# Effect of cyclodextrins as encapsulating agents for stimulating mycelium growth of the desert truffle

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## INTRODUCTION AND OBJECTIVE

The erratic and slow growth of *T. claveryi* mycelium, an edible desert truffle with important gastronomic, nutritional and antioxidant properties, in vitro, represents an impairment to obtaining mycorrhizal plants and it makes necessary to find a new culture medium able to overcome these drawbacks. In this work, we analyze the effect of encapsulant agents as cyclodextrins (CDs) on the growth of *T. claveryi* mycelium. Different parameters, including colony diameter, growth rate and colony fresh weight, were evaluated, both in the presence and absence of these encapsulant agents. The results obtained confirm the ability of CDs to stimulate the growth of *T. claveryi* mycelium when present in the culture medium. Three natural (α-, β-, and γ) and two modified (hydroxypropil-β and methyl-β) CDs were assayed. The best results were obtained with β- CD, but no improvement was observed with its chemically modified derivatives. CDs complex the different compounds present in the culture medium which impair mycelial growth.

## MATERIAL AND METHODS

### *Chemicals and reagents*

Natural and modified CDs were purchased from Sigma-Aldrich (Madrid, Spain) and used as received.

### *Fungal growth in pure culture*

Mycelium from *T. claveryi* was isolated from ascocarps collected in the south-eastern of Spain, under H. almeriense as host plant. Both mycelium cultures were maintained at 23ºC on MMN medium without malt extract, at pH 7.0 and solidified with 7 g/l Panreac agar, which was selected as the best medium for in vitro mycelium growth.

### *Effect of CDs*

First, several CDs at two different concentrations were tested to select those that promote mycelium growth. Mycelial colonies were grown in Petri dishes on cellophane filters saturated with 4 ml of liquid medium but placed on autoclaved 3 mm-glass beads (Sigma) to avoid submersion. After inoculation, the Petri dishes were sealed with Parafilm and maintained in the dark at 23ºC for 9 weeks. Secondly, after selecting β-CDs as the most effective CDs for stimulating mycelium growth of T. *claveryi*, two types of modified β-CDs (HP-β-CD and methyl-β-CD) were studied. This assay was carried out using mycelium grown on MMN agar medium for 12 weeks.

## RESULTS AND DISCUSSION

### *Mycelial growth of T. claveryi in the presence of CDs*

In a period of five weeks, the *T. claveryi* mycelium grew very little in MMN medium. However, the results obtained indicate in our investigation show that the addition of 8 mM α-CD to the culture medium led to a significant increase in mycelium growth compared with the control assay, confirming the ability of CDs to stimulate the mycelial growth of *T. claveryi*. The encapsulating activity of CDs is clearly demonstrated by the fact that the brown pigmentation of the culture medium observed in control assays was not observed in the presence of CDs. This effect is clearly the result of by the encapsulation of phenolic compounds some of which may be substrates of tyrosinase.

### *Addition of glucidic molecules to culture medium on the growth of T. claveryi mycelium*

To rule out the possibility that the stimulation of growth observed in *T. claveryi* mycelium is due to the hydrolysis of CDs and the subsequent consumption of the glucose obtained, the effect of its addition to the growth medium was analyzed. Two concentrations of D-glucose (48 mM and 56 mM), equivalent in the number of glucose units to 8 mM of both, α-CD and β-CD, were assayed (Table 1). The results obtained after five weeks confirm the ability of CDs to stimulate mycelial growth. This effect was more evident with 8 mM β-CD, which produced a 6.5-fold increase in growth compared with a control. The addition of a D-glucose concentrations equivalent to the glucose content in the CDs assayed produced only a slight increase in colony diameter.

