



- Gene cloning, characterization, and molecular
- 2 simulations of a novel recombinant chitinase from
- 3 Chitinibacter tainanensis CT01 appropriate for chitin
- 4 enzymatic hydrolysis

5 Yeng-Tseng Wang^{1,2,3,4} and ^{5*} and Po-Long Wu⁶

- ¹ Department of Biochemistry, College of Medicine, Kaohsiung Medical University, Kaohsiung City 80708, Taiwan, ROC
- 8 ² Drug Development and Value Creation Research Center, Kaohsiung Medical University, Kaohsiung, 7 Taiwan, ROC
- 10 ^{3.} Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC
- 11 ⁴ Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, ROC
- 12 ⁵ School of Post-Baccalaureate Medicine, College of Medicine, Kaohsiung Medical University, Taiwan
- Biotech Business Center, Refining & Manufaturing Research Institute, CPC Corporation, Chiayi City 60051,
 Taiwan, ROC. 078760@cpc.com.tw
- 15 * Correspondence: c00jsw00@kmu.edu.tw or c00jsw00@gmail.com; Tel.: +886- 07-3121101
- 16 Received: 07 June 2020; Accepted: 20 July 2020; Published: date





Figure 1. The phylogenetic tree with the 12 chitinase-encoding genes sequences of Chitinibactertainanensis CT01 and the other chitinase genes sequences.

M S H F N R F A V A A I P A A L M A V A S F S Y A A D A W K E G tcgacctataacgcaggcactgttgtttcatatgccggtaacgactaccaagctctggtgactcacactgcctacgtgggcgctaactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggaaccctggtgacctactggtgacctggtgacctactgS T Y N A G T V V S Y A G N D Y Q A L V T H T A Y V G A N W N P A S T P T L W K L V A T G S A T P A P T A T P V T T A K P T T A ccaacaactgcgccaaccaccgcaccgacaactgctccaactaccgcgccaactactggcccagtagcaggttgtggctctgtagcggcttggaatPTTAPTTAPTTAPTTAPTTGPVAGCGSVAAWN S T A A Y S G G A V V A Y N G G K Y S A K W W T Q G Q A P S A T D Q W G P W K Y E G E C G P A V T A T P T V A P T P T P V G M T ccagcaccaaccgtagcgccaactccaactgcagttccaactgcagcgccaacaccagttgctaccttggctccaggtcaagaagtgcctccacctgcagcgccaactgcagttgctacctgcagcgccaactgcagttgctacctgcagcgccaactgcagttgctacctgcagcgccaactgcagttgctacctgcagcgccaactgcagttgctacctgcagttgcagttgccagttgcagttgccagttgcagttgcagttgccagttgcagtP A P T V A P T P T A V P T A A P T P V A T L A P G Q E V P P P A Q A Q V G S Y F T Q W G V Y G R D Y Q V A D I I S S G A A Q Q ctgacette at caacet acgette cgg taacate taccagaaa aatgg tgg tacgg tg gg ta cgg tg accaa actgg aaccagg tg caactga tg accaa actgg aaccagg tg caactga tacgg tg accaa actgg aaccagg tg caactga tacgg tg accaa actgg accagg tg accag tg accagg tg accag tg acL T F I N Y A F G N I Y Q K N G G Y E C G I V N K L E P G A T D A N A P G A G T G G D A W A D F G L T A K R R V D P A D Q I K W D D K L A G N F R E F Q A Y K K K F P D T K L F I S L G G W T W tcgaaatggttctetgctgcatcgaaaaccgacgcctgcgtaaacagctggttaaatcgtgtatcgacatctacatcaaaggtaacttgccagtgtaaggtaactgccagtgtaaggtaactgccagtgtaaggtaactgccagtgtaaggtaactgccagtgtaaggtaactgccagtgtaaggtaactgccagtgtaaggtaggtaaggtaggtaaggtaggtaaggtaggtaaggtaggtaaggtaggtaaggtaggtaaggtaggtaggtaaggtS K W F S A A S K T D A L R K Q L V K S C I D I Y I K G N L P V V D G R G G A G S A A N I F D G I D I D W E F P G V Q G V G Y N T V A P E D K Q N F T L L L A E F R K Q L D E L A A A N Q K K Y Y L T V A I G V G R D K I E M T E P R E Y A R Y L D W I N M M T Y D Y N G G W N A Q G P T D F Q S H L F A D P S N P Q Y K C S G a a a c c a g c c g a c c a g c t g a c c g c g g g g c c t g g t g t t t a c t a c a a c a c c g a t g a c c g g t g a c c g g t g a c c g g t g a t c c g g t g a t c c g g t g a t c c g t g a t c c a g c t g g t g a t c cK P A D K C Y G D R S L V S Y Y N T D D A V N L L I Q A G V N P K K L V V G I P K Y G R G W T G V T N V N N G L Y Q K A T D A A R G T Y E K G I E D F K V L K N A A G T V Y V H P V T K Q S Y K F D G S T F W S Y D T P E V I Q T K I D Y A K A K G L N G G V F SWSLDGDDSAATLSKAMGKARQKGELNSKLEG aagcctatccctaaccctctcctcggtctcgattctacgcgtaccggtcatcatcaccatcacK P I P N P L L G L D S T R T G H H H H H

20

21

Figure 2. Amino acid and DNA sequence of chitinase 1198.



22

23

24

Figure 3. (A) Secondary structures of chitinase 1198. (B) Predicted 3D structures of chitinase 1198.



25 **Figure 4.** Multiple sequence alignment of the active site (green box: DXXDXDXE one letter amino acid

- 26 motif) of the deduced protein sequence of chitinase 1198 with chitinase from Arthobacter (PDB ID:
 27 1KFW), (PDB ID: 4W5U), Bacillus circulans (PDB ID: 1ITX), Serratia marcescens (PDB ID:2WLY) and
- 28 chitinase from nematophagous fungus (PDB ID: 3G6L).



Figure 5. Comparing the Chi1198 3D structures (colored in green) with chitinases. from Arthobacter
 (PDB ID: 1KFW and colored in purple), (PDB ID: 4W5U and colored in gray), Bacillus circulans (PDB
 ID: 1ITX and colored in cyan), Serratia marcescens (PDB ID:2WLY and colored in tints) and chitinase

33 from nematophagous fungus (PDB ID: 3G6L and colored in blue).



34

29

Figure 6. Chi1198 with chitin oligomers (repeat unit: 12) and Family 18 chitinase: DXXDXDXE amino acid
 motif. (The distance between the Asp440 of chitinase1198 and the nitrogen atom of the 2nd repeat unit
 from the nonreducing end).



39 Figure 7. Chi1198 with chitin oligomers (repeat unit: 12) and Family 18 chitinase: DXXDXDXE amino acid 40 motif. (The distance between the Asp440 of chitinase1198 and the nitrogen atom of the 3rd repeat unit 41





motif. (The distance between the Asp440 of chitinase1198 and the nitrogen atom of the 2nd repeat unit

42

38

43 44

45



from the nonreducing end).

© 2018 by the authors. Submitted for possible open access publication under the terms and Attribution (CC license conditions of the Creative Commons BY) (http://creativecommons.org/licenses/by/4.0/).