



## Antifungal metabolites from *Streptomyces* sp. as potential biomolecules to combat multi-resistant *Candida* sp. responsible of vaginal candidiasis

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# CONTEXT

## FUNGAL INFECTIONS:

- global public health issue
- affect more than a billion people (11 million cases per year).
- responsible of significant morbidity and mortality worldwide (immunocompromised patients)

## *Candida albicans*:

- the causative agent of candidiasis in humans.
- part of the normal microbiota of healthy humans (mucous membranes of the oral cavity, gastrointestinal tract and vagina)
- causes of superficial and systemic infections in immunocompromised and susceptible individuals)



## WARNING:



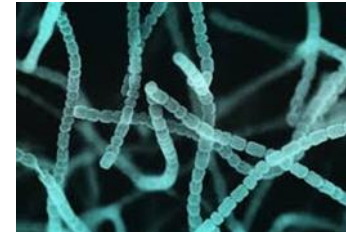
- Increase of the number of *Candida albicans* strains resistant to currently used antifungal drugs.
- Antimicrobial resistance (AMR) is a One Health challenge

## SOLUTIONS:

- Search of new antifungal molecules to treat effectively mycotic infections via microorganisms (Actinomycetes: *Streptomyces*)

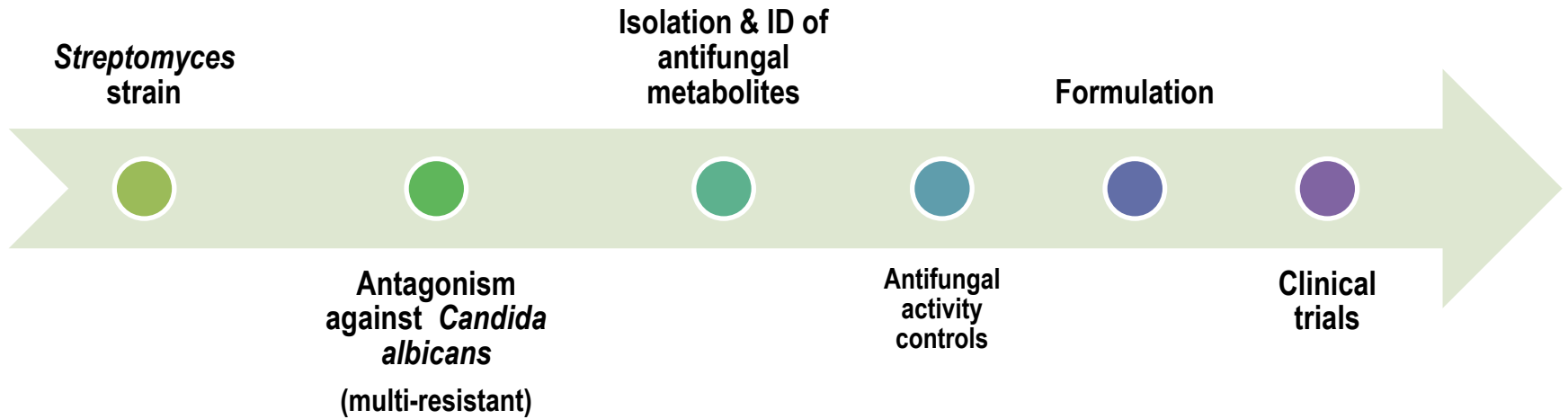


## *Streptomyces:*

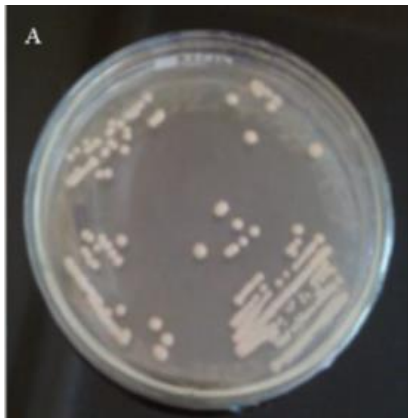


- Gram positive and filamentous bacteria
- Capacity for producing multiple bioactive molecules and natural antibiotics
- Potential candidates for producing new natural drugs to combat the emergence of AMR

