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Article

Targeting of the Essential *acpP*, *ftsZ*, and *rne* Genes in Carbapenem-Resistant *Acinetobacter baumannii* by Antisense PNA Precision Antibacterials

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Supplementary

Supplementary Tables and Figures:

Table S1. (**A**) Clinical and phenotypic characteristics of the CRAB in this study (MIC μg/mL). (**B**) Genotypic characteristics of the CRAB strains in this study. The production of carbapenemases is an important mechanism responsible for the carbapenem resistance in *A. baumannii*. The genes encoding carbapenem-hydrolyzing class D ß-lactamases (CHDLs), class B metallo-ß-lactamases (MBLs) are shown here.

(A)

Clinical strains.	Source	Imipenem	Meropenem	Colistin	Ciprofloxacin	Tigecycline	Tetracycline	Ceftazidime	Gentamicin
AC1	Wound	8	4	1	8	0.5	16	64	8
AC2	Wound	16	16	1	16	1	64	64	16
AC3	Sputum	32	4	1	8	0.5	16	64	8
AC4	Trachea	8	16	1	16	1	64	64	16
AC5	Urine	16	16	1	16	1	64	32	16
AC6	Blood	16	16	1	32	1	16	32	16
AC7	Blood	16	16	1	16	1	64	64	16
AC8	Sputum	16	16	1	32	1	32	64	16
AC9	CSF	16	16	1	8	1	32	32	16
AC44	Urine	16	16	0.5	32	1	256	256	16
AC46	Sputum	16	16	1	32	0.5	128	128	16
SSI1104	NA	NA	> 16	1	> 8	1	64	> 8	32
ATCC 19606	Type strain	1	1	1	1	0.5	1	8	4

(B)

Clinical Isolates No	*bla*IMP	*bla*VIM	*bla*SPM	*bla*GES	*bla*OXA23	*bla*OXA-24	Is/ *bla*OXA23	Is/*bla*oxa51
AC1	_	+	+	+	+	_	_	_
AC2	_	+	_	+	+	_	+	+
AC3	+	+	_	_	+	+	_	_
AC4	_	+	_	_	+	+	+	_
AC5	_	+	_	_	+	+	+	+
AC6	_	+	_	_	+	+	+	+
AC7	_	+	_	+	+	+	+	+
AC8	_	+	_	+	+	+	+	+
AC9	_	+	_	+	+	+	+	+
AC44	_	+	_	+	+	+	+	+

AC46	_	+	_	_	+	+	+	+
SSI1104	ND	ND	ND	ND	+	ND	ND	ND

Table S2. *Acinetobacter baumannii* strains that were examined for designing antisense in this study. The target genes ID obtained from GenBank database updated January 2019.

Strains	*ftsZ* Gene Bank AC number	*acpP* Gene Bank AC number.
A. baumannii AYE	NC_010400.1	NC_010410.1
A.baumannii ATCC17978	A1S_3331	CP000521
A. baumannii ACICU	NC_010611.1	NC_010611.1
A. baumannii SDF	ABSDF3467	NC_010400.1
A.baumannii ATCC 19606	CP046654.1	CP046654.1

Table S3. Anti-*ftsZ* BPP-PNA used in this study.

		Mass(found/calculate)
5612	H-(KFF)$_3$K-eg1-GAGGCCATGAC-NH$_2$	4572(4568)
5615	H-(KFF)$_3$K-eg1-GAGTCCAGGAC-NH$_2$	4568(4568)
5613	H-(R-Ahx-R)$_4$-Ahx-GAGGCCATGAC-NH$_2$	4914(4917)
5616	H-(R-Ahx-R)$_4$-Ahx-GAGTCCAGGAC-NH$_2$	4914(4917)
5657	H-(KFF)$_3$K-eg1-ATGAGGCCATG-NH$_2$	4583(4580)
5658	H-(KFF)$_3$K-eg1-GGCCATGACCT-NH$_2$	4583(4580)

Table S4. Anti-*acpP* BPP-PNA used in this study.

		Mass(found/calculated)
5652	H-(KFF)$_3$K-eg1-ATATCGCTCAC-NH$_2$	4478(4477)
5653	H-(KFF)$_3$K-eg1-ATACCGCTTAC-NH$_2$	4478(4477)
5639	H-(KFF)$_3$K-eg1-TGATTTGCCAC-NH$_2$	4512(4509)
5640	H-(KFF)$_3$K-eg1-TGACTTGTCAC-NH$_2$	4510(4509)
5823	H-(kff)$_3$k-eg1-TGATTTGCCAC-NH$_2$	4509(4507)
5824	H-(kff)$_3$k -eg1-TGACTTGTCAC-NH$_2$	4509(4507)

Table S5. Anti-*rne* BPP-PNA used in this study.

		Mass(found/calculated)
5637	H-(KFF)$_3$K-eg1-ACGTTTCATGG-NH$_2$	4549(4540)
5638	H-(KFF)$_3$K-eg1-ACGATTCTTGG-NH$_2$	4549(4548)
5654	H-(KFF)$_3$K-eg1-ATACGTTTCAT-NH$_2$	4508(4507)
5821	H-(KFF)$_3$K-eg1-ATCCGTTTAAT-NH$_2$	4508(4509)
5656	H-(KFF)$_3$K-eg1-TTTCATGGGTG-NH$_2$	4580(4582)
5822	H-(KFF)$_3$K-eg1- TTTGATGTGTG -NH$_2$	4580(4580)
5655	H-(KFF)$_3$K-eg1-GTTTCATGGGT-NH$_2$	4580(4578)

Table 6. Primers used in Real time RT-PCR.

