Wolbachia

Subjects: Microbiology
Contributor: Ann Fallon

Wolbachia is an intracellular bacterium that occurs in arthropods and in filarial worms. First described nearly a century ago in the reproductive tissues of *Culex pipiens* mosquitoes, *Wolbachia* is now known to occur in roughly 50% of insect species, and has been considered the most abundant intracellular bacterium on earth. In insect hosts, *Wolbachia* modifies reproduction in ways that facilitate spread of the microbe within the host population, but otherwise is relatively benign. In this “gene drive” capacity, *Wolbachia* provides a tool for manipulating mosquito populations. In mosquitoes, *Wolbachia* causes cytoplasmic incompatibility, in which the fusion of egg and sperm nuclei is disrupted, and eggs fail to hatch, depending on the presence/absence of *Wolbachia* in the parent insects. Recent findings demonstrate that *Wolbachia* from infected insects can be transferred into mosquito species that do not host a natural infection. When transinfected into *Aedes aegypti*, an important vector of dengue and Zika viruses, *Wolbachia* causes cytoplasmic incompatibility and, in addition, decreases the mosquito’s ability to transmit viruses to humans.

Keywords: alpha-proteobacteria ; reproductive parasite ; symbiont ; mosquito ; insect cell lines ; genetic manipulation ; cell culture

1. Introduction

Wolbachia is an obligate intracellular microbe first described in reproductive tissues of *Culex pipiens* mosquitoes nearly a century ago [1][2]. Like *Escherichia coli* , *Wolbachia* is a Gram-negative bacterium in the phylum Proteobacteria: the purple bacteria and their relatives. Proteobacteria include nine monophyletic classes representing tremendous biodiversity. Among these, the genera *Ehrlichia* and *Anaplasma* , which can cause disease in humans, are classified with *Wolbachia* as members of the alpha-proteobacteria, in the order *Rickettsiales*, family *Anaplasmataceae*. *Wolbachia* is uniquely associated with invertebrates, does not infect vertebrate hosts, and replicates only within a eukaryotic host cell. In contrast, *E. coli* and many familiar Gram-negative pathogens of humans classified as gamma-proteobacteria can be cultured in liquid medium and plated on solid media as free-living microbes.

Knowledge of well-studied free-living bacteria provides an important framework for investigating the genetics and physiology of *Wolbachia* , now known to infect a high proportion of insect species, in addition to other arthropods and filarial worms, all members of the *Ecdysozoa*. Because of its widespread distribution among insects [3][4], *Wolbachia* provides a model system for exploring biological interactions between an intracellular microbe, the invertebrate host cells in which it resides, and the diversity of reproductive phenotypes with which it is associated [5][6]. In species that harbor *Wolbachia*, the bacterium is transmitted vertically, from mother to offspring, which retain the infection. In most arthropods, *Wolbachia* alters reproduction in diverse ways that favor its invasion of naive populations, and is sometimes considered a reproductive parasite. In contrast, *Wolbachia* is an essential symbiont in filarial worms [7][8][9]. In mosquitoes, *Wolbachia* causes a reproductive distortion called cytoplasmic incompatibility (CI), which has important applications in vector control [10].

2. Wolbachia in Insect Cell Lines

Wolbachia ’s obligate intracellular lifestyle complicates the biochemical and genetic analyses that could advance pest control and anti-filarial applications. Even with hosts amenable to laboratory rearing, maintenance of colonies, dissection of infected tissues, and embryonic microinjection are labor-intensive and time-consuming. Moreover, many existing laboratory colonies are highly inbred, complicating cage studies that address fitness. The utility of *Wolbachia* in control applications would be enhanced if the microbe could be experimentally manipulated by genetic engineering to express selectable markers, which in turn will be advanced by improving manipulation of *Wolbachia* in cell lines and expanding the diversity of *Wolbachia* strains that can be investigated in culture. A modest advance would be adaptation of a filarial strain of *Wolbachia* to a cell line; at present, *Wolbachia* -infected insect cell lines are used as a surrogate to identify new drugs that target *Wolbachia* for treatment of filarial diseases [11][12][13].

