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Research Paper

DISTRIBUTION, DIVERSITY AND ANTAGONISTIC ABILITY OF ACTINOBACTERIA FROM BLACK PEPPER (*Piper nigrum* L.) RHIZOSPHERE

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Abstract

A survey was undertaken in the major black pepper growing belts of Kerala and Karnataka for studying the distribution and diversity of actinobacteria in the rhizosphere of black pepper. A total of 129 morphologically different actinobacteria were isolated from 123 samples collected from different agro climatic niche. The 129 actinobacteria were morphologically characterized on the basis of the colour of aerial mycelia, substrate mycelia, spore chain morphology and production of diffusible pigments. The isolates were also tested for their antagonistic ability against major pathogens of black pepper. Morphologically the isolates fall into 7 groups in which majority were found to be in the genus *Streptomyces* (100). Other groups are *Actinoplanes* (10), *Micromonospora* (10), *Nocardiopsis* (2), *Actinomyces* (3), *Actinopolyspora* (1), and *Dactylosporium* (1). Functionally, more than 50% of the isolates showed 51-94% inhibition against the major pathogens.

Key words: Actinobacteria, Antagonism, Black pepper, Diversity, *P. capsici*, Rhizosphere, Spore chain morphology.

INTRODUCTION

Actinobacteria, the well known source of antimicrobial secondary metabolites, are Gram- positive filamentous free living saprophytic bacteria. They are widely distributed in soil and also colonizing plants. Although the genus *Streptomyces* are the most successfully exploited source for secondary metabolites, other genera of Actinobacteria like *Saccharopolyspora, Amycolatopsis, Micromonospora* and *Actinoplanes* are also major producers of commercially important biomolecules (Solanki *et al*., 2008). These actinobacterial secondary metabolites have wide range of biological functions which include antibacterial (streptomycin), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin), immunosuppressive (rapamycin), antitumor (actinomycin,), enzyme inhibitory (clavulanic acid) and diabetogenic (bafilomycin, streptozotocin) activities. Various approaches are continuing to explore new and particular ecosystem

for the actinobacterial diversity to open an entry to the discovery of unknown biomolecules (Park *et al*., 2014).

In the diverse rhizosphere microbial community, actinobacteria represents a large part. (Strobel *et al*., 2004), which contributes a major role in the turnover of recalcitrant plant organic matter producing a steadiness in the ecosystem (Miyadoh, 1997). According to Kimura and Asakawa (2006), most of the actinobacteria are isolated from soil, rhizosphere region and rice fields. The interactions such as nitrogen fixation, plant growth hormone production and protection of plants against infection by rhizosphere actinobacteria have been demonstrated by Crawford *et al* (1993). The production of lytic enzymes by the actinobacteria make the survival of plants easier by degrading diverse substrates occurring in plant litter and soil, which has the ability to inhibit the growth of phytopathogens (Priyadharsini *et al*., 2015). The derivative power of actinobacteria is also well known for carbon cycle and humus formation in the environment which ultimately improve the soil nutrition for plant growth. Above all these, actinobacteria especially *Streptomyces* are inexhaustible producers of secondary metabolites, used as biocontrol agents to control soil-borne and seed borne diseases of plants. According to Burkholder *et al* 1954, the microscopic characterization based on aerial and substrate mycelium and spore chain morphology are very useful in identification of actinobacteria. The sporophore morphology was found to be most constant and clearly mentioned feature used for actinobacterial classification. The method followed by International Streptomyces Projects (ISP) was mainly used for the actinobacterial characterization (Shrilling and Gottlieb 1966). The characteristic features of actinobacteria such as colour of aerial spore mass, reverse side colour, diffusible pigment production, production of melanoid pigments are also have been played an important role.

In the present study, it is aimed to evaluate the genus diversity of actinobacteria in rhizosphere of black pepper in the major cultivated areas of Southern states of India *viz*., Kerala and Karnataka and to discover their antagonistic activity against major pathogens of black pepper *viz*., *Phytophthora capsici, Sclerotium rolfsii* and *Collectotrichum gloeosporioides*.

MATERIALS AND METHODS

Survey and Sample collection:

Suvrvey for the collection of rhizosphere soil sample from black pepper was undertaken in the major black pepper growing tracts of Kerala and Karnataka during the monsoon period (May-September) of 2012 and 2013. The locations covered include Kasaragod, Kannur, Kozhikode, Wayanad, Thrissur, Ernakulam, Kottayam, Idukki and Malappuram districts in Kerala and Coorg district in Karnataka. During the survey, rhizosphere soil samples were collected from healthy black pepper vines encompassing different ecological niche where black pepper is extensively cultivated. Sampling was done district wise and tried to cover all the taluk of each district.

Sampling was done as per Yanai *et al*. 2003. The soil adhered to the roots of black pepper was collected by removing the layer of top soil and subsequent brushing of rhizosphere soil (1-5mm from the root surface since soil farther than 1cm from the root was considered as non rhizosphere soil) since the soil texture and actual soil moisture strongly influence the amount of soil adhering to the root system. The soil samples were

collected in polythene bags, tied and labelled, and brought to laboratory and stored at 4^0C for further studies.

Isolation of Actinobacteria

Isolation was done as per the procedure of Matsukawa *et al*., 2007 and Hong *et al*., 2009. Different physical pretreatments were done to facilitate the isolation of actinobacteria by eliminating unwanted fungal and bacterial populations. Drying and heating pre-treatment of soil given in the procedure enhances the chances of getting actinobacterial colony while eliminating most unwanted gram negative bacteria (Hong *et al*. 2009; Kavitha *et al*., 2010, and Matsukawa *et al*., 2007). The soil samples were dried for one week and sieved through 2mm pore sieve. One gram of soil was diluted to 10^{-2} dilution using sterile water and incubated in an orbital shaker at 28^0C at 170rpm for 30 min and further incubated at 50^0C in a water bath for 15-20 min to reduce the bacterial community. Serial dilutions of the soil were made up to 10^{-7} and 1 ml of 10^{-5} and 10^{-7} dilutions were plated in selective media *viz*., Actinomycetes Isolation Agar (Himedia) by pour plate method. There were three replications for each dilution. Plates were incubated at 28^0C for 8–15 days and typical colonies of actinobacteria were isolated on the basis of traditional morphological criteria. The actinobacterial colonies were hard to touch and leathery in nature compared to the bacterial or fungal colonies. The individual colonies were then purified by sub culturing on ISP-2 (International Streptomyces Project-2) medium.

Morphological characterization

Purified colonies of actinobacterial isolates were grown in ISP2 medium (Shirling and Gottlieb, 1966) at 28^0C for 7 days. Colony morphology such as appearance, colour of areal hyphae, growth of vegetative hyphae, diffusible pigment production etc were observed and documented. The colour of spore masses and diffusible pigment production were visually estimated with the help of RHS-colour code (RHS colour chart, Fifth edition-Royal Horticultural Society).

Microscopic characterization

Spore chain morphology was studied using modified cover slip culture method (Hopwood, 1960 modified). The isolates were inoculated on PDA agar block and then a sterile cover slip was placed over it and incubated at 28^0C for 3 days. The mycelium grown on the cover slip was stained with crystal violet. Spore chain morphology was observed under light microscopy (100X magnification) using Leica DM5000B Research microscope.

***In vitro* testing for antagonism**

The 129 isolates of morphologically different actinobacteria were assayed for their antagonistic /inhibitory activity against major pathogens of black pepper *viz*., *P. capsici*, *S. rolfsii* and *C.gloeopsorioides* by dual culture method (Khamna *et al*., 2009), with some modifications. Single colonies of actinobacteria were streaked on both sides of the Potato Dextrose Agar (PDA) plate at a distance of 3.5 cm away from the each other. After sufficient growth attained by the bacterial colony, 5 mm mycelial plugs taken from the growing edge of 72 h old culture of the target pathogen was inoculated at the centre of the plate in between the actinobacterial streaking and incubated at 26^0C for 4–5 days. The experiment was repeated three times for conformation and three independent replicates were used. Plates inoculated with pathogens alone served as controls. The

percentage of inhibition was calculated by measuring the radial growth of the pathogen using the formula.

I= C – T/C X 100

Where I = % inhibition, C = radial growth (mm) of the pathogen in control and T = radial growth of the pathogen in treatment.

Statistical Analysis

The data was statistically analyzed using analysis of variance (ANOVA) with the statistical package SAS software (Version 9.3) and subjected to mean separation by Least Significant Difference (LSD) test, $P<0.05$.

RESULTS

Survey and Sample collection

The samples were collected during the monsoon season (May-September) of 2012 and 2013. A total of 123 soil samples were collected from different ecological niche were black pepper is cultivated in a large and marginal scale (Table 1). The samples were collected from 9 districts of Kerala which includes 24 samples from Wayanad, 21 samples each from Idukki and Kozhikode, 17 samples from Ernakulam, 8 samples from Kannur, 7 samples from Kottayam, 6 samples from Thrissur, 4 samples from Kasaragod, and 2 samples from Malappuram. Thirteen samples
were collected from Coorg district of Karnataka which is major black pepper growing belt of India.

Isolation of actinobacteria from the collected soil samples

A total of 129 actinobacteria with different morphological characters were isolated from the collected soil samples (Table 1). Maximum isolates (49) were obtained from nine locations of Ernakulam district which include 17 from Kunnathunad, five each from Keezhillam Meempara,and Ramamangalam, 3 each from Thiruvaniyoor and Mannur, 4 from Mulanthuruthy, six from Maneed, and 1from Amballoor. This is followed by Waynad (23). From other places, the number of isolates obtained ranged from 4 to 18. All the isolates were maintained on ISP2 medium.

