Isolation and Characterization of *kinC*, a Gene That Encodes a Sensor Kinase Homologous to the Sporulation Sensor Kinases KinA and KinB in *Bacillus subtilis*

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Phosphorylation of the transcription factor encoded by spo0A is required for the initiation of sporulation in *Bacillus subtilis*. Production and accumulation of Spo0A~P is controlled by histidine protein kinases and the spo0 gene products. To identify additional genes that might be involved in the initiation of sporulation and production of Spo0A~P, we isolated genes which when present on a multicopy plasmid could suppress the sporulation defect of a spo0K mutant. kinC was one gene isolated in this way. A multicopy plasmid containing kinC completely or partially suppressed the sporulation defect caused by mutations in spo0K, kinA, spo0F, and spo0B, indicating that at least when overexpressed, KinC is capable of stimulating phosphorylation of Spo0A independently of the normal phosphorylation pathway. The predicted product of kinC is 428 amino acids long and is most similar to KinA and KinB, the histidine protein kinases involved in the initiation of sporulation. In otherwise wild-type strains, kinC null mutations caused little or no defect in sporulation under the conditions tested. However, in the absence of a functional phosphorelay (spo0F or spo0B), KinC appears to be the kinase responsible for phosphorylation of the sof-1 and rvtA11 forms of Spo0A.

Cells of the gram-positive soil bacterium *Bacillus subtilis* differentiate to form heat-resistant metabolically dormant spores under appropriate conditions. The initiation of sporulation depends upon activation of the Spo0A transcription factor by phosphorylation (20), and a threshold concentration of Spo0A~P appears to be required (11). Spo0A~P is involved in the transcriptional regulation of many sporulation genes. It directly activates transcription of *spoIIA*, *spoIIE*, and *spoIIG*, which are necessary for sporulation (4, 6, 48, 49, 57, 62). Also, Spo0A~P represses transcription of *abrB* (56), which encodes a repressor of several genes involved in sporulation (44, 55, 59, 64).

Spo0A receives phosphate, albeit indirectly, from KinA (2, 38) and KinB (58), histidine protein kinases that belong to a conserved family of proteins known as sensor kinases. Sensor kinases are generally involved in signal transduction; they autophosphorylate and donate phosphate to particular proteins in response to changes in the environment, modifying the activity of those proteins. The proteins that receive phosphate from sensor kinases belong to a conserved family of proteins known as response regulators, which are often involved in transcriptional regulation. Together, a sensor protein and its cognate response regulator form a two-component system. There are many examples of two-component systems involved in signal transduction in bacteria (1, 37), and similar systems in plants (10) and yeasts (36) have recently been identified.

Although Spo0A belongs to the response regulator family of proteins, it does not normally obtain phosphate directly from a histidine protein kinase. Rather, Spo0A receives phosphate through a multicomponent phosphorelay (9). KinA (and other kinases) first donates phosphate to response regulator Spo0F. The phosphate is then transferred from Spo0F to Spo0B and

finally to Spo0A. A major function of the phosphorelay seems to be to integrate multiple developmental signals that regulate the initiation of sporulation (9, 22, 24–26).

We have identified and characterized a third kinase gene, kinC, that is involved in the phosphorylation of Spo0A. This gene was identified as a multicopy suppressor of a *spo0K* mutant. The *spo0K* operon encodes an oligopeptide permease that is required for efficient initiation of sporulation (39, 43, 45) and the development of genetic competence (45, 46) and appears to affect activation of Spo0A (45). We describe the cloning and characterization of *kinC*, whose predicted product is most similar to the sensor kinases KinA and KinB.

Certain altered function mutations in *spo0A*, e.g., *sof-1* (*sur0F1*), *sur0B20*, and *rvtA11* (21, 29, 51–53), can bypass the need for *spo0F* and *spo0B* in sporulation. Presumably, in the absence of *spo0F* or *spo0B*, these altered forms of Spo0A obtain phosphate directly from some other source, probably one or more histidine protein kinases (26, 53). We show that KinC is one such kinase.

kinC was identified independently by Y. Kobayashi and his colleagues in a screen for mutations that abolished the suppressing activity of *sur0B20* (31).

MATERIALS AND METHODS

Media. Routine growth and maintenance of *Escherichia coli* and *B. subtilis* was done in LB medium (34). The nutrient sporulation medium used was $2\times$ SG medium (32) or DS medium (50). Media in plates were solidified with 15 g of agar (Difco Laboratories) per liter. Sporulation proficiency was visualized on DS or $2\times$ SG plates. Antibiotics were used at the following concentrations: ampicillin at 100 µg/ml, chloramphenicol (Cm) at 5 µg/ml, spectinomycin (Spec) at 100 µg/ml, neomycin at 5 µg/ml, and erythromycin and lincomycin together (MLS) at 0.5 and 12.5 µg/ml, respectively, to select for the *em* gene. Cells were made competent in S7 minimal medium as described previously (33, 45).

Strains and plasmids. Standard *E. coli* strains were used for cloning and maintaining plasmids, as previously described (23, 45). The *B. subtilis* strains used are listed in Table 1, and all are derived from strain 168. The JH642 (42) or PB2 (7, 45) strains were used as the wild type, as indicated. The plasmids used are listed in Table 2, and some are illustrated in Fig. 1 and 2.

The *AspolE*::*spc* allele contains the *spc* cassette from pUS19 (5) inserted into a deletion of *spo0E* from 13 bp upstream of the *spo0E* start codon to the G in the

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JH642 $trpC2 pheA1$ 42 PB2 $trpC2$ 7AG676JH642 $spo0B\Delta Pst pheA^+$ $spon$ JRL237JH642 $pHP13$ pH JRL357JH642 $\Delta spo0K357::neo$ pH JRL358JH642 $\Delta spo0K358::erm$ ($\Delta spo0K::erm$)JRL407PB2 $spo0K::pJL58$ JRL408PB2 Pspac- $spo0K$ JRL417PB2 $\Delta spo0K::erm$ JRL459JRL459PB2 Pspac- $spo0K$ spoIIA+::($spoIIA-lacZ neo$) pJL52 $spon$ JRL530JH642 $spo0A9V$ pLK2 $spon$ JRL550JH642 $spo0A9V$ pLK2 $spon$ JRL550JH642 $spo0A9V$ pLK2 $spon$ JRL555JH642 $\Delta spo0K::erm$ pLK2 $spon$ JRL660JH642 $\Delta kinC::spc$ $spon$ JRL65PB2 $\Delta kinC645::spc$ $\Delta spo0A^+-cat$ JRL763JH642 $spo0B\Delta Pst pheA^+ sof-1-cat$ sof -	$\partial B\Delta$ allele cotransformed with ∂heA^+ (59)
PB2 $trpC2$ 7 AG676 JH642 $spo0B\Delta Pst pheA^+$ $spon JRL237 JH642 pHP13 pH JRL357 JH642 \Delta spo0K357::neo p JRL358 JH642 \Delta spo0K357::neo pH JRL407 PB2 spo0K::pJL58 pH JRL407 PB2 spo0K::pJL58 pH JRL417 PB2 \Delta spo0K spoIIA^+:(spoIIA-lacZ neo) pJL52 spon JRL530 JH642 spo0A9V pLK2 spon JRL532 JH642 spo0A9V pLK2 spon JRL550 JH642 spo0A9V pLK2 spon JRL555 JH642 spo0A9V pHP13 JRL555 JH642 \Delta spo0K::erm pLK2 JRL555 JH642 \Delta spo0K::erm pHP13 JRL555 JH642 \Delta spo0K::erm pLK2 JRL555 JH642 \Delta spo0K::erm pHP13 JRL555 JH642 \Delta spo0K::erm pLK2 JRL645 PB2 \Delta kinC645::spc (\Delta kinC::spc) JRL645 PB2 \Delta kinC645::spc (\Delta kinC::spc) JRL600 JH642 \Delta kinC::spc JH642 \Delta spo0B\Delta Pst pheA^+ sof-1-cat sof- $	$0B\Delta$ allele cotransformed with $bheA^+$ (59)
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JRL552 JH642 spo0J93 pHP13 JRL555 JRL555 JH642 Δspo0K::erm pLK2 JRL555 JH642 Δspo0K::erm pHP13 JRL595 JH642 Δspo0K::erm pHP13 JRL645 PB2 ΔkinC645::spc (ΔkinC::spc) JRL660 JH642 ΔkinC::spc JRL753 JH642 ΔkinC::spc spo0A ⁺ -cat JRL763 JH642 spo0BΔPst pheA ⁺ sof-1-cat	0.0003(43)
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JRL645PB2 $\Delta kinC645$::spc ($\Delta kinC$::spc)JRL645JH642 $\Delta kinC$::spcJRL753JH642 $\Delta kinC$::spc spo0A ⁺ -catJRL763JH642 spo0B Δ Pst pheA ⁺ sof-1-cat	
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JRL000JH042 $\Delta kinC::spc$ $sp00A^+$ -catJRL763JH642 $sp00B\Delta Pst$ $pheA^+$ sof -	
JRL763JH642 sp00B Δ Pst phe A^+ sof-1-catsof-	
tr	-1 (21, 29) \sim 90% linked to <i>cat</i> by
JRL764 JH642 spo0B\DeltaPst pheA ⁺ ryt411-cat ryt4	<i>411</i> (51)
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IRL767 IH642 spoBAPst pheA ⁺ sof-1-cat AkinC::spc	
IRL768 IH642 spoBAPst phe4 ⁺ rvt411-cat AkinC::spc	
IRL770 $H642 \operatorname{spo}BAPst pheA^+ rvtA11-cat kinA::Tn917 kin.$	A::Tn917 (2, 47)
IRL783 IH642 spo0FAS spo0BAPst phe A^+ rvtA11-cat	$00F\Lambda S(29)$
IRL790 JH642 AkinC::spc sof-1-cat	
JRL791 JH642 AkinC::spc rvt411-cat	
JRL792 JH642 $spo0F\Delta S$ $spo0B\Delta Pst$ $pheA^+$ $rvtA11$ -cat $\Delta kinC$::spc	
JRL794 JH642 spo0F Δ S spo0B Δ Pst phe A^+ rvtA11-cat kinA::Tn917	
IRL796 IH642 spopEAS spopEAP phet + pLA^+ pLK2	
IRI 797 IH642 spoRAS spoRAPt $pheA^+$ pHP13	
IRI 812 H642 thrC·(kinC-locZ erm)	
IRL923 IH642 sno0FAS pHP13	
IRL925 H642 spoRFAS pL K2	
IRL 951 PB2 Aspol/K-serm pL K2	
[RI 902 H642 snotF11 n] K2 s	0F11 (35 40 41)
IRL 903 IH642 spolE 11 pHP13	0211 (55, 40, 41)
IRL 905 IH642 spoRAPst $nke4^+$ pL K2	
$\frac{1}{104} \frac{1}{2900} \frac{1}{1042} \frac{1}{2900} \frac{1}{1042} \frac{1}{1042}$	
$\frac{111}{1010} \frac{11042}{1000} \frac{1}{1000} 1$	
$\frac{1}{1011} \qquad \qquad \frac{1042}{1042} \frac{1}{1017} $	
JIL 1124 JIL 4 $M(A_1, \Pi(B^2) / \mu L X)$	
JRC1124 JR042 $\Delta spoot_{A}spoot_{A}spoot_{A}spineA = prr13$ JRC1125 JR042 $\Delta spoot_{A}spineA = spineA^{+} = V2$	
JRC112.) JH042 ΔSp00E::spc sp00F Δsp00bΔrst pneA pLR2	
K1044 JH042 spotE11 kin4::1191/ VI1501 IH642 papelE221 mt 411 setures	
K11321 JT1042 spoor 221 rvtA11-cut::spc SpO	05221 (12)

^a BGSC, Bacillus Genetic Stock Center.

TAG stop codon and was constructed by K. Ireton. The *rvtA11* (51) and *sof-1* (29, 53) alleles of *spo0A* that were used are \sim 90% linked by transformation to *cat* or to *spc* (16, 22, 24), with the *spc* gene inserted at the *Nco*I site of the *cat* gene by using pJL62 (Table 2).

We made a null mutation in *spo0K* by deleting the first four genes of the *spo0K* operon, from *spo0KA* codon 18 (at the *Esp*1 site) to *spo0KD* codon 127 (at the *Bg*/II site) (45), and inserting an *erm* cassette. The plasmid that contains this mutation is pJL50 (Table 2) and has the *cat* gene in the plasmid backbone. This mutation was introduced into the chromosome by transforming wild-type cells with linearized pJL50 and selecting for MLS^r. One of the MLS^r Cm^{*} transformants, resulting from a double crossover, was chosen as the $\Delta spo0K::erm$ mutant. A similar deletion-insertion mutation was made by inserting a *neo* cassette ($\Delta spo0K::neo$) instead of *erm*.

A null mutation in *kinC* was made by deleting the region from 25 bp upstream of the putative *kinC* start codon (the *PstI* site in Fig. 2) to codon 211 (the *ClaI* site in Fig. 2) and inserting the *spc* cassette from pJL74. This mutation, contained on pLK25, was recombined into the chromosome by double crossover by transforming the linearized plasmid into PB2 and selecting for Spec^r and screening for Cm^s.

We constructed a *kinC-lacZ* transcriptional fusion that contains the sequence from ~1.5 kb upstream of the putative *kinC* start codon to codon 211 and introduced it into the *thrC* locus of the chromosome, using *lacZ* fusion vector pDG793 (provided by P. Stragier). The plasmid containing this fusion is pLK114 (Table 2 and Fig. 2). pLK114 was linearized and transformed into wild-type cells selecting for MLS^r. Double-crossover events that resulted in the introduction of the *kinC-lacZ* fusion at *thrC* caused a Thr⁻ phenotype. Making the multicopy plasmid library. Two different multicopy plasmid li-

Making the multicopy plasmid library. Two different multicopy plasmid libraries were made in the *B. subtilis-E. coli* shuttle vector pHP13 (8, 18). pHP13 has two drug markers suitable for selection in *B. subtilis: erm* (which confers MLS^r) and *cat* (which confers Cm^r). In *B. subtilis*, pHP13 has a copy number of approximately five or six per cell (8, 18). Chromosomal DNA from JH642 was partially digested with *Sau3A* and electrophoresed on an agarose gel. DNA was isolated from the size ranges of approximately 2 to 4.5 kb (library A) and approximately 4.5 to 9 kb (library B), on the basis of size markers run in parallel. Size-fractionated DNA was ligated into pHP13 that had been linearized with *Bam*H1 and treated with phosphatase before transformation into *E. coli* selecting for Cm^r. The transformatis were pooled and plasmid DNA was prepared for transformation into *B. subtilis*.

