Supplementary Materials for

A superefficient ochratoxin A hydrolase with promising potential for industrial applications

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[¶]These authors contributed equally to this work (HL purified and characterized the enzyme ADH3, GW illustrated the efficient catalytic mechanism, and NC isolated the strain CW117 and screened the enzyme ADH3).

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Supplementary Text:

Text S1 genomic analysis

Based on OTA degradation product by *S. acidaminiphila* CW117, amido bond hydrolases are considered as the degradation enzymes (1, 2). Currently, few known examples of hydrolases, such as peptidase, amidohydrolase and carboxypeptidase were characterized as OTA degradation enzyme (3-6). In order to obtain the OTA degradation genes from the strain CW117, we assembled and analyzed the complete genome sequence of *S. acidaminiphila* CW117, and the general genomic information was outlined in Table S1-Table S3. After genomic data assemble, gene prediction and annotation were performed by BLAST searches in 10 databases (Fig.S2 – Fig.S3). More than 3800 genes were annotated by the database of COG, KEGG, NR, Pfam and Swissprot, and a complete genome map of *S. acidaminiphila* CW117 was produced by assembled and annotation results (Fig.S4). By genome sequence analysis, 53 amido bond hydrolase including 5 amidases, 14 amidohydrolases, 5 carboxypeptidases and 29 peptidases' family (Table S4) were screened from complete genome sequence of CP062156.1 (*S. acidaminiphila* CW117).

Table S1 Results for genome sequencing on the *S. acidaminiphila* CW117

Type	Illumina	PacBio
RawReads	9463494	307993
RawBases	1.4 G	3.7 G
CleanReads	9279424	221027
CleanBases	1.3 G	2.7 G
Meanlength (bp)	-	12375
N50 (bp)	-	12616

Table S2 Genome assembly, ORF determination and basal information on the *S. acidaminiphila* CW117.

Type	Number/character	Average length (or Percentage in genome)	Total length
Contigs/Genome	1 (Circular)	4090129 bp	4090129 bp
Gene	3844	954.38 bp (89.7%)	3668652 bp
G+C (%)	-	68.75	-
sRNA	11	144.182 bp	1586
23S rRNA	3	2875 bp	8625
16S rRNA	3	1534 bp	4602
5S rRNA	3	114 bp	342
tRNA	61	77.541 bp	4730
SINEs	14	0.02%	912 bp
LINEs	6	0.01%	500 bp
DNA transposon	5	0.01%	342 bp
Tandem repeats	234	0.42%	17229 bp
Num. of Prophage	2	68681.5	137363 bp
Num. of CRISPR	2	984 bp	1968 bp
LTR elements	0	0%	
Unclassified	0	0%	0

Table S3 The genomics island (GIs) predictions in the *S. acidaminiphila* CW117 genome.

GIs number	Start position	End position	GI length
GI1	135821	145627	9807 bp
GI2	167433	205407	37975 bp
GI3	361511	371271	9761 bp
GI4	639600	643913	4314 bp
GI5	1468321	1482892	14572 bp
GI6	1493582	1503040	9459 bp
GI7	1545600	1558567	12968 bp

Table S4. The candidates of OTA degradation gene screened from the *S. acidaminiphila* strain CW117 genome (53 candidates).

