JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2417-2424

Antimycotic Activity of Acidophilic Bacteria from Acid Soil of Odisha against Some Phytopathogens

S.K. Nayak and B.B. Mishra

Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha 751003, India.

(Received: 08 September 2013; accepted: 12 November 2013)

In view of control of phytopathogens by soil bacteria an attempt was taken in the present investigation to screen and isolate antifungal bacteria from lateritic soil zone (20.11 to 20.85 latitude and 85.01 to 85.54 longitude) acid soil (pH 4.9-5.6) region of Odisha covering four districts. About 6 bacterial species isolated from two districts were found to be promising antimycotic *in vitro*. The organisms were subjected to various biochemical tests and were identified as *Bacillus subtilis* and *Bacillus azotoformans* from Dhenkanal district and *Bacillus farraginis* and *Bacillus amyloliquefaciens* from Nayagarh district as per ABIS online software. *B. amyloliquefaciens* showed significant inhibition zone (18mm) against the pathogen *P. notatum*.

Key words: Acid soil, Antifungal bacteria, Phytopathogens, Antimycotic activity, ABIS ONLINE.

Control of plant disease is a pressing need for agriculture in the 21st century. The increasing demand for a steady, healthy food supply by a burgeoning human population requires controlling crop diseases that reduce crop yield[1]. Biological control, using microorganisms to suppress plant disease offers a powerful alternative to the use of synthetic chemicals. The rich diversity of the microbes provides a seemingly endless resource for this purpose. Increasing the abundance of a particular strain in the vicinity of a plant can suppress disease without producing lasting effects on the rest of the microbial community or other organisms in the ecosystem[2,3].

Most bacteria produce antimicrobial compounds, such as broad spectrum classical antibiotics, posses biologically active fungicidal properties. Although many *Bacillus* species synthesize relatively abundant antimicrobial compounds[4], the possibility for screening of a new species of *Bacillus* being antimycotic producer is considered to be one of the major interests in bacteriocins research[5]. A strain of *Bacillus subtilis* is highly effective for crop protection from the pathogens *Fusarium* sp. and *Rhizoctonia* sp., as well as in stimulating plant growth[6,7].

Mycotoxins are toxic secondary metabolites produced under appropriate environmental conditions by filamentous fungi, mainly by species of *Aspergillus*, *Penicillium* and *Fusarium*[8]. The discovery and characterization of antimycotic compounds produced by organisms isolated from extreme environments are not only of interest of the day, but also potentially important to industry. Such organisms, may provide a new and more efficient means for the inhibition of target microorganisms, with production of unique compounds.

One of the modern approaches is screening of bacteria for antimicrobial activities from varied environments. In this regard acid soil (lateritic soil) of Odisha carries a significant importance as little or no microbiological study has been done in past with a view to their biotic potential. On account of that an attempt was made

* To whom all correspondence should be addressed.
E-mail: bb_mishra58@yahoomail.com

in the present study for the isolation, screening and identification of antifungal bacteria from lateritic soil region of Odisha.

MATERIALS AND METHODS

Soil sampling and isolation of bacteria

Experimental soil samples were collected from rice ploughed field of Acid soil regions (pH 4.9-5.6) of Odisha during early spring season. Top 0–15 cm of soil was collected aseptically, covered with plastic bags, air dried for 48hrs powdered and used for the study. 10gms soil sample was mixed with double distilled water (soil: water = 1:2 w/v) for soil pH measurement. Standard isolation method was followed taking specimens randomly, without any prior knowledge of the microbial composition of the source under investigation. Traditional *in vitro* cultivation method was followed for heterotrophic bacterial isolation on Nutrient Agar (NA) media (Himedia, India) plates in triplicate with pH 5.4, 5.0, 4.9 and 5.6 for Dhenkanal, Cuttack, Jajpur and Nayagarh samples respectively. A total of thirty six (serial 1-36) bacteria were isolated from Acid soils (Dhenkanal, Cuttack, Jajpur and Nayagarh) in Odisha.

Microscopic identification

Identification of bacterial isolates was carried out by colony characteristics on NA plate and Gram's reaction.

