JOURNAL OF BACTERIOLOGY, Oct. 1989, p. 5347–5353
0021-9193/89/105347-07$02.00/0

Vol. 171, No. 10

# Cloning and Characterization of *srfB*, a Regulatory Gene Involved in Surfactin Production and Competence in *Bacillus subtilis*

MICHIKO M. NAKANO†* AND PETER ZUBER†

*Department of Botany and Microbiology, Oklahoma State University, Stillwater, Oklahoma 74078*

Received 1 March 1989/Accepted 26 June 1989

**A Tn*917* insertion mutation *srfB* impairs the production of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. *srfB* is located between *aroG* and *ald* in the *B. subtilis* genome, as determined by phage PBS1 transduction mapping, and is not linked to the previously described surfactin loci *sfp* or *srfA*. A *srfB* mutant was found to be also deficient in the establishment of competence. SPβ phage-mediated complementation analysis showed that both competence and surfactin production were restored in the *srfB* mutant by a single DNA fragment of 1.5 kilobase pairs. The sequence of the complementing DNA revealed that the *srfB* gene is *comA*, an early competence gene which codes for a product similar to that of the activator class of bacterial two-component regulatory systems. The *srfB* mutation impaired the expression of a *srfA-lacZ* fusion, suggesting that surfactin production is positively regulated at the transcriptional level by the *srfB* (*comA*) gene product.**

A cyclic lipopeptide, surfactin, which is produced by *Bacillus subtilis* ATCC 21332, is one of the most powerful biosurfactants known (2). Biosynthesis of surfactin occurs nonribosomally by a multifunctional enzyme system (14) similar to those used in the synthesis of other peptide antibiotics of bacilli (12, 13).

In a previous paper (19), we reported the identification of two loci, *sfp* and *srf*, which are involved in the production of surfactin and which are tightly linked in the *B. subtilis* genome. *sfp* is the locus which, when transferred from the surfactin-producing ($Srf^+$) strain ATCC 21332 to JH642, a derivative of the standard *B. subtilis* strain 168 and a nonproducer ($Srf^-$), renders JH642 cells $Srf^+$. It is not known whether the product of *sfp* serves a regulatory role in surfactin production or whether it encodes an enzyme that completes the surfactin biosynthetic pathway. The *srf* locus was identified as a Tn*917* insertion mutation (32) which rendered *sfp*-bearing cells $Srf^-$. Evidence from previous studies has shown that the *srf* locus is present in the cells of the nonproducing strain (19). The *srf* locus encompasses more than 15 kilobase pairs (kb) of DNA, suggesting that *srf* contains the genes encoding the enzymes that catalyze the biosynthesis of surfactin. This is in accordance with the observation that the enzymes which catalyze the synthesis of the peptide antibiotics tyrocidine and gramicidin have very high molecular weights and that the genes which code for them are clustered into large operons (M. A. Marahiel, M. Krause, G. Mittenhuber, and R. Weckermann, unpublished data). The transcription of *srf*, as judged by the *srf-lacZ* fusion experiment (19), increased dramatically after the beginning of the stationary phase, which is in keeping with the observed increase of antibiotic production in stationary-phase cultures of *Bacillus* cells.

In this report we describe the identification of a third locus, called *srfB*, that is required for surfactin production. We also show that *srfB* is necessary for the expression of *srfA-lacZ* (formerly *srf-lacZ*) and is identical to *comA*, an early competence gene isolated by D. Dubnau and coworkers (7, 29).

* Corresponding author.

† Present address: Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, 1501 Kings Highway, Shreveport, LA 71130.

## MATERIALS AND METHODS

**Strains and plasmids.** The *B. subtilis* strains used in this study are listed in Table 1. *Escherichia coli* AG1574 [*araD139* Δ(*ara leu*)*7697* Δ*lacX74 galUK* $r^-$ $m^+$ *strA recA56 srl*) was obtained from A. Grossman and was used for cloning *B. subtilis* DNA. *E. coli* GM119 (*dam*) was obtained from R. Nutter and was used as a host to prepare plasmids for restriction endonuclease cleavage by using *Bcl*I and *Cla*I. Plasmids pTV20 and pTV21 were provided by P. Youngman (33) and were used to clone *B. subtilis* chromosomal DNA adjacent to the Tn*917* insertion. pMMN7 and pMMN13 are derivatives of pGEM4 (Promega Biotech) and carry the chloramphenicol resistance ($Cm^r$) gene from plasmid pMI1082 (constructed by M. Igo) (34) in opposite orientations.

