Applied Biological Research 20(3): 234-243; (2018)
DOI: 10.5958/0974-4517.2018.00032.0

In vitro BIOEFFICACY OF RHIZOBACTERIA, ISOLATED FROM WALNUT (*Juglans regia* L.) RHIZOSPHERE IN NORTH-WESTERN HIMALAYAS, AGAINST FIVE FUNGAL PHYTOPATHOGENS

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(Received 11 January, 2018; accepted 24 June, 2018)

ABSTRACT

The present study was aimed to assess the bioefficacy of rhizobacteria isolated from walnut (*Juglans regia*) grown in North Western Himalayas against five fungal phytopathogens so that they may, in future, be exploited as biocontrol agents against fruit crop diseases. Ninety eight rhizobacterial isolates were isolated from walnut rhizosphere in major walnut growing areas of Kashmir. The siderophore & HCN production and chitinase activity were detected in 53, 82 and 35 isolates, respectively. *In vitro* assessment of rhizobacteria for biocontrol efficacy against 5 fungal pathogens *viz., Dematophora necatrix, Alternaria solani, Pythium aphanidermatum, Fusarium oxysporum* and *Phytophthora capsici* revealed that most of the rhizobacterial isolates exhibiting growth inhibition of various fungal pathogens belonged to the genus *Bacillus*. In present study, 23, 20, 19, 21 and 20 isolates were found antagonistic to *D. necatrix, A. solani, F. oxysporum, P. aphanidermatum* and *P. capsici,* respectively, with maximum inhibition in each respective case by isolate WI 90 (66%), WI 63 (55.6%), WI 62 (43.8%), WI 63 (45.5%) and WI 65 (49%). Twelve most effective isolates were morpho-biochemically and molecularly characterized and on the basis of 16S rDNA sequencing were identified as *Bacillus licheniformis* (isolates WI 90, *Bacillus subtilis* (isolates WI 63 and WI 65), *Bacillus tequilensis* (isolate WI 62), *Bacillus cereus* (isolate WI 36), *Micrococcus luteus* (isolates WI 12, WI 41 and WI 80), *Micrococcus yunnanensis* (isolates WI 30 and WI 60) and *Micrococcus* sp. (isolates WI 11 and WI 91).

Key words: Biocontrol agents, fungal phytopathogens, PGP attributes, rhizobacteria, walnut

INTRODUCTION

The Persian walnut (*Juglans regia* L.) is a temperate deciduous broadleaf tree which is considered native to South-East Europe to South-West China. It is an important nut tree grown in North Western Himalayan belt expanding from Darjeeling and Sikkim to Jammu & Kashmir in India. The Jammu and Kashmir State is a major producer of walnut in India covering an area of 89,339 ha with a production of 2,66,280 metric tonnes (Anonymous, 2016). Walnut cultivation is generally confined to less wet or dry sites adjacent to undulating forests and the plant is grown at an altitude of 900-3550 m masl (Wani *et al.,* 2014). Due to nutritionally poor soils, highly fragile environment and undulating topography in North-West Himalayas, walnut plant is often subject to biotic and abiotic stress which impairs its growth and yield. Reports reveal that the total microbial population,

root exudates and microbial biomass C and N in walnut rhizospheric soils decreased remarkably because of prevailing drought and other stress conditions (Liu *et al.*, 2014). The rhizosphere of a plant is a microecological zone in direct proximity with plant roots. About 15% root surface area is generally covered by rhizosphere-specific microbes so provide many sites for biological interactions (Berendsen *et al*., 2012).

A range of interactions from symbiotic relationships to deleterious interactions do occur in rhizosphere (Beattie, 2006). Plant growth promoting rhizobacteria (PGPR) promote growth through production of growth regulators or through solubilization of mineral phosphates/other nutrients or fixation of atmospheric nitrogen or through antagonistic action against phytopathogenic microbes by siderophores, antibiotics and cyanide production (Sarvanakumar *et al.*, 2007; Chen, 2011). PGPR belong to diverse genera especially *Alcaligenes, Azospirillum, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia* which are capable of exerting beneficial influence on plant growth (Aravind *et al*., 2009). Some rhizobacteria show the ability to antagonize phytopathogens through competition, antibiotics production or lytic enzymes secretion (Van Loon and Bakker, 2003) that make them a potent tool for reducing damages through prevention of deleterious effects of phytopathogens.

