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Supporting information for article:

Expression, purification and crystallization of the *N*-acetyl-(*R*)-β-phenylalanine acylases derived from *Burkholderia* sp. AJ110349 and *Variovorax* sp. AJ110348 and structure determination of the *Burkholderia* enzyme

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Supplementary Table S1.

Macromolecule-production information of *N*-acetyl-(*R*)-β-phenylalanine acylase derived from *Variovorax* sp. AJ110348.

	V-His-β-FAA	V-β-FAA-His
Source organism	*Variovorax* sp. AJ110348	*Variovorax* sp. AJ110348
DNA source	*Variovorax* sp. AJ110348 genomic DNA	−
Forward primer[#]	5'-CGGTAAGCTT AAGGAGGAACGCGGC ATG ACG ATG-3'	−
Reverse primer[#]	5'-GAGGCCGGTCTCTAGA GTTCTGAAAGGTCGG-3'	−
Cloning vector	pUC19	−
DNA source	pUC19 plasmid harboring the *Variovorax* β-FAA gene	pColdI-V-His-β-FAA
Forward primer[#]	5'-CGCCAT ATG ACG ATG CAG CAG CAG AAG ATC-3'	5'-ATCACAAAGTGCAT ATG ACG ATG CAG CAG CAG AAG ATC C-3'
Reverse primer[#]	5'-AGTGAAT TCA TGG TGC TGC GTG CTC CAG GGA-3'	5'-TCGACAAGCTTGAATTC TCA **ATG ATG ATG ATG ATG ATG** TGG TGC TGC GTG CTC CAG GG-3'
Expression vector	pColdI	pColdIII
Expression host	*Escherichia coli* strain BL21	*Escherichia coli* strain BL21
Complete amino-acid sequence of the construct produced[*]	MNHKVHHHHHHIEGRHMTMQQQKILPPHRS LQVPGVHGYTDRKSVAAGEVVRFHISSDVP YTLSVCQLGSDTEGRTDDAVLHTFEPSAPR VHPIHPGSYVRVERGLDQPLRALTLECWVQ VWSLGTRQSVIGQFDLPGACGYGLFIDEDG RAVFHLGDGGAFRAEGQLSGGALQPRRWHH VVATWNGSETVLWLDGEAVASGRFDGPLQP GRAPLRLGSSGIDGLADAFLEGDIVMPAIY SHALQADEVKARFADRGLHTPRGRTVLACW PLREERGDVVADASGHQRTGRIVNHGTWMI VGPAFEPHRVNEFSDEGYDPLTDPTRGHGL RLASDDLYDCRWPESHAFRMPADAKSGVYV GRVSFLLDGRSAEYDITFIVRRAANRAPAP VLVLCATNSWLAYAATPFAKNVASDPVWPR RSAGLQNSHPEAPAFCSYTYHRGGQPTYQV GLRMPWPNASPNALYDPADAGFSQWTRLER RLHVWLDRCGYEYDVVSDLDLHRDPGLLKA YGTVFINGHSEYWSQPACDGLDDYLSNGGT AIVLSGNTMYLRVSYDEECTVMEQRKVRGP GDEDGAESVELRPPAGPYGEQYHSQDWARG GQFRQAGRSCADLIGLESAGWAFADGDDFG VYHATQPGHFLFTQPHPLGLEEGSTFGHAP GGGLPRAIGHEWDLSVATLRRMTRTLPAGE RLPEPHRGIQVIAEGRRQRPGRLDAYLDYY SQPTDSLGGLSAEMIYWERPQGGRVFNAGA VGASWVLGADPSFEGLLRNVLHHFGVRPAT GADLADQPSLEHAAP	MNHKVHMTMQQQKILPPHRSLQVPGVHGYT DRKSVAAGEVVRFHISSDVPYTLSVCQLGS DTEGRTDDAVLHTFEPSAPRVHPIHPGSYV RVERGLDQPLRALTLECWVQVWSLGTRQSV IGQFDLPGACGYGLFIDEDGRAVFHLGDGG AFRAEGQLSGGALQPRRWHHVVATWNGSET VLWLDGEAVASGRFDGPLQPGRAPLRLGSS GIDGLADAFLEGDIVMPAIYSHALQADEVK ARFADRGLHTPRGRTVLACWPLREERGDVV ADASGHQRTGRIVNHGTWMIVGPAFEPHRV NEFSDEGYDPLTDPTRGHGLRLASDDLYDC RWPESHAFRMPADAKSGVYVGRVSFLLDGR SAEYDITFIVRRAANRAPAPVLVLCATNSW LAYAATPFAKNVASDPVWPRRSAGLQNSHP EAPAFCSYTYHRGGQPTYQVGLRMPWPNAS PNALYDPADAGFSQWTRLERRLHVWLDRCG YEYDVVSDLDLHRDPGLLKAYGTVFINGHS EYWSQPACDGLDDYLSNGGTAIVLSGNTMY LRVSYDEECTVMEQRKVRGPGDEDGAESVE LRPPAGPYGEQYHSQDWARGGQFRQAGRSC ADLIGLESAGWAFADGDDFGVYHATQPGHF LFTQPHPLGLEEGSTFGHAPGGGLPRAIGH EWDLSVATLRRMTRTLPAGERLPEPHRGIQ VIAEGRRQRPGRLDAYLDYYSQPTDSLGGL SAEMIYWERPQGGRVFNAGAVGASWVLGAD PSFEGLLRNVLHHFGVRPATGADLADQPSL EHAAPHHHHHH

