## Production of citric acid by locally isolated Asperigillus niger from Saladdin province

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#### Abstract:

Three locally isolated *Asperigillus niger* from three selective location of Saladdin province (Alam, Tikrit, Al-Dhuloiya) were studied for its ability to produce citric acid in synthetic medium (sucrose salts medium). The results showed that the culture of *Asperigillus niger* from (Al-Dhuloiya) was found to be the best producer of citric acid as compare with other two isolates.

The results indicated that the maximum production of citric acid was obtained when the volume of fermentation medium was (50 ml) in (250 ml) Erlenmeyer conical flask and the maximum production of citric acid was achieved after (7) days of inoculation.

#### **Introduction:**

Citric acid is the first stable intermediate compound of Kerb,s cycle (4). It is white solid at room temperature, melts at 153c and decomposes at higher temperature into other products. Citric acid is produced during trophase as a metabolic product (8). Because of its high solubility, palatability and low toxicity, citric acid has now become one of the most commonly used acid, as food acidulate and in pharmaceutical industries. Most of citric acid produced commercially comes from Asperigillus niger (1). The objective of this study was planed to obtain an efficient isolate for citric acid production.

## Material and Methods:

#### Isolation :

The fungus of *Asperigillus niger* were isolated from soil samples of Saladdin Province (Tikrit , Alam , and Al-Dhuloiya) by serial dilution method (2). The isolates were mainted on Czapex Dox medium slants. Incubated at (30c) and stored at (5c). The conidial suspension was prepared from 3-5 days, old cultures in sterilized distilled water (10m) wire loop and then it was shaken vigorously for breaking the clumps of conidia.

#### Fermentation technique :

Twenty– five ml of salt medium (containing sucrose 15%,  $H_2PO_4$  0.1, MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.025 and NH<sub>4</sub>NO<sub>3</sub> 0.2 mg/l) at pH 3.5 was taken in 250 ml cotton plugged conical flask. The flask were sterilized in autoclave at (121c°) for (20) min and cooled at room temperature. The flasks were inoculated and then incubated at (30c°) for (7) days. The fermented broth was filtered and was used for the estimation of citric acid.

#### Assay Method:

Dry cell mass was determined by filtering the culture medium through weighed whatmann filter paper no. (44) . Mycelia were thoroughly washed with tap water and dried at (105 $^{\circ}$ ), over night and mycelial dry weight was calculated.

The concentration of citric acid was estimated by two methods :

<u>**a.**</u> Titration method , by taking 10 ml of fermented broth against 0.1N NaOH using phenolphthalein as indicator (5).

Titre\* Normality of alkalix\* Equivalent wt. of acid

Total Acid=----\*100 Volume of Sample\*1000

**<u>b.</u>** Standard addition method, by using spectrophotometer (UV-visible spectrophotometer, centra -5 -) at 260 nm.

Cs \* Vs

Cx=-----

- Vx Vx • The concentration of aitric acid in the se
- **Cx :** The concentration of citric acid in the sample .
- **Vx** : The volume of addition sample solution .
- Cs : The concentration of standard citric acid solution .
- Vs: The volume of sample, equivellant to standard volume solution .

#### **Results and Discussion:**

In this study, culture of Asperigillus niger isolated from different soil samples produced citric acid as shown in (Table 1). The culture of Asperigillus niger from (Al-Dhuloiva) was found to be the best produce of citric acid , and this may be attributed to the effect of environmental conditions of each location on Asperigillus niger, strains and this finding is in agreement with (5). The effect of volume of fermentation medium (25,50,75,100) on citric acid production was studied (Table 2 and Figure 1-4) the maximum production of citric acid was obtained when the volume of fermentation medium was (50 ml) the mycelia dry weight and citric acid were (13.9g/l) and (68g/l) respectively. The production of citric decreased with the increasing the volume of the medium, this may be due to the reduction of air supply and also due to the limiting of hyphal growth as well as diffusional restriction (6). The effect of different incubation periods on the production of citric acid by isolated strain of Asperigillus niger was investigated (Table 3 ad figure 5-14). The fermentation was carried out from (1-10 days). After (1 day) of incubation the amount of citric acid produced was (15.2, 14.96 g/l) . (Table and Figer 1) respectively, and maximum production (72.7, 96 g/l) (Table and figure 1) respectively was achieved after (7 days) of inoculation and mycelial dry weight was (14.8 g/l) Further increase in incubation period did not show any enhancement in citric acid production. This it might be due to decrease in amount of available Nitrogen in the medium, the age of fungi, the presence of inhibitors produced by fungi itself and the depletion of sugar contents(7). Finally we need more studied on this local isolation of Asperigillus niger through make an improvement on cultural condition may also increase the biosynthesis of citric acid.

