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SK Munnysha
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Nitisha Gahlot
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Mamta Beniwal
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Smriti Akodia
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

L Pdiangburom
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Corresponding Author:
SK Munnysha
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Evaluation of antagonistic and plant growth promoting activities of endophytes against *Sclerotium rolfsii* in French bean: A review

SK Munnysha, Nitisha Gahlot, Mamta Beniwal, Smriti Akodia and L Pdiangburom

Abstract
French bean (*Phaseolus vulgaris* L.) is the most important pulse and also an export crop in India. It suffers heavily from pod rot disease which is incited by *Sclerotium rolfsii* Sacc. *S. rolfsii* is well known polyphagous, devastating soil borne pathogenic fungus. It is very difficult to control by chemicals, because fungus survives as sclerotia, which persist in soil for several years. Hence, endophytes as biocontrol agents are used for managing the disease pathogen. Endophytes ubiquitously colonize the internal tissues of plants. The endophytes frequently secrete antimicrobial compounds, plant growth hormones, solubilize mineral phosphate and chelate toxic metals. Endophytes as biocontrol agents are great momentum in recent years due to an increasing awareness of pesticide hazards and environmental pollution.

Keywords: French bean, *Sclerotium rolfsii,* sclerotia, endophytes, pesticide, pollution

Introduction

French bean is an important vegetable food crop grown throughout the world and contribute nearly 30% of the total production of food legumes (Vasishtha and Srivastava, 2012) [46]. French bean is a rich source of nutrient and minerals. The French bean seeds (Rajma) contains 17.8-28.7% protein, green pods contain 1.4-2.5% protein, 11.7% fat, 70% carbohydrates and 340-450 Kcal energy (Sardana *et al*., 2000) [36]. French bean is an important cool season legume crop grown for its tender pods, shelled green bean and dry beans. The ideal temperature range for proper growth of this crop is 10-27°C. Above 30°C, the flower drop is a serious problem. Similarly, below 5°C, the flowers and developing pods and branches are damaged. French bean crop prone to many diseases such as anthracnose, pod rot, bean rust, white mold and fusarium rot. Among these, pod rot incited by *S. rolfsii* Sacc. causes 40-50% yield loss in India (Dasgupta *et al*., 2005) [11]. *S. rolfsii* Sacc. Is a necrotrophic soil borne pathogen with wide host range (Punja, 1985) [29]? The pathogen was first reported by Rolfs (1892) [31] as a causal agent of southern blight of tomato in Florida. Further, in India Mundkar successfully isolated the perfect stage of *S. rolfsii*. The fungus produces white cottony mycelial growth on potato dextrose agar medium and the colony resembles compact and fluffy. Initially, it produces white colored sclerotia later their color changes from white to dark brown at maturity stage (Punja 1985) [29]. Stephen (1992) [40] described the symptoms produced by *S. rolfsii* in appearance of progressive yellowing and wilting of the plants. The fungus produces abundant white fluffy mycelium on infected tissues and soil. *S. rolfsii* infects more than 500 plant species including brinjal, bean, cucumber, groundnut, maize, soybean, tomato and water melon (Sharma *et al.*, 2002) [38]. *S. rolfsii* has wide host range and difficult to control by chemicals alone, because the fungus produces sclerotia and survives in soil for a longer period of time. Hence, alternative method of using bacterial endophytes as biocontrol agents for managing the disease pathogen. Biocontrol agents (BCAs) are getting momentum in recent years due to an increasing awareness of pesticide hazards, subsequent environmental pollution, the failure of current fungicides due to resistant races or pathotypes of specific diseases of crop plants, higher cost of development, greater difficulties associated with findings of new fungicides and many diseases posed to give more attention on alternative methods, like biocontrol. Bacterial endophytes are those bacteria which asymptomatically inhabit the internal tissues of plants and they colonize the same ecological niches as disease causing organisms (Chen *et al.*, 1995) [8].

Endophytes are biocontrol agents often effective against plant diseases (Hutberg *et al*., 2010) [20].

