



Universität Hamburg
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Attachment of *Listeria monocytogenes* to corn salad

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Microbiology

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Foto: UHH/Lutsch

Importance of *Listeria monocytogenes*

- Gram-positive, rod-shaped
- Psychrophilic $\Rightarrow$ Food-borne pathogen
- Intracellular parasite
- Gastroenteritis, encephalitis, meningitis
- YOPI are potential risk group
- High hospitalization and mortality rate
- Meat, fish, dairy, (leafy) vegetables

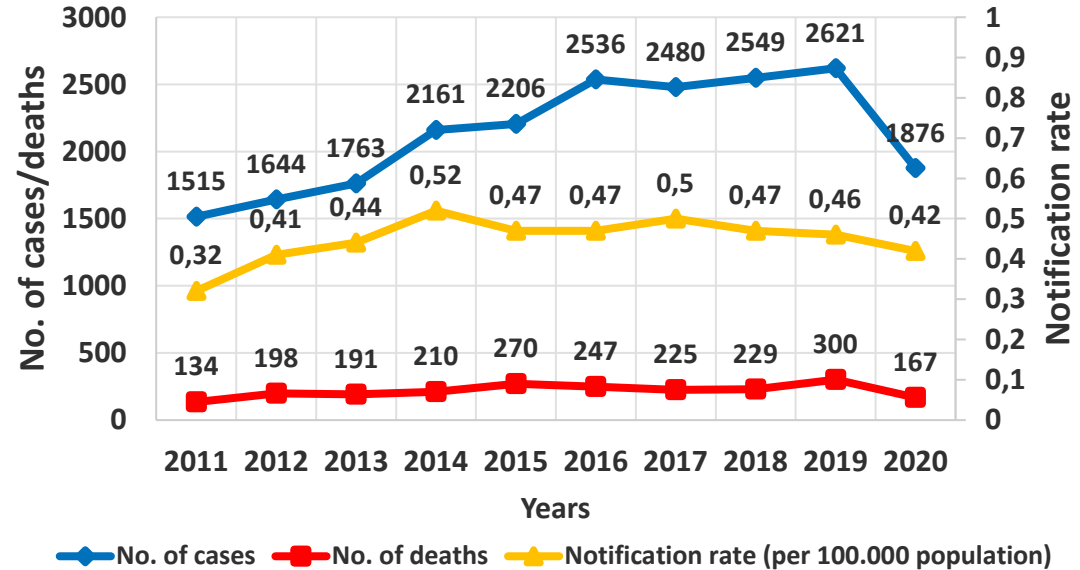


Fig. 1: Annual listeriosis incidence in the EU between 2011 and 2020. UK for 2020 not included

Aim of this study

In what way does *L. monocytogenes* interact with corn salad and where?

Strains under investigation:

L. monocytogenes NCTC 10887 serovar 1/2a from chinchilla

L. monocytogenes 15-L-06447-1-1 serovar 4b from mung bean sprouts

L. monocytogenes 20-L-06460-1-1 serovar 1/2a from RTE salad



Fig. 2: Sterile cultivated corn salad

-> SEM to visualize colonization

-> Tagging of *L. monocytogenes* to study spacial and temporal dimensions of colonization *via* LSM

-> Investigation of *L. monocytogenes* under static conditions forming biofilm

SEM of *L. monocytogenes* colonizing sterile corn salad

Contamination of sterile *V. locusta*
with *L. monocytogenes* NCTC 10887
(5 μ l, OD₆₀₀ = 2.0)
Incubation 48 h, 22 °C

- Fixation with
2.5 % glutaraldehyde
- Dehydration with EtOH
- Crit. point drying

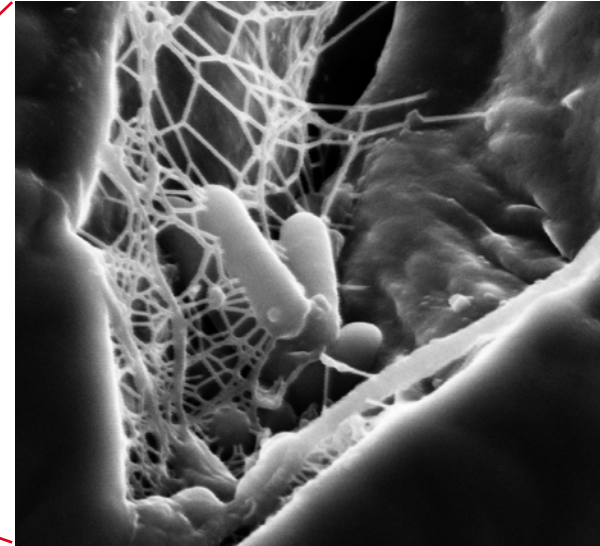
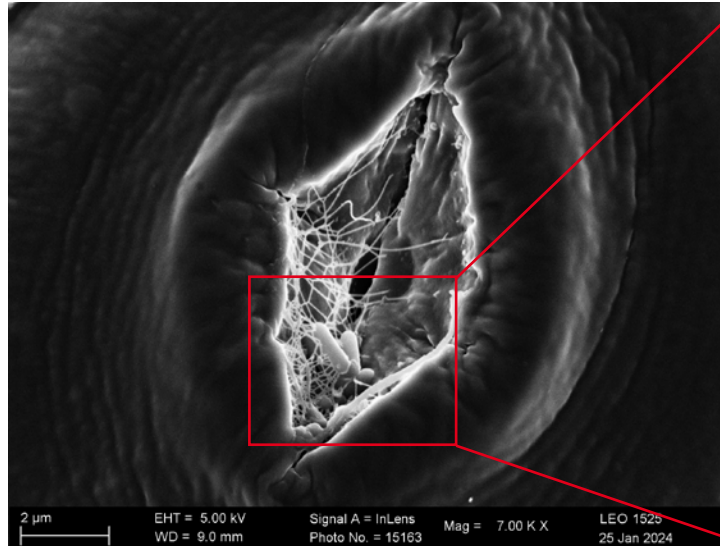


