

Biological control of *Aspergillus niger* and *Aspergillus flavus* isolated from strawberry (*Fragaria x ananassa* Duch) by streptomycetes sp.

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Abstract

Actinobacteria are major producers of antifungal metabolites especially for use in agriculture biocontrol. In this study, the identification of *Streptomyces* sp. isolated from saline soil was carried out using morphological and biochemical studies. Optimization the culture conditions to produce metabolite was done. Two fungal samples were isolated from decaying strawberry. Their molecular identity was determined. Effects of Antifungal activity of *Streptomyces* sp. activities was determined by agar dilution method and inhibitory percentage of growth evaluated. Results showed the strain of actinomyces isolate, strongly belongs to the genus *Streptomyces*. The highest metabolite weight (1.4 gram) at 32 °C, at pH 6.0, an incubation time of 120h under aerobic conditions with culture medium compounds (yeast extract 0.4%, Dextrose 0.4 %, Pepton 0.5%, NaCl 0.5%) happened. Bioactive metabolite *Streptomyces* sp. effected on two isolates of *Aspergillus* (*A. niger* and *A. flavus*) isolate. Concentration of 6 mg / ml of metabolites has 100% growth inhibition, In contrast, mancozeb fungicide had 100% growth inhibition at the same concentration too. Therefore, based on the results achieved, metabolite produced by *Streptomyces* sp. can be used as a biological agent to control the fungi infecting strawberry and it is a suitable alternative to chemical fungicides.

Keyword: *Aspergillus*, Biological fungicides, *Streptomyces*, strawberry

Introduction

Strawberry (*Fragaria x ananassa* Duch.) belongs to the family Rosaceae (Li, 2023) and widely consumed worldwide [14]. The fruit of this plant becomes juicy over time and turns from acidic to sugary. Owing to its physiological characteristics and high respiration rate, strawberry is one of the most sensitive and easily spoiled fruits, and even if there are no spoilage substances in the environment, the metabolic activities of this fruit will start after harvesting [16]. Fungal contamination is a major challenge for the horticultural crops. Fungi are present all over in the environment and can easily infect horticultural products. Due to juicy texture and delicate nature, strawberries are one of the most decayable fruits, with a very limited storage time. Fungal infections are often the main cause of post-harvest erosion in strawberries. Several fungal strains commonly affect strawberries and causing decay and reducing fruit quality [4]. Microscopic filamentous fungi produce large amounts of extracellular enzymes like pectinases and hemicellulases, which are influencing the deterioration of fruits. Contamination of fruits by fungi not only causes high post-harvest waste, but also fruits can also be a source of toxic substances harmful to humans [2]. Common fungal infections species which contaminate strawberries are *Aspergillus*, *Penicillium*, *Fusarium* [14] and *Botrytis* [4]. These contaminations may not only compromise the organoleptic quality of strawberries, but also their safety, when mycotoxin production happen [14]. *Aspergillus* is one of the largest and most intensively investigated taxons of fungi. There are more than 180 species in this genus, including *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreu* [6]. Among the fungal species, *Aspergillus flavus* is of concern due to its aflatoxin-producing ability and posing health risks to consumers. *Aspergillus flavus* found all over the place that poses a significant threat to plants as well as humans. This fungi can produces pectinases, which accelerate spoilage and quality loss [14]. *Aspergillus niger* is a wide spread fungus in the world. Observing black mold on the fruits can be the reason for the growth of this fungus [18]. *Aspergillus niger* as a pathogen not only causes remarkable losses but also produces a large number of mycotoxins, making it one of the main agent of post-harvest decay in berries [10]. In recent years, use of biological compounds to control contaminated fungal is notable. Research review revealed that streptomycetes have an inherent potentiality to produce antimicrobial agents. Sustainable agriculture is increasingly related to biological agents as alternatives to chemicals [19]. Soil bacteria have received major notice due to their extreme population diversity and production of a variety antifungal compounds. They mostly belong to the genera *Streptomyces* [1]. *Streptomyces* is the largest genus of Actinomycetota. Actinomycetes are the principal source of antifungal agents. The antagonistic activity of actinomycetes is used for the bio-control of fungal infections in herbs [7]. *Streptomyces* produces approximately 75-80% of the total antibiotics produced by microbes, such as nystatin, amphotericin B, natamycin, bafilomycin A₁, concanamycin, and 3-phenylpropionic acid [1]. The genus *Streptomyces* is famous for being a never-ending source of bioactive secondary metabolites belonging to various chemical classes such as alkaloids, aminoglycosides, terpenoids, glycopeptides, tetracyclines, polyketides, β -lactams, macrolides, and others [3]. According to what has been stated, in this study, we evaluate the antifungal potential of metabolites obtained from *Streptomyces* spp against *A. niger* and *A. flavus* isolated from strawberries. In the following, the results obtained from *Streptomyces* metabolite as a biological compound have been compared with mancozeb poison as a chemical compound.

