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Possible Mechanisms Underlying the Slow Lactose Fermentation Phenotype in *Shigella* spp.

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A Southern hybridization analysis revealed that the region homologous to *Escherichia coli lacZ* was present on the chromosomal DNAs of β-galactosidase-positive *Shigella* strains, such as *Shigella dysenteriae* serovar 1 and *Shigella sonnei* strains, whereas this region was absent from chromosomal DNAs of β-galactosidase-negative strains of *Shigella flexneri* and *Shigella boydii*. We found that the *lacY-A* region was deficient in *S. dysenteriae* serovar 1 and believe that this is the reason for the slow fermentation of lactose by this strain. *S. sonnei* strains possessed the region which hybridized with *E. coli lacY-A* despite their slow hydrolysis of lactose. The whole lactose-fermenting region was cloned from *S. sonnei* and compared with the cloned *lac* operon of *E. coli* K-12. Both clones directed the synthesis of β-galactosidase in an *E. coli* K-12 strain lacking indigenous β-galactosidase activity (strain JM109-1), and we observed no difference in the expression of β-galactosidase activity in *S. sonnei* and *E. coli*. However, *E. coli* JM109-1 harboring the lactose-fermenting genes of *S. sonnei* exhibited the slow lactose fermentation phenotype like the parental strain. *S. sonnei* strains had no detectable lactose permease activities. *E. coli* JM109-1 harboring the lactose-fermenting genes of *S. sonnei* had a detectable permease activity, possibly because of the multicopy nature of the cloned genes, but this permease activity was much lower than that of strain JM109-1 harboring the *lac* operon of *E. coli* K-12. From these results we concluded that slow lactose fermentation by *S. sonnei* is due to weak lactose permease activity.

Lactose fermentation is a biochemical property that is used for identification of *Shigella* spp. isolated from specimens from patients suffering from or suspected of dysentery. The lactose fermentation phenotype is used to distinguish *Shigella* spp. from *Escherichia coli* because almost no strains of *Shigella* spp. ferment lactose (10). Specimens from patients are first streaked onto differential media, such as deoxycholate citrate agar and shigella-salmonella agar, and after overnight incubation at 37°C nonfermenting colonies are selected for further examination. *Shigella* and *E. coli* strains are often extremely difficult to distinguish on the basis of biochemical phenotypes because there are aerogenic (gas-producing) *Shigella* strains (10) and lactose-negative, anaerogenic, nonmotile *E. coli* strains (8). Moreover, there are some β-galactosidase-positive *Shigella* strains, including *Shigella dysenteriae* serovar 1 and *Shigella sonnei* strains (10), and this phenotype makes species identification tentative. However, these lactose-fermenting *Shigella* strains usually ferment lactose relatively slowly, and the colonies change to lactose-fermenting colonies after cultivation for 2 or more days. In this study we investigated lactose fermentation by *Shigella* spp. genetically to clarify the mechanisms of slow fermentation of lactose by *S. dysenteriae* serovar 1 and *S. sonnei*.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The bacterial strains, bacteriophage, and plasmids which we used are listed in Table 1. Unless otherwise stated, the bacteria were grown in LB medium (10 g of tryptone per liter, 5 g of yeast extract per liter, and 5 g of NaCl per liter; pH 7.0). Lactose fermentation was determined on O-F medium (2.7 g of peptone per liter, 5 g of NaCl per liter, 0.3 g of K_2HPO_4 per liter, 0.03 g of bromthymol blue per liter, and 3 g of agar per liter; pH 7.1) containing 1% lactose. M9 medium (6 g of Na_2HPO_4 per liter, 3 g of KH_2PO_4 per liter, 0.5 g of NaCl per liter, 1 g of NH_4Cl per liter, 2 ml of 1 M $MgCl_2$ per liter, 0.1 ml of 1 M $CaCl_2$ per liter, and 5 ml of 80% glycerol per liter or 10 ml of 20% glucose per liter; pH 7.4) supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Mich.) was used as the basal medium for cultivation of bacteria that were to be tested for β-galactosidase and lactose permease activities. M9 medium containing glycerol was designated M9 glycerol medium, and M9 medium containing glucose was designated M9 glucose medium.

