

## Levels of Metabolic Intermediates in *Streptococcus lactis* Grown on Different Carbon Sources and the Effect on Product Formation \*

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The growing interest in using fermentation processes for producing both high value chemicals and chemical feed stocks, points out the necessity of understanding how the metabolism of the microbial cell is regulated.<sup>1</sup> With a deeper knowledge about these mechanisms it will be possible to direct fermentation processes towards higher yields of desired products, which is of utmost importance in situations where the price of the raw material often makes up much more than 50 % of the price of the product.

There are essentially two ways of gaining an understanding of the regulation of microbial metabolism: one is to identify the various enzymes along a route, show their specificity and regulatory function; the other is to quantify intermediary metabolites and from there identify which steps are regulatory. This communication describes the latter approach.

Methods for determining intermediary metabolite levels were developed by Lowry and Passonneau.<sup>2</sup> These methods preferably use the glycolytic and Krebs cycle enzymes to "pull" a certain metabolite towards a reduction of NAD or oxidation of NADH. This can then be measured fluorimetrically. These methods have so far only been used in a few applications. The metabolism of *E. coli* grown on various carbon and nitrogen sources has been studied.<sup>3</sup> Homo- and heterolactic fermentation in *S. lactis* has also been elucidated.<sup>4</sup>

This communication deals with the application of these fluorimetric methods to investigate the shift in product formation when *S. lactis* is grown on maltose as compared to glucose. A previous

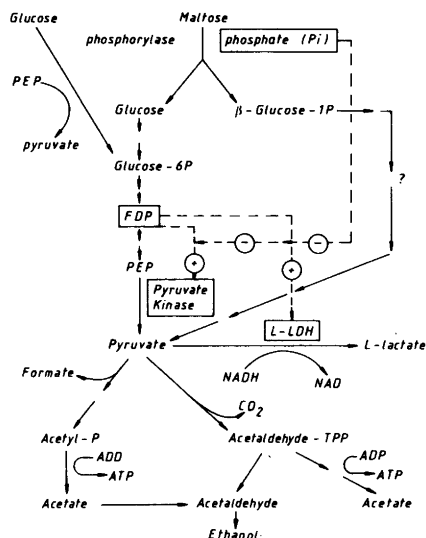


Fig. 1. Effects of metabolic regulation on product formation in *Streptococcus lactis* as suggested by Häggström (1981).

investigation had shown a pronounced heterofermentative product formation when *S. lactis* was grown in maltose, whereas glucose grown cells gave a homofermentative pattern.<sup>5</sup> In Fig. 1 the proposed metabolic routes for *S. lactis* grown on glucose and maltose, respectively, are shown. *S. lactis* 65.1 from the Swedish Dairies Association, Malmö, was grown on a complex media containing tryptone (5 g/l), yeast extract (5 g/l), caseamino acids (1 g/l), phosphate, magnesium sulfate and carbohydrates, (20 g/l glucose or 20 g/l maltose).

In a preliminary investigation it was found that the complex medium interfered with the measurements of pyruvate (PYR) and phosphoenolpyruvate (PEP), the disturbing components being tryptone and yeast extract. The concentration of these components was then decreased to 1/20 of the original values without decline in growth rate. At these concentrations they did not interfere with the measurements of metabolite levels.

The other problem involved when determining intracellular metabolite concentrations was to find a sampling technique which enabled the metabolism to be stopped in less than 2 s so that the pools of metabolites would not be changed due to starvation. Several sampling techniques were compared in the present study (Table 1). The reliability of these methods was tested by measuring intracellular levels of fructose - 1,6-

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Table 1. A comparison of the sampling techniques. All perchloric acid extracts were vortexed vigorously and were placed on ice for 5 min before centrifugation and neutralization to pH 7.0 with solid  $K_2CO_3$ . The clarified supernatants were stored at  $-80^\circ C$  until analyzed, usually the next day. Values for FDP were calculated on the basis that 1 mg dry weight of cells contains  $1.67 \mu l$  cytoplasmic fluid.<sup>6</sup>

Method	Glucose grown cells FDP (mM)	Maltose grown cells FDP (mM)	Advantages	Dis-advantages
Syringe <sup>a</sup>	30.1	20.5	Fastest immediate stop in metabolism	Cells dilute Medium present
Filter <sup>b</sup>	25.6	14.7	Fast Removes medium Concentrates cells	Vacuum may affect metabolism
Lyophilization <sup>c</sup>	26.7	13.6	Fast Immediate stop in metabolism Concentrates cells	Concentrates medium
Centrifuge <sup>d</sup>	7.3	4.5	Removes medium Concentrates cells	Slow

<sup>a</sup> With the syringe method samples are taken up in a syringe containing enough concentrated perchloric acid to give a 0.6 M final concentration. <sup>b</sup> The samples are filtered and the filter is transferred to liquid nitrogen in less than 2 seconds. Filters are subsequently extracted with 0.6 M perchloric acid. <sup>c</sup> The samples are dropped into liquid nitrogen and then lyophilized. The residue is then extracted as in b. <sup>d</sup> The samples are centrifuged for 5 min at 10 000 g, the supernatant decanted, and the pellet immediately extracted as in b.

Table 2. Effect of carbon source on metabolite levels and product patterns.

Carbon source	FDP(mM)	PEP(mM)	PYR(mM)	g/l EtOH	g/l HOAc	Lactate (mM)
Glucose	33	11.8	<3	N.D. <sup>a</sup>	N.D. <sup>a</sup>	0.246
Maltose	15	13.6	<3	0.199	0.133	0.206

<sup>a</sup> N.D.=not detectable.

diphosphate (FDP) in both glucose and maltose grown cells. Within experimental error the filter, syringe and lyophilization methods gave the same results (Table 1), whereas centrifugation appeared to interfere with metabolism, most likely in that it was the slowest method. Because of its simplicity and rapidity, the filter method was utilized in all further experiments.

Intracellular levels of fructose-1,6-diphosphate (FDP), phosphoenolpyruvate (PEP) and pyruvate (PYR) were then determined using batch fermentations of *S. lactis* grown on glucose or maltose, since these metabolites were considered key intermediates in understanding the shift from

homo to heterolactic product formation. At least eight samples were taken during the exponential growth phase and analyzed in duplicate. The data given in Table 2 are the average values. The extracellular products lactate, ethanol and acetate were also determined.<sup>5</sup>

When *S. lactis* was grown on glucose, the intracellular concentration of FDP was about double compared to when the organism was grown on maltose (Table 2). The levels of PEP and PYR, however, were about the same for both carbon sources.

The FDP data support the hypothesis that the two sugars in maltose are metabolized along

different metabolic routes (Fig. 1) and that only half of the maltose molecule is processed along the Embden Meyerhof Pathway. As such, the decreased level of FDP in maltose grown cells may not be high enough to stimulate the L-lactate dehydrogenase to completely convert pyruvate to L-lactate, thus, opening the possibility for pyruvate to be metabolized to other end-products. Phosphate involved in the internal splitting of maltose has also been proposed as a regulatory factor.<sup>5</sup>

Although PEP in the maltose grown cells would also be expected to be one half of that found in glucose grown cells, the fact that PEP is used in the transport of glucose, but not necessarily of maltose,<sup>5</sup> may explain why this metabolite has a similar concentration in both cases (Table 2). Pyruvate concentrations are very low in the two situations, due to rapid funneling of this metabolite into the various pathways.

In conclusion, this study shows that it is possible to measure concentrations of intracellular intermediary metabolites even in fermentations based on complex media, and that these measurements can contribute to the understanding of how microbial metabolism is regulated.

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