The author's research focuses on systematic exploration of *Wolbachia* propagation in cultured cells as a substitute for the differentiated host tissues, such as ovaries and testes, in which *Wolbachia* is most abundant. Cell lines used to propagate *Wolbachia* are listed in **Table 1** , wherein supergroup designations are noted after the strain name; for example, w Pip_B indicates that w Pip is classified in supergroup B. With the exception of a single member of supergroup F from the cat flea [14], only members of supergroups A and B, sometimes called the "pandemic" supergroups, have been maintained in insect cell lines. The reader should note that, in some cases, an infected cell line may have been sub-cultured only a limited number of times and/or has a very long doubling time, and that the same cell line may have been infected with the same strain of *Wolbachia* by different investigators, and given a different name. An important incentive for employing cell lines was the possibility that preadaption to cultured cells might improve the likelihood that *Wolbachia* would establish in novel hosts infected by embryonic microinjection, and towards this end, a few lines have been maintained for several years [15]. In other cases, which are not reviewed in detail here, infected cell lines have been used to test effects of *Wolbachia* on viral replication in efforts that generally validate the anti-pathogen responses seen in transinfected mosquitoes. Finally, as with *Wolbachia* itself, a uniform descriptive label for infected cell lines remains to be developed.

Table 1. Cell lines in which *Wolbachia* strains have been propagated.

Cell Line Designation	*Wolbachia* Strain_Supergroup	Source of *Wolbachia*	Reference	Comments
Dipteran cell lines				
***Aedes albopictus* (mosquito)**				
Aa23	*w*AlbB_B	*Aedes albopictus* embryos	[16]	First infected cell line; established from naturally infected *Ae. albopictus*; one of two *Wolbachia* strains
Aa23(T)	*w*Mel_A	infected RML-12 cells	[17]	12 passages
Aa23(T)	*w*Ri_A *w*Cof_A *w*AlbB_B *w*Pip_B *w*CauA_A *w*CauB_B	*D. simulans* eggs *D. simulans* eggs infected Aa23 cells *Cx. pipiens* eggs *Cadra cautella* eggs *Cadra cautella* eggs	[18]	Demonstration of shell vial technique; details focus on *w*Ri
Aa23(T)	*w*MelPop	w^{1118} embryos	[15]	Generated *w*MelPop-CLA
NIAS-AeAl-2	*w*Stri_B *w*Kue_A *w*CauA_A	*L. striatellus* ovary *Ephestia kuehniella* eggs *Cadra cautella* eggs	[19]	Infected from small inoculum; one ovary, or 80–100 eggs; Infected AeAl-2 cells form aggregates; occasional addition of uninfected cells to infected cultures
NIAS-AeAl-2	*w*Cau_A *w*CauB_B *w*Kue_A	*Ephestia kuehniella* eggs *Ephestia kuehniella* eggs *Ephestia kuehniella* eggs	[20]	Two stages: infection and maintenance
RML-12	*w*MelPop-CLA_A	infected Aa23 cells	[15]	*w*MelPop transferred to cells; serial passage; reintroduction into original host by microinjection; some loss of virulence; "genetic adaptation" to improve transfer to new hosts
RML-12	*w*Mel_A	O'Neill et al.; cited in [17] personal communication	[17]	Maintained for 3 years
C6/36	*w*Ri_A	*D. simulans* eggs	[18]	
C6/36	*w*Mel_A	infected RML-12 cells	[17]	Stable; higher density than RML-12 cells
C6/36	*w*AlbB_B	infected Aa23 cells	[21]	
C6/36	*w*AlbB_B	infected Aa23 cells	[22]	

