CHAPTER 8
APPENDICES

Appendix A – Genes significantly up-regulated in *A. baumannii* strain ATCC 17978 under iron-limiting conditions

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2382	BasD	7.4
A1S_2380	putative acinetobactin biosynthesis protein	7.4
A1S_2381	putative acinetobactin biosynthesis protein (KEGG; EC:2.7.7.58)	7.3
A1S_2383	putative acinetobactin biosynthesis protein	7.2
A1S_2581	isochorismate synthetase	7.2
A1S_2379	putative acinetobactin biosynthesis protein (KEGG; EC:4.1.1.22)	7.2
A1S_2388	putative ferric acinetobactin transport system permease protein	7.1
A1S_2390	putative acinetobactin biosynthesis protein	7.1
A1S_2387	BauE	7.0
A1S_2372	putative acinetobactin biosynthesis protein	6.9
A1S_2389	putative ferric acinetobactin transport system permease protein	6.9
A1S_1647	putative siderophore biosynthesis protein	6.9
A1S_2384	BasC	6.8
A1S_2378	putative ABC transporter	6.7
A1S_2392	putative acinetobactin utilization protein	6.7
A1S_1648	putative lysine/ornithine N-monooxygenase	6.6
A1S_2567	putative thioesterase	6.6
A1S_2386	putative ferric acinetobactin binding protein	6.6
A1S_2566	putative ferric siderophore receptor protein	6.6
A1S_2580	23-dihydro-2,3-dihydroxybenzoate synthetase, isochorismatase (KEGG; EC:3.3.2.1)	6.5
A1S_1649	putative RND family drug transporter	6.4
A1S_2385	putative ferric acinetobactin receptor	6.4
A1S_2578	putative non-ribosomal peptide synthetase	6.3
A1S_2377	putative ABC transporter	6.3
A1S_2577	putative non-ribosomal peptide synthetase	6.2
A1S_2077	putative outer membrane porin receptor for Fe(III)-coprogen, Fe(III)-ferrioxamine B and Fe(III)-rhodotrulic acid uptake (FhuE)	6.2
A1S_2579	23-dihydro-2,3-dihydroxybenzoate dehydrogenase (KEGG; EC:1.3.1.28)	6.2
A1S_1657	putative siderophore biosynthesis protein; putative acetyltransferase	6.1
A1S_2376	putative ABC transporter	6.1
A1S_2375	putative ABC transporter	6.1
A1S_1650	hypothetical protein	6.0
A1S_2576	putative non-ribosomal peptide synthetase	6.0
A1S_2575	putative nonribosomal peptide synthetase	5.9
A1S_2574	23-dihydroxybenzoate-AMP ligase	5.8

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2573	23-dihydroxybenzoate-AMP ligase	5.8
A1S_2568	phosphopantetheinyl transferase component of siderophore synthetase	5.8
A1S_2374	putative acinetobactin biosynthesis protein	5.7
A1S_1652	hypothetical protein	5.6
A1S_1651	hypothetical protein	5.6
A1S_2373	putative acinetobactin biosynthesis protein	5.5
A1S_2391	putative acinetobactin biosynthesis protein	5.5
A1S_2572	putative siderophore biosynthesis protein	5.4
A1S_2565	putative MFS transport protein	5.2
A1S_2571	putative ornithine decarboxylase	5.1
A1S_3174	putative regulatory or redox component complexing with Bfr in iron storage and mobility (Bfd)	5.1
A1S_2563	putative siderophore-interacting protein	5.0
A1S_1986	fumarase C (KEGG; EC:4.2.1.2)	4.8
A1S_2564	putative siderophore-interacting protein	4.7
A1S_1653	hypothetical protein	4.6
A1S_2123	putative signal peptide	4.6
A1S_2076	putative outer membrane porin receptor for Fe(III)-coprogen, Fe(III)-ferrioxamine B and Fe(III)-rhodotrulic acid uptake (FhuE)	4.5
A1S_1667	putative ferric hydroxamate siderophore receptor	4.3
A1S_3339	putative ferric siderophore receptor protein	4.1
A1S_2562	putative multidrug resistance pump	4.0
A1S_3043	hypothetical protein	4.0
A1S_1654	putative demethylmenaquinone methyltransferase	3.9
A1S_0452	hypothetical protein	3.8
A1S_2183	putative signal peptide	3.7
A1S_0980	ferric enterobactin receptor precursor	3.6
A1S_0981	ferric enterobactin receptor precursor	3.6
A1S_0169	hypothetical protein	3.5
A1S_0804	trehalose-6-phosphate phophatase	3.4
A1S_2809	bacteriolytic lipoprotein entericidin B	3.2
A1S_0453	putative biopolymer transport protein (ExbB)	3.2
A1S_0242	putative ferrous iron transport protein A	3.2
A1S_1666	hypothetical protein	3.2
A1S_0454	putative biopolymer transport protein (ExbD)	3.1
A1S_0170	putative outer membrane copper receptor (OprC)	2.9
A1S_1687	transcriptional regulator	2.8
A1S_3324	putative ferric siderophore receptor protein	2.7
A1S_2734	putative phosphatidylglycerophosphatase B	2.7
A1S_2278	putative hydrolase of the alpha/beta superfamily	2.7
A1S_2696	hypothetical protein	2.6
A1S_2820	hypothetical protein	2.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1655	putative ferric siderophore receptor protein	2.5
A1S_1950	putative universal stress protein	2.5
A1S_2102	aldehyde dehydrogenase 1 (KEGG; EC:1.2.1.3)	2.5
A1S_2093	hypothetical protein	2.4
A1S_1383	surface antigen	2.4
A1S_1063	TonB-dependent siderophore receptor	2.4
A1S_2570	putative siderophore biosynthesis protein; putative acetyltransferase	2.4
A1S_3325	putative ferric siderophore receptor protein	2.3
A1S_3303	hypothetical protein	2.3
A1S_0243	putative ferrous iron transport protein B	2.2
A1S_1656	hypothetical protein	2.2
A1S_1932	hypothetical protein	2.2
A1S_2798	hypothetical protein	2.2
A1S_0171	hypothetical protein	2.2
A1S_1680	hypothetical protein	2.2
A1S_0683	putative sigma(54) modulation protein RpoX	2.1
A1S_0416	putative transcriptional regulator (LysR family)	2.1
A1S_1703	dihydrolipoamide dehydrogenase	2.0
A1S_2080	putative siderophore receptor	2.0
A1S_3301	hypothetical protein	2.0
A1S_0570	hypothetical protein	2.0
A1S_0244	hypothetical protein	2.0
A1S_2582	putative transcriptional regulator (AraC family)	2.0
A1S_1701	dihydrolipoamide acetyltransferase	2.0
A1S_1751	AdeA membrane fusion protein	2.0
A1S_1699	acetoin:26-dichlorophenolindophenol oxidoreductase alpha subunit	2.0
A1S_1220	putative threonine efflux protein	1.9
A1S_0803	trehalose-6-phosphate synthase	1.9
A1S_0779	hypothetical protein	1.9
A1S_2230	hypothetical protein	1.9
A1S_3283	gamma-aminobutyrate permease	1.9
A1S_1456	putative chromate transport protein	1.9
A1S_1385	hypothetical protein	1.8
A1S_1505	YyaM	1.8
A1S_0474	putative ferric siderophore receptor protein	1.8
A1S_1697	putative transcriptional regulator	1.8
A1S_2159	hypothetical protein	1.8
A1S_2081	TonB-dependent siderophore receptor	1.7
A1S_2975	hypothetical protein	1.7
A1S_1008	isocitrate lyase	1.7
A1S_1750	AdeB	1.7
A1S_2041	hypothetical protein	1.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1700	acetoin:26-dichlorophenolindophenol oxidoreductase beta subunit (KEGG; EC:1.2.4.1)	1.7
A1S_1204	hypothetical protein	1.7
A1S_2318	hypothetical protein	1.6
A1S_1890	3-carboxy-ciscis-muconate cycloisomerase (KEGG; EC:5.5.1.2)	1.6
A1S_1933	hypothetical protein	1.6
A1S_1954	serine proteinase	1.6
A1S_1736	hypothetical protein	1.6
A1S_1702	dihydrolipoamide dehydrogenase	1.6
A1S_2735	AdeI	1.6
A1S_2098	putative alcohol dehydrogenase (KEGG; EC:1.1.1.1)	1.6
A1S_0210	transposase	1.6
A1S_2484	hypothetical protein	1.6
A1S_1730	short-chain fatty acid transporter (scFAT family)	1.6
A1S_3300	putative sodium:solute symporter	1.5
A1S_2667	hypothetical protein	1.5
A1S_1951	hypothetical protein	1.5
A1S_0978	hypothetical protein	1.5
A1S_0168	zinc(II) binding peptide deformylase 1 (KEGG; EC:3.5.1.88)	1.5
A1S_2466	hypothetical protein	1.5
A1S_2331	putative acyl-CoA dehydrogenase	1.5
A1S_1752	AdeA membrane fusion protein	1.5
A1S_1984	D-amino acid dehydrogenase small subunit	1.5
A1S_1577	putative flavin-binding monooxygenase	1.5
A1S_0184	hypothetical protein	1.5
A1S_1698	lipoate synthase	1.5
A1S_3329	EsvJ	1.5
A1S_2538	OMP CarO precursor	1.5
A1S_1193	OmpA/MotB	1.5
A1S_0979	putative membrane-bound protein in GNT I transport system (GntY)	1.4
A1S_1920	putative metalloprotease	1.4
A1S_2330	putative acyl-CoA dehydrogenase	1.4
A1S_1919	putative phospholipase A1 precursor (PldA)	1.4
A1S_0470	methionine biosynthesis protein	1.4
A1S_1395	hypothetical protein	1.4
A1S_0207	hypothetical protein	1.4
A1S_2654	putative periplasmic binding protein of transport/transglycosylase	1.4
A1S_2317	putative lipoprotein precursor (RlpA-like)	1.4
A1S_1228	cold shock protein	1.4
A1S_1467	putative glutamate symport transmembrane protein	1.4
A1S_1732	acetoacetyl-CoA transferase alpha subunit	1.4

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2889	putative signal peptide	1.4
A1S_0408	putative glutathione S-transferase	1.4
A1S_0209	transposase	1.4
A1S_0895	ferric uptake regulator	1.4
A1S_2261	putative cold shock protein	1.4
A1S_1672	hypothetical protein	1.4
A1S_0771	hypothetical protein	1.3
A1S_1713	putative transcriptional regulator (AraC family)	1.3
A1S_1658	putative hydrolase haloacid dehalogenase-like family	1.3
A1S_2569	hypothetical protein	1.3
A1S_1141	carbon storage regulator	1.3
A1S_1934	hypothetical protein	1.3
A1S_0657	transposase	1.3
A1S_1909	hypothetical protein	1.3
A1S_1856	p-hydroxyphenylacetate hydroxylase C1:reductase component	1.3
A1S_0147	ATP synthase protein I	1.3
A1S_3463	YPPCP.09C-like protein	1.3
A1S_1526	hypothetical protein	1.3
A1S_0360	30S ribosomal protein S15	1.3
A1S_2135	putative signal peptide	1.3
A1S_3171	RNA polymerase omega subunit	1.3
A1S_2178	putative transglutaminase	1.3
A1S_1731	acetoacetyl-CoA transferase beta subunit	1.3
A1S_3281	4-aminobutyrate aminotransferase PLP-dependent	1.3
A1S_1988	putative intracellular sulfur oxidation protein (DsrE-like)	1.2
A1S_1634	*iscRSUA* operon repressor	1.2
A1S_0546	hypothetical protein	1.2
A1S_0745	hypothetical protein	1.2
A1S_2483	hypothetical protein	1.2
A1S_1573	integration host factor beta subunit	1.2
A1S_2906	putative sensory transduction histidine kinase	1.2
A1S_1896	putative cell division protein (FtsB-like)	1.2
A1S_1889	3-oxoadipate enol-lactonase I	1.2
A1S_2503	putative outer membrane lipoprotein	1.2
A1S_2101	putative transcriptional regulator	1.2
A1S_3462	hypothetical protein	1.2
A1S_0631	hypothetical protein	1.2
A1S_2485	putative glycosyltransferase	1.2
A1S_1632	cysteine desulfurase	1.2
A1S_1457	putative chromate transport protein	1.2
A1S_1671	PltJ	1.2
A1S_2106	ethanolamine ammonia-lyase light chain	1.2
A1S_0634	hypothetical protein	1.2

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1704	acetoin dehydrogenase (KEGG; EC:1.1.1.5)	1.2
A1S_2724	putative hemagglutinin/hemolysin-related protein	1.2
A1S_1726	aspartate ammonia-lyase (aspartase) (KEGG; EC:4.3.1.1)	1.2
A1S_2985	hypothetical protein	1.2
A1S_1987	putative UDP-galactose 4-epimerase (GalE-like)	1.2
A1S_1620	hypothetical protein	1.2
A1S_0549	hypothetical protein	1.1
A1S_1633	cysteine desulfurase	1.1
A1S_0481	phosphate acetyltransferase	1.1
A1S_0251	thiamine hydroxymethylpyrimidine moiety synthesis	1.1
A1S_1269	putative allophanate hydrolase subunit 1 and 2	1.1
A1S_2785	putative protease	1.1
A1S_1178	ATP phosphoribosyltransferase	1.1
A1S_3272	putative MFS transport protein	1.1
A1S_1403	putative cysteine desulfurase 1 (Csd)	1.1
A1S_1931	hypothetical protein	1.1
A1S_1224	transposase	1.1
A1S_1756	transcriptional regulator AraC family	1.1
A1S_2560	hypothetical protein	1.1
A1S_0597	50S ribosomal protein L20	1.1
A1S_1390	hypothetical protein	1.1
A1S_1077	hypothetical protein	1.1
A1S_0894	outer membrane lipoprotein	1.1
A1S_3231	putative acetyl-CoA hydrolase/transferase (KEGG; EC:3.1.2.1)	1.1
A1S_2888	hypothetical protein	1.1
A1S_0820	putative peptidoglycan-binding LysM	1.1
A1S_1833	hypothetical protein (KEGG; EC:1.11.1.10)	1.1
A1S_1222	possible exonuclease	1.1
A1S_1268	hypothetical protein	1.1
A1S_1735	hypothetical protein	1.1
A1S_2231	gamma-aminobutyrate permease	1.1
A1S_2823	hypothetical protein	1.1
A1S_1540	putative transcriptional regulator (TetR family)	1.1
A1S_2446	high-affinity phosphate transport protein	1.1
A1S_1578	putative transcriptional regulator	1.0
A1S_1881	porin	1.0
A1S_1088	hypothetical protein	1.0
A1S_2843	hypothetical protein	1.0
A1S_2362	putative xanthine dehydrogenase protein	1.0
A1S_2598	putative RNA polymerase sigma factor	1.0
A1S_2892	putative TonB-dependent receptor protein	1.0
A1S_1617	30S ribosomal protein S20	1.0

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0630	hypothetical protein	1.0
A1S_0603	integration host factor alpha subunit	1.0
A1S_0421	protein chain initiation factor IF-1	1.0
A1S_2559	hypothetical protein	1.0
A1S_2736	putative RND family drug transporter	1.0
A1S_1255	lipid A biosynthesis lauroyl acyltransferase	1.0
A1S_0203	hypothetical protein	1.0
A1S_1897	hypothetical protein	1.0
A1S_3248	glycerol uptake facilitator	1.0
A1S_1329	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	1.0
A1S_0997	hypothetical protein	1.0
A1S_0786	putative signal peptide	1.0
A1S_2601	putative outer membrane protein A	1.0
A1S_2447	EsvD	1.0
A1S_1747	anthranilate dioxygenase reductase	1.0
A1S_0482	acetate kinase (propionate kinase) (KEGG; EC:2.7.2.1)	1.0
A1S_2666	putative 3'5'-cyclic-nucleotide phosphodiesterase	1.0
A1S_1853	hypothetical protein	1.0
A1S_0788	hypothetical protein	1.0
A1S_1528	hypothetical protein (KEGG; EC:1.5.99.8)	1.0
A1S_1426	phosphoribosyl-dephospho-CoA transferase	1.0
A1S_1908	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (KEGG; EC:2.5.1.54)	1.0
A1S_0815	hypothetical protein	0.9
A1S_1645	hypothetical protein	0.9
A1S_2119	putative acetyltransferase	0.9
A1S_1384	CinA-like protein	0.9
A1S_1075	D-amino-acid dehydrogenase	0.9
A1S_2311	putative transport protein	0.9
A1S_1911	putative protease	0.9
A1S_3126	peptidase S8 and S53 subtilisin, kexin, sedolisin	0.9
A1S_1695	putative two-component response regulator	0.9
A1S_2978	hypothetical protein	0.9
A1S_1921	ferrichrome-iron receptor	0.9
A1S_1601	malate synthase G	0.9
A1S_1299	hypothetical protein	0.9
A1S_0136	glutathione S-transferase	0.9
A1S_2640	putative oxidoreductase molybdopterin	0.9
A1S_0902	lactoylglutathione lyase-related protein	0.9
A1S_1523	putative signal peptide	0.9
A1S_2078	predicted acetyltransferase	0.9
A1S_1435	hypothetical protein	0.9
A1S_2527	putative thioesterase protein	0.9

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0635	hypothetical bacteriophage protein	0.9
A1S_0928	bet gene repressor	0.9
A1S_0633	hypothetical protein	0.9
A1S_1412	glutathione S-transferase-like protein	0.9
A1S_2105	ethanolamine ammonia-lyase	0.9
A1S_2623	hypothetical protein	0.9
A1S_2557	hypothetical protein	0.9
A1S_2737	AdeK	0.9
A1S_1618	hypothetical protein	0.9
A1S_2266	hypothetical protein	0.9
A1S_2075	putative outer membrane protein	0.9
A1S_3475	hypothetical protein	0.9
A1S_1744	putative electron transfer flavoprotein	0.9
A1S_1773	putative RND family drug transporter	0.9
A1S_3385	hypothetical protein	0.9
A1S_1880	pyrroloquinoline-quinone QuiA (KEGG; EC:1.1.99.25)	0.9
A1S_1477	putative amino acid transporter	0.9
A1S_3470	regulatory protein LysR	0.9
A1S_0389	hypothetical protein	0.9
A1S_1530	SSS family major sodium/proline symporter	0.9
A1S_1411	putative glutathione S-transferase protein	0.9
A1S_0334	hypothetical protein	0.9
A1S_0495	putative glycosyl transferase	0.9
A1S_2042	putative transcriptional regulator (TetR family)	0.9
A1S_3028	putative tRNA-i(6)A37 modification enzyme	0.8
A1S_3150	putative signal peptide	0.8
A1S_0654	regulatory protein ArsR	0.8
A1S_2177	putative proteasome protease	0.8
A1S_1710	hypothetical protein	0.8
A1S_1805	putative MFS transport protein	0.8
A1S_2840	OmpA	0.8
A1S_1298	hypothetical protein	0.8
A1S_1681	putative methyltransferase	0.8
A1S_0787	putative signal peptide	0.8
A1S_1673	inner membrane permease protein	0.8
A1S_1636	putative poly(hydroxyalcanoate) granule associated protein	0.8
A1S_2950	hypothetical protein	0.8
A1S_1263	L-2-haloalkanoic acid dehalogenase	0.8
A1S_0836	putative signal peptide	0.8
A1S_0128	hypothetical protein	0.8
A1S_2849	putative glucose-sensitive porin (OprB-like)	0.8
A1S_2507	hypothetical protein	0.8
A1S_1641	alkane 1-monooxygenase	0.8

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0816	50S ribosomal protein L32	0.8
A1S_2859	putative hemolysin III (HLY-III)	0.8
A1S_1328	putative oxidoreductase	0.8
A1S_1278	allophanate hydrolase subunit 2	0.8
A1S_3144	hypothetical protein	0.8
A1S_2682	cell division protein	0.8
A1S_0996	hypothetical protein	0.8
A1S_2031	hypothetical protein	0.8
A1S_1123	putative flavin-binding monooxygenase	0.8
A1S_1086	hypothetical protein	0.8
A1S_2208	putative transcriptional regulator	0.8
A1S_0802	putative transport protein (permease)	0.8
A1S_2765	putative intracellular protease/amidase	0.8
A1S_0652	putative ferrous iron transport protein A	0.8
A1S_1749	ABC transporter-like protein	0.8
A1S_1290	hypothetical protein	0.8
A1S_0661	phage integrase family protein	0.8
A1S_2659	hypothetical protein	0.8
A1S_1907	putative peroxidase	0.8
A1S_2171	30S ribosomal protein S6	0.8
A1S_1910	ATP-binding protease component	0.8
A1S_1474	uridylyltransferase	0.8
A1S_0672	resolvase	0.8
A1S_1288	putative VGR-related protein	0.8
A1S_0889	hypothetical protein	0.8
A1S_1809	putative hydrolase transmembrane protein	0.8
A1S_1430	malonate utilization transcriptional regulator (LysR family)	0.8
A1S_0659	hypothetical protein	0.8
A1S_1494	putative protein (DcaP-like)	0.8
A1S_2491	putative signal peptide	0.8
A1S_0918	hypothetical protein	0.8
A1S_2558	putative transposase	0.8
A1S_1810	putative tartrate transporter	0.8
A1S_2249	hypothetical protein	0.8
A1S_2132	putative outer membrane protein	0.8
A1S_2121	hypothetical protein	0.8
A1S_2490	UDP-N-acetyl glucosamine-2-epimerase	0.8
A1S_2082	putative transcriptional regulator	0.8
A1S_0281	preprotein translocase IISP family membrane subunit	0.7
A1S_2267	hypothetical protein	0.7
A1S_2394	putative threonine ammonia-lyase	0.7
A1S_2473	transcriptional regulator LysR family	0.7
A1S_1257	putative MFS transport protein	0.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2881	putative fatty acid desaturase	0.7
A1S_2363	xanthine dehydrogenase large subunit	0.7
A1S_2749	hypothetical protein	0.7
A1S_2131	hypothetical protein	0.7
A1S_1393	putative two-component sensor kinase	0.7
A1S_2924	putative rhodanese-related sulfurtransferase	0.7
A1S_0919	putative permease transmembrane protein	0.7
A1S_1979	putative transcriptional regulator	0.7
A1S_3474	hypothetical protein	0.7
A1S_2262	EsvH	0.7
A1S_0357	exonuclease V beta chain	0.7
A1S_1138	putative cytochrome	0.7
A1S_1785	putative iron transport protein	0.7
A1S_0656	hypothetical protein	0.7
A1S_1630	hypothetical protein	0.7
A1S_1778	putative methylenetetrahydrofolate reductase	0.7
A1S_2104	ethanolamine ammonia-lyase heavy chain	0.7
A1S_1431	malonate utilization transcriptional regulator (LysR family)	0.7
A1S_1696	hypothetical protein	0.7
A1S_1616	hypothetical protein	0.7
A1S_1755	AdeT	0.7
A1S_1311	hypothetical protein	0.7
A1S_0801	putative transport protein (permease)	0.7
A1S_1216	LysR regulator	0.7
A1S_1574	hypothetical protein	0.7
A1S_1597	phage tail tape meausure protein lambda family	0.7
A1S_2445	high-affinity phosphate transport protein (KEGG; EC:3.6.3.27)	0.7
A1S_3114	hypothetical protein	0.7
A1S_1427	malonate decarboxylase epsilon subunit	0.7
A1S_2350	chorismate pyruvate lyase	0.7
A1S_0783	hypothetical protein	0.7
A1S_2151	putative transcriptional regulator (AraC family)	0.7
A1S_1816	putative long-chain fatty acid transport protein	0.7
A1S_1814	putative transporter	0.7
A1S_1790	6-phosphogluconate dehydrogenase NAD-binding	0.7
A1S_1361	ABC-type spermidine/putrescine transport system ATPase component	0.7
A1S_0469	hypothetical protein	0.7
A1S_2600	hypothetical protein	0.7
A1S_2152	putative transcriptional regulator (AraC family)	0.7
A1S_1271	putative chloride transport protein	0.7
A1S_3374	positive pho regulon response regulator	0.6
A1S_0444	hypothetical protein	0.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1163	hypothetical protein	0.6
A1S_1282	CopG	0.6
A1S_2599	hypothetical protein	0.6
A1S_1425	malonate decarboxylase gamma subunit	0.6
A1S_2604	putative permease (PerM family)	0.6
A1S_0637	DNA-directed DNA polymerase	0.6
A1S_0692	FilC	0.6
A1S_1815	acyl-CoA synthetase	0.6
A1S_1690	putative ATPase	0.6
A1S_0391	50S ribosomal protein L31 type B	0.6
A1S_1316	putative MFS transport protein	0.6
A1S_0354	exonuclease V gamma chain	0.6
A1S_1999	molybdopterin biosynthesis protein A	0.6
A1S_1522	predicted redox disulfide bond formation protein OsmC-like protein	0.6
A1S_2847	glucose dehydrogenase	0.6
A1S_1356	p-hydroxybenzoate hydroxylase transcriptional activator (KEGG; EC:1.14.13.2)	0.6
A1S_1740	benzoate transport	0.6
A1S_1596	hypothetical protein	0.6
A1S_1512	putative ferredoxin	0.6
A1S_0591	putative AMP-dependent synthetase/ligase	0.6
A1S_1626	putative adenylate or guanylate cyclase	0.6
A1S_1734	hypothetical protein	0.6
A1S_2792	putative esterase of the alpha-beta hydrolase superfamily	0.6
A1S_2099	hypothetical protein	0.6
A1S_2489	UDP-N-acetyl glucosamine-2-epimerase(EC:5.1.3.14)	0.6
A1S_1739	putative MFS transport protein	0.6
A1S_2440	putative purine metabolism protein	0.6
A1S_2205	paraquat-inducible protein A	0.6
A1S_2757	hypothetical protein	0.6
A1S_0857	hypothetical protein	0.6
A1S_1428	malonate transporter	0.6
A1S_0971	methionine synthase (KEGG; EC:2.1.1.13)	0.6
A1S_1312	hypothetical protein	0.6
A1S_1689	hypothetical protein	0.6
A1S_2365	xanthine dehydrogenase small subunit	0.6
A1S_1346	phenylacetyl-CoA ligase	0.6
A1S_1598	hypothetical protein	0.6
A1S_2086	putative short chain dehydrogenase	0.6
A1S_1625	putative adenylate or guanylate cyclase	0.6
A1S_1955	hypothetical protein	0.6
A1S_3264	putative transcriptional regulator	0.6
A1S_2120	16S rRNA pseudouridylate 516 synthase	0.6

Locus-tag	**Gene product**	**Differential expression** (Log_2)
A1S_2146	molybdopterin biosynthesis protein	0.6
A1S_2986	hypothetical protein	0.6
A1S_1759	short-chain dehydrogenase/reductase SDR	0.6
A1S_0709	putative cation efflux system protein	0.6
A1S_1691	hypothetical protein	0.6
A1S_2907	hypothetical protein	0.6
A1S_1480	hypothetical protein	0.6
A1S_1238	hypothetical protein	0.5
A1S_2002	nitrate reductase large subunit	0.5
A1S_2087	putative glutathione S-transferase	0.5

Appendix B – Genes significantly down-regulated in *A. baumannii* strain ATCC 17978 under iron-limiting conditions

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2297	putative 4Fe-4S ferredoxin	-4.8
A1S_1925	cytochrome d terminal oxidase polypeptide subunit II	-4.6
A1S_1924	cytochrome d terminal oxidase polypeptide subunit I	-4.6
A1S_2296	putative protease	-4.4
A1S_0292	putative outer membrane protein W	-4.0
A1S_1926	hypothetical protein	-4.0
A1S_0891	hemerythrin-like metal-binding protein	-3.7
A1S_0480	fumarate hydratase	-3.2
A1S_3108	coproporphyrinogen III oxidase (KEGG; EC:1.3.3.3)	-3.0
A1S_3219	putative RND family drug transporter	-3.0
A1S_1510	fimbrial protein	-2.6
A1S_3218	EsvF1	-2.6
A1S_3273	putative peptide signal	-2.5
A1S_3402	arginase/agmatinase/formimionoglutamate hydrolase (KEGG; EC:3.5.3.8)	-2.4
A1S_3207	sulfate transport protein	-2.1
A1S_1442	taurine ABC transporter periplasmic taurine-binding protein	-2.1
A1S_3403	imidazolonepropionase (KEGG; EC:3.5.2.7)	-2.0
A1S_0371	hypothetical protein	-2.0
A1S_1443	taurine ATP-binding transport system component	-1.9
A1S_3038	hypothetical protein	-1.9
A1S_2426	lactoylglutathione lyase (KEGG; EC:4.4.1.5)	-1.9
A1S_0157	hypothetical protein	-1.9
A1S_0921	arginine/ornithine antiporter	-1.9
A1S_1509	pili assembly chaperone	-1.9
A1S_2970	hypothetical protein	-1.8
A1S_0099	D-serine/D-alanine/glycine transport protein	-1.8
A1S_3405	histidine ammonia-lyase (KEGG; EC:4.3.1.3)	-1.8
A1S_3407	urocanase (KEGG; EC:4.2.1.49)	-1.8
A1S_3406	urocanate hydratase	-1.8
A1S_0302	hypothetical protein	-1.8
A1S_0781	putative MTA/SAH nucleosidase	-1.7
A1S_0922	putative homocysteine S-methyltransferase family protein	-1.7
A1S_2531	sulfate transport protein	-1.7
A1S_3252	putative DNA/RNA non-specific endonuclease G protein	-1.7
A1S_1147	site-specific DNA methylase-like protein	-1.7
A1S_2959	Hsp 24 nucleotide exchange factor	-1.7
A1S_1397	ArtM protein	-1.7
A1S_3404	proline transport protein (APC family)	-1.7
A1S_2812	methyl-accepting chemotaxis protein	-1.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0809	dethiobiotin synthetase x	-1.6
A1S_3297	putative outer membrane protein	-1.6
A1S_2919	hypothetical protein	-1.6
A1S_2911	uncharacterised membrane protein LemA family	-1.6
A1S_0378	EsvG	-1.6
A1S_3194	putative membrane protein (ComN)	-1.6
A1S_3418	4-hydroxyphenylpyruvate dioxygenase (KEGG; EC:1.13.11.27)	-1.6
A1S_3410	putative acyltransferase	-1.6
A1S_1396	ABC-type amino acid transport system	-1.6
A1S_3355	hypothetical protein	-1.6
A1S_0525	hypothetical protein	-1.6
A1S_3085	putative flavohemoprotein	-1.5
A1S_3175	bacterioferritin	-1.5
A1S_0114	acyl carrier protein	-1.5
A1S_0094	lrp regulon transcriptional regulator (AsnC family)	-1.5
A1S_3455	dihydroxy-acid dehydratase (KEGG; EC:4.2.1.9)	-1.5
A1S_1444	ABC taurine transporter permease subunit	-1.5
A1S_3443	heat shock protein Hsp40	-1.5
A1S_2665	chaperone Hsp10	-1.5
A1S_0167	hypothetical protein	-1.5
A1S_1053	hypothetical protein	-1.5
A1S_0087	short-chain dehydrogenase/reductase SDR	-1.5
A1S_2532	sulfate transport protein	-1.4
A1S_3047	oligopeptidase A	-1.4
A1S_0105	putative acyl-CoA dehydrogenase (KEGG; EC:1.3.99.2)	-1.4
A1S_0115	amino acid adenylation	-1.4
A1S_3192	putative lipoprotein (ComL)	-1.4
A1S_0526	hypothetical protein	-1.4
A1S_3023	hypothetical protein	-1.4
A1S_1116	vanillate O-demethylase oxygenase subunit (KEGG; EC:1.14.13.82)	-1.4
A1S_3128	succinylglutamate desuccinylase	-1.4
A1S_0028	FMNH(2)-dependent alkanesulfonate monooxygenase (KEGG; EC:1.14.14.5)	-1.4
A1S_1800	putative RND family drug transporter	-1.3
A1S_1905	24-dienoyl-CoA reductase (KEGG; EC:1.3.1.34)	-1.3
A1S_1799	putative MFS transport protein	-1.3
A1S_0425	hypothetical protein	-1.3
A1S_0113	acyl-CoA dehydrogenase	-1.3
A1S_3046	oligopeptidase A	-1.3
A1S_3255	putative transcriptional regulator AraC/XylS family protein	-1.3
A1S_0753	NADH dehydrogenase I chain B (KEGG; EC:1.6.5.3)	-1.3
A1S_2960	chaperone Hsp70	-1.3

