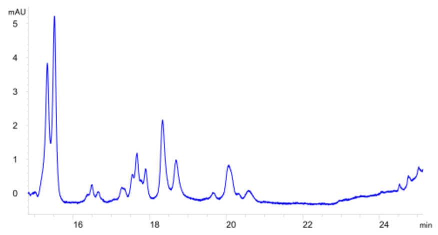
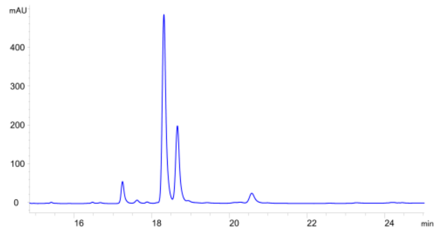
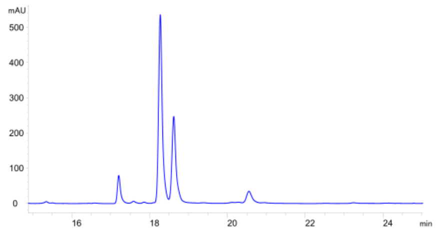
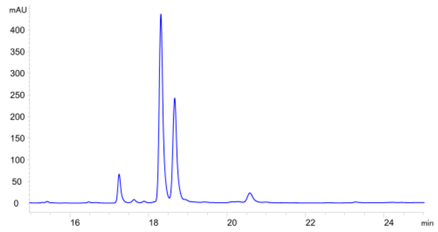
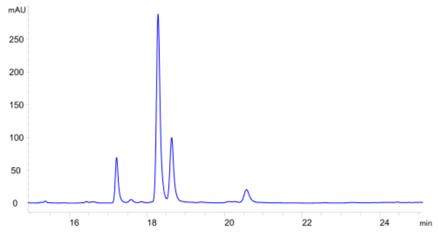
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *54 | Le4678 | HK | Hybrid | ALN56502.1 | 951302265 | 0.0 | 98 | Histidine kinase/ response regulator hybrid protein |
| *55 | Le4679 | HK | Hybrid | ALN56501.1 | 951302264 | 0.0 | 99 | Histidine kinase-, DNA gyrase B-, and HSP90-like atpase |
| 56 | Le4778 | RR | LytTR | ALN59894.1 | 951305657 | 4e-39 | 39 | Alginate biosynthesis regulatory protein |
| | Le4779 | HK | Classic | ALN59895.1 | 951305658 | 4e-26 | 40 | Two-component system sensor protein |
| 57 | Le4788 | HK | Classic | ALN56382.1 | 951302145 | 0.0 | 99 | Sensor histidine kinase |
| | Le4789 | RR | OmpR | ALN56381.1 | 951302144 | 5e-168 | 100 | Transcriptional regulatory protein, C terminal |
| 58 | Le4844 | HK | Classic | ALN59682.1 | 951305445 | 0.0 | 99 | Sensor histidine kinase |
| | Le4845 | RR | OmpR | ALN59683.1 | 951305446 | 9e-170 | 100 | DNA-binding response regulator |
| 59 | Le5176 | RR | NtrC | ALN56052.1 | 951301815 | 0.0 | 99 | C4-dicarboxylate transport transcriptional regulatory protein DctD |
| | Le5177 | HK | Classic | ALN56051.1 | 951301814 | 0.0 | 99 | Histidine kinase |
| 60 | Le5230 | RR | OmpR | ALN55989.1 | 951301752 | 6e-165 | 97 | DNA-binding response regulator |
| | Le5231 | HK | Hybrid | ALN55990.1 | 951301753 | 0.0 | 97 | Sensory box histidine kinase |

