Resultados e Discussão das Aulas Práticas

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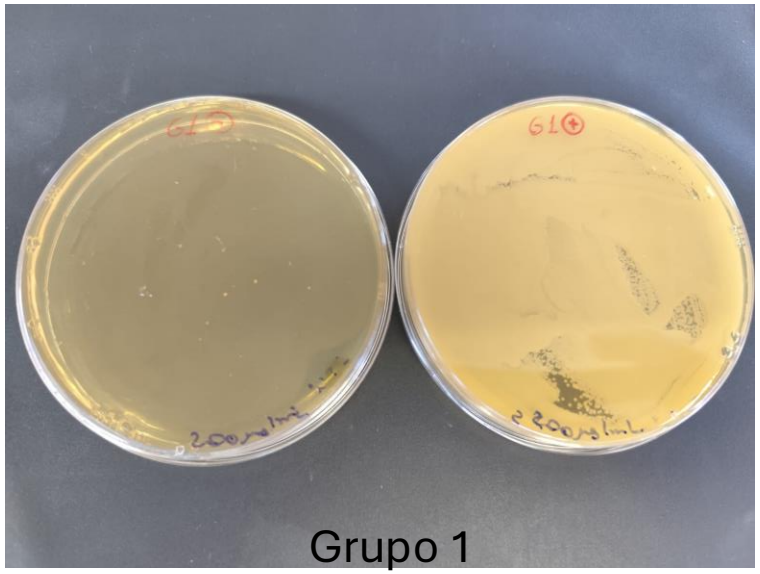
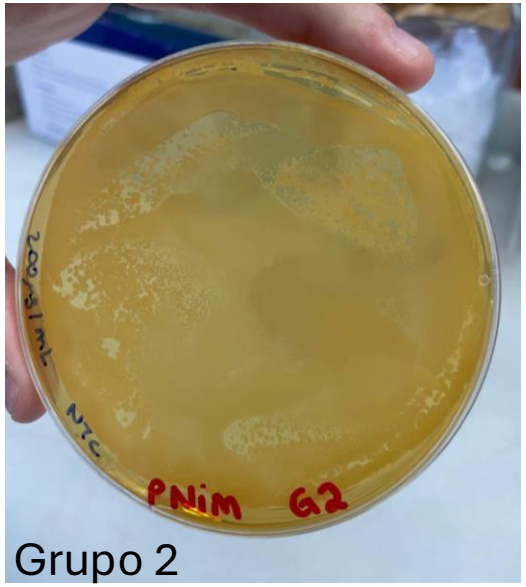
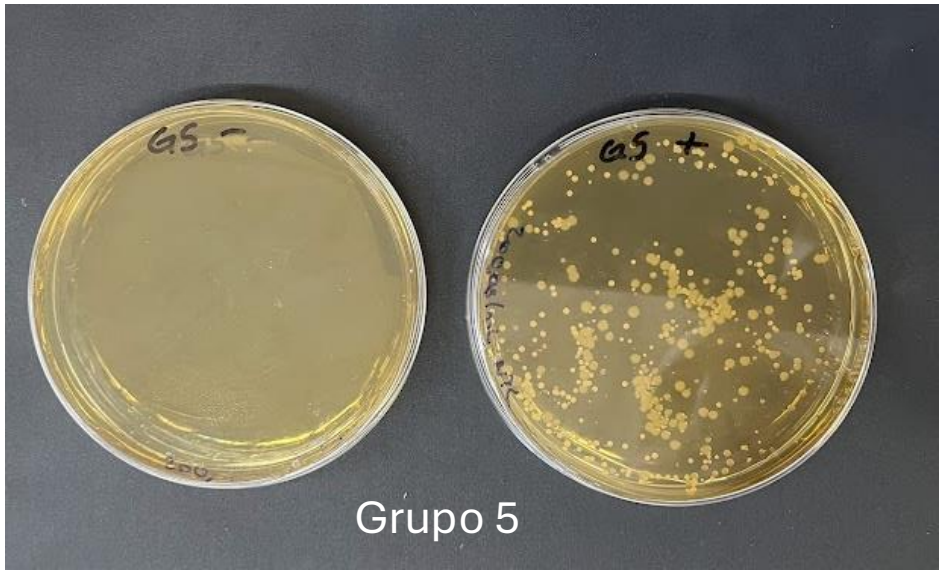
Dr. Daniel Felipe Freitas de Jesus

