

Supplementary Material

A new and promiscuous α/β hydrolase from *Acinetobacter tandoii* DSM 14970^T inactivates the mycotoxin ochratoxin A

Ana Sánchez-Arroyo¹, Laura Plaza-Vinuesa¹, María Claudia Abeijón-Mukdsi¹, Blanca de las Rivas¹, José Miguel Mancheño^{2,*}, and Rosario Muñoz^{1,*}

¹ Institute of Food Science, Technology and Nutrition (ICTAN), CSIC, José Antonio Novais 6, 28040 Madrid, Spain

² Department of Crystallography and Structural Biology, Institute of Physical Chemistry Blas Cabrera, CSIC, Serrano 119, 28006 Madrid, Spain

*Corresponding authors:

Rosario Muñoz
r.munoz@csic.es

José Miguel Mancheño
jm.mancheno@csic.es

Figure S1

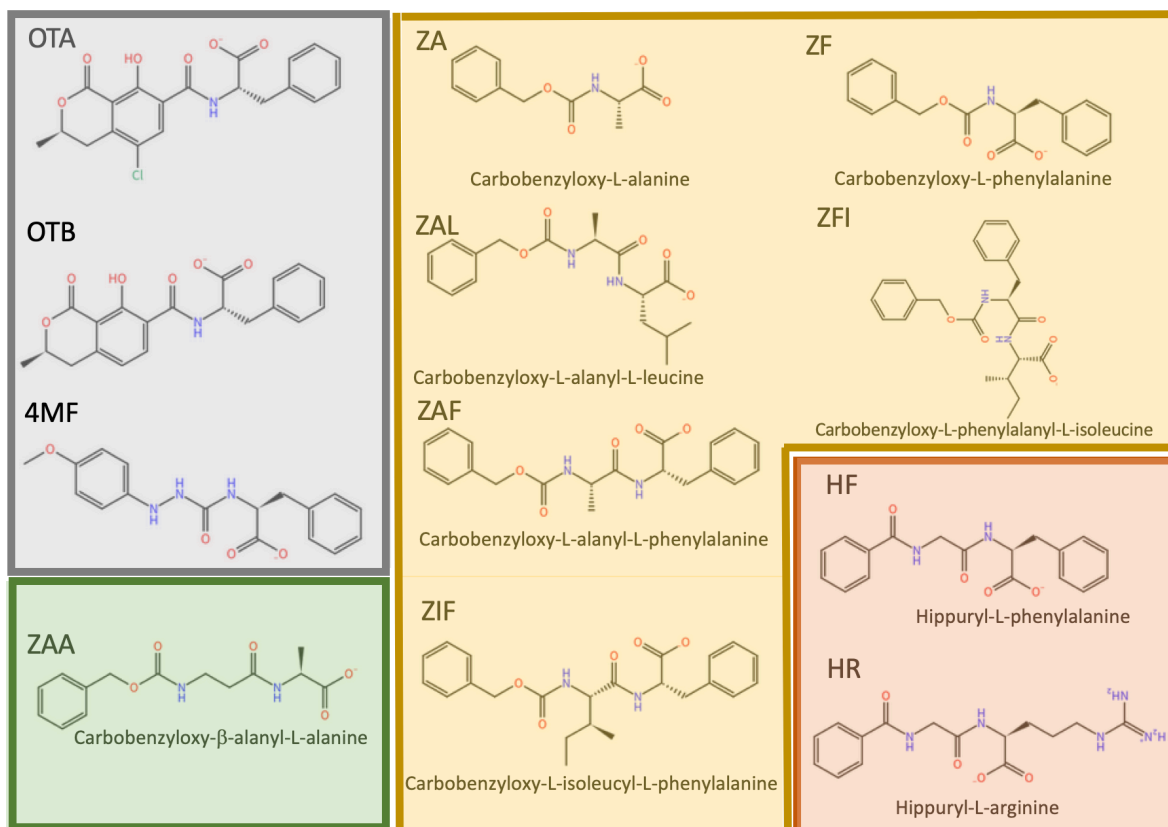


Fig. S1 2D structures of the synthetic carboxypeptidase substrates used in this work together with the structures of ochratoxins A and B, and its analog *N*-(4-methoxyphenylazoformyl)-phenylalanine (4MF).

