Supplementary Information

Indole-induced Reversion of Intrinsic Multi-antibiotic Resistance in *Lysobacter enzymogenes*

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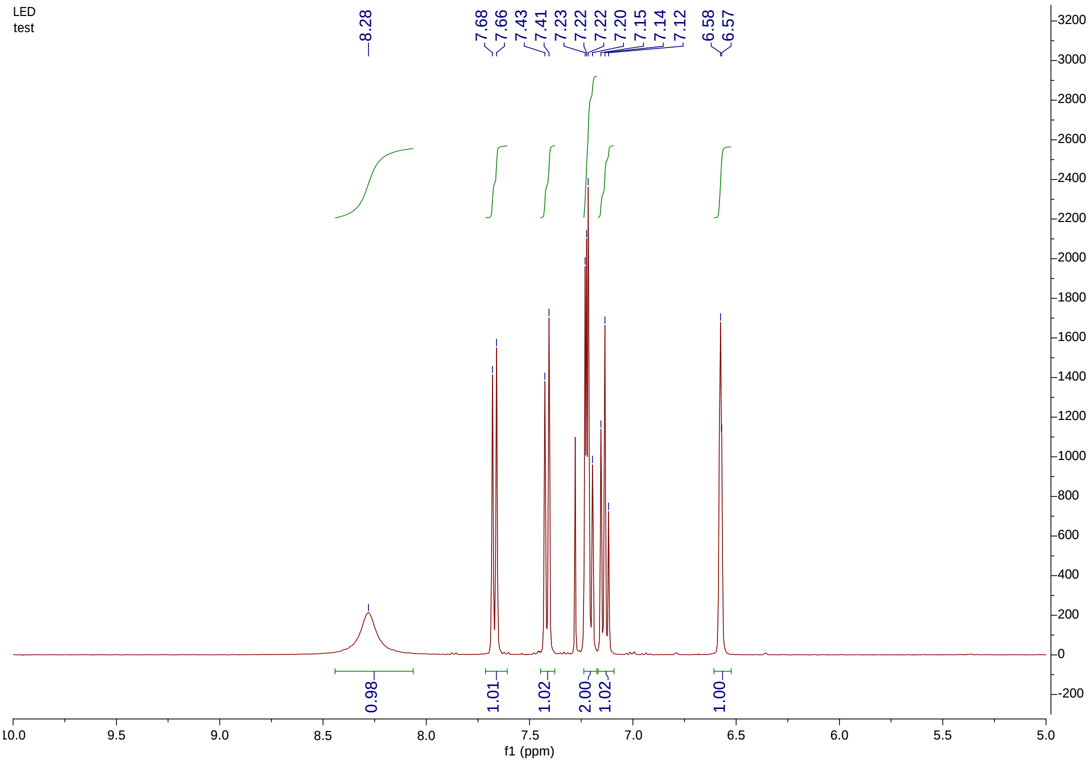
TABLE S1. Solvent system used for analytic HPLC. Solvent A, acetonitrile containing 0.1% formic acid; Solvent B, water containing 0.1% formic acid; Flow rate, 1 mL/min; Detect wavelength, 280 nm.

Time (min)	Solvent A (%)	Solvent B (%)
0	40	60
4	40	60
17	70	30
20	70	30
25	100	0
28	100	0
29	40	60

FIG S1. ^{1}H-NMR and ^{13}C-NMR of the compound isolated from the LED209 treated *Lysobacter enzymogenes* OH11. The data are consistent with that of standard indole.



