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INDUCTION OF CEPHALOSPORIN C IN *Cephalosporium acremonium* USING U.V. LIGHT AND SELECTION OF A MUTANT THAT SHOWED HIGHEST FERMENTATION CAPABILITY

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ABSTRACT: ***Ultraviolet-light mutagenesis had been used in the induction of cephalosporin C (CPC) production in Cephalosporium acremonium ATCC 11550. Selection of high antibiotic-producers among the mutants was carried out by agar diffusion method using Bacillus subtilis ATCC 6633, and then final selection of the highest CPC producing mutant was carried out in shake flasks in which it produced 900 mg/L. The newly-isolated mutant (M104) produced 2160 mg/L CPC in a batch process in NBS stirred-tank bioreactor (working volume 4L). The fermentation and sugar assimilation behavior of the strain M104 have been investigated. It has been observed that adequate aeration is critically important to supply oxygen that is necessary for antibiotic biosynthesis. The diauxic phenomenon has been observed in this fermentation process in which a mixture of an easily metabolized sugar (glucose) with another more difficult to be assimilated (sucrose) is used to build up the antibiotic-producing biomass (by glucose) and to make the fungus attain the physiological state of antibiotic production (by sucrose). The maximum CPC concentration was obtained at 96 hours of fermentation time, the maximum specific productivity was 186 mg CPC/g dry weight at 136 hours, and the maximum rate of CPC production was 31 mg/L.hr at 64 hours. This work is considered a model of strain genetic improvement that is carried out to enhance the antibiotic production in industry to increase the profit with a reduction of the cost of the final product.***

Key Words*: Cephalosporium acremonium; ultraviolet-light mutagenesis; cephalosporin C; diauxic phenomenon***.**

INTRODUCTION

The discovery of antibiotics started with the discovery of the efficacy of a β-lactam compound and is perhaps the most important discovery in the history of therapeutic medicine (Brakhage, 1998). The application of antibiotics to treat infectious diseases may conceivably have saved more lives than any other medical development (Heatly, 1990).

Cephalosporins were developed to overcome the allergic problems associated with penicillins (Lowe, 2001), also they have broad antibacterial spectrum, and in addition was highly resistant to β-lactamase (Carlile *et al.*, 2001). Cephalosporins are made from cephalosporin C, the fermentation product of *Cephalosporium acremonium*, which after extraction and purification, is hydrolyzed, either enzymatically or chemically, to the active nucleus, 7-aminocephalosporanic acid (7-ACA), which serves as substrate for the chemical synthesis of injectable and oral semi-synthetic cephalosporins (Lowe, 2001).

Due to the outgrowth of human population in Egypt, and consequent increasing need to afford sufficient quantities of antibiotics for treatment of infectious disease, there is a new trend to produce these chemicals locally. This will reduce the imported quantities of raw materials which may contribute to a reduction of the cost of such medications, and therefore, a reduction of the cost of health care. Despite the importance of such work, very few attempts have been carried out to improve cephalosporin C production by mutation in Egypt. El-Bondokly (1997) isolated a U.V. induced mutant that produced about 500 mg/L cephalosporin C from the strain *A. chrysogenum* ATCC 11550 which produced about 200 mg/L.

The objective of this work is to induce cephalosporin C production in the strain *Acremonium chrysogenum* ATCC 11550. This work is considered as a model of strain genetic improvement by U.V. mutagenesis and investigating the newly-isolated mutant's fermentation behavior and sugar assimilation in lab scale stirred-tank bioreactor.

MATERIALS

This work was carried out in the laboratories of the Industrial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofia University during 2006 to 2008.

MICROORGANISMS:

The fungus used in this work was *Cephalosporium acremonium* (syn. *Acremonium chrysogenum*) ATCC 11550 (DSM 880, CBS 779.69, or CMI 49137) as the parent strain. This strain is the original Brotzu's strain (Perez-Martinez and Peberdy, 1985, and Velasco *et al.,* 1999), which was first isolated from sea water near a sewage outlet at Cagliari, Sardinia, Italy in 1948 (Brakhage, 1998). It has many applications, such as production of cephalosporin C, and production of deacetoxycephalosporin C according to the US patent no. 3,979,260 (Nakao *et al.*, 1976).

The bacterial strain *Bacillus subtilis* ATCC 6633, which is sensitive to the cephalosporin antibiotics, was used for screening of the fungal mutants antibiotic production capacity by using the agar diffusion method (as suggested by Hebiraw Co. for Pharmaceutical Raw Materials).

Microbiological media:

Complete medium (CM): (Vialta *et al.,* 1997)

This medium was used for the propagation and maintenance of the mutants and for screening for the antibiotic productivity.

Seed medium (SM): (Vialta *et al.,* 1997)

It was used for propagation of the inoculum, used to inoculate the fermentation medium.

Fermentation medium 1 (FM 1): (Vialta *et al.,* 1997)

It was used in the final selection of the highest producing mutant in shake flasks, based on inhibition zone diameters on plates.

Fermentation medium 2 (FM 2): (Lee *et al*., 2001)

It was the medium in which cephalosporin C production and sugars assimilation of the highest producing mutant was monitored in batch fermentation in the stirred-tank bioreactor.

Sucrose	**36 g/l**
Glucose	**27 g/l**
Corn steep liquor	**50 g/l**
KH_2PO_4	**3.0 g/l**
K_2HPO_4	**5.0 g/l**
$(NH_4)_2SO_4$	**8.0 g/l**
DL-methionine	**5.0 g/l**
Antifoam	**1 ml/l**
Trace element solution*	**50 ml/l**

***. Trace element solution: (Cruz *et al*., 1999)**

Na_2SO_4	**16.2 g/l**
$MgSO_4.7H_2O$	**7.68 g/l**
$CaCl_2.2H_2O$	**7.68 g/l**
$MnSO_4.H_2O$	**0.64 g/l**
$ZnSO_4.7H_2O$	**0.64 g/l**
$CuSO_4.5H_2O$	**0.004 g/l**

- Sugars and $(NH_4)_2SO_4$ were sterilized separately, and aseptically added.

