Supplementary Materials for

Elucidation of new sulfamethoxazole catabolic pathways in whole-cell catalyst of bacterium *Kocuria rhizophila* SA117

Method S1. Spectrophotometric analyses of SMX

Preparation of samples for spectrometric determination was performed as follows: 1 mL samples (pellet of a culture or reaction mixture supernatant) were extracted with 1 mL of tert-butyl methyl ether under vigorous shaking (10 mL vials, vortex mixer, 20 min). Organic and water phases were separated by centrifugation (7 000×*g*, 10 min, 6 °C). The organic phase was evaporated, and sample dissolved in aqueous ethanol (1:1) for determination of residual concentration of SMX. SMX standard stock solution (1 mg/mL) was prepared by dissolving 10 mg of SMX in 10 mL of aqueous ethanol (1:1). Spectrophotometric measurements were performed using a Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation-Gruppe, Kyoto, Japan). The spectrum was measured from 200 to 400 nm and maximum of absorbance was determined at 264 nm. Working samples were prepared by appropriate dilution of 2 – 10 times to meet calibrated range for SMX. Quantities of 10-50 µg SMX corresponded to absorbance of 0.46-2.08. The analyses were performed in triplicate with the LOD and LOQ for SMX as 2.1 and 7.5 µg/mL.

Method S2. Pyrolysis-gas chromatography-mass spectrometry

Py-GC-MS was performed using a 5000 Pyroprobe (CDS Analytical, Inc., Oxford, PA, USA) fitted via a CDS 1500 valve interface. Around 0.2 mg of lyophilized bacteria were loaded into a pyrolysis tube, and samples were heated to 650 °C at a rate of 20 °C/ms and held there for 15 s in a helium flow. The pyrolysis unit was coupled to a GC-MS. The pyrolyzed compounds were then transferred to a gas chromatograph at 280 °C for further separation and identification. The volatile compounds were analyzed using a high-performance 7890 A gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a fused silica capillary column (SPB® 5 Capillary GC Column, L × I.D. 30 m × 0.25 mm × 0.25 µm, bonded; poly(5% diphenyl/95% dimethyl siloxane phase)) coupled to an Agilent 7000 MS/MS (Agilent) with EI (70 eV). The temperature program was as follows: 40 °C for 1 min, subsequently increasing at 5 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. The mass range used for the mass-selective detector was 30–200 Da. The mass spectra were examined by comparing the mass spectrum library attached to the GC-MS and the experimental mass spectra.