# RESEARCH STRATEGY



Multi-resistant *Candida albicans* [1]



*Streptomyces* sp. 3400 JX826625 [2]



[1] Andriambeloson et al. American Journal of Life Sciences. 2019 7(6): 164

[2] Andriambeloson et al. American Journal of Life Sciences. 2016 4(6): 152

# METHODOLOGY

## Antagonistic tests

- *Streptomyces* sp. 3400 JX826625
- Ketoconazole & Nystatine (standards)

## Antifungal metabolite production

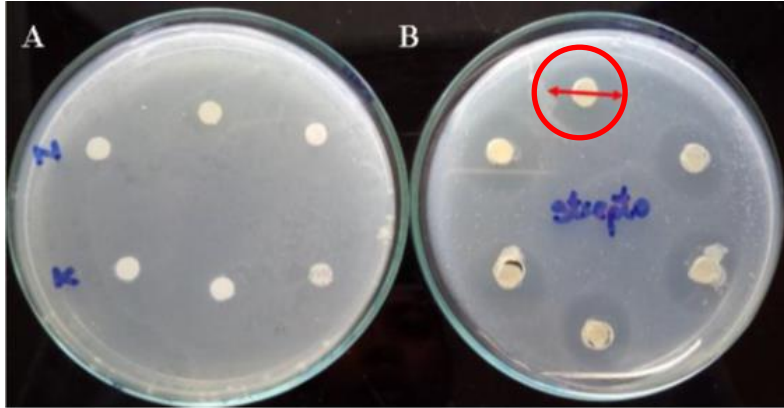
- Culture media, pH, temperature, and extraction optimization

## Isolation & chemical characterization

- Solvent fractionation
- Thin Layer Chromatography (analytical & preparative)
- Spectra UV-Visible (200-500nm)
- Nature of antifungal metabolites
- Stability of active molecules

# RESULTS

## 1. ANTAGONISTIC ACTIVITY OF *Streptomyces* sp. 3400 JX826625 AGAINST *Candida albicans*



N: Nystatine (NY 100 000)

K: Ketoconazole (KET 50 )

*Streptomyces* sp.: 25mm

## 2. OPTIMIZATION OF ANTIFUNGAL METABOLITE PRODUCTION

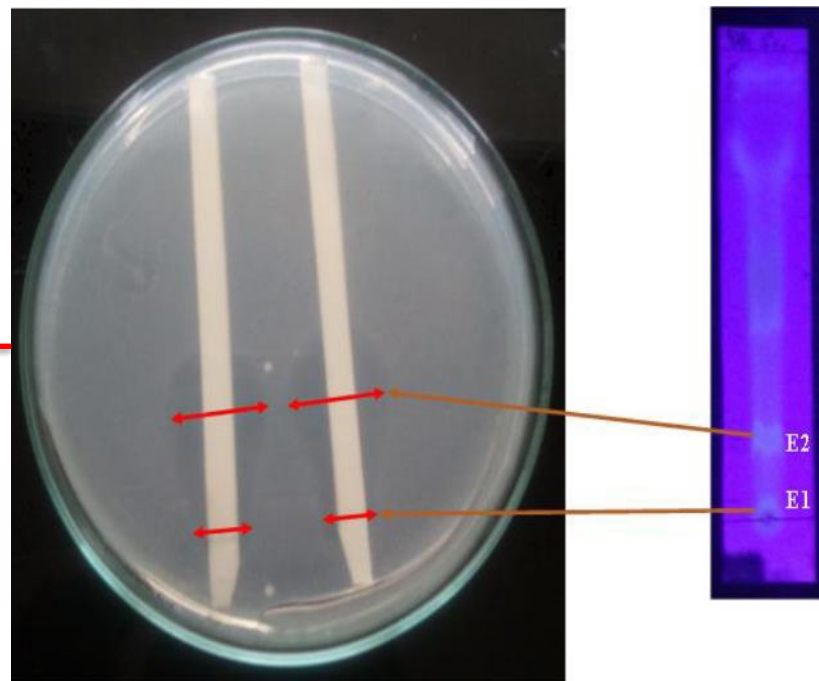
OPTIMAL CONDITIONS	
Culture medium	Bennett – ISP2 – AS1 – SCA – AFM – Sporulation agar
pH	5,13 – 7,01 – 8,97 – 11,02
Temperature (°C)	4 – 25 – 30 – 37 – 44
Extraction solvent	Ethanol – Butanol – Hexane – Ethyl acetate

