Production of beta-carbonic anhydrases (β-CA) from *Pseudomonas aeruginosa* and biothermodynamical analysis of β-CA interaction with potential inhibitors

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ABSTRACT

Pseudomonas aeruginosa is one of the most commonly isolated life-threatening opportunistic pathogens and is globally recognized as a serious threat because of its immunity to nearly all known antibiotics. Three beta-carbonic anhydrases (β-CAs) psCA1, psCA2, and psCA3 are known to be vitally important for the survival of this pathogen making them a promising group of antimicrobial drug targets.

OBJECTIVES

The two main objectives of this study would involve producing and characterizing recombinant CAs of *P. aeruginosa*, as well as screening a library of potential inhibitors to find the molecules that inhibit the activity of target CAs.

METHODS

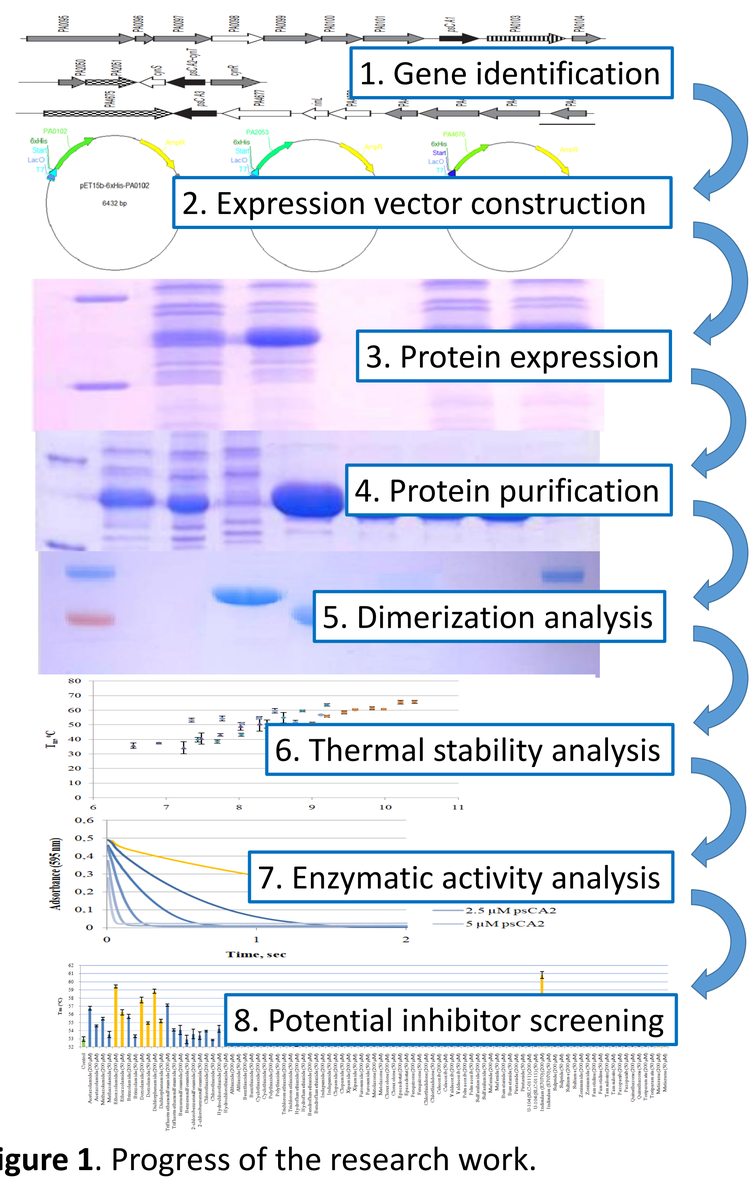

Figure 1. Progress of the research work.