Revival of fungal pathogens

The phytopathogens (*Aspergillus fumigatus*, *Penicillium notatum* and *Fusarium* sp.) were grown in different medium. *A. fumigatus* and *P. notatum* were grown aerobically for 48hr at 25°C in PDA (Potato Dextrose Agar)[9]. *Fusarium* sp. was grown on RBCA (Rose Bengal Chloramphenicol agar) (Himedia, India) and successfully revived prior to experimental use.

Bacterial inocula preparation

Bacterial crude cultures used for bioassay were prepared in NB (Nutrient Broth) with appropriate acidic condition. Cultures were incubated at 37°C for 24hr on a shaker incubator. For testing antimicrobial activity, PDA (Potato dextrose agar) medium was used.

In vitro Antimycotic assays

Antimycotic activity of the bacterial isolates was determined by agar diffusion technique. Antimycotic assays were performed on 9cm Petri plates containing 25ml of PDA medium. A well was made by sterile Cork Borer (diameter, 6mm) in the centre of the Petri plate. 100-µl aliquot of 24hr old culture was pipetted into the well. Plates were incubated at 30°C for 24-48hr. Effectivity of the isolates was assessed by growth inhibition of pathogenic fungi. The results were reported by measuring the diameter of inhibition zone. Three plates with equal measurement were used for each sample and the experiment was repeated thrice. The data was computed and statistically analysed. Antimycotic activities of the isolates were measured in terms of percent inhibition (P.I.) of fungal growth.

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

Where 'C' is the diameter of fungal growth in control plate and 'T' is diameter of fungal growth in test plate[10,11]. The total P.I. (TPI) can be calculated from the below formula.

[TPI=100-P.I.]

Biochemical Characterization

After the microscopic examination the Gram negative and Gram Positive bacteria were subjected to biochemical test for identification. Enzymatic and tests for sugar utilization were conducted as per the requirement of the bacterial identification software ABIS (Advanced Bacterial Identification Software) online[12]. Gram +ve and -ve (rods & cocci) bacteria were identified from the Gram's reaction, morphology and colony characteristics on basal media up to generic level. For all biochemical tests 24hr old cultures were used. A control was run separately for comparison.

RESULTS

Experimental soil samples collected from lateritic soil regions of Odisha (Dhenkanal, Cuttack, Jajpur and Nayagarh) were tested acidic in nature with pH ranging from 4.8-6.2. The area ranges to a lowest of 19m to highest of 90m altitude (Table-1). Ten samples were collected from each of 4 places adapting standard sampling technique weighing 150gm each.

A total of 36 bacteria were isolated from acid soil viz. 8 independent colonies from Dhenkanal coded as DOD-1 to DOD-8, 5 bacteria from Cuttack district starting from COD-1 to COD-5, 8nos. bacterial colonies from Jajpur district coded

as JOD-1 to JOD-8 and 15nos. of bacterial colonies were isolated from acid soil of Nayagarh district coded as NOD-1 to NOD-15. On evaluation of antifungal activity of the bacterial isolates out of 36 isolates in total, 5 isolates inhibited growth of *Aspergillus fumigatus in vitro*. Rest of the bacteria failed to produce any inhibition zone against the pathogen. Bacteria isolated from Cuttack and Jajpur were proved to be impotent for antifungal properties. Among the isolates NOD-14 was the most significant inhibitor (Fig. 1) as compared to the control (C.D. at $p<0.05=0.91$) (Table 2). Amongst the isolates from the acid soil region, five isolates registered zone of inhibition against *Penicillium notatum*; 2 from Dhenkanal and 3 from Nayagarh district. From plate assay it is confirmed that the bacteria from Jajpur and Cuttack district were unable to inhibit the growth of the fungus. Maximum inhibition was reported in NOD-14 followed by NOD-10 and NOD-5. Other organisms didn't produce any insignificant difference between the control and the treatment and found at par with control 89.9±0.6 (C.D. at $p<0.05=0.62$) (Table 2). The inhibitory strength of the bacteria from the lateritic soil region was tested against *Fusarium* sp., the producers of fumonisins. Only 4nos were able to exhibit antifungal activity in plate assay by agar diffusion method. NOD-10 produced 16mm zone of inhibition followed by NOD-14 (Fig.-2). Only a single bacterium (DOD-4) from Dhenkanal district produced inhibition zone against *Fusarium* sp. There was no significant difference between the control and the treatment as regards inhibition by other bacteria 89.9±0.6 (C.D. at $p<0.05=1.003$) (Table 2).

