CALORIMETRIC MEASUREMENTS OF ENERGY OF MAINTENANCE OF STREPTOCOCCUS FAECALIS

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Many aerobic micro-organisms exhibit respiratory activity in the absence of external substrates. This endogenous metabolism is readily observed by the classical methods of manometry. In contrast, most anaerobic organisms have no such convenient property; their endogenous metabolism is a virtually unexplored domain (Dawes and Ribbons 1962).

Calorimetric observation of suspensions of washed cells of S. faecalis show that heat is produced by such preparations in the absence of added energy sources. The rate of heat production is correlated both with the environment of the microbes and with their degree of organization.

Experimental

Washed suspensions - Streptococcus faecalis (ATCC4083) was grown on a medium containing 2.0% each of sodium citrate, glucose, dried peptone and dried yeast extract. 17-hour cells were harvested by centrifugation, washed twice with deaerated potassium phosphate buffer 0.1M, pH 6.0, and suspended in the same buffer.

Calorimetry - The technique has been previously described (Forrest Walker and Hopgood 1961). Endogenous heat production was observed in 250 ml samples of washed suspension under a gas phase of nitrogen.

Glycolytic activity -

a) Warburg manometry - Washed suspensions were centrifuged, the cell paste so obtained suspended in 0.025M - $NaHCO_3$ and 0.5 ml of this suspen-

sion containing 2 – 5 mg of cells added to the side-bulb of the Warburg cup. The central compartment contained 0.1 ml of 0.1M glucose plus 2 ml of 0.025M – $NaHCO_3$. The gas phase was N_2 – 20% CO_2, and glycolytic activity was determined by rate of CO_2 evolution.

b) pH-stat – The cells were suspended in 10 ml of 0.01M phosphate buffer, 0.5 ml of 10% glucose added, and the pH maintained at 6.0 by titration of the lactic acid produced with 0.1M – NaOH.

Dry weights – The optical density of suspensions was measured at 650 mμ and dry weight obtained by reference to a standard curve.

Temperature – All incubations, calorimetry and glycolytic activity assays were carried out at 37°C.

Soluble carbon compounds were assayed by the method described by Halliwell (1960) on supernatants obtained after lysis by heating the cell suspensions for 10 minutes on a boiling water bath.

Amino acids were determined by the ninhydrin method of Moore and Stein (1948) after release of intracellular material as above.

Results

Figure 1 shows a typical calorimetric record from a suspension in which the redox potential was not controlled. Heat is produced at a constant rate for several hours after the suspension is first prepared, then the heat production falls off slowly till its rate is immeasurable. The rates of release of soluble carbon compounds and amino acids follow the pattern of heat production (Figure 2). The glycolytic activity also remains at its initial value for several hours then falls slowly till the suspension becomes inactive. Figure 3 shows the reduction in glycolytic activity of aliquots of suspension, one aerated and the other maintained at a low E_h under nitrogen by the addition of sodium thioglycollate (final concentration 0.05%). Manometric measurements show a very small oxygen uptake by aerated suspensions, 70 μl/hour/g.

The rate of loss of glycolytic activity in suspensions is markedly affected by the cell density (Figure 4).

Figure 5 shows the calculated specific rate of heat production (endogenous heat production rate divided by weight of cells) plotted against cell density.

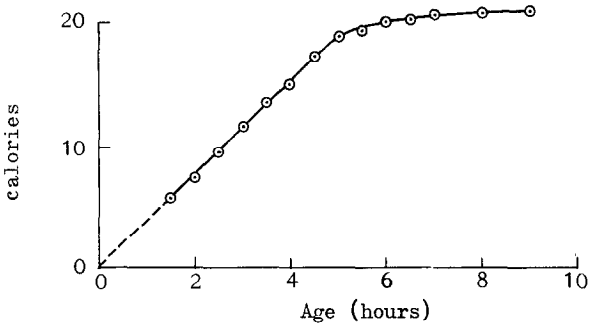

Figure 1 - Endogenous heat production by washed suspensions of S. faecalis. 450 mg cells. Gas phase N_2, not reduced.

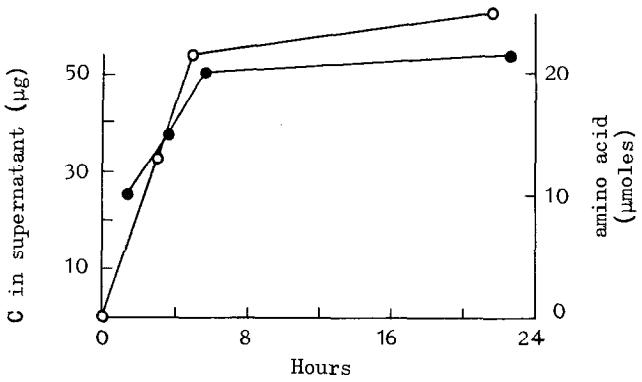

Figure 2 - Production of soluble carbon compounds and amino acids by washed suspensions of S. faecalis. Gas phase N_2, not reduced.
o soluble carbon, 2.3 mg cells, ● amino acids, 45 mg cells.

