Int.J.Curr.Microbiol.App.Sci (2018) *7*(9): 1980-1989

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 09 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.709.240

Growth Inhibition of Pathogenic Fungi and Salt Tolerance Ability of Rhizosphere Bacteria

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Keywords

Rhizosphere bacteria, *R. solanii*, *N. indica*, Growth inhibition, Salt tolerance

Article Info

Accepted:
12 August 2018
Available Online:
10 September 2018

A B S T R A C T

Management of crop diseases by biological control is an alternative and effective approach with minimum deleterious effect on environment and human health for ecosystem stability leading to improvement in crop yields. The present study was undertaken to characterize antagonistic rhizosphere bacteria against phytopathogenic fungi i.e., *Neovossia indica* and *Rhizoctonia solani*, which cause Karnal bunt and root rot disease, respectively. Twenty two rhizobacterial isolates out of total 88 isolates obtained from wheat rhizosphere, 25% isolates inhibited the growth of both *R. solani* and *N. indica* fungi. Individually, 27 isolates showed growth inhibition of *R. solani* whereas 49 isolates showed growth inhibition of *N. indica*. The results suggested that different bacterial metabolites are involved in antagonism of the two fungi. Thirty four antagonistic rhizobacterial isolates were further tested for salt tolerance at 2, 4, 6 and 8% salt concentrations. Thirteen isolates showed tolerance to 2% salt concentrations and twelve isolates showed salt tolerance upto 6% concentration. Only 10 bacterial isolates showed moderate growth at 8% salt concentration. The selected antagonistic rhizobacterial isolates having salt tolerance and plant growth promoting traits could help in improving crop productivity for sustainable agriculture.

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important agricultural commodities to feed ever-increasing human population. Though the production of wheat has increased after green revolution but the scarcity of nutrients (N, P and K) and salinity of the soil has been found to affect its yield and quality. Moreover, the attack of various diseases like head blight, powdery mildew, root rots, rusts, smuts, take-all and Karnal bunt greatly affect its yield and quality. Karnal bunt disease of wheat is caused by fungus *Neovossia indica* (Mitra) (Syn. *Tilletia indica*) and it is a threatening floral disease in most of the wheat-growing regions of the world (Carris *et al.*, 2006). Another root rot disease in wheat is caused by *Rhizoctonia solani* (Ryder *et al.*, 1998; Gill *et al.*, 2001). The pathogenic fungi *R. solani* causes destruction of roots and increased transpiration, thereby plants are weakened and killed or more generally recover with delayed maturity.

Soil salinity is another major threat to agricultural crop production in arid and semiarid regions, where rainfall is limited. In saline soils, the damaging effect of salt accumulation has become an important environmental concern. Salinity affects about 800 Mha of arable lands worldwide (Munns and Tester, 2008). The major inhibitory effects of salinity on the growth and metabolism of plants may be due to osmotic inhibition, toxic effects of salts and ions, nutritional imbalance caused by salinity, endogenous hormonal imbalance or energy losses resulting from diversion of photosynthates from growth functions to osmotic regulation and tissue retardation mechanism. Many approaches have been used to alleviate salinity stress in various crops and inoculation with plant growth promoting rhizobacteria has been found to ameliorate the salt stress leading to improved crop productivity (Plaut *et al.,* 2013; Wang *et al.,* 2016; Sehrawat *et al.,* 2018).

Wheat is a source of staple food in many countries of the world. Seed bacterization with beneficial microorganisms usually results in enhanced germination, plant growth and yield in wheat (Sangwan *et al.,* 2012; Chaudhary *et al.,* 2013). Therefore, the present study was conducted to isolate antagonistic bacteria, which inhibited the growth of pathogenic fungi *N. indica* and *R. solani* on medium plates. Some of the antagonistic bacteria even showed tolerance to 6-8% salt concentration. These salt tolerant antagonistic bacteria could be exploited as biofertilizer for plant growth promotion of wheat in salinity affected soils under field conditions.

Materials and Methods

Isolation of bacteria from rhizosphere

Soil samples were collected from the rhizosphere of wheat at 60 and 90 days of plant growth grown in field areas of Bawal, Rewari and Hisar in Haryana state. Serial dilutions of soil samples (upto 10^{-4}) were made and 0.1 ml of diluted soil suspension was placed on King's B and Luria Bertani (LB) medium plates. The plates were incubated at 28±2°C in BOD incubator for 3-4 days. Rhizobacterial isolates were selected based on typical morphological and pigment production characteristics.