Nutrient agar: (Atlas and Parks, 1997)

It was used for propagation and maintenance of *Bacillus subtilis* ATCC 6633, also for screening of fungal mutants by agar diffusion method.

Experimental methods

Ultraviolet-light mutagenesis and strain selection:

Irradiation of *Cephalosporium acremonium* spores with ultraviolet-light: (according to Drew and Demain (1975), and Lee *et al*. (2001), but with modifications)

A spore suspension was prepared by pouring NaCl solution (0.9% w/v) onto a complete medium agar slant, on which *Cephalosporium acremonium* ATCC 11550 had been grown for 7 days and mixed well. Spore concentration was determined microscopically with a Neubauer® haemocytometer (depth 0.1 mm- small-square area 0.0025 mm^2). Three ml aliquots of conidial suspension was poured into autoclaved Petri dishes, and irradiated with U.V. light for 0, 2, 4, 6, 8, 10 and 12 minutes at a distance of 20 cm from the U.V. lamp (Phillips® T UV-15W/G15 T8 (UV-C)). Suspensions were agitated during irradiation by rotating the Petri dishes on an orbital shaker OS-20 (Boeco, Germany) at 150 rpm. After irradiation, plates were kept in dark for 2 hours. Viable counts were determined by plating 0.2 ml of appropriately diluted samples on CM agar plates. They were incubated turned upside-down at 28^O C. for 5 days. Colonies that appeared on the plates were isolated and transferred into CM agar slants, and incubated for 7 days at 28^O C. for further examination.

Screening of the mutgenized isolates' antibiotic production capacity by agar diffusion method:

Mutagenized isolates were screened for cephalosporin production by agar diffusion method using *Bacillus subtilis* ATCC 6633 as the test organism. Thirty centimeters CM agar plates were subdivided into 21 sections and each section is inoculated with an isolate (figure (1)). Plates were incubated turned upside-down at 28^O C. for 4 days, as the maximum cephalosporin C (CPC) production occurs at the 5^{th} day of incubation (according to Perez-Martinez and Peberdy (1985), Basak *et al*. (1995), Vialta *et al*. (1997), and Silva *et al* (1998)). Nutrient agar seeded with the test organism *B. subtilis* ATCC 6633 was poured over the grown colonies of every isolate. Plates were left for the agar to solidify, then were incubated turned upside-down for 24 hours. *Bacillus subtilis* grew as a loan with clear inhibition zones around the fungal colonies. Colony diameter was subtracted from the inhibition zone diameter for each isolate. This experiment was repeated up to six times depending on the variation between individual determinations of inhibition zone diameters for each isolate to minimize the error.

Inhibition zone diameters of all the survivors from U.V. light exposure were statistically compared to that of the original strain, using the Completely Randomized Design (Steel and Torrie, 1960), where it does not necessitate an equal number of repetitions for each isolate.

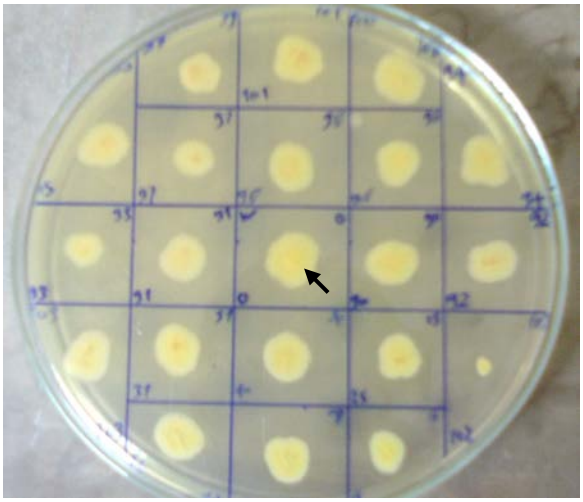

Figure (1): A 30 cm CM agar plate subdivided into 21 sections, each section is inoculated with an isolate. The arrow points to the wild strain which has the same place in each plate.

Selection of the highest cephalosporin C-producer mutant in liquid medium:

This was the next step in selection of a high antibiotic-producer mutant to be used in the next fermentation experiment. The ten highest productive mutants were tested for CPC production in liquid fermentation medium by the method described by Vialta *et al*. (1997). 500-ml Erlenmeyer flasks containing 50 ml SM were used to allow for adequate aeration. The flasks were shaken at 250 rpm on a digital orbital shaker (VWR DS2-500-1, VWR International, Henry Troemner LLC, 201 Wolf Drive, Thorofare, NJ, USA) at 28^{O} C. as specified by Karaffa *et al*. (2003), for 2 days. Then, 500-ml Erlenmeyer flasks containing 40 ml FM 1 were inoculated by 1.6 ml SM, and shaken at the same conditions as with SM, but for 7 days. The culture broth samples at the end of the fermentation were centrifuged at 4000 rpm for 20 minutes and the clear supernatants were used in assay of CPC, deacetylcephalosporin C (DAC), and deacetoxycephalosporin C (DAOC) with HPLC.

Cephalosporin C production by the mutant strain M 104:

Batch fermentation of the mutant strain M 104 in the stirred-tank bioreactor:

This batch fermentation was carried out to determine the cephalosporin C production, and to investigate sugars metabolic behavior of the mutant strain M 104.

Growth from two 1-inch diameter 7-day old CM agar slants of the mutant strain M 104 was suspended in 10 ml of normal saline for each slant. 2.5 ml were transferred to eight 500-ml Erlenmeyer flasks containing 50 ml SM, and shaken on the orbital shaker (VWR) at 250 rpm at 28^{O} C. for 2 days, as described by Vialta *et al*. (1997).