Primer	Genes	PCR Product
acpP F: AACAACTTGGTCTTAAAG acpP R: GATTGAACTGTAGTGATT	acpP	163 bp
ftsZ F: CAGCACATGGTACAGTCT ftsZ R: GGCATTCATACAGTCAAGTG	ftsZ	84 bp
zipA-F : GAGCAGATTATCGCATTA zipA-R : ATACGCAATACAGAGAAC	zipA	170bp
polA-F : AACATTCCAATTCGTAGAC polA-R : TGAACTAAGGCATCATCT	polA	143bp
16s rRNA-F: CAAACAGGATTAGATACC 16s rRNA-R: GTCAATTCATTTGAGTTT	16srRNA	146 bp

A

Query	1	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	40
CP054302.1	1665017	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1664978
CP053391.1	1657749	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1657788
CP048827.1	3682037	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	3681998
CP041148.1	1880027	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1880066
CP050385.1	3532759	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	3532720
CP050388.1	3772341	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	3772302
CP050390.1	1612700	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613091
CP050400.1	3550417	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	3550378
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CP050403.1	2197071	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	2197462
CP051474.1	1612342	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1612733
CP050421.1	1624941	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1625332
CP050416.1	1613866	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613457
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CP050415.1	29189229	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	29189668
CP050425.1	1784559	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1784998
CP050432.1	1783833	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1784224
CP050523.1	1623855	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1624244
CP050526.1	1624941	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1625332
CP051862.1	3563902	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	3563863
CP051866.1	1688818	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1688857
CP051875.1	1631103	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1631142
CP051869.1	11107951	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	11107912
CP050916.1	1613151	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613541
CP050914.1	1709461	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1709851
CP050911.1	1613511	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613901
CP050904.1	1613161	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613551
CP050907.1	1613241	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1613631
CP049806.1	1663251	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1663641
CP049363.1	36790901	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	36790511
AP022836.1	1596941	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1597331
CP035109.1	7030631	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	7030241
CP045428.1	33881251	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	33880861
CP046654.1	11614171	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	11613781
CP046536.1	35854331	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	35853941
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CP033243.1	1675751	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1676141
CP045528.1	27576181	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	27575791
CP045541.1	2399811	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	2399421
CP045110.1	38399931	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	38399541
CP042556.1	250671	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	250281
CP032743.1	27120721	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	27121111
CP044356.1	36474761	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	36474371
CP040911.1	1788681	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1789071
CP043953.1	1614301	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1614691
CP043910.1	12166791	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	12166401
CP043418.1	1665621	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1666011
CP043419.1	1664621	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1665011
CP043417.1	1664441	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1664831
CP043180.1	6404321	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	6403931
CP043052.1	1667611	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1668001
CP042994.1	1653481	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1653871
CP042841.1	1663981	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1664371
CP026089.2	1689141	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1689531
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CP027250.2	1657501	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1657891
CP027254.3	1922321	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1922711
CP027246.2	1664501	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1664891
CP026750.2	1625031	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	1625421
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CP039028.2	12177251	TATTAGGAACCCTAAAGGTCATGGCCTCATTTGAATTTAT	12176861
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B

