

Transcriptional landscape of the *gacA* mutant in *P. stutzeri* A1501 during growth curve.

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Supplementary material:

Supplementary Figure S1: Homology model for GacA, a protein implicated in the regulation of metabolic pathways. The **A)** Ribbon and **B)** Globular structures depict the N- and C-terminal domains. The figure was generated with the Swiss-Model software (<https://swissmodel.expasy.org/>) and the Chimera software (<https://www.cgl.ucsf.edu/chimera/>).

Supplementary Figure S2: The gene of interest, as well as mutator fragments of **A)** up regulatory genes, **B)** the Chloramphenicol resistant gene, and **C)** down regulatory genes, were amplified using a standard polymerase chain reaction (PCR) procedure. The band representing the up and down regulatory genes has a length of 500 base pairs, whereas the Chloramphenicol resistant gene has a length of 660 base pairs. **D)** After the successful transformation and generation of the mutant $\Delta gacA$ strain, the individual colonies were confirmed by colony PCR.

Supplementary Figure S3: The transfer of resistance genes from plasmids to the genome of *Pseudomonas stutzeri* A1501 involves the substitution of $\Delta gacA$ genes with Chloramphenicol-resistant genes.

Supplementary Figure S4: Quality of RNA isolation used for the transcriptome Analysis

Supplementary Table-S1. Primers employed in the present study were utilized in the $\Delta gacA$ mutant.

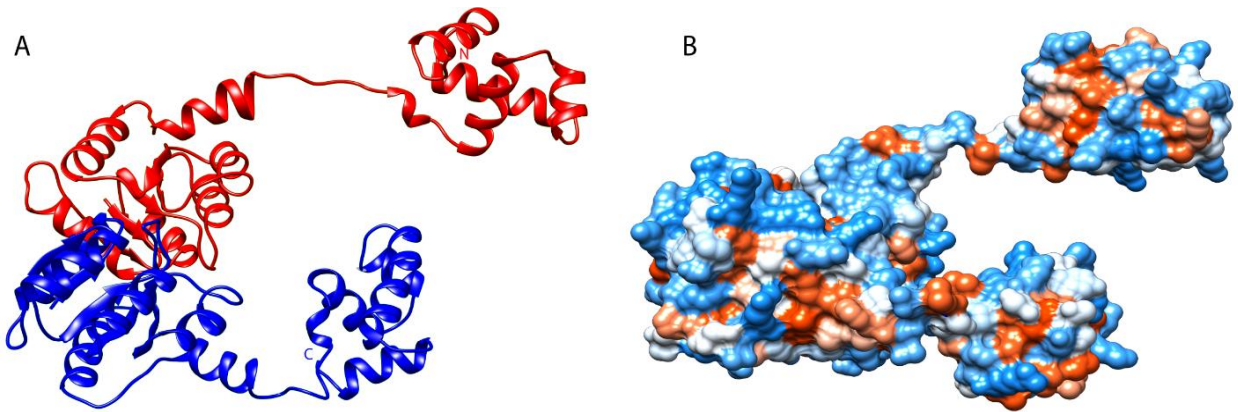
Supplementary Table-S2. qRT-PCR primers that were utilized in the study.

