Supplementary Information (SI) for RSC Sustainability. This journal is © The Royal Society of Chemistry 2024

## **Supporting Information**

Rapid screening of toxicity to thermotolerant yeasts: inhibition of growth and fermentation by ionic liquids and zwitterions

Mayu Shibata, Ayumi Hachisu, Souta Uemori, Hitomi Tobe, Kazuaki Ninomiya, and Kosuke Kuroda\*

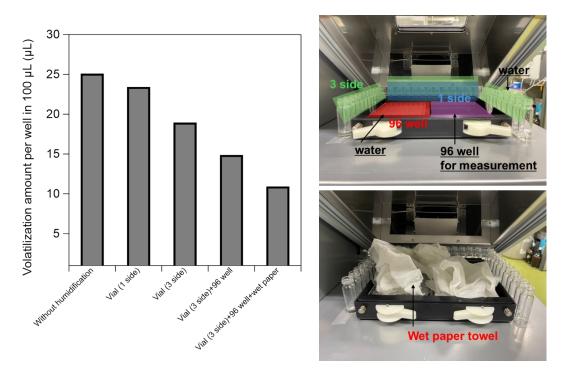


Fig. S1 Volatilization amount per well after 6h incubation with various humidification methods. For humidification, the vials and wells in the 96-well plate (left one in the picture) were filled with water, and paper towels were always kept wet. The experimental for this estimation is shown below.

Water (100  $\mu$ L) was added to each well of the 96-well plate. The plate was placed in an incubator under the respective humidification conditions and incubated for 6 hours.

Volatilisation was measured according to the equation:

Volatilization amount per well (mg)
\_ Weight 96 well before incububation (mg) – Weight 96 well after incub

96

The density of water was calculated as 1  $\mu$ L/mg.

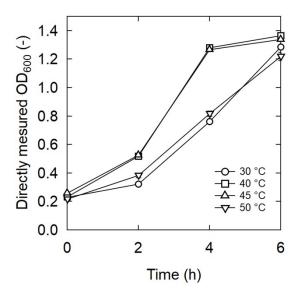


Fig. S2  $OD_{600}$  after incubation in media at 30, 40, 45, and 50 °C. Strain: *K. marxianus* DMKU3-1042.

Initial OD<sub>600</sub>: 0.2.

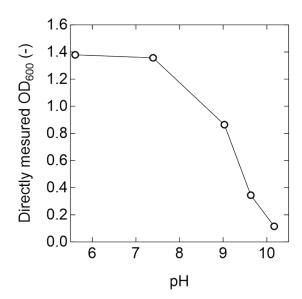


Fig. S3  $OD_{600}$  of *K. marxianus* (DMKU3-1042) solution after incubation at 40 °C at various pH, adjusted with NaOH.

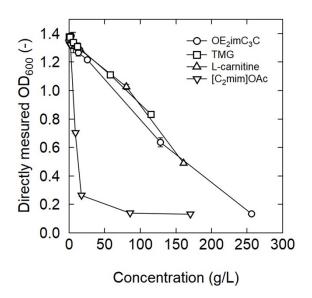


Fig. S4  $OD_{600}$  after 6h incubation at 40 °C in media supplemented with  $OE_2imC_3C$ , TMG, L-carnitine, and  $[C_2mim]OAc$ . Strain: *K. marxianus* DMKU3-1042. Initial  $OD_{600}$ : 0.2.

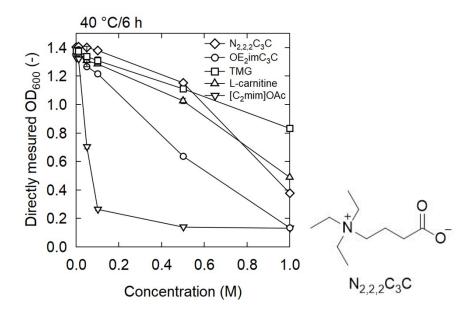
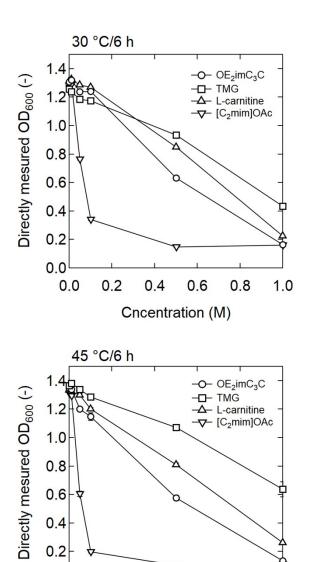