Amongst the temperate fruit trees, the rhizosphere of pome fruits *viz*., apple, pear, cherry, apricot, etc. have extensively been explored (Preeti Mehta *et al.*, 2015; Shweta Gupta *et al*., 2016) while the rhizosphere of nut crops like walnut has rarely been studies (Dar *et al*., 2009; Shiwani Guleria *et al.,* 2014). Walnut trees exhibit allelopathy so only some specific types of microbes thrive under its tree canopy thus it is expected to harbour quite different microbial flora than those observed other temperate fruit crops. Zang *et al.* (2015) identified 11 strains of phosphate-solubilizing bacteria in walnut rhizosphere by 16 S rDNA which belonged to 5 genera namely *Pseudomonas*, *Staphylococcus*, *Planomicrobium*, *Microbacterium*, and *Acinetobacter*. Fungal pathogens cause many diseases in horticultural crops so influence the quality and yield of fruits (Kader, 2002). Chemical control involves health hazards and it is advocated to minimize its use through integrated disease management including the use of biocontrol agents. Dalal and Kulkarni (2013) isolated 31 bacteria belonging to *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Acetobacter*, *Burkholderia*, *Rhizobium* and *Xanthomonas* from soybean and screened them *in vitro* for antagonistic activity against soil-borne fungal pathogens *viz*., *R. solani, F. oxysporum, Sclerotium rolfsii, Collectotrichum truncatum, Macrophomina phaseolina* and *Alternaria alternata*. Five isolates, JDB 3 (*Pseudomonas* spp.), JDB 5 (*Pseudomonas* spp.), JDB 9 (*Bacillus* spp.), JDB 11 (*Bacillus* spp.) and JDB 14 (*Bacillus* spp.) were found to exhibit maximum number of PGP traits *viz*., production of plant growth regulators (auxins, gibberellins and cytokinins), siderophores and HCN, so were considered efficient isolates possessing dual abilities i.e. antagonistic and plant growth promotion. Geetha *et al.* (2014) isolated and screened 180 PGPR strains from green gram rhizosphere for their antifungal activity against *M. phaseolina, Colletotrichum capscici, R. solani* and *F. oxysporum.* Perusal of literature has revealed that with the exception of a preliminary report (Dar *et al.,* 2009), no studies on rhizosphere microbes of walnut with respect to their biological control attributes have been conducted in Western Himalayas especially in Jammu & Kashmir state. Hence, the present study was aimed to assess rhizobacteria, isolated from walnut rhizosphere in Western Himalaya, for their biocontrol ability against five plant pathogenic fungi.

MATERIALS AND METHODS

The rhizosphere soil samples along with root samples were collected in June 2015 from the canopy of young actively growing walnut trees from major walnut growing areas of Kashmir valley spread over four districts namely Kupwara, Baramulla, Budgam and Shopian. In each district three commercially walnut growing blocks were selected and in each block three sites chosen for the

purpose. Composite rhizospheric soil samples (1 g) from each site were serially diluted (10^3-10^7) and 0.1 mL suspension spread on pre-poured nutrient agar medium. The plates were incubated at 25±1°C for 48 h. The isolated colonies on nutrient agar medium were replica plated (Roberts, 1959) onto selective media *viz*., CAS medium for assessing their siderophore producing ability qualitatively and quantitatively (Schwyn and Neilands, 1987), King's B medium amended with 4.4 g L^{-1} glycine for hydrogen cyanide production (Baker and Schippers (1987) and chitinase assay as per Robert and Selitrennikoff (1988). The percent siderophore was calculated as under:

$$\text{Per cent siderophore unit} = (A_r - A_s) \div A_s \times 100$$

Wherein A_r and A_s show the absorbance of reference and test isolates, respectively.