The isolates were nomenclatured by giving the prefix IISRBPAct where IISR stands for the parent institute (Indian Institute of Spices Research) and BP for black pepper. The isolates were serially numbered as IISRBPAct1, IISRBPAct2, and IISRBPAct3 likewise. The isolates were stored in PDA slant at 4^0C and in 20% glycerol at $^{-}20^0$C for further use. The PDA slants were used as working culture while glycerol stock is used for long term preservation

Morphological characterization of collected actinobacteria

All the 129 actinobacterial isolates were morphologically characterized by growing on ISP2 medium (Table 2&3). For the *Streptomyces* sp., the colour of aerial mycelium varied from Greyed-white, white or grey-brown. The *Micromonospora* sp. exhibited aerial mycelial mass and colour ranges from Greyed-white to Greyed-brown while substrate mycelium was Greyed- yellow and brown/ black. In *Actinoplanes* sp. the aerial mycelia was Greyed-white to Greyed- brown with Greyed-yellow to brown substrate mycelium. *Dactylosporangium* sp. was grey-brown to grey-white whereas *Actinopolyspora* sp was Greyed-white to Greyed-yellow. *Actinomyces* colonies were found in Greyed-white to brown in colour while *Nocardiopsis* is Greyed- white in colour. Only limited growth of aerial mycelium was observed in five isolates (IISRBPAct82,

IISRBPAct62, IISRBPAct75, IISRBPAct93 and IISRBPAct43). The colour of substrate mycelium was observed to be Greyed-white to brown or black. Five isolates showed diffusible pigment production (Creyed-yellow, Greyed-purple and brown) in the media. Colony morphology of selected *Streptomyces* and non-*Streptomyces* actinobacteria isolated from rhizosphere region of black pepper is given Fig 2 and 3

Microscopic characterization of isolates actinobacteria

The 129 isolates of actinobacteria were microscopically characterized as belonged to the groups *Streptomyces*, *Actinoplanes*, *Micromonospora*, *Actinomyces*, *Nocardiopsis*, and *Actinopolyspora* (Table 2&3). Out of 129 actinobacterial isolates, 100 isolates were identified in the genus *Streptomyces* (spore chain with coiling, spiral and looped), 10 in *Actinoplanes* (spores in sporangia having spherical shape), 10 as *Micromonospora* (clusters of single conidia on substrate mycelium), 2 as *Nocardiopsis* (aerial mycelium totally sporulated), 3 as *Actinomyces* (branching vegetative mycelium), 1 as *Actinopolyspora* (long chains of spores on aerial hyphae) and 1 as *Dactylosporangium* (Oligosporous, claviform sporangia on aerial mycelium). Spore chain morphology of selected *Streptomyces* and non- *Streptomyces* isolates are shown in Fig. 4 and Fig 5.

The occurrence and allocation of different genera of actinobacteria in different rhizosphere soil samples of black pepper are represented in Table 4. The *Streptomyces* are widely distributed in all the locations due to their wide adaptability to different habitats. *Actinoplane* sp are frequently isolated from the Waynad, and Kozhikode followed by Coorg and Idukki where black pepper is cultivated in fairly large scale. *Micromonospora* sp. also reported from Meppadi and Pulpally in Waynad followed by Malappuram, Kottayam, Kannur and Coorg. *Nocardiopsis* sp.was isolated from Kanjangad of Kasaragod and Maneed of Ernakulam. *Actinomyces* sp. was isolated from Ernakulam and Kozhikode, while *Dactylopsorium* sp. and *Actinopolyspora* sp were obtained from Vellamunda and Adikkolli of Waynad district. From Kunnathunad of Ernakulam district, 17 isolates were obtained; among them 16 were *Streptomyces* sp. one *Actinomyces* sp. Two Actinobacterial isolates remained unidentified from Kalangali of Kozhikode, suspect for rare actinobacterial species.

Distribution frequency actinobacteria in the rhizosphere of black pepper

Based on the morphological identification, frequencies of the occurrence of different genera of actinobacteria in different locations were calculated. The frequency of the genus *Streptomyces* was 77.51% followed by *Actinoplanes* (7.75%), *Micromonospora* (7.75%), *Actinomyces* (2.32%), *Nocardiopsis* (1.55%), *Actinopolyspora* (0.77%) and *Dactylopsorium* (0.77%). The genera such as *Actinopolyspora* and *Dactylopsorium* were recorded in low percentage of frequency (0.77%) (Fig 6). From the present study, *Streptomyces* was the most predominant genera when compared to other genera. Besides *Streptomyces*, the genera most commonly appeared on media were *Actinoplanes*, *Micromonospora*, and *Nocariopsis*.

***In vitro* evaluation of *Actinobacteria* for its antagonistic potential**

The 129 actinobacterial isolates were studied for *in vitro* antagonistic activity against three major pathogens of black pepper (Table 5). Among them 72 isolates showed more than 50% of inhibition against *Phytophthora capsici* in which 4 isolates coming under *Micromonospora* sp, 6 isolates under *Actinoplane* sp. and 2 isolates under *Nocardiopsis* sp., while all others are *Streptomyces sp.* of which 18 isolates showed more than 80%

inhibition against *P. capsici*. These isolates belonged to two genera (1*Micromonospora* and 6 *Streptomyces*).

58 isolates showed more than 50% of inhibition towards *Sclerotium rolfsii* in which five isolates belonged to *Actinoplane* sp., four *Micromonospora* sp. and one *Nocardiopsis* sp., and the remaining are *Streptomyces* sp. (48). Twelve isolates showed more than 80% of inhibition towards *Sclerotium rolfsii* in which 10 are *Streptomyces* sp., and one each of *Actinoplane* sp. *Micromonospora* sp. Two *Streptomyces* sp isolates showed more than 90% inhibition towards *S. rolfsii*.

68 isolates showed more than 50% of inhibition towards *Colletotrichum gloeosporioides* of which four are *Micromonospora* sp., two are *Actinomyces* sp., five are *Actinoplane* sp. and one *Dactylosporium* sp., The remaining are *Streptomyces sp*. 14 isolates showed more than 80% of inhibition towards *Colletotrichum gloeosporioides* of which two belong to *Micromonospora* sp., and 12 of *Streptomyces* sp. Three *Streptomyces* isolates showed more than 90% of inhibition against *C. gloeosporioides*.

Only one isolate (IISRBP Act1) showed more than 90% (92-94%) inhibition to all the tested pathogens showing its great potential. This is followed by IISRBPAct25 and IISRBPAct42. The shortlisted actinobacterial isolates were identified by 16S rDNA sequencing using actinomycetes specific primers S-C-Act-235-S-20 (50-CGCGGCCTATCAGCTTGTTG-30), S-C-Act-878-A-19 (50-CCGTACTCCCCAGGCGGGG-30) (Stach *et al*., 2003a). NCBI BLAST search of the 640 nucleotide position of 16S-rDNA gene sequence of potential isolates showed 97-99% homology to the known *Streptomyces* spp.

The 16S-rDNA gene sequence of the promising isolates IISRBPAct1, IISRBPAct25 and IISRBPAct42 were deposited in NCBI Gen-Bank with accession numbers KM361516, KM361514 and KM361515 respectively.

Table 1. Details of locations surveyed for sample collection, no of samples collected, and no of Actinobacterial isolates obtained from them

District	Place of collection	Latitude and Longitude	Number of samples collected	No of isolates obtained 3	Isolates obtained
Coorg	Suntikoppa	12°28' N/ 75°50'E	6	2	IISRBPAct32, 33
	Virajpet	12°11'N/ 75°55'E	3	4	IISRBPAct34, 35, 36, 37
	Kathalakkadu	12°22'N / 75°42'E	4	1	IISRBPAct38
Kasaragod	Kanjangad	12°19'N/75°5' E	4	2	IISRBP Act69, 70
Kannur	Taliparamba	12°2'N/75°22'E	4	2	IISRBP Act71, 72
	Panniyur	12°4'N/75°24'E	4	2	IISRBP Act73, 74
Waynad	Vellamunda	11°44' N / 75°56'E	10	5	IISRBPAct9, 10, 11, 12, 13
	Meppadi	11°33'N /76°8'E	3	7	IISRBPAct14, 15, 16, 17, 18, 19, 20
	Adikkolli	11°47'N /76°10'E	1	6	IISRBPAct21, 22, 23, 24, 25, 26

	Pulpally	11°44'N /76°12'E	10	5	IISRBPAct27, 28, 29, 30, 31
Kozhikode	Narikkuni	11°21'N/75°51'E	4	1	IISRBP Act51
	Nanminda	11°25'N/75°50'E	4	2	IISRBP Act52, 53
	Thalayad	11°30'N/ 75°53'E	5	4	IISRBP Act54, 55, 56, 57
	Kallanode	11°32' N/75°52'E	6	3	IISRBP Act58, 59, 60
	Kalangali	11°32'N/75°50'E	2	8	IISRBP Act61, 62, 63, 64, 65, 66, 67, 68
Malappuram	Perinthalmanna	10°57'N/ 76°17'E	2	4	IISRBP Act 39, 40, 41, 42
Thrissur	Irinjalakuda	10°20'N/76°12'E	2	3	IISRBP Act124, 125, 126
	Thalappilly	10°39'N /76°14'E	4	3	IISRBP Act127, 128, 129
Ernakulam	Kunnathunad	09°53'N/76°23'E	2	17	IISRBP Act75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91
	Keezhillam	10°3'N/76°31'E	3	5	IISRBP Act92, 93, 94, 95, 96
	Ramamangalam,	09°56'N/76°29'E	2	5	IISRBP Act97, 98, 99, 100, 101
	Thiruvaniyoor	09°56'N/76°25'E	1	3	IISRBP Act102, 103, 104
	Mulanthuruthy	09°53'N/ 6°23'E	2	4	IISRBP Act105, 106, 107, 108
	Mannur	10°2'N/76°32'E	2	3	IISRBP Act109, 110, 111
	Maneed	09°54'N/76°27'E	2	6	IISRBP Act112, 113, 114, 115, 116, 117
	Amballoor	10°26'N/76°15'E	1	1	IISRBP Act118
	Meempara	09°55'N/76°27'E	2	5	IISRBP Act119, 120, 121, 122, 123