**Media.** Preparation of 2× yeast tryptone (2×YT), LB, and DS media have been described previously (19). Surfactin production was assayed on a blood agar plate as described previously (19). An assay for antibiotic production was performed by replica plating colonies onto LB agar overlayed with LB soft agar containing Spo0A (ZB515) or *Staphylococcus aureus* cells. Protease production was assayed on an LB agar plate overlayed with LB soft agar containing 3% skim milk. Macrolide-lincosamide-streptogramin B-resistant ($MLS^r$) and chloramphenicol-resistant ($Cm^r$) cells were selected as described in a previous report (19).

**Transformation and transduction.** Transformation and phage PBS1 transduction procedures have been described previously (19). Development of competence was determined at time intervals after 5 ml of cells cultured in GM1 medium (20) was transferred to 45 ml of GM2 medium (20). One milliliter of each culture was assayed for competence by using 0.3 μg of chromosomal DNA from strain IS251, and a number of $Phe^+$ transformants were scored.

**SPβ phage technique.** Complementation analysis of the *srfB*::Tn*917* mutant was performed by using SPβ, a temperate phage of *B. subtilis*. Construction of SPβ derivative phage SPβ*c2del2*::Tn*917*::pSK10Δ6 has been described previously (35). Cells of strain ZB307A, a lysogen of pro-

TABLE 1. *B. subtilis* strains used in this study

| Strain | Genotype | Source or reference |
|---|---|---|
| JH642 | *trpC2 pheA1* | J. Hoch |
| IS251 | *trpC2 spo0BΔPst* | I. Smith |
| IA9 | *ald-1 aroG932 leuA8 trpC2* | Bacillus Genetic Stock Center |
| IA10 | *hisA1 thr-5 trpC2* | Bacillus Genetic Stock Center |
| ZB307A | Lysogen SPβ*c2del2*::Tn*917*::pSK10Δ6 | 35 |
| ZB449 | *trpC2 pheA1 abrB703* SPβ cured | |
| ZB515 | *trpC2 pheA1 spo0AΔ204* | |
| OKB105 | *pheA1 sfp* | 19 |
| OKB125 | *pheA1 sfp srfA*::Tn*917*::*lac-55* | 19 |
| OKB143 | *pheA1 srfA*::Tn*917*::*lac-55* | 19 |
| OKB167 | *pheA1 sfp srfB*::Tn*917lac* | This work |
| OKB171 | *pheA1 sfp srfB*::Tn*917lac*::pTV21 | This work |
| OKB173 | *pheA1 sfp srfB*::Tn*917lac*::pTV20 | This work |
| OKB192 | *trpC2 pheA1 srfB*::Tn*917lac* | This work |
| OKB195 | *pheA1 sfp*; p167-20H plasmid integrant | This work |
| OKB206 | *trpC2 pheA1*; lysogen SPβ*c2del2*::Tn*917*::pSK10Δ6::p195S | This work |
| OKB207 | *trpC2 pheA1*; lysogen SPβ*c2del2*::Tn*917*::pSK10Δ6::pMMN11 | This work |
| OKB210 | *pheA1 sfp srfA*::Tn*917*::*lac-55 srfB*::Tn*917lac* | This work |
| OKB211 | *pheA1 srfA*::Tn*917*::*lac-55 srfB*::Tn*917lac* | This work |

phage SPβ*c2del2*::Tn*917*::pSK10Δ6, was transformed with pMMN7 or pMMN13 plasmids containing *B. subtilis* chromosomal DNA. Circular plasmid DNA recombined into the SPβ prophage of ZB307A by a Campbell-like recombination mechanism at the regions of pBR322 homology present in both SPβ*c2del2*::Tn*917*::pSK10Δ6 DNA and the pMMN derivatives. To isolate SPβ derivatives carrying *B. subtilis* DNA, Cm$^r$ transformants were pooled and an SPβ lysate was generated by heating a mid-log-phase culture of pooled transformants in 2×YT medium at 52°C for 5.5 min. Incubation was continued at 37°C until cells were lysed. The lysate (primary lysate) was filtered through a 0.2-μm-pore-size filter and stored at 4°C. Mid-log-phase cultures of an SPβ-cured strain, ZB449, were infected with 40 μl of the primary phage lysate that was obtained as described above and incubated for 1 h in 2×YT medium containing levels of chloramphenicol (0.5 μg/ml) which induced the expression of the *cat* gene within the plasmid constructs. Lysogens were selected for chloramphenicol resistance, and the secondary lysate was prepared as described above for preparation of the primary lysate. The secondary lysate was used as a source of phage for complementation analysis.

**β-Galactosidase assay.** Assay of β-galactosidase specific activity in cells cultured in DS medium was done as described previously (19).

**DNA sequencing.** DNA sequencing was done by the dideoxy chain-termination method of Sanger et al. (25). Single-stranded templates were prepared by using M13 vectors (17), and [α-$^{35}$S]dATP and T7 DNA polymerase (Sequenase; U.S. Biochemical Corp.) were used in the sequencing reactions (26). M13 clones that were used in the sequencing reactions contained the two *Bcl*I-*Cla*I fragments of the *comA* (*srfB*) region (see Fig. 4).

