JOURNAL OF BACTERIOLOGY, June 1987, p. 2685–2690
0021-9193/87/062685-06$02.00/0

Vol. 169, No. 6

Expression and Regulation of a *Vibrio alginolyticus* Sucrose Utilization System Cloned in *Escherichia coli*

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Received 19 December 1986/Accepted 10 March 1987

A halotolerant collagenolytic *Vibrio alginolyticus* strain isolated from salted hides had intracellular sucrase activity and did not secrete sucrase into the medium. The strain actively transported sucrose by a sucrose-inducible, Na^+-independent process. A 10.4-kilobase DNA fragment of *V. alginolyticus* DNA was cloned into *Escherichia coli*. The recombinant *E. coli*(pVS100) could utilize sucrose as a sole carbon source. In contrast to *V. alginolyticus*, the recombinant *E. coli* produced both intra- and extracellular sucrase activities. Up to 20% of the total sucrase activity was in the supernatant. Sucrase synthesis in *E. coli*(pVS100) was inducible and was subject to glucose repression, which was relieved by cyclic AMP. Sucrose was actively transported by a sucrose-inducible, Na^+-independent system in *E. coli*(pVS100). Sucrose uptake was inhibitied by the addition of a proton conductor. The maximum velocity and apparent K_m values of sucrose uptake for the *V. alginolyticus* strain and *E. coli*(pVS100) were 130 nmol/mg of protein per min and 50 μM and 6 nmol/mg of protein per min and 275 μM, respectively.

Vibrio alginolyticus is an aerobic, halotolerant, gram-negative bacterium, which has been isolated from marine environments (20) and from damaged salted hides (16, 29, 30). The ability of *V. alginolyticus* to utilize sucrose has been used to distinguish it from *Vibrio parahaemolyticus*, which causes food-borne gastroenteritis (20). Kakinuma and Unemoto (5) investigated the initial step of the sucrose catabolic pathway in a marine *V. alginolyticus* strain and showed that sucrose is actively transported by the Na^+ electrochemical potential and then hydrolyzed intracellularly to glucose and fructose by sucrase.

The pathway of sucrose metabolism in the gram-negative marine *V. alginolyticus* strain differs from that in gram-positive *Bacilus subtilis* (6), *Streptococcus lactis* (26), and *Streptococcus mutans* (21, 24), for which sucrose is accumulated in the cell by the phosphoenolpyruvate-dependent phosphotransferase system. Sucrose 6-phosphate, which is the intracellular product of this transport system, is then hydrolyzed to glucose 6-phosphate and fructose by sucrose-6-phosphate hydrolase.

We investigated the sucrose metabolism in the collagenolytic *V. alginolyticus* strain and report here the cloning, expression, and regulation of the *V. alginolyticus* sucrose utilization system in *Escherichia coli*. Evidence is presented for the active transport of sucrose by a Na^+-independent process in *E. coli*, as well as for the appearance of extra- and intracellular sucrase enzyme activities.

MATERIALS AND METHODS

Plasmids, bacterial strains, and growth conditions. *V. alginolyticus* (16, 29, 30) was used as the source of chromosomal DNA. *E. coli* JA221 *recA1 leuB6 trpE5* (11) was used as the recipient strain. Plasmid pEcoR251, a gift from M. Zabeau, Plant Genetic Systems, Ghent, Belgium, is a positive selection vector containing the *E. coli Eco*RI endonuclease gene under the control of the bacteriophage λ rightward promoter, the ampicillin resistance gene, and the plasmid pBR322 origin of replication. It was derived from the pCL plasmids described by Zabeau and Stanley (32). The *Eco*RI endonuclease gene product, expressed at high levels by the λ promoter on pEcoR251, is lethal unless insertionally inactivated or regulated by plasmid pCI857, which contains a temperature-sensitive λ repressor gene (17). The *Eco*RI gene has a single *Bgl*II cloning site.

The *V. alginolyticus* strain was grown at 30°C in Luria broth and on Luria agar (14) containing 0.4 M NaCl and minimal media (8, 27) supplemented with 15 μM thiamine and 6 mM sucrose as the sole source of carbon.

