

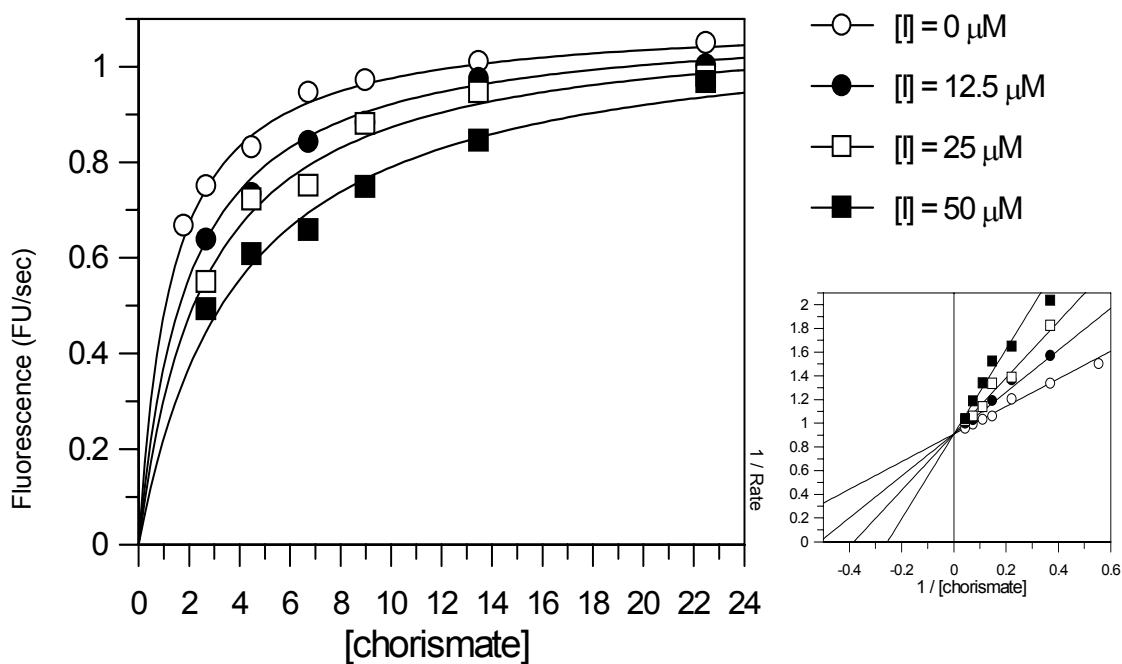
Supplementary data

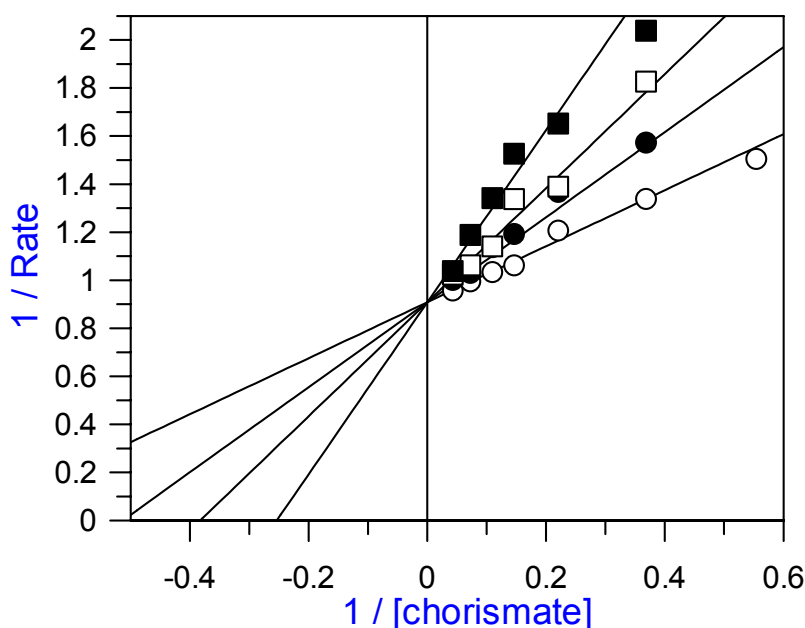
Irp 9 Enzyme assays.

Steady-state kinetic experiments were performed spectrophotometrically on a microplate reader by monitoring the formation of the product salicylate fluorimetrically (excitation 305 nm, emission 440 nm)^{5,6}. The assays contained 1.7 μM enzyme and were performed in duplicate at 25°C in 100 mM potassium phosphate, 10 mM MgCl_2 , pH 7.0. Chorismate concentrations were varied from 2.7 – 22.5 μM and inhibitor concentrations were varied depending on the potency of the compound. The amount of salicylate formed was determined from a standard curve obtained using 0-25 μM salicylate. Data were fitted using GraFit (version 5.0.10; Erithacus software).

