A

```
79%   RsmA(PAO1)        MLILTRRVGETLMVGD-DVTVTVLGVKGNQVRIGVNAPKEVAVHREEIYQRIQKEKDQEPNH
      PP_4472(KT2440)   MLILTRRCAESLIIGDGEITVTVLGVKGNQVRIGVSAPKEVAVHREEIYLRIKKEKDEEPSL
95%   RsmA(F113)        MLILTRRCAESLIIGDGEITVTVLGVKGNQVRIGVNAPKEVAVHREEIYLRIKKEKDDEPSH

61%   RsmA(PAO1)        MLILTRRVGETLMVGDDVTVTVLGVKGNQVRIGVNAPKEVAVHREEIYQRIQKEKDQEPNH
      PP_3832(KT2440)   MLILTRKVGESIVINDDIKVTILGVKGMQVRIGIDAPKDVQVHREEIFKRIQAGSPAPEKHEDTH
72%   RsmE(F113)        MLILTRKVGESINIGDDITITILGVSGQQVRIGINAPKNVAVHREEIYQRIQAGLTAPDKPQ-TP

43%   RsmA(PAO1)        MLILTRRVGETLMVGDDVTVTVLGVKGNQVRIGVNAPKEVAVHREEIYQRIQKEKDQEPNH
      PP_1746(KT2440)   MLVIGREVGEVIVIGDDIRIMVVETRDGVVRFGVAAPREVPVHRAEVYKRI---KASKQSKA
59%   RsmI(F113)        MLVLSRAVGELISIGDDISVRVLSVSGGTVRFGVEAPRHVDVHRSEIYDKIQKRKALATRKACSVE
```

B

```
RsmA(PP_4472)  MLILTRRCAESLIIGDGEITVTVLGVKGNQVRIGVSAPKEVAVHREEIYLRIKKEKDEEPSL
RsmE(PP_3832)  MLILTRKVGESIVINDDIKVTILGVKGMQVRIGIDAPKDVQVHREEIFKRIQAGSPAPEKHEDTH
RsmI(PP_1746)  MLVIGREVGEVIVIGDDIRIMVVETRDGVVRFGVAAPREVPVHRAEVYKRIKASKQSKA
```

C

rsmY P. protegens Pf-5 vs. intergenic PP_0370-PP_0371

```
ATGGACGTCGCGCAGGAAGCGCAAAGCAACAACACGGACACGTAGGATT-CC-GCCAGGATGGTGGAGTGAAAAGGATGTCA--GGGA
TTGCGCAGGAAGCGCAAAGGACAGATCAAGGACGACGAC--GAAGGACCACCTGCCAGGATGGCGGGGCGATACGGATGTCACAGGGA

AACAGTCTGCAAAGCCCCGCTTCGGCGGGGTTTT
AACAGTCTGGAAAACCCCGCTTCGGCGGGGTTTT
```

rsmZ P. protegens Pf-5 vs. intergenic PP_1624-PP_1625

```
TGTCGACGGATAGACACAGCCATCAAGGACGATGGTCA-GGACATCGCAGGAAGCGA-TTCATCAGGACGATGAAAAGGAACA-CAG
TGCACAGGGACATGCACAGGCTTTCAGGATGAAGGCCAGGGACATCGCAAGAAGCGATTTCATCAGGATGATGTTTGGGACAAGCAG

GGACTAGGGAAAAA--TGTGGGCGGGTCATACCGCCCCTTTTTTT
GGACTACGGAAAAAAATGTGGGCGGGTCAAACCGCCCCTTTTTTT
```

