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# Expression of *Serratia marcescens* Extracellular Proteins Requires *recA*

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**A previously described regulatory mutation which abolishes expression of the extracellular nuclease of *Serratia marcescens* is shown to be a mutation of the *Serratia recA* gene. The defect in nuclease expression could be restored by introducing a plasmid carrying the *recA* gene of *Escherichia coli*. The DNA sequence of the *Serratia* gene is very similar to that of the *E. coli* gene. The putative LexA-binding site of the *Serratia recA* gene is almost identical to that of *E. coli*, along with the promoter. A similar LexA-binding site can also be found upstream of the nuclease gene. As expected from this finding, we show that nuclease expression can be induced by SOS-inducing agents such as mitomycin C. Although inducible in *S. marcescens*, the nuclease was expressed only at the uninduced levels in *E. coli* and could not be induced by mitomycin C. The extracellular chitinase and lipase were similarly affected by the mutations altering nuclease expression and were also induced by mitomycin C.**

Extracellular proteins can be produced by a variety of microbial systems. They tend to fall into a few major classes of proteins, such as bacterial toxins and virulence factors as well as degradative enzymes. Despite the importance of these proteins, the process of extracellular secretion, or excretion, is not well characterized in gram-negative bacteria (18, 21).

Our laboratory has been interested primarily in using the extracellular nuclease of the gram-negative bacterium *Serratia marcescens* (6) as a model system to study extracellular proteins and their excretion. When this protein is expressed in *Escherichia coli*, it is secreted extracellularly without any accessory gene products from *S. marcescens*. This is similar to other self-secreting proteins (1, 4, 11, 18) which are also found extracellularly when expressed in *E. coli*. Many other extracellular proteins from *S. marcescens* have also been cloned and characterized. The chitinase (10), a phospholipase A (7), and a serine protease (32) can also be excreted from *E. coli*, although the major metalloprotease gene does not express active protease from *E. coli* (17; S. C. Braunagel and M. J. Benedik, unpublished observations). An extracellular lipase (8) is also produced from *S. marcescens*, but it has not been well characterized, nor has its gene been identified.

Many of these degradative enzymes are regulated, usually by the substrate upon which they act. The metalloprotease from *S. marcescens* sp. is induced by the presence of proteins such as casein or gelatin in the growth medium (2), and chitinase is induced by exogenous chitin (16). No known regulation of the nuclease has been described; there is no effect on nuclease expression caused by the addition of nucleic acid to the growth medium or the addition or removal of glucose (31). Despite an apparent lack of regulation, mutations have been described which, paradoxically, can only be assumed to be regulatory mutations.

Nuclease-superexpressing mutants of *S. marcescens* have been isolated after nitrosoguanidine mutagenesis (29, 31). Wild-type *S. marcescens* produces an abundant level of extracellular nuclease activity, but the nuclease-superexpressing mutants [*nuc*(Su)] express at least 10-fold-higher levels. These mutants are pleiotropic; in addition to nuclease, they overexpress lipase, a *Serratia* colicin equivalent called marcescin, and possibly β-lactamase (31). The *nuc*(Su) mutants arose at about the same frequency as nuclease-defective mutants; similar mutants could also be isolated with the frameshift mutagen ICR-191, suggesting that gene inactivation mutations can result in this phenotype (29).

A second regulatory mutation has been previously described by us (9). Isolated by transposon mutagenesis, this mutation abolishes nuclease expression. The mutation is not complemented by plasmids carrying the nuclease gene and promoter; however, nuclease expression can be restored if the nuclease gene is expressed from a *lac* promoter carried by the plasmid. The nuclease promoter appears to function, although only at a low level, in *E. coli* (1). In this work, we demonstrate that the nuclease gene is regulated by an SOS-like system of *S. marcescens* and that it can be induced by the DNA-damaging agent mitomycin C. We further describe the nature of the regulatory mutations previously presented.

## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this work are listed in Table 1. Our wild-type *S. marcescens* is strain SM6. A nuclease-deficient derivative, SM6 *nucB*::Tn*5*, was previously described (9). This strain was shown to be a regulatory mutant which prevents nuclease expression from its own promoter but in which nuclease can be expressed from a *lac* promoter. The strain W1050 [*nuc*(Su)] (31) is a derivative of strain W1128 [SM6(F′ *lac*)] isolated after nitrosoguanidine mutagenesis as a super nuclease producer. This mutant also overexpresses lipase, marcescin (the *S. marcescens* colicin equivalent), and possibly β-lactamase (31). Strain TT392 (26) is a derivative of a different *S. marcescens* wild-type strain, Sr41. It carries a mutation in the nuclease structural gene (9) and expresses no detectable nuclease under any of our assay conditions. This strain also carries mutations making it restriction defective and more sensitive to ampicillin and kanamycin than wild-type *S. marcescens*.

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TABLE 1. *S. marcescens* strains[a]

| Strain | Relevant markers | Source or reference |
|---|---|---|
| SM6 | Wild type | 1, 8 |
| SM6 *nucB*::Tn*5* | Sm6 Nuc$^-$ | 8 |
| W1128 | SM6(F' *lac*) | U. Winkler |
| W1050 | W1128 *nuc*(Su) | 28, U. Winkler |
| TT392 | Nuc$^-$ $R_{SM}^-$ Amp$^s$ Kan$^s$ | 8, 23 |

[a] Described further in Materials and Methods.

