Complementation of the protein transport defect of an *Escherichia coli secY* mutant (*secY24*) by *Bacillus subtilis secY* homologue

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Bacillus subtilis SecY homologue shares 41.3% homology with that of *E. coli* and remarkably higher homologous regions (more than 80%) are present in the four cytoplasmic regions [(1990) J. Biochem. 107, 603–607]. Based on the formation of the mature form of OmpA in *E. coli*, we have shown that the protein transport defect of the *E. coli secY* mutant (*secY24*) is complemented by the gene product from the *B. subtilis secY* homologue, which is expressed under the *lac* promoter control. However, *B. subtilis* SecY could not restore growth of the *E. coli* mutant at non-permissive temperature.

Bacillus subtilis; SecY homologue; Functional conservation; Protein export

1. INTRODUCTION

Protein secretion is considered to be complex and requires the function of a number of different gene products [1]. In *Escherichia coli*, several genes whose products are necessary for protein translocation have been defined [2–6]. One of them, the *secY (prlA)* gene product is an essential component of the *E. coli* cytoplasmic membrane with multiple spanning membrane domains [7], and it plays an apparent pivotal role in protein translocation across the cytoplasmic membrane of *E. coli* [2,8]. In order to analyse the protein secretion system in *Bacillus subtilis*, we have identified the *secY* homologue of *B. subtilis* within the promoter-distal portion of the *spc-α* ribosomal protein operon [9]. The SecY homologue had a high primary sequence identity, similar hydropathic profile and similar distribution of charged amino acid residues to *E. coli* SecY and it is suggested that the SecY protein of *B. subtilis* shares a similar function in the translocation of secretory proteins across the cytoplasmic membrane with that of *E. coli*. In this study, to examine this possibility, *B. subtilis* SecY was expressed in the *E. coli secY* mutant. The results suggest that the *B. subtilis* SecY can complement the protein transport defect of the *E. coli secY* mutant (*secY24*) on the basis of the formation of mature OmpA.

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2. MATERIALS AND METHODS

2.1. *Bacterial strains*

The isogenic derivatives of *E. coli* MC4100, strains KI297 (*secY24*) and KI298 (*secY*$^+$) which carry *lacI*^{q}, *lacZ*$^+$ and *pro*$^+$ were constructed by introducing F′ *lacI*^{q} *lacZ*$^+$*pro*$^+$ factor into IQ85 and IQ86 [2], respectively, and served as recipients for complementation analysis. The mutant (*secY24*) is temperature-sensitive. On minimal agar plates, it can grow below 40°C but not at 42°C. The pulse-labeling analysis indicates that the mutant (*secY24*) has a defect in the conversion of precursors of periplasmic and outer membrane proteins to mature forms at 42°C; precursors were located inside the cell [2].

2.2. *Plasmid construction*

The expression vector, pNO1575 [8], was a derivative of pBR322 containing a 425 bp *Hae*II fragment of pUC9, which included the *lac* promoter and the multiple cloning site. pKY3 was a derivative of pNO1575 carrying the *E. coli secY* gene and most of the X reading frame. The *E. coli secY* in pKY3 is transcribed from the *lac* promoter [2]. For the controlled expression of the *B. subtilis secY* in *E. coli*, a series of pKY3-based plasmids, pTUE804, pTUE806 and pTUE808 were constructed as illustrated in Fig. 1. A 3.5 kb *Hin*dIII fragment of pTUE801 [9], in which five open reading frames for L30, L15, SecY, Adk and Map of *B. subtilis* were included, was inserted into the *Hin*dIII site of pNO1575 which is located just downstream of the *lac* promoter. The plasmid constructed above was designated as pTUE804. For constructing pTUE808, pTUE804 was digested with *Eco*RV and *Sac*I and religated after the treatment with T4 DNA polymerase. All the SecY coding sequences and the N-terminal region of adenylate kinase were deleted in pTUE808. pTUE806 was constructed by inserting a 1.5 kb *Dra*I-*Sac*I fragment of pTUE804 into the *Sma*I-*Sac*I site of pNO1575. The 1.5 kb *Dra*I-*Sac*I fragment contains the entire *secY* gene including its ribosome binding site, as well as the 5′ end of *adk*, encoding its NH_2-terminal 57 amino acids.