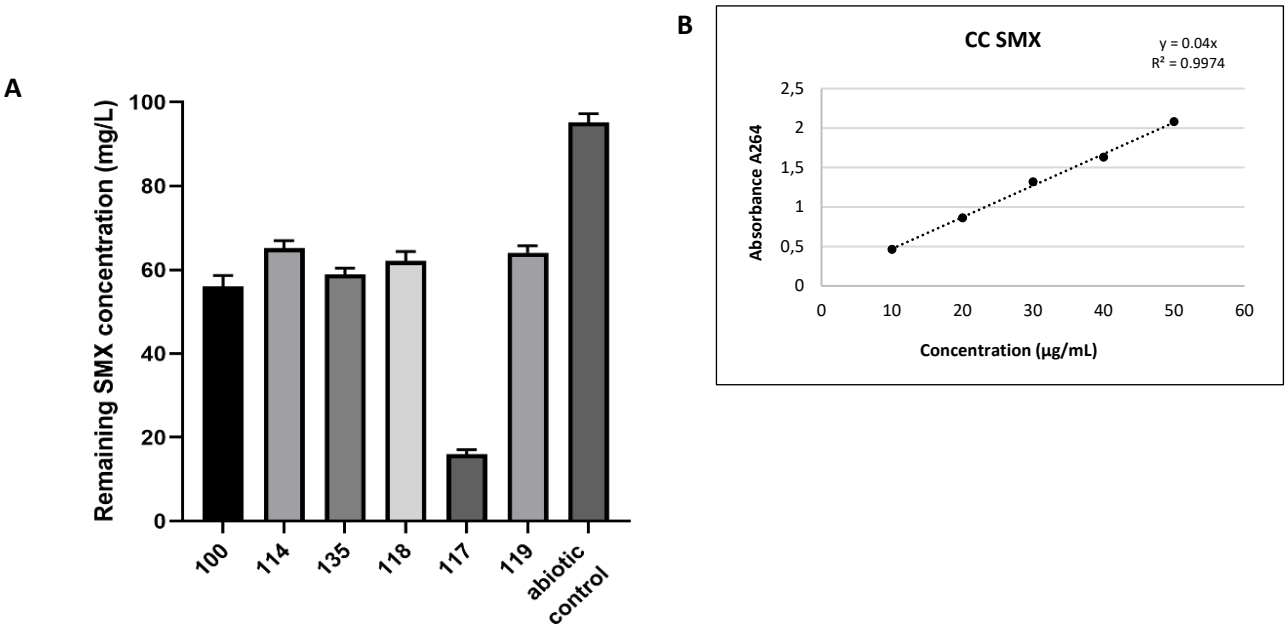


Figure S1. A. SMX-degrading activity of six tolerant isolates incubated in M9TEYE liquid medium supplemented with SMX (100 mg/L) for 72 h at 28 °C and abiotic control of SMX (100 mg/L). Values represent the average of three independent experiments ± SD. **B**. Calibration curve for SMX at 264 nm.

