

Original Research Article

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Exploring of Salt tolerant Biocontrol Agent (STBA) against Phytopathogen of Mustard Crop (*Brassicae juncea*)

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ABSTRACT

Plant diseases have a major adverse effect on the crop yield and these can controlled by the biological approach by using PGPRs. Mustard crop suffers due to the increased number of abiotic and biotic stressors which affects its growth and yield. Now a day's PGPR are being widely used worldwide, due to their eco-friendly nature and is considered as traditional agricultural technique. Keeping in view the above problem, the present study has been conducted to explore the biocontrol agents along with their Plant Growth Promoting activities. A total of 300 bacterial strains were isolated from the rhizospheric soil of mustard crop. Further, twenty five bacterial strains were identified as salt tolerant rhizobacterial strains in 10% of NaCl supplemented Nutrient Agar Media. Only eight bacterial strains B1, B3, B9, B10, B11, B1, B14, B17 and B22 were found promising for their antagonistic activity against *Alternaria brassicae* plant pathogen. The percentage of inhibition varied from 31% to 42% under in-vitro condition. These bacterial strains were tested for other traits like HCN, Siderophore and Amylase production. Results showed that all the eight bacterial strains produced HCN, ranging from 20% to 60%. Out of eight bacterial strains, six bacterial strains showed potentiality for siderophore production ranging from 28% to 63% and five produced amylase ranging from 20% to 60%.

Keywords

Biocontrol agent,
Abiotic stress,
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Introduction

Rapeseed-mustard is the one of the most important post-rainy (winter, October-March) oilseed crop in India. Mustard is a glycophyte crop cultivated in the arid and semi-arid areas of the world and is adversely affected by saline stress (Srivastava *et al.*, 2015). It has

been reported that growth and yield of the crop is considerably affected due to several abiotic and biotic stress factors, (Prasad *et al.*, 2011; Atkinson *et al.*, 2013; Narsai *et al.*, 2013; Prasad and Sonnewald, 2013; Suzuki *et al.*, 2014; Mahalingam, 2015; Pandey *et al.*, 2015a; Ramegowda and Senthil Kumar, 2015). It was found that influence of

occurrence and pathogen spread was due to the salinity an abiotic stress factor (Triky-Dotan *et al.*, 2005; Roubtsova and Bostock, 2009). Phytopathogen *Alternaria brassicae* is one of the causative agent of alternaria blight diseases which is a serious threat to mustard (*Brassica juncea*) cultivation (P. Chomoczynski, and N. Sacchi, 1987). This diseases can infects the plants at all the stages of their growth and negatively affect both quality and quantity oil yielding of the crops (Meena *et al.*, 2010). It is reported that in India *Alternaria blight* is a most common destructive diseases of mustard crop (S. J. Kolte, 1985) causes yield loss of varies from 10.0 to 70.0 per cent (Kumar and Kolte, 2001). Infectious seed causes oil yield loss ranges between 15%-36% has been reported by A.N. Ansari (1988). Worldwide to control the plant diseases occur due to the ingestion of synthetic pesticides, a huge amount of money is being spent. PGPR with combinations of different mechanisms of action in plants allowing the improved crop yields and is also helpful for good health of crops. PGPRs associated with plant roots offer enhanced plant growth and stress alleviation by various means.

Plant growth promoting rhizobacteria found on the plant root surface can enhance plant growth and abiotic and biotic stress alleviation by the various mean. A practice use rhizobacteria in agricultural field can overcome the harmful effect of synthetic fungicide on environmental and health concerns (Raupach and Kloepper 1998; Kobayashi *et al.*, 2002). PGPR-mediated Biocontrol strains even under saline condition against an array of phytopathogens can reduce several plant diseases have been reported (Paul and Nair 2008; Triky-Dotan *et al.*, 2005). Inhibition of infectious pathogens by using another potential microorganism is Biocontrol mechanism (Cook, 1993; Baker, 1991). Potential biological control agents were

remaining same even under stress conditions indicate Biocontrol can sustain even under advers conditions also (Diby *et al.*, (2005a).