Idukki	Venmani	10°59'N/ 75°59'E	6	3	IISRBP Act43, 44, 45,
	Cardamom Research Centre	09°53'N/ 77°09'E	2	2	IISRBP Act46, 47
	Pulianmala	09°42'N/ 77°10'E	2	0	
	Peerumedu	09°36'N/ 77°09'E	3	2	IISRBP Act48, 49
	Pampadumpara	09°47'N/ 77°09'E	4	0	
	Devikulam	09°58'N/ 77°02'E	4	1	IISRBP Act50
	Vechoor	09 °35'N /76°20'E	4	3	IISRBP Act1, 2, 3
	Thalayalam	09°39'N /76°20'E 39' N /76° 20' E	2	2	IISRBP Act4, 5
Kottayam	Vaikom	09°33'N /76°23'E	1	3	IISRBP Act6, 7, 8
	Kalangali	11°32'N/75°50'E	2	8	IISRBP Act61, 62, 63, 64, 65, 66, 67, 68

Table 2. Cultural characteristics of *Streptomyces* isolates

Isolate Code	Colour of aerial mycelium	Reversible side colour	Diffusible pigment	Spore chain morphology
IISRBPAct1	White	Greyed- yellow		Spira
IISRBPAct3	Grey- white	Greyed- yellow	_	Spira
IISRBPAct4	White	Grey-brown	Creyed-yellow	Rectiflexibiles
IISRBPAct5	Grey-brown	Brown	_	Rectiflexibiles
IISRBPAct6	Grey- brown	Brown	_	Spira
IISRBPAct7	Greyed-yellow	Brown	_	Rectiflexibiles
IISRBPAct8	Greyed-purple	Brown	_	Retinaculiaperti
IISRBPAct9	White	Brown	_	Retinaculiaperti
IISRBPAct11	White	Greyed-yellow	_	Spira
IISRBPAct14	White	Brown	_	Fragmented aerial hyphae
IISRBPAct15	White	Greyed –white	_	Spira
IISRBPAct16	White	Greyed-white	_	Fragmented aerial hyphae
IISRBPAct19	Greyed- purple	Brown	Greyed-purple	Fragmented hyphae
IISRBPAct21	Greyed-white	Yellow-white	_	Rectiflexibiles
IISRBPAct22	Greyed-white	Brown	_	Spira
IISRBPAct23	White	White	_	Rectiflexibiles
IISRBPAct24	Greyed-yellow	Brown	_	Fragmented aerial hyphae
IISRBPAct25	White	Greyed –white	_	Spira

IISRBPAct30	White	Creyed orange	Creyed yellow	Spira
IISRBPAct33	Greyed-white	Brown	_	Fragmented branched aerial hyphae
IISRBPAct35	Greyed white	Brown	_	Rectiflexibiles
IISRBPAct36	Greyed- white	Greyed- orange	_	Spira
IISRBPAct37			_	Rectiflexibiles
IISRBPAct40	Greyed-white	Greyed-white	_	Spira
IISRBPAct42	Grey-brown	Black	Creyed-yellow	Spira
IISRBPAct44	Grey-brown	Black	_	Spira
IISRBPAct45	Greyed- white	Brown	_	Retinaculiaperti
IISRBPAct46	White	Creyed white	_	Rectiflexibiles
IISRBPAct47	Greyed- white	Creyed white	_	Spira
IISRBPAct48	Grey-brown	Brown	_	Spira
IISRBPAct49	Greyed- white	Greyed-purple	_	Rectiflexibiles
IISRBPAct50	White	Greyed- white	_	Rectiflexibiles
IISRBPAct51	Grey-brown	White	_	Rectiflexibiles
IISRBPAct52	White	Brown	_	Rectiflexibiles
IISRBPAct53	White	Brown	_	Spira
IISRBPAct54	White	Greyed- white	_	Spira
IISRBPAct55	Grey-brown	Black	_	Retinaculiaperti
IISRBPAct56	Grey-brown	Brown	_	Retinaculiaperti
IISRBPAct58	Greyed-white	Black	_	Fragmented aerial hyphae
IISRBPAct59	Grey-brown	Black	_	Rectiflexibiles
IISRBPAct61	Greyed- white	Black	_	Fragmented aerial hyphae
IISRBPAct63	Greyed- white	Brown	_	Arthrospores
IISRBPAct64	Grey-brown	Brown	_	Arthrospores
IISRBPAct65	Greyed- white	Greyed-yellow	_	Spira
IISRBPAct66	Greyed- white	White	_	Rectiflexibiles
IISRBPAct67	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct69	Grey-brown	Black	_	Spira
IISRBPAct72	Greyed- white	Black	_	Rectiflexibiles
IISRBPAct73	Greyed- white	Creyed- white	_	Spira
IISRBPAct74	White	Greyed- white	_	Retinaculiaperti
IISRBPAct76	Grey- brown	Brown	_	Spira
IISRBPAct77	Greyed- white	Creyed-white	_	Retinaculiaperti
IISRBPAct78	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct79	White	Brown	_	Rectiflexibiles
IISRBPAct80	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct81	Grey-brown	Brown	_	Retinaculiaperti
IISRBPAct82	Greyed- white	Brown	_	Fragmented aerial mycelium
IISRBPAct83	White	Greyed- white	_	Rectiflexibiles
IISRBPAct84	Grey- brown	Brown	_	Retinaculiaperti
IISRBPAct85	Greyed- white	Brown	_	Retinaculiaperti
IISRBPAct86	Greyed- white	Greyed-white	_	Rectiflexibiles
IISRBPAct87	White	Greyed- white	_	Retinaculiaperti
IISRBPAct88	Greyed- white	Dark- brown	_	Spira
IISRBPAct89	Dark -brown	Black	_	Spira
IISRBPAct90	White	Greyed- white	_	Retinaculiaperti

IISRBPAct91	White	Greyed- white	_	Rectiflexibiles
IISRBPAct92	White	Greyed- white	_	Rectiflexibiles
IISRBPAct94	Grey-brown	Black	_	Spira
IISRBPAct95	White	Greyed- white	_	Fragemented aerial hyphae
IISRBPAct96	White	Greyed- white	_	Retinaculiaperti
IISRBPAct97	Grey-brown	Brown	_	Retinaculiaperti
IISRBPAct98	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct99	White	White	_	Spira
IISRBPAct100	White	Creyed-yellow	_	Spira
IISRBPAct101	Greyed- white	Greyed- white	_	Retinaculiaperti
IISRBPAct102	Grey-brown	Brown	_	Rectiflexibiles
IISRBPAct103	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct104	White	Greyed- white	_	Rectiflexibiles
IISRBPAct105	Grey-brown	Black	_	Rectiflexibiles
IISRBPAct106	Greyed- white	White	_	Rectiflexibiles
IISRBPAct107	White	Greyed- white	_	Rectiflexibiles
IISRBPAct108	Brown	Brown	_	Rectiflexibiles
IISRBPAct109	White	Greyed- white	_	Retinaculiaperti
IISRBPAct110	Greyed- white	Creyed-white	_	Spira
IISRBPAct111	Grey-brown	Brown	_	Spira
IISRBPAct113	Greyed- white	White	_	Spira
IISRBPAct114	White	Grey- brown	_	Spira
IISRBPAct115	White	Greyed- white	_	Rectiflexibiles
IISRBPAct116	White	Brown	_	Rectiflexibiles
IISRBPAct117	White	Dull white	_	Rectiflexibiles
IISRBPAct118	White	Greyed –white	_	Rectiflexibiles
IISRBPAct119	Greyed-white	Greyed-yellow	_	Rectiflexibiles
IISRBPAct120	Grey-brown	Black	_	Rectiflexibiles
IISRBPAct121	Greyed-white	Brown	_	Retinaculiaperti
IISRBPAct122	Greyed white	Greyed-yellow	_	Spira
IISRBPAct123	White	Creyed orange	_	Spira
IISRBPAct124	Grey-brown	Blak	_	Retinaculiaperti
IISRBPAct125	Greyed-white	Brown	_	Rectiflexibiles
IISRBPAct126	White	Greyed- white	_	Rectiflexibiles
IISRBPAct127	Grey-brown	Brown	_	Rectiflexibiles
IISRBPAct128	Greyed- white	Brown	_	Rectiflexibiles
IISRBPAct129	White	White	_	Rectiflexibiles

Table 3. Cultural characteristics of non-*Streptomyces* isolates

Isolate Code	Colour of aerial mycelium	Reversible side colour	Diffusible pigment	Spore chain morphology
***Micromonospora* sp.**				
IISRBPAct2	Grey-white	Greyed- yellow	_	Single spore
IISRBPAct17	Greyed-white	White	_	Single spore
IISRBPAct18	White	Greyed-white	_	Single spore
IISRBPAct27	Grey-brown	Black	_	Single spore
IISRBPAct31	Grey-brown	Blak	_	Single spore
IISRBPAct32	Greyed-white	Brown	_	Spores on short spore chain

IISRBPAct34	White	Brown	_	Single spore
IISRBPAct39	Greyed-white	Greyed-yellow	_	Single spore
IISRBPAct41	Grey- brown	Brown	Brown	Single spore
IISRBPAct71	Brown	Creyed-white	_	Spores on short spore chain
***Actinoplanes* sp.**				
IISRBPAct10	Greyed-white	Greyed-yellow	_	Sporangia
IISRBPAct12	Grey- brown	Brown	_	Sporangia
IISRBPAct20	Grey-brown	Brown	_	Sporangia
IISRBPAct28	Greyed-white	Brown	_	Sporangia
IISRBPAct29	Greyed-white	Greyed-yellow	_	Sporangia on substrate mycelium
IISRBPAct38	Grey-brown	Brown	_	Sporangia on substrate mycelium
IISRBPAct43	Greyed-white	Brown	_	Sporangia
IISRBPAct57	Grey-brown	Brown	_	Sporangia on substrate mycelium
IISRBPAct60	Greyed- white	Creyed white	_	Sporangia
IISRBPAct68	Greyed- white	Greyed-yellow	_	Sporangia
***Nocardiopsis* sp.**				
IISRBPAct70	Greyed- white	Greyed-white	_	Sporulated aerial mycelium
IISRBPAct112	Greyed- white	Creyed-white	_	Characteristic spore chain
***Dactylosporangium* sp.**				
IISRBPAct13	Grey- brown	Greyed-white	_	Sporangia
***Actinomyces* sp.**				
IISRBPAct62	Greyed- white	Creyed white	_	No aerial mycelium
IISRBPAct75	Brown	Greyed- white	_	Limited aerial mycelium
IISRBPAct93	Brown	Brown	_	Only substrate mycelium
***Actinopolyspora* sp.**				
IISRBPAct26	Greyed-white	Greyed-yellow	_	Fragmented aerial hyphae

