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# Utilization of Cellobiose and D-Glucose by *Clostridium thermocellum* ATCC-27405

P. E. Hernández *, J. A. Ordóñez and B. Sanz

Departamento de Higiene y Microbiología de los Alimentos
Facultad de Veterinaria, Universidad Complutense
28040 Madrid (Spain)

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Cultures of *Clostridium thermocellum* ATCC-27405, maintained on cellulose and not adapted to grow on glucose utilize cellobiose preferentially over D-glucose, and are only able to initiate growth on D-glucose when the cellobiose has been exhausted from the growth medium. However, D-glucose is the carbon source preferentially utilized when cultures of this microorganism, previously adapted for growth on glucose, are transferred to a medium with equivalent concentrations of both sugars. One reason for the preferential utilization of glucose over that of cellobiose might be the competitive inhibition of cellobiose phosphorylase by intracellular glucose accumulation. When in the glucose-adapted cultures the pressure to grow on glucose as the sole carbon source is again released, both sugars can be simultaneously utilized.

**Key words:** *Clostridium thermocellum,* Thermophilic bacteria, Utilization of cellobiose, Utilization of D-glucose.

*Clostridium thermocellum* is an anaerobic, thermophilic and cellulolytic microorganism (9, 11), potentially useful for the direct conversion of cellulosic biomass to ethanol (2, 8, 12, 13). *C. thermocellum* can grow on cellulose, cellobiose or D-glucose as sole sources of carbon and energy (14), but an extended lag time is observed before growth on D-glucose occurs. By successive transfers on glucose, cultures are adapted to grow on glucose without that extended lag period (14). When *C. thermocellum* grows on cellulose, cellobiose and D-glucose are always present in the fermentation broth as end products of the extracellular enzymatic degradation of this complex substrate, and it could be, therefore, that cellobiose and D-glucose regulated the utilization of each other through an effect on their uptake and/or metabolism (5-7). We have investigated the kinetics of utilization of cellobiose and D-glucose when cultures of *C. thermocellum* were exposed to a mixture of these sugars at equivalent concentrations.

* To whom all correspondence should be addressed.

## Materials and Methods

*Clostridium thermocellum*, strain ATCC-27405, was obtained from the American Type Culture Collection, Rockville, Maryland. The growth of the microorganism was carried out as previously reported (4). The glucose-adapted cultures were obtained by growth of *C. thermocellum* for many generations on D-glucose as the sole source of carbon and energy. After three more transfers on CM-4 glucose, tubes containing CM-4 medium and any of the carbon sources were inoculated. The CM-4 medium contains (g/l): yeast extract, 10.0; $(NH_4)_2SO_4$, 1.3; $MgCl_2$, 0.75; $CaCl_2$, 0.1; $K_2HPO_4$, 2.9; $KH_2PO_4$, 1.5; sodium thioglycolate, 0.5; $FeSO_4$, $1.25 \times 10^{-6}$ and resazurin $2 \times 10^{-6}$. The cellobiose phosphorylase activity was measured by the method of ALEXANDER (2), with some modifications. The composition of the reaction mixture was (per ml): 3,3-dimethylglutaric acid buffer, pH 6.0, 64 mM; $MgSO_4$, 10 mM; EDTA, 0.015 mM; glucose-1-6-bisphosphate, 1.22 $\mu$M; NADP, 0.3 mM; phosphoglucomutase, 10 $\mu$g; glucose-6-p-phosphate dehydrogenase, 1.2 U; $Na_2HPO_4$, 25 mM; cellobiose, 0.5 to 20 mM, and the appropriate concentration of cell free extract. The cell free extracts were obtained according to HERNÁNDEZ (6). The reduction of NADP was measured by the absorbance increase at 50°C and 340 nm. When specified, the experimental data were subjected to multiparametric nonlinear regression analysis. The growth and utilization of sugars by *C. thermocellum* growing in a mixture of cellobiose and D-glucose was measured when cultures of this microorganism, either non-adapted to grow on glucose, or glucose-adapted, were inoculated into CM-4 medium containing cellobiose and D-glucose each at a concentration of 1.5 g/l. The kinetics of growth and the concentrations of cellobiose and D-glucose in the medium were determined periodically. D-glucose in the medium was determined by the colorimetric assay of D-glucose oxidation with glucose oxidase (Sigma, Diagnostic Kit N.° 510). Total reducing sugars were calculated according to the dinitrosalicylic acid (DNS) method (10). All values were corrected for the levels of non-utilizable reducing sugars present in CM-4 medium.

