Identification, Mapping, Cloning and Characterization of a Gene (*sbmA*) Required for Microcin B17 Action on *Escherichia coli* K12

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We have identified mutations in three different chromosomal genes of *Escherichia coli* K12 which reduce sensitivity to microcin B17. Mutations in *ompF* and *ompR* genes affected production of an outer membrane porin protein, OmpF, and resulted in reduced sensitivity to a number of other agents (colicins, bacteriophages) besides microcin B17. The third class of mutants were specifically and highly resistant to microcin B17. The mutations in these strains were mapped to a gene (*sbmA*), located at 8·7 min on the *E. coli* K12 chromosome, which is closely linked to *phoA*. The wild-type *sbmA* allele was cloned into multiple copy number plasmids, and its location within the cloned DNA fragment was further defined by mutagenesis with MiniMudII1681. These insertion mutations resulted in in-frame fusions between the *sbmA* and *lacZ* genes, thereby allowing us to determine the direction of *sbmA* gene transcription. Plasmids carrying these gene fusions produced low levels of β-galactosidase, indicating that the *sbmA* gene is poorly expressed. We have been unable to identify the *sbmA* gene product, but indirect evidence indicates that it might be an envelope protein involved in microcin uptake.

INTRODUCTION

Microcin B17 is a polypeptide antibiotic of approximately 4000 M_r produced by *Escherichia coli* strains harbouring plasmid pMccB17 (pRYC17) (Asensio *et al.*, 1976; Baquero *et al.*, 1978; Herrero & Moreno, 1986). The genetic determinants involved in microcin B17 production reside in a 3·8 kb stretch of plasmid DNA that contains four cistrons, all of which are required for antibiotic production (San Millán *et al.*, 1985*a*, *b*). Microcin B17 production also requires the expression of three chromosomal loci, one of which is the *ompR* gene (Hernández-Chico *et al.*, 1982; Baquero & Moreno, 1984). Unlike the production of most colicins, that of microcin B17 is not lethal and is not induced by DNA-damaging agents (Baquero & Moreno, 1984; Pugsley, 1984).

The activity spectrum of microcin B17 is restricted to a few species of bacteria related to *E. coli*. The antibiotic specifically inhibits DNA synthesis in susceptible *E. coli* K12 strains; DNA is degraded, and the cells die. Consequently, the so-called SOS system is derepressed in microcin B17-treated cells. This expression requires the products of the *recA* and *recBC* genes and an active replication fork. Mutations in *recA* or *recBC* enhance microcin B17 sensitivity, whereas *lexA* (Def) mutations reduce it (Herrero & Moreno, 1986).

As another approach to the study of microcin B17 action, we have isolated and characterized microcin B17 insensitive mutants of *E. coli* K12. In this article, we identify three chromosomal genes whose products are required for microcin action, and report the cloning and characterization of one of them which seems to be specifically involved in microcin action.

Abbreviations: $B17^R$, microcin B17 resistance; XP, 5-bromo-4-chloro-3-indolylphosphate; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; AU, arbitrary antibiotic unit; IPTG, isopropyl-β-D-galactopyranoside.

METHODS

Bacterial strains, plasmids and bacteriophages. Those used are listed in Table 1.

Media and chemicals. LB medium and M63 minimal medium (Miller, 1972) were used throughout. M63 medium was supplemented with thiamin (1 μg ml^{-1}), glucose (0·2%) or lactose (0·2%), and, where necessary, with L-amino acids (20 μg ml^{-1}). Antibiotics were used at the following concentrations: ampicillin (Ap), 50 μg ml^{-1}; tetracycline (Tc), 20 μg ml^{-1}; streptomycin (Sm), 100 μg ml^{-1}; sodium nalidixate (Nal), 40 μg ml^{-1}; kanamycin (Km), 30 μg ml^{-1}. Phage P1 was grown on LB agar supplemented with 2·5 mM-$CaCl_2$, 0·2% glucose and 1·2% Difco agar. Alkaline phosphatase-producing ($PhoA^+$) strains were scored on MOPS/glucose medium supplemented with 10^{-4} M-potassium phosphate (Neidhardt *et al.*, 1974) and containing 40 μg XP (5-bromo-4-chloro-3-indolylphosphate) ml^{-1} as chromogenic alkaline phosphatase substrate. Production of β-galactosidase ($LacZ^+$) was detected on LB agar plates containing 20 μg X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) ml^{-1} (Miller, 1972). Microcin insensitive mutants were selected on M63 glucose or LB agar plates containing crude microcin (50 AU ml^{-1}). Microcin was prepared using the overproducing strain RYC893 and assayed as described below.