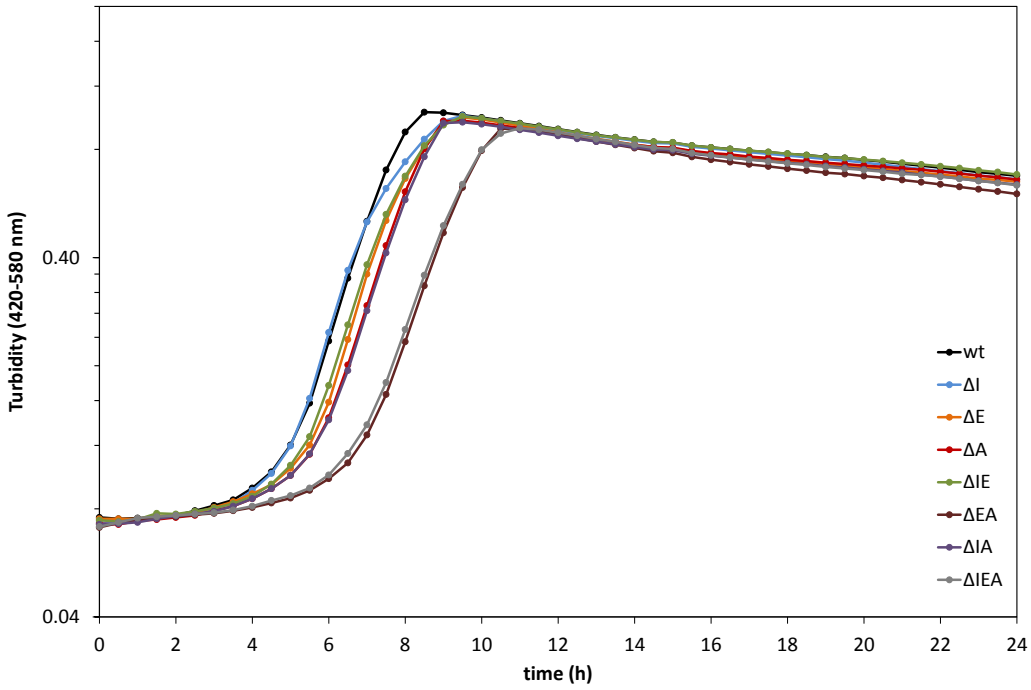
rsmX P. fluorescens F113 vs. intergenic PP_0214-PP_0215 (reverse complement.)

```
                                        TGGACT-CATCCACTGAAGCACAGGAAGTGCTCAGGATCAGGGACGA-TCGACC
←PP_0215  TGGGGGCTGCAGCGCTCATCGCCAACTACTCCTGAATAACCTGACACACAGTCCGT-CGCAGCTTCCAAAGCCAGTCCTCC

TTGCAAGGAAAGCTATCGACAGGGAGTCGTAATGGTCTTGGAAAAACCCGCTTCGGCGGGTTTTTTT
-GATAACCATAGC--GCCACACGGCTT-ATCTGGGCATAAAAAAAACCCGCCTAGGCGGGTTTTTTCTGAAGCGCGTCACATCA ←PP_0214
```

Figure S1. A) Alignment and percent identities of each Rsm protein of *P. putida* KT2440 with RsmA of *P. aeruginosa* and their respective homolog in *P. fluorescens* F113. **B)** Amino acid sequence comparison between the three Rsm family proteins of *Pseudomonas putida* KT2440. In both panels A and B, yellow and turquoise shading indicate identical residues and conservative changes, respectively. Residues involved in mRNA recognition are shown in magenta boldface (**Schubert M, Lapouge K, Duss O, Oberstrass FC, Jelesarov I, Haas D, Allain FH.** 2007. Molecular basis of messenger RNA recognition by the specific bacterial repressing clamp RsmA/CsrA. Nat Struct Mol Biol **14:**807-813). **C)** Identification of chromosomal regions encoding small RNAs *rsmY*, *rsmZ* and putative *rsmX* in *P. putida* KT2440, by comparison with the corresponding sequences of *P. protegens* Pf-5 and P. *fluorescens* F113. Identical nucleotides are shaded in yellow. Start and stop codons of PP_0215 and PP_0214 are underlined and shown in blue and red, respectively.

