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Isolation and Identification of Endophytic Microorganisms of Soybean (*Glycine max (L.)* Merril)

N.S. KULKARNI* and J.M. DALAL

Microbiology Research Laboratory, Department of Microbiology,
R. A. Mahavidyalaya, Washim 444 505, India.
*Corresponding author E-mail: nskulkarni29@rediffmail.com

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ABSTRACT

Endophytic microorganisms live within host plants without causing any noticeable symptoms of disease. Endophytes are usually symptomless, but may produce beneficial to pathogenic effects. Present investigation describes the isolation and identification of endophytic bacteria from soybean. During this investigation 21 endophytic isolates were obtained that included 12 bacterial, 05 fungal and 04 actinomycteal isolates. Among the bacterial genera *Pseudomonas, Rhizobium, Enterobaer, Baciillus and Klebsiella spp*. were obtained. Fungal genera were *Aspergillus, Fusarium, Rhizoctonia* and *Alternaria spp.* and actinomycetal were *Streptomyces spp.* Endophytic microorganisms received considerable attention due to their biotechnological utility in various agricultural, industrial and medical fields.

Key words: Endophytes, Soybean, Endophytic population.

INTRODUCTION

Endophytic microorganisms live within host plants without causing any noticeable symptoms of disease[1]. They are ubiquitous in most plant species, especially in field-grown plants and have been found in almost all vascular plants species examined to date and include bacteria, actinomycetes and fungi[2]. Endophytes are usually symptomless, but may produce beneficial to pathogenic effects[3-4]. Among the plant associated microorganisms, endophytes are regarded as a largely untapped resource for the discovery of isolates with novel traits[5]. Endophytes have been shown to confer fitness benefits to host plants including tolerance to herbivory, heat, salt, disease, and drought, and increased below- and aboveground biomass. Endophytic colonization may also improve the ecological adaptability of the host by enhancing tolerance to biotic and abiotic stresses[6]. Intimate associations between endophytes and host plants can be formed without harming the plant and they have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens due to induction of systemic resistance (ISR) and/or various environmental stresses[2]. Endophytic microorganisms received considerable attention due to their biotechnological utility in various agricultural, industrial and medical areas[7, 8].

In present investigation we describe the isolation and identification of endophytic microorganims of soybean (*Glycine max* (L) Merril). It also provide the information on natural occurrence and colonization of endophytic bacteria, fungi and actinomycetes.

MATERIALS AND METHODS

Isolation of endophytic microorganisms

Five healthy plants at R4-R5 stage (9) were screened and were carefully uprooted, washed under tap water and further separately cut into sections of 2-3 cm long. All the sections were surface sterilized by washing with distilled water followed by rinse with 70% ethanol for 30 seconds.

Sterilization with 0.1% $HgCl_2$ upto 3 minutes was carried out for roots and nodules, whereas, upto 5 minutes for leaves and stems respectively. The tissue was then washed ten times using sterile distilled water. Sterility checks after surface sterilization was carried out by monitoring separately the section impressions and rinse wash water for the presence or absence of microbial growth incubated for 6 days in selective media. The absence of growth was taken into consideration as positive test for surface sterilization[4].

Surface-sterilized tissue was aseptically macerated with homogenizers. Macerated tissue (1 gm) was suspended in sterile phosphate buffer. Serial dilution were made up to 10^{-6} dilution and were spreaded separately on different media, viz. nutrient agar for bacteria, Cazpex-Dox agar supplemented with 10 µgm of chlorotetracycline for fungi and glycerol-yeast agar supplemented with aureomycin for actinomycetes[10].

The petri plates for bacterial isolation were incubated at 37°C upto 48hrs., whereas for fungal cultures the plates were incubated at 28°C upto 7 days and at room temperature (28°C ± 5°C) upto 15 days in dark for actinomycetes isolation. Pure cultures were subsequently isolated and maintained[4,11,12].

The bacterial and actinomycetal isolates were identified to genus level according to *Bergey's Manual of Determinative Bacteriology*[13] on the basis morphological, cultural and biochemical characteristics and the fungal isolates were on the basis of morphological and cultural characteristics.

RESULTS AND DISCUSSION

In present investigation a range of endophytic microorganisms were isolated from soybean. During this investigation 21 endophytic isolates were obtained that included 12 bacterial, 05 fungal and 04 actinomycteal isolates. Maximum number of endophytic isolates were obtained from roots (47.61 %), followed by leaves (33.33 %), stem (19.04 %) and root nodules (9.52 %). Present studies revealed that the dominance of endophytic bacteria over fungi and actinomycetes. The maximum isolation frequency was found to be in roots is in support that the endophytic population emerges from the rhizospheric microflora.

From all the isolates the bacterial isolates belonged to 6 genera, fungal 04 and actinomycetes 01 genera. Among the bacterial genera *Pseudomonas, Rhizobium, Enterobaer, Baciillus and Klebsiella spp*. were obtained. Fungal genera were *Aspergillus, Fusarium, Rhizoctonia* and *Alternaria spp.* and actinomycetal were *Streptomyces spp.*

Soybean shows a remarkable diversity because of its long history of cultivation and selection under various climatic, edaphic and biotic environments in geographically diverse areas[4]. As plant matures all the nutritional requirements are optimum and a stable endophytic population is obtained thus, there appear to be coincidence of plant maturity and endophytic population. The endophytic population is influenced by several factors viz., the developmental stage during which the plant is sampled, environmental conditions and the location of the plant. Moreover the genotype as well as cultivar of plant also affects significantly as the endophytic population is naturally selected by host (11 and 14).Our findings are in accordance with earlier studies of Kuklinsky-Sobral *et. al.*[15], Pimental *et. al.*[11] and Hung and Annapurna[4].

An understanding of the factors affect-ing the population of endophytes is necessary to achieve a consistent application of these microorganisms in relation to plant health and biocontrol. We are currently investing the role of the isolated endophytic microorganisms in relation to induction systemic resistance in soybean.

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