Preparation of DNA. Plasmid DNA was prepared by using the rapid alkaline extraction method and was purified by using NA agarose (Pharmacia, Uppsala, Sweden) gel electrophoresis followed by electroelution (6). Bacterial chromosomal DNA was extracted as described previously (12).

Southern hybridization analysis. A Southern hybridization analysis was carried out to investigate the presence of genes corresponding to the *lac* operon of *E. coli* K-12 in *Shigella* spp. We transferred genomic DNA fragments, which were separated on an agarose gel by electrophoresis, onto a nylon membrane (Hybond-N; Amersham, Buckinghamshire, United Kingdom) by using the method of Southern, as described by Maniatis et al. (6). The original amount of DNA applied for electrophoresis was 5 μg per well. Probe DNA was prepared from plasmid pKM005, which carries the *lac* structural genes in the absence of control (*o*, p, i) elements (7). A 1.82-kbp *Hin*cII fragment, which included about 60% of the C-terminal DNA sequence for β-galactosidase, was used as the *lacZ* probe. A 2.15-kbp *Pvu*II fragment, which included the whole *lacY-A* region, was used as the probe for *lacY-A*. The regions which were used as DNA probes were determined by using the DNA sequence data for the *lac*

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TABLE 1. Bacterial strains, bacteriophage, and plasmids used

Strain, phage, or plasmid	Characteristics[a]	Source or reference[b]
Strains		
S. dysenteriae 1	Clinical isolate	Saito
S. flexneri 1b	Clinical isolate	Saito
S. flexneri 3a	Clinical isolate	Saito
S. boydii 2	Clinical isolate	Saito
S. boydii 4	Clinical isolate	Saito
S. sonnei 85-8	Clinical isolate	Saito
S. sonnei 85-181	Clinical isolate	Saito
S. sonnei 85-152	Clinical isolate	Saito
S. sonnei 86-8	Clinical isolate	Saito
S. sonnei 86-85	Clinical isolate	Saito
S. sonnei 86-121	Clinical isolate	Saito
S. sonnei 87-81	Clinical isolate	Saito
S. sonnei 88-86	Clinical isolate	Saito
E. coli C600	F^- *thi-1 leuB6 lacY1 tonA21 supE44* λ^-	Ohta
E. coli JM109	*recA*Δ(*lac-proAB*) *endA1 gyrA96 thi hsdR17 SupE44 relA1* F′ (*traD36 proA*$^+$*B*$^+$ *lacI*^{q}*ZΔM15*)	Ohta
E. coli JM109-1	JM109 lacking the F′ plasmid	This study
E. coli F379	Wild-type strain	
Phage clone 10A6	Lactose operon	5
Plasmids		
pACYC184	Cp^r Tc^r; cloning vector	6
pKM005	Ap^r *lacZ lacY lacA*; promoter research vector (selection vector)	Mizuno
pLOE10A-6	Cp^r; recombinant plasmid which carries 12-kbp chromosomal DNA fragment of *E. coli* K-12, including the *lac* operon; vector is pACYC184	This study
pLOS10B-1	Cp^r; recombinant plasmid which carries 30-kbp chromosomal DNA fragment of *S. sonnei*, including the lactose-fermenting genes; vector is pACYC184	This study
pLOS10B-2	Cp^r; recombinant plasmid which carries 8-kbp chromosomal DNA fragment of *S. sonnei*, including the lactose-fermenting genes; vector is pACYC184	This study

[a] Abbreviations: Ap, ampicillin; Cp, chloramphenicol; Tc, tetracycline.
[b] Saito, M. Saito, Aichi Prefectural Institute of Public Health; Ohta, A. Ohta, Department of Biochemistry, Saitama University; Mizuno, T. Mizuno, Laboratory of Microbiology, Faculty of Agriculture, Nagoya University.

operon of *E. coli* K-12 in the SDC-GENETYX data base (Software Development Co., Tokyo, Japan). DNA fragments were excised from a low-melting-temperature agarose gel (International Biotechnologies, Inc., New Haven, Conn.) after electrophoresis. Each gel slice was labeled directly with [α-^{32}P]dCTP by using a multiprime labeling kit (Amersham). The procedures used for labeling DNA segments and the conditions used for hybridization have been described previously (1).

