PHOSPHORILASES INDUCTION BY DIFFERENT CARBON SOURCES IN FOUR PREDOMINANT RUMEN BACTERIA
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ABSTRACT

The activities of some phosphorylase enzymes, e.g. cellulobiose phosphorylase, celllobiose phosphorylase, maltose phosphorylase and lactose phosphorylase, have been examined in some rumen bacteria e.g. R. flavefaciens 17, B. fibrisolvens OR77, P. bryantii B4 and S. bovis A30 in vitro. The cellbiose phosphorylase activity was observed in R. flavefaciens 17 and B. fibrisolvens OR77. The activity of cellbiose phosphorylase was detected in R. flavefaciens 17, B. fibrisolvens OR77 and P. bryantii B4. Lactose phosphorylase activity was found in B. fibrisolvens OR77, P. bryantii B4 and S. bovis A30. While maltose phosphorylase activity was detected only in S. bovis A30.

The induction of phosphorylases by some carbon sources was investigated. Cellulobiose phosphorylase in R. flavefaciens 17 and P. bryantii B4 is a constitutive enzyme but in B. fibrisolvens OR77, it is inducible. Maltose has induced the maltose phosphorylase in S. bovis A30. The time-course experiments revealed that the cell growth and the pattern of enzyme production in R. flavefaciens 17 occurred within 24 hr incubation time. The relationship between endoglucanase and cellbiose phosphorylase was observed in R. flavefaciens 17 and it was concluded that the production of cellbiose phosphorylase is parallel with the production of endoglucanase. All phosphorylases differ in their affinity and specificity for substrate and physiological roles. The maximum growth was observed in the period between 18-21 hr incubation in all carbon sources.

The high level of cellbiose phosphorylase activity was present in cellulose medium after 24 hr of incubation at 37°C. Cellulobiose phosphorylase activity was found in cellbiose, cellolodextrins, xylan or cellulose-grow cells. The high level of activity was with cellbiose, cellolodextrins and xylan.

Keywords: Rumen bacteria, phosphorylase, fiber, cellulose, cellbiose, cellolodextrins, xylan.

INTRODUCTION

The rumen bacteria are members of a microbial consortium that perform several functions vital to the well-being of the host animal such as the degradation of fiber and other polymeric plant material which are not degraded by the host animal, synthesis of microbial protein, synthesis of certain vitamins and degradation of some toxic compounds of the diet.

Since bacterial energy sources in the rumen are often limited and the yield of ATP is generally low in anaerobic rumen bacteria, efficient utilization of the substrate is important for growth of rumen bacteria (Martin and Wani, 2000).

Considerable research efforts have been devoted to the manipulation of rumen metabolism to improve the transformation of poor quality feeds into
milk and meat. The developments in recombinant DNA techniques and molecular biology have led rumen microbiologists to apply these techniques to the rumen microflora (Smith and Hespell, 1983). The main aims of the manipulation of the rumen microbial ecosystem are:

1. To improve fiber digestion.
2. To reduce protein degradation or to produce amino acids.
3. To modify the ratio of the fermentation products.
4. To inhibit the growth and metabolic activities of undesirable organisms.
5. To obtain bacterial production of substances that are of benefit to the host metabolism.

Plant cell wall is the most abundant energy source in the world. Improvements of the plant materials utilization may play an important role for narrowing the energy gap. Ruminants can change the plant cell walls to protein food (meat, milk, cheese etc.) by the help of microorganisms, which inhabit their rumen. Contribution of the cellulolytic bacteria to this function is especially important. Cellulose degradation is realized by a mixture of cellulolytic enzymes, such as endo-1,4-glucanase (E.C. 3.2.1.4), cellobiohydrolase (E.C. 3.2.1.91) and β-glucosidase. It is important to clarify the enzyme system employed in the control of cellulose degradation in the rumen. Unlike soil clostridium or soil fungi that are used for industrial cellulase production, little is known about the enzyme system of the rumen cellulolytic bacteria.