*E. coli* JM101 (27) was used for most plasmid manipulations; strain MM294 was used when a restrictionless *E. coli* strain was needed.

**Media and growth conditions.** LB medium was used for routine growth of both *E. coli* and *S. marcescens*. For plasmid maintenance, 50 μg of ampicillin per ml was used for both *E. coli* and *S. marcescens* TT392, while 1 mg of ampicillin per ml was used with SM6 derivatives. Kanamycin was used at 25 μg/ml for *E. coli* or strain TT392 and at 100 μg/ml for SM6 strains. *E. coli* was routinely grown at 37°C, and *S. marcescens* was grown at 30°C. Nuclease indicator medium was DNase test agar from Difco Laboratories or GIBCO Laboratories. This medium was supplemented with 50 μg of methyl green per ml. The commercial media available with methyl green already added gave too much batch-to-batch variability. Inductions were done by adding mitomycin C (Sigma Chemical Co.) to a final concentration of 0.1 μg/ml to cultures in mid-log phase and allowing growth to continue either until the cells entered stationary phase or overnight. Chitin plates were made as previously described (9).

For UV killing, the cells were grown in LB medium, washed once, and suspended in an equal volume of 10 mM NaCl. They were then exposed to UV irradiation for the times listed. Dilutions were made in LB medium, and the mixtures were plated out for viable cells at 30°C. All procedures after irradiation were performed in the dark.

**Plasmids.** Most plasmid constructions were based on the pUC vector system (27). Transcriptional *lac* fusions were made with pTrp-Lac, a vector based on pBR322 which carries a *trp-lac* operon fusion in place of the tetracycline gene (T. K. Ball and M. J. Benedik, unpublished). This vector expresses *lacZ* efficiently only when a promoter is cloned upstream at the *Eco*RI or *Hin*dIII restriction sites.

Plasmids pNuc1 and pNuc4 were described previously (1). They both carry the nuclease gene from SM6. In pNuc1, the nuclease gene is on a 2.5-kilobase (kb) *Eco*RI fragment in pUC18; nuclease is expressed only from its own promoter in *S. marcescens* and at a low basal level in *E. coli*. A subclone carrying a 1.4-kb *Rsa*I fragment inserted into the *Sma*I site of pUC18 was used to make pNuc4 and pNuc4-R. These plasmids have the same fragment in opposite orientations, so that pNuc4 can express nuclease from both the plasmid *lac* promoter and the nuclease promoter; pNuc4-R expresses nuclease only from its own promoter. About 120 bases are upstream from the start of the nuclease gene in these two plasmids.

A *nuc-lacZ* transcriptional fusion was created by subcloning an *Eco*RI-to-*Eco*RV fragment from pNuc1 into pUC18 digested with *Eco*RI and *Sma*I. This fragment carries the 1-kb upstream region which we have shown contains both the nuclease regulatory region and a transcription terminator upstream which prevents plasmid promoters from transcribing the nuclease gene (1). The *Eco*RV site is within the nuclease structural gene. The *Eco*RI-to-*Hin*dIII fragment was isolated from this subclone in pUC18 and inserted into pTrp-Lac, which carries a *trp-lac* transcriptional fusion replacing the tetracycline gene of pBR322 and oriented in the same direction, to create plasmid pNuc-lacZ.

Plasmids pGE516 and pGE106 were obtained from George Weinstock. pGE516 carries the *E. coli recA* gene inserted as a *Bam*HI fragment in pBR322. Plasmid pGE106 carries the *E. coli lexA* gene as an *Eco*RI-*Pst*I fragment inserted in pBR322. This fragment was moved from pGE106 into pUC18 with the same enzymes to create an ampicillin-resistant vector for transforming into *S. marcescens*.

The *recA-lacZ* fusion was made by cloning an *Eco*RI-to-*Eco*RV fragment carrying the regulatory region and 5' end of the *Serratia recA* gene between the *Eco*RI and *Sma*I sites of pUC18. The entire polylinker was moved as an *Eco*RI-to-*Hin*dIII fragment into the pTrp-Lac plasmid described above to create pSMrecA-lacZ. The *lexA-lacZ* fusion was made in an identical fashion to the *recA-lacZ* fusion described above, also by using the *Eco*RI and *Eco*RV sites proximal and internal to the *lexA* gene.

*S. marcescens* was transformed essentially as described elsewhere (22).

**DNA sequencing of the *S. marcescens recA* gene.** The *nucB*::Tn*5* mutation was cloned by digesting DNA from the mutant strain with *Eco*RI and ligating it to similarly digested pUC18. Transformants in strain MM294 were selected on kanamycin plates to obtain inserts carrying the Tn*5* transposon, which encodes kanamycin resistance and has no internal *Eco*RI sites. The two transformants that arose contained plasmids with inserts identical in size and restriction pattern, carrying the entire transposon and about 1.7 kb of flanking DNA. One of these was saved and called pNucB-Tn*5*. The DNA sequence was determined by the dideoxy sequencing method, using Sequenase from U.S. Biochemicals.

Briefly, the sequencing strategy was to sequence in from the terminal *Eco*RI sites and to sequence out from the Tn*5* by using an oligonucleotide primer homologous to the ends of Tn*5*. Oligonucleotide primers deduced from newly sequenced regions were then used to complete the sequence and verify that of the other strand. The sequence presented below is the sequence deduced from the Tn*5* insertion mutant by removing the 9-base duplication caused by the Tn*5* transposition.

