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In vitro and in silico studies on biocontrol agent of bacterial strains against *Fusarium oxysporum* f. sp. *lycopersici*

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The tomato is botanicaly known as *Lycopersicum esculentum* belongs to the family *Solanacae*. A survey was conducted to assess the intensity of *Fusarium* wilt disease incidence in Dindigul and Coimbatore Districts of Tamil Nadu. The wilt disease maximum incidence of 30 percent was recorded in Thondamuthur village of Coimbatore District followed by Ayyalur village of Dindigul District. The pathogen was isolated from the infected material and it was identified as *Fusarium oxysporum* f.sp. *lycopersici*. Among the thirty isolates isolated from rhizosphere soil collected from two Districts, *Pseudomonas* sp. (PV2) recorded the maximum inhibition zone of 16 mm and 66.16 per cent inhibition; *Bacillus* sp. (BVE1) recorded 18mm of inhibition zone, 68.33 percent over control and *Serratia* sp. (SM1) recorded the maximum inhibition zone of 15.20 mm and 64.44 per cent inhibition of mycelia growth over control *in vitro*. The highest inhibition 88.33 per cent over control was observed in BVE1 isolates revealed that the isolate inhibited the growth of *Fusarium oxysporum* f .sp. *lycopersici*. The efficiency of *in silico* studies of Protein protein docking clearly demonstrated that *Bacillus* antifungal compound proteins have an inhibitory activity towards receptor fungal protein of Polygalacturonase. *Bacillus* sp. can also be used as the biocontrol agent in agricultural field to reduce the wilt incidence of tomato.

Key words: Survey, Pathogen and antagonism isolation, *In vitro* antifungal and *In silico* analysis.

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and important commercial vegetable crops grown throughout the world. In India, Tamil Nadu occupies seventh position in production of tomato among the states (Anitha and Rabeeth, 2009). Tomato occupies around 0.25 lakhs hectares in the state with an average yield of 12,500 kg per hectare. The major production Districts are Dharmapuri, Coimbatore, Salem, Krishnagiri, Theni, Dindigul and Vellore. Coimbatore is the second largest District in tomato acreage and production (Anonymus, 2009). Many diseases and disorders can affect tomatoes during the growing season. *Fusarium oxysporum* f.sp. *lycopersici* (FOL) is a highly destructive pathogen which affects the crop production worldwide and cause chronic threat to agricultural food production. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield and eventually crop loss (Rajappan and Ramarej, 1999).

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Management of soil-borne diseases such as wilt caused by *Fusarium* species has always been problematic. Applying chemical fungicides is considered to be the most effective for plant disease management. However, excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution and development of pathogen resistance to fungicide. Because of the worsening problems in fungal disease control, a serious search is needed to identify alternative methods for plant protection. In this context, biocontrol is an eco-friendly way of managing fusarium wilt in tomato which offers an alternative to fungicides (Asha *et al.,* 2011). In recent year Plant Growth Promoting Rhizhobacteria (PGPR) has been suggested as an attractive alternative for disease management approach (Fravel, 2005). Antagonistic bacteria that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front line defense for roots against attack by pathogens (Clayet-Marcel *et al.,* 2001; Choudary and Johri, 2008; and Berg, 2009). Hence, the present investigation was undertaken to exploit the utility of rhizosphere microorganism isolated from Tamil Nadu State against *Fusarium oxysporum* induced wilt disease in tomato *in vitro*. Further *In silico* analysis of antifungal compounds produced by antagonistic bacterial strains was carried out.

MATERIAL AND METHODS

Survey

A survey was conducted in major tomato growing areas of Dindigul and Coimbatore Districts of Tamil Nadu to assess the incidence of Fusarium wilt disease. Plant showing the symptoms of wilt were identified and the percentage disease incidence was calculated based on number of plants infected/m^2. The infected samples were collected and pathogens were isolated.

