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Effects of mimosine on *Wolbachia* in mosquito cells: cell cycle suppression reduces bacterial abundance

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Abstract

The plant allelochemical L-mimosine (β -[*N*-(3-hydroxy-4-pyridone)]- α -aminopropionic acid; leucenol) resembles the nonessential amino acid, tyrosine. Because the obligate intracellular alphaproteobacterium, *Wolbachia pipientis*, metabolizes amino acids derived from host cells, the effects of mimosine on infected and uninfected mosquito cells were investigated. The EC₅₀ for mimosine was 6–7 μ M with *Aedes albopictus* C7-10 and C/wStr cell lines, and was not influenced by infection status. Mosquito cells responded to concentrations of mimosine substantially lower than those used to synchronize the mammalian cell cycle; at concentrations of 30–35 μ M, mimosine reversibly arrested the mosquito cell cycle at the G1/S boundary and inhibited growth of *Wolbachia* strain wStr. Although lower concentrations of mimosine slightly increased wStr abundance, concentrations that suppressed the cell cycle reduced *Wolbachia* levels.

Keywords

Mimosine; Amino acid metabolism; *Wolbachia*; *Aedes albopictus*; Cell cycle

Introduction

Wolbachia pipientis is an obligate intracellular alphaproteobacterium that occurs in arthropods and nematode worms. Its streamlined genome and reduced metabolic capabilities make it dependent on its host for nutrients essential for survival and replication (Wu *et al.* 2004; Hotopp *et al.* 2006). Insect-associated *Wolbachia* are most abundant in reproductive tissues and are maternally transmitted in infected eggs. In populations comprising infected and uninfected individuals, reproductive distortions such as parthenogenesis, feminization, or cytoplasmic incompatibility differentially enhance reproduction of infected females and microbial invasion of uninfected populations. Understanding the molecular mechanisms by which *Wolbachia* induces these reproductive distortions has important applications for pest control (Sinkins and Gould 2006).

Strains of *Wolbachia* such as wAlbB from *Aedes albopictus* mosquitoes (O'Neill *et al.* 1997; Fallon 2008) and wStr from the planthopper, *Laodelphax striatellus* (Noda *et al.* 2002; Fallon *et al.* 2013a), replicate in insect cell lines. In particular, the C/wStr1 *A. albopictus* mosquito cell line maintains a robust and persistent infection with wStr (Fallon *et al.* 2013a)

that provides an in vitro system for exploring metabolic interdependencies that would be difficult to investigate in infected reproductive tissues. Our goal is to develop mosquito cell lines to maximize in vitro production of *Wolbachia* and to serve as recipients optimized for selection of transformed bacteria. Here, C/wStr1 cells were used to investigate effects of L-mimosine (β -[*N*-(3-hydroxy-4-pyridone)]- α -aminopropionic acid; leucenol), a plant allelochemical that resembles the nonessential amino acid, tyrosine, which *Wolbachia* obtains from host cells (Hotopp *et al.* 2006).

Materials and Methods

Cells and culture conditions

A. albopictus wild-type C7-10 and *Wolbachia*-infected C/wStr1 mosquito cells were maintained at 28°C in a 5% CO₂ atmosphere in modified Eagle's medium containing 5% heat-inactivated fetal bovine serum as detailed previously (Shih *et al.* 1998; Fallon *et al.* 2013a). Cells were counted after resuspension in phosphate-buffered saline (Dulbecco and Vogt 1954) using a Coulter electronic cell counter. C/wStr1 cells were at passages 50 to 111 during the course of these experiments.

Mimosine

Mimosine (Sigma M-0253, St. Louis, MO; 50 mM) was prepared in 0.5 M sodium bicarbonate, sterilized by filtration, and diluted to 1.0 mM for addition to culture medium at maximum concentrations of 50 μ M. MTT assays were based on conversion of methylthiazole tetrazolium to a colored product as described previously (Fallon and Hellestad 2008).