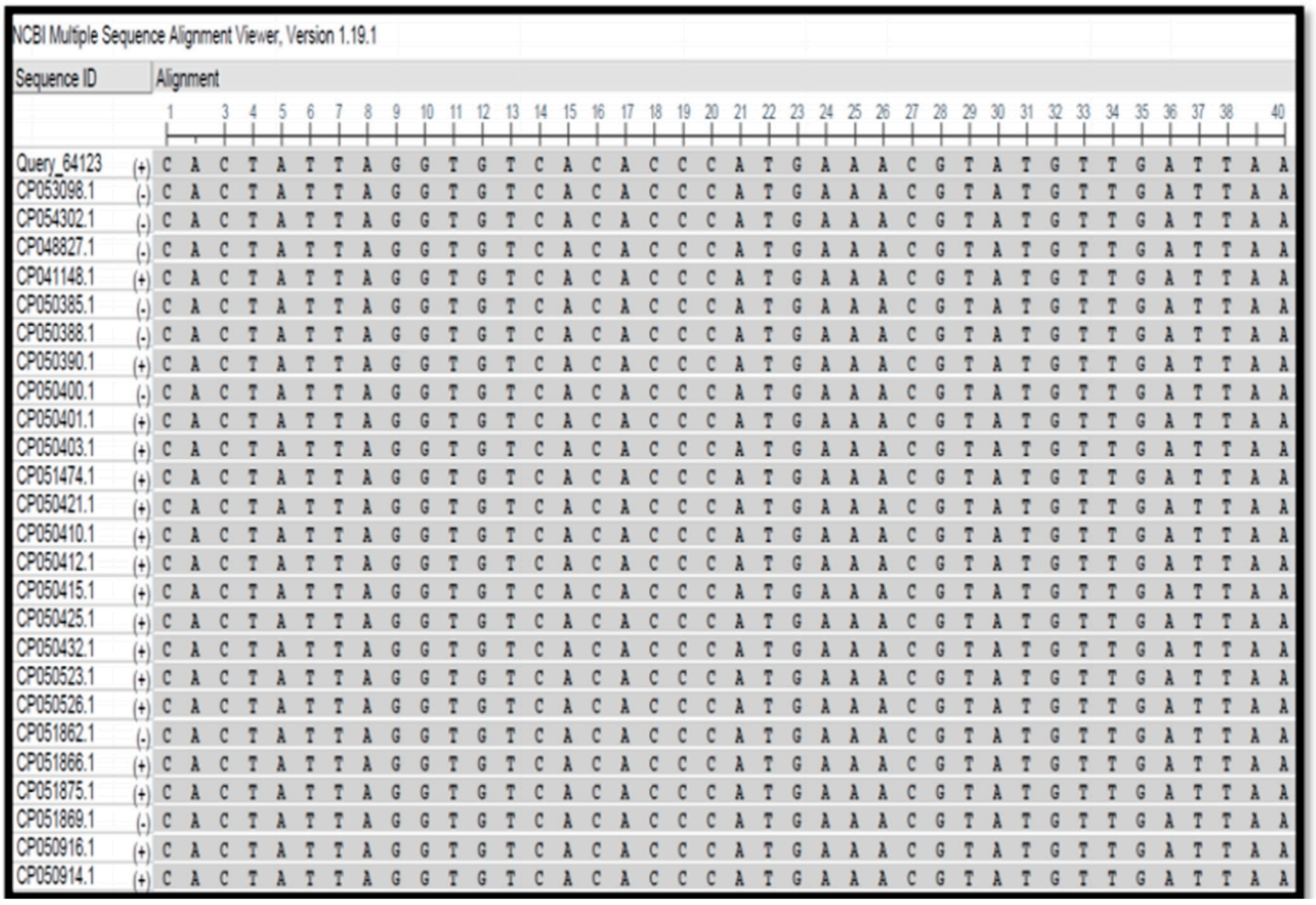


Figure S1. (**A**) Multiple sequence alignment of *ftsZ* gene, region -20 to +20, using Basic Local Alignment Search Tool (BLAST) on the NCBI website. The BLAST results confirmed the 5′ terminal region of the *ftsZ* gene including the ribosomal binding site and the start ATG codon is conserved among *A.baumannii* species. (**B**) Multiple sequence alignment of the rne gene, region -20 to +20, using BLAST on the NCBI website. The BLAST results confirmed the 5′ terminal region of the rne gene including the ribosomal binding site and the start ATG codon is conserved among *A.baumannii* species.

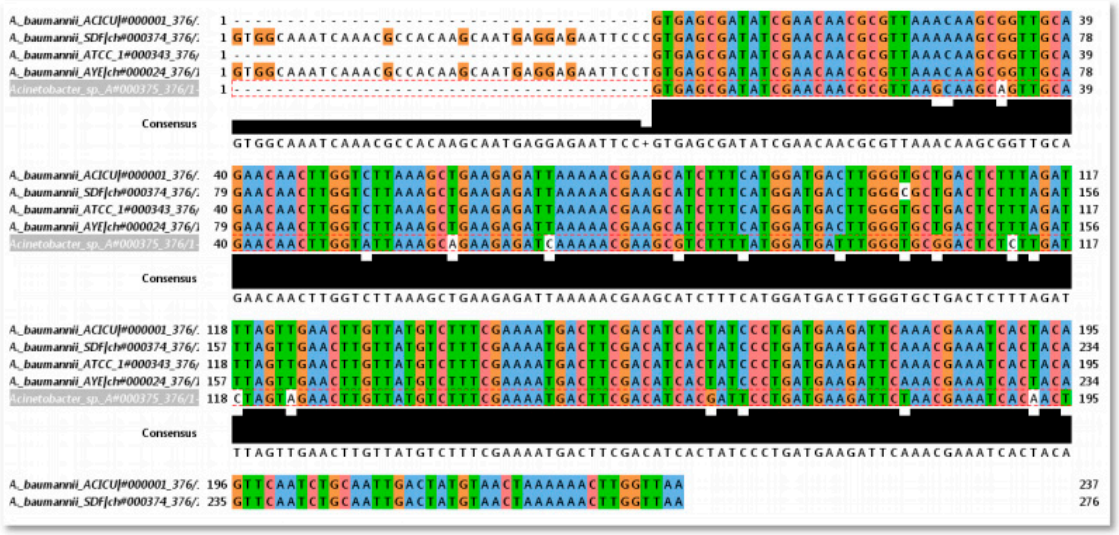


Figure S2. A multiple sequence alignment of *acpP* gene (GenBank database updated January 2019) using the Basic Local Alignment Search Tool (BLAST) on the NCBI website in *A. baumannii* AYE,*A.baumannii* SDF, *A. baumannii* ACICU, *A. baumannii* ATCC 17978 and *Acinetobacter* sp ADP1 (Sequence ID was represented in Table S2)[32]. The *acpP* gene has two lengths in different strains of *A.baumannii*.The *acpP* gene in *A.baumannii* ATCC19606, AYE and SDF strains was 276 bp in length while that was 237 bp in length on both ACICU and ATCC17978 strains.