A



B

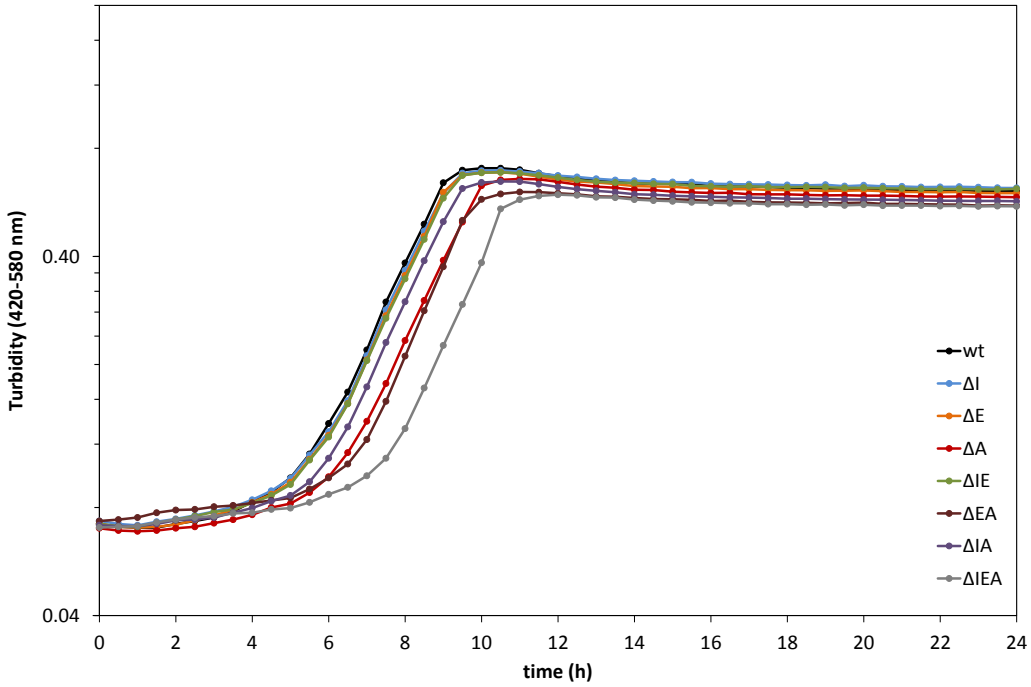


Figure S2. Growth of wild type KT2440 (wt) and *rsm* mutants in minimal medium with 10 mM citrate (**A**) or 5 mM glucose (**B**) as carbon source. Cultures grown overnight were inoculated in multiwell plates at an initial OD_{600} of 0.05, in triplicate. Turbidity was monitored every 30 minutes during growth at 30°C in a BioScreen. Average growth curves of each strain are shown, from three technical replicas. Error bars are omitted for clarity, in all cases the standard error was below 5%.

