Pedosphere **26**(5): 768–773, 2016
doi:10.1016/S1002-0160(15)60080-7
ISSN 1002-0160/CN 32-1315/P
© 2016 Soil Science Society of China
Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Letter to the Editor

A Modified Plate Assay for Rapid Screening of Potassium-Solubilizing Bacteria

Mahendra Vikram Singh RAJAWAT, Surender SINGH, Satya Prakash TYAGI and Anil Kumar SAXENA*

Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012 (India)

(Received July 29, 2015; revised May 25, 2016)

ABSTRACT

The utility of microorganisms for solubilizing the unavailable forms of potassium (K) from soil has led to renewed interest in fabrication of rapid and sensitive plate assays for their isolation and screening. The present study developed a modified plate assay and compared it with previously reported methods for the isolation and screening of K-solubilizing bacteria. The newly developed plate assay is based on improved visualization of halo zone formation around the colonies on agar plates, through inclusion of an acid-base indicator dye, bromothymol blue (BTB), to modify the previously reported Aleksandrov medium. The halo zone exhibited a significant correlation ($R = 0.939$) with K released in liquid medium. The visualization of potential K solubilizers was improved using this method, which would help in detection of weak/non-acid producers based on secretion of organic acids in the medium. Organic acids in plate diffuse radially and form halo zones in response to reaction with the acid-base indicator dye BTB. Furthermore, K solubilization on plates with this method can be observed within 48–72 h, against the incubation time of 4–5 d needed in the earlier method. Therefore, the newly developed protocol for the plate assay was time saving, more sensitive, and beneficial in comparison to the previously reported Aleksandrov plate assay.

Key Words: bromothymol blue (BTB), Aleksandrov medium, halo zone formation, indicator dye, K solubilizers, microorganisms

Citation: Rajawat M V S, Singh S, Tyagi S P, Saxena A K. 2016. A modified plate assay for rapid screening of potassium-solubilizing bacteria. *Pedosphere*. **26**(5): 768–773.

INTRODUCTION

Potassium (K), a major macronutrient for plant growth and development, is essential for many functions in the plant system. The significant role of K on growth, development, yield, and disease resistance has been illustrated in a wide variety of plant species (Ma, 2004; Ahmad and Maathuis, 2014). It remotes many activities such as the metabolism of carbohydrates, organic acids, fats, and nitrogenous compounds, besides involvement in protein synthesis, photosynthesis, resistance to drought, and water use efficiency. The concentration of soluble K in soil is very less, ranging from 4.0 to 30.0 g kg^{-1} (Sparks and Huang, 1985); hence, there is a growing interest in the role of microorganisms in the dissolution of K-bearing minerals. There are four pools of K in soil, *i.e.*, mineral K, available K or exchangeable K, non-exchangeable K, and soluble K (Goldstein, 1994; Zarjani *et al.*, 2013). Most of the K deposits in soil (90%–98%) are in the form of soil minerals, which can not be taken up directly by plants (Huang and Kiang, 1972; Goldstein, 1994). Several investigations showed that microorganisms increase the available K in culture medium. *Bacillus mucilaginosus* enhances the decomposition rate of aluminosilicates and dissolves K and SiO_2 from insoluble minerals by producing organic acids (Welch and Vandevivere, 1994; Ehrlich *et al.*, 2010). Several microorganisms are able to solubilize insoluble forms of K-bearing minerals such as feldspar, mica, illite, and orthoclase, by secreting organic acids that either directly decompose K or chelate the silicon ion to release K in solution (Toba *et al.*, 1991; Bennett *et al.*, 1998). Capsular polysaccharides and carboxylic acids such as citric acid, tartaric acid, and oxalic acids are known to be involved in the solubilization of feldspar by *Bacillus mucilaginosus* and *Bacillus edaphicus* (Richards and Bates, 1989; Lin *et al.*, 2002).

At present, no rapid assay system is available to identify K-solubilizing microorganisms directly on the plates, similar to those available for the identification of phosphorus (P) solubilizers on Pikovskaya agar pla-

*Corresponding author. E-mail: saxena461@yahoo.com.

tes which contain insoluble sources of P. Potassium-solubilizing bacteria are generally screened by a plate assay using Aleksandrov agar medium (Aleksandrov *et al.*, 1967), based on exopolysaccharide production; however, the reliability of this exopolysaccharide-based screening for K solubilizers is questionable. Several bacterial isolates solubilize various types of insoluble K minerals in medium, but do not produce any exopolysaccharide on agar plates. Therefore, an assay for screening, based on better visualization techniques, needs to be developed as a prerequisite for identification of promising K solubilizers, as biofertilizer options for K-deficient soils. The present investigation was focused towards the development of rapid plate-based assay system for screening K solubilizers from soil samples.