Materials and Methods

In vitro evaluation of bacterial endophytes against *Sclerotium rolfsii*

Six bacterial endophytes *viz*., BS80 (E1), BS118 (E2), BS178 (E3), BS1032 (E4), BS78 (E5) and BS179 (E6) were tested for their biocontrol potential against the pathogen on PDA medium using the Dual culture technique (Ganesan and Gnanamanickam, 1987) [14].

Dual culture assay

The fungal culture was grown on PDA plate for 3 days. With the help of sterilized cork borer 5 mm diameter fungal discs were cut from the periphery of the culture plate and placed at the center of the fresh PDA plates. 24 hr old culture of bacterial strains were then streaked parallel on both the side of the fungal disc 1 cm away from the disc. Three replications were maintained. The plates without bacterial streak served as control. The plates were then kept for incubation at 28±1°C till the control plate fully covered by the pathogen. Per cent inhibition was recorded by following the method described by Vincent (1927) [49] as

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where,

"C" is the maximum growth of the fungal mycelia under control

"T" is fungal mycelial growth in dual culture

Compatibility among the bacterial endophytes

After the 6 bacterial isolates being screened against *Sclerotium rolfsii* Sacc. causing pod rot of French bean, 3 potential bacterial antagonists were selected, based on per cent inhibition on growth of pathogen. The 3 screened efficient endophytes were tested for their compatibility among each other by following method of Fukui *et al.* (1994) [13]. The bacterial strains were streaked horizontally and vertically to each other. The plates were incubated at room temperature (28±1°C). After 72 h of incubation, the plates were observed for the absence of inhibition zone at the point of contact between two isolates.

Preparation of microbial consortia

Microbial consortia was an approach to combat plant disease. Application of microbial consortium consisting of efficient strains for biological control may be a superior technique compared to application of individual microbes for managing plant diseases. Guetsky *et al.* (2002) and Jetiyanon and Kloepper (2002) [18, 21] also suggested that, consortial application of different bioagents is required for improved and stable control against a complex of disease and noticed reduction in population of pathogen and improvement in plant growth characters with co-inoculation of bioagents as compared to individual application and control. Use of different species of microbes in combination may further have the advantage of enhancing biocontrol efficacies as different microbes occupy different niches in the root zone and thereby restrict competition among them. Additionally, diversity in biocontrol mechanisms offered by each microbial component may also help in enhancing disease suppressiveness. Some earlier studies showed that different microorganisms namely Trichoderma, Bacillus, Pseudomonas, Rhizobium, Glomus, etc. were used to develop microbial consortia (Jetiyanon, 2007) [21]. Later on microbes capable of inducing systemic resistance (Bakker *et al.*, 2007) [5] as well as enhancing nutrient use efficiency were also included in the microbial mixtures (Harman, 2011) [19]. Akanksha *et al.* (2012) [2] studied the impact of triple microbial consortium consisting of *Pseudomonas fluorescens* (PHU094), *Trichoderma* (THU0816) and *Rhizobium* (RL091) alleviation of biotic stress in chickpea through enhanced anti-oxidant and phenyl propanoid activities. The results thus suggest an augmented elicitation of stress response in chickpea under *S. rolfsii* stress by the triple microbial consortium. Compatible microbes i.e., microbial strains that have no suppressive effect on other strains when co-cultured in a common medium in consortium may have an enhanced impact on plant growth promotion or disease suppression (Singh *et al.*, 2013) [39]. These microbes are able to do their work individually. However, when compatible strains of these microbes are applied together as a consortium, the crop plants are expected to get a combined benefit of high N and P availabilities for uptake leading to better plant health and yield. Combining an antagonist bioagent may further facilitate disease free growth of the plants. Therefore, applying microbes as a consortium has great potentiality particularly in modern agriculture where minimization of chemical fertilizers and pesticides is one of the priorities.

