



Article

Benefits and Drawbacks of Harboring Plasmid pP32BP2, Identified in Arctic Psychrophilic Bacterium *Psychrobacter* sp. DAB_AL32B

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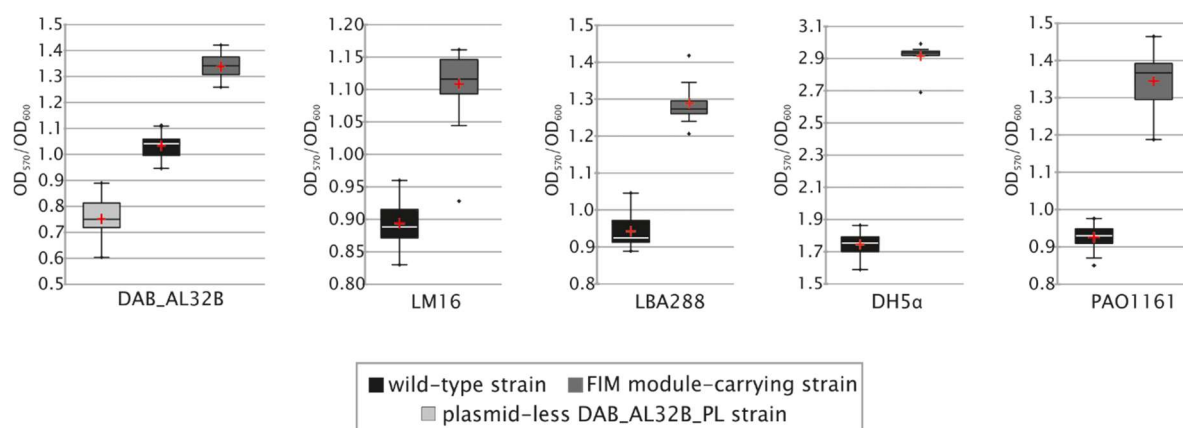


Figure S1. Box plots of OD₅₇₀/OD₆₀₀ ratios for the results of the crystal violet staining test performed for *Psychrobacter* spp. wild-type, pP32BP2-less, and plasmid-less carrying pBBR-Ps-FIM strains as well as heterologous wild-type and FIM module-carrying strains. DAB_AL32B – *Psychrobacter* sp. DAB_AL32B, LM16 – *Achromobacter* sp. LM16, LBA288 – *A. tumefaciens* LBA288, DH5α – *E. coli* DH5α, PAO1161 – *P. aeruginosa* PAO1161. The red crosses correspond to the means. The central horizontal bars are the medians. The lower and upper limits of the box are the first and third quartiles, respectively. Points above and below the whiskers' upper and lower bounds are outliers.