Locus-tag	Gene product	Differential expression (Log_2)
A1S_3193	putative membrane protein (ComO)	-1.3
A1S_3421	putative transporter; putative sodium/bile acid transporter family protein	-1.3
A1S_1032	hypothetical protein	-1.3
A1S_3382	catalase (KEGG; EC:1.11.1.6)	-1.3
A1S_3130	succinylglutamic semialdehyde dehydrogenase	-1.3
A1S_1279	hypothetical protein	-1.3
A1S_2815	twitching motility protein	-1.3
A1S_2753	putative protein (DcaP-like)	-1.3
A1S_0029	ABC-type nitrate/sulfonate/bicarbonate transport systems	-1.3
A1S_3351	putative deacetylase	-1.3
A1S_1092	succinylornithine transaminase (carbon starvation protein C)	-1.3
A1S_0524	hypothetical protein (KEGG; EC:1.1.1.100)	-1.3
A1S_1674	hypothetical protein	-1.3
A1S_1106	hypothetical protein	-1.3
A1S_1121	lipase/esterase	-1.3
A1S_2708	hypothetical protein	-1.3
A1S_2968	hypothetical protein	-1.2
A1S_0737	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	-1.2
A1S_0974	hypothetical protein	-1.2
A1S_0106	putative enoyl-CoA hydratase/isomerase	-1.2
A1S_1367	hypothetical protein	-1.2
A1S_1108	acyl coenzyme A dehydrogenase	-1.2
A1S_0995	rubredoxin	-1.2
A1S_1033	putative antigen	-1.2
A1S_0365	putative amino-acid efflux transmembrane protein	-1.2
A1S_3129	succinylarginine dihydrolase	-1.2
A1S_1104	chlorogenate esterase	-1.2
A1S_3323	putative flavoprotein	-1.2
A1S_0936	hypothetical protein	-1.2
A1S_1338	hypothetical protein	-1.2
A1S_2533	putative esterase	-1.2
A1S_2793	putative amino-acid transport protein	-1.2
A1S_2713	succinate dehydrogenase flavoprotein subunit (KEGG; EC:1.3.5.1)	-1.2
A1S_3146	multidrug efflux transport protein	-1.2
A1S_2944	hypothetical protein	-1.2
A1S_3361	hypothetical protein	-1.2
A1S_1399	ArtI protein	-1.2
A1S_0386	putative methyltransferase	-1.1
A1S_3127	putative signal peptide	-1.1
A1S_1801	putative RND family drug transporter	-1.1

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2221	sodium/glutamate symport carrier protein	-1.1
A1S_0473	iron uptake factor	-1.1
A1S_3363	membrane metalloendopeptidases proteins	-1.1
A1S_2343	superoxide dismutase (KEGG; EC:1.15.1.1)	-1.1
A1S_0067	L-lactate permease	-1.1
A1S_0109	homoserine lactone synthase	-1.1
A1S_2198	putative multidrug resistance protein	-1.1
A1S_2334	S-adenosyl-L-homocysteine hydrolase (KEGG; EC:3.3.1.1)	-1.1
A1S_0289	hypothetical protein	-1.1
A1S_3217	RND divalent metal cation efflux transporter	-1.1
A1S_2790	microcin B17 transport protein	-1.1
A1S_0727	putative substrate-binding protein	-1.1
A1S_3419	hypothetical protein	-1.1
A1S_3211	putative APC family S-methylmethionine transporter (MmuP)	-1.1
A1S_2128	aconitate hydratase 2	-1.1
A1S_1678	putative histidine triad family protein	-1.1
A1S_2535	putative sulfate permease	-1.1
A1S_1715	(Acyl-carrier protein) phosphodiesterase (KEGG; EC:3.1.4.14)	-1.1
A1S_0705	D-ribulose-5-phosphate 3-epimerase (KEGG; EC:5.1.3.1)	-1.1
A1S_3342	putative arsenate reductase	-1.1
A1S_1079	dichlorophenol hydroxylase (KEGG; EC:1.14.13.20)	-1.1
A1S_2305	cation/multidrug efflux pump	-1.1
A1S_0030	alkanesulfonate transport protein	-1.1
A1S_2304	putative RND family drug transporter	-1.1
A1S_0460	prolipoprotein diacylglyceryl transferase	-1.1
A1S_2977	cation diffusion facilitator family transporter	-1.1
A1S_3305	NADH-dependent FMN reductase	-1.1
A1S_0112	acyl-CoA synthetase/AMP-acid ligases II	-1.1
A1S_1094	D-serine/D-alanine/glycine transporter	-1.1
A1S_0310	EsvL	-1.1
A1S_0520	putative oxidoreductase protein; putative dehydrogenase (flavoprotein)	-1.1
A1S_0735	transcriptional regulator (LysR family)	-1.1
A1S_1947	phosphotransferase system fructose-specific IIBC component	-1.1
A1S_0318	putative FusE-MFP/HlyD membrane fusion protein	-1.1
A1S_0742	iron-regulated protein	-1.1
A1S_2670	glycerol kinase (KEGG; EC:2.7.1.30)	-1.1
A1S_0534	NADH-dependent enoyl-ACP reductase (KEGG; EC:1.3.1.9)	-1.1
A1S_0107	putative enoyl-CoA hydratase/isomerase family protein	-1.1
A1S_1781	putative ribose-phosphate pyrophosphokinase	-1.1
A1S_2534	sulfate transport protein	-1.1
A1S_1232	EsvB	-1.1
A1S_1398	GlnQ protein	-1.1

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2799	hypothetical protein	-1.1
A1S_3414	fumarylacetoacetase (KEGG; EC:3.7.1.2)	-1.1
A1S_0975	hypothetical protein	-1.1
A1S_0800	bacterioferritin	-1.1
A1S_3240	hypothetical protein	-1.1
A1S_3053	acyl coenzyme A dehydrogenase (KEGG; EC:1.3.99.13)	-1.1
A1S_1445	taurine dioxygenase (KEGG; EC:1.14.11.17)	-1.1
A1S_2526	L-aspartate oxidase (KEGG; EC:1.4.3.16)	-1.1
A1S_3134	glutamate dehydrogenase ($NAD(P)^+$) oxidoreductase protein (KEGG; EC:1.4.1.3)	-1.1
A1S_2470	putative protease	-1.1
A1S_3257	hypothetical protein Patl_1499	-1.1
A1S_1336	hypothetical protein	-1.1
A1S_0161	putative MFS transport protein	-1.1
A1S_0738	putative flavoprotein oxidoreductase	-1.1
A1S_0395	Na^+-driven multidrug efflux pump	-1.1
A1S_1337	phenylacetic acid degradation B	-1.1
A1S_2748	putative ammonium transporter	-1.1
A1S_3176	hypothetical protein	-1.1
A1S_2814	twitching motility protein	-1.0
A1S_2825	putative thiol:disulphide interchange protein (DsbC-like)	-1.0
A1S_1400	putative ABC transporter	-1.0
A1S_1151	hypothetical protein	-1.0
A1S_2124	putative MFS transport protein	-1.0
A1S_3123	putative transcriptional regulator	-1.0
A1S_2775	3'-phosphoadenylylsulfate reductase	-1.0
A1S_3132	succinylornithine transaminase	-1.0
A1S_0233	type 4 fimbriae expression regulatory protein	-1.0
A1S_3048	hypothetical protein	-1.0
A1S_0728	citrate-proton symporter (MFS)	-1.0
A1S_0962	putative glutaminase	-1.0
A1S_0886	deoxyuridine 5'-triphosphate nucleotidohydrolase	-1.0
A1S_2126	aconitate hydratase 2	-1.0
A1S_0925	choline dehydrogenase	-1.0
A1S_3238	phosphoribosylformimino-5-aminoimidazole carboxamide isomerase (KEGG; EC:5.3.1.16)	-1.0
A1S_0402	putative very-long-chain acyl-CoA synthetase	-1.0
A1S_3121	hypothetical protein	-1.0
A1S_2451	transcriptional regulator AsnC family	-1.0
A1S_0462	putative phosphatase	-1.0
A1S_2215	CsuC	-1.0
A1S_0585	3-methyl-2-oxobutanoate hydroxymethyltransferase	-1.0
A1S_0433	transport protein Uup	-1.0

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2966	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl- meso-diaminopimelate ligase	-1.0
A1S_0521	hypothetical protein	-1.0
A1S_0912	ribosomal protein L22	-1.0
A1S_2852	putative thiolase; putative acyl-CoA thiolase (KEGG; EC:2.3.1.9)	-1.0
A1S_3286	putative inner membrane protein	-1.0
A1S_2127	aconitate hydratase 2	-1.0
A1S_3413	APC family aromatic amino acid transporter	-1.0
A1S_3359	topoisomerase IV subunit B	-1.0
A1S_2433	survival protein (acid phosphatase)	-1.0
A1S_2537	putative LysR-type transcriptional regulator	-1.0
A1S_0537	putative RND family drug transporter	-1.0
A1S_1985	hypothetical protein	-1.0
A1S_0046	putative virulence factor MviN family	-1.0
A1S_0166	putative Rossmann-fold nucleotide-binding DNA uptake protein (Smf)	-1.0
A1S_0135	hypothetical protein	-1.0
A1S_2251	amidophosphoribosyltransferase (KEGG; EC:2.4.2.14)	-1.0
A1S_0915	putative MFS transport protein	-1.0
A1S_0586	3-methyl-2-oxobutanoate hydroxymethyltransferase	-1.0
A1S_1764	putative transcriptional regulator	-1.0
A1S_2880	putative signal peptide	-1.0
A1S_3195	putative membrane protein ComM	-1.0
A1S_1105	hypothetical protein	-1.0
A1S_0048	FKBP-type peptidyl-prolyl cis-trans isomerase (rotamase)	-1.0
A1S_2791	threonine dehydratase	-1.0
A1S_0618	hypothetical protein	-1.0
A1S_2290	putative secretion pathway ATPase	-1.0
A1S_0984	putative carbonic anhydrase	-1.0
A1S_3004	hypothetical protein	-0.9
A1S_2611	transport protein of outer membrane lipoproteins	-0.9
A1S_3227	putative RNA binding protein	-0.9
A1S_1146	site-specific DNA-methyltransferase	-0.9
A1S_0916	dihydrodipicolinate synthetase	-0.9
A1S_2472	putative transport protein	-0.9
A1S_2821	putative alkylphosphonate uptake protein (PhnA) in phosphonate metabolism	-0.9
A1S_3372	putative short-chain dehydrogenase	-0.9
A1S_0523	putative 3-hydroxylacyl-(acyl carrier protein) dehydratase	-0.9
A1S_2427	putative transporter	-0.9
A1S_0616	secretion protein XcpR	-0.9
A1S_0038	putative transcriptional regulator	-0.9

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0367	glutathione-regulated potassium-efflux system protein (K^+/H^+ antiporter)	-0.9
A1S_2714	succinate dehydrogenase iron-sulfur subunit (KEGG; EC:1.3.5.1)	-0.9
A1S_0522	hypothetical protein	-0.9
A1S_0042	ribonuclease PH (KEGG; EC:2.7.7.56)	-0.9
A1S_2789	putative metallopeptidase	-0.9
A1S_0568	pyridine nucleotide transhydrogenase beta subunit	-0.9
A1S_0164	hypothetical protein	-0.9
A1S_1765	hypothetical protein	-0.9
A1S_0007	putative transport protein	-0.9
A1S_1717	transcriptional regulator GntR family	-0.9
A1S_1334	iron-sulfur-dependent L-serine dehydratase single chain form	-0.9
A1S_0291	hypothetical protein	-0.9
A1S_2586	deoxyguanosinetriphosphate triphosphohydrolase	-0.9
A1S_1233	hypothetical protein	-0.9
A1S_3270	putative permease	-0.9
A1S_0756	NADH dehydrogenase I chain F (KEGG; EC:1.6.99.5)	-0.9
A1S_1072	hypothetical protein	-0.9
A1S_2836	putative transporter	-0.9
A1S_2664	chaperone Hsp60	-0.9
A1S_1828	putative transcriptional regulator MerR family	-0.9
A1S_2441	adenylosuccinate lyase	-0.9
A1S_3109	dehydroshikimate reductase NAD(P)-binding	-0.9
A1S_0587	pantoate--beta-alanine ligase (KEGG; EC:6.3.2.1)	-0.9
A1S_0519	hypothetical protein	-0.9
A1S_0027	alkanesulfonate transport protein	-0.9
A1S_0059	putative glycosyltransferase	-0.9
A1S_3386	phosphoserine phosphatase	-0.9
A1S_2979	tRNA modification GTPase	-0.9
A1S_1441	putative signal peptide	-0.9
A1S_1415	transcriptional regulator	-0.9
A1S_3354	xanthine/uracil permease family	-0.9
A1S_0069	L-lactate dehydrogenase FMN linked (KEGG; EC:1.1.2.3)	-0.9
A1S_3451	putative uracil transport protein (NCS2 family)	-0.9
A1S_3256	enhancing lycopene biosynthesis protein 2	-0.9
A1S_0429	glutamate:aspartate symport protein (DAACS family)	-0.9
A1S_3200	phospho-N-acetylmuramoyl-pentapeptide transferase	-0.9
A1S_2701	putative amino acid transport protein (APC family)	-0.9
A1S_1339	phenylacetate-CoA oxygenase PaaJ subunit	-0.9
A1S_1145	putative Cro protein	-0.9
A1S_3243	putative cold shock protein	-0.9
A1S_0134	pirin-related protein	-0.9
A1S_2295	putative transcriptional regulator	-0.9

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2612	transport protein of outer membrane lipoproteins	-0.9
A1S_0755	NADH dehydrogenase I chain E	-0.9
A1S_3447	putative RND family drug transporter	-0.9
A1S_1449	transcriptional regulatory protein	-0.9
A1S_1452	arsenate reductase	-0.9
A1S_2643	oxidoreductase short chain dehydrogenase/reductase family	-0.9
A1S_1115	vanillate O-demethylase oxidoreductase	-0.9
A1S_2008	DNA repair protein	-0.9
A1S_3381	AnkB protein	-0.9
A1S_0536	macrolide transport protein	-0.9
A1S_3037	putative ribonuclease (Rbn)	-0.9
A1S_0535	putative RND family drug transporter	-0.9
A1S_2068	putative benzoate membrane transport protein	-0.9
A1S_0518	hypothetical protein	-0.9
A1S_1005	putative hemolysin-related protein	-0.9
A1S_3440	putative MFS transport protein	-0.9
A1S_0799	DNA ligase (KEGG; EC:6.5.1.2)	-0.9
A1S_0461	hypothetical protein	-0.9
A1S_1836	hypothetical protein	-0.9
A1S_1093	arginine/ornithine N-succinyltransferase beta subunit	-0.9
A1S_3233	hypothetical protein	-0.9
A1S_1898	enolase (KEGG; EC:4.2.1.11)	-0.9
A1S_3244	putative homoserine kinase (ThrB)	-0.9
A1S_3311	hypothetical protein	-0.9
A1S_1139	putative signal peptide	-0.9
A1S_1091	succinylornithine transaminase (carbon starvation protein C)	-0.8
A1S_1796	aldehyde dehydrogenase	-0.8
A1S_2353	dipeptide/tripeptide permease	-0.8
A1S_0181	transcriptional regulator SoxR-family	-0.8
A1S_0324	putative tRNA/rRNA methyltransferase	-0.8
A1S_0110	hypothetical protein	-0.8
A1S_3411	putative GTPases (G3E family)	-0.8
A1S_0323	hypothetical protein	-0.8
A1S_2370	uroporphyrinogen decarboxylase (KEGG; EC:4.1.1.37)	-0.8
A1S_1946	1-phosphofructokinase	-0.8
A1S_1538	transporter sodium-dicarboxylate symporter family	-0.8
A1S_0567	pyridine nucleotide transhydrogenase (proton pump) alpha subunit (part2)	-0.8
A1S_2525	putative serine protease	-0.8
A1S_0725	hypothetical protein	-0.8
A1S_2429	putative ATP-dependent protease	-0.8
A1S_2917	hypothetical protein	-0.8
A1S_2688	transcription elongation factor	-0.8

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0351	hypothetical protein	-0.8
A1S_1536	putative nucleoprotein/polynucleotide-associated enzyme	-0.8
A1S_3054	putative monooxygenase flavin-binding family	-0.8
A1S_3433	putative peptidase	-0.8
A1S_0293	putative signal peptide	-0.8
A1S_2588	holliday junction helicase subunit B	-0.8
A1S_0398	putative short-chain dehydrogenase	-0.8
A1S_0245	UDP-N-acetylmuramoylalanine-D-glutamate ligase	-0.8
A1S_0538	putative RND family drug transporter	-0.8
A1S_0097	hypothetical protein	-0.8
A1S_1511	biotin synthase (KEGG; EC:2.8.1.6)	-0.8
A1S_1665	hypothetical protein	-0.8
A1S_2428	putative ATP-dependent protease	-0.8
A1S_1679	putative signal peptide	-0.8
A1S_3289	putative integral membrane protein	-0.8
A1S_3434	hypothetical protein	-0.8
A1S_2712	succinate dehydrogenase hydrophobic subunit	-0.8
A1S_0096	alanine racemase 2 PLP-binding, catabolic	-0.8
A1S_1045	Co/Zn/Cd efflux system	-0.8
A1S_0946	hypothetical protein	-0.8
A1S_0219	ammonium transport protein (Amt family)	-0.8
A1S_0766	putative cold shock protein	-0.8
A1S_2298	SMR family efflux pump	-0.8
A1S_0390	putative type III effector	-0.8
A1S_2550	Tn7 transposase A	-0.8
A1S_0794	hypothetical protein	-0.8
A1S_1175	phage integrase	-0.8
A1S_3399	VirP	-0.8
A1S_0584	2-amino-4-hydroxy-6- hydroxymethyldihydropteridine pyrophosphokinase	-0.8
A1S_3135	putative APC family S-methylmethionine transporter (MmuP)	-0.8
A1S_0329	type 4 fimbrial biogenesis protein	-0.8
A1S_1454	transmembrane arsenate pump protein	-0.8
A1S_2817	NolF secretion protein	-0.8
A1S_0321	recombination and DNA repair protein	-0.8
A1S_3156	hypothetical protein	-0.8
A1S_3011	putative transcriptional regulator	-0.8
A1S_1161	hypothetical protein	-0.8
A1S_0825	aspartate 1-decarboxylase precursor	-0.8
A1S_2471	putative epimerase	-0.8
A1S_0531	putative GTPase	-0.8
A1S_0483	phosphogluconate dehydratase (KEGG; EC:4.2.1.12)	-0.8
A1S_1582	transcriptional regulator Cro/CI family	-0.8

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0066	hypothetical protein	-0.8
A1S_1062	putative FMN oxidoreductase	-0.8
A1S_0494	putative glycosyl transferase	-0.8
A1S_0235	sensor protein	-0.8
A1S_1795	dihydroxy-acid dehydratase	-0.8
A1S_3439	aldehyde reductase (KEGG; EC:1.1.1.274)	-0.8
A1S_3254	potassium transport system low affinity (KUP family)	-0.8
A1S_3133	bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase protein	-0.8
A1S_1280	transcriptional regulator DUF24 family	-0.8
A1S_3198	23S ribosomal RNA G745 methyltransferase	-0.8
A1S_1084	glycine/D-amino acid oxidases (deaminating)	-0.8
A1S_0270	putative general secretion pathway protein	-0.8
A1S_0566	pyridine nucleotide transhydrogenase (proton pump) alpha subunit (part1)	-0.8
A1S_1050	putative signal peptide	-0.8
A1S_2528	thymidylate kinase	-0.8
A1S_0290	hypothetical protein	-0.8
A1S_0485	high-affinity gluconate permease (GntP family)	-0.8
A1S_3157	hypothetical protein	-0.8
A1S_2294	hypothetical protein	-0.8
A1S_0180	hypothetical protein	-0.8
A1S_2613	transport protein of outer membrane lipoproteins	-0.8
A1S_3460	putative glutathione S-transferase	-0.8
A1S_0793	putative transcriptional regulator	-0.8
A1S_1137	adenosine deaminase	-0.8
A1S_3110	hypothetical protein	-0.8
A1S_0060	hypothetical protein	-0.8
A1S_3268	hypothetical protein	-0.7
A1S_3290	EsvF2	-0.7
A1S_1518	putative suppressor of F exclusion of phage T7	-0.7
A1S_1418	putative MFS transport protein	-0.7
A1S_3422	hypothetical protein	-0.7
A1S_2645	hypothetical repressor protein	-0.7
A1S_0492	beta-N-acetyl-D-glucosaminidase (KEGG; EC:3.2.1.52)	-0.7
A1S_1945	phosphotransferase system fructose-specific EI/HPr/EIIA components	-0.7
A1S_0393	putative acyl-CoA dehydrogenase	-0.7
A1S_1537	hypothetical protein	-0.7
A1S_1416	negative transcriptional regulator	-0.7
A1S_1140	RNAse HII (KEGG; EC:3.1.26.4)	-0.7
A1S_3131	arginine succinyltransferase (KEGG; EC:2.3.1.109)	-0.7
A1S_0163	hypothetical protein	-0.7
A1S_2705	hypothetical protein	-0.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2347	hypothetical protein	-0.7
A1S_0129	hypothetical protein	-0.7
A1S_0855	dioxygenase beta subunit	-0.7
A1S_1551	chromosome partitioning protein	-0.7
A1S_1042	hypothetical protein	-0.7
A1S_0968	putative phthalate transporter	-0.7
A1S_3398	glutamate racemase	-0.7
A1S_0682	RNA polymerase sigma-54 factor	-0.7
A1S_3450	putative uracil transport protein (NCS2 family)	-0.7
A1S_0049	protein tyrosine kinase	-0.7
A1S_1247	putative O-methyltransferase protein	-0.7
A1S_1191	aspartate carbamoyltransferase non-catalytic chain	-0.7
A1S_1117	vanillate transporter (MFS)	-0.7
A1S_0752	NADH dehydrogenase I chain A	-0.7
A1S_3446	putative RND family drug transporter	-0.7
A1S_0316	putative transcriptional regulator	-0.7
A1S_0014	tyrosyl-tRNA synthetase	-0.7
A1S_1068	argininosuccinate synthetase	-0.7
A1S_1136	putative transporter	-0.7
A1S_0897	twitching motility protein	-0.7
A1S_0611	putative integral membrane resistance protein	-0.7
A1S_0767	excalibur	-0.7
A1S_0320	hypothetical protein	-0.7
A1S_2648	hypothetical protein	-0.7
A1S_2729	outer-membrane lipoproteins carrier protein	-0.7
A1S_3306	putative sulfonate monooxygenase (MsuD)	-0.7
A1S_1419	anti-sigm factor ChrR	-0.7
A1S_3214	cation efflux system protein	-0.7
A1S_0896	twitching motility protein	-0.7
A1S_0373	apolipoprotein N-acyltransferase copper homeostasis protein	-0.7
A1S_3383	D-tyrosyl tRNA(tyr) deacylase	-0.7
A1S_2897	UDP-N-acetylglucosamine 2-epimerase (KEGG; EC:5.1.3.14)	-0.7
A1S_3095	ATP-dependent DNA helicase	-0.7
A1S_2946	hypothetical protein	-0.7
A1S_0608	acetyl-coenzyme A carboxylase carboxyl transferase (alpha subunit) (KEGG; EC:6.4.1.2)	-0.7
A1S_0994	rubredoxin reductase	-0.7
A1S_3136	hypothetical protein	-0.7
A1S_0941	enoyl-CoA hydratase/isomerase	-0.7
A1S_1015	urease accessory protein	-0.7
A1S_2430	putative ATP-dependent protease	-0.7
A1S_0348	2-octaprenylphenol hydroxylase of ubiquinone biosynthetic pathway	-0.7
A1S_1964	putative signal peptide	-0.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2134	putative ubiquinone biosynthesis protein	-0.7
A1S_2869	acetylCoA carboxylase beta subunit	-0.7
A1S_2974	hypoxanthine phosphoribosyltransferase	-0.7
A1S_1371	transcriptional regulator LysR family	-0.7
A1S_0200	inorganic pyrophosphatase (KEGG; EC:3.6.1.1)	-0.7
A1S_2516	pyridoxal phosphate biosynthetic protein	-0.7
A1S_1993	regulatory protein GntR HTH	-0.7
A1S_3269	hypothetical protein	-0.7
A1S_0073	putative carboxyphosphonoenolpyruvate phosphonomutase or putative methylisocitrate lyase (PrpB) (KEGG; EC:4.1.3.30)	-0.7
A1S_3187	3-dehydroquinate synthase (KEGG; EC:4.2.3.4)	-0.7
A1S_0068	L-lactate utilization transcriptional repressor (GntR family)	-0.7
A1S_0614	putative small conductance mechanosensitive ion channel	-0.7
A1S_3457	16S rRNA m5C967 SAM-dependent methyltransferase	-0.7
A1S_1078	hypothetical protein	-0.7
A1S_0024	putative MTA/SAH nucleosidase	-0.7
A1S_1466	glutaminase-asparaginase (KEGG; EC:3.5.1.38)	-0.7
A1S_0399	putative transcriptional regulator (LysR family)	-0.7
A1S_0973	D-serine deaminase (dehydratase) (EC:4.3.1.18)	-0.7
A1S_0713	quinolinate synthetase A	-0.7
A1S_0569	dehydrogenase/reductase	-0.7
A1S_1001	ATP-sulfurylase subunit 1 (KEGG; EC:2.7.7.4)	-0.7
A1S_2813	twitching motility protein	-0.7
A1S_0189	hypothetical protein	-0.7
A1S_0555	hypothetical protein	-0.7
A1S_0026	alkanesulfonate transport protein	-0.7
A1S_0927	NAD^+-dependent betaine aldehyde dehydrogenase (KEGG; EC:1.2.1.8)	-0.7
A1S_0486	thermoresistant gluconokinase	-0.7
A1S_0754	NADH dehydrogenase I chain CD	-0.7
A1S_0262	porphobilinogen deaminase	-0.7
A1S_1076	transcription regulator LysR family	-0.7
A1S_0100	putative transcriptional regulator (LysR family)	-0.7
A1S_3285	hypothetical protein	-0.7
A1S_1417	putative amino-acid acetyltransferase	-0.7
A1S_3221	putative transport protein	-0.7
A1S_2515	putative tRNA hydroxylase	-0.7
A1S_2702	putative alcohol dehydrogenase	-0.7
A1S_2658	heat shock protein	-0.7
A1S_0308	beta-hydroxylase	-0.7
A1S_1070	24-dienoyl-CoA reductase (KEGG; EC:1.3.1.34)	-0.7
A1S_1904	hypothetical protein	-0.7
A1S_0795	hypothetical protein	-0.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0062	putative UTP-glucose-1-phosphate uridylyltransferase (KEGG; EC:2.7.7.9)	-0.7
A1S_1111	enoyl-CoA hydratase/lyase	-0.7
A1S_0440	hypothetical protein	-0.7
A1S_0849	tartrate dehydrogenase (KEGG; EC:1.1.1.93)	-0.7
A1S_0528	protein exporting molecular chaperone	-0.7
A1S_3201	phospho-N-acetylmuramoyl-pentapeptide transferase	-0.7
A1S_0232	type 4 fimbriae expression regulatory protein	-0.7
A1S_0383	hypothetical protein	-0.7
A1S_2630	putative LysE family transporter	-0.7
A1S_3314	xenobiotic reductase	-0.7
A1S_2971	putative vanillate O-demethylase oxygenase subunit	-0.7
A1S_0362	putative magnesium $Mg(2^+)$/cobalt $Co(2^+)$ transport protein	-0.7
A1S_2479	putative D-ala-D-ala-carboxypeptidase penicillin-binding protein	-0.7
A1S_2109	peptidyl-prolyl cis-trans isomerase precursor	-0.7
A1S_0747	ribonucleoside diphosphate reductase alpha subunit	-0.7
A1S_0080	beta-ketoacyl-ACP synthase I	-0.7
A1S_2176	alanine racemase 1	-0.7
A1S_2609	putative lipid A biosynthesis lauroyl acyltransferase	-0.7
A1S_2967	hypothetical protein	-0.7
A1S_2011	biotin carboxylase (A subunit of acetyl-CoA carboxylase) (KEGG; EC:6.3.4.14)	-0.6
A1S_2040	putative phage integrase	-0.6
A1S_3022	hypothetical protein	-0.6
A1S_0926	choline dehydrogenase	-0.6
A1S_2157	putative signal peptide	-0.6
A1S_1639	peptidyl-prolyl cis-trans isomerase precursor	-0.6
A1S_1974	ribosome releasing factor	-0.6
A1S_0515	histidine ammonia-lyase protein	-0.6
A1S_3319	pyridoxamine 5'-phosphate oxidase (KEGG; EC:1.4.3.5)	-0.6
A1S_1285	ABC transporter related (KEGG; EC:3.6.3.25)	-0.6
A1S_3276	hypothetical protein	-0.6
A1S_2866	hypothetical protein	-0.6
A1S_3111	putative acyl-CoA dehydrogenase	-0.6
A1S_0862	hypothetical protein	-0.6
A1S_2811	chemotactic signal transduction system component	-0.6
A1S_1496	hypothetical protein	-0.6
A1S_2738	hypothetical protein	-0.6
A1S_0424	putative L-asparaginase I (AnsA)	-0.6
A1S_0382	ferrochelatase	-0.6
A1S_1130	pirin-like:cupin 2 barrel	-0.6
A1S_1580	integrase	-0.6
A1S_0458	thymidylate synthase	-0.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2898	hypothetical protein	-0.6
A1S_2838	lysine-specific permease	-0.6
A1S_1225	peptidase S24 S26A and S26B	-0.6
A1S_0510	putative acyl carrier protein (ACP)	-0.6
A1S_0366	heat shock protein Hsp33	-0.6
A1S_2587	holliday junction helicase subunit A	-0.6
A1S_0949	putative dioxygenase	-0.6
A1S_0394	putative acyl-CoA dehydrogenase	-0.6
A1S_0191	hypothetical protein	-0.6
A1S_1148	hypothetical protein	-0.6
A1S_0961	peptide deformylase 2 (KEGG; EC:3.5.1.88)	-0.6
A1S_0698	putative anhydratase	-0.6
A1S_0517	hypothetical protein	-0.6
A1S_0596	putative transporter	-0.6
A1S_0272	hypothetical protein	-0.6
A1S_3435	3-methyl-adenine DNA glycosylase I (KEGG; EC:3.2.2.20)	-0.6
A1S_2010	biotin carboxyl carrier protein of acetyl-CoA carboxylase (BCCP)	-0.6
A1S_0863	beta-ketoacyl-ACP synthase I	-0.6
A1S_2342	hypothetical protein	-0.6
A1S_0723	L-carnitine dehydrogenase	-0.6
A1S_1860	ring hydroxylating dioxygenase Rieske (2Fe-2S) protein	-0.6
A1S_3456	methionyl-tRNA formyltransferase (KEGG; EC:2.1.2.9)	-0.6
A1S_2361	hypothetical protein	-0.6
A1S_0047	FKBP-type 22KD peptidyl-prolyl cis-trans isomerase (rotamase)	-0.6
A1S_1103	putative transcriptional regulator (GntR family)	-0.6
A1S_2912	queuine tRNA-ribosyltransferase (KEGG; EC:2.4.2.29)	-0.6
A1S_2265	thiamine biosynthesis protein thiazole moiety	-0.6
A1S_1124	transcriptional regulator AraC family	-0.6
A1S_1182	cyclic AMP receptor protein	-0.6
A1S_2956	esterase	-0.6
A1S_1980	ornithine carbamoyltransferase (KEGG; EC:2.1.3.3)	-0.6
A1S_2853	putative 3-hydroxybutyryl-CoA epimerase	-0.6
A1S_2862	preprotein translocase secretion protein of IISP family	-0.6
A1S_3448	putative transcriptional regulator	-0.6
A1S_0478	putative signal peptide	-0.6
A1S_2302	ABC lysine-arginine-ornithine transporter periplasmic ligand binding protein	-0.6
A1S_2419	elongation factor P	-0.6
A1S_2397	MoeA	-0.6
A1S_2703	hypothetical protein	-0.6
A1S_2197	putative transcriptional regulator protein TetR family	-0.6
A1S_2894	aspartyl-tRNA synthetase	-0.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0037	alkali-inducible disulfide interchange protein	-0.6
A1S_2069	putative magesium transporter transmembrane protein	-0.6
A1S_0359	putative beta-lactamase	-0.6
A1S_1192	aspartate carbamoyltransferase non-catalytic chain	-0.6
A1S_3271	putative transcriptional regulator (LysR family)	-0.6
A1S_2493	putative 2-nitropropane dioxygenase	-0.6
A1S_2819	prolyl-tRNA synthetase	-0.6
A1S_0908	putative RND family drug transporter	-0.6
A1S_2504	excinuclease ABC subunit B	-0.6
A1S_2963	phosphoribosylaminoimidazole carboxylase ATPase subunit	-0.6
A1S_0266	hypothetical protein	-0.6
A1S_0234	type 4 fimbriae expression regulatory protein	-0.6
A1S_1264	putative class A beta-lactamase	-0.6
A1S_3263	putative cytoplasmic protein	-0.6
A1S_0031	N-alpha-acetylglutamate synthase (KEGG; EC:2.3.1.1)	-0.6
A1S_0883	putative phospholipid/glycerol acyltransferase	-0.6
A1S_2432	lipoprotein precursor	-0.6
A1S_2973	guanine deaminase	-0.6
A1S_1226	hypothetical protein	-0.6
A1S_0322	hypothetical protein	-0.6
A1S_3033	hypothetical protein	-0.6
A1S_0924	choline dehydrogenase	-0.6
A1S_3459	hypothetical protein	-0.6
A1S_0512	hypothetical protein	-0.6
A1S_0343	pH adaptation potassium efflux system transmembrane protein	-0.6
A1S_3393	hypothetical protein	-0.6
A1S_0271	putative general secretion pathway protein	-0.6
A1S_1550	glucose-inhibited division protein B	-0.6
A1S_0475	trigger factor septum formation molecular chaperone	-0.6
A1S_0484	hypothetical protein	-0.6
A1S_0420	3-isopropylmalate dehydrogenase (KEGG; EC:1.1.1.85)	-0.6
A1S_2918	DNA repair protein	-0.6
A1S_1935	hypothetical protein	-0.6
A1S_0050	putative protein tyrosine phosphatase	-0.6
A1S_2770	fatty acid desaturase	-0.6
A1S_2915	preprotein translocase IISP family, membrane subunit	-0.6
A1S_3251	transporter LysE family	-0.6
A1S_2271	RtcB	-0.6
A1S_2536	putative ATPase	-0.6
A1S_1559	type 4 fimbrial biogenesis protein	-0.6
A1S_2468	hypothetical protein	-0.6
A1S_1281	TPR domain protein	-0.6
A1S_0439	DNA topoisomerase type I omega protein	-0.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1410	putative LysR-family transcriptional regulator	-0.6
A1S_2354	peptidase M24	-0.6
A1S_2990	putative acyltransferase	-0.6
A1S_1529	leucine-responsive regulatory protein	-0.6
A1S_3003	stringent starvation protein B	-0.6
A1S_2927	phage integrase	-0.6
A1S_0807	8-amino-7-oxononanoate synthase	-0.6
A1S_1335	phenylacetic acid degradation protein PaaN	-0.6
A1S_1357	alanine racemase	-0.6
A1S_3191	putative outer membrane protein (ComQ)	-0.6
A1S_0165	putative Sua5/YciO/YrdC/YwlC family protein	-0.6
A1S_2674	hypothetical protein (KEGG; EC:3.5.4.9)	-0.5
A1S_1095	aldehyde dehydrogenase (KEGG; EC:1.2.1.68)	-0.5
A1S_0493	carboxy-terminal protease	-0.5
A1S_2335	510-methylenetetrahydrofolate reductase	-0.5
A1S_3083	putative cystathionine beta-lyase PLP-dependent	-0.5
A1S_3202	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D- alanine ligase	-0.5
A1S_2340	HtrA-like serine protease	-0.5
A1S_1135	putative transporter	-0.5
A1S_0892	hypothetical protein	-0.5
A1S_0255	putative RND family drug transporter	-0.5
A1S_3032	TonB-like protein	-0.5
A1S_1440	putative MFS transport protein	-0.5
A1S_3432	A/G specific adenine glycosylase	-0.5
A1S_2482	deoxyribodipyrimidine photolyase (photoreactivation) FAD-binding	-0.5
A1S_2641	glycerate kinase	-0.5
A1S_0765	uracil phosphoribosyltransferase	-0.5
A1S_0951	putative ferredoxin reductase subunit of phenylpropionate dioxygenase	-0.5
A1S_0205	hypothetical protein	-0.5
A1S_2501	glyceraldehyde-3-phosphate dehydrogenase	-0.5
A1S_3416	glyoxalase/bleomycin resistance protein/dioxygenase	-0.5
A1S_1044	Co/Zn/Cd efflux system	-0.5
A1S_0239	homoserine dehydrogenase	-0.5
A1S_0931	high-affinity choline transporter (BCCT family)	-0.5
A1S_0053	MviM protein	-0.5
A1S_3099	putative toluene-tolerance protein (Ttg2E)	-0.5
A1S_0808	putative biotin biosynthesis protein (BioC)	-0.5
A1S_2412	hypothetical protein	-0.5
A1S_3288	putative MFS transport protein	-0.5
A1S_1420	regulatory protein LysR:LysR, substrate-binding	-0.5
A1S_2981	inner membrane protein (IMP) integration factor	-0.5
A1S_3357	allantoicase	-0.5