For HCN production, the isolates were streaked on King's B medium amended with 4.4 g L^{-1} glycine. Whatman No.1 filter paper discs were dipped in 0.5% picric acid in 2% sodium carbonate solution, placed in the lid of each Petriplate and sealed. Plates were incubated for 4 days at 28±2°C. Colour change of filter paper from deep yellow to orange and orange to brown indicated the production of HCN.

The isolates were characterized for various morpho-biochemical properties as per *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994). On the basis of initial screening, the most efficient isolates were characterized at molecular level on the basis of 16S rRNA sequencing (Dar *et al.*, 2018).

For assessing the antifungal activity of rhizobacterial isolates, five fungal pathogens *viz*., *Dematophora necatrix*, *Alternaria solani*, *Pythium aphanidermatum*, *Fusarium oxysporum* and *Phytophthora capsici* were procured from the Division of Plant Pathology, SKUAST-Kashmir, Srinagar (J&K). To assess bioefficacy of rhizobacterial antagonists, a loopful of 48 h old culture of each isolate was streaked a little below the centre of pre-poured Petri-plates containing malt extract agar (MEA) medium and then incubated overnight at 37°C. The mycelial discs of 4 days old culture of test fungal pathogens were placed simultaneously on one side of the streak. A check inoculated with test pathogens alone was also maintained for comparison. The experiment was conducted in a completely randomized design with each treatment replicated four times. The plates were incubated at 24±1°C for 7 days and mycelial growth inhibition calculated as per Vincet (1947).

$$I = \frac{C-T}{C} \times 100$$

wherein I is % growth inhibition and C and T are fungal growth in control and treatments, respectively.

The data was analyzed statistically using analysis of variance technique (Narayanan and Adorisio, 1983). The significance of treatments was tested at 5% probability level and treatment values compared using Duncan's multiple range test as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The rhizobacterial population in soil samples, collected from the selected sites in Western Himalayan state of Jammu & Kashmir and raised on nutrient agar medium, ranged from 25.0 x 10^5 to 95.0 × 10^5 cfu g^{-1}. Lamado *et al.* (2013) have suggested that rhizosphere bacterial population can vary between 7.3 x 10^4 to 1.3 x 10^5 cfu g^{-1}. Isolation of different bacterial groups from rhizosphere samples from different plants on specific media have been reported earlier by Ahmed *et al*. (2014).

Of the 98 morphologically dissimilar bacterial isolates, isolated from walnut rhizosphere in present study, 65 bacterial isolates were Gram positive rods and had variable colony morphology. On the basis of morpho-biochemical characters, 65 isolates were found to belong to genus *Bacillus*, 12 to genus *Azotobacter*, 17 to genus *Micrococcus* and 4 to *Pseudomonas***.** These findings are supported by Vedan *et al*. (2010) who reported predominance of *Bacillus* sp. and *Pseudomonas* sp.

Table 1: Siderophore, chitinase and HCN production by the rhizobacterial isolates, isolated from walnut rhizosphere in North-Western Himalayas

