Growing Asaia

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Introduction

This document provides you with all the protocols you need to grow Asaia, namely how to prepare medium for Asaia and the optimum temperature and pH for growing Asaia. We describe in more detail we will describe our results on using antibiotics because we found Asaia to be naturally resistant against some commonly used antibiotics.

Medium

Asaia does not grow in LB medium, like E. coli, but in GLY medium. Here you can find recipe for GLY medium for liquid culture and GLY Agar medium to make plate.

For 1 litre of Glycerol medium

- *25g of Glycerol*
- *10 g of Yeast extract*
- *1L distilled H_2O*

1. *Mix all components together*
2. *Adjust the pH to 5*
3. *Sterilize by autoclaving*

For 1 litre of Glycerol Agar medium

- *25g of Glycerol*
- *10 g of Yeast extract*
- *20g of Agar*
- *1L distilled H_2O*
- *1 Magnetic stirrer*

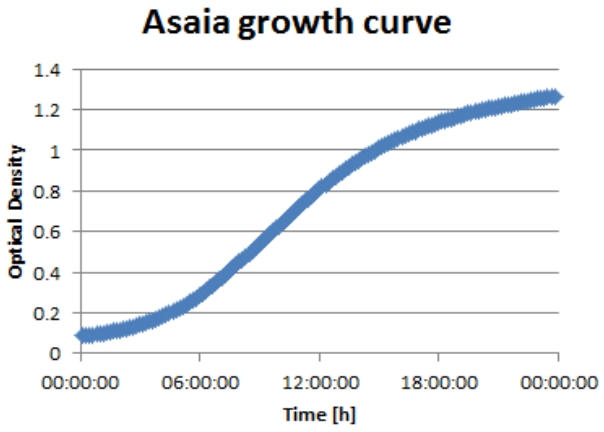
1. *Mix all components together*
2. *Adjust the pH to 5*
3. *Add the Agar*
4. *Add the stirrer*
5. *Sterilize by autoclaving*

Temperature

To make Asaia grow as fast as possible, you need to set-up the incubation temperature at 30°C. Note that when you put Asaia in the fridge, they will grow more quickly than E-coli at 4°C.

Growth curves

The doubling time of Asaia is about 2-3 hours. Here you can see the grow curve of Asaia.



pH

The right pH for Asaia is

pH = 5

To adjust the pH of all your medium, use HCl to decrease the pH and NaOH to increase.

ANTIBIOTICS

Asaia is naturally resistant to many antibiotics. For example, You cannot use Chloramphenicol or Ampicillin.

In the table bellow you will find the antibiotics we have tested.

Abbreviation	Full name
Amp	Ampicillin
Kan	Kanamycin
Gm	Gentamycin
St	Streptomycin
Ery	Erythromycin
Ch	Chloramphenicol
Rif	Rifampicin
Tet	Tetracyclin

First, we did some tests with filter paper. We grew Asaia on GLY agar plates with filters soaked in antibiotics at different concentrations. Figure 2 is an example of a plate. The next table shows you the diameter of ring around the lens. The diameter of the lens is 1cm. If the antibiotic has no effect, the diameter is set to 0. The concentration is in ug/ml and the diameter in cm.

Conc.	Amp	Kan	Gm	St	Sp	Ery	Ch	Rif	Tet
5000	0.0	2.1	2.3	4.2	2.6	0.0	0.0	1.8	4.4
2000	0.0	1.4	1.7	3.5	1.6	0.0	0.0	0.0	3.3
1000	0.0	0.0	1.2	3.2	0.0	0.0	0.0	0.0	2.9
500	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0

Then we did experiment in liquid culture. In the following table you will find the doubling time in function of the concentration of the antibiotic. If Asaia didn't grow, it is mark with a "-". The concentration is in ug/ml and the doubling time in hour.

Conc.	K-	Kan	Gm	St	Ery	Ch	Rif	Tet
500.0	5.42	-	-	-	-	-	-	-
250.0	2.68	-	-	-	-	-	-	-
125.0	2.48	-	-	-	3.37	-	-	-
62.5	2.76	-	-	-	2.59	-	-	-
31.3	2.43	-	-	-	2.9	-	-	-
15.6	2.38	5.77	-	-	3	2.7	3.7	-
7.8	2.47	3.01	3.09	-	2.93	2.42	2.57	-
3.9	2.52	2.88	2.13	-	2.61	2.34	2.27	-

You can found in figure 1 same values plot on a graphic. When Asaia didn't' grow, the doubling time is set at 10h.