7. **Gaille, C., C. Reimmann, and D. Haas.** 2003. Isochorismate synthase (PchA), the first and rate-limiting enzyme in salicylate biosynthesis of *Pseudomonas aeruginosa*. *J. Biol. Chem.* **278**:16893-16898. **Gaille, C.,**
8. **P. Kast, and D. Haas.** 2002. Salicylate biosynthesis in *Pseudomonas aeruginosa* - Purification and characterization of PchB, a novel bifunctional enzyme displaying isochorismate pyruvate-lyase and chorismate mutase activities. *J. Biol. Chem.* **277**:21768-21775.

Least squares fitting and Lineweaver-Burk plot for the reversible competitive inhibition of *Y. enterocolitica* Irp9 by 19





Analytical data

5: ν_{\max} (ATR): 2989, 2532 br. (O-H acid str.), 1682, 1610 (C=O, str), 1583, 1492 (C=C, ar) cm^{-1} ; ^1H NMR (CD_3OD) δ 1.58 (3H, d, J 6.8 Hz, CH_3), 4.82 (1H, q, J 6.8 Hz, CH), 7.08 (1H, ddd, J 0.8, 2.5, 8.0 Hz, ArH), 7.31 (1H, t, J 8.0 Hz, ArH), 7.51 (1H, dd, J 1.3, 2.5 Hz, ArH), 7.61 (1H, dt, J 1.3, 8.0 Hz, ArH); ^{13}C NMR (CD_3OD) δ 18.7, 73.4, 116.6, 121.1, 123.7, 130.5, 133.0, 158.9, 169.5, 175.5; HRMS calcd for $\text{C}_{10}\text{H}_{14}\text{O}_5\text{N}$: MNH_4^+ , 228.0866. Found: MNH_4^+ , 228.0869.

6: ν_{\max} (ATR): 2845, 2631 (br, acid OH stretch), 1726, 1713, 1678 (C=O, str), 1583, 1508 (C=C, ar); ^1H NMR (CD_3OD) δ 1.62 (3H, d, J 6.0 Hz, CH_3), 2.30 (3H, s, CH_3), 4.83 (1H, q, J 6.0 Hz, CH), 7.21 (1H, d, J 7.8 Hz, H-5), 7.39 (1H, d, J 1.5 Hz, H-2), 7.62 (1H, dd, J 7.8, 1.5 Hz, H-6); ^{13}C NMR (CD_3OD) δ 17.0, 19.2, 73.8, 113.6, 124.1, 130.8, 132.0, 134.5, 157.4, 170.0, 175.9; HRMS calcd for $\text{C}_{11}\text{H}_{12}\text{O}_5\text{Na}$: MNa^+ , 247.0582. Found: MNa^+ , 247.0578.

7: ν_{\max} (ATR): 2989 (br, acid OH stretch) 2596 (Ar C-H stretch), 1718, 1676 (C=O), 1603, 1583 (C=C, ar) cm^{-1} ; ^1H NMR (d^6 -acetone) δ 1.60 (3H, d, J 6.8 Hz, CH_3), 3.91 (3H, s, OCH_3), 4.85 (1H, q, J 6.8 Hz, CH), 7.07 (1H, d, J 8.4 Hz, ArH, H-5), 7.55 (1H, d, J 2.0 Hz, ArH, H-2), 7.69 (1H, dd, J 8.4, 2.0 Hz, ArH, H-6); ^{13}C NMR (d^6 -acetone) δ 19.2, 56.7, 74.6, 112.8, 117.8, 124.0, 126.1, 148.2, 155.5, 167.7, 173.5; HRMS calcd for $\text{C}_{11}\text{H}_{12}\text{O}_6\text{Na}$: MNa^+ , 263.0532. Found: MNa^+ , 263.0529.

8: ^1H NMR (d^6 -acetone) δ 1.64 (3H, d, J 6.9 Hz, CH_3), 4.90 (1H, q, J 6.9 Hz, CH), 6.92 (1H, d, J 8.3 Hz, ArH, H-5), 7.60 (1H, d, J 1.9 Hz, ArH, H-2), 7.62 (1H, dd, J 8.3, 1.9 Hz, ArH, H-6); ^{13}C NMR (d^6 -acetone) δ 17.0, 73.7, 114.4, 116.2, 120.9, 124.4, 144.5, 151.3, 165.2, 172.2; HRMS calcd for $\text{C}_{10}\text{H}_{10}\text{O}_6$: M^+ , 226.0477. Found: M^+ , 226.0484.