The volume used for inoculation was 10% of the media volume of the main fermentation culture, as previously suggested by several workers; Silva *et al*. (1998) Cruz *et al*. (1999), Lee *et al*. (2001), and Karrafa *et al*. (2003). The SM flasks were pooled and used to inoculate 7.5L stirred-tank bioreactor (Bioflo® 310, New Brunswick Scientific Co., Inc., Edison, NJ, USA), with instruments for temperature control, dissolved oxygen sensor (In Pro 6800®, Mettler-Toledo GmbH, process analytics, industrie Nord, CH-8902 Urdorf), and a gel-filled pH meter (Ingold® pH electrodes, Mettler-Toledo GmbH, process analytics, industrie Nord, CH-8902 Urdorf, Switzerland) filled with 4 L FM 2, and pH was controlled between 6.2-6.8 (as stated by Kim *et al.*, 2007) by the automatic addition of 2 M NaOH or 2 M H_2SO_4, while the temperature was maintained at 27^{O} C. Antifoam was added when needed.

Samples of 20 ml were taken every 8 hours during fermentation, starting from the time of inoculation (zero time). Ten milliliters of each sample was centrifuged at 6000 rpm for 15 min, and the clear supernatant was assayed for CPC, DAC, DAOC with HPLC, and the pellet was used for measuring dry weight of the cells.

Analytical methods:

Fungal cells counts:

Microscopical spore counting using Neubauer® haemocytometer:

The counts of both sides were averaged, and the average was multiplied by 10^4 ($1\ mm^2$ (square area) X 0.1 mm (depth) X 10^{-3} (conversion to cm^3)) = $count/cm^3$

Original plate viable count:

Colonies were counted on the plate, and the original viable count was calculated by the following formula:

$$\frac{\text{Colony count X dilution factor}}{\text{Volume of inoculum}} = \text{Colony forming units/ml}$$

Sugars assays:

Glucose determination:

It was assayed colorimetrically using enzyme colorimetric GOD-POD (glucose oxidase- peroxidase) kit (Spinreact®, S.A. Ctra. Santa Coloma, 7 E-17176 Sant Esteve De Bas (GI), Spain). Measurement was carried out at room temperature after 15 minutes of mixing the samples with the reagent, and then the color intensities were measured versus a standard using a

spectrophotometer (Model 390, Buck Scientific, Inc.). Procedures of measurement were carried out according to manufacturer's instructions.

Sucrose determination:

Sucrose was assayed as described previously for glucose after being hydrolyzed by 10% HCl in a boiling water bath for 10 minutes, as described by Timberlake, (2002). The result was the total sugars concentration; sucrose concentration was determined by subtracting glucose concentration from the total sugar concentration.

Cephalosporin C (CPC) assay:

CPC in the culture supernatants was determined by HPLC system with automatic autosampler (HP model 1100). The column was Lichrosorb® C8 (L= 250 mm, Ø= 4.6 mm), mobile phase was acetate buffer pH 4.75 (980 ml): acetonitrile (20 ml) with a flow rate 2ml/min. Detection was carried out by a U.V detector at 254 nm. CPC in samples was calculated by the following equation:

$$\mu g/ml = \frac{\text{Sample peak area} \times \text{standard conc.} \times \text{dil. factor}}{\text{Standard peak area}}$$

Biomass determination:

Dry weight:

The dry cell weight of mycelium was estimated, as described by Nigam *et al*. (2007), by centrifuging 10 ml of fermentation broth, washed three times with distilled water, recentrifuged, and kept for drying at 80^{O} C. till a constant weight.

Percentage mycelium volume (PMV):

10 ml samples of culture broth were centrifuged and the volume of cells was measured and expressed as a percentage (Lim *et al*., 2002).

RESULTS AND DISCUSSION:

Ultraviolet-light mutagenesis and strain selection:

Irradiation of *Cephalosporium acremonium* spores with ultraviolet-light:

The wild-type strain was mutagenized by exposure to U.V. light. The survival curve is shown in Figure (2).

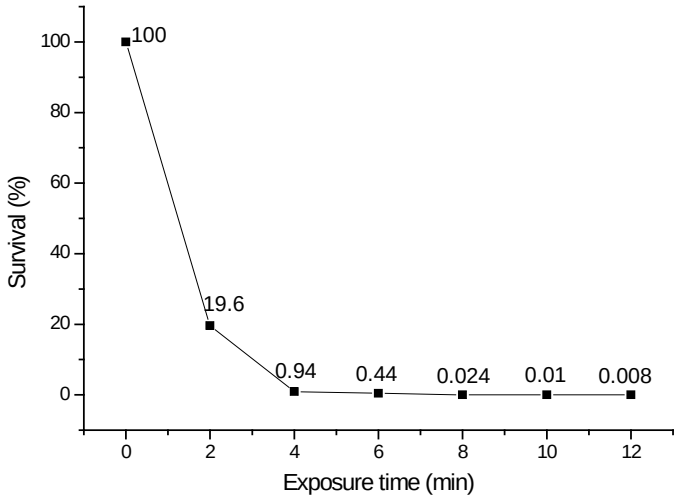

Figure (2): U.V. light exposure survival rate.

It is observed from Figure (2) that killing by U.V. light was faster at the early exposure until 2 minutes (more steep line), and then killing rate is slowed down with increasing exposure time. The possible explanation is that the highly sensitive fungal cells (the majority of the population) had been killed at the early times of exposure. The less sensitive and resistant cells were killed at a slower rate with elongated U.V. exposure time.

Screening of the mutagenized isolates for antibiotic production by agar-diffusion method:

Isolates were screened for cephalosporin production using *Bacillus subtilis* ATCC 6633 as the test organism in terms of inhibition zone diameter. Mutagenesis by exposure to U.V. light gave rise to 107 colonies. The genetic character of concern is the antibiotic-production capacity of the strain, which had been tested in terms of inhibition zone diameter on a cephalosporin-sensitive strain. Statistical design divided the 107 strains, in comparison to the original strain, to 10 as highly significant antibiotic producers, 4 significant, 67 non-significantly different from the original strain and 24 were significantly less than the original.

Screening of the highest ten mutants in liquid medium:

The highest ten mutants were screened in FM 1 (Vialta *et al*., 1997). Cephalosporins were assayed by HPLC, and the results are shown in table (1).

Table (1): HPLC assay results of the highest ten mutants.

