

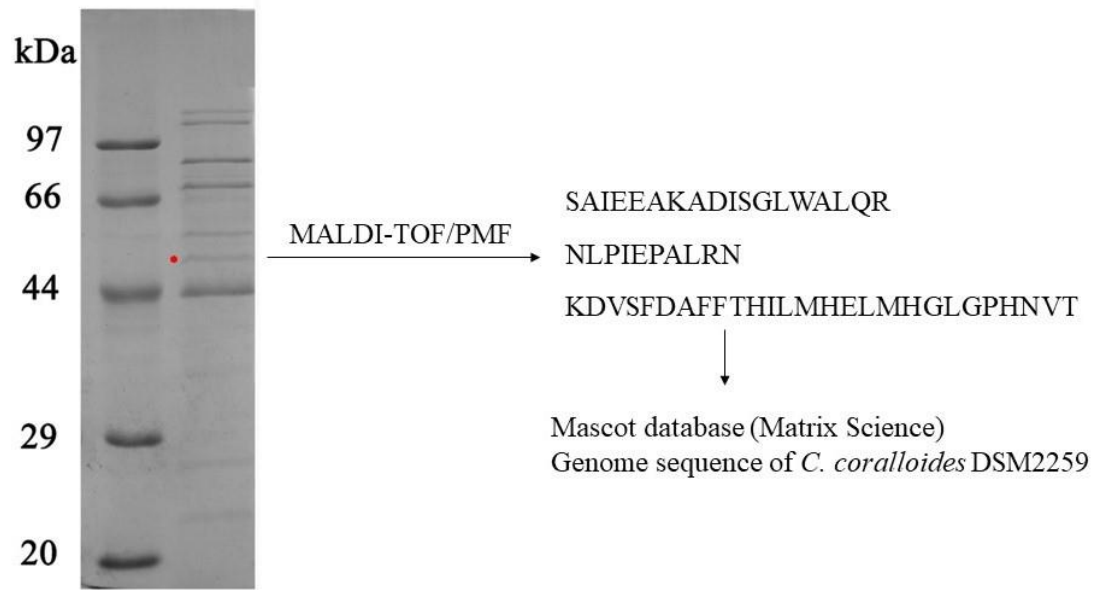
# **Structural and Functional Characterization of a New Bacterial Dipeptidyl Peptidase III Involved in Fruiting Body Formation in Myxobacteria**

**Si-Bo Chen<sup>1</sup>, Han Zhang<sup>1</sup>, Si Chen<sup>1</sup>, Xian-Feng Ye<sup>1</sup>, Zhou-Kun Li<sup>1</sup>, Wei-Dong Liu<sup>2</sup>, Zhong-Li Cui<sup>1</sup> and Yan Huang<sup>1,\*</sup>**

<sup>1</sup> Key Laboratory of Agricultural Environmental Microbiology, Ministry of Agriculture and Rural Affairs, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China

<sup>2</sup> Industrial Enzymes National Engineering Laboratory, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

\* Correspondence: [huangyan@njau.edu.cn](mailto:huangyan@njau.edu.cn)

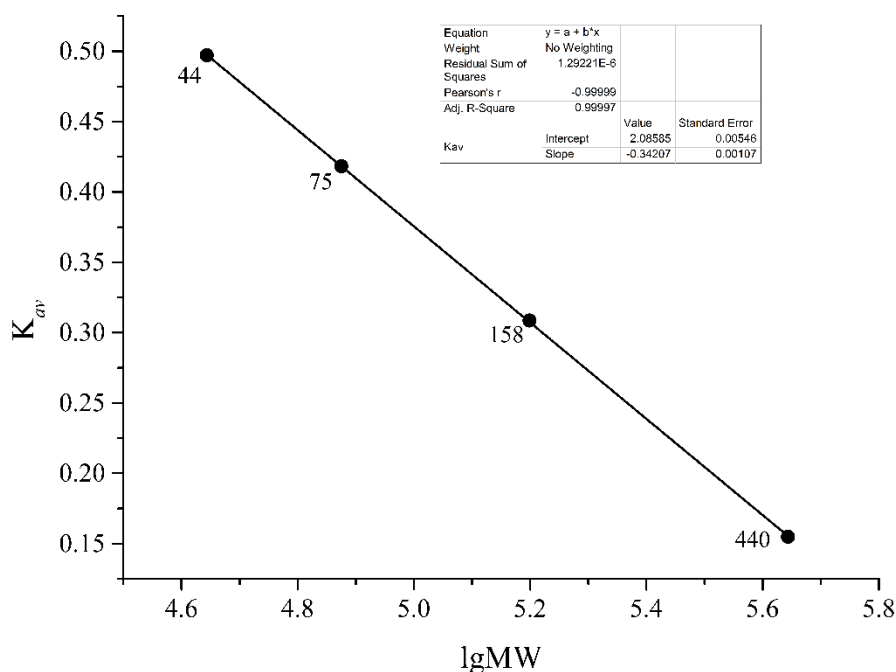


**Figure. S1** The MALDI-TOF result of the extracellular protein from *Coralloccoccus* sp. EGB

[illegible]