In the primers, restriction enzyme sites (*Hind*III, *Xba*I, *Nde*I, *Eco*RI) are underlined. An additional sequence coding His-tag is indicated in bold.

* Amino acid sequence coded by the translation enhancing element derived from pCold vectors are underlined.

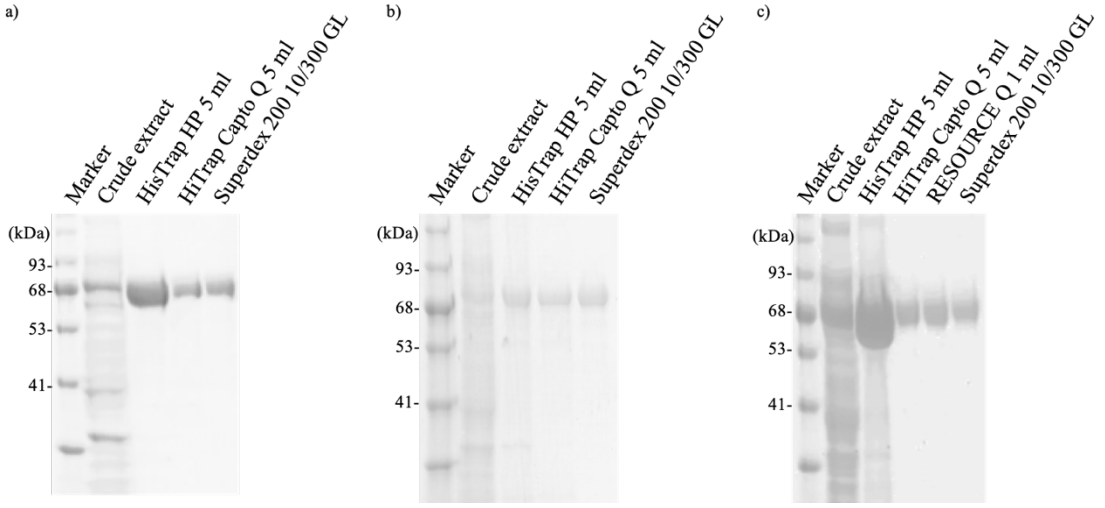
Supplementary Table S2.

Summary of data collection and processing.

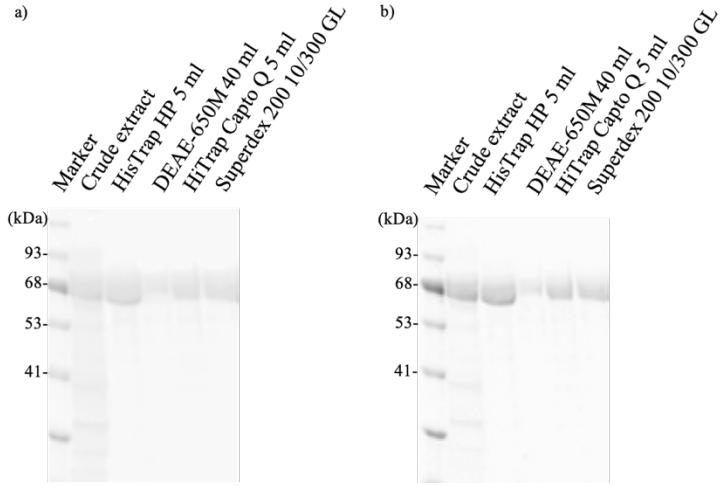
Values for the outer shell are given in parentheses.

