TABLE 1 Summary of infection assay methods

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Reference | C. *jejuni* Strains | Growth | Source | Housing | Inoculation | Incubation | Survival |
| Champion 2010 | 11168H  81-176  G1 | CAB with HB 5%-9% or Skirrow Suppl. MHB at MA1/shaking | Livefoods Direct Ltd (UK) | Wood chips, 15°C | 10 μL injection in PBS, OD590=1.0A, 106 CFU into right foreleg | 37°C, 24 hrs, assume aerobicF | Plate Grown  81-176 – 43%  11168H – 36%  G1 – ~25%  Broth Grown  11168H - 0% |
| Senior 2011 | 11168H  11168-O  81116  81-176 | MHB with CPK/shaking | Livefoods Direct Ltd (UK) | Wood chips, 15°C | 10 μL injection in PBS, OD590=1.0, 106 CFUB into right foreleg | 37°C, 24 hrs, aerobic | 11168H – 0%  11168-O – 27%  81116 – 90%  81-176 – 90% |
| Gundogdu 2011 | 11168H | CAB with HB 7% and CSS, BB at MA1/shaking | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS, OD600=0.1B, 106 CFU into right foreleg | 37°C, 24 hrs, aerobic | 11168H – ~45% |
| Elmi 2012 | 11168H | CAB + HB 7% and CSS, BB at MA1/shaking | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS, OD600=0.1B, 106 CFU into right foreleg | 37°C, 24 hrs, aerobic | 11168H – ~70%  OMVs from:  11168H – 70% |
| van Alphen 2014 | 11168H  81-176 | MHA, MHB at MA1/shaking | Bug Order Inc. (CA) | Wood chips, 4°C | 5 μL injection in MgSO4, OD550=1.0, 109 CFUB in left hindmost pro-leg | 37°C, 24 hrs, aerobic | 11168H – ~12%  81-176 – ~30% |
| Humphery 2015 | 11168H  M1  13126  12662  DBM1 | CAB with DHB (5%), MHB at 5% O2/ 12% CO2/ 3% H2/ 80% N2 | Livefoods Direct Ltd (UK) | Not specified - Ref Gundogdu 2011 | 10 μL injection in PBS, 106 CFUB into haemocoel (unspecified) | 37°C, 48 hrs, aerobic | 11168H – 67%  M1 – 88%  13126 – 63%  12662 – 95%  DBM1 – 78% |
| Gundogdu 2015 | 11168H | CAB with HB 7% and CSS, BB at MA1/shaking | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS, OD600=0.1B, 106 CFU into right foreleg | 37°C, 24 hrs, aerobic | 11168H – ~45% |
| Handley 2015 | 11168 | BA, Blood Agar Base Agar with Skirrows Suppl., BB at MA1/shaking | Livefoods Direct Ltd (UK) | Not described | 10 μL injection in PBS, OD600=0.1B, 106 CFU into right foremost pro-leg. | 37°C, 24 hrs, aerobic | 11168 – ~20% |
| Reuter 2015 | 11168 | Blood Agar Base Agar with Skirrows Suppl. at MA1 | Livefoods Direct Ltd (UK) | Not Specified | 10 μL injection in PBS, OD600=1.0, 106 CFUB into right foremost pro-leg | 37°C, 24 hrs, aerobic | 11168 – ~30% |
| Jowiya 2015 | 11168H  81-176 | MHB, BA, CAB with DHB 7% with CPK | Cornish Crispa Co. (UK) | Wood chips, 12°C | 10 μL injection in PBS, 106 CFUB into right forelegs | Unspecified temp for 24 hrsG, aerobic | 11168H – ~90%H  81-176 – ~80% |
| Gundogdu 2016 | 11168H  81-176  81116  M1 | CAB with HB 7% and CSS, BB at MA1/shaking | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS, OD600=0.1B, 106 CFU into right foreleg | 37°C, 24 hrs, aerobic | 11168H – ~45%  81-176 – ~65%  81116 – ~50%  M1 – ~50% |
| Askoura 2016 | 11168 | Biphasic MHA/MHB at 8% O2, 5% CO2, 4% H2, 83% N2 | Gecko Gurl (CA) | 10-15°C, wood chips assumed | 10 μL injection in PBS, 105-107 CFUA into left hindmost pro-leg | 25°C for 144 hrs on wood chips | 11168 106 CFU:  24 hrs – ~80%  48 hrs – ~60%  72 hrs – ~40%  120 hrs – ~20%  144 hrs – ~0% |
| Askoura 2017 | Selected strain(s) | Biphasic MHA/MHB at 8% O2, 5% CO2, 4% H2, 83% N2 | “trusted vendor” | Wood chips, 10-15°C | 10 μL injection in PBS, 105-107 CFUA into left hindmost pro-leg | Room temp, aerobic | N/A |
