Role of RsmA in the regulation of swarming motility and virulence factor expression in Proteus mirabilis

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Swarming by Proteus mirabilis involves differentiation of typical short vegetative rods into filamentous hyper-flagellated swarm cells that undergo cycles of rapid and co-ordinated population migration across surfaces and exhibit high levels of virulence gene expression. RsmA (repressor of secondary metabolites) and CsrA, its homologue in Escherichia coli, control many phenotypic traits, such as motility and pathogenesis in Erwinia species, glycogen biosynthesis, cell size and biofilm formation in Escherichia coli and swarming motility in Serratia marcescens. To investigate the role of RsmA in Proteus mirabilis, the rsmA gene from Proteus mirabilis (hereafter referred to as Pm) was cloned. Pm showed high sequence similarity to Escherichia coli CarA and RsmA cloned from Erwinia carotovora subsp. carotovora, Serratia marcescens, Haemophilus influenzae and Bacillus subtilis and could complement an Escherichia coli csrA mutant in glycogen synthesis. A low-copy-number plasmid carrying Pm was expressed from its native promoter caused suppression of swarming motility and expression of virulence factors in Proteus mirabilis. mRNA stability assays suggested that Pm inhibited virulence factor expression through promoting mRNA degradation. RsmA homologues cloned from Serratia marcescens and Erwinia carotovora subsp. carotovora could also inhibit swarming and virulence factor expression in Proteus mirabilis.

INTRODUCTION

Swarming motility is a type of population migration behaviour characteristic of some bacteria on solid media (Eberl et al., 1999; Harshey, 1994; Harshey & Matsuyama, 1994). It is a cyclic process and is closely correlated with the expression of virulence factors in both Proteus and Serratia (Allison & Hughes, 1991; Allison et al., 1992; Givskov et al., 1997; Liaw et al., 2000). In these bacteria, swarming migration involves the co-ordinated differentiation of short, motile vegetative cells bearing a few peritrichous flagella into multinucleate, aseptate swarm cells of up to 40 times the vegetative cell length and with a much greater surface density of flagella (Allison et al., 1992; Givskov et al., 1997). Swarming is influenced by multiple environmental signals, such as cell density and the presence of particular amino acids and possibly peptides (Allison et al., 1993; Gaiser & Hughes, 1997; Rauprich et al., 1996). Characterization of swarming-defective Proteus transposon mutants has indicated that many proteins are involved in the regulation of differentiation and subsequent swarming migration (Belas et al., 1991, 1995; Gygi et al., 1995a, b, 1997; Fraser & Hughes, 1999). These include FlhA, a protein involved in flagellum assembly and swarm-cell differentiation (Gygi et al., 1995a), FlgN, a substrate-specific flagellar chaperone that prevents oligomerization of hook-associated proteins and thus facilitates flagellum assembly (Fraser et al., 1999), FlhD2C2 (heterotetramers of FlhD and FlhC), a transcription activator that regulates the expression of the flagellar regulon (Fraser & Hughes, 1999), Umo proteins that upregulate the flhDC operon during differentiation (Fraser & Hughes, 1999), Lrp, a global translational regulator that links physiological signals to swarming differentiation (Frazer & Hughes, 1999), CcmA, a protein that regulates cell shape and thus influences multicellular swimming (Hay et al., 1999), and RhaA, a putative bacterial two-component sensor kinase involved in the regulation of swarming (Liaw et al., 2001). Of these, FlhDC, Lrp and the Umo proteins probably function as part of a broader regulatory network that may include bacterial two-component systems and the chemotaxis phosphorelay (Fraser & Hughes, 1999).

The nucleotide and deduced amino acid sequences of Proteus mirabilis rsmA are available as supplementary material in JMM Online (http://jmm.sgmjournals.org/).

