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Improved method for effective screening of ACC (1-aminocyclopropane-1-carboxylate) deaminase producing microorganisms

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ABSTRACT

Aminocyclopropane-1-carboxylate deaminase (ACCD) producing microorganisms support plant growth under a variety of biotic and abiotic stress conditions such as drought, soil salinity, flooding, heavy metal pollution and phyto-pathogen attack. Available screening methods for ACCD give idea only about its primary microbial ACCD activity than the actual potential. In the present investigation, we have simply improved screening method by incorporating pH indicator dyes (phenol red and bromothymol blue) in ACC containing medium. This modification is based on the basic principle that ACCD action releases ammonia which can be detected by color change and zone around the bacterial colony. High color intensity and zone around the colony indicates most potent producer, colony showing only a color change indicates moderate potential and no change in colony color indicates least efficiency. Enzymatic bioassays as well as root elongation studies revealed that ACC-deaminase activity of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Bacillus subtilis* clearly corresponds to their growth on dye incorporated ACC medium. This method could be used to complement the existing screening methods and to speed up the targeted isolation of agriculturally important microorganisms.

1. Introduction

Aminocyclopropane-1-carboxylate (ACC) is an immediate precursor in ethylene biosynthesis which is hydrolyzed to α-ketobutyrate and ammonium ions by the activity of enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) (EC 3.5.99.7) (Glick et al., 2007). Regulation of ethylene concentration is one of the key mechanisms in normal plant growth and development, such as aging process, fruit ripening and flower withering. Moreover ethylene concentration coordinates the stress signal transduction process. The microorganisms containing ACC deaminase, act as an ethylene controller by utilizing ACC, secreted by plant cells and avoid ethylene mediated inhibition of plant growth (Glick et al., 1998). Several recent studies identified ACC deaminase producing microbes, *Pseudomonas* sp. (Honma and Shimomura, 1978), *Mesorhizobium* spp. (Nascimento et al., 2013), *Achromobacter* sp. (Mayak et al., 2004), *Actinobacteria* (Hontzeas et al., 2005; Siddikee et al., 2010), *Firmicutes* (Siddikee et al., 2010; Ghosh et al., 2003; Timmusk et al., 2011), and *Bacteroidetes* (Maimaiti et al., 2007; Marques et al., 2010) that are suitable for plant growth in different stress conditions. These microorganisms are basically important to develop sustainable and environment friendly biological solutions to meet ever increasing food demands (Glick, 2014). Thus it is sensible to increase the screening spectrum and isolate potential ACCD producing microbes from diverse eco-climatic conditions. This necessitates the effective screening method to indicate most potential microorganisms at primary screening level and thus to narrow down number of samples in the subsequent stages. In this study we are suggesting an addition of pH indicator dye (phenol red and bromothymol blue) as a simple improvement in existing screening method to isolate potential ACCD producing microbes for the effective screening at primary level. (See Fig. 1.)

2. Material and methods

2.1. Modified ACC medium and isolation of bacteria

Rhizosphere soil samples from agriculture field of *Arachis hypogaea* near Jalgaon, Maharashtra, India were collected and screened for ACCD positive microorganisms as per Glick et al. (1995) with slight modifications. The minimal medium (in grams, $CaCO_3$ 4.0, Glucose 2.0, Sodium citrate 2.0, Potassium gluconate 2.0 in 1000 ml dH_2O) and minimal ACC medium agar plates with a final ACC concentration of 3.0 mmol l^{-1} were prepared as described by Penrose and Glick

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