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Derepression of LamB Protein Facilitates Outer Membrane Permeation of Carbohydrates into *Escherichia coli* under Conditions of Nutrient Stress

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The level of LamB protein in the outer membrane of *Escherichia coli* was derepressed in the absence of a known inducer (maltodextrins) under carbohydrate-limiting conditions in chemostats. LamB protein contributed to the ability of the bacteria to remove sugar from glucose-limited chemostats, and well-characterized *lamB* mutants with reduced stability constants for glucose were less growth competitive under glucose limitation than those with wild-type affinity. In turn, wild-type bacteria were less growth competitive than *lamB* mutants with enhanced sugar affinity. In contrast to an earlier report, we found that LamB$^-$ bacteria were less able to compete in carbohydrate-limited chemostats (with glucose, lactose, arabinose, or glycerol as the carbon and energy sources) when mixed with LamB$^+$ bacteria. The transport K_m for [^{14}C]glucose was affected by the presence or affinity of LamB, but only in chemostat-grown bacteria, with their elevated LamB levels. The pattern of expression of LamB and the advantage it confers for growth on low concentrations of carbohydrates are consistent with a wider role in sugar permeation than simply maltosaccharide transport, and hence the well-known maltoporin activity of LamB is but one facet of its role as the general glycoporin of *E. coli*. A corollary of these findings is that OmpF/OmpC porins, present at high levels in carbon-limited bacteria, do not provide sufficient permeability to sugars or even glycerol to support high growth rates at low concentrations. Hence, the sugar-binding site of LamB protein is an important contributor to the permeability of the outer membrane to carbohydrates in habitats with low extracellular nutrient concentrations.

The general porins OmpF and OmpC of *Escherichia coli* permit glucose permeation at high rates when studied in vitro (16). A commonly held extrapolation is that glucose (and all monosaccharide) transport across the outer membrane is therefore OmpF/C porin dependent. Some evidence for this was that a lack of porins affects the "growth K_m" for solutes, including glucose, when studied in batch culture (25). It is also generally assumed that glycerol, which is less than 100 molecular weight, should have no difficulty diffusing across the outer membrane through OmpF/C pores with their notional but misleading "cutoff molecular weights" of about 600 (16). Yet there is little experimental evidence that permeation of carbohydrates across the outer membrane is dependent only on OmpF/C porins at low, micromolar extracellular concentrations. Indeed, the surprising conclusion in this communication is that growth on most carbohydrates at micromolar concentrations is LamB dependent.

This conclusion is in conflict with another commonly held view, namely, that LamB protein or maltoporin is physiologically significant only in maltose and maltosaccharide permeation across the outer membrane. LamB was shown to be regulated coordinately with the well-studied *mal* regulon (20, 21), and previous results with *lamB* mutants pointed to an essential role only in maltodextrin utilization; lack of LamB prevents growth on sugars larger than maltotriose in batch culture (26). However, von Meyenburg and Nikaido did show that LamB protein permits significant permeation of other sugars in the absence of porins (25). Also, it has been generally ignored that the sugar-binding site of LamB has a weak but measurable affinity for glucose and other mono- and disaccharides (2), which could facilitate the permeation of these substrates across the outer membrane. Also influential have been results (24) showing that the presence of LamB protein confers no advantage over LamB$^-$ bacteria in growth competition experiments in chemostats with glycerol or lactose as the substrate, in contrast to strong selection in favor of LamB$^+$ bacteria on maltose. However, a slight advantage of LamB$^+$ cells growing on limiting glucose was observed (24).

We had to reexamine the view that LamB was unimportant in the transport of other sugars after finding that LamB affinity was a considerable selective determinant in competition for low concentrations of glucose and other carbohydrates in growth experiments with LamB sugar-binding site mutants. The study presented here provides evidence that LamB indeed functions as a broad specificity glycoporin under conditions of carbohydrate limitation. This function of LamB could not have been predicted from batch culture data, since LamB protein confers little or no transport advantage on porin-containing cells under sugar-rich (>0.2 mM) conditions of growth.

If indeed LamB functionally contributes to growth on glucose, how can this be reconciled with the classic view of *mal* regulation (21), namely, that LamB expression is repressed by glucose and dependent on maltodextrins for induction? This point has also been reexamined. We find that, in contrast to growth in glucose batch cultures, glucose-limited growth in chemostats leads to a striking derepression of LamB. This derepression can be elicited in bacteria growing in the presence of low concentrations of nutrients other than maltodextrins. Hence, LamB levels respond to nutrient deprivation. However, this phenomenon can only be loosely, if at all, categorized as a starvation response or a stationary-phase response (11, 22), since derepression oc-

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TABLE 1. Bacterial strains used in this study

Strain	Relevant genotype	Parent strain	Origin or reference
BW1022	HfrG6 *metA trpE aroB*		4
pop1080	HfrG6 *metA trpE lamB102*		26
pop1306	Hfr *metA trpE gal rpoB lamB506*(Am)		M. Hofnung
BW1500	HfrG6 *metA trpE aroB lamB1500*		6
BW2644	HfrG6 *trpE aroB lamB375*		6
BW2900	HfrG6 *trpE*	BW1022	This study
BW2901	HfrG6 *metA*	BW1022	This study
BW2902	HfrG6 *aroB lamB375*	BW2644	This study
BW2903	HfrG6 *trpE lamB375*	BW2644	This study
BW2906	Hfr *trpE lamB1500*	BW1500	This study
BW2907	Hfr *metA lamB1500*	BW1500	This study
BW2909	Hfr *trpE lamB102*	pop1080	This study
BW2912	Hfr *trpE gal rpoB lamB506*(Am)	pop1306	This study
LA5731	F$^-$ *ptsF lacY arg mgl-515 zee-700*::Tn*10*(P1 *cml clr1000*)		W. Boos

curs in exponentially growing bacteria with rapid doubling times.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were derivatives of *E. coli* K-12 and are shown in Table 1. The strains with individual auxotrophic markers were derived from *lamB* affinity mutants in a BW1022 background (4, 6), with one or two of the three amino acid markers removed by P1-mediated transduction, with P1 *cml clr1000* grown on LA5731 as the donor. Wild-type and high-affinity mutants with single-amino-acid markers were constructed by transducing the parent strain (BW1022 or BW1500) to Aro$^+$. These Aro$^+$ derivatives were then transduced to either Met$^+$ or Trp$^+$. For the other *lamB* mutants, the parent strains (BW2644, pop1080, and pop1306) were transduced to Aro$^+$, Trp$^+$, or Met$^+$ as required. The maximum growth rate was estimated for all constructs in batch culture; there was no detectable difference in the maximal growth rates of the mutant strains on 0.2% glucose.