Fig. 3: *L. monocytogenes* NCTC 10887 on sterile *V. locusta*, 48 h, 22 °C

Tagging of *L. monocytogenes* NCTC 10887

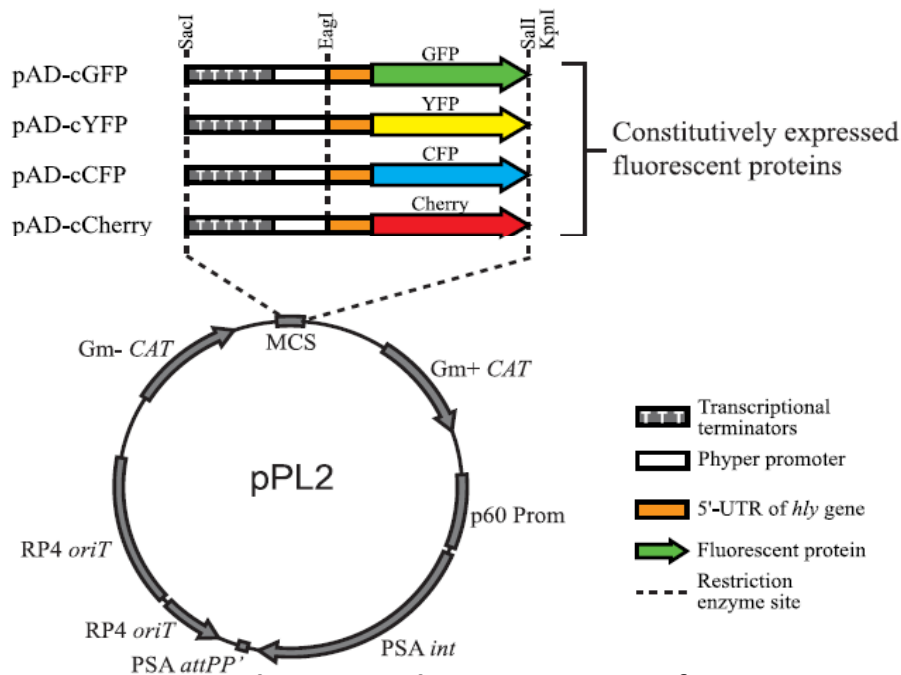


Fig. 4: pPL2 chromosomal integration vector for *L. monocytogenes* (Balestrino et al. (2010), modified)

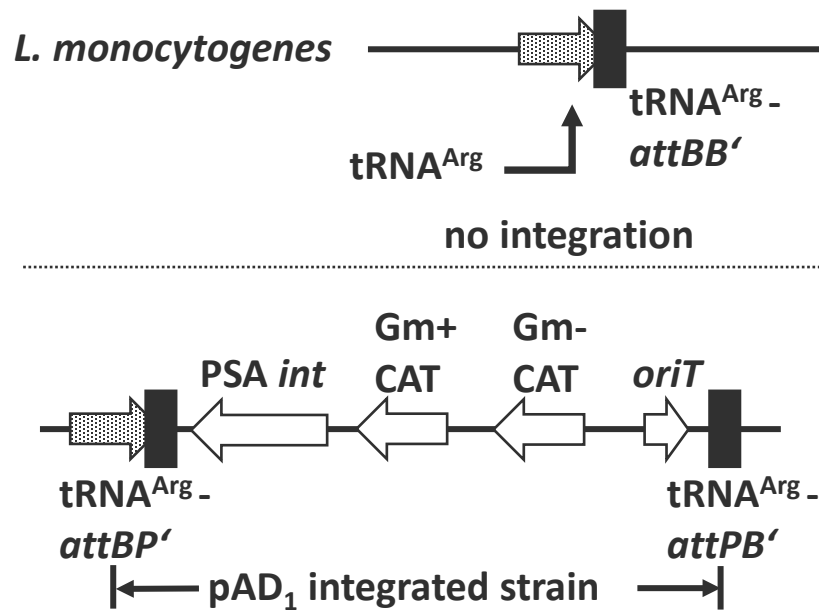


Fig. 5: Chromosomal integration site of pPL2 vector (Lauer et al. (2002), modified)

