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The study inhibition activity of *Penicillium rubrum* crude extract to some pathogenic bacteria

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Abstract:

The antibacterial activities of *Penicillium rubrum* crude extract to six pathogenic bacteria were investigated. Results indicated that, the inhibition activities were increased according to the concentration of fungal filtrate. A highest inhibition activity was observed against *Streptococcus pyogens*, the inhibition zones were 29, 33, 37, and 40mm at concentrations 70, 80, 90, and 100% respectively. The inhibition zones against other bacterial isolates were 15, 17, 25, 27, and 33mm for *Klebsiella* sp., *Staph. aureus, Proteus mirabills, E.coli,* and *Salmonella* sp. At concentration 100% respectively in comparison with standard E. *coli (TET res)* when inhibition zone was 13mm.

دراسة الفعالية التثبيطية للمستخلص الخام للعفن *Penicillium rubrum* لبعض عزلات البكتريا الممرضة

الخلاصة:

هدفت الدراسة الى التحري عن الفعالية التثبيطية للمستخلص الخام من العفن Penicillium rubrum لستّ عزلات من البكتريا الممرضة. أشارت النتائج الى أن الفعالية التثبيطية قد زادت طبقاًلزيادة تركيز الراشح الخام. أعلى فعالية تثبيطية لوحظت ضد *Streptococcuspyogens*، حيث كانت الفعالية التثبيطية 29, 33, 37 و 40 مليمتر عند استخدام التراكيز 70, 80, 90 و ٪100 على التوالي. اما الفعالية التثبيطية ضد العزلات البكتيريا الأخرى كانت 15, 17, 25, 27 و 33 مليمتر لكل من *Klebsiella sp., Staph.aureus, Proteus mirabills, E.coli* و *Salmonella sp.* بتركيز ٪100 على التوالي بالمقارنة مع العزلة القياسية (E.coli TET res) حيث كانت الفعالية التثبيطية 13 مليمتر.

Introduction

Penicillium is an ascomycetous fungal genus with widespread occurrence in most terrestrial environments. About two hundred species are well described and most of them are soil inhabitants, food borne contaminants or food ingredients used in the preparation of cheese and sausages (1,2).The maximum number soil *Penicillium* sp. were found in the of during summer seasons rather than dry or winter season. The associated mycobiota of *Penicillium* had also a higher population in rainy seasons (3). Morphological Characterization of *Penicillium* species was carried out based on fungal morphology (macroscopic and microscopic). Macroscopic study was done by studying growth rate, color texture and topography of colony using two standard media namely Potato Dextrose Agar (PDA) and Czapek Dox Agar. Microscopic study of different *Penicillium* species was done by preparing slide mount with lacto phenol cotton blue stain and observed under light microscope (4). The *Penicillium* species isolated from the virgin soils had potential antimicrobial activity against clinical bacterial pathogens which may further be utilized for production of novel fungal compounds (5). Primary metabolism encompasses reactions involving those compounds which are formed as a part of the normal anabolic and catabolic processes (6). Microbial secondary metabolites are compounds produced mainly by actinomycetes and fungi, usually late in the growth cycle (idiophase). Although antibiotics are the best known secondary metabolites, there are others with an enormous range of other biological activities mainly in field like: pharmaceutical and cosmetics, food, agriculture and farming. These include compound with anti-inflammatory, hypotensive, antitumour, anticholesterolemic activities, and also insecticides, plant growth regulators and environmental friendly herbicides and pesticides. These compounds are usually produced by liquid submerged fermentation, but some of these metabolites could be advantageously produced by solid-state fermentation (7). *Penicillium rubrum* was the toxigenic fungi isolated by Burnside *et al* (8). This fungal isolate can produce both primary and secondary metabolites which exhibits antimicrobial activities (9,10), So the present work aimed to obtained these metabolites and test its activity against six pathogenic bacteria.

Material and Methods

Fungal and Bacterial isolates:

Fungal and bacterial isolates were obtained from Bghdad university Biotechnology Department, Colloge of Science, and these isolates were:

◈ *Penicillium rubrum, E. coli, Salmonella sp. Staphylococcus aureus, Streptococcus pyogens, Proteus mirabilis, Klebsiella sp.*

E. coli (TET res)

Maintenance to fungal and bacterial isolates: Fungal isolates were inoculated in potato dextrose agar medium as slants, incubated for 7 days at 28°C and then stored at 4°C until use. Bacterial isolates were inoculated in nutrient agar medium as slants,then incubated for 24h at 37°C and stored at 4°C until use. Wilson and (11). And to achieve this aim the below steps were followed: Production of *Penicillium rubrum* metabolites using submerge culture. Separation and sterilization of metabolites by filtration. Antibacterial assay of metabolites against six pathogenic bacteria.