Fig. S5 OD<sub>600</sub> after 6h incubation in media supplemented with N<sub>2,2,2</sub>C<sub>3</sub>C, OE<sub>2</sub>imC<sub>3</sub>C, TMG, L-carnitine, and [C<sub>2</sub>mim]OAc at 40 °C. Strain: *K. marxianus* DMKU3-1042. Initial OD<sub>600</sub>: 0.2.



0.2

0.0 0.0

0.2

0.4

0.6

Concentration (M)

8.0

Fig. S6 OD<sub>600</sub> after 6h incubation in media supplemented with OE<sub>2</sub>imC<sub>3</sub>C, TMG, L-carnitine, and [C<sub>2</sub>mim]OAc at 30 and 45 °C. Strain: K. marxianus DMKU3-1042. Initial OD<sub>600</sub>: 0.2

1.0

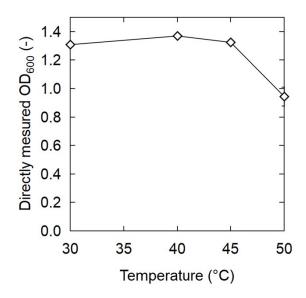
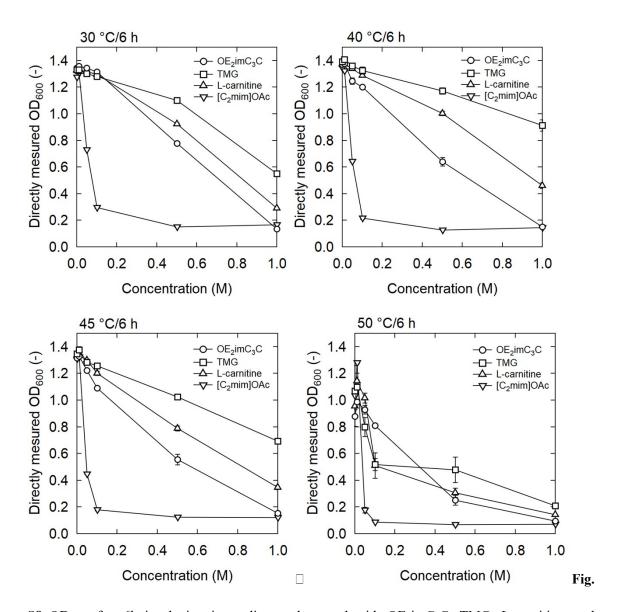


Fig. S7 OD<sub>600</sub> after 6h incubation without zwitterions/ionic liquids. Strain: K. marxianus NBRC1777.



**S8** OD<sub>600</sub> after 6h incubation in media supplemented with OE<sub>2</sub>imC<sub>3</sub>C, TMG, L-carnitine, and [C<sub>2</sub>mim]OAc at 30, 40, 45 and 50 °C. Strain: *K. marxianus* NBRC1777. Initial OD<sub>600</sub>: 0.2.

Table S1. pH of the 1.0 M zwitterion solutions.

	рН
Medium only	6.1
$OE_2imC_3C$	7.0
TMG	6.1
L-carnitine	6.5
[C <sub>2</sub> mim]OAc	6.4

Table S2. Water concentration and zwitterion/water ratio in the 0.5 and 1.0 M zwitterion solutions.

Water concentration was roughly calculated with the density of 1.0 g/mL.

	Water conc. (mol/L)		zwitterion/water	
			(mol/mol)	
zwitterion conc.	0.5 M	1.0 M	0.5 M	1.0 M
OE <sub>2</sub> imC <sub>3</sub> C	48.4	41.3	0.0103	0.0242
TMG	52.3	49.0	0.0096	0.0204
L-carnitine	51.0	46.6	0.0098	0.0215