Supplementary Table 1. Culture conditions of microbes and cancer cells.

	Microbes	Seed OD_{620nm} [A]	Culture time	OD_{620nm} (+)[B]	OD_{620nm} (-)[B]	Medium[C]	HPLC solvent[D]
a	*Escherichia coli* K-12	0.268±0.003	18 h	0.771±0.003	0.708±0.005	Nutrient	Standard
b	*Serratia marcescens* B-0524	0.181±0.002	18 h	0.386±0.010	0.359±0.024	Nutrient	Standard
c	*Enterococcus faecalis* JCM5803	0.130±0.002	18 h	0.172±0.004	0.131±0.001	HI	Standard
d	*Aspergillus niger* F16	0.212±0.001	72 h	0.263±0.007	0.054±0.009	RPMI	TRH, LTA, LTC: Standard, LTB: 80%
e	*Salmonella enteritidis* 1891	0.169±0.002	18 h	0.327±0.010	0.244±0.009	HI	Standard
f	*Mycobacterium smegmatis* ATCC 607	1.496±0.003	18 h	0.341±0.057	0.329±0.006	Nutrient, 2% glycerol, (IS uridine)	Standard
g	*Bacillus subtilis* 168	0.066±0.002	24 h	0.693±0.045	0.518±0.0314	LB	TRH, LTA, LTC: Standard, LTB: 90%
h	*Pseudomonas aeruginosa* A3	0.415±0.002	18 h	1.232±0.015	1.209±0.008	Nutrient	Standard
i	*Micrococcus luteus* IFO3333	1.508±0.007	18 h	0.093±0.006	0.072±0.003	Nutrient	Standard
j	*Bacteroides fragilis* JCM11019	0.843±0.021	24 h	0.875±0.013	0.891±0.875	GAM	TRH, LTA, LTC: Standard, LTB: 90%
k	*Saccharomyces cerevisiae* F-7	0.498±0.007	72 h	0.025±0.028	0.009±0.002	RPMI	TRH, LTA, LTC: Standard, LTB: 80%
l	*Candida albicans* 3147	0.263±0.010	72 h	0.050±0.014	0.024±0.004	RPMI	TRH, LTA, LTC: Standard, LTB: 80%
	Cell line	**Cell concentration**					
m	Mewo	3.2 x 10^4 cells/0.2 ml	48 h	Not determined	Not determined	10% FBS-DMEM	TRH, LTA: Standard, LTB, LTC: 80%
n	OVK18	3.2 x 10^4 cells/0.2 ml	48 h	Not determined	Not determined	10% FBS-DMEM	TRH, LTA: Standard, LTB, LTC: 80%

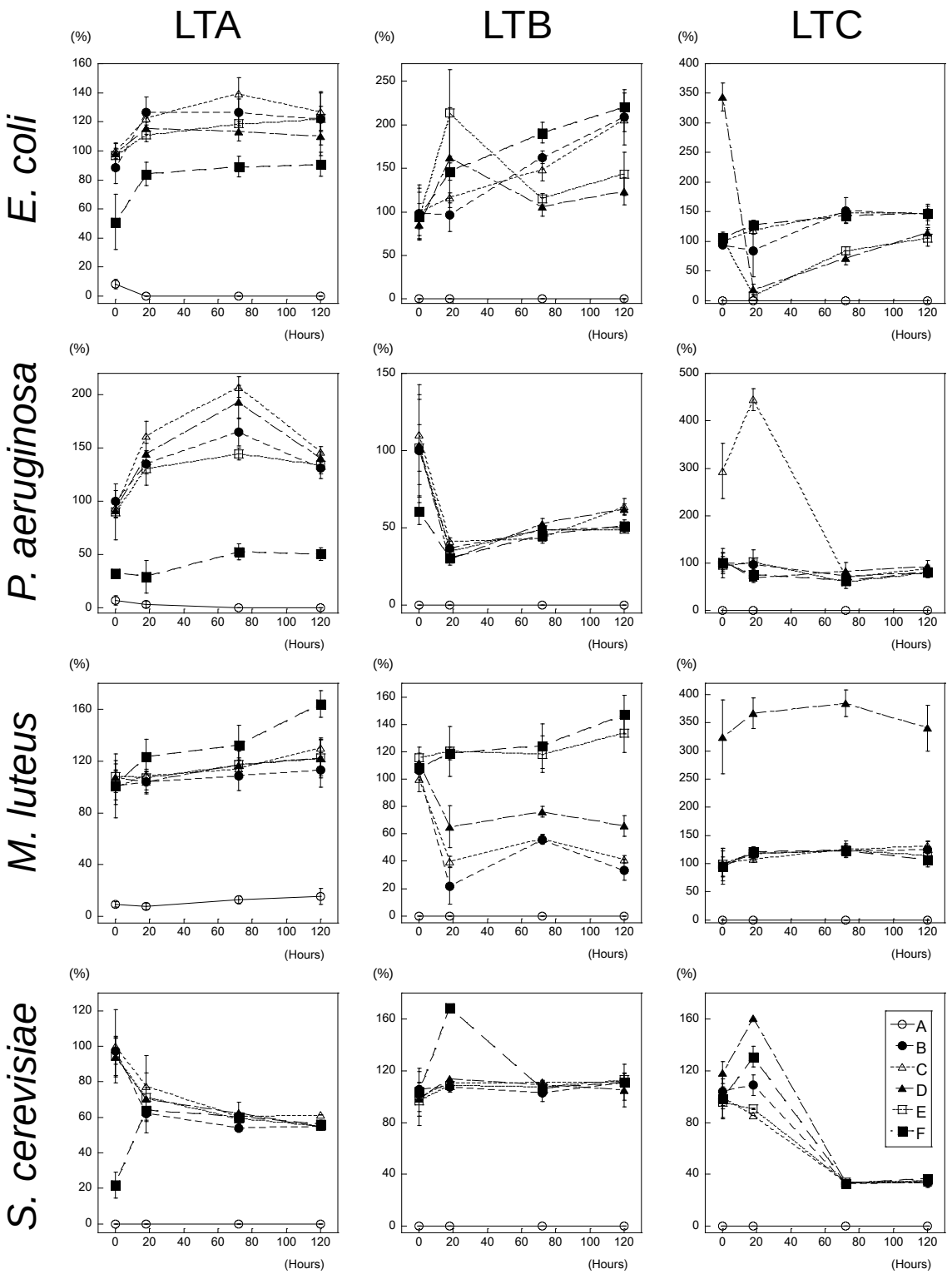
[A] Seed culture of microbes were diluted at 1/1000 to start the culture.