Table 4. Occurrence and distribution of actinobacteria in black pepper rhizosphere

District	Place n	No of Actinobacterial colonies	Streptomyces sp	Actinoplanes sp	Micromonospora sp	Nocardiopsis sp.	*Actinomyces sp*	*Dactylosporangium.*sp	*Actinopolyspora* sp	Unidentified
Kottayam	Vechoor	3	2		1					
	Thalayalam	2	2							
	Vaikom	3	3							
Waynad	Vellamunda	5	2	2				1		
	Meppadi	7	4	1	2					
	Adikkolli	6	5						1	
	Pulpally	5	1	2	2					
Coorg	Suntikoppa	2	1		1					
	Virajpet	4	3		1					
	Kathalakkadu	1		1						
Malappuram	Perinthalmanna	4	2		2					
Idukki	Venmani	3	2	1						
	Cardamom Research Centre	2	2							
	Pulianmala	0	0							
	Peerumedu	2	2							
	Pampadumpara	0	0							
	Devikulam	1	1							
Kozhikode	Narikkuni	1	1							
	Nanminda	2	2							
	Thalayad	4	3	1						
	Kallanode	3	2	1						
	Kalangali	8	4	1			1			2
Kasaragod	Kanjangad	2	1			1				
Kannur	Taliparamba	2	1		1					
	Panniyur	2	2							
Ernakulam	Kunnathunad	17	16				1			
	Keezhillam	5	4				1			
	Ramamangalam,	5	5							
	Thiruvaniyoor	3	3							
	Mulanthuruthy	4	4							
	Mannur	3	3							
	Maneed	6	5			1				
	Amballoor	1	1							
	Meempara	5	5							
Thrissur	Irinjalakuda	3	3							
	Thalappilly	3	3							

Table 5. *In vitro* evaluation of actinobacteria against major pathogens of black pepper

Isolates	*P. capsici*	*S. rolfsii*	*Collectotrichum*
IISRBPAct1	91.80(73.36)^{A}	94.03(75.87)^{A}	93.70(75.46)^{A}
IISRBPAct2	73.23(58.85)^{NOPQ}	39.63(39.02)^{WXYZ}	50.40(45.23)$^{(2)BCD}$
IISRBPAct3	87.97(69.71)^{BCD}	87.80(69.57)^{BC}	66.63(54.72)^{PQRST}
IISRBPAct4	90.20(71.76)^{AB}	65.93(54.29)^{IJKL}	91.63(73.20)^{AB}
IISRBPAct5	39.87(39.15)$^{(2)MNOPQR}$	54.43(47.54)^{PQRS}	73.33(58.91)^{KLMNO}
IISRBPAct6	32.73(34.89)$^{(2)VWXYZ(3)A}$	24.07(29.37)$^{(2)CDEF}$	41.23(39.95)$^{(2)GHIJ}$
IISRBPAct7	11.97(20.17)$^{(3)JK}$	5.50(13.56)$^{(2)NO}$	12.07(20.32)$^{(2)VW}$
IISRBPAct8	75.90(60.62)^{KLMNO}	53.70(47.12)^{QRST}	36.20(36.98)$^{(2)IJKLMN}$
IISRBPAct9	77.57(61.75)^{JKLMN}	85.57(67.68)^{BCD}	78.73(62.54)^{HIJK}
IISRBPAct10	74.83(59.89)^{MNOP}	55.20(47.99)^{OPQR}	36.67(37.25)$^{(2)IJKLMN}$
IISRBPAct11	91.80(73.36)^{A}	64.43(53.40)^{JKLMN}	85.80(67.87)^{CDEF}
IISRBPAct12	40.93(39.77)$^{(2)LMNOPQ}$	65.57(54.07)^{IJKLM}	52.07(46.18)$^{Z(2)ABCD}$
IISRBPAct13	4.87(12.27)$^{(3)L}$	0.00(0.00)$^{(2)P}$	64.57(53.47)^{RSTUV}
IISRBPAct14	2.13(8.28)$^{(3)M}$	0.00(0.00)$^{(2)P}$	32.07(34.48)$^{(2)LMNO}$
IISRBPAct15	4.87(12.62)$^{(3)L}$	0.00$^{(0.00)(2)P}$	2.73$^{(8.63)(3)BC}$
IISRBPAct16	75.37(60.25)^{LMNOP}	28.50$^{(32.22)(2)BCD}$	81.23(64.34)^{FGHI}
IISRBPAct17	25.63(30.41)$^{(3)CDEF}$	10.00$^{(18.42)(2)KLMN}$	38.30$^{(38.23)(2)IJKL}$
IISRBPAct18	79.73(63.25)^{GHIJKL}	57.07(49.07)^{NOPQ}	82.90(65.68)^{EFGH}
IISRBPAct19	81.93(64.86)^{FGHI}	46.30(42.88)^{STUVWX}	85.80(67.89)^{CDEF}

IISRBPAct20	35.47(36.54)$^{(2)RSTUVWX}$	9.63$^{(18.04)(2)LMNO}$	52.07(46.18Z)$^{(2)ABCD}$
IISRBPAct21	89.57(71.17)^{ABC}	74.80(59.89)^{FGH}	37.07(37.50)$^{(2)IJKLMN}$
IISRBPAct22	63.87(53.06)$^{WXYZ(2)A}$	69.63(56.56)^{GHIJ}	40.00(39.23)$^{(2)HIJK}$
IISRBPAct23	91.80(73.36)^{A}	68.17(55.68)^{GHIJK}	89.57(71.16)^{BC}
IISRBPAct24	78.10(62.11)^{IJKLM}	47.40(43.51)^{RSTUVW}	49.17(44.52)$^{(2)CDE}$
IISRBPAct25	86.30(68.31)^{CDE}	90.37(71.94)^{AB}	87.90(69.65)^{BCD}
IISRBPAct26	22.37(28.22)$^{(3)FG}$	31.83$^{(34.34Z)(2)ABC}$	35.40(36.51)$^{(2)JKLMN}$
IISRBPAct27	32.20(34.56)$^{(2)WXYZ(3)AB}$	46.30(42.88)^{STUVWX}	19.13(25.94)$^{(2)STU}$
IISRBPAct 28	84.67(66.95)^{DEF}	57.43(49.28)^{MNOPQ}	75.80(60.53)^{IJKLM}
IISRBPAct 29	37.30(37.63)$^{(2)QRSTUV}$	24.80$^{(29.87)(2)CDE}$	38.33(38.25)$^{(2)IJKL}$
IISRBPAct 30	87.37(69.19)^{BCD}	87.43(69.25)^{BC}	87.50(69.30)^{CDE}
IISRBPAct31	91.80(73.36)^{A}	84.07(66.49)^{CDE}	72.50(58.37)^{LMNOP}
IISRBPAct32	50.23(45.13)$^{(2)EFGHI}$	63.33(52.74)^{JKLMNO}	31.23(33.91)$^{(2)NOP}$
IISRBPAct33	24.53(29.68)$^{(3)DEF}$	55.20(47.99)^{OPQR}	37.50(37.76)$^{(2)IJKLMN}$
IISRBPAct34	44.23(41.69)$^{(2)JKLMN}$	0.00$^{(0.29)(2)P}$	42.07$^{(40.43)(2)FGHI}$
IISRBPAct35	37.70(37.88)$^{(2)PQRSTUV}$	16.33$^{(23.83)(2)GHIJ}$	37.07$^{(37.50)(2)IJKLMN}$
IISRBPAct36	91.80(73.36)^{A}	51.87(46.07)^{QRSTUV}	85.40(67.54)^{CDEFG}
IISRBPAct37	90.67(72.23)^{AB}	13.70(21.66)$^{(2)HIJKL}$	50.00(45.00)$^{(2)BCD}$
IISRBPAct38	89.03(70.67)^{ABC}	47.80(43.74)^{RSTUVW}	77.50(61.68)^{IJKL}
IISRBPAct39	32.17(34.55)$^{(2)WXYZ(3)AB}$	0.00(0.29)$^{(2)P}$	37.50(37.76)$^{(2)IJKLMN}$
IISRBPAct40	83.03(65.68)^{EFGH}	60.00(50.77)^{KLMNOPQ}	50.40(45.23)$^{(2)BCD}$
IISRBPAct41	32.20(34.56)$^{(2)WXYZ(3)AB}$	0.00(0.29)$^{(2)P}$	37.50(37.76)$^{(2)IJKLMN}$
IISRBPAct42	68.80(56.06)^{QRSTUVW}	94.40(76.31)^{A}	93.70(75.46)^{A}
IISRBPAct43	67.17(55.04)^{STUVWX}	0.00(0.29)$^{(2)P}$	54.13(47.37)$^{YZ(2)ABC}$
IISRBPAct44	21.27(27.45)$^{(3)FGH}$	0.00(0.29)$^{(2)P}$	50.80(45.46)$^{(2)ABCD}$