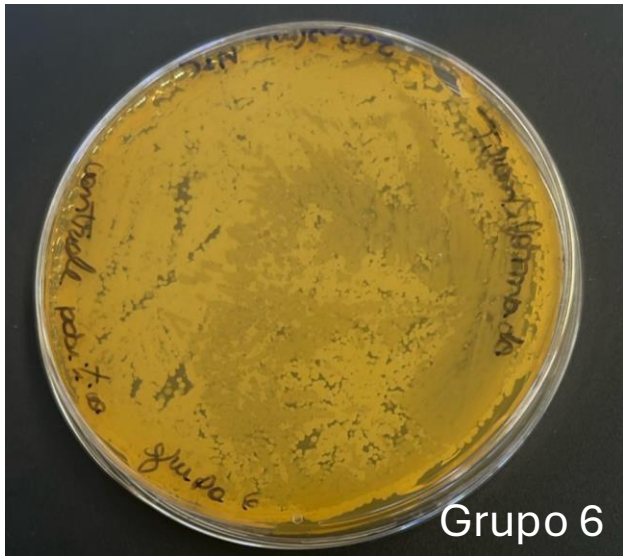
Tatiana Alves dos Reis

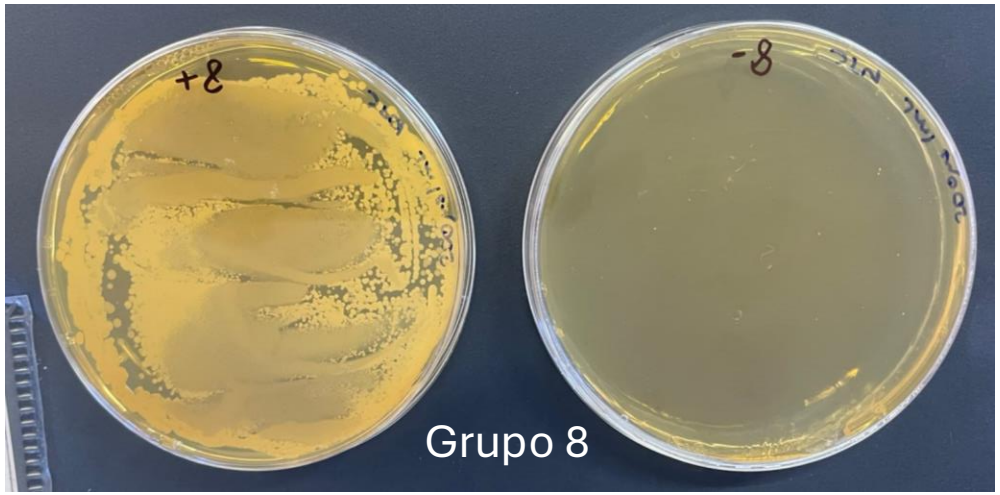
Transformação



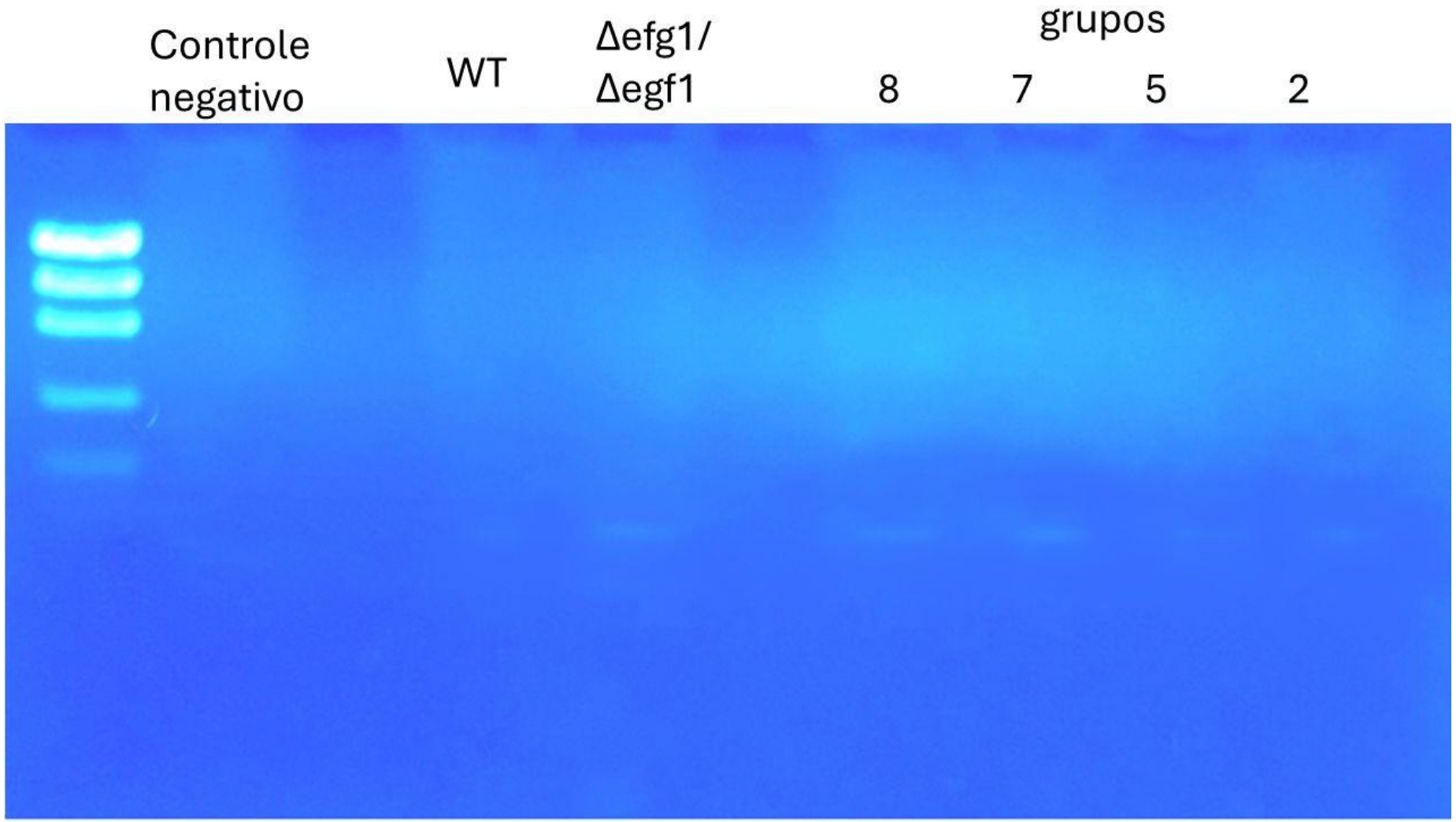








Confirmação por PCR



Teste de Sensibilidade aos Antifúngicos

	AMB (CI_{90})	FLC (CI_{50})	ANF (CI_{50})
WT (G1)	0,12	0,12	≤ 0,008
Δ (G1)	0,06	0,12	≤ 0,008
CP (G1)	0,06	0,25	-
WT (G2)	0,06	0,25	0,007
Δ (G2)	0,06	0,125	0,007
CP (G2)	0,06	1	-
WT (G3)	0,125	0,125	<0,008
Δ (G3)	0,03	<0,06	< 0,008
CP (G3)	0,125	1	-
WT (G4)			
Δ (G4)			
CP (G4)			

QC for EUCAST antifungal testing

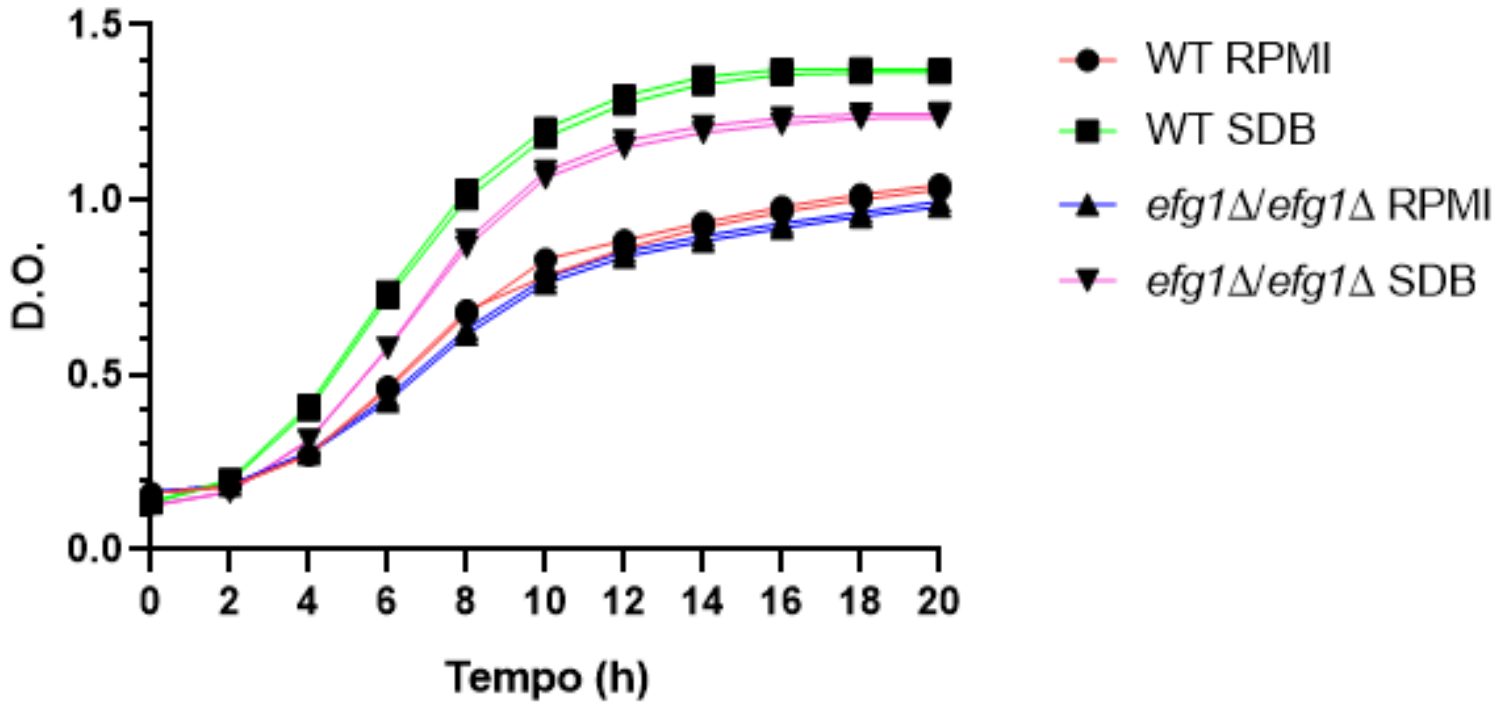
***Candida parapsilosis* ATCC 22019**[a]
Version 7.0, valid from 2023-08-14

See EUCAST E.Def 7.4 for MIC methodology

Antimicrobial agent	MIC[b] (mg/L)	
	Target	Range
Amphotericin B	0.25-0.5	0.125-1.0
Anidulafungin	0.5	0.25-1.0
Caspofungin	NA[c]	NA[c]
Fluconazole	1	0.5-2
Flucytosine	0.25	0.125-0.5
Isavuconazole	0.016	0.008-0.03
Itraconazole	0.06	0.03-0.125
Micafungin	1	0.5-2
Posaconazole	0.03	0.016-0.06
Rezafungin[##]	0.25-0.5	0.125-1
Voriconazole	0.03	0.016-0.06