Isolation and identification of pathogen

The pathogen was isolated by tissue segment method (Schulz *et al.,* 1993) on potato dextrose agar medium. Infected stems and roots were cut into small pieces of 1 to 1.5 cm, surface sterilized with 1% sodium hypochlorite for one min, washed in sterile distilled water thrice and then aseptically transferred to sterile Potato Dextrose Agar (PDA) plates. The plates were incubated at 25^{0}C for 5-7 days. The hypal tips of fungi growing from the pieces were transferred aseptically to PDA slants for further studies. The identification was based the morphological characters of conidia (observed under a compound microscope). Pure cultures were maintained on Potato Dextrose Agar (PDA) slants at 4°C.

Isolation and identification of bacterial strains

Bacterial strains were isolated from the rhizosphere soil of tomato collected from different places of Dindigul and Coimbatore Districts, Tamil Nadu. One gram of rhizosphere soil near the root surface was collected and transferred to a 250 ml conical flask containing100 ml of sterile water. After thorough shaking for 15 min in shaker, 1 ml of 10^{-7} and 10^{-8} dilution were inoculated into Nutrient agar medium for isolation of *Bacillus* Sp. and in King's B medium for isolation for *P. fluorescens*, for each dilution three plates were maintained., incubated at 30°C for 24 hrs. Pure cultures of PGPR strains were identified using morphological and physiological characteristics as well as biochemical characterization was done by procedures outlined by Cappucino and Sharman (1992).

Evaluation of PGPR antagonistic activity *In vitro* against *F. oxysporum* f. sp. *lycopersici*

The Preliminary screening of antagonistic efficacy of all the isolated PGPR was tested in YMA medium against *F. oxysporum* f.sp. *lycopersici* by dual culture

technique. A mycelial disc of the pathogen was placed at one end of the petriplate and the bacterial antagonists were streaked at the opposite. The plates were incubated at 28±2°C for 7 days and after incubation the mycelial growth of the pathogen, inhibition was measured and percent inhibition (PI) was calculated (Dennis and Webster, 1971; Kucuk and Kivance, 2004).

Similarly, antifungal activity of isolated bacterial cultures were tested in agar well diffusion method against phyto-pathogen (Sandhu *et al*., 2004). A loopful of individual PGPR strain (PM1, BVE1and SN1) was inoculated into the Nutrient broth and incubated in a rotary shaker at for 48 h at room temperature. Bacterial supernatant (0.2 ml) was prepared by centrifuging at 7000 rpm for 10 min at 4°C. Equally spaced and sized wells were cut in the pathogen swabbed Potato Dextrose Agar plates. In to each well 10 µl of supernatants obtained from PM1, BVE1and SN1 and for control distilled water was added, incubated at 27°C for 3-7 days and inhibition of mycelia growth was measured.

In silico studies - Docking

Ligand Preparation

The three dimensional structures of inhibitor proteins were downloaded in pdb format from Protein Data Bank. Hydrogen Bonds were added and the energy was minimized using CHARMM force field.

Protein Preparation

The crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMM force field.

ZDOCK

A number of algorithms have been developed to address the initial stage of unbound protein-protein docking. The program used individual protein structures determined by experimental or computational methods as inputs and predicts the structure of a number of protein complexes (i.e., the top 2000 complexes). ZDOCK uses a simple shape complementarily method called Pairwise Shape Complementarity (PSC).

DOCK PROTEINS

Protein-protein docking was the task of assembling separate protein components into protein-protein complexes or assemblies using computational methods. Typically such methods start with the three-dimensional (3D) structures of the individual components, often referred to as a protein receptor and a protein ligand, Predictive information may also be provided in the absence of complete experimental data (Chen *et al*., 2003b).

The divide-and-conquer strategy is used widely in the docking field (Sternberg *et al*., 2000, Smith *et al*., 2002). This strategy starts with an initial-stage algorithm focused on retaining reasonably small number of near-native complexes (hits), followed by a refinement algorithm that is used to rank the hits.