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2322	protein chain elongation factor EF-Ts	-0.5
A1S_0769	ferredoxin-NADP+ reductase	-0.5
A1S_2634	DNA repair protein	-0.5
A1S_2514	short-chain alcohol dehydrogenase of unknown specificity	-0.5
A1S_0806	adenosylmethionine-8-amino-7-oxononanoate aminotransferase (KEGG; EC:2.6.1.62)	-0.5
A1S_0527	putative holo-(acyl carrier protein) synthase 2	-0.5
A1S_3430	putative dienelactone hydrolase	-0.5
A1S_0116	putative RND family drug transporter	-0.5
A1S_2035	hypothetical protein	-0.5
A1S_1101	5-dehydro-4-deoxyglucarate dehydratase (KEGG; EC:4.2.1.41)	-0.5
A1S_0369	general secretion pathway protein F	-0.5
A1S_1544	fructose-16-bisphosphate aldolase, class II	-0.5
A1S_2620	putative RND family drug transporter	-0.5
A1S_2301	amino acid ABC transporter permease protein	-0.5
A1S_0052	WecC protein	-0.5
A1S_3390	transcription termination L factor	-0.5
A1S_1144	merops peptidase family S24	-0.5
A1S_0438	hypothetical protein	-0.5
A1S_3100	putative toluene tolerance protein (Ttg2D)	-0.5
A1S_0241	site-specific tyrosine recombinase	-0.5
A1S_2125	putative potassium channel VIC family	-0.5
A1S_2832	peptidyl-prolyl cis-trans isomerase precursor	-0.5
A1S_1976	hypothetical protein	-0.5
A1S_0247	putative glutamyl t-RNA synthetase	-0.5
A1S_0051	putative outer membrane protein	-0.5
A1S_0706	putative hydrolase	-0.5
A1S_0190	hypothetical protein	-0.5
A1S_2511	phenylacetic acid degradation-related protein	-0.5
A1S_3212	hypothetical protein	-0.5
A1S_0257	hypothetical protein	-0.5
A1S_0070	D-lactate dehydrogenase NADH independent, FAD-binding domain (KEGG; EC:1.1.1.28)	-0.5
A1S_0265	hypothetical protein	-0.5
A1S_1940	methionine aminopeptidase (KEGG; EC:3.4.11.18)	-0.5
A1S_1389	DNA polymerase V component	-0.5
A1S_0615	hypothetical protein	-0.5
A1S_0117	hypothetical protein	-0.5
A1S_1102	2-ketoglutarate semialdehyde dehydrogenase (KEGG; EC:1.2.1.4)	-0.5
A1S_2945	hypothetical protein	-0.5
A1S_1028	hypothetical protein	-0.5
A1S_2508	aspartate aminotransferase A (KEGG; EC:2.6.1.1)	-0.5
A1S_3106	1-deoxyxylulose-5-phosphate synthase (KEGG; EC:2.2.1.7)	-0.5

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2980	hypothetical protein	-0.5
A1S_2900	putative lipopolysaccharide core biosynthesis glycosyl transferase LpsC	-0.5
A1S_3036	tryptophan repressor binding protein	-0.5
A1S_1662	hypothetical protein	-0.5
A1S_0349	hypothetical protein	-0.5
A1S_0851	putative lipase	-0.5
A1S_0446	putative transport protein (CPA2 family)	-0.5
A1S_0423	tRNA-pseudouridine synthase I	-0.5
A1S_1507	fimbrial protein	-0.5
A1S_2768	bacitracin resistance protein (KEGG; EC:2.7.1.66)	-0.5
A1S_0342	pH adaptation potassium efflux system C transmembrane protein	-0.5
A1S_0258	argininosuccinate lyase	-0.5
A1S_0989	monofunctional biosynthetic peptidoglycan transglycosylase	-0.5
A1S_0409	hypothetical protein	-0.5
A1S_3353	putative transthyretin-like protein precursor	-0.5
A1S_0130	GMP synthetase	-0.5
A1S_2856	putative esterase	-0.5
A1S_2303	transcriptional regulator LysR family	-0.5
A1S_1488	putative Acyl-CoA dehydrogenase	-0.5
A1S_2872	hypothetical protein	-0.5
A1S_1172	putative transposase	-0.5
A1S_1461	hypothetical protein	-0.5
A1S_3282	putative transcriptional regulator (GntR family)	-0.5
A1S_0327	type 4 prepilin-like proteins leader peptide processing enzyme (KEGG; EC:3.4.23.43)	-0.5
A1S_1194	hypothetical protein	-0.5
A1S_0848	transcriptional regulator	-0.5
A1S_0249	cyclic 3'5'-adenosine monophosphate phosphodiesterase	-0.5
A1S_2796	hypothetical protein	-0.5
A1S_2456	transcriptional regulator LysR family	-0.5
A1S_2637	erythronate-4-phosphate dehydrogenase	-0.5
A1S_1863	hypothetical protein	-0.5
A1S_1638	peptidyl-prolyl cis-trans isomerase precursor	-0.5
A1S_0913	hypothetical protein	-0.4
A1S_1918	putative potassium uptake protein (TrkH)	-0.4
A1S_0063	putative UDP-glucose 6-dehydrogenase	-0.4
A1S_2763	aromatic amino acid transporter (APC family)	-0.4
A1S_3452	putative transcriptional regulator	-0.4
A1S_3040	putative fumarylacetoacetate hydrolase family protein	-0.4
A1S_0864	beta-ketoacyl-ACP synthase I	-0.4
A1S_3387	hypothetical protein	-0.4
A1S_2901	putative polysaccharide deacetylase	-0.4

Appendix C – ATCC 17978 FUR binding sites

Locus-tag	p-value	Expression (Log_2)	Gene product
A1S_0416[a]	1.10E-10	2.1	putative transcriptional regulator (LysR family)
A1S_2382[a]	1.90E-10	7.4	BasD
A1S_2391[a]	7.00E-10	5.5	putative acinetobactin biosynthesis protein
A1S_2582[a]	7.80E-10	2.0	putative transcriptional regulator (AraC family)
A1S_2278[a]	8.70E-10	2.7	putative hydrolase of the alpha/beta superfamily
A1S_2372[a]	1.60E-09	6.9	putative acinetobactin biosynthesis protein
A1S_0242[a]	3.10E-09	3.2	putative ferrous iron transport protein A
A1S_3174[a]	3.40E-09	5.1	putative regulatory or redox component complexing with Bfr in iron storage and mobility (Bfd)
A1S_1647[a]	5.20E-09	6.9	putative siderophore biosynthesis protein
A1S_1657[a]	9.80E-09	6.1	putative siderophore biosynthesis protein; putative acetyltransferase
A1S_1667[a]	1.40E-08	4.3	putative ferric hydroxamate siderophore receptor
A1S_3339[a]	1.90E-08	4.1	putative ferric siderophore receptor protein
A1S_2566[a]	2.00E-08	6.6	putative thioesterase
A1S_2080[a]	2.50E-08	2.0	putative siderophore receptor
A1S_0980[a]	2.80E-08	3.6	ferric enterobactin receptor precursor
A1S_3324[a]	2.80E-08	2.7	putative ferric siderophore receptor protein
A1S_2392[a]	3.00E-08	6.7	putative acinetobactin utilization protein
A1S_2077[a]	3.90E-08	6.2	putative outer membrane porin receptor for Fe(III)-coprogen, Fe(III)-ferrioxamine B and Fe(III)-rhodotrulic acid uptake (FhuE)
A1S_2078	3.90E-08	0.9	predicted acetyltransferase
A1S_0474	4.10E-08	1.8	putative ferric siderophore receptor protein
A1S_2123[a]	8.60E-08	4.6	putative signal peptide
A1S_2667	8.60E-08	1.5	hypothetical protein
A1S_2581[a]	1.90E-07	7.2	isochorismate synthetase
A1S_1510	8.30E-07	-2.6	fimbrial protein
A1S_2650	1.20E-06	-0.2	GCN5-related N-acetyltransferase
A1S_1384	1.30E-06	0.9	CinA-like protein
A1S_1385	1.30E-06	1.8	hypothetical protein
A1S_1921	1.70E-06	0.9	ferrichrome-iron receptor
A1S_1922	1.70E-06	0.1	putative sugar kinase protein
A1S_0630	1.80E-06	1.0	hypothetical protein
A1S_0171[a]	2.80E-06	2.2	hypothetical protein
A1S_0371	3.50E-06	-2.0	hypothetical protein
A1S_0736	5.10E-06	-0.6	hypothetical protein
A1S_2271	5.10E-06	-0.6	RtcB protein
A1S_2567[a]	5.70E-06	6.6	putative ferric siderophore receptor protein
A1S_0634	6.10E-06	1.2	bacteriophage
A1S_0452[a]	6.50E-06	3.8	TonB family protein
A1S_2710	6.50E-06	0.6	hypothetical protein (KEGG; EC:2.3.3.1)

Locus-tag	p-value	Expression (Log_2)	Gene product
A1S_2711	6.50E-06	-0.5	succinate dehydrogenase cytochrome b556 subunit
A1S_2559	7.50E-06	1.0	cupin 4 transcription factor
A1S_2798[a]	7.50E-06	2.2	iron uptake factor
A1S_1896	8.00E-06	1.2	putative cell division protein (FtsB-like)
A1S_1897	8.00E-06	1.0	hypothetical protein
A1S_1607	8.50E-06	-0.1	TonB-dependent receptor
A1S_1608	8.50E-06	0.0	heme-binding protein A precursor
A1S_0799	8.80E-06	-0.9	DNA ligase (KEGG; EC:6.5.1.2)
A1S_0800	8.80E-06	-1.1	bacterioferritin
A1S_0858	1.00E-05	-0.1	putative glutamine-dependent $NAD(^+)$ synthetase $NAD(^+)$ synthase
A1S_0859	1.00E-05	0.3	putative glutamine-dependent $NAD(^+)$ synthetase $NAD(^+)$ synthase
A1S_1865	1.00E-05	-0.1	Glu-tRNA amidotransferase
A1S_1866	1.00E-05	-0.5	putative transcriptional repressor of for multidrug resistance pump (MarR family)
A1S_0944	1.10E-05	-0.2	putative transcriptional regulator (PcaU-like)
A1S_0945	1.10E-05	-0.3	putative ferredoxin
A1S_1488	1.10E-05	-0.5	putative acyl-CoA dehydrogenase
A1S_1489	1.10E-05	-0.3	putative glutathione S-transferase
A1S_3175	1.10E-05	-1.5	bacterioferritin
A1S_0110	1.20E-05	-0.8	hypothetical protein
A1S_0111	1.20E-05	-0.1	eR transcriptional regulator
A1S_1004	1.20E-05	-0.5	citrate transporter
A1S_1005	1.20E-05	-0.9	putative hemolysin-related protein
A1S_0812	1.40E-05	0.2	segregation and condensation protein A
A1S_2359	1.40E-05	0.3	anthranilate phosphoribosyltransferase (KEGG; EC:2.4.2.18)
A1S_0141	1.50E-05	-0.3	putative dyp-type peroxidase
A1S_0142	1.50E-05	0.5	homoserine/homoserine lactone efflux protein
A1S_3388	1.60E-05	-0.1	hypothetical protein
A1S_1027	2.00E-05	-0.5	putative signal peptide
A1S_2444	2.20E-05	0.0	putative protease (SohB)
A1S_1063	2.30E-05	2.4	TonB-dependent siderophore receptor
A1S_2475	2.50E-05	0.4	isocitrate dehydrogenase
A1S_2476	2.50E-05	-0.1	putative pseudouridine synthase
A1S_2157	2.60E-05	-0.6	putative signal peptide
A1S_0224	2.70E-05	-0.1	hypothetical protein
A1S_0938	2.70E-05	0.0	putative lipoprotein
A1S_2230	2.70E-05	1.9	hypothetical protein
A1S_1406	3.10E-05	-0.5	major membrane protein I (MMP-I)
A1S_1407	3.10E-05	-0.5	serine acetyltransferase
A1S_2357	3.90E-05	-0.3	putative TonB-dependent receptor
A1S_2358	3.90E-05	-0.2	putative TonB-dependent receptor

Locus-tag	p-value	Expression (Log_2)	Gene product
A1S_3231	4.10E-05	1.1	putative acetyl-CoA hydrolase/transferase (KEGG; EC:3.1.2.1)
A1S_3232	4.10E-05	0.6	putative acyltransferase
A1S_2286	6.20E-05	0.1	thiamine monophosphate synthase

[a] Examples of MAST hists used for iterative refinement of the scoring matrix of the *A. baumannii* ATCC 17978 FUR box motif as described in Section 3.2.3

Appendix D – Genes more than 2-fold up-regulated in *A. baumannii* strain 17978hm compared to strain ATCC 17978

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0114	acyl carrier protein	9.5
A1S_0115	amino acid adenylation	9.3
A1S_0112	acyl-CoA synthetase/AMP-acid ligases II	9.1
A1S_0113	acyl-CoA dehydrogenase	9.1
A1S_0116	putative RND family drug transporter	7.9
A1S_0117	hypothetical protein	7.7
A1S_1357	alanine racemase	6.1
A1S_0118	hypothetical protein	5.8
A1S_1292	putative signal peptide	5.6
A1S_0119	phosphopantethiene-protein transferase	5.6
A1S_0628	putative transposase	5.4
A1S_1293	hypothetical protein	5.2
A1S_1294	hypothetical protein	5.1
A1S_0109	homoserine lactone synthase	4.9
A1S_2554	putative transposase	4.8
A1S_0745	hypothetical protein	4.4
A1S_1256	putative transcriptional regulator	4.4
A1S_1508	fimbrial biogenesis outer membrane usher protein	4.2
A1S_1509	pili assembly chaperone	4.1
A1S_1439	putative coenzyme F420-dependent N5N10-methylene tetrahydromethanopterin reductase	4.1
A1S_1507	fimbrial protein	3.8
A1S_1295	hypothetical protein	3.8
A1S_1438	putative coenzyme F420-dependent N5N10-methylene tetrahydromethanopterin reductase	3.7
A1S_1510	fimbrial protein	3.6
A1S_1079	dichlorophenol hydroxylase (KEGG; EC:1.14.13.20)	3.4
A1S_1032	hypothetical protein	3.3
A1S_1751	AdeA membrane fusion protein	3.1
A1S_1033	putative antigen	3.0
A1S_0110	hypothetical protein	3.0
A1S_1699	acetoin:26-dichlorophenolindophenol oxidoreductase alpha subunit	2.9
A1S_1700	acetoin:26-dichlorophenolindophenol oxidoreductase beta subunit (KEGG; EC:1.2.4.1)	2.9
A1S_2649	putative regulatory protein	2.8
A1S_2648	hypothetical protein	2.8
A1S_1703	dihydrolipoamide dehydrogenase	2.7
A1S_1078	hypothetical protein	2.7
A1S_1702	dihydrolipoamide dehydrogenase	2.6
A1S_1081	putative transcriptional regulator	2.6
A1S_1406	major membrane protein I (MMP-I)	2.5
A1S_1297	hypothetical protein	2.5

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1405	putative cysteine desulfurase 1 (Csd)	2.5
A1S_0111	eR transcriptional regulator	2.5
A1S_1384	CinA-like protein	2.5
A1S_1701	dihydrolipoamide acetyltransferase	2.5
A1S_1770	hypothetical protein	2.4
A1S_1408	putative rhodanese-related sulfurtransferase	2.4
A1S_1769	putative RND family drug transporter	2.4
A1S_1272	putative transcriptional regulator	2.3
A1S_3273	putative peptide signal	2.3
A1S_1404	putative cysteine desulfurase 1 (Csd)	2.2
A1S_1304	hypothetical protein	2.2
A1S_1296	hypothetical protein	2.2
A1S_3104	putative ATP-dependent RNA helicase	2.2
A1S_0921	arginine/ornithine antiporter	2.2
A1S_1407	serine acetyltransferase	2.1
A1S_0922	putative homocysteine S-methyltransferase family protein	2.1
A1S_3120	hypothetical protein	2.1
A1S_1440	putative MFS transport protein	2.1
A1S_3146	multidrug efflux transport protein	2.0
A1S_2396	putative transcriptional regulator	2.0
A1S_2647	putative transcriptional regulator	2.0
A1S_0739	putative transcriptional regulator	2.0
A1S_1704	acetoin dehydrogenase (KEGG; EC:1.1.1.5)	2.0
A1S_1698	lipoate synthase	2.0
A1S_1752	AdeA membrane fusion protein	1.9
A1S_1366	transporter LysE family	1.9
A1S_1750	AdeB	1.9
A1S_3217	RND divalent metal cation efflux transporter	1.9
A1S_2304	putative RND family drug transporter	1.9
A1S_2082	putative transcriptional regulator	1.9
A1S_1303	hypothetical protein	1.9
A1S_1768	hypothetical protein	1.9
A1S_2929	putative cation efflux system protein	1.9
A1S_1302	hypothetical protein	1.9
A1S_3061	secretion protein	1.8
A1S_1298	hypothetical protein	1.8
A1S_1305	putative outer membrane lipoprotein	1.8
A1S_1301	hypothetical protein	1.8
A1S_3218	EsvF1	1.8
A1S_1300	hypothetical protein	1.8
A1S_3446	putative RND family drug transporter	1.8
A1S_0547	putative transcriptional regulator	1.8
A1S_3075	30S ribosomal protein S3	1.8
A1S_2509	putative chaperone	1.7
A1S_3447	putative RND family drug transporter	1.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1771	hypothetical protein	1.7
A1S_0807	8-amino-7-oxononanoate synthase	1.7
A1S_1306	hypothetical protein	1.7
A1S_1774	putative hydrolase	1.7
A1S_0663	putative DNA helicase	1.6
A1S_3174	putative regulatory or redox component complexing with Bfr in iron storage and mobility (Bfd)	1.6
A1S_3060	50S ribosomal protein L36	1.6
A1S_2930	putative ferrous iron transport protein B	1.6
A1S_0253	transcriptional regulator	1.6
A1S_1077	hypothetical protein	1.6
A1S_0808	putative biotin biosynthesis protein (BioC)	1.6
A1S_1403	putative cysteine desulfurase 1 (Csd)	1.6
A1S_3073	50S ribosomal protein L29	1.6
A1S_2305	cation/multidrug efflux pump	1.5
A1S_3079	50S ribosomal protein L4	1.5
A1S_3077	50S ribosomal protein L2	1.5
A1S_1832	oxidoreductase FMN-binding	1.5
A1S_1310	hypothetical protein	1.5
A1S_2208	putative transcriptional regulator	1.5
A1S_0806	adenosylmethionine-8-amino-7-oxononanoate aminotransferase (KEGG; EC:2.6.1.62)	1.5
A1S_2789	putative metallopeptidase	1.5
A1S_3072	30S ribosomal protein S17	1.5
A1S_1954	serine proteinase	1.5
A1S_2319	hypothetical protein	1.5
A1S_3063	50S ribosomal protein L30	1.5
A1S_2141	potassium-transporting ATPase A chain	1.5
A1S_1094	D-serine/D-alanine/glycine transporter	1.4
A1S_2643	oxidoreductase short chain dehydrogenase/reductase family	1.4
A1S_1763	putative transcriptional regulator	1.4
A1S_1824	hypothetical protein	1.4
A1S_1210	putative MFS transport protein	1.4
A1S_0338	ribosome-binding factor A	1.4
A1S_0099	D-serine/D-alanine/glycine transport protein	1.4
A1S_2474	putative MFS transport protein	1.4
A1S_1823	transcriptional Regulator TetR family	1.4
A1S_0285	50S ribosomal protein	1.4
A1S_1307	putative ClpA/B-type chaperone	1.4
A1S_0525	hypothetical protein	1.4
A1S_0672	resolvase	1.4
A1S_0809	dethiobiotin synthetase x	1.4
A1S_1309	hypothetical protein	1.4
A1S_1242	putative ABC family drug transporter	1.4
A1S_3078	50S ribosomal protein L23	1.4
A1S_2124	putative MFS transport protein	1.4

