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Antibacterial activities secondary metabolites from endophytic fungus *Fusarium solani*.

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Abstract

An endophytic fungus isolated from root of tomato plantdisplayed considerable antibacterial activity. The fungus was identified as *Fusarium solani* based on morphological characterization. Fungal extraction was carried out by ethyl acetate solvent. The metabolite showed activity against both bacterial testes. Bioactivity of fungal extract against *E. coli*(ATCC25922) *and S. aureus* (NCTC6571)by using a disc diffusion technique was examined. The inhibition zones exhibited by fungal extract were 28 mm diam of *E. coli* and 45 mm diam of *S. aureus* . MIC test revealed that the extract of *Fusarium solani* exhibited a minimal inhibition values (12.5 and 6.25 ug/L) against *E. coli and S. aureus*, respectively.A verification of non-toxicity of the fungal extract against human blood revealed a negative test. The chemical analysis of the fungal crude extract showed that extract of*F. solani* contains Tannins group and phenol compounds, absent amino acid andflavonoide.The metabolite produced by theendophytic fungus could be an alternative source of antimicrobial agents against clinical pathogens.

Keywords: Antibiotics, bacteria , tannin , bioactive metabolites, Endophytic fungi.

1.Introduction

Endophytic fungi are defiend as microbes capable of living in host plants tissue without causing any symptoms [1]. To date, endophytic fungi have been separated into four classes based on host range, type of tissue(s) colonized, colonization in planta, diversity in planta, transmission and fitness benefits [2].Endophytes belong to diverse groups of bacteria and fungi [3]. Endophytic fungi are extremely ubiquitous; it is thought that the vast majority of plant species in natural ecosystems (if not all of them) harbor fungal endophytes[2]. Endophytic fungi are estimated to be represented by at least one million species residing in plants [4]. Research on endophytes dates back to over one hundred years[5]. During this period,

several aspects of endophyte biology were thoroughly studied, including the diversity, taxonomy, reproduction, host ecology and effects on the host [6].The ubiquity of endophytes in the plant kingdom is well investigatedspecies so far [7].The analysis of any plant material may result in the discovery of a range of different endophytic fungi. Many of these fungi might appear to be specific to a particular host. In addition environmental and edaphic conditions are expected to affect the nature and the population of endophytes.They are an important and novel source of natural bioactive compounds with great potential applications in agriculture, medicine and the food industry [8][9]. Since the "gold" bioactive compound paclitaxel was successfully discovered in the endophytic fungus *Taxomyces andreanae* in 1993, many scientists of interested instudying fungal endophytes as potential producers of novel and bioactive compounds, and over the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered in endophytic fungi. These bioactive compounds could be mainly classified as alkaloids, terpenoids, steroids, quinones, isocoumarins, lignans, phenylpropanoids, phenols and lactones [10][11].

2.Materials and methods.

2.1Isolation of endophytic fungi

The fungus used in this study was obtained as an endophyte collected from Farms intheprovinceofMaysan .The isolation of endophyticfungus*Fusariumsolani* was carried out as described by [12] with minor modifications.Briefly, plant sample, which included root washed under running tap water for 10 min, and then airdried.Before surface sterilization, roots were cut into small fragments using sterile surgical blades into 1 cm long segments. Sample of fragments were successively surface sterilizedby immersion in 70% ethanol for 1 min,5.25% sodium hypochlorite solution for 5 min, 70% ethanol for 30 s and sterile distilled water for3 to 5 s. The cut surfaces of the segments were placed on Petridishes containing malt extract agar (MEA) (oxoid) supplementedwith chloramphenicol, Merck) and streptomycinsulphateto suppress bacterial growth and incubated at 28°C until the outgrowth of endophytic fungi wasdiscerned. Pure cultures were then transferred to malt extract agar (MEA) plates freeof antibiotics and incubated three weeks on PDA plates at 28°C. fungal culture were identified of fungi wasconfirmed according to the availabletaxonomic literature [13] [14] [15].

2.2Fungal culture extraction

Five discs (0.5 mm diam.) were cut from the axenic fungal culture of each isolate by using a cork borer which amended into PD liquid medium in 500 ml flasks (with triplicates) and incubated at 25 C for 2 weeks on a rotary shaker. Fungal cultures were filtered on Whatman No 1 filter paper and the pH was adjusted at 3 by drops of HCl for fungal filtrate. Filtrate was extracted in ethyl acetate (1:1 vol:vol) by using separating funnel. The organic layer was collected by dehydration of water by using Na_2SO_4. The filtrate was filtered again and placed in Petri dishes then left to dry at room temperature. 100 ug of the dried extract was dissolved in 1 ml ethanol as stock extract solution to be used for further experiments.

2.3Antimicrobial bioactivity assay.

