BIOTRANS
LA ROCHELLE 2023

N°1139 / PC
TOPIC(s) : Enzyme discovery and engineering / Industrial biocatalysis

# Discovery of a novel ultra-thermostable Carbonic Anhydrase for enzymatic CO2 sequestration using high-throughput metagenomic analysis

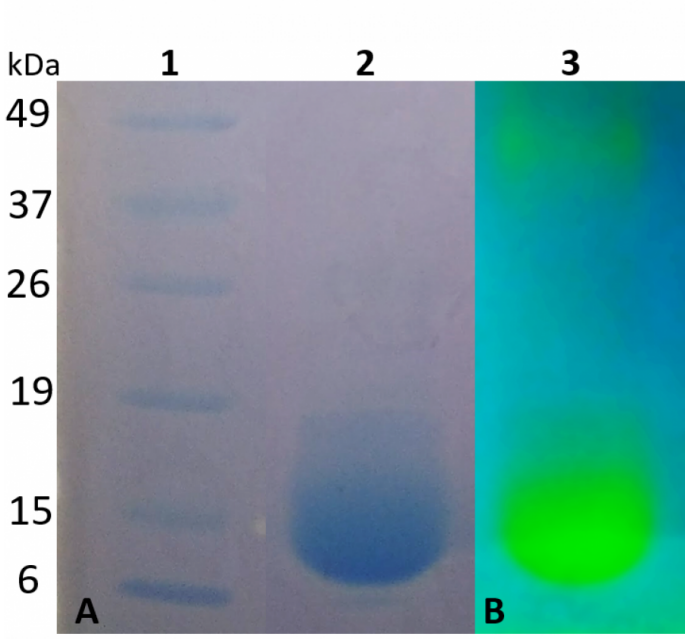
## AUTHORS

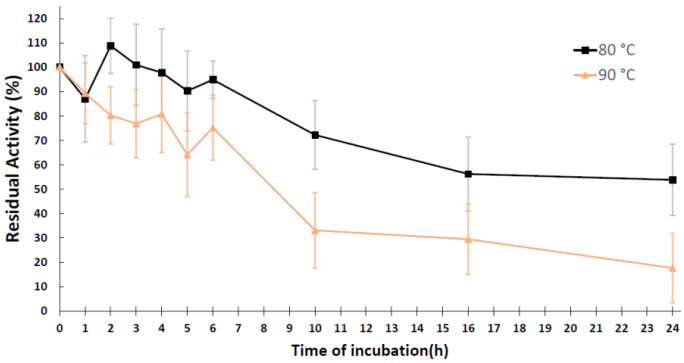
KONSTANTINOS RIGKOS / NATIONAL HELLENIC RESEARCH FOUNDATION (N.H.R.F), VASILEOS KONSTANTINOU 48, ATHENS
GEORGIOS FILIS / NATIONAL HELLENIC RESEARCH FOUNDATION (N.H.R.F), VASILEOS KONSTANTINOU 48, ATHENS
PAVLOS SARIDIS / NATIONAL HELLENIC RESEARCH FOUNDATION (N.H.R.F), VASILEOS KONSTANTINOU 48, ATHENS
DIMITRA ZARAFETA / NATIONAL HELLENIC RESEARCH FOUNDATION (N.H.R.F), VASILEOS KONSTANTINOU 48, ATHENS
GEORGIOS SKRETAS / BIOMEDICAL SCIENCES RESEARCH CENTRE “ALEXANDER, FLEMING “, FLEMINGK 34, VARI
Corresponding author : DIMITRA ZARAFETA / dzarafeta@eie.gr