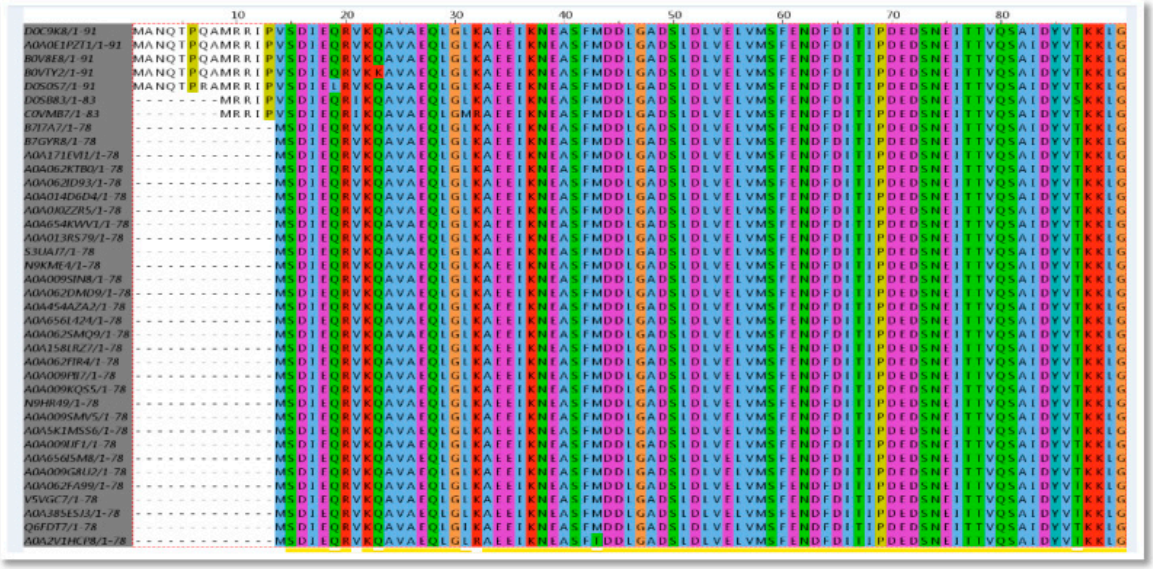


Figure S3. A multiple sequence alignment of AcpP proteins (UniProt updated 2019/01).using UniProt website (*A. baumannii* AYE UniProt ID: B0V8E8, *A. baumannii* SDF UniProt ID: B0VTY2 and *A. baumannii* ATCC 19606 UniProt ID: D0C9K8.

A

21 Genes

Accession Number	Gene Name	Organism	Replicon	Gene Length	Location (kb)	Description
ABAYE2991	***acpP***	*A. baumannii* AYE	*chromosome*	276	3018.68	Acyl carrier protein
JW1080	***acpP***	*E. coli* W3110	*chromosome*	237	1153.19	Acyl carrier protein
UTI89_C1220	***acpP***	*E. coli* UTI89	*chromosome*	237	1196.58	Acyl carrier protein
SbBS512_E2230	***acpP***	*S. boydii* CDC 3083-94	*chromosome*	237	2030.10	Acyl carrier protein
SBO_1969	***acpP***	*S. boydii* Sb227	*chromosome*	237	1943.72	Acyl carrier protein
SCH_1144	***acpP***	*S. choleraesuis* enterica sv Choleraesuis SC-B67	*chromosome*	237	1260.17	Acyl carrier protein
SeD_A2173	***acpP***	*S. dublin* sv Dublin CT_02021853	*chromosome*	237	2086.89	Acyl carrier protein
SDY_2056	***acpP***	*S. dysenteriae* Sd197	*chromosome*	237	1868.37	Acyl carrier protein
SEN1853	***acpP***	*S. enteritidis* P125109	*chromosome*	237	1958.59	Acyl carrier protein
SFV_1114	***acpP***	*S. flexneri* 5 8401	*chromosome*	237	1157.41	Acyl carrier protein
S1178	***acpP***	*S. flexneri* 2a 2457T	*chromosome*	237	1142.22	Acyl carrier protein
CP0124	***acpP***	*S. flexneri* 2a 301	*pCP301*	237	107.17	Acyl carrier protein
SF1098	***acpP***	*S. flexneri* 2a 301	*chromosome*	237	1139.33	Acyl carrier protein
SG1926	***acpP***	*S. gallinarum* 287/91	*chromosome*	237	1991.33	Acyl carrier protein
SPAB_02327	***acpP***	*S. paratyphi* enterica sv Paratyphi B SPB7	*chromosome*	237	1928.49	Acyl carrier protein
SPA1655	***acpP***	*S. paratyphi* enterica sv Paratyphi A ATCC 9150	*chromosome*	237	1720.08	Acyl carrier protein
SeSA_A1266	***acpP***	*S. schwarzengrund* sv Schwarzengrund CVM19633	*chromosome*	237	1238.41	Acyl carrier protein
SSON_1114	***acpP***	*S. sonnei* Ss046	*chromosome*	237	1192.91	Acyl carrier protein
STY1235	***acpP***	*S. typhi* CT18	*chromosome*	237	1189.78	Acyl carrier protein
t1724	***acpP***	*S. typhi* enterica sv Typhi Ty2	*chromosome*	237	1791.40	Acyl carrier protein
STM1196	***acpP***	*S. typhimurium* LT2	*chromosome*	237	1280.11	Acyl carrier protein