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1172	putative transposase	1.3
A1S_1089	hypothetical protein	1.3
A1S_2139	hypothetical protein (KEGG; EC:3.6.3.12)	1.3
A1S_1627	hypothetical protein	1.3
A1S_3074	50S ribosomal protein L16	1.3
A1S_3080	50S ribosomal protein L3	1.3
A1S_2321	hypothetical protein	1.3
A1S_1308	hypothetical protein	1.3
A1S_0804	trehalose-6-phosphate phophatase	1.3
A1S_3056	RNA polymerase alpha subunit	1.3
A1S_0080	beta-ketoacyl-ACP synthase I	1.3
A1S_1228	cold shock protein	1.3
A1S_2171	30S ribosomal protein S6	1.3
A1S_0666	TrbL/VirB6 plasmid conjugal transfer protein	1.3
A1S_3059	30S ribosomal protein S13	1.3
A1S_1147	site-specific DNA methylase-like protein	1.3
A1S_1953	putative sulfate transporter	1.3
A1S_3445	putative RND family drug transporter	1.3
A1S_0868	protein chain elongation factor EF-G GTP-binding	1.3
A1S_2312	putative transport protein	1.3
A1S_3400	hypothetical protein	1.3
A1S_1539	putative transcriptional regulator (ArsR family)	1.3
A1S_3062	50S ribosomal protein L15	1.3
A1S_0254	permease (drug/metabolite transporter) superfamily	1.3
A1S_1799	putative MFS transport protein	1.3
A1S_3410	putative acyltransferase	1.3
A1S_0742	iron-regulated protein	1.3
A1S_1350	putative transcriptional regulator	1.3
A1S_0284	50S ribosomal protein	1.3
A1S_2271	RtcB protein	1.3
A1S_0781	putative MTA/SAH nucleosidase	1.3
A1S_3411	putative GTPases (G3E family)	1.3
A1S_1312	hypothetical protein	1.3
A1S_1311	hypothetical protein	1.3
A1S_2142	hypothetical protein	1.3
A1S_0149	membrane-bound ATP synthase F0 sector, subunit a	1.3
A1S_3057	30S ribosomal protein S4	1.2
A1S_3068	30S ribosomal protein S14	1.2
A1S_1497	putative acyltransferase	1.2
A1S_1772	putative MFS transport protein	1.2
A1S_2220	hypothetical protein	1.2
A1S_2074	hypothetical protein	1.2
A1S_1628	heat shock protein	1.2
A1S_0805	putative hydrolase biotin biosynthesis (BioH)	1.2
A1S_1499	hypothetical protein	1.2

Locus-tag	Gene product	Differential expression (Log_2)
A1S_3148	NADPH specific quinone oxidoreductase	1.2
A1S_3067	30S ribosomal protein S8	1.2
A1S_0523	putative 3-hydroxylacyl-(acyl carrier protein) dehydratase	1.2
A1S_2838	lysine-specific permease	1.2
A1S_0673	putative transposase	1.2
A1S_1299	hypothetical protein	1.2
A1S_2009	3-dehydroquinate dehydratase type II (KEGG; EC:4.2.1.10)	1.2
A1S_0081	putative signal peptide	1.2
A1S_0524	hypothetical protein (KEGG; EC:1.1.1.100)	1.2
A1S_2140	potassium-transporting ATPase B chain	1.2
A1S_0302	hypothetical protein	1.2
A1S_1826	NADH dehydrogenase FAD-containing subunit	1.2
A1S_2173	50S ribosomal protein L9	1.2
A1S_0519	hypothetical protein	1.2
A1S_1441	putative signal peptide	1.2
A1S_2968	hypothetical protein	1.2
A1S_2836	putative transporter	1.1
A1S_3058	30S ribosomal protein S11	1.1
A1S_2162	hypothetical protein YcdS precursor	1.1
A1S_0866	30S ribosomal protein S12	1.1
A1S_3156	hypothetical protein	1.1
A1S_1385	hypothetical protein	1.1
A1S_3157	hypothetical protein	1.1
A1S_0027	alkanesulfonate transport protein	1.1
A1S_0520	putative oxidoreductase protein; putative dehydrogenase (flavoprotein)	1.1
A1S_3275	putative methyltransferase	1.1
A1S_1137	adenosine deaminase	1.1
A1S_2744	SAM-dependent methyltransferase	1.1
A1S_0662	phage integrase family protein	1.1
A1S_2161	putative hemin storage signal peptide protein	1.1
A1S_0521	hypothetical protein	1.1
A1S_1834	hypothetical protein	1.1
A1S_3069	50S ribosomal protein L5	1.1
A1S_3219	putative RND family drug transporter	1.1
A1S_3064	30S ribosomal protein S5	1.1
A1S_0007	putative transport protein	1.1
A1S_1757	alpha/beta hydrolase	1.1
A1S_1258	hypothetical protein	1.1
A1S_3136	hypothetical protein	1.1
A1S_3270	putative permease	1.0
A1S_3397	hypothetical protein	1.0
A1S_1590	peptidase U35 phage prohead HK97	1.0
A1S_3070	50S ribosomal protein L24	1.0
A1S_2311	putative transport protein	1.0

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2160	haemin storage system HmsR protein	1.0
A1S_2013	putative MFS transport protein	1.0
A1S_0365	putative amino-acid efflux transmembrane protein	1.0
A1S_1739	putative MFS transport protein	1.0
A1S_0974	hypothetical protein	1.0
A1S_1773	putative RND family drug transporter	1.0
A1S_0867	30S ribosomal protein S7	1.0
A1S_0522	hypothetical protein	1.0
A1S_3081	30S ribosomal protein S10	1.0
A1S_1281	TPR domain protein	1.0
A1S_3076	50S ribosomal protein L22	1.0
A1S_1588	phage terminase-like protein large subunit	1.0
A1S_2535	putative sulfate permease	1.0
A1S_3071	50S ribosomal protein L14	1.0
A1S_3440	putative MFS transport protein	1.0
A1S_2313	EsvE1	1.0
A1S_0241	site-specific tyrosine recombinase	1.0
A1S_2369	hypothetical protein	1.0
A1S_3055	50S ribosomal protein L17	1.0
A1S_2219	transcriptional regulator GntR family	1.0
A1S_1583	hypothetical protein	1.0
A1S_2654	putative periplasmic binding protein of transport/transglycosylase	1.0
A1S_1946	1-phosphofructokinase	1.0
A1S_1831	hypothetical protein	1.0
A1S_0093	hypothetical protein	1.0
A1S_0741	hypothetical protein	1.0
A1S_2016	phage-related lysozyme	1.0
A1S_0086	hypothetical protein	1.0
A1S_1083	aromatic amino acid transporter (APC family)	1.0
A1S_1585	putative replicative DNA helicase	1.0
A1S_1398	GlnQ protein	1.0
A1S_0978	hypothetical protein	1.0
A1S_0936	hypothetical protein	1.0
A1S_1945	phosphotransferase system fructose-specific EI/HPr/EIIA components	1.0
A1S_0251	thiamine hydroxymethylpyrimidine moiety synthesis	1.0
A1S_2534	sulfate transport protein	1.0

Appendix E – Genes more than 2-fold down-regulated in *A. baumannii* strain 17978hm compared to strain ATCC 17978

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1186	ATP-dependent protease Hsp 100	-4.7
A1S_2183	putative signal peptide	-4.4
A1S_1950	putative universal stress protein	-4.3
A1S_0095	D-amino acid dehydrogenase (KEGG; EC:1.4.99.1)	-4.1
A1S_0771	hypothetical protein	-4.0
A1S_3113	hypothetical protein	-4.0
A1S_3350	hypothetical protein	-3.8
A1S_2195	hypothetical protein	-3.6
A1S_1708	beta-lactamase-like protein	-3.6
A1S_3317	putative outer membrane protein	-3.6
A1S_1030	DNA-binding ATP-dependent protease La	-3.6
A1S_1932	hypothetical protein	-3.4
A1S_2960	chaperone Hsp70	-3.4
A1S_0096	alanine racemase 2 PLP-binding, catabolic	-3.3
A1S_2093	hypothetical protein	-3.2
A1S_0558	aconitate hydratase 1 (KEGG; EC:4.2.1.3)	-3.1
A1S_1193	OmpA/MotB	-3.1
A1S_3175	bacterioferritin	-3.1
A1S_2070	P-type ATPase Mg^{2+} ATPase transporter (KEGG; EC:3.6.3.2)	-3.1
A1S_1031	DNA-binding ATP-dependent protease La	-3.0
A1S_1687	transcriptional regulator	-3.0
A1S_1338	hypothetical protein	-3.0
A1S_0800	bacterioferritin	-3.0
A1S_1046	lysine exporter protein (LysE/YggA)	-3.0
A1S_3023	hypothetical protein	-3.0
A1S_1984	D-amino acid dehydrogenase small subunit	-3.0
A1S_0363	hypothetical protein	-2.9
A1S_0683	putative sigma(54) modulation protein RpoX	-2.8
A1S_2538	outer membrane protein CarO precursor	-2.8
A1S_1266	hypothetical protein	-2.8
A1S_1337	phenylacetic acid degradation B	-2.7
A1S_2820	hypothetical protein	-2.7
A1S_1339	phenylacetate-CoA oxygenase PaaJ subunit	-2.7
A1S_3277	putative pirin-like protein	-2.7
A1S_0301	hypothetical protein	-2.7
A1S_1267	putative lactam utilization protein	-2.7
A1S_1910	ATP-binding protease component	-2.7
A1S_1246	putative universal stress protein	-2.6
A1S_0445	hypothetical protein	-2.6
A1S_0097	hypothetical protein	-2.6
A1S_1270	hypothetical protein	-2.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_3046	oligopeptidase A	-2.5
A1S_2296	putative protease	-2.5
A1S_1269	putative allophanate hydrolase subunit 1 and 2	-2.5
A1S_1340	phenylacetate-CoA oxygenase/reductase PaaK subunit	-2.5
A1S_2809	bacteriolytic lipoprotein entericidin B	-2.5
A1S_1343	PaaC	-2.5
A1S_1268	hypothetical protein	-2.5
A1S_2616	hypothetical protein	-2.5
A1S_0210	transposase	-2.5
A1S_2664	chaperone Hsp60	-2.4
A1S_2291	hypothetical protein	-2.4
A1S_1342	putative enoyl-CoA hydratase II	-2.4
A1S_2450	putative pyruvate decarboxylase	-2.4
A1S_2259	putative signal peptide	-2.4
A1S_2840	OmpA	-2.4
A1S_2959	Hsp 24 nucleotide exchange factor	-2.4
A1S_1925	cytochrome d terminal oxidase polypeptide subunit II	-2.4
A1S_1433	ubiquinol oxidase subunit II	-2.3
A1S_0207	hypothetical protein	-2.3
A1S_0646	IcmB protein	-2.3
A1S_2449	aromatic amino acid transporter (APC family)	-2.3
A1S_0172	hypothetical protein	-2.3
A1S_2072	putative universal stress protein family	-2.3
A1S_1518	putative suppressor of F exclusion of phage T7	-2.3
A1S_1390	hypothetical protein	-2.3
A1S_1862	hypothetical protein	-2.2
A1S_0496	putative phosphatidylglycerophosphatase B	-2.2
A1S_0412	catalase (KEGG; EC:1.11.1.6)	-2.2
A1S_1926	hypothetical protein	-2.2
A1S_0884	putative outer membrane protein	-2.2
A1S_1726	aspartate ammonia-lyase (aspartase) (KEGG; EC:4.3.1.1)	-2.2
A1S_2416	hypothetical protein	-2.2
A1S_3180	putative signal peptide	-2.2
A1S_1335	iron-sulfur-dependent L-serine dehydratase single chain form	-2.2
A1S_1924	cytochrome d terminal oxidase polypeptide subunit I	-2.1
A1S_1861	benzoate dioxygenase large subunit	-2.1
A1S_0627	hypothetical protein	-2.1
A1S_2417	starvation-induced peptide utilization protein	-2.1
A1S_0642	hypothetical protein	-2.1
A1S_1336	hypothetical protein	-2.1
A1S_1859	aromatic-ring-hydroxylating dioxygenase beta subunit	-2.1
A1S_2696	hypothetical protein	-2.1
A1S_3122	hypothetical protein	-2.1
A1S_2092	aminopeptidase N	-2.1
A1S_0299	hypothetical protein	-2.1

Locus-tag	Gene product	Differential expression (Log_2)
A1S_2886	acyl-CoA dehydrogenase	-2.0
A1S_0465	Sec-independent protein translocase protein	-2.0
A1S_0634	hypothetical protein	-2.0
A1S_3246	hypothetical protein	-2.0
A1S_2504	excinuclease ABC subunit B	-2.0
A1S_3047	oligopeptidase A	-2.0
A1S_0015	hypothetical protein	-2.0
A1S_2444	putative protease (SohB)	-2.0
A1S_2640	putative oxidoreductase molybdopterin	-2.0
A1S_1346	phenylacetyl-CoA ligase	-2.0
A1S_2331	putative acyl-CoA dehydrogenase	-2.0
A1S_0647	IcmO protein	-1.9
A1S_0873	putative arginine-tRNA-protein transferase	-1.9
A1S_2665	chaperone Hsp10	-1.9
A1S_2418	starvation-induced peptide utilization protein	-1.9
A1S_1008	isocitrate lyase	-1.9
A1S_0750	hypothetical protein	-1.9
A1S_0654	regulatory protein ArsR	-1.9
A1S_2975	hypothetical protein	-1.9
A1S_1344	thiolase (KEGG; EC:2.3.1.9)	-1.9
A1S_0366	heat shock protein Hsp33	-1.9
A1S_0303	putative peptide signal	-1.9
A1S_0632	DNA primase	-1.9
A1S_2834	mechanosensitive channel	-1.9
A1S_2452	NAD-dependent aldehyde dehydrogenases	-1.9
A1S_3473	hypothetical protein	-1.8
A1S_2635	hypothetical protein	-1.8
A1S_0871	putative metal-dependent hydrolase	-1.8
A1S_0464	Sec-independent protein translocase protein	-1.8
A1S_2699	putative transcriptional regulator	-1.8
A1S_2486	hypothetical protein	-1.8
A1S_2598	putative RNA polymerase sigma factor	-1.8
A1S_1670	secretion protein HlyD	-1.8
A1S_0472	2-isopropylmalate synthase	-1.8
A1S_1573	integration host factor beta subunit	-1.8
A1S_2297	putative 4Fe-4S ferredoxin	-1.8
A1S_2330	putative acyl-CoA dehydrogenase	-1.8
A1S_2548	putative enoyl-CoA hydratase/isomerase	-1.8
A1S_3024	hypothetical protein	-1.8
A1S_1858	short-chain dehydrogenase/reductase SDR	-1.8
A1S_1505	YyaM	-1.8
A1S_1349	thioesterase domain protein	-1.8
A1S_2705	hypothetical protein	-1.8
A1S_2551	Tn7-like transposition protein B	-1.7
A1S_3229	two-component response regulator	-1.7

Locus-tag	Gene product	Differential expression (Log_2)
A1S_3449	phosphoenolpyruvate carboxylase	-1.7
A1S_1863	hypothetical protein	-1.7
A1S_0889	hypothetical protein	-1.7
A1S_0820	putative peptidoglycan-binding LysM	-1.7
A1S_1856	p-hydroxyphenylacetate hydroxylase C1:reductase component	-1.7
A1S_1345	PaaK	-1.7
A1S_1434	ubiquinol oxidase subunit I	-1.7
A1S_0645	hypothetical protein	-1.7
A1S_0034	putative oxoacyl-(acyl carrier protein) reductase	-1.7
A1S_1864	acyl-CoA dehydrogenase-like protein	-1.7
A1S_3343	hypothetical protein	-1.7
A1S_0779	hypothetical protein	-1.7
A1S_2669	FAD dependent oxidoreductase	-1.7
A1S_2552	ATPase	-1.7
A1S_0641	hypothetical protein	-1.7
A1S_1865	Glu-tRNA amidotransferase	-1.7
A1S_0640	hypothetical protein	-1.7
A1S_1181	putative oxidoreductase aldo/keto reductase family	-1.7
A1S_0639	IncC protein	-1.7
A1S_1205	alkyl hydroperoxide reductase C22 subunit	-1.7
A1S_2737	AdeK	-1.7
A1S_0305	3-ketoacyl-CoA thiolase	-1.6
A1S_2550	Tn7 transposase A	-1.6
A1S_0624	putative lipoprotein	-1.6
A1S_1933	hypothetical protein	-1.6
A1S_1857	vanillate O-demethylase oxidoreductase	-1.6
A1S_0530	hypothetical protein	-1.6
A1S_3108	coproporphyrinogen III oxidase (KEGG; EC:1.3.3.3)	-1.6
A1S_0629	hypothetical protein	-1.6
A1S_2006	response regulator protein	-1.6
A1S_2847	glucose dehydrogenase	-1.6
A1S_3352	hypothetical protein	-1.6
A1S_2599	hypothetical protein	-1.6
A1S_0297	hypothetical protein	-1.6
A1S_1356	p-hydroxybenzoate hydroxylase transcriptional activator (KEGG; EC:1.14.13.2)	-1.6
A1S_0463	putative alkaline phosphatase	-1.6
A1S_2773	putative long-chain fatty acid transport protein	-1.6
A1S_2406	hypothetical protein	-1.6
A1S_2233	hypothetical protein	-1.6
A1S_3208	putative peptide signal	-1.6
A1S_3280	$NADP^+$-dependent succinate semialdehyde dehydrogenase (KEGG; EC:1.2.1.16)	-1.6
A1S_1347	PaaX	-1.6
A1S_1833	hypothetical protein (KEGG; EC:1.11.1.10)	-1.6

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1348	carbonic anhydrases/acetyltransferases isoleucine patch superfamily	-1.6
A1S_0651	TraB protein	-1.6
A1S_0643	hypothetical protein	-1.5
A1S_0650	conjugal transfer protein	-1.5
A1S_0571	hydroxypyruvate isomerase	-1.5
A1S_1645	hypothetical protein	-1.5
A1S_1637	DNA-binding protein HU-beta	-1.5
A1S_2481	FkbP-type peptidyl-prolyl cis-trans isomerase	-1.5
A1S_1369	putative oxidoreductase protein	-1.5
A1S_2487	hypothetical protein	-1.5
A1S_1641	alkane 1-monooxygenase	-1.5
A1S_2180	hypothetical protein	-1.5
A1S_0529	glutaredoxin	-1.5
A1S_0649	putative phage primase	-1.5
A1S_0655	putative efflux protein	-1.5
A1S_0644	hypothetical protein	-1.5
A1S_0803	trehalose-6-phosphate synthase	-1.5
A1S_1119	choline dehydrogenase and related flavoproteins	-1.5
A1S_0040	putative oxidoreductase	-1.5
A1S_0441	hypothetical protein	-1.5
A1S_1043	putative transcriptional regulator	-1.5
A1S_0603	integration host factor alpha subunit	-1.5
A1S_0037	alkali-inducible disulfide interchange protein	-1.4
A1S_2681	cell division protein	-1.4
A1S_3428	putative glucose dehydrogenase precursor	-1.4
A1S_1044	Co/Zn/Cd efflux system	-1.4
A1S_1368	pyruvate ferredoxin/flavodoxin oxidoreductase	-1.4
A1S_3278	hydrolase isochorismatase family	-1.4
A1S_0449	coniferyl aldehyde dehydrogenase (CALDH) (KEGG; EC:1.2.1.68)	-1.4
A1S_2600	hypothetical protein	-1.4
A1S_1109	coenzyme A ligase	-1.4
A1S_2503	putative outer membrane lipoprotein	-1.4
A1S_1647	putative siderophore biosynthesis protein	-1.4
A1S_2459	putative oxidoreductase	-1.4
A1S_1648	putative lysine/ornithine N-monooxygenase	-1.4
A1S_0371	hypothetical protein	-1.4
A1S_2553	transposition site target selection protein D	-1.4
A1S_2218	CsuA/B	-1.4
A1S_2906	putative sensory transduction histidine kinase	-1.4
A1S_0209	transposase	-1.4
A1S_2102	aldehyde dehydrogenase 1 (KEGG; EC:1.2.1.3)	-1.4
A1S_2168	cytochrome o ubiquinol oxidase subunit III	-1.4
A1S_2692	putative universal stress protein A (UspA)	-1.4
A1S_2061	putative short-chain dehydrogenase	-1.4

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0580	putative short-chain dehydrogenase	-1.4
A1S_1574	hypothetical protein	-1.4
A1S_2232	methylmalonate-semialdehyde dehydrogenase (KEGG; EC:1.2.1.27)	-1.4
A1S_1254	hypothetical protein	-1.4
A1S_3327	dihydrolipoamide S-acetyltransferase E2 component of the pyruvate dehydrogenase complex	-1.4
A1S_3301	hypothetical protein	-1.4
A1S_1987	putative UDP-galactose 4-epimerase (GalE-like)	-1.4
A1S_0364	curved DNA-binding protein	-1.4
A1S_2924	putative Rhodanese-related sulfurtransferase	-1.4
A1S_1677	putative porin precursor	-1.4
A1S_3231	putative acetyl-CoA hydrolase/transferase (KEGG; EC:3.1.2.1)	-1.4
A1S_0631	hypothetical protein	-1.4
A1S_1220	putative threonine efflux protein	-1.4
A1S_2848	glucose dehydrogenase	-1.4
A1S_2628	electron transfer flavoprotein beta-subunit	-1.4
A1S_2230	hypothetical protein	-1.3
A1S_0626	hypothetical protein	-1.3
A1S_3121	hypothetical protein	-1.3
A1S_3111	putative acyl-CoA dehydrogenase	-1.3
A1S_0573	putative enoyl-CoA hydratase/isomerase	-1.3
A1S_0748	two-component regulatory activator (OmpR family)	-1.3
A1S_1671	PltJ	-1.3
A1S_0421	protein chain initiation factor IF-1	-1.3
A1S_0661	phage integrase family protein	-1.3
A1S_2343	superoxide dismutase (KEGG; EC:1.15.1.1)	-1.3
A1S_0533	hypothetical protein	-1.3
A1S_1961	heat shock protein 15	-1.3
A1S_1180	putative Zn-dependent protease with chaperone function	-1.3
A1S_0182	hypothetical protein	-1.3
A1S_3300	putative sodium:solute symporter	-1.3
A1S_3418	4-hydroxyphenylpyruvate dioxygenase (KEGG; EC:1.13.11.27)	-1.3
A1S_1650	hypothetical protein	-1.3
A1S_2887	acyl-CoA dehydrogenase A	-1.3
A1S_0237	D-alanyl-D-alanine endopeptidase penicillin-binding protein 7 and penicillin-binding protein 8	-1.3
A1S_0170	putative outer membrane copper receptor (OprC)	-1.3
A1S_2222	putative poly(R)-hydroxyalkanoic acid synthase	-1.3
A1S_0016	site-specific tyrosine recombinase	-1.3
A1S_3414	fumarylacetoacetase (KEGG; EC:3.7.1.2)	-1.3
A1S_3171	RNA polymerase omega subunit	-1.3
A1S_1523	putative signal peptide	-1.3
A1S_0090	hypothetical protein	-1.3

Locus-tag	Gene product	Differential expression (Log_2)
A1S_0630	hypothetical protein	-1.3
A1S_1680	hypothetical protein	-1.3
A1S_1111	enoyl-CoA hydratase/lyase	-1.3
A1S_2710	hypothetical protein (KEGG; EC:2.3.3.1)	-1.3
A1S_1470	glutathione peroxidase (KEGG; EC:1.11.1.9)	-1.3
A1S_2889	putative signal peptide	-1.3
A1S_2785	putative protease	-1.3
A1S_0635	hypothetical bacteriophage protein	-1.3
A1S_1867	putative MFS transport protein	-1.3
A1S_2484	hypothetical protein	-1.3
A1S_1321	putative hemolysin	-1.3
A1S_1709	hypothetical protein	-1.3
A1S_0469	hypothetical protein	-1.3
A1S_0091	hypothetical protein	-1.2
A1S_2627	electron transfer flavoprotein alpha-subunit	-1.2
A1S_3139	putative signal peptide	-1.2
A1S_2069	putative magesium transporter transmembrane protein	-1.2
A1S_1042	hypothetical protein	-1.2
A1S_2658	heat shock protein	-1.2
A1S_0659	hypothetical protein	-1.2
A1S_1524	hypothetical protein	-1.2
A1S_1341	enoyl-CoA hydratase/carnithine racemase	-1.2
A1S_2458	putative fatty acid desaturase	-1.2
A1S_1199	putative glutathionine S-transferase	-1.2
A1S_1651	hypothetical protein	-1.2
A1S_1738	putative transcriptional regulator	-1.2
A1S_2719	succinyl-CoA synthetase alpha chain (KEGG; EC:6.2.1.5)	-1.2
A1S_0928	bet gene repressor	-1.2
A1S_1204	hypothetical protein	-1.2
A1S_3474	hypothetical protein	-1.2
A1S_1986	fumarase C (KEGG; EC:4.2.1.2)	-1.2
A1S_0094	lrp regulon transcriptional regulator (AsnC family)	-1.2
A1S_0865	hypothetical protein	-1.2
A1S_0353	hypothetical protein	-1.2
A1S_1931	hypothetical protein	-1.2
A1S_1649	putative RND family drug transporter	-1.2
A1S_0528	protein exporting molecular chaperone	-1.2
A1S_3298	putative high affinity choline transport protein (bet-like)	-1.2
A1S_1911	putative protease	-1.2
A1S_1141	carbon storage regulator	-1.1
A1S_0477	ATP-dependent Clp protease ATP-binding subunit	-1.1
A1S_2639	formate dehydrogenase formation protein	-1.1
A1S_2164	phosphoenolpyruvate synthase (KEGG; EC:2.7.9.2)	-1.1
A1S_3469	diaminopimelate decarboxylase	-1.1
A1S_2485	putative glycosyltransferase	-1.1

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1490	glutamate/aspartate transport protein	-1.1
A1S_1197	putative extracellular nuclease	-1.1
A1S_2891	putative phospholipase D endonuclease domain	-1.1
A1S_1860	ring hydroxylating dioxygenase Rieske (2Fe-2S) protein	-1.1
A1S_0218	nitrogen assimilation regulatory protein P-II 2	-1.1
A1S_2167	cytochrome o ubiquinol oxidase subunit I	-1.1
A1S_3458	putative Na^+-dependent transporters of the SNF family	-1.1
A1S_0747	ribonucleoside diphosphate reductase alpha subunit	-1.1
A1S_0466	Sec-independent protein translocase protein	-1.1
A1S_2234	hypothetical protein	-1.1
A1S_2823	hypothetical protein	-1.1
A1S_0676	putative transposase	-1.1
A1S_1093	arginine/ornithine N-succinyltransferase beta subunit	-1.1
A1S_0638	hypothetical protein	-1.1
A1S_0658	transposase	-1.1
A1S_1370	oxidoreductase	-1.1
A1S_0620	putative anti-anti-sigma factor	-1.1
A1S_3179	hypothetical protein	-1.1
A1S_1962	DNA strand exchange and recombination protein	-1.1
A1S_2758	putative membrane protease subunit	-1.1
A1S_0462	putative phosphatase	-1.1
A1S_3030	phosphate starvation-inducible protein (PhoH-like)	-1.1
A1S_0633	hypothetical protein	-1.1
A1S_0746	ribonucleoside-diphosphate reductase beta subunit	-1.1
A1S_2667	hypothetical protein	-1.1
A1S_0916	dihydrodipicolinate synthetase	-1.1
A1S_0236	response regulator	-1.1
A1S_1944	alpha/beta hydrolase	-1.1
A1S_1935	hypothetical protein	-1.1
A1S_3443	heat shock protein Hsp40	-1.1
A1S_0671	protein tyrosine phosphatase	-1.1
A1S_1652	hypothetical protein	-1.1
A1S_1451	hypothetical protein	-1.1
A1S_2098	putative alcohol dehydrogenase (KEGG; EC:1.1.1.1)	-1.1
A1S_2448	putative phosphate transporter	-1.1
A1S_2166	cytochrome o ubiquinol oxidase subunit II	-1.1
A1S_3416	glyoxalase/bleomycin resistance protein/dioxygenase	-1.1
A1S_1737	3-hydroxybutyrate dehydrogenase	-1.1
A1S_0009	putative RND family drug transporter	-1.1
A1S_0041	putative linoleoyl-CoA desaturase	-1.1
A1S_2880	putative signal peptide	-1.1
A1S_2685	hypothetical protein	-1.1
A1S_0764	NADH dehydrogenase I chain N	-1.1
A1S_0315	DNA repair system	-1.0
A1S_3031	hypothetical protein	-1.0

Locus-tag	Gene product	Differential expression (Log_2)
A1S_1187	CinA-like protein	-1.0
A1S_2843	hypothetical protein	-1.0
A1S_2595	peptidoglycan-associated lipoprotein precursor	-1.0
A1S_3368	hypothetical protein	-1.0
A1S_2546	secreted trypsin-like serine protease	-1.0
A1S_1326	hypothetical protein	-1.0
A1S_1388	hypothetical protein	-1.0
A1S_1203	o-methyl transferase	-1.0
A1S_2385	putative ferric acinetobactin receptor	-1.0
A1S_1827	hypothetical protein	-1.0
A1S_0606	hypothetical protein	-1.0
A1S_1868	porin for benzoate transport (BenP)	-1.0
A1S_0290	hypothetical protein	-1.0
A1S_2395	hypothetical protein	-1.0
A1S_1471	putative transcriptional regulator (AraC family)	-1.0
A1S_1469	peptide methionine sulfoxide reductase (KEGG; EC:1.8.4.6)	-1.0
A1S_1948	putative transcriptional repressor of for multidrug resistance pump (MarR family)	-1.0
A1S_0201	putative outer membrane protein	-1.0
A1S_1526	hypothetical protein	-1.0
A1S_0227	aminopeptidase A (KEGG; EC:3.4.11.1)	-1.0
A1S_2318	hypothetical protein	-1.0
A1S_0470	methionine biosynthesis protein	-1.0
A1S_0292	putative outer membrane protein W	-1.0
A1S_2317	putative lipoprotein precursor (RlpA-like)	-1.0
A1S_0422	putative transcriptional regulator (AraC family)	-1.0
A1S_3367	hypothetical protein	-1.0
A1S_2935	copper resistance protein B precursor	-1.0
A1S_3178	hypothetical protein	-1.0
A1S_0493	carboxy-terminal protease	-1.0
A1S_1012	EsvC (KEGG; EC:3.5.1.5)	-1.0
A1S_2682	cell division protein	-1.0
A1S_2325	putative outer membrane protein	-1.0
A1S_2904	branched-chain amino acid transferase	-1.0
A1S_1869	putative porin for benzoate transport (BenP)	-1.0
A1S_0623	DNA mismatch repair enzyme	-1.0
A1S_0067	L-lactate permease	-1.0
A1S_1672	hypothetical protein	-1.0
A1S_0648	hypothetical protein	-1.0
A1S_2477	isocitrate dehydrogenase (KEGG; EC:1.1.1.42)	-1.0
A1S_2210	hypothetical protein	-1.0
A1S_0171	hypothetical protein	-1.0
A1S_0428	hypothetical protein	-1.0
A1S_1653	hypothetical protein	-1.0
A1S_0637	DNA-directed DNA polymerase	-1.0

Locus-tag	**Gene product**	**Differential expression** (Log_2)
A1S_2604	putative permease (PerM family)	-1.0

Appendix F – Eijkelkamp *et al.* 2011; BMC Genomics

Eijkelkamp *et al. BMC Genomics* 2011, **12**:126
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BMC Genomics

RESEARCH ARTICLE Open Access

Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions

Bart A Eijkelkamp[1], Karl A Hassan[2], Ian T Paulsen[2], Melissa H Brown[1*]

Abstract

Background: Iron acquisition systems are important virulence factors in pathogenic bacteria. To identify these systems in *Acinetobacter baumannii*, the transcriptomic response of the completely sequenced strain ATCC 17978 under iron limiting conditions was investigated using a genomic microarray that contained probes for all annotated open reading frames.