Utilization of plant materials by ruminants depends upon the microbial consortium of the rumen. This process is slow and incomplete, and enhancement of the fiber-hydrolyzing activity of the rumen bacteria to increase the efficiency of ruminal metabolism and animal reproduction. The microbial degradation of plant cell wall material in the rumen is the key to the nutrition of ruminant animal. The energy source in forages exists primarily as structural polysaccharides. The cellulose microfibrils are linked to xylans by hydrogen bonds and therefore the accessibility of the cellulotic materials to hydrolytic enzymes requires the removal of the matrix embedding the cellulose microfibrils. Thus, ruminal bacteria are challenged with a complex heterogeneous substrate requiring the elaboration of an array of enzymes, notably cellulases and xylanases. The predominant cellulose-degrading bacteria in the rumen are *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens*, whereas the major hemicellulose utilizers include the *Ruminococcus* sp., *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*. The physiology, enzymology, and genetics of cellulose degradation by rumen bacteria has been studied, but many questions remain unanswered. The cellulolytic enzyme system has been examined in some depth and has been shown to be complex, involving numerous enzymes. Therefore, the objectives of this study are investigating:

a) the distribution of some phosphorylases enzymes in some rumen bacterial species
b) phosphorylases induction by some carbon source and
c) selecting of the highest activity for cellobiose phosphorylase in selected predominant rumen bacteria.
MATERIALS AND METHODS

Ruminococcus flavefaciens as a cellulolytic rumen bacteria, Butyrivibrio fibrisolvens as a hemicellulolytic rumen bacteria, Prevotella bryantii as a hemicellulolytic rumen bacteria and Streptococcus bovis as an amylolytic rumen bacteria are four of the most predominant bacteria isolated from the rumen were used in this present work in order to investigate a) the distribution of some phosphorylases enzymes in some rumen bacterial species b) phosphorylases induction by some carbon source and c) selecting of the highest activity for cellobiose phosphorylase in selected predominant rumen bacteria all bacteria are tested in vitro.

1. Bacterial strains and culture media
1.1. Ruminococcus Flavefaciens 17
Ruminococcus flavefaciens 17 kindly provided by H. J. Flint, Rowett Research Institute, Greenburn, Bucksburn, Aberdeen AB2 9SB, UK, as a source of genomic DNA. It was grown anaerobically at 37°C in M2 medium (Bryant, 1972) as described previously (Flint et al., 1989) whose composition it is as follows: mineral solution (a) 150 ml, mineral solution (b) 150 ml, rumen fluid 150 ml, deionized water 550 ml, bacto casitone 10 g, yeast extract 10 g, cellobiose 0.2% and resazurin 100 µl. The pH is adjusted to 6.8 in addition to 0.5 cysteine-HCl and 2.5 g NaHCO₃ were added to the medium.

1.2. Prevotella bryantii B4
Prevotella bryantii B4 is provided kindly from H. J. Flint, Rowett Research Institute, Greenburn, Bucksburn, Aberdeen AB2 9SB, UK.

1.3. Butyrivibrio fibrisolvens OR77 and Streptococcus bovis A30
These strains were provided from Dr H. Minato, Ibaraki University, Japan. Prevotella bryantii B4, Butyrivibrio fibrisolvens OR77, and Streptococcus bovis A30 were grown anaerobically at 37°C on Ruminal Glucose Medium (RGM) which is composed of 170 ml Hungate solution A (6.0 g NaCl, 3.0 g (NH₄)₂SO₄, 3.0 g KH₂PO₄, 0.6 g MgSO₄, 0.6 g CaCl₂ per liter). 170 ml Hungate solution B (3.0 g K₂HPO₄ / l), 300 ml rumen fluid, 500 ml distilled H₂O, 1.0 g trypticase, 0.5 g yeast extract, 0.5 g L-cysteine, 5.0 g NaHCO₃, 100 µl resazurin, 2.0 g glucose. The carbon sources were added as separate sterile solutions, and cultures were grown at 37°C.