2.3. *Media, labeling, immunoprecipitation and electrophoresis*

Cells were grown in medium E [10] supplemented with thiamine (2 μg/ml), and 18 amino acids (20 μg/ml), excluding methionine and cysteine. 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and am-

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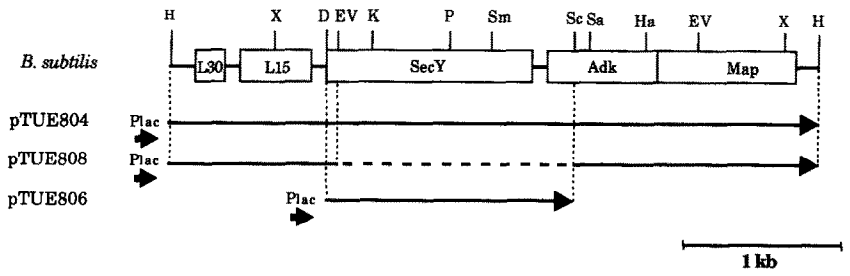

Fig. 1. Gene alignment around *secY* in the promoter-distal portion of the *B. subtilis spc* operon and the plasmids used for the complementation analysis. The five open reading frames are represented by boxes labeled with gene products. The DNA regions of the chromosome carried in each plasmid are indicated by horizontal arrows (——→). The broken line represents the deleted region. The directions of transcription initiated by the *lac* promoter are indicated by small arrows (➡). The positions of restriction sites are indicated for *Dra*I (D), *Eco*RV (EV), *Hin*dIII (H), *Hae*III (Ha), *Kpn*I (K), *Pst*I (P), *Sma*I (Sm), *Sac*I (Sc), *Sal*I (Sa) and *Xba*I (X).

picillin (50 μg/ml) were added as required. For pulse-chase experiments, cells were first grown at 30°C and, when cultures reached a reading of 20–30 in a Klett colorimeter, they were shifted to 42°C. After 120 min, synthesis and formation of the precursor and mature forms of OmpA were examined by pulse-labeling the cells with 20 μCi/ml of [^{35}S]methionine (Amersham Int. PLC) for 30 s followed by chase with 200 μg/ml of unlabeled L-methionine. The labeled culture was processed as described previously [8] for immunoprecipitation of OmpA and SDS-polyacrylamide gel electrophoresis. Protein bands on the autoradiographs were quantified densitometrically with a Molecular Dynamics 300SXE Scanning Imager.

2.4. *Northern blotting analysis*

For Northern hybridization, *E. coli* KI297 cells transformed with appropriate plasmids were grown to mid-log phase in YT medium containing 50 μg/ml of ampicillin and 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Total RNA was extracted by the method of Summers [11] using diethylpyrocarbonate, electrophoresed on a 1% agarose gel containing 6% (v/v) formaldehyde [12], and transferred onto Gene Screen Plus membrane as described by Shilo and Weinberg [13]. A ^{32}P-labeled 1.0 kb *Eco*RV-*Sma*I internal fragment of the *B. subtilis secY* gene was used as the probe.

3. RESULTS AND DISCUSSION

To determine whether the *B. subtilis* SecY can complement *E. coli* SecY, cells of KI297 (*secY24*) were transformed with the plasmids expressing the *B. subtilis secY*$^+$ under the *lac* promoter control. The transformant cells were subjected to pulse-chase with [^{35}S]methionine, immunoprecipitation and SDS-polyacrylamide gel electrophoresis. The autoradiogram in Fig. 2 indicates that the precursor of OmpA protein was completely processed to the mature form within 4 min in the *secY24* mutant carrying pTUE804 (lanes 1–4]. In contrast, processing was not observed in the mutant carrying either pTUE808 (lanes 5–8), or pNO1575 (lanes 17–20). Densitometric scanning of the autoradiogram showed that almost all the OmpA precursor in the pTUE808-transformant remained after a 4 min chase. Furthermore, processing of the precursor to the mature forms of OmpA in the *secY24* mutant harboring pTUE806 (Fig. 2, lanes 9–12) was almost as rapid as that in the mutant harboring pKY3 (Fig. 2, lanes 13–16). Fig. 3 shows the results of quantitation of the autoradiogram bands by densitometric scanning. In the pKY3- and pTUE806-transformants, 40–50% of the total OmpA has already been processed to the mature form, and hence exported to the periplasm, even at 0 min. These results indicate that the *B. subtilis* SecY was able to promote translocation of OmpA that had otherwise been retarded due to the *secY24* mutation, and that *B. subtilis* SecY may replace *E. coli* SecY in *E. coli* cells. Although virtually all OmpA in the pTUE806-transformant was processed to the mature form within 1 min, about 50% remained unprocessed in the pTUE804-transformant. This difference between the two plasmids could be due to a difference in the translational level of SecY protein from the *secY* mRNA, since the length of the flanking region around the *secY* genes is different in the two plasmids (Fig. 1).

