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Catabolite Regulation in a Diauxic Strain and a Nondiauxic Strain of *Streptococcus bovis*

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Abstract. *Streptococcus bovis* JB1 utilized glucose preferentially to lactose and grew diauxically, but *S. bovis* 581AXY2 grew nondiauxically and used glucose preferentially only when the glucose concentration was very high (greater than 5 mM). As little as 0.1 mM glucose completely inhibited the lactose transport of JB1. The lactose transport system of 581AXY2 was at least tenfold less sensitive to glucose, and 1 mM glucose caused only a 50% inhibition of lactose transport. Both strains had phosphotransferase systems (PTSs) for glucose and lactose. The glucose PTSs were constitutive, but little lactose PTS activity was detected unless lactose was the energy source for growth. JB1 had approximately threefold more glucose PTS activity than 581AXY2 (1600 versus 600 nmol glucose (mg protein)$^{-1}$(min)$^{-1}$. The glucose PTS of JB1 showed normal Michaelis Menten kinetics, and the affinity constant (K_s) was 0.12 mM. The glucose PTS of 581AXY2 was atypical, and the plot of velocity versus velocity/substrate was biphasic. The low capacity system had a K_s of 0.20 mM, but the K_s of the high capacity system was greater than 6 mM. On the basis of these results, diauxic growth is dependent on the affinity of glucose enzyme II and the velocity of glucose transport.

Streptococcus bovis is a rapidly growing and opportunistic ruminal bacterium that outgrows other bacteria, decreases ruminal pH, and causes acute indigestion [6, 7, 17, 19]. Recent work also indicates that *S. bovis* can be a significant inhabitant of the human bowel, and its proliferation has been correlated with the onset of colon cancer [1, 4, 9]. Lactose is not normally a substrate for ruminal fermentation, but significant amounts of lactose can enter the human large intestine [10]. From the fact that all strains of *S. bovis* can utilize lactose [6] and the observation that lactose intolerance and colon cancer are both late-onset human diseases [15], it appeared that lactose utilization might be a key factor influencing the growth of *S. bovis* in the human bowel.

Previous work indicated that the JB1 strain of *S. bovis* had a glucose phosphotransferase system (PTS) [12], PTS systems for maltose, sucrose, and cellobiose [13], a facilitated diffusion mechanism for glucose [16], and a lactose PTS [3]. Glucose inhibited the lactose uptake of *S. bovis* JB1 and allowed it to grow diauxically [3], but a mutant that lacked glucose PTS activity used glucose and lactose simultaneously and grew in a nondiauxic fashion [8]. This latter result supported the idea that glucose enzyme II was inhibiting the lactose PTS. *S. bovis* JB1 and 581AXY2 are phenotypically similar [18], and both strains have a glucose PTS [14].

Preliminary experiments indicated that 581AXY2 used glucose preferentially to lactose only when the glucose concentration was very high (>5 mM). The following experiments were designed to determine if JB1 and 581AXY2 had a different mechanisms of catabolite regulation.

Materials and Methods

Cell growth. *Streptococcus bovis* JB1 and 581AXY2 were previously described [18], and 581AXY2 was originally obtained from Colin Stewart (Rowett Research Institute, Aberdeen, Scotland). Cultures were grown anaerobically at 39°C in basal medium containing (per liter) 292

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mg K_2HPO_4, 292 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2 \cdot 2H_2O$, 600 mg cysteine hydrochloride, 1 g Trypticase (BBL Microbiology Systems, Cockeysville, Md., USA), 0.5 g yeast extract, and either 10 mM glucose or 5 mM lactose. Carbon sources and glucose analogs were all prepared anoxically and added from separately sterilized stock solutions to obtain the desired final concentration. The medium was adjusted to pH 6.7, and the final pH was never less than 6.5. Growth was monitored by the increase in optical density (1 cm light path, 600 nm). The relationship between optical density and cell protein was 160 mg protein (liter)$^{-1}$ (optical density unit)$^{-1}$.