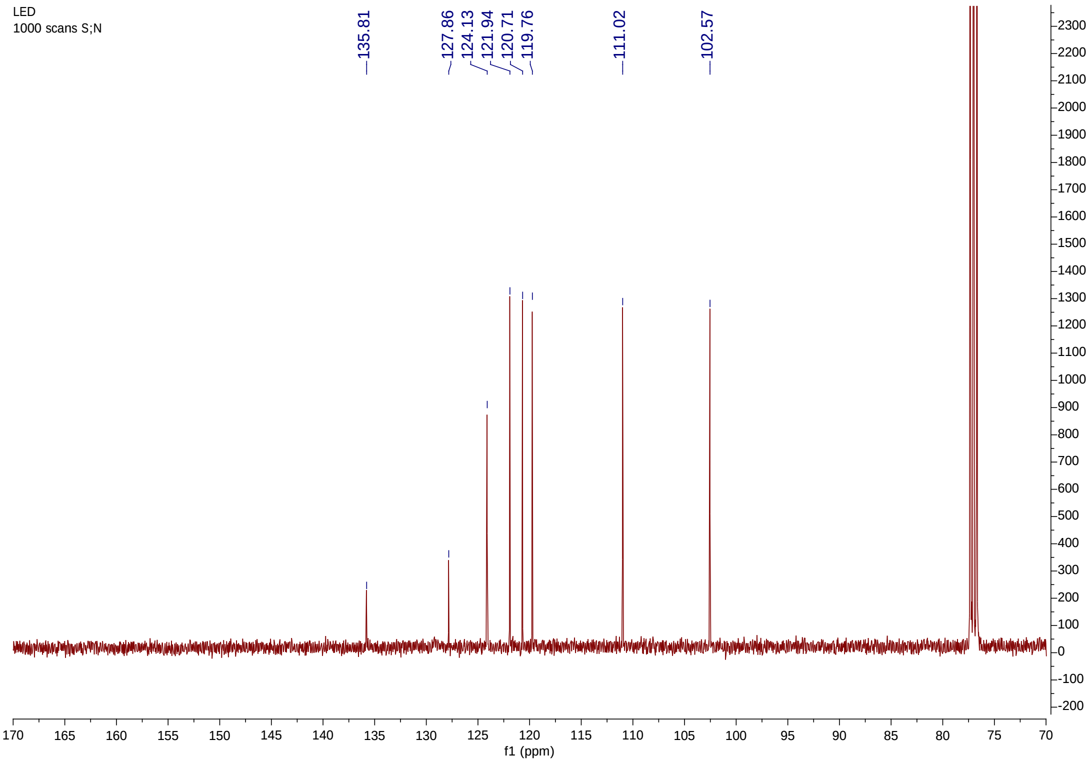

LED
1000 scans S;N
135.81
127.86
124.13
121.94
120.71
119.76
111.02
102.57
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
f1 (ppm)

FIG S2. Sequence alignment between Le0754/Le0752 in *Lysobacter enzymogenes* OH11 and the two-component regulatory system QseC/QseB from *E. coli*. The original sequences in *E. coli* K12 are QseB (or YgiX, AAC76061.1) and QseC (or YgiY, AAC76062.1),[1] and the sequences in *E. coli* O157:H7 are QseB (AAG58159.1) and QseC (AAG58160.1).[2] The sequence analysis was done by using CLUSTAL O (1.2.1) multiple sequence alignment. Le0754 shows 31.1/43.3% identity/similarity to QseC through the whole sequence, and its histidine phosphorylation site is underlined and in red color. Le0752 has 47.5/62.0% identity/similarity to QseB through the whole sequence, and its aspartate phosphorylation site is underlined and red color. Also red-color highlighted are four conserved QseC residues for generating four single point-mutants, D85V, F126A, F154A, W164E, at the N-terminal ligand-binding domain, but not the C-terminal kinase domain.