Keeping in view the economic value of the crop for the region and extensive damaged caused by *Alternaria* blight diseases, the present study was conducted to find out the suitable eco- friendly strategy which can act as Biocontrol agents (PGPR) against the *Alternaria* blight diseases.

Materials and Methods

Soil sampling

Mustard rhizosphere soil samples for the isolation of halotolerant Biocontrol agents were collected from the salt affected area of four districts of U.P. viz Mau, Ballia, Gorakhpur and Varanasi during winter season (Nov-Dec) of 2016. Four villages from the each district were selected for the soil sampling and total of 16 soil samples were collected from the different locations. Uprooted mustard plants were kept in the sterile polythene bags, labelled and tied it. Subsequently soils were dried under shade condition and make pooled them locations wise.

Isolation of bacteria

Isolation of bacteria from the rhizospheric soil was done by serial dilution method. From the rhizospheric soil 0.1 g of soil is suspended in 1 ml of distilled water and makes further dilutions from 10^{-1} to 10^{-5} after that 0.1 ml of diluted soil suspension (10^{-4} and 10^{-5}) is spread over the surface of the Nutrient agar plate. The inoculated plates are incubated at 37°C for 24-48 hours. During the incubation period bacterial cell on the agar plate grows and multiplies rapidly to produce a mass of bacteria cells called a 'colony'. All the visible single colony of bacterium was separate out

by streaking method and each pure bacterial isolate in 40% of glycerol stock was kept in -20 refrigerator for the long term storage.

Screening of Salt Tolerant Bacterial Isolates

Screening of Salt Tolerant Bacterial isolates was done in the medium containing NaCl. A salt amended modified media was prepared by adding 10% of NaCl in Nutrient Broth (NB) and Nutrient Agar (NA) medium. A 48 hrs old fresh bacterial culture of 50 µl was inoculated in the NB medium and for the plate assay a pure colony of bacteria was streaked on modified NA medium.

Both bacterial streaked plates and Nutrient Broth was incubated at 37⁰ C with for 48-72 hrs. After completion of incubation period bacterial growth in NB was recorded by taking OD at 600nm and in NA plate Salt tolerant bacteria (STB) was screened by the visible different colony of bacteria.

Dual culture assay

The antagonistic activity of Potential PGPR against *Alternaria* species was tested by dual culture technique. Two bacterial strains were streaked on the both sides of PDA plates with the test fungal pathogen which was kept in the centre of the plate and a fungal disc alone in a separate Petri-dish served as control and kept it for the incubation at 24 ± 2⁰C for 5-7 days. Percentage of inhibition zone was recorded on the basis of the mycelia growth of fungal pathogen by using the formula Per cent inhibition (I) = $C-T/C \times 100$ (Riungu *et al.*, 2008) :

Where, C- mycelial growth of pathogen in absence of antagonists

T- mycelial growth of pathogen in presence of antagonists.

Extracellular Enzyme production

All the bacterial isolates were tested for starch hydrolysis as described by Bernfeld. *et al.*, 1955. Bacterial streaked starch enriched agar (1% pH 7.2) plates were incubated at 37⁰C for 24 to 48 hrs. After then Lugol's iodine solution in ratio of (1: 5 Lugol's iodine: dist. H₂O) was flooded over the plates. Appearance of a transparent zone around the bacterial colony indicated production of amylase. This halo was measured for subsequent calculation of the enzymatic index by dividing halo size by colony size (Alves *et al.*, 2002).

Hydrogen Cyanide Production

HCN production test of potential bacterial isolates was examined as per the method described by Bakker and Schipper 1987. King's B Agar medium amended with glycine (4.4 g/L) was prepared and overnight kept it for the solidification. Each bacterial isolates were streaked on a single separate plate and lid of each Petri plate was covered with Whatman no.1 filter paper soaked in 0.5% picric acid in 2% sodium carbonate and incubated at 30-35⁰C for 5-7 days. Results was observed due to the Change of colour from yellow to orange and then to dark brown in the filter paper indicated as positive reaction and the absence of change colour as negative reaction.

