Exocellular Succinogluan Production by *Agrobacterium radiobacter* NCIB 11883

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The efficiency of growth and exopolysaccharide production by *Agrobacterium radiobacter* NCIB 11883 was examined in both carbon- and nitrogen-limited chemostat cultures. Under carbon limitation this organism exhibited two distinct \( Y_{O2}^{max} \) values, one below \( D = 0.15 \) h\(^{-1}\) (40 g mol\(^{-1}\)) and the other above this dilution rate (84 g mol\(^{-1}\)). Under nitrogen limitation optimum exopolysaccharide production occurred at low dilution rates and under these conditions accounted for virtually all the product carbon excreted. The maximum observed yield of exopolysaccharide was 3.5 g (g O\(_2\))\(^{-1}\) and 0.65 g (g glucose)\(^{-1}\). These observed yields when corrected for the cellular requirement for glucose and oxygen gave values very similar to the theoretical value if the ATP/O quotient of carbon-limited cultures grown at corresponding dilution rates was used. Thus, the efficiency of growth of both carbon- and nitrogen-limited cultures was similar once an allowance for exopolysaccharide production was made. Under conditions optimum for polysaccharide production virtually all the respiratory activity occurring over and above that required for growth was utilized in polysaccharide production. Exopolysaccharide production is a major event in energetic terms and the rate of ATP utilization for its synthesis can be equivalent to 90% of that required for cell production. Nevertheless, because of the relationship between the structure of the polysaccharide and the ATP/O quotient extant in *A. radiobacter* succinogluan production supplies up to approximately 56% of its own ATP demand during the synthesis of the acid moieties that comprise this polymer.

INTRODUCTION

Succinogluans are heteropolysaccharides containing glucose, galactose, succinate, pyruvate and in some cases acetate. They are produced by a number of different genera of bacteria, such as *Alcaligenes faecalis* (Harada & Yoshimura, 1964), *Agrobacterium radiobacter*, *A. rhizogenes*, *A. tumefaciens*, *Rhizobium meliloti* (Hisamuta *et al.*, 1977, 1978) and *Pseudomonas* (Williams & Wimpenny, 1978, Cripps *et al.*, 1984). The exact composition of these heteropolysaccharides and the specific rates at which they are excreted can vary considerably. Succinogluans are an economically interesting group of polysaccharides and a number of patents concerning their production and application have been filed (William & Lawson, 1978; Steenbergen & Young, 1981; Yin, 1982; Cripps *et al.*, 1984). The organism used in this study, *Agrobacterium radiobacter* NCIB 11883 is used in a commercial process for the production of succinogluan (Linton *et al.*, 1984b) marketed by Shell under the trade name Enorlo-S.

There have been a number of theoretical studies concerning the energetic requirements of exopolysaccharide production by bacteria. The maximum carbon to carbon conversion efficiency for curdlan (\( \beta \)-1,3-glucan) production by *Alcaligenes faecalis* was calculated to be

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demonstrated that the yield from oxygen, unlike that from the carbon source, was strongly reported by Jarman polymer synthesis. Moreover, there is also a paucity of data concerning the growth efficiency of exopolysaccharide production contain data regarding the efficiency of oxygen utilization for the growth efficiency of a succinoglucan-producing strain of Agrobacterium radiobacter in order to assess how close experimental yields approach those theoretically predicted and to allow a rational means of assessing the scope for further yield improvements in the production of this exopolysaccharide.

**METHODS**

**Micro-organism.** Agrobacterium radiobacter was isolated from activated sludge using continuous enrichment methods with methanol as the sole source of carbon and energy. Although this organism was originally isolated on methanol, polysaccharide production is only observed during growth on glucose. Indeed, after serial incubation on glucose all attempts to get this isolate to grow on methanol have failed. This isolate has been examined at the laboratory of the National Collection of Industrial Bacteria; it was tentatively identified as Agrobacterium tumefaciens/radiobacter and given the accession number NCIB 11883. A detailed description of the organism was published by Linton et al. (1984b). This strain exhibits a number of features that are atypical of the genus Agrobacterium, e.g. branching, the ability to grow at 37 °C and the inability to produce 3-ketolactose or to utilize opine or nopaline (Dr J. W. Drozd, personal communication) as the sole source of nitrogen, whereas these two compounds are utilized by most strains of A. tumefaciens.

