

Correlation between Tolerance to 2-deoxy-D-glucose and Productivity of Strains of *Pleurotus ostreatus*

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ABSTRACT: The effect of several concentrations of the toxic analogue of glucose, 2-deoxy-D-glucose (2-DG), was observed on the rate of apical growth of strains of *Pleurotus ostreatus* using different carbon sources. The growth of the strains under these conditions distinguished between tolerant and sensitive strains. It was observed that the phenotype which was tolerant to 2-DG was correlated with high productivity of the strains in pilot production farms.

1 INTRODUCTION

Edible mushrooms degrade ligninocellulolytic substrates; they are important for their economic and nutritional value. *Pleurotus ostreatus* is one of the cultivated edible mushrooms produced in the largest quantity around the world, and the second most cultivated after *Agaricus bisporus* (Oliver and Delmas 1987, Guinberteau 1990). Given its industrial importance, several studies have been carried out in order to find out more about the relationship between the behaviour of the strains at the laboratory level and at the production level (Macaya-Lizano 1975).

In previous research, strains of other genera with derepressed phenotypes were detected by their growth on 2-deoxy-D-glucose (2-DG). It has been reported that 2-DG resistant strains of *Saccharomyces cerevisiae* have a derepressed level of several enzyme systems (Heredia and Sols 1964, Entian 1981, Entian and Mecke 1982). In *Neurospora crassa*, it has been found that *dgr* mutants were pleiotropic because they showed increased levels of both glucose and also of saccharase and amylase activ-

ities (Allen *et al.* 1989) and some *dgr* strains of *Aspergillus niger* produced citric acid faster than the parental, 2-DG sensitive strain (Kirimura *et al.* 1993). On the basis of these findings, it may be possible to use this toxic analogue to detect strains of *Pleurotus ostreatus* with increased metabolism which might be more productive. Such derepressed phenotypes in the strains could be detected by observing fungal growth differences supported either by complex carbon sources or by simple ones, in the presence of various levels of the analogue 2-DG. It is hoped that derepressed phenotypes could have a shorter and more productive reproductive cycle (Sanchez and Viniestra-Gonzalez 1996).

In *Coprinus cinereus*, the value of mutants resistant to growth inhibitions caused by sugar analogues has been discussed and changes during hyphal differentiation similar to that which occurs in tissues of the fruiting body has been observed (Moore and Stewart 1971, Moore 1981).

A procedure for detecting 2-deoxy-D-glucose (2-DG) tolerant strains of *P. ostreatus* in starch medium is described in this work. It has been found that 2-DG tolerance of the strains is correlated with high productivity of 'oyster mushrooms' in pilot scale mushroom production farms.

2 MATERIALS AND METHODS

2.1 Strains

The strains of *P. ostreatus* studied were the following: 32783, 38537 and 58052 from the ATCC collection (Peoria, IL U.S.A.) and UAT PO3, UAT PO4 and UAT PO7 from the collection at Universidad Autónoma de Tlaxcala (Tlaxcala, México).

2.2 Inocula source

The strains were cultured in Czapek-Dox medium at 25°C for 7 days in Petri dishes. The mycelial mass developed on the agar plates was cut using a borer of approximately 4 mm diam and used as the source of inocula.

2.3 Culture media

The culture media were prepared using the following carbon sources: 10.5 g l⁻¹ maize starch (from J. T. Baker in Mexico and from Fluka in England), 10.5 g l⁻¹ maize starch + 0.01 g l⁻¹ 2-DG, 10.5 g l⁻¹ maize starch + 0.1 g l⁻¹ 2-DG, 10.5 g l⁻¹ maize starch + 1.0 g l⁻¹ 2-DG, 10.6 g l⁻¹ glucose, 10.6 g l⁻¹ glucose + 0.01 g l⁻¹ 2-DG, 10.6 g l⁻¹ glucose + 0.1 g l⁻¹ 2-DG, 10.6 g l⁻¹ glucose + 1.0 g l⁻¹ 2-DG, 10.0 g l⁻¹ fructose (crystalline, Sigma), 10.0

g l⁻¹ fructose + 0.01 g l⁻¹ 2-DG, 10.0 g l⁻¹ fructose + 0.1 g l⁻¹ 2-DG, 10.0 g l⁻¹ fructose + 1.0 g l⁻¹ 2-DG.

Furthermore, all of them also contained a mineral salts medium, which was modified from that of Macaya-Lizano (1975) and Eger (1978); it consisted of (g l⁻¹): (NH₄)₂SO₄, 1.0; KH₂PO₄, 0.5; MgSO₄.7H₂O, 0.5; CaH₄(PO₄)₂.H₂O, 0.3; FeSO₄.7H₂O, 0.02; ZnSO₄.7H₂O, 0.02; MnSO₄.H₂O, 0.02. The pH of the medium was adjusted to 6.5 before and after sterilisation using KOH. Agar plates were made using a final concentration of 17 g l⁻¹ agar.