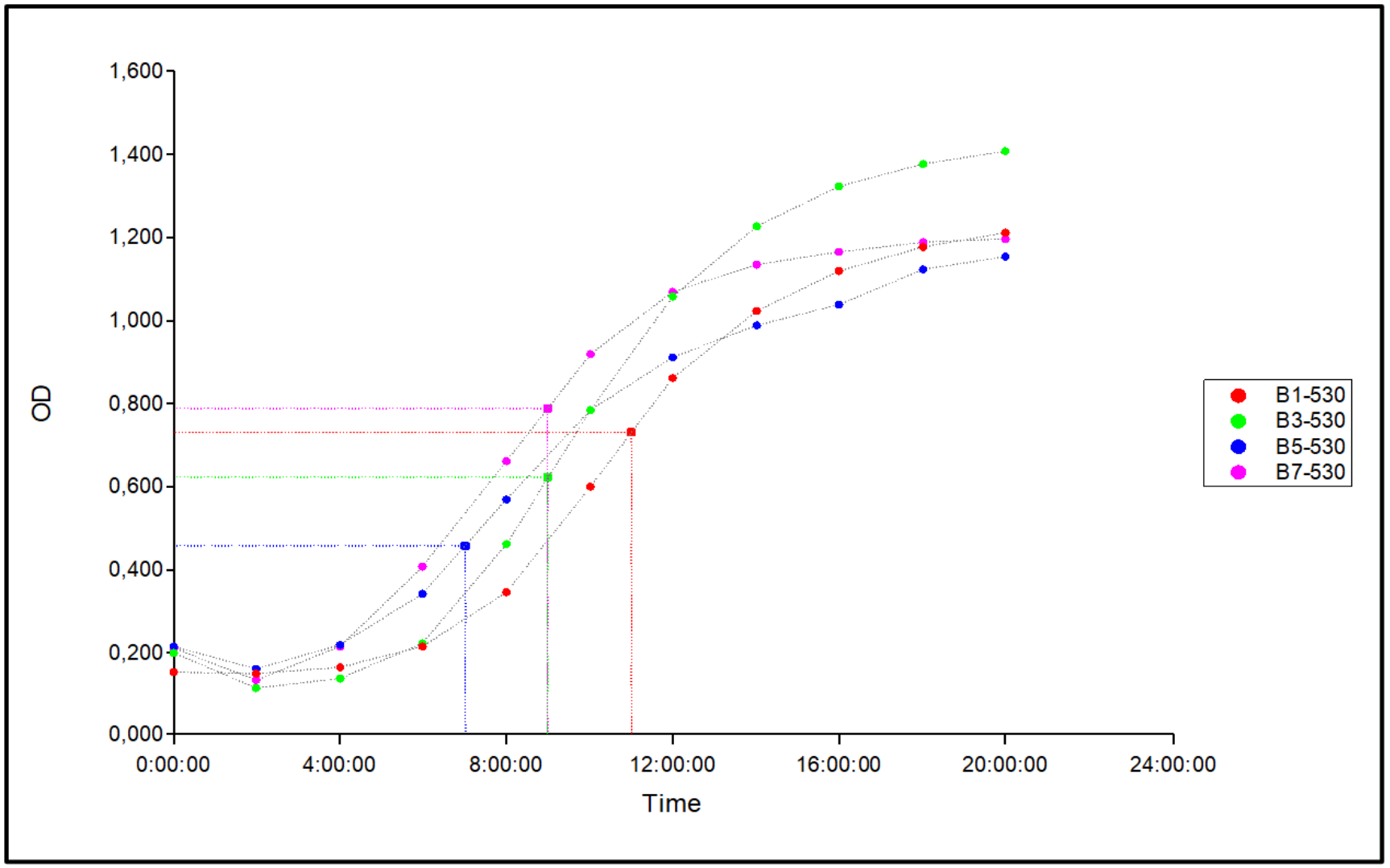
	AMB (CI_{90})	FLC (CI_{50})	ANF (CI_{50})
WT (G5)	0,007	0,06	<0,07
Δ (G5)	<0,03	<0,25	<0,03
CP (G5)	0,06	0,125	-
WT (G6)	0,007	0,125	0,03
Δ (G6)	0,007	0,06	0,03
CP (G6)	0,007	0,5	-
WT (G7)	0,125	0,06 / 0,125 (?)	< 0,008
Δ (G7)	0,03	0,06	< 0,008
CP (G7)	0,03	1	-
WT (G8)	0,03	0,125	0,015
Δ (G8)	0,03	0,125	0,015
CP (G8)	0,06	1	-