[B] OD_{620nm} after the culture with (+) or witout (-) 500 μg each of trehalose, lentztrehaloses A, B, and C.

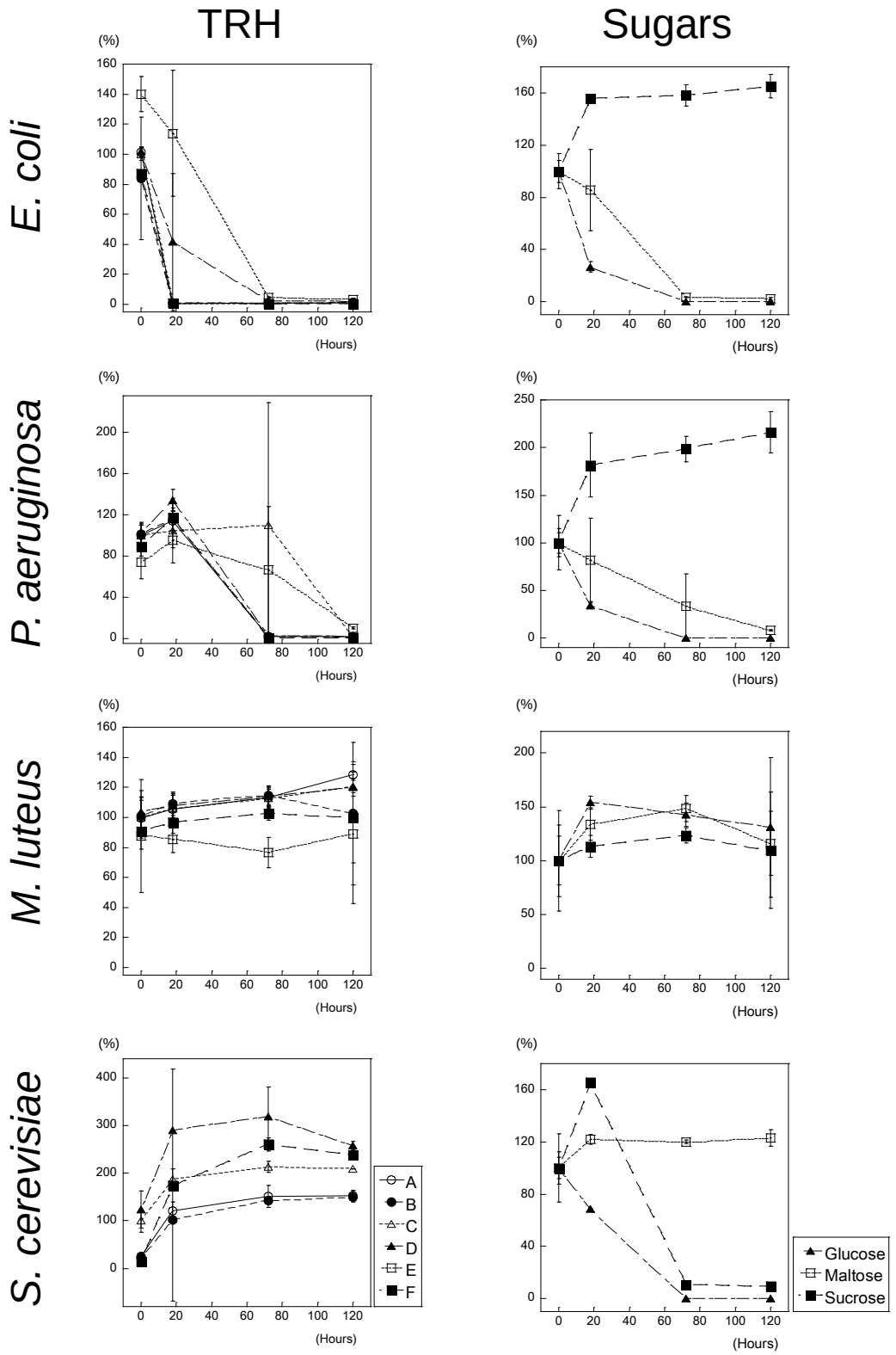
[C] Nutrient: 1% polypeptone (Nihon Pharmaceutical Co., LTD., Tokyo), 1% fish meat extract (Kyokuto Pharmaceutical Industrial Co., LTD., Tokyo), 0.2% NaCl
HI: Heart infusion broth (Becton, Dickinson and Company, Franklin Lakes, NJ)
RPMI: RPMI 1640 (Nissui Pharmaceutical Co.)
LB: LB BROTH DAIGO (Nihon Pharmaceutical Co., LTD., Tokyo)
GAM: Gifu anaerobic medium (GAM broth: Nissui Pharmaceutical Co., Tokyo)
10% FBS-DMEM: 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), Dulbecco's Modified Eagle Medium (Nissui Pharmaceutical Co.
In the culture of *M. smegmatis* ATCC 607, uridine was used as the internal standard (IS). Glycerol was used as the IS in other cultures

