

## Supplement

### The carnitine degradation pathway of *Acinetobacter baumannii*

#### and its role in virulence

Jennifer Breisch<sup>1</sup>, Clemens Schumm<sup>1</sup>, Anja Poehlein<sup>2</sup>, Rolf Daniel<sup>2</sup> and

Beate Averhoff<sup>1\*</sup>

<sup>1</sup>*Department of Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences,  
Goethe-University Frankfurt am Main, Germany*

<sup>2</sup>*Department of Genomic and Applied Microbiology and Göttingen Genomics Laboratory,  
Institute of Microbiology and Genetics, Georg-August University of Göttingen, Germany*

*Running title: Carnitine degradation pathway of *Acinetobacter baumannii**

*Key words: carnitine degradation, TMA formation, metabolic adaptation, D-malate  
utilization*

\*Correspondence to: Beate Averhoff, Department of Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe-University Frankfurt am Main, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany. Tel.: + 49 69 79829507. E-mail address: [averhoff@bio.uni-frankfurt.de](mailto:averhoff@bio.uni-frankfurt.de)

Suppl. Tab. 1. Primers used in this study

Primer	Sequence 5'→3'	Function
<i>carR_for_SacI</i>	GATCGAGCTCGTGAATAATCTACCTAACCTATC	purification <i>CarR</i>
<i>carR_rev_EcoRI</i>	GCGCGAATTCTTATAGAAAGACTTTTTCAGGCGG	purification <i>CarR</i>
<i>mdh_up_for_NotI</i>	CTGAGCGGCCGCGCAATGGATGTTCTTAGCAG	deletion <i>mdh</i>
<i>mdh_up_rev_BamHI</i>	CTGAGGATCCGCTTCCTTCACACCTTCGAC	deletion <i>mdh</i>
<i>mdh_do_for_BamHI</i>	CTGAGGATCCCGCTATGGAATAACGCCATC	deletion <i>mdh</i>
<i>mdh_do_rev_PstI</i>	CTGACTGCAGCAGAATAATCACGATGAATG	deletion <i>mdh</i>
<i>mdh_ctr_for</i>	CGAAGATGGACACCGCAAATCATG	sequencing $\Delta$ <i>mdh</i>
<i>mdh_ctr_rev</i>	CTTTGGCATAATCCTGTATGAG	sequencing $\Delta$ <i>mdh</i>
<i>hyd_up_for_NotI</i>	ATTAGCGGCCGCCAGTGGACGACATCG	deletion <i>hyd</i>
<i>hyd_up_rev_BamHI</i>	ATACGGATCCCCGTTCCCTCTAGCTG	deletion <i>hyd</i>
<i>hyd_do_for_BamHI</i>	CAGAGGATCCGGATTAAAGCAAGTTCATCGG	deletion <i>hyd</i>
<i>hyd_do_rev_PstI</i>	AGCTCTGCAGCCGAGTCATTTGGACGAAAAG	deletion <i>hyd</i>
<i>hyd_ctr_for</i>	CTTGCAAGCTCAATCAAACCTGG	sequencing $\Delta$ <i>hyd</i>
<i>hyd_ctr_rev</i>	GTAATTTTTCAACTGCACTCATG	sequencing $\Delta$ <i>hyd</i>
<i>carR_up_for_XbaI</i>	GACTTCTAGAACCGATGTCGTCCACTGCATC	deletion <i>carR</i>
<i>carR_up_rev_NotI</i>	GCATGCGGCCGCCACGATTGCAGGCGATGGC	deletion <i>carR</i>
<i>carR_do_for_NotI</i>	GCATGCGGCCGCCTGTACTCTTGTGCAAGGCC	deletion <i>carR</i>
<i>carR_do_rev_SacI</i>	GACTGAGCTCCGGCTATGTAGCTCAGTTG	deletion <i>carR</i>
<i>carR_ctr_for</i>	CAGTAATACTGGGGTTGTA	sequencing $\Delta$ <i>carR</i>
<i>carR_ctr_rev</i>	GCTTATAGGCTCACTTCACCTC	sequencing $\Delta$ <i>carR</i>
01344 <sup>a</sup> - <i>carR_for</i>	GATTATCCGTATCAAGTTGC	bridging PCR
01344 <sup>a</sup> - <i>carR_rev</i>	ACTTGCCATAGAAGGTCAC	bridging PCR
<i>carR_mdh_for</i>	CCATACGACCCGTAGGCGTAC	bridging PCR + EMSA
<i>carR_mdh_rev</i>	AACCACGACGGAAGTGGAGC	bridging PCR + EMSA
<i>tdh_aci01347_for</i>	CCATTGGTTTAGCGGCATCG	bridging PCR
<i>tdh_aci01347_rev</i>	CATGACATTGTGCTGTATTG	bridging PCR
<i>aci01347_hyd_for</i>	GTTCTGGTGTGTCATGCTGAC	bridging PCR
<i>aci01347_hyd_rev</i>	GTGAATCGAGTCCACCTACC	bridging PCR
<i>hyd_cntA_for</i>	GCACGCCCATGCGCCGTTGT	bridging PCR
<i>hyd_cntA_rev</i>	TGCTTTACCACTGCCACTT	bridging PCR
<i>cntA_msd_for</i>	GGCAGCAACTTCATGACAGT	bridging PCR

