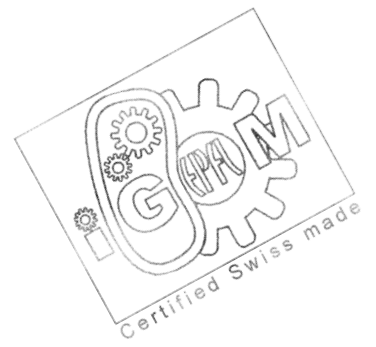


Growing Asaia



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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 optimal tem-

perature and pH for growing Asaia. We describe in more detail 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 the recipe for GLY medium for liquid culture and GLY Agar medium to make plates.

For 1 L of Glycerol medium

- ◆ 25 g of Glycerol
 - ◆ 10 g of Yeast extract
 - ◆ 1 L distilled H₂O
1. Mix all components together
 2. Adjust the pH to 5
 3. Sterilize by autoclaving

For 1 L of Glycerol Agar medium

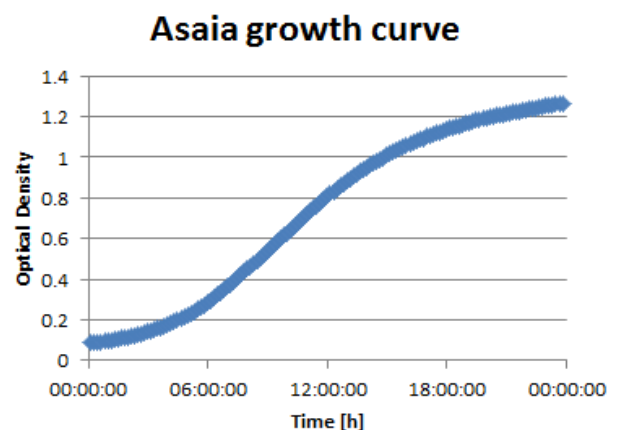
- ◆ 25 g of Glycerol
 - ◆ 10 g of Yeast extract
 - ◆ 20 g of Agar
 - ◆ 1 L distilled H₂O
 - ◆ 1 Magnetic stirrer
1. Mix all components together
 2. Adjust the pH to 5
 3. Add the Agar
 4. Add the stirrer
 5. Sterilize by autoclaving

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 growth curve of Asaia.



PH

The right pH for Asaia is

$$pH = 5$$

To adjust the pH of 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 below 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 1 cm. 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 experiments in liquid culture. In the following table you will find the doubling time in function of the antibiotic concentration. If Asaia didn't grow, it is marked

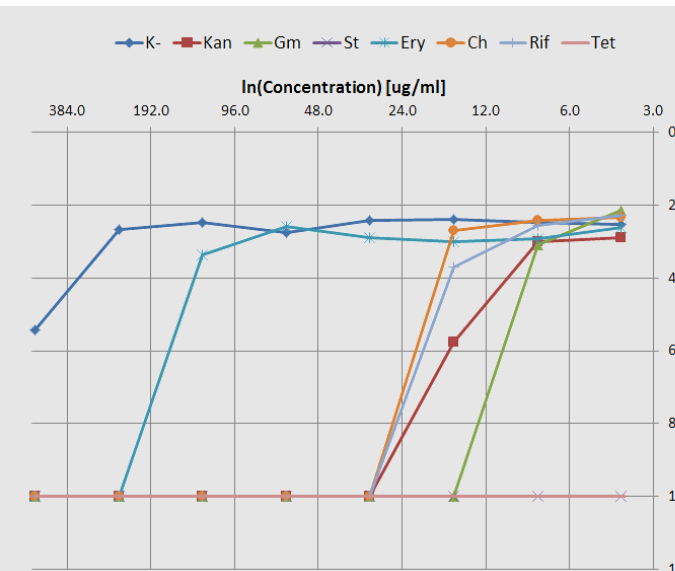


Figure 1

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	-

We plotted the same values in Figure 1. Please note that when Asaia didn't grow, the doubling time is set to 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.

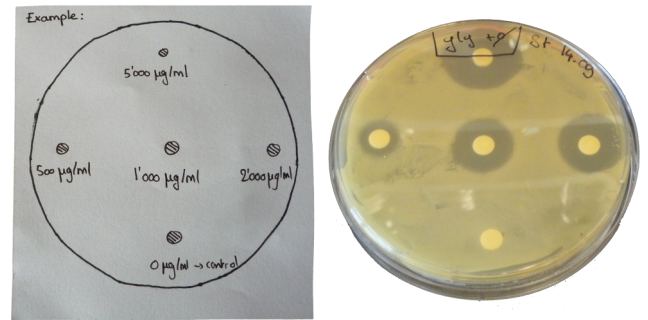
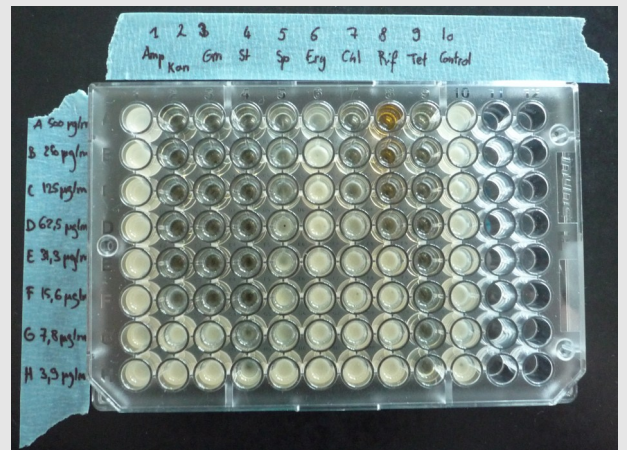


Figure 2. Filter paper experiment with Streptomycin

This figure is the result of the growth experiment, you have different concentrations in rows and different antibiotics in columns. Green holes are filled with grown up Asaia.



CULTURES

Liquid culture: To set up a liquid culture, you have to fill a 15 ml aeration tube with 5 ml of GLY medium with or without antibiotic, then just either add 50 μ l of another liquid culture or throw a tip into the tube which contains a bacterial pellet picked up from a plate.

Plate growth: generate GLY plates with or without antibiotics. Put some beads and add 100 μ l of liquid culture. Then shake the plate. You can inoculate 5 plates at once.

After inoculation, put them at 30°C. The incubation time depends on culture type and antibiotics (see Table below).

Putting the plates at 4°C renders Asaia pink after 4 days.

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



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



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)