**Table 1. Effect of different glucidic molecules on the colony diameter of T. claveryi mycelium after five weeks.**

| Colony diameter (cm) | | | | |
|---|---|---|---|---|
| *Control* | *8 mM α-CD* | *8 mM β-CD* | *48 mM Glucose* | *56 mM Glucose* |
| 0.6 a | 2.8 b | 3.9 b | 1.1 a | 1.0 a |

### *T. claveryi growth in the presence of natural CDs*

Three types of natural CDs with GRAS status (α-, β-, and γ-CDs) were used to this end (Fig.1). In the absence of CDs, the mycelium grew very little and, after five weeks, the colony diameter measured only 0.65 cm. Interestingly, γ-CD was not only unable to stimu-

late mycelium growth but even produced a slight decrease in colony diameter, although the difference with respect to the control assay was not statistically significant . These results suggest that the size of CDs, and their capacity to encapsulate molecules of a different size, is essential for a significant effect, which indicates that a molecular interaction exists between CDs and specific molecules present in the culture medium. However, the presence of the other two natural CDs produced an important increase in colony diameter. After five weeks of cultivation, the colony diameters reached 3.90 cm and 2.80 cm in the presence of 8 mM of β-CD and α-CD, respectively (Fig. 1).

On the other hand, the mycelium growth curve observed in the presence of α-CD was in agreement with the growth model proposed for several ectomycorrhizal fungi and similar to the growth curves reported by other authors (Navarro–Ródenas 2011) for *T. claveryi* in different culture conditions. These growth curves showed an initial lag phase followed by an exponential growth phase and a maximum rate phase, before growth slowed and the colony finally became inactive.

***T. claveryi growth in the presence of modified CDs***

To compare the effect on mycelium growth of the addition of modified CDs with that observed using natural β-CDs, three parameters (final colony diameter, growth rate and colony fresh weight) were determined after five weeks of culture. Although the colonies cultivated in the presence of 8 mM methyl-β-CD reached the maximum final diameter and growth rate, followed by β-CD and HP-β-CD, the differences between the three treatments were not statistically significant. With respect to the colony fresh weight, the results are even more similar, although a slightly higher fresh weight was recorded when β-CD was added to the culture medium.

The results, together with the fact that only the natural CD has GRAS status and, moreover, it is cheaper than methyl-β-CD confirm that the most appropriate CD to stimulate the growth of *T. claveryi* mycelium is β-CD.

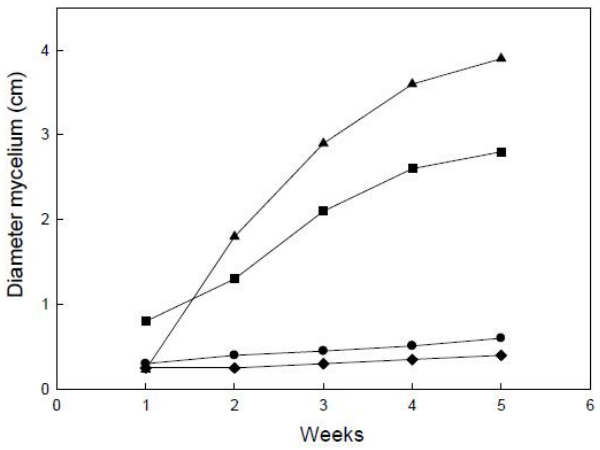


**Figure 1. Effect of the addition of different types of CDs to the culture medium on the mycelial growth (cm) of T. claveryi. (●) control; (♦) γ-CD; (■) α-CD and (▲) β-CD.**

## CONCLUSIONS

Encapsulant agents CDs are able of stimulate the mycelium growth of the desert truffle *T. claveryi*, increasing the values of colony diameter, growth rate and colony fresh weight after cultivation.

The increase in mycelium growth observed when CDs are added to the culture medium must be due to the formation of an encaspsulation complex and not to the use of CDs as a carbon source.

β-CD is seen to be the most effective natural CD to stimulate the mycelium growth of the T. claveryi. The inner diameter of the hydrophobic cavity of β-CD, corresponding to a structure formed by seven molecules of glucose, leads to a more favorable interaction between the CDs and the different molecules present in the culture medium that otherwise hinder the correct growth of this desert truffle.

Although modified CDs are more widely used than natural CDs to complex different guest molecules, the data presented in this work indicate that the chemical modification of β-CD does not improve the growth of *T. claveryi* mycelium.

## REFERENCES


- Navarro-Ródenas A. et al. (2011) *Effect of water stress on in vitro mycelium cultures of two mycorrhizal desert truffles*. Mycorrhiza 21, 247–253


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