Filter paper discs (0.6 mm) after being sterilized by autoclave were socked in fungal crude extract solution for 5 min., filter paper discs with extract were placed on the surface of Muller-Hinton agar medium in Petri-dishes streaked with 0.2 ml of bacterial suspension of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). Plates were incubated at 37 ◦C for 24 hr, an appearance of inhibition zones around the filter paper disc indicated the bioactivity of crude metabolites of the tested fungal isolates [16]. The diameters of the clear zones were measured and compared with control agar plates containing discs with solvent only (control), triplicates were made.

2.4 Minimal inhibitory concentration test.

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined by the standard serial dilution assay [17]. The MIC value in this assay was indicated by the absence of bacterial growth at the minimal concentration of the extract. Crude extracts fungs *Fusarium solani* isolate were selected for this test. The inhibitory test was carried out on Muller-Hinton agar medium.

2.5 Chemical analysis of fungal crude extracts.

Fungal culture extracts of *Fusarium solani* were chemically analyzed for alkaloids, phenols, amino acids, flavenoides and tannins according to method described by [18].

2.6 Toxicity test

Cytotoxicity of the fungal crud extract was examined by using human RBC following the method of [19].

2.7 Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) between pair of variables.

3.Results

The strain was isolated from the root of *Lycopersicum esculentus.* The isolated endophyte typically possessed small hyphae, as a white colony mycelium, when young. The mycelia are thread-like, branched, septate and slow-growing; spores werecylindrical to oval resembled *Fusarium solani*.

The preliminary screening of *Fusarium solani*. for their bioactivityagainst both bacteria *E. coli* and *S*of clearzones ranged between 22 and 28 mm diam(Table 1) (Figs. 1).

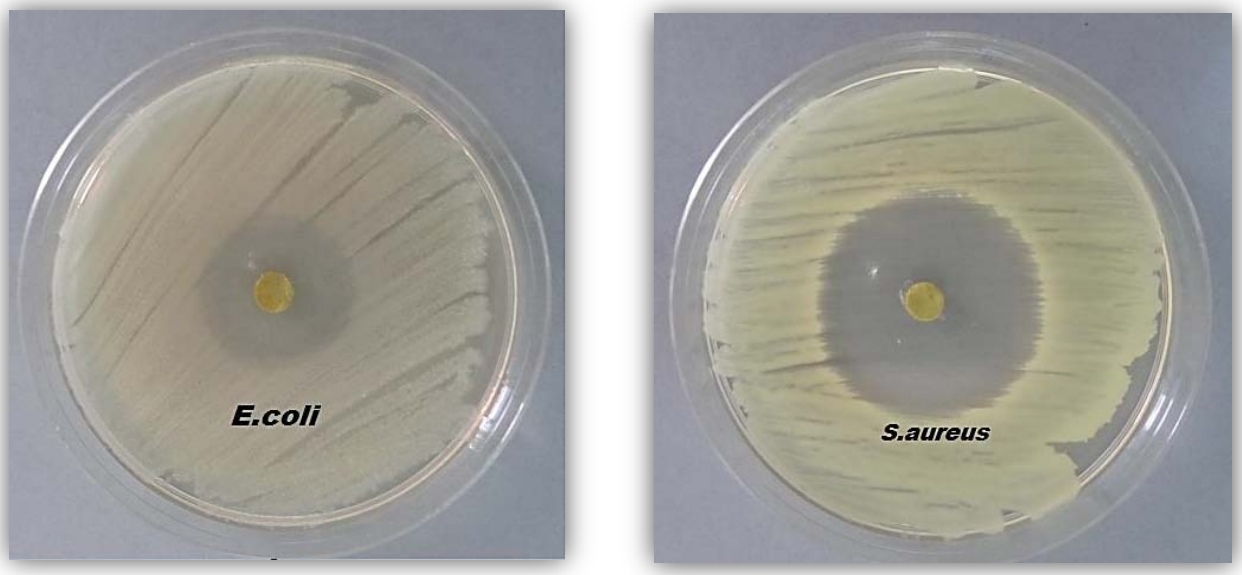

Fig. 1.: Inhibition zones(mm) exhibited by fungal crude extracts against *E. coli and S . aureus*

Table 1. Growth inhibition zones (mm) exhibited by the fungal culture filtrate extract against two isolates of bacteria.

Fungal species	*E. coli*	*S. aureus*
Fusarium solani	28	45

Numbers represent average of three replicates$P \leq 0.05$

The MIC andMBC tests indicated that the extract of *Fusarium solani* exhibited the minimal values of MIC (12.5 and 6.5 ug/L)and MBC (25 and 12.5 ug/L) against *E. coli*and *S. aureus*, respectively (Table 2).

Table2. The minimum inhibitory concentrations (MIC) and minimum Bactericidalconcentrations (MBC) of fungal crude extracts against two isolates of bacteria.