Siderophore Production

Siderophore Production test was performed by following the method given by Schwyn and Neilands (1997). Universal CAS assay medium on agar plate was prepared and each bacterial strain was inoculated in the middle of the plate and incubate at 30⁰C for 2-5 days. Siderophore producing bacteria was identified by CAS-agar colour changed from blue to orange, purple, or magenta. Un-inoculated control plates of CAS-agar was incubated

under the same conditions as described above was found with no colour change in the CAS-blue agar. Change of colour from orange, purple, or magenta colour indicated as positive reaction for siderophore Production test and the absence as negative reaction.

PCR amplification

The PCR amplification of 16S rRNA genes of bacterial strains was done with an universal 16s rRNA forward primer PA (5'-AGAGTTTGATCCTGGCTCAG-3') 1 µl reverse primer PH (5'-AAGGAGGTGATCCAGCCGCA-3'). 25µl of PCR mixture contained 1µl forward primer PA 1 µl reverse primer 0.5 µl of bacterial lysis and 10 µl of nuclease free water in a PCR tube for each bacterial strain. Thermal cycling consisted of the following steps: 94°C for 3 min, followed by 35 cycles of 94°C for 1 mint, 52°C for 1mint, 72°C for 1 mint, and a final step at 72°C for 10 min. PCR products were electrophoreses through a 1.2% agarose gel in 0.5X Tris-borate-EDTA buffer and visualized under the UV light in a gel documentation system (BIORAD, India). PCR products of all potential bacterial isolates were outsourced for partial 16s sequencing from omega biotech limited (Kanpur (India) Pvt. and identification of bacterial strains at genus and species level was done by using NCBI BLAST searches.

Results and Discussion

Out of 300 bacterial isolates from rhizospheric soil samples, twenty five could grow in 10% of NaCl medium and out of these 25 isolates, only eight bacterial strains showed antagonistic activity against *A. Brassicae* under in-vitro condition.. All the eight bacterial strains were tested for other Biocontrol activities like HCN production, Siderophore production and Starch solubilisation. These eight bacterial strains were identified as genus *Bacillus* and

Enterobacter by using 16s sequencing. Bacterial strains B3 and B22 were identified as genus *Bacillus* (Table 1) and rest other bacterial strains B8, B9, B12, B15, B17 and B20 were identified as genus *Enterobacter* (Table 1). The sequences of the isolates were submitted to Gen Bank under accession numbers MK 418219, MK 4711330, MK 463942, MK 463943, MK 463952, MK 478371, and MK530649 (Table 1).

PGPR traits

Dual culture method

All the 25 Salt Tolerant Rhizobacteria (STR) bacterial strains were tested for their antagonistic activity against *Alternaria Brassicae*. It was found that out of 25 STR strains 8 bacterial strains B1, B3, B9, B10, B11, B14, B17 and B22 identified as genus enterobacter and *Bacillus* (Table 1) were found to be antagonistic to *Alternaria Brassicae* (fig and Table 2). Bacterial strains were found to inhibit pathogen and the inhibition ranged from 22.75% to 44.4% under in-vitro condition. Maximum zone of inhibition (Table 2 and Fig 1, 2, 3, 4) was shown by bacterial strains B4 and B20 identified as genus *Bacillus* and *Enterobacter* (Table 1).

Siderophore production

The ability of rhizobacteria to produce siderophores has been the focus of many studies dedicated to investigating PGPR (Maksimov *et al.*, 2011). All the 25 bacterial strains were tested for the siderophore production and it was found that 64% of STRs are able for the production of Sidereophore and only 16% bacterial strains were found positive for both siderophore production and antagonistic activity. The siderophore production by four bacterial strains viz B3, B8, B15 and B17 ranged from 52.38% to

63.15% (table 1). Bacterial strain B17 identified as genus *Enterobacter* was found to exhibit the highest (63.15%) siderophore production activity (table 1).