QseC alignment

```
Le0754          MSAVDGRDAAAPGEREARWRDRDRERESQRSGWRERCRQSREFPAGRSLRWKLTWVLFKA    60
K12             ----------------------------------------MKFTQRLSLRVRLTLIFLIL    20
O157            ----------------------------------------MKFTQRLSLRVRLTLIFLIL    20
                                                         :*    *** :** :::

Le0754          VLLAWFVWLSCQIWQLGRERT-GMLDHSLREIAEQVLGSMP----EGLERLPSRDPRRAT    115
K12             ASVTWLLS-SFVAWKQTTDNVDELFDTQLMLFAKRL-STLDLNEINAADR-MAQTPNRLK    77
O157            ASVTWLLS-SFVAWKQTTDNVDELFDTQLMLFAKRL-STLDLNEINAADR-MAQTPNKLK    77
                . ::*::  *   *:   :..  ::* .*  :*::: .::     :. :*  :: *.: .

Le0754          VPVHADQKMSFQVWAHGRNVVYSAAAPLQPLN-PEFKDGFARRVI--DGERWQVYTLTDK    172
K12             HGHVDDDALTFAIFTHDGRMVLNDGDNGEDIPYSYQREGFADGQLVGEDDPWRFVWMTSP    137
O157            HGHVDDDALTFAIFTHDGRMVLNDGDNGEDIPYSYQREGFADGQLVGDKDQWRFVWMTSP    137
                     *: ::* :::*  .:* . .   : :     ::***   :  : : *:.  :*.

Le0754          ARGLVVQVGRTKRMAIEELQGWIVGSLLAAALILVVFALATWLVIGRSLRPITALRRTLQ    232
K12             DGKYRIVVGQEWEYREDMALAIVAGQLIPWLVALPIMLIIMMVLLGRELAPLNKLALALR    197
O157            DGKYRIVVGQEWEYREDMALAIVAGQLIPWLVALPVMLIIMMVLLGRELAPLNKLALALR    197
                     : **:  .   :   . :.*.*:   : * :: :   :::**.* *:. *  :*:
```

```
Le0754      QRQPLDLTPLPTHPLPSEFHPLVEAFNGQLERVDAAVQHERRFISDAAHELRTPLAVLST   292
K12         MRDPDSEKPLNATGVPSEVRPLVESLNQLFARTHAMMVRERRFTSDAAHELRSPLTALKV   257
O157        MRDPDSEKPLNATGVPSEVRPLVESLNQLFARTHAMMVRERRFTSDAAHELRSPLTALKV   257
             *:* . .** :  :***.:****::*  : *..* : :**** ********:**:.*..

Le0754      HAELAL-RATTLEAKNAALQKLNAGVQRSARLSEQLLDLARLDAGEESVRLAPLDLSDLI   351
K12         QTEVAQLSDDDPQARKKALLQLHSGIDRATRLVDQLLTLSRLDSLDNLQDVAEIPLEDLL   317
O157        QTEVAQLSDDDPQARKKALLQLHSGIDRATRLVDQLLTLSRLDSLDNLQDVAEIPLEDLL   317
            ::*:*       :*:: ** :*.:*::*::** :*** *:***: ::   :* : *.**:

Le0754      VLVIRDFETLARER--RQRISLRAEPTRLLGDVDQLGILLRNLIDNAVRHAGADGQVAVS   409
K12         QSSVMDIYHTAQQAKIDVRLTLNAHSIKRTGQPLLLSLLVRNLLDNAVRYSPQGSVVDVT   377
O157        QSSVMDIYHTAQQAKIDVRLTLNVQGIKRTGQPLLLSLLVRNLLDNAVRYSPQGSVVDVT   377
               : *:   *::     *::*...  :  *:   *.:*:***:*****::   . * *:

Le0754      CSAEAGGAVLRVADNGPGVATDDCERIFDRFYRAPGSPDGGSGIGLSLVARIAQTHGARI   469
K12         L----NADNFIVRDNGPGVTPEALARIGERFYRPPGQTATGSGLGLSIVQRIAKLHGMNV   433
O157        L----NADNFIVRDNGPGVTPEALARIGERFYRPPGQTATGSGLGLSIVQRIAKLHGMNV   433
                  .  : * ******: :   ** :**** **.   ***:***:* ***: ** .:

Le0754      ECGQGLERAGDDPRGPGRGFEVCVRFPPVSP      500
K12         EFGNAEQ----------GGFEAKVSW-----      449
O157        EFGNAEQ----------GGFEAKVSW-----      449
            * *:. :           ***. * :
```