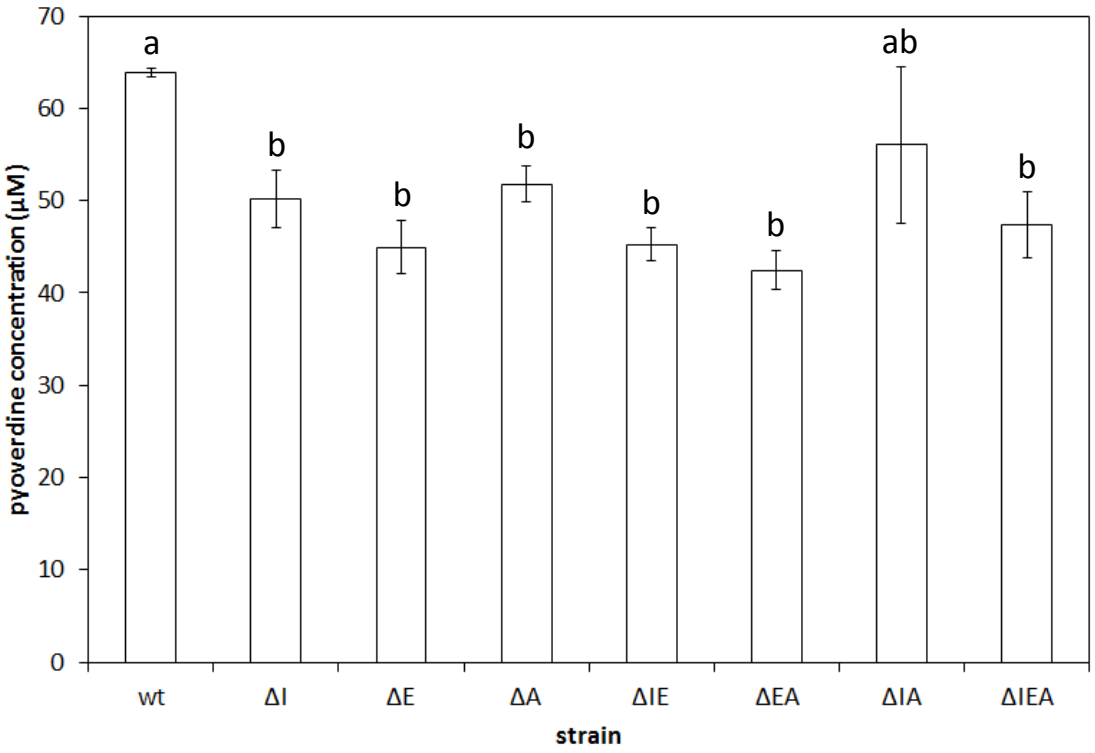


Figure S3. Pyoverdine concentration in supernatants of cultures grown overnight in King's B medium, calculated by measuring absorbance at 405 nm (molar extintion coefficient of pyoverdine = $1{,}9\times10^{-4}$ $M^{-1}\times cm^{-1}$). Data are averages and standard deviations of three biological replicas with three technical replicas each, normalized to OD_{660} = 1 to correct for potential growth differences between cultures. Different letters are indicative of statistically significant differences between datasets (1-way ANOVA, $p<0.05$).