## PURPOSE OF THE ABSTRACT

Anthropogenic CO2 emissions have been dramatically increasing for the past 30 years, an outcome that has led to an enhanced greenhouse effect, causing the Earth's temperature to rise at an accelerated rate. This phenomenon, known as Global Warming, is expected to have devastating effects on humanity if not addressed. This is well reflected in the commitment the European Commission made in 2019 to achieve net-zero emissions by 2050. In this landscape, the need for novel, low cost and sustainable CO2 Capture and Storage Technologies (CCS) is urgent. Today, the go-to technology relies on the use of amines for the chemical separation of CO2 from flue gas. Unfortunately, amines have a negative environmental impact due to high toxicity and high-energy consumption related to their regeneration process. Consequently, more eco-friendly technologies to mitigate the negative impact of CO2 rising emissions are pursued by researchers worldwide. An alternative green technology for CO2 sequestration involves the use of carbonic anhydrase (CA), an enzyme that catalyzes the reversible hydration of CO2 to HCO3- and H+ ions. This catalytic activity is vital for many biological processes, including respiration and photosynthesis and because of this, CAs are found in all living organisms. Despite the fact that CAs are abundant in nature and can speed up CO2 absorption kinetics in aqueous media, the harsh conditions present in industrial setups (high temperatures, alkaline pH and high salinity) compromise the stability of common enzymes. A promising approach to overcome this issue is the identification of thermostable enzymes which belong to the protein repertoire of extremophilic organisms. A bottleneck to this strategy is the fact that the protein space of such organisms is not accessible through classic microbiology, as 99% of them cannot be cultured. Metagenomic analysis can overcome this limitation by giving access to this huge, unexplored protein space, and driving the discovery of extremozymes of interest, such as thermostable CAs. In the present study, the discovery and characterization of a novel carbonic anhydrase is presented. High-throughput bioinformatic analysis of large, metagenomic data sets found in NIH's Sequence Read Archive (SRA) and in the Joint Genome Institute (JGI) open-access databases, resulted in the identification of an ultra-thermostable CA that was produced recombinantly in E. coli and studied. The novel CA-KR1 carbonic anhydrase, shares low similarity to known enzymes and exhibits very high thermostability, which, to our knowledge ranks it amongst the most thermostable CAs reported to date. This trait renders CA-KR1 a very strong candidate biocatalyst for enzymatic industrial CO2 sequestration.

## FIGURES




### FIGURE 1

SDS-PAGE & Protonography analysis of CA-KR1 protein.

(A) Unboiled sample of purified CA-KR1 protein (Lane 2) and protein standard (Lane 1). (B) Protonography analysis of CA-KR1 (Lane 3). The color shift from blue to yellow indicates CA activity.

### FIGURE 2

CA-KR1 Thermostability

The residual activity of CA-KR1 was measured following incubation of the enzyme at 80oC and 90oC for up to 24 h. The reported values correspond to the mean value from five independent experiments performed at least in triplicate +/- SD.


## KEYWORDS

Enzyme discovery | CO2 Capture | Carbonic Anhydrase | Bioinfomatic Analysis


## BIBLIOGRAPHY


[1] Ozensoy Guler O, Capasso C, Supuran CT. J Enzyme Inhib Med Chem. 2016, 31(5):689-94.

[2] Angeli A, Supuran CT. J Enzyme Inhib Med Chem. 2023, 38(1):2166503.

[3] Hanifa M, Agarwal R, Sharma U, Thapliyal PC, Singh LP. J CO2 Util. 2023, 67:102292.

[4] Olabi AG, Wilberforce T, Sayed ET, Shehata N, Alami AH, Maghrabie HM, et al. Energies. 2022, 15(22):8639.

[5] Dutcher B, Fan M, Russell AG. ACS Appl Mater Interfaces. 2015, 7(4):2137-48.

[6] Anderson C, Harkin T, Ho M, Mumford K, Qader A, Stevens G, et al. Energy Procedia. 2013, 37:225-32.

[7] Barzagli F, Giorgi C, Mani F, Peruzzini M. J CO2 Util. 2017, 22:346-54.

[8] Talekar S, Jo BH, Dordick JS, Kim J. Curr Opin Biotechnol. 2022, 74:230-40.