TABLE 2	Plasmids	used	in	this study

Vectors Philoscriptilit KS+ Ap; used for subcloning and sequencing (Stratagene) pAGS8 Ap Cm (28) Ap Cm (28) pHilot Ap TC, Cm, integrative vector (15) Ap TC, integrative vector (3) pUC18:som Ap Neo, source of <i>no</i> cassette (3) Ap Neo, source of <i>no</i> cassette (3) pBIST501 Ap Neo, source of <i>no</i> cassette (3) Ap Spec: 11-kb Bgfl (blanted)-Mdfl (blunted) yer, cassette from pUS19 cloned into Neol (blunted) of cat gene in pH1101; used to convert Cm 'Spec' strains to Cm' Spec' pL73 Ap Spec: 11-kb Bgfl (blanted)-Mdfl (blunted) yer, cassette from pUS19 cloned into Neol (blunted) of pBisecript SK- pL74 Ap Spec: 11-kb Bgfl (blanted)-Mdfl (blunted) yer cassette from pUS19 cloned into Neol (blunted) of PBIP13 pL74 Ap Spec: 11-kb Bgfl (blanted)-Mdfl (blunted) yer cassette from pUS19 cloned into Smal site of pBisecript SK- pL73 Ap Spec: 11-kb Bgfl (blanted)-Mdfl (blunted) deficin of pH173; helper plasmid used in conjunction with pH181 for homology assistance pDG793 Ap Cm; clone of gno0KB-E in pJH101 (45) pDR8 Ap Cm; clone of gno0KB-E in pJH101 (45) pDR14 Ap Cm; Sch EaxRI fragment from pDR2 cloned into EczRI of pDR18, putting all of spo0K4-D on a gno0K promoter region (rain pDR18) in pBluescriptII SK + (45) pJL45 Ap Cm; Sch EaxRI fragment from pDR2 cloned into EczRI of pDR18, putti	Plasmid	Description (source or reference) ^{a}	
pBluescriptIL KS+ pAGS8 Ap cn and for subcloning and sequencing (Stratagene) pAGS8 Ap Cn (28) pH1101 Ap Tc Cm; integrative vector (15) pDCIM32(H)::::::::::::::::::::::::::::::::::::	Vectors		
pAGS8 The Derivative of the product of product pro	pBluescriptII KS+	Ap: used for subcloning and sequencing (Stratagene)	
pH101 Ap Tc Cm ² integrative vector (15) pCEM3Z47L;csarl (pGEMcar) Ap Cm ² , integrative vector (3) pDCU3.scm Ap Cm ² , integrative vector (3) pDCU3.scm Ap Nucl. source of <i>noc</i> assette (3) pDL51 Ap Spec; integrative vector, source of <i>noc</i> cassette (5) pL52 Ap Spec; integrative vector, source of <i>noc</i> cassette (5) pL73 Ap Spec; integrative vector, source of <i>noc</i> cassette (5) pL74 Ap Spec; integrative vector, source of <i>noc</i> cassette (5) pL73 Ap Spec; integrative vector, source of <i>noc</i> cassette (5) pL74 Ap Spec; integrative vector, source (7, 8, 18) pL74 Ap Spec; integrative vector, source (7, 8, 18) pL74 Ap Spec; some as pL73 except <i>spc</i> cassette is cloned in opposite orientation pL74 Ap Spec; some as pL73 except <i>spc</i> cassette (5, 18) pL152 ML53, USA Bam11 (butted) Voc1 (butted) dection of ptiP13; helper plasmid used in conjunction with pHP13 (butted) voc1 (butted) dection of ptiP13; helper plasmid used in conjunction with pHP13 (butted) dection of ptiP13; helper plasmid used in conjunction with pHP13 (butted) voc1 (butted) dection of ptiP13; helper plasmid used in conjunction with pHP13 (butted) dection of ptiP13; helper plasmid used in conjunction a single integrational plasmid pDR9 Ap Cm; clone of <i>spoRR-E</i> in pHH01 (45) pDR14 Ap Cm; Cl	pAG58	An Cm (28)	
μ200 MaZ(f +) :ccat-l (pGEMcat) Ap Cm: integraphies sectors (30) pUC18:zerr Ap Nor: source of mic cassets (20) pUC18:zerr Ap Nor: source of mic cassets (20) pLS19 Ap Socies of mic cassets (20) pLS2 Ap Socies of mic cassets (20) pLT3 Ap Socies of mic cassets (20) pH713 MLS Socies of mic cassets (20) pH714 Ap Socies as pLT3 except yc cassets is cloned in opposite orientation pH713 MLS Socies of socitX-receins (3) pLS2 MLS Socies of socitX-receins (3) pDR3 Ap Cm: clone of socitX-receins (3) pDR4 Ap Cm: clone of socitX-receins (3)	pH050	Ap Tr (20)	
pDC18.2017 pJC18, source of ann cassette (3) pDC18.2017 Ap ML18, source of ann cassette (2) pDC18.2017 Ap Source of ann cassette (2) pDL73 Ap Source of ann cassette (2) pDL74 Ap Source of ann cassette (2) pDL75 Ap MEX control of ann bill bill bill bill bill bill bill bi	$p_{\text{CEM}27f(+)}$ uset 1 ($p_{\text{CEM}aat}$)	Ap Te chi, integrative vector (2)	
pUC.B:2777 Ap MLS; source of are cassette (20) pDEST501 Ap Noc: source of are cassette (27) pDS19 Ap Spec; integrative vector, source of are cassette (5) pJL73 Ap Spec; integrative vector, source of are cassette from pUS19 cloned into Nool (blunted) of car gene in pH101; used to convert Cm' Spec' strains to Cm' Spec' pJL74 Ap Spec; integrative vector, source of are cassette is cloned in opposite orientation pH173 MLS Cm; B: subdisk E coil shuttle vector (8, 18) pJL74 Ap Spec; same as pLJ3 except spc cassette is cloned in opposite orientation pH173 MLS Cm; B: subdisk E coil shuttle vector (8, 18) pJL74 Ap Spec; casme as pLJ3 except spc cassette is cloned in opposite orientation pJL75 MLS, vector used to construct Lac2. transcriptional fusions and recombine into the chromosome at drC (gift from P. Stragier) Other plasmids pDR1 pDR18 Ap Cm; clone of spo0K2E in pJH101 (45) pJL81 Ap Cm; clone of spo0K2E for promoter region from Pvull site upstream of promoter to EcoRI site in spo0K3 in gpB110 (45) pJL45 Ap Cm; clone of spo0K2E for gpL101 (45) pL47 Ap Cm; clone of spo0K2E for gpL101 (45) pJL47 Ap Cm; clone of spo0K2E for gpL101 (45) pJL47 Ap Cm; clone discof gpL101 (45) <td< td=""><td>pGEMI3ZI(+)::cal-1 (pGEMical)</td><td>Ap Cm; integrative vector (65)</td></td<>	pGEMI3ZI(+)::cal-1 (pGEMical)	Ap Cm; integrative vector (65)	
pBEST501 Ap Noc; source of <i>noc</i> cassette (7) pUS19 Ap Spec; integrative vector, source of <i>spc</i> cassette fon pUS19 doned into Neol (blunted) of car genin pHR10 used to convert. Only Spc* strains to Cm* Spc* pL73 Ap Spec; 1.1-kb Rgf (blunted)-Add (blunted) spc cassette from pUS19 doned into Neol (blunted) of car genin pHR110 used to convert. Cm* Spc* strains to Cm* Spc* pL74 Ap Spc; 1.1-kb Rgf (blunted)-Add (blunted) spc cassette for on pUS19 doned into Neol (blunted) pBESTS01 pL74 Ap Spc; same ap LJ2 secept spc cassette is cloned in opposite orientation pHR13 pL174 Ap Spc; same ap LJ2 secept spc cassette is cloned in opposite orientation with PH35 for homology asistance pDG793 Ap MLS; vector used to construct lac2 transcriptional fusions and recombine into the chromosome at <i>dbC</i> (glt from P. Stragter) Other plasmids PDR9 pDR14 Ap Cm; clone of <i>spo0KB</i> -E in pH101 (45) pDR15 Ap Cm; clone of <i>spo0K</i> promoter region from Pw11 site upstream of promoter to EcoRI site in <i>spo0KA</i> in pH1101 (45) pDR14 Ap Cm; clone of <i>spo0K</i> promoter region (an in DR18) in pBluescript11 S+ (45) pL145 Ap Cm; 1-kb EcoRI fugment from pDR2 cloned into <i>EcoRI</i> of pDR18, putting all of <i>spo0KA-D</i> on a single integrational plasmid pL44 Ap Cm; 1-kb CaRI (blunted)-Saf fragment, containing Pape, from pAG58 cloned into <i>EcoRI</i> of pL10; used to make <i>App00K::neo</i>	pUC18::erm	Ap MLS; source of <i>erm</i> cassette (30)	
pDIS9 Ap Spec: integrative vector, source of pc cassette from pUS19 cloned into $kcol$ (blunted) of cat gene in pH101; used to convert Cm' Spec' strains to Cm' Spec' pIL73 Ap Spec: integrative vector, source of pc cassette from pUS19 cloned into $Snal$ site of pBilescript SK+ pIL74 Ap Spec: same as pL73 except spc cassette is cloned in opposite orientation pHP13 MLS Cm; B: subdisk-E: coli shuttle vector (8, 18) pJL52 Sk-kb BamHI (blunted)-Ncc (blunted) bace cassette from pUS19 cloned into Smal site of $pBilescript SK+$ pJD6793 MLS, Ci-Sk-b BamHI (blunted)-Ncc (blunted) bace (blunted) b	pBEST501	Ap Neo; source of <i>neo</i> cassette (27)	
p1L2 Ap Spec; 1.1-kb Bgfl (blunted)-Ndel (blunted) spc cassette from pUS19 cloned into Neol (blunted) of cat gene in pH1011 (sed to convert Cm' Spce' strains to Cm' Spce' p1L73 Ap Spec; 1.1-kb Bgfl (blunted)-Ndel (blunted) spc cassette from pUS19 cloned into Smal site of pBluescript ISK + the Reg Network (8, 18) p1L74 Ap Spec; Snn eas p1L73 except spc cassette is cloned in opposite orientation with pHP13 for homology assistance pDG793 MLS: 0.5-kb BamHI (blunted)-Ncol (blunted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance pDG793 Ap Cm; clone of spo0KB-E in pH101 (45) pDR18 Ap Cm; clone of spo0KB-E in pH101 (45) pDR21 Ap; clone of spo0KB promoter region from Pvull site upstream of promoter to EcoRI site in spo0KA in pH101 (45) pL10 Ap Cm; clone of spo0KB promoter region from pvull Site upstream of promoter to EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sall for pH101 pL45 Ap Cm; 3E-Akb EcoRI (hunted)-Sall fragment, containing Pspac, from pAG58 cloned into M263 by consist that from Pspac pJL49 Ap Cm; 0.9-kb Xmn1-Cla1 (blunted)-fragment from pDR21 5 by downstream from spo0K promoter cloned into EcorI-Bg/II (sites lost) of pL10; used to make Aspo0K::em pJL51 Ap Cm; 0.9-kb Xmn1-Cla1 (blunted)-fragment from pDR21 5 by downstream from spo0K promoter cloned into EcoRI of pL10; used to make Aspo0K::em pLK2 ML52 Mc Marchase-Aspo0K promoter cloned	pUS19	Ap Spec; integrative vector, source of <i>spc</i> cassette (5)	
pl.73 Ap Spec: 11-kb Bd [0 lunted)-Net (blunted) spc cassette from pLS19 cloned into Smal site of pBluescript SK + pl.74 Ap Spec: same as pl.73 except spc cassette is cloned in opposite orientation pHP13 MLS Cm; B: subtilis-E: coli shuttle vector (B; 18) pl.75 MLS, Oct-M BamHI (blunted)-Xcol (blunted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance pDG793 Ap MLS; vector used to construct lac2 transcriptional fusions and recombine into the chromosome at thrC (gift from P. Stragier) Other plasmids pDR9 pDR12 Ap Cm; clone of spo0K promoter region from Prull site upstream of promoter to EcoRI site in spo0K/A in pHI101 (45) pDR18 Ap: Cone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) plL10 Ap Cm; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) plL11 Ap: Cone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) plL45 Ap: Cone of spo0K Promoter region (as in pDR18) in pBluescriptII SK+ (45) plL46 Ap Cm; 3.4-b EcoRI (blunted)-SaI fragment rom pDR2 cloned into EcoRI of pDR18, putting all of spo0K-4-D on a single integrational plasmid plL47 Ap Cm; 1.4-b Ab more assette from pBEST501 cloned into Exp1-BgIII (sites lost) of plL10; used to make App0K::zem plL50 Ap Cm; 0.5-b A Xmn/C clut (blunted) fragment from pDR21 bonownstream from spo0K promoter cloned into B	pJL62	Ap Spec; 1.1-kb BglI (blunted)-NdeI (blunted) spc cassette from pUS19 cloned into NcoI (blunted) of	
pl.73 Ap Spec; 1.1-kb Bgfl (blutted)-Mdcl (blutted) spc cassette from pUS19 cloned into Smal site of pPluescript SK+ pl.74 Ap Spec; same as pl.73 except spc cassette is cloned in opposite orientation pHP13 pl.52 MLS: 0.5-kb BamHI (blutted)-Ncol (blutted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance. pDG793 Ap MLS: vector used to construct lacZ transcriptional fusions and recombine into the chromosome at thrC (gift from P. Stragier) Other plasmids pDR9 pDR11 Ap Cm; clone of spo0KB-E in pJH101 (45) pDR21 Ap: clone of spo0K promoter region from PvuII site upstream of promoter to EcoRI site in spo0K4 in pJH101 (45) pDR21 Ap: clone of spo0K promoter region from PvuII site upstream of promoter to EcoRI site in spo0K in game in tom pDR9 cloned into EcoRI of pDR18, putting all of spo0K4-D on a single integrational plasmid pIL49 Ap Cm; clone of public fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0K4-D on a single integrational plasmid pIL47 Ap Cm; 31-bp EcoRI (blutted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blutted)-Sall fragment from pDR21 (blutted)-BamHI-SphI fragment containing lacI from pAG58 cloned into ExpL-BgII (sites lost) of pJL10; used to make Δspo0K:::em pIL50 Ap Cm; 0.3-kb EcoRI (blutted) site of pIL47 pL51 Ap Cm; 0.9-kb XmnL-Call (blutted)-fragment from pDR21 containing a 36-bp fragment upstream (23 bp) of the spo0K promoter cloned into Call (blu	-	<i>cat</i> gene in pJH101; used to convert Cm ^r Spec ^s strains to Cm ^s Spec ^r	
p174 Ap Spec: same is ab IL73 except spc cassette is cloned in opposite orientation p1F913 MLS (m. 5. subfile-E. coli shutle vector (8, 18) p1L52 MLS: 0.5-kb BamH (blunted)-Ncot (blunted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance pDG793 Ap MLS: vector used to construct McZ transcriptional fusions and recombine into the chromosome at thrC (gift from P. Stragjer) Other plasmids pDR19 Ap Cm; clone of spo0KB-E in pJH101 (45) pDR19 Ap Cm; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL10 Ap Cm; 36-kb EcoRI fragment from pDR2 (onced into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmid pJL45 Ap Cm; Clone Of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL47 Ap Cm; Soka EcoRI (futured)-Sall fragment, containing Psac, from pAG58 cloned into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmid pJL47 Ap Cm; Psac reglexement vector (Fig. 1); 1.3-kb BamHL-SphI fragment containing lacl from pAG58 cloned into EcoRI of pJL10; used to make 2xpo0K::new pJL49 Ap Cm; 0.9-kb ArmH-Cla1 (blunted)-SpiI fragment from pDR21 S bp downstream from spo0K promoter cloned into Exp1-Bg/II (sites lost) of pJL10; used to make 2xpo0K::new pJL51 Ap Cm; 0.6-kb BamHI (blunted)-S	pJL73	Ap Spec; 1.1-kb <i>BgI</i> I (blunted)- <i>Nde</i> I (blunted) <i>spc</i> cassette from pUS19 cloned into <i>Sma</i> I site of pBluescript SK+	