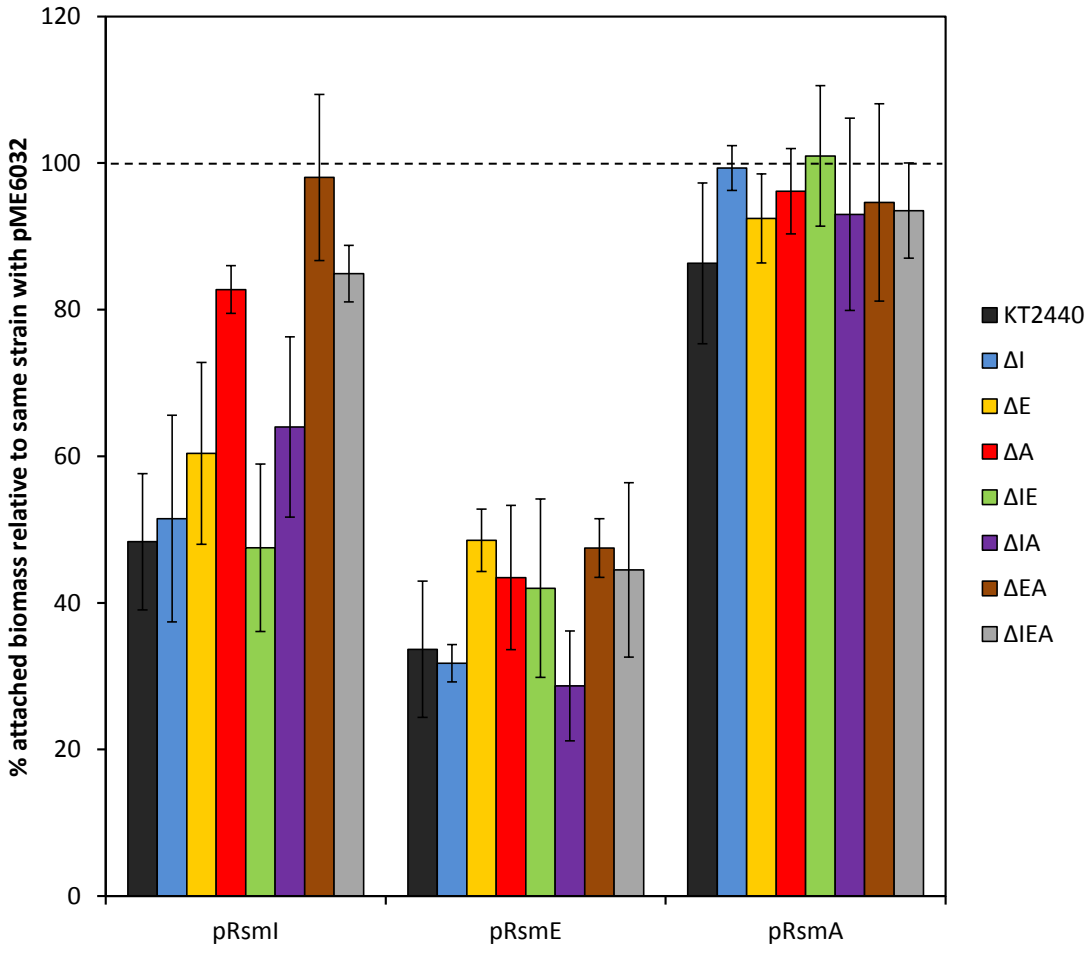


Figure S4. Quantitative analysis of the influence of overexpressing each Rsm protein on biofilm formation by KT2440 and *rsm* mutant backgrounds. Attached biomass (see Fig. 5) was quantified by measuring absorbance at 580 nm after crystal violet staining and solubilization of the dye with 30% acetic acid. Values are given in percentage relative to the same strain carrying the empty vector pME6032, which corresponds to a value of 100% (Averages and S.D. from three different experiments).

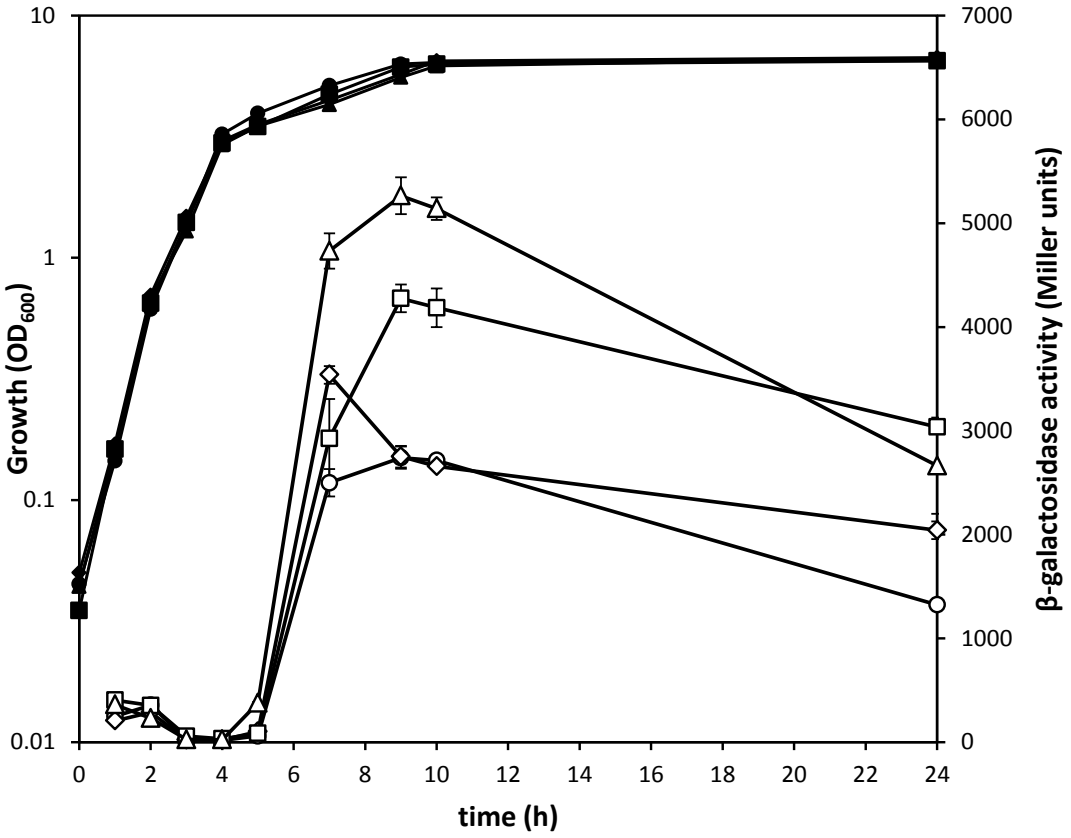


Figure S5. Influence of Rsm proteins on *lapF* expression. Growth (closed symbols) and β-galactosidase activity (open symbols) of KT2440 (circles) and mutants ΔI (diamonds) ΔE (squares) and ΔA (triangles), carrying the *lapF::lacZ* fusion in pMMG1. Data are averages and standard deviations from two biological replicas with two technical replicas.