Flow cytometry

Flow cytometry was done on a Coulter EPIC S XL-MCL flow cytometer with propidium iodide staining under conditions that allowed simultaneous detection of *Wolbachia* and host cell nuclei described previously (Fallon 2014). Briefly, cells were resuspended from 35-mm plates using 20 strokes with a Pasteur pipet. The resuspension (0.3 ml) was diluted by addition of 0.3 ml of 20 mM Tris-HCl, pH 7.5, 0.4% Triton X-100, 0.2 mg/ml propidium iodide, and 0.1 mg/ml boiled RNase A. For cell cycle analyses of uninfected C7-10 cells, voltage settings were as follows: forward scatter, 193 V, gain=1.0; side scatter, 400 V, gain=1.0; FL3, 675 V, gain=1.0; AUX (FL3 peak height) 500 V; gain= 1.0. The discriminator was set to AUX=30, and 15,000 events were recorded at a flow rate of 60 μ l/min. For simultaneous analysis of *Wolbachia* peaks (WP) and cell peaks (CP), voltage settings were as follows: forward scatter, 193 V, gain=1.0; side scatter, 800 V; gain=5.0; FL3, 773 V, gain=1.0; AUX (FL3 peak height), 155 V, gain=1.0. The discriminator setting was reduced to AUX=3, the flow rate was 60 μ l/min, and total events were collected over a fixed time of 1 min. The WP underestimates *Wolbachia* abundance by approximately 200-fold (Fallon 2014). Raw (uncorrected) events were used to calculate ratios of WP/CP (events in the *Wolbachia* peak divided by events in the cell peak) in Fig. 5.

Experimental design and reproducibility

All results are based on a minimum of three biological replicates using cells at different passage levels; figures show representative experiments. Within experiments, samples were assayed in duplicate, and conclusions are based on a series of concentrations and/or time points as shown. Agreement between duplicate samples was typically less than 5%. In Fig. 5, which showed the most variability, bars indicate the range of values. Quantitative details specific to each experiment are provided in the legends.

Results

Effects of mimosine on mosquito cell growth

Effects of mimosine on growth of uninfected control *A. albopictus* mosquito C7-10 cells and a *Wolbachia*-infected sister line, C/wStr1 were similar. When cells were plated at low density and allowed to grow to confluency, approximately 6 μ M mimosine inhibited C7-10 cell growth by 50% (EC₅₀) relative to untreated controls, with close agreement between numerical cell counts and MTT assays as a measure of metabolic activity (Fig. 1A). Likewise, the EC₅₀ for mimosine was approximately 7 μ M in C/wStr1 cells harboring a persistent *Wolbachia* infection, regardless of the presence of antibiotics that suppress *Wolbachia* growth (Fig. 1B). To compensate for higher cell densities attained with antibiotic suppression of *Wolbachia* (Fallon *et al.* 2013a, b), cell counts were normalized by defining values for mimosine-free controls as 100% for each population. Visual inspection by microscopy indicated that the abundance of fluorescently stained *Wolbachia* appeared unaffected by mimosine (not shown), and metabolic activity assessed by MTT values of both control and mimosine-treated C/wStr1 cells reached maximal levels at about 5 d after plating (Fig. 2). The EC₅₀ value indicates that mimosine affects mosquito cell growth at concentrations well below the 0.2- to 0.4-mM concentrations used with mammalian cells for cell cycle arrest (Hughes and Cook 1996; Park *et al.* 2012).

Cell cycle arrest

MTT values between days 3 and 5 after treatment of C/wStr1 cells with 20 μ M mimosine were unchanged, relative to modest increases at 5 and 10 μ M (Fig. 2), consistent with an arrest of the cell cycle at higher concentrations. Flow cytometry was used to estimate percentages of the cell population in G1, S, and G2 as a function of mimosine concentration (Fig. 3A). After 48 h in 30 μ M mimosine, the increased percentage of cells in S (closed circles) was compensated by an approximately equivalent decrease in G1 (open circles). These transitions were accompanied by a modest increase in G2 (shaded squares) at 20 μ M mimosine. In aggregate, these data suggest that the arrest is close to the G1/S boundary. The percentage of cells in S changed little from 48 to 72 h (Fig. 3B), but the modest decrease in the proportion of cells in G1 and increase in G2 suggested that cells already in S at the time of treatment may complete the cycle in progress and/or that mimosine may be metabolized or degraded during extended culture. When cells were washed and refed with fresh medium after 72 h in 35 μ M mimosine, flow cytometry histograms showed that accumulation of cells at the G1/S boundary was followed by progression through S by 5 h after mimosine was removed (Fig. 4). By 48 h after refeeding, the proportion of cells in S dropped to 13% (data not shown).