2.4. Rate of apical growth

The culture media inoculated were incubated at 25°C and each treatment was measured in three different Petri dishes. The radius of each colony was measured daily from the second to the sixth day of incubation, using a Shadomaster projector at magnification X10 (J. E. Baty & Co. Ltd. Burgess Hill, Sussex, England), these data were analysed by linear regression obtaining the rate of apical growth in mm hr⁻¹.

2.5 Evaluation at pilot production level

The experiments at the pilot production level were done using polyethylene bags filled with 5 kg of pasteurised wheat straw at 85°C during 1 h and inoculated with mycelial spawn grown in wheat grains. Each strain was inoculated separately in 10 bags and incubated at 25°C in a dark room for 15 days, then they were moved to the production room at 21°C and humid atmosphere. Observations were made in order to assess the day of emergence of the fruiting bodies on the cultures. The basidiomes produced were collected and weighed (during three harvests) and their data was recorded for each sample and date.

2.6 Data analysis

Analysis of variances (ANOVA) and Duncan tests for mean values were carried out using the SAS program. The equations that were used for estimating biological efficiency of mushroom production, *E*, and productivity, *PR*, were as follows:

$$E = \Delta P / M_0 \quad (1)$$

Where, ΔP was the amount in kg of edible mushrooms produced as fresh product and M_0 the initial amount (kg) of raw material introduced in the bag (as dry material). Efficiencies were evaluated after three successive harvests in the same bag.

$$PR = (E/\Delta t) \times 100g \text{ kg}^{-1} \quad (2)$$

where, Δt , was the sum of all the harvesting times.

3 RESULTS

3.1 Rates of apical growth of *P. ostreatus* using starch, glucose and fructose as different carbon source and several concentration of 2-DG

Figure 1 shows the effect of the 2-DG on the rate of apical growth of several strains of *P. ostreatus*, obtained in superficial cultures made with maize starch as the main carbon source. The growth of the strains in maize starch plus 0.1 g l⁻¹ of 2-DG enabled them to be distinguished because three strains UAT PO4, UAT PO7 and ATCC 38537 that grew on this culture medium also had a high level of productivity (Table 1). These were twice as productive as three other strains UAT PO3, ATCC 58052 and ATCC 32783 that were 2-DG sensitive failing to grow in this medium.

Figure 2 shows the effect of the 2-DG on the rate of apical growth, but in this case glucose was used as the main carbon source.

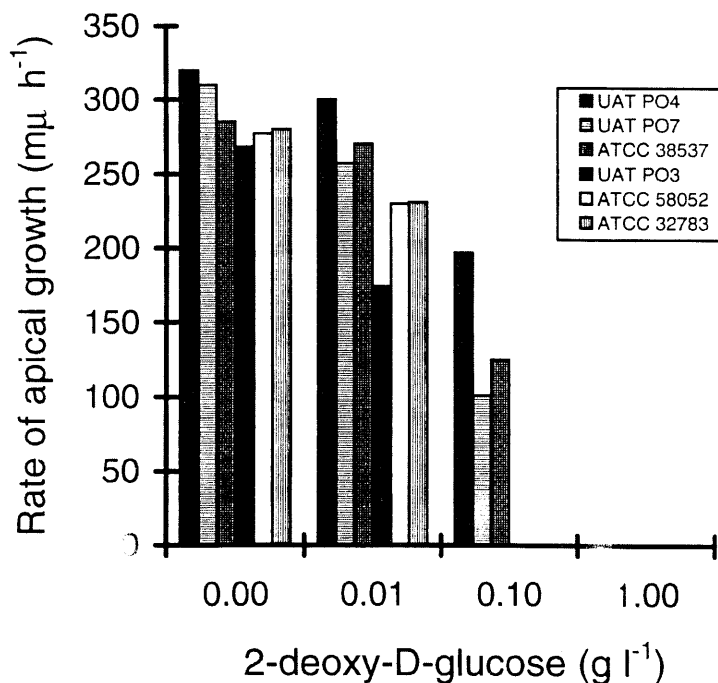


Fig. 1. Rate of apical growth of several strains of *Pleurotus ostreatus* on maize starch as carbon source.

Table 1. Production indices of several strains of *Pleurotus ostreatus* grown on wheat straw.