Computer analysis of the sequence and comparisons with the *E. coli* homolog were carried out by using the University of Wisconsin Genetics Computer Group software on a MicroVAX computer system. The sequence has been submitted to GenBank with the accession number M22935.

**Enzyme assays.** Levels of nuclease production were determined from halo sizes on DNase test agar medium. A microwell dish assay modified from that described by Burke and Slinker (3) was used for routine quantitative analysis. The wells of a microwell dish were filled with 100 μl of a solution of 50 mM Tris (pH 8)–10 mM $MgCl_2$–100 μg of DNA per ml–10 μg of ethidium bromide per ml. A 50-μl sample of nuclease was applied to the top well of each column, and 50 μl was removed and added to the next well. These threefold dilutions were continued for the eight wells in the column. Dilutions of all 12 samples were made concurrently by using a 12-tip multichannel pipettor. A standard nuclease solution was used in one column of each plate for calibration. After incubation at room temperature for 10 to 60 min, the plate was placed on a UV light box and photographed through an orange filter. The loss of fluorescence due to degradation of the DNA was visualized, and each sample was compared

with the standard solution. Many other nucleases are inhibited by the presence of ethidium bromide in this assay, but the *S. marcescens* nuclease worked well. Nuclease activities are presented in a bar chart (see Fig. 1) as the dilution factor required to show no loss of fluorescence for a certain incubation period.

β-Galactosidase levels from the *lacZ* fusions were assayed by the method of Miller (14). Chitinase levels were determined by using tritiated chitin, following the protocol of Molano et al. (15), and protease was measured with azocasein as described previously (9). The serine protease was measured after the addition of 50 mM EDTA to the culture supernatants to inhibit the metalloprotease. Lipase assays were done by using *p*-nitrophenyl palmitate as a colorimetric substrate, as described elsewhere (30). Levels of nuclease, chitinase, or protease production could be estimated from halo sizes on appropriate indicator media (9). Nuclease indicator medium is especially sensitive and can detect nuclease levels less than $10^{-3}$ that of uninduced SM6.

## RESULTS

**Mitomycin C induction of nuclease expression.** Mutants of *S. marcescens* were previously isolated which overexpress the extracellular nuclease (29, 31). These mutants turned out to be pleiotropic in that a number of other activities are also overexpressed, including the extracellular lipase, the colicin equivalent called marcescin, and possibly β-lactamase. Although no regulation of nuclease expression had been described, the existence of these mutants and of the regulatory mutant we previously described (9) suggested that some mechanism of regulation exists. Since colicins are regulated by the SOS system of *E. coli* and since their expression can be induced by DNA-damaging agents, we speculated that the marcescin of *S. marcescens* might be similarly regulated. Since mutants of *S. marcescens* overexpress both marcescin and nuclease, these two activities could be coordinately regulated. This hypothesis was tested by measuring extracellular nuclease from wild-type strain SM6 after mitomycin C induction. Nuclease levels were greatly induced after mitomycin C induction (Fig. 1). We also tested the known regulatory mutants of nuclease expression. Strain W1050 is a nuclease-overproducing mutant [*nuc*(Su)] of strain SM6. Its basal level of expression is elevated relative to that of SM6, but it also is induced by mitomycin C to slightly higher levels.

Plasmids carrying the SM6 nuclease gene were introduced into the nuclease-defective strain TT392. Figure 1 shows that nuclease expression can be induced from pNuc4-R, which carries only about 120 bases of upstream DNA. This confirms that the nuclease regulatory region is present in this 120-base region, as we previously suggested (1). However, no induction of nuclease activity from pNuc4-R was seen in *E. coli*.

We have previously shown that the SM6 *nucB* mutant carries a regulatory mutation abolishing nuclease expression (9). Furthermore, no nuclease is expressed when this strain carries a plasmid-encoded nuclease gene, such as pNuc4-R, or when induced by mitomycin C.

**Transcriptional fusions expressing *lacZ*.** A transcriptional fusion expressing the *lacZ* gene was created so that β-galactosidase could be used to monitor transcription from the nuclease regulatory region. This operon fusion plasmid, pNuc-lacZ, was transformed into the different *S. marcescens* and *E. coli* strains shown in Table 2. In both SM6 and W1128 wild-type strains, β-galactosidase activity was induced 20- to 30-fold by mitomycin C. However, no induction was observed in either the *nucB* mutant or in *E. coli*. The *nuc*(Su) mutant W1050 also overexpressed β-galactosidase from the nuclease promoter without induction, confirming that this mutant carries a regulatory mutation for nuclease expression. Expression of β-galactosidase can be further induced in this strain by mitomycin C to a level about twofold higher than the induced level of the parent strain, W1128.

