

Thermostable hemicellulolytic enzymes secreted by a microbiome isolated from a local anaerobic digester

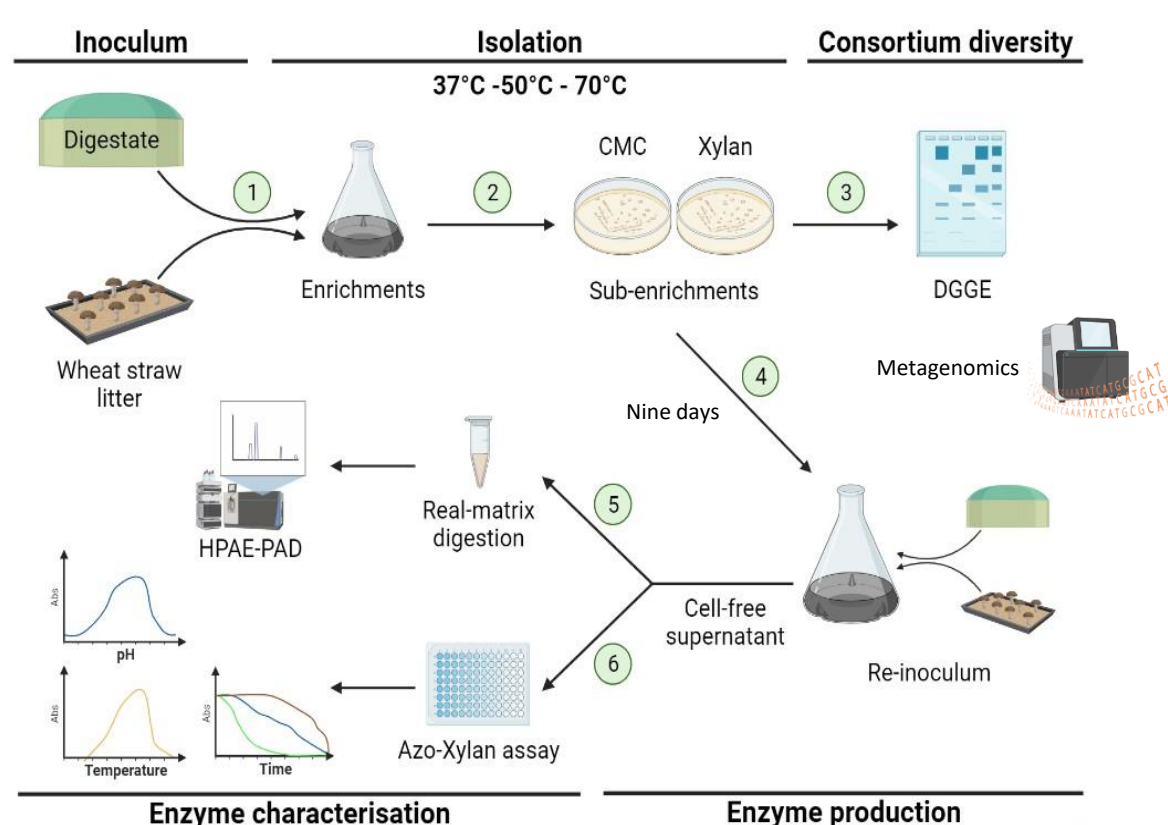
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INTRODUCTION & AIM