Effects on *Wolbachia* levels

Flow cytometry conditions have been developed for examining cell number and *Wolbachia* abundance in the same sample by electronically shifting the position of propidium iodide-stained cell nuclei to the extreme right of a histogram showing events as a function of fluorescence on a log scale (inset, Fig. 5; Fallon 2014). As detailed previously, when plated at low density, cell numbers and *Wolbachia* abundance reach maximum levels as cells reach confluency, typically 7–10 d after plating. In Fig. 5, the ratio of events in the *Wolbachia* peak (WP) divided by events in the cell peak (CP) is shown as a function of days after plating and mimosine concentration, without correction for an approximately 200-fold underestimation of *Wolbachia* numbers (Fallon 2014). For control cells without mimosine (open bars), maximum WP/CP occurred on day 7 and was about 20% lower than levels with 5 μ M mimosine. With 10 μ M mimosine, the maximum attained on day 8 was again slightly higher than the control on day 7. These modest differences suggest that a decrease in the growth rate of the host cell delays the time at which maximal *Wolbachia* levels are reached and may marginally increase microbial abundance. However, the effects are small and would not be useful experimentally for improving *Wolbachia* yields on a large scale. Note that higher mimosine concentrations (20 and 35 μ M) approaching those that induce cell cycle arrest proportionately decrease the increases in WP/CP ratios with time and suggest that *Wolbachia* do not increase when host cells are prevented from cycling.

Discussion

In vitro approaches for investigating host-microbe interactions in *Wolbachia*-infected mosquito cells will contribute to eventual genetic manipulation of *Wolbachia* for pest control. Ideally, it is desirable to define culture conditions that will enhance recovery of cells infected with genetically modified *Wolbachia*. Such a recovery system would offset the low frequency with which intracellular microbes can be transformed (Beare *et al.* 2011). The ω Str infection in C/ ω Str1 cells is remarkably benign, despite an approximately twofold reduction in maximal cell densities relative to populations in which *Wolbachia* is suppressed by antibiotic treatment. The infection has persisted for more than 120 subcultures without supplementation with uninfected cells, and infected cells starved experimentally for serum for up to 10 d, or isolated clones that occasionally persist in flasks containing spent cultures invariably retain (rather than lose) the infection (Fallon *et al.* 2013a). Although isolated clones that occur in spent medium may scavenge nutrients from dead and dying cells, the present results suggest that measurable increases in *Wolbachia* abundance per cell may require cycling, and in this context, it will be of interest to examine long-term effects of serum starvation.

A criterion for evaluating potential compounds that might differentially improve recovery of infected cells has emerged from the limited studies available thus far. For example, based on increased expression of host antioxidant proteins in *Wolbachia*-infected cells (Brennan *et al.* 2008), treatment of cells with the oxidizing agent, paraquat, was tested for selective potential. Likewise, genomic indications that *Wolbachia* retains pathways for riboflavin biosynthesis suggested that infection might supplement host requirements for this essential nutrient (Hosokawa *et al.* 2010). However, both paraquat (Fallon *et al.* 2013b) and lumiflavin

(Fallon *et al.* 2014) are more toxic to *w*Str than to the host cells, precluding their development as useful selective agents. These agents, and presumably others that are preferentially toxic to *Wolbachia*, relative to the host cell, result in cell densities that exceed those of untreated infected cells and approach those achieved in the presence of rifampicin and tetracycline.

In the context of our long-term goals, the amino acid analog mimosine was an attractive test candidate because *Wolbachia* obtains amino acids from host cells. Aside from potential effects on tyrosine pools, mimosine interferes with folate metabolism by inhibiting serine hydroxymethyltransferase (Oppenheim *et al.* 2000), a *Wolbachia* enzyme with relatively high expression in C/*w*Str1 cells (Baldrige *et al.* 2014). Mimosine has also been shown to inhibit prolyl hydroxylases and oxygen homeostasis (Appelhoff *et al.* 2004; Smith and Talbot 2010). Although the importance of proline in *Wolbachia* physiology remains unknown, *w*Str retains a pathway for proline biosynthesis, which may supplement its availability from host cells (Baldrige *et al.* 2014). Mimosine has also been associated with DNA strand breakage (Mladenov and Anachkova 2003) and apoptosis (Hallak *et al.* 2008), scavenging of peroxynitrates (Choi *et al.* 2002), iron chelation (Kulp and Vulliet 1996; Szuts and Krude 2004), and inhibition of zinc-finger protein binding activities (Perry *et al.* 2005). Finally, mimosine has differential effects in neoplastic cells (Chang *et al.* 1999) and has been used for cell cycle synchronization (Hughes and Cook 1996; Park *et al.* 2012).

