

SUPPLEMENTARY MATERIAL

Genome-wide analysis of targets for post-transcriptional regulation by Rsm proteins in *Pseudomonas putida*

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Tables S1. Targets of Rsm proteins in *P. putida* KT2440 above the indicated cutoff parameters. Start and end genome coordinates for each RNA species in the different biological replicas, and the average fold enrichment values are shown, along with the loci covered in each case. The number of targets indicated in the Venn diagram in Figure 2 is the number shown in square brackets in boldface, after removing redundancies (see notes). Loci orientation is indicated by > (+strand) and < (-strand). When the target includes sequences corresponding to more than one locus, the ones with the longest overlap are shown in boldface. Targets specifically mentioned in the text are shown in red. When long intergenic sequences are included, these are indicated in parentheses.

Colors indicate common targets as follows:

A+E+I
A+E
A+I
E+I

Notes:

- a: 1 target in RsmE and RsmI; taken as 1 target in the Venn diagram
- b: *ISPpu8* and the adjacent intergenic region are considered as 1 target in all cases
- c: when 2 targets correspond to the same gene, they are taken as 1

RsmA; -10logPval > 130; fold enrichment ≥ 2.15					
RsmA1		RsmA2		average Fold enr.	245 [241] targets
start	end	start	end		locus PP_
14181	15830	14354	15830	2.77	0013>
61840	62547	61839	62515	2.74	0053>
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94454	94930	94462	94939	3.09	0084>< 0085
95034	95923	95028	95913	2.65	<0085 86 > 87 ><88
147520	148125	147475	148138	3.12	< 0139 <0140
168416	170838	168416	170834	2.32	0158>0159>
171386	172939	171359	172941	6.21	16SA (rRNA)>
173232	176106	173071	176108	4.64	23SA (rRNA)>
176821	178369	176800	178370	7.40	5SA (rRNA)>
178673	181522	178511	181537	4.70	23SB (rRNA)>
225090	225629	225117	225618	4.41	<0173
253817	254334	253874	254318	3.15	<0203
313343	313943	313343	313945	2.26	0258 ><0259
314667	315358	314708	315367	3.03	0260 >0261>
333211	333697	333212	333676	3.64	5435> 0274 >
339227	340248	339227	340259	2.20	<0281< 0282 (art) <0283
351734	352280	351724	352265	3.75	0292 >0293>
355137	356457	355067	356454	2.34	<0295< 0296
358290	360006	358257	360006	2.34	5441> 0298 ><0299