Siderophore production [SP] (%siderophore unit)				Chitinase activity [CA] (units mL^{-1})				Hydrogen cyanide production [HCNP]*					
Walnut isolates	SP	Walnut isolates	SP	Walnut isolates	CA	Walnut isolates	CA	Walnut isolates	HCNP	Walnut isolates	HCNP	Walnut isolates	HCNP
WI 1	4.75	WI 52	12.80	WI 2	11.21	WI 51	9.08	WI 1	+	WI 35	+	WI 71	+
WI 2	8.20	WI 54	10.80	WI 3	9.80	WI 52	13.17	WI 2	++	WI 36	+++	WI 72	+
WI 3	10.42	WI 56	13.90	WI 4	12.43	WI 53	14.55	WI 3	++	WI 37	+	WI 73	++
WI 4	12.38	WI 58	13.00	WI 11	24.05	WI 54	15.76	WI 4	+++	WI 38	+	WI 74	+
WI 5	8.60	WI 60	16.50	WI 12	18.2	WI 60	19.75	WI 5	++	WI 41	++	WI 75	+
WI 8	12.50	WI 62	22.30	WI 13	10.88	WI 62	25.33	WI 6	+	WI 42	+	WI 76	+
WI 9	14.20	WI 63	16.60	WI 14	9.65	WI 63	30.47	WI 7	+++	WI 44	++	WI 77	+
WI 11	18.66	WI 65	18.83	WI 15	20.13	WI 65	25.14	WI 9	++	WI 45	+	WI 78	+
WI 12	27.21	WI 67	12.00	WI 16	17.88	WI 73	10.92	WI 11	++	WI 46	+	WI 79	+
WI 13	14.80	WI 68	14.26	WI 17	12.00	WI 80	17.67	WI 12	++++	WI 47	+	WI 80	++
WI 14	12.30	WI 70	7.70	WI 18	8.90	WI 81	13.45	WI 13	++	WI 48	+	WI 81	+
WI 16	14.50	WI 72	7.60	WI 30	16.45	WI 82	10.23	WI 14	+++	WI 49	+	WI 82	+
WI 18	14.20	WI 74	9.80	WI 36	18.30	WI 83	12.75	WI 15	++	WI 50	+	WI 83	+
WI 19	10.70	WI 76	9.60	WI 37	11.36	WI 84	14.98	WI 16	++	WI 51	+	WI 84	+
WI 22	10.20	WI 78	11.20	WI 38	9.46	WI 89	16.15	WI 17	++	WI 52	+	WI 85	++
WI 24	13.65	WI 79	11.50	WI 41	21.03	WI 90	23.18	WI 18	++	WI 53	+	WI 86	+
WI 27	9.20	WI 80	16.00	WI 44	19.00	WI 91	17.50	WI 19	+++	WI 54	+	WI 87	+
WI 30	17.70	WI 82	10.00	WI 50	11.05	-	-	WI 20	++	WI 57	+	WI 88	+
WI 33	14.64	WI 83	8.50	-	-	-	-	WI 21	++++	WI 58	+	WI 89	+
WI 36	17.50	WI 85	12.33	-	-	-	-	WI 22	+++	WI 59	++	WI 90	+++
WI 39	14.80	WI 86	8.00	-	-	-	-	WI 25	+	WI 60	+++	WI 91	+++
WI 41	22.45	WI 88	10.50	-	-	-	-	WI 26	+	WI 61	++	WI 92	++
WI 44	22.50	WI 90	25.00	-	-	-	-	WI 28	+	WI 62	+++	WI 94	+
WI 46	9.00	WI 91	15.00	-	-	-	-	WI 29	+	WI 63	++	WI 95	+
WI 48	11.00	WI 95	11.20	-	-	-	-	WI 30	++	WI 64	+	WI 96	+
WI 50	14.00	WI 98	10.00	-	-	-	-	WI 31	+	WI 65	++	WI 98	++
WI 51	14.00	-	-	-	-	-	-	WI 32	++	WI 66	+	-	-
				-	-	-	-	WI 33	++	WI 69	+	-	-
Mean			13.18				15.71						
$CD_{0.05}$			2.16				0.04						
SE(m)			0.77				0.01						
CV (%)			5.09				1.16						

Rest of the isolates not indicated under each parameter did not show any respective activity;
* + = low production; ++ = medium production; and +++ = high production

in plant rhizosphere. Vega *et al*. (2005) reported that the high number of bacteria isolated from coffee rhizosphere belonged to genera *Bacillus*, *Burkholderia*, *Clavibacter*, *Curtobacterium*, *Escherichia*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Serratia* and *Stenotrophomonas*.

