METABOLISM OF LACTIC ACID BACTERIA

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University, New Zealand.

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ABSTRACT

Streptococcus lactis organisms were grown in lactose-limited batch culture and the survival characteristics of washed organisms were examined at the growth temperature. Washed suspensions had high initial viabilities (>99%) which were maintained for varying periods depending on the presence of certain added materials in the buffer and the conditions of incubation. Added Mg^{2+} markedly prolonged survival, while high bacterial concentrations also extended survival times, probably because Mg^{2+} was excreted by the bacteria. Surviving organisms in some conditions showed prolonged division lags, especially in the absence of Mg^{2+}. Addition of trace amounts of EDTA decreased the death rate by removing toxic cation impurities, while the buffer salt concentration had little effect on survival within wide limits. The optimum pH value for survival was near 7.0 and survival times increased considerably at lower temperatures. Agitation and aeration tended to decrease survival and the death rate was not influenced by the phase of growth at which the organisms were harvested from a lactose-limiting medium.

Addition of casamino acids increased survival times markedly in the presence of Mg^{2+}; arginine was almost as effective as the complete mixture of amino acids while other individual amino acids tested gave only slight increases in survival times. Fermentable carbohydrates accelerated death of starved organisms irrespective of the growth phase from which they were harvested and of the limiting nutrient; the accelerated death rate was reduced by addition of Mg^{2+}. Glucose metabolism proceeded at a much faster rate than arginine metabolism, theoretically producing about 7.5 times as much ATP. This rapid generation of ATP may be responsible for the more rapid

death rates with added carbohydrates. Arginine substantially reduced the lethal effect of adverse pH values and suppressed the leakage of free intracellular amino acids into the external medium.

Survival studies were followed by an investigation of the changes which took place in starved organisms and their relation to survival. No polyglucose or poly-β-hydroxybutyrat was detected and starved organisms had a negligible respiration rate. Soluble protein was released from viable organisms into the suspending buffer and the intracellular free amino acid pool declined steadily with the components appearing in the suspending buffer; a net increase in the total amount of free amino acid indicated some protein hydrolysis. Chloramphenicol reduced the death rates in some environments, possibly by suppressing protein degradation. RNA was hydrolysed with the release of u.v.-absorbing bases and ribose from the organisms. Conditions which promoted rapid RNA breakdown also produced rapid death rates and long cell division lags in surviving organisms. There was no appreciable degradation of carbohydrate or DNA. After 28 hr. starvation in buffer containing Mg^{2+}, the bacterial dry wt. decreased by 26%; loss of RNA, protein and free amino acids accounted for 10.3%, 7.3% and 2.7% of the total bacterial mass loss. The products of polymer hydrolysis appeared to be released in an undegraded form into the external buffer and there was no appreciable formation of lactate, ammonia or volatile fatty acids possibly indicating the absence of any important endogenous energy sources.

Protein synthesis, determined by the incorporation of valine-^{14}C into TCA-insoluble material, was barely detectable when organisms were starved in buffer containing Mg^{2+}. Addition of an energy source allowed limited protein synthesis while glucose produced a much higher rate of valine-^{14}C uptake and incorporation than arginine.

Although arginine prolonged survival this was not due to the limited protein synthesis which took place. The survival capacity of starved organisms could be correlated with the ability to synthesize protein which in turn may be correlated with RNA stability.

A new method was developed for the assay of glycolytic activity in microorganisms. Organisms were incubated with glucose-U-^{14}C and samples removed at intervals. Samples were chromatographed on DEAE-cellulose paper strips in deionized water which separated the radioactive anionic products of glycolysis (lactate, acetate and formate) from the unfermented glucose. The activity of the two fractions was then determined by liquid scintillation counting. The glycolytic activity of starved organisms declined steadily and was not correlated with survival.

Phospholipid was broken down on prolonged starvation and the permeability properties of the organism were gradually lost. Addition of spermine gave enhanced survival and suppressed the release of u.v.-absorbing material. Lactic dehydrogenase and DNA were released as the death rate increased in buffer containing Mg^{2+} and eventually, well after death, cell lysis occurred. Electron micrographs indicated that addition of amino acids maintained cell structures for a much longer period and in this system cell lysis occurred as the death rate increased. It was concluded that the death rate of starved S. lactis organisms in phosphate buffer was partly dependent on the presence of Mg^{2+}, which probably acted by promoting polymer stability, particularly that of RNA. In this environment, a suitable exogenous energy source further enhanced survival which may ultimately be a function of cell wall and membrane stability.

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PREFACE

A major function of the Microbiology Department of the New Zealand Dairy Research Institute is the maintenance of active cultures of various lactic acid bacteria for use in the cheese industry. This work is vital for the efficient functioning of one of the country's major industries. The most important cheese 'starter' organisms are the lactic streptococci. This group consists of Streptococcus lactis, Streptococcus cremoris and variants. No detailed measurements have been reported on factors affecting the survival and activity of lactic streptococci, or closely related bacteria, at growth temperatures. Accordingly, it was decided to undertake such an investigation which could possibly produce results of considerable practical significance.

The Introduction is divided into two parts. Part I contains a literature review on the metabolism of lactic acid bacteria which provides a background for subsequent discussions. Part II contains a summary of the reports most relevant to studies on the survival of S. lactis.

Publications to date from results presented in this thesis are entitled:
'Survival of Streptococcus lactis in starvation conditions', J. gen. Microbiol., 50, 367, (1968),
'A new method for the assay of glycolytic activity with special reference to microorganisms', Anal. Biochem., in Press.

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