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Standardization of Isolation Technique of *Rhizobium* from Root Nodules of Lentil

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A B S T R A C T

Native plant growth promoting rhizobacteria are unable to compete with other bacterial strains and hence cannot express their full potential in realising the beneficial effects. Hence, inoculation of plants with target microorganism is necessary to exploit full potential for yield enhancement. This study was aimed at standardizing the technique for isolation of *Rhizobium* species from root nodules of lentil so as to explore and maximize their contributions to soil nitrogen fertilization in place of synthetic fertilizers. A total of five nodulated healthy plants collected from North-eastern part of Uttar Pradesh, India were sterilized and macerated under aseptic condition followed by streaking one loop full suspension in a zig-zag manner using the inoculation loop on YEMA medium in laminar air flow and incubated in a B.O.D. incubator at 28±2ºC for 24 hours. Growth on YEMA plate was observed after the said incubation period and then maintained at 4ºC in 20% (w/v) glycerol at -80ºC for long-term storage. Vigorous growth of bacteria was seen in next 24 to 48 hours. Bacterial colonies were found to have distinct colour, morphology, appearance and texture on agar plates.

Introduction

Lentil (*Lens culinaris* L.), a member of leguminosae family, having origin in Middle East and Egypt, later spreaded to Latin America (Cubero, 1981 and Duke, 1981) is one of the oldest cultivated legume plants (Bahl *et al.*, 1993 and Rehman *et al.*, 1994), grown commercially for its edible seeds. It is a self-pollinating, annual cool season crop with disomic chromosome number of 14 (Muehlbauer, 1991) with a haploid genome size of an estimated 4063 Mbp (Arumuganathan and Earle, 1991). It can be grown to an altitude of 3000 meters above mean sea level. However, seed yield decreases with increasing altitude (Whyte *et al.*, 1953).

The total cultivated area in the world accounts around 4.6 million hectares producing 4.2 million tons of seeds with an average productivity of 1095 kg/ha (FAO, 2010). India accounts the largest global area with 1.48 million ha and 1.01 million ha production (AICRP, 2010-2011).

Yield levels can be enhanced by efficient use of synthetic fertilizers. However on account of increasing environmental degradation, synthetic fertilizers need to be replaced by a more effective, sustainable and cost-effective method through the use of biofertilizer. Plant Growth Promoting Rhizobacteria (PGPR) residing in rhizosphere in association with plant root surface has positive effects on growth and yield of different crops in a sustainable manner. These beneficial microorganisms are important components of plant productivity and soil fertility because of their participation in several key processes by release of different plant growth regulators (like IAA, GA *etc*.), by solubilising inorganic phosphates, by biological nitrogen fixation, control of plant pathogens *via* synthesis of antibiotics, by nutrient cycling and seedling establishment (Jeffries *et al.,* 2003 and Rosas *et al.,* 2009). They have been also reported to confer upon the plants ability to tolerate different abiotic stresses (Yang *et al.,* 2009 and Lim and Kim, 2013). Native plant growth promoting rhizobacteria are unable to compete with other bacterial strains and hence cannot express their full potential in realising the beneficial effects. Hence, inoculation of plants with target microorganism is necessary to exploit full potential for yield enhancement. This study was aimed at standardizing the isolation of *Rhizobium* species from root nodules of lentil so as to explore and maximize their contributions to soil nitrogen fertilization in place of synthetic fertilizers.

Materials and Methods

A total of five nodulated plants were collected from North-eastern part of Uttar Pradesh, India. Healthy plants were uprooted carefully and those plants possessing healthy nodules with pink colour were selected and transported to the P.G.P.R. laboratory, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University Varanasi, India in polythene bags for immediate processing**.** Yeast extract mannitol agar (YEMA) with pH range of 6.8-7.0 was prepared before *Rhizobium* isolation as described by Rajendran *et al.,* In this, healthy, unbroken, firm and pink nodules were selected and washed under tap water to remove adhering mud and soil particles, followed by treating it carefully with 5% hydrogen peroxide for surface sterilization. Repeated washing of nodules in sterile water for 3- 4mins was made to get rid of the sterilant and then treated with 70% ethyl alcohol for about one minute and 0.1% $HgCl_2$ for two minutes.

Again they were washed with sterile water (3 successive times) under aseptic conditions and crushed with sterile crucible. A suspension made of the crushed nodules, streaked one loop full suspension in a zig-zag manner using the inoculation loop on YEMA medium under aseptic condition in laminar air flow and incubated in a B.O.D. incubator (Narang Scientific, India) at 28±2 ^{0}C (Vincent, 1970) for 24 hours.

Table.1 Selected rhizobial strains and their sources

Strains	Source
LR1	North-eastern part of Uttar Pradesh, India
LR 2	
LR 3	
LR 4	
LR 5	

Growth on YEMA plate was observed after the said incubation period and then maintained at 4ºC in 20% (w/v) glycerol at -80ºC for long-term storage. Glass vessels containing nutrient media were sterilized and autoclaved at 15 lbs square^{-1} inch pressure at 121ºC for 15 minutes. Double distilled water was used throughout the experiment for preparation of media, reagents *etc*.

Results and Discussion

Bacterial colonies of *Rhizobium* strains (Table 1) isolated from the samples collected from regions of north-eastern part of Uttar Pradesh, India were visible on agar plates after 12 hours of inoculation. Vigorous growth of bacteria was seen in next 24 to 48 hours. Bacterial colonies were found to have distinct colour, morphology, appearance and texture on agar plates. Further bacterial colonies were transferred in the bakelite tube having YEMA media through streaking for medium term storage. Interestingly these isolates could be used in future for further testing the biochemical, physiological, morphological characteristics of bacteria as well as their potential for providing the crop better growth in biotic and abiotic stress conditions.

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