Results Discussion

In this study, two fungal strains, *Aspergillus niger* and *Aspergillus flavus*, were isolated from decaying strawberry fruits. Macroscopic and microscopic characteristics of fungal isolates were examined "Figure 1".

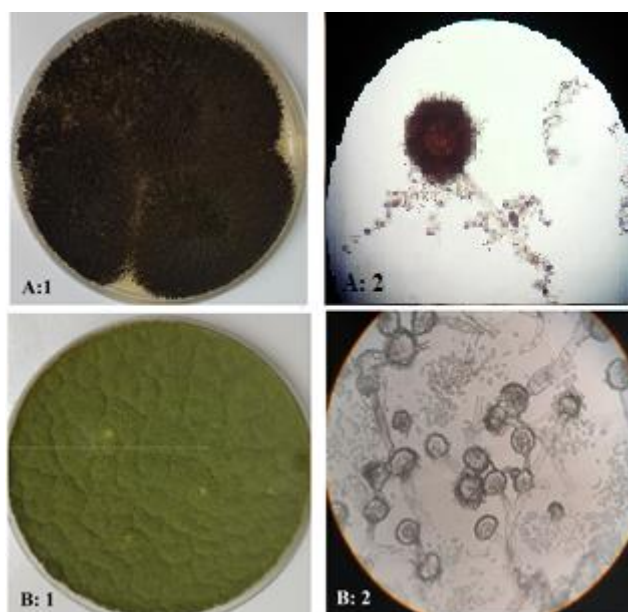


Figure (1) A:1 and A:2 Macroscopic and microscopic picture of *A. niger*, B:1 and B:2 Macroscopic and microscopic picture of *A. flavus*

To identify molecular identity, polymerase chain reaction (PCR) using ITS primers was done [15]. Based on ITS gene sequence analysis, the closest phylogenetic neighbour of isolates were *Aspergillus niger* "Figure 2" and *Aspergillus flavus* "Figure 3"

