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ISOLATION, CHARACTERIZATION OF GIBBERELLIC ACID PRODUCING RHIZOACTERIA FROM VARIOUS PLANT ROOT

Aparna A. Bhairappa and V.B. Nannavare
Assistant professor and student
Department of Biotechnology
Walchand College of arts and science, solapur, Maharashtra, India

Abstract - Gibberellic acid (GA3) considered as one of the important plant hormone in growth and development of plant. It has wide applications in agriculture area as well as in horticulture. Bacteria have symbiotic association with plant thus they utilize atmospheric nitrogen and help plant for growth .one of the important metabolite known as gibberellic acid synthesised by bacteria utilized by plant for their growth and development. This study aims at isolation of gibberelic acid producing bacteria and its characterization .Total 25 isolates were isolated from rhizospheric soil of various local plants such as Rice, sugarcane, jowari, mug bean from agricultural field of solapur area .these isolates were screened for gibberellic acid production in artificial nutrient media and analysed by spectrophotometric method. Among these isolates RR5 (Rice Rizobacteria) and SR2 (Sugar Rhizobacteria) produce gibberellic acid in high amount as compare to other isolates and thus used for further study. The filtrate was bio assayed on wheat plant and show significant growth after treatment with organic solution of rhizobacterial filtrate.

Keywords - isolation, gibberellic acid producing, rhizobacteria, separating, chromatography

Introduction

Bacteria are ubiquitous in environment and are abundant in soil and they show symbiotic association with plants and help for their growth and development.one of the secondary metabolite produced by bacteria is used by plant gibberellin have various role in breaking seed dormancy, initiation of flowering stem elongation and promotion of seed germination[1]. As they are also important to show their response to change in environmental conditions .gibberellin also acts as principle agent that regulate expression of genetic potential.gibberellines are group of important diterpenoidacid among commercial phytoharmone. The rhizobacteria *Azotobacter, Azospirillum, pseudomonas* are known as potential for gibberelline production [2]. About 70 different gibberellins are known GA3 is considered as original gibberellic acid [3]. The plant enzymes such as protease, ribonuclease, and nitroreductase are induced by GA. As growing demand for GA leads to production by solid state fermentation .There is no standard protocol for purification but liquid-liquid extraction method is used where ethyl acetate is used as extraction solvent [4]. The present study have been aimed at isolation, characterization of gibberellic acid producing rhizobacteria and check their effect on growth of plant.

Material and methods

Sample collection

Soil sample were collected from agricultural field of vicinity of solapur, Maharashtra. Four different plant root soil sample were collected in the sterile plastic bag and stored at 4 ºC for further use.

Isolation and screening for gibberellic acid producing bacteria

For isolation 1gm of rhizospheric soil was mixed in 9ml sterile D/W and shaken for 15 min to get rhizospheric suspension. This

Obtained rhizospheric suspension is serially diluted up to 10^{-6}. T0 get isolated colonies 0.1 ml from each dilution was plated on yeast Manitol agar. The plates were incubated at 30ºC for 24-48 hrs. For screening GA producing bacterial isolates. The isolated bacteria was inoculated in 100ml yeast manitol broth / nutrient broth for 7 days at 30ºC at 100rpm. After 7 days broth was centrifuged at 5000rpm for 7 min and supernatant was used to analysis amount of GA produced by bacterial isolated by following method

GA3 estimation by DNPH (2, 4 – Dinitrophenyl hydrazine) method (Graham and Thomas, 1961)

For GA estimation 5ml cell free extract and 5ml ethyl acetate was taken in test tube and shake vigorously for 10 min and separate ethyl acetate layer and remaining ethyl acetate was evaporated from organic layer and this organic layer was dissolved in alcohol. 1 ml of DNPH was mixed with 2ml organic suspension and incubated in water bath at 100 °C for 5 min and cooled[6] .And in cooled extract add 5 ml of 10% potassium hydroxide and wait until red wine color developed. Add 15ml of sterile distilled water the content was diluted to 1:2 using sterile distilled water. Color intensity was measured at 430nm in UV-VIS spectrophotometer. For standard GA (0.8 mg/ml) was prepared in absolute alcohol and estimated.

Production of gibberellic acid by submerge fermentation

For production of gibberellic acid submerged fermentation is used where sterile yeast manitol broth /nutrient broth is used. Inoculated isolated single colony in separate 250 ml yeast manitol broth / nutrient broth and incubated for 7 days at 30ºC at 120 rpm samples were removed aseptically and analyzed for GA production at regular interval.