**Media.** The medium used for carbon limitation was prepared in four portions that were sterilized separately and mixed when cool. Constituents (g 1-I): (1) (NH₄)₂SO₄, 3-0 g; KH₂PO₄, 0-75 g. (2) MgSO₄.7H₂O, 0-4; CaCl₂.2H₂O, 0-012; trace element mixture, 2-5 ml 1-I. (3) glucose, 6-0. (4) FeSO₄, 1 M solution. 0-05 ml 1-I. The trace elements stock solution contained (g 1-I): CuSO₄.5H₂O, 0-249; MnSO₄.4H₂O, 0-223; ZnSO₄.7H₂O, 0-287; CoCl₂.6H₂O, 0-118; H₃BO₃, 0-03; Na₂MoO₄.2H₂O, 0-124; KI, 0-083.

The requirement of micro-organisms for magnesium is known to increase with growth rate (Tempest et al., 1965). Consequently, for carbon-limited growth at dilution rates >0-15 h⁻¹ the MgSO₄.7H₂O concentration was increased to 0-6 g 1⁻¹ in order to prevent this nutrient becoming growth limiting. However, the chemostat is maintained at pH 7-0 and micro-precipitation of magnesium and other ions occurs and this may reduce their availability to micro-organisms. To overcome this problem the chelator nitrilotriacetic acid (0-147 g l⁻¹) was also added as the presence of a chelator increases the availability of these ions because the adjustment of the equilibrium subsequent to the removal of an ion from such a system is instantaneous and this is contrast to the time required for solution from a precipitate (Spencer, 1957; Linton et al., 1977).

For nitrogen-limited growth the (NH₄)₂SO₄ concentration was reduced to 0-75 g l⁻¹ and the glucose concentration increased to between 10 and 15 g l⁻¹ depending on the growth rate. This was necessary to maintain approximately 2-5 g excess glucose l⁻¹ in the culture at all growth rates examined.

**Continuous culture.** A Biotech fermenter of working volume approximately 3-5 l was operated with virtually no head space, as described by Linton et al. (1984a).

**Bacterial and exopolysaccharide dry weight.** The dry weight of bacteria and exopolysaccharide was determined by centrifugation (20000 r.p.m. for 30 min) of a suitably diluted sample obtained directly from the fermenter. The pellet was washed, re-centrifuged and then dried to constant weight in a vacuum oven. The polymer present in the supernatant was precipitated by adding 4 vols propan-2-ol, recovered and then dried to constant weight as described above.

**Analysis of metabolic products.** Culture supernatants were examined for the presence of sugars and organic acids by HPLC as described below. Culture supernatant samples were also subjected to FAB mass spectroscopic analysis as described by Linton et al. (1986).

**Chemical characterization of exopolysaccharides.** Exocellular polysaccharide was dialysed, freeze-dried and then vacuum dried at 50 °C for 16 h. Duplicate samples were weighed (20 mg) into glass tubes, 3-0 ml of 0-5 M H₂SO₄ (Aristar) added, the tubes sealed and then heated to 95 °C for 16 h. After cooling, the samples were filtered through disposable 0-45 μm microfilters and divided into two portions. One was used for the analysis or organic acids and the other neutralized and then analysed for sugars. Sugar and acid contents of hydrolysates were determined using a Varian 5000 liquid chromatograph. For organic acid determination a BioRad HPX-87 Aminex column, 300 x 7-8 mm was used at 40 °C. The eluent was 0-00625 M H₂SO₄ in purified water at a flow rate of 0-6 ml min⁻¹. The sample size was 50 μl and detection was by UV light at 210 nm. Sugars were determined on a BioRad Aminex
RESULTS AND DISCUSSION