2. Cell fractionation
The cells were harvested by centrifugation (15000 rpm for 10 min at 4°C). The pellet was washed twice with either 50 mM Tris- HCl buffer plus 2 mM DTT (dithiothreitol) (pH 6.8, 4°C) for endoglucanase assay. The pellet was suspended in 1 ml of 50 mM Tris-HCl buffer plus 2 mM DTT (pH 6.8, 4°C) and 25 µl of toluene was added for the cells disruption. Then the endoglucanase activity was measured. For cellobiose-phosphorylase (Cellobiose, orthophosphate-D-glycosyltransferase, EC2.4.1.20), cellobiosephosphorylase (1,4-β-D-triglucan:orthophosphate-D-glycosyltransferase, EC2.4.1.49). β-
glucosidase, the pellets suspended in the buffer (Sml). The suspension ultrasonicated (20 times, 30 sec), and centrifuged for 10 min, 15000rpm at 4°C. The precipitate discarded and the supernatant (cell extract fraction) was kept at 4°C for enzyme assays (Helaszek and White, 1991; Lou et al., 1997).

3. Enzyme assays.
Cellulose and cellobiose phosphorylase activities assays were performed by measuring Pi (inorganic phosphorus) formation (Alexander, 1968).
Maltose phosphorylase and Lactose phosphorylase activities were assayed according to the procedure of Lou et al., 1996.
Endoglucanase (CMCase) was assayed according to procedure of Matsushita et al., 1990, Pittepher and Latham, 1979) (α-amylase activity was measured by adding rice starch instead of carboxymethyl cellulose in CMCase reaction mixture (Peterson and Porath, 1966). The protein was determined by the procedure of Lowery et al., (1951). This study is carried out at the Kyoto Prefectural University, Laboratory of Animal Science, Kyoto, Japan.

Cellodextrins preparation

Cellodextrins were prepared by a modification of the method of Freer and Detroy (1982). Sigmoid Cell 20 microcrystalline cellulose (10g) was first mixed with 100 ml of ice-cold concentrated HCl in 500 ml flask and stirred to give a uniform suspension. Ice-cold fuming HCl (100ml) was then poured into the flask to completely dissolve cellulose. A slightly yellow, viscous solution formed after 2 hr of incubation at the room temperature. The HCl was partially removed by application of vacuum for 30 minutes. Cellodextrins were precipitated by the addition of 10 volumes of acetone. The precipitate was washed four times with 5 volumes of acetone and collected by centrifugation (10,000 rpm, 0°C for 15 minutes). The pellet was suspended in 600 ml of deionized distilled water to extract water-soluble cellodextrins, and the solution was stirred overnight in a chemical hood to remove residual acetone. The supernatant was collected after centrifugation, neutralized by passage through an anion exchange column (1.7 by 7.5 cm; Dowex AG1-X8; Bio-Rad) and concentrated to 10-15 ml on a rotary evaporator (45°C).

Statistical analysis:
The statistical analysis was carried out according to Snedecor and Cochran (1967).

RESULTS

1. Bacterial growth

Using several carbon sources, the bacterial cells growth and phosphorylase induction system were tested. The used carbon sources were glucose, cellobiose, lactose, or maltose. P. flavefaciens 17 grew on cellobiose as a growth substrate but no growth was found in glucose, lactose or maltose medium. P. broynati B14 is one of the most numerous ruminal
bacteria and it is a noncellulolytic bacteria rather, hemcellulolytic. It utilized glucose, cellobiose, lactose or maltose as a sole energy source. *B.fibrilsolvens* OR77 is among the predominant species present in the rumen and extensively solubilised the hemcellulose more than cellulose. It was able to grow on glucose, cellobiose, lactose or maltose medium. *S.bovis* A30 is among the most amylytic bacteria found in the rumen. It was able to grow in glucose, cellobiose, lactose or maltose medium.