<b>Primer</b>	<b>Sequence 5'→3'</b>	<b>Function</b>
<i>cntA_msd_rev</i>	AGGCTTGCCTTGCTCAATGG	bridging PCR
<i>msd_cntB_for</i>	CCGTACAATGGAAATTGTCC	bridging PCR
<i>msd_cntB_rev</i>	GAAATCTGGATTTACAGCC	bridging PCR
<i>cntB_01352<sup>b</sup>_for</i>	CGCATGTGTATGTATGTGGC	bridging PCR
<i>cntB_01352<sup>b</sup>_rev</i>	ATCAAAGCTCAAAAACACTC	bridging PCR
<i>betA_up_for</i>	GAATGCGGCCGCAACGTGTATCAATATGTGGTACAG	EMSA control
<i>betA_up_rev</i>	GACTGGATCCTTGCCGAGAAAATTGCAGAC	EMSA control

<sup>a</sup> Open reading frame HMPREF0010\_01344

<sup>b</sup> Open reading frame HMPREF0010\_01352

Suppl. Tab. 2. Carnitine, acetylcarnitine or D-malate induced genes. Given is the log<sub>2</sub> fold change in comparison to cells grown in the presence of succinate. Phenylacetic acid pathway (*paa* genes) and homogentisate pathway (*hmg* genes) are highlighted with light grey and carnitine degradation pathway is highlighted in dark grey.

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_0013	aldehyde-activating protein	3.08	5.51	4.58
HTZ92_0051	acetyltransferase	5.22	4.21	5.17
HTZ92_0052	acetyl-CoA hydrolase	1.42	6.28	2.77
HTZ92_0060	Sell-„repeat“ protein	3.82	3.48	5.47
HTZ92_0152	hypothetical protein	4.69	4.75	5.00
HTZ92_0293	acyl-CoA thioesterase	5.12	4.58	4.38
HTZ92_0469	putative transporter	6.37	0.66	0.68
HTZ92_0729	pyruvate decarboxylase	6.23	2.07	3.54
HTZ92_1139	aldehyde dehydrogenase	6.04	0.27	2.49
HTZ92_1292	histidine kinase	4.54	7.29	6.76
HTZ92_1302	cytochrome- BD-oxidase subunit II	1.67	4.67	2.84
HTZ92_1303	cytochrome-BD-II oxidase subunit I	2.44	5.84	3.79
HTZ92_1344	4-carboxymuconolacton decarboxylase	3.91	3.95	5.23
HTZ92_1352	transporter component	6.10	6.44	5.74
HTZ92_1353	YeeE/YedE family protein (sulphur transport protein)	6.58	6.96	6.82
HTZ92_1552	α-ketoglutarate transporter	3.45	3.79	5.23
HTZ92_1572	2,3-butandiole dehydrogenase	6.47	-0.60	2.75
HTZ92_1573	diacetyl reductase	6.70	-0.82	2.81
HTZ92_1574	dihydrolipoamide dehydrogenase	7.15	-1.00	3.00