The transformed *E. coli* JA221 strain was grown at 37°C in Luria broth containing ampicillin (50 μg/ml) and minimal media (8, 27) supplemented with 86 mM NaCl, 15 μM thiamine, 6 mM sucrose, L-tryptophan and L-leucine (20 μg/ml each), 2.5 mg of vitamin-free Casamino Acids (Difco Laboratories) per ml, and 50 μg of ampicillin per ml.

The recipient strain *E. coli* JA221 was grown under identical conditions to those for the *E. coli* transformant, except that sucrose was replaced by 10 mM glucose and the ampicillin was omitted.

Cloning the *V. alginolyticus* sucrase system. *V. alginolyticus* DNA was partially digested with nuclease *Sau*3A and fractionated on a sucrose density gradient. Fragments of 4 to 10 kilobases (kb) were ligated with *Bgl*II-digested pEcoR251 DNA and used to transform competent cells of *E. coli* JA221. Ap^r transformants were selected on Luria agar containing 100 μg of ampicillin per ml. Ap^r colonies able to utilize sucrose as a sole carbon source were selected by being plated on minimal medium containing sucrose and ampicillin.

Restriction endonuclease mapping and DNA hybridization. Plasmid DNA was isolated by the method of Ish-Horowicz and Burke (4), digested with a variety of restriction endonucleases, and resolved by electrophoresis in Tris–borate–EDTA-buffered 0.8% agarose gels. *V. alginolyticus* chromosomal DNA, prepared by the method of Marmur (12), was digested with restriction endonucleases and separated by electrophoresis in Tris–acetate-buffered 1.2% agarose gels. The DNA was transferred to GeneScreen membranes (New England Nuclear Corp.) (22). DNA probes were prepared by nick translation with [α-^{32}P]dCTP as

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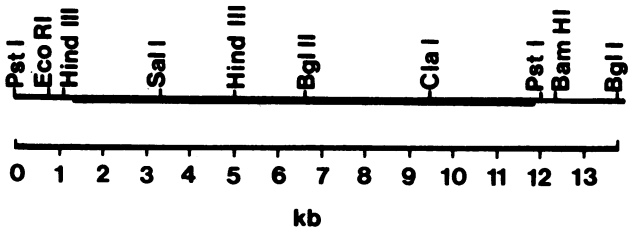

FIG. 1. Restriction endonuclease map of pVS100. ▬, *V. alginolyticus* insert; —, plasmid vector DNA.

described by Rigby et al. (18). The hybridization conditions were chosen as recommended by Maniatis et al. (11).

Cell fractionation. Periplasmic and cytoplasmic cell fractions were prepared by the osmotic-shock method (31). Cell extracts were prepared by sonicating washed cells for three 30-s bursts at 4°C in 0.05 M potassium phosphate buffer (pH 7.0). The sonicated cells were centrifuged at 4°C for 15 min in an Eppendorf microfuge, and the supernatant fluid was retained for enzyme assays.

Enzyme assays. Sucrase, β-lactamase, and β-galactosidase were assayed in cell-free culture supernatants, sonicated cell extracts, and cytoplasmic and periplasmic cell fractions. Sucrase was determined by incubating 400 μl of 0.88 M sucrose in 0.05 M potassium phosphate buffer (pH 7.0) with 1 ml of the appropriate enzyme dilution at 37°C for 30 min. Glucose-dinitrosalicylic acid (13) was used to determine the amount of reaction product formed. β-Galactosidase and β-lactamase were determined by the methods of Pardee et al. (15) and Sykes and Nordström (25), respectively. Protein concentrations in cell extracts or whole cells were assayed by the method of Lowry et al. (9) with bovine serum albumin as a standard. Sucrase activity was expressed as micromoles of reducing sugar produced per milliliter per minute.

Uptake of [^{14}C]sucrose. *V. alginolyticus*, *E. coli* JA221, and *E. coli* JA221(pVS100) were grown to late stationary phase on supplemented minimal medium. Cells were harvested by centrifugation, washed twice, and resuspended in the same volume of minimal medium at a protein concentration of approximately 0.4 mg/ml. The uptake reaction was started by the addition of 0.5 mM [^{14}C]sucrose (specific activity, 10 Ci/mol). Samples (50 μl) were withdrawn at intervals, filtered through membrane filters (pore size, 0.45 μm; Millipore Corp.), and rinsed once with 2 ml of minimal medium. After being dried, the filters were counted in 5 ml of toluene scintillation fluid with a scintillation spectrometer (Packard Tri-Carb 460).