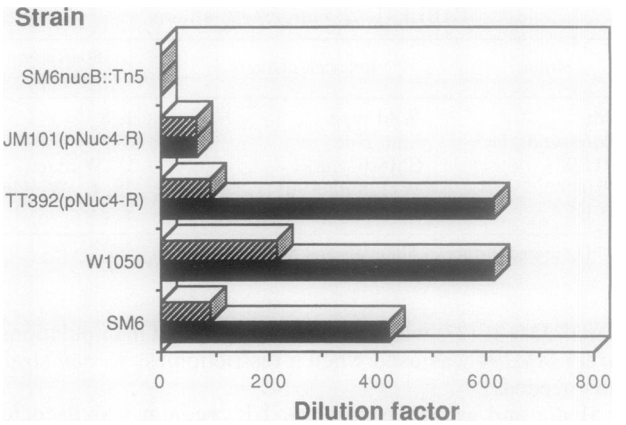


FIG. 1. Mitomycin C induction of nuclease expression from *S. marcescens* and *E. coli* strains. Cultures were grown overnight in LB medium, and the cells were removed by centrifugation. The nuclease found in the growth medium was measured with the microwell dish assay, and relative activities are presented as the dilution required for no loss of fluorescence. Results for uninduced cultures (▨) and cultures grown in the presence of 0.1 μg of mitomycin C per ml (■) are shown.

**Nature of the *nucB* mutation.** Since nuclease expression appears to be under SOS-like regulation by virtue of its mitomycin C inducibility, the *nucB* mutant may be defective in some component of the SOS system. We had noted that colonies of the *nucB* mutant were smaller and that the cultures grew more slowly than the wild type. To further analyze this observation, a UV survival experiment was conducted to determine whether this mutant was more sensitive to UV than was the wild type. In Fig. 2, the UV

TABLE 2. *lacZ* transcriptional fusions to the nuclease promoter

| Strain | Plasmid | β-Galactosidase activity[a] | |
|---|---|---|---|
| | | Without mitomycin C | With mitomycin C |
| JM101 | pNuc2-lacZ | 50 | 70 |
| Sm6 | | <20 | <20 |
| Sm6 | pNuc2-lacZ | 40 | 1,300 |
| Sm6 *nucB*::Tn*5* | pNuc2-lacZ | <20 | <20 |
| W1128 | | 190 | 200 |
| W1050 | | 200 | 200 |
| W1128 | pNuc2-lacZ | 250 (60) | 1,300 (1,100) |
| W1050 | pNuc2-lacZ | 980 (780) | 2,500 (2,300) |

[a] Cells were grown in LB medium to an $A_{600}$ of 0.5. Mitomycin C was added to a final concentration of 0.1 μg/ml, and the cells were grown for 2 h. They were then washed, resuspended in fresh medium, and grown for an additional 3 h. β-Galactosidase activity was measured as described in Materials and Methods. The results presented are an average of at least two experiments. Numbers in parentheses are the corrected values after the background level of β-galactosidase from the F′ *lac* in the W1128 and W1050 strains was subtracted.

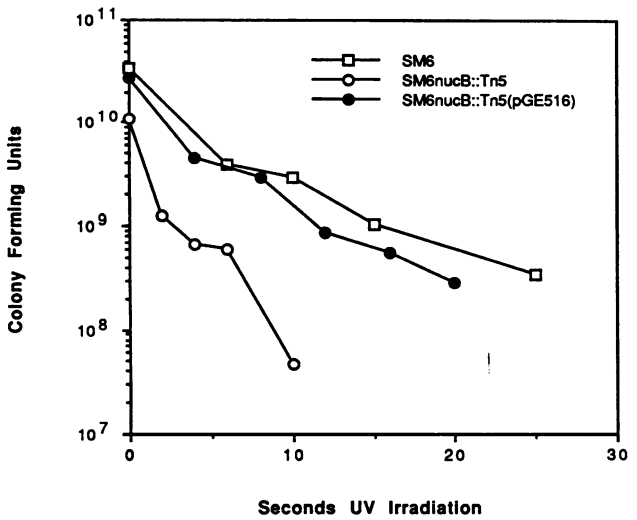


FIG. 2. Survival curve for *S. marcescens* SM6, SM6 *nucB*::Tn*5*, and SM6 *nucB*::Tn*5*(pGE516) carrying the *E. coli recA* gene after exposure to UV irradiation.

survival curve is shown. The *nucB* mutant was clearly more UV sensitive than its parent strain, SM6. These data suggested that the *nucB* mutant was a *recA* mutant. To test this possibility, the *recA* gene of *E. coli* was introduced into the *S. marcescens nucB* mutant, and we show that the gene restored most of the UV resistance of wild-type SM6, confirming that the *nucB* mutant is defective in RecA. The *E. coli recA* gene also restores nuclease expression in the *nucB*::Tn*5* mutant, as well as mitomycin C inducibility, to wild-type levels (data not shown).

**Cloning and sequencing of *S. marcescens recA*.** The *nucB* mutant was isolated after Tn*5* mutagenesis. Since there are no *Eco*RI sites in Tn*5*, an *Eco*RI fragment would carry the entire transposon, with flanking DNA on both sides. Genomic DNA was prepared from the mutant strain, digested with *Eco*RI, and ligated to pUC18 plasmid DNA similarly digested. Transformants were selected on kanamycin medium, since Tn*5* encodes kanamycin resistance. Two colonies were isolated, and both carried the same size insert.

The insert from one of these clones was prepared and used to probe a Southern blot of wild-type SM6 and the *nucB*::Tn*5* mutant genomic DNAs. For SM6, an *Eco*RI fragment of slightly less than 2 kb was observed. In the mutant this fragment was not found, but a single fragment of about 7 kb was found which had the same apparent size on the Southern blot as the plasmid insert (data not shown). This was important for confirming the presence of only a single Tn*5* insert in this mutant strain and that our clone represented the mutation.