Gene no.	enzyme	Gene locus_tag	Expression primers
cp1	Dipeptidyl carboxypeptidase II	H7691_17490	F:CCG**GAATTC**ATGTCGCGCACCCTCATTC R:CCG**CTCGAG**TTACTTCAGCCCGCGGTTC
cp2	LD-carboxypeptidase	H7691_05885	F:CCG**GAATTC**ATGGACAGGCGGCGATTC R:CCG**CTCGAG**TCAGCTGCCGGCGAACAC
cp4	D-alanyl-D-alanine carboxypeptidase	H7691_02910	F:CGC**GGATCC**ATGAAATTCCGCTTTGCCG R:CCG**CTCGAG**TCAGGACTTCCACCACATC
cp5	D-alanyl-D-alanine carboxypeptidase	H7691_13635	F:CCG**GAATTC**ATGCCCCAAGCTACCGCC R:CCG**CTCGAG**TCAGGCAGGCGTGCCGC
cpm14	Metallocarboxypeptidase M14	H7691_05930	F:CCG**GAATTC**ATGACCACCGCCTTCTATCC R:CCG**CTCGAG**TCAGCGGTGCGCCAGCG
ad1	amidase	H7691_03865	F:CGC**GGATCC**ATGCGCGCCCTCCACACC R:CCG**CTCGAG**TCAACGGGTCTGCGCGGTAT
ad2	*N*-acetylmuramoyl-*L*-alanineamidase	H7691_13045	F:CGC**GGATCC**ATGCCCGCGATCCACATC R:CCG**CTCGAG**CTACATGCGCTGCAGGGGT
ad3	N-acetylmuramoyl-L-alanine amidase	H7691_01300	F:CGC**GGATCC**ATGAAGATGTCCACCAAGC R:CCG**CTCGAG**TCAGCGGTGGGTGGCC
ad4	N-acetylmuramoyl-L-alanine amidase	H7691_01285	F:CCG**GAATTC**ATGACGCATCGGAAAACCC R:CCG**CTCGAG**TTACGGCGTATCGGTCGTG
ad5	N-acetylmuramoyl-L-alanine amidase	H7691_08545	F:CCG**GAATTC**ATGCTCAAGGGAAGCCGCC R:CCG**CTCGAG**TTACGGCGTATCGGTCGTG
adh1	Amidohydrolase	H7691_08865	F:CCG**GAATTC**ATGAATGACCTCCGTATTTCAC R:CCG**CTCGAG**TCAGCCCAGGGTGAAGG
adh2	Amidohydrolase	H7691_03195	F:CCG**GAATTC**ATGAGCGCGTCCATCGTGT R:CCG**CTCGAG**TCAGCGGCGGTAGGCCG