The Total percentage of inhibition was determined. The whole spent broth (WSB) of NOD-14 inhibited 22.50% growth of *P. notatum* in comparison to 17.50% by NOD-10 followed by 15.00% by DOD-2. The highest percentage of inhibition against *A. fumigatus* was shown by WSB of NOD-14 up to 21.25% followed by 17.50% and 15.00% by NOD-10 and DOD-4 respectively. *Fusarium* sp. inhibited the least amongst the pathogens up to 15.00% by DOD-4, 17.50% by NOD-14 and maximum up to 20.00% by WSB of NOD-10(Table 3).

The colony characteristics ranged from circular medium to large size and irregular spreading configuration with colour ranged between off white

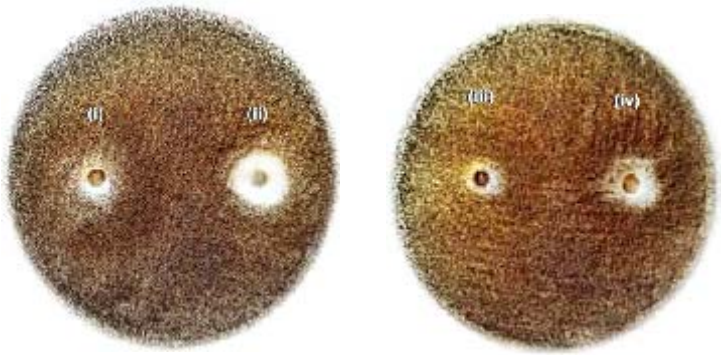

Fig. 1. *In vitro* antimycotic assay of whole spent broth antifungal bacterial isolates (i), (ii), (iii) & (iv) shows DOD-2, NOD-14, DOD-4 & NOD-10 against *Aspergillus fumigatus*

Table 1. Geographical distribution of different acid soil sampling region of Odisha

Sampling site	Geographical Indications			pH	Samples collected in g
	Latitude	Longitude	Altitude MSL in m		
Mahishapat, Dhenkanal	20.67	85.54	76	5.3-6.2	150
Barang, Cuttack	20.27	85.52	23.5	5.0-5.1	150
Chandikhol, Jajpur	20.85	86.33	19	4.8-5.5	150
Ranpur, Nayagarh	20.11	85.01	90	5.3-5.9	150

MSL- Mean Sea Level

Table 2. Inhibitory zone of the bacterial isolates from varying sampling sites of different agro eco regions (sub-regions) against *A. fumigatus*, *P. notatum* and *Fusarium* sp.

Soil Sampling (Sl. No.)	Bacteria Isolated		Zone of Inhibition(mm)		
			A. fumigatus	*P. notatum*	*Fusarium* sp.
Sampling site-1'a'	1	DOD-1	-	12	-
	2	DOD-2	<10	12	-
	3	DOD-3	-	-	-
	4	DOD-4	12	-	12
	5	DOD-5	-	-	-
	6	DOD-6	-	-	-
	7	DOD-7	-	-	-
	8	DOD-8	-	-	-
Sampling site-1'b'	9	COD-1	-	-	-
	10	COD-2	-	-	-
	11	COD-3	-	-	-
	12	COD-4	-	-	-
	13	COD-5	-	-	-
Sampling site-1'c'	14	JOD-1	-	-	-
	15	JOD-2	-	-	-
	16	JOD-3	-	-	-
	17	JOD-4	-	-	-
	18	JOD-5	-	-	-
	19	JOD-6	-	-	-
	20	JOD-7	-	-	-
	21	JOD-8	-	-	-
Sampling site-1'd'	22	NOD-1	-	-	-
	23	NOD-2	-	-	-
	24	NOD-3	-	-	-
	25	NOD-4	-	-	-
	26	NOD-5	12	14	16
	27	NOD-6	-	-	-
	28	NOD-7	-	-	-
	29	NOD-8	-	-	-
	30	NOD-9	-	-	-
	31	NOD-10	14	14	16
	32	NOD-11	-	-	-
	33	NOD-12	-	-	-
	34	NOD-13	-	-	-
	35	NOD-14	17	18	14
	36	NOD-15	-	-	-
	C.D. at p<0.05		0.91	0.81	1.003