Measurement of β-galactosidase activity. The assay for β-galactosidase activity was carried out by using a previously described method (9). Briefly, an overnight culture of each of the strains which were to be tested was prepared in M9 glycerol medium supplemented with Casamino Acids and 1 mM isopropyl-β-D-thiogalactoside (IPTG) as an inducer of β-galactosidase. Overnight cultures in the same medium without IPTG were also prepared. The overnight cultures were then subcultured in the same media with or without IPTG until the optical density at 600 nm of each culture reached 0.5. After the bacterial membranes were disrupted by incubation with toluene, β-galactosidase activity was measured by using *o*-nitrophenyl-β-D-galactoside as the substrate. Enzyme activities were expressed as units per milligram of protein (9). The β-galactosidase activities of bacterial cells cultured in M9 glucose media with and without IPTG were also determined. To compare the function of the cloned lactose-fermenting genes of *S. sonnei* with the function of the *lac* operon of *E. coli* K-12, *E. coli* JM109 lacking the F′ plasmid (strain JM109-1) was used as the host strain. Strain JM109-1, which required proline for growth on the synthetic medium as a result of the loss of the F′ plasmid, was isolated after JM109 was cultivated in LB medium. Strain JM109-1 was transformed by using pLOS10B-1, pLOS10B-2, or pLOE10A-6 (see below).

Western blot analysis. The molecular weight and immunological cross-reactivity of β-galactosidase from *S. sonnei* were determined by a Western blot analysis. Sodium dodecyl sulfate protein extracts of the whole cells were prepared by the method described by Silhavy et al. (11) and were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred from polyacrylamide gels to nitrocellulose membranes in 25 mM Tris–glycine buffer (pH 8.3) containing 20% methanol and stained as previously described (4). Rabbit anti-β-galactosidase serum (Chemicon International, Inc., El Segundo, Calif.) was used for staining.

Cloning of lactose-fermenting genes of *S. sonnei*. Chromosomal DNA from *S. sonnei* 85-8 was digested with *Sal*I and ligated into the same cloning sites of cloning vector pACYC184, or chromosomal DNA from the same strain was digested with *Bgl*II and ligated with *Bam*HI-opened pACYC184. The former plasmid, which carried an 8-kbp *Sal*I fragment, was designated pLOS10B-2, and the latter plasmid, which carried a 30-kbp *Bgl*II fragment, was designated pLOS10B-1. The lactose-fermenting clones were selected on M9 glycerol agar plates containing 30 μg of chloramphenicol per ml, 0.1 mM 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and 0.2 mM IPTG. The methods used for plasmid extraction and analysis by agarose gel electrophoresis have been described previously (6).