Screening of rhizobacterial isolates for growth inhibition of pathogenic fungi

The interaction of rhizobacterial isolates with *Neovossia indica* and *Rhizoctonia solani* was studied by the spot test method (Sindhu *et al.,* 1999) on PDA medium plates. *Neovossia indica* and *Rhizoctonia solani* were grown on PDA slants. Spore suspensions of fungi were prepared in three ml sterilized water. About 0.2 ml of fungal spore suspension was spread over PDA medium plates. A loopful of 48-hour old growth of the rhizobacterial isolates was spotted on preseeded plates. Six cultures were spotted on each plate. Plates were incubated for 48 hours at 28±2°C and growth inhibition of fungi was recorded. Measurement of antagonistic activity of rhizobacterial isolates was based upon the ability of bacterial strains to inhibit fungal growth on PDA medium plates. The isolates showing zone of fungal growth inhibition were further tested for salt tolerance ability.

Screening of rhizobacterial isolates for growth at different salt concentrations

Selected rhizobacterial isolates were analyzed for their ability to grow at different concentrations of NaCl i.e., 0, 2, 4, 6 and 8% (w/v) on LB medium plates (Chaudhary and Sindhu, 2017). Salt amended medium plates were spotted with a 20 µl growth suspension of different bacterial isolates and incubated for 3-4 days at 28±2ºC in a BOD incubator. The susceptibility or tolerance to NaCl was

recorded by observing the growth on salt incorporated medium plates and colony size was measured in different salt concentrations.

Results and Discussion

Isolation of bacterial isolates from wheat rhizosphere

Seventy five bacterial isolates were obtained from soil samples collected from the rhizosphere of wheat. Thirteen reference cultures were obtained from Microbiology department for antagonistic and salt tolerance studies. Bacterial isolates were selected based on morphological and pigment production characteristics. In earlier studies, Fatima *et al.,* (2009) isolated bacterial strains from the rhizoplane and rhizosphere of wheat from four different locations. Some of the strains showed production of indole acetic acid, phosphorous solublization capability and inhibited the growth of *Rhizoctonia solani* on rye agar medium. Kumar *et al.,* (2012) obtained seven bacterial isolates from rhizosphere of common bean, which showed plant growth promoting and antagonistic activities.

Islam *et al.,* (2016) isolated 66 rhizobacteria from cucumber rhizosphere, out of which 10 bacteria (PPB1, PPB2, PPB3, PPB4, PPB5, PPB8, PPB9, PPB10, PPB11 and PPB12) were selected based on their *in vitro* plant growth promoting attributes and antagonism of phytopathogens.

Screening of rhizobacteria for antagonistic activity against *R. solani* and *N. indica*

All eighty eight bacterial isolates were screened for their antagonistic activity by spot test method using modified LB medium plates. Detection of antagonistic activity of rhizobacterial isolates was based upon the ability of rhizobacterial strains to inhibit fungal growth on modified LB medium plates. Only 27 bacterial isolates inhibited the growth of *Rhizoctonia solani* and different isolates varied in their inhibition of fungi. Isolates BWA36, RWA42, RWA48, RWA53, HCA3, HCA61 and RCA3 showed significant inhibition (measuring more than 2.0 mm) against *R. solani* (Table 1 and Fig. 1). Isolates BWA6, BWA7, BWA14, BWA27, BWA40, RWA59, SYB101, SB153 and JMM24 also showed moderate inhibition (measuring 1.1-2.0 mm) against *R. solani.* Sixty one isolates did not inhibit the growth of *R. solani*.

Further, screening of all the 88 bacterial isolates for antagonistic activity against *Neovossia indica* showed that seven bacterial isolates BWA6, BWA19, BWA23, RWA48, RWA53, HCA61 and RCA3 caused maximum inhibition (measuring more than 2.0 mm) against *N. indica* (Table 2 and Fig. 2). Moderate inhibition (measuring 1.1-2.0 mm) of *N. indica* was caused by 20 rhizobacterial isolates. Thirty nine rhizobacterial isolates did not inhibit growth of *N. indica*. Twenty two isolates i.e., BWA2, BWA6, BWA7, BWA8, BWA14, BWA19, BWA23, BWA27, BWA29, BWA36, BWA38, BWA40, RWA42, RWA48, RWA53, RWA59, HMM8, HCA3, HCA61, RCA3, SB153 and JMM24 inhibited the growth of both *R. solani* and *N. indica* fungi.