The GenBank/EMBL/DDBJ accession number for the DNA sequence of Proteus mirabilis rsmA reported in this paper is AF403736.
RsmA is a homologue of CsrA (for carbon storage regulator) (Romeo et al., 1993; Cui et al., 1995), a critical component of the Escherichia coli Csr system, a global regulatory system that represses a variety of stationary-phase genes (Romeo et al., 1993). CsrA inhibits glycogen biosynthesis and catabolism, gluconeogenesis and biofilm formation in Escherichia coli (Romeo et al., 1993; Romeo, 1998). CsrA represses glycogen synthesis by causing rapid mRNA decay (Liu et al., 1993). This leads to a decrease in intracellular levels of glycogen biosynthesis enzymes, which, in turn, decreases the rate of glycogen biosynthesis. A second component of the Csr regulatory system, CsrB, a non-coding RNA molecule, acts as an antagonist of CsrA, presumably by sequestering it (Liu et al., 1997; Liu & Romeo, 1997). Searches in the GenBank databases have shown that homologues of csrA can be found in many Gram-negative bacteria and some Gram-positive bacteria (White et al., 1996). RsmA, a homologue of CsrA, represses stationary-phase genes in Pseudomonas fluorescens (Blumer et al., 1999) and negatively controls several genes involved in motility, secondary metabolism, pathogenesis and quorum-sensing in Erwinia carotovora subsp. carotovora (Cui et al., 1995; Liu et al., 1998; Mukherjee et al., 1996).

Proteus mirabilis is an important pathogen of the urinary tract, especially in patients with indwelling urinary catheters (Warren, 1982). It is believed that the ability of Proteus mirabilis to colonize the urinary tract is associated with its swarming motility. Moreover, the ability of Proteus mirabilis to express virulence factors, including urease, protease, haemolysin and flagellin, and to invade human uroepithelial cells is coupled to swarming differentiation (Allison & Hughes, 1991; Allison et al., 1992; Liaw et al., 2000). RsmA has been shown to repress swarming motility and the expression of virulence factors in many Erwinia species (Cui et al., 1995; Liu et al., 1998; Mukherjee et al., 1996). However, in Escherichia coli, CsrA, a homologue of RsmA, positively regulates swarming motility and flhDC expression (Wei et al., 2001). In this study, the rsmA allele of Proteus mirabilis has been identified and cloned and the effect of RsmAm on the swarming behaviour and the expression of virulence factors, including haemolysin, protease, urease and flagellin, in Proteus mirabilis has been investigated. The trans-acting effects of RsmAm on (RsmA encoded by Erwinia carotovora subsp. carotovora) and RsmAm (RsmA encoded by Serratia marcescens) (Ang et al., 2001) on swarming-related traits of Proteus mirabilis were also examined.

**METHODS**

**Enzymes and chemicals.** DNA restriction and modification enzymes were purchased from Boehringer. Taq polymerase and PCR-related products were from Perkin Elmer. Other chemicals were purchased from Sigma.

**Bacterial strains, plasmids and culture conditions.** The bacterial strains and plasmids used are described in Table 1. Bacteria were cultured at 37 °C in Luria–Bertani (LB) medium. Swarming motility was examined on swarming-agar plates (LB solidified with 1-5 % agar) by inoculating 5 μl of an overnight broth culture on to the centre of the agar plate. The plates were dried before inoculation and incubated at 37 °C. Hourly increases in the optical density of broth cultures at 600 nm were taken as a measurement of the growth rate.

**Recombinant DNA techniques.** Standard protocols were used for Southern hybridization, isolation of plasmid and chromosomal DNA, transformation, electroporation, PCR, restriction endonuclease digestion, agarse gel electrophoresis, recovery of DNA from agarose gels and the ligation of DNA fragments. DNA sequence analysis and DNA similarity searches were performed using a Perkin-Elmer Autossequencer model 373A with a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). The DNA sequence of PCR products was confirmed by sequencing both strands. DNA sequence similarity searches of GenBank were performed using programs of the University of Wisconsin Genetics Computer Group (Devereux et al., 1984). Protein primary sequence comparisons were performed using BLAST via the NCBI internet homepage (http://www.ncbi.nlm.nih.gov/).