Screening for tagged *L. monocytogenes* NCTC 10887 cells

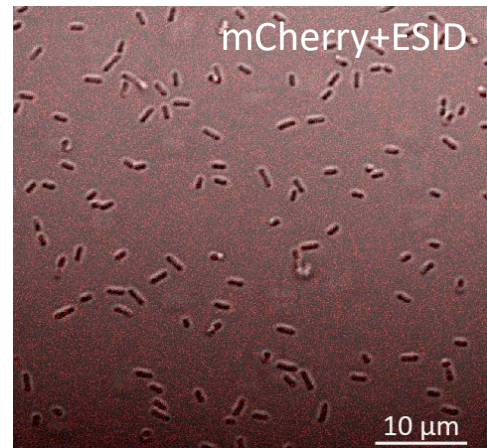


Fig. 6: *L. monocytogenes*
NCTC 10887 wildtype

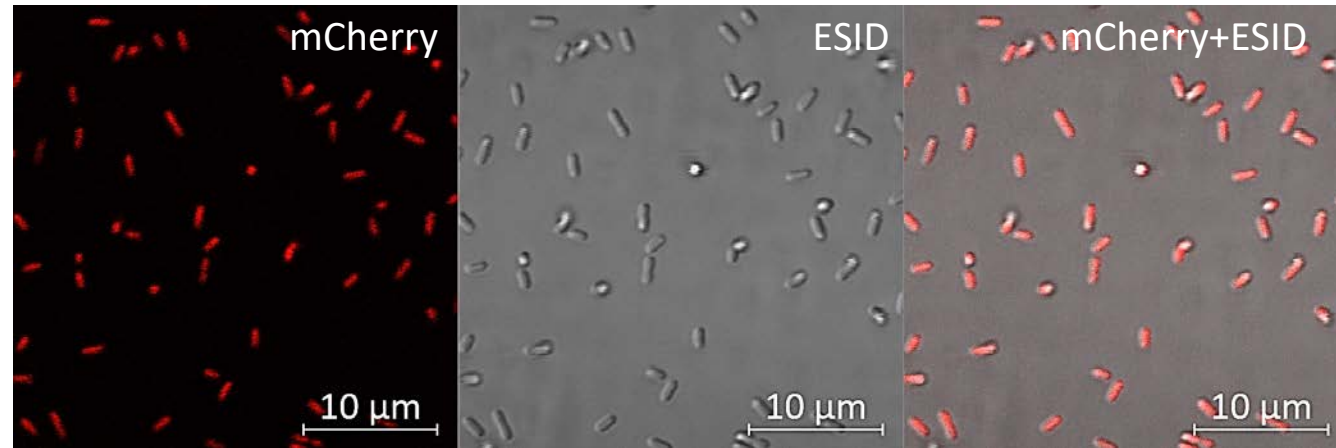
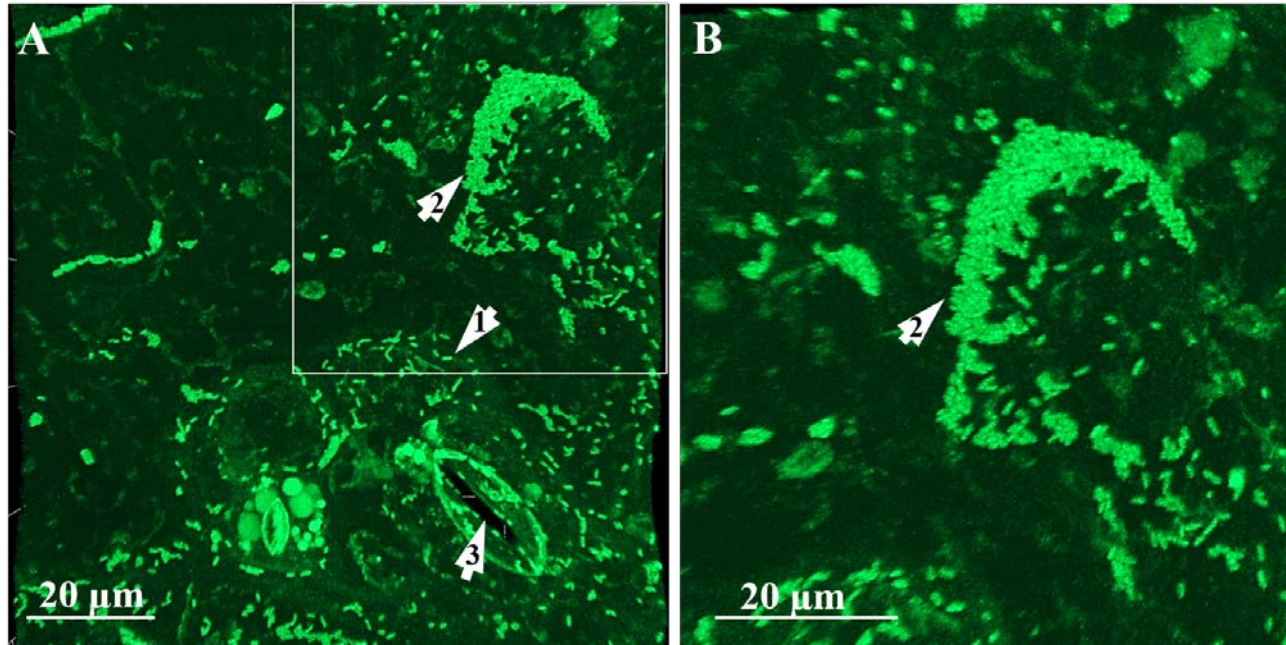


Fig. 7: *L. monocytogenes* NCTC 10887 pAD1::phyper::mCherry

Attachment of *L. monocytogenes* NCTC 10887 pAD1::phyper::GFP to corn salad



1. Single cells
2. Layer formation
3. Stoma colonization

Fig. 8: *L. monocytogenes* NCTC 10887 pAD1::phyper::GFP incubated on corn salad, 22°C, 48 h

L. monocytogenes NCTC 10887 growth in different media

Corn salad medium (SM):

M9 - salt solution 200 ml

Corn salad extract 250 ml

H₂O ad 1000 ml

Fornfeld et al. (2017)

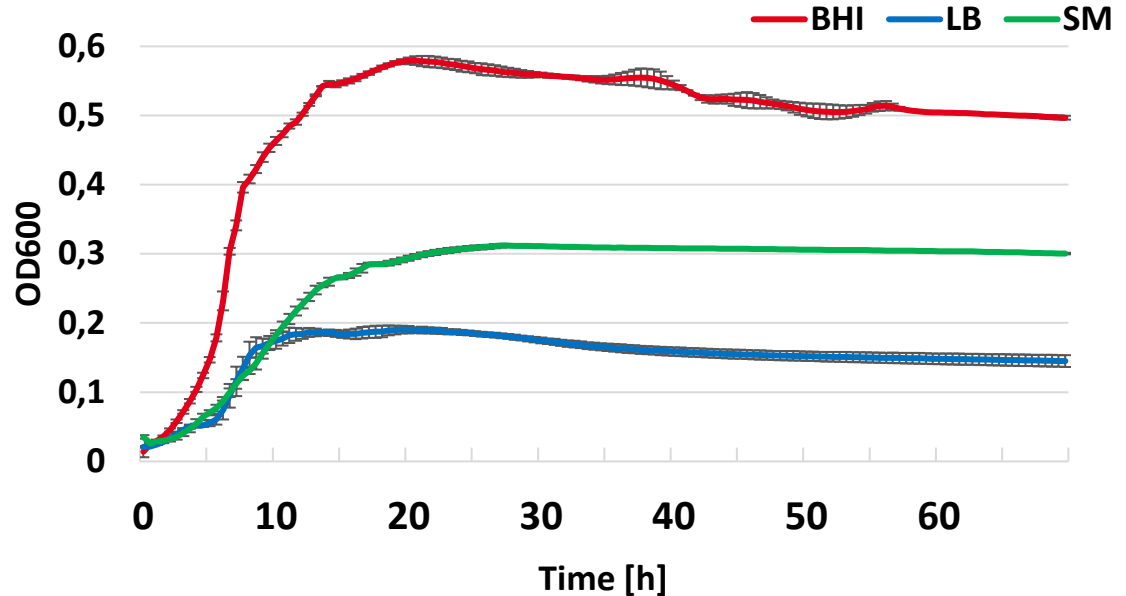
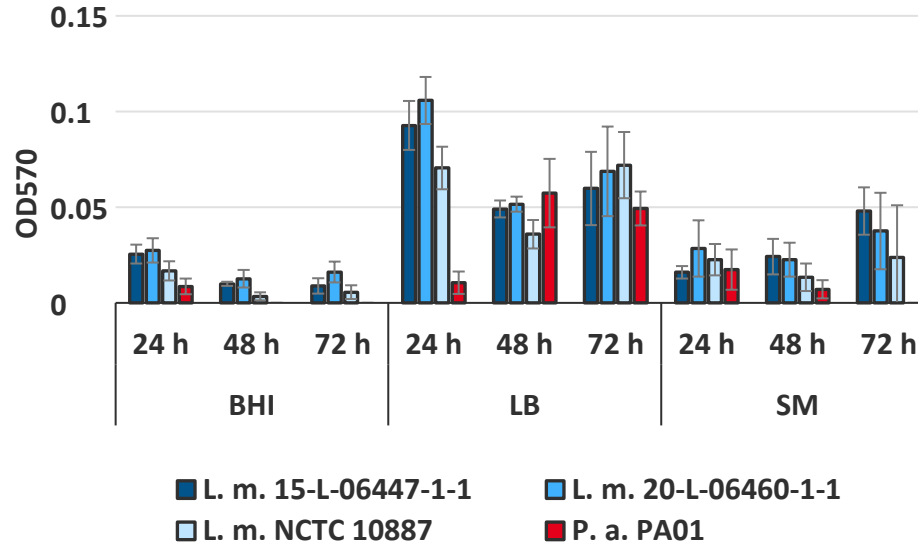