Mutant number	CPC peak area	DAC percentage	DAOC percentage	CPC percentage	CPC concentration
0 (wild)	0	51.4%	37%	0	0
16	0	59%	41%	0	0
56	0	17.7%	40.4%	0	0
57	35	7%	32.2%	12.8%	304 µg/ml
107	0	34.3%	13.5%	0	0
26	0	46.5%	41.6%	0	0
105	0	61.4%	22.7%	0	0
104*	103	16.5%	7%	30%	900 µg/ml
46	64	32%	16.7%	25%	556 µg/ml
20	88.7	20.5%	14%	25%	770 µg/ml
31	0	40.7%	27.1%	0	0

***The mutant 104 was the highest CPC-producer.**

It is observed from table (1) that the original strain *C. acremonium* ATCC 11550 did not produce cephalosporin C, which was the strain that Brotzu discovered to produce CPC, as stated earlier, and also it produced 200mg/L according to El-Bondokly (1997). This discrepancy may indicate that this strain lost its cephalosporin C production ability possibly due to genetic events or mutations that occurred during storage. A similar situation was previously found and explained by Vialta *et al*. (1997). While this strain did not produce cephalosporin C, it was found to accumulate DAOC and DAC. This indicates that the DAC acetyltransferase (DAC-AT) is not functional. It is not known if this is due to formation of inactive enzyme or whether it is blocked in transcription or translation of the gene encoding actyl-CoA:DAC o-acetyltransferase (DAC-AT). Velasco *et al.* (1999) investigated this phenomenon in the mutant *A. chrysogenum* ATCC 20371 that does not produce CPC while it accumulates DAC. They found that this mutant shows, however, a normal transcript of the DAC-AT gene and, therefore the mutation probably results in a truncated protein or in the inability to translate the mRNA.

From Table (1), the mutant no. 104 (named M 104 in the context) was the highest significantly improved mutant statistically (data not shown), also it produces the highest concentration of cephalosporin C; 900 µg/ml in shake flask fermentation. This could be explained by the higher potency of CPC to kill *B. subtilis* ATCC 6633, thus produced larger inhibition zones, than its intermediates; DAOC and DAC. This agreement of statistical and practical results indicated that this statistical design could be a useful tool with an acceptable accuracy in this type of experiments. The mutant strain M 104, which was induced by 10 min U.V. exposure of the parent strain, was used in the next fermentation experiment for CPC production.

Cephalosporin C production by the mutant strain M 104:

Batch fermentation of the mutant strain M 104 in the stirred-tank bioreactor:

Batch fermentation was carried out in the stirred-tank bioreactor to demonstrate the mutant's behavior in terms of cephalosporin C production, biomass growth, and sugars consumption. Figure (3) (a) and (b)shows the operating dissolved oxygen control; agitation and airflow, (c) shows the dissolved oxygen (DO) concentration in the fermentation broth allover the process, (d) shows the pH changes during the fermentation process, (e) illustrates the mutant's behavior during the fermentation time, while (f) illustrates the cephalosporin nucleus production pattern.

pH was automatically controlled initially at 6.5 as described by Seidel *et al*. (2002a, b), then controlled between 6 and 7, which is in the optimal range for fungal growth and antibiotic-production as stated previously by Kim *et al.* (2007). pH had a tendency to decline at the beginning of the process as demonstrated from Figure (3) (d). Lowe (2001), Sàndor *et al.* (2001), and Seidel *et al*. (2002a) previously explained this phenomenon to be due to the culture's sugar metabolism that produces acids that lower the pH. On contrary, after sugars depletion till the end of the cultivation the pH tended to increase (Figure (3) (d)), which explained by Seidel *et al*. (2002a) to be due to lysis of the cells.

Dissolved oxygen (DO) concentration declined in the early growth phase to a value close to 20% (Figure (3) (c)). This is due to the fast oxygen consumption during the early growth phase of the biomass, in addition that most of the biomass was in the form of filamentous hyphae that forms a network-like structure that caused an increase in the viscosity of the fermentation broth (as demonstrated in Figure (6)), which impair oxygen transfer inside the broth leading to low dissolved oxygen concentration as explained by (Basak *et al.,* 1995; Sàndor *et al.,* 2002; Lim *et al.,* 2002). At about 13 hours, dissolved oxygen increased by manually increasing agitation and air flow, and then they could be decreased as dissolved oxygen tended to rise after about 26 hours due to fragmentation of hyphae to the arthrospore shape (Figure (3) (c) and (6)), which results in the reduction in the viscosity of the broth and leads to a greater oxygen transfer inside the broth, consistent with the findings of Lim *et al.* (2001), and Elander (2003).

Many workers used the percentage mycelium volume (PMV) as a measure of biomass concentration such as Sohn *et al*. (1994), and Lim *et al*. (2002). Figure (4) illustrates the changes in PMV during the batch fermentation to be correlated with cephalosporin C production.