RESULTS

P. aeruginosa β-CAs were cloned, heterologously expressed and purified. Monomeric psCA1, psCA2 and psCA3 yield molecular monomeric masses of 27, 23 and 24 kDa. Proteins were analyzed by SDS PAGE under non-reducing conditions, the results of the analysis have shown that psCAs form dimers with molecular masses of 58, 51, and 53 kDa.

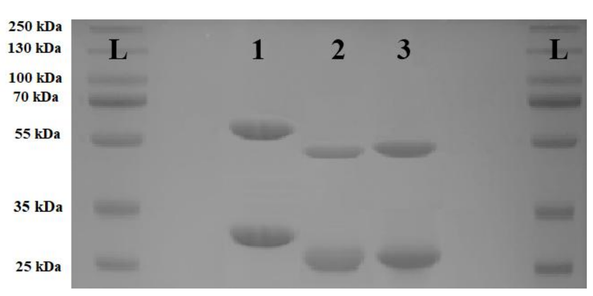

Figure 2. SDS PAGE analysis of psCA1, psCA2 and psCA3 under non-reducing conditions. Sample order: L - protein ladder; 1 – psCA1; 2 – psCA2; 3 – psCA3.

RESULTS

The native structure of a protein is sensitive to a variety of external influences: temperature, pH, salts, mechanical stress, and other factors. The stability of P. aeruginosa β-CAs was studied in buffer systems of various compositions and pH.

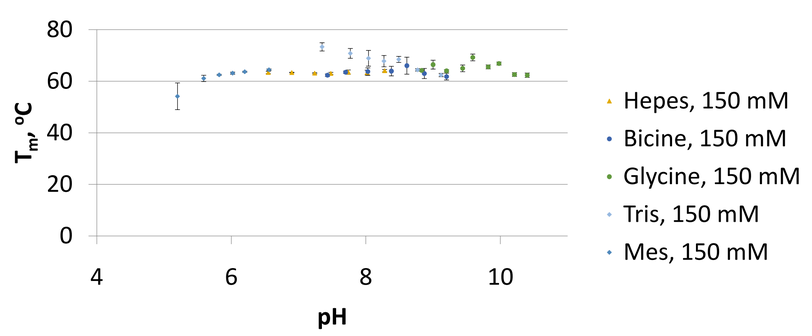

Figure 3. Graph illustrating the dependence of psCA1 melting temperature (T_m) on ambient pH values in different buffers.

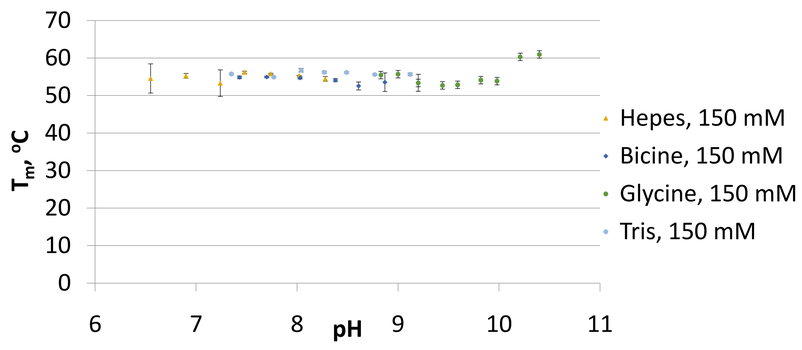

Figure 4. Graph illustrating the dependence of psCA2 melting temperature (T_m) on ambient pH values in different buffers.

