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Research Article

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Production of Cephalosporin C from *Acremonium chrysogenum*, and its Antimicrobial Activity against Some Pathogenic Bacteria

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Abstract The Production of cephalosporin C by *Acremonium chrysogenum*in flask contain fermentation medium, after that its extraction, purification and estimation was performed by Spectrophotometric Method**.** The antimicrobial activities of both the crude and purified cephalosporin C were performed against the test organisms by Agar Well Diffusion Method. Agar well diffusion method and the inhibition zones were recorded and evaluated the effectiveness of the antibiotic against gram positive bacteria (*Staphylococcus aereus* and *Bacillus subtilis*) and no effectiveness against gram negative bacteria (*Pseudomonas aeruginosa* and *Proteus mirabilis*).

Keywords Cephalosporin C, *Acremonium chrysogenum*, Antimicrobial, Well diffusion method

Introduction

Cephalosporin Cfungal metabolite is produced industrially in batch process by *Acremonium chrysogenum* (formerly named *Cephalosporium acremonium*) was isolated from seawater close to a sewage at Cagliari (Sardinia, Italy) in 1945 by Giuseppe Brotzu [1]. Over the past 50 years, β-lactam antibiotics were industrial produced by fermentation, which lead to enhanced productivity and substantial cost reduction [2-3].

Today, cephalosporin derivatives are widely used in the treatment of infectious diseases, and are one of the world's major biotechnological products [1].Cephalosporin C area bactericidal agents used in treatment of infections caused by bacteria susceptible to this particular form of antibiotic and less toxic but are less susceptible to penicillinases [4]. Fed-batch fermentations are used for production of cephalosporin C (CPC) by using complex media. The presence of CPC in fermentation broth was determined by a combination of biological and spectroscopic methods [5].The technology for separating and purification of biological products from fermentation broth is difficult because used complex medium [6].

In this present paper, *A. chrysogenum* was isolated from soil to produce CPC by using a semisynthetic medium in shake flask, and the antimicrobial effect of CPC against some Gram positive and Gram negative bacteria was investigated.

Materials and Methods

Materials

Soil Sample Collection

The samples of soil were collected from fertile soil of agricultural land in Gaza Strip.

Microorganisms

Pathogenic strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus mirabilis* were obtained from the biology department of the Islamic University-Gaza (IUG) as tested organisms in this study.

Antibiotics

Antibiotic was used: Cefaclor (cj30), Cephadroxil (CDX30), and Cefixime (CFM5) as biochemical test in this study.

Methods

Sample Collection and Isolation

For each collected sample, 3 g soil was diluted in 100 ml of distilled water and allowed to stand for 15 min. An aliquot of 0.1 ml of each suspension was plated on potato dextrose agar (PDA) as selective medium. Plates were incubated at 25°C, and monitored after 5days. Representative colonies were selected and streaked on new plates of selective medium PDA. The isolated Acremonium species were preserved on PDA plates at 4°C until further use [7].

Morphological Characteristics

The macroscopic and microscopic features were investigated according to [8]. The texture and colour of the colonies and their distribution was observed.

Biochemical Characterization

The inhibitory effect of different antibiotics on the isolated fungus was observed. These antibiotics include Cefaclor (cj30), Cephadroxil (CDX30), and Cefixime (CFM5).

Production of Cephalosporin C

The medium used for the production of antibiotic was 2.7 g glucose, 3.6 g sucrose, 0.7 g NaCl, 4 ml salt solution A (composed of 11.5 g KH_2PO_4, 15.6 g K_2HPO_4, 0.16 g Na_2SO_4, 0.13 g $MgSO_4.7H_2O$, 0.022 g $ZnSO_4.7H_2O$, and 0.055 g $CuSO_4$ per 250 ml of distilled water) and 0.75 ml salt solution B made up of 2 % ferrous ammonium sulfate [9]. 5 ml media was prepared in test tube and 1-2 loop full of *Acremonium chrysogenum* culture was inoculated into broth and incubated at 28 °C, 200 rpm for 24 hr in shaker incubator. Then, added to fresh 50 ml media and incubated for 72 hr in shaker incubator. 200 ml media was prepared and inoculated with the 50 ml broth present in 250 ml Erlenmeyer flask and incubated for 4 days in shaker incubator at the same conditions. Then we took 20 ml from the 250 ml flask and put it in 200 ml fresh medium. Estimation of cephalosporin C was done after each inoculation by spectrophotometric method [10].