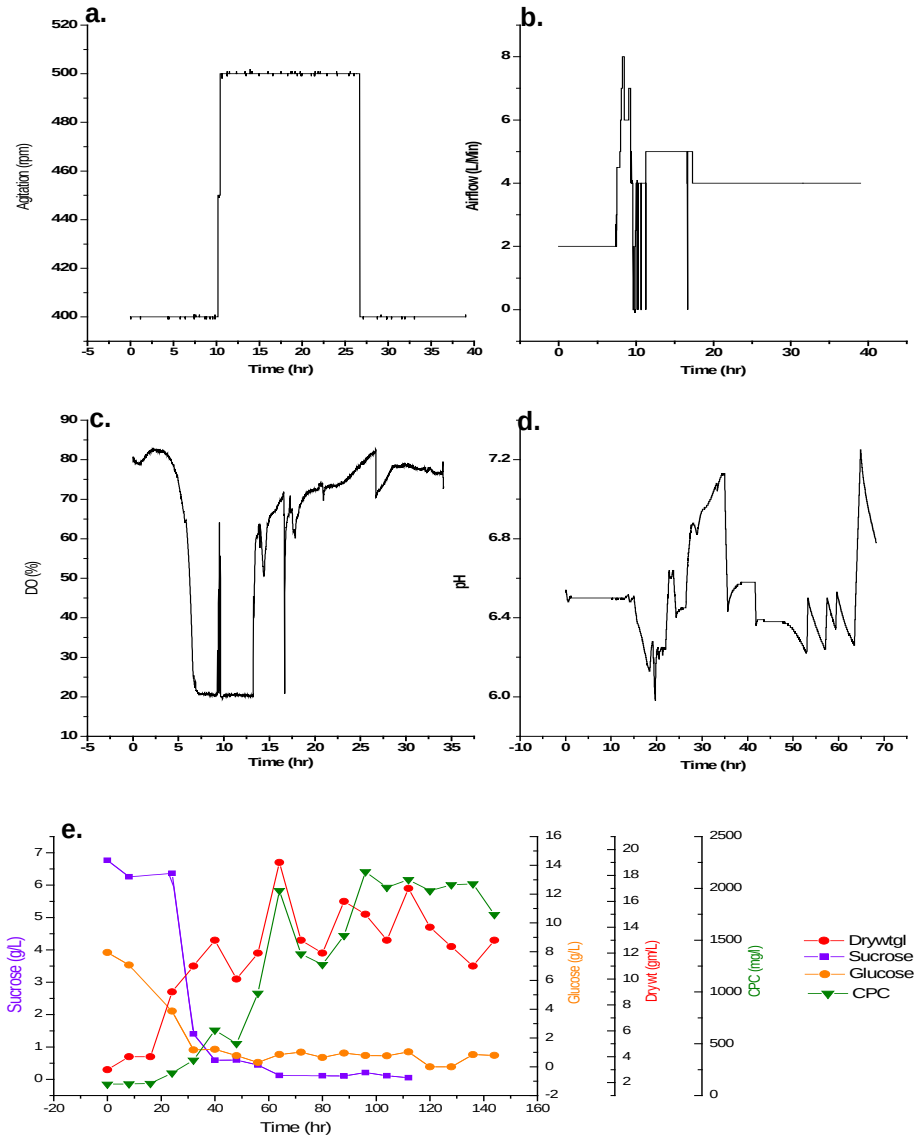

Figure (3) (to be continued): Time course of batch culture of the mutant M 104 (a) agitation; (b) airflow; (c) dissolved oxygen (DO); (d) pH changes of the fermentation broth; (e) cephalosporin C production, biomass growth, and residual sugars concentrations; (f) cephalosporin C intermediates-production pattern.

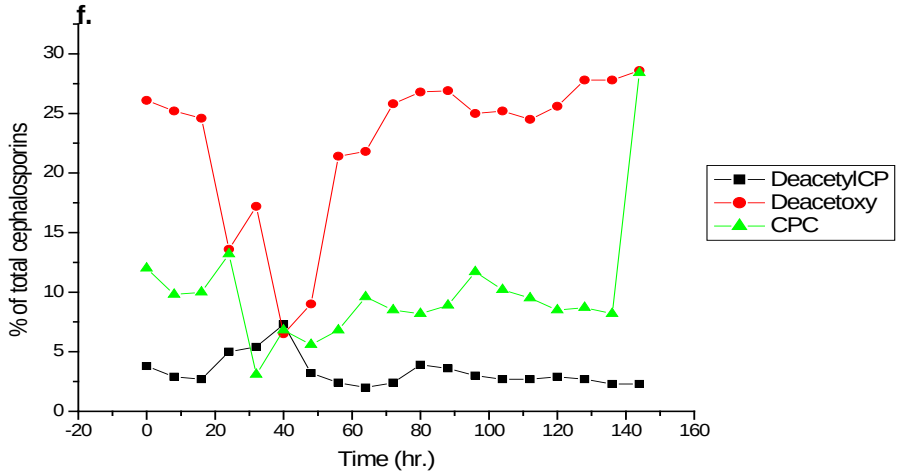

Figure (3) (cont.): Time course of batch culture of the mutant M 104 (a) agitation; (b) airflow; (c) dissolved oxygen (DO); (d) pH changes of the fermentation broth; (e) cephalosporin C production, biomass growth, and residual sugars concentrations; (f) cephalosporin C intermediates-production pattern.

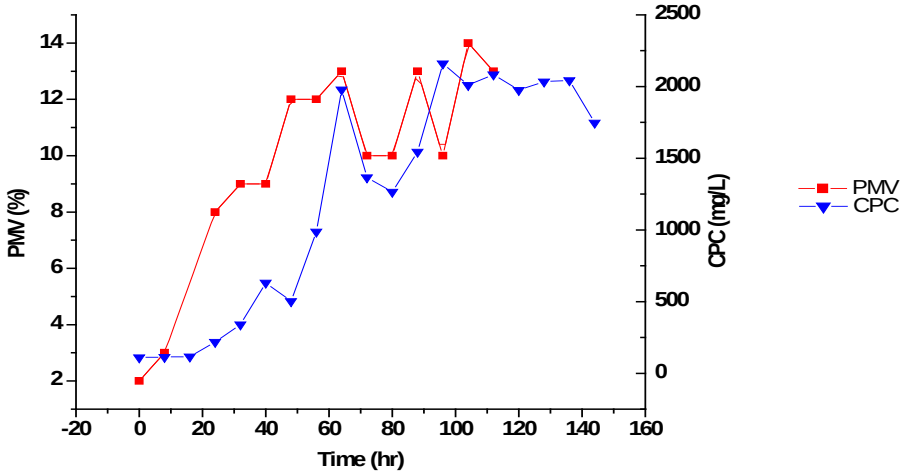

Figure (4): Correlation between cephalosporin C concentration and biomass in terms of packed mycelium volume (PMV).

By comparison between Figures (3) (d) and (4), it is observed that a better correlation exists between the dry weight as a measure of biomass concentration and the cephalosporin C production. This means that dry weight measurement is a better indication of the biomass concentration than percentage mycelium volume. Dry weight measurement was used by many workers such as Cruz *et al.* (1999), Sàndor *et al.* (2001), Karaffa *et al.* (2003), Kim *et al.* (2007), and Nigam *et al.* (2007).