The mutant gene, excluding most of the transposon, was sequenced by the dideoxy method, using a variety of synthetic oligonucleotides as primers. In Fig. 3, the DNA sequence of the 1.7-kb *Eco*RI fragment is shown, along with the predicted amino acid translation. The sequence shown is the predicted sequence of the wild-type gene deduced from the mutant sequence by removing the Tn*5* sequence and 9-base-pair duplication caused by the transposon.

When compared with the *E. coli recA* gene, the *Serratia* gene was found to be about 85% homologous over the coding region. The homology is quite strong over most of the gene; however, it is significantly reduced for the terminal 150 bases of the coding region. Downstream of the gene there is little obvious similarity between the *E. coli* and the *Serratia* sequences. In Fig. 3, the *E. coli* amino acid differences only are shown below the *Serratia* translation. The first 306 amino acids are very homologous (>95%), but the carboxyl-terminal 49 amino acids are not. The *Serratia* RecA protein is one amino acid longer than the *E. coli* protein.

In the 250 bases upstream which can be compared, there are regions of strong homology and regions of no homology. One of the regions of very strong homology begins at 50 (for *E. coli*) or 70 (for *S. marcescens*) bases upstream of the coding region. This corresponds to the promoter and LexA repressor-binding region, which is strongly conserved between the two bacteria. These regulatory regions are compared in Fig. 4. The LexA-binding site region of the *E. coli* gene is identical in *S. marcescens* except for a single base. The upstream region of the nuclease gene was also scanned for a LexA-binding site. A site closely fitting the consensus *E. coli* LexA-binding site can be seen.

**Investigating the nuclease-superexpressing mutant.** The *nuc*(Su) mutant strain W1050 carries a pleiotropic mutation causing the overexpression of nuclease, lipase, marcescin, and possibly β-lactamase. Presumably the mutation inactivates a common repressor regulating these extracellular proteins. Either this repressor could be LexA, suggested by the fact that the nuclease gene has a consensus LexA-binding site upstream, or the repressor identified by the *nuc*(Su) mutation could be a different molecule, specific, for example, for extracellular proteins. Two experimental approaches were used to differentiate between these possibilities.

First we introduced pSMrecA-lacZ carrying a *recA-lacZ* transcriptional fusion into strains W1128 and W1050. The experimental conditions were identical to those described for Table 2. β-Galactosidase expression was mitomycin C inducible in both W1050 and W1128 (β-galactosidase activities of 9,100 and 7,200 U, respectively), but W1050 had about a twofold-elevated uninduced level relative to its parent (2,700 versus 1,200 U). These β-galactosidase values can be adjusted by subtracting the background from the values in Table 2, but this has no significant effect. The mutation in W1050 appears to have a slight effect on RecA expression but not as significant an effect as on nuclease expression. We had previously shown (1) that overexpression of the nuclease from a plasmid in *E. coli* leads to a slight induction of the SOS system, as measured with a *recA-lacZ* fusion. Since W1050 overexpresses the nuclease, we are probably observing the same effect on RecA expression in *S. marcescens*.

Next we introduced the *E. coli lexA* gene into strains SM6 and W1050. The gene was subcloned as an *Eco*RI-*Pst*I fragment from pGE106 into pUC18 to create an ampicillin-resistant derivative for transformation into *S. marcescens*. With either the wild-type SM6 and W1128 strains or the overexpressing mutant W1050, we observed no change in nuclease expression with or without the *lexA* plasmid. Both halo sizes on nuclease indicator plates and nuclease levels in supernatants from overnight cultures with or without mitomycin C induction were analyzed.

A *lexA-lacZ* transcriptional fusion, made in an identical fashion to pSMrecA-lacZ by using the upstream *Eco*RI site and the internal *Eco*RV site in *lexA*, was used to verify *lexA* expression in *S. marcescens*. In strain SM6 this operon fusion expressed 700 U of β-galactosidase and could be induced by mitomycin C to 1,500 U.

