

Supplementary Information

for

Dependence of the Staphylococcal Volatilome Composition on Microbial Nutrition

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Table S1 See **Supplementary File**. Volatile compounds detected in the headspace of *in vitro* cultures of *S. aureus* (Sa) or *S. epidermidis* (Se) cultured in brain heart infusion (BHI), lysogeny broth Lennox (LB), Mueller-Hinton broth (MHB), or tryptic soy broth (TSB).

Table S2 Number of volatiles produced in 16 chemical classes by culturing *S. aureus* (Sa) or *S. epidermidis* (Se) in brain heart infusion (BHI), lysogeny broth (LB), Mueller-Hinton broth (MHB), or tryptic soy broth (TSB). A list of abbreviations for the chemical classes is provided in Table S1.

	NIT-ARO	A/E-ARO	KET-ARO	ALD-ARO	ALC-ARO	HC-ARO	HET-ARO	HALO	SULF	NIT	A/E	ETH	KET	ALD	ALC	HC	Total
BHI, Sa	0	0	0	0	0	0	6	1	3	1	6	1	13	1	2	13	47
BHI, Se	0	0	1	1	0	3	4	3	7	0	3	4	13	2	5	30	76
LB, Sa	1	0	0	0	1	1	10	2	7	2	2	0	13	0	5	19	63
LB, Se	1	1	0	1	1	2	6	2	5	0	3	0	11	2	3	25	63
MHB, Sa	0	0	0	0	0	0	1	1	5	1	0	1	6	1	4	18	38
MHB, Se	1	0	0	2	0	3	2	2	2	1	1	0	4	0	2	17	37
TSB, Sa	0	0	0	0	0	2	10	3	3	2	5	0	11	0	2	16	54
TSB, Se	0	0	0	1	0	0	7	0	0	3	1	0	4	2	1	8	27
BHI	0	0	1	1	0	3	7	4	7	1	9	5	20	3	5	31	97
LB	1	1	0	1	1	2	11	2	10	2	3	0	16	2	7	30	89
MHB	1	1	0	2	0	3	3	3	4	2	1	1	8	1	5	24	59
TSB	0	0	0	1	0	2	10	3	3	3	5	0	12	2	2	19	62
Sa	1	0	0	0	1	3	12	4	11	3	10	2	18	1	7	29	102
Se	1	1	1	2	1	5	9	4	9	3	4	4	16	6	7	37	110
Total	1	1	1	2	1	7	13	5	12	4	11	5	23	6	12	42	146

Table S3 Number of volatiles produced in 16 chemical classes by culturing *S. aureus* (Sa) or *S. epidermidis* (Se) in brain heart infusion (BHI), lysogeny broth (LB), Mueller-Hinton broth (MHB), or tryptic soy broth (TSB), normalized to the total number in each row in Table S2. A list of abbreviations for the chemical classes is provided in Table S1.

	NIT-ARO	A/E-ARO	KET-ARO	ALD-ARO	ALC-ARO	HC-ARO	HET-ARO	HALO	SULF	NIT	A/E	ETH	KET	ALD	ALC	HC
BHI, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.02	0.06	0.02	0.13	0.02	0.28	0.02	0.04	0.28
BHI, Se	0.00	0.00	0.01	0.01	0.00	0.04	0.05	0.04	0.09	0.00	0.04	0.05	0.17	0.03	0.07	0.39
LB, Sa	0.02	0.00	0.00	0.00	0.02	0.02	0.16	0.03	0.11	0.03	0.03	0.00	0.21	0.00	0.08	0.30
LB, Se	0.02	0.02	0.00	0.02	0.02	0.03	0.10	0.03	0.08	0.00	0.05	0.00	0.17	0.03	0.05	0.40
MHB, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.13	0.03	0.00	0.03	0.16	0.03	0.11	0.47
MHB, Se	0.03	0.00	0.00	0.05	0.00	0.08	0.05	0.05	0.05	0.03	0.03	0.00	0.11	0.00	0.05	0.46
TSB, Sa	0.00	0.00	0.00	0.00	0.00	0.04	0.19	0.06	0.06	0.04	0.09	0.00	0.20	0.00	0.04	0.30
TSB, Se	0.00	0.00	0.00	0.04	0.00	0.00	0.26	0.00	0.00	0.11	0.04	0.00	0.15	0.07	0.04	0.30
BHI	0.00	0.00	0.01	0.01	0.00	0.03	0.07	0.04	0.07	0.01	0.09	0.05	0.21	0.03	0.05	0.32
LB	0.01	0.01	0.00	0.01	0.01	0.02	0.12	0.02	0.11	0.02	0.03	0.00	0.18	0.02	0.08	0.34
MHB	0.02	0.02	0.00	0.03	0.00	0.05	0.05	0.05	0.07	0.03	0.02	0.02	0.14	0.02	0.08	0.41
TSB	0.00	0.00	0.00	0.02	0.00	0.03	0.16	0.05	0.05	0.05	0.08	0.00	0.19	0.03	0.03	0.31
Sa	0.01	0.00	0.00	0.00	0.01	0.03	0.12	0.04	0.11	0.03	0.10	0.02	0.18	0.01	0.07	0.28
Se	0.01	0.01	0.01	0.02	0.01	0.05	0.08	0.04	0.08	0.03	0.04	0.04	0.15	0.05	0.06	0.34
Total	0.01	0.01	0.01	0.01	0.01	0.05	0.09	0.03	0.08	0.03	0.08	0.03	0.16	0.04	0.08	0.29