Cell Line Designation	*Wolbachia* Strain_Supergroup	Source of *Wolbachia*	Reference	Comments
C6/36	*w*MelPop-CLA_A	RML-12-CLA	[23]	C6/36.*w*MelPop-CLA
C6/36	*w*AlbB_B	infected Aa23 cells	[24]	Virus screen
C7-10	*w*Stri_B	NIAS-AeAl-2	[25]	Called C/*w*Stri1 line
C7-10	*w*AlbB_B	infected Aa23 cells	[26]	Infected line: C7-10B
C7-10	*w*Ri_A	*D. simulans* eggs	[26]	Infected line: C7-10R C7-10R more stable, uniform than C7-10B
TK-6 (C7-10)	*w*Alb_B	infected Aa23 cells	[27]	Stable 5 months
Mtx-5011-256	*w*Stri_B	C/*w*Stri1 cells	[28]	Lower MOI than C7-10; aneuploidy a factor?
Aedes aegypti mosquito				
Aag2	*w*AlbB_B	infected Aa23 cells	[29]	Line called Aag2.*w*AlbB
Aag2	*w*AlbB_B	infected Aa23 cells	[30]	Line called *w*-Aag2
Aag2	*w*Mel_A	*D. melanogaster* embryos	[31][32]	Line called Aag-2*w*Mel
Aag2	*w*Mel_A *w*MelPop-CLA_A	Infected RML-12 cells Infected RML-12 cells	[33]	[15]
Aa-20	*w*MelPop-CLA_A	Not stated	[34]	Mos 20; CVCL_Z353; [35]
Anopheles gambiae mosquito				
Mos-55	*w*MelPop-CLA_A	infected Aa23 cells	[15]	
Sua5B	*w*AlbB_B *w*Ri_A	infected Aa23 cells *D. simulans* eggs	[36]	Best was $1/10^3$ cells infected
Drosophila melanogaster				
S2	*w*Ri_A	*D. simulans* eggs	[18]	
S2	strain from Dm2008Wb1cells	infected, *D. melanogaster*	[37]	(from abstract; Russian)
Dm2008Wb1	primary cell culture	infected, *D. melanogaster*	[37]	(from abstract; Russian)
JW-18	*w*Mel-Pop_A	infected, *D. melanogaster*	[13]	Albendazole sulfone inhibits
1182-48	*w*MelPop_A	infected JW-18 cells	[38]	Acentriolar haploid line
S2R+	*w*MelPop_A	infected JW-18 cells	[38]	Tetraploid male cells; higher *Wolbachia* titers
Lutzomyia longipalpis (sandfly)				
LL5	*w*MelPop-CLA_A *w*Mel_A	infected RML-12 cells infected RML-12 cells	[39]	Immune activation unstable; no effect on *Leishmania*
Lulo	*w*MelPop-CLA_A *w*Mel_A (unstable)	infected RML-12 cells infected RML-12 cells	[39]	
Culicoides sonorensis (Biting midge)				
W3	*w*AlbB_B	infected Aa23 cells	[40]	Line W3
W8	*w*AlbB_B	infected Aa23 cells	[40]	Higher density than W3

Cell Line Designation	*Wolbachia* Strain_Supergroup	Source of *Wolbachia*	Reference	Comments
Hematobia irritans **(Horn fly)**				
HIE-18	***w*AlbB_B** ***w*Mel_A** ***w*MelPop_A**	**infected Aa23 cells** **infected Aag2 cells** **infected Aag2 cells**	[41]	**50 passages**
Lepidopteran				
BCIRL-HZ-AM1-G5 ***Heliothis zea***	***w*Stri_B**	***L. striatellus* ovary**	[19]	
Sf9 ***Spodoptera frugiperda***	***w*Ri_A**	***D. simulans* eggs**	[18]	
Sf9 ***Spodoptera frugiperda***	***w*CauB_B**	***Ephestia kuehniella* eggs**	[20]	
Tick				
Ixodes scapularis	***w*AlbB_B, *w*Stri_B** ***w*Cfe_F**	**infected mosquito cells** **cat fleas**	[14]	***w*Stri_B, 29 passages** ***w*Cfe_F, 2 passages**
Ixodes ricinus	***w*AlbB_B, *w*Stri_B**	**infected mosquito cells**	[14]	
Riphicephalus microplus	***w*AlbB_B, *w*Stri_B**	**infected mosquito cells**	[14]	
Mammal				
L929 (mouse)	***w*Stri_B**	***L. striatellus* ovary**	[19]	**Cells maintained at 28 °C**
Filarial screening				
Aa23	***w*AlbB_B**		[11]	**Anti-filarial screen**
C6/36	***w*AlbB_B**	**infected Aa23 cells**	[12]	**Macrofilaricides**
JW-18	***w*MelPop_A**	***D. melanogaster w*^{1118}**	[13]	**Anti-filarial screen**

Insect cell lines in which *Wolbachia* has been maintained. Columns from left to right show: (1) cell lines, arranged in groups according to species from which the cell line was derived; (2) *Wolbachia* strain_supergroup; (3) source of the material introduced into the cell line; (4) reference; (5) brief comments.