piPr Pipel, smith as pD-2 (skep) by centor (Sing) Pipel, smith as pD-2 (skep) by centor (Sing) piPr MLS, 0.5-kb ban/H1 (blunted)-Nool (blunted) deletion of pIP13; helper plasmid used in conjunction with pIP13 for monology assistance pDG793 Ap MLS; vector used to construct <i>lac2</i> transcriptional fusions and recombine into the chromosome at <i>thrC</i> (gift from P. Stragier) Other plasmids Ap Cm; clone of <i>spo0KB-E</i> in pJH101 (45) pDR9 Ap Cm; clone of <i>spo0KB</i> promoter region from Pvull site upstream of promoter to <i>EcoRI</i> site in <i>spo0KA</i> in pJH101 (45) pDR1 Ap Cm; clone of <i>spo0KB</i> promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL10 Ap Cm; 36-kb <i>EcoRI</i> (fragment from pDR9 cloned into <i>EcoRI</i> of <i>pp00K-A</i> -D on a single integrational plasmid pJL45 Ap Cm; 13-bp <i>EcoRI</i> (hounted)- <i>SalI</i> fragment, containing Pspat, from pAG58 cloned into <i>EcoNI</i> (blunted)- <i>SalI</i> of <i>pp116</i> ; ph16 fragment containing <i>lacI</i> from pAG58 cloned into <i>EcoNI</i> (blunted)- <i>SalI</i> of <i>pp116</i> ; ph16 fragment containing <i>lacI</i> from pAG58 cloned into <i>Ba/pDK</i> ::rem pJL49 Ap Cm Neo; 1.3-kb <i>neo</i> cassette from pDES1501 cloned into <i>Exp1-BgIII</i> (sites lost) of pJL10; used to make <i>App0K</i> ::rem pJL50 Ap Cm; 0.4-kb AmII-Cla1 (blunted)-SaII fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the <i>spo0K</i> promoter cloned into <i>EcoI</i> -Bg/II (blunted) of pJL51; used to make <i>App0K</i> ::rem pLK2 MLS Cm; original <i>klnC</i> clone, ~3-5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into <i>Ba/HI</i> of	nH 74	An Speci some as pll 73 except snc cassette is cloned in opposite orientation	
p1152 MLS 0.15. Mollink-2: Coil Shittle Vector (6, 16) p1252 MLS 0.5.4b BamHI (blunted)-Neol (blunted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance pDG793 Ap MLS (m.5; Nobility-E. coil Shittle Vector (blunted) deletion of pHP13; helper plasmid used in conjunction with pHP13 for homology assistance pDR9 Ap Cm; clone of spo0KB-E in pH1101 (45) pDR18 Ap Cm; clone of spo0K promoter region from Pvul1 site upstream of promoter to EcoRI site in spo0KA in pH1101 (45) pDR21 Ap; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL45 Ap Cm; 3.6.kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmid pJL45 Ap Cm; 1.3.kb neo cassette from pDE3 cloned into Exp1-BgHI (sites lost) of pL1.0; used to make Δppo0K::neo pJL50 Ap Cm (1.5, 2.3.kb neo cassette from pDE21 S bp downstream from spo0K promoter cloned into Sp1.6k;:neo pJL51 Ap Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH62 chromosomal DNA cloned into BarHI of pH113 pLK2 MLS Cm; 1.4-kb Pst fragment from pDK21 containing a 58-bp fragment upstream (23 bp) of the spo0K promoter cloned into EcoRI of pH123 pL51 Ap Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH64 chromosomal DNA cloned into BarHI of pH113 pLK2 MLS Cm; 1.4-kb Pst fragment from pLK2 cloned into Pst1 (blunted) of pGEMcat Pspac-sp00K <td>pjL/4</td> <td>Ap species, same as $p_{21/2}$ except species set is toned in opposite orientation</td>	pjL/4	Ap species, same as $p_{21/2}$ except species set is toned in opposite orientation	
pL52 MLS; 0:340 BamH1 (billing)-Acol (pluncid) deletion of pH21; helper plasmid used in conjunction with pHP13 for homology assistance pDG793 AP MLS; vector used to construct lac2 transcriptional fusions and recombine into the chromosome at thrC (gift from P. Stragjer) Other plasmids pDR9 pDR18 Ap Cn; clone of spo0KB-E in pJH101 (45) pDR10 Ap Cn; clone of spo0K promoter region from PvuI site upstream of promoter to EcoRI site in spo0K4 in pJH101 (45) pDR21 Ap; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL45 Ap Cn; 36-kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0K4-D on a single integrational plasmid pJL45 Ap Cn; 31-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sal fragment from pDR21 for phane constraining lac1 from pAG58 cloned into Exp1-BgII (sites lost) of pJL10; used to make App0K::rree pJL49 Ap Cn; 0:6-kb ManH1 (blunted)-Sapl fragment from pDR21 bp downstream from spo0K promoter cloned into Sall (blunted)-Sapl fragment from pDR21 bp downstream from spo0K promoter cloned into Sall (blunted)-Sapl fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K income form pLK2 cloned into Ps1 (blunted) of pELS1; used to make App0K::rree pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BanH1 of pHP13 pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of pHF13 pLK2		MLS Chi, B. subulus-E. Con shuttle vector (6, 16)	
pDG793 Ap MLS; vector used to construct <i>lacZ</i> transcriptional fusions and recombine into the chromosome at <i>dnC</i> (gift from P. Stragier) Other plasmids pDR9 Ap Cm; clone of <i>spo0KB</i> - <i>E</i> in pJH101 (45) pDR18 Ap Cm; clone of <i>spo0K</i> promoter region from <i>PvII</i> site upstream of promoter to <i>Eco</i> RI site in <i>spo0KA</i> in pH101 (45) pDR10 Ap Cm; clone of <i>spo0K</i> promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL10 Ap Cm; 30- <i>kb</i> EcoRI fragment from pJR9 cloned into <i>Eco</i> RI of pDR18, putting all of <i>spo0K4-D</i> on a single integrational plasmid pJL45 Ap Cm; Pape replacement vector (Fig. 1); 13- <i>kb</i> BamH1-SpiI fragment containing <i>lacI</i> from pAG58 cloned into <i>Eco</i> NI (blunted)-SalI afgment, containing <i>lacI</i> from pAG58 cloned into <i>Eco</i> NI (blunted)-SalI <i>is to no</i> cassette from pDEST501 cloned into <i>Exp1-Bg</i> III (sites lost) of pJL10; used to make <i>App0K:srew</i> pJL50 Ap Cm; N0- <i>kb Bam</i> HI (blunted) fragment from pDR21 bp downstream from <i>spo0K</i> promoter cloned into <i>SalI</i> (blunted) site of flagment from pDR21 so transing a 368-bp fragment upstream (23 bp) of the <i>spo0K</i> gromoter cloned into <i>EvaI</i> -BgIII (blunted) of pJL51; used to make <i>App0K:srew</i> pLK21 MLS Cm; criginal <i>kinC</i> clone, ~35- <i>kb Sau3</i> A partial digest of JH622 chromosomal DNA cloned into <i>BamHI</i> (blunted)- <i>BamHI</i> (blunted) <i>ag</i> fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the <i>spo0K</i> gromoter cloned into <i>DH</i> 145 and <i>Clan</i> (blunted) <i>fag</i> pLK21 MLS Cm; criginal <i>kinC</i> clone, ~35- <i>kb Sau3</i> A partial digest of JH642 chromosomal DNA cloned into <i>BamHI</i> (blunted) <i>fag</i>	pJL52	with pHP13 for homology assistance	
Other plasmids pDR9 Ap Cm; clone of spo0KB-E in pJH101 (45) pDR18 Ap Cm; clone of spo0K promoter region from PvuII site upstream of promoter to EcoRI site in spo0KA in pJH101 (45) pDR21 Ap; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL10 Ap Cm; 3.6-kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmid pJL45 Ap Cm; 311-bp EcoRI (blunted)-SaII fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-SaII of pJH101 pJL47 Ap Cm; Space replacement vector (Fig. 1); 1.3-kb BamHI-Sph1 fragment containing lacI from pAG58 cloned into Expl-BgII (sites lost) of pJL10; used to make dxpo0K::neo pJL50 Ap Cm Neci, 1.3-kb neo cassette from pBEST501 cloned into Expl-Bg/II (sites lost) of pJL10; used to make dxpo0K::neo pJL51 Ap Cm (0.9-kb MmI-Cd1 (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into SaII (blunted)-SaII fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into Cla1 (blunted)-BamHI (blunted) of pL151; used to make Spacespo0K pLK2 MLS Cm; riginal kinC clone, ~3.5-kb EcoRI into Clane, into Cla1 (blunted) of pL51; used to make Spacespo0K pLK2 MLS Cm; 1.4-kb Pril fragment from pLK2 cloned into PrI (blunted) of pGEMcat pLK2 MLS Cm; 1.4-kb Pril fragment from pLK2 cloned into PrI cla1 (blunted) of pGEMcat pLK21 M	pDG793	Ap MLS; vector used to construct <i>lacZ</i> transcriptional fusions and recombine into the chromosome at <i>thrC</i> (gift from P. Stragier)	
Ap Communic pDR0Ap Cm; clone of spo0KB-E in pJH101 (45)pDR18Ap Cm; clone of spo0K promoter region from PvuII site upstream of promoter to EcoRI site in spo0KA in pJH101 (45)pDR21Ap; clone of spo0K promoter region (as in pDR18) in pBluescriptII SK+ (45)pJL10Ap Cm; 36.46 EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmidpJL45Ap Cm; 16.46 EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0KA-D on a single integrational plasmidpJL47Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb BamHI-SphI fragment containing lac1 from pAG58 cloned into pIL45 so transcription of lac1 is opposite that from PspacpJL49Ap Cm Neo; 1.3-kb neo cassette from pUC18:zenn cloned into EspI-BgIII (sites lost) of pJL10; used to make Expo0K:zmopJL50Ap Cm Neo; 1.3-kb mc cassette from pUC18:zenn cloned into EspI-BgIII (sites lost) of pJL10; used to make Expo0K:zmopJL51Ap Cm; 0.9-kb Xnn1-Cla1 (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into SaII (blunted)-saI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into Cla1 (blunted)-BamHI (blunted) of pJL51; used to make Bapac/SMOKpLK2MLS Cm; 2.1-kb PsH fragment from pLK2 cloned into PsH site of PHP13pLK21MLS Cm; 3.5-kb EcoRI (fragment from pLK2 cloned into PsH (blunted) of pGEMcat BamHI of pHP13pLK24MLS Cm; 3.5-kb EcoRI (fragment from pLK2 cloned into PsH (blunted) of pGEMcat PLK24pLK25Ap Cm; 3.5-kb EcoRI (fragment from pLK2 cloned into PsH (blunted) of pGEMcatpLK24MLS Cm; 1.1-kb PsH fragment from pLK2 cloned into PsH (blunted) of <br<< td=""><td>Other plasmids</td><td></td></br<<>	Other plasmids		
pDR18 Ap Cm; clone of spotK promoter region (as in pDR18) in pBluescriptII SK+ (45) pDR21 Ap Cm; clone of spotK promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL10 Ap Cm; 36-kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spotKA-D on a single integrational plasmid pJL45 Ap Cm; 311-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sall of pJH10 pJL47 Ap Cm; 13-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into pJL45 so transcription of lacl is opposite that from Pspac pJL49 Ap Cm; 0.1-3-kb nec cassette from pBEST501 cloned into Esp1-BgIII (sites lost) of pJL10; used to make Aspo0K::neo pJL50 Ap Cm; 0.3-kb mc cassette from pDC115::erm cloned into Esp1-BgIII (sites lost) of pJL10; used to make Aspo0K::neo pJL51 Ap Cm; 0.4-kb BamH1 (blunted) fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the sp0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Aspo0K::neo pJL58 Ap Cm; 0.4-kb BamH1 (blunted)-Ssp1 fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the sp0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Pspac.sp0K pLK2 MLS Cm; 1.4-kb ClaI (blunted)-fragment of pLK2 cloned into PsI ite of PH13 pLK2 MLS Cm; 1.4-kb ClaI (blunted) fragment of pLK2 cloned into PsI (blunted) of pGEMcat pLK2 MLS Cm; 1.4-kb ClaI (blunted) irggment of pLK2 cloned into PsI ClaI (bl	nDR9	An Cm: clone of $spa0KB-F$ in nIH101 (45)	
pDR21 Ap Cini, Choice option (Jointer Fegion Hond Funds are optication of product to Leord site in spotRA in pH101 (45) pJL10 Ap; Clone of spotR promoter region (as in pDR18) in pBluescriptII SK+ (45) pJL11 Ap Cin; 3.64 & EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spotRA-D on a single integrational plasmid pJL45 Ap Cin; 3.64 & EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spotRA-D on a single integrational plasmid pJL47 Ap Cin; 9.64 & EcoRI fragment from pDR9 cloned into EcoRI of pDR16, particle and the spot of the pDR26 cloned into EspI-BgII (sites lost) of pJL10; used to make AspotR:::new pJL49 Ap Cm Nec; 1.3-kb erm cassette from pUC18::erm cloned into EspI-BgIII (sites lost) of pJL10; used to make AspotR:::new pJL51 Ap Cm; 0.9-kb XmnL-ClaI (blunted) fragment from pDR21 5 bp downstream from spotR promoter cloned into SaII (blunted) site of pJL47 pJL58 Ap Cm; 0.9-kb ZmnL-ClaI (blunted) fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spotR promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make AspotR::mo pLK2 MLS Cm; 1.4-kb PstI fragment from pLK2 cloned into PstI site of pHP13 pLK21 MLS Cm; 1.4-kb PstI fragment from pLK2 cloned into PstI (blunted) of pGEMcat pLK23 MLS Cm; 1.4-kb PstI fragment from pLK2 cloned into PstI (blunted) of pGEMcat pLK24 MLS Cm; 1.4-kb PstI fragment from pLK2 cloned into PstI (blunted) of pGEMcat	pDR18	An Cm: clone of spolK promoter region from PwIII site upstream of promoter to EcoRI site in	
pDR21Ap: clone of <i>spolK</i> promoter region (as in pDR18) in pBluescriptII SK+ (45)pJL10Ap Cm; 3.6-kb <i>Eco</i> RI fragment from pDR9 cloned into <i>Eco</i> RI of pDR18, putting all of <i>spolK4-D</i> on a single integrational plasmidpJL45Ap Cm; 31-bp <i>Eco</i> RI (blunted)- <i>Sall</i> fragment, containing Pspac, from pAG58 cloned into <i>Eco</i> NI (blunted)- <i>Sall</i> of pJH101pJL47Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb <i>BamHI-SphI</i> fragment containing <i>lacl</i> from pAG58 cloned into pJL45 so transcription of <i>lacl</i> is opposite that from PspacpJL49Ap Cm Neo; 1.3-kb <i>neo</i> cassette from pBEST501 cloned into <i>EspI-Bg/II</i> (sites lost) of pJL10; used to 	purio	spo0KA in pJH101 (45)	
