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Functional Identification of the Product of the *Bacillus subtilis* *yvaL* Gene as a SecG Homologue

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Protein export in *Escherichia coli* is mediated by translocase, a multisubunit membrane protein complex with SecA as the peripheral subunit and the SecY, SecE, and SecG proteins as the integral membrane domain. In the gram-positive bacterium *Bacillus subtilis*, SecA, SecY, and SecE have been identified through genetic analysis. Sequence comparison of the *Bacillus* chromosome identified a potential homologue of SecG, termed *YvaL*. A chromosomal disruption of the *yvaL* gene results in mild cold sensitivity and causes a β -lactamase secretion defect. The cold sensitivity is exacerbated by overexpression of the secretory protein α -amylase, whereas growth and β -lactamase secretion are restored by coexpression of *yvaL* or the *E. coli* *secG* gene. These results indicate that the *yvaL* gene codes for a protein that is functionally homologous to SecG.

Bacillus subtilis, a gram-positive bacterium, has arisen next to *Escherichia coli* as a paradigm for studies on protein secretion primarily because bacilli have a high capacity for the production of exoenzymes. Protein secretion across the cytoplasmic membrane of *B. subtilis* is thought to be catalyzed by a system that is homologous to the precursor protein translocase of *E. coli* (22, 34). In *E. coli*, precursor protein translocation is mediated by a cytosolic chaperone, SecB; the translocation ATPase, SecA; and a large integral membrane protein complex with SecY, SecE, and SecG (9). SecD and SecF are accessory subunits that are not essential for translocation but that add to the fidelity and, possibly, the specificity of the reaction. Only SecA, SecE, and SecY are essential for viability, and homologues have been identified genetically in *B. subtilis*. SecA is encoded by the *divA* gene (1, 24) and was originally found in a set of mutants conditionally defective in division or unable to sporulate. The integral membrane proteins SecY (5, 26, 30) and SecE (11) were identified after nucleotide sequence analysis of the chromosomal regions that contain the ribosomal *spc* operon and *nusG*, respectively. The analogous regions in *E. coli* contain *secY* and *secE*, respectively. Complete sequence analysis of the *B. subtilis* chromosome has also revealed the presence of a single protein that is homologous to both SecD and SecF (3), but a homologue of the SecB protein has not been found (17).

In addition to the high similarity of the precursor protein translocases of *B. subtilis* and *E. coli*, some marked differences have been noted. *B. subtilis* contains not one but multiple signal peptidases with different specificity towards various secretory proteins (2). PrsA, a protein that is membrane bound through the presence of an amino-terminal fatty acyl anchor and is itself a secretory protein, has a profound effect on the secretion of some proteins in *B. subtilis* but is absent in *E. coli*

(15, 16). PrsA is thought to function as a peptidyl-prolyl isomerase, but this activity has not yet been demonstrated in vitro. Another point of interest is the degree of host specificity of the components of the secretory apparatus. *E. coli* SecA cannot complement a *B. subtilis* *divA* mutant (28), whereas *B. subtilis* SecA can complement conditionally lethal *secA* mutations but only under specific sets of conditions (2, 13, 18, 28). Also, the *E. coli* and *B. subtilis* SecY proteins do not appear to be exchangeable (26).

The purification of the *E. coli* precursor protein translocase (6) has, in addition to SecY and SecE, given rise to the copurification of a protein termed band 1 (8). This protein is identical to P12, which was identified as a proteinaceous factor that stimulates SecYE-mediated protein translocation in vitro (19). The gene for P12 has been cloned via reverse genetics, and its chromosomal inactivation renders some *E. coli* strains cold sensitive for growth (20). Suppressor mutations have been found that are linked to this gene and that rescue cells from the toxic effects of the expression of heterologous mammalian secretory proteins (4). Based on these observations, the gene coding for P12 or band 1 was termed *secG*. SecG is the third, but nonessential, component of the heterotrimeric integral membrane domain of the precursor protein translocase. SecD and SecF can functionally replace SecG (10). SecG harbors two transmembrane segments that are thought to reverse their topology when SecA initiates translocation at the expense of ATP (21). SecG has been proposed to facilitate the membrane insertion of SecA, more or less acting as grease, which might explain why *secG* is not an essential gene under all conditions.