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	390819	391513	390819	391644	2.45	0325>
	394576	395854	394588	395840	2.34	0328>
	406290	408007	406290	408005	2.18	<0338
	432981	435388	432962	435392	2.37	<0356
a	448788	450486	448793	450452	3.61	0370>
	450517	451034	450513	451049	8.36	mr05 (rsmY) ><0371
	524940	526464	524928	526469	6.07	16SC (rRNA)>
	526802	529660	526632	529683	4.43	23SC (rRNA)>
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	627312	629948	627322	630285	2.27	< 0541<0542<0543
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	641025	642518	641053	642923	2.56	<0552<0553
	655922	656371	655893	656381	4.42	< 0563 (gcbA)
	672573	673831	672652	673856	2.32	0573><0574 <0575
	697826	699335	697803	699337	5.40	16SD (rRNA)>
	699875	702746	699861	702788	4.36	23SD (rRNA)>
	731735	733043	731795	733080	2.25	0625>
	734169	735287	734162	735289	2.84	<0626
	751774	752773	751762	752797	2.48	5451><0641
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	958559	959213	958536	959222	2.67	0820>0821>
	1026884	1027651	1026650	1027643	2.37	<0884<0885
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	1048261	1048900	1048287	1048910	4.04	<0906< 0907
	1084145	1084974	1084147	1084952	2.53	0938>0939>
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	1149910	1151059	1149882	1151081	2.32	<1009
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	1177549	1178761	1177513	1178772	3.52	1032>
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	1242584	1242946	1242607	1242936	3.18	<1083
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	1284031	1285573	1284037	1285630	2.15	1121>1122>
	1316197	1316515	1316188	1316503	3.83	>mr_15
	1325493	1327014	1325484	1327035	5.98	16SE (rRNA)
	1327335	1330214	1327345	1330221	4.60	23SE (rRNA)
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	1556616	1557427	1556722	1557248	4.16	1367>5468><1368
	1566286	1567285	1566295	1567284	3.74	1375>
	1606506	1607728	1606526	1607758	2.44	<t64< 1408
	1635349	1635782	1635348	1635764	3.64	1434>
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	1707234	1708652	1707235	1708677	2.62	<1502<1503
	1712253	1712984	1712278	1712974	2.52	1506>
	1818338	1819856	1818329	1819847	2.20	1622> 1623 (rps5)
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	1835561	1836387	1835569	1836388	2.56	<1640
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	1966194	1968002	1966189	1968117	2.42	1762><1763
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	2063621	2064532	2063624	2064379	2.26	1840><1841
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	2126927	2127555	2126932	2127745	3.70	1887>
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2439257	2440414	2439264	2440284	2.65	2137>
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2501704	2502448	2501869	2502460	3.94	2195>
2507033	2508097	2507624	2508096	3.52	2200>< 2201
2526550	2529551	2526877	2529510	3.05	2215> 16 > 17 >(405bp)18>
2530422	2530940	2530425	2530952	4.88	2218> 2219 >
2548691	2550200	2548661	2550209	5.59	16SF (rRNA)
2550746	2553605	2550754	2553649	4.46	23SF (rRNA)
2624383	2624965	2624379	2624961	2.99	2296 >2297>
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2668138	2672391	2668152	2672546	2.46	2338>< 2339
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2783732	2784188	2783699	2784134	3.32	2437>
2786580	2786930	2786570	2786954	3.46	<2438
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2850955	2851307	2850943	2851315	4.63	2504 >5530>
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3070606	3071644	3070593	3071642	3.36	2681 >2682>
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3575447	3576499	3575446	3576480	2.15	<3156
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3924796	3925304	3924810	3925292	3.37	<3461< 5602
4013159	4013712	4013161	4013721	2.87	mr44>
4022139	4023114	4022125	4023093	6.04	<3547(666bp) <3548
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4114287	4114797	4114285	4114747	4.09	<3620
4144163	4145054	4144145	4145096	2.72	3648> 3649 >
4160099	4160858	4160146	4160882	2.59	3662 ><3663
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4197055	4197785	4197048	4197814	4.04	<5620
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4244409	4245111	4244630	4245148	2.99	<3721<3722
4272704	4273098	4272712	4273117	3.85	3743 ><3744
4289421	4290858	4289279	4290161	3.04	< 3761 (<i>cfcA</i>)
4295633	4296371	4295634	4296298	2.56	3765>
4302943	4304486	4303221	4304468	2.97	< 3775 <3776
4306075	4306926	4306072	4306929	3.00	<3778

