

A novel potent crystalline chitin decomposer: chitin deacetylase from

***Acinetobacter schindleri* MCDA01**

Guang Yang^{a,b,c,d}, Yuhan Wang^{a,d}, Yaowei Fang^{a,b,c,d}, Jia An^{a,d}, Xiaoyue Hou^{a,b,c,d},

Jing Lu^{a,b,c,d}, Rongjun Zhu^{a,d}, Shu Liu^{a,b,c,d} *

^a *Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang, 222005, China*

^b *Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005, China*

^c *Jiangsu Marine Resources Development Research Institute, Jiangsu Ocean University, Lianyungang, 222000, China*

^d *College of food science and engineering, Jiangsu Ocean University, Lianyungang, 222005, China*

* Corresponding author:

Shu Liu: E-mail: jdliushu@163.com

Tel.: +860515861246008

Fax: +860515861246008

Supplementary Table S1:**Table S1** CDAs with characterized activity on crystalline chitin.

Enzyme	Organism	GenBank No.	PDB ID	Deacetylation of crystalline chitin	Reference
<i>AsCDA</i>	<i>Acinetobacter schindleri</i>	MW295944.1	-	63.05%	This study
<i>AnCDA</i>	<i>Aspergillus nidulans</i>	EAA66447.1	2Y8U	0.50%	23
<i>ScCDA2</i>	<i>Saccharomy cescerevisiae</i>	NM001182196.1	-	9.16%	20
<i>ArCE4</i>	<i>Arthrobacter</i> sp.	LT630322.1	5LFC	0.01%	55
<i>ClCDA</i>	<i>Colletotrichum lindemuthianum</i>	AAT68493.1	2IW0	0.50%	19
<i>SbCDA</i>	<i>Scopulariopsis brevicaulis</i>	-	-	3.7%	55
<i>NaCDA</i>	<i>Nitratireductor aquimarinusMCD</i>	-	-	32.37%	48
<i>MeCDA</i>	<i>Microbacterium esteraromaticum</i>	-	-	32.75%	18

Supplementary Table S2:

Table S2 Chitin degrading enzymes in *Acinetobacter schindleri* MCDA01 identified by the CAZy database.

Function	Gene ID	Enzymes	CAZy family	Number of genes
Deacetylase	MCDA01GL002828	N-acetylglucosamine deacetylase	CE11	1
	MCDA01GL001582	Diacetylchitobiose deacetylase	CE14	1
	MCDA01GL002213	Acetylxyln esterase	CE3	1
	MCDA01GL002854	Chitin deacetylase		
Hydrolase	MCDA01GL002176		CE4	3
	MCDA01GL002179			
	MCDA01GL001269	Chitinases of classes I, II, and IV	GH19	1
	MCDA01GL000141	Modules of approx for		
	MCDA01GL000167	chitinases		4
	MCDA01GL001985		CBM50	
	MCDA01GL002353			

Supplementary Table S3:

Table S3 General genome features of seventeen strains in this study.