## □ TLC of butanol fraction

DCM/MeOH/water (8/2/0,2)

N° of bands	UV 254 nm		UV 365 nm	
	Rf	Color	Rf	Color
10	0,95	Grey	0,95	Fluorescent blue
9	–	–	0,93	Orange
8	0,86	White	0,86	White
7	0,75	White	0,75	Yellow
6	0,53	Black	0,53	Red brown
5	0,42	Grey	0,42	Yellow
4	–	–	0,21	Fluorescent yellow
3	0,17	Blue-black	0,17	Orange brown
2	–	–	0,15	Fluorescent purple
1	0,02	Orange	0,02	Yellow

## □ Bioautography of the chromatogram



E1 : Rf= 0,02 ; Ø= 10mm  
E2 : Rf= 0,15 ; Ø= 19mm

**TWO ACTIVE FRACTIONS**

## ❑ Chemical screening of the butanol fraction from *Streptomyces* sp. 3400 JX826625

Chemical families	Reagents	Observations		Rf
		Colorations	Results	
Terpenes	Anisaldehyde sulfuric 5%	Red brown	+	0,75
Triterpenes	Liebermann Burchard	-	-	-
Alkaloids	Dragendorff	Orange	+	0,53
		Orange	+	0,21
Anthracene derivatives and coumarins	Ethanollic solution of KOH 5%	Fuorescent blue	+ (coumarins)	0,95
		Fluorescent yellow	+ (anthracene derivatives)	0,21
		<b>Purple</b>	<b>?</b>	<b>0,15*</b>
		<b>Fluorescent yellow</b>	<b>+ (anthracene derivatives)</b>	<b>0,02*</b>
Flavonoïds	Neu Reagent	-	-	-

(\*) : Rf with activity against *C. albicans*, + : presence, - : absence

## ❑ Purification of the active compounds by TLC preparative + MeOH extraction

 Two active purified fractions against multiresistant *C. albicans* ( $\varnothing$  = 17 mm & 19 mm)



## Minimum inhibitory concentration (MIC) of antifungal metabolites

Dilution  
method

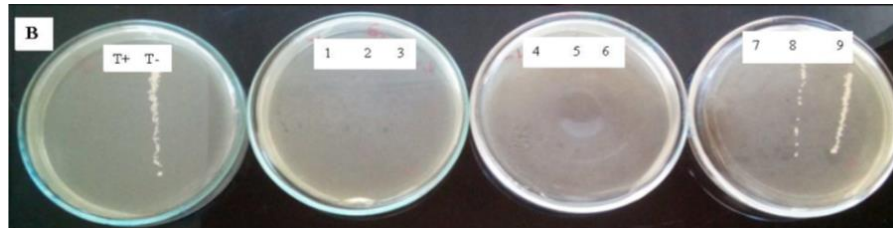


FUNGISTATIC  
ACTIVITY

Tube	T+	T-	1	2	3	4	5	6	7	8	9	10	
E2 (µg/ml)	0	0	100	50	25	12.5	6.250	3.125	<b>1.562</b>	0.781	0.390	0.195	
Aspect de la culture	Limpide	Trouble	Limpide						<b>CMI</b>	Trouble			