Growth media and culture conditions. The chemostat medium consisted of minimal medium A (MMA [12]) supplemented with one or more of the appropriate amino acids (methionine, 40 μg/ml; tryptophan, 40 μg/ml; or aromatic acids [tryptophan, 20 μg/ml; tyrosine, 20 μg/ml; phenylalanine, 20 μg/ml; and shikimic acid, 4 μg/ml]). In chemostats, one carbon source was present at the following concentrations in the feed medium: glucose (0.02 or 0.1% [wt/vol], as stated), lactose (0.02%, wt/vol), arabinose (0.02%, wt/vol), succinate (0.02%, wt/vol), glycerol (0.02 or 0.1% [wt/vol], as stated), guanosine (0.06%, wt/vol), and maltooligosaccharide (0.02 or 0.1% [wt/vol], as stated). For batch cultures, 60 ml of MMA was supplemented with the above concentrations of required amino acids and the carbon source at 0.2% (wt/vol).

Chemostat cultures. Positive-pressure chemostats were used for continuous culture, with the outlet adjusted to maintain a culture volume of 80 ml. Air from a Hy-Flo pump (Medcalf Bros. Ltd., Hertfordshire, England) was sterilized by passage through an Acrodisc filter (Gelman Sciences, Ann Arbor, Mich.) and humidified by being bubbled through sterile water held in a flask. A sparger bubbled filtered, humidified air through the culture and, together with a magnetic stirrer, provided aeration and mixing. The temperature was maintained at 37°C by incubating the culture vessel in a water bath. A peristaltic pump was used to supply input medium at a dilution rate (D) of 0.28 to 0.3 h^{-1} for the experiments reported in this article.

To start growth in continuous cultures, the 80-ml culture vessel was inoculated with 2 to 3 ml of an exponential-phase batch culture of the appropriate strain on the same carbon source. Growth continued at a dilution rate of 0.28 to 0.3 h^{-1} for at least 5 culture volumes before sampling for transport or competition experiments. Chemostats generally reached the steady state within 12 h after inoculation, as indicated by determining the residual glucose concentration and optical density of cultures. The culture was sampled by removing the desired volume via a sampling port with a sterile syringe. The residual glucose and saccharide concentrations in the chemostat cultures were determined by the anthrone assay for carbohydrates (24) after immediate filtration of withdrawn samples through microfilter units (0.2-μm pore size).

Growth competition experiments. Pure steady-state cultures (D = 0.28 h^{-1}; input sugar concentration as specified) of two separate strains with different amino acid markers and *lamB* alleles were established as described above. The cultures were mixed in a 1:1 ratio by aseptically transferring 40 ml from one culture vessel to the second culture vessel (similarly, 40 ml was removed from the second vessel for transfer to the first), maintaining 80-ml volume chemostats. Growth continued at the same dilution rate in each vessel. At the times indicated, chemostats were sampled by removing 2 ml of culture and determining the optical density, while a 100-μl sample was diluted in MMA and the proportion of each strain in the mixed culture was determined by a plate count on MMA plates containing the appropriate amino acid. Dilutions of the mixed culture were also plated onto nutrient agar (NA) plates and incubated for 24 to 48 h at 37°C. Colony counts were performed to check that the total CFU were equivalent to the sum of the colony count on selective plates.

Estimation of LamB levels in the outer membrane by immunoassay. The reactivity of LamB in the outer membrane to surface-specific anti-LamB monoclonal antibody was tested with intact bacteria filtered onto bacteriological filters. Bacteria harvested from culture were washed and resuspended in phosphate-buffered saline (PBS) buffer, pH 7.4, containing 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 in 1 liter of H_2O to a density of 2×10^8 bacteria per ml. Then, 10 μl of bacterial suspension was applied to a marked square on a cellulose-nitrate disk (Micro Filtration Systems, Dublin, Calif.) under gentle suction and air dried for 10 min. Additional protein sites were blocked by

gently shaking the disk for 15 min in 20 ml of blocking solution (0.2 g of instant nonfat dried milk [Diploma Skim Milk] dissolved in 20 ml of PBS). Excess blocking solution was removed by blotting the disk between two filter papers. Four portions (2.5 μl each) of 1:500-diluted monoclonal anti-LamB antibody (5) were pipetted at 5-min intervals directly onto the square on the disk containing antigen. The filter disk was then washed three times for 3 min each in 100 ml of fresh PBS to remove any unbound antibody. Four 2.5-μl portions of 1:50-diluted enzyme (anti-mouse immunoglobulin G [IgG]-peroxidase [Amersham, Sydney, Australia] diluted in PBS) were added to each square at 5-min intervals. The disk was again washed with 15 ml of PBS under suction, followed by three 3-min rinses, each time in 100 ml of PBS, and then developed in 10 ml of substrate solution (0.4 mg of 4-chloro-1-naphthol per ml plus 0.03% H_2O_2 in PBS).

Estimation of LamB levels in the outer membrane by gel electrophoresis. To estimate the amount of LamB in the outer membrane of batch- and chemostat (0.1% input carbon source)-grown cells, the outer membrane was extracted as described previously (6) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10). Samples were boiled for 5 min before loading.

Transport assays. Samples (10 ml) from chemostat or batch cultures were harvested, washed twice in MMA, and resuspended to an identical optical density (A_{580} = 0.2). To start assays, 60 μl of bacterial suspension was added to 12 μl of [^{14}C]glucose (0.5 μM final concentration) at room temperature (20 to 25°C). Radioactive sugars were from Amersham. Samples (20 μl) were removed at 10, 20, 30, 40, and 120 s, immediately filtered through 0.45-μM cellulose-nitrate membrane filters (Whatman Ltd., Maidstone, England), and washed with 10 ml of MMA. The filters were counted in liquid scintillant (Aqueous Counting Scintillant; Amersham), and the initial rate of sugar uptake was calculated from the slope derived from the initial time points. Transport kinetics at different sugar concentrations were measured in the same way except that bacteria were resuspended to a higher bacterial density (A_{580} = 0.5) and 60 μl of bacterial suspension was added to 12 μl of glucose solutions ranging from 0.2 to 30 μM.