Fig. 9: Growth of *L. monocytogenes* NCTC 10887, static conditions, 22 °C

Crystal – violet assays for biofilm quantification

- Incubation at 22 °C
- For 72 h
- *P. aeruginosa* PA01 as positive control



Most biofilm formation:

- In LB broth
- During first 24 h
- Followed by SM, BHI

Fig. 10: CV-Assays for different *L. monocytogenes* strains at ambient temperature (22 °C)

Biofilm formation of *L. monocytogenes* NCTC 10887 pAD1::phyper::mCherry

- Quantification with „BiofilmQ“
- Three biological replicates averaged
- 1D histogram of cell count plotted against biofilm height

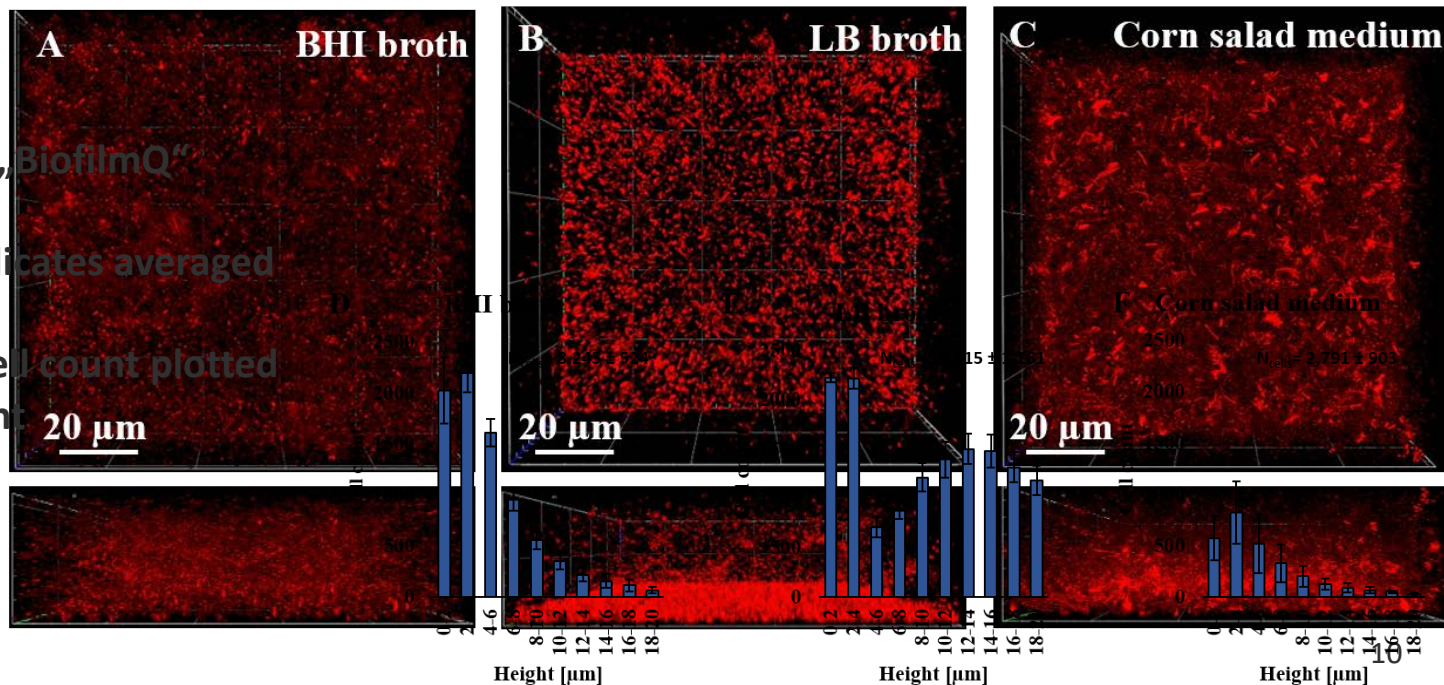


Fig. 11: *L. m.* NCTC 10887, 48 h, 22 °C (A, B, C) and BiofilmQ quantification (D, E, F)

Conclusion and outlook



SEM and LSM imaging shows *L. monocytogenes* is able to colonize *V. locusta*, produces structures within the stomata.



CV-Assays/LSM imaging shows most biofilm is formed in LB-broth at 22 °C during the first 24 h of incubation.