Results: Under low iron conditions, transcription levels were more than 2-fold up-regulated for 463 genes, including 95 genes that were up-regulated more than 4-fold. Of particular significance, three siderophore biosynthesis gene clusters, including one novel cluster, were highly up-regulated. Binding sites for the ferric uptake regulator were identified in the promoter regions of many up-regulated genes, suggesting a prominent role for this regulator in the *Acinetobacter* iron acquisition response. Down-regulation under iron limitation was less dramatic as the transcription of only 202 genes varied more than 2-fold. Various genes involved in motility featured prominently amongst the genes down-regulated when iron was less readily available. Motility assays confirmed that these transcriptional changes are manifested at the phenotypic level. The siderophore biosynthesis gene clusters were further investigated by means of comparative genomic analysis of 10 sequenced *Acinetobacter* isolates. These analyses revealed important roles for mobile genetic elements in shaping the siderophore meditated iron acquisition mechanisms between different *Acinetobacter* strains.

Conclusions: *A. baumannii* grown under iron limited conditions resulted in major transcriptional changes of not only many iron acquisition related genes, but also genes involved in other processes such as motility. Overall, this study showed that *A. baumannii* is well adaptable to growth in an environment which has limiting iron availability.

Background

An increasing prevalence of infections caused by *Acinetobacter baumannii* has been observed in the clinical setting throughout the last 10 to 15 years [1,2]. *A. baumannii* is able to persist in the hospital environment and in particular intensive care units, due to its wide variety of resistance mechanisms and high survival rate on abiotic surfaces [3-6]. Some clinical *A. baumannii* strains have been shown to be naturally competent for the uptake of genetic material, which facilitates acquisition of novel resistance and virulence genes [7-9].

Free iron is a limited micronutrient in hosts where it is typically tightly bound within a range of biomolecules, such as heme. As such, iron acquisition systems are important factors for the virulence of pathogenic organisms. Bacteria can adapt to iron limited host environments through the expression of a range of iron acquisition mechanisms. One pathway for uptake of iron involves direct binding of Fe^{2+} or heme to receptors or transport proteins on the cell surface [10]. A second more energy intensive mechanism of iron uptake involves the production and secretion of high-affinity iron chelating siderophores, which compete with host cells for iron [11,12]. The genes involved in the production of a siderophore are usually clustered within the genome of the producing organism. In addition to biosysnthesis genes, many of these gene clusters also

* Correspondence: melissa.brown@flinders.edu.au
[1]School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia
Full list of author information is available at the end of the article

encode efflux pumps with putative roles in siderophore export. Transporters classified within the ATP-binding cassette (ABC) superfamily, major facilitator superfamily (MFS) and resistance-nodulation-cell division (RND) family have been associated with siderophore extrusion [13]. However, the ability to transport siderophores into the extracellular space has been shown for only two pumps, both MFS members. EntS of *Escherichia coli* transports enterobactin [14] and YmfE of *Bacillus subtilis* is involved in transport of bacillibactin [15]. Inactivation of these pumps results in decreased efflux of the fully synthesized siderophore, but increased extrusion of siderophore precursor products [14,15].

The uptake and reduction of iron-loaded ferric siderophores involves the TonB-ExbB-ExbD energy transduction system in combination with a ferric siderophore complex receptor [16,17]. Bacteria often contain numerous ferric siderophore complex receptors. Some of these are encoded within siderophore biosynthesis gene clusters and are likely to display specificity for the locally encoded siderophore. However, various other ferric siderophore receptors can be scattered throughout the genome and may have the ability to recognize exogneously produced siderophores that are structurally unrelated to endogenous siderophores [18,19].

Most *A. baumannii* strains have the ability to grow under iron limiting conditions, which assists in the colonization of a host, however, a diversity of iron acquisition mechanisms has been shown between different *Acinetobacter* strains [20]. To date, three different siderophore biosynthesis gene clusters have been described in *A. baumannii* [21-23]. Of these, the cluster encoding the siderophore acinetobactin has been the most extensively studied. Knockout experiments have confirmed the functions of both a siderophore biosynthesis protein and receptor from the acinetobactin gene cluster [24]. Furthermore, acinetobactin is the only siderophore produced by *Acinetobacter* to have been structurally characterized [25]. A second siderophore biosynthesis gene cluster found only in *A. baumannii* 8399 has been characterized using complementation experiments in an *E. coli* mutant strain [21]. Finally, a putative siderophore biosynthesis gene cluster has been described in strain ATCC 17978 and subjected to limited quantitative reverse transcription PCR (qRT-PCR) analyses under iron limiting conditions [23].

To comprehensively identify mechanisms of iron acquisition and low iron adaptation in *A. baumannii*, the affect of iron starvation on *A. baumannii* cells was investigated at the global level. The transcriptomic responses of *A. baumannii* ATCC 17978 cells to low iron conditions were examined using a whole genome microarray.

Results and Discussion

Optimization of test conditions for transcriptomics

Iron is an essential micronutrient and depletion in the growth medium is likely to have an impact on cell viability. Therefore, growth of *A. baumannii* ATCC 17978 was investigated under varying iron concentrations to determine optimal conditions for whole transcriptome analysis. Reduction of available iron in Mueller-Hinton (MH) medium was achieved by supplementation of 2,2'-dipyridyl (DIP), a synthetic iron chelator. This compound had no effect on the pH of the medium (data not shown). No significant change in the growth rate of *A. baumannii* strain ATCC 17978 was observed after addition of 100 μM DIP, whereas, supplementation with 200 μM DIP resulted in a growth delay of approximately 45 min at mid-log phase (OD_{600} = 0.7) (Figure 1). Moreover, the total biomass was reduced by more than 10% at stationary phase (> 240 min). Addition of 300 μM DIP had a major impact on growth and resulted in more than 70% biomass reduction compared to cultures under iron replete conditions during stationary phase. Due to the moderate inhibitory, but non-lethal effect of 200 μM DIP, this concentration was chosen to study transcriptional changes under iron limitation. Preliminary qRT-PCR was performed to confirm transcriptional adaptation in response to iron limitation by assaying the level of transcription of the ferric uptake regulator (FUR), which is known for auto-up-regulation when iron is limited [26]. *A. baumannii* grown in the presence of 200 μM DIP expressed *FUR* at levels increased more than 2-fold compared to cells grown under iron replete conditions (data not shown). Therefore, the genome wide transcriptional changes of ATCC 17978 grown in MH medium and MH

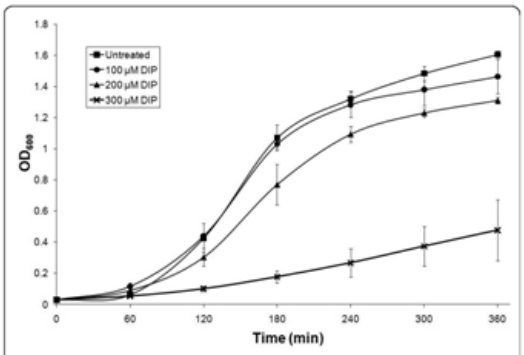

Figure 1 Growth curves of *A. baumannii* with varying iron concentrations. Growth under different iron concentrations was tested in Mueller-Hinton (MH) broth and MH supplemented with 2,2'-dipyridyl (DIP) to final concentrations of 100, 200 and 300 μM. Absorbance was measured every hour at OD_{600} for 6 hours; the data represent the average of three separate experiments and the error bars show the standard deviation.

supplemented with 200 μM DIP during mid log-phase were compared by microarray analysis.

Global transcriptional changes of *A. baumannii* to iron starvation

Iron limitation had far reaching transcriptional effects on *A. baumannii* cells (Figure 2). Significance analysis [27] of the microarray data showed that 1207 genes were significantly differentially expressed under iron limiting as compared to iron replete conditions (Additional file 1). Transcript levels were more than 2-fold higher for 463 genes, of which 95 genes were up-regulated more than 4-fold (Figure 2). The maximum overexpression observed was 165-fold for the siderophore biosynthesis gene *basD*. Fewer genes were down-regulated under iron limitation; only 202 genes were more than 2-fold underexpressed with a maximum down-regulation of 29-fold (A1S_2297). The array results were validated by qRT-PCR analysis on a subset of differentially expressed genes (Figure 3). There was

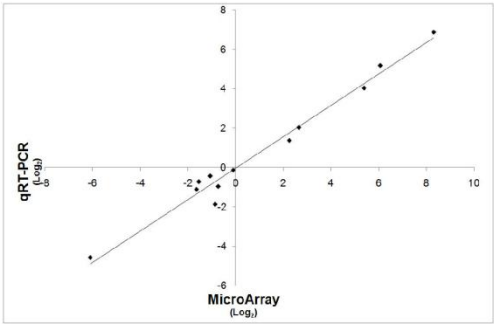

Figure 3 Validation of the microarray results. The transcriptomic results obtained by microarray hybridisation were validated by quantitive RT-PCR (qRT-PCR) analysis. The level of differential expression of 12 genes was compared and showed a correlation between microarray (Y-axis) and qRT-PCR analysis (X-axis). The level of differential expression between iron replete and iron limitation is given in Log_2-values.

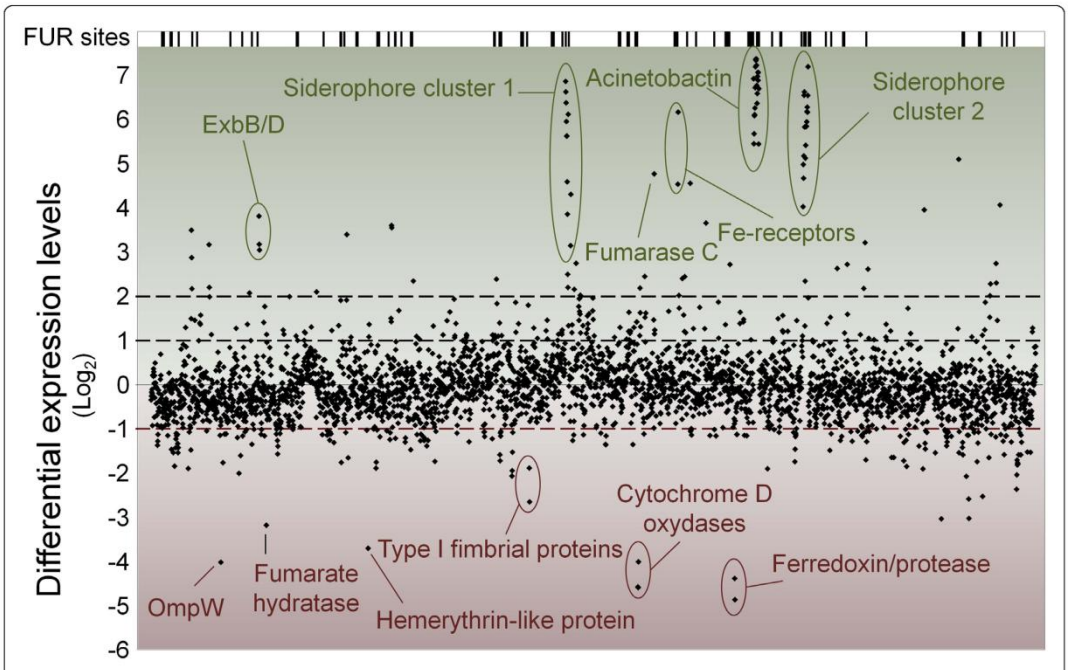

Figure 2 Overview of transcriptional responses to iron starvation. The *A. baumannii* ATCC 17978 transcriptome was compared under iron replete and iron limiting conditions (200 μM DIP). All 3367 genes of the *A. baumannii* ATCC 17978 genome are represented on the X-axis, ordered according locus tag. Differential expression levels between iron replete and iron limiting conditions are displayed in Log_2-values on the Y-axis. Up- and down-regulated genes under iron limiting conditions are displayed in the green and red sections, respectively. Gene names or functions have been provided for various highly differentially expressed genes, such as siderophore biosynthesis genes and the fumarases. The putative FUR binding sites displayed in the top section were identified as described in Methods and the nucleotide sequences are listed in Additional file 2.

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good correlation between data from the qRT-PCR and the microarray analyses, although the qRT-PCR data generally showed higher fold changes than the microarray expression data. The tendency for microarrays to underestimate fold changes relative to qRT-PCR is well established [28].

Microarray data displayed by clusters of orthologous groups (COG) functional categories showed that 27% of the genes up-regulated under iron limited conditions encode proteins involved in secondary metabolite biosynthesis, transport and catabolism (Figure 4). The majority of these genes are located within three large overexpressed gene clusters, each of which is known or predicted to synthesize a siderophore (Figure 2). Siderophore cluster 1 (A1S_1647-1657) is a novel putative siderophore gene cluster, having not been previously identified. The two other highly overexpressed siderophore clusters identified, siderophore cluster 2 (A1S_2562-2581) and the acinetobactin cluster (A1S_2372-2392), have been described previously [21,23]. Many other overexpressed genes within this COG category encode ferric siderophore receptors, which are widely dispersed across the genome.

In addition to siderophore-related genes, a high percentage, 22%, of genes up-regulated under iron limitation, encoded proteins categorized within the defence mechanism COG. A number of these genes encode transporter proteins that are classified as defence proteins due to their predicted roles in the export of metabolic waste or other toxic compounds. Interestingly, our analysis of several of these proteins, primarily those encoded within siderophore biosynthetic loci, suggested that they function in the extrusion of siderophores (Hassan *et al.*, unpublished data).

Various genes related to cell motility were down-regulated when *A. baumannii* was grown under iron limiting conditions (Figure 4). These included biosynthesis genes homologous to both type IV pili and chaperone-usher pili assembly systems, or type I pili. Another heavily down-regulated gene encoded a hemerythrin-like

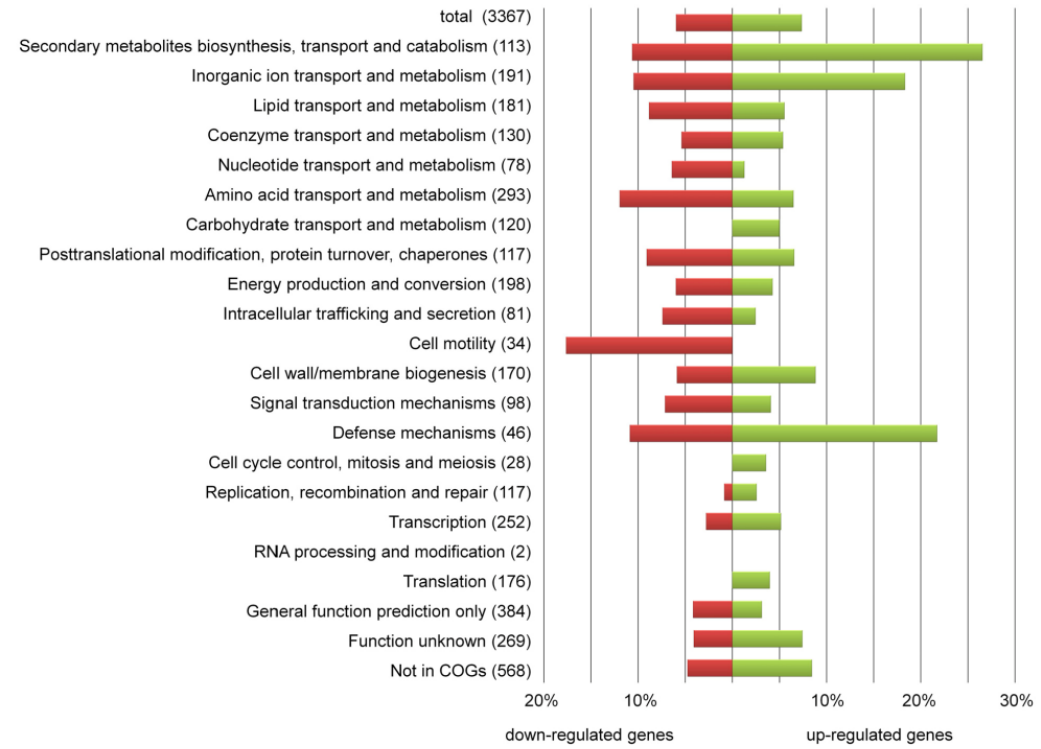

Figure 4 Microarray results displayed by COG-function. Depiction of cluster of orthologous groups (COG) and the percentage of up-regulated (green) and down-regulated genes (red) within such group determined by microarray. The total number of genes per COG is shown in parentheses.

protein (A1S_0891). Various functions have been suggested for these proteins with an iron containing centre, including detoxification, transport and storage of iron and/or oxygen, or a role as a sensory protein [29]. The cytochrome D genes, part of the respiratory system, and 4Fe-4S-ferredoxin, which facilitates electron transport in various metabolic processes, were also down-regulated more than 4-fold. The iron-dependent Class I fumarate hydratase was found to be 9-fold down-regulated. In contrast, fumarase C, which belongs to the iron-independent Class II was 27-fold up-regulated, suggesting a physiological shift to the Class II protein under iron limitation (Figure 2). These findings correlated with results observed in a study on fumarase A (Class I) and fumarase C in the tri-carboxylic acid cycle of *Pseudomonas fluorescence* [30]. In conclusion, *A. baumannii* showed strong transcriptional responses to iron starvation, predominantly in up-regulation of iron acquisition mechanisms. However, many genes related to other processes than iron acquisition, such as respiration and motility, were also transcriptionally affected under the conditions tested.

A. baumannii FUR box optimization

Bioinformatic analyses were performed to identify motifs within the promoter regions of iron responsive genes that could serve regulatory functions. The multiple em for motif elicitation (MEME) tool and the multiple alignment and search tool (MAST) were used to identify motifs and search for these motifs across the *A. baumannii* ATCC 17978 genome, respectively [31,32]. MEME-based analyses of the upstream regions of all genes overexpressed by at least 4-fold under iron limitation identified a motif bearing strong similarity to the FUR binding sites of *E. coli* and *Pseudomonas* spp. [33]. To confirm the importance of FUR in regulating iron adaptation responses in *A. baumannii*, a scoring matrix was created using the experimentally determined *E. coli* FUR binding sites [34]. This scoring matrix was used to screen the ATCC 17978 genome using MAST. Hits obtained using MAST, which were found upstream of genes that were more than 4-fold up-regulated in response to iron limitation and with a p-value less than $10e^{-5}$, were selected for iterative refinement of the scoring matrix, until no new hits were obtained (Table 1). The resulting *A. baumannii* FUR box motif showed a 25 nucleotide palindromic sequence (Figure 5).

In a previous study, the upstream regions of *FUR* genes from different *A. baumannii* isolates were aligned to obtain a motif representing the FUR binding site [26]. Some differences exist between this sequence and the FUR box sequence shown in Figure 5, most notably, the previously described motif lacked the typical FUR box palindomic structure. FUR is known to auto-regulate its own expression, however, as seen in the current study, up-regulation of *FUR* under iron limiting conditions is at lower levels than that of other FUR regulated genes, e.g., siderophore biosynthesis genes, a phenomenon that could reflect a lower binding affinity of the FUR protein for the *FUR* promoter region [35-38]. Therefore, the FUR binding sequences found upstream of the *FUR* gene may have predicted a less than optimal *A. baumannii* FUR box consensus motif, which does not show the typical palindromic structure. Moreover, FUR motifs of different bacterial genera show a high level of homology, whereas the previously described *A. baumannii* FUR motif is more distant.

A MAST search (parameters; E < 100, p < $10e^{-4}$) using the optimized *A. baumannii* FUR motif showed 81 hits

Table 1 Putative FUR binding sequences in the ATCC 17978 genome

Locus tag	Putative FUR binding sequence (5'-3')
A1S_0242	TTATTTGGTAATTATTCTCATTTAT
A1S_0416	GGATTTGTTAATGATTATCATTTGC
A1S_0474	GCGAATAATAATAATTCTTATTTAT
A1S_0980	GATATTGTTAATAATTATCATTATT
A1S_1647	TGAAATGATAATAATTATCATTAAT
A1S_1657	ATAATTGATAATGATAATCATTTTT
A1S_1667	GATAATGTAAATAATTCTCATTTAT
A1S_2077	TCATTTGATACTGATTATCAATATT
A1S_2080	ATAAATGAGAATGATTTTAATTAAT
A1S_2123	GATAATAAGAATTATTTTTATTTGT
A1S_2278	TTATTTGATAATGATTTTCATTTAT
A1S_2372	GTTATTGATAATAATAATCATTTGC
A1S_2382	GCAACTGGTAATCATTTTCATTTGT
A1S_2391	GTAATTGTAAATGATTATCATTTAT
A1S_2392	GTAAATAATAATCATTATTAATTGT
A1S_2567	TTACTTGAGAATGATTCTTGATAAC
A1S_2581	TTAAATGAGAATCATTTTCATTTAT
A1S_2582	TTAAATGAGAATCATTTTCATTTAT
A1S_2667	TTTTTTGAGAATTATTATTGATTAT
A1S_3174	ATTATTGATAATTATTATCGTTTGT
A1S_3324	GATAATGAGAATTATTTTAATTTAT
A1S_3339	TTAAATGATTATAATTATCATTTAT

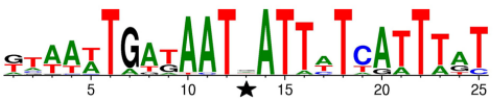

Figure 5 The optimized *A. baumannii* FUR motif. Per position, the size of the nucleotide (T in red, A in green, C in blue and G in black) indicates its prevalence in the 22 included sequences from Table 1. The motif shows a palindrome with a central non-conserved nucleotide in position 13 which is indicated by the star. The figure of the *A. baumannii* ATCC 17978 FUR motif was created using WebLogo 3.0 [64].

to the *A. baumannii* ATCC 17978 genome (Additional file 2). Over 80% of the genes with a well conserved FUR box upstream ($p < 10e^{-5}$, n = 41) showed more than 2-fold up-regulation. Furthermore, of the 95 genes up-regulated more than 4-fold under iron limiting conditions, 75 were preceded by putative FUR binding sites. These studies highlight a significant correlation between the level of conservation of a putative FUR binding site and the level of up-regulation under iron limiting conditions.

Extracytoplasmic function (ECF) transcription factors are sigma-70 family proteins that are responsive to environmental changes such as iron starvation [39]. These proteins play an important role in regulating iron-uptake mechanisms in several bacterial genera. For example, one of the best characterized ECF sigma factors, PvdS, controls the genes required for biosynthesis and transport of the siderophore pyoverdine in *P. aeruginosa* [40]. Expression of *pvdS* and various other sigma-70 factors in *Pseudomonas* is regulated by FUR [40]. However, in this study no predicted sigma-70 factors were identified in the list of genes containing a putative *A. baumannii* FUR binding site (Additional file 2). Moreover, no significant differentially expressed sigma-70 factors were found under the iron limited conditions, suggesting that in *Acinetobacter*, these proteins do not function in iron-uptake regulation. Another regulatory mechanism involved in iron homeostasis includes small RNA molecules, such as *ryhB* from *E. coli* or *prrF* from *P. aeruginosa*. However, sequences homologous to either of these small RNAs were not found in the *A. baumannii* ATCC 17978 genome. Nonetheless, a role for small RNAs in iron homeostasis can not be ruled out, since the *A. baumannii* ATCC 17978 genome contains a gene encoding the RNA chaperone Hfq (A1S_3785), which is required for the functionality of numerous small RNAs involved in iron homeostasis in various Gram-negative bacteria [41,42]. The results of this study suggest that FUR is the primary regulator of iron uptake in *A. baumannii*.

Transcriptional profiling of the siderophore mediated iron acquisition mechanisms

Genes involved in the biosynthesis, efflux and uptake of a siderophore are often clustered within bacterial genomes. To date, three putative siderophore gene clusters have been identified in *A. baumannii* [21-23], of which two can be found in strain ATCC 17978. A significant finding from the microarray results was the detection of the novel putative *A. baumannii* siderophore gene cluster (Figure 2; Siderophore cluster 1: A1S_1647 - A1S_1657). Several genes within siderophore cluster 1 were more than 100-fold overproduced under iron limitation, highlighting their potential importance in iron uptake (Figures 2 and 6a). Siderophore cluster 1 contains eight genes with a putative function in siderophore biosynthesis, A1S_1647, A1S_1648, A1S_1650-1654 and A1S_1657. Siderophore extrusion is most likely facilitated by an MFS efflux pump (A1S_1649). A receptor (A1S_1655) and PepSY-associated transmembrane helix family protein (A1S_1656) are likely to be involved in recognition and reduction of ferric siderophores, respectively. FUR boxes for transcriptional regulation of the unidirectional, operon-like gene cluster could be identified upstream of A1S_1647 and A1S_1657 (Figure 6a).

Siderophore gene cluster 2 (A1S_2562-2581) [23] showed similarly high levels of overexpression as cluster 1 (Figure 6b). This cluster contains 15 genes involved in siderophore biosynthesis (A1S_2567-2581), three genes involved in recognition and uptake of the ferric siderophore (A1S_2563, A1S_2564 and A1S_2566) and two genes encoding putative efflux pumps. One efflux pump gene, A1S_2565, encodes a putative MFS efflux pump. As mentioned previously, members of this family have been identified in various siderophore gene clusters in other bacteria and have proven to play a role in the efflux of enterobactin [14]. The second efflux pump, A1S_2562 is a member of the multidrug and toxic compound extrusion (MATE) family [43]. To our knowledge this is the first report of a bacterial MATE pump having a putative role in siderophore efflux. Similar to siderophore cluster 1, FUR appears to be the main transcriptional regulator, since binding sites could be identified upstream of A1S_2566, A1S_2567 and A1S_2581 (Figure 6b).

The most extensively characterized *Acinetobacter* siderophore gene cluster is that responsible for biosynthesis of acinetobactin [22]. Acinetobactin is synthesized from a 2,3-dihydroxybenzoic acid, threonine and hydroxyhistamine, and contains catecholate and hydroxamate groups that provide a high affinity for iron [25]. The acinetobactin biosynthesis genes include *basA-D*, *basF- J* and *entE* (Figure 6c). Gene pair *barA* and *barB* encodes a siderophore efflux system of the ABC-superfamily, the products of *bauA-F* form a receptor for recognition of ferric acinetobactin and the products of *bauB-E* are involved in translocation of ferric acinetobactin (Figure 6c). All of these genes showed high levels of overexpression, ranging from 43-fold to 165-fold. The cluster contains putative FUR boxes upstream of *basJ*, *entE*/*basD*, *basA*/*bau* and *bauD*/*basD*. These same FUR binding sites have been experimentally identified using a FUR titration assay [22], validating the FUR analysis described here.

Interestingly, transcriptional up-regulation gradually decreased in all three siderophore clusters when distance from the FUR box increased, demonstrating the importance of FUR in regulating siderophore production

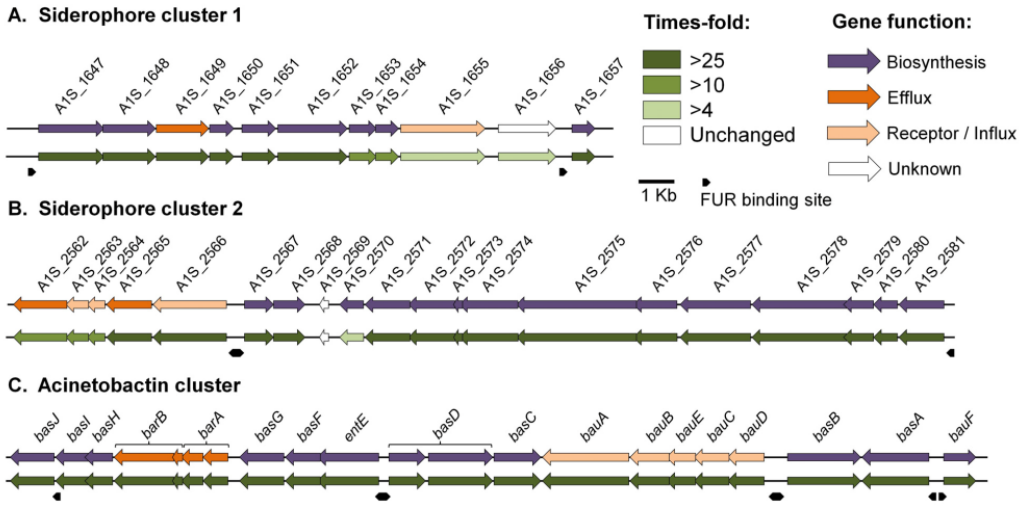

Figure 6 Transcriptional profiling of three siderophore gene clusters identified in *A. baumannii* ATCC 17978. Transcriptional alteration of the three siderophore gene clusters to low iron conditions are shown, (A) siderophore gene cluster 1 (A1S_1647-1657), (B) siderophore cluster 2 (A1S_2562-2581) and (C) the acinetobactin gene cluster (A1S_2372-2392). The top arrows show predicted gene function; siderophore biosynthesis in purple, receptors and uptake mechanisms in light orange, efflux pumps in orange and genes of unknown function in white. The relative transcriptional differences between *A. baumannii* grown under iron replete and iron limiting conditions are depicted in the bottom set of arrows according to the green color scale bar, all values are in times-fold difference. Genes depicted in white were not differentially expressed and those in dark green were overexpressed more than 25-fold. No significant down-regulation was observed within the siderophore gene clusters. Putative FUR boxes are shown as black arrows.

at the level of gene transcription. This is the first time that a full transcriptional profile has been provided for the siderophore gene clusters in *A. baumannii* under iron limiting conditions. Most importantly, a novel putative siderophore gene cluster was identified.

Siderophore receptors expressed on the surface of the bacterial outer membrane play a crucial role in the recognition of iron-loaded siderophores and therefore iron uptake. These receptors are likely to recruit the TonB-ExbB-ExbD translocation system for transport of ferric siderophores from the extracellular space to the cytoplasm [16,44]. The *A. baumannii* ATCC 17978 genome contains 22 putative siderophore receptors. Analysis demonstrated that 15 of these are located downstream of a putative FUR box (Additional file 2) of which 11 were significantly up-regulated under iron limiting conditions.