adh3	Amidohydrolase	H7691_12935	F:CGC**GGATCC**ATGCCGATCCGCCGCCGC R:CCG**CTCGAG**TCACTGCTTGTAGATCACCCCG
adh4	Amidohydrolase	H7691_09595	F:CCG**GAATTC**ATGAACCCGCTGACCGCC R:CCG**CTCGAG**TCAGGGGGCCACATTGCGT
adh5	Amidohydrolase	H7691_11440	F:CCG**GAATTC**ATGAAAACCACGCTCTGCCTC R:CCG**CTCGAG**TCAGCGGGCGGCTTCGG
adh6	Amidohydrolase	H7691_08445	F:CCG**GAATTC**ATGGGCGCGAGGCGGGC R:CCG**CTCGAG**TCATCGCGACGAAGGCTTG
adh7	Amidohydrolase	H7691_14245	F:CGC**GGATCC**ATGCGCCGTACCGCCGC R:CCG**CTCGAG**TCAGCCCTCGCTGGCCG
adh8	Amidohydrolase	H7691_13545	F:CGC**GGATCC**ATGAAACTGCTGTTGGCCC R:CCG**GAATTC**TTATTTCCCCGCTTGCTCC
adh9	Amidohydrolase	H7691_08440	F:CGC**GGATCC**ATGAACATGAACCCCCGC R:CCG**CTCGAG**TCAGTAGAAGCCCACGTTG
adh10	Amidohydrolase	H7691_03200	F:CCG**GAATTC**ATGCGACACCGACTGCTG R:CCG**CTCGAG**TCATGGCCGCACCTCCTG
adh11	Amidohydrolase	H7691_03430	F:CCG**GAATTC**ATGAGCCGGCTCGACAAC R:CCG**CTCGAG**CTAGCCGAGCTGCAGC
adh12	Amidohydrolase	H7691_05890	F:CCG**GAATTC**ATGCGCCCGCTGTCGTTG R:CCG**CTCGAG**TCAGTCGGCGCCCTTG
adh13	Amidohydrolase	H7691_11430	F:CCG**GAATTC**ATGTTCGACCACCTGTTCAC R:CCG**CTCGAG**TCAGGACGCGGTTGCGTC
naa	amidohydrolase	H7691_01355	F:CGC**GGATCC**ATGATCCGCAAGACCGTTCTGT R:CCG**CTCGAG**TCAGCCGGCGCCGCCGT
pd1	peptidase	H7691_03070	F:CCG**GAATTC**ATGGCGGTGGCGGTGTG R:CCG**CTCGAG**TCAAGGGAACTGGGCCCC
pd2	peptidase	H7691_12755	F:CGC**GGATCC**ATGAAGAACGCCACCGG R:CCG**CTCGAG**TTACCAGATCACTACCTGC