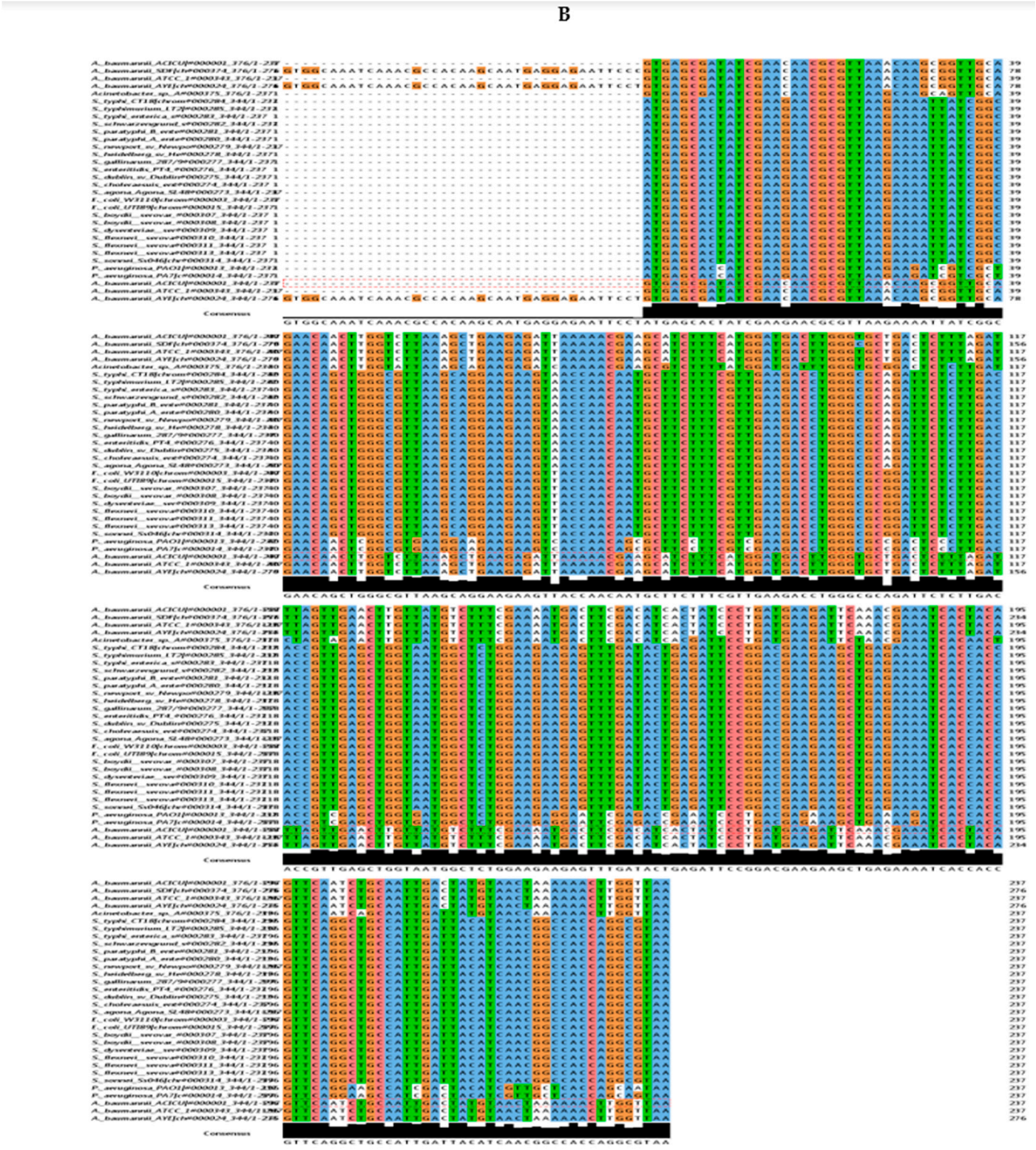
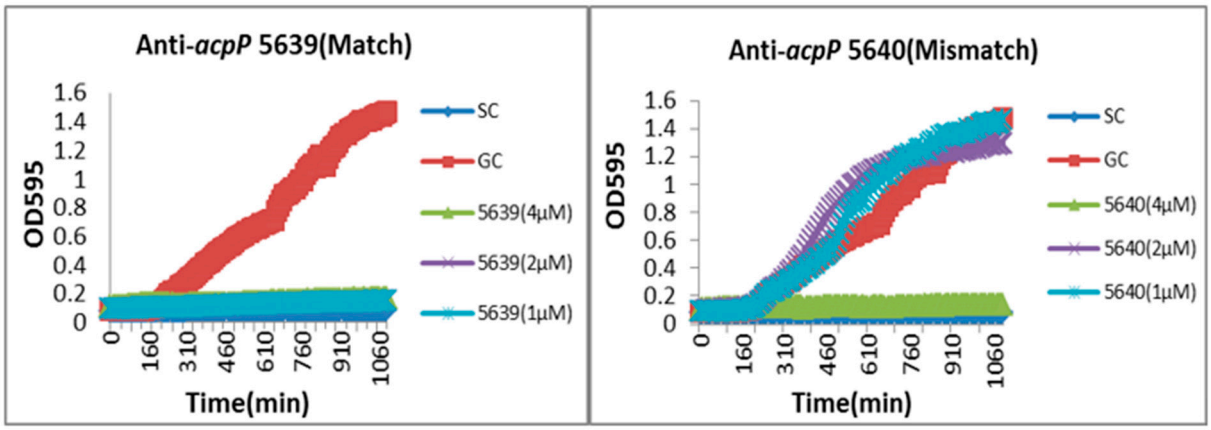


Figure S4. (**A**)The sequence of the *acpP* gene in *A.baumannii* and the other gram negative bacteria was obtained from genolist database[32] .(**B**)Multiple sequence alignment of *acpP* gene was done for determination of conserved regions.

(A)


Anti-acpP 5639(Match)
OD595
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
160
310
460
610
760
910
1060
Time(min)
SC
GC
5639(4μM)
5639(2μM)
5639(1μM)
Anti-acpP 5640(Mismatch)
OD595
Time(min)
SC
GC
5640(4μM)
5640(2μM)
5640(1μM)