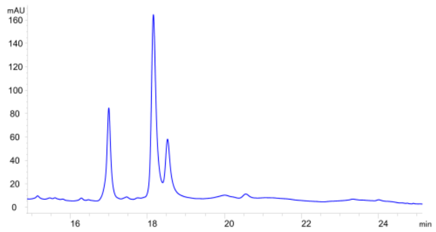
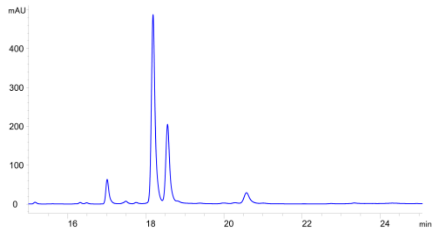
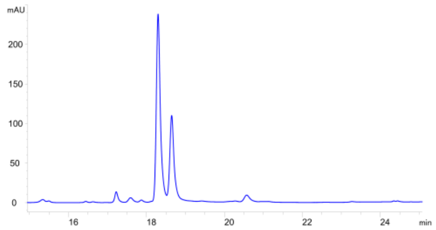
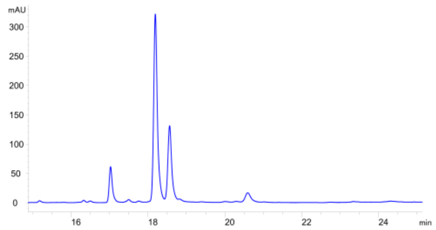
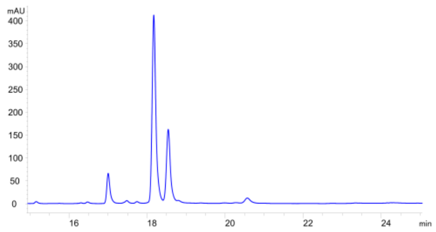
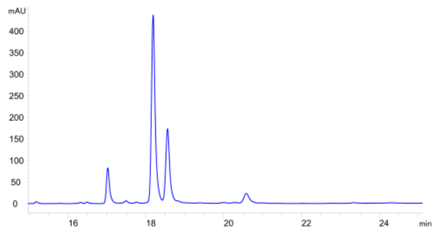
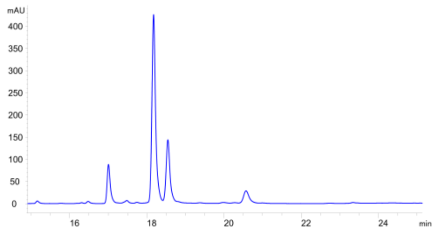
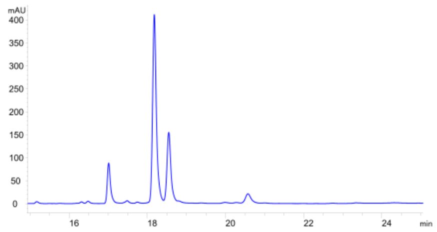
[a] The asterisks indicate that these two-component systems are orphans in *Lysobacter enzymogenes*.

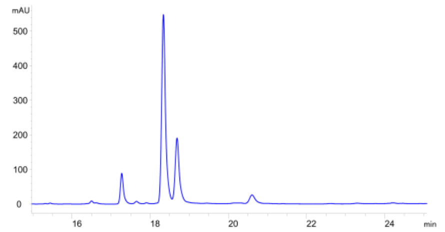
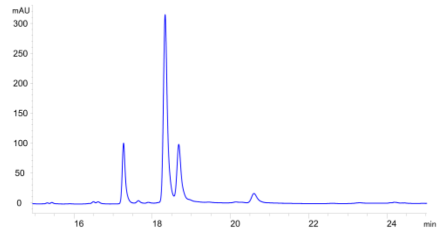
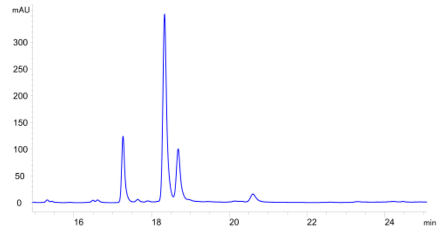
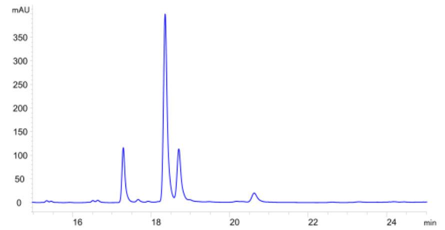
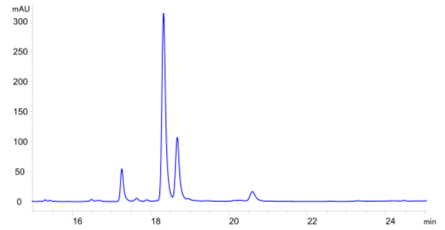
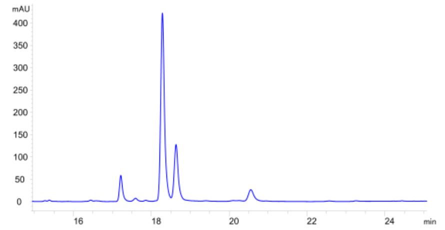
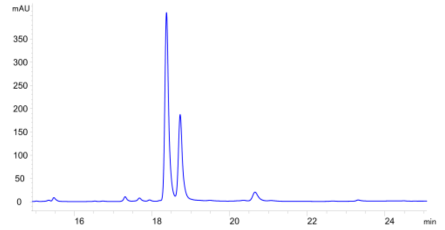
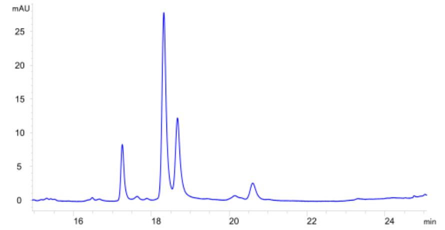
[b] Data for the genome information of *L. enzymogenes* strain C3 was downloaded from NCBI with an accession number of CP013140.