RESULT

Survey, Isolation and identification of pathogen

A survey was conducted to assess the intensity of *Fusarium* wilt disease incidence in Dindigul and Coimbatore Districts of Tamil Nadu. The maximum wilt disease incidence of 30% was recorded in Thondamuthur village of Coimbatore District. Similarly maximum wilt disease incidence (25.4) was observed in Ayyalur village of Dindigul District (Table 1). The pathogen was isolated and it was identified as *Fusarium oxysporum* f.sp. *lycopersici* from the infected samples of tomato (Plate 1).

Isolation and identification of antagonistic bacteria

Thirty isolates of bacteria were isolated from the rhizosphere soil of tomato collected from different tomato growing areas in Dindigul and Coimbatore District, Tamil Nadu. They were identified as different bacterial culture like *Bacillus* sp. and *Pseudomonas* sp, *Serratia* sp. by using the suitable biochemical test.

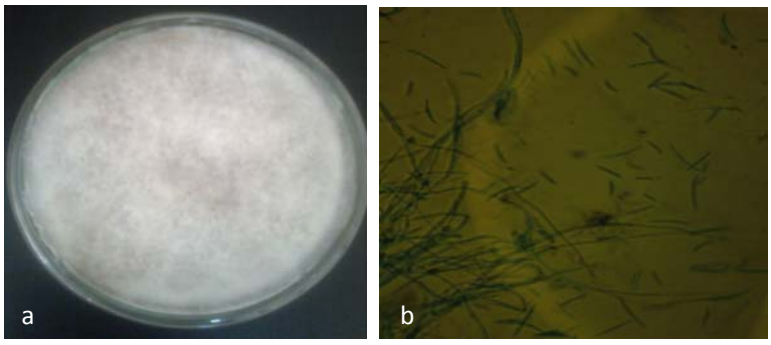


Plate 1. a) Morphological and cultural characteristic of plant pathogenic fungi b) Microscopic identification of pathogenic fungi

Table 1. Survey for the occurrence of tomato wilt complex disease incidence

S. No	Location		Per cent Disease Incidence
1.	Coimbatore District	Pollachi	17.3
		Perur	11.2
		Thondamuthur	30.5
2.	Dindigul District	Vadamadurai	16.5
		Ayyalur	25.4
		Vaiyampatty	12.6

Table 2. Screening of *Pseudomonas* isolates against *Fusarium oxysporum* f.sp. *lycopersici*

S. No	*Pseudomonas* isolates	Place of collection	Mycelia growth (mm)	Inhibition zone (mm)	Per cent inhibition
1	PT1	Thirumalayam palayam	54.00	12.20	40.00
2	PV1	Velanthavalam	65.50	10.00	27.22
3	PP1	Pichanur	88.50	2.20	1.66
4	PVE1	Veerapanur	40.40	14.30	55.05
5	PN1	Navakari	38.50	12.30	50.16
6	PM1	Manaparai	45.00	15.2	57.00
7	PV2	Velanthavalam	30.40	16.00	66.16
8	PN2	Navakari	80.55	8.60	10.5
9	PM2	Manaparai	66.50	10.30	26.11
10	PP1	Pichanur	88.00	5.00	2.22

Screening of PGPR antagonistic activity under *In vitro* conditions

All the isolates were screened against *Fusarium oxysporum* f. sp. *lycopersici* for determine antagonistic activity. Among the ten isolates *Pseudomonas* (PV2) recorded the maximum inhibition zone of 16 mm and 66.16 per cent inhibition of mycelia growth over control followed by PM1 isolate (Table 2). Among the various isolates of *Bacillus* sp, isolate BVE1 significantly reduced the linear mycelia growth of *F. oxysporum* f.sp. *lycopersici* to an extent of 68.33 percent over control with an inhibition zone 18 mm under *In vitro*. It was followed by BV1 isolate (Table 3). Similarly, *Serratia* sp. (SM1)

recorded the maximum inhibition zone of 15.20 mm and 64.44 per cent inhibition of mycelia growth followed by SN1 isolate (Table 4). From the results higher percent inhibition producing PM1, BVE1and SN1 isolates were selected for further studies.

