Convenient carbon source and precursor substances for improving the growth regulators production by plant growth promoting rhizobacteria

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ABSTRACT

An experiment was carried out to determine the converable carbon sources , tryptophane and adenine concentrations for growth regulators production by *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* . Obtained results showed that the mannitol and glucose were the best carbon sources for PGRs production by *A. chroococcum* and *B. megaterium* var. *phosphaticum*, respectively. Also, the produced amounts of indole butyric acid (IBA) was higher than IAA with different applied carbon sources. *A. chroococcum* produced higher amounts of zeatin and kinetin compared to those produced by *B. megaterium* var. *phosphaticum*. While, *B. megaterium* var. *phosphaticum* produced higher amounts of (9R) benzyl adenine and (9G) benzyl adenine compared with those produced by *A. chroococcum* . Production of auxins, gibberellic acid (GA_3) and cytokinins was increased with increasing tryptophan concentration. The highest amounts of PGRs produced by the two strains were obtained with tryptophan addition at 10^{-3} molar .

Highest amounts of PGRs were produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* when adenine was applied with 10^{-5} and10^{-4} molar to the both strains, respectively. The two strains produced IBA amounts higher than IAA. Also, *A. chroococcum* produce higher amount of gibberellic acid and cytokinin rather than that given by *B. megaterium* var. *phosphaticum*. Generally, obtained data from investigation showed that the application of the optimal conditions together gave highest amounts of PGRs as compared with the other individual treatments. This result is logic and was anticipated.

Keywords: PGPR , PGRs , carbon sources , tryptophane , adenine , TLC, GLC, *Azotobacter* , *Bacillus* .

Abbreviations: **PGRs: plant growth regulators ; PGPR: plant growth promoting rhizobacteria ; IAA: Indole acetic acid ; IBA: Indole butyric acid ; GA_3: Gibberellic acid ; Z: Zeatin ; KIN: Kinetin ; (9R)BAP: (9R)Benzyl adenine ; (9G)BAP: (9G)Benzyl adenine ; IP : Isopentyl alcohol ; *M*: Molar**

INTRODUCTION

L-tryptophan is considered as the precursor for indole-3- acetic acid and other auxins production by microorganisms. Furthermore, L-tryptophan is considered as a substrate for GA_3 production. Adenine is considered the most suitable one among some precursors for cytokinins production by microorganisms.

Vessey (2003) and Morsy (2005) stated that glucose at 10 g L^{-1} was the best carbon source for PGRs production by PGPR . L-tryptophan serves as a substrate for auxins and GA_3 production (**Khalid *et al* , 2004**) and **Zahir *et al* (2005).** Adenine is considered the most suitable one among some precursors for cytokinins production by microorganisms (**Arshad and Frankenberger,**

1991). PGPR isolated from rhizosphere of various crops have the ability to produce auxins as secondary metabolities , various metabolic pathways such as :(1) Indole -3- acetamide pathway (2) Indole-3-pyrovic acid pathway (3) Tryptamide pathway (4) Tryptophan side chain pathway and (5) Indole -3- acetonitrile pathway are involved in the production of IAA **(Pallai , 2005)**.

One of the common biosynthetic utilize adenine as the precursor of free cytokinins production in PGPR (*Azotobacter* spp). Also addition of precursors such as adenine and isopentyl alcohol to the culture medium of *A. chroococcum* resulted in enhanced growth of *Raphanus sativus* under genotobiotic and greenhouse conditions (**Pallai , 2005**).

Therefore , this research aimed to determine the best additional from precursors substances for maximum growth regulators production by *A. chroococcum* and *B. megaterium* var. *phosphaticum.*

MATERIALS AND METHODS

Effect of different carbon sources on PGRs production

Specific media prepared for either *A. chroococcum* and *B. megaterium* var. *phosphaticum* and supplemented with different carbon sources namely fructose, sucrose, mannitol, glucose and a mixture of them .Concentration of each applied sugar was 2 and 0.5 % for *A. chroococcum* and *B. megaterium* var. *phosphaticum* , respectively .

Ashbey's medium was inoculated with 24–48 hrs old culture of *A. chroococcum* and incubated at 32°C for 4 days whereas, modified Bunt and Rovira medium was inoculated with *B. megaterium* var. *phosphaticum* and incubated at 30°C for 2 days. The produced PGRs were detected by TLC and determined by GLC to detect out the most suitable carbon source which gives maximum PGRs production .

Effect of different concentrations of DL - tryptophan on PGRs production

Specific media for the investigated strains were provided with different concentrations of tryptophan (10^{-3} – 10^{-8} *M*) then , sterilized , inoculated with strains and incubated at 32°C for 4 days and at 30°C for 2 days for *A. chroococcum* and *B. megaterium* var. *phosphaticum*, respectively. The produced PGRs were detected by TLC and determined by GLC according to **Mazur and Homme (1993)** to determine the most suitable concentration of tryptophan can give high PGRs production .

Effect of different concentrations of adenine on PGRs production

Flasks containing of specific media were supplemented with the different concentrations of adenine (10^{-3} *M* to 10^{-10} *M)*. Then, sterilized and inoculated with the tested strains of (24-48 hrs old culture) and incubated at 32°C for 4 days and at 30°C for 2 days for *A. chroococcum* and *B. megaterium* var. *phosphaticum*, respectively. The produced PGRs was detected by TLC and determined by GLC as mentioned before to limit the most suitable concentration of adenine can give high production of PGRs.

Optimal conditions for PGRs production.