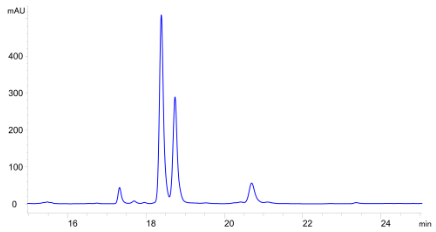
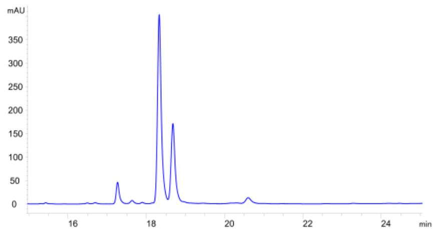
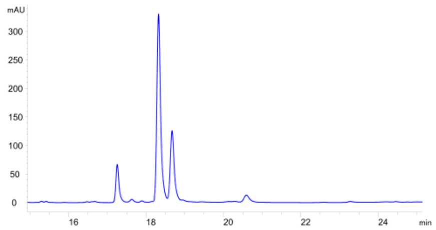
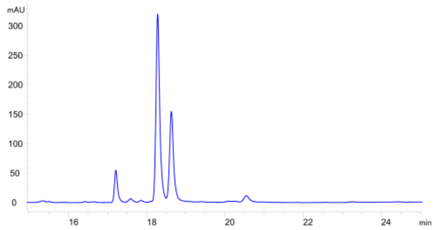
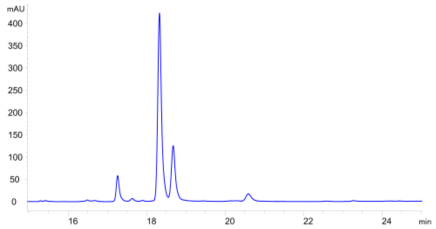
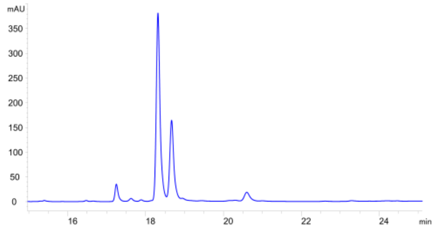
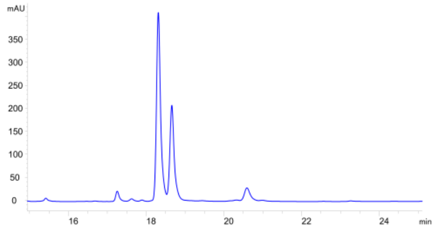
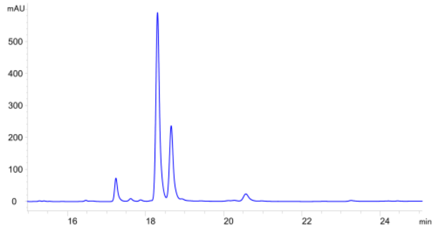
[c] The domain architecture of each family was presented in Figure 1.