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	4329856	4330807	4329980	4330799	2.27	3801>3802>
	4357839	4358524	4357838	4358524	2.53	3832(rsmE) ><3833
	4415756	4416152	4415762	4416153	4.06	3901>
	4418225	4419212	4418122	4419200	3.18	3908><3909
	4458536	4458988	4458505	4459004	3.32	3952>(266bp)3953>
a	4472735	4473269	4472724	4473293	4.08	3962>(665bp)
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	4583557	4584376	4583534	4584141	2.34	<4060
	4634949	4636282	4635010	4636299	2.56	<4098< 99 <100
	4643129	4644681	4643116	4644693	2.47	4108 ><4109
	4645433	4647635	4645696	4647631	2.51	4111>
	4679897	4680813	4680062	4680799	2.71	<4143
	4698169	4698690	4698164	4698682	4.18	<4158
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	4739415	4740721	4739432	4740824	2.28	4194>
	4765303	4767845	4765312	4767833	2.69	4217>
	4839441	4842928	4839431	4842902	2.52	4254>55>56>57>58
	4850156	4851247	4850154	4851293	2.19	4265 > 4266>
	4855177	4855801	4855185	4855801	2.56	4270 >
	4858749	4859210	4858705	4859220	3.00	<mr49
	4866695	4867460	4866720	4867443	3.02	4278 >
b	4907783	4908137	4908163	4908682	4.46	<4317(277bp) 4318 >
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	5180332	5181109	5180378	5181100	3.75	4558>< 4559 <4560
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	5307693	5310555	5307688	5310568	5.02	235G (rRNA)
	5311157	5312725	5311125	5312725	6.36	165G (rRNA)
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	5338209	5338748	5338204	5338748	3.93	mr53>
	5344571	5346807	5344587	5346814	2.22	4702>
	5351298	5351797	5351288	5351823	3.20	<4707
	5351979	5353807	5351953	5353858	2.15	<4708
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	5425060	5425437	5425082	5425440	3.85	4764>(147pb)
	5437583	5437900	5437606	5437900	8.19	<mr57
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	5483693	5484213	5483695	5484190	3.29	4822 >
	5533169	5534481	5533192	5534480	2.44	<4866<4867
	5542726	5545081	5542713	5545108	2.15	<4874<75<76<77
	5554163	5555573	5554156	5555561	3.06	<4885< 4886 < 4887
	5557919	5559481	5557921	5559500	2.19	<4889<4890
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	5667891	5669578	5667863	5669590	2.42	mr59 > 4976 >4977>
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	5695783	5696600	5695784	5696591	2.75	4999 >
	5699356	5700384	5699356	5700535	2.44	5003 >
	5704485	5705484	5704480	5705477	2.46	< 5007 <5008
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	5801087	5801596	5801067	5801584	2.77	< 5079
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	5852162	5852598	5852172	5852593	4.09	<5129<5720
	5892769	5893243	5892789	5893262	3.16	<5167
	5896282	5896649	5896275	5896571	3.90	<5171

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5930846	5931762	5930842	5931780	2.67	<5198<5199
5936428	5937988	5936420	5938023	3.39	5206>5207>
5941844	5942543	5941988	5942556	3.73	<5209
5965490	5966129	5965507	5966108	2.29	5231><5232
6027907	6029544	6027908	6029541	2.39	<5278
6060952	6061450	6060951	6061470	2.45	<5313
6114030	6115621	6114043	6115616	2.20	5364><5365
6130950	6131902	6131168	6131913	3.26	<5379<5732<5380
6145339	6147414	6145376	6147403	2.36	<5389<90<91<92
6150919	6153185	6150923	6153156	2.21	5395>5737>5738