The optimal conditions for *A. chroococcum* namely: incubation temperature (32°C); incubation period (4 days); tryptophan concentration (10^{-3} molar) ; adenine concentration (10^{-5} molar) and mannitol as a suitable carbon source. Whereas, The optimal conditions for *B.*

megaterium var. *phosphaticum* namely: incubation temperature (30°C); incubation period (2 days); tryptophan concentration (10^{-3} molar) ; adenine concentration (10^{-4} molar) and glucose as a suitable carbon source.

Specific media were inoculated with standard inoculum of 24 – 48 hrs old culture of *A. chroococcum* or *B. megaterium* var. *phosphaticum,* respectively. Then incubated at 32°C for 4 days and at 30°C for 2 days for both cultures , respectively. Then PGRs were extracted and determined by GLC as mentioned before .

Analysis methods

The analysis of plant growth regulators were identified by thin layer chromatography and determined by gas liquid chromatography in Soil Microbiology Department , Soil , Water and Environment Res. Ins. , Agric. Res. Center , Giza .

- **Thin layer chromatography (TLC) assessment**

Detection of IAA using TLC was accomplished by spotting 10 µL of the methanol extracts on fluorescent silica gel plates 0.25 mm thickness (Merch, Germany). The spots of the respective culture extracts and phytohormone standard solutions were developed in the following solvent systems:

A) Freshly prepared solvent mixture of chloroform : ethyl acetate: acetic acid (60: 40: 5) for auxins and gibberellins **(Salamone *et al*., 2001; Ahmad *et al*., 2005).**

B) Freshly prepared solvent mixture of: chloroform: methanol (9: 1) for cytokinins. (**Holl *et al.*, 1988).**

After the development the TLC plates were dried at 50 °C for 5 minutes , then they were exposed to UV radiation at 350 nm wave length to detect the compounds and measure their R_f values **(Torres-Rubio *et al.*, 2000)**.

- **Gas-liquid chromatography (GLC) assessment .**

Extracted samples were silylated before GLC analysis as follow:

Aliquots of 0.5 to 1 ml of each fraction sample and standard solutions were placed individually in 5 ml test tubes and evaporated to dryness, then 100µL of N,O-bis-(trimethylsilyl)acetamide (BSA) [sigma] was added to prepare trimethylsilyl derivatives (TMSi). The test tubes were immediately capped and heated to (50 – 60°C) for 30 minutes and evaporated to dryness to remove the access of (TMSi), then the residue was dissolved in 0.2 ml absolute methanol alcohol **(Mazur and Homme, 1993; Rahal *et al.*, 2006).**

One µL of each TMS derivative sample was injected into a split-splitless HP 5890 series II Gas chromatography equipped with a flame ionization detector, data analysis using chemostation software running of HP Vectra 486 computer and capillary column HP_5 (0.32 mm internal diameter, 25 m long and 0.1 µm film thikness of methyl silica gum).

The GC programal temperature was (185°C - 250°C) at rate of 10°C min^{-1}, then 2 min at 250°C, injector and detector temperatures were 250°C and 270°C, respectively . The nitrogen carrier gas flow rate of at 5 ml . min^{-1} and the flow rate of hydrogen and air for the flame ionization detector was 30 and 300 ml. min^{-1}, respectively .

Retention time (**R_t**) for each peak of the authentic standard materials was recorded and the amounts of separated compounds were calculated automatically by computer unit and printed .

Standard compounds used in TLC and GLC analysis

Indole -3- acetic acid (IAA) , Indole-3- butyric acid (IBA) and *t*-Zeatin were obtained from (Sigma) ; Adenine (Merch , Germany); Tryptophan was obtained from (Lopa, India) and GA_3, (9R)Benzyl adenine {(9R)BAP}, (9G)Benzyl adenine {(9G)BAP} and isopentyl alcohol (IP) were obtained from Genetic Engineering Research Inc. (El-Sadat City).

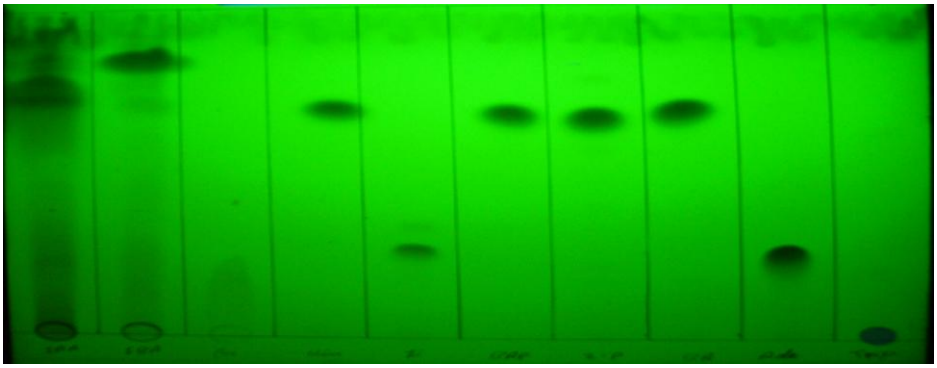
RESULTS AND DISCUSSION

Retention flow of pure growth regulators, adenine and tryptophan

Technical growth regulator agents, adenine and tryptophan were developed by TLC to limit their retention flow (R_f) values to be as reference to those obtained from sample extracts **(Martinez-Toledo *et al.*, 1988 ; Srinivasan *et al.*, 1996; Salamone *et al.*, 2001 and Torres-Rubio *et al.*, 2000)**. The obtained values are presented in **Table (1)** and shown in **Figs (1 a & 1 b**).