pd3	peptidase	H7691_03710	F:CCG**GAATTC**ATGTTGCGAGCAGTGGG R:CCG**CTCGAG**TCATTCCGGGTCGGCCC
pds8	peptidase S8	H7691_05865	F:CCG**GAATTC**ATGCGCAGCACGTTCAGGG R:CCG**CTCGAG**TCAGAACCCGCGCATGAAC
pds9	peptidase S9	H7691_00630	F:CCG**GAATTC**ATGGGAAGGGGATGGTG R:CCG**CTCGAG**TCAGCTCCCGATGTGCTC
pds10	peptidase S10	H7691_07065	F:CCG**GAATTC**ATGAAACACCTGCTGTACGT R:CCG**CTCGAG**TCAGTTGCGCTGGTACATG
pds41	peptidase S41	H7691_01105	F:CCG**GAATTC**ATGCGTGTAGCCGGCCTT R:CCG**CTCGAG**TCACTTGCCGCCGTCGAC
pds46	peptidase S46	H7691_18370	F:CCG**GAATTC**ATGAAGCGCACACCGCT R:CCG**CTCGAG**TTACTCCCGCGGCAGGC
pds49	peptidase S49	H7691_11860	F:CGC**GGATCC**ATGACCCTGTTACCGCATATG R:CCG**GAATTC**TCACTTCTCCTGTCCGGC
c-pds	carboxy terminal-processing peptidase	H7691_02940	F:CCG**GAATTC**ATGAACTACCGAGTACCCG R:CCG**CTCGAG**TCAGTCGGCCCAGCGG
mepds	metalloendopeptidase	H7691_08660	F:CCG**GAATTC**ATGCGCTCGATGCTCCTG R:CCG**CTCGAG**CTACTTGCCGCGTTCGAG
pdm1	M1 family metallopeptidase	H7691_02850	F:CGC**GGATCC**ATGCGTTCACCCTTCCTG R:CCG**GAATTC**TTACGGCTTCGGCGCGG
pdm2	M2 family metallopeptidase	H7691_13720	F:CGC**GGATCC**ATGTACCCGGAAATGACCTC R:CCG**GAATTC**TTACGGCGCGGCCGGCT
pdm3	M3 family metallopeptidase	H7691_09315	F:CGC**GGATCC**ATGGCTTTGCAACAGCAGGCG R:CCG**CTCGAG**TTACTTGCTTTCGGCGCCGG
pdm13	peptidase M13	H7691_05295	F:CCG**GAATTC**ATGACCCTTTCCAAGCTCG R:CCG**CTCGAG**TTACCAGATGACCACGCG
pdm15	M15 family metallopeptidase	H7691_01310	F:CCG**GAATTC**ATGCGCAGAGCCATTGCC R:CCG**CTCGAG**CTAGCGCACCGGGAAGTC
pdm20	peptidase M20	H7691_14450	F:CCG**GAATTC**ATGGACAGCGCCAAGCTC R:CCG**CTCGAG**TCAGCAGCAGCCGTGGC
pdm23	peptidase M23	H7691_14040	F:CCG**GAATTC**ATGCACCGCCTGACGCTC R:CCG**CTCGAG**TCAGGGCGGGGCGCCC
pdm28	peptidase M28	H7691_14695	F:CCG**GAATTC**ATGCGCCGCCTCACCTTC R:CCG**CTCGAG**TCATTCCTTGGGCGGCAC
pdm48	peptidase M48	H7691_04110	F:CCG**GAATTC**ATGATGCTGCGCGAACTG R:CCG**CTCGAG**TCAGCGGCGCTGCATGT
pdm61	peptidase M61	H7691_00400	F:CCG**GAATTC**ATGTACGCGCACAAGTGGT R:CCG**CTCGAG**TCAACGCCGCGGCGCG
pdc13	peptidase C13	H7691_10490	F:CGC**GGATCC**ATGCCTGCCGCCATCACC R:CCG**GAATTC**TCAGCGCGTCCCGGGG
pdc40	peptidase C40	H7691_12795	F:CGC**GGATCC**ATGCACATCACGCCAGCT R:CCG**GAATTC**TCAGCGCAGCACGCGCTT
pdp1	peptidase P1	H7691_05170	F:CCG**GAATTC**ATGATCAAGCGCTGGTCCC R:CCG**CTCGAG**TCAGCGTGGCGCCGGC
pdu32	peptidase U32	H7691_02725	F:CCG**GAATTC**ATGAAGATCAGCCTTGGCC R:CCG**CTCGAG**TCAGGCCTGCATGCGCAT
dpd-1	dipeptidase	H7691_18780	F:CGC**GGATCC**ATGCCGCTGTGTCGTCTG R:CCG**GAATTC**TTACTTGGCGCCGTTCTTC
dpd-2	dipeptidase	H7691_15105	F:CGC**GGATCC**ATGACCAATGGCCTGCTG R:CCG**GAATTC**TTACTCCAGCGCCGCCTT
pdz	zinc-dependent peptidase	H7691_17225	F:CGC**GGATCC**ATGGCACCGCCTGCTGC R:CCG**CTCGAG**CTATGGCTGTTGCAGCGC
m-hd	Metallo-hydrolase	H7691_05370	F:CGC**GGATCC**ATGATCAAGCGGTGCTTGCT R:TCC**CCCGGG**TCAGTCTTCCAGCAGCGG