**Table S2 Representative HPLC results for HSAF analysis of RR mutants**

| Strains | HPLC[a] | Peak area | OD$_{600}$ | Area/ OD$_{600}$ | Strains | HPLC | Peak area | OD$_{600}$ | Area/ OD$_{600}$ |
|---|---|---|---|---|---|---|---|---|---|
| OH11 |  | 2805.2 | 1.26 | 2226.3 | Δ*rpfG*$_{Le}$ |  | 18.8 | 1.428 | 13.2 |
| Δ*Le0041* |  | 3335.8 | 1.132 | 2946.8 | Δ*Le0371* |  | 3611.5 | 1.262 | 2861.7 |
| Δ*Le0445* |  | 3010.4 | 1.07 | 2813.5 | Δ*Le0537* |  | 1992.2 | 1.26 | 1581.1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le0598* |  | 1432.2 | 1.214 | 1179.7 | Δ*Le0760* |  | 3308.3 | 1.072 | 3086.1 |
| Δ*Le0872* |  | 1750.2 | 1.199 | 1459.7 | Δ*Le0906* |  | 2192.2 | 1.201 | 1825.3 |
| Δ*Le0916* |  | 2799.5 | 1.191 | 2350.5 | Δ*Le0979* |  | 2958.3 | 1.152 | 2568.0 |
| Δ*Le1110* |  | 2869.4 | 1.135 | 2528.1 | Δ*Le1120* |  | 2799.2 | 1.415 | 1978.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le1130* |  | 3815.2 | 1.7 | 2244.2 | Δ*Le1234* |  | 2317.3 | 1.249 | 1855.3 |
| Δ*Le1263* |  | 2498.5 | 1.24 | 2014.9 | Δ*Le1423* |  | 2747 | 1.139 | 2411.8 |
| Δ*Le1446* |  | 2159.8 | 1.223 | 1766.0 | Δ*Le1610* |  | 2873.6 | 1.444 | 1990.0 |
| Δ*Le1647* |  | 2842.7 | 1.285 | 2212.2 | Δ*pilR* |  | 216.4 | 1.097 | 197.3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le1910* |  | 3850.9 | 1.283 | 3001.5 | Δ*Le1921* |  | 2794.1 | 1.3 | 2149.3 |
| Δ*Le1936* |  | 2222 | 0.876 | 2536.5 | Δ*Le2134* |  | 2206 | 1.305 | 1690.4 |
| Δ*Le2333* |  | 2928.2 | 1.237 | 2367.2 | Δ*Le2666* |  | 2636.1 | 1.213 | 2173.2 |
| Δ*Le2949* |  | 3078.4 | 1.21 | 2544.1 | Δ*Le3126* |  | 4015 | 1.063 | 3777.0 |

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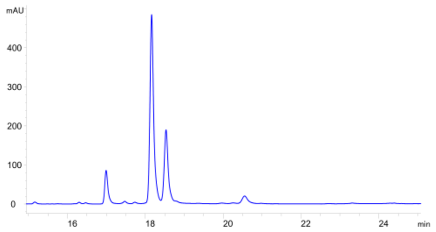
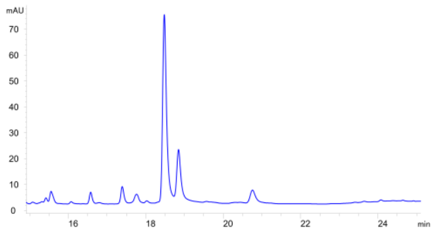
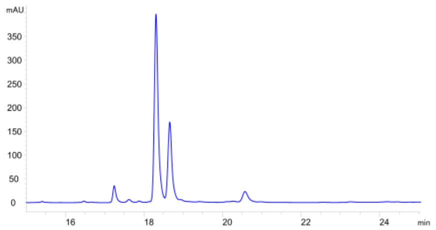
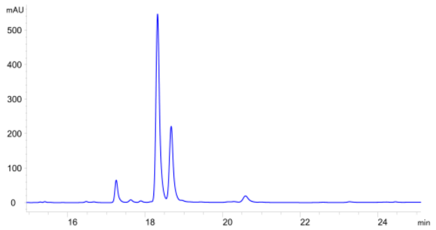
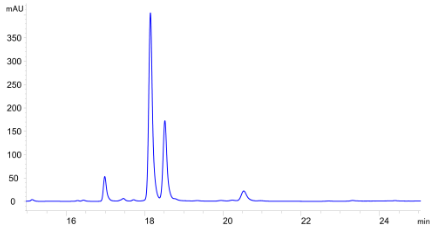
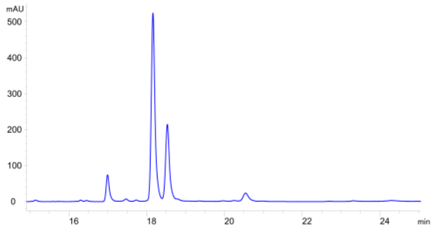
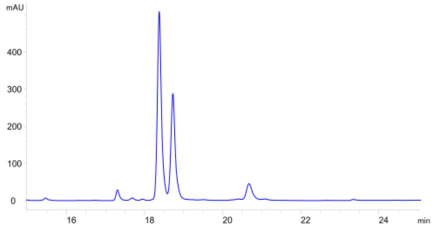
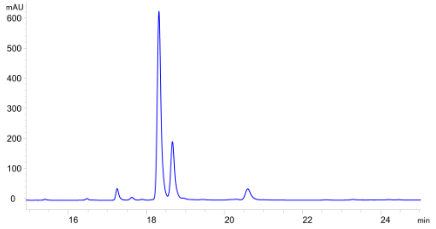
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le3199* |  | 3295.2 | 1.344 | 2451.8 | Δ*Le3200* |  | 529.9 | 1.422 | 372.6 |
| Δ*Le3343* |  | 2741.2 | 1.119 | 2449.7 | Δ*Le3450* |  | 3723.3 | 1.063 | 3502.6 |
| Δ*Le3590* |  | 2762.4 | 1.022 | 2702.9 | Δ*Le3696* |  | 3546.3 | 1.174 | 3020.7 |
| Δ*Le3816* |  | 3796.5 | 1.39 | 2731.3 | Δ*Le4011* |  | 4302.2 | 1.443 | 2981.4 |

