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Isolation and Characterization of an *Escherichia coli* Mutant Lacking the Major Serine Transporter, and Cloning of a Serine Transporter Gene[1]

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L-Serine as well as L-valine inhibits the growth of *Escherichia coli* cells, and L-isoleucine releases this growth inhibition. We isolated an *E. coli* mutant (designated as WAT9) that was able to grow on lactate (or glucose) as a carbon source even in the presence of L-serine, the parent not being able to. Cells of WAT9 were not able to grow on L-serine as a carbon source even if L-isoleucine was present in the culture medium, while the parental cells grew. This mutant was shown to lack the principal L-serine transporter in *E. coli*, the Na^+/serine symporter. This mutant is useful for analysis of the role(s) of the Na^+/serine symporter in cell physiology and as a host for the cloning of L-serine transporter gene(s). In fact, we cloned a gene encoding a serine transporter from chromosomal DNA of *E. coli* using WAT9 as the host. The gene enabled the mutant cells to grow on L-serine. Transport activity for L-serine was restored in the mutant cells harboring a plasmid carrying the gene. We partially sequenced the gene and found that it was the *tdcC* gene. We showed that TdcC is an H^+/serine symporter.

Key words: *Escherichia coli*, gene cloning, serine, *tdcC*, transport mutant.

Cells of *Escherichia coli* possess multiple transport systems for L-serine (hereafter, serine and other amino acids mentioned in this paper are of the L-form) (*1*). Previously, we reported that a major serine transport system in *E. coli*, the serine-threonine system, is a Na^+-coupled symporter (*2*). This system is a constitutive one (*3*). We also reported that another major serine transport system, the serine-specific system, is an H^+-coupled symporter (*1*). Gene expression of this second system is regulated by the leucine-responsive regulatory protein (Lrp) (*4*). The transport activity of this system is low when cells are grown in the absence of leucine, and high when cells are grown in the presence of leucine (*1*). The gene (*sdaC*) encoding this second system has been cloned and sequenced (*4*). One leucine-isoleucine-valine transport system (LIV-1) also transports serine (*5*). Another threonine-serine transport system, TdcC, is not induced when cells are grown under aerobic conditions (*6*). Since there are several transport systems for serine in *E. coli* cells, it is generally difficult to isolate serine transport-defective mutants.

Serine inhibits the growth of wild type *E. coli* cells under certain conditions (*7, 8*). For example, although *E. coli* cells can grow on glucose as a carbon source, the cells are not able to grow if a low concentration of serine (less than 1 mM) is added to the growth medium (*9*). Similar growth inhibition of *E. coli* by valine is a well-known phenomenon (*10*). We found that the growth inhibition by serine is caused by inhibition of homoserine dehydrogenase I, which is involved in the biosynthesis of isoleucine by serine (*9*). Thus, concomitant addition of isoleucine counteracts the growth inhibition by serine (*9*) as well as the inhibition by valine (*11*), although the targets of the inhibition by these amino acids are different. On the other hand, *E. coli* cells are able to grow on serine as a carbon source when a small amount of isoleucine (and glycine and threonine) is added to the culture medium (*12*). These properties could be utilized for the isolation of mutants lacking major serine transport system(s). Furthermore, once such mutants are obtained, they should be useful for analysis of the role(s) of the major serine transporter(s) in cell physiology and for cloning of gene(s) encoding serine transporter(s). Here we report the isolation of such a mutant and the cloning of a gene encoding an H^+/serine symporter.

MATERIALS AND METHODS

Bacterium and Growth—*E. coli* W3133-2 (*13*), a serine-sensitive strain (*10*), was used as the parental strain. Cells were grown in a minimal salt medium (*14*) (Na^+ salts were replaced with K^+ salts) supplemented with either 40 mM potassium lactate, or 40 mM serine, 1 mM glycine, 1 mM isoleucine, and 1 mM threonine (*12*), at 37°C under aerobic conditions. Cell growth was monitored turbidimetrically at 650 nm.

