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Recombinant LPMOs and the *Aspergillus nidulans* role as expression system

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1. INTRODUCTION

Lytic polysaccharide monooxygenases (LPMOs) are enzymes capable of carrying out an oxidative attack on abundant polymers in nature such as cellulose, hemicellulose, chitin and starch. These proteins are classified as auxiliary activity (AA) enzymes in CAZy database and grouped into eight families AA9-AA11 and AA13-AA17 (www.cazy.org). The AA9 act mainly on cellulose and can also act on xyloglucan being the most studied family with 34 members characterized according to CAZy. Despite their mechanism of action has not been fully elucidated, these metalloproteins are positioned in the industrial market and incorporated into commercial enzyme cocktails used to saccharify lignocellulosic biomass (Harris et al., 2014). The vertiginous growth in research and application of LPMOs is due to its ability to boost the saccharification process, acting in synergism with celulases (Hu et al., 2015). To be studied, the enzymes must be secreted at high levels by an organism, being this requirement very difficult to occur in nature, which makes necessary the use of recombinant DNA technology to heterologously produce LPMOs. In this scenario *Aspergillus nidulans* emerges as a model organism since it has the whole genome sequenced and tools that facilitates its manipulation to be used as protein expression system. In 2012 was reported a system based in a strain of *A. nidulans* using the pEXPYR expression/secretion vector to produce high levels of heterologous protein (Segato et al., 2012). Since that *A. nidulans+pEXPYR* has been an efficient system in expression/production of many CAZymes including LPMOs. This work shows the heterologous production of six LPMOs from *Thermothielavioides terrestris*, seven from *Aspergillus fumigatus* and one from *Thermothelomyces thermophilus* in its active form using *A. nidulans*+pEXPYR as expression system, among the characterized AA9-LPMOs was observed a collaborative effect with cellulases increasing up to a 29% saccharification processes, participation in photobiocatalysis and lignin modification.

2. MAIN RESULTS

The genes encoding AA9-LPMOs were efficiently cloned into pEXPYR by high throughput method, and incorporated into *A. nidulans* genome, each transformant strain was able to efficiently secrete LPMOs in its active form (Figure 1). In addition, *A. nidulans* was used as an expression system for *Tt*LPMO9H a LPMO from *T. thermophilus* already characterized by our group together with *Af*AA9A and *Af*AA9B from *A. fumigatus* (Higasi et al., 2021; Velasco et al., 2021), and it has been possible to describe characteristics of great interest such as the boosting effect of *Af*AA9B and *Tt*LPMO9H increasing biomass saccharification in 20 and 29% respectively. Also, was showed an increase in the oxidative activity of *Tt*LPMO9H in presence of photopigments-light and the capacity of *Af*AA9B to modify lignin.

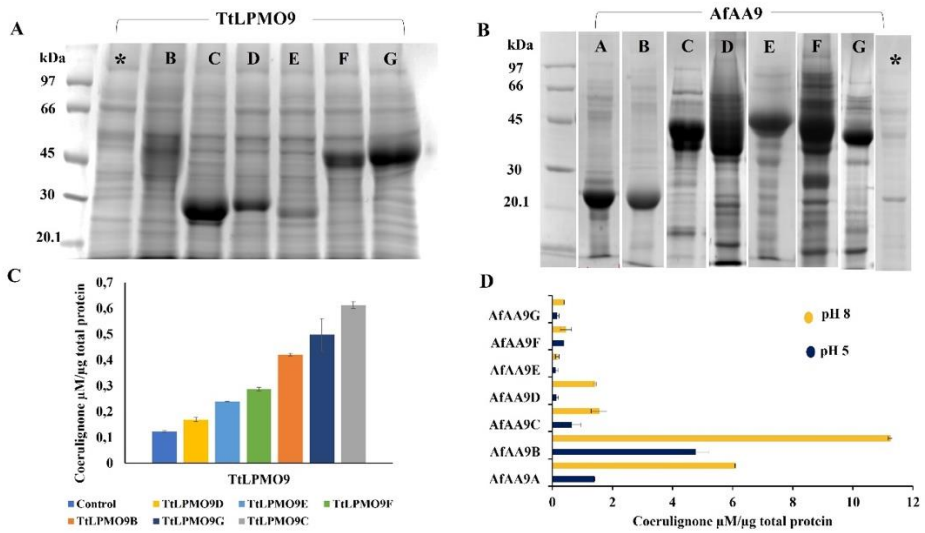

Figure 1. *Recombinant LPMOs produced in A. nidulans*. SDS-PAGE showing 6 LPMOs from *T. terrestris* (A) and 7 from *A. fumigatus* (B). (*) control: non-recombinant *A. nidulans* for LPMOs. The 13 LPMOs showed peroxidase activity using the 2,6-DMP method, (C) activity profile of crude enzymatic extracts of the TtLPMOs, (D) activity of the AfAA9s purified by ionic exchange. Reactions were performed for 5 min at 40°C, pH 8 for TtLPMOs, pH 5 and pH 8 for AfAA9s.

3. CONCLUSION

The LPMOs discovery have brought development to the classic industrial procedures of plant biomass saccharification favoring biorefinery projects. *A. nidulans* has shown as an efficient expression and secretion system for these metalloenzymes, offering the opportunity to produce new LPMOs with interesting characteristics to promote the green economy.

5. REFERENCES

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