Determination of gibberellic acid by spectrophotometer

After 7 days incubation 15 ml broth was taken and centrifuged at 8000 rpm for 7min to remove biomass. That centrifuged broth was mixed with 2ml zinc acetate and kept for 2min thereafter, 2ml potassium ferocynide was added and centrifuged at 8000 rpm for 15 minutes. 5 ml of supernatant was taken and mixed with 5ml 30% hydrochloric acid and incubated for 1.5 hrs.[8] Absorbance was measured at 254nm using standard prepared standard from 10-100µg/ml

Reagents	**Blank**	**[1]**	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**
Gibberellic acid (ml)	-	1	2	3	4	5	6	7	8	9	10
Phosphomolybdic acid (ml)	15	15	15	15	15	15	15	15	15	15	15
Distilled water	10	9	8	7	6	5	4	3	2	1	-
Conc.of GA in µg/ml	-	10	20	30	40	50	60	70	80	90	100
O.D at 780nm	0.01	0.04	0.08	0.12	0.16	0.21	0.24	0.28	0.32	0.35	0.48

Quantification of gibberellic acid by thin layer chromatography (TLC)

TLC of extracted GA was done along with standard gibberellic acid for quantitative estimation. The TLC plate of appropriate size (20×15) was used. The extracted organic solvent and standard GA was dissolved in acetonitrile and spotted on TLC plate with glass capillary allowed to dry then these different plates was kept in solvent system containing benzene**:**nbutanol**:**acetic acid (6**:**3**:**1) the developed spots were visualized under UV after spraying with ethanol **:**sulfuric acid (95**:**5).

Purification of gibberellic acid

After 7 days culture broth was filtered through whatman no 42 filter paper pH was adjusted by 0.1N HCL or KOH to 2.5-3.0 ethyl acetate was used as extraction solvent[9] .culture filtrate was extracted by three time ethyl acetate (450+150+150 mL) in separating funnel (1L) organic layer was evaporated at room temperature for 2-3 hrs.

RESULTS AND DISCUSSION

The predominant and desirable rhizobacteria were isolated. Specific media was used for isolation, identification and characterization i.e. yeast manitol agar and nutrient agar. The difference in rhizobacteria is due to difference in rhizosphere environment microbes show symbiosis towards root due to root provides important organic factors and nutrient for growth and development of microbes, thus ,microbes use sugar, amino acid , and various organic acid released by plant and provide them required essential components. One of the important factor released by microbe is growth hormone utilized by plant**.**

Isolation and screening of gibberellic acid producing bacteria

Total 25 visibly different isolated colonies were obtained with distinct morphology the media used for isolation was yeast manitol agar. The most isolates were obtained from rice (Oryza sativa) and sugarcane.(*Saccharum officinarum*) 12 and 8 respectively while in jowari(Sorghum) and mungbean (Vigna radiata) were found least i.e. 3and 2 respectively as shown in below image(fig 1)

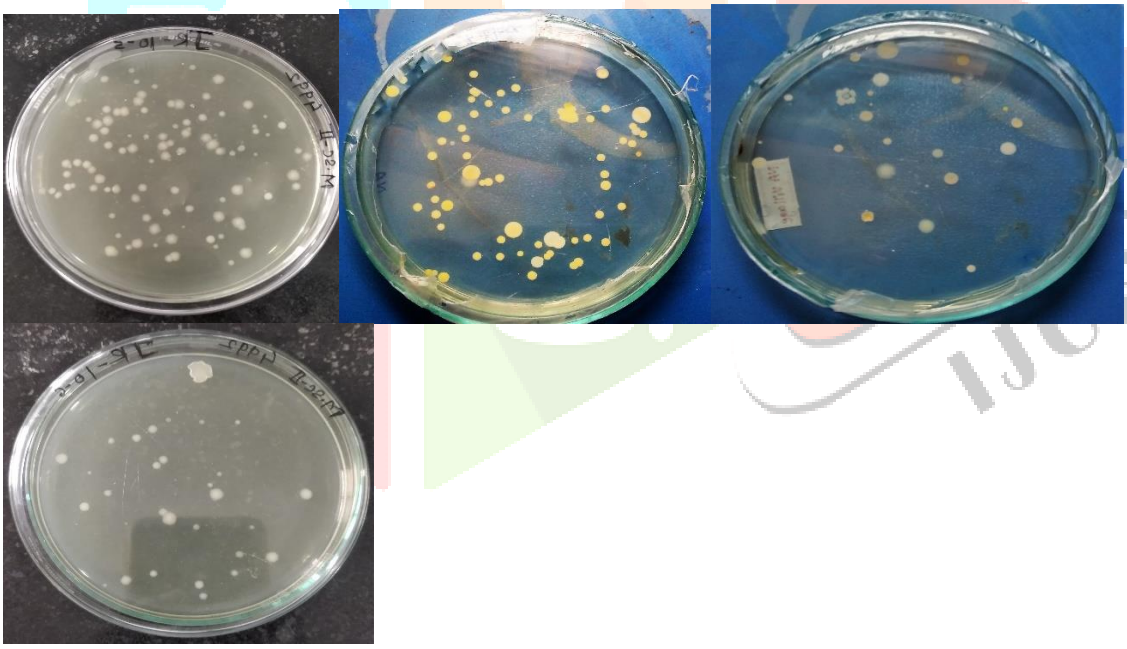

Figure.no 1

Screening of rhizobacteria for GA

Twenty five isolates of bacteria from rhizosphere of rice, sugarcane, mungbean, jowari were tested for their activity for GA's production out of them 11 bacteria were identified to produce GA's. Among the all Rice Rhizobium (RR5) And sugarcane rhizobium (SR2) produce higher amount of gibberellic acid 60µg/ml and 72µg/ml respectively after 7 day incubation. The least production activity was noticed in rhizobacteria from mungbean and jowari which produced in between 20-23µg/ml concentration. The concentration was analyzed by various methods mentioned below.