Differences in biofilm architecture, dependend on the incubation media.
Most cells in first layers.

Outlook:

- Transcriptomic data and reporter fusions
- Create a time series *via* LSM

Contact



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Sources:

- Fig 1: Osek J, Wieczorek K. *Listeria monocytogenes*-How This Pathogen Uses Its Virulence Mechanisms to Infect the Hosts. *Pathogens*. 2022 Dec 7;11(12):1491. doi: 10.3390/pathogens11121491. PMID: 36558825; PMCID: PMC9783847.
- Fig 2: Source: Blattgemüse - Weltweit, Deutschland. (n.d.). Zugriff am 23. April 2024, vo: <https://de.statista.com/outlook/cmo/lebensmittel/gemuese/frisches-gemuese/blattgemuese/custom?currency=EUR&locale=de&token=pihJ63N0Y35pkGFRbYvphxrl0EmFzok6tYcw8ylejWbeEWoyv2d7H0QbW7wij3IJ81AM0Q85WNzDNEi6EHb0ZRTQMhewDa1yQ-33zw%3D%3D>
- Fig 5: Balestrino D, Hamon MA, Dortet L, Nahori MA, Pizarro-Cerda J, Alignani D, Dussurget O, Cossart P, Toledo-Arana A. Single-cell techniques using chromosomally tagged fluorescent bacteria to study *Listeria monocytogenes* infection processes. *Appl Environ Microbiol*. 2010 Jun;76(11):3625-36. doi: 10.1128/AEM.02612-09. Epub 2010 Apr 2. PMID: 20363781; PMCID: PMC2876438.
- Fig 6: Lauer P, Chow MY, Loessner MJ, Portnoy DA, Calendar R. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J Bacteriol*. 2002 Aug;184(15):4177-86. doi: 10.1128/JB.184.15.4177-4186.2002. Erratum in: *J Bacteriol*. 2003 Feb;185(4):1484. PMID: 12107135; PMCID: PMC135211.

Crystal – violet assays for biofilm quantification

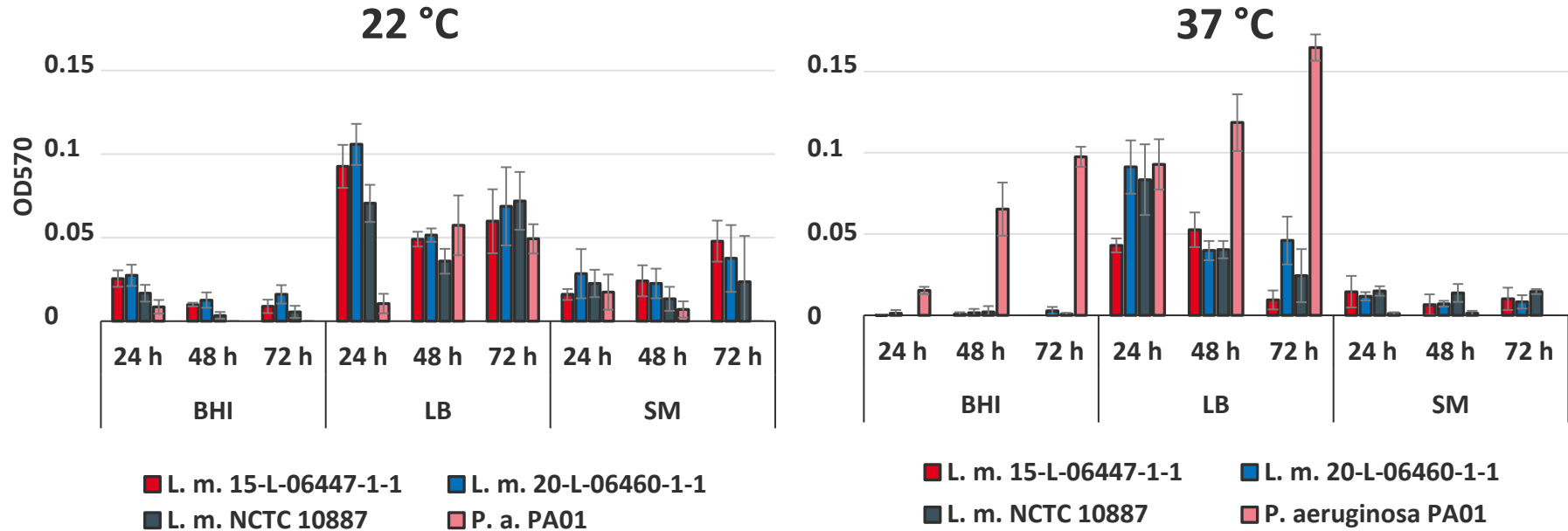
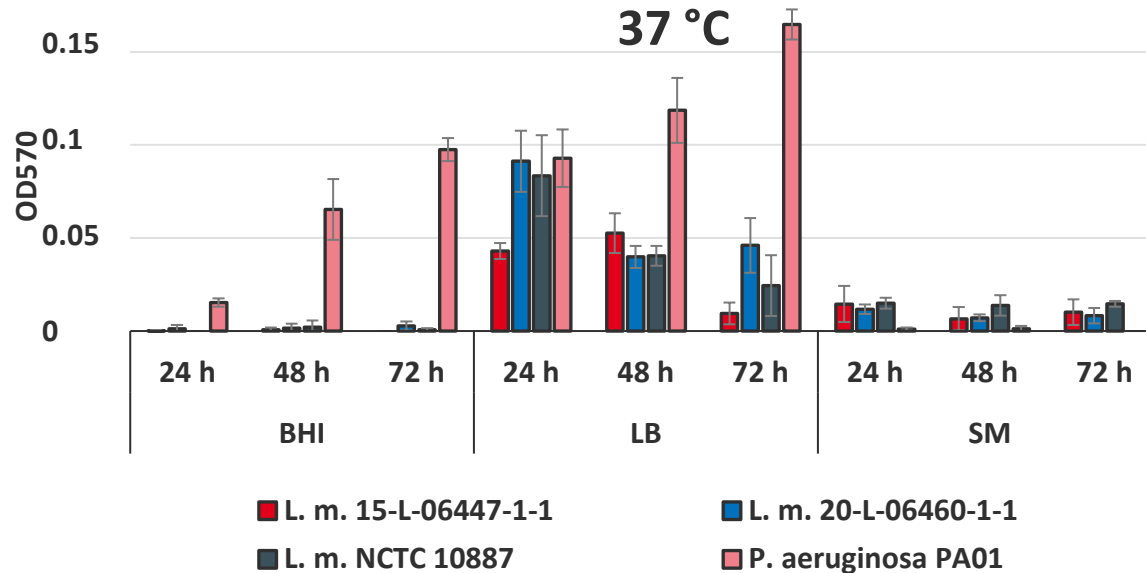