Growth efficiency of A. radiobacter NCIB 11883

As a prerequisite to studies on the energetics of exopolysaccharide production it is necessary to determine the growth efficiency of the producing strain. A. radiobacter was therefore grown in glucose-limited chemostat culture maintained at steady state at various growth rates. The culture was shown to be glucose limited as a linear relationship was found between the steady state bacterial dry weight and the reservoir glucose concentration over the range 1 to 10 g l\(^{-1}\). In order to ensure glucose-limited growth at all dilution rates the input glucose concentration to the chemostat was maintained at a lower value of 6 g l\(^{-1}\). Growth is unequivocally carbon limited; indeed, the linear relationship between input glucose concentration and bacterial dry weight can be maintained over a glucose input of 1 to 25 g l\(^{-1}\) by increasing the concentration of only (NH\(_4\))\(_2\)SO\(_4\) and K\(_2\)HPO\(_4\) to 10 g l\(^{-1}\) and 1.5 g l\(^{-1}\) respectively. At each growth rate the molar growth yield from both glucose and oxygen at growth rates above 0.15 h\(^{-1}\) was found to exhibit two distinct linear phases as a function of growth rate (Fig. 1). In both cases the rate of glucose (\(q_{\text{glc}}\)) and oxygen (\(q_{\text{O}}\)) consumption was found to be linear

\[
\frac{\text{d} \text{Glucose}}{\text{d} \text{Time}} = \frac{\text{Glucose}}{\text{Time}} = \text{Constant}
\]

\[
\frac{\text{d} \text{Oxygen}}{\text{d} \text{Time}} = \frac{\text{Oxygen}}{\text{Time}} = \text{Constant}
\]

This increase in growth efficiency was not observed when the chelator was present in the medium at growth rates >0.15 h\(^{-1}\). It therefore appears that at high growth rates some inorganic ion, probably Mg or Fe, affects growth efficiency; however, it is unclear why there should be such a relatively large increase in growth efficiency when the availability of these metals is increased by adding a chelator to cultures growing at growth rates >0.15 h\(^{-1}\). Metabolites like acetate were not detected at high growth rates as has been found for Klebsiella aerogenes (Stouthamer & Bettenhausen, 1975) and Beneckea natriegens (Linton et al., 1977) so the depression in the \(q_o\) is not caused by a significant rate of substrate level phosphorylation. Moreover, if such phosphorylation occurred then the rate of glucose consumption would increase and not decrease in conjunction with the respiration rate (Fig. 1). The low values of residual carbon in the culture at various dilution rates support these observations (Table 1).

The maximum yields from glucose and oxygen, corrected for cellular maintenance energy requirements, were obtained from the slopes of the \(q_{\text{glc}}\) and \(q_o\) curves shown in Fig. 1. Two distinct \(Y_{\text{glc}}^{\text{max}}\) values were obtained. At dilution rates <0.15 h\(^{-1}\) the \(Y_{\text{glc}}^{\text{max}}\) was found to be 40 g mol\(^{-1}\) whereas at \(D\geq0.15\) h\(^{-1}\) a value of 84 g mol\(^{-1}\) was obtained. Assuming that the \(Y_{\text{ATP}}^{\text{max}}\) falls between 12.4 and 14.0 g dry wt (mol ATP)\(^{-1}\) (Jones, 1977), a relative ATP/O quotient of 1.4 to 1.6 is obtained from the \(Y_{\text{O}}^{\text{max}}\) at \(D <0.15\) h\(^{-1}\) and 2.8 to 3.2 from the \(Y_{\text{O}}^{\text{max}}\) at \(D >0.15\) h\(^{-1}\).
Fig. 1. Rate of glucose (○) and oxygen (●) consumption as a function of dilution rate for a glucose-limited chemostat culture of *A. radiobacter* NCIB 11883 growing at pH 7.0 and 30 °C.