Toluene-treated cells. Cells were harvested (10 ml) during exponential growth (optical density approximately 1.0 at 600 nm) by centrifugation (10,000 *g*, 10 min, 4°C) and washed twice in 100 mM sodium-potassium phosphate buffer (pH 7.2) containing 5 mM $MgCl_2$ and 2 mM dithiothreitol. The cells from 10 ml culture were then resuspended in 2 ml of the same buffer and stored on ice. The cell suspension (500 µl) was treated with 15 µl of toluene:ethanol mixture (1:9, vol/vol) as previously described [12].

PTS assays. PEP-dependent sugar phosphorylation was assayed enzymatically by a method that measured pyruvate production via lactate dehydrogenase [12]. The PTS assays were performed at 39°C, and the activities at 39°C were at least tenfold higher than the ones measured at room temperature. Corrections were made for nonspecific NADH oxidase activity, and specific activities were determined under first order conditions (protein concentration versus activity was linear). Each assay typically contained 10 µl of the toluenized cells, 0.5 units L-lactate dehydrogenase, 0.15 µmol DTT, 1.0 µmol NADH, 20 µmol PEP, 20 µmol sugar, 33 µmol potassium phosphate, 33 µmol sodium phosphate per ml. All assays were performed in triplicate with appropriate controls. The coefficient of variation was always less than 10%.

Transport of radioactive sugars. Cells were harvested during exponential growth by centrifugation (10,000 *g*, 5 min, 4°C), washed twice in aerobic 100 mM sodium-potassium phosphate buffer (pH 7.2) containing 5 mM $MgCl_2$ and 2 mM dithiothreitol, and incubated (approximately 80 µg protein in 200 µl) aerobically with [^{14}C]lactose (60 µM final concentration; 50 mCi mmol^{-1}). Transport was terminated by the addition of 2 ml ice-cold 0.1 M LiCl to the reaction mixture and rapid filtration through 0.45 µm pore size cellulose nitrate membrane filters. Filters were washed with 2 ml of 0.1 M LiCl, dried for 30 min at 120°C, and radioactivity was determined by liquid scintillation counting. The transport kinetics were first order (protein versus activity was linear).

Other assays. The sugars were assayed by high pressure liquid chromatography (87H BioRad column, 0.5 ml min^{-1} 0.17 N H_2SO_4, refractive index detector, 50°C). Cells were treated with 0.2 *N* NaOH (100°C, 10 min), and protein was determined by the Lowry method [11].

Materials. All chemicals were analytical reagent grade. [Methyl-^{14}C]β-thiogalactopyranoside ([^{14}C]TMG), [^{14}C] glucose, and [^{14}C] 2-deoxyglucose were obtained from Amersham International (Amersham Laboratories, Arlington Heights, Illinois, USA).

Results

Diauxic growth. *S. bovis* JB1 and 581AXY2 grew rapidly on glucose and had high rates of phosphoenolpyruvate-dependent glucose phosphorylation (Table 1). Lactose-grown cells also had high glucose PTS activities, but 581AXY2 had more glucose PTS activity when it was grown on glucose. Both strains grew less rapidly on lactose than on glucose, and the lactose PTS activities were lower. Neither strain had significant lactose PTS activity unless lactose was the energy source for growth. *S. bovis* JB1 grew in a diauxic fashion, and there was a long lag time in growth before the second phase of growth (Fig. 1b). JB1 used glucose in a sequential fashion, and lactose utilization was not observed until all of the glucose was depleted (Fig. 1a). When glucose and lactose were provided at similar concentrations (approximately 2.5 mM), no diauxie was observed with 581AXY2 (Fig. 1a), and glucose and lactose were utilized simultaneously (Fig. 1b). When 581AXY2 was provided with eight- and fourfold higher glucose and lactose, respectively, glucose was used preferentially to lactose (Fig.

Table 1. Growth and PTS activity of *S. bovis*

Strain	Growth substrate	Growth rate (h^{-1})	Glucose PTS activity[a]	Lactose PTS activity[a]
JB1	Glucose	1.85 ± 0.07	1696 ± 63	10 ± 3
	Lactose	1.02 ± 0.04	953 ± 40	415 ± 29
581AXY2	Glucose	1.84 ± 0.14	626 ± 86	26 ± 7
	Lactose	1.36 ± 0.09	201 ± 10	368 ± 93

[a] All activities are expressed as nmol substrate converted to product per min per mg protein by toluene-treated cells at 39°C. Results are the means of triplicates ± range.

