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Growth of Streptococcus bovis and a Butyrivibrio in batch and continuous culture and the relationship of molar growth yield to intermicrobial competition

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ABSTRACT

Cell growth yield of Streptococcus bovis and Butyrivibrio were determined in batch cultures where growth was separately limited by glucose, CO_2 and trypticase. With S. bovis, glucose limited growth and a Yg of 39.6 g / M in the presence of excess CO_2 was determined. S. bovis grew in the absence of CO_2, but the Yg was reduced to 16.5 g / M. In the presence of excess CO_2, the Yg determined for Butyrivibrio was 55 g / M. Butyrivibrio was strictly limited by CO_2 and the Y_{CO_2} was equal to Yg. This led to the suggestion that CO_2metabolism allows the generation of at least two additional ATP when combined with glucose metabolism for both organisms.

Monod growth constants were determined for both organisms in continuous culture under glucose limitation. Ks and μ_{max} for S. bovis were 0.429 mM / l and 2.47 hr^{-1}, respectively. For Butyrivibrio, Ks and μ_{max} were 0.332 mM / l and 0.704 hr^{-1}, respectively. The cell growth yields for S. bovis and Butyrivibrio were determined to be 39.6 g / M and 69.1 g / M, respectively. At growth rates less than 0.2 hr^{-1} colony forming units and total cell counts of S. bovis decreased, but cell yield did not. Colony forming units, total counts and cell growth yield of Butyrivibrio did not decrease at low growth rates.

When S. bovis and Butyrivibrio were grown in continuous mixed culture, Butyrivibrio dominated at growth rates below 0.5 hr^{-1} and growth of S. bovis was strongly depressed. That Butyrivibrio dominated mixed cultures supports the proposition that an organism deriving more ATP per mole of substrate that another will dominate in environments comparable with continuous culture. The roles of maintenance energy, Ks and μ_{max} and cell yield in competition are considered.