RESULTS

LamB protein levels under conditions of carbohydrate limitation. Chemostat cultures limited by the input of various carbon sources were established, and the level of LamB was monitored in wild-type *E. coli*. To prevent any possible limitation by nutrients other than sugars, low densities of bacteria were used in our chemostats, with an input of 0.02 to 0.1% carbon source in the feed medium. The strain used was a derivative of the HFr G6 strains used in early studies of *lamB* regulation (20). As described in the early literature, LamB in this strain was expressed significantly in batch cultures of wild-type bacteria only in maltose-containing medium, but with a higher basal level on glycerol than on glucose. Figure 1 confirms this pattern of expression of LamB protein extracted from the outer membrane of bacteria grown in standard batch culture with 0.2% carbohydrate input. In stark contrast, a different pattern was found in bacteria grown under sugar limitation in chemostats over a wide range of dilution rates (0.1 to 0.7 h^{-1}). A band corresponding to LamB was expressed to high levels in glucose-limited cultures as well as in maltose-grown cultures (Fig. 1, lane C); expression in chemostats with limiting glycerol (lane A) was lower than that in chemostats with limiting glucose. Relevant to this finding was that *Klebsiella aerogenes*, which has a LamB-like protein, also derepressed a 47,000-molecular-weight protein on glucose limitation in chemostats, but its identity to LamB was not established (23).

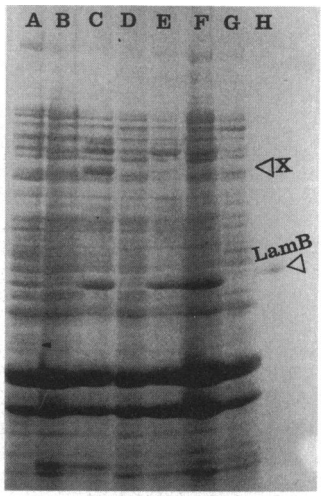

FIG. 1. Outer membrane pattern of batch- and chemostat-grown bacteria analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Bacteria were grown in batch (0.2% [wt/vol] carbon source) or chemostat cultures limited by carbohydrate (0.1%, wt/vol) at a dilution rate of 0.3 h^{-1}. Outer membranes were extracted from wild-type cells (BW2901) cultured in maltooligosaccharide batch (lane F), maltooligosaccharide chemostat (E), glucose batch (D), glucose chemostat (C), glycerol batch (B), and glycerol chemostat (A) cultures. Lane G contains membrane from BW2909 (*lamB*) grown in glucose-rich conditions. Lane H shows purified LamB monomer. Band X corresponds to an additional unidentified protein in BW2901 present in lane C under glucose limitation.

It is evident from Fig. 1 that glucose limitation strongly induces at least one other unidentified outer membrane protein (band X) not found in batch cultures or after growth under other conditions. To confirm whether the derepressed outer membrane protein band migrating as LamB was indeed LamB and not a novel protein, we also measured LamB levels in intact bacteria with an immunoassay. These assays were done with one of the surface epitope-specific monoclonal anti-LamB antibodies (5). As shown in Fig. 2, the pattern of induction of LamB as an antigen was entirely consistent with the pattern in Fig. 1, and there was a drastic difference in LamB response between glucose-limited and glucose-rich (batch) cultures. Hence, LamB could be derepressed in the absence of maltose, but curiously, limiting glucose was a better substrate than glycerol or succinate for LamB derepression. Other results (not shown) indicate that concentrations of glucose, below 0.2 mM in the medium result in derepression. The genetic basis of this regulation is under investigation.

Contribution of LamB to defining the growth affinity of *E. coli* for glucose. If the elevated level of LamB under glucose limitation is physiologically important for outer membrane permeability, it would be expected that LamB mutants would exhibit different growth and transport properties for glucose and perhaps other small carbohydrates. Well-characterized *lamB* mutants with altered channel properties were available from earlier studies, and as shown in Table 2, altered LamB proteins with greater than 20-fold differences

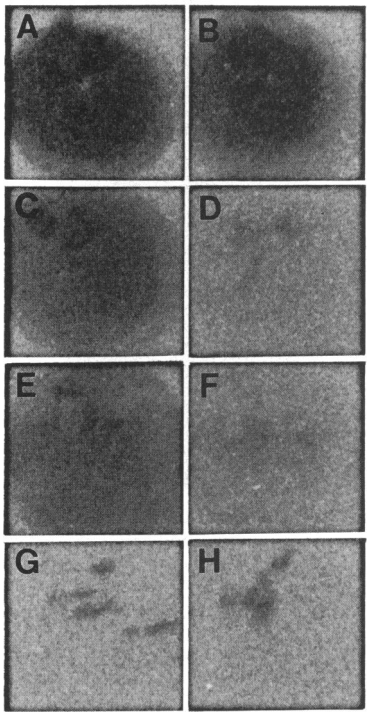

FIG. 2. Immunoassay of LamB protein in bacteria grown under sugar limitation. Bacteria were grown in batch (0.2% [wt/vol] carbon source) or chemostat (0.1% [wt/vol] carbon source) cultures and filtered directly onto bacteriological filters as described in the text. With monoclonal anti-LamB antibody (5) and anti-mouse rabbit immunoglobulin G antibody conjugated to horseradish peroxidase, LamB was detectable in bacteria grown in maltooligosaccharide batch culture (B), maltooligosaccharide chemostat culture (A), glucose batch culture (D), glucose chemostat culture (C), glycerol batch culture (F), and glycerol chemostat culture (E). No LamB was detected in the outer membrane of BW2909 (*lamB*) grown in glucose-rich (H) or glucose-limited (G) conditions.

in glucose-binding affinity have been described (1). These mutants were used in several experiments to establish the role of LamB protein; the first of these approaches tested the correlation of growth affinity with the sugar-binding affinity of LamB channels.