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_1575	diaminohydroxyphosphoribosyl-aminopyrimidine deaminase	8.28	-0.11	4.13
HTZ92_1576	TPP-dependent acetoine dehydrogenase	7.44	-0.59	3.52
HTZ92_1577	ABC-transporter substrate binding protein	6.80	-1.08	2.98
HTZ92_1578	lipoyl synthase	5.34	-1.68	2.39
HTZ92_1579	regulator	4.42	-0.37	2.35
HTZ92_1713	hypothetical protein	5.12	4.42	3.93
HTZ92_1749	hypothetical protein	5.48	-0.21	2.42
HTZ92_1805	MsrB (peptide-methionine (R)-S-oxide reductase)	4.93	5.26	5.84
HTZ92_1858	LrgA (murein hydrolase transporter)	7.45	1.51	1.80
HTZ92_1954	MFS transporter permease	6.10	2.17	3.89
HTZ92_1955	hydroxymethylglutaryl-CoA lyase	5.44	0.85	1.81
HTZ92_1956	carnitine dehydratase	5.49	0.29	1.65
HTZ92_1973	PaaC 3-hydroxy-acyl-CoA dehydrogenase	3.66	0.10	0.90
HTZ92_1974	enoyl-CoA hydratase	3.96	-0.07	1.10
HTZ92_1975	enoyl-CoA hydratase	3.81	-0.96	1.26
HTZ92_1976	PaaK (phenylacetate-CoA oxygenase/reductase)	4.62	-0.84	1.62
HTZ92_1977	PaaJ (phenylacetate-CoA oxygenase)	4.74	-0.15	2.01
HTZ92_1978	PaaI (phenylacetic acid degradation protein)	5.49	0.12	2.75
HTZ92_1979	PaaB (phenylacetate-CoA oxygenase subunit)	3.29	-1.70	1.03
HTZ92_1980	PaaA (phenylacetate-CoA oxygenase subunit)	5.64	-0.20	3.28
HTZ92_1981	PaaN (enoyl-CoA hydratase)	5.94	0.07	3.48
HTZ92_2060	4-hydroxybenzoate transporter	6.11	4.63	4.71

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_2117	hypothetical protein	3.40	5.16	3.50
HTZ92_2171	regulator (Cu-Export)	5.09	4.72	4.83
HTZ92_2207	GNAT family N-acetyltransferase	5.10	3.66	4.48
HTZ92_2358	MerC	7.96	6.64	7.53
HTZ92_2363	Cd(II)/Pb(II)-responsible transcriptional regulator	5.62	4.82	5.14
HTZ92_2449	esterase	-0.16	5.21	2.41
HTZ92_2581	lactoylglutathion lyase	3.01	3.87	5.01
HTZ92_2589	SmpA/OmlA family (bacterial outer membrane lipoprotein)	5.73	4.49	4.47
HTZ92_2625	CntB	4.86	5.04	5.25
HTZ92_2626	malic semialdehyde dehydrogenase	5.47	5.50	5.85
HTZ92_2627	CntA	6.08	6.17	6.50
HTZ92_2628	acetylcarnitine hydrolase	5.34	5.39	6.26
HTZ92_2629	Aci01347	6.47	6.54	7.46
HTZ92_2630	malate/tartrate dehydrogenase	8.49	8.25	8.87
HTZ92_2632	phosphate porine	6.03	3.93	6.62
HTZ92_2881	rhodanese	5.55	6.22	5.44
HTZ92_2981	hypothetical protein	4.36	5.42	6.10
HTZ92_3059	cation:proton antiporter	4.31	4.92	5.13
HTZ92_3387	ABC-transporter substrate binding protein	1.71	4.50	3.38
HTZ92_3388	sulfonate ABC-transporter substrate binding protein	1.82	4.83	4.64
HTZ92_3389	alkansulfonate monooxygenase	1.37	4.09	3.98
HTZ92_3390	ABC-transporter permease	2.13	4.22	4.57

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_3497	HmgA (glyoxylase)	6.52	5.05	4.61
HTZ92_3498	HmgC (maleylacetoacetate isomerase)	5.39	3.89	3.06
HTZ92_3499	HmgB (fumarylacetoacetase)	5.65	4.65	3.70
HTZ92_3500	amino acid transporter	6.00	4.94	4.10
HTZ92_3606	hypothetical protein	4.91	4.52	4.49
HTZ92_3607	hybrid histidine kinase/response regulator	2.73	3.53	2.18
HTZ92_3608	hypothetical protein	1.33	6.33	3.40
HTZ92_3609	cation:acetate symporter	2.76	7.64	4.43
HTZ92_3610	ion channel	1.81	1.64	2.73

Suppl. Tab. 3. Carnitine, acetylcarnitine or D-malate repressed genes. Given is the log<sub>2</sub> fold change in comparison to cells grown in the presence of succinate.