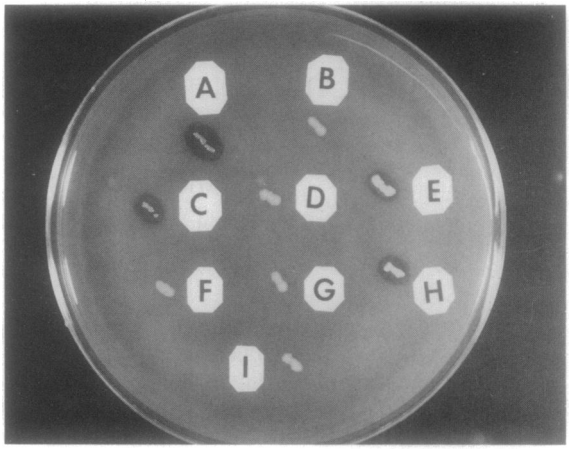



FIG. 1. Surfactin production of various derivatives assayed on MM blood agar plate. The MM blood agar plate was prepared as described in a previous report (19). (A) OKB105; (B) OKB167; (C to I) OKB167-lysogenized SPβ derivatives containing p195S (C), pMMN10 (D), pMMN11 (E), pMMN14, (F), pMMN15 (G), pMMN16 (H), and pMMN18, (I).

## RESULTS

**Isolation of surfactin-nonproducing (*srf*) mutant.** In a previous study (19), we constructed a surfactin-producing derivative (OKB105) of *B. subtilis* 168, which was then used to identify Srf$^-$ Tn*917* insertion mutants. To search for more Srf$^-$ mutants, competent cells of OKB105 were transformed with chromosomal DNA prepared from a Tn*917lacZ* library (21) of strain YB886, which was obtained from R. Yasbin (16). Transformants bearing Tn*917lacZ* insertions were obtained by selecting for MLS$^r$, which was conferred by Tn*917*. Among 280 MLS$^r$ transformants, two *srf* mutants were isolated by screening the transformant colonies on blood agar plates (19). A mutant, OKB167, showed drastically reduced surfactin activity on a blood agar plate (Fig. 1) and was chosen for further characterization.

The effect of the mutation on other activities associated with the stationary phase was examined. Colonies of OKB167 cells were observed to sporulate and to produce both protease and antibiotics against antibiotic-sensitive *B. subtilis* Spo0A cells and *S. aureus* cells to wild-type levels. Of the stationary-phase-associated phenotypes, besides surfactin production, only competence was affected by the mutation in OKB167 cells (see below). The new locus required for surfactin production was designated *srfB*, and the locus identified previously, *srf* (19), was renamed *srfA*.

**Genetic mapping of *srfB*::Tn*917*.** By taking advantage of the MLS$^r$ marker of the *srfB*::Tn*917* insertion, PBS1 transduction mapping was performed to determine the genomic location of the *srfB* locus. By using strain IA9 as a recipient, *srfB*::Tn*917* was found to map to an area near 280° on the genetic map (22) with 74% cotransduction frequency to *ald* and with 14% cotransduction frequency to *aroG*. By using strain IA10 as a recipient, *srfB*::Tn*917* cotransduced 57% with *thrA*. Thus, we found that *srfB* was unlinked to the two previously identified loci required for surfactin production; the genomic location of these two loci were identified near 40° on the genetic map.

**Precise location of *srfB*::Tn*917*.** The technique developed by Youngman et al. (33) was used to clone chromosomal DNA adjacent to *srfB*::Tn*917*. Plasmids pTV20 and pTV21 are pBR322 derivatives which carry ampicillin resistance

