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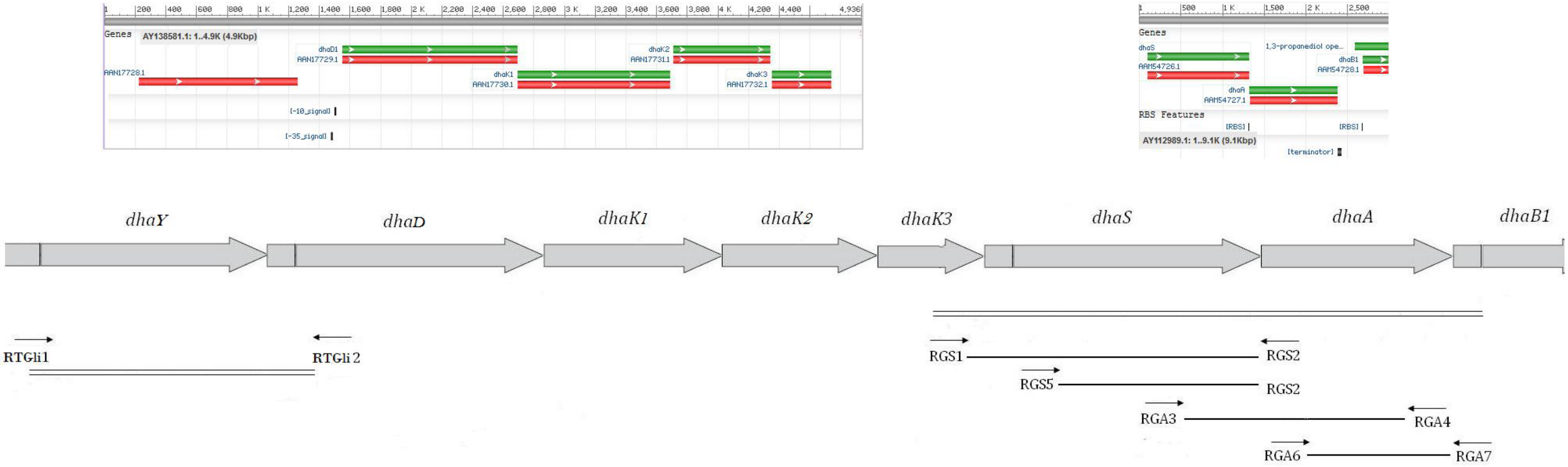
SUPPLEMENTARY MATERIAL 1-3

Computational analysis of 1,3-propanediol operon transcriptional regulators: insights into Clostridium sp. glycerol metabolism regulation

Supplement 1. Designed primers for amplifying and sequencing chromosomal upstream region of the *Clostridium* sp. IBUN 13A strain's 1,3-PD operon, using the thermocycler conditions according to each primers pair. The figure below shows a map of the amplified chromosomal region using the sequence of *C. butyricum* as template. *D-Forward, R-Reverse

Primer	**Sequence (5´-->3´)**	**Template position (bp) (Acc Cod GenBank)**	**Region**
RGS1 -D*	GAGAAAGCAATAAATGCCTG	4857 – 4876 (AY 138581)	Intergenic *dha*K3-*dha*S
RGS2 -R	TTTGCACTTTGTAACTCACC	1440 – 1421(AY112989.1)	*dha*A
RGS5 -D	ACTTCCAATAATGTCACTGG	532 - 551 (AY 112989.1)	*dha*S
RGA3 -D	GCGGAATAGGCATATCAAAGG	1122 – 1142 (AY112989.1)	*dha*S
RGA4 -R	ATTTCTGTAGGCTGATGGTG	32 - 13 (DQ 901408.5) 2359 – 2340 (AY 112989.1)	*dha*A
RGA6 -D	TGGAATTATTGCTCTTATCC	1719 - 1738 (AY 112989.1)	*dha*A
RGA7 -R	CTCCAATCACATTCTTACTTGG	354 - 333 (DQ 901408.5) 2681 – 2660 (AY 112989.1)	Intergenic *dha*A-*dha*B1
RTGli1 D	GTTATATATGAGTATTCAATGGG	15 – 37 (AY 138581)	putative transcriptional regulator (*dha*Y)
RTGli2 -R	AGCTGATTCTCCAAAACTTC	1645 – 1626 (AY 138581)	*dha*D