Purification of Cephalosporin C

Ten milliliters crude filtrate was taken and its pH was adjusted at 2.1 with HCl & stirred for 30 min with 0.025 gm of activated charcoal then the mixture was filtered on filter paper. The cake was then washed with 1 ml DW (distilled water) and again the pH of filtrate was adjusted at pH 7 with NaOH. Further it was stirred with 0.1 gm of activated charcoal for 30 min. Then it was filtered and washed with 1 ml of DW twice. Further it was extracted with 30 ml of 10 % chloroform in separation funnel and then put it in oven overnight [11].

Antimicrobial Activity Test of the Cephalosporin C

Antibiotic sensitivity test for the cephalosporin C was carried out to check the purity of the antibiotic produced.The antibacterial activity was again performed for the pure samples against the same test organisms as it was done earlier by well diffusion method [12].

Results and Discussion

Morphological Characteristics

Macroscopic Features

The texture of the colony is compact, flat or folded, and occasionally raised in the centre. It is glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed. By aging, the surface of the colony may become cottony due to the overgrowth of loose hyphae. The colour of the colony is white, pale grey or pale pink on the surface (Figure 1). These findings were in consistent with the reported macroscopic characteristics of *Acremonium chrysogenum* [8].

Microscopic Features

They usually appear in clusters, in balls or rarely as fragile chains. The conidia are bound by a gelatinous material. They may be single or multicellular, fusiform with a slight curve or resemble a shallow crescent**(Figure 2)**. These structural properties of conidia vary depending on the species [8].

Figure 1: The macroscopic features of the isolated fungus

Figure 2: The microscopic features of the isolated fungus

Biochemical Characterization

There was not any inhibitory effect observed of the tested antibiotics on the isolated fungus. These antibiotics include Cefaclor (cj30), Cephadroxil (CDX30), and Cefixime (CFM5).So, according to the morphological and biochemical characterization it is confirmed that the isolated fungus was *Acremonium chrysogenum*[8].

Growth of*Acremonium chrysogenum*

The growth rate of *Acremonium chrysogenum* was moderately rapid and reach the maximum growth rate within 50-55 hours as shown in the curve **(Figure 3)**.

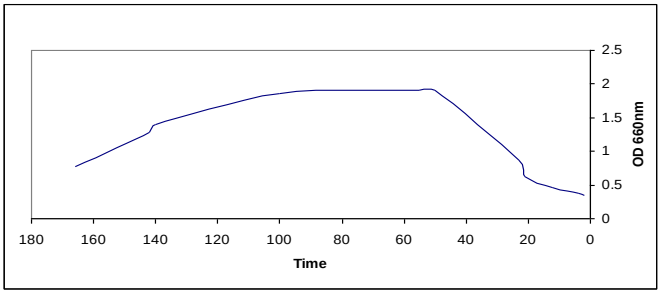

Figure 3: The growth curve of the isolated Fungus Acremonium chrysogenum

Cephalosporin C Activity Test

The antibacterial activity of the crude and purified cephalosporin C against the tested pathogenic bacteria was investigated (**Figure 4**). It has been observed that gram positive (*Staphylococcus areau s*and *Bacillus subtilis*) was more susceptible to the employed cephalosporin C than gram negative (*Pseudomonas aeruginosa* and *Proteus mirabilis*) as shown in **Table 1**.


A


B

Figure 4: Crude cephalosporin C (A) and purified (B)

Table 1: The effect of cephalosporin C against testedbacteria

Staphylococcus aureus	Inhibition
Proteus mirabilis	No inhibition
Pseudomonas aeruginosa	No inhibition
Bacillus subtilis	Inhibition

Conclusion

The results of this study were encouraging and suggested the possibility of more researches to find another organism for treating bacterial infections.

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