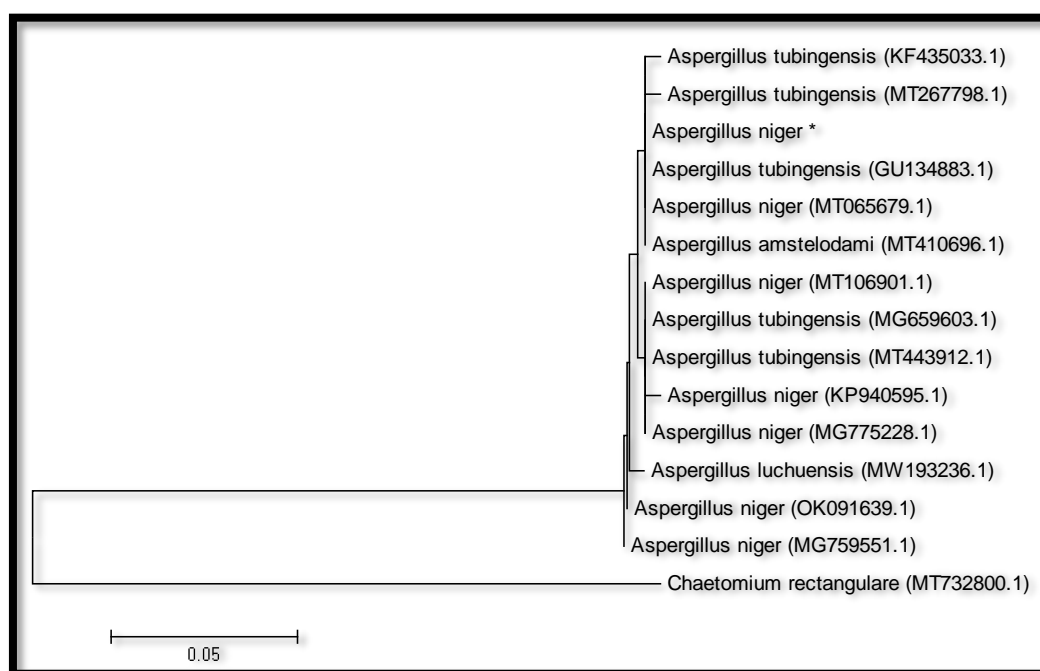


Figure (2) Phylogeny tree of *Aspergillus niger* isolates

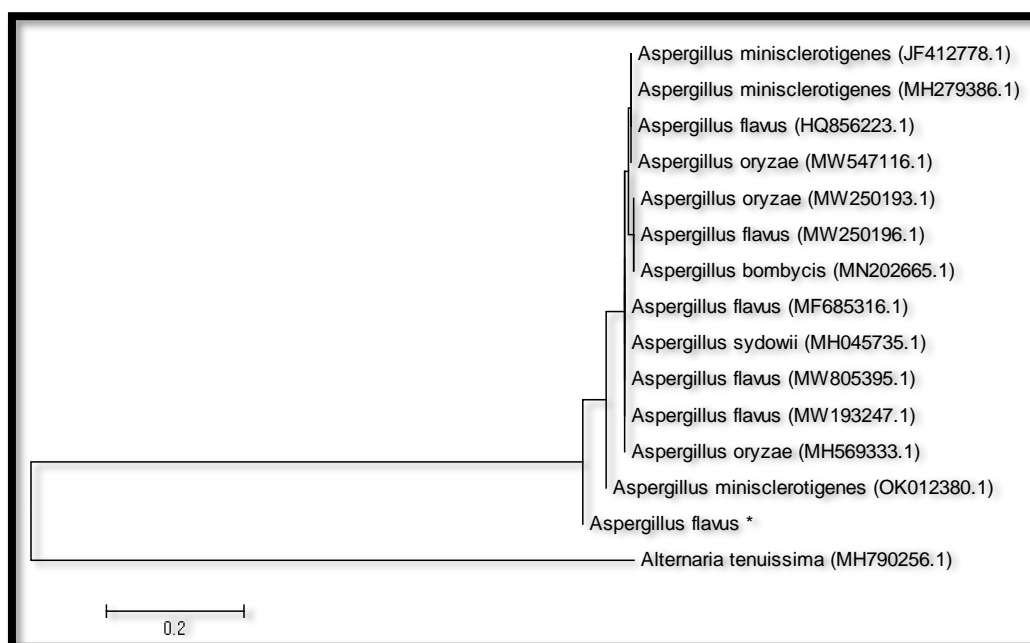


Figure (3) Phylogeny tree of *Aspergillus flavus* isolates

In the current study, an aerobic, Gram-positive, acid-fast negative (Figure 4) and non-motile actinomycete was isolated from a salty soil sample collected in the Kerman, Iran. Morphological and biochemical characteristics showed a high similarity to the genus *Streptomyces*.