2. Phosphorylases induction by substrates.

The activity of cellobiose phosphorylase and cellobiose phosphorylase were detected in *R.flavefaciens* 17 in the cellobiose-grown cells. On the other hand, maltose phosphorylase and lactose phosphorylase were not produced in this organism (Table 1). The release of cellobiose phosphorylase in glucose, lactose or maltose-grown cells in *P.bryantii* B.4 was low, the activity was decreased 75% for the cells grown on glucose and lactose, 45% for the cells grown on maltose compared to cellobiose-grown cells. No activity was detected for cellobiose phosphorylase or maltose phosphorylase in this organism. Lactose phosphorylase activity was observed in lactose-grown cells or maltose-grown cells but no activity in the glucose or cellobiose-grown cells. The lactose phosphorylase activity was twice higher in the lactose grown-cells than in maltose grown-cells (Table 2).

Table 1: Effect of carbon source on CEP, CTP, MalP or LacP activities (nmole/min/mg protein) in R.flavefaciens 17

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>CEP</th>
<th>CTP</th>
<th>MalP</th>
<th>LacP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>212.80±1.24</td>
<td>22.66±0.12</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>Lactose</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>Maltose</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

Table 2: Effect of carbon source on CEP, CTP, MalP or LacP activities (nmole/min/mg protein) in P.bryantii B.4.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>CEP</th>
<th>CTP</th>
<th>MalP</th>
<th>LacP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.10±0.20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>16.30±0.08</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.41±0.38</td>
<td>ND</td>
<td>ND</td>
<td>33.23±0.07</td>
</tr>
<tr>
<td>Maltose</td>
<td>9.21±0.38</td>
<td>ND</td>
<td>ND</td>
<td>17.74±0.05</td>
</tr>
</tbody>
</table>

*B.fibrilsolvens* OR77 showed the cellobiose phosphorylase activity in cellobiose, lactose or maltose grown-cells but the activity was too low in glucose-grown cells. The highest activity for cellobiose phosphorylase was found in case of lactose grown-cells followed by cellobiose grown-cells. The
activity was about half that of \textit{R. flavofaciens} 17. Cellulose phosphorylase activity was observed in glucose, cellobiose, lactose or maltose medium and the highest activity was observed in lactose or cellobiose-grown cells of \textit{B. fibrisolvens} OR77. This organism has shown high activity for cellulose phosphorylase than expected. Its activity was greater than twofold the activity in \textit{R. flavofaciens} 17 which grew on cellobiose. Lactose phosphorylase activity was detected in \textit{B. fibrisolvens} OR77, which grew on glucose, cellobiose, lactose or maltose as a sole energy source. The highest specific activity for lactose phosphorylase was found in lactose or maltose-grown cells but the lowest activity was in cellobiose medium. This bacterium did not produce maltose phosphorylase (Table 3).

Table 3: Effect of carbon source on CBP, CTP, MalP or LacP activities (nmole/min/mg protein) in \textit{B. fibrisolvens} OR77.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>CBP</th>
<th>CTP</th>
<th>MalP</th>
<th>LacP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>9.20 ±0.05</td>
<td>54.00 ±3.38</td>
<td>ND</td>
<td>62.26 ±1.74</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>91.37 ±0.60</td>
<td>75.88 ±0.11</td>
<td>ND</td>
<td>15.06 ±1.76</td>
</tr>
<tr>
<td>Lactose</td>
<td>123.43 ±0.62</td>
<td>134.13 ±1.48</td>
<td>ND</td>
<td>137.79 ±0.28</td>
</tr>
<tr>
<td>Maltose</td>
<td>31.39 ±0.33</td>
<td>71.71 ±0.16</td>
<td>ND</td>
<td>136.14 ±0.53</td>
</tr>
</tbody>
</table>

NG, no growth. Mean ± SE

CBP, Cellulose phosphorylase CTP, Cellotriose phosphorylase MalP, Maltose phosphorylase LacP, Lactose phosphorylase

The cellobiose phosphorylase and cellotriose phosphorylase activities were not detected in \textit{S. bovis} A30. This organism produced the lactose phosphorylase and maltose phosphorylase. The activity of lactose phosphorylase was found only in lactose-grown cells. Maltose phosphorylase activity was much higher on maltose-grown cells than glucose, cellobiose or lactose grown-cells (Table 4).