QseB alignment

```
K12         MRILLIEDDMLIGDGIKTGLSKMGFSVDWFTQGRQGKEALYSAPYDAVILDLTLPGMDGR   60
O157        MRILLIEDDMLIGDGIKTGLSKMGFSVDWFTQGRQGKEALYSAPYDAVILDLTLPGMDGR   60
Le0752      MNILLVEDDAMLAEAVRTGLGHDGWRVDWVADAPLAKTALVDHDFDAVVLDLGLPGGSGL   60
            *.***:*** ::.:.::***.: *: ***.::.  .* ** .  :***:*** *** .*

K12         DILREWREKGQREPVLILTARDALAERVEGLRLGADDYLCKPFALIEVAARLEALMRRTN   120
O157        DILREWREKGQREPVLILTARDALEERVXGLRLGADDYLCKPFALIEVAARLEALMRRTN   120
Le0752      GVLGALRNRYDATPVLIVTARDKLSERIAGLDAGADDYIVKPFQLDELCARLRAVMRRSQ   120
             :*   *:: :  ****:**** * **: **  *****: *** * *:.***.*:***::

K12         GQASNELRHGNVMLDPGKRIATLAGEPLTLKPKEFALLELLMRNAGRVLSRKLIEEKLYT   180
O157        GQASNELRHGNVMLDPGKRIATLAGEPLTLKPKEFALLELLMRNAGRVLPRKLIEEKLYT   180
Le0752      GRVSPVLSCGAVVLDPARRLVTRDGEPVALSGHEFRTLTLLLERQGRVVTREQLEEAVYG   180
            *:.*  *  * *:***.:*:.*  ***::*. :**  * **:.. ***: *: :** :*
```

```
K12         WDEEVTSNAVEVHVHHLRRKLGSDFIRTVHGIGYTLGEK--     219
O157        WDEEVTSNAVEVHVHHLRRKLGSDFIRTVHGIGYTLGEK--     219
Le0752      SSGTIESNTIAVYVHQLRRKLGEQLIVTVHGYGYRVGGGPN     221
             .  : **:: *:**:******.::* **** ** :*
```

FIG S3. Quantitative PCR (qPCR) analysis of the expression of *Le-qseC* and *Le-qseB* in the wild type *L. enzymogenes* OH11. Black bars are results for *Le-qseC* expression; grey bars are for *Le-qseB* expression. A and D. wild type untreated; B and E. wild type treated with 200 μM indole; C and F. wild type treated with 10 pM LED209.