Table 3. Screening of *Bacillus* isolates against *Fusarium oxysporum* f .sp. *lycopersici*

S.No	*Bacillus* isolates	Place of collection	Mycelial growth (mm)	Inhibition zone (mm)	Per cent inhibition
1	BVE1	Veerapanur	28.50	18.00	68.33
2	BN1	Navakari	88.50	0.00	1.38
3	BM1	Manaparai	75.00	9.00	16.66
4	BT1	Thirumalayam palayam	82.20	2.00	8.66
5	BV1	Velanthavalam	55.50	15.00	38.33
6	BP1	Pichanur	85.50	1.50	4.94
7	BVE2	Veerapanur	65.50	10.00	27.22
8	BV2	Velanthavalam	72.50	8.00	19.44
9	BM2	Manaparai	72.00	8.20	20.00
10	BN2	Navakarai	82.20	2.00	8.66

Table 4. Screening of *Serratia* isolates against *Fusarium oxysporum* f .sp. *lycopersici*

S.No	*Serratia* isolates	Place of collection	Mycelial growth (mm)	Inhibition zone (mm)	Per cent inhibition
1	SP1	Pichanur	38.00	12.00	57.77
2	SVE1	Veerapanur	85.50	3.20	5.00
3	SN1	Navakari	53.50	14.00	40.55
4	SM1	Manaparai	32.00	15.20	64.44
5	ST1	Thirumalayam palayam	75.20	6.00	16.44
6	SV1	Velanthavalam	72.50	8.00	19.44
7	SP2	Pichanur	38.00	12.00	57.77
8	SVE2	Veerapanur	81.00	4.00	10.00
9	SP2	Velanthavalam	45.00	10.00	50.00
10	SM2	Manaparai	72.00	8.00	20.00

Table 5. Antagonists against *Fusarium oxysporum* f .sp. *lycopersici* (Dual culture method)

S.No	Antagonists	Place of collection	Days	Mycelia growth (mm)	Inhibition zone (mm)	Per cent inhibition
1	PV2	Velanthavalam	10 Days	30.40	16.00	66.16
			15 Days	28.40	17.50	68.44
			20 Days	20.50	18.50	77.22
2	BVE1	Veerapanur	10 Days	28.50	18.00	68.33
			15 Days	22.20	19.50	75.33
			20 Days	10.50	20.00	88.33
3	SM1	Navakari	10 Days	32.00	15.20	64.44
			15 Days	30.00	17.20	66.66
			20 Days	25.50	17.60	71.66

Evaluation of PGPR Antagonists against *Fusarium oxysporum* f.sp. *lycopersici In vitro*

For dual culture method, Three isolates were screen for *in vitro* antagonism against *Fusarium oxysporum* f .sp. *lycopersici* by dual culture technique (Plate 2). The efficacy of *in vitro* antagonism of three isolates (PV2, BVE1, and SM1) were found increase per cent inhibition over control. The maximum inhibition percentage was observed in BE1 isolates revealed 88.33 per

cent inhibition of growth of *Fusarium oxysporum* f. sp. *lycopersici,* over control (Table 5). **In agar well diffusion method,** antagonist bacteria BVE1 recorded the zone of inhibition (6.2 mm) at 10 µl concentration followed by PV2, SM1 were recorded the zone of inhibition of 5.5mm and 3.5mm respectively (Plate 3).