'a'- sub humid to humid eastern & south eastern upland; 'b'- Eastern Ghats hot moist sub-humid eco-sub-region; 'c'- Eastern plateau (chhotanagpur) and Eastern Zone; 'd'- Eastern Ghats, hot moist sub humid eco sub region; Unidentified bacteria are numbered where alphabet represents their respective places of sample collected; "D"- Dhenkanal, "N"- Nayagarh, "J"-Jajpur, "C"- Cuttack, "OD"-Odisha.

Table 3. Percentage of Inhibition by whole spent broth of antifungal bacterial isolates

Phytopathogens	Bacterial Inhibition (%)			
	DOD-2	DOD-4	NOD-10	NOD-14
A. fumigatus	-	15.00	17.50	21.25
P. notatum	15.00	-	17.50	22.50
Fusarium sp.	-	15.00	20.00	17.50

Table 4. Morphological characteristic of bacterial isolates from lateritic acid soil of Odisha

Sl. No.	Colony Characteristic	Gram's Variable
DOD-2	Circular, Medium size, Fade yellow colour, Entire margin, Lustreless surface, Flat colonies, Opaque	Positive, Rods
DOD-4	Circular, Medium size, Off white colour, Erose margin, Lustreless surface, Convex colonies, Opaque	Positive, Rods
NOD-5	Circular, Large size, Fade Yellow Colour, Entire margin, Convex colonies, Opaque	Negative, Rods
NOD-10	Circular, Large size, White Colour, Entire margin, Flat appearance, Opaque	Positive, Rods
NOD-14	Off white Colour, Irregular and spreading configuration, Lobate margin, Flat, Opaque	Positive, Rods

"D" denotes Dhenkanal District; "N" denotes Nayagarh District and Odisha abbreviated as "OD"

and fade yellow. Colonies were with entire, erose or lobate margin. All 5 colonies were opaque with 1 negative and rest positive to Gram's reaction and all are rod shaped in micrograph (Table-4).

There are two bacteria from Dhenkanal district which were active against almost all pathogens and these were biochemically characterized. Following the biochemical result in Advanced bacterial identification software (ABIS) online the bacterium coded DOD-1 was found to be *Bacillus subtilis* and DOD-2 was *Bacillus azotoformans* (Table-5). The bacteria isolated from Cuttack and Jajpur failed to prove their activity against the fungal pathogens. The isolates from acid soil of Nayagarh were also identified by ABIS online. Out of 15 bacteria only 3 were antifungal bacteria and amongst them 2 were most potent and it is found that the bacterium NOD-10 was *Bacillus farraginis* and NOD-14 was *Bacillus amyloliquefaciens* (Table-6).