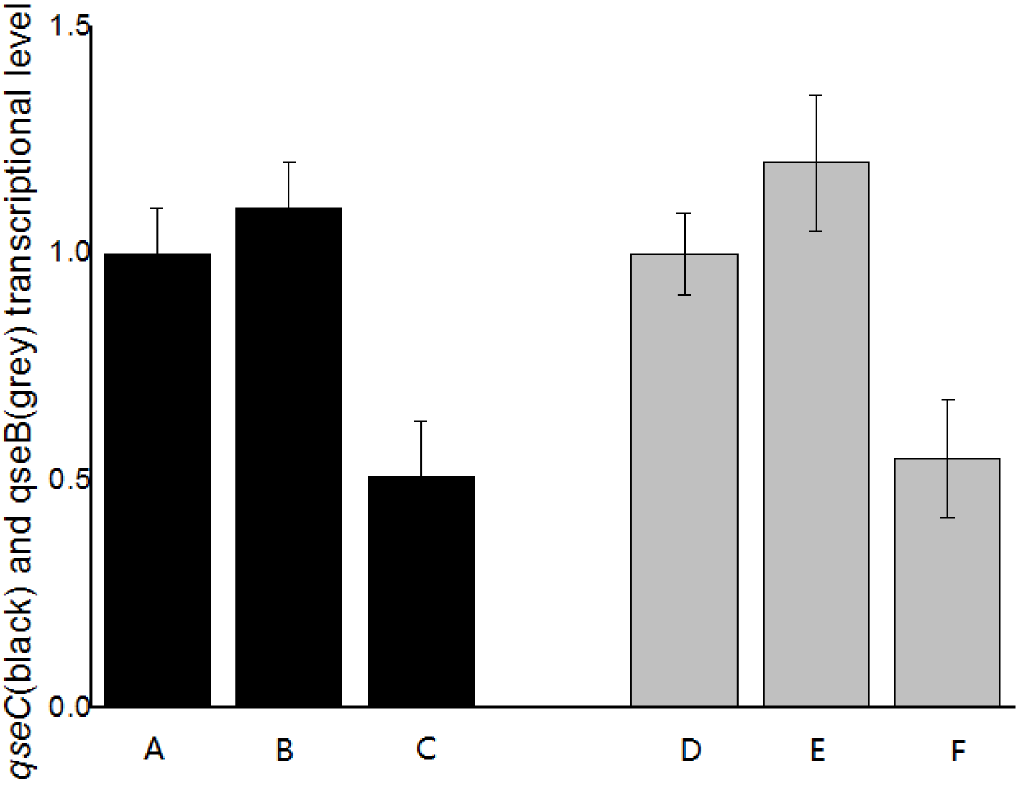


FIG S4. ^{1}H-NMR of compound **3** in the synthesis of fluorescent indole probe.