Isolates BWA14, RWA42 and SYB101 inhibited only *R. solani* but did not show antagonistic activity against *N. indica*, whereas eleven isolates BWA1, BWA18, BWA20, BWA25, RWA52, RWA55, RWA63, RWA69, RWA71, RWA72 and HMM97 isolates caused growth inhibition of only *N. indica*. Out of 61 isolates which lacked the ability to inhibit *R. solani*, twenty eight isolates caused growth inhibition of *N. indica*. These results suggested that different bacterial metabolites are involved in antagonism of the two fungi.

Table.1 Rhizobacterial isolates showing antagonistic activity against *Rhizoctonia solani*

Bacterial isolates	Inhibition category
BWA2, BWA8, BWA19, BWA23, BWA29, BWA31, BWA33, BWA34, BWA38, RWA72, HMM8	+
BWA6, BWA7, BWA14, BWA27, BWA40, RWA59, SYB101, SB153, JMM24	++
BWA36, RWA42, RWA48, RWA53, HCA3, HCA61, RCA3	+++
BWA1, BWA3, BWA4, BWA5, BWA9, BWA10, BWA11, BWA12, BWA13, BWA15, BWA16, BWA17, BWA18, BWA20, BWA21, BWA22, BWA24, BWA25, BWA26, BWA28, BWA30, BWA32, BWA35, BWA37, BWA39, RWA41, RWA43, RWA44, RWA45, RWA46, RWA47, RWA49, RWA50, RWA51, RWA52, RWA54, RWA55, RWA56, RWA57, RWA58, RWA60, RWA61, RWA62, RWA63, RWA64, RWA65, RWA66, RW67, RWA68, RWA69, RWA70, RWA71, RWA73, RWA74, RWA75, HMM26, HMM21, HMM73, JMM19, JMM11, HMM97	-

+: inhibition zone 0.5-1.0 mm; ++: inhibition zone 1.1-2.0 mm; +++: inhibition zone > 2.0 mm; -: No inhibition of fungi growth

Table.2 Rhizobacterial isolates showing antagonistic activity against *Neovossia indica*

Bacterial isolates	Inhibition category
BWA2, BWA3, BWA10, BWA14, BWA25, BWA30, BWA36, BWA38, BWA39, RWA45, RWA51, RWA54, RWA57, RWA60, RWA65, RWA68, RWA70, RWA71, RWA74, HMM26, JMM19, JMM11	+
BWA1, BWA7, BWA8, BWA18, BWA20, BWA27, BWA29, BWA40, RWA41, RWA42, RWA52, RWA55, RWA59, RWA63, RWA69, HCA3, HMM97, HMM8, SB153, JMM24	++
BWA6, BWA19, BWA23, RWA48, RWA53, HCA61, RCA3	+++
BWA4, BWA5, BWA9, BWA11, BWA12, BWA13, BWA15, BWA16, BWA17, BWA21, BWA22, BWA24, BWA26, BWA28, BWA31, BWA32, BWA33, BWA34, BWA35, BWA37, RWA43, RWA44, RWA46, RWA47, RWA49, RWA50, RWA56, RWA58, RWA61, RWA62, RWA64, RWA66, RWA67, RWA72, RWA73, RWA75, SYB101, HMM21, HMM73	-

+: inhibition zone 0.5-1.0 mm; ++: inhibition zone 1.1-2.0 mm; +++: inhibition zone > 2.0 mm; -: No inhibition of fungi growth