Table 4: Effect of growth substrates on CBP, CTP, MalP or LacP activities (nmole/min/mg protein) in \textit{S. bovis} A30.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>CBP</th>
<th>CTP</th>
<th>MalP</th>
<th>LacP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>ND</td>
<td>ND</td>
<td>43.13 ±0.57</td>
<td>ND</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>ND</td>
<td>ND</td>
<td>48.32 ±0.78</td>
<td>ND</td>
</tr>
<tr>
<td>Lactose</td>
<td>ND</td>
<td>ND</td>
<td>28.52 ±0.11</td>
<td>75.48 ±0.28</td>
</tr>
<tr>
<td>Maltose</td>
<td>ND</td>
<td>ND</td>
<td>102.04 ±1.39</td>
<td>ND</td>
</tr>
</tbody>
</table>

NG, no growth. Mean ± SE

CBP, Cellulose phosphorylase CTP, Cellotriose phosphorylase MalP, Maltose phosphorylase LacP, Lactose phosphorylase

\textit{R. flavofaciens} 17 showed the highest activity for cellulose phosphorylase compared to \textit{B. fibrisolvens} OR77 and \textit{P. bryantii} B4. The activity in \textit{R. flavofaciens} 17 was two fold or 13 fold greater than \textit{B. fibrisolvens} OR77 and \textit{P. bryantii} B4, respectively when the cellobiose was used as a growth substrate. Cellotriose phosphorylase activity was detected in \textit{R. flavofaciens} 17 and \textit{B. fibrisolvens} OR77. The activity of cellulose
phosphorylase in *B. fibrisolvens* ORT7 was 6 fold greater than in *R. flavefaciens* 17.

3. Production of endo-glucanase

Endoglucanase (1,4-β-D-glucan gluco-oxidase, EC 3.2.1.4) activity was examined in *R. flavefaciens* 17, *B. fibrisolvens* ORT7, and *P. bryantii* B4 under various growth conditions. The results reported that all these bacterial strains have the endo-glucanase activity but the activity in *R. flavefaciens* 17 was higher than those in *B. fibrisolvens* ORT7, and *P. bryantii* B4 (Table 5).

<table>
<thead>
<tr>
<th>Carbon source</th>
<th><em>R. flavefaciens</em></th>
<th><em>P. bryantii</em></th>
<th><em>B. fibrisolvens</em></th>
<th><em>S. bovis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* + growth - no growth

4. Effect of carbon sources and time course on both phosphorylase induction and endo-glucanase activities in *Ruminococcus Flavefaciens* 17

From previous results, *Ruminococcus Flavefaciens* 17 was selected to study some physiological aspects, which are related to substrate regulation (specificity), phosphorylase induction system and the relation ship between phosphorylases and endo-glucanase. Cellulose, cellobextrins, cellulbiose or xylan was used as a sole energy source for the growth of *R. Flavefaciens* 17. The composition of prepared cellobextrins was shown in (Fig 1). The maximum growth was observed in the period between 18-21 hr incubation with all carbon sources (Fig 2). The highest growth was observed in cellulbiose medium. *Ruminococcus Flavefaciens* 17 preferentially utilized cellulbiose and xylan then cellobextrins and cellulose as a sole energy source.

The activity of cellulbiose phosphorylase were found in cell extract in *R. Flavefaciens* 17 and influenced by the fiber source. The high level of activity was present in cellulose medium after 24 hr of incubation (Fig 3). Cellulbiose phosphorylase activity was found in cellulbiose, cellobextrins, xylan or cellulbiose-grown cells. The high level of activity was found with cellulbiose, cellobextrins and xylan (Fig 4, 5 and 6).

The β-glucosidase activity was detected in cellulose ,cellobextrins, cellulbiose or xylan-grown cells. The maximum activity of the β-glucosidase was found at 24 hr in cellulose or cellobextrins grown cells at 18-21 hr in cellulbiose or xylan grown cells. The activity in cellulbiose medium was twice in cellulbiose medium as much as cellulose medium. The production of β-glucosidase in cellobextrins or xylan medium was less than in cellulbiose medium by 20%.

6045
Fig. 1: Thin layer chromatography (TLC) for the composition of prepared cellobextrins, a; glucose and cellobiose standard, b; prepared cellobextrins.