Strain	Level	Size (Mb)	GC %	Scaffolds	CDs	BioSample	BioProject
<i>Acinetobacter schindleri</i> HZE30-1	Complete	3.33719	42.5	9	3023	SAMN12478519	PRJNA558439
<i>Acinetobacter schindleri</i> HZE23-1	Complete	3.37611	42.7	11	3053	SAMN12478518	PRJNA558439
<i>Acinetobacter schindleri</i> HZE33-1	Complete	3.3207	42.6	9	3004	SAMN12478520	PRJNA558439
<i>Acinetobacter schindleri</i> SGAir0122	Complete	3.24466	42.6	2	2826	SAMN08222738	PRJNA388547
<i>Acinetobacter schindleri</i> ACE	Complete	3.26805	42.6	7	2959	SAMN04956557	PRJNA320745
<i>Acinetobacter schindleri</i> H3	Complete	3.2587	42.5	5	2904	SAMN09464373	PRJNA477264
<i>Acinetobacter schindleri</i> CIP 107287	Scaffold	3.39456	42.3	18	3081	SAMN01828163	PRJNA183293
<i>Acinetobacter schindleri</i> S00243	Scaffold	3.17198	42.7	65	2895	SAMN14908301	PRJNA632052
<i>Acinetobacter schindleri</i> GTC3703	Contig	3.24409	42.4	98	2965	SAMD00019892	PRJDB1754
<i>Acinetobacter schindleri</i> DRN	Contig	3.15797	42.7	105	2848	SAMN08942761	PRJNA450622
<i>Acinetobacter schindleri</i> NIPH900	Scaffold	3.4149	42.4	12	3096	SAMN01087915	PRJNA183303
<i>Acinetobacter schindleri</i> TG19614	Contig	3.19703	42.4	159	2914	SAMN02471245	PRJNA172718
<i>Acinetobacter schindleri</i> UBA3129	Scaffold	2.76116	42.7	309	0	SAMN06456231	PRJNA348753
<i>Acinetobacter schindleri</i> UBA10059	Scaffold	2.51164	41.2	290	2284	SAMN08020004	PRJNA417962
<i>Acinetobacter schindleri</i> MCDA01	Complete	3.33239	43.1	1	3107	SAMN19459915	PRJNA733816
<i>Bacillus anthracis</i> Ames	Complete	5.22729	35.4	2	5072	SAMN02603432	PRJNA309
<i>Bacillus cereus</i> ATCC10876	Scaffold	5.99608	34.8	2	5753	SAMN02736987	PRJNA243525

Supplementary Figure S1:

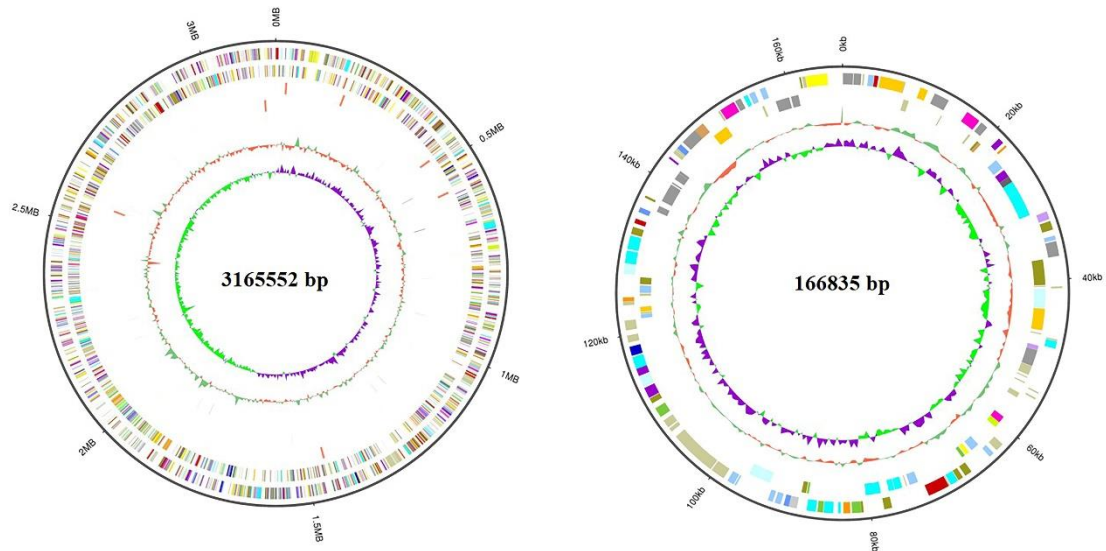


Figure S1 Circular graph of the *Acinetobacter schindleri* MCDA01 complete genome. Circles (from inside to outside): circle 1, GC skew; circle 2, GC content; circle 3, tRNA and rRNA genes on the reverse strand; circles 4, tRNA and rRNA genes on the forward strand; circle5, protein-coding genes on the forward reverse strand; circle 6, protein-coding genes on the forward strand; circle 7, genome location information.