## Minimum fungicidal concentration (MFC) of antifungal metabolites

Enumeration  
on Petri plate

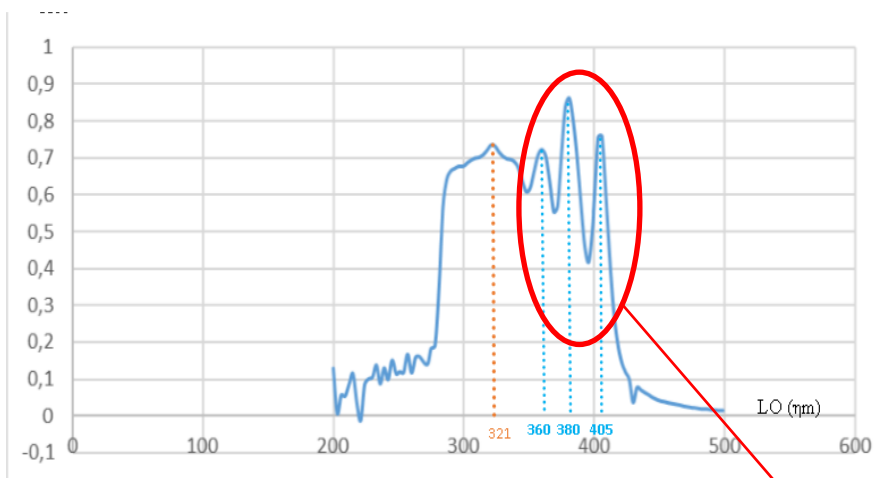


FUNGICIDAL  
ACTIVITY

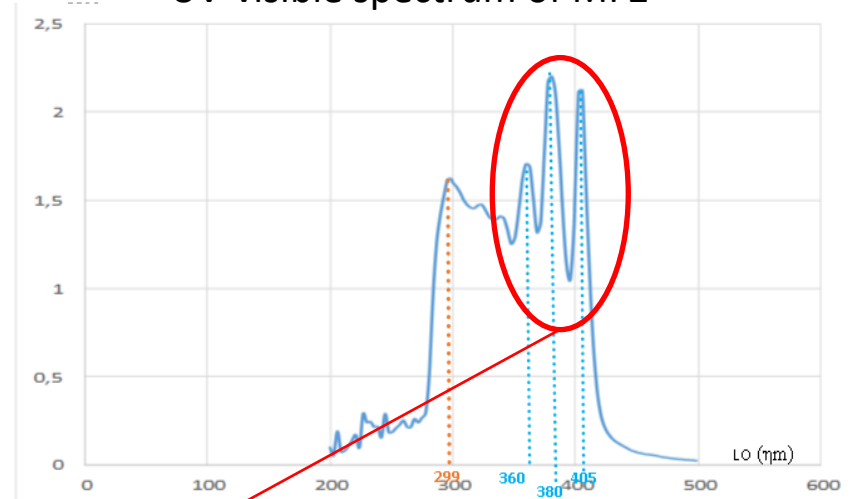
	FM1	FM2
<b>MIC (µg/ml)</b>	1,562	17
<b>MFC (µg/ml)</b>	3,125	34
<b>MFC/MIC</b>	2	2
<b>ACTION</b>	<b>Fungicidal</b> (Fauchère et Avril, 2002)	