pJL10 Ap Cm; 3.6-kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0K4-D on a single integrational plasmid pJL45 Ap Cm; 311-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sall of pJH101 pJL47 Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb BamHI-SphI fragment containing lacl from pAG58 cloned into pL45 so transcription of lacl is opposite that from Pspac pJL49 Ap Cm Neo; 1.3-kb neo cassette from pBE5T501 cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Asp00K::neo pJL50 Ap Cm (0.9-kb XmnI-ClaI (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into Sall (blunted) site of pJL47 pJL58 Ap Cm; 0.9-kb XmnI-ClaI (blunted) SpI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into ClaI (blunted) of pJL51; used to make Asp00K::rem pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 pLK2 MLS Cm; 1.4-kb PaI fragment from pLK2 cloned into PsI (blunted) of pGEMcat pLK24 Ap Cm; 3.5-kb EcoRI (blunted)-FindHI (blunted) spc cassette of pJL74 cloned into PsI-ClaI (blunted) of pLK24 pLK25 Ap Cm; 4.4-kb PaI-BamHI (blunted) spc cassette of pJL74 cloned into PsI-ClaI (blunted) of pLK24; used to make AkinC::spc pLK24 Ap Cm; 3.5-kb EcoRI (fragment from pLK2 cloned into PsI-ClaI (blunted) of pLK24; used to make AkinC::spc pLK24 Ap Cm; 4.4-kb PaI-BamHI (blu	pDR21	Ap; clone of $spo0K$ promoter region (as in pDR18) in pBluescriptII SK+ (45)	
pIL45 a single integrational plasmid integrational plasmid pJL45 Ap Cm; 311-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sall of pJH101 pJL47 Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb BamHI-Sph1 fragment containing lac1 from pAG58 cloned into pL45 so transcription of lac1 is opposite that from Pspac cloned into pL45 so transcription of lac1 is opposite that from Pspac cloned into pL45 so transcription of lac1 is opposite that from Pspac cloned into pL45 so transcription of lac1 is opposite that from Pspac cloned into pL45 kb em cassette from pUE18:em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Asp00K::rem pJL50 Ap Cm NLS; 2.3-kb em cassette from pUE18:em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Asp00K::rem pJL51 Ap Cm; 0.6-kb BamHI (blunted) fragment from pDR21 so pdownstream from spo0K promoter cloned into Sall (blunted)-San fragment must make (23 bp) of the spo0K promoter cloned into Clal (blunted)-BamHI (blunted) of pL51; used to make Pspac-spo0K pLK2 MLS Cm; 0: original kinC Clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 pLK21 MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2 pLK23 MLS Cm; 1.4-kb PsrI deletion of pLK2 cloned into PsrI (blunted) of pGEMcat pLK24 Ap Cm Spec; 1.1-kb PsrI-BamHI (blunted) spc cassette of pJL74 cloned into PsrI-ClaI (blunted) of pLK24 used to make AkinC::spc pLK25 Ap Cm Spec; 1.1-kb PsrI-BamHI (blunted) spc cassette of pLK2 cloned into PsrI-ClaI (b	pJL10	Ap Cm; 3.6-kb EcoRI fragment from pDR9 cloned into EcoRI of pDR18, putting all of spo0KA-D on	
pJL45 Ap Cm; 311-bp EcoRI (blunted)-Sall fragment, containing Pspac, from pAG58 cloned into EcoNI (blunted)-Sall of pJH101 pJL47 Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb BamHI-Sph1 fragment containing lac1 from pAG58 cloned into pJL45 so transcription of lac1 is opposite that from Pspac pJL49 Ap Cm, Ney, 1.3-kb neo cassette from pBEST501 cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Aspo0K:::eo pJL50 Ap Cm, 0.9-kb Xmn1-Cla1 (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into Sall (blunted) site of pJL47 pJL58 Ap Cm; 0.9-kb Xmn1-Cla1 (blunted) site of pJL47 pJL58 Ap Cm; 0.6-kb BamHI (blunted)-SapI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into Cla1 (blunted)-BamHI (blunted) of pJL51; used to make Pspac-spo0K pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 pLK21 MLS Cm; 1.4-kb Cla1 (blunted)-HamHI (blunted) of pGEMcat pLK23 MLS Cm; 1.4-kb Cla1 (blunted) fragment of pLK2 cloned into Pst1 (blunted) of pGEMcat pLK24 Ap Cm Ss-kb EcoRI (blunted) fragment for pLK2 cloned into Pst1 (blunted) of pGEMcat pLK24 MLS Cm; 1.4-kb Cla1 (blunted) fragment of pLK2 cloned into Pst1-Cla1 (blunted) of pEMcat pLK21 MLS Cm; 2.1-kb Pst1 fragment from pLK2 cloned into EcoRI of pBLecat pLK22 MLS Cm; 1.4-kb Cla1 (blunted) fragment of pLK2 cloned into Ps	I · · ·	a single integrational plasmid	
pJL47(butted) space replacement vector (Fig. 1); 1.3-kb BamHI-SphI fragment containing lac1 from pAG58 cloned into pJL45 so transcription of lac1 is opposite that from PspacpJL49Ap Cm Neo; 1.3-kb neo cassette from pBEST501 cloned into EspI-Bg/II (sites lost) of pJL10; used to make Δspo0K:.neopJL50Ap Cm MLS; 2.3-kb erm cassette from pUC18::erm cloned into EspI-Bg/II (sites lost) of pJL10; used to make Δspo0K:.neopJL51Ap Cm; 0.9-kb XmI-ClaI (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into Sal1 (blunted) site of pJL47pJL58Ap Cm; 0.6-kb BamHI (blunted)-SspI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Pspac-spo0KpLK2MLS Cm; 0.4-kb BamHI (blunted)-HindIII (blunted)-BamHI (blunted) of pJL51; used to make Pspac-spo0KpLK2MLS Cm; 1.4-kb Cla (blunted)-HindIII (blunted) deletion of pLK2 pLK23pLK24MLS Cm; 1.4-kb PsII fragment from pLK2 cloned into PsII site of pHP13 pLK24pLK25MLS Cm; 1.4-kb PsII deletion of pLK2 pLK24pLK26Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PsII cloned into PsII-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK56Ap : 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ pLK102pLK104Ap : 0.4-kb EcoRV-Smal deletion of pLK26pLK114Ap MLS; kicle clacz; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.4-kb EcoRV Smal deletion of pLK102pLK121Ap Cm; 0.4-kb ApaI (blunted)-ScaII (blunted) fragment of pLK120 </td <td>pJL45</td> <td>Ap Cm; 311-bp <i>Eco</i>RI (blunted)-<i>Sal</i>I fragment, containing Pspac, from pAG58 cloned into <i>Eco</i>NI (blunted)-<i>Sal</i>I of pH101</td>	pJL45	Ap Cm; 311-bp <i>Eco</i> RI (blunted)- <i>Sal</i> I fragment, containing Pspac, from pAG58 cloned into <i>Eco</i> NI (blunted)- <i>Sal</i> I of pH101	
pJL49 Ap Cm Neo; 1.3-kb neo cassette from pBEST501 cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Δsp00K::neo pJL50 Ap Cm MLS; 2.3-kb em cassette from pUC18::em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Δsp00K::neo pJL51 Ap Cm MLS; 2.3-kb em cassette from pUC18::em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Δsp00K::em pJL51 Ap Cm; 0.9-kb Xmn1-ClaI (blunted) fragment from pDR21 5 bp downstream from sp0K promoter cloned into SaII (blunted) ssp1 fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the sp0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Pspac-sp0K pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 pLK21 MLS Cm; 1.4-kb PsII fragment from pLK2 cloned into PsII site of pHP13 pLK22 MLS Cm; 1.4-kb PsII deletion of pLK2 pLK23 MLS Cm; 1.4-kb PsII deletion of pLK2 pLK24 Ap Cm; 3.5-kb EcoRI (blunted) spc cassette of pJL74 cloned into PsII-ClaI (blunted) of pLK24; used to make ΔkinC::spc pLK56 Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ pLK104 Ap Cn; 0.7-kb EcoRV-SmaI deletion of pLK102 pLK104 Ap Cn; 0.7-kb EcoRV-SmaI deletion of pLK102 pLK56 Ap; 0.4-kb EcoRV-SmaI deletion of pLK102 pLK104 Ap Cn; 0.7-kb EcoRV fragment from pLK2 cloned into EcoRI bamHI	pJL47	Ap Cm; Pspac replacement vector (Fig. 1); 1.3-kb <i>Bam</i> HI- <i>Sph</i> I fragment containing <i>lac1</i> from pAG58	
pJL49Ap Cm Neo; 1.3-kb neo cassette from pBEST501 cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Δsp0K::neopJL50Ap Cm MLS; 2.3-kb em cassette from pUC18::em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Δsp0K::empJL51Ap Cm; 0.9-kb Xmn1-ClaI (blunted) fragment from pDR21 5 bp downstream from sp0K promoter cloned into SaII (blunted) site of pJL47pJL58Ap Cm; 0.6-kb BamHI (blunted)-Ssp1 fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the sp0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Bspac-sp0KpLK2MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13pLK21MLS Cm; 1.4-kb PsI fragment from pLK2 cloned into PsI bit of pHP13pLK22MLS Cm; 1.4-kb PsI fragment of pLK2pLK23MLS Cm; 1.4-kb PsI fragment of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PsI-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK25Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PsI-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK26Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS + Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pLK2pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lac2; 2.3-kb EcoRI cloluted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make tinC::(kinC-lacZ)pLK120Ap Cm; 0.2-kb MaeI HI-PsI fragment of pLK102 cloned into EcoRI-BamHI (blunted) of pDG793; used to make tinC::(kinC-lacZ)pLK121Ap Cm; 0.2-kb MaeI HI-PsI fragment of pLK104 cloned into SaI (blunte		cloned into pJL45 so transcription of <i>lacI</i> is opposite that from Pspac	
pJL50Ap Cm MLS; 2.3-kb em cassette from pUC18::em cloned into Esp1-Bg/II (sites lost) of pJL10; used to make Asp00K::empJL51Ap Cm; 0.9-kb Xmn1-Cla1 (blunted) fragment from pDR21 5 bp downstream from sp0K promoter cloned into Sal1 (blunted) site of pJL47pJL58Ap Cm; 0.6-kb BamHI (blunted) site of pJL47pJL58Ap Cm; 0.6-kb BamHI (blunted) Ssp1 fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the sp0K promoter cloned into Cla1 (blunted)-BamHI (blunted) of pJL51; used to make Bpac-sp0KpLK2MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13pLK21MLS Cm; 1.4-kb Ps1 fragment from pLK2 cloned into Ps1 site of pHP13pLK22MLS Cm; 1.4-kb Ps1 deletion of pLK2pLK24Ap Cm 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into Ps1 (blunted) of pGEMcatpLK25Ap Cm Spc; 1.1-kb Ps1-BamHI (blunted) spc cassette of pJL74 cloned into Ps1-ClaI (blunted) of pLK24; used to make AkinC:spcpLK102Ap; 2.2-kb ClaI deletion of pLK20pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap Cm; 0.2-kb ZoaRV fragment from pLK2 cloned into EcoRI of pBluescriptII KS + pLK102pLK124Ap Cm; 0.2-kb ZoaRV fragment from pLK2 cloned into for LCoaI (blunted) of pDG793; used to make thrc::(kinC-lacZ)pLK121Ap Cm; 0.2-kb MeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb M	pJL49	Ap Cm Neo; 1.3-kb <i>neo</i> cassette from pBEST501 cloned into <i>EspI-BgIII</i> (sites lost) of pJL10; used to make Asno0K::neo	
 pJL50 pJL51 pJL51 Ap Cm; 0.9-kb Xmn1-ClaI (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into Sall (blunted) site of pJL47 pJL58 Ap Cm; 0.6-kb BamHI (blunted) fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Aspo0K. pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BaHII of pHP13 pLK21 MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2 pLK23 MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2 pLK24 Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcat pLK25 Ap Cm Spec; 1.1-kb PstI-BamHI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) of pLK24; used to make ΔkinC:spc pLK102 Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ pLK104 Ap; 0.2-kb EcoRV-SmaI deletion of pLK102 pLK114 Ap MLS; kinC-lac2; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK22 cloned into EcoRI-BamHI (blunted) of pGF93; used to make thrC::(kinC-lacZ) pLK121 pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK2 cloned into pGEMcat; contains kinC promoter pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK2 linto SmaI of pGEMcat; contains kinC promoter pLK124 Ap Cm; 0.7-kb EcoRV fragment of pLK120 cloned into GEMcat pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK2 linto SmaI of pGEMcat; contains kinC promoter pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK20 cloned into pGEMcat pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK20 cloned into GEMcat pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK20 cloned into GEMcat pLK124 Ap Cm; 0.7-kb EcoRV fragment from pLK2	nH 50	An C M MIS 2.2.3 th arm cascatte from nUC18: arm cloned into Fent Rall (sites loct) of nU 10: used	
pJL51Ap Cm; 0.9-kb XmnI-ClaI (blunted) fragment from pDR21 5 bp downstream from spo0K promoter cloned into SalI (blunted) site of pJL47pJL58Ap Cm; 0.9-kb XmnI-ClaI (blunted)-SspI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Pspac-spo0KpLK2MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13pLK21MLS Cm; 2.1-kb PstI fragment from pLK2 cloned into PstI site of pHP13pLK22MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2pLK23MLS Cm; 1.4-kb PstI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm; 3.5-kb EcoRI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS + pLK104pLK104Ap; 0.2-kb ClaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 not smal of pGEMcat; contains kinC promoter pLK121pLK124Ap Cm; 0.2-kb Mcd (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb Mcd (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb Mcd (blunted)-SalI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb Mcd (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK	pJL50	to make Ample Ample and the make and the make and the make Ample and t	
