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Effect of Low *p*H on Thiomethyl-β-D-Galactoside Uptake by *Streptococcus lactis*

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Maximal β-galactosidase activity in *Streptococcus lactis* was obtained at *p*H 7, but the maximal rate of thiomethyl-β-D-galactoside uptake was observed at *p*H 3.6 to 4. It is concluded that the decrease in β-galactosidase activity in intact cells at lowered *p*H is not due to diminished transport of β-galactoside.

Most streptococcal species produce acid at a rapid rate in the presence of carbohydrate; hence, the acid sensitivity of metabolic systems essential to these microorganisms should be important for their survival. β-Galactoside transport and β-galactosidase activity have been intensively studied in *Escherichia coli* (3, 4, 6) and have also been investigated in *Streptococcus lactis* (1, 2). By comparing the effect of environmental *p*H on the β-galactoside transport with that on β-galactosidase activity in *S. lactis*, it would be possible to show whether these systems are affected. For the present study, *S. lactis* ATCC 7962 was used, as β-galactosidase in this strain is not labile to toluene treatment (1). The hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) was used to measure β-galactosidase activity in intact cells, and the nonhydrolyzable substrate ^{14}C-thiomethyl-β-D-galactoside (^{14}C-TMG) was used to measure β-galactoside transport. A comparison of these activities can be made on the assumption that TMG is transported by the same permease that transports ONPG into the cells (3, 6).

Ten-hour cultures of *S. lactis*, induced for β-galactosidase with lactose, were used to prepare cell suspensions for the enzyme assay (1). The growth medium and the cell suspensions were prepared in phosphate-citrate buffer (0.1 M Na_2HPO_4 plus 0.05 M citric acid) at *p*H 6. β-Galactosidase activity in intact and toluene-treated cells was measured by adding 1 ml of the cell suspension to 4 ml of 0.005 M ONPG in the buffer at *p*H 4, 4.6, 5, 6, 7, and 8. The assay procedure was as described by Citti et al. for *S. lactis* (1).

To study the uptake of TMG, *S. lactis* was grown for 10 hr at 30 C in 300 ml of mineral medium M63 (6) in the phosphate-citrate buffer (*p*H 6) supplemented with 0.1% peptone and 1% lactose. The cells were washed and resuspended in 10 ml of the same medium at *p*H 6 (250 to 300 μg of bacterial nitrogen per ml), but lactose was replaced by 0.3% maltose. After incubation for 3 hr in the presence of maltose as the sole source of energy, the cells were centrifuged at 30 C and used for the measurement of ^{14}C-TMG uptake at *p*H 3, 3.6, 4, 4.6, 5, 6, 7, and 8.

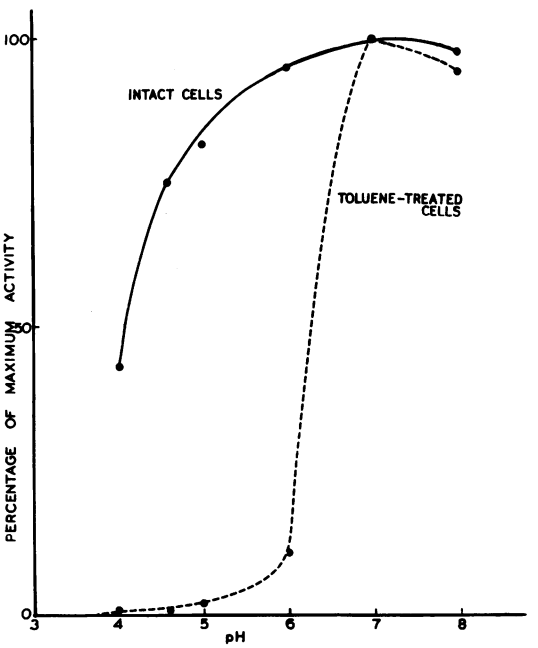

FIG. 1. *Effect of pH on β-galatosidase activity in intact and toluene-treated cells of Streptococcus lactis. The ordinate refers to the percentage of maximal activity obtained at pH 7.*