**Cloning of Proteus mirabilis P19 rsmA.** The PCR primers csrA-R and csrAF (Table 2), designed according to conserved regions in Escherichia coli csrA and Erwinia carotovora subsp. carotovora rsmA, were used to amplify a conserved DNA fragment from Proteus mirabilis P19 chromosomal DNA. A single DNA band with the expected size of 156 bp was detected. This 156-bp DNA fragment, which showed high sequence identity to Escherichia coli csrA and Erwinia carotovora subsp. carotovora rsmA, was used as a hybridization probe in the following cloning protocol (Fig. 1). Chromosomal DNA prepared from Proteus mirabilis P19 was first digested with EcoRV, followed by ligation to EcoRV-digested pZEO2.1 (Table 1). The 5′ half and upstream region of rsmAm (2600 bp) was amplified by PCR using the primers M13R and csrAF (Table 2) and the 3′ half and downstream region (500 bp) was amplified using the primers csrAF and M13F (Table 2). Southern hybridization using the labelled 156-bp DNA fragment as a probe and DNA sequencing confirmed that the amplified DNA fragments contained the rsmAm-like sequence. The full-length rsmAm was subsequently amplified using the primers rsmAF and rsmAR (Table 2), designed from the 5′ and 3′ flanking regions of rsmAm. The amplified rsmAm and its flanking sequences were then cloned into pCR2.1 to generate p55.

**Complementation of an Escherichia coli csrA mutant with rsmAm.** The csrA-defective Escherichia coli mutant TR1-5BW3414 (Romeo et al., 1993) was transformed with pACYC184 containing rsmAm, pPS1 (pCR2.1 containing rsmAm) or pUC19 containing csrA. Chloramphenicol- and ampicillin-resistant colonies were selected and subjected to plasmid preparation to confirm the presence of the appropriate plasmid. These transformed strains together with the wild-type strain were grown in LB medium containing 37 °C, the plates were treated with iodine solution (0-01 M I2, 0-03 M KI) to detect the accumulation of glycogen produced by these strains.

**Measurement of cell length, flagellin level and haemolysin, urease and protease activities.** Measurements of cell length, flagellin level and haemolysin, urease and protease activities were performed as described previously (Liaw et al., 2001). Briefly, 150 μl aliquots of stationary-phase LB cultures from pM13-containing and pACYC184-containing Proteus mirabilis were spread on to LB agar plates and incubated at 37 °C for various lengths of time. After incubation, cells from the entire surface were harvested by washing with 5 ml LB. These
cells were then subjected to several assays. For cell-elongation measurements, bacteria were fixed in 4 % paraformaldehyde and examined by light microscopy at a magnification of 1000× under oil-immersion using an Olympus BH2 microscope equipped with a graticule. The lengths of 100 cells in each sample were determined and the mean calculated. Cell membrane-associated haemolysin activity was assayed as described previously (Liu et al., 2001). Bacterial strains and plasmids

Table 1. Bacterial strains and plasmids

<table>
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<th>Strains</th>
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<tr>
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<td>Romeo et al. (1993)</td>
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Plasmids

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<td>TcR, Cmr, replicon P15A</td>
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<tr>
<td>pSJ</td>
<td>Full-length <em>rsmA</em> in pCR2.1</td>
</tr>
<tr>
<td>pRsmA</td>
<td>Full-length <em>rsmA</em> in pACYC184</td>
</tr>
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<tr>
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<td>pCSR10</td>
<td>Full-length csrA in pUC19</td>
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Table 2. Nucleotide sequences of primers used in PCR and sequencing