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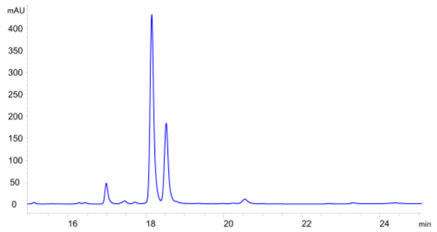
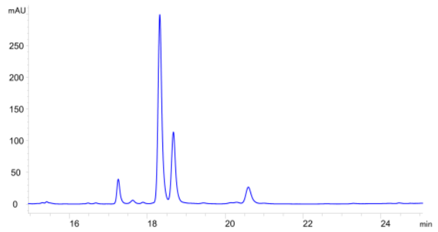
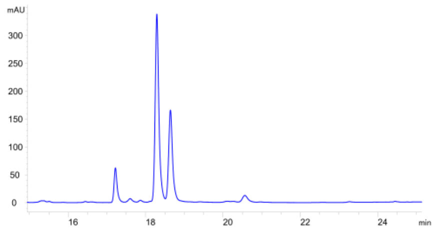
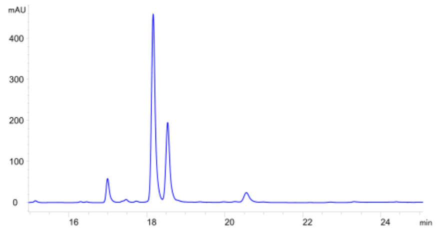
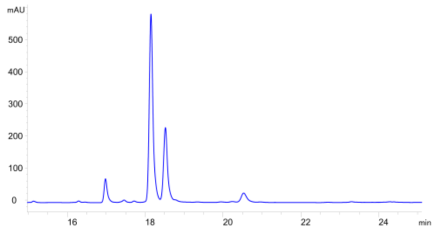
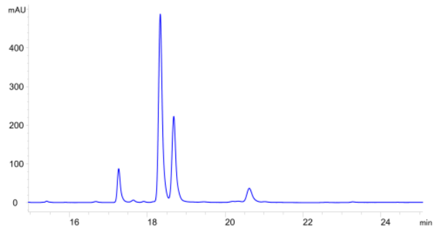
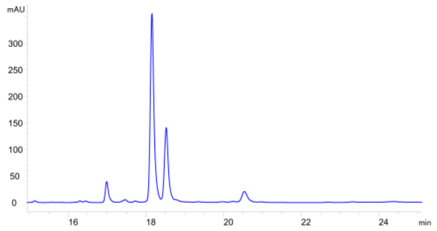
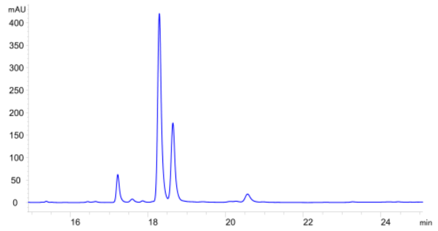
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le4034* |  | 2957 | 1.03 | 2870.9 | Δ*Le4042* |  | 2103.3 | 1.218 | 1726.8 |
| Δ*Le4189* |  | 2285.2 | 1.205 | 1896.4 | Δ*Le4215* |  | 3125.1 | 1.234 | 2532.5 |
| Δ*Le4260* |  | 3987.5 | 1.049 | 3801.2 | Δ*Le4303* |  | 3359.8 | 1.223 | 2747.2 |
| Δ*Le4778* |  | 2411 | 1.152 | 2092.9 | Δ*Le5176* |  | 2854.1 | 1.266 | 2254.4 |

