The genes of the sulphoquinovose catabolism in *Escherichia coli* are also associated with a previously unknown pathway of lactose degradation

Anna Kaznadzey¹, Pavel Shelyakin^{1,2}, Evgeniya Belousova³ Aleksandra Eremina⁴, Uliana Shvyreva⁵, Darya Bykova³, Vera Emelianenko³, Anastasiya Korosteleva⁶, Maria Tutukina ^{5,7,*} Mikhail S Gelfand^{1,3,7,8}

1 – A. A. Kharkevich Institute for Information Transmission Problems, RAS, Bolshoy Karetny per. 19, Moscow, 127051, Russia

2 - N. I. Vavilov Institute of General Genetics, RAS, ul. Gubkina 3, Moscow, 119991, Russia

3 – M. V. Lomonosov Moscow State University, Vorobievy Gory 1-73, Moscow, 119991, Russia

4 – The University of Edinburgh, Alexander Crum Brown Rd, Edinburgh, Scotland, EH9 3FF, UK

5 – Institute of Cell Biophysics, RAS, Institutskaya 3, Pushchino, 142290, Russia

6 – Novosibirsk State University, ul. Pirogova 2, Novosibirsk, 630090, Russia

7 – Center for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Moscow, 143028, Russia

8 – Faculty of Computer Science, Higher School of Economics, Kochnovsky pr. 3, Moscow, 125319, Russia

* Corresponding author. E-mail: m.tutukina@skoltech.ru

Strains	Description	Reference
K-12 MG1655	Wild type F- lambda- <i>ilvG- rfb</i> -50 rph-1	47
M182	⊿lac derivative of K-12 MG1655	40
MG1655∆ <i>vihW</i>	$\Delta yihW$ derivative of K-12 MG1655	This work
MG1655∆ <i>crp</i>	Δcrp derivative of K-12 MG1655	48
, M182∆ <i>yihW</i>	$\Delta yihW$ derivative of M182	This work
M182Acrp	Acrp derivative of M182	49
BL21*(DE3)	F- ompT hsdSB (rB-mB-) gal dcm rne131 (DE3)	50
Plasmids	, , , , , , , , , , , , , , , , , , , ,	
pET_CRP	Vector based on pET28b (Invitrogen) overproducing the CRP protein, Kan ^R	This work

Table S1. Escherichia coli strains and plasmids used in this study

Primer name	Primer sequence (5'-3')
yihU/V_F	CGTTCACATCAAAGACGCGA
yihU/V_R	GTCGGTAACCCTTCCACGTA
yihV/W_F	TCAACCGGCCTTCAAAGTTG
yihV/W_R	GCGATCAGCATGAGGAGTTG
yihS/R_F	AGCTGGATGCGGACAATAAG
yihS/R_R	GGCATCTCTTCGGGTTTGTG
yihW_RT	CCGTATTAACGACGCTGGAA
yihW_PCR	GCCGAGCGTGGGTATATGAA
yihV_RT	TCATCACCTACGCGACCAAT
yihV_PCR	TTCGTGTTGCTTGTGTAGGT
yihU_RT	GGAGTCGCACCTTTGTCTAC
yihU_PCR	CGCGTTTATCGGTTTAGGAC
yihT_RT	ATTGTTGATCTACCAGAATCG
yihT_PCR	CGAAGCCATGCGCATGATGT
yihS_RT	CCGTGATCAACCAACGAGTA
yihS_PCR	GGTTTTGGCTGGTTAGGCAA
hns_RT	ATTTAACGGCAGCAAGGCTATT
hns_PCR	GAAGTTGAAGAGCGCACTCG
hns_Bgl_263	AGGGAGATCTCGTAAACACAACTA
hns_Xba	GTTGTCTAGAATTTTAAGTGCTTCG
CRP_Ndel	ACCGCATATGGTGCTTGGCAAACCGCAA
CRP_Bpu1102	CCACGCTGAGCGGATTAACGAGTGCCGTA

Table S2. Oligonucleotide primers used in this study.



Fig. S1. CRP induction and purification steps. 1 and 4 – total lysate after 16h induction with 20μ M IPTG; 2 – soluble fraction; 3 – insoluble fraction; 5 – flow-through after loading of the final lysate (shown on lane 6) onto a column with cAMP-agarose; 6 – cleared cell lysate after treatment with BugBuster reagent (Novagen) followed by sonication; 7 and 8 – pure CRP eluted with 5mM cAMP. Blue prestained protein ladder (11-190 kDa, New England Biolabs) was used as a standard.



Fig. S2 Growth of the *Escherichia coli* M182 wild type strain, and its $\Delta yihW$ and Δcrp derivatives on MacConkey agar.



Fig. S3. Effects of *yihW* and *crp* deletions on growth in the presence of 0.2 % glucose or galactose. Squares: the parent strain; triangles: the *yihW* mutant. Dashed lines correspond to cultures with deleted *crp*.