Homologues of *secG* have been found in other gram-negative bacteria, but none have been demonstrated in gram-positive bacteria. Since *secG* codes for a nonessential component of the precursor protein translocase, its genetic identification is complicated. Recently, the sequencing of the *B. subtilis* chromosome has been completed (17). We now report on the identification of an open reading frame, *yvaL*, that bears significant sequence similarity to the *E. coli* *secG* gene. Our data demonstrate that *yvaL* codes for a protein that is functionally homologous to SecG.

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TABLE 1. Plasmids used in this study

Plasmid	Replicon(s)	Resistance(s)	Relevant expression
pDELG2	ColE1	Amp ^r Cam ^r	— ^a
pPR111	ColE1, <i>repR</i>	Amp ^r Phle ^r	—
pET470	ColE1, <i>repR</i>	Amp ^r Phle ^r	<i>E. coli</i> SecG
pET471	ColE1, <i>repR</i>	Amp ^r Phle ^r	<i>B. subtilis</i> YvaL
pET468	<i>repA</i>	Ery ^r	AmyQ
pET472	<i>repA</i>	Ery ^r	AmyQ, <i>E. coli</i> SecG
pET473	<i>repA</i>	Ery ^r	AmyQ, <i>B. subtilis</i> YvaL
pET304	ColE1	Amp ^r	<i>E. coli</i> SecG
pET820	ColE1	Amp ^r	<i>B. subtilis</i> YvaL

^a —, deletion vector.

MATERIALS AND METHODS

Bacterial strains and growth media. Strains were grown in Luria-Bertani broth or on Luria-Bertani agar. When necessary, the medium was supplemented with relevant antibiotics as indicated. Construction of vectors was done with *E. coli* DH5 α [*supE44* Δ *lacU169* (ϕ 80*lacZ* Δ M15) *hsdR17* *recA1* *endA1* *gyrA96* *thi-1* *relA1*]). Chromosomal deletions and growth experiments were done with *B. subtilis* DB104 (*nprE18 aprE* Δ 3) (35) or *E. coli* KN370 (20).

Construction of plasmids. All of the relevant plasmids are listed in Table 1. The *E. coli* *secG* and *B. subtilis* *yvaL* genes, including suitable ribosome binding sites, were amplified as *Bam*HI-*Xba*I cassettes by PCR from chromosomal DNA of strains DH5 α and DB104, respectively, and cloned into pBluescript SK+ by using the primers listed in Table 2. The sequences of both open reading frames were determined and compared against the relevant databases. For expression in *E. coli*, the genes were cloned into pET324 (31), yielding pET304 (*E. coli* *secG*) and pET820 (*B. subtilis* *yvaL*).

Vectors pPR111, a pUB110 derivative (7), and pBEY13 a gift from R. Breitling (4a), are shuttle vectors using a ColE1 origin for replication in *E. coli* and *repR* for replication in gram-positive organisms. These plasmids encode ampicillin resistance (Amp^r) markers for *E. coli* and phleomycin resistance (Phle^r) markers for *B. subtilis*. Vector pBEY13 expresses the *B. subtilis* *secY* and *secE* genes from the constitutive staphylococcal *sak* promoter. Plasmids pET470 and pET471 were formed by replacing the *secYE* cassette with *E. coli* *secG* and *B. subtilis* *yvaL*, respectively.

Vector pAMP21 is a pGK13 (14)-based broad-host-range vector containing the *p32* promoter derived from *Lactococcus lactis* (32) with a synthetic ribosome binding site and an *Nco*I site overlapping the start codon. The *B. amyloliquefaciens* α -amylase gene was isolated by PCR from plasmid pKTH10 (23) as an *Nco*I-*Bam*HI cassette and ligated into *Nco*I-*Bam*HI-digested pAMP21. The resulting vector, named pET468, harbors the *amyQ* gene under the control of the constitutive *p32* promoter. Vectors pET472 and pET473 were generated by ligating the *secG* and *yvaL* gene-containing *Bam*HI-*Bss*HIII fragments, respectively, from the pBluescript derivatives into *Bam*HI-*Mlu*I-digested pET468. The resulting vectors express *B. amyloliquefaciens* α -amylase and *secG* or *yvaL* as a tandem operon from the single *p32* promoter.