Curva de Crescimento



Grupo 1

Curva de Crescimento



Grupo 2

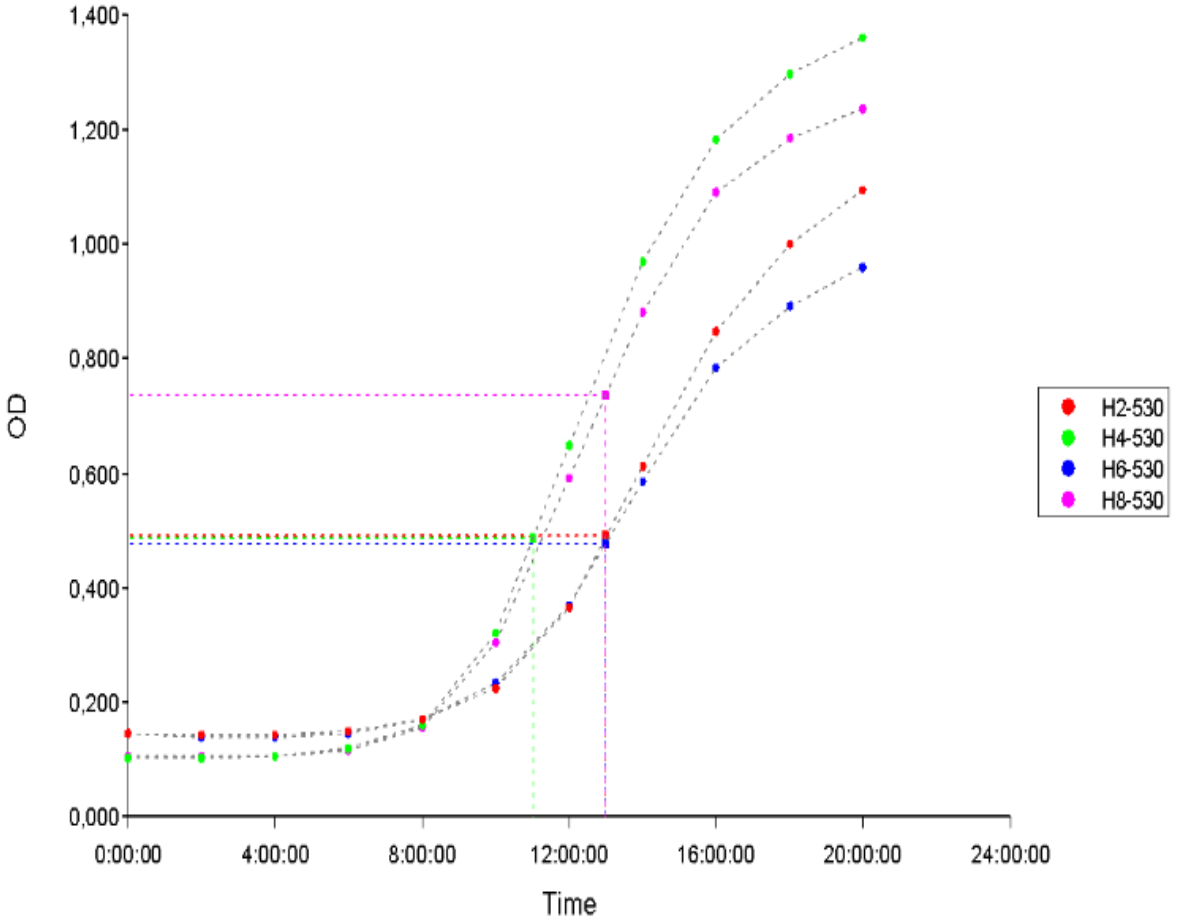
WT SDA

Δefg1/efg1 SDA

WT RPMI

Δefg1/efg1RPMI

Curva de Crescimento

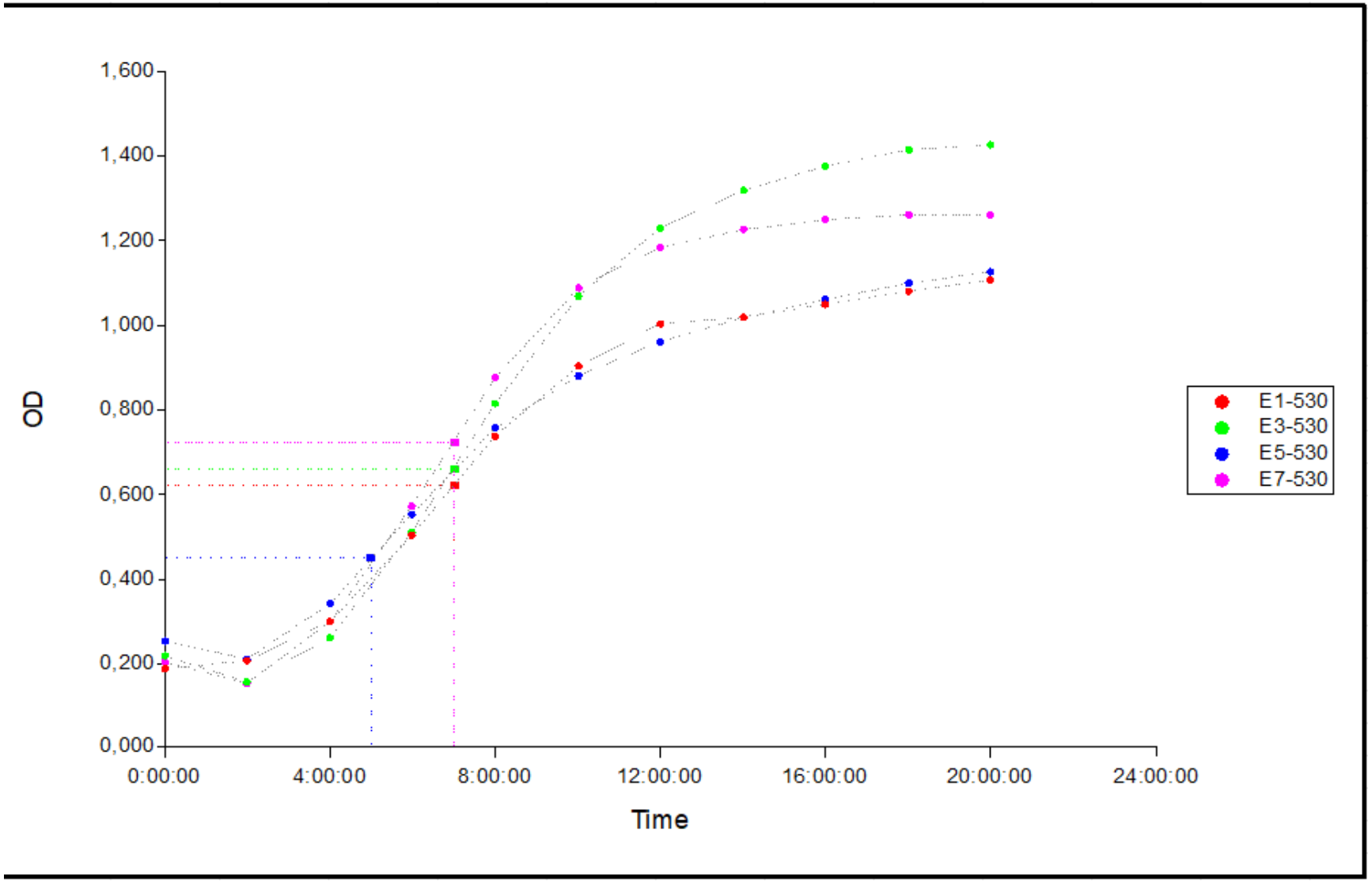


WT SDA
Δefg1/efg1 SDA
WT RPMI
Δefg1/efg1RPMI

G3
O meio SDA é rico em glicose e peptona, o que resulta em crescimento rápido e robusto.

O RPMI é um meio definido mais pobre em nutrientes, o que resulta num crescimento lento, moderado com formação de filamentos. A levedura detecta as limitações nutricionais do meio através de receptores membranares que desencadeiam no se citoplasma a expressão de proteínas envolvidas com o metabolismo adaptativo em resposta ao estresse para ativação, por exemplo, de mecanismos para utilização de outras fontes de carbono ou a produção de hifas para invasão de novos ambientes que possam suprir tais carências.