Table.3 Salt tolerance of different rhizobacterial isolates

Colony size	Growth of bacteria at different salt concentrations				
	0%	2%	4%	6%	8%
Group I (0.5-2.0 mm)	-	-	BWA23	BWA19, BWA23, BWA36, SB153, JMM24	RWA72, SYB101, HMM97, HMM8, BWA1, BWA6, BWA14, BWA18, BWA19, BWA20, BWA23, BWA27, BWA36, BWA40, RWA42, RWA52, RWA53, RWA63, RWA69, SB153, JMM24
Group II (2.0-5.0 mm)	BWA14, BWA19, BWA20, BWA23, BWA27, BWA36, BWA40, RWA52, RWA53, RWA55, RWA59, RWA63, RWA72, HCA61, SB153	HCA61, HMM97, SB153, JMM24, BWA1, BWA6, BWA14, BWA19, BWA20, BWA23, BWA27, BWA36, BWA40, RWA52, RWA53, RWA59, RWA63, RWA69, RWA72, SYB101, HCA3	JMM24, HCA3, RWA59, RWA63, RWA69, RCA3, HMM97, BWA1, HCA61, HMM8, SB153, BWA6, BWA8, BWA14, BWA19, BWA20, BWA27, BWA36, BWA40, RWA52, RWA53	RWA59, HCA3, RWA63, HCA61, RCA3, HMM97, BWA1, BWA6, HMM8, BWA7, BWA8, BWA14, BWA20 BWA27, BWA40, RWA52, RWA53	BWA2, HCA3, HCA61, RCA3, BWA7, BWA8, BWA25, BWA29, BWA38, RWA48, RWA55, RWA59, RWA71
Group III (5.0-10.0 mm)	RCA3, HMM8, HMM97, BWA1, BWA6, BWA7, BWA18, BWA38, RWA42, RWA48, RWA69, RWA71, SYB101, HCA3	BWA7, BWA8, BWA18, BWA38, RWA42, RWA48, RWA71, RCA3, HMM8	BWA7, BWA18, BWA25, BWA29, BWA38, RWA42, RWA48, RWA71, RWA72, SYB101	BWA18, BWA25, BWA29, BWA38, RWA42, RWA48, RWA55, RWA69, RWA71, RWA72, SYB101	-
Group IV (10.0-15.0 mm)	BWA2, BWA25, BWA29	BWA2, BWA25, BWA29	BWA2, RWA55	BWA2	-
Group V (15.0-20.0 mm)	BWA8	RWA55	-	-	-

Fig.1 Rhizobacterial isolates showing inhibition zone against *Rhizoctonia solani*