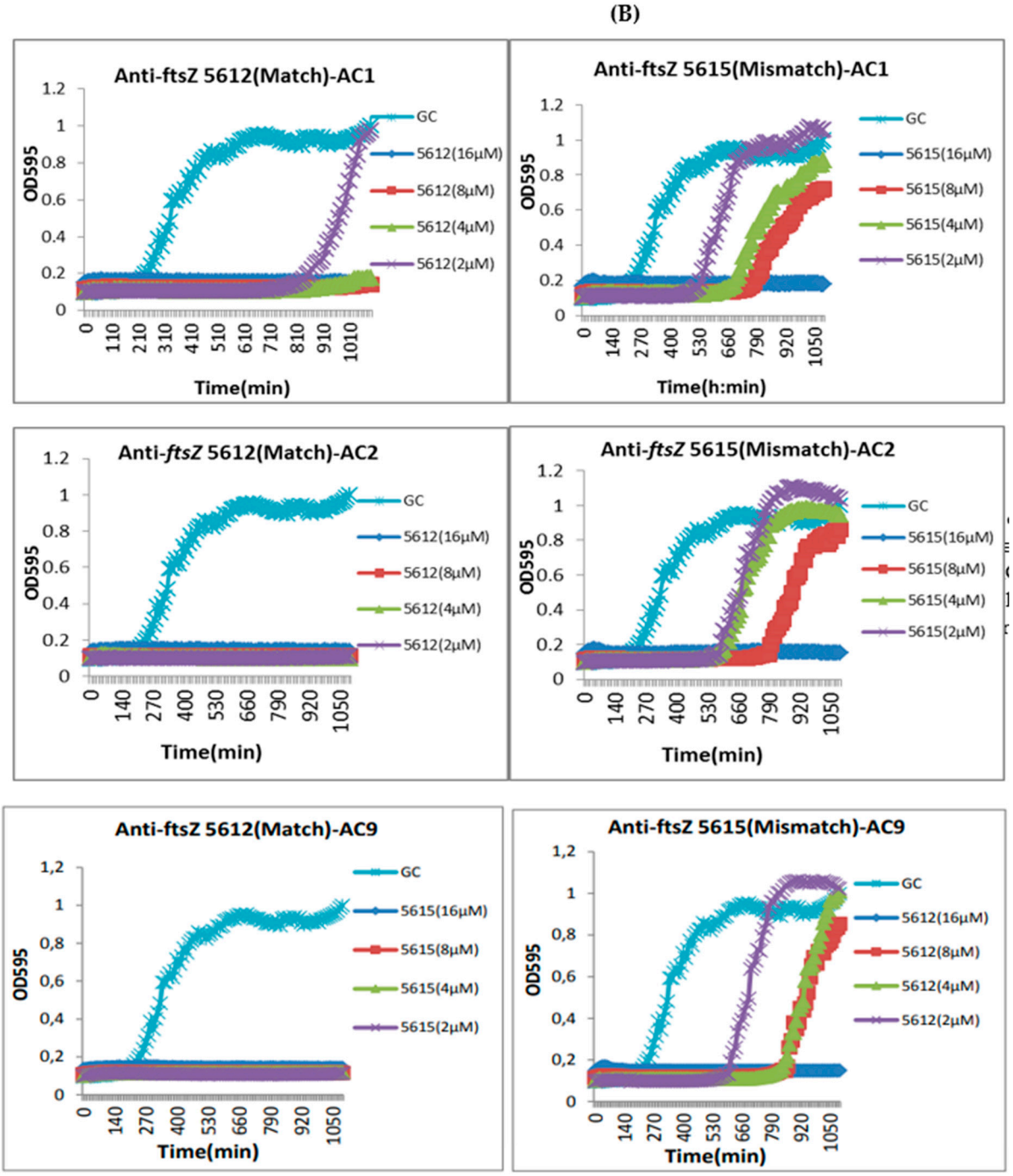


Figure S5. (**A**) Growth inhibition activity curve of the most active anti-*acpP* $(KFF)_3K$-PNA(5639) and a corresponding mismatch control (5640) against CRAB strain (AC44).(**B**) Growth inhibition activity of the most active anti-*ftsZ* $(KFF)_3K$-PNA(5612) and a corresponding mismatch control (5615) against three CRAB strains(AC1,AC2 and AC9). Concentration-dependent growth inhibition of anti-*ftsZ* $(KFF)_3K$-PNA(5612) and a corresponding mismatch controls on the growth of three CRAB strains (AC1,AC2 and AC9). Bacteria were grown in the absence of PNA (GC) and different doses of peptide–PNA conjugates (2μM to 16 μM).