The Na^+ dependence of sucrose uptake in *E. coli* JA221(pVS100) was determined by treating the cells twice with 50 mM diethanolamine hydrochloride–0.4 M CsCl (pH 8.0) for 10 min (28) and then washing them twice in 0.05 M potassium phosphate buffer (pH 6.9) containing 0.1 mM $MgSO_4$. *V. alginolyticus* cells were treated with choline chloride salts by the method of MacLeod and MacLeod (10). Samples (50 μl) of the *E. coli* cell suspension (2.5 mg of protein per ml) were added to 330 μl of the potassium phosphate buffer containing 12.5 mM glucose, 12.5 mM fructose, and either 0.1 M NaCl, KCl, or LiCl or no additional salt. Samples (25 μl) of the *V. alginolyticus* cell suspension (2.5 mg of protein per ml) were added to 365 μl of incubation medium (10) containing the same salts as the wash solution, except that the choline chloride was replaced by 0.3 M NaCl, KCl, or LiCl. After 30 s, the uptake experiments were started by the addition of 0.25 mM [^{14}C]sucrose. The uncoupler carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP) (10 μM), dissolved in methanol, was added 7 min before the sucrose was added.

Values for the K_m and V_{max} for sucrose uptake of *V. alginolyticus* and *E. coli* cells were determined by using 7.5 mM sodium succinate minimal medium salts containing chloramphenicol (100 μg/ml) and 0.05 M potassium phosphate–0.1 mM $MgSO_4$ (pH 6.9) buffer. Because *E. coli* JA221(pVS100) cells produce extracellular sucrase, the uptake of sucrose was determined after an adaption period of 30 s in the presence of a 50-fold molar excess of glucose and fructose to [^{14}C]sucrose. Uptake was determined at room temperature within 20 s after the addition of [^{14}C]sucrose. The rates of sucrose uptake were linear over 20 s, and the maximum amount of [^{14}C]sucrose taken up was less than 10% of the amount of [^{14}C]sucrose added. One nanomole of [^{14}C]sucrose was represented by 20,000 cpm.

RESULTS

Cloning of *V. alginolyticus* sucrase system in *E. coli*. Transformation of *E. coli* JA221 with pEcoR251 containing *V. alginolyticus* DNA fragments resulted in the isolation of six colonies which were able to utilize sucrose as a sole carbon source. Plasmids isolated from these colonies all retransformed *E. coli* JA221, and colonies able to utilize sucrose were obtained at the same frequency as Ap^r transformants. One plasmid was chosen for further study and designated pVS100. Propagation of the *E. coli* transformant on MacConkey agar containing sucrose resulted in red colonies, which indicated the production of acid following sucrose hydrolysis.

Restriction endonuclease mapping and DNA hybridization. The restriction endonuclease map of pVS100 was obtained by complete single, double, and triple digestions with a variety of restriction endonucleases (Fig. 1). The origin of the 10.4-kb insert in pVS100 was determined by Southern blotting and DNA hybridization with ^{32}P-labeled pVS100 (Fig. 2). Labeled pEcoR251 did not hybridize to *V. alginolyticus* DNA (data not shown). *V. alginolyticus* total cellular DNA was digested with *Eco*RI, *Hin*dIII, *Bgl*I, and