IISRBPAct45	47.50(43.56)$^{(2)GHIJK}$	30.37(33.43)$^{(2)ABC}$	49.13(44.50)$^{(2)CDE}$
IISRBPAct46	83.60(66.11)^{EFG}	88.90(70.60)^{BC}	65.40(53.97)^{QRSTU}
IISRBPAct47	54.07(47.34)$^{(2)CDE}$	44.40(41.78)^{UVWX}	48.73(44.27)$^{(2)CDEF}$
IISRBPAct48	33.83(35.57)$^{(2)TUVWXYZ}$	22.20(28.11)$^{(2)DEFG}$	34.57(36.00)$^{(2)KLMNO}$
IISRBPAct49	59.53(50.50)$^{Z(2)AB}$	44.03(41.57)^{UVWX}	70.80(57.30)^{MNOPQR}
IISRBPAct50	79.77(63.34)^{GHIJK}	40.37(39.44)^{WXY}	83.75(66.24)^{DEFGH}
IISRBPAct51	71.10(57.49)^{PQRS}	41.47(40.08)^{WXY}	25.40(30.25)$^{(2)PQR}$
IISRBPAct52	37.80(37.94)$^{(2)PQRSTU}$	85.17(67.37)^{BCDE}	54.53(47.60)$^{XYZ(2)ABC}$
IISRBPAct53	28.90(32.51)$^{(3)ABCD}$	28.13(32.02)$^{(2)BCD}$	69.70(56.61)^{NOPQR}
IISRBPAct54	14.43(22.30)$^{(3)IJ}$	36.63(36.11)$^{YZ(2)AB}$	7.23(15.53)$^{(2)XYZ}$
IISRBPAct55	78.90(62.70)^{HIJKLM}	54.77(47.74)^{PQRS}	3.00(6.01)$^{(3)C}$
IISRBPAct56	40.03(39.24)$^{(2)MNOPQR}$	47.77(43.72)^{RSTUVW}	75.77(60.53)^{IJKLM}
IISRBPAct57	65.00(53.73)^{UVWXY}	62.97(52.53)^{JKLMNOP}	24.20(29.44)$^{(2)QRS}$
IISRBPAct58	2.20(6.98)$^{(3)M}$	0.00(0.29)$^{(2)P}$	10.27(18.67)$^{(2)WX}$
IISRBPAct59	45.57(42.45)$^{(2)HIJKL}$	53.67(47.10)^{QRST}	69.07(56.21)^{NOPQRS}
IISRBPAct60	27.77(31.78)$^{(3)BCDE}$	83.67(66.17)^{CDE}	37.00(37.46)$^{(2)IJKLMN}$
IISRBPAct61	35.53(36.58)$^{(2)RSTUVW}$	12.20(20.43)$^{(2)IJKL}$	75.73(60.54)^{IJKLM}
IISRBPAct62	23.90(29.25)$^{(3)EF}$	45.17(42.23)^{TUVWX}	56.33(48.64)$^{WXYZ(2)AB}$
IISRBPAct63	77.77(61.89)^{IJKLM}	68.53(55.88)^{GHIJ}	25.40(30.23)$^{(2)PQR}$
IISRBPAct64	34.43(35.93)$^{(2)STUVWXY}$	0.00(0.29)$^{(2)P}$	80.60(63.89)^{GHI}
IISRBPAct65	69.47(56.47)^{QRSTU}	44.03(41.51)^{UVWX}	61.80(51.83)^{TUVW}
IISRBPAct66	68.33(55.76)^{RSTUVWX}	30.73(33.65)$^{(2)ABC}$	12.07(20.24)$^{(2)VW}$
IISRBPAct67	81.67(64.66F)^{GHIJ}	40.37^{YZ}(39.44)^{WXY}	21.20(27.38)$^{(2)RST}$
IISRBPAct68	63.33(52.75)$^{XYZ(2)A}$	0.00(0.29)$^{(2)P}$	6.67(14.86)$^{(2)XYZ}$
IISRBPAct69	29.43(32.82)$^{(2)Z(3)ABC}$	24.80(29.84)$^{(2)CDE}$	37.60(37.82)$^{(2)IJKLM}$
IISRBPAct70	65.57(54.08)^{TUVWX}	62.93(52.50)^{JKLMNOP}	46.03(42.72)$^{(2)DEFGH}$

IISRBPAct71	30.53(33.53)$^{(2)XYZ(3)AB}$	57.40(49.26)^{MNOPQ}	80.00(63.45)^{HIJ}
IISRBPAct72	45.00(42.12)$^{(2)IJKLM}$	57.03(49.05)^{NOPQ}	57.53(49.34)$^{WXYZ(2)A}$
IISRBPAct73	76.67(61.15)^{KLMNO}	0.00(0.29)$^{(2)P}$	9.63(17.97)$^{(2)WX}$
IISRBPAct74	11.10(19.38)$^{(3)JK}$	17.80(24.94)$^{(2)EFGHI}$	35.17(36.37)$^{(2)JKLMNO}$
IISRBPAct75	1.67(5.99)$^{(3)MN}$	31.10(33.88)$^{(2)ABC}$	24.80(29.86)$^{(2)QR}$
IISRBPAct76	53.33(46.91)$^{(2)DEF}$	22.20(28.11)$^{(2)DEFG}$	0.00(0.29)$^{(3)D}$
IISRBPAct77	12.77(20.75)$^{(3)JK}$	40.00(39.23)^{WXYZ}	74.53(59.72)^{JKLMN}
IISRBPAct78	48.33(44.04)$^{(2)FGHIJ}$	59.63(50.56)^{LMNOPQ}	6.00(11.73)$^{(2)Z(3)AB}$
IISRBPAct79	31.67(34.24)$^{(2)WXYZ(3)AB}$	43.30(41.15)^{VWX}	6.67(14.86)$^{(2)XYZ}$
IISRBPAct80	77.23(61.52)^{KLMNO}	0.00(0.29)$^{(2)P}$	62.40(52.18)^{TUVW}
IISRBPAct81	76.67(61.12)^{KLMNO}	30.37$^{(33.43)(2)ABC}$	42.40$^{(40.62)(2)EFGHI}$
IISRBPAct82	33.33(35.25)$^{(2)UVWXYZ(3)A}$	78.53(62.40)^{EF}	53.30$^{(46.89)YZ(2)ABC}$
IISRBPAct83	66.10(54.40)^{TUVWX}	6.63(14.83)$^{(2)MNO}$	78.80(62.62)^{HIJK}
IISRBPAct84	63.33(52.74)$^{XYZ(2)A}$	64.43(53.40)^{JKLMN}	47.90(43.80)$^{(2)CDEFG}$
IISRBPAct85	18.33(25.34)$^{(3)GHI}$	0.00(0.29)$^{(2)P}$	0.00(0.29)$^{(3)D}$
IISRBPAct86	72.77(58.56)^{OPQR}	38.17$^{(38.15)XYZ(2)A}$	4.83(10.44)$^{(3)AB}$
IISRBPAct87	1.10(3.49)$^{(3)N}$	84.80(67.08)^{BCDE}	31.50(34.10)$^{(2)MNOP}$
IISRBPAct88	4.43(12.10)$^{(3)L}$	10.73(19.09)$^{(2)JKLM}$	69.70(56.61)^{NOPQR}
IISRBPAct89	64.43(53.39)^{VWXYZ}	48.17(43.95)^{RSTUVW}	3.67(9.14)$^{(3)BC}$
IISRBPAct90	30.00(33.19)$^{(2)YZ(3)ABC}$	69.43(56.45)^{GHIJ}	0.00(0.29)$^{(3)D}$
IISRBPAct91	56.63(48.82)$^{(2)BCD}$	0.00(0.29)$^{(2)P}$	65.47(54.01)^{QRSTU}
IISRBPAct92	65.00(53.73)^{UVWXY}	59.63(50.56)^{LMNOPQ}	25.43(30.26)$^{(2)PQR}$
IISRBPAct93	2.20(6.98)$^{(3)M}$	22.20(28.11)$^{(2)DEFG}$	63.00(52.54)^{STUVW}
IISRBPAct94	68.90(56.12)^{QRSTUV}	67.03(54.96)^{HIJKL}	21.20(27.38)$^{(2)RST}$
IISRBPAct95	69.43(56.44)^{QRSTU}	52.57(46.47)^{QRSTU}	76.37(60.94)^{IJKLM}
IISRBPAct96	56.10(48.51)$^{(2)BCD}$	0.00(0.29)$^{(2)P}$	59.37(50.41)^{UVWXY}

IISRBPAct97	60.43(51.03)$^{YZ(2)AB}$	29.27(32.75)$^{(2)BCD}$	23.00(28.58)$^{(2)RS}$
IISRBPAct98	71.10(57.49)^{PQRS}	59.23(50.34)^{LMNOPQ}	73.30(58.89)^{KLMNO}
IISRBPAct99	75.00(60.01)^{MNOP}	55.53(48.18)^{OPQR}	0.00(0.29)$^{(3)D}$
IISRBPAct100	68.33(55.76)^{RSTUVWX}	0.00(0.29)$^{(2)P}$	6.03(13.99)$^{(2)YZ(3)A}$
IISRBPAct101	17.23(24.49)$^{(3)HI}$	67.43(55.21)^{GHIJKL}	77.60(61.76)^{IJKL}
IISRBPAct102	43.33(41.17)$^{(2)JKLMNO}$	80.37(63.73)^{DEF}	58.17(49.71)^{VWXYZ}
IISRBPAct103	58.87(50.11)$^{(2)ABC}$	46.27(42.85)^{STUVWX}	4.20(9.56)$^{(3)BC}$
IISRBPAct104	71.13(57.54)^{PQRS}	15.57(23.21)$^{(2)GHIJK}$	66.63(54.73)^{PQRST}
IISRBPAct105	38.87(38.57)$^{(2)OPQRST}$	47.03(43.30)^{RSTUVW}	7.83$^{(16.16)(2)XY}$
IISRBPAct106	43.90(41.49)$^{(2)JKLMNO}$	74.83(59.91)^{FGH}	16.97$^{(24.30)(2)TU}$
IISRBPAct107	80.00(63.48)^{GHIJK}	64.07(53.18)^{JKLMN}	65.47(54.02)^{QRSTU}
IISRBPAct108	52.20(46.26)$^{(2)DEFG}$	5.90(13.51)$^{(2)NO}$	29.03(32.60)$^{(2)OPQ}$
IISRBPAct109	72.77(58.56)^{OPQR}	66.30(54.52)^{IJKL}	61.80(51.83)^{TUVW}
IISRBPAct110	76.10(60.77)^{KLMNO}	64.07(53.18)^{JKLMN}	72.70(58.51)^{LMNOP}
IISRBPAct111	33.90(35.60)$^{(2)TUVWXYZ}$	75.20(60.16)^{FG}	67.27(55.12)^{OPQRST}
IISRBPAct112	50.53(45.31)$^{(2)EFGH}$	10.37(18.76)$^{(2)KLM}$	12.07(20.24)$^{(2)VW}$
IISRBPAct113	68.33(55.76)^{RSTUVWX}	0.00(0.29)$^{(2)P}$	15.17(22.91)$^{(2)UV}$
IISRBPAct114	31.67(34.24)$^{(2)WXYZ(3)AB}$	20.00(26.53)$^{(2)EFGH}$	81.20(64.34)^{FGHI}
IISRBPAct115	66.10(54.40)^{TUVWX}	75.20(60.16)^{FG}	25.40(30.23)$^{(2)PQR}$
IISRBPAct116	68.90(56.11)^{QRSTUV}	65.57(54.07)^{IJKLM}	56.37(8.66)$^{WXYZ(2)AB}$
IISRBPAct117	18.90(25.75)$^{(3)GH}$	75.20(60.16)^{FG}	69.67(56.60)^{NOPQR}
IISRBPAct118	68.90(56.11)^{QRSTUV}	55.20(47.99)^{OPQR}	61.20(51.48)^{TUVWX}
IISRBPAct119	1.67(5.99)$^{(3)MN}$	70.37(57.02)^{GHIJ}	71.47(57.72)^{MNOPQ}
IISRBPAct120	39.47(38.91)$^{(2)NOPQRS}$	78.53(62.40)^{EF}	74.53(59.72)^{JKLMN}
IISRBPAct121	77.23(61.52)^{KLMNO}	65.93(54.29)^{IJKL}	70.90(57.47)^{MNOPQ}