Table 1. Molar growth yields, bacterial protein contents and carbon balances for glucose-limited cultures of *A. radiobacter* NCIB 11883 growing in a chemostat at various growth rates

<table>
<thead>
<tr>
<th>Dilution rate, D (h⁻¹)</th>
<th>Rate of glucose consumption (mmol g⁻¹ h⁻¹)</th>
<th>Rate of oxygen consumption (mmol g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.043</td>
<td>9.0</td>
<td>0.9</td>
</tr>
<tr>
<td>0.05</td>
<td>9.5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.09</td>
<td>9.8</td>
<td>1.1</td>
</tr>
<tr>
<td>0.1</td>
<td>9.9</td>
<td>1.2</td>
</tr>
<tr>
<td>0.11</td>
<td>9.7</td>
<td>1.0</td>
</tr>
<tr>
<td>0.21</td>
<td>10.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.25</td>
<td>10.8</td>
<td>1.8</td>
</tr>
<tr>
<td>0.32</td>
<td>11.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

respectively. It should be stressed that these quotients are not precise values and are only of use as yardsticks of comparison between different organisms. Clearly, the ATP/O quotients determined will be very sensitive to the *Y* value used.

**Cytochrome analysis**

The cytochrome complement of *A. radiobacter* grown under carbon or nitrogen limitation was qualitatively the same and consisted of cytochromes *c*₅₅₀, *c*₅₅₅, *b*₅₆₀ and the terminal oxidase *aa₃*. This cytochrome composition is consistent with a potential ATP/O quotient of 3 (Jones, 1977).

A detailed study of the respiratory system of this organism (accompanying paper: Cornish et al., 1987) indicates that it possesses a branched electron transport system. Although straight lines have been drawn through the *q*₀ vs *D* curve (Fig. 1) the relationship is probably a curve with both branches of the electron transport system functioning simultaneously but at varying extents depending on the availability of metal ions and the growth rate.

**Exopolysaccharide production**

*A. radiobacter* NCIB 11883 was grown at various dilution rates under nitrogen limitation at a constant nitrogen input of 0.75 g (NH₄)₂SO₄ l⁻¹. Sufficient glucose was added to ensure that excess unmetabolized glucose remained in the culture at all dilution rates examined (Table 2). Electron micrographs of thin sections prepared from cells grown under these conditions revealed the presence of large amounts of intracellular storage granules (not shown). The
Succinoglucan production by A. radiobacter

Table 2. Effect of growth rate on the molar growth yield from glucose and oxygen, the rate of extracellular polysaccharide production and the metabolic fate of glucose in an ammonia-limited chemostat culture of A. radiobacter NCIB 11883

<table>
<thead>
<tr>
<th>Dilution rate, D (h⁻¹)</th>
<th>0-041</th>
<th>0-047</th>
<th>0-08</th>
<th>0-13</th>
<th>0-265</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concn (g l⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input</td>
<td>20-57</td>
<td>19-2</td>
<td>16-02</td>
<td>13-81</td>
<td>9-51</td>
</tr>
<tr>
<td>Output</td>
<td>5-72</td>
<td>2-3</td>
<td>3-3</td>
<td>5-12</td>
<td>6-48</td>
</tr>
<tr>
<td>Yₚ₝ (g dry wt mol⁻¹)</td>
<td>22-4</td>
<td>17-3</td>
<td>23-0</td>
<td>30-8</td>
<td>84-1</td>
</tr>
<tr>
<td>Yₒₒ (g dry wt mol⁻¹)</td>
<td>23-0</td>
<td>17-3</td>
<td>21-8</td>
<td>30-4</td>
<td>33-5</td>
</tr>
<tr>
<td>Intracellular glucose (% dry wt)</td>
<td>25</td>
<td>31</td>
<td>26-1</td>
<td>14-7</td>
<td>4-5</td>
</tr>
<tr>
<td>Exopolysaccharide concn (g l⁻¹)</td>
<td>8-0</td>
<td>9-8</td>
<td>5-36</td>
<td>2-5</td>
<td>0-58</td>
</tr>
<tr>
<td>qₑ (g exopolysaccharide g⁻¹ h⁻¹)</td>
<td>0-18</td>
<td>0-26</td>
<td>0-28</td>
<td>0-20</td>
<td>0-11</td>
</tr>
<tr>
<td>Carbon balance (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell carbon</td>
<td>13-46</td>
<td>10-5</td>
<td>14-02</td>
<td>19-7</td>
<td>51-5</td>
</tr>
<tr>
<td>Soluble product carbon</td>
<td>72-1</td>
<td>69-35</td>
<td>66-9</td>
<td>71-5</td>
<td>12-3</td>
</tr>
<tr>
<td>CO₂ carbon</td>
<td>17-9</td>
<td>17-0</td>
<td>15-75</td>
<td>16-78</td>
<td>43-5</td>
</tr>
<tr>
<td>Recovery</td>
<td>103-5</td>
<td>97-25</td>
<td>96-6</td>
<td>109-9</td>
<td>107-9</td>
</tr>
</tbody>
</table>