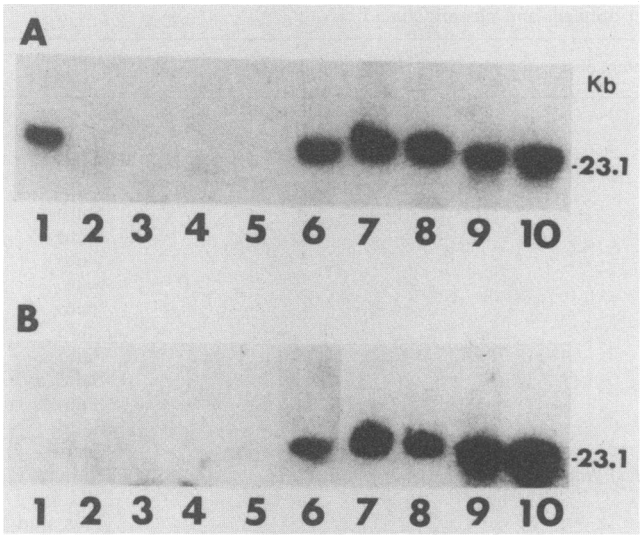

FIG. 1. Southern hybridization analysis of the chromosomal DNAs of *Shigella* spp. Chromosomal DNA preparations were transferred from 0.9% agarose to nitrocellulose membranes after gel electrophoresis. The probes used were the *lacZ* (A) and *lacY-A* (B) regions of the *lac* operon of *E. coli* K-12. Chromosomal DNAs from *S. dysenteriae* serovar 1 (lanes 1), *S. flexneri* 1b and 3a (lanes 2 and 3, respectively), *S. boydii* 2 and 4 (lanes 4 and 5, respectively), *S. sonnei* 85-152, 85-181, and 87-81 (lanes 6 through 8, respectively), and *E. coli* C600 and F379 (lanes 9 and 10, respectively) were analyzed.

Cloning of the *lac* operon of *E. coli* K-12. The *lac* operon of *E. coli* K-12 was subcloned from a genomic library of *E. coli* W3110, which was constructed by Kohara et al. (5). Lambda phage clone 10A6 carrying the entire *lac* operon was digested with *Pst*I and ligated with *Hin*cII-opened pACYC184. The resulting plasmid carried a 12-kbp *Pst*I fragment and was designated pLOE10A-6.

Measurement of lactose permease activity. Lactose permease activities were determined by using the method of Brooker et al. and ^{14}C-labeled lactose (3).

RESULTS

Southern hybridization analysis of chromosomal DNAs of *Shigella* spp. in which *E. coli lacZ* and *lacY-A* regions were used as probes. Among the *Shigella* strains examined, the chromosomal DNAs from *S. dysenteriae* serovar 1 and *S. sonnei* strains hybridized with the *lacZ* probe with almost the same intensity as the positive control DNAs from *E. coli* K-12 and F379 (Fig. 1A, lanes 1 and 6 through 10). However, the chromosomal DNAs from *Shigella flexneri* 1b and 3a and *Shigella boydii* 2 and 4 did not hybridize with the *lacZ* probe at all (Fig. 1A, lanes 2 through 5). These results corresponded well with the positive and negative β-galactosidase phenotypes of *Shigella* spp. Next, we examined the presence of DNA homologous to the *lacY-A* region of *E. coli* K-12 in *Shigella* spp. The chromosomal DNAs of *Shigella* strains whose chromosomal DNAs did not hybridize with the *lacZ* probe also did not hybridize with the *lacY-A* probe (Fig. 1B, lanes 2 through 5). DNA from the chromosome of *S. dysenteriae* serovar 1 did not hybridize with the *lacY-A* probe (Fig. 1B, lane 1). DNAs from the chromosomes of *S. sonnei* strains contained DNA which hybridized with the *lacY-A* region of *E. coli* K-12 (Fig. 1B, lanes 6 through 8).

TABLE 2. β-Galactosidase activities of *Shigella* spp. and *E. coli* strains

Expt	Strain	β-Galactosidase activity (U/mg of protein) of cells cultured in[a]: M9 glucose medium	M9 glycerol medium	M9 glucose medium + IPTG	M9 glycerol medium + IPTG
1	*S. dysenteriae* 1	ND[b]	7.2	ND	196.6
	S. sonnei 85-8	ND	1.6	ND	418.4
	S. sonnei 85-181	ND	5.6	ND	219.7
	S. sonnei 85-152	ND	13.5	ND	1,486.4
	S. sonnei 86-8	ND	9.2	ND	212.5
	S. sonnei 86-85	ND	8.8	ND	1,143.7
	S. sonnei 86-121	ND	4.9	ND	224.4
	S. sonnei 87-81	ND	8.7	ND	762.5
	S. sonnei 88-96	ND	5.3	ND	428.2
	E. coli F379	ND	0	ND	1,651.2
2	*S. dysenteriae* 1	5.4	11.0	147.7	150.6
	S. sonnei 85-152	13.4	11.6	202.5	386.4
	S. sonnei 87-81	38.0	14.0	125.9	250.1
	E. coli C600	22.1	15.7	87.8	556.1
	E. coli F379	8.0	51.0	76.2	616.3

[a] β-Galactosidase activities were measured after bacterial cells were disrupted with toluene.
[b] ND, not done.