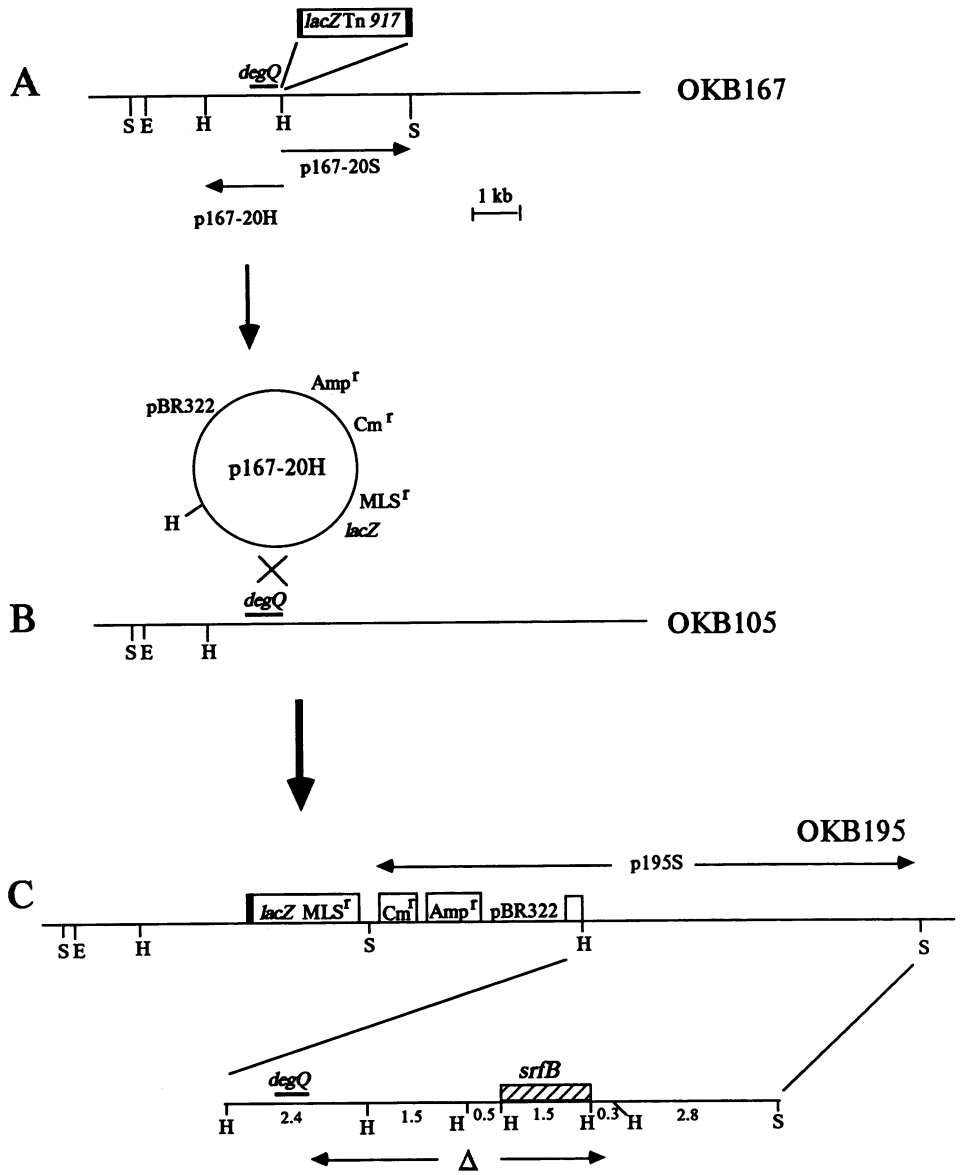
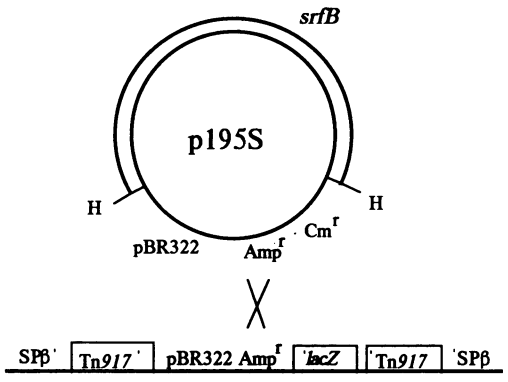



FIG. 2. Molecular cloning of *srfB* and restriction map of the *srfB* region. (A) *degQ* region of the OKB167 chromosome. The locations of *degQ* and *srfB*::Tn*917lacZ* are shown. (B) Diagram of the recombination event between plasmid p167-20H and the *degQ* region of OKB105, which gave rise to the plasmid integrant OKB195 (see panel C). (C) Restriction enzyme map of OKB195 chromosomal DNA between *Hin*dIII and *Sph*I. The numbers below the restriction enzyme map indicate the sizes (in kilobases) of each *Hin*dIII fragment. The hatched box shows the *srfB* gene determined by the SPβ phage complementation experiment (see Fig. 4). Δ designates the region deleted from OKB167 chromosomal DNA. Restriction enzyme recognition sites; E, *Eco*RI; H, *Hin*dIII; S, *Sph*I.

(Amp$^r$) and Cm$^r$ genes in opposite orientations with Tn*917* DNA. pTV20 and pTV21 were made linear by *Pst*I digestion and were then used to transform OKB167 competent cells. The plasmid integrated into the Tn*917* locus of OKB167 chromosomal DNA by marker replacement recombination, thereby placing the pBR322 moiety within the middle of the transposon. Chromosomal DNAs from two transformants, OKB173 (with integrated pTV20) and OKB171 (with integrated pTV21), were digested with various restriction enzymes, diluted, and ligated under conditions that promoted intramolecular ligation. This procedure resulted in pBR322 derivatives bearing chromosomal DNA that flanked the transposon. The ligation mixtures were used to transform *E. coli* to Amp$^r$.