MATERIALS AND METHODS

Isolation of bacteria and screening of K solubilizers

Soil samples were collected from the rhizosphere of vegetable crops and isolation of bacteria was done on a nutrient agar medium (pH 7.0 ± 0.2) containing 5 g L^{-1} peptone, 3 g L^{-1} beef extract, 5 g L^{-1} sodium chloride, and 15 g L^{-1} agar, using standard spread plate technique. A total of 85 bacterial isolates exhibiting different morphological characteristics were purified and spot inoculated on Aleksandrov agar medium plates (Hu *et al.*, 2006). The plates were incubated for 5 d at 30 °C and observed for the formation of halo zones around the colonies. Cultures positive for K solubilization based on plate assay were grown in Aleksandrov broth individually and solubilized K in culture supernatant was quantified with a flame photometer. All chemicals used were of analytical grade.

Qualitative analyses of K solubilization

Qualitative analysis of K solubilization was carried out using the Aleksandrov medium (pH 7.2 ± 0.2) containing 5.0 g L^{-1} glucose, 0.5 g L^{-1} magnesium sulphate, 0.005 g L^{-1} ferric chloride, 0.1 g L^{-1} calcium carbonate, 2 g L^{-1} calcium phosphate, and 2 g L^{-1} K-bearing minerals (Hu *et al.*, 2006). Potassium aluminosilicates were purchased from HiMedia Labs, Mumbai (India). All chemicals used were of analytical grade.

Optimization of assay using dyes

A modified Aleksandrov medium was prepared by amending the Aleksandrov medium with different concentrations of 3 different acid-base indicator dyes, bromocresol purple, phenol red, and bromothymol blue (BTB), from stock solutions (5 g L^{-1}) prepared in 70% (weight/volume) ethanol. Different amounts of stock dye solution ranging from 0.25 to 2.5 mL were mixed in 100 mL of Aleksandrov agar medium to achieve final concentrations of 12.5, 25.0, 37.5, 50.0, 75.0, 100.0 and 125.0 mg L^{-1}. After adding the measured amounts of dye solution, the medium was autoclaved and poured into Petri plates. Plates containing Aleksandrov medium without dye solution served as a control. The halo zone size and colony diameter were measured after 72 h. The halo zone size was calculated by subtracting the diameter of colony from the total diameter (Fig. 1).

Quantitative analysis of K solubilization

Quantitative assay of K solubilization was carried out in the same Aleksandrov medium as the qualitative assay of K solubilization. The cultures were inoculated in 150 mL conical flasks containing 40 mL of Aleksandrov broth and incubated for 5 d at 30 °C on a rotary shaker at 100 r min^{-1}. Autoclaved broth served as a control. The pH of the medium was checked at the end of incubation. After incubation, medium was centrifuged at 10 000 r min^{-1} for 10 min, and the superna tant was used for estimation of soluble K with a flame photometer. Different concentrations (20, 30, and 40

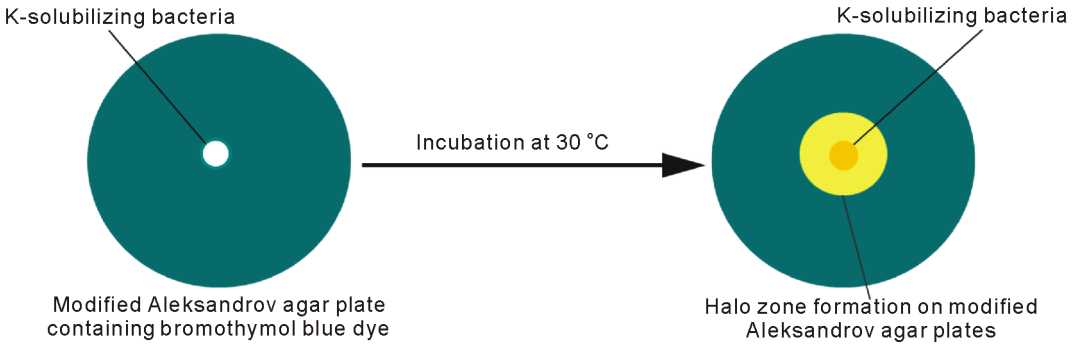

Fig. 1 Schematic diagram of plate assay for rapid screening of K-solubilizing bacteria.

Download English Version:

https://daneshyari.com/en/article/4581171

Download Persian Version:

https://daneshyari.com/article/4581171

Daneshyari.com