Table S4 Relative abundances of volatiles produced in 16 chemical classes by culturing *S. aureus* (Sa) or *S. epidermidis* (Se) in brain heart infusion (BHI), lysogeny broth (LB), Mueller-Hinton broth (MHB), or tryptic soy broth (TSB). A list of abbreviations for the chemical classes is provided in Table S1.

	NIT-ARO	A/E-ARO	KET-ARO	ALD-ARO	ALC-ARO	HC-ARO	HET-ARO	HALO	SULF	NIT	A/E	ETH	KET	ALD	ALC	HC	Total
BHI, Sa	0	0	0	0	0	0	40	11	284	68	161709	4	41786	3	28	126053	329986
BHI, Se	0	0	19953	11	0	8	15857	12983	778	0	266498	10	179	3164	730	20131	340302
LB, Sa	2	0	0	0	3	2	1505	39	39936	39816	204	0	35495	0	44	2259	119305
LB, Se	2	7	0	5	2	4838	22635	96	145	0	80099	0	1224	5	40173	59903	209134
MHB, Sa	0	0	0	0	0	0	7	45	1656	5	0	2	63912	3	16	8671	74317
MHB, Se	3	0	0	642	0	7	14	1266	255	5	3162	0	34	0	25	176	5589
TSB, Sa	0	0	0	0	0	1587	301531	2041	215	416	704277	0	20646	0	81	8751	1039545
TSB, Se	0	0	0	3	0	0	9616	0	0	809	100000	0	1276	44	32	26	111806
BHI	0	0	19953	11	0	8	15889	12995	659	68	342286	15	41844	3168	730	138096	575722
LB	2	0	0	5	3	4838	23600	96	40380	39816	80099	0	36093	5	40210	61935	327082
MHB	3	7	0	642	0	7	22	1311	1638	11	3162	2	63936	3	38	8654	79436
TSB	0	0	0	3	0	1587	301536	2041	215	1211	704277	0	20656	44	99	8760	1040429
Sa	2	0	0	0	3	1589	301551	2110	41960	40227	830188	6	142016	3	121	143233	1503009
Se	3	7	19953	642	2	4845	38064	13067	1132	811	295816	10	2514	3272	40581	72876	493595
Total	3	7	19953	642	3	6433	323680	13111	42190	41022	912931	15	142624	3272	40668	207642	1754196

Table S5 Relative abundances of volatiles produced in 16 chemical classes by culturing *S. aureus* (Sa) or *S. epidermidis* (Se) in brain heart infusion (BHI), lysogeny broth (LB), Mueller-Hinton broth (MHB), or tryptic soy broth (TSB), normalized to the total abundance in each row in Table S4. A list of abbreviations for the chemical classes is provided in Table S1.

	NIT-ARO	A/E-ARO	KET-ARO	ALD-ARO	ALC-ARO	HC-ARO	HET-ARO	HALO	SULF	NIT	A/E	ETH	KET	ALD	ALC	HC
BHI, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.13	0.00	0.00	0.38
BHI, Se	0.00	0.00	0.06	0.00	0.00	0.00	0.05	0.04	0.00	0.00	0.78	0.00	0.00	0.01	0.00	0.06
LB, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.33	0.33	0.00	0.00	0.30	0.00	0.00	0.02
LB, Se	0.00	0.00	0.00	0.00	0.00	0.02	0.11	0.00	0.00	0.00	0.38	0.00	0.01	0.00	0.19	0.29
MHB, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.86	0.00	0.00	0.12
MHB, Se	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.23	0.05	0.00	0.57	0.00	0.01	0.00	0.00	0.03
TSB, Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.68	0.00	0.02	0.00	0.00	0.01
TSB, Se	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.01	0.89	0.00	0.01	0.00	0.00	0.00
BHI	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.59	0.00	0.07	0.01	0.00	0.24
LB	0.00	0.00	0.00	0.00	0.00	0.01	0.07	0.00	0.12	0.12	0.24	0.00	0.11	0.00	0.12	0.19
MHB	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.02	0.00	0.04	0.00	0.80	0.00	0.00	0.11
TSB	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.68	0.00	0.02	0.00	0.00	0.01
Sa	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.03	0.03	0.55	0.00	0.09	0.00	0.00	0.10
Se	0.00	0.00	0.04	0.00	0.00	0.01	0.08	0.03	0.00	0.00	0.60	0.00	0.01	0.01	0.08	0.15
Total	0.00	0.00	0.01	0.00	0.00	0.00	0.18	0.01	0.02	0.02	0.52	0.00	0.08	0.00	0.02	0.12