Disruption of the *yvaL* gene. The *yvaL* gene was disrupted in *B. subtilis* DB104 as follows. Regions immediately upstream and downstream of *yvaL* were amplified from chromosomal DNA from strain DB104 as *Bam*HI-*Xba*I and *Kpn*I-*Hinc*II cassettes, respectively, and cloned into pBluescript SK+. Subsequently, a *Bgl*II-*Pvu*II digested chloramphenicol resistance (Cam^r) marker was placed between the *Bam*HI and *Hinc*II sites, yielding pDELG2. This vector contains the DB104 chromosomal region with the *yvaL* gene replaced with the Cam^r marker. Vector pDELG2 was linearized with *Pvu*II to yield a 2.8-kb fragment containing the *yvaL::cam* region and subsequently transformed into *B. subtilis* DB104 by natural competence (36). Cam^r colonies resulting from a double crossover were selected. The correct position of the chromosomal replacement was confirmed by PCR. In the resulting strain, DB104 Δ *yvaL*, the Cam^r-encoding gene replaced the *yvaL* gene while leaving the flanking regions intact. Since the mutations cause a complete deletion, no selective pressure is needed after the initial selection.

Growth experiments. *B. subtilis* DB104 and DB104 Δ *yvaL* were transformed with each of six plasmids constructed for testing, i.e., pPR111, pET470, pET471, pET468, pET472, and pET473. After transformation, plates were incubated at 30°C overnight. Selective pressure using the appropriate antibiotics was applied from this point onward. No chloramphenicol was used at this stage. A single colony was picked for each transformant and cultured overnight at 30°C in liquid medium. Subsequently, cells were streaked on plates and incubated at temperatures ranging from 15 to 30°C. Plates were inspected daily, and the occurrence and size of the colonies were noted and scored when the wild-type strain reached a diameter of several millimeters.

For expression in *E. coli*, plasmids pET304 and pET820 were transformed to *E. coli* KN370 (*secG::kan*) as described before (20) and assayed for the formation of single colonies on agar plates at either 20 or 37°C, with or without induction by 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG).

Vesicle preparation and Western blotting. Overnight cultures of *E. coli* KN370 transformed with pET304 or pET820 were diluted 1:50 into fresh medium and grown to an optical density at 600 nm (OD₆₀₀) of 0.6, at which point 1 mM IPTG was added and growth was allowed to resume for another 3 h. Cells were harvested by centrifugation, resuspended in TN buffer (25 mM Tris-Cl [pH 7.5], 100 mM NaCl), and subjected to French pressure treatment (three times at 8,000 lb/in²). Cells debris was removed by centrifugation at 10,000 \times g for 10 min, and vesicles were collected by centrifugation at 150,000 \times g for 45 min. Vesicles were resuspended in TN buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using antibodies directed against SecG and YvaL.

Analysis of cellular and secreted proteins. *B. subtilis* DB104 and DB104 Δ *yvaL* were grown overnight at 30°C in liquid medium. The overnight cultures were diluted 1:50 into fresh medium and grown to mid-logarithmic phase at different temperatures. Cultures were cooled on ice and fractionated into cellular and medium fractions by centrifugation. The medium fraction was precipitated with 10% (wt/vol) (final concentration) trichloroacetic acid, washed twice with cold acetone, and analyzed by SDS-PAGE. Cellular pellets were resuspended in sample buffer, sonicated, and analyzed by SDS-PAGE.

β -Lactamase activity was determined in strain DB104 transformed with plasmid pPR111 and in strain DB104 Δ *yvaL* transformed with pPR111, pET470, or pET471. Overnight cultures of transformants were diluted 1:50 into fresh medium, and cells were grown to mid-logarithmic phase at 30°C. Cells were removed by centrifugation, and the culture supernatants were used for determination of β -lactamase activity with nitrocefin (25). Aliquots of 100 μ l of culture supernatant were added to a reaction mixture (100 mM potassium phosphate [pH 7.0], 0.5-mg/ml nitrocefin), which was then incubated at 20°C. The OD₄₈₆ was measured after 5 and 250 min.

Miscellaneous methods. A peptide polyclonal antibody directed against an internal YvaL sequence (³⁹Ala-Glu-Gln-Leu-Phe-Gly-Lys-Gln-Lys-Ala-Arg-Gly-Leu-Asp⁵²) with an amino-terminal Tyr for coupling to keyhole limpet hemocyanin was produced in rabbits in accordance with standard procedures by NEOSYSTEM, Strasbourg, France. A peptide polyclonal antibody directed against the internal SecG sequence ⁸⁹Ala-Pro-Ala-Lys-Thr-Glu-Gln-Thr-Gln-Pro⁹⁸ was produced in rabbits in accordance with standard procedures by Research Genetics, Huntsville, Ala.