Fig. 10: CV-Assays for different *L. monocytogenes* strains at ambient and body temperature

Crystal – violet assays for biofilm quantification



Most biofilm formation:

- In LB – medium
- During first 24 h
- Little to no formation in SM, BHI

Fig. 10: CV-Assays for different *L. monocytogenes* strains at body temperature (37 °C)

Table 1. Confirmed listeriosis cases and rates per 100 000 population by country and year, EU/EEA, 2018–2022

Country	2018		2019		2020		2021		2022	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
Austria	27	0.31	38	0.43	41	0.46	38	0.43	47	0.52
Belgium	74	0.81	66	0.72	54	0.59	68	0.74	87	0.94
Bulgaria	9	0.13	13	0.19	4	0.06	3	0.04	5	0.07
Croatia	4	0.10	6	0.15	5	0.12	8	0.20	5	0.13
Cyprus	1	0.12	1	0.11	2	0.23	1	0.11	1	0.11
Czechia	31	0.29	27	0.25	16	0.15	24	0.22	48	0.46
Denmark	49	0.85	61	1.05	43	0.74	62	1.06	86	1.46
Estonia	27	2.05	21	1.59	3	0.23	5	0.38	11	0.83
Finland	80	1.45	50	0.91	94	1.70	70	1.26	70	1.26
France	338	0.50	373	0.56	334	0.50	435	0.64	451	0.66
Germany	678	0.82	571	0.69	546	0.66	562	0.68	548	0.66
Greece	19	0.18	10	0.09	20	0.19	21	0.20	7	0.07
Hungary	24	0.25	39	0.40	32	0.33	35	0.36	64	0.66
Iceland	2	0.57	4	1.12	4	1.10	5	1.36	2	0.53
Ireland	21	0.43	17	0.35	6	0.12	14	0.28	17	0.34
Italy	178	0.29	202	0.34	155	0.26	230	0.39	345	0.58
Latvia	15	0.78	6	0.31	8	0.42	10	0.53	8	0.43
Liechtenstein	NDR	NRC	NDR	NRC	NDR	NRC	0	0.00	0	0.00
Lithuania	20	0.71	6	0.21	7	0.25	7	0.25	13	0.46
Luxembourg	5	0.83	3	0.49	4	0.64	4	0.63	4	0.62
Malta	1	0.21	5	1.01	5	0.97	0	0.00	1	0.19
Netherlands	69	0.40	103	0.60	90	0.52	86	0.49	94	0.53
Norway	24	0.45	27	0.51	37	0.69	20	0.37	30	0.55
Poland	128	0.34	121	0.32	57	0.15	120	0.32	142	0.38
Portugal	64	0.62	56	0.54	47	0.46	57	0.55	63	0.61
Romania	28	0.14	17	0.09	2	0.01	11	0.06	14	0.07
Slovakia	17	0.31	18	0.33	7	0.13	13	0.24	25	0.46
Slovenia	10	0.48	20	0.96	26	1.24	19	0.90	20	0.95
Spain	370	NRC	504	NRC	191	NRC	355	0.77	437	0.95
Sweden	89	0.88	113	1.10	88	0.85	107	1.03	125	1.20

Biofilm formation of *L. monocytogenes* NCTC 10887 pAD1::phyper::mCherry

- Quantification with „BiofilmQ“
- Three biological replicates averaged
- 1D histogramm of cell count plotted against biofilm height