Siderophore production

Siderophore production was detected in 53 isolates i.e. 54.1% isolates. The isolate WI 12 produced highest %siderophore units (27.2) and isolate WI 1 produced minimum %siderophore units (4.75) [Table 1]. Significantly highest and lowest siderophore zones were produced by isolates WI 36 (16.3 mm) and WI 86 (3.0 mm). Baharucha *et al*. (2013) detected 41% isolates able to produce siderophore. Shobha and Kumudhini (2012) reported various bacterial isolates as efficient siderophore producers and reported that *Bacillus* isolate JUMB7 produced 10% siderophore. Pal and Gokarn (2010) reported that *Klebsiella* sp. were able to produce 3.2 and 12.0% siderophore units which falls within our observed range. Similarly, Kaushal and Kaushal (2011) reported that isolate MK7 produced a zone of 13.33 mm.

Hydrogen cyanide production

Hydrogen cyanide is a secondary metabolite produced commonly by rhizobacteria and is postulated to play a role in biological control of pathogens (Defago and Haas, 1990). In present study, 82 isolates (83.7%) showed the ability to produce HCN though in varying amounts (Table 1). The rhizobacterial isolates WI 12, WI 21, WI 36, WI 60, WI 62, WI 90 and WI 91 exhibited high HCN production. HCN production reportedly is a common trait of *Bacillus* (88.9%) and *Pseudomonas* (50%) in rhizospheric soil (Heydari *et al*., 2008). HCN antibiosis has widely been accepted as a PGP property (Cazorla *et al.,* 2006; Pliego *et al.,* 2011), and siderophores, HCN and antibiotic production reportedly plays vital role in disease suppression (Voisard *et al.,* 1989). Malleswari and Bagyanarayana (2013) isolated 219 rhizobacterial strains from different locations of Andhra Pradesh (India) and reported that 58 isolates (37.8%) showed HCN production.

Chitinase activity

Among different lytic enzymes, chitinases are particularly useful in agriculture as biocontrol agents against various fungal pathogens owing to their ability to hydrolyze chitinous fungal cell wall (Suresh *et al*., 2010; Wahyudi *et al*., 2011). In present study, 35 isolates (35.7%) exhibited chitinase activity and of these isolate WI 63 showed maximum production of 30.47 units mL^{-1} and isolate WI 8 showed minimum production of 8.90 units mL^{-1} (Table 1). The results are in agreement with Bhatt and Vyas (2014) who isolated and screened bacterial isolates for various PGPR activities *viz.,* P-solubilization and production of IAA, HCN, chitinase, amylase, etc. found 27% strains lipase producers, 53% amylase producers and 50% chitinase producers. Wang *et al*. (2013) also found chitinase activity in 30% bacterial isolates. Chitinase production is induced in a colloidal chitin containing environment (Mahadevan and Crawford, 1997).

Antifungal activities of rhizobacterial isolates against various fungal pathogens