Protein	V-β-FAA-His
Composition of reservoir solution	0.1 M MES-NaOH pH 6.3, 29.4 % (w/v) polyethylene glycol monomethyl ether 5000, 0.2 M ammonium sulfate
Diffraction source	AR-NE3A, PF
Wavelength (Å)	1.0000
Temperature (K)	100
Detector	Dectris Pilatus 2M-F
Crystal-detector distance (mm)	364.492
Rotation range per image (°)	0.2
Total rotation range (°)	360
Exposure time per image (s)	0.2
Space group	$P222_1$
a, *b*, *c* (Å)	86.04, 100.36, 180.49
α, *β*, *γ* (°)	90, 90, 90
Mosaicity (°)	0.24
Resolution range (Å)	49.31–3.07 (3.26–3.07)
Total No. of reflections	386831 (64265)
No. of unique reflections	29971 (4750)
Completeness (%)	100 (100)
Redundancy	12.9 (13.5)
$<I/\sigma(I)>$	11.8 (2.5)
R_{meas}	0.199 (1.096)
Overall *B* factor from Wilson plot (Å^2)	35.4
Twin fraction	0.406

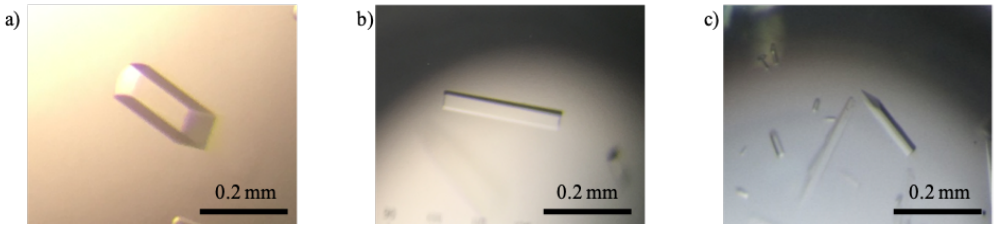
Note: This data was collected at beamline AR-NE3A in Photon Factory of KEK. Diffraction were recorded on Dectris Pilatus 2M-F detector.



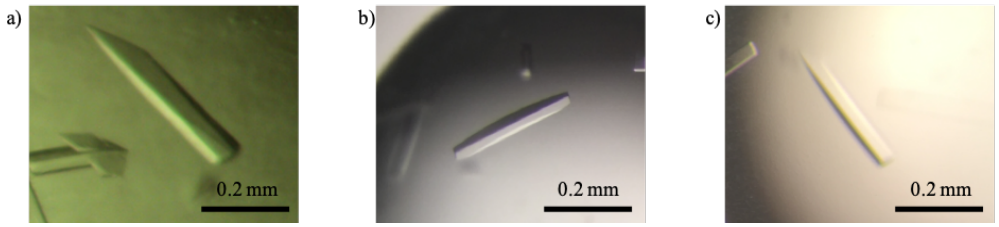
Supplementary Fig. S1. SDS-PAGE analysis of the purified β-FAAs derived from *Burkholderia* sp. AJ110349. a) B-His-β-FAA, b) B-β-FAA-His, c) B-Se-β-FAA-His. Proteins in the collected fractions were separated on NuPAGE 4 to 12 % Bis-Tris gels (Invitrogen) with NuPAGE MOPS SDS running buffer (Invitrogen), and stained with Coomassie Brilliant Blue G250. On the most left well in each gel, FastGene BlueStar Prestained Protein Marker (FastGene) were applied; their molecular masses are indicated in kDa.



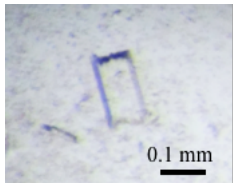
Supplementary Fig. S2. SDS-PAGE analysis of the purified β-FAAs derived from *Variovorax* sp. AJ110348. a) V-His-β-FAA, b) V-β-FAA-His. Proteins in the collected fractions were separated on NuPAGE 4 to 12 % Bis-Tris gels (Invitrogen) with NuPAGE MOPS SDS running buffer (Invitrogen), and stained with Coomassie Brilliant Blue G250. On the most left well in each gel, FastGene BlueStar Prestained Protein Marker (FastGene) were applied; their molecular masses are indicated in kDa.