[illegible]

**Figure. S2** Multiple sequence alignment of DPP III from various species. The organisms and UniProt sequence identifiers are *Monodelphis domestica* (F6ZXH8), *Sarcophilus harrisii* (A0A7N4P2W4), *Homo sapiens* (Q9NY33), *Rattus norvegicus* (O55096), *Crotalus adamanteus* (J3RZ30), *Xenopus laevis* (Q6DE90), *Salmo salar* (B5X435), *Drosophila mojavensis* (B4K999), *Glossina morsitans* (D3TMQ7), *Daphnia pulex* (E9GTX0), *Necator americanus* (W2SLT7), *Caenorhabditis elegans* (G5ECW7), *Syphacia muris* (A0A0N5AXJ5), *Schizophyllum commune* (D8PVF6), *Saccharomyces cerevisiae* (Q08225), *Aureobasidium pullulans* (A0A074XX23), *Flavobacteriales bacterium* (A4AQA4), *Croceibacter atlanticus* (A3U8C5), *Bacteroides cellulosilyticus* (E2NMV7), *Prevotella marshii* (E0NQE0), *Bacteroides thetaiotaomicron* (A0A139KC05), *Porphyromonas gingivalis* (Q7MX92), *Anaeromyxobacter dehalogenans* (Q2IN78), *Corallococcus* sp. EGB (A0A5H1ZR28), *Caldithrix abyssi* (H1XW48), *Setaria italica* (K3YPW2), *Physcomitrella patens* (A9TLP4), *Arabidopsis thaliana* (Q8L831). Dots indicate gaps. Identical amino acid residues at the same position are shaded.



**Figure. S3** The calibration curve  $\lg MW$  vs  $K_{av}$ . The equation is  $K_{av} = 2.08585 - 0.34207 \lg MW$ , where the  $K_{av}$  is distribution coefficient,  $MW$  is the molecular weight of the standard samples. The  $V_e$ ,  $K_{av}$  and  $\lg MW$  for CoDPP III is 71.95 mL, 0.3513 and 5.0708, respectively.

**Table S1** Effect of metal ions on CoDPP III activity

Metal ions	Relative activity (%)		
	0.1 mM	0.5 mM	1.0 mM
No addition	100	100	100
Zn <sup>2+</sup>	78.15±0.6	10.6±0.1	-
Co <sup>2+</sup>	141.06±1.2	121.19±2.2	105.2±2.4
Mn <sup>2+</sup>	129.14±3.3	111.92±2.1	98.92±2.1
Mg <sup>2+</sup>	119.21±1.6	129.8±2.7	109.7±1.5
Ba <sup>2+</sup>	121.19±0.9	123.18±1.3	105.93±1.2
Ca <sup>2+</sup>	116.56±0.8	121.19±2.1	114.21±2.1
Cu <sup>2+</sup>	4.64±0.1	-	-
Ni <sup>2+</sup>	7.95±0.2	5.96±0.1	-

**Table S2** Strains and plasmids used in this study

Strains and plasmids	Relevant genotype or characteristic(s)	Reference
<i>Corallococcus</i> sp. EGB	wild type	[45]
<i>E. coli</i>		
<i>E. coli</i> BL21(DE3)	F- <i>ompT hsdS</i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ) <i>gal dcm lacY1</i> (DE3)	Novagen
<i>E. coli</i> B834(DE3)	F- <i>ompT hsdS</i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ) <i>gal dcm met</i> (DE3)	Novagen
<i>M. xanthus</i>		
DK1622	wild type	[46]
DK1622- <i>Codpp</i> III	<i>attB</i> ::pET29a- <i>attP</i> - <i>Codpp</i> III	This study
DK1622-Δ <i>asgA</i>	DK1622 mutant with <i>asgA</i> gene disrupted	This study
<b>Plasmids</b>		
pET29a	pBR322 ori, Kan <sup>r</sup>	Novagen
pBJ113	pBR322 ori, galK; Kan <sup>r</sup>	[47]
pET29a- <i>Codpp</i> III	<i>Codpp</i> III inserted into <i>Nde</i> I/ <i>Xho</i> I site of pET29a	This study
pET29a- <i>attP</i>	pET29a derivative carrying the MX8 <i>attP</i> region, Kan <sup>r</sup>	This study
pET29a- <i>attP</i> - <i>Codpp</i> III	A 2.7 kb fragment containing <i>Codpp</i> III gene and its promoter inserted into <i>Hind</i> III/ <i>Xho</i> I site of pET29a- <i>attP</i>	This study
pBJ113- <i>asgA</i>	1073 bp internal fragment of <i>asgA</i> inserted into <i>Hind</i> III/ <i>Eco</i> R I site of pBJ113	This study