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<td><em>rsmA</em></td>
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mRNA stability assay. *Proteus mirabilis* P19 cells transformed with the pACYC184 vector or pRsmA were grown at 37 °C in LB to an OD600 of 0.5. At 0, 2, 4, 6 and 8 min after addition of rifampicin to a final concentration of 400 μg ml⁻¹ to block further transcription, aliquots (10 ml) were collected in tubes containing 1 ml freshly prepared stop solution (5 % phenol in ethanol). Total RNA was extracted by the hot-phenol method (Magni et al., 1995) and Northern blot analysis was performed to detect the haemolysin mRNA as described previously (Liu et al., 1998). RNA samples were loaded on formaldehyde (6 %)/agarose (1·2 %) gels and electrophoresed in MOPS buffer (0·02 M MOPS, 0.005 M sodium acetate, 0·001 M EDTA). After transfer to a nitrocellulose membrane which was washed in 2× SSC (0·3 M NaCl, 0·03 M trisodium citrate), the RNAs were cross-linked to the membrane by UV irradiation. The haemolysin gene (*hpmA*) probe (1 kb) was amplified from genomic DNA with a PCR DIG Probe Synthesis kit (Boehringer Mannheim) using primers HpmF and HpmR (Table 2), designed from the *hpmA* sequence (Uphoff & Welch, 1990). Pre-hybridization (4 h at 58 °C) and hybridization (1 h at 58 °C) were performed in DIG Easy Hyb* buffer (Boehringer Mannheim). After hybridization, membranes were washed twice for 5 min at 50 °C in 2× SSC, 0·1 % SDS, then twice for 15 min at 50 °C in 0·1 % SSC, 0·1 % SDS, and examined by using the DIG DNA Luminescence Detection kit (Boehringer Mannheim). A 0·24–9·5 kb RNA ladder (Gibco-BRL) was used as a size marker.

**RESULTS**

Cloning of the *rsmA* gene from *Proteus mirabilis*

In order to know whether *rsmA* exists in *Proteus mirabilis* and might play a role in the regulation of *Proteus mirabilis* swarming and virulence factor expression, *rsmA* was first cloned. The PCR primers *csrAF* and *csrAR* (Table 2), designed according to conserved regions in *Escherichia coli* *csrA* and *Erwinia carotovora* subspp. *carotovora* *rsmA*, amplified a conserved DNA fragment of *Proteus mirabilis* P19 chromosomal DNA to give a single DNA band with the expected size of 156 bp. This had high DNA sequence similarities with the sequences of RNA binding motif and the phylogenetically conserved region of the *rsmA* gene.
Identification to sequences of Escherichia coli csrA and Erwinia carotovora subsp. carotovora rsmA (unpublished observations). Despite repeated attempts, it was not possible to clone the complete rsmA<sub>Pm</sub> gene via a colony hybridization protocol (a genomic library had been constructed in the high-copy-number plasmid pZErO2.1 and it is possible that overexpression of rsmA<sub>Pm</sub> was lethal). Therefore, PCR was used to clone the complete rsmA<sub>Pm</sub> gene as described. The nucleotide sequence and the deduced amino acid sequence of the open reading frame (ORF) of the rsmA<sub>Pm</sub> gene-containing fragment are available as supplementary material in JMM Online (http://jmm.sgmjournals.org/). The fragment contains 1021 bp in total and an ORF (nt 555–743) that could encode a 6.8-kDa polypeptide of 62 amino acid residues. A putative Shine–Dalgarno sequence (5'-AGGAG-3') is located 6 bp upstream of the ATG start codon. Putative -10 and -35 regions are also shown. Fig. 2 shows the alignment of deduced amino acid sequences among Escherichia coli CsrA, Proteus mirabilis RsmA<sub>Pm</sub>, Erwinia carotovora subsp. carotovora RsmA<sub>Bac</sub>, and other RsmA homologues in Serratia marcescens, Haemophilus influenzae and Bacillus subtilis.

Amino acid sequence comparison revealed that RsmA<sub>Pm</sub> showed 96% identity to Escherichia coli CsrA and RsmA<sub>Ec</sub>, 94% identity to RsmA<sub>Bac</sub>, and respectively 70 and 45% identity to RsmA from H. influenzae and B. subtilis. As with Escherichia coli CsrA (Romeo, 1998), the predicted product of rsmA<sub>Pm</sub> also contains a putative RNA-binding domain (see supplementary material) that is similar to the KH (K protein homology) motif found only in proteins associated with RNA (Stoumi et al., 1994).