Supplementary Table-S3. Software and tools for statistical analysis

Supplementary Table-S4. Reagents

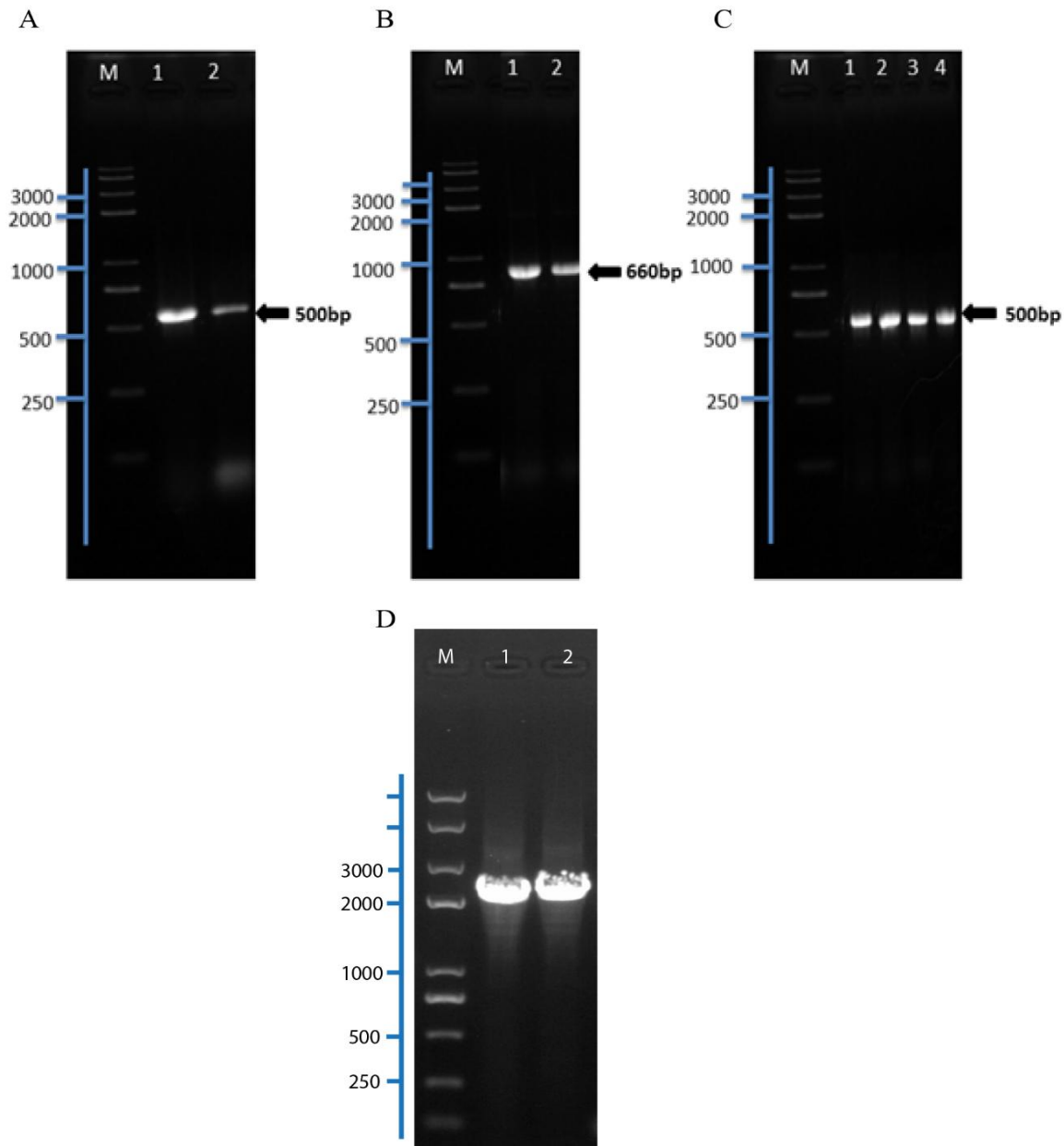
Supplementary Table-S5. Data Summary

Supplementary Figure S1:



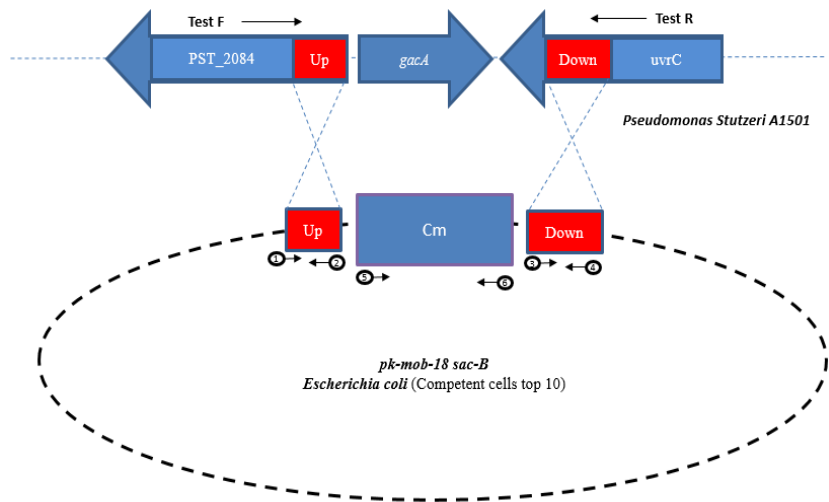
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Supplementary Figure S2:



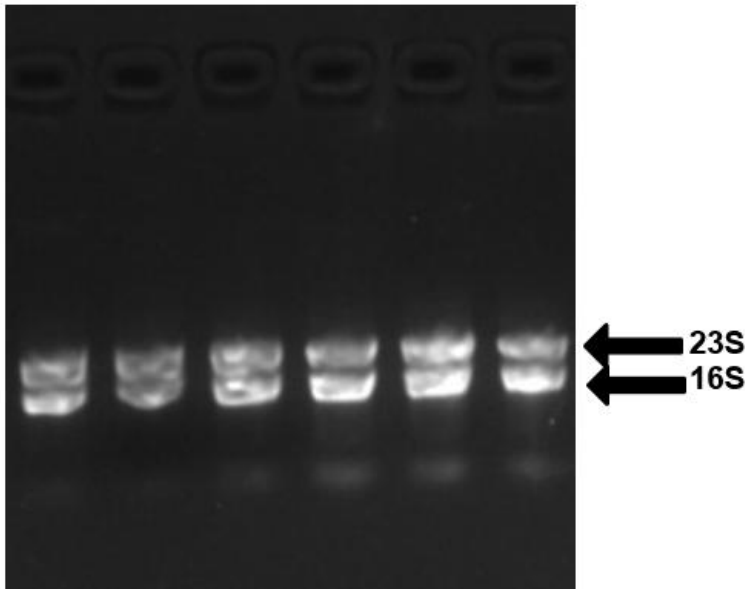
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Supplementary Figure S4:



Supplementary Figure S4: Quality of RNA isolation used for the transcriptome Analysis

Supplementary Table

Supplementary Table-S1. Primers employed in the present study were utilized in the $\Delta gacA$ mutant.

Description	Sequence (5'-3')
Upstream Forward primer of <i>gacA</i>	GCGGATCCGATTGATCAGCCGCTCCAGCCCGT
Upstream Reverse primer of <i>gacA</i>	AAGGGCCCTCGGTCTCCATGCCAGCACCCCTAATCAAGC AGACACC
Forward Primer for Chloramphenicol resistance	TGTCTGCTTGATTAGGGTGCTGGCATGGAGACCGAGGG CCCTTGA
Reverse Primer for Chloramphenicol resistance	GAGGAATCGAAGGCTTCTGTCATATTACGCCCCGCCCTG CCACTCAT
Downstream Forward primer of <i>gacA</i>	TGAGTGGCAGGGCGGGGCGTAATATGACAGAAGCCTT CGATTCCTCG
Downstream Reverse primer of <i>gacA</i>	GGAAGCTTGCTGTCCTCGCATTGCCGCACGAAG

Confirmation test forward primer for <i>gacA</i> mutation	CCATGATTACGAATTCCCAGGGGATCCGATTTGATCAGCC GCTCCAGCC
Confirmation test reverse primer for <i>gacA</i> mutation	GTAAAACGACGGCCAGTGCCAAGCTTGAACATGCGATA GACGCCCCGGC

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Supplementary Table-S2. qRT-PCR primers that were utilized in the study.