Supplementary Figure S2:

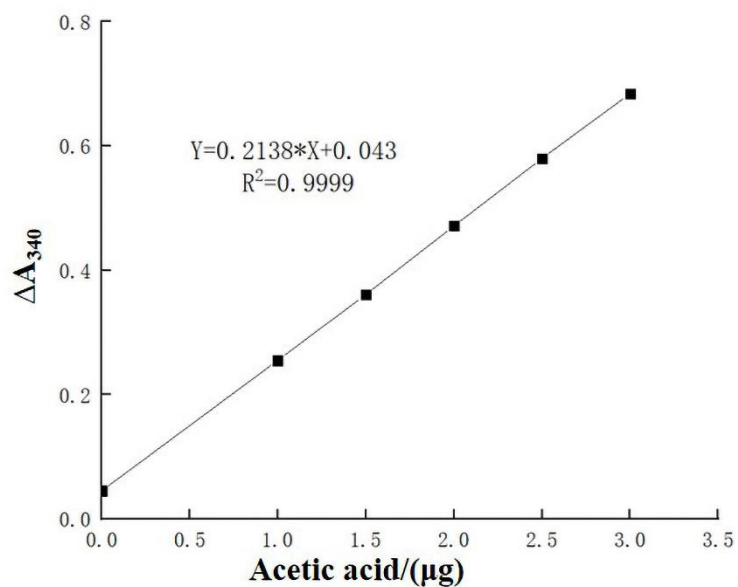


Figure S2 Standard curve of acetic acid for enzyme assay of *AsCDA*.

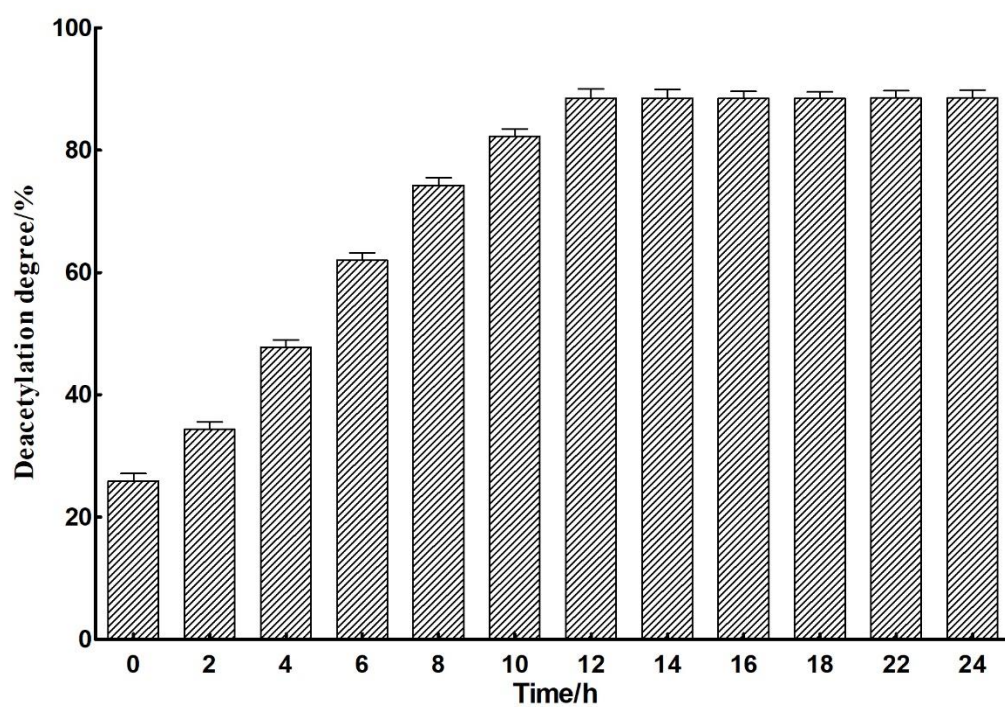


Figure S3 The determination of the degree of deacetylation of α-chitin treated by *AsCDA* with different time points (0 h, 2 h, 4 h, 6 h, 8h, 10 h, 12 h, 14 h, 16 h, 18h, 22

h and 24 h.

Supplementary informations:

Amino acid sequences of 14 members of the deacetylase superfamily were shown below to perform the multiple sequence alignment.