Microcin, colicin and phage sensitivity. Microcin B17 sensitivity was determined by allowing drops of a suspension of strain RYC893 to run in a straight line on M63 glucose plates. After 30 h incubation, strains to be tested were cross-streaked and the plates reincubated. Only resistant clones grew in the vicinity of the microcinogenic bacteria. The critical dilution method (Mayr-Harting *et al.*, 1972) was used for more accurate determinations of microcin sensitivity. Colicin sensitivity was also determined by the cross streak method using the strains listed by Pugsley (1985). Phage sensitivity was determined by cross-streaking using phages TuIa (5×10^7 p.f.u. ml^{-1}) and λVh434 (1×10^8 p.f.u. ml^{-1}).

Transposon Tn5 mutagenesis. Five cultures of strain BM21 were incubated at 30 °C with phage λ 467 (*rex*::Tn*5*) according to Berg (1977). After 20 min, the mixture was diluted into 9 vols L broth and incubated for 2 h at 42 °C to allow expression of kanamycin resistance. Samples from each culture were then plated on M63 glucose medium containing Km and microcin B17, and incubated at 42 °C.

Genetic techniques. Conjugation and P1 transduction were done as described by Miller (1972). Plasmid DNA was extracted from overnight LB cultures. Cleared lysates were obtained and DNA was precipitated as described by Maniatis *et al.* (1982). Digestion with restriction endonucleases, ligation with T4 DNA ligase and transformation were all done as described by Davis *et al.* (1980). Agarose gel electrophoresis was done in a vertical slab apparatus using Tris/phosphate buffer (Davis *et al.*, 1980). The sizes of restriction endonuclease fragments were determined by comparing their mobilities with *Hin*dIII fragments of phage λ DNA (Sanger *et al.*, 1982) or with *Hae*III fragments of phage φX174 replicative DNA (Sanger *et al.*, 1978) in 0·6–1·2% agarose gels.

Construction of pMM100. The expression vector pUC13 (Messing, 1983) was used to clone *sbmA*$^+$ under *lacZp* control. In order to avoid problems caused by the poor growth of cells carrying this pUC13 derivative, we first constructed a compatible, multiple copy number plasmid carrying the *lacI*^{q1} allele of the lactose repressor gene (Calos & Miller, 1981). An *Eco*RI fragment of pLi7 (Table 1) containing *lacI*^{q1} was extracted from an agarose gel and ligated into the *Eco*RI site of pACYC184. The resulting plasmid, pMM100, was shown to repress *lacZ*$^+$ expression in cells devoid of the chromosomal *lacI* gene. *lacZ* expression remained inducible by IPTG.

sbmA::MudII1681 gene fusions in pMM73-4. Strain POII1681(pMM73-4) was used to produce transducing particles. These were used to infect strain RYC761 as described by Castilho *et al.* (1984). The transduction mixture was incubated for 3 h at 30 °C to allow expression of kanamycin resistance, and was then plated onto LB Km Tc X-gal agar containing microcin B17. Nine experiments were done in order to obtain independent insertions. β-Galactosidase was assayed according to Miller (1972).

RESULTS

Isolation of microcin B17-resistant mutants. Spontaneous resistant mutants were obtained at frequencies of about 1×10^{-6} when sensitive *E. coli* K12 BM21 or RYC10 cells were plated on selective agar containing microcin B17. The mutants appeared at about 10 times this frequency when Tn*5*-mutagenized bacteria were used.