The *yvaL* gene was found in the *Subtilist* database by using the Blast search program included in reference 10a; other searches were done by using the Blast server at the National Center for Biotechnology Information (18a). Sequence alignments were done with ClustalX (29).

RESULTS

Identification of a *secG* homologue in *B. subtilis*. The *Subtilist* database of the *B. subtilis* chromosome was scanned with the *E. coli* *secG* gene by using the Blast search program included in reference 10a. This search yielded the *yvaL* gene (accession no. BG14067) as the only likely candidate (Fig. 1), with an E value of 2.4×10^{-8} . The E value of the next best score was 0.65. *YvaL* is a 228-bp gene located at 295° of the genetic map of the *B. subtilis* chromosome (Fig. 2), in a region that bears many

TABLE 2. PCR amplification primers used in this study

Primer	Sequence (restriction enzyme) ^a
<i>B. amyloliquefaciens</i> <i>amyQ</i> forward	<u>CGCCATGGTTCAAAAACGAAAGC</u> (<i>Nco</i> I)
<i>B. amyloliquefaciens</i> <i>amyQ</i> reverse.....	<u>GCGGATCCTTATTTCTGAACATA</u> (<i>Bam</i> HI)
<i>B. subtilis</i> <i>yvaL</i> forward	<u>AAAGGATCCTAGTCTGGAGGTGTATGGGATGC</u> (<i>Bam</i> HI)
<i>B. subtilis</i> <i>yvaL</i> reverse	<u>AAATCTAGATTCTCGAGCCCTATAGGATATAAGCAAGC</u> (<i>Xba</i> I)
<i>E. coli</i> <i>secG</i> forward.....	<u>CCCGATCCGGAGGTTTAATTCATGTATGAAGCTCTTT</u> (<i>Bam</i> HI)
<i>E. coli</i> <i>secG</i> reverse.....	<u>CCCTCTAGACTCGAGTTAGTTCGGGATATCGC</u> (<i>Xba</i> I)

^a Recognition sites of the restriction enzymes used are underlined. Ribosome binding sites and start and stop codons are in boldface.



	tms 1	tms 2	
Bs	76
Ml	77
Mt	77
Cg	77
Ec	TNKG.S.EWENLSAPAKTEQTQPAAPAKP.....	TSDIPN.....	104
Hi	GNVQKGTFFDLSQAAEQVQQQ.AAPAKDNK.....	NSDIPQ.....	104
Ps	AQQLT.QVGLPDPVAVLEVKQKPAADDVPVLEGQKPAAVPADVPQAPEKK		105
Af	GSVVEKSVQTEQSEGGKTTQESGK.....		100

FIG. 1. Multiple-sequence alignment of *secG* genes and potential homologues. Organisms are indicated as follows: Bs, *B. subtilis* (EMBL accession no. E1186051); Ml, *Mycobacterium leprae* (SwissProt accession no. P38388); Mt, *M. tuberculosis* (EMBL accession no. Z95844); Cg, *Corynebacterium glutamicum*. (GenBank accession no. M25819); Ec, *E. coli* (PIR accession no. S40402); Hi, *Haemophilus influenzae* (PIR accession no. H64068); Ps, *Pseudomonas syringae*; (EMBL accession no. U85643); Af, *Aquifex aeolicus* (TREMBLNEW accession no. G2982840). Conserved residues are shaded according to the number of sequences in which the residue is conserved, and potential transmembrane segments (tms) are underlined.

genes whose functions are unknown. *yvaL* seems to be the first gene in an operon, since the upstream gene *yvaM* is transcribed from the opposite strand. Downstream of *yvaL*, five genes can be identified without obvious promoter or terminator sequences in between but followed by a clear terminator structure. *YvaL* codes for an integral membrane protein of 76 amino acids that, in analogy to SecG, is predicted to span the membrane twice. It is 33% identical and 57% similar to *E. coli* SecG. Further searches with the *B. subtilis yvaL* and *E. coli secG* genes using the Blast server at reference 18a revealed the presence of homologues in other gram-positive bacteria. A multiple sequence alignment of these putative SecG proteins is shown in Fig. 1. Overall, the putative SecG homologues of gram-positive bacteria appear to be shorter than their counterparts in gram-negative bacteria. Although the *Mycobacterium leprae secG* gene is indicated in the data banks as such, no

functional evidence is available that this open reading frame is, indeed, functionally homologous to SecG.