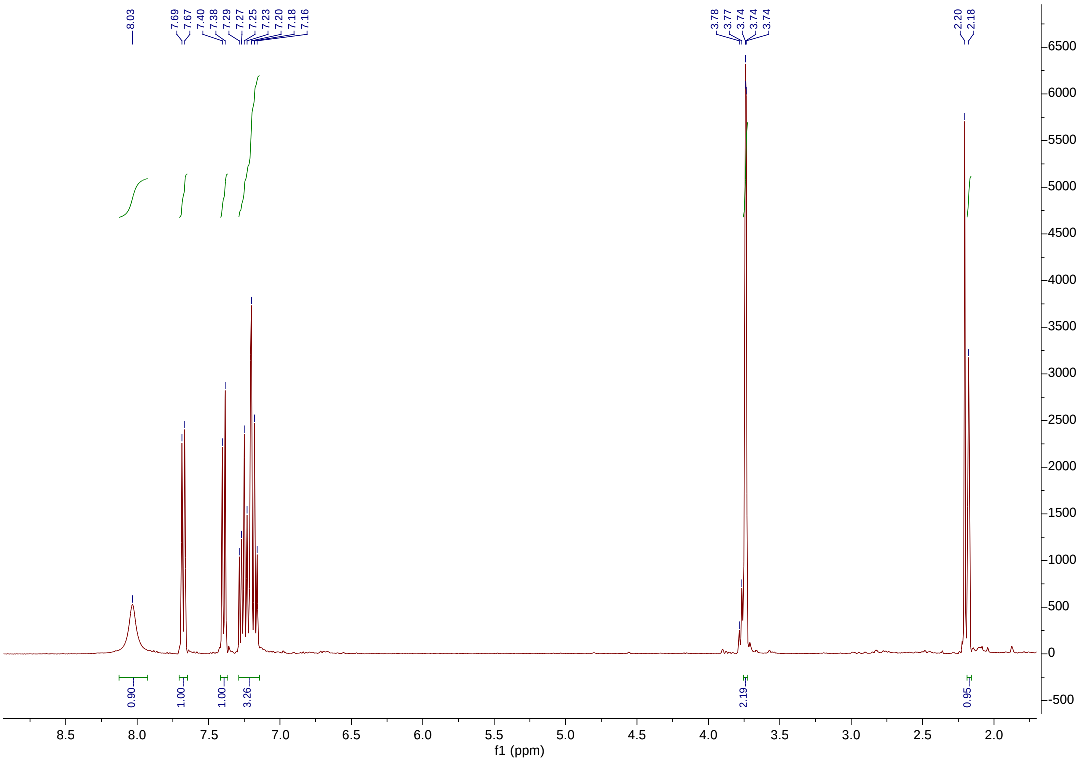
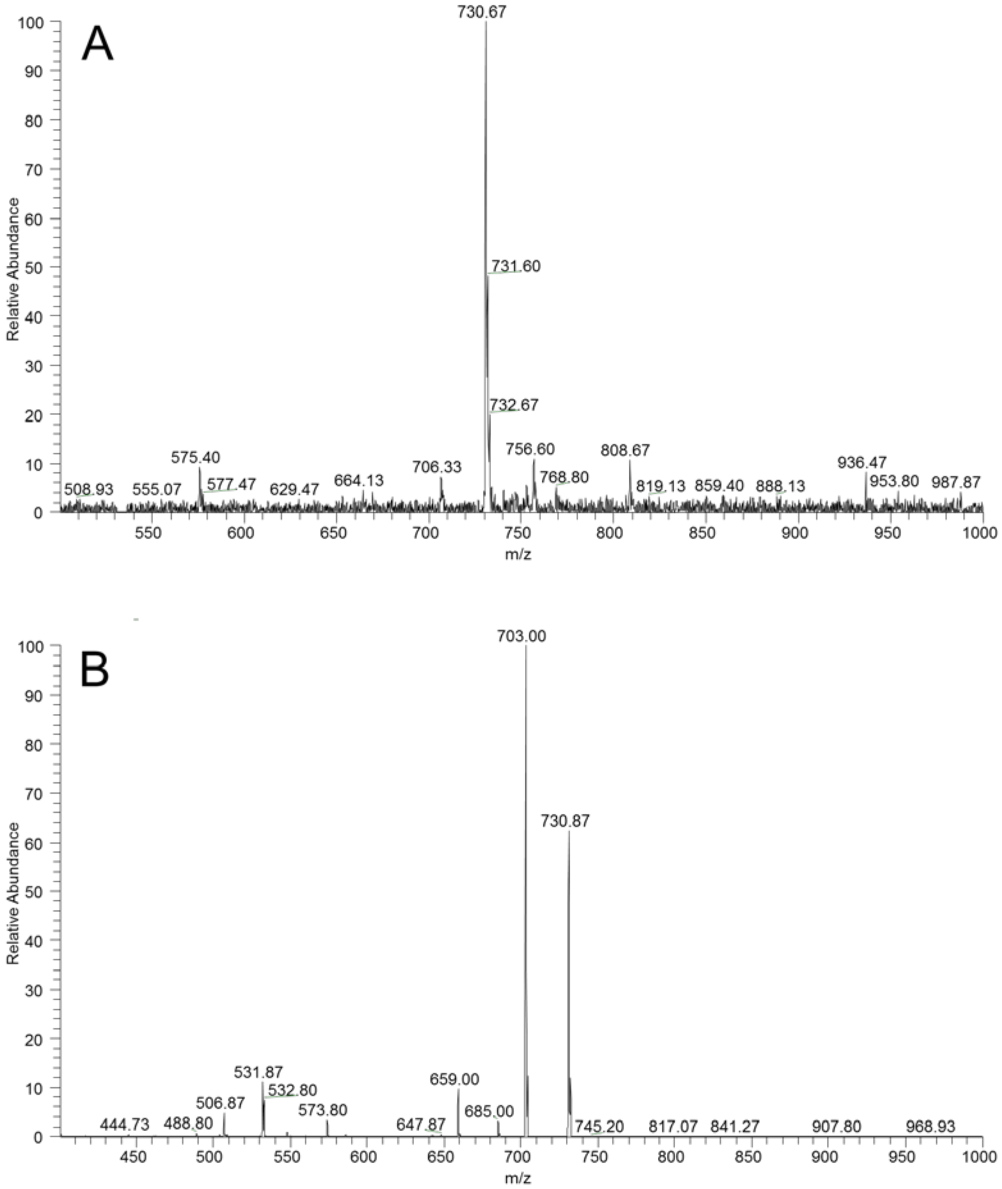
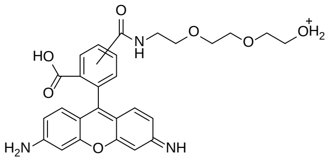


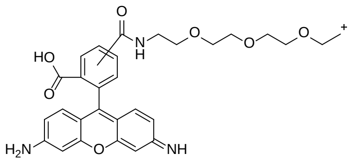
FIG S5. MS of compound **1**, the fluorescent indole probe. A. Full Scan; B. MS-MS data; C. possible structure of the MS-MS fragments.