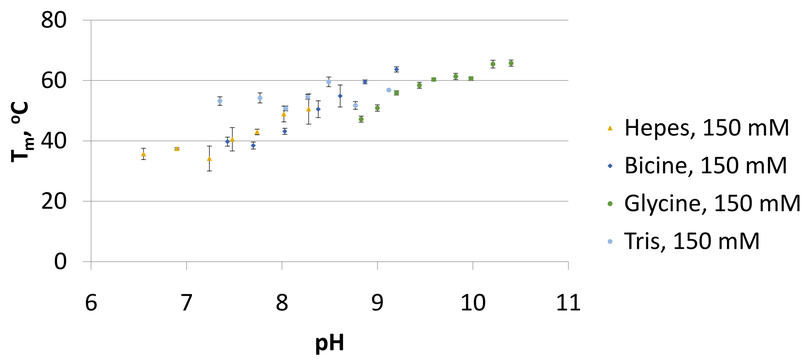

Figure 5. Graph illustrating the dependence of psCA3 melting temperature (T_m) on ambient pH values in different buffers.

Enzymatic activity of the purified recombinant β-CAs of *P. aeruginosa* was measured by the CO_2 hydration method using the stopped-flow technique. The results showed that all recombinant proteins were able to catalyze the CO_2 hydration reaction. Relative catalytic activities of investigated CAs can be arranged in the order of a decreasing catalytic activity: psCA2, psCA1, psCA3.

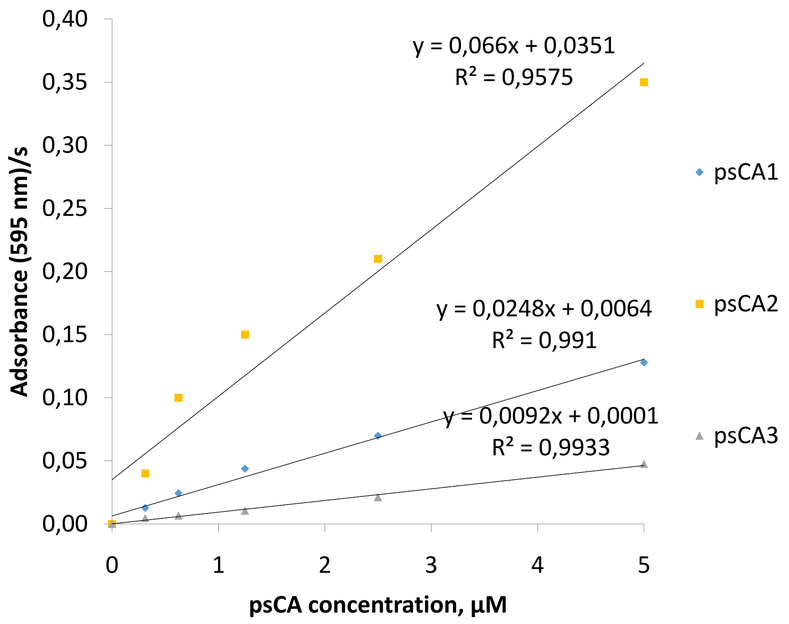

Figure 6. Relative activity of psCAs. Initial velocity dependence on CA concentration at constant CO_2 concentration.

RESULTS

The interaction of *P. aeruginosa* CAs with commercial CA inhibitors was investigated by the FTSA. Specific inhibitors stabilize the target protein when binding it causing an increase in melting temperature. The analysis followed the principle that compounds capable of elevating the protein melting temperature by 2 °C or more when used in 50 μM concentration could be considered as potentialy effective inhibitors. psCA1 and psCA2 did not bind any of the commercial ligands studied, whereas psCA3 was binded by ethoxzolamide, dorzolamide, dichlorophenamide, indisulam and methicrane.