Figure S2

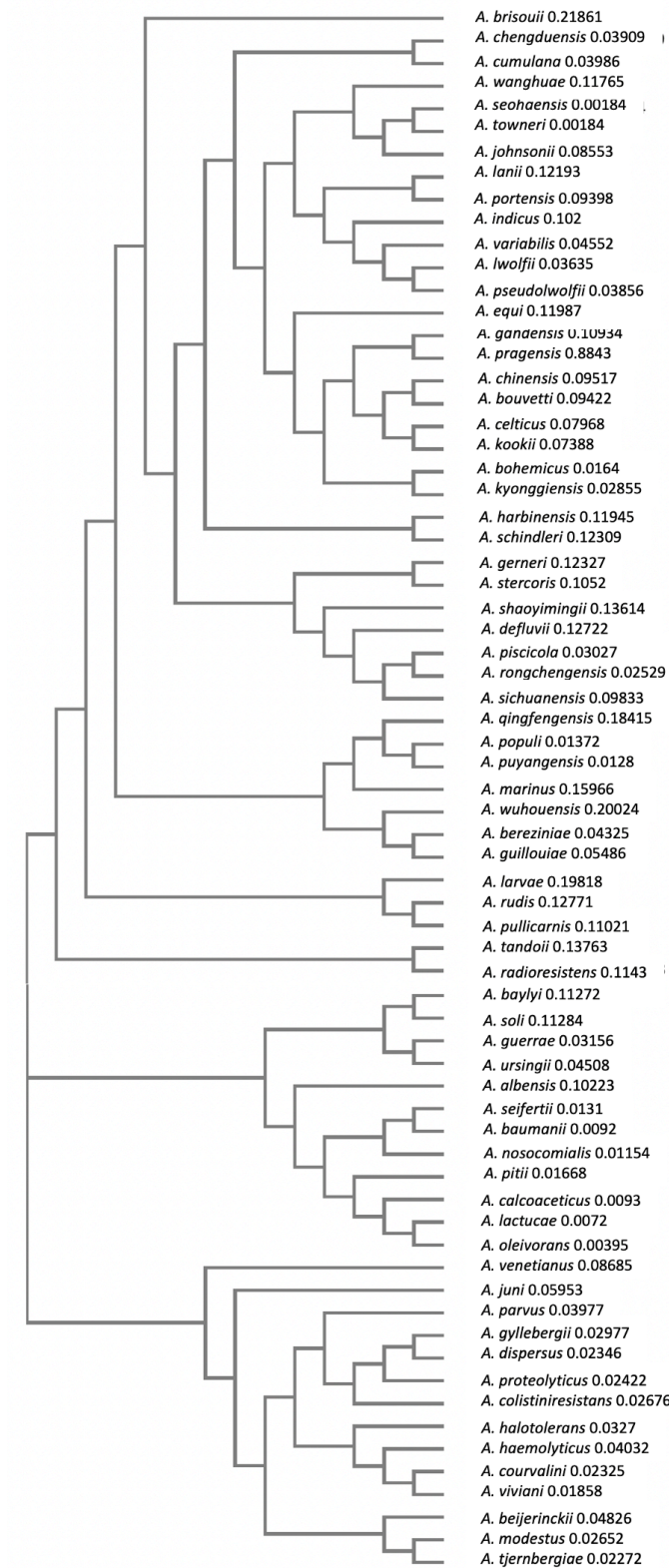


Fig. S2 Cladogram showing the distribution of the *AtABH* hydrolase within the *Acinetobacter* genus (69 different species). Distances between sequences were calculated with Clustal Omega Software.