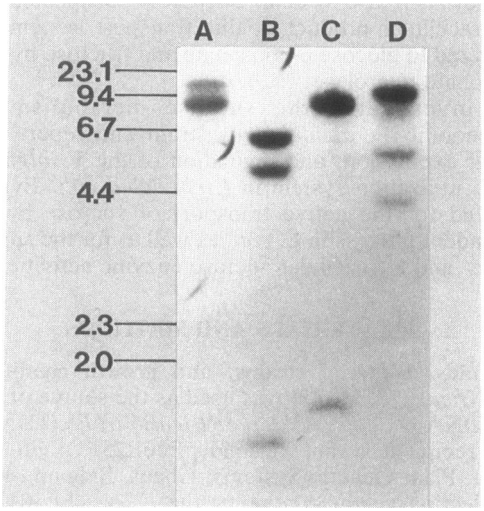

FIG. 2. Hybridization of ^{32}P-labeled pVS100 DNA with *V. alginolyticus* cellular DNA. Lanes A to D show an autoradiogram of various restriction endonuclease digestion products of *V. alginolyticus* cellular DNA after electrophoresis and transfer to a membrane hybridized with ^{32}P-labeled pVS100: A, *Eco*RI; B, *Hin*dIII; C, *Bgl*I; D, *Sal*I.

*Sal*I restriction endonucleases and probed with ^{32}P-labeled pVS100. From the restriction map, hybridization of *V. alginolyticus* chromosomal DNA digested with *Eco*RI and *Bgl*I would be expected to yield single bands on the autoradiograph, but two bands were observed (Fig. 2). Similarly, hybridization of *V. alginolyticus* chromosomal DNA digested with *Hin*dIII and *Sal*I would be expected to yield two bands, but three bands were observed. This suggests that either a region within the cloned 10.4-kb fragment might be duplicated in the *V. alginolyticus* genome or the *V. alginolyticus* DNA was only partially digested in the four experiments with the different restriction enzymes. Control experiments with plasmids and phages indicated that the enzymes were all active and that they totally digested the control DNA.

Regulatory studies. Supernatant and intracellular sucrase activities were assayed throughout the growth cycle of *V. alginolyticus* and *E. coli* JA221(pVS100) (Fig. 3). Both bacteria synthesized intracellular sucrase activity throughout the growth cycle in sucrose minimal medium. No extracellular sucrase activity was detected in the *V. alginolyticus* cultures, whereas the *E. coli* JA221 transformant produced extracellular sucrase activity during growth. The levels of intracellular sucrase in *E. coli* containing pVS100 were approximately fivefold higher than in the *V. alginolyticus* strain. This could be due to an effect of the gene copy number.

Expression of sucrase activity in *E. coli* JA221(pVS100)

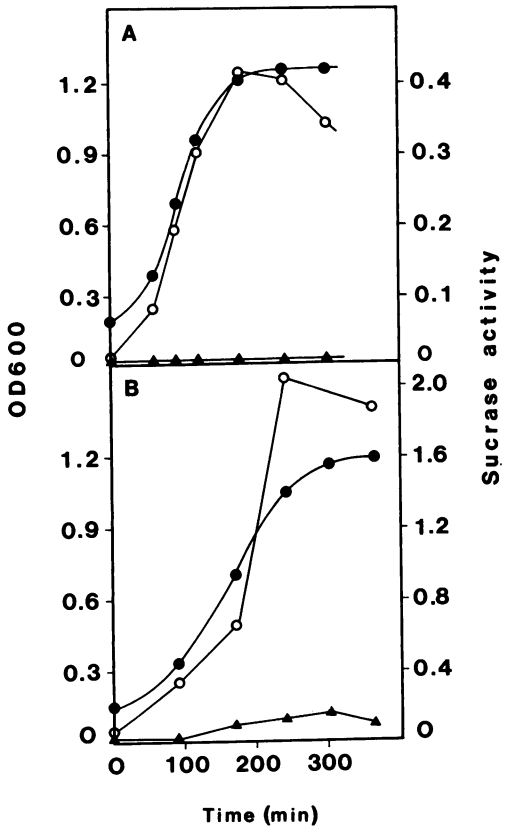

FIG. 3. Sucrase activities as a function of growth in *V. alginolyticus* (A) and *E. coli* JA221(pVS100) (B). Sucrase activities are expressed as micromoles of reducing sugar produced per milliliter per minute. Symbols: ●, cell growth curve, (optical density at 600 nm [OD_{600}]; ○, intracellular sucrase; ▲, extracellular sucrase.

