CHARACTERIZATION OF PHOTODECARBOXYLASES CAPABLE OF PRODUCING PETROLEUM-LIKE MOLECULES

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ABSTRACT

Fatty acid decarboxylases have emerged as a powerful alternative for the biological production of alkanes, providing a sustainable route to produce drop-in fuels, such as aviation biokerosene. This study focused on the discovery and partial characterization of new photodecarboxylases prospected by sequence similarity network (SSN). We successfully purified two photoenzymes that exhibited significant activity and promiscuity even when compared to the counterpart CvFAP, highlighting them as key candidates for further molecular investigation. The newly prospected FAPs exhibited initial velocity in kinetic experiments higher than CvFAP under both sunlight and blue light conditions. Their capacity to produce alkanes from a broad range of fatty acids at different conditions underscored them as potential targets to be applied in cell-free or in vivo processes to produce sustainable advanced fuels.

Keywords: Decarboxylase. Photoenzyme. FAP. Fatty Acid. Biofuels.

1 INTRODUCTION

Over the past decade, drop-in biofuels have emerged as a promising alternative to first-generation biofuels, such as bioethanol and biodiesel. Waste oils and fats are the primary raw materials used to produce drop-in biofuels. Since these materials are primarily composed of triglycerides, they can be converted into fatty acids through hydrolysis.[1]

A group of enzymes responsible for the decarboxylation of fatty acids into hydrocarbons are known as fatty acid photodecarboxylases (FAPs). In the presence of light, these enzymes can promote the decarboxylation of free fatty acids into alkanes and CO2. Currently, only a few FAPs are described in the literature, with emphasis on the FAP derived from the microalga Chlorella variabilis NC64A, also known as CvFAP.[2]

Therefore, the discovery and characterization of new enzymes with photodecarboxylase activity is essential to speed up the biobased production of hydrocarbons. In addition to that, it is known that the use of light can cause enzyme deactivation due to the formation of free radicals at the catalytic site, also highlighting the need to increase the arsenal of FAPs that present more light tolerance.

2 MATERIAL & METHODS

Heterologous Expression in E. coli

The genes in Table 1 were identified through Sequence Similarity Network (SSN). They were acquired in pET28a vectors for transformation and expression in E. coli BL21 (DE3), except for M1VK13 that was expressed in E. coli p-RARE2, following established protocols. The process involves growing transformed clones in TB medium, inducing expression with 0.5-1 mM IPTG.

Table 1: Selected target genes

Protein	% ID to CvFAP	Molecular Weight (KDa)
NV16	55,1	65,2
C4N3	55,1	63,5
M1VK13	41,3	71,6
ZTH3	41,1	67,2
NVI0	44,5	67,0

Protein Purification

Proteins fused to a 6His-tag were purified via affinity chromatography on nickel-Sepharose columns, followed by SDS-PAGE verification. Pure samples were further tested for activity performance.

Decarboxylation Activity

Activity assays were conducted under various conditions, including light sources and different substrates (C10 to C20, including unsaturated ones) to assess their impact on the enzymatic activity of FAP´s. The reactions were carried out for 1 hour under blue LED light, and sunlight at different periods of day, then quenched by the addition of HCl.

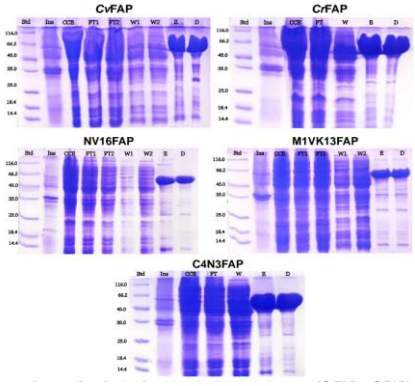
GC-FID. Analysis for alkanes determination

Alkanes present in the reaction mixtures were identified using gas chromatography with a flame ionization detector (GC-FID). The samples were prepared through centrifugation and subsequently analyzed using precise chromatographic settings.