HCN Production

68% of bacterial strains were found to be positive for HCN production test. Only five bacterial strains viz B3, B4, B, B15 and B17 were found to be positive for HCN production and antagonism. Maximum HCN production was seen in bacterial strain B15 in plate assay method.

Starch solubilisation Test

Starch solubilisation was determined by the appearance of halo zone and is indicative of areas of hydrolysis by bacterial strains. 36% bacterial strains were found to have starch solubilisation activity and only five, out of eight bacterial strains viz B3, B4, B9 B12 and B17 showed both the traits of antagonism and starch hydrolysis. The range for starch solubilisation for all the five bacterial strains was calculated between 20% to 60%, B4 and B12 strains showed highest starch solubilisation activity (60%) as compared to other bacterial strains.

In order to alleviate the adverse effects of biotic and abiotic stresses on plants, we need to have the biocontrol agents having salt tolerant activities. Bacterial isolates B1, B3, B9, B10, B11, B1, B14, B17 and B22 showed similarity with the genus *Enterobacter* and *Bacillus*. Biocontrol agents have been reported to be effective against an array of pathogens even under saline stress conditions (Elmer 2003; Paul and Nair 2008; Rangarajan *et al.*, 2003; Triky-Dotan *et al.*, 2005). Bacterial colonisation in plants can induce systemic resistance against various kind of phytopathogens (van Loon *et al.*, 1998). It has been reported that many of rhizobacterial

strains act by inducing the systemic resistance in plants against the several plant diseases (Leeman *et al.*, 1995; Park *et al.*, 2009). Antagonistic activity of bacterial strains against fungal pathogens can be confirmed by the formation of inhibition zones between the bacterial isolates and the fungal isolates (Ji *et al.*, 2014 or it can be measured by the decrease in mycelia growth of fungal pathogens (Lee *et al.*, 2017). This inhibition of mycelial growth may be due to the production of antifungal volatile compound (VOCs) by the bacterial strains. It has been reported by Raza *et al.*, (2016) that *P. fluorescens* WR-1 in Biocontrol because they produce VOCs. Recently the similar result of antifungal activities was also reported by Kandel *et al.*, (2017) and Lee *et al.*, (2017).

Production of siderophores by microbes can enhance iron uptake indicate bacterial ferric-siderophore complex (Masalha *et al.*, 2000; Katiyar and Goel, 2004; Dimkpa *et al.*, 2009). Siderophore production activity can act as Biocontrol activity to suppress the phytopathogens. The role of siderophore production by the microbes in their Biocontrol activity has been studied previously by Solans *et al.*, 2016.

HCN production by the potential microbes can be influenced by several environmental factors (Castric, 1975, 1983;). It was reported by Defago *et al.*, 1990 that Hydrocyanic acid (HCN) produced by many rhizobacterial strains has biological control activity. Voisard *et al.*, (1989) found role of HCN in biological control. The mechanisms of biocontrol viz antagonistic action toward plant pathogens, production of siderophores, cyanide, and hydrolytic enzymes have been reported by the many of researchers (Shaikh and Sayyed 2015). Productions of hydrolytic enzymes against various phytopathogens also play a major role in bio-control (Shaikh and Sayyed 2015).