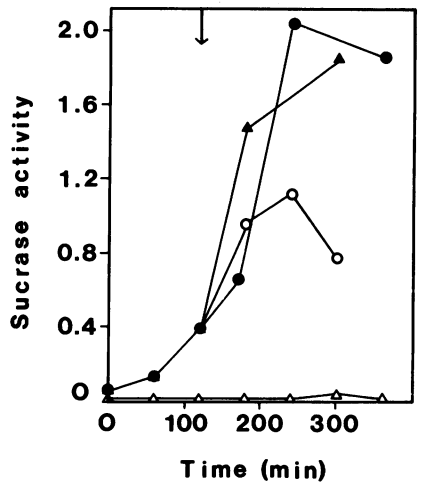

FIG. 4. Induction and repression of intracellular sucrase synthesis in *E. coli* JA221(pVS100) by sucrose and glucose, respectively. An *E. coli* JA221(pVS100) culture grown in glucose minimal medium was resuspended in glucose minimal medium (△). *E. coli* JA221(pVS100) cultures grown in sucrose minimal medium were resuspended in sucrose minimal medium (●). After 120 min (arrow), 10 mM glucose (○) or 10 mM glucose–5 mM cyclic AMP (▲) was added. Sucrose activities of the cultures are expressed as micromoles of reducing sugar produced per milliliter per minute.

required the presence of sucrose as an inducer (Fig. 4), and no sucrase activity was detected in glucose minimal medium. The effect of glucose on sucrase activity in *E. coli* JA221(pVS100) was determined (Fig. 4). Sucrase synthesis in mid-exponential-phase cultures growing in 6 mM sucrose minimal medium was inhibited by the addition of 10 mM glucose. This glucose-induced repression of sucrase synthesis in mid-exponential-phase *E. coli* JA221(pVS100) cells was reversed by the simultaneous addition of 5 mM cyclic AMP. In *V. alginolyticus*, sucrase synthesis was also induced by sucrose and repressed by glucose, and the repression was reversed by cyclic AMP (data not shown).

Production of sucrase in *E. coli* JA221(pVS100) was not regulated by ammonia repression. The addition of vitamin-free Casamino Acids (2.5 mg/ml) to mid-exponential-phase *E. coli* JA221(pVS100) cells growing on sucrose minimal medium did not affect the production of sucrase.

Cellular distribution of sucrase in *E. coli* JA221 containing pVS100. The localization of the sucrase activity of *E. coli* JA221(pVS100) was determined (Table 1). The localization of β-galactosidase and β-lactamase, as control cytoplasmic and periplasmic enzymes, repectively, was determined by using the same cultures. The majority of the β-galactosidase

TABLE 1. Localization of the sucrase activity in *E. coli* JA221(pVS100) cells[a]

Enzyme	Enzyme activity[b]		
	Cytoplasm	Periplasm	Extracellular supernatant fluid
β-Lactamase	12.5–15.3	55.0–62.6	24.6–29.7
β-Galactosidase	90.8–94.4	2.4–3.5	3.2–6.1
Sucrase	67.7–84.9	2.3–20.8	9.2–21.5

[a] Cell fractions were prepared and enzyme assays were performed as described in Materials and Methods.

[b] Enzyme activities are expressed as a percentage of the total enzyme activity. Experiments were performed in triplicate, and the range of values is given.