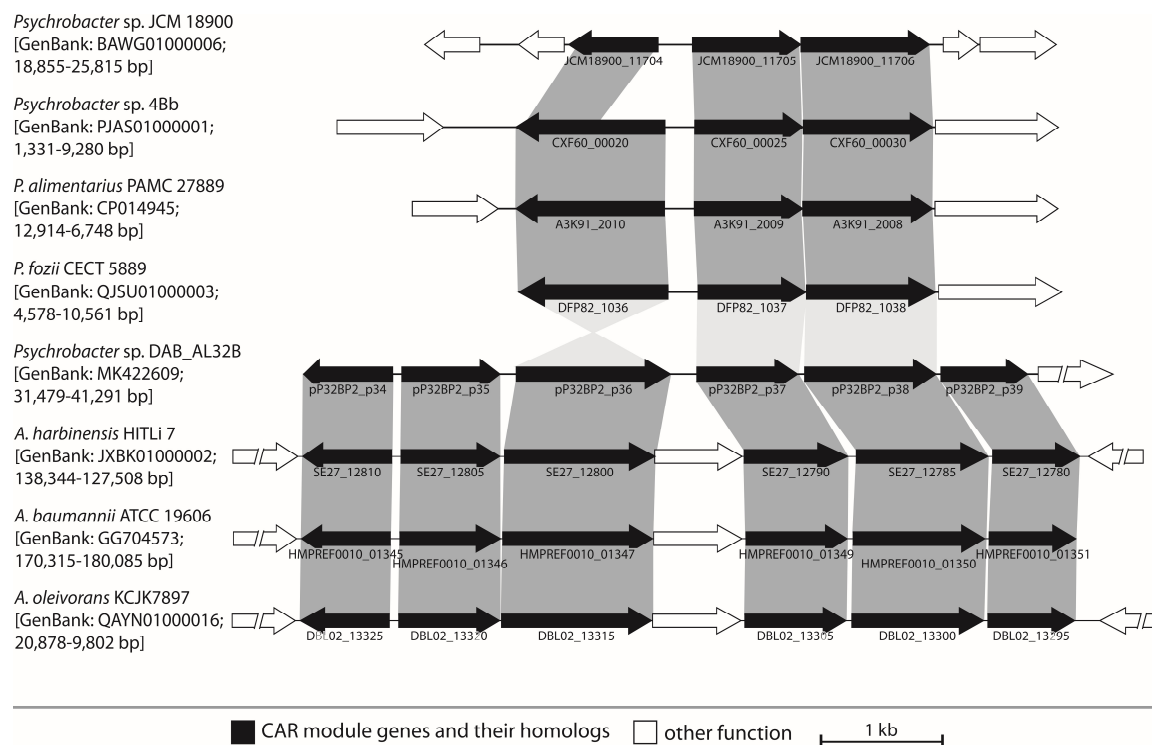


Figure S2. Comparison of the CAR module of pP32BP2 and related modules found in *Psychrobacter* and *Acinetobacter* genomes. Arrows indicate genes and their transcriptional orientation. GenBank accession numbers and coordinates of the gene clusters are shown in parentheses. Locus tags are shown below genes homologous to the CAR genes. The gray-shaded areas connect genes encoding proteins sharing $\geq 59\%$ (dark gray) or 23–33% (light gray) aa identity, respectively.