**Construction of a *recA nuc*(Su) double mutant.** To determine whether either mutation is dominant, which would help

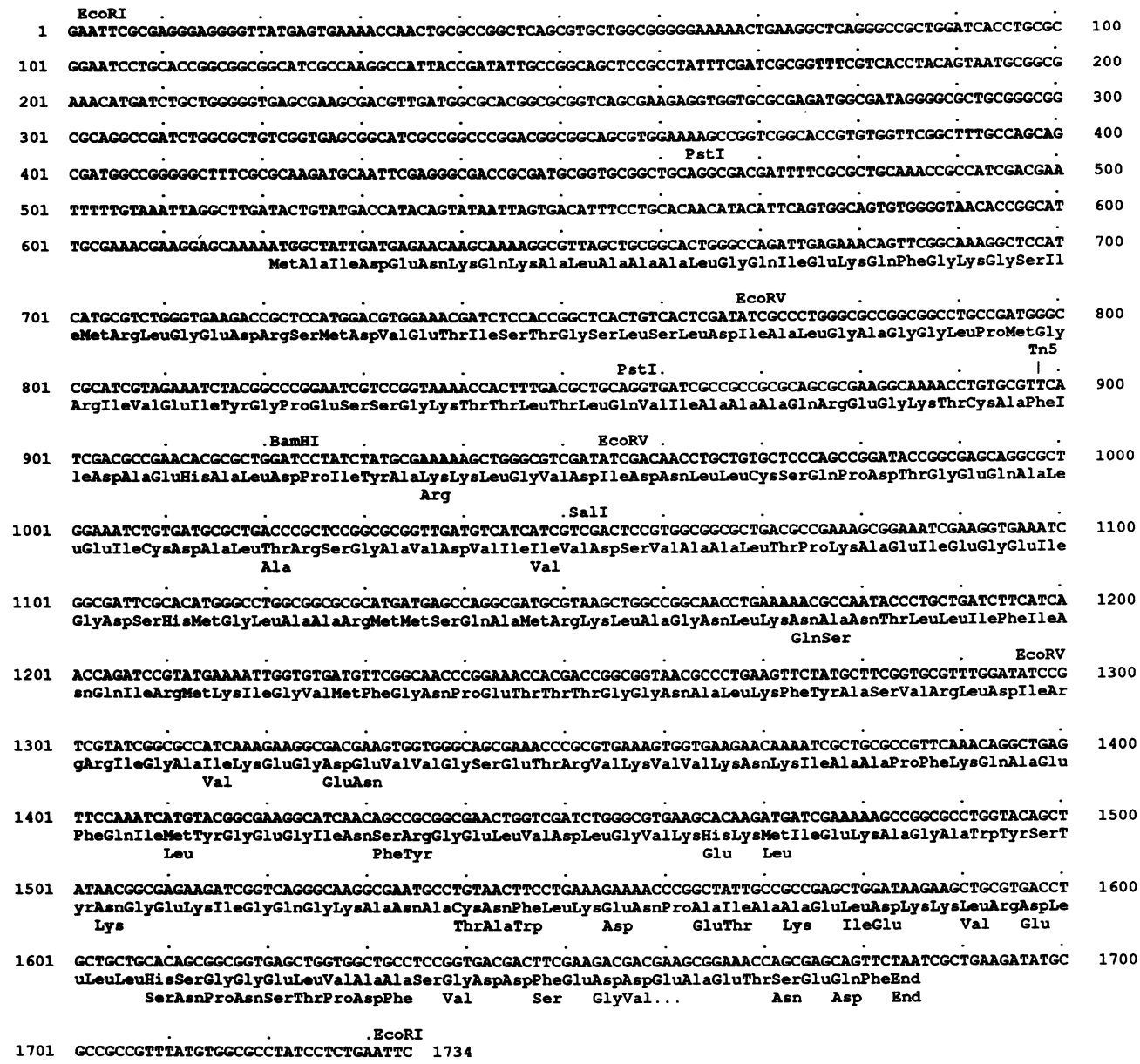


FIG. 3. DNA sequence of the *recA* gene from *S. marcescens* SM6. The predicted amino acid sequence based on homology to the *E. coli* sequence is shown below the DNA sequence. The amino acids which are different in the *E. coli* sequence are shown below the *S. marcescens* amino acid sequence. The Tn*5* insertion at position 898 is displayed, as are common restriction endonuclease cleavage sites. Starting at position 1659, the *S. marcescens* sequence has a 3-base insertion relative to the *E. coli* sequence.

characterize the interaction between the regulatory mutants, a double-mutant strain was constructed by generalized transduction. The *S. marcescens*-transducing bacteriophage PS20 (12) was grown on the *nucB*::Tn*5* mutant, and the lysate was used to transduce W1050 to kanamycin resistance. About half of the kanamycin-resistant colonies produced no halo on DNase indicator medium. These colonies could be shown to be sensitive to UV irradiation, as expected for *recA* mutants.

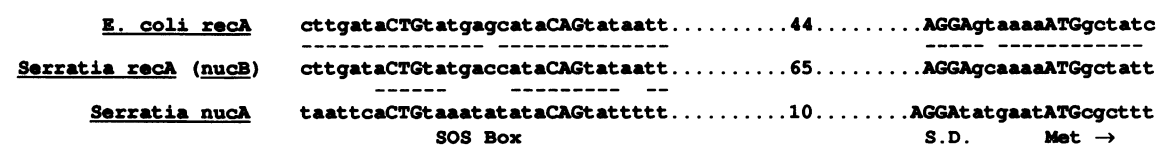


FIG. 4. Comparison of the upstream regulatory regions from the *E. coli recA* gene, the *S. marcescens recA* gene, and the *S. marcescens* nuclease gene. Identical bases are indicated (-). The highly conserved bases from the consensus SOS box are displayed in capital letters (CTG and CAG), as are the Shine-Dalgarno ribosome-binding site region (AGGA) and the Met start codon (ATG).

TABLE 3. Mitomycin C induction of other extracellular proteins

| Strain | Induction treatment | Enzyme activity[a] | | | |
|---|---|---|---|---|---|
| | | Lipase | Chitinase | Metallo-protease | Serine protease |
| SM6 | | 0.10 | 170 | 0.06 | 0.03 |
| SM6 | Mit-C | 0.33 | 1,500 | 0.06 | 0.03 |
| SM6 | Chitin | ND | 310 | ND | ND |
| SM6 | Chitin + Mit-C | ND | 6,800 | ND | ND |
| W1050 | | 0.16 | 2,000 | 0.06 | 0.03 |
| W1050 | Mit-C | 0.52 | 4,600 | 0.06 | 0.03 |

[a] Measured from the growth media of overnight cultures grown in LB medium with or without 0.1 μg of mitomycin C (Mit-C) per ml and with or without chitin added to the medium. Lipase, chitinase, and protease were measured as described in Materials and Methods. Metalloprotease was measured in the presence of 0.0125 M phenylmethylsulfonyl fluoride to inhibit the serine protease. Serine protease was measured in the presence of 0.04 M EDTA to inhibit the metalloprotease. ND, Not determined.