Here, it is shown that *A. albopictus* mosquito cells are sensitive to micromolar concentrations of mimosine, that sensitivity is not affected by infection with *Wolbachia*, and that mimosine treatment causes a reversible G1/S arrest in the mosquito cell cycle at concentrations substantially lower than those used with vertebrate cells. Although this investigation did not attempt to distinguish rigorously between effects in late G1 vs. early S, the results are consistent with studies with mammalian cells, in which most investigators suggest that mimosine arrests the cell cycle late in G1 (Dong and Zhang 2003, Perry *et al.* 2005), while in permeabilized HeLa cells, mimosine arrests the cell cycle early in S (Hughes and Cook 1996). Concentrations of mimosine that are effective with mosquito cells are about 10-fold lower than those used with mammalian cells and are also approximately 10-fold lower than concentrations of hydroxyurea used for synchronization of mosquito cells (Gerenday *et al.* 1997). Although mimosine has been little used with insect cells, we note that Thumbi *et al.* (2007) reported inhibition of *Autographa californica* nucleopolyhedrosis virus replication in *Spodoptera frugiperda* Sf21 (Lepidoptera) cells, in which a 50% cell toxicity was defined at 0.8 μ M after a 24-h treatment. Although mosquito cells are substantially more sensitive to mimosine, concentrations below the EC₅₀ for host cells have minimal effects on *Wolbachia*.

