



Effect of different nutrient sources on the production of plant growth regulators (PGRs) by fluorescent *Pseudomonas* species

KEYWORDS

Fluorescent *Pseudomonas*, Plant growth regulators, Auxins, Gibberellins, Cytokinins

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ABSTRACT Plant growth regulators are important secondary metabolites produced by fluorescent *Pseudomonas* sp. such as auxins, gibberellic acid and cytokinins which play significant role in increasing the root surface and length that help in plant growth promotion and development. Two strains of fluorescent *Pseudomonas* sp. An-1-kul and An-13-kul were found to produce plant hormone i.e. auxins, gibberellins and cytokinins. The plant hormone production was further evaluated by using different media (succinate, King's B, nutrient, peptone water and trypticase soya broth) and nutrient sources i.e. carbon (Sucrose, D-Glucose, Meso-inositol) and nitrogen sources (L-tryptophan). In the studies it was found that nutrient broth was best among all the media used for plant hormone production and media amended with tryptophan was found best for auxins production and D-glucose was found best for production of gibberellins and cytokinins.

Introduction

The microbial synthesis of plant growth regulators (auxins, gibberellins and cytokinins) is an important factor in soil fertility (Kampert and Strzelczyk, 1975). Plant growth regulators are the organic substances that influence physiological processes of plants at very low concentrations when produced endogenously by plants, they are referred to as phytohormones (plant hormones), where as the term plant growth regulators (PGRs) include many synthetic and naturally occurring compounds. Nickell (1982) defined this term i.e. plant growth regulators as " either natural or synthetic compounds including microbial plant growth regulators that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields or facilitate harvesting". The production of plant growth regulators induces additional root hair and lateral root formation (Tien et al., 1979). Thereby enhancing the plant's ability to take up nutrients from soil and increasing the yield. Rapid establishment of roots, whether by elongation of primary roots or by proliferation of lateral and adventitious roots, is advantageous for young seedlings as it increases their ability to anchor themselves to the soil and to obtain water and nutrient from the environment, thus enhancing the chance of survival. Most root promoting bacteria synthesize indole acetic acid (IAA) and their effects on plant mimic that of exogenous indole acetic acid (Alvares et al., 1989; Meuwley and Pilet, 1981). Microorganisms inhabiting rhizosphere of various plants are likely to synthesize and release auxins as secondary metabolites. Improved plant growth via plant associated bacteria has been reported in many plants microbes interactions. Bacteria that inhabit the rhizosphere may influence plant growth directly by contributing to host plants endogenous pool of plant growth regulators; indole acetic acid (Lee et al., 2004) via phosphate solubilization activity, by enhancing bacterial root and production of siderophores. Microbial synthesis of plant growth regulators is an important factor of soil fertility (Kampert and Sterezyk, 1975). Gibberellins, cytokinins and indole acetic acid are secondary metabolites which are important biotechnology and economically products produced commercially from fungi for agricultural and horticultural industry (Decan, 1984).

The ability of microorganism to synthesize various metabolites can effect the soil properties, the growth in chemical composition of plants and considered as one of the most important factor of soil fertility (Vancera and Jandera, 1986). Numerous *Pseudomonas* strains display plant growth promoting activities and have been investigated due to their widespread distribution in soil, ability to colonize the rhizosphere of host plants and ability to produce a range of compound antagonistic to a number of serious plant pathogens (Ross et al., 2000) Present aim of study is to evaluate different media and nutrient sources for the production of plant growth regulators.

Material methods

Pseudomonas sp. isolated from the rhizosphere soil of apple orchards were screened out for the production of plant growth regulators viz., auxins, gibberellins and cytokinins. Standard methods were used for estimation of plant growth regulators i.e. auxins (Gordon and Weber, 1951), gibberellins (Holbrook et al., 1961) and cytokinins (Letham, 1971) grown on different media and carbon and nitrogen source.

Effect of different media on the production of plant growth regulators by *Pseudomonas aeruginosa* (An-1-kul and An-13-kul) at different time of incubation

Effect of media on the production of plant growth regulators i.e. auxins, gibberellins and cytokinins were studied by two *Pseudomonas* strains An-1-kul and An-13-kul in five different broth; succinate, King's B, nutrient, peptone water and trypticase soya broth. 0.5ml of inoculum of 18 h. old of culture was used to inoculate 100 ml of broth in 250 ml of Erlenmeyer flask. Flasks were incubated at 28° C for 72 h under shaken conditions (90rpm). Supernatant were harvested by centrifugation at 10,000 rpm for 30 minute at 4° C and were used for estimation of auxins, gibberellins and cytokinins.