Quantification of gibberellic acid by thin layer chromatography

The gibberellic acid extracted from culture of various plant rhizobacteria using TLC was performed. The gibberellins compounds obtained from rhizobium extract of RR5, RS2, RJ8, and RM5 confirmed presence of gibberellins with rf values of 0.79, 0.75, 0.72 and 0.69 respectively near to standard GA (0.83). The RF values of respective gibberellic acid was shown in table no 1

Sr. No	Names of Rhizobacteria	RF(TLC) Value
1	Standard Gibberellic Acid	0.83
2	Rice Rhizobium(RR5)	0.79
3	Sugarcane Rhizobium (SR2)	0.75
4	Jowari Rhizobium (JR8)	0.72
5	Mungbean Rhizobium (MR5)	0.69

Table No:1 Determination of gibberellic acid by spectrophotometer

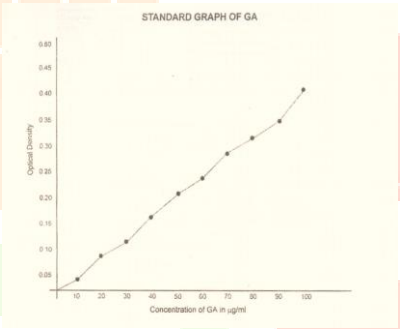

Figure No: 2

sr.no	Organism	Optical density	Concentration in µg/ml
1	RR5	0.25	65
2	RS2	0.30	72
3	RJ2	0.16	40
4	RM3	0.08	20
5	RR3	0.10	23
6	RS7	0.09	22
7	RM1	0.08	20
8	RR8	0.15	37
9	RJ1	0.12	32
10	RS9	0.20	50
11	RR12	O15	37

Amount of gibberellic acid produced by various plant rhizobacteria are shown in Figure No3

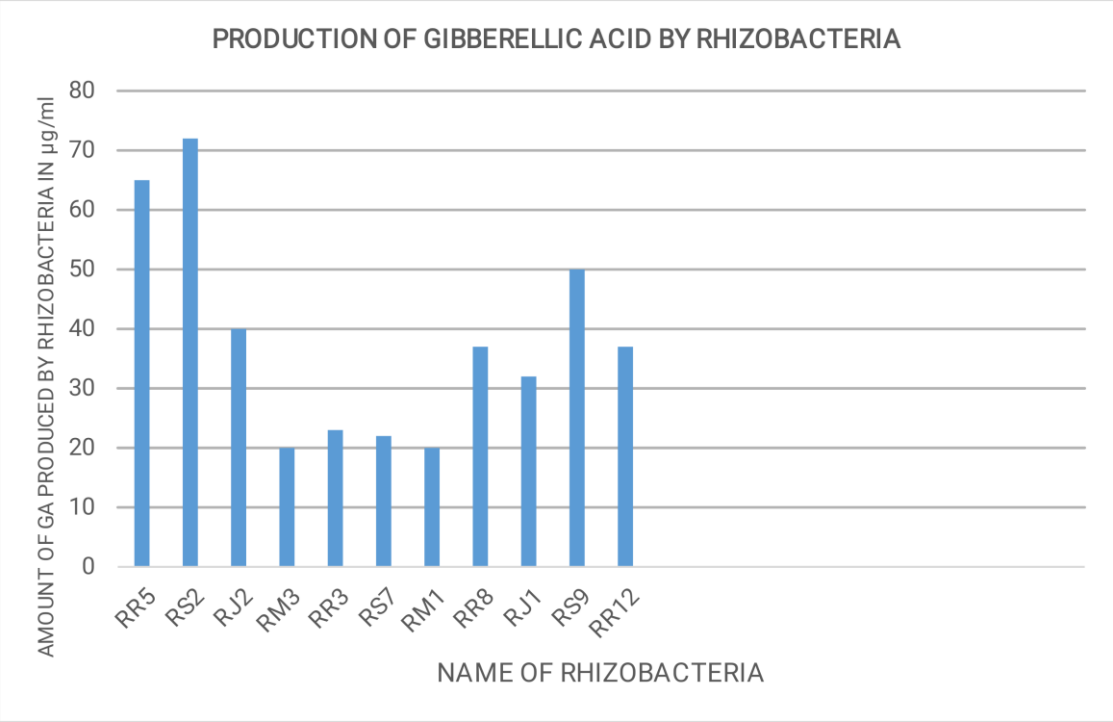

Figure No: 3

CONCLUSION

The gibberellic acid producing rhizobacteria isolated among which 11 have most potent ability to produce GA. The rhizobacteria RM3, RM1, RS7, RR3 had lowest activity for gibberellic acid production i.e. 20µg/ml, 20µg/ml, 22µg/ml, 23 µg/ml respectively while highest GA produced by RS2 and RR5 in 72µg/ml and 65µg/ml respectively. The produced GA was detected using the spectrophotometric method and analyzed by various methods mentioned above.

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