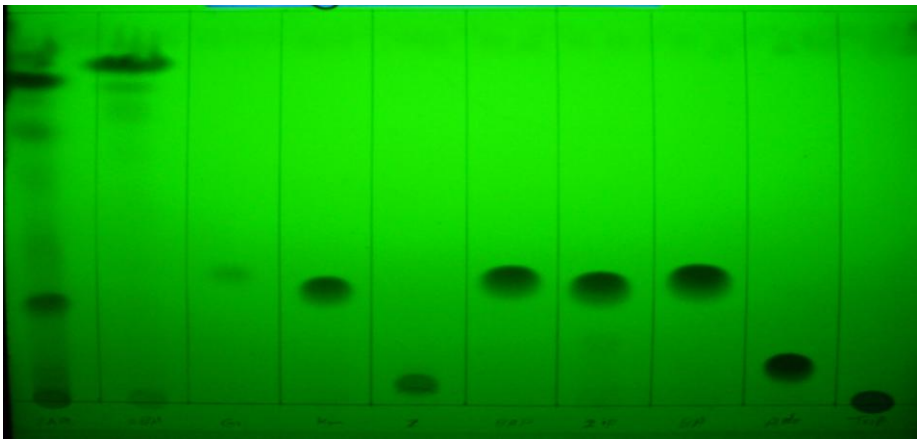
Table 1. Retention flow (R_f) values of the pure phytohormones – like substances developed by Thin Layer Chromatography (TLC) with two different development systems.

Compounds	Retention flow (R_f)	
	In auxins development system (1)	In cytokinins development system (2)
Indole acetic acid (IAA)	0.25 , 0.38 , 0.55 , 0.69	0.71 , 0.75
Indole butyric acid (IBA)	0.86	0.81
Gibberellic acid (GA_3)	0.33	0.16
Zeatin (Z)	0.05	0.3
Kinetin (KIN)	0.28	0.66
(9R) Benzyl adenine (BAP)	0.31	0.65
(9G) Benzyl adenine (BAP)	0.29	0.64
Isopentyl alcohol (IP)	0.28	0.63
Adenine (ADE)	0.12	0.24
Tryptophan (TRY)	zero	zero



1- IAA : Indole acetic acid
2- IBA : Indole butyric acid .
3- GA_3 : Gibberellic acid .
4- KIN : Kinetin .
5- Z : Zeatin
6- (9G) BAP : Benzyl adenine .
7- IP :Isopentyl alcohol
8- (9R) BAP : Benzyl adenine
9- ADE : Adenine
10- TRY : Tryptophan

Fig 1.a. TLC separation for authentic phytohormones- like substances using auxins separation development system.



1-	IAA : Indole acetic acid	6-	(9G) BAP : Benzyl adenine .
2-	IBA : Indole butyric acid .	7-	IP :Isopentyl alcohol
3-	GA_3 : Gibberellic acid .	8-	(9R) BAP : Benzyl adenine
4-	KIN : Kinetin .	9-	ADE : Adenine
5-	Z : Zeatin	10-	TRY : Tryptophan

Fig 1.b. TLC separation for authentic phytohormones – like substances using cytokinins separation development system .

Effect of applied carbon sources on PGRs production

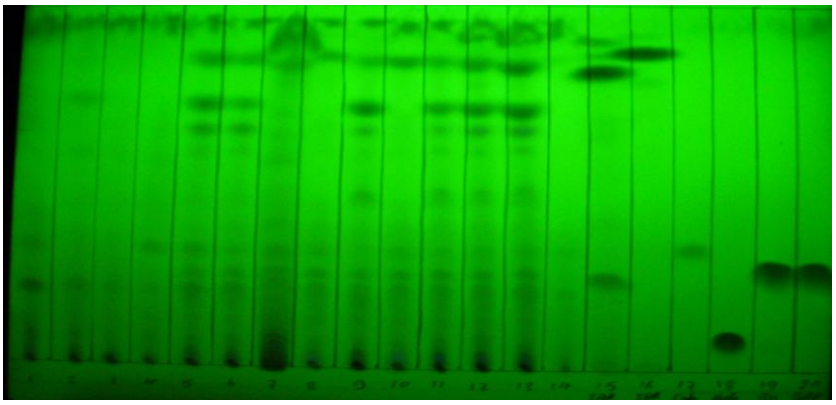
A)Qualitative analysis of PGRs produced in presence of different carbon sources

Fig (2 a) show the TLC separation of growth regulators extracted from cultures ammended with different sugars as a carbon source the results indicated that *A. chroococcum* produced IAA, IBA and GA_3 when mannitol or sucrose was used individually as a carbon source. Whereas, IAA and GA_3 were the only product when glucose or fructose was used.

Concerning the PGRs produced by *B. megaterium* var. *phosphaticum* , data in **Fig (2 a)** revealed that three compounds having R_f 0.24 , 0.33and 0.86 these compounds were identified as IAA , IBA and GA_3 were the main product when glucose was used as a carbon source. While, IAA and GA_3 were the main products when mannitol, fructose and sucrose were used as a carbon source.

