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Biological control of wood decay fungi by *Streptomyces* strains's extract

Dounia Mortabit[1], Mourad Zyani[1], and Saad Ibnsouda Koraichi[1-2]

[1]Microbial Biotechnology Laboratory, SMBA University,
Faculty of Sciences and Technology, Route Immouzer, P. O. Box 2202, Fez, Morocco

[2]Centre Universitaire Régional d'Interface, Université Sidi Mohamed Ben Abdellah - Fès, Morocco

ABSTRACT: The ability of actinomycetes to produce extracellular antifungal metabolites against fungal strains causing damages in the wood of the old Medina of Fez has been investigated. Two *Streptomyces sp* were screened for the inhibition of wood decay. Screening for antifungal activity of actinomycetes was performed with dual methods: the method of disk diffusion and the method of agar blocks. The result indicates that the crude extract of *Streptomyces sp* had a broad spectrum against fungi causing wood decay and inhibits their growth for more than 15 weeks; therefore it's could be an interesting source of antifungal bioactive substances.

KEYWORDS: Biological control, Actinomycetes *Streptomyces sp*, antifungal, wood decay fungi.

1 INTRODUCTION

Fungal pathogens pose serious problems worldwide in the preservation of patrimonies, especially those wooden. In the city of Fez, the heritage of the old medina experiencing a remarkable deterioration due to various fungal species belonging mainly to the genus: *Thielavia, Penicillium* and *Cladosporium*. Chemical fungicides are extensively used in various domains. However, excessive use of chemical fungicides has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide… Because of the worsening problems in fungal disease control, a serious search is needed to identify alternative methods for wood protection, which are less dependent on chemicals and are more environmentally friendly. Microbial antagonists are widely used for the biocontrol of fungal diseases. Various microbial antagonists have been investigated as potential antifungal biocontrol agents. Many species of actinomycetes, especially those belonging to the genus *Streptomyces* (Gram-positive, myceliaforming soil bacteria), are well known as biocontrol agents that inhibit several pathogenic fungi ([1], [2], [3]). Some actinomycetes can play a protection role by inhibiting the development of potential fungal pathogens by producing enzymes, which degrade the fungal cell wall or produce antifungal compounds [4].

In this article, the ability of actinomycetes to produce extracellular antifungal metabolites against fungal strains causing damages in the wood of the old Medina of Fez has been reported. This antifungal potential could be exploited for its future use as a biofungicide.

2 MATERIEL AND METHODS

2.1 FUNGAL STRAINS AND CULTURE CONDITIONS

In this study, four white rot fungi (*Thielavia hyalocarpa, Penicillium commune, Penicillium chrysogenum, Penicillium expansum*) and one brown rot fungi (*Cladosporium cladosporioides*) were used. These fungi were isolated from a damaged wood of an old house in the medina of Fez in our previous study [5]. These fungi were used in the antifungal assay as we have been previously described [6]. They were maintained and grown on 2% malt agar at 25°C.

Corresponding Author: Dounia Mortabit

2.2 CULTIVATION CONDITIONS AND METABOLITES EXTRACTION

Two *Streptomyces sp* (S1 et S2) were used in this study, they are already been characterisated and identified in our laboratory [7]. They were cultivated on ISP2 medium: yeast extract 4%, malt extract 10%, glucoe 4%, and agar 18%. The pH is adjusted to 7.2. The plates were incubated at 28°C from 3 to 4 weeks.

For the preparation of actinimycet's extract the cultivation was carried out in liquid medium in 500 ml flasks with 100 ml media at 28 °C and 240 rpm for 7 days under agitation (250 rpm). Mycelium was separated from the supernatant at 8000 rpm for 15 min, then a solution of ethyl acetate was added and shaken for 2h [8]. Ethanol extracts of the mycelium were prepared. Two variants of the agar plate diffusion method were applied.

2.3 METHOD OF DISK DIFFUSION

The antifungall activity was determined by disk diffusion bioassays ([9], [10]) against *Thielavia hyalocarpa, Penicillium commune, Penicillium chrysogenum, Penicillium expansum* and *Cladosporium cladosporioides.* The test plates for fungi were prepared by pouring 15 ml of potato dextrose agars (PDAs) as the base layer. After solidifying, each plate was inoculated with each corresponding fungal spores (1×10^6 spores/ ml) as the seed layer. The sterilized paper disc (6 mm) was placed on the middle of the plate. An aliquot (50 µl) of the actinomycete's extract was added on the paper disc. Plates were incubated à 25°C for 72h. After incubation, the diameters of inhibition zones were measured.

2.4 METHOD OF AGAR BLOCKS

Cylindrical pieces were cut out from well grown and sporulated culture of the actinomycete strain on solid nutrition medium [11]. The blocks were placed on the Petri dishes deep inoculated with a fixed amount of test-microorganisms (10^8cells/ml). The cultures stayed for 7 days at 25 °C 5. The antifungal activity was measured in mm sterile zone on the 7th day.