RsmE; -10logPval > 170; fold enrichment ≥ 4							
RsmE1		RsmE2		RsmE3		average Fold	270 [261] targets
start	end	start	end	start	end	enr.	locus PP_
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73573	74445	73544	74458	73676	74440	6.90	0063><64
74489	74890	74489	74900	74459	74888	7.18	<0064<0065
94555	94940	94507	94961	94511	94940	8.58	0084><0085
105204	106008	105182	106008	105204	106002	4.32	0100>
107617	108469	107624	108479	107615	108453	4.75	0101><5426
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155538	155919	155549	155974	155543	155780	8.65	<0146
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224752	225652	224764	225633	224752	225680	4.70	<0173
233559	234386	233595	234393	233547	234374	5.40	0180><0181<0182
236999	238376	236996	238288	237057	238405	5.01	0185>
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344515	344969	344455	344936	344643	344945	8.75	<0285<0286<0287
351586	352596	351614	352597	351599	352495	7.81	0292>0293>
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448800	451299	448799	451290	448829	451258	16.46	0370>mr05 (<i>rsmY</i>)><0371
456576	457049	456578	457039	456570	457007	7.71	<0375<0376
470467	470964	470465	470989	470465	470969	6.03	<0386
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514196	516151	514267	516103	514265	516118	5.47	<0426<0427<0428
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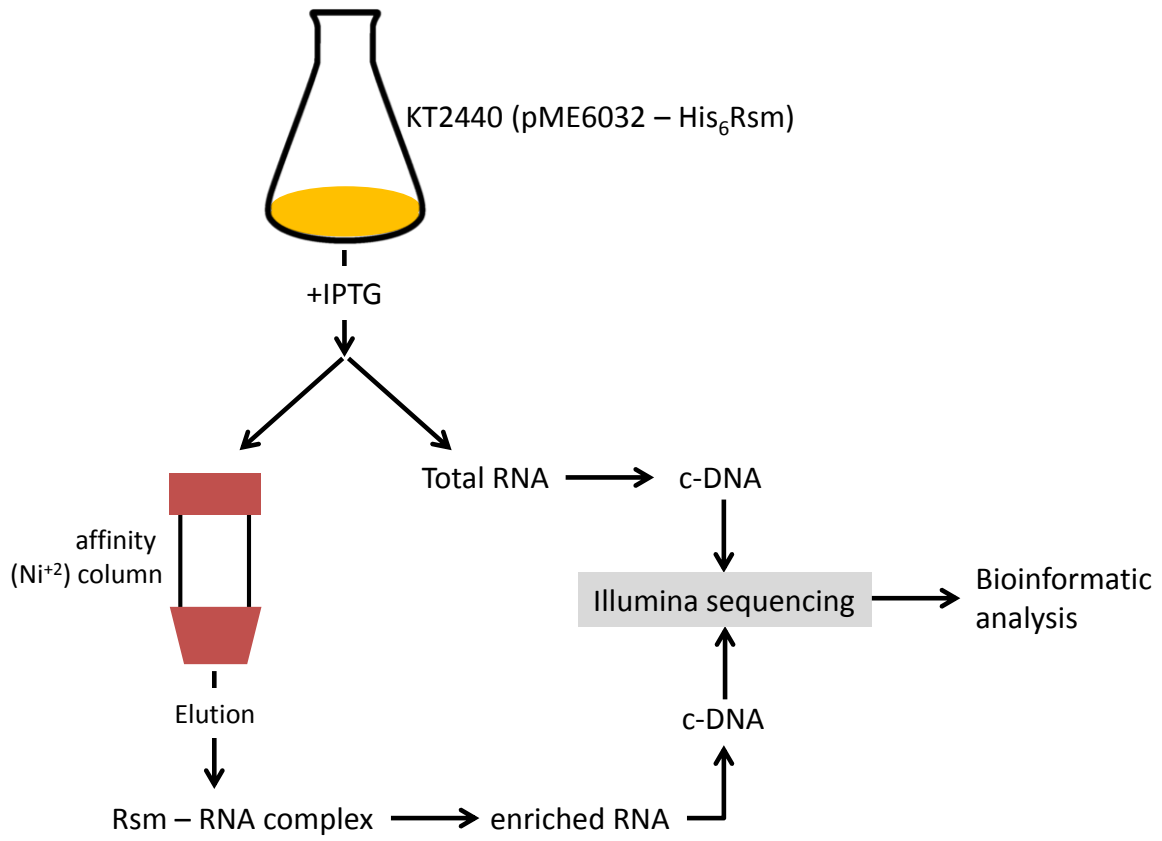


Figure S1. Schematic representation of the method used to identify Rsm-bound RNA sequences. See text for details.