Effect of few additional nutrient sources on growth and production of plant growth regulators by *Pseudomonas* strain An-1-kul and An-13-kul

Effect of additional nutrient sources viz., Sucrose, L-tryptophan, D-Glucose, Meso-inositol on the production of

plant growth regulators i.e. auxins, gibberellins and cytokinins were studied. Two strains of *Pseudomonas* sp. (An-1-kul and An-13-kul) were grown in nutrient broth having 1% of additional nutrient sources. 0.5 ml of 18 h. old inoculum of each *Pseudomonas* sp. (An-1-kul and An-13-kul) was inoculated in 100ml broth in 250 ml of Erlenmeyer flask and incubated at 28°C for 72 h. under shaken conditions (90rpm). Supernatant were harvested by centrifugation at 10,000 rpm for 30 minute at 4°C and were used for estimation of auxins, gibberellins and cytokinins.

Result and discussion

Effect of different media on growth of *Pseudomonas* sp. and production of plant growth regulators

Effect of Media

Different organisms are able to grow in different types of growth media but the growth and biological activity may vary since organisms face different environmental factors affecting the production of biological activity by an organism and provide information for possible parameters affecting biological activity production by organisms in vitro. The present experiment focused on the identification of a suitable growth medium for *Pseudomonas aeruginosa* An-1-kul, An-13-kul that could induce higher levels of plant growth regulators production. The two selected isolates of *Pseudomonas aeruginosa* preferred same media for optimum production of plant growth regulators.

The best media for the growth and production of plant growth regulators viz., auxins, gibberellins and cytokinins by *Pseudomonas aeruginosa* (An-1-kul, An-13-kul) was nutrient broth. They result revealed that (Table 1 and 2) all the three growth regulators viz., auxins, gibberellins and cytokinins were produced maximally by An-1-kul and An-13-kul in nutrient broth after 72h at 28°C as compared to production in other broth like succinate, trypticase soya and peptone water. Although good growth may occur in many media secondary metabolites may only be produced in a specific medium (Bentley and Keil, 1962). Sometimes a given organisms may produce one metabolite on one medium and a totally different one on another medium Variation in the chemical composition of the medium and its relationship to yield and types of secondary metabolites is well known (Oxford et al., 1935). Development of medium that produce high yields of a desired secondary metabolite is still empirical to a considerable degree (Demain, 1972 and 1973). Distribution of precursors into various branched pathways may vary and may depend on the growth environment and the genotype of the organism. The flow of precursors can be increased in the desired direction by mutation or medium manipulation. The presence or absence of certain ions of carbon and nitrogen sources can inhibit, activate, induce and suppress certain enzymes per-

it can be concluded from the present study that fluorescent *Pseudomonas* sp. can be further used for biofertilizer development.

Table.1. Effect of different media and time of incubation on the growth and production of auxins, gibberellins and cytokinins produced by *Pseudomonas aeruginosa* An-1-kul

Media/ broth	Growth (A_{540})				Production of plant growth regulators ($\mu\text{g/ml}$)											
					Auxins*				Gibberellins**				Cytokinins***			
	Time interval(h)				Time interval(h)											
	24h	48h	72h	Mean	24h	48h	72h	Mean	24h	48h	72h	Mean	24h	48h	72h	Mean
Succinate	0.21	0.25	0.30	0.25	7.27	9.32	11.93	9.50	143.5	160.6	168.0	157.4	0.58	45.45	122.7	56.24
King's B	0.27	0.31	0.35	0.30	6.00	8.66	10.98	8.54	138.7	150.3	161.6	150.2	1.17	90.9	154.5	82.19

turbing the normal channeling of key intermediates that support balanced growth (Weinberg, 1978; Malik, 1982).