**Fig. 2.** Alignment of the deduced amino acid sequences of RsmA homologues in Escherichia coli K-12 (E. coli; Romeo et al., 1993), Serratia marcescens (S. m.; Ang et al., 2001), Erwinia carotovora subsp. carotovora (E. c. c.; Cui et al., 1995), Proteus mirabilis (P. m.), Haemophilus influenzae (H. i.; Fleischmann et al., 1995) and Bacillus subtilis (B. s.; Mirel & Chamberlin, 1989). Vertical lines indicate 100% identity to RsmA from H. influenzae and B. subtilis. As with Escherichia coli CsrA (Romeo, 1998), the predicted product of rsmA<sub>Pm</sub> also contains a putative RNA-binding domain (see supplementary material) that is similar to the KH (K protein homology) motif found only in proteins associated with RNA (Stoumi et al., 1994).

**Complementation of an Escherichia coli csrA mutant with rsmA<sub>Pm</sub>**

To investigate whether RsmA<sub>Pm</sub> had similar functions to CsrA, an Escherichia coli csrA-defective mutant, TRI-1BW3414 (Romeo et al., 1993), was transformed respectively with the vectors pACYC184, pRsmA (rsmA<sub>Pm</sub> in low-copy-number vector pACYC184), pSJ (rsmA<sub>Pm</sub> in high-copy-number vector pCR2.1) or pCSR10 (csrA in high-copy-number vector pUC19). The ability of RsmA<sub>Pm</sub> and CsrA to suppress the glycogen-overproducing activity of the TRI-1BW3414 mutant was tested. As shown in Fig. 3, the TRI-1BW3414 mutant and its pACYC184 transformant stained dark-brown due to excess glycogen accumulation. The TRI-1BW3414 mutant transformed with pRsmA stained yellowish brown, similar to the staining...
pattern of the wild-type strain BW3414, indicating that the glycogen-overproducing phenotype of the TR1-5BW3414 mutant was suppressed by RsmA Pm. The TR1-5BW3414 mutants transformed with pSJ or pCSR10 showed a yellow staining pattern, suggesting that RsmAPm and CsrA had similar abilities to suppress glycogen production by the mutant. These results suggested that rsmA Pm could complement the glycogen-excess phenotype of the csrA mutant and that RsmAPm had similar functions to CsrA.

Effect of RsmAPm on the swimming and swarming activities of Proteus mirabilis

The above results suggested that the cloned rsmA Pm gene was a true csrA homologue. Because CsrA and its homologue RsmA regulate motility in Escherichia coli and Erwinia species, the effect of RsmAPm on the swimming and swarming activities of Proteus mirabilis was examined. Because expression of rsmAPm from a high-copy-number vector, such as pZErO2.1 or pCR2.1, in Proteus mirabilis, resulted in inhibition of growth (unpublished observations), the rsmAPm gene and its flanking sequences were cloned into the low-copy-number vector pACYC184 (Table 1) to generate pRsmA. The pRsmA plasmid was transformed into Proteus mirabilis P19 to establish an RsmAPm-expressing strain. As shown in Fig. 4, while Proteus mirabilis P19 transformed with the pACYC184 vector swarmed normally, the RsmAPm-expressing Proteus mirabilis strain, which expresses RsmAPm from its native promoter, lost its ability to swarm. In contrast, the swimming motility of Proteus mirabilis was not affected by the presence of RsmAPm (unpublished observations). These results indicated that RsmAPm could specifically inhibit the swimming activity of Proteus mirabilis P19.

Fig. 3. Complementation of an Escherichia coli csrA mutant with rsmA Pm detected by iodine staining. Cultures were streaked on to Kornberg medium and incubated overnight at 37 °C before treatment with iodine solution. Plate segments were streaked with: 1–3, mutant strain TR1-5BW3414 transformed with pACYC184 (1), pSJ (2) and pCSR10 (3); 4, wild-type strain BW3414; 5, mutant strain transformed with pRsmA; 6, mutant strain TR1-5BW3414.