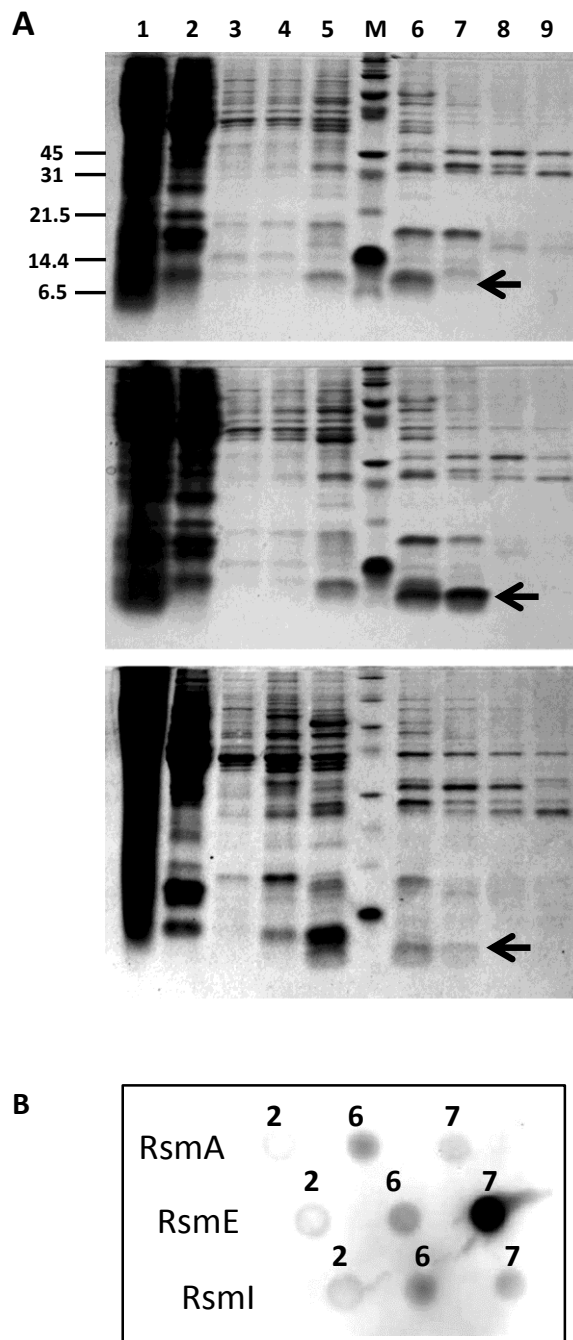


Figure S2. A. SDS-PAGE of fractions recovered by affinity purification of His₆-Rsm proteins from IPTG-induced KT2440 cultures harboring pME6032-*rsmA* (top) pME6032-*rsmE* (middle), and pME6032-*rsmI* (bottom). Lanes: 1, samples before injection in HisTrap columns (Amersham Bioscience); 2, elution with 7% imidazole; 3-9, fractions recovered in the 7-100% range of imidazole concentrations. Arrows indicate the bands possibly corresponding to Rsm proteins. **B.** Detection of His₆-Rsm proteins by dot blot. Twenty μ L of the indicated samples (equivalent to the lanes in A) were spotted on an activated PVDF membrane and a monoclonal His-tag antibody (Invitrogen), diluted 1/5000 was used for chemiluminescence detection in a Bio-Rad ChemiDoc with ECL (Pierce) after incubation with a HRP-conjugated secondary antibody.