 Ap Cm; 0.9-K0 Amin-Cal (blunted) fragment from pDR21 5 bp downstream from spook promoter cloned into <i>Sall</i> (blunted) site of p1L47 pJL58 Ap Cm; 0.6-kb <i>Bam</i>HI (blunted)-<i>Ssp</i>I fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the <i>spo0K</i> promoter cloned into <i>Cla</i>I (blunted)-<i>Bam</i>HI (blunted) of pJL51; used to make Pspac-<i>spo0K</i> pLK2 MLS Cm; original <i>kinC</i> clone, ~3.5-kb <i>Sau</i>3A partial digest of JH642 chromosomal DNA cloned into <i>Bam</i>HI of pHP13 pLK21 MLS Cm; 1.1-kb <i>Psi</i>I fragment from pLK2 cloned into <i>Psi</i>I site of pHP13 pLK22 MLS Cm; 1.4-kb <i>Psi</i>I deletion of pLK2 pLK24 pLK24 Ap Cm Soc, 1.1-kb <i>Psi</i>I deletion of pLK2 cloned into <i>Psi</i>I (blunted) of pGEM<i>cat</i> pLK25 Ap Cm Soc, 1.1-kb <i>Psi</i>I fragment from pLK2 cloned into <i>Psi</i>I (blunted) of pGEM<i>cat</i> pLK26 pLK26 pLK27 pLK27 pLK28 pLK29 pLK29 pLK29 pLK29 pLK20 pLK29 pLK20 p	- II <i>5</i> 1	W make $\Delta pown.em$	
 pJL58 Ap Cm; 0.6-kb BamHI (blunted)-SspI fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the spo0K promoter cloned into Cla1 (blunted)-BamHI (blunted) of pJL51; used to make Pspac-spo0K pLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 pLK21 MLS Cm; 2.1-kb PsII fragment from pLK2 cloned into PstI site of pHP13 pLK22 MLS Cm; 1.4-kb Cla1 (blunted)-HindIII (blunted) deletion of pLK2 pLK23 MLS Cm; 1.4-kb PsII deletion of pLK2 pLK24 Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcat pLK25 Ap Cm; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ pLK102 pLK104 Ap; 3.5-kb EcoRI regment detion of pLK102 pLK114 Ap Cm; 0.4-kb EcoRV fragment from pLK2 cloned into Fuk2 cloned into EcoRI of pLK2 cloned into EcoRI of pLK102 pLK120 Ap Cm; 0.7-kb EcoRV fragment from pLK2 cloned into Fuk2 cloned into EcoRI of pLK24 pLK120 Ap Cm; 0.7-kb EcoRV fragment from pLK2 cloned into Fuk2 cloned into EcoRI-BamHI (blunted) of pGF9/3; used to make thrC::(kinC-lacZ) pLK120 Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into GEMcat pLK124 Ap Cm; 0.4-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK120 pLK125 MLS Cm; 0.6-kb ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277 	pJL51	Ap Cm; 0.9-kb <i>Xmn1-Cla1</i> (blunted) fragment from pDR21 5 bp downstream from <i>spou</i> K promoter	
pJL58Ap Cm; 0.6-kb BamHI (blunted)-SpI fragment from pJR21 containing a 368-bp fragment upstream (23 bp) of the sp00K promoter cloned into ClaI (blunted)-BamHI (blunted) of pJL51; used to make Pspac-sp00KpLK2MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13pLK21MLS Cm; 2.1-kb PsI fragment from pLK2 cloned into PsI site of pHP13pLK22MLS Cm; 1.4-kb PsI deletion of pLK2pLK23MLS Cm; 1.4-kb PsI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PsI (blunted) of pGEMcatpLK56Ap Cm; 3.5-kb EcoRI (blunted) fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK104Ap; 0.4-kb EcoRV fragment from pLK2 cloned into fragment of pLK2 cloned into EcoRI of pBluescriptII KS+pLK114Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK120pLK125Ap Cm; 0.2-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK2pLK126Ap Cm; 0.2-kb		cloned into Sall (blunted) site of pJL4/	
 Pspac-spo0K PLK2 MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13 PLK21 MLS Cm; 2.1-kb Pstl fragment from pLK2 cloned into Pstl site of pHP13 PLK22 MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2 PLK23 MLS Cm; 1.4-kb Pstl deletion of pLK2 PLK24 Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into Pstl (blunted) of pGEMcat PLK25 Ap Cm Spec; 1.1-kb Pstl-BamHI (blunted) spc cassette of pJL74 cloned into Pstl-ClaI (blunted) of pLK24; used to make \LakinC::spc PLK56 Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ PLK102 Ap; 0.4-kb EcoRV-Smal deletion of pLK102 PLK114 Ap Cm; 0.7-kb EcoRV fragment from pLK2 into Smal of pGEMcat; contains kinC promoter PLK120 Ap Cm; 0.7-kb EcoRV fragment from pLK2 cloned into pLK120 PLK121 Ap Cm; 0.4-kb BamHI-PsrI fragment of pLK120 cloned into pGEMcat PLK124 Ap Cm; 0.4-kb BamHI-PsrI fragment of pLK120 cloned into pGEMcat PLK125 MLS Cm; 0.4-kb ApaI (blunted)-EcoRI (blunted) deletion of pLK2 PLK126 Ap Cm; 102-bp ApaI (blunted)-Sall (blunted) deletion of pLK2 Ap Cm; 02-bp ApaI (blunted)-Sall (blunted) cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277 	pJL58	Ap Cm; 0.6-kb <i>Bam</i> HI (blunted)- <i>Ssp</i> I fragment from pDR21 containing a 368-bp fragment upstream (23 bp) of the <i>spo0K</i> promoter cloned into <i>Cla</i> I (blunted)- <i>Bam</i> HI (blunted) of pJL51; used to make	
pLK2MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into BamHI of pHP13pLK21MLS Cm; 2.1-kb PstI fragment from pLK2 cloned into PstI site of pHP13pLK22MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2pLK23MLS Cm; 1.4-kb PstI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK56Ap; 3.5-kb EcoRV fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 2.2-kb ClaI deletion of pLK102pLK14Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK124Ap Cm; 0.2-kb Mal (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-SalI (blunted) deletion of pLK2		Pspac-spo0K	
BamHI of pHP13pLK21MLS Cm; 2.1-kb PsrI fragment from pLK2 cloned into PsrI site of pHP13pLK22MLS Cm; 1.4-kb Clal (blunted)-HindIII (blunted) deletion of pLK2pLK23MLS Cm; 1.4-kb PsrI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PsrI (blunted) of pGEMcatpLK25Ap Cm Spec; 1.1-kb PsrI-BamHI (blunted) spc cassette of pJL74 cloned into PsrI-ClaI (blunted) ofpLK26Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 2.2-kb ClaI deletion of pLK56pLK114Ap (S. icon V-SmaI deletion of pLK102pLK124Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 0.2-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 0.2-kb ApaI (blunted)-BamHI fragment of pLK104 cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK2	MLS Cm; original kinC clone, ~3.5-kb Sau3A partial digest of JH642 chromosomal DNA cloned into	
pLK21MLS Cm; 2.1-kb PstI fragment from pLK2 cloned into PstI site of pHP13pLK22MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2pLK23MLS Cm; 1.4-kb PstI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm Spec; 1.1-kb PstI-BamHI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) ofpLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI(blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK12pLK126Ap Cm; 102-bp ApaI (blunted)-SaII (blunted) deletion of pLK2	•	BamHI of pHP13	
pLK22MLS Cm; 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2pLK23MLS Cm; 1.4-kb ClaI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm Spec; 1.1-kb PstI-BamHI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) ofpLK26Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 3.5-kb CaI deletion of pLK56pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI(blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.2-kb MaI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-SaII (blunted) cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK21	MLS Cm: 2.1-kb PstI fragment from pLK2 cloned into PstI site of pHP13	
pLK23MLS Cm; 1.4-kb PstI deletion of pLK2pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm Spec; 1.1-kb PstI-BamHI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) ofpLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 2.2-kb ClaI deletion of pLK56pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lac2; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	nLK22	MIS Cm: 1.4-kb ClaI (blunted)-HindIII (blunted) deletion of pLK2	
pLK24Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI (blunted) of pGEMcatpLK25Ap Cm; 3.5-kb EcoRI (blunted) fragment of pLK2 cloned into PstI-ClaI (blunted) ofpLK26Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 2.2-kb ClaI deletion of pLK56pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI(blunted) of pDG793; used to make hrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-SaII (blunted) cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	pL K23	MIS Cm: 14-kb PyrI deletion of pIK2	
pLK24Ap Cin, 3.5-kb EcoRI (bilined) magnetic of pLK2 cloned into 7str (bilined) of pOEMetalpLK25Ap Cin Spec; 1.1-kb PstI-BamHI (blunted) spc cassette of pJL74 cloned into PstI-ClaI (blunted) of pLK24; used to make ΔkinC::spcpLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+ Ap; 2.2-kb ClaI deletion of pLK56pLK102Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoter pLK121pLK124Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK25 pLK24	An Cm: 3.5 the Food (clound of pLike)	
pLK23Ap Chi Spec, 1.1-64 Dispec, 1.1-74 cloned into Fsh-Carl (blunted) of pLK24; used to make ΔkinC::spcpLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS + Ap; 2.2-kb ClaI deletion of pLK56pLK102Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoter 	pLK24	Ap Cin, 5.5-K below (outned) hagnen of plaze concerning in outned, of polarization of the first outned) of	
pLK24; used to make \(\Delta kmC:\Spc\)pLK56Ap; 3.5-kb \(\Delta coRI\) fragment from pLK2 cloned into \(\Delta coRI\) of pBluescriptII KS+pLK102Ap; 2.2-kb \(\Delta I\) deletion of pLK56pLK104Ap; 0.4-kb \(\Delta coRV-Smal\) deletion of pLK102pLK114Ap MLS; \(\kinC-lacZ; 2.3-kb \(\Delta coRI-ClaI\) (blunted) fragment of pLK2 cloned into \(\Delta coRI-BamHI\)(blunted) of pDG793; used to make \(\text{thrC::}(kinC-lacZ)\)pLK120Ap Cm; 0.7-kb \(\Delta coRV\) fragment from pLK2 into \(\Smal\) of pGEMcat; contains \(\kinC\) promoterpLK121Ap Cm; 0.2-kb \(\Ndel\) (blunted)-\(\Delta coRI\) (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb \(\BamHI-PstI\) fragment of pLK120 cloned into \(\Beta Cat\)pLK125MLS Cm; 0.6-kb \(\ApaI\) (blunted)-\(\Sat\) (blunted) deletion of pLK2pLK126Ap Cm; 102-bp \(ApaI\) (blunted)-\(\BamHI\) fragment of pLK104 cloned into \(\Sat\) (blunted)-\(\BamHI\) of pGEMcat; used to disrupt \(\sit\) of 277	plk25	Ap Cin spec, 1.1-k01 sin-bannin (binned) spe cassene of pJL/4 cloned into I sin-Cun (blunded) of	
pLK56Ap; 3.5-kb EcoRI fragment from pLK2 cloned into EcoRI of pBluescriptII KS+pLK102Ap; 2.2-kb ClaI deletion of pLK56pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoter Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcat PLK125pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	11/5/	$p_{L} \times a_{i}$ used to make $\Delta kinC:spc$	
pLK102Ap; 2.2-kb Cla1 deletion of pLK56pLK104Ap; 0.4-kb EcoRV-SmaI deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoter Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SaII (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SaII (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK56	Ap; 3.5-kb <i>Eco</i> RI fragment from pLK2 cloned into <i>Eco</i> RI of pBluescriptII KS+	
pLK104Ap; 0.4-kb EcoRV-Smal deletion of pLK102pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into Smal of pGEMcat; contains kinC promoter Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK102	Ap; 2.2-kb Clal deletion of pLK56	
pLK114Ap MLS; kinC-lacZ; 2.3-kb EcoRI-ClaI (blunted) fragment of pLK2 cloned into EcoRI-BamHI (blunted) of pDG793; used to make thrC::(kinC-lacZ)pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoter Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK104	Ap; 0.4-kb <i>Eco</i> RV- <i>Sma</i> I deletion of pLK102	
pLK120Ap Cm; 0.7-kb EcoRV fragment from pLK2 into SmaI of pGEMcat; contains kinC promoterpLK121Ap Cm; 0.7-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK114	Ap MLS; <i>kinC-lacZ</i> ; 2.3-kb <i>Eco</i> RI- <i>Cla</i> I (blunted) fragment of pLK2 cloned into <i>Eco</i> RI- <i>Bam</i> HI (blunted) of pDG793; used to make <i>thrC</i> ::(<i>kinC-lacZ</i>)	
pLK125Ap Cm; 0.7-Kb Eddw Hagnett Holin pLK2 into smart of pDEMtar, contains kmc prolificierpLK121Ap Cm; 0.2-kb NdeI (blunted)-EcoRI (blunted) deletion of pLK120pLK124Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pI K120	An Cm: 0.7-th EcoRV fragment from nI K2 into Swal of nGEMeat: contains kinC promotor	
pLK121 Ap Cm; 0.2-kb Warl (bilinted)-ECOKI (bilinted) deletion of pLK120 pLK124 Ap Cm; 0.4-kb BamHI-PstI fragment of pLK120 cloned into pGEMcat pLK125 MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) eletion of pLK2 pLK126 Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLA120 pL K121	Ap Cm, 0.7 K0 Etory magnetic non pEx2 into Small of pOEWtat, contains kine promote $A_{\rm P}$ Cm, 0.2 kb Ndd (bluntad) Eco PI (bluntad) deletion of FLV120	
pLK124Ap Cm; 0.4-KD BamHI-FSII fragment of pLK120 cloned into pGEMcatpLK125MLS Cm; 0.6-kb ApaI (blunted)-SalI (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	PLA121	Ap Cm, 0.2-K0 Nucl (bluncu)-ECOKI (bluncu) detection of pLK120	
pLK125MLS Cm; 0.6-kb Apa1 (blunted)-Sal1 (blunted) deletion of pLK2pLK126Ap Cm; 102-bp ApaI (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	PLK124	Ap Cir; 0.4-k0 BamHi-Fsii iragment of pLK120 cloned into pGEMcat	
pLK126 Ap Cm; 102-bp Apa1 (blunted)-BamHI fragment of pLK104 cloned into SalI (blunted)-BamHI of pGEMcat; used to disrupt orf277	pLK125	MLS Cm; U.o-kb Apa1 (blunted)-Sal1 (blunted) deletion of pLK2	
	pLK126	Ap Cm; 102-bp <i>Apa</i> 1 (blunted)- <i>Bam</i> HI tragment of pLK104 cloned into <i>Sal</i> 1 (blunted)- <i>Bam</i> HI of pGEM <i>cat</i> ; used to disrupt <i>orf</i> 277	