Figure (5) shows that the forms present in the seed were conidia and germinating conidia, which upon inoculation, the germ tubes grew to yield long slender smooth hyphae (Figure (6)). Between 8 and 24 hours, hyphae were prevalent in the medium forming a network-like structure that caused the decline in dissolved oxygen level (Figure (3) (c)) in addition to the high oxygen consumption with fast growth (trophophase). Extensive fragmentation of the hyphae took place between 24 and 40 hours yielding conidia and germinating conidia (Figure (6)), which coincides with the increase in dissolved oxygen level and the start of CPC production (Figure (3) (e)), which is consistent with the explanation of Elander (2003) that arthrospore stage leads to greater oxygen availability to the organism (which is critical for maximal expression of the important biosynthetic cyclase and expandase enzymes) and results in rapid cephalosporin production (Figure (3) (e)).

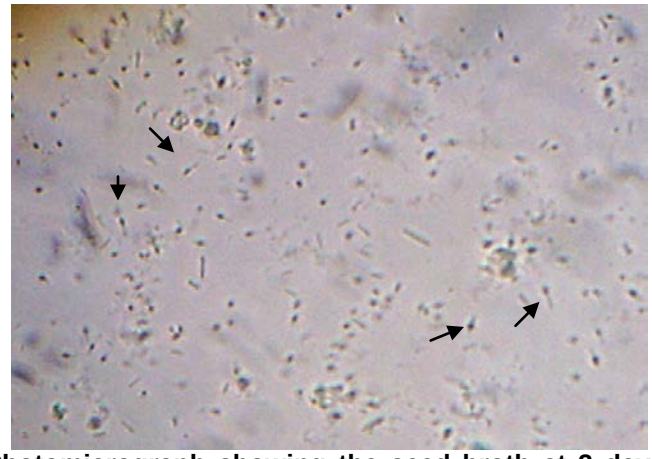

Figure (5): Photomicrograph showing the seed broth at 2 days (at the time of inoculation). The major morphological forms are conidia and germinating conidia (arrows are showing germinating conidia).

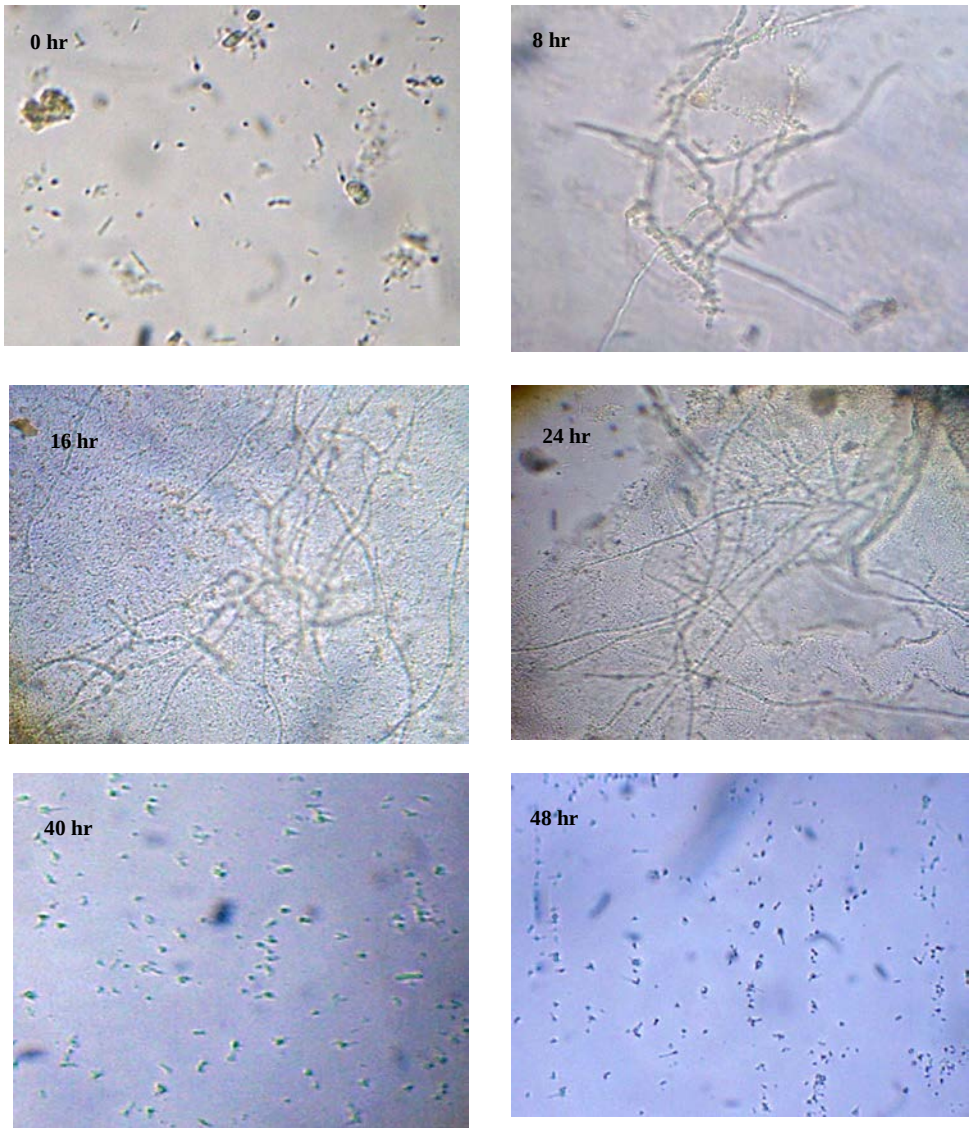

Figure (6) (to be continued): Morphological changes of the mutant M 104 throughout the batch-fermentation.