Mutations in ompR and ompF cause microcin resistance. Mutants obtained by the above procedure were tested for their sensitivity to various bacteriophages and colicins. A small proportion were resistant to bacteriophage TuIa or TuIa plus λVh434, and exhibited substantially reduced sensitivity to colicins A, E2 through E8, K, L, S4 and N. An examination of their outer membrane protein profiles by SDS–PAGE revealed that they were devoid of major outer membrane proteins OmpF (TuIa^R, λVh434^S) or OmpF plus OmpC (TuIa^R, λVh434^R), which agrees with the fact that these phages use OmpF or OmpC respectively as their receptors

Table 1. *Bacterial strains, bacteriophages and plasmids*

E. coli strain	Genotype/phenotype	Source and/or reference*
pop3000	HfrH	M. Schwartz
GM247	HfrH ProC⁻ PyrD⁻ Sp^R†	R. D'Ari
RYC22	F⁻ *his-4* Thr⁻ Leu⁻ ProA⁻ ArgE⁻ Gal⁻ Xyl⁻ LacY⁻ Tsx^R ThiA⁻ Str^R Nal^R	Laboratory collection
RYC893	F⁻ *araD139* Δ*lacU169* Δ*malB1* Str^R RelA⁻ ThiA⁻ (pMM102)	San Millán *et al.* (1985*a*)
BM21	F⁻ Nal^R (λ^+)	Hernández-Chico *et al.* (1982)
RYC10	BM21 RpoB⁻	Laboratory collection
RYC816	BM21 *recA56 srl*::Tn*10*‡	Hernández-Chico *et al.* (1982)
MC4100	F⁻ *araD139* Δ*lacU169* Str^R ThiA⁻ RelA⁻	Casadaban (1976)
SM125	MC4100 PhoA⁻ *proC*::Tn*5*	S. Michaelis
MH150	MC4100 *ompC*::Tn*5*	Hall & Silhavy (1979)
RYC514	MC4100 *ompR101*	Laboratory collection
MH407	MC4100 *malQ7 ompF7*::Mucts	Hall & Silhavy (1981)
SM547	F⁻ Leu⁻ PhoR⁻ Δ(*sbmA phoA proC*) *tsx*::Tn*5*	S. Michaelis
RYC717	BM21 *sbmA1*	This work; spontaneous mutant
RYC714	BM21 *sbmA11*::Tn*5*	Tn*5*-generated $B17^R$ mutant
RYC726	RYC10 Δ(*phoA sbmA*)14	Spontaneous $B17^R$ mutant
RYC730	MC4100 *sbmA1* PhoA⁻	Pro^+ PhoA⁻ $B17^R$ transductant of SM125 via P1 grown on RYC717
RYC760	RYC730 *recA56 srl*::Tn*10*	Tc^R Rec⁻ transductant of RYC730 via P1 grown on RYC816
RYC745	RYC22 *sbmA11*::Tn*5*	Km^R $B17^R$ transductant of RYC22 via P1 grown on RYC714
RYC761	MC4100 Δ(*phoA sbmA*)*14* $MuctsAp^R$	Mu lysogen of Pro^+ PhoA⁻ $B17^R$ transductant of SM125 via P1 grown on RYC726
RYC953	BM21 Δ(*sbmA phoA proC*) *tsx*::Tn*5*	Km^R Pro⁻ $B17^R$ transductant of BM21 via P1 grown on SM547
POI1681	AraD⁻ Δ(*ara leu*)*7697* Δ(*proAB argF lac*)*X111* Str^R Mucts MudII1681	Castilho *et al.* (1984)
Bacteriophage		
λ 467	λcI857 *rex*::Tn*5*§ *O*am29 *P*am80 *b*221	Berg (1977)
$MuctsAp^R$	Mucts carrying *bla* derived from Tn*3*	W. Schuman
Plasmid		
pMM102	pBR322::$MccB17^+$ $MccB17^{imm}$	San Millán *et al.* (1985*b*)
pL719	ColE1 *sbmA*⁺ *phoA*⁺ *proC*⁺ *phoB*⁺	Clarke and Carbon collection via R. Portalier
pLi7	pBR322 $lacI^{q1}$ *lacZ′*	B. Müller-Hill
pUC13	*bla lacZ′*	Messing (1983)
pAPIP502	F′ *lacZM15* $lacI^{q1}$ Tn*10*	Laboratory collection

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† Sp^R, spectinomycin resistant.

