The NucE and NucD lysis proteins are not essential for secretion of the *Serratia marcescens* extracellular nuclease

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The nuclease of *Serratia marcescens* is an extracellular protein encoded by the *nucA* gene. Pre-nuclease carries a typical 21-amino-acid N-terminal signal sequence that interacts with the Sec machinery to allow the translocation of nuclease to the periplasm. In *Escherichia coli* the nuclease remains in the periplasm; however, *S. marcescens* has the capacity to secrete nuclease extracellularly. The *nucC* operon carrying the *nucEDC* genes of *S. marcescens* has been identified previously. NucC is a transcriptional activator necessary for expression of nuclease as well as the extracellular bacteriocin 28b. NucE resembles and can act as a bacteriophage holin, whereas NucD has homology to bacteriophage lysozyme-like proteins. When present on a multicopy plasmid, the *nucC* operon, and specifically the *nucED* genes, appeared to allow extracellular secretion of nuclease from *E. coli*. Here experiments are reported which demonstrate that, when the *nucC* operon was placed in the *E. coli* chromosome in single copy, nuclease secretion was lost and nuclease remained periplasmic. The converse experiment, deletion of the *nucE* and *nucD* genes from the chromosome of *S. marcescens*, likewise had no effect on nuclease secretion by *S. marcescens*. It is concluded therefore that NucD and NucE are not necessary for nuclease secretion.

Keywords: nuclease, protein secretion, *Serratia marcescens*, lytic enzymes

INTRODUCTION

The Gram-negative enteric bacterium *Serratia marcescens* is a prolific producer of extracellular enzymes which serve to exploit its environment for nutrients. These proteins, which include proteases, chitinases, a lipase and phospholipase, siderophores, haemolysin, bacteriocins and a nuclease, utilize differing secretory systems to gain their release from the cell. Here we continue our studies on the extracellular nuclease. It is initially synthesized as a pre-protein of 266 amino acids with a signal peptide consisting of 21 amino acid residues. Nuclease secretion is a two-step process in *S. marcescens*, in which the pre-enzyme is first rapidly exported to the periplasm, the result of typical Sec-dependent signal sequence processing, and then is translocated slowly across the outer membrane (Benedik & Strych, 1998; Suh *et al.*, 1996).

Nuclease expression as well as its secretion are regulated by growth phase (Suh *et al.*, 1996). Transcription of the *nucA* gene is also SOS-regulated (Chen *et al.*, 1992) and

requires, in addition, the transcriptional activator NucC (Jin *et al.*, 1996). NucC is a member of the bacteriophage P2 Ogr family of transcriptional activators and likely interacts with the α subunit of RNA polymerase. NucC binds to a region between –82 and –51 upstream of the transcriptional start of *nucA*, which includes a copy of the TGT–N12–ACA activator recognition motif, whose deletion obliterates activation (Winslow *et al.*, 1998).

Interestingly, *nucC* lies in an operon with two other genes, *nucD* and *nucE*, which bear strong resemblance to genes encoding phage proteins. NucE shows homology to phage holin proteins involved in releasing lysozyme to the peptidoglycan of Gram-negative bacteria. It possesses the characteristic two transmembrane domains and a positively charged C-terminus. Moreover, it is capable of acting as a holin in *Escherichia coli* and functionally substituting for the S protein of bacteriophage λ (Berkmen *et al.*, 1997). Its closest homologue is a putative holin found in the genome of '*Haemophilus somnus*' (Pontarollo *et al.*, 1997). NucD shows significant homology to the V type lysozymes

Table 1. Bacterial strains, plasmids and bacteriophages

Construction of some strains is further described in Results.