presence of these storage granules correlated with the content of intracellularly stored glucose which was found to be inversely related to growth rate (Table 2). Exopolysaccharide was produced at all growth rates examined; however, the specific rate of production decreased at high growth rates. At dilution rates between 0-04 and 0-08 h⁻¹ exopolysaccharide was the major metabolite excreted and accounted for between 70 and 90% of the product carbon. No other metabolites could be detected by HPLC analysis of culture supernatants for sugars and organic acids as described in Methods. At higher growth rates the proportion of glucose carbon converted into soluble metabolic products decreased markedly as expected (Linton et al., 1983) and there was a marked increase in the bacterial growth yield from glucose and oxygen (Table 2).

Efficiency of exopolysaccharide production

Based on experimental measurements a unit of succinoglucan was found to be composed of glucose/galactose/pyruvate/succinate/acetate (molar ratio 7:1:1:1:0-1). Assuming the elimination of 1 mol H₂O per hexose polymerized and organic acid esterified the Mᵣ of a unit of succinoglucan is 1470-2 (i.e. C₃₅₅H₇₅₇O₄₅₁).

In the biosynthesis of succinoglucan, polymerization of the sugar backbone is an energy-requiring process whereas synthesis of the acid moieties results in the net production of energy. The energetic contribution of the acid moieties to the overall energetic requirements for succinoglucan synthesis will depend on the mode of glucose catabolism. The Entner–Douderoff pathway and the pentose cycle are known to be present in Agrobacterium (Arthur et al., 1975); however, the relative importance of these pathways for exopolysaccharide synthesis is not known. The following stoichiometry for exopolysaccharide production has been calculated assuming the operation of the Entner–Douderoff and tricarboxylic acid pathways.

1 Pyruvate

\[
0-5 \text{ Glucose} + 0-5 \text{ ATP} + 0-5 \text{ NAD} = \text{ phosphoenolpyruvate} + 0-5 \text{ NADPH}_2 + 0-5 \text{ NADH}_2 \\
+ 0-5 \text{ NAD} + 0-5 \text{ ATP} + 0-5 \text{ P}_i
\]

1 Succinate

\[
\text{Glucose} + 3 \text{ NAD} + 2 \text{ NADP} + \text{HS-CoA} = \text{succinyl-CoA} + 2 \text{ CO}_2 + 3 \text{ NADH}_2 + 2\text{NADPH}_2
\]

0-1 Acetate

\[
0-05 \text{ Glucose} + 0-1 \text{ HS-CoA} + 0-15 \text{ NAD} = \text{acetyl-CoA} + 0-05 \text{ NADPH}_2 + 0-15 \text{ NADH}_2 \\
+ 0-05 \text{ NAD} + 0-05 \text{ ATP} + 0-1 \text{ CO}_2
\]
Table 3. *Experimental stoichiometry for growth and exopolysaccharide production by* *A. radiobacter* *NCIB 11883 as a function of dilution rate in a nitrogen-limited chemostat*

Theoretical yields for exopolysaccharide production are compared with the observed yield values after an allowance has been made for the glucose and oxygen requirements of cell biosynthesis.