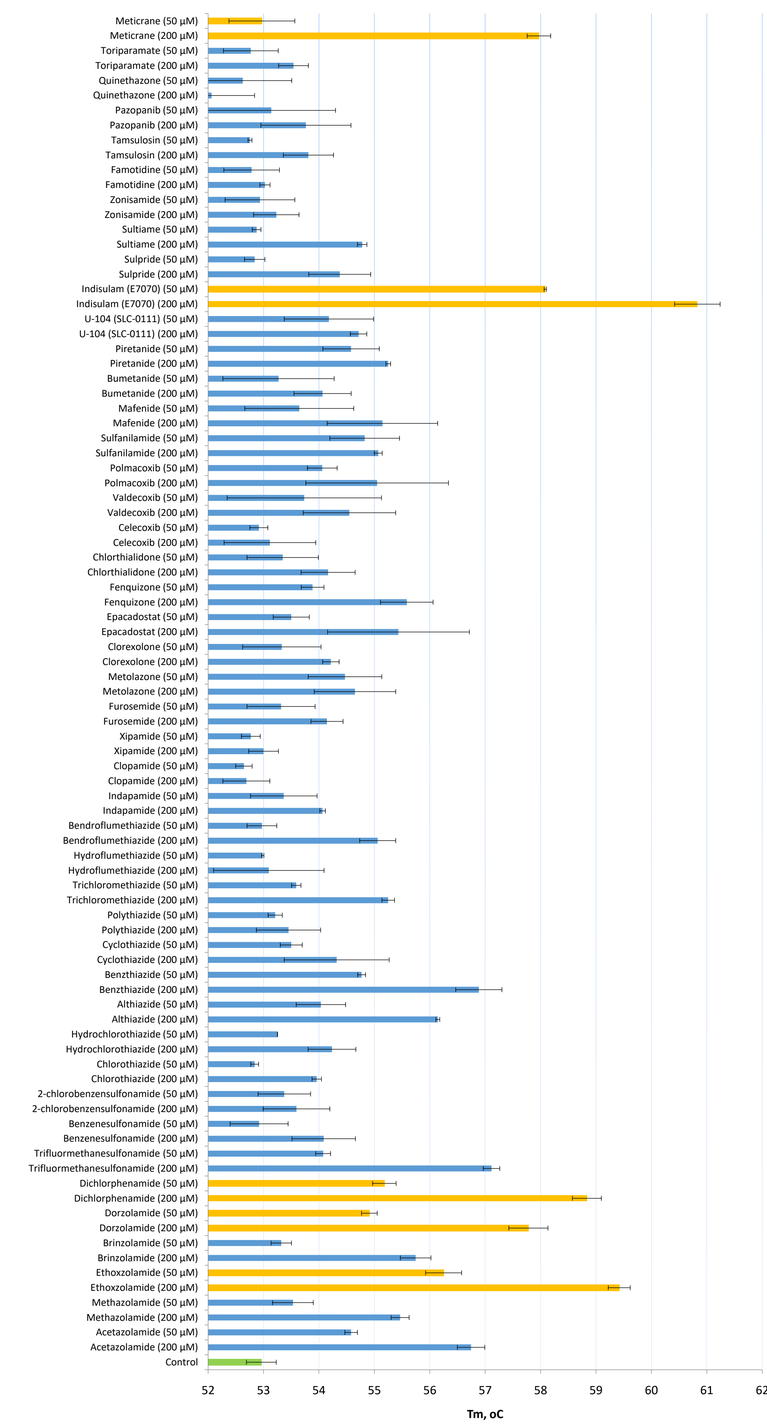

Figure 5. Bar chart illustrating the effect of 45 commercial CA ligands on the melting temperature (T_m) of psCA3 at different ligand concentrations (200 μM and 50 μM).

CONCLUSIONS

Recombinant *P. aeroginosa* CAs were obtained in the study, their partial characterization was performed, and the proteins were used in screening experiments of commercial inhibitors. No molecules were found to bind sufficiently strongly to psCA1 and psCA2, but four potential inhibitors of psCA3 were identified.

REFERENCES

Lotlikar, S.R., Hnatusko, S., Dickenson, N.E., Choudhari, S.P., Picking, W.L., Patrauchan, M.A., 2013. Three functional β-carbonic anhydrases in Pseudomonas aeruginosa PAO1: role in survival in ambient air. Microbiology 159, 1748–1759. https://doi.org/10.1099/mic.0.066357-0

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