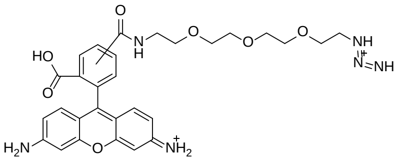
C



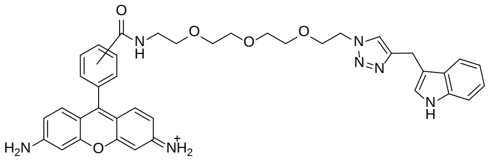
m/z = 506



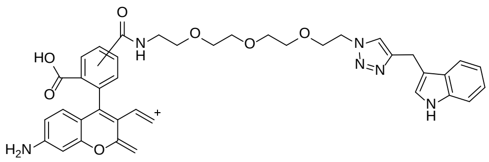
m/z = 531



m/z = 573



m/z = 685



m/z = 703

FIG S6A. Effect of indole and LED209 on the production of the antifungal HSAF in *L. enzymogenes* OH11. A. treated with 200 μM indole; B. treated with 100 μM indole; C. treated with 50 μM indole; D. treated with 5 pM LED209; E. untreated control. The identity of the peaks: a) HSAF, b) Alteramide A, c) 3-deOH-HSAF, d) 3-deOH-Altermide A (see Figure S6B for structures).