## □ Additional analyses of the active compounds

UV-visible spectrum of MF1



UV-visible spectrum of MF2



**Polyenes:** 3 successive pics between 290nm and 405nm (360nm, 380nm, 405nm)

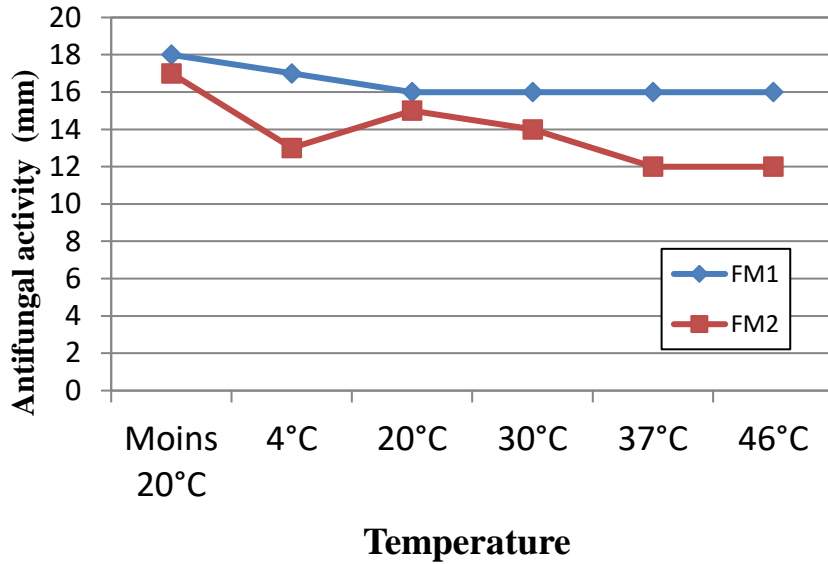
Absorption spectrum of polyenes (Lindenfelser *et al.*, 1964)

Types of polyene	Maximum absorption spectrum (nm)
Tetraene	290 - 291, 303 - 306, 318 - 320
Pentaene	318 - 324, 333 - 338, 346 - 358
Hexaene	339 - 341, 356 - 358, 377 - 380
Heptaene	358 - 365, 376 - 380, 399 - 405

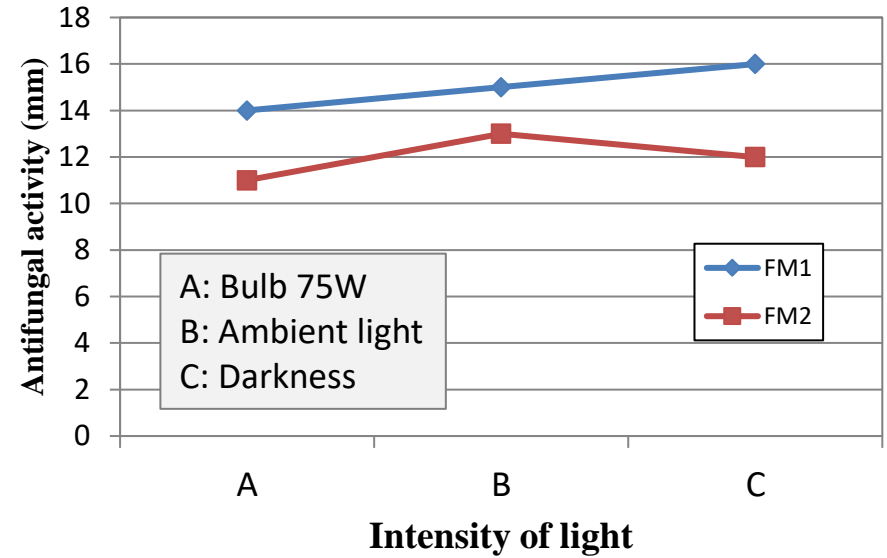
**Non-polyenes?:** 321 nm (MF1) ~ Rf = 0,02 – 299 nm (MF2) ~ Rf = 0,15

## ☐ Stability of antifungal compounds

**Temperature effect on antifungal activity**



**Light effect on antifungal activity**



## CONCLUSION

- Antifungal compounds produced by *Streptomyces* sp. 3400 JX826625 are active against a multi-resistant *Candida* sp.
- Potential use for treating multi-resistant candidiasis

## PERSPECTIVES

- Chemical structure identification of the two antifungal compounds
- Study of their action mechanisms
- Formulation type ointment and toxicity assay
- Clinical assay

**THANK YOU FOR YOUR  
ATTENTION**