The chromosomal DNA adjacent to *srfB*::Tn*917* thus cloned was subcloned into M13 phage vectors and was subject to nucleotide sequence analysis. The sequencing data of chromosomal DNA from plasmid p167-20H (obtained from *Hin*dIII-digested OKB173 chromosomal DNA) showed that the Tn*917* insert in OKB167 was located downstream of the *sacQ* gene (renamed *degQ* by D. Henner et al. [9]). Tn*917* was inserted about 40 base pairs downstream of the inverted repeat (possible transcription terminator) following the *degQ*-coding region (the sequence is presented in Fig. 1 of reference 31). Chromosomal DNA adjacent to the *lacZ* distal end of Tn*917* was also sequenced and was not found to be homologous to the *degQ* downstream sequence reported by Yang et al. (31). The sequencing results suggested that the *srfB* mutation is a deletion of the DNA adjacent to the Tn*917* insertion.




SPβ *c2del2* ::Tn*917* ::pSK10Δ6

ZB307A

FIG. 3. Construction of phage SPβ*c2del2*::Tn*917*::pSK10Δ6:: p195S. The diagram shows the recombination event between the SPβ prophage of strain ZB307A and the *srfB*-bearing plasmid p195S. The open box on the plasmid diagram indicates the location of the *B. subtilis* chromosomal DNA containing the *srfB* gene. H, *Hin*dIII.

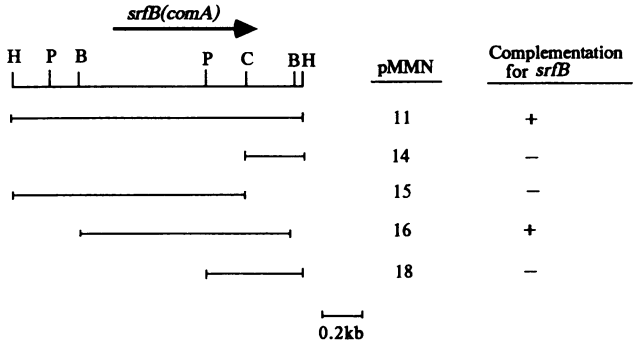



FIG. 4. Complementation by SPβ specialized transduction for *srfB*. The entire *Hin*dIII fragment or subfragments of it were recloned into pMMN7 or pMMN13. The resultant plasmids designated as pMMN numbers on the right side of each map were inserted into SPβ*c2del2*::Tn*917*::pSK10Δ6 through the recombination event shown in Fig. 3 and were tested for the ability of surfactin production to rescue strain OKB167 (*srfB*). The assay of strains on the blood agar plate is shown in Fig. 1. The arrow shows the location and orientation of the *srfB* open reading frame, as determined by nucleotide sequence analysis. Restriction enzyme recognition sites: B, *Bcl*I; C, *Cla*I; H, *Hin*dIII; P, *Pvu*II.

**Restriction enzyme map of the *degQ-srfB* region.** The next step in the identification of the *srfB* locus was the construction of a restriction enzyme map of the *degQ-srfB* region. The DNA deleted from the *srfB* mutant was first cloned from cells of OKB105 (Srf$^+$). Competent OKB105 cells were transformed with p167-20H with selection for Cm$^r$ and MLS$^r$. A Cm$^r$ (MLS$^r$) transformant, OKB195, incorporated p167-20H into its chromosome by a Campbell-like recombination (Fig. 2B), which resulted in a partial diploid strain bearing an intact copy of the *srfB* region. Plasmid p195S, which contained the region that was deleted in OKB167, was isolated by digesting OKB195 chromosomal DNA with *Sph*I followed by intramolecular ligation as described above (Fig. 2C). A comparison of the restriction enzyme maps and partial sequence data of p167-20S (Fig. 2A) and p195S revealed the extent of the *srfB* deletion. The deletion endpoint distal to Tn*917lacZ* insert was identified by comparing the sequence of the OKB167 DNA contained within p167-20S with that of the 0.3-kb *Hin*dIII fragment (the sequence was provided by Y. Weinrach and D. Dubnau) shown in Fig. 2C. About 5 kb of DNA adjacent to the Tn*917* insert was deleted in OKB167 (Fig. 2).