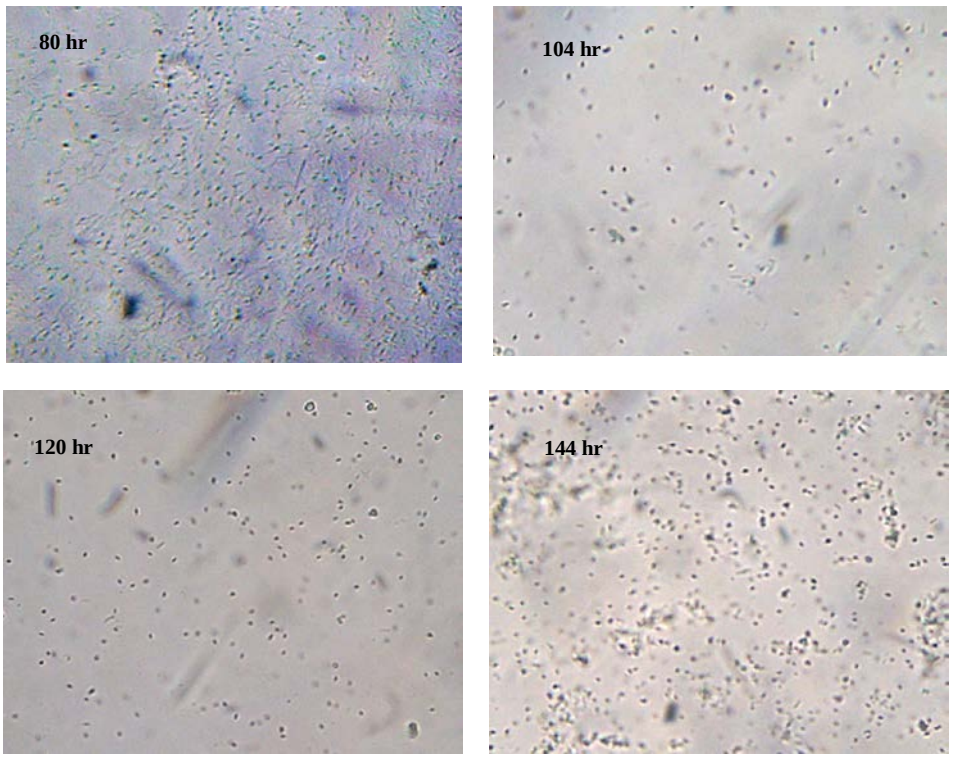

Figure (6) (cont.): Morphological changes of the mutant M 104 throughout the batch-fermentation.

It was observed from figure (6) that fragmentation took place when sugars (carbon source) had become at low level, as illustrated in Figure (3) (e), which is consistent with the finding of Sàndor *et al*. (2001) that both cephalosporin C production and fragmentation are responses to more fundamental physiological changes caused by poor availability of carbon with subsequent decrease in growth rate (idiophase). The suboptimal growth rate, resulting from low nutrition sources, is the signal for a high antibiotic production by the fungus according to Brakhage (1998).

The well-known diauxic phenomenon has been clearly defined in this fermentation. As illustrated in Figure (3) (c), Glucose, the easily metabolized carbon source, is preferentially and rapidly consumed by the growing fungal cells that were rapidly propagating (the two lines are seen characteristically intersecting in the figure). Shortly before glucose depletion, the more difficult carbohydrate to assimilate, sucrose, started to be consumed by fungal cells promoting slower cell growth and synchronous with the onset of hyphal fragmentation and antibiotic production. It was observed that when glucose was being consumed by the cells, the majority of morphological forms are

long, slender hyphae (Figures (3) (e) and (6)). The amount of cephalosporin C produced prior to complete depletion of glucose is low (Figure (3) (e)). Immediately after the depletion of glucose, production of CPC started, accompanied by extensive fragmentation of hyphae into short swollen forms that ultimately became differentiated into arthrospores and conidia (Figure (6)).

Just before sugars depletion, CPC started to rise after about 50 hours to reach a maximum concentration of 2160 mg/L at 96 hours of fermentation time. This time of maximum production was one day earlier than the 5 days that was stated by Perez-Martinez and Peberdy (1985), Basak *et al*. (1995), Vialta *et al*. (1997), and Silva *et al.* (1998), which was used for screening of mutants on agar plates, but this did not impair the screening process, as pouring of nutrient agar seeded with *Bacillus subtilis* on the colonies of mutants occurred after it had produced the maximum amount of cephalosporin C ensuring largest possible inhibition zones due to inhibition of bacteria by cephalosporin C.

To know how much CPC produced by each biomass unit, specific productivity is calculated and plotted against time of fermentation as illustrated in Figure (7).

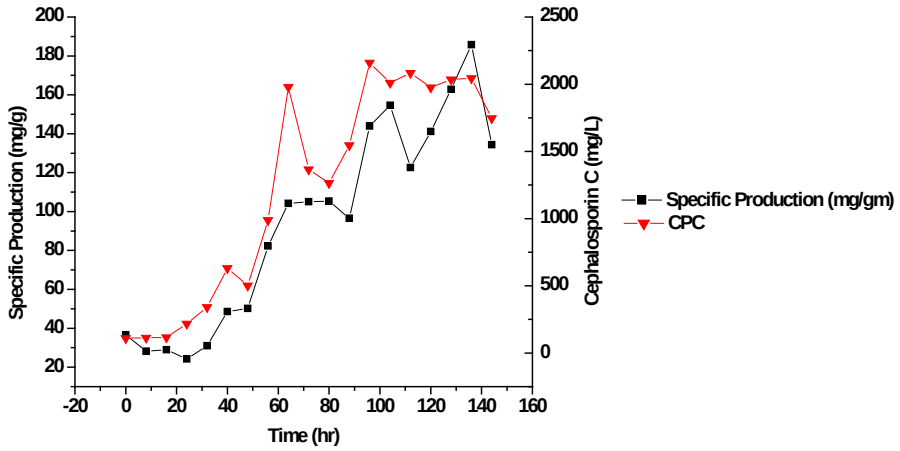

Figure (7): Time profile of specific production of cephalosporin C per unit biomass and cephalosporin C production.