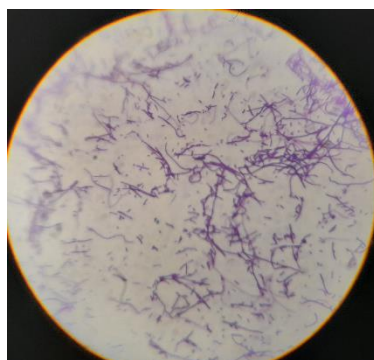


Figure (4) Acid-fast staining of streptomyces isolate

Optimization of growth condition for maximum production of metabolite by *Streptomyces* Sp. were examined. Testing different solutions to optimal biomass and metabolite product, the streptomyces Sp, exhibited maximum metabolite weight (1.4 gram) at 32 °C, at pH 6.0, an incubation time of 120h under aerobic conditions with culture medium compounds (yeast extract 0.4%, Dextrose 0.4 %, Pepton 0.5%, NaCl 0.5%). Almost Similar results were obtained in the study of Oskay (2011) in the effects of environmental conditions on biomass production by *Streptomyces* Sp. Which showed the highest antimicrobial activities under aerobic conditions at temperature 30° C and pH at 7.5. Kavitha and Vijayalakshmi (2009) in the determination of the effect of various cultural parameters on the metabolite activity of *Nocardia levis*, found medium containing 2% sucrose supported high levels of biomass and bioactive metabolite production by the strain. In this research, antifungal test of metabolite was investigated by agar dilution method. The concentration used for test were 6 mg/ml, 3 mg/ml and 2 mg/ml. Then, the inhibitory percentage of growth was evaluated by the following formula [11]. Finally, The results of test compared with Moncozeb chemical fungicide which used in agriculture.

$$I = 100 (C-T)/C$$

I: Inhibitory percentage of growth

C: Diameter of control colony

T: Diameter of treatment colony

The test results showed metabolite of streptomyces Sp. has the ability for the inhibition of *A. niger* and *A. flavus* growth in 6 mg /ml concentration "Table 1" and "Table 2". Both metabolite (biological antifungal) and mancozeb (chemical fungicide) at concentration of 1 mg /ml had no antifungal effect on any *Aspergillus* isolates.

Table 1- Percentage inhibition of *A. niger* growth

	Inhibitory percentage of growth		
	6 mg /ml (Concentration)	3 mg /ml (Concentration)	1 mg /ml (Concentration)
Metabolite	100 %	0	0
Mancozeb	100 %	50 %	0

Table 2- Percentage inhibition of *A. flavus* growth

	Inhibitory percentage of growth		
	6 mg /ml (Concentration)	3 mg /ml (Concentration)	1 mg /ml (Concentration)
Metabolite	100 %	0	0
Mancozeb	100 %	67 %	0

Related studies have confirmed that many biological factors have been found to control fungi causing spoilage of agricultural products. Giacomelli Ribeiro (2024) confirmed actinobacteria from genus *Amycolatopsis*, *Curtobacterium*, *Kocuria*, *Nocardioideis*, *Nocardioipsis*, *Saccharopolyspora*, *Streptovericillium* and especially *Streptomyces* showed a broad antifungal spectrum through several antibiosis mechanisms such as the production of natural antifungal compounds, siderophores, extracellular hydrolytic enzymes and activation of plant defense system. Park (2024) found a strain of *Streptomyces* with remarkable antifungal activity against multiple phytopathogenic fungi. This strain not only inhibited seven phytopathogenic fungi including *Fusarium oxysporum* and *Aspergillus niger* and but also showed a control effect against *F. oxysporum* infected red pepper, strawberry, and tomato in the in vivo test. Shen (2024) worked on antifungal bacterium, *Streptomyces graminearus* STR-1, which showing antagonistic activity to diverse fungal pathogens including *Magnaporthe oryzae*, *Rhizoctonia solani*, *Fusarium graminearum*, *Ustilaginoidea virens*, and *Bipolaris maydis*. Its antifungal activity was relatively stable and less affected by temperature and pH.

Conclusions

Postharvest decay is one of the main factors that determine losses and reduce food quality of strawberry. *Aspergillus* is a group of fungi that easily grow on strawberries and cause them to spoil quickly. Chemical fungicides resulted in environmental pollution, expanded risk of health problems, development of pathogenic fungi resistance, reduce soil quality, and produced hazardous agricultural products. It is evident from the results of this investigation that bioactive metabolite *Streptomyces* sp. effected on two isolates of *Aspergillus* (*A. niger* and *A. flavus*) isolated from strawberries. Concentration of 6 mg / ml of metbolites has 100% growth inhibition. In contrast, mancozeb fungicide had 100% growth inhibition at the same concentration too. Based on what was obtained from the results of this research, it seems logical to use the metabolite streptomyces sp. as a biological agent to control and eliminate fungi that could corruption.

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