Table. 1. Cost estimate of combo-kit for rapid screening of plant growth-promoting bacteria.

Particulars	Chemical cost (Quantity)*	Kit expenditure#[α]
Plate cost (@ Rs. 110/-)	Per piece	Rs. 110.00
IAA production		
L-Tryptophan	Rs. 245.00 (5 g)	Rs. 49.00
Luria Bertani agar	Rs. 3545.00 (500 g)	Rs. 2.67
Salkowski reagent $HClO_4$ $FeCl_3.6H_2O$	Rs. 1485.00 (500 mL) Rs. 580.00 (500 g)	Rs. 5.80 Rs. 74.25
Membrane filter paper	Rs. 785.00 (100 nos.)	Rs. 23.55
Ammonia production		
NaCl	Rs. 175.00 (500 g)	Rs. 0.35
Peptone	Rs. 2285.00 (500 g)	Rs. 4.57
Agar	Rs. 3805.00 (500 g)	Rs. 11.42
Nessler's reagent	Rs. 175.00 (125 mL)	Rs. 1.4
Membrane filter paper	Rs. 785.00 (100 nos.)	Rs. 23.55
Phosphate solubilization		
Pikovskaya media	Rs. 3995.00 (500 g)	Rs. 25.00
Siderophore production		
Chromazurol	Rs. 3025.00 (10 g)	Rs. 18.33
$FeCl_3.6H_2O$	Rs. 580.00 (500 g)	Rs. 5.80
HCl	Rs. 295.00 (500 mL)	Rs. 0.59
C-TAB	Rs. 2333.00 (500 g)	Rs. 0.34
Luria Bertani agar	Rs. 3545.00 (500 g)	Rs. 2.67
Total cost		**Rs. ~ 360/-**

**Chemicals price may vary, # Cost excluding labour and facilities,*

[α]It could save ~ 60-80% cost expenditure from regular used methodology.

Tech NRRI

Combo-kit for Rapid Screening of Plant Growth Promoting Bacteria

NRRI Research/Technical Brief - 5

Editing and layout : GAK Kumar & Sandhyarani Dalal

Photography: Prakash Kar & Bhagaban Behera

Laser typeset at the National Rice Research Institute, Indian Council of Agricultural Research, Cuttack (Odisha) 753 006, India and printed at Printech offset, BBSR. Published by The Director, for the National Rice Research Institute, Cuttack (Odisha) 753 006.

Combo-kit for Rapid Screening of Plant Growth-promoting Bacteria

U Kumar, P Panneerselvam, M Kaviraj and AK Nayak

Plant growth-promoting bacteria (PGPB) is one of the important contributors in agriculture to improve plant and soil health by supplying both macro- and micro-nutrients to the plants and also by producing plant hormones, siderophore, ammonia, hydrogen cyanide and other secondary metabolites etc. Though the numbers of methodology and technique for screening of efficient PGPB are available, most of them are not rapid and economic. It is also labour intensive particularly for screening of large number of environmental samples. Therefore, a simple combo technique, comprising specific media and modified methodology for rapid screening of large number of PGPB for assessing their functional traits like indole acetic acid, ammonia, siderophore and phosphate solubilisation, is developed.

Technology Description

A 12 wells containing transparent plate has been selected for developing this combo-kit. In each well the specific medium related to screening of plant growth-promoting traits of bacteria *viz.,* indole acetic acid, ammonia, siderophore productions and phosphate solubilization are used with two replications along with control.

Media Composition for PGP Traits with Modified Methodology

1. **IAA (g/L)**
   Casein enzyme hydrolysate (10), Meat extract (5), NaCl (5), Agar (15), Tryptophan (5 mM) and Salkowski reagent. pH should be 7.5 ± 0.2.

2. **Ammonia production (g/L)**
   Peptone (10), NaCl (5), Agar (15) and Nessler's reagent. pH should be 7.5 ± 0.2.

3. **Siderophore production(g/L)**
   Solution A: Chromazurol (60.6 mg). **Solution B:** 1 mM $FeCl_3.6H_20$ in 10 mM HCl. **Solution C:** C-TAB (72.9 mg). Adding Solution A into B and mixed the resulting solution in C (pH 4-5). Mixed the final solution into the media comprises with Peptone (5), Beef extract (3) and Agar (15). pH should be 7.4 ± 0.2.