Isolation of Mutants—To isolate mutants lacking major serine transporter(s), we first isolated mutants that were

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resistant to serine. We tried to disrupt the transporter gene(s) by inserting the Mu phage. Cells of *E. coli* W3133-2 were infected with the Mud(Ap^R, *lac*)I phage (*15, 16*), and mutants that grew on a solid medium containing 40 mM lactate and 1 mM serine were isolated. We isolated 25 mutants at this stage. The parental cells did not grow at all on this plate. We measured the serine transport activity in these mutants. Eight of them showed very low serine transport activity (data not shown). We then examined the growth of these 8 mutants on serine as a carbon source (40 mM) plus glycine (1 mM), isoleucine (1 mM), and threonine (1 mM). One of the mutants, designated as WAT9, seemed to be the best one for our purpose, judging from its growth properties and serine transport activity. Afterward, the inserted Mu DNA was removed from the chromosome of WAT9 cells by heat induction (*15, 16*).

Cloning and Sequencing—Chromosomal DNA was prepared from cells of *E. coli* W3133-2 by the method of Berns and Thomas (*17*). The DNA was partially digested with the restriction enzyme, *Sau*3AI, and then fragments of 4 to 10 kbp were separated by sucrose density gradient centrifugation. The DNA fragments were ligated into pBR322 (which had been digested with *Bam*HI and dephosphorylated with bacterial alkaline phosphatase) using T4 DNA ligase. Competent cells of *E. coli* WAT9 were transformed with the ligated hybrid plasmids, and then spread on agar plates containing the minimal medium, 40 mM serine, 1 mM glycine, 1 mM isoleucine, 1 mM threonine, 40 μg/ml ampicillin, and 1.5% agar. The plates were incubated at 37°C for 3 days and then the colonies that formed were picked up. Plasmids were prepared from the transformants, and competent cells of *E. coli* WAT9 were retransformed and spread on the plates again. The plates were incubated at 37°C for 3 days. Plasmids in the retransformants were isolated. We obtained 5 candidate hybrid plasmids. Judging from their restriction patterns, all 5 plasmids seemed to carry a common region of the *E. coli* chromosomal DNA. These 5 plasmids were further characterized.

Although we also tried to clone gene(s) which enable WAT9 cells to grow on serine using pUC18 as the vector, we were unable to obtained any candidate.

The nucleotide sequence was determined by the dideoxy chain termination method (*18*) using a DNA sequencer (Pharmacia Biotech, ALF express).

Sequence data were analyzed with GENETYX sequence analysis software (Software Development). The SwissProt and Genbank databases were screened for sequence similarities.

Transport Assays—For the transport of [^{14}C]serine, cells were grown in the minimal medium supplemented with 40 mM potassium lactate, and transport was measured as described previously (*2*). For measurement of H^+ or Na^+ entry into cells elicited by serine influx, cells were grown in the minimal medium supplemented with 40 mM glycerol. The ion movement was measured with an H^+- or Na^+-electrode as described previously (*1, 2*).

RESULTS

Isolation of a Mutant and Its Growth Properties—To isolate a mutant with a defective serine transporter, we first isolated a serine-resistant mutant. The rationale is that serine transporter-defective cells should survive in the presence of serine because enough serine for inhibition of homoserine dehydrogenase I can not get into cells. Figure 1 (A and B) shows the effects of serine (1 mM) on growth of the parental cells (W3133-2) and mutant cells (WAT9). In this experiment, cells were shaken in the minimal medium supplemented with 40 mM potassium lactate as a carbon source. The parental cells grew well in the absence of serine, but not in its presence. On the other hand, cells of WAT9 grew fairly well even in the presence of serine. Similar growth was observed when glucose, instead of lactate, was added as a carbon source (data not shown). These observations are consistent with our anticipation that the major constitutive serine transporter is defective in WAT9 cells. It is highly likely that a sufficient amount of serine was not taken up by the mutant cells because of the lack of the major constitutive serine transporter, so sufficient inhibition of homoserine dehydrogenase I by serine did not take place in the mutant.

If the major constitutive serine transporter is defective in mutant WAT9, cells of WAT9 will not grow on serine as a carbon source even in the presence of 1 mM isoleucine (and glycine and threonine). In fact, WAT9 cells did not grow under such conditions, although the parental cells grew well (Fig. 1C). It seems that the mutant cells were unable to take up enough serine to support cell growth because the major uptake system was lost. The mutant cells, however, grew fairly well on serine as a carbon source when leucine (1 mM) in addition to isoleucine (and glycine and threonine) (1 mM), was added to the culture medium (data not shown). This indicates that the second serine transporter in *E. coli*, the H^+/serine symporter, which is induced by leucine (*1*), is normal in WAT9 cells.