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Table of Contents

Abstract ii

Acknowledgements iii

Table of Contents iv

List of Figures vii

List of Tables viii

List of Plates ix

Section I: Introduction and Statement of Purpose 1

Section II: Methods 6

1. Medium: Constituents and Preparation 6

1.1. Standard Medium 6

1.2. Preparation of Medium for Continuous Culture 7

2. Batch Culture Apparatus 8

2.1. Standard Roll Tubes 8

2.2. Nephelometer Flasks 8

2.3. Largh Batch Culture Flasks 8

3. Continuous Culture Apparatus 11

3.1. Carbon Dioxide System 11

3.2. Medium Reservoir 14

3.3. Pump 14

3.4. Temperature Control 15

3.5. Growth Vessel 16

4. Analytical Methods 18

4.1. Glucose 18

4.2. Lactic Acid 18

4.3. Volatile Fatty Acids 22

3.1. Thin Layer Chromatography 22

3.2. Steam Distillation 22

4.4. Determination of Dry Weight 23

4.5. Determination of Colony Forming Units 23

4.6 Direct Microscopic Counts of Cells 24

II.4.7. Radiometric Methods 25

7.1. Scintillation Counting and Method of Correcting Counts (CPM to DPM) 25

7.2. Methods of Sample Collection 26

2.1. Collection of Cells 26

2.2. Collection of Gasses 26

2.3. Collection of Spots from Thin Layer Chromatography 26

7.3. Scintillation Fluid: Composition and Sample Mixing 27

4.8. Autoradiography 28

8.1. Acid Hydrolysis of Cells 27

8.2. Thin Layer Chromatography of Acid Hydrolysates 28

Section III: Results

III.1. Batch Culture Results

1.1. Batch Culture of Sb1

1.1. Cultural Characteristics 29

1.2. Growth Yield of Sb1 with Glucose 33

1.3. Growth Yield of Sb1 with Trypticase 38

1.4. Growth Yield of Sb1 with CO_2 43

5,1, Retention of ^{14}C Glucose and $^{14}CO_2$ 47

5.2. Account of Total Label of ^{14}C Glucose and $^{14}CO_2$ fed cells 48

1.2. Batch Culture of WV1

2.1. Cultural Characteristics 55

2.2. Growth Yield of WV1 with Glucose 55

2.3. Growth Yield of WV1 with Trypticase 58

2.4. Growth Yield of WV1 with CO_2 58

2.5. Retention of ^{14}C Glucose and $^{14}CO_2$ by WV1 62

III.2. Continuous Culture Results

2.1. Continuous Culture of <u>Streptococcus bovis</u>

1.1. Initiation of Continuous Culture 70

1.2. Determination of Equilibration Time 70

1.3. Equilibrium Results for Sb1 71

1.4. Cell Dry Weight Results for Sb1 78

1.5. Determination of Monod Constants for Sb1 78

2.2. Continuous Culture of Butyrivibrio

2.1. Equilibrium Results for WV1 78

2.3. Cell Dry Weight Results for WV1 83

2.4. Determination of Monod Constants for WV1 83

2.3. Continuous Mixed Culture of Sb1 and WV1

3.1. Inoculation Procedure and Results of Growth at D = 0.36 hr^{-1} 92

3.3. Mixed Continuous Culture at Low Growth Rates 95

Section IV: Discussion and Conclusions

IV.1. Culture Identities 112

IV.2. Batch Culture 112

IV.3. Continuous Monocultures 116

IV.4. Continuous Mixed Cultures 118

IV.5. Conclusions 119

. . . .

Bibliography

List of Figures

Figure 1: Diagrams of Batch & Continuous Culture Fittings

Figure 2: Diagram of Continuous Culture Apparatus

Figure 3: Standard Curve for Glucose Estimation

Figure 4: Standard Curve for Lactate Estimation

Figure 5: Batch Growth of Sb1: Cell Yield with Glucose

Figure 6: Batch Growth of Sb1: Cell Yield and Other Parameters of Growth with Glucose

Figure 7: Visible Light Absorption Spectrum of Pigment Produced by Sb1

Figure 8: Batch Growth of Sb1: Cell Yield with Trypticase

Figure 9: Batch Growth of WV1: Cell Yield with Glucose

Figure 10: Batch Growth of WV1: Cell Yield with Trypticase

Figure 11: Continuous Culture of Sb1: Summary of CFU with D

Figure 12: Continuous Culture of Sb1: Summary of Total Counts

Figure 13: Continuous Culture of Sb1: Summary of Cell Yield

Figure 14: Continuous Culture of Sb1: Lineweaver-Burke Plot

Figure 15: Continuous Culture of WV1: Summary of CFU and Total Cell Counts

Figure 16: Continuous Culture of WV1: Summary of Cell Yield

Figure 17: Continuous Culture of WV1: Lineweaver-Burke Plot

Figure 18: Continuous Culture of WV1: Summary of Glucose Determination

Figure 19: Continuous Mixed Cultures of Sb1 and WV1: D = 0.36 hr^{-1}

Figure 20: " " " " " " " 0.34 "

Figure 21: " " " " " " " 0.36 "

Figure 22: " " " " " " " 0.018 "

Figure 23: " " " " " " " 0.058 "

Figure 24: " " " " " " " 0.058 "

Figure 25: " " " " " " " 0.057 & 0.58 hr^{-1}

List of Tables

Table 1: Growth of Sb1 and WV1 with a Variety of Substrates

Table 2: Growth of Sb1 with CO_2 at 3 mM PO_4

Table 3: Growth of Sb1 with CO_2 at 25 mM PO_4

Table 4: ^{14}C glucose Retention by Sb1 at Differing Initial Levels of Glucose

Table 5: ^{14}C Labelled Glucose and CO_2 Retention by Sb1

Table 6: ^{14}C Labelled Glucose and CO_2 Retention by Sb1

Table 7: TLC of Steam Distillate of Culture Supernatant of Sb1

Table 8: TLC of Supernatant of ^{14}C Labelled Cultures of Sb1.