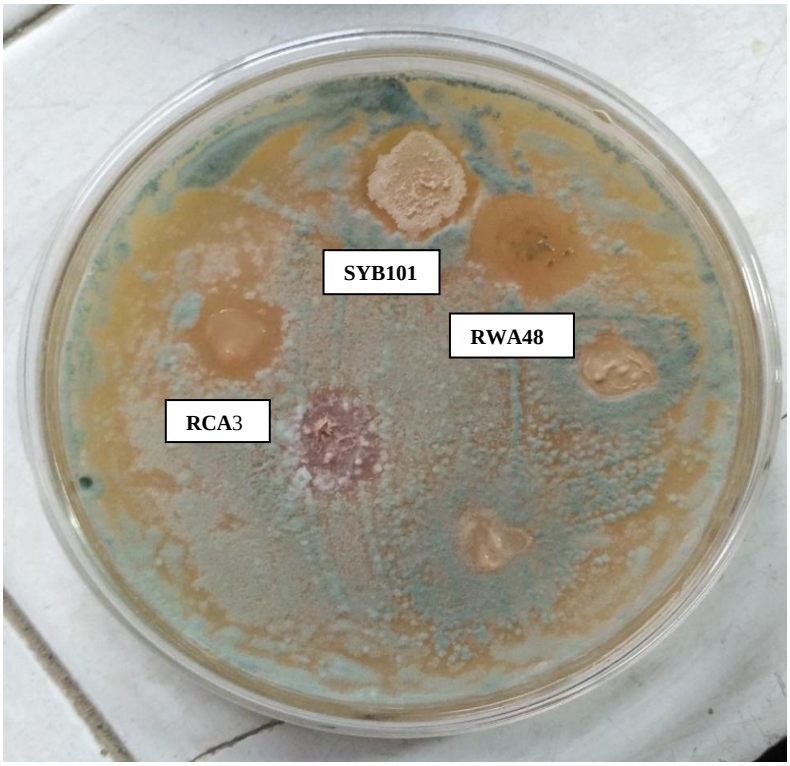


Fig.2 Rhizobacterial isolates showing inhibition zone against *Neovossia indica*

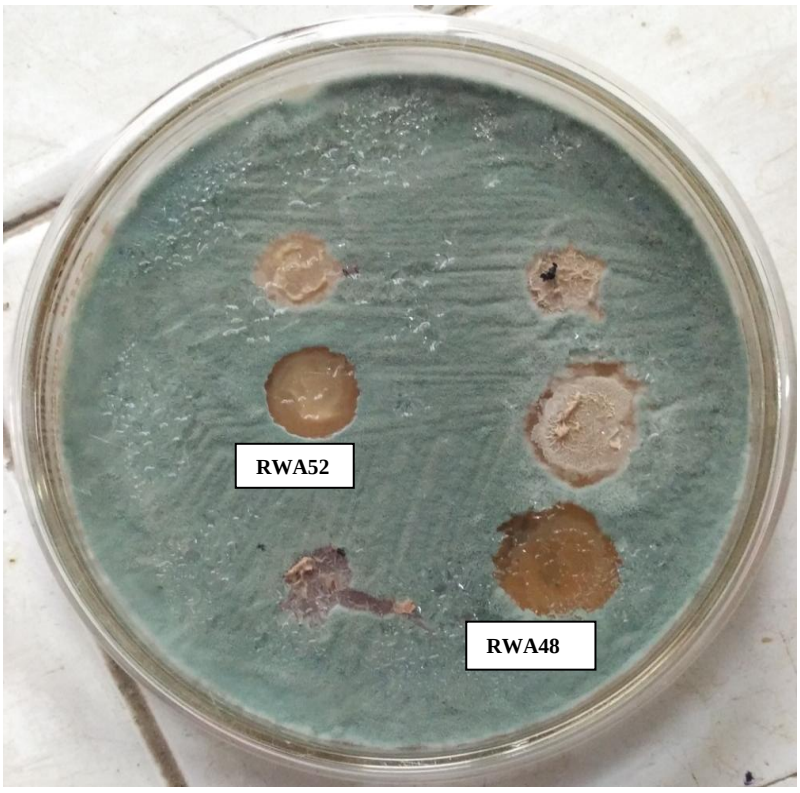
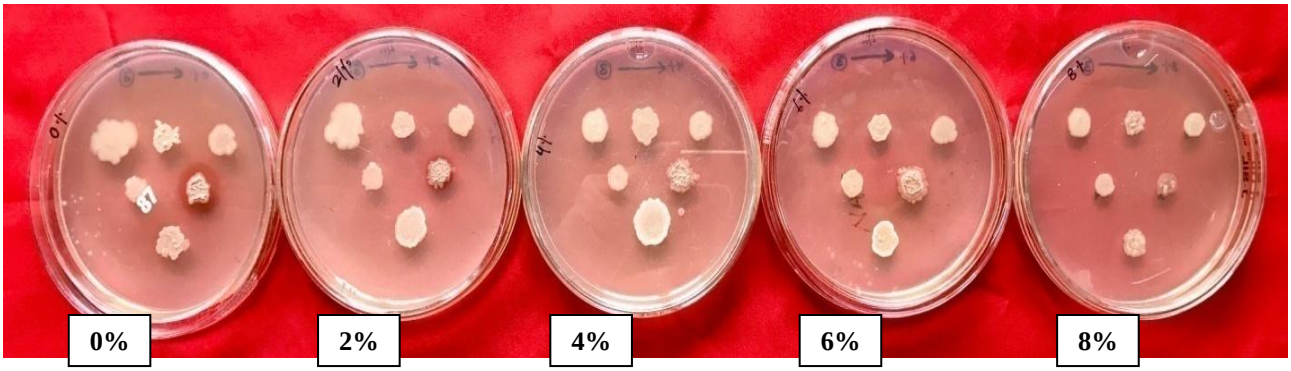


Fig.3 Growth of different bacterial isolates at different salt concentrations



In similar studies, Lee *et al.,* (2008) obtained forty one bacterial isolates from rhizosphere, soil out of which 12 isolates exhibited maximum antagonistic activity in dual culture assay against *Phytophthora* fungi under *in vitro* and *in vivo* conditions. All the antagonistic bacterial isolates showed varying levels of antagonism, whereas the isolates R33 and R13 exhibited the maximum (86.8 and 71%) ability to reduce the Phytophthora blight disease severity under *in vivo* conditions on red pepper plants. Similarly, Gajbhiye *et al.,* (2010) isolated *Bacillus subtilis* from cotton rhizosphere and 38% isolates showed competitive activity against *Fusarium oxysporum,* and exhibited more than 50% mycelial inhibition in dual culture bioassay. Kumar *et al.,* (2012) reported that out of seven bacterial isolates, the *Bacillus* sp. strain BPR7 strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina, Fusarium oxysporum, F. solani, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Colletotricum* sp. under *in vitro* conditions. Sasirekha and Srividya (2016) screened *Pseudomonas aeruginosa* strain FP6 for its *in vitro* antagonistic activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* on King's B medium, with and without $FeCl_3$. Strain FP6 showed significant reduction in *R. solani* growth with $FeCl_3$ supplementation as compared to the control (without $FeCl_3$), suggesting the role of siderophore mediated antagonism of *R. solani*. Saleemi *et al.,* (2017) tested three IAA producing plant growth promoting rhizobacteria (PGPR) isolates (WPR-42, WPR-51 and WM-1) out of total 63 bacterial isolates, for their antifungal activity and for seed germination of two wheat varieties. Isolate WPR-51 and mixture of three isolates completely neutralized the harmful effects of *Rhizoctonia solani* as 100% of the seeds of both varieties germinated in these treatments.