Fig 2: Effect of carbon source on cell growth in R. flavofaciens 17. 
* cellulose □ cellobextrins ▲ cellobiose • xylan
Fig. 3: Specific activities (m mol/mg protein) of CTP, CBP, β-glucosidase and CMCase in R. flavefaciens 17 grown on cellulose.

X CTP ■ CBP ▲ β-glucosidase ● CMCase

Fig. 4: Specific activities (m mol/mg protein) of CTP, CBP, β-glucosidase and CMCase in R. flavefaciens 17 grown on cellobextrins.
X CTP ■ CBP ▲ β-glucosidase ● CMCase

6047
Fig. 5: Specific activities (n mol/mg protein) of CTP, CBP, β-glucosidase and CMCase in R. flavefaciens 17 grown on xylanose.

X CTP ■ CBP ▲ β-glucosidase ● CMCase

Fig. 6: Specific activities (n mol/mg protein) of CTP, CBP, β-glucosidase and CMCase in R. flavefaciens 17 grown on xylan.

X CTP ■ CBP ▲ β-glucosidase ● CMCase

6048
DISCUSSION

Phosphorylases are omnipresent enzymes that are found in many organisms, including bacteria, yeast, slime mold, plants and vertebrates. There are many phosphorylases identified, e.g., maltodextrin phosphorylase, maltose phosphorylase, cellobiose phosphorylase, cellobextrins phosphorylase, sucrose phosphorylase and glycogen phosphorylase. These enzymes catalyse the reversible cleavage of polysaccharides, oligosaccharides, or disaccharides into α-D-glucose-1-phosphate and hence play a central role in carbohydrate metabolism. Phosphorylase enzymes from plant, mold, yeast and human origins have been studied in some details but a few studies have been focused on phosphorylases in rumen bacteria. Therefore, information on phosphorylases will help elucidate its potential role in cellulolysis and is also necessary for a comprehensive understanding of carbohydrate utilization by rumen bacteria. Cellulolytic bacteria usually degrade cellulose by the synergistic action of endo- and exo-glucanase (Chang et al., 1982/87), but this process is potentially inhibited by accumulation of soluble end products e.g. cellobiose and cellulose (Johnson et al., 1982).

Therefore, the activity of cellobiose, cellobiose, maltose and lactose phosphorylases were investigated in this study in some rumen bacteria e.g. R. flavefaciens 17, P. bryantii B4, B. fibrisolvens OR77, or S. bovis A30. These bacterial species were selected in this study to represent cellulolytic, hemi-cellulolytic, non-cellulolytic and amylolytic bacteria in the rumen. The induction system of cellulases still remains unclear. Recently, it was found that most of induction system are carried out by two components regulatory system. When sensor and regulatory protein perceives an inducer (input signal), histidine residue of the protein is phosphorylated. Then, the phosphorylated system is transferred to the regulatory protein. Finally, the phosphorylated regulatory protein is converted into active form and induces the target gene expression. This experiment also, investigated the phosphorylases induction in various rumen bacteria by some carbon sources. We have hypothesized that celluloligosaccharides induced cellulase production in R. flavefaciens and phosphorylases are related to polymer degradation and bacterial growth. R. flavefaciens 17 cannot utilize glucose, lactose or maltose as growth substrates but it was grown on cellobiose, cellobextrins, cellobiose or xylan as a sole energy source. Helaszek and White (1991) reported that R. flavefaciens FD-1 is unable to transport and utilize extracellular glucose. The highest growth rate was observed in cellobiose medium indicating that this bacterium had affinity and preference for cellobiose. Ayers (1958) reported that cellobiose is taken up by the cell of R. flavefaciens and undergoes a phosphorylolytic cleavage yielding glucose-1-phosphate and glucose, both of which are metabolised. Also, Hungate (1963) predicted that high growth yield on cellobiose could be attributed to the action of cellobiose phosphorylase and this is in agreement with the current results which showed that this organism has cellobiose phosphorylase activity. B. fibrisolvens OR77, P. bryantii B4 and S. bovis A30
were grown and utilized glucose, cellobiose, lactose or maltose as a sole energy source.