Introducing the *E. coli recA* gene into a transductant restored nuclease expression to the *nuc*(Su) level. We presume that the other transductants arose by secondary transposition events, which we also observed when transducing wild-type SM6 to kanamycin resistance. This result also confirms that the mutation in W1050 is not in *lexA*, since the overexpression phenotype would be expected to be dominant in that case.

**Mitomycin C regulation of other excreted proteins.** Since both the lipase and nuclease are affected by the pleiotropic mutation in W1050 and the nuclease is regulated by an SOS-like system, it seemed logical to question whether the other extracellular proteins from *S. marcescens* are similarly regulated. The proteins include chitinase and both a metalloprotease and a serine protease. These different enzymes were measured from uninduced and mitomycin C-induced cultures. Neither protease was regulated by mitomycin C, but both lipase and chitinase activities were induced (Table 3). Strain W1050 overproduced both chitinase and lipase but not protease. Chitinase expression is also known to be induced by chitin. This regulation is superimposed upon mitomycin C regulation (Table 3).

In Fig. 5 the extracellular protein profile from induced and uninduced SM6 cultures, as well as W1050, is shown after resolution by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A number of proteins appear in the culture supernatant from mitomycin C-induced cultures, confirming that many extracellular proteins are regulated by this SOS-like system. This result also suggests that *S. marcescens* produces more extracellular proteins than have been identified to date, at least after mitomycin C induction.

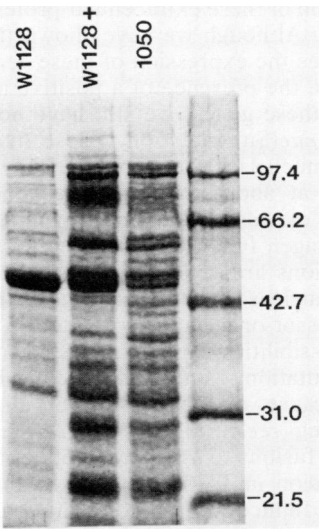


FIG. 5. Extracellular proteins found in the supernatant of *S. marcescens*. Cells were grown overnight with (+) or without mitomycin C and removed by centrifugation. The growth medium supernatant was precipitated with acetone and fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% gel. The gel was stained with Coomassie blue. Molecular weight markers (in thousands) are shown in the right-hand lane.

## DISCUSSION

Regulation of the extracellular nuclease from *S. marcescens* has been a mystery despite the availability of regulatory mutants previously described (9, 29, 31). In this work we show that the nuclease and other extracellular proteins are regulated by an SOS-like system. A transposon mutation inactivating the *Serratia recA* gene abolishes nuclease expression and greatly reduces chitinase expression.

We presume that the SOS system from *Serratia* sp. works in a fashion similar to that in *E. coli*, in which a number of genes coordinately regulated by the SOS system are all repressed by the LexA repressor (28). Similarly, certain other repressors, such as the *c*I protein of bacteriophage λ, can be inactivated by the activated RecA protein. In response to DNA damage the RecA protein is converted to an active form, leading to an autocatalytic cleavage of LexA and other repressors. This allows a number of different repressed systems to coordinate the expression of their proteins, depending on the state of RecA in the cell.

The *recA* gene of *S. marcescens* is shown to be highly homologous to the *E. coli* gene, with significant divergence only at the carboxyl-terminal region. It is not clear whether these differences suggest altered specificity or properties of RecA or whether this region is less critical. The putative LexA-binding sites from the nuclease gene and the *recA* gene of *S. marcescens* both fit the consensus for a LexA-binding site from *E. coli*, suggesting that the LexA proteins should be similar in both of these organisms.

One striking paradox remains. Although nuclease expression is mitomycin C inducible in *S. marcescens*, and although critical components such as RecA can be provided by *E. coli* to complement *Serratia* mutants, the nuclease gene is not mitomycin C inducible in *E. coli*. Plasmids carrying the nuclease gene, or carrying transcriptional fusions to *lacZ*, were expressed at a low constitutive level in *E. coli* and could not be induced by mitomycin C. It certainly is possible that the nuclease promoter does not work well in *E. coli*. A variety of other *Serratia* promoters do work well in *E. coli* (4, 10, 19), but expression of the *Serratia* serine protease is suggested to come only from a plasmid promoter (32). If an unidentified activator protein is required for nuclease promoter activity, then this promoter would not be expected to work in either *S. marcescens* or *E. coli* in its absence. In this case, nuclease expression in *E. coli* may originate from a plasmid promoter, from another low-level promoter elsewhere on the DNA fragment, or from low-level expression of the native nuclease promoter. If nuclease expression does come from some fortuitous promoter, then mitomycin C induction should not be observed; however, if expression originates from the nuclease promoter, then the basal expression should still show some mitomycin C regulation.

The regulation of these extracellular proteins may be even more complex. Although we have shown that an SOS-like system regulates the expression of these gene products and we hypothesize the presence of a positive activator for the expression of these genes, we still have not explained the nature of the *nuc*(Su) mutation. These mutations are not changes in the nuclease promoter, since they are pleiotropic, can be found at about the same frequency as nuclease mutations, and can also be found after mutagenesis with the frameshift mutagen ICR-191 (31). Therefore, at least some *nuc*(Su) mutations are gene knockout mutations. The elevated level of nuclease expression suggests that the mutation defines a repressor or negative regulator of this system.