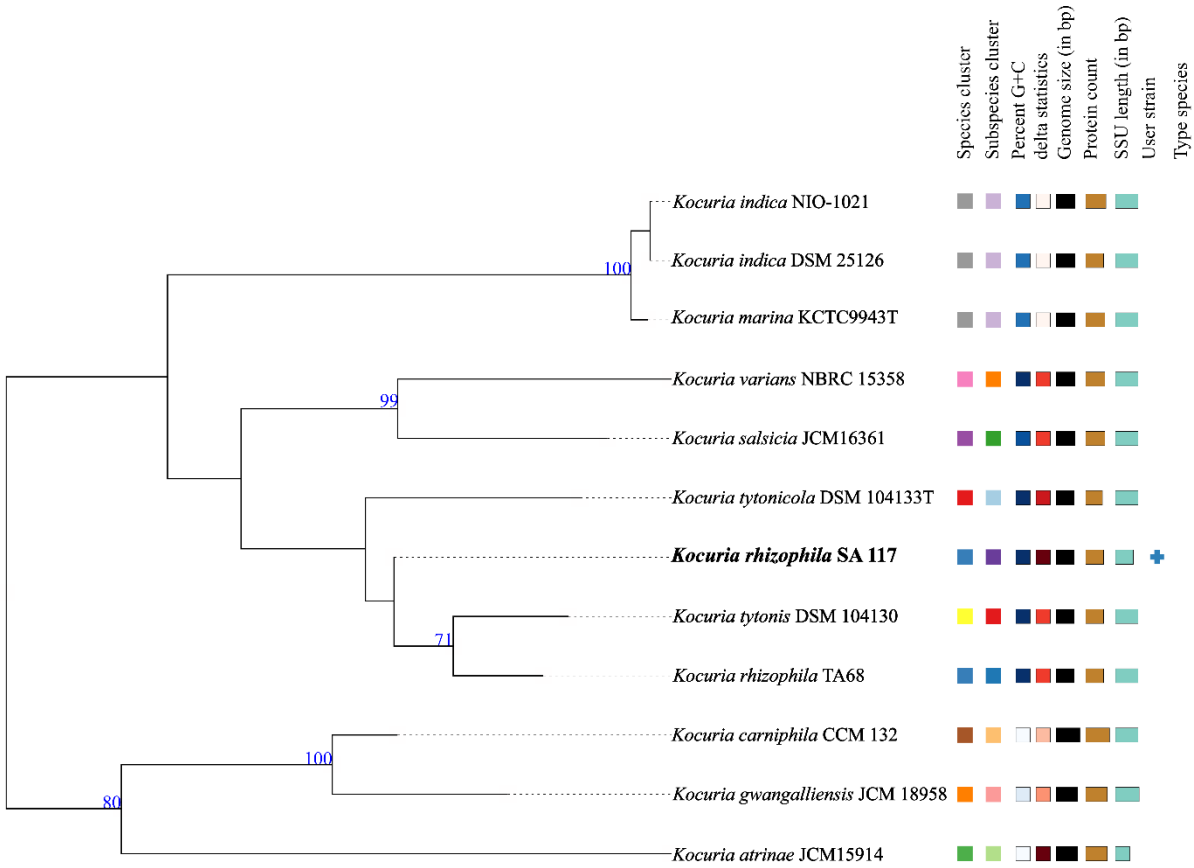


Figure S2. 16S-based phylogenetic tree. Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from 16S rRNA gene sequences. Branch lengths are scaled in terms of GBDP distance formula d_5. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 69.8%. The tree was rooted at the midpoint. *K. rhizophila* SA117 is depicted in bold. The same color indicates the same species cluster.

Table S1: ^{1}H and ^{13}C NMR data of 3,5,4′-[$^{2}H_3$]-sulfamethoxazole ($^{2}H_3$-SMX) (399.83 MHz for ^{1}H, 100.54 MHz for ^{13}C, CD_3OD, 30 °C)

Atom	δ_C	δ_H	n_H	m	HMBC	δ_C^{lit}	δ_H^{lit}
1	126.52	-	0	-	2,6 w	126.4	-
2,6	130.14	7.546	2	s	2,6	130.2	7.58
3,5	113.77-114.25	-	0	-	-	113.6-114.4	-
4	154.63	-	0	-	2,6	154.7	-
3'	159.55	-	0	-	6' w	159.6	-
4'	95.93-96.50	-	0	-	6'	95.8-96.7	-
5'	171.68	-	0	-	6'	171.7	-
6'	12.21	2.296	3	s	-	12.3	2.32

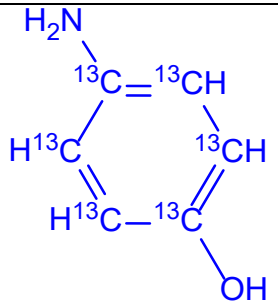
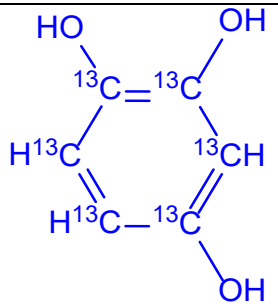
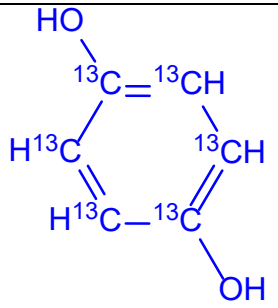
^{lit} … previously published data
^{w} … weak correlation

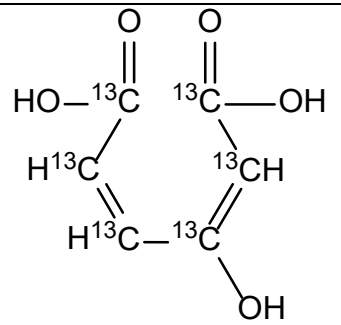
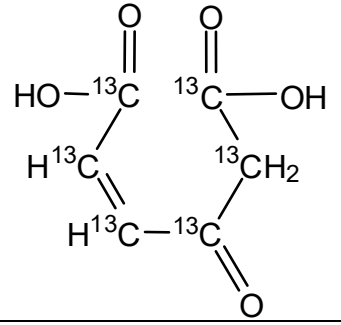
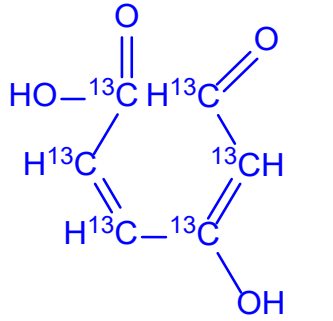
Table S2. Morphological and biochemical characteristics of the strain SA117.