Identification of the Defective Serine Transporter in the Mutant—Threonine as well as serine is a good substrate for the Na^+/serine symport system (*2, 3*). The high serine transport activity in the parental W3133-2 cells was strongly inhibited by excess (50-fold) threonine (Fig. 2A). The WAT9 cells, on the other hand, showed very low serine transport activity, and the low activity was not significantly affected by excess threonine (Fig. 2B). This is convincing evidence that the WAT9 cells lacked the major serine-

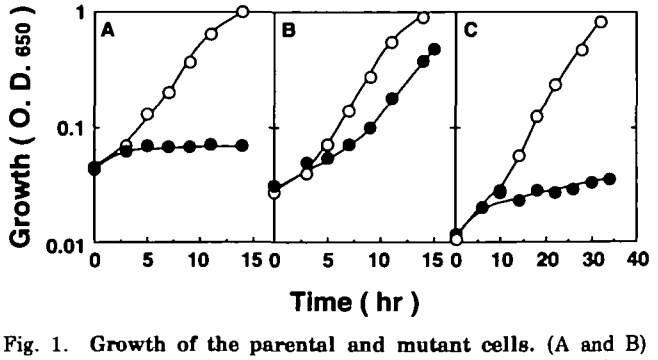

Fig. 1. **Growth of the parental and mutant cells.** (A and B) Effects of serine on the growth of parental cells, W3133-2 (A), or mutant cells, WAT9 (B). Cells were shaken in the minimal medium supplemented with 40 mM potassium lactate in either the absence (○) or presence (●) of 1 mM serine at 37°C under aerobic conditions. (C) Growth on serine. Cells of W3133-2 (○) or WAT9 (●) were shaken in the minimal medium supplemented with 40 mM serine, 1 mM isoleucine, 1 mM glycine, and 1 mM threonine at 37°C under aerobic conditions. Cell growth was monitored turbidimetrically at 650 nm.

threonine transporter (Na^+/serine symporter). It should be pointed out that the serine transport in the parental cells was greatly stimulated by Na^+ (*2*), but the low serine transport activity in WAT9 cells was not stimulated by Na^+ at all (data not shown). We detected serine transport activity, which was not inhibited by excess threonine, in WAT9 cells grown in the presence of leucine, an inducer of the H^+/serine symporter (data not shown). We also detected H^+ entry into the leucine-induced WAT9 cells elicited by serine influx (data not shown). These results confirm that the second serine transporter, the serine-specific H^+/serine symporter, is normal in WAT9.

To confirm that the WAT9 cells had lost the activity of the Na^+/serine symporter, we measured Na^+ entry into cells elicited by serine influx using an Na^+-electrode as described previously (*2*). The parental *E. coli* W3133-2 cells showed Na^+ entry when serine was added to the cell suspension under anaerobic conditions (Fig. 3). As expected, the serine-induced Na^+ entry was completely absent in cells of WAT9. Similarly, although W3133-2 cells showed

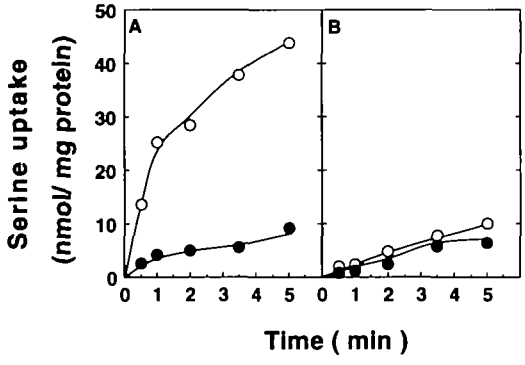

Fig. 2. **Serine transport in the parental and mutant cells.** Cells of the parent, W3133-2, and the mutant, WAT9, were grown in the minimal medium supplemented with 40 mM potassium lactate at 37°C under aerobic conditions. Serine transport in W3133-2 cells (A) or WAT9 cells (B) was measured, at a final concentration of 0.1 mM, in either the absence (○) or presence (●) of a 50-fold molar excess (5 mM) of threonine.