**Complementation by SPβ specialized transduction for *srfB*.** SPβ specialized transduction was carried out to determine whether the plasmid p195S could restore the Srf$^+$ phenotype to the *srfB* mutant by complementation. ZB307A, a lysogen of the phage SPβ*c2del2*::Tn*917*::pSK10Δ6 was transformed with p195S with selection for Cm$^r$ to construct SPβ*c2del2*:: Tn*917*::p195S (Fig. 3), as described in the Materials and Methods. After purification of the SPβ*c2del2*::Tn*917*::p195S phage by passage through ZB449 cells, the specialized transducing lysate was used to lysogenize cells of strain OKB167 with selection for Cm$^r$. Lysogens were subsequently screened on blood agar plates for surfactin production (Fig. 1). Each of the Cm$^r$ lysogens with a p195S-bearing SPβ prophage was found to produce surfactin.

To precisely identify the DNA of the *srfB* locus, which is required for surfactin production, DNA fragments from *Hin*dIII-digested plasmid p195S were subcloned into pMMN7 or pMMN13, which are pBR322 derivatives that carry Amp$^r$ and Cm$^r$ genes (the latter marker was used for selection in *B. subtilis*). Each plasmid carrying one of the *Hin*dIII fragments was introduced into SPβ phage as described above and was tested for SPβ phage-mediated complementation of *srfB*. A 1.5-kb *Hin*dIII fragment (Fig. 2) was found to complement *srfB* and was used to further define the *srfB* region. The subcloning-complementation experiments that were performed with the *Hin*dIII fragment to determine the minimum essential region required to complement *srfB* are summarized in Fig. 4. The *Bcl*I fragment of pMMN16 was the smallest fragment which could complement *srfB*. Figure 1 shows the surfactin production of various derivatives assayed on blood agar plates. OKB167 strains lysogenized with SPβ containing pMMN11 and pMMN16 produced a slightly lower amount of surfactin than did OKB105 and OKB167 lysogenized with SPβ*c2del2*::Tn*917*::pSK10Δ6:: p195S, as judged by the size of the zone of lysis on blood agar. This partial complementation was observed when the plasmids were tested for their ability to confer a competence-positive phenotype in the *srfB* mutant (see below). Although the assay was rather qualitative, it suggests that all of the DNA that was deleted in OKB167 is necessary for the full expression of *srfB*.

**Complementation of the competence defect by SPβ specialized transduction.** In the process of constructing OKB171 and OKB173, OKB167 was observed to be much less competent than OKB105. To determine whether the competence defect was caused by the *srfB* deletion, *srfB*::Tn*917* was transferred to JH642 cells by transformation with selection for MLS$^r$. A transformant with *srfB*::Tn*917* DNA, OKB192, was also shown to be competence defective. Transformation frequencies of JH642 and OKB192 cells were determined during the growth of cultures in GM2 competence medium (Table 2). Both strains showed patterns of competence development characteristic of late-growth-phase-associated activity; however, the transformation frequencies of OKB192 were $10^{-3}$ those of the parental JH642 strain. OKB206 and OKB207 carrying SPβ-borne p195S and pMMN11, respectively, were observed to restore the capacity to establish competence, although not to the level observed in the parent (Table 2). OKB206 lysogenized with SPβ(p195S) showed a higher level of competence than did OKB207, which harbored the prophage SPβ(pMMN11).

TABLE 2. Development of competence of *srfB* and SPβ*srfB* lysogens

| Time (h)[a] | No. of transformants/ml of culture | | | |
|---|---|---|---|---|
| | JH642 | OKB192 | OKB206 | OKB207 |
| 1 | $2.2 \times 10^4$ | $4.0 \times 10$ | $8.0 \times 10^2$ | $2.0 \times 10^2$ |
| 2 | $6.1 \times 10^4$ | $4.0 \times 10$ | $3.7 \times 10^3$ | $1.7 \times 10^3$ |
| 2.5 | $3.4 \times 10^4$ | $5.0 \times 10$ | $5.5 \times 10^3$ | $1.0 \times 10^3$ |
| 3 | $3.5 \times 10^4$ | 2.0 | $4.4 \times 10^3$ | $9.0 \times 10^2$ |
| 3.5 | $1.1 \times 10^4$ | 2.0 | $5.8 \times 10^3$ | $2.0 \times 10^2$ |
| 4 | $6.0 \times 10^3$ | 1.0 | $2.6 \times 10^3$ | $2.0 \times 10^2$ |

[a] Time after introduction into GM2 competence medium (20).

This was similar to the result of the experiment described above in which we examined the Srf phenotype of the pMMN11- and p195S-bearing SPβ lysogens.

**Nucleotide sequence of the *srfB* locus.** The 1.5-kb *Hin*dIII fragment (insert of pMMN11) was sequenced by the dideoxy chain-termination method (see Materials and Methods). Sequencing analysis of the *Bcl*I fragment containing the *srfB*-complementing DNA (Fig. 4) showed that *srfB* is identical to *comA*, the early competence gene that has been isolated and characterized by Dubnau and co-workers (1, 7, 8, 29). Neither pMMN14 nor pMMN15 could complement the *srfB* mutation, indicating that the region around the *Cla*I site is required for *srfB* activity. Sequence analysis of this region showed that the *Cla*I site is located within the carboxy-terminal-coding end of *comA*. Based on the sequence data and the phenotype of the *srfB* mutation, we conclude that *srfB* is *comA*.