Under iron limited conditions, high levels of overexpression were also determined for the *tonB-exbB-exbD* gene cluster (A1S_0452-0454) which contained a predicted FUR box. Previously, a second TonB-ExbB-ExbD energy transduction system in strain ATCC 17978 (A1S_1603-1605) was described [23]. The cluster restored enterobactin utilization in *E. coli exbBD* mutants, but not in *tonB* mutants. However, in our study, no significant transcriptional up-regulation of the genes within this cluster (A1S_1603-1613) was observed under iron limiting conditions. It is possible that the cluster is related to heme acquisition rather than siderophore mediated iron uptake, since genes related to hemophore utilization were located adjacent to this cluster. A heme receptor/reduction mechanism may not be required under the conditions tested in our study, since no hemophores are being synthesized by *A. baumannii* ATCC 17978 and no exogenous hemophores were present.

Investigation of motility under iron limiting conditions

It is well established that pili play an important role in the pathogenicity of bacteria due to their roles in motility, adherence, invasion and resistance [45,46]. Grouping transcriptome results from this study by COG function showed that 18% of the genes related to motility were significantly down-regulated under iron limiting conditions (Figure 4). The down-regulated genes from this group are part of the chaperone-usher pili assembly systems (type I pili) and type IV pili that have been previously identified in *A. baumannii* [47].

Homologous features have been associated with biofilm formation and motility in various organisms, including *E. coli* and *P. aeruginosa* [48-50].

In *A. baumannii*, biofilm formation on abiotic surfaces has been linked to a type I pili encoded by *csuAB-E* [6]. The CsuA/B, CsuA, CsuB and CsuE proteins are predicted to form part of the type I pili rod [6,46]. CsuC forms a periplasmic chaperone protein that accelerates folding of the pilus rod subunits and CsuD is an outer membrane protein (OMP) responsible for assembly and extension of the pilus [6,46]. CsuD shares 40% and 45% amino acid sequence similarity with the OMP of two other type I pili mechanisms in strain ATCC 17978, A1S_1508 and A1S_2089, respectively. *csuC* and homolog A1S_1509 were both down-regulated under iron starvation, by 2.0-fold and 3.7-fold, respectively (Figure 7a). The *csuB* and *csuE* homologs within this second cluster, A1S_1507 and A1S_1510, respectively, were also significantly down-regulated under iron limitation. There were no genes down-regulated in the third type I pili cluster (A1S_2088-2091). It has been shown that transcription of the *csu* cluster in *A. baumannii* is controlled by the BfmRS two-component regulator [51]. However, no significant differential expression was observed for either gene encoding this system in this study. Biofilm assays were performed under iron replete and iron limited conditions in order to assess the impact of down-regulation of the *csu* cluster in the formation of these structures. However, no significant differences were observed between planktonic growth and biofilm formation under iron limiting or replete conditions (data not shown). A similar study with *P. aeruginosa* on the effect of iron limitation on biofilm formation showed that growth as a biofilm was impaired to greater extent than planktonic growth [52]. Interestingly, it was also shown that twitching motility was enhanced when iron was less readily available [52], which could correlate with the overexpression of the type IV pili observed in *Moraxella catarrhalis* under iron limitation [53]. These findings indicate that binding and adherence characteristics follow different regulatory pathways in *A. baumannii*.

Various genes involved in the biosynthesis of type IV pili, including *pilB-D*, *pilT*, *pilU*, *comM-O*, *comL*, *comQ* and genes that play a role in chemosensory and regulation of this complex, *pilG-J*, *pilR*, *pilS* and the *chpA*-like, were down-regulated under iron limitation in strain ATCC 17978 (Figure 7b). In *P. aeruginosa*, type IV pili have proven to play a role in swarming motility, a form of migration over nutrient rich semi-solid surfaces [54,55]. Previous studies on the type IV secretion mechanism in *Acinetobacter* have been predominantly related to its function in DNA acquisition [56,57]. A swarming phenotype on Luria-Bertani medium containing a low percentage (0.25%) of agar was determined for *A. baumannii* strain ATCC 17978 (Eijkelkamp *et al.*, unpublished data). The effect of iron limitation on swarming motility was investigated by supplementation of DIP to the swarming medium. Strain ATCC 17978 was found to be incapable of migrating over the surface of the semi-solid medium when 200 μM DIP was supplemented in the medium, whereas non-migrational growth remained largely unchanged (Figure 8). Since bacterial motility is a high energy consuming process, the inability to migrate may be a stress response of *A. baumannii* ATCC 17978 to low iron levels.

Comparative analysis of the iron acquisition mechanisms of sequenced *Acinetobacter* isolates

The iron uptake machinery encoded by different *A. baumannii* strains may differ, as variation in the composition of siderophore mediated iron uptake proteins in the outer-membrane has been shown in a study on different *Acinetobacter* strains [20]. Moreover, a siderophore gene cluster found in *A. baumannii* 8399 (*om73 - entD*) could not be identified in any other sequenced *Acinetobacter* strain [21]. To explore this possibility in more detail comparative analyses of siderophore gene clusters were conducted using 10 fully sequenced *Acinetobacter* genomes, including *A. baumannii* strains ATCC 17978, ATCC 19606, AYE, AB0057, ACICU, 307-0294, D1279779, WM99c and SDF, and *A. baylyi* strain ADP1.

The novel putative siderophore cluster 1 (A1S_1647-1657) was found to be well conserved between strains ATCC 17978, ATCC 19606, AYE, AB0057, ACICU, 307-0294, D127 and WM99c (Table 2). Interestingly, the boundaries of this cluster, including genes orthologous to A1S_1647 and A1S_1657 that encode proteins with homology to the siderophore biosynthesis proteins IucA/IucC and acetyltransferase, respectively, were identified in strain SDF but the intervening genes appear to have been replaced by a 3.5 kb transposon encoding a transposase of the IS5 family (Figure 9). Well over 100 copies of this insertion sequence are found throughout the SDF genome and, along with other insertion sequence elements, are known to have played a major role in genome reduction in this environmental *A. baumannii* strain [47]. No other putative siderophore biosynthesis gene clusters were identified in strain SDF. Therefore, as a result of this insertion, this strain does not appear to encode any siderophore mediated iron acquisition mechanisms. This may be one reason that this strain has a higher requirement for soluble iron than other *A. baumannii* strains, as outlined below. Cluster 1 could also be found in *A. baylyi* APD1, however, it lacked a putative membrane protein (A1S_1656)

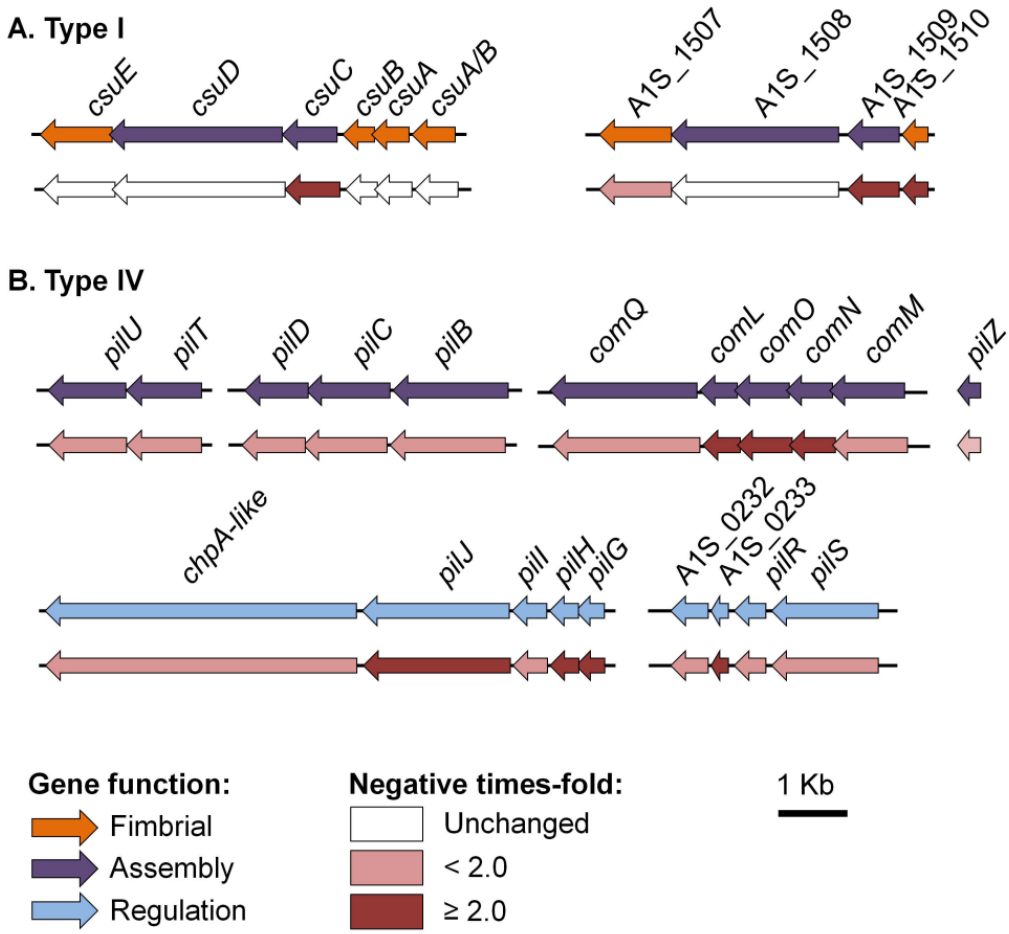

Figure 7 Gene clusters with a putative role in motility. Many genes in the motility COG were found down-regulated including genes of (A) type I and (B) type IV pili. The top set of arrows show predicted gene function; pilus proteins in orange, pilus assembly proteins in purple, and regulatory proteins in light blue. The relative transcriptional differences between *A. baumannii* grown under iron rich and iron limiting conditions is depicted in the bottom set of arrows, all values are in times-fold. Genes depicted in white were not differentially expressed, those shaded in light red were down-regulated less than 2-fold, whereas those in dark red were down-regulated 2-fold or more. No significant up-regulation was observed within gene clusters related to motility.

and acetyltransferase (A1S_1657). Instead, the ADP1 cluster contained a putative acetyltransferase (ACIAD2117) inserted between orthologs of A1S_1654 and A1S_1655 (Figure 9). The putative membrane protein A1S_1657 is most likely involved in recognition of the chelated siderophore, however, this role could be fulfilled by other ferric siderophore receptors, encoded distally in the genome. Whereas, cluster 1 is in the same genomic position in all *A. baumannii* strains, in *A. baylyi* ADP1 it appears to have been subjected to genomic rearrangement as it is located in a different position.

Of the 10 *Acinetobacter* strains surveyed, siderophore cluster 2 was only detected in ATCC 17978 and ADP1. Additionally, no positive hits for A1S_2562, a gene within this siderophore cluster, were identified in 59 clinical *Acinetobacter* isolates from widespread locations in Australia using PCR screening (data not shown). Therefore, this cluster appears to be relatively rare

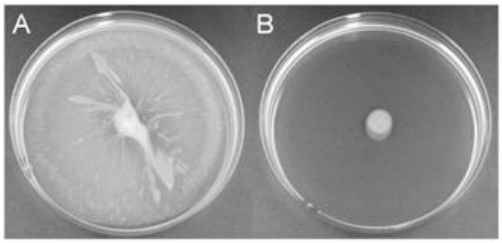

Figure 8 Swarming of *A. baumannii* ATCC 17978. *A. baumannii* colony material was spotted on Luria-Bertani medium containing 0.25% agar. Swarming motility (A) is visible as the channel-like growth around the dense white colony material. The absence of halo growth around the colony (B) indicates lack of swarming motility of *A. baumannii* when available iron is limited.

across the *Acinetobacter* genus. The average homology between the ATCC 17978 and ADP1 siderophore cluster 2 genes was 75%, which is high compared to orthologous genes elsewhere within these two genomes, e.g. 53% within siderophore cluster 1. Transposases were found at the termini of siderophore cluster 2 in both ATCC 17878 and ADP1, suggesting that this gene cluster may have been horizontally acquired. Nonetheless, these transposases are distinct and have inserted into distinct genomic positions in the two strains, suggesting that siderophore cluster 2 was incorporated into the two genomes in separate transfer events. No other hits were obtained in a BLASTn search of the GenBank database with cluster 2, therefore, the origin of this cluster remains unknown.

A high level of conservation was observed for the acinetobactin gene cluster among most *A. baumannii* isolates (Table 2). However, this cluster was not seen in the SDF and ADP1 strains. A fifth cluster was identified by BLASTp searches in several sequenced *A. baumannii* strains (considering the *A. baumannii* 8399 siderophore cluster as the fourth *Acinetobacter* siderophore gene cluster) (Table 2). The genes are represented in strain AYE by ABAYE1888 and ABAYE1889. A putative FUR box could be identified upstream of ABAYE1889 and high expression levels were observed under iron limiting conditions using qRT-PCR analysis (data not shown). The two genes encode proteins that were found to be homologous to an isochorismatase and a 2,3-dihydro-2,3-hydroxybenzoate dehydrogenase. The product synthesized by these two enzymes in this cluster, 2,3-dihydroxybenzoate, is an iron binding compound, but also a precursor component for more complex siderophores, like acinetobactin. This cluster is well conserved between strains AYE, AB0057, ACICU, 307-0294, ATCC 19606, D1279779 and WM99c.

The minimum inhibitory concentration (MIC) of DIP was determined for seven of the strains included in the genetic comparison (*A. baumannii* strains ATCC 17978, ATCC 19606, AYE, D1279779, WM99c and SDF, and *A. baylyi* strain ADP1). Growth of *A. baylyi* strain ADP1 and *A. baumannii* SDF was inhibited at lower levels compared to other strains. ATCC 17978 did not show higher MIC values for DIP compared to AYE, WM99c or D1279779, despite having three highly expressed siderophore gene clusters. Therefore, viability of *Acinetobacter* strains under varying iron concentrations does not appear to directly correlate with the presence or absence of siderophore gene clusters. Strains with higher OD_{600} values under iron replete conditions showed higher MIC levels for DIP.

A second FUR-like transcription repressor

Various bacterial genomes contain multiple *FUR*-like genes. These *FUR* homologs often encode repressors with similar domains but with higher affinity for metals

Table 2 Genomic comparison of siderophore gene clusters in sequenced *Acinetobacter* isolates

		Cluster 1 A1S_1647-1657	Cluster 2 A1S_2562-2581	Acinetobactin A1S_2372-2392	Cluster 4 *om73-entD*	Cluster 5 ABAYE1888 and ABAYE1889
A. baumannii	ATCC 17978	+	+	+	-	-
	ATCC 19606	+	-	+	-	+
	AYE	+	-	+	-	+
	AB0057	+	-	+	-	+
	ACICU	+	-	+	-	+
	307-0294	+	-	+	-	+
	D1279779	+	-	+	-	+
	WM99c	+	-	+	-	+
	SDF	-	-	-	-	-
	8399	nd	nd	nd	+	nd
A. baylyi	ADP1	+	+	-	-	-

nd = not determined.

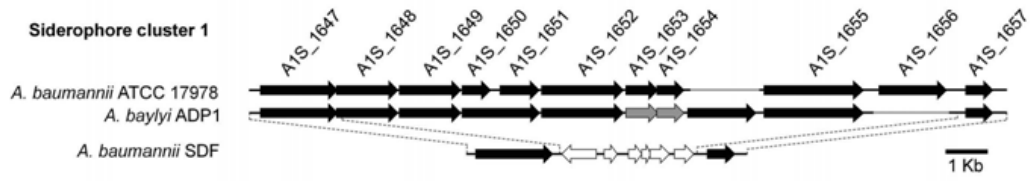

Figure 9 Comparison of siderophore cluster 1 in sequenced *Acinetobacter* isolates. The alignment of siderophore cluster 1 between strain ATCC 17978, ADP1 and SDF. Arrows indicate open reading frames, in black genes with high homology (> 50% identity) and in grey genes with low homology (< 50% identity). White genes represent a 3.5 Kb transposon insertion, which replaced the A1S_1648-1656 orthologs in strain SDF.

other than iron, such as zinc, manganese or nickel [58]. The *A. baumannii* AYE genome also encoded a second FUR-like regulator that has not yet been characterized, ABAYE1887. This gene was located adjacent to the putative siderophore genes described above (ABAYE1888 and ABAYE1889). Conserved domain (CD)-searches showed the highest homology with cd07153 (E = $1e^{-10}$), which can be found in FUR and other metalloregulatory proteins. A putative zinc uptake regulator (ZUR) can be found in a zinc-uptake gene cluster in strain AYE (ABAYE3726). Pairwise alignment of ABAYE1887 and *FUR* (ABAYE2920), and ABAYE1887 and *ZUR*, indicated higher homology for ABAYE1887 to *FUR* than to *ZUR*, showing 46% and 33% similarity, respectively. Little is known about auto-regulation of *FUR* homologs. In the case of ABAYE1887, a FUR box with a low p-value can be found less than 200 bp upstream of the start codon using the ATCC 17978 optimized FUR motif. Moreover, qRT-PCR analysis demonstrated that growth of *A. baumannii* AYE under iron limiting conditions resulted in approximately 164-fold up-regulation of ABAYE1887, whereas, *FUR* only showed 1.6-fold up-regulation. This second FUR-like gene can also be found in strains ATCC 19606, D1279779 and WM99c, and a truncated form in SDF. Further experimental work is required to determine if ABAYE1887 plays a role in transcription of genes related to iron acquisition.

Conclusions

This study defined the global transcriptional response of *A. baumannii* to iron starvation. The up-regulation of three siderophore mediated iron acquisition systems was the predominant feature of this transcriptional response. The high level of overexpression of these systems under iron limitation, suggests that each is active in mediating iron uptake and therefore likely to be of importance to *A. baumannii* for survival in iron limited environments, such as human hosts. Several genes involved in other processes, such as respiration and electron transport were also significantly differentially expressed. Our data corroborate results from a recently published proteomic study of *A. baumannii* under iron rich and iron limiting conditions [59], such as up-regulation of the iron acquisition mechanisms and *fumC*, and the down-regulation of *fumA* and *ompW*. The abundance of putative FUR binding sites identified upstream of up-regulated genes highlighted a major role for this regulator in transcriptional up-regulation under iron limiting conditions. Various genes of the type IV pili were down-regulated under iron limiting conditions. This may in fact explain the inability of strain ATCC 17978 to migrate on semi-solid surfaces under low iron concentrations. Overall, the results indicated that *A. baumannii* is adaptable to an environment with limiting iron availability.

Methods

Bacterial strains and growth conditions

Acinetobacter strains were obtained from the following sources: ATCC 17978 [GenBank: NC_009085] and 19606 [GenBank: NZ_ACQB00000000] from the American Type Culture Collection (ATCC); SDF [GenBank: NC_010400] from the Collection de Souches de l'Unité des Rickettsies (CSUR), Marseille, France; AYE [GenBank: NC_010410] from Patrice Nordmann, Dept. Bacteriologie-Virologie, Hopital de Bicetre, Le-Kremlin-Bicetre, France; WM99c and ADP1 [GenBank: NC_005966] from Jon Iredell, Westmead Millennium Institute, Sydney, Australia; and D1279779 from The Menzies School of Health Research, Darwin, Australia. The ATCC 19606 genomic sequence was obtained from the NCBI REFSEQ database. *A. baumannii* strains D1279779 and WM99c were sequenced recently by 454 pyrosequencing (Paulsen *et al.*, unpublished data). These Whole Genome Shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers AERY00000000 for WM99c and AERZ00000000 for D1279779. The versions described in this paper are the first versions, AERY01000000 and AERZ01000000, respectively. The scaffold sequences of these three strains were tiled to the ATCC 17978 genome using Mauve [60].

Iron limitation was achieved by growing *Acinetobacter* strains in Mueller-Hinton (MH) medium supplemented with 2,2'-dipyridyl (DIP). The growth curves were obtained by culturing *A. baumannii* ATCC 17978 with different concentrations of available iron in Mueller-Hinton (MH) medium; untreated, 100 μM DIP, 200 μM DIP and 300 μM DIP. In all following experiments, cultures in MH were supplemented with 200 μM DIP.

Microarray development

An 8 × 15 K custom genomic microarray was developed for *A. baumannii* ATCC 17978 on the Agilent platform using the Agilent eArray package http://earray.chem.agilent.com/earray/. At least four 60 mer DNA oligonucleotides with an average GC % of 41.5, were incorporated into the design for each of the protein coding genes annotated in the ATCC 17978 genome sequence [61]. The array also included a set of intra array controls; 132 probes replicated at least 10 times in the design, and the Agilent control spots.

RNA isolation

Cells cultured under iron replete, untreated MH, and iron limited conditions of MH with 200 μM DIP, were grown until they reached mid-log phase (OD_{600} = 0.7). The cells were pelleted and lyzed in 1 mL TRIzol® reagent (Invitrogen, Australia). Following phase separation, RNA was extracted from the aqueous phase using the PureLink™ Micro-to-Midi Total RNA Purification kit (Invitrogen), incorporating an on-column DNAseI (Invitrogen) digestion, as per manufacturer's recommendations.

cDNA synthesis and microarray hybridization

For the microarray analyses, the cDNA synthesis, labelling and hybridizations were conducted at the Ramaciotti Centre for Gene Function Analysis, University of NSW, Australia. Total RNA was reverse transcribed and labelled with either Cy3 or Cy5 using the Agilent Fairplay Microarray Labelling kit (Stratagene). Labelled cDNA samples were hybridized to a custom designed 8 × 15 K two colour gene expression microarray slide. The results reported are based on three biological and four technical repeats, including one dye-swap experiment. Statistical analysis was performed on $\log_2$-transformed signal ratios of the replicate spots using the SAM algorithms [27]. All results described were found to be significant using a false discovery rate of less than 5% unless otherwise indicated. All microarray data presented are in accordance with the Microarray Gene Expression Data Society's minimum information about microarray experiment recommendations [62]. Descriptions of the microarray experiments, quantification data and array design have been deposited into GEO http://www.ncbi.nlm.nih.gov/geo/ and can be accessed using the accession number GSE24921.

Quantitative RT-PCR

Validation of the microarray results, preliminary experiments with *FUR* and transcription level measurements of *A. baumannii* strain AYE were performed using a two-step quantitive RT-PCR (qRT-PCR). RNA isolation was performed as described above. cDNA was synthesized using random hexamers and SuperscriptII (Invitrogen). Primers were designed to generate 100 - 150 bp amplicons and are listed in Table 3. qPCR was performed using Sybr Green mastermix (Invitrogen). Transcriptional differences were calculated using the $\Delta\Delta C_t$ method [63].

A. baumannii FUR binding site analysis

A scoring matrix was defined from the 48 experimentally determined *E. coli* FUR binding sites [34] using the Multiple Em for Motif Elicitation (MEME) tool. The *A. baumannii* ATCC 17978 genome was analysed with the resulting scoring matrix using the Motif Alignment and Search Tool (MAST). Putative FUR binding site sequences that are located within the 200 bp region upstream a start codon, with a p-value of less than $10e^{-5}$ and of which the downstream gene showed more than 4-fold up-regulation were further investigated. Subsequent MEME and MAST analyses with the described criteria were performed until no new positive hits were obtained. The resulting 21 putative FUR binding site

Table 3 Oligonucleotides used in the study

Locus tag	Forward primer (5'-3')	Reverse primer (5'-3')
A1S_r01*+	CAGCTCGTGTCGTGAGATGT	CGTAAGGGCCATGATGACTT
A1S_2501+	CAACACTGGTAAATGGCGTG	ACAACGTTTTTCATTTCGCC
A1S_0395	TCATGCTCTTGTTCAGTGGC	GCATTGCCAATACCCCTAGA
A1S_3420	GCCTTGCTTTACTTGTTCCG	GCATCAGTAAATGGGCAGGT
A1S_2562	TTGCCATCAGTAGTGCAACC	TCCTGCAATCACAACACCAT
A1S_3371	CAGATCCAACTGTGGTGGTG	TCAGCATCGGTACGGTTACA
A1S_0895	GCGCAAAGCTGGACTTAAAG	CGGTAAACTGTCGCAAGTCC
A1S_2565	TGGCTCGATATTCAACGTCA	TAACAGCAAACCACCACCAA
A1S_0897	CCGCGAGCGACTAAGC	TGTCGCAGCCCATGAA
A1S_1647	GGACGCCATCGTCTCG	GCGTCCCGGCTTTGTA
A1S_1925	GGTGGCGCGCTATTTG	GTTGCGCCATTGGGTA
A1S_2080	GGTCGATGGCGTTCCA	CAGCCGCTTTCGTGGT
A1S_3195	GCGCTCAACCGCGTAA	TGCCGGATCGTCTTGC
A1S_1509	CCAAGGAAGGCGCTGT	TTGGGGAATGGCTTGC
ABAYE1887	CCCTTTTGATGATTTTACGG	CAAGGCTTAAGCGCGGTA
ABAYE1888	CCAGCGCATCACCACA	TCCGCTCGAACAACTCA
ABAYE1889	GGGGCGATTTCAAGTGC	TCGCGATCAGCCAACA

* Primer sequences obtained from Higgins *et al.*, 2004 [66].
+ Oligonuduotides used as references.

sequences were aligned using Weblogo 3.0 [64] to create the optimized *A. baumannii* FUR motif.

Swarming motility assay

Swarming motility assays were performed at 37°C on Luria Bertani (LB) medium containing 0.25% agar. Positive swarmers showed a halo growth zone with channel-like structures.

Static biofilm formation assay

The static biofilm formation assay was performed as described previously [65] with minor modifications. MH broth was inoculated with bacterial colony material and incubated overnight at 37°C. The cultures were subsequently diluted 1:100 in fresh MH broth in polystyrene microtiter trays and incubated ON at 37°C. Adherent cells were washed once with PBS, stained by incubation with 0.1% crystal violet for 30 min at 4°C, and washed 3X with PBS. Dye was released from the cells using ethanol:acetone (4:1) and shaking at 200 rpm for 30 min at RT. Absorbance was measured at 595 nm on a Fluostar Omega spectrometer (BMG Labtech, Offenburg, Germany). The biofilm data represent the average of at least 3 independent experiments of triplicate wells.

Additional material

Additional file 1: Significance analysis of the microarray results. Significant differentially expressed genes in the microarray, determined by SAM [27].

Additional file 2: ATCC 17978 FUR binding sites. A list of putative FUR binding sites in the *A. baumannii* ATCC 17978 genome determined by MAST with E < 100.

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Author details

[1]School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia. [2]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia.

Authors' contributions

BAE, KAH, ITP and MHB designed the research project. BAE and KAH carried out the experiments and BAE, KAH, ITP and MHB wrote the manuscript. All authors have read and approved the final manuscript.

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Appendix G – Eijkelkamp *et al.* 2011; FEMS Microbiology Letters

FEMS MICROBIOLOGY LETTERS

RESEARCH LETTER

Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates

Bart A. Eijkelkamp[1], Uwe H. Stroeher[1], Karl A. Hassan[2], Michael S. Papadimitrious[1], Ian T. Paulsen[2] & Melissa H. Brown[1]

[1]School of Biological Sciences, Flinders University, Adelaide, SA, Australia; and [2]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia

Correspondence: Melissa H. Brown, School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia. Tel.: +61 8 8201 2747; fax: +61 8 8201 3015; e-mail: melissa.brown@flinders.edu.au

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twitching; swarming; eukaryotic cell adherence; biofilm formation; type IV pili; Australian clinical strains.

Abstract

Acinetobacter baumannii continues to be a major health problem especially in hospital settings. Herein, features that may play a role in persistence and disease potential were investigated in a collection of clinical *A. baumannii* strains from Australia. Twitching motility was found to be a common trait in *A. baumannii* international clone I strains and in abundant biofilm formers, whereas swarming motility was only observed in isolates not classified within the international clone lineages. Bioinformatic analysis of the type IV fimbriae revealed a correlation between PilA sequence homology and motility. A high level of variability in adherence to both abiotic surfaces and epithelial cells was found. We report for the first time the motility characteristics of a large number of *A. baumannii* isolates and present a direct comparison of *A. baumannii* binding to nasopharyngeal and lung epithelial cells.

Introduction

Acinetobacter baumannii is an emerging opportunistic pathogen widely distributed in hospital settings. Its ability to survive in adverse conditions and expression of significant levels of antibiotic resistance have made this a difficult pathogen to treat (Bergogne-Berezin & Towner, 1996; Dijkshoorn *et al.*, 2007; Peleg *et al.*, 2008). To date, little is known about the survival and persistence strategies of this organism or whether these strategies are universally applied in all clinical isolates. Three clonal groups designated international clone I, II and III, have been defined and together form the majority of clinical *A. baumannii* strains found in Europe. The existence of international clone I and II *A. baumannii* isolates in Australia has previously been shown (Post & Hall, 2009; Post *et al.*, 2010; Runnegar *et al.*, 2010), however, no data are available in respect to the prevalence of these widespread lineages throughout Australia.

Although, historically the *Acinetobacter* genus is described as non-motile, which is related to the lack of flagella and therefore its inability to swim (Baumann *et al.*, 1968), various studies have shown motility of isolates that belong to the *Acinetobacter calcoaceticus-baumannii* complex (Barker & Maxted, 1975; Henrichsen, 1975, 1984; Mukerji & Bhopale, 1983). More recently, motility of *A. baumannii* strain ATCC 17978 was found to be inhibited by blue light and by iron limitation (Mussi *et al.*, 2010; Eijkelkamp *et al.*, 2011). Interestingly, reduced iron levels resulted in down-regulation of several genes that encode the type IV pili system (Eijkelkamp *et al.*, 2011), a system that may function in *A. baumannii* motility. Indeed, a study by Henrichsen and Blom demonstrated a correlation between the presence of fimbriae and motility exhibited by isolates belonging to the *Acinetobacter calcoaceticus-baumannii* complex (Henrichsen & Blom, 1975). Bacterial motility has been linked to increased virulence in various bacteria, such as *Pseudomonas aeruginosa* and *Dichelobacter nodosus* (Han *et al.*, 2008; Alarcon *et al.*, 2009). Nonetheless, to date, the role of motility in virulence of *A. baumannii* has not been described.