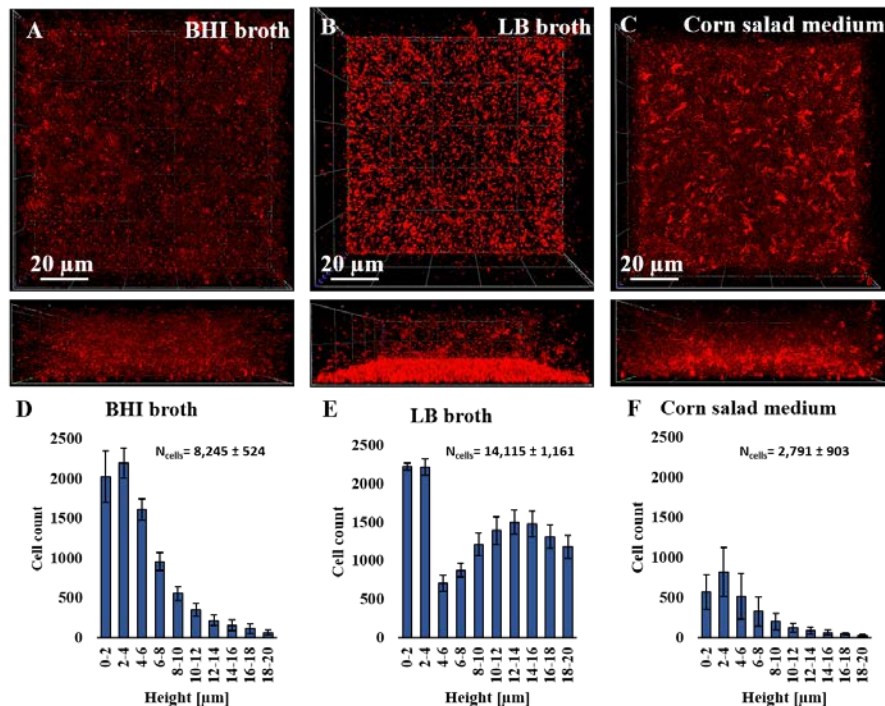
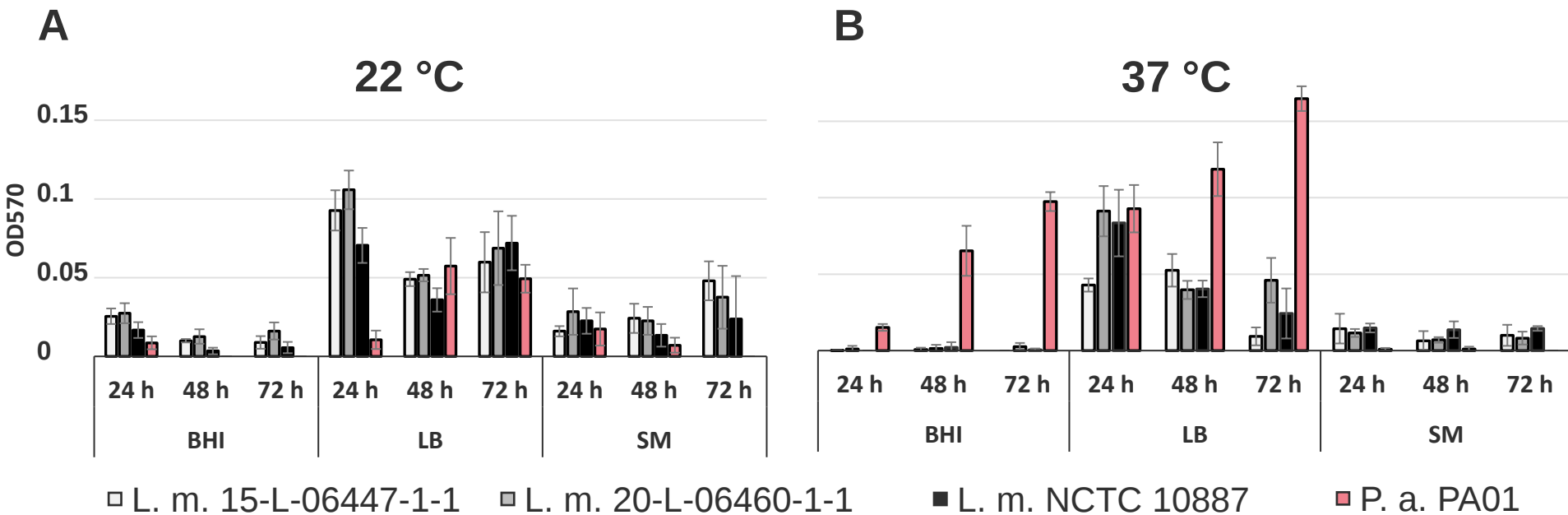
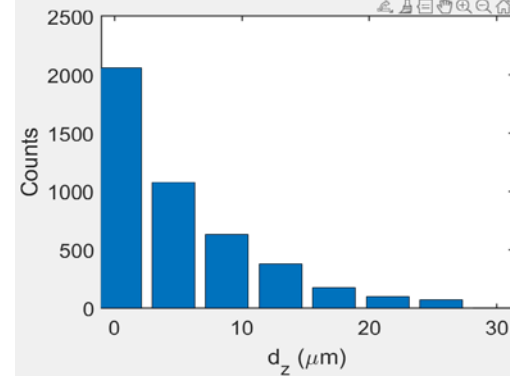
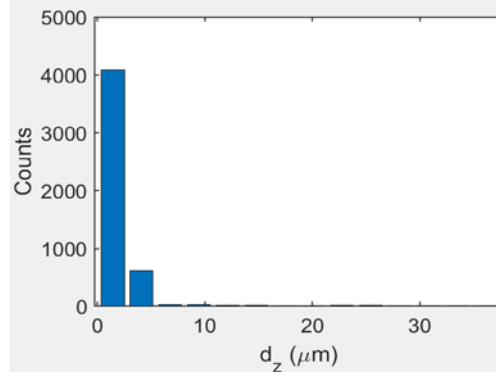
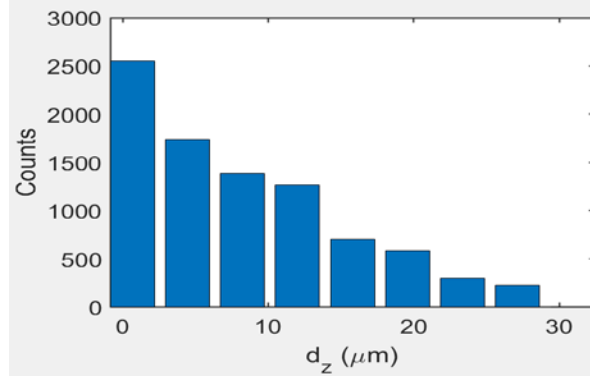
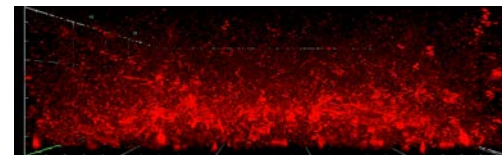
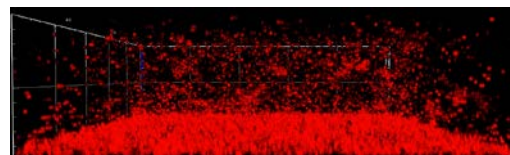
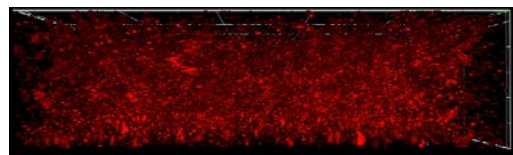
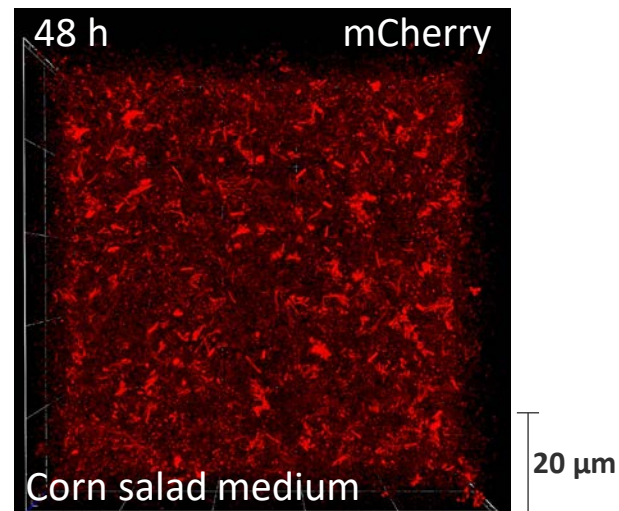
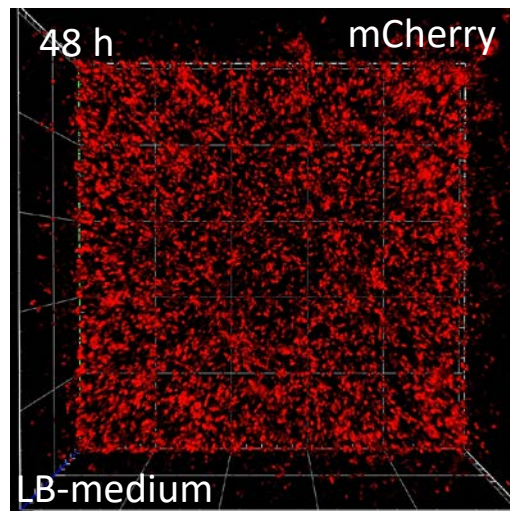
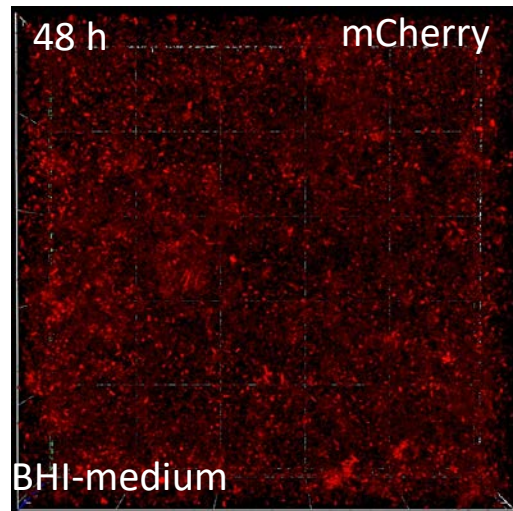


