P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(5): 1147-1150

© 2020 IJCS

Received: 05-08-2020

Accepted: 09-09-2020

Sushanti Thokchom

Department of Plant Pathology,
School of Crop Protection,
College of Post Graduate
Studies in Agricultural Sciences,
Central Agricultural University
(Imphal), Umiam, Meghalaya,
India

Pranab Malakar

Department of Plant Pathology,
School of Crop Protection,
College of Post Graduate
Studies in Agricultural Sciences,
Central Agricultural University
(Imphal), Umiam, Meghalaya,
India

RK Tombisana Devi

Department of Plant Pathology,
School of Crop Protection,
College of Post Graduate
Studies in Agricultural Sciences,
Central Agricultural University
(Imphal), Umiam, Meghalaya,
India

Corresponding Author:**Sushanti Thokchom**

Department of Plant Pathology,
School of Crop Protection,
College of Post Graduate
Studies in Agricultural Sciences,
Central Agricultural University
(Imphal), Umiam, Meghalaya,
India

Endophytic bacteria associated with cultivars of *Brassica juncea* and their antagonistic potentials against *Alternaria brassicae*

Sushanti Thokchom, Pranab Malakar and RK Tombisana Devi

DOI: <https://doi.org/10.22271/chemi.2020.v8.i5p.10449>

Abstract

The yield potential of *Brassica juncea* is constrained by many fungal diseases among which *Alternaria* blight poses a major challenge worldwide. Endophytic bacteria are increasingly recognized as a promising source of novel organic natural metabolites for a variety of biological activities. However endophytic bacteria belonging to the group *Bacillus* and fluorescent *Pseudomonads* are being reported as superior groups exploited for their role in combating phytopathogens. Therefore, the bacteria belonging to the above groups were primarily targeted for this study. In this study, fifty six endophytic bacteria were selectively isolated from seeds, roots and leaves of 20 different cultivars of *Brassica juncea* growing in various parts of North Eastern Region of India. Out of all isolates tested BS8, BS15, BS21 and FP13 show inhibition of 59.63%, 65.93%, 58.52% and 63.33 % respectively. When tested for functional properties, BS21 produced IAA as well as ammonia, BS15 and FP13 produced only Ammonia whereas BS8 solubilised Phosphate. BS8(*Bacillus cereus*), BS15(*Bacillus subtilis*), BS21 (*Bacillus subtilis*) and FP13 (*Pseudomonas fluorescens*) were selected on the basis of antagonistic as well as plant growth promoting properties for formulating a microbial consortium and subsequent evaluation in the field.

Keywords: Endophytes, bacillus, fluorescent pseudomonads, *Brassica juncea*

Introduction

Brassica juncea is one of the important oilseed crops grown extensively in the Indian sub-continent, Europe, Australia, and Canada. *Alternaria* blight of oilseed mustard is caused by a seed borne necrotrophic fungal pathogen of *Alternaria* spp. belonging to the family Pleosporaceae. Both *A. brassicicola* and *A. brassicae* have been reported to cause the disease, yet *A. brassicae* is known to be more virulent, causing major damage to oilseed Brassicas. The fungus is capable of infecting the aerial plant parts at all the stages of growth. The disease symptoms are manifested as black/ brown necrotic lesions on the leaves, esp. older ones, stem and siliques.

Accumulating evidences suggest that the organisms under most scrutiny for potential use in biological control of pest and diseases are bacteria belonging to the genera *Pseudomonas* and *Bacillus* (Ramamoorthy *et al.*, 2001) [5]. The positive effect of a single microbial species on plant growth in low-fertility soils was improved by the application of mixtures of different microbial inocula. Microbial consortia are much more efficient than single strains of organisms with diverse metabolic capabilities. Therefore, the isolation, identification and formulation of bacterial endophyte based microbial consortium from mustard plant and their ability to produce antimicrobial compounds and plant growth promoting factors can replace the use of toxic chemicals for management of *Alternaria* blight disease and reduce contamination of the environment in the raising scenario of promotion of organic farming in North East India.

Materials and Methods**Isolation of bacterial endophytes**

Seeds of 20 cultivars of Mustard (*Brassica juncea*) were collected from different parts of North East India and are grown separately using same type of soil in pots. Isolation of the bacterial endophytes were performed according to the method described by Zinneal *et al.*, 2002 [12] with minor modifications.

