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Molar Growth Yields in *Streptococcus faecalis* var. *liquefaciens*

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The term molar growth yield coefficient (Y) proposed by T. Bauchop and S. R. Elsden (J. Gen. Microbiol. **23**:457, 1960) defines microbial growth during energy-limiting synthesis, and was selected to compare growth yields with adenosine triphosphate (ATP) formed from the energy source(s) employed. Data obtained experimentally have indicated that Y_{ATP} is 10.5 g of cells (dry weight) per mole of ATP generated (I. C. Gunsalus and C. W. Shuster, p. I, *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 2, Academic Press, Inc., New York 1961).

W. W. Forrest and D. J. Walker (J. Bacteriol. **89**:1448, 1965) reported growth yield coefficients ($Y_{glucose}$) for *Streptococcus faecalis* ATCC 4083 obtained under conditions where growth was limited by the concentration of glucose. They found $Y_{glucose}$ to be 32 at concentrations of sugar less than 6 μmoles/ml, and 21 at higher concentrations of sugar. The amount of glucose consumed was not reported, and the data were apparently calculated with the assumption of complete utilization of the sugar under the specified conditions. We have observed the cessation of growth of cultures of *S. faecalis* and *S. faecalis* var. *liquefaciens* as a result of the depletion of arginine while sugar was still present in the growth medium (*unpublished data*). Forrest and Walker did not measure the consumption of arginine, although they did report growth in the complex medium devoid of glucose.

In view of these facts, we have evaluated the Y_{ATP} coefficient during active growth of two strains of *S. faecalis* var. *liquefaciens* strain R64A (L. R. Shugart and R. W. Beck, J. Bacteriol. **88**: 586, 1964) and ATCC strain 13398.

Data were collected during active growth of these organisms in a complex medium containing 1.0% Casamino Acids (Difco), 0.1% yeast extract (Difco), and varying concentrations of glucose and arginine. The finished medium, which had a *p*H of 6.7 and was 0.075 M with respect to phosphate buffer, was dispensed in 100-ml quantities into 250-ml flasks.

An inoculum of 1 ml of washed cells (18- to 24-hr culture) suspended in saline to an optical density of one (measured at 500 mμ) was employed in all experiments. Incubation was at 37 C in stationary culture. Dry weight was determined by measuring the absorbancy of suitably diluted cultures at 500 mμ and referring to a standard curve. Glucose was estimated with anthrone reagent [N. U. Fairbairn, Chem. Ind. (London, p. 86, 1953], and arginine was measured by a modified Sakaguchi procedure (p. 150, *in* Litwach [ed.], Experimental biochemistry, John Wiley & Sons, Inc., New York, 1960). These procedures proved to be satisfactory for quantitative measurements of glucose and arginine in the complex medium.

The data in Table 1 show that the cell crops obtained were larger than could be accounted for by assuming 2 moles of ATP per mole of glucose fermented (homolactic fermentation via the Embden-Meyerhof pathway) and 1 mole of ATP per mole of arginine utilized (via the arginine dihydrolase system). This yield of ATP from arginine has been established by V. A. Knivett (Biochem. J. **56**:602, 1954), N. E. Jones, L. Spector, and F. Lipmann (J. Am. Chem. Soc. **77**:819, 1955, and by T. Bauchop and S. R. Elsden (J. Gen. Microbiol. **23**:457, 1960). The last column of the table shows that one additional mole of ATP from the catabolism of 1 mole of glucose is required to explain the cell yields obtained in experiments with both strains of *S. liquefaciens*.

These data confirm the assertion of W. W. Forrest and D. J. Walker (J. Bacteriol. **89**:1448, 1965), that up to 3 ATP per mole of glucose is produced. It is clear that this additional yield of energy can occur at concentrations of sugar up to seven times greater than the 6 μmole/ml limit suggested by these authors.

W. W. Forrest, D. J. Walker, and M. F. Hopgood (J. Bacteriol. **82**:685, 1961) reported

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TABLE 1. *Substrate catabolism and ATP yield during growth of Streptococcus liquefaciens*

Strain	Expt no.	Energy sources				Cell yield	Total ATP yield		Increased ATP yield per μmole of glucose[c]
		Glucose		Arginine			Calculated[a]	Required[b]	
		Initial amt	Amt used	Initial amt	Amt used				
		μmoles/ml		μmoles/ml		μg/ml	μmoles/ml	μmoles/ml	μmoles
R64A	1	40.6	22.4	12.4	9.8	850	54.6	80.9	1.17
	2	43.1	15.4	2.4	2.3	540	33.1	51.4	1.19
	3	12.9	12.7	18.8	13.1	540	38.5	51.4	1.01
	4	12.9	12.9	29.4	13.4	560	39.2	53.3	1.09
	5	23.5	13.6	35.3	6.0	530	33.2	50.5	1.27
13398	1	35.7	22.8	8.8	6.9	810	52.5	77.1	1.08
	2	43.1	22.5	2.4	2.4	680	47.4	64.8	0.77
	3	12.9	12.9	18.8	13.5	630	39.3	60.0	1.60
	4	12.9	12.6	29.4	18.2	660	43.4	62.8	1.54
	5	23.5	19.4	35.3	14.8	740	53.6	70.5	0.87
Avg.....									1.16

[a] Obtained by assuming 2 μmoles of ATP generated per μmole of glucose utilized and 1 μmole of ATP generated per μmole of arginine utilized.

[b] Obtained by dividing the cell yield by the energy yield coefficient (10.5).

[c] Obtained by subtracting the total ATP yield calculated from the total ATP yield required and dividing by the total amount of glucose utilized.

that during exponential growth of *S. faecalis* the fermentation of glucose is not homolactic (up to 50% steam-volatile acids being produced), and W. W. Forrest and D. J. Walker (J. Bacteriol. **89**:1448, 1965) suggested that some of the pyruvate produced during the fermentation of glucose may undergo a dismutation type of reaction, leading to the formation of acetate, CO_2, and up to 1 mole of ATP per mole of pyruvate. We are currently investigating the fermentation pathway involved under the experimental conditions employed.

ERRATUM

Characterization of "Clearance" Factor and "Cell-bound" Antibody in Experimental Typhoid

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Volume 91, no. 5, page 1705, first line of Abstract: Change "Daizo, Ushiba" to "Ushiba, Daizo."