To confirm that the *B. subtilis secY* gene clones were actually transcribed in *E. coli* cells, total RNAs were extracted from cells in the mid-log phase of growth and the transcripts were analysed by Northern hybridization

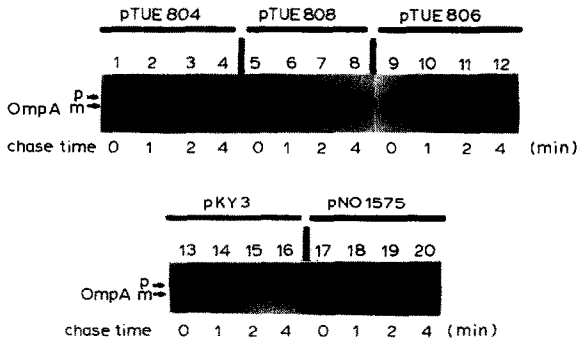

Fig. 2. Autoradiograms of SDS-polyacrylamide gels resolving immunoprecipitates from *E. coli* mutant (*secY24*) harboring recombinant plasmids. *E. coli secY24* mutant harboring pTUE804 (lanes 1–4), pTUE808 (lanes 5–8), pTUE806 (lanes 9–12), pKY3 (encoding the intact *E. coli* SecY, lanes 13–16) or pNO1575 (lanes 17–20) was pulse-labeled with [^{35}S]methionine and chased with non-radioactive methionine for 0 min (lanes 1, 5, 9, 13 and 17), 1 min (lanes 2, 6, 10, 14 and 18), 2 min (lanes 3, 7, 11, 15 and 19), and 4 min (lanes 4, 8, 12, 16 and 20). Samples were immunoprecipitated with antiserum against OmpA. p = precursor of OmpA protein; m = mature form of OmpA.

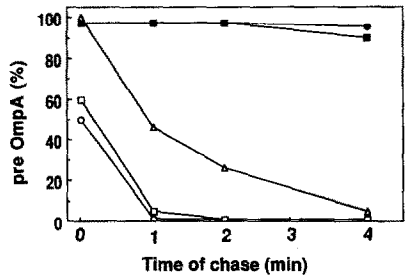

Fig. 3. Kinetics of the processing of precursor of OmpA protein in transformants of *E. coli secY24* mutant. Autoradiograms obtained in Fig. 2 were quantitated densitometrically. The percentage of the precursor OmpA observed in each lane was plotted. (Δ—Δ) pTUE804-transformant; (□—□) pTUE806-transformant; (■—■) pTUE808-transformant; (○—○) pKY3-transformant; (●—●) pNO1575-transformant.

using the ^{32}P-labeled *B. subtilis secY* fragment (the 1.0 kb *Eco*RV-*Sma*I fragment) as the probe. As shown in Fig. 4, positively hybridizing bands were detected in cells harboring pTUE804 and pTUE806 but not pTUE808. The size of the mRNA from pTUE804 and pTUE806 was 3.7 knt and 1.7 knt, respectively, as predicted. Therefore the *secY* gene of *B. subtilis* in the recombinant plasmids was transcribed in *E. coli* transformants.

To judge whether the presence of *B. subtilis* SecY could restore the growth of the *secY24* mutant, transformants were grown on polypeptone agar plates at permissive and non-permissive temperatures in the presence of 1 mM IPTG (Table I). Small colonies of the pTUE804- and pTUE806-transformants were observed at the non-permissive temperature after 24 h incubation. However, they disappeared during prolonged incubation (48 h). Furthermore, the presence of the *B. subtilis secY* gene in the *SecY*$^+$ *E. coli* strain (KI298) inhibited cell growth at 42°C, and KI298 containing pTUE804 and pTUE806 showed 'ghost like' colonies such as those of the *secY24* mutant after 48 h incubation at 42°C. These results suggest that accumulation of *B. subtilis* SecY causes some perturbation of physiological functions in *E. coli* cells.

Suh et al. [14] reported that *B. subtilis* SecY could not complement the protein export defect in the *E. coli secY24* mutant (IQ85) when the *B. subtilis secY* gene region was placed under the control of the *tac* promoter in an expression vector. Precursors of OmpA and maltose binding protein were not processed in the presence of *B. subtilis* SecY. Although the reason for the discrepancy between the results of ours and Suh et al. is not known, the following may be considered. Since the *secY24* mutant strain IQ85 carries Δ*lacI* and since the *tac* promoter is stronger than the *lac* promoter, the *B. subtilis secY* gene in IQ85 might have been expressed at a higher level, leading to a higher accumulation of *B. subtilis* SecY in the *E. coli* cells. Such continuous overproduction of *B. subtilis* SecY may cause