>MW295944.1 [*Acinetobacter schindleri* MCDA01]

VTAYLRGKQLIETIQKQEYSRDLIGYHGKPPHAQWPNAARIAVQFVLNY
EEGGENHVEHGDSSSEQFLSEIVGAASFPAVHRSMDSMYEYGSRAGFWRIHEE
FQKRGWPMTIFGVGMALARNPYIVEAIKAADYDVVSHGQRWLHYQDMEIET
ERQHMDQALSVLTELFGETPIGWYTGRDSPNTRQLLAEFSSQIKYDSDYGGDD
LPFWSTLTEPNGQKRPHLIIPYTLECNDMKFSSPGGFNSGEQFYQYLKDAFDV
LYSEGETAPKMMSIGMHCRLLGRPGRFKALQRFMDYVQSHDKVWICCRNDI
AQHWYTHHFPAHQD

active sites: Glu⁵¹, Glu⁵², His¹⁴⁰, Trp¹⁴⁴ and His²⁷⁶; Glu⁵¹: base catalytic site; His²⁷⁶: acid catalytic site.

>APH81274.1 [*Pestalotiopsis* sp.]

MLAPLFAALLAGAATASPIQERQSSVPVGTIITACTVPNTFALTDDGPFAY
TSELLDLLSSNGVKATFFLNGQNWGSIYDYTSVVTRMDAEGHQIGSHTWSHA
DLATLDAAGITSQMTQLETALTSILGKVPTYMRPPYFSTNALALSTLGGLGYH
VINANIDTLDYEHDDDTIGVAFTNFQNGLASGGTVSLMHDVHAQTVHVLVQE
AINAIKAKGLTPVTVGTC LGDASANWYKSGGGSGTTPPATGGPSPDDTCGGS
NGYVCQNSQCCSQGWGCGTTSEYCAAGCQAAYGPCT

>CAP60162.1 [*Podospira anserina*]

MINVPVTLLSLSAVATAAALADANSQSTSPNPSRQHARDTLQKRAN
CGAGIGNCPSGQCCSQYGWCGVTSEHCGTGCQSGFGTCTGGGGGNNEE
TLSTPRPKFGSIPYGVITITNCNAAGTIALTFDDGPFLYTNQLLDLLAQQQV
KATFFTNGLNWGDATQAPYPDVLRRIVNDGHQLGSHTYNHPDLNTLTTA
ARRSNMAQNEKIFKDALGGYFPTYMRPPYGSCTGQCLTDLGDLDGYHVIN
WNIDTLDYQGNIPNSQNIFNSAVSTNAAANKYIALAHDVHQATVQQLAL
GLIQTAKNRGYRLVTVGECLGDAPVNWYRDATTGNARTGGGGGSGGNP
NPGPITSTNGLCGSTNGNMNCLNSGFGNCCSQWGFCGSTAEYCGANCQR
AFGNCN

>EAA66447.1 [*Aspergillus nidulans* FGSC A4]

MFATLALVFTALASNALTTPLPLVRRVPTGQVITQCTTPNTIALTFDD
GPSEYTPQLLDLLSRY SARATFFVLGDAAAQNPGLLQRM RDEGHQVGA
HTYDHSVSLPSLGYDGIASQMTRLEEVIRPALGVAPAYMRPPYLETNELVL
QVMRDLDYRVISASVDTKDYENQDADAIINTSFQLFLDQLDAGGNIVLA
HDIHYWTVASLAERMLQEVNARGLIATTVGDCLDGEIAWYH

>QNP99526.1 [*Yarrowia lipolytica*]

MIWRALFLFVLPVATQANFTVKDLLQHKDNEWWAPCSAQGGTLD
QQSIMAAEPFPPWLTAFTGLTAWPQDNPPYIPLDYVDLATVPNHIKREL
GICDGVERTACSFDCSLCIAFDDIRTCNKISQTFDDGPSPSTPKLLEMLPSK
TTFFVQGVNVVRFPEIFREQHRQGHELLASHTWSHPNLASLSNEEIVAQLQ
WTNWAMNATAGIIPRYFRPPYGAIDNRVRAIVRMLGMQSVLWDRDFTD
WKVNAGIKTSPEVVVEEVQDWKLQGGGWGLILEHDTTIKTVNVGLDVAK