An estimate of the influence of LamB affinity on growth affinity could be approximated in the following way. As described by Monod (13), bacteria show saturable growth rates with respect to nutrient concentration, described by the relationship:

$$V = V_{max} \cdot C/(C + K_s) \quad (1)$$

where V is the rate of growth, V_{max} is the maximal growth rate, C is the concentration of limiting nutrient in the medium, and K_s is the substrate half-saturation or affinity constant. In a chemostat, V is set by the dilution rate and was fixed at 0.3 h^{-1}. The V_{max} at the saturating sugar concentration for wild-type and LamB mutant strains was also shown to be identical in growth experiments in batch culture (results not shown). Hence, if relationship 1 is rearranged to

$$V/V_{max} = C/(C + K_s) \quad (2)$$

and because V/V_{max} is identical for each culture at a constant dilution rate, then

$$V/V_{max} = C_{wt}/(C_{wt} + K_{s_{wt}}) =$$

$$C_{m1}/(C_{m1} + K_{s_{m1}}) = C_{m2}/(C_{m2} + K_{s_{m2}}) \quad (3)$$

where C_{wt} is the residual sugar concentration in wild-type cultures, $K_{s_{wt}}$ is the wild-type growth affinity, C_{m1} is the residual sugar concentration in the first mutant culture, $K_{s_{m1}}$ is the growth affinity of mutant 1, etc., equation 3 can be simplified to

$$K_{s_{wt}}/K_{s_{m1}} = C_{m1}/C_{wt} \quad (4)$$

Hence, measurable differences in residual glucose concentration between wild-type and mutant cultures should reflect a difference in growth affinity.

Chemostats limited by glucose were established for LamB affinity mutant and wild-type strains, and the concentration of remaining carbohydrate was estimated during steady-state growth at the same set dilution rate (0.3 h^{-1}) and with the same glucose input (0.02%). As shown in Table 2, the residual sugar concentration in at least three independent chemostats for each of the strains was consistently highest in the continuous culture of the $LamB^-$ strain, next highest with the low-affinity LamB mutant, and lowest with the high-affinity mutant. This suggested that the growth affinity for glucose in otherwise similar *E. coli* strains was indeed influenced by LamB affinity. The ratios of growth affinities were roughly in line with the sugar-binding properties of LamB determined in vitro, as shown in Table 2.

It should be noted that equations 1 through 4 are likely an oversimplification, as more than one system may be in-

TABLE 2. Glucose-scavenging ability and glucose transport rates in bacteria containing LamB proteins with different sugar affinities

Strain	Binding dissociation constant for glucose[a] (mM)	Glucose concn in chemostat[b] (μM)	[^{14}C]glucose transport[c] (pmol/min/10^8 bacteria)	
			Batch culture	Chemostat culture
Wild type (*lamB*$^+$)	120	30.7 ± 7.3 (7)	68 ± 2.9	257 ± 43
$LamB^-$ [*lamB506*(Am)]	—[d]	121 ± 15 (4)	65	86.6 ± 16
Low-affinity (*lamB375*)	>1,000	75 ± 18 (5)	69.5 ± 0.71	134 ± 31
High-affinity (*lamB1500*)	80	17 ± 1.5 (4)	71 ± 1.41	655 ± 35

[a] Binding constants were derived from channel-blocking experiments with the LamB proteins reconstituted in black lipid membranes (1).

[b] Residual glucose levels in steady-state chemostats were measured when the bacteria were growing at a dilution rate of 0.3 h^{-1}, with an input glucose concentration of 0.02% (1.1 mM). Values are means ± standard deviations. Numbers in parentheses represent the number of independent determinations on different chemostats.

[c] Transport of 0.5 μM input [^{14}C]glucose. Values are means ± standard deviations for three determinations, except for $LamB^-$, which was done once.

[d] —, no protein.

volved in glucose entry during growth in chemostats. For example, if LamB permeates only half the glucose and (nonsaturable) porins permeate the rest, growth saturation may not follow simple saturation kinetics, and the above analysis would not allow a direct comparison of *K*s and LamB binding constants. The transport data with washed bacteria below suggest that at 0.5 μM external glucose, about two-thirds of glucose transport in the wild type is LamB dependent (Table 2). We have no data on the proportion of glucose entering via LamB or other channels under chemostat conditions.

Selective advantage of sugar affinity in the LamB channel in competition for glucose. To refine the conclusion that the LamB binding site was important for growth on glucose, the *lamB* mutants were grown in competition with each other and with the wild type in chemostats to test the influence of binding affinity on growth competition at low external sugar concentrations. In these experiments, the *lamB* variants were each grown in independent glucose-limited chemostats and mixed 1:1 for competition in the same medium, with growth continuing at the same dilution rate. The bacteria in the mixed culture were counted by plate counts with different auxotrophic markers in combination with the *lamB* allele. The results of these experiments are shown in Fig. 3, with two reciprocal pairings of auxotrophic and *lamB* markers used to check for possible non-LamB-dependent selection between strains. In control experiments, each *lamB* mutant also competed against itself in the two auxotrophic backgrounds; no selection based on non-*lamB* markers was found within the time frame of these experiments (results not shown). As shown in Fig. 3B for competition between the wild-type $LamB^+$ and low-affinity mutant strains, the wild-type population became predominant regardless of the marker background. Likewise, strains with the high-affinity *lamB* allele outcompeted both the wild type and low-affinity mutants. It is also evident from Fig. 3 that the magnitude of the selection is in line with the difference in LamB glucose affinities of the strains noted in Table 2. Hence, the sugar affinity of LamB has a significant selective advantage in growth on low concentrations of glucose.

Selective advantage of LamB in growth on sugars besides glucose. Growth competition in chemostats was also performed with other limiting nutrients, as shown in Fig. 4. Chemostats were inoculated with an equal population of wild-type and $LamB^-$ bacteria, both previously adapted to nutrient-limited chemostats at the same dilution rate. Consistent with the data in Fig. 3, competition for glucose resulted in selection in favor of wild-type bacteria. Selection in favor of $LamB^+$ bacteria was also found in mixed cultures containing arabinose, maltose, and lactose as well as glycerol. Strong selection was found only with carbohydrates; selection was not observed with aspartate, succinate, or guanosine as the limiting substrate. Previous studies showed that aspartate was a very poor substrate for LamB pores (7). In the presence of guanosine, the $LamB^-$ strain is actually at an advantage, possibly because Tsx and OmpF/C are better expressed in the absence of LamB. Among the carbohydrates, the selective advantage of LamB was less marked with the smaller sugars arabinose and glycerol. Nevertheless, the most unexpected feature of these experiments is that LamB influences competition for glycerol, indicating that even glycerol diffusion is outer membrane limited. No previous evidence for glycerol binding by LamB has been reported in channel blocking experiments, but extremely high concentrations may be required to observe this interaction in vitro.