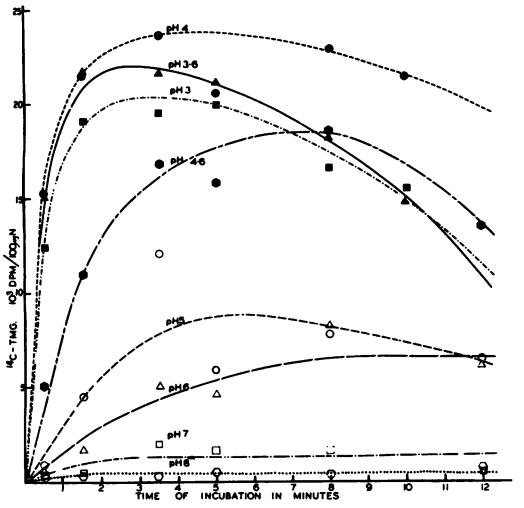

FIG. 2. *Effect of pH on ^{14}C-TMG uptake by Streptococcus lactis. Cells were suspended in 10 ml of medium M63 (10^{-3} M $MgSO_4$, 2×10^{-2} M NH_4Cl and 10^{-5} M $FeCl_3$ in phosphate-citrate buffer) at the indicated pH containing 0.3% maltose, 0.1% peptone, and 40 μg of chloramphenicol. At zero time, 5 μc of ^{14}C-TMG (specific activity, 7.9 mc/mmole) was added, and 0.5-ml samples were withdrawn at intervals, filtered, and washed three times with 2-ml portions of the cold buffer. After drying the filter, the cells were counted by the liquid scintillation technique. Total disintegrations per min (DPM) per 100 μg of bacterial nitrogen (N) are presented on the ordinate.*

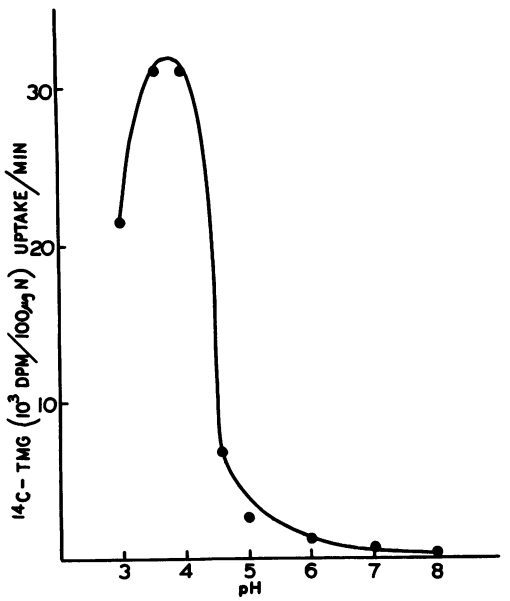

FIG. 3. *Effect of pH on the initial rate of ^{14}C-TMG uptake by Streptococcus lactis. The initial rates were obtained from the data presented in Fig. 2.*

Maximal β-galactosidase activity in intact cells was obtained at *p*H 7, and this activity was found to increase by several-fold after toluene treatment. In toluene-treated cells there was a sharp decrease in activity as the *p*H was lowered, compared to that observed with intact cells (Fig. 1). Similar results were reported by Lederberg for *E. coli* (4). A simple interpretation of these results would be that the intact cytoplasmic membrane of the cell protects the internal environment from the external acid environment.

The maximal rate of TMG uptake was observed at *p*H 3.6 to 4 within 5 min of incubation (Fig. 2). On further incubation up to 60 min, a decrease in cellular ^{14}C-TMG was found below *p*H 6. Thus, *S. lactis* is able to carry out active transport of TMG in a highly acidic environment at 30 C. It can be concluded that the decrease in β-galactosidase activity in intact cells at a *p*H below 6 (Fig. 1) is not due to diminished transport of β-galactoside. It seems that the transport system compensates for the effect of lowered *p*H on the β-galactosidase activity by making a larger quantity of substrate available to the enzyme in intact cells under acidic conditions. It is often assumed that enzyme activities would decrease at low *p*H, but this report reveals that lowered *p*H results in an increase in β-galactoside permease activity in *S. lactis*.

Recently, in studies on the phosphoenolpyruvate in lactose utilization, McKay et al. (5), working at *p*H 7, reported a slower rate of ^{14}C-TMG uptake by *S. lactis* 7962 when compared to strain C_2F. In the present work, the high uptake observed with strain 7962 would be dependent on the presence of an exogenous energy source.

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