Table S6 Media composition and characteristics

	BRAIN HEART INFUSION		LYSOGENY BROTH LENNOX		MUELLER HINTON BROTH		TRYPTIC SOY BROTH	
Manufacturer	Bacto		Bacto		Difco		Bacto	
Part Number(s)	237500		211705, 288620		275730		211825	
Protein source(s)	Proteose Peptone	10.0 g	Tryptone	10.0 g	Acid Digest of Casein	17.5 g	Pancreatic Digest of Casein	17.0 g
	Calf Brain Infusion	7.7 g			Beef Extract Powder	2.0 g	Papaic Digest of Soybean	3.0 g
	Beef Heart Infusion	9.8 g						
Sugar/Starch	Dextrose	2.0 g	Starch (amylopectin)		1.5 g	Dextrose	2.5 g	
Salts	NaCl	5.0 g	NaCl		5.0 g	NaCl	5.0 g	
Buffer	Na ₂ HPO ₄	2.5 g				K ₂ HPO ₄	2.5 g	
Other			Yeast extract		5.0 g			
Notes			• No cholesterol • No saturated fatty acids • Yeast extract total carbohydrates = 816 mg		• Not cation-adjusted (Ca ²⁺ , Mg ²⁺) • Low thymine and thymidine			

Table S7 Parameters for HS-SPME volatiles sampling, GC×GC-TOF analysis, and data processing and alignment

Autosampler Method	
Instrument description	Gerstel® MPS Pro®
Software description	Gerstel® Maestro® (version 1.5.3.2)
Sampling Parameters	
Cooled tray temperature	4 °C
Solid-phase microextraction (SPME)	Manufacturer: Supelco® Fiber type: PDMS/CAR/DVB (2 cm; 50/30 µm)
Incubation time	5 min
Agitator parameters, incubation	Temperature: 50 °C On time: 10 s Off time: 1 s Speed: 600 rpm
Agitation, sampling	On
Vial penetration	21 mm
Extraction time	10 min
Injection penetration	67 mm
Desorption time	180 s
Inlet (CIS) Parameters	
Initial temperature	250 °C
Equilibrium time	0.05 min
Initial time	0.10 min
Ramp rate	12 °C·s ⁻¹
End temperature	250 °C
Hold time	10.5 min
GC×GC Method	
Instrument description	Agilent® 7890B
Column configuration	Column 1: Rxi®-624Sil MS, 60 m × 0.25 mm × 1.4 µm Column 2: Stabilwax®, 1 m × 0.25 mm × 0.5 µm
Carrier gas	Helium, 2 mL·min ⁻¹ (constant)
Front inlet type	Gerstel®
Front inlet mode	Splitless
Front inlet septum purge flow	1 mL·min ⁻¹
Front inlet septum purge time	300 s
Front inlet purge flow	50 mL·min ⁻¹
Front inlet total purge flow	52 mL·min ⁻¹
Oven equilibration time	5 s
Primary oven temperature ramp	Initial temperature: 35 °C Initial time: 0.5 min Ramp rate: 5 C·min ⁻¹ Final temperature: 230 °C Hold time: 5 min

Secondary oven temperature offset	+5 °C (relative to primary oven)
Modulator temperature offset	+15 °C (relative to secondary oven)
Modulation timing	Modulation period: 2.00 s Hot pulse time: 0.50 s Cold pulse time: 0.50 s
Transfer line temperature	250 °C
Mass Spectrometry Method	
Instrument description	LECO® Pegasus® 4D
Use GC method total time for MS method total time	Yes
Acquisition delay	180 s
Filament active time	180 s to end of run
Start mass/End mass	35/400
Acquisition rate	100 spectra·s ⁻¹
Optimized voltage offset	+50 V
Electron energy	-70 eV
Ion source temperature	250 °C
Acquisition masses	TIC
Data Processing Method	
Software description	LECO® ChromaTOF® and Statistical Compare (version 4.71.0.0)
Baseline tracking/Offset	Entire run/0.5 (through middle of noise)
Data points averaged for smoothing	Auto
First dimension peak width	12 slices
Mass spectral match required to combine peaks	600
Second dimension peak width	0.15
Min. subpeak signal-to-noise (S/N) for retention	6
Integration approach	Traditional
Peak finding	S/N: 50 Min. number of apexing masses: 2
Mass to use for area/height calculation	Unique mass
Alignment analyte match criteria	Spectral match mass threshold: 10 Minimum spectral similarity match: 600 Max. number of modulation periods apart: 3 Max. retention time difference: 0.2 S/N for second peak find: 5
Criteria for inclusion of analytes	Min. number of samples that contain analyte: 1