The results in Fig. 4 differ from those for glycerol and lactose reported earlier in competition between wild-type and $LamB^-$ bacteria (24). There is no clear explanation for the difference between these results. One explanation we can offer is that because the residual sugar levels were not monitored in the earlier study, it is possible that the chemostats were growth limited by factors other than carbohydrate limitation. If the chemostats were not sugar limited in the experiments of Szmelcman and Hofnung, no derepression of LamB, no growth difference, and no difference in glucose transport rate (see below) would have been observed. It may also be relevant that, in contrast to our experiments, in which cultures were adapted to chemostats before mixing, the earlier experiments were based on inoculating mixed batch cultures directly into chemostats. In such an inoculum, LamB protein was probably repressed in the wild type, which would have no immediate growth advantage.

Glucose transport at micromolar concentrations is influenced by LamB affinity. Given the above results in growth competition, it was also important to test whether higher transport rates at low concentrations account for the advantage of $LamB^+$ bacteria compared with $LamB^-$ mutants. Indeed, as in earlier studies, we found no detectable difference in glucose transport rates between mutant and wild-type batch culture-grown bacteria (Table 2). However, the strictly glucose-limited, chemostat-grown bacteria show a considerably higher glucose uptake rate in the presence of a high- or wild-type-affinity LamB protein (Table 2); the high-affinity protein permits a sevenfold-greater transport rate than found in bacteria with no LamB and dependent only on porins. The major difference between these outcomes can be ascribed to the much higher LamB level in the chemostat cultures; the batch cultures and probably those of Szmelcman and Hofnung (24) were not derepressed for LamB to contribute to transport rates.

The presence of LamB influenced the transport K_m for glucose, as shown in Fig. 5. Glucose transport appeared to show simple saturation kinetics with and without LamB, but there was a decrease in the K_m from 13 μM in wild-type bacteria grown in batch culture (or $LamB^-$ bacteria in chemostats) to 6 μM with wild-type or 4 μM with high-affinity LamB mutant bacteria grown in chemostats. This result confirms the contribution of LamB to the overall affinity of glucose uptake and suggests that glucose transport kinetics are not determined solely by the phosphoenolpyruvate: glucose phosphotransferase system or other cytoplasmic membrane transporters, as is usually assumed (17, 19).

DISCUSSION

Novel aspects of LamB regulation. Given the seemingly well established role of LamB in outer membrane physiology, several results in this study were unexpected. The finding that LamB is derepressed to high levels by glucose limitation was a surprise, as was the independence of induction from exogenous maltodextrins. These results could not have been predicted from the current theory of *mal* regulation (21). A more complete view is that LamB has a dual role, with the level of LamB responding to the presence of maltodextrins under carbon-rich conditions (i.e., in batch culture) as well as to a general limitation for other carbohydrates. Recent work with *lamB-lacZ* fusions showed there is at least a 30-fold increase in *lamB* expression in chemostat versus batch cultures on glucose (16a).

Induction of LamB in the absence of exogenous maltosaccharides under sugar limitation may mean that an alternative

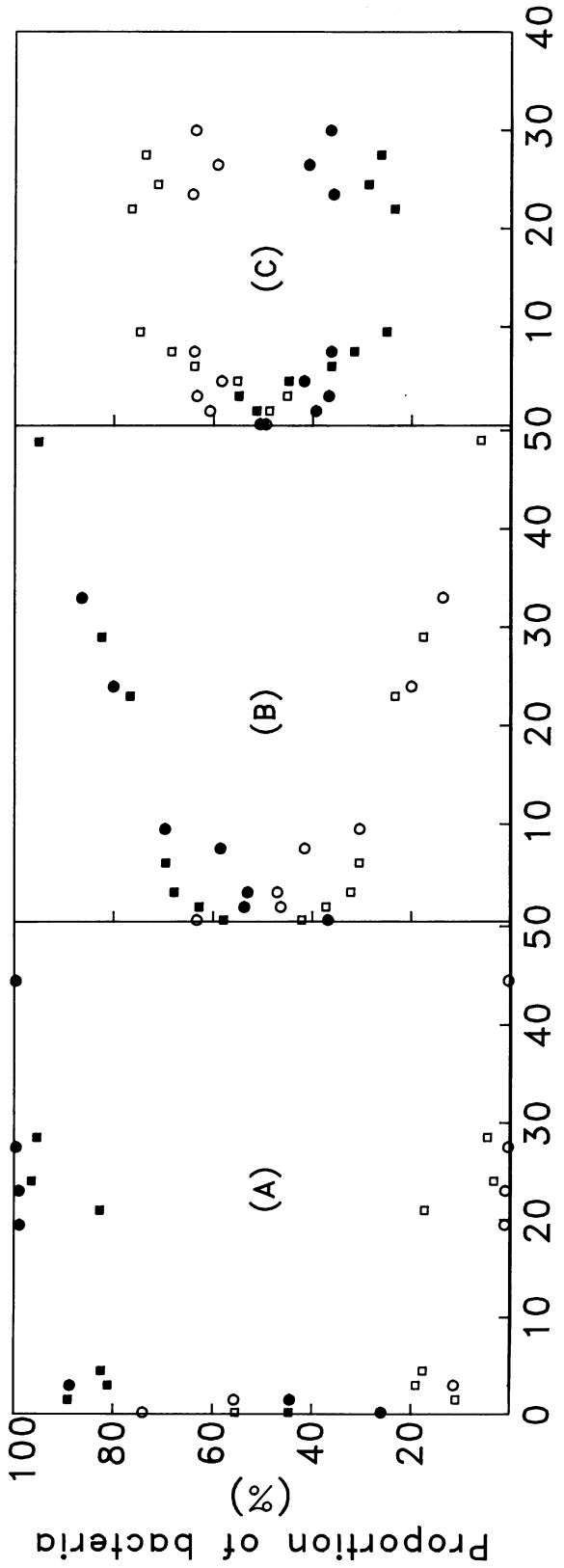

FIG. 3. Competition between bacteria with different LamB sugar affinities for limiting glucose in chemostats. (A) Mixture of high-affinity *lamB* mutant BW2907 (●) and low-affinity *lamB* mutant BW2903 (○) or mixture of high-affinity *lamB* mutant BW2906 (■) and low-affinity *lamB* mutant BW2902 (□). (B) Mixture of wild-type BW2901 (■) and low-affinity *lamB* mutant BW2903 (□) or mixture of wild-type BW2900 (●) and low-affinity *lamB* mutant BW2902 (○). (C) Mixture of high-affinity *lamB* mutant BW2907 (□) and wild-type BW2900 (■) or mixture of high-affinity *lamB* mutant BW2906 (○) and wild-type BW2901 (●).

