# Supporting information of " Identification of the Catalytic Triad of Family S46 Exopeptidases, Closely Related to Clan PA Endopeptidases "

Yoshiyuki Suzuki<sup>1</sup>, Yasumitsu Sakamoto<sup>2</sup>, Nobutada Tanaka<sup>3</sup>, Hirofumi Okada<sup>1</sup>, Yasushi Morikawa<sup>1</sup> & Wataru Ogasawara<sup>1</sup>

<sup>1</sup>The Department of Bioengineering, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan, <sup>2</sup>School of Pharmacy, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba, Iwate 028-3694, Japan and <sup>3</sup>School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.

#### **Supplementary Figures**



Supplemental Figure S1. Phylogenetic tree of 16S rRNA from related species of *Pseudoxanthomonas*. The phylogenetic tree of 16S rRNA was constructed by MUSCLE multiple sequence alignment and phylogenetic analysis by maximum likelihood. Bootstrap values above 50% are shown at branch points.



**Supplemental Figure S2. Circular dichroism spectra.** (A) Spectra of DAP BII and mutants were obtained with a circular dichroism spectrophotometer. The spectra predict an alpha-helical content of about 47% for these proteins. (B) Spectra of DAP BII wild-type and alpha-chymotrypsin (*Bos taurus*).

## Supplementary Tables

Supplemental Table S1. DNA/DNA hybridization					
·	% homology with				
	WO24 <sup><i>a</i></sup>	P. mexicana <sup>a</sup>	P. japonensis <sup>a</sup>		
Strain WO24 <sup>b</sup>	100	100	59		
Pseudoxanthomonas mexicana JCM11524 <sup>b</sup>	71	100	59		
Pseudoxanthomonas japonensis JCM11525 <sup>b</sup>	46	59	100		

### Supplemental Table S1. DNA/DNA hybridization

*a* filter-bound

b labeled

Strain	Pseudoxanthomonas mexicana WO24
Sampling place	A manufactory of tofu
Morphology	Rod
Length	2.0 - 3.0 μm
Width	0.7 μm
Motility	-
Spore formation	-
Gram-reaction	-
Oxidase	+
Catalase	+
Urease	+
Nitrate reductase	+
Indole formation	-
Starch hydrolysis	-
Acid from glucose	+
Oxidative-fermetative test	+
Growth temperature	Growth between 12 - 42 °C
GC content	67.9 mol%

Supplemental Table S2. Morphological and physiological properties of *Pseudoxanthomonas* mexicana WO24

Mutation	Primer sequences (5'→3')			
	Front	Reverse		
For Mutan-Super Express Km (5' end is phosphorylated)				
pK8b2-SacI	GCCGtCCAGAgCtCCCAGG			
pK8b3-SacI	AGGCCGaGCtcGtCGCGCC			
1stMet-NdeI	AGGAAACACATAAAcatATGCGCCACCCCGC			
For PrimeSTAR Mutagenesis Basal Kit				
H86A	CAACCATgcCTGCGCCTATGGCGCCAT	GCGCAGgcATGGTTGGTGACCACCAG		
S657A	CGGCAACgCCGGTTCGCCGGTGCTC	GAACCGGoGTTGCCGCCGGTGATGTCC		
D195A	ATCAAGGcCGTGCGCCTGGCCTACG	GCGCACGgCCTTGATCTCCAGGTTCT		
D214A	GACATCGcCAACTGGATGTGGCCGCG	CCAGTTGgCGATGTCGCCGCCGAACT		
D224A	CGGCGcCTTCGCGTTCTACCGCGC	AACGCGAAGgCGCCGGTGTGGCGC		
D522A	AGCAACGeTCCGGCCATCCAGTATGCG	GGCCGGAgCGTTGCTGGCTTCGAAC		
D574A	TATCCCGeTGCCAACCTGTCGCTGC	GTTGGCAgCGGGATAGACGAACTCGC		
D195N	ATCAAGaACGTGCGCCTGGCCTACG	GCGCACGTtCTTGATCTCCAGGTTCTTG		
D214N	CGACATCaACAACTGGATGTGGCCGC	CAGTTGTtGATGTCGCCGCCGAACTTG		
D224N	CACCGGCaACTTCGCGTTCTACCGCGCC	GCGAAGTtGCCGGTGTGGCGCGGCCT		
D522N	CAGCAACaATCCGGCCATCCAGTATGC	GCCGGATtGTTGCTGGCTTCGAACGC		
D574N	CTATCCCaATGCCAACCTGTCGCTGC	TTGGCATtGGGATAGACGAACTCGCCC		

#### Supplemental Table S3. Primers used in this study