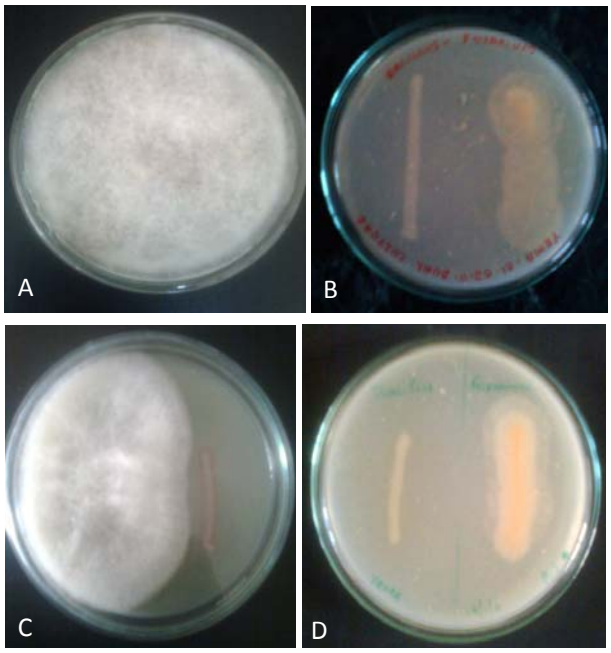


Plate 2. Antifungal activity screening of antagonist bacteria against *Fusarium oxysporum* f.sp. *lycopersici* – Dual culture test A. Control; B. **BVE1 - *Bacillus* sp.; C. SM1- *Serratia* sp.; D. PV2 - *Pseudomonas* sp.**

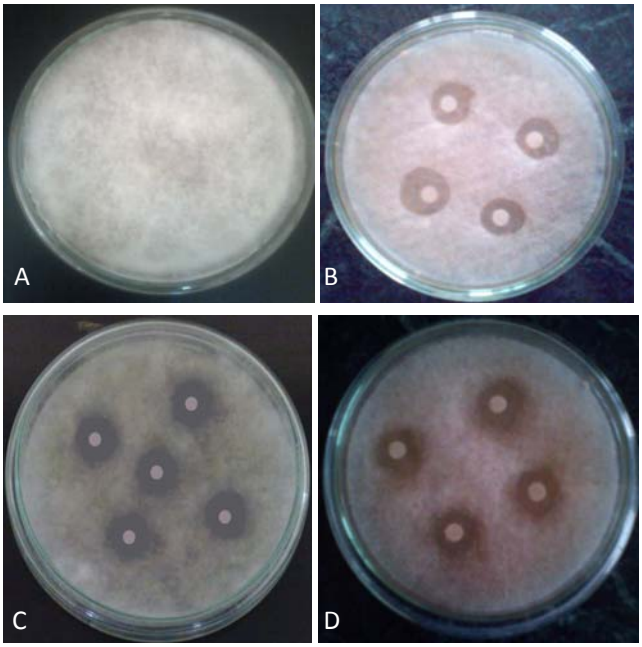
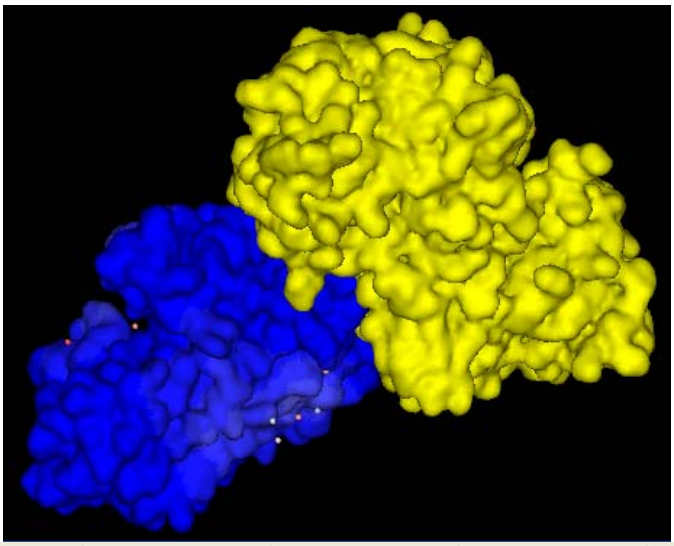


Plate 3. Antifungal activity of antagonist bacterial compounds against *Fusarium oxysporum* f. sp. *lycopersici* – Disc method A. Control B. PV2 - *Pseudomonas* sp. C. BVE1 - *Bacillus* sp. D. SM1 - *Serratia* sp.