^a Ap, Tc, Cm, Neo, Spec, MLS refer to resistance to the particular drugs. All sizes in kilobases are approximate.

Approximately 80 to 90% of the plasmids in library A had inserts, and the chance that any particular fragment of the average 3.2-kb size is present in the library is ~87%, assuming a random distribution of *Sau3A* sites, no bias in selection in *E. coli*, and an average insert size of ~3.2 kb (probably an overestimate). For library B, ~60 to 70% of the plasmids had inserts and assuming an average 5.4 b size the chance that a particular fragment of average circ is the size that average average 5-kb size the chance that a particular fragment of average size is represented is ~50%.

conditional mutation of *spo0K* by replacing the normal *spo0K* promoter with the LacI-repressible, isopropyl- β -D-thiogalactopyranoside (IPTG)-inducible promoter Pspac (19, 28, 61), using the vector pJL47 (Fig. 1A). pJL47 can be used to replace the promoter of a gene with the *lacI* cassette and Pspac in a two-step process without leaving behind any drug resistance marker. pJL58 contains DNA from immediately upstream and downstream of the *spo0K* promoter cloned into pJL47 (Fig. 1B and Table 2) and was used to replace the normal *spo0K* promoter with Pspac in two steps. Transformation of pJL58

Construction of a conditional spo0K mutant (Pspac-spo0K). We constructed a



FIG. 1. Making a LacI-repressible, IPTG-inducible allele of *spo0K*. (A) pJL47 contains *lacI* under control of the constitutive P_{pen} promoter and the LacI-repressible, IPTG-inducible promoter Pspac (19, 61). (B) pJL58 contains sequences from both upstream (stippled box) and downstream (striped box) of the *spo0K* promoter cloned upstream and downstream, respectively, of the *lacI*-Pspac cassette of pJL47. When transformed into wild-type cells selecting for Cm⁷ (integration step), pJL58 can recombine either with the upstream *spo0K* sequence (shown), which would result in a phenotypically Spo⁺ transformant, or with the downstream *spo0K* sequence (not shown), which would result in a transformant which was Spo⁺ only in the presence of IPTG. Cells that had undergone a second recombination event that left behind the *lacI*-Pspac cassette in place of the *spo0K* promoter (excision step) were isolated as described in Materials and Methods. These cells were Cm^s and Spo⁻ in the absence of IPTG and Spo⁺ in the presence of IPTG. Cells Har High Spo⁺ transformant, spo18, sequence (shown), the combination event that left behind the *lacI*-Pspac cassette in place of the *spo0K* promoter (excision step) were isolated as described in Materials and Methods. These cells were Cm^s and Spo⁻ in the absence of IPTG and Spo⁺ in the presence of IPTG. Cells Har High Spo⁺ transformant spo18, so 19, so 10, so 10,

into wild-type cells vielded two types of transformants. One class was Spo⁺ and most likely resulted from recombination with sequences upstream of the promoter. The other class was Spo⁻ in the absence of IPTG and Spo⁺ in the presence of IPTG. This class most likely resulted from recombination with sequences downstream of the promoter. Three Spo+ transformants were chosen and used to screen for a second recombination event that would leave behind the lacI-Pspac cassette in place of the spo0K promoter (Fig. 1B). The Spo+ transformants were grown to stationary phase in LB medium in the absence of selection for the integrated plasmid, that is, in the absence of Cm. We screened ${\sim}13{,}000$ colonies on sporulation plates (lacking IPTG and Cm) and identified two Spo⁻ colonies that were also Cm^s. The sporulation phenotypes of both of these colonies were completely dependent on IPTG; they were Spo+ in the presence of 1 mM IPTG, and Spo- in the absence of IPTG (data not shown). The sporulation and competence phenotypes in the absence of IPTG were indistinguishable from those of a spo0K mutant. One isolate, JRL408 (Pspacspo0K), was chosen for further experiments. Strains containing the Pspac-spo0K fusion as the only copy of spo0K could be made competent under permissive conditions (in the presence of IPTG), and transformants could be screened under nonpermissive conditions (in the absence of IPTG).

Plasmid marker rescue transformation. To facilitate transformation of either of the libraries into *B. subtilis*, we constructed a helper plasmid that could be used in combination with pHP13 (8). The helper plasmid, pJL52, was made by deleting pHP13 from *Bam*HI to *Ncol*. This deletes part of *cat*, and pJL52 does not confer Cm^r but still confers MLS^r.

Conditions for the suppressor screen. Since *spo0K* mutations cause a more severe sporulation defect in the PB2 strain background than in the JH642 background (45), it seemed that a screen for multicopy suppressors of *spo0K* might be more sensitive in the PB2 background. Accordingly, we constructed a PB2 derivative that contained the Pspac-*spo0K* fusion, the helper plasmid pJL52, and a *spoIIA-lacZ* transcriptional fusion (24). This strain, JRL459, was grown in the presence of MLS to maintain pJL52. JRL459 was made competent in S7 minimal medium (plus tryptophan at 40 µg/ml) in 1 mM IPTG to allow expression of *spo0K*. Competent cells were transformed with the pHP13-based multicopy plasmid libraries, and Cm^r transformants were selected on 2×SG plates containing Cm or containing Cm and 5-bromo-4-chloro-3-indolyl-β-p-galactopy-ranoside (X-Gal; 120 µg/ml). Transformants that had a more pronounced Spo⁺ morphology or that were darker blue on X-Gal (indicating increased expression of *spoIIA-lacZ*) than the parent strain were chosen for further analysis.

Sporulation assays. Cells were grown in DS or $2 \times SG$ medium at $37^{\circ}C$ unless otherwise indicated, and spores were assayed approximately 20 h after the end of exponential growth. The number of viable cells per milliliter of culture was determined as the total number of CFU on LB plates. The number of spores per milliliter of culture was determined as the number of CFU after heat treatment ($80^{\circ}C$ for 20 min). Sporulation frequency is the ratio of spores per milliliter.

DNA sequencing. The Sequenase V 2.0 kit (U.S. Biochemical Corp.) was used to sequence double-stranded plasmid DNA. The sequence of one strand or the other was determined from different subclones of pLK2 inserted into pBlue-scriptII KS by using either the universal or reverse primer. To determine the sequence of the opposite strand, primers complementary to the sequence determined with the pBluescript subclones were made, and these primers were used to sequence pLK2.

Primer extension analysis. JRL951 (Δ*spo0K::erm* pLK2) was grown in 2×SG medium, and 50-ml samples were taken at various times for preparation of RNA essentially as described previously (3, 23). The sequence of the primer LKP16 was 5'-TTCTTCAGAAAGCTGTTTATACTTCCATTC-3', and its complement is one of the underlined sequences in Fig. 3. The primer was end labeled with ³²P, essentially as described previously (3), and purified with a NICK spin column by the protocol supplied (Pharmacia). The protocol for hybridization extension



FIG. 2. Map of the kinC region and plasmids. Restriction site abbreviations: R, EcoRV; N, NdeI; P, PstI; C, ClaI; A, ApaI; E, EcoRI.

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EcoRV

1	CTAAAAAAAGGCGGAGTGATATCATCTCCCCCCTTTTTTCGTTGCCCCATTTTTGATTACCGCCGCCTAAGATATCAAGACATCAAGATATTTGGT
01	
91 101	
VinC	
271	
271	
261	
201	
451	
451	V F O T N A K G F I T Y I. N O A W A S I T G F S I S E C M G
541	
241	T M Y N D Y F T K F K H V A D H T N T O T O N K A S S G M F
631	TTACGGCAAAATACGGCACAAAAAAACGGCACGACTTTTTTGGGGAGAGAGTCATTATAAACTTTACGACGAGAGAGA
001	TAKYVTKNGTIFWGEVHYKLYYDRDDOFTG
721	GCAGCCTGGGTACAATGTCAGATATCACTGAGCGGAAAGAGGCTGAAGAGTGAGCTCATTGAGATTAATGAACGGCTGGCGAGGGAATCCC
	SLGTMSDITERKEAEDELIEINERLARESO
811	AGAAACTATCAATCACAAGTGAACTTGCCGCAGGTATTGCTCATGAGGGTCAGAAACCCTTTAACATCTGTCAGCGGTTTTCCTCCAGATTA
	K L S I T S E L A A G I A H E V R N P L T S V S G F L Q I M
901	TGAAAACACAATATCCGGACAGAAAAGACTATTTTGACATCATCTTTTCAGAGATTAAAAGAATCGATTTAGTGCTCAGCGAGCTGCTGC
	K T Q Y P D R K D Y F D I I F S E I K R I D L V L S E L L L
991	TGCTTGCAAAAACCGCAGGCAATCACATTTAAAAACACACCAGCTTAATGAGATCTTGAAACAAGTCACGACATTGCTTGATACCAATGCAA
	LAKPQAITFKTHQLNEILKQVTTLLDTNAI
1081	TTCTGTCCAATATCGTCATAGAGAAAAATTTCAAAGAGACAGATGGCTGTATGATTAATGGAGACGAAAATCAGCTGAAGCAGGTCTTTA
	L S N I V I E K N F K E T D G C M I N G D E N Q L K Q V F I
1171	TCAACATCATTAAAAACGGAATTGAGGCAATGCCAAAGGGCGGTGTCGTAACCATTTCAACTGCTAAAACCGCCTCTCATGCAGTGATAA
	N I I K N G I E A M P K G G V V T I S T A K T A S H A V I S
1261	GCGTAAAGGATGAAGGAAACGGCATGCCGCAGGAAAAGCTGAAGCAGATTGGCAAACCTTTTTATTCAACAAAAAGAAAAGGGCACTGGAC
	V K D E G N G M P Q E K L K Q I G K P F Y S T K E K G T G L
1351	TGGGACTTCCCATTTGTTTGAGAATCCTGAAGGAACATGACGGGGAATTGAAAATCGAAAGTGAAGCTGGAAAAGGCAGCGTCTTTCAAG
	G L P I C L R I L K E H D G E L K I E S E A G K G S V F Q V
1441	TGGTTTTGCCTTTAAAATCAGACAGCTG <u>AGAGGAG</u> AAAAATAAA GTG AACTCGCTTCTGTTTGTATACGGGACATTAAGAAAGCATGAAA
	VLPLKSDS* OFf277 MINSLLFVYGTLRKHEK
1531	AAAACCATCATTTGCTGGCACAATCGGCATGTATCAATGAGCAGGCGAGAACAAAGGGAAGTTTGTTT
	N H H L L A Q S A C I N E Q A R T K G S L F A A K E G P T V
1621	TTGTTTTCAATGATGAAGATGAAGGCTATATTATATGGCCAAGTATATGAAGCAGATGAATTGTGTATACATAAGCTCGATCAATTTTTTTC
1 17 1 1	V F N D E D E G Y I Y G E V Y E A D E L C I H K L D Q F F Q
1/11	AAGGATATCATAAACAGACGITTTTTTTTTTTTTTTTTTT
1001	
1801	
1001	
1031	
1001	
1901	
2071	
2011	A T C V T V V D F C V F C T V F C T V P D A F V D V F A C C D V V
2161	
2T01	\mathbf{K} \mathbf{D} \mathbf{C} \mathbf{L} \mathbf{T} \mathbf{F} \mathbf{L} \mathbf{V} \mathbf{L} \mathbf{O} \mathbf{K} \mathbf{F} \mathbf{A} \mathbf{F} \mathbf{T} \mathbf{A} \mathbf{P} \mathbf{P} \mathbf{O} \mathbf{H} \mathbf{Y} \mathbf{O} \mathbf{T} \mathbf{F} \mathbf{T} \mathbf{F} \mathbf{F} \mathbf{C} \mathbf{A} \mathbf{F}
2251	
	LYLSPETTEKLKRHMNSLPKG
23/1	