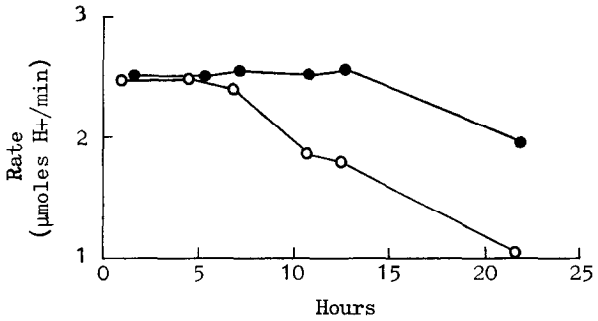

Figure 3 - Glycolytic activity of cells incubated in aerated and reduced washed suspensions. 16 mg cells, activity determined by pH-stat.
o gas phase air, ● gas phase N_2, reduced with sodium thioglycollate.

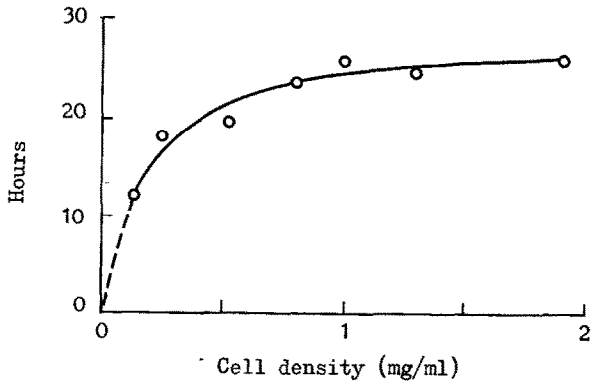

Figure 4 - Time for glycolytic activity of cells from suspensions of differing cell densities to drop to 50% of original. Manometric assay of activity. Gas phase N_2, not reduced.

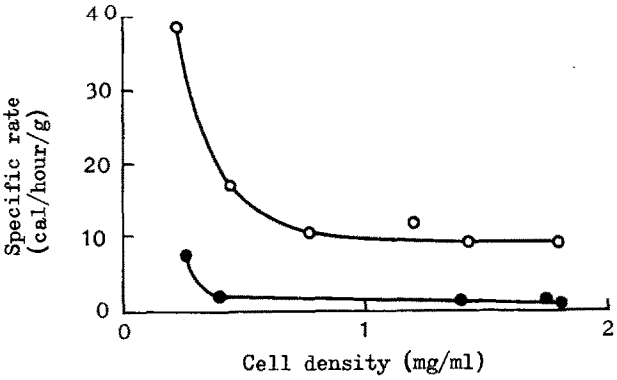

Figure 5 - Specific rate of endogenous heat production by washed suspensions of varying cell densities. Gas phase N_2. o E_h not controlled, ● reduced with sodium thioglycollate.

The lower concentrations of organisms produce heat at a greater rate per unit dry weight than the higher concentrations. The effect of reducing the E_h of the suspension by adding sodium thioglycollate (final concentration 0.05%) is striking.

Discussion

The glycolytic activity of S. faecalis grown under standardised conditions is a very well-defined property of the organisms (Forrest Walker and Hopgood 1961). It is a reasonable assumption from this that loss of glycolytic activity represents loss of organization of the microbes. (Nothing is assumed about viability). The loss of activity is a rate process beginning after heat produc-

tion ceases; the rate is influenced both by the redox potential of the suspension and cell density, similarly to the rate of heat production by the organisms.

The effect on both glycolytic activity and heat production of controlling the environment of the cells with thioglycollate, together with the more rapid loss of glycolytic activity and increased rate of heat production by dilute suspensions, indicate that the favourable environment for the cells is one of low E_h and high cell density. When they are placed in a less favourable situation more work is required for their maintenance, so that their heat production is increased and their finite reserves of internal energy sources are used more quickly.

This effect gives a physical basis for the common observation that the inoculum age and size determines to some extent the length of the lag phase in a bacterial culture. The previous history of the cells in an inoculum will affect their reserves; when the cells are then subjected to conditions of low cell density, they may have to produce energy rapidly to survive, thus reducing the proportion of their available energy directed to the process of multiplication.

The minimal uptake of oxygen by the suspensions under a gas phase of air shows that the heat production observed under nitrogen cannot be due to reaction with traces of oxygen. However, the observed production of amino acids also seems insufficient to explain all the observed heat. The soluble carbon compounds appearing concurrently with endogenous heat production can all be accounted for as amino acids (assuming 4 carbon atoms per acid) but the rate of production is only 110 μmoles per hour per g dry weight of cells. Sturtevant's (1953) calorimetric determinations of the heat of hydrolysis of peptide bonds give a value for ΔH of -2 kilocalories per mole; thus anaerobic production of amino acids by splitting of peptide bonds would produce only 0.2 calories per g per hour, compared with the minimum observed heat output in a favourable environment of 1.5 calories per g per hour.

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