‡ The *recA56* allele was introduced into several strains by contransduction with *srl*::Tn*10* using P1 grown on strain RYC816. Tc^R transductants were selected, and RecA⁻ clones were detected by their increased sensitivity to UV light.

§ Tn*5* confers kanamycin resistance.

(Datta *et al.*, 1977; Hankte, 1978). Mutations which reduce production of OmpF or OmpF plus OmpC proteins are also known to cause reduced sensitivity to several colicins (Pugsley, 1984).

Mutations in two different genes are known to produce the phenotype(s) we have described above for the microcin resistant mutants: mutations in *ompF*, the structural gene for the OmpF protein, cause specific loss of OmpF, whereas mutations in *ompR*, a positive regulator gene, often result in failure to produce OmpF and OmpC proteins (Hall & Silhavy, 1981). The genetic location of two of each class of mutations selected by their microcin B17 resistance phenotype was confirmed as *ompF* or *ompR* by P1 transduction experiments. The $TuIa^R$ $B17^R$ (microcin B17 resistance) mutation was 50% cotransduced with *pyrD* (21 min), and the $TuIa^R$ $B17^R$

Table 2. *Transduction mapping of sbmA11::Tn5 mutation*

Phage P1 grown on strain RYC745 (Lac⁻ *sbmA11*::Tn*5* ProC⁺ Tsx⁻) was used to infect strain GM247 (Lac⁺ SbmA⁺ ProC⁻ Tsx⁺). Km^R and Pro⁺ transductants were selected and scored for unselected markers. The Sbm⁺ phenotype was checked by cross-streaking, and the Tsx⁺ phenotype by sensitivity to bacteriophage T6 (Tsx⁻ mutants are T6^R).

Km^R (*sbmA11*::Tn*5*) transductants (97 analysed)				Pro⁺ transductants (148 analysed)			
Non-selected markers				Non-selected markers			
Lac	ProC	Tsx	No. of transductants	Lac	SbmA	Tsx	No. of transductants
−	−	−	0	−	−	−	0
−	−	+	3	−	−	+	4
−	+	−	0	−	+	−	0
−	+	+	1	−	+	+	0
+	−	−	2	+	−	−	28
+	−	+	28	+	−	+	101
+	+	−	15	+	+	−	3
+	+	+	48	+	+	+	12

Table 3. *Transduction mapping of the spontaneous sbmA1 mutation*

Strain SM125 (SbmA⁺ PhoA⁻ ProC⁻) was transduced with phage P1 grown on strain RYC717 (*sbmA1* PhoA⁺ ProC⁺) with selection for Pro⁺ transductants; 199 of these transductants were tested.

Unselected markers		
SbmA	PhoA	No. of transductants
+	+	18
+	−	10
−	+	170
−	−	1

λVh434^R mutation was 80% cotransduced with *malA* (75 min). These are the expected linkages for *ompF* and *ompR* loci respectively (Bachmann, 1983). In addition, well-characterized mutations in *ompR* and in *ompF* were also found to confer microcin B17 resistance (see Table 4).

Mutants specifically resistant to microcin B17. Approximately 80% of the mutants we obtained were specifically resistant to microcin B17, and remained sensitive to all other agents tested. We first studied mutants of this type obtained after transposon Tn*5* mutagenesis. Absolute linkage of Tn*5*-encoded kanamycin resistance and microcin B17 resistance was first tested by P1 phage transduction, thereby confirming that Tn*5* was inserted in the gene required for microcin sensitivity. One such strain used for further analysis was RYC714, and the locus affected was termed *sbmA* (*s*ensitivity to *B*17 *m*icrocin). The *sbmA11*::Tn*5* mutation was transduced into strain pop3000 (HfrH), which was then conjugated with strain RYC22 (Thr⁻ Leu⁻ ProA⁻ LacY⁻ Tsx⁻ Nal^R) with selection for Km^R and counter selection for Nal^R (GyrA⁻). This experiment demonstrated a close linkage between *sbmA11*::Tn*5* and *lacY*. The results of P1 transduction experiments reported in Table 2 confirmed this linkage, and showed that the *sbmA11*::Tn*5* mutation was 90% cotransduced with the *proC* gene being located between this marker and *lac*. Other transduction experiments showed that the spontaneous *sbmA1* mutation was also cotransduced with *proC* (86%) and was located to the left of *phoA* (Table 3). All these data indicate that *sbmA* maps at approximately 8·7 min on the *E. coli* linkage map with the following order of markers: *lac–sbmA–phoA–proC*.