Metabolites production: Metabolites were produced in submerged medium, by inoculating each flask with loop filled with *penicillium rubrum* spores and incubated at 25-30°C for 4-5 days, for growing and producing metabolites that have antimicrobial activity.

Extraction and Sterilization of fungal filtrate: Separation by filter paper (1.5µm) to separate extract from mycelium and culture medium.

1. Centrifugation at 1000 rpm at 20min to separate the extract from any remaining cells.
2. Sterilization by Millipore filter (0.22µm) to purify the extract.

Antibacterial activity assay: The crude extract of *P. rubrum* was investigated for antimicrobial activity against bacterial pathogens *Klebsiella* sp*., Staph. aureus, Proteus mirabills, E.coli,* and *Salmonella* sp. using the holes or well method with

Petri dish template system inoculated with 0.1ml of the assayed pathogenic bacteria, followed by incubation at 37°C for 24 h. The bacterial cultures grown in nutrient broth were inoculated on the surface of the nutrient agar media and gently spread through the help of glass spreader. After drying 0.1µl of the crude extract was placed within hole with three replicate that was making by using Cork borer instrument.

Results and Discussion:

The rubratoxin B (Fig 1.3), a toxic secondary metabolite of *P. rubrum* (11). Rubratoxin B is mutagenic, hepatotoxic, nephrotoxic and splenotoxic to several animals (8, 12,13,14,15). Even though rubratoxin B has negative health effects, it has potential as an anti-tumor agent (16,17). Mycotoxins are nearly all cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis. Of course they are also toxic to the cells of higher plants and animals, including humans (18).

The antimicrobial activity (mm) of *P. rubrum* crude extract against six pathogenic bacteria e.g. *E.coli, Salmonella* sp., *Staph. aureus, Strep. pyogens, P. mirabillis* and *Klebsiella* sp. where investigated in comparison with standard *E.coli (TET res) provided from Baghdad university - colleg of science*. The results in table (1) indicated that gradual concentration

of *P. rubrum* crude extract against bacterial isolates has extrusive proportional, the inhibitory activity increase with increasing in *P rubrum* crude extract concentration, the inhibitory activities of crude extract at conc. 70% toward, *E.coli, Salmonella* sp., *Staph.aureus, Strep.pyogens, Proteus mirabillis, Klebsiella* sp. and *E coli (TET res)* were 20mm, 17mm, 15mm, 29mm, 12mm 7mm and 5mm. While at conc. 100%, where 27mm, 33mm, 17mm, 40mm, 25mm, 15mm, and 13mm, the highest inhibitory activity of crude extract was toward bacteria *Strep. pyogens* (40mm), and the lowest inhibitory activity was toward, *Klebsiella sp.* (15mm). The antibacterial activity against *p. mirabilis* and *Klebsiella sp* were illustrated in as in figures (1) and (2).

Table 1: Inhibition activities (mm) of *Penicillium rubrum* crude extract against pathogenic bacteria.

Bacterial isolate	*Filtrate concentration (%)*			
	70	80	90	100
	Inhibition zone mm			
E. coli	20	22	23	27
Salmonella sp.	17	21	25	33
Staphylococcus aureus	15	17	17	17
Streptococcus pyogens	29	33	37	40
Proteus mirabillis	12	19	22	25
Klebsiella sp.	7	13	15	15
E. coli (*TET res*)	5	7	13	13