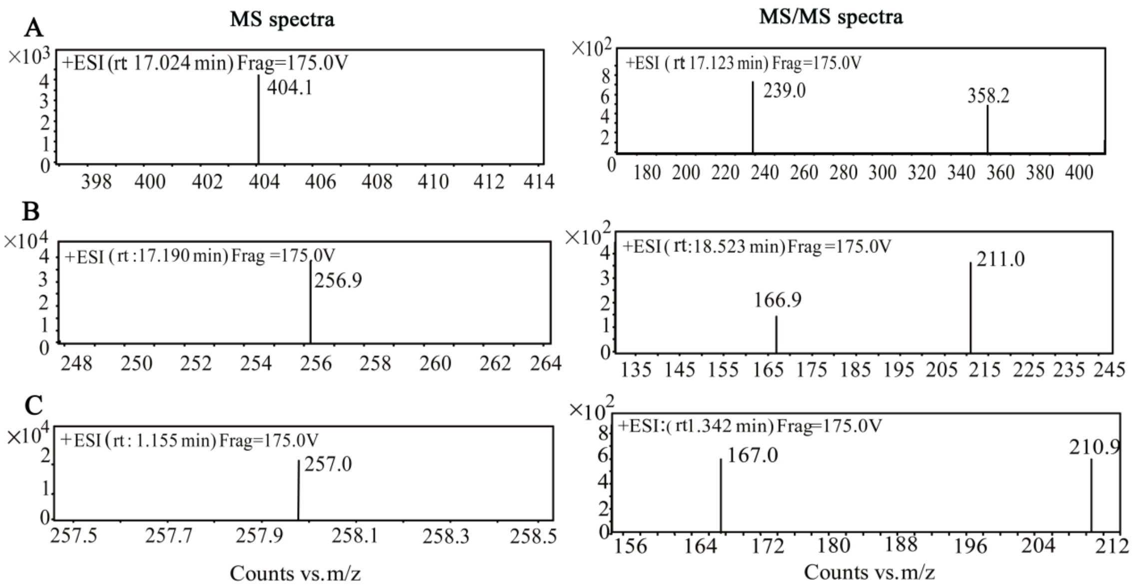

Figure S1. The liquid chromatography-tandem mass analysis of OTA degradation product by the strain CW117 under the positive ionization mode. A, the spectra of OTA standard; B, the spectra of OTα standard; C, the spectra of OTA degradation product. The OTA standard produced [M+H$^+$] at m/z 404 as precursor ion in MS spectrum, and product ions at m/z 239 and 358 in MS/MS spectrum. The OTα standard produced [M+H$^+$] at m/z 256.9 as precursor ion in MS spectrum, and product ions at m/z 167 and 211 as in MS/MS spectrum.

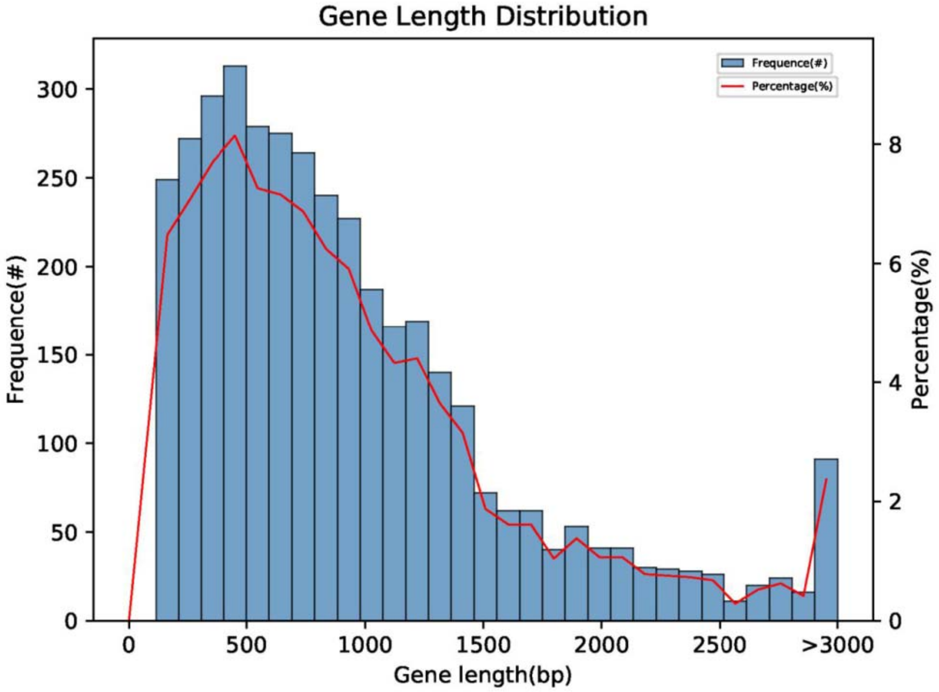

Figure S2. The gene length distribution of CW117 genome. The gene length of CW117 was mainly distributed in the range of 100 - 1500 bp.