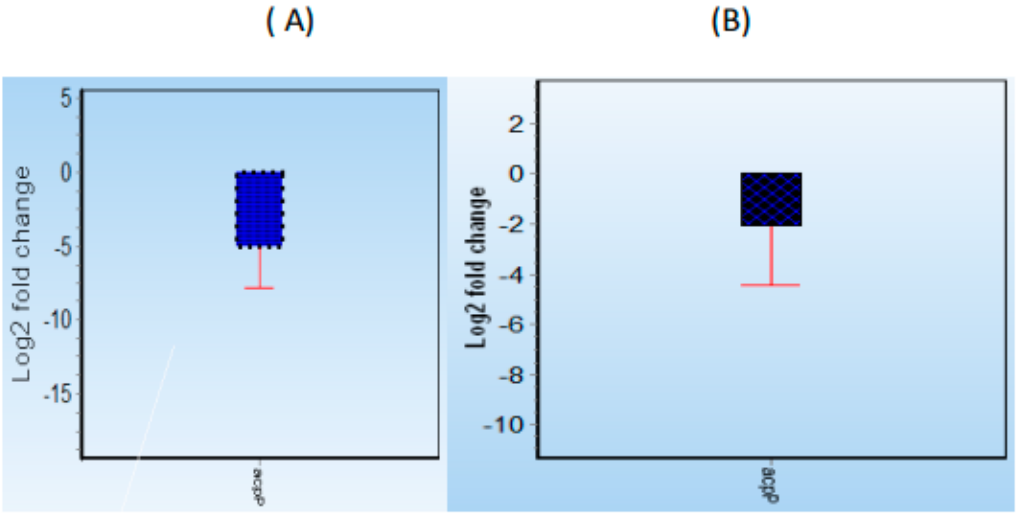
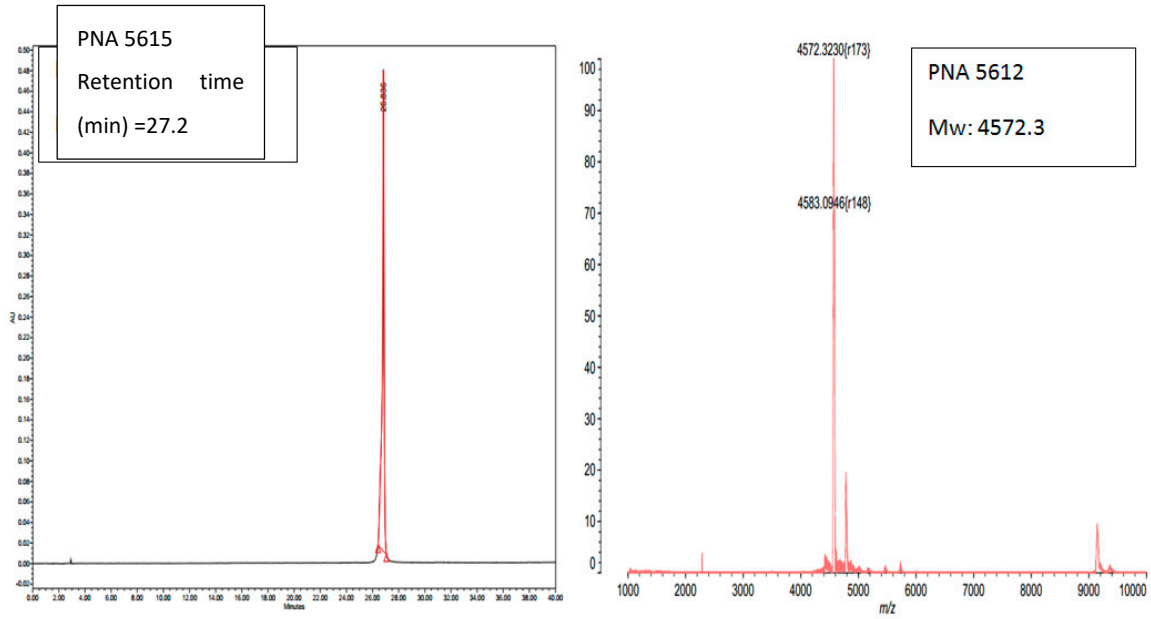
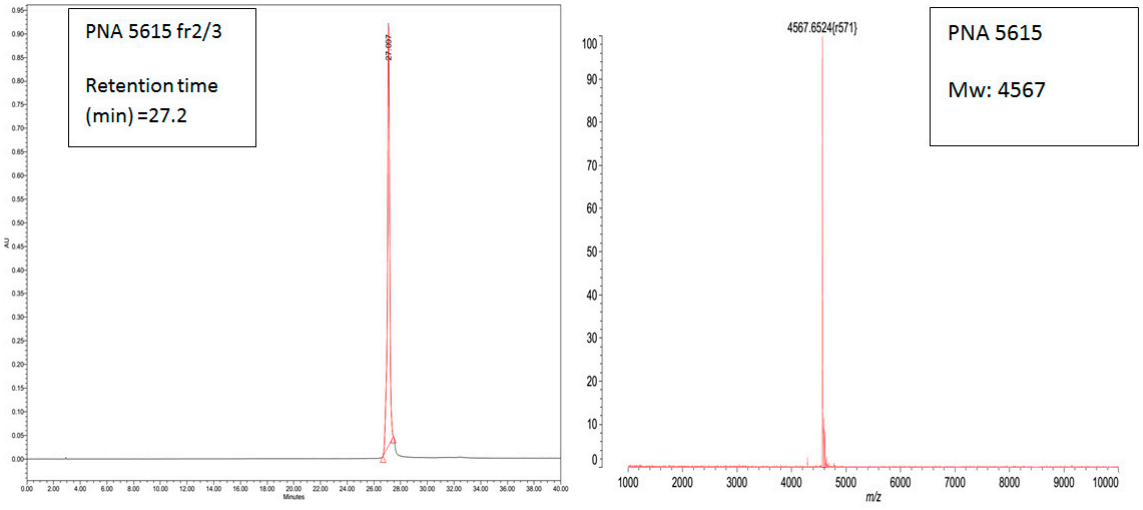
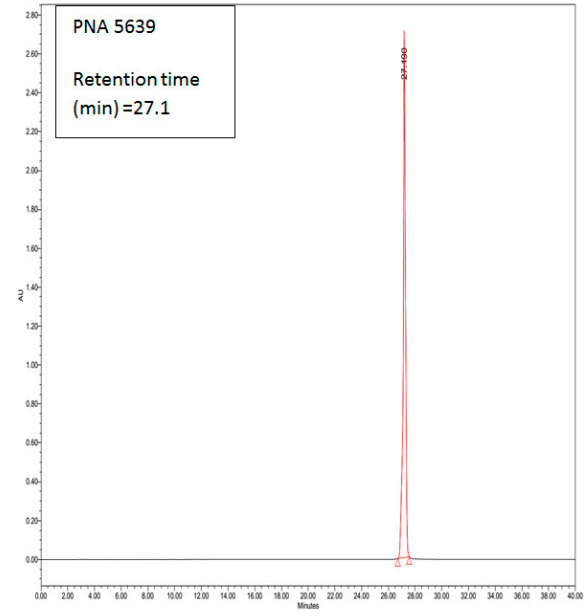
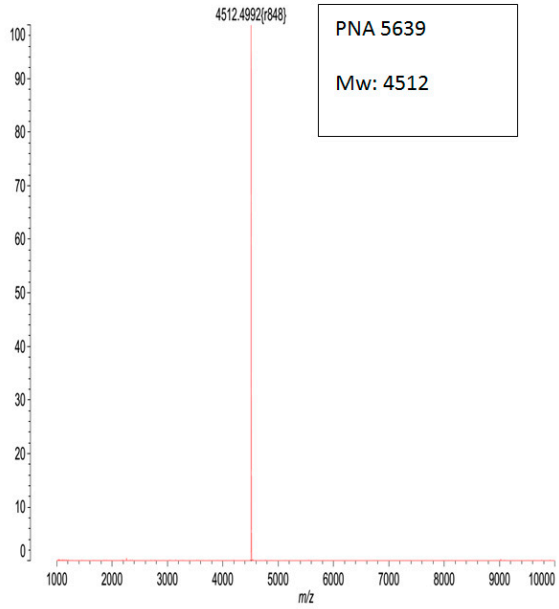


Figure S6. (**A**) Log2-fold change values determined by qPCR for *acpP* mRNA in CRAB AC44 strain, 1 hour treated with different concentration of anti-*acpP* $(KFF)_3K$-PNA 5639 (match) compared to the untreated sample. (B) Log2-fold change values determined by qPCR for *acpP* mRNA in CRAB AC44 strain, 1 hour treated with different concentration of anti-*acpP* $(KFF)_3K$-PNA 5640 (Mismatch) compared with the untreated sample. P values were calculated between (A) match and untreated sample (P-Value = 0.01) and (**B**) Mismatch and untreated sample (P-Value = 0.059) using twotailed Student's t-test ($P<0.05$ was considered as significant). Values shown in the graphs are the mean of three replicates. Error bars depict standard error of the mean.


