

## Molar Growth Yield of *Streptococcus faecalis* on Pyruvate

HASSAN H. MOUSTAFA AND E. B. COLLINS

Department of Food Science and Technology, University of California, Davis, California 95616

Received for publication 7 December 1968

Y(pyruvate) was 17.3, similar to Y(arginine), for *Streptococcus faecalis* 6783 grown statically in a complex medium in 1 atm of air.

The concept developed by Bauchop and Elsden (1) on dry-weight yields (Y) per mole of energy source, Y(substrate) and Y[adenosine triphosphate (ATP)], has been used widely in microbial biochemistry and physiology. A Y(ATP) value of 10.5 was reported for *Streptococcus faecalis* NCTC 6783 by Bauchop and Elsden (1). Moustafa and Collins (6) recently reported that the Y(ATP) value for this organism growing on arginine is 17.8, and that the average Y(ATP) for species of *Streptococcus* (including *S. faecalis*) growing on glucose, galactose, lactose, or maltose was 17.0. It is important to test the value of Y(ATP) for different microorganisms growing on limiting concentrations of various energy sources. This paper reports on Y(pyruvate) for *S. faecalis* NCTC 6783.

Deibel and Niven (2) reported that *S. faecalis* can utilize pyruvate as the sole source of energy. Since the oxidation of pyruvate to acetate and CO<sub>2</sub> or formate by certain lactic acid bacteria yields 1 mole of ATP per mole of pyruvate oxidized (4, 5), Y(pyruvate) should equal Y(arginine) if oxidative phosphorylation does not occur. A. J. Smalley et al. (Bacteriol. Proc., p. 109, 1967) found Y(glucose) for *S. faecalis* growing aerobically to be 55 and, by assuming that Y(ATP) equals 10.5, concluded that the high result for Y(glucose) substantiated the occurrence of oxidative phosphorylation.

*S. faecalis* 6783 failed to utilize pyruvate as the sole source of energy in BEO medium, reported by Moustafa and Collins (6), but it grew on pyruvate in the complex medium of Deibel and Niven (2) adjusted to pH 6.8. Figure 1 shows the growth of *S. faecalis* in the complex medium with different concentrations of glucose or pyruvate. Y(glucose) determined from the data is 46.4 for concentrations of glucose below 6  $\mu$ moles/ml and 34.4 for glucose concentrations of 6 to 10  $\mu$ moles/ml. Y(pyruvate) is 17.3 for pyruvate concentrations up to 15  $\mu$ moles/ml. Growth was significant

without added energy source, and straight-line curves relating the dry weight of cells to amounts of utilized substrate passed above the origin, indicating that the complex medium contained an unknown energy source.

The value of 34.4 for Y(glucose) determined with the higher limiting concentrations of glucose

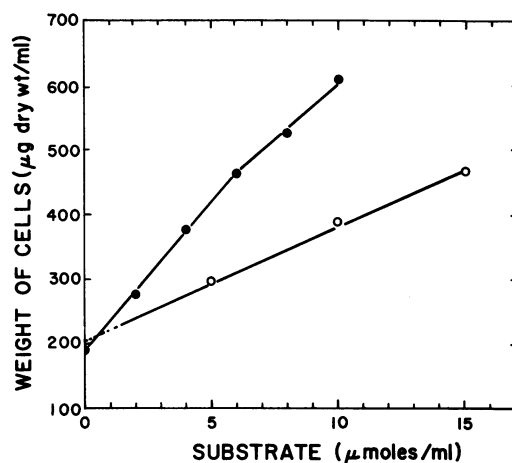


FIG. 1. Yields of *S. faecalis* 6783 growing on glucose or pyruvate. The organism was grown statically in 1 atm of air at 37 C in quantities of the complex medium of Deibel and Niven (2) at pH 6.8. Dry weight was determined from a standard curve relating optical density to dry weight. A 1-mg amount (dry weight) corresponded to 2.91 optical density units at 600 nm. Symbols: ●, glucose; ○, pyruvate.

in this complex medium is similar to the Y(glucose) value found by Moustafa and Collins (6) for the same organism in BEO medium, and the value of 17.3 for Y(pyruvate) is similar to the Y(arginine) value for the organism in BEO medium (6). These in vivo results support indications of in vitro results (4, 5) that only 1 mole of ATP per mole of pyruvate is produced in the oxi-

dation of pyruvate to acetate and CO<sub>2</sub> or formate. Similarity of Y(pyruvate) to Y(arginine) suggests that the hydrogens formed in pyruvate oxidation by *S. faecalis* (grown statically in an atmosphere of air) were not used for oxidative phosphorylation.

The high Y(glucose) value of 46.4 suggested that of the pyruvate formed during the fermentation of glucose at concentrations of less than 6  $\mu$ moles/ml, about 50% was oxidized to acetate plus CO<sub>2</sub> or formate, with the formation of 1 mole extra of ATP per mole of glucose. Gunsalus (4) reported a similar observation for *S. faecalis* growing aerobically on glucose. This 50% increase in Y(glucose) at low levels of glucose was observed only in the complex medium containing 0.5% yeast extract (2), not in the BEO medium. When the BEO medium (pH 6.8) was supplemented with 0.1% yeast extract, however, the organism was able to utilize pyruvate as the sole source of energy, which indicated that the BEO medium lacked some growth factor required for pyruvate oxidation.

Yeast extract is known to contain lipoic acid, originally called the pyruvate-oxidizing factor, which is required for pyruvate oxidation to acetate and CO<sub>2</sub> (2, 5) and utilization of citrate (R. H. Deibel et al., *Bacteriol. Proc.*, p. 114, 1958) and

serine (R. H. Deibel and C. F. Niven, Jr., *Bacteriol. Proc.*, p. 164, 1960) by *S. faecalis*. Addition of sodium lipoate to the BEO medium (64  $\mu$ g/ml) resulted in erratic utilization of pyruvate by the organism. Thiamine (1  $\mu$ g/ml) added to the BEO medium besides the sodium lipoate enabled *S. faecalis* to use added pyruvate as the sole source of energy.

This investigation was supported by Public Health Service research grant UI-00101 from the National Center for Urban and Industrial Health.

#### LITERATURE CITED

1. Bauchop, T., and S. R. Elsdon. 1960. The growth of microorganisms in relation to their energy supply. *J. Gen. Microbiol.* 23:457-469.
2. Deibel, R. H., and C. F. Niven, Jr. 1964. Pyruvate fermentation by *Streptococcus faecalis*. *J. Bacteriol.* 88:4-10.
3. Gunsalus, I. C. 1954. Oxidative and transfer reactions of lipoic acid. *Federation Proc.* 13:715-722.
4. Gunsalus, I. C. 1960. Energy metabolism of lactic acid bacteria. *Proc. Intern. Congr. Biochem.* 4th (Vienna), 1958. 13:444-449.
5. Gunsalus, I. C., and C. W. Shuster. 1961. Energy yielding metabolism in bacteria, p. 1-58. In I. C. Gunsalus and R. Y. Stanier (ed.), *The bacteria*. Vol. 2. Academic Press, Inc., New York.
6. Moustafa, H. H., and E. B. Collins. 1968. Molar growth yields of certain lactic acid bacteria as influenced by autolysis. *J. Bacteriol.* 16:117-125.