S.No	Primer Name	Primer sequence (5'to3')	S.No	Primer Name	Primer sequence (5'to3')
1	RT16S-F	CCTACGGGAGGCAGCAG	35	RT-PST_0690-R	CGGCTGGTGAAAACAATAGG
2	RT16S-R	ATTACCGCGGCTGCTGG	36	RT-PST_0690-F	GGGTCAAGGGTATCCACTCA
3	RT- <i>ntrB</i> -R	GATATGGCCGATGGTGAAC	37	RT-PST_1127-R	ACGCCATGGTCCTGTAGAG
4	RT- <i>ntrB</i> -F	AGGGAAGCATCATTTTGGTG	38	RT-PST_1127-F	CATCGAAGCCAACATCCAG
5	RT-PST_1981-R	TGGGTGTGTACGAATCTCCA	39	RT-PST_2797-R	CGTGACGGTGGAGTAGGAGT
6	RT-PST_1981-F	ATAACCTGTCCCAGGTGTG	40	RT-PST_2797-F	TGATCATCGAGCTGAACCTG
7	RT-PST_2278-R	GCAGGCTATCGGTAAGTTCG	41	RT-PST_3852-R	AGGTTGTCGAGGTTTTCACG
8	RT-PST_2278-F	CTGCTGGTGGCACTGTTCT	42	RT-PST_3852-F	GCAAGGGTCTGTCGAGCTAC
9	RT-PST_1916-R	GCGAAGGCTTCATTGATCTC	43	RT-PST_3494-R	ACCTGGTTGACGAAAGCATC
10	RT-PST_1916-F	CCAATTCAGTTCGATCTCC	44	RT-PST_3494-F	CGTCCATGCGTGTCTATCAC
11	RT-PST_1054-R	AACGCAATAATTTGGCCTTG	45	RT-PST_3495-R	GACGCACATCACATTGGACT
12	RT-PST_1054-F	CGCAGCAAAAACCCCTATTA	46	RT-PST_3495-F	GGAAATCATGCTCGGTTACG
13	RT- <i>narG</i> -R	TCTTCCACTCGGGATTGTTC	47	RT-PST_3496-R	GAGGCTGATGGTGACCTTG
14	RT- <i>narG</i> -F	AGCGAGTACTTCCGCGACTA	48	RT-PST_3496-F	TAATCTCGATCTGCCTGTGC
15	RT- <i>narH</i> -R	TCAGTCCTCCACAGCTGGA	49	RT-PST_3497-R	GCCTTGAAGTGGATGACGAT
16	RT- <i>narH</i> -F	ATGAAGATTCGTTCAACAAGT	50	RT-PST_3497-F	GGAAAACCTCAACACCCAGA
17	RT- <i>narI</i> -R	TCATACCGGGCTCTTGCTCT	51	RT-PST_3500-R	CAGCTGATCGGAGAAGATCC
18	RT- <i>narI</i> -F	ATGTCTAACTTCAATTTCCT	52	RT-PST_3500-F	GCTATACCCAGGAGCCCTTC
19	RT- <i>narJ</i> -R	TTAGACATTGCGCACCTCCT	53	RT-PST_3898-R	GGTCGATCTGGATCTCGAAG
20	RT- <i>narJ</i> -F	ATGCGCATCCTTAAAGTGAT	54	RT-PST_3898-F	GCAACAAGGTCATCGCCTAT
21	RT- <i>narK</i> -R	TCGCTGAAGATGGTGAAGT	55	RT- <i>algA</i> -F	ATGATCCCGGTTATTCTTTC
22	RT- <i>narK</i> -F	CAAGATTCTCGGCCTCTACG	56	RT- <i>algA</i> -R	CTATCGCGCCACTTTGGCCT
23	RT- <i>narM</i> -R	CACGGTGTGAAGGGGTACT	57	RT-PST_3946-R	AGCGGTGCAAGTTCTACGTC
24	RT- <i>narM</i> -F	CGTATCGAGGGTCTGGGTTA	58	RT-PST_3946-F	CCTGCCATTGCTGTCATTC
25	RT- <i>zwf</i> -R	CTTCGAGCACCTTCACCTTC	59	RT-PST_3948-R	GCCTTGCGACATAGAAGAG
26	RT- <i>zwf</i> -F	TCGATCACGTGCAGATTACC	60	RT-PST_3948-F	TGGTGGTGTAAGCAACGGTA
27	RT- <i>gltA</i> -R	GATTCTCTGCCAGCTGCTC	61	RT-PST_2491-R	ATAACTGAACAGGCCCATGC
28	RT- <i>gltA</i> -F	AGGGCCTGATGTAATTGACG	62	RT-PST_2491-F	GACTACTCCTGCTGGCGTTC
29	RT-PST_2311-R	GTCGTACTCGACGCTCATCA	63	RT-PST_1890-R	TGCCGACAACCTTTATGCTG
30	RT-PST_2311-F	AGATCAAGGCCATGGTTGAC	64	RT-PST_1890-F	TCATCGATCTCAATCGCAAG
31	RT-PST_3898-R	GGTCGATCTGGATCTCGAAG	65	RT-PST_2958-R	GATCAGGCTGTTACGGGTGT
32	RT-PST_3898-F	GCAACAAGGTCATCGCCTAT	66	RT-PST_2958-F	ACAGGGATCGATCAGAATGC
33	RT-PST_0689-R	GGTGGGAATGACCCATACTG	67	RT-PST_2280-R	GCTTGCTCTGGGTGTCGTAT
34	RT-PST_0689-F	GGACTGGACGCACTGGTATT	68	RT-PST_2280-F	AAGAGTCAGCCGCACTCAA