3. Why Cultured Cells?

If the streamlined *Wolbachia* genome can be genetically engineered in the future, propagation of the altered genome will require efficient reintroduction into a host cell to allow replication and expansion of transformant populations. Use of cell lines offers a practical means of producing the large quantities of *Wolbachia* that will be needed to develop transformation protocols that are sufficiently robust for use in basic research and pest control applications. Although isolated examples of successful transformation of intracellular microorganisms such as *Coxiella burnetti*, the pathogen that causes Q fever, have been achieved, these remain labor intensive and have low frequencies of success [42]. Nevertheless, over the past two decades, remarkable progress towards cell-free culture of *Coxiella* has been achieved, despite its streamlined 2 Mb genome [43][44]. These successes underscore the importance of detailed attention to culture conditions and metabolic activities of obligate intracellular microbes. *Wolbachia* lacks pathogenicity to humans, and its genome is more extensively streamlined, relative to that of *Coxiella*. Nevertheless, the long evolutionary history of *Wolbachia's* interaction with invertebrate hosts and its adaptations for germline transmission contribute to the value of *Wolbachia* as a model system for understanding the biology of obligate intracellular bacteria in invertebrate cells and manipulating their biology for control of insect pests.

References

1. Hertig, M.; Wolbach, S.B. Studies on Rickettsia-like micro-organisms in insects. J. Med. Res. 1924, 44, 329–374.
2. Hertig, M. The rickettsia, Wolbachia pipientis (Gen. Et SP.N.) and associated inclusions of the mosquito Culex pipiens. Parasitology 1936, 28, 453–486.
3. Hilgenboecker, K.; Hammerstein, P.; Schlattmann, P.; Telschow, A.; Werren, J.H. How many species are infected with Wolbachia? —A statistical analysis of current data. FEMS Microbiol. Lett. 2008, 281, 215–220.
4. Zug, R.; Hammerstein, P. Still a host of hosts for Wolbachia: Analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE 2012, 7, e38544.
5. Werren, J.H.; Baldo, L.; Clark, M.E. Wolbachia: Master manipulators of invertebrate biology. Nat. Rev. Microbiol. 2008, 6, 741–751.
6. Landmann, F. The Wolbachia Endosymbionts. Microbiol. Spectr. 2019, 7.
7. Taylor, M.J.; Bandi, C.; Hoerauf, A. Wolbachia bacterial endosymbionts of filarial nematodes. Adv. Parasitol. 2005, 60, 245–284.
8. Fenn, K.; Conlon, C.; Jones, M.; Quail, M.A.; Holroyd, N.E.; Parkhill, J.; Blaxter, M. Phylogenetic relationships of the Wolbachia of nematodes and arthropods. PLoS Pathog. 2006, 2, e94.
9. Johnston, K.L.; Ford, L.; Umareddy, I.; Townson, S.; Specht, S.; Pfarr, K.; Hoerauf, A.; Altmeyer, R.; Taylor, M.J. Repurposing of approved drugs from the human pharmacopoeia to target Wolbachia endosymbionts of onchocerciasis and lymphatic filariasis. Int. J. Parasitol. Drugs Drug Resist. 2014, 4, 278–286.