Table 9: Autoradiogram of Sb1 Labelled With ^{14}C Glucose

Table 10: Growth of WV1 with CO_2 at 3 mM PO_4

Table 11: Growth of WV1 with CO_2 at 25 mM PO_4

Table 12: Retention of ^{14}C-labelled Glucose and CO_2 by WV1

Table 13: TLC of Steam Distillate of Culture Supernatant of ^{14}C labelled Cultures of WV1

Table 14: TLC of Supernatant of ^{14}C Labelled Cultures of WV1

Table 15: Autoradiogram of WV1 Labelled with ^{14}C Glucose

List of Plates

Plate 1: Surface and Sub-surface Colony of SB1

Plate 2: Capsule of Sb1

Plate 3: Sb1 Grown Without CO_2

Plate 4: Sb1 Grown With CO_2

Plate 5: Sub-surface Multi-lobed Colony of WV1

Plate 6: Complete Surface Colony of WV1

Plate 7: Rhizoid Surface Colony of WV1

Plate 8: WV1 Grown on Low CO_2

Plate 9: WV1 Grown on High CO_2

Plate 10: Continuous Culture of Sb1, Pigment Production

Plate 11: Cells From Continuous Mixed Culture at Low Dilution Rate

Inroduction

The bovine rumen is a semi-continuously fed culture which contains a wide variety of protozoa and bacteria. These microorganisms obtain their energy and nutrients by fermenting the food eaten by the animal and the animal in turn obtains energy by absorbing the end products of the microbial fermentation and digests the microorganisms themselves. Volatile fatty acids which cannot be metabolized anaerobically in the rumen are absorbed by the ruminant and metabolized aerobically to provide energy for the synthesis of glucose (Hungate, 1966). The microorganisms are digested in the abomasum and duodenum and constitute a major source of protein for the animal.

The stoichiometry of the rumen fermentation has been a topic of interest because of its direct implications in the bovine nutrition. Approximately 75 % of the available carbohydrate is converted to fatty acids, which are utilized by the animal (Barcroft, et al, 1944). That microbial protein synthesis is capable of supporting ruminant growth has been shown (Loosli, et al, 1949; Virtanen, 1966). The actual amount of protein available to the cow in the form of microbial cells has been estimated by a number of means but the precise amount remains indefinite, chiefly due to the difficulty of separating the microbes from the other rumen contents (Walker & Nader, 1968). The 10 g of microbial protein synthesized per 100 g of carbohydrate estimated as maximum by Hungate (1966) has been increased by later authors using label incorporation (Walker and Nader, 1968; Al-Rabbat et al, 1971; Pilgrim, et al, 1970) and phospholipid synthesis (Bucholtz & Bergen, 1973). From these increases has come the suggestion that the average yield of cell material per hexose, and hence the number of ATP derived from each hexose, in the rumen should be increased.

Butyrivibrio fibrisolvens is a rumen organism that ferments carbohydrates to

CO_2, H_2, ethanol, and acetic, butyric, formic and lactic acids (Hungate, 1966). It is a common rumen organism which usually occurs in the rumen at a concentration of at least $10^8 ml^{-1}$ (Bryant & Burkey, 1953). The production of significant quantities of butyric acid and the numbers present in the rumen indicate that Buyrivibrio contributes significantly to metabolism in the rumen (Bryant & Small, 1956).

Streptococcus bovis is also a rumen organism, which is not normally found in the ruminant diet. Although S. bovis can always be isolated from rumen contents, its numbers seldom exceed $10^7 ml^{-1}$, and its main fermentation product is lactate (Hungate, 1966). It has been shown that the lactate pool in rumen contents is normally small and turns over slowly (Jayasuriya & Hungate, 1959). Consequently, S.bovis has been considered an organism not contributing greatly to ruminant metabolism (Hungate, 1966). However, conversion of lactate to volatile fatty acids in whole rumen contents has been demonstrated (Nakamura & Takahashi, 1971) and lactate may be considered a normal intermediate in the rumen fermentation.

Under certain conditions, when the ruminant diet is shifted from low to high carbohydrate, the production of lactic acid can increase to such an extent that acid indigestion results due to the inability of the digesta to metabolize lactic acid as rapidly as it is produced. When this occurs, the concentration of S.bovis is found to have increased to the vicinity of $5 \times 10^9 ml^{-1}$ and is considered one of the most significant contributors to acid indigestion. The ability of S.bovis to generate such high numbers appears to be due to its high maximum specific growth rate (a doubling time of 20 minutes).