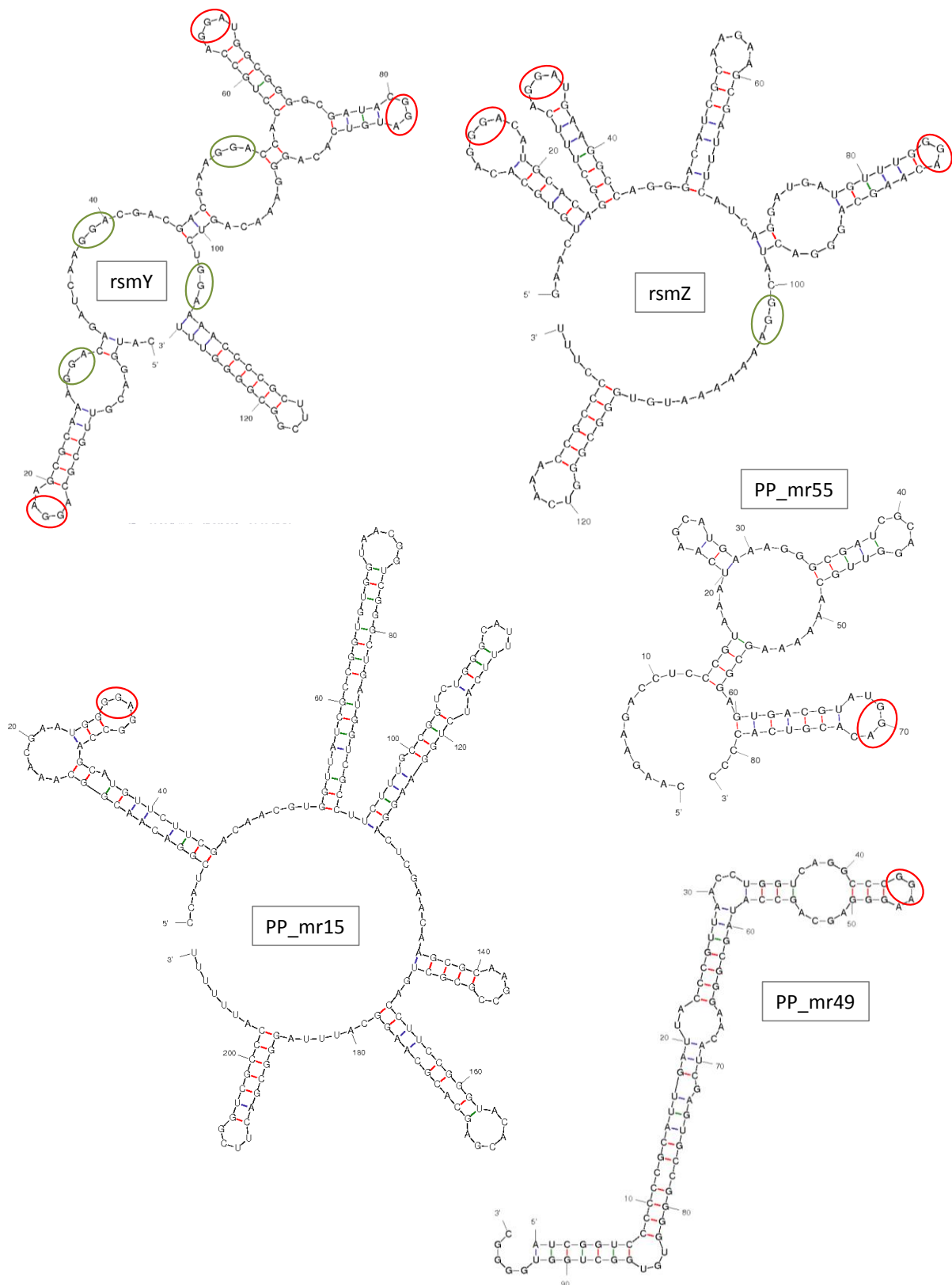


Figure S3. Secondary structure predictions for several ncRNAs of *P. putida*. Unpaired GGA motifs and those in stem-loop structures are indicated in green and red, respectively. Predictions were done using the Mfold web server (M. Zuker. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31** (13), 3406-3415, 2003).

TGTAAG--N₆--CTTACA

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```

Figure S4. Putative GacA binding sites in the promoter regions of rsmY, rsmZ and PP_mr55. Bases identical to the consensus (indicated above the sequences) are highlighted in yellow; additional ones conserved in the three sequences are highlighted in blue and those conserved in two of the sequences in grey.