Surface sterilization

The plant parts (seed, root and leaf) were taken and processed separately. Healthy and undamaged leaves (from 45 day old plant) and root (from 30 day old plant) were collected from the plant grown in pot. These were then washed under running water and dried followed by surface sterilization. Surface sterilization was done first by washing in 70% ethanol for 1 min followed by immersing in 2% sodium hypochlorite solution for 3 mins and again in 70% ethanol for 30 sec followed by rinsing in sterile double distilled water 4-5 times.

Isolation

The samples (root and leaf) were cut into small pieces and macerated using Phosphate Saline Buffer (pH 7.2) with a sterile mortar and pestle. For specific isolation of *Bacillus* spp. 1.5 ml of the aliquot from the total extract were suspended in microcentrifuge tubes and were given heat shock treatment at 80°C for 5 mins to eliminate other unwanted microorganisms and tissue extract were then prepared for serial dilution upto 10⁻⁵. 0.1 ml from this final aliquot was plated with Nutrient Agar medium by using Pour plate method. For isolation of fluorescent *Pseudomonads* specific media i.e. King's Medium B was used.

Detection of targeted bacterial colonies and its purification

Colonies of *Bacillus* spp. were observed and for fluorescent *Pseudomonads*, fluorescent colonies were detected by viewing the culture plates under UV light (365nm) in UV transilluminator. The colony morphologies of all the isolates were recorded. The single colonies were then picked up and streaked in a fresh NA medium and KMB medium respectively with the help of a sterile loop to obtain pure culture. They were incubated at 28 ± 1°C for 24 h and maintained by regular sub culturing at an interval of two months and kept at 4±1°C in refrigerator.

In vitro evaluation of antagonistic potential of the endophytes

Characteristic symptoms of Alternaria blight were collected from nearby mustard growing fields of Ri Bhoi district (Meghalaya) for isolation of the pathogen. These selected infected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 2% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Excess of moisture was removed by putting these pieces in between two folds of sterilized blotting paper under aseptic conditions in the inoculation chamber. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for seven days.

Dual Culture Assay

All the bacterial isolates were tested for their inhibitory activity against *Alternaria brassicae* by dual culture technique (Ganesan and Gnanamanickam, 1987) ^[1]. The fungal pathogen was grown on a PDA plate till it covered the whole

surface of the agar. With the help of a sterile cork borer, a disc of fungal growth from this plate was taken and placed at the center of a fresh PDA plate. Twenty four hours old culture of each bacterial strain was then streaked parallelly on both side of the fungal disc 3 cm away from the disc. The plates were kept for incubation at 28±1°C for 7 days. Visual observations on the inhibition of growth of fungal pathogen were recorded after 5th, 7th and 9th days after incubation and compared with the PDA plate simultaneously inoculated with only the fungal pathogen and inhibition percent was recorded.

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

Where “C” is the maximum growth of the fungal mycelia under control conditions and “T” is fungal mycelia growth in treatment” (Vincent, 1947) ^[11].

Characterization of the potential bacterial antagonists

The potential bacterial antagonists were characterised morphologically as well as biochemically by comparing the guidelines given in the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) ^[3]. Colony morphology like shape, elevation, texture and pigmentation were observed. Biochemical test like Catalase test, Citrate Utilisation, Gelatin hydrolysis, Methyl Red (MR) Test, Voges Proskauer Test (VP), Arginine Dehydrolase, Levan Production, Nitrate Reduction, Casein Hydrolysis, Starch Hydrolysis were conducted for tentative identification of the isolates upto species level.

Results

Isolation of the bacterial endophytes: Thirty one bacterial endophytes belonging to the genus *Bacillus* and 25 Fluorescent *Pseudomonads* were obtained from seed, root and leaf portion of the plant. The isolates were preliminary confirmed as belonging to the two genus by observing the colony morphology like flat, rough, dry and creamy white colony colour and KOH solubility test (Vishunavat and Kolte, 2005) (specific to the genus *Bacillus*) and bluish green fluorescent colony which were observed under UV of 650 nm (specific for fluorescent *Pseudomonads*).

In vitro evaluation of antagonistic potential of the endophytes

Among the isolates BS15 showed minimum of 31.2mm mycelial growth of *Alternaria brassicae* accounting to inhibition per cent of 65.55% over control. This was followed by isolate FP13 with inhibition per cent of 63.33. Isolate BS8 and BS21 also showed inhibition of 59.63 and 58.24 per cent respectively. The other isolates were less effective in inhibiting the mycelial growth of *Alternaria brassicae* under *in vitro*.