Several other independent Tn*5* and spontaneous microcin B17-resistant mutants were also analysed by P1 transduction to localize their mutations. They all showed high cotransduction (85–90%) with *proC*. In addition, a significant proportion of mutants were also unable to

Table 4. *Microcin B17 sensitivity of E. coli K12 strains carrying recA, sbmA and mutations affecting porin production*

Microcin sensitivity was measured by the critical dilution assay on LB agar plates. The strains used were MC4100 (wild-type), RYC514 (OmpR$^-$), MH407 (OmpF$^-$), MH150 (OmpC$^-$) and RYC730 (SbmA$^-$), and their *recA56 srl*::Tn*10* derivatives. The experiments were done at 30 °C.

Phenotype					
RecA	OmpR	OmpF	OmpC	SbmA	Microcin titre
+	+	+	+	+	8
−	+	+	+	+	128
+	−	+	+	+	<1
−	−	+	+	+	1
+	+	−	+	+	1
−	+	−	+	+	16
+	+	+	−	+	4
−	+	+	−	+	32
+	+	+	+	−	<1
−	+	+	+	−	<1

produce alkaline phosphatase on XP plates, indicating that they probably carried deletion mutations affecting *sbmA* and *phoA*. We conclude that all these mutants are affected in the same locus, *sbmA*.

Two F′ factors (F′W3747 and X573; Low, 1972) carrying the 8–9 min region of the *E. coli* K12 chromosome were introduced into strain RYC760 (SbmA$^-$ PhoA$^-$ Lac$^-$ RecA$^-$). They complemented the *sbmA* mutation, and the normal level of microcin B17 sensitivity was recovered. The *sbmA* mutation is, therefore, recessive to the wild-type allele.

Levels of microcin B17 resistance. Strains carrying *recA* mutations are about 10 times more sensitive to microcin B17 than strains with the *recA*$^+$ allele (Herrero & Moreno, 1986). The critical dilution method was used to evaluate the level of microcin B17 resistance afforded by *ompF*, *ompC*, *ompR* and *sbmA* mutations in strains also carrying *recA*$^+$ or *recA* alleles. Strains carrying *ompR* or *ompF* mutations were only partially resistant to microcin B17, whereas strains carrying the *sbmA* mutation were completely resistant. *ompC* mutations resulted in a slightly decreased sensitivity (Table 4).

Cloning of sbmA into pBR322. Plasmid pL719, from the Clarke and Carbon collection (Clarke & Carbon, 1976), carries the *phoA*$^+$, *proC*$^+$ and *phoB*$^+$ alleles (R. Portalier, personal communication), and was found to complement *sbmA* mutations. It was transferred by F-duction to strain RYC953 (SbmA$^-$ ProC$^-$), with selection on minimal medium without proline. All but one of the transconjugants were microcin B17 sensitive and synthesized alkaline phosphatase. The plasmid from the microcin B17-resistant clone was purified and analysed with restriction endonucleases. It was found to lack a 6·8 kb stretch of DNA from within an 8·8 kb *Pst*I fragment of pL719. This fragment was cloned into the *Pst*I site of pBR322. Strain RYC953 was transformed with a ligation mixture containing *Pst*I digests from pL719 and pBR322, and Tc^R clones were selected. Plasmid pMM70 was found in one of the Tc^R transformants which was sensitive to Ap and microcin B17. This plasmid carried both *phoA*$^+$ and *sbmA*$^+$ alleles. A physical map of pMM70 is presented in Fig. 1.