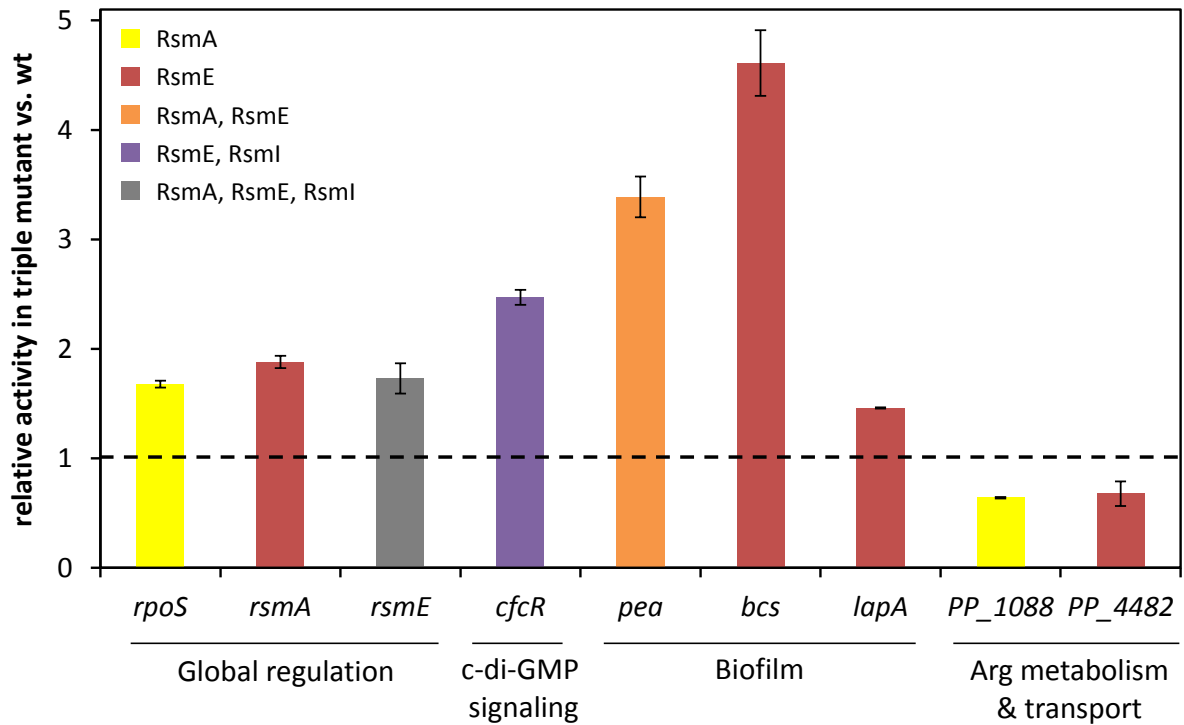


Figure S5. Influence of Rsm proteins on expression of some of the identified targets. Translation fusions to the reporter *'lacZ* were analyzed in KT2440 and the triple *rsm* mutant after 5h of growth. Data are presented as relative β -galactosidase activity in the mutant with respect to the wild type, and are averages and standard deviations of two independent experiments with three technical replicas each. A value of 1 (broken line) indicates that there is no difference between the two strains. Color codes indicate the Rsm protein(s) for which each RNA has been identified as target. Constructs have been described elsewhere (Martínez-Gil et al., 2010; Huertas-Rosales et al., 2016, 2017; Barrientos-Moreno et al., manuscript in preparation).