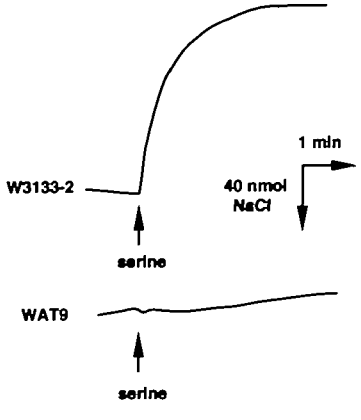

Fig. 3. **Entry of Na^+ into cells elicited by serine influx.** Cells of W3133-2 and WAT9 were grown in the minimal medium supplemented with 40 mM glycerol at 37°C under aerobic conditions. Entry of Na^+ into cells was measured with an Na^+-electrode at 25°C. At the time points indicated by the arrows, serine (final concentration, 1 mM) was added to the cell suspensions under anaerobic conditions. Upward deflection in the chart represents entry of Na^+ into cells.

threonine-induced Na^+ uptake, WAT9 cells did not (data not shown). In a control experiment, we measured Na^+ entry elicited by proline influx into cells mediated by the Na^+/proline symporter (*19*), and observed no difference between the parental cells and the WAT9 cells (data not shown).

Thus, it became clear that mutant WAT9 is defective in the major constitutive serine transporter, the Na^+/serine symporter. Since the second major serine transporter in *E. coli*, the H^+/serine symporter (SdaC), is an inducible system, WAT9 cells grown in the absence of the inducer, leucine, possess only minor transport systems for serine. Mutant WAT9, therefore, must be useful as a host for the cloning of serine transporter gene(s).

Cloning of the Gene Restoring the Growth on Serine, and Serine Transport Activity—Using an *E. coli* mutant,

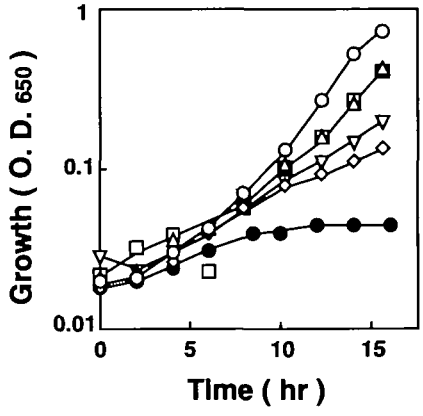

Fig. 4. **Growth of WAT9 cells harboring the plasmids.** Cells of WAT9 (●), WAT9/pSET122 (△), WAT9/pSET1814 (□), WAT9/pSET1815 (▽), WAT9/pSET1817 (○), or WAT9/pSET1821 (◇) were grown in the minimal medium supplemented with 40 mM serine, 1 mM isoleucine, 1 mM glycine, and 1 mM threonine at 37°C under aerobic conditions. Cell growth was monitored turbidimetrically at 650 nm.

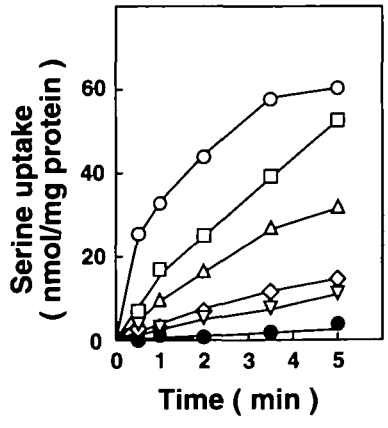

Fig. 5. **Serine transport in cells of WAT9 harboring the plasmids.** Parental cells and mutant cells were grown in the minimal medium supplemented with 40 mM potassium lactate at 37°C under aerobic conditions. The transport of [^{14}C]serine was measured in cells of WAT9/pSET122 (△), WAT9/pSET1814 (□), WAT9/pSET1815 (▽), WAT9/pSET1817 (○), or WAT9/pSET1821 (◇) at 25°C. In one experiment, [^{14}C]serine transport was measured in the presence of a 50-fold molar excess (5 mM) of threonine for WAT9/pSET1817 (●).