Effect of additional nutrient sources on plant growth regulator production

All organisms require an energy source for cell synthesis. The nutritional (carbon and protein) source is frequently the energy source, in addition to its role as a major constituent of cellular material. As a source of cellular material carbon forms approximately 50 per cent of the dry weight of bacteria and fungi. Carbohydrates, particularly glucose, are very popular choices for growth media, but, depending upon the particular nutritional versatility of the organisms under study suitable carbon sources also include carbohydrate derivatives, fatty acids, alcohols, organic acids, hydrocarbons and organic nitrogenous compounds. The use of glucose resulted in dramatic increase of pyochelin and salicylic acid, indicating that the ratio of carbon source to nutrient concentration played a key role in the metabolic flow (Duffy and Defago, 1999). Our study showed that plant growth regulators production was maximum when media supplemented with galactose followed by meso-ionositol, tryptophan and minimum plant growth regulators production was found in the media containing sucrose as carbon source. Maximum plant growth regulators production in case of auxins was recorded in media having additional carbon L-tryptophan and minimum in meso-ionositol containing medium this trend of plant growth regulators production in all the two strains was similar with all the substrates. The production of auxins (Table 3 and 4) by *Pseudomonas aeruginosa* An-1-kul was found maximum in L-tryptophan containing broth (23.6 $\mu\text{g/ml}$) than the other nutrient sources. Results revealed that production of gibberellins by *Pseudomonas aeruginosa* was found maximum in D-glucose containing broth 257.4 $\mu\text{g/ml}$ the average production of cytokinins (Table 3) by *Pseudomonas aeruginosa* was found maximum in D-glucose containing broth (130.2 $\mu\text{g/ml}$). Similarly in case An-13-kul, the production of auxins (Table 4) was found maximum in L-tryptophan containing broth (22.3 $\mu\text{g/ml}$) than the other nutrient sources. Results revealed that production of gibberellins was found maximum L-tryptophan containing broth 242.7 $\mu\text{g/ml}$ followed by D-glucose containing broth i.e. 229.8 $\mu\text{g/ml}$. The production of cytokinins was found maximum in D-glucose containing broth (204.5 $\mu\text{g/ml}$).

CONCLUSION

Present study suggested that fluorescent *Pseudomonas* sp. have capacity to produce plant growth regulators and its production further enhanced by growing in suitable growth medium and nutrients. Tryptophan amended nutrient broth was found more suitable for the production of auxins. So

Nutrient	0.30	0.51	0.95	0.59	9.27	12.26	17.82	12.17	207.7	222.2	229.3	207.2	2.35	181.8	227.2	130.9
Trypticase soy	0.22	0.27	0.31	0.26	5.83	8.54	11.10	8.48	137.1	148.0	160.6	148.8	1.76	113.6	136.3	84.11
Peptone water	0.19	0.21	0.25	0.21	5.50	7.49	10.21	7.73	129.3	144.8	156.4	144.9	0.35	45.45	113.6	53.13
Mean	0.23	0.31	0.43	0.32	6.77	9.18	12.41	9.45	151.3	165.2	176.2	164.2	1.24	95.44	150.8	82.51

Effect CD _{0.05}																
Media (T)					0.009				1.00				0.094			0.87
Interval (I)					0.007				0.07				0.072			0.67
Interaction (T X I)					0.014				0.17				0.161			0.151

Production of plant growth regulators i.e. auxins*, gibberellins** and cytokinins*** produced in supernatant and expressed in terms of concentration (µg/ml) as calibrated from the standard curve of indole acetic acid (IAA); 10-100(µg/ml) , gibberellic acid (GA₃) ; 100-1000(µg/ml) , kinetin; 100-1000 (µg/ml)

Table.2. Effect of different media and time of incubation on the growth and production of auxins, gibberellins and cytokinins produced by Pseudomonas aeruginosa An-13-kul

Media/ broth	Growth (A ₅₄₀)				Production of plant growth regulators (µg/ml)											
					Auxins*				Gibberellins**				Cytokinins***			
	Time interval(h)				-Time interval(h)											
	24h	48h	72h	Mean	24h	48h	72h	Mean	24h	48h	72h	Mean	24h	48h	72h	Mean
Succinate	0.19	0.25	0.30	0.24	4.77	6.93	12.26	7.98	114.81	147.06	183.18	148.3	0.47	68.18	95.45	55.09
King's B	0.20	0.28	0.45	0.31	2.49	6.38	9.88	6.25	106.10	136.4	175.76	139.4	0.58	81.81	100.0	60.80
Nutrient	0.25	0.35	0.81	0.47	7.49	12.48	16.53	13.0	171.57	210.92	238.98	219.7	1.76	186.36	204.54	137.4
Trypticase soy	0.17	0.27	0.32	0.25	1.89	6.22	9.27	5.79	111.27	133.83	165.12	136.7	1.17	104.54	113.63	73.11
Peptone water	0.28	0.35	0.48	0.37	1.83	5.43	8.04	5.1	103.2	130.61	157.70	130.5	0.35	63.63	90.9	51.53
Mean	0.22	0.30	0.47	0.33	7.48	3.69	11.20	7.46	121.4	151.8	184.1	152.43	0.866	101.1	120.8	74.25

Effect CD_{0.05}

Media (T)	0.0096	0.96	0.962	0.092
Interval (I)	0.0074	0.745	0.074	0.071
Interaction (T X I)	0.0165	0.165	0.166	0.159

• Production of plant growth regulators i.e. auxins*, gibberellins** and cytokinins*** extracellularly in supernatant and expressed in terms of concentration (µg/ml) and calibrated from the standard curve of indole acetic acid (IAA); 10-100(µg/ml) , gibberellic acid (GA₃) ; 100-1000(µg/ml) , kinetin; 100-1000 (µg/ml)