It is obvious from Figure (7) that specific productivity began to rise at about 24 hours prior to glucose depletion, and then attained a plateau between 40 and 48 hours that was the period at which glucose had been fully depleted (Figure (3) (e)) and fragmentation had taken place (Figure (6)). Between 48 and 64 hours, specific productivity was doubled, and attained another plateau between 64 and 80 hours when sucrose was depleted at 64

hours (Figure (3) (e)). After depletion of carbon sources, specific production underwent fluctuation and had a maximum of 186 mg CPC/g dry weight at 136 hours, and then a reduction occurred due to shortage of carbon sources at the end of the fermentation process.

Figure (8) shows the rate of accumulation of CPC in the medium, which had three peaks at 40, 64, and 94 hours. The maximum CPC production rate was at 64 hour at the time when sucrose was depleting and the biomass was at its maximum (Figure (3) (e)). The maximum production rate was earlier than that of the strain *A. chrysogenum* (C3) used by Seidel *et al*. (2002a), which had the maximum rate at 80 hours. This is consistent with the finding that maximum CPC concentration in the culture broth was at 96 hours that is one day earlier than that stated in literature. This consistency indicates that this mutant (M 104) produces CPC in a shorter time than that stated previously in the literature, which is an advantageous trait of the strain to be used in fermentation to reduce the process time, as stated by Carlile *et al*. (2001).

Deacetylcephalosporin C (DAC) was the lowest in the compounds with cephalosporin nucleus. Deacetoxycephalosporin C (DAOC) was the highest, and this explains why the original strain ATCC 11550 was used in synthesis of DAOC according to the US patent no. 3,979,260 (Nakao *et al.*, 1976). Cephalosporin C (CPC) was in-between.

In conclusion, a U.V. mutant was isolated that produced 2160 mg/L CPC in a batch process, after irradiating *Cephalosporium acremonium* ATCC 11550, which did not produce cephalosporin C (Table (1)). A substantial proportion of intermediates (DAOC and DAC) produced by the parent strain has been shifted to production of cephalosporin C (Table (1) and Figure (3) (f)).

The fermentation experiment carried out in stirred-tank bioreactor pointed out that oxygen availability for the culture is of critical importance for cephalosporin C production, but with less importance for biomass growth. This oxygen availability can be ensured by proper agitation and aeration of the culture and by the proper inoculum concentration as well.

A mixture of an easily metabolized sugar (glucose) with another more difficult to be assimilated (sucrose) is used to build up the antibiotic-producing biomass (by the easily metabolized sugar) and to make the fungus attain the physiological state of antibiotic production (the more difficult sugar to be assimilated).

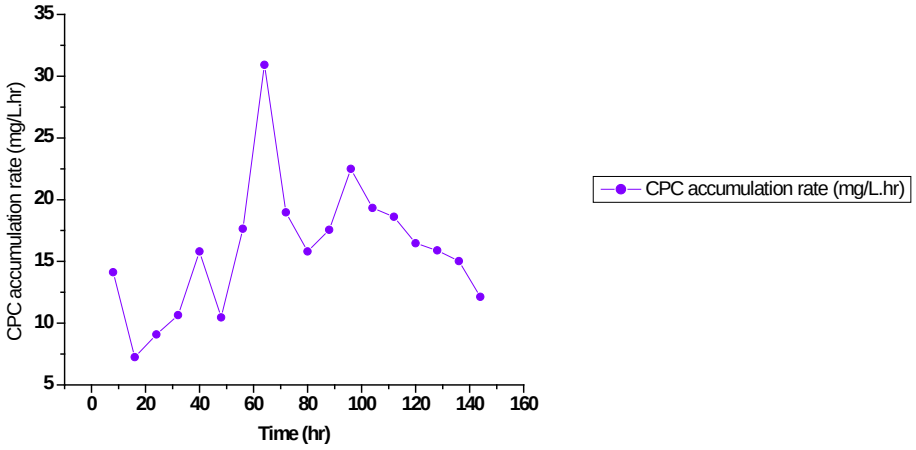

Figure (8): Rate of cephalosporin C accumulation in the fermentation medium versus time.

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حث إنتاج السيفالوسبورين من فطر السيفالوسبوريوم أكريمونيوم بواسطة الأشعة فوق البنفسجية وإنتخاب طفرة ذات أعلى قدرة تخمر

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الملخص العربي

هذا البحث يتناول إستخدام الأشعة فوق البنفسجية كعامل مطفرلحث إنتاج المضاد الحيوى السيفالوسبورين من السلالة ١١٥٥٠ من الفطرسيفالوسبوريوم أكريمونيوم. و لقد تم إختيار أعلى السلالات إنتاجا من بين السلالات المطفرة بإستخدام السلالة ٦٦٣٣ من الباسيلاس. أما السلالة الجديدة (M 104) ذات الإنتاجية الأعلى فقد أنتجت ٩٠٠ مجالتر من السيفالوسبورين فى الفلاسكات الزجاجية و ٢١٦٠ مجالتر فى المخمر المعملى. تم أيضا دراسة السلوك الأيضى و التخمرى للسلالة الجديدة، فقد لوحظت ظاهرة التفضيل الغدائى خلال عملية التخمرإذ تم إستخدام خليط من سكر سهل الهضم كالجلوكوز مع آخر أصعب فى الهضم كالسكروز ليتم تكوين كتلة حيوية من الفطر(بواسطة الجلوكوز) ثم للوصول للحالة الفسيولوچية المهيئة لإنتاج المضاد الحيوى (بواسطة السكروز). وقد لوحظ أيضا الإحتياج الشديد للتهوية الكافية لتوفير الأكسجين الضرورى لعملية تكوين المضاد الحيوى. كان أعلى تركيز للسيفالوسبورين عند ٩٦ ساعة من زمن التخمر، وأعلى إنتاجية للجرام من خلايا الفطر ١٨٦ مجاجم عند ١٣٦ ساعة، ثم أعلى معدل زمنى للإنتاج ٣١ مجالتر.ساعة عند ٦٤ ساعة. هذا البحث يعتبر نموذج لعملية تحسين السلالة المنتجة للمضاد الحيوى فى الصناعة لزيادة الربح و تقليل سعر المنتج النهائى.