Figure S3

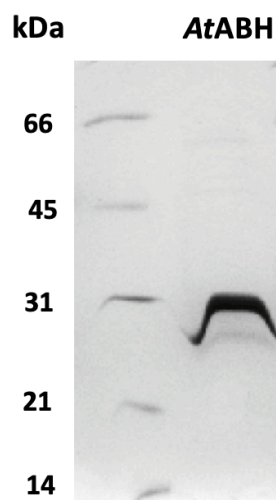


Fig. S3 SDS-PAGE analysis of the purification of *AtABH* recombinant protein. The 12.5% gel was stained with Coomassie blue. Molecular mass markers are located on the left (SDS-PAGE standards, Bio-Rad).

Figure S4

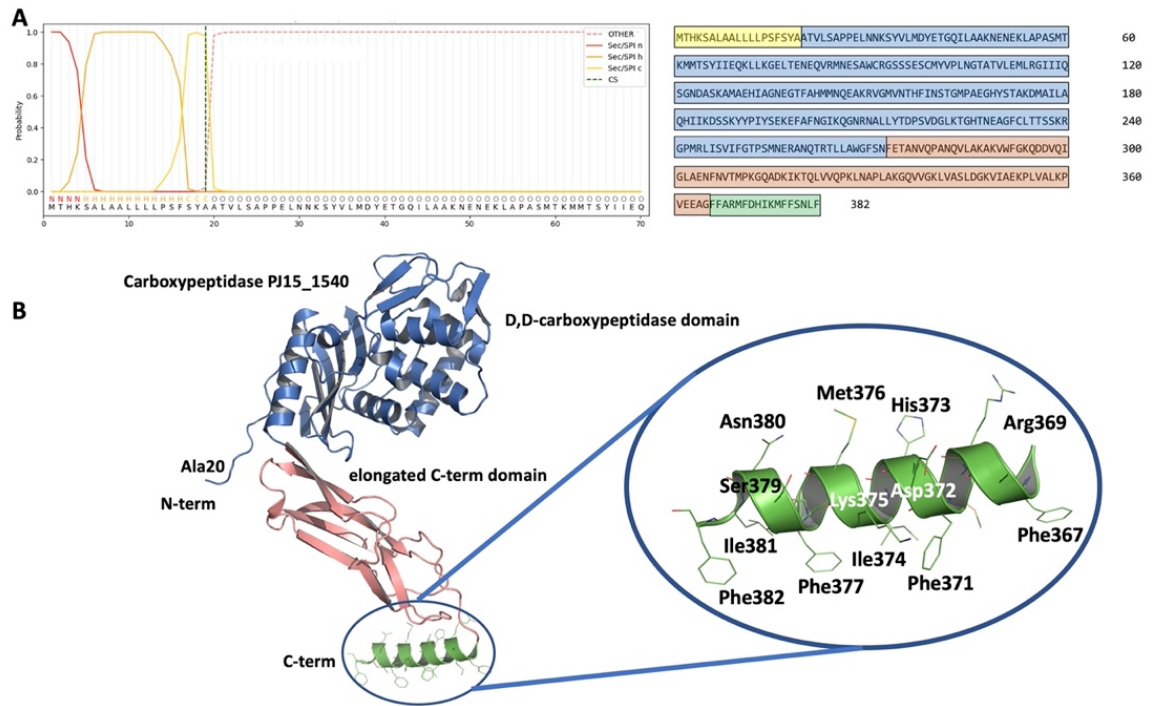


Fig. S4 Predicted structure of the carboxypeptidase PJ15_1540 from *Acinetobacter* sp. *negl*. The structural model of PJ15_1540 obtained with AlphaFold2 reveals that the enzyme is a penicillin-binding protein with an N-terminal D,D-carboxypeptidase domain (blue ribbon), an elongated C-terminal domain (orange ribbon), and a C-terminal amphipathic α -helix (green ribbon). The close-up view of this helix remarks its markedly amphipathic character, which is consistent with the putative membrane-associated nature of the protein.

Table S1

Table S1. Structurally similar proteins to PJ15_1540 determined with DALI

Protein	Z-score	rmsd (Å)	lali ¹	%id ²
6osu	34.0	1.3	251	41
1xp4	30.7	1.6	245	27
1tvf	29.6	1.8	247	30
6muq	26.2	2.3	239	24

¹lali: number of aligned residues; ²%id: sequence identity to PJ15_1540