10. Xi, Z.; Joshi, D. Genetic control of dengue and malaria using Wolbachia. In Genetic Control of Malaria and Dengue; Adelman, Z.N., Ed.; Academic Press: London, UK, 2016; Chapter 14; pp. 305–333.
11. Hermans, P.G.; Hart, C.A.; Trees, A.J. In vitro activity of antimicrobial agents against the endosymbiont Wolbachia pipientis. J. Antimicrob. Chemother. 2001, 47, 659–664.
12. Clare, R.H.; Cook, D.A.; Johnston, K.L.; Ford, L.; Ward, S.A.; Taylor, M.J. Development and validation of a high-throughput anti-Wolbachia whole-cell screen: A route to macrofilaricidal drugs against onchocerciasis and lymphatic filariasis. J. Biomol. Screen 2015, 20, 64–69.
13. Serbus, L.R.; Landmann, F.; Bray, W.M.; White, P.M.; Ruybal, J.; Lokey, R.S.; Debec, A.; Sullivan, W.A. cell-based screen reveals that the albendazole metabolite, albendazole sulfone, targets Wolbachia. PLoS Pathog. 2012, 8, e1002922.
14. Khoo, J.J.; Kurtti, T.J.; Husin, N.A.; Beliavskaia, A.; Lim, F.S.; Zulkifli, M.M.S.; Al-Khafaji, A.M.; Hartley, C.; Darby, A.C.; Hughes, G.L.; et al. Isolation and propagation of laboratory strains and a novel flea-derived field strain of Wolbachia in tick cell lines. Microorganisms 2020, 8, 988.
15. McMeniman, C.J.; Lane, A.M.; Fong, A.W.; Voronin, D.A.; Iturbe-Ormaetxe, I.; Yamada, R.; McGraw, E.A.; O'Neill, S.L. Host adaptation of a Wolbachia strain after long-term serial passage in mosquito cell lines. Appl. Environ. Microbiol. 2008, 74, 6964–6969.
16. O'Neill, S.L.; Pettigrew, M.M.; Sinkins, S.P.; Braig, H.R.; Andreadis, T.G.; Tesh, R.B. In vitro cultivation of Wolbachia pipientis in an Aedes albopictus cell line. Insect Mol. Biol. 1997, 6, 33–39.
17. Voronin, D.; Tran-Van, V.; Potier, P.; Mavingui, P. Transinfection and growth discrepancy of Drosophila Wolbachia strain wMel in cell lines of the mosquito Aedes albopictus. J. Appl. Microbiol. 2010, 108, 2133–2141.
18. Dobson, S.L.; Marsland, E.J.; Veneti, Z.; Bourtzis, K.; O'Neill, S.L. Characterization of Wolbachia host range via the in vitro establishment of infections. Appl. Environ. Microbiol. 2002, 68, 656–660.
19. Noda, H.; Miyoshi, T.; Koizumi, Y. In vitro cultivation of Wolbachia in insect and mammalian cell lines. Vitr. Cell. Dev. Biol. Anim. 2002, 38, 423–427.
20. Furukawa, S.; Tanaka, K.; Fukatsu, T.; Sasaki, T. In vitro infection of Wolbachia in insect cell lines. Appl. Entomol. Zool. 2008, 43, 519–525.
21. Raquin, V.; Valiente Moro, C.; Saucereau, Y.; Tran, F.-H.; Potier, P.; Mavingui, P. Native Wolbachia from Aedes albopictus blocks Chikungunya virus infection in cellulo. PLoS ONE 2015, 10, e0125066.
22. Fenollar, F.; La Scola, B.; Inokuma, H.; Dumler, J.S.; Taylor, M.J.; Raoult, D. Culture and phenotypic characterization of a Wolbachia pipientis isolate. J. Clin. Microbiol. 2003, 41, 5434–5441.
23. Frentiu, F.D.; Robinson, J.; Young, P.R.; McGraw, E.A.; O'Neill, S.L. Wolbachia-mediated resistance to dengue virus infection and death at the cellular level. PLoS ONE 2010, 5, e13398.