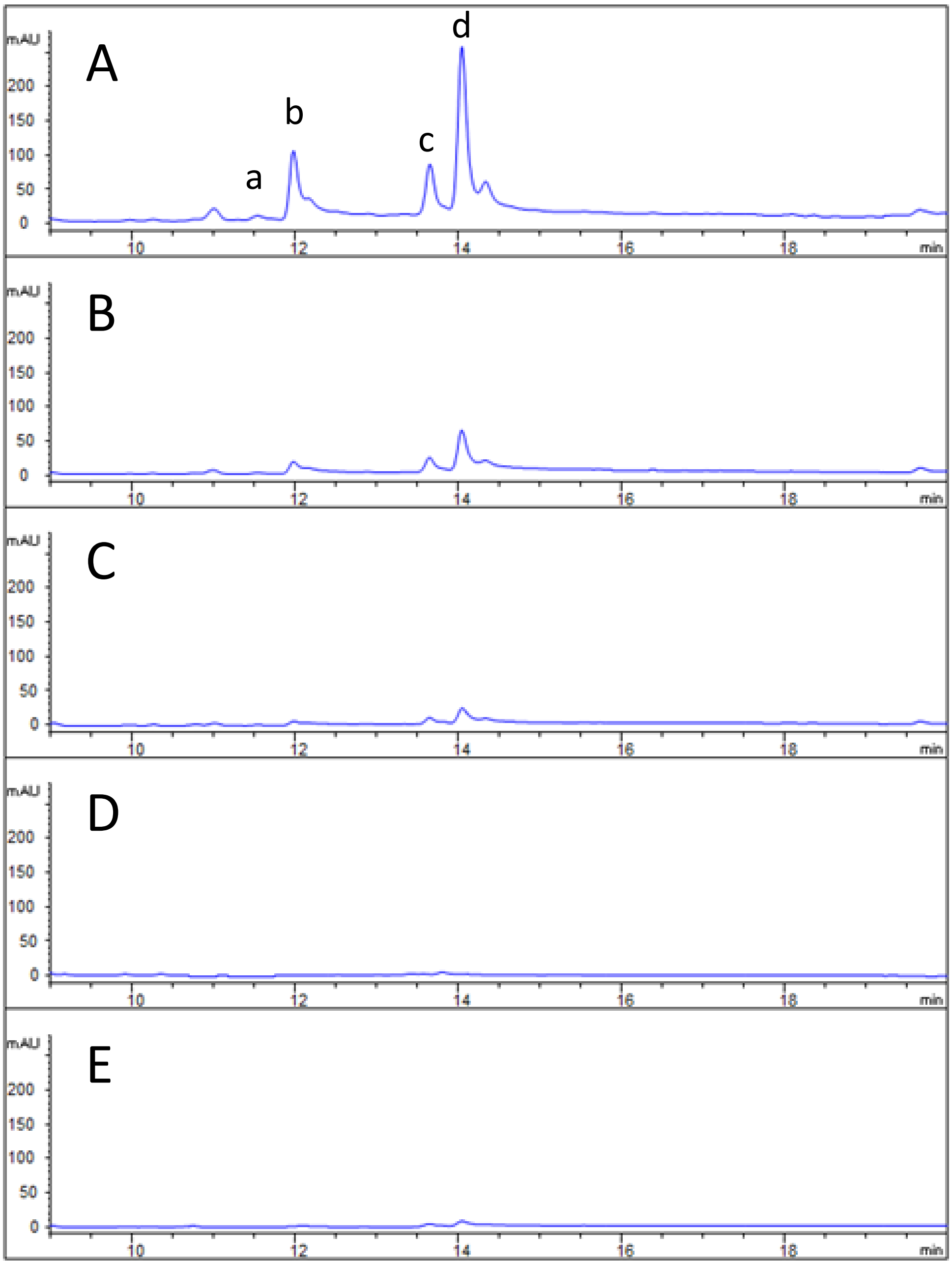
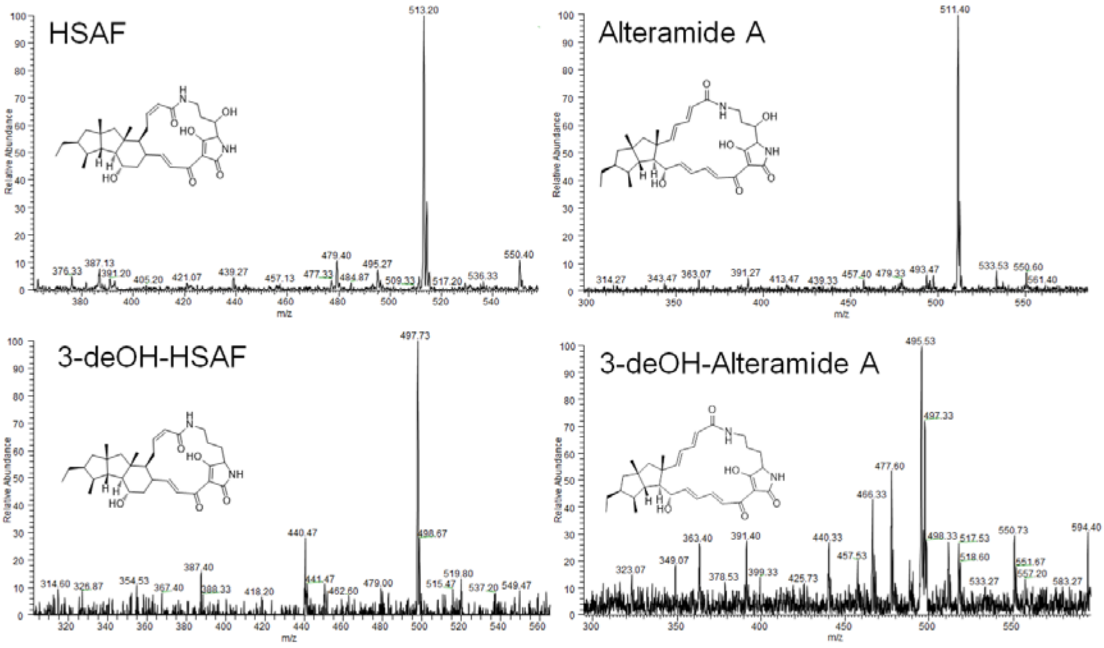


FIG S6B. Mass spectra and chemical structure of HSAF, Alteramide A, 3-deOH-HSAF and 3-deOH-Alteramide A.



REFERENCES

1. **Blattner, F.R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., and Shao, Y.** (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.
2. **Perna, N.T., Plunkett, G., Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F., Evans, P.S., Gregor, J., Kirkpatrick, H.A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamousis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J., Yen, G., Schwartz, D.C., Welch, R.A., and Blattner, F.R.** (2001) Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* **409**: 529-533.