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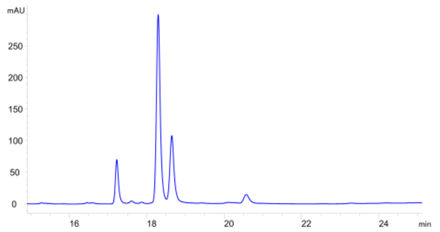
|---|---|---|---|---|---|---|---|---|---|
| Δ*Le5230* |  | 2098.2 | 1.38 | 1520.4 | | | | | |

[a]The peak of HSAF produced by *Lysobacter enzymogenes* OH11 was indicated by a red arrow.

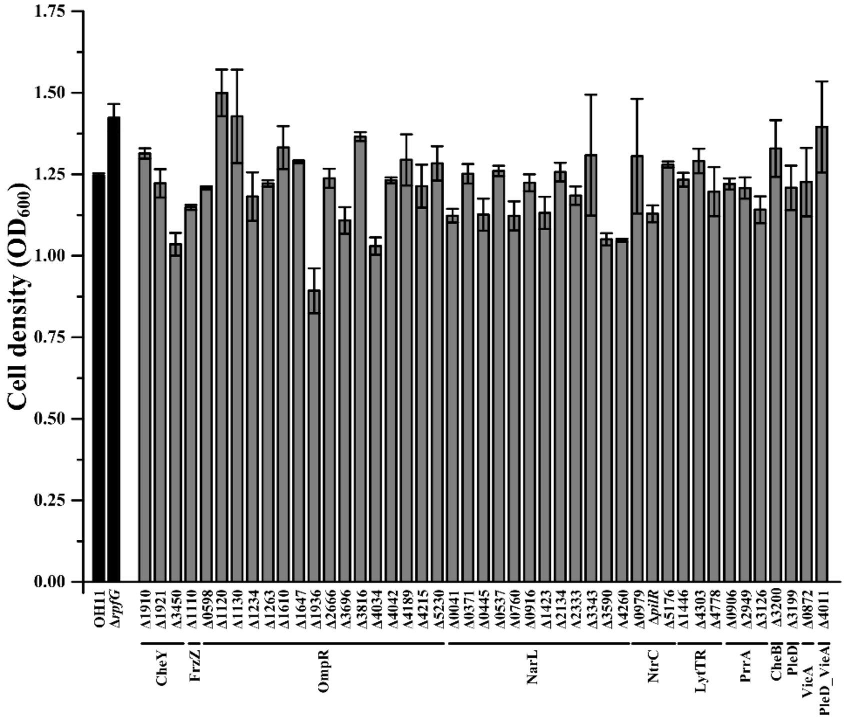


**Figure S1. *L. enzymogenes* RR deletion mutants do not have significant growth defects in the HSAF-inducing medium.** The same $OD_{600}$ of bacterial culture of each mutant was used as original inoculum, and these inoculated cultures were grown at 28°C for 48 h, followed by measuring $OD_{600}$.