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_0025	hypothetical protein	-4.07	-5.95	-4.01
HTZ92_0029	hypothetical protein	-3.14	-0.21	-5.86
HTZ92_0102	(2Fe-2S)-binding protein	-5.38	-4.07	-3.79
HTZ92_0115	30S ribosomal protein S16	-4.81	-5.53	-4.70
HTZ92_0214	50S ribosomal protein L30	-6.75	-7.58	-7.31
HTZ92_0217	ribosomal protein L36	-6.85	-7.57	-7.63
HTZ92_0225	flavinator of succinate dehydrogenase	-5.21	-4.77	-3.89
HTZ92_0227	hypothetical protein	-9.54	-9.94	-9.03
HTZ92_0325	glycosyl transferase	-1.66	-1.67	-7.10
HTZ92_0364	methyltransferase	-7.20	-5.40	-3.22
HTZ92_0413	bacteriolytic lipoprotein entericidin B	-8.17	-7.29	-5.92
HTZ92_0423	hypothetical protein	-5.88	-6.58	-6.99
HTZ92_0485	50S ribosomal protein L27	-4.96	-5.91	-5.24
HTZ92_0533	hypothetical protein	-2.91	-4.50	-5.43
HTZ92_0630	hypothetical protein	-6.22	-4.97	-5.38
HTZ92_0660	hypothetical protein	-5.23	-5.55	-5.79
HTZ92_0753	50S ribosomal protein L31	-5.74	-6.73	-5.76
HTZ92_0854	hypothetical protein	-5.22	-5.84	-6.70
HTZ92_0855	hypothetical protein	-8.26	-7.73	-5.48
HTZ92_0861	hypothetical protein	-5.29	-3.61	-3.36