The effective endophytes were selected and then prepared formulation by the method described by Nandakumar *et al.* (2002) [27]. Each of the selected potential three bacterial endophytes was grown separately in LB broth which were followed by incubation in a rotary shaker at 150 rpm for 48 h at 28±1°C. After 48 h of incubation, the bacterial cultures were mixed equally (v/v) to make 4 microbial consortia namely, MC1 (E1+E2), MC2 (E2+E3), MC3 (E3+E1) an MC4 (E1+E2+E3).

In vitro evaluation of endophytes alone and in consortia on vigour index of French bean seeds

The French bean seeds (15 numbers) were soaked in 50 ml of the microbial consortia (10^6cfu/ml) of E1, E2, E3, E1 + E2, E2 + E3, E3 + E1 and E1 + E2 + E3 respectively and the treated seeds were kept inside the Petri plates lined with the moistened filter paper. The seeded plates were kept in room temperature. Each treatment was replicated into 3 times, seeds soaked in sterile distilled water only served as control. Observation were taken after 7 days of incubation for germination per cent and shoot length (cm) and root length (cm) and Vigour Index (VI) were calculated after 21 days of incubation by following the method described by Gopalakrishnan *et al.* (2012) [17] as

$$\text{Vigour index (Vi)} = (RL + SL) \times GP$$

Where,

RL = mean root length (cm)
SL = mean shoot length (cm)
GP = Germination per cent

The bacterial endophytes appear to have originated from the rhizosphere population and some of these developed the capability to colonize internal plant tissues (Germida *et al.*, 1998) [15]. The close association of endophytes with internal tissues of host plant has increasingly gained them scientific and commercial interest due to their potential to improve plant quality and growth (Schulz *et al.*, 1999) [37]. Endophytic bacteria are thought to interact closely with their host plants, and therefore could be used as biological control agents in sustainable crop production potentially (Sturz and Nowark, 2000) [43]. Endophytes are plant-associated prokaryotes that form association with their host plants by colonizing the internal tissues, which has made them valuable for agriculture as a tool in improving crop performance (Azevedo *et al.*, 2000) [4]. They are ubiquitous, colonize most of the plants, and have been isolated from almost all of the plants till date. The study of plant-associated bacteria and the evaluation of their antagonistic potential is important in understanding their ecological role and interaction with plants besides biotechnological applications (Emmert and Handelsman, 1999; Bloemberg and Lugtenberg, 2001) [12, 7]. Plant growth promoting endophytes differ from biocontrol strains that do not necessarily inhibit pathogens but increase plant growth through phosphate solubilisation activity (Verma *et al*., 2001) [48]. It has been reported that the proportion of endophytes able to suppress disease symptoms are high in comparison to that observed for rhizosphere bacteria (Reiter *et al*., 2002) [30]. Despite different ecological niches, free-living rhizobacteria and endophytic bacteria use the same mechanisms to promote plant growth and control pathogens (Compant *et al.*, 2005) [9]. Vega *et al*. (2005) [47] reported that population of endophytes inside plants varies from plant to plant and even some endophytic bacteria show tissue specificity. However, mostly the endophytic population in plant tissues ranges between $5.6x10^3$ and $6.9x10^5$ cfu/g. One of the most commonly recognized rhizobacterium, *B. subtilis*, has strong antimicrobial properties and 4-5% of its genome is devoted to synthesis of antibiotic compounds (Stein, 2005) [40-41]. Endophytes have been isolated from almost all plant parts, including fruits, leaves, stems, seeds, nodules and roots (Rosenblueth and Martinez, 2006) [32]. Ziedan (2006) [52] obtained 25 bacterial isolates from inner tissue of peanut plant roots (90 days old). Three isolates of *Bacillus subtilis* and one *Pseudomonas fluorescens* were identified as best antagonizing isolates. Endophytic growth promoting bacteria facilitate plant growth when used as bio-inoculant formulations or for environmental cleanup (phytoremediation) (Ryan *et al*., 2008) [33]. There are many *Bacillus* sp. reported to show plant growth promotion capability (Wang *et al.*, 2009 [51]. Bacterial strains isolated from watermelon roots reduced *Didymella bryoniae* infection up to 70% when applied to watermelon seeds before planting (Nga *et al.*, 2010) [25]. Dalpati *et al.* (2010) [10] evaluated four different bio-agents *viz., T. harzanium*, *T. viride*, *P. fluorescens* and *B. subtilis* against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*. Among the four bio-agents, *T. harzanium* was found superior as compared to others by inhibition of the growth (76.66%) followed by *Bacillus subtilis* (73.66%). The microbes which resides inside the plant tissues are known as endophytes (Orole and Adejumo, 2011) [28]. Wahyudi *et al*. (2011) [50] reported the ability of *Bacillus* sp. to root length, shoot length of seedlings (seed germination bioassay) *in vitro.* An endophyte is an endosymbiont, often a bacterium or fungus that lives within a plant part for at least part of its life cycle without causing apparent disease (Thyagarajan and Namasivayam, 2011) [45]. Amaresan *et al.* (2012) [3] studied the efficacy of eight isolates of putative endophytes, isolated from surface- sterilized tomato and chilli seeds, observed that the isolates BETS14, BETL13, BECS6 and BECS7 exhibited high antagonistic activity *in vitro* and significantly inhibited the growth of *S. rolfsii*, *F. oxysporum*, *C. capsici*and *Pythium* sp. followed by BECS1, BECL8 and BETS11, which showed only significant growth inhibition in *F. oxysporum*, *C. capsici* and *Pythium* sp. *Bacillus* sp. are also reported as endophytic bacteria in higher plants (Li *et al*., 2012) [22]. Plant growth promoting bacteria also frequently secrete antimicrobial compounds, plant growth hormones, solubilize mineral phosphate and chelate toxic metals in the rhizosphere (Ahemad and Kibret, 2014) [1]. Endophytic population is known to vary from plant to plant and also from species to species. Same species of plant may also show different endophytic population occurring at different regions. Hence temporal and climatic changes affect occurrence of endophytes (Nair and Padmavathy, 2014) [26]. Gao *et al.* (2015) [16] studied the biocontrol efficacy of 14 endophytic bacterial strains against *Blumeria graminis* f. sp. *tritici* under greenhouse conditions and found that *Bacillus subtilis* strain E1R-j significantly reduced disease index and exhibited best control (90.97%). Bacterial endophytes ubiquitously colonize the internal tissues of plants almost in all the plants and generally promote plant growth (Santoyo *et al.*, 2016) [35].