## Results and Discussion

When cultures of *Clostridium thermocellum* maintained on cellulose were exposed to a mixture of cellobiose and D-glucose (fig. 1), the first carbon source utilized was cellobiose and no glucose was consumed during the first 8 hr of growth. In these cultures, the ability to utilize glucose appeared after an extended lag time, if the pH of the medium after the comsumption of cellobiose was adequate for the expression of this function (results not shown). In contrast, when the glucose-adapted cultures were exposed to the same mixture of cellobiose and D-glucose, the preferred carbon source was glucose (fig. 2). Moreover, cellobiose was not utilized until all the glucose was exhausted from the medium.

Further experiments were carried out to study the nature of the regulatory process by which D-glucose could control the utilization of cellobiose in the glucose-adapted cultures. D-glucose may affect the utilization of cellobiose either by interfering with its uptake or its metabolism. The mechanism by which cellobiose is transported in this microorganism is not yet known and in the present work we examined the effect of D-glucose on the activity of cellobiose phosphorylase, the first enzyme involved in the metabolism of cellobiose (1, 2). The cellobiose phosphorylase activity (fig. 3), was strongly inhibited by the presence of D-glucose in the cell free

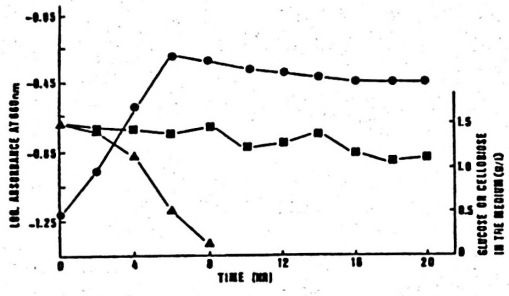

Fig. 1. *Growth (●), glucose utilization (■) and cellobiose utilization (▲), by* C. thermocellum *maintained on cellulose, on a mixture of cellobiose and glucose at 1.5 g/l each.*

extracts of the glucose-adapted cultures. Data analysis by non-linear regression indicated that the pattern of inhibition corresponded to a competitive one, the Ki of inhibition by D-glucose being $0.41 \times 10^{-3}$ M. These data indicates that at least one of the mechanisms by which glucose is utilized preferentially to cellobiose may be by intracellular accumulation of D-glucose which inhibits competitively the activity of cellobiose phosphorylase. This inhibition may be due to the fact that glucose is one of the products of the cellobiose phosphorylase reaction (1, 2).

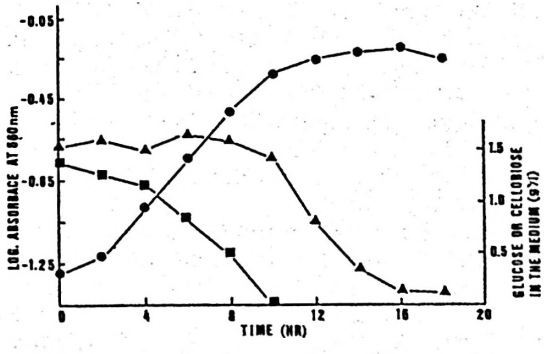

Fig. 2. *Growth (●), glucose utilization (■) and cellobiose utilization (▲), by glucose-adapted* C. thermocellum, *on a mixture of cellobiose and D-glucose at 1.5 g/l each.*

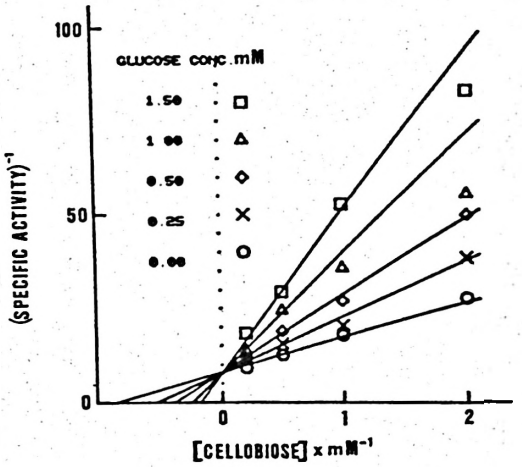


Fig. 3. *Inhibition by D-glucose of the cellobiose phosphorylase activity on cell free extracts of glucose-adapted* C. thermocellum. Data analysis by non-linear regression. The specific activity is expressed as micromoles of NADPH/min × mg cell dry weight.