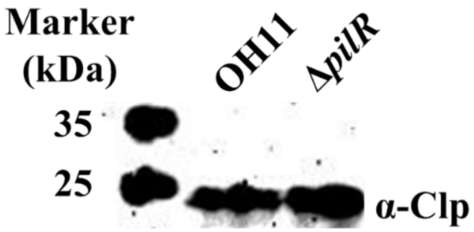


**Figure S2 Mutation of *pilR* did not significantly alter protein level of Clp in *Lysobacter enzymogenes*.** Cells were harvested at $OD_{600}$ of 1.0 that were grown in the HSAF-producing medium (1/10 TSB). At this $OD_{600}$ value (1.0), *L. enzymogenes* has been shown to produce HSAF according to our earlier study (1). The 25-KD band corresponding to the predicated size of Clp was detected by the polyclonal antibody α-Clp.

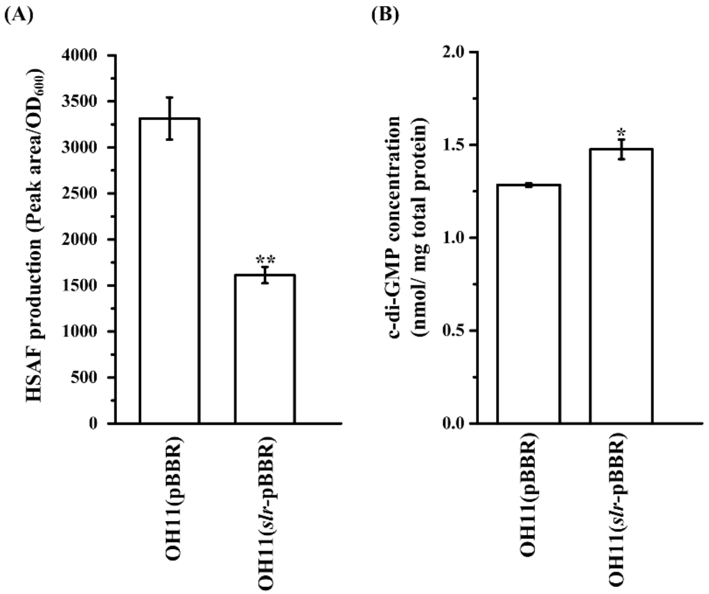


**Figure S3 Increased c-di-GMP levels suppressed HSAF production at wild type of *Lysobacter enzymogenes*. (A)** The c-di-GMP diguanylate cyclase, Slr1143, significantly reduces HSAF production **(A)**, while remarkably increases c-di-GMP levels **(B)** at the wild-type OH11. OH11(pBBR) and OH11 (*slr*-pBBR) indicate the wild-type OH11 containing empty vector and the plasmid-borne *slr1143*, respectively. Three technical replicates for each treatment were used, and the biological experiment was performed three times. Vertical bars represent standard errors. *P<0.05 or **P<0.01 relative to the wild-type OH11 containing empty vector.

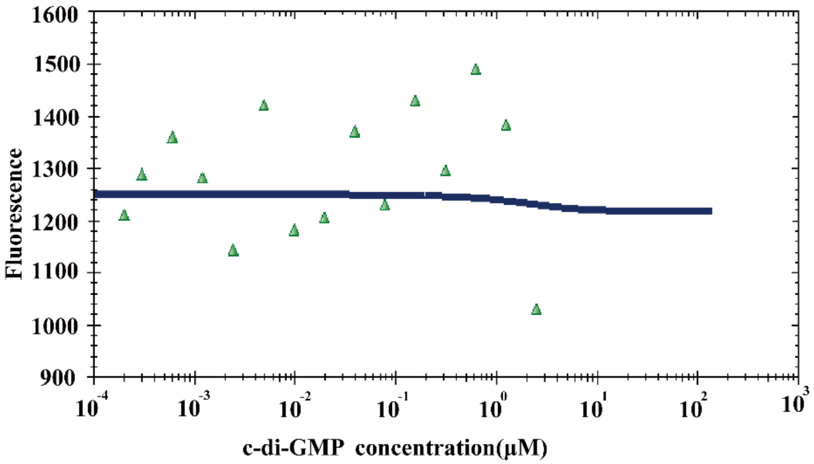


**Figure S4 The binding of c-di-GMP and PilR measured by Microscale Thermophoresis.** No binding could be observed for c-di-GMP and the His-tagged PilR.

## Reference

1. **Wang RP, Xu HY, Du LC, Chou SH, Liu HX, Liu YZ, Liu FQ and Qian GL.** 2016. A TonB-dependent receptor regulates antifungal HSAF biosynthesis in *Lysobacter*. Sci Rep **6:**26881.