<table>
<thead>
<tr>
<th>Dilution rate, $D$ (h$^{-1}$)</th>
<th>Glucose + O$_2$ + NH$_3$ =</th>
<th>Experimental stoichiometry</th>
<th>Recovery (%)</th>
<th>Observed yields for cell production (g g$^{-1}$)</th>
<th>Yields corrected assuming an ATP/O quotient of 1.5</th>
<th>Theoretical yields assuming an ATP/O quotient of 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(C$<em>{646}$H$</em>{70}$N$<em>{2}$O$</em>{57}$)</td>
<td>cells + intracellular polysaccharide + CO$_2$ + exopolysaccharide + H$_2$O</td>
<td></td>
<td>C</td>
<td>H</td>
<td>O</td>
</tr>
<tr>
<td>0.041</td>
<td>6 + 6.15 + 0.81 = 4.06 + 0.193 + 7.6 + 3.67 + 15.36 96</td>
<td>100</td>
<td>107.5</td>
<td>0.57</td>
<td>3.14</td>
<td>0.73</td>
</tr>
<tr>
<td>0.047</td>
<td>6 + 6.49 + 0.615 = 3.07 + 0.218 + 6.29 + 4.35 + 12.69 101</td>
<td>100</td>
<td>100</td>
<td>0.67</td>
<td>3.5</td>
<td>0.73</td>
</tr>
<tr>
<td>0.08</td>
<td>6 + 7.77 + 1.06 = 5.33 + 0.26 + 7.48 + 3.50 + 15.34 96.8</td>
<td>100</td>
<td>100</td>
<td>0.55</td>
<td>2.41</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Hexose backbone

\[ 8 \text{ Glucose} + 16 \text{ ATP} = [8 \text{ hexose unit}] \]
\[ 8 \text{ Hexose unit} + \text{ ATP} = [8 \text{ hexose}] \text{ on polymer} \]

Net

\[ 9.55 \text{ Glucose} + 2.55 \text{ NADP} = [7 \text{ glucose} : 1 \text{ galactose} : 1 \text{ pyruvate} : 1 \text{ succinate} : 0.1 \text{ acetate}] + 2.55 \text{ NADPH}_2 \]
\[ + 3.65 \text{ NADH}_2 + 2.1 \text{ CO}_2 + 1.1 \text{ ADP} + 17.45 \text{ P}_i \]

In addition to the ATP requirements shown above there is an additional requirement for sugar transport. \textit{A. radiobacter} NCIB 11883 is an obligate aerobe and the phosphotransferase system of glucose uptake is unlikely to be operational (Romano, 1986). This organism has been shown to possess a shockable binding protein system when grown under carbon limitation (A. Cornish & C. W. Jones, personal communication). The precise energetic requirement for this glucose uptake system is not known but is probably 1 ATP per glucose transported (Romano, 1986). It should be noted, however, that the extent to which this high affinity (Wilson & Smith, 1978) system is operational under conditions of glucose excess (nitrogen limitation) remains to be established. Thus, the extent of the energetic contribution made by the synthesis of the acid moieties will depend on the ATP/O quotient and the precise requirements for glucose transport. If no ATP is required for transport then at an ATP/O quotient of 1.5 and 3.0 approximately 56% and 100% of the energetic requirement of exopolysaccharide synthesis is supplied during the production of the acid moieties that comprise the molecule. However, if 1 ATP is required per glucose transported this energetic contribution falls to 35% and 70% at an ATP/O quotient of 1.5 and 3 respectively.