Screening of rhizobacterial isolates for salt tolerance

All the selected rhizobacterial isolates were tested for salt tolerance at different salt concentrations i.e., 2, 4, 6 and 8% on medium plates. Out of 34 selected antagonistic rhizobacterial isolates, thirteen isolates showed good growth measuring 5.0-20.0 mm colony growth at 2% salt concentrations (Table 3 and Fig. 3). Rhizobacterial isolate RWA55 showed more growth (colony size measuring between 15.0-20.0 mm) at 2% salt concentration and colony size measured 12.0 mm at 4% salt concentration. Twelve isolates i.e., BWA2, BWA18, BWA25, BWA29, BWA38, RWA42, RWA48, RWA55, RWA69, RWA71, RWA72 and SYB101 showed salt tolerance upto 6% NaCl concentration with colony size measuring between 5.0-15.0 mm. Only 13 bacterial isolates showed moderate growth at 8% salt

concentration, out of which ten rhizobacterial isolates i.e., BWA2, BWA7, BWA8, BWA29, BWA38, RWA48, RWA59, HCA3, HCA61 and RCA3 also inhibited the growth of both *R. solani* and *N. indica* fungi (Table 1 and 2). In earlier studies, Upadhyay *et al.,* (2009) isolated 130 rhizobacteria from a saline infested zone of wheat rhizosphere and 24 rhizobacterial isolates were found tolerant at 8% NaCl concentrations. Upadhyay *et al.,* (2012) observed that two plant growth- promoting rhizobacterial isolates, *Bacillus subtilis* SU47 and A*rthrobacter* sp. SU18 could tolerate upto 8% NaCl concentration. Upadhyay and Singh (2015) isolated nine salt- tolerant rhizobacteria that significantly improved the growth and yield of wheat crops in saline soil. Chen *et al.,* (2016) reported that *Bacillus amyloliquefaciens* strain SQR9 could help maize plants to tolerate salt stress. After exposure to salt stress for 20 days, SQR9 significantly promoted the growth of maize seedlings under salt stress and enhanced the chlorophyll content of the plants as compared with the control. Chaudhary and Sindhu (2017) obtained 55 rhizobacterial isolates from the chickpea rhizosphere soil and 41.8% rhizobacterial isolates formed colonies varying from 0.5-10 mm size at 3% NaCl concentration, and only 10.9% isolates showed growth at 4% NaCl concentration.

Management of crop diseases by chemical, physical and cultural methods may cause negative impacts on soil ecosystems. On the other hand, biological control involves the use of soil- and plant-associated beneficial rhizosphere microorganisms, which improves the yield and quality of grain crops (Sindhu *et al*., 2011; 2017). Currently, 20% of the world's irrigated land is salt affected and irrigated with water containing elevated salt levels. Many approaches including use of plant growth promoting rhizobacteria have been used to alleviate salinity stress of various crops. The use of biocontrol agents in agricultural fields effectively control various plant diseases and improves the quality of soil by mineralization of nutrients making them fertile for years, and thereby affects the growth of plants (Sindhu *et al*., 2009). This approach is economical and environment-friendly for sustainable agriculture. The rhizobacterial isolates obtained in this study possessing antagonistic activity against phytopathogenic fungi along with salt tolerance ability could be used as biofertilizer for improving the growth and yield of wheat crops under field conditions.

Acknowledgement

Authors thank the Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India for providing necessary facilities for this work.

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How to cite this article:

Anupma Dahiya, Ruchi Sharma, Swati Sindhu and Satyavir Singh Sindhu. 2018. Growth Inhibition of Pathogenic Fungi and Salt Tolerance Ability of Rhizosphere Bacteria. *Int.J.Curr.Microbiol.App.Sci.* 7(09): 1980-1989. doi: https://doi.org/10.20546/ijcmas.2018.709.240