To localize the *sbmA* locus on pMM70, we constructed a series of *in vitro* deletion derivatives. All but one of them, pMM73-3, complemented chromosomal *sbmA* mutations, indicating that *sbmA* is located within the 2·6 kb *Ava*I–*Pst*I fragment of pMM70 (Fig. 1).

lacZ gene fusions in sbmA. In order to determine the direction of *sbmA* transcription, we constructed *sbmA–lacZ* gene protein fusions in plasmid pMM73-4 using miniMudII1681 (Castilho *et al.*, 1984). Plasmid pMM73-4 was introduced into strain POII1681, and a phage lysate was obtained from the resulting transformant. Strain RYC761 Δ(*phoA sbmA*) was transduced with the lysate in nine separate experiments. Transductants which had received

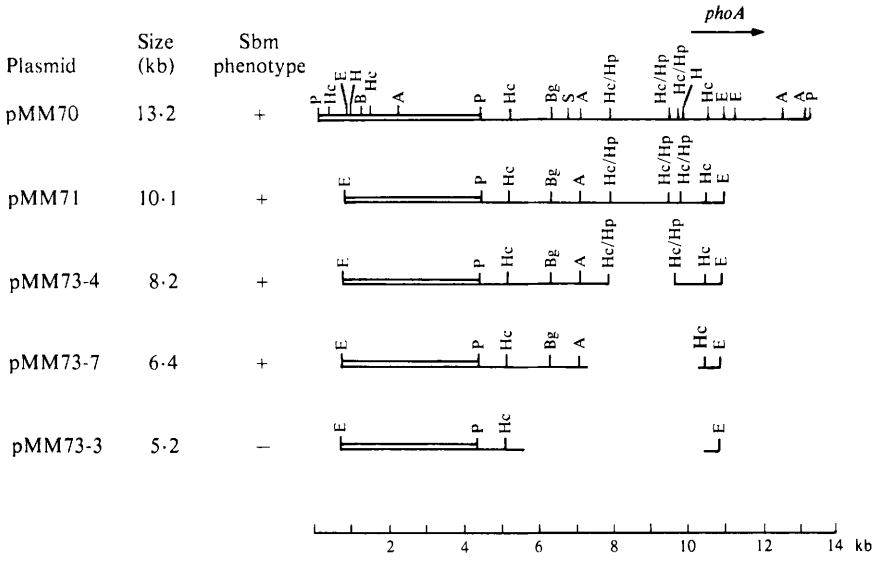

Fig. 1. Physical map of pMM70 and its derivatives. pMM70 was digested with *Eco*RI and religated to obtain pMM71. pMM73-4 is a deletion derivative of pMM71 obtained by digestion with *Hpa*I. pMM73-7 and pMM73-3 were obtained in the same way, but are smaller than pMM73-4 due to an exonuclease activity in the *Hpa*I preparation. The arrow indicates the position and direction of transcription of the *phoA* gene (Sarthy *et al.*, 1981). The restriction pattern of this region coincides with those already described for the *phoA* locus (Berg, 1981; Inouye *et al.*, 1981; Boidol *et al.*, 1982). Only pMM70 is able to complement *phoA* mutations. ——, pBR322; ▭, cloned DNA; P, *Pst*I; Hc, *Hin*cII; Bg, *Bgl*II; S, *Sac*II; A, *Ava*I; Hp, *Hpa*I; H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI.

pMM73-4 (Tc^R) and MudII1681 (Km^R) and which retained their microcin B17 resistance were selected on M63 glucose X-gal plates supplemented as indicated in Methods. Approximately 17% of the transductants were Lac^+ and, therefore, likely to carry *sbmA–lacZ* gene fusions created by insertion of MudII1681 into *sbmA*. Nine independent Lac^+ clones were selected for further analysis. Each contained a plasmid of about 22·4 kb (comprising the 8·2 kb of pMM73-4 and the 14·2 kb of MudII1681) which could transform $LacZ^-$ strains to Lac^+, but which could not complement *sbmA* mutations. These plasmids were digested with *Pst*I and *Bam*HI restriction endonucleases in order to determine the orientation and position of the MudII1681 insertions (Fig. 2). All of the insertions were within a 1050 bp length of DNA within the *Pst*I–*Bgl*II fragment of pMM73-4. They were all orientated in the same direction, with *lacZ* being read from the *Pst*I end of the fragment towards the *Bgl*II end. By relating these results to the known positions of *phoA* and *sbmA* in pMM70 and in the *E. coli* K12 chromosome (Fig. 1) (Bachmann, 1983), we can deduce that *sbmA* is transcribed in a clockwise direction in the *E. coli* chromosome.