Characterization of the potential antagonists

Thirty one isolates of *Bacillus* and 25 isolates of fluorescent *Pseudomonads* were isolated from seed, root and leaf of different cultivars of mustard indicating that the two genus are the most predominant and easy culturable endophytes.

Table 1.

	Shape	Texture	Colour	3 % KOH	Citrate	Gelatin	Levan	Nitrate	Starch	Arginine	Methyl Red	Voges proskauer	Result
BS8	Rd	R	CW	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	<i>Bacillus cereus</i>
BS15	Rd	R	CW	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	<i>B.subtilis</i>
BS21	Rd	R	CW	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	<i>B.subtilis</i>
FP13	Rd	S	GY	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	<i>Pseudomonas fluorescens</i>

Rd= Rod, F=Flat, R= Rough, S=Smooth, CW=Creamy White, GY=Greenish Yellow

Fig.1 *In vitro* evaluation of antagonistic potentials of the endophytes against *Alternaria brassicae*

Fig 2: Gelatin hydrolysis

Fig 3: Levan Production

Fig 4: Arginine dehydrolase

Fig 6: VP test

Fig 7: Starch hydrolysis

Fig 8: Nitrate Reduction Test

(a): Colonies resembling *Bacillus* spp. obtained after serial dilution
(b): Colonies of fluorescent *Pseudomonad* detected under UV.

Discussion

Diversity has been found at the cultivar level as well. Antagonistic test revealed the ability of the isolates to suppress the mycelial growth of the fungus indicating differences within the isolates of the genus which was later confirmed by the biochemical tests. Similarly studies conducted on endophytic colonization of 11 field-grown pea cultivars showed significant differences in the level of endophyte colonisation between cultivars at the mature stage. It is also known that tomato cultivars differ in their endophytic flora (Samish *et al.*, 1961)^[7]. Further, Siciliano *et al.*, (1999)^[8] have shown that endophytic populations are different in the roots of *Brassica napus* cultivars Excel, Parkland and a transgene of Excel, Quest. Thus, there seems to be evidence in favour of a relationship between plant genotype and colonisation of endophytes. Similar line of works like isolation of endophytes and evaluation against pathogens were carried out in nutmeg and cinnamon by earlier workers like Sopalaun *et al.*, (2003)^[9] and Gary *et al.*, (2003)^[2] respectively. *Bacillus spp.* and fluorescent Pseudomonads are found to be the most predominating groups of endophytes and are isolated from mostly all the compartments of the plant. The bacterial antagonists that were found capable of inhibiting the mycelial growth of the fungus may be further tested for their plant growth promotion properties and may be tested for their compatibility among each other for developing a consortium and later examined for its efficacy in combating the disease in field condition.

References

1. Ganesan P, Gnanamanickam SS. Biological control of *Sclerotium rolfsii* in pea nut by inoculation with *P. fluorescens*. *Soil Bio. Biochem.* 1987; 19:35-38.
2. Gary AS. Endophytes as sources of bioactive products. *Microbes Infect.* 2003; 5:535-544.
3. Holt JA, Kreig NR, Sneath PHA. *Bergey's Manual of Determinative Bacteriology*. Baltimore, MD, 1994.
4. Karuna V, Kolte SJ. *Essentials of Phytopathological Techniques*. Kalyani Publishers, New Delhi, 2005, 210 P.
5. Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and disease. *Crop Protect.* 2001; 20:1-11.
6. Rosenblueth M, Martinez RE. Bacterial endophytes and their interactions with hosts. *Mol. Plant Microbe Interact.* 2006; 19:827-37.
7. Samish Z, Etinger-Tulczynska R, Bick M. Micro flora within healthy tomatoes. *Appl. Microbiol.* 1961; 9:20-25.
8. Siciliano SD, Germida JJ. Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. *Fems. Microbiol. Ecol.* 1999; 29:263-272.
9. Sopalaun K, Strobel GA, Hess WM, Worapon J. A record of *Muscodoralbus*, a novel endophytic from *Myristica fragrans* in Thailand. *Mycotaxon.* 2003; 88:239-247.
10. Sturz AV, Nowak J. Endophytic communities of rhizobacteria and strategies required to create yield enhancing associations with crops. *Appl. Soil Ecol.* 2000; 15:183-190.
11. Vincent JH. Distortion of fungi hyphae in the presence of certain inhibitors. *Nat.* 1947; 159:850.

12. Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P *et al.* Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.* 2002; 68:2198-2208.