However, the level of DNA homology in this region seemed relatively low, because the signal intensities of these strains were weak compared with the signal intensity of the positive control (Fig. 1B, lanes 9 and 10).

Comparison of β-galactosidase activities of *Shigella* spp. and *E. coli*. We assayed the β-galactosidase activities of *S. dysenteriae* serovar 1 and *S. sonnei* strains whose chromosomal DNAs hybridized with the *E. coli lacZ* probe, *E. coli* C600, and strain F375 (Table 2). The specific activities of the β-galactosidases of these *Shigella* strains were inducible by IPTG, like those of *E. coli* strains. Although the intensities of the enzyme activities of the *S. sonnei* strains were diverse, there were many strains of *S. sonnei* whose β-galactosidase activities were as potent as those of *E. coli*. The intensity of the enzyme activity of the *S. dysenteriae* serovar 1 strain was low compared with that of *E. coli*. We also investigated the effect of adding glucose on the induction of β-galactosidase by IPTG in these strains (Table 2). Enzyme induction by IPTG was suppressed by the addition of glucose (catabolite repression) in *E. coli*. In contrast, suppression of enzyme induction by glucose was not significant in *S. dysenteriae* serovar 1 and *S. sonnei*.

Western blot analysis of β-galactosidases of *S. sonnei* and *E. coli*. The molecular weights and immunological cross-reactivities of the β-galactosidases of *S. sonnei* and *E. coli* were studied by performing a Western blot analysis. Whole-cell extracts from *S. sonnei* strains were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with anti-β-galactosidase rabbit serum after blotting onto a nitrocellulose membrane (Fig. 2). The β-galactosidases of *S. sonnei* strains were cross-reactive with the β-galactosidase of *E. coli*, and the molecular weights of the β-galactosidases of *S. sonnei* strains were very similar to the molecular weight of *E. coli* β-galactosidase (116,000).

Cloning of the lactose-fermenting genes of *S. sonnei* and the *lac* operon of *E. coli* K-12. After *S. sonnei* 85-8 chromosomal DNA was digested with various restriction enzymes, Southern hybridization analyses were carried out with *E. coli lacZ* and *lacY-A* probes. The *Bgl*II and *Sal*I fragments were

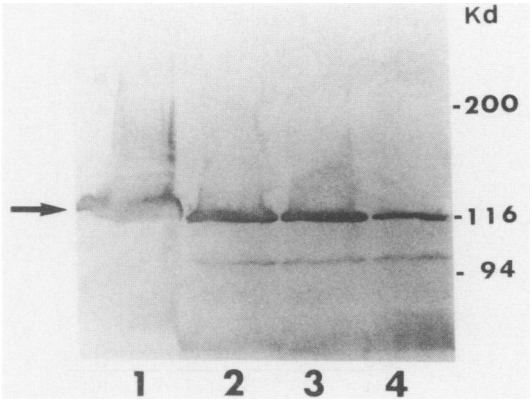

FIG. 2. Western blot analysis of whole-cell extracts of *E. coli* F379 (lane 1) and *S. sonnei* 85-152, 85-181, and 87-81 (lanes 2 through 4, respectively). Anti-β-galactosidase rabbit serum was used for staining. The position of β-galactosidase is indicated by an arrow. Kd, kilodaltons.

ligated into pACYC184, and the resulting constructions were designated pLOS10B-1 and pLOS10B-2, respectively. The *Pst*I fragments containing the entire *lac* operon of *E. coli* K-12 were also ligated into pACYC184, and the resulting construction was designated pLOE10A-6. Figure 3 shows the restriction enzyme maps of these clones. The restriction enzyme maps of the *lacZ* regions of *S. sonnei* and *E. coli* were identical. However, the maps of the *lacY-A* regions of the two organisms were different; the *lacY-A* region of *S. sonnei* had *Eco*RI, *Hin*dIII, *Bam*HI, *Sal*I, and *Pst*I sites, whereas in the *lacY-A* region of *E. coli* there were no such restriction sites. It has been shown that the *lacY-A* region of *E. coli* does not have these restriction sites but has *Pvu*II, *Kpn*I, and *Eco*RV sites (5).