Deletion of *yvaL* causes mild cold sensitivity of growth. Disruption of the *secG* gene has been shown to result in a cold-sensitive phenotype of *E. coli* MC4100-derived strains (20) at temperatures of 25°C and below. Assuming that SecG and YvaL function in the same manner, deletion of *yvaL* is expected to render *B. subtilis* cold sensitive as well. Therefore, the *yvaL* gene was deleted completely from the chromosome of *B. subtilis* DB104 by homologous recombination and replaced with a Cam^r marker. The correct position of the chromosomal replacement was confirmed by PCR. The resulting strain, DB104Δ*yvaL*, was normally viable at 37°C when grown on either rich or minimal medium. Incubation below 20°C revealed mild cold sensitivity, and the strain showed progressively slower growth than DB104 (data not shown). The cold-

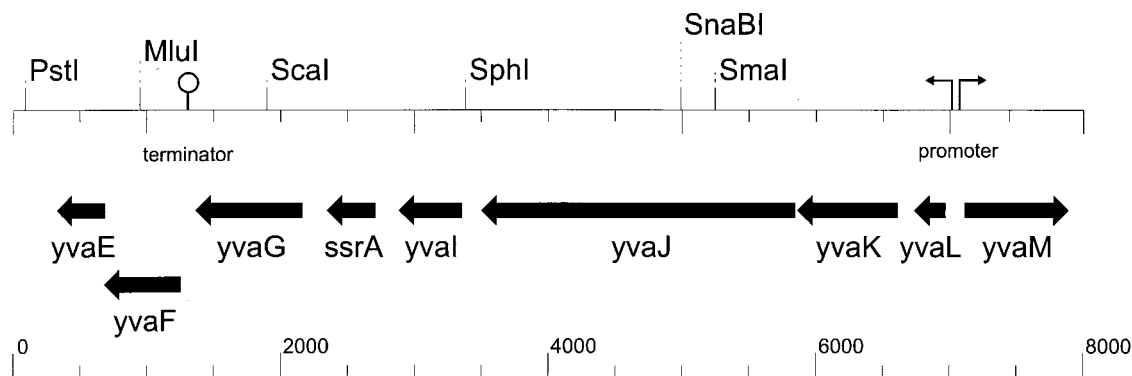


FIG. 2. Analyses of the chromosomal region containing *yvaL*. The sequence runs from bp 3447500 to bp 3455500 of the *B. subtilis* chromosome. Open reading frames are represented by straight arrows and named as in the *Subtilist* database. The promoter of the *yvaL* operon is depicted as a broken arrow, and the terminator is shown as a loop.

TABLE 3. Results of growth experiments

Strain	Expression	Growth ^a at:		
		15°C	20°C	25°C
DB104::111		++	++	++
DB104::470	<i>E. coli</i> SecG	±	±	++
DB104::471	<i>B. subtilis</i> YvaL	++	++	++
$\Delta yvaL$::111		±	±	++
$\Delta yvaL$::470	<i>E. coli</i> SecG	-	-	++
$\Delta yvaL$::471	<i>B. subtilis</i> YvaL	++	++	++
DB104::468	α -Amylase	++	++	++
DB104::472	α -Amylase, <i>E. coli</i> SecG	±	±	++
DB104::473	α -Amylase, <i>B. subtilis</i> YvaL	++	++	++
$\Delta yvaL$::468	α -Amylase	-	-	++
$\Delta yvaL$::472	α -Amylase, <i>E. coli</i> SecG	±	±	±
$\Delta yvaL$::473	α -Amylase, <i>B. subtilis</i> YvaL	±	±	±

^a Growth was scored as follows: ++, growth like that of the reference; ±, growth slower than that of the reference; -, no growth.

sensitive phenotype is not absolute. Compared to that of the wild type, growth was retarded more severely when the temperature was further lowered, but after the cells were shifted again to higher temperatures, growth resumed at a rate comparable to that of the wild type.

