Low Cost Growth Media for Mass Multiplication of Phosphate Solubilizing Rhizobacteria Isolated from Rice Rhizosphere

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Abstract
Mass multiplication of beneficial microorganisms is considered to be important as they could be used as bio inoculants in agriculture. Commercial media available for growth of microorganisms are expensive. Therefore, identification of suitable low cost growth media for mass multiplication of microorganisms is important. The study was conducted to identify suitable low cost growth media for mass multiplication of three phosphate solubilizing rhizobacterial isolates isolated from the rice rhizosphere. Four media namely coconut water, coconut water supplemented with sugar (15 g/L), rice porridge and rice porridge supplemented with sugar (15 g/L) were tested for their suitability as growth media for growing three selected bacterial isolates. Nutrient broth was used as the control. Results revealed that all four media used for the study supported the growth and higher cell yield of three bacterial isolates after incubating at room temperature (30 ± 2 °C) for 24 hours. However, the medium that gave the maximum cell yield varied with the bacterial isolate. Significantly higher growth of three bacterial isolates was observed in the medium contained rice porridge supplemented with sugar (15 g/L) than rice porridge alone. The study revealed that the suitability of rice porridge and coconut water based media for mass multiplication of phosphate solubilizing rhizobacteria.

Key words: Growth media, Mass multiplication, Rhizobacteria
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Introduction

There are several evidences for identification of beneficial microorganisms with plant growth promoting activities through isolation and *in vitro* screening processes in scientific literature. However, these beneficial microbes rarely appear commercial microbial inoculants that can be used in agriculture (Nita *et al.*, 2012). Delivery of identified beneficial microorganisms into the field is the most important aspect in terms of their commercial exploitation (Anith *et al.*, 2014). It is necessary to deliver viable microorganisms as formulated inoculants and it has become a focus of research and industrial development in search for alternatives to agrochemicals.

To formulate microbial products, identified microorganisms should be multiplied in large scale either in solid form or in liquid form and a suitable carrier material should be identified (Princy *et al.*, 2014).Beneficial microorganisms are produced as either as carrier based inoculants or as liquid bio formulations. Use of liquid bio formulations are advantageous compared to carrier based inoculants due to the ease of application, enhanced survival and improved protection against environmental stresses. Commonly used commercial liquid growth media for multiplication of bacteria are prohibitively expensive for industrial scale microbial inoculums production. Therefore, identification of suitable growth media for mass multiplication of microorganisms is extremely important when considering the commercial production of microbial inoculants. The media used for the mass multiplication should; support fast multiplication of beneficial microorganisms, cost effective and abundant.

Coconut water is considered as a waste material of copra industry. Mature coconut water consists of 5.4% total solids, 3 % soluble sugars, 0.5% minerals, 0.1% proteins, making it an ideal medium for growth of many microorganisms (Mathew *et al.*, 2010). It is used as a supplement in many bacteriological media and tissue culture media (Anith *et al.*, 2014).The other growth medium, rice porridge is the liquid collected after boiling rice. It contains carbohydrate and minor amounts of phosphorus and potassium and used as a growth media for microorganisms (Princy *et al.*, 2014). The objective of the study was to identify suitable low cost growth media using rice porridge and coconut water for the mass multiplication of efficient phosphate solubilizing rhizobacteria isolated form rice rhizosphere.

Materials and Methods

Phosphate solubilizing rhizobacterial isolates used for the study

Three phosphate solubilizing rhizobacterial isolates were selected to test the suitability of

low cost growth media for bacterial mass multiplication which promoted the growth of rice plants under greenhouse condition. These bacteria were isolated from rhizosphere of two month old rice plants of Bw 367and Bw 372 varieties grown in a paddy field at rice research station, Bentota. Phosphate solubilizing ability of these bacterial isolates was determined by inoculating on Pikovskaya's agar medium.

Culture media

Two rice porridge based media and two coconut water based media were tested for the growth of each phosphate solubilizing rhizobacterial isolate. Following 4 media were used in the experiment.

1. Rice porridge
2. Rice porridge + Sugar (15 g/l)
3. 100% Coconut water
4. 100% Coconut water+ Sugar (15 g/l)

Nutrient broth medium, which is normally used commercial growth medium for bacterial multiplication was also used as the positive control. Rice porridge was prepared by boiling 250 g of rice in 1L of water. Filtrate was taken after boiling. Coconut water was obtained from mature coconuts. Culture media were prepared and sterilized at 121 ^{0}C for 20 minutes. Each sterilized medium (100 mL) was inoculated with a loop full of 24 hours old bacterial culture grown on nutrient agar medium. The initial bacterial cell concentration in each medium after inoculation was determined by using serial dilution plate technique on nutrient agar medium. Inoculated media were incubated at room temperature (30 ± 2 ^{o}C) for 24 hours. After incubation period, viable bacterial cell concentration in each medium was determined by using serial dilution plate technique on nutrient agar medium. To obtain the colony count, 10^{-5} dilution was used. Growth of each bacterial isolate in each growth medium was tested six times. Data was statistically analyzed using SAS statistical software package.