E. coli JM109-1 harboring pLOS10B-1 or pLOS10B-2 produced non-lactose-fermenting colonies on O-F medium after 1 day of culture, but the colonies changed to lactose-fermenting colonies after 2 days of culture. Thus, these organisms expressed the slow lactose fermentation phenotype of the parental strain (*S. sonnei* 85-8). On the other hand, strain JM109-1 harboring pLOE10A-6 produced lactose-fermenting colonies after 1 day of culture.

The β-galactosidase activities of *E. coli* JM109-1(pLOS10B-1), JM109-1(pLOS10B-2), and JM109-1(pLOE10A-6) were determined (Table 3). The enzyme activities directed by these organisms were almost the same. The enzyme activities of JM109-1(pLOS10B-1), JM109-1(pLOS10B-2), and JM109-1

TABLE 3. β-Galactosidase activities of *E. coli* JM109-1 carrying the lactose-fermenting genes of *S. sonnei* or the *E. coli* K-12 *lac* operon

Strain	β-Galactosidase activity (U/mg of protein) of cells cultured in[a]:	
	M9 glycerol medium	M9 glycerol medium + IPTG
JM109-1(pACYC184)	6.8	9.9
JM109-1(pLOE10A-6)	38.4	5,372.1
JM109-1(pLOS10B-1)	34.4	4,704.5
JM109-1(pLOS10B-2)	27.3	4,384.8

[a] β-Galactosidase activities were measured after bacterial cells were disrupted with toluene.

(pLOE10A-6) were much higher than those of *S. sonnei* and *E. coli* C600 and F379, possibly because of the presence of the genes encoding the synthesis of β-galactosidase on the multicopy plasmids (Table 2).

Comparison of *lacY* function (lactose permease activities) of *Shigella* spp. and *E. coli*. The lactose permease activities of *S. dysenteriae* serovar 1 and *S. sonnei* were compared with the lactose permease activity of *E. coli*. *S. dysenteriae* serovar 1 and *S. sonnei* 85-152 and 88-96 had no detectable lactose permease activities, whereas *E. coli* F379 had a high level of permease activity (Fig. 4). The permease activities of *E. coli* JM109-1(pLOS10B-1), JM109-1(pLOS10B-2), and JM109-1(pLOE10A-6) were also determined (Fig. 5 and 6). The level of permease activity of *E. coli* JM109-1(pLOE 10A-6) was the same as the level of permease activity of *E. coli* F379 (Fig. 4). Strain JM109-1 had no detectable lactose permease activity. Strains JM109-1(pLOS10B-1) and JM109-1 (pLOS10B-2) had very low but definite permease activities (Fig. 6). *S. sonnei* 85-8, the donor of the lactose-fermenting genes for the plasmids, also had no detectable permease activity.

DISCUSSION

In this paper we show that (i) a DNA sequence homologous to *lacZ* of *E. coli* is present in β-galactosidase-positive *Shigella* strains, such as *S. dysenteriae* serovar 1 and *S. sonnei* strains, (ii) a DNA sequence homologous to the *lacY-A* region of *E. coli* is also present in *S. sonnei* but is not present in *S. dysenteriae* serovar 1, (iii) the molecular weight of the β-galactosidase of *S. sonnei* is very similar to the molecular weight of the β-galactosidase of *E. coli*, and (iv) the β-galactosidase of *S. sonnei* is antigenically cross-reac-