Another factor that may influence the success of *A. baumannii* as a pathogen is its ability to adhere to abiotic surfaces, which has been examined by a number

of groups (Cevahir *et al.*, 2008; Lee *et al.*, 2008; de Breij *et al.*, 2010). Initial attachment to abiotic surfaces is the first step for colonization and subsequent biofilm formation on medical devices, such as ventilator tubing and catheters. The Csu type I pilus, the biofilm-associated protein, outer membrane protein A (OmpA) and production of poly-beta-1-6-*N*-acetylglucosamine appear to be involved in this process (Tomaras *et al.*, 2003; Loehfelm *et al.*, 2008; Choi *et al.*, 2009; Gaddy *et al.*, 2009). Another critical step in the pathogenesis of *A. baumannii* is the ability to adhere to eukaryotic cells; studies examining adherence to cell lines have revealed a high level of variability between isolates in their binding capacity (Lee *et al.*, 2008; de Breij *et al.*, 2010).

In this study the clonal groupings of 50 clinical *A. baumannii* strains isolated from diverse settings were determined and two distinct forms of motility, twitching and swarming, were investigated. Furthermore, the capacity of these isolates to adhere to both abiotic and biotic surfaces is reported. Within the fully sequenced strains, this phenotypic information was examined in the context of gene content in an attempt to delineate the molecular factors directing these characteristics.

Materials and methods

Strain collection

The 52 clinical Australian *Acinetobacter* strains (50 *A. baumannii*, 1 *Acinetobacter* gen. sp. 13TU and 1 *Acinetobacter* gen. sp. 3) were isolated and identified by hospital-associated diagnostic laboratories including; Flinders Medical Centre, Flinders Private Hospital, Royal Adelaide Hospital, Westmead Hospital, Prince of Wales Hospital, Royal Brisbane & Women's Hospital and The Menzies Darwin. Two *A. baumannii* isolates, D1279779 and WM99c, were recently sequenced by our groups (D Farrugia, KA Hassan, LDH Elbourne, BA Eijkelkamp, MH Brown & IT Paulsen, unpublished data) and whole genome shotgun sequence data are available from the NCBI WGS database under the accession numbers AERZ00000000 and AERY00000000, respectively. The following *A. baumannii* reference strains were included in the characterization; AB0057 (CP001182) (Adams *et al.*, 2008), AYE (CU459141) (Fournier *et al.*, 2006), ATCC 19606 (NZ_ACQB00000000) and ATCC 17978 (CP000521) (Smith *et al.*, 2007). The ATCC strains 17978 and 19606 were purchased from the American Type Culture Collection. Strain AB0057 and AYE were obtained from A/Prof. Robert A. Bonomo (Veterans Affairs Medical Center, Cleveland, Ohio, USA) and Prof. Patrice Nordmann (Hopital de Bicetre, Le-Kremlin-Bicetre, France), respectively.

International clone determination

Identification of *ompA*, *OXA51*-like and *csuE* allelic variants was performed as described previously (Turton *et al.*, 2007), using a multiplex PCR-based screening method. Strains were assigned to the international clone complex based on the obtained PCR pattern as defined by Turton *et al.* (2007).

Motility assays

Twitching motility was investigated as previously described (Semmler *et al.*, 1999). In brief, one overnight (ON) grown colony was collected with a sterile toothpick and stabbed through Mueller-Hinton (MH) medium containing 1% agar to the bottom of the Petri dish. Plates were subsequently incubated ON at 37 °C. Positive twitchers were defined as those strains that showed a zone of >10 mm around the site of inoculation. To investigate swarming motility, one colony was transferred to Luria-Bertani (LB) medium containing 0.25% agar and incubated ON at 37 °C. Positive swarmers showed a halo growth zone of >20 mm. The motility assays were repeated for those strains where no motility phenotype was observed.

Adherence assays

The static biofilm formation assay was performed as described previously (O'Toole *et al.*, 1999), with minor modifications. MH broth was inoculated with one colony and incubated ON at 37 °C. Cultures were subsequently diluted 1 : 100 in fresh MH broth in polystyrene microtitre trays and incubated ON at 37 °C. Adherent cells were washed once with phosphate buffered saline (PBS), stained by incubation with 0.1% crystal violet for 30 min at 4 °C, and washed three times with PBS. Dye was released from the cells using ethanol:acetone (4 : 1) and shaking at 200 rpm for 30 min at room temperature. Absorbance was measured at 595 nm on a FLUOstar Omega spectrometer (BMG Labtech, Offenburg, Germany). The biofilm data represent the average of at least three independent experiments of triplicate wells. Planktonic-growing bacteria were removed and the $OD_{600\ nm}$ was determined to ensure strains did not show a growth defect.

Adherence of *A. baumannii* strains to A549 cells (human type 2 pneumocytes) (Giard *et al.*, 1973) and Detroit 562 cells (human nasopharyngeal cells) (Peterson *et al.*, 1968) was determined essentially as described elsewhere (Talbot *et al.*, 1996). Cell lines were grown in Dulbecco's Modified Eagle medium (Invitrogen, Australia) supplemented with 10% foetal bovine serum (Bovogen,

Australia). Prior to use, the cell monolayer was examined microscopically to ensure >95% coverage. Washed A549 or Detroit monolayers, in 24-well tissue culture plates, were subsequently infected with a bacterial inoculum containing ~1 × 10^7 colony forming units (CFU). The inoculum numbers were subsequently determined by viable count assays. After incubation at 37 °C for 4 h, culture medium was removed, and the monolayers washed three times with 1 mL of PBS. The cell monolayers were detached from the plate by treatment with 100 μL of 0.25% trypsin and 0.02% EDTA in PBS. Eukaryotic cells were subsequently lysed by the addition of 400 μL 0.025% Triton X-100, and serial 10-fold dilutions thereof were plated on LB agar to determine the number of CFU of adherent bacteria per well. The collated data for the adherence assay were obtained from at least three independent experiments and represent the data points for each experiment of quadruplicate wells.

Statistical analysis

All statistical comparisons were based on the Student's *t*-test (two-tailed).

Results

Strain selection and clonality

A total of 52 randomly selected Australian clinical *Acinetobacter* strains were used in this study of which 50 were *A. baumannii* isolates, one *Acinetobacter* gen. sp. 13TU (WM98b) and one *Acinetobacter* gen. sp. 3 (WM97b). Four non-Australian *A. baumannii* reference strains were also included in the characterization; AB0057 (Adams *et al.*, 2008), AYE (Fournier *et al.*, 2006), ATCC 19606 and ATCC 17978 (Smith *et al.*, 2007).

The clonal groupings amongst clinical *A. baumannii* strains were investigated by determining the presence of *ompA*, *csuE* and $bla_{OXA\text{-}51\text{-}like}$ allelic variants as described previously (Turton *et al.*, 2007). Interpretation of the amplification profiles obtained using the two multiplex PCRs showed that 12% of the *A. baumannii* isolates studied belonged to international clone group I ($n = 6$), 64% to international clone group II ($n = 32$) and 24% were found to not be part of either of these clonal lineages ($n = 12$) (Fig. 1). No strains were found to belong to international clonal lineage III.

Motility of *A. baumannii*

It was found that three noninternational clone type *A. baumannii* strains and the *Acinetobacter* gen. sp. 13TU strain WM98b had the ability to migrate on semi-solid agars (Fig. 1). This form of surface translocation was designated as swarming, as proposed by Kaiser (Kaiser, 2007). Swarming motility was investigated on different media, LB, MH and M9, and at varying temperatures, 25, 30 and 37 °C. All swarming strains displayed a more pronounced motile phenotype on semi-solid LB media incubated at 37 °C. We also found that swarming occurred at a higher rate on media with lower agar percentages. The lowest tested concentration of agar was 0.25%.

Various other *Acinetobacter* strains, including AYE and AB0057 showed no motility on semi-solid media, however, these strains migrated in the medium-plastic interface of solid media, referred to as twitching motility (Semmler *et al.*, 1999). All strains were investigated for twitching on both LB and MH media. Although some strains had the ability to twitch on LB media, a greater proportion of strains were able to twitch on MH media, no strains were found to only twitch on LB media. Twitching of various representative strains was studied at temperatures of 25, 30 and 37 °C and using varying agar percentages, 0.25%, 0.5%, 0.75% and 1%. These results revealed that twitching occurred at an optimal rate in MH containing 1% agar incubated at 37 °C. All eight international clone I isolates showed a twitching zone of more than 10 mm (defined to be the minimum in this study). Of the strains which exhibited twitching motility, only a subset also displayed swarming motility, and *vice versa* (Fig. 1), highlighting that twitching and swarming represent two distinct phenotypes in *Acinetobacter*.

Adherence to abiotic surfaces and biofilm formation

Using a microtitre plate biofilm assay, a significant level of variation, greater than 10-fold, was observed in the ability of different strains to form biofilms on abiotic surfaces (Fig. 1). Analysis of the biofilm data using a two-tailed Student's *t*-test revealed that international clone I isolates formed less developed biofilms compared to international clone II and noninternational clone isolates ($P < 0.005$ and $P < 0.05$, respectively). No significant difference in biofilm formation was observed between international clone II and noninternational clone isolates.

Adherence to eukaryotic cell surfaces

Two distinct eukaryotic cell types were used to examine adherence, and potentially invasion and intracellular replication, of a selected number of *A. baumannii* isolates. Detroit 562 human nasopharyngeal cells were chosen to mimic adherence/carriage of *A. baumannii* strains in the nasal pharyngeal cavity. The second cell line employed

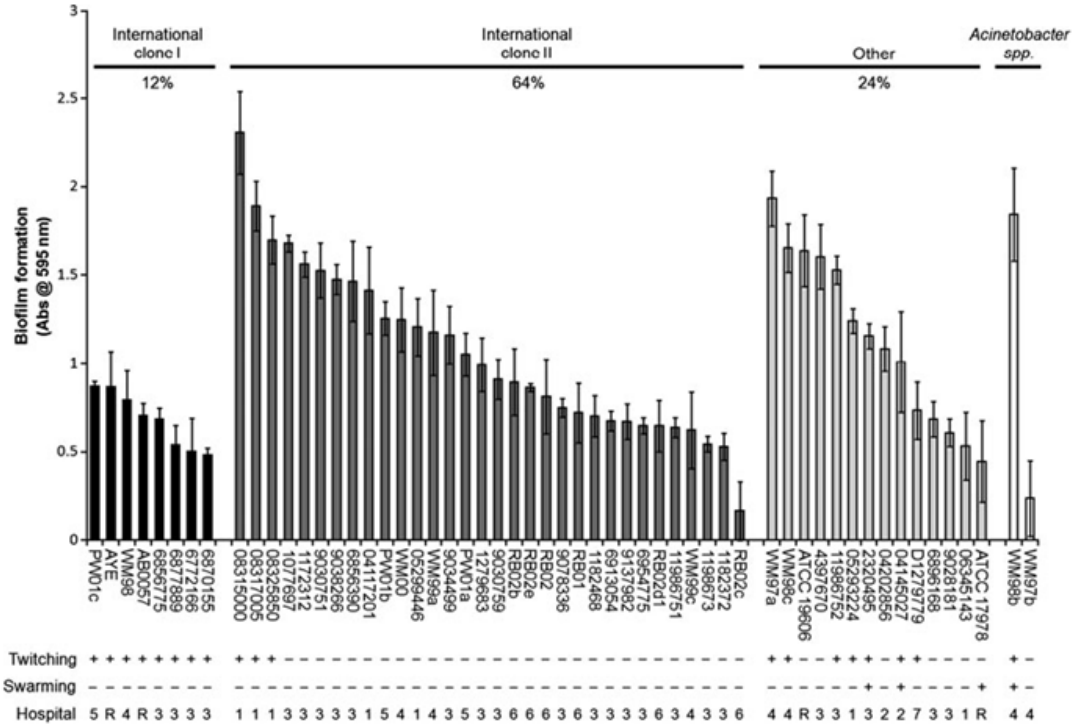

Fig. 1. Characterization of *Acinetobacter* isolates. The graph represents the level of biofilm formation (absorbance at 595 nm), which was investigated in a 96-well microtiter tray using a crystal violet stain method. The error bars represent the standard deviation. All 56 strains were sorted based on species, clonality and biofilm formation; *A. baumannii* international clone I (black), *A. baumannii* international clone II (dark grey), noninternational (other) *A. baumannii* isolates (light grey) and *Acinetobacter* spp. (white). Strains were provided by the following hospitals/hospital-associated laboratories; Flinders Medical Centre (1), Flinders Private Hospital (2), Royal Adelaide Hospital (3), Westmead Hospital (4), Prince of Wales Hospital (5), Royal Brisbane & Women's Hospital (6), The Menzies Darwin (7) and (R) represents the non-Australian reference strains.

was A549 human type 2 pneumocytes, that has previously been used to mimic adherence to the human lung and as such represents a potential model for pneumonia caused by *A. baumannii* (March *et al.*, 2010). The *A. baumannii* isolates selected for cell adherence studies displayed differential abiotic surface adherence and motility characteristics. These studies also included a number of previously studied and published strains. Similar to our data on abiotic adherence, there were significant differences between *Acinetobacter* strains in their capacity to adhere to eukaryotic cells (Fig. 2). For example, differences of more than 17-fold were seen between ATCC 19606 and WM99c when investigating binding to A549 cells. A more than 60-fold difference in adherence to Detroit 562 cells was observed between strains D1279779 and WM97a. Examination of the ability of differing clonal groups to adhere to the eukaryotic cells revealed no clonal specific trends. In this study, a significant difference between binding to A549 and Detroit 562 cells was observed for *A. baumannii* strains D1279779 and ATCC 17978 ($P < 0.05$, two-tailed Student's *t*-test). Both of these *A. baumannii* strains showed a higher level of adherence to lung epithelial cells compared to nasopharyngeal cells. All other strains examined have similar levels of binding to the two distinct epithelial cell lines.

Genomic analysis of *A. baumannii* motility and adherence features

The complete genome of a number of *A. baumannii* strains has been sequenced and six of these fully sequenced strains were included in this study. Genomic comparison may prove useful for the identification of the molecular mechanisms involved in the characteristics studied herein. Although limited information is available on the molecular mechanism, type IV pili may play a role

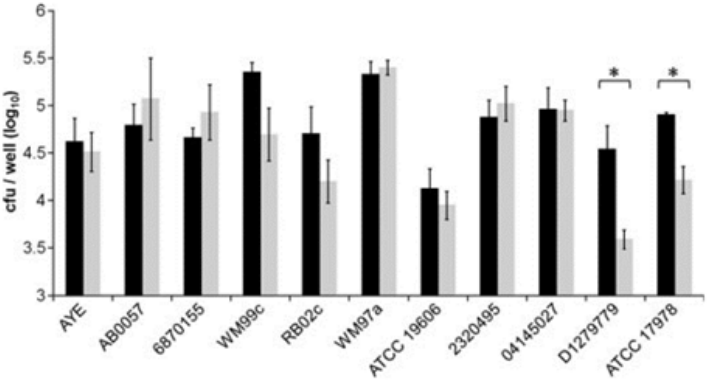

Fig. 2. Adherence to eukaryotic epithelial cells. Depicted are the colony forming units (CFU) in log_{10} values of *Acinetobacter baumannii* cells that adhered to A549 cells (black) and Detroit 562 cells (grey). Strains D1279779 and ATCC 17978 showed significantly lower binding to Detroit 562 cells compared to A549 cells ($P < 0.05$) and are indicated by an asterisk. Statistical analysis was carried out using a two-tailed Student's *t*-test. Error bars show the standard error of the mean.

in *A. baumannii* motility, based on for example, the correlation between the presence of fimbriae and motility in *A. calcoaceticus* (Henrichsen & Blom, 1975) and transcriptional and phenotypic analysis of *A. baumannii* under iron limiting conditions (Eijkelkamp *et al.*, 2011). Moreover, a role for type IV pili in motility of other nonflagellated gamma-proteobacteria, such as *Xylella fastidiosa*, has been reported (Meng *et al.*, 2005; De La Fuente *et al.*, 2007). Comparative genomic analysis using Mauve (Darling *et al.*, 2004) showed that the genes encoding different subunits or regulators as part of the type IV pili were present in all fully sequenced *A. baumannii* isolates included (data not shown). Most genes encoding type IV pili showed a high level of conservation, except for *pilA*, the gene encoding the pilin subunit PilA. In *P. aeruginosa*, a distinctive type of PilA has been linked to an enhanced twitching phenotype and virulence (Stewart *et al.*, 2011). In our study, amino acid sequence analysis revealed the presence of different *A. baumannii* PilA groups (Fig. 3). The isolates within these PilA groups were clonally related and exhibited the same motility characteristics, e.g. the international clone I isolates shared a highly similar PilA amino acid sequence and all exhibited a twitching phenotype. Interestingly, the PilA sequences from other motile bacterial species clustered with PilA from the motile *A. baumannii* isolates, e.g. the *P. aeruginosa* and *D. nodosus* PilA shared the highest homology levels with PilA from international clone I isolates and *X. fastidiosa* PilA with that from ATCC strain 17978.

Linking adherence phenotypes to genotypes was also attempted, as multiple adherence mechanisms have been identified. Although Bap (Loehfelm *et al.*, 2008) showed major sequence variation, no direct link between adherence characteristics and sequence homology could be established. The *pgaABCD* cluster responsible for production of poly-beta-1-6-*N*-acetylglucosamine (Choi *et al.*, 2009),

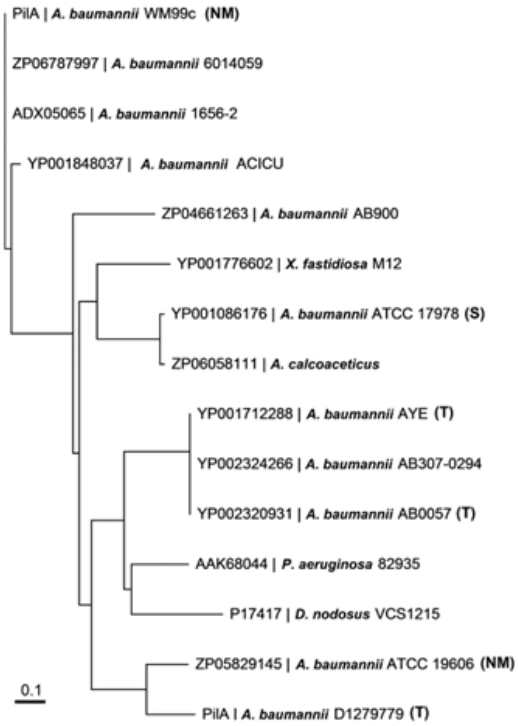

Fig. 3. PilA similarity analysis. PilA amino acid sequences of various sequenced *Acinetobacter* strains were investigated. The PilA sequences from other bacterial species that showed a high level of similarity, *Pseudomonas aeruginosa*, *Xylella fastidiosa* and *Dichelobacter nodosus*, were also included. The sequences were aligned using ClustalW2 and a tree was generated using the Neighbour-joining clustering method. Six strains were investigated for their motility phenotype; twitching (T), swarming (S) or nonmotile (NM).

and *ompA* (Gaddy *et al.*, 2009) displayed a high level of conservation between the investigated strains, therefore, sequence differences that may be linked to a phenotype could not be observed. In total, four different type I pili clusters were identified in the six sequenced strains included in this study; AB57_1744-1747, AB57_2565-2570 (*csu* cluster) (Tomaras *et al.*, 2003), AB57_2420-2423 and AB57_2003-2007. The *csu* gene cluster was well conserved between the strains investigated; however, *csuB* of ATCC 17978 contained a single base-pair (bp) insertion, which resulted in a truncation of the open reading frame. Subsequently, the gap between the *csuB* and *csuC* open reading frames increased from 5 bp to 96 bp. Although transcription is unlikely to be influenced by the single bp insertion, the increase between *csuB* and *csuC* may affect translation of *csuC* and other downstream genes in this operon. Interestingly, this strain showed the lowest level of binding to abiotic surfaces of all *A. baumannii* strains investigated, with the exception of strain RB02c (Fig. 1). The first open reading frame of the AB57_1744-1747 and AB57_2420-2423 polycistronic gene clusters contained homopolymeric tracts of varying lengths, and were therefore reanalysed by Sanger sequencing. Sequence differences were rebutted for AB57_1744_1747 using Sanger sequencing, however, strains ATCC 17978 and ATCC 19606 appeared to have an additional thymine in AB57_2423, which resulted in a frame-shift. However, even with this additional information, no direct correlation could be determined between the presence of type I pili clusters AB57_1744-1747, AB57_2420-2423 or AB57_2003-2007 and adherence to either biotic or abiotic surfaces.

Discussion

The Australian clinical *A. baumannii* isolates showed a similar clonal distribution to that found in Europe, viz., a high prevalence of international clone I and II strains (reviewed in Peleg *et al.*, 2008). However, no international clone III isolates were identified in this study. Since bacterial motility is a known virulence factor in numerous bacterial species (Han *et al.*, 2008; Alarcon *et al.*, 2009; Proft & Baker, 2009), the motility potential of our 52 clinical isolates was examined. The motility phenotypes in this study were determined using the general classifications for both swarming and twitching (Semmler *et al.*, 1999; Kaiser, 2007). Our data revealed that all international clone I isolates showed significant twitching. A number of other twitching isolates, not part of this clonal lineage, had the ability to form well developed biofilms compared to the international clone I isolates (see below), with the exception of *A. baumannii* strain D1279779. This relatively poor biofilm former ($OD_{595\ nm}$<1) also showed a small twitching zone (approximately 12 mm). Swarming motility was observed in three noninternational clone isolates, including *A. baumannii* ATCC 17978, a fully sequenced reference strain. Studies using MH and LB media showed that twitching and swarming phenotypes are largely medium dependent. Furthermore, twitching and swarming were demonstrated to be distinct characteristics, as many twitchers did not swarm, and *A. baumannii* strain ATCC 17978 swarmed, but did not twitch. PilA showed a high degree of amino acid sequence conservation within twitching isolates, indicating that type IV pili may play a role in motility in this species. Examination of biofilm formation showed that there was a significant difference between international clone I and II isolates, correlating with previously published data (de Breij *et al.*, 2010). We also found a significant difference ($P < 0.05$) between international clone I and noninternational clone isolates, indicating that in general international clone I isolates are limited in their ability to form biofilms.

We determined the adherence of selected *A. baumannii* isolates to eukaryotic cells of nasopharyngeal (Detroit 562) and alveolar (A549) origin. Not only were significant differences observed between strains, two isolates, D1279779 and ATCC 17978, showed significantly lower adherence to nasopharyngeal cells compared to lung epithelial cells.

Comparison of the ability to form biofilms and eukaryotic cell adherence revealed no relationship between these two phenotypes in the strains tested. This suggests that the mechanism of adherence to either abiotic or biotic surfaces appears to be different and draws a parallel with the results from other studies (Lee *et al.*, 2008; de Breij *et al.*, 2010). Moreover, previous studies have shown that adherence to abiotic surfaces is in part mediated by the *csu* type I pili cluster in strain ATCC 19606 (Tomaras *et al.*, 2003), however, in a subsequent study using the same *csu* knockout strain, no difference was observed in the ability to bind bronchial cells (de Breij *et al.*, 2009). Our data corroborates the previously published reports on biotic and abiotic adherence. A potentially critical mutation was found in the *csuB* open reading frame of strain ATCC 17978, a strain displaying lower levels of binding to abiotic surfaces compared to the other fully sequenced strains. No direct correlation could be established between the presence or absence of other type I pili clusters and adherence.

Overall, these studies demonstrate the significant diversity in phenotypic characteristics of clinical *Acinetobacter* isolates. Comparative analyses of the type IV pili genes between the sequenced strains examined revealed a potential role in motility. However, further investigation is required to fully delineate the mechanisms of motility and adherence in *A. baumannii* and the role of these phenotypes in promoting virulence of this important pathogen.

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Appendix H – Eijkelkamp *et al.* 2011; Journal of Molecular Microbiology and Biotechnology

Short Communication

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Development of a High-Throughput Cloning Strategy for Characterization of *Acinetobacter baumannii* Drug Transporter Proteins

Bart A. Eijkelkamp[a] Karl A. Hassan[b] Ian T. Paulsen[b] Melissa H. Brown[a]

[a]School of Biological Sciences, Flinders University, Adelaide, S.A., and [b]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, N.S.W., Australia

Key Words

Acinetobacter baumannii · Efflux · Transport proteins · MATE transporter · RND transporter · Gateway cloning · Multidrug resistance

Abstract

Heterologous expression of membrane proteins in *Escherichia coli* often requires optimization to overcome problems with toxicity of the recombinant protein to the host cell. A number of Gateway-based destination vectors were constructed to investigate expression of membrane proteins using a high-throughput approach. These vectors were tested using putative drug transporter proteins from the multidrug and toxic compound extrusion (MATE) family and the resistance-nodulation-cell division superfamily encoded by the human pathogen *Acinetobacter baumannii*. Active transport of antibiotics and antiseptics mediated by efflux proteins contributes to the high level of multidrug resistance observed in *A. baumannii*. Substrates for 4 of the 5 putative efflux proteins investigated were identified using the expression vectors designed in this study. Additionally, a Gateway-based suicide vector was designed for construction of specific *A. baumannii* insertion disruption mutants. This knockout cloning strategy was tested and shown to be successful in inactivating AbeM4, a putative MATE family protein. Therefore, we have shown that the Gateway-based vectors constructed in this study are versatile tools that can be used for manipulation and characterization of membrane proteins.

It is common to determine the function of a protein in a recombinant host, such as *Escherichia coli*. Additionally, heterologous expression in *E. coli* is widely used where a large amount of pure protein is required for functional and structural characterization, which may not be available from the native host. A common problem related to overexpression of recombinant membrane proteins is compromised membrane integrity, which could lead to toxicity of the overexpressed protein to the host cell [Montigny et al., 2004; Noirclerc-Savoye et al., 2003]. Many different expression systems have been developed throughout the years [Ward et al., 2001]. However, there does not appear to be an expression vector or *E. coli* host that is suitable for all membrane proteins. Therefore, the optimal expression level of a given transport protein, typically requires a number of different expression systems to be tested, which is a time-consuming process.

Acinetobacter baumannii is a common cause of infections in patients in intensive care units. High levels of resistance to antibiotics and antiseptics allow this emerging Gram-negative pathogen to sustain in the hospital environment and spread from patient to patient via medical staff or equipment [Dijkshoorn et al., 2007]. Once an infection with *A. baumannii* has been established, it is often difficult to treat due to the limited availability of effective antibiotics. Several different resistance mechanisms have been studied in *A. baumannii* [Gordon and Wareham, 2010] of which active efflux is a known contributor to development of multidrug resistant *A. bau-*

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Melissa H. Brown
School of Biological Sciences, Flinders University
GPO Box 2100
Adelaide, SA 5001 (Australia)
Tel. +618 8201 2747, E-Mail melissa.brown@flinders.edu.au

mannii (MDRAB) [Zhang et al., 2010]. As observed in other bacterial genera, resistance in MDRAB mediated by drug transport is often associated with over-expression of a relevant efflux system [Higgins et al., 2004]. To date, transport proteins that have been functionally characterized in *Acinetobacter* include AbeS [Srinivasan et al., 2009] from the small multidrug resistance (SMR) family, AmvA [Rajamohan et al., 2010], CraA [Roca et al., 2009], AedC and AedF [Hassan et al., 2011] and Tet(A) [Ribera et al., 2003] from the major facilitator superfamily (MFS), AbeM [Su et al., 2005] from the multidrug and toxic compound extrusion (MATE) family, and AdeB [Magnet et al., 2001], AdeE [Chau et al., 2004] and AdeJ [Damier-Piolle et al., 2008] from the resistance-nodulation-cell division (RND) superfamily. This represents only a fraction of the total number of predicted efflux proteins encoded by clinical *A. baumannii* strains. For example, 55 putative drug efflux systems have been identified in *A. baumannii* strain ATCC 17978 using the transporter automatic annotation pipeline (www.membranetransport.org).

Here, we developed a high-throughput Gateway-based cloning strategy suitable for heterologous expression of *A. baumannii* efflux systems in *E. coli*. The expression vectors were tested with transport proteins from different families and used for the functional characterization of five putative efflux proteins. The cloning strategy was extended to generate an *A. baumannii* knockout system, and was shown to be successful for the disruption of a gene encoding a MATE family protein.

Annotation and Isolation of the *A. baumannii* Efflux Systems

In this study, transport proteins classified in the MATE and RND superfamilies from *A. baumannii* strain ATCC 17978 [Smith et al., 2007] were investigated (table 1). Proteins classified within these families of transport proteins display vastly different structural characteristics and were chosen to test the limits of the expression system developed. First, the open reading frames (ORFs) of all predicted MATE genes, A1S_0395, A1S_2562, A1S_3371 and A1S_3420, and two predicted RND genes, A1S_3445 and A1S_3446, were manually annotated as differences were observed in multiple sequence alignments with homologues from other fully sequenced *A. baumannii* strains (data not shown). Sequence analysis suggested that A1S_3446 and A1S_3445 of *A. baumannii* ATCC 17978 were actually a single ORF that had been split by a sequencing error. This was confirmed by resequencing of the chromosomal DNA by Sanger sequencing (data not shown). Using Glimmer3 [Delcher et al., 1999], alternative start codons were identified for all MATE ORFs and A1S_3445/6. The protein sequences encoded by the manually annotated transporter genes were examined using the topology prediction program TMHMM [Krogh et al., 2001] and found to adhere to the regular topological organizations of their respective transporter families, i.e. 12 predicted transmembrane helices for both the MATE and RND proteins, as well as long periplasmic loops between helices 1 and 2, and 7 and 8, of the RND family transporter. RND proteins are known to have the ability to form tripartite efflux complexes that span both the inner and outer membranes of Gram-negative bacteria. These protein complexes consist of the RND protein, a pore forming outer membrane protein (OMP) and a membrane fusion protein (MFP) [Pos, 2009]. The ORF of A1S_3445/6 is preceded by a gene encoding a predicted MFP and a divergently transcribed regulator. This gene cluster does not contain an OMP. However, the RND transport protein and MFP could form a complex with an OMP transcribed elsewhere on the genome to form the tripartite complex. Interaction of an RND with a distally encoded OMP has been observed for other RND tripartite systems, such as the AcrAB (MFP and RND) and TolC (OMP) proteins that form a major drug efflux system encoded in *E. coli* [Ma et al., 1993].