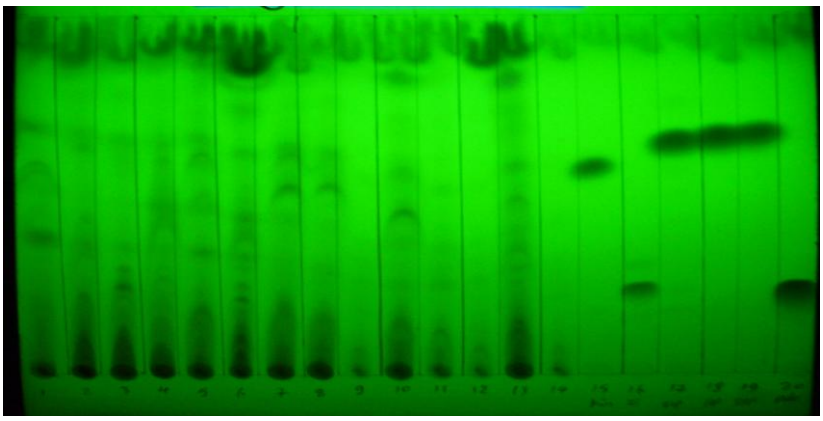
Data in **Fig (2 b)** showed the TLC separation of PGRs produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* in presence of different carbon sources using cytokinins development system .TLC chromatogram show that *A. chroococcum* produced kinetin compound when glucose or fructose was added as a carbon source . While , zeatin was produced when mannitol or sucrose was used. IBA compound was produced only by *A. chroococcum* when the mixture of either (mannitol + glucose) or (sucrose + fructose) were used as carbon sources.

Concerning the PGRs produced by *B. megaterium* var. *phosphaticum*, data in **Fig (2 b)** indicated that three compounds having R_f 0.33, 0.65 and 0.66 were produced when glucose was used as a carbon source. These compounds were identified as Z, (9R)BAP and KIN, respectively. Zeatin was the only detected compound when mannitol was used as a carbon source.



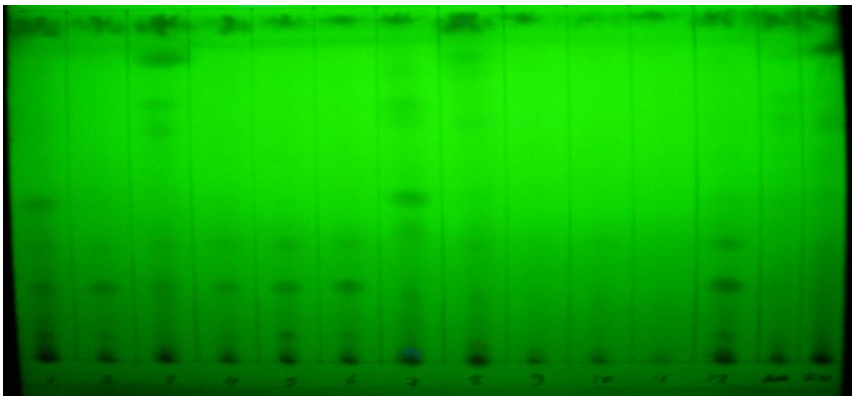
1- *Bacillus* (glucose)
2- *Bacillus* (sucrose)
3- *Bacillus* (mannitol)
4- *Bacillus* (fructose)
5- *Azotobacter* (sucrose)
6- *Azotobacter* (mannitol)
7- *Azotobacter* (fructose)
8- *Azotobacter* (glucose)
9- *Azotobacter* (sucrose + mannitol)
10- *Azotobacter* (sucrose + fructose)
11- *Azotobacter* (sucrose + glucose)
12- *Azotobacter* (mannitol + fructose)
13- *Azotobacter* (mannitol + glucose)
14- *Azotobacter* (glucose + fructose)

Fig 2.a. TLC separation of auxins and gibberellins produced by *A. chroococcum and B. megaterium var. phosphaticum* grown in presence of different carbon sources using auxins development separation system



1- *Bacillus* (glucose)
2- *Bacillus* (sucrose)
3- *Bacillus* (mannitol)
4- *Bacillus* (fructose)
5- *Azotobacter* (sucrose)
6- *Azotobacter* (mannitol)
7- *Azotobacter* (fructose)
8- *Azotobacter* (glucose)
9- *Azotobacter* (sucrose + mannitol)
10- *Azotobacter* (sucrose + fructose)
11- *Azotobacter* (sucrose + glucose)
12- *Azotobacter* (mannitol + fructose)
13- *Azotobacter* (mannitol + glucose)
14- *Azotobacter* (glucose + fructose)

Fig 2.b. TLC separation of cytokinins produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* grown in presence of different carbon sources using cytokinins development separation system



1- *Bacillus* (10^{-3})
2- *Bacillus* (10^{-4})
3- *Bacillus* (10^{-5})
4- *Bacillus* (10^{-6})
5- *Bacillus* (10^{-7})
6- *Bacillus* (10^{-8})
7- *Azotobacter* (10^{-3})
8- *Azotobacter* (10^{-4})
9- *Azotobacter* (10^{-5})
10- *Azotobacter* (10^{-6})
11- *Azotobacter* (10^{-7})
12- *Azotobacter* (10^{-8})

Fig 3.a. TLC separation of auxins and gibberellins produced by *Azotobacter chroococcum* and *Bacillus megaterium* var. *Phosphaticum* grown in presence of different tryptophan concentrations using auxins development separation system

B)Quntitative analysis of the produced PGRs .

Table (2) show the PGRs amounts produced as a result for different sugar application the results indicated that the two investigated strains produced PGRs under all applied carbon sources. Data also showed that mannitol was the more convenient carbon source for PGRs production by *A. chroococcum.* Whereas, glucose was the best carbon source for PGRs production by *B. megaterium var. phosphaticum.*

Table 2. Quntitative analysis of the PGRs produced under application of different carbon sources (mg . L^{-1}).