Fig. 4. Swarming of pRsmA-transformed (a) and pACYC184-transformed (b) Proteus mirabilis P19 on LB swarming plates. Aliquots (5 μl) of bacterial culture were inoculated centrally on to LB swarming plates. The plates were incubated at 37 °C and observed after 10 h incubation.
Effect of RsmAPm on mRNA stability

Because both RsmAEcc and CsrA regulate gene expression by affecting the stability of mRNA (Liu et al., 1995, 1998), it was possible that RsmA Pm inhibited virulence-gene expression through a similar mechanism. Therefore, the stability of haemolysin mRNAs isolated from the vector-transformed Proteus mirabilis P19 and pRsmA-transformed Proteus mirabilis P19 was compared. As shown in Fig. 6, the haemolysin mRNA in the RsmA Pm-transformed cells was completely degraded 8 min after rifampicin treatment, while that in the vector-transformed cells was not. These results indicated that RsmA Pm promoted mRNA degradation in Proteus mirabilis P19. Therefore, RsmA Pm, like CsrA and RsmA Ecc, could inhibit virulence factor expression by affecting mRNA stability.

RsmAEcc and RsmASm trans-suppress swarming and virulence factor expression in Proteus mirabilis P19

To investigate whether swarming and virulence factor expression in Proteus mirabilis could also be inhibited by other RsmA homologues, the pSA1 plasmid (Table 1), which contained the rsmA Ecc gene (rsmA from Erwinia carotovora subsp. carotovora) under the control of its native promoter, was transformed into Proteus mirabilis. As shown in Figs 7 and 8, while the vector-transformed Proteus mirabilis could differentiate, swarm and express virulence factors normally, the pSA1-transformed Proteus mirabilis strain lost its abilities to differentiate, swarm and express normal levels of virulence factors. Similarly, RsmASm (RsmA encoded by Serratia marcescens) also inhibited the ability of Proteus mirabilis to swarm and express virulence factors (unpublished observations). Together, these data confirmed that swarming and virulence factor expression in Proteus mirabilis could be suppressed by RsmA Pm and its homologues.

DISCUSSION

In many host–pathogen interactions, disease development requires co-ordinated expression of sets of genes in response to various signals and environmental cues (Gottor & Miller,
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1998). Regulation of these genes is subject to both transcriptional and post-transcriptional control. RsmA–RsmB constitutes an important regulatory system responsible for post-transcriptional regulation (Liu et al., 1998; Cui et al., 1999). RsmA, an RNA-binding protein, promotes the decay of many mRNA species (Liu et al., 1998). RsmB, an untranslated regulatory RNA, on the other hand, neutralizes the effect of RsmA by forming a ribonucleoprotein complex with the latter (Liu et al., 1998). The RsmA regulatory system has been conserved in many enterobacterial species (Romeo et al., 1993; Cui et al., 1995; White et al., 1996) and has been shown to control diverse phenotypes including motility and expression of virulence factors (Cui et al., 1995; Mukherjee et al., 1996). In this study, we cloned the rsmAp gene from Proteus mirabilis and investigated the effect of RsmAPm on swarming and virulence factor expression in Proteus mirabilis. rsmAPm was found to complement the defectiveness of an Escherichia coli csrA (rsmA homologue) mutant (Romeo et al., 1993) and could act as rsmB Ec (Ang et al., 2001) and rsmASm (Ang et al., 2001) to suppress swarming and virulence factor expression in Proteus mirabilis.

Several lines of evidence suggest that the rsmAp gene we cloned is a true rsmA homologue. Firstly, the DNA sequence and the predicted product of rsmAp showed high similarity to rsmA cloned from other bacteria (Fig. 2). Secondly, like other RsmAs, the predicted rsmAPm product bore a putative RNA-binding motif (Sioni et al., 1994). Thirdly, rsmAPm could complement the glycogen-excess phenotype of an Escherichia coli csrA mutant (Fig. 3). Furthermore, complementation of the csrA mutant with rsmAp also restored the cell length to wild-type and abolished its formation of a biofilm (unpublished observations). Fourthly, RsmAPm could act as RsmApEc and RsmApSm to suppress swarming and virulence factor expression in Proteus mirabilis.