 Table 1: Isolates of Asperigillus niger from different

location		
Location	Mycelial dry	Citric
of isolate	weight g/l	acid g/l
Al- Dhuloiya	13.47	52.3
Tikrit	12.9	22.8
Alam	10.1	8.1

 Table 2: Effect of volume on citric acid production by

 Asperigillus niger (Al- Dhuloiya)

Volume of Fermentation medium (ml)	Mycelial dry weight g/l	Citric acid g/l
25	11.8	23.6
50	13.9	68
75	13.2	45.8
100	12.7	40.3

 Table 3: Effect of incubation period on citric acid by

 Asperigillus niger (Al- Dhuloiya)

Days	Mycelial dry	Citric acid g/l
	weight g/l	
1	4.4	15.2
2	4.6	15.2
3	8.6	20
4	9.2	24
5	10.3	29.3
6	11.3	38.4
7	14.8	72.7
8	13.2	44
9	16.1	38.9
10	15.3	32.8



Volume of added citric acid	Absorbance
0	0.58
1	0.78
2	0.95
3	1.14
4	1.3
5	1.47

Figure 1: Effect of volume on citric acid production by Asperigillus niger (Al-Dhuloiya). Volume 25 ml, citric acid 34.08g/l.



Volume of added citric acid	Absorbance
0	1.17
1	1.34
2	1.51
3	1.68
4	1.82

**Figure 2**: Effect of volume on citric acid production by *Asperigillus niger* (Al-Dhuloiya). Volume 50 ml, citric acid 68g/l.



Volume of added Citric acid

Volume of added citric acid	Absorbance
0	0.89
1	1.07
2	1.26
3	1.44
4	1.59
5	1 75

Figure 3: Effect of volume on citric acid production by Asperigillus niger (Al-Dhuloiya). Volume 75 ml, citric acid 50.3g/l.



Volume of added citric acid	Absorbance
0	1.11
1	1.38
2	1.69
3	1.88
4	2.08
5	2.27

Figure 4: Effect of volume on citric acid production by *Asperigillus niger* (Al-Dhuloiya). Volume 100 ml, citric acid 40.3g/l.



Volume of added citric acid	Absorbance
1	0.75
2	1.013
3	1.37
4	1.68
5	1.96
6	2.24

Figure 5: Effect of incubation periods on citric acid production

by Asperigillus niger (Al-Dhuloiya). 1 day, citric acid

14.96 g/l.



Volume of added citric acid	Absorbance
0	0.57
1	0.7
2	0.93
3	1.14
4	1.34
5	1.43

Figure 6: Effect of incubation periods on citric acid production by *Asperigillus niger* (Al-Dhuloiya). 2 days, citric acid 29.32 g/l.



Volume of added citric acid	Absorbance
1	0.79
2	1.03
3	1.21
4	1.46
5	1.61
6	1.79

Figure 7: Effect of incubation periods on citric acid production

by Asperigillus niger (Al-Dhuloiya). 3 days, citric acid

32.92 g/l.



Volume of added citric acid	Absorbance
1	0.93
2	1.14
3	1.29
4	1.49
5	1.69
6	1.89

Figure 8: Effect of incubation periods on citric acid production

by Asperigillus niger (Al-Dhuloiya). 4 days, citric acid

#### 38.92 g/l.



Volume of added citric acid	Absorbance
0	0.77
1	0.95
2	1.17
3	1.34
4	1.5
5	1.6

Figure 9: Effect of incubation periods on citric acid production

by Asperigillus niger (Al-Dhuloiya). 5 days, citric acid





Absorbance
1.21
1.38
1.53
1.7
1.85
1.98

Figure 10: Effect of incubation periods on citric acid

production by Asperigillus niger (Al-Dhuloiya). 6

days, citric acid 65.6 g/l.



Volume of added citric acid	Absorbance
0	1.49
1	1.67
2	1.81
3	1.96
4	2.09
5	2 24

Figure 11: Effect of incubation periods on citric acid

production by *Asperigillus niger* (Al-Dhuloiya). 7 days, citric acid 96 g/l.



Volume of added citric acid	Absorbance
1	1.08
2	1.25
3	1.43
4	1.56
5	1.69
6	1.83

Figure 12: Effect of incubation periods on citric acid production by *Asperigillus niger* (Al-Dhuloiya). 8 days, citric acid 67.04 g/l.



Volume of added citric acid	Absorbance
0	0.69
1	0.89
2	1.07
3	1.26
4	1.43
5	1.57

Figure 13: Effect of incubation periods on citric acid

production by *Asperigillus niger* (Al-Dhuloiya). 9 days, citric acid 38.4 g/l.



6 1.41 Figure 14: Effect of incubation periods on citric acid

production by Asperigillus niger (Al-Dhuloiya). 10

days, citric acid 24.36 g/l.

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# انتاج حامض الستريك بوساطة عزلة محلية من الفطر في محافظة صلاح الدين

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### الملخص:

دراسة قابلية إنتاج ثلاث عزلات محلية للفطر Asperrgillus niger من ثلاثة مواقع منتخبة في محافظة صلاح الدين بإنتاجها لحامض الستريك في بيئة مغذية صناعية ، وأظهرت النتائج أن عزلة الفطر Asperrgillus niger من موقع الضلوعية هي الأحسن في إنتاجها لحامض الستريك

مقارنة بالعزلتين الأخيرتين . ودلت النتائج أن أعلى إنتاج لحامض الستريك تم الحصول عليه عندما كان حجم الوسط المستخدم للتخمير (٥٠ مل) وفي دوارق مخروطية سعة (٢٥٠ مل) ، كذلك فإن أعلى إنتاج لحامض الستريك تم إنجازه كان بعد مرور (٧ أيام) من التلقيح .