Carbon sources	Auxins		Gibberellin	Cytokinins				
	IAA	IBA	GA_3	Z	KIN	(9R) BAP	(9G) BAP	IP
Azotobacter chroococcum								
Glucose	14.0	37.7	38.3	53.9	82.9	30.7	ND	ND
Fructose	8.40	26.7	44.8	ND	82.6	52.2	ND	ND
Sucrose	15.0	34.3	25.7	54.7	81.5	39.5	ND	5.90
Mannitol	18.6	39.3	66.2	64.7	97.4	79.2	0.72	7.30
Bacillus megaterium* var. *phosphaticum								
Glucose	18.6	46.2	94.6	55.9	64.6	66.4	ND	4.40
Fructose	3.40	38.3	6.13	37.9	45.6	55.2	1.97	3.90
Sucrose	5.30	45.4	21.1	35.9	56.7	60.0	3.90	ND
Mannitol	ND	ND	0.00	41.2	64.6	66.4	ND	4.40

Generally, the data show that *A. chroococcum* produced higher amounts of IAA rather than *B. megaterium* var. *phosphaticum* with application of any carbon sources. Whereas the contrary was occurred for IBA production by the two strains. Respecting the effect of carbon sources on cytokinins production, data revealed that *A. chroococcum* produced higher amounts of zeatin and kinetin than that produced by *B. megaterium* var. *phosphaticum*. Also, the produced amounts of cytokinins by the two strains under application of different carbon sources were higher than those produced from auxins and gibberellins (GA_3) .

Similar results were observed by **Martinez-Toledo *et al* (1988)** who found that *A. chroococcum* grown on both of the two medium states (supplemented with 0.5% glucose or amended with maize root exudates) stimulated the production of all phytohormones.

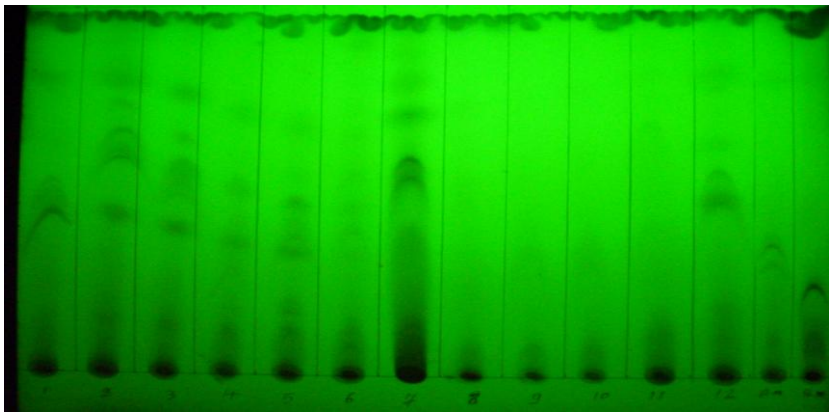
Morsy (2005) studied the effect of different carbon sources (glycerol, glucose, fructose, mannose, xylose, arabinose and sucrose) for maximum IAA and GA_3 production by three strains of *B. subtilis* 47, 82 and 104 . The author found that glucose, fructose and glycerol were the best carbon sources , respectively.

Effect of tryptophan ammendment on PGRs production

A) Qualitative analysis of PGRs produced in presence of different tryptophan concentrations.

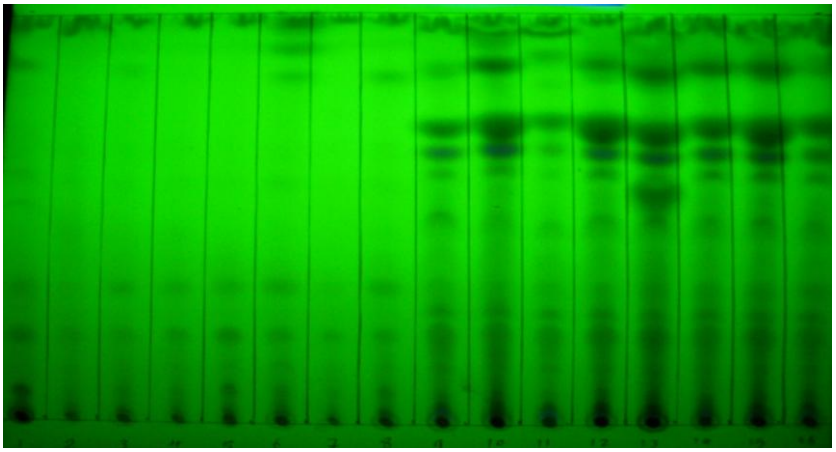
Fig (3 a) show the separation of PGRs compounds produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* under application of different tryptophan concentrations using auxins development separation system.

Obtained data showed that the application of tryptophan with concentrations (10^{-3} – 10^{-4} *M*) activated the production of IAA and IBA by *A. chroococcum*. The application of tryptophan with 10^{-8} *M* increased the production of IAA and GA_3. On the other hand, *B. megaterium* var. *phosphaticum* produced Z , IAA , GA_3 and IBA when tryptophan was applied with concentration 10^{-5} molar. Also, the same compounds exept IBA were detected with the application of other tryptophan concentrations.