ALGPNQLTVAECVGQTQWYQDPARLGNEPRRGHRAASYQRS

>BAP71097.1 [*Kluyveromyces marxianus*]

MILTCWIVNIWISIVLVSAAAIDMLDVESNSLSISAEDASKEATLSEQSIFPI
GRSPRSQAKTPFFAWLTQFTGLTEWPGANPPYIPLDFIDFNKIEKYEPYKEGRC
NKNPRESCSFDCHKCVAHDDVHSCAKLSQTFDDGPSVHTLTLLDKLQHKTF
FTLGMNVVERPEIYHTVMERGHGHTWHPFLPSLTNEQIIAQLEWSIWAM
NATGNHLPKWWRPPYGGIDNRVRSIARQFGMQAVLWDHDTLDWLAASNPFQ
RTESAILKDVTEWKRTSNGLILEHDGSKKTVDIAMKVNDIIGSDQMTAAQCV
GGIDYIKEFKV

>NP_013411.1 [*Saccharomyces cerevisiae* S288C]

MRIQLNTIDLQCIIALSCLGQFVHAEANREDLKQIDFQFPVLERAATKTPF
PDWLSAFTGLKEWPGLDPPYIPLDFIDFSQIPDYKEYDQNHCDSPRDSCSFD
CHHCTEHDDVYTCSKLSQTFDDGPSASTTKLLDRLKHNSTFFNLGVNIVQHP
DIYQRMQKEGHLIGSHTWSHVYLPNVSNEKIIAQIEWSIWAMNATGNHTPKW
FRPPYGGIDNRVRAITRQFGLQAVLWDHDTFDWSLLLNDSVITEQEILQNVIN
WNKSGTGLILEHDSTEKTVDLAIKINKLIGDDQSTVSHCVGGIDYIKEFLS

>KJD58109.1 [*Bacillus amyloliquefaciens*]

MKWFCSICCMVLLAGGINSAEAVPNERINWGFKRSVNHQPPDAGRQLD
NLVKKYDAFYLGNTKEKTIYLTFDNGYENGYTPKVLDVLKKHHVTGTFFVTG
HFVKEQPDLIKRMSKEGHIIGNHSFHHPDLTTKTGDEIQDELDSVTEAVYNITG
KRDONLYLRPPRGVFSEYVLKETKRLGYQTVFWSVAFVDWKVNEQKGKQYAY
DHMIKQAHPGAIYLLHTVSKDNSEALDDAITDLKKEGYTFKSIDDLMFEEKEM

KLPRL

>WP_019713628.1 [*Bacillus spizizenii*]

MKWMCSICCAAVLLAGGAAQAGAVANEPINWGFKRSVNHQPPDAG
KQLNRLIEKYDAFYLGHTKEKTIYLTFDNGYENGYTPKVLDVLKKHRVT
GTFFVTGHFVKDQPELIK RMSDEGHIIGNHSFHHPDLTTKTADQIQDELDS
VNEEVYKITGEQDNLYLRPPRGVFSEYVLKETKRLGYQTVFWSVAFVDW
KINNQKGKKYAYDHMIKQAHPGAIYLLHTVSRDNAEALDDAITDLKKQ
GYTFKSLDDLMFEKEMKLPSL

>AFQ56715.1 [*Bacillus subtilis* QB928]

MAMKWMCSICCAAVLLAGGAAQAEAVPNEPINWGFKRSVNHQPPD
AGKQLNSLIEKYDAFYLGNTKEKTIYLTFDNGYENGYTPKVLDVLKKHR
VTGTFFVTGHFVKDQPQLIK RMSDEGHIIGNHSFHHPDLTTKTADQIQDE
LDSVNEEVYKITGKQDNLYLRPPRGVFSEYVLKETKRLGYQTVFWSVAF
VDWKINNQKGKKYAYDHMIKQAHPGAIYLLHTVSRDNAEALDDAITDL
KKQGYTFKSIDDLMEFEKEMRLPSL