The fact that S.bovis has a high maximum rate of growth and yet normally exists in low numbers has been explained in two ways. In animals shifted to high grain diets, high concentrations of S.bovis occur for a period of time, but in well adapted animals, the numbers are similar to those found in animals receiving a low grain diet. This has been

attributed to the establishment of a new equilibrium population in which it is possible that the S. bovis serve as food for an enlarged protozoan population. An alternative suggestion for the normally low concentration of S. bovis has been its poor ability to compete due to its relatively inefficient energy yielding metabolism in contrast to other rumen bacteria.

It has been generally accepted that established pathways of energy metabolism generate predictable yields of high energy intermediates, such as ATP, and that these are used to synthesize new cell material with constant efficiency. This constant, as determined by Bauchop & Elsden (1960) is 10.5 g per mole of ATP. This constant has been verified for a variety of microorganisms (Forrest & Walker, 1971) and used widely for its predictive value. In 1966, Hungate suggested that "..... in the competition to achieve maximum growth, selection is against lactic acid and ethanol production. Formation of each of these products entails loss of available ATP. Conversion of pyruvate to acetyl CoA and the reduction of acetyl CoA to ethanol similarly entails loss of a potential ATP. The acetyl-CoA can yield an ATP unless it must be used for hydrogen disposal... according to this view, the propinionate formed in the rumen represents additional synthesis of ATP. This is also true for acetate on the hypothesis that pyruvate is split to acetyl CoA. A production of ATP in butyrate formation has been surmised, but efforts to demonstrate it have been unsuccessful. If there is a selection for maximum biochemical work, butyrate should also represent an end product accompanying additional energy conservation by the cell.

According to these views, the carbon dioxide, methane, acetate, propionate and butyrate, final products in the rumen fermentation, are formed because pathways leading to them provide the most efficient conversion of fermentable substrate into microbial cells."

The purpose of the research reported in this thesis was to test the proposition that an organism which derives a relatively low yield of two ATP per mole of glucose fermented will not be able to compete with an organism which can derive additional ATP by metabolism of pyruvate to acetate, propionate or butyrate. Two organisms were chosen for the experiments, S. bovis, which is a homofermenter and was expected to derive only two ATP per mole of glucose (Hungate, 1966) and Butyrivibrio fibrisolvens, which produces primarily formic and butyric acid and was expected to derive a larger number of ATP per mole of glucose fermented.

Continuous culture must be used to study competition based on substrate utilization because the outcome in batch culture will depend simply on the maximum specific growth rate and the duration of the lag phase for each organism. The organism with the larger maximum specific growth rate and shorter lag phase will always dominate if growth of both organisms is limited by the same substrate. Continuous culture in a chemostat allows this type of competition to be studied by limitation of the culture to a specific growth rate. Both organisms will have the same specific growth rate, and assuming equal affinity for substrate, dominance will depend on the efficiency with which the organism utilizes the substrate to synthesise new cell material.

The equations of continuous culture were originally derived by Monod (1950) and subsequently by Herbert et al (1956). Equations of significance to the work in this thesis are the following:

A. Dependance of specific growth rate on substrate concentration

$\mu = \mu_{max} S \ / \ (K_s + S)$ equation (1)

μ = specific growth rate

μ_{max} = maximum specific growth rate

K_s = substrate concentration at which $= \frac{1}{2}\ _{max}$

S = substrate concentration

B. The inverse of equation (1) provides a graphical means of determining the constants $_{max}$ and K_s. A plot of equation (2) is called a Lineweaver-Burke plot.

$$1 / \mu = 1 / \mu_{max} + (K_s / \mu_{max}) 1 / S \qquad \text{equation (2)}$$

C. Molar growth yield

$$Yg = x / (Sr - S) \qquad \text{equation (3)}$$

Yg = grams cells produced per mole of substrate consumed

x = dry weight of cells in grams per liter

Sr = original or reservoir concentration of substrate

S = final or growth vessel concentration of substrate

Variations on these equations (Van Uden, 1969; Powell, 1967) due to the inconstancy of Yg and the occurrence of maintenance metabolism will be considered in the discussion in regard to the results shown in Section III of this thesis. Awareness that these variations can occur led me to measure parameters necessary for the determination of viability (total and colony forming units (CFU) counts) and yield (dry weight) in continuous culture experiments.