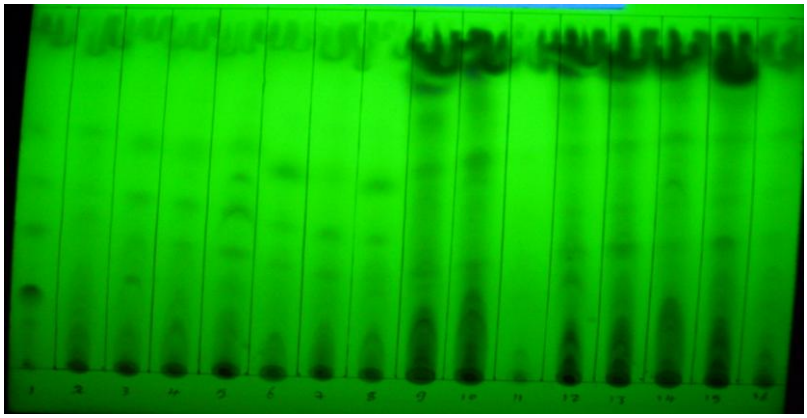
1- *Bacillus* (10^{-3})
2- *Bacillus* (10^{-4})
3- *Bacillus* (10^{-5})
4- *Bacillus* (10^{-6})
5- *Bacillus* (10^{-7})
6- *Bacillus* (10^{-8})
7- *Azotobacter* (10^{-3})
8- *Azotobacter* (10^{-4})
9- *Azotobacter* (10^{-5})
10- *Azotobacter* (10^{-6})
11- *Azotobacter* (10^{-7})
12- *Azotobacter* (10^{-8})

Fig 3.b. TLC separation of cytokinins produced by *A. chroococcum* and *Bacillus megaterium* var. *Phosphaticum* grown in presence of different tryptophan concentrations using cytokinins development separation system



1- *Bacillus* (10^{-3})
2- *Bacillus* (10^{-4})
3- *Bacillus* (10^{-5})
4- *Bacillus* (10^{-6})
5- *Bacillus* (10^{-7})
6- *Bacillus* (10^{-8})
7- *Bacillus* (10^{-9})
8- *Bacillus* (10^{-10})
9- *Azotobacter* (10^{-3})
10- *Azotobacter* (10^{-4})
11- *Azotobacter* (10^{-5})
12- *Azotobacter* (10^{-6})
13- *Azotobacter* (10^{-7})
14- *Azotobacter* (10^{-8})
15- *Azotobacter* (10^{-9}).
16- *Azotobacter* (10^{-10}).

Fig 4.a. TLC separation of auxins and gibberellins produced by *A .chroococcum* and *B. megaterium* var. *phosphaticum* grown in presence of different adenine concentrations using auxins development separation system



1- *Bacillus* (10^{-3})
2- *Bacillus* (10^{-4})
3- *Bacillus* (10^{-5})
4- *Bacillus* (10^{-6})
5- *Bacillus* (10^{-7})
6- *Bacillus* (10^{-8})
7- *Bacillus* (10^{-9})
8- *Bacillus* (10^{-10})
9- *Azotobacter* (10^{-3})
10- *Azotobacter* (10^{-4})
11- *Azotobacter* (10^{-5})
12- *Azotobacter* (10^{-6})
13- *Azotobacter* (10^{-7})
14- *Azotobacter* (10^{-8})
15- *Azotobacter* (10^{-9}).
16- *Azotobacter* (10^{-10}).

Fig 4.b. TLC separation of cytokinins produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* grown in presence of different adenine concentrations using cytokinins development separation system

Fig (3 b) emphasize the PGRs produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* under different tryptophan concentration detected by using cytokinin development separation system . Obtained results showed that IP , (9R)BAP , KIN and IAA compounds were produced by *A. chroococcum* when tryptophan was applied with 10^{-3} molar. The same compoundes exept KIN were detected when tryptophan was applied with 10^{-8} molar .

From **Figs (3 a&b)** we can observed that growth regulators GA_3, Z and KIN produced by *B. megaterium* var. *phosphaticum*, when tryptophan was applied with 10^{-3} molar. whereas its application with 10^{-4} molar tryptophan, GA_3,Z, (9G)BAP and IBA were produced with the other applied concentrations of tryptophan *B. megaterium* var. *phosphaticum* produced Z, (9R)BAP, IP, IAA and KIN. Identification of these PGRs were achieved by TLC according to their retention flow rates.

B) Quantitative analysis of thePGR produced s under of different tryptophan concentrations.

Data in **Table (3)** clearly showed that the production of auxins, gibberellic acid and cytokinins increased with increasing tryptophan concentration. These results was observed with both *A. chroococcum and B. megaterium* var. *phosphaticum* strains.

These results are in harmony with those obtained by **Khalid *et al* (2004) and Zahir *et al* (2005)** who found that L-tryptophan serves as a physiological precursor for auxins in plant and microbes of the rhizobacterial isolates (*Azospirillum* sp, *Bacillus* sp and *Pseudomonas* sp), 83% produced auxins in absence of L- tryptophan, whereas 100% produced auxins in presence of L-tryptophan. Most isolates of rhizobacteria were likely to be due to L- tryptophan serving as an auxins precursor rather than any precursor.

The highest produced amounts of auxins , gibberellic acid and cytokinins by the two strains were recorded when 10^{-3} molar of tryptophan was applied. While , the reverce was observed when 10^{-8} molar of tryptophan was used.

Table 3. Quantitative analysis of the PGRs produced in presence of different tryptophan concentrations. (mg . L^{-1}).