To analyze in more detail the phenotype of the deletion strain compared to that of the wild type, cells were transformed with plasmids expressing *E. coli* SecG or *B. subtilis* YvaL, as well as a control plasmid (Table 3). After preincubation at temperatures that do not affect the growth of the deletion strain, cells were plated and incubated at various temperatures. Growth of the colonies was monitored over a period of sev-

eral days. Wild-type cells were not affected, and mutant cells transformed with the control plasmid behaved like their non-transformed counterparts, showing retarded growth but not a complete stop at lower temperatures. Transformation of the deletion strain with pET471 expressing the *yvaL* gene product relieved the retardation of growth, showing that the phenotype of the mutant was caused not by any polar effects but by the deletion of *yvaL* alone. Surprisingly, when the mutant was transformed with pET470 expressing *E. coli* SecG, growth stopped completely at temperatures of 20°C or lower. Also in wild-type cells, expression of *E. coli* SecG caused some interference with growth at low temperatures, possibly due to competition for SecYE with YvaL. These data indicate that disruption of *yvaL* from the *B. subtilis* chromosome causes mild cold sensitivity of growth. However, the effect is much weaker than that reported for *E. coli* KN370 (20).

Cold sensitivity of the growth of a *B. subtilis* $\Delta yvaL$ strain is exacerbated by overexpression of preAmyQ. Since no complete cold sensitivity could be demonstrated for the DB104 $\Delta yvaL$ strain, cells were transformed with high-copy plasmid PET468 and derivatives. These plasmids express the precursor form of α -amylase (preAmyQ) to high levels, thereby invoking secretory stress. Derivatives pET472 and pET473 express preAmyQ in combination with SecG or YvaL, respectively. Expression of preAmyQ did not retard the growth of the deletion mutant at 30°C, the temperature used to preculture the cells. The level of secreted α -amylase was the same for the wild type and the deletion mutant, as judged by halo formation on starch-containing plates and analysis of culture supernatants (data not shown). When pET468 transformants of *B. subtilis* DB104 $\Delta yvaL$ were shifted to lower temperatures, clear and complete cold sensitivity was evident. Already at 20°C, cells stopped growing

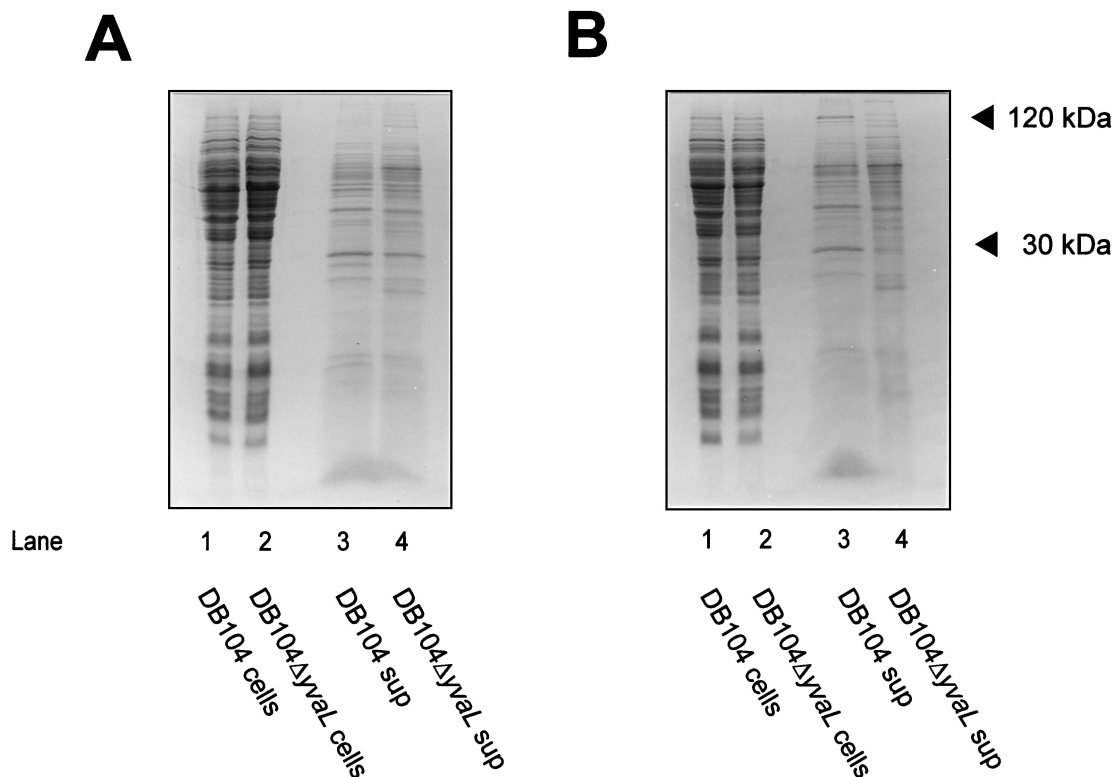


FIG. 3. Coomassie-stained SDS-PAGE of cellular and medium fractions of *B. subtilis* DB104 and DB104 $\Delta yvaL$ cultures grown to mid-logarithmic phase at 37°C (A) or 20°C (B). Positions of 30- and 120-kDa proteins absent in the supernatant of the *yvaL* mutant are indicated.