In this study, the activity of cellobiose, cellobiose phosphorylases was found in _R. flavofaciens_ 17, _P. bryantii_ B14 and _B. fibrisolvens_ OR77. In addition, the activity of lactose phosphorylase was detected in _P. bryantii_ B14, _B. fibrisolvens_ OR77 and _S. bovis_ A30. _S. bovis_ A30 is the bacterium that produced the maltose phosphorylase. The cellobiose phosphorylase activity in _R. flavofaciens_ 17 was detected as measured by release of Pi. Furthermore, cellulose or cellobextrins were found to be required for maximum production of cellobiose phosphorylase in _R. flavofaciens_ 17 but xylan or cellulose cannot induce the production of cellobiose phosphorylase indicating that cellobextrins induced the production of cellobiose phosphorylase. The explanation of induction of cellobiose phosphorylase in the cellulose-grown cells, the cellobiose was initially metabolised by endo-
glucanases producing cellobio-oligosaccharides which induced the production of cellobiose phosphorylase in _R. flavofaciens_ 17. The highest activity of cellobiose phosphorylase was found after 24 hr incubation time which supported this explanation. The activity of cellobiose phosphorylase was low in cellobiose or xylan-grown cells due to substrate preference and specificity. This is in agreement with Schenkl and Nidetzky, (1999) who stated that bacterial phosphorylases differed in their substrate specificity. Surprisingly, cellobiose phosphorylase activity in _B. fibrisolvens_ OR77 was twofold greater than the activity in _R. flavofaciens_ 17 which grew on cellobiose. Lactose or cellobiose induced the production of cellobiose phosphorylase in _B. fibrisolvens_ OR77 may be due to phosphorylase enzyme playing an important role in the growth of this bacterium by preventing the accumulation of soluble end products. This enzyme was semi-purified. This is first trial for the prediction and semi-purification of cellobiose phosphorylase in _R. flavofaciens_ 17.

When cellobiose, glucose, lactose or maltose were used as a sole energy source, the cellobiose phosphorylase activity was induced by cellobiose in _R. flavofaciens_ 17. _B. fibrisolvens_ OR77 has the cellobiose phosphorylase activity in cellobiose, lactose, or maltose-grown cells but the activity was low in glucose-grown cells indicating that the enzyme was regulated by the carbon source and induced by either lactose or cellobiose. Then, cellulose, cellobextrins, cellobiose or xylan were used as a sole energy source in _R. flavofaciens_ 17 and showed that the activity of cellobiose phosphorylase in cellulose-grown cells of _R. flavofaciens_ 17 was low. Highest activity was found in cellobiose-grown cells indicating that _R. flavofaciens_ 17 had high affinity and preference for cellobiose, while _B. fibrisolvens_ OR77 had higher affinities for cellobiose or lactose than for glucose or maltose and the phosphorylase can improve the growth of these bacteria. Another explanation is due to substrate specificity and cellobiose is required for the maximum production of cellobiose phosphorylase. The highest bacterial growth rate was found in cellobiose-grown cells after 18 hr incubation and at the same time from 18-21 hr incubation, the highest activity for cellobiose phosphorylase was observed indicating that production of such enzyme encourages the bacterial growth rate. Hungate, (1963) predicted that high
growth yield on cellulose could be attributed to the action of cellulose phosphorylase, and the results indicated that such activity was presented. In cellulose medium, the activity of cellulose phosphorylase was high because the prepared cellobiose contains large amounts of cellulose resulting from the production of cellulose phosphorylase in R. flavofaciens 17.

The results concluded that R. flavofaciens 17 has the highest activity of cellulose phosphorylase comparing to B. fibrisolvens OR77 and P. bryantii B4. Surprisingly, the cellulose phosphorylase production in B. fibrisolvens OR77 was higher than in R. flavofaciens 17 and it was induced by glucose notably.