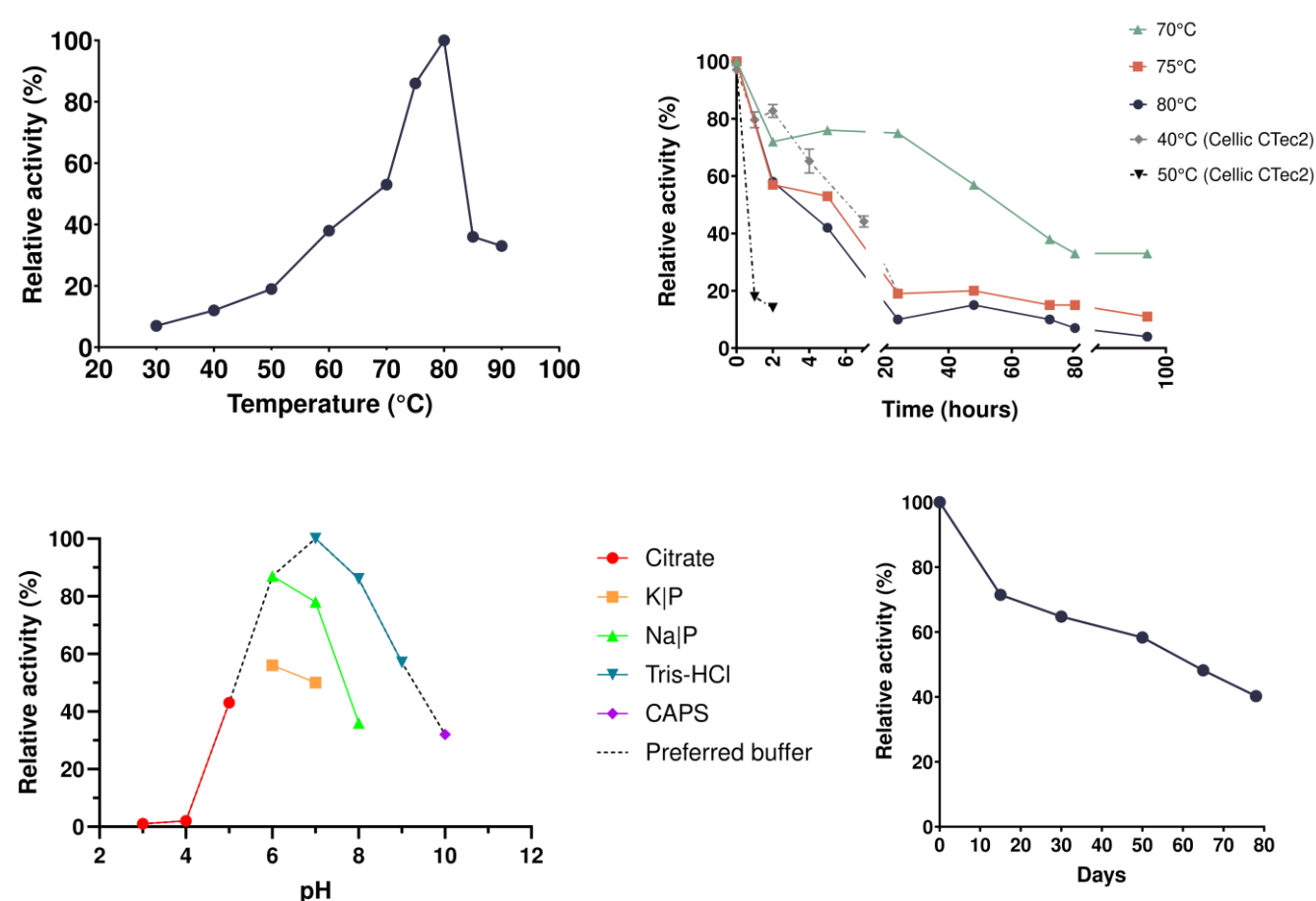
Lignocellulose biomasses (LCB) are abundant reservoir of value –added chemicals^[1]. Thermophilic enzymes represent valuable tools to setup effective biomass deconstruction protocols, aiding circular economy-inspired industrial applications. Here, we report the characterisation of enzymes secreted by a microbiome, namely CMC-70, previously enriched at 70°C on spent mushroom substrate (SMS) and then selected on a pure carbon source (CMC)^{[2],[3]}. The microbiome was proliferated on SMS as the sole carbon source to induce enzymes secretion, revealing the presence in the supernatant of thermostable xylanases. Xylanases in their cell-free supernatants (CFS) were characterised in terms of temperature and pH optima, thermal stability and types of products released upon hydrolysis of pure and complex hemicellulose-derived polymers. These insights provide a framework for future formulations of more effective enzymatic cocktails for biotechnological applications. The research was funded by Next Generation EU in the framework of National Biodiversity Future Center (NBFC).

