Article

Adaptive Laboratory Evolution for Multistress Tolerance, including Fermentability at High Glucose Concentrations in Thermotolerant *Candida tropicalis*

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Supplementary Materials

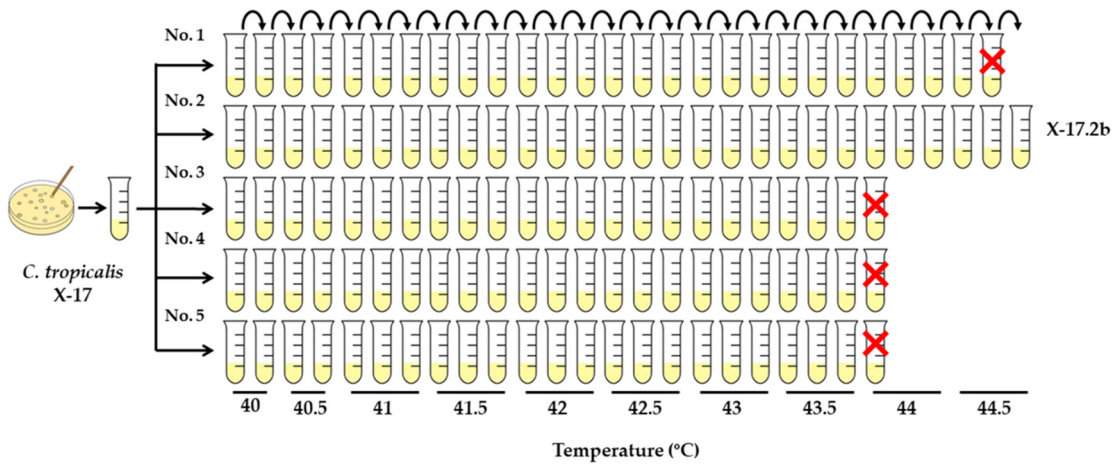

Figure S1: Schematic diagram of RLCGT for *C. tropicalis* X-17. *C. tropicalis* X-17 was cultured in five tubes in parallel in a 5-ml YP liquid medium containing 200 $g \cdot L^{-1}$ of glucose under a shaking condition at 100 rpm for 7 days and the cultivation was repeated with gradually increasing temperature from 40 °C to 44.5 °C. At each step, cells were transferred to a fresh medium at the initial OD_{660} value of 0.1.