Finding of cellulose phosphorylase and cellulose phosphorylase is logically acceptable and essential because the cellulose and cellotriose are the main end products of cellulolysis by the cellulose complex of R. flavofaciens (Rasmussen et al., 1985 and Russell, 1985). On the other hand, the activity of cellulose phosphorylase in P. bryantii B4 was low indicating that the simple hydrolysis of disaccharides by production of β-glucosidase is much greater than phosphorylolytic activity by cellulose phosphorylase.

Enzymes capable of degrading cellulose and cellotriose were found as essential components of microbial cellulolytic enzyme systems. They converted the cellobiose and cellotriose formed during the enzymatic degradation of cellulose by the synergistic action of endoglucanase and exoglucanases to fermentable sugars e.g. glucose and glucose-1-phosphate is the Embden-Meyerhoff-Parnas pathway. As cellulolytic enzymes are generally subject to end product inhibition and the cellotriose and cellulose are the main end products for cellulose degradation by R. flavofaciens (Rasmussen et al., 1988 and Russell, 1985) so, an adequate level of cellulose and cellotriose phosphorylases are required for efficient breakdown of cellulose.

The metabolism of soluble cellulose degradation products involves phosphorylolytic and hydrolytic cleavage (Coughlan and Mayer, 1992). Hydrolysis is catalyzed by β-glucosidases releasing glucose from the disaccharides and the non-reducing ends of the oligosaccharides. Phosphorylolytic is energetically disadvantageous and constitute the primary route of disaccharides and cellobiose utilization in particular anaerobic environments. The cellulosidase degradation was investigated by either hydrolytic cleavage through β-glucosidase or phosphorylolytic cleavage in R. flavofaciens 17. The phosphorylolytic cleavage was about threefold greater than hydrolytic cleavage activity in R. flavofaciens 17 indicating that phosphorylases were key enzymes in the initial metabolism of the soluble products of cellulose degradation. The metabolic pathways, which use phosphorylolytic cleavage, conserve more energy, through investment of ATP, than those utilizing simple hydrolysis. Since bacterial energy sources in the rumen are often limited and the yield of ATP is generally low in anaerobic microorganisms, efficient utilization of the substrate is important for the growth of ruminal bacteria. On the other hand Lou et al. (1997) reported that the hydrolytic cleavage of cellulose was three-folds greater than the
phosphorylolytic activity in P. bryantii B1, as measured by an enzymatic assay suggesting that this organism degraded cellulose mainly through hydrolytic rather than phosphorylolytic cleavage. The results concluded that there are two factors affecting cellulose cleavage, either hydrolytic or phosphorylolytic in R. flavefaciens 17, including the carbon source and lyme course.

REFERENCES


تعزز إنزيمات الفسفرة بواسطة مصادر كروبية في أربع أنواع من بكتيريا الكرش

1. جمع العلوم الفيزيائية، معمال ومواد السكر، كوكاموس وباشا، الفصل، 1979

2. جمع كوكاموس، معمل العلوم الفيزيائية، باشا، الفصل، 1979

3. حذف إنزيمات الفسفرة بواسطة مصادر كروبية في أربع أنواع من بكتيريا الكرش


تحفز الإنزيمات الفسفرة بواسطة مصادر كروبية في أربع أنواع من بكتيريا الكرش.

Cellulose

phosphorylase, cellobiose phosphorylase, maltose phosphorylase and lactose phosphorylase

على بعض البكتيريا الكرش مثل

R. flavus, B. fibrisolvens OR77, P. bryantii B.4 and S. bavosi

Cellulbiose phosphorylase

R. flavus and B. fibrisolvens OR77, P. bryantii B.4, and S. bavosi

ملاحظة: إنزيمات الفسفرة في كل زمن وتوجد في بكتيريا الكرش

Induction of phosphorylases

لا يمكن تحفيز إنزيمات الفسفرة بواسطة مصادر كروبية في كل زمن وتوجد في بكتيريا الكرش

R. flavus and B. fibrisolvens OR77, P. bryantii B.4, and S. bavosi

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وإلى ذلك ما زالت الحالات تجدر بالبحث في البلاغ عن تأثير الإنزيمات على نتائج التجربة...