9: ν_{\max} (ATR) 3340 (NH), 1668 (C=O), 1610 (C=C, ar) cm^{-1} ; ^1H NMR (D_2O) δ 1.16 (3 H, d, J 6.8 Hz, CH_3), 3.74 (1 H, q, J 6.8 Hz, CH), 6.75 (1 H, d, J 8.1 Hz, H-5), 7.19 (1 H, d, J 1.8 Hz, H-2), 7.25 (1 H, dd, J 8.1, 1.8 Hz, H-6); ^{13}C NMR (DEPT , D_2O) δ 17.1 (CH_3), 51.4 (CH), 116.0 (CH), 118.1 (CH), 122.1 (CH), 131.2 (C), 133.7 (C), 134.4 (C), 175.0 (C), 175.7 (C).

10: ν_{\max} (ATR): 2927, 2542 (br, acid OH stretch), 1686 (C=O, str), 1581, 1506 (C=C, ar); ^1H NMR (CD_3OD) δ 1.62 (3H, d, J 6.8 Hz, CH_3), 4.68 (1H, d, J 14.6 Hz, CHH), 4.79 (1H, d, J 14.6 Hz, CHH), 4.90 (1H, q, J 6.8 Hz, CH), 7.43 (1H, d, J 1.3 Hz, H-2), 7.50 (1H, d, J 7.8 Hz, H-5), 7.65 (1H, dd, J 7.8, 1.3 Hz, H-6); ^{13}C NMR (CD_3OD) δ 21.5, 62.8, 76.4, 116.1, 126.6, 131.2, 134.3, 139.6, 158.6, 172.1, 178.2; HRMS calcd for $\text{C}_{11}\text{H}_{11}\text{O}_6\text{Na}$: MNa^+ , 263.0532. Found: MNa^+ , 263.0537.

11: ν_{\max} . (ATR): 2949, 2570 (br OH acid), 2093, 2068 (N₃ stretch), 1703, 1692 (C=O, str), 1584 (C=C, ar) cm^{-1} ; ¹H NMR (CD₃OD) δ 1.64 (3H, d, *J* 6.8 Hz, CH₃), 4.39 (1H, d, *J* 14.0 Hz, *CHH*), 4.55 (1H, d, *J* 14.0 Hz, *CHH*), 4.92 (1H, q, *J* 6.8 Hz, CH), 7.37 (1H, d, *J* 7.8 Hz, H-5), 7.49 (1H, d, *J* 1.3 Hz, H-2), 7.62 (1H, dd, *J* 7.8, 1.3 Hz, H-6); ¹³C NMR (CD₃OD) δ 17.2, 49.2, 72.3, 112.3, 122.2, 129.2, 129.7, 131.5, 155.4, 167.5, 173.6; HRMS calcd for C₁₁H₁₁N₃O₅Na: MNa⁺, 288.0596. Found: MNa⁺, 288.0603.

12: ν_{\max} . (ATR): 3044, 2297, 1555, 1405 cm^{-1} ; ¹H NMR (D₂O) δ 1.64 (3H, d, *J* 6.8 Hz, CH₃), 4.07 (1H, d, *J* 13.3 Hz, *CHH*), 4.32 (1H, d, *J* 13.3 Hz, *CHH*), 4.83 (1H, q, *J* 6.8 Hz, CH), 7.40 (1H, d, *J* 7.8 Hz, H-5), 7.49 (1H, d, *J* 1.4 Hz, H-2), 7.62 (1H, dd, *J* 7.8, 1.4 Hz, H-6); ¹³C NMR (D₂O) δ 18.8, 39.6, 75.8, 113.3, 122.4, 124.9, 131.4, 138.4, 156.4, 173.9, 180.5; HRMS calcd for C₁₁H₁₂N₃O₅Na₂: MNa₂²⁺, 284.0511. Found: MNa₂²⁺, 284.0494.

13: ν_{\max} . (ATR): 2987, 2901 br. (O-H acid str.), 1727, 1693 (C=O, str), 1583, 1507 (C=C, ar) cm^{-1} ; ¹H NMR (CD₃OD) δ 1.65 (3H, d, *J* 6.8 Hz, CH₃), 3.72 (1H, d, *J* 13.8 Hz, *CHH*), 3.78 (1H, d, *J* 13.8 Hz, *CHH*), 4.92 (1H, q, *J* 6.8 Hz, CH), 5.02 (1H, s, SH), 7.36 (1H, d, *J* 7.8 Hz, H-5), 7.42 (1H, d, *J* 1.4 Hz, H-2), 7.58 (1H, dd, *J* 7.8, 1.4 Hz, H-6); ¹³C NMR (CD₃OD) δ 18.9, 24.0, 73.6, 113.8, 124.0, 130.6, 131.7, 137.4, 156.2, 169.3, 175.2; HRMS calcd for C₁₁H₁₆O₅NS: MNH₄⁺, 274.07443. Found: MNH₄⁺, 274.0749.