**Effect of *srfB* on the *srfA-lacZ* fusion.** *comA* has been described as a regulatory gene that is required for competence gene expression (7); hence, we examined the affect of *srfB* on the expression of the putative *srf* biosynthesis genes. This was accomplished by using the previously described *srfA-lacZ* fusion (19). OKB125 carrying *sfp* and *srfA-lacZ* was transformed with OKB167 DNA with selection for MLS$^r$. A transformant, OKB210 (carrying *sfp srfA-lacZ srfB*::Tn*917*), showed a drastic reduction in β-galactosidase activity on LB–X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates compared with the *srfB*$^+$ parent. Expression of *srfA-lacZ* was monitored by assaying β-galactosidase activity in OKB125 and OKB210 cultured in DS liquid medium (Fig. 5). As shown previously (19), the specific activity of β-galactosidase directed by the putative *srfA* promoter in OKB125 increased gradually through the exponential growth phase and then sharply increased after the beginning of the stationary phase. Introduction of the *srfB* mutation into cells of the *srfA-lacZ*-bearing strain almost entirely eliminated the *srfA*-directed β-galactosidase activity (Fig. 5). The residual activity (maximum of 2 units) detected in OKB210 was close to the activity of OKB167 (OKB167, which contained a *srfB*::Tn*917lacZ* insertion, was found to contain a low level of β-galactosidase activity, possibly as a result of transcriptional readthrough from the *degQ* promoter). A *srfB* mutant derivative (OKB211) of OKB143 (*sfp*$^0$ *srfA-lacZ* [19]) was also shown to have greatly reduced β-galactosidase activity compared with OKB143 (data not shown).

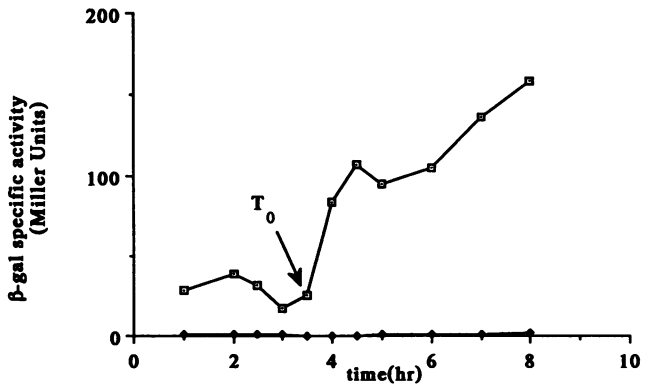



FIG. 5. β-Galactosidase (β-gal) activity of *B. subtilis* cells grown in DS medium. $T_0$ represents the end of the exponential growth phase. Symbols: ⊡, OKB125 (*srfA-lacZ sfp*); ◆, OKB210 (*srfA-lacZ sfp srfB*::Tn*917lacZ*).

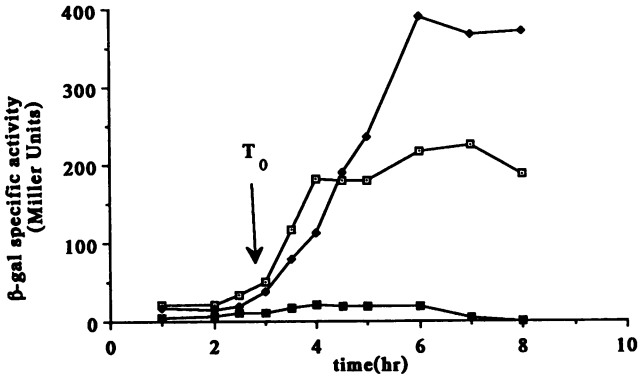



FIG. 6. Effect of glucose and glutamine on *srfA-lacZ* expression. $T_0$ represents the end of the exponential growth phase. OKB105 cells were grown overnight in DS medium and were transferred into DS medium (□), DS medium with 0.5% glucose (◆), or DS medium with 0.5% glucose and 0.2% glutamine (■).