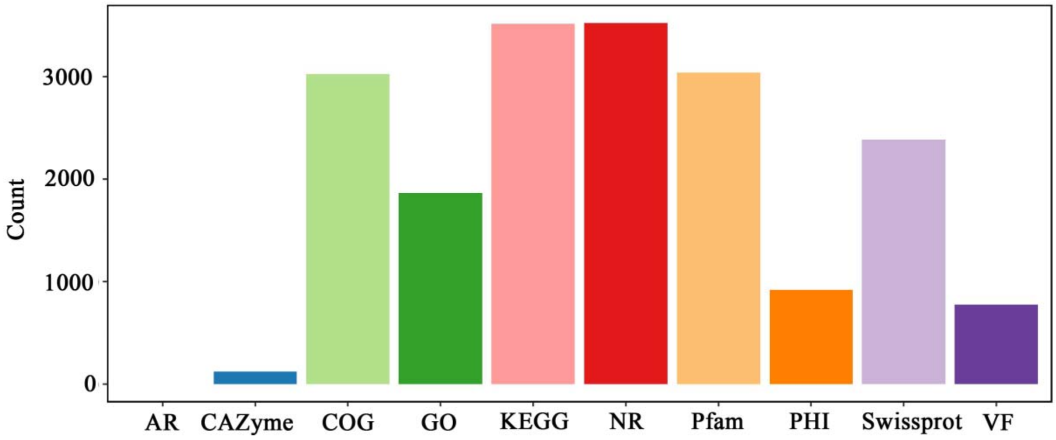

Figure S3. The statistics of gene annotation in different database. NR, Non-Redundant Protein Database; Swiss-prot, Swiss-prot Database; COG, Cluster of Orthologous Group of Proteins; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; Pfam, Pfam Database; CAZyme, Carbohydrate-Active enZYmes Database; PHI, Pathogen Host Interactions; VF, Virulence Factors of Pathogenic Bacteria; AR, Antibiotic Resistance Genes Database.

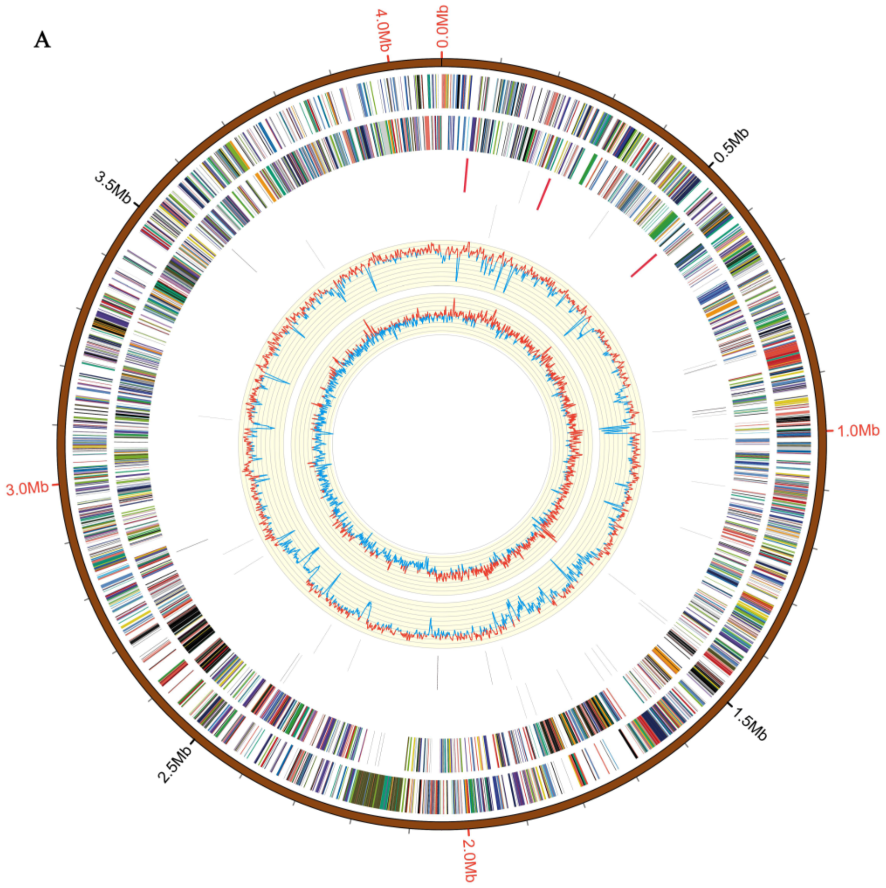

Figure S4. The complete genome of *Stenotrophomonas acidaminiphila* CW117. Rings from the outside to inside 1) scale marks of the genome; 2) protein-coding genes on the forward strand; 3) protein-coding genes on the reverse strand; 4) tRNA (black) and rRNA (red) genes on the forward strand; 5) tRNA (black) and rRNA (red) genes on the reverse strand; 6) GC content; 7) GC skew. Protein-coding genes are color coded according to their COG categories.