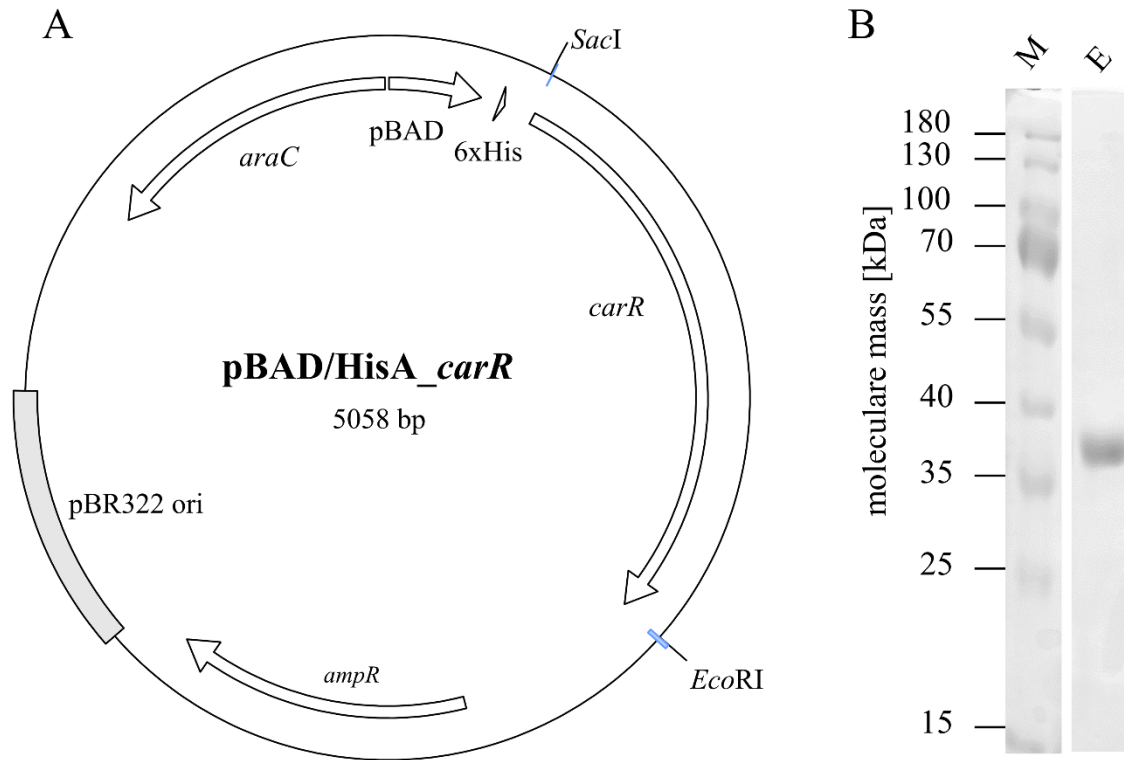


Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_0863	hypothetical protein	-5.94	-5.13	-5.62
HTZ92_0864	hypothetical protein	-8.33	-7.76	-6.40
HTZ92_0867	hypothetical protein	-6.13	-4.14	-4.68
HTZ92_0868	hypothetical protein	-4.39	-8.01	-6.97
HTZ92_0878	hypothetical protein	-5.82	-6.10	-8.20
HTZ92_0901	Regulatory protein mnt	-3.45	-3.88	-5.04
HTZ92_0905	phage anti-repressor protein	-5.69	-6.15	-7.79
HTZ92_0964	cold-shock protein	-6.15	-5.79	-5.02
HTZ92_0980	30S ribosomal protein S21	-4.57	-5.29	-4.25
HTZ92_0990	hypothetical protein	-6.69	-3.35	-4.18
HTZ92_1004	hypothetical protein	-5.22	-4.70	-4.41
HTZ92_1054	DNA-binding protein for site-specific recombination, transcription of rRNA and tRNA operons and DNA replication	-5.00	-5.49	-3.92
HTZ92_1067	30S ribosomal protein S18	-5.11	-6.31	-5.98
HTZ92_1099	hypothetical protein	-5.22	-3.67	-4.34
HTZ92_1173	hypothetical protein	-4.10	-1.97	-6.78
HTZ92_1176	hypothetical protein	-1.67	-5.60	-2.49
HTZ92_1209	3-dehydroquinate dehydratase	-4.79	-5.74	-4.04
HTZ92_1229	DsrH like protein (sulphur oxidation)	-1.82	-5.64	-1.99
HTZ92_1263	hypothetical protein	-5.60	-1.72	-3.01
HTZ92_1276	hypothetical protein	-4.79	-4.52	-5.14
HTZ92_1417	hypothetical protein	-3.66	-3.33	-5.80

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_1452	hypothetical protein	-6.89	-7.17	-6.16
HTZ92_1453	hypothetical protein	-5.43	-3.75	-1.07
HTZ92_1477	hypothetical protein	-4.83	-5.93	-10.63
HTZ92_1548	hypothetical protein	-5.95	-6.23	-9.11
HTZ92_1549	hypothetical protein	-2.78	-4.62	-5.75
HTZ92_1592	signal peptide protein	-4.63	-4.44	-5.94
HTZ92_1640	transcriptional regulator HU subunit alpha	-5.65	-4.79	-4.84
HTZ92_1658	30S ribosomal protein S20	-5.36	-5.66	-4.72
HTZ92_1711	hypothetical protein	-7.29	-7.76	-7.55
HTZ92_1889	hypothetical protein	-6.06	-5.54	-3.27
HTZ92_1891	hypothetical protein	-5.19	-2.25	-4.31
HTZ92_1895	hypothetical protein	-7.75	-5.09	-7.20
HTZ92_1994	hypothetical protein	-5.65	-5.99	-4.86
HTZ92_2012	PAAR domain-containing protein family protein	-4.54	-4.83	-5.77
HTZ92_2055	hypothetical protein	-3.57	-3.51	-5.15
HTZ92_2059	hypothetical protein	-5.61	-2.12	-3.57
HTZ92_2080	hypothetical protein	-4.07	-3.32	-6.10
HTZ92_2093	hypothetical protein	-4.57	-4.30	-5.26
HTZ92_2126	hypothetical protein	-5.71	-4.07	-2.57
HTZ92_2134	DNA-binding protein	-4.43	-2.89	-5.17
HTZ92_2137	hypothetical protein	-3.72	-6.00	-6.87
HTZ92_2147	peptidoglycan-binding protein	-4.29	-4.50	-6.96