Strain, phage or plasmid	Description	Source or reference
Strains		
<i>S. marcescens</i>		
MB841	Wild-type Sm6	
MB991	SM6 <i>nucD</i> ::Tn5	Chen <i>et al.</i> (1992)
MB1325	SM6 <i>nucEDC</i> ΔBgl	This study
MB1365	SM6 <i>nucEDC</i> ΔNco2-3	This study
MB1437	SM6 <i>nucEDC</i> ΔEcoRVBgl	This study
<i>E. coli</i>		
BT333	<i>endA</i> ::tet ^r	Cherepanov & Wackernagel (1995)
GE43	MC4100 Δ(<i>gal</i> -G ^h) <i>b2</i> ^λ ::(Km ^r <i>lac</i> 'ZY ⁺)	Weisemann <i>et al.</i> (1984)
MB503	<i>supE thi hsdΔ5</i> Δ(<i>lac</i> - <i>proAB</i>) <i>endA</i> ::Tn10 F'[<i>traD36 proAB lacI^a lacZ</i> ΔM15]	Laboratory stock
MC1000.1	<i>araD139</i> Δ(<i>ara</i> - <i>leu</i>)7679 <i>galU galK</i> Δ(<i>lac</i>)174 <i>rpsL thi-1</i>	Laboratory stock
MB1310	MC1000.1 PhoA ^{cs}	Spontaneous PhoA ^{cs}
MB1318	MB1310 <i>endA</i> ::tet	Transductant from BT333
MB1324	MB1318 <i>endA</i> :: <i>tet</i> Δ	EndA ⁻ Tet ^s cured
MB1371	MB1324 Δ(<i>gal</i> -G ^h) <i>b2</i> ^λ ::(Km ^r <i>lac</i> 'ZY ⁺)	Km ^r transductant from GE43
MB1440	MB1371 <i>lacZYA</i> ⁺	Lac ⁺ from Hfr mating
MB1464	MB1440 <i>lacZ</i> :: <i>nucA</i> ⁺	Lysogenized and cured with λNuc2R, Km ^s
MB1477	MB1464 <i>lacZ</i> :: <i>nucEDC</i> ⁺	This study
MB1478	MB1464 <i>lacZ</i> :: <i>nucEDC</i> ΔNco2-3	This study
Bacteriophages		
λplac5	λcI857Sam100 <i>plac5nin5</i>	Laboratory stock
λNuc2R	λcI857Sam100 <i>plac5lacZ</i> :: <i>nucA-2Rnin5</i>	This study
Plasmids		
pAC9	Km ^r p15 plasmid with pUC9 polylinker	Devine <i>et al.</i> (1989)
pBS	pBluescript SK II(+)	Stratagene
pMAK705	Temperature-sensitive pSC101 vector, Cm ^r	Hamilton <i>et al.</i> (1989)
pZ150Bam3.3	pZ150 containing the <i>S. marcescens</i> <i>nucEDC</i> operon on a 3.3 kb <i>Bam</i> HI fragment	Jin <i>et al.</i> (1996)
pMAKBam3.3	pMAK705 with <i>Bam</i> HI insert from pZ150Bam3.3	This study
pZ150Bam3.3ΔBgl	Frameshift at <i>Bgl</i> II site of C-terminus of NucE	Jin <i>et al.</i> (1996)
pMAKBam3.3ΔBgl	pMAK705 with <i>Bam</i> HI insert from pZ150Bam3.3(ΔBgl)	This study
pZ150Bam3.3ΔNco2-3	Deletion of <i>Nco</i> I ₂ - <i>Nco</i> I ₃ in Bam3.3	Jin <i>et al.</i> (1996)
pMAKBam3.3ΔNco2-3	pMAK705 with <i>Bam</i> HI insert from pZ150Bam3.3ΔNco2-3	This study
pAC9Bam3.3	pAC9 with <i>Bam</i> HI insert from pZ150Bam3.3	This study
pAC9Bam3.3ΔEcoRVBgl	Deletion of <i>Eco</i> RV- <i>Bgl</i> II in Bam3.3	This study
pMAKBam3.3ΔEcoRVBgl	pMAK705 with <i>Bam</i> HI insert from pAC9Bam3.3ΔEcoRVBgl	This study

* The superscript c represents constitutive expression of PhoA.