PNA 5615
Retention time
(min) =27.2
4572.3230(r173)
4583.0946(r148)
PNA 5612
Mw: 4572.3
m/z


PNA 5615 fr2/3
Retention time
(min) =27.2
4567.6524(r571)
PNA 5615
Mw: 4567
m/z


PNA 5639
Retention time (min) =27.1
27.190
AU
Minutes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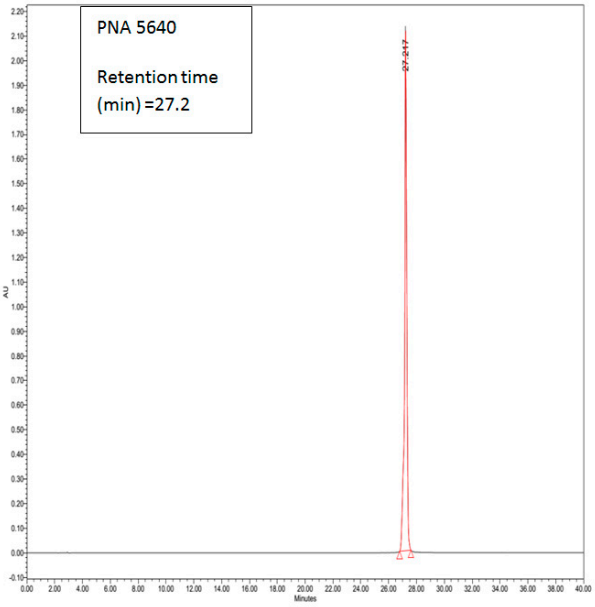
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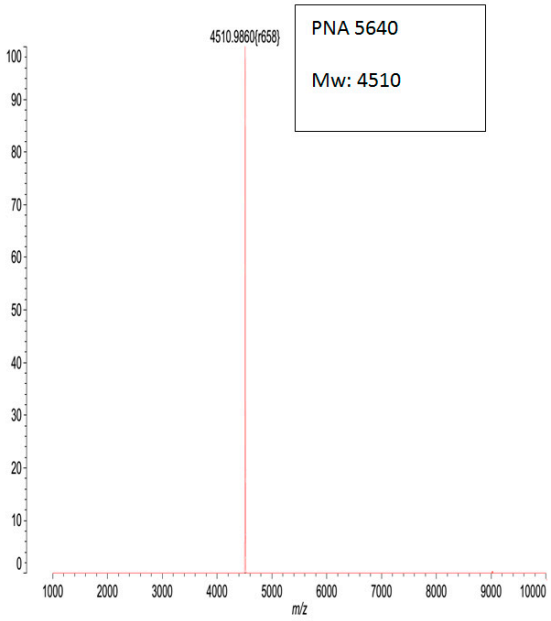
AU
Minutes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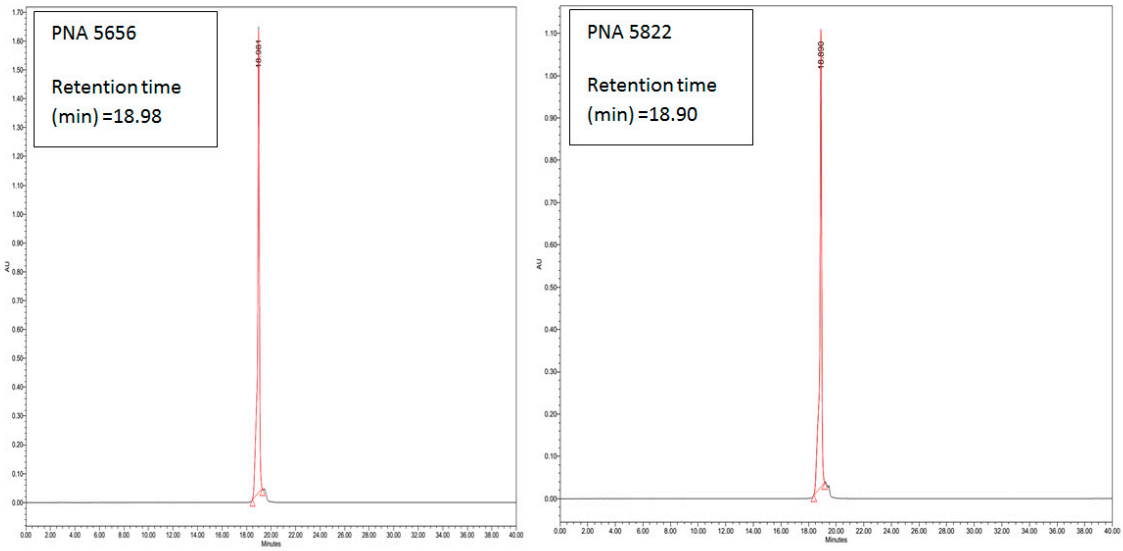

Figure S7. Analytical HPLC and MALDI-TOF mass spectrometry results of the most active BPP-PNAs.