Antagonistic activity of potential bacterial stains against



Fig1: B9 and B10

Fig2: B21 and B22

Fig3: B4 and B9

Fig4: B13 and B14

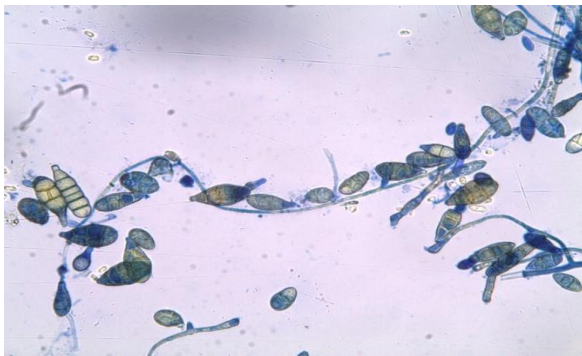


Fig5: Microscopic of *Alternaria Brassicae*



Fig 6: Pathogen *Alternaria Brassicae*

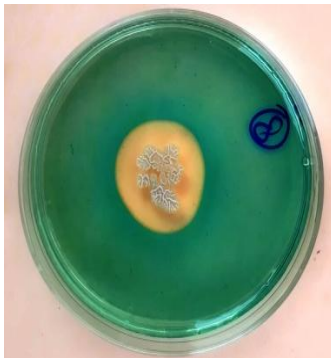


Fig7: Siderophore production



Fig 8: HCN Production and Control



Fig9: Starch hydrolysis

Table.1 Identification of potential bacterial strains by 16s RNA

Table Molecular characterization of STBA strain to genomic level by 16s rRNA partial sequencing and their NCBI accession no			
Isolate code	Nucleotide base length (base pair)	NCBI accession no.	Species
B1	700	MK 446245	<i>Enterobacter xianfangensis</i>
B3	650	MK 418219	<i>Bacillus FZB42</i>
B9	430	MK 471330	<i>Enterobacter bugandensis</i>
B10	530	MK 463942	<i>Enterobacter Xianfangensis</i>
B11	600	MK 463943	<i>Enterobacter Xianfangensis</i>
B14	815	MK 463952	<i>Enterobacter Xianfangensis</i>
B17	600	MK 478371	<i>Enterobacter Xianfangensis</i>
B22	515	MK530649	<i>Paenibacillus alvei</i>

Table.2 Plant Growth promoting attributes

Bacterial strain code	Growth at 10% NaCl	Antagonistic activity (%)	HCN Production (%)	Siderophore Production (%)	Amylase production (%)
B1	0.33	31.3	20	56	0
B3	0.174	31.33	20	55	20
B9	0.131	22.75	20	0	40
B10	0.23	44.2	20	63	60
B11	0.6	39.91	20	55	20
B14	0.157	39.91	20	28	0
B17	0.72	35.6	20	63.15	20
B22	0.39	44.2	40	0	0

Studies showed that Biocontrol agents synthesized extracellular hydrolytic enzymes and the enzymes have the potential of inhibiting phytopathogens (Pal and Gardener 2006). Starch hydrolysis can metabolize sugars which can help in better plant growth by increasing germination of seeds (Beck and Ziegler, 1989; Akazawa and Nishimura, 2011).

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References

- Akazawa, T., and Nishimura, H.(2011). Topographic aspects of bio synthesis, extracellular secretion, and intracellular storage of proteins in plant cells. *Annu. Rev. Plan Physiol.* 36, 441–472.
- ALVES, M. H. *et al.*, Screening of *Mucor* spp. for the production of amylase, lipase, poly galacturonase and protease. *Brazilian Journal of Microbiology*, v. 33, n. 4, p. 225-230, 2002.
- Ansari, A. N., Khan, M. W. and Muheet, A. 1988. Effect of *Alternaria* blight on