>pdb:1W1A [*Bacillus subtilis*]

GPLGSVLLAGGAAQAEAVPNEPINWGFKRSVNHQPPDAGKQLNSLI
EKYDAFYLGNTKEKTIYLTFDNGYENGYTPKVLDVLKKHRVTGTFFVTG
HFVKDQPQLIK RMSDEGHIIGNHSFHHPDLTTKTADQIQDELDSVNEEVY
KITGKQDNLYLRPPRGVFSEYVLKETKRLGYQTVFWSVAFVDWKINNQK
GKKYAYDHMIKQAHPGAIYLLHTVSRDNAEALDDAITDLKKQGYTFKSI
DDLMEFEKEMRLPSL

>NP_001342829.1 [*Schizosaccharomyces pombe*]

MYETRDLTGNAGKPVDNTPWPNNNSKIAVSFVVNYEEGGERSLLYEDEGF
ETFLTEAGLMPFPNRPVRERSIESCFEYGSRCGFWRILNLFKKHKVPFTCWAIG
QAVEKNPVVVGAMEEAGCEVGSHSRWINYEGVPPETEYEHKKSVQAIQKA
SPSNSAPRSWYTGRASLNTRKLVCQVYKDLGLPQPFDSDEYNDDYPYWVAD
PLASKPGAEDDKGLLIVPYTLEVNDMKYAVAPGFCNSDDFYTYARDAFDVLY
EEGLEGAPKMMTIGLHCRLTGRPGRFRGLQKLMEHITSKEGVWVATREQIAQ
AWSAKHPYKA

>pdb:3CL6 [*Pseudomonas fluorescens*]

MSVDYPRDLIGYGSNPPHPHWPGKARIALSFVLNYEEGGERNILHGDKE
SEAFLSEMVSAQPLQGERNMSMESLYEYGSRAGVWRILKLFKAFDIPLTIFAVA
MAAQRHPDVIRAMVAAGHEICSHGYRWIDYQYMDEAQEREHMLEAIRITEL
TGERPLGWYTGRTGPNTRRLVMEEGGFLYDCDTYDDDLPYWEPNNPTGKPH
LVIPYTLDTNDMRFTQVQGFKGDDFFEYLKDAFDVLYAEGAEAPKMLSIGL
HCRLIGRPARLAALQRFIEYAKSHEQVWFTRRVDIARHWHATHPYTGAAK

>ARW18113.1 [*Komagataeibacter europaeus*]

MSGTGVYPRDLVGYGGNPPDAKWPGGARIAVQFVINYEEGAENSVLHG
DRGSEAFLSEMIGAQSITGARAIAMESLYEYGSRAGFWRLHRIFTQRKQPLTVF
GVAAAMARNPAAVAAMKEAGWEIASHGLRWIDYQHVPEAVERAHIQECIAL
HTKLTGSRPLGWYQGRTSPNTARLIAEEGGFVYDADSYADDLPYYDRSHGRA
QLIVPYTLDVNDMRFVAINGFTEGEQFFTYLRDTFDELYEEGADRPRMMSVG
LHCRVAGRPGRARAVARFLDYIATREKVWVVTRLDIARHWLETHPA

>CDI21894.1 [*Klebsiella pneumoniae*]

MGENQEHYPRDLRGYAGEPPHARWPGGARIAVQFVLNYEEGAENH
VLHGDAGSEQFLSDIIGAASYPARHMSMDSLYEYGSRAGFWRIHREFSQR
GLPLTVFGVAMALARHPEIVAAIKAADYDVVSHGWRWIHYQHMDIAEER
EHLQKAVQVLTDLFGKPPTGWYTGRDSPNTRQLVVEHGGFDYDSYYG
DDLFWSEVACSDGSQRPHLIVPYTLDANDMRFATAQGFNTAEQFYTYL
KDSFDVLYAEGETAPKMMSVGMHCRLGRPGRFRALQRFLDYIQQHDK
VWVCTRQQIADHWRETHPYRG