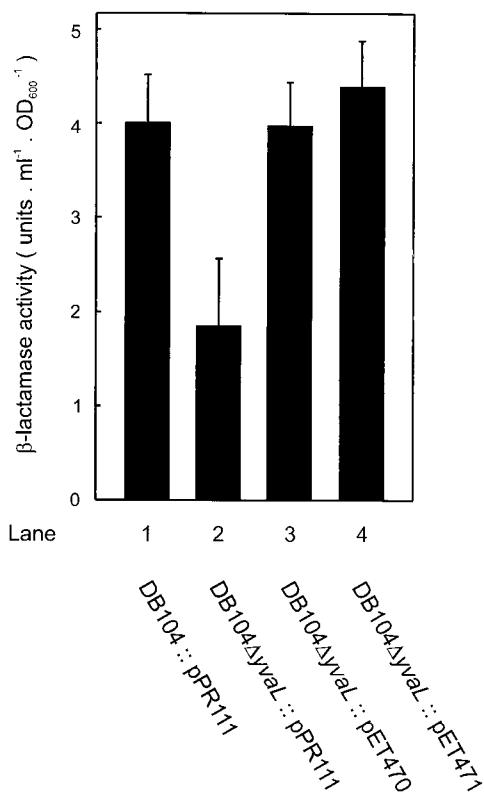


FIG. 4. Restoration of β -lactamase secretion by YvaL and SecG. β -lactamase activities were determined in culture supernatants as described in Materials and Methods. One unit of activity is defined as the amount of enzyme needed to give an increase in OD₆₀₀ of 10^{-3} /min. Means and standard errors from three experiments are shown.

completely (Table 3), and when the bacteria were transferred back to the permissive temperature of 30°C after prolonged incubation at 20°C, growth was not resumed. Apparently, the deletion mutant is capable of sustaining a basic level of secretion even at lower temperatures but cannot handle the over-expression of a secretory protein within a broad temperature range. When coexpressed, both *secG* and *yvaL* complemented the deletion mutant, albeit the growth level of the transformants did not reach that of the wild type. Also, in this case, expression of SecG, but not that of YvaL, interferes with the growth of the wild type in a temperature-dependent way.

Effect of the *yvaL* deletion on the secretion of proteins. To investigate more directly the involvement of the precursor protein translocase in the cold-sensitive phenotype of the $\Delta yvaL$ deletion strain, the polypeptide patterns of wild-type and mutant cells were analyzed. In the culture supernatants of cells grown at 37°C, the yields of secreted proteins appeared generally to be similar for the wild type and mutants (Fig. 3A), although some cell lysis seems to occur in the deletion strain. However, the supernatant of cultures grown at 20°C showed some differences in the polypeptide pattern (Fig. 3B), e.g., at 30 and 120 kDa. Also, some secreted but cell-associated proteins appeared to be absent even at 37°C in the deletion mutant, as judged by proteinase K accessibility (data not shown). Since some, but not all, extracellular proteins are absent in the *yvaL* deletion strain, it seems that YvaL is needed for the secretion of a specific subset of proteins. The size of the 30-kDa protein may correspond to that of mature endogenous β -lactamase. The β -lactamase activity was about two- to three-

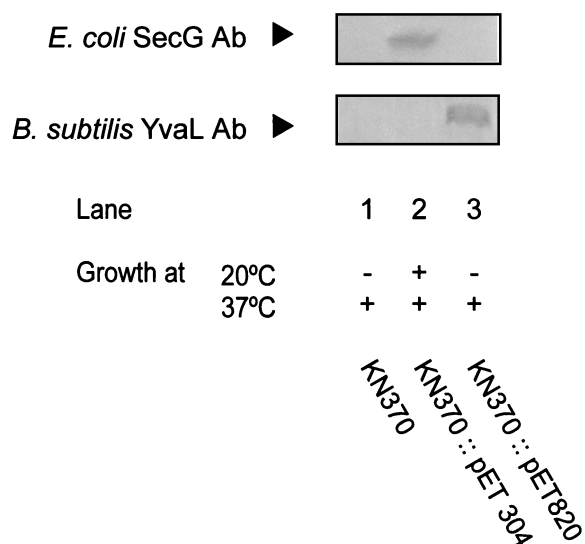


FIG. 5. Complementation of the growth of *E. coli* KN370 by SecG or YvaL. The top panels show immunodetection of SecG or YvaL in *E. coli* KN370 bearing the plasmids indicated and after induction with IPTG. Results of the growth experiments at the indicated temperatures and in the presence of IPTG are listed below. Ab, antibody.