4. **Phosphate solubilization (g/L)**
   Media A: Glucose (10), $Ca_3(PO_4)_2$ (5), $MgCl_2.H_2O$ (5), $MgSO_4.7H_2O$ (0.25), KCl (0.2),$(NH_4)_2SO_4$ (0.1) and Agar (15).
   Media B: Glucose (10), $Ca_3(PO_4)_2$ (5), $(NH_4)_2SO_4$(0.5), NaCl (0.2), $MgSO_4.7H_2O$ (0.1), KCl (0.2), Yeast extract (0.5), $MnSO_4.H_2O$ (0.002), $FeSO_4.7H_2O$ (0.002) and Agar (15). pH should be 7±0.2.

Rapid Screening of PGPB

- Inoculate 50 µL of the bacterial inoculum on the surface of two vertical wells in each plate, keeping one well as uninoculated control (adding 50 µL of sterile water).
- Temperature of incubation: 35 ± 2 ºC.
- Duration of incubation: 3-4 days (for the best result).

Salient Features

- Plant growth promoting traits like indole acetic acid, ammonia, siderophore productions and phosphate solubilization can be assessed within four days.
- Shelf-life of this kit is about 6-8 months under proper storage (4 ºC) condition.
- Economic compared to commercial individual media.
- Easy to handle.
- Easy to discard.
- Sensitive to very low concentration (<10^3 cells/ml) of bacterial inoculums.

Mode of Screening of PGPB Traits

- Open the kit aseptically.
- Peel off the sealing foil.
- Inoculate 50 µL of the bacterial inoculum on the surface of two vertical wells in each plate, keeping one well as uninoculated control (adding 50 µL of sterile water).
- Temperature of incubation: 35 ± 2 ºC.
- Duration of incubation: 3-4 days (for the best result).
- The presence and absence of halo for phosphate solubilization and colour appearance for indole acetic acid (light to dark pink), ammonia (orange to red), siderophore (blue to green with halo) are visualized for the positive results.

Precautions

- Should open the kit aseptically (inside laminar air flow).
- Should wear protective gear (goggles, masks, gloves etc.) to handle the kit for avoiding any contamination.
- Should avoid direct eye and skin contact during bacterial inoculation process.
- Should avoid eating /drinking /smoking during inoculation process.
- In case of contact with eyes: should flush with water profusely for 20 minutes.
- Should sanitize hand before and after the work.

Technology Validation

- The combo-kit technology for PGP screening has been evaluated in Microbiology lab of ICAR-NRRI, Cuttack for several times to check its reproducibility.
- The presence and absence of halo for phosphate solubilization and color appearance for indole acetic acid (light to dark pink), ammonia (orange to red), siderophore (blue to green with halo) are visualized for the positive results (Fig.1).

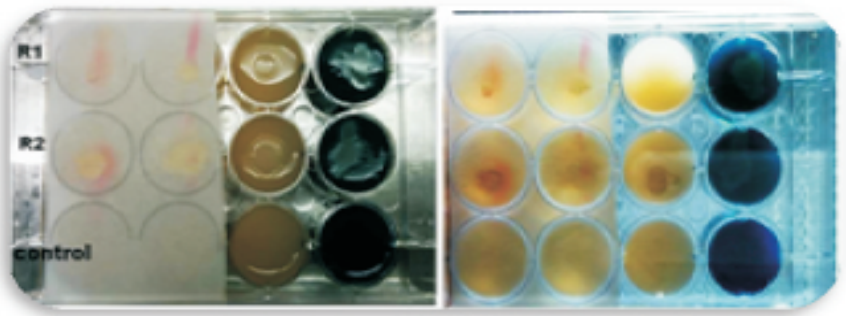

Fig. 1. Combo kit for detection of plant growth-promoting bacteria from environmental samples.

Significance of Technology

- Screening more number of bacterial samples within a short time span.
- Economic as compared to regular individual laboratory methodology (Table 1).
- Labour-saving.
- User-friendly.