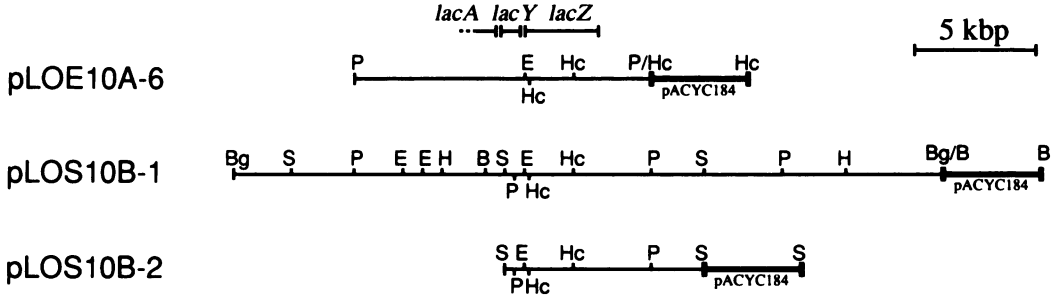

FIG. 3. Comparison of the restriction enzyme maps of the *E. coli* K-12 *lac* operon and the lactose-fermenting genes (*lacZ* and *lacY-A*) of *S. sonnei*. The regions corresponding to *lacZ* and *lacY-A* are indicated. Restriction enzyme abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; P, *Pst*I; S, *Sal*I.

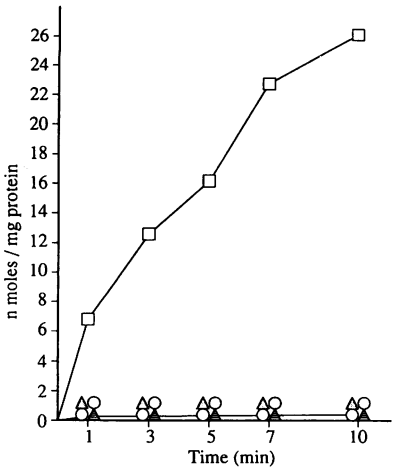

FIG. 4. Lactose uptake in *Shigella* spp. and *E. coli*. Symbols: ▲, *S. dysenteriae* serovar 1; ○, *S. sonnei* 85-8; ◬, *S. sonnei* 85-152; ◎, *S. sonnei* 88-96; □, *E. coli* F379.

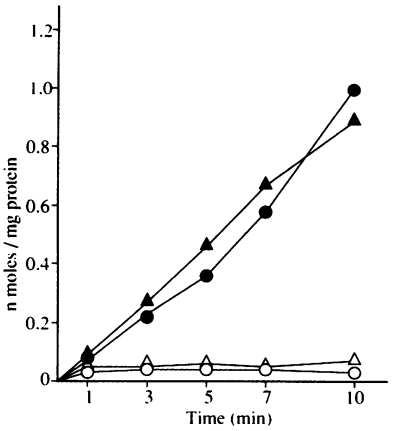

FIG. 6. Lactose uptake in *E. coli* JM109-1(pLOS10B-1) and JM109-1(pLOS10B-2). Symbols: ▲, strain JM109-1(pLOS10B-1); ●, strain JM109-1(pLOS10B-2); △, strain JM109-1; ○, *S. sonnei* 85-8. The data for strains JM109-1(pLOS10B-1), JM109-1(pLOS10B-2), and JM109-1 are the same as the data shown in Fig. 5, but the scale of the ordinate has been magnified.

tive with the β-galactosidase of *E. coli*. From these results we concluded that on the chromosomal DNA of *S. sonnei* there are genes that correspond to the *lac* operon of *E. coli*, although we do not know whether these genes are an operon, and that *S. dysenteriae* serovar 1 possesses a region that corresponds to *lacZ* but lacks a region that corresponds to *lacY-A*.