IISRBPAct122	65.00(53.73)^{UVWXY}	0.00(0.29)$^{(2)P}$	61.80(51.83)^{TUVW}
IISRBPAct123	42.77(40.84)$^{(2)KLMNOP}$	79.27(62.92)^{DEF}	72.13(58.21)^{LMNOP}
IISRBPAct124	33.90(35.60)$^{(2)TUVWXYZ}$	73.33(58.92)^{FGHI}	61.80(51.83)^{TUVW}
IISRBPAct125	67.23(55.09)^{STUVWX}	31.83(34.34)$^{Z(2)ABC}$	75.77(60.56)^{IJKLM}
IISRBPAct126	79.43(63.10)^{GHIJKL}	5.53(13.15)$^{(2)O}$	58.17(49.72)^{VWXYZ}
IISRBPAct127	70.00(56.81)^{QRST}	17.40(24.62)$^{(2)FGHI}$	0.00(0.29)$^{(3)D}$
IISRBPAct128	18.33(25.34)$^{(3)GHI}$	70.00(56.80)^{GHIJ}	59.37(50.41)^{UVWXY}
IISRBPAct129	10.53(18.86)$^{(3)K}$	44.43(41.80)^{UVWX}	20.00(26.52)$^{(2)RSTU}$

Notes: Data are mean of three replications. Figures in parentheses are arc sine transformed values. Data followed by the same letter in a column are not significantly different (p = 0.05) from each other according to DMRT.

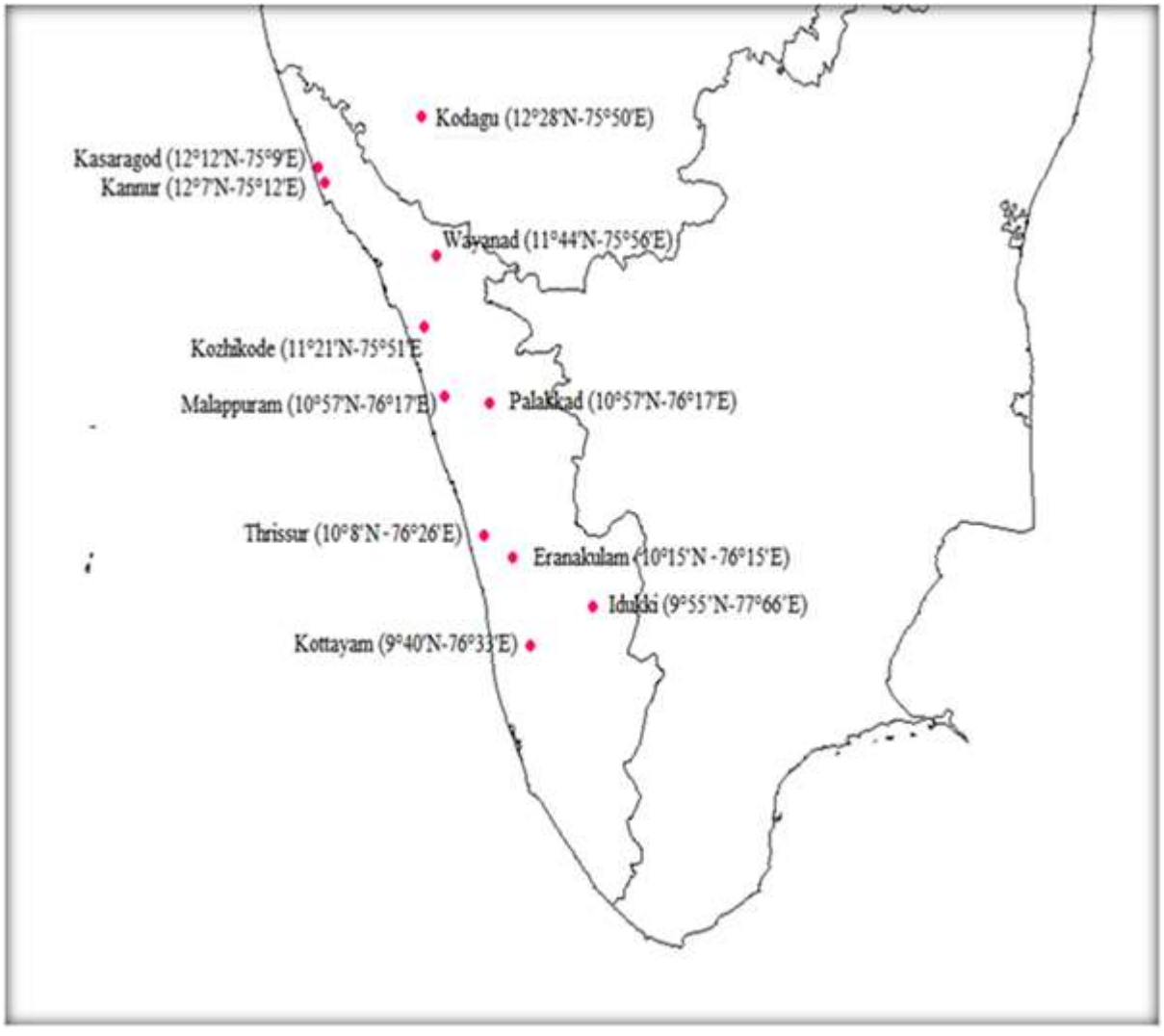

Fig. 1 Map showing Kerala and parts of Karnataka for soil sample collection

Fig 2. Colony morphology *Streptomyces* isolates on ISP2 (A) IISRBPAct45 (B) IISRBPAct47 (C) IISRBPAct5 (D) IISRBPAct9 (E) IISRBPAct16 (F) IISRBPAct 21 (G) IISRBPAct35 (H) IISRBPAct40 (I) IISRBPAct44 (J) IISRBPAct1 (K) IISRBPAct25 (L) IISRBPAct3 (M) IISRBPAct15 (N) IISRBPAct50 (O) IISRBPAct 73 (P) IISRBPAct42.

Fig 3. Colony morphology of non- *Streptomyces* isolates on ISP2 *Actinomyces* sp. (A) IISRBPAct75. *Actinopolyspora* sp. (B) IISRBPAct26. *Dactylosporangium* sp. (C) IISRBPAct 13. *Nocaridopsis* sp. (D) IISRBPAct112. *Actinoplanes* sp. (E) IISRBPAct60, (F) IISRBPAct 68, (G) IISRBPAct62. (H) IISRBPAct28 *Micromonospora* sp. (I) IISRBPAct2 (J) IISRBPAct41 (K) IISRBPAct39 (L) IISRBPAct27 (M) IISRBPAct34 (N) IISRBPAct18 (O) IISRBPAct 17 (P) IISRBPAct27.