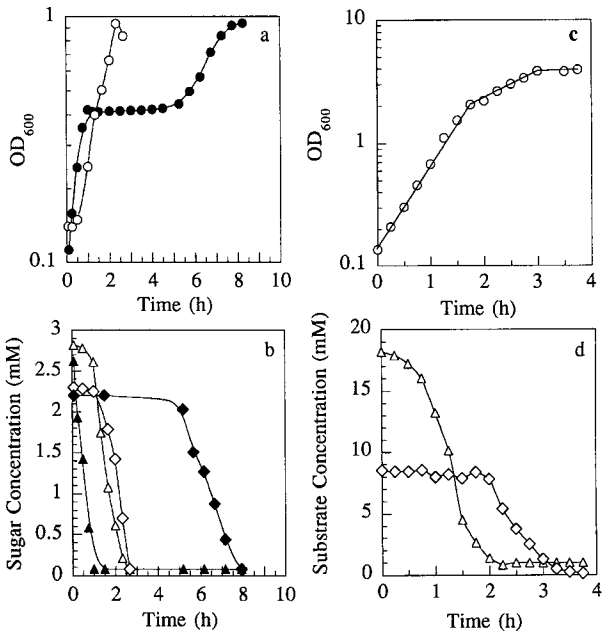

Fig. 1. (a) The growth by *S. bovis* of JB1 (closed symbols) and 581AXY2 (open symbols) and (b) their patterns of glucose (△, ▲) and lactose (◇, ◆) utilization. In parts (c) and (d) 581AXY2 was grown with higher concentrations of glucose and lactose.

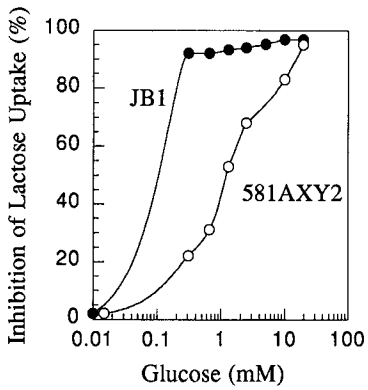

Fig. 2. The effect of external glucose on the uptake of [^{14}C]lactose by *S. bovis* of JB1 (closed symbols) and 581AXY2 (open symbols).

1d). No diauxie was observed, although growth was slower when lactose was utilized (Fig. 1c).

Inducer exclusion. Even very low concentrations of glucose (as little as 0.1 mM) completely inhibited [^{14}C] lactose uptake by JB1 (Fig. 2). Glucose also excluded [^{14}C] lactose from 581AXY2, but much higher concentrations were required. A glucose concentration of 1 mM caused only a 50% inhibition of [^{14}C] lactose uptake.

Kinetics glucose PTS. JB1 had more glucose PTS activity than 581AXY2 (Fig. 3a). The glucose PTS of JB1 followed typical Michaelis-Menten saturation kinetics, and the K_s and K_{max} values were 0.13 mM glucose and 1600 nmol glucose (mg protein)$^{-1}$(min)$^{-1}$, respectively (Fig. 3b). 581AXY2 had atypical kinetics, and plot of velocity versus velocity was biphasic (Fig. 3c). The high capacity system had a V_{max} of only 900 nmol glucose (mg protein)$^{-1}$(min)$^{-1}$, and the K_s was greater than 6 mM. Strain 581AXY2 also had a high affinity glucose PTS (K_s of 0.20 mM), but the V_{max} of this system was only 200 nmol glucose (mg protein)$^{-1(min)^{-1}}$.