Mass balances for growth and polysaccharide production

The stoichiometry of growth and exopolysaccharide production was measured under nitrogen limitation at three different growth rates (Table 3). In all cases good carbon recoveries were obtained which suggests that no metabolic product other than exopolysaccharide was produced under these conditions. In order to compare the observed yields of exocellular polysaccharide with those theoretically expected it is necessary to make an allowance for the amount of glucose and oxygen utilized for cell production. This was achieved by using the observed \( Y_{\text{glc}} \) and \( Y_{\text{o}} \), values of carbon-limited cultures grown at corresponding growth rates to correct the yields of exopolysaccharide production under nitrogen limitation. The corrected yields from both glucose and oxygen were remarkably similar at the three different growth rates. Moreover, the \( Y_{\text{glc}} \) and \( Y_{\text{o}} \), for polymer obtained after making a correction for cell growth are close to the theoretical values calculated assuming an ATP/O quotient of 1.5 (obtained from growth under carbon limitation) (Table 3). If the observed ATP/O quotient of the corresponding carbon-limited cultures grown at the same growth rate is used then the theoretical yields from glucose and oxygen are 0.79 g (g glucose\(^{-1}\)) and 5.7 g (g O\(_2\)\(^{-1}\)) respectively, which are virtually identical to the experimentally derived values (Table 3). These results suggest that the energetic requirement for glucose uptake under nitrogen limitation is probably less than 1 ATP per glucose transported as the theoretical yield calculated assuming this requirement is approximately 3.2 g expolysaccharide (g O\(_2\)\(^{-1}\)) at an ATP/O quotient of 1.5.

These results indicate that although the overall \( Y_{\text{o}} \), values of nitrogen-limited cultures are considerably lower than those of corresponding carbon-limited cultures the efficiency of growth of both remain similar provided the ATP utilized for exopolysaccharide production is taken into account. Moreover, as the corrected yields are very close to the theoretical yields virtually all the respiratory activity occurring over and above that required for growth is utilized for exopolysaccharide production. Under conditions optimum for exopolysaccharide production the rate of ATP utilization for succinoglucan production accounts for up to 90% of that required for cell production at the same growth rate. As the growth rate is increased the impact of exopolysaccharide production on the overall rate of ATP utilization becomes progressively smaller (Table 4). High rates of ATP utilization for exopolysaccharide production have also
been reported for *Xanthomonas campestris* (xanthan) and *Pseudomonas aeruginosa* (alginate) by Jarman & Pace (1984) and for the methylophroph *Methylophilus* sp. by Linton et al. (1986). In energetic terms polysaccharide production is a major event but as yet its physiological role remains obscure. The results reported here indicate that very little energy is dissipated under conditions of carbon excess and most of the ATP produced over and above that required for cell turnover over and above that required for cellular biosynthesis. It was shown previously (Linton et al., 1986) that in methylo trophic bacteria the composition of the exopolysaccharide produced appears to be related to the energetic constraints imposed by the various pathways of *C*\textsubscript{1} fixation. In *A. radiobacter* there appears to be a similar relationship between energetic efficiency (ATP/O quotient) and the chemical composition of the exopolysaccharide excreted. Further work is needed in order to determine whether the results reported here are specific to the two organisms studied or whether they are relevant to other exopolysaccharide-producing organisms.

**Table 4. Rate of ATP production for cell and exopolysaccharide production as a function of growth rate for *A. radiobacter* NCIB 11883 growing under nitrogen limitation in a chemostat**

<table>
<thead>
<tr>
<th>Dilution rate, D (h(^{-1}))</th>
<th>(q_{\text{ATP}}) for cell production* (mmol ATP g(^{-1}) h(^{-1}))</th>
<th>(q_{\text{ATP}}) for exopolysaccharide synthesis† (mmol ATP g(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.041</td>
<td>2.92</td>
<td>2.08</td>
</tr>
<tr>
<td>0.047</td>
<td>3.35</td>
<td>3.0</td>
</tr>
<tr>
<td>0.08</td>
<td>5.71</td>
<td>3.23</td>
</tr>
<tr>
<td>0.13</td>
<td>9.28</td>
<td>2.3</td>
</tr>
<tr>
<td>0.265</td>
<td>18.92</td>
<td>1.27</td>
</tr>
</tbody>
</table>

* Assuming a \(Y_{\text{ATP}}\) value of 14.
† Assuming 17 ATP per unit of succinoglucan.

**REFERENCES**


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