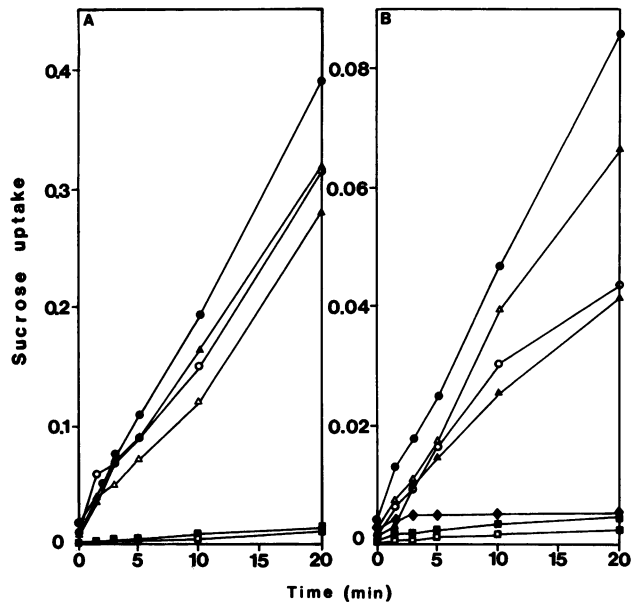

FIG. 5. [^{14}C]sucrose uptake (micromoles per milligram of protein) by *V. alginolyticus* (A) and *E. coli* JA221(pVS100) (B). Sucrose-induced washed cells (approximately 0.4 mg of protein per ml) were added to 0.5 mM [^{14}C]sucrose at time zero. Nonradioactive 15 mM sucrose (■), 28 mM fructose (△), 28 mM glucose (○), 28 mM glucose–28 mM fructose (▲), or 28 mM glucose–28 mM fructose–15 mM sucrose (□) was added at time zero. [^{14}C]sucrose uptake was determined after various time intervals. Other symbols: ● no additions; ◆, [^{14}C]sucrose uptake by *E. coli* JA221.

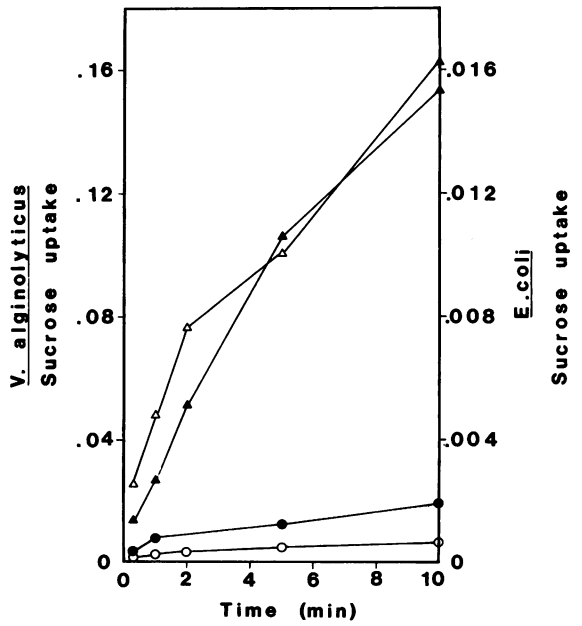

FIG. 6. Induction of [^{14}C]sucrose uptake in *V. alginolyticus* and *E. coli* JA221(pVS100). *V. alginolyticus* cells grown in glucose (○) and sucrose (△) minimal media and *E. coli* JA221(pVS100) cells grown in glucose (●) and sucrose (▲) minimal media were used. [^{14}C]sucrose uptake in *E. coli* was determined in the presence of 12.5 mM glucose–12.5 mM fructose. [^{14}C]sucrose uptake (0.5 mM) is expressed in micromoles per milligram of protein.

activity was detected in the cytoplasm, whereas the majority of the β-lactamase activity was present in the periplasm (Table 1). The majority of the sucrase activity was in the cytoplasm, and the remainder of the sucrase activity (average, 24%) appeared to be variably distributed between the periplasm and extracellular supernatant fluid. Up to approximately 20% of the sucrase activity in *E. coli* JA221(pVS100) cultures was detected in the extracellular supernatant fluid.

Uptake of [^{14}C]sucrose. *E. coli* JA221 did not take up sucrose (Fig. 5). [^{14}C]sucrose experiments with *E. coli* JA221(pVS100) cells were complicated, since well-washed mid-exponential cells contained extracellular sucrase. This extracellular sucrase immediately degraded sucrose to glucose and fructose, which could be taken up by the cell. Washed *V. alginolyticus* cells did not contain extracellular sucrase. The addition of nonradioactive sucrose or sucrose plus glucose and fructose to *V. alginolyticus* and *E. coli* JA221(pVS100) reduced the uptake of labeled sucrose by more than 90% (Fig. 5). The addition of nonradioactive glucose, fructose, or both reduced the uptake of [^{14}C]sucrose by *E. coli* JA221(pVS100) more than by *V. alginolyticus* (compare Fig. 5A and B), probably because the extracellular sucrase present in the *E. coli* resuspended cells had hydrolyzed some of the [^{14}C]sucrose to glucose and fructose. It was concluded that sucrose is specifically taken up by *E. coli* JA221(pVS100) and that the label does not enter primarily via glucose or fructose uptake systems. The sucrose uptake system in *V. alginolyticus* and *E. coli* JA221(pVS100) was induced by sucrose (Fig. 6).