***In silico* studies**

Antagonist might be produced by antifungal compound. 3-D structural of compound were identified using PDB. *Bacillus* sp., produces some compounds like iturin, bacilysin, surfactin, fengycin etc, Like wise *Pseudomonas* sp., produces some compounds like Zwittermicin, 2,4-diacetyl-phloroglucinol etc where as *Serratia* sp., produces compound like pyrrolnitrin.

The results of protein protein docking clearly demonstrated that ligand proteins having the activity against the given receptor fungal protein Polygalacturonase. The study was carried out with given input of receptor protein from fungus and ligand proteins from three different bacterial sources. The docking study predictions provide a quantitative energetic measure that supports the *Bacillus* sp. express the polygalacturonase-inhibiting proteins that have been shown to inhibit a variety of Polygalacturonase, followed by *Pseudomonas* sp. towards Polygalacturonase was also observed (Plate 4). Similar studies has been reported by Francesca *et al.,* (2005).



ClusterSize	Density	Rank	ZDock Score	ZRank Score
1	1	1	18.9	-72.272
1	1	2	18.08	-61.098
1	1	3	17.62	-52.57
1	1	4	19.22	-45.215
1	1	5	17.38	-42.456
1	1	6	18.24	-42.136
1	1	7	17.28	-39.56
1	1	8	17.54	-32.792
1	1	9	18.06	-29.087
1	1	10	19.02	-27.874

Plate 4. Docking of 3H7Y –Bacilysin (inhibitor) with Polygalacturonase (Receptor). The fungal protein Polygalacturonase (Receptor) was docked with bacterial protein Bacilysin (inhibitor) of *Bacillus* sp. The top ranked pose 18.9 is the best docking pose.

DISCUSSION

During the last decade Fusarium wilt in tomato has become a serious problem worldwide. Due to the economic importance of yield losses caused by the pathogen, the objective of our study was to

find biological solutions to suppress the fungus. A new approach in crop protection to reduce the disease damage level and several PGPR strains and microorganisms were reported in many crops for the control of fungal pathogens (Bharathi *et al*., 2004).

In the present study, rhizosphere soil of tomato was collected from different tomato growing areas of Dindigul and Coimbatore District, Tamil Nadu. Different bacterial strains were isolated and higher percent inhibition producing PM1, BVE1and SN1 isolates was identified as *Bacillus* sp. and *Pseudomonas* sp, *Serratia* sp. Similarly in earlier reports biocontrol strains has been isolated from rhizosphere soil (Lottmann *et al*., 2000; Kurze *et al*., 2001; Scherwinski *et al*., 2008). Likewise *B. cereus* X16, a halophilic strain isolated from salty soils, inhibited strongly the growth of *F. roseum var. sambucinum* on solid medium as well as on wounded potato tubers (Sadfi *et al*., 2001)

Fusarium is relatively common within subtropical and tropical environments and some species can be pathogenic to several plant species (Bokshi *et al*., 2003). *Fusarium oxysporum* f. sp. *lycopersici*, the fungus that causes Fusarium wilt, attacks only certain tomato cultivars. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remainder of the plant. Diseased plants show yellowing of lower leaves and pronounced vascular discoloration. The vascular discoloration often extends in the upper stem. Growers often cut the stem to see vascular discoloration to confirm the field diagnosis. Fusarium can be easily isolated from stem tissue.

As per the survey conducted in Coimbatore and Dindigul Districts, incidence of wilt complex disease was observed higher in Thondamuthur (Coimbatore), Ayyalur villages (Dindigul District) as compare to other places. From the infected plant material, the pathogen was isolated and identified as *Fusarium oxysporium* f.sp. *lycoperisici*. *In vitro* antifungal activity studies (Dual culture) showed that, three bacterial strains namely PM1, BVE1and SN1 showed biocontrol activity against the disease complex causing Fusarium wilt under laboratory conditions. Production of antibiotics such as iturin, bacillomycin, zwittermycin A and surfactin is responsible for their antifungal action of antagonistic organisms (Sangita and Shah, 2000). The mechanism through which the antagonistic bacteria, act on the plant pathogens includes the production of antagonistic proteins, antibiotics, lytic enzymes etc. In the present studies for *in vitro* antagonism of bacterial antagonists revealed that the *Pseudomonas* sp. (PV2), *Bacillus* sp. (BVE1) *Serratia* sp. (SM1) inhibited the mycelia growth of *Fusarium oxysporium* f.sp. *lycoperisici* compared to control. The inhibition of pathogen might be due to the production of antifungal metabolites. Similarly production of antibiotics or other inhibitory substances by *B. cereus*, *B. licheniformis* and *B. subtilis* against several avocado post-harvest pathogens was demonstrated by using the dual culture technique as well as using the indirect agar-plate method by Upadhyay and Jayaswal, (1992).