All the 98 rhizobacterial isolates were screened for their antifungal activities against five fungal pathogens *viz*., *Dematophora necatrix*, *Alternaria solani*, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Phytophthora capsica.* Only 23 isolates (23.5%) were found antagonistic to *D. necatrix.* Amongst these, isolate WI 90 exhibited maximum fungal growth inhibition of 66%, followed by isolate WI 60 (43.0% inhibition), WI 11 (41.4% inhibition), WI 36 (38.4% inhibition) and WI 62 (38.2% inhibition) [Table 2]. Minimum growth inhibition in *D. necatrix* was observed in isolate WI 4 (7.6%). Amongst rhizobacterial isolates, only 20 isolates (20.4%) showed antagonistic activity against *A. solani*. The isolate WI 36 showed maximum growth inhibition of 67% against this fungus, followed by isolate WI 63 (55.6% inhibition), WI 11 (45.5% inhibition) and WI 65 (44.0% inhibition). Minimum growth inhibition against *A. solani* was depicted by isolate WI I7 (8.9%). Only 19 isolates inhibited mycelial growth of *F. oxysporum*, with maximum inhibition by isolate WI 62 (43.8%) and least by isolate WI 2 (10.7%). Similarly, only 21 isolates were found antagonistic to *P. aphanidermatum* with maximum mycelial growth inhibition depicted by isolate WI 63 (45.5%), followed by statistically at par isolate WI 60 (43.2%) and least inhibition was shown by isolate WI 49 (15.4%). Only 20 isolates exhibited growth inhibition of *P. capsici* with maximum inhibition by isolate WI 65 (49.0%) and minimum by isolate WI 38 (8.8%). The growth inhibition of phytopathogens may be attributed to the production of some specific siderophores, antibiotics, secondary metabolites or hydrolytic enzymes (Kirner *et al*., 1998; Srivastav *et al*., 2004). The clear zone of inhibition produced in *in vitro* experiment is an indicative of antibiosis by biocontrol agent against the fungal pathogens. Geetha *et al*. (2014) isolated and screened 180 PGPR strains from green gram rhizosphere for their antifungal activity against *Macrophomina phaseolina*, *Colletotrichum capscici*, *Rhizoctonia solani* and *F. oxysporum* and found 6 isolates most effective in enhancing the growth of green gram due to the production of ammonia, IAA and HCN, phosphate solubilization and antifungal activity against pathogenic fungi. Dalal and Kulkarni (2013) reported that out of 31 rhizobacterial isolates, five isolates JDB 3 (*Pseudomonas* spp.), JDB 5 (*Pseudomonas* spp.), JDB 9 (*Bacillus* spp.), JDB 11 (*Bacillus* spp.) and JDB 14 (*Bacillus* spp.)

Table 2: Antifungal activity of rhizobacterial isolates, collected from walnut rhizospehere in North Western Himalayas, against various fungal phytopathogens

Dematophora necatrix		*Alternaria mali*		*Fusarium oxysporum*		*Pythium aphanidermatum*		*Phytophthora* sp.	
Isolates	% Inhibition	Isolates	% Inhibition	Isolates	% Inhibition	Isolates	% Inhibition	Isolates	%Inhibition
WI 2	15.5	WI 4	17.3	WI 2	10.7	WI 2	22.5	WI 2	18.8
WI 3	11.7	WI 7	8.9	WI 3	18.8	WI 3	35.3	WI 3	17.5
WI 4	7.6	WI 11	45.5	WI 11	39.7	WI 7	21.2	WI 4	12.7
WI 7	16.0	WI 12	30.8	WI 12	43.8	WI 11	30.4	WI 11	47.0
WI 11	41.4	WI 14	16.6	WI 19	13.2	WI 12	45.5	WI 12	35.3
WI 12	34.7	WI 18	31.5	WI 30	37.0	WI 13	19.2	WI 13	21.5
WI 13	12.0	WI 30	21.4	WI 36	32.3	WI 21	26.0	WI 22	10.0
WI 14	17.3	WI 36	27.0	WI 41	33.3	WI 30	35.3	WI 30	38.7
WI 30	29.6	WI 37	22.5	WI 44	12.3	WI 36	38.2	WI 36	25.4
WI 36	38.4	WI 41	26.6	WI 50	19.2	WI 38	18.5	WI 38	8.8
WI 37	16.0	WI 42	12.9	WI 51	12.4	WI 41	26.0	WI 41	23.2
WI 38	29.2	WI 51	17.7	WI 60	20.3	WI 49	15.4	WI 50	18.8
WI 41	23.3	WI 59	17.5	WI 62	22.2	WI 51	24.7	WI 51	24.3
WI 44	12.0	WI 60	29.8	WI 65	36.6	WI 60	43.2	WI 60	27.1
WI 50	10.0	WI 62	39.6	WI 85	16.5	WI 62	38.4	WI 62	29.8
WI 51	17.3	WI 65	44.0	WI 90	33.9	WI 63	15.4	WI 63	21.4
WI 60	23.0	WI 87	19.5	WI 91	25.0	WI 65	10.0	WI 65	27.8
WI 62	38.2	WI 90	21.8	WI 98	13.3	WI 85	17.5	WI 86	16.0
WI 63	36.5	WI 91	23.3	-	-	WI 89	13.7	WI 90	17.3
WI 65	25.5	-	-	-	-	WI 90	21.5	WI 91	22.5
WI 73	8.7	-	-	-	-	WI 91	19.5	-	-
WI 89	16.5	-	-	-	-	-	-	-	-
WI 90	46.0	-	-	-	-		-	-	-
Mean	22.88		24.95		24.47	-	25.58		23.19
$CD_{0.05}$	0.62		3.66		3.54		4.66		6.40
SEM+	0.33		1.64		1.60		2.60		3.12
CV (%)	3.54		2.45		2.45		2.18		3.40