METHOD

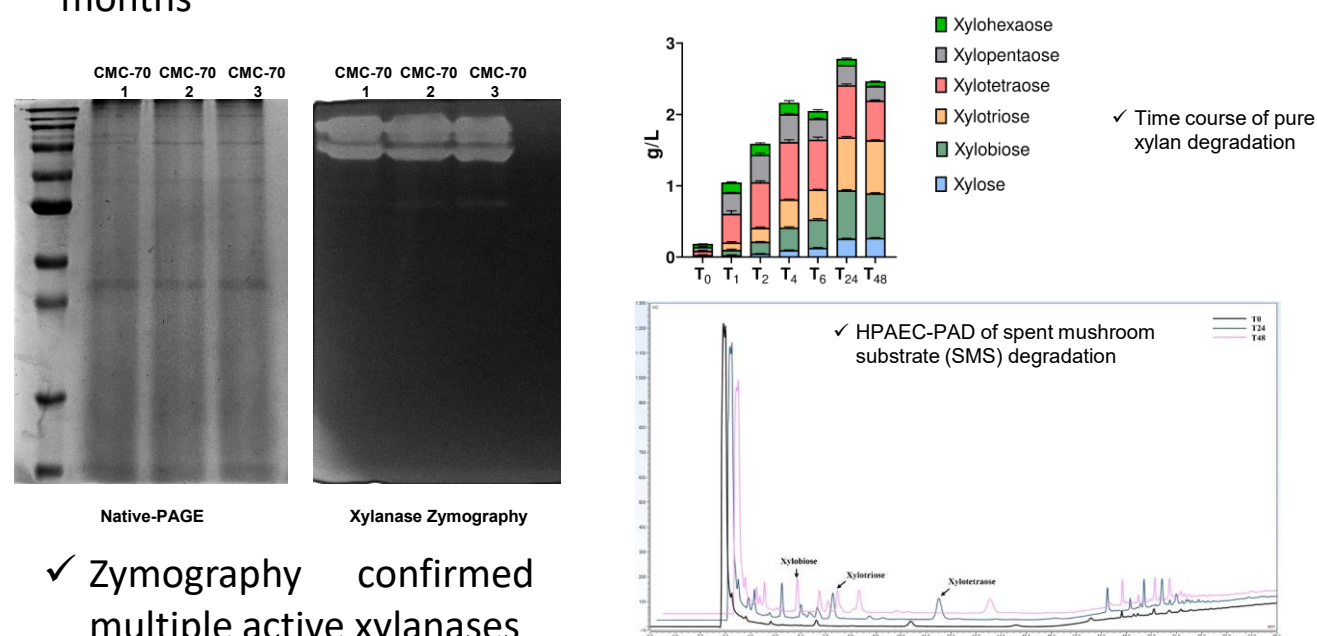


- ✓ Enrichment cultures of lignocellulose-degrading microbes were set up in a cheap medium composed of digestate as microbial inoculum and SMS as sole carbon source
- ✓ Five sub-enriched consortia were isolated based on three temperatures (37°C, 50°C, and 70°C) and two carbon sources (CMC and xylan). Sub-enriched microbes were proliferated in the presence of sterile SMS and digestate to induce lignocellulolytic enzyme secretion
- ✓ The presence of endo-1,4-β-D-xylanase in the secretomes was tested using the chromogenic substrate Azo-xylan (Megazyme)
- ✓ Variations in the Azo-xylan assay were used to obtain a complete biochemical characterization of the CMC-70 secretome
- ✓ The hydrolytic potential of cell-free supernatants towards LCB-derived pure polysaccharides and complex matrices (SMS and rice husk) was assessed via HPAEC-PAD

RESULTS & DISCUSSION



- ✓ Enzymes were active over a wide pH range, with an optimum at pH 7
- ✓ Maximal activity was reached at 80°C and > 50% residual activity was maintained at 70°C for 48h, outperforming the commercial cocktail Cellic CTec2® (Novozymes)
- ✓ Secretomes retained > 50% activity after storage at 4°C for two months



- ✓ Zymography confirmed multiple active xylanases

CONCLUSION

- ✓ Microbiomes selected at 70°C using carboxymethylcellulose exhibited the secretion of thermostable hemicellulolytic enzymes that demonstrated a broad range of temperature and pH activity
- ✓ Assays on pure and complex LCBs revealed diverse glycoside hydrolase (GH) activities
- ✓ Anaerobic digester microbiomes are valuable reservoirs of thermostable LC hydrolases

REFERENCES

- [1] Zuliani et al. *Processes* 2021, 9, 1583.
- [2] Bombardi et al. *International Journal of Molecular Sciences*, 2024, 25, 1090.
- [3] Bombardi et al. *International Journal of Molecular Sciences*, 2024, 25(18), 9887.