- oilcontent of rapeseed-mustard, *Current Science*. 57: 1023-1024.
- Atkinson, Nicky J., Catherine J. Lilley, and Peter E. Urwin. "Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. *Plant physiology* 162.4 (2013): 2028-2041.
- Bernfeld, P. (1955) X Amylases,. and B in : *Methods in enzymology*. 5 P Colowick and N D Kaplan eds. Vol. I, Academic Press, New York, pp 149-150.
- Castric P A 1975 Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. *Can. J. Microbiol.* 21, 613–618
- Cattelan, A. J., P. G. Hartel, and J. J. Fuhrmann. "Screening for plant growth-promoting rhizobacteria to promote early soybean growth." *Soil Science Society of America Journal* 63.6 (1999): 1670-1680.
- Chomoczynski, P. N. and Sacchi, 1987 Single step method of RNA isolation by acid-guanidinium thiocyanate phenol-chloroform extraction, *Analytical Biochemistry*. 162: 156-159.
- Defago, G. AND HAAS, D., 1990, Pseudomonads as antagonists of soil borne plant pathogens: mode of action and genetic analysis. *Soil Biochemistry*, (Eds) Bolley, J.M. and Stortzky, G. New York, Baul, 6: 249-291 diazotrophic bacterial community. *Plant and Soil*, v. 356, n. 1, p. 35-49, 2012.
- Diby P, Bharathkumar S, Sudha N (2005a) Osmotolerance in Biocontrol strain of *pseudomonas pseudoalcaligenes* MSP-538: a study using osmolyte, protein and gene expression profiling. *Ann Microbiol* 55(4):243–47
- Elmer WH (2003) Local and systemic effects of NaCl on root composition, rhizobacteria, and *Fusarium* crown and root rot of asparagus. *Phytopathol* 93(2):186–92. doi:10.1094/PHYTO.2003.93.2.186
- Dimkpa, Christian O., *et al.*, "Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. *Soil Biology and Biochemistry* 41.1 (2009): 154-162.
- Ji SH, Gururani MA, Chun SC (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol Res* 169:83–98. <https://doi.org/10.1016/j.micres.2013.06.003>
- Katiyar V and Goel R (2004) Siderophore mediated plant growth promotion at low temperature by mutant of fluorescent pseudomonad. *Plant Growth Regul* 42:239-244.
- Kolte, S. J. 1985. Disease of annual edible oilseed crops, in: *Rapeseed Mustard and Sesame Diseases*, II: CRC Press, Florida. pp.
- Kumar, B., and Kolte S J 2001. Progression of *Alternaria* blight of mustard in relation to components of resistance. *Indian Phytopathology* 54: 329-3
- Lee T, Park D, Kim K, Lim SM, Yu NH, Kim S, Kim HY, Jung KS, Jang JY, Park JC, Ham H, Lee S, Hong SK, Kim JC (2017) Characterization of *Bacillus amyloliquefaciens* DA12 showing potent antifungal activity against mycotoxigenic *Fusarium* species. *Plant Pathol J* 33:499–507. <https://doi.org/10.5423/PPJ.FT.06.2017.0126>
- Mahalingam, Ramamurthy. "Consideration of combined stress: a crucial paradigm for improving multiple stress tolerance in plants." *Combined stresses in plants*. Springer, Cham, 2015. 1-25.
- Maksimov IV, Abizgil'dina RR and Pusenkova LI (2011) Plant growth promoting rhizobacteria as alternative