Tryptophan concentrations *(molar)*	Auxins		Gibberellin	Cytokinins				
	IAA	IBA	GA_3	Z	KIN	(9R) BAP	(9G) BAP	IP
Azotobacter chroococcum								
10^{-8}	2.80	ND	12.4	23.0	13.2	ND	ND	ND
10^{-7}	3.34	12.6	12.7	24.4	18.5	ND	ND	ND
10^{-6}	5.52	10.4	17.8	30.0	22.3	ND	ND	ND
10^{-5}	5.71	18.0	23.3	31.9	33.8	ND	ND	0.91
10^{-4}	6.64	25.3	45.6	33.7	48.1	ND	0.30	1.92
10^{-3}	10.3	37.1	85.5	42.5	49.4	17.6	1.44	4.75
Bacillus megaterium var. phosphaticum								
10^{-8}	ND	ND	ND	14.8	14.0	12.0	ND	ND
10^{-7}	8.11	10.7	16.3	18.5	18.2	24.0	ND	ND
10^{-6}	8.53	18.0	21.4	19.2	26.6	29.3	0.46	0.87
10^{-5}	9.30	18.8	44.0	23.1	33.7	41.9	0.94	1.24
10^{-4}	11.2	18.9	62.2	36.5	54.2	49.6	2.32	2.22
10^{-3}	13.6	24.9	76.4	41.3	93.5	68.3	2.81	6.32

Concerning the effect of different tryptophan concentrations on cytokinins production, data in **Table** (**3)** emphasized that *A. chroococcum* produced higher amounts of zeatin compared to those

produced by *B. megaterium* var. *phosphaticum* . On the contrary, *B. megaterium* var. *phosphaticum* strain produced higher amounts of kinetin , (9R) benzyl adenine , (9G) benzyl adenine and isopentyl alcohol compared to those produced by *A. chroococcum* under all applied different concentration of tryptophan.

Effect of adenine concentrations on PGRs production

A) Qualitative analysis of the PGRs produced in presence of different adenine concentrations

Fig (4 a) show different PGRs compounds produced by *A. chroococcum* and *B. megaterium* var. *phosphaticum* under application of different adenine concentrations which were detected by Thin layer chromatography using auxin development separation system.

A. chroococcum grown in presence of 10^{-3} molar adenine, three compounds having R_f values (0.24 , 0.32 and 0.86) were appeared and identified as IAA, GA_3 and IBA, respectively . Also, IAA and IBA were detected when applied the other concentrations of adenine.

Moreover, data in **Fig (4 a)** showed that *B. megaterium* var. *phosphaticum* produced IAA , GA_3 and IBA when adenine was applied at concentrations of 10^{-3} and 10^{-5} molar . Whereas , Z , IAA , GA_3 and IBA were produced when it was added with 10^{-8} and 10^{-10} molar . On the other hand, the separation of thebextract components with of cytokinins development system indicated that the produced PGRs compounds by *A. chroococcum* and *B. megaterium* var. *phodsphsticum* had many different compounds shown in **Fig (4 b)** .

Obtained results indicated that the compounds (9G)BAP and IAA were detected in the extracted culture of *A. chroococcum* when 10^{-3} molar of adenine was applied. While, Z and KIN detected when 10^{-4} molar of adenine was applied . Two another compounds were produced have R_f values (0.3 and 0.63) can be identified as Z and IP , respectively .

Concerning the produced compounds by *B. megaterium* var. *phosphaticum*, **Fig (4 b)** show that, zeatin was produced under all investigated concentrations of adenine except the concentration 10^{-6} molar. Since, KIN was the only produced compound. Also, Z , IP , ADE and (9G)BAP were detected when adenine was applied with 10^{-3} molar. On reverse, the production of IP was clearly detected under the most applied adenine concentrations.

B) Quantitative analysis of the PGRs produced in presence of different adenine concentrations

It is obvious from data in **Table (4)** that, the highest amounts of PGRs produced by *A. chroococcum* strain were detected when 10^{-5} molar of adenine was used. Whereas, the highest amounts produced by *B. megaterium* var. *phosphaticum* strain were detected when 10^{-4} molar of adenine was used .

In addition, the lowest production of PGRs by the two strains were detected when the application of adenine was decreased to 10^{-10} molar. Moreover, at the optimum adenine concentration (10^{-5} and 10^{-4}) molar the production of IBA by *A. chroococcum* and *B. megaterium* var. *phosphaticum* was higher than IAA .

Also, the data indicated that *A. chroococcum* and *B. megaterium* var. *phosphaticum* strains produced higher amounts of kinetin and (9R) benzyl adenine than other cytokinins compounds. Similar trends of results was recorded with the different adenine concentrations. It is important to mention that *A. chroococcum* strain gave higher records of gibberellic acid rather than those given by *B. megaterium* var. *phosphaticum* strain. This result was observed at all applied adenine

concentrations . Also, *A. chroococcum* showed higher values of cytokinin compounds at most applied adenine concentration rather than these produced by *B. megaterium* var. *phosphaticum* strain.

Table 4. Quantitative analysis of the PGRs produced in presence of different adenine concentrations (mg . L^{-1}).