β-Galactosidase activities in strains carrying plasmids with *sbmA–lacZ* gene fusions were relatively low (Fig. 2).

Cloning of sbmA in pUC13. We have studied the protein profiles of cell envelope and soluble fractions from strains carrying wild-type or mutant *sbmA* alleles, and from strains carrying pMM70 and its deletion derivatives, by SDS–PAGE, but have consistently failed to identify any protein which could correspond to the *sbmA* gene product. The presence of pMM70 and its $SbmA^+$ derivatives did not increase sensitivity to microcin B17, suggesting that either the *sbmA* gene product was not overproduced or it was not limiting for microcin B17 action. In order to increase SbmA production, we cloned the 1·75 kb *Pst*I–*Bgl*II fragment of pMM73-4 into pUC13 in the hope of expressing *sbmA* from the *lacZ* promoter. Transformants carrying this recombinant plasmid (pMM90) were indeed about four times more sensitive to microcin B17

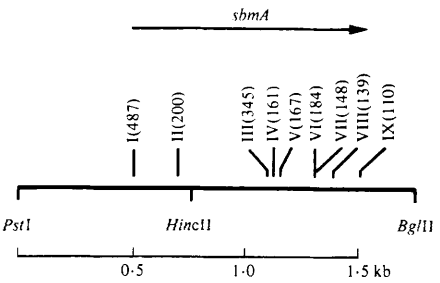

Fig. 2. Localization of MudII1681 insertions producing *sbmA*::*lacZ* gene fusions in pMM73-4. The 1·75 kb *Pst*I–*Bgl*II fragment of pMM73-4 is shown. The fusions are numbered I to IX, and the pointers indicate the sites where MudII1681 was inserted. The sites were determined by digestion with restriction endonucleases *Bam*HI and *Pst*I and are based on the known positions of these sites in pMM73-4 (this study) and in MudII1681 (Castilho *et al.*, 1984; O'Conner & Malamy, 1983; Jorgensen *et al.*. 1979). Values in parentheses are β-galactosidase activities (Miller units); for comparison, *lacZ* gene fusions to the colicin E2 structural gene promoter (*ceaBp*) in pBR322 derivatives yield 8000–30000 units of β-galactosidase.

than wild-type cells, suggesting that an increase in the level of *sbmA* gene expression had been achieved. However, we were still unable to detect a putative *sbmA* gene product. All these experiments were done in cells carrying pMM100, a multiple copy number plasmid carrying the $lacI^{q1}$ repressor allele, and in the absence of induction by IPTG. Clones carrying pMM90 in the absence of *lacI*-encoded repressor, or even in the presence of pAPIP502 ($F' lac^{q1}$), were unstable, grew poorly and tended to produce microcin-resistant subclones. IPTG induction of cells carrying pMM90 and pMM100 also caused a rapid loss of viability. We conclude that overexpression of *sbmA* may be lethal.

DISCUSSION

Mutations in three chromosomal genes, *ompF*, *ompR* and the previously undescribed gene which we have named *sbmA*, affect sensitivity to microcin B17. If we consider that *sbmA* constitutes a single cistron, it could encode a protein of $\geqslant 40$ kDa. Experiments with *sbmA–lacZ* gene fusions indicate that *sbmA* is probably poorly expressed, which is consistent with the fact that we have so far been unable to detect the *sbmA* gene product, either by examining fractionated cells by SDS–PAGE, or by labelling plasmid-encoded proteins in maxicell or minicell systems (data not shown).