14: ¹H NMR (CDCl₃) δ 1.57 (3H, d, *J* 6.8 Hz, CH₃), 4.81 (1H, q, *J* 6.8 Hz, CH), 6.77 (2H, t, *J* 8.0 Hz, ArH), 7.11 (1H, d, *J* 8.0 Hz, ArH), 7.51 (1H, d, *J* 8.0 Hz, ArH); ¹³C NMR (CDCl₃) 18.8, 75.4, 115.1, 119.3, 123.3, 124.8, 147.5, 154.4, 173.7, 175.7.

15: ν_{\max} . (ATR): 1727, 1689 (C=O, str), 1609, 1582, 1546 (C=C, ar) cm^{-1} ; ¹H NMR (CDCl₃) δ 1.55 (3H, d, *J* 6.5 Hz, CH₃), 4.94 (1H, q, *J* 6.5 Hz, CH) 7.29 (1H, d, *J* 8.2 Hz, ArH), 7.47 (1H, t, *J* 8.2 Hz, ArH, H-5), 7.58 (1H, d, *J* 8.2 Hz, ArH); ¹³C NMR (CDCl₃) δ 17.8, 74.5, 119.4, 123.4, 125.1, 131.2, 142.1, 149.9, 165.2, 173.3; LCMS: ret. time = 3.34 min, M-H = 254.0; HRMS calcd for C₁₀H₁₃N₂O₇: MNH₄⁺, 273.0717. Found: MNH₄⁺, 273.0715.

16: ν_{\max} . (ATR): 3322 (br. NH str.), 1583, 1522 (C=C, ar) cm^{-1} ; ¹H NMR (D₂O) δ 1.48 (3H, d, *J* 6.8 Hz, CH₃), 4.47 (1H, q, *J* 6.8 Hz, CH) 6.63 (1H, t, *J* 7.9 Hz, ArH, H-5), 6.72 (1H, dd, *J* 7.9, 1.3 Hz, ArH), 7.25 (1H, dd, *J* 7.9, 1.3 Hz, ArH); ¹³C NMR (D₂O) δ 18.8, 76.2, 114.6, 117.2, 121.6, 123.4, 138.3, 146.9, 161.0, 176.3, 181.1; LCMS: M+H = 226.1, M-H = 224.0, ret. time = 2.41 min; HRMS calcd for C₁₀H₁₂NO₅: MH⁺, 226.0710. Found: MH⁺, 226.0705.

17: ν_{\max} . (ATR): 3270 br. (acid O-H str.), 1659, 1558 (C=O, str) cm^{-1} ; ¹H NMR (D₂O) δ 2.12-2.80 (4H, m, H-3 + H-6), 3.54 (1H, m, H-4), 3.87 (1H, m, H-5), 3.96 (1H, d, ²*J* = 15.8 Hz, *CHH*), 4.12 (1H, d, ²*J* = 15.8 Hz, *CHH*), 6.41 (1H, m, H-2); ¹³C NMR (D₂O) δ 29.8, 31.9, 68.4, 68.5, 79.7, 131.3, 132.3, 175.8, 178.5.

18: ν_{\max} . (ATR): 3331 br. (acid O-H str.), 1579 (C=O, str), 1400 (C=C) cm^{-1} ; ¹H NMR (CDCl₃) δ 2.56-2.79 (4H, m, H-3 + H-6), 3.67 (1H, m, H-4), 3.92 (1H, m, H-5), 4.33 (1H, d, ²*J* = 12.7 Hz, *CHH*), 4.38 (1H, d, ²*J* = 12.7 Hz, *CHH*), 5.63 (1H, d, ²*J* = 1.1 Hz, CH), 5.91 (1H, d, ²*J* = 1.1 Hz, CH), 6.42 (1H, m, H-2); ¹³C NMR (CDCl₃) δ 29.4, 31.4, 68.2, 69.5, 78.0, 122.8, 131.3, 132.2, 142.6, 174.9, 175.8.

19: ν_{\max} . (ATR): 3304 br. (O-H str.), 1656 (C=O, str), 1554 (C=C), 1395 (C=C, ar) cm^{-1} ; ¹H NMR (D₂O) δ 2.06-2.95 (4H, m, H-6_{ax}, H-6_{eq}, H-3_{ax}, H-3_{eq}), 3.99 (1H, m, CH), 4.12 (1H, m, CH), 4.63 (1H, d, ²*J* = 2.4 Hz, C=*CHH*), 5.12 (1H, d, ²*J* = 2.4 Hz, C=*CHH*), 6.43 (1H, m, H-2); ¹³C NMR (D₂O) δ 28.5, 31.5, 67.9, 76.9, 92.4, 131.2, 132.1, 153.6, 171.0, 175.6.