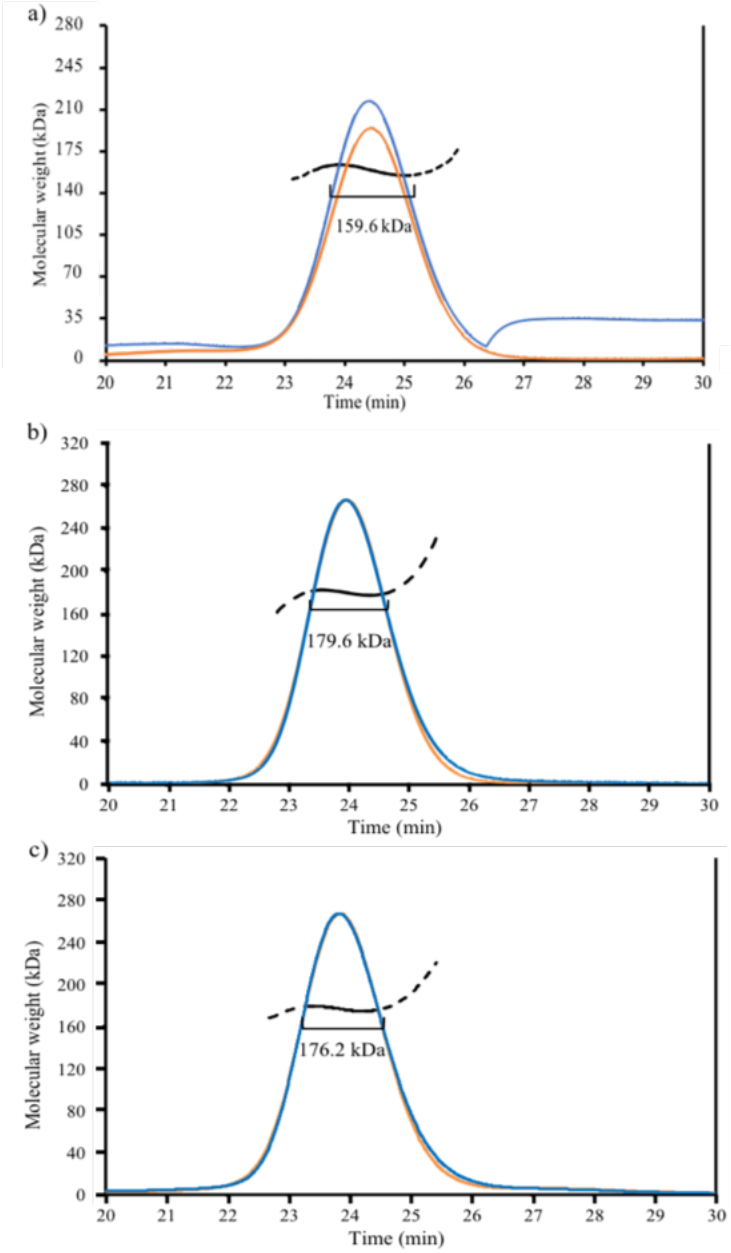
Supplementary Fig. S3. B-His-β-FAA crystals. Each panel corresponds to the individual crystallization conditions with Reservoir solutions (a), (b) and (c).



Supplementary Fig. S4. B-Se-β-FAA-His crystals. Each panel corresponds to the individual crystallization conditions with Reservoir solutions (a), (b) and (c).



Supplementary Fig. S5. V-β-FAA-His crystal obtained with the reservoir solution containing 0.1 M MES-NaOH pH 6.5–6.8, 25–30 % (w/v) polyethylene glycol monomethyl ether 5000, 0.2 M ammonium sulfate.



Supplementary Fig. S6. SEC-SLS analysis of β-FAAs. a) B-His-β-FAA, b) V-β-FAA-His, c) V-His-β-FAA. The chromatograms of the absorbance at 280 nm and the static light-scattering at 90° are scaled into an arbitrary unit and shown in orange line and blue line, respectively. The weight averaged molecular weights are shown in black line and dots. Regarding B-His-β-FAA, the averaged value calculated from the intensities from 23.5 min to 25.1 min was 159.6 kDa (± 1.03 %). Regarding V-β-FAA-His, the averaged value calculated from the intensities from 23.4 min to 24.6 min was 179.6 kDa (± 0.71 %). Regarding V-His-β-FAA, the averaged value calculated from the intensities from 23.3 min to 24.5 min was 176.2 kDa (± 0.73 %).