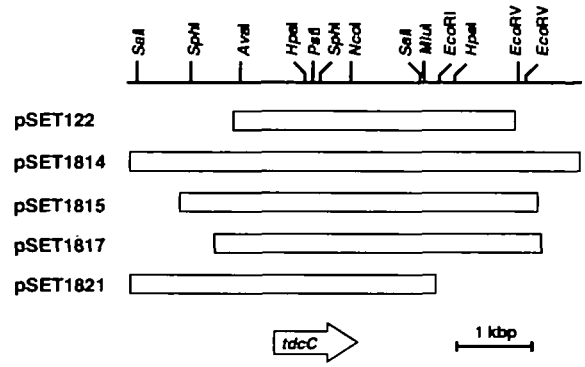

Fig. 6. **Plasmids and restriction maps of the cloned DNA containing the serine transporter gene.** Physical maps of the DNA inserts derived from the *E. coli* chromosome in pSET122, pSET1814, pSET1815, pSET1817, and pSET1821 are shown. The inserts are aligned so that restriction sites are at the same horizontal position in each insert. The location and direction of the *tdcC* gene are shown at the bottom.

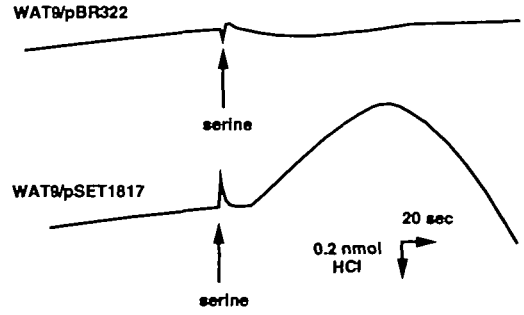

Fig. 7. **Entry of H^+ driven by downhill serine influx into cells.** Cells of WAT9/pBR322 and WAT9/pSET1817 were grown in the minimal medium supplemented with 40 mM glycerol as a carbon source at 37°C under aerobic conditions. Changes in the H^+ concentration in the assay medium were measured using an H^+-electrode at 25°C. At the time point indicated by an arrow, serine was added to the cell suspension to give a final concentration of 0.1 mM under anaerobic conditions. Upward deflection in the chart indicates uptake of H^+ into cells.

WAT9, which cannot grow on serine as the host, we obtained 5 candidate hybrid plasmids (pSET122, pSET1814, pSET1815, pSET1817, and pSET1821) which enabled the host cells to grow on agar plates containing serine as a carbon source. Cells harboring the plasmids showed differences in the sizes of the colonies formed on the plates. We measured the growth rate of each transformant in a liquid medium. WAT9 cells harboring pSET1817 (WAT9/pSET1817) showed the fastest growth in the minimal medium supplemented with 40 mM serine, 1 mM glycine, 1 mM isoleucine, and 1 mM threonine, followed by WAT9/pSET1814 and WAT9/pSET122 (Fig. 4). Cells of WAT9/pSET1815 and WAT9/pSET1821 showed the slowest but significant growth on serine (Fig. 4).

We measured the serine transport activity in these transformed cells. As shown in Fig. 5, cells of WAT9/pSET1817 showed the highest serine transport activity, followed by WAT9/pSET1814 and WAT9/pSET122. Cells of WAT9/pSET1815 and WAT9/pSET1821 showed the lowest but significant activity. Thus, there is a rough correlation between the rate of growth on serine and serine transport activity among the cells harboring these plasmids. The serine transport activity detected in cells of WAT9/pSET1817 was strongly inhibited by the presence of excess (50-fold) threonine in the assay medium (Fig. 5). Similar strong inhibition by threonine was observed for cells of WAT9/pSET1814, WAT9/pSET122, WAT9/pSET1815, and WAT9/pSET1821 (data not shown). Therefore, it seems that threonine is also a substrate for the transporter cloned here. No other amino acid showed significant inhibition of the serine transport when present in a 50-fold excess (data not shown). Thus, the transport system(s) cloned is (are) specific for serine and threonine. This property is the same as that of the Na^+/serine (threonine) system (*2, 3*), and that of the threonine-serine system, TdcC (*6*). The serine transport activity observed in these cells was not stimulated by Na^+ at all (data not shown). Thus, it seems that the transport system(s) cloned here is (are) not the Na^+/serine symporter but the TdcC system or a system so far unidentified.

Identification of the Cloned Gene—To determine whether these plasmids carry a common DNA insert or not, we constructed restriction maps of plasmids pSET122, pSET1814, pSET1815, pSET1817, and pSET1821 (Fig. 6). The maps revealed that a common DNA region is present in all of these plasmids. Furthermore, the maps of the insert DNAs were identical with that of the region including *tdcC* (*20*). Thus, the serine transporter gene carried by these plasmids must be *tdcC*.

