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Biodegradation modelling of polylactic acid-based biopolymer by *Thermobifida* consortium

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Introduction

The extensive production and use of petroleum-based plastics casuses a significant threat to the environment because they cannot or very slowly degrade causing serious ecological pollution to natural life, therefore they are not sustainable. Biopolymers are becoming widespread as substituents for petroleum-derived plastics due to their biodegradability and production of biobased monomers. Polylactic acid (PLA) is one of the most promising of the biobased and biodegradable polymers currently in the market, produced from renewable feedstock (corn, wheat, rice). PLA is mostly resistant to microbial attacks under ambient conditions, meanwhile very few information are available regarded to its biological degradation.

Results

Strain selection

The strain selection (45 ±2°C , 3 days) was performed from thermophilic bacteria by using screening method. For the proteolityc enzyme activity testing (Fig. 1a) the used media was a casein agar plate (Radha *et al.*, 2012) and for the esterase enzyme activity (Fig. 1b) it was the Tween 80 agar plate (Rhiani *et al.*, 2018). The most promising results were selected to create microbial consortium and were used for biodegradation modelling.

a. b.

Thermobifida fusca B2355

Figure 1. Strain selection

Biodegradation modelling

The shredded and cleaned PLA based cutlery (PLA-spent grain blend knives) was examined (150RPM, 45 ±2°C and 60 ±2°C , 21 days) in a submerged (TPY +5% olive oil) environment for biodegradation and the inoculation rate of the pre-cultured bacteria strains (*Thermobifida fusca* B2355, *Thermobifida cellulosilytica* B1997) were 10 (w/w) %.

Figure 2. Polylactic acid based cutlery life-cycle

Depolymerase enzyme activity

According to certain studies the degradation of natural polymers are coherent with proteolytic and esterase type (cutinase) enzyme activity properties of certain microorganisms. The **protease** activity (A660nm) was determined with casein as a substrate (Ire *et. al.*, 2011) and **cutinase** (A415nm) activity was determined with p-nitrophenol-butyrate as a substrate (Castro-Ochoa *et. al.*, 2012).