fold lower in the culture supernatant of strain DB104ΔyvaL than in that of strain DB104 (Fig. 4). Interestingly, the β -lactamase activity could be restored to normal levels by the expression of either *B. subtilis* YvaL or *E. coli* SecG. It is important to note that the *E. coli* β -lactamase present as an Amp^r marker on the plasmids used is not expressed in *B. subtilis* (33). The β -lactamase activity of strain DB104 was the same with or without plasmid pPR111. These data strongly suggest that the *B. subtilis yvaL* deletion strain is impaired in the secretion of some proteins.

YvaL does not complement the cold sensitivity of the *E. coli secG* null strain. The *secG* disruption mutant *E. coli* KN370 shows a cold-sensitive phenotype (20). At 37°C, no growth defect is observed, while at 20°C, the strain is no longer able to form single colonies on agar plates. Upon induction with IPTG, plasmid pET304 expressing *E. coli* SecG was able to restore growth at the nonpermissive temperature of 20°C (Fig. 5). However, when *E. coli* KN370 was transformed with pET820 containing *yvaL*, no growth restoration was observed at the nonpermissive temperature, not even when the expression was induced by IPTG. On the other hand, growth was normal at the permissive temperature of 37°C. These data demonstrate that YvaL cannot functionally replace SecG in *E. coli*.

DISCUSSION

To facilitate functional studies on the precursor protein translocase of the gram-positive bacterium *B. subtilis*, we have cloned a homologue of SecG termed YvaL. The gene was identified on the basis of sequence similarity with its gram-negative counterpart. Although the overall identity is low, there is clear similarity between the YvaL and SecG proteins. Both proteins harbor two putative transmembrane segments that are connected via a glycine-rich loop. The YvaL protein is shorter than the SecG protein and lacks the carboxyl-terminal extension. This property is shared with other SecG homologues of gram-positive bacteria present in the databases. Like that of *secG* in *E. coli* (20), disruption of the *yvaL* gene in the

chromosome of *B. subtilis* DB104 results in a cold-sensitive growth defect. However, this effect is mild compared to that in the *E. coli* *secG* null strain but is elevated when secretory stress is imposed by overexpression of the precursor form of α -amylase. The cold sensitivity can be overcome by expression of YvaL or SecG in *trans*, although growth is not restored to the level observed with the wild type only. Despite the care that was taken to disrupt only the *yvaL* open reading frame, the integration of the resistance marker could modulate the expression of the downstream genes and thereby affect physiology. It has been noted that the cold sensitivity of *E. coli* growth is strain dependent (10, 20). The reason for this is not entirely clear, but it may well relate to differences in growth physiology, secretion demand, and/or the level of translocase components in the various strains. Analysis of the profile of secreted proteins in wild-type *B. subtilis* and the $\Delta yvaL$ strain most notably reveals that two major proteins are absent in the latter strain. However, in the culture supernatant containing the secreted proteins, only certain polypeptides are affected. Therefore, it appears that deletion of *yvaL* does not result in a strong pleiotropic secretion defect but rather affects the secretion of a subset of proteins. No direct analyses of total secreted proteins has been performed with the *E. coli* *secG* null strain, but the *in vitro* translocation of the precursors proOmpA and proOmpF-Lpp demonstrates a clear difference in SecG dependence (19). Direct evidence that SecG and YvaL have the same function is provided by the observation that the secretion of β -lactamase in the *B. subtilis* $\Delta yvaL$ strain is restored not only by expression of YvaL but also by that of SecG. On the other hand, YvaL cannot complement the *E. coli* *secG* null strain. In conclusion, our results demonstrate that *B. subtilis* YvaL is a functional homologue of *E. coli* SecG. It is concluded that the heterotrimeric organization of the integral membrane domain of the translocase is conserved well in bacteria.

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