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ORIGINAL ARTICLE

MODIFIED MEDIUM FOR ISOLATION AND PRELIMINARY SCREENING OF INDOLE ACETIC ACID (IAA) PRODUCING BACTERIA FROM SOIL SAMPLES

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ABSTRACT

General classical method of detecting IAA production by PGPR bacteria under *in vitro* conditions was by colorimetric method using Salkowski's reagent. The present study involves development of a novel modified medium KUIAAM to detect and isolate IAA producing bacteria (BIPs) by incorporating the indicator in the medium, wherein BIPs are characterized by the development of pink colour over the colonies after a period of incubation. This medium will surely serve as an preliminary alternate for isolation and screening of IAA producing bacteria.

Key words: seasonal variations, physico- chemical parameters, coastal waters, Mallipattinam.

1.INTRODUCTION

Plant Growth Promoting Rhizobacteria can be classified according to their beneficial effects as biofertilizers, phytostimulators and biocontrol agents. The phytostimulators enhance plant growth through direct mechanisms by production of phytohormones such as auxins, gibberellins and cytokinins. Indole acetic acid (IAA) is one of the most physiologically active auxins. It is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch, 1985; Frankenberger and Brunner, 1983). IAA is the main auxin, controlling many physiological functions including cell enlargement and division, tissue differentiation and responses to light and gravity with a great impact on the root development. Root tissues are most sensitive to IAA fluctuations and the root responses range from cell enlargement and elongation to development of lateral and adventitious roots (Finnie and Van Staden, 1985). Therefore the bacterial IAA producers are highly valuable as PGPRs.

2.MATERIALS AND METHODS

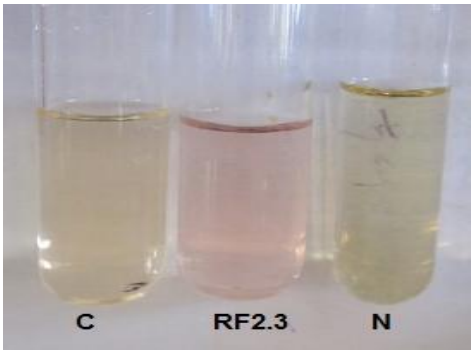
Thirty bacterial isolates isolated from forest soils of Siruvani Hills (Western Ghats) was screened for their IAA production in NA broth by usual colorimetric method using Salkowski's reagent (Shahab *et al.*, 2009) in which 2 ml of bacterial culture supernatant was acidified with 2 drops of 10 mM Orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml 35% Perchloric acid and 1ml 0.5 M Ferric chloride). The development of pink colour indicates the presence of IAA (Ahmad *et al*, 2005). The intensity of the colour developed is dependent on the concentration of IAA (Thanuja and Ambika, 2010). Concentration of IAA produced by cultures was measured with the help of standard graph of IAA (Patten and Glick, 2002).

Out of 30 isolates screened, 3 isolates (RF2.3, RF2.8, RF2.7) and 2 isolates (RF1.10 & RF2.5) showed high and low level of IAA production when tested with Salkowski's reagent (Table 1) (Fig. 1).

Table.1. Details of IAA produced by the bacterial isolates

S.No.	Isolate	Concentration of IAA (µg/ml)
1	RF2.3	0.8
2	RF2.8	0.4
3	RF2.5	0.15
4	RF2.7	0.1
5	RF1.10	0.25

Fig.1. Characteristic pink colour for IAA production exhibited by isolate RF2.3



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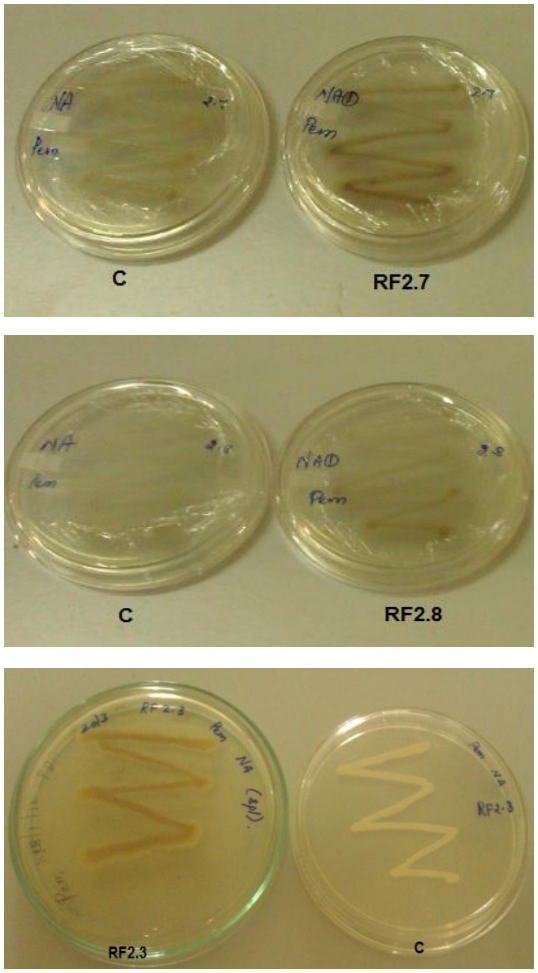
A new medium by name Karunya University Indole Acetic Acid Medium (KUIAAM) for direct detection of IAA production by the bacteria was formulated. KUIAAM medium was prepared with NA medium containing Tryptophan (0.1 gm/l), Perchloric acid and Ferric Chloride in three different ratio (Table 2) and Orthophosphoric acid (20 µl/100 ml) and the pH was determined and sterilized at 121°C for 15 min at 15 lbs pressure. All the five isolates (RF2.3, RF2.8, RF1.10, RF2.7 & RF2.5) which exhibited promising IAA production in normal colorimetric method was inoculated in KUIAAM to detect the IAA production efficacy characterized by pink colour development over the culture in the medium after 72 hr of incubation. NA medium amended with Tryptophan alone served as control.

Table 2. Details of Salkowski's reagent amended in modified Medium (KUIAAM)

S. No.	Concentration of Perchloric Acid and $FeCl_3$ (v/v Ratio)	Volume of reagent added to medium (µl/100ml)	pH of the medium
1	2:1	150	5.84
2	2:1.5	130	6.04
3	2:1	100	6.34

Fig. 2 Characteristic Pink colour exhibited by isolates RF2.7, RF2.3, RF2.8 in KUIAAM medium



3.RESULTS AND DISCUSSION

The isolates RF2.3, RF2.7 and RF2.8 which exhibited promising IAA production in normal colorimetric method exhibited clear characteristic pink colour formation over the colonies in the KUIAAM which confirmed the ability of the reagent and the indicator to react with the IAA produced by the isolates (Fig. 2). The other two isolates RF2.5 and Rf1.10 which showed low level of IAA production in the normal colorimetric method exhibited very little variation ie. Pink colour formation over culture compared to control. No characteristic pink colour formation was exhibited by the cultures inoculated over the normal NA medium which served as control. Among the 3 different concentrations of the Salkowski's reagent used, 2:1 ratio of Perchloric acid & $FeCl_3$ (150 µl in 100 ml) proved to be most efficient in production of pink colour over the bacterial colonies. As the duration of incubation increased (3 days to one week) the appearance of pink colour over the culture also increased.

The newly developed medium KUIAAM can effectively be used as alternate medium to qualitatively identify bacteria producing promising level of IAA when compared to the normal NA broth Salkowski's reagent method. Based on the results obtained further standardization of the medium is under process which can be effectively used for isolation of IAA producing bacteria for crop growth enhancement.

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