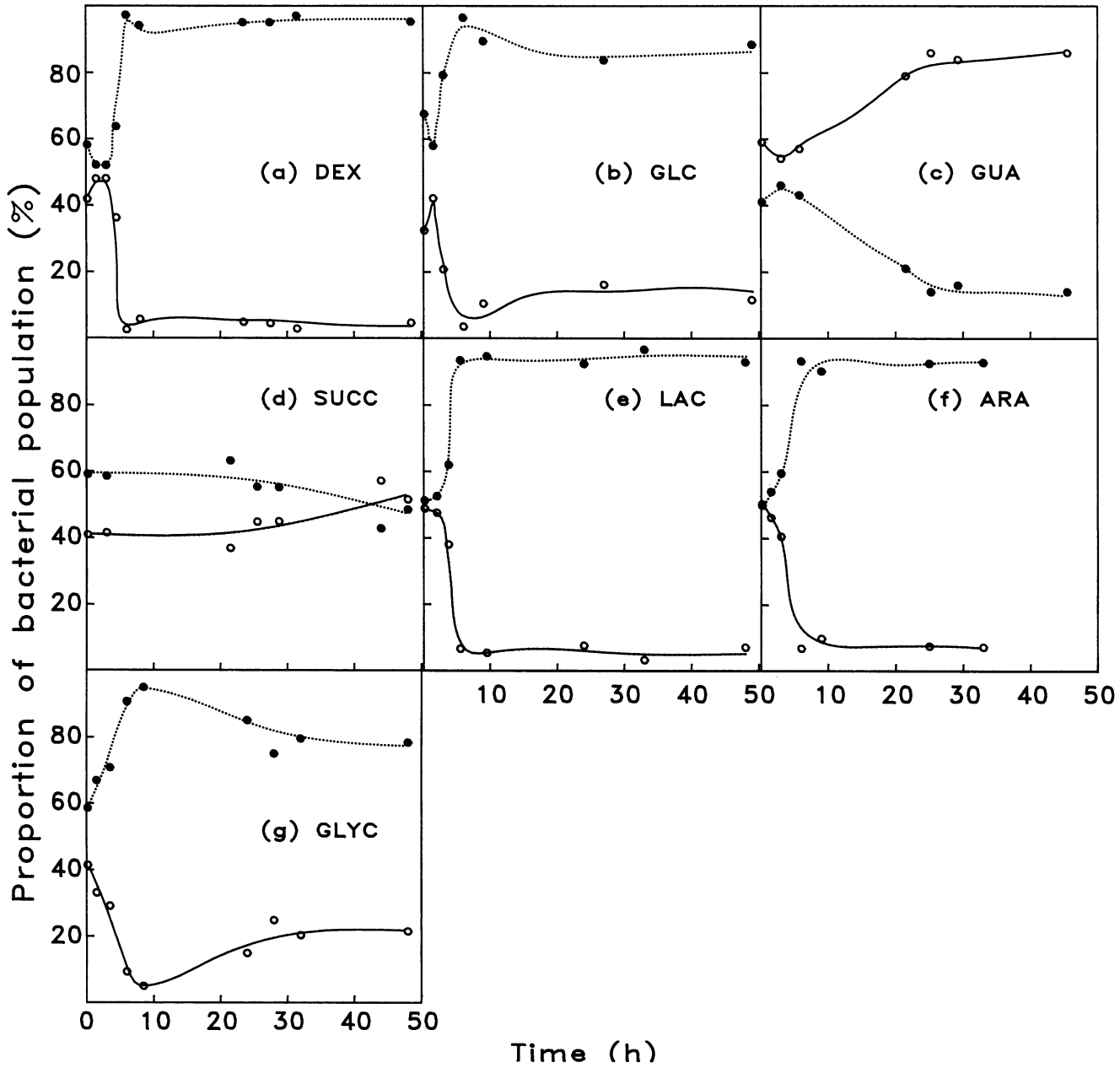

FIG. 4. Competition between LamB$^+$ and LamB$^-$ strains for different limiting nutrients. Experiments were done in chemostats limited for (a) maltooligosaccharide, (b) glucose, (c) guanosine, (d) succinate, (e) lactose, (f) arabinose, and (g) glycerol. In each case, the mixed culture was inoculated with wild-type BW2901 (●) and strain BW2912 [*lamB506*(Am)] (○). The bacteria in mixed culture were differentiated by plate counts with methionine and tryptophan to select the wild-type and mutant strains, respectively.

route to inducer synthesis exists; this may involve some of the products affecting basal levels of *mal* regulation described by Ehrmann and Boos (3). The higher level of induction on glucose than on glycerol may be explained by alternative inducers synthesized from glucose but not from succinate or glycerol. Alternatively, induction in the absence of maltodextrins may be due to the overexpression of MalT from its cyclic AMP receptor protein-dependent promoter (21) in the presence of high cyclic AMP levels under nutrient limitation. There are indeed elevated cyclic AMP levels under the glucose-limiting conditions used in this study (10a). Given the high levels of MalT, perhaps enough of the protein is in an activator conformation to stimulate expression from the *malK-lamB* promoter, which is itself cyclic AMP receptor protein dependent (21). Further studies will be required to clarify these points. Preliminary data suggest that at least the transporter genes in the *mal* regulon are controlled in parallel with LamB (16a).