Medium used: Malt extract agar, readings taken after 7-14 days
Rest of the isolates showed no fungal inhibition or contact inhibition

showed maximum PGP traits *viz*., production of plant growth regulators (auxins, gibberellins and cytokinins), siderophores and HCN, so were considered as efficient isolates possessing dual abilities *i.e.* antagonistic and plant growth promotion with the view of plant health and productivity.

Most of the rhizobacterial isolates exerting mycelial growth inhibition in various soil borne pathogens in present study, on morph-biochemical basis, belonged to the genus *Bacillus*. Twelve bacterial isolates *viz.*, WI 11, WI 12, WI 30, WI 36, WI 41, WI 60, WI 62, WI 63, WI 65, WI 80, WI 90 and WI 91, on the basis of preliminary screening based on qualitative tests for siderophore, hydrogen cyanide and chitinase production (Shakeela Sofi, 2017), were identified by amplifying their 16S rRNA gene sequences of different lengths. The partial sequences of nucleotides were compared with the available sequences from NCBI database and the sequences showing >99% similarity were retrieved by BLAST-N program (NCBI; *www.ncbi.nlm.nih.gov/BLAST*). The bacterial isolates were identified on the basis of maximum sequence homology and phylogeny with the global reference sequences. The isolates were identified as *Micrococcus luteus* (isolates WI 12, WI 41 and WI 80), *Micrococcus yunnanensis* (isolates WI 30 and WI 60), *Micrococcus* sp. (isolates WI 11 and WI 91), *Bacillus subtilis* (isolates WI 63 and WI 65), *Bacillus tequilensis* (isolate WI 62), *Bacillus cereus* (isolate WI 36) and *Bacillus licheniformis* (isolate WI 90). These rhizobacterial species are reported for the first time from walnut rhizosphere. Previously Dar *et al.* (2009) have reported the presence of genera *Azotobacter, Azospirillum, Bacillus, Pseudomonas, Aspergillus* and *Penicillium* in walnut rhizosphere but they did not identify them upto species level.

A relatively wide range of antagonistic performances among the isolates was observed in present study and similar observation have previously been reported in other studies involving the same or different fungi (Idris *et al*., 2007; Calvo *et al*., 2010). In present study, almost all the selected bacterial isolates showed antifungal activity against *F. oxysporum, P. aphanidermatum, P. capsici, A. solani* and *D. necatrix*. This is in agreement with those of Ramirej *et al*. (2004) and Cazorla *et al*. (2007) who reported that *Bacillus* strain UCR 236 and *Bacillus* spp. produced antifungal substances against a number of mycelial fungi. Malleswari and Bagyanarayana (2013) found 43 isolates out of 219 (19.6%) exhibited antifungal activity against *Macrophomina phaseolina* and four of these isolates were identified as *Pantoea* sp., *Bacillus* sp. and *Pseudomonas* sp. In agreement to our observations, Sharma *et al.* (2015) reported biocontrol potential of *Bacillus* species against *Phytophthora capsica* and *Fusarium oxysporum* and found that under *in vitro* conditions 9 isolates (45%) inhibited *P. capsici* while ten isolates (50%) inhibited *F. oxysporum.*

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