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263 **Supplementary Table-S3.** Software and tools for statistical analysis
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Description	Source	Identifier
GraphPad Prism	https://www.graphpad.com/scientific-software/prism/	Version 9.0.0
Chimera	https://www.cgl.ucsf.edu/chimera/	Alpha version 1.14
Cluspro	https://cluspro.org/login.php	Kozakov et al., 2017
Alphafold	https://alphafold.ebi.ac.uk/	Jumper et al., 2021
Sppider	http://sppider.cchmc.org/	Porollo et al., 2012
Expasy	https://web.expasy.org/translate/	Schwede et al., 2003
R-Studio	https://www.rstudio.com/	2021.09.0
Adobe Illustrator	https://www.adobe.com/cn/products/illustrator.	Version 2020 24.0.1
Adobe Photoshop	https://www.adobe.com/cn/products/photoshop/	Version 21.0.2
MEGA 7	https://www.megasoftware.net	Version 7
Snapgene 3.2.1	https://www.snapgene.com/	Version 3.2.1

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Description	Source	Identifier
<i>Xba</i> I	NEB	Cat# R0145S
<i>Sph</i> I-HF	NEB	Cat# R3182S
<i>Nhe</i> I-HF	NEB	Cat# R3131S
<i>EcoR</i> I-HF	NEB	Cat# R3101S
<i>BamH</i> I -HF	NEB	Cat# R3136S
<i>Hind</i> III-HF	NEB	Cat# R3104S
<i>Nde</i> I	NEB	Cat# R0111S
<i>Spe</i> I-HF	NEB	Cat# R3133S
<i>Xho</i> I	NEB	Cat# R0146S
<i>Nco</i> I-HF	NEB	Cat# R3193S
Yeast Extract Powder	Oxoid	Cat# LP0021B
DMSO	Invitrogen	Cat# D12345
Trypton	Oxoid	Cat# LP0042B
Ethanol	Thermo Scientific	Cat# 611050040
Ampicillin	Sigma-Aldrich	Cat# A5354
Gentamicin	Sigma-Aldrich	Cat# G1264-5G
Hygromycin B	Sigma-Aldrich	Cat# SBR00039
Kanamycin	Sigma-Aldrich	Cat# E004000-5G
Tetracycline hydrochloride	Thermo Scientific	Cat# 233100025
HEPES buffer	GIBCO	Cat# 15630080
Trimethylamine	Sigma-Aldrich	Cat# 471283
b-Mercaptoethanol	Sigma-Aldrich	Cat# 444203
DTT	Thermo Scientific	Cat# R0861
RNAlater solution	Invitrogen	Cat# AM7020

Supplementary Table-S5. Data Summary

<u>Accession</u>	<u>Title</u>	<u>BioProject</u>	<u>BioSample</u>	<u>Library ID</u>	<u>Files</u>	<u>Status</u>
SRR21626850	Sample collected after 6 hours	PRJNA880443	SAMN30904711	GC_A1501_6	3	Released
SRR21626849	Sample collected after 6 hours	PRJNA880443	SAMN30904812	GC_gacA_6	3	Released
SRR21626848	Sample collected after 8 hours	PRJNA880443	SAMN30904714	GC_A1501_8	3	Released
SRR21626847	Sample collected after 8 hours	PRJNA880443	SAMN30904815	GC_gacA_8	3	Released