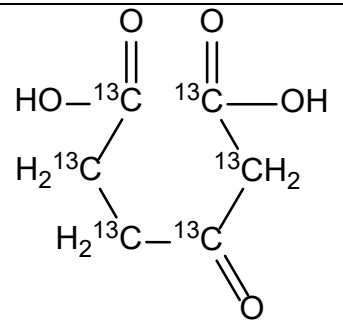
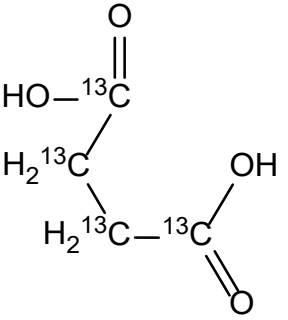
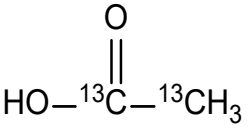
Test	Results
Colony color/shape	Yellow, circular
Surface	Smooth, glossy
Growth at 37 °C	+
Growth at 15 °C	+
Catalase	+
Urease	+
Trehalose	+
Ornithine decarboxylase	-
Raffinose	-
Sorbitol	-
Cellobiose	-
Xylose	+
Gelatinase	+
Maltose	+
Tween hydrolases	+
Lipase	+
Nitrate reduction	-
Arginin hydrolase	-
Esculin hydrolysis	-
Fructose	+

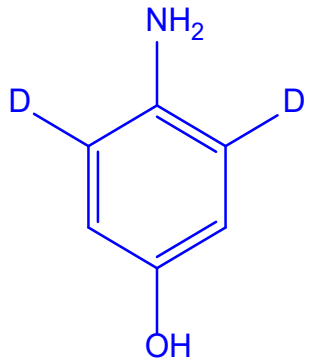
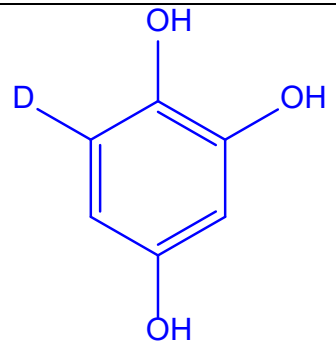
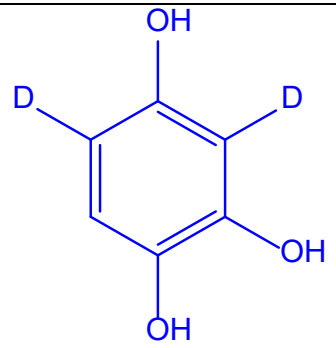
+ positive characteristic; − negative characteristic

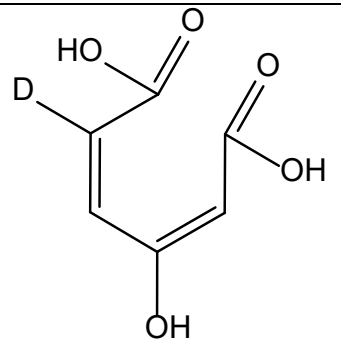
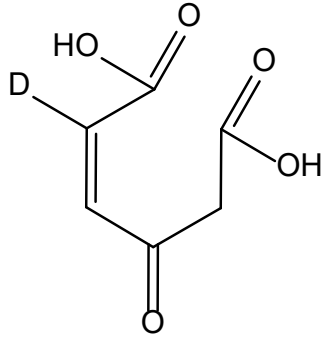
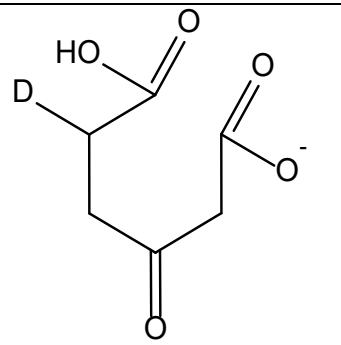
Table S3. Overview of the products found during the SMX degradation by whole-cell catalyst of *K. rhizophila* SA 117 determined by accurate mass measurements.