>*rsmI*

[GCGGCTGTATGACGAGATGGCCGAAATGGCGGTGAAAATGCGGGCAAAAGCCCCGTCGCGATGATTGGTCTGTAGGA
GTAGTTACTCAAAAGTGTAAGAGGCTTCTGAATCGCAGTGGAACTATTGAGTAGCTGAATGCCTACGGGGCAGGCTCC
GGTTTTCCCTTTCGCAGGAGGATCGGAACATGCTGGTAATAGGGCGCGAAGTAGGG]

>*rsmE*

[GGCCTTGCTGTTGTTTCTTCAGGCACTTGAGGAACAGGAGGACCATTGAGCTTGTCTATTCAGCCCATGATGCGAAG
AAATTTCCCATTAGCCGCCAACCCCCCTATTCTTGCCAGCGAGCACCTTGGCCGACAAA[CCGTTGACGGTTTGCTTT
GGCACCTGCAACTGAAGACACGCAACAGAATTTGTCTGTCGGGGAATTACTATCCGCCGAAAGCGTCTTTAATGAGAA
GGAAGAGGGACAAGCTCCCGCCGCCGCGGCAACTAGCCCAGCGGACTTATACATCACCAAGGAGAACTACATGCTGAT
ACTCACCCGTAAGGTTGGC]

>*rsmA*

[CTTCCAGGGCGTCGACGAGCACGGCAGCATCACTACCCTGGGTCGTGGTGGCTCTGATACCACCGGCGTGGCCCTGG
CGGCGGCGCTGAAGGCTGATGAGTGCCAGATCTACACTGATGTTGATGGCGTCTACACCACCGACCCGCGCGTCGTGC
CACAGGCCCGTCGCCTGGAGAAGATCACCTTCGAAGAGATGCTGGAAATGGCCAGCCTTGGTTCCAAGGTGCTGCAGA
TCCGTTCGGTGGAGTTCGCCGGCAAGTACAACGTTCCGCTGCGCGTGCTGCACAGCTTCAAGGAGGGTCCGGGTACCC
TCATTACCATTGATGAAGAGGAATCCATGGAACAGCCGATCATTTCCGGTATCGCCTTCAACCGTGATGAAGCAAAGC
TGACCATTCGCGGCGTGCCGGATACCCCGGGCGTGGCCTTCAAGATCCTCGGCCCGATCAGCGCTTCGAACATCGAAG
TCGACATGATCGTGCAGAACGTTGCGCACGATAACACCACCGACTTCACCTTCACCGTGCACCGCAATGAGTACGAGA
AGGCGCAGAGCGTGCTGGAAAACACCGCGCGCGAAATCGGTGCCCGTGAAGTGATCGGCGACACCAAGATCGCCAAGG
TCTCGATCGTCGGCGTCGGCATGCGTTCGCACGCCGGTGTTGCCAGCTGCATGTTCGAAGCCCTGGCCAAGGAGAGCA
TCAACATCCAGATGATCTCCACTTCCGAGATCAAGGTTTCGGTGGTGCTCGAAGAGAAGTACCTGGAGCTGGCCGTAC
GCGCGCTGCATACCGCGTTCGATCTGGATGCTCCTGCCCGACAGGGCGAGTAAGGCGCTGCCTGGAAGGGCGCGGCTT
GCCGCGCCCTTCGCGTTTCTGCTCGCCGCGCAGGTCCTGTCCTGCCTTGTGCGGCGAGTAACCGGGGCCATGGTCGTC
GTCCGCGACCCGGGCCCGATATCCAAGACTGTTACCCCTGAATGTTTTGCGTAAGGAGAAAGCTATGTTGATTCTGAC
TCGTCGG]

Figure S6. Transcriptional start sites (highlighted in turquoise) identified in *rsmI, rsmE* and *rsmA* by RACE. The ribosomal binding sites are underlinded and start codons are highlighted yellow. Stop codons of upstream genes are shown in red. Fragments included in translational fusions with LacZ are indicated by purple square brackets. Sequences compatible with σ^{54}- and σ^{70}-dependent promoters in *rsmI* and *rsmE* are shaded in grey, with conserved bases in bold.