Different possibilities can be suggested for the nature of the *nuc*(Su) mutation. Mutations affecting LexA would be expected to cause overexpression of all SOS-regulated genes, including *recA*. Measuring *recA* expression with transcriptional fusions to *lacZ* shows about a twofold elevation of expression in W1050 relative to that in its parent strain, W1128. Induction by mitomycin C further increases *recA* expression another three- to fourfold. This would not be expected in a LexA-deficient strain, although it is compatible with a leaky *lexA* mutation. These results are also compatible with another explanation. We had previously shown (1) that expression of the nuclease gene in *E. coli* results in a slight induction of the SOS system, as measured by a *recA-lacZ* transcriptional fusion. This is presumably due to DNA damage caused by the nuclease itself. Since W1050 overexpresses nuclease, we might expect an increased basal-level expression of SOS-regulated genes.

Even more germane is the introduction of the cloned *E. coli lexA* gene on a multicopy plasmid. It has no effect on nuclease expression from strain W1050, suggesting that the *E. coli lexA* gene cannot complement the *nuc*(Su) lesion. This could either mean that the mutation is not in *lexA* or that the *E. coli* gene is not expressed in *S. marcescens*. By using *lexA-lacZ* transcriptional fusions, we have shown that the *E. coli* gene is transcribed in *S. marcescens* but have not proven that it is functional. In other enteric bacteria, such as *Erwinia*, *Citrobacter*, *Shigella*, and *Klebsiella* spp., the *E. coli* LexA protein has actually been shown to function (13, 24).

Lastly, we created a double-mutant strain carrying both the *nuc*(Su) mutation and the *nucB*::Tn*5* mutation, which we now know is a *recA* null mutant. If the *nuc*(Su) mutation was a leaky *lexA* mutation, then this double mutant would be expected to overexpress nuclease. Instead, the *recA* mutation was dominant.

Although we cannot totally exclude the possibility that the mutation is a leaky *lexA* allele, these experiments, taken together, do suggest that the *nuc*(Su) mutation defines another regulatory locus. This regulator, presumably a repressor, is probably specific to a regulatory network for many of the extracellular proteins of *S. marcescens*, since the only observed phenotype is overexpression of a variety of extracellular proteins. The regulatory protein defined by the *nuc*(Su) mutation need not act directly on the extracellular protein genes themselves but may instead act by repressing another regulatory molecule, such as our suggested activator protein. Bear in mind that if the *nuc*(Su) mutation were actually a leaky *lexA*, we would not have to postulate this second repressor. Conversely, since we have not ruled out or directly implicated *lexA* in the mitomycin C induction of extracellular proteins, we cannot claim that this regulatory system is strictly identical to SOS regulation of *E. coli*. Therefore, we refer to an SOS-like form of regulation.

The reason for such a complex mode of regulation may be that the extracellular nuclease is growth phase regulated; it is expressed only as the cells enter stationary phase (T. K. Ball and M. J. Benedik, unpublished observations). Similar regulation has been observed for the *Serratia* phospholipase (7). Growth phase-regulated promoters have been found for microcin expression in *E. coli* (5) and have been well studied in *Staphylococcus aureus*, in which mutations have been isolated and an accessory gene for the expression of growth phase-regulated proteins has been identified (20). This growth phase regulation may be distinct from the SOS-like regulation, implying that the nuclease gene is regulated separately by two different systems. Alternatively, these two regulatory modes may be intertwined. These questions are presently under investigation.

Although it is perhaps surprising to find an SOS-like system of regulation for *Serratia* extracellular proteins, it is not unique to *Serratia* spp. The pectin lyase and carotovoricin of *Erwinia carotovora* have also been shown to be regulated by the SOS system (33). It is not clear why extracellular proteins are controlled by this mechanism of gene regulation. For colicin expression, it has been shown that the basal production of colicin in uninduced cultures is due to the complete induction of only a few cells in the culture (25). This result may be due to spontaneous induction of the SOS system (23). The same phenomenon may hold true for nuclease expression; we cannot rule out the possibility that the basal-level nuclease expression does come from a few cells expressing high levels of nuclease, as opposed to all cells expressing a low level. The phenotype of *recA* mutants is also similar; both with colicin expression (23) and nuclease expression, the *recA* mutation essentially shuts off the basal level of expression.

With toxin and colicin production, there is a certain logic to utilizing SOS regulation. Colicin expression leads to cell death; therefore, those cells which have sustained DNA damage to the extent requiring maximal SOS induction are logically the cells in the population with the lowest survival fitness. However, this is not the case with extracellular catabolic enzymes, such as chitinase, lipase, and nuclease. We know that chitinase and nuclease can be expressed at high levels from plasmids under control of the *lac* promoter with no loss of viability. Therefore, unlike the colicin model, a rationale for SOS regulation of these extracellular enzymes must be sought elsewhere.

One factor common to both *Erwinia* and *Serratia* spp. is that, rather than being strictly enteric, both can be found free-living in the environment. An SOS-like regulatory system may provide a means for the bacteria to sense the environmental location. Bacteria present on leaf surfaces, on fruit, or on top of the soil should be constantly bombarded by UV irradiation from the sun. These may be precisely the environments where the bacteria would find substances they can metabolize by using extracellular degradative enzymes.

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