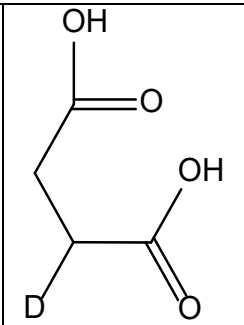
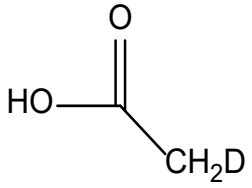
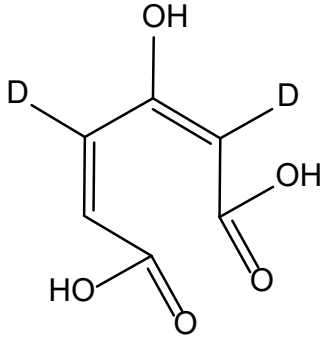
No.	Name	Measured	Mass error (ppm)	Elementary composition	Structure
M1	4-aminophenol-$^{13}C_6$	116.0807	0.5	$[^{13}C_6H_8NO]^+$	
M2	benzene-1,2,4-triol-1,2,3,4,5,6-$^{13}C_6$	131.0441	0.4	$[^{13}C_6H_5O_3]^-$	
M3	hydroquinone-$^{13}C_6$	117.0647	0.5	$[^{13}C_6H_7O_2]^+$	

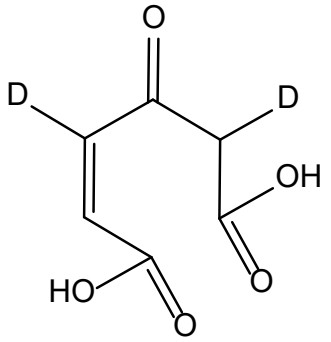
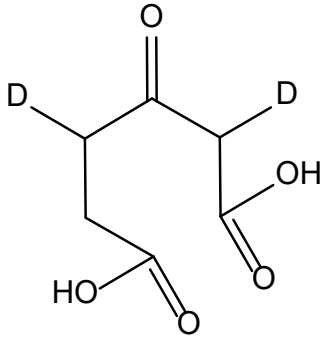
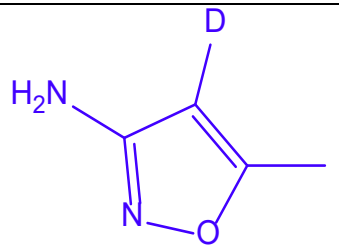
M4a	(2*E*,4*Z*)-3-hydroxyhexa-2,4-dienedioic-1,2,3,4,5,6-$^{13}C_6$ acid	163.0339	0.5	$[^{13}C_6H_5O_5]^-$	
M4b	(*Z*)-4-oxohex-2-enedioic-1,2,3,4,5,6-$^{13}C_6$ acid	tautomer of **4a**	-	-	
M5	(2*Z*,4*E*)-4-hydroxy-6-oxohexa-2,4-dienoic-1,2,3,4,5,6-$^{13}C_6$ acid	147.0395	0.5	$[^{13}C_6H_5O_4]^-$	

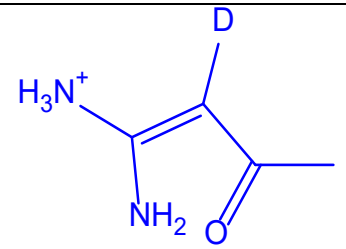
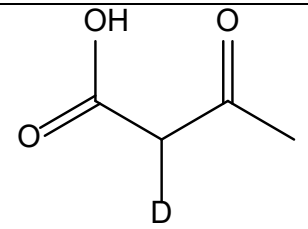
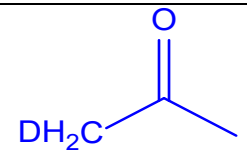
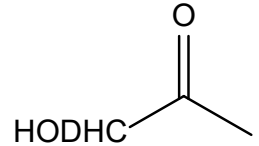
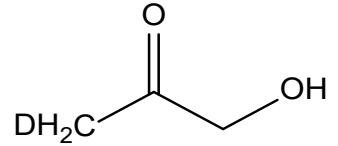
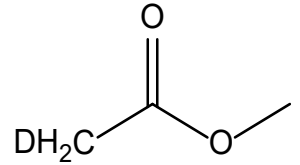
M6	3-oxohexanedioic-1,2,3,4,5,6-$^{13}C_6$ acid	165.0498	0.6	$[^{13}C_6H_7O_5]^-$	
M7	succinic-$^{13}C_4$ acid	121.0326	0.3	$[^{13}C_4H_5O_4]^-$	
M8	acetic-$^{13}C_2$ acid	61.0205	0.3	$[^{13}C_2H_4O_2]^-$	