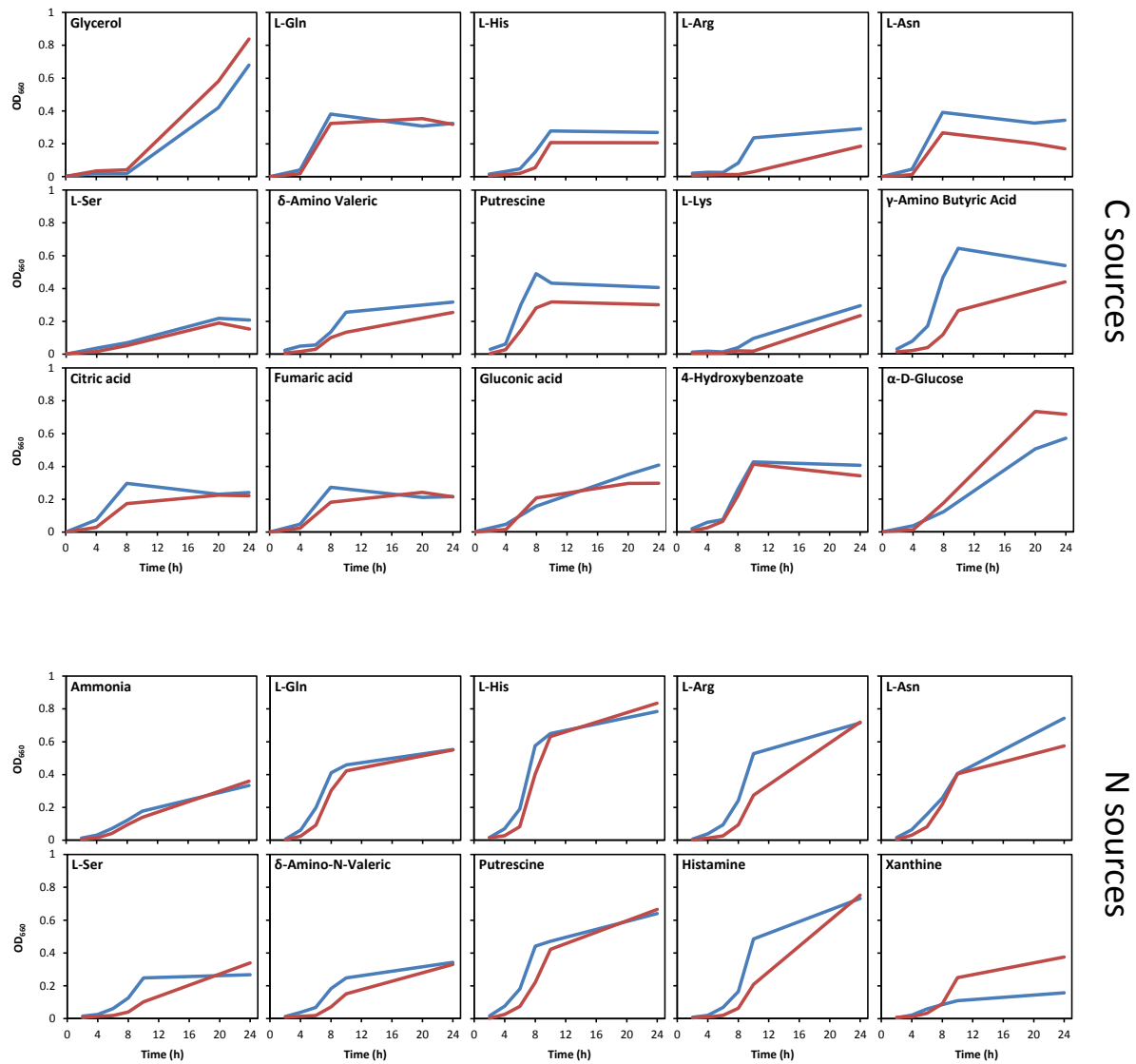


Figure S6. Growth of *P. putida* KT2440 (blue lines) and the triple *rsm* mutant (red lines) in BIOLOG plates during 24h of incubation at 30°C, with some of the compounds tested as sole source of carbon (top graphs) or nitrogen (bottom graphs).