Table 3. Effect of additional nutrient sources and time of incubation on the growth and production of auxins, gibberellins and cytokinins produced by Pseudomonas aeruginosa An-1-kul

Nutrient sources(1% each in NB)	Growth (A ₅₄₀)				Production of plant growth regulators (µg/ml)														
					Auxins*				Gibberellins**				Cytokinins***						
	Time interval(h)				Time interval(h)														
	24h	48h	72h	Mean	24h	48h	72h	%inc./ decr. over NB*	Mean	24h	48h	72h	%inc./ decr. over NB*	Mean	24h	48h	72h	%inc./ decr. over NB*	Mean
Sucrose	0.19	0.24	0.30	0.25	1.95	5.35	9.15	94.75↓	5.48	176.3	204.7	252.9	10.29↑	211.3	91.5	104.8	113.6	10.0↓	103.3
L-Tryptophan	0.2	0.30	0.45	0.31	9.75	16.78	23.6	32.4 ↑	16.71	184.9	196.3	257.4	12.25↑	212.9	102.2	116.7	150.0	51.46↓	123.0
D-Glucose	0.25	0.34	0.81	0.47	4.56	10.73	14.48	23.06↓	9.92	189.3	206.9	258.3	12.64↑	218.8	110.3	121.3	159.1	42.80↓	130.2
Meso-inositol	0.17	0.26	0.32	0.25	1.48	3.16	7.21	147.1↓	3.96	161.5	182.1	203.5	12.67↓	182.4	88.3	100.1	68.2	223.1↓	98.87
Mean	0.20	0.285	0.47		4.44	9.00	13.61			178.0	197.5	243.5			98.07	110.7	132.7		

CD _{.05}				
T	0.0097	0.976	0.972	0.972
I	0.0084	0.846	0.842	0.842
T X I	0.017	1.692	1.692	1.67

• Production of plant growth regulators i.e. Auxins*, Gibberellins** and Cytokinins*** in supernatant and expressed in terms of concentration (µg/ml) as calibrated from the standard curve of indole acetic acid (IAA); 10-100(µg/ml) , gibberellic acid (GA₃) ; 100-1000(µg/ml) , kinetin;100-1000 (µg/ml) at different incubation with additional source of carbon in nutrient broth

Table 4. Effect of additional nutrient sources and time of incubation on the growth and production of auxins, gibberellins and cytokinins produced by *Pseudomonas aeruginosa* An-13-ukl

Nutrient sources (1% in NB)	Growth (A ₅₄₀)				Production of plant growth regulators (µg/ml)															
					Auxins*				Gibberellins**				Cytokinins***							
	Time interval(h)				Time interval (h)															
	24h	48h	72h	Mean	24h	48h	72h	%inc./decr. over NB*	Mean	24h	48h	72h	%inc./decr. over NB*	Mean	24h	48h	72h	%inc./decr. over NB*	Mean	
Sucrose	0.16	0.24	0.31	0.24	3.79	6.98	9.71	70.23 ↓	6.83	181.91	232.7	253.5	6.07 ↑	222.7	85.5	95.8	104.5	95.7 ↓	95.27	
L-Tryp- tophan	0.19	0.30	0.45	0.31	16.6	19.7	22.3	34.9 ↑	19.03	196.4	242.7	256.4	7.28 ↑	231.6	169.3	192.4	204.5	-	164.0	
D-Glu- cose	0.22	0.34	0.83	0.46	3.81	8.46	12.26	34.8 ↓	8.18	202.3	229.8	262.8	9.96 ↑	232.2	135.7	168.9	186.4	9.73 ↓	189.1	
Meso- ionositol	0.18	0.26	0.32	0.26	2.32	6.78	10.4	58.9 ↓	6.50	189.4	206.9	226.1	5.69 ↓	207.1	85.3	96.2	102.0	100.5 ↓	94.83	
Mean	0.187	0.285	0.480		6.63	10.48	13.67			192.5	228.0	249.7			119.2	138.6	149.6			

CD _{.05}			
T	0.0097	0.972	0.972
I	0.0084	0.842	0.842
T X I	0.017	1.68	1.68

• Production of plant growth regulators i.e. Auxins*, Gibberellins** and Cytokinins*** in supernatant and expressed in terms of concentration (µg/ml) calibrated from the standard curve of indole acetic acid (IAA); 10-100(µg/ml) , gibberellic acid (GA₃) ; 100-1000(µg/ml) , kinetin;100-1000 (µg/ml) at different incubation periods with additional carbon sources in nutrient broth

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