The results we have obtained do not allow us to define the roles of the *ompF*, *ompR* and *sbmA* gene products in microcin B17 action. It is possible that OmpF protein plays a role in the penetration of the *E. coli* K12 outer membrane by microcin B17, as it does for several colicins (Konisky, 1979; Pugsley, 1984). Our results show that OmpC protein is only partially able to replace OmpF protein with respect to microcin B17 action, as is the case with colicins such as E2 and E3 (Mock & Pugsley, 1982). These major outer membrane proteins may either function as pores to allow the passage of the microcin/colicin across the membrane, or may help to stabilize or activate the microcin/colicin receptor. The former hypothesis seems more likely in the case of microcin B17 than for colicins such as E2 or E3, because microcins are considerably smaller (Baquero & Moreno, 1984).

The role of the *sbmA* gene product in microcin B17 action remains to be defined. Mutations affecting *sbmA*, and even deletions of the entire gene, do not affect cell growth, indicating that the *sbmA* product is not essential for cell viability. The fact that *sbmA* mutations lead to very high levels of microcin B17 resistance indicates that it codes for a key product for microcin action. The following results indicate that the *sbmA* gene may code for, or is required for, the synthesis of an envelope protein, possibly the microcin B17 receptor. (*a*) Exogenous microcin B17 kills susceptible bacteria, and induces genes involved in the SOS repair system in susceptible and immune strains (Herrero & Moreno, 1986; Herrero *et al.*, 1986). These effects

are not observed in *sbmA* mutants. (*b*) Microcin-producing cells exhibit levels of SOS gene expression (measured with a *sfiA–lacZ* operon fusion) which are higher than those in cells without plasmids or in cells harbouring non-producing plasmid derivatives. When cells carried the wild-type plasmid pMccB17, in which microcin production is balanced with immunity, the level of Φ(*sfiA–lacZ*) expression was twice the basal level. This stimulation in SOS expression was completely abolished when the producer cells carried the *sbmA1* mutation (Herrero *et al.*, 1986). A similar situation has been described previously with cells carrying plasmids encoding colicin E2, which has endodeoxyribonuclease activity, and hence induces the SOS system. Mutations affecting colicin E2 uptake (*btuB* or *ompF*/*ompR*) in the colicin producer cells eliminated the SOS stimulation effect. It was concluded that the effect was due to colicin, which, after being released into the medium, entered other cells in the culture, overcoming their immunity and thus exerting its effects (Pugsley, 1983; Pugsley *et al.*, 1983). (*c*) When cells harboured pMM102, a plasmid in which the balance between microcin production and immunity was altered, during its construction, in favour of production (unpublished), Φ(*sfiA–lacZ*) was expressed at levels as high as 15 times the basal value (Herrero, 1984). In this case, the introduction of the *sbmA1* mutation reduced, but did not abolish, the activation of Φ(*sfiA–lacZ*), which was expressed at four times the basal level (Herrero, 1984). These results indicate that *sbmA1* did not prevent the action of endogenous microcin that had not been neutralized because of the inbalance between production and immunity. (*d*) Further support for this view came from the behaviour of strains carrying pMM102 and a *recA* mutation. As indicated in the Introduction, *recA* and *recBC* mutants are extremely sensitive to microcin. When these cells carry pMM102 they cannot grow in minimal medium in which microcin production is 'optimal'. This property, which is due to the inbalance between production and immunity (San Millán *et al.*, 1985*a*; unpublished), could neither be suppressed by the introduction of *sbmA* mutations, nor by *ompR* mutations. Hence these mutations protect cells from exogenous, but not from endogenous, microcin. We are, at present, attempting to select chromosomal mutations which prevent the action of endogenous microcin B17.

In conclusion, our results indicate that *sbmA* mutants remain sensitive to microcin produced by and acting within the producer cell. The product of the *sbmA* gene is therefore needed only for the action of exogenous microcin, and hence is likely to play a role in microcin uptake. The preparation of radio-labelled microcin for use in binding assays will be required to demonstrate whether the *sbmA* product is indeed the microcin B17 receptor. The low level of microcin activity so far recovered, even from cultures of overproducing strains, is insufficient for this purpose.

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