[D] Standard solvent condition was a gradient of 90–50% acetonitrile, or a 90% or 80% isocratic concentration of acetonitrile
when the peaks of LTB and/or LTC were overlapped with the other components in the medium
TRH: trehalose, LTA,LTB, LTC: lentztrehaloses A, B, and C.

Supplementary Figure 1



Supplementary Figure 2



Captions to the supplementary figures.

Supplementary Figure 1. Stability of lentztrehaloses A, B, and C in microbial cultures. *Escherichia coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Saccharomyces cerevisiae* were cultured in the media A–F as follows: (A) Nutrient (for the bacteria) or RPMI (for the yeast). (B) medium A + 5 μg/ml each of lentztrehaloses A, B, and C. (C) medium B + 5 μg/ml trehalose. (D) medium C + 0.5% glucose. (E) medium C + 0.5% maltose. (F) medium C + 0.5% sucrose. The bacteria were cultured at 37 °C and the yeast was cultured at 30 °C up to 120 h. The culture broths were harvested at the indicated time points and diluted to 1/10 with methanol, followed by centrifugation at 21,000 × g for 5 min. The amounts of lentztrehaloses in the supernatants were measured by LC-MS using a HILIC column. Each graph shows the detection pattern of one lentztrehalose (LTA, B, or C) in the microbial culture indicated at the top. The ratio (%) of the detected amount to that of medium C at 0 hour was plotted. Because the detection of lentztrehaloses, especially B and C, were affected by the existence of other sugars in the medium, lentztrehaloses appeared to decrease at some early culturing time points. However, after long

culture times when many of the other sugars and components in the medium have been digested, the detected amounts of lentztrehaloses became stable as shown in each graph from 72–120 h and further decreases were not clearly observed. Thus, we concluded that lentztrehaloses A, B, and C were only minimally digested in the single microbial culture and even extending the culturing time and the presence of other sugars such as glucose, maltose, and sucrose did not affect it.

Supplementary Figure 2. Digestion of trehalose and other sugars in microbial cultures. The microbes were cultured in the media A–F and the amounts of trehalose and other sugars were measured as described in the legend of Supplementary Figure 1. For trehalose (TRH), the ratio (%) of the detected amount to that of medium C at 0 h was plotted. For other sugars (Sugars), only the results of the cultures D–F supplemented with excess amounts of glucose, maltose, or sucrose are shown. The ratio (%) of each sugar amount to that at 0 h was plotted.