FIG. 3. Nucleotide and amino acid sequences of *kinC*. The DNA sequence was determined from both strands as described in Materials and Methods. An inverted repeat surrounding the *Eco*RV site upstream of *kinC* is indicated by a pair of arrows. A putative -10 region for a sigma-A promoter is shown with a thick underline. The transcriptional start site is indicated with an arrow at nucleotide 158. Putative ribosome binding sites for KinC and Orf277 are underlined, and putative translational start sites for KinC and Orf277 are in boldface type. A sequence complementary to primer LKP16, which was used for primer extension analysis, is underlined with a single arrow.

reactions was essentially as described previously (3, 23) except that we used 200 μg of RNA in each sample instead of 50 $\mu g.$

Nucleotide sequence accession number. The *kinC* sequence shown in Fig. 3 has been assigned the data bank accession number L34803.

Transduction. We grew a PBS1-transducing lysate on JRL660 ($\Delta kinC::spc$ trpC2 pheA1) and used it to transduce some of the mapping kit strains (13) essentially as described earlier (12). In the three-factor cross between $\Delta kinC::spc$, spo0E11, and kinA::Tn917, transductants were tested for MLS^r and the colony morphology was analyzed to distinguish between the different classes of recombinants. It was relatively easy to distinguish spo0E11 from kinA::Tn917 from the spo0E11 kinA::Tn917 double mutant.

β-Galactosidase assays. For determination of β-galactosidase specific activity, cells were grown in 2×SG medium and samples were taken at appropriate times. Prior to the enzyme assay, cells were removed by centrifugation and resuspended in Spizizen salts (54). β-Galactosidase specific activity is expressed as ($\Delta 4_{20}$ per minute per milliliter of culture per unit of optical density at 600 nm) × 1,000 (34).

RESULTS

Isolation of genes which, when present on a multicopy plasmid, suppress *spo0K* mutants. *spo0K* null mutants are defective in competence development and have a decreased frequency of transformation. We constructed a conditional *spo0K* mutation so that a multicopy plasmid library could be introduced into the mutant under permissive conditions and be screened for the desired clones under nonpermissive conditions. The conditional allele replaced the normal *spo0K* promoter with the LacI-repressible, IPTG-inducible promoter, Pspac, such that the Pspac-*spo0K* fusion was present as a single copy in the chromosome and was the only copy of *spo0K* (see Materials and Methods; Fig. 1B). Cells containing the Pspac-*spo0K* fusion (JRL408) were Spo⁻ in the absence of IPTG and Spo⁺ in the presence of IPTG. The sporulation frequency in the absence of IPTG was similar to that of a *spo0K* null mutant, while the frequency in the presence of IPTG was similar to that of wild-type cells. In addition, the Pspac-*spo0K* mutant was partly defective in transformation (Com⁻) in the absence of IPTG and Com⁺ in the presence of IPTG.

B. subtilis takes up transforming DNA as a linear single strand, making establishment of a multicopy circular plasmid rather inefficient. However, incoming DNA is able to recombine with homologous sequences very efficiently. To facilitate introduction and establishment of a multicopy plasmid library in *B. subtilis*, we constructed a helper plasmid, pJL52, that could be used in combination with the cloning vector pHP13 (see Materials and Methods). When a competent cell containing pJL52 takes up a plasmid monomer from a pHP13-based library and linearizes it, pJL52 provides the homology necessary for the plasmid to recombine and form a closed circular plasmid. One can select for these recombination events by using the Cm^r marker that is present on pHP13 but not on pJL52. This general process is known as plasmid marker rescue transformation or homology assistance (8, 17).

Two different multicopy libraries (see Materials and Methods) were used to screen for genes which when overexpressed could suppress the sporulation defect caused by mutations in *spo0K*. Strain JRL459 (Pspac-*spo0K* pJL52 *spoIIA-lacZ*) was grown and made competent in the presence of IPTG (phenotypically Spo0K⁺), transformed with plasmid DNA from each library, and screened for sporulation phenotypes in the absence of IPTG (Spo0K⁻). We screened for transformants that had a Spo⁺ colony morphology on sporulation plates or that had increased expression of a *spoIIA-lacZ* fusion, as judged by their blue color on sporulation plates containing X-Gal.

We screened approximately 11,000 transformants (~4,300 from library A and ~6,700 from library B) and found 9 that contained plasmids that reproducibly suppressed the sporulation defect caused by the conditional *spo0K* mutation. To test whether these plasmids suppressed the sporulation defect caused by a true *spo0K* null mutation ($\Delta spo0K::erm$), we isolated plasmid DNA from the candidates and introduced it into the wild type (PB2), a Pspac-*spo0K* mutant (JRL408), and a $\Delta spo0K::erm$ mutant (JRL417). All nine plasmids suppressed the sporulation as well as that caused by the Pspac-*spo0K* mutation.

Restriction mapping of the nine plasmids showed that they fell into two classes, exemplified by the plasmids pLK2 and pLK11. The genes contained on these plasmids were called *mskA* and *mskB*, respectively, where *msk* stands for multicopy suppressor of *spo0K*. These two plasmids suppressed the sporulation defect but not the competence defect caused by null mutations in *spo0K*. Below, we describe the characterization of pLK2.

pLK2 contains a gene (*kinC*) **encoding a histidine protein kinase homologous to KinA and KinB.** Various fragments from the insert in pLK2 were subcloned into the multicopy vector (pHP13) to yield pLK21, pLK22, pLK23, and pLK125 (Fig. 2 and Table 2). These clones were used to identify the region responsible for suppression of the *spo0K* sporulation phenotype. The insert in pLK125 extends from the left end of pLK2 (as drawn in Fig. 2) to the *ApaI* site and completely suppressed the *spo0K* sporulation phenotype. pLK21 contains

```
KinA ...KSEKLSI AGQLAAGIAH EIRNPLTAIK GFLQLM.... KPTMEGNEHY
                                               431
       LARESQKLSI TSELAAGIAH EVRNPLTSVS GFLQIM.... KTQYPDRKDY
KinC
                                                250
       KinB
    .LIHSEKMTI VSELAASVAH EVRNPLTVVR GFVQLLFNDE TLQNKSSADY
                                                252
    FDIVFSELSR IELILSELLM LAKPQQNAVK EYLNLKKLIG EVSALLETQA
                                               481
KinA
    KinC
                                                299
    .:::||:.| : ::.:| :||.| .. | |..::| :...!: . |
KKLVLSELDR AQGIITNYLD MAKQQLYE.K EVFDLSALIK ETSSLMVSYA
                                               301
KinB
KinA
    NLNGIFIRTS Y.EKDSIYIN GDQNQLKQVF INLIKNAVES MPDG.GTVDI
                                                529
      ......
             ILSNIVIEKN FKETDGCMIN GDENQLKQVF INIKNGIEA MPKG.GVVTI
KinC
                                                348
              ...: :| ||...|||.
                             KinB
    NYKSVTVEAE ... TEPDLLIY GDATKLKQA. INLMKNSIEA VPHGKGMIHI
                                                348
    IITEDEHSVH VTVKDEGEGI PEKVLNRIGE PFLTTKEKGT GLGLMVTFNI 579
KinA
             STAKTASHAV ISVKDEGNGM PQEKLKQIGK PFYSTKEKGT GLGLPICLRI
KinC
                                                398
     . .
           SAKRNGHTIM INITDNGVGM TDHQMQKLGE PYYSLKTNGT GLGLTVTFSI
                                                398
KinB
KinA
    IENHQGVIHV DSHPEKGTAF KISFPKK*
                               606
      .:|:| :.: :|.::||..|
    LKEHDGELKI ESEAGKGSVF QVVLPLKSDS* 428
KinC
    KinB
```

FIG. 4. Comparison of the C-terminal region of KinC to the C-terminal regions of KinA and KinB. The sequences are aligned according to the PILEUP program of the University of Wisconsin Genetics Computer Group (14). The first residues shown are amino acids 389, 204, and 205 for KinA, KinC, and KinB, respectively. In the regions shown, KinC is 51% identical to KinA and 36% identical to KinB. Residues that are highly conserved in the family of sensor kinases (1, 37) are indicated in slightly enlarged boldface type. In KinC, these residues include the conserved histidine that is thought to be the site of phosphorylation at position 223, the asparagine at position 331, and the two glycinerich regions at positions 363 to 367 and 387 to 391.

the right end of the insert in pLK2 and ends at the *Pst*I site and partially suppressed the sporulation phenotype. pLK22 and pLK23 did not suppress the *spo0K* mutant. These results indicate that the suppressing activity is located upstream of the *Apa*I site.

We determined the DNA sequence from the leftmost *Eco*RV site shown in Fig. 2 to the right end of the pLK2 insert. Analysis of the sequence (Fig. 3) revealed two open reading frames. The predicted protein product of the first open reading frame, kinC, is 428 amino acids long and is homologous to histidine protein kinases. KinC is most similar to the two histidine protein kinases involved in sporulation, KinA (2, 38) and KinB (58). In the carboxy-terminal 213 amino acids of the proteins, KinC has 111 residues (51%) that are identical to those of KinA, and 81 (36%) that are identical to those of KinB (Fig. 4). The ability to suppress the spo0K sporulation defect was localized to the kinC open reading frame (as discussed above). pLK21, which partially suppresses the sporulation defect, is truncated in the 5' end of the open reading frame and probably produces a protein fragment that is likely expressed from a plasmid promoter.

Just downstream of kinC, there is an open reading frame predicted to encode a protein of 277 amino acids, Orf277 (Fig. 3). The *orf277* gene product does not appear to be similar to any other protein in the database. In particular, it does not seem to be a member of the response regulator family of proteins (sensor proteins and their cognate response regulators often occur in the same operon [1]), nor is it similar to KapB, the product of the gene downstream of *kinB* (58).

Effects of overexpression of *kinC* in different *spo0* mutants. We tested the ability of multicopy *kinC* (pLK2) to suppress the sporulation defect caused by *spo0* mutations. In all cases, the

TABLE 3. Suppression of spo0 mutants by multicopy kinC

Delevent construct of strain	Sporulation frequency ^a		
harboring plasmid	pHP13 (vector)	pLK2 (multicopy kinC)	
WT ^b (JH642)	0.41	0.81	
$\Delta spo0K::erm$	$6.0 imes 10^{-3}$	0.63	
kinA::Tn917	$9.8 imes 10^{-2}$	0.28	
spo0A9V	$< 9.0 imes 10^{-8}$	$< 8.2 \times 10^{-8}$	
spo0J93	$2.1 imes 10^{-4}$	0.21	
spo0E11	$2.5 imes 10^{-3}$	$6.9 imes 10^{-3}$	
$spo0F\Delta S$	$<\!\!2.4 imes 10^{-7}$	9.8×10^{-3}	
$spo0B\Delta Pst$	$< 4.1 \times 10^{-7}$	1.1×10^{-2}	
$spo0F\Delta S spo0B\Delta Pst$	${<}1.8 imes10^{-7}$	$2.5 imes 10^{-2}$	
$spo0F\Delta S spo0B\Delta Pst \Delta spo0E::spc$	8.5×10^{-7}	0.22	

^a Cells were grown in DS medium with Cm, and sporulation frequency was determined as described in Materials and Methods.

^b WT, wild type.

sporulation frequency was measured in otherwise isogenic strains containing the specific sporulation mutation and either pLK2 or the cloning vector, pHP13. pLK2 significantly suppressed the sporulation defect caused by *spo0K*, *spo0J*, *spo0F*, and *spo0B* mutations (Table 3). In addition, it also suppressed the sporulation defect of a *kinA* mutant (Table 3). Multicopy *kinC* did not suppress the sporulation defect of the *spo0A9V* mutant or that of the *spo0E11* mutant. Because multicopy *kinC* is able to significantly bypass the need for *spo0F* and *spo0B* in sporulation, it appears that KinC, at least when overexpressed, is able to act directly on Spo0A.