Adenine concentrations *(Molar)*	Auxins		Gibberellin	Cytokinins				
	IAA	IBA	GA_3	Z	KIN	(9R)BAP	(9G)BAP	IP
				Azotobacter chroococcum				
10^{-10}	4.70	ND	22.6	20.7	ND	36.9	0.50	ND
10^{-9}	9.20	ND	35.7	57.3	45.6	70.4	1.40	ND
10^{-8}	8.30	22.6	42.6	11.0	46.6	72.5	1.70	ND
10^{-7}	10.6	23.7	45.3	71.7	52.8	71.9	2.80	ND
10^{-6}	8.00	33.7	52.3	37.7	56.6	76.2	3.00	5.10
10^{-5}	14.3	41.8	59.8	79.3	92.8	96.2	4.50	9.46
10^{-4}	10.6	ND	48.0	51.7	86.6	82.3	2.00	9.20
10^{-3}	5.90	ND	42.7	76.8	69.9	70.3	1.00	12.0
				Bacillus megaterium var. phosphaticum				
10^{-10}	2.40	10.4	7.01	8.90	ND	13.7	ND	ND
10^{-9}	0.40	11.3	9.43	16.4	12.3	21.3	ND	ND
10^{-8}	1.40	22.3	13.0	17.3	61.5	30.1	ND	ND
10^{-7}	1.00	22.4	13.3	39.7	63.9	35.2	ND	1.10
10^{-6}	2.30	25.1	17.2	46.7	76.7	43.9	0.67	2.00
10^{-5}	12.9	28.4	23.8	58.5	82.6	66.7	0.80	2.00
10^{-4}	5.40	40.4	30.4	69.3	89.7	85.2	1.60	2.20
10^{-3}	4.50	ND	13.1	43.1	73.1	19.8	ND	ND

Similar trend of results was observed by **Nieto and Frankenberger (1989, a)** who studied the effect of various concentrations (10, 1, 0.1 mM) of adenine on biosynthesis of cytokinins by *A. chroococcum*. He found that 10 mM was the best concentration for zeatin production

Also , **Arshad and Frankenberger (1991)** found that the application of adenine at 10^{-5} *M* to *Azotobacter chroococcum* culture was the best one among several applied concentrations (10^{-3} - 10^{-6} *M*) for cytokinins production .

Optimal conditions and PGRs production by *Azotobacter chroococcum* and *Bacillus megaterium var. phosphaticum*

Obtained results recorded in **Table (5)** showed that the optimal conditions gave the highest production of PGRs . This result is logic and was anticipated . Data recorded in **Table (5)** clearly indicated that *B. megaterium* var. *phosphaticum* produced higher amounts of auxins rather than *A. chroococcum*. While , the reverse occurred with gibberellic acid production .

Table 5. Optimal conditions and PGRs Production by *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* (mg. L^{-1}).

Compounds / Strains	Auxins		Gibberellin	Cytokinins				
	IAA	IBA	GA_3	Z	KIN	(9R) BAP	(9G) BAP	IP
Azotobacter chroococcum	71.8	56.7	145.6	107.9	93.9	100.4	9.3	17.0
Bacillus megaterium var. phosphaticum	79.6	102.0	102.3	98.1	97.4	69.3	10.0	11.3

Regarding the cytokinins production, obtained data revealed that zeatin and (9R) benzyl adenine were produced with higher amounts by *Azotobacter chroococcum* compared to those produced by *Bacillus megaterium* var. *phosphaticum* .

CONCLUSION AND RECOMMENDATION

In view of the obtained results, it could be concluded that the nutritional substances affecting the PGRs production since the tryptophan is considered as a precursor for auxins and gibberellins biosynthesis. As well as adenine is considered the most suitable precursor for cytokinins biosynthesis .

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اختيار أفضل مصادر الكربون والمواد المحفزة لإنتاج منظمات النمو بواسطة البكتريا المشجعة لنمو النبات

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فى هذا البحث تم دراسة تأثير مصادر الكربون والتربتوفان والآدينين على معدل إنتاج منظمات النمو بواسطة جنسى البكتريا *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* .

ولقد أوضحت النتائج أن أنسب مصدر كربون للحصول على أعلى إنتاج من منظمات النمو بواسطة *A. chroococcum* هو المانيتول ، بينما كان الجلوكوز هو أنسب مصدر كربونى لميكروب *B. megaterium* var. *phosphaticum* . أيضا أوضحت النتائج أن أعلى إنتاج من منظمات النمو وخصوصا الإندولات بواسطة كلا منهما لوحظ عند إضافة التربتوفان بتركيز $١٠^{-٣}$ مولر بينما تناقص بإنخفاض تركيز التربتوفان فى بيئة النمو . كذلك أوضحت النتائج المتحصل عليها أن إضافة الآدينين بتركيز $١٠^{-٥}$ مولر و $١٠^{-٤}$ مولر إلى بيئة نمو كل من *A. chroococcum* و *B. megaterium* var. *phosphaticum* على التوالى أدى إلى الحصول على أعلى إنتاج من منظمات النمو.

عند إستخدام الظروف المثلى المتحصل عليها من التجارب السابقة فى إنتاج منظمات النمو بواسطة السلالتين تحت الدراسة تم الحصول على أعلى إنتاج من كل منظمات النمو التى درست وذلك بالمقارنة بإستخدام كل عامل بمفرده . أثبتت الدراسة أن ميكروب *B. megaterium* var. *phosphaticum* أنتج كمية أكبر من الأوكسينات مقارنة بميكروب *A. chroococcum* . بينما أنتج ميكروب *A. chroococcum* كمية أكبر من حامض الجبريلليك مقارنة بميكروب *B. megaterium* var. *phosphaticum* . كذلك وجد أن ميكروب *Azotobacter chroococcum* أنتج كمية كبيرة من الزياتين والبنزيل آدينين مقارنة بميكروب *Bacillus megaterium* var. *phosphaticum* بينما كان إنتاج *Azotobacter chroococcum* من الكينيتين أعلى من.