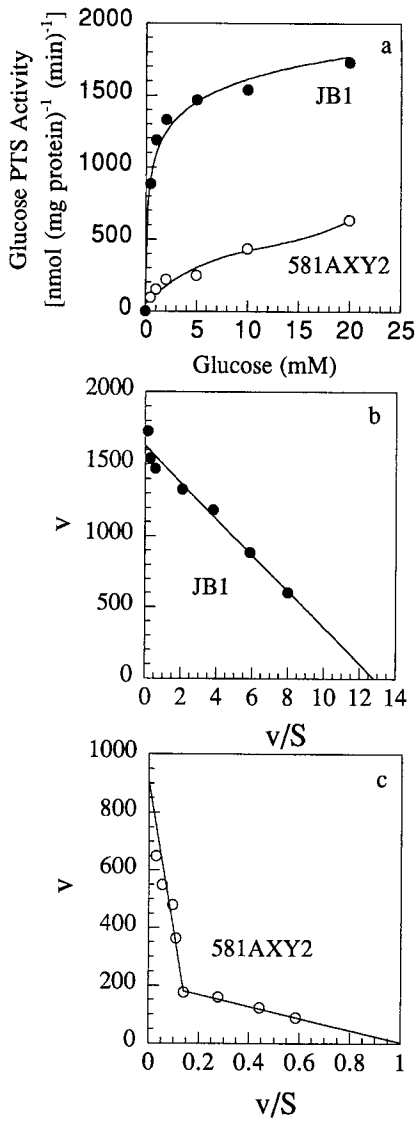

Fig. 3. The effect of glucose concentration (a) on the rate of phosphoenolpyruvate-dependent glucose phosphorylation by *S. bovis* of JB1 (closed symbols) and 581AXY2 (open symbols). The relationships between the velocity and velocity/substrate are shown in part (b) and (c). v has the units of [nmol (mg protein)$-$1(min)$-$1] and v/S has the units of [nmol (mg protein)$-$1(min)$-$1(μM glucose)$-$1].

Discussion

Streptococcus bovis JB1 has two transport systems for glucose, a PTS [12] and a facilitated diffusion mechanism [16]. These mechanisms can be differentiated by their method of phosphorylation. PTS activity is phosphoenolpyruvate dependent, but glucose translocated via the facilitated diffusion mechanism must be phosphorylated by an ATP-dependent glucokinase. Early work indicated that the specific activity of the glucose PTS was approximately 15-fold lower than the glucose consumption rate of exponentially growing cells [12, 16], but later work indicated that the glucose PTS had unusual temperature sensitivity. When the assay temperature was increased from 22° to 39°C, the normal growth temperature, the PTS activity increased more than tenfold [2]. Previous work [3] indicated that glucose PTS of JB1 had a velocity of approximately 1000 nmol glucose (mg protein)$^{-1}$(min)$^{-1}$. By increasing the substrate concentration from 5 to 20 mM and making sure that the cells never reached stationary phase, it was possible to get a rate as high as the glucose consumption rate [1600 nmol glucose (mg protein)$^{-1}$(min)$^{-1}$].

S. bovis JB1 has a fructose 1,6 diphosphate-dependent inducer expulsion mechanism for the nonmetabolizable lactose analog, thiomethylgalactoside [3], but this mechanism alone could not mediate preferential glucose utilization and diauxic growth [8]. A mutant

lacking glucose PTS activity used glucose and lactose simultaneously, and this result indicated that the glucose PTS was responsible for lactose exclusion [8]. In Gram-positive lactic acid bacteria, inducer exclusion has been explained by the hypothetical ability of glucose enzyme II to bind most of the phosphorylated HPr [20]. It has long been recognized that preferential sugar utilization and diauxic growth are the properties of batch cultures [5]. When bacteria are grown in sugar-limited continuous cultures at slow dilution rates, the concentration of extracellular sugar is low, the rate of sugar transport is slow, enzyme II glucose can no longer bind all of the phosphorylated HPr, and all available sugars are utilized simultaneously.

The comparison of *S. bovis* JB1 and 581AXY2 indicates that the K_s and V_{max} of glucose enzyme II are both important features of inducer exclusion. JB1 had a high-capacity, high-affinity glucose transport system, and this PTS allowed JB1 to use glucose preferentially, even if the external glucose concentration was low. The glucose PTS of 581AXY2 was a less active transport system, and its glucose enzyme II was only able to mediate inducer exclusion when the external glucose concentration was high.

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