It was previously shown (5) that the sucrose uptake system in a marine *V. alginolyticus* strain requires Na^+. The specific requirement for Na^+ cannot be met by other cations (K^+, Li^+). Sucrose uptake by the collagenolytic *V. alginolyticus* strain and *E. coli* JA221(pVS100) was determined (Fig. 7). In the presence or absence of Na^+, sucrose

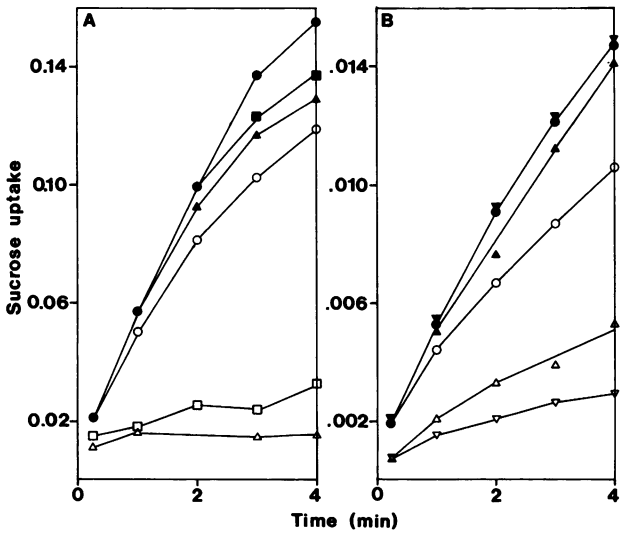

FIG. 7. Salt dependence of [^{14}C]sucrose uptake by *V. alginolyticus* (A) and *E. coli* JA221(pVS100) (B). *V. alginolyticus* and *E. coli* cells (2.5 mg of protein per ml) were added to the incubation medium containing 0.3 M or 0.1 M salts, respectively, and 10 μM CCCP was added after 7 min. [^{14}C]sucrose uptake in *E. coli* was determined in the presence of 12.5 mM glucose and 12.5 mM fructose. [^{14}C]sucrose uptake is expressed in micromoles per milligram of protein. Symbols: ●, NaCl, KCl; ▲, LiCl; ■, choline chloride; ▼, no salts; ○, methanol; △, NaCl plus CCCP; □, choline chloride plus CCCP; ▽, no salts plus CCCP.

uptake occurred in both *V. alginolyticus* and *E. coli* JA221(pVS100). These strains also transported sucrose in the presence of K^+ and Li^+. The uptake of sucrose by *V. alginolyticus* and *E. coli* JA221(pVS100) was markedly inhibited by the uncoupler CCCP (Fig. 7). The CCCP was added as a solution in methanol, which only slightly inhibited sucrose uptake.

The maximum velocity and apparent K_m value of sucrose uptake for the *V. alginolyticus* strain and *E. coli* JA221(pVS100) were 130 nmol/mg of protein per min and 50 μM and 6 nmol/mg of protein per min and 275 μM, respectively.

DISCUSSION

The collagenolytic *V. alginolyticus* strain isolated from salted hides had intracellular sucrase activity and did not secrete sucrase into the medium. This halotolerant bacterium is able to utilize and actively transport sucrose. We cloned a 10.4-kb DNA fragment from *V. alginolyticus* and showed that it enables *E. coli* to utilize sucrose as a sole source of carbon. The appearance of an extra hybridization band on the Southern blot (Fig. 2) in addition to the band(s) predicted from the restriction map of pVS100 (Fig. 1) may result from the presence of related sequences elsewhere on the *V. alginolyticus* chromosome. Gene amplification in *Vibrio cholerae* has been demonstrated (3). Alternatively, small repetitive extragenic sequences such as those reported for *E. coli* and *Salmonella typhimurium* (23) may be present in *V. alginolyticus*. In biotechnology processes, *E. coli* is often the organism of choice for the production of cloned gene products. There may be practical advantages in having *E. coli* strains which are able to utilize cheap sucrose substrates (e.g., molasses), particularly for the production of commodity products.