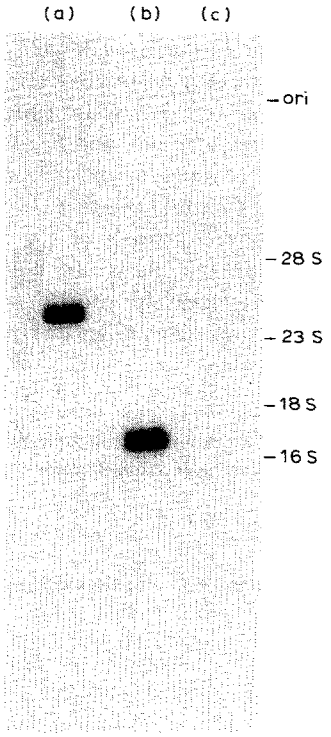

Fig. 4. RNA blot analysis of the transcripts from the plasmid-borne *B. subtilis secY* genes expressed in the *E. coli secY24* mutant. Total RNAs were extracted from *E. coli secY24* harboring pTUE804 (a), pTUE806 (b), or pTUE808 (c). Ten μg of each total RNA was electrophoresed and blotted onto a Gene Screen Plus hybridization membrane. Thereafter *secY* mRNA was analysed by the DNA-RNA hybridization, using ^{32}P-labeled *B. subtilis secY* gene as a probe.

physiological derangement of the *E. coli* cells and *B. subtilis* SecY would not be able to function in promoting export of the OmpA and maltose binding protein. Even protein export in the wild-type cells (IQ86) can be inhibited by the expression of *B. subtilis* SecY [14] and the growth of *E. coli* KI298 is inhibited by pTUE804 and pTUE806 (our present results).

Table I

Effect of the expression of the *B. subtilis secY* gene on the growth of the *E. coli* wild-type (KI298) and mutant (KI297) cells

		30°C		42°C	
Strain (genotype)	plasmids carried	24 h	48 h	24 h	48 h
E. coli KI297 (*secY24*)	pTUE804	+ + +	−	+	−
	pTUE806	+ + +	−	+	−
	pKY3	+ + +	+ + +	+ + +	+ + +
E. coli KI298 (*secY*$^+$)	pTUE804	+ + +	−	+	−
	pTUE806	+ + +	−	+	−
	pKY3	+ + +	+ + +	+ + +	+ + +

E. coli transformants carrying each plasmid were incubated on polypeptone agar plates supplemented with IPTG. Duplicates were placed at the permissive (30°C) and non-permissive (42°C) temperature, respectively. After 24 h and 48 h, the growth was scored: + + +, normal growth compared to that of recipient wild-type; +, weak growth; −, 'ghost like' colonies.

We have shown that *B. subtilis* SecY complemented the OmpA export in *E. coli secY24* cells but could not restore the growth at non-permissive temperature. The former finding suggested that some functions of SecY protein are conserved in *E. coli* and *B. subtilis*. The latter result, on the other hand, suggests that either the expression level of SecY should precisely be tuned in order to sustain the balanced growth or the SecY proteins from the two organisms have some important difference in substrate specificity, coordination with other cellular components, or some other unknown aspects of the their functions.

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REFERENCES

[1] Riggs, P.D., Derman, A.I. and Beckwith, J. (1988) Genetics 118, 571-579.
[2] Shiba, K., Ito, K., Yura, T. and Cerretti, D.P. (1984) EMBO J. 3, 631-635.
[3] Bankatis, V. and Bassford, P. (1985) J. Bacteriol. 161, 169-178.
[4] Gardel, C., Benson, S., Hunt, J., Michaelis, S. and Beckwith, J. (1987) J. Bacteriol. 169, 1286-1290.
[5] Kumamoto, C. and Beckwith, J. (1985) J. Bacteriol. 163, 267-274.
[6] Oliver, D. and Beckwith, J. (1981) Cell 25, 765-772.
[7] Akiyama, Y. and Ito, K. (1987) EMBO J. 6, 3465-3470.
[8] Ito, K., Wittekind, M., Nomura, M., Shiba, K., Yura, T., Miura, A. and Nashimoto, H. (1983) Cell 32, 789-797.
[9] Nakamura, K., Nakamura, A., Takamatsu, H., Yoshikawa, H. and Yamane, K. (1990) J. Biochem. 107, 603-607.
[10] Vogel, H.J. and Bonner, D.M. (1956) J. Biol. Chem. 218, 97-106.
[11] Summers, W.C. (1970) Anal. Biochem. 33, 459-463.
[12] Maniatis, T., Fritsh, E.F. and Sambrook, J. (1982) Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[13] Shilo, B. and Weinberg, R.A. (1981) Proc. Natl. Acad. USA 78, 6789-6792.
[14] Suh, J.-W., Boylan, S.A., Thomas, S.M., Dolan, K.M., Oliver, Oliver, D.B. and Price, C.W. (1990) Mol. Microbiol. 4, 305-314.