The example of *Bacillus* sp. confirmed strain-specificity, which was found for antagonistic mechanisms of plant-associated bacteria (Berg *et al*., 2002). Here, we demonstrated that *Bacillus* isolates (BVE1) was produced some unidentified compounds, in which significantly reduced the phytotoxicity of culture of *F.oxysporum* f.sp. *lycopersici.* Antimicrobial activity of such type of lipopeptide antibiotics produced by *Bacillus* sp. has been described by Yakinov, (1995).

The Protein Data Bank (PDB) was a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data typically

obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world and can be accessed at no charge on the internet. The structure of compound was identified using PDB. Protein-protein docking is the task of assembling separate protein components into protein-protein complexes or assemblies using computational methods. Typically such methods start with the three-dimensional structures of the individual components, often referred to as a protein receptor and a protein ligand. Such predictions can help researchers gain early insights into cellular function and aid rational approaches to disease treatment and drug design.

Understanding the interactions that proteins make with other proteins in a cell is of critical importance to understanding processes such as signal transduction, cell regulation, and molecular recognition. However, experimental determination of the structures of such complexes is not trivial and recent efforts have focused on computational methods.

The interactions between bacterial compound and receptor compound of fungal compound have been studied by ZDOCK docking program. The docking study predictions provide a quantitative energetic measure that supports the *Bacillus* sp. proteins have an inhibitory activity towards receptor Polygalacturonase and inhibitory activity of *Pseudomonas* sp. towards Polygalacturonase. Further it is concluded that *Bacillus* sp. proteins have inhibition activity towards fungal protein.

From the study it was revealed that the *Bacillus* sp. can also be used as the biocontrol agent in agricultural field to reduce the wilt incidence of tomato and for obtaining a better growth and yield. However, more detailed and specialized studies, such as on the interaction with indigenous soil microorganisms and application method of the agent, are needed for effective and secure control of Fusarium in the fields.

REFERENCES

Anitha, A., and Rabeeth, M. 2009. Control of fusarium wilt of tomato by bioformulation of *Streptomyces griseus* in green house condition. *African J. of Basic and Applied Science*. 1(1-2): 9 – 14.

Anonymus. 2009. *Science Reporter*.

Asha, B. B., Chandra Nayaka, S., Udaya Shankar, A. C., Srinivas, C., and Niranjana, S. R. 2011. Biological control of *F. oxysporum* f. sp. l*ycopersici* causing wilt of tomato by *Pseudomonas fluorescens*. *Int. J. Microbiology Research.* 3(2): 79-84.

Berg, G. 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol*. 84: 11-18.

Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. *Appl. and Envt. Microb.* 68: 3328–3338.

Bharathi, R., Vivekananthan R., Harish, S., Ramanathan A., and Samiyappan, R. 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Protection*. 23: 835-843.

Bokshi, A. I., Morris, S. C., and Deverall, B. J. 2003. Effects of benzothiadiazole and acetylsalicylic acid on beta-1,3-glucanase activity and disease resistance in potato. *Plant Pathol*. 52: 22-27.

Cappucino, J. G., and Sherman, N. 1992. *Microbiology, a laboratory manual*. The Benjamin/Cummings Publishing Company Inc, California.

Chen, R., Mintseris, J., Janin, J., and Weng, Z. 2003b. A protein-protein docking benchmark. *Proteins*. 52: 88-91.