For your experiment you can use our biobricks, Asaia's origin + KanR or Asaia's origin + TetR and just add your own parts to that. For more information, see the "Cloning in Asaia" sheet.

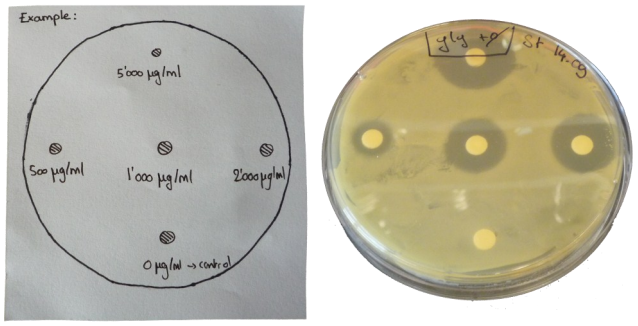


Figure 2. Filter paper experiment on Streptomycin

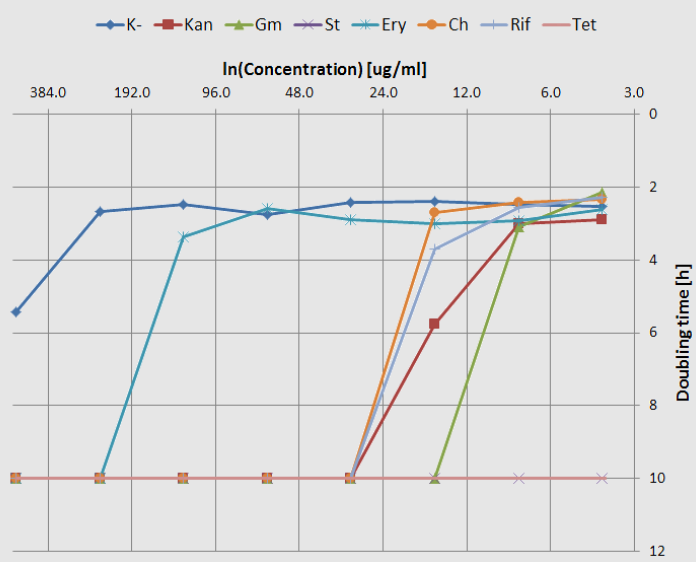
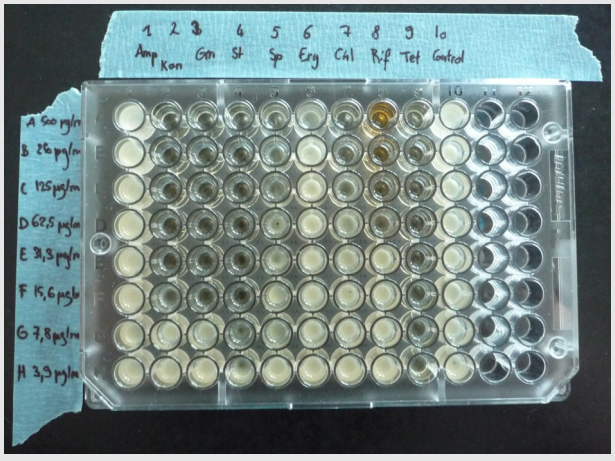


Figure 1

This figure is the result of the grow experiment, you have different concentration in vertical and, horizontally, different antibiotics. Green holes are filled with grown up Asaia.



CULTURES

First liquid culture. To do a liquid culture you have to fill a 15ml aeration tube with 5ml of GLY medium with or without antibiotic, then just either add 50ul of an other liquid culture or throw the tip you use to pick up a colony from a plate.

For plate culture you need to do GLY plate with or without antibiotics. Put some beads and add 100µl of liquid culture. Then shack the plate. You can do 5 plates at once.

When your culture are ready, put them at 30°C. The incubation time depend of culture type and antibiotics. The table shows you all incubation time you need.

	With antibiotics	without antibiotics
Liquid culture	3 days	2 days
Plate	4 days (1st colonies at 3 days)	3 days

Figure 3. Asaia plate culture after 3 days in the incubator at 30°C

After growing, if you put your colonies at 4°C Asaia will became pink after 4 days.

Figure 4. Asaia after 4 days at 4°C

REFERENCES

Guido et al, *Bacteria of the genus Asaia stably associate with Anopheles stephensi, an Asian malarial mosquito vector* (2007)