

Supplementary Material

Enzymatic Properties of *Bacillus velezensis* YB1534 Chitosanase and Antibacterial Activity of Its Oligosaccharide Products

Yiwei Dai^{1,*}, Huiru Zhao¹, Yingxi Chen¹, Xinping Lin¹, Sufang Zhang¹, Chaofan Ji¹

1 State Key Laboratory of Marine Food Processing & Safety Control, National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian, Liaoning, 116034, China

* Corresponding author:

Yiwei Dai, Tel.: +86-182-6228-4612; E-mail address: ywdai6228@126.com

Table S1. 16S rDNA sequence-based identification of chitosan-degrading strains isolated from shrimp samples.

Strains	Scientific name	Identity
XX-1	<i>Acinetobacter calcoaceticus</i> strain A46	100.00%
XX-2	<i>Acinetobacter baumannii</i> strain B	99.86%
XX-3	<i>Acinetobacter sp.</i> strain TH-S-11-1	99.86%
XX-4	<i>Bacterium</i> strain QLS201807OPB3	99.79%
XX-5	<i>Bacterium</i> strain XFZ4	99.65%
XX-6	<i>Bacterium</i> strain BS0171	99.72%
XX-7	<i>Bacterium</i> strain BS1876	99.51%
XX-8	<i>Bacterium</i> strain BS0508	100.00%
XX-9	<i>Cedecea davisae</i> DSM 4568	99.63%
XX-10	<i>Bacterium</i> strain WG90917	99.79%
YB1534	<i>Bacillus velezensis</i> strain K-2	99.93%
YB1536	<i>Bacillus amyloliquefaciens</i> strain B10	100.00%
YB1531	<i>Bacillus velezensis</i> strain B49	100.00%
YB2433	<i>Bacillus amyloliquefaciens</i> subsp. <i>plantarum</i> strain HK-2-26	100.00%
YB1514	<i>Bacillus cereus</i> strain BA6-1	100.00%
MJD11B	<i>Aspergillus minisclerotigenes</i>	99.19%
MJD28B	<i>Aspergillus oryzae</i> isolate TURS11	99.67%

Table S2. Comparison of enzymatic properties of chitosanases from different sources.

Chitosanase source	Molecular weight	Optimal temperature	Optimal pH	Enzyme activity	Minimum DP of products	Antimicrobial activity of products (MIC)	Reference
<i>B. paramycoides</i> BP-N07	37 kDa	50°C	6.0	8133.17±47.83 U/mg	2	-	[14]
<i>Kitasatospora setae</i> KM-6054	31.07 kDa	60°C	5.0	241.39 U/mg	2	-	[16]
<i>Aquabacterium</i> sp. A7-Y	50.7 kDa	40°C	5.0	18 U/mg	3	<i>Magnaporthe oryzae</i> <i>Fusarium oxysporum</i>	[26]
<i>B. thuringiensis</i> var. <i>dendrolimus</i> B-387	40 kDa	55°C	6.5	-	2	<i>E. coli</i> (70 ± 8 µg/mL) <i>Pseudomonas aeruginosa</i> (> 2000 µg/mL) <i>Enterobacter cloacae</i> (60 ± 3 µg/mL) <i>B. cereus</i> (1610 ± 150 µg/mL)	[24]
<i>Trichoderma harzianum</i>	42 kDa	-	-	2000 U/g	Average Mw 6-	<i>S. epidermidis</i> (62.5 µg/mL)	[27]
<i>Myceliophthora thermophila</i>	43 kDa			1500 U/g	21 kDA	<i>E. coli</i> (> 500 µg/mL)	
Commercial chitosanase	-	50 °C	5.2	102.53 U/g	2	<i>Salmonella</i> (64 mg/mL)	[28]

Commercial chitosanase from	-	-	5.7	-	-	<i>Campylobacter jejuni</i>	[29]
<i>Streptomyces griseus</i>							
<i>B. velezensis</i> YB1534	31 kDa	50°C	6.0	338.2 U/mg	2	<i>E. coli</i> (0.625 mg/mL)	This study
						<i>S. aureus</i> (0.313 mg/mL)	
						<i>S. typhi</i> 50071 (1.25 mg/mL)	
						<i>A. hydrophila</i> (0.625 mg/mL)	

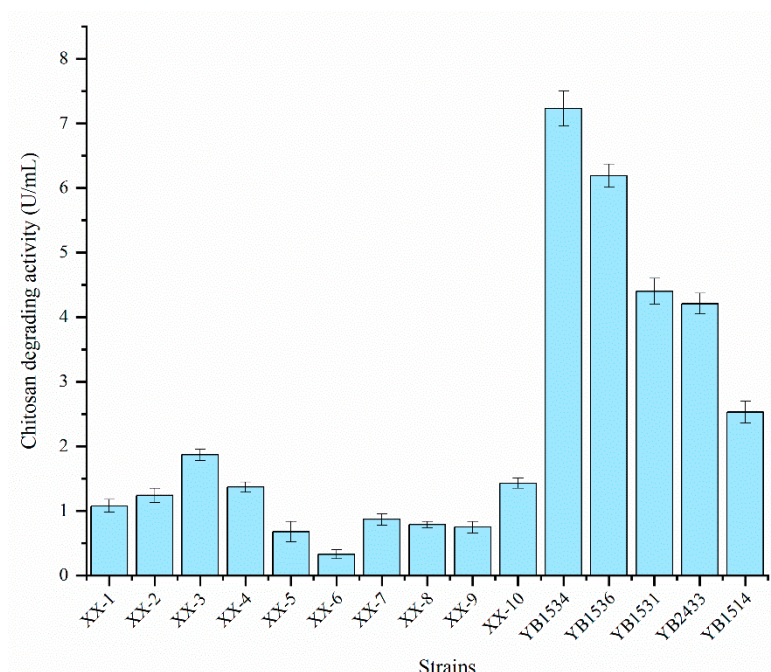


Figure S1. The chitosanase activities of isolated strains.