RGS1-RGS2:	1 X (94°C/3 min);	30 X (94°C/30 s,	59°C /30 s, 72°C/90 s);	1 X (72ºC/3 min)
RGS5-RGS2:	1 X (94°C/3 min);	30 X (94°C/30 s,	59°C /30 s, 72°C/50 s);	1 X (72ºC/3 min)
RGA3-RGA4:	1 X (94°C/5 min);	30 X (94°C/30 s,	58°C /30 s, 72°C/70 s);	1 X (72ºC/3 min)
RGA6-RGA7:	1 X (94°C/5 min);	30 X (94°C/30 s	56°C /30 s, 72°C/50 s);	1 X (72ºC/3 min)
RTGli1- RTGli2:	1 X (94°C/5 min);	30 X (94°C/30 s	55°C /30 s, 72°C/105 s);	1 X (72ºC/3 min)



Images at the top are downloaded from NCBI. Graphic display of sequences AY112989.1 and AY138581.1 from *C. butyricum*.

Supplement 2. Selected sequences for multiple sequence alignment (MSA)
Sequences have been organized according to domain homology. *characterised proteins †proteins selected for MSA. ◊Number of cluster means number of organisms identified with the same consensus sequence (www.uniprot.org/).

Code Number	Name	Microorganism	Cluster◊
POCR SUPERFAMILY SECUENCES			
Q05587.2	PocR	*Salmonella typhimurium*	8
D2P159	PocR	*Listeria monocytogenes*	7
B7NC30	Transcriptional regulator: propanediol utilization	*Escherichia coli*	4
HISTIDIN-KINASE SUPERFAMILY SECUENCES			
O31516.1	YesM†	*Bacillus subtilis*	-
C1HQL6	Histidine kinase†	*Escherichia coli*	4
B0K3C6	Histidine kinase†	*Thermoanaerobacter sp*	4
P94513.1	LytS	*Bacillus Subtilis*	-
Q4A009.1	LytS†	*Staphylococcus saprophyticus*	-
Q4L8V3.	LytS	*Staphylococcus haemolyticus*	-
Q81JL2 *	LytS†	*Bacillus anthracis*	9
Q814J0.1	LytS	*Bacillus cereus*	-
Q82Z75*	LytS†	*Enterococcus faecalis*	4
Q53705*	LytS	*Staphylococcus aureus*	36
Q8E7H4*	LytS†	*Streptococcus agalactiae*	7
P0AA94*	YpdA†	*Escherichia coli*	43
C3NWE4	Autolysin sensor kinase†	*Vibrio cholerae*	11
A1UV04	Histidine kinase†	*Burkholderia mallei*	7
A9R5L7	Sensor histidine kinase†	*Yersinia pestis*	18
Q0TNM2	Sensor histidine kinase†	*Clostridium perfringens*	6
D1JYM2	Two-component system sensor†	*Bacteroides sp.*	4
B5XP86	Sensor histidine kinase†	*Klebsiella sp.*	4
B0U2X0	Two-component system sensor protein†	*Xylella fastidiosa*	3
P58363*	ArcB	*Escherichia coli*	13
P0AE84*	Sensor protein CpxA	*Escherichia coli*	53
A7WWQ7*	Sensor protein kinase WalK	*Staphylococcus aureus*	38
Q57QC4*	Virulence sensor histidine kinase PhoQ	*Salmonella enterica*	25
P0A4I6*	Sensor protein CiaH	*Streptococcus pneumoniae*	22
P0A5Z9*	Sensor-type histidine kinase PrrB	*Mycobacterium tuberculosis*	16
P09384*	CheA	*Salmonella typhimurium*	8
B1QUC8	Two-component sensor histidine kinase	*Clostridium butyricum*	2
Q48768*	CheA	*Listeria monocytogenes*	6
P33639*	Sensor protein PilS	*Pseudomonas aeruginosa*	5
A5I0M8	Sensor histidine kinase	*Clostridium botulinum*	4
C7T955	Sensor transduction histidine kinase	*Lactobacillus rhamnosus*	4
C2GVB6	Histidine kinase sensor	*Bifidobacterium longum*	4