Curva de Crescimento



WT SDA

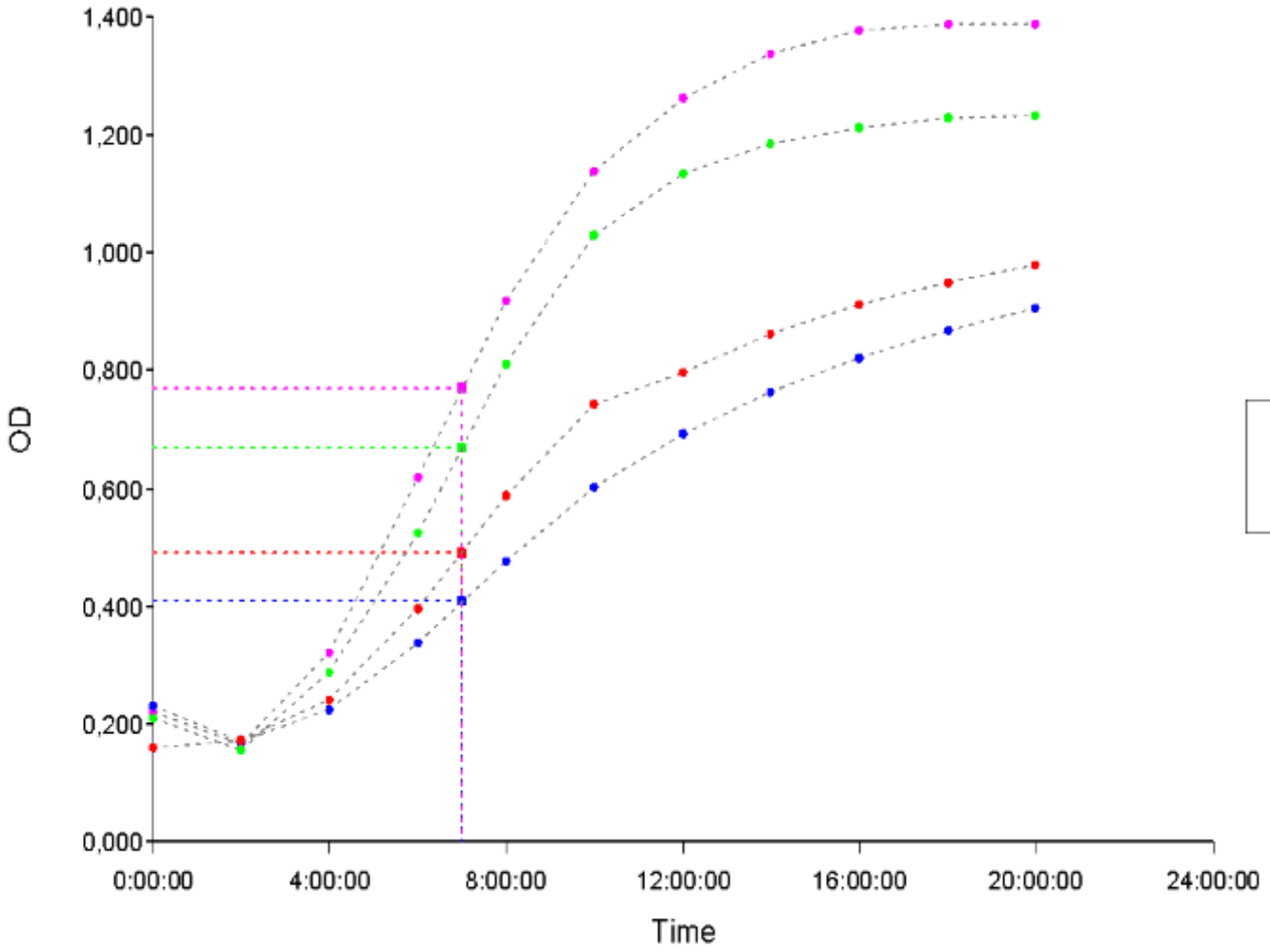
Δefg1/efg1 SDA

WT RPMI

Δefg1/efg1RPMI

Grupo 5

Curva de Crescimento



Grupo 6

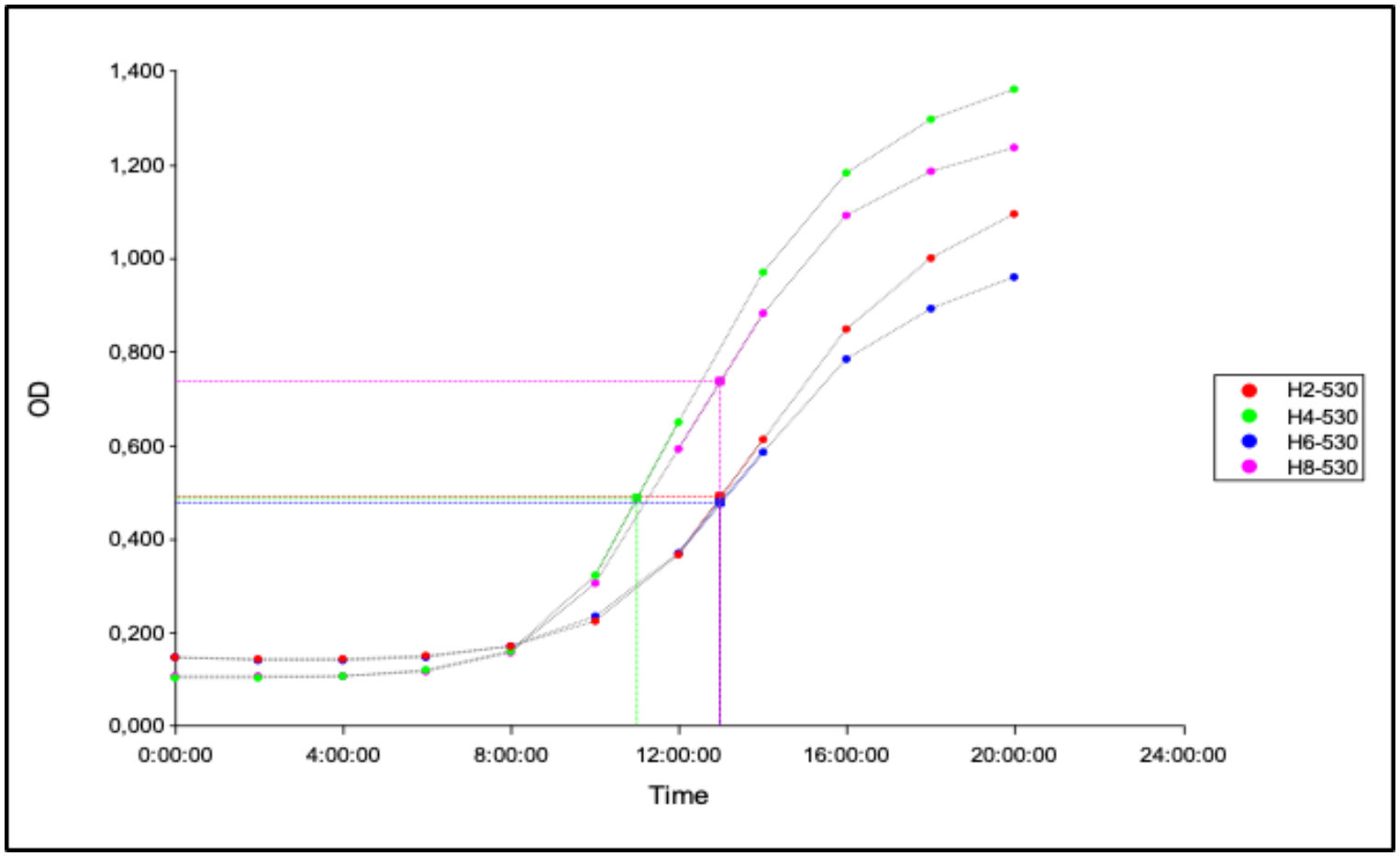
WT SDB

Δefg1/efg1 SDB

WT RPMI

Δefg1/efg1 RPMI

Curva de Crescimento



Grupo 7

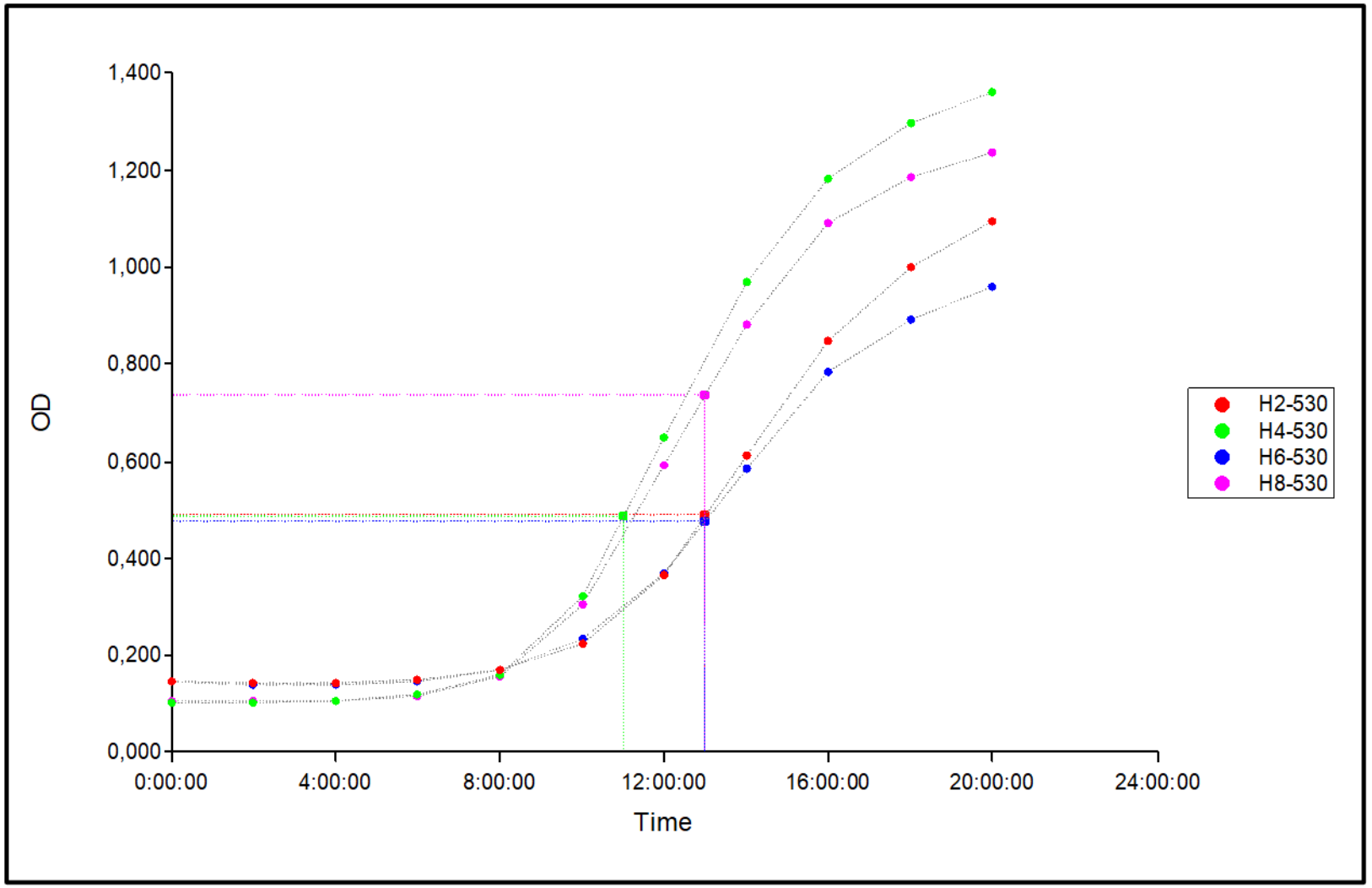
WT SDA

Δefg1/efg1 SDA

WT RPMI

Δefg1/efg1RPMI

Curva de Crescimento



Grupo 8

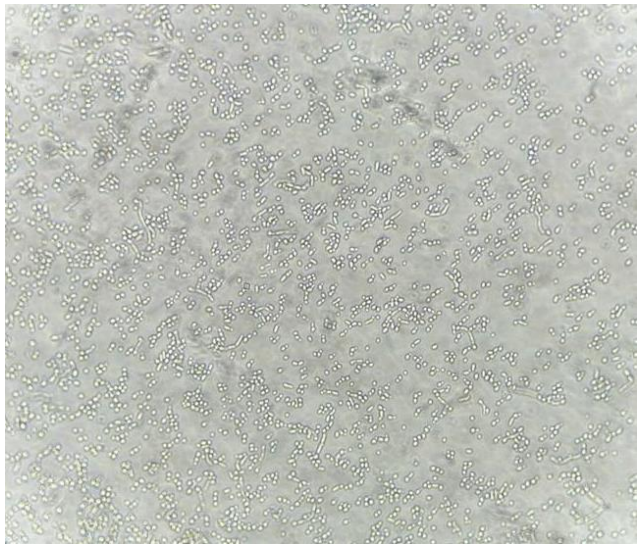
WT SDA

Δefg1/efg1 SDA