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AfOTase    IKAALETMPGYQI----------QTGIAQTGVKAVL---------KGGKPGPVVALRADMDALPVQERND —117
ADH3       RVVDLGDKVCLPG----------WTDLHVH---------------LGSQSSP-QSYSEDFRLDPVDH--- —106
OTase      ALVISDKIIAFVGSEADIPKKYLRSTQSTHRVPVLMPGLWDCHMHFGGDDDYYNDYTSGLATHPASSG-- —136
CP         IASMTKMMTEYLL------------LEAIQ----------------EGKVKWDQTYTPDDYVYEISQDN- —106
PJ15_1540  PASMTKMMTSYII-----------EQKLLK-----------------GELTENEQVRMNESAWCRGSSSE —97
CP A       FPSLQAVKVFLEA-----------HGIRYR-----------------IMIEDVQSLLDEEQEQMFASQS- —109

AfOTase    VVAAAETVVALNNIIAQRTNPQDGTTVVTVGSLQSGNRPNVLPESADISGTVR------SLSKQNQATAH —314
ADH3       IKAVVDTARDYGFRVAAHAHGTEGMKRAVQAGVTSIEHGTYMDDEVMRLMKQHGTWYVPTFYAGRFVTEK —303
OTase      LKVIVEEAARQNRIVSAHVHGKAGIMAAIKAGCKSLEHVSYADEEVWELMKEKG-----ILYVATRSVIE —334
CP         WNFMLKGLVSEYPGVDGLKTGSTDSAGSCFTSTAQRNGMRVITVVLNAKGNLHTG----RFDETKKMLDY —304
PJ15_1540  --ALLYTDPS----VDGLKTGHTNEAGFCLTTSSKRGPMRLISVIFGTPSMNER------ANQTRTLLAW —268
CP A       R-SVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKSIVD------FVKDHGNFKAF —302

AfOTase    ELIQRYAQNIAANHDLKATVRIDT--GYEVLVSDPKATQTVIPALDLATDGIGAKEVAPGMG-SEDFGAF —381
ADH3       AAIDGYFPEVVRPKAARIGALISQ---TAAKAYRNGVRIAFGTDQGVGPHGDNAREFVYMVE-AGIPAAY —369
OTase      IFLASNGEGLVKESWAKLQALADSHLKAYQGAIKAGVTIALGTDT--APGGPTALELQFAVERGGMTPLE —402
CP         AFNSNFSMKDLYPEGSQVKGHKTID-VEKGKDKQVDIVTDKALSIPVKSGDEKNYKAEVTLDKKEITAPV —373
PJ15_1540  GFSN-FETANVQPANQVLAKAKVW----FGKQDDVQIGLAENFNVTMPKGQADKIKTQLVVQ-PKLNAPL —332
CP A       LSIHSYSQLLLYPYGYTTQSIPDK---TELNQVAKSAVEALKSLYGTSYKYGSIITTIYQASGGSIDWSY —369
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Figure S5. Multiple sequences alignments of ADH3 and other OTA detoxify enzymes with known polypeptide sequences. The catalytic residues for ADH3 are shown in *white* character with a *black* background. The result of multiple sequences alignments showed that the sequences of the identified peptidases (detoxify enzymes) responsible OTA detoxification showed high diversity, and ADH3 showed the closest relative to OTase indentified from *Aspergillus niger*. The amino acid sequences used in multiple sequences alignments were as follows: ADH3 (QOF97534.1), AfOTase (OSZ37025.1), PJ15_1540 (KHF78480.1), OTase (AIG55189.1), CP (AKA44618.1), CPA (NP_777175.1).