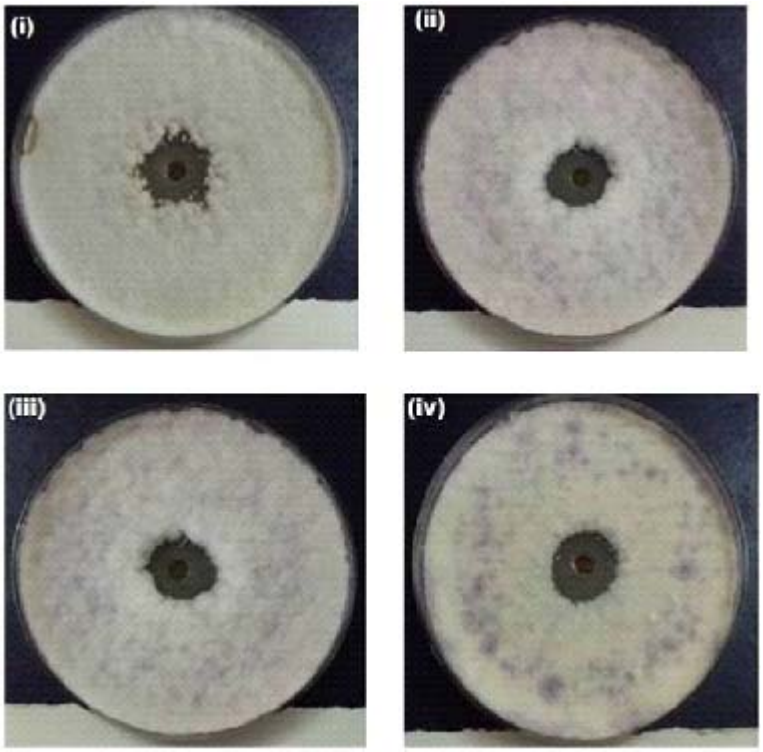

Fig. 2. *In vitro* antimycotic assay of whole spent broth of acidophilic antifungal bacteria against *Fusarium* sp.

Table 5. Biochemical characteristics of the most potent bacterial isolates from Dhenkanal district active against Phytopathogens

Sl. No.	IMVIC Tests				Enzyme Activity (Extracellular and Intracellular)								Salt Tolerance	Sugar Utilization							
	MR	VP	IP	CU	SD	EH	GL	PH	Oxi	Cat	Ure	Nit	(NaCl)	Glu	Suc	Ara	Fru	Gal	Lac	Mal	Rib
DOD-2	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
DOD-4	+	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-

"D" denotes Dhenkanal District and Odisha abbreviated as "OD": MR- Methyl Red, VP- Voges-Proskauer, IP- Indole Production, SD- Starch Degradation, EH- Esculin Hydrolysis, CU- Citrate Utilization, GL- Gelatin Liquefaction, PH- Pectin Hydrolysis, OXi- Oxidase, Cat-Catalase, Ure- Urease, Nit- Nitrate. Glu- Glucose, Suc- Sucrose, Fru- Fructose, Gal- Galactose, Lac-Lactose, Mal-Maltose, Rib-Ribose.

Table 6. Biochemical characteristics of the most potent bacterial isolates from Nayagarh District against Phytopathogens

S.	IMVIC Tests			Enzyme Activity (Extracellular and Intracellular)								Mot	Sugar Utilization																Antibiogram Profile						
No.	MR	VP	IP	CU	SD	EH	Ure	Oxi	Cat	PH	CH		A	L	R	M	S	T	Mo	Su	C	F	D	G	Gy	I	X	Rh	PB	C	CT	G	NA	CIP	ST
NOD-10	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	R	R	R	S	S	S	R
NOD-14	+	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	R	R	S	R	R	S	R

"N" denotes Nayagarh District and Odisha abbreviated as "OD" '+'symbolizes Utilizes Sugar; '-' symbolizes unable to utilize sugar; MR- Methyl Red, VP- Voges-Proskauer, IP- Indole Production, CU- Citrate Utilization, SD- Starch Degradation, EH- Esculin Hydrolysis, Ure- Urease, Oxi- Oxidase, Cat-Catalase, PH- Pectin Hydrolysis, CU- Casein Hydrolysis Mot- Motility Test; A- Arabinose; L-Lactose; R-Rhamnose; M-Maltose; S-Salicin; T-Trehalose; Mo-Mannose; Su-Sucrose; C-Cellobiose; F-Fructose; D-Dextrose; G- D Glucose; Gy- Glycerol; I- Inositol; X- Xylose; Rh- Raffinose. S-Sensitive; R-Resistance; PB-Polymyxin-B; C-Chloramphenicol; CT-Co-Trimaoxazole; G-Gentamycin; NA-Nalidixic Acid; CIP- Ciprofloxacin; ST-Streptomycin

DISCUSSION

Microbial diversity of extreme environment is of significant importance to soil microbiologist as they play an important role being acclimatized to the environment. Soil is considered one of the most suitable environments for microbial growth[13]. The acid soil (pH 4.8-6.2±0.10) favours growth and sustenance of variety of microbes especially bacteria having adaptability to acidic environment[9].

