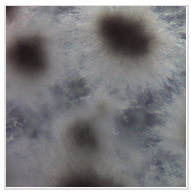

Dec 24, 2021

Candida tropicalis filamentation assay with fluconazole

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BIOSALUD

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This protocol was used to obtain enough quantity of RNA of a nonsusceptible strain of *Candida tropicalis,* with the goal to do a transcriptomic analysis to demonstrate the degree of differential expression of genes under filamentation and non-filamentation conditions and against a non-filamenting strain, susceptible to fluconazole (*Candida tropicalis* ATCC750)

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Aug 19, 2021

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RPMI PREPARATION

1 MEDIA PREPARATION

1.1 Medium to inhibit filamentation (RPMI+NAC)
RPMI1640: 10g
3-[N-morpholino]propane sulfonic acid buffer (MOPS): 35 g
N-acetyl glucosamine: 20 g
Distilled water 500 ml

Adjust pH at 7.0
Adjust volume at 1000 ml
Esterilize by filtration

1.2 MEDIUM TO PROMOTE THE FILAMENTATION (RPMI+SFB)

RPMI1640: 10g
3-[N-morpholino] propane sulfonic acid buffer (MOPS): 35g
Glucose: 20 g
Distilled water: 500 ml
Adjust pH at 7.0
Adjust volume at 1000 ml
Add 10% of Fetal bovine serum

2 Fluconazole preparation

Weigh 6.4 mg of Fluconazole (Cat Sigma: F8929)
Dissolve in 1 ml of Dimethyl sulfoxide (DMSO)
aliquot and freeze at -80°C

2.1 FLUCONAZOLE (FLU) DILUTION

USE A V or U bottom 96 wells plate

REAGENTS	FLU 6400 µg/mL	FLU 3200 µg/mL	FLU 1600 µg/mL	FLU 800µg/mL	FLU 400 µg/mL	FLU 200 µg/mL	FLU 100 µg/mL	FLU 50 µg/mL	FLU 25 µg/mL	FLU 12,5 µg/mL
DMSO		50	75	175						
FLU 6400 µg/mL		50	25	25						
DMSO					50	75	175			
FLU 800 µg/mL					50	25	25			
DMSO								50	75	175
FLU 100 µg/mL								50	25	25

Susceptibility test 1d

3 INOCOLUM DILUTION

Cultivate *Candida tropicalis* in Potato dextrosa agar o in Liquid Sabouraud
Incubate for 24 h at 35°C
Dilute one or two colonies in sterile saline solution
Vortex until homogeinity
Adjust the turbidity according to the 0.5 MacFarland Standard ($1x10^6$-$5x\ 10^6$ CFU)

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Make a 1:100 dilution of the inocolum using each media (filamentation and No fiamentation)
Vortex for 15 seg
Make a 1:20 dilution using the 1:100 dilution, using the same media (Final yeast concentration: $5x10^2$ to $3x10^3$ FCU

4 MIC ASSAY USING MEDIA TO FILAMENTATION (SFB) AND MEDIUM TO INHIBIT THE FILAMENTATION (NAC) 1d

USE A STERILE 24 WELLS PLATE

RPMI + NAC

REAGENTS	64 µg/ml	32 µg/mL	16µg/mL	8 µg/mL	4 µg/mL	2 µg/mL
RPMI+NAC	90 µL	90 µL	90 µL	90 µL	90 µL	90 µL
FLU (dil)	10 µL (6400 µg/mL)	10 µL (3200 µg/mL)	10 µL (1600 µg/mL)	10 µL (800 µg/mL)	10 µL (400 µg/mL)	10 µL (200 µg/mL)
INOCULUM (dil 1:20)	900 µL	900 µL	900 µL	900 µL	900 µL	900 µL
REAGENTS	1 µg/ml	0.5 µg/ml	0.250 µg/ml	0.125 µg/ml	C+	
RPMI+NAC	90 µL	90 µL	90 µL	90 µL	90 µL	1000 µL
FLU (dil)	10 µL (100 µg/mL)	10 µL (50 µg/mL)	10 µL (25 µg/mL)	10 µL (12.5 µg/mL)		
DMSO					10 µL	
INOCULUM (dil 1:20)	900 µL	900 µL	900 µL	900 µL	900 µL	

C+: Positive control; C-: negative control

RPMI+SFB

REAGENTS	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL
RPMI +SFB	90 µL	90 µL	90 µL	90 µL	90 µL	90 µL
FLU (dil)	10 µL (6400 µg/mL)	10 µL (3200 µg/mL)	10 µL (1600 µg/mL)	10 µL (800 µg/mL)	10 µL (400 µg/mL)	10 µL (200 µg/mL)
INOCULUM (dil 1:20)	900 µL	900 µL	900 µL	900 µL	900 µL	900 µL
REAGENTS	1 µg/mL	0.5 µg/mL	0.25 µg/mL	0.125 µg/mL	C+	C-
RPMI+SFB	90 µL	90 µL	90 µL	90 µL	90 µL	1000 µL
FLU (dil)	10 µL (100 µg/mL)	10 µL (50 µg/mL)	10 µL (25 µg/mL)	10 µL (12.5 µg/mL)		
DMSO						
INOCULUM (dil 1:20)	900 µL	900 µL	900 µL	900 µL	900 µL	

C+: Positive control; C-: Negative Control

Incubate at 35°C for 24 hrs with constant agitation (200 rpm approx.)
Read using an inverted microscope, comparing controls with each well
The Minimal inhibitory concentration will be where was observed 50% or more of growth
Also, register the filamentation grade in each well

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5 CULTURE OF *C. tropicalis* TO HARVEST YEAST FOR TRANSCRIPTOMIC ANALYSIS

Obtain a Fluconazole Strain and a certificate strain (ATCC750)
Establish the susceptibility grade of both strains
Culture both strains with filamentation (RPMI+SFB) and Non-filamentation (RPMI+NAC) media, using Fluconazole at subinhibitory concentrations
Incubate at 35°C by 24 hrs with constant agitation (200 rpm)
Check the growth degree and filamentation degree
Harvest the colonies with a sterile Pasteur pipet
Centrifuge at high speed
Extract the supernatant
Add RNA later (Sigma Aldrich Ref0901)
Freeze at -80°C until the RNA extraction

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