To prove this, we partially sequenced the DNA region which seemed to be the coding region for the *tdcC* gene, and the nucleotide sequence determined was exactly the same as that of the *tdcC* gene (*20*).

TdcC Is an H^+/Serine Symporter—The serine transport activity observed in cells of WAT9/pSET1817 was completely inhibited by an H^+ conductor, carbonyl cyanide *m*-chlorophenylhydrazone, CCCP (data not shown). As described above, the serine transport was not stimulated by Na^+ at all. Datta and collaborators reported that threonine transport *via* the TdcC system was strongly inhibited by CCCP or KCN, but not by monensin, an Na^+/H^+ exchanger (*6*). They suggested that a proton gradient was most likely the driving force for threonine transport *via* the TdcC system (*6*). All of these results suggest that transport *via* the TdcC system proceeds through H^+/serine (or threonine) symport. In fact, we detected H^+ entry into cells of WAT9/pSET1817 (alkalinization of the assay medium) elicited by serine influx (Fig. 7). After a short while, acidification of the medium was observed, perhaps due to the acid production resulting from serine metabolism, as reported previously (*1*). No such H^+ entry was detected for cells of WAT9/pBR322. Thus, it became clear that TdcC is an H^+/serine (threonine) symporter.

DISCUSSION

Isolation of mutant WAT9, which lacks the Na^+/serine symporter, revealed the importance of the Na^+/serine symporter in several aspects of the cell physiology of *E. coli*. The cells of WAT9 were unable to grow on serine as a carbon source. The WAT9 cells were resistant to serine when lactate or glucose was used as a carbon source. The serine transport activity in WAT9 cells was greatly reduced compared with that in the parental cells. These

results clearly show that the Na^+/serine symporter is the sole major constitutive transport system for serine in *E. coli*. Thus, the Na^+/serine symporter has sufficient ability to taking up enough serine to support growth on serine. Furthermore, the serine-sensitivity of *E. coli* cells depends on the Na^+/serine symporter. It should be pointed out, however, that the activity of the H^+/serine symporter (SdaC system) is high enough to support cell growth on serine when the inducer of the system, leucine, is present.

We cloned a gene which enabled WAT9 cells to grow on serine as a carbon source. The gene was *tdcC*. The TdcC transporter was found to be an H^+/serine (threonine) symporter. The activity of the TdcC system in *E. coli* is detectable only when cells are grown in an amino acid-rich medium under anaerobic conditions (*6*). In fact, we detected no H^+/serine symport activity in cells of W3133-2 or WAT9 grown in the minimal medium supplemented with lactate as a carbon source. On the other hand, we observed high activity of the TdcC system in cells of WAT9/pSET1817 grown in the medium with no amino acids under aerobic conditions. Plasmid pSET1817 possesses a promoter for the *tet* gene derived from pBR322 in the upstream region of the *tdcC* gene in the proper direction. Thus, it seems that the *tdcC* gene carried on pSET1817 was expressed from this promoter, and therefore cells of WAT9/pSET1817 showed high serine transport activity.

Although there are several transport systems for serine in *E. coli*, all of the plasmids we obtained only carried the *tdcC* gene, *i.e.* not other serine transporter genes, when pBR322 was used as the vector. We were unable to clone even the *tdcC* gene when pUC18 was used as the vector. Since the copy number of pUC18 is much larger than that of pBR322, it seems that too high production of TdcC is harmful for cells. The gene for the Na^+/serine symporter has not been cloned yet, so we are interested in cloning it. It is likely that since the activities of the Na^+/serine symporter and the SdaC H^+/serine symporter are very high, cloning of their genes into a high copy plasmid and expression at a high level may be harmful for cells. If the activity of the Na^+-coupled symporter or the H^+-coupled symporter is extremely high, proper Na^+ circulation or H^+ circulation in cell membranes would be disturbed. If these ideas are correct, we should use a low copy plasmid as a vector for cloning of the gene encoding the Na^+/serine symporter. Indeed, recently we succeeded in the cloning of the Na^+/serine symporter gene using a low copy plasmid as the vector and WAT9 as the host.

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