Fig. 11: *L. m.* NCTC 10887, 48 h, 22 °C (A, B, C) and BiofilmQ quantification (D, E, F)





Biofilm formation of *L. monocytogenes* NCTC 10887

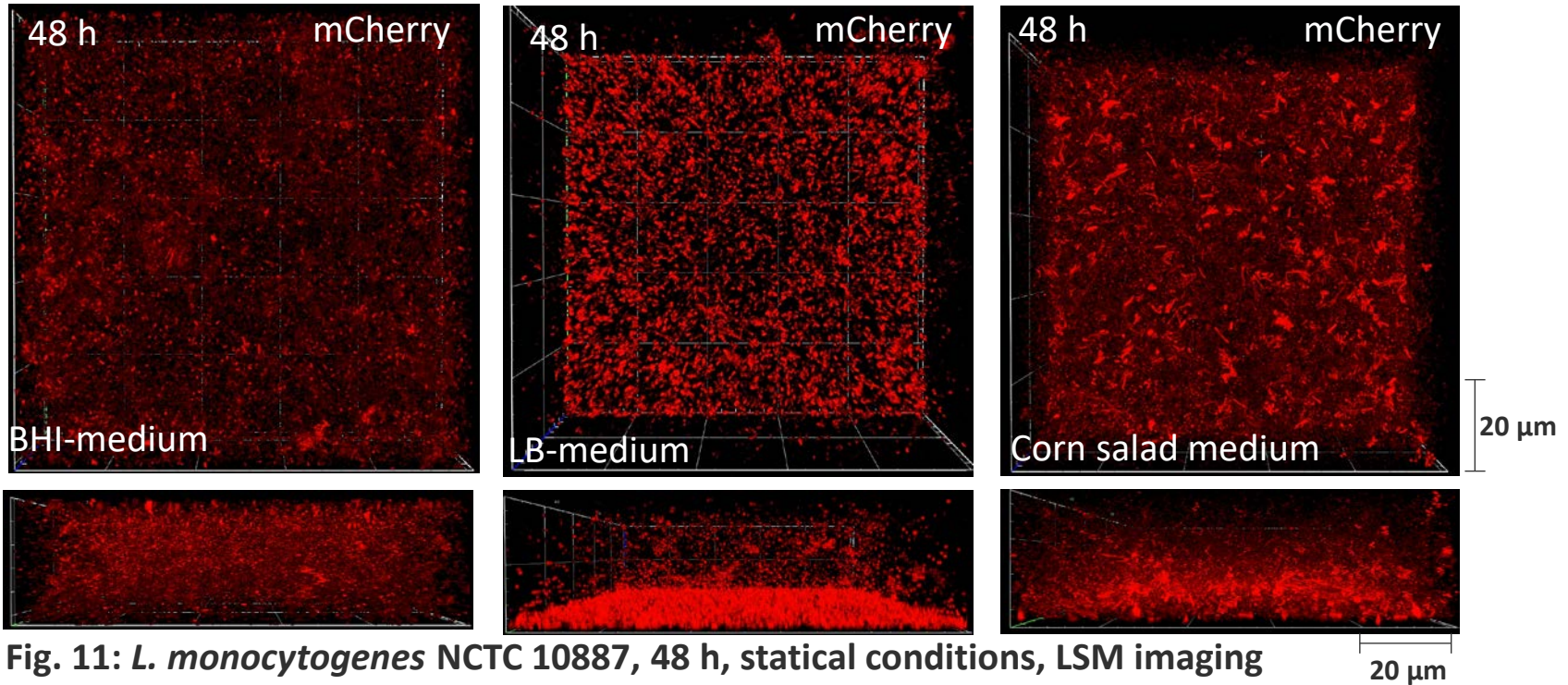


Fig. 11: *L. monocytogenes* NCTC 10887, 48 h, static conditions, LSM imaging