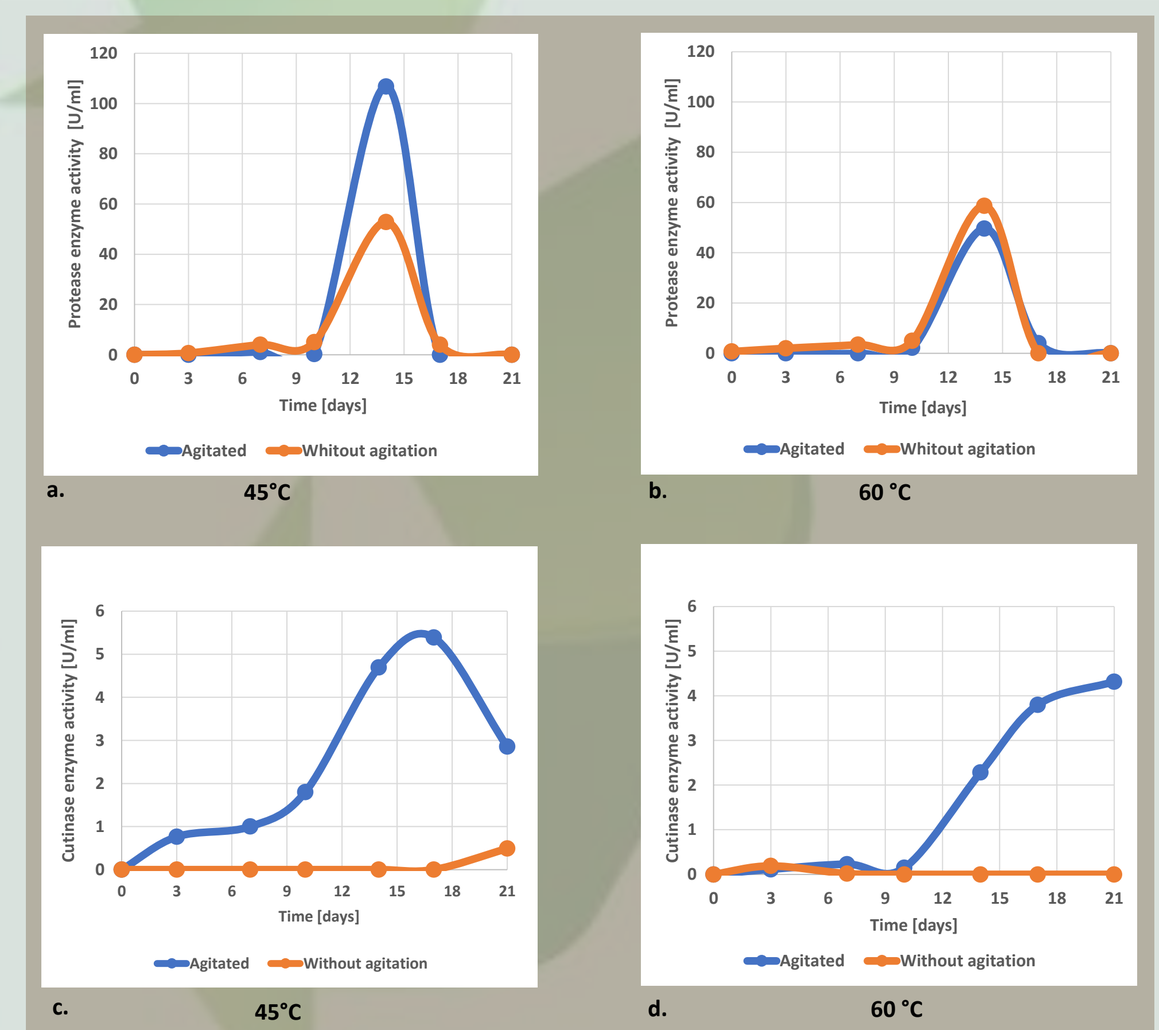

Figure 3. Effects of protease enzyme activity (a,b) and cutinase enzyme activity (c,d) at 45 ±2°C and 60 ±2°C

During the experiment on 5% olive oil supplemented TPY medium the *Thermobifida* consortium synthetized **protease** and **cutinase** enzyme activity amount was higher at 45°C and agitated environment. The maximum enzyme titer of the **protease** (Fig. 3a) was more than **100 U/ml** and the highest **cutinase** enzyme amount (Fig. 3c) was **5 U/ml** at 45°C and agitated environment. At the end of the biological treatment, these enzyme amounts resulted **15 % PLA mass degradation**.

At 60 °C the highest **protease** enzyme amount was **58 U/ml** without agitation (Fig. 3b) and **4 U/ml** (Fig. 3d) was the highest synthetized **cutinase** during the experiment. The PLA lost about **10%** in its weight at the end of biological degradation process.

This results presents a good opportunity to apply further microorganism consortium and inducers for the effective biodegradation.

References

1. Castro-Ochoa D., Pena-Montes ., Gonzalez-Canto A., Alva.Gasca A., Esquivel-Bautista R., Navarro-Ocana A., Farres A. (2012) ANCUT2, an extracellular cutinase from *Aspergillus nidulans* induced by olive oil, Appl. Biochem Biotechnol 166:1275 -1290
2. Ire F.S., Okolo B.N. , Moneke A.N., Odibo F.J., (2011), Influence of cultivation conditions on the production of protease from *Aspergullus carbonarius* using submerged fermentation, African Journal of Food Science Vol.5(6), pp.353-364
3. Radha S., Sridevi A., Nithya V.J., Prasad N.B.L., Narasimha G., (2012) Isolation and screening of proteolytic fungal cultures from soil contaminated with abattoir waste, Biochemistry an Indian Journal BCAIJ 6(7) 226-230
4. Rihani A., Tichati L., Soumati B., (2018) Isolation and identification of lipase producing fungi drom local olive oil manufacture in East od Algeria, St. Cerc. St. 14 CICBIA 2018 19 (1), pp. 013 – 022

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