24. Ekwudu, O.; Devine, G.J.; Aaskov, J.G.; Frentiu, F.D. Wolbachia strain wAlbB blocks replication of flaviviruses and alphaviruses in mosquito cell culture. Parasites Vectors 2020, 13, 54.
25. Fallon, A.M.; Baldridge, G.D.; Higgins, L.-A.; Witthuhn, B.A. Wolbachia from the planthopper Laodelphax striatellus establishes a robust, persistent, streptomycin-resistant infection in clonal mosquito cells. Vitr. Cell. Dev. Biol. Anim. 2013, 49, 66–73.
26. Venard, C.M.; Crain, P.R.; Dobson, S.L. SYTO11 staining vs. FISH staining: A comparison of two methods to stain Wolbachia pipientis in cell cultures. Lett. Appl. Microbiol 2011, 52, 168–176.
27. Fallon, A.M.; Witthuhn, B.A. Proteasome activity in a naïve mosquito cell line infected with Wolbachia pipientis wAlbB. Vitr. Cell. Dev. Biol. Anim. 2009, 45, 460–466.
28. Fallon, A.M. Conditions facilitating infection of mosquito cell lines with Wolbachia, an obligate intracellular bacterium. Vitr. Cell. Dev. Biol. Anim. 2019, 55, 120–129.
29. Bishop, C.; Parry, R.; Asgari, S. Effect of Wolbachia wAlbB on a positive-sense RNA negev-like virus: A novel virus persistently infecting Aedes albopictus mosquitoes and cells. J. Gen. Virol. 2020, 101, 216–225.
30. Lu, P.; Bian, G.; Pan, X.; Xi, Z. Wolbachia induces density-dependent inhibition to dengue virus in mosquito cells. PLoS Negl. Trop. Dis. 2012, 6, e1754.
31. Koh, C.; Audsley, M.D.; Di Giallonardo, F.; Kerton, E.J.; Young, P.R.; Holmes, E.C.; McGraw, E.A. Sustained Wolbachia-mediated blocking of dengue virus isolates following serial passage in Aedes aegypti cell culture. Virus Evol. 2019, 8, vez012.
32. Terradas, G.; Joubert, D.A.; McGraw, E.A. The RNAi pathway plays a small part in Wolbachia-mediated blocking of dengue virus in mosquito cells. Sci. Rep. 2017, 7, 43847.
33. McLean, B.J.; Dainty, K.R.; Flores, H.A.; O'Neill, S.L. Differential suppression of persistent insect specific viruses in trans-infected wMel and wMelPop-CLA Aedes-derived mosquito lines. Virology 2019, 527, 141–145.
34. Etebari, K.; Asad, S.; Zhang, G.; Asgari, S. Identification of Aedes aegypti long intergenic non-coding RNAs and their association with Wolbachia and dengue virus infection. PLoS Negl. Trop. Dis. 2016, 10, e0005069.
35. Kurtti, T.J.; Munderloh, U.G. Mosquito cell culture. In Advanced Cell Culture; Maramorosch, K., Ed.; Academic Press: London, UK, 1984; pp. 259–302.
36. Rasgon, J.L.; Ren, X.; Petridis, M. Can Anopheles gambiae be infected with Wolbachia pipientis? Insights from an in vitro system. Appl. Environ. Microbiol. 2006, 72, 7718–7722.
37. Andrianova, B.V.; Goriacheva, I.I.; Aleksandrov, I.D.; Gorelova, T.V. Establishment of a new continuous cell line of Drosophila melanogaster strain infected by the intracellular endosymbiotic bacterium Wolbachia pipientis under natural conditions. Genetika 2010, 46, 14–17. (In Russian)
38. White, P.M.; Pietri, J.E.; Debec, A.; Russell, S.; Patel, B.; Sullivan, W. Mechanisms of horizontal cell-to-cell transfer of Wolbachia spp. in Drosophila melanogaster. Appl. Environ. Microbiol. 2017, 83, e03425-16.
39. Da Silva Gonçalves, D.; Iturbe-Ormaetxe, I.; Martins-da-Silva, A.; Tellieria, E.L.; Rocha, M.N.; Traub-Cseko, Y.M.; O'Neill, S.L.; Sant'Anna, M.R.V.; Moreira, L.A. Wolbachia introduction into Lutzomyia longipalpis (Diptera: Psychodidae) cell lines and its effects on immune-related gene expression and interaction with Leishmania infantum. Parasites Vectors 2019, 12, 33.
40. Ghosh, A.; Jasperson, D.; Cohnstaedt, L.W.; Brelsford, C.L. Transfection of Culicoides sonorensis biting midge cell lines with Wolbachia pipientis. Parasites Vectors 2019, 12, 483.
41. Madhav, M.; Brown, G.; Morgan, J.A.; Asgari, S.; McGraw, E.A.; Munderloh, U.G.; Kurtti, T.J.; James, P. Wolbachia successfully replicate in a newly established horn fly, Haematobia irritans irritans (L.) (Diptera: Muscidae) cell line. Pest Manag. Sci. 2020, 76, 2441–2452.
42. Beare, P.A.; Howe, D.; Cockrell, D.C.; Omsland, A.; Hansen, B.; Heinzen, R.A. Characterization of a Coxiella burnetii ftsZ mutant generated by Himar1 transposon mutagenesis. J. Bacteriol. 2009, 191, 1369–1381.
43. Omsland, A.; Beare, P.A.; Hill, J.; Cockrell, D.C.; Howe, D.; Hansen, B.; Samuel, J.E.; Heinzen, R.A. Isolation from animal tissue and genetic transformation of Coxiella burnetii are facilitated by an improved axenic growth medium. Appl. Environ. Microbiol. 2011, 77, 3720–3725.
44. Sanchez, S.E.; Vallejo-Esquerra, E.; Omsland, A. Use of axenic culture tools to study Coxiella burnetii. Curr. Protoc. Microbiol. 2008, 50, e52.