- to chemical crop protectors from pathogens (Review). *Appl Biochem Microbiol* 47:333-345
- Masalha J, Kosegarten H, Elmaci Ö and Mengel K (2000) The central role of microbial activity for iron acquisition in maize and sunflower. *Biol Fert Soils* 30:433-439.
- Meena, P. D., Awasthi, R. P., Chattopadhyay, C., Kolte, S. J. And Kumar, A. 2010. *Alternaria* blight: a chronic disease in rapeseedmustard. *J. Oilseed Brassica*. 1: 1-11.
- Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11, 15–19. doi: 10.1016/j.tplants.2005. 11.002
- Narsai, Reena, *et al.*, "Antagonistic, overlapping and distinct responses to biotic stress in rice (*Oryza sativa*) and interactions with abiotic stress." *BMC genomics* 14.1 (2013): 93.
- Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *J Basic Microbiol* 48(5):378–84. doi:10.1002/jobm. 200700365
- Prasch, Christian Maximilian, and Uwe Sonnewald. "Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks." *Plant physiology* 162.4 (2013): 1849-1866.
- Ramegowda, Venkategowda, and Muthappa Senthil-Kumar. "The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination." *Journal of plant physiology* 176 (2015): 47-54.
- Rangarajan S, Saleena LM, Vasudevan P, Nair S (2003) Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. *Plant Soil* 251(1):73–82. doi :10.1023 A:1022950811520
- Raupach, Georg S., and Joseph W. Kloepper. "Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens." *Phytopathology* 88.11 (1998): 1158-1164.
- Raza W, Ling N, Liu D, Wei Z, Huang Q, Shen Q (2016) Volatile organic compounds produced by *Pseudomonas fluorescens* WR-1 restrict the growth and virulence traits of *Ralstonia solanacearum*. *Microbiol Res* 192:103–113. <https://doi.org/10.1016/j.micres.2016.05.014> reducing sugar. *Anal Chem* 31: 426-428.
- Riungu, G. M., Muthorni, J. W., Narla, R. D., Wagacha, J. M., and Gathumbi, J. K. (2008). Management of *Fusarium* head blight of wheat and deoxynivalenol accumulation using antagonistic microorganisms. *Plant Pathol. J.* 7, 13–19. doi: 10.3923/ppj.2008.13.19
- Roubtsova, T.V., and Bostock, R.M. (2009) Epi sodic abiotic stress as a potential contributing factor to onset and severity of disease caused by *Phytophthora ramorum* in *Rhododendron* and *Viburnum*. *Plant Dis* 93: 912–918
- Solans, Mariana, *et al.*, "Potential biocontrol actinobacteria: rhizospheric isolates from the Argentine Pampas lowlands legumes." *Journal of basic microbiology* 56.11 (2016): 1289-1298.
- Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, 203(1), 32-43.
- Triky-Dotan S, Yermiyahu U, Katan J, Gamliel A (2005) Development of crown and root rot disease of tomato

- under irrigation with saline water. *Phytopathol* 95(12):1438–44. doi:10.1094/PHYTO-95-1438
- VOISARD, C., KEEL, O., HAAS, P. AND DEFAGO, G., 1989, Cyanide production by *Pseudomonas fluorescens* helps to suppress black root rot of tobacco under gnotobiotic condition. *European Microbiological Journal*, 8: 351-358.
- Kobayashi, Daisuke, *et al.*, "Endogenous reactive oxygen species is an important mediator of miconazole antifungal effect." *Antimicrobial Agents and Chemotherapy* 46.10 (2002): 3113-3117.
- Cook, R. James. "Making greater use of introduced microorganisms for biological control of plant pathogens." *Annual review of phytopathology* 31.1 (1993): 53-80.
- Baker, Ralph. "Induction of rhizosphere competence in the biocontrol fungus *Trichoderma*." *The rhizosphere and plant growth*. Springer, Dordrecht, 1991. 221-228.
- Shrivastava, Pooja, and Rajesh Kumar. "Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation." *Saudi journal of biological sciences* 22.2 (2015): 123-131.
- Bakker and Schippers 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biology and Biochemistry* 19: 451-457
- Schwyn, B., Neilands, J.B., 1997. Universal chemical assay for the detection and determination of siderophores. *Anal. BioPesquisa do Estado de Sao Paulo (FAPESP) and ~ chem.* 160, 46–56
- Van Loon LC, Pierpont WS, Boller T and Conejro V (1994) Recommendations for naming plant pathogenesis-related proteins. *Plant Mol Biol Rep* 12: 245-264
- Kandel, Shyam L., *et al.*, "An in vitro study of bio-control and plant growth promotion potential of Salicaceae endophytes." *Frontiers in microbiology* 8 (2017): 386.
- Leeman, M., *et al.*, "Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*." *Phytopathology* 85.9 (1995): 1021-1027.
- Shaikh, S. S., and R. Z. Sayyed. "PGPR: A BOON FOR AGRICULTURE." *Ф94 Фундаментальные и прикладные аспекты создания* (2015): 54.
- Park, Chang-Jin, and Young-Su Seo. "Heat shock proteins: a review of the molecular chaperones for plant immunity." *The plant pathology journal* 31.4 (2015): 323.

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