When lactose is used as an inducer, the following four steps are necessary for induction to take place (2): (i) lactose must enter the cell through the lactose permease; (ii) lactose must be converted to the true inducer via the transgalactosidation activity of β-galactosidase; (iii) the true inducer must be able to interact with the *lac* repressor; and (iv) the inducer must cause an allosteric transformation of the repressor. Because IPTG induced the β-galactosidase in *S. dysenteriae* serovar 1 and *S. sonnei*, steps iii and iv took place normally in these strains. The β-galactosidase activity of *S. dysenteriae* serovar 1 cultivated in the presence of IPTG was low compared with that of *E. coli*. Active transport of lactose or IPTG into the cells through lactose permease does not occur in *S. dysenteriae* serovar 1 because of the deletion of *lacY*. This is considered to be the reason for the low β-galactosidase activity and also the slow lactose fermentation phenotype of this strain. The level of β-galactosidase activity of *E. coli* JM109-1 harboring the cloned lactose-fermenting genes of *S. sonnei* was almost the same as the level of β-galactosidase activity of JM109-1 harboring the cloned *lac* operon of *E. coli* K-12. In contrast, the level of lactose permease activity directed by the cloned genes of *S. sonnei* was much lower than the level of lactose permease activity directed by the cloned *lac* operon of *E. coli* K-12. The lactose permease activity of *S. sonnei* was so weak that it was not detectable within the experimental time period (10 min), whereas strain JM109-1 harboring the cloned genes of *S. sonnei* exhibited low but detectable permease activity. The difference in the intensities of the permease activities between wild-type *S. sonnei* strains and strain JM109-1 harboring the cloned genes of *S. sonnei* may have been due to the fact that the cloned genes were integrated into the multicopy plasmid. From these results we concluded that the weak function of lactose uptake must be the major reason for the slow lactose fermentation phenotype of *S. sonnei* strains. These results also suggest that it is better to use a substrate whose uptake into cells is independent of *lacY* function, such as 5-bromo-4-chloro-3-indolyl-β-D-galactoside, for rapid determinations of β-galactosidase activities in *S. dysenteriae* serovar 1 and *S. sonnei*.

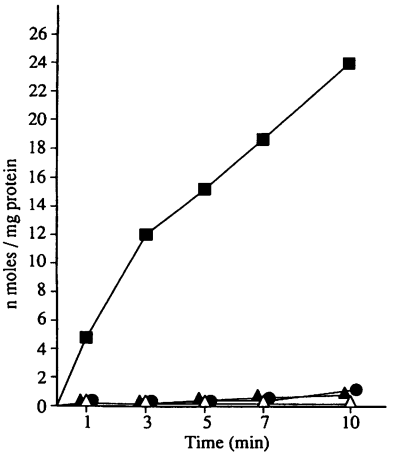

FIG. 5. Lactose uptake in *E. coli* JM109-1(pLOS10B-1), JM109-1 (pLOS10B-2), and JM109-1(pLOE10A-6). Symbols: ▲, strain JM109-1 (pLOS10B-1); ●, strain JM109-1(pLOS10B-2); ■, strain JM109-1 (pLOE10A-6); △, strain JM109-1.

It is interesting that there was no catabolite repression of β-galactosidase in *S. dysenteriae* serovar 1 and *S. sonnei*, in contrast to *E. coli*, whose β-galactosidase exhibited marked catabolite repression. Although the true reason for the lack of catabolite repression of β-galactosidase in these *Shigella* strains is unknown, this phenomenon may be related to the slow lactose fermentation phenotype of these organisms.

In this study we demonstrated that *S. sonnei* has a gene corresponding to *lacY* of *E. coli*, but its *lacY* function is very weak compared with that of *E. coli*. The reason for the weak *lacY* function of *S. sonnei* is unknown. The level of DNA homology in the *lacY-A* regions of *S. sonnei* and *E. coli* seemed to be relatively low on the basis of the results of a

hybridization experiment. This observation is compatible with the finding that the restriction enzyme maps of the *lacY-A* regions of *S. sonnei* and *E. coli* were different. It is possible that the *lacY-A* region of *S. sonnei* has some functional defect. A genetic analysis of the *lacY-A* region of *S. sonnei* is now under way in our laboratory.

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