2.5 IN VITRO ANTAGONIST ASSAY

In the Petri dish test chamber, sterilized specimens of cedar wood were inoculated by fungal spores. After 72h, each specimen was sprayed with 1 ml of *Streptomyces's* metabolite. Petri dish chamber were then incubated for 20 weeks at 25°C.

3 RESULTS AND DISCUSSION

The two actinomycetes were isolated from deteriorated wood in an old house built 450 years ago located in the former Derb Lamté in the Medina of Fez. They are already cited by Jihani et a.,l [7] and identified as *Streptomyces sp*. The crude extract of antifungal compounds isolated from the strains S1 and S2 was used to check the antifungal activity against fungi causing wood decay in the old medina of Fez (*T. hyalocarpa, P. commune, P. chrysogenum, P. expansum* and *C. cladosporioides*. Table 1 summarizes the antifungal activity of the actinomycetes S1 and S2.It has been observed that both of S1 and S2 inhibits fungal growth and the growth of fungal mycelium decreases with the increase in the concentration of compound extracted from the two strains of *Streptomyces sp* (S1 and S2).It has been also noted that 100 µl of the actinomycetes extract allowed a total inhibition of *T. hyalocarpa* and *P. chrysogenum* growth. Similar results have been reported by [12] who reported that the crude extract of *Streptomyces sp* was active against *Aspergillus niger* and *Staphylococcus aureus.* In an author study, Harpreet et al., [13] has determinate the antifungal activity of stremptomyces isolates from soil against *Aspergillus niger, Aspergillus flavus*, *Alternaria sps*, *Fusarium sps* and *Rhizopus stolonifer*

Table 1. Antifungal activity of Streptomyces sp's extract

Fungal species	Diameter of Fungal Growth					
	Extract of actinomycetes isolate (50 µl) (cm)		Extract of actinomycetes isolate (75 µl) (cm)		Extract of actinomycetes isolate (100 µl) (cm)	
	S1	S2	S1	S2	S1	S2
T. hyalocarpa	1.5	1.8	0.9	0.6	No growth	No growth
P. commune	2.0	2.0	1	1.4	0.2	0.2
P. chrysogenum	2 .0	1.8	1.2	1.0	No growth	No growth
P. expansum	1.4	1.2	0.8	0.6	0.4	0.3
C.cladosporioides	2.3	2.2	1.6	1.5	0.5	0.5

The Method of agar blocks was used to confirm precedent result. Antagonism was measured by zone of inhibition of fungal growth and results are shown in table 2. From the findings, the crude exctract of *streptomyces sp* (S1 and S2) displayed an antifungal activity against all the fungi studied. *T. hyalocarpa and P. chrysogenum* were more sensitive to the crude extract and presents the maximum diameter of clear zone (more than 2.5 cm); while *C.cladosporioides* was more resistant to the crude extract and presents the minimum diameter of clear zone (less than 1.6 cm). The results showed that the crude extract of *Stremptomyces sp* (S1 and S2) had a wide range of antifungal activities toward many species of fungi. Thus this extract might represent a good alternative of biocontrol against fungi causing wood decay.

The study of antifungal activities of the two strains of *Streptomyces sp* (S1 and S2) against wood decay fungi was also investigated. We note that S1 and S2 retard the growth of all the five fungi for more than 15 weeks. Furthermore, an encouraging result was obtained: *T. Hyalocarpa, P. commune* and *P. chrysogenum* growth's was retarded for 20 weeks. However, *P. expansum* growth's was retarded for 18 weeks by S1 and 17 weeks by S2; while *C.cladosporioides* growth's was retarded for only 15 weeks byS1 and S2. One more test must be performed in vivo to confirm the results obtained in vitro. In this way, we suppose that this work could be a good way to perform furher experiments in order to use the crude extract of *Steptomyces sp* as an environmentally friendly bioactive alternative against fungi causing wood decay.

Table 2. Antifungal activity of the actinomycete strains determined by the agar block method

Fungal species	Diameter of inhibition zone (cm)	
	S1	S2
T. hyalocarpa	2,5	2,5
P. commune	2	1,8
P. chrysogenum	2,5	2,4
P. expansum	1,8	1,6
C.cladosporioides	1,6	1,4

4 CONCLUSION

During our study, we found that the crude extract of *Streptomyces sp* could inhibit the growth of *T. hyalocarpa, P. commune, P. chrysogenum, P. expansum* and *C. Cladosporioides*: main destructive agents of wood in the old Médina of Fez. This suggested that the crude extract of *Streptomyces sp* had a broad spectrum against fungi causing wood decay and deserved further study as a useful biological control agent preservation of historical monuments. Several field tests using the crude extract of *Streptomyces sp* are therefore necessary.

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