

## Supplementary Figure S2- Growth of different *Bacillus licheniformis* strains on agar plate, at 37°C, 55°C and 60°C

The  $OD_{600nm}$  of the ON cultures in LB media was measured and used to inoculate 5 ml of fresh LB medium to the  $OD_{600nm}$  0.05. Cultures were then grown at 37 °C, 200 rpm until  $OD_{600nm}$  0.5. This step was performed to get endospores-free culture. 1 ml of cells from the day culture in LB medium were collected by centrifugation at 10000 x g for 2 min (5430 R Eppendorf centrifuge) and resuspended in 1 ml sterile 0.9 % NaCl solution. The  $OD_{600nm}$  of the cells was measured and was adjusted to 1.0 with the 0.9 % NaCl solution. A 10-fold dilution series in the NaCl solution up to  $10^{-9}$  was prepared. 3 µl of cells from each dilution tube was spotted on LB agar plates and dried under the flow cabinet. The plates were incubated at 37 °C, 55 °C, and 60 °C.



LB agar plate, 37°C

LB agar plate,55°C

LB agar plate, 60°C

#### Supplementary Figure S3A- Hemeolysis Test for different *Bacillus licheniformis* strains

Growth of all 10 *B. licheniformis* food isolates and the type strain DSM13<sup>T</sup> on Columbia blood agar, showing hemolytic activity. The clearing zone on the blood agar indicates the lysis of erythrocytes, indicative of the presence of the biosurfactant lichenysin. A small clearing zone indicated weak hemolytic activity and a big clearing zone indicated strong hemolytic activity.



### Supplementary Figure S3B- Hemeolysis Test for different Bacillus licheniformis strains

Growth of all 10 *B. licheniformis* food isolates and the type strain DSM13<sup>T</sup> on Columbia blood agar, showing hemolytic activity. The clearing zone on the blood agar indicates the lysis of erythrocytes, indicative of the presence of the biosurfactant lichenysin. A small clearing zone indicated weak hemolytic activity and a big clearing zone indicated strong hemolytic activity.



## Supplementary Figure S4 – Growth of different *Bacillus licheniformis* strains in TSB medium under different conditions







#### **Figure S5- Chromatographs of lichenysin A variants**

Chromatographs of C11 to C17- lichenysin A variants detected for B4094 via RP-HPLC-QTOF-ESI/MS. 5  $\mu$ L sample was injected on a C8 analytical column (Phenomenex) thermostated at 50° C. Lichenysin A was eluted at a flow rate of 0.2 mL/min with a linear gradient of 0.10% formic acid in 40% water + 55% acetonitrile + 5% tetrahydrofuran to 0.1% formic acid in 75% acetonitrile + 25% tetrahydrofuran in 20 min. Lichenysin A variants were screened according to the masses in Table S1. Quantitative analyses were carried out using lichenysin A standard (range 10 – 40 000  $\mu$ g/L) (Lipofabrik).

Chromatographs of lichenysin A variants   +ESI EIC(979.6438) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C11-Lichenvsin:
2	[M + H] <sup>+</sup> = 979.6438 Da
+ESI EIC(993.6595) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C12-Lichenysin; [M + H] <sup>+</sup> = 993.6595 Da
+ESI EIC(1007.6751) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C13-Lichenysin; [M + H] <sup>+</sup> = 1007.6751 Da
+ESI EIC(1021.6908) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C14-Lichenysin; [M + H] <sup>+</sup> = 1021.6908 Da
+ESI EIC(1035.7064) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C15-Lichenysin; [M + H] <sup>+</sup> = 1035.7064 Da
+ESI EIC(1049.7221) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d	C16-Lichenysin; [M + H] <sup>+</sup> = 1049.7221 Da
+ESI EIC(1063.7377) Scan Frag=150.0V HY244, SM, B-B4094, R1 - T72, T 37C.d 2	C17-Lichenysin; [M + H] <sup>+</sup> = 1063.7377 Da
6 7 8 9 10 11 12 13 14 Counts vs. Acquisition Time (min)	15 16 17 18 19

# Figure S6- Bacillus licheniformis growth and lichenysin production in different matrices