**Expression of *srfA-lacZ* under altered nutritional conditions.** Surfactin production and competence appear to share a common mechanism of regulation that is dependent on the *srfB* (*comA*) gene. It is reasonable, therefore, to propose that there is a particular physiological or metabolic event to which surfactin production and competence establishment serve as a response, with this response being mediated through a *srfB*-dependent mechanism. To address this issue, we examined the effect of altered growth conditions on the expression of the *srfA* gene. It has been reported previously (1) that *com* gene expression is stimulated by glucose and is repressed by exogenously added glutamine. Glucose and glutamine were added to test their effects on *srfA*-directed β-galactosidase activity. OKB125 cells were grown in DS medium containing 0.5% glucose, 0.5% glucose and 0.2% glutamine, or no addition. Figure 6 shows that *srfA-lacZ* expression was stimulated by growth in glucose; however, it was strongly repressed by the addition of glutamine in the presence of glucose. The repression was caused synergistically by glucose and glutamine, because glutamine addition alone did not repress *srfA-lacZ* expression (data not shown).

## DISCUSSION

We identified a Tn*917* insertion mutation, *srfB*, which impaired surfactin production. The mutation mapped to a locus that was unlinked to the two previously identified loci *srfA* and *sfp*, both of which are required for surfactin production. The *srfB* mutation was discovered to be caused by a 5-kb deletion of DNA adjacent to a Tn*917* transposon

which integrated immediately downstream of *degQ*. Such deletions that lie immediately adjacent to Tn*917* insertions have been described by Vandeyar and Zahler (28). The Srf$^-$ and Com$^-$ phenotypes of the *srfB* mutation, the complementation analysis for surfactin production and competence, and the nucleotide sequence analysis of the *srfB* gene, together, showed that *srfB* is actually *comA*. *comA*, an early competence gene, has been identified by the Tn*917* insertion mutation *com-124* (1, 7, 8, 29) and is possibly the same gene which is identified by the *com-9* mutation (5).

Analysis of the deduced product of the *comA* gene (29) showed that the product has significant homology with the so-called activator class of two-component regulatory proteins (23) such as OmpR (30) and NtrC (3). There have been three other proteins reported so far in *B. subtilis* which show homology to the two-component regulators Spo0A (6), Spo0F (27), and DegU (SacU) (9, 15). Both *spo0A* and *spo0F* mutations exhibit a pleiotropic phenotype which includes a defect in the initiation of sporulation. *degU* also exhibits a pleiotropic phenotype that effects the expression of genes that encode degradative enzymes. As for the other class of the two-component regulatory proteins known as the sensors, the only candidate reported so far in *B. subtilis* is a product of *degS* which forms a single transcriptional unit with *degU* (15). In this report we showed that surfactin production is under ComA (SrfB)-dependent regulation which operates at the level of transcription. It is not known whether ComA (SrfB) acts directly or indirectly to activate *srfA* transcription. The homology between known transcriptional activators (NtrC) and the ComA (SrfB) protein and the requirement of ComA (SrfB) for the transcription of *srfA-lacZ* suggests that it may be a positive activator of *srf* gene transcription, but proof awaits experiments that can provide more direct evidence. It is notable that synthesis of microcin B17, another peptide antibiotic that is produced by members of the family *Enterobacteriaceae*, has been shown to occur in cells in the stationary phase of growth and is dependent on the product of the *ompR* gene, a transcriptional activator that is part of the EnvZ-OmpR two-component regulatory system (10).

The transcription of the *srfA* and *com* genes was stimulated by glucose but was repressed by the addition of glutamine together with glucose to the growth medium. The glucose- and glutamine-dependent regulation of *srfA-lacZ* expression resembles that of the *citB* gene which codes for the tricarboxylic acid cycle enzyme aconitase (4, 24). Other *B. subtilis* genes whose expression is stimulated by glucose include the *ctc* gene (11) and the *menCD* genes (menaquinone biosynthesis genes [18]); however, in these cases the addition of glutamine results in the further enhancement of gene expression. The presence of glucose and glutamine in growth medium is known to repress tricarboxylic acid cycle enzymes. However, it is not known whether *srfA* transcriptional regulation is coupled with the tricarboxylic acid cycle.

Several questions remain to be answered. Is there a protein(s) of the sensor class of two-component regulators which is involved in the regulation of competence and surfactin gene expression, and is the same sensor used for both? What is the stimulus that activates ComA (SrfB) and its putative two-component partner? What other activities are regulated by ComA (SrfB)? Does the *comA* (*srfB*) product interact directly with *srfA* DNA to activate transcription, and if so, where is the exact target site? An examination of the effect of exogenous glucose and glutamine on the expression of *srfA* and *com* genes and the identification of the *srfA* promoter region may be keys to answering some of these questions.

## ACKNOWLEDGMENTS

We thank Y. Weinrauch and D. Dubnau for helpful discussions and sharing unpublished results. We also thank R. Yasbin for the Tn*917lac* library and P. Youngman, A. Grossman, I. Smith, and R. Nutter for providing strains and plasmids.

This work was supported by Public Health Service grant GM39479-01 from the National Institutes of Health (to P.Z.) and grant HR8-3342 from the Oklahoma Center for the Advancement of Science and Technology (to P.Z.).

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