Production index	UAT PO4	UAT PO7	ATCC 38537	UAT PO3	ATCC 58052	ATCC 32783
Fructification ^a	19	1	23	26	38	53
	±2.2	±2.6	±1.4	±4.3	±3.5	±1.6
1st harvest ^b	28	26	29	35	44	59
	±2.4	±2.3	±2.6	±2.8	±5.6	±1.0
2nd harvest	39	43	43	57	55	0
	±4.2	±4.06	±7.1	±9.7	±2.9	
3rd harvest	56	63	52	81	63	0
	±6.8	±7.4	±2.1	±19.3	±1.2	
Efficiency ^c	1.00	0.97	0.85	0.93	0.35	0.36
Productivity ^c	17.9	15.	16.3	11.5	5.5	6.1

^a Expressed in days (including time of incubation).

^b Expressed in kg of edible mushroom per kg of wheat straw (Eq. 1).

^c Expressed as average daily mushroom production in g per kg of wheat straw (Eq. 2).

Complete inhibition of mycelial growth was not observed in any of the media assayed here. On the other hand, the 2-DG tolerant strains showed an increase of the rate of apical growth when 2-DG was added to glucose medium at concentration of 0.01 or 0.1 g l⁻¹, with respect to those without 2-DG. Stimulation of apical growth by 2-DG was not observed in the strains that did not grow well in starch + 2-DG mixtures (strains UAT PO3, ATCC 58052 and ATCC 32783).

Figure 3 shows the effect of adding increasing concentrations of 2-DG on the rate of apical growth of different strains of *P. ostreatus*, and using fructose as the carbon source. The strains UAT PO4, UAT PO7 and ATCC 38537 (tolerant strains) grew in the medium that contained 0.1 g l⁻¹ of 2-DG, while the strain UAT PO3 (sensitive strain) did not grow and the strains ATCC 58052 and ATCC 32783 (sensitive strains) grew but with a day delay.

3.2 Pilot production of basidiomes of *P. ostreatus* on ground wheat straw

Table 1 shows the production indices of several strains of *P. ostreatus*. The strains with the phenotype 2-DG tolerant (UAT PO4, UAT PO7 and ATCC 38537) had the shortest fructification times (19, 17 and 23 days, respectively); whereas the 2-DG sensitive strains (UAT PO3, ATCC 58052, ATCC 32783) had longer fructification times (26, 38 and 53 days, respectively). Also the values of the biological efficiency, E, of 2-DG tolerant strains were in the range from 85% to 100%, whereas the sensitive strains had E values in the range from 35 to 93%. Productivity, PR, was

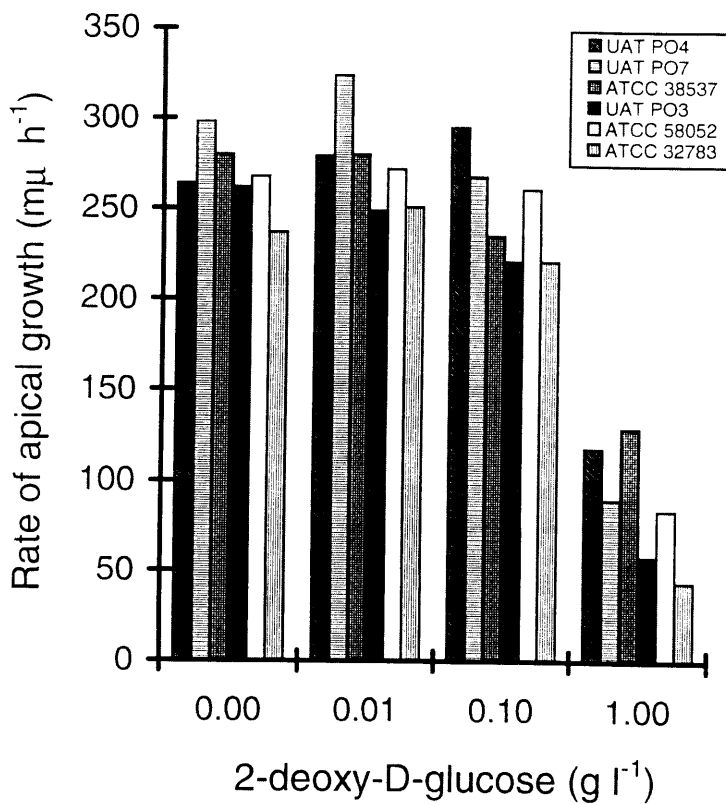


Fig. 2. Rate of apical growth of several strains of *Pleurotus ostreatus* on glucose as a carbon source.

measured in terms of g of edible biomass (basidiomes) per day and per kg of raw material (wheat straw). Clearly, average PR was superior for 2-DG tolerant strains (PR = 16.5 (1.2 g kg⁻¹ d⁻¹) as compared to average PR of 2-DG sensitive strains (PR = 7.7 (3.3 g kg⁻¹ d⁻¹).