| Tang 2017 | 11168-GSI  111680-O | Sheep Blood Agar + Skirrows Suppl., Biphasic MHB/MHA at 7.5% CO2/ 7.5% O2/ 5% N2/ remainder not stated | Livefoods Direct Ltd (UK) | Wood chips, 15°C | 10 μL injection in PBS, OD600=0.6, 106 CFUB into right foremost pro-leg | 37°C and 42°C for 24 hrs, aerobic | 37°C:  11168-GS – 83%  11168-O – ~70%  42°C:L  11168-GS – 23%  11168-O – ~65% |
| Mehat 2018 | 81116  M1 | MHA + Sheep blood 5%, MHB with CPK | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS OD590=1.0B,106 CFU into right foremost pro-leg | 37°C for 24 hrs, aerobic | 81116 – ~10%  M1 – ~5% |
| Elmi 2018 | 11168HC | BB + 0.1 or 0.2% Na taurocholate (ST), MA1, 37°CD | Livefoods Direct Ltd (UK) | Wood chips, 16°C | 10 μL injection in PBS, 5 mg of OMVs (no bacteria injected), right foreleg | 37°C for 72 hrs, aerobic | OMVs from:  11168H – 55%  + 0.1% ST - ~35%  + 0.1% ST - ~30% |
| Brunner 2018 | 11168H | Blood agar no.2 with 0.5% yeast extract and DHB (5%) with CPK | UK Waxworms Ltd. (UK) | No specified | 10 μL injection in PBS, 107 CFUB into second foreleg | 37°C for 120 hrs, aerobic | 11168H:  24 hrs – ~90%  48 hrs – ~80%  72 hrs – ~60%  96 hrs – ~50%  120 hrs – ~35% |
| Liaw 2019 | 488  81-176 | CAB with HB (7%) and Skirrows Suppl. at MA1 | Livefoods Direct Ltd (UK) | Not Specified | 10 μL injection in PBS OD600=0.1B,noCFU approximation, into right foremost foreleg | 37°C for 120 hrs, aerobic | 488:  24 hrs – ~90%  120 hrs – ~50%  81-176:  24 hrs – ~100%  120 hrs – ~75% |
| Whelan 2019 | 11168  81-176 | MHB with CPK | Livefoods Direct Ltd (UK) | Wood chips, 15°C | 20 μL injection in PBS, OD600=1.0, 101-108 CFUA into right hindmost pro-leg | 37°C for 72 hrs, aerobic | 11168 106 CFU:  24 hrs – ~90%  48 hrs – ~90% |
| Bojanic 2020 | *Campylobacter spp.* (34 strains *- C. jejuni*) | Columbia blood agar at 10% CO2/ 3% O2/ 82% N2/ 5% H2 | Biosuppliers (NZ) | Woodchips and honeycomb, 25°C | 10 μL injection in PBS, OD590=.05, 104, 106, and 108 CFUA into left hindmost pro-leg | 37°C for 288 hrs, microaerobicJ | Aggregated C. jejuni:  24 hrs – ~95%  72 hrs – ~73%  120 hrs – ~60%  240 hrs – ~50% |
| Barnawi 2020 | 11168 | MHA, MHB at MA1 | Livefoods Direct Ltd (UK) | Not Specified | 10 μL injection in PBS, OD600 = 1.0, 106 CFUB intohindmost pro-leg | 32 - 37°C for 144 hrs, aerobic | 11168 106 CFU at 37 °C:  24 hrs – ~50%  72 hrs – ~20%  120 hrs – ~0%  11168 107 CFU at 32 °C  24 hrs – ~100%  72 hrs – ~95%  120 hrs – ~90% |
| Emery 2021 | BAA-224 | MHB or MHA with Anaerocult PK | Livefoods Direct Ltd (UK) | On SD, 28-30°C | 10 μL injection in PBS, 1 & 3x106 CFUA into left hindmost pro-leg and orally | 30°C for 72 hrs, aerobic | BAA-224 106 Force Fed:  SD – ~70%  BAA-224 106 injected:  SD – ~85% |
| Benoit 2022 | 11168  81-176 | BA with defibrinated sheep blood 10% at 10% CO2/ 4% O2/ 86% N2 | Bug Company (US) | Wood chips, 4°C | 5 μL injection in PBS, OD600 = 1.0, 2.7x107 CFUA intoleft hindmost pro-leg | 37°C for 144 hrs, aerobic | 11168:  24 hrs – ~50%  72 hrs – ~40%  120 hrs – ~20%  81-176:  24 hrs – ~60%  72 hrs – ~40%  120 hrs – ~20% |
| Myles 2024 | 11168 | Tryptic soy agar with sheep blood 5%, Tryptic soy broth, BB at MA1/shaking | Recorp Inc. (US) | Wood chips, 15°C | 10 μL injection in 0.85% saline, OD600=3.0, 5x109 CFUA, intoright foremost foreleg | 37°C for 144 hrs, aerobic | 11168:  25 hrs – ~25%  42 hrs – ~12% |
| Gomes 2024 | ATCC 33291E | Not specified | Not specified | Glass, 28°C, fed (but food not described) | 5 μL injection in PBS, approximated 5x106 CFUB, intoright hindmost pro-leg | 37°C for 192 hrs (8 days), aerobic | ATCC 33291:  48 hrs – ~100%  72 hrs – ~80%  192 hrs – ~80% |