Results and Discussion

In the present study all four media which were tested, supported the growth of three phosphate solubilizing rhizobacterial isolates used for the study, and gave a sufficient cell yield for each bacterial isolate after 24 hours of incubation period. However, the growth medium that gave the maximum colony count varied with the bacterial isolate. Coconut water and rice porridge was supplemented with sugar to increase the amount of nutrients in the media. Table 1 shows colony count of each bacterial isolate on each medium after incubating at room temperature (30 ± 2 ^{o}C) for 24 hours.

The growth medium consisted of rice porridge supplemented with sugar (15g/L) gave a significantly higher cell yield compared to the growth medium which contained only rice porridge, considering the growth of studied three bacterial isolates. However, there was no significant difference in the cell yield in growth medium that contained coconut water and coconut water supplemented with sugar (15g/L) when considering the growth of bacterial isolate 01 and isolate 02. In contrast, when considering the growth of bacterial isolate 03,a significantly higher colony count was observed in the medium comprised of coconut water supplemented with sugar than the medium contained solely coconut water. Peighami-Ashnaei *et al.*(2009) reported that the accurate incorporation of nutrients in growth media improved the biomass production of microorganisms. According to the results of the study, cell yield of studied bacterial isolates

Table 1: Growth of phosphate solubilizing rhizobacterial isolates on different growth media

Growth Medium	Bacterial population after 24 hours (*10^{8} CFU/ml)		
	Bacterial isolate 01	Bacterial isolate 02	Bacterial isolate 03
Rice porridge	3.0^{c}	97.0b	7.7^{c}
Rice porridge +Sugar (15g/L)	10.8^{b}	247.0^{a}	17.0^{a}
Coconut water (100%)	13.1^{ab}	73.0b	7.2^{c}
(100%) Coconut water + Sugar (15g/L)	13.8^{ab}	50.00^{b}	15.8^{a}
Nutrient Broth	14.7^{a}	229.0^{a}	11.1^{b}
Initial bacterial population(*10^{8} CFU/mL)	0.015	0.047	0.027

a,b,c Significantly different samples according to the statistical analysis

could be improved by supplementing rice porridge with sugar. It has been reported that coconut water is a cheap and excellent multiplication medium for plant growth promoting rhizobacteria. Further, the growth of rhizobacteria in coconut water resulted in increased seed and root colonization due to the production of mucoidal that in turn improved plant growth (Anith *et al.*, 2014). Mathew et al. (2010) reported the 25% coconut water supplemented with 15 g/L sugar and 50% coconut water as cheap liquid media for mass multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens*. Princy *et al.* (2014) reported the use of rice porridge for mass multiplication of phosphate solubilizing bacteria and potassium solubilizing bacteria isolated from tea plantation soils in Southern India. This study also revealed the suitability of rice porridge and coconut water as low cost environmentally friendly growth media for mass multiplication of beneficial bacteria.

In large scale multiplication, the suitable growth media should allow a maximum concentration of biomass of beneficial microorganism at a low price. Since the population level of the bacterial isolates in tested low cost media gone up to 10^{8-10} cfu/mL, the same preparation could be further diluted and could be applied to plants in the form of spray or soil drench as a bio fertilizer under field condition. In the study bacteria inoculated culture media were incubated at room temperature (30 ± 2 °C) for 24 hours. Higher colony count of bacteria can be obtained by incubating the bacteria inoculated media at 37°C for longer time period. Considering the cost of production, coconut water and rice porridge are very cheap and it is highly economical compared to commercially available bacteriological growth media. Production cost for 1L of nutrient broth is about Rs. 500.00, whereas the production cost of 1L of rice porridge is just around Rs. 30.00 and coconut water is considered as a waste product of copra industry. Therefore, four growth media tested in this study could be used as suitable low cost growth media for mass multiplication of phosphate solubilizing rhizobacteria. Most importantly, since these media do not exert any harmful impact on environment, they can be used in sustainable agriculture.

The entire process of microbial inoculant production consists of several steps. These include identification of suitable media for mass multiplication of microorganisms, correct formulation to extend the shelf life, mode of application and industrial consideration regarding production of commercial inoculants. Therefore, the study needs to be extended to examine the bio efficacy of rice porridge and coconut water based phosphate solubilizing rhizobacterial formulation under filed condition for the growth promotion of rice cultivation. The low-cost growth media identified from this study can also be tested for mass multiplication of other beneficial microorganisms which are important in agriculture.

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