4 DISCUSSION

The use of different carbon sources and increasing concentrations of 2-deoxy-D-glucose allowed the strains of *Pleurotus ostreatus* to be categorised for sensitivity or tolerance to this compound. The growth of the strains depends on the relative abilities of the carbon source to inhibit uptake of the analogue into the cell (Moore 1981).

The use of maize starch as a carbon source and 0.1 g l⁻¹ of 2-DG revealed a clear difference in terms of 2-DG tolerant phenotype. Clearly, the strains with this phenotype (UAT PO4, UAT PO7 and ATCC 38537) selected

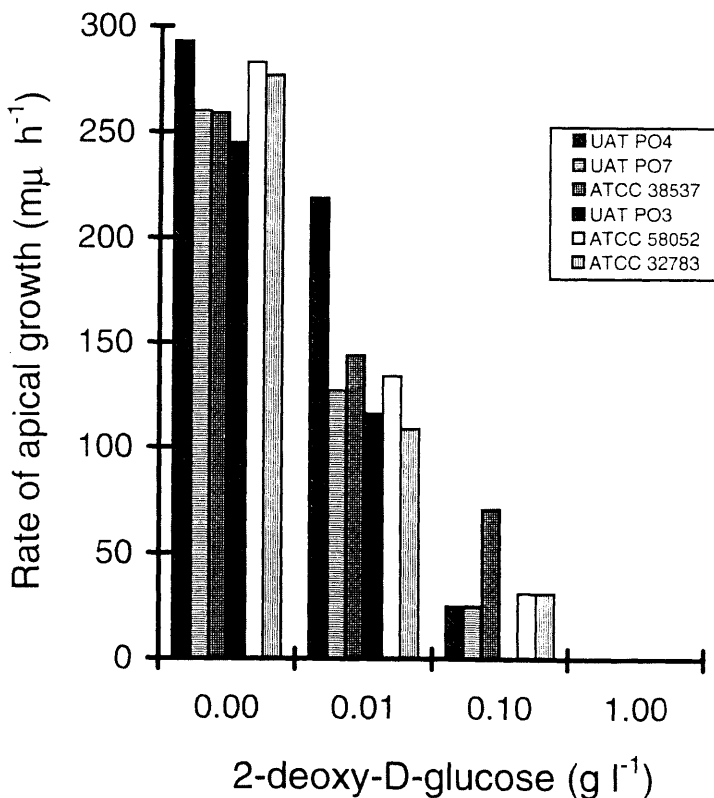


Fig. 3. Rate of apical growth of several strains of *Pleurotus ostreatus* with fructose as a carbon source.

on agar plates were the most productive at the level of basidiome production. Similar results on tolerance of the strains were obtained when they were grown on fructose as carbon source, however in this case the difference between both phenotypes were not so evident, because although the UAT PO3 strain (2-DG sensitive phenotype) did not grow when the 2-DG concentration was 0.1 g l⁻¹, the ATCC 58052 and ATCC 32783 strains (2-DG sensitive phenotype) grew after a delay of one day. Using glucose as carbon source, tolerant strains showed a slight increase of rate of apical growth at the concentration of 0.01 or 0.1 g l⁻¹ of 2-DG with respect to those values without 2-DG. This effect was not observed in the sensitive strains.

The statistical analysis showed that the UAT PO4 strain was significantly different, presenting the highest mean values of rate of apical growth in all the culture medium tested and was also the most productive in a production plant. Therefore, determining the tolerance to 2-DG at laboratory level of several strains could be a way of finding those more

productive at a production level. This may be a very practical method for selecting strains to be cultivated in the mushroom production industry.

A possible explanation for this is that 2-DG produces a metabolic stress, which could possibly be used to identify the mutant strains to this compound and detect those that could have in some way increased their metabolic activities. However, the reasons for such a correlation between 2-DG tolerant phenotype and productivity in the *P. ostreatus* strains, are unclear. It might be assumed that the 2-DG tolerant strains observed in starch are partly derepressed for producing amylases and grow faster in complex substrates than those that are more sensitive to this analogue. Alternatively, the response may be related to interaction between glucose analogues and wall synthesis, which is probably linked to fungal morphogenesis (Gooday 1972, Craig *et al.* 1981, de Rousset-Hall and Gooday 1975, Moore 1981, Wessels *et al.* 1990).

These studies have shown that 2-DG affects morphogenesis in some way, but the precise way is not clear. Therefore, our future experiments will be focused on fungal wall and cell morphogenesis, given that the major effect of the 2-DG is on the cell wall (Moore 1981) and that studies carried out in *C. cinereus* and *A. bisporus* have demonstrated that the chitin synthesis is essential for the elongation of the stipe hyphal wall.

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