(Berkmen *et al.*, 1997; Fastrez, 1996); its closest homologues are the lysozyme of bacteriophage P1 (Schmidt *et al.*, 1996) and again the lysis cassette of '*H. somnus*'.

The holin-lysozyme system is used by many bacteriophages to release their progeny from infected cells (Young, 1992). The viral nature of these genes implies that their bacterial homologues, such as *nucED* or those of '*H. somnus*', originated as part of a cryptic prophage, either arising from phage or perhaps giving rise to phage lysis genes. The NucC analogue of *S. marcescens* strain 2170 (Ferrer *et al.*, 1996), RegC, functions as an activator of bacteriocin 28b (*bss*) transcription as it does for *nucA*. It is tempting to speculate, but it has not been demonstrated, that the NucED homologues of this strain are important for bacteriocin release.

The aim of this study was to clarify the role of NucE and NucD with respect to nuclease secretion in *S. marcescens*. Since the expression of NucE and NucD is coordinated with expression of NucA, they may play a role in the secretion of the NucA nuclease. If so, then it is likely that the extracellular appearance of this nuclease is non-specific and likely due to cell lysis. In fact, when the *nucEDC* operon was carried on a multicopy plasmid in an *E. coli* strain carrying *nucA*, not only was nuclease expression increased, but the enzyme was also found in the growth medium (Jin *et al.*, 1996). Here we create *E. coli* strains carrying the *nucA* and *nucEDC* genes in single copy on the chromosome and expressed from their native promoter. We also create mutant strains of *S. marcescens* deleted for *nucE* and *nucD* which form the basis for our determination that NucE and NucD play no significant role in nuclease secretion. Therefore, as we have earlier proposed, it seems likely that nuclease is secreted by a specific transport system (Suh *et al.*, 1996).

METHODS

Bacterial strains, plasmids and growth conditions. The plasmids and strains used in this study are described in Table 1. *E. coli* and *S. marcescens* were routinely grown in LB medium (Miller, 1992) at 37 °C and 30 °C, respectively. When necessary, the growth medium was supplemented with 100 µg ampicillin ml⁻¹, 25 µg kanamycin ml⁻¹ or 30 µg chloramphenicol ml⁻¹ for *E. coli*, and 1 mg ampicillin ml⁻¹, 100 µg kanamycin ml⁻¹ or 200 µg chloramphenicol ml⁻¹ for *S. marcescens*. Nuclease indicator plates were made by adding 80 µg methyl green (Sigma) ml⁻¹ to DNase Test Agar (Difco Laboratories).

DNA manipulations. Chromosomal and plasmid DNA isolation and manipulation were performed as described previously (Jin *et al.*, 1996). DNA transformation into *S. marcescens* was done by electroporation (Miller, 1994) and into *E. coli* strain MB503 by the one-step method (Chung *et al.*, 1989). DNA sequencing was performed on an ABI Prism 373 sequencer using the dye terminator cycle sequencing kit as recommended by the manufacturer (Perkin-Elmer).

Southern hybridization. DNA probes were labelled radioactively with [α -³²P]dCTP using the Prime-A-Gene kit (Promega). After digestion with an endonuclease, the DNA was separated on an agarose gel and transferred to a Gene Screen Plus Membrane. Hybridization was performed under stringent conditions at 68 °C (Sambrook *et al.*, 1989).

PCR. PCR was performed on a GeneAmp 2400 thermal cycler (Perkin-Elmer) using *Taq* polymerase. For the amplification of the *nucEDC* operon the following oligonucleotides (Life Technologies) were used: NucE-1 (5' TTGAT GCCTC CGGCG CTCCC G); NucE-2 (5' GTGAC TGCGG GTGGA ATTCG G); NucC-1 (5' CGGGT GCGGC TGCGC CGGGA C).

Gene replacement. Chromosomal gene replacement was performed following integration of a temperature-sensitive plasmid as described by Hamilton *et al.* (1989). P1 transductions and other genetic methods were performed according to standard methods (Miller, 1992).

Enzyme assays. Nuclease activity was measured by a microtitre dish assay and β -lactamase by a spectrophotometric assay as described by Jin *et al.* (1996). The extracellular fraction was prepared by removing cells by centrifugation at high speed in a microcentrifuge. The cellular fraction was prepared by freeze-thaw lysis (Suh *et al.*, 1996).

RESULTS

Construction of *nucEDC* mutants

The plasmid pZ150Bam3.3 is a pBR322-based plasmid carrying the *nucEDC* operon (Fig. 1) on a 3.3 kb *Bam*HI fragment (Jin *et al.*, 1996). Mutations in the *nucE* and *nucD* genes were previously constructed (Jin *et al.*, 1996) as follows. The *nucE* mutant, Bam3.3 Δ Bgl, is a frame-shift mutation created by filling in the *Bgl*II restriction site within *nucE*. Bam3.3 Δ Nco2-3 is a deletion of *nucED* constructed by deleting the DNA fragment between *Nco*I sites 2 and 3. The *nucE* deletion mutant Bam3.3 Δ EcoRVBgl has the DNA between *Eco*RV and *Bgl* II sites deleted, removing the first 228 nt of the N-terminus of *nucE*.

Chromosomal allele replacement

The mutants described above were subcloned into the temperature-sensitive replication vector pMAK705 (Hamilton *et al.*, 1989) which was used to exchange these alleles between plasmids and the *S. marcescens* chromosome. Strain MB991 was the recipient for such allele exchanges. It carries a Tn5 insertion in the *nucD* gene which has been shown to dramatically reduce *nucA* transcription due to the polar effect of Tn5 on transcription of *nucC* (Jin *et al.*, 1996). After electroporation of pMAKBam3.3EC, pMAKBam3.3 Δ Nco2-3, pMAKBam3.3 Δ Bgl and pMAKBam3.3 Δ EcoRVBgl into MB991 at the permissive temperature of 30 °C, nuclease activity of the plasmid-bearing strains was measured by the microtitre dish assay to verify successful transformation. Introduction of an expressed *nucC* gene restores nuclease expression.

Plasmid integrants were selected on chloramphenicol agar at the non-permissive temperature of 42 °C where plasmid replication does not occur. Integrants were purified and resolved in a second recombination event at 30 °C. After a final round of growth at 42 °C without selective pressure, loss of the plasmid was determined by replica-picking colonies for loss of chloramphenicol resistance. Allele replacement was confirmed by loss of

Fig. 1. Map of the *nucEDC* deletion mutants used in this study. The relevant features of the 3.3 kb *Bam*HI fragment containing the *nucEDC* operon are depicted at the top. The name of the plasmid construct is given on the left of the figure and the corresponding strain is named on the right. The insertion site of the Tn5 transposon in MB991 is indicated by a large inverted triangle. Δ, Deletions generated by restriction digestion and religation; X, frameshift mutation in *nucE*; P, *nucEDC* promoter region.

Fig. 2. Detection of nuclease activity on DNase Test Agar medium with methyl green. A single colony of wild-type strain MB841 and four *nucEDC* mutants was picked to a plate and grown overnight at room temperature. The size of the halo around the *S. marcescens* colonies is indicative of the level of nuclease expression.

kanamycin resistance and concurrent restoration of nuclease activity. These mutants are strains MB1325, MB1365 and MB1437 summarized in Table 1.

Physical verification of the chromosomal gene replacements

The replacement of the *nucD*::Tn5 allele of strain MB991 with the *nucE* and *nucD* mutations was verified by PCR analysis and Southern hybridization. For the

diagnostic amplification of the *nucEDC* operon from the wild-type strain MB841 and from the mutants MB1325 and MB1365, primers NucE-1 and NucC-1 hybridizing upstream of *nucE* and downstream of *nucC*, respectively, were used. For the wild-type MB841 and the *Bgl*II frameshift mutant MB1325, the predicted 1 kb band was detected by agarose gel electrophoresis and analysed by *Bgl*II restriction digestion for the presence or absence of that site. For the Δ*Nco*I deletion mutant MB1365, the expected 370 bp band was observed. Primers NucE-2 and NucC-1 were used for strain MB1437, which is deleted for the NucE-1 primer binding site, and yielded the correct amplification product of 1200 nt.

To exclude the presence of duplications of the *nucEDC* region on the chromosome, a Southern hybridization, using the 3.3 kb *Bam*HI fragment from pZ150Bam3.3 as a probe, was performed. After digesting the genomic DNAs with *Bam*HI, there was only a single band of the predicted size with each of the mutants.

Nuclease secretion by the *nucED* mutants

The *nucED* mutants MB991, MB1325, MB1365 and MB1437, as well as the *S. marcescens* wild-type strain MB841, were analysed for nuclease production. In Fig. 2 a DNase Test Agar plate is shown of these strains. The halo sizes are good representations of the amount of nuclease produced and released by the bacteria. There is no significant difference among the *nucE* and *nucED* mutant strains; however, as expected, MB991 has greatly reduced halo size. These strains were further tested for nuclease activity found within the cells or released to the culture supernatant after overnight growth to stationary phase, and these data are presented

Table 2. Localization of nuclease activity in *S. marcescens* mutant strains

Strains were grown to stationary phase in LB medium. Intracellular and extracellular fractions were prepared. Nuclease activity (arbitrary units representing the dilution factor required to make the activity undetectable for a given time period) was assayed by the microtitre dish assay and the values are rounded to the nearest 10.

Strain	Nuclease activity	
	Intracellular	Extracellular
MB841	1	30
MB991	<1	<1
MB1325	1	30
MB1365	1	30
MB1437	1	30

in Table 2. Other than MB991, these strains all expressed and secreted similar levels of nuclease activity. These experiments demonstrate that NucE and NucD are not essential for nuclease production or secretion by *S. marcescens*. As a further confirmation that these enzyme activities are representative of the protein levels, the NucA protein was analysed by Western blotting. The band intensities (Western blot not shown) found in the cellular and extracellular fraction correlated well with the enzymic activities found.

The above experiments present only a static picture of nuclease activity found after prolonged growth. It is possible that NucE or NucD plays a role in the rate by which nuclease is released by the cell. A further experiment was undertaken to address this issue. In Fig. 3 profiles of nuclease activity found in the cellular and extracellular fraction are presented at different times during growth of the culture. The profiles of the NucE

Fig. 3. Nuclease expression and secretion during growth of *S. marcescens*. Wild-type strain MB841 and three *nucEDC* mutants were grown in LB medium and samples were taken at different time points during growth. The OD₆₀₀ (●) was measured spectrophotometrically and nuclease activity (the arbitrary units represent the dilution factor required to make the activity undetectable for a given time period) was measured by a microtitre dish assay (Jin *et al.*, 1996) and is plotted against the time in hours after inoculation. Extracellular nuclease is represented by solid bars, and intracellular activity by hatched bars.

Table 3. Localization of nuclease activity in *E. coli*

Strains were grown overnight in LB medium supplemented with antibiotics as needed. Enzymes were measured as described in Methods and the values are rounded to the nearest 10. ND, Not determined.

Strain	Nuclease*		β -Lactamase†	
	Intracellular	Extracellular	Intracellular	Extracellular
MB1464	< 1	< 1	ND	ND
MB1477(pBS)	60	30	20	< 1
MB1478(pBS)	60	30	20	< 1
MB1464(pMAK705)	< 1	< 1	ND	ND

* Activity is measured in arbitrary units which represent the dilution factor required to make the activity undetectable for a given time period.

† Activity is measured as the $\Delta A_{420} \text{ min}^{-1} \times 100$.

and NucD mutant strains are essentially identical to the wild-type strain; the ratio of extra- to intracellular nuclease activity increased throughout the growth phase reaching its maximum at the last time point. This confirms that nuclease is predominantly secreted to the extracellular medium in all strains, and at essentially the same rate, indicating that the presence of NucD or NucE cannot be required for nuclease secretion. From these results it can be concluded that NucC, as described earlier, strongly increases nuclease expression, but that NucD and NucE do not contribute to either expression or secretion of nuclease in *S. marcescens*.

Nuclease secretion from *E. coli*

Previously it was observed that NucED could allow the extracellular release of NucA from *E. coli*, in the absence of apparent lysis, using strains carrying the genes encoding these proteins on multicopy plasmids (Jin *et al.*, 1996). Was this observation an artifact of the higher copy number or do NucED allow NucA release from *E. coli*? In order to test this, an *E. coli* model strain was built carrying *nucA* and *nucEDC* in single copy on the chromosome.

Briefly, *E. coli* MC1000.1 was deleted for the periplasmic nuclease gene *endA* as described by Cherepanov & Wackernagel (1995). A defective lambda prophage carrying a fragment of *lacZ* and marked by a Km^r cassette was transduced into this strain from GE43 (Weisemann *et al.*, 1984). This was made wild-type for the *lac* operon by an Hfr mating to yield MB1440 and then lysogenized with λNuc2R . This phage was constructed by growing $\lambda\text{cl857Sam100plac5nin5}$ on a strain carrying the entire *nucA* gene on plasmid pNuc2R (Ball *et al.*, 1987) and allowing recombination to occur between the *lacZ* of the phage and the *lacZ* cassette of the pUC18 vector. This exchange yielded the $\text{Lac}^- \text{Nuc}^+$ phage λNuc2R .

Nuc^+ lysogens were isolated in host MB1440 infected with λNuc2R on DNase Test Agar. Temperature-

resistant survivors were isolated at 42 °C, to yield MB1464, which carries *nucA* crossed into *lacZ* of the defective prophage but remains *lacZYA*⁺ at the normal site. This strain produced low levels of nuclease, which was not secreted into the growth medium.

The *nucEDC* operon and *nucED* $\Delta\text{Nco2-3}$ deletion (NucC^+) were crossed into *lacZ* at the *lac* operon by the same method described for *S. marcescens* using plasmids pMAKBam3.3 and pMAKBam3.3 $\Delta\text{Nco2-3}$, respectively. This recombination used the *lacZ* region flanking the insert of the pMAK705 plasmid. Confirmation of the resulting strains, MB1477 and MB 1478, was performed by PCR, as was done previously for *S. marcescens* strains.

The levels of intra- and extracellular nuclease activity were determined for the above strains and the results obtained are shown in Table 3. From these results we conclude the following. (i) *E. coli* carrying only the *nucA* structural gene neither expresses nor secretes measurable amounts of nuclease. (ii) When *nucC* was present in single copy on the chromosome, intracellular nuclease activity increased approximately 60-fold. In addition, both strains, either carrying *nucEDC* or *nucC* alone, were unable to efficiently secrete the nuclease with about two-thirds of the nuclease activity remaining within the cell. (iii) Random cell lysis was not occurring; there was negligible β -lactamase activity, expressed from pBS, found in the extracellular fraction (<1%). Therefore NucE and NucD do not affect the localization of nuclease in *E. coli* either.

DISCUSSION

The *nucE* and *nucD* gene products closely resemble bacteriophage holin and lysis proteins. These proteins are responsible for lysing an infected host after lytic growth of the phage (Young, 1992). The lytic proteins, such as lysozymes, specifically cleave the peptidoglycan to allow osmotic lysis of the host, whereas the holin is needed to allow the lytic enzyme to gain access to the

Gram-negative peptidoglycan. Phage lytic proteins are not secreted, and hence remain in the cytoplasm where there is no substrate, until their release by the holin.

It has recently been demonstrated that NucE and NucD are able to function as lytic proteins; they are capable of complementing lysis-defective mutants of bacteriophage λ to allow plaque formation and release of phage. Their expression, even from medium-copy-number vectors, results in cell lysis (Berkmen *et al.*, 1997). The aim of this study was to determine whether NucE and NucD perform an additional function in *S. marcescens*, such as by aiding the release of nuclease into the extracellular medium. The working hypothesis was that the lysozyme NucD gained access to the periplasm to allow cleavage of the murein mesh and eventually cause partial lysis and release of nuclease across the outer membrane. This hypothesis was based on earlier experiments (Jin *et al.*, 1996) where, in a plasmid-based system in *E. coli*, the *nucEDC* operon had a positive effect on the appearance of extracellular nuclease. These genes are both found in an operon with the gene for the transcriptional activator NucC essential for nuclease expression, providing the first suggestion that they might play a nuclease-related role.

To directly test the hypothesis, deletions of various combinations of the *nucE* and *nucD* genes were constructed and recombined back to the *S. marcescens* chromosome to construct mutant strains defective in them. Strains deleted for either *nucE* or both *nucE* and *nucD* were tested for NucA production and were fully competent to express and secrete nuclease extracellularly. The final ratio of extracellular to intracellular nuclease was no different in these strains than it was in the wild-type, nor was the rate of its secretion. From this we conclude that normally NucE and NucD do not play a significant role in nuclease secretion by *S. marcescens*.

Why do the results of this work differ from previous observations (Jin *et al.*, 1996)? The likeliest explanation is the difference in gene dosage, although differences between *E. coli* and *S. marcescens* are also possible. These can be distinguished by looking at the behaviour of NucA in *E. coli* strains carrying either single or multiple copies of *nucEDC*. Strains carrying a single copy of *nucEDC* and also of *nucA* in their genome fail to efficiently secrete nuclease. Likewise, deletions of *nucE* or *nucED* have no effect. However, if a plasmid carrying *nucEDC* is introduced into *E. coli* strain MB1464 expressing NucA from a chromosomal locus, some increase is observed in the amount of nuclease activity found extracellularly. The amount of extracellular β -lactamase also increases (Jin *et al.*, 1996; U. Strych, unpublished). This suggests that when *nucE* and *nucD* were supplied in higher copy number their observed effect of apparent secretion can be seen, but is likely due to cell lysis. Overexpression of NucC significantly slows down the growth of *E. coli* strains (U. Strych, unpublished; G. Christie, personal communication) and overexpression of NucE and NucD can result in cell lysis (M. Berkmen, unpublished).

As we have ruled out the role of NucED in nuclease secretion in *S. marcescens*, what function might they have? Certainly they could be the vestige of a cryptic prophage and have no function. They may be maintained by virtue of their linkage with *nucC*, which does have a role. However this is unlikely. Carrying these genes is not obviously deleterious; introducing them to the *E. coli* chromosome or deleting them from the *S. marcescens* chromosome has no noticeable effect on the growth of either strain. It would appear logical that bacteria should not encode and express such genes; however they do, and they are found in other bacteria as well, such as '*H. somnus*' (Pontarollo *et al.*, 1997). Another intriguing model is that they may play a role, for example to allow release of bacteriocin. Bacteriocin 28b is expressed by many strains of *S. marcescens* and its expression likewise requires NucC, as does NucA (Ferrer *et al.*, 1996). There is no lysis gene found downstream of the 28b gene, therefore NucED may take this role. Although *nucED* are not in the 28b operon, they are coordinately regulated and therefore in the same regulon. Unfortunately our strains do not express 28b and hence we are unable to easily test this attractive model with our *nucED*-deleted strains of *S. marcescens*.

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REFERENCES

- Ball, T. K., Saurugger, P. N. & Benedik, M. J. (1987). The extracellular nuclease gene of *Serratia marcescens* and its secretion from *Escherichia coli*. *Gene* **57**, 183–192.
- Benedik, M. J. & Strych, U. (1998). *Serratia marcescens* and its extracellular nuclease. *FEMS Microbiol Lett* **165**, 1–13.
- Berkmen, M., Benedik, M. J. & Bläsi, U. (1997). The *Serratia marcescens* NucE protein functions as a holin in *Escherichia coli*. *J Bacteriol* **179**, 6522–6524.
- Chen, Y. C., Shipley, G. L., Ball, T. K. & Benedik, M. J. (1992). Regulatory mutants and transcriptional control of the *Serratia marcescens* extracellular nuclease gene. *Mol Microbiol* **6**, 643–651.
- Cherepanov, P. P. & Wackernagel, W. (1995). Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158**, 9–14.
- Chung, C. T., Niemela, S. L. & Miller, R. H. (1989). One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. *Proc Natl Acad Sci USA* **86**, 2172–2175.
- Devine, J. H., Shadel, G. S. & Baldwin, T. O. (1989). Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744. *Proc Natl Acad Sci USA* **86**, 5688–5692.
- Fastrez, J. (1996). Phage lysozymes. *EXS* **75**, 35–64.
- Ferrer, S., Viejo, M. B., Guasch, J. F., Endefaque, J. & Regue, M. (1996). Genetic evidence for an activator required for induction of

- colicin-like bacteriocin 28b production in *Serratia marcescens* by DNA damaging agents. *J Bacteriol* **178**, 951–960.
- Hamilton, C. M., Aldea, M. A., Washburn, B. K., Babitzke, P. & Kushner, S. R. (1989).** New method for generating deletions and gene replacements in *Escherichia coli*. *J Bacteriol* **171**, 4617–4622.
- Jin, S., Chen, Y. C., Christie, G. E. & Benedik, M. J. (1996).** Regulation of the *Serratia marcescens* extracellular nuclease: positive control by a homolog of P2 Ogr encoded by a cryptic prophage. *J Mol Biol* **256**, 264–278.
- Miller, J. F. (1994).** Bacterial transformation by electroporation. *Methods Enzymol* **235**, 375–385.
- Miller, J. H. (1992).** *A Short Course in Bacterial Genetics: a Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Pontarollo, R. A., Rioux, C. R. & Potter, A. A. (1997).** Cloning and characterization of bacteriophage-like DNA from *Haemophilus somnus* homologous to phages P2 and HP1. *J xBacteriol* **179**, 1872–1879.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schmidt, C., Velleman, M. & Arber, W. (1996).** Three functions of bacteriophage P1 involved in cell lysis. *J Bacteriol* **178**, 1099–1104.
- Suh, Y., Jin, S., Ball, T. K. & Benedik, M. J. (1996).** Two-step secretion of the *Serratia marcescens* extracellular nuclease. *J Bacteriol* **178**, 3771–3778.
- Weisemann, J. M., Funk, C. & Weinstock, G. M. (1984).** Measurement of *in vivo* expression of the *recA* gene of *Escherichia coli* by using *lacZ* gene fusions. *J Bacteriol* **160**, 112–121.
- Winslow, R. H., Julien, B., Calendar, R. & Christie, G. E. (1998).** An upstream sequence element required for NucC-dependent expression of the *Serratia marcescens* extracellular nuclease. *J Bacteriol* **180**, 6064–6067.
- Young, R. (1992).** Bacteriophage lysis: mechanism and regulation. *Microbiol Rev* **56**, 430–481.
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