BA, Brucella Agar. BB, Brucella Broth. BHIA, Brain Heart Infusion Agar. CAB, Columbia Agar Base. CSS, Campy Selective Supplement. DHB, Defibrinated Horse Blood. HB, Horse Blood. MHA, Mueller-Hinton Agar. MHB, Mueller-Hinton Broth. MA1 = microaerobic one (5% O2, 10% CO2, 85% N2), CPK = 5% O2, 10% CO2, approximately generated by CampyPak (Becton Dickinson). SD (Standard Diet) = corn meal (90 g), wheat flour (50 g), dried milk (50 g), beeswax (50 g), honey (50 g), glycerol (50 g), dry yeast (22 g), and distilled water (50 g).

ADoses confirmed by serial dilution and plating onto CBA (data not shown). Stated as “approximately 106 CFU” per worm.

BDoses rely on absorbance values and are not stated to be confirmed by plating.

C11168H was the strain that OMVs were derived from.

DCulture conditions under which OMVs were generated.

EAlso tested 18 *C. coli* strains, data not shown here.

FAtmospheric aerobic conditions are assumed for the worms, although in most studies that is not stated.

GWorm incubation conditions not given, but since authors refer to Champion 2010, it is likely 37°C.

HSurvival of worms injected with 11168H exposed to amylase was 15% and amylase-exposed 81-176 was 10%.

I11168-GS is the isolate that was genomically sequenced, while 11168-O is the initial clinical isolate (Gaynor, EC et al.,*J Bacteriol* 2004).

JMicroaerobic was 10% CO2/ 3% O2/ 82% N2/ 5% H2.

KMerck product literature states that the Anaerocult P system is supposed to generate environments with no oxygen and some CO2.

LThe bacteria were cultured at 42°C. After injection, the worms were incubated at 37°C.