Code Number	Name	Microorganism	Cluster◊
REC SUPERFAMILY SECUENCES			
O31517.1	YesN†	*Bacillus subtilis*	-
Q4L8V4.1|	LytR	*Staphylococcus haemolyticus*	-
P0AE68*	CheY†	*Escherichia coli*	49
P0AA18*	OmpR†	*Escherichia coli*	100
P0A9Q3	ArcA†	*Escherichia coli*	66
Q2G2G2*	Response regulator SaeR†	*Staphylococcus aureus*	57
B2SB45*	Polar-differentiation RR DivK	*Brucella abortus*	37
P0A2D6*	CheY	*Salmonella typhimurium*	32
P60611*	LytR	*Staphylococcus aureus*	30
P0A4H8*	Transcriptional regulatory CiaR	*Streptococcus pneumoniae*	27
A4TIX9	Two-component regulatory system†	*Yersinia pestis*	25
C4KXK1	CheY†	*Burkholderia pseudomallei*	25
C3M734	CheY†	*Vibrio cholerae*	24
Q81JL3	LytT†	*Bacillus anthracis*	23
A9M2D6	Two-component system regulator†	*Neisseria sp.*	20
P95193*	Transcriptional regulatory protein DevR†	*Mycobacterium tuberculosis*	19
P0A4H6*	CheY†	*Listeria monocytogenes*	16
Q06239*	Regulatory protein VanR†	*Enterococcus faecalis*	13
A0Q7W8	Two-component response regulator†	*Francisella tularensis*	12
A3M2P4	Two-component regulatory activator OmpR†	*Acinetobacter sp.*	11
A5I5I	CheY†	*Clostridium botulinum*	10
P44845*	Nitrate/nitrite response regulator†	*Haemophilus influenzae*	9
B0RXW	Two-component system regulatory protein†	*Xanthomonas sp.*	9
P71403	CheY†	*Helicobacter pylori*	8
B1QYY4	Two-component response regulator†	*Clostridium butyricum*	2
A0PYZ2	Two-component response regulator†	*Clostridium novyi*	-
Q97KQ2	Two-component response regulator†	*Clostridium acetobutylicum*	-
P29369	Glycerol metabolism activator AgmR†	*Pseudomonas aeruginosa*	5
P0ACZ6*	Positive transcription regulator EvgA†	*Escherichia coli*	60
B1BNB8	DNA-binding response regulator	*Clostridium perfringens*	5
HTH_ARAC SUPERFAMILY SECUENCES			
A4WG91.1	Transcriptional activator RhaS	*Enterobacter sp.*	-
B5FPP5.1	Transcriptional activator RhaS	*Salmonella enterica*	-
P07642.1*	AraC†	*Erwinia chrysanthemi*	-
P0A9E1.1*	AraC†	*Escherichia coli*	-
P0A9E4*	Regulatory protein SoxS†	*Escherichia coli*	60
P63202*	Transcriptional regulator GadW†	*Escherichia coli*	28
Q56143*	Regulatory protein SoxS†	*Salmonella typhimurium*	27
Q1C0W2*	Transcriptional activator RhaR†	*Yersinia pestis*	21
P68913*	Transcriptional regulator Rv1395†	*Mycobacterium tuberculosis*	16
B5QWY5*	RhaR†	*Salmonella sp.*	13
P45008*	Transcriptional regulator HI1052†	*Haemophilus influenzae*	8
Q48413*	Transcriptional activator RamA†	*Klebsiella pneumonia*	7
P0A2S7*	Transcriptional regulator MxiE†	*Shigella sp.*	5
P72171*	Ornithine utilization regulator†	*Pseudomonas aeruginosa*	4

Supplement 3. Comparison of DhaS and DhaY secondary structure using Psi-Pred with MSA obtained by Anantharaman and Aravind (2005). A. Prediction of secondary structure and consensus sequence made for PocR by Anantharaman and Aravind (2005), the script E represents prediction of β-sheet and H for α-helix. B. DhaS and DhaY protein's sequences and secondary structure, in green is shown the conserved G residue and in red boxes are shown the three highly conserved cysteine residues.