ORIGIN

```

1      ATGAAAATCA GCTTGAAGAA AAAAGCAGGT TTTTGAAGA AGACGGCGGT TTCGTCACCT
61     ATTTTCACCA TGTTTTTAC CCTGATGATG AGCGGTACGG TTTTTCGCGC CGGGCTGAAT
121    ACGGATCAGA AGCGCCGGGC GGAACAGCTG ACCAGCATCT TTGAAAACGG AAAGACGGAA
181    ATCCAATACG GATATGTTGA AGCGTTGGAT GACGGAAGAG GTTACACTTG CGGGCGGGCC
241    GGCTTTACGA CGGCTACCGG AGATGCGCTG GAAGTAGTCG AAGTATACAC GAAAGCGGTG
301    CCGAATAACA AATTGAAAAA GTATTGCGCT GAATTGCGGC GTCTTGCGAA GGACGAAAGC
361    GATGACATCA GCAATCTGAA AGGATTGCTT TCTGCGTGGC GCTCACTTGG CAATGATAAA
421    GCCTTCCGCG CTGCCCAAGA TAAGGTAAAT GACAGCTTGT ATTATCAGCC GCGGATGAAA
481    CGTTCAGAAA ATGCCGGACT GAAAACGGCC TTGGCAAAAG CAGTGATGTA CGATACGGTG
541    ATTCAGCATG GCGACGGCGA TGATCCAGAC TCCTTTTATG CCCTGATTAA ACGCACGAAC
601    AAAAAAATGG GCGGGTCACC GAAAGACGGA ACTGACGAGA AGAAATGGCT CAATAAATTG
661    TTGGATGTGC GCTATGACGA TCTGATGAAT CCGTCAGATG AGGACACTCA GGATGAATGG
721    AGAGAATCGG TTGCCCGTGT CGACGTTTTC CGCGATATTG TCAAAGAGAA GAACTACAAT
781    TTAAACGGGC CGATTCATGT CCGGTCAAGC GAATACGGTA ATTTCACTAT TCAATAA

```

ORIGIN

```

1      MKISLKKKAG FWKKTAVSSL IFTMFFTLMM SGTVFAAGLN TDQKRRAEQL TSIFENGKTE
61     IQYGYVEALD DGRGYTCGRA GFTTATGDAL EVVEVYTKAV PNNKLKKYLP ELRRLAKDES
121    DDISNLKGFA SAWRSLGNDK AFRAAQDKVN DSLYYQPAMK RSENAGLKTA LAKAVMYDTV
181    IQHGDGDDPD SFYALIKRTN KKMGGSPKDG TDEKKWLNKF LDVRYDDL MN PSDEDTQDEW
241    RESVARVDVF RDIVKEKNYN LNGPIHVRSS EYGNFTIQ

```

Figure S2 The DNA sequences and the amino acids sequence of ByChi used in this study (the sequence in red represents the signal peptide).



Figure S3 The predicted signal peptide by SignalP analysis.

Reference

- [14] Wang, Y., Mo, H., Hu, Z., Liu, B., Zhang, Z., Fang, Y., Hou, X., Liu, S., & Yang, G. Production, characterization and application of a novel chitosanase from marine bacterium *Bacillus paramycoides* BP-N07. *Foods* 2023, 12, 3350.
- [16] Xu, Y., Wang, H., Zhu, B., & Yao, Z. Biochemical characterization and elucidation action mode of a new endolytic chitosanase for efficient preparation of chitosan oligosaccharides. *Biomass Convers Bior* 2024, 14, 18897-18905.
- [24] Aktuganov, G. E., Safina, V. R., Galimzianova, N. F., Gilvanova, E. A., Kuzmina, L. Y., Melentiev, A. I., Baymiev, A. H., & Lopatin, S. A. Constitutive chitosanase from *Bacillus thuringiensis* B-387 and its potential for preparation of antimicrobial chitooligomers. *World J Microb Biot* 2022, 38, 167.
- [26] Wang, Y., Li, D., Liu, M., Xia, C., Fan, Q., Li, X., Lan, Z., Shi, G., Dong, W., Li, Z., & Cui, Z. Preparation of active chitooligo-saccharides with a novel chitosanase Aq CoA and their application in fungal disease protection. *J Agric Food Chem* 2021, 69, 3351-3361.
- [27] Khayrova, A., Lopatin, S., Shagdarova, B., Sinitsyna, O., Sinitsyn, A., & Varlamov, V. Evaluation of antibacterial and antifungal properties of low molecular weight chitosan extracted from *Hermetia illucens* relative to crab chitosan. *Molecules* 2022, 27(2).
- [28] Zhao, X. P., Liu, J., Sui, Z. J., Xu, M. J., & Zhu, Z. Y. Preparation and antibacterial effect of chitooligosaccharides monomers with different polymerization degrees from crab shell chitosan by enzymatic hydrolysis. *Biotechnol Appl Bioc* 2022, 70(1), 164-174.
- [29] Mengíbar, M., Ganan, M., Miralles, B., Carrascosa, A. V., Martínez-Rodríguez, A. J., Peter, M. G., & Heras, A. Antibacterial activity of products of depolymerization of chitosans with lysozyme and chitosanase against *Campylobacter jejuni*. *Carbohydr Polym* 2022, 84(2), 844-848.