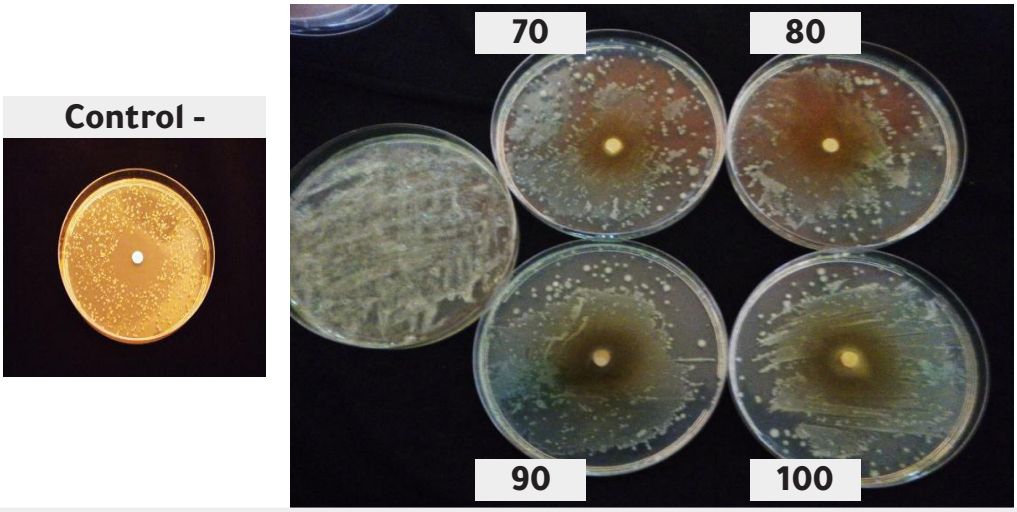

Fig. 1: The antibacterial activity of *Penicillium rubrum* crude extract against *proteus mirabilis* growing on nutrient agar medium under incubation at 37°C for 24h and using chloramphenicol as control.

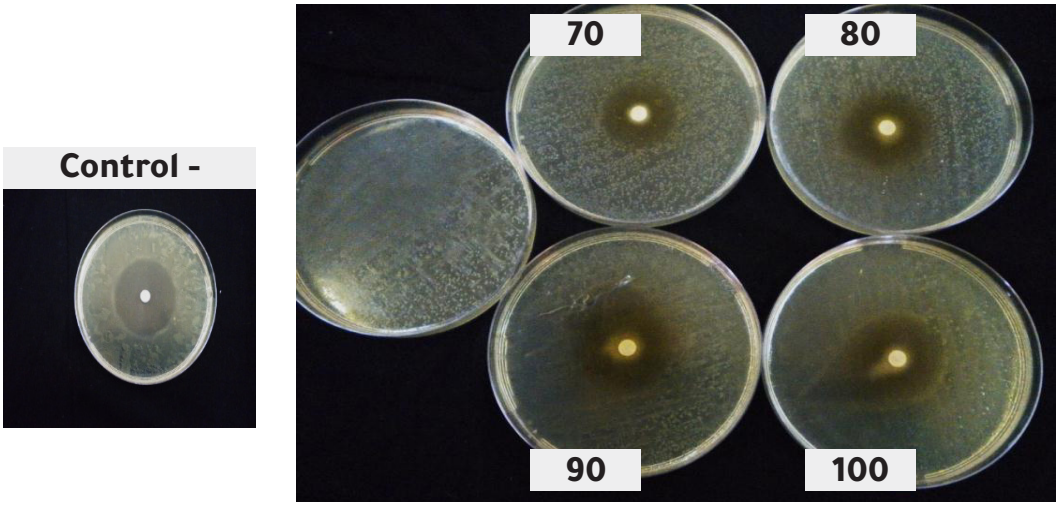

Fig. 2: The antibacterial activity of *Penicillium rubrum* crude extract against *Klebsiella* sp growing on nutrient agar medium under incubation at 37°C for 24h and using chloramphenicol as control-

From the results which obtained in current work we can concluded that, the crude extract of *P. rubrum* has inhibitor activity against six pathogenic bacteria *E.coli, Salmonella.sp., Staph. aureus, Strep. pyogens, P. mirabillis* and *Klebsiella* sp., the crude extract has extrusive proportional against bacterial isolates the inhibitory activity increased with the increasing in *P. rubrum* crude extract, the highest inhibitory activity of crude extract at concentration 100% toword bacterial isolate *Strep. Pyogens* isolates (40mm) and the lowest inhibitory activity of crude extract was to ward *Klebsiella* sp. (15mm), this due that crude extract was contain several compounds have inhibitory activity against organisms, for example phoenicin inhibit the growth of *Staph. aureus*. Hepatotoxic substance has inhibitory activity against higher animals (8). Rubratoxin B has limited toxicity to microorganisms (8,11) therefore; the results were varying against pathogenic bacteria, Rubratoxin B has no effect on the algae, fungi, or gram negative bacteria . Antiprotozoal activity of Rubratoxin B was on the growth response, nucleic acids, and protein content (19). Rubratoxin B appear to be toxic to variety of higher animal (8,11). Natural S.M, Methyl 6-acetyl-u-methoxy-5,7,8-trihydroxy naphthalene, showed to be the most active compounds, possessing expressive activity against *Candida albicans, Listeria monocitogenes* and *Bacillus* (20).

Conclusions The crude extract of *penicillium rubrum* showed activity against pathogenic bacteria, and the inhibitory activity was extrusive proportion with the crude extract concentration.The highest inhibitory

activity of crude extract was toward *Strep. pyogens* (40 mm), and the lowest inhibitory activity was toward bacteria *Klebsiella sp*. (15mm), and inhibitory activity toward standard isolate *E.coli (TET res)* was (13mm).

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