Evaluation of bacterial endophytes in field condition

Santiago *et al.* (2007) [34] suggested that compatibility among bacterial inoculants is important for efficient plant growth promotion. Mukherji *et al.* (2008) [24] found that inoculation of microbial consortium resulted in an increase in plant height and biomass 4 weeks post infection (WPI). Stockwell *et al*. (2011) [42] suggested the application of microbe in a consortium may improve efficacy, reliability and consistency of the microbes under diverse soil and environmental conditions. Suprata (2012) reported the microbial antagonists is an alternative to synthetic chemicals in controlling plant diseases. Berg and Ayyasamy (2014) [6] reported bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents. The screened potential bacterial endophytes were evaluated against pod rot of French bean under field conditions. Different treatments were followed *viz.,* seed treatment, soil application, seed + soil application with different strains *Bacillus subtilis*, mancozeb @0.2% as chemical check and control were followed during the field evaluation. The Per cent disease index was recorded with the method given by Mayee and Datar (1986) [23].

Conclusion

Biocontrol agents or bacterial endophytes are microorganisms that decrease the harmful effects of pathogens. These microorganisms acts as a plant growth promoting bacteria (PGPB) which exert beneficial effects on plants. Bacterial endophytes promote plant growth and yield and would acts as biocontrol agents. Ultimately, exclusive studies on the endophytic bacteria would reveal useful information for effective disease management without causing harm to other biosystem. Hence, endophytic antagonistic bacteria emerge as a potential strategy for preventing the crop diseases.

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