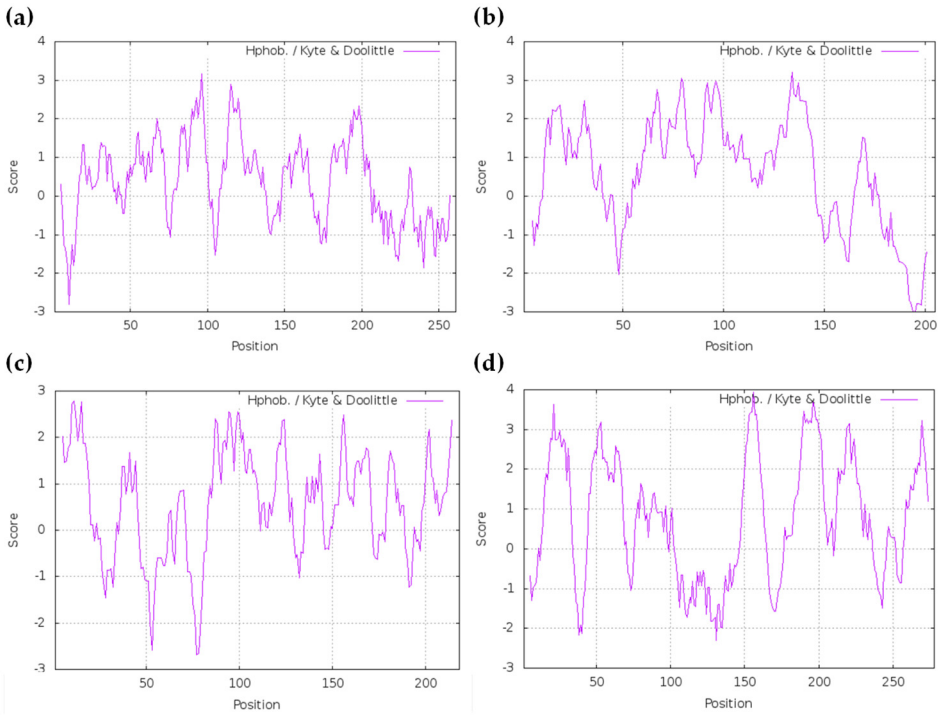

Figure S2: Hydropathy analysis of four hypothetical proteins of which genes were up-regulated in X-17.2b. Hydropathy profiles of a glucose transporter encoded by *CTRG_06250* **(a)**, a hypothetical protein encoded by *CTRG_06056* **(b)**, a hypothetical protein encoded by *CTRG_00691* **(c)** and a hypothetical protein encoded by *CTRG_01139* **(d)** are shown. Hydropathy analysis was performed by the method of Kyte and Doolittle [36].

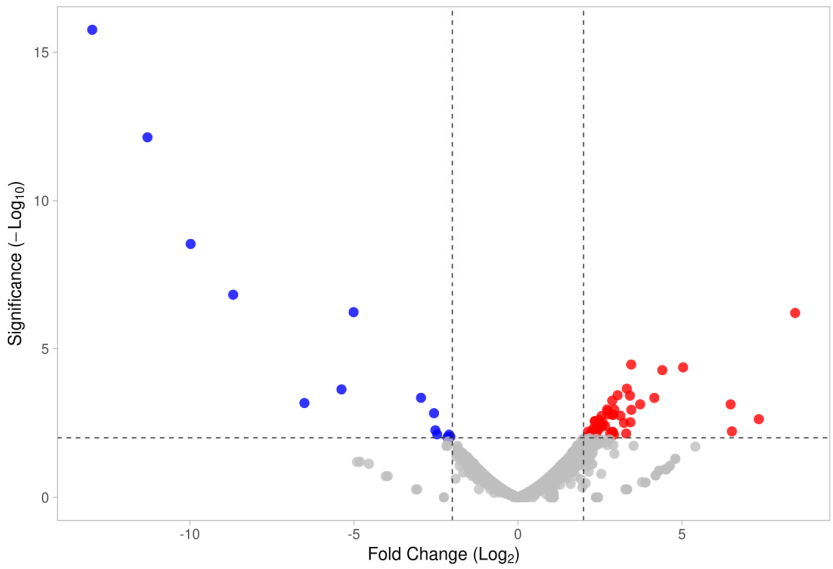

Figure S3: Volcano plots of differentially expressed genes (DEGs) of adapted strain X-17.2b versus the parental strain X-17 at 37 ˚C for 12 h. Genes with adjusted *P*-values less than 0.01 and $\log_2$ (fold change) values greater than 2 and less than -2 were assigned as genes with differential expression. Red symbols, significantly up-regulated genes; blue symbols, significantly down-regulated genes.

Table S1. Significantly down-regulated genes ($log_2 < -2$) in the adapted strain.

Name	Log_2 Fold Change	Product
CTRG_04849	-2.1	Oxidoreductase
CTRG_00109	-2.1	Hypothetical protein
CTRG_00211	-2.1	Fructose-bisphosphate aldolase
CTRG_05493	-2.2	RNA polymerase II transcription factor B subunit 5
CTRG_01742	-2.2	Transporter protein SMF1/ESP1
CTRG_05850	-2.2	Hypothetical protein
CTRG_05940	-2.3	Meiotic sister chromatid recombination protein 1
CTRG_04226	-2.3	Hypothetical protein
CTRG_00580	-2.3	Putative diacetyl reductase [(R)-acetoin forming] 2
CTRG_00882	-2.5	NAD-dependent alcohol dehydrogenase
CTRG_00284	-2.5	Glutathione S-transferase 1
CTRG_05790	-2.5	Respiratory supercomplex factor 2, mitochondrial
CTRG_01828	-2.6	Hypothetical protein
CTRG_01856	-2.7	Hypothetical protein
CTRG_03259	-2.8	Hypothetical protein
CTRG_03258	-3.3	Hypothetical protein
CTRG_04826	-5.2	Hypothetical protein
CTRG_04987	-5.8	Sodium transport ATPase 2