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_2156	hypothetical protein	-4.27	-3.36	-5.14
HTZ92_2157	cold-shock protein	-3.89	-4.41	-5.23
HTZ92_2161	hypothetical protein	-6.04	-6.38	-5.29
HTZ92_2163	transposase	-6.55	-6.56	-6.39
HTZ92_2165	exodeoxyribonuclease VII	-3.54	-6.61	-3.87
HTZ92_2232	hypothetical protein	-4.78	-3.85	-5.03
HTZ92_2237	hypothetical protein	-3.98	-2.23	-5.10
HTZ92_2254	hypothetical protein	-6.10	-6.42	-5.26
HTZ92_2265	Cro/C1 family transcriptional regulator	-1.66	-2.22	-6.53
HTZ92_2411	hypothetical protein	-4.14	-6.39	-5.40
HTZ92_2446	hypothetical protein	-5.46	-9.72	-8.81
HTZ92_2451	rubredoxin	-7.05	-6.72	-6.82
HTZ92_2470	lipoprotein precursor	-6.07	-3.00	-1.87
HTZ92_2512	hypothetical protein	-6.26	-4.67	-3.32
HTZ92_2530	hypothetical protein	-2.83	-3.10	-5.92
HTZ92_2538	hypothetical protein	-4.97	-4.54	-6.40
HTZ92_2559	hypothetical protein	-4.79	-3.88	-6.95
HTZ92_2566	hypothetical protein	-6.60	-7.01	-6.67
HTZ92_2592	hemerythrin	-0.97	-1.03	-5.43
HTZ92_2655	acyl carrier protein	-4.95	-5.50	-5.37
HTZ92_2658	50S ribosomal protein L32	-5.68	-6.46	-5.12
HTZ92_2729	hypothetical protein	-5.48	-8.73	-7.78

Locus-tag	Function	Carnitine [log <sub>2</sub> ]	Acetylcarnitine [log <sub>2</sub> ]	D-malate [log <sub>2</sub> ]
HTZ92_2738	hypothetical protein	-0.66	-1.32	-5.77
HTZ92_2819	50S ribosomal protein L35	-4.37	-5.69	-4.57
HTZ92_2952	50S ribosomal protein L28	-4.09	-5.03	-4.98
HTZ92_2953	50S ribosomal protein L33	-6.90	-7.71	-7.86
HTZ92_3123	acyl-CoA thioester hydrolase YbgC/YbaW family	-3.30	-3.91	-6.36
HTZ92_3169	membrane fusogenic activity	-3.57	-5.11	-4.21
HTZ92_3201	hypothetical protein	-2.30	-3.26	-5.12
HTZ92_3342	hypothetical protein	-5.57	-5.96	-2.06
HTZ92_3343	hypothetical protein	-4.38	-4.86	-5.26
HTZ92_3352	hypothetical protein	-4.95	-5.44	-4.97
HTZ92_3370	hypothetical protein	-4.93	-5.83	-5.41
HTZ92_3424	hypothetical protein	-7.90	-6.09	-3.54
HTZ92_3434	ribosomal protein L34	-5.26	-6.45	-5.66
HTZ92_3644	hypothetical protein	-6.75	-6.68	-9.73
HTZ92_3645	hypothetical protein	-6.29	-6.56	-8.73
HTZ92_3646	cold-shock protein	-7.09	-7.84	-8.89
HTZ92_3649	hypothetical protein	-3.50	-3.84	-7.91



**Suppl. Fig. 1. Recombinant plasmid for production of CarR in *E. coli* (A) and purification of the regulator CarR.** N-terminal His<sub>6</sub>-tagged CarR was produced in *E. coli* BL21 STAR using the pBAD/HisA expression vector. The *carR* gene was integrated in pBAD/HisA using SacI and EcoRI restriction sites (A). CarR was purified *via* affinity chromatography using Ni-NTA (elution at 150 mM imidazole) and analyzed on a 12.5 % SDS gel. 20 µg of protein was applied to the gel and stained with Coomassie Brilliant Blue R-250 (B).