A substrate profile for AbeM (A1S_0395) has been described previously [Su et al., 2005]. Therefore, this protein was used as a positive control in the assays described below. The three other putative MATE genes were named *abeM2* (A1S_2562), *abeM3* (A1S_3371) and *abeM4* (A1S_3420), and the RND family member *adeM* (A1S_3445/6). All genes were amplified from *A. baumannii* strain ATCC 17978 with a 5′ CACC-overhang sequence and without a stop codon (table 2). The amplicons were inserted into the pENTR™/DS/D-TOPO vector (Invitrogen) using the TOPO reaction, generating so-called entry clones which contain an *E. coli* optimized ribosome-binding site (table 1).

Construction of a Gateway-Based Cloning System for Heterologous Expression of Membrane Proteins

In this study, various expression vectors based on Gateway technology (Invitrogen) were designed to allow 'shuffling' of the gene of interest into different expression

Table 1. Strains and plasmids used in this study

Strains and plasmids	Genotype or description	Source or reference
A. baumannii ATCC 17978	reference strain	[Smith et al., 2007]
A. baumannii ATCC 17978_abeM4::Gm	insertion disruption of *abeM4* in *A. baumannii* ATCC 17978	this study
E. coli DH5α	*fhuA2* Δ(*argF-lacZ*)U169 *phoA glnV*44 Φ80 Δ(*lacZ*)M15 *gyrA96 recA1 relA1 endA*1 *thi*-1 *hsdR*17	Invitrogen
E. coli AG100A	Δ*acrAB*	[Okusu et al., 1996]
E. coli BL21(DE3)	B *dcm ompT hsdS*($r_B^-m_B^-$) *gal*	Invitrogen
E. coli TOP10	*mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), Δ*lac*X74, *deo*R, *rec*A1, *ara*D139Δ(*ara-leu*)7697, *gal*K, *rps*L, *end*A1, *nup*G	Invitrogen
E. coli ccdB Survival	F– *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*ΔM15 Δ*lacX*74 *recA1 ara*Δ139 Δ(*ara-leu*)7697 *galU galK rpsL* (StrR) *endA*1 *nupG fhuA*::IS2	Invitrogen
pPS856	Amp^R Gm^R	[Hoang et al., 1998]
pENTR™/DS/D-TOPO	Kan^R; Entry vector	Invitrogen
pENTRabeM	Kan^R; pENTR™/DS/D-TOPO containing PCR product using abeM_TOPO primers	this study
pENTRabeM2	Kan^R; pENTR™/DS/D-TOPO containing PCR product using abeM2_TOPO primers	this study
pENTRabeM3	Kan^R; pENTR™/DS/D-TOPO containing PCR product using abeM3_TOPO primers	this study
pENTRabeM4	Kan^R; pENTR™/DS/D-TOPO containing PCR product using abeM4_TOPO primers	this study
pENTRabeM4::Gm	Kan^R Gm^R; pENTR_abeM4 containing the *Xba*I fragment from pPS856	this study
pENTRadeM	Kan^R; pENTR™/DS/D-TOPO containing PCR product using adeM_TOPO primers	this study
pET-DEST42	Amp^R Cm^R *ccdB*; Destination vector	invitrogen
pETabeM4	Amp^R; LR reaction pET-DEST42 and pENTR_abeM4	this study
pETadeM	Amp^R; LR reaction pET-DEST42 and pENTR_adeM	this study
pBAD30	Amp^R; Expression vector	[Guzman et al., 1995]
pBADgw	Amp^R Cm^R *ccdB*; pBAD30 containing PCR product from pET-DEST42 using Gateway2 primers (*Kpn*I-*Xba*I)	this study
pBADgw_abeM4	Amp^R; LR reaction pBADgw and pENTR_abeM4	this study
pBADgw_adeM	Amp^R; LR reaction pBADgw and pENTR_adeM	this study
pBluescriptII	Amp^R; Expression vector	[Alting-Mees and Short, 1989]
$pBSgwP_{T7}$	Amp^R Cm^R *ccdB*; pBluescriptII containing PCR product from pET-DEST42 using Gateway2 primers (*Kpn*I-*Xba*I)	this study
$pBSgwP_{T7}$_abeM	Amp^R; LR reaction $pBSgwP_{T7}$ and pENTR_abeM	this study
$pBSgwP_{T7}$_abeM2	Amp^R; LR reaction $pBSgwP_{T7}$ and pENTR_abeM2	this study
$pBSgwP_{T7}$_abeM3	Amp^R; LR reaction $pBSgwP_{T7}$ and pENTR_abeM3	this study
$pBSgwP_{T7}$_abeM4	Amp^R; LR reaction $pBSgwP_{T7}$ and pENTR_abeM4	this study
$pBSgwP_{T7}$_adeM	Amp^R; LR reaction $pBSgwP_{T7}$ and pENTR_adeM	this study
$pBSgwP_{lac}$	Amp^R Cm^R *ccdB*; pBluescriptII containing PCR product from pET-DEST42 using Gateway1 primers (*Xba*I-*Xho*I)	this study
$pBSgwP_{lac}$_abeM	Amp^R; LR reaction $pBSgwP_{lac}$ and pENTR_abeM	this study
$pBSgwP_{lac}$_abeM2	Amp^R; LR reaction $pBSgwP_{lac}$ and pENTR_abeM2	this study
$pBSgwP_{lac}$_abeM3	Amp^R; LR reaction $pBSgwP_{lac}$ and pENTR_abeM3	this study
$pBSgwP_{lac}$_abeM4	Amp^R; LR reaction $pBSgwP_{lac}$ and pENTR_abeM4	this study
$pBSgwP_{lac}$_adeM	Amp^R; LR reaction $pBSgwP_{lac}$ and pENTR_adeM	this study
pEX18Tc	Tet^R; Suicide vector	[Hoang et al., 1998]
pEXgwTc	Tet^R Cm^R *ccdB*; Gateway features cloned from $pBSgwP_{lac}$ into pEX18Tc using *Xba*I and *Xho*I	this study
pEXgwTc_abeM4::Gm	Tet^R Gm^R; LR reaction pEXgwTc and pENTRabeM4::Gm	this study

systems previously shown to be successful in the overexpression of membrane transport proteins from various transporter classes. The vectors included pET-DEST42, pBAD30 and pBluescriptII, which have differences in their copy number, the promoter and regulation of transcription. The pET and pBAD plasmids are propagated at a low to medium copy number in the cell, but expression is driven by strong promoters [Dubendorff and Studier, 1991; Guzman et al., 1995]. Gene expression can be controlled by the LacI/O and AraC regulators for pET and

Table 2. Oligonucleotides used in the study

Name	Forward	Reverse
abeM_TOPO	5′-CACCATGTCGAATGTCACGTCGTT-3′	5′-GGTTTGACTTAAACGTTTGGTATT-3′
abeM2_TOPO	5′-CACCATGAATATGCTCAAAGACAT-3′	5′-GGTTGAAATGGTCTCACCAACTGG-3′
abeM3_TOPO	5′-CACCATGGGCGATGGAGAATACAT-3′	5′-AATAAGAACTTTGATCTTCTTTTT-3′
abeM4_TOPO	5′-CACCATGAACCAGATTTTTAAATT-3′	5′-AAAACCTCTTAACTGCTTTCTAAA-3′
adeM_TOPO	5′-CACCATGAAATTTAATCTCTCTGAATG-3′	5′-TGCTGTTTTTTTCACCTTAAACC-3′
Gateway1	5′-GAGATCTAGAACAAGTTTGTACAAAAAAGC-3′	5′-GAGACTCGAGTCAATGGTGATGGTGATGAT-3′
Gateway2	5′-GAGAGGTACCACAAGTTTGTACAAAAAAGC-3′	5′-GAGATCTAGATCAATGGTGATGGTGATGAT-3′

pBAD, respectively, and both plasmids also contain transcriptional terminators. These expression systems are considered suitable for obtaining high levels of recombinant protein for in vitro investigation [Hassan et al., 2009]. In contrast, the lower expression levels obtained by using pBluescriptII [Alting-Mees and Short, 1989] behind the T7 promoter (pBS_P_{T7}) in *E. coli* appear more suitable for in vivo characterizations of membrane transporters. Uninduced expression from pBluescriptII in *E. coli* strain DH5α has previously been used for in vivo characterization of heterologously expressed membrane transporters [Hassan et al., 2006; Xu et al., 2006]. However, in this study, leaky expression of T7 RNA polymerase in *E. coli* strain BL21(DE3) may be responsible for transcription of the gene of interest from pBluescriptII. Cloning the gene in the opposite direction allows transcription to be directed by the lac promoter (pBS_P_{lac}). This is a native promoter site for *E. coli* RNA polymerases. Therefore, this expression system allows the user to select any *E. coli* host background, e.g. the hypersusceptible *E. coli* strain AG100A [Okusu et al., 1996] when performing drug resistance studies.

The pET-DEST42 Gateway vector was obtained commercially (Invitrogen) and the Gateway features from this plasmid were isolated by PCR using the primers listed in table 2. The fragment was subsequently cloned into pBAD30, generating pBADgw, and in pBluescriptII in both orientations, generating pBSgwP_{T7} and pBSgwP_{lac} (fig. 1; table 1). The Gateway features include *att*R1, *att*R2, *cat* and *ccdB*, and two affinity tags, His_6 and the V5-epitope. The *att*R sites are the sequences that undergo recombination during the LR-reaction, in which the *att*L sites from the entry clone (pENTR™/DS/D-TOPO-based constructs) recombine with the *att*R sites from the destination clone, generating an expression clone. During this process the gene of interest will be transferred from the entry clone to the destination clone. The product of the *ccdB* gene located between the *att*R1 and *att*R2 sites is lethal to most *E. coli* strains, therefore, the *ccdB* Survival *E. coli* strain (Invitrogen) was used for propagation of the destination clones. Additional negative selection can be achieved by incubation with chloramphenicol, as a *cat* gene is also present between the *att*R sites. The two affinity tags and a stop codon will be introduced when the gene of interest is being transferred from the entry clone to the destination clone.

Genes encoding representatives of the MATE and RND family, *abeM4* and *adeM*, respectively, were recombined into all four destination vectors to investigate protein expression. The expression clones obtained were then transformed into appropriate *E. coli* host strains; the pET clones into BL21(DE3), pBAD into TOP10, pBS_P_{T7} into BL21(DE3) and pBS_P_{lac} into DH5α or AG100A (for drug resistance assays). Overnight cultures in Luria-Bertani (LB) medium were diluted and expression was induced at OD_{600} = 0.5 for the pET and pBAD expression systems, with 0.5 mM IPTG and 0.004% L-arabinose, respectively. All cultures were harvested when OD_{600} reached 0.7. The membrane fraction of the cultured cells was isolated using methods essentially described elsewhere [Hassan et al., 2009] and equal amounts of total protein, 60 μg, were loaded onto 12% SDS-PAGE gels. Western blot analysis using anti-X6 HIS epitope tag antibodies (Rockland) was employed to detect recombinant protein in the isolated membrane fraction (fig. 2). Both AdeM and AbeM4 were detected at their expected sizes, ~110 and ~50 kDa, respectively (fig. 2). AdeM synthesis was at similar levels when using pBADgw_adeM, pBSgwP_{T7}_adeM or pBSgwP_{lac}_adeM; however, the levels appeared higher in the sample from pET_adeM. AbeM4 protein was detected when using pET_abeM4, pBADgw_abeM4 and pBSgwP_{T7}_abeM4 expression systems; however, no recombinant protein could be detected with pBSgwP_{lac} _abeM4 as an expression vector. Expres-

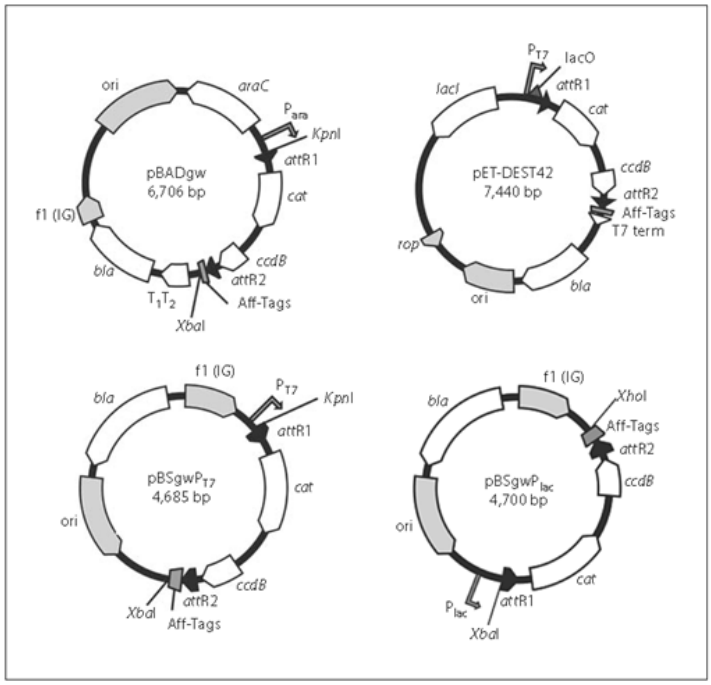

Fig. 1. The Gateway-based expression systems used for *A. baumannii* efflux protein expression. The Gateway features from pET-DEST42 were cloned into pBAD30 downstream of P_{ara} and into pBluescriptII behind both P_{T7} and P_{lac}, generating pBADgw, pBSgwP$_{T7}$ and pBSgwP$_{lac}$, respectively. All plasmid backbones contain the *bla* gene for ampicillin selection. The replication features, including the origin of replication (ori), the intergenic region of phage f1 [f1(IG)] and the copy number repression site *(rop)* of the plasmids are represented in light grey. The promoter and direction of transcription are indicated by a narrow arrow (P_{T7}, P_{ara} or P_{lac}). The *lacI* gene encodes a product that binds to the *lacO* site for transcriptional inhibition from P_{T7} in pET-DEST42. Both pET-DEST42 and pBADgw contain transcription termination sites, T7 term and T_1T_2, respectively. The *att*R1 and *att*R2 sites form the site-specific recombination sequences, which allow the gene of interest to be inserted in-frame with the His_6 and V5-epitope tags (Aff-Tags) and a stop codon. Two negative selection genes are located between the *att*R sites, *cat* for chloramphenicol selection and the *ccdB* gene that encodes a lethal product to most *E. coli* strains.

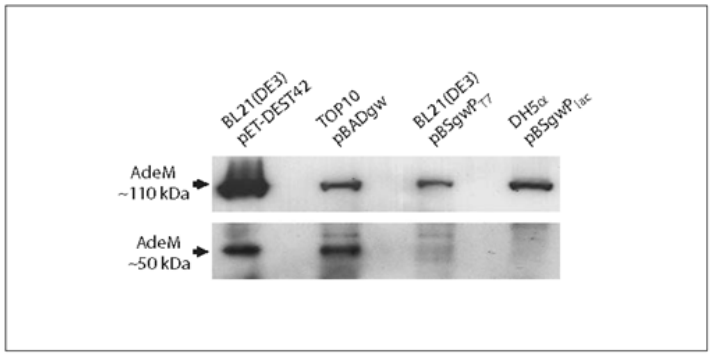

Fig. 2. Western blot detection of heterologously expressed *A. baumannii* efflux proteins in *E. coli*. Expression of AdeM and AbeM4 in the four different expression systems was investigated by Western blot analysis. The membrane fraction of cells expressing each construct was isolated and an equal amount of total protein (60 µg) was loaded onto a 12% SDS-PAGE gel. Recombinant protein was detected using a anti-X6 HIS antibody.

sion levels of AbeM4 obtained with pBSgwP$_{T7}$_abeM4 were lower than those observed using the pET_abeM4 and pBADgw_abeM4 expression clones. The respective negative controls for the four different expression systems showed no reactive signal using the anti-X6 HIS antibody.

Functional Characterization of *A. baumannii* Efflux Systems

In order to identify substrates of the efflux systems, minimum inhibitory concentration (MIC) assays were carried out on *E. coli* cells expressing the putative efflux

Table 3. Substrate profile of heterologously expressed *A. baumannii* efflux proteins in *E. coli*

Efflux protein	Destination clone/*E. coli* host[a]	Substrates[b] (increase in times-fold over vector control)
AdeM	pBSgwP$_{T7}$/BL21(DE3)	norfloxacin (2), chlorhexidine (2)
AbeM	pBSgwP$_{T7}$/BL21(DE3)	norfloxacin (4), ciprofloxacin (4), trimethoprim (2), chlorhexidine (4), ethidium (2), DAPI (2)
AbeM2	pBSgwP$_{T7}$/BL21(DE3)	ciprofloxacin (2)
AbeM3	pBSgwP$_{T7}$/BL21(DE3) pBSgwP$_{lac}$/AG100A	–
AbeM4	pBSgwP$_{T7}$/BL21(DE3)	ciprofloxacin (2)

[a] MIC assays were performed at least three times.
[b] Compounds tested: tetracycline, ciprofloxacin, norfloxacin, kanamycin, colistin, erythromycin, trimethoprim, gentamicin, chloramphenicol, rifampicin, triclosan, benzalkonium, chlorhexidine, sodium dodecyl sulfate, tetraphenylphosphonium, ethidium, 4′,6-diamidino-2-phenylindole (DAPI), rhodamine 6G.

pump genes from *A. baumannii*. The microdilution method [Wiegand et al., 2008] was performed at least three times using freshly transformed cells. All four MATE proteins, AbeM, AbeM2, AbeM3 and AbeM4, and the RND member AdeM were investigated in this study. Resistance to a wide range of compounds was tested, including various antibiotics (tetracycline, ciprofloxacin, norfloxacin, kanamycin, colistin, erythromycin, trimethoprim, gentamicin, chloramphenicol and rifampicin), antiseptics (triclosan, sodium dodecyl sulphate, chlorhexidine, benzalkonium and tetraphenylphosphonium) and dyes (ethidium, 4′,6-diamidino-2-phenylindole and rhodamine 6G) (table 3). The pBSgwP$_{T7}$ and pBSgwP$_{lac}$ destination clones were used for the in vivo functional characterization of the putative efflux proteins, as protein expression from the pET-DEST42 and pBADgw vectors was significantly detrimental to cell growth, possibly as a result of the high expression levels obtained from these vectors (fig. 2). Western blot analyses indicated that all recombinant proteins were present in the membrane fraction using either pBSgwP$_{T7}$ or pBSgwP$_{lac}$, except for AbeM4 when using pBSgwP$_{lac}$ as described above. Interestingly, the relative resistance observed for AbeM was the same when using pBSgwP$_{T7}$_*abeM* in *E. coli* BL21(DE3) or pBSgwP$_{lac}$_*abeM* in the hypersusceptible *E. coli* strain AG100A. The relative resistance was measured as the difference in the MIC value between the expressing clone and its respective vector control, e.g. this was 4-fold in both systems for ciprofloxacin. The MIC values were consistent across three independent experiments, therefore, a 2-fold increase was considered to be the contribution of the recombinantly expressed protein. An extensive substrate profile was obtained for AbeM, as resistance was observed to the fluoroquinolones norfloxacin and ciprofloxacin, trimethoprim, chlorhexidine, ethidium and 4′,6-diamidino-2-phenylindole. This confirmed that AbeM is a multidrug efflux system, as it has the ability to transport structurally diverse compounds, correlating with a previous study [Su et al., 2005]. Therefore, the same approach was applied to characterize the three other MATE proteins and AdeM. AbeM2 and AbeM4 were found to confer resistance to ciprofloxacin, which appears to be a common substrate of MATE proteins. Of note, the *abem2* gene is located in a putative siderophore biosynthesis cluster and may play a role in iron homeostasis [Eijkelkamp et al., 2011]. Expression of AbeM3 did not result in increased resistance to any of the 18 compounds tested in either *E. coli* BL21(DE3) using pBSgwP$_{T7}$ or in *E. coli* AG100A using pBSgwP$_{lac}$. Therefore, the function of AbeM3 in *A. baumannii* requires further investigation. Ciprofloxacin and chlorhexidine were positively identified as substrates of AdeM by in vivo characterization of the recombinant protein in *E. coli*. Although it was shown that AdeM is a multidrug transporter, the resistance levels observed in the heterologous expression system may only represent a partial potential of this pump. In its native environment in *A. baumannii*, it is likely to form a tripartite complex with MFP and OMP. Such a fully assembled efflux system is likely to potentiate higher levels of drug resistance than the resistance observed in this study on the heterologously expressed RND protein only.

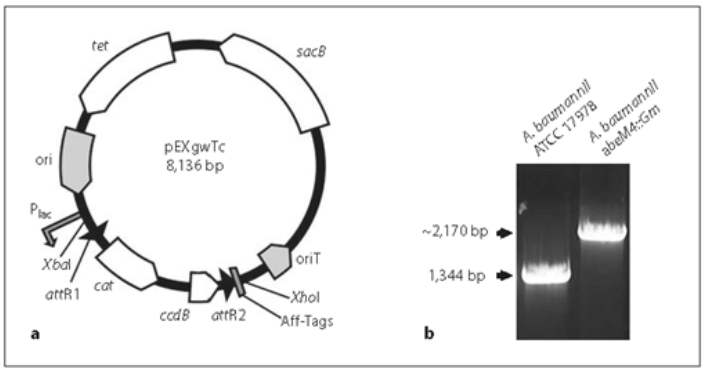

Fig. 3. The *A. baumannii* insertion disruption strategy developed in this study. Schematic representation of the suicide Gateway construct generated in this study (**a**), with the Gateway features (described in fig. 1) inserted into pEX18Tc using *Xba*I and *Xho*I, to generate pEXgwTc. The *abeM4* insertion disruption strain *A. baumannii* ATCC 17978_abeM4::Gm showed an amplification product of increased size when using *abeM4*-specific primers (**b**).

Constructing Directed *A. baumannii* Knockout Strains

The Gateway-based cloning system was extended from heterologous expression for use in *A. baumannii* knockout construction. The Gateway suicide vector constructed in this study was based on the *Pseudomonas aeruginosa* pEX vectors, which have shown to be successful in other *A. baumannii* knockout studies [Dorsey et al., 2004; Roca et al., 2009]. We selected pEX18Tc, a derivative of pEX100T, in which the *bla* gene has been replaced with a *tet* gene and therefore, its use in *A. baumannii* is more widely applicable, since this species is typically resistant to ampicillin [Hoang et al., 1998; Schweizer and Hoang, 1995]. The Gateway features were cloned from pBSgwP$_{lac}$ using *Xba*I and *Xho*I and inserted into pEX18Tc behind the lac promoter, generating pEXgwTc (fig. 3a).

The existing entry clone harboring *abeM4* was used as a template and the gentamicin resistance gene *aacC1* isolated from pPS856 [Hoang et al., 1998] using *Xba*I and was subsequently inserted into a native *Xba*I site in *abeM4*. This site is located close to the middle of the ORF (position 618 of 1344), leaving >600 bp of flanking DNA on both sides for a double crossover recombination event to occur. The *abeM4::Gm* insertion disruption construct was cloned into pEXgwTc using the LR reaction. Importantly, the use of Gateway cloning in preparation of a knockout construct is advantageous as multiple cloning steps in the procedure can limit the availability of endonuclease restriction sites. This novel method requires only one conventional cloning step. The suicide construct, pEXgwTc_abeM4::Gm, was transformed into *A. baumannii* strain ATCC 17978 by electroporation, as described previously [Dorsey et al., 2002]. The transformed cells were then cultured overnight with appropriate antibiotic selection to allow the double crossover recombination event to take place. The *sacB* gene, located on the suicide vector, allows for selection of cells that have lost the plasmid by replacing glucose for sucrose as the sole carbon source in M9 medium. Colonies were washed in phosphate buffered saline and cultured overnight on M9 medium with sucrose (0.5%) and gentamicin (12.5 μg/ml). Over 30 colonies were analyzed by PCR with abeM4_TOPO forward and reverse primers which are located at the termini of the *abeM4* ORF. A single PCR product of ~2,170 bp indicated that insertion disruption of *abeM4* by *aacC1* had taken place in the 30 screened colonies (fig. 3b). The colonies were subsequently tested for tetracycline susceptibility, which showed that the suicide vector was removed from the clones investigated. Disruption of *abeM4* was confirmed by DNA sequencing of two representative clones.

The resulting *A. baumannii* mutant strain ATCC 17978_abeM4::Gm was phenotypically characterized. Growth curves showed no significant differences between the growth rate of the mutant and wild-type strain (data not shown), moreover, the colony size and morphology appeared unaffected on nonselective LB agars. A drug susceptibility profile was compared between ATCC 17978 and ATCC 17978_abeM4::Gm. Interestingly, whereas ciprofloxacin was shown to be a substrate of AbeM4 in the heterologous expression studies, no differences in susceptibility to ciprofloxacin were observed for the abeM4 disrupted mutant. In fact, no significant differences in susceptibility were observed to any of the 18 compounds listed in table 3. Although low, *abeM4* transcription levels in *A. baumannii* grown in broth cultures

were similar to those found for other genes encoding transporters, such as *abeM* and A1S_2305 (data not shown). The genes encoding efflux proteins may show transcriptional responses upon exposure to its substrates, as observed for *mexXY*, an operon encoding a *P. aeruginosa* RND transporter system [Poole, 2008]. However, qRT-PCR analysis showed no transcriptional upregulation of *abeM4* when ATCC 17978 cells were exposed to sub-inhibitory concentrations of ciprofloxacin (data not shown). In a study on the effect of increased NaCl levels to *A. baumannii*, it was shown that numerous transporter proteins were significantly upregulated, including *abeM4* (>12-fold), and also increased resistance to several different antibiotics was observed [Hood et al., 2010]. Subsequently, in this study, drug resistance of ATCC 17978 and ATCC 17978_abeM4::Gm was investigated under high salt conditions (MH supplemented with 150 μM NaCl); however, no differences between the two strains were seen. The role of AbeM4 as a transporter of, for example, ciprofloxacin in *A. baumannii*, may be overshadowed by other transporters with similar substrate profiles and for that reason, mutants with multiple inactivated transporters would have to be constructed to investigate the native function of AbeM4.

Conclusions

The Gateway-based cloning and expression systems designed in this study were proven successful for heterologous expression of distinct *A. baumannii* membrane transport proteins. Functional characterization of all putative *A. baumannii* ATCC 17978 MATE proteins and AdeM, a member of the RND family, was carried out and their function as drug transport proteins was confirmed for four of the five investigated. A novel Gateway-based suicide vector was constructed and used to generate an *abeM4* insertion disruption in *A. baumannii* ATCC 17978. The described cloning strategy could also be applied as a versatile tool for characterization of various other membrane proteins.

Acknowledgements

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Appendix I – Abbreviations

Å	Angstrom
A	Adenine
ABC	(Adenosine triphosphate) binding cassette
Aff-Tags	Affinity-tags
AFLP	Amplified fragment length polymorphism
AHL	Acyl homoserine lactones
Amp	Ampicillin
ARDRA	Amplified *16SrDNA* restriction analysis
ATCC	American type culture collection
ATP	Adenosine triphosphate
Bap	Biofilm-associated protein
Blastn	Basic local alignment search tool for nucleotide sequences
Blastp	Basic local alignment search tool for protein sequences
bp	Base pair
C	Cytosine
°C	Degree Celsius
CD	Conserved domain
cDNA	Complimentary deoxyribonucleic acid
CFU	Colony forming units
Chx	Chlorhexidine
Cip	Ciprofloxacin
Cm	Chloramphenicol
cm	Centimetre
Cm^R	Chloramphenicol resistance
COG	Clusters of orthologous groups
Col	Colistin
DAPI	4',6-diamidino-2-phenylindole
DC-BCA	Detergent compatible bicinchoninic acid
DEPC	Diethylpyrocarbonate
dH_2O	Distilled H_2O
DHA	Drug:H^+ antiporter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECF	Extracytoplasmic function
FDA	US food and drug administration
FUR	Ferric uptake regulator
g	Centrifugal force
G	Guanine
GEO	Gene expression omnibus
Gm	Gentamicin
Gm^R	Gentamicin resistance
HI	Hydrophobicity index

ICU	Intensive care unit
IPTG	Isopropyl β-D-1-thiogalactopyranoside
Kan	Kanamycin
Kan^R	Kanamycin resistance
Kb	Kilo base
kDa	Kilo Dalton
KEGG	Kyoto encyclopaedia of genes and genomes
l	Litre
LB	Luria-Bertani
LPS	Lipopolysaccharides
M	Molar
mA	Milliampere
MAST	Motif alignment and search tool
MATE	Multidrug and toxic compound extrusion
MATH	Microbial adhesion to hydrocarbons
MDRAB	Multidrug resistant *Acinetobacter baumannii*
MEME	Multiple em (expectation maximisation) for motif elicitation
MFP	Membrane fusion protein
MFS	Major facilitator superfamily
mg	Milligram
MH	Mueller-Hinton
MIC	Minimal inhibitory concentration
ml	Millilitre
MLST	Multi-locus sequence typing
mM	Millimolar
mm	Millimetre
mQ	MilliQ
mRNA	Messenger ribonucleic acid
NCBI	National center for biotechnology information
ng	Nanogram
nm	Nanometre
OD	Optical density
OMP	Outer membrane protein
ON	Overnight
ORF	Open reading frame
ori	Origin of replication
p-value	Probability value
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PNAG	Poly-β-(1-6)-N-acetylglucosamine
psi	Pounds per square inch
PVDF	Polyvinylidene fluoride
PUM	Potassium urea magnesium

qRT-PCR	Quantitive reverse transcription polymerase chain reaction
QS	Quorum-sensing
rDNA	Ribosomal deoxyribonucleic acid
rep	Replication
RNA	Ribonucleic acid
RND	Resistance-nodulation-cell division
rop	Copy-number repression site
rpm	Rotations per minute
RT	Room temperature
SAM	Statistical analysis of microarrays
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SMP	Small multidrug protein
SMR	Small multidrug resistance
SNP	Single nucleotide polymorphism
Sug	Suppressor of *groEL*
T	Thymine
TAE	Tris-base acetic acid ethylenediaminetetraacitic acid
TTBS	Tween Tris-buffered saline
Tc	Tetracycline
Tet^R	Tetracycline resistance
TMS	Transmembrane segments
TransAAP	Trans automated annotation pipeline
TTBS	Tween tris-buffered saline
U	Units
UT	Untreated
V	Volt
v/v	Volume/volume
VNTR	Variable-number tandem repeats
w/v	Weight/volume
WT	Wild-type
ZUR	Zinc uptake regulator
µg	Microgram
µl	Microlitre
µM	Micromolar