Application of this screening procedure gives useful information for the identification of possible potent organisms. These methods suffer from the limitation of not specifically demonstrating bactericidal activity. The antifungal activities of bacteria were evaluated by agar diffusion method as it is simple and cost effective[14]. The present study emphasizes on screening of bacterial isolates from Acid soil region of Odisha and biochemical characterization of most potent isolates against some plant pathogens.

A no. of soil bacteria isolated from rhizospheric region were able to inhibit the *Fusarium* sp. in dual culture method up to 60.00%[15]. However, the bacteria isolated from Jajpur and Cuttack districts were unable to inhibit the phytopathogen *A. fumigatus*, *P. notatum* and *Fusarium* sp. a serious rice pathogen. Out of five bacteria 3 bacteria from Nayagarh and 2 from Dhenkanal districts were inhibiting the fungus in the range of 12 to 18mm. NOD-14 produces the maximum of 18mm inhibition zone against *P. notatum* followed by 17mm against *A. fumigatus*. The maximum zone of inhibition with 16mm was registered by NOD-5 while DOD-4 comes with the lowest inhibition of 12mm against *Fusarium* sp. which is the first report from the acid soil region of Odisha. However, the biocontrol potential of *B. subtilis*, have been reported as effective against a broad spectrum of plant diseases caused by soil borne[16] and foliar fungal pathogens[17].

Amongst the 36 isolates obtained in primary screening, four potent antifungal isolates were gone through biochemical characterizations. 2 isolates form Dhenkanal district (DOD-2 and DOD-4) and 2 isolates from Nayagarh districts were inhibiting almost all pathogens. Biochemical characters of 2 Dhenkanal isolates revealed that they belongs to *Bacillus* sp. were identified as *Bacillus subtilis* (probability 94%) and *Bacillus azotoformans* (probability 92%). The biochemical characteristics of Nayagarh isolates showed that NOD-10 as *Bacillus farraginis* (probability 80%) and NOD-14 as *Bacillus amyloliquefaciens* (probability 82%) by ABIS ONLINE software[18].

Antifungal activity of *Bacillus* sp. isolates against *A. fumigatus*, *P. notatum* and *Fusarium* sp. was related to its ability to produce antifungal compounds similar as previously reported by other *Bacillus* sp. The growth of *Penicillium* was inhibited by >50% by 14 strains of *Bacillus* sp. *in vitro*[19]. *B. subtilis* strain GA1 has potential to control disease of apple caused by fungus[20].

Most of the *Bacillus* are non-pathogenic, with high secretion capacity and are competent in producing various biological substances such as secretory proteins, enzymes, biofilms, biosurfactants and antibiotics. Screening of different antibiotics from natural sources is increasingly essential to agricultural realm as pathogenic bacteria develop resistance against commonly used therapeutic agents. On comparison, the zone of inhibition against *P. notatum* by *B. amyloliquefaciens* was more and superior amongst 5 isolates. The zone of inhibition of other isolates didn't produce insignificant difference between control and treatment and found at par with control 89.9±0.6 (C.D. at p=0.05=0.81) (Table-2).

ACKNOWLEDGEMENTS

Financial support by UGC, New Delhi is duly acknowledged.

REFERENCES

1. Emmert, A.B., Handelsman, J. Biocontrol of plant disease: a (Gram-) positive perspective. *FEMS Microbiol.*, 1999; **171**: 1-9.
2. Gilbert, G.S., Parke, J.L., Clayton, M.K., Handelsman, J. Effects of an introduced bacterium on bacterial communities on roots. *Ecology*,1993; **74**: 840-854.
3. Osburn, R.M., Milner, J.L., Oplinger, E.S., Smith, R.S., Handelsman, J. Effect of Bacillus cereus UW85 on the yield of soybean at two field sites in Wisconsin. *Plant Dis.*, 1995; **79**: 551-556.
4. Lisboa, M.P., Bonatto, D., Bizani, D.,

Henriques, J.A.P., Brandelli, A. Characterisation of a bacteriocin-like substances produced by produced by Bacillus amyloliquefaciens isolated from the Brazilian Atlantic forest. *Int. Microbiol.*, 2006; **9**: 111-118.

