Importance of carbon source for the cellulase activity in Trichoderma strains isolated from kitchen waste compost

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In face of the sustainability challenge, one of the major issues in urban areas is the production of kitchen waste residues. This type of solid waste is rich in lipids and lignocellulosic compounds that are hard to decompose due to the complexity of their molecules. In this scenario, one of the most interesting solutions is the composting process since it allows the treatment and reuse of household waste in an effective way in terms of both cost and time.

In order to increase the composting efficiency and improve the quality of the final compost, recent studies have suggested the enrichment of the waste with specific microorganisms such as fungi and bacteria that are capable of degrading complex macromolecules (Gautam *et al.* 2011). Making use of microorganisms multifunctionality could enable a faster and more efficient composting process. In the present study, our aim is to isolate and identify fungi strains able to degrade cellulose and to understand how different carbon sources could affect their cellulolytic potential.

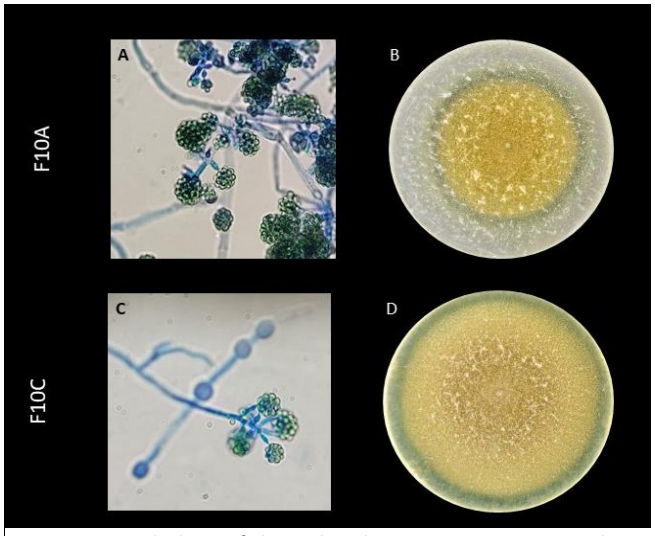

Figure 1. Morphology of the isolated strains. Fungi F10A and F10C were photographed under the light microscope at 40x (A and C) and growing in PDA plates (B and D).

To isolate potential cellulolytic fungi, samples were collected from a kitchen waste composting pile, then mixed with NaCl 0,9% and plated in PDA medium after a serial dilution. Isolated colonies were then grown in agar-water medium for hyphal tip isolation and then characterized according to their morphology using the slide culture technique. The two strains isolated were called F10A and F10C and they were identified as *Trichoderma spp* (Figure 1).

After the first identification, the strains were plated in carboxymethylcellulose (CMC) medium to evaluate their capacity to produce CMCase enzyme. Following the method proposed by Carder (1986), the plates were stained with Congo red after a three-day incubation to check for halo formation. In order to test substrate influence in the enzyme production, strains were also submitted to 5 growing cycles in CMC medium and then checked for halo formation. The results presented in Figure 2 show that although no halo was formed in the first test (A and C), after growing repeatedly in the presence of CMC substrate, the two strains were induced to produce CMCase enzymes after the three-day incubation period.

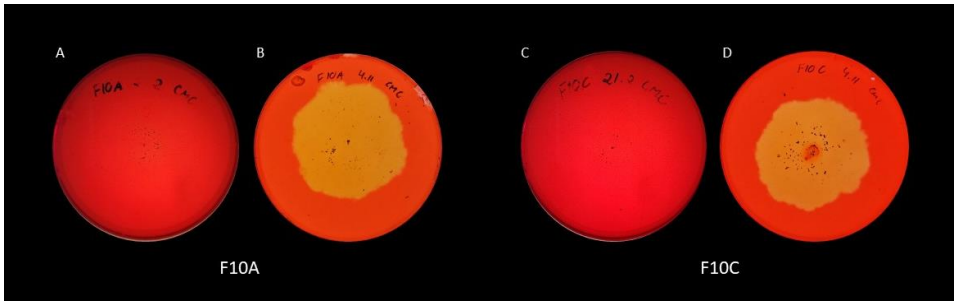

Figure 2. Cellulase production in CMC medium. Fungi F10A and F10C growing in CMC medium after isolation (A and C) and after growing repeatedly in the presence of CMC substrate (B and D). All experiments were conducted after a three-day incubation period.

After the plate test, the Trichoderma strains were submitted to a submerged-state fermentation (SmF) experiment using basal medium with the addition of different carbon sources that are commonly used in composting piles: wood shaving, rice straw and dry Tifton 85 grass (*Cynodon* spp.). The SmF was conducted for 21 days at 28˚C in triplicates and the filter paper activity (FPase) was determined according to Miller (1959) to measure the total cellulase activity. The results are shown in Figure 3.

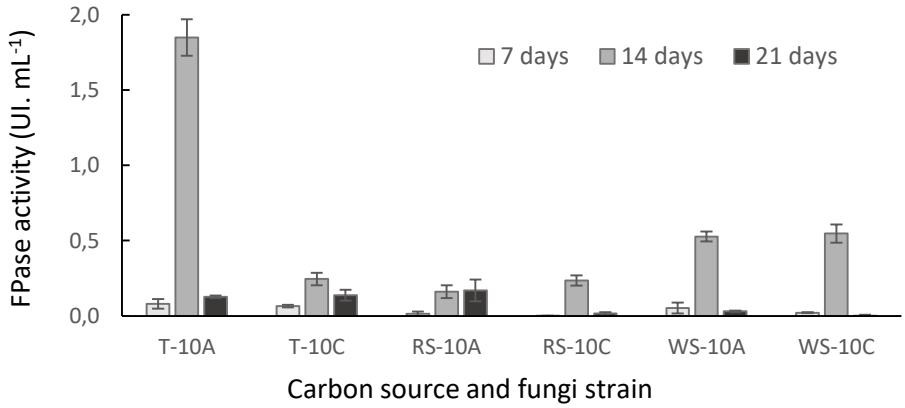

Figure 3. FPase activity of fungi F10A and F10C in submerged-state fermentation with different carbon sources. FPase activity was measured after 7, 14 and 21 for fungi incubated with Tifton 85 grass (T), rice straw (RS) and wood shavings (WS). The results are presented in means and standard deviation.

In agreement with other recent findings, our results show that the carbon source has an influence on the enzyme production (Amadi *at al.* 2020). The combination of strain F10A growing with Tifton 85 for 14 days resulted in the higher FPase activity. Both strains showed similar results in the presence of wood shavings with the higher means also after 14-day incubation (Figure 3). Other culture conditions need to be optimized in order to achieve the full potential of enzyme production of our isolates (Su *at al.* 2017). Nevertheless, our results point to the potential use of Trichoderma strains to enrich composting piles and improve the composting process of kitchen waste.

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