The effect of pLK2 on the *spo0B* mutant makes it possible to determine the likely target of Spo0E in vivo. Recent in vitro experiments demonstrate that Spo0E is a phosphatase that removes phosphate from Spo0A \sim P (35). *spo0E11* is a non-sense mutation that produces an N-terminal fragment of Spo0E and inhibits sporulation (41), most likely because of increased phosphatase activity (35).

If the in vivo target of Spo0E is one of the components of the phosphorelay (Spo0F or Spo0B), then *spo0E* mutations should have little or no effect in the absence of a functioning phosphorelay. On the other hand, if the in vivo target of Spo0E is Spo0A, as suggested by the in vitro results (35), then *spo0E* mutations are likely to have effects in the absence of the phosphorelay, if they can be measured. pLK2 partially suppressed the sporulation defect of a *spo0F spo0B* double mutant (Table 3), and this suppression was enhanced by a null mutation in *spo0E* (Table 3; *spo0F spo0B* Δ *spo0E*::*spc*). Since the *spo0E* mutation causes a phenotype in the absence of Spo0F and Spo0B, Spo0E is probably inhibiting Spo0A directly, consistent with the in vitro results.

kinC null mutations have no significant effect on sporulation. To characterize the phenotypes caused by loss of kinC, we constructed a strain (JRL660) containing a deletion-insertion mutation in kinC, $\Delta kinC::spc$ (Fig. 2; see Materials and Methods). The $\Delta kinC::spc$ mutation had little or no effect on the sporulation frequency, compared with that of the wild type (Table 4). Sporulation conditions tested included 2×SG medium (Table 4), DS medium, minimal exhaustion medium, and minimal medium with decoyinine (data not shown). In addition, the $\Delta kinC::spc$ mutation did not alter the sporulation defect caused by mutations in kinA, spo0K, or spo0J (data not shown). The $\Delta kinC::spc$ mutation also had little or no effect on competence development or expression of comG-lacZ compared with that of otherwise isogenic kinC⁺ cells (data not shown).

TABLE 4. *kinC* requirements of strains for suppression of *spo0B*\Delta*Pst* by *sof-1* or *rvtA11*

Strain	Relevant genotype	Sporulation frequency ^a
JH642	WT ^b	0.59
JRL753	$\Delta kinC::spc$	0.92
JRL791	$\Delta kinC::spc \ rvtA11$	0.51
JRL790	$\Delta kinC::spc \ sof-1$	0.70
JRL766	$spo0B\Delta Pst$	$< 4.1 \times 10^{-8}$
JRL763	$spo0B\Delta Pst \ sof-1$	$3.6 imes 10^{-4}$
JRL767	spo0B Δ Pst sof-1 Δ kinC	$1.4 imes 10^{-7}$
JRL764	$spo0B\Delta Pst \ rvtA11$	2.1×10^{-3}
JRL768	spo0B Δ Pst rvtA11 Δ kinC	${<}2 imes10^{-8}$
JRL783	$spo0F\Delta S \ spo0B\Delta Pst \ rvtA11$	0.73
JRL792	$spo0F\Delta S$ $spo0B\Delta Pst$ $rvtA11$ $\Delta kinC$	$< 1 \times 10^{-7}$
JRL770	$spo0B\Delta Pst$ rvtA11 kinA	$1.2 imes 10^{-5}$
JRL794	spo $0F\Delta S$ spo $0B\Delta Pst$ rvt $A11$ kin A	0.34

 a Cells were grown in 2×SG medium, and sporulation frequency was determined as described in Materials and Methods.

^b WT, wild type.

KinC is required for sof-1 and rvtA11 to suppress the sporulation defect of spo0B mutants. The sof-1 and rvtA11 alleles of spo0A bypass or partially bypass the sporulation defect caused by mutations in *spo0F* and *spo0B*, possibly because these altered forms of Spo0A will accept phosphate from kinases independently of Spo0F and Spo0B. To determine whether KinC is one such kinase, we introduced the $\Delta kinC$::spc mutation into a spo0B mutant that had either the sof-1 or rvtA11 allele of Sp00A. The kinC mutation completely abolished the suppressing effect of either allele (Table 4). In addition, kinC was required for sof-1 and rvtA11 to suppress spo0F mutants (data not shown). kinC, and not orf277 or any other gene downstream of kinC, was responsible for this phenotype. Disruption of orf277 by integrating pLK126 (Fig. 2) by a single crossover had no effect on the ability of rvtA11 to suppress spo0F221 (data not shown). These results indicate that KinC is the major kinase that donates phosphate to the sof-1 and rvtA11 forms of Spo0A in the absence of Spo0F or Spo0B.

Additional results suggest that KinC can also act on Spo0F and, in some conditions, might prefer Spo0F to Spo0A. The incomplete suppression of the spo0B mutant by sof-1 or rvtA11 was due to the presence of $spo0F^+$. rvtA11 allowed spo0F*spo0B* double mutants to sporulate at a much higher frequency than the otherwise isogenic *spo0B* single mutant, and this suppression was completely dependent on kinC (Table 4). Null mutations in kinA significantly reduced the sporulation frequency of the spo0B rvtA11 strain but had no significant effect on sporulation of the otherwise isogenic spo0F spo0B rvtA11 strain (Table 4). Together, these results indicate that KinC probably interacts with Spo0F and that the presence of Spo0F inhibits the ability of KinC to donate phosphate to Spo0A^{*vtA11*}. Furthermore, the absence of KinA probably increases the amount of unphosphorylated Spo0F, increasing the KinC diverted from Spo0A^{rvtA11} and decreasing the ability of the cells to sporulate. These results suggested that KinC normally can interact with Spo0F and probably contributes to the initiation of sporulation.

Genetic mapping of *kinC*. We used generalized transduction with PBS1 to determine the chromosomal map location of *kinC*. A PBS1 lysate was made from JRL660 ($\Delta kinC::spc$) and used to transduce the mapping kit strains (13) to Spec^r. The initial results indicated that *kinC* was ~15 to 20% cotransduced with *pyrD*⁺. A series of two-factor crosses indicated that *kinC* was ~50% linked to *spo0E* and ~50% linked to *kinA*. To map *kinC* with greater resolution, we did a three-factor cross



FIG. 5. Primer extension analysis of *kinC* mRNA. RNA was obtained as described in Materials and Methods, and extensions were performed with primer LKP16 (Fig. 3). The sequencing ladder (lanes G, A, T, and C) was constructed from pLK2 by using primer LKP16. The KinC band is indicated by an arrow.

with $\Delta kinC$::spc, spo0E11, and kinA::Tn917. Strain KI644 (kinC⁺ spo0E11 kinA::Tn917) was transduced to Spec^r with a PBS1 lysate grown on JRL660 ($\Delta kinC$::spc spo0E⁺ kinA⁺). spo0E⁺ was cotransduced with $\Delta kinC$::spc in 107 of 199 transductants, and kinA⁺ was cotransduced with $\Delta kinC$::spc in 147 of 199 transductants. Every spo0E⁺ transductant was also kinA⁺ (MLS^s), indicating that the gene order is spo0E-kinA-kinC.

Localization of the promoter and primer extension analysis. Preliminary analysis indicated that the kinC promoter was between the EcoRV and NdeI sites upstream of the kinC open reading frame (Fig. 2). This was determined by integrating various plasmids into a spo0F rvtA11 strain (KI1521). If the insert in the plasmid was internal to the kinC transcription unit, then integrating the plasmid would disrupt kinC and the strain would become Spo⁻. If the plasmid extended past the 5' end of the transcription unit (or past the 3' end of kinC), then integrating the plasmid would not disrupt kinC and the cells would remain Spo⁺. Integration of pLK121 and pLK124 resulted in a Spo⁻ phenotype, while integration of pLK120 maintained the Spo⁺ phenotype. These results indicated that the promoter region of kinC was probably between the upstream endpoint of pLK120 (EcoRV) and the upstream endpoint of pLK121 (NdeI).

We performed primer extension analysis, using primer LKP16 (see Materials and Methods; Fig. 3), to localize the 5' end of the *kinC* mRNA. RNA was prepared from strains grown in $2\times$ SG medium. Despite repeated attempts, we could not detect *kinC*-specific RNA from wild-type cells. However, a *kinC* transcript was easily detected from a strain (JRL951) with multicopy *kinC* (Fig. 5). The 5' end of the mRNA is ~25 nucleotides upstream of the *kinC* start codon (Fig. 3 and 5). Just upstream of the putative start site is a sequence that matches the consensus for the -10 region of sigma-A promoters in five of six positions (Fig. 3). However, sequences located in the -35 region do not show a striking resemblance to the -35 consensus sequence for sigma-A promoters, perhaps explaining the evidently low level of expression of *kinC*. Just



FIG. 6. Expression of *kinC-lacZ*. Strain JRL812 [*thrC::(kinC-lacZ erm*)] was grown in $2\times$ SG medium, and samples were taken at the indicated times for determination of β -galactosidase specific activity. Similar results were obtained with cells grown in DS medium, except that specific activity levels were reduced (data not shown).

upstream of the putative -10 region is a perfect 0A box, TGNCGAA, the consensus binding site for Spo0A (56). On this basis, one might expect Spo0A or Spo0A~P to act as a repressor of *kinC*. However, a *spo0A* null mutation had relatively little effect on *kinC-lacZ* expression (as discussed below). This could indicate that Spo0A does not control expression of *kinC* or that the effect of the *spo0A* mutation was masked by other regulatory factors.

kinC is expressed as cells enter stationary phase. To determine how kinC is normally expressed, we made a kinC-lacZ fusion and introduced it into the chromosome by double-crossover recombination at the *thrC* locus by using pLK114 (Fig. 2; see Materials and Methods). The kinC-lacZ fusion contains all of the sequences upstream of the kinC open reading frame that are present in pLK2. The level of expression of kinC-lacZ was low during exponential growth in 2×SG medium and increased as the cells approached stationary phase (Fig. 6). The low level of β-galactosidase specific activity made it difficult to reliably determine quantitative effects of various regulatory mutations on kinC-lacZ expression. However, there seemed to be no significant effect of mutations in spo0A, spo0B, spo0F, spo0H, spo0K, kinA, abrB, comP, comA, and sin on expression of the kinC-lacZ fusion (data not shown). Of course, we cannot rule out the possibility that there is an effect under other conditions or in other strain backgrounds.

DISCUSSION

We have isolated and characterized *B. subtilis kinC*, a gene which when present on a multicopy plasmid suppresses the sporulation defect caused by a null mutation in *spo0K*. Identification of genes on the basis of phenotypes caused by increased expression is a relatively general approach that is easier than and can sometimes be used in place of isolating gain-of-function mutations. In addition, genes that might be difficult to identify on the basis of phenotypes caused by loss-of-function mutations are sometimes easier to identify on the basis of phenotypes caused by loss-of-function mutations are sometimes easier to identify on the basis of phenotypes caused by overexpression.

The *kinC* gene product is homologous to histidine protein kinases and is most similar to KinA and KinB, the histidine protein kinases involved in the initiation of sporulation (2, 38, 58). On the basis of this homology and the phenotypes caused by overexpression and deletion of *kinC*, we infer that the *kinC* gene product is a histidine protein kinase. *kinC* in multicopy partly bypasses the need for *spo0F* and *spo0B* in sporulation, suggesting that KinC, at least when overexpressed, is able to donate phosphate directly to Spo0A. Mutations in *spo0A* (*sof-1* and *rvtA11*) that bypass the need for the phosphorelay require *kinC* in order to support sporulation. Thus, KinC appears to be the primary kinase responsible for phosphorylation of the *sof-1*

and *rvtA11* forms of Spo0A and for sporulation in these strains in the absence of the phosphorelay.

We suspect that KinC is also able to donate phosphate to Spo0F. Spo0F is a response regulator protein and presumably obtains phosphate in vivo from KinA and KinB. We found that the presence of $spo0F^+$ inhibited the ability of a spo0B rvtA11 mutant to sporulate (Table 4). A simple interpretation of this finding is that Spo0F can compete with Spo0A^{rvtA} for access to KinC~P. This would suggest that KinC might normally play a role in donating phosphate to Spo0F in wild-type cells.

In addition to the three kinases and Spo0F and Spo0B, other proteins also control accumulation of Spo0A~P in vivo, either by regulating activity of the phosphorelay or by acting on Spo0A directly. *spo0E* encodes a phosphatase that is an inhibitor of the initiation of sporulation (35, 41). In vitro, the target of the Spo0E phosphatase is Spo0A~P, suggesting that in vivo Spo0A~P might also be the primary target (35). We used multicopy *kinC* to show that Spo0E can function independently of the phosphorelay (Table 3), indicating that the in vivo target is probably Spo0A~P, consistent with the in vitro results.

One function of the phosphorelay is to integrate the multiple developmental signals that regulate the initiation of sporulation (9, 22, 24–26). One of the developmental signals controlling the activity of the phosphorelay is related to the state of DNA replication. The activity of the phosphorelay is inhibited when DNA replication is inhibited. We were able to use multicopy *kinC* and a *kinC* mutant (previously called *mskA*) to show that the target of the DNA replication control is not Spo0A but probably Spo0F or Spo0B (24).

A role for KinC in sporulation? Null mutations in kinC do not cause any obvious defect in sporulation under standard laboratory conditions, suggesting that KinC might not normally be involved in sporulation. However, as discussed above, we suspect that KinC normally contributes some phosphate to Spo0F and is partly redundant with KinA and KinB. While KinA and KinB appear to be responsible for most of the Spo0A~P that accumulates during the initiation of sporulation, there is some Spo0A~P in even a *kinA kinB* double mutant (58) and we suspect that this comes from KinC. In addition, there are many ways for cells to deplete nutrients and enter the sporulation pathway. It seems possible that the relative contributions of the individual kinases might vary depending on the specific condition that induces sporulation.

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