5. Nayak, S.K., Mishra, B.B. Antimicrobial activity of *Bacillus* sp. metabolites extracted in polarity gradient based solvent system against *T. mentagrophytes*, a dermatophyte. *The Ecoscan*, 2012; **6**(1&2): 57-60.
6. Turner, J.T., Backman, P.A. Factors relating to peanut yield increases after seed treatment with Bacillus subtilis. *Plant Dis.*, 1991; **75**: 347-353.
7. Backman, P.A., Brannen, P.M., Mahaffee, W.F.: Plant response and disease control following seed inoculation with Bacillus subtilis. In: *Proceedings of the Third International Workshop on Plant Growth-Promoting Rhizobacteria* (Ryder, M.H., Stephens, P.M. and Bowen, G.D., Eds.) CSIRO, South Australia: 1994; pp. 3-8.
8. Bennett, J.W., Klich, M. Mycotoxins. *Clinical Microbiol. Rev*., 2003; **16**: 497.
9. Fateixa, S., Neves, M.C., Adelaide, A., João, O., Tito, T. Antifungal activity of SiO_2/Ag_2S nanocomposites against *Aspergillus niger*. *Colloids and Surfaces B: Biointerfaces*, 2009; **74**: 304-308.
10. Jeyalakshmi, C., Durairaj, P., Seetharaman, K., Sivaprakasam. Biocontrol of fruit rot and dieback of chilli using antagonistic microorganisms. *Indian Phytopathology*, 1998; **51**(2): 180-183.
11. Nayak, S.K., Santra, G.H., Mishra, B.B. Antimycotic Potential of Bacterial Ethanolic Extract Isolated from Acid Soil of Mahishapat, Odisha. *J. of Pure. and Appl. Microbiol.*, 2012; **6**(4): 1853-1858.
12. Costin, S., Ionut, S. *ABIS online* - Bacterial identification software, http:// www.tgw1916.net/bacteria _logare.html 2007-2012.
13. Cavalcanti, M.A., Oliveira, L.G., Fernandes, M.J., Lima, D.M. Filamentous fungi isolated from soil in districts of the Xingo´region, Braz. *Acta Bot. Bras*, 2006; **20**(4): 831-837.
14. Scorzoni, L., Benaducci, T, Almeida, A.M.F., Silva, D.H.S., Bolzani, V.S., Mendes, M.J.S. Comparative study of disk diffusion and Comparative study of disk diffusion and micro dilution methods for evaluation of antifungal activity of natural compounds against medical yeasts *Candida* spp and *Cryptococcus* spp. *Rev Ciênc Rev Ciênc Farm Básica Apl.*, 2007; **28**(1): 25-34.
15. Sandikar, B.M., Awasthi, R.S. Screening of antagonistic *Bacillus* sp. against phytopathogenic *Pythium* and *Fusarium* sp. *Asian J. of Microbiol., Biotech. & Environ. Sc. P.*, 2010; (3): 527-532.
16. Asaka, O., Shoda, M. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Appl. Environ. Microbiol*, 1996; **62**: 4081-4085.
17. Romero, D., de Vicente, A., Rakotoaly, R., Dufour, S., Veening, J., Arrebola, E. Cazorla, F., Kuipers, O., Paquot, M., Pérez-García, A. The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward Podosphaera fusca. *Mol. Plant- Microbe Interact*, 2007; **20**: 430-440.
18. Thangaraj, N., Sharan, S., Suneetha, V. Role of *Proteus mirabilis* in Caffeine Degradation – A Preliminary Bioinformatics Study. *Rs. J. of Recent Sc.*, 2013; **2**: 33-40.
19. Arras, G., D'hallewin, G. In vitro and in vivo control of *Penicillium digitatum* and *Botrytis cinerea* in citrus fruit by *Bacillus subtilis* strains. *Ag. Mediterr.*, 1994; **124**: 56-61.
20. Toure, Y., Ongena, M., Jacques, P., Guiro, A., Thonart, P. Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. *J. of Appl. Microbiol.*, 2004; **96**: 1151-1160.