Studies of the starvation response have identified a number of proteins that are derepressed under conditions of nutrient stress (11). Some of these proteins were derepressed in a cyclic AMP- and cyclic AMP receptor protein-dependent manner, and LamB protein probably belongs to this category. However, it should be noted that limitation for

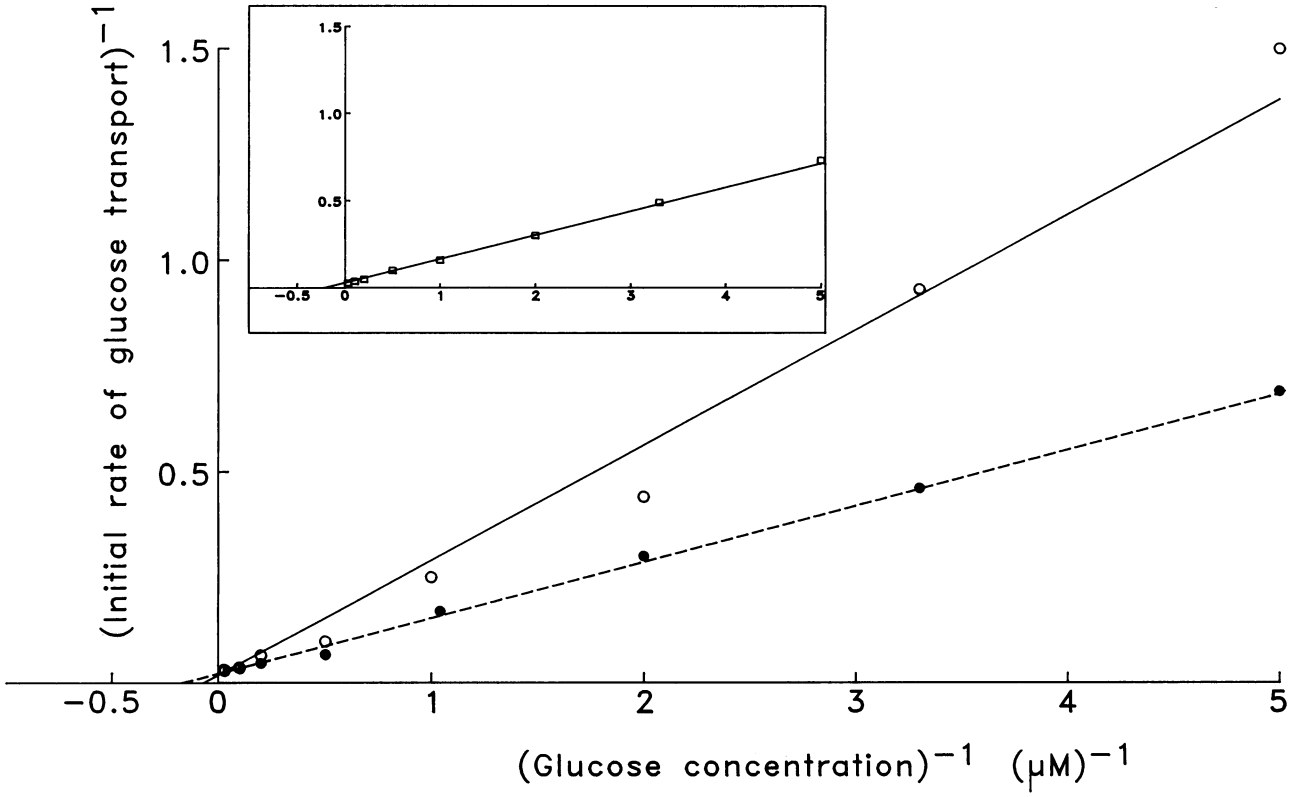

FIG. 5. Transport kinetics for glucose and the influence of LamB affinity. The initial rate of glucose transport was assayed at various glucose concentrations with wild-type (BW2901, ●) and LamB$^-$ (BW2912, ○) strains. The inset shows the kinetics of high-affinity LamB (BW2906, □) bacteria. Results are plotted as a Lineweaver-Burk plot, with the y axis in units of nanomoles of glucose taken up per minute per 10^8 bacteria.

sugars in these chemostats involved cells in an exponential growth phase with respectable doubling times (2.3 to 2.5 h). Glucose-limited bacteria are not starving, and these results suggest that a more precise descriptive analysis of what is commonly called the starvation response should be undertaken.

Role of the sugar-binding site in transport through LamB. The low-affinity sugar-binding site of LamB was found to be an important determinant in influencing growth affinity (Table 2) and hence the ability to compete for scarce carbohydrates (Fig. 3 and 4). The contribution of the binding site was also reflected in the relative rates of micromolar glucose transport in mutants with different binding affinities (Fig. 5). As pointed out earlier in relation to maltose transport, the advantage of a binding site is particularly significant for improving permeation rates at low extracellular concentrations (2). These conditions are met in the chemostats used in this study as well as in the most common natural environments of *E. coli* (8). Also, at saturating concentrations, LamB is a faster glucose channel than a maltose channel (9), so a sudden availability of sugar would permit a rapid transition to the catabolite-repressed state.

The previous emphasis on LamB protein as a maltoporin has tended to mask the significance of the finding that LamB has a measurable affinity not just for maltodextrins but also for other sugars. For example, the LamB channel is half-saturated by glucose at approximately 0.1 M and has similar affinities for several mono- and disaccharides (2). Even though the glucose concentration in a chemostat is nearly 10^4-fold lower, this does not indicate a lack of importance of the binding site in transport physiology at low concentrations. It is recognized that LamB is important in transport at well below its binding half-saturation concentration for maltosaccharides; as described for maltose (in reference 24 and many other publications), the significance of LamB in transport is most apparent at micromolar maltose concentrations, even though the binding K_d for maltose is in the 10 mM range. Therefore, there is nothing different in LamB contributing to the transport of glucose in the chemostat at 20 to 30 μM glucose when its K_d is 100 mM. The same is true for lactose but with the proviso that the chemostat concentration of lactose is much higher in experiments such as that shown in Fig. 4 (0.4 to 0.5 mM lactose at steady state), given the poor affinity of the LacY transporter (transport K_m, 0.2 mM). The observation that LamB contributes significantly to lactose permeation at these concentrations means that lactose flux through porins must be very poor at submillimolar concentrations.