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References

- Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, Gleadle JM. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Biol Chem*. 2004; 279:38458–38465. [PubMed: 15247232]
- Baldrige GD, Baldrige AS, Higgins LA, Witthuhn BA, Markowski TW, Fallon AM. Proteomic profiling of a robust *Wolbachia* infection in an *Aedes albopictus* mosquito cell line. *Mol Microbiol*. 2014; 94:537–556. [PubMed: 25155417]
- Beare PA, Sandoz KM, Omsland A, Rockey DD, Henzein RA. Advances in genetic manipulation of obligate intracellular bacterial pathogens. *Front Microbiol*. 2011; 2:1–13. [PubMed: 21716958]
- Brennan LJ, Keddie BA, Braig HR, Harris HL. The endosymbiont *Wolbachia pipientis* induces the expression of host antioxidant proteins in an *Aedes albopictus* cell line. *PLoS One*. 2008; 3(5):e2083. doi:10.1371/journal.pone.0002083. [PubMed: 18461124]
- Chang HC, Lee TH, Chuang LY, Yen MH, Hung WC. Inhibitory effect of mimosine on proliferation of human lung cancer cells is mediated by multiple mechanisms. *Cancer Lett*. 1999; 145:1–8. [PubMed: 10530763]
- Choi JJ, Oh YK, Kim HS, Kim HC, Ko KH, Kim WK. Mimosine prevents the death of glucose-deprived immunostimulated astrocytes by scavenging peroxynitrite. *GLIA*. 2002; 39(1):37–46. [PubMed: 12112374]
- Dong Z, Zhang JT. EIF3 p170, a mediator of mimosine effects on protein synthesis and cell cycle progression. *Mol Biol Cell*. 2003; 14:3942–3951. [PubMed: 12972576]
- Dulbecco R, Vogt M. Plaque formation and isolation of pure lines with poliomyelitis virus. *J Exp Med*. 1954; 99:167–182. [PubMed: 13130792]
- Fallon AM. Cytological properties of an *Aedes albopictus* mosquito cell line infected with *Wolbachia* strain wAlbB. *In Vitro Cell Dev Biol-Anim*. 2008; 44:154–161. [PubMed: 18401667]
- Fallon AM. Flow cytometric evaluation of the intracellular bacterium, *Wolbachia pipientis*, in mosquito cells. *J Microbiol Methods*. 2014; 107:119–125. [PubMed: 25300665]
- Fallon AM, Baldrige GD, Carroll EM, Kurtz CM. Depletion of host cell riboflavin reduces *Wolbachia* levels in cultured mosquito cells. *In Vitro Cell Dev Biol-Anim*. 2014; 50:707–713. [PubMed: 24789726]
- Fallon AM, Baldrige GD, Higgins LA, Witthuhn BA. *Wolbachia* from the planthopper *Laodelphax striatellus* establishes a robust, persistent, streptomycin-resistant infection in clonal mosquito cells. *In vitro Cell Dev Biol-Animal*. 2013a; 49:66–73.
- Fallon AM, Kurtz CM, Carroll EM. The oxidizing agent, paraquat, is more toxic to *Wolbachia* than to mosquito host cells. *In vitro Cell Dev Biol-Anim*. 2013b; 49:501–507. [PubMed: 23719839]
- Fallon AM, Hellestad VJ. Standardization of a colorimetric method to quantify growth and metabolic activity of *Wolbachia*-infected mosquito cells. *In Vitro Cell Dev Biol-Anim*. 2008; 44:351–356. [PubMed: 18622662]
- Gerenday A, Blauwkamp TS, Fallon AM. Synchronization of *Aedes albopictus* mosquito cells using hydroxyurea. *Insect Mol Biol*. 1997; 6:191–196. [PubMed: 9099583]
- Hallak M, Vazana L, Shpilberg O, Levy I, Mazar J, Nathan I. A molecular mechanism for mimosine-induced apoptosis involving oxidative stress and mitochondrial activation. *Apoptosis*. 2008; 13:147–155. [PubMed: 18058236]
- Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci USA*. 2010; 107:769–774.
- Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen JA, et al. Comparative genomics of emerging human ehrlichiosis agents. *PLoS Genet*. 2006; 2(2):e21. doi:10.1371/journal.pgen.0020021. [PubMed: 16482227]
- Hughes TA, Cook PR. Mimosine arrests the cell cycle after cells enter S-phase. *Exp Cell Res*. 1996; 222:275–280. [PubMed: 8598214]
- Kulp KS, Vulliet PR. Mimosine blocks cell cycle progression by chelating iron in asynchronous human breast cancer cells. *Toxicol Appl Pharmacol*. 1996; 139:356–364. [PubMed: 8806853]

- Mladenov E, Anachkova B. DNA breaks induction by mimosine. Zeitschrift fur Naturforschung. Section C. J Biosci. 2003; 58:732–735.
- Noda H, Miyoshi T, Koizumi Y. In vitro cultivation of *Wolbachia* in insect and mammalian cell lines. In Vitro Cell Dev Biol-Anim. 2002; 38:423–427. [PubMed: 12534342]
- O'Neill SL, Pettigrew MM, Sinkins SP, Braig HR, Andreadis TG, Tesh RB. In vitro cultivation of *Wolbachia pipientis* in an *Aedes albopictus* cell line. Insect Mol Biol. 1997; 6:33–39. [PubMed: 9013253]
- Oppenheim EW, Nasrallah IM, Mastro MG, Stover PJ. Mimosine is a cell-specific antagonist of folate metabolism. J Biol Chem. 2000; 275:19268–19274. [PubMed: 10766749]
- Park SY, Im JS, Park SR, Kim SE, Wang HJ, Lee JK. Mimosine arrests the cell cycle prior to the onset of DNA replication by preventing the binding of human Ctf4/And-1 to chromatin via Hif-1 α activation in HeLa cells. Cell Cycle. 2012; 11:761–766. [PubMed: 22374673]
- Perry C, Sastry R, Nasrallah IM, Stover PJ. Mimosine attenuates serine hydroxymethyltransferase transcription by chelating zinc. Implications for inhibition of DNA replication. J Biol Chem. 2005; 281:396–400. [PubMed: 15531579]
- Shih KM, Gerenday A, Fallon AM. Culture of mosquito cells in Eagle's medium. In Vitro Cell Dev Biol-Anim. 1998; 34:629–630. [PubMed: 9769146]
- Sinkins SP, Gould F. Gene drive systems for insect disease vectors. Nat Rev Genet. 2006; 7:427–435. [PubMed: 16682981]
- Smith TG, Talbot NP. Prolyl hydroxylases and therapeutics. Antioxid Redox Signal. 2010; 12:431–433. doi:10.1089/ars.2009.2901. [PubMed: 19761407]
- Szuts D, Krude T. Cell cycle arrest at the initiation step of human chromosomal DNA replication causes DNA damage. J Cell Sci. 2004; 117:4897–4908. [PubMed: 15456844]
- Thumbi DK, Arif B, Krell PJ. Susceptibility of *Autographa californica multiple nucleopolyhedrosis* to inhibitors of DNA replication. J Virol Methods. 2007; 146:209–217. [PubMed: 17706798]
- Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, et al. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. PLoS Biol. 2004; 2:E69. [PubMed: 15024419]