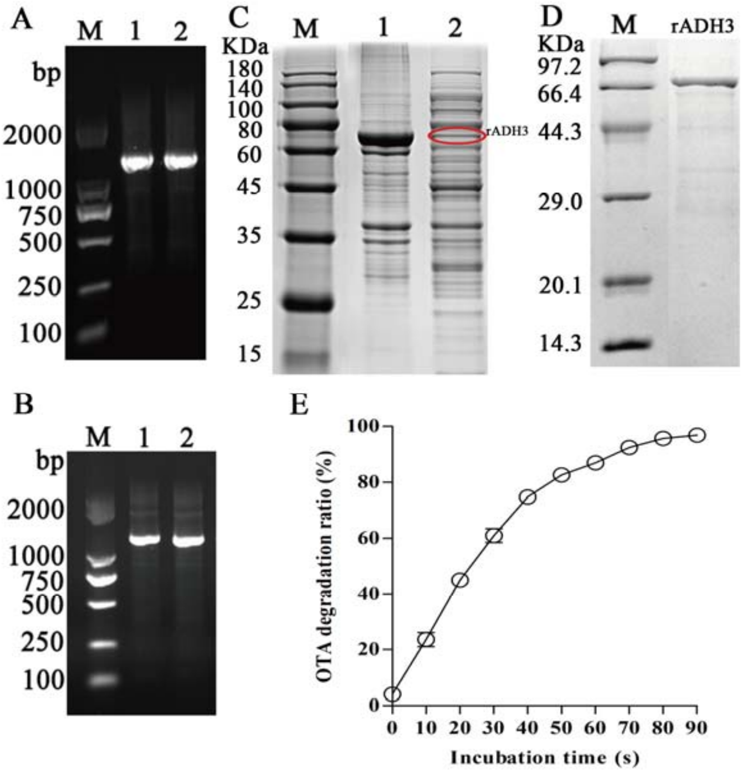

Figure S6. Gene cloning, protein expression, purification and activity assay of ADH3. A, the PCR product of gene *adh3* from the CW117 genome; B, The PCR verification of *gene adh3* from *E. coli* BL21 transformant pGEX-4T-1/*adh3*; C, SDS-PAGE analysis of the heterologous expressed rADH3 (M, marker; 1, the expressed rADH3 in precipitant; 2, the expressed rADH3 in supernatant); D, SDS-PAGE analysis of the purified rADH3 (M, marker; rADH3, the purified rADH3 protein); E, OTA degradation assays on purified rADH3 by 1.2 μg/mL active protein.

Reference

1. Stander MA, Steyn PS, van Der Westhuizen FH, Payne BE. 2001. A kinetic study into the hydrolysis of the ochratoxins and analogues by carboxypeptidase A. Chem Res Toxicol 14:302-4.
2. Wu Q, Dohnal V, Huang L, Kuca K, Wang X, Chen G, Yuan Z. 2011. Metabolic pathways of ochratoxin A. Curr Drug Metab 12:1-10.
3. Chang X, Wu Z, Wu S, Dai Y, Sun C. 2015. Degradation of ochratoxin A by Bacillus amyloliquefaciens ASAG1. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32:564-71.
4. Dobritzsch D, Wang H, Schneider G, Yu S. 2014. Structural and functional characterization of ochratoxinase, a novel mycotoxin-degrading enzyme. Biochem J 462:441-52.
5. Liuzzi VC, Fanelli F, Tristezza M, Haidukowski M, Picardi E, Manzari C, Lionetti C, Grieco F, Logrieco AF, Thon MR, Pesole G, Mule G. 2016. Transcriptional Analysis of Acinetobacter sp. neg1 Capable of Degrading Ochratoxin A. Front Microbiol 7:2162.
6. Zhang H, Zhang Y, Yin T, Wang J, Zhang X. 2019. Heterologous Expression and Characterization of A Novel Ochratoxin A Degrading Enzyme, N-acyl-L-amino Acid Amidohydrolase, from Alcaligenes faecalis. Toxins (Basel) 11.