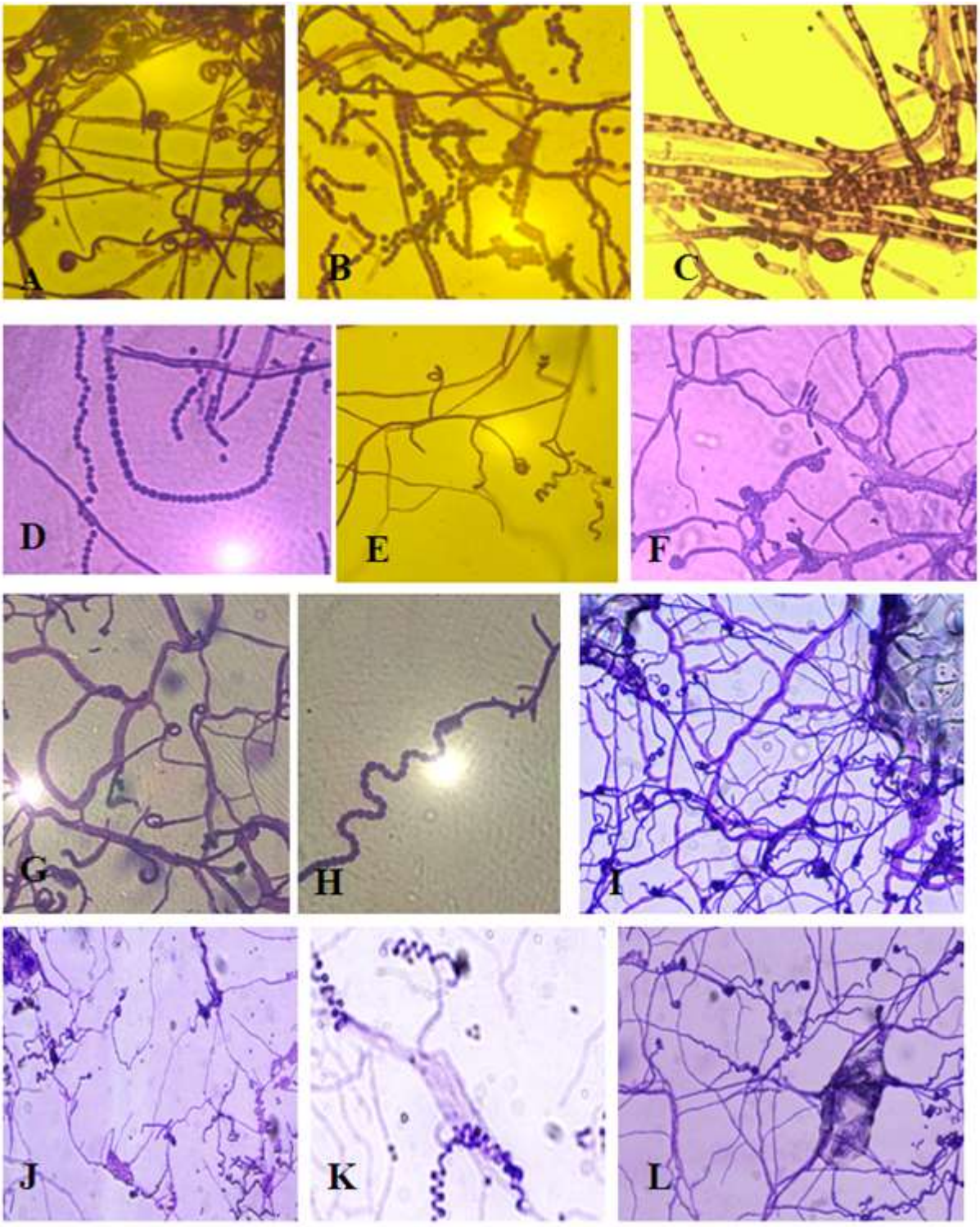

Fig 4 Microscopy of *Streptomyces* under light microscopy (A) IISRBPAct30 spira type (B) IISRBPAct21 *Rectiflexibiles* type (C) IISRBPAct16 Arthrospore formation in mycelia (D) IISRBPAct46 *Rectiflexibiles* type (E) IISRBPAct54 Spira type (F) IISRBPAct 73-closed spira (G) IISRBPAct36-closed spira (H) IISRBPAct40- *Retinaculiaperti* type (I) IISRBPAct44- *Retinaculiaperti* type (J) IISRBPAct101- spira (K) IISRBPAct25- spira (L) IISRBPAct3- spira

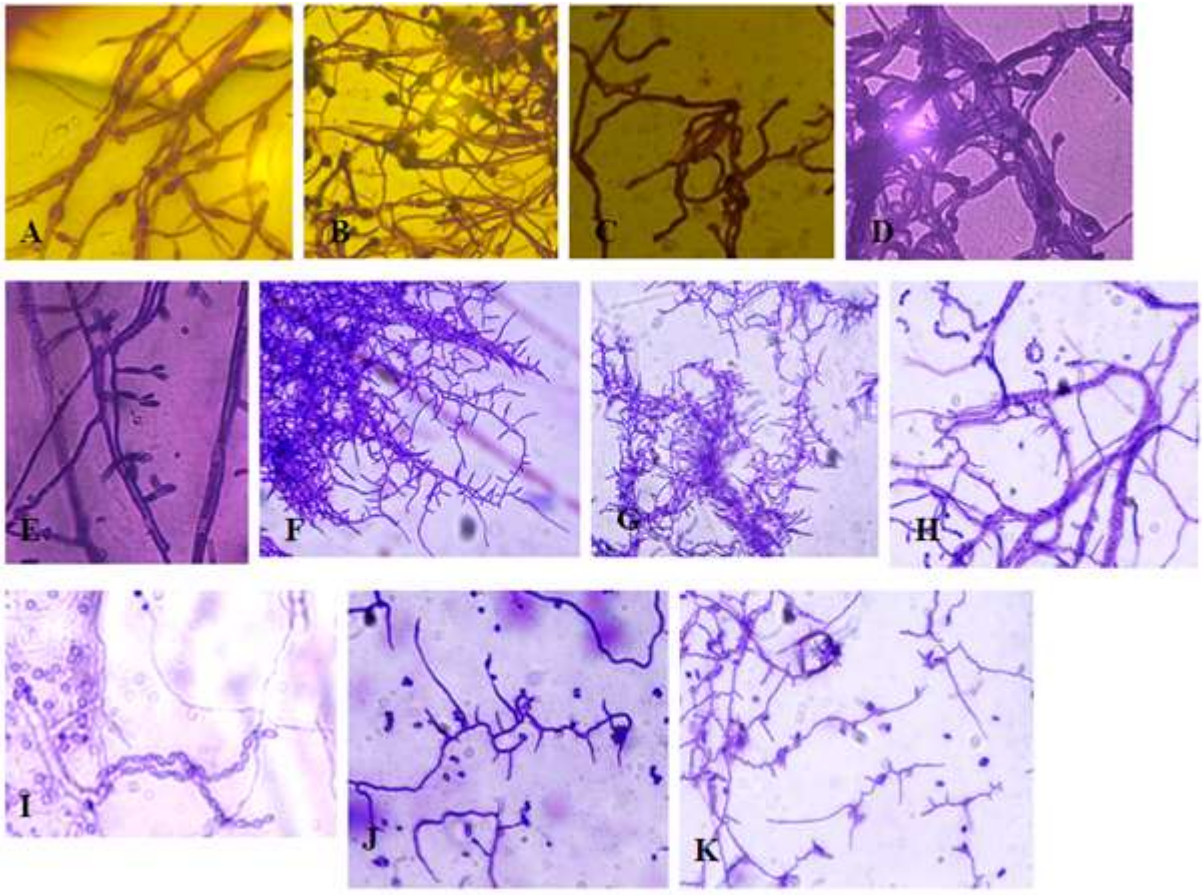

Fig 5 Microscopy of non - *Streptomyces* the isolates *Actinoplanes* sp. (A) IISRBPAct12, (B) IISRBPAct 28, (C) IISRBPAct57 (D) IISRBPAct20 (Sporangia formation on aerial and substrate mycelium) *Dactylosporangium* sp. (E) IISRBPAct13 (oligosporous, claviform sporangia developed on aerial mycelium). *Actinomyces* sp. (F) IISRBPAct 93 (G) IISRBPAct62 (Substrate mycelium) *Actinopolyspora* sp (H) IISRBPAct26 (long chains of spores on aerial hyphae), *Nocaridopsis* sp. (I) IISRBPAct112 (Fragmenting branched aerial hyphae) *Micromonospora* sp. (J) IISRBPAct27, (K) IISRBPAct71 (hyphae along with single, non-motile and smooth spores).

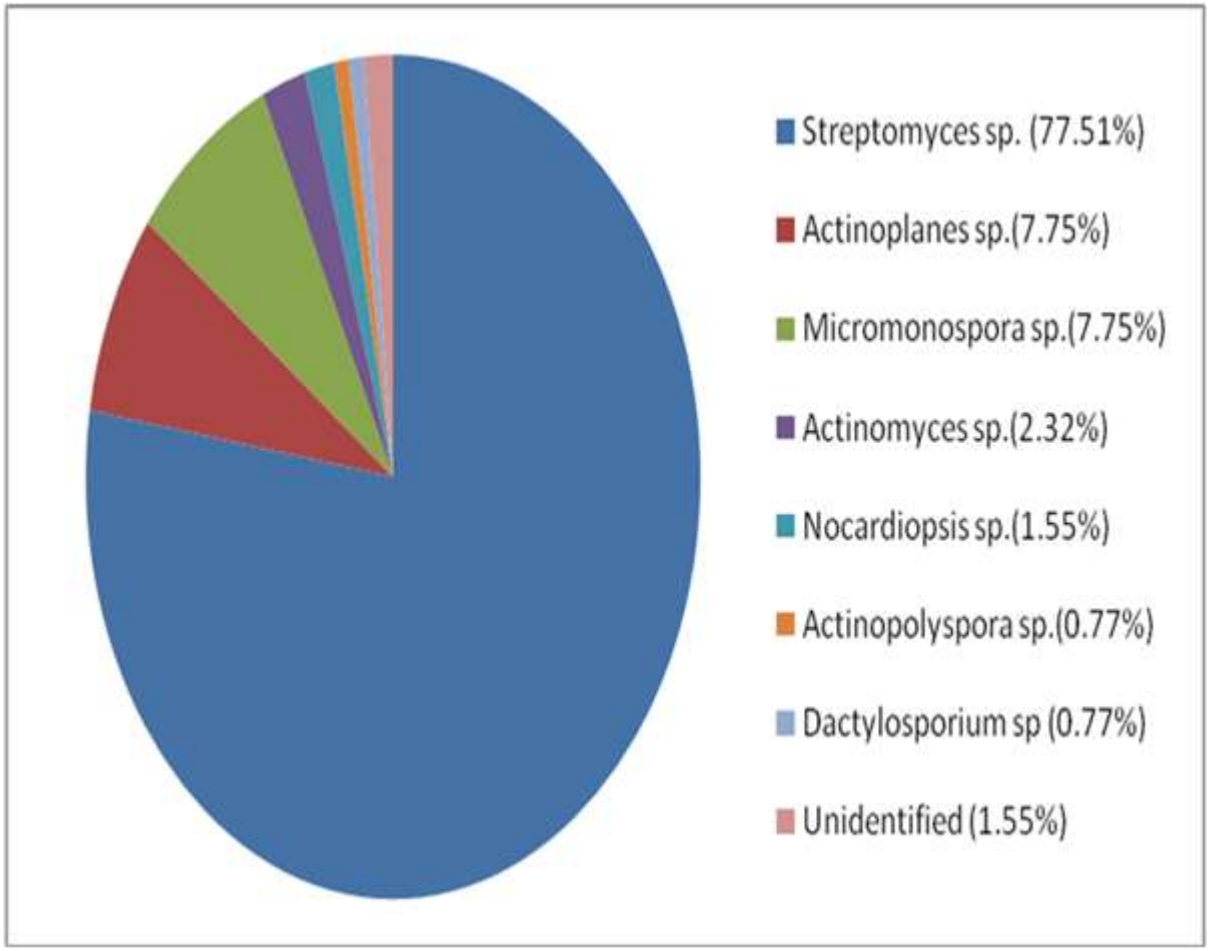

Fig 6. Percentage frequency of isolated actinomycete genera

DISCUSSIONS

The black pepper cultivation is mainly concentrated to the Southern States *i.e.*, Kerala and Karnataka. Microorganisms in the rhizosphere region of the black pepper play an important role in its growth and development through nutrient regeneration by biological processes either decomposition or by fixing the nitrogen and phosphorous. Evaluation of microbial diversity in the rhizosphere ecosystem of black pepper is not attempted so far. The present study has been undertaken to find out the diversity and function of actinobacteria in the rhizosphere soil of black pepper.