Figure 1.

Effect of mimosine on growth and metabolic activity of mosquito cells. (A) Uninfected C7-10 cells ($1 \times 10^5/\text{ml}$) were plated in the indicated concentrations of mimosine. After 5 d, cells were assayed by MTT (closed circles) or counted with a Coulter electronic cell counter (open symbols). Values are expressed as percentage of controls without mimosine. Maximum values were 3.2 OD_{570} for the MTT assay and 14.5×10^5 cells/ml for Coulter counts. For Coulter counts, the background of 1×10^5 cells/ml (the number of cells initially plated) was subtracted prior to calculation. (B) C/wStr1 cells (passage 63) were plated at 1×10^5 cells/ml in the presence (open squares) or absence (closed squares) of rifampicin and tetracycline, which suppress growth of *Wolbachia* and enhance growth of host cells. On day 2, when cells had reached a density of 1.4×10^5 cells/ml, mimosine was added at the indicated concentrations. On day 6, cells were counted with a Coulter electronic cell counter, and values are expressed as percentage of control plates without mimosine. Control counts in the presence of antibiotics were 15.2×10^5 cells/ml; in the absence of antibiotics, $8.3 \times 10^5/\text{ml}$. Microscopic examination confirmed suppression of *Wolbachia* in antibiotic-treated cells. Results were confirmed with MTT assays (not shown).

Figure 2.

C/wStr1 cells (passage 51) were diluted to 1×10^5 cells/ml, and 2 ml was plated in 35-mm culture dishes. After 2 d (cell density 3.4×10^5 cells/plate), mimosine (*dashed lines*) was added to final concentrations of 5 μ M (*gray triangles*), 10 μ M (*gray diamonds*), and 20 μ M (*gray circles*). Control plates included rifampicin and tetracycline in the absence of mimosine (*open squares*) and C/wStr1 cells without antibiotics or mimosine (*solid squares*). After 3, 5, 6, and 8 d, duplicate plates were assayed by MTT.

Figure 3.

C7-10 cells (1.1×10^6 cells per 35-mm plate) were treated with indicated concentrations of mimosine, and cell cycle phases were analyzed by flow cytometry at 24, 48, and 72 h. Panel (A) shows the 48-h data set as a function of mimosine concentration. Panel (B) shows the untreated and 30-μM mimosine samples at 24, 48, and 72 h. *Open symbols/bars, G1; closed symbols/bars, S; gray symbols/bars, G2.*

Figure 4.

Effect of mimosine removal on cell cycle progression. Flow cytometry histograms show untreated C7-10 cells (*left*), C7-10 cells treated with 35 μ M mimosine for 72 h and assayed immediately (*middle*), or rinsed with phosphate-buffered saline and assayed after 5 h in fresh E-5 medium (*right*).

Figure 5.

Effects of mimosine on *Wolbachia* replication. *C/wStr1* cells (passage 111) were seeded in 35-mm plates at a concentration of 1.5×10^5 cells/ml (2 ml/plate). On day 3, mimosine was added at concentrations of 0, 5, 10, 20, and 35 μM , indicated by increasing levels of shading in the columns shown in Fig. 5. For plates without mimosine, day 4 CP values were $11,758 \pm 351$; WP values were $11,466 \pm 3178$, and the WP/CP ratio was 0.97. Values for days 5 to 10 are shown. Bars indicate the range of WP/CP ratios between duplicate plates.