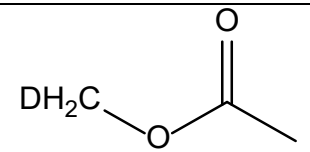
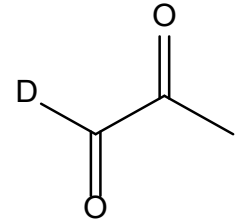
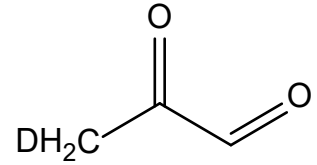
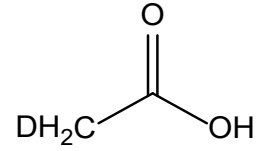
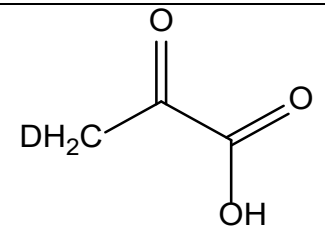
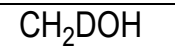
M9	4-aminophen-3,5-D_2-ol	112.0731	0.5	$[C_6H_6D_2NO]^+$	
M10	benzene-6-D-1,2,4-triol	126.0305	0.2	$[C_6H_4DO_3]^-$	
M11	benzene-3,5-D_2-1,2,4-triol	127.0367	0.3	$[C_6H_3D_2O_3]^-$	

M12a	(2*E*,4*Z*)-3-hydroxyhexa-2,4-dienedioic-5-D acid	158.0198	0.7	$[C_6H_4DO_5]^-$	
M12b	(*Z*)-4-oxohex-2-enedioic-2-D acid	tautomer of M12a			
M13	3-oxohexanedioic-5-D acid	160.0356	0.6	$[C_6H_6DO_5]^-$	

M14	succinic-2-D acid	118.0251	0.5	$[C_4H_4DO_4]^-$	
M15	acetic-2-D acid	60.0196	0.5	$[C_2H_2DO_2]^-$	
M16a	(2*E*,4*Z*)-3-hydroxyhexa-2,4-dienedioic-2,4-D_2 acid	159.0261	0.7	$[C_6H_3D_2O_5]^-$	

M16b	(*Z*)-4-oxohex-2-enedioic-3,5-D_2 acid		tautomer of 16a		
M17	3-oxohexanedioic-2,4-D_2 acid	161.0419	0.6	$[C_6H_5D_2O_5]^-$	
M18	5-methylisoxazol-4-D-3-amine	100.0621	0.5	$[C_4H_6DN_2O]^+$	

M19	(*E*)-1-amino-3-oxobut-1-en-2-D-1-aminium	102.0773	0.2	$[C_4H_8DN_2O]^+$	
M20	3-oxobutanoic-2-D acid	102.0301	0.6	$[C_4H_4DO_3]^-$	
M21	propan-2-one-1-D	59.0	-	C_3H_5DO	
M22	1-hydroxypropan-2-one-1-D	75.0	-	$C_3H_5DO_2$	
M23	1-hydroxypropan-2-one-3-D	75.0	-	$C_3H_5DO_2$	
M24	methyl acetate-2-D	75.0	-	$C_3H_5DO_2$	

M25	methyl-D acetate	75.0	-	$C_3H_5DO_2$	
M26	2-oxopropanal-1-D	72.0196	0.6	$[C_3H_2DO_2]^-$	
M27	2-oxopropanal-3-D	72.0196	0.5	$[C_3H_2DO_2]^-$	
M28	acetic-2-D acid	60.0196	0.3	$[C_2H_2DO_2]^-$	
M29	2-oxopropanoic-3-D acid	88.0152	0.2	$[C_3H_2DO_3]^-$	
M30	methan-D-ol	33.0	-	CH_3DO	

[xx] Compounds labeled in **blue** have already been described