Choudary, D., and Johri, B. 2008. Interactions of *Bacillus* spp. and plants-with special reference to Induced Systemic Resistance (ISR). *Microbiol. Res*.10:1016-1019.

Clayet-Marcel, J., Larcher, M., Bartrand, H., Rapior, S., and Pinochet, X. 2001. Plant growth enhancement by rhizobacteria. In: JF Morot-Gaudry (ed) Nitrogen assimilation by plant: physiological, biochemical, and molecular aspects. *Science publishers Inc*. 4: 145-152.

Dennis, C., and Webester, J. 1971. Antagonist properties of species group of *Trichoderma*. 3. Hyphal interaction. *Trans. Br. Mycol. Soc*. 57: 263- 369.

Francesca Sicilia, Juan Fernandez-Recio, Claudio Caprari, Giulia De Lorenzo, Demetrius Tsernoglou, Felice Cervone., and Luca Federici, 2005. The poly-galacturonase-inhibiting protein PGIP2 of *Phaseolus vulgaris* has evolved a mixed mode of inhibition of Endo-polygalacturonase PG1 of *Botrytis cinerea*. *Plant Physiology*. 139:1380–1388.

Fravel, D. R. 2005. Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol*. 43: 337-359.

Kucuk, C., and Kivanc, M. 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turk. J. Biol*. 28: 111-115.

Kurze, S., Bahl, H., Dahl, R., and Berg, G. 2001. Biological control of fungal strawberry diseases by *Serratia plymuthica* HRO-C48. *Plant Disease*. 85: 529–534.

Lottmann, J., Heuer, H., de Vries, J., Mahn, A., Düring, K., and Wackernagel, W. 2000. Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. *FEMS Microbiology Ecology*. 33: 41–49.

Rajappan, K., and Ramarej, B. 1999. Evaluation of fungal and bacterial antagonists against *Fusarium monili-forme* causing wilt of cauliflower. *Annals of Plant Protection Sciences*. 7: 205-207.

Sadfi, N., Cherif, M., Fliss, I., Boudabbous, A., and Antoun, H. 2001. Evaluation of *Bacillus* isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of Fusarium dry rot of potato tubers. *J. Plant. Pathol*. 83: 101-118.

Sandhu, S. S., Kanojiya, A., Mishra, P. K., and Vikrant, P. 2004. Antifungal activity of *Bacillus thuringiensis* var *kurstaki* on *Trichoderma viridae. Proceeding in National Academic science* (USA) 74B.

Sangita Bapat, and Shah, A. K. 2000. Biocontrol of fusarium wilts of pigeon pea by *Bacillus brevis*. *Can. J. Microbiol*. 46: 125-132.

Scherwinski, K., Grosch, R., and Berg, G. 2008. Effect of bacterial antagonists on lettuce: active biocontrol of *Rhizoctonia solani* and negligible, short-term effect on non-target microbes. *FEMS Microbiology Ecology*. 64: 106–116.

Schulz, B., Wanke, S., Draeger, S., and Aust, H. J. 1993. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycol. Res.*, 97:1447-1450.

Smith, G. R., and Sternberg, M. J. 2002. Prediction of protein-protein inter-actions by docking methods, Curr. Opin. *Struct. Biol.*12(1): 28-35.

Sternberg, M. J., Gabb, H. A., Jackson, R. M., and Moont, G. 2000. Protein-protein docking. Generation and filtering of complexes. *Methods Mol. Biol*. 143: 399-415.

Upadhyay, R. S., and Jayaswal, R. K. 1992. *Pseudomonas cepacia* causes mycelial deformities and inhibition of conidiation in phytopathogenic fungi. *Current Microbiology*. 24: 181 - 187.

Yakinov, M. M., Timmis, K. N., Wray, V., and Fredrickson, H. L. 1995. Character-ization of a new lipopeptide surfactant produced by thermotolerant and halo-tolerant subsurface *B. licheniformis*. *Appl Environ Microbiol*. 61: 1706.