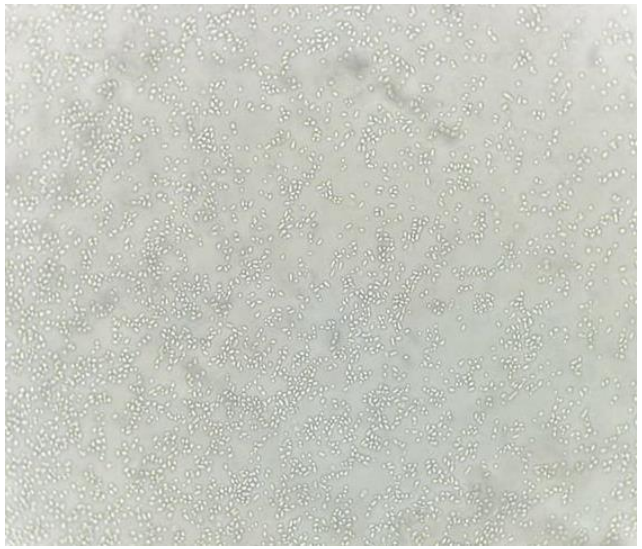
WT RPMI

Δefg1/efg1RPMI

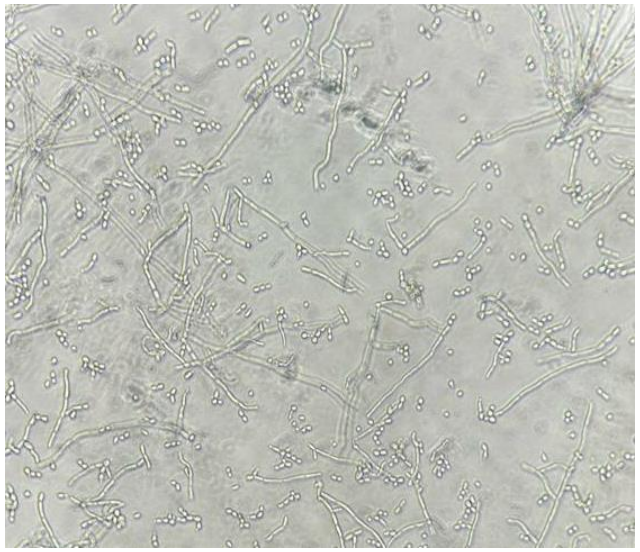
Filamentação - RPMI/SDB/Spider



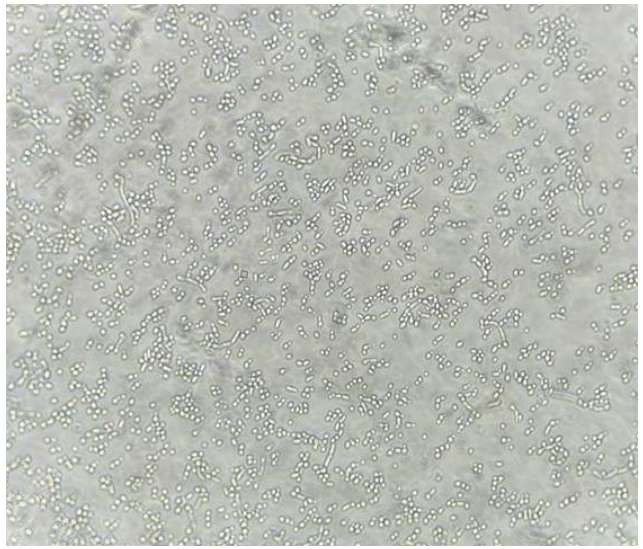
WT - SDB



*efg1*Δ/*efg1*Δ - SDB



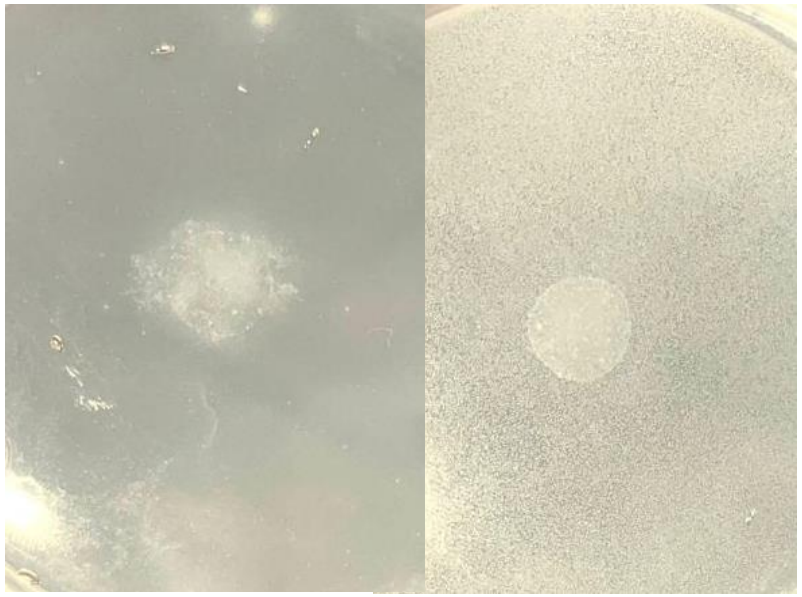
WT - RPMI



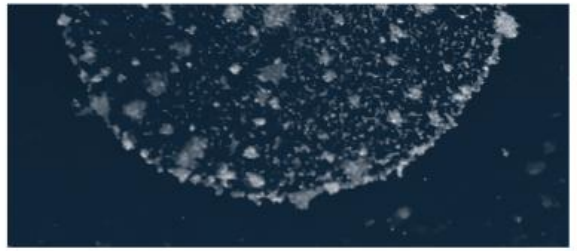
*efg1*Δ/*efg1*Δ - RPMI

Filamentação - RPMI/SDB/Spider

Spider – Grupo 2



C.albicans WT

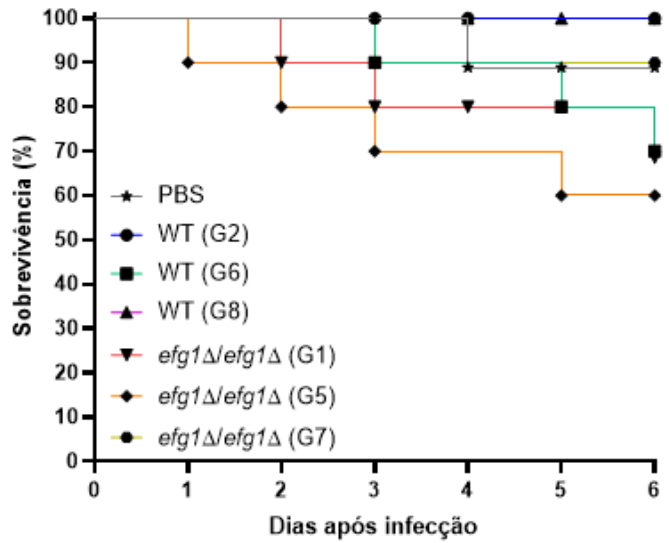