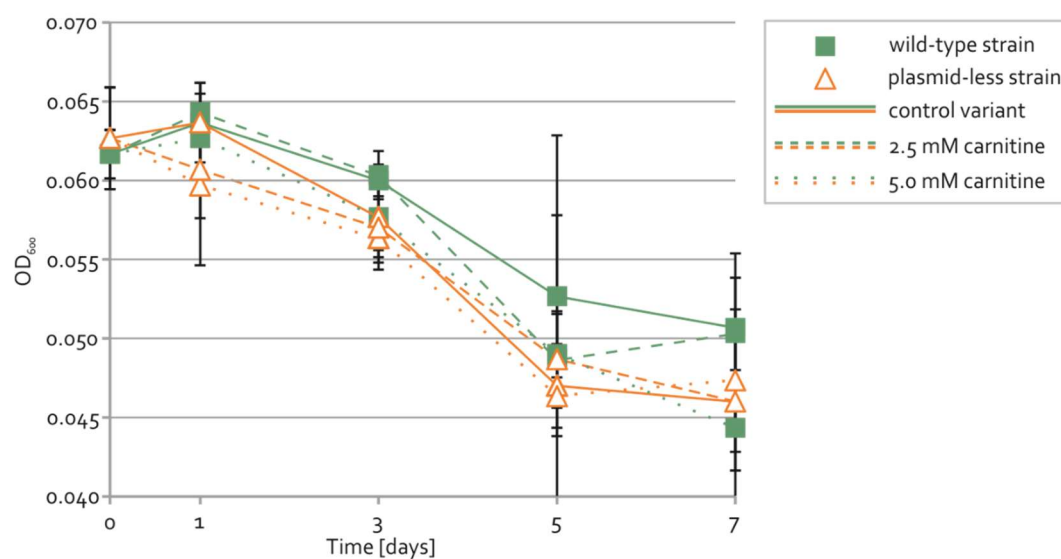


Figure S3. Growth of *Psychrobacter* sp. DAB_AL32B wild-type (green) and the plasmid-less (orange) strains under anaerobic conditions on sodium succinate (solid line) and carnitine (dashed and dotted lines, depending on final carnitine concentration). Control variant – bacteria cultivated in minimal medium supplemented with sodium succinate at a final concentration of 0.5% (w/v); 2.5 mM and 5.0 mM carnitine – bacteria cultivated in minimal medium additionally supplemented with carnitine at final concentrations of 2.5 or 5.0 mM, respectively. The mean values for three replicate cultures are plotted with error bars representing the standard deviations.

Table S1. Genes located within the pP32BP2 plasmid.

Gene	Coordinates	Protein length (aa)	Predicted function
<i>pP32BP2_p01</i>	571-1485	305	replication initiation protein RepA, COG5527
<i>pP32BP2_p02</i>	1654-2292	213	partitioning protein ParA, COG1192
<i>pP32BP2_p03</i>	2296-2505	70	partitioning protein ParB
<i>pP32BP2_p04</i>	2741-3955	405	transposase of <i>ISPssp4</i> , IS256 family, COG3328
<i>pP32BP2_p05</i>	4395-4057	112	hypothetical protein
<i>pP32BP2_p06</i>	4868-6043	392	putative competence protein, CoiA-like family, COG4469
<i>pP32BP2_p07</i>	6225-7148	308	hypothetical protein
<i>pP32BP2_p08</i>	7577-8167	197	serine recombinase, COG1961
<i>pP32BP2_p09</i>	8241-8486	82	transposase, IS3 family, partial
<i>pP32BP2_p10</i>	9566-8553	337	glycosyltransferase, COG0463 [EC: 2.4.1.-]
<i>pP32BP2_p11</i>	10408-11988	527	Fic family protein, COG3177
<i>pP32BP2_p12</i>	12257-12523	89	transposase, IS3 family, partial, COG2963
<i>pP32BP2_p13</i>	12475-13002	176	transposase, IS3 family, partial
<i>pP32BP2_p14</i>	13027-13344	106	transposase, IS3 family, partial, COG2801
<i>pP32BP2_p15</i>	18768-13498	1756	protein of unknown function
<i>pP32BP2_p16</i>	19844-19656	62	protein of unknown function (DUF1643)
<i>pP32BP2_p17</i>	20272-20039	77	transposase, IS3 family, partial, COG2801
<i>pP32BP2_p18</i>	20448-20269	59	transposase, IS3 family, partial
<i>pP32BP2_p19</i>	20559-20921	121	first part of transposase of <i>ISPssp5</i> , IS5 family, COG3293
<i>pP32BP2_p20</i>	20897-21307	145	second part of transposase of <i>ISPssp5</i> , IS5 family, COG3293
<i>pP32BP2_p21</i>	21557-21300	79	transposase, IS3 family, partial, COG2801
<i>pP32BP2_p22</i>	21703-21542	53	transposase, IS3 family, partial, COG2963
<i>pP32BP2_p23</i>	22050-21754	98	transposase, IS3 family, partial, COG2963
<i>pP32BP2_p24</i>	22908-22174	244	hypothetical protein
<i>pP32BP2_p25</i>	23496-23669	58	transposase, IS630 family, partial, COG3415
<i>pP32BP2_p26</i>	24110-23808	100	serine recombinase, partial, COG1961
<i>pP32BP2_p27</i>	24247-24074	57	serine recombinase, partial, COG1961
<i>pP32BP2_p28</i>	24403-24257	48	serine recombinase, partial, COG1961
<i>pP32BP2_p29</i>	24818-25348	177	major structural fimbrial subunit, COG3539
<i>pP32BP2_p30</i>	25427-26152	242	fimbriae assembly chaperone, COG3121
<i>pP32BP2_p31</i>	26223-28778	852	outer membrane usher protein, COG3188
<i>pP32BP2_p32</i>	28775-29791	339	putative adhesin
<i>pP32BP2_p33</i>	30346-30065	93	hypothetical protein
<i>pP32BP2_p34</i>	32462-31479	327	transcriptional regulator, LysR family, COG0583
<i>pP32BP2_p35</i>	32563-33651	363	malate dehydrogenase, COG0473 [EC:1.1.1.38]
<i>pP32BP2_p36</i>	33826-35532	569	carnitine transporter, BCCT (betaine/carnitine/choline) family, COG1292 [TC:2.A.15]
<i>pP32BP2_p37</i>	35813-36928	372	two component carnitine monooxygenase, oxygenase component, COG4638 [EC:1.14.13.239]
<i>pP32BP2_p38</i>	37003-38454	484	malic semialdehyde dehydrogenase, COG1012 [EC:1.2.1.4]
<i>pP32BP2_p39</i>	38499-39458	320	two component carnitine monooxygenase, reductase component, COG1018 [EC:1.14.13.239]
<i>pP32BP2_p40</i>	39579-41291	571	acetolactate synthase, large subunit, COG0028 [EC:2.2.1.6]
<i>pP32BP2_p41</i>	41518-42528	337	NADPH:quinone oxidoreductase, COG0604 [EC:1.6.5.5]
<i>pP32BP2_p42</i>	42655-44166	504	betaine aldehyde dehydrogenase, COG1012 [EC:1.2.1.8]
<i>pP32BP2_p43</i>	45257-44430	275	transcriptional regulator, IclR family, COG1414
<i>pP32BP2_p44</i>	45405-46589	395	crotonbetainyl-CoA reductase, COG1960 [EC:1.3.8.13]
<i>pP32BP2_p45</i>	46765-48003	413	γ -butyrobetainyl-CoA:carnitine CoA transferase, COG1804 [EC:2.8.3.16]
<i>pP32BP2_p46</i>	48119-48382	88	transposase, IS3 family, partial, COG2963
<i>pP32BP2_p47</i>	48355-48603	83	transposase, IS3 family, partial