Actinobacteria especially *Streptomyces*, have been reported from rhizosphere region of paddy field (Priyadharsini *et al*., 2015). *Streptomyces* was the most predominant genera recorded in the present study when compared to other genera. The superiority of *Streptomyces* among the actinobacteria especially in soils has also been described by many workers (Dhanasekaran *et al.* 2009; Vijayakumar *et al*., 2007; Moncheva *et al*. 2002; Balagurunathan *et al*., 1996; Mansour *et al*., 2003; Sharma *et al*., 2012; Sirisha *et al*., 2013). The frequency of the genus *Streptomyces* was 57.4% which is lower as compared to our studies (77.51%), followed by *Actinopolyspora* (14.7%), *Saccharopolyspora* (10.3%), *Actinomadura* (5.9%), *Nocardiopsis* (4.4%) and *Micromonospora* (2.9%). The other genera such as *Microbispora*, *Actinomyces* and *Actinoplanes* were recorded in low percentage of frequency (1.5%). The superiority of *Streptomyces* sp. other than soil habitat was proved by Jami *et al* in 2015, They revealed

significant actinobacterial diversity in the fresh water fish gut microbiota based on classical cultivation techniques, repetitive sequence-based PCR (rep-PCR), 16S rDNA sequencing succeeded by phylogenetic analysis. In their study, 72% of the total isolates belong to the family *Streptomycetaceae*. The most leading group could be allocated to the family of *Pseudonocardiaceae* followed by the members of *Micromonosporaceae*. Besides *Streptomyces*, the non-*Streptomyces* genera most commonly appeared on media were *Actinopolyspora*, *Saccaharopolyspora*, *Actinomadura*, *Nocardiopsis*, *Micromonospora*, *Actinoplanes* and *Microbispora* and *Actinomyces*. The predominance of *Streptomyces* was also found in deep waters of Arctic origin and deep-sea coral reef invertebrates (Sarmiento-Vizcaíno *et al.,* 2017). Actinobacteria isolated from species of gorgonian corals (Zhang *et al*., 2013) where *Streptomyces* and *Micromonospora* were the dominant ones. In actinobacterial strains isolated from male cones of Scots pine trees (Pinus sylvestris) also, more frequent representative strains were *Streptomyces* (Axenov-Gribanov *et al*., 2016).

During the study of endophytic actinobacterial diversity in the native herbaceous plant species of Korea, Kim *et al*., 2012 found that the genus *Streptomyces* comprised 45.9% of the total isolates, followed by *Micromonospora* (18.8%), *Rhodococcus* (6.6%), *Microbispora* (4.9%), and *Micrococcus* (4.9%). Most of the isolates showed hydrolytic enzyme activity and antagonistic activity against at least one fungal pathogen *Phytophthora capsici, Rhizoctonia solani, Colletotrichum gloeosporioides*, *Fusarium solani* and *Alternaria alternata.* They got two *Micromonospora* spp. that showed antagonistic activity towards *Colletotrichum gloeosporioides.* In our study also we got five *Micromonospora sp*. which showed more than 50% of inhibition towards *P. capsici*, two showed more than 50% inhibition against *S. rolfsii* and five showed more than 50% *inhibition* against *C. gloeosporioides*. According to Gangwar *et al*., 2014, endophytic *Micromonospora* sp. isolated from medicinal plants showed 16% inhibition, *Streptomyces albosporus* O11 showed 53.5% of inhibition, and *Saccharopolyspora* O9 showed 56.4% of inhibition towards *Phytophthora drechsleri*.
In vitro evaluation method was used as the primary screening procedure for selecting the potential isolates (Awla *et al*., 2017; Xue *et al*., 2013. Among the non-*Streptomyces*, *Actinoplane* sp. also showed antifungal activity. According to El-Tarabily (2009), *Actinoplanes capanulatus* showed 32.30mm diameter of clear zone of inhibition against *Pythium aphanidermatum*, a soil borne pathogen belongs to oomycetes. Here four *Actinoplanes* sp. showed more than 50% inhibition towards the oomycete *P. capsici*. *Actinoplanes philippinensis* exhibited only 11.35mm of lesion diameter on performing *in vitro* bioassay against *Pythium coloratum* while in control; it was 16.5mm (El-Tarabily *et al*., 1997). Sabu *et al*. 2017 reported the antimicrobial activity of endophytic *Nocardiopsis* sp. from *Zingiber officinale* rhizome towards *Pythium myriotylum*, *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholerae* and *Bacillus substilis*. In our studies, two *Nocardiopsis* sp. showed more than 50% inhibition towards *P. capsici*.
Kawato and Shinobu (1979) used cover slip culture method for characterizing actinobacteria morphologically along with many biochemical tests. Hensyl *et al*., 1994 provided the morphological features of *Streptomyces* as white and yellowish grey coloured and light yellowish brown coloured reverse side on ISP media without diffusible pigments. The *Actinopolyspora* sp with elongated and circular shaped 20-30 spore chain structures was observed by Xiao-Yang *et al.* (2000). In the present study, the isolates were characterized based on spore chain morphology. Kokare *et al* (2004a) characterized four isolates of *Acinopolyspora* (*A. halophila, A. mortivalis, A. iraqiensis* and

Actinopolyspora sp. AH1) using slide culture technique and scanning electron microscopy.

Morphological, microscopical, biochemical and physiological characters are used for characterizing and identifying *Streptomyces* sp. (Augustine *et al.,* 2004 ; Dhanasekaran *et al.,* 2005a; Narayana *et al.,* 2005; Augustine and Kapadnis, 2005; You *et al.,* 2005; Dhevagi and Poorani, 2006). Panaiyadiyan and Chellaia (2011) followed colony morphology (color of aerial spore mass, reverse side and diffusible pigments) and microscopic morphology (spore, sporangium) for the characterization and identification of actinobacteria.

Subhasish *et al (*2013) characterized and identified *Nocardiopsis* sp. from Namakkal and Tiruchirappalli soil samples using morphological, biochemical and molecular methods. Upon the Phase contrast microscopic study of *Nocardiopsis* sp. (Vimal *et al*., 2009) the spores are smooth, appeared in long chain and oblong in shape. The same type of spore chain morphology was observed in IISRBPAct112, the *Nocardiopsis* sp. obtained in our study. Chiaraphongphon *et al*., 2010, isolated a novel *Dactylosporangium* sp. from tropical forest soil in Nakhon Sawan Province, Thailand and found that the strain possessed finger-shaped sporangia on short sporangiophores that developed directly from substrate hyphae under scanning electron microscopy. Using light microscopy, we also identified the same structure in IISRBPAct13. Morphological characterization of *Micromonospora* sp. (Thawai *et al*., 2005), showed the colonies are yellowish white and turn greyish black after sporulation in ISP2 medium. Single spores are formed on substrate hyphae and aerial mycelium was not well developed. In our studies, we got the *Micromonospora* cultures that exhibited aerial mycelial mass colour ranges from Greyed-white to Greyed-brown while substrate mycelium found in Greyed-yellow, brown or black.

Morphological features such as aerial colour mass, substrate mycelium, melanoid pigments, and spore chain morphology are critical in the genus level identification of the *Streptomyces* sp. According to the type of spore chain observed under light microscopy, the isolates were categorized as Rectiflexibiles where spores are straight chains, Spira which is spiral in shape may be closed or opened or Retinaculiaperti as compact coils or extended open coils. Taddei *et al*., in 2006 studied morphological characters of 71 isolates retrieved from soil samples and found different strains of *Streptomyces* sp. with different spore chain morphology. In our study also a number of *Streptomyces* sp. were identified only with the spore chain morphology. Different type of sporangial formation especially on substrate mycelia are the main characteristic feature of *Actinoplanes* sp. (Li *et al*., 2016). Sporangum having sac like structure in which the spore become matured. In our study, 10 isolates were categorized under *Actinoplanes* sp. based on the development of sporangia. *Actinomyces* sp. is characterized by their high degree of branching of the vegetative hyphae and the only genus associated with human normal microbial flora (Sarkonen *et al*., 2001). We categorized three isolates for the genus because of the true branching of the vegetative mycelium.

From our study on occurrence of different genera of *Actinobacteria* in different rhizosphere soil samples of black pepper, it was observed that Wayanad soil samples are richer in actinobacterial diversity compared to the other districts of Kerala. According to Gopal *et al*., 2015, actinomycetes diversity was less; only *Streptomyces* sp. was isolated from rhizosphere of major horticultural crops. According to Hamedani *et al* (2012) forest and lake soils of Wayanad maintain a diverse population of potent actinomycetes. They isolated *Streptomyces lomondensis* strain CK63 having exceptional

enzymatic potential and antibacterial properties that would found a variety of industrial and pharmaceutical applications. Smitha *et al*., in 2016**,** isolated *Streptomyces sp*, *Nocardia sp.* and *Pseudonocardia sp*. with phosphate solubilizing capability from Wayanad District and thus found that Wayanad is a good region of biodiversity and has been sufficiently acceptable due to its immense floral diversity and also microbial diversity.

CONCLUSION

The present study showed that, the black pepper rhizosphere in Southern states of India is eminently a suitable ecosystem for the diversity of potential actinobacteria which could further enhance crop yield and quality. Though there are 129 isolates of actinobacteria reported in the present study, it does not give a complete picture of actinobacterial diversity. It needs periodic visits to the field, isolation from different substrates collected from the surroundings and the usage of different media.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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