3 RESULTS & DISCUSSION

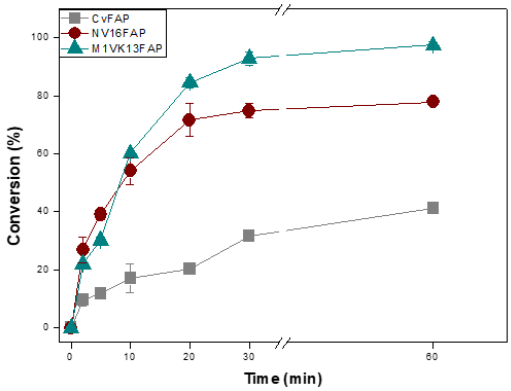
All enzymes were expressed in different strains of E. coli and subsequently purified. Among them, only ZTH3 and NVI0 exhibited low expression levels in the soluble fraction. The remaining enzymes were primarily expressed in the soluble fraction and were thus selected for further characterization. Only NV16 and M1VK13 showed conversions that surpassed CvFAP (see Figure 1).

Figure 1: SDS.PAGE. Expression and purification of literature FAP´s and three new FAP´s discovery by SSN (NV16FAP, M1VK13FAP and C4N3FAP)



In order to achieve a better understanding of the catalytic efficiency of NV16 and M1VK13 FAPs, the kinetic assay was conducted at a fixed concentration of the enzymes. Thus, over a 1-hour reaction period and in the presence of blue light, the conversion of the substrate C16:0 into pentadecane at different reaction times was evaluated (Figure 2).

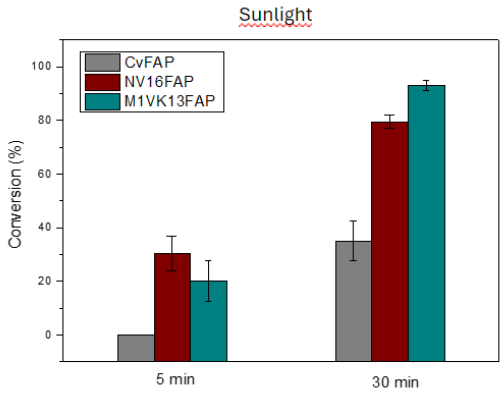
Figure 2: Comparison of the catalytic performance of different FAPs as a function of reaction time in the presence of blue light. Reaction conditions: 100 mM Tris-HCl (pH 8.5), 500 µM substrate, 0.5 µM FAP, 400 rpm, room temperature, presence of blue light (450 nm).



As observed in Figure 2, the new FAPs presented higher catalytic activity compared to CvFAP under same conditions. After 1 hour of reaction, CvFAP converted approximately 40% of the substrate into alkane. In contrast, the NV16 and M1VK13 FAPs converted 78% and 98% of the substrate, respectively, within the same period.

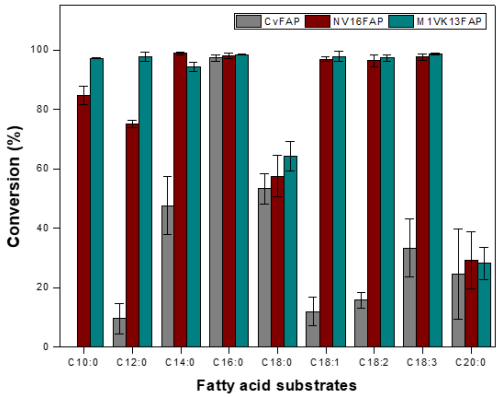
Influence of sunlight on the catalytic activity was also investigated. Thus, decarboxylation reactions of the substrate C16:0 in the presence of these enzymes were conducted on sunny days, at differents periods of time. Results shows similar conversion between M1VK13 and NV16 FAPs at 5 minutes and 30 minutes of reaction (Figure 3). Remarkably, both enzymes displayed an improved performance comparing to CvFAP. This is an important advance in the search of others sources of light to avoid photodeactivation under blue light.

Figure 3: Comparison of catalytic activity of differents FAP´s in presence of sunlight using palmitic acid as substrate.



We further followed by investigating the specificity of newly discovered FAPs. For this, fatty acids of varying chain lengths as well as saturation were evaluated.

Figure 4: Conversion of different fatty acids substrates into alkanes.



According to results illustrated in Figure 4, while CvFAP is more specific for mid-chains (C14:0, C16:0 and C18:0), NV16 and M1VK13 exhibited excellent conversions (> 90%) for mostly of substrates tested, including unsaturated ones.

4 CONCLUSION

The partial characterization of different FAPs prospected by SSN revealed potential photoenzymes to be applied in drop-in biofuel to obtain alka(e)nes.

Furthermore, we aim to evaluate their structural aspects to better understand the observed catalytic enhancement in addition to propose variant mutants.

REFERENCES

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ACKNOWLEDGEMENTS

This work was supported by grant nº 2024/13770-0, São Paulo Research Foundation (FAPESP).