Glucose-adapted cultures of *C. thermocellum* were also grown for at least 100 generations in CM-4 cellobiose, and for 15 generations in CM-4 cellulose to determine if the release of adaptation to growth on glucose as the sole carbon source affected the glucose utilization system previously acquired. The cultures were further transferred to a mixture of cellobiose and glucose each at 1.5 g/l and, as seen from figure 4, although glucose was still preferentially utilized, both sugars were utilized simultaneously. To explain the latter result two hypotheses may be advanced. One is that the growth of the glucose-adapted cultures for many generations on cellobiose and D-glucose was gradually selecting for a culture that was overcoming the effect of D-glucose over the cellobiose phosphorylase activity. The second hypothesis has to assume that the culture which utilizes simultaneously cellobiose and D-glucose consists, in fact, of a mixed population of cells independently utilizing either cellobiose or D-glucose. For-

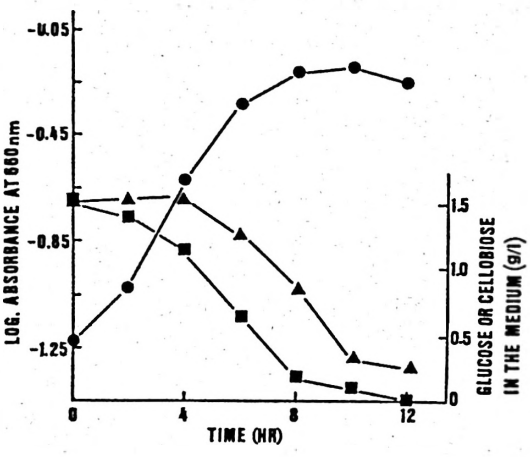


Fig. 4. *Growth (●), glucose utilization (■) and cellobiose utilization (▲), by* C. thermocellum *cultures first adapted to grow on glucose, then maintained for 100 generations on cellobiose and finally for 15 generations on cellulose.* Cells were finally transferred to a mixture of cellobiose and D-glucose at 1.5 g/l each.

mally, this would be possible if a turn on-turn off kind of genetic switch could regulate the ability of *C. thermocellum* to grow on D-glucose.

Taken together these results suggest that even in the glucose-adapted cultures, *C. thermocellum* ATCC-27405 tends in the presence of cellobiose, to switch towards the progressive utilization of this carbohydrate over D-glucose to sustain its growth. We have previously demonstrated that this may be due (6, 7) to the fact that cellobiose is energetically more favorable than D-glucose to sustain the growth of this microorganism. Such a preferential utilization of cellobiose over D-glucose is of practical importance in the fermentation industry. With the results obtained by us it is possible to predict that, if this microorganism is employed in the industrial transformation of cellulosic biomass to ethanol and cellulolytic enzymes, the D-glucose should be either considered as a by-product of the fermentation process or as a substrate of fermentation if a strictly controlled glucose-adapted inoculum and/or continuous fermentation programs are employed.

## Resumen

Los cultivos de *Clostridium thermocellum* ATCC-27405, mantenidos en celulosa y no adaptados a crecer en glucosa, utilizan preferencialmente como sustrato de crecimiento celobiosa y no glucosa. Sin embargo, la glucosa es la fuente de carbono preferencialmente utilizada cuando cultivos de este microorganismo adaptados a crecer en glucosa, crecen en un medio de concentraciones equivalentes de estos azúcares. La utilización preferencial de la glucosa sobre la celobiosa se debe a la inhibición competitiva de la enzima celobiosa fosforilasa por la acumulación intracelular de glucosa. Cuando en los cultivos adaptados a crecer en glucosa se reduce la presión ejercida para crecer en glucosa como única fuente de carbono entonces estos cultivos utilizan celobiosa y glucosa simultáneamente.

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