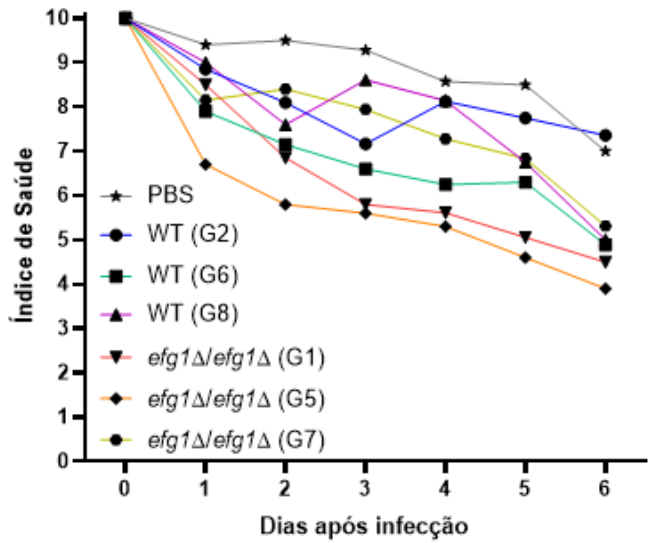
C.albicans △efg1

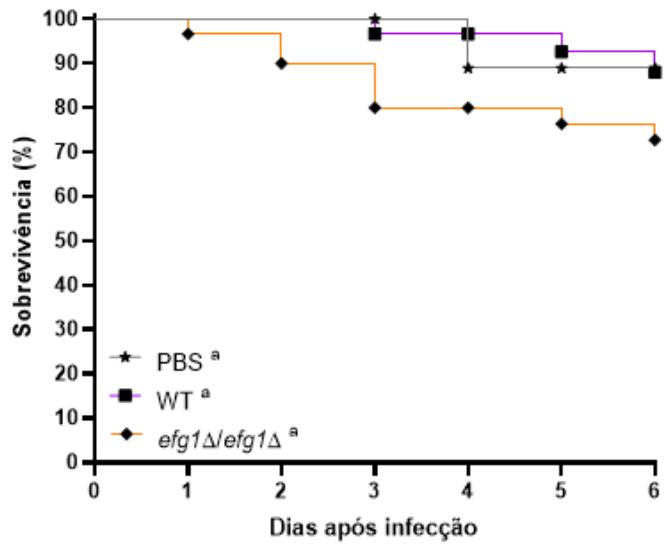
Spider – Grupo 3

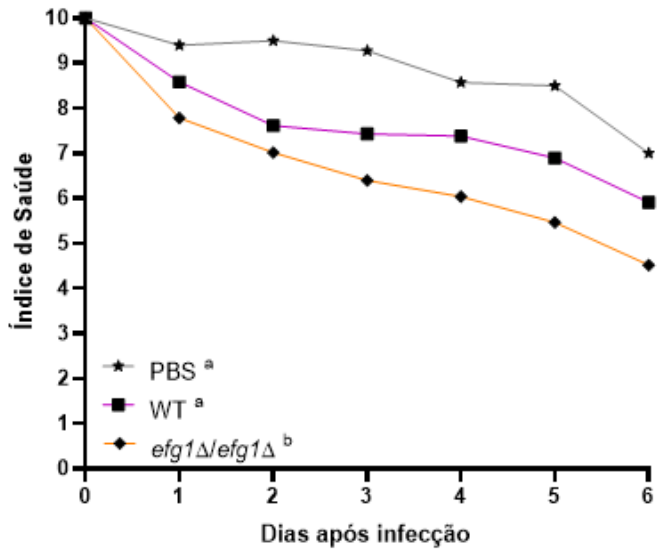
Spider – Grupo 6

Virulência em *Galleria mellonella*

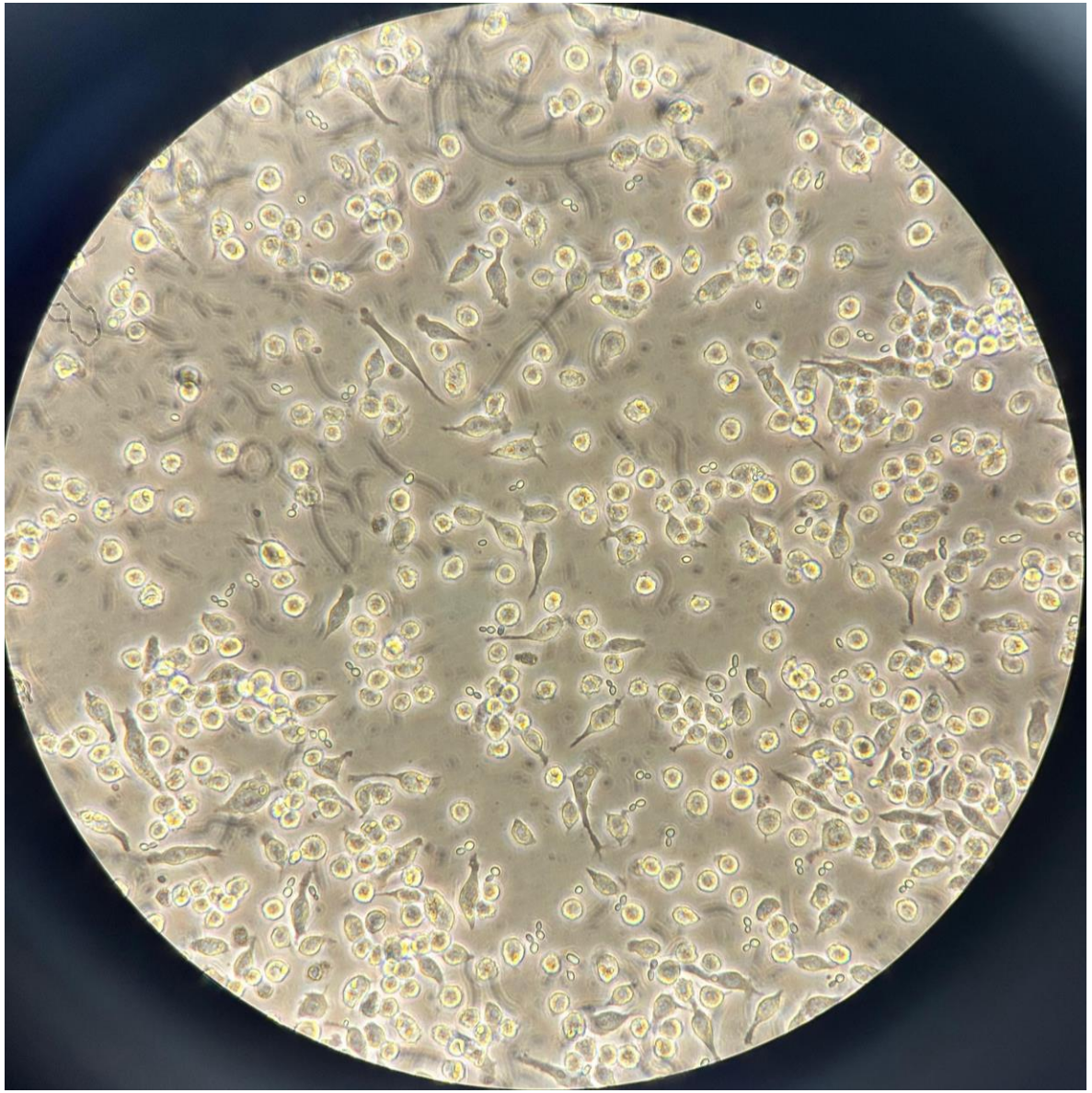








Ensaio de Fagocitose



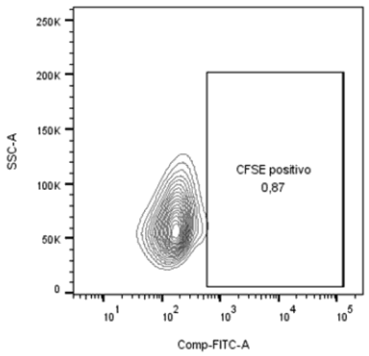
Ensaio de Fagocitose

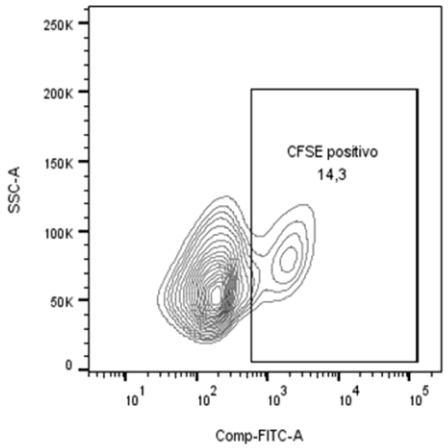
Controle

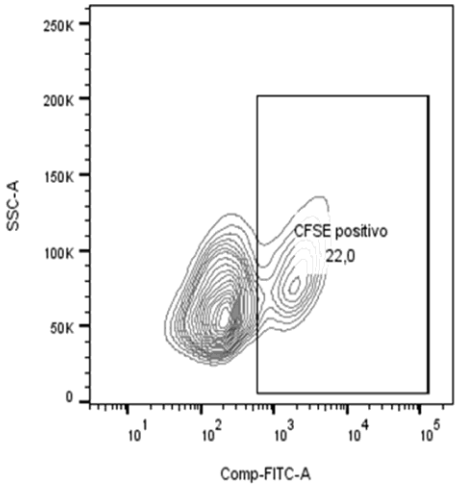
1:1

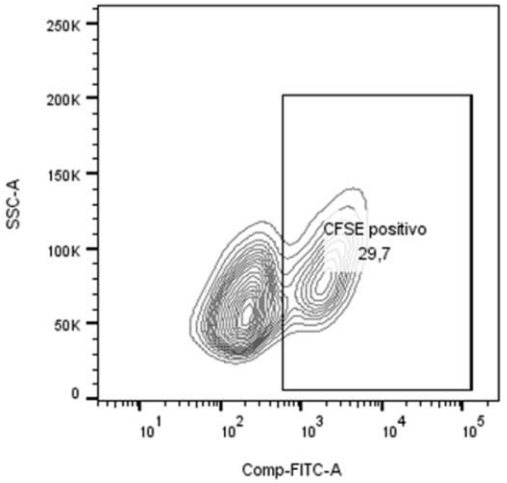