<i>pP32BP2_p48</i>	48587-49066	160	transposase, IS3 family, partial, COG2801
<i>pP32BP2_p49</i>	49115-49285	57	putative transposase, IS3 family, partial
<i>pP32BP2_p50</i>	49973-50317	115	transporter EmrE, COG2076 [TC:2.A.7.1]
<i>pP32BP2_p51</i>	50412-50567	52	transcriptional regulator, AcrR family, partial, COG1309
<i>pP32BP2_p52</i>	52114-51581	177	transposase, IS1 family, partial, COG3677 and COG1662
<i>pP32BP2_p53</i>	54149-52899	416	hypothetical protein
<i>pP32BP2_p54</i>	54229-54438	70	XRE-family like protein

Table S2. Primers used in this study.

Primer	Sequence¹	Position²
L32REP	ataccggtGCGAACCACACTGTGAGTATTG	54,185-54,204
R32REP	ataccggtTTAATTCTATCGCCCGCCTG	1,510-1,491
L232BREP	caaccggtTCTCCACCAATACTTATCACAG	54,069-54,090
R232BREP	gaaccggtTAGGGTCTTGATCAACTAGAC	2,647-2,627
F1	GGCATGGTTGCTCAAGGTAG	28,605-28,624
R1	CTATTGTACTGGTGGTATGCCG	30,182-30,161
F2	GCGATTCGATGCCAATGACT	30,649-30,668
R2	GAGCTTAATTCTTCGCCTGC	32,381-32,362
F3	ACCACTTGCTGAGGCATTAG	37,305-37,324
R3	GGTAAGCTGTTCAACTTCGG	15,903-15,884

¹ Sequences are shown in the 5' to 3' orientation. Sequence not complementary to the plasmid are shown in lowercase. Introduced restriction sites are underlined.

² Position of primers in pP32BP2 plasmid.