Fungal species	MIC (ug/L)		MBC (ug/L)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Fusarium solani	12.5	6.5	25	12.5

Numbers represent average of three replicates

The chemical analysis of the fungal crude extract showed that extract *Fusarium solani* contains Tannins group and phenol compounds, but lacksamino acids and flavonoid (Table3).

Table 3. Chemical compound groups of the fungal crude extract

Fungal species	Alkaloides	Amino acids	Flavonoids	Phenols	Tanins
Fusarium solani	+	-	-	+	+

+ present – absent

4.Discussion

The endophyte fungus from Iraq was identified as *F. solani* by morphological characterization. Identification of*Fusarium solani*., at the subgenus level by morphological traits is difficult because of unstable mycelial pigmentation, shape and sizeof conidia feature[20].Most *Fusarium*spp.are soil-borne and widely distributed in natureas saprophytes and pathogens. Some species cause plant diseases with important economic. impacts while others cause severe human infections [21]. *Fusarium* spp. have been reported as endophytes from several plants with diverse biological activity [22].

Fungi in general are a good source for antimicrobial agents [23]. Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds, which are of pharmaceutical, industrial and agricultural importance have been reported and characterized from fungal endophytes. [24] suggested studying endophytic fungi since such plants may harbourunique andrare endophytes capable of producing important bioactive metabolites with multiple applications.*Taxus*, a gymnosperm is an important anticancer plant. Several endophytic fungi isolated from *Taxus* spp., worldwide have been reported to produce important bioactive metabolites[25].the production of metabolic substances by fungi, in general, is often affected by various growth conditional factors mainly the fermentation medium [26]. In the present study a liquid state fermentation medium used is efficient for a mass production of bioactive metabolites by the selected fungi.The crude filtrate extract of the examined specie of *Fusarium solani* exhibited an inhibitory action against both bacterial strains *E. coli* and *S. aureus.* However, a variation in the inhibitory action, based on the inhibition zones diameter , among the examined fungus was noticed. Extract higher inhibition zones against *S. aureus* while the extract exhibited lower inhibitory action against *E. colli*.The Chemical analysis of the crude extract indicated that*Fusariumsolani*possesse tannin compounds and phenol compounds .The antimicrobial inhibitory impact of these extracts can be related to the bioactivity of these compounds. It has been reported that several phenolic compounds including tannin are potent inhibitors of microbial enzymes like protease [27]. Other studies showed that tannin inhibits the growth of both *E. coli and S. aureu*s and has been attributed to the mechanism of tannin binding with the protein of the bacterial cell walls [28].A verification of non toxicity of the fungal extract against human blood revealed a negative test [29]. It can be recommended here that the extract should be kept in capsulated polymeric substances so it may last longer and maintains its bioactivity. A conclusion can be derived from this preliminary screening

of endophytic fungus *Fusarium solani* that thisfungus possesses a potential secondarychemical compounds that can be ofsignificance and a promising asantimicrobial agents. However, a furtherinvestigation is needed to examine thebioactivity of these extracts against morepathogenic bacteria and pathogenic fungi aswell.

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الفعالية الضد البكتيرية للأيض الثانوي من فطرالنابوت الداخلي *Fusarium solani*

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الخلاصة

تضمنت الدراسة عزل وتشخيص الفطر *Fusarium solani* من النابوت الداخلي Endophytic fungus من داخل جذور نبات الطماطه وتنقيته وتنميته في وسط PDB واستخلص الراشح والذي اظهر فعالية ضد بكتيرية عاليه,استخدم المذيب اثيل استيت للحصول على المستخلص الفطري. وقد اظهر هذا المستخلص فعاليه تجاه كلتا العزلتين القياسيتين*E.coli*(ATCC25922) و*Staphylococcus aureus*(NCTC6571)بأستخدام تقنيه الانتشار بالاقراص وكانت اقطار مناطق التثبيط للمستخلص الفطري 28 ملم تجاه *E.coli*و45 ملم تجاه *St.aureus* . وكشف اختبار التركيز المثبط الادنى (MIC) للمستخلص الفطري قيم تثبيط هي (12.5 و6.25ميكروكرام/لتر) ضد بكتريا*E.coli* و*St.aureus*على التوالي.كما لوحظ انخفاض فعالية المستخلص عند خزنه بدرجه حرارة (8) ولمدة ستة اشهر.كما لم يظهر المستخلص الفطري أي سميه تجاه كريات الدم الحمر للإنسان. اظهرت الكشوفات الكيميائية للمستخلص الفطري احتواءه على التانيناتوالفينولات وعدم احتواءه على احماض امينيه وفلافونيدات.ان الايض المنتج من فطريات Endophytic يمكن ان يكون مصدرا بديلا للمضادات البكتيرية .

الكلمات المفتاحية: المضادات ألحيوية البكتريا , التانينات , الايوض الفعالة , فطريات الاندوفايت.