2:1

3:1

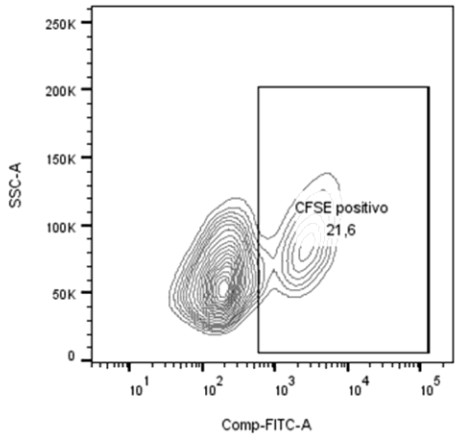


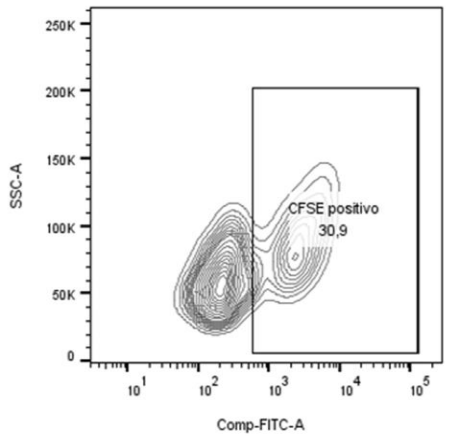


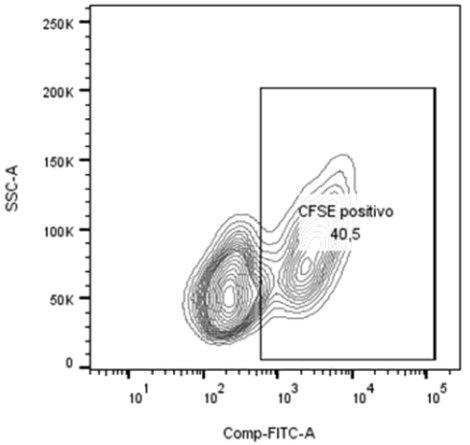




Cepa 1

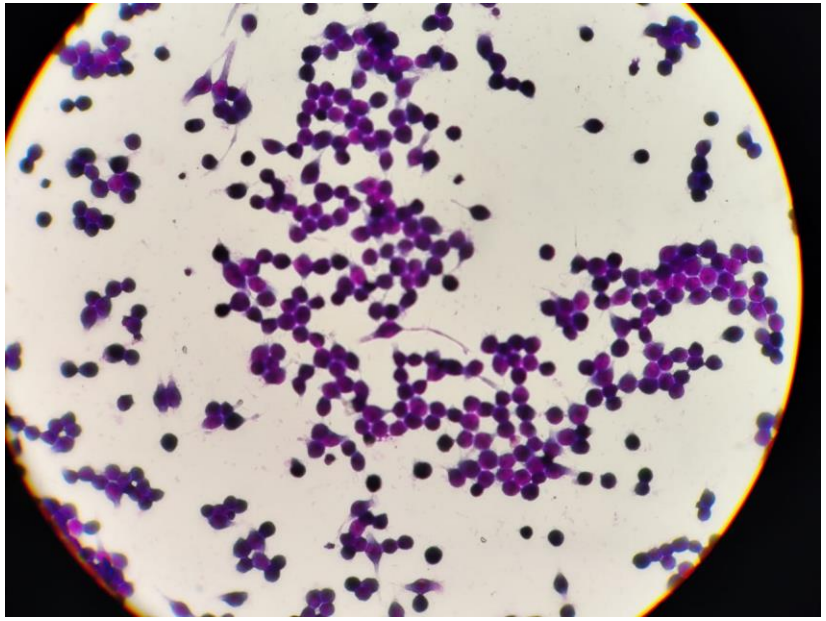






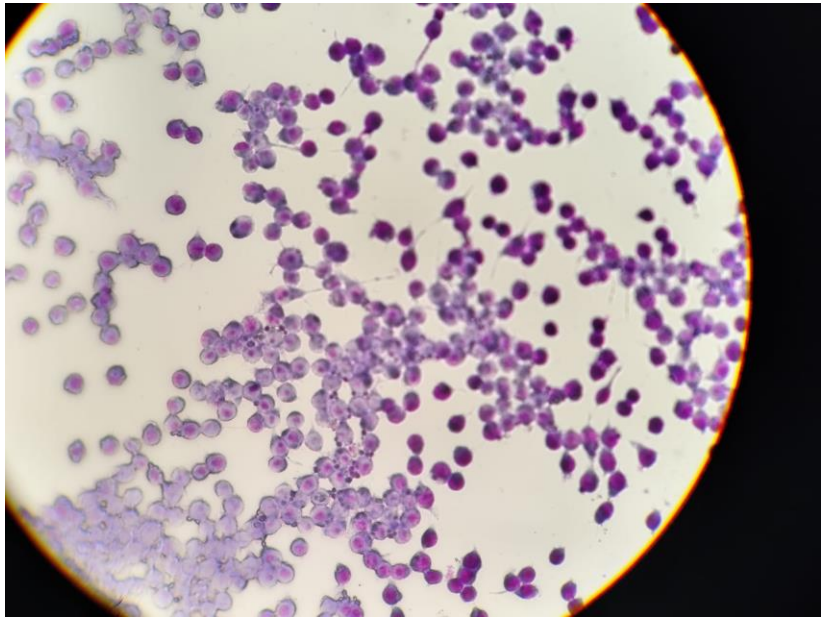
Cepa 2

Ensaio de Fagocitose



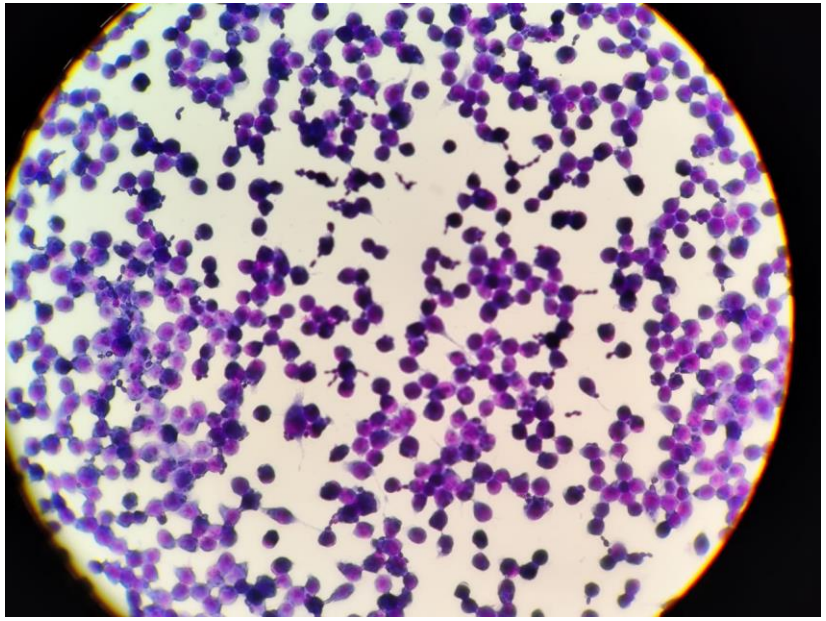
Apenas macrófagos

Poços 1 e 2



Macrófagos + *Candida* misteriosa 1

Poços 3 e 4



Macrófagos + *Candida* misteriosa 2

Poços 5 e 6