In contrast to the situation with *V. alginolyticus*, *E. coli* JA221(pVS100) produced both intra- and extracellular sucrase activities. Although the majority of the sucrase activity was cytoplasmic, up to approximately 20% of the enzyme was detected in the supernatant fluid. The distribution of sucrase in the periplasm and supernatant varied from culture to culture.

Sucrase production by *E. coli* JA221(pVS100) was inducible by sucrose. Perhaps the cloned DNA fragment from *V. alginolyticus* includes a regulatory gene whose action allows sucrose to activate sucrase production. It is possible that *E. coli* JA221 contains an analogous fortuitous repressor protein which recognizes the cloned DNA fragment and is able to complex with sucrose. Sucrase production was subject to glucose repression, which was relieved by cyclic AMP, and the cloned *V. alginolyticus* fragment presumably contains a CAP binding site. Since no genes on the vector pEcoR251 are regulated by sucrose, we concluded that sucrase production in *E. coli* is expressed from a *V. alginolyticus* regulation region on pVS100.

The uptake studies indicate that the cloned *V. alginolyticus* fragment also contained the gene(s) required for the transport of sucrose. Kakinuma and Unemoto (5) reported that a marine *V. alginolyticus* strain transports sucrose by a Na^+-dependent system. In contrast, the halotolerant collagenolytic *V. alginolyticus* strain transported sucrose by a Na^+-independent system. There is no doubt that the collagenolytic bacterium isolated from hides by Welton and Woods (29, 30) is a *V. alginolyticus* strain. The strain was originally classified as an *Achromobacter iophagus* strain. The identification was confirmed by the National Collection of Industrial Bacteria, Aberdeen, Scotland, but has since been reinvestigated by M. Hendrie, National Collection of Industrial Bacteria, and the strain has been reclassified as *V. alginolyticus*. Recently, the 5S rRNA sequence of the collagenolytic *V. alginolyticus* strain was shown to be similar to that of other marine *V. alginolyticus* strains (M. MacDonell, personal communication). The cloned sucrose uptake system was Na^+ independent and inducible in *E. coli*, which was unable to transport sucrose without the cloned DNA. The V_{max} for sucrose transport in *V. alginolyticus* is appreciably higher than in the transformed *E. coli* strain, and the K_m is lower. We conclude that in *E. coli* JA221(pVS100) cells, sucrose is actively transported and then is hydrolyzed to glucose and fructose by the intracellular sucrase. The extracellular sucrase can also degrade the sucrose to glucose and fructose, which are taken up by the glucose and fructose transport systems. This dual system suggests that *E. coli* strains carrying pVS100 may be adept at scavenging and utilizing sucrose in low-grade industrial molasses media.

B. subtilis has at least eight different loci involved in sucrose metabolism (7), and three structural proteins are specifically induced by sucrose: intra- and extracellular sucrase enzymes known as sucrase and levansucrase, respectively, and a membrane component of the phosphoenolpyruvate-dependent phosphotransferase system of sucrose transport. Although the sucrase and levansucrase genes have been cloned and expressed in *E. coli* (1, 2), they do not allow *E. coli* to utilize sucrose as a sole carbon source. Furthermore, the *E. coli* containing the cloned *B. subtilis* levansucrase gene did not secrete levansucrase into the medium (2).

The conjugative plasmid pUR400, which enables *E. coli* K-12 to utilize sucrose as the sole carbon source (19), contains *srcA* and *srcB* genes. The *srcA* gene encodes a sucrose-specific, membrane-bound protein which facilitates sucrose uptake and transport, and the *srcB* gene produces an intracellular sucrose-6-phosphate hydrolase. pUR400 does not produce an extracellular sucrase.

Our results indicate that the inducible sucrase gene(s) and the inducible uptake system are linked on a 10.4-kb *V. alginolyticus* DNA fragment. This fragment will enable us to define and characterize the *V. alginolyticus* sucrase production and sucrose transport systems, as well as the mechanism of sucrase secretion in *E. coli*.

ACKNOWLEDGMENT

We thank Simon Silver for suggestions regarding the sucrose uptake studies and for constructive criticism of the manuscript.

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