Is LamB a maltoporin or a glycoporin? The frequently used designation of LamB as a maltoporin actually describes only part of the function of this protein, namely, that which is important under conditions of *mal* induction by maltose and maltodextrins. The name maltoporin does not do justice to the full binding or permeation properties of LamB, and neither does it describe its role in the physiological response to sugar limitation. The name glycoporin would be more descriptive of the role of LamB in carbohydrate permeation across the outer membrane of *E. coli*. In disagreement with

the recent suggestion that outer membrane proteins with a binding site, like LamB protein, should not be called porins (15), the highly conserved β-barrel channel structure in porins as well as in LamB (18) indicates that all these proteins belong to the same structural family. Also, the wide spectrum of sugars recognized suggests that the -porin suffix is justified and stresses the role of LamB in the outer membrane permeation of a wide range of carbohydrates.

Are the general porins important only in nutrient-rich conditions? The data of von Meyenburg and Nikaido (25) demonstrated the contribution of porins to growth in batch cultures with low sugar concentrations. However, as probably with the Szmelcman and Hofnung (24) cultures, LamB was not optimally induced with the conditions of inoculum growth used in these experiments. Our transport data suggest that at submicromolar concentrations, less than one-third of the flux of glucose into nutrient-stressed *E. coli* is mediated independently of LamB, presumably through the general porins OmpF and OmpC. This result suggests that the contribution of general porins to nutrient uptake is not very significant under conditions of nutrient limitation. In turn, this raises the question of whether limitation for any class of nutrient elicits a mechanism of outer membrane permeation that is independent of general porin function. It is evident that PhoE, LamB, and iron transporters are derepressed by different classes of nutrient limitation, and other types of limitation may well induce yet undefined outer membrane proteins, such as band X in Fig. 1.

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REFERENCES

1. **Benz, R., G. Francis, T. Nakae, and T. Ferenci.** 1992. Investigation of the selectivity of maltoporin channels using mutant LamB proteins: mutations changing the maltodextrin binding site. Biochim. Biophys. Acta **1104**:299–307.
2. **Benz, R., A. Schmid, and G. H. Vos-Scheperkeuter.** 1987. Mechanism of sugar transport through the sugar-specific LamB channel of *Escherichia coli* outer membrane. J. Membr. Biol. **100**:21–29.
3. **Ehrmann, M., and W. Boos.** 1987. Identification of endogenous inducers of the *mal* regulon in *Escherichia coli*. J. Bacteriol. **169**:3539–3545.
4. **Ferenci, T., and K.-S. Lee.** 1982. Directed evolution of the lambda receptor of *Escherichia coli* through affinity-chromatographic selection. J. Mol. Biol. **160**:431–444.
5. **Gabay, J., S. A. Benson, and M. Schwartz.** 1983. Genetic mapping of antigenic determinants in a membrane protein. J. Biol. Chem. **258**:2410–2414.
6. **Heine, H.-G., G. Francis, K. S. Lee, and T. Ferenci.** 1988. Genetic analysis of sequences in maltoporin that contribute to binding domains and pore structure. J. Bacteriol. **170**:1730–1738.
7. **Ingham, C., M. Buechner, and J. Adler.** 1990. Effect of outer membrane permeability on chemotaxis in *Escherichia coli*. J. Bacteriol. **172**:3577–3583.
8. **Koch, A. L.** 1971. The adaptive responses of *Escherichia coli* to a famine and feast existence. Adv. Microb. Physiol. **6**:147–217.
9. **Luckey, M., and H. Nikaido.** 1980. Specificity of diffusion channels produced by λ phage receptor protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:167–171.
10. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane protein of Escherichia coli K12 into four bands. FEBS Lett. **58**:254–258.

10a. **Marshall, T., and T. Ferenci.** Unpublished data.

11. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in non-differentiating bacteria. Annu. Rev. Microbiol. **43**:293–316.
12. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Monod, J.** 1949. The growth of bacterial cultures. Annu. Rev. Microbiol. **3**:371–394.
14. **Muir, M., L. Williams, and T. Ferenci.** 1985. Influence of transport energization on the growth yield of *Escherichia coli*. J. Bacteriol. **163**:1237–1242.
15. **Nikaido, H.** 1992. Porins and specific channels of bacterial outer membranes. Mol. Microbiol. **6**:435–442.
16. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49**:1–32.

16a. **Notley, L., and T. Ferenci.** Unpublished data.

17. **O'Brien, R. W., O. M. Neijssel, and D. W. Tempest.** 1980. Glucose phosphoenolpyruvate phosphotransferase activity and glucose uptake rate of *Klebsiella aerogenes* growing in chemostat culture. J. Gen. Microbiol. **116**:305–314.
18. **Pauptit, R. A., T. Schirmer, J. N. Jansonius, J. P. Rosenbusch, M. W. Parker, D. Tsernoglou, M. S. Weiss, and G. E. Schulz.** 1991. A common channel-forming motif in evolutionarily distant porins. J. Struct. Biol. **107**:136–145.
19. **Ruijter, G. J. G., P. W. Postma, and K. Van Dam.** 1990. Adaptation of *Salmonella typhimurium* mutants containing uncoupled enzyme II^{Glc} to glucose-limited conditions. J. Bacteriol. **172**:4783–4789.
20. **Schwartz, M.** 1976. The adsorption of coliphage lambda to its host: effect of variations in the surface density of receptor and in phage-receptor affinity. J. Mol. Biol. **103**:521–536.
21. **Schwartz, M.** 1987. The maltose regulon, p. 1482–1502. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
22. **Siegele, D. A., and R. Kolter.** 1992. Life after log. J. Bacteriol. **174**:345–348.
23. **Sterkenburg, A., E. Vlegels, and J. T. M. Wouters.** 1984. Influence of nutrient limitation and growth rate on the outer membrane proteins of *Klebsiella aerogenes* NCTC 418. J. Gen. Microbiol. **130**:2347–2355.
24. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
25. **von Meyenburg, K., and N. Nikaido.** 1977. Outer membrane of gram-negative bacteria. XVI. Specificity of transport process catalyzed by the λ-receptor protein in *Escherichia coli*. Biochem. Biophys. Res. Commun. **78**:1100–1107.
26. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140**:1–13.