The role of RsmA/CsrA in regulating bacterial virulence is of considerable interest and has been established in Erwinia species that are plant pathogens. A transposon-insertion mutation in rsmA in Erwinia caused hypervirulence, over-production of lytic enzymes and elevation of their corresponding transcripts (Cui et al., 1995; Chatterjee et al., 1995). Motility is an important survival mechanism and a distinct advantage for host-adapted species. Overexpression of rsmA has been demonstrated to suppress motility and flagella production in Erwinia carotovora subsp. carotovora (Mukherjee et al., 1996). Our finding that RsmAp could suppress swarming and virulence factor expression in Proteus mirabilis is consistent with the above observations. Our results also suggest that RsmAp may play an important role in regulating virulence in Proteus mirabilis.

RsmA regulates gene expression by controlling mRNA stability and, thus, is a global regulator of gene expression in many bacteria (Romeo, 1998). RsmAp also regulated gene expression through regulating mRNA stability and could promote mRNA degradation (Fig. 6). It is possible that the activity of RsmAPm must be rigorously controlled in the cells (Cui et al., 1999; Mukherjee et al., 1998). Overexpression of rsmA from high-copy-number plasmids or artificial strong promoters is generally detrimental to cell physiology and, in certain hosts, is even lethal (Cui et al., 1999). In our study, overexpression of rsmAPm from the high-copy-number plasmids pZErO2.1 and pCR2.1 was inhibitory to the growth of Proteus mirabilis and Escherichia coli, whereas rsmAPm expressed from the low-copy-number pACYC184 was not (unpublished observations). These observations suggest that the level of RsmAPm must be delicately regulated in Proteus mirabilis and are supported by findings implying that the rsmAp gene is essential for Proteus mirabilis. Repeated attempts to isolate rsmAp−.
knockout Proteus mirabilis mutants by using the pKO3 gene-replacement vector (Link et al., 1997) and the screening of ~10,000 clones have proved unsuccessful. rsmA/csrA is not essential in Escherichia coli or Serratia marcescens (Romeo et al., 1993; Ang et al., 2001). However, a csrA-knockout mutation is deleterious in Salmonella enterica serovar Typhimurium (Altier et al., 2000) and possibly also in Yersinia and Legionella species (T. Romeo, personal communication). Further experiments from construction of rsmA Pm temperature-sensitive mutants should be performed to determine the essentiality of rsmA Pm in Proteus mirabilis.

Regulation of swarming and virulence factor expression is a complex process and involves many factors and regulatory pathways (Fraser & Hughes, 1999). Previously, we demonstrated that RsbA, a sensor kinase homologue of the bacterial two-component system, was a negative regulator of swarming and virulence factor expression in Proteus mirabilis (Liaw et al., 2001). rsbA-defective Proteus mirabilis mutants exhibit a super-swarming phenotype and overproduce virulence factors. When rsmA Pm was transformed into these mutants, swarming was drastically suppressed (unpublished observations). These results suggest that the RsbA regulatory pathway and the RsmA Pm regulatory pathway may cross-talk to each other. In Pseudomonas fluorescens and Erwinia carotovora subsp. carotovora, the two-component system GacA/GacS has been shown to control the production of extracellular enzymes by regulating the expression of the rsmA/rsmB system (Mukherjee et al., 1998; Cui et al., 2001). It is possible that RsbA may also regulate swarming and virulence factor expression through modulating the expression of the rsmA/rsmB system. Experiments aimed at elucidating the relationship between RsbA and RsmA Pm are in progress.

In addition to rsmA Pm, we also found that rsmA homologues exist in many bacteria including Pseudomonas aeruginosa, Vibrio parahaemolyticus, Morganella morganii, Klebsiella pneumoniae, Aeromonas hydrophila, Citrobacter diversus, Helicobacter pylori and Streptococcus sanguis (unpublished observations). Whether these rsmA homologues also regulate virulence in these bacteria is of interest and warrants further investigation.

**ACKNOWLEDGEMENTS**

We thank Dr Tony Romeo for providing us with Escherichia coli strains TR1-5BW3414 and BW3414 and for helpful discussions. This work was supported by grants from the National